

The anticancer effects a new benzimidazole on HCC1937 breast cancer cell line

Lamia F. Abu-Marzoq¹, El-Muaiz Qasm Al-Bari Mohamed Ali², Saeb H. Aliwaini³

¹ Faculty of Postgraduate Studies and Scientific Research, University of AL-Butana, Ruffaa, Sudan

² Department of Biochemistry, Faculty of Medicine, University of AL-Butana, Ruffaa, Sudan

³ Department of Biological Sciences and Biotechnology, Faculty of Sciences, Islamic University of Gaza, P.O. Box 108, Gaza, Palestine

ABSTRACT

Background: The lack of selectivity of the present medications and the decreased sensitivity to tumor therapies are the two main barriers to the treatment of solid tumors. This study's goal was to evaluate the anticancer activity of newly created benzimidazole medication compounds against the HCC1937 cell line, which has been the subject of numerous studies suggesting benzimidazole compounds and their derivatives as prospective targeted treatments and EGFR inhibitors.

Methods: Clonogenic survival assay used to inhibit the colony formation, A trypan blue assay was used to determine BA586's capacity to cause cell death in the HCC1937 cell line. MTT was used to assess the cytotoxicity of BA586 on HCC1937 triple-negative cells. To evaluate the BA586's capacity to prevent migration, a scratch motility assay was employed. The western blotting technique was performed to quantify the presence of several cell cycle arrest and apoptotic markers, which was also used to understand how the BA586 chemical works. **Results:** With IC₅₀ values of 42 M, our research showed that BA586 had a potent cytotoxic effect against HCC1937 cells in an amount- and time-dependent manner when applied in vitro. According to a trypan blue dye exclusion assay, the BA586 chemical significantly increases cell mortality in the HCC1937 cell line. The BA586 chemical significantly lowered the HCC1937. More than 68% of cells survive at 1000 cell concentrations, while 60% do so at 500 cell concentrations, according to clonogenic and mammosphere investigations. Western blotting analysis revealed that elevating the concentrations of p53 and p21 in treated cells, the chemical BA586 induces cell cycle arrest. Importantly, this chemical greatly (by more than 70%) inhibited the migration of the HCC1937 cell line. The findings also demonstrate that BA586 can cause DNA fragmentation, which increases p53 levels. **Conclusions:** The newly discovered BA586 benzimidazole compound may be an effective anticancer agent against the HCC1937 cell line.

Conclusions: The newly discovered BA586 benzimidazole compound may be an effective anticancer agent against the HCC1937 cell line.

Keywords: p-AKT, HCC1937, benzimidazole, cleaved PARP, apoptosis

Address for correspondence:

Lamia. F. Abu. Marzoq, Faculty of Postgraduate Studies and Scientific Research, University of AL-Butana, Ruffaa, Sudan
E-mail: L.marzoq@gmail.com

Word count: 3772 **Tables:** 00 **Figures:** 08 **References:** 17

Received: 28 September, 2023, Manuscript No. OAR-24-115137

Editor Assigned: 18 November, 2023, Pre-QC No. OAR-24-115137 (PQ)

Reviewed: 03 December, 2023, QC No. OAR-24-115137 (Q)

Revised: 09 January, 2024, Manuscript No. OAR-24-115137 (R)

Published: 12 January, 2024, Invoice No. J-115137

INTRODUCTION

The type with the greatest incidence is breast cancer among women and the one that is most common in general. WHO predicted 2.26 million more instances of breast tumors among women in 2020 [1]. Additionally, it has a significant global impact on the death and morbidity of women. The incidence of breast cancer varies significantly around the world [2]. The number of newly diagnosed cases of breast cancer is expected to increase by over 40% by 2040, reaching around 3 million cases annually. Similar plans call for a more than 50% rise in breast cancer fatalities, from 685,000 in 2020 to 1 million in 2040 [3]. The metastatic stage of breast cancer remains the main cause of death from the disease, which continues to claim the majority of victims. There are five subtypes of it depending on the amounts of PR and ER, HER2, cytokeratins 6 and 5, and claudins 3/4/7 expression: basal-like, claudin-low, luminal A/B, (HER⁺), and typical breast-like [4]. The triple-negative breast cancer which makes up 15%-20% of all breast cancers and is prone to metastasizing, lacks expression of the HER2 gene, the progesterone receptor, or the estrogen receptor [5].

