ความเป็นพิษต่อเซลล์ของเมลาโทนินและอนุพันธ์ ต่อเซลล์เพาะเลี้ยงชนิดเซลล์ตับ

Cytotoxicity of Melatonin and Derivatives on Human Liver Cell Lines

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ข้อมูลบทความ

ประวัติบทความ:

รับเพื่อพิจารณา: 23 พฤศจิกายน 2565

แก้ไข: 20 มีนาคม 2566

ตอบรับ: 20 มีนาคม 2566

คำสำคัญ:

เมลาโทนิน/อนุพันธ์เมลาโทนิน/ความเป็นพิษต่อเซลล์/เซลล์ตับปกติ/เซลล์มะเร็งตับ

บทคัดย่อ

เมลาโทนินเป็นฮอร์โมนที่ถูกสังเคราะห์และหลั่งโดยต่อมไพเนียล ซึ่งพบว่าเมลาโทนินเป็นสารที่มีคุณสมบัติในการเหนี่ยวนำให้เกิดการตายของเซลล์มะเร็งได้ อย่างไรก็ตามข้อจำกัดของเมลาโทนินคือเป็นฮอร์โมนที่มีค่าครึ่งชีวิตค่อนข้างสั้นและออกฤทธิ์กว้าง งานวิจัยนี้มีวัตถุประสงค์เพื่อสังเคราะห์อนุพันธ์เมลาโทนินโดยมุ่งเน้นศึกษาการแทนที่ที่ N-atom บนวงแหวนอินโดลของเมลาโทนินและทำการทดสอบอนุพันธ์ของเมลาโทนินโดยทดสอบในเซลล์เพาะเลี้ยงชนิดเซลล์ตับปกติของมนุษย์ (Chang liver) และเซลล์มะเร็งตับของมนุษย์ (HepG2) ศึกษาความเป็นพิษต่อเซลล์โดยใช้การทดสอบ MTT ผลการศึกษาพบว่าผลผลิตร้อยละ (percent yield) ของสารเอ็นเมทิลเมลาโทนิน อะซิเตตเมลาโทนิน เบนโซอิลเมลาโทนิน และเนปโทอิลเมลาโทนิน ร้อยละ 43.41, 44.78, 54.55 และ 30.50 ตามลำดับ ผลการทดสอบ MTT ไม่พบความเป็นพิษต่อเซลล์ของเมลาโทนินทั้งในเซลล์ตับปกติและเซลล์มะเร็งตับ หลังจากได้รับสารทดสอบ 24 ชั่วโมง ส่วนอนุพันธ์เมลาโทนินที่สังเคราะห์ได้พบว่ามีความเป็นพิษต่อเซลล์ทั้งสองชนิดแต่ อยู่ในระดับที่แตกต่างกัน ซึ่งผลการทดสอบดังกล่าวสามารถใช้เป็นข้อมูลเบื้องต้นในการศึกษาและพัฒนาสารอนุพันธ์เมลาโทนินไปเป็นยาแผนปัจจุบันใช้ในการรักษาโรคมะเร็งได้ต่อไป

Article Info

Article History:

Received: November 23, 2022

Revised: March 20, 2023

Accepted: March 20, 2023

Keywords:

Melatonin/Melatonin derivatives/Cytotoxicity/ Chang liver cell line/ HepG2 cell lines

Abstract

Melatonin (MT) is a hormone synthesized and secreted by the pineal gland that has been shown to directly induce cell death as well as exhibit oncostatic activity. However, its short biological half-life and the lack of selectivity of melatonin at the target sites limit its potential as an anticancer agent. This study aimed to develop a new class of anticancer agents by N-substituting the indole ring of melatonin and testing its derivatives on human liver (Chang) and human liver carcinoma (HepG2) cell lines for their cytotoxicity using MTT assay. The percent yield of N-methyl melatonin, Acetate melatonin, Benzoyl melatonin, and Napthoyl melatonin derivatives were 43.41%, 44.78%, 54.55% and 30.50%, respectively. Interestingly, melatonin showed no cytotoxicity on both Chang liver and HepG2 cell lines after 24 h exposure, while all its derivatives showed cytotoxicity on both cell lines at varying levels. Therefore, these findings provide promising insight for the development of melatonin derivatives as effective anticancer agent, which can be further improved for its selectivity in future study.

