

4-Methylthiazole Triggers Apoptosis and Mitochondrial Disruption in HL-60 Cells

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Abstract

Thiazole derivatives are gaining prominence in cancer research due to their potent anti-cancer effects and multifaceted biological activities. Notably, in leukemia research, these compounds are studied for their ability to induce apoptosis, disrupt mitochondrial membrane potential (MMP), and modulate cell signaling pathways. This study focuses on 4-Methylthiazole's effectiveness in inducing apoptosis in HL-60 leukemia cells. It significantly disrupts MMP, activates caspase-3, and promotes cytochrome c release, which are key indicators of apoptosis (**** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05). Additionally, 4-Methylthiazole reduces CD45 and CD123 surface markers, altering the phenotypic characteristics of leukemia cells (**** p < 0.0001).

A high-dose treatment with 4-Methylthiazole significantly increases ROS levels, pointing to elevated oxidative stress and intracellular free radicals, contributing to its cytotoxic effects (*p < 0.05). The study also observed a significant rise in TNF- α levels post-treatment, indicating a pro-inflammatory response that may further inhibit leukemia cell viability. While IL-6 levels remained unchanged, a dose-dependent decrease in IL-10 levels was noted, suggesting a reduction in immunosuppressive conditions within the tumor microenvironment (*p < 0.05).

Overall, 4-Methylthiazole targets leukemia cells through multiple apoptotic mechanisms and modifies the immune landscape of the tumor microenvironment, enhancing its therapeutic potential. This study highlights the need for further clinical investigation to fully exploit the potential of thiazole derivatives in leukemia treatment.

1. Introduction

Thiazole has been the subject of extensive research due to its potential biological activities, including its association with cancer. The molecular structure of the thiazole has been elucidated by millimeter wave spectroscopy and quantum chemical calculations, providing valuable information about its chemical properties (Khemissi et al., 2022). Collectively, these findings highlight the importance of 4-methyl thiazole in the field of cancer research and its potential as a pharmacologically active compound. In addition to thiazole and cancer, the relationship between thiazole and leukemia has also been examined in the scientific literature. Thiazole derivatives have shown significant potential as anticancer agents, and several studies have been conducted demonstrating their cytotoxic effects on various cancer cell lines, including leukemia. Thiazole antibiotics have been shown to inhibit FoxM1 transcriptional activity, downregulate FoxM1 expression, and induce cell death in neuroblastoma, leukemia, and liver cancer cells (Bhat et al., 2009). Similarly, substituted thiazole-5-carboxamides have been identified as potent Src/Abl kinase inhibitors with excellent antiproliferative activity against hematological and solid tumor cell lines, including leukemia (Lombardo et al., 2004). The cytotoxicity of some newly synthesized thiazole derivatives against human cancer cell lines including HL-60 (promyelotic leukemia) and Jurkat (acute lymphoblastic leukemia) was investigated (Petrou et al., 2021). 2-aminothiazole analogs have been found against a wide variety of human cancer cell lines, including leukemia (Alizadeh & Hashemi,

2021). It has also been demonstrated that synthetic analogues of bacilamide-type compounds with structural modifications in the thiazole ring can suppress the proliferation of leukemia cell lines (Zhang et al., 2022). As a Whole, these studies highlight the potential of thiazole derivatives in targeting leukemia and other types of cancer. Several studies have focused on the synthesis and evaluation of new thiazole derivatives for their anticancer properties. Imidazo2,1-b-thiazole derivatives have demonstrated significant antiproliferative activity against a panel of cancer cells, including leukemia cells (Hussein & Al-Lami, 2022). Likewise, a thiazole analog has been identified as a potential liver-specific anticancer agent that can interfere with apoptotic signaling pathways in liver cancer (*A Thiazole Analogue Exhibits an Anti-Proliferative Effect in Different Human Carcinoma Cell Lines and Its Mechanism Based on Molecular Modeling*, n.d.). The thiazole antibiotic thiostreptone was shown to selectively induce cell cycle arrest and cell death in breast cancer cells by down-regulating FoxM1 expression (Kwok et al., 2008). The cytotoxic effects of thiazole derivatives have been evaluated on specific cancer cell lines. For example, 11-phenethyl-3,11-dihydro-2H-benzothiochromeno2,3-dthiazole-2,5,10-trione has demonstrated cytotoxic effects on leukemia cells (Lozynskyi et al., 2022). Chloro-substituted (S)-6-(substituted arylthiourea)-2-amino-4,5,6,7-tetrahydrobenzodithiazole molecules have been identified as important antiproliferative agents against leukemia cells (Shivaramu et al., 2009). The potential of thiazole derivatives to target leukemia has been investigated through the synthesis of new compounds. For example, synthesized pyrazole derivatives containing thiazole moieties and evaluated their anti-leukemia activities (Moustafa, 2019). These studies provide strong evidence for the potential of thiazole derivatives to target leukemia and other types of cancer.

The newly synthesized thiazole derivative 2-[(4,5-dihydrothiazol-2-yl)thio]-N-(4-methylthiazol-2-yl) acetamide compound was used in the study. The compound contains 2-thiazoline group. Cyclooxygenase enzyme inhibition experiments of the compound were performed and COX-1 inhibitory activity was found to be quite high. *In vitro* cell analyses of the compound were also performed. After the compound was applied to NIH/3T3 mouse fibroblast cell line, the IC₅₀ value of the cells at the end of 24 hours as a result of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) analysis was found to be 90.00 ± 21.79 µg/mL. Since the cytotoxicity of the compound was also low, it was used in our study to investigate its anti-cancer activity on leukemia cell line (Erdaş, 2020)

2. Materials and Methods

2.1. Propagation of Leukemia Cell Line

HL60 cell lines, sourced from the American Type Culture Collection (ATCC®) in Manassas, VA, USA, were cultured in a growth medium enriched with 10% fetal bovine serum and 2 mM L-glutamine. The medium was further supplemented with 1% PSA, containing 10,000 units/ml of penicillin and 10,000 µg/ml of streptomycin, along with 25 µg/ml of amphotericin B. The cells were incubated at 37°C in a humidified 5% CO₂ atmosphere. Appropriate culture containers were utilized based on the volume of cells, with daily monitoring to ensure optimal growth conditions.

2.2. Cell viability and proliferation analysis in HL-60 Cells

A 4-methylthiazole stock solution was prepared by initial dissolution in DMSO, followed by subsequent dilution with RPMI medium to achieve the required concentrations. HL60 cells were seeded in 96-well plates at a density of 2×10^3 cells per well in 200 μ l of RPMI medium, which was supplemented with 10% fetal bovine serum, 1% PSA (penicillin at 10,000 units/ml and streptomycin at 10,000 μ g/ml), and 25 μ g/ml of Amphotericin B. The cells were then treated with various concentrations of 4-methylthiazole (25, 50, 100, 200, and 500 μ M), with each concentration tested in quadruplicate. A 0.1% DMSO solution served as the solvent control, while untreated cells were used as the negative control. Cell viability was assessed at 24, 48, and 72 hours post-treatment by adding a cell viability reagent (Promega's CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS), Cat. No: G3580) to each well, following the manufacturer's instructions. The cells were incubated for 3 hours at 37°C in darkness, after which the absorbance was measured at a wavelength of 490 nm. The IC_{50} value, the concentration at which 4-methylthiazole reduced the absorbance of treated cells by 50% relative to the negative control, was determined from these measurements.

