Journal of Applied Pharmaceutical Science Vol. 5 (Suppl 1), pp. 007-011, May, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.54.S2
ISSN 2231-3354

In vitro Cytotoxic Activity of New Triphenyltin (IV) Alkyl-isopropyl-di-thiocarbamate Compounds on Human Acute T-Lymphoblastic Cell Line

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ARTICLE INFO

Article history:
Received on: 09/02/2015
Revised on: 17/02/2015
Accepted on: 11/03/2015
Available online: 15/05/2015

Key words:
apoptosis, triphenyltin(IV), dithiocarbamate, MTT assay, cytotoxic.

ABSTRACT

Cancer is one of the leading causes of mortality and morbidity worldwide. Various new metal-based compounds including organotin (IV) compounds have been actively synthesised due to their good cytotoxicity in cancerous cells. In this study, we tested the cytotoxicity of new triphenyltin (IV) dithiocarbamate compounds (triphenyltin (IV) benzylisopropyldithiocarbamate, compound 1; triphenyltin (IV) methylisopropyldithiocarbamate, compound 2; and triphenyltin (IV) ethylisopropyldithiocarbamate, compound 3) on the human acute T-lymphoblastic cell line, Jurkat E6.1 by MTT assay at three periods of time. The triphenyltin (IV) methylisopropyldithiocarbamate compound showed the lowest IC_{50} values (0.03 µM) after 24 h of treatment on Jurkat E6.1 cell lines. The IC_{50} values obtained for the three compounds for 24 h of treatment were 0.18 µM, 0.03 µM, and 0.42 µM, respectively. Next, for 48 h and 72 h of treatment, the IC_{50} values were 0.15 µM, 0.18 µM, and 0.40 µM and 0.18 µM, 0.19 µM, and 0.41 µM, respectively. These tested compounds were found to give cytotoxic activity against Jurkat E6.1 cell lines at micromolar doses. Observation on morphological changes of Jurkat E6.1 cell lines treated with IC_{50} values at 24 h of treatment showed morphological changes such as cell shrinkage and membrane blebbing, characterising the mode of cell death as apoptosis. Thus, further studies on the specific mechanisms of action of these compounds in human cells should be carried out to elucidate their potential as anticancer agents.

INTRODUCTION

Dithiocarbamates are versatile ligands capable of forming complexes with most existing elements. Dithiocarbamates are also able to stabilise transition metals in various oxidation states (Nabipour *et al.*, 2010). The ability of these ligands to stabilise high oxidation states in metal complexes reflects the strong o-bonding characteristic. Although the sulphur atoms of dithiocarbamate ligands possess o-donor and n-back-donation characteristics of the same order of magnitude, these ligands also possess a special feature in that there is an additional n-electron flow from nitrogen to sulphur via planar de-localised π-orbital system, as shown below:

This special feature results in strong electron donation, hence a high electron density on the metal, leading to its next higher oxidation state.

However, while dithiocarbamate complexes have been known for over a century, with many thousands are being prepared, the vast majority of these complexes contain only simple alkyl substituents such as methyl and ethyl. A developing interest in the area of dithiocarbamate chemistry is the functionalisation of the backbone such that new applications and interactions can be developed. This area is still in its early stages, but interesting and potential applications have already been noted, including the functionalisation of gold nanoparticles, the stepwise build-up of multimetallic arrays, the synthesis of dithiocarbamate-containing

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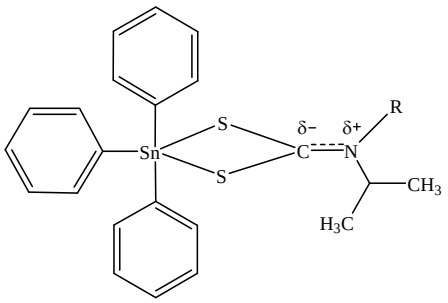
supramolecular systems that may be used for anion binding, and the development of technetium radiopharmaceuticals. Dithiocarbamates are a class of metal-chelating antioxidant compounds that have various applications in medicine, such as for the treatment of bacterial and fungal infections and possibly the treatment of AIDS (Buac *et al*., 2012).

