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Evaluation of Cytotoxicity of 2-(4-phenylthiazol-2-yl) benzo[de]isoquinoline-1,3-dione Derivatives

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ABSTRACT

Background and Objective: This study aims to evaluate the cytotoxic effects of 2-(4-phenylthiazol-2-yl) benzo[de]isoquinoline-1,3-dione derivatives. These compounds were investigated for their potential to induce apoptosis in fibroblast, KB, MCF-7, and PC3 cell lines.

Materials and Methods: The cytotoxicity of the synthesized derivatives was assessed on fibroblast, KB, MCF-7, and PC3 cell lines using the MTT assay. Additionally, mitochondrial membrane potential and reactive oxygen species (ROS) levels were measured in these cells. Data were analyzed using Prism software.

Results: Among methoxy-substituted derivatives at meta and para positions, the para-methoxy substituted compound exhibited higher cytotoxicity against fibroblast cells with an IC50 of 9.5 ± 0.004 µM compared to the meta-substituted derivative. This increased toxicity is attributed to the electron-donating capacity of the para-methoxy group, which facilitates electron donation to the aromatic rings. In the KB cell line, the derivative bearing a para-methyl substituent showed significant cytotoxicity with an IC50 of 8.3 ± 0.005 µM. Both para-methoxy and para-methyl derivatives demonstrated acceptable cytotoxic effects against MCF-7 cells.

Conclusion: The methyl substituent, acting as an electron-donating group, exhibited notable cytotoxicity against the tested cell lines. Based on these in vitro anti-cancer findings, further studies are recommended to evaluate the effects of these derivatives on caspase activity and to explore their cytotoxic potential against other cancer cell lines.

Key words: Thiazole derivatives, Apoptosis, Anti-cancer

INTRODUCTION

Cancer is a major global public health concern. The mortality rate caused by cancer is particularly high in developing countries. Among women, malignant breast cancer is the most prevalent cancer and the leading cause of cancer-related deaths. Although chemotherapy remains the primary treatment for cancer, its use is limited due to adverse side effects and the increasing development of drug resistance. The growing incidence of chemotherapy drug resistance poses a significant challenge to effective cancer treatment. Therefore, there is an urgent need to develop new classes of chemotherapeutic agents. DNA-alkylating agents represent an important category of anticancer drugs used in cancer therapy.

Epidemiology of cancer can contribute to the understanding of the origins of cancer. By utilizing epidemiological studies that link specific environmental, racial (genetic), and cultural influences to the incidence of particular neoplasms, significant insights into the causes of cancer can be gained. Certain diseases that are associated with increased health risks (pre-neoplastic disorders) also provide key understanding of cancer pathogenesis, as they help explain the ultimate causes of cancer development at the cellular and molecular levels, resulting in disruptions to cell growth and behavior [4]. Cancer refers to a group of diseases characterized by abnormal cells that exhibit uncontrolled and extensive growth. Cancer cells do not follow the normal regulatory processes that control cell growth, survival, and proliferation, and they cannot perform physiological functions similar to those of their differentiated counterparts. Most cancer cells are either undifferentiated or poorly differentiated. Another hallmark of cancer cells is their ability to detach from the primary tumor, invade adjacent tissues, and spread through the blood or lymphatic system to colonize other tissues (metastasis). They can also stimulate the formation of new blood vessels (angiogenesis) and possess unlimited capacity for DNA transcription, enabling continued growth and survival. Cancers can arise in any tissue of the body and form either benign or malignant tumors. If the growth of a malignant tumor proceeds uncontrollably, it ultimately leads to the death of the patient, whereas benign tumors do not metastasize or invade other tissues [5, 6]. Cancer is a lifestyle-related disease, and environmental factors that cause somatic mutations are the main determinants of cancer in most cases. This view is supported by geographical differences in cancer incidence and mortality rates of specific cancer types [7].

By identifying cancer treatment methods, one-third of patients have been treated with local methods (surgery or radiotherapy), which are truly effective; of course, this is only the case if the tumor has not metastasized at the time of treatment. In other cases, the diagnosis of early micrometastases is a characteristic feature of neoplasms that necessitates chemotherapy for effective cancer management [8]. Compounds containing thiazole have shown a wide range of biological effects, such as antitumor, antibacterial, anti-inflammatory, and antifungal activities. In the past decade, amides containing heterocycles have served as the basis for designing many pharmaceutical compounds due to their extensive effects. Although most research attention has been focused on pyridine, pyrazole, and oxadiazole, notable studies have also been conducted on compounds containing the thiazole ring, such as 2-phenyl-4-trifluoromethyl thiazole-5-carboxamide, which confirms the anticancer effects of the thiazole ring [9].

