KIF15 inhibitor suppresses the proliferation and migration, and induces the apoptosis of breast cancer cells

ALI HAIDER ALHAMMER[1], SUAD GAZI ALKUFI[1], SHAYMAA ISMAEL AL-JUBOORI[2] and SHAHAD ALI MUDHAFAR[1]

[1]Department of Medical and Molecular Biotechnology, Biotechnology Research Center, Al-Nahrain University, Jadriya, Baghdad 10072, Iraq; [2]Department of Biology, College of Science for Women, Baghdad University, Jadriya, Baghdad 10071, Iraq

Received July 9, 2024; Accepted October 15, 2024

DOI: 10.3892/wasj.2024.291

Abstract. Recent studies have shown that motor proteins, known as the kinesin superfamily proteins (KIFs), are associated with the pathogenesis of several types of cancer. Kinesin family member 15 protein (KIF15) is overexpressed in multiple malignancies, including breast cancer (BC), and could be exploited therapeutically. Therefore, to the best of our knowledge, the present study, for the first time, examined the cytotoxic effects of the KIF15 small molecule inhibitor, Kif15-IN-1, on BC cell lines derived from triple-negative BC (TNBC) and estrogen receptor-positive (ER^+) BC *in vitro* in addition to normal cells. The viability of MDA-MB231 (TNBC), MCF7 (ER^+), and normal rat embryo fibroblasts (REF) was examined using an MTT assay following treatment of the cells with Kif15-IN-1. The morphological alterations and migration potential were examined under an inverted microscope. Apoptosis was detected via fluorescence microscopy. Reverse transcription-quantitative polymerase chain reaction was used to measure gene expression. The results revealed an anti-proliferative effect of Kif15-IN-1 in both MDA-MB231 and MCF7 cells and this effect was associated with increased apoptosis, the suppression of migration and increased cell size. Furthermore, Kif15-IN-1 significantly reduced *KIF15* expression in both cell lines. Taken together, in the present study, to the best of our knowledge, Kif15-IN-1 was explored in BC for the first time, and was found to inhibit the proliferation of BC cell lines, regardless of the subtype and status of ER expression. The cytotoxic effect was associated with increased apoptosis, a decreased capacity for migration and the downregulation of *KIF15* expression. However, the preclinical confirmation of these results using *in vivo* models is necessary. The present study demonstrates however, a novel possible treatment strategy for several breast cancer subtypes, including ER^+ and TNBC, which may include the inhibition of KIF15.

Correspondence to: Dr Ali Haider Alhammer, Department of Medical and Molecular Biotechnology, Biotechnology Research Center, Al-Nahrain University, Jadriya, Al Jadriyah Bridge, Baghdad 10072, Iraq
E-mail: ali.alhammer@nahrainuniv.edu.iq; alialhammer@gmail.com

Abbreviations: KIFs, kinesin superfamily proteins; BC, breast cancer; TNBC, triple-negative breast cancer; ER, estrogen receptor; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; KIF15, kinesin family member 15; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GI_{50}, growth inhibitory concentration that reduces viability by 50%; AO/EB, acridine orange-ethidium bromide dual staining; cDNA, complementary DNA

Key words: triple-negative breast cancer, estrogen receptor-positive, MDA-MB231, mitotic spindle inhibitor, KIF15, Kif15-IN-1

Introduction

Breast cancer (BC) is among the most common malignancies affecting women and is expected to cause 42,250 related deaths and 310,000 new cases in the United states in 2024 (1). Based on the expression of the hormone receptors, progesterone receptor (PR), estrogen receptor (ER) and human epidermal growth factor 2 (HER2), currently, the most widely used and accepted approach for categorizing BC is immunohistochemistry. Consequently, the four most well-known subtypes of BC are luminal A, luminal B, HER2-positive and triple-negative BC (TNBC) (2,3). The TNBC subtype, an aggressive disease, accounts for 10 to 20% of all BC cases (4). TNBC is characterized by the absence of the expression of all three receptors, high relapse rates, resistance to current endocrine therapies and short survival rates (5); therefore, the treatment of this subtype is challenging. TNBC arises from the complex interactions between targets and factors (6). This heterogeneity is associated with the reduced efficacy of therapies, a decreased overall survival, and a more aggressive disease course (5,7). Furthermore, ER and PR are present in 70% of luminal A malignancies, which also lack HER2, exhibit low levels of the cell proliferation marker Ki-67 (<20%) and lack HER2. In terms of clinical characteristics, these tumors are low-grade, slow growing and are associated with the optimal prognosis, a greater survival rate and a lower recurrence rate. In addition, the treatment efficiency of the most prevalent subtype of BC (ER^+) has improved over the past few years following the introduction of endocrine-targeted therapies, such as tamoxifen.

However, approximately one third of patients may suffer from metastasis and relapse due to the development of resistance to endocrine therapies (8,9).

Kinesin superfamily proteins (KIFs) are motor proteins that hydrolyze adenosine triphosphate as an energy source and mediate the intracellular transportation of macromolecules, such as chromosomes, organelles, RNA, vesicles and proteins (10). Kinesin family member (KIF15), also known as kinesin 12, plays an essential role in cell division (mitosis), cytoskeleton structure and the transportation of cellular molecules. KIF15 is a microtubule-protein complex that maintains the bipolar geometry of the mitotic spindle and facilitates chromosome segregation in collaboration with KIF11 (11,12). Recently, multiple studies have shown that KIF15 is highly expressed in various malignancies, such as BC, gallbladder cancer and gastric cancer, suggesting that KIF15 is a biomarker that could be exploited therapeutically (13-16).