Chemotherapy, hormone therapy, immunotherapy, radiotherapy, surgery, and targeted therapy are among the common cancer treatment options [6]. One of the best and most efficient first-line treatments for removing and managing cancers among these is chemotherapy. The vast majority of chemotherapy drugs work to restrict cell division, target vital biological systems, and ultimately stop the growth of cancer cells [7]. Adverse effects and the potential for drug resistance [8]. There's a risk of weariness, an accelerated risk of infection, nausea, vomiting, hair loss, a decrease in appetite, diarrhea, constipation, tingling and numbness, discomfort, early menopause, and other side effects. Additionally, a single-drug strategy for treating BC is linked with a high failure rate as a result of factors such individual variability in pharmaceutical response, non-specific target action, toxic effects of drugs, and/or the development of resistance [9]. In this case, the use of combined cancer therapy is indicated. When adopting non-cross-resistant therapies, polytherapy that concurrently targets many pathways is less likely to fail than monotherapy [10]. The use of combined treatments faces a number of obstacles while being a promising one, including a lack of medication alternatives or access to them and increased treatment-related toxicity when combining chemotherapies [11].

Consequently, it is essential to develop and produce new anticancer

medicines that perform better than the current chemotherapeutic techniques while also having less adverse effects. Here Finding innovative, specifically targeted cancer treatments is crucial to battling this deadly illness.

The current study's primary objective was to determine if BA586 suppresses the EGFR/AKT pathway, cell cycle arrest, and induction of apoptosis, as well as the drug's anticancer effects on the HCC1937 cell line.

METHODS

Synthesis of benimidazole compound

Preparation of cells treatments: The Islamic University of Gaza's chemistry department's laboratory, under the direction of Prof. Bassam Abu Thaher, created the BA586 substance chemically (11). Which demonstrated in vitro the anticipated anticancer efficacy against the HCC1937 cell lines. The chemical BA586 was dissolved in DMSO (at 25°C) to yield 10 mM (stock), and no more than 7 days were allowed for storage at room temperature. During usage and to get the final concentration of between 10 and 100 M in ten Eppendorf tubes.

HCC1937 cell line culture and treatments:

Dulbecco's Modified Eagle Medium will be used for the HCC1937 cell culture (Biological Industries). 10% fetal bovine serum, 100 U/mL antibiotics were then added to the DMEM media as a supplement ,then added in incubator with 95% air humidity and 5% CO₂, cells were kept at 37°C. Each two to three days, the media was altered. Dimethyl sulfoxide was used to dissolve the BA586 compound, resulting in a stock solution with a 10 mM concentration. Only seven days were used to store the stock solution. Subsequent dilutions in DMSO media were made in preparation for use and to reach the desired concentration. Cells that had been given the vehicle were incubated in DMEM with DMSO.DMSO was applied to control cells at the same quantities as it was to the experimental cells.

Cell morphology:

To achieve a confluency of 60% to 70% on the day of treatment, the HCC1937 cells were implanted at the proper count. After being exposed to the BA586 compound, using an inverted light microscope, the morphological alterations in the cells were observed and documented on video. (Olympus 1X71, USA).

Tests for cytotoxicity:

In order to achieve a confluency of at least 70%, the HCC1937 cells were planted in 96-well plates at a density of 6.5×10^3 cells each well then incubated for 48 hours at 37°C in 95% air humidity and 5% CO₂ environment. After initial 48 hours, cells were exposed to BA586 chemical concentrations ranging from 0 to 100 M for an additional 48 hours. According to the manufacturer's instructions, on the third day, ten milliliters of MTT were added to every well. (Roche Diagnostics, Mannheim, Germany) [12]. Taking a single set of wells into account as the control (MTT using cells only, untreated). After 16 hours of dark incubation at 37°C in an incubator with 5% CO₂, 100 L of 10% Sodium Dodecyl Sulfate (SDS), which acts as a solubilizing buffer, was added to the plate. Using an ELISA reader, the absorbance in each well was calculated. (CF-fiocchetti, Italy),

and the mean cell viability was calculated proportion to the average vehicle control [13].

