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CHEMICAL SYNTHESIS OF 1,6-DIOXYGENATED XANTHONES AND THEIR CYTOTOXIC ACTIVITIES

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CHEMICAL SYNTHESIS OF 1,6-DIOXYGENATED XANTHONES AND THEIR CYTOTOXIC ACTIVITIES

By

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ABSTRACT

In this project, a total of three 1,6-dioxygenated xanthones including a xanthone block and its two derivatives, namely 1,6-dihydroxyxanthone, 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one, and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one were successfully synthesized and characterized. These pure compounds were isolated by using column chromatography and their structures were identified through UV-Vis, IR, 1D- and 2D-NMR spectroscopic analyses. The xanthone block was synthesized using 2,6-dihydroxycarboxylic acid in the presence of Eaton's reagent and the produced xanthone block was used as starting material for prenylation in both organic and aqueous media.

Cytotoxic assays were carried out to all these xanthones isolated against HeLa and MDA-MB-231 cancer cell lines by using MTT colorimetric method. The effects of these compounds on the *in vitro* growth of both cancer cell lines were evaluated for their structure-activity relationship (SAR) study. Among the three xanthhones tested, the block 1,6-dihydroxyxanthone was found to elicit strong inhibitory activity towards HeLa cancer cell with IC_{50} value of 7.0 μg/mL. O-prenylation of xanthone block gave 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one which were found to give no significant growth inhibitory activity

against the two cancer cell lines tested. This suggests that the presence of hydroxyl group at carbon C-6 position was crucial for significant growth inhibitory activity against HeLa cancer cell line where etherification to this group had led to the total loss of activity.

ABSTRAK

Dalam projek ini, satu siri xanthone iaitu 1,6-dihidroksixanthone, 1-hidroksi-6-(3-metil-but-2-eniloksi)-xanthen-9-one, dan 1-(3-metil-but-2-eniloksi)-6-(3-metil-but-2-eniloksi)-xanthen-9-one telah berjaya dihasil dan dikenalpasti. Hasil sintesis ini telah dipisah melalui kromatografi kolom dan identitinya telah dikenalpasti melalui kaedah spektroskopi seperti UV-Vis, IR, 1D- dan 2D-NMR. Blok xanthone disintesis menggunakan 2,6-dihidroksi asid karboksilik dan hasil sintesis tersebut digunakan sebagai bahan permulaan untuk prenilasi secara organik dan akueus.

Untuk menilai kegiatan sitotoksik xanthone, ketiga-tiga jenis xanthone ini telah diuji terhadap sel-sel kanser HeLa dan MDA-MB-231 dengan menggunakan kaedah MTT. Kesan sebatian xanthone terhadap pertumbuhan *in vitro* bagi kedua-dua jenis sel kanser telah dinilai dengan menghubungkaitkan struktur dengan aktiviti. Di antara sebatian-sebatian di atas, didapati hanya 1,6-dihidroksixanthone menunjukkan aktiviti yang mampu menghalang pertumbuhan *in vitro* sel kanser HeLa dengan nilai IC_{50} 7.0 μg/mL. O-prenylasi xanthone seperti 1-hidroksi-6-(3-metil-but-2-enil)-xanthen-9-one dan 1-(3-metil-but-2-eniloksi)-6-(3-metil-but-2-eniloksi)-xanthen-9-one tidak memberi kesan terhadap kedua-dua jenis sel kanser. Ini mencadangkan bahawa kumpulan hydroksi dalam kedudukan ke-6 adalah sangat penting untuk menentu keupayaan dalam menghalang sel kanser.

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APPROVAL SHEET

I certify that, this project report entitled "**CHEMICAL SYNTHESIS OF 1,6-OXYGENATED XANTHONES AND THEIR CYTOTOXIC ACTIVITIES**" was prepared by BAK JOR YEE and submitted in partial fulfillment of the requirement for the degree of Bachelor of Science (Hons.) in Chemistry at Universiti Tunku Abdul Rahman.

Approved by

Supervisor

_____ Date:_____

(Dr. Lim Chan Kiang)

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UNIVERSITI TUNKU ABDUL RAHMAN

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PERMISSION SHEET

It is hereby certified that **BAK JOR YEE** (ID No: **08ADB05721**) has completed this report entitled "**CHEMICAL SYNTHESIS OF 1,6-DIOXYGENATED XANTHONES AND THEIR CYTOTOXIC ACTIVITIES**" under the supervision of Dr. Lim Chan Kiang from the Department of Chemical Science, Faculty of Science.

I hereby give permission to my supervisor to write and prepare manuscript of these research findings for publishing in any form, if I did not prepare it within six (6) month time from this date provided that my name is included as one of the authors for this article. Arrangement of my name depends on my supervisor.

DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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(BAK JOR YEE)

Date:

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LIST OF ABBREVIATIONS

α	Alpha
δ	Chemical shift in ppm
λ_{max}	Wavelength maxima in nm
^{1}H	Proton
^{13}C	Carbon-13
μL	Microlitre
ATCC	American Type Culture Collection
CuI	Copper(I) iodide
d	Doublet
DMSO	Dimethyl sulfoxide
DDQ	Dichloro dicyano quinone
FBS	Fetal Bovine Serum
h	Hour
HCl	Hydrochloric acid
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
IC_{50}	50% Inhibitory Concentration
IR	Infrared
J	Coupling constant in Hz
KBr	Potassium bromide

K_2CO_3	Potassium carbonate
$KMnO_4$	Potassium permanganate
KOH	Potassium hydroxide
m/z	Mass-to-charge ratio
MHz	Megahertz
min	Minute
mmol	Milimole
mol	Mole
MS	Mass Spectroscopy
nm	nanometer
NMR	Nuclear Magnetic Resonance
2D-NMR	Two dimensional Nuclear Magnetic Resonance
o	Ortho
PPA	Polyphosphoric acid
ppm	Parts per million
$POCl_3$	Phosphorus oxychloride
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
UV-Vis	Ultraviolet-Visible

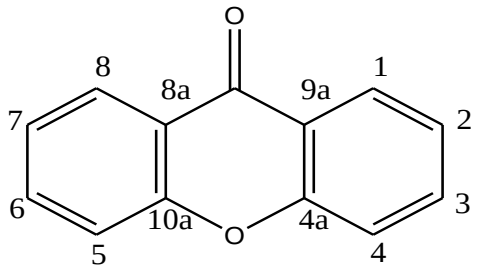
CHAPTER 1

INTRODUCTION

1.1 Background

The term “xanthone” is derived from the Greek word, xanthos, which means yellow (Roberts, 1961). Xanthones are a class of heterocyclic compounds with simple three-membered ring that are mainly found as secondary metabolites in higher plants and microorganisms. They have been classified into five groups: (a) simple oxygenated xanthones, (b) xanthone glycosides, (c) prenylated xanthones, (d) xanthonolignoids and (e) miscellaneous xanthones (Sultanbawa, 1980; Jiang *et al*., 2004).

Xanthones or xanthen-9H-ones, is an organic compound with molecular formula $C_{13}H_8O_2$ containing oxygen with a yellow coloration and all of them have a dibenzo-γ-pyrone as the basic skeleton (Figure 1.1).



Dibenzo-γ-pyrone

Figure 1.1: Basic skeleton of xanthone

These xanthonic compounds show interesting biological activities and pharmacological importance associated with their tricyclic scaffold, depending on the nature and the position of different substituents (Pinto *et al*., 2005). Xanthones interact to a different extent with various pharmacological targets based on the substituents on their core ring. For instance, the anticancer activities of xanthones are strongly dependent to the ring substituents and their positions.

In the past, extensive research has been conducted to obtain xanthone derivatives from natural resources as well as synthetic approaches. The great interest on xanthones is mainly due to their abundance in nature and also their significant biological properties such as anti-microbial activities (Bennet *et al*., 1989), anti-thrombotic (Lin *et al*., 1996a), anti-inflammatory (Lin *et al*., 1996b), cytotoxic and anti-tumor properties (Pedro *et al*., 2002b; Yoshimi *et al*., 2001).

In general, the two major sources of xanthone derivatives are synthesis and isolation from natural resources such as higher plants, lower fungi, and lichens (Vieira and Kijjoa, 2005). However, the xanthonic diversity of natural origin is relatively limited due to the limited type and position of the substituents imposed by the biosynthetic pathways. Therefore, chemical synthesis of xanthones is established to expand the possibilities of having different nature and positions of the substituents on the xanthonic nucleus.

1.2 Distribution of Xanthones in Nature

Early in 1960's, xanthones were reported to be isolated from lower fungi, lichens and only three families of flowering plants including Gentianaceae, Guttiferae and Anacardiaceae (Roberts, 1961). In 1992, about 20 families of plants have been found to produce xanthones. Among these families of flowering plants, Gentianaceae and Guttiferae were found to be the principal sources of xanthone derivatives (Mandal *et al*., 1992).

Currently, it has been observed that a growing number of plant species containing xanthones exhibit various biological properties and are used as chemotherapeutic agents in indigenous medicine for the treatments of many diseases. A typical

example of plant containing xanthones is the fruit of mangosteen, *Garcinia mangostana*, which is one of the widely consumed tropical fruits.

The Mangosteen tree is fairly widespread in Southeast Asian countries and the products developed from *Garcinia mangostana* fruit are now highly popular because of the biological activity of its phytochemicals. The fruit hull of *Garcinia mangostana* has been used as a traditional medicine in Southeast Asia for the treatment of inflammation, skin infections, ulcers, and wound healing (Farnsworth *et al*., 1992).