Over the past two decades, the development of various selective kinesin spindle inhibitors has become an attractive avenue (17). Several studies have investigated the role of KIF15 in carcinogenesis. Wang *et al* (18) reported that KIF15 was overexpressed in patients with pancreatic cancer and that KIF15 silencing was associated with a reduced proliferation both *in vitro* and *in vivo*. Similarly, another research group suggested that KIF15 is a candidate biomarker for glioma and could be used as a therapeutic target (19). More importantly, KIF15 has been identified as a risk factor for TNBC, and its downregulation by lentiviral infection has been shown to be associated with the induction of apoptosis (20). Notably, scientists have identified 10 candidate kinesin small-molecule chemical inhibitors for clinical evaluation, two of which are the most promising KIF11 inhibitors: Filanesib (Arry-520) and ispinesib (SB-715992) (21). In addition, a recent study utilized the small-molecule KIF15 inhibitor, Kif15-IN-1, as a combination therapy with ispinesib in gastric cancer cells. This combination was found to exert synergistic effects both *in vitro* and *in vivo* (12).

Previous molecular and functional analyses have suggested that KIF15 motor proteins contribute to cell division and, consequently, malignant growth (22). Therefore, targeted therapies are warranted to provide effective treatments with increased efficacy and low toxicity. The present study aimed to investigate the impact of the KIF15 inhibitor, Kif15-IN-1, on the viability, apoptosis induction, and migration of BC cell lines with different statuses of ER expression.

Materials and methods

Cells, cell culture and drug preparation. In the present study, two human breast adenocarcinoma cell lines were used: MCF7, which expresses ER (ER^+) and MDA-MB231, which lacks ER (ER^-) and belongs to the TNBC subtype (23,24). In addition, non-transformed rat embryo fibroblasts (REF) were used as a normal control (25). The MDA-MB231 (ATCC HTB-26) cells were kindly supplied by the Faculty of Science, Baghdad University, and the MCF7 (ATCC HTB-22) cells were purchased from ATCC. In addition, REF were kindly provided by the Biotechnology Research Center at Al-Nahrain University. REF were originally established by the Experimental Therapy Department of the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR), which is affiliated with Mustansiriyah University in Baghdad, Iraq. As described in a previous study by the authors (26), the cells were cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640) supplemented with L-glutamine (Capricorn Scientific GmbH). To create a complete medium, 1% penicillin/streptomycin solution (100X; Euroclone S.p.A.) and 10% fetal bovine serum (FBS) were added to the RPMI-1640 medium. The cells were kept in an incubator with 5% CO_2 and 95% humidity at 37˚C, as previously described (27).

The potent KIF15 inhibitor, Kif15-IN-1, was purchased from MedChemExpress (cat. no. HY-15948). For *in vitro* assays, a stock solution of 20 mM was created by dissolving it in 100% dimethyl sulfoxide (DMSO). The required concentrations for subsequent experiments were prepared in complete medium from the stock solution.

Cytotoxicity assay. Cytotoxic effects were assessed using an *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (28,29). A total of 10,000 cells were seeded in each well of a 96-well plate and incubated overnight to ensure cell adhesion. MDA-MB231, MCF7 and REF cell lines were then exposed to increasing concentrations of Kif15-IN-1 (10-100 μM) three replicate wells were used for each treatment. Following incubation (24, 48 h for MDA-MB231 and MCF7 and 24, 48 and 96 h for REF), the medium was removed from the plate, and 20 μl MTT solution (5 mg/ml) (Shanghai Macklin Biochemical Co., Ltd.) was added to each well and incubated for 3 h at 37˚C in the dark. To dissolve the MTT, 50 μl DMSO (Bio Basic Inc.) was added, followed by 10 min of shaking (30). A microplate reader (BioTek Instruments, Inc.) was used to measure the absorbance at 490 nm. The following equation was used to determine the percentage of viable cells from the raw absorbance data:

$$\text{Viability \%} = \frac{\text{A test} - \text{blank}}{\text{A control} - \text{A blank}} \text{x100},$$

where 'A' represents the absorbance. The dose-response curve was generated using GraphPad Prism software version 6 (Dotmatics), and the growth inhibitory concentration that reduces viability by 50% (GI_{50}) was determined via the same curve.

Morphological analysis. The present study investigated the effects of Kif15-IN-1 treatment on cell morphology. The cell lines (MDA-MB231, MCF7 and REF), in a 24-well plate, were seeded at a seeding density of $26x10^3$ cells per well. The cell lines were treated with their relevant GI_{50} doses of Kif15-IN-1 for 24 h at 37˚C in 5% CO_2 and 95% humidified air. The cells were then analyzed at a magnification of x200 using an inverted microscope (Meiji Techno). Subsequently, images were captured using digital camera (Canon, Inc.), as previously described (31).

Scratch assay. The MDA-MB231 cells were seeded in 24-well plates under the same conditions used in the morphological study. Although serum starvation is necessary for scratch assay experiments, serum starvation has been shown to significantly influence the migration, proliferation and migration-associated genes of MDA-MB-231 breast cancer cells (32). Moreover, serum deprivation has been demonstrated to affect the cell

cycle (33,34), which may subsequently influence the efficacy of the Kif15 inhibitor. Therefore, the present study utilized complete medium (containing 10% FBS) to reduce the possible effects of serum deprivation. In addition, we aimed to perform this test in more physiological settings. Therefore, the MDA-MB231 cells were cultured using complete medium (10% FBS) for 24 h until they became nearly confluent, and then a scratch was made using a pipette tip in the middle of the well before washing with PBS. Fresh complete medium (10% FBS) was replaced with the relevant wells containing DMSO (Control) or Kif15-IN-1 (58 μM). Images were captured using an inverted microscope (MEIJI, Japan) (x100 magnification) with a digital camera (Canon, Japan) before the plate was returned to a humidified, warm incubator (37˚C). Following incubation for 48 h, images were obtained. In addition, ImageJ™ software version 1.46r was used for the analysis of wound healing images.

