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APOPTOTIC CELL DEATH VIA MAPK SIGNALLING PATHWAY INDUCED BY TRIPHENYLTIN(IV) DITHIOCARBAMATE COMPOUNDS IN CHILDHOOD B-ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) CELL LINES, REH

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ABSTRACT

Childhood acute lymphoblastic leukemia (ALL) remains the most frequently diagnosed paediatric blood cancer, yet current chemotherapy drugs are constrained by severe toxicities, including neurotoxic effects. This study evaluated two novels organotin(IV) dithiocarbamate derivatives: triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM) and triphenyltin(IV) *N*-ethylbenzyldithiocarbamate (TPNE) for anti-leukemia activity against B-ALL (Reh) cells and non-cancerous WIL2-NS cells. MTT assays revealed potent cytotoxicity (IC_{50}: TPNM 0.18 ± 0.04 µM; TPNE 0.14 ± 0.01 µM) with selectivity indices >2, indicating selective targeting of leukaemia cells. Annexin V-FITC/PI flow cytometry revealed apoptosis rates above 50-70% in treated Reh cells ($p < 0.05$). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant. ELISA assays were used to measure the expression of proteins involved in apoptosis and MAPK signalling pathways. Protein expression analysis indicated activation of the intrinsic apoptotic pathway, evidenced by higher caspase-9 activation relative to caspase-8, upregulation of the pro-apoptotic protein Bax, and downregulation of the anti-apoptotic protein Bcl-2. Furthermore, both compounds significantly activated the JNK and p38 MAPK signalling pathways, implicating oxidative stress-mediated MAPK activation in their cytotoxic mechanism ($p < 0.05$). These findings suggest that TPNM and TPNE exhibit strong potential as anti-leukaemia candidates for childhood B-ALL by inducing cell death through intrinsic apoptosis via MAPK signalling pathway activation.

Keywords: Triphenyltin(IV) dithiocarbamate, Childhood B-leukaemia, Apoptosis, Intrinsic pathway, MAPK signalling pathway

1. INTRODUCTION

Childhood acute lymphoblastic leukaemia (ALL) is the most prevalent pediatric malignancy globally, characterised by the abnormal proliferation of lymphoid progenitor cells. Among its subtypes, B-cell acute lymphoblastic leukaemia (B-ALL) represents most cases. While advancements in multi-agent chemotherapy regimens have significantly improved the five-year survival rates to approximately 90%, the clinical management of B-ALL remains a challenge (Munir et al. 2023). Approximately 15-20% of patients experience disease relapse, which is often associated with a poorer diagnosis and resistance to conventional therapies. Chemotherapy drugs remain the primary treatment for cancer, as chemotherapeutic agents can enter the bloodstream and reach the entire body. These agents act by killing cancer cells through specific mechanisms of action. Furthermore, standard treatment involving vincristine or anthracyclines is frequently limited by severe systemic toxicities and long-term adverse effects, including neurotoxicity and cardiotoxicity, highlighting an urgent need for more selective and less toxic therapeutic alternatives.

In recent years, synthetic metal-based anticancer agents have emerged as promising candidates in medicinal chemistry due to their diverse structural geometries and potent biological activities. Among these, organotin(IV) compounds have gained significant attention for their remarkable anti-proliferative and pro-apoptotic properties against various human cancer cell lines (Abdul Razak et al. 2025; Rasli et al. 2023; Waseem et al. 2022; Haezam et al. 2021) Organotin(IV) refers to a class of organometallic compounds in which a tin atom is covalently bonded to hydrocarbon groups, conferring unique chemical properties that may be exploited for anticancer activity (Abdul Razak et al., 2025). This metal has been studied and found to exhibit solubility in biological systems through the loss of electrons to form positively charged species (Adeyemi et al., 2018). The biological efficacy of organotin(IV) derivatives is often attributed to their ability to interact with cellular targets, including mitochondrial membranes and DNA. Specifically, the incorporation of dithiocarbamate ligands into the organotin framework has been shown to stabilise the metal centre and enhance its lipophilicity, potentially improving cellular uptake and selectivity toward malignant cells compared to normal cells.

