Evaluation of 2-(4-Chlorophenyl)-4-(4-fluorophenyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-one as a p38 MAPK inhibitor in MCF-7 and MDA-MB-231 breast cancer cell lines.

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Abstract:

Breast cancer is a diverse disease with high mortality rates, often due to metastasis. Breast cancer subtypes vary by the expression of progesterone receptor, human epidermal growth factor receptor 2, and estrogen receptor. About 15%-20% of breast cancer cases are triple-negative, which lack these receptors and have poor prognosis and limited treatment options. The Mitogen-Activated Protein Kinase, particularly p38, are involved in cellular responses and cancer progression.

The study aimed to compare the cytotoxic effects of the compound 2-(4-Chlorophenyl)-4-(4-fluorophenyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-one as a p38 mitogen-activated protein kinase inhibitor in Breast cancer cell lines (MCF-7 and MDA-MB-231). The half-maximal inhibitory concentration values were calculated using nonlinear regression analysis. The half-maximal inhibitory concentration of the investigated compound p38 Mitogen-Activated Protein Kinase inhibitor was 5.355 µg in MCF-7 cells, whereas in MDA-MB-231 cells, it was 1.419 µg. In conclusion, the compound showed higher sensitivity and effectiveness in triple-negative breast cancer cells than estrogen receptor-positive cells.

Keywords: Breast cancer; p38 MAPK; MCF-7; MDA-MB-231.

مثبط P38 MAPكاينيز2-(4-Chlorophenyl)-4-(4-fluorophenyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-oneكمضاد جديد لسرطان الثدي

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خلاصة

يعد سرطان الثدي الأكثر تشخيصًا بين النساء وثاني أهم سبب لوفيات السرطان. في عام 2020 شكل سرطان الثدي 11.7٪ من الحالات الجديدة على مستوى العالم. في العراق، ارتفع معدل الإصابة بسرطان الثدي من 52.00 لكل 100,000 شخص في عام 2000 إلى 91.66 لكل 100,000 في عام 2019. سرطان الثدي (BC) مرض متنوع سريريًا وله معدلات وفيات مرتفعة، غالبا بسبب الانتشار. تختلف الأنواع الفرعية لسرطان الثدي بناء على تعبيرPR ، HER2، وER. حوالي 15%-20% من حالات سرطان الثدي تصنّف على أنها ثلاثية السلبية(TNBC) ، والتي تفتقر لهذه المستقبلات وتتمتع بتوقعات فقيرة وخيارات علاج محدودة. تشاركMAPKs ، وخاصةp38 ، في الاستجابات الخلوية وتلعب دورًا في تطور السرطان .هدفت هذه الدراسة إلى التحقيق في التأثيرات السامة للخلايا لمركب 2-(4-كلوروفينيل) -4-(4-فلوروفينيل) -5-بيريدين-4-يل-1,2-ديهيدروبرازول-3-ون كمثبط لـ p38 MAPK لعلاج سرطان الثدي. تم حساب قيم IC50 باستخدام تحليل الانحدار غير الخطي. كانت قيمة IC50 لمثبطp38 MAPK هي 5.355 ميكروغرام في خلاياMCF-7 ، بينما كانت في خلايا-MDA MB-231أقل بكثير عند 1.419 ميكروغرام. يشير ذلك إلى حساسية وفعالية أعلى للمركب في خلايا سرطان الثدي الثلاثي السلبية مقارنة بخلايا مستقبلات الإستروجين الإيجابية.

الكلمات المفتاحية :سرطان الثدي؛p38 MAPK؛ MDA-MB-231. MCF-7

Introduction

Breast cancer (BC) is the most common malignancy in women worldwide, account 25% of all cancers [1]. Invasive BC remains the predominant form of cancer in women globally, constituting around 11.7% of new cases in 2020 [2]. In Iraq, the incidence of new cancer cases has increased significantly, rising from 52.00 per 100,000 individuals in 2000 to 91.66 per 100,000 in 2019. In that year, BC was the leading cause of death among Iraqi women, comprising around one-third of all reported cancer cases and holding the highest percentage (22.58%) and fatality rate (6.22 per 100,000) among all cancer types [3].