1. Introduction

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020. Human liver carcinoma is the most common cancer in the world and is responsible for more than 600,000 deaths annually. It is the third leading cause of cancer death in 2020 (830 000 deaths) [1-2]. The majority of patients with hepatocellular carcinoma die within 1 year after the diagnosis. At present, the treatment of hepatocellular carcinoma mainly includes surgery and chemotherapy, but the curative effects of the existing chemotherapeutic drugs are not good enough, and they have numerous side effects. Therefore, searching for highly efficient antitumor drugs remains a hot research area [3].

Melatonin (N-acetyl-5-methoxy tryptamine) is a hormone synthesized and secreted by the human pineal gland mainly during the night since light exposure suppresses its production. It has been shown to directly induce the cell death of different types of human tumors such as MCF-7 breast cancer cell line and prostatic tumor cell line LNCaP [4-6]. Moreover, melatonin exhibits natural oncostatic activity and inhibits cancer cell growth [7]. However, the use of melatonin as a drug is limited despite its low

toxicity. It also has some pharmacokinetic issues such as limited oral bioavailability and short half-life, which limit its tissue availability [8-9]. Therefore, many researchers try to modify its structure to improve pharmacology properties and also therapeutic effects.

This study aimed to develop a new class of anticancer agents from melatonin core structure, focusing on N-substitution at the indole ring and to test the cytotoxic effect of melatonin and its derivatives on human liver cell lines: Chang liver and human liver carcinoma cell line (HepG2).

2. Methodology

2.1 Chemicals and reagents

Human liver carcinoma (HepG2) cells were purchased from Cell Lines Service (CLS; Germany). Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM) and Fetal Bovine Serum (FBS) were obtained from Gibco Invitrogen (Grand Island, NY, USA). The chemical 3-(4,5-dimethylthiazol-2yl)-2,5 diphenyltetrazolium bromide (MTT) and antibiotic (streptomycin and penicillin) used for cell culture were acquired from Gibco Invitrogen (Grand Island, NY, USA). Chemicals and solvents for the preparation of melatonin derivatives are melatonin, methyl iodide (for N-methyl melatonin), acetic anhydride (for Melatonin acetate), benzoyl chloride (for benzoyl melatonin), napthoyl chloride (for napthoyl melatonin), DMAP (4 - Dimethylaminopyridine), dichloromethane, pyridine, methanol, hexane and ethyl acetate.

2.2 Preparation of melatonin derivatives

Weigh melatonin and DMAP were dissolved in a round bottle flask with 1 mL pyridine under CaCl2–tube to avoid moisture. The acid chloride was slightly added and stirred for 5 min in an ice bath. After that, it is continuously stirred at room temperature overnight to ensure the reaction is complete. Thin-layer chromatography was used for determining whether the reaction was completed. The reaction mixture was concentrated afterward by a rotary evaporator. Finally, crude products were further purified by column chromatography (CC) under various eluent systems, which started from a non-polar solvent (ex. 100% hexane) to a more polar solvent by the combination between hexane and ethyl acetate to give products as shown in Table 1.

2.3 Cell Culture

HepG2 and Chang liver cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin and penicillin. Cells were maintained at 37 °C in a 5% CO_2 incubator to allow the cells to grow and form a monolayer in the flask. In all experiments, the cells were grown to 70-80% confluence.

2.4 Measurement of cell viability (MTT assay)

The effects of melatonin and its derivatives on cell viability were determined using the MTT assay. HepG2 and Chang liver cells were cultured in a 24-well plate, and the cells were incubated for 24 h at 37 °C in a CO2 incubator of 5% and 95% air mixture. After 24 h, cells were

treated with various concentrations (12.5, 25, 50, 100, or 200 µM) of melatonin, and its derivatives (1% DMSO (Sigma-Aldrich, Germany) was used as a negative control. Cultured solutions were then removed and replaced by 200 µl of MTT solution (0.5 mg/mL in a culture medium). The cells were incubated for 20-30 min at 37 °C under CO_2. After that, the medium was removed, and the formazan crystals produced in the cells were dissolved by DMSO (500 µL/well). The absorbance was measured at 550 nm using a microplate reader (Bio-Rad, Model 680, USA).