Apoptosis detection by fluorescence microscopy. As described in a previous study by the authors (31), apoptosis analysis was performed after 25x10^3 MDA-MB231 cells were exposed to the control (DMSO) or Kif15-IN-1 (58 μM) for 48 h in a 24-well plate. The cells were then trypsinized, rinsed with PBS, and collected in 1.5 ml tubes (Eppendorf Germany). A total of 9 μl of the cell suspension was mixed with 1 μl of acridine orange-ethidium bromide (AO/EB) staining dye [100 μg/ml AO and 100 μg/ml EB (Fluka Chemie GmbH)]. Subsequently, 100 cells were scored for different stages of apoptosis using a fluorescence microscope (Human Diagnostics), and images of randomly selected representative fields were obtained.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the MDA-MB231 and MCF7 cells using TRIzol™ reagent 2023 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The ProtoScript® First Strand complementary DNA (cDNA) Synthesis kit (E6300S) from New England Biolabs was used according to the vendor's instructions, and cDNA was obtained by the reverse transcription of total RNA. This assay is highly specific for double-stranded DNA (dsDNA) over RNA. To measure the mRNA levels, Luna Universal qPCR Master Mix (M3003S) from New England Biolabs (SYBR®/FAM channel) was used. In addition, the thermocycling conditions were as follows: 30 sec of denaturation at 94˚C, 40 cycles of denaturation at 94˚C for 5 sec, 15 sec of annealing at 58˚C, and 10 sec of extension at 72˚C. *KIF15* gene expression was measured in the treated and untreated cells using RT-qPCR and relative cycle threshold ($2^{-\Delta\Delta Cq}$) methodology (35). *GAPDH* was used as the internal control (housekeeping gene). The primers used were as follows: *KIF15* forward, 5'-CTGCCTGGGCCAAGTGATTA-3' and reverse, 5'-CGGGATTCCTTGTGGAGCTT-3'; and *GAPDH* forward, 5'-GGTGTGAACCATGAGAAGTATGA-3' and reverse, 5'-GAGTCCTTCCACGATACCAAAG-3'.

Statistical analysis. The data were analyzed using GraphPad Prism software (version 6.0.0; Dotmatics) and Microsoft Excel 2019. The majority of the experiments were conducted at least twice. The unpaired t-test was used to compare two means. The data were analyzed according to the standard error of the mean (SEM). A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Kif15-IN-1 inhibits BC cell proliferation in a concentration-dependent manner regardless of the ER status. The present study investigated the cytotoxic effects of a KIF15 inhibitor (Kif15-IN-1) on BC cells. The MDA-MB231 (ER^-) and MCF7 (ER^+) cell lines were treated with increasing concentrations of Kif15-IN-1 for 24 and 48 h (Fig. 1A and B). In addition, a normal cell line (REF) was also incubated with Kif15-IN-1 for 24, 48 and 96 h. Subsequently, cell viability was evaluated using an MTT assay. Of note, the MDA-MB231 cells exposed for 48 h were more sensitive to Kif15-IN-1 (mean GI_{50}=57.95±3.99 μM) than were the cells incubated for 24 h (mean GI_{50}=85.94±4.75 μM) (Fig. 1A). The MCF7 cells were less sensitive following incubation for 24 h (GI_{50}=93.17±3.82 μM), but were more sensitive after 48 h (mean GI_{50}=61.9±0.8 μM) (Fig. 1B). Notably, the REF were resistant (GI_{50} >100 μM) to Kif15-IN-1 at the different time points examined (Fig. 1C).

Kif15-IN-1 treatment alters the morphology and suppresses the migratory potential of BC cells. To further examine the effects of Kif15-IN-1, both MDA-MB231 and MCF7 cells were exposed to their relevant GI_{50} concentrations, and after 24 h, the morphology of the cells was examined under an inverted microscope. Of note, in the MDA-MB231 cells, the impact on viability was associated with a decrease in cell number, and the size of the treated cells was greater than that of the control cells. In addition, some cells floated in the medium, which may indicate that these cells were dead (Fig. 1D). However, in the MCF7 cells, the treatment was associated with a decreased cell number and size; all treated cells were rounded compared with those in the control group (Fig. 1E). Notably, no obvious morphological changes were observed in the REF treated with Kif15-IN-1 compared with the untreated cells (Fig. 1F).

Additionally, the effects of Kif15-IN-1 on the migratory potential of MDA-MB231 cells were examined using a scratch assay (Fig. 2). As illustrated in Fig. 2A, there was less wound healing in the Kif15-IN-1-treated cells than in the control-treated cells, and the bar chart indicates a significant decrease of ~39% (P=0.011) after 48 h of exposure (Fig. 2B). Of note, small gaps are present in the cells treated with the KIF15 inhibitor in the wound healing image at 48 h. As indicated in Fig. 3, Kif15-IN-1 promotes apoptosis. This may explain the tiny gaps observed in the cell monolayer of the Kif15-IN-1-treated cells (Fig. 2A).

Kif15-IN-1 induces BC cell apoptosis and significantly reduces the expression of KIF15. To better understand the effects of Kif15-IN-1, the induction of the apoptosis of MDA-MB231 and MCF7 cells was estimated using AO/EB staining to confirm the cytotoxic ability of Kif15-IN-1. BC cells were co-cultured with the indicated treatments for 48 h before apoptosis was detected (Fig. 3). As shown in Fig. 3A and B, the majority of the MDA-MB231 cells underwent early apoptosis (~75%), and ~80% of the cells treated with the GI_{50} dose of Kif15-IN-1 were