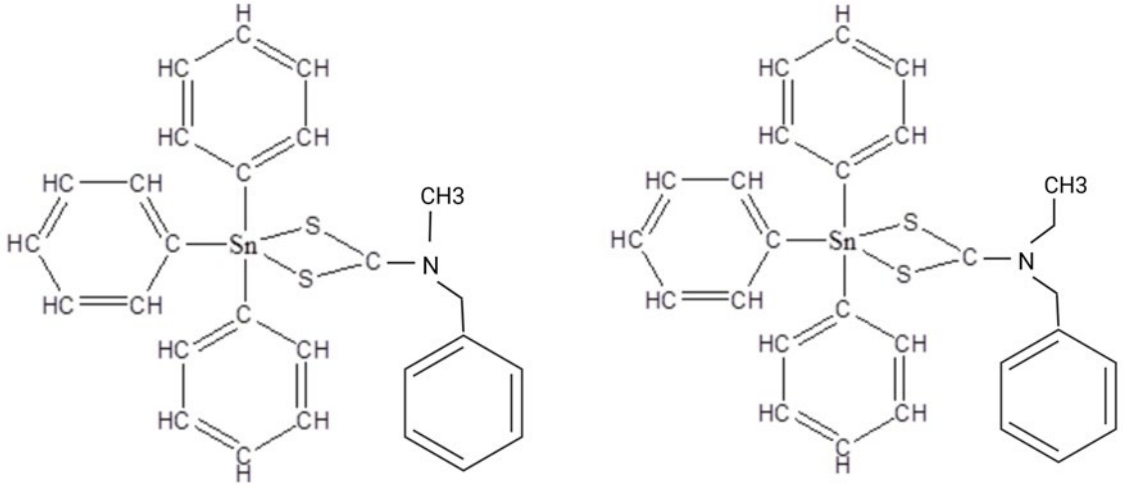
Numerous previous studies have shown that triorganotin compounds possess potent cytotoxic activity against cancer cells. However, yet their molecular mechanisms remain poorly defined, particularly in leukaemia. Moreover, their roles in modulating the cell death pathway have not been fully elucidated. This study synthesised two novel triphenyltin(IV) dithiocarbamate derivatives: triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM) and triphenyltin(IV) *N*-ethylbenzyldithiocarbamate (TPNE) and evaluated their cytotoxicity against a human pre-B acute lymphoblastic leukaemia (Reh) cell line, with selectivity assessed with non-cancerous WIL2-NS cells. We also investigated their mechanisms of action, including apoptosis induction and activation of the mitogen-activated protein kinase (MAPK) signalling pathway. This comprehensive evaluation aims to elucidate the anti-leukaemia potential of both triphenyltin(IV) compounds to clarify their molecular mechanisms of action of cell death pathways and provide insight into their therapeutic relevance as safer and more targeted treatment options for childhood B-ALL.

2. METHODS

2.1 Synthesis of Triphenyltin(IV) Dithiocarbamate Compounds

Both triphenyltin(IV) dithiocarbamate compounds, namely triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM) and triphenyltin(IV) *N*-ethylbenzyldithiocarbamate (TPNE), were newly-synthesised and characterised at the Chemistry Laboratory, Faculty of Health Sciences, Universiti

Kebangsaan Malaysia, following established procedures. The chemical structures are presented in **Figure 1.**



Triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM)

Triphenyltin(IV) *N*-ethylbenzyldithiocarbamate (TPNE)

Figure 1. Chemical structure of triphenyltin(IV) dithiocarbamate compounds.

2.2 Stock Preparation

Both triphenyltin(IV) dithiocarbamate compounds were prepared at a concentration of 10 mM in 1mL dimethyl sulfoxide (DMSO) (ChemAR®) and stored at -20 °C until use. Prior to each experiment, fresh working solutions were prepared by serial dilution in RPMI-1640 medium to achieve final concentrations of 5 µM, 2.5 µM, 1.25 µM, 0.63 µM, 0.32 µM, and 0.16 µM. Vincristine (VCR) was included as a positive control to validate cytotoxic responses.

2.3 Cell Culture

The human pre-B acute lymphoblastic leukaemia cell line Reh (CRL-8286™) and the non-cancerous human B lymphoblast cell line WIL2-NS (CRL-8155™) were obtained from the American Type Culture Collection (ATCC). Both cell lines, which grow in suspension, were cultured in Roswell Park Memorial Institute Medium 1640 (RPMI-1640) (Capricorn Scientific, Germany) (Cat# RPMI-A) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Capricorn Scientific, Germany) (Cat# FBS-16A) and 1% penicillin-streptomycin solution to prepare a complete growth medium. Cultures were maintained in a humidified incubator at 37 °C with 5% CO_2 and routinely examined under an inverted microscope to monitor morphology and viability. Subculturing was performed every 2–3 days to maintain a density of 2×10^5 to 1×10^6 cells/mL. Prior to experimentation, cell viability was confirmed to be greater than 80% using the trypan blue exclusion method. All assays were conducted using cells at passages 3–10 to minimise variability. The WIL2-NS cell line served as a normal control to evaluate the selectivity of the compounds towards malignant versus non-malignant cells.

2.4 Cytotoxicity Study (MTT Assay)

Both Reh and WIL2-NS cells were seeded at a density of 5 × 10^5 cells/mL in 96-well plates. Cells were exposed to a series of TPNM and TPNE concentrations (5, 2.5, 1.25, 0.63, 0.32, and 0.16 µM) and incubated for 24 hours at 37 °C in a humidified 5% CO_2 incubator. Vincristine (VCR) was used as a positive control, while untreated cells served as a negative control. Both compounds were dissolved in DMSO to prepare stock solutions, which were then diluted in RPMI-1640 medium. To ensure that the observed cytotoxic effects were solely due to the compounds and not to the solvent, the final DMSO concentration in all treatment groups was kept below 0.1% (v/v). Previous studies have demonstrated that DMSO at concentrations ≤ 0.1% does not interfere with cell growth or metabolism.

