Archives of Razi Institute Journal

Volume 80, NO. 3, 2025

Journal homepage: https://archrazi.areeo.ac.ir

Short Communication

Research on the Synergistic Effect of A Novel Apoptosis Inducer Combined with AKT and HSP90 Selective Inhibitors on Hormone-Sensitive and Hormone-Resistant Breast Cancer Cell Lines

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Article Info:

Received: 3 June 2024
Revised: 2 October 2024
Accepted: 6 October 2024

Keywords:

MCF7 Breast Cancer Cell Line, AKT Inhibitor IV, Isatin-5-Sulfonamides, Synergism, Resistance.

ABSTRACT

One of the frequent malignant tumors affecting women is breast cancer. This tumor develops and occurs due to several internal and external factors. Resistance remains a key challenge in modern breast cancer therapy. Novel 1-substituted isatin-5-sulfonamides with antiproliferative effects based on isatin-core-containing antitumor compounds were synthesized in three stages via alkylation using benzyl chlorides. The study focuses on the synergistic effect of the obtained 1-substituted isatin-5-sulfonamides, exhibiting pro-apoptotic activity and is combined with heat shock protein 90 (HSP90) and protein kinase B (AKT) selective inhibitors in breast cancer cell lines, which are sensitive and resistant to antiestrogens.To create resistance, 4-hydroxytamoxifen (HT) was applied to create a resistant cell subline (MCF7/HT), achieving a resistance index of 2. MCF7/p53-LUC cell subline was obtained through transfection using the p53-responsive luciferase reporter plasmid. The lead compound *LCTA-3344*, exhibited the most significant antiproliferative effect, with a lower half-maximal inhibitory concentration (IC_{50}) in MCF7/HT (1.4±0.1 μM) compared to MCF7 (2.6±0.3 μM). Synergistic effects were observed when Combining the apoptosis inducer *LCTA-3344* and AKT Inhibitor IV in both MCF7 and MCF7/HT, demonstrating the combination index (CI) values of 0.8 and 0.4, respectively(indicating higher activity). Apoptosis inducer *LCTA-3344* combined with AKT Inhibitor X and HSP90 inhibitor did not show such significant activity with a minimal CI value of 0.9. Notably,Compound *LCTA-3344* did not enhance luciferase activity in the MCF7/p53-LUC cell subline, while chemotherapeutic agent doxorubicin has been determined to be its potent inducer. In conclusion, apoptosis inducer *LCTA-3344* was 1.9-fold more active toward MCF7/HT in comparison to the parental cell line. Compound *LCTA-3344* together with AKT Inhibitor IV was the most active drug combination on the MCF7/HT subline, with a CI of 0.4. Compound *LCTA-3344* induced apoptosis through a p53-independent mechanism, which holds promise as a novel therapy for hormone-resistant breast cancers. AKT Inhibitor IV caused apoptosis of MCF7 cells to a greater extent than compound *LCTA-3344*, and their combination resulted in a synergistic effect.

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How to cite this article: **Salnikova DI, Krymov SK, Bogdanov FB, Sorokin DV, Andreeva OE, Khamidullina AI, Shchekotikhin AE, Scherbakov AM. Research on the Synergistic Effect of A Novel Apoptosis Inducer Combined with AKT and HSP90 Selective Inhibitors on Hormone-Sensitive and Hormone-Resistant Breast Cancer Cell Lines. *Archives of Razi Institute*. 2025;80(3):791-800.** DOI: 10.66224/ARI.2025.80.3.791

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1. Introduction

Breast cancer is a complex and heterogeneous malignant tumor that affects both women and men. It is characterized by the uncontrolled growth of breast tissue cells. According to worldwide statistics, breast cancer is the leading cause of cancer-related deaths among women (1). In 2020 alone, it was estimated that more than 2 million new diagnoses were made, and more than 685,000 women worldwide died from breast cancer. These alarming figures highlight the urgent need for effective treatment strategies (2, 3).

