Sains Malaysiana 52(5)(2023): 1513-1521
http://doi.org/10.17576/jsm-2023-5205-14

Diorganotin(IV) *N*-methyl-*N*-phenethyldithiocarbamate Compounds Induce Cytotoxicity via Apoptosis in K562 Human Erythroleukaemia Cells

(Sebatian Diorganostanum(IV) *N*-metil-*N*-fenetilditiokarbamat Mengaruh Sitotoksisiti melalui Apoptosis terhadap Sel Eitroleukemia Manusia, K562)

SHARIFAH NADHIRA SYED ANNUAR, NURUL FARAHANA KAMALUDIN[1,*], NORMAH AWANG[1], KOK MENG CHAN[1] & NORRAPHAT UTTRAPHAN PIM[1]

Center for Toxicology and Health Risk Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Received: 20 September 2022/Accepted: 11 April 2023

ABSTRACT

Imatinib mesylate (IM), a leading treatment for chronic myeloid leukaemia (CML), has sparked worries about the possibility of CML patients developing a resistance to it. As a result, researchers are becoming more interested in organotin(IV) compounds due to their strong potential to be developed as anticancer agents and employed as an option to address the issues regarding IM-resistance therapy. Generally, this study is to determine the cytotoxicity induced by diorganotin(IV) dithiocarbamate compounds in K562 human erythroleukaemia cells. The two novel diorganotin(IV) compounds namely diphenyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate **(C1)** and dibutyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate **(C2)** were assessed their cytotoxicity via MTT [3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and mode of cell death via Annexin V-FITC/PI assay with the duration treatment of 24 h. Both compounds displayed strong cytotoxicity in K562 cells. At concentration of 4.2 µM for **C1** and 1.6 µM for **C2**, both compounds were able to induce 49.70% and 46.83% apoptotic events, respectively. The changes in cells' morphological can also be seen 24 h after being exposed to the compounds at their respective IC_{50} doses. The findings demonstrated that the morphology of the cells was similar to apoptotic features, including cell shrinkage and the production of apoptotic bodies, meanwhile, the low levels of necrotic cells (<1%) also can be seen via cell lysis. In conclusion, both compounds possess the potential as antileukaemia drugs nevertheless, further studies on their action mechanism are required to ratify their qualities and suitability in the research of anticancer drugs development.

Keywords: Cancer; carbamate; cell death; organotin(IV); toxicity

ABSTRAK

Imatinib mesylate (IM) yang merupakan rawatan utama bagi Leukemia Mieloid Kronik (CML) telah mendatangkan kebimbangan kerana pesakit CML berkemungkinan menunjukkan kesan rintangan terhadap penggunaan IM. Oleh yang demikian, sebatian organostanum(IV) telah menarik minat para penyelidik kerana berpotensi tinggi untuk dibangunkan sebagai agen antikanser dan alternatif bagi menangani isu rintangan terhadap IM. Secara amnya, kajian ini adalah untuk menentukan kesitotoksikan aruhan sebatian diorganostanum(IV) ditiokarbamat terhadap sel K562 eritroleukemia manusia. Dua sebatian baharu diorganostanum(IV) iaitu difenilstanum(IV) *N*-metil-*N*-fenetilditiokarbamat (**C1**) dan dibutilstanum(IV) *N*-metil-*N*-fenetilditiokarbamat (**C2**) telah dinilai kesan ketoksikannya melalui asai 3-(4-5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromida (MTT) dan mod kematian sel melalui asai Annexin V-FITC/PI dalam tempoh 24 jam rawatan. Kedua-dua sebatian menunjukkan kesan sitotoksisiti yang kuat terhadap sel K562. Pada kepekatan 4.2 µM bagi **C1** dan 1.6 µM bagi **C2**, masing-masing mampu mengaruh 49.70% dan 46.83% sel apoptotik. Malahan, perubahan pada morfologi sel yang menunjukkan ciri-ciri apoptosis antaranya termasuk pengecutan sel dan pembentukan jasad apoptotik, juga dapat dilihat selepas 24 jam rawatan mengikut dos IC_{50} sementara itu, tahap sel nekrotik yang rendah (<1%) dapat dilihat melalui lisis sel. Kesimpulannya, kedua-dua sebatian berpotensi untuk dibangunkan sebagai dadah antileukemia, namun kajian lanjutan berkaitan mekanisme tindakannya adalah perlu bagi mengesahkan kualiti dan tahap kesesuaiannya dalam penyelidikan pembangunan dadah antikanser.

Kata kunci: Kanser; karbamat; kematian sel; ketoksikan; organostanum(IV)

INTRODUCTION

Cancer can be described as the uncontrolled growth and spread of abnormal cells, which is a major public health issue (Demir & Değim 2018). Chronic myeloid leukaemia (CML) occurs when Philadelphia (Ph) chromosome is formed. This takes place when the long arms of chromosome 9 and 22 break off and trade places (Hochhaus et al. 2009). In depth, Ph chromosome emerged when a piece of chromosome 9 attaches on chromosome 22 forming BCR-ABL gene (National Cancer Institute 2021). These CML cells (K562) increase its growth which accumulated in bone marrow and blood (Martinez-Castillo et al. 2016).