Following incubation, 20 µL of MTT (Merck, Germany) (Cat# US1475989-1GM) solution was added to each well, and the plate was incubated for an additional 4 hours, protected from light. During this period, metabolically active cells reduced MTT to insoluble formazan crystals. The medium was then carefully aspirated, and 200 µL of DMSO was added to dissolve the crystals. After 15 minutes of incubation, absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed relative to untreated controls, and IC_{50} values were calculated from dose–response curves generated using GraphPad Prism 10.0.

2.5 Selectivity Index (SI)

The selectivity index (SI) was used to assess compound selectivity, calculated as the ratio of the IC_{50} in WIL2-NS cells to that in Reh cells. An SI > 2 was considered indicative of selective cytotoxicity toward cancer cells. WIL2-NS was chosen as the normal comparator because of its lineage similarity to Reh, providing biologically relevant comparisons among lymphocyte-derived cells. The calculation is done with a formula as shown below:

$$SI = \frac{IC50\ WIL2 - NS}{IC50\ Reh}$$

2.6 Mode of Cell Death (Annexin V-FITC/PI Assay)

Apoptosis and necrosis were evaluated using the Annexin V-FITC/PI detection kit (Cat# MAB556547). Reh cells (5 × 10^5/mL) were seeded in 6-well plates and treated with TPNM or TPNE at IC_{50} concentrations. Vincristine (VCR) and untreated cells served as positive and negative controls, respectively. After 24 h, cells were collected, washed with PBS, and resuspended in 1× binding buffer. Cells were stained with 5 µL Annexin V-FITC for 15 min in the dark, followed by 5 µL PI for 5 min. Samples were diluted with 400 µL binding buffer and analyzed on a BD FACS Canto II flow cytometer within 1 hour. For each sample, a minimum of 10,000 events were recorded. The gating strategy involved an initial gate on a forward scatter (FSC) versus side scatter (SSC) plot to exclude cell debris and doublets, focusing on the viable cell population. The quadrants were set based on the untreated control to distinguish between viable (Annexin V−/PI−), early apoptotic (Annexin V+/PI−), late apoptotic (Annexin V+/PI+), and necrotic (Annexin V−/PI+) cells.

2.7 Protein Expression Analysis (ELISA)

Reagents Section:

Human ELISA kits for Caspase-8 (Cat# E4991Hu), Caspase-9 (Cat# E4994Hu), BAX (Cat# E4977Hu), Bcl-2 (Cat# E1832Hu), JNK/SAPK (Cat# E3409Hu), and p38 MAPK (Cat# E7590Hu) were purchased from BT Lab (Shanghai, China).

Procedure Section:

To quantify proteins involved in apoptosis and MAPK signalling, ELISA was performed according to the manufacturer's instructions. Reh cell lysates from TPNM- and TPNE-treated groups were prepared, and total protein concentration was determined by BCA assay. Each sample was first standardized to 0.7 mg/mL, and a 1:20 dilution was used for ELISA. Briefly, 50 µL of standard and 40 µL of sample were added to wells, followed by 10 µL of anti-protein antibody and 50 µL streptavidin-HRP (except blanks). Plates were incubated at 37 °C for 60 min, washed five times, and developed with 50 µL of substrates A and B for 10 min in the dark. Reactions were stopped with 50 µL stop solution, and absorbance was measured at 450 nm. Protein levels were analysed using MyAssays software. Results were normalised to the untreated control and expressed as relative fold change to facilitate comparison between signalling markers.

2.8 Statistical Analysis

All experimental data are presented as mean ± standard error of the mean (SEM) from three independent experiments (n = 3). Statistical assumptions for parametric analysis were verified prior to hypothesis testing; the normality of data distribution was confirmed using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variances was assessed using Brown-Forsythe and Bartlett's tests. Statistical comparisons between treatment groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to identify significant differences among groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. All statistical analyses were conducted using GraphPad Prism version 10.0 (GraphPad Software, USA).

3. RESULTS

3.1 Cytotoxic Effects and Selectivity Index

The MTT assay was employed as a preliminary screening to assess the cytotoxicity of TPNM and TPNE in Reh and WIL2-NS cell lines, with vincristine (VCR) as a positive control. This colorimetric assay quantifies cell viability based on the reduction of MTT by mitochondrial dehydrogenases in metabolically active cells, where decreased absorbance corresponds to reduced viability. Cells were treated with the compounds for 24 h, and IC_{50} values were determined from dose–response curves generated by plotting percentage viability against compound concentration.

As shown in **Figure 2**, both compounds exhibited potent cytotoxicity against Reh cells. The IC_{50} of TPNM was 0.18 ± 0.04 µM, while TPNE demonstrated slightly greater activity with an IC_{50} of 0.14 ± 0.02 µM. These values were significantly lower ($p < 0.05$) than those of vincristine (VCR; 0.41 ± 0.08 µM), indicating that both triphenyltin(IV) dithiocarbamate derivatives were more effective than the standard chemotherapy drug in inhibiting Reh cell proliferation. In contrast, cytotoxicity was less pronounced in the non-cancerous WIL2-NS cell line, with IC_{50} values of 0.41 ± 0.09 µM for TPNM, 0.36 ± 0.30 µM for TPNE, and 0.52 ± 0.50 µM for VCR. A summary of IC_{50} values for both compounds and controls in Reh and WIL2-NS cells is presented in Table 1.

