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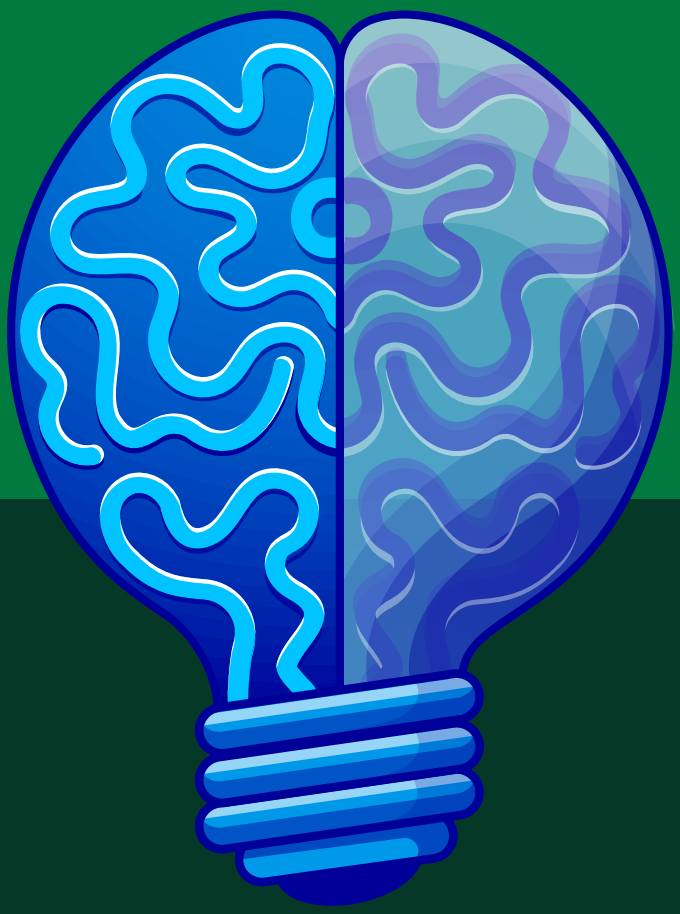

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Preface

The Scientific Project Colloquium offers a platform for publishing Diploma Science final year projects (FYP). The objective is to effectively distribute research findings throughout all scientific disciplines. The primary objective of including final year projects into the course curriculum is to encourage students to put their theoretical knowledge into practical applications.

We would like to express our gratitude to our primary establishment, the Faculty of Applied Sciences and Universiti Teknologi MARA, Perak Branch, for their invaluable assistance.

Lastly, we would like to express our gratitude to all of the authors for the tremendous help in preparing the articles, without which this undertaking would not have been completed.

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CYTOTOXIC EVALUATION OF 6-METOXY TETRAHYDRO-β-CARBOLINE DERIVATIVES (6MTHβC) IN HUMAN NEUROBLASTOMA (SK-N-SH) CELLS

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Abstract: Tetrahydro-β-carboline (THβC) alkaloids, a varied group of indole-derived chemicals, demonstrate pharmacological actions including anticancer, antibacterial, antioxidant, and neuroactive effects in nature. In this study, 6-methoxy-tetrahydro-β-carboline (6MTHβC) has been synthesized and evaluated for its toxicity against the human neuroblastoma cell line (SK-N-SH) using the MTT assay. The assay was conducted at a concentration of 50 µM of each compound. The findings showed that four compounds exhibited toxicity toward SK-N-SH cells, while eight compounds demonstrated more than 50% cell viability. This study provides fundamental toxicological insights into these compounds and lays the groundwork for pharmacological and potential new treatment possibilities related to neurodegenerative disease.

Keywords: *Cytotoxicity, SK-N-SH, Tetrahydro-β-carboline, Human neuroblastoma cell*

INTRODUCTION

Tetrahydro-β-carboline (THβC) alkaloids, a diverse class of indole-based compounds, exhibit pharmacological activities such as anticancer, antimicrobial, antioxidant, and neuroactive properties throughout nature (Herraiz & Guillén, 2018; Cao et al., 2007). Wang et al. (2021) found that structural modifications to the THβC scaffold, particularly substitutions at critical locations, enhance bioactivity and selectivity. Methoxy-substituted derivatives may interact with biomolecular targets of cancer therapy, making them interesting. The varied clinical behaviour and resistance to traditional treatments make neuroblastoma, one of the most frequent extracranial solid tumours in children, a serious therapeutic challenge (AlKhazal et al., 2025). The SK-N-SH human neuroblastoma cell line is commonly used to test the cytotoxic and neuropharmacological effects of synthetic and natural chemicals *in vitro* (Zhou et al., 2021). Thus, identifying novel small compounds that induce cytotoxicity in neuroblastoma cells is crucial for discovering new treatment options.