Several subtypes of breast cancer exist, including inflammatory breast cancer, invasive lobular carcinoma, invasive ductal carcinoma, and others. These subtypes differ in their growth patterns, genetic alterations, and response to treatment (4). The hormone receptor-positive subtype is one of the major forms of breast cancer, in which tumor cells possess the estrogen receptor alpha (ERα) and/or progesterone receptor (PR), making these cells dependent on hormone signaling for growth and survival (5, 6).

Estrogens are steroid hormones that play a critical role in the development and progression of breast cancer (7, 8). There are three main types of estrogens: 17β-estradiol, estrone, and estriol. 17β-Estradiol is the primary estrogen hormone produced mainly in the ovaries. Estrone is the second most abundant estrogen in the body and is produced primarily after menopause, while estriol is present during pregnancy. Estrogens bind to ERα, a protein found in most types of breast cancer (9). Activation of ERα signaling promotes cell proliferation and survival, contributing to tumor growth. Therefore, targeting ERα has become a key therapeutic approach (9).

Anticancer therapy that decreases estrogen levels, such as inhibitors of estrogen synthesis (a.k.a. aromatase inhibitors) and gonadotropin-releasing hormone analogs, have been proven effective in treating ERα-positive breast cancer. These drugs reduce estrogen production or block its action, leading to tumor regression. In addition, antiestrogens such as tamoxifen and HT, one of its major active metabolites, function as selective estrogen receptor modulators. They block ERα signaling in breast cancer cells, inhibiting their growth (10, 11). However, despite initial positive responses to hormone therapies, many patients eventually develop endocrine resistance to these treatments, leading to cancer recurrence, disease progression, and metastasis (12). One of the mechanisms underlying this resistance involves mutations in the estrogen receptor gene 1. These mutations result in a constitutively active form of ERα that is no longer inhibited by antiestrogens (9). The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway has emerged as a promising therapeutic route, due to the pivotal role of AKT, a serine/threonine kinase, in cell survival, proliferation, and differentiation (13).

AKT kinase shares significant similarity to protein kinases A and C (14). Also, this kinase is closely related in structure to the retroviral protein akt (v-akt) (15). Structurally, AKT consists of three domains: an amino-terminal (N-terminal), a central, and a carboxyl-terminal fragment (C-terminal).

The N-terminal domain, homologous to pleckstrin (PH), consists of 100 amino acids and is similar to others identified in molecules that bind 3-phosphoinositides by interacting with membrane lipid products such as phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and phosphatidylinositol 4,5-bisphosphate (PIP2). The kinase domain of AKT is similar to the corresponding domain of the AGC protein kinases (16). Phosphorylation of Thr308 activates AKT kinase. The C-terminal groove of AKT consists of 40 amino acids, forming a hydrophobic region, which contains an important regulatory residue, which is Ser473.

HSP90 belongs to a group of chaperone proteins andis often hyperexpressed in cancer and participates in the folding, stabilization, and activation of proteins necessary for intracellular signaling, such as AKT. Targeting either AKT or HSP90 has shown promise in preclinical studies, and several AKT inhibitors and HSP90 inhibitors, such as luminespib (a second-generation HSP90 inhibitor), are currently under investigation in clinical trials (17, 18).

AKT inhibitors, including antibodies and small molecules, have demonstrated potent antiproliferative effects in hormone-sensitive breast cancer cells. Having acted on the AKT signaling pathway, these inhibitors disrupt cell cycle progression, induce apoptosis, and reduce AKT-mediated cell survival signals (19). Numerous studies have reported the efficacy of AKT inhibitors, such as MK-2206, GDC-0068, and AZD5363, in preclinical hormone-sensitive breast cancer models. These inhibitors havereduced the proliferation of hormone-sensitive breast cancer cell lines, suppressed downstream AKT signaling, and sensitized cells to

hormone therapy, thus leading to more effective treatment strategies (20). Here, we investigate the antiproliferative activity of AKT Inhibitor IV and AKT Inhibitor X in hormone-resistant and hormone-sensitive breast cancer cell sublines.

To address endocrine resistance and improve treatment outcomes, new therapeutic strategies are being developed. The discovery of selective estrogen receptor degraders, such as fulvestrant, has shown promise in targeting ERα for degradation and effectively blocking its activity (21). In conclusion, breast cancer remains a significant global health issue. Understanding the role of estrogens, ERα signaling, and the mechanisms of endocrine resistance have led to improved targeted treatment (20). Ongoing research and the exploration of novel treatment approaches are needed to further improve the outcome for breast cancer patients worldwide (2).

