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BIIB021, an orally available and small-molecule inhibitor of HSP90, activates intrinsic apoptotic pathway in human cervical adenocarcinoma cell line (HeLa)

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Abstract. – OBJECTIVE: Heat shock protein 90 (HSP90) is a highly conserved ATP-dependent chaperone protein that plays a vital role in tumorigenesis. This study aims to investigate the apoptosis inducer role of BIIB021 (orally available HSP90 inhibitors) compound *via* inhibition of HSP90 activity in the human cervical cancer cell line (HeLa).

PATIENTS AND METHODS: The anticancer potential of BIIB021 was determined by XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)] cell proliferation assay against the human cervical cancer cell line (HeLa). ATPase and luciferase aggregation assays were carried out to detect the HSP90 inhibitor potential of BIIB021. To determine the antiproliferative mechanism of the BIIB021, the expression level of the pro-apoptotic and antiapoptotic markers was determined by reverse transcription polymerase chain reaction (RT-PCR) and ELISA experiments.

RESULTS: BIIB021 exhibited a cytotoxic effect on HeLa cell proliferation and the inhibitory concentration $(IC)_{50}$ dose of BIIB021 was found to be 14.79 nM at 48 h. BIIB021 decreased the ATP hydrolysis rate of HSP90 and blocked the refolding of the desaturated luciferase in the presence of ATP. To understand the antiproliferative mechanism of the BIIB021 in HeLa cells, the mRNA and protein expression levels of the apoptotic markers [BCL-2 associated X (BAX), B-cell lymphoma 2 (BCL-2), cytochrome-c (CYT-c), and caspase-3 (CAS-3)] were determined by RT-PCR and ELISA experiments. The results obtained indicated that BIIB021 decreased BCL-2 levels and increased BAX, CYT-c, and CAS-3 levels in human cervical cancer cells.

CONCLUSIONS: These results confirmed that BIIB021 inhibited the chaperone activity of HSP90, resulting in anti-proliferating effects in cervical cancer cells *via* the induction of the intrinsic apoptotic pathways.

Key Words:
Chemotherapy, Cervical cancer, HSP90, BIIB021, Apoptosis, HeLa.

Introduction

Cervical cancer is the fourth most common cancer type among women worldwide. In 2020, approximately 600,000 women were diagnosed with cervical cancer and about half of these cases died globally[1]. Decreased incidence of squamous cell cervical cancer, the most common histopathological subtype, has been determined in the last three decades. However, an increase in the incidence of cervical adenocarcinoma, the second most common histological type, has been detected from 5% to 20%. Human papillomavirus (HPV) infection is the most important risk factor for cervical cancer, accounting for 99% of the squamous type and 85% of adenocarcinoma types. Moreover, smoking, a weakened immune system, and the use of diethylstilbestrol (an artificial form of estrogen) by mothers of patients during pregnancy are other risk factors for the development of cervical cancer[1-6]. Chemotherapy is one of the therapeutic options for the treatment of cervical cancer, particularly in advanced stages. Platinum-based drugs (cisplatin and carboplatin), paclitaxel (Taxol), topotecan, and bevacizumab are widely used to treat locally advanced and metastatic cervical cancer[7,8]. To improve treatment outcomes of cervical cancer, researchers[9-11] have been focused on the development of effective target-specific chemotherapeutics. In this context, HSP90 has a big biological potential for designing next-generation target-specific drugs for the treatment of cervical cancer.

HSP90 is an important member of the chaperone protein family, and its expression is extremely increased in cancer cells. HSP90 stabilizes and activates oncogenic client proteins (growth factor receptors, steroid hormone receptors, tyrosine kinases, cell cycle regulators, and transcriptional

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factors) and stimulates malignant progression[12-15]. Especially, apoptotic machinery is closely associated with HSP90 activity in cancer cells. Up-regulation of HSP90 promotes cancer cell survival and protects cells against apoptosis. To suppress intrinsic and extrinsic apoptotic pathways in tumors, HSP90 decreases the release of cytochrome-c (CYT-c) by activating B-cell lymphoma 2 (BCL-2) and blocks the activation of caspase 8/10 in the death-inducing signaling complex[15-18]. Inhibition of HSP90 activity disrupts oncogenic signaling pathways *via* the degradation of oncogenic client proteins and stimulates apoptosis. Therefore, inhibition of HSP90 has been a significant pharmaceutical strategy for the treatment of cancer[19-22].

HSP90 is composed of three conserved domains: N-terminal domain (NTD), middle domain (MD), and C-terminal domain (CTD). NTD contains ATP binding pocket, and ATP hydrolysis energy provides proper conformation of HSP90 for the proper folding mechanism of oncogenic client proteins. Therefore, the ATP binding pocket of the NTD has been an important target in developing effective HSP90 inhibitors. Most HSP90 inhibitors have higher binding affinity than ATP for NTD[15,23,24]. The anticancer potential of HSP90 inhibitors has been extensively evaluated against a wide variety of cancer types in experimental and clinical studies[12,14,15] for nearly 20 years. In clinical studies[25,26], first-generation HSP90 inhibitors [geldanamycin, 17-N-allyl-17-demethoxygeldanamycin (17-AAG), radicicol, and retaspimycin HCl (IPI-504)] did not display therapeutic efficiency on cancer types for their solubility problems and adverse effects. Therefore, orally available next-generation HSP90 inhibitors have been designed for therapeutic applications on cancer types[27].

BIIB021 (also known as CNF2024) (3',5'-dimethyl-4'-methoxy-2'-pyridyl derivative) is the first orally administrated and fully synthetic HSP90 inhibitor that has been tested in *in-vitro*, *in-vivo* and clinical studies[28-30] to understand its anticancer activities against cancer types. BIIB021 selectively and potently blocks HSP90 ATPase activity, thereby inhibiting the proper folding of oncogenic client proteins (Figure 1). Experimental studies[28,29] reported that BIIB021 shows a higher affinity for the NTD region of HSP90 compared to ATP and other inhibitors (BIIB021: ~1.7nM and ~17-AAG: 4.6 nM). Therefore, BIIB021 is a promising HSP90 inhibitor in next-generation cancer drug discovery.