At least three duplicates for each sample were used to determine the cell viability. Under these conditions, DMSO was not toxic, and cell survival in negative control was assumed to be 100%. The percentage of cell viability was calculated on the basis of the following formula:

$$\% \text{ Viability} = \frac{\text{Mean Absorbance of sample x 100}}{\text{Mean Absorbance of negative control}} \quad \text{.....(1)}$$

2.5 Statistical Analysis

The data were displayed as a mean ± S.E of results obtained from at least three samples. One-way analysis of variance (ANOVA) was used to assess the significant difference between the treatment and the control samples, and a p-value < 0.05 was considered statistically significant.

3. Results

In this study, we tested new compounds including N-methyl derivatives and ester derivatives (acetate, benzoyl, and napthoyl derivatives). There are two substitutions for ester derivatives: alkyl group (acetate derivatives) and aryl group (benzoyl and napthoyl derivatives). All of the derivative compounds and structures are shown in Figure 1.

Table 1 shows the percentage yield of the products that we found. After obtaining the melatonin derivatives, all compounds were determined for effects on cell viability by using an MTT assay.

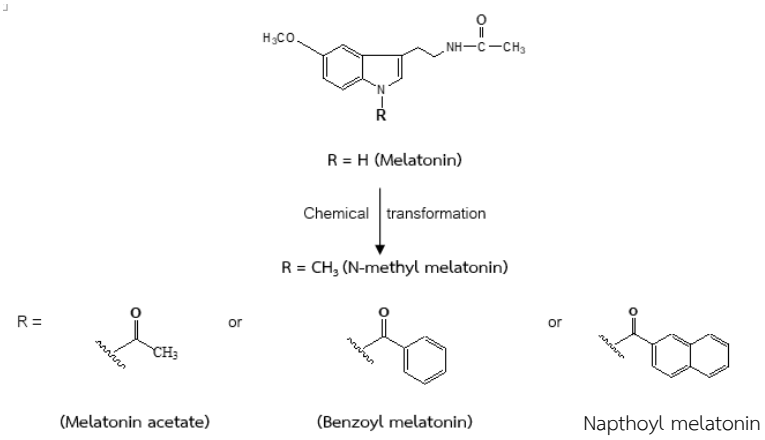

Figure 1 Chemical structures of melatonin and Its derivatives

Table 1 Percent yield of melatonin derivatives.

Melatonin derivatives	Yield (%)
N-methyl melatonin	43.41
Melatonin acetate	44.78
Benzoyl melatonin	54.55
Napthoyl melatonin	30.50

The result showed no cytotoxicity of melatonin on both Chang liver and HepG2 cell lines after 24 h exposure when compared to a control group (Figure 2). For the result of melatonin derivative, all its derivatives showed cytotoxicity on both cell lines after 24 h exposure when compared to a control group. At low concentrations (12.5 – 25 µM), N-methyl melatonin showed no cytotoxicity to Chang liver cells. However, at higher concentrations (50 – 200 µM), it showed cytotoxicity, which was significantly lower in % viability when compared to the control group ($p < 0.05$) (Figure 3). The result showed acetate melatonin at all concentrations had no cytotoxicity on Chang liver cells, but it showed significant cytotoxicity to HepG2 cells ($p < 0.05$) at high concentrations (100 – 200 µM) (Figure 4). Finally, for benzoyl and napthoyl derivatives, the results were similar on both cell lines. Benzoyl melatonin was significantly lower in % viability at all concentrations when compared to the negative control group ($p < 0.05$) (Figure 5). At low concentrations, napthoyl melatonin had no significant differences ($p > 0.05$) among the control group, while higher concentrations showed significantly different results when compared to the control group ($p < 0.05$) (Figure 6).