Apart from that, imatinib mesylate (IM), the native inhibitor of BCR-ABL (nonreceptor) tyrosine kinase (Demir & Değim 2018) has been used as front-line treatment of CML patients since 1998. However, IM treatment is associated with mild to moderate toxic effects where reverse reaction occurs when the dose is reduced or treatment is stopped (Mughal & Schrieber 2010). The first clinical metal-based drug called cisplatin that was introduced in 1965 by Rosenberg has developed side effects towards cancer patients. Hence, this raised attention to the development of non-platinum metal and organometallic compounds as anticancer agents (Alama et al. 2009). Organometallic compounds have been widely studied because of their potential as an antitumor agent. Among these organometallic compounds, organotin(IV) compounds exerted an interesting antitumor property (Gómez, Contreras-Ordoñez & Ramírez-Apan 2006).

Organotin(IV) derivatives are widely synthesized and its potential as anticancer agents are extensively being studied (Annuar et al. 2021; Awang et al. 2016). Besides, the cytotoxic effects of organotin(IV) compounds have been explored in diverse human cell lines and animal origins. (Kamaludin et al. 2017). Generally, the majority of organotin(IV) compounds are extremely toxic even at low concentrations, and they display considerable biological action. Additionally, the existence of at least one covalent C-Sn bond distinguishes organotin(IV) compounds from other types (Pellerito et al. 2006). In this study, the cytotoxic potency of the two diorganotin(IV) dithiocarbamate compounds which are diphenyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate [$C_{32}H_{36}N_2S_4Sn$] (**C1**) and dibutyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate [$C_{28}H_{42}N_2S_4Sn$], (**C2**) against human leukemic cell line namely K562 cells were assessed. Figures 1 and 2 depict the chemical structures of both compounds. **C1** consists of two phenyl groups bond to central tin atom, whereas **C2** consists of two butyl groups that are also bond to central tin atom. Both compounds possess the same ligand, which is *N*-methyl-*N*-phenethyl dithiocarbamate with semi-double bond between carbon and nitrogen, or known as thioreide bond.

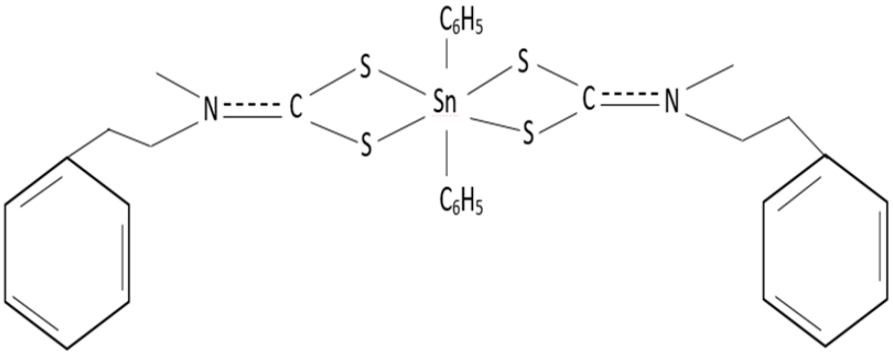


FIGURE 1. Chemical structure of **C1**

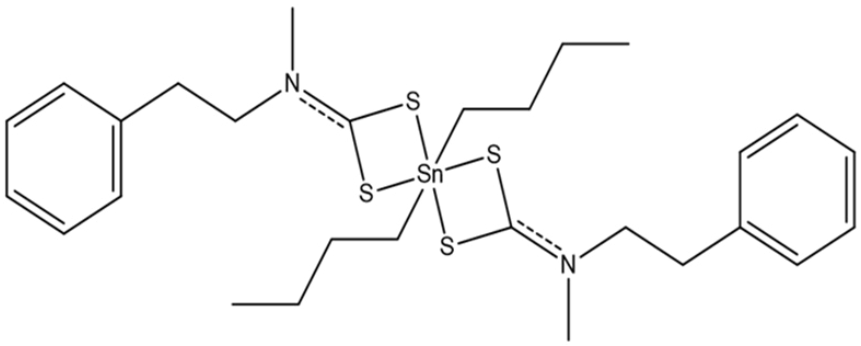


FIGURE 2. Chemical structure of **C2** (Pim et al. 2018)

Materials and Methods

Research compounds

Diorganotin(IV) dithiocarbamate compounds, which are diphenyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate (**C1**) and dibutyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate (**C2**) were synthesized as previously described by Pim et al. (2018).

Cell culture and reagents

Human leukemic cell line, K562 cells was purchased from the American Type Culture Collection (ATCC) and cultured at 37 °C in a humidified atmosphere with 5% CO_2. The K562 cells were maintained in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with L-glutamine, sodium bicarbonate ($NaHCO_3$), 1% penicillin and streptomycin and 10% of Fetal Bovine Serum (FBS) (GIBCO, USA).