In experimental and clinical studies, the anticancer potential of BIIB021 has not been studied on cervical cancer yet. Thus, in this study, we investigated the underlying mechanisms of BIIB021 on inhibition of cell proliferation with induction of apoptotic pathway in the human cervical adenocarcinoma cell line (HeLa). This study posited the hypothesis that HSP90 inhibition by BIIB021 might be a new therapeutic strategy for the treatment of cervical cancer.

Materials And Methods

Materials

BIIB021 (6-Chloro-9-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-9H-purin-2-amine) was supplied from Adooq (Irvine, CA, USA). HeLa cell line was from ATCC (American Type Culture Collection, Manassas, VI, USA). Eagle's Minimum Essential Medium (EMEM), trypsin-ethylenediaminetetraacetic acid (EDTA), penicillin-streptomycin solution, heat-inactivated fetal bovine serum (FBS), and L-glutamine were from Capricorn (Ebsdorfergrund, Germany). XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)] cell proliferation kit was supplied by Biological Industries (Kibbutz Beit-Haemek, Israel). ELISA kits were purchased from Sunred Biological Technology Co., Ltd (Shanghai, China). Primers were synthesized from Macrogen Corp (Seoul, South Korea). Total RNA isolation kit was from Thermo Scientific (Waltham, MA, United States). The one-step EvaGreen qRT-PCR kit was from SNP Biotechnology (Ankara, Turkey). BCA total protein kit was from Serva (Heidelberg, Germany). HSP90 expression plasmid was synthesized from Biomatik (Ontario, Canada).

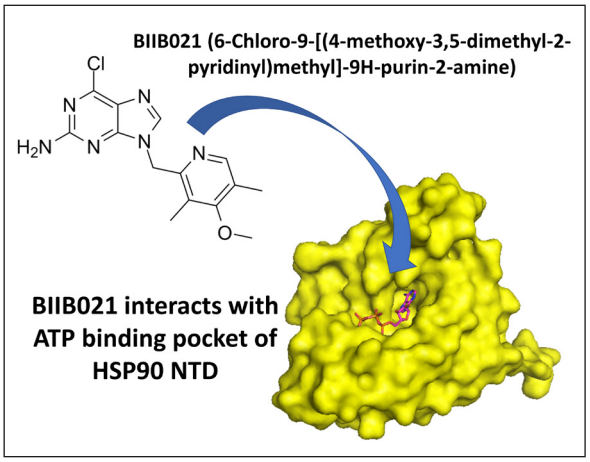

Figure 1. Schematic representation of the interaction of BIIB021 with HSP90 NTD.

XTT Assay

HeLa cells were cultured in EMEM medium containing 10% FBS, 1% L-glutamine, 100 IU/mL penicillin, and 10 mg/mL streptomycin at 37ºC in a humidified atmosphere of 95% air and 5% CO_2. The cells were plated at a density of 2×10^5 cells/well in 96-well plates. After overnight incubation, the cells were treated with different concentrations of BIIB021 (100 nM, 50 nM, 25 nM, 12.5 nM, 6.25 nM, 3.125 nM, 1.5625 nM), and the cells were cultured for further 48 h. After 48 h incubation, a colorimetric XTT cell proliferation kit was applied according to the manufacturer's protocol. The absorbance was measured at 450 nm using an ELISA reader (Santa Clara, CA, USA) and the inhibitory concentration $(IC)_{50}$ value of BIIB021 was calculated with GraphPad Prism 8.0 software (La Jolla, CA, USA).

Expression and Purification of HSP90

Human HSP90 gene-inserted plasmid DNA was transformed into BL21 Star™(DE3) cells, and they were spread on Lysogeny Broth-Agar (LB-Agar) plates containing ampicillin. The single colony on plates was selected, and cells were grown in 10 ml of LB solution overnight. Mini-culture was transferred were into 1 L of LB containing ampicillin and protein expression was induced by isopropyl-β-D-thiogalactoside (IPTG). After incubation, the cells were harvested and lysed by ultrasonicator in buffer solution (pH 7.4, 20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole). HSP90 was purified by affinity chromatography using nickel-nitrilotriacetic acid (Ni-NTA) resin.

ATPase Assay

The inhibition of ATPase activity of HSP90 with BIIB021 was determined by coupled enzyme assay (pyruvate kinase/lactate dehydrogenase)[31]. Recombinant HSP90 protein (10 µg/µl) was incubated at 37°C for 5 minutes in 500 µl of buffer solution [pH 7.4, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 50 mM NaCl, 4 mM MgCl2, 0.2 mM NADH, 0.5 mM phosphoenolpyruvate, 18 Unit lactate dehydrogenase (LDH), 24 Unit pyruvate kinase/1 ml]. IC_{50} dose of BIIB021 (14.79 nM) and 0.5 mM ATP were added to the mixture, and the formation of adenosine diphosphate (ADP) from ATP was measured at 340 nm.

Luciferase Aggregation Assay

The effect of BIIB021 on HSP90 chaperone activity was determined by luciferase aggregation assay[31]. Luciferase was denatured using urea and diluted with buffer solution (pH: 7.4, 25 mM HEPES, 50 mM KCl, 5 mM MgCl2, 2 mM ATP, and 5 mM dithiothreitol). HeLa cell lysate (cell lysate contains HSP90 and its co-chaperones) was added to the reaction mixture. To increase HSP90 chaperone activity, ATP and recombinant HSP90 were added to cell lysate-containing reaction mixture and the IC_{50} dose of BIIB021 (14.79 nM) was applied to the mixture for monitoring the inhibition of HSP90 luciferase reactivation. The level of reactivated luciferase aggregates was measured spectrometrically at 320 nm.