This study aims to develop novel 1-substituted isatin-5-sulfonamides with pro-apoptotic activity and study their combination with HSP90 and AKT inhibitors on hormone-resistant and hormone-sensitive breast cancer cell sublines.

2. Materials and Methods

2.1. Synthesis

Previous successful examples of the design and synthesis of isatin derivatives with antiproliferative activity provide a foundation for further research on the scaffold in the development of new antitumor drugs. Sulfonamides'ability to inhibit tumor-associated carbonic anhydrases IX and XII (22) and to block ERα expression (23) has increased interest in this pharmacophore group for cancer therapy. Despite limited research on isatinsulfonamides in medicinal chemistry, the available evidence points to their possible potential for anti-cancer therapy and diagnosis. In particular, several investigations have shown their ability to inhibit caspases 3 and 7, which aere key executors of apoptosis.

More recently, using molecular hybridization method, based on the structures of an activator of apoptosis and a selective carbonic anhydrase IX inhibitor, 22 1-substituted isatin-5-sulfonamides were designed, synthesized, and tested. A straightforward approach to isatin-5-sulfonamide 2 turned out to be the heterocyclization of the isatin core by the Sandmeyer method. At the first stage, sulfanilamide reacted with chloral hydrate and hydroxylammonium chloride to give intermediate α-isonitrosoacetanilide, which was then condensed at 90 °C in sulfuric acid to afford isatin-5-sulfonamide 2 in a satisfactory overall yield.

Subsequently, isatin-5-sulfonamide alkylation through diverse benzyl chlorides was the basis for the synthesis of a previously undiscovered group of 1-substituted isatin-5-sulfonamides (24).

Notably, 1-(4-((trifluoromethyl)thio) benzyl) isatin-5-sulfonamide (compound *LCTA-3344*, Figure 1a) was *identified* as a leading compound and apoptosis inducer during the subsequent biological experiments.

2.1.1. Isatin-5-Sulfonamide 2

1. To 2,2,2-trichloroethane-1,1-diol (0.053 mol, 8.63 g) dissolved in water (125 mL), a hydroxylamine hydrochloride (0.116 mol, 8 g), anhydrous sodium sulfate (0.231 mol, 32.7 g), and concentrated sulfuric acid (9.6 mL in 20 mL of water) were added.

This mixture was heated to 40°C and a solution of 4-aminobenzenesulfonamide (0.035 mol, 6 g) was added all at once while stirring. The resulting mixture was stirred, heated to the boiling point, then cooled to 25°C. The white precipitate was filtered off, washed with cold water, and dried in a vacuum oven. This process yielded 6.10 g of anilide. with a yield of 72%. The crude product is pure enough for further reaction.

2. Anilide (8.23 mmol, 2.00 g) was gradually added to the stirring concentrated sulfuric acid (8 mL) at 70 °C. After addition, the mixture was heated to 90°C and stirred for 2 hours. The solution was then cooled to 25°C and poured into ice (80 g). The brown precipitate was filtered off. Mother liquor was extracted with EtOAc (3 × 20 mL). The organic phases were combined, washed with saturated $NaHCO_3$ solution and water, dried over sodium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (EtOAc – toluene 3:1, silica gel) to obtain 0.85 g of isatin-5-sulfonamide, with a yield of46%. HPLC: LW = 244 nm, t_R = 7.3 min, gradient B 10 → 50% (30 min); powder of a yellow color; mp > 250 C; purity: 98%; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.03 (d, J^1 = 8.2 Hz, 1H, Ar), 7.38 (s, 2H, NH_2), 7.83 (d, J^2 = 2.0 Hz, 1H, Ar), 7.96 (dd, J^1 = 8.2, J^2 = 2.0 Hz, 1H, Ar), 11.36 (br s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 112.7, 118.3, 122.2, 135.5, 138.9, 153.1, 160.1, 183.8 ppm; HRMS (ESI) (m/z) $[M-H]^-$: calculated for $C_8H_5N_2O_4S$ 224.9976, found 224.9925; IR ν max, (film) cm^{-1} 903 w, 914 w, 986 s, 1065 s, 1131 m, 1156 s, 1190 m, 1212 m, 1296 s, 1335 s, 1397 m, 1456 m, 1536 w, 1613 s, 1743 s, 3088 w, 3255 m, 3343 s.

