ORIGINAL ARTICLE

Cytotoxic effect and apoptotic induction of tricyclohexyltin p-methoxycinnamate on HT-29 colorectal cancer cells: Implications for anticancer therapeutics

Abdah Md Akim, Gul-e-Saba Chaudhry[1], Zeenia[1], Tan Weay Ken, Yeong Yik Sung[1], Tengku Sifzizul Tengku Muhammad[1]

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, [1]Institute of Climate adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

J. Adv. Pharm. Technol. Res.

ABSTRACT

Colorectal cancer's escalating prevalence in Malaysia prompts the exploration of innovative anticancer agents; amidst this backdrop, tricyclohexyltin p-methoxycinnamate emerges as a synthesized organotin complex with unique bioactive properties. Notably, the novelty of this research lies in its groundbreaking investigation into the hitherto unexplored anticancer potential and mode of cell death induced by tricyclohexyltin p-methoxycinnamate on colon cancer (human colorectal adenocarcinoma cell line (HT-29)) cell lines. This study pioneers the assessment of tricyclohexyltin p-methoxycinnamate's cytotoxic effects through the "(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay," revealing a dose- and time-dependent cytotoxicity with IC_{50} values of 1.2 × 10-6M for 24 h, 1.0 × 10-6M for 48 h, and 5.0 × 10-7M for 72 h. The mode of cell death through "AO/PI" staining alongside cell cycle analysis, highlighting apoptosis as the predominant mode of cell death in the HT-29 cell line, accompanied by substantial cell cycle arrest at the sub-"G0" phase. The tricyclohexyltin p-methoxycinnamate's shown potential antiproliferative properties, cell cycle arrest, and apoptosis in HT-29 cancer cells. This novel insight into the compound's mode of action positions it as a promising candidate for future anticancer therapeutics. The study underscores the urgency of investigating innovative approaches amidst the rising colorectal cancer rates, emphasizing the compound's potential through further in-depth studies and preclinical trials.

Key words: Anticancer, apoptosis, cancer, cell cycle, tricyclohexyltin p-methoxycinnamate

Address for correspondence:
Dr. Gul-e-Saba Chaudhry,
Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Terengganu 21030, Terengganu, Malaysia.
E-mail: sababiochem@gmail.com
Prof. Dr. Abdah Md Akim,
Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia.
E-mail: abdah@upm.edu.my

Submitted: 03-Sep-2023 **Revised:** 16-Apr-2024
Accepted: 21-Oct-2024 **Published:** 14-Feb-2025

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/JAPTR
	DOI: 10.4103/JAPTR.JAPTR_427_23

INTRODUCTION

The "organotin" compound was first synthesized by Lowiq in "1852," and the series of the compounds was studied by Franklin in 1853.[1] The organotin compound was composed of tin and hydrocarbon substituents and was part of organometallic chemistry.[2] Organotin compounds have

How to cite this article: Akim AM, Chaudhry G, Zeenia, Ken TW, Sung YY, Tengku Muhammad TS. Cytotoxic effect and apoptotic induction of tricyclohexyltin p-methoxycinnamate on HT-29 colorectal cancer cells: Implications for anticancer therapeutics. J Adv Pharm Technol Res 2025;16:1-5.

been used as "bactericides," "fungicides," "insecticides," "herbicides," and "wood preservatives," demonstrating the biologically active properties of organotin compounds.[1] In 1929, scientists found that organotin complexes had shown antitumor activity against cancer cells,[3] and this caused increasing interest in organotin compounds due to their possibility of being used as an antitumor agent.[4] The antiproliferative effect of the organotin complex was due to its ability to interact with organometallic compounds with the DNA because it could recognize specific DNA sequences. This caused "modification" of the "DNA structure" or sequence changes. Variation of DNA structure or sequence caused affection of the gene expression effect because of the inability to access the "activator" or "repressor" protein.[5] The coordination number and types of groups linked to the central tin atom were necessary for DNA binding in organotin complexes. Anchoring to the phosphate group in the DNA sugar backbone was unnecessary because of the solid binding for nitrogen in the DNA bases. Therefore, thanks to this interaction, the tin core was maintained and was octahedrally stable.[3] Apoptosis held a pivotal role in upholding equilibrium between cellular demise and proliferation. Evasion of apoptosis disrupted this balance, drove unregulated cell proliferation, and contributed to various ailments, including cancer.[6] The p53 tumor suppressor, "TRAIL" receptor, caspases pathway, and "Bcl-2" family of proteins were examples of the apoptotic pathway, and some metal compounds could activate the apoptotic process through those pathways.[3] The organotin complex might have initiated the apoptotic pathway by releasing the metal compounds, which caused the metal-induced reactive oxygen species to be induced. This reactive oxygen species would thus cause metal-induced intracellular apoptosis.[7] For example, organotin complexes such as "di-n-butyltin" and "tri-n-butyltin" could induce apoptosis in rat thymocytes by inhibiting DNA synthesis. These organotin complexes could cause the release of Ca^2+ ions and then the release of cytochrome c from the mitochondrion. This would lead to caspase pathway activation and then cause DNA fragmentation. DNA fragmentation would cause the cells to undergo the apoptotic pathway.[8] Our current investigation showed the antiproliferative activity, mode of cell death, and cell cycle study of the organotin compounds, tricyclohexyltin p-methoxycinnamate, on the HT-29 cell line.