2.3 Analysis of the Effect of 4 methyl thiazole on Cancer Cell Number and Viability

Apoptosis Analysis

HL-60 cells were seeded into 96-well plates at a concentration of 2000 cells per well. These cells were treated with 4-methylthiazole at the IC_{50} concentrations previously established (46 μ M and 89 μ M), as well as with 0.1% DMSO as a control, with each condition tested in triplicate. The plates were then incubated in a humidified environment at 37°C and 5% CO₂ for three days. Following the incubation, cells were collected from the plates and centrifuged at 1500 rpm for 5 minutes. The resulting cell pellet was resuspended in 1X binding buffer to prepare for apoptosis evaluation. For the detection of apoptotic cells, staining was performed using FITC Annexin V and propidium iodide (PI), according to the manufacturer's protocol (ABP, FITC Annexin V and PI Apoptosis Kit, Cat. No: A026). Flow cytometric analysis was conducted on a Cytoflex S Flow Cytometer (Beckman, Cat. No: B47903, USA), following methodologies outlined in (Meriç et al., 2024)

Assessing Leukemia Marker Modulations in Monoclonal Antibodies

The quantification and percentage of leukemia cells in HL-60 cultures treated with 1% DMSO and the IC_{50} concentration of 4-methylthiazole were assessed. This evaluation involved analyzing surface markers—specifically, CD34, CD45, CD33 and CD123—using a flow cytometer. The cells were first stained with fluorochrome-conjugated antibodies, a technique consistent with methodologies applied in prior research (Meriç et al., 2024)

Evaluation of Mitochondrial Membrane Potential ($\Delta\Psi_m$) Dynamics

Leukemia cells underwent treatment with 4-methylthiazole at the previously established IC₅₀ values. At the 72-hour mark, the MMP was assessed using the "Elabscience, Mitochondrial Membrane Potential Assay Kit (with JC-1), Cat. No: E-CK-A301" kit, following the manufacturer's instructions.

Assessment of Caspase-3 Activation and Cytochrome c Release

Leukemia cells received treatment with 4-methylthiazole at the established IC₅₀ concentrations. 72 hours later, caspase-3 activity was evaluated using the "BD Pharmingen™ FITC Active Caspase-3 Apoptosis Kit" (Cat. No: 550480), adhering to the provided guidelines. Additionally, to assess the release of cytochrome c after 72 hours of treatment, the procedure outlined for the "GMP FITC anti-Cytochrome c Antibody" (Cat. No: 260100) was employed.

2.4. Evaluating the Impact of 4-Methyl Thiazole on Apoptosis, Inflammatory Responses, and Oxidative Stress Proteins

The concentrations of CYT-C, CASP-3, ROS, TNF- α , IL-6, and IL-10 in the experimental groups were measured using ELISA kits. The assays were conducted with a Thermo Scientific Multiscan SkyHigh ELISA reader and a Thermo Scientific Wellwash plate washer. The results are presented as mean \pm standard deviation, with each assay performed in triplicate.

Assessing Reactive Oxygen Species Levels

The levels of ROS in cell samples were quantified using the ROS ELISA kit (Catalog No. E0304Ra) from BTLAB, China. The kit features a plate pre-coated with ROS antibodies. When the sample containing ROS is added, it binds to these antibodies. The assay is concluded by adding an acidic stop solution, and the absorbance is read at 450 nm. The ROS concentrations were expressed in units per milliliter (U/mL).

Cytochrome C Somatic and Caspase-3 Assays

To assess apoptotic markers, the activities of CASP-3 and CYT-C in the cell samples were determined using kits from BT LAB (China), catalog numbers E1648Ra and E0627Ra, respectively. The concentrations of CASP-3 and CYT-C were expressed in nanograms per milliliter (ng/mL).

Pro-inflammatory and Anti-inflammatory Factors Assay

The concentrations of TNF- α , IL-6, and IL-10 in cell lysates were measured using specific kits from BT LAB (China), with catalog numbers E0764Ra, E0135Ra, and E0108Ra, respectively. The levels of TNF- α were expressed in nanograms per liter (ng/L), while IL-6 and IL-10 were reported in picograms per milliliter (pg/mL). The concentrations were determined by comparing the optical density readings with a standard curve.

2.5. Statistical Analysis

All data were statistically analyzed using one-way ANOVA or a two-tailed Student's t-test. GraphPad Prism (version 8.0.1) software was utilized for performing the statistical analyses and plotting the graphs. Error bars represent the standard error of the mean (SEM) from a minimum of three independent experiments. Statistical significance was defined as *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, and ****P ≤ 0.0001.

3. Results

3.1. Evaluation of Cell Survival and Growth in HL-60 Cells

The control group represents baseline cell viability, which is considered to be 100% valid throughout the experiment. Cell viability at 24 hours, shown by red bars, is high at all doses. At 48 hours, a noticeable decrease in cell viability occurs at doses of approximately 100 μ M and 200 μ M, represented by gray bars. Cell viability at 72 hours, shown by blue bars, further decreases at doses higher than 50 μ M (Fig. 1)

The purple horizontal line in the graph indicates the threshold of 50% cell viability. Any bar falling below this line indicates a dose and time point at which more than half of the cells are no longer viable (Fig. 1)

Microsoft Excel was used for linear regression to calculate IC₅₀ values from sigmoidal inhibition curves. 48 hours after treatment with 4-methylthiazole, IC₅₀ value was 89.08, 72 hours after treatment with 4-methylthiazole, the IC₅₀ value was 46.0277. The IC₅₀ value we determined was used in the entire experimental set.

3.2. Assessment of Cell Apoptosis

Statistically significantly lower cell viability was observed at both concentrations (46 μ M and 89 μ M) compared to DMSO, indicating that the drug reduces cell viability. Both concentrations of the drug were found to induce significantly post-apoptotic cell death compared to DMSO. Likewise, it was observed that both concentrations of the drug induced significantly pre-apoptotic cell death compared to DMSO (Fig. 2A,B).