MTT cytotoxicity assay

The viability of K562 cells was determined using MTT assay (Mosmann 1983) with slight modifications. Dimethyl sulphoxide (DMSO) was used to dissolve both **C1** and **C2**. Following treatment, both compounds were diluted in culture media before being induced into the cells (5×10^5 cells mL^{-1}) at various doses (0.16, 0.31, 0.63, 1.25, 2.50, 5.00 and 10.00 µM) for 24 h. Next, each well was added with 20 µL of a 5 mg mL^{-1} MTT solution before a 4 h incubation period. Approximately, 180 µL media in each well was removed, followed by, the addition of 180 µL of DMSO to dissolve the crystal formazan, and further incubated for 15 min. By using an ELISA Microplate Reader, the optical density (OD) of each well was measured at a wavelength of 570 nm (Labsystem Multiscan Multisoft, Finland). The inhibitory concentration, or IC_{50}, was determined and used as a metric to compare the relative cytotoxicity of each compound. The inhibitory concentration is the concentration that inhibits 50% growth of a cell population (Thati et al. 2007).

Annexin V-FITC/propidium iodide labelling

The mode of cell death in K562 cells was determined via Annexin V-FITC/PI (Leong et al. 2016). A sterile 6-well microplate was used to seed the cells at a density of 1×10^6 cells mL^{-1}. Then, both compounds were used to treat the cells at IC_{50} concentrations (cells density 5×10^5 cells mL^{-1}) and further incubated for 24 h. 200 µL of compounds-treated K562 cells were harvested and collected into a microcentrifuge tube. The cells were centrifuged at 220 x g for 5 min, 4 °C. The supernatant was removed. The pellet was washed with chilled PBS and centrifuged at 220 x g for 5 min, 4 °C. The pellet was then resuspended in 150 µL of Annexin V Binding Buffer followed by mixing the cell suspension with 5 µL of Annexin V-FITC (Sigma Aldrich, USA) for 13 min at room temperature. Subsequently, 10 µL of Propidium Iodide (PI) (50 µg mL^{-1}) was added into the cells and further incubated for 2 min. The entire staining procedure was done in a dark environment. Further, 350 µL of Annexin V binding buffer was added prior to analysis with FACSCanto II flow cytometry (Becton Dickinson, USA).

Morphological changes

The cells' morphology of 24 h-treated cells using both compounds at their respective IC_{50} doses were scrutinised using a 40× inverted light microscope.

Statistical analysis

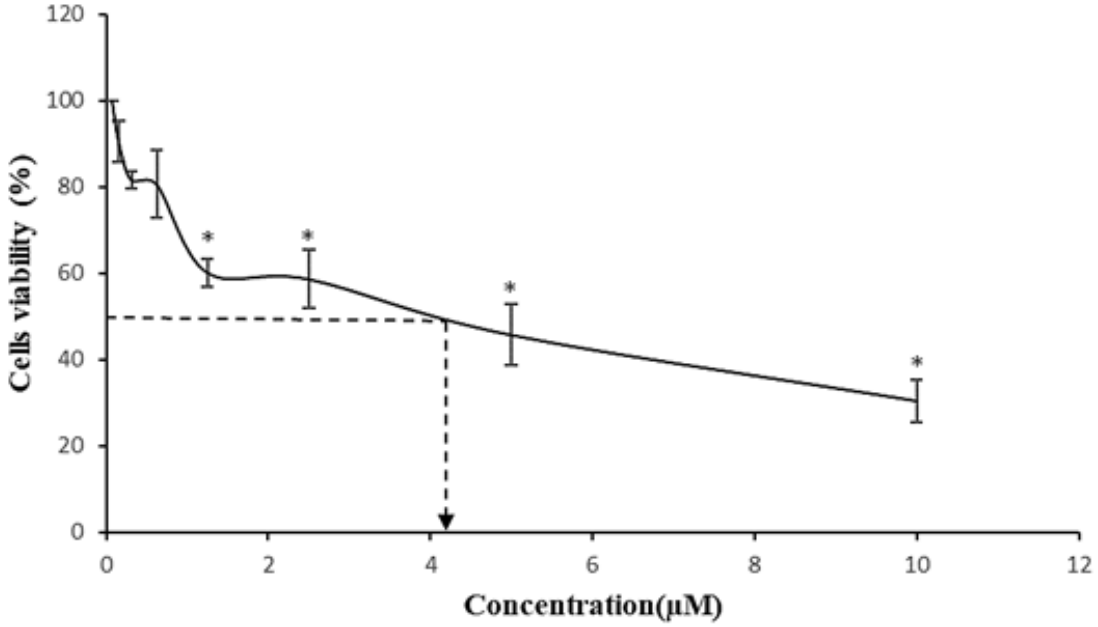
The data were expressed as the mean ± standard error of mean (SEM). Statistical Package for Social Sciences (SPSS) version 23.0 was used to perform a one-way ANOVA statistical analysis of the untreated and treated cells. From the analysis, the data were considered statistically significance when compared to the untreated cells (negative control) when $p<0.05$.