MATERIALS AND METHODS

The tricyclohexyltin p-methoxycinnamate was synthesized and provided by the "Department of Chemistry," Faculty of Sciences, "University Malaya." The HT-29 (colon cancer) cell line was acquired from the "American Type Culture Collection," USA. The cell lines medium '"RPMI 1640" purchased from Nacalai Tesque, Kyoto, Japan. The "FBS" and trypsin purchased from PAA Laboratories, Austria. However, the phosphate buffer, RNAase A reagent, (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) reagent solution, acridine orange (AO), and propidium iodide (PI) purchased from Sigma, USA. All reagents were of analytical grades.

Cytotoxic analysis

The cell culture was grown in "RPMI 1640" culture media complemented by 10% "FBS," and "cell culture" antibiotics. The analysis was performed through previously optimized and established method.[9] The cancer and normal cells were grown in a 5% CO_2 at 37°C. The cytotoxicity was evaluated through using MTT reagent. Ultimately, the absorbance was measured using an "enzyme-linked immunosorbent assay (ELISA)" 96-well plate reader at a wavelength of 490 nm.

Apoptosis analysis through using acridine orange/propidium iodide staining

The HT-29 cells were seeded into six-well plate and incubated at for 24 h in humidified environment. Subsequently, the old medium was removed from each well, and 2 mL of tricyclohexyltin p-methoxycinnamate at concentrations of 3.5 × 10-7M (IC_{30}), 1.2 × 10-6M (IC_{50}), and 2.25 × 10-6M (IC_{70}) was added into individual cells. The six-well plate was then incubated at 37°C with 5% CO_2 for an additional 24 h. The supernatant was removed, and the HT-29 cells were stained with 50 µL of AO (1 mg/mL) and 50 µL of PI (1 mg/mL). The stained cells were transferred onto a slide and examined using fluorescence microscope.

Cell cycle analysis through flow cytometry

Initially, 1×10^{-6} cells were seeded into each well of a "six-well" plate, then incubated at 37°C with 5% CO_2 for 24 h. Following incubation, 2 mL of tricyclohexyltin p-methoxycinnamate with concentrations of 3.5 × 10-7M (IC_{30}), 1.2 × 10-6M (IC_{50}), and 2.25 × 10-6M (IC_{70}) were added to each well. Briefly, the cells were fixed in "70% ethanol" overnight at −20°C. Subsequently, 5 µL of RNAase A was added, followed by 50 µL of 1 mg/mL PI, and the mixture was incubated for 30 min. Finally, the stained samples were analyzed using a flow cytometer.

Statistical analysis

All the determinations were expressed in mean ± standard error of the mean. The dose–response relationship of tricyclohexyltin p-methoxycinnamate toward HT-29 cancer cell line was evaluated using the "Statistical Package for the Social Science" (SPSS) version 13.0 (SPSS Chicago (Ill., USA)). The different concentration of tricyclohexyltin p-methoxycinnamate in the AO/PI assay was analyzed through "one-way analysis of variance" and "Dunnett's multiple-range" tests. A "$P < 0.05$" was considered significant in all the statistical analyses.

RESULTS AND DISCUSSIONS

The cytotoxicity assay revealed that escalating concentrations and

prolonged treatment with tricyclohexyltin p-methoxycinnamate led to a reduction in the viability of the HT-29 cancer cell line [Figure 1]. Notably, tricyclohexyltin p-methoxycinnamate did not demonstrate significant toxicity toward Vero cells or healthy cells. The IC_{50} values of tricyclohexyltin p-methoxycinnamate at 24, 48, and 72 h, along with those of the positive control, 5-fluorouracil, are detailed in Table 1.