3.3. Changes in Leukemia Cell Surface Markers

Before treatment, CD45 + level in HL-60 cells was very high at 94.48%; This indicates that the majority of leukemia cells express this common leukocyte antigen, which is typical for white blood cells, including leukemic cells. After treatment with 4-Methylthiazole, CD45 + levels decrease to an average of 2.17%, indicating a significant reduction in the presence of this antigen on the cell surface. Similarly CD123 + level was also high before treatment, at 98.34%; this is characteristic of acute myeloid leukemia cells because CD123 is frequently overexpressed in such cells. After 4-Methylthiazole treatment, CD123 + levels decreased significantly to an average of 15.02% (Fig. 3A,B)

3.4. Evaluation of Mitochondrial Membrane Potential ($\Delta\Psi_m$) Dynamics

The bar graph shows the results of MMP analysis in HL-60 cells following treatment with DMSO (control), CCCP+ (positive control for MMP loss), and two concentrations of 4-Methylthiazole. Y axis measures the percentage of HL-60 cells showing changes in MMP; The x axis is indicative of apoptotic in the early stage (pre-apoptotic, JC-1+) or late stage (post-apoptotic, JC-1-). DMSO-treated cells (blue bars) show low levels of both early and late apoptotic changes and serve as baseline controls. Cells treated with CCCP+ (red bars), a compound known to degrade MMP, were used as a positive control. Treatment with 46 μ M 4-Methylthiazole (green bars) results in a significant increase in late apoptotic changes compared to control and a smaller but statistically significant effect on early-stage apoptosis ($*p < 0.05$). At a higher concentration of 89 μ M (purple bars), 4-Methylthiazole shows a statistically significant increase in late apoptotic changes compared to both DMSO and CCCP + control; this indicates a dose-dependent effect on the induction of apoptosis ($***p < 0.0001$). On the other hand, at the concentration of 49 μ M (green bars), 4-Methylthiazole showed a statistically significant increase in early apoptotic changes compared to the DMSO control ($*p < 0.05$). Briefly, our data show that 4-Methylthiazole is effective in disrupting mitochondrial function and causes apoptosis in HL-60 cells (Fig. 4A,B)

3.5. Assessment of Caspase-3 Activation and Cytochrome-C Release

Caspase-3 activity appears to increase in a dose-dependent manner with 4-Methylthiazole. While there is around 20% activation at the 46 μ M dose, this rate increases to 50% at the 89 μ M dose. When looking at the control groups (UNT and DMSO), it is observed that there is a very high level of Caspase-3 activation (Fig. 5A). Cytochrome C activity shows a more significant increase (approximately 55%) at the 46 μ M dose of 4-Methylthiazole compared to 89 μ M (Fig. 5B).

According to ELISA experiment analysis results, a statistically significant difference in Caspase-3 protein levels was observed between UNT and DMSO after treatment ($**p < 0.01$). Caspase-3 protein levels have significantly increased after treatment with 4-Methylthiazole at 89 μ M compared to UNT ($***p < 0.001$). It has also been found that Caspase-3 protein levels increased significantly in a dose-dependent manner (Fig. 6A). Although treatment with 4-Methylthiazole at 46 μ M appears to have caused an increase in Cytochrome-C levels compared to UNT and DMSO, this increase was not found to be statistically significant (Fig. 6B)

3.6. Evaluating Reactive Oxygen Species (ROS) Concentrations

Both doses of 4-Methylthiazole significantly increased ROS levels compared to the control, but the 89 μ M dose raised ROS levels to a statistically significant extent compared to the 46 μ M dose (* $p < 0.05$). According to these results, the higher dose of 4-Methylthiazole enhances oxidative stress, leading to an increase in the levels of free radicals and ROS within the cells. HL-60 cells undergo apoptosis in response to the rise in ROS levels (Figs. 7 and 2).

3.7. Assessment of Pro-inflammatory and Anti-inflammatory Biomarkers

It is observed that TNF- α levels at the 46 μ M dose of 4-Methylthiazole decreased slightly compared to the UNT control, but increased statistically significantly at the 89 μ M dose. It is observed that TNF- α levels increase statistically significantly depending on the dose (* $p < 0.05$). In addition, compared to UNT and DMSO, the 89 μ M dose caused a significant increase in TNF- α levels, and this difference was found to be statistically significant (* $p < 0.05$) (Fig. 8A). On the other hand, no significant effect of the 46 μ M dose was observed on TNF- α levels compared to UNT and DMSO (Fig. 8A). Compared to the UNT and DMSO groups, the effect of 4-Methylthiazole on IL-6 levels was minimal, with levels remaining similar at both doses (Fig. 8B). At the 46 μ M dose, IL-10 levels decreased compared to the control group, and at the 89 μ M dose, they decreased significantly (* $p < 0.05$) (Fig. 8C).

4. Discussion

In our study of 4-Methylthiazole, we observed significant pre-apoptotic and post-apoptotic cell death in HL-60 cells across both tested doses. A dose-dependent increase in Caspase-3 degradation, a recognized marker of apoptosis, was noted. Intriguingly, the activity of Cytochrome C was significantly elevated at a lower dose of 46 μ M compared to 89 μ M, indicating a complex dose-response relationship. This was confirmed by eliza analyses of Caspase-3 and Cytochrome C, ensuring consistency across experimental outcomes. Such findings are supported by extensive literature indicating the role of thiazole derivatives in inducing apoptosis. For example, (Dasari & Tchounwou, 2014) have noted that cisplatin, which includes a thiazole ring, interrupts DNA repair mechanisms and triggers DNA damage, leading to apoptosis in cancer cells. Similarly, (Dawood et al., 2021) have demonstrated that bis-thiazole derivatives promote apoptosis across various cancer cell lines, and (Alizadeh & Hashemi, 2021) have detailed the apoptosis-inducing capabilities of specific thiazole derivatives through flow cytometry and DNA fragmentation analyses. Additional studies bolster these observations (Bimoussa et al., 2022) investigated the cytotoxic mechanisms of thiazole analogues, revealing common initiation of apoptosis via Caspase-3/7 activation, induction of G2/M phase arrest, and disruption of MMP in cancer cells. This body of research collectively clarifies the molecular pathways utilized by thiazole derivatives to exert their cytotoxic effects, offering valuable insights for the development of targeted cancer therapies. Furthermore, (Al-Warhi et al., 2023) support the role of Caspase 3 in the apoptotic impacts of thiazole derivatives. ("Novel Endoperoxide Steroid Derivatives Containing Thiazole Moiety as Potential Antitumor Agents," 2023) suggest that the molecular mechanism of thiazole derivatives might involve the mitochondrial apoptotic pathway, closely associated with Caspase activation, including Caspase 3.

Moreover, (Dutta et al., 2018) demonstrated that thiazole peptides inhibit c-MYC expression by specifically targeting the c-MYC G-quadruplex, which correlates with the downregulation of c-MYC expression in cancer cells, leading to the release of Cytochrome C and subsequent activation of apoptotic pathways. (Dos Santos et al., 2016) also shed light on the antitumor and immunomodulatory activities of thiosemicarbazones and 1,3-thiazoles in Jurkat and HT-29 cells, noting the potential immunomodulatory effects of thiazole compounds, including activities related to Cytochrome C and apoptosis induction. The activation of Caspase-3 and the release of Cytochrome C from mitochondria to the cytosol are crucial steps in the apoptosis pathway, indicating that the thiazole derivative activates the intrinsic (mitochondrial) pathway of apoptosis, leading to cancer cell death, consistent with the reported literature (Bimoussa et al., 2022).

