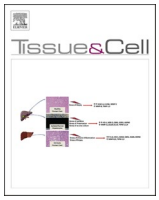
Tissue and Cell 95 (2025) 102937

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice



Pro-apoptotic and mitochondria-disrupting effects of 4-methylthiazole in K562 leukemia cells: A mechanistic investigation

Neslihan Meriç [a,*], Ezgi Kar [b], Fatih Kar [c]

[a] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Kutahya Health Sciences University, Kütahya, Turkey
[b] Department of Nutrition and Dietetics, Faculty of Health Sciences, Kutahya Health Sciences University, Kütahya, Turkey
[c] Department of Medical Biochemistry, Faculty of Medicine, Kutahya Health Sciences University, Kütahya, Turkey

ARTICLE INFO

Key Words:
4-methylthiazole
Apoptosis
Mitochondrial disruption
S-phase arrest

ABSTRACT

Thiazole derivatives have garnered attention for their anticancer potential. This study investigates the antileukemic effects of 4-methylthiazole on K562 chronic myeloid leukemia (CML) cells, focusing on apoptosis induction and mitochondrial dysfunction. Cell viability was assessed using MTS assays; apoptosis and necrosis were analyzed via Annexin V/PI staining and flow cytometry; mitochondrial membrane potential changes were evaluated with JC-1 dye; gene expression levels were measured by qRT-PCR; and levels of apoptosis- and cytokine-related proteins were quantified using ELISA. Treatment with 4-methylthiazole led to selective cytotoxicity in K562 cells while sparing healthy peripheral blood mononuclear cells (PBMNCs). Apoptotic induction was evidenced by Caspase-3 (CASP-3) activation, Cytochrome-C (CYT-C), release, and mitochondrial depolarization. Gene expression analysis showed upregulation of pro-apoptotic markers such as *TP53* (tumor suppressor protein 53), *BAX* and *BAK* (pro-apoptotic Bcl-2 family proteins), while upregulation of *CASP3* (caspase-3) expression was not statistically significant. Conversely, levels of *GPX4* (glutathione peroxidase 4, involved in oxidative stress protection) remained unchanged, indicating an apoptosis mechanism independent of oxidative stress. Additionally, *SEMA3A* (Semaphorin 3 A, involved in tumor progression and cell signaling) was significantly downregulated. Cytokine profiling revealed a dose-dependent modulation of IL-6, while TNF-α and IL-10 levels remained unaffected. These findings suggest that 4-methylthiazole induces apoptosis through mitochondrial pathways, affects cytokine signaling, and selectively targets leukemia cells, supporting its potential as a therapeutic candidate for CML treatment.

1. Introduction

Thiazole derivatives have attracted attention in cancer research in recent years due to their diverse biological activities and potential as anticancer agents. The thiazole ring system is a prominent structural motif in many bioactive compounds, and its derivatives have been shown to exhibit potent anticancer properties through various mechanisms. A key mechanism through which thiazole derivatives exert their anticancer effects involves the suppression of oncogenes and regulatory proteins implicated in cancer progression. Dutta et al. showed that cell-penetrating thiazole peptides can inhibit c-MYC expression by targeting the G-quadruplex structure, leading to reduced cell proliferation in cancer cell lines such as HeLa (cervical cancer) and A549 (lung carcinoma) (Dutta et al., 2018). Similarly, Korkmaz emphasized that amino thiazole compounds effectively inhibit thioredoxin reductase 1 (TrxR1) and other enzymes that are vital for cancer cell survival, thus demonstrating their potential as therapeutic agents against glioblastoma (a type of brain tumor) (Korkmaz, 2024). The synthesis of novel thiazole derivatives has also been a focus of research, and studies have shown that specific structural modifications can enhance their anticancer activity. For example, Mabkhot et al. reported to design, synthesis, and biological assessment of new thiazole derivatives that showed significant cytotoxicity against the HCT-116 colon carcinoma cell line, outperforming established anticancer drugs such as vinblastine (Mabkhot et al., 2018). Furthermore, Hosny et al. investigated a series of thiazole derivatives against breast cancer cells and confirmed their excellent antitumor activity (Hosny et al., 2019). In addition to direct cytotoxic effects, thiazole derivatives have been shown to trigger apoptotic pathways in cancer cells. Amin and colleagues discussed the antiproliferative effects of a thiazole analog in various human carcinoma cell

* Corresponding author.
E-mail address: neslihan.meric@ksbu.edu.tr (N. Meriç).

https://doi.org/10.1016/j.tice.2025.102937
Received 5 March 2025; Received in revised form 22 April 2025; Accepted 23 April 2025
Available online 27 April 2025

lines and attributed its efficacy to its ability to induce apoptosis (Amin et al., 2017). This apoptotic mechanism is crucial as it reduces tumor growth and metastasis by inducing programmed cell death.

Furthermore, thiazole compounds have been investigated for their ability to inhibit key signaling pathways involved in cancer progression. Orujova and colleagues described a series of thiazole-hydrazone hybrids targeting the Akt pathway, a critical regulator of cell viability and proliferative capacity in non-small cell lung cancer (Orujova et al., 2023a, 2023b). This highlights the versatility of thiazole derivatives in targeting multiple cancer-related pathways. The structural diversity of thiazole derivatives allows for the exploration of various modifications to improve their therapeutic profile. For example, Zahran and colleagues synthesized a thiazole derivative compound that showed significant antitumor activity against Ehrlich ascites carcinoma in vivo, demonstrating the potential of thiazole scaffolds in developing effective cancer treatments (Mohamed et al., 2018). In addition, studies by Zheng and colleagues revealed that thiazole derivatives can inhibit metastatic cancer cell migration and invasion, further supporting their role in cancer treatment (Zheng et al., 2013).

In addition to all these, the relationship between thiazole and cancer, additionally, the association between thiazole derivatives and leukemia has been extensively investigated in the scientific literature. One of the main mechanisms by which thiazole derivatives show their anticancer effects is the induction of apoptosis in leukemia cells. Popovych and colleagues reported that thiazole derivatives complexed with nanoscopic polymeric carriers caused significant morphological changes in apoptosis in mouse lymphoma cells (hematological malignancy). This was associated with changes in the levels of pro-apoptotic and anti-apoptotic proteins, such as an increase in Bim and a decrease in Bcl-2, along with the activation of poly (ADP-ribose) polymerase 1 and caspase 3 cleavage ((PDF) Effect of thiazole derivative complexed with nanoscale polymeric carriers on cellular ultrastructure of murine lymphoma cells in vivo, 2024). Similarly, Meriç demonstrated that 4-methylthiazole triggered apoptosis and mitochondrial degradation in HL-60 cells (human promyelocytic leukemia), further supporting the pro-apoptotic potential of thiazole derivatives in the treatment of leukemia (Meriç et al., 2024).

Moreover, structural modifications of thiazole derivatives have been shown to increase their anticancer activity. Zhang et al. emphasized that synthetic analogues of bacilliamide compounds with modifications in the thiazole ring effectively suppressed the proliferation of acute lymphoblastic leukemia (ALL) and acute promyelocytic leukemia cell lines (Zhang et al., 2022). This finding coincides with the observations of Finiuk et al., who noted that novel thiazole derivatives exhibited significant antiproliferative activity against various human tumor cell lines, including those associated with leukemia (Finiuk et al., 2022). The ability of thiazole derivatives to inhibit important cellular processes such as histone acetylation and cell cycle progression has also been demonstrated, and compounds such as CPTH6 have demonstrated the capacity to induce histone hypoacetylation and apoptosis in human leukemia cells (Trisciuoglio et al., 2012). In addition to their apoptotic effects, thiazole derivatives have been reported to affect the activity of various enzymes and cellular pathways critical for cancer progression. For example, Abbas et al. investigated thiazolyl-hydrazone derivatives as inhibitors of histone lysine acetyltransferases, which play an important role in regulating gene expression and cancer cell proliferation (Abbas et al., 2022). Moreover, thiazole derivatives have been suggested to play a role in regulating antioxidant enzyme activity and may affect the oxidative stress response in cancer cells (Ivan Franko National University of Lviv, 4 Hrushevsky str., Lviv 79005, Ukraine et al., 2023). This multifaceted approach to target leukemia highlights the therapeutic promise of thiazole derivatives.

The synthesis of novel thiazole compounds remains an active area of research with ongoing efforts to optimize their structures for enhanced anticancer activity. Studies have shown that thiazole derivatives can exhibit selective cytotoxicity against leukemia cells and protect normal cells, indicating a favorable therapeutic index (Ivan Franko National University of Lviv, Ukraine et al., 2021). The diverse biological activities of thiazole derivatives, including their ability to act on multiple molecular targets, position them as valuable candidates for further development in the treatment of leukemia.

The association between thiazoles and their derivatives and leukemia is characterized by a robust body of evidence supporting their anticancer properties. Through mechanisms such as induction of apoptosis, modulation of histone acetylation, and selective cytotoxicity, thiazole derivatives represent a promising class of compounds in the ongoing search for effective leukemia treatments. Further research should aim to unravel the precise molecular mechanisms of their action and refine their pharmacological properties for enhanced clinical applicability. This study builds upon such evidence by exploring the apoptotic effects of 4-methylthiazole specifically in K562 leukemia cells.

In this study, a newly synthesized thiazole derivative, 2-[(4,5-dihydrothiazol-2-yl)thio]-N-(4-methylthiazol-2-yl) acetamide, was utilized. This compound features a 2-thiazoline moiety. Cyclooxygenase (COX) enzyme inhibition assays demonstrated that the compound exhibits a high inhibitory activity against COX-1. Furthermore, its biological effects were assessed through in vitro cell-based analyses. Following treatment of the NIH/3T3 mouse fibroblast cell line with the compound, cytotoxicity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, revealing an IC50 value of 90.00 ± 21.79 μg/mL at 24 hours post-treatment. Given its low cytotoxicity profile, this compound was selected for further investigation regarding its anticancer potential in leukemia cell line (Erdaş, 2020).

2. Materials and methods

2.1. Propagation of leukemia cell line

K562 cells, obtained from the American Type Culture Collection (ATCC®, Manassas, VA, USA), were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum (FBS; Sigma-Aldrich, USA, Cat. No. 12103 C) and 2 mM L-glutamine. To prevent microbial contamination, the medium was further enriched with 1 % penicillin-streptomycin-amphotericin B (PSA), comprising 10,000 units/mL penicillin, 10,000 μg/mL streptomycin, and 25 μg/mL amphotericin B. The cells were maintained at 37°C in a humidified atmosphere containing 5 % CO_2. Culture vessels were selected based on cell density, and the cultures were monitored daily to ensure optimal growth conditions.

2.2. Isolation and characterization of peripheral blood mononuclear cells (PBMNCs)

PBMNCs used as healthy control cells in this study were commercially obtained from the American Type Culture Collection (ATCC®, Manassas, VA, USA). These PBMNCs were provided in a ready-to-culture format and were not freshly isolated from human donors by the authors. As primary cells, PBMNCs provide a physiologically relevant baseline for evaluating the selective cytotoxicity of therapeutic agents. Their inclusion allows for a direct comparison of the effects of 4-methylthiazole between normal and leukemic cells, ensuring that observed cytotoxic effects are selective to malignant cells.

PBMNCs were cultured in RPMI-1640 medium supplemented with 20 % FBS, 2 mM L-glutamine, and 1 % penicillin-streptomycin-amphotericin B (PSA), containing 10,000 units/mL penicillin, 10,000 μg/mL streptomycin, and 25 μg/mL amphotericin B. All cultures were maintained at 37°C in a humidified atmosphere containing 5 % CO_2, ensuring optimal cell viability and physiological relevance throughout the experiments.

2.3. Assessment of cell viability and proliferation in K562 cells

2.3.1. Preparation of 4-methylthiazole stock solution and treatment protocol

A 4-methylthiazole stock solution was prepared by initially dissolving the compound in dimethyl sulfoxide (DMSO), followed by serial dilution in RPMI-1640 medium to achieve the desired working concentrations.