Prashanth and his colleagues, in 2014, synthesized a series of 2-(4-benzyl-phenoxy)-N-(4-phenyl-thiazol-2-yl)acetamide compounds and examined their cytotoxic effects on two cell lines, EAC and DLA. Compound 10h showed the strongest anti-proliferative effects by inhibiting the translation of VEGF-A [10]. Turan-Zitouni G and colleagues, in 2016, synthesized bis-thiazole derivatives and investigated the toxicity of these derivatives on the cancer cell lines A549 and C6. The bis-thiazole derivatives inhibited DNA synthesis, and compound 5 exhibited the most significant effects on the cell lines [11]. De Santana TI and his team, in 2017, aiming to develop compounds with high anticancer activity and low toxicity to other organs, synthesized 22 new thiazole derivatives. They ultimately concluded that these derivatives induce DNA fragmentation and mitochondrial depolarization, thereby exerting their anticancer effects [12]. Kaviarasan and colleagues, in 2020, synthesized 4,3,1-thiadiazole derivatives and evaluated the anticancer effects of these compounds. Compounds c3 and j3 showed acceptable effects on MDA-MB-231 cells; compounds c3 and e3 on MCF-7 cells; and compounds j3 and a3 on Vero cells [13].

Removal of the cancerous tumor and the surrounding tissue through surgery is often effective and is considered the primary method for tumors that are sufficiently large and accessible. However, complete surgical removal is difficult, and usually some cancer cells remain. Surgery may have the undesirable side effect of accelerating a metastatic process by altering the growth rate of the residual cancer cells. In many cases, patients die from metastatic cancer after the successful removal of the primary tumor. Clinical observations indicate that the larger the tumor at the time of removal, the greater the likelihood

of patient death from metastatic cancer that was undetectable during surgery [14, 15]. Multifaceted treatment, which follows surgery and includes radiotherapy, chemotherapy, immunotherapy, and other therapeutic methods, offers a higher chance of eliminating cancer cells or at least keeping them in remission. Chemotherapy, radiotherapy, immunotherapy, or their combinations have become the main treatment methods under these conditions. Today, new methods and techniques are under development that have attracted attention in modern medicine, science, and technology [16, 17].

Research has shown that certain factors such as old age, smoking, lack of physical activity or being overweight, poor diet, alcohol consumption, and ultraviolet radiation increase the risk of developing cancer. Given that this disease is considered one of the deadliest diseases in the world in the present century, researchers have continually been seeking new approaches to treat it. There are four standard methods for cancer treatment: surgery, chemotherapy, radiotherapy, and biologic therapy. In recent years, due to the increasing prevalence of cancer, research aimed at discovering and improving the effects of anticancer drugs has intensified. In the pursuit of discovering new molecules with cytotoxic properties and potential anticancer effects, it has been established that derivatives of isoquinoline and thiazole, in addition to their antifungal, antibacterial, and other effects, also exhibit cytotoxic effects. Therefore, based on recent research, it was decided to investigate the cytotoxicity of derivatives of 2-(4-phenyl-thiazol-2-yl)-benzo[de]isoquinoline-3,1-dione as apoptosis inducers with potential anticancer effects.

Materials and Methods

Since studying cells and examining their changes *in vivo* (within the living organism) is impossible, in cell culture methods, cells are grown *in vitro* (outside the living organism) in the laboratory, which facilitates their study. Cell lines were obtained from the cell bank of the Pasteur Institute of Iran (Table 1).

Table 1: Cell lines used in this study and their tissue of origin

origin tissue	cell line name
human dermal normal fibroblast	Fibroblast
Human epithelial carcinoma Cell Lines	KB
Human Breast Cancer Cell Lines	MCF-7
Human Prostate Cancer Cell Lines	PC_3

In cell culture, cells usually grow either on a solid substrate (adherent to the surface) or suspended in a liquid medium (in suspension) inside a culture vessel containing the necessary materials for their growth and proliferation. For growth and proliferation, cells require a complete culture medium with specific physicochemical properties and special components.

To prepare the complete culture medium for the growth and maintenance of the cells, a medium containing the following components was used:

DMEM/F12 medium	89 ml (89%)
FBS Serum	10 ml (10%)
Penicillin-streptomycin 100 x	1ml (1%)
NaCl	8 g (137mM)
KCl	0.2 g (2.7mM)

KH_2PO_4	0.24g (2mM)
$Na_2HPO_4.12\ H_2O$	1.44 g (10mM)

The mentioned materials were carefully weighed, brought to a volume of 1000 milliliters with deionized water, sterilized by autoclaving, and stored at 4 degrees Celsius. This buffer, due to its appropriate pH for the cells, is used as a washing solution before trypsinization of the cells as well as a diluent for 10X concentrated trypsin.