Determination of BAX, BCL-2, CYT-c, and CAS-3 Gene Expression

The gene expression levels of the *BAX, BCL-2, CYT-c,* and *CAS-3* were determined in BIIB021-treated HeLa cells. HeLa cells were exposed to IC_{50} dose of BIIB021 (14.79 nM) for 48 h and total RNA was extracted using a commercial kit according to the manufacturer's instructions. The concentrations of the RNA samples were measured using the nanodrop instrument. Then, the reverse transcription polymerase chain reaction (RT-PCR) experiment was set up with *BAX, BCL-2, CYT-c,* and *CAS-3* primers using a one-step EvaGreen qRT-PCR kit. *GAPDH* was used as a housekeeping gene. The sequences primers of human *BAX*, *BCL-2, CYT-c, CAS-3*, and *GAPDH* were as follows: *BAX* sense, 5'-TCAGGATGCGTCCACCAAGAAG-3' and antisense, 5'-TGTGTCCACGGCGGCAATCATC-3'; *BCL-2* sense, 5'-ATCGCCCTGTGGATGACTGAGT-3' and antisense, 5'-GCCAGGAGAAATCAAACAGAGGC-3'; *CYT-c* sense, 5'-CGTTGTGCCAGCGACTAAAAA-3' and antisense, 5'-GATTTGGCCCAGTCTTGTGC-3'; *CAS-3* sense, 5'-ACATGGAAGCGAATCAATGGACTC-3' and antisense, 5'-AAGGACTCAAATTCTGTTGCCACC-3'; *GAPDH* sense, 5'-GTCTCCTCTGACTTCAACAGCG-3' and antisense, 5'-ACCACCCTGTTGCTGTAGCCAA-3'. Briefly, PCR reactions were amplified for 1 cycle: 50°C for 30 min, 1 cycle: 95°C for 3 min, and 40 cycles: 95°C for 15 s. and 60°C for 1 min. PCR amplification was performed in a final volume of 25 µL using the Roche Light Cycler 480 instrument (Rotkreuz, Switzerland). The relative expression level of genes was calculated using the $2^{-\Delta\Delta Ct}$ method.

Determination of BAX, BCL-2, CYT-c, and CAS-3 Protein Expression

To analyze the protein expression levels of the *BAX, BCL-2, CYT-c,* and *CAS-3* in treated HeLa

cells with IC_{50} values of the BIB021 (14.79 nM), commercial ELISA kits were used. After treatment, HeLa cell lysate was prepared using radioimmunoprecipitation assay (RIPA) lysis buffer, and the protein concentration of cell lysates was measured using a BCA protein assay kit. The cells *BAX, BCL-2, CYT-c,* and *CAS-3* protein levels were measured using human ELISA kits and were performed according to the manufacturer's instructions. The protein level of the control group was accepted as 100%, and the results of the BIIB021 group were determined as a percentage compared to the control group.

Statistical Analysis

Statistical analysis and comparable data groups were assessed using GraphPad Prism 7.0 (Boston, MA, USA) software by Student's *t*-test and one-way ANOVA test. Probability values of $p<0.05$ were considered to be statistically significant.

Results

XTT Assay

The anticancer activity of BIIB021 was tested on the human cervical cancer cell line (HeLa) by XTT cell proliferation assay. IC_{50} value of BIIB021 was calculated to be 36.15 nM and 14.79 nM on the HeLa cell line at 24 h and 48 h, respectively. As shown in Figure 2, BIIB021 decreased HeLa cell proliferation in a time and dose-dependent manner.

ATPase Assay

To investigate the inhibitory effect of the IC_{50} dose of BIIB021 on the ATPase function of HSP90, an ATP hydrolysis experiment was performed. As shown in Figure 3, the IC_{50} dose of BIIB021 dramatically decreased the ATP hydrolysis rate of HSP90.

Luciferase Aggregation Assay

The inhibitory effect of BIIB021 on the chaperone activity of HSP90 was evaluated using a luciferase aggregation assay. Refolding level of denatured luciferase by HSP90 was measured in HeLa cells treated with BIIB021. In this assay, cell lysate showed weak refolding activity of HSP90, and the addition of ATP in the reaction mixture increased the refolding of denatured luciferase. However, the IC_{50} dose of BIIB021 decreased the luciferase refolding activity of HSP90 in the presence of ATP (Figure 4).

Determination of BAX, BCL-2, CYT-c, and CAS-3 Gene and Protein Expression in HeLa Cells

To understand the cytotoxicity mechanism of the BIIB021, the alteration of expression levels of the apoptosis-related markers (*BAX, BCL-2, CYT-c,* and *CAS-3*) were determined by RT-PCR and ELISA experiments in the HeLa cell line (Figure 5 and Figure 6). The mRNA and protein levels of the BAX and CYT-c increased in HeLa cells after exposure to the BIIB021. When cervical cancer cells were treated with

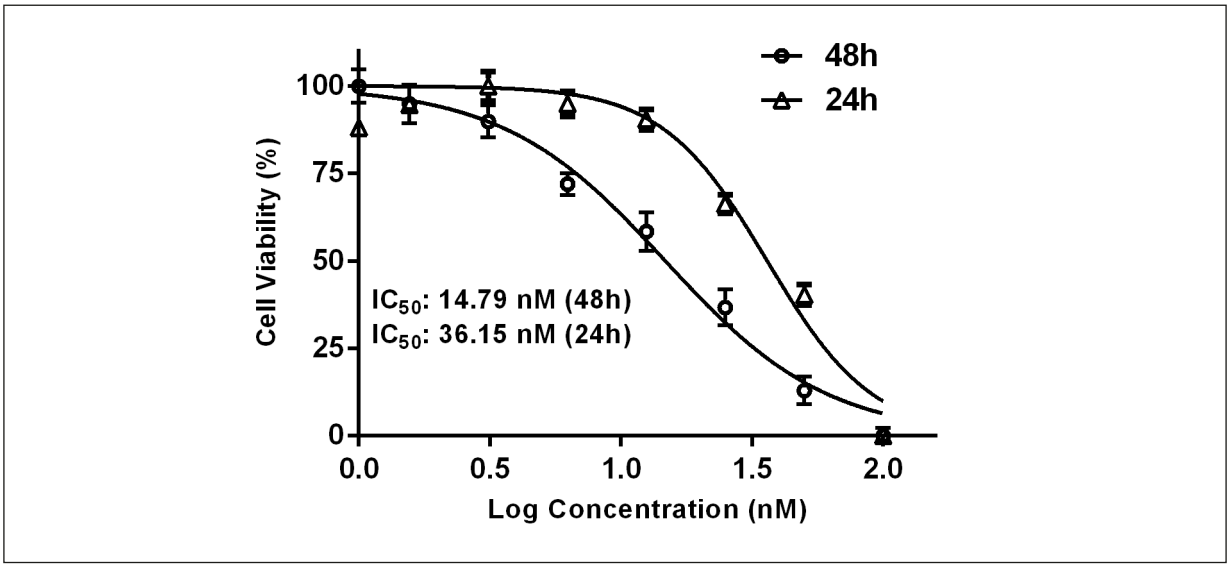

Figure 2. XTT cell proliferation result of BIIB021 in HeLa cell line. IC_{50} doses of BIIB021 were calculated to be 36.15 nM and 14.79 nM on HeLa cell line at 24 h and 48 h, respectively. Vertical bars indicate the standard deviation. Values are expressed mean±SEM (n>3).