The intricate relationship between mitochondrial membrane activity and cancer cell physiology has been a focal point of research, illuminating the critical role mitochondria play in energy production, apoptosis, and cellular signaling. Their dysfunction is increasingly recognized as pivotal in the onset and progression of various diseases, particularly cancer. Research demonstrates that disrupted mitochondrial dynamics are linked to the development and progression of cancer, underlining the significance of mitochondrial morphology in the broader context of cancer biology, as outlined by studies from (De Rrasmo et al., 2023; Xing et al., 2022). The stability of MMP ($\Delta\Psi_m$) is vital for cellular homeostasis and has been identified as crucial in cancer development. Variations in this potential are correlated with the anti-cancer properties of various therapeutic agents, as evidenced by the works of (Park et al., 2019; Zhao et al., 2020). Moreover, the disruption of MMP is a well-documented trigger for apoptosis in cancer cells, which places mitochondrial function at the heart of decisions regarding the fate of cancer cells. This connection is supported by findings from (Moustapha et al., 2015; Peng et al., 2021). Our own research using JC-1 assay data confirms the critical impact of mitochondrial dysfunction in apoptosis induced by thiazole derivatives. This loss of MMP underscores the mechanism through which these compounds exert their cytotoxic effects, aligning with established research that highlights the essential role of mitochondrial health in managing cancer cell survival and death.

In previous studies, flow cytometry was used to measure the expression of some specific surface markers in cancer cells (Hussein & Al-Lami, 2022; Sun et al., 2017). However, no studies investigating the effects of thiazole and its derivatives on the expression of specific surface markers were found. The changes in surface marker expression in our findings suggest that the thiazole derivative not only induces apoptosis, but may also alter the differentiation status or viability of leukemia cells and potentially affect their aggressiveness or sensitivity to other treatments.

ROS are pivotal in various physiological processes, including cell signaling and immune responses. While integral to cellular function, excessive ROS can induce oxidative stress, damaging cellular components. Research into thiazole derivatives has shown their potential to modulate ROS levels, suggesting both anti-inflammatory and antioxidant properties. For example, (Ilkiv et al., 2022) explored the effects of thiazole derivatives complexed with polyethylene glycol (PEG)-based nanoparticles on ROS production in NK/Ly lymphoma cells and mouse hepatocytes. Their findings underscore the complex interplay

between thiazole derivatives and ROS levels, illustrating the compounds' capabilities to modulate oxidative stress. Further investigations have connected thiazole derivatives with epigenetic regulation. (Chimenti et al., 2009) demonstrated that these compounds inhibit histone acetyltransferases, an action that might indirectly affect ROS levels by altering gene expression. Additionally, (Kupczyk et al., 2020) examined new 2-(isopropylamino) thiazol-4(5H)-one derivatives, highlighting their inhibitory effect on 11 β -HSD1 and 11 β -HSD2 enzymes and underscoring their potential in cancer prevention. Although this study didn't directly focus on ROS, it provided insights into the diverse biological activities of thiazole derivatives, further encouraging exploration of their role in oxidative stress modulation. Moreover, thiazole derivatives have been noted for their anti-inflammatory properties, which may relate to their effects on ROS levels (Hassan et al., 2021). In our study, a significant increase in ROS activity was observed following treatment with high doses of 4-Methylthiazole, indicative of rising oxidative stress and intracellular free radicals. This response aligns with the known ability of thiazole derivatives to regulate cellular ROS levels. Intriguingly, contrary to the general assumption that high ROS levels are detrimental, this increase may contribute to the anticancer effects of thiazole derivatives by inducing oxidative damage and cell death. Thus, the elevated ROS levels in response to high doses of 4-Methylthiazole are crucial for inhibiting cancer cell growth, highlighting the potential of thiazole derivatives to trigger cancer cell death through enhanced oxidative stress.

Thiazole derivatives have garnered significant interest in oncological research due to their interactions with tumor necrosis factor-alpha (TNF- α), a cytokine pivotal in both inflammation and the progression of cancer. TNF- α is known to enhance cancer-related processes such as cell migration, invasion, and metastasis, as demonstrated in studies by (Cai et al., 2017; Wolczyk et al., 2016). Furthermore, thiazole derivatives, including 4-methylthiazole, have demonstrated promising anticancer properties by modulating TNF- α activity and subsequently inducing apoptosis in cancer cells, which hampers tumor growth, as evidenced by repeated findings in studies by (Mohamed et al., 2019). Moreover, these compounds have been shown to block TNF- α -mediated signaling pathways, which play a crucial role in the survival and proliferation of cancer cells (Srinivasan et al., 2010). TNF- α typically exhibits a dual role in oncology; while it promotes tumor progression through enhancing cellular mechanisms like migration and metastasis, under certain conditions, it can also trigger apoptosis in tumor cells. The observed increase in TNF- α levels following treatment with high doses of 4-methylthiazole suggests a multifaceted mechanism of anticancer action. This rise in TNF- α may contribute to the compound's anticancer efficacy by enhancing immune responses, promoting apoptosis, and inhibiting cancer cell proliferation. Overall, the interaction of thiazole derivatives with TNF- α and their ability to influence related biological pathways highlight their potential as effective agents in cancer treatment. This underscores their versatility and capacity to manipulate critical cytokines such as TNF- α , making them promising candidates for future therapeutic applications. The interplay between thiazole derivatives and interleukins, particularly IL-6 and IL-10, in the context of cancer merits significant attention. IL-6, recognized as a pro-inflammatory cytokine, is implicated in enhancing cancer progression and contributing to chemotherapy resistance. Conversely, IL-10, an anti-inflammatory cytokine, has a multifaceted role in cancer by regulating immune responses and facilitating tumor immune escape. This

nuanced relationship underscores the critical nature of understanding how thiazole derivatives might influence IL-10 signaling within the realm of cancer therapy. Studies have shown that thiazole derivatives possess immunomodulatory properties and can alter the cytokine environment within the tumor microenvironment, potentially affecting levels of cytokines like IL-10 (Chahal et al., n.d.). In our own investigations, we observed no significant alteration in IL-6 levels after treating HL-60 cells with 4-Methylthiazole, suggesting that the anticancer mechanisms of this derivative do not directly engage IL-6 modulation. This implies that thiazole derivatives may exert their anticancer effects through pathways other than those involving IL-6. Interestingly, a dose-dependent reduction in IL-10 levels was noted following treatment with 4-Methylthiazole. This decrease is critical as it suggests a shift in the tumor microenvironment's cytokine milieu towards a state that is less supportive of immunosuppression. The reduction in IL-10 could lead to enhanced immune system activation against the tumor, potentially heightening the efficacy of immune-mediated tumor destruction. This finding aligns with the reported immunomodulatory capabilities of thiazole derivatives and underscores a promising area for further exploration and application in cancer therapy, highlighting the potential of these compounds to modulate critical immune pathways in oncology.