Results and Discussion

The half-maximal inhibitory concentration, IC_{50} was measured to determine the cytotoxicity concentration for testing the behavior of a potential therapeutic drug (Kathiresan & Ramakrishnan 2021). The graph of cytotoxic effect against K562 cells after 24 h treatment using both compounds are presented in Figures 3 and 4, respectively. Based on the graph, both compounds effectively reducing the viability of K562 cells. The IC_{50} values of **C1**, **C2** and positive control (menadione) against K562 cells were tabulated in Table 1.

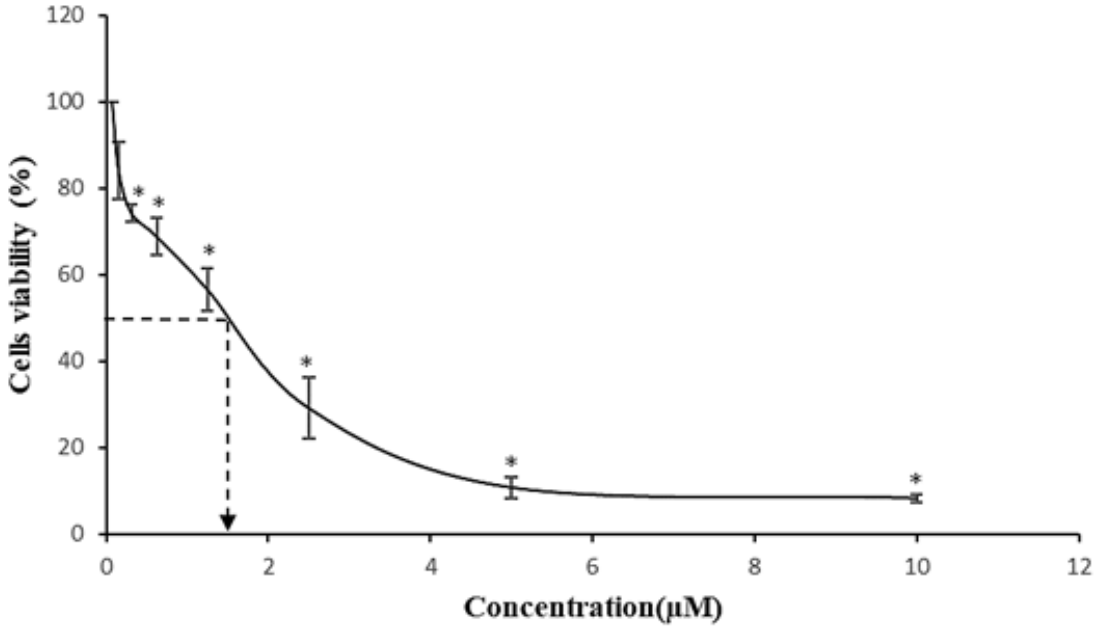
The results obtained from this study indicate that both diorganotin(IV) dithiocarbamate compounds investigated have the potential to reduce the cell viability (%) of treated human leukemic cell lines, particularly K562 cells. This effect was observed during a 24-hour treatment period at the highest concentration of 10.00 µM. Moreover, based on the IC_{50} value obtained from MTT assay, both **C1** and **C2** are toxic against K562 cells. Overall, **C2** showed a stronger cytotoxic effect as compared to **C1** when treated in K562 cells within 24

h duration. This proven by the IC_{50} value obtained for both compounds where 1.6 µM for **C2** and 4.2 µM for **C1**. Both compounds can be classified as a highly toxic compound as described by How et al. (2008) due to IC_{50} value for both compounds were less than 5.0 µg cm^{-3} (<7.34 µM).



*Difference was statistically significant (p<0.05) compared with the negative control

FIGURE 3. The cytotoxicity of **C1** after 24 h treatment in K562 cells. Values are the cell viability (%) ± S.E.M of three separate repeated experiments



*Difference was statistically significant (p<0.05) compared with the negative control

FIGURE 4. The cytotoxicity of **C2** after 24 h treatment in K562 cells. Values are the cell viability (%) ± S.E.M of three separate repeated experiments

TABLE 1. IC_{50} values for **C1**, **C2** and menadione on K562 cells

Compounds	IC_{50} value (μM)
C1	4.2
C2	1.6
Menadione	15.4

According to Biplob, Baul and Chatterjee (2008) and Ray, Sarma and Antony (2000), the cytotoxicity of a molecule is substantially impacted by its molecular structure, with the shorter the length of the alkyl substitution group, the greater the cytotoxicity of the compound. Some studies have also suggested that the ligand and the R group connected to the central tin atom determine the relationship between a compound's structure and its action (Shang et al. 2011; Yin et al. 2012). In addition, both compounds possess the same ligand which is *N*-methyl-*N*-phenethyldithiocarbamate. The role of the ligand itself is important in determining the cytotoxicity properties of a compound. Besides, ligands possess the capability to modulate the reactivity, lipophilicity, oral/systemic bioavailability of metal ions, as well as stabilize their oxidation state and substitutional inertness, all in accordance with the specific demands of chemotherapy (Jaiswal 2017).