Trypan- blue dye exclusion viability assay:

This test was performed to see how BA586 affected the survival of HCC1937 cells. In order to achieve a confluency of at least 70%, cells were grown in a 6-well plate at a density of 300000 HCC1937 cells/well-400000 HCC1937 cells/well and kept with 95% air humidity and 5% CO₂ for 24 hours at 37°C. Following that, cells received treatment the BA586 compound in accordance with the IC50 and incubated for 24 hours in the same conditions. The media was then withdrawn, the cells were isolated by trypsinization, and they were then twice-washed in phosphate-buffered saline. The cells were then scored using a light microscope after being diluted with 0.4% trypan blue (Sigma) at a 1:9 ratio. The total number of alive and dead cells was estimated after counting viable (not stained) and nonviable (blue-stained) cells. The following equation was used to compute the percentage of viable cells [14].

$$\% \text{ Cell viability} = \frac{\text{total viable cells (unstained)} \times 100}{\text{total cells (stained + unstained)}}$$

Clonogenic survival assay:

Before being replaced at low density (500 and 1000 cells), the HCC1937 cells were pre-cultured to 70% to 80% confluence in 35 mm tissue culture dishes, treated with 40 M of BA586 compounds for 24 hours, and then incubated for 2 weeks in an incubator with 5% CO₂ and 95% air humidity. Every 2 to three days, the media was often updated. The remaining cells were then rinsed with PBS, three times, fixed for 20 minutes in a 3:1 solution of methanol and acetic acid, and the excess fixative was washed off and stained for 20 minutes at room temperature with 10% Giemsa stain (Sigma, USA). Four phosphate buffer saline washes were performed on the stained colonies. Using the following equation, the change in the proportion of colonies that have survived was estimated [15].

$$\frac{\text{Total number of colonies the experiment}}{\text{Total number of colonies in the control}} \times 100$$

Westren blot analysis:

After harvesting the HCC1937 cells after 48 hours of BA586 treatment, the protein was extracted in 2X boiling blue and heated for 10 minutes to inhibit protease action transferred electrophoretically to PVDF membrane after being resolved on gels with 8%-15% SDS-PAGE [16]. The membranes were incubated nightly at 4°C with the appropriate primary antibodies after being blocked for an hour at 25°C in PBS containing 5% non-fat dried milk. After membranes had been cleaned in PBS containing 0.1% Tween, they were incubated for an hour at 25°C with gentle shaking in either goat anti-mouse IgG or goat's anti-rabbit IgG peroxidase-conjugated secondary antibodies. The membranes were seen using an enhanced chemiluminescence kit following a second PBS/T wash (SC-2048, Santa Cruz, USA). The first antibodies were diluted as follows: rabbit polyclonal anti-PARP-1/2 (1:1000; sc-7150); mouse monoclonal anti-p53 (sc126); mouse monoclonal. A Electro Chemi-Luminescence reaction (ECL) detection equipment was used to detect antibody-

reactive proteins in the darkroom as a band [17].

Statistical analysis

The data are means with appropriate replicates' standard errors of the means (SEM). t-test and Excel were used to examine the data. P 0.01 was considered statistically significant.

RESULT

BA586 has a strong cytotoxic effect on HCC1937 cell line

HCC1937 cell line was exposed to various concentrations of the BA586 compound (0.0 M to 50.0 M) for forty-eight hours in order to investigate any potential harmful effects. Cell viability was assessed using the MTT test. The findings demonstrated that BA586 caused cytotoxic effects in cell lines with IC50s of 42 M. Figure 1 shows that 10 μ M of the BA586 inhibited more than 10% of HCC1937 cell proliferation. Furthermore, 25 μ M of the compound inhibited more than 30% of breast cancer cells.