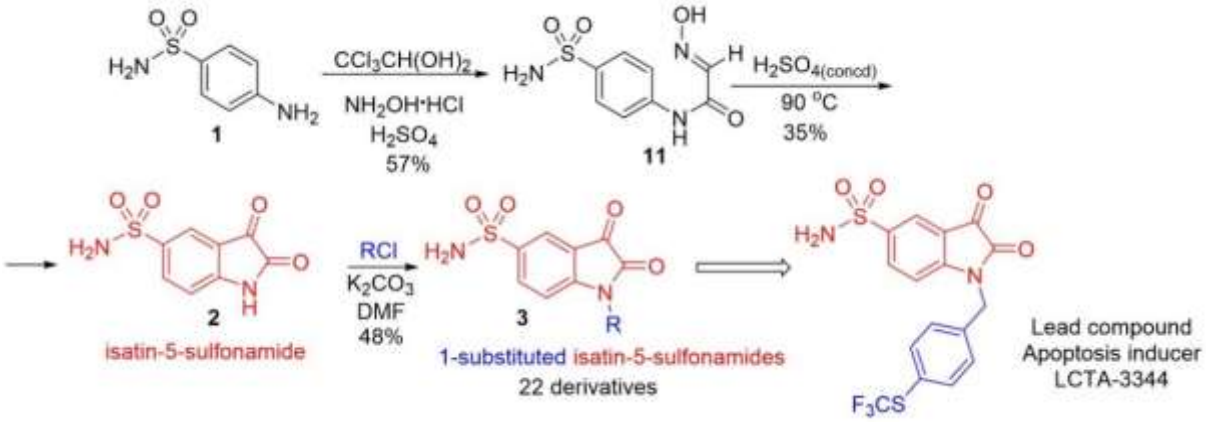

Figure 1. Synthesis of the 1-substituted isatin-5-sulfonamides series. RCl – alkyl chloride; K_2CO_3 – potassium carbonate; DMF– dimethylformamide.

3. To a solution of K_2CO_3 (2.4 mmol, 0.33 g) and isatin-5-sulfonamide (0.80 mmol, 0.18 g) in DMF (5 mL), a 4-(trifluoromethylthio)benzyl chloride (1.04 mmol, 0.16 g) was added at room temperature. The solution was stirred for 6 hours. After completion of the reaction monitored by TLC, 30 mL of the cold water and 2M HCl were added to the reaction mixture to adjust the pH to 4–5. After that, the mixture was extracted with EtOAc (3×15 mL). The organic phases were combined, washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified using column chromatography (EtOAc – toluene 1:2, silica gel) to obtain LCTA-3344 (183 mg). Yield 48%. Purity: 98%; mp = 209-211 °C; Yellow powder; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.03 (*s*, 2H, CH_2), 7.10 (*d*, J^1 = 8.2 Hz, 1H, Ar), 7.41 (*s*, 2H, NH_2), 7.62 (*d*, J^1 = 7.8 Hz, 2H, Ar), 7.69 (*d*, J^1 = 7.8 Hz, 2H, Ar), 7.92 (*s*, 1H, Ar), 7.96 (*d*, J^1 = 8.2 Hz, 1H, Ar) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 182.1, 159.2, 152.4, 139.6, 136.9 (3C), 135.0, 43.1, 111.3, 118.6, 121.9, 122.5, 129.2 (2C), 129.6 (q J^1 = 110.4 Hz, CF_3) ppm; HRMS (ESI) (m/z) $[M+Na]^+$: calculated for $C_{16}H_{11}F_3N_2NaO_4S_2$, found 439.0005; IR ν max, (film) cm^{-1} 720 m, 733 m, 756 m, 822 s, 873 s, 914 s, 952 w, 962 w, 1017 s, 1074 s, 1105 s, 1126 s, 1148 s, 1304 w, 1332 s, 1370 s, 1408 m, 1441 m, 1474 s, 1494 w, 1613 s, 1736 s, 3253, 3336.