Cancer is a prevalent cause of death worldwide. Every year, several natural and synthetic compounds are tested for various anticancer activities. Currently, research on the synthesis and application of metal-based antitumour drugs is considered one of the areas in biomedical and inorganic chemistry that are expanding at a quite fast rate (Gomez-Ruiz *et al*., 2008). More recently, various studies on the *in vitro* antitumour properties of organotin compounds against a wide panel of human tumour cell lines have been reported.

These compounds have been proven to be as effective as, or even better than, traditional heavy metal-based anticancer drugs (Kaluderovic *et al*., 2010).

One of the chemotherapeutic drugs currently used is the platinum-based compound, cisplatin, which is the first metal-containing anticancer drug. However, its use in patients can potentially result in severe side effects. This disadvantage of cisplatin has spurred interest among researchers to identify safer metal-based compounds (Jamieson and Lippard, 1999). As platinum and tin atoms possess common chemical properties (Gielen, 2002), tin complexes have been proposed to be potential therapeutic alternatives to cisplatin and similar anticancer agents.

In our previous works, we have reported several new triphenyltin(IV) dithiocarbamate compounds and their cytotoxic effects on a few human cancerous cell lines (Awang *et al*., 2012; Awang *et al*., 2014). Interestingly, all of these compounds have a good potential to become anticancer agents against the cancerous cell lines, with their IC_{50} less than 1 µM.



R = benzyl, ethyl, or methyl

Fig. 1: General chemical structure of the three compounds.

Therefore, this study was conducted to evaluate the in vitro cytotoxicity of the newly synthesised triphenyltin(IV) dithiocarbamate compounds namely triphenyltin(IV) benzylisopropyldithiocarbamate (compound 1), triphenyltin(IV) methylisopropyldithiocarbamate (compound 2), and triphenyltin(IV) ethylisopropyldithiocarbamate (compound 3). The general chemical structure proposed for the three compounds is shown in Figure 1, where R = *N*-benzyl (compound 1); *N*-methyl (compound 2); and *N*-ethyl (compound 3). In this study, the cytotoxicity study of the compounds was done in human leukaemic cell lines, Jurkat E6.1. The observation of the morphological changes in the cell was also carried out.

MATERIALS AND METHOD

Method for Compounds Synthesis

Triphenyltin(IV) alkylisopropyldithiocarbamate compounds were synthesised via *in situ* method using the respective secondary amines (*N*-benzyl-*N*-isopropylamine, *N*-methyl-*N*-isopropylamine, and *N*-ethyl-*N*-isopropylamine), carbon disulphide, and triphenyltin(IV) chloride salts (Awang *et al.*, 2012). The formation of the compounds was confirmed via elemental analysis (C, H, N, and S), infrared, and nuclear magnetic resonance (^{1}H and ^{13}C) spectroscopies. These experimental data have been published by Awang *et al*. (2010).

Stock Preparation of the Three Compounds

The three compounds (0.12 µmol) were dissolved in 1.2 mL of dimethyl sulphoxide (DMSO) in order to obtain 100 mM of stock solution. This stock was stored at −20 °C, and serial dilution was freshly prepared using the culture media.

Jurkat E6.1 Cell Culture

Jurkat E6.1 cell lines were purchased from the American Type Culture Collection (ATCC). These cells were cultured in a T-75 culture flask containing Roswell Park Memorial Institute (RPMI 1640) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin to obtain complete growth media. The cell culture was incubated in an incubator at a temperature of 37 °C and humidified atmosphere containing 5% CO_2. The subculture of cells was conducted every 2 to 3 days to maintain cell growth and healthiness.

MTT Cytotoxicity Assay

The viability of Jurkat E6.1 cells was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazholium bromide (MTT) assay (Mossman 1983). The cells were treated with the compounds at three different times of exposure (24, 48, and 72 h) and different concentrations through the employment of the serial dilution method. Cells without treatment solution represented the negative control, whereas etoposide was used as the positive control. The cells were seeded in a sterile 96-well microplate at a density of 1×10^6 cells mL^{-1} with a fresh medium containing tested compounds at various concentrations ranging from 0 to 2 µM. The cells were incubated at 37 °C in 5% CO_2 for 24, 48, or 72 h. At the end of the treatment, 20 µL of 5 mg/mL MTT solution was added to each well prior to 4 h incubation. Approximately 180 µL media in each well were removed and replaced with 180 µL of DMSO to dissolve the crystal formazan. After 15 min of incubation, the plate was agitated using an orbital shaker for 5 min to ensure the crystal formazan was completely dissolved. The

Optical Density (OD) of each well was measured at a 570 nm wavelength using an Elisa Microplate Reader (iMark). The inhibitory concentration that killed 50% of the cell population (IC_{50}) was calculated and used as a parameter to compare the relative cytotoxicity of each compound (Thati *et al.*, 2007).