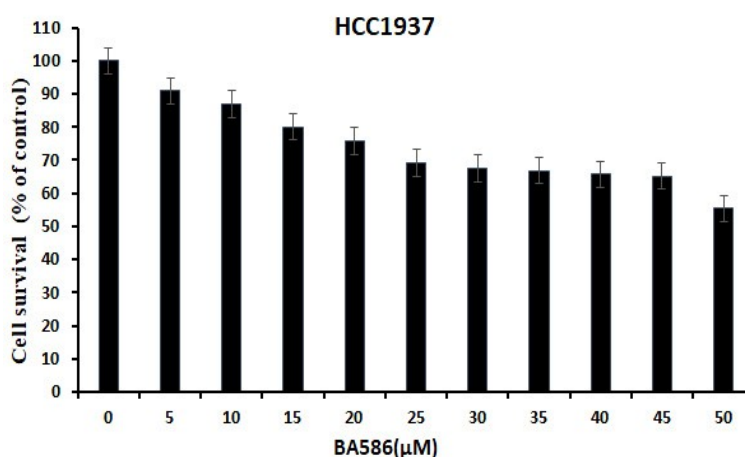


Fig. 1. The cytotoxic effect of BA586 on HCC1937 cell line

BA586 induces cell death in HCC1937 cell line

Using the trypan blue assay, BA586's impact on the viability of HCC1937 cells was evaluated. For 48 hours, cells were exposed

to various doses of BA586. The outcomes demonstrate that both breast cancer cell lines' cell viability was reduced in a dose-dependent manner. Increased by nearly 45% at 20 M after 48 hours from 25% at 15 M (Figure 2).

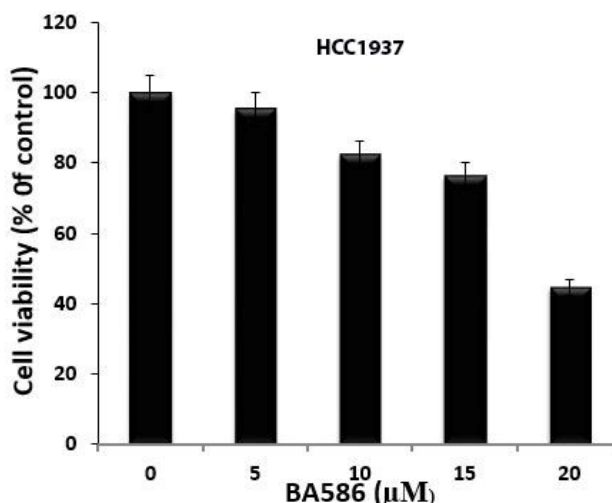


Fig. 2. BA586 inhibits HCC1937 cell viability

BA586 compound inhibits the growth HCC1937 cell line

Using a growth curve test, BA586's impact on HCC1937 was investigated. As a result, BA586 at IC50 was applied to HCC1937 cell lines over an extended period of time. From a linear equation,

Microsoft Office 2010's Excel program estimated the Population-Doubling Time (PDT) in hours. Results showed that 10 M of BA586 raised PDT from 40 hours for control cells to 79.2 hours for treated cells and reduced the proliferation of HCC1937 cells for about 39 hours (Figure 3).

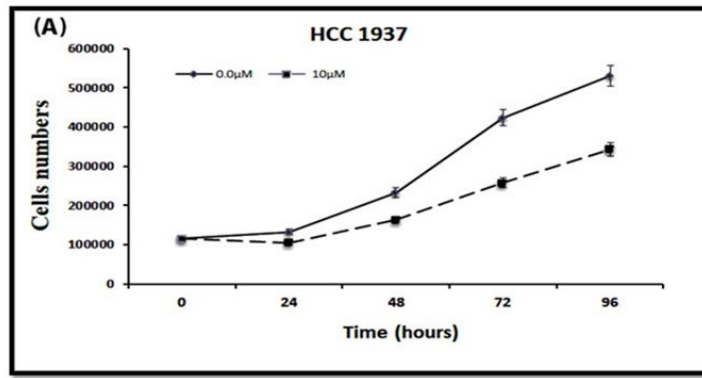


Fig. 3. Growth curve of HCC1937 cells with and without treatment

BA586 induces a long-term cytotoxic effect on HCC1937 cell line

Using a clonogenic survival experiment, prolonged cytotoxicity was investigated of BA586 on breast cancer cells. This test assesses a cell's capacity to maintain its reproductive integrity over an extended period of time, typically between 7 days and 21 days after exposure (Franken et al., 2006). Breast cancer cell HCC1937 was treated with BA586 for 24 hours and reseeded with relatively