4-Methylthiazole distinguishes itself from other thiazole derivatives used in leukemia treatment due to its unique biological effects. Research focusing on its impact on HL-60 leukemia cells has demonstrated its capacity to disrupt mitochondrial membrane potential, initiate ROS production, activate caspase-3, and facilitate the release of cytochrome c. These actions underscore the potential of 4-methylthiazole to induce apoptosis, effectively promoting programmed cell death in leukemia cells. A particularly noteworthy aspect of 4-methylthiazole is its impact on leukemia cell surface markers, specifically CD45 and CD123. The significant reduction in these markers suggests a profound effect on cell differentiation and behavior, potentially enhancing the therapeutic efficacy of 4-methylthiazole against leukemia cells. The observed decrease in CD45 expression following treatment with this thiazole derivative might reflect a crucial change in the cells' phenotype. Given that CD45 is a critical tyrosine phosphatase necessary for antigen receptor signaling on leukocytes, its downregulation could indicate a loss of leukemic cell viability or a shift toward a less differentiated state. Moreover, CD123—the alpha chain of the interleukin-3 receptor—plays a pivotal role in the treatment of leukemia due to its high expression in leukemic blasts. A reduction in CD123 + cells after treatment with 4-methylthiazole suggests a significant phenotypic alteration. This could imply that the thiazole derivative not only reduces the population of leukemic stem cells but might also impact crucial survival signaling pathways. In the clinical context of leukemia treatment, a decrease in CD123 expression post-treatment is viewed positively, as it could correspond to a reduced leukemic cell population or a decrease in the disease's aggressiveness. Considering that CD123 is associated with a poor prognosis in acute myeloid leukemia (AML), strategies that diminish its expression are likely to improve clinical outcomes. Thus, 4-methylthiazole's ability to lower CD123 expression highlights its potential as an effective agent in leukemia therapy, offering hope for better management of this challenging disease.

These results may indicate the anti-cancer potential of 4-Methylthiazole. By inducing oxidative stress and apoptosis, it promotes cell death and modulates inflammatory responses. These properties may be

particularly useful in cancer treatment. However, the side effects and off-target effects of such substances should be carefully examined. In particular, it should be taken into account that pro-inflammatory and oxidative effects can have detrimental effects on healthy cells. Therefore, further research into the clinical use of 4-Methylthiazole is required and these effects are needed to be evaluated in a broader biological and clinical context.

Declarations

Author Contributions

NM formulated the study designs, devised the experiments and gathered as well as analyzed the data. EK and FK supported the experimental stages and contributed to analyzed the data.

Ethical Approval

This study did not involve any human or animal subjects that required ethical approval.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

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Conflict of interest

The authors have no conflict of interest to declare

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Figures

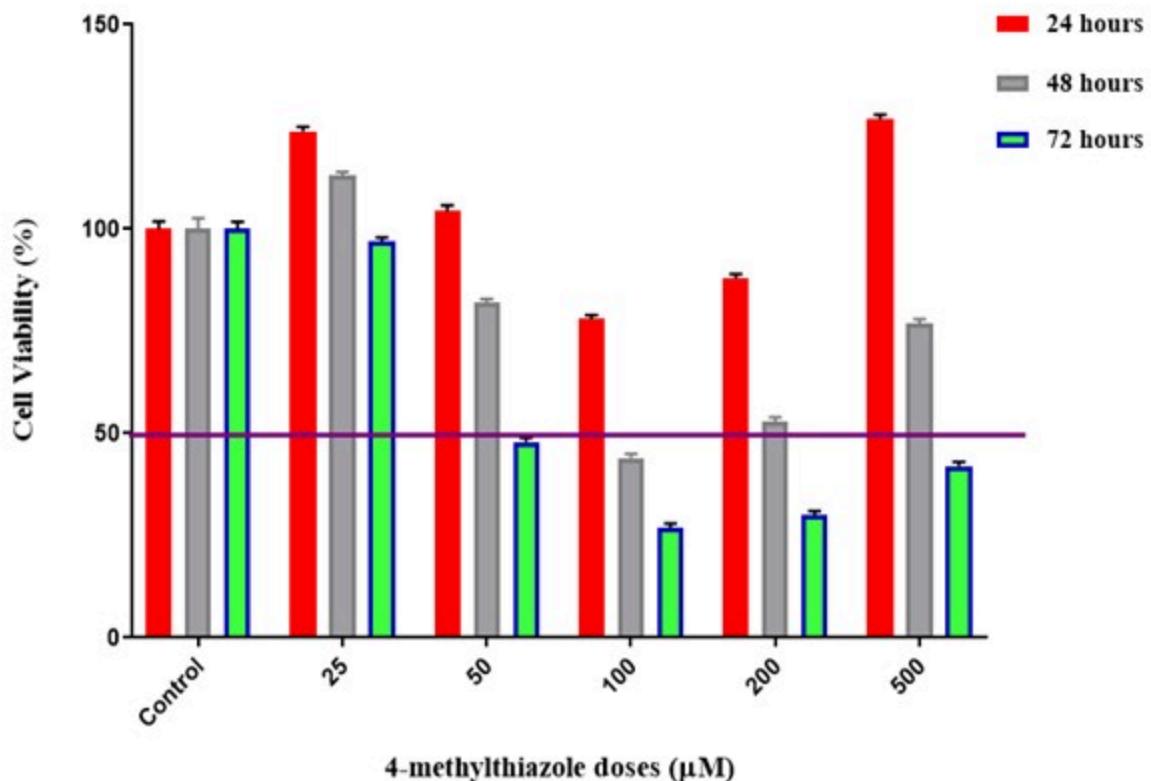
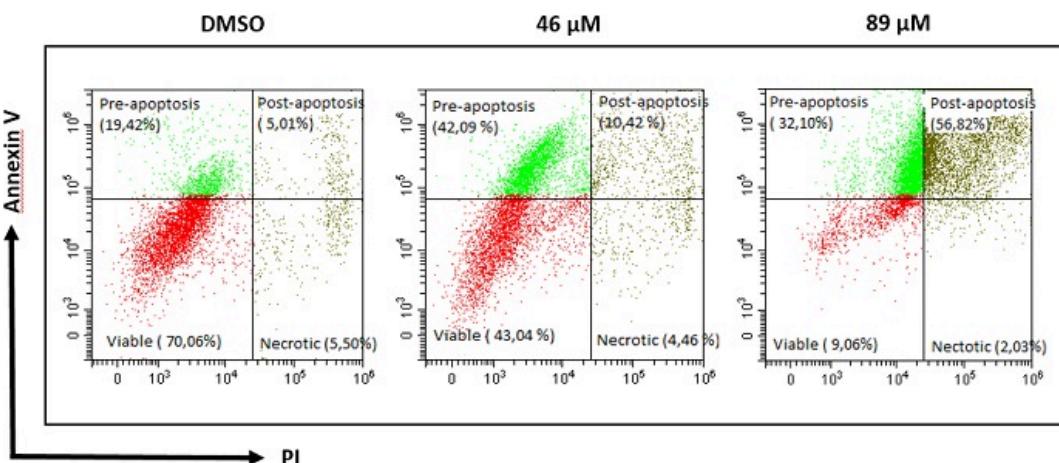
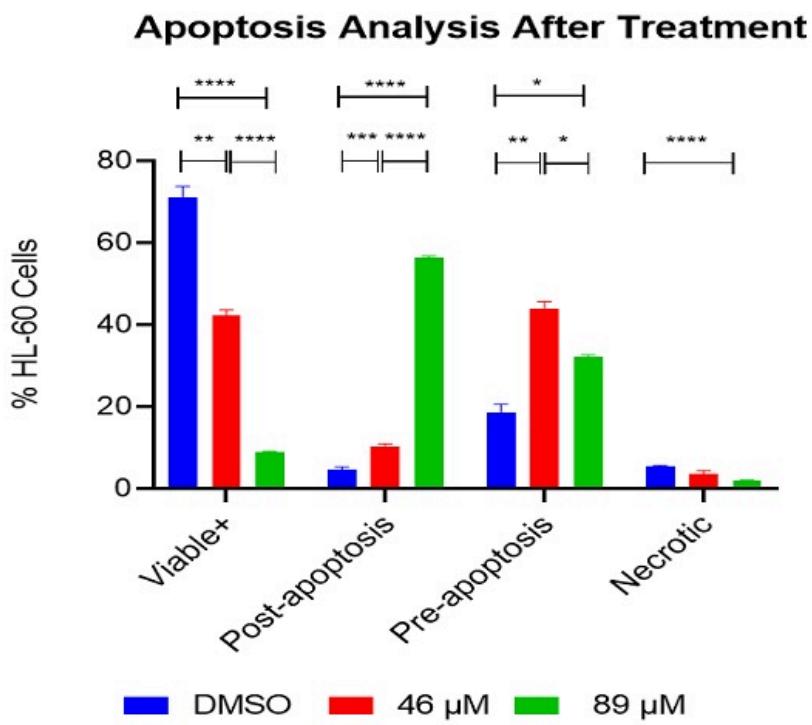