2.2. Cells and Reagents

The MCF7 breast cancer cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). To establish a resistant subline, MCF7 cells underwent prolonged HT treatment, as previously discussed in our work (25, 26). Further experiments were carried out three weeks following the drug withdrawal. The resistance index, calculated as the ratio of the IC_{50} of HT on the MCF7/HT subline to its IC_{50} on the parental MCF7 cell line, was 2. The HT-resistant subline was termed MCF7/HT.

HT (4-[(1Z)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenyl-1-buten-1-yl]-phenol), HSP90 inhibitor (luminespib, NVP-AUY922;

5-[2,4-dihydroxy-5-(1-methylethyl)phenyl]-N-ethyl-4-[4-(4-morpholinylmethyl)phenyl]-3-isoxazolecarboxamide), AKT Inhibitor X (2-chloro-N,N-diethyl-10H-phenoxazine-10-butanamine, monohydrochloride),

AKT Inhibitor IV (6-(2-benzothiazolyl)-1-ethyl-2-[2-(methylphenylamino)ethenyl]-3-phenyl-1H-benzimidazolium, monoiodide) and MTT (2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2H-tetrazolium, monobromide) were procured from Cayman Chemical (Ann Arbor, MI, USA). Reagents for cell cultivation were acquired from PanEco (Moscow, Russia).

2.3. Estimation of Antiproliferative Activity

A density of $4{\cdot}10^4$ cells per well was chosen to seed the cells onto 24-well plates (TPP), and the cell suspension was added to 900 µL of medium. The derivatives were added in 100 µL of the DMEM medium after 24 hours of cultivation in the NuAire CO_2 incubator (Leeds, MN, USA) at 37 °C and 80-90% relative humidity. The range of compound concentration values was 0; 3.1; 6.3; 12.5; 25; and 50 µM. Cell survival was evaluated using the MTT assay after 72 hours, previously described in the literature (27).

2.4. Search for Synergistic Combinations

An investigation was conducted to identify effective combinations of *LCTA-3344* with HSP90 inhibitor, AKT Inhibitor X, and AKT Inhibitor IV in various concentrations on the MCF7 cell line and MCF7/HT subline.

The component concentrations for drug combinations were selected so that cell survival was approximately 80% in the presence of a less active compound in a pair and about 60% with the addition of a more active substance. To evaluate the effectiveness of these combinations, the CI index was calculated using the CompuSyn program by the method of Chou and Talalay (28). A combination was considered synergistic if CI was less than 0.9, additive when CI was in the range of 0.9 to 1.1 and antagonistic if CI was greater than 1.1. The compounds *LCTA-3344* and AKT Inhibitor IV were combined in doses of 0.46 and 0.049 μM, respectively.

2.5. Reporter Gene Assay

The transcriptional activity of p53 was evaluated using a reporter assay, as discussed in the previous research (29). MCF7 cell line was transfected with Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and plasmids: p53-LUC, bearing the luciferase gene under the control of p53-responsive elements, and a plasmid for the β-galactosidase expression (30) to monitor the effectiveness of transfection. The transfected cells were designated as MCF7/p53-LUC. Luciferase activity in MCF7/p53-LUC cell subline was calculated on a Tecan Infinite M200 Pro microplate reader (Tecan Group, Männedorf, canton of Zürich, Switzerland) in compliance with the Promega procedure. Relative luciferase activity was regarded as the relation of luciferase activity to β-galactosidase activity.

2.6. Immunoblotting

Modified immunoblotting was carried out as previously detailed (31). The expression levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), B-cell leukemia/lymphoma 2 protein (Bcl-2), phosphorylated Bcl-2 (p-Bcl-2, Ser70), and cleaved poly(ADP-ribose) polymerase (PARP) expression were assessed with Cell Signaling Technology antibodies (Danvers, MA, USA).

