http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (17): 833-838, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.833.838

Cytotoxicity of Triphenyltin(IV) Methyl- and Ethylisopropyldithiocarbamate Compounds in Chronic Myelogenus Leukemia Cell Line (K-562)

[1]N. Awang, [2]N.F. Kamaludin, [2]A. Hamid, [1]N.W.N. Mokhtar and [2]N.F. Rajab
[1]Environmental Health and Industrial Safety Programme, [2]Biomedical Science Programme,
School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences,
Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Abstract: Studies on the discovery of new cancer treatment by using metal-based compounds such as tin (Sn) has now greatly being synthesized and evaluated to identify their effectiveness and suitability to be developed as a new anticancer drug. Approach: This study was carried out to evaluate the cytotoxicity of triphenyltin(IV) methylisopropyldithiocarbamate (compound 1) and triphenyltin(IV) ethylisopropyldithiocarbamate (compound (2) on chronic myelogenus leukemia cells. The determination of their cytotoxicity (IC_{50}) at different time of exposure and concentration was carried out through the employment of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay. Results: The IC_{50} values obtained for compound 1 and 2 following treatment at 24, 48 and 72 h were 0.660, 0.223, 0.370 μM and 0.677, 0.306, 0.360 μM, respectively. Cell morphological changes such as apoptotic and necrotic features were also been observed. Conclusion: The compounds tested were found to give cytotoxic effect against chronic myelogenus leukemia (K-562) cell at a micromolar dose. Thus, further study on their specific mechanism of actions in the human cells should be carried out to elucidate their potential as an anticancer agent.

Key words: Tin compound, cytotoxicity, K-562, morphological changes, dithiocarbamate

INTRODUCTION

Metals are beneficial in variety of applications in human daily life including agricultures, manufacturing industries and medicine. In medicine, various types of compounds are currently being synthesized and studied world wide with the aim of evaluating their potential to be developed as a new anticancer agent. Nowadays, the organometallic compounds such as aurum and tin derivatives compounds are the most potent candidates to possess anticancer property as they showed a promising anti tumor effect among variety of human cancer cell lines (Alama *et al*., 2009). Organotin(IV), is one of the examples on metal-based compounds used in previous studies which gave a very strong cytotoxic effect towards cancer cells tested, thus shows potential to be developed as an anti tumor drug (Pellerito *et al*., 2006). Some examples of the organotin(IV) derivatives compounds found to give a good anti proliferating effect towards cancer cells are diphenyltin(IV) and triphenyltin(IV).

Diphenyltin demonstrates high anti proliferative effect against several human tumor cell lymphoma tested *in vitro* (Syng-ai *et al*., 2002). Besides, *in vitro* studies by using several organotin compounds also showed better antitumor effects against HeLa tumor cells, CoLo205 and MCF-7 as compared to cisplatin (Tian *et al*., 2005). Furthermore, Kaludjerovic *et al*. (2010) who used triphenyltin(IV) chloride found that this compound gave higher cytotoxicity than cisplatin when tested in several types of tumor cells including ovarian, lung, head and neck cancer cells.

Other than organotin, dithiocarbamate compounds also used in variety of applications especially in agricultures, industries and medicines. In agriculture, these compounds are currently used as fungicides, herbicides and insecticides (Johnson *et al*., 2003). Maneb, Zineb and Mancozeb are several examples of dithiocarbamate compounds applied as fungicides. At the cellular level in the *in vitro* model, dithiocarbamate acts as a pro-oxidant or antioxidant, inhibitor or inducer

Corresponding Author: N. Awang, Environmental Health and Industrial, Safety Programme,
School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences,
Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lnmpur,
Malaysia Tel: +60326878126

of apoptosis and also enzyme inhibitors (Gessner and Gessner, 1992). Interestingly, the combination of organotin and dithiocarbamate compounds showed potential to be developed as anticancer agents, antimicrobial agent and also insecticide (Pratt, 1994).