The cell dissociation solution was prepared by diluting the concentrated stock solution according to the following formula:

PBS buffer	90 ml
Trypsin- EDTA (10 X, 2.5 % Trypsin)	10ml (1X, 0.25 %)

Continuous and repeated culturing of cell lines is not a suitable method for maintaining cells because with ongoing culturing, the risk of microbial contamination of the cell strains increases. To freeze cells in liquid nitrogen, after the final cell count and centrifugation, the supernatant was discarded and the cell pellet was gently resuspended several times to achieve uniformity. Then, freezing medium containing 10% DMSO and 90% culture medium was added dropwise slowly. Dimethyl sulfoxide (DMSO) is a protective agent that prevents ice crystal formation and is necessary for freezing cells. Generally, cells should be frozen at a rate of about -1 to -3 degrees Celsius per minute. One important point during cell freezing is that the cells must be free of any microbial contamination. Also, the cells should be young, healthy, in the logarithmic growth phase, and have a viability of over 90%. It is preferable to change the culture medium 24 hours before freezing to prevent toxic metabolites from damaging the cells and reducing viability. The freezing medium consists of 90% FBS and 10% DMSO. For every one million cells, 1 cc of freezing medium was added, and then the cells were transferred to cryotubes. These cryotubes were placed in a -20°C freezer for three hours. Then, the vials were transferred to a -70°C freezer, and after 24 hours, placed inside a liquid nitrogen tank.

Cell Viability (Cytotoxicity) Assay

To monitor the condition of cells in culture, assess their survival, and evaluate their status after various drug treatments, different methods are used. One of these methods is the MTT assay, which quantitatively measures cell viability by determining the amount of a metabolic product. This assay reflects the activity of cellular mitochondria, which is directly related to cell survival. MTT is a yellow tetrazolium salt that is reduced by dehydrogenase enzymes present in the mitochondria of active living cells to form a purple-colored, crystalline, and insoluble metabolite called formazan. The formed crystals are then dissolved in DMSO, and the optical density (absorbance) of the resulting colored solution is measured by a spectrophotometer at wavelengths between 500 and 600 nm. A decrease in the number of viable cells in the test sample leads to a reduction in total metabolic activity. This reduction is directly related to the decreased formation of purple crystals, indicating lower mitochondrial activity and thus reflecting the proportion of live or dead cells [18].

MTT Dye Solution

This solution is prepared by the following method and is used in the MTT assay:

MTT	89 ml, 89%
PBS buffer	100 ml

After preparation, this solution was aliquoted into containers covered with aluminum foil (due to light

sensitivity) and stored at –20 degrees Celsius.

Cell Toxicity Assessment

To evaluate cell toxicity, different concentrations of 12 synthesized derivatives were prepared and tested on four cell lines. The method used was the MTT assay, in which, after several steps and the addition of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), the intensity of the color produced by formazan formation was measured at an appropriate wavelength. The color intensity is proportional to the number of viable cells. The IC50 values were calculated using Prism software, and finally, the structure-activity relationship of the synthesized compounds was analyzed.

To evaluate the cytotoxic effects, various concentrations of 12 synthesized derivatives were prepared and tested on three cell lines using the MTT method. MTT is a yellow solution that serves as a substrate for the mitochondrial enzyme succinate dehydrogenase. Living cells with active mitochondria can reduce MTT through a series of reactions to produce purple formazan crystals. The formation of formazan is an indicator of cell viability.

The synthesized derivatives were dissolved in DMSO and prepared at various concentrations. Cells were seeded at a density of 20,000 cells per well in wells containing 200 microliters of culture medium. The cells were incubated for 24 hours to allow them to adhere to the bottom of the plate, stabilize, and establish proper cell-to-cell connections. One day after seeding, the cells were exposed to different concentrations (0, 5, 25, 50, and 100 micromolar) of the synthesized derivatives dissolved in DMSO. The plates were then incubated in an incubator with 5% CO2, 95% humidity, and at 37°C for 24 hours. After the incubation period, the plates were removed from the incubator, and the supernatant in each well was gently discarded. Then, 20 microliters of MTT solution were added to each well, and the plates were incubated for three hours. After three hours, 100 microliters of DMSO were added to each well to dissolve the formed formazan crystals. The absorbance of each well was measured at 490 nm using a plate reader. The IC50 values for each compound were calculated using Prism software.