Another natural xanthone analogue are phomoxanthones, a structurally unique xanthone dimer obtained from the endophytic fungus *Phomopsis* species. According to the research, these xanthone dimer analogues have shown an excellent cytotoxic activity against tested tumor cell lines (Isaka *et al*., 2001).

The biological activities of xanthones are known to be dependent on the various types and position of substituents on the core ring. This leads to the limited usefulness of xanthones that arise from natural origin because they have restricted nature and position of substituents. Due to this reason, various synthesis techniques had been developed to produce differently substituted xanthones which have different biological functions.

1.3 Chemical Synthesis of Xanthones

Synthetic xanthones are xanthones that produced by reacting one compound with another through chemical reaction in laboratory to serve various purposes. The derivation of xanthones involves attaching different chemical groups to the core ring. The type and position of the attached substituents to the ring determine the specific properties of xanthones (Vennetier, 2009).

The limitation of natural xanthones can be overcome through chemical synthesis of xanthones. The chemical synthesis of new compounds could extend the possibilities of having different nature and position of substituents on the xanthonic nucleus. This allows the rationalization and characterization of the different xanthonic structural features which have important biological activities.

Since structural modifications with side chain substituents on the oxygenated xanthone core provide a wide spectrum of biological activity, most synthetic xanthone derivations have been focused on the introduction of various substituents onto the aromatic moieties in the xanthone core.

One of the examples of synthetic xanthones is epoxyxanthone. Xanthones with an epoxy group had been shown to effectively inhibit cancer cells growth. It was

observed that when two epoxy groups were tethered to the 3,5-position of xanthone core ring, its cytotoxic activity was dramatically increased (Liou *et al*., 1993). Epoxide seems to have an important role in the biological action since the ring-opened epoxide xanthones lost some of their cytotoxic activities (Lin *et al*., 1996c).

1.4 Prenylated Xanthones

Many naturally occurring xanthones and their prenylated derivatives are found to exhibit significant biological and pharmalogical properties, such as antibacterial, antifungal and antitumor activities. It can be inferred that the presence of prenyl groups helps to improve the potency and selectivity for some of these properties (Pinto *et al*., 2005).

For this reason, a series of prenylated xanthone derivatives were synthesized in this project to evaluate for their effect on the cytotoxic activity. The synthesis of prenylated xanthone derivatives was carried out by introduction of the prenyl side chain to the hydroxyxanthone core ring in a vigorous condition.

Prenylated xanthones, including furan and pyran derivatives, have been reported to mediate several interesting biological activities, concerning a large variety of

targets with therapeutic value (Pinto *et al*., 2005). Although the oxygenation pattern of these derivatives plays an important role in their biological activity, the presence of the prenyl side chains also seems to enhance the interaction with biological membranes when compared with their non-prenylated analogs (Epifano *et al*., 2007).

1.5 Current Anticancer Drug Development

Cancer is one of the leading causes of death in the world today. The reason cancers are so difficult to treat is because there are various forms of cancers, such as leukemia cancer, skin cancer, lung cancer, etc. In addition, tumor cells are greatly similar to non-tumor cells which make it hard to target the tumor cells specifically in a cancer patient. By definition, cancer cells are cells that have lost their cell cycle control and go on proliferating uncontrollably. Then, these overgrown cells start to metastasize and invade other tissues, thus spreading the cancers to other part of the body. Eventually, it grows into huge cell masses that block the normal bodily function and cause death to the host.

One of the concerns for chemotherapy in treating cancer is that the tumor cells are targeted non-specifically and thus generate side effects to the patient. Tumor cells are physiologically similar to normal body cells in structure except for its

uncontrolled division. So, treatment that usually kills the tumor cells will have some effects upon the normal cells to a certain extent. For example, methotrexate, hydroxyurea and 5-fluororacil are currently used as antitumor drugs that inhibit the DNA synthesis of the tumor cells. However, as mentioned, they have negative impacts upon normal cells too and thus they are used with precaution in cancer treatment. So, much effort has been put into searching and synthesizing new potential specific-acting antitumor drugs with fewer side effects. Recently, researchers have found interesting compounds such as ferrocene (Wu *et al.*, 2010), doxorubicin (Herringson and Altin, 2010), eribulins (Taur *et al*., 2010) and xanthones that exhibit interesting cytotoxic activity in killing tumor cells. Among those, xanthone is one of the compounds that have attracted the greatest attentions from many scientists due to its promising bioactivities.

1.6 Bioactivities of Xanthones

Recently, there has been a great deal of interest in the potential cytotoxic activity of xanthones. Chemically, the xanthone nucleus comprises an important class of oxygenated heterocycles whose role is well-known in medicinal chemistry. In fact, furanoxanthone derivatives have been shown to be the most potent inhibitors of tumour cell growth among xanthones. For instance, psorespermine, a dihydrofuranoxanthone, exhibited significant anti-tumour activity in the cells

culture derived from a human carcinoma of the nasopharinx *in vitro* system and also on leukaemia of mice *in vivo* system (Kupchan *et al*., 1980).

Prenylated xanthones from Guttiferae have been shown *in vitro* to have great inhibitory activity compared with 5-fluorouracil on colon cancer cells culture (Sordat *et al*., 1992). Besides anti-tumoral properties, some prenylated xanthones isolated from *G.cola* have also been shown to some extent to possess considerable anti-malarial property against *Plasmodium falciparum* (Likhitwitayawuid *et al*., 1998).

Other than cytotoxic and anti-tumour activities of xanthones, numerous reports have appeared in the literature concerning the interesting pharmacological properties of naturally occurring and synthetic xanthones. Among all the pharmacological properties of xanthones, the most studied one is the stimulation of central nervous system (CNS) by the inhibition of monoamine oxidase, MAO. Inhibitors of MAO are used as anti-depressant drugs. The inhibition *in vitro* and *in vivo* of MAO by the plants extracts rich in xanthones or by the pure xanthones has been reported in many works (Fowler and Ross, 1984).

Besides, anti-microbial activities have also been demonstrated by prenylated xanthones (Bennet *et al*., 1989). Synthetic heptacyclic xanthones exhibited

antibiotic activity against anaerobic bacteria, mycoplasma, and some Gram-positive bacteria (Mehta *et al*., 1994).

The objectives of this study are:

- To synthesize xanthone block and its derivatives.

- To purify the xanthone block and its derivatives through various chromatographic methods.

- To identify and characterize the pure xanthone block and its derivatives through 1D- & 2D-NMR, UV-Vis, and FT-IR spectroscopic analyses.

- To determine the cytotoxic activities of xanthone block and its derivatives against HeLa (cervical carcinoma) and MDA-MB-231 (human estrogen receptor negative breast cancer) cancer cell lines.

CHAPTER 2

LITERATURE REVIEW

Xanthones are tricyclic dibenzopyrans with various physicochemical and pharmacological properties. Although a large number of xanthone-based natural products have been isolated and characterized, the therapeutic agents that utilize this unique heterocyclic structure are still very limited due to its restricted diversity. Thus, chemical synthesis of xanthone derivatives has been developed to overcome this issue.

Other than to develop more diverse bioactive compounds for therapeutic purposes, xanthones chemical synthesis is also aimed for other applications such as in the medicinal chemistry and in the fluorescent probes preparation that utilizes the photochemical properties of xanthones.

2.1 Biosynthesis of Xanthones

Xanthones and its derivatives can be abundantly found in the nature. They are not only found in the lower fungi and lichens, but also in higher plants such as flowering plants and fruiting trees (Roberts, 1961). For example, the main sources of xanthone derivatives in the past had been the flowering plants Gentianaceae and Guttiferae (Mandal *et al*., 1992). Another important xanthones source that has gained popularity recently is the fruit of the tropical plant *Garcinia mangostana.*

For decades, the biosynthetic pathway of xanthones in the plants has been actively studied by many authors *in vivo* (Fujita and Inoue, 1980) and *in vitro* (Groger *et al*., 1968; Locksley and Murray., 1970; Gupta and Lewis., 1971). All these researchers attempted to inter-relate the observed oxygen patterns in natural xanthones and correlate them with the recognized oxygen patterns. They had proposed two different processes that are involved in the biosynthesis of xanthones:

i. Acetate Polymalonic Route (Figure 2.1)
ii. Mixed Shikimate Acetate Pathway (Figure 2.2)

2.1.1 Acetate Polymalonic Route

According to McMaster and co-researchers, the synthesis of some xanthones in lower plants such as micro-organisms and lichens was totally acetate-derived from seven acetate units. This observation was then further proven by Birch and co-researchers in 1976. They proposed a biosynthetic mechanism of ravelenin from *Helminthosporium ravenelii* and then illustrated the acetate polymalonic route. In the biosynthetic mechanism of ravelenin, benzophenone is involved as an intermediate.