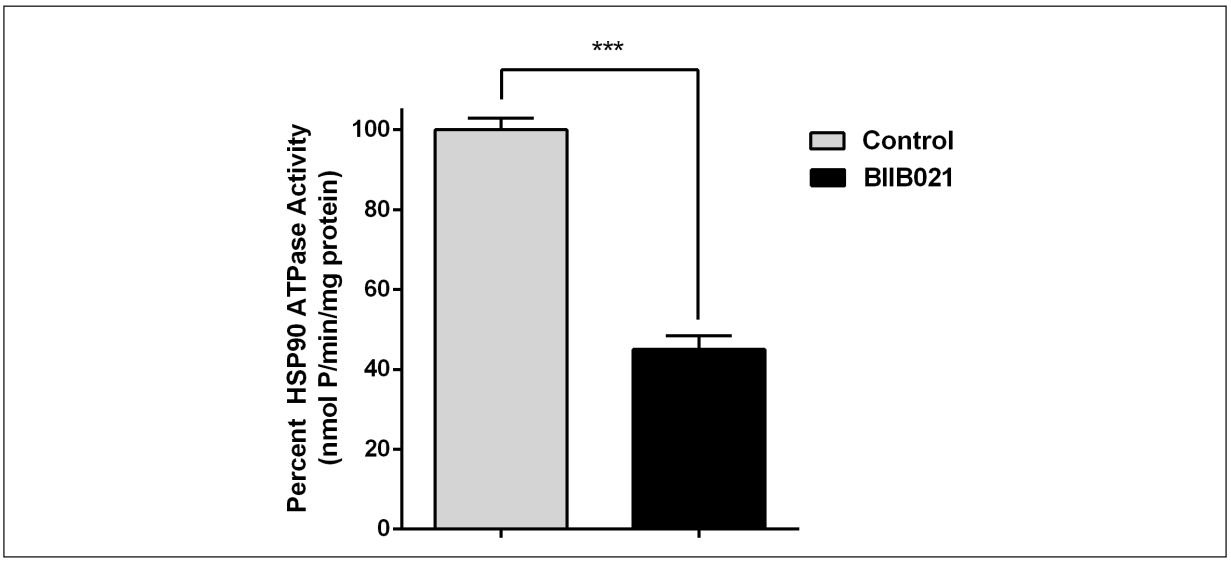

Figure 3. Measurement of HSP90 ATPase activity in the presence of BIIB021. Values are expressed mean±SEM (n>3). ***p<0.001 compared to control.

the IC_{50} value of BIIB021, the protein expression of CAS-3 significantly increased, while no statistically significant change was observed in the mRNA expression level.

Discussion

Overexpression of HSP90 suppresses apoptotic pathways for stimulation of cancer cell proliferation. Therefore, the inhibition of HSP90 activity has been a significant strategy in cancer treatment. BIIB021 is a fully synthetic orally available HSP90 inhibitor that interacts with the ATP binding region of HSP90, resulting in HSP90 chaperone dysfunction in cancer cells[11,15,22]. In the current study, the apoptotic inducer role of BIIB021 was evaluated on a human cervical cancer cell line (HeLa) with assays.

In the literature, the anticancer potential of BIIB021 has been evaluated against a wide variety of cancer types. Lundgren et al[29] reported that BIIB021 exhibited an antiproliferative effect on human breast cancer (BT474 and MCF-7), gastric cancer (N87), colon cancer (HT-29), non-small

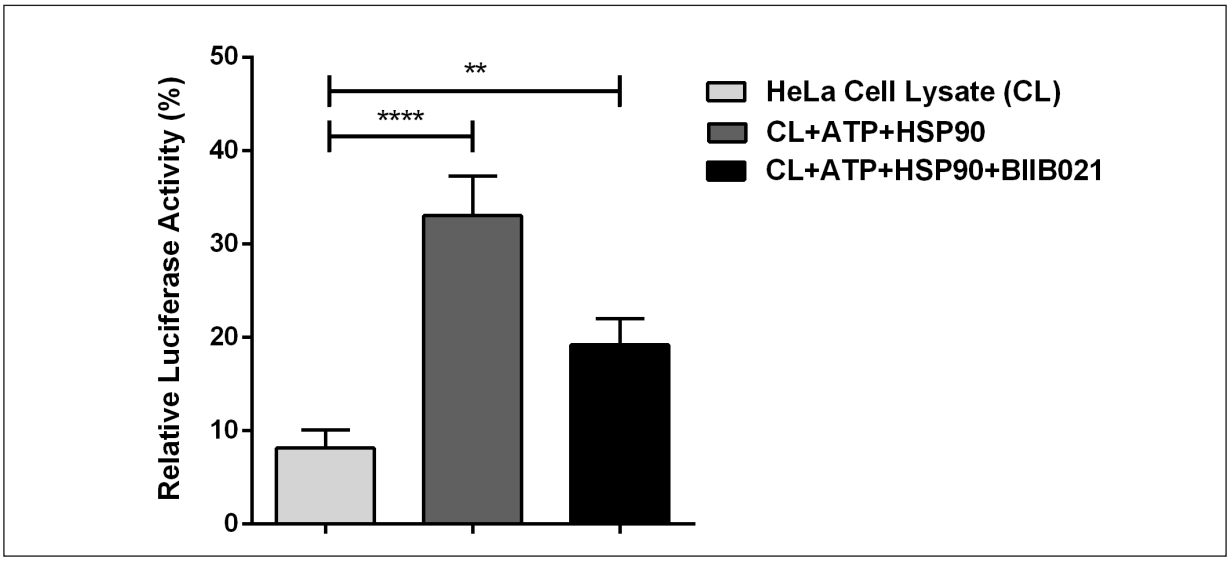

Figure 4. Luciferase aggregation assay in the presence of BIIB021. Values are expressed mean±SEM (n>3). **p<0.01, ****p<0.0001 compared to control.