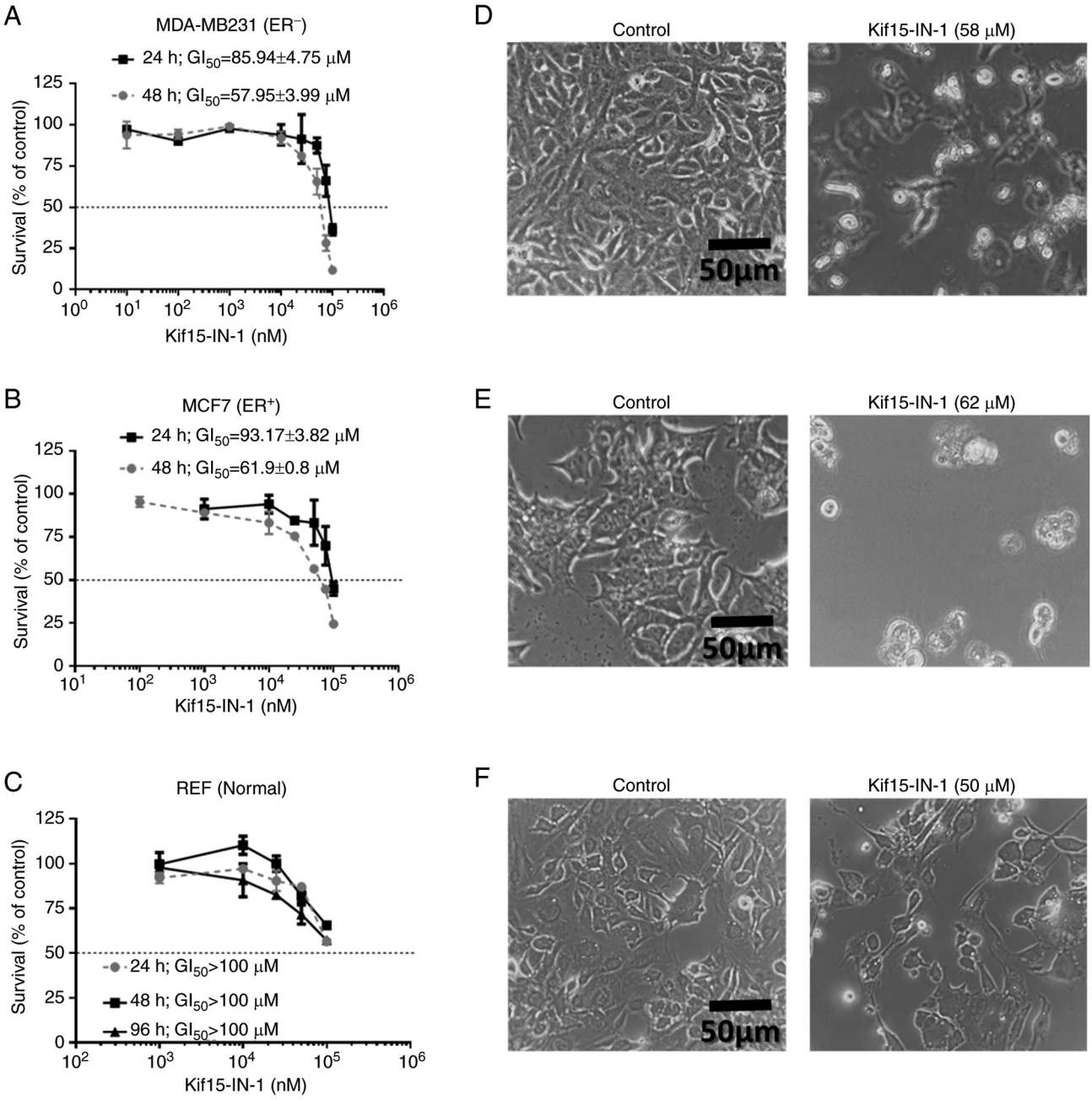

Figure 1. Kif15-IN-1 inhibits the proliferative activity and alters the morphology of BC cells. (A) MDA-MB231, (B) MCF7, and (C) REF were exposed to increasing concentrations of Kif15-IN-1 for the indicated durations; only the BC cell lines were relatively sensitive to 48 h of incubation. Compared with those of the control cells, the data are presented as the mean percentage survival ± SEM, and at least two replicates were performed with triplicate measurements, which produced the findings. The GI_{50} values are indicated. In addition, (D) MDA-MB231, (E) MCF7, and (F) REF were incubated with the indicated treatments for 24 h, and images of morphological alterations were obtained using an inverted microscope at x200 magnification; the images are representative of two replicates. BC, breast cancer; KIF15, kinesin family member 15; REF, rat embryo fibroblasts; GI_{50}, growth inhibitory concentration that reduces viability by 50%.

apoptotic (early and late apoptosis). In addition, the counts of MCF7 cells treated with Kif15-IN-1 at the GI_{50} dose revealed that ~15% of the cells were apoptotic at the late stage; however, no significant difference in the total number of apoptotic cells (early and late apoptosis) was observed (Fig. 3C and D).

In addition, RT-qPCR was used to investigate the effects of Kif15-IN-1 on *KIF15* mRNA levels. Notably, it was found that, compared with the control, Kif15-IN-1 significantly reduced the expression of the *KIF15* gene in both the MDA-MB-231 (18.8-fold) (Fig. 4A) and MCF7 (9.7-fold) cells after 24 h (Fig. 4B).

Discussion

BC is the leading cause of cancer-related mortality among women worldwide; several subtypes of BC, such as TNBC, are challenging to treat (36). In addition, the emergence of resistance to current therapeutics is a matter of concern; therefore, novel therapies targeting aberrant cellular mechanisms are warranted to overcome resistance and improve treatment efficacy. Multiple studies have emphasized the role of mitotic motor proteins in the carcinogenesis of various malignancies, including BC (16,18,20,37). Of note, recent reports have