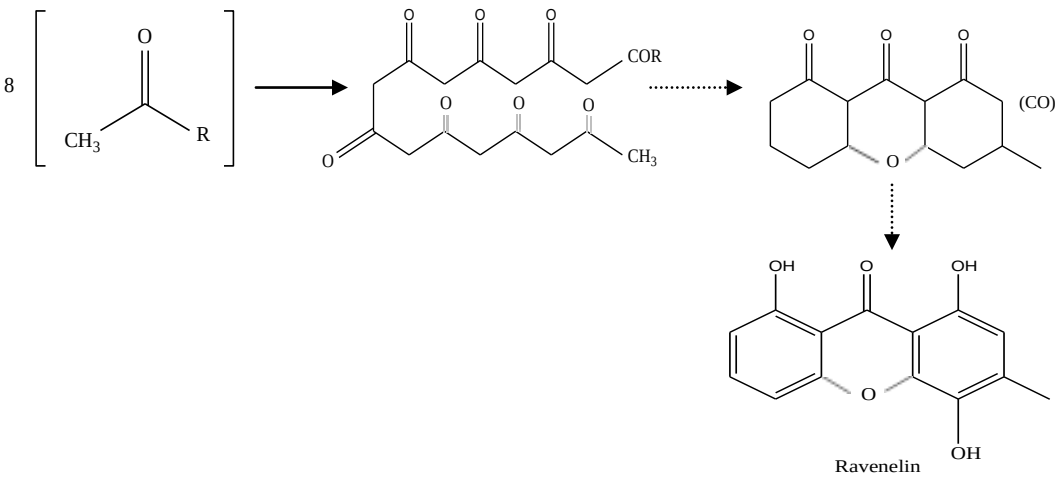


Figure 2.1: Biosynthesis of xanthone through acetate polymalonic route

2.1.2 Mixed Shikimate Acetate Pathway

It is believed that the oxygenation patterns of all xanthones in higher plants are formed by a pathway known as the mixed shikimate acetate pathway. In this mixed shikimate acetate pathway, ring A with C=O group attached comes from

the shikimic acid pathway while ring B arises from acetate-malonate polyketide route (Locksley and Muray, 1970; Afzal and Al-Hassan, 1980; Sultanbawa, 1980). These two moieties (ring A and ring B) then condense to form benzophenone or benzophenone-like intermediates. The intermediates formed react intramolecularly to give rise to xanthones.

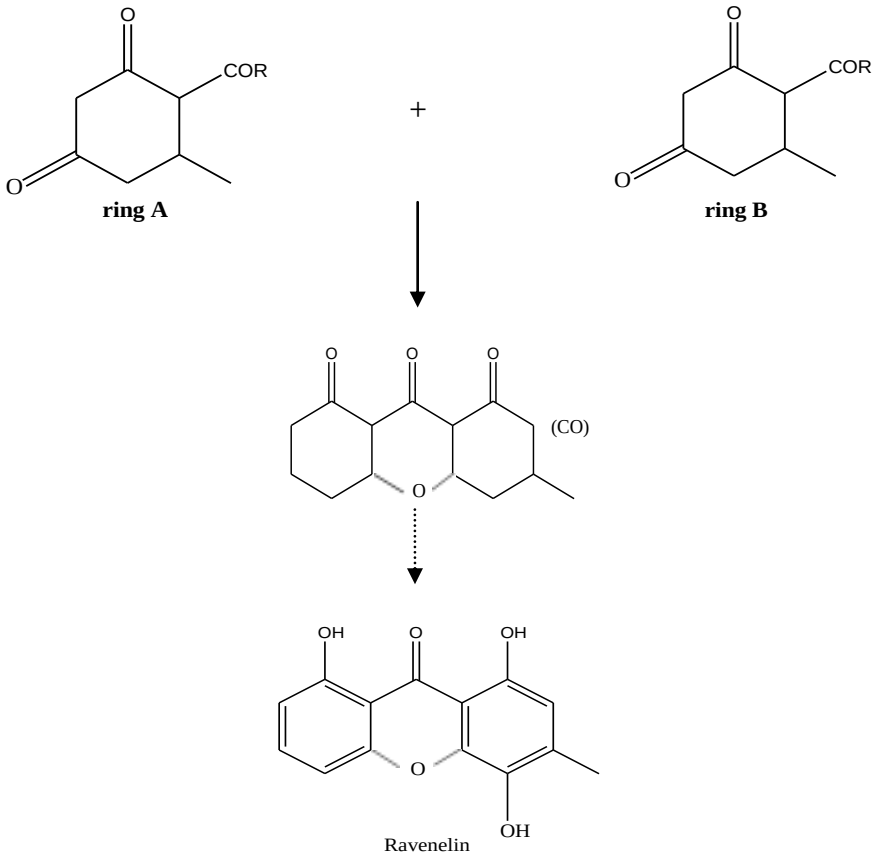


Figure 2.2: Biosynthesis of xanthone through mixed shikimate acetate pathway

The mechanism of this reaction involves either phenol oxidative coupling (Lewis, 1963), quinone addition (Ellis *et al*., 1967), dehydration between hydroxyl group on the acetate and shikimate-derived rings or spirodienone formation and then

followed by subsequent rearrangements that form the xanthone, ravenelin (Gottlieb, 1968).

2.2 Chemical Synthesis of Xanthones

In order to utilize xanthone and its derivatives in a large scale, chemists have tried to synthesize them chemically or extract them from the abundantly available natural products. In line with this, chemists have tried to develop several methods to synthesize xanthones with high yields. Generally, there are two methods to synthesize xanthones chemically, which are:

i) Grover, Shah and Shah classic method

ii) Ullmann method

2.2.1 Grover, Shah and Shah Classic Method

The first known chemical synthesis of xanthone was invented by Kostanecki in 1892 and this pathway was known as Michael-Kostanecki method. In this method, an equimolar mixture of a polyphenol and salicylic acid are heated in the presence of a dehydrating agent such as acetic anhydride or zinc chloride. However, this method produces a simple hydroxyxanthone with low yield due to the harsh

condition of experiment. Besides that, high possibility of auto-condensation, decarboxylation and many other side reactions are also the shortcoming of this synthesis method.

This synthetic approach was then improved by Grover, Shah and Shah in 1955. In Grover, Shah and Shah classic method, the xanthones are obtained by the condensation between an *ortho*-oxygenated benzoic acid and an activated polyphenol such as phloroglucinol. The usage of Eaton's reagent (phosphorus pentoxide and methanesulfonic acid: P_2O_5/ CH_3SO_3H) as the coupling agent has resulted in the high yield of xanthone.

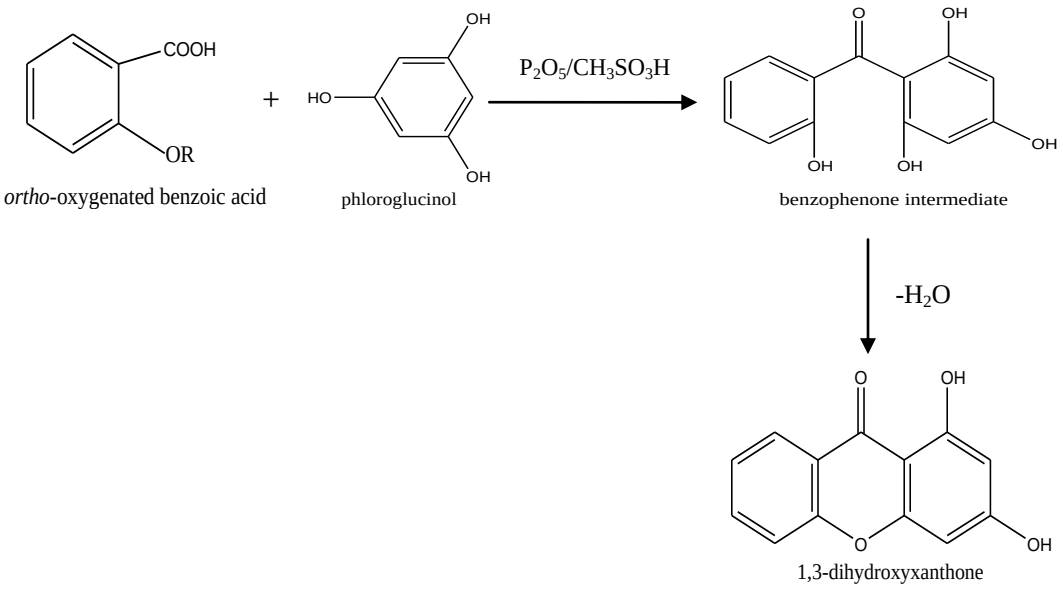


Figure 2.3: Synthesis of xanthone (Grover, Shah and Shah, 1955)

In Figure 2.3, the intermediate benzophenone derivative is involved in cyclization through a dehydrative or oxidative process that yields a simple xanthone.

The advantage of this modified synthesis method is that the reaction temperature required is very low. Nonetheless, there are still a number of limitations in this method. When hydroquinone, resorcinol or pyrogallol is used as polyphenol in reaction, poor yields are obtained. Other than this, there is no direct promotion of cyclization in this method even though the formation of benzophenone intermediate must occur in the mechanism. Although it is possible for the benzophenone intermediate to cyclize during the heating process, the resulted yield is still very low.

2.2.2 Ullmann Method

In 1906, an alternative synthesis method for xanthone had been developed. Ullmann and Pauchaud proposed that the condensation of a phenol and *ortho*-chlorobenzoic acid leads to the formation of 2-phenoxybenzoic acid. The intermediate is then treated with polyphosphoric acid (PPA) and phosphorus oxychloride ($POCl_3$), respectively, and thus cyclize to xanthone blocks under Friedel-Crafts condition which gives high yields (Figure 2.4).

The Ullmann reaction requires a careful optimization of the temperature, the type of base, and of the copper catalyst, respectively. The best yield resulted by dry

heating of the reaction mixture from 180°C to 200°C with potassium carbonate and copper bronze/copper(I) iodide as catalysts.