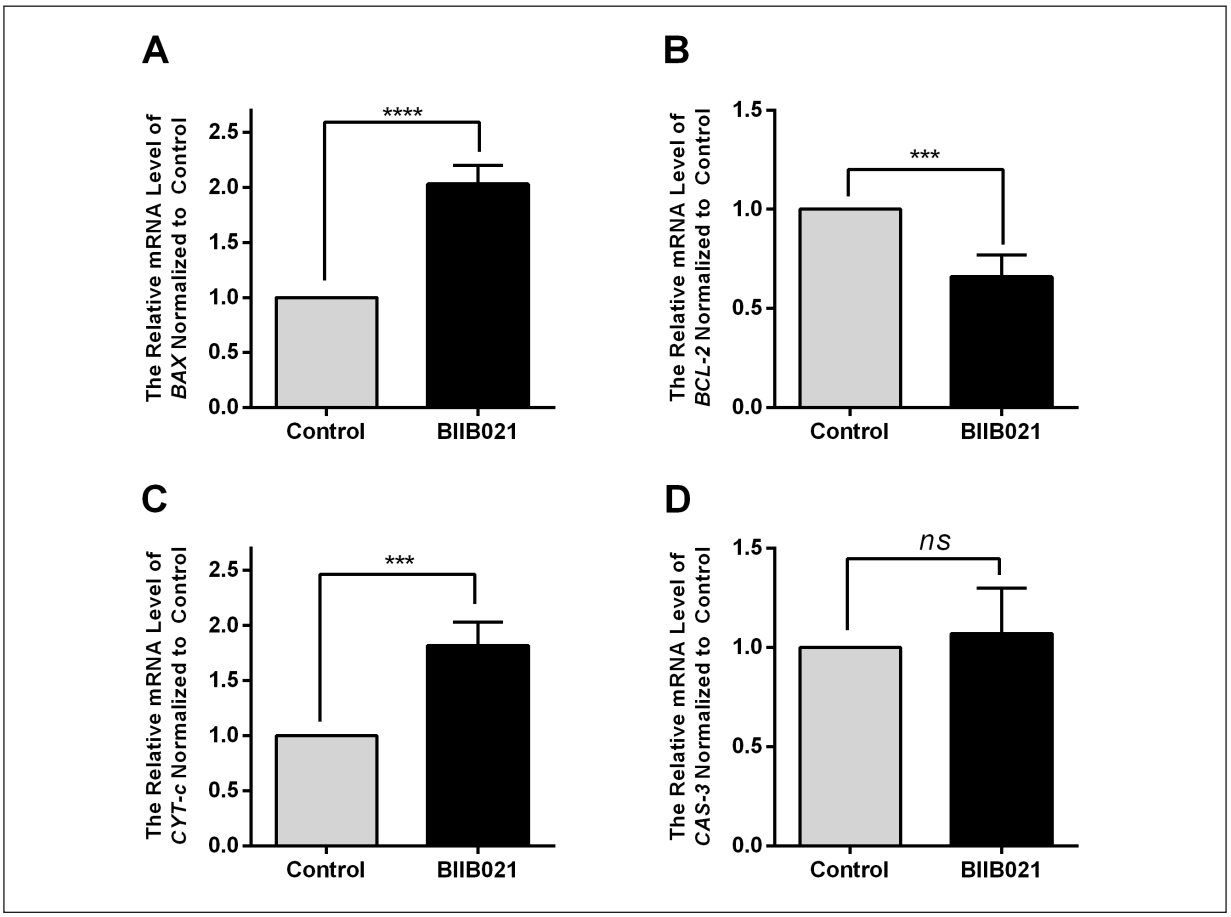

Figure 5. The effect of BIIB021 on level of apoptosis related gene expressions in HeLa cells. Alteration of mRNA levels of *BAX* (**A**), *BCL-2* (**B**), *CYT-c* (**C**), and *CAS-3* (**D**) were determined by using RT-PCR analysis. Values are expressed mean±SEM (n>3). ***p<0.001, ****p<0.0001 compared to control and ns: not significant.

cell lung cancer (H1650 and H1299), and small cell lung cancer (H69 and H82) cell lines with IC_{50} value ranging from 60 nM to 310 nM. In another study[32], BIIB021 decreases Eca109 and Eca9706 (esophageal squamous cell carcinoma) cell proliferation with IC_{50} values of 661.10 nM and 53.31 nM, respectively. Kim et al[33] investigated the cytotoxic potential of BIIB021 on human thyroid carcinoma cells (TPC-1 and SW1736). Administration of cells with BIIB021 at 10 µM exhibited significant toxicity against TPC-1 and SW1736 cell lines. According to these toxicity values, BIIB021 effectively decreased proliferation at the nM level in HeLa cervical cancer cell line.

HSP90 requires ATP hydrolysis energy to provide proper folding of oncogenic client proteins. HSP90 is a dimeric protein, and its crystal structure is similar to the letter "V" (open conformation) in the absence of ATP. In this state, oncogenic client proteins interact with hydrophobic residues in the interior of MD for proper folding. Hydrolysis of ATP in the NTD triggers the change of the open conformation of HSP to the closed conformation. After the dimerization of CTD, the nascent substrate protein acquires its proper folded structure. Therefore, designing an inhibitor with a higher affinity for the ATP binding site of HSP90 than ATP is an important therapeutic approach in cancer treatment. This assay supports that BIIB021 is a potent HSP90 inhibitor by inhibiting ATP hydrolysis function[34,35].