Results

In the MTT assay, the effects of a compound after entering the cell and its impact on mitochondria and cellular metabolic activity are evaluated. After synthesizing the desired compounds, various concentrations of the synthesized derivatives were prepared to select the most potent compound for cytotoxicity testing. These were tested on fibroblast, KB, MCF-7, and PC3 cell lines. The IC50 values of the synthesized compounds are reported in Table 2. The percentage of survival of the cancer cell lines after 24 hours of exposure to different concentrations of the synthesized derivatives was assessed using the MTT method. The IC50 values are expressed in micromolar units, and the statistical data are presented as Mean ± SEM, based on three independent experiments. Statistical analyses were performed using Prism software. P-values less than 0.001 were considered statistically significant.

Table 2: Cell toxicity results based on IC50 (µM ± SEM) in four cancer cell lines

کد ترکیبات	R	Cell lines			
		Fibroblast	KB	MCF-7	PC_3
4a (A)	H	9.5±0.009	7.3±0.007	5±0.013	9.5±0.01
4b (MH)	3-OH	17.7±0.02	10±0.01	12±0.037	35.5±0.04
4c (MMO)	3-O	10.3±0.05	10.3±0.09	6±0.015	21.7±0.03

	Me				
4d (PMO)	4-O Me	9.5±0.004	34±0.017	5±0.012	9.5±0.015
4e (PC)	4-Cl	9.5±0.006	8±0.01	5±0.039	9.5±0.015
4f (PB)	4-Br	26.7±0.006	8±0.009	24±0.037	40±0.02
4g (PF)	4-F	38.3±0.013	21.3±0.01	24±0.069	72.3±0.03
4h (PI)	4-I	9.5±0.005	8±0.006	5±0.02	9.5±0.012
4i (PM)	4-Me	8±0.003	8.3±0.005	5±0.01	6.2±0.012
4j (PN)	4-NO_2	9.5±0.008	38±0.008	5±0.013	9.5±0.015
Doxorubicin	-	2.6	1.4	2.1	2.9

The survival percentage of fibroblast cells after 24 hours of exposure to various concentrations of the synthesized compounds (4a-4j) was assessed using the MTT assay. The IC50 results and the plot of the compounds against the logarithm of concentration are presented in Table 2 and Figure 1, respectively, using the MTT method. This study showed that the synthesized compounds (4a-4j) did not exhibit significant cytotoxicity in this cell line. Among the synthesized compounds (4a-4j), compound (4i) with a para-methyl substitution demonstrated the highest effectiveness in inhibiting the growth of fibroblast cancer cells, with the lowest IC50 value of 8 ± 0.003 micromolar compared to the other compounds.

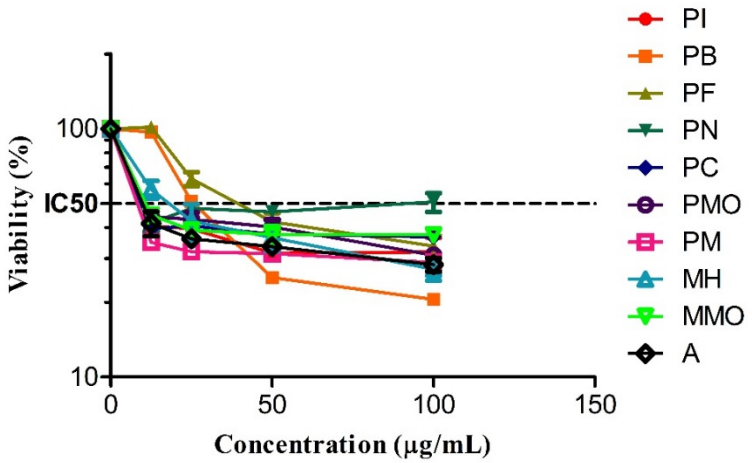

Figure 1. Effect of compounds (4a-4j) on the survival percentage of fibroblast cell line.

Cell viability was measured using the MTT assay. The IC50 values were determined by plotting concentrations (0, 5, 25, 50, and 100 µg/mL) of the compounds against the percentage of viable cells. Results are presented as Mean ± SEM from three independent experiments.

The survival percentage of KB cells after 24 hours of exposure to various concentrations of the synthesized compounds (4a-4j) was evaluated using the MTT assay. The IC50 results and the plot of the

compounds against the logarithm of their concentrations are presented in Table 2 and Figure 2, respectively, using the MTT method. This study showed that the synthesized compounds (4a-4j) did not exhibit significant cytotoxicity in this cell line. Among the synthesized compounds (4a-4j), compound (4a) with a hydrogen substitution demonstrated the highest effectiveness in inhibiting the growth of KB cancer cells, with the lowest IC50 value of 7.3 ± 0.007 micromolar compared to the other compounds.