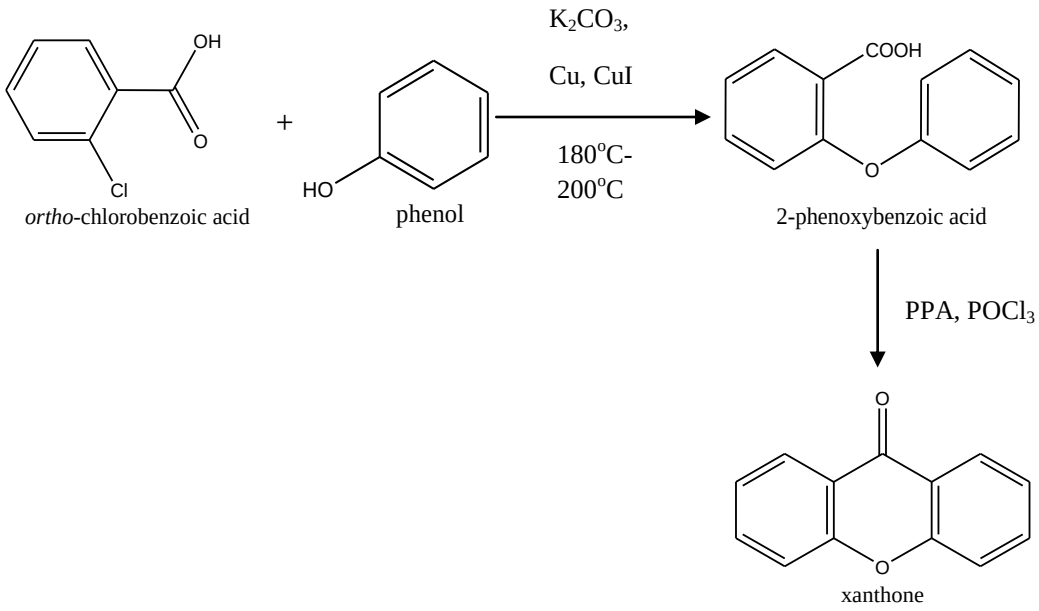


Figure 2.4: Synthesis of xanthone (Ullmann and Pauchaud, 1906)

The attachment of functional groups to different positions at the xanthone ring can be carried out by using the appropriate substituted phenol. Through this, various types of xanthone derivatives can be made depending on the position of substituents on phenol. This example is illustrated in Figure 2.5 where methyl group, CH_3 is introduced to the phenol ring. Xanthonecarboxylic acid was obtained by oxidation of the methyl group on phenol with potassium permanganate in alkaline solution (i). This process was then followed by intramolecular Friedel-Crafts acylation with polyphosphoric acid (PPA) (ii).

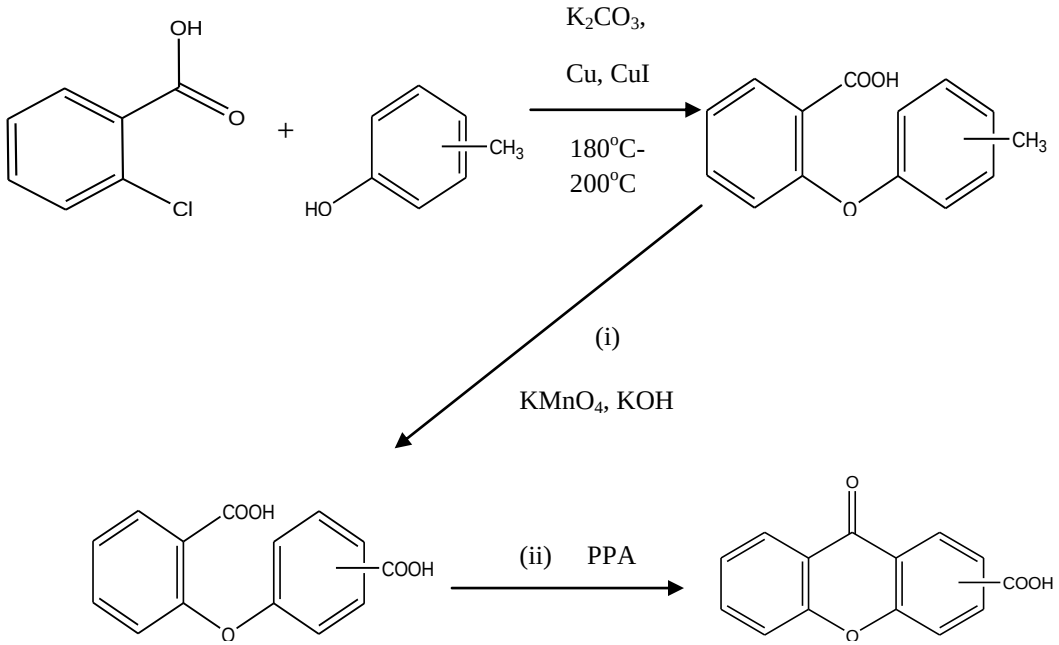


Figure 2.5: Synthesis of xanthonecarboxylic acid

Undeniably, Ullmann and Pauchaud had developed an efficient method for xanthones synthesis. However, the procedures are very tedious and involve various factors that are difficult to regulate. The synthesis procedures are extremely dependent and are very sensitive to various factors such as temperature, type of base, and catalysts used. If any of those conditions are not optimized or regulated properly, poor yield of xanthone synthesis may be obtained as a result.

2.3 Chemical Synthesis of Prenylated Xanthones

Xanthones with different substitution groups have different biological functions. Due to the limited diversity of substituted xanthones in the nature, chemists had come out with various methods to introduce prenylated groups onto the xanthone blocks. These unconventional xanthones derivatives have different properties in their anti-tumor activities as well as different applications in the industry such as in the medicinal chemistry. The two major prenylated xanthones are oxyprenylated (O-prenylation) and carboprenylated (C-prenylated) xanthones. Oxyprenylated xanthones are xanthones with prenyl groups attached to the oxygen atoms that are directly bonded to the xanthone ring while carboprenylated xanthones have their prenyl groups attached to the carbon atoms in the ring system.

O-prenylated xanthones are biologically active natural products and are often used as important precursors for the synthesis of furano- and pyranoxanthone derivatives (Jain *el al*., 1974). These furano- and pyrano- derivatives have been reported to show interesting biological activities.

2.3.1 O-prenylated and C-prenylated Xanthones

In general, O-prenylation and C-prenylation are the two major prenylated xanthones. O-prenylated and C-prenylated xanthone can be synthesized through the prenylation of a hydroxyxanthone with prenyl bromide in basic medium such as potassium carbonate in acetone (Castanheiro *et al*., 2009). Basically, the prenylation takes place either on the aromatic ring or the hydroxyl group where the prenyl bromide reacts with the naturally or synthetic xanthones (Figure 2.6).

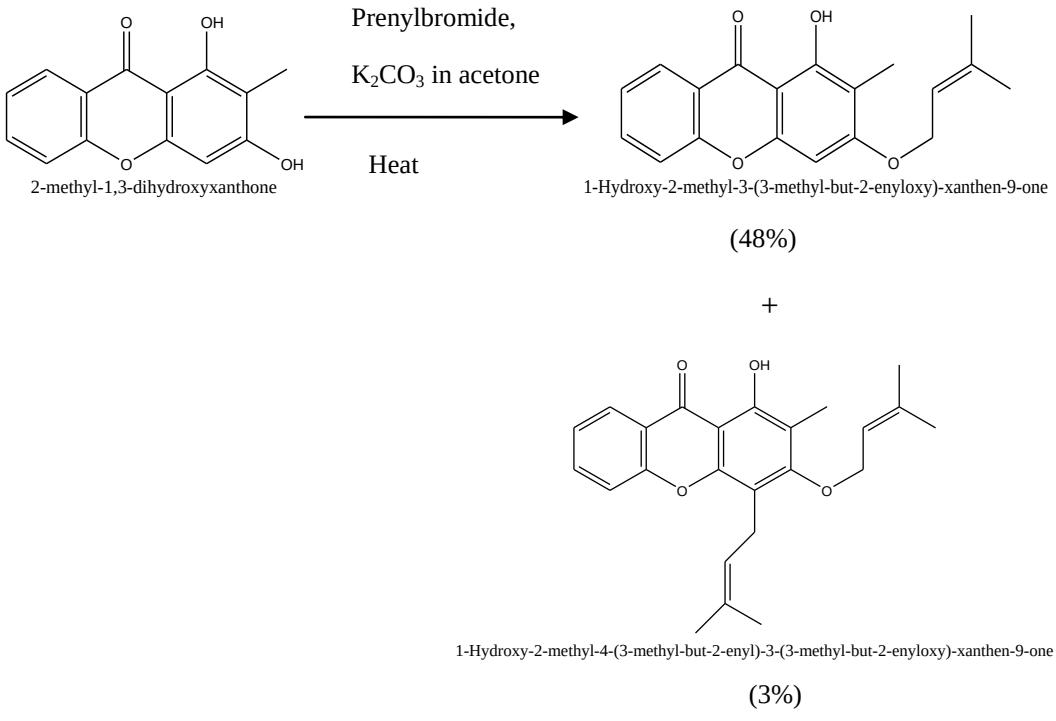


Figure 2.6: O-prenylation and C-prenylation of xanthones

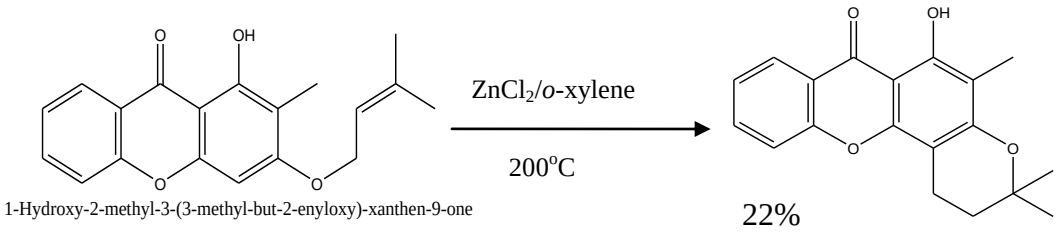
The classic synthesis method of the prenylated xanthone usually involved toxic reagents (Castanheiro *et al*., 2007). Consequently, this method is considered to be environmental unfriendly and therefore highly undesirable. Later on, an alternative approach to synthesize prenylated xanthones has been developed to resolve this issue. This new technique —microwave-assisted organic synthesis (MAOS) has been demonstrated to not only accelerate many organic reactions, but also to improve the yield percentage and the selectivity (Oliver, 2004; Kappe, 2005). With this breakthrough, many reaction parameters such as reaction temperature, time, variations in solvent, and catalysts are now able to be manipulated to optimize the desired chemistry (Oliver, 2006). For instance, the reaction time was reduced from 8 hour in the conventional heating to only 1 hour in the MAOS method.

