SYNTHESIS, CHARACTERIZATION AND MOLECULAR DOCKING OF HYDROXYXANTHONE AND PRENYLATED XANTHONE DERIVATIVES AS POTENTIAL AROMATASE INHIBITOR

By

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Xanthone is an important scaffold for the development of new drugs due to its various positions and types of attached substituents with specific functions. This makes xanthone and their derivatives capable of exhibiting a variety of cytotoxic and biological activities. By discovering new lead compounds, the threats of existing and emerging diseases can be managed and synthesis is one of the most important approaches to this.

This research project aimed to design and synthesize a series of xanthones with different types of attached substituents that were evaluated for their anti-cancer activity against breast cancer cell lines for their potential as aromatase inhibitors. These compounds were *in vitro* tested against breast cancer cell lines, MCF-7 and MDA-MB-231 cell lines which can lead to the discovery of new anticancer drug candidates for future drug discovery research. A total of twenty xanthones were synthesized where a series of hydroxyxanthone (**45-52**) were firstly prepared *via* modified Grover, Shah and Shah method, before further reacted with alkyl halide under reflux to obtain 3-*O* alkylated xanthone derivatives (**53** and **54**), while reacted with prenal to obtain prenylated xanthone derivatives (**55-58**) and then further reacted with alkyl halide to obtain alkylated prenylxanthone derivatives (**59-64**). The structures of synthesized xanthones were analyzed using spectroscopic methods such as Direct injection-mass spectroscopy (DI-MS), Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR). Molecular docking studies were performed to study the binding affinity of these compounds (**45-64**) towards breast cancer enzyme, aromatase (PDB: 3EQM) . Based on the *in vitro* results, most of the compounds did not show any significant activity against the breast cancer cell lines except compounds **51** and **55** which showed moderate activity against the MCF-7 cell line with an IC_{50} value of 50±0.69 µM and 50±1.66 µM, respectively. However, only compound **51** showed moderate activity against the MDA-MB-231 cell line with an IC_{50} value of 60±0.67 µM, while compound **55** did not show any activity. These two compounds were then tested against VERO (African Green Monkey Kidney) and BEAS-2B (Lung Epithelial) cell lines to study their toxicity against

normal cells. From the results obtained, compound **55** showed lesser toxicity against VERO and BEAS-2B cell lines with an IC_{50} value of 60±1.41 µM and 60±0.85 µM respectively, while compound **51** showed higher toxicity against normal cell VERO and BEAS-2B cell lines with an IC_{50} value of 35±1.51 µM and 40±0.70 µM compared to cancer cells.

Hence, compounds **51** and **55** were chosen to study their binding interactions with aromatase (PDB ID: 3EQM), where the binding affinities of compounds **51** and **55** were -7.9 kcal/mol and -9.4 kcal/mol, respectively. Based on the results obtained, a series of interactions with the most of the amino acid residues in aromatase were involved in compound **51** and compound **55** which could play an important role in binding in the aromatase enzyme.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SINTESIS, PENCIRIAN DAN PENGEDOKAN MOLEKUL BAGI DERIVATIF HIDROKSIXANTON DAN TERFRENIL XANTON SEBAGAI POTENSI PERENCAT AROMATASE

Oleh

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Xanton merupakan antara kumpulan yang penting dalam proses pencarian ubat-ubatan yang baharu disebabkan oleh kepelbagaian kedudukan dan jenis kumpulan kimia yang terikat kepada xanton yang mempunyai fungsi tertentu. Ini menjadikan xanthone dan derivatifnya mempunyai pelbagai aktiviti sitotoksik dan biologi. Dalam pencarian sebatian yang baharu, ancaman penyakit yang sedia ada dan yang baru muncul boleh ditangani dan sintesis adalah salah satu pendekatan yang paling penting bagi hal ini.