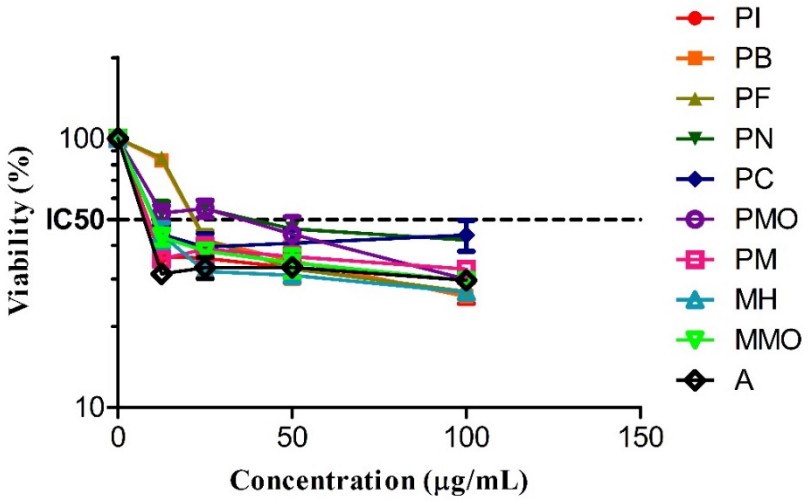

Figure 2. Effect of compounds (4a-4j) on the survival percentage of KB cell line.

Cell viability was measured using the MTT assay. The IC50 values were determined by plotting concentrations (0, 5, 25, 50, and 100 µg/mL) of the compounds against the percentage of viable cells. Results are presented as Mean ± SEM from three independent experiments.

The survival percentage of MCF-7 cells after 24 hours of exposure to various concentrations of the synthesized compounds (4a-4j) was evaluated using the MTT assay. The IC50 results and the plot of the compounds against the logarithm of their concentrations are presented in Table 2 and Figure 3, respectively, using the MTT method.

This study showed that the synthesized compounds (4a-4j) did not exhibit significant cytotoxicity in this cell line. Among the synthesized compounds (4a-4j), compounds (4a, 4d, 4e, 4h, 4i, 4j) with hydrogen, para-methoxy, para-chloro, para-iodo, para-methyl, and para-nitro substitutions showed the highest effectiveness in inhibiting the growth of MCF-7 cancer cells, each with the lowest IC50 value of 5.0 micromolar compared to the other compounds.

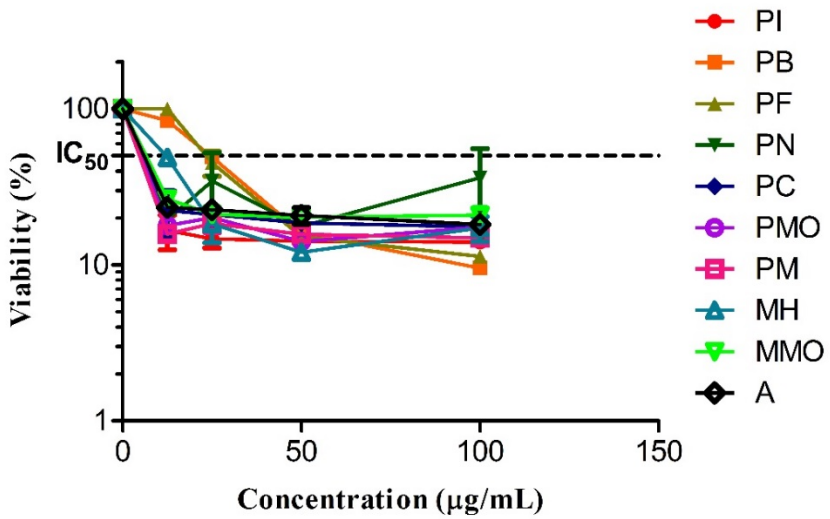

Figure 3. Effect of compounds (4a-4j) on the survival percentage of MCF-7 cell line.

Cell viability was measured using the MTT assay. The IC50 values were determined by plotting concentrations (0, 5, 25, 50, and 100 µg/mL) of the compounds against the percentage of viable cells. Results are presented as Mean ± SEM from three independent experiments.

The survival percentage of PC3 cells after 24 hours of exposure to various concentrations of the synthesized compounds (4a-4j) was evaluated using the MTT assay. The IC50 results and the plot of the compounds against the logarithm of their concentrations are presented in Table 2 and Figure 4, respectively, using the MTT method.