Figure 1

This bar graph presents the impact of various concentrations of 4-methylthiazole on cell viability, measured at different time points (24, 48, and 72 hours). The x-axis represents the doses of 4-methylthiazole used, given in micromolar (μM) units, while the y-axis shows the percentage of cell viability compared to the control group. The error bars represent the standard error of the mean.

A)**B)****Figure 2**

Apoptosis Analysis After Treatment in HL-60 Cells **A)** The flow cytometry plots of apoptosis, and **B)** Its quantification in HL-60 Cells. The data suggest that 4-Methylthiazole at both concentrations significantly reduces the percentage of viable cells and increases the percentage of post-apoptotic and pre-apoptotic cells compared to the control. (Untreated (UNT), solvent control (DMSO), **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$). The error bars represent the standard error of the mean.

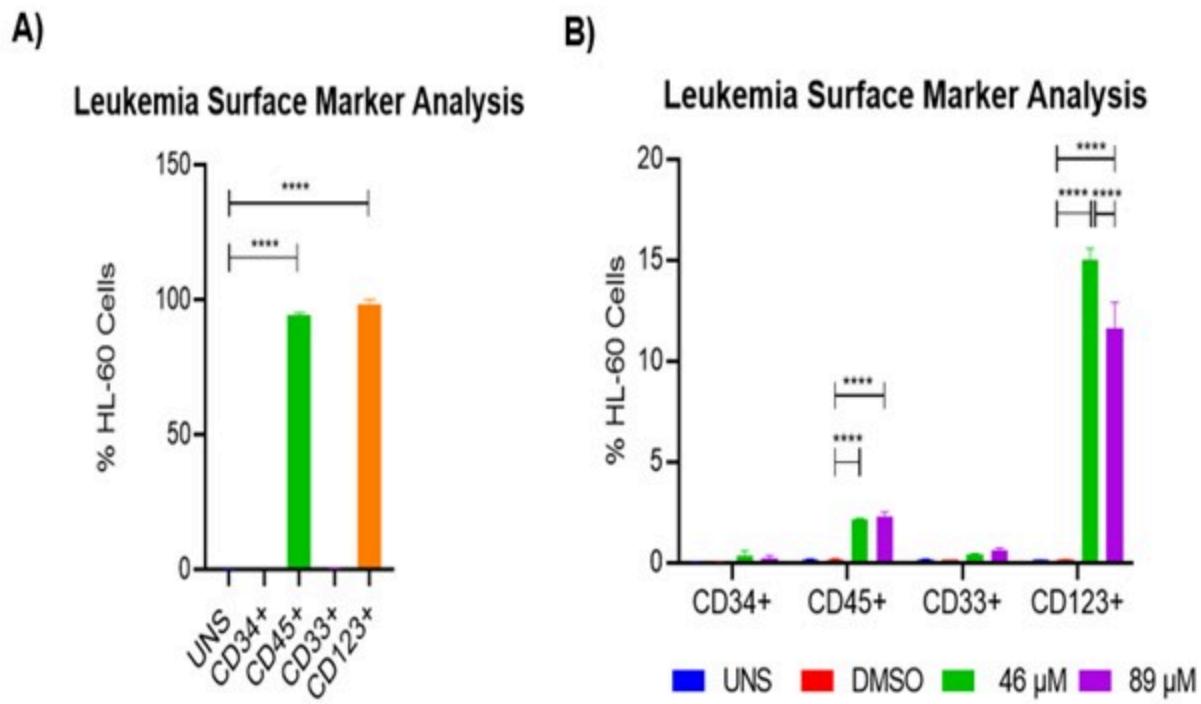
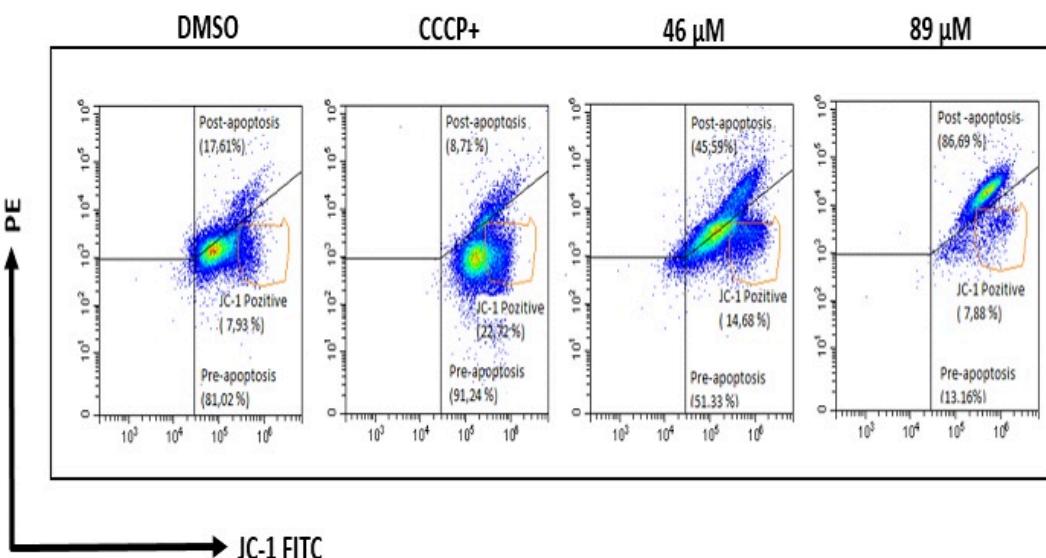
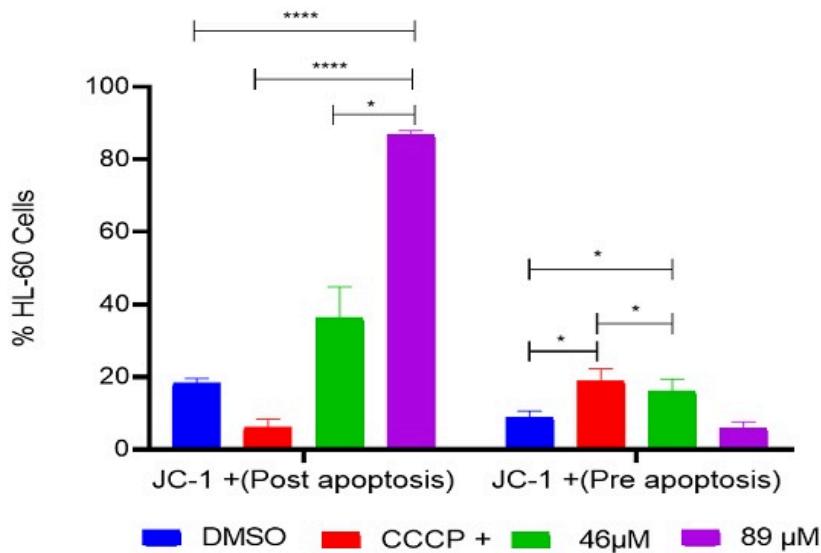