Detection was carried out with horseradish peroxidase-conjugated rabbit immunoglobulin secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) and an ImageQuant LAS 4000 imager (GE Healthcare, Chicago, IL, USA), as indicated in Mruk's procedure (32). GAPDH served as a control for sample loading. PARP cleavage allowed the detection of apoptosis. Bcl-2 was assessed as a negative apoptosis regulator.

2.7. Statistical Evaluation

Results were analyzed using GraphPad Prism 9.0 (Boston, MA, USA) and Microsoft Excel . Each assay was completed a minimum of three independent times to maintain repeatability, calculate mean values, and provide the standard deviation (S.D.) values. Student's *t*-test at $p<0.05$ represented a statistically relevant result.

3. Results

According to the MTT assay, compound *LCTA-3344* demonstrated the most potent antiproliferative effect, inhibiting cancer cells growth at low micromolar doses. The *LCTA-3344* IC_{50} value on the HT-resistant subline was 1.4±0.1 μM ,which was 1.9 times lower than the appropriate parameter on the MCF7 line (2.6±0.3 μM) (5, 24). Previously, we described a development of the MCF7/HT resistant subline with a resistance index equal to 2 (17). Effective combinations of *LCTA-3344* with HSP90 inhibitor, AKT Inhibitor X, and AKT Inhibitor IV were searched for. The combinations of AKT Inhibitor IV (0.049 μM) and *LCTA-3344* (0.46 μM) on HT-sensitive and HT-resistant sublines were synergistic, and CI values were 0.8 and 0.4 (higher activity), respectively. In comparison , *LCTA-3344* combinations with luminespib and AKT Inhibitor X did not show such significant activity with the lowest CI value, 0.9 (Figure 2).

Using the MCF7/p53-LUC cells obtained by transfection and applied for assessing the p53 activity, compound *LCTA-3344* did not enhance luciferase activity, whereas doxorubicin, as a reference drug has been determined to be its potent inducer (Figure 3).

Immunoblotting results showed that apoptosis, revealed by the PARP cleavage, was greater following the addition of AKT Inhibitor IV compared to *LCTA-3344*, and the effect of the combination was synergistic. Bcl-2 has been identified as a negative regulator of apoptosis (Figure 3). Three AKT inhibitors were assessed, including luminespib, AKT Inhibitor IV, and AKT Inhibitor X. Previously, we demonstrated the high antiproliferative activity of the compounds on HT-resistant cells (17). The IC_{50} value of AKT Inhibitor X was 4.2 μM, while AKT Inhibitor IV demonstrated higher activity, showing an IC_{50} value equal to 390 nM. Luminespib exhibited the greatest effect against MCF7 and MCF7/HT cell lines, showing an IC_{50} value as 14 and 18 nM, respectively.

The AKT-mediated signaling pathway represents a crucial regulator of cellular processes involved in cancer progression, including enhanced cell survival, proliferation, and resistance to apoptosis (31).

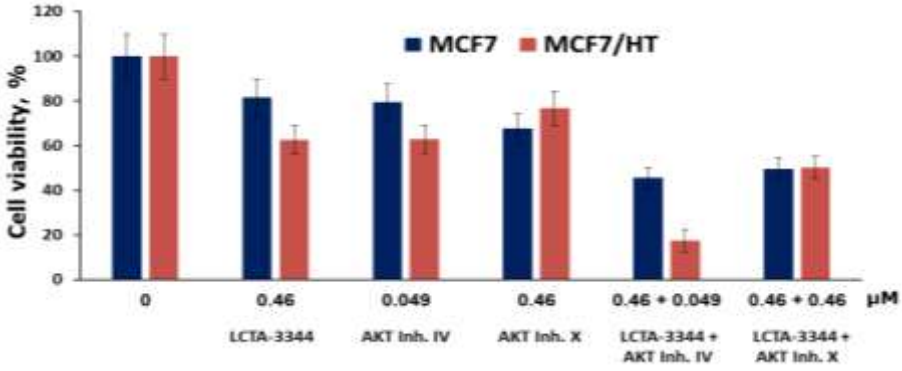

Figure 2. Synergistic effect of apoptosis inducer *LCTA-3344* combined with AKT Inhibitor IV in comparison with an additive effect of *LCTA-3344* and AKT Inhibitor X combination on MCF7 HT-sensitive and HT-resistant cell sublines.