In order to rationalize the inhibitory effects of both compounds in the tested cell lines, the Annexin V-FITC/PI assay was conducted to identify whether cells died via necrosis or apoptosis. The percentage of the externalization of phosphatidylserine showed that the K562 cells died via apoptosis upon being treated with the interest compounds. Both compounds induced apoptotic events by 49.70 ± 1.15 % for **C1** and 46.83 ± 1.50 % for **C2** at their respective IC_{50} doses as compared with 16.03% ± 1.7% of apoptotic cells in the negative control ($p<0.05$). The results indicate that both compounds decreased the proliferation of K562 cells followed by inducing apoptosis (Figure 5). As indicated by Girasolo et al. (2014), necrosis is typically associated with cell death that leads to pronounced inflammation. On the other hand, apoptosis involves the transformation of cells into smaller vesicles known as apoptotic bodies, which remain attached to the membrane. These bodies are subsequently engulfed by macrophages without triggering an inflammatory response. Therefore, induction of apoptosis is important in producing anticancer agents. The results demonstrated that both compounds were capable to induce apoptotic cell death for about 48% with concentrations of IC_{50} value in 24 h duration treatment. These findings showed that the obtained IC_{50} values of both compounds and the percentage of apoptotic cells were in agreement.

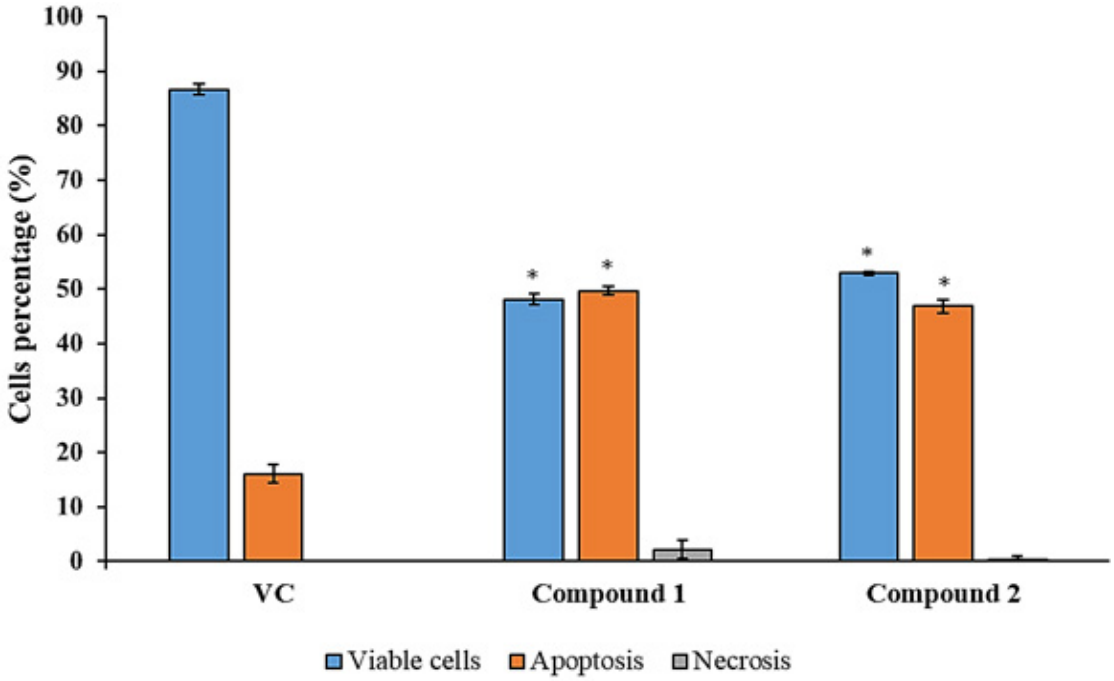
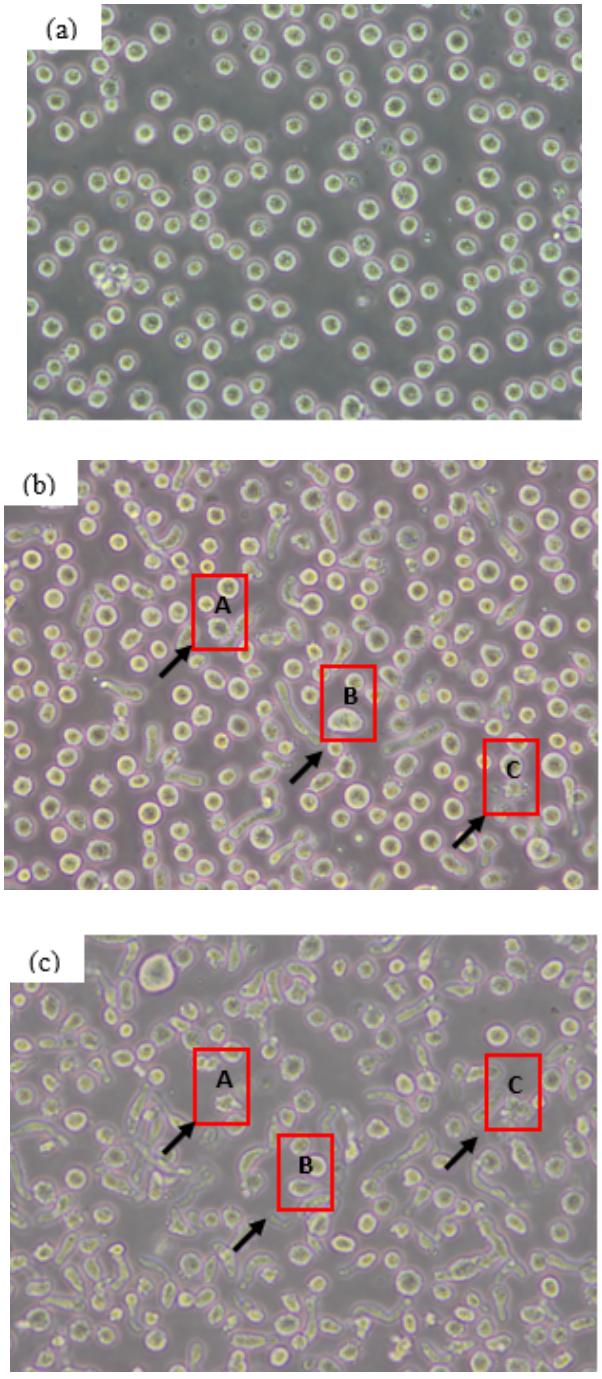


