Brief Report

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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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 is a key challenge in modern breast cancer therapy. Novel 1-substituted isatin-5-sulfonamides with antiproliferative effects on the basis of isatin-core-contained antitumor compounds were synthesized in three stages via alkylation with benzyl chlorides. The work aims at the synergistic effect of the obtained 1-substituted isatin-5-sulfonamides exhibiting pro-apoptotic activity and 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. 4-hydroxytamoxifen (HT) was applied to create a resistant cell subline (MCF7/HT); a resistance index amounted to 2. MCF7/p53-LUC cell subline was obtained through transfection using the p53-responsive luciferase reporter plasmid. Lead compound *LCTA-3344* exhibited the most significant antiproliferative effect; its half-maximal inhibitory concentration (IC_{50}) on MCF7/HT (1.4±0.1 μM) was less compared to that on MCF7 (2.6±0.3 μM). Combinations of apoptosis inducer *LCTA-3344* and AKT Inhibitor IV were synergistic on MCF7 and MCF7/HT, demonstrating the combination index (CI) values of 0.8 and 0.4 (a higher activity), respectively. 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. Compound *LCTA-3344* did not enhance luciferase activity in 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.

Keywords: MCF7 breast cancer cell line; AKT Inhibitor IV; Isatin-5-sulfonamides; Synergism; Resistance

1. Introduction

Breast cancer is a complex and heterogeneous malignant tumor that affects both women and men. It is described by the uncontrolled growth of breast tissue cells. According to worldwide statistics, breast cancer is the main reason for cancer deaths in the female population (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 breast cancer subtypes 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 strategies (4). The hormone receptor-positive subtype is one of the major subtypes 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 perform a critical function in breast cancer development and progression (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 that is found in most types of breast cancer (9). ERα signaling activation 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, has been effective in treating ERα-positive breast cancer. These drugs reduce estrogen production or prevent 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, a significant proportion of patients eventually develop endocrine resistance to these treatments, leading to cancer recurrence, disease progression, and metastasis (12). One of the mechanisms underlying this resistance is the acquisition of 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 become a promising therapeutic route due to the pivotal role of AKT, a serine/threonine kinase, in cell survival, proliferation, and differentiation (13). AKT kinase is characterized by significant similarity to protein kinases A and C (14). Also, this kinase is close 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 found 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 containing an important regulatory residue, Ser473.

HSP90 belongs to a group of chaperone proteins. HSP90 is often hyperexpressed in cancer and participates in the folding, stabilization, and activation of proteins necessary for intracellular signaling, such as AKT. Targeting 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 now under investigation in clinical trials (17, 18).

AKT inhibitors involving antibodies and small molecules have demonstrated potent antiproliferative effects in hormone-sensitive cells of breast cancer. Acting 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 breast cancer models sensitive to hormones. These inhibitors have been demonstrated to reduce the proliferation of hormone-sensitive breast cancer cell lines, suppress downstream AKT signaling, and sensitize cells to hormone therapy, thus facilitating more effective treatment strategies (20). Here, we explore the antiproliferative activity of AKT Inhibitor IV and AKT Inhibitor X targeting hormone-resistant and hormone-sensitive cell sublines of breast cancer.

To overcome 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, effectively blocking its activity (21). In conclusion, breast cancer is a significant global health issue. Understanding the role of estrogens, ERα signaling, and the mechanisms of endocrine resistance has resulted in improved targeted treatment (20). Continued research and the exploration of novel treatment approaches are needed to further improve the outcome for breast cancer patients worldwide (2).

This investigation 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. Methods

2.1. Synthesis

Successful examples of the design and synthesis of isatin derivatives with antiproliferative activity establish the basis for further research on the scaffold in the development of new antitumor drugs. The ability of sulfonamides to inhibit tumor carbonic anhydrases IX and XII (22) and to block ERα expression (23) contributes to the increased interest in this

pharmacophore group for the treatment of cancer diseases. 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, well-known executors of apoptosis.

More recently, with the use of the 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 **1** reacted with chloral hydrate and hydroxylammonium chloride to give intermediate α-isonitrosoacetanilide, which was condensed at 90 °C in sulfuric acid to isatin-5-sulfonamide **2** in a satisfactory overall yield.

