SYNTHESIS AND ANTIOXIDANT ACTIVITIES OF HYDROXYLATED COUMARINYL CHALCONES

MOHAMAD SHAFIQ BIN SULEIMAN

UNIVERSITI TEKNOLOGI MALAYSIA

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MOHAMAD SHAFIQ BIN SULEIMAN

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ABSTRACT

Hydroxylated coumarins and chalcones are known to possess potent antioxidant activities. The present study highlights the synthesis of two hydroxycoumarins namely 3-acetyl-7-hydroxycoumarin and 3-acetyl-6-hydroxycoumarin. Both compounds were synthesized using the Knoevenagel condensation method with respective hydroxybenzaldehydes under basic conditions. The synthesized hydroxycoumarins served as an intermediate for the subsequent coupling reaction to produce three new coumarinyl chalcones known as 7-hydroxy-3-[3-(4′-hydroxyphenyl)prop-2-enoyl]-2*H*-1-benzopyran-2-one, 6-hydroxy-3-[3-(4′-hydroxyphenyl)prop-2-enoyl]-2*H*-1-benzopyran-2-one and 7-hydroxy-3-[3-(3′,4′-dihydroxyphenyl)prop-2-enoyl]-2*H*-1-benzopyran-2-one respectively. The target products were synthesized *via* the Claisen-Schmidt condensation reaction utilizing the Lewis acid, boron trifluoride-etherate (BF_3-Et_2O) in 1,4-dioxane. The structures of the synthetic compounds were confirmed by spectroscopic techniques which includes the Infrared (IR) and Nuclear Magnetic Resonance (NMR) (^1H, ^{13}C and DEPT) Spectroscopies. The antioxidant activities of all synthesized compounds were evaluated using three antioxidant assays known as the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric Reducing Ability of Plasma (FRAP) assays using the Bio-Tek Epoch microplate reader. Among the compounds tested, the coumarinyl chalcones exhibited a better antioxidant activity compared to the hydroxycoumarins. 7-Hydroxy-3-[3-(3′,4′-dihydroxyphenyl)prop-2-enoyl]-2*H*-1-benzopyran-2-one proved to be the best antioxidant in all assays with SC_{50} values of 0.57 mM and 0.036 mM in the ABTS and DPPH assays respectively. The FRAP value of this compound falls between the range of 0.09 mM to 1.67 mM. The values were comparable with the positive control used in these assays known as butylated hydroxyanisole (BHA).

ABSTRAK

Hidroksi kumarin dan kalkon terkenal dengan aktiviti antioxidan yang kuat. Kajian ini menonjolkan hasil sintesis bagi dua hidroksikumarin yang bernama 3-asetil-7-hidroksikumarin dan 3-asetil-6-hidroksikumarin. Kedua-dua sebatian ini disintesis menggunakan kaedah kondensasi Knoevenagel dengan hidroksibenzaldehid masing-masing dalam keadaan bes. Dua hidroksikumarin yang disintesis ini digunakan sebagai sebatian perantara untuk tindak balas penggandingan yang dilakukan berikutnya untuk menghasilkan tiga sebatian kumarinil kalkon yang masing-masing dikenali sebagai 7-hidroksi-3-[3-(4′-hidroksifenil)prop-2-enoil]-2*H*-1-benzopiran-2-on, 6-hidroksi-3-[3-(4′-hidroksifenil)prop-2-enoil]-2*H*-1-benzopiran-2-on dan 7-hidroksi-3-[3-(3′,4′-dihidroksifenil)prop-2-enoil]-2*H*-1-benzopiran-2-on. Ketiga-tiga sebatian sasaran disintesis melalui reaksi kondensasi Claisen-Schmidt dengan menggunakan sejenis asid Lewis, boron-trifluorida eterat (BF_3-Et_2O) dalam 1,4-dioksan. Struktur sebatian sintetik ini disahkan menggunakan teknik spektroskopi termasuk Spektroskopi Inframerah (IR) dan Resonans Magnet Nukleus (NMR) (^1H, ^{13}C dan DEPT). Penilaian antioksidan ke atas sebatian yang disintesis juga dijalankan menerusi tiga ujian antioksidan iaitu ujian 2,2-difenil-1-pikrilhidrazil (DPPH), ujian 2,2'-azinobis-(3-etilbenzotiazolin-6-asid sulfonik) (ABTS) dan ujian Kemampuan Plasma Menurunkan Ferik (FRAP) menggunakan Bio-Tek pembaca mikroplat Epoch. Antara semua sebatian yang diuji, sebatian kumarinil kalkon menunjukkan aktiviti antioksidan yang lebih tinggi berbanding sebatian hidroksikumarin.7-Hidroksi-3-[3-(3′,4′-dihidroksifenil)prop-2-enoil]-2*H*-1-benzopiran-2-on terbukti menjadi antioksidan terbaik dalam semua ujian antioksidan yang dijalankan dengan bacaan SC_{50} 0.57 mM dan 0.036 mM masing-masing dalam ujian ABTS dan ujian DPPH. Nilai FRAP sebatian ini ialah antara 0.09 mM hingga 1.67 mM. Nilai-nilai ini dapat dibandingkan dengan kawalan positif yang digunakan dalam ujian-ujian ini yang dikenali sebagai butil hidroksianisol (BHA).