Therefore, this study was carried out to evaluate the cytotoxicity of triphenyltin(IV) methylisopropyldithiocarbamate and triphenyltin(IV) ethylisopropyldithiocarbamate against chronic myelogenus leukemia cell (K-562). The cell morphological changes during treatment have also been observed. Thus, their potential as anticancer agent can be identified.

MATERIALS AND METHODS

Stock preparation of compound 1: An amount of 0.0598 g of compound 1 was fully dissolved in 1.2 mL Dimethyl Sulphoxide (DMSO) to obtain a 100 mM of stock solution. This stock was stored at 4°C and serial dilution was freshly prepared using the culture media.

Stock preparation of compound 2: The stock solution for compound 2 was prepared by using the same procedure for compound 1. An amount of 0.0615 g of compound 2 was fully dissolved in 1.2 mL Dimethyl Sulphoxide (DMSO) and stored at 4°C.

K-562 cell culture: K-562 cell line was purchase from American Type Culture Collection (ATCC). These cells were cultured in a culture flask (T-75) containing Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 20% Foetal Bovine Serum (FBS) and 1% penicillin/streptomycin to obtain complete growth media. Cell culture was incubated in an incubator at temperature 37°C with humidified atmosphere containing 5% CO_2. Subculture of cells was done every 2-3 days to maintain cells growth and healthiness.

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltin-tetrazolium bromide] assay: The cells were treated with both compounds at three different times of exposure (24, 48 and 72 h) and different concentrations through the employment of serial dilution method. Cells without treatment solution represent the negative control, whereas doxorubicin was used as positive control. Cisplatin was used as a comparison to the tested compounds. After each treatment time points (24, 48 and 72 h), each well was added with 0.5% MTT solution and incubated for 4 h.

Then the supernatant in each well was removed and added with DMSO solution to dissolve the formazan crystals formed. The color intensity in each well was analyzed using ELISA micro plate reader at 570 nm. The IC_{50} values were obtained from the data plotted in the graph.

Cell morphology: K-562 cells were cultured in 75 cm^3 flask until confluent. An amount of 2×10^6 cells/mL were transferred into 6-well culture plates. Cells were prepared for observation at three different times of exposure (24, 48 and 72 h). The concentration used for both compounds and doxorubicin were based on the IC_{50} values obtained from MTT assay. The concentration used for cisplatin was 40 μM. Cells without treatment were used as negative control. Cells were then incubated according to each time points and the morphological changes in each different time points were observed under inverted microscope.

RESULTS

Based on the experiment, both compound 1 and 2 showed cytotoxic effects against K-562 cells at three different times of exposure (Table 1). The IC_{50} values obtained for 24 h of treatment for compound 1 and 2 were 0.660 and 0.677 μM, respectively. At 48 h of treatment, the IC_{50} value for compound 1 was 0.223 μM, while compound 2 was 0.306 μM. For 72 h of treatment, the IC_{50} values found were 0.370 μM for compound 1 and 0.360 μM for compound 2. This clearly shows that there was no significant difference between IC_{50} values obtained between both compounds.

The morphological observation under inverted microscope was done on K-562 cells during treatment using compound 1 and 2 at IC_{50} concentration for each time point. As compared to cells without treatment (Fig. 1), cells upon 24 h of treatment began experiencing changes on their structure (Fig. 2). It was found that both compounds caused necrosis to K-562 in which cells were swelling and also lysed. At the same time apoptosis was also induced by these compounds with features such as cell shrinkage, membrane blebbing and apoptotic bodies clearly seen. The morphological changes for cells upon 48 and 72 h of treatment for both compounds were showed in Fig. 3-4, respectively. After 48 h of treatment, cells were lysed and more cells died whereas upon 72 h of exposure, almost all cells died.