K562 cells were plated in 96-well plates at a density of 2×10^3 cells per well in a final volume of 200 µL of complete RPMI-1640 medium. Cells were subsequently treated with increasing concentrations of 4-methylthiazole (25, 50, 100, 200, and 500 µM), and each condition was tested in quadruplicate. A 0.1 % DMSO solution served as the vehicle control, while untreated (UNT) cells were used as the negative control.

2.3.2. Cell viability assessment

Cell viability was evaluated at 24, 48, and 72 hours after treatment using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS, Cat. No: G3580, Promega), in accordance with the manufacturer's instructions. The MTS reagent was added to each well, and the cells were incubated for 3 hours at 37°C in darkness. Absorbance was subsequently measured at 490 nm using a microplate reader. The IC50 value, defined as the concentration of 4-methylthiazole that resulted in a 50 % reduction in absorbance relative to the negative control, was determined from these measurements.

The IC_{50} values were determined by plotting dose-response curves and applying a four-parameter logistic (4PL) regression model defined by the equation $y = d + [(a - d) / (1 + (x/c)^b)]$, where **y** represents cell viability percentage, **x** is the concentration of 4-methylthiazole, **a** and **d** denote the upper and lower asymptotes of the curve respectively, ccc corresponds to the IC_{50} value, and b indicates the slope factor. Curve fitting and statistical analyses were performed using GraphPad Prism software.

2.4. Evaluation of the impact of 4-methylthiazole on cancer cell count and viability

2.4.1. Apoptosis analysis of K562 cells treated with 4-methylthiazole

K562 cells were seeded into 96-well plates at a density of 2000 cells per well and subsequently treated with 4-methylthiazole at its previously determined IC50 concentrations (162 µM and 230 µM). A 0.1 % DMSO solution was used as a control, and each experimental condition was tested in triplicate. The plates were incubated in a humidified environment at 37°C with 5 % CO_2 for 48 hours.

After incubation, the cells were collected, centrifuged at 1500 rpm for 5 minutes, and the obtained cell pellet was resuspended in 1X binding buffer for subsequent apoptosis analysis. Apoptotic cell detection was performed using the FITC Annexin V and Propidium Iodide (PI) Apoptosis Kit (ABP, Cat. No: A026) according to the manufacturer's protocol. Flow cytometric analysis was performed using a CytoFLEX S Flow Cytometer (Beckman, USA, Cat. No: B47903) in accordance with previously established protocols (Meriç et al., 2024).

2.4.1.1. Flow cytometry-based apoptosis analysis. During the staining process, cells were first labeled with PI, followed by FITC Annexin V staining. Flow cytometry data analysis was performed using an appropriate gating strategy to distinguish different cell populations based on apoptosis status:

Live cells (Annexin V^-/PI^-)

Early apoptosis cells (Annexin V^+/PI^-)

Late apoptosis or necrotic cells (Annexin V^+/PI^+)

Necrotic cells (Annexin V^-/PI^+)

The obtained data were analyzed to classify cells into different apoptotic stages, including early apoptosis and late apoptosis. Each sample was analyzed in triplicate to ensure the accuracy and reproducibility of the results. A minimum of 10,000 events were acquired and gated per sample during flow cytometry analysis to ensure statistical reliability.

2.4.2. Mitochondrial membrane potential ($\Delta\Psi m$) analysis in leukemia cells treated with 4-methylthiazole

Leukemia cells were treated with 4-methylthiazole at its previously determined IC50 concentrations and incubated for 48 hours under standard culture conditions (37°C, 5 % CO_2, humidified atmosphere). Cells were cultured in 6-well plates prior to mitochondrial membrane potential (MMP) analysis. Following treatment, mitochondrial membrane potential (MMP) was assessed using the Mitochondrial Membrane Potential Assay Kit (with JC-1) (Elabscience, Cat. No: E-CK-A301) according to the manufacturer's protocol. The cationic fluorescent dye JC-1 selectively accumulates in mitochondria based on membrane potential, forming J-aggregates with red fluorescence (excitation: 585 nm, emission: 590 nm) in healthy mitochondria, while remaining in its monomeric form with green fluorescence (excitation: 514 nm, emission: 529 nm) in depolarized mitochondria. At the end of the incubation period, cells were harvested by centrifugation (1500 rpm, 5 min, room temperature), resuspended in fresh RPMI-1640 medium, and stained with JC-1 dye. After incubation at 37°C in the dark for 20–30 minutes, cells were washed with 1X JC-1 buffer solution, and fluorescence intensities were measured using a fluorescence plate reader or flow cytometer. The ratio of red-to-green fluorescence was calculated to assess mitochondrial depolarization, with a decreased ratio indicating mitochondrial dysfunction. Fluorescence intensity values were normalized against UNT control cells, and all experiments were performed in triplicate to ensure reproducibility and statistical significance (Meriç et al., 2024).

2.4.3. Quantitative real-time PCR (qRT-PCR) analysis of gene expression

Gene expression levels were assessed by seeding K562 leukemia cells at a density of 5×10^5 cells per well in six-well plates, followed by treatment with effective concentrations of 4-methylthiazole. A vehicle control group received 0.1 % DMSO. After 48 hours of incubation, total RNA was extracted using the PureLink™ RNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Cat. No: 12183025), in accordance with the manufacturer's instructions.

The isolated RNA was then reverse-transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cat. No: 4368814). Quantitative real-time PCR (qRT-PCR) was performed using the Maxima™ SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Cat. No: K0221) with gene-specific primers (Oligomer, Turkey), as listed in Table 1. Amplification and fluorescence detection were carried out using the Bio-Rad CFX96 Touch™ Real-Time PCR Detection System.

2.4.4. ELISA-based cytokine and protein quantification

After 48 hours of incubation with 4-methylthiazole or the vehicle control, culture supernatants were harvested by centrifugation at 1500 rpm for 5 minutes. Before performing ELISA, all samples were brought to room temperature, and the assays were carried out in accordance with the manufacturers' protocols. The levels of CYT-C, CASP-3, TNF-α, IL-6, GPX4, SEMA3A, and IL-10 in the experimental groups were measured using ELISA kits. The assays were carried out with a Thermo Scientific Multiscan SkyHigh ELISA reader and a Thermo Scientific Wellwash plate washer. All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation. Potential variations in cell number across treatment groups were addressed by normalizing all ELISA data to the total protein content of each sample, which was determined using the Bradford protein assay prior to cytokine and apoptotic marker quantification.

Table 1
List of primers used for real-time PCR.

Gene	Forward (5' to 3')	Reverse (5' to 3')	Function
GAPDH	TTTTGCGTCGCCAGCC	ATGGAATTTGCCATGGGTGGA	Housekeepingene
B-ACTIN	GCCTCGCCTTTGCCGAT	AGGTAGTCAGTCAGGTCCCG	Housekeepingene
VEGF	TAAGTCCTGGAGCGTTCCCT	ACGCGAGTCTGTGTTTTTGC	Triggers the growth of new blood vessels
p53	AAGTCTAGAGCCACCGTCCA	ACCATCGCTATCTGAGCAGC	p53 mutations are associated with cancers and affect chemotherapy response
PTEN	AGCTGGAAAGGGACGAACTG	ACACACAGGTAACGGCTGAG	It inhibits tumors by suppressing a pathway that increases cancer cells' proliferation and drug resistance.
BCL−2	AAAAATACAACATCACAGAGGAAGT	TCCCGGTTATCGTACCCTGT	It helps cancer cells escape death by preventing apoptosis.
AKT1	CCAGGATCCATGGGTAGGAAC	CTCCTCCTCCTCCTGCTTCT	PI3K/AKT/mTOR pathways enable cancer cells to resist death and grow.
BAX	AGGGGCCCTTTTGCTTCAG	TGTCCAGCCCATGATGGTTC	Promotes apoptosis
BAK	GCAGGCTGATCCCGTCC	GGCTAAGGAGGTCCCAGAGA	
NFKB	AACAGCAGATGGCCCATACC	AATAGGCAAGGTCAGGGTGC	It promotes tumor growth and spread, leads to chemotherapy resistance and increased inflammatory responses, and can also prevent death.
CAS−3	TGCTATTGTGAGGCGGTTGT	TCTGTTGCCACCTTTCGGTT	It drives tumor cells to death.
MMP−9	TCTATGGTCCTCGCCCTGAA	TTGTATCCGGCAAACTGGCT	It destroys the extracellular matrix, causing the tumor to grow, spread, and spread to distant tissues.

2.4.4.1. Apoptosis-related proteins. Apoptotic markers were evaluated by determining the activities of SEMA3A, GPX4, CASP-3, and CYT-C in cell samples using ELISA kits from BT LAB (China) with catalog numbers E2251Ra, E1787Ra, E1648Ra, and E0627Ra, respectively. The concentrations of SEMA3A, CASP-3, and CYT-C were quantified in nanograms per milliliter (ng/mL), whereas GPX4 levels were expressed in milliunits per liter (mU/L).

2.4.4.2. Evaluation of pro-inflammatory and anti-inflammatory factors. The levels of TNF-α, IL-6, and IL-10 in cell lysates were measured using specific ELISA kits from BT LAB (China), with catalog numbers E0764Ra, E0135Ra, and E0108Ra, respectively. TNF-α concentrations were expressed in nanograms per liter (ng/L), while IL-6 and IL-10 levels were reported in nanograms per liter (ng/L) and picograms per milliliter (pg/mL), respectively. Quantification was conducted by comparing the optical density values of the samples against a standard calibration curve.

2.5. Statistical analysis

Statistical analyses were performed using one-way ANOVA or a two-tailed Student's *t*-test. GraphPad Prism (version 8.0.1) software was employed for data analysis and graphical representation. Error bars indicate the standard error of the mean (SEM) from at least three independent experiments. Statistical significance thresholds were set at *P ≤ 0.05, * *P ≤ 0.01, * **P ≤ 0.001, and * *** *P ≤ 0.0001.

3. Results

3.1. Cytotoxic effects of 4-methylthiazole on K562 cells

The control group represents the baseline cell viability, which is standardized as 100 % throughout the experiment. The effect of 4-methylthiazole on K562 cell viability was assessed at 24, 48, and 72 hours following treatment with increasing concentrations (25, 50, 100, 200, and 500 μM) **(**Fig. 1**A).** At 24 hours (blue bars), cell viability remained relatively high across all doses, with minimal cytotoxic effects observed.

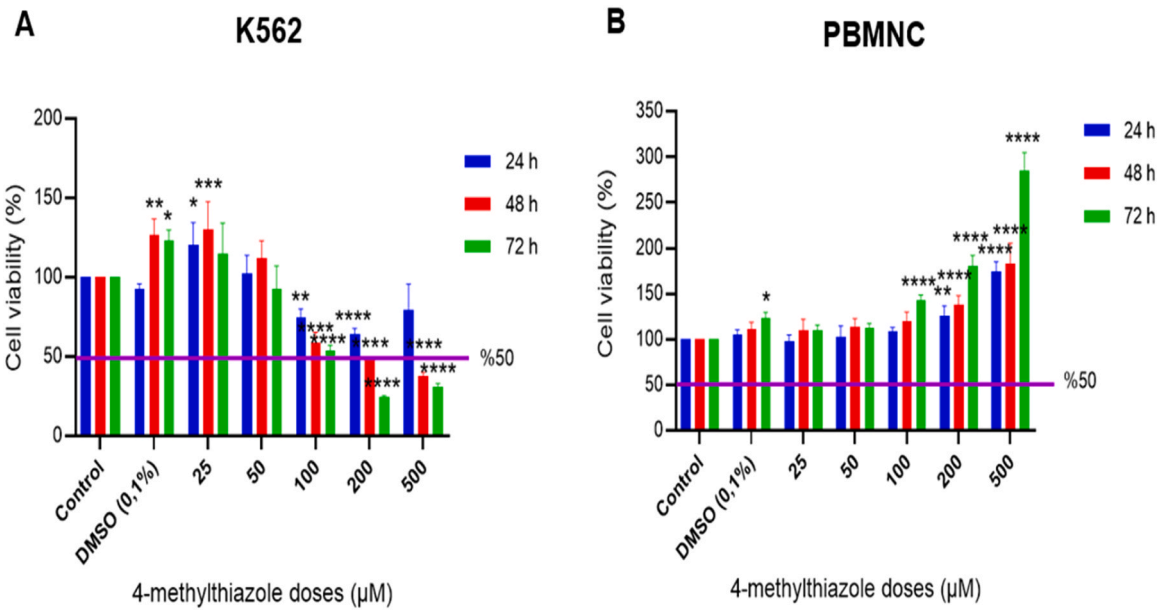


Fig. 1. This bar graph illustrates the effect of different concentrations of 4-methylthiazole on cell viability in K562 **(A)** and PBMNC **(B)** cell lines at 24, 48, and 72 hours. The x-axis represents the concentrations of 4-methylthiazole (μM), while the y-axis indicates the percentage of cell viability relative to the control group. The blue, red, and green bars correspond to 24, 48, and 72 hours**,** respectively. The horizontal magenta line marks the 50 % viability threshold. Error bars denote the standard error of the mean (SEM). Statistical significance is denoted as follows: * ** * $p < 0.0001$, * ** $p < 0.001$, * * $p < 0.01$, * $p < 0.05$. Data are presented as mean ± standard error (SE) from at least three independent experiments (h=hours).