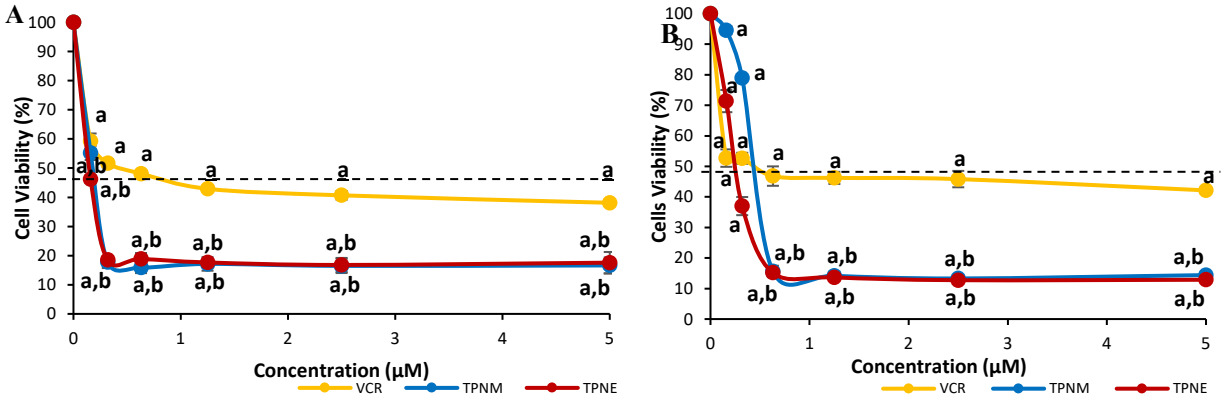


Figure 2. Dose-Response Curves Showing IC_{50} Values of Triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM), Triphenyltin(IV) *N*-ethylbenzyldithiocarbamate (TPNE) and Vincristine (VCR) in (A) Reh and (B) WIL2-NS Cell Lines.

Results are presented as mean ± SEM from three independent replicates (n = 3). The curves demonstrate the cytotoxicity profiles of each compound after 24 hours of treatment. [a]Significance differences compared to negative control ($p<0.05$), [a,b]Significance differences compared to vincristine (VCR) ($p<0.05$)

The Selectivity Index (SI) was used to evaluate the selectivity of TPNM and TPNE between cancerous and non-cancerous cells. Compounds with SI values greater than 2 are considered to exhibit selective anticancer activity. As shown in Table 1, both compounds achieved SI values above this threshold, indicating preferential selectivity toward Reh cells over WIL2-NS cells. These results suggest that TPNM and TPNE possess promising selectivity and may offer safer therapeutic potential compared to conventional treatment.

Table 1. Summary of IC_{50} and Selectivity Index (SI) Values of Triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM), Triphenyltin(IV) *N*-ethylbenzyldithiocarbamate (TPNE) and Vincristine (VCR) in Reh and WIL2-NS Cell Lines.

Compounds	IC_{50} values (µM)		SI values
	Reh	WIL2-NS	
TPNM	0.18 ± 0.04	0.41 ± 0.09	2.28
TPNE	0.14 ± 0.01	0.36 ± 0.30	2.57
VCR (positive control)	0.41 ± 0.10	0.59 ± 0.50	1.44

3.2 Mode of Cell Death

To clarify the mechanism of cytotoxicity, the mode of cell death induced by TPNM and TPNE in Reh cells was assessed using Annexin V-FITC/PI staining. This approach distinguished viable, apoptotic, and necrotic populations, and flow cytometry was used to quantify the effects. **Figure 3 (A)** shows that 24-hour treatment with TPNM, TPNE, and VCR predominantly induced apoptosis in Reh cells, with apoptotic populations of 52.20%, 70.37%, and 59.27%, respectively. Necrosis remained below 1% across all treatments, confirming apoptosis as the primary mode of cell death. **Figure 3 (B)** Morphological examination under an inverted microscope showed that TPNM-, TPNE-, and VCR-treated cells exhibited

hallmark apoptotic features, including shrinkage, membrane blebbing, chromatin condensation, and apoptotic body formation, corroborating the flow cytometry results.