Inducing apoptosis in cancer cells represents an optimal strategy for cancer treatment, as it avoids causing damage to the surrounding tissue, unlike cell death through necrosis, which may lead to injury in the adjacent tissue.[10] Previous investigations on organotin complexes have provided evidence suggesting that these complexes can induce apoptosis in cells, with a study demonstrating p53-dependent mediation of apoptosis in tumor cells.[3] Our study treated the "HT-29" colon cancer cell line with varying concentrations of IC_{30}, IC_{50}, and IC_{70} of tricyclohexyltin p-methoxycinnamate. Fluorescent images of HT-29 cells following exposure to tricyclohexyltin p-methoxycinnamate revealed a concentration-dependent impact, as illustrated in Figure 2. Observable changes included DNA fragmentation, membrane blebbing, and apoptotic body formation, indicative of apoptosis in the treated cells. The negative control (without treatment) exhibited a predominance of green viable cells compared to red apoptotic cells. Notably, IC_{70}, featuring a higher concentration of tricyclohexyltin p-methoxycinnamate, displayed more "red color" apoptotic cells than "green color" viable cells. Meanwhile, IC_{50} demonstrated a nearly equal distribution of "red apoptotic" cells and "green viable" cells, and IC_{30}, with a lower concentration of tricyclohexyltin p-methoxycinnamate, exhibited a lower number of red apoptotic cells than green viable cells [Table 2]. Analysis of the treated cancer cell lines indicated a reduction in cell viability, with a higher abundance of red apoptotic cells compared to the control group. This substantiates the antiproliferative or apoptotic effect of tricyclohexyltin p-methoxycinnamate on cancer cells. Furthermore, the dose-dependent relationship was evident, as a higher dose of tricyclohexyltin p-methoxycinnamate resulted in an increased number of apoptotic cells. This confirmation supports the notion that tricyclohexyltin p-methoxycinnamate influences cell viability in a concentration-dependent manner. Statistical analysis using SPSS affirmed a significant difference in the dose–response relationship of tricyclohexyltin p-methoxycinnamate toward the HT-29 colon cancer cell line, as demonstrated by the AO/PI assay. This substantiates that tricyclohexyltin p-methoxycinnamate exerts its apoptotic effect on cells across different concentrations.

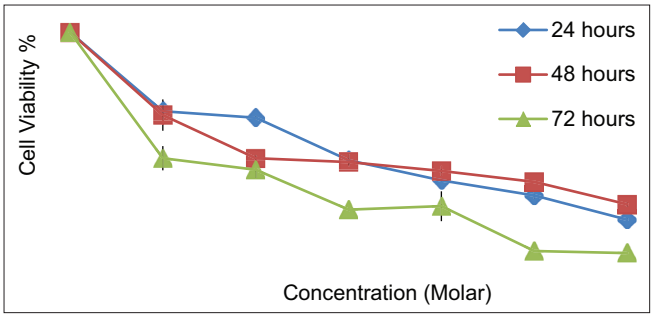

Figure 1: Graph percentage of HT-29 cancer cell line viability versus tricyclohexyltin p-methoxycinnamate concentration for specific screening after 24, 48, and 72 h of incubation

Cell cycle phase distribution analysis using RNAase A/propidium iodide assay

To further validate the apoptogenic effect on HT-29 cells, an RNAase/PI assay was conducted. The RNAase A/PI assay is a valuable and sensitive technique for assessing cell cycle progression or DNA content within cells. The "propidium iodide" PI is a "DNA-intercalating" fluorescent dye capable of permeating through a permeabilized membrane and intercalating into DNA. The intensity of the PI signal correlates directly with the "DNA" content.[11] RNAase

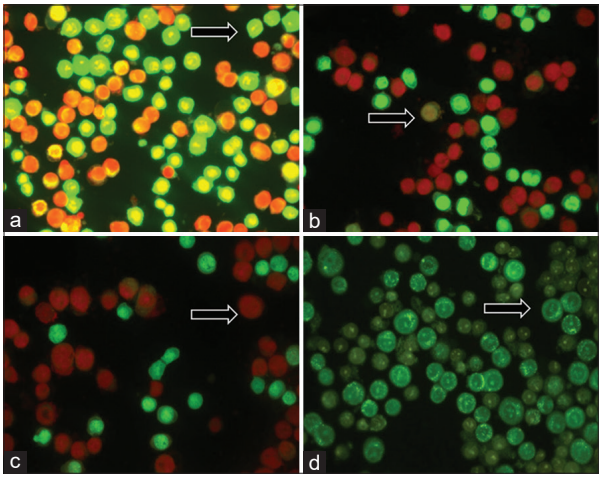

Figure 2: Fluorescent images of acridine orange and propidium iodide staining. The arrows represent increase in IC value dependant change in cells. HT-29 Colorectal cancer cell line was treated with Tricyclohexyltin p-methoxycinnamate in a dose-dependent manner; (a) treatment of IC_{30}, (b) treatment of IC_{50}, (c) treatment of IC_{70}, and (d) untreated cells