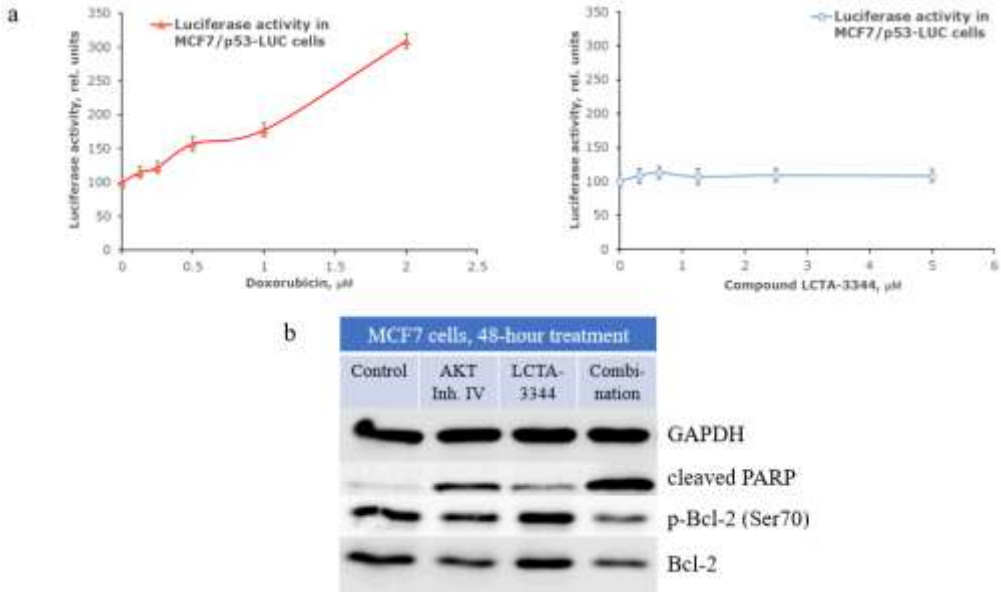

Figure 3. P53 activity and apoptosis. (a) Comparison of the effects of *LCTA-3344* and doxorubicin on luciferase activity in the MCF7/p53-LUC cell subline. (b) The effects of AKT Inhibitor IV, *LCTA-3344*, and their combination on the induction of apoptosis of MCF7 cells. Cleaved PARP and p-Bcl-2/Bcl-2 have been used as markers of apoptosis pathways.

Activation of AKT occurs downstream of multiple receptor tyrosine kinases and intracellular signaling molecules, leading to phosphorylation and subsequent activation of the AKT protein. Once activated , influences various cellular events by phosphorylation of downstream targets that regulate various cellular events, including glucose metabolism, protein synthesis, cell cycle progression, and apoptosis evasion (33).

Hormone-sensitive breast cancers dependon estrogen and/or progesterone signaling for their growth and survival. Current therapies for hormone-sensitive breast cancer aim to suppress hormone signaling using selective ERα modulators, selective ERα degraders or aromatase inhibitors. The greatest advances in this area of oncology have been driven by Prof. V. Craig Jordan's efforts (11, 34). In the 1970s, he introduced tamoxifen (ICI 46,474), the best-known selective estrogen receptor α modulator. This drug was first developed as a contraceptive, but a series of experiments led to its classification as an anti-estrogen for breast cancer therapy. Today,tamoxifen is the main hormonal drug to treat women with ERα (+) breast cancer (35). However, resistance to these treatment modalities remains a major problem. Preclinical trials have shown an association of aberrant AKT pathway activation with resistance to hormone therapy in ERα-positive breast cancer cell lines (36).