Table 1: IC_{50} values for K-562 after treatment

	Time (h)		
IC_{50} values (μM)	24	48	72
Compound 1	0.660	0.223	0.370
Compound 2	0.677	0.306	0.360
Cisplatin	-	-	-
Doxorubicin 31.04	0.760	0.590	

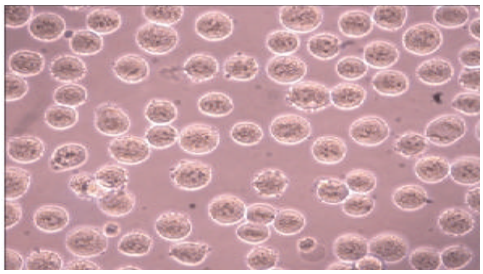

Fig. 1: Morphology of K-562 cells without treatment (400x)

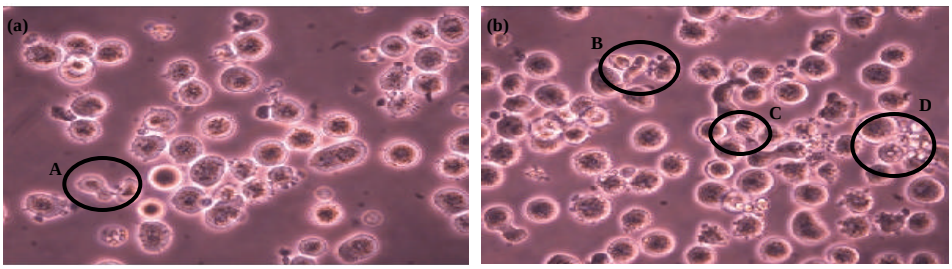

Fig. 2(a-b): Morphology of K-562 cells, (a) With treatment of compound 1 and (b) With treatment of compound 2 after 24 h treatment by using IC_{50} value A: Membrane blebbing, B: Lysed cells, C: Cell shrinkage, D: Apoptotic bodies (400x)

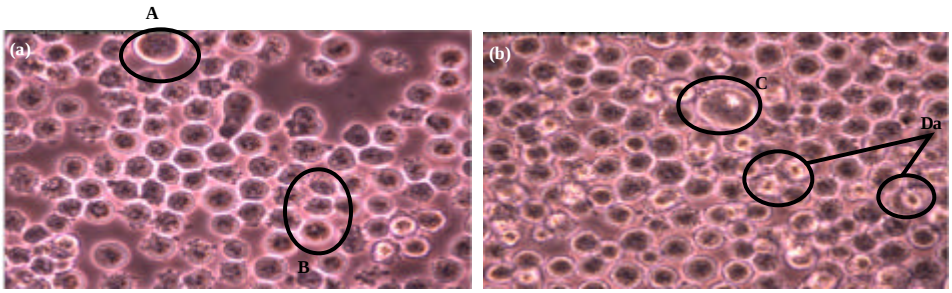

Fig. 3(a-b): Morphology of K-562 cells, (a) With treatment of compound 1 and (b) With treatment of compound 2 after 48 h treatment by using IC_{50} value A and C: Cell swelling, B: Cell shrinkage, D: Nuclear condensation (400x)

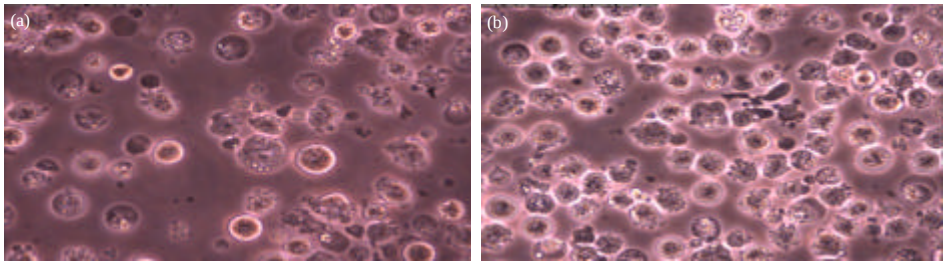

Fig. 4(a-b): Morphology of K-562 cells, (a) With treatment of compound 1 and (b) With treatment of compound 2 after 72 h treatment by using IC_{50} value (400x)