Table 1: Half-maximal inhibitory concentration values of tricyclohexyltin p-methoxycinnamate and positive control (5-fluorouracil) on HT-29 colon cancer cell line after 24-, 48- and 72-h incubation

Duration of incubation (h)	Tricyclohexyltin p-methoxycinnamate, IC_{50} ± SEM	5-fluorouracil (IC_{50} ± SEM)
24	$1.25\times10^{-6}\pm5.77\times10^{-8}$ M	-
48	$1.17\times10^{-6}\pm2.21\times10^{-7}$ M	-
72	$4.58\times10^{-7}\pm9.61\times10^{-8}$ M	$2.43\times10^{-4}\pm1.45\times10^{-5}$ M

SEM: Standard error of mean, IC_{50}: Half-maximal inhibitory concentration

A introduced to degrade RNA, as PI also binds to RNA, thereby preventing false-positive results. In the RNAase A/PI assay [Figure 3], the untreated group exhibited 55.56% of cells accumulated in the "sub-G0" phase. Conversely, the IC_{30} treated group demonstrated an accumulation of 63.41% of cells in the "sub-G0" phase, the IC_{50} treated group displayed 66.00%, and the IC_{70}-treated group showed 71.58% in the "sub-G0" phase. The increased percentage of cells in the "sub-G0" phase from IC_{30} to IC_{70} signifies a higher incidence of apoptosis at elevated concentrations of tricyclohexyltin p-methoxycinnamate. A higher percentage of cells in the "sub-G0" phase indicates DNA cleavage and apoptosis during programmed cell death. Cellular endonucleases degrade DNA during apoptosis, resulting in apoptotic cell nuclei containing less DNA than healthy cell nuclei. Consequently, the treatment-inducing apoptosis led to fewer cells in the G0/G1 phase, with evident sub-G0 peaks in the fluorescence histograms. These sub-G0 peaks in the histogram facilitate quantifying apoptotic cells in the samples. Organotin (IV) compounds have garnered significant attention for their anticancer properties, comparable to other metallodrugs.[12] Diorganotin (IV) and triorganotin (IV) compounds have been extensively studied for their *in vitro* antitumor properties against various cancers. The quantity and type of alkyl or aryl substituents, along with the number of Sn-C bonds in an organotin molecule, may influence the biological effects of the product.[13-15] These substances exhibit fewer adverse effects, superior excretion qualities, and heightened antiproliferative activity at low concentrations than other platinum-based medications.[16]

Table 2: Quantitative analysis mean number of viable and apoptotic cells observed with different concentrations of tricyclohexyltin p-methoxycinnamate under fluorescent microscope

	Apoptotic cells			Viable cells		
	Mean	SD	SEM	Mean	SD	SEM
Control	0.00	0.00	0.00	108.00	15.72	9.07
IC30	51.00	5.57	3.21	59.67	5.69	3.28
IC50	38.33	1.53	0.88	31.67	0.58	0.33
IC70	54.67	13.01	7.51	24.33	3.06	1.76

SEM: Standard error of mean, SD: Standard deviation, IC: Inhibitory concentration

The two prominent mechanisms through which cancer cells undergo cell death are apoptosis and necrosis. Initiating apoptosis, characterized by programmed cell death, or inducing cell lysis leading to inflammation – a characteristic feature of necrosis, are significant avenues in cancer treatment.[17-19] Our research underscores the inhibitory effect of tricyclohexyltin p-methoxycinnamate on the growth of the HT-29 colon cancer cell line, resulting in cell death by inducing cell arrest. This cell arrest may activate specific cell death pathways, potentially leading to programmed cell death. Notably, our findings strongly indicate that apoptosis is the primary cause of cell death. However, verification through caspase investigations is crucial to determine whether the process is intrinsic or extrinsic. The process of apoptosis is well-defined, involving