few cells to conduct this experiment (1000 cells per well and 500 cells per well). After that, the cells were given 14 days to grow without BA586. Results demonstrated a considerable reduction in the number of colonies in breast cancer cell lines treated with HCC1937, with nearly no colonies remaining at the IC50 concentrations of BA586 (Figure 4). When compared to the control cell colonies, there was a marked tendency for BA586-treated cells to have smaller colonies.

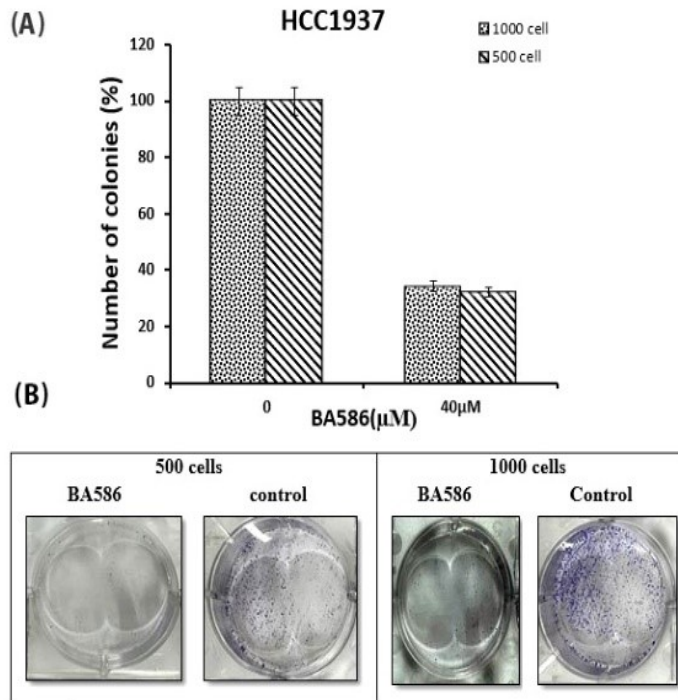


Fig. 4. The long-term cytotoxicity induced by BA586 on HCC1937 cell line

BA586 inhibits the migration of HCC1937 cell line

Using a scratch motility assay, the anti-migration activity of BA586 compound was investigated. HCC1937 cell line was exposed to

20 µM of BA586. Comparing treated HCC1937 cell lines to control cells, a notable decrease in cell migration rate was seen Figure 5. At 72 hours, BA586 reduces the HCC1937 cell line's capacity for migration by about 75%.

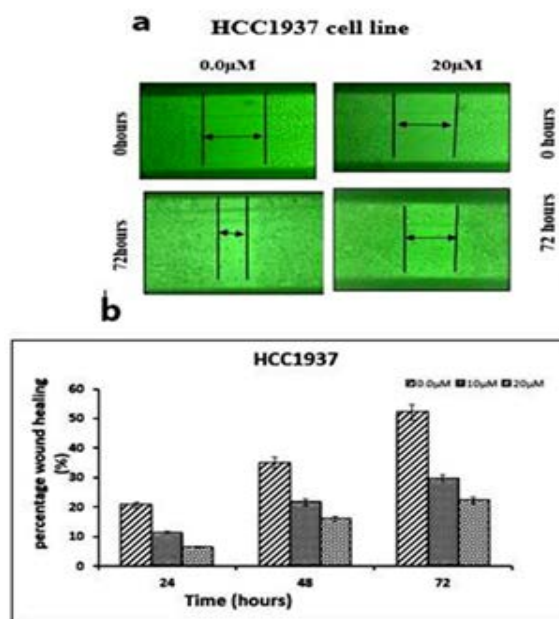


Fig. 5. BA586 inhibits the migration of HCC1937 cell line

In the HCC1937 cell line, BA586 causes cell cycle arrests and activates the p53-p21 pathway

Determining whether BA586 caused a cell cycle arrest, levels of cell cycle regulators p53 and p21 were evaluated in BA586 treated

cells by western blotting experiment. HCC1937 cells were exposed to 40 μM of BA586 for twenty-four hours & forty-eight hours. The outcomes showed that therapy with BA586 increased the concentrations of the cell cycle inhibitor proteins p21 and p53 in HCC1937 cell lines (Figure 6).