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LIST OF ABBREVIATIONS

λ	-	Wavelength
ABTS	-	2,2′-Azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)
Ac_2O	-	Acetic Anhydride
BHA	-	Butylated Hydroxy Anisole
CC	-	Column Chromatography
COSY	-	Correlation Spectroscopy
^{13}C-DEPT	-	Carbon-13 Distortionless Enhancement by Polarization Transfer
^{13}C-NMR	-	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
ESR	-	Electron Spin Resonance
EtOAc	-	Ethyl Acetate
EtOH	-	Ethanol
FRAP	-	Ferric Reducing Ability of Plasma
^{1}H-NMR	-	Proton Nuclear Magnetic Resonance Spectroscopy
IR	-	Infrared Spectroscopy
J	-	Coupling Constant
EtOK	-	Potassium Acetate
EtONa	-	Sodium Acetate
ORAC	-	Oxygen Radical Absorbance Capacity
PAL	-	Phenylalanine Ammonia Lyase
PMN	-	Polymorphonucleate
TLC	-	Thin Layer Chromatography
UDP	-	Uridine Diphosphate
UV	-	Ultraviolet

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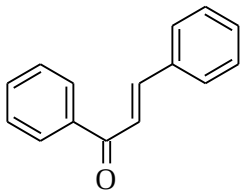
CHAPTER 1

INTRODUCTION

1.1 Background of Study

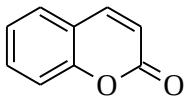
Naturally occurring compounds are produced as a result of biodiversity in which organisms interact with their surroundings to yield various chemical entities necessary for the organisms to survive [1]. Chalcone is a class of natural compounds that can be isolated from various plant species and their existence in the plant kingdom is vast. Chalcones are also known by the name of 1,3-diaryl-2-propen-1-one **(1)** [2]. There are quite a number of natural and synthetic chalcones that display useful bioactivities which includes cytotoxicity, chemoprotective, antibacterial, antifungal, antiviral and antiflammatory properties [3]. Based on the biosynthesis of flavonoids in plants, chalcones exist as an intermediate as well as the final product [2].



(1)

Another class of naturally occurring compounds is known as coumarin **(2)** or 1,2-benzopyrone. Naturally occurring coumarins are some of the most abundant chemicals in natural products [4]. It is the parent molecule of the compound dicoumarol. Compound **(2)** has the simplest structure within a huge class of phenolic compounds and they consist of a benzene ring fused to an α-pyrone ring [5].

Previous reports have showed that compound **(2)** and its several simple derivatives have antitumor properties. The biological and pharmacological effects of these compounds largely depends on the position and the type of substituents attached to the compound [6]. Some reports have shown that coumarins possessed antimicrobial, inhibited lipooxygenase and cyclooxygenase metabolic pathways, antioxidant, inflammatory and antitumoral activities [7, 8].



(2)

A wide range of natural products such as the ones mentioned above can be taken as chemical scaffolds as they are able to provide templates with high potential for combinatorial chemistry since they have the ability to display chemical information in a three-dimensional space. These countless drug classes aids therapeutic areas of infectious diseases and oncology as they are able to interact with numerous specific targets within the cell. Also, for many years, they have been deliberated as the base molecules in the process of drug discovery and development. Libraries are constructed with a basis of those scaffolds thus having the potential for both lead discovery and lead optimization. In lead discovery the compound is hoped to have an effect against targets unrelated to the original activity of the natural product and in lead optimization the compound is derivatized with the hope of improving its properties over the natural product [9].

Nowadays, hybrid molecules are the current trend in drug development. Several biological characteristics are able to be observed with these hybrid molecules. Recently, several reports proposed that coumarins having coupled with other molecules possessing a different biological activity will exhibit dual bioactivities. The coupling process will also enhance the properties of compounds to exhibit activities such as antiplatelet, antioxidant and anti-inflammatory activities [10 - 12].