FIGURE 5. **C1**,**C2** - induced apoptosis in K562 cells at their respective IC_{50} doses for 24 h of treatment. Data represent the mean (± SEM) of at least three independent experiments

According to Alabsi et al. (2012), phosphatidylserine (PS), which is normally on the inside of the plasma membrane, moves to the outside of the membrane during apoptotic cell death, exposing PS to extracellular action. With the presence of calcium ions (Ca^{2+}), PS able to bind to the Annexin V-FITC with high affinity indicating the cells died via apoptosis (Engeland et al. 1998; Hammill et al. 1999). During late apoptotic and necrotic cells, the integrity of the cell membrane is not intact thus it loses its integrity which allowing PI to access nuclei and intercalate between DNA bases (Girasolo et al. 2014). The bonded alkyl groups to the central tin atom affected the biological activities of organotin(IV) compounds, which in turn affected the lipophilicity of the pertinent compounds in biological cells (Adeyemi et al. 2019).

Figure 6 shows the morphological changes of K652 cells after being treated for 24 h using IC_{50} value of **C1**, **C2** as well as non-treated cells (negative



A: Cells shrinkage, B: Cells blebbing, C: Cells lysis

FIGURE 6. The changes in the K562 cells' morphology (40x) - (a) Untreated, (b) treated with **C1** for 24 h and (c) treated with **C2** for 24 h

control). The observations include cells shrinkage, cells swelling, formation of apoptotic bodies and cells lysis, nevertheless for non-treated K562 cells, there is no significant changes was observed in morphology of the cells. According to Pellerito et al. (2006), apoptosis is categorised as a type of controlled cell death that is characterised by the development of apoptotic bodies, nuclear chromatin condensation, cell shrinkage, and blebbing of the plasma membrane. Necrosis, which includes cell swelling can also cause cell death; this has been observed (<1%) in tested cells following **C1** and **C2** treatment.

As a whole, both **C1** and **C2** exert cytotoxic effects against K562 cells in 24 h of exposure with IC_{50} dose of less than 5 μM. The incubation period that is normally selected for toxicity study of organotin(IV) compounds in cell culture is between 24 and 48 h where the condition of cells itself show a maximum reaction (Pellerito et al. 2006). Interestingly, both diorganotin(IV) dithiocarbamate compounds used in this study produced around 48% apoptotic cell death, which is consistent with the concentration determined from the MTT assay, which inhibits 50% cell growth. The results of statistical analysis also showed no significant difference (p>0.05) in apoptotic cell death induced by both **C1** and **C2**. The results obtained from this study align with the findings of recent studies (Attanzio et al. 2020; Haezam et al. 2021), which propose the potential utilization of organotin(IV) compounds as metallodrugs with anticancer.

Conclusions

Diphenyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate (**C1**) and dibutyltin(IV) *N*-methyl-*N*-phenethyldithiocarbamate (**C2**) have been shown to have a significant level of cytotoxicity towards K562 cells, manifesting their ability to decrease cell viability and induce apoptosis at low concentrations. In comparison between these two compounds, dibutyltin(IV) (**C2**) exerts a higher cytotoxicity in K562 cells as compared to diphenyltin(IV) (**C1**). These cytotoxicity assessments support the potential of **C1** and **C2** to be developed into anti-leukemic drugs; however, more research is required to identify the exact mechanism of action of these compounds.