Figure 3

Leukemia Surface Marker Analysis on HL-60 Cells **A)** Pre-treatment surface marker expression. The graph shows the percentage of HL-60 cells expressing various leukemia surface markers (CD34+, CD45+, CD33+, CD123+) **B)** Post-treatment surface marker expression: This graph illustrates the impact of 4-Methylthiazole at concentrations of 46 μ M and 89 μ M on the expression levels of leukemia surface markers (CD34, CD45, CD33, CD123) in HL-60 cells. The error bars represent the standard error of the mean (Unstained (UNS), solvent control (DMSO), **** p < 0.0001)

A)**B)**

MMP Analysis After Treatment

**Figure 4**

Effects of 4-Methylthiazole on MMP in HL-60 Cells. **A)** The flow cytometry plots of mitochondrial membrane potential, and **B)** its quantification in HL-60 Cells. The error bars represent the standard error of the mean (Untreated (UNT), solvent control (DMSO), positive control (CCCP+), *p < 0,05, **** p < 0,0001).

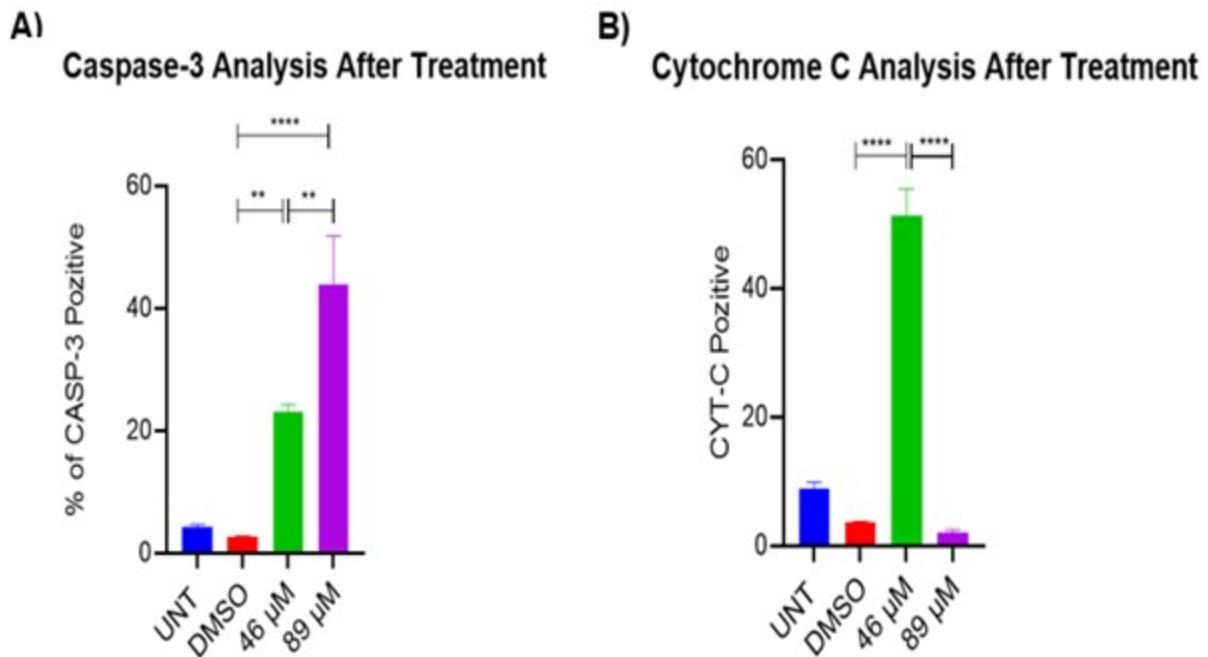


Figure 5

Evaluation of Caspase-3 and Cytochrome C Activity in HL-60 Cells Following Treatment with 4-Methylthiazole **A)** Percentage of Caspase-3 Activation. This graph illustrates that treatment with 4-Methylthiazole at both tested concentrations results in a significant increase in Caspase-3 activity compared to the DMSO control, as indicated by statistical analysis. **B)** Percentage of Cytochrome C Activation. This graph demonstrates a notable increase in Cytochrome C activation at the 46 μ M concentration (approximately 55%) compared to the DMSO control, as supported by statistical analysis. The error bars represent the standard error of the mean (Untreated (UNT), solvent control (DMSO), **p < 0.01, ****p < 0.0001).