Introducing a methoxy group to 6-methoxy-tetrahydro-β-carboline (6MTHβC) derivatives (Figure 1) may alter their electrical characteristics, lipophilicity, and receptor-binding affinity, thereby affecting their lethal potential. Despite their well-documented bioactivities, limited research exists on methoxy-substituted analogues of THβC alkaloids against neuroblastoma. In this work, 6MTHβC derivatives will be tested for cytotoxicity *in vitro* utilising the SK-N-SH cell line. This study provides fundamental toxicological insights into these compounds and lays the groundwork for pharmacological, mechanistic, and SAR studies.

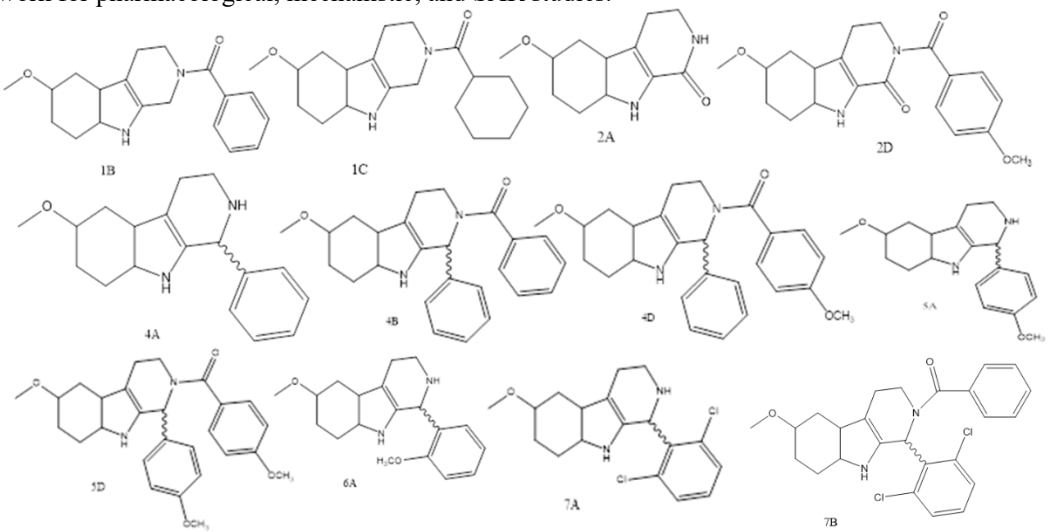

Figure 1 Structure of 6-methoxy-tetrahydro-β-carboline (6MTHβC) derivative

METHODOLOGY

The toxicity study of twelve 6MTHβC compounds was carried out using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, as described by Mosmann (1983). The cell culture experiments were performed using aseptic techniques in a sterile biosafety cabinet. The human neuroblastoma cell line (SK-N-SH) was cultured in Eagle's Minimum Essential Medium, EMEM (ATCC 30-2003), supplemented with 10% heat-inactivated fetal bovine serum and 1% of 10,000 U/ml penicillin and 10 mg/mL streptomycin per mL. Other solutions, such as phosphate buffer saline (PBS) and distilled water, were autoclaved before use.

The cells were grown as a monolayer at 37 °C in a humidified atmosphere containing 5% CO_2. Cells with 80% confluency were harvested from a 75 cm2 culture flask. The cells were rinsed with 10 mL of PBS, trypsinized using 5 mL of Trypsin-EDTA 1x, diluted in growth medium, and then centrifuged at 800 g. Cells were then counted using a hemocytometer and plated at a density of 2×10^4 cells per well in a 96-well plate. The cells were incubated for 24 hrs at 37 °C to confluency, with 5% CO_2 in a humidified atmosphere. All the 6MTHβC compounds were dissolved in 100% DMSO (stock solution) at 20 mM concentration. For the working solution, 5 µL of stock solution was diluted with fresh growth medium to 500 µL.

100 µL of a working solution containing compounds in triplicate at a final concentration of 50 µM in a medium was added to the cells and incubated for another 48 hrs. As a negative control, cells were treated with 200 µL of the vehicle in growth media. Cisplatin was used as a positive control drug. The final concentration of DMSO was kept at 0.5 % v/v in this experiment. After 48 hrs, 20 µL of aqueous MTT solution (5 mg/mL in PBS) was added to each well, and the plate was covered with aluminum foil and incubated at 37 °C for 3 hrs in a CO_2 incubator in the dark. After 3 hrs, the MTT solution was carefully decanted off, and dark blue formazan crystals were extracted from the cells with 100 µL of DMSO in each well. The optical density (OD) was measured with a 96-well ELISA microplate reader at 570 nm, with the reference filter set to 630 nm. All MTT assays were repeated three times. The cell viability of the tested compounds was estimated by directly comparing the OD value of the treated cells with that of the untreated cells. Percentage cell viability was calculated using the formula (Kyung et al., 2005):

$$\%\ Cell\ Viability = \frac{OD\ treated\ cell\ -\ blank}{OD\ untreated\ cell\ -\ blank} \times 100$$