In the literature, BIIB021 inhibited luciferase refolding across lung cancer (A549), colon cancer (HCT116), and prostate cancer (PC3-MM2) cell lines in the range of 40-70 nM. Furthermore, NVP-AUY922 (a highly potent HSP90 inhibitor) exhibited inhibition of luciferase refolding in A549 and PC3-MM2 cells at around 500 nM and 60 nM, respectively[36]. Geldanamycin is the first natural HSP90 inhibitor that prevented refolding of luciferase at a concentration of about 200 nM[37]. Compared to these results, the low concertation

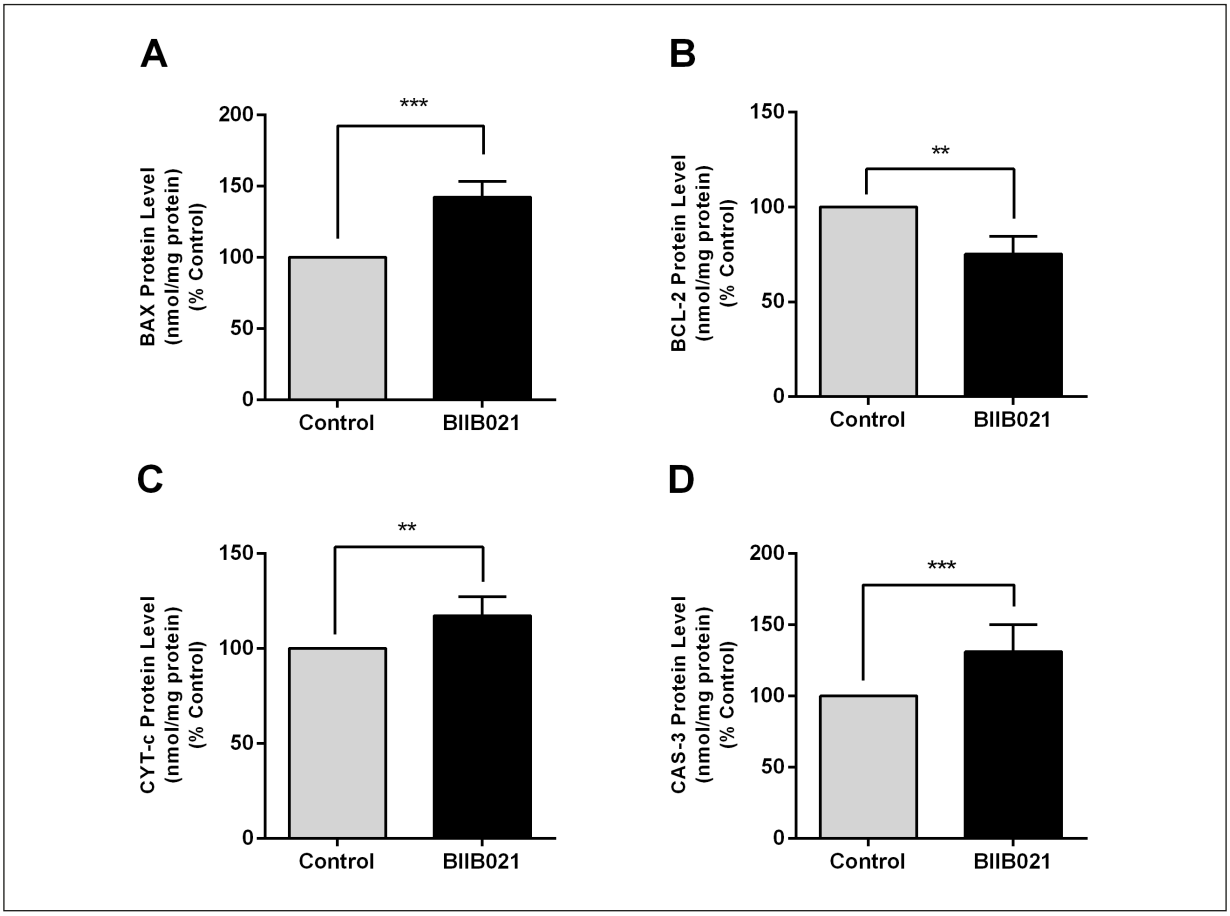

Figure 6. The effect of BIIB021 on level of apoptosis related protein expressions in HeLa cells. Alteration of protein levels of *BAX* (**A**), *BCL-2* (**B**), *CYT-c* (**C**), and *CAS-3* (**D**) were determined by using RT-PCR analysis. Values are expressed mean±SEM (n>3). $^{**}p<0.01$, $^{***}p<0.001$ compared to control.

of BIIB021 (14.79 nM) inhibited luciferase refolding activity in HeLa cells in the presence of ATP. It should be noted that BIIB021 is a highly potent HSP90 inhibitor in HeLa cells, according to luciferase aggregation assay.

Avoidance of apoptosis is a vital biological process for the survival of cancerous cells, regardless of cancer type. Therefore, the main mechanism of many anticancer drugs is the activation of apoptotic signaling pathways in cancer cells. Basically, stimulation of pro-apoptotic markers and inhibition of anti-apoptotic markers involved in intrinsic pathways have been significant approaches for cancer drug design[38,39]. The intrinsic mechanism of apoptosis is mainly regulated by the mitochondria. The pro-apoptotic proteins [BCL-2 associated X (BAX), BCL-2 antagonist/killer (BAK), BH3 interacting domain death agonist (BID), BCL-2 family apoptosis regulator (BOK), etc.] provide disruption of mitochondrial outer membrane permeability (MOMP), resulting in the release of CYT-c into the cytosol. Then, CYT-c binds to the apoptotic protease activating factor 1 (APAF-1) to form an apoptosome complex. The apoptosome activates CAS-9 to initiate the CAS-3-dependent proteolytic cascade. The antiapoptotic proteins, including BCL-2, inhibit disruption of MOMP by interacting with pro-apoptotic proteins. The upregulation of BCL-2 promotes cancer cell survival by abrogating apoptosis. Therefore, the inhibition of BCL-2 and activation of BAX is known as significant molecular mechanisms to provoke intrinsic apoptotic pathways in cancer cells[40-42].