This study showed that the synthesized compounds (4a-4j) did not exhibit significant cytotoxicity in this cell line. Among the synthesized compounds (4a-4j), compound (4i) with a para-methyl substitution demonstrated the highest effectiveness in inhibiting the growth of PC3 cancer cells, with the lowest IC50 value of 6.2 ± 0.012 micromolar compared to the other compounds.

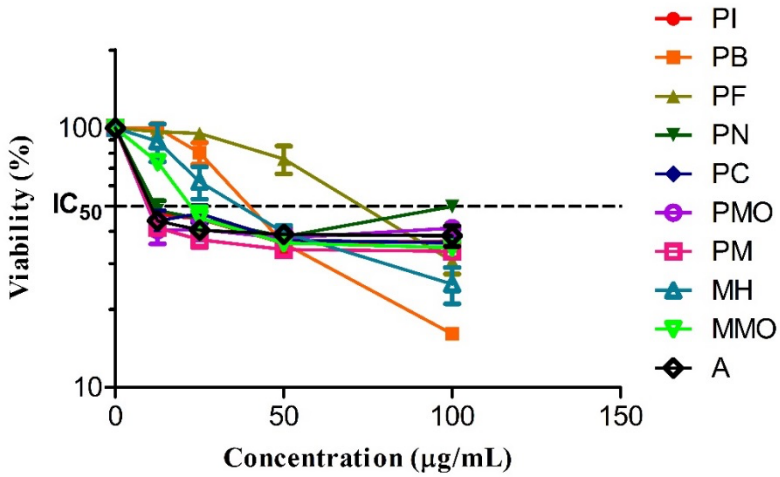

Figure 4: Effect of compounds (4a-4j) on the survival percentage of PC3 cell line.

Cell viability was measured using the MTT assay. The IC50 values were determined by plotting concentrations (0, 5, 25, 50, and 100 µg/mL) of the compounds against the percentage of viable cells. Results are presented as Mean ± SEM from three independent experiments.

In this study, the effects of the compounds that showed the highest cytotoxicity in the MTT assay were evaluated on the mitochondrial membrane potential as one of the indicators of apoptosis. To measure mitochondrial membrane potential, the fluorescent dye Rhodamine 123 was used. This assay is based on the ability of the mitochondrial membrane to retain Rhodamine 123 dye. The cationic fluorogenic dye Rhodamine 123 has a high affinity for accumulation in mitochondria. Therefore, tracking the lipophilic Rhodamine 123 dye, which enters the cytosol by diffusion, and evaluating the fluorescence intensity of this compound after entering the cell, compared with control cells, is considered an indicator of changes in mitochondrial membrane potential. Given the negative charge of the mitochondrial matrix in healthy cells, the positively charged fluorochrome Rhodamine 123 accumulates within the mitochondria. Consequently, the intracellular fluorescence level reflects the degree of changes in mitochondrial membrane potential. Thus, the mitochondrial capacity to uptake Rhodamine 123, determined as the difference in Rhodamine 123 fluorescence intensity between control and treated cells and the percentage of these changes, was used as an index to assess mitochondrial membrane potential loss. Based on this, the mitochondrial membrane permeability effects of the compounds strophanthin (4a), meta-methoxy (4c), para-methoxy (4d), para-chloro (4e), para-iodo (4h), para-methyl (4i), and para-nitro (4j) were measured according to the MTT assay evaluation. In this assay, reduction or no reduction in mitochondrial membrane potential by these derivatives serves as an important indicator for determining apoptosis induction via the intrinsic pathway in MCF-7 cells.

The results shown in Figure 5 indicate that in the MCF-7 cell line, the compounds strophanthin (4a), meta-methoxy (4c), para-methoxy (4d), para-chloro (4e), para-iodo (4h), para-methyl (4i), and para-nitro (4j) significantly decreased the mitochondrial membrane potential compared to the control group. Among these, the compound strophanthin (4a) demonstrated the greatest reduction in mitochondrial membrane potential in the MCF-7 cell line compared to the control.