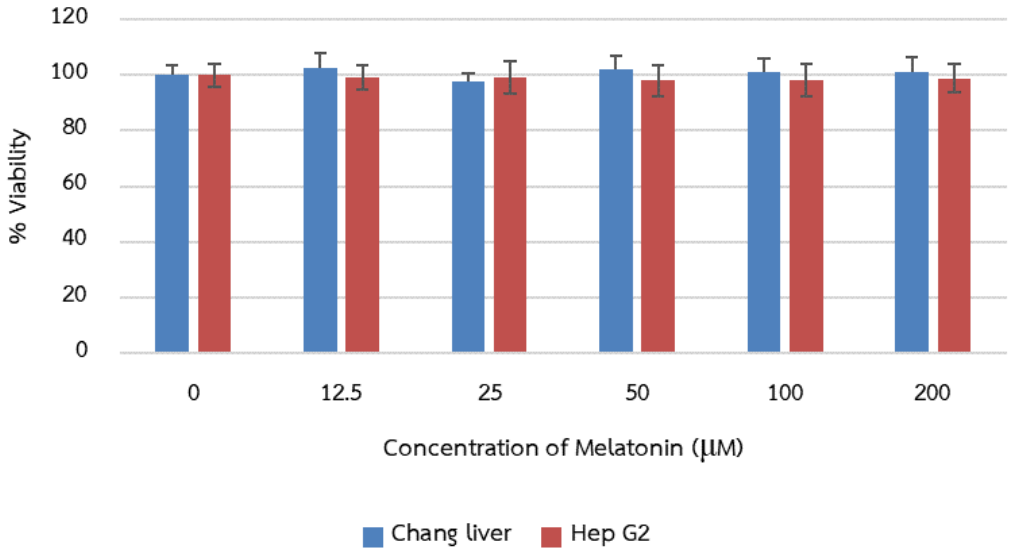

Figure 2 Viabilities (%) of Chang liver and HepG2 cell lines after 24 h exposure to melatonin at various concentrations. All data are expressed as mean ± S.E. (n=3).
*Groups are significantly different from control values ($p < 0.05$).

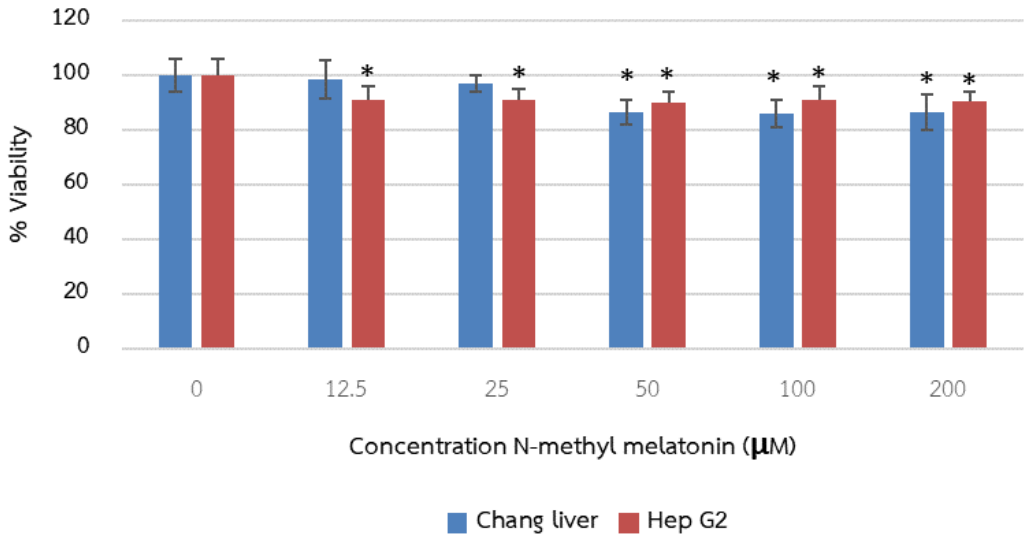

Figure 3 Viabilities (%) of Chang liver cell and HepG2 after 24 h exposure to N-methyl melatonin derivatives at various concentrations. All data are expressed as mean ± S.E (n = 4). *Groups are significantly different from control values ($p < 0.05$).