However, at 48 hours (red bars), a marked reduction in viability was evident at doses of 100 μM and above. By 72 hours (green bars), cell viability further declined, particularly at doses exceeding 50 μM. The magenta horizontal line at 50 % cell viability indicates the threshold below which more than half of the cells were non-viable, demonstrating a dose-dependent cytotoxic effect of 4-methylthiazole**(**Fig. 1**A).** Notably, DMSO (0.1 %), used as the vehicle control, did not significantly affect K562 cell viability compared to the untreated (UNT) control group at any time point, confirming its suitability as a solvent.

The IC_{50} values were determined using linear regression analysis on sigmoidal inhibition curves in Microsoft Excel. At 48 hours post-treatment, the IC_{50} value was calculated as 230 μM, whereas at 72 hours, the IC_{50} value was reduced to 162 μM. The determined IC_{50} values were subsequently utilized for further experimental assays.

3.2. *Selective toxicity of 4-methylthiazole in peripheral blood mononuclear cells (PBMNCs)*

The effect of 4-methylthiazole on the viability of peripheral PBMNCs was assessed at 24, 48, and 72 hours following treatment with increasing concentrations (25, 50, 100, 200, and 500 μM) **(**Fig. 1**B).** At 24 hours (blue bars), PBMNCs exhibited minimal response to 4-methylthiazole treatment, maintaining viability across all tested concentrations. This observation indicates limited cytotoxicity at early time points. Similarly, at 48 hours (red bars), PBMNCs continued to display stable viability, demonstrating resilience to the treatment. However, at 72 hours (green bars), a marked increase in viability was observed, particularly at higher concentrations (200–500 μM), indicating a potential proliferative effect rather than cytotoxicity **(**Fig. 1**B).** The increase in viability observed in PBMNC cells at higher concentrations after 72 hours might be attributed to cellular stress responses or interference specific to the MTT assay used. In the literature, it has been reported that, in some cases, in vitro cytotoxicity assays can show

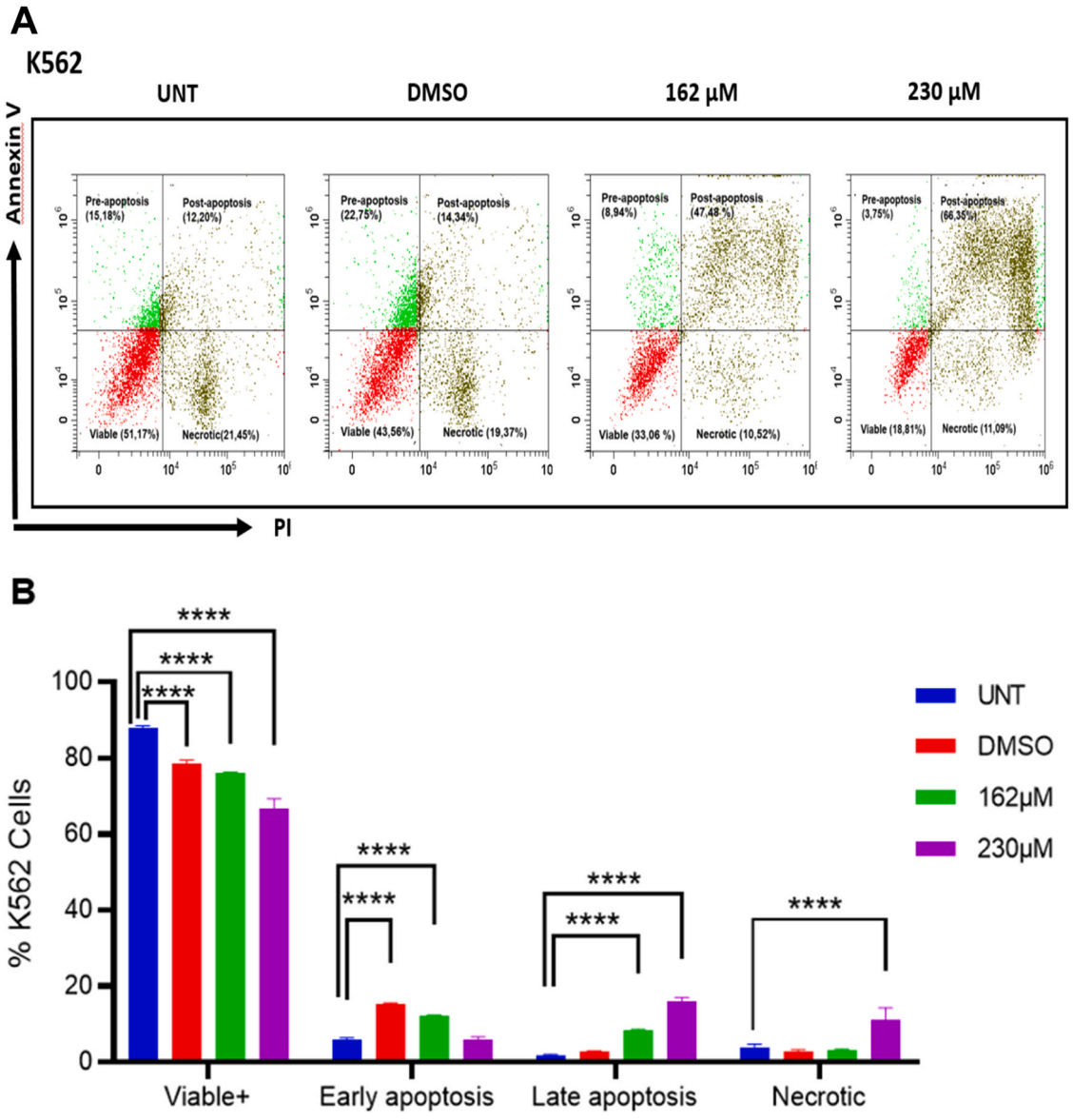


Fig. 2. Apoptosis Analysis After Treatment in K562 Cells, **A)** The flow cytometry plots of apoptosis, and B) Quantification of apoptotic and necrotic cell populations in K562 cells following treatment with 4-methylthiazole. The data indicate that 4-methylthiazole at both 162 μM and 230 μM concentrations significantly reduces the percentage of viable cells while increasing early apoptosis, late apoptosis and necrotic cell populations compared to UNT (UNT: Untreated, DMSO: Solvent control, **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$). The error bars represent the standard error of the mean.

temporary increases in cellular metabolic activity or measurement artifacts due to methodological limitations (Berridge et al., 2005; Fulda et al., 2010).

The magenta horizontal line represents the 50 % viability threshold, below which cell viability is considered significantly impaired. Throughout the experiment, PBMNC viability remained well above this threshold across all tested doses and time points, highlighting the non-toxic nature of 4-methylthiazole on healthy cells. In contrast to leukemia cell line, which demonstrated a dose- and time-dependent cytotoxic response, PBMNCs remained largely unaffected, further emphasizing the selective effect of 4-methylthiazole on cancer cells while sparing normal cells. These findings indicating that 4-methylthiazole exhibits selective cytotoxicity against leukemia cells while maintaining the viability of healthy PBMNCs, supporting its potential as a targeted therapeutic agent.

3.3. Induction of apoptosis and necrosis in K562 cells

The effects of 4-methylthiazole on K562 cell viability and apoptotic progression were analyzed following treatment with 162 μM and 230 μM concentrations, compared to the UNT and DMSO control groups. A statistically significant reduction in viable cells was observed at both treatment concentrations (162 μM and 230 μM) compared to the UNT control (****$p < 0.0001$), indicating that 4-methylthiazole significantly decreases K562 cell viability in a dose-dependent manner **(**Fig. 2**B).**

Additionally, both drug concentrations induced a significant increase in late apoptosis cell death compared to the UNT group (****$p < 0.0001$), highlighting the cytotoxic effect of 4-methylthiazole. Additionally, a significant increase in the early apoptosis cell population was observed only at 162 μM compared to the UNT control (****$p < 0.0001$), indicating that early apoptosis events are dose-dependent. Furthermore, necrotic cell populations were significantly higher at 230 μM (****$p < 0.0001$), indicating that at higher doses, 4-methylthiazole induces not only apoptosis but also necrosis **(**Fig. 2**B).** These findings collectively suggest that 4-methylthiazole effectively reduces K562 cell viability by promoting both apoptotic and necrotic cell death in a dose-dependent manner. A statistically significant increase was observed only in the early apoptosis cell population in the DMSO-treated group compared to the UNT. In contrast, treatment with 4-methylthiazole led to a pronounced and statistically significant increase in both pre and late apoptosis populations.

Microscopic analysis in Fig. 3 demonstrates dose-dependent morphological changes and apoptotic features in K562 cells following treatment with 4-methylthiazole. In the UNT and DMSO-treated control groups, cells exhibit a dense and uniform morphology, indicative of healthy cell populations. However, treatment with 162 μM 4-methylthiazole results in a reduction in cell density, along with the appearance of early apoptosis features, such as membrane blebbing and cell shrinkage. These apoptotic characteristics become more pronounced at 230 μM, where a significant decrease in cell density, increased cellular debris, and enhanced morphological changes suggest advanced apoptosis. These findings highlight the concentration-dependent apoptotic effects of 4-methylthiazole on K562 cells, supporting its potential as an anticancer agent.