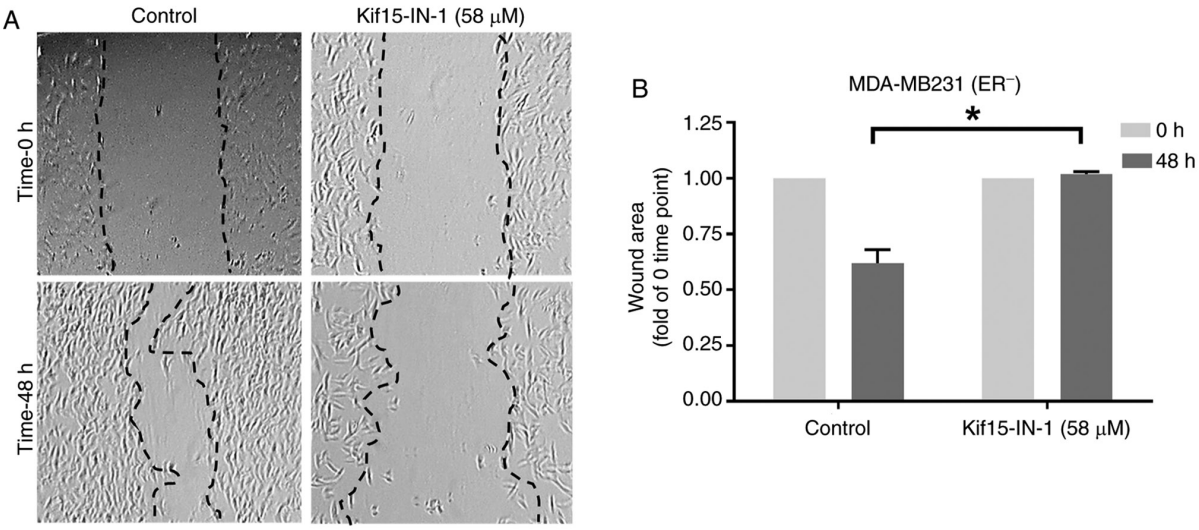

Figure 2. Kif15-IN-1 hinders the migratory potential of MDA-MB231 cells. (A) In cells treated with the growth inhibitory concentration that reduces viability by 50% and untreated cells used as controls, wound healing experiments were carried out in MDA-MB231 cells at 0 and 48 h. Representative inverted microscopy images at x100 magnification illustrate the area covered by the cells at 0 and 48 h after wounding. (B) A significant reduction in wound healing was observed after treatment with Kif15-IN-1. The scratch area was measured using ImageJ™ software and normalized to that at 0 h. The error bars indicate the mean ± SEM of two to three replicates. *P<0.05. KIF15, kinesin family member 15.

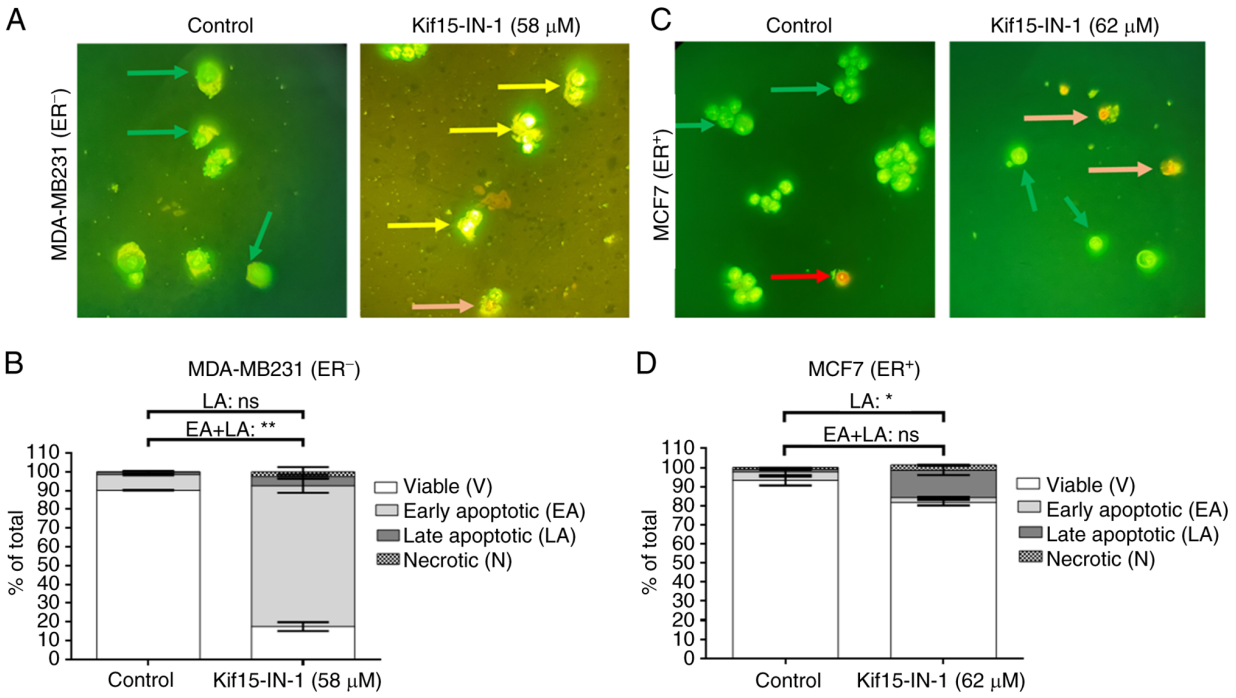

Figure 3. Kif15-IN-1 enhances the induction of BC cell apoptosis. BC cells were treated with the indicated concentrations of Kif15-IN-1 for 48 h before being stained with AO/EB and examined under a fluorescence microscope; representative images of (A) MDA-MB231 and (C) MCF7 cells were obtained at x100 magnification. Bar charts indicate the percentage of (B) MDA-MB231 and (D) MCF7 cells at different stages and treatments indicated. (A and C) Green arrows indicate viable cells, yellow arrows indicate early apoptotic cells, orange arrows indicate late apoptotic cells and red arrows indicate necrotic cells. Error bars represent the mean ± SEM of more than one replicate; the statistical symbols indicate significant differences in apoptotic cells between the treatment and control groups. *P<0.05 and **P<0.01; ns, not significant; BC, breast cancer; KIF15, kinesin family member 15.

investigated the function and structure of the motor kinesin protein KIF15 in several tumors, suggesting that this protein could be exploited therapeutically (12,15,22).