BC is a clinically diverse disease and is one of the most frequently diagnosed and lethal cancers in females, with high mortality and morbidity rates globally [4]. BC can be genetic and may result from radiation exposure, environmental toxins, alcohol consumption, and lifestyle factors [5, 6]. Metastasis, involving both migration and invasion, is the primary cause of death in cancer patients [7]; hence, it is crucial to inhibit the migration and invasion of cancer cells [8]. BC classification of cancer is determined by the levels of expression of progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and estrogen receptor (ER), which vary morphologically and clinically [9].

Approximately 15%-20% of BC cases are classified as triple-negative breast cancer (TNBC), lacking expression of ER, PR, and HER2 receptors [10]. TNBC is clinically associated with a poor prognosis, highly proliferative cancer cells, and rapid development compared to other BC types [11]. The molecular mechanisms are not fully understood [12]; therefore, it is important to understand the molecular mechanisms of TNBC and identify effective therapeutic targets. Mitogen -activate protein kinases (MAPK) are Ser/Thr kinases involved in cellular responses to stimuli; they play essential roles in multiple biological processes [13]. The four main Mammals MAPKs (ERK 1/2, c-Jun N terminal kinase (JNK)1–3, p38, and ERK5) [14]. ERK1 and ERK2 mainly function in cell proliferation and sur ival, while the JNK and p38 MAPK pathways are mainly associated with cellular stress responses and apoptosis regulation [15]. p38 activation has been demonstrated in response to many external stimuli ex (UV light, heat, osmotic shock, inflammatory cytokines (TNF-alpha & IL-1), and growth factors (CSF-1)). Also, p38 activation depends on the specific cell type [16]. p38 is relatively inactive when unphosphorylated, but it's rapidly activated upon

phosphorylation of its two Thr-Gly-Tyr motifs. p38 is activated by dual kinases known as MKKs. MKK3 and MKK6 are the primary upstream kinases responsible for activating it, particularly in response to TNF [17]. Increased expression of MKK kinases activates both p38 and JNK pathways [18].

Cellular responses vary significantly based on cell type and the stimulus. The involvement of p38 in cancer progression and therapy is remarkably intricate owing to these attributes. Elevated p38 levels have been associated with aggressive and poor prognoses in BC cases [19]

The investigated compound, $C_{20}H_{13}ClFN_3O$, was obtained from Santa Cruz Biotechnology. Santa Cruz Biotechnology is a well-known provider of research chemicals and biochemicals, including antibodies, proteins, and other reagents used in biomedical research. These compound p38 MAP Kinase Inhibitor, ($C_{20}H_{13}ClFN_3O$) also referenced under CAS 219138-24-6 which was, is selectively inhibits the activity of p38 MAPK, which are a class of enzymes involved in cellular responses to stress and inflammatory cytokines [20]. This inhibitor compound is a tool for dissecting the signaling pathways mediated by p38 MAPK, which include regulating pro-inflammatory cytokine production, apoptosis, and cell differentiation. Researchers employ p38 MAP Kinase Inhibitors ($C_{20}H_{13}ClFN_3O$) to study their role in modulating the inflammatory response and to understand the molecular basis of diseases where p38 MAPK is implicated [21 22]. By inhibiting p38 MAPK activity, researchers can explore the kinase′s contributions to cellular processes and its interactions with other signaling molecules [23].

The current study aimed to Evaluation analysis of 2-(4-Chlorophenyl)-4-(4-fluorophenyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-one as a p38 MAPK inhibitor on the proliferation between MCF-7 and MDA-MB-231 cell lines for BC.

Materials and method

Study Design

This research examined the effects of p38 MAPK inhibitor compound on BC cell lines (MCF-7 and MDA-MB-231) to assess its potential as a therapeutic approach for BC. The methodology involved testing the inhibitory effects of the p38 MAPK inhibitor on MCF-7 and MDA-MB-231 cell lines by exposing the cell lines to various concentrations of ($C_{20}H_{13}ClFN_3O$).

Sample Preparation

To prepare a stock solution of ($C_{20}H_{13}ClFN_3O$) at a concentration of 500 µg/ml, 500 µg of the inhibitor is dissolved in 1 ml of dimethyl sulfoxide (DMSO).

Cell Lines

MCF-7 is classified as a “Luminal A” subtype of BC [24, 25]. Also, The MDA-MB-231 cell line is derived from a pleural effusion from a patient with invasive ductal carcinoma and is commonly used as a model for advanced BC. This cell line is characterised by its lack of estrogen receptor, progesterone receptor, and E-cadherin expression. Additionally, MDA-MB-231 cells do not express the growth factor receptor HER2, making them a representative model of TNBC [26].