3.4. Cell cycle disruptions induced by 4-methylthiazole in K562 cells

As shown in Fig. 4**,** treatment of K562 cells with different concentrations of 4-methylthiazole (162 μM and 230 μM) resulted in statistically significant changes in cell cycle phases compared to the UNT group. In the G0-G1 phase, treatment with 162 μM 4-methylthiazole led to a significant decrease compared to the UNT group (****$p < 0.0001$). This finding suggests that 4-methylthiazole prevents cells from being arrested in the G0-G1 phase, promoting cell cycle progression and

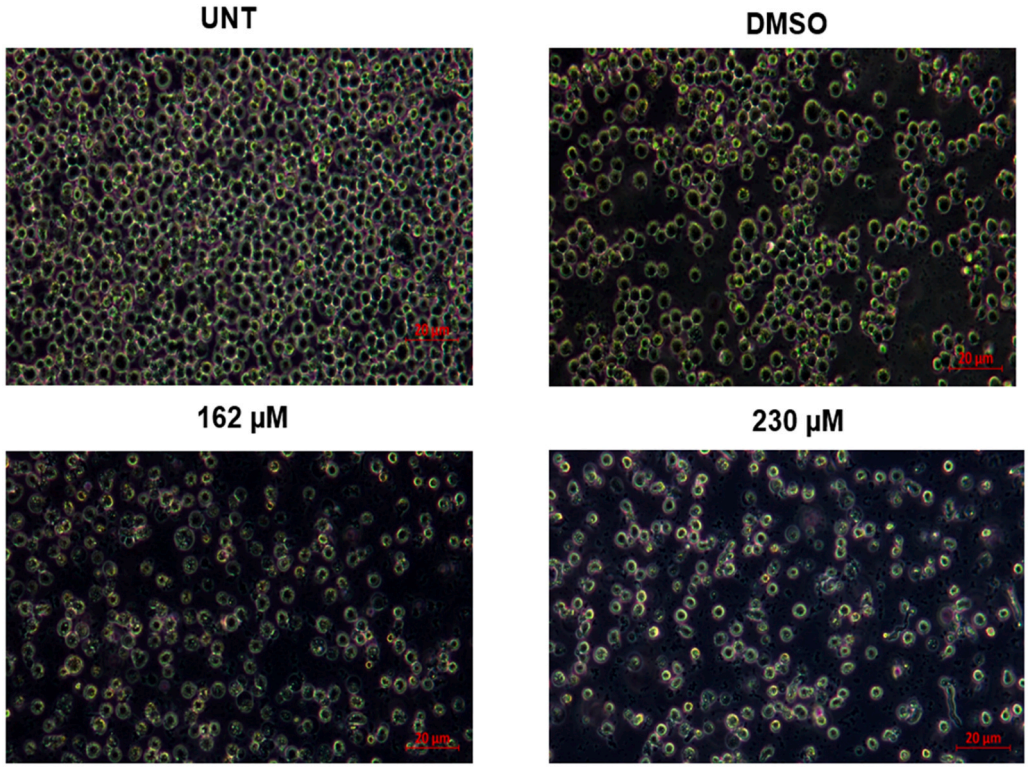


Fig. 3. Microscopic images of K562 cells treated with different concentrations of 4-methylthiazole. Representative phase-contrast microscopy images of K562 cells under different treatment conditions**:** untreated (UNT), solvent control (DMSO), and cells treated with 162 μM and 230 μM 4-methylthiazole. The images illustrate dose-dependent morphological changes, including reduced cell density, membrane blebbing, and signs of apoptosis at higher concentrations. Images were captured using a ZEISS microscopy system under 20X magnification.

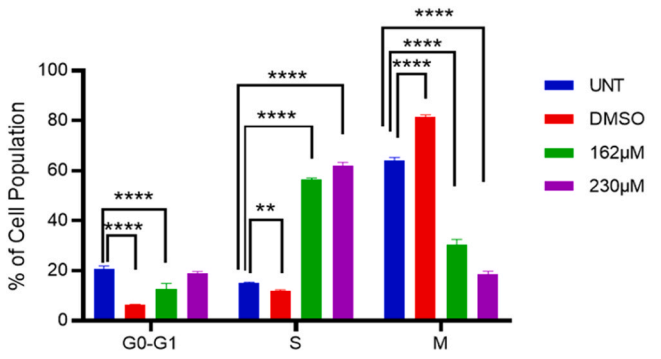


Fig. 4. Cell cycle distribution analysis of K562 cells treated with 4-methylthiazole at different concentrations. K562 cells were treated with vehicle control (DMSO), untreated control (UNT), or 4-methylthiazole at 162 μM and 230 μM, and their cell cycle distribution was analyzed. A significant reduction in the G0-G1 phase population was observed upon 4-methylthiazole treatment compared to UNT. Treatment with 4-methylthiazole led to a significant reduction in the G0-G1 phase population compared to UNT. Additionally, a dose-dependent increase in the S phase was observed, whereas the M phase population decreased at both concentrations relative to the UNT control. Statistical significance is denoted as follows: * ** * $p < 0.0001$, * * $p < 0.01$. Data are presented as mean ± standard error (SE) of at least three independent experiments.

potentially enhancing proliferation. The S phase population significantly increased at both concentrations (162 μM and 230 μM) compared to the UNT group (****$p < 0.0001$). This increase indicates that 4-methylthiazole induces the accumulation of cells in the S phase and may affect the DNA synthesis process. This finding suggests that the compound may cause replication stress, leading to an arrest in the replication phase and impairing mitotic progression. The M phase population showed a significant decrease at both doses compared to the UNT group (****$p < 0.0001$). In particular, treatment with 230 μM 4-methylthiazole caused a pronounced reduction in the M phase, demonstrating that the compound may inhibit the transition to mitosis and induce mitotic blockade. Interestingly, DMSO treatment led to a significant increase in the M phase cell population compared to both untreated and 4-methylthiazole-treated groups, accompanied by a marked decrease in the G0-G1 and S phases. These findings indicating that DMSO, despite being a vehicle control, may exert a measurable effect on cell cycle progression, particularly by promoting mitotic entry.

3.5. Regulation of SEMA3A expression following 4-methylthiazole treatment

As shown in Fig. 5**A**, the expression of SEMA3A+ protein was found to be significantly higher in comparison to the unstained (UNS) control group (*$p < 0.05$). This finding indicates that SEMA3A is naturally expressed at high levels in K562 cells. In Fig. 5**B**, a statistically significant reduction in SEMA3A+ expression was observed following treatment of K562 cells with different concentrations of 4-methylthiazole (162 μM and 230 μM). Specifically, treatment with 162 μM 4-methylthiazole resulted in a significant decrease in SEMA3A+ expression compared to both the UNT and DMSO control groups (**$p < 0.01$, ***$p < 0.001$). At 230 μM, the reduction in SEMA3A+ expression was even more pronounced compared to 162 μM, with a highly significant decrease observed (***$p < 0.001$). SEMA3A expression in DMSO-treated cells was slightly but significantly higher compared to untreated controls (*$p < 0.05$), indicating that DMSO may exert a moderate enhancing effect on basal SEMA3A levels.

These findings demonstrate that 4-methylthiazole inhibits SEMA3A+ expression in K562 cells in a dose-dependent manner. The observed reduction in SEMA3A+ expression suggests that 4-methylthiazole may exert a regulatory effect on cell proliferation or differentiation. In conclusion, 4-methylthiazole appears to modulate SEMA3A expression in K562 cells through an inhibitory mechanism, highlighting the need for further mechanistic studies to investigate its potential biological implications.

3.6. Mitochondrial membrane potential ($\Delta\Psi m$) alterations in K562 cells treated with 4-methylthiazole

As illustrated in Fig. 6, the analysis of mitochondrial membrane potential (MMP) in K562 cells following treatment with UNT, DMSO (vehicle control), CCCP+ (positive control for MMP loss), and different concentrations of 4-methylthiazole (162 μM and 230 μM) revealed significant changes in apoptotic progression. In the JC-1 + (early apoptosis) population, both 162 μM and 230 μM 4-methylthiazole treatments resulted in a significant increase compared to the UNT control groups (****$p < 0.0001$). This suggests that 4-methylthiazole disrupts mitochondrial membrane integrity, leading to early apoptosis

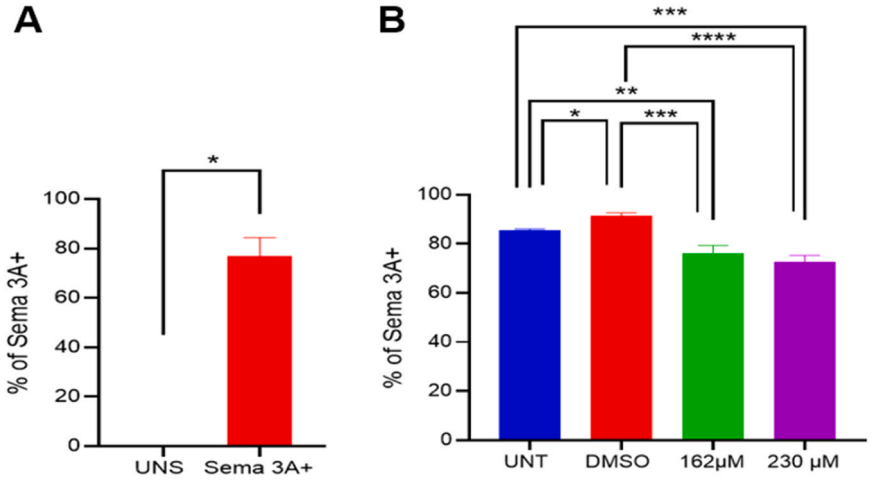


Fig. 5. Analysis of Sema 3 A+ protein expression in K562 cells after treatment with 4-methylthiazole **A)** Baseline levels of Sema 3 A+ protein expression in K562 cells, showing a significant increase in the Sema 3 A+ population compared to the unstained (UNS) control B) Sema 3 A+ protein expression in K562 cells following treatment with vehicle control (DMSO), untreated control (UNT), or 4-methylthiazole at 162 μM and 230 μM. Treatment with 4-methylthiazole resulted in a dose-dependent decrease in the percentage of Sema 3 A+ cells compared to both DMSO and UNT controls, with statistical significance indicated. Statistical significance is denoted as follows: * ** * $p < 0.0001$, * ** $p < 0.001$, * * $p < 0.01$, * $p < 0.05$. Data are presented as mean ± standard error (SE) from at least three independent experiments.

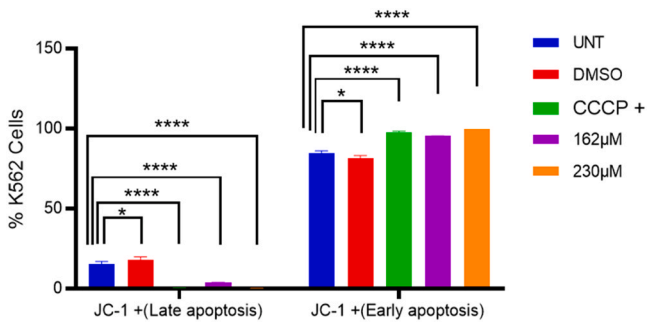


Fig. 6. Analysis of mitochondrial membrane potential (MMP) in K562 cells after 4-methylthiazole treatment using JC-1 staining. K562 cells were treated with vehicle control (DMSO), untreated control (UNT), 4-methylthiazole at 162 μM and 230 μM. Mitochondrial membrane potential was assessed via JC-1 staining, which distinguished early apoptosis and late apoptosis cell populations. Compared to UNT control, 4-methylthiazole treatment caused a significant decrease in JC-1 positive cells at the late apoptosis stage, indicating mitochondrial membrane depolarization. Conversely, the early apoptosis JC-1 positive cell population increased in cells after 4-methylthiazole treatment compared to the UNT group. Statistical significance is indicated as follows: * ** * $p < 0.0001$, * $p < 0.05$. Data are presented as the mean ± standard error (SE) from at least three independent experiments.

events. The CCCP+ treatment, which is a known MMP disruptor, exhibited a similar but slightly stronger effect, serving as a positive control for mitochondrial depolarization. Conversely, in the JC-1 + (late apoptosis) population, a statistically significant decrease was observed following 4-methylthiazole treatment (both 162 μM and 230 μM) compared to UNT groups (****$p < 0.0001$). This indicates a dose-dependent reduction in late apoptosis mitochondrial activity, demonstrating that 4-methylthiazole-induced apoptosis occurs primarily in the early apoptosis phase rather than post- apoptosis. The DMSO control group exhibited MMP levels similar to untreated cells, confirming that the solvent did not alter mitochondrial integrity (Fig. 6**).**

Overall, these findings demonstrate that 4-methylthiazole significantly alters mitochondrial membrane potential in K562 cells, predominantly inducing pre- apoptotic rather than late apoptosis events. The observed mitochondrial dysfunction and apoptotic induction highlight the potential of 4-methylthiazole as an antileukemic agent targeting mitochondrial pathways. Further studies are necessary to elucidate the underlying molecular mechanisms of 4-methylthiazole-mediated apoptosis in leukemia cells.