Cell Morphological Observation

Cells were seeded into a sterile 96-well microplate at a density of 1×10^6 cells mL^{-1} and treated with the compounds using their respective IC_{50} concentrations. The cells were incubated for 24 h at 37 °C in 5% CO_2 incubator. Finally, the cells were observed under light inverted microscope at 400× magnification.

RESULTS

Cytotoxicity of Triphenyltin (IV) *N*-alkyl-*N*-isopropyldithiocarbamate Compounds on Jurkat E6.1

As summarised in Table 1, the three compounds showed high cytotoxicity (0.03–0.42 µM) towards Jurkat E6.1 cell lines at three periods of exposure (24 h, 48 h, and 72 h). The IC_{50} values of the three compounds at 24 h of treatment were 0.18 µM, 0.03 µM, and 0.42 µM, respectively. The highest sensitivity among the three compounds was towards compound 2. At 48 h of treatment, the IC_{50} value for compound 1 was 0.15 µM, for compound 2 was 0.18 µM, and for compound 3 was 0.40 µM. Interestingly, the IC_{50} of etoposide was almost similar to compound 1 at the three periods of exposure. Meanwhile, at 72 h of treatment, the tested compounds showed almost the same value of IC_{50}.

Table 1: IC_{50} values of the three compounds after treatment at 24 h, 48 h, and 72 h against Jurkat E6.1

	Treatment time (hours)		
IC_{50} value (µM)		**48**	**72**
Compound 1	0.18	0.15	0.18
Compound 2	0.03	0.18	0.19
Compound 3	0.42	0.40	0.41
Etoposide	0.12	0.18	0.18

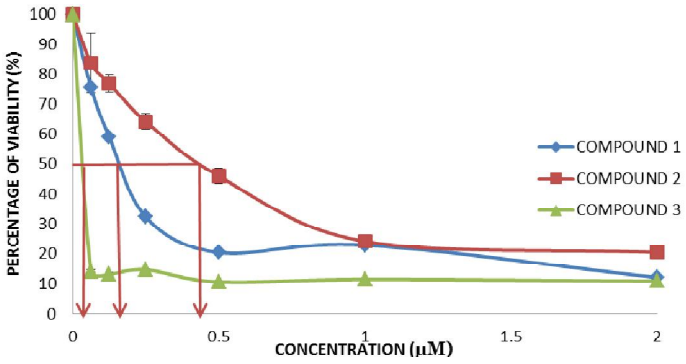


Fig. 2: The cytotoxicity of the three compounds against Jurkat E6.1 cells upon 24 h of treatment using MTT assay. Data represent the mean (± SEM) of at least three independent experiments.

At 24 h of treatment, the statistical analysis for the three compounds revealed significant differences in terms of percentage of viability between treated cells and untreated cells ($p<0.05$) at all concentrations (see Figure 2).

At 48 h of treatment, only compound 1 revealed significant differences in terms of percentage of viability between treated cells and untreated cells ($p<0.05$) at all concentrations (see Figure 3).

At 72 h of treatment, the statistical analysis for the three compounds revealed that the percentage of cell viability for treated cells from 0.13 to 2.00 µM, from 0.25 to 2.00 µM, and from 0.5 to 2.00 µM, respectively, was significantly different ($p<0.05$) compared to the percentage of cell viability exhibited by untreated cells (see Figure 4). These results revealed that these three compounds showed a high potential to act as anticancer agents.