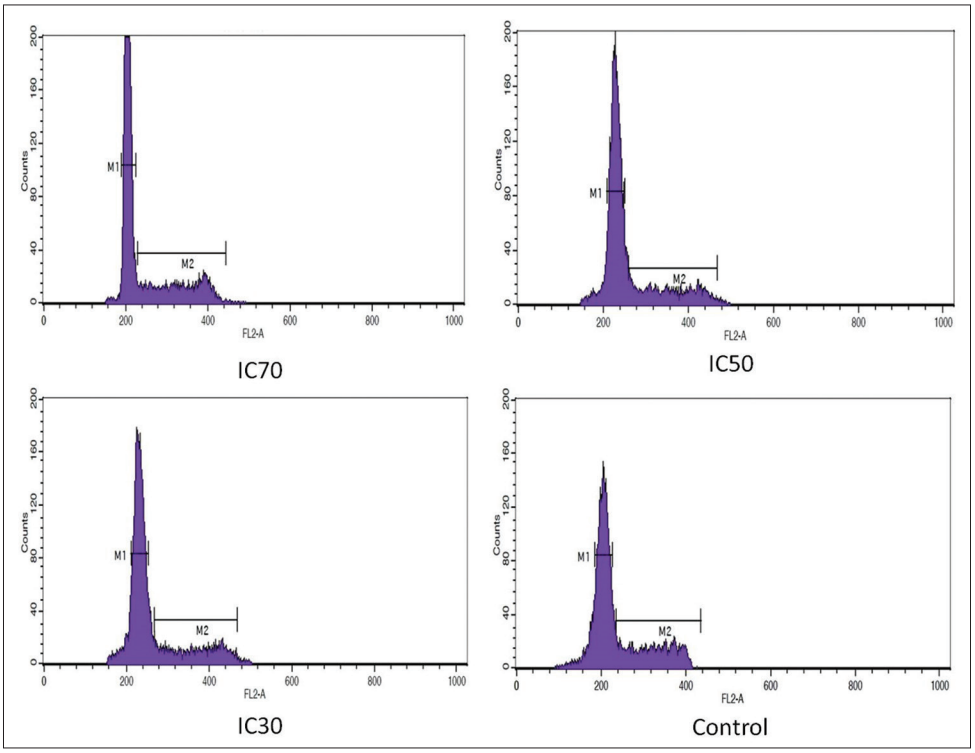

Figure 3: Cell cycle analysis of treated HT-29 colon cancer cell line. Untreated and Tricyclohexyltin p-methoxycinnamate with IC_{70}, IC_{50}, and IC_{30} values

a cascade of enzymatic processes within cells. According to Jan and Chaudhry,[6] phosphatidylserine (PS) translocates from the cytoplasm to the cell's surface during the initial phases of apoptosis induction. The distinctive characteristic of cytotoxic agents lies in their ability to induce apoptosis while maintaining membrane integrity. Further research is necessary to fully validate apoptosis by delineating each step of cell death, from DNA fragmentation to PS exposure, triggered during this cellular event. Future research investigations may delve into the activation of caspases, the mitochondrial route, and the role of death receptors. These factors will strengthen the conclusion regarding the apoptotic process of tricyclohexyltin p-methoxycinnamate in HT-29 colon cancer cells and contribute to advancing our understanding of the apoptotic process. Considering these results, research into the cellular and molecular impacts of tricyclohexyltin p-methoxycinnamate is warranted to ensure its potential as a therapeutic agent.