3.7. Gene expression modulations induced by 4-methylthiazole in K562 cells

Fig. 7 shows the gene expression changes in K562 cells treated with 4-methylthiazole at concentrations of 162 μM and 230 μM for 48 hours compared to untreated cells (UNT). The results indicate statistically significant upregulation of the apoptotic genes *P53, BAX,* and *BAK* specifically at the concentration of 162 μM (*$p < 0.05$), suggesting enhanced apoptotic signaling at this dose. Although *CAS3* expression also increased at 162 μM concentration, this increase was not statistically significant. The expression of the anti-apoptotic gene *BCL-2* showed a trend of downregulation, especially at 230 μM, but this change was not statistically significant. Additionally, the survival-related genes *AKT-1* and *PTEN* exhibited decreases, particularly noticeable at 230 μM, yet these reductions were not statistically significant either. Although an increase was observed in the *NF-κB* expression level at 162 μM and 230 μM, this upregulation did not reach statistical significance. This suggests limited activation of inflammatory or stress-related signaling pathways. Similarly, no statistically significant changes were observed in the expression levels of angiogenesis-related *VEGF* or metastasis-associated *MMP-9*, indicating that 4-methylthiazole likely does not significantly influence these processes in K562 cells under the tested conditions. DMSO did not induce any statistically significant alterations in the expression levels of the analyzed genes compared to the untreated control group.

Overall, these findings suggest that 4-methylthiazole exerts potential pro-apoptotic and anti-cancer effects by significantly increasing apoptosis-related gene expression without activating survival or metastatic pathways, making it a promising candidate for leukemia therapy.

3.8. Effects of 4-methylthiazole on apoptotic and oxidative stress markers

As illustrated in Fig. 8**A**, CASP-3 activity exhibited an increase following 4-methylthiazole treatment, with a significant elevation at 162 μM compared to the UNT group (*$p < 0.05$). However, at 230 μM, CASP-3 levels increased in a non-statistically significant manner.

In Fig. 8**B,** CYT-C levels showed a dose-dependent change. Treatment with 162 μM 4-methylthiazole caused a slight decrease in CYT-C levels that was not statistically significant compared to UNT and DMSO, while a significant increase was observed at 230 μM (*$p < 0.05$), indicating mitochondrial involvement in apoptosis induction.

Conversely, SEMA3A protein levels **(**Fig. 8**C)** did not show significant changes across the different conditions, indicating that 4-methylthiazole does not directly modulate SEMA3A expression in K562 cells. Similarly, Glutathione Peroxidase 4 (GPX4) activity **(**Fig. 8**D)** remained relatively

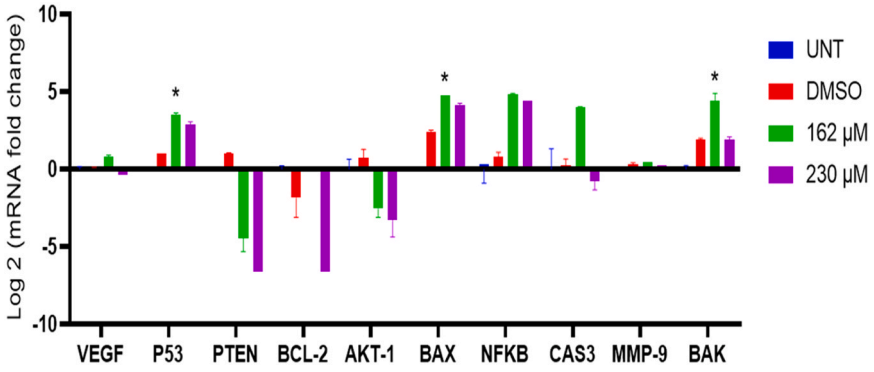


Fig. 7. Gene expression analysis of K562 cells treated with 4-methylthiazole for 48 hours. Log2-transformed mRNA fold change values were assessed for key genes involved in apoptosis (P53, BAX, BAK, CAS3), cell survival (BCL-2, AKT-1, PTEN), inflammation (NFKB), angiogenesis (VEGF), and metastasis (MMP-9). Cells were treated with 162 μM and 230 μM concentrations of 4-methylthiazole, and results were compared to untreated (UNT). Data represent mean ± SEM from three independent experiments. Statistical significance was determined using Tukey's multiple comparisons test (* $p < 0.05$).

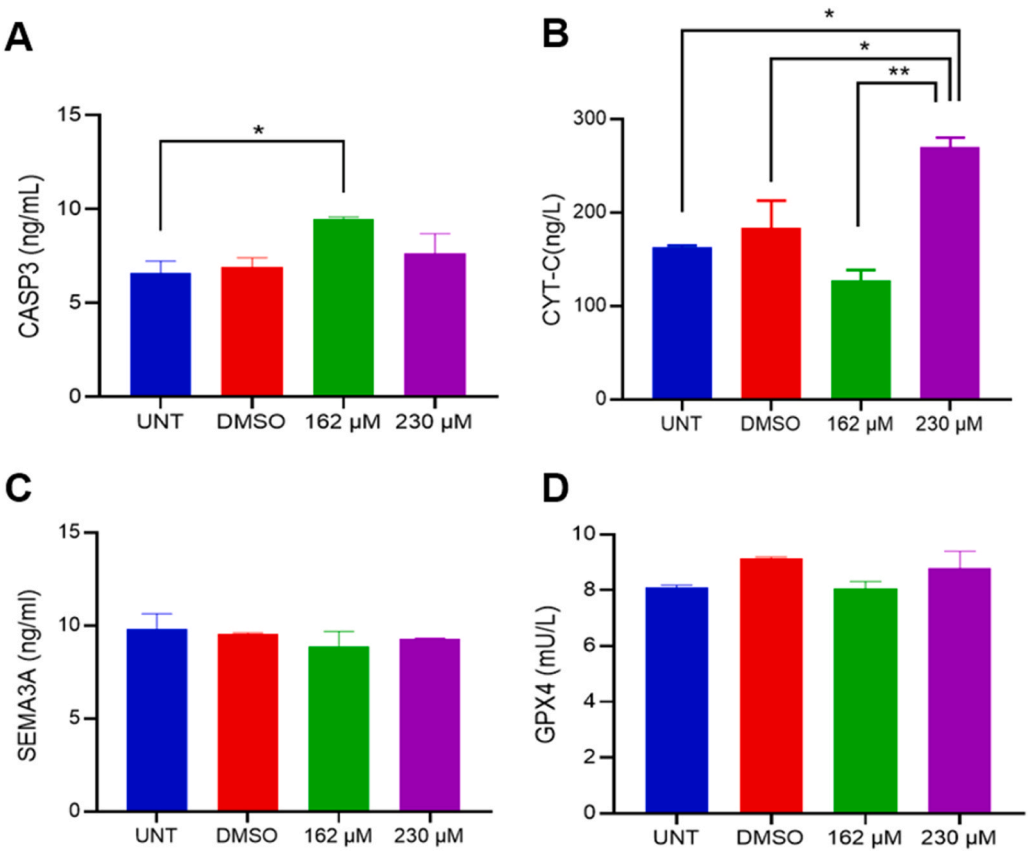


Fig. 8. Changes in the levels of apoptosis and oxidative stress-related proteins (CASP3, Cytochrome C, SEMA3A, and GPX4) in K562 cells after 4-methylthiazole treatment determined by ELISA **A)** CASP3 levels were significantly increased at 162 µM compared to UNT control, indicating activation of apoptotic pathways **B)** Cytochrome C levels showed a dose-dependent change with a significant decrease at 162 µM, whereas 230 µM treatment led to a significant increase compared to UNT control, suggesting mitochondrial involvement in apoptosis regulation **C)** SEMA3A levels remained unchanged in all treatment groups, indicating no significant effect of 4-methylthiazole on this protein in K562 cells **D)** GPX4 levels were stable across treatments, indicating that 4-methylthiazole did not significantly alter the oxidative stress response via GPX4 modulation. Statistical significance is indicated as: * *p < 0.01, *p < 0.05. Data are presented as mean ± standard error (SE) from at least three independent experiments.

stable across all conditions, indicating that 4-methylthiazole does not significantly impact antioxidant defense mechanisms at the tested concentrations. Protein levels measured in the DMSO group were statistically indistinguishable from the UNT groups, indicating that the solvent had no detectable effect on apoptosis- or oxidative stress-related proteins.

Overall, these findings suggest that 4-methylthiazole induces apoptosis in K562 cells through the activation of CASP-3 and the release

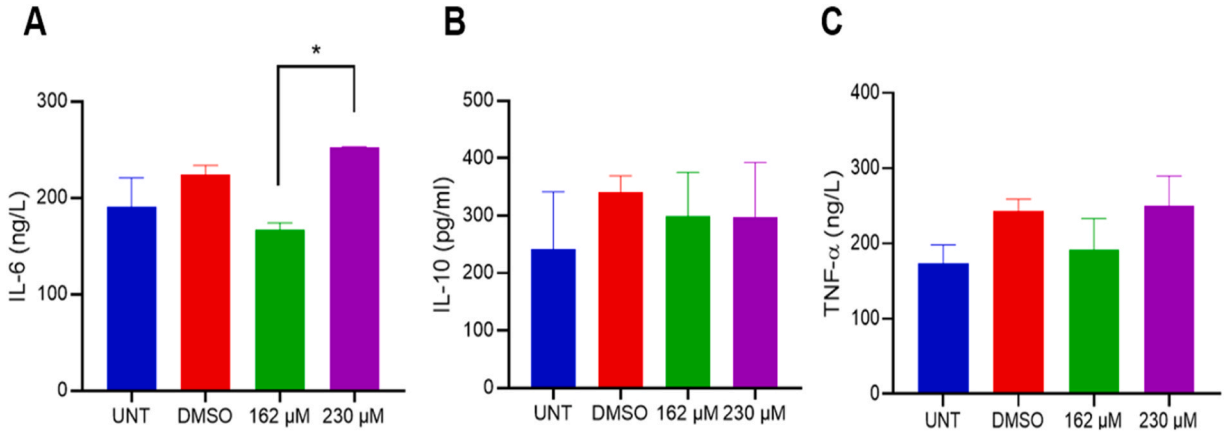


Fig. 9. Effects of 4-methylthiazole treatment on IL-6, IL-10 and TNF-α levels in K562 cells determined by ELISA analysis A) IL-6 levels showed a significant increase in 230 µM 4-methylthiazole treatment compared to 162 µM, while other comparisons showed no statistically significant changes B) IL-10 levels remained relatively constant in all treatment groups, with no significant difference observed between untreated (UNT), DMSO and 4-methylthiazole treated groups C) TNF-α levels showed a slight increase in the DMSO-treated group compared to UNT, but no statistically significant changes were detected following 4-methylthiazole treatment at either concentration.Statistical significance is indicated as: * p < 0.05. Data are presented as the mean ± standard error (SE) from at least three independent experiments.

of CYT-C. However, its effect on oxidative stress markers such as GPX4 and SEMA3A remains limited, and further studies are required to determine its precise mechanism of action in apoptosis regulation.

3.9. Cytokine modulation in K562 cells following 4-methylthiazole exposure

As illustrated in Fig. 9**A**, treatment with 4-methylthiazole at 162 μM resulted in a slight decrease in IL-6 levels compared to the UNT and DMSO groups. However, at 230 μM, IL-6 levels significantly increased compared to 162 μM treatment ($^*p < 0.05$), indicating a dose-dependent modulation of IL-6 secretion. The observed biphasic response in IL-6 levels - a decrease at 162 μM and a significant increase at 230 μM - may reflect a concentration-dependent change in cellular stress or inflammatory signaling pathways. While at low concentrations 4-methylthiazole may suppress IL-6 expression through apoptosis induction or anti-inflammatory effects, higher concentrations may have triggered a secondary inflammatory response due to increased cellular stress or sublethal injury. IL-10 levels did not show a statistically significant change across the different experimental groups, indicating that 4-methylthiazole does not exert a strong regulatory effect on this anti-inflammatory cytokine in Fig. 9**B.** As shown in Fig. 9**C**, TNF-α levels remained relatively stable across all groups, with no significant changes observed following 4-methylthiazole treatment. Overall, these findings suggest that 4-methylthiazole influences IL-6 expression in a dose-dependent manner, whereas its effects on IL-10 and TNF-α remain limited. There were no statistically significant differences in IL-6, IL-10, or TNF-α levels between DMSO-treated and UNT groups, indicating that the solvent exerted negligible immunomodulatory effects under the experimental conditions.