HSP90 is accepted as an important prognostic factor in cancer cells and is closely related to the intrinsic apoptotic pathway. Overexpression of HSP90 prevents caspase activation and inhibits apoptotic pathways in different cellular models. Particularly, HSP90 controls intrinsic apoptotic pathways in tumors by regulating mitochondrial membrane permeabilization

and CYT-c release. Many experimental studies[16,43-45]. reported that the interaction between HSP90 and BCL-2 blocks the up-regulation of pro-apoptotic factors and prevents the release of CYT-c by the inactivation of CAS-3. Therefore, inhibition of HSP90 provokes an intrinsic apoptotic pathway in tumor cells

The apoptotic potential of BIIB021 and other HSP90 inhibitors has been extensively evaluated in many experimental studies[18,45-48]. Li et al[45] investigated the anticancer activity of BIIB021 against human T-cell acute lymphoblastic leukemia cells (Molt-4). BIIB021 inhibited the proliferation of Molt-4 cells and stimulated apoptotic cell death *via* caspase activation. In another study[46], the anticancer potential of Debio-0932 (next-generation HSP90 inhibitor) was investigated against human breast cancer cell lines (MCF-7 and MDA-MB-231). Debio-0932 triggered the intrinsic apoptotic pathway by increasing the expression ratio of BAX/BCL-2 in these cancer cell lines. STA-9090 (a potent small-molecule inhibitor of HSP90) prevented MDA-MB-231 cell proliferation *via* up-regulation of apoptotic markers poly(ADP-ribose) polymerase (PARP) and BCL-2-like protein 11 (BIM)[47]. According to all these results, next-generation HSP90 inhibitors exhibit great potential for stimulating apoptotic cell death pathways in cancer cells.

Conclusions

In conclusion, BIIB021 interrupted the chaperone function of HSP90 by inhibiting the ATPase process. BIIB021 decreased HeLa cell proliferation by inducing intrinsic apoptotic pathways. BIIB021 provided down-regulation of BCL-2 and increased the expression of BAX, CYT-c, and CAS-3 in cervical cancer cells. These results suggest a promising anticancer activity of BIIB021 against human cervical cancer cells.

Data Availability
Data may be provided on reasonable request to the corresponding author.

Funding
None.

Conflict of Interest
The authors declare that there is no conflict of interest.

Ethics Approval
Not applicable.

Informed Consent
Not applicable.

Authors' Contributions
CMG: Project development, data sampling, investigation, manuscript writing. AÖ: Support writing, Data analysis, manuscript reviewing, statistical analysis. All authors read and approved the final manuscript.

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References

1) Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-249.
2) Berek JS, Hacker NF, Fu YS, Sokale JR, Leuchter RC, Lagasse LD. Adenocarcinoma of the uterine cervix: histologic variables associated with lymph node metastasis and survival. Obstet Gynecol 1985; 65: 46-52.
3) Rositch AF, Levinson K, Suneja G, Monterosso A, Schymura MJ, McNeel TS, Horner MJ, Engels E, Shiels MS. Epidemiology of Cervical Adenocarcinoma and Squamous Cell Carcinoma Among Women Living With Human Immunodeficiency Virus Compared With the General Population in the United States. Clin Infect Dis 2022; 74: 814-820.
4) Allahqoli L, Dehdari T, Rahmani A, Fallahi A, Gharacheh M, Hajinasab N, Salehiniya H, Alkatout I. Delayed cervical cancer diagnosis: a systematic review. Eur Rev Med Pharmacol Sci 2022; 26: 8467-8480.
5) Frega A, Gentili C, Proietti S, Lepore E, Unfer V, Fuso A. Epigallocatechin gallate, folic acid, vitamin B12, and hyaluronic acid significantly increase apoptosis and p53 expression in HeLa cells. Eur Rev Med Pharmacol Sci 2023; 27: 5240-5245.
6) Azer SA, AlSaleem A, Albassam N, Khateeb R, Alessa A, Aljaloud A, Alkathiri S. What do university students know about cervical cancer and HPV vaccine? Eur Rev Med Pharmacol Sci 2022; 26: 3735-3744.
7) Regalado Porras GO, Chávez Nogueda J, Poitevin Chacón A. Chemotherapy and molecular therapy in cervical cancer. Rep Pract Oncol Radiother 2018; 23: 533-539.
8) Kumar L, Harish P, Malik PS, Khurana S. Che-

motherapy and targeted therapy in the management of cervical cancer. Curr Probl Cancer 2018; 42: 120-128.