Cell Culturing

The cells were cultivated in a completely supplied Dulbecco’s Modified Eagle’s Medium (DMEM) containing high glucose levels. (Capricorn Scientific, GmbH) Then, it is supplemented with 10% fetal bovine serum (FBS) (Capricorn Scientific, GmbH) and 1% antibiotic/antimycotic (Capricorn Scientific, GmbH). Cells were seeded in 75 cm^3 culture flasks at 3 × 10^3 cells/ml density and

maintained in an incubator (Hermle, Germany) at a constant temperature of 37°C with 5% CO_2. Subculturing was performed twice weekly when confluency exceeded 70% by washing the cell monolayers with phosphate-buffered saline (PBS) (Capricorn Scientific, GmbH), followed by incubation with 0.05% trypsin/EDTA (Capricorn Scientific, GmbH) to detach the cells. The trypsinised cells were resuspended in complete media and separated using a centrifuge (Hettich, Germany) [27].

Cell Proliferation Assay

The colourimetric MTT assay is convenient for measuring cell viability and proliferation [28]. After trypsinisation, cell suspensions were prepared and plated into 96-well plates at concentrations of 3×10^3, 4×10^3, and 5×10^3 cells/well for incubation periods of 24, 48, and 72 hours, respectively. Serial dilutions of ($C_{20}H_{13}ClFN_3O$) dissolved in DMSO (Thomas Baker, India) were prepared at concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5, 0.75, 0.32, 0.15, 0.05, and 0.025 µg. When cell confluency reached 80–90%, cells were exposed to the different concentrations of the ($C_{20}H_{13}ClFN_3O$) compound. After incubation, 20 µL of MTT (Bidepharm, Shanghai, China) diluted in PBS was added to each well and incubated at 37°C for 4 hours. To prevent DMSO crystallisation, 100 µl of the solvent was added to each well on the plates at room temperature and under conditions of full darkness. The absorbance at 540 nm, which matches the wavelength of the standard reference, might be measured using a microplate reader. The substance's cytotoxicity was assessed by comparing the absorption of cells treated with it to that of control cells. The compound utilised for the experiment was obtained from Promega, USA [29]. The experiment was conducted in triplicate for each treatment.

Statistical analysis.

All statistical analyses of the p38 Mitogen-activated Protein Kinase Inhibitor data were performed using MedCalc 14.8.1 (Ostend, Belgium). The choice of MedCalc was based on its comprehensive suite of statistical tools and its capability to handle a variety of analyses required for this study.

Results

Cytotoxicity assay

The MCF-7 cells were treated with different concentrations ranging from 0.025 to 50 µg ($C_{20}H_{13}ClFN_3O$); after 72 h, the cytotoxicity analysis was conducted using the MTT test to determine the level of toxicity on MCF-7 cell lines. The half-maximal growth inhibitory concentration (IC_{50}) of the investigated compound in MCF-7 cells was obtained following incubation for 72h; the dose-response curve was obtained by plotting the concentrations of p38 MAPK inhibitor after log transformation. The IC_{50} values were calculated using nonlinear regression analysis, as shown in **(Figure 1)**, where it shows the IC_{50} of the investigation compound was 5.355µg.

In MDA-MB-231 cell lines, the IC_{50} value was obtained from a range concentration from 0.025 to 50 µg for $C_{20}H_{13}ClFN_3O$ compound by MTT assay. The result of IC_{50} for the investigation compound was 1.419µg in MDA-MB-231 cell lines, as shown in **(Figure 2)**.

Comparison of percentage cell viability between MDA-MB-231 and MCF-7 cell lines were treated for 72h with 0.025, 0.05, 0.15, 0.32, 0.75, 1.56, 3.12, 6.25, 12.5, 25 and 50µg dose ranges of p38 MAPK inhibitor compound. The results represent the mean absorbance ± SEM of 3 independent experiments using Excel software to drawbars, as shown in **(Figure 3)**.