4. Discussion

Our study investigates the antileukemic effects of 4-methylthiazole on K562 cells, revealing its important role in apoptosis induction, mitochondrial dysfunction, and cytokine modulation. Consistent with previous findings on thiazole derivatives as anticancer agents (Dawood et al., 2021; Synthesis and Biological Evaluation of Novel Thiazole Analogs with Both Anti-Proliferative and Mechanistic Analyses and Molecular Docking Studies—Bimoussa—2022—ChemistrySelect—Wiley Online Library, n.d.), our results emphasize that 4-methylthiazole exerts its cytotoxic effects primarily through mitochondria-mediated apoptosis, a hallmark of chemotherapeutic agents targeting leukemia cells.

One of the key findings of our study is the significant increase in CASP-3 activity (1.8-fold increase, $^*p < 0.05$) and CYT-C release (1.5-fold increase at 230 μM, $^*p < 0.05$) at 162 μM treatment. These findings are in line with previous studies showing that thiazole derivatives can trigger apoptosis via caspase activation and mitochondrial membrane depolarization (Al-Warhi et al., 2023; Synthesis and Biological Evaluation of Novel Thiazole Analogs with Both Anti-Proliferative and Mechanistic Analyses and Molecular Docking Studies—Bimoussa—2022—ChemistrySelect—Wiley Online Library n.d.). Dutta et al. (2018) previously reported that thiazole-containing peptides can disrupt mitochondrial integrity and lead to caspase-dependent apoptosis in various cancer cell lines. Our findings further validate this mechanism, reinforcing the mitochondrial apoptotic pathway as the primary mode of action of 4-methylthiazole in K562 cells. Interestingly, CYT-C activity was significantly higher at a lower dose (46 μM) compared to 89 μM in previous studies (Mohamed et al., 2018), demonstrating a complex dose–response relationship. In our study, 162 μM treatment did not significantly change CYT-C levels, but a significant increase was observed at 230 μM, demonstrating that 4-methylthiazole may exert biphasic effects on mitochondrial pathways.

Although the IC_{50} value of 230 μM was determined based on metabolic activity using the MTS assay after 48 hours of treatment, flow cytometric analysis of viable and apoptotic cell populations at the same concentration and time point revealed a slightly higher percentage of viable cells (approximately 65–70 %). This apparent discrepancy is most likely attributable to methodological differences between the two assays. The MTS assay measures mitochondrial enzymatic activity as an indirect indicator of cell viability, whereas flow cytometry detects phosphatidylserine externalization and membrane integrity, which may reflect apoptotic changes at a different temporal resolution. Such variations are commonly observed and do not undermine the overall cytotoxic profile of the compound.

The 4-methylthiazole concentrations used in our study (162 μM and 230 μM) are consistent with concentration ranges reported in similar studies investigating thiazole derivatives in the literature and were selected to determine the mechanism of action under in vitro conditions; for instance, (Coşkun et al., 2023) reported an effective concentration of 175.02 μM for benzimidazole-thiazole hybrids, (Başoğlu et al., 2021). identified effective concentrations up to 275 μM for imidazo[2,1-*b*] thiazole derivatives.

Mitochondria play a central role in the regulation of apoptosis, and disruption of mitochondrial membrane potential (MMP, $\Delta\Psi m$) is an early indicator of apoptotic commitment (De Rasmo et al., 2023). Our JC-1 staining results showed a 2.3-fold decrease in the red/green fluorescence ratio, confirming that 4-methylthiazole causes mitochondrial depolarization in a dose-dependent manner. The positive control CCCP, a well-known mitochondrial disruptor, exhibited a similar but slightly stronger effect, confirming that loss of MMP is an important factor in 4-methylthiazole-induced apoptosis. These results are consistent with those reported by (Peng et al., 2021) and (Moustapha et al., 2015) showed that thiazole analogs disrupt MMP and lead to caspase activation and mitochondrial-mediated apoptosis. In addition, our study is consistent with (Park et al., 2019) and (Zhao et al., 2020) who suggested that MMP degradation is an important mechanism for anti-cancer activity in various thiazole derivatives.

Recent studies have demonstrated that ceRNA-mediated regulatory pathways, such as the lncRNA-00742/miR-116 axis targeting CD74, play a critical role in mitochondrial apoptosis triggered by environmental stressors like vanadium. These findings highlight the importance of post-transcriptional regulatory mechanisms in metal-induced apoptosis, supporting our observations on 4-methylthiazole–induced mitochondrial dysfunction (J et al., 2024).

The results of the study show that 4-methylthiazole significantly affected important apoptotic markers in K562 cells, which are consistent with previous findings on the use of thiazole derivatives as anticancer agents (Finiuk et al., 2022; Lozynskyi et al., 2022). PCR analysis confirmed the upregulation of *TP53, BAX, BAK,* and *CAS3,* confirming previous study results that thiazole derivatives promote apoptosis by increasing the expression of these genes (Meriç et al., 2024). In addition, further transcriptomic analyses revealed that exposure to toxic metals such as vanadium induced specific molecular responses and transcriptional reprogramming in kidney cells (Qiao et al., 2024). Such findings suggest that transcriptome-level profiling in future studies could help unravel the broader impact of 4-methylthiazole on gene networks beyond apoptosis. K562 cells do not express functional p53 protein due to the loss of one *TP53* allele and a frameshift mutation in exon 5 of the other allele. These mutations result in the formation of a premature stop codon in the N-terminal region of the p53 protein, leading to the production of a truncated, non-functional protein. Therefore, the presence of *TP53* mRNA in K562 cells does not indicate the production of functional p53 protein (Law et al., 1993). In our study, we observed a significant increase in *TP53* transcript levels following 4-methylthiazole treatment. However, assuming that elevated mRNA levels reflect the presence or activity of functional p53 protein may be misleading. Thus, to clarify the biological significance and cellular effects of *TP53* induction, further analyses at the protein level (e.g., Western blot) and functional assays (e.g., evaluation of transcriptional activity of p53 target genes) are necessary.

The increased *CASP-3* activation observed in this study (1.8-fold at

162 μM, *$p < 0.05$) further supports its role as an apoptosis executioner, consistent with previous studies emphasizing caspase-mediated cell death induced by thiazole compounds (Choi et al., 2012). Unlike some studies reporting decreased *BCL-2* expression (Altıntop et al., 2018), the present findings did not show significant changes in *BCL-2* levels, implying a different apoptotic mechanism that may be independent of anti-apoptotic modulation. Moreover, *AKT-1* and *PTEN* levels remained unchanged, indicating that 4-methylthiazole may function by means of alternative apoptotic pathways in K562 cells, unlike previous reports of AKT pathway inhibition by thiazole derivatives (Orujova et al., 2023a, 2023b). The observed upregulation of *NF-κB* contradicts previous studies reporting its suppression (Altıntop et al., 2018), which may indicate a potential compensatory stress response. Although the upregulation of *NF-κB* observed in our study appears to contradict earlier reports indicating its suppression by other thiazole derivatives, this discrepancy may stem from differences in cell lines (e.g., K562 vs. solid tumor lines), compound structure (simple thiazoles vs. thiazole-hydrazone hybrids), or experimental parameters such as dose and exposure duration. Given the role of *NF-κB* in stress response and inflammation, its upregulation in our system may reflect a compensatory mechanism to mitochondrial disruption or cytokine modulation induced by 4-methylthiazole, rather than a direct pro-survival effect. Further mechanistic studies, particularly involving NF-κB pathway inhibition, are warranted to clarify this context-specific regulation. *NF-κB* Moreover, *VEGF* and *MMP-9* levels remained unchanged, indicating that 4-methylthiazole does not significantly affect angiogenesis or metastasis-related pathways, unlike other thiazole derivatives known to inhibit these processes (Sharma et al., 2023).

Cell cycle analysis revealed that 4-methylthiazole induced S phase accumulation (35 % increase, ****$p < 0.0001$) and mitotic blockade (60 % decrease in M phase, ****$p < 0.0001$), indicating replication stress and impaired proliferation. This effect is comparable to (Zhang et al., 2022) who showed that thiazole analogs inhibit cell cycle progression by way of G2/M phase arrest in leukemia cells. The observed increase in S-phase population suggests that 4-methylthiazole may interfere with DNA synthesis, potentially through mechanisms similar to cisplatin-induced DNA damage (Dasari and Tchounwou, 2014). These findings are particularly important considering that (Meriç et al., 2024) previously reported that 4-ethylthiazole impaired leukemia cell cycle progression, further supporting the potential therapeutic role of this compound.

Inflammatory cytokines play an important role in cancer progression and apoptosis regulation. Our cytokine profiling revealed a dose-dependent modulation of IL-6, with a 40 % decrease at 162 μM but a 25 % increase at 230 μM (*$p < 0.05$). This biphasic response suggests that low-dose 4-methylthiazole may suppress inflammatory signaling, while higher doses may activate compensatory immune responses. This observation is in line with studies by (Chahal et al., n.d.) and (Ilkiv et al., 2022) that described the dual role of thiazole derivatives in cytokine regulation. Interestingly, TNF-α and IL-10 levels remained unaffected, contradicting previous findings that thiazole derivatives modulate these cytokines in cancer models (Cai et al., 2017; Wolczyk et al., 2016). However, our data support the work of (dos Santos et al., 2016) who reported that some thiazole compounds selectively modulate IL-6 without significantly altering TNF-α or IL-10 levels.

Our results show a 32 % decrease in SEMA3A expression at 230 μM, while GPX4 levels remained unchanged (***$p < 0.001$). The role of SEMA3A in cancer biology varies depending on the cancer type and cellular context. Some studies have reported that SEMA3A plays a tumor suppressor role and its downregulation can promote the proliferation and migration of cancer cells ("(PDF) Synthetic Approaches toward Certain Structurally Related Antimicrobial Thiazole Derivatives, 2010–2020, 2024"). However, it has also been shown that SEMA3A may have tumor-promoting effects through different signaling pathways in some hematological malignancies and certain solid tumors (Kupczyk et al., 2020). In this context, the changes observed in SEMA3A levels after 4-methylthiazole application in our study should be evaluated in line with cell type and context-specific mechanisms. Our findings suggest that 4-methylthiazole may also regulate apoptosis by a mechanism independent of SEMA3A.

Similarly, GPX4, an important regulator of oxidative stress and ferroptosis, was not affected by 4-methylthiazole, even at the highest concentration tested that its cytotoxic effects are independent of ferroptotic pathways. These results are consistent with studies by (Chimenti et al., 2009) and (Kupczyk et al., 2020), who found that some thiazole analogs triggered apoptosis without affecting oxidative stress markers.

Involvement of lncRNA-mediated ferroptosis pathways has been recently identified in metal-induced toxicity, such as the copper-induced lncRNA-TCONS-6251/miR-novel-100/TC2N axis in kidney cells (Copper as the driver of the lncRNA-TCONS-6251/miR-novel-100/TC2N axis: Unraveling ferroptosis in duck kidney—ScienceDirect, n.d.). Whether 4-methylthiazole can similarly induce ferroptosis in leukemia models may be the subject of future research.

Natural compounds such as Ginkgo biloba extract have shown protective effects against chemical-induced organ toxicity by modulating cell cycle regulators such as SKP2 and inhibiting autophagy (Ginkgo biloba extract alleviates deltamethrin-induced testicular injury by upregulating SKP2 and inhibiting Beclin1-independent autophagy—ScienceDirect n.d.). Although our study focused on pro-apoptotic mechanisms, future research could investigate whether 4-methylthiazole also interacts with autophagic or protective survival pathways.