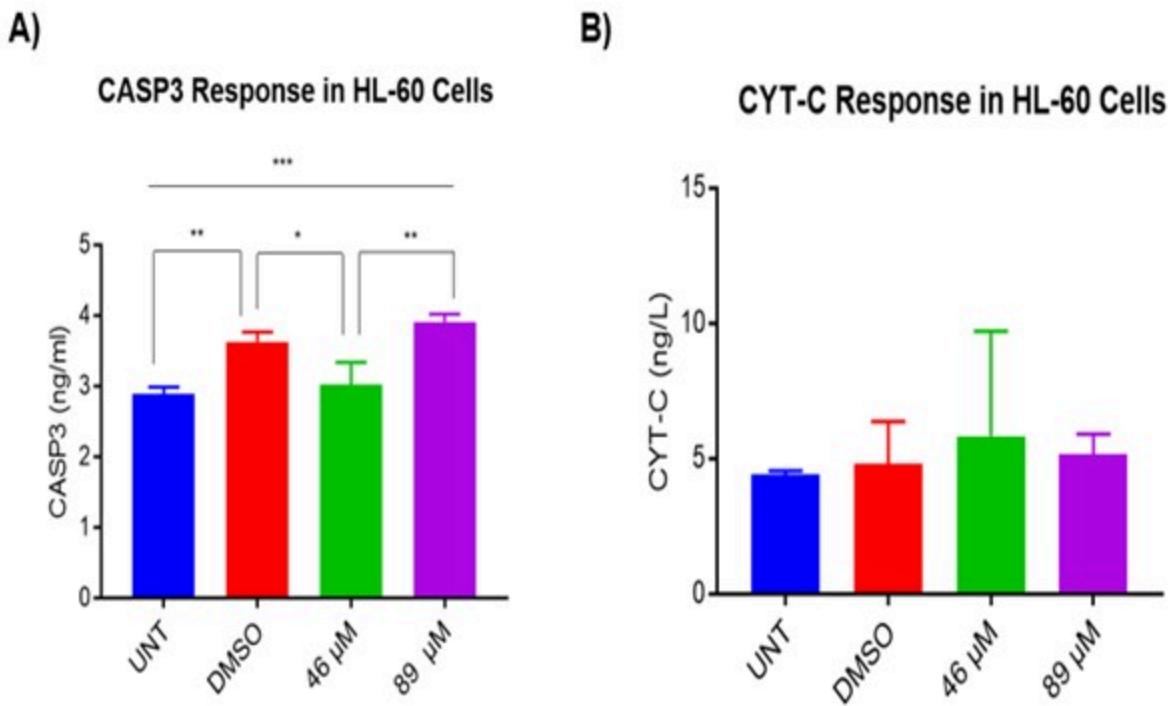


Figure 6

Effects of two different concentrations of a 4-Methylthiazole (46 μ M and 98 μ M) and DMSO vehicle control on **A**) CASP3 protein levels in HL-60 Cells. **B**) CYT-C protein levels in HL-60 Cells. The error bars represent the standard error of the mean (Untreated (UNT), solvent control (DMSO), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

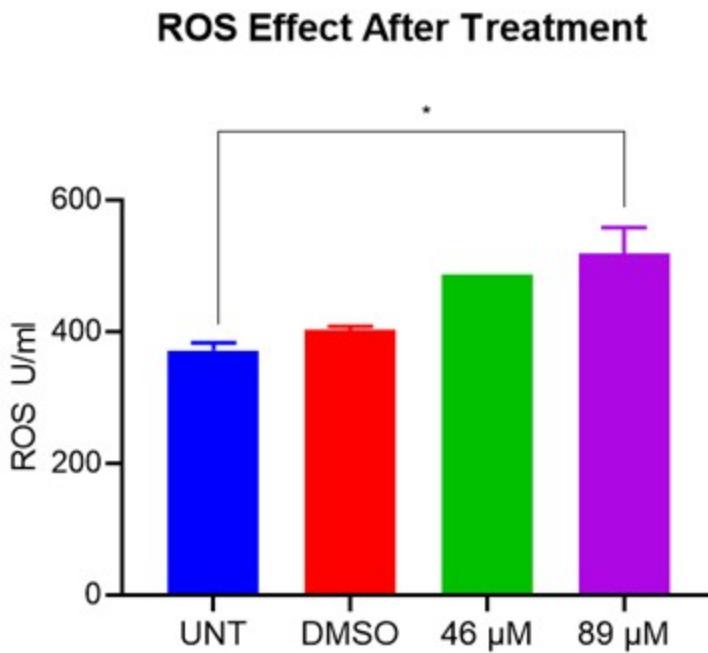


Figure 7

This graph shows the effects of 4-Methylthiazole at doses of 46 μ M and 89 μ M applied to HL-60 cells on ROS levels. Error bars represent standard error of mean values (Untreated (UNT), solvent control (DMSO), *p < 0,05).

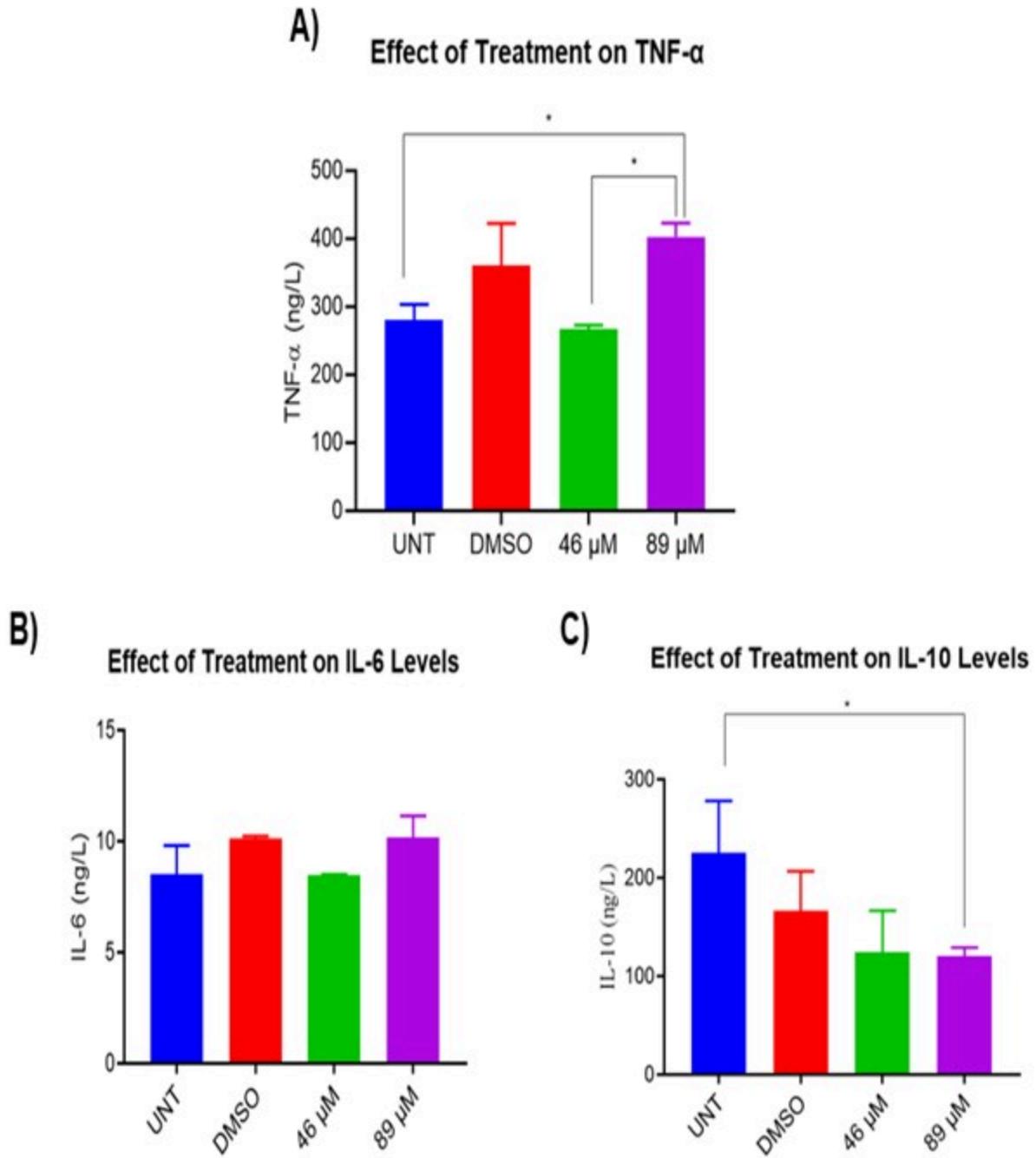


Figure 8

The effects of 4-Methylthiazole at doses of 46 μ M and 89 μ M applied to HL-60 cells on **A) TNF- α** , **B) IL-6** and **C) IL-10** levels are shown. The error bars represent the standard error of the mean (Untreated (UNT), solvent control (DMSO), *p < 0,05).