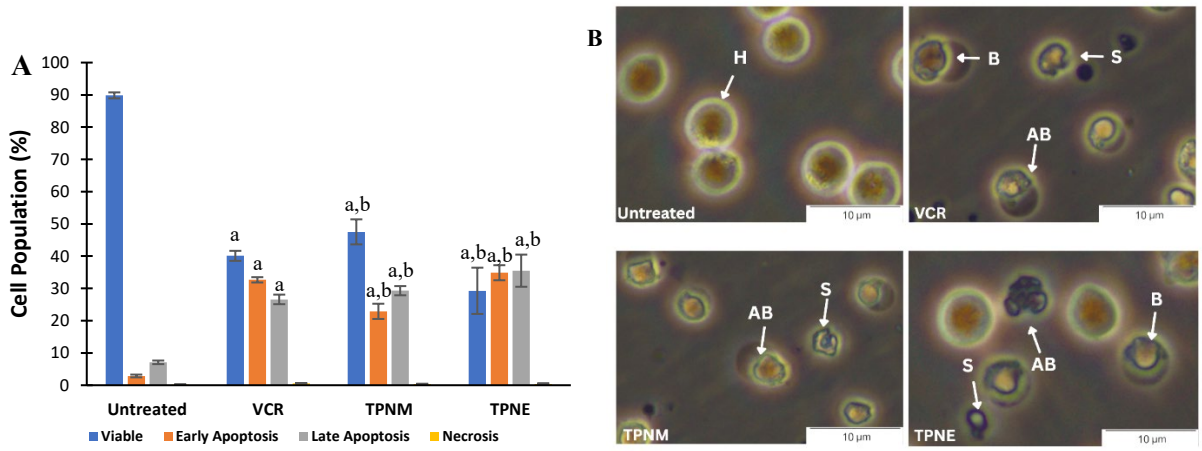


Figure 3. Effects of Triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM), Triphenyltin(IV) N-ethylbenzyldithiocarbamate (TPNE), and Vincristine (VCR) on Reh cells: apoptosis (A) and cell morphology (B) (magnification 400x).

The data show the percentage of cells (%) ± SEM obtained from three individual experiments. Healthy cell (H), membrane blebbing (B), shrinkage cell (S), apoptotic body (AB). [a]Significance differences compared to negative control ($p<0.05$), [a,b]Significance differences compared to vincristine (VCR) ($p<0.05$).

Morphological changes in Reh cells following treatment with TPNM and TPNE were observed using an inverted microscope (Olympus CKX 41) equipped with a digital camera. Images were captured at 400x total magnification. For each sample, multiple fields of view were examined to ensure representative imaging. Scale bars of 10 µM were calibrated and applied to the images using ImageJ.

3.3 Expression of Apoptosis and MAPK Signalling Pathway

3.3.1 Apoptotic Molecular Pathways-Related Proteins (Caspase-8, Caspase-9, Bax, Bcl-2)

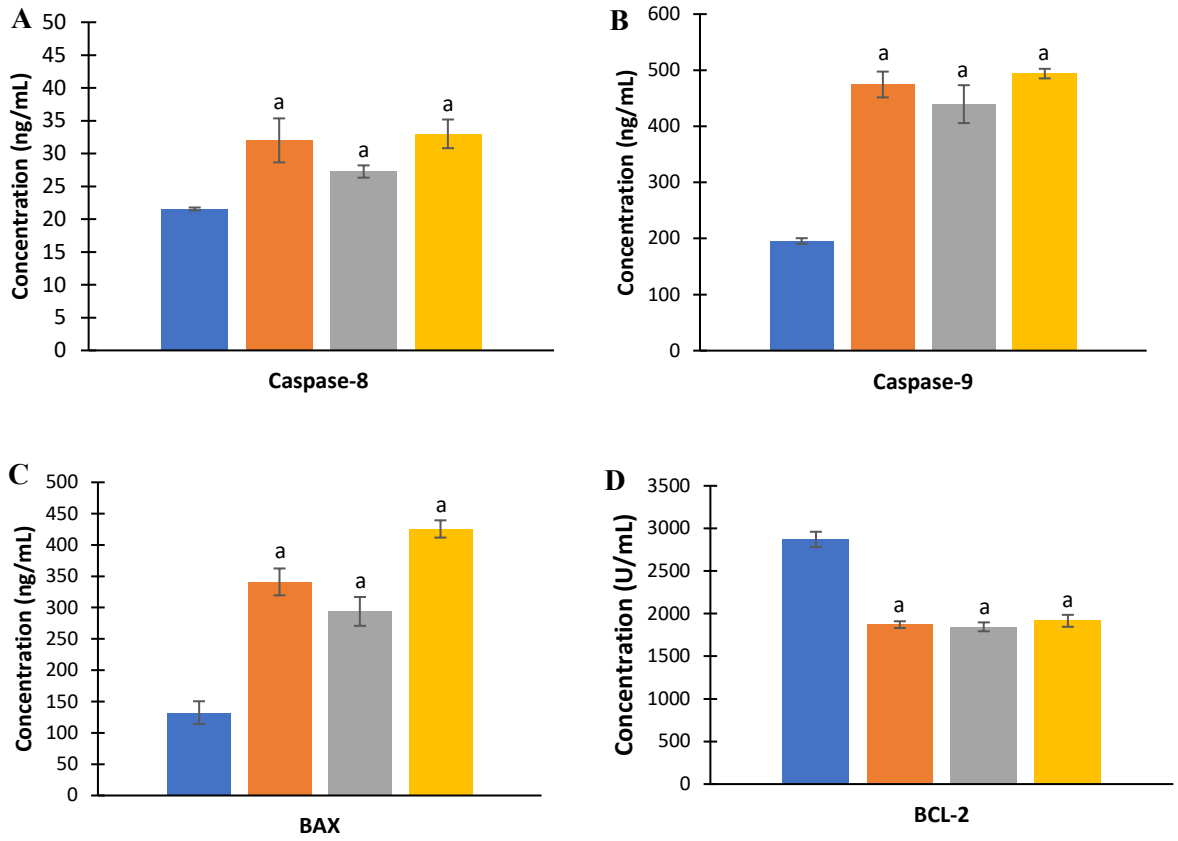
To elucidate the apoptotic mechanism induced by TPNM and TPNE, the levels of key regulatory proteins (Caspase-8, Caspase-9, Bax, and Bcl-2) were quantified using ELISA. Data were normalized to the untreated control and expressed as a relative fold change.