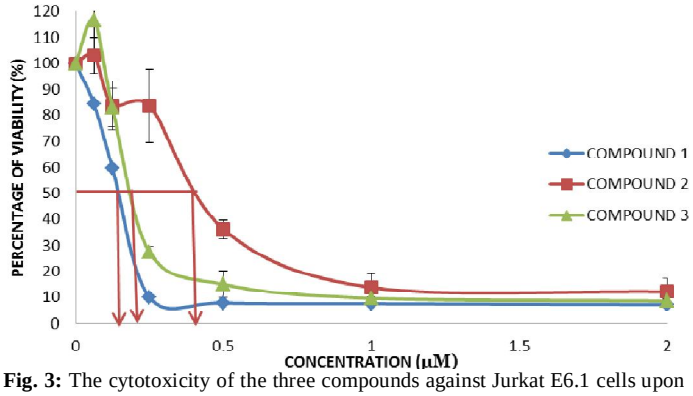


Fig. 3: The cytotoxicity of the three compounds against Jurkat E6.1 cells upon 48 h of treatment using MTT assay. Data represent the mean (± SEM) of at least three independent experiments.

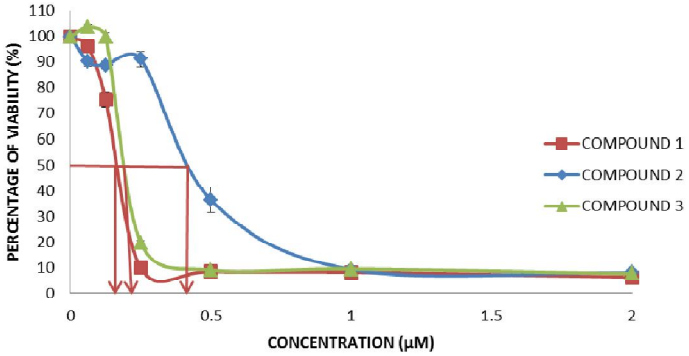


Fig. 4: The cytotoxicity of the three compounds against Jurkat E6.1 cells upon 72 h of treatment using MTT assay. Data represent the mean (± SEM) of at least three independent experiments.

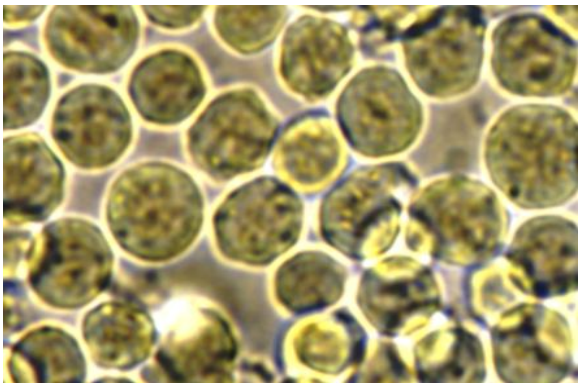
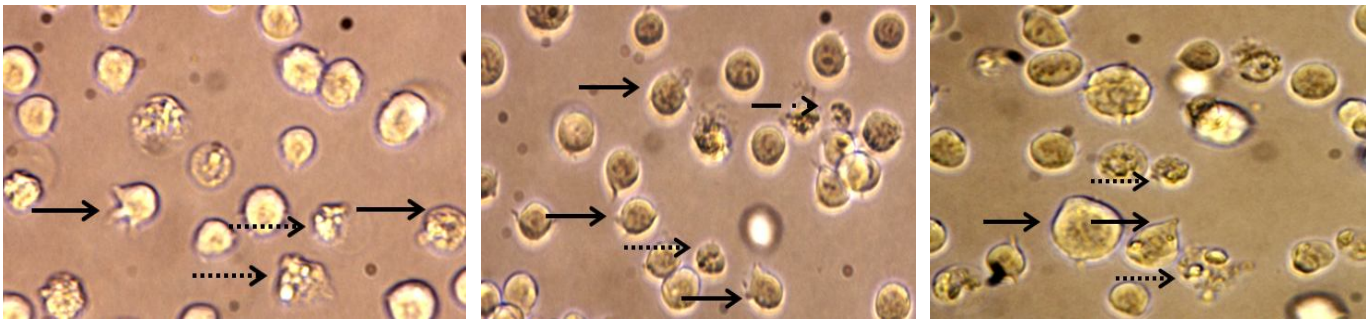


Fig 5. Morphology of Jurkat E6.1 cells without treatment



A B C

Fig. 6(a–c): Morphology of Jurkat E6.1 cells, (a) with treatment of compound 1, (b) with treatment of compound 2, and (c) treatment with compound 3 after 24 h of treatment using IC_{50} value.