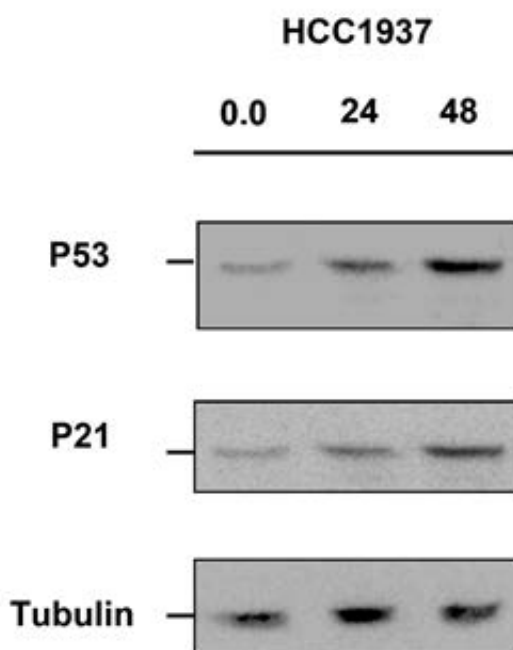


Fig. 6. BA586 activates P53-P21 pathway. HCC1937 was treated with IC50 of BA586 for twenty-four and forty-eight hours

Apoptosis is induced in HCC1937 breast cancer cells by BA586

A potent apoptosis signal known as PARP breakage was measured using a Western blot., in order to ascertain whether BA586's cytotoxicity involves the induction of apoptosis. For 24 hours and 48

hours, BA586 at IC50 concentrations was applied to breast cancer cell line HCC1937. Results demonstrated that after 48 hours of BA586 treatment, PARP cleavage increased in HCC1937 (Figure 7). Together, our results demonstrated that BA586 therapy caused apoptosis in HCC1937 cell line.

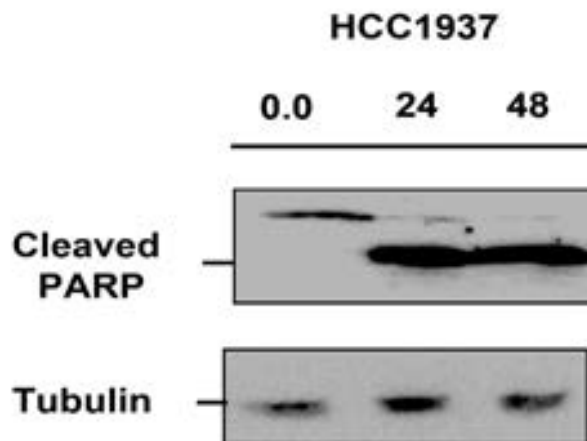


Fig. 7. BA586 induces apoptosis in HCC1937 cell line

The impact of BA586 on p-EGFR and p-AKT levels

A western blotting experiment was used to look into the concentration of p-EGFR protein in order to determine BA586's capacity to block EGFR activation and phosphorylation. The presence of p-AKT protein, an essential kinase that is activated downstream of the active EGFR receptor, was also discovered. It is important for the persistence and growth of tumor cells. The data in figure 8 showed that treated cells have lower levels of p-EGFR than control cells. Since p-EGFR is the activated form of EGFR, the decreased level

of p-EGFR suggested that BA586 inhibits the phosphorylation of the EGFR, p-EGFR downstream proteins including p-AKT, which are crucial in the survival and growth of cancer cells, are also inhibited.