Recent studies have shown that KIF15 expression is upregulated in patients with BC and is abnormal in accelerating cell cycle progression (13,20). In addition, Alfarsi *et al* (38) reported that KIF18A was highly expressed in >2,000 patients with ER⁺ BC and that it was a significant predictor of a poor response to endocrine treatment. Of note, multiple attempts to downregulate KIF15 protein expression via RNA interference techniques in different types of cancer have yielded promising results *in vitro* and *in vivo* (16,20). However, despite the potential therapeutic advantages of siRNAs, several issues have made it difficult to employ siRNA-based antiviral treatments,

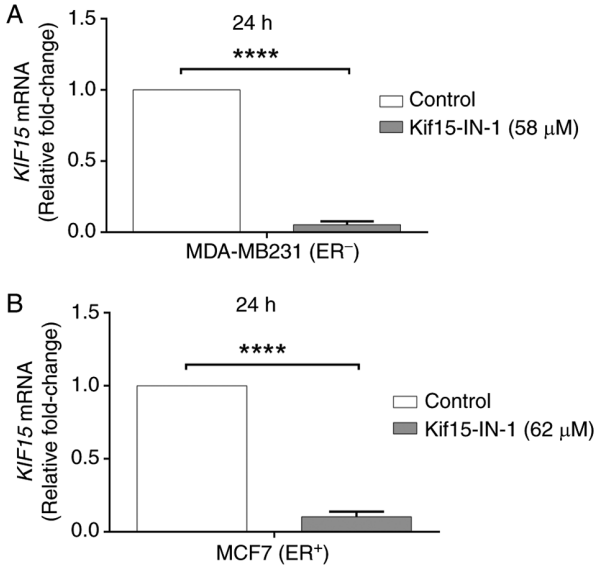

Figure 4. Expression of the KIF15 gene is downregulated following treatment with Kif15-IN-1. mRNA levels were measured using reverse transcription-quantitative polymerase chain reaction in (A) MDA-MB231 and (B) MCF7 cells following 24 h of incubation with the indicated concentrations of Kif15-IN-1; the bars reflect the fold change in relation to the control treatment. The reference gene used was GAPDH. Asterisks indicate significant differences between the indicated and control bars, and error bars indicate the mean ± SEM. $^{****}P \leq 0.0001$. KIF15, kinesin family member 15.

including off-target effects, siRNA instability, poor long-term protein expression, drug resistance and immunological reactions (39). Therefore, the present study aimed to explore the impact of a small-molecule KIF15 inhibitor (Kif15-IN-1) on BC cell lines derived from different subtypes (ER^+ and TNBC) in an attempt to identify a chemical-targeted therapy that may enhance the treatment efficacy of existing therapies for BC and overcome resistance.

To explore the effects of KIF15 inhibition in BC cells, MDA-MB231 and MCF7 cells were treated with Kif15-IN-1 monotherapy *in vitro*. Interestingly, viability was inhibited, and the KIF15 inhibitor was cytotoxic in both cell lines, regardless of mutational status and subtype. Considering that KIF15 is highly expressed in BC cells (13,20), it was hypothesized that Kif15-IN-1 would inhibit the target protein KIF15, as evidenced by the significant downregulation of *KIF15* in both cell lines (MDA-MB231 and MCF7). Additionally, the normal cells used, REF, were resistant to Kif15-IN-1, unlike the BC cell lines, which further supports the assumption of target (KIF15) inhibition. The downregulation of the *KIF15* gene expression may be attributed to the off-target effect of the KIF15 inhibitor or the mechanism of a negative feedback network between protein and gene expression through the binding of proteins to RNA regulatory motifs of mRNAs (40).

In addition, cytotoxicity is associated with the induction of programmed cell death and a decreased migratory potential. Of note, a similar effect was previously observed following *KIF15* knockdown in BC (13,20), gallbladder cancer (15), pancreatic cancer (18) and Burkitt lymphoma (41). Additionally, bioinformatics and experimental studies have revealed that *KIF15* knockdown induces programmed cell death in ovarian cancer by triggering the interaction of several pathways (42). Additionally, recent research in prostate cancer cells has revealed that the knockdown of *KIF15* was associated with the inhibition of proliferation and migration potential from one side and the induction of programmed cell death by decreasing the activity of the PI3K/Akt pathway (43). Another recent study revealed that KIF15 may modulate apoptotic pathways to reduce the production of seven anti-apoptotic proteins in gastric cancer (16).

KIF15 plays a vital role in clustering microtubules into bundles to mediate the movement of cells (44); therefore, the suppression of migration is a consequence of KIF15 inhibition. In addition, PI3K/Akt activity is associated with the regulation of cell movement (43,45). Notably, in addition to the cytotoxic effect, herein, the microscopic examination of BC cells via both inverted light and fluorescence microscopy revealed greater enlargement in cells treated with Kif15-IN-1 than in the control-treated cells. This result may indicate that KIF15 inhibition is associated with the inhibition of both chromosomal segregation and cell cycle progression (20,46). KIF15 is vital in driving centrosome separation and promotes bipolar spindle assembly during cell division. In addition, the change in cell shape may support the assumption of KIF15 protein inhibition, as KIF15 is involved in microtubule generation and maintaining cell shape and the skeleton (47).

Of note, Dumas *et al* (48) assumed that KIF15 may partially compensate for the effect of mitotic spindle inhibitors (e.g., Eg5 inhibitors) and thereby induce resistance. Therefore, to overcome this resistance, researchers (49,50) have explored the addition of KIF15 inhibitors to mitotic spindle inhibitors and obtained successful combinations. However, further experimentation is required for a more in-depth understanding of the effect mechanisms, the primary limitation of which is the lack of financial support and instrumentation.

In conclusion, in the present study, KIF15 expression was found to be upregulated in BC. Of note, the present study revealed that the small-molecule inhibitor, Kif15-IN-1, was cytotoxic to ER^+ and TNBC BC cell lines. This resulted in a significant decrease in the ability to migrate, along with morphological alterations and the activation of apoptosis. The effects were linked mechanistically to the downregulation of the *KIF15* gene. Therefore, further *in vivo* studies are warranted to confirm these findings, and exploring the combination of Kif15-IN-1 with existing chemotherapies in BC is advisable.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