In 2009, Castanheiro and co-researchers had found that by using MAOS, the yield of oxyprenylated xanthone (in Figure 2.6), 1-hydroxy-2-methyl-3-(3-methyl-but-2-enyloxy)-xanthene-9-one, was dramatically increased from 48% to 83%. The only flaw of this technique is that the yield of the diprenylated by-product could not be improved significantly as desired.

2.3.2 Cyclization of Prenylated Xanthones

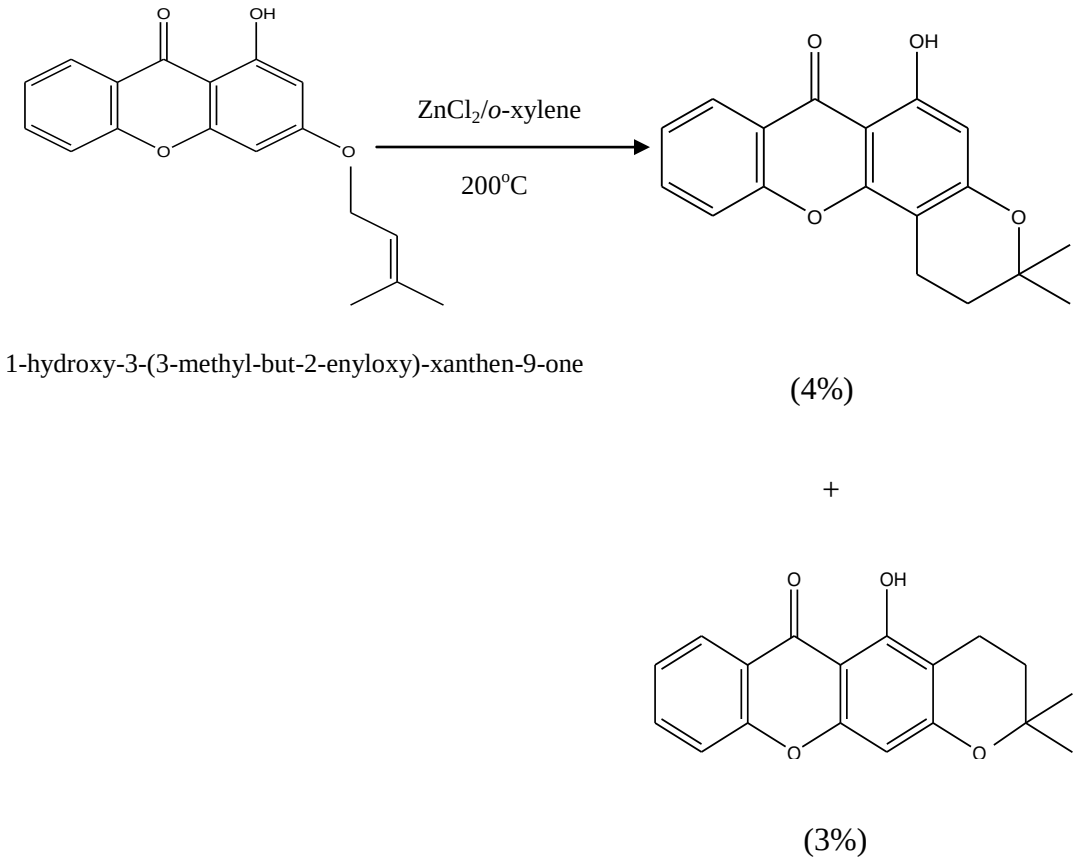
In 2007, Castanheiro *et al.* proposed that prenylation of xanthone with prenyl bromide followed by cyclization of the respective monoprenylated products were found to be more selective in showing their growth inhibitory effects when compared with their building blocks. Thus, it is desirable to chemically cyclize prenylated xanthones to create compounds with high efficiency of anti-tumor activity.

In 2009, Castanheiro and co-researchers have used the oxyprenylated xanthones as precursors for the synthesis of dihydropyranoxanthone. In the synthesis, the oxyprenylated xanthones were heated with zinc chloride, $ZnCl_2$ in *o*-xylene at 200^{o}C for 21 hour. However, Castanheiro *et al.* found that the yield percentage decreases dramatically when there is more than one possibility of cyclization available (Figure 2.7 and Figure 2.8).



1-Hydroxy-2-methyl-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

Figure 2.7: Cyclization of 1-hydroxy-2-methyl-3-(3-methyl-but-2-enloxy)-xanthen-9-one



1-hydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

(4%)

+

(3%)

Figure 2.8: Cyclization of 1-hydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one

According to Castainheiro, in order to improve the yield, one-pot synthesis was designed by using Montmorillonite K10 clay to catalyze direct condensation of both types of xanthones with prenyl bromide in various conditions. As expected, the yield of product of cyclization of 1-hydroxy-2-methyl-3-(3-methyl-but-2-enloxy)-xanthen-9-one was improved from 22% to 51%. However, this method is very selective and it only works on cyclization of 1-hydroxy-2-methyl-3-(3-methyl-but-2-enloxy)-xanthen-9-one but not on 1-hydroxy-3-(3-methyl-but-2-enyloxy)-xanthen-9-one that has more than one possible cyclization outcome.

The advantages of using Montmorillonite K10 clay are not only that the reaction occurs under milder conditions, but also results in better yield, shorter reaction time and higher selectivity. In addition, the purification procedures are also made relatively simpler because this type of clay catalyst can easily be separated out and regenerated from the reaction mixture. However, not all types of prenylated xanthones can be synthesized by using this technique. Hence, its usefulness is limited only to certain types of prenylated xanthones synthesis.

2.4 Cytotoxic Activities of Xanthones

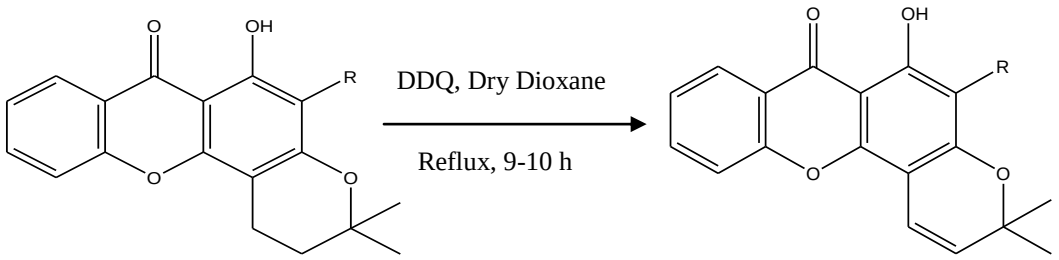
The planar structure of xanthones is recognized as an efficient DNA intercalator. In addition, a number of xanthones derivatives have been proven to show anticancer activities via non-covalent DNA interaction. Thus, xanthonic compounds have attracted numerous scientific interests ever since their discovery because of the excellent cytotoxic profiles.

2.4.1 Pyranoxanthones

Among the xanthone derivatives, pyranoxanthones is one of the xanthone groups that has been studied extensively because of its promising cytotoxic activities. Recent researches had shown that pyranoxanthones are more biologically active

than dihydropyranoxanthones. This leads to many researchers to modify their rigidification strategy in order to improve the anti-tumor activity of xanthone derivatives. Therefore, dehydrogenation strategy was employed and applied to the dihydropyran ring of dihydropyranoxanthones to produce pyranoxanthones.

In 2001, Ho *et al* demonstrated that pyranoxanthones can be obtained through dehydrogenation of the respective dihydropyranoxanthones with dichloro dicyano quinone (DDQ) in refluxing dry dioxane. In fact, they found out that pyranoxanthone with a methyl group substituted at carbon C-2 obtained the highest yield as compared to pyranoxanthone with hydrogen attached at carbon C-2 (Figure 2.9).



R = CH_3 R= CH_3 (67%)

R = H R = H (41%)

Figure 2.9: Synthesis of pyranoxanthones (Ho *et al*., 2001)

2.4.2 Epoxyxanthones

Xanthones with attached epoxy group were proven to inhibit cancer growth effectively. In 1996, Lin *et al* had proposed that epoxide has an important role in the biological action since its epoxide ring-opened dihydroxy compounds lost some of their cytotoxicity. Although the mechanism of action of these epoxide groups had not been systematically studied, it was suggested that the increased DNA interaction might have contributed to this observation.

In fact, when two epoxy groups were tethered to the 3, 5-position of xanthone, the cytotoxic activity was dramatically increased (Liou *et al*., 1993). According to Woo *et al.* in 2007, 1,3-bisepoxyxanthone showed the most active cell growth inhibition capacity due to the presence of two epoxy group attached on the xanthone ring. In addition, Woo *et al* also reported that a two-epoxy group substitution on xanthone building block generated a better cytotoxic activity than a single epoxy substitution. The synthetic method for obtaining epoxyxanthones is described in Figure 2.10.