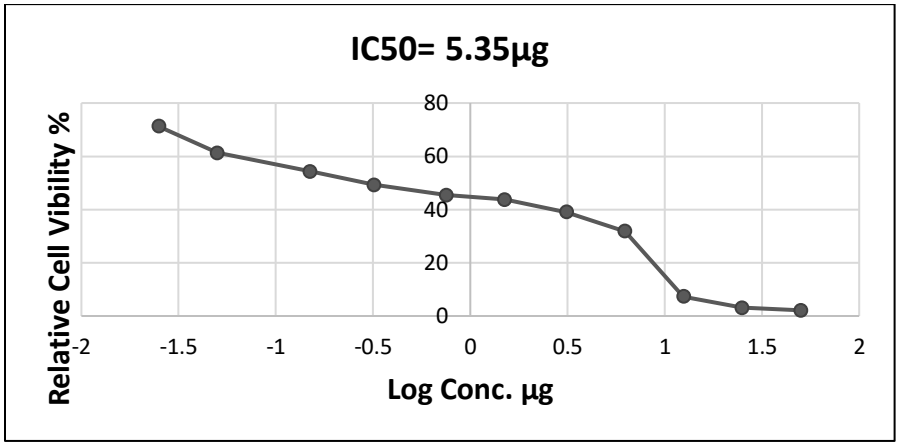


Figure 1: The IC50 for ($C_{20}H_{13}ClFN_3O$) P38 MAPK inhibitor in MCF-7

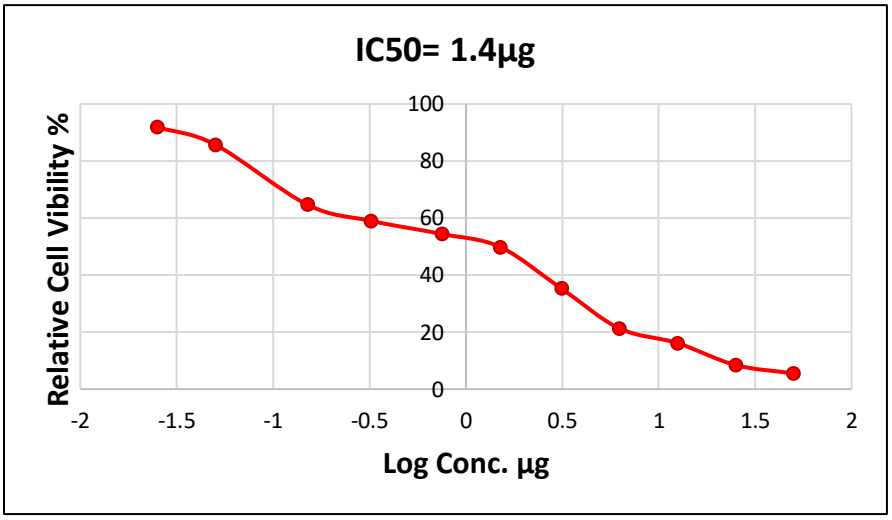
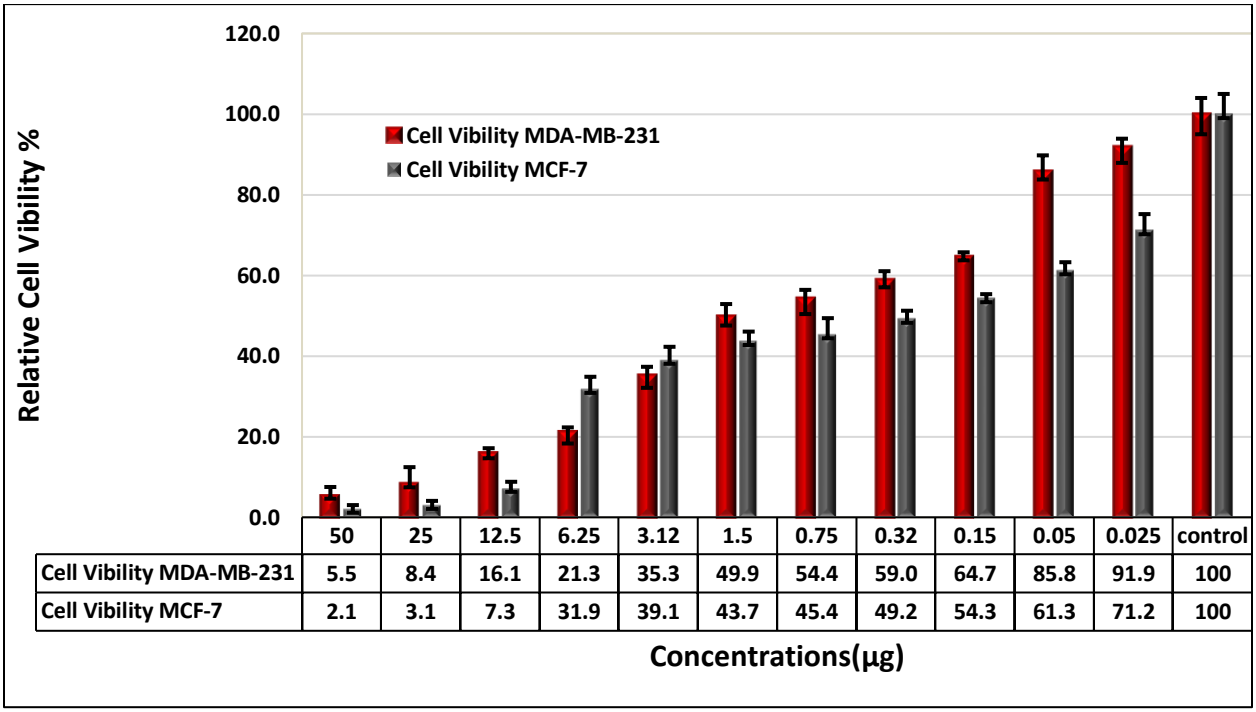