Interestingly, 4-methylthiazole K562 exhibited dose-dependent cytotoxicity in leukemia cells, while a significant increase in cell viability was observed in PBMNCs at the highest concentration tested (500 μM). This differential response may reflect different metabolic adaptations and stress tolerance mechanisms between malignant and non-malignant cells. Many studies have shown that some anticancer agents can inhibit the proliferation of cancer cells while exhibiting minimal or even supportive effects on normal cells. Such selective behavior suggests the ability of thiazole compounds to activate compensatory survival pathways in non-transformed cells, potentially through hormetic mechanisms or immune-modulatory signaling. Furthermore, the observed increase in mitochondrial activity in PBMNCs may be due to mild oxidative or metabolic stimulation triggered by the compound, which does not occur in leukemic cells due to their altered redox balance. In support of this, some anti-cancer molecules have been shown to promote tissue regeneration and modulate immune responses by reducing inflammation.

A key limitation of this study is its reliance solely on in vitro experiments using a single leukemia cell line, which may not fully reflect the complex nature of the tumor microenvironment or systemic responses in a living organism. Therefore, further advanced studies involving multiple cell lines and animal models are necessary to evaluate the therapeutic efficacy and pharmacokinetic properties of 4-methylthiazole under physiological conditions.

DMSO (0.1 %) was used as a vehicle control throughout the study; however, its effects were not entirely negligible. In specific assays, such as cell cycle distribution and SEMA3A expression, DMSO induced statistically significant changes compared to untreated controls. These findings emphasize that even commonly used solvents may exert mild biological effects, which can influence the interpretation of experimental outcomes. Although appropriate untreated controls were included in all experiments, the observed solvent effects must be acknowledged as a limitation of the study. Future experiments using alternative solvents or additional solvent controls may help further delineate the compound-specific effects of 4-methylthiazole.

5. Conclusion and future perspectives

Our study demonstrates that 4-methylthiazole induces apoptosis in K562 leukemia cells via CASP-3 activation, CYT-C release,

mitochondrial dysfunction, and S-phase arrest. These findings support the growing evidence that thiazole derivatives are promising anticancer agents with selective cytotoxicity against leukemia cells.

Our data show that 4-methylthiazole exhibits selective toxicity, with healthy PBMNCs retaining > 80 % viability at the highest concentration (500 μM). This is in sharp contrast to previous chemotherapy agents such as cisplatin, which cause significant off-target toxicity (Dasari and Tchounwou, 2014).

Evaluation of the therapeutic efficacy of 4-methylthiazole in in vivo leukemia odels is critical to understanding the potential of this compound for clinical use. Furthermore, investigation of its interactions with key apoptosis regulators such as p53 and Bcl-2 will provide a more detailed understanding of its effects on apoptotic mechanisms. Furthermore, investigation of the possible synergistic effects of 4-methylthiazole with standard chemotherapeutic agents such as imatinib and daunorubicin may contribute to the development of combination treatment strategies. In this context, comprehensive elucidation of the molecular mechanisms of 4-methylthiazole will enable the creation of more targeted and effective treatment approaches for leukemia patients.

Although statistically significant increases in both IL-6 secretion and necrotic cell population were observed at the highest concentration of 4-methylthiazole (230 μM), these changes remained within the biologically acceptable ranges reported in the literature for in vitro studies. The elevation in IL-6 may reflect a mild stress or immunomodulatory response rather than a proinflammatory or pathological effect. Similarly, the degree of necrosis observed may not represent uncontrolled cell lysis but rather a limited secondary response accompanied by apoptosis. However, these findings underscore the need for further evaluation of the immunological and cytotoxic profiles of the compound in more complex in vivo models.

Author Contributions

NM conceptualized the study design, designed the experiments, and collected and analyzed the data. EK and FK assisted in the experimental procedures and contributed to data analysis.

CRediT authorship contribution statement

Kar Fatih: Writing – review & editing, Writing – original draft, Visualization, Validation. **Meriç Neslihan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Data curation. **Kar Ezgi:** Writing – review & editing, Writing – original draft, Visualization.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Ethical Approval

This research did not include human or animal subjects requiring ethical approval.

Funding

This study was not supported by any specific grant from public, commercial, or non-profit funding agencies

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abbas, E.M.H., Farghaly, T.A., Sabour, R., Shaaban, M.R., Abdallah, Z.A., 2022. Design, synthesis, cytotoxicity, and molecular docking studies of novel thiazolyl-hydrazone derivatives as histone lysine acetyl-transferase inhibitors and apoptosis inducers. Arch. Der Pharm. 355 (7), e2200076. https://doi.org/10.1002/ardp.202200076.

Altıntop, M.D., Sever, B., Akalın Çiftçi, G., Özdemir, A., 2018. Design, synthesis, and evaluation of a new series of thiazole-based anticancer agents as potent Akt inhibitors. Mol. (Basel, Switz.) 23 (6), 1318. https://doi.org/10.3390/molecules23061318.

Al-Warhi, T., Almahli, H., Maklad, R.M., Elsayed, Z.M., El Hassab, M.A., Alotaibi, O.J., Aljaeed, N., Ayyad, R.R., Ghabour, H.A., Eldehna, W.M., El-Ashrey, M.K., 2023. 1-Benzyl-5-bromo-3-hydrazonoindolin-2-ones as novel anticancer agents: synthesis, biological evaluation and molecular modeling insights. Mol. (Basel, Switz.) 28 (7), 3203. https://doi.org/10.3390/molecules28073203.

Amin, H.K., El-Araby, A.M., Eid, S., Nasr, T., Bondock, S., Leheta, O., Dawoud, M.E., 2017. A thiazole analogue exhibits an anti-proliferative effect in different human carcinoma cell lines and its mechanism based on molecular modeling. Article 1 Adv. Biol. Chem. 7 (1). https://doi.org/10.4236/abc.2017.71005.

Başoğlu, F., Ulusoy-Güzeldemirci, N., Akalın-Çiftçi, G., Çetinkaya, S., Ece, A., 2021. Novel imidazo[2,1-b]thiazole-based anticancer agents as potential focal adhesion kinase inhibitors: synthesis, in silico and in vitro evaluation. Chem. Biol. Drug Des. 98 (2), 270–282. https://doi.org/10.1111/cbdd.13896.

Berridge, M.V., Herst, P.M., Tan, A.S., 2005. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. In: In *Biotechnology Annual Review*, 11. Elsevier, pp. 127–152. https://doi.org/10.1016/S1387-2656(05)11004-7.

Cai, X., Cao, C., Li, J., Chen, F., Zhang, S., Liu, B., Zhang, W., Zhang, X., Ye, L., 2017. Inflammatory factor TNF-α promotes the growth of breast cancer via the positive feedback loop of TNFR1/NF-κB (and/or p38)/p-STAT3/HBXIP/TNFR1. Oncotarget 8 (35), 58338–58352. https://doi.org/10.18632/oncotarget.16873.

Chahal, S., Punia, J., Rani, P., Singh, R., Mayank, Kumar, P., Kataria, R., Joshi, G., & Sindhu, J. (n.d.). Development of thiazole-appended novel hydrazones as a new class of α-amylase inhibitors with anticancer assets: An in silico and in vitro approach. *RSC Medicinal Chemistry*, *14*(4), 757–781. https://doi.org/10.1039/d2md00431c.

Chimenti, F., Bizzarri, B., Maccioni, E., Secci, D., Bolasco, A., Chimenti, P., Fioravanti, R., Granese, A., Carradori, S., Tosi, F., Ballario, P., Vernarecci, S., Filetici, P., 2009. A novel histone acetyltransferase inhibitor modulating Gcn5 network: cyclopentylidene-[4-(4'-chlorophenyl)thiazol-2-yl)hydrazone. J. Med. Chem. 52 (2), 530–536. https://doi.org/10.1021/jm800885d.

Choi, M.-J., Lee, H., Lee, J.-H., Jung, K.H., Kim, D., Hong, S., Hong, S.-S., 2012. The effect of HS-111, a novel thiazolamine derivative, on apoptosis and angiogenesis of hepatocellular carcinoma cells. Arch. Pharmacal Res. 35 (4), 747–754. https://doi.org/10.1007/s12272-012-0420-4.

Copper as the driver of the lncRNA-TCONS-6251/miR-novel-100/TC2N axis: Unraveling ferroptosis in duck kidney—ScienceDirect. (n.d.). Retrieved April 2, 2025, from ⟨https://www.sciencedirect.com/science/article/abs/pii/S0141813024076062?via%3Dihub⟩.

Coşkun, G., Erdoğan, Ö., CeviK, O., ŞahiN, Z., DemiRayak, Ş., 2023. Benzimidazole-thiazole hybrids; synthesis, structure elucidation and cytotoxic properties. Acta Med. Nicomedia 6 (1), 65–70. https://doi.org/10.53446/actamednicomedia.1229450.

Dasari, S., Tchounwou, P.B., 2014. Cisplatin in cancer therapy: molecular mechanisms of action. Eur. J. Pharmacol. 740, 364–378. https://doi.org/10.1016/j.ejphar.2014.07.025.

Dawood, K.M., Raslan, M.A., Abbas, A.A., Mohamed, B.E., Abdellattif, M.H., Nafie, M.S., Hassan, M.K., 2021. Novel bis-thiazole derivatives: synthesis and potential cytotoxic activity through apoptosis with molecular docking approaches. Front. Chem. 9, 694870. https://doi.org/10.3389/fchem.2021.694870.

De Rasmo, D., Cormio, A., Cormio, G., Signorile, A., 2023. Ovarian Cancer: A Landscape of Mitochondria with Emphasis on Mitochondrial Dynamics. Int. J. Mol. Sci. 24 (2), 1224. https://doi.org/10.3390/ijms24021224.

dos Santos, T.A.R., da Silva, A.C., Silva, E.B., Gomes, P.A.T., de, M., Espíndola, J.W.P., Cardoso, M.V., de, O., Moreira, D.R.M., Leite, A.C.L., Pereira, V.R.A., 2016. Antitumor and immunomodulatory activities of thiosemicarbazones and 1,3-Thiazoles in Jurkat and HT-29 cells. Biomed. Pharmacother. 82, 555–560. https://doi.org/10.1016/j.biopha.2016.05.038.

Dutta, D., Debnath, M., Müller, D., Paul, R., Das, T., Bessi, I., Schwalbe, H., Dash, J., 2018. Cell penetrating thiazole peptides inhibit c-MYC expression via site-specific targeting of c-MYC G-quadruplex. Nucleic Acids Res. 46 (11), 5355–5365. https://doi.org/10.1093/nar/gky385.

Erdaş, D. (2020). *Metiltiyazol türevlerinin antikanser ve antiinflamatuvar aktivitelerinin değerlendirilmesi* [masterThesis, Sağlık Bilimleri Enstitüsü]. ⟨https://acikbilim.yok.gov.tr/handle/20.500.12812/328401⟩.

Finiuk, N.S., Drapak, I.V., Zimenkovsky, B.S., Stoika, R.S., 2022. Study in vitro of the anticancer activity of [3-allyl-4-(41-methoxyphenyl)-3H-thiazole-2-ylidene]-(32-trifluoromethylphenyl)amine hydrobromide toward human tumor cells. Biopolym. Cell 38 (3), 195–204. https://doi.org/10.7124/bc.000A7E.

Fulda, S., Gorman, A.M., Hori, O., Samali, A., 2010. Cellular stress responses: Cell survival and cell death. Int. J. Cell Biol. 2010, 214074. https://doi.org/10.1155/2010/214074.

Ginkgo biloba extract alleviates deltamethrin-induced testicular injury by upregulating SKP2 and inhibiting Beclin1-independent autophagy—ScienceDirect. (n.d.). Retrieved April 2, 2025, from ⟨https://www.sciencedirect.com/science/article/abs/pii/S0944711324009024?via%3Dihub⟩.