Then, 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). 1-(4-((trifluoromethyl)thio)benzyl)isatin-5-sulfonamide (compound *LCTA-3344*, Fig. 1a) was defined as a leading compound and apoptosis inducer during the following biological experiments.

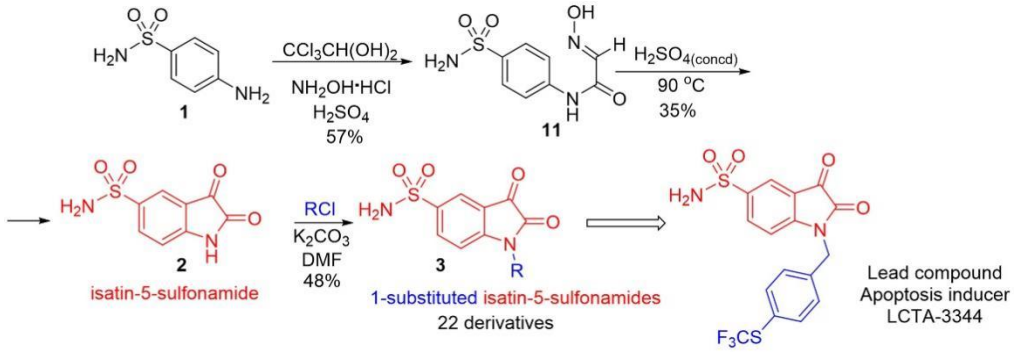

Fig. 1a. Synthesis of the 1-substituted isatin-5-sulfonamides series. RCl – alkyl chloride; K_2CO_3 – potassium carbonate; DMF – dimethylformamide

2.1.1. Isatin-5-sulfonamide 2

1.1. 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 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. Yield: 72%. The crude product is pure enough for further reaction.

1.2. Anilide (8.23 mmol, 2.00 g) was portionally added to the stirring concentrated sulfuric acid (8 mL) at 70 °C. After addition, the mixture was heated to 90 °C and stirred during 2 h. The solution was 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). 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. Yield: 46%. 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.

2.1.2. 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 were exposed to 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, determined 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 obtained 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, as earlier reported in the research (27).

2.4. Search for Synergistic Combinations

A search for 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 was performed. The component concentrations for drug combinations were selected so that cell survival was 80% in the presence of a less active compound in a pair and 60% with the addition of a more active substance. To evaluate the combination effectiveness, the CI index was measured in the CompuSyn program by the method of Chou and Talalay (28). A combination was considered synergistic in the event that CI was less than 0.9, additive when CI was in the range of 0.9 to 1.1, and antagonistic in the event that 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 estimated using a reporter assay, as discussed in the 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). 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 was assessed with Cell Signaling Technology antibodies (Danvers, MA, USA). Detection was accomplished by use of 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 was 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

GraphPad Prism 9.0 (Boston, MA, USA) and Microsoft Excel were applied for result interpretation. Each assay was completed minimum three individual 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 & Discussion

According to the MTT assay, compound *LCTA-3344* demonstrated the most potent antiproliferative effect by inhibiting cancer cells using low micromolar doses. The *LCTA-3344* IC_{50} value on the HT-resistant subline was 1.4±0.1 μM and 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. To compare, *LCTA-3344* combinations with luminespib and AKT Inhibitor X did not show such significant activity with the lowest CI value, 0.9 (Fig. 1b).

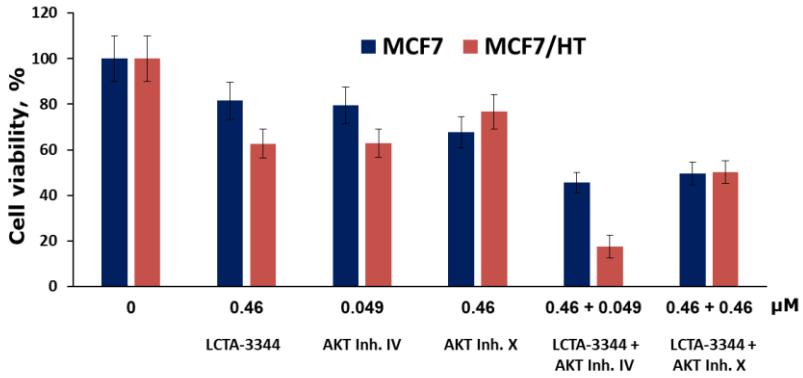

Fig. 1b. Synergistic effect of apoptosis inducer *LCTA-3344* combined with AKT Inhibitor IV in comparison with an additive effect of *LCTA-3344* and AKT Inhibitor X combination on MCF7 HT-sensitive and HT-resistant cell sublines

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 (Fig. 2a).