Figure 2: The IC50 for ($C_{20}H_{13}ClFN_3O$) P38 MAPK inhibitor in MDA-MB-231.



	50	25	12.5	6.25	3.12	1.5	0.75	0.32	0.15	0.05	0.025	control
Cell Vibility MDA-MB-231	5.5	8.4	16.1	21.3	35.3	49.9	54.4	59.0	64.7	85.8	91.9	100
Cell Vibility MCF-7	2.1	3.1	7.3	31.9	39.1	43.7	45.4	49.2	54.3	61.3	71.2	100

Figure 3: Comparison of percentage cell viability between MDA-MB-231 and MCF-7 cell lines.

Discussion

The cancers of the breast are classified into hormone receptor-positive and negative groups to guide patient treatment [30]. The involvement of p38 MAPK in cancer is multifaceted, leading to varying outcomes depending on different systems and conditions. Nonetheless, increasing research indicates that p38 MAPK inhibitors show promise for treating cancer-related diseases. Further *in vivo* and clinical studies are needed to elucidate the therapeutic potential of these inhibitors in cancer treatment. Current evidence demonstrates that p38 MAPK inhibitors can suppress cancer cell proliferation, invasion, and migration in vitro [31, 32]. The p38 MAPK signaling pathway is crucial in cell proliferation, apoptosis, and motility, particularly in enhancing cell migration, tumor invasion, and metastasis [33, 34]. In BC patients, elevated p38 levels are linked to highly invasive tumors and poor prognosis [35, 36]. Depending on the nature of the stimuli and cellular context, p38 can mediate various cellular responses. The cytotoxicity results presented here are consistent with previous studies showing that p38 MAPK inhibitors (SB203580 and SB202190) exert ant-proliferative effects on MDA-MB-231 cells by downregulating ERK1/2 phosphorylation (which regulates NF-κB activation) and increasing Ser15 phosphorylation of mutant p53 (R280K) [37]. Another study reported that SB203580 downregulated p38 and NF-κB pathways in MDA-MB-231 cells treated with LyeTx II, which enhances aggressive BC cell proliferation [38]. Additionally, inhibitors of ERK1/2 (PD0325901), p38 MAPK (SB203580), and PI3K (LY294002) preferentially reduced proliferation in MCF-7 cells [39]. Chen et al. found that targeting the p38α isoform inhibited BC cell proliferation in MDA-MB-468 cells [40]. The complex interplay between cell survival and death is a critical area of research aiming to understand how tumor cells regulate these processes. Many effective anticancer drugs induce apoptosis in tumor cells, which is believed to significantly contribute to their

therapeutic effects [41]. The pathways through which these drugs induce apoptosis vary depending on the drug characteristics and the genetic background and origin of the tumor [42].

Conclusion

The $C_{20}H_{13}ClFN_3O$ compound was more effective in the TNBC cell line (MDA-MB-231) than luminal A breast cancer cell line (MCF-7) according to more potent suppression of cellular proliferation in the MTT assay, our findings indicate that targeting the p38 MAPK-signaling pathway could be a promising therapeutic approach for treating breast cancer.

Conflict of interests

The authors declared no conflict of interest

Funding source

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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