As shown in Figure 4, both compounds significantly modulated the expression of proteins associated with the apoptotic cascade. Treatment with TPNM and TPNE led to an increase in the protein levels of both initiator caspases; however, the induction of caspase-9 was notably higher compared to caspase-8 across all treated groups. While the elevation in caspase-8 suggests some involvement of the extrinsic pathway, the predominance of caspase-9 expression indicated that cell death is primarily mediated through the intrinsic mitochondrial route. This is further supported by the significant upregulation of Bax and the concomitant downregulation of Bcl-2, shifting the cellular balance toward a pro-apoptotic state. While both compounds effectively triggered these changes, TPNE had a more pronounced effect on Caspase-9 expression than TPNM.

3.3.2 MAPK Signalling Pathway (JNK, p38 MAPK)

The MAPK signalling pathway serves as a central regulator of cellular responses to external stimuli and stress. Within this framework, the p38 MAPK and JNK kinases are specifically associated with the induction of programmed cell death in response to cellular damage.

In this study, 24-hour treatment with TPNM and TPNE resulted in a significant increase in the protein levels of both p38 MAPK and JNK in Reh cells (Figure 4(E) and (F)). The upregulation of these signalling molecules suggests that triphenyltin(IV) derivatives induce a state of cellular stress that correlates with the observed pro-apoptotic response. TPNE and the positive control, VCR, elicited higher expression levels of both MAPK proteins compared to TPNM. These results indicate that the MAPK pathway plays an integral role in the anti-leukemic action of these triphenyltin(IV) dithiocarbamate compounds, likely by translating cellular stress signals into the apoptotic cascade.



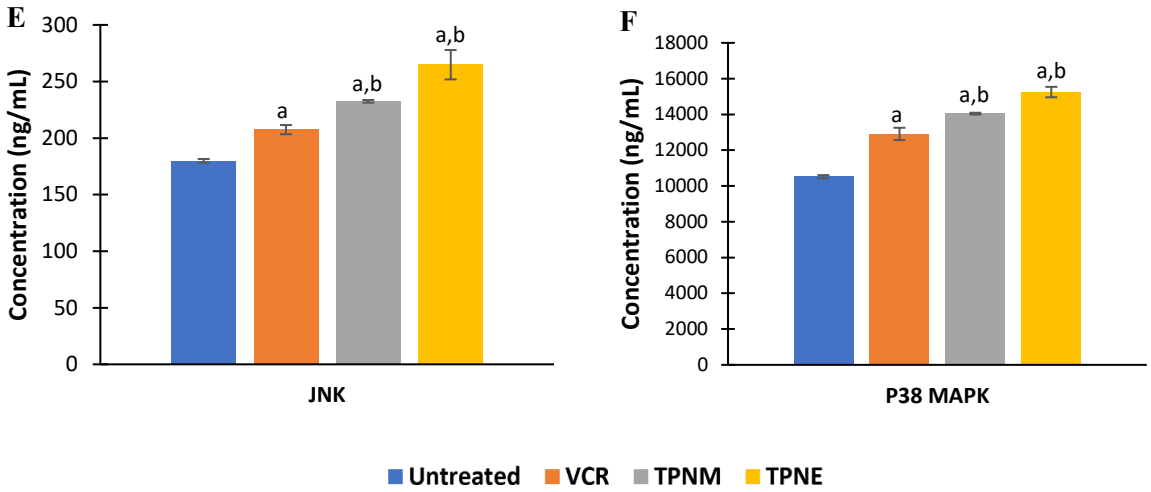


Figure 4. Expression of apoptosis- and MAPK-related proteins in Reh cells after treatment (24H) with Triphenyltin(IV) *N*-methylbenzyldithiocarbamate (TPNM), Triphenyltin(IV) *N*-ethylbenzyldithiocarbamate (TPNE) and Vincristine (VCR): Caspase-8 (A), Caspase-9 (B), Bax (C), Bcl-2 (D), JNK (E), and p38 MAPK (F).

The data show the percentage of cells (%) ± SEM obtained from three individual experiments. [a]Significance differences compared to negative control ($p<0.05$), [a,b]Significance differences compared to vincristine (VCR) ($p<0.05$).

The concentration of each protein marker was calculated based on the standard curve generated for each plate using MyAssays software. To account for biological variation and to highlight the magnitude of the response, data were expressed as fold-change relative to the untreated control. Normalization was performed using the formula:

$$Fold\ change\ = \frac{Concentration\ of\ Treated\ Sample}{Mean\ Concentration\ of\ Untreated\ Control}$$

An untreated control value is therefore represented as 1.0 (or 100%).