Hosny, M.A., Zaki, Y.H., Mokbel, W.A., Abdelhamid, A.O., 2019. Synthesis of novel thiazole, pyranothiazole, thiazolo[4,5-b]pyridines and thiazolo[5′,4′:5,6]pyrano[2,3-d]pyrimidine derivatives and incorporating isoindoline-1,3-dione group. BMC Chem. 13 (1), 37. https://doi.org/10.1186/s13065-019-0559-x.

Ilkiv, M.V., Shalai, Y.R., Manko, B.O., Ostapiuk, Y.V., Mitina, N.E., Zaichenko, A.S., Babsky, A.M., 2022, May 1. Generation of ROS under the influence of thiazole derivative and its complexes with PEG-based polymeric nanoparticles. | EBSCOhost. https://doi.org/10.7124/bc.000A7D.

Ivan Franko National University of Lviv, 4 Hrushevsky str., Lviv 79005, Ukraine, Omeliukh, B., Shalai, Ya, Ivan Franko National University of Lviv, 4 Hrushevsky str., Lviv 79005, Ukraine, Bura, M., Ivan Franko National University of Lviv, 4 Hrushevsky str., Lviv 79005, Ukraine, Ilkiv, M., Ivan Franko National University of Lviv, 4 Hrushevsky str., Lviv 79005, Ukraine, Ostapiuk, Yu, Ivan Franko National University of Lviv, 4 Hrushevsky str., Lviv 79005, Ukraine, Mitina, N., Lviv Polytechnic National University, 9 St. Yura sq., Lviv 79013, Ukraine, Zaichenko, O., Lviv Polytechnic National University, 9 St. Yura sq., Lviv 79013, Ukraine, Babsky, A., & Ivan Franko National University of Lviv, 4 Hrushevsky str., Lviv 79005, Ukraine. (2023). Activity of antioxidant enzymes in hepatocytes of mice with lymphoma under the action of thiazole derivative in complex with polymeric nanocarrier. *The Animal Biology*, *25*(3), 3–7. https://doi.org/10.15407/animbiol25.03.003.

Ivan Franko National University of Lviv, Ukraine, Shalai, Ya.R., Popovych, M.V., Ivan Franko National University of Lviv, Ukraine;, Mandzynets, S.M., Ivan Franko National University of Lviv, Ukraine;, Hreniukh, V.P., Ivan Franko National University of Lviv, Ukraine;, Finiuk, N.S., Ivan Franko National University of Lviv, Ukraine;, Babsky, A.M., & Ivan Franko National University of Lviv, Ukraine; (2021). Prooxidant and antioxidant processes in the liver homogenate of healthy and tumor-bearing mice under the action of thiazole derivatives. *The Ukrainian Biochemical Journal*, *93*(3), 61–67. https://doi.org/10.15407/ubj93.03.061.

J, C., X, D., C, X., Y, Z., H, C., G, H., X, G., X, G., P, L., F, Y., 2024. Cooperative application of transcriptomics and ceRNA hypothesis: lncRNA-00742/miR-116 targets CD74 to mediate vanadium-induced mitochondrial apoptosis in duck liver. J. Hazard. Mater. 480. https://doi.org/10.1016/j.jhazmat.2024.135904.

Korkmaz, I.N., 2024. Investigation of the effects of thiazole compounds on thioredoxin reductase 1 (TrxR1), glutathione S-transferase (GST), and glutathione reductase (GR) targeted human brain glioblastoma cancer (U-87 MG). Biotechnol. Appl. Biochem. 71 (4), 948–959. https://doi.org/10.1002/bab.2589.

Kupczyk, D., Studzińska, R., Bilski, R., Baumgart, S., Kołodziejska, R., Woźniak, A., 2020. Synthesis of novel 2-(isopropylamino)thiazol-4(5H)-one derivatives and their inhibitory activity of 11β-HSD1 and 11β-HSD2 in aspect of carcinogenesis prevention. Mol. (Basel, Switz.) 25 (18), 4233. https://doi.org/10.3390/molecules25184233.

Law, J.C., Ritke, M.K., Yalowich, J.C., Leder, G.H., Ferrell, R.E., 1993. Mutational inactivation of the p53 gene in the human erythroid leukemic K562 cell line. Leuk. Res. 17 (12), 1045–1050. https://doi.org/10.1016/0145-2126(93)90161-d.

Lozynskyi, A., Senkiv, J., Ivasechko, I., Finiuk, N., Klyuchivska, O., Kashchak, N., Lesyk, D., Karkhut, A., Polovkovych, S., Levytska, O., Karpenko, O., Boshkayeva, A., Sayakova, G., Gzella, A., Stoika, R., Lesyk, R., 2022. 1,4-naphthoquinone motif in the synthesis of new thiopyrano[2,3-d]thiazoles as potential biologically active compounds. Article 21. Molecules 27 (21). https://doi.org/10.3390/molecules27217575.

Mabkhot, Y.N., Alharbi, M.M., Al-Showiman, S.S., Ghabbour, H.A., Kheder, N.A., Soliman, S.M., Frey, W., 2018. Stereoselective synthesis, X-ray analysis, computational studies and biological evaluation of new thiazole derivatives as potential anticancer agents. Chem. Cent. J. 12 (1), 56. https://doi.org/10.1186/s13065-018-0420-7.

Meriç, N., Albayrak, E., Gülbaş, Z., Kocabaş, F., 2024. MEIS inhibitors reduce the viability of primary leukemia cells and Stem cells by inducing apoptosis. Leuk. Lymphoma 65 (2), 187–198. https://doi.org/10.1080/10428194.2023.2275532.

Meriç, N., Kar, E., Kar, F., 2024. 4-Methylthiazole triggers apoptosis and mitochondrial disruption in HL-60 cells. Mol. Biol. Rep. 51 (1), 997. https://doi.org/10.1007/s11033-024-09939-y.

Mohamed, F.Z., Elghreeb, M.S., Abdelhamid, M.S., Elbaz, H.A.Synthesis and Evaluation of the Antitumor Activity of (E)-3-((2-(5-(4-chlorophenyl) thiazol-2-yl) hydrazono) methyl) benzene-1,2-diol "In vitro and In vivo Study." Asian Journal of Research in Biochemistry, 1–16. https://doi.org/10.9734/ajrb/2018/v3i229826.

Moustapha, A., Pérétout, P.A., Rainey, N.E., Sureau, F., Geze, M., Petit, J.-M., Dewailly, E., Slomianny, C., Petit, P.X., 2015. Curcumin induces crosstalk between autophagy and apoptosis mediated by calcium release from the endoplasmic reticulum, lysosomal destabilization and mitochondrial events. Cell Death Discov. 1, 15017. https://doi.org/10.1038/cddiscovery.2015.17.

Orujova, T., Ece, A., Akalın Çiftçi, G., Özdemir, A., Altıntop, M.D., 2023a. A new series of thiazole-hydrazone hybrids for Akt-targeted therapy of non-small cell lung cancer. Drug Dev. Res. 84 (2), 185–199. https://doi.org/10.1002/ddr.22022.

Orujova, T., Ece, A., Akalın Çiftçi, G., Özdemir, A., Altıntop, M.D., 2023b. A new series of thiazole-hydrazone hybrids for Akt-targeted therapy of non-small cell lung cancer. Drug Dev. Res. 84 (2), 185–199. https://doi.org/10.1002/ddr.22022.

Park, S.K., Kang, J.Y., Kim, J.M., Yoo, S.K., Han, H.J., Chung, D.H., Kim, D.-O., Kim, G.-H., Heo, H.J., 2019. Fucoidan-Rich Substances from Ecklonia cava Improve Trimethyltin-Induced Cognitive Dysfunction via Down-Regulation of Amyloid β Production/Tau Hyperphosphorylation. Mar. Drugs 17 (10), 591. https://doi.org/10.3390/md17100591.

(PDF) Effect of thiazole derivative complexed with nanoscale polymeric carriers on cellular ultrastructure of murine lymphoma cells in vivo. (2024). ResearchGate. https://doi.org/10.30970/sbi.1502.653.

(PDF) Synthetic Approaches toward Certain Structurally Related Antimicrobial Thiazole Derivatives (2010-2020). (2024). ResearchGate. https://doi.org/10.3987/REV-21-956.

Peng, Y., Lu, J., Li, R., Zhao, Y., Hai, L., Guo, L., Wu, Y., 2021. Glucose and triphenylphosphonium co-modified redox-sensitive liposomes to synergistically treat glioma with doxorubicin and lonidamine. ACS Appl. Mater. Interfaces 13 (23), 26682–26693. https://doi.org/10.1021/acsami.1c02404.

Qiao, N., Dai, X., Chen, J., Cao, H., Hu, G., Guo, X., Liu, P., Xing, C., Yang, F., 2024. Single nucleus RNA sequencing reveals cellular and molecular responses to vanadium exposure in duck kidneys. J. Hazard. Mater. 480, 136492. https://doi.org/10.1016/j.jhazmat.2024.136492.

Sharma, D., Singh, M., Joshi, J., Garg, M., Chaudhary, V., Blankenberg, D., Chandna, S., Kumar, V., Rani, R., 2023. Design and Synthesis of thiazole scaffold-based small molecules as anticancer agents targeting the human lactate dehydrogenase A enzyme. ACS Omega 8 (20), 17552–17562. https://doi.org/10.1021/acsomega.2c07569.

Synthesis and Biological Evaluation of Novel Thiazole Analogs with Both Anti-Proliferative and Mechanistic Analyses and Molecular Docking Studies—Bimoussa—2022—ChemistrySelect—Wiley Online Library. (n.d.). Retrieved February 8, 2025, from ⟨https://chemistry-europe.onlinelibrary.wiley.com/doi/abs/10.1002/slct.202104270⟩.

Trisciuoglio, D., Ragazzoni, Y., Pelosi, A., Desideri, M., Carradori, S., Gabellini, C., Maresca, G., Nescatelli, R., Secci, D., Bolasco, A., Bizzarri, B., Cavaliere, C., D'Agnano, I., Filetici, P., Ricci-Vitiani, L., Rizzo, M.G., Del Bufalo, D., 2012. CPTH6, a thiazole derivative, induces histone hypoacetylation and apoptosis in human leukemia cells. Clin. Cancer Res.: Off. J. Am. Assoc. Cancer Res. 18 (2), 475–486. https://doi.org/10.1158/1078-0432.CCR-11-0579.

Wolczyk, D., Zaremba-Czogalla, M., Hryniewicz-Jankowska, A., Tabola, R., Grabowski, K., Sikorski, A.F., Augoff, K., 2016. TNF-α promotes breast cancer cell migration and enhances the concentration of membrane-associated proteases in lipid rafts. Cell. Oncol. (Dordr., Neth.) 39 (4), 353–363. https://doi.org/10.1007/s13402-016-0280-x.

Zhang, S., Croppi, G., Hu, H., Li, Y., Zhu, C., Wu, F., Zhang, F., Li, Z., 2022. Bacillamide F, extracted from marine bacillus atrophaeus c89, preliminary effects on leukemia cell lines. Biology 11 (12), 1712. https://doi.org/10.3390/biology11121712.

Zhao, X., Zhang, E., Ren, X., Bai, X., Wang, D., Bai, L., Luo, D., Guo, Z., Wang, Q., Yang, J., 2020. Edaravone alleviates cell apoptosis and mitochondrial injury in ischemia-reperfusion-induced kidney injury via the JAK/STAT pathway. Biol. Res. 53 (1), 28. https://doi.org/10.1186/s40659-020-00297-0.

Zheng, S., Zhong, Q., Jiang, Q., Mottamal, M., Zhang, Q., Zhu, N., Burow, M.E., Worthylake, R.A., Wang, G., 2013. Discovery of a series of thiazole derivatives as novel inhibitors of metastatic cancer cell migration and invasion. ACS Med. Chem. Lett. 4 (2), 191–196. https://doi.org/10.1021/ml300322n.