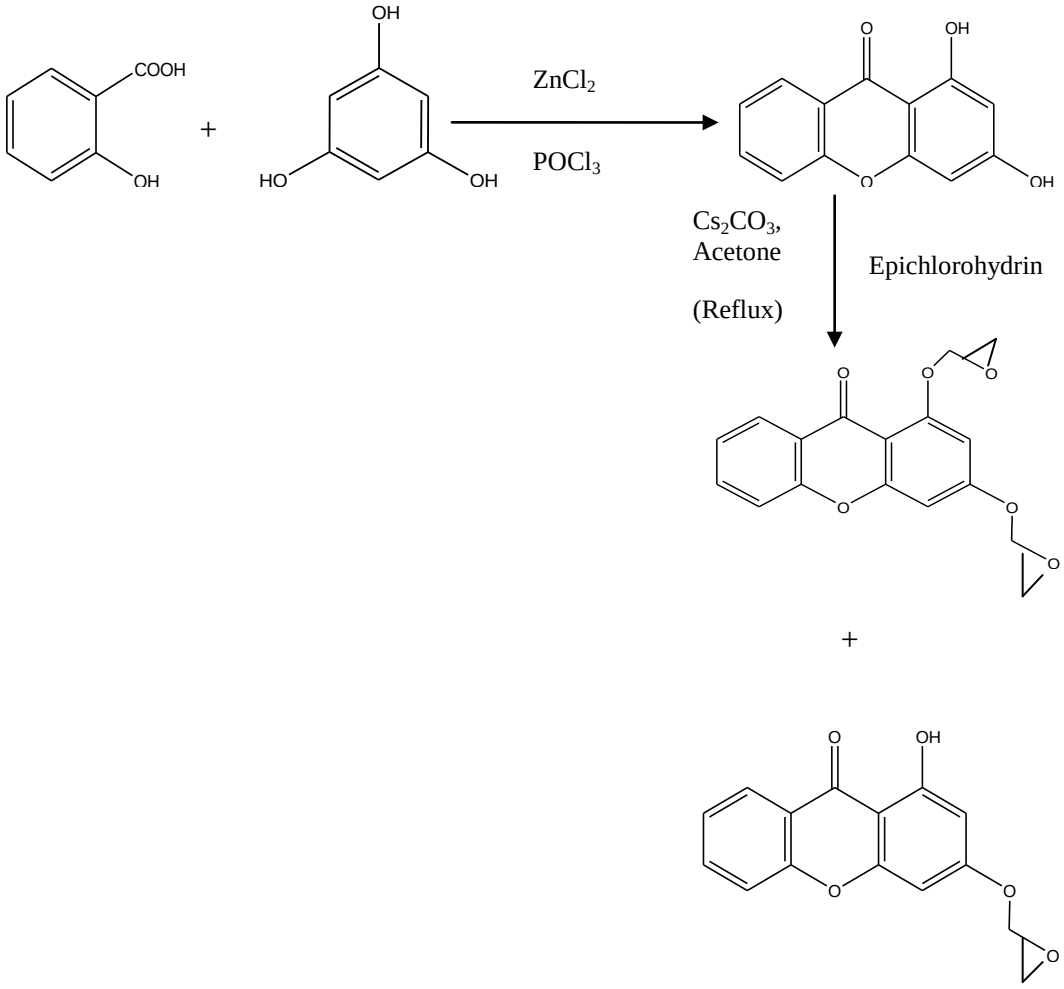


Figure 2.10: Synthesis pathway of epoxyxanthone (Woo *et al*., 2007)

2.4.3 Furanoxanthones

Other than epoxyxanthone, furanoxanthone derivatives were also found to be one of the most potent inhibitors of tumour cell growth among the xanthone compounds. This fact was further supported by Abou-Shoer *et al* in 1988. Abou-Shoer and co-researchers reported that many furanoxanthones were found to

exhibit cytotoxic property on cells cultured from human edenocarnoma in the colon. Psorespermine, a dihydrofuranoxanthone was proven to exhibit a significant level of anti-tumor activity in the cells culture derived from a human carcinorma of the nasopharinx (K.B) *in vitro* system and also on leukaemia P338 of mice *in vivo* system (Kupchan *et al*., 1980).

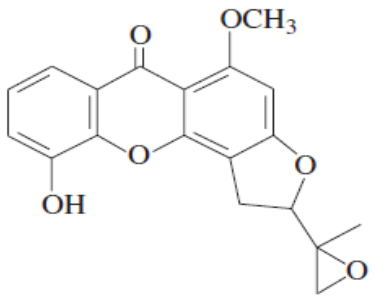


Figure 2.11: Structure of psorespermine

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemical Reagents

The chemicals used for the synthesis of xanthone block are listed in Table 3.1.

Table 3.1: List of chemical reagents for synthesis of xanthone block

Chemical reagents	Molecular formula	Molecular weight, M_w (g mol^{-1})	Source, Country
2,6-dihydroxybenzoic acid	$C_7H_6O_4$	154.12	Acros Organics, Belgium
Eaton's reagent	$P_2O_5/MeSO_3H$	-	Acros Organics, Belgium

The chemicals used for the prenylation of xanthone block (both organic and aqueous media) are listed in Table 3.2.

Table 3.2: List of chemical reagents for prenylation of xanthone block

Chemical reagents	Molecular formula	Molecular weight, M_w (g mol^{-1})	Source, Country
Acetone	CH_3COCH_3	58.08	QREC, Malaysia
Ethyl Acetate	$CH_3COOC_2H_5$	88.11	LAB-SCAN, Ireland
Hydrochloric acid (37%)	HCl	36.46	Fisher Scientific, UK
Potassium carbonate	K_2CO_3	138.21	John Kollin Corporation
Prenyl bromide (3,3-dimethylallyl bromide)	C_5H_9Br	149.09	Sigma-aldrich, USA

The solvents used in purification by using column chromatography are listed in Table 3.3.

Table 3.3: List of solvents and materials used in purification

Solvents/Materails	Molecular formula	Density, ρ (g ml^{-1})	Source, Country
Acetone	CH_3COCH_3	0.791	QREC, Malaysia
Dichloromethane	CH_2Cl_2	1.325	Fisher Scientific, UK
Ethyl Acetate	$CH_3COOC_2H_5$	0.902	LAB-SCAN, Ireland
n-Hexane	$CH_3(CH_2)_4CH_3$	0.659	Merck, Germany
Methanol	CH_3OH	0.791	Mallinckrodt Chemicals, Phillipsburg
Petroleum Ether	-	-	Fisher Scientific, UK

All the deuterated solvents and materials used in chemical analyses are listed in Table 3.4.

Table 3.4: Deuterated solvents and materials used in chemical analysis

Deuterated solvents/Materials	Source, Country
Acetone-d_6	Acros Organis, Belgium
Deuterated chloroform ($CDCl_3$)	Acros Organis, Belgium
Methanol-d_4	Acros Organis, Belgium
Silica gel (60 Å)	a) Silicycle, Canada b) Merck, Germany
TLC silica gel 60 F_{254}	Merck, Germany

All the chemical reagents and materials used in bioassay are listed in Table 3.5.

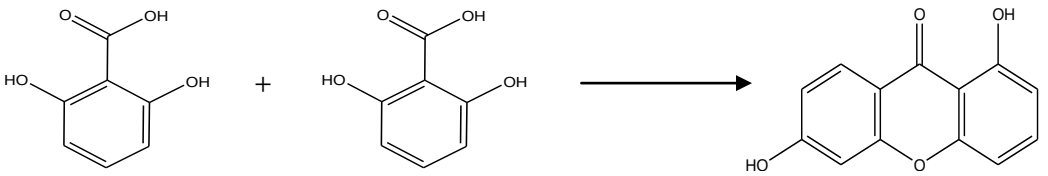
Table 3.5: List of materials and reagents used in bioassay

Reagents/Materials	Source, Country
96-cell plate	TPP, Europe
Dimethyl sulfoxide (DMSO)	Fisher Scientific, UK
Fetal Bovine Serum (FBS)	Hyclone Thermo Scientific, South America
HeLa cell	America Type Culture Collection (ATCC), USA
MDA-MB-231 cell	America Type Culture Collection (ATCC), USA
MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide)	Sigma-Aldrich, USA
RPMI 1640 media	Cellgro, Manassas

3.2 Methodology

3.2.1 Chemical Synthesis of 1,6-Dihydroxyxanthone

75 mmol (11.559 g) of 2,6-dihydroxybenzoic acid was prepared and added with 100 ml of Eaton's reagent in a 250 ml flat-bottom flask. The mixture was added with boiling chips and was refluxed for 30 minutes at 90^{o}C in water bath with constant stirring. The reaction mixture was then cooled to room temperature and was introduced into 1 L beaker initially filled with some ice. It was stirred and kept cool by adding ice from time to time for 1 hour. The precipitates formed were filtered using Buchner filtration, washed with cold water and lastly dried in oven at 50^{o}C for overnight. The filtrate collected in Buchner filtration was extracted by using ethyl acetate. The upper layer which is organic layer was separated and dried by using rotary evaporator. Both the precipitate and the extract from filtrate were combined and subjected to column chromatography with gradient elution (100% hexane followed by 90% hexane: 10% dichloromethane).