Notes; ——▶ : membrane blebbing; - - - - - - - - - ▶ : lysed cells; ——— - ▶ : cell shrinkage (400×)

Morphological Changes of Jurkat E6.1 Induced by Triphenyltin (IV) *N*-alkyl-*N*-isopropyldithiocarbamate Compounds

Figure 5 shows the morphology of the untreated Jurkat E6.1 cells. The cell morphology changes were assessed using the IC_{50} concentration (0.18 µM, 0.03 µM, and 0.42 µM) of each compound (the three compounds) upon 24 h of treatment. The results of the observation showed that the cells shrank, and consequently apoptotic bodies formed (see Figure 6).

DISCUSSION

The organotin derivatives are greatly being synthesised, and their anticancer properties are extensively being studied. Organotin compounds have marked cytotoxicities in various types of cell lines of both human and animal origins. Several organotin compounds synthesised by our group also show significant cytotoxicity towards HepG2 hepatocarcinoma, Jurkat T lymphoblastic, chronic myelogenous leukaemia (K562), and thymoma murine (WEHI 7.2) cell lines (Awang *et al.*, 2012; Awang *et al.*, 2014).

Previous findings reveal that triphenyltin(IV) dithiocarbamate compounds display high toxicity towards chronic myelogenous leukaemia (K562) cells with IC_{50} values of less than 0.7 µM (Awang *et al.*, 2012). Therefore, these triphenyltin(IV) dithiocarbamate compounds have been classified as very toxic (How *et al*., 2008).

Similarly, the triphenyltin (IV) *N*-alkyl-*N*-isopropyldithiocarbamate compounds may also be classified as very toxic due to their efficacy to inhibit the growth of the Jurkat E6.1 cell population at as low as 0.03 µM (for compound 2) at 24 h of treatment. As revealed by this present study, all the compounds showed cytotoxicity in Jurkat E6.1 cells, with the highest cytotoxicity was reported by compound 2 (0.03 µM) at 24 h of treatment. It is suggested that the molecular structure of the compound plays a significant role in determining its cytotoxicity. The shorter length of an alkyl substitution group in the compound increases its cytotoxicity (Biplob *et al.*, 2008; Ray *et al.*, 2000). This explanation probably reflects our findings that revealed compounds with methyl group showed higher cytotoxicity compared to compounds with benzyl and ethyl group as their dithiocarbamate ligand.

To further evaluate the cell changes upon treatment, the cell morphological observation was conducted in Jurkat E6.1 cells. The cells began to appear in visibly different form than the control at IC_{50} concentration for the tested compounds. These results showed that these compounds gave a cytotoxic effect towards Jurkat E6.1 cells at very low concentrations. The cells started to lose their shape with more intercell spaces observed.

CONCLUSION

In conclusion, triphenyltin(IV) dithiocarbamate compounds tested in this study demonstrated cytotoxic properties. This finding warranted further investigation on the compounds as potential anticancer agents.

The synthesised compounds were next evaluated for their cytotoxicity against Jurkat E6.1 cell lines, and they were found to be highly potential. All of these compounds had high efficacy towards the tested cells. We conclude that the presence of aryl groups attached to the tin atom would be critical for their biological activities. Further studies are currently in progress in an attempt to define the important mechanisms of action of the mentioned compounds.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the research grant provided by the Ministry of Higher Education (FRGS/2/2013/SKK10/UKM/02/1) and the laboratory assistants from the Biomedical Science Programme and Environmental Health and Industrial Safety Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia for the technical support.

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How to cite this article:

Normah Awang, Nor Syaidatul Akmal Mohd Yousof, Nor Fadilah Rajab and Nurul Farahana Kamaludin. *In vitro* Cytotoxic Activity of New Triphenyltin(IV) Alkylisopropyldithiocarbamate Compounds on Human Acute T-Lymphoblastic Cell Line. J App Pharm Sci, 2015; 5 (Suppl 1): 007-011.