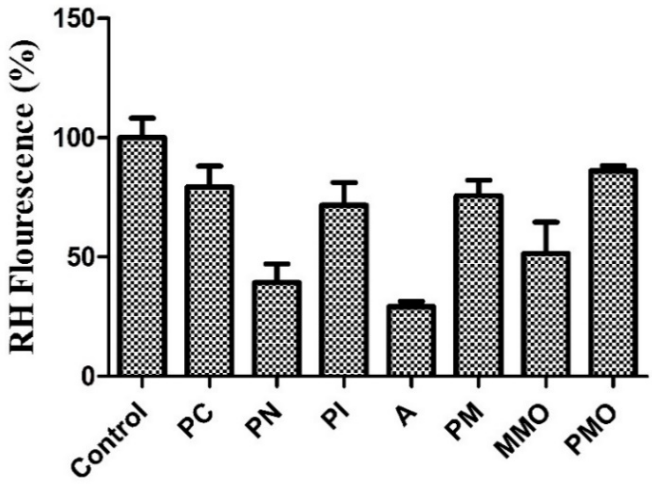

Figure 5: Effect of strophanthin (4a), meta-methoxy (4c), para-methoxy (4d), para-chloro (4e), para-iodo (4h), para-methyl (4i), and para-nitro (4j) derivatives at IC50 concentration on mitochondrial membrane potential in MCF-7 cell line.

Results are presented as Mean ± SEM from three independent experiments, compared to the control group (MCF-7 cells treated with DMSO).

Regarding the determination of reactive oxygen species (ROS), the effect of the derivatives on oxidative stress in MCF-7 cells was investigated using a colorimetric method. Intracellular ROS accumulation was measured using 2',7'-dichlorofluorescin diacetate (DCFH-DA). DCFH-DA is a non-fluorescent

compound that can easily pass through the cell membrane. This compound is hydrolyzed by intracellular esterases to dichlorofluorescin (DCFH), which can then be oxidized in the presence of ROS to the highly fluorescent compound dichlorofluorescein (DCF). This compound can be used as a qualitative and quantitative indicator to measure oxidizing species such as ROS. Fluorescence intensity was measured using a Biotek microplate reader at an excitation wavelength of 488 nm and an emission wavelength of 528 nm. Consequently, the intracellular fluorescence level can indicate the amount of free radicals formed. In this assay, free radical production induced by strophanthin (4a), meta-methoxy (4c), para-methoxy (4d), para-chloro (4e), para-iodo (4h), para-methyl (4i), and para-nitro (4j) compounds at IC50 concentrations was tested in the MCF-7 cell line. The derivatives showed no increase in oxygen free radicals or induction of oxidative stress compared to the control group (Figure 6).

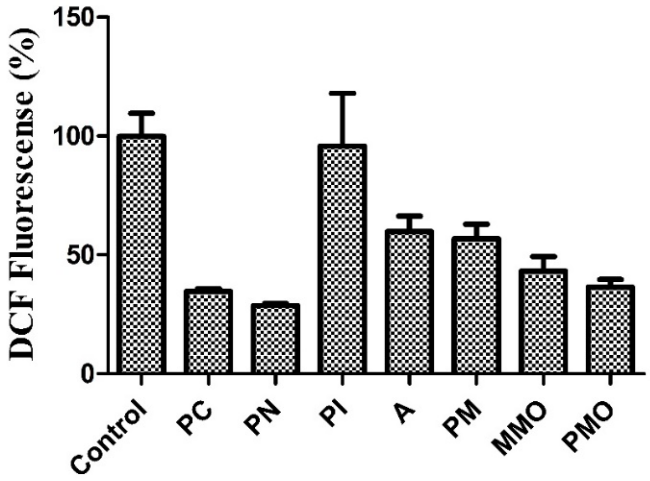

Figure 6: Levels of reactive oxygen species produced after exposure of MCF-7 cells to strophanthin (4a), meta-methoxy (4c), para-methoxy (4d), para-chloro (4e), para-iodo (4h), para-methyl (4i), and para-nitro (4j) derivatives at IC50 concentration.

Results are presented as Mean ± SEM from three independent experiments compared to the control group (MCF-7 cells treated with DMSO).

Discussion

Regarding the cytotoxicity results, a new series of thiazole derivatives were synthesized and their anticancer activities were evaluated in vitro. Four cell lines, namely fibroblast, KB, MCF-7, and PC3, were used to assess cytotoxicity using the MTT assay. Various substitutions with different electronic properties were made on the phenyl ring to elucidate the structure-activity relationship (SAR) of the synthesized derivatives. Overall, to investigate the structure-activity relationship (SAR) of these compounds, electron-withdrawing groups such as chlorine, fluorine, and nitro, as well as electron-donating groups like methoxy and methyl, were introduced on the phenyl ring. The effects of these substituents at different positions on the ring were examined in three cell lines based on the compounds' potency expressed by IC50 values.