2,6-dihydroxybenzoic acid 1,6-dihydroxyxanthone

Figure 3.1: Chemical synthesis of 1,6-dihydroxyxanthone

3.2.2 Prenylation of 1,6-Dihydroxyxanthone in Organic Medium

4 mmol (0.9135 g) of xanthonic block and 12 mmol (1.6587 g) of potassium carbonate, K_2CO_3 were mixed in a 250 ml flat-bottom flask and stirred with 90 ml of acetone for 5 minutes at room temperature. 16 mmol (2.3864 g) of prenyl bromide was prepared in a 5 ml measuring cylinder and was added dropwise into the reaction mixture by using Pasteur pipette. The measuring cylinder was then rinsed with 10 ml of acetone and poured into the mixture. The mixture was then refluxed at 65^{o}C for 6 hours. The reaction mixture was cooled to room temperature and the precipitate was filtered by using filtering funnel. The precipitate was dried in an oven at 50^{o}C while the filtrate collected in beaker was dried under reduced pressure. Both the products and filtrate were combined and subjected to column chromatography with gradient elution by using 100% hexane.

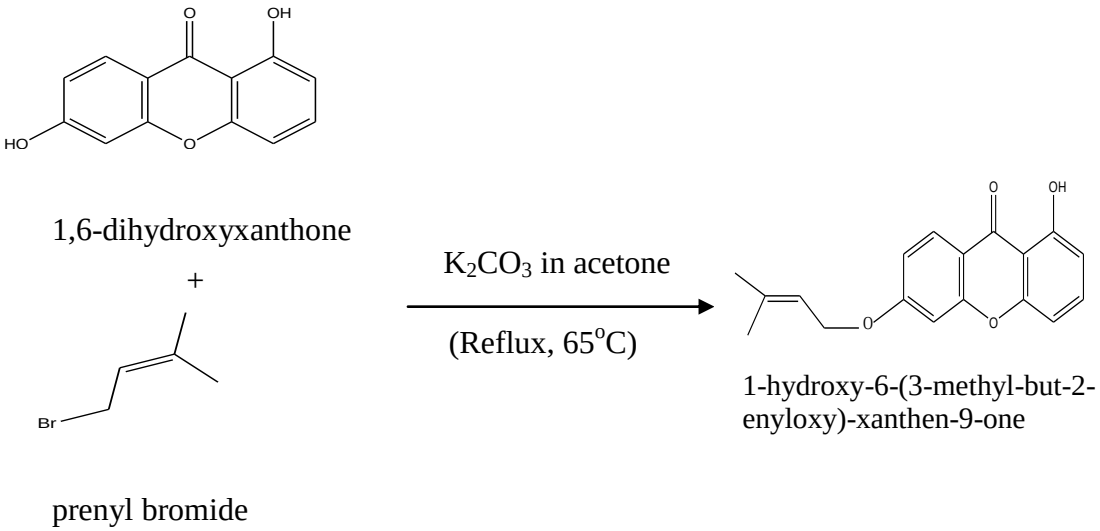
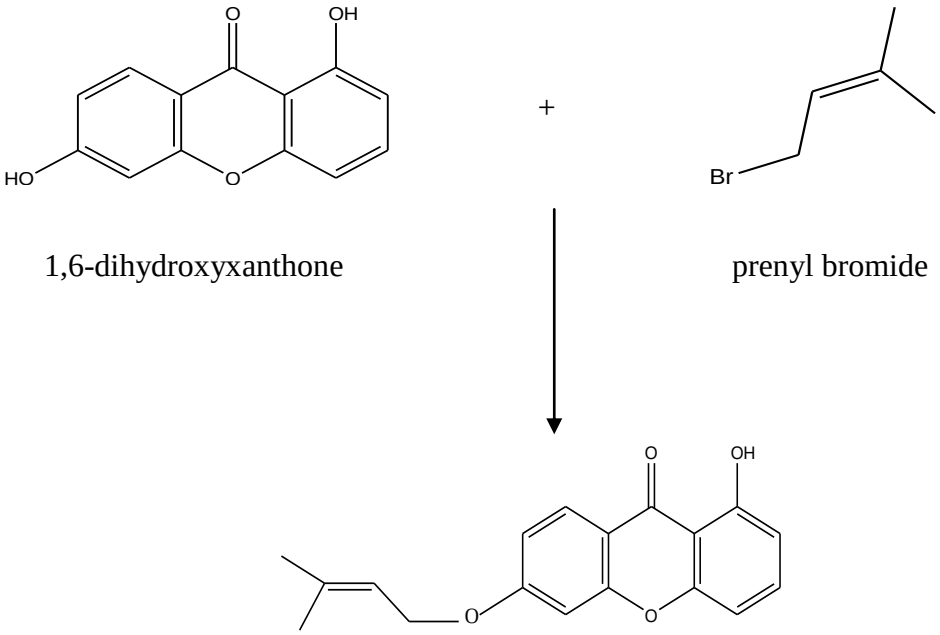


Figure 3.2: Prenylation of xanthone block in organic medium

3.2.3 Prenylation of 1,6-Dihydroxyxanthone in Aqueous Medium

Potassium carbonate solution was prepared by dissolving 35 g of potassium carbonate, K_2CO_3 was dissolved in 100 ml of deionized water. 4 mmol (0.912 g) of xanthonic block was prepared in a 250 ml flat-bottom flask and added with 100 ml of potassium carbonate solution as prepared above, and stirred for 5 minutes. 16 mmol (2.384 g) of prenyl bromide was measured in a 5 ml measuring cylinder and added with 4 ml of acetone. By using a syringe, the mixture of acetone and prenyl bromide was slowly added into the xanthonic mixture. The measuring cylinder was rinsed with 1 ml of acetone and poured into the reaction mixture. The reaction mixture was stirred for 16 hours at room temperature. After that, 100 ml of 10% hydrochloric acid, HCl was added dropwise by using Pasteur pipette whilst stirring at room temperature until the mixture turned acidic. The reaction mixture was extracted with 50 ml of ethyl acetate in a 250 ml separatory funnel. The upper organic layer was collected and then dried using rotary evaporator. The crude product was subjected to column chromatography with gradient elution (100% hexane, followed by 90% hexane: 10% dichloromethane).

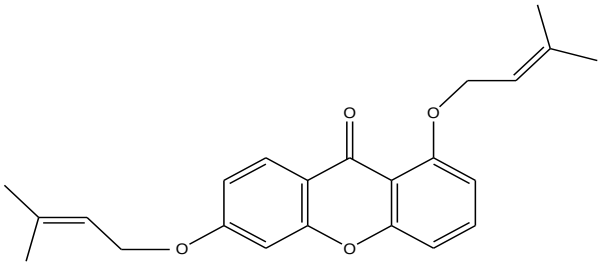


1,6-dihydroxyxanthone

prenyl bromide

1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

+



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Figure 3.3: Prenylation of 1,6-dihydroxyxanthone in aqueous medium

3.2.3.1 Preparation of 10% Hydrochloric Acid

77.3 ml of deionized water was added into a 100 ml measuring cylinder. Then, with a clean Pasteur pipette, 22.7 ml of 37% hydrochloric acid, HCl was slowly transferred out from analytical grade bottle and introduced into the measuring cylinder containing the deionized water. The measuring cylinder was stirred slowly so that homogeneous solution can be obtained.

3.2.4 Purification by Using Chromatographic Methods

The sample for column chromatography was prepared by using dry packing method in which the sample was dissolved in a small amount of solvent and droplets of the sample solution were mixed with silica gel. The prepared sample mixture was left to dry in order for the sample to coat onto the silica gel. The column packing material used was Silicycle or Merck silica gel. The gravity column was first half-filled with non-polar solvent, and then slurry of silica gel was introduced into the column until a desired length. The packing material in the gravity column was allowed to settle down by continuously eluting a the non-polar solvent. After the silica gel was densely packed, the dried sample was introduced into the column. Separation of the compounds was carried out through continuous elution of solvents in increasing polarity (hexane-dichloromethane,

dichloromethane-ethyl acetate, ethyl acetate-acetone, acetone-methanol). The fractions collected from column chromatography were analyzed using Thin Layer Chromatography (TLC) plates.

3.2.5 Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a chromatographic method used to separate compounds based on difference in their polarity. In this study, Thin Layer Chromatography was carried out by using an aluminium plate of 5 cm x 5 cm dimensions coated with Merck brand silica gel 60 F_{254}. A thin capillary tube was dipped into the sample solution and was spotted onto the baseline drawn on the plate. At the same time, a chamber with appropriate solvent system was prepared. After the chamber was saturated with the vapor of mobile phase, the TLC plate was placed into the chamber for development. After the solvent reached the marked solvent front on TLC plates, it was removed from the chamber and the spots developed were visualized under ultra-violet lamp with both short wavelength (254 nm) and long wavelength (366 nm). Each spot can be identified based on their value of retention factor, R_f. Compound with high R_f value indicates that it is relatively non-polar while compound with low R_f value is more polar. The R_f value is a ratio obtained from the equation below:

$$R_f = \frac{\text{distance traveled by the compound (cm)}}{\text{distance tranveled by the solvent front (cm)}}$$