FINDINGS

Twelve compounds were successfully screened for toxicity using the MTT assay. The reduction of the MTT assay was conducted to assess the cell viability. MTT is a water-soluble tetrazolium salt that is reduced to a coloured water-insoluble salt by metabolically viable cells. The number of living cells present is proportional to the transformed atoms of MTT tetrazolium salt into the blue-coloured product. The percentage of cell viability for each compound is summarized in Table 1. The MTT assay was conducted for twelve 6MTHβCs derivatives on SK-N-SH at a concentration of 50 µM. The positive control of the experiment is cisplatin. The results indicated that the eight 6MTHβCs derivatives showed more than 50% cell viability. Four compounds (2D, 4B, 4D, 5D) showed a percentage cell viability of less than 50% and are considered toxic at a concentration of 50 µM, as suggested by the previous report (Akundi et al., 2004).

Table 1 Percentage of cell viability for each compound

Compound	% Cell viability
Cisplatin	1.67 ± 0.48
1B	92.44 ± 3.12
1C	62.20 ± 2.21
2A	101.21 ± 4.05
2D	16.75 ± 1.74
4A	92.62 ± 2.18
4B	39.91 ± 4.66
4D	49.01 ± 7.62
5A	100.97 ± 1.59
5D	50.90 ± 0.63
6A	72.54 ± 4.11
7A	91.26 ± 0.53
7B	80.97 ± 0.23

Cell viability and/or cell proliferation are good indicators of cell health. Physical and chemical agents can influence cell health and metabolism. Cell toxicity can be caused by various processes, including cell membrane disintegration, protein synthesis suppression, irreversible binding to receptors, inhibition of polydeoxynucleotide

elongation, and enzymatic reactions (Aslantürk, 2018). In this study, in vitro toxicity against SK-N-SH was conducted to screen the 6MTHβCs derivatives. Generally, all the 6MTHβCs derivatives have the same skeleton (parent) but different substituents at C1 and N2. Using an indicator reported by Akundi and coworkers (2004), eight compounds (1B, 1C, 2A, 4A, 5A, 6A, 7A, and 7B) were considered nontoxic, as they had a percentage cell viability of more than 50%. At the same time, four compounds, namely 2D, 4B, 4D, and 5D, were toxic as their percentage of cell viability was less than 50%. The results clearly showed that different substituents at the C1 and N1 affect the properties of the compounds. From the results, it is evident that the compounds with substituents B (phenyl) and D (methoxyphenyl) exhibit toxicity effects on the SK-N-SH cells. The 2D was the most toxic to the cells since cell viability was the lowest (17%).

However, for the same series of compounds, such as 4B and 4D, the 4B compound is more toxic, possibly due to the absence of the methoxy group at the benzene ring attached to the N2. In contrast, the 4A compound with a hydrogen atom substituent at position N2 was safe. Compared across the series, but with similar substitutions in C1, such as between 1B and 4B, only 4B exhibits toxic properties due to the phenyl substituent attached to the C1 atom. When comparing 2D, 4D, and 5D, 2D was the most toxic compound, suggesting that the presence of a carbonyl group at the C1 position caused the toxic effect. This toxicity study involved only a limited number of compounds; therefore, a larger number of compounds are required to produce meaningful information. The previous report showed that tetrahydro-β-carbolines and 1,3,5-triazine hybrids are anticancer agents against eight human cancer cell lines and the normal human fibroblast (NIH3T3) cell line (Kumar et al., 2010). Other studies have reported that THβCs derivatives have toxicity in both in vitro and in vivo models, including neurodegeneration and Parkinson-type symptoms (Akundi et al., 2004). Thus, there is a possibility that the 6MTHβCs derivatives may exhibit toxic effects against specific cell lines.

CONCLUSIONS

The current research revealed that 6-methoxy-tetrahydro-β-carboline (6MTHβC) derivatives display significant cytotoxic effects on the SK-N-SH human neuroblastoma cell line. These findings underscore the promise of methoxy-substituted THβC scaffolds as viable options for advancing anticancer research. The observed cytotoxicity offers initial indications of biological activity; nevertheless, further research is necessary to clarify the mechanisms of action, assess selectivity for cancer cells compared to normal cells, and investigate structure–activity correlations. This research establishes a basis for progressing 6MTHβC derivatives into extensive pharmacological and toxicological studies.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

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