DISCUSSION

At highest concentration of 2 μM, it was found to not causing the entire cells population death. Pratt (1994) concludes that in some tissues including both with normal differentiation and tumor, there was a stage where no proliferation occurred in living cells and the cells will leave the cycle temporarily. This stage was referred as G_0 phase. In this phase, the cells were considered to be in resting phase (Pratt, 1994) where the cells will not divide normally to be performed live during chemotherapy but later will be generated back after treatment. This could be the reason why certain cells were still alive during treatment at high concentration.

Another possible reason that contributes to this phenomenon is resistance. Cells resistance to organotin(IV) compounds will still alive even treated at a high concentration and exposed for a longer period. Besides, the nature of these compounds that showed cytostatic activity also prevent the entire cells death at 2 μM concentrations. Cytostatic can be described as a process retarding the growth of cells and preventing the cells division.

Previous study has proven that triphenyltin(IV) complexes with carboxylic acid derivatives through the addition of methylene groups, showed that the IC_{50} values obtained when tested on promyelocytic leukemia cells HL-60 were lower than etoposide (Yip *et al*., 2006). The triphenyltin(IV) carboxylate complex gave lower IC_{50} value than diphenyltin(IV) on cervical adenocarcinoma cells (HeLa), chronic myelogenus leukemia (K-562) and malignant melanoma conducted by Gomez-Ruiz *et al*. (2008). Other than that, the organotin dithiocarbamate compounds were also found to exhibit high potential to be developed as an anticancer agent. A research conducted using organotin complex like aromatic diorganotin(IV) dithiocarbamate showed a high cytotoxic effect against HL-60 and MCF-7 cells (Sanuddin *et al*., 2005).

Besides that, both compounds were found to be in dose and time dependent manner. This was further corroborated by a study performed by Chikahisa *et al*. (1998) on the effects of triphenyltin on growth and viability of K-562 leukemia cells. The study showed that the inhibition on the growth of K-562 cells depend on the dose and duration of exposure at specific concentration for 72 h. Comparative effects of triphenyltin compounds with other compounds such as diphenyltin and monophenyltin were also presented. The results showed that the triphenyltin concentration inhibit 50% of K-562 cells growth with lower IC_{50} value as compared to diphenyltin and monophenyltin (Chikahisa *et al*., 1998).

Prior to this study, a research conducted on triphenyltin(IV) benzylisopropyldithiocarabamate tested on WEHI 7.2 cells was able to kill 50% of cells population with IC_{50} values 0.6 μM for 24, 48 and 72 h of exposure (Normah *et al*., 2011).

Hence, the potential of these compounds to induce cytotoxicity on tumor cell lines has already been proven. However, it must be noted that a good anticancer agent should give selective effects by giving maximum effect on cancer cells and at the same time giving the least effect on non cancer cells (Salmon and Sartorelli, 2001). An interesting fact that giving the additional value on organotin(IV) dithiocarbamate compounds is the ability of their molecular structure to be modified. The modification on their molecular structures is likely possible to reduce their toxic affects on non cancerous cells and simultaneously still have a good potential as anticancer agents. Substitution of organotin(IV) with other chemical structures or breaking its linear carbon chain bonded to tin atom will reduce the toxicity level.

In order to compare the cytotoxic potential of these compounds with established clinical drugs, cisplatin was used. Cisplatin was chose as it share similar characteristic with organotin(IV) compound that is a metal based compound. In this study, cisplatin did not show cytotoxic effect on K-562 cells. This is because cisplatin is a chemotherapeutic drug used for solid tumors and not for blood cancers like leukemia. Hussain *et al*. (2008) reported that cisplatin is widely used for treatment of tumors such as testicular, ovarian, cervical, endometrial, head, neck, lung and gastroesophagus cancers. Cisplatin is also used as a second or third treatment for prostate and breast cancer including melanoma and glioma (Oh *et al*., 2007).