Projek penyelidikan ini bertujuan untuk mereka bentuk dan menghasilkan satu siri xanton dengan pelbagai jenis kumpulan kimia yang dinilai untuk aktiviti anti-kanser terhadap sel kanser payudara sebagai perencat aromatase. Sebatian ini telah diuji secara *in vitro* terhadap sel kanser payudara, menggunakan sel MCF-7 dan MDA-MB-231 yang mempunyai kebolehan sebagai calon ubat antikanser yang baharu untuk penyelidikan ubat masa hadapan. Di dalam kajian ini, sebanyak dua puluh xanton telah disintesis di mana siri hidroksi xanton (**45-52**) telah dihasilkan terlebih dahulu melalui kaedah Grover, Shah dan Shah yang diubah suai, sebelum bertindak balas dengan alkil halida di bawah refluk untuk menghasilkan derivatif xanton teralkil 3-*O* (**53** dan **54**), manakala hidroksi xanton telah bertindak balas dengan prenal untuk menghasilkan derivatif terprenil xanton (**55-58**) dan bertindak balas dengan alkil halida untuk menghasilkan xanthon alkil terprenil (**59-64**). Struktur xanton yang telah disinthesis, telah dianalisis menggunakan kaedah spektroskopi seperti spektroskopi jusim-suntikan terus (DI-MS), spesktroskopi inframerah-penjelmaan fourier (FTIR) dan resonans nuklear magnetik (NMR). Pengedokan molekul dilakukan untuk mengkaji pertalian pengikatan sebatian (**45-64**) terhadap enzim kanser payudara, aromatas (PDB: 3EQM). Berdasarkan keputusan *in vitro*, kebanyakan sebatian tidak menunjukkan sebarang aktiviti yang ketara terhadap garisan sel kanser payudara kecuali sebatian **51** dan **55** yang menunjukkan aktiviti yang sederhana terhadap garis sel

MCF-7 dengan nilai IC_{50} 50±0.69 µM dan 50±1.66 µM. Walau bagaimanapun, hanya sebatian **51** menunjukkan aktiviti sederhana terhadap sel MDA-MB-231 dengan nilai IC_{50} 60±0.67 µM, di mana sebatian **55** tidak menunjukkan sebarang aktiviti. Kedua-dua sebatian ini kemudiannya diuji terhadap saluran sel VERO (buah pinggang monyet hijau africa) dan BEAS-2B (epitelium paru-paru) untuk mengkaji ketoksikannya terhadap sel normal.

Daripada keputusan yang diperoleh, sebatian **51** menunjukkan ketoksikan yang lebih rendah terhadap garisan sel normal, VERO dan BEAS-2B dengan nilai IC_{50} 60±1.51 µM dan 60±0.85 µM, manakala sebatian **55** menunjukkan ketoksikan yang lebih tinggi terhadap garisan sel normal, VERO dan BEAS-2B dengan nilai IC_{50} 35±1.51 µM dan 40±0.70 µM berbanding sel kanser. Oleh itu, sebatian **51** dan **55** dipilih untuk mengkaji interaksi pengikatannya dengan aromatase (PDB ID: 3EQM) di mana afiniti pengikatan sebatian **51** dan **55** ialah sebanyak -7.9 kcal/mol dan -9.4 kcal/mol. Berdasarkan keputusan yang diperolehi, satu siri interaksi dengan majoriti sisa asid amino dalam aromatase terlibat dalam sebatian **51** dan sebatian **55** yang boleh memainkan peranan penting dalam mengikat enzim aromatase.

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LIST OF ABBREVIATIONS

γ	gamma
α	alpha
δ	chemical shift in ppm
UV	ultraviolet
AChE	acetylcholinesterase
BuChE	butyrylcholinesterase
μM	micromolar
mmol	millimolar
ml	milliliter
mg	milligram
^{o}C	degree celcius
%	percentage
^{13}C	carbon-13
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
cm^{-1}	per centimeter
d	doublet
dd	doublet of doublet
DI-MS	Direct Injection-Mass Spectroscopy
DMSO	dimethyl sulfoxide
FT-IR	Fourier Transform-Infrared spectroscopy
GC-MS	Gas Chromatograph-Mass Spectroscopy
^{1}H	proton
Hz	Hertz
IC_{50}	Inhibitor concentration at which the enzyme reaction velocity is 50% of the uninhibited reaction
m	multiplet

m/z	Mass-to-charge ratio
m.p.	melting point
NMR	Nuclear Magnetic Resonance
PDB	Protein data bank
ppm	Parts per million
Rf	retention factor
rt	room temperature
SAR	Structure-activity relationship
s	singlet
t	triplet
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
HMBC	Heteronuclear Multiple Bond Connectivity by 2D
HSQC	Heteronuclear Single Quantum Coherence
IR	Infrared

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Xanthones are secondary metabolites that are commonly found in higher plant families, fungi, and lichens (Vieira & Kijjoa, 2005). Xanthones (9*H*-xanthen-9-ones) are a class of yellow-coloured heterocyclic organic compounds containing oxygen, whereby their molecular formula is $C_{13}H_8O_2$ and they depict a dibenzo-γ-pyrone framework as shown in **Figure 1.1**. Xanthone is an important scaffolding material in the development of new drugs due to its promising biological activities.