9) Huo Z, Liu K, Zhang X, Liang Y, Sun X. Discovery of pyrimidine-bridged CA-4 CBSIs for the treatment of cervical cancer in combination with cisplatin with significantly reduced nephrotoxicity. Eur J Med Chem 2022; 235: 114271.
10) Hu J, Zhang Y, Tang N, Lu Y, Guo P, Huang Z. Discovery of novel 1,3,5-triazine derivatives as potent inhibitor of cervical cancer via dual inhibition of PI3K/mTOR. Bioorg Med Chem 2021; 32: 115997.
11) Karademir D, Özgür A. The effects of STA-9090 (Ganetespib) and venetoclax (ABT-199) combination on apoptotic pathways in human cervical cancer cells. Med Oncol 2023; 40: 234.
12) Miyata Y, Nakamoto H, Neckers L. The therapeutic target Hsp90 and cancer hallmarks. Curr Pharm Des 2013; 19: 347-365.
13) Birbo B, Madu EE, Madu CO, Jain A, Lu Y. Role of HSP90 in Cancer. Int J Mol Sci 2021; 22: 10317.
14) Neckers L, Workman P. Hsp90 molecular chaperone inhibitors: are we there yet? Clin Cancer Res 2012; 18: 64-76.
15) Özgür A, Tutar Y. Heat Shock Protein 90 Inhibition in Cancer Drug Discovery: From Chemistry to Futural Clinical Applications. Anticancer Agents Med Chem 2016; 16: 280-290.
16) Wang X, Chen M, Zhou J, Zhang X. HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy (Review). Int J Oncol 2014; 45: 18-30.
17) Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science 1997; 275: 1129-1132.
18) Peng C, Zhao F, Li H, Li L, Yang Y, Liu F. HSP90 mediates the connection of multiple programmed cell death in diseases. Cell Death Dis 2022; 13: 929.
19) Ren X, Li T, Zhang W, Yang X. Targeting Heat-Shock Protein 90 in Cancer: An Update on Combination Therapy. Cells 2022; 11: 2556.
20) Zhang J, Li H, Liu Y, Zhao K, Wei S, Sugarman ET, Liu L, Zhang G. Targeting HSP90 as a Novel Therapy for Cancer: Mechanistic Insights and Translational Relevance. Cells 2022; 11: 2778.
21) Mahalingam D, Swords R, Carew JS, Nawrocki ST, Bhalla K, Giles FJ. Targeting HSP90 for cancer therapy. Br J Cancer 2009; 100: 1523-1529.
22) Özgür A. Investigation of anticancer activities of STA-9090 (ganetespib) as a second generation HSP90 inhibitor in Saos-2 osteosarcoma cells. J Chemother 2021; 33: 554-563.
23) Hoter A, El-Sabban ME, Naim HY. The HSP90 Family: Structure, Regulation, Function, and Implications in Health and Disease. Int J Mol Sci 2018; 19: 2560.
24) Tutar L, Tutar Y. Heat shock proteins; an overview. Curr Pharm Biotechnol 2010; 11: 216-222.
25) Gorska M, Popowska U, Sielicka-Dudzin A, Kuban-Jankowska A, Sawczuk W, Knap N, Cicero G, Wozniak F. Geldanamycin and its derivatives as Hsp90 inhibitors. Front Biosci (Landmark Ed) 2012; 17: 2269-2277.
26) Sharp S, Workman P. Inhibitors of the HSP90 molecular chaperone: current status. Adv Cancer Res 2006; 95: 323-348.
27) Sanchez J, Carter TR, Cohen MS, Blagg BSJ. Old and New Approaches to Target the Hsp90 Chaperone. Curr Cancer Drug Targets 2020; 20: 253-270.
28) He W, Hu H. BIIB021, an Hsp90 inhibitor: A promising therapeutic strategy for blood malignancies (Review). Oncol Rep 2018; 40: 3-15.
29) Lundgren K, Zhang H, Brekken J, Huser N, Powell RE, Timple N, Busch DJ, Neely L, Sensintaffar JL, Yang YC, McKenzie A, Friedman J, Scannevin R, Kamal A, Hong K, Kasibhatla SR, Boehm MF, Burrows FJ. BIIB021, an orally available, fully synthetic small-molecule inhibitor of the heat shock protein Hsp90. Mol Cancer Ther 2009; 8: 921-929.
30) Dickson MA, Okuno SH, Keohan ML, Maki RG, D'Adamo DR, Akhurst TJ, Antonescu CR, Schwartz GK. Phase II study of the HSP90-inhibitor BIIB021 in gastrointestinal stromal tumors. Ann Oncol 2013; 24: 252-257.
31) Koca İ, Özgür A, Er M, Gümüş M, Açikalin Coşkun K, Tutar Y. Design and synthesis of pyrimidinyl acyl thioureas as novel Hsp90 inhibitors in invasive ductal breast cancer and its bone metastasis. Eur J Med Chem 2016; 122: 280-290.
32) Wang XT, Bao CH, Jia YB, Wang N, Ma W, Liu F, Wang C, Wang JB, Song QX, Cheng YF. BIIB021, a novel Hsp90 inhibitor, sensitizes esophageal squamous cell carcinoma to radiation. Biochem Biophys Res Commun 2014; 452: 945-950.
33) Kim SH, Cho YK, Huh JH, Kang JG, Ihm SH, Choi MG, Lee SJ. Heat Shock Protein 90 Inhibitors AUY922, BIIB021 and SNX5422 Induce Bim-mediated Death of Thyroid Carcinoma Cells. Anticancer Res 2020; 40: 6137-6150.
34) Pearl LH. Review: The HSP90 molecular chaperone-an enigmatic ATPase. Biopolymers 2016; 105: 594-607.
35) Meyer P, Prodromou C, Hu B, Vaughan C, Roe SM, Panaretou B, Piper PW, Pearl LH. Structural and functional analysis of the middle segment of hsp90: implications for ATP hydrolysis and client protein and cochaperone interactions. Mol Cell 2003; 11: 647-658.
36) Sadikot T, Swink M, Eskew JD, Brown D, Zhao H, Kusuma BR, Rajewski RA, Blagg BS, Matts RL, Holzbeierlein JM, Vielhauer GA. Development of a high-throughput screening cancer cell-based luciferase refolding assay for identifying Hsp90 inhibitors. Assay Drug Dev Technol 2013; 11: 478-488.
37) Stebbins CE, Russo AA, Schneider C, Rosen

N, Hartl FU, Pavletich NP. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 1997; 89: 239-250.

38) Pfeffer CM, Singh ATK. Apoptosis: A Target for Anticancer Therapy. Int J Mol Sci 2018; 19: 448.

39) Sellers WR, Fisher DE. Apoptosis and cancer drug targeting. J Clin Invest 1999; 104: 1655-1661.

40) Lossi L. The concept of intrinsic versus extrinsic apoptosis. Biochem J 2022; 479: 357-384.

41) Jan R, Chaudhry GE. Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. Adv Pharm Bull 2019, 9: 205-218.

42) Matthews GM, Newbold A, Johnstone RW. Intrinsic and extrinsic apoptotic pathway signaling as determinants of histone deacetylase inhibitor antitumor activity. Adv Cancer Res 2012; 116: 165-197.

43) Kennedy D, Jäger R, Mosser DD, Samali A. Regulation of apoptosis by heat shock proteins. IUBMB Life 2014; 66: 327-338.

44) Pandey P, Saleh A, Nakazawa A, Kumar S, Srinivasula SM, Kumar V, Weichselbaum R, Nalin C, Alnemri ES, Kufe D, Kharbanda S. Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. EMBO J 2000; 19: 4310-4322.

45) Li M, Zhang X, Zhou WJ, Chen YH, Liu H, Liu L, Yang CM, Qan WB. Hsp90 inhibitor BIIB021 enhances triptolide-induced apoptosis of human T-cell acute lymphoblastic leukemia cells in vitro mainly by disrupting p53-MDM2 balance. Acta Pharmacol Sin 2013; 34: 1545-1553.

46) Özgür A, Kara A, Gökşen Tosun N, Tekin Ş, Gökçe İ. Debio-0932, a second generation oral Hsp90 inhibitor, induces apoptosis in MCF-7 and MDA-MB-231 cell lines. Mol Biol Rep 2021; 48: 3439-3449.

47) Friedland JC, Smith DL, Sang J, Acquaviva J, He S, Zhang C, Proia DA. Targeted inhibition of Hsp90 by ganetespib is effective across a broad spectrum of breast cancer subtypes. Invest New Drugs 2014; 32: 14-24.

48) Li ZN, Luo Y. HSP90 inhibitors and cancer: Prospects for use in targeted therapies (Review). Oncol Rep 2023; 49: 6.