CONCLUSION

Overall, the rising incidence of colorectal cancer in Malaysia highlights the critical need to address this worldwide health issue. Due to its diverse bioactive properties, tricyclohexyltin p-methoxycinnamate is promising as a potential therapeutic agent. This work investigated the cytotoxic effects and mechanisms of cell death induced by tricyclohexyltin p-methoxycinnamate on colon cancer (HT-29) cell lines. This study emphasizes tricyclohexyltin p-methoxycinnamate's antiproliferative qualities and capacity to cause apoptosis in HT-29 cancer cells. Its potential use in anticancer treatments is highlighted by the requirement for additional research into its mechanisms of action through thorough studies and preclinical trials. The future of improving cancer treatment potentially lies in further investigating tricyclohexyltin p-methoxycinnamate's mechanism in detail, including signaling pathways triggered in various cell types and toxicity of a compound, safety profile keeps in check to consider it with having therapeutic potential.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Alexander R. Industrial applications of organotin compounds. Ann N Y Acad Sci 2006;1:107-23.
2. Thoonen SH, Deelman BJ, van Koten G. Synthetic aspects of tetraorganotins and organotin (IV) halides. J Organomet Chem 2004;689:2145-57.
3. Tabassum S, Pettinari C. Chemical and biotechnological developments in organotin cancer chemotherapy. J Organomet Chem 2006;691:1761-6.
4. Syed Annuar SN, Kamaludin NF, Awang N, Chan KM. Cellular basis of organotin (IV) derivatives as anticancer metallodrugs: A review. Front Chem 2021;9:657599.
5. Al-Allaf TA, Rashan LJ, Stelzner A, Powell DR. Organotin (IV) complexes with various donor ligands and their cytotoxicity against tumour cell lines. Part (I): R_2SnCl_2 with Schiff bases; unusual C=N bond cleavage of the bases and X-ray structures of the ionic products formed. Appl Organomet Chem 2003;17:891-7.
6. Jan R, Chaudhry GE. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. Adv Pharm Bull 2019;9:205-18.
7. Chen F, Vallyathan V, Castranova V, Shi X. Cell apoptosis induced by carcinogenic metals. Mol Cell Biochem 2001;222:183-8.
8. Gennari A, Viviani B, Galli CL, Marinovich M, Pieters R, Corsini E. Organotins induce apoptosis by disturbance of [Ca (2+)] (i) and mitochondrial activity, causing oxidative stress and activation of caspases in rat thymocytes. Toxicol Appl Pharmacol 2000;169:185-90.
9. Mehmood R, Sadiq A, Alsantali RI, Mughal EU, Alsharif MA, Naeem N, *et al*. Synthesis and evaluation of 1,3,5-triaryl-2-pyrazoline derivatives as potent dual inhibitors of urease and α-Glucosidase together with their cytotoxic, molecular modeling and drug-likeness studies. ACS Omega 2022;7:3775-95.
10. Byron KM, Brita B, Philip MS, David PT, Kaedi RG, Bronwyn GH, *et al*. Induction of apoptosis in HT-29 human colon adenocarcinoma cells by 13-cis-retinoic acid and Vitamin E succinate. Nutr Res 2006;26:186-92.
11. Ramos-Gómez M, Seiz EG, Martínez-Serrano A. Optimization of the magnetic labeling of human neural stem cells and MRI visualization in the hemiparkinsonian rat brain. J Nanobiotechnology 2015;13:20.
12. Gielen M, Dalil H, Biesemans M, Mahieu B, De Vos D, Willem R. Di-and tri-organotin derivatives of 3S, 4S-3-[(R)-1-(tert-butyl-dimethylsilyloxy) ethyl] -4-[(R)-1-carboxyethyl] -2-azetidinone: Synthesis, characterization and *in vitro* antitumour activity. Appl Organomet Chem 1999;13:515-20.
13. Abd Aziz NA, Awang N, Chan KM, Kamaludin NF, Mohamad Anuar NN. Organotin (IV) dithiocarbamate compounds as anticancer agents: A review of syntheses and cytotoxicity studies. Molecules 2023;28:5841.
14. Alama A, Tasso B, Novelli F, Sparatore F. Organometallic compounds in oncology: Implications of novel organotins as antitumor agents. Drug Discov Today 2009;14:500-8.
15. Hadjikakou SK, Hadjiliadis N. Antiproliferative and anti-tumor activity of organotin compounds. Coord Chem Rev 2009;253:235-49.
16. Attanzio A, D'Agostino S, Busà R, Frazzitta A, Rubino S, Girasolo MA, *et al*. Cytotoxic activity of organotin (IV) derivatives with triazolopyrimidine containing exocyclic oxygen atoms. Molecules 2020;25:859.
17. Chaudhry GE, Md Akim A, Sung YY, Sifzizul TMT. Cancer and apoptosis: The apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. Front Pharmacol 2022;10;13:842376. doi: 10.3389/fphar.2022.842376.
18. Chaudhry GE, Jan R, Akim A, Zafar MN, Sung YY, Muhammad TST. Breast Cancer: A Global Concern, Diagnostic and Therapeutic Perspectives, Mechanistic Targets in Drug Development. Adv Pharm Bull 2021;11:580-94.
19. Chaudhry GeS, Zeenia, Akim AM, Sung YY, Muhammad TST. Green and Blue Solutions: Natural Compounds from Plants and Marine Organisms Therapeutic Role in Induction of Cancer Cell Death. In: Interdisciplinary Cancer Research. Springer, Cham; 2024. doi: 10.1007/16833_2024_310.