Besides cisplatin, another established clinical drug used in this study is doxorubicin. In this study, doxorubicin was used as a positive control. It was found that the viability of K-562 cells significantly decrease when the concentration of doxorubicin increase. At low concentration, doxorubicin already showed its IC_{50} values. Hatano *et al*. (1993) reported that in terms of cytotoxicity, doxorubicin showed strong cytotoxic effect on leukemia cells K-562.

Cells treated will experience morphological changes. Based on the morphological observation, it can be confirmed that the IC_{50} values obtained, which is at low concentration, was able to cause cell death and proved that compound 1 and 2 can give toxic effect to K-562 cells. A study conducted by Hoti *et al*. (2003) indicated that the triphenyltin benzimidazolethiol induced apoptosis in human cervical cancer cells. In addition, the study also found that this compound was better than cisplatin in inducing apoptosis.

Morphological observation using IC_{50} value confirmed that both compounds gave cytotoxic effect towards K-562 cells at very low concentrations. Previous study found that at concentration of 4 μmol L^{-1}, triphenyltin acetate induced early and late apoptosis phenomenon. While at the highest concentration up to 8 μmol L^{-1}, cells were driven to undergo necrosis rather than apoptosis (Bollo *et al*., 2006).

CONCLUSION

In conclusion, the newly synthesized triphenyltin(IV) methylisopropyldithiocarbamate and triphenyltin(IV) ethylisopropyldithiocarbamate showed cytotoxic characteristic due to their ability to inhibit K-562 cells growth at very low doses. Further studies should be carried out on the mechanism of action of triphenyltin(IV) methylisopropyldithiocarbamate and triphenyltin(IV) ethylisopropyldithiocarbamate on K-562 cells, thus confirm its potential to be developed as anticancer agent.

ACKNOWLEDGMENTS

We would like to thank the Malaysian of Higher Education (MOHE) for the financial support of the FRGS grant (Project code: UKM-NN-06-FRGS0003-2007). Technical support from laboratory assistants of Compatibility and Toxicology Laboratory, Universiti Kebangsaan Malaysia is gratefully acknowledged.

REFERENCES

Alama, A., B. Tasso, F. Novelli and F. Sparatore, 2009. Organometallic compounds in oncology: Implications of novel organotins as antitumor agents. Drug Discovery Today, 14: 500-508.

Bollo, E., R. Guglielmino, S. Sant, P. Pregel and F. Riondata *et al*., 2006. Biochemical, ultrastructural and molecular characterization of the triphenyltin acetate (TPTA)-induced apoptosis in primary cultures of mouse thymocytes. Cell Biol. Toxicol., 22: 275-284.

Chikahisa, L., Y. Oyama, Y. Iguchi, E. Okazaki, N. Miyoshi, M. Satoh and Y. Yamada, 1998. Effects of triphenyltin on growth and viability of K562 leukemia cells. Environ. Toxicol. Pharmacol., 6: 209-215.

Gessner, P.K. and T. Gessner, 1992. Disulfiram and its Metabolite Diethyldithiocarbamate: Pharmacology and Status in the Treatment of Alcoholism, HIV Infections, AIDS and Heavy Metal Toxicity. Chapman and Hall, New York, USA., ISBN-13: 9780412360107, Pages: 452.

Gomez-Ruiz, S., G.N. Kaluderovic, S. Prashar, E. Hey-Hawkins, A. Eric, Z. Zizak and Z.D. Juranic, 2008. Study of the cytotoxic activity of di and triphenyltin(IV) carboxylate complexes. J. Inorg. Biochem., 102: 2087-2096.