4. DISCUSSION

The results obtained from the MTT assay, the median IC_{50} values for the compounds TPNM and TPNE were below 2 µM, indicating that both tested compounds can exert potent cytotoxic effects on B-ALL Reh cells. Both tested compounds contain phenyl groups bonded to the tin atom, which facilitates interactions with biological molecules and enhances their activity (Ruan et al. 2011). These phenyl groups also increase the lipophilicity of the organotin structure, enabling the compounds to more readily penetrate biological and cellular systems via the cell membrane (Hussain et al. 2015; Alouch & Whalen, 2005). In addition, the dithiocarbamate ligands improve solubility in organic solvents, further supporting transport across cell membranes (Kamaludin et al. 2017). The two sulphur atoms in the dithiocarbamate ligands confer strong metal-binding properties, allowing them to inhibit enzymes, disrupt biological processes, and contribute to the cytotoxic effects on cells (Onwudiwe & Ajibade, 2012; Mohamad et al. 2016). Together, the phenyl-tin moiety and the dithiocarbamate ligands enable TPNM and TPNE to efficiently cross cell membranes and effectively interfere with the biological environment of leukemia cells. A study by Hamid et al. (2020) demonstrated that triphenyltin(IV) compounds exhibited the

strongest cytotoxic effects on K-562 leukaemia cells compared to dibutyltin(IV) and dimethyltin(IV) compounds. This enhanced activity was attributed to the greater number of functional groups bound to the tin atom in triphenyltin(IV). Additionally, the length of the carbon-tin bonds was suggested to play a role in increasing the compound's cytotoxic potential.

Based on the selectivity index (SI), both triphenyltin(IV) dithiocarbamate compounds, TPNM and TPNE, were found to be more selective toward B-ALL Reh cells, with selectivity index values greater than 2.00. A higher selectivity index indicates a greater preference of the compound for cancer cells (Badisa et al. 2009). Furthermore, a high selectivity index is essential for an effective anticancer agent, allowing maximum effects on cancer cells while minimizing effects on non-cancerous cells (Rashidi et al. 2017). While conventional therapies such as vincristine (VCR) and Daunorubicin remain the backbone of B-ALL treatment, their clinical utility is frequently hampered by dose-limiting neurotoxicity and bone marrow suppression. In this study, TPNM and TPNE exhibited IC_{50} values in the sub-micromolar range (0.18 μM and 0.14 μM, respectively), comparable in potency to VCR. However, a critical advantage observed here is the SI value. While VCR often lacks high specificity between malignant and rapidly dividing healthy cells, TPNM and TPNE demonstrated SI values greater than 2 against non-cancerous WIL2-NS cells. This suggests a more favourable therapeutic potential, reducing the 'off target' effects that lead to the long-term morbidities seen in pediatric survivors.

Next, the compounds evaluated in this study are proposed to induce apoptosis rather than necrosis. Apoptosis is a tightly regulated form of programmed cell death that allows the elimination of damaged or unwanted cells without triggering an inflammatory response. In contrast, necrosis is an uncontrolled mode of cell death that can release intracellular contents into surrounding tissues, leading to inflammation and potential tissue damage (Awang et al. 2022). By promoting apoptosis, these compounds are more likely to achieve targeted cytotoxic effects on leukaemia cells while minimising adverse effects on surrounding healthy tissues. This study showed that both tested compounds may induce apoptosis, with TPNE being the highest percentage of cells that undergo cell death via apoptosis. Organotin compounds are known to induce apoptosis, as both triphenyltin compounds possess phenyl structures and lipophilic properties. Their lipophilicity enhances solubility in lipids, allowing the phenyl groups to effectively interact with phosphatidylserine on cell membranes (Hussain et al. 2015; Arur et al. 2003). Cell death morphology was also observed and induced morphological features of apoptosis in both leukemia cell lines. Observed characteristics included membrane blebbing, cell shrinkage, and formation of apoptotic bodies. According to Elmore (2007), hallmarks of apoptosis include cell shrinkage, formation of apoptotic bodies, membrane blebbing, and condensed cytoplasm.

In this study, the apoptotic cell death pathways activated by TPNM and TPNE were investigated through the measurement of caspase-8 and caspase-9, which are key initiator caspases in the extrinsic and intrinsic apoptosis pathways, respectively. Treatment of Reh cells with these compounds resulted in a significant upregulation of caspase-9, while caspase-8 levels remained relatively low or unchanged. This indicates that the intrinsic mitochondrial pathway is the primary route through which these compounds induce cell death. Activation of caspase-9 triggers a cascade involving the cleavage of downstream executioner caspases such as caspase-3, leading to hallmark apoptotic events including chromatin condensation, membrane blebbing, and formation of apoptotic bodies. The preferential activation of the intrinsic pathway suggests that TPNM and TPNE may induce mitochondrial outer membrane permeabilization, potentially mediated by pro-apoptotic proteins like Bax, and disrupt mitochondrial integrity, thereby amplifying the apoptotic signal and ensuring efficient programmed cell death. Previous studies reported that a novel organotin supramolecular coordination polymer (OSCP) was able to inhibit proliferation and induce apoptosis in human hepatocellular carcinoma (HepG2) cells. This effect was

associated with an increase in the pro-apoptotic protein Bax and a decrease in the anti-apoptotic protein Bcl-2 (Alallah et al., 2017).