Consequently, inhibitors of AKT have emerged as potential therapeutic agents to overcome resistance and

inhibit breast cancer cell growth (21).The development of novel synergistic combinations of AKT inhibitors with chemotherapeutic agents in cell models and preclinical and clinical trials is being actively investigated. Hopcroft L. *et al.* developed and tested an effective combination of the selective AKT inhibitor capivasertib and fulvestrant , a selective ERα degrader (SERD), in preclinical studies of ERα-positive, palbociclib-resistant breast cancer (21). The combination improved progression-free survival in the Phase III clinical trial (CAPItello-291), involving patients with hormone receptor-positive breast cancer treated with aromatase inhibitors, with or without CDK4/6 inhibitors. Additionally, Cocco S. *et al.* studied whether ipatasertib and taselisib, the PI3K/AKT inhibitors, might cause autophagy in breast cancer models and whether chloroquine, a known autophagy inhibitor, would enhance the anti-cancer effect of ipatasertib and taselisib combined with conventional chemotherapy (37). The induction of autophagy after treatment with ipatasertib and taselisib was assessed in MCF7, MDA-MB-231, MDA-MB-361, MDA-MB-468, and SKBR3 breast cancer cells. The data showed that ipatasertib and taselisib triggered enhanced autophagy signaling in breast cancer; the activity was especially noticeable in PI3K/AKT-resistant triple-negative breast cancer cell sublines. Autophagy inhibition by chloroquine enhances the therapeutic effect of PI3K/AKT inhibitors in *in vitro* and *in vivo* models of triple-negative breast cancer cells, synergizing with taxane-associated chemotherapy. Similarly, Balasis M. *et al.* demonstrated that farnesyltransferase inhibitors combined with AKT synergistically act on cell lines of breast cancer and induce considerable mammary cancer regression in ErbB2 transgenic mice (38). All these results yielded CI values less than 1, indicating synergy.

The progression of resistance to hormone therapy remains a significant clinical challenge in breast cancer management. Emerging evidence suggests that AKT pathway activation performs a pivotal function in mediating hormone therapy resistance in both initial and late disease phases (33). In hormone-resistant breast cancer cells, AKT inhibitors have exhibited promising antitumor activity, potentially overcoming the resistance mechanisms. Preclinical data suggest that AKT inhibitors can sensitize hormone-resistant breast cancer cells to hormone therapies by suppressing AKT-mediated cell survival signaling ,and restoring the efficacy of hormonal interventions (36)

4. Conclusion

The AKT signaling pathway represents an attractive therapeutic target for hormone-resistant and hormone-sensitive cell breast cancer sublines. AKT inhibitors have shown promising antiproliferative effects in preclinical models, demonstrating their potential as adjunct therapeutic agents for overcoming hormone therapy resistance and inhibiting tumor growth. Further investigations, including clinical trials, are warranted to fully understand the potential of AKT inhibitors in improving treatment outcomes for breast cancer patients, opening the possibility for individualized and specialized therapeutic approaches (39).

In this study, we described the HT-resistant cell subline that appeared to partly preserve hormone signaling. MCF7/HT cell subline was sensitive to selective AKT inhibitors. The strongest effect was observed with the HSP90-AKT blocker luminespib, whose IC_{50} is approximately 20 nM. AKT inhibitors combined with drugs interacting with other molecular targets can improve the efficacy of anti-cancer treatment and be regarded as a possible option to overcome resistance in ERα-positive breast cancer cell lines.

Acknowledgment

The authors would like to thank the Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Institute of Gene Biology, Russian Academy of Sciences, for providing the equipment and research facilities (microplate spectrophotometer).

Authors' Contribution

Study concept and design: A. M.S, A. E.S.

Acquisition of data: D. I.S, S. K.K, D. V.S, F. B.B, A. I.K, O. E.A.

Analysis and interpretation of data: D. I.S, S. K.K, D. V.S, F.B.B., O. E.A, A. I.K.

Drafting of the manuscript: D. I.S.

Critical revision of the manuscript for important intellectual content: A. M.S, S. K.K.

Statistical analysis: A .M.S, S. K.K.

Administrative, technical and material support: A. M.S, A. E.S.

Methodology: D. I.S, S. K.K, D. V.S, O. E.A, A. M. S, A. E.S,

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the study design; data collection, analysis, interpretation; manuscript writing, or in the decision to publish.

Funding

This research was partly funded by the Russian Science Foundation, agreement 20-13-00402, https://rscf.ru/project/23-13-45035/, accessed on September 15, 2024)

Data Availability

All relevant data are reported within the paper. Additional information can be obtained upon request from the authors.

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