Hatano, T., K. Ohkawa and M. Matsuda, 1993. Cytotoxic effect of the protein-doxorubicin conjugates on the multidrug-resistant human myelogenous leukemia cell line, K562, *in vitro*. Tumor Biol., 14: 288-294.

Hoti, N., J. Ma, S. Tabassum, Y. Wang and M. Wu, 2003. Triphenyl tin benzimidazolethiol, a novel antitumor agent, induces mitochondrial-mediated apoptosis in human cervical cancer cells via suppression of HPV-18 encoded E6. J. Biochem., 134: 521-528.

Hussain, S.A., Y.T. Ma and M.H. Cullen, 2008. Management of metastatic germ cell tumors. Expert Revision Anticancer Ther., 8: 771-784.

Johnson, D.J., V. Amarnath, K. Amarnath, H. Valentine and W.M. Valentine, 2003. Characterizing the influence of structure and route of exposure on the disposition of dithiocarbamates using toluene-3,4-dithiol analysis of blood and urinary carbon disulfide metabolites. Toxicol. Sci., 76: 65-74.

Kaludjerovic, G.N., H. Kommera, E. Hey-Hawkins, R. Paschke and S. Gomez-Ruiz, 2010. Synthesis and biological applications of ionic triphenyltin(IV) chloride carboxylate complexes with exceptionally high cytotoxicity. Metallomics, 2: 419-428.

Normah, A., K.N. Farahana, B. Ester, H. Asmah, N. Rajab and A. Abdul Halim, 2011. Cytotoxic and genotoxic effect of triphenyltin(IV) benzylisopropyldithiocarbamate in thymoma murine cell line (WEHI 7.2). Res. J. Chem. Environ., 15: 544-549.

Oh, W.K., M.H. Tay and J. Huang, 2007. Is there a role for platinum chemotherapy in the treatment of patients with hormone-refractory prostate cancer? Cancer, 109: 477-486.

Pellerito, C., L. Nagy, L. Pellerito and A. Szorcsik, 2006. Biological activity studies on organotin(IV)$^{n+}$ complexes and parent compounds. J. Organomet. Chem., 691: 1733-1747.

Pratt, W.B., 1994. The Anticancer Drugs. 2nd Edn., Oxford University Press, USA., ISBN-13: 9780195067385, pp: 69-235.

Salmon, S.E. and A.C. Sartorelli, 2001. Cancer Chemotheraphy. In: Basic and Clinical Pharmacology, Katzung, B.G. (Ed.). 8th Edn. McGraw-Hill Companies, New York, USA., ISBN-13: 9780838505984, pp: 923-956.

Sanuddin, M., Y. Farina, B.M. Yamin and I. Baba, 2005. Sintesis dan Pencirian Sebatian Kompleks Diorganostanum(IV) ditiokarbamat [Synthesis and characterization of diorganotin(IV) dithiocarbamate complexes]. Sains Malaysiana, 34: 107-112.

Syng-ai, C., T.S.B. Baul and A. Chatterjee, 2002. Antiproliferative and cytotoxic effect of a novel organotin compound on mammalian cells both *in vitro* and *in vivo*. Mutation Res./Genet. Toxicol. Environ. Mutagen., 513: 49-59.

Tian, L.J., Y.X. Sun, G.M. Yang, B.C. Qian and Z.C. Shang, 2005. Formation, structure and *in vitro* antitumour activity of a novel binuclear organotin complex. Chin. Chem. Lett., 16: 1584-1586.

Yip, F.W., Y. Farina, S.G. Teoh, S.H. Inayat-Hussain, B.M. Yamin, I. Baba and A.M. Ali, 2006. Effects of additional methylene groups of triphenyltin(IV) complex derivatives of dicarboxylic acid on cytotoxicity tests on human promylocytic cells and 119Sn NMR resonance. Malaysia J. Pharm. Sci., 4: 23-47.