Mitogen-activated protein kinase (MAPK) is a critical signalling pathway that mediates various cellular networks involved in cell survival, death, cell cycle progression, and growth. MAPK plays a key role in translating extracellular stimuli into diverse cellular responses, including cell proliferation, differentiation, and apoptosis (Yue et al. 2020). In the present study, treatment with TPNM and TPNE led to a marked increase in JNK and p38 MAPK protein levels in and Reh cells. As integral components of the MAPK signalling framework, these proteins regulate cellular response to stress, DNA damage, and apoptotic stimuli (Feng et al. 2017). While JNK and p38 MAPK are typically activated by upstream initiators such as oxidative stress or inflammatory signals, our findings specifically demonstrate a significant upregulation of these kinases at the protein level. This shift suggests that TPNM and TPNE modulate pro-apoptotic signalling, thereby contributing to the leukemic cell death observed.

This study demonstrated that VCR has higher levels of pro-apoptotic protein expression (such as caspase-8, -9, and Bax) than TPNM. This high potency is coupled with a lower selectivity index (SI < 2) compared to TPNM. This discrepancy highlights the fundamental difference between cytotoxic absolute and therapeutic selectivity. As a tubulin-binding agent, VCR interferes with the mitotic spindle, a mechanism that affects all rapidly dividing cells, including healthy hematopoietic stem cells and neurons, leading to its well-documented clinical toxicities.

Conversely, the triphenyltin(IV) derivative TPNM, which showed slightly lower protein expression than VCR, maintained an SI > 2. This suggests that the dithiocarbamate ligands may facilitate a more selective interaction with leukaemia-specific metabolic or signalling vulnerabilities, such as mitochondrial membrane sensitivity or specific MAPK triggers. For the treatment of childhood B-ALL, a compound that achieves a 'threshold' level of apoptosis in malignant cells while sparing healthy tissue is often more clinically desirable than a highly potent but non-selective agent.

Furthermore, compared to other organotin-dithiocarbamate complexes studied in recent years, our compounds demonstrated a more robust activation of the p38 MAPK pathway, suggesting that both TPNM and TPNE play a crucial role in directing the signalling response toward apoptosis rather than non-specific necrosis.

5. CONCLUSION

Both triphenyltin(IV) dithiocarbamate compounds, TPNM and TPNE, exert potent cytotoxicity and reduce cell proliferation. Both compounds may exert potent anti-leukemic effects by inducing apoptotic cell death through the intrinsic pathway, involving the activation of MAPK-mediated signalling mechanisms. These findings support further investigation of both compounds as promising candidates for childhood B-ALL therapy.

6. LIMITATIONS and FUTURE DIRECTIONS

While TPNM and TPNE demonstrate promising anti-leukemic activity, several limitations concern their acknowledgement. This study was conducted exclusively *in vitro* using Reh cell line, thus it does not account for the complex pharmacokinetic and systemic toxicities present in *in vivo* study. Mechanistically, while apoptosis was confirmed through Annexin V-FITC/PI and the upregulation of initiator Caspase-8 and -9, the downstream executioner phase involving Caspase-3 and PARP cleavage was not directly quantified. Furthermore, although MAPK pathway modulation (p38 and JNK) indicates a cellular stress response, specific upstream

triggers such as Reactive Oxygen Species (ROS) production and mitochondrial membrane potential (Δψm) remain to be elucidated. Future research utilizing *in vivo* models and high-resolution assays will be essential to define the precise molecular targets and therapeutic safety of these triphenyltin(IV) derivatives.

7. ACKNOWLEDGEMENTS

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8. FUNDING

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9. AUTHOR'S CONTRIBUTION

Conceptualization: Hamid, A. – Developed the research idea and designed the study.

Methodology: Hamid, A., Abdul Razak, NH. – Designed experiments.

Investigation: Abdul Razak, NH. – Performed the experiments and collected the data.

Data Analysis: Abdul Razak, NH. – Analyzed and interpreted the experimental results.

Writing – Original Draft: Abdul Razak, NH. – Wrote the initial manuscript draft.

Visualization: Abdul Razak, NH. – Prepared figures and tables for the manuscript.

Supervision: Hamid, A., Awang, N., Kamaludin, NF. – Provided oversight and guidance throughout the project.

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All authors actively contributed to providing critical feedback, shaping the research, guiding the analysis, and refining the manuscript.

10. CONFLICT OF INTEREST DECLARATION

We affirm that there is no Conflict of Interest among the author(s) concerning the subject matter or materials discussed in this manuscript. We further certify that the article represents the original work of the Authors and Co-Authors. The manuscript has not been previously published and is not currently under consideration for publication elsewhere. This research/manuscript has neither been submitted for publication nor published in whole or in part elsewhere. We attest that all authors have made significant contributions to the work, ensuring the validity and legitimacy of the data and its interpretation, thereby warranting its submission to the Malaysian Journal of Clinical Biochemistry.

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