Acknowledgements

We would like to thank the Ministry of Higher Education, Malaysia for the financial support through the Fundamental Research Grant Scheme (FRGS/1/2019/STG04/UKM/03/3). We would also offer the utmost sincere thanks to the laboratory assistants of Environmental Health and Industrial Safety programme and Biomedical Science programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia for providing the essential laboratory facilities which enabled this study to take place. The authors declare that there are no conflicts of interest.

References

Adeyemi, J.O., Onwudiwe, D.C., Ekennia, A.C., Anokwurud, C.P., Nundkumar, N., Singh, M. & Hosten, E.C. 2019. Synthesis, characterization and biological activities of organotin(IV) diallyldithiocarbamate complexes. *Inorganica Chimica Acta* 485: 64-72. http://dx.doi.org/10.1016/j.ica.2018.09.085

Alabsi, A.M., Ali, R., Al-Dubai, S.A.R., Harun, H., Abu Kasim, N.H. & Alsalahi, A. 2012. Apoptosis induction, cell cycle arrest and *in vitro* anticancer activity of gonothalamin in a cancer cell lines. *Asian Pacific Journal of Cancer Prevention* 13(10): 5131-5136. https://doi.org/10.7314/APJCP.2012.13.10.5131

Alama, A., Tasso, B., Novelli, F. & Sparatore, F. 2009. Organometallic compounds in oncology: Implications of novel organotins as antitumor agents. *Drug Discovery Today* 14(9-10): 500-508. https://doi.org/10.1016/j.drudis.2009.02.002

Annuar, S.N.S., Kamaludin, N.F., Awang, N. & Chan, K.M. 2021. Cellular basis of organotin(IV) derivatives as anticancer metallodrugs: A review. *Frontiers in Chemistry* 9: 1-15. https://doi.org/10.3389/fchem.2021.657599

Attanzio, A., D'Agostino, S., Busà, R., Frazzitta, A., Rubino, S., Girasolo, M.A., Sabatino, P. & Tesoriere, L. 2020 Cytotoxic activity of organotin(IV) derivatives with triazolopyrimidine containing exocyclic oxygen atoms. *Molecules* 25(4): 1-16. https://doi.org/10.3390/molecules25040859

Awang, N., Kamaludin, N.F., Baba, I., Chan, K.M., Rajab, N.F. & Hamid, A. 2016. Synthesis, characterization and antitumor activity of new organotin(IV) methoxyethyldithiocarbamate complexes. *Oriental Journal of Chemistry* 32(1): 101-107. http://dx.doi.org/10.13005/ojc/320110

Biplob, K., Baul, T.S.B. & Chatterjee, A. 2008. p53-dependent antiproliferative and anticancer effect of novel alkyl series of diorganotin (IV) compounds. *Investigational New Drugs* 27: 319-326. https://doi.org/10.1007/s10637-008-9176-6

Demir, G.M. & Değim, I.T. 2018. Determination of imatinib mesylate and erlotinib hydrochloride in mice plasma using ultrahigh performance liquid chromatography method. *Kuwait Journal of Science* 45(3): 57-63.

Engeland, M.V., Nieland, L.J.W., Ramaekers, F.C.S., Schuttle, B. & Reutelingsperger, C.P.M. 1998. Annexin V-affinity assay: A review on an apoptosis detection system based on phosphatidylserine exposure. *Journal of Quantitative Cell Science* 31: 1-9. https://doi.org/10.1002/(SICI)1097-0320(19980101)31:1%3C1::AID-CYTO1%3E3.0.CO;2-R

Girasolo, M.A., Attanzio, A., Sabatino, P., Tesoriere, T., Rubino, S. & Stocco, G. 2014 Organotin(IV) derivatives with 5,7-disubstituted-1,2,4-triazolo [1,5-a]pyrimidine and their cytotoxic activities: The importance of being conformers. *Inorganica Chimica Acta* 423: 168-176. http://dx.doi.org/10.1016/j.ica.2014.07.015

Gómez, E., Contreras-Ordoñez, G. & Ramírez-Apan, T. 2006. Synthesis, characterization and *in vitro* cytotoxicity of pentacoordinated tin(IV) complexes derived from aminoalcohols. *Chemical and Pharmaceutical Bulletin* 54(1): 54-57. https://doi.org/10.1248/cpb.54.54

Haezam, F.N., Awang, N., Kamaludin, N.F. & Mohamad, R. 2021. Synthesis and cytotoxic activity of organotin(IV) diallyldithiocarbamate compounds as anticancer agent towards colon adenocarcinoma cells (HT-29). *Saudi Journal of Biological Sciences* 28(5): 3160-3168. https://doi.org/10.1016/j.sjbs.2021.02.060

Hammill, A.K., Uhr, J.W. & Scheuermann, R.H. 1999. Annexin V staining due to loss of membrane asymmetry can be reversible and precede commitment to apoptotic death. *Experimental Cell Research* 251(1): 16-21. https://doi.org/10.1006/excr.1999.4581

Hochhaus, A., O'Brien, S.G., Guilhot, F., Druker, B.J., Branford, S., Foroni, L., Goldman, J.M., Mu¨ller, M.C., Radich, J.P., Rudoltz, M., Mone, M., Gathmann, I., Hughes, T.P. & Larson, R.A. 2009. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Journal of Leukemia* 23: 1054-1061. https://doi.org/10.1038/leu.2009.38

How, F.N.F., Crouse, K.A., Tahir, M.I.M., Tarafder, M.T.H. & Cowley, A.R. 2008. Synthesis, characterization and biological studiesof S-benzyl-&beta-N-(-benzoyl) dithiocarbazate and its metal complexes. *Polyhedron* 27(15): 3325-3329. https://doi.org/10.1016/j.poly.2008.07.022

Jaiswal, N. 2017. Organotin(IV) complexes and DNA interaction: A promising future for tin based metallodrugs. *International Journal of ChemTech Research* 10: 495-499.