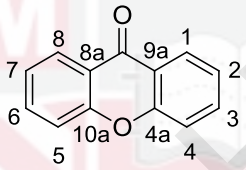

Figure 1.1: Xanthone scaffold

In the 1960's, xanthones were isolated from lower fungi, lichens, and only three families of flowering plants, including Gentianaceae, Clusiaceae Lindl, which also known as Gutifferae, and Anacardiaceae (Roberts, 1961). While in 1992, Mandal *et al*. reported that plants from 20 other families also produce xanthones. However, Gentianaceae and Guttiferae were identified as the main sources of xanthone derivatives among flowering plant families that year (Mandal, Das, & Joshi, 1992). Clusiaceae is also one of the families that highly produces xanthones (Perest & Nagem, 1997; Peres, Nagem, & de Oliveira, 2000). *Garcinia mangostana* L., which belongs to the Clusiaceae family, commonly known as mangosteen, can be found in the region of Southeast Asia and is now highly popular due to the biological activity of its phytochemicals. **Figure 1.2** shows a total of 1225 xanthones were successfully isolated between 2012 and 2019 from 23 different plant families (L. Klein-Júnior *et al*., 2020).

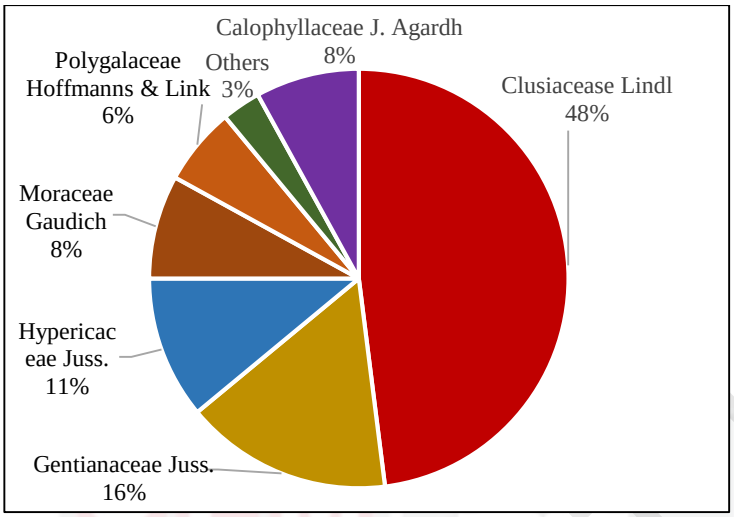

Figure 1.2: Pie chart of xanthone distribution by plant family (period of isolation 2012-2019).

1.2 Synthetic approaches to xanthones

There were various xanthones have been successfully isolated from natural resources with different patterns of substitution, which leads to a wide variety of compounds with biological interest. However, isolation of xanthones from natural resources is difficult to obtain since the concentration of xanthones in the plants are present in low yield. Hence, due to this limitation, the synthesis of xanthones has been explored. New xanthones with different positions and natures of substituents on the building block can be obtained *via* synthetic methods. Synthetic methods are divided into two methods, which are biosynthesis and chemical synthesis. Chemical synthesis of xanthones involves catalytic reactions that are carried out in the laboratory, while biosynthesis is carried out in living organisms where an enzymatic reaction is used to produce numerous xanthone derivatives.

1.3 Biological activities of xanthones

Xanthones are an important class of biologically active heterocycles that have wide potential in the medicinal chemistry field due to their significant biological and pharmacological applications. Hence, many researchers are focusing on the synthesis of various xanthone derivatives for the development of potent and novel drugs.

Most of the studies conducted earlier clearly showed that the privileged structure of xanthones displays a wide range of biological activities such as anticancer *(Pedro et al*.,

2002) anti-tumoural (Luo *et al*., 2013), anticholinesterase (Menéndez *et al*., 2017), α-glucosidase inhibiton (Liu *et al*., 2008) and melanogenesis inhibition (Rosa *et al*., 2021) activities.