The results demonstrate that, in contrast HCC1937 cell line that had not been treated, BA586-treated cells had decreased levels of pEGFR expression. According to these results, pAKT was significantly decreased in the treated cells. As a result, it is clear that BA586 has the ability to block the EGFR/AKT pathway. Additionally, the data demonstrate that, in contrast to untreated breast cancer cells, BA586-treated cells display very low levels of pERK1/2

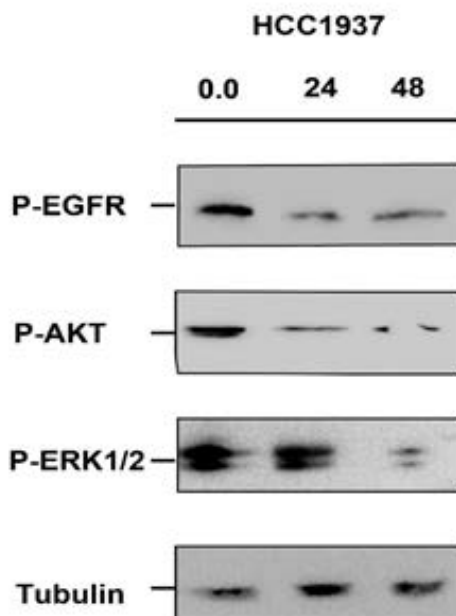


Fig. 8. P-EGFR and p-AKT levels are changed by BA586

DISCUSSION AND CONCLUSION

The antiproliferative activities of freshly synthesized benzimidazole compounds were investigated in the current study using the MTT technique BA586, which were evaluated against triple-

negative HCC1937 cell lines. Following a 48-hour treatment with varying concentrations of BA586 (0.0 M to 50.0 M), this anti-growth action was identified. According to our research, BA586 significantly damages HCC-1937 cell lines, with IC50 values of 42 M. Additionally, the substance has decreased cells' capac-

ity to survive and create colonies. This pattern of outcomes is consistent with earlier research (2) which presented three novel copper-benzimidazole complexes. The MTT assay revealed that these complexes exhibit less potency, with an IC₅₀ of 45.2.6 M in HepG2 cells. (liver cancer cell line). Additionally, the complex 3's IC₅₀ for the colon cancer HT018, breast cancer cell line MDA-MB 231 were 51.3 2.3 and 54.6 2.6 M, respectively. Overall, these findings support the BA586 compound's modestly potent action. Another study found that benzimidazole Compounds 285 b, 285 d, and 285 g, which were more potent than doxorubicin and had IC₅₀ values of 24.95, 26.36, and 22.59 nM against MCF-7 cells, had substantial anti-proliferative activity.

In The present study, cell death induced by BA586, on Tnegative (HCC1937) was first assayed by trypan blue. In addition, the mode of cell death was evaluated morphologically (inverted and fluorescence microscopy) and biochemically PARP cleavage. At the molecular level, western blotting confirmed that in both breast cancer cell lines, BA586 induced (PARP) cleavage which is a robust marker for apoptosis (Figure 7). PARP-1 protein causes cell death by releasing apoptosis inducing factor, and the inactivation of this protein by proteolytic cleavage is used as a marker for cells that are going through apoptosis. This study is significant since it is the first to examine the novel benzimidazole

compound BA586 in vitro and to show how it interacts with the HCC1937 cell line., which has anticancer properties. There are several indications from the most recent study that the anticancer compound benzimidazole is a promising one. In vitro, the HCC1937 cell line is significantly cytotoxic to the BA586 in a time- and concentration-dependent way., where the IC₅₀ number is significantly reduced by these substances. According to the trypan blue dye exclusion test, the BA586 compound also In the HCC1937 cell line, it induces the death of cells and has an anti-proliferative impact. Furthermore, at concentrations of 1000 cells and 500 cells, respectively, these compounds reduced the survival rate of HCC1937 cells by over 70% and 60%, respectively. Treatment with the new BA586 causes stop cell cycle in HCC1937 cells, as evidenced by the increasing levels of p21, p53, and PARP cleavage to in BA586exposed cells. The treated cells also displayed a notable decrease in pAKT. BA586 effectively inhibits the PI3K/ Akt signaling pathway by suppressing the EGFR/AKT pathway. To better understand how these compounds affect both cancerous and non-cancerous cells, we recommend more research into how benzimidazole molecules interact with other anticancer drugs to determine whether they have any anticancer effects. To completely understand the potential effects of benzimidazole molecules in vivo, more study is required.