Kamaludin, N.F., Zakaria, S.A., Awang, N., Mohamad, R. & Pim, N.U. 2017. Cytotoxicity assessment of organotin(IV) (2-metoxyethyl) methyldithiocarbamate compounds in human leukemia cell lines. *Oriental Journal of Chemistry* 33: 1756-1766. http://dx.doi.org/10.13005/ojc/330420

Kathiresan, K. & Ramakrishnan, M. 2021. Biosynthesis of iron oxide nanoparticles from dates, characterization, and investigation of anticarcinogenic and antimicrobial properties. *Kuwait Journal of Science* 49: 4. https://doi.org/10.48129/kjs.13181

Leong, L.M., Chan, K.M., Hamid, A., Latip, J. & Rajab, N.F. 2016. Herbal formulation C168 attenuates proliferation and induces apoptosis in HCT 116 human colorectal carcinoma cells: role of oxidative stress and DNA damage. *Evidence-Based Complementary and Alternative Medicine* 2016: Article ID 2091085. https://doi.org/10.1155/2016/2091085

Martinez-Castillo, M., Bonilla-Moreno, R., Aleman-Lazarini, L., Meraz-Rios, M.A., Orozco, L., Cedillo-Barron, L., Cordova, E.J. & Villegas-Sepulveda, N. 2016. A subpopulation of the K562 cells are killed by curcumin treatment after G2/M arrest and mitotic catastrophe. *PLoS ONE* 11(11): e0165971. https://doi.org/10.1371/journal.pone.0165971

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65(1-2): 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

Mughal, T.I. & Schrieber, A. 2010. Principal long-term adverse effects of imatinib in patients with chronic myeloid leukemia in chronic phase. *Biologics: Targets and Therapy* 4: 315-323. https://doi.org/10.2147/BTT.S5775

National Cancer Institute. 2021. Philadelphia chromosome. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/philadelphia-chromosome. Accessed 10 December 2021

Pellerito, C., Nagy, L., Pellerito, L. & Szorcsik, A. 2006. Biological activity studies on organotin(IV)$^{n+}$ complexes and parent compounds. *The Journal of Organic Chemistry* 691(8): 1733-1747. https://doi.org/10.1016/j.jorganchem.2005.12.025

Pim, N.U., Awang, N., Mohamad, R., Abdul Aziz, Y.F. & Kamaludin, N.F. 2018 Synthesis and characterisation of novel diorganotin(IV) complexes derived from *N*-Methyl-phenethyl dithiocarbamate and Bis(2-ethoxyethyl) dithiocarbamate. *Asian Journal of Chemistry* 30(6): 1364-1368. http://dx.doi.org/10.14233/ajchem.2018.21253

Ray, D., Sarma, K.D. & Antony, A. 2000. Differential effects of tri-n-butylstannyl benzoates on induction of apoptosis in K562 and MCF-7 cells. *IUBMB Life* 49(6): 519-525. https://doi.org/10.1080/15216540050167061

Shang, X., Meng, X., Alegria, E.C.B.A., Li, Q., Fátima, M., Guedes da Silva, C., Kuznetsov, M.L. & Pombeiro, A.J.L. 2011. Syntheses, molecular structures, electrochemical behavior, theoretical study, and antitumor activities of organotin(IV) complexes containing 1-(4-Chlorophenyl)-1-cyclopentanecarboxylato ligands. *Inorganic Chemistry* 50(17): 8158-8167. https://doi.org/10.1021/ic200635g

Thati, B., Noble, A., Creaven, B.S., Walsh, M., McCann, M., Kavanagh, K., Devereux, M. & Egan, D.A. 2007. *In vitro* anti-tumour and cyto-selective effects of coumarin-3-carboxylic acid and three of its hydroxylated derivatives, along with their silver-based complexes, using human epithelial carcinoma cell line. *Cancer Letters* 248(2): 321-331. https://doi.org/10.1016/j.canlet.2006.08.009

Yin, H., Yue, C., Hong, M., Cui, J., Wu, Q. & Zhang, X. 2012. Synthesis, structural characterization and *in vitro* cytotoxicity of diorganotin (IV) diimido complexes. *European Journal of Medicinal Chemistry* 58: 533-542. https://doi.org/10.1016/j.ejmech.2012.05.021

*Corresponding author; email: nurulfarahana@ukm.edu.my