Cancer is known as a disease that is characterized by the division of cells uncontrollably which able to invade other tissues by metastasis (Suphavenich *et al.*, 2009). Metastasis is known as the spreading of the cancer cell to the neighbouring cell. Due to the structural diversity and biological aspects, researchers are attracted to xanthone in search for potent and new anticancer drug candidates (Ito *et al*., 2003). Over the last years, studies on anticancer and anti-inflammatory activities of xanthones showed significant findings with their efficacy and low toxicity in normal cells (Gutierrez-Orozco & Failla, 2013; Pérez-Rojas et al., 2016).

All over the world, one of the severest problems among the aged population is Alzheimer's disease (AD), which is a progressive and degenerative disorder. The most predominant treatment strategy for AD is the use of cholinesterase inhibitor drugs. In recent research, it was found that dihydroxyxanthone Mannich base derivatives were potential dual inhibitors of both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) for the treatment of AD (Qin *et al*., 2013).

In a recent study in 2019, new xanthone-triazole derivatives were designed and synthesized as anti-diabetic agents that showed great activity towards α-glucosidase inhibition (Ye *et al*., 2019). α-Glucosidase is an enzyme that catalyzes the final step of carbohydrate digestion in the biological system. Therefore, α-glucosidase inhibitors have a wide range of applications in medicinal chemistry. They play an important role in elucidating the action mechanism of α-glucosidase at molecular levels, and in the development of chemotherapeutics for clinical use in the treatment of carbohydrate mediated diseases, such as diabetes, cancer, HIV, hepatitis, hyperlipoproteinemia, and obesity.

UV radiation is one of the main external factors that affect the integrity of human skin. Excessive exposure to UV radiation increases the production of Reactive Oxygen Species (ROS), which initiate the processes involved in photoaging. Repeated exposure to this radiation also increases melanin production, which leads to various pigmentation diseases. A series of synthesized xanthones were reported to prevent the harmful effects of UV radiation and high production of ROS. Most of the compounds showed better activity compared to the reference compound, kojic acid (Rosa *et al*., 2021).

Therefore, this study was aimed to synthesize the xanthone scaffold followed by incorporating the cyclic ring into the system and study its biological activities, specifically on breast cancer. Furthermore, the structure-activity relationship (SAR) of the selected xanthones that show better activity towards the *in vitro* testing were analyzed too. Hence, by modifying xanthone to create a series of novel derivatives, new anticancer

drug, which clearly demonstrated its potential for development as a new antitumour agent may be able to discover from this study (García-Niño et al., 2017).

1.4 Problem Statement

Cancer is a leading cause of death around the world, accounting for nearly 10 million deaths in 2020. The most common type of cancer in 2020 was breast cancer, where 2.26 million new cases were reported. Based on previous studies, xanthones isolated from plants showed promising bioactivity towards anti-cancer (Klein-Júnior *et al*., 2020). However, since xanthones from natural resources are practically limited in position and type of the substituents, the synthetic approach to producing xanthones may lead to the possibility of producing xanthones with various positions and natures of the substituents on the xanthone nucleus.

Therefore, a series of hydroxyxanthones, 3-*O*-alkylated xanthones, prenylated xanthones and alkylated prenylxanthone were designed and synthesized with different functional groups on the xanthone scaffold to enhance its bioactivity towards breast cancer. Later, docking studies will be carried out in order to understand the binding mode of the synthesized xanthone derivatives with the breast cancer enzyme. Since aromatase, a cytochrome P450 enzyme complex present in breast tissues, is highly expressed in the MCF-7 cell line, the binding affinity of these synthesized compounds to aromatase was investigated using the docking method (Elekofehinti, 2015).

1.5 Objectives of the study

- To synthesize a series of hydroxyxanthones, 3-*O*-alkylated xanthones, prenylated xanthones and alkylated prenylxanthone.
- To structurally characterize the synthesized compounds *via* spectroscopic methods.
- To screen and evaluate the *in vitro* cytotoxic activity of the derivatives on breast cancer cell lines, MCF-7 and MDA-MB-231 and normal cell lines VERO and BEAS 2B.
- To study the binding affinity and the interactions of the most active compound with the aromatase enzyme using molecular docking.

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