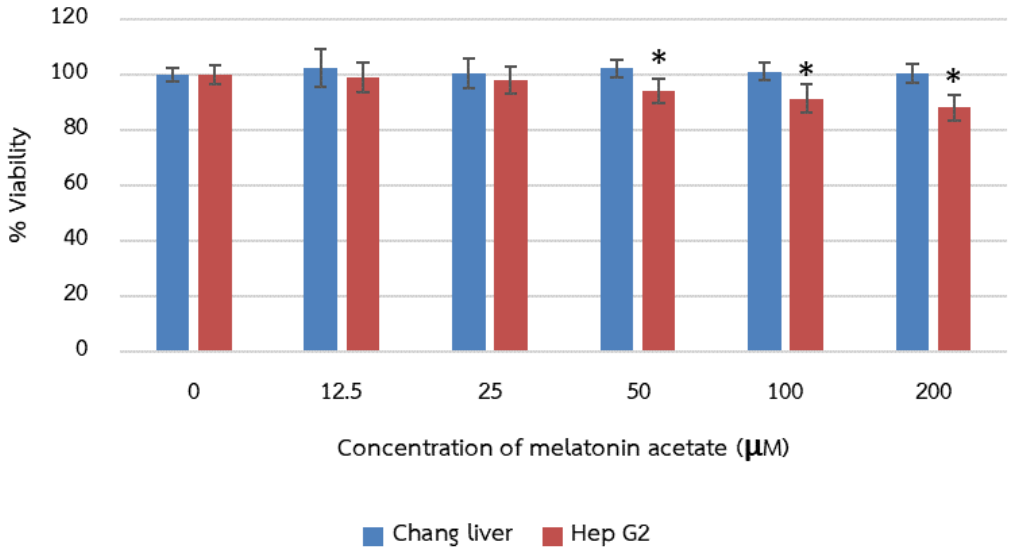

Figure 4 Viabilities (%) of Chang liver cell and HepG2 after 24 h exposure to melatonin acetate derivatives at various concentrations. All data are expressed as mean ± S.E (n = 4). *Groups are significantly different from control values ($p < 0.05$).

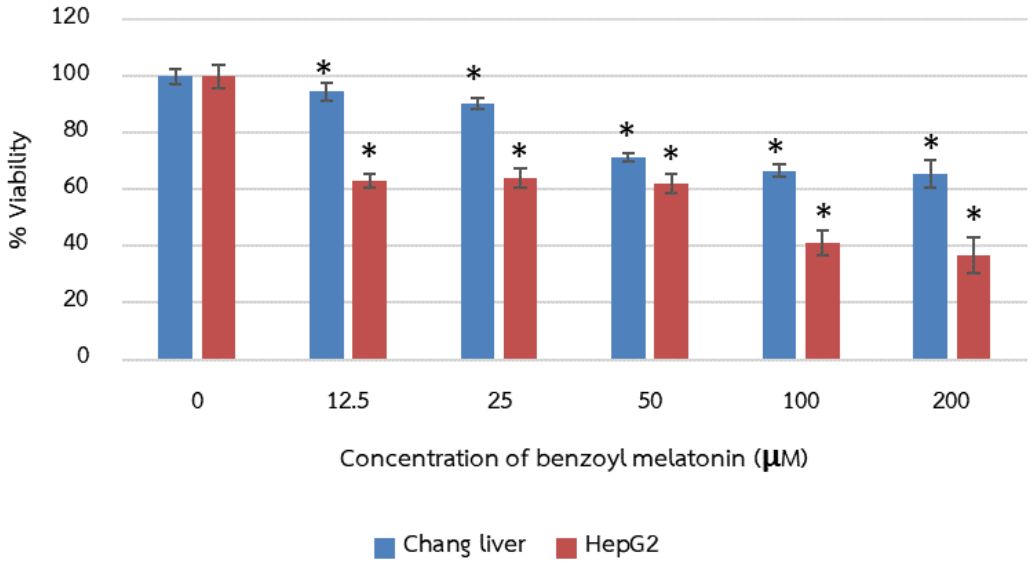

Figure 5 Viabilities (%) of Chang liver cell and HepG2 after 24 h exposure to benzoyl melatonin derivatives at various concentrations. All data are expressed as mean ± S.E (n = 4). *Groups are significantly different from control values ($p < 0.05$).