REFERENCES

1. Rudnicka E, Napierała P, Podfigurna A, Męczekalski B, Smolarczyk R et al. The World Health Organization (WHO) approach to healthy ageing. *Maturitas*. 2020;139.
2. Siegel RL, Jakubowski CD, Fedewa SA, Davis A, Azad NS et al. Colorectal cancer in the young: epidemiology, prevention, management. *Am. Soc. Clin. Oncol. Educ. Book*, 2020; 21;40:75-88.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: cancer j. clin.* 2021;71:249.
4. Perou CM, Sørlie T, Eisen MB, Van De Rijn M, Jeffrey SS et al. Molecular portraits of human breast tumours. *nature*. 2000 17;406(6797):747-752.
5. Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO et al. Breast cancer subtypes and the risk of local and regional relapse. *J. clin. oncol.* 2010 1;28:1684-1691.
6. Arruebo M, Vilaboa N, Sáez-Gutierrez B, Lambea J, Tres A et al. Assessment of the evolution of cancer treatment therapies. *Cancers*. 2011 12;3:3279-3330.
7. Mokhtari RB, Homayouni TS, Baluch N, Morgatskaya E, Kumar S et al . Combination therapy in combating cancer. *Oncotarget*. 2017 6;8:38022.
8. De Silva F, Alcom J. A tale of two cancers: A current concise overview of breast and prostate cancer. *Cancers*. 2022 15;14:2954.
9. Hasan M, Leak RK, Stratford RE, Zlotos DP, Witt-Enderby PA et al. Drug conjugates—an emerging approach to treat breast cancer. *Pharmacol. Res. Perspect.* 2018;6:e00417.
10. Chatterjee N, Bivona TG et al. Polytherapy and targeted cancer drug resistance. *Trends in cancer*. 2019 1;5:170-182.
11. Thaher BA, Al-Masri I, Wahedy K, Morjan R, Aliwaini S et al. Synthesis and bioassay of 3-Aryl-1-(pyridin-4-yl) benzo [4, 5] imidazo [1, 2-d][1, 2, 4]-triazin-4 (3 H)-ones as anti-cancer agents. *Naunyn-Schmiedeb. Arch. Pharmacol.* 2023;1:1-4.
12. Aliwaini S, Lubbad AM, Shourfa A, Hamada HA, Ayesh B et al. Overexpression of TBX3 transcription factor as a potential diagnostic marker for breast cancer. *Mol clin. oncol.* 2019 1;10:105-12.
13. Dhameliya TM, Patel KI, Tiwari R, Vagolu SK, Panda D et al. Design, synthesis, and biological evaluation of benzo [d] imidazole-2-carboxamides as new anti-TB agents. *Bioorg Chem.* 2021 1;107:104538.
14. Yamasaki F, Zhang D, Bartholomeusz C, Sudo T, Hortobagyi GN et al. Sensitivity of breast cancer cells to erlotinib depends on cyclin-dependent kinase 2 activity. *Mol cancer ther.* 2007 1;6:2168-2177.
15. Franken NA, Rodermond HM, Stap J, Haveman J, Van Bree C. Clonogenic assay of cells in vitro. *Nat protoc.*, 2006 ;1:2315-2319.
16. Al-Qatati A, Aliwaini S. Combined pitavastatin and dacarbazine treatment activates apoptosis and autophagy resulting in synergistic cytotoxicity in melanoma cells. *Oncol lett.* 2017 1;14:7993-7999.
17. Aliwaini S, Awadallah AM, Morjan RY, Ghunaim M, Alqaddi H et al. Novel imidazo [1, 2-a] pyridine inhibits AKT/mTOR pathway and induces cell cycle arrest and apoptosis in melanoma and cervical cancer cells. *Oncol Lett.* 2019 1;18:830-837.