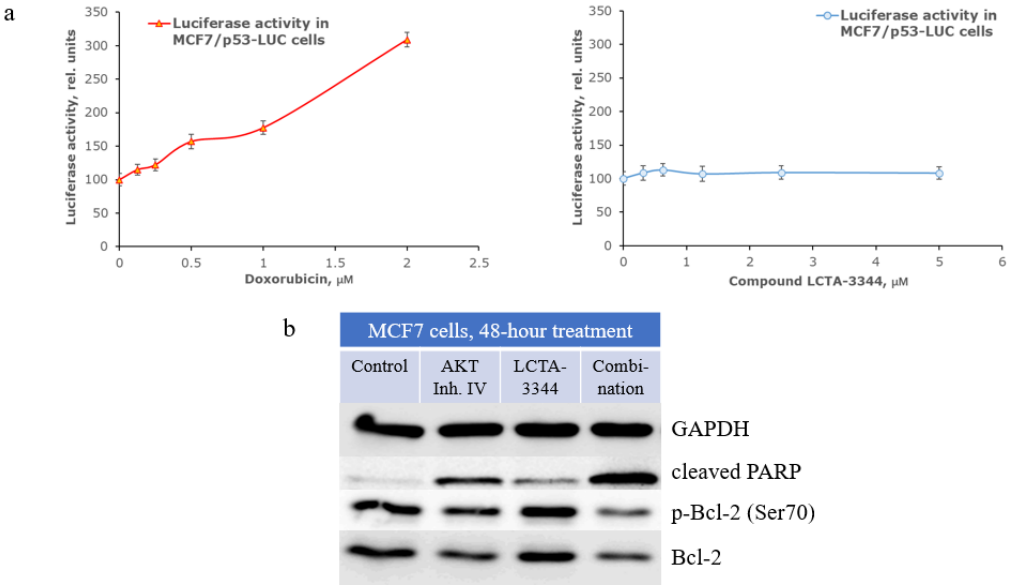

Fig. 2. P53 activity and apoptosis. (a) Comparison of the effects of *LCTA-3344* and doxorubicin on luciferase activity in the MCF7/p53-LUC cell subline. (b) The effects of AKT Inhibitor IV, *LCTA-3344*, and their combination on the induction of apoptosis of MCF7 cells. Cleaved PARP and p-Bcl-2/Bcl-2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

Immunoblotting results showed that apoptosis revealed by the PARP cleavage was greater with the addition of AKT Inhibitor IV rather than *LCTA-3344*, and the effect of the combination was synergistic. Bcl-2 has been identified as a negative regulator of apoptosis (Fig. 2b). 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 amounted to 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, correspondingly.

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). AKT activation occurs downstream of multiple receptor tyrosine kinases and intracellular signaling molecules, leading to phosphorylation and subsequent activation of the AKT protein. Upon activation, AKT mediates its effects through the 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 rely on estrogen and/or progesterone signaling for their growth and survival. Current therapies for hormone-sensitive breast cancer aim to suppress hormone signaling with selective ERα modulators, selective ERα degraders or aromatase inhibitors. The greatest advances in this area of oncology have been due to Prof. V. Craig Jordan's efforts (11, 34). In the 1970s, he launched the era of 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. Now 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). As such, 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 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 a Phase III clinical trial (CAPItello-291) involving patients with hormone receptor-positive breast cancer who received aromatase inhibitors in the presence or absence of CDK4/6 inhibitors. Cocco S. *et al.* studied whether ipatasertib and taselisib, the PI3K/AKT inhibitors, might cause autophagy in breast cancer models and if 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 cells of breast cancer. 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. 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 empirical data have CI values less than 1.

The progression of resistance to hormone treatment 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 findings 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 sublines of breast cancer. 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 highest effect was found for 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 conquer resistance in ERα-positive cell lines of breast cancer.

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Data availability All the relevant data are reported within the paper. For additional details, data are available on request to the authors.

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