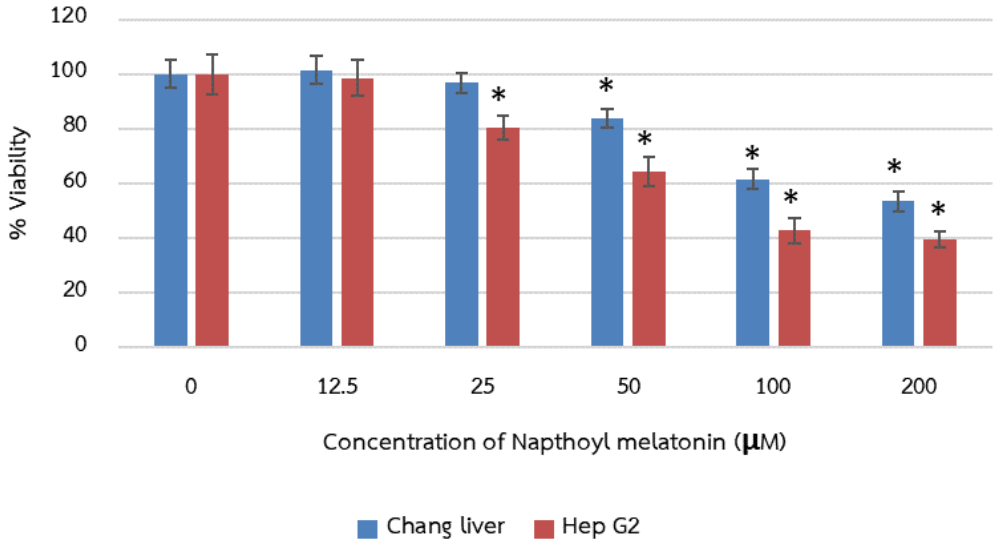

Figure 6 Viabilities (%) of Chang liver cell and HepG2 after 24 h exposure to napthoyl melatonin derivatives at various concentrations. All data are expressed as mean ± S.E (n = 4). *Groups are significantly different from control values ($p < 0.05$)

4. Discussion

A considerable amount of evidence has been documented on the efficacy of melatonin in reducing tumor growth. While most of these data are from studies on experimental animals [10-11], trials in humans [12-13] with a wide variety of different cancers are also suggestive of the oncostatic

actions of melatonin. Moreover, in vitro model found melatonin inhibited the growth of prostate cancer cells (LNCaP). [7] In addition, many studies suggest that natural bioactive compounds and their synthetic derivatives could be used in combination with traditional chemotherapeutic agents as potential anti-cancer therapies. [14-15] In this study, we employed melatonin derivatives and studied the ability to promote cell death in human liver carcinoma (HepG2) cells. According to these studies, all newly synthesized compounds were tested for cytotoxicity in normal human liver cell lines (Chang liver) and human liver carcinoma cell lines (HepG2) using an MTT assay. The results showed no cytotoxicity of melatonin (12.5, 25, 50, 100, 200 μM) on both Chang liver and HepG2 cell lines after 24 h exposure when compared to a control group. The results of this study are consistent with a study by Fan et al. (2010), which tested the cell toxicity of melatonin by MTT assay by raising HepG2 and Bel-7402 liver cancer cells in a cell culture diet containing melatonin concentrations of 10-8-10-5 mol/L for 48 h. [16] However, all of the melatonin derivatives showed cytotoxicity on both cell lines after 24 h exposure when compared to the control group. Interestingly, all compounds, especially nathoyl- and benzoyl-melatonins, exhibited slight selectivity against HepG2 cells over Chang liver cells, and melatonin derivatives showed higher potency than melatonin, especially the ester derivatives. The Aryl group (benzoyl and napthoyl melatonin derivatives) showed high cytotoxicity on both cell lines ($p<0.05$) and more than N-methyl melatonin derivatives (benzoyl melatonin > napthoyl melatonin > acetate melatonin > N-methyl melatonin> melatonin).

5. Conclusion

According to these results, melatonin derivatives showed higher potency than melatonin, especially the ester derivatives. The Aryl group (benzoyl and napthoyl melatonin derivatives) showed high cytotoxicity on both cell lines ($p<0.05$) and more than N-methyl melatonin derivatives. These results suggest that newly synthesized melatonin derivatives, N-methyl melatonin, acetyl melatonin, and benzoyl melatonin have cytotoxicity to HepG2 cell lines more potent than its parent compound. Melatonin and Its cytotoxicity potency may be partially associated with more lipophilicity of new synthesis compounds, leading to higher cell membrane penetration. Lipophilicity is an important drug property, which impacts drug uptake and metabolism. It also plays a dominant role in promoting off-target binding or promiscuity, with increased lipophilicity leading to an increased likelihood of binding to unwanted cellular targets. Therefore, these derivatives should be studied for their specific actions on cells. However, the results of this study could be used as preliminary data for the future development of melatonin structure for better selectivity.

6. Acknowledgements

Financial support from The National Research Council of Thailand (NRCT) in FY and Melatonin research group Khon Kaen University (KKU), Thailand.

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