AHA designed and executed the experiments (MTT, cell culture, apoptosis analysis, scratch, morphological assays

and statistical analysis). AHA was also involved in the conceptualization of the study, in material collection, in the writing, reviewing and editing of the original draft of the manuscript, in study supervision, and in project administration. SGA was involved in the conceptualization of the study, in the study methodology, in the validation of the RT-qPCR results, in data curation, and in study supervision. SGA was also involved in the writing of the original draft of the manuscript and in material collection. SIAJ was involved in the conceptualization and design of the study, study execution, and in the analysis of the RT-qPCR results. SIAJ was also involved in the writing of the original draft of the manuscript and in material collection. SAM maintained the cells and participated in the experiments (MTT, cell culture, apoptosis, scratch, and morphological assays and material collection). AHA and SGA confirm the authenticity of all the raw data. All the authors have read and approved the final version of the manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Giaquinto AN and Jemal A: Cancer statistics, 2024. CA Cancer J Clin 74: 12-49, 2024.
2. Al-Juboori SI, Vadakekolathu J, Idri S, Wagner S, Zafeiris D, Pearson JR, Almshayakhchi R, Caraglia M, Desiderio V, Miles AK, *et al*: PYK2 promotes HER2-positive breast cancer invasion. J Exp Clin Cancer Res 38: 210, 2019.
3. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R and Stanisławek A: Breast cancer-epidemiology, risk factors, classification, prognostic markers, and current treatment Strategies-an updated review. Cancers (Basel) 13: 4287, 2021.
4. Larkin L: Breast cancer genetics and risk assessment: An overview for the clinician. Climacteric 26: 229-234, 2023.
5. Park JH, Jonas SF, Bataillon G, Criscitiello C, Salgado R, Loi S, Viale G, Lee HJ, Dieci MV, Kim SB, *et al*: Prognostic value of tumor-infiltrating lymphocytes in patients with early-stage triple-negative breast cancers (TNBC) who did not receive adjuvant chemotherapy. Ann Oncol 30: 1941-1949, 2019.
6. Aine M, Boyaci C, Hartman J, Häkkinen J, Mitra S, Campos AB, Nimeus E, Ehinger A, Vallon-Christersson J, Borg Å, *et al*: Molecular analyses of triple-negative breast cancer in the young and elderly. Breast Cancer Res 23: 20, 2021.
7. Lehmann BD, Pietenpol JA and Tan AR: Triple-negative breast cancer: Molecular subtypes and new targets for therapy. Am Soc Clin Oncol Educ Book: e31-e39, 2015.
8. Zboril EK, Grible JM, Boyd DC, Hairr NS, Leftwich TJ, Esquivel MF, Duong AK, Turner SA, Ferreira-Gonzalez A, Olex AL, *et al*: Stratification of tamoxifen synergistic combinations for the treatment of ER+ breast cancer. Cancers (Basel) 15: 3179, 2023.
9. Höller A, Nguyen-Sträuli BD, Frauchiger-Heuer H and Ring A: 'Diagnostic and prognostic biomarkers of luminal breast cancer: Where are We Now?' Breast Cancer (Dove Med Press) 15: 525-540, 2023.
10. Hirokawa N, Noda Y, Tanaka Y and Niwa S: Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 10: 682-696, 2009.
11. Miki H, Okada Y and Hirokawa N: Analysis of the kinesin superfamily: Insights into structure and function. Trends Cell Biol 15: 467-476, 2005.
12. Sun RF, He N, Zhang GY, Yu ZY, Li LS, Ma ZJ and Jiao ZY: Combined inhibition of KIF11 and KIF15 as an effective therapeutic strategy for gastric cancer. Curr Cancer Drug Targets 23: 293-306, 2023.
13. Zeng H, Li T, Zhai D, Bi J, Kuang X, Lu S, Shan Z and Lin Y: ZNF367-induced transcriptional activation of KIF15 accelerates the progression of breast cancer. Int J Biol Sci 16: 2084-2093, 2020.
14. Gao X, Zhu L, Lu X, Wang Y, Li R and Jiang G: KIF15 contributes to cell proliferation and migration in breast cancer. Hum Cell 33: 1218-1228, 2020.
15. Wang J, Wang D, Fei Z, Feng D, Zhang B, Gao P, Hu G, Li W, Huang X, Chen D, *et al*: KIF15 knockdown suppresses gallbladder cancer development. Eur J Cell Biol 100: 151182, 2021.
16. Ding L, Li B, Yu X, Li Z, Li X, Dang S, Lv Q, Wei J, Sun H, Chen H, *et al*: KIF15 facilitates gastric cancer via enhancing proliferation, inhibiting apoptosis, and predict poor prognosis. Cancer Cell Int 20: 125, 2020.
17. Al Kufi SGJH, Emmerson J, Rosenqvist H, Garcia CMM, Rios-Szwed DO and Wiese M: Absence of DEATH kinesin is fatal for Leishmania mexicana amastigotes. Sci Rep 12: 3266, 2022.
18. Wang J, Guo X, Xie C and Jiang J: KIF15 promotes pancreatic cancer proliferation via the MEK-ERK signalling pathway. Br J Cancer 117: 245-255, 2017.
19. Wang Q, Han B, Huang W, Qi C and Liu F: Identification of KIF15 as a potential therapeutic target and prognostic factor for glioma. Oncol Rep 43: 1035-1044, 2020.
20. Sheng J, Li C, Dong M and Jiang K: Identification by comprehensive bioinformatics analysis of KIF15 as a candidate risk gene for Triple-negative breast cancer. Cancer Manag Res 12: 12337-12348, 2020.
21. Shahin R and Aljamal S: Kinesin spindle protein inhibitors in cancer: From high throughput screening to novel therapeutic strategies. Future Sci OA 8: FSO778, 2022.
22. Gibbs B, Douglas J, Wates R, McDonald P, Whitaker A, Ndi C, Pathak H, Harned L, Neuenswander S, Broward M, *et al*: Abstract 5334: Targeting the KIF15-TPX2 PPI to overcome KIF11 inhibitor resistance in epithelial ovarian cancer. Cancer Res 83: 5334-5334, 2023.
23. Holliday DL and Speirs V: Choosing the right cell line for breast cancer research. Breast Cancer Res 13: 215, 2011.
24. Fedele P, Orlando L and Cinieri S: Targeting triple negative breast cancer with histone deacetylase inhibitors. Expert Opin Investig Drugs 26: 1199-1206, 2017.
25. Fathi SM, Alhammer AH and Ali IA: Testing the cytotoxic potential of biosynthesized nanoparticles using Conocarpus erectus Leaves against human breast cancer cells. AIP Conf Proc 2922: 040008, 2024.
26. Zaki NH, Ali AM, Al-Rubaiee GH and Alhammer AH: Malaysian Anti-bacterial and Anti-tumoral activities of spirulina platensis extracellular extract. J Med Health Sci 18: 11-16, 2022.
27. Ali SM, Al-Karam LQ and Alhammer AJN: In Vitro cancer cells therapy with nano-gold depending on its optical properties. NeuroQuantology 20: 57-61, 2022.
28. Lafta FM, Mohammed RK, Alhammer AH and Ahmed ME: Cytotoxic potential of Neem (Azadirachta indica A. Juss) Oil. Tropical J Natural Product Res 7: 5436-5440, 2023.
29. Alhammer AH, Al-juboori SI and Mudhafar SA: APR-246 enhances the anticancer effect of doxorubicin against p53-mutant AsPC-1 pancreatic cancer cells. Baghdad Sci J 21: 2551-2560, 2024.
30. Nashaan FA, Al-Rawi MS, Alhammer AH, Rabie AM and Tomma JH: Synthesis, characterization, and cytotoxic activity of some imides from galloyl hydrazide. Eurasian Chem Commun 4: 966-975, 2022.
31. Mohammed I, Alhammer AH and Arif IS: The p53 reactivator PRIMA-1^{MET} synergises with 5-fluorouracil to induce apoptosis in pancreatic cancer cells. Invest New Drugs 41: 587-595, 2023.
32. Ahmadiankia N, Bagheri M and Fazli MJAB: Differential migration-related gene expression and altered cytokine secretion in response to serum starvation in cultured MDA-MB-231 cells. Asian Biomed 13: 123-129, 2019.
33. Chen M, Huang J, Yang X, Liu B, Zhang W, Huang L, Deng F, Ma J, Bai Y, Lu R, *et al*: Serum starvation induced cell cycle synchronization facilitates human somatic cells Reprogramming. PLoS One 7: e28203, 2012.
34. Aghababazadeh M and Kerachian MA: Cell fasting: Cellular response and application of serum starvation. J Fasting Health 2: 147-150, 2014.

35. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
36. Lei S, Zheng R, Zhang S, Wang S, Chen R, Sun K, Zeng H, Zhou J and Wei W: Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020. Cancer Commun (Lond) 41: 1183-1194, 2021.
37. Qiao Y, Chen J, Ma C, Liu Y, Li P, Wang Y, Hou L and Liu Z: Increased KIF15 expression predicts a poor prognosis in patients with lung adenocarcinoma. Cell Physiol Biochem 51: 1-10, 2018.
38. Alfarsi LH, Elansari R, Toss MS, Diez-Rodriguez M, Nolan CC, Ellis IO, Rakha EA and Green AR: Kinesin family member-18A (KIF18A) is a predictive biomarker of poor benefit from endocrine therapy in early ER+ breast cancer. Breast Cancer Res Treat 173: 93-102, 2019.
39. Kang H, Ga YJ, Kim SH, Cho YH, Kim JW, Kim C and Yeh JY: Small interfering RNA (siRNA)-based therapeutic applications against viruses: Principles, potential, and challenges. J Biomed Sci 30: 88, 2023.
40. Stapleton JA, Endo K, Fujita Y, Hayashi K, Takinoue M, Saito H and Inoue T: Feedback control of protein expression in mammalian cells by tunable synthetic translational inhibition. ACS Synth Biol 1: 83-88, 2012.
41. Wang Z, Chen M, Fang X, Hong H, Yao Y and Huang H: KIF15 is involved in development and progression of Burkitt lymphoma. Cancer Cell Int 21: 261, 2021.
42. Sun X, Chen M, Liao B and Liang Z: Knockdown of KIF15 promotes cell apoptosis by activating crosstalk of multiple pathways in ovarian cancer: Bioinformatic and experimental analysis. Int J Clin Exp Pathol 14: 267-291, 2021.
43. Bi H, Hou X, Shen Q, Liu Z, Zhu X, Ma L and Lu J: Knockdown of KIF15 suppresses proliferation of prostate cancer cells and induces apoptosis through PI3K/Akt signaling pathway. Cell Death Discov 9: 326, 2023.
44. Mann BJ, Balchand SK and Wadsworth P: Regulation of Kif15 localization and motility by the C-terminus of TPX2 and microtubule dynamics. Mol Biol Cell 28: 65-75, 2017.
45. Xue G and Hemmings BA: PKB/Akt-dependent regulation of cell motility. J Natl Cancer Inst 105: 393-404, 2013.
46. Malaby HLH, Dumas ME, Ohi R and Stumpff J: Kinesin-binding protein ensures accurate chromosome segregation by buffering KIF18A and KIF15. J Cell Biol 218: 1218-1234, 2019.
47. Klejnot M, Falnikar A, Ulaganathan V, Cross RA, Baas PW and Kozielski F: The crystal structure and biochemical characterization of Kif15: A bifunctional molecular motor involved in bipolar spindle formation and neuronal development. Acta Crystallogr D Biol Crystallogr 70: 123-133, 2014.
48. Dumas ME, Chen GY, Kendrick ND, Xu G, Larsen SD, Jana S, Waterson AG, Bauer JA, Hancock W, Sulikowski GA and Ohi R: Dual inhibition of Kif15 by oxindole and quinazolinedione chemical probes. Bioorg Med Chem Lett 29: 148-154, 2019.
49. Solon AL, Zaniewski TM, O'Brien P, Clasby M, Hancock WO and Ohi R: Synergy between inhibitors of two mitotic spindle assembly motors undermines an adaptive response. Mol Biol Cell 33: ar132, 2022.
50. Milic B, Chakraborty A, Han K, Bassik MC and Block SM: KIF15 nanomechanics and kinesin inhibitors, with implications for cancer chemotherapeutics. Proc Natl Acad Sci USA 115: E4613-E4622, 2018.

Copyright © 2024 Alhammer et al. This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) License.