Regarding the effect of halogen substitutions (chlorine, bromine, fluorine, and iodine) on the four cell lines—fibroblast, KB, MCF-7, and PC3—the results showed that the synthesized derivatives with bromine and fluorine substitutions did not exhibit acceptable cytotoxicity against these cell lines. Among these compounds, para-iodo (4h) and para-chloro (4e) derivatives demonstrated higher cytotoxicity against these cell lines with IC50 values of 9.5 ± 0.005 and 9.5 ± 0.006 micromolar, respectively, compared to the para-bromo and para-fluoro derivatives. In the KB cell line, the fluorine-substituted derivative with an IC50 of 21.3 ± 0.01 micromolar showed low cytotoxicity, whereas the chlorine, bromine, and iodine substituted compounds exhibited higher toxicity. In the MCF-7 cell line, the

derivatives substituted with chlorine and iodine showed acceptable sensitivity against the cancerous cells. Among the halogens, bromine and fluorine substituted derivatives did not show sufficient cytotoxicity. The para-iodo (4h) and para-chloro (4e) compounds had IC50 values of 5 ± 0.02 and 5 ± 0.039 micromolar, respectively, demonstrating greater cytotoxicity compared to the para-bromo and para-fluoro derivatives. In the PC3 cell line, the results were similar to those obtained for the fibroblast and MCF-7 cell lines. Bromine and fluorine substituted derivatives did not show acceptable cytotoxicity, whereas the para-iodo (4h) and para-chloro (4e) derivatives exhibited higher toxicity against this cell line compared to the para-bromo and para-fluoro derivatives.

Regarding the effect of hydrogen (acetophenone) and nitro substitutions on the four cell lines—fibroblast, KB, MCF-7, and PC3—the results showed that in the fibroblast cell line, the derivatives synthesized with acetophenone and nitro substitutions exhibited acceptable cytotoxicity against this cell line. Both derivatives demonstrated nearly equal IC50 values of approximately 9.5 ± 0.009 micromolar against this cell line. In the KB cell line, the derivative with the hydrogen substitution showed acceptable cytotoxicity with an IC50 of 7.3 ± 0.007 micromolar, whereas the nitro-substituted compound did not show acceptable toxicity against this cell line. In the MCF-7 cell line, derivatives with acetophenone and nitro substitutions exhibited acceptable cytotoxicity. Both derivatives showed nearly equal IC50 values of approximately 5 ± 0.013 micromolar against this cell line. In the PC3 cell line, derivatives with acetophenone and nitro substitutions also showed acceptable cytotoxicity, with both derivatives demonstrating nearly equal IC50 values of approximately 9.5 ± 0.015 micromolar against this cell line.

Evaluation of the effect of meta-hydroxy, meta-methoxy, para-methoxy, and para-methyl substitutions (electron-donating groups) on four cell lines—fibroblast, KB, MCF-7, and PC3—showed the following: In the fibroblast cell line, among the synthesized derivatives with electron-donating substitutions, para-methyl exhibited the highest cytotoxicity against this cell line, with an IC50 of 8 ± 0.003 micromolar. Between the two positions meta and para for the methoxy substitution, the para-methoxy derivative with an IC50 of 9.5 ± 0.004 micromolar showed greater cytotoxicity compared to the meta position. The electron-donating ability of the methoxy group at the para position, by providing electrons to the aromatic ring, explains the higher cytotoxicity induced by this compound relative to other positions. In the KB cell line, the derivative with the para-methyl substitution showed acceptable cytotoxicity with an IC50 of 8.3 ± 0.005 micromolar. Among the two methoxy positions, meta showed higher toxicity (IC50 = 10.3 ± 0.009 micromolar) than para against this cell line. It is suggested that steric effects, lipophilicity, or the hydrogen bonding ability of the oxygen atom in the methoxy group may contribute to this reduction in cytotoxicity. Additionally, it is proposed that spatially, the ability to form hydrogen bonds by the oxygen atom is only feasible at the meta position. In the MCF-7 cell line, derivatives with para-methoxy and para-methyl substitutions exhibited acceptable cytotoxicity. Both derivatives showed nearly equal IC50 values of approximately 5 ± 0.013 micromolar against this cell line. In the PC3 cell line, among the derivatives with electron-donating substitutions, para-methyl showed the highest cytotoxicity with an IC50 of 6.2 ± 0.012 micromolar. Between the two positions meta and para, the para substitution showed greater potential than the meta substitution.

Conclusion

In this study, the derivatives 4a-4j were used as apoptosis inducers with potential anticancer effects. The structure-activity relationship among these compounds showed that both electron-withdrawing and electron-donating substituents were effective in enhancing the cytotoxic effects of these compounds. The methyl substituent, as an electron-donating group, exhibited acceptable cytotoxicity against the tested cell lines. Considering the results of the anticancer effects of these compounds in the in vitro environment, it is recommended to further investigate the effects of these derivatives on caspase activity and to evaluate their effects on other cancer cell lines.

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