In recent years, the demands for natural product inspirited drug-like molecules or their libraries have hiked and therefore, it imposes the necessity for the development of reaction sequences and linking strategies that allow complex and assorted target molecules to be constructed in a more facile and reliable manner [13]. Therefore, the overall mission or target in any organic synthesis is to build or construct any organic molecules that are desired. Synthesizing bioactive natural compounds are among the goal in doing organic synthesis. Presently, organic synthesis is considered to be very important since natural products synthesis is not the only pathway to synthesis compounds having useful properties anymore. Organic synthesis has also evolved that some useful properties are able to be discovered due to the synthetic studies conducted [2].

1.2 Problem Statement

Recent developments in pharmacology are looking into the coupling or combination of two pharmacophores within a molecule. These combinations allow the availability of active sites that are able to accommodate two different targets within the same molecule. The coupling of these pharmacophores provides a way to surpass drug resistance [14] and lowering the emergence of new resistant strains [15]. Cellular oxidative stress are created due to a rise in free radicals, it plays an important role in the aging process through pathogenesis apart from other diseases which includes cancer, atherosclerosis, diabetes and Alzheimer's disease [16, 17]. Hence, it would be intriguing to synthesize hybrid molecules and investigate whether these molecules are able to relieve oxidative stress as the research and development of antioxidants have drawn a great deal of attention in recent years [18].

1.3 Objectives of Study

This study was designed based on the following objectives:

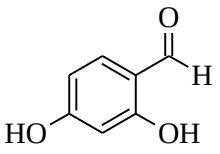
(a) To synthesize hydroxycoumarins and hydroxylated coumarinyl chalcones.

(b) To characterize the structure of synthetic compounds using Nuclear Magnetic Resonance (NMR) and Infrared Spectroscopies (IR).

(c) To evaluate the antioxidant properties of all synthesized compounds.

1.4 Scope of Study

The synthesis of hydroxycoumarins involved the usage of 2,4-dihydroxybenzaldehyde **(3)**, 2,5-dihydroxybenzaldehyde **(4)** and ethyl acetoacetate **(5)** as the starting materials. Piperidine and glacial acetic acid acted as the catalyst and co-catalyst respectively. The subsequent reaction to produce the desired coumarinyl chalcones utilized the synthetic hydroxycoumarins with either 4-hydroxybenzaldehyde **(6)** or 3,4-dihydroxybenzaldehyde **(7)** as the starting materials. The catalyst used was the Lewis acid, boron-trifluoride etherate (BF_3-Et_2O) and dioxane acted as the solvent. The reactions were monitored by means of the thin layer chromatographic (TLC) technique and column chromatography (CC) was adapted for the purification process.



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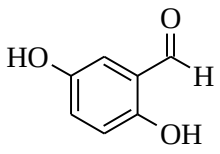

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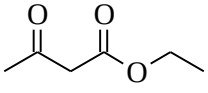

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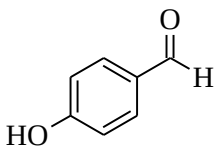

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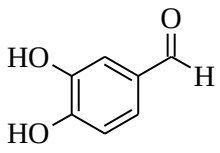

(7)

The synthetic compounds were analysed using several spectroscopic methods which consisted of infrared (IR) spectroscopy, 1D NMR (^{1}H, ^{13}C and DEPT) and 2D NMR (COSY). All products are tested for their antioxidant activities using the Ferric Reducing Ability of Plasma (FRAP), the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2 diphenyl-1-picrylhydrazyl (DPPH) assays. All of these assays were monitored using the Bio-Tek Epoch microplate reader.

1.5 Significance of Study

Coumarins are a type of heterocyclic molecules that positively impact the human health. The effects imposed by these compounds are due to the radical scavenging properties which are mostly connected with their antioxidant activities [19]. A number of publications reported the antioxidant activity of chalcones, particularly hydroxylated chalcones [20-24]. These studies indicate that hydroxychalcones are a potent radical scavenger [18]. The antioxidant properties are related closely to the radical scavenging potentials of these compounds and they are affected greatly by the substituents attached to the compounds. Much of this significant property is observed in compounds having hydroxyl groups or oxygenated substituents [25]. Therefore, this research is dedicated to synthesize several derivatives of natural hydroxycoumarins and also hybrid molecules known as coumarinyl chalcone which will have hydroxyl moieties. These compounds are expected to possess potent antioxidant properties. Through the development of these antioxidants, a cure for all free radical related diseases could be found or at least the risks of inflicting them could be minimized.

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