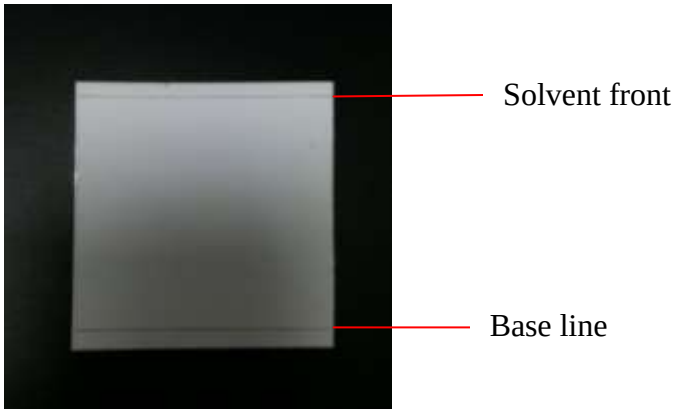


Figure 3.4: Thin Layer Chromatography (TLC) plates

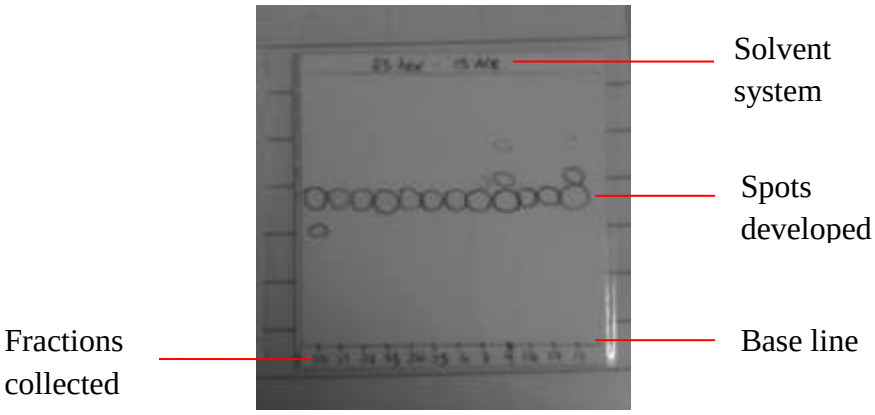


Figure 3.5: Developed Thin Layer Chromatography (TLC) plates

3.3 Instruments

3.3.1 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is applicable to nucleus possessing spin. Much information can be obtained from an NMR spectrum such as the number and type of chemical entities in a molecule. In this project, JEOL

JNM-ECX 400 MHz spectrometer was use to obtain ^{1}H-NMR, ^{13}C-NMR, HMQC (Heteronuclear Multiple Quantum Coherence), and HMBC (Heteronuclear Multiple Bond Coherence) for sample analysis. The solvents used are methanol-d_4, acetone-d_6, and deuterated-chloroform ($CDCl_3$) while tetramethylsilane (TMS) was used as the internal standard and reference. NMR samples were prepared by dissolving samples in a small amount of deuterated-solvent that filled the NMR tube up to a height of approximately 4 cm. The NMR tube was labeled and the cap was sealed with parafilm tightly to avoid solvent evaporation. Then the prepared samples were analyzed by NMR spectrometer.

3.3.2 Infrared (IR) Spectroscopy

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used to identify the chemical functional groups present in a sample and to provide unique characteristic identification of the compound. Different chemical functional groups absorb at different frequencies in the Infrared (IR) region. Therefore, IR spectroscopy has provided useful information for structural elucidation and compound identification in this project. In this study, Perkin Elmer 2000-FTIR spectrophotometer was used for sample analysis in the absorption range of 4000 cm^{-1} to 400 cm^{-1}. The IR sample pellet was prepared by grinding small amount of sample with potassium bromide, KBr powder in ratio of

1:10 and the mixture was then compressed under high pressure to form KBr sample pellet.

3.3.3 Ultraviolet-Visible (UV-Vis) Spectroscopy

Ultraviolet-visible spectroscopy utilizes the light in the visible and adjacent (near-UV and near-IR) ranges to provide qualitative information for highly conjugated organic compounds. The energy absorption in the visible light range results in the electronic transition of the respective molecules which gives rise to different color intensity. Perkin-Elmer Lambda (25/35/45) UV-Vis spectrophotometer was used in this project for sample analysis. A small amount of sample was dissolved in ethanol and was measured by using quartz cuvette in the range of 100 nm to 800 nm.

3.3.4 Melting Point Apparatus

Melting point of a compound is the temperature at which the material changes from solid state into liquid state. Pure crystalline substance has a clear and sharp defined melting point because it melts at a precisely defined temperature. During the melting process, the heat energy supplied consumed by the compound as heat of fusion and hence, the temperature remains constant. In this project, melting

point determination of samples was carried out to determine the purity of samples by comparing the exact melting point with the literature melting point of pure compound. Measuring melting point is a simple yet important test for gauging the purity of organic compound. Barnstead Electrothermal 9100 melting point apparatus was used to determine the melting point of the samples. Before melting point measurement system was put into operation, its temperature scale was calibrated using appropriate reference substances with melting point that is known exactly. The sample was ground into fine powder and was introduced into the haematocrit capillaries. The range of temperature at which the solid started to melt and the solid completely melted was recorded.

3.4 Bioassay

Bioassay was carried out to investigate the cytotoxic activity of the xanthone samples against HeLa and MDA-MB-231 cancer cell lines. The colorimetric 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assay was used to determine the cell viability after cytotoxic treatments. In the bioassay, both the HeLa cells (0.75×10^5 cells/ml) and MDA-MB-231 cells (3.5×10^5 cells/ml) were cultured in 96-well plates with 0.1 % dimethyl sulfoxide (DMSO) containing the xanthone samples (series of concentration at 50 μg/mL, 25 μg/mL, 12.5 μg/mL, 6.25 μg/mL, 3.125 μg/mL, and 1.563 μg/mL) at 37 ^{o}C for 72 hours. At the same time, blank cell control and blank medium control were also prepared.

Then, the cells were added with 20 μL of 5 mg/mL MTT and were incubated at 37 ^{o}C for 3 hours. After the incubation, 70 % of supernatant was removed and 150 μL of DMSO was added into each well. Optical density values of the cells were measured using Model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) at wavelength 550 nm. The cell viabilities were then calculated from the obtained optical density values.

3.5 Calculation

3.5.1 Percentage Yield of Xanthones

The yield percent of each synthesized xanthone can be calculated by using the formula given below:

$$\text{Yield percent of xanthone} = \frac{\text{experimental yield of xanthone (g)}}{\text{theoretical yield of xanthone (g)}} \text{ x } 100\%$$

3.5.2 Cell Viability

Cell viabilities after cytotoxic treatments can be calculated from the formula given below:

$$\text{Cell viability} = \frac{x-y}{z-y} \times 100\%$$

Where x = average optical density value of cell treated with compound

y = average optical density value of blank medium

z = average optical density value of cell control

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Percentage Yield of Xanthone Block and Its Derivatives

The synthesis approach for the xanthone block involved the condensation of 2 mol of 2,6-dihydroxybenzoic acid in the presence of Eaton's reagent and this had resulted in a relatively low yield of 21.0 % for 1,6-dihydroxyxanthone.

Prenylation of xanthone block, 1,6-dihydroxyxanthone separately in organic medium and aqueous medium were found to produce 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one in different percentage yields of 50.5% and 17.3%, respectively. 1,6-Dihydroxyxanthone has two hydroxyl groups attached to carbon positions C-1 and C-6 on the xanthone nucleus. Hydroxyl group at position C-6 is found to be the more reactive because it is non-chelated as compared to chelated hydroxyl group at position C-1. Thus, the prenyl groups are more likely to react with the non-chelated hydroxyl group at C-6 than the chelated hydroxyl group at C-1 which is relatively less reactive. Therefore, 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one is the major compound from the two different prenylation syntheses.

Besides 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one, 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one was also obtained from the prenylation of xanthone block in aqueous medium with a lower percentage yield of 6.1% as compared to the formal compound. The percentage yields of xanthone and its derivatives are tabulated in Table 4.1.

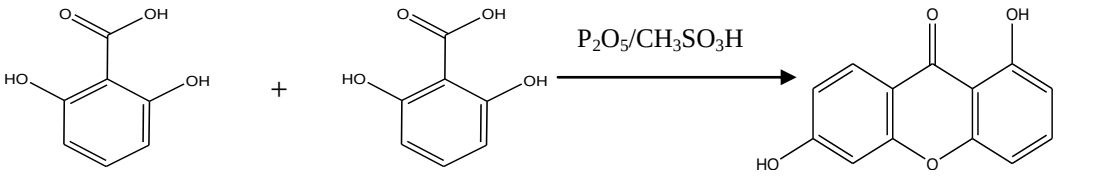
Table 4.1: Percentage yield of xanthone block and its derivatives

Compounds	Percentage Yield,%
1,6-dihydroxyxanthone	21.0
Organic medium: 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one	50.5
Aqueous medium: 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one	17.3
1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one	6.1

4.2 Synthesis and Isolation of Xanthone Block

1,6-dihydroxyxanthone was prepared from the reaction of 75 mmol (11.559 g) of 2,6-dihydroxybenzoic acid in the presence of Eaton's reagent as the coupling agent. Boiling chips were added to prevent the reaction from being overheated. A

magnetic stirrer bar was also added to mix the mixture homogeneously so that reaction can occur more efficiently. The total crude product obtained was 7.0993 g and after purification by using column chromatography, the pure 1,6-dihydroxyxanthone obtained was 1.7713 g, which was 21.0% of the percentage yield compared with the literature percentage yield, 19% (Liu *et al*., 2006). The equation of reaction for the synthesis of 1,6-dihydroxyxanthone is shown in Figure 4.1.



2,6-dihydroxybenzoic acid

1,6-dihydroxyxanthone

Figure 4.1: Equation of reaction for synthesis of 1,6-dihydroxyxanthone

4.2.1 Structure Elucidation of 1,6-Dihydroxyxanthone

In this synthesis, 1,6-dihydroxyxanthone was isolated as the major compound and it appears in the form of yellowish solids with a melting point range of 247^{o}C to 249^{o}C. When the TLC was developed with solvent system of 90% dichloromethane and 10% acetone, the collected fractions from 28 to 41

containing the compound gave a single spot with R_f value of 0.50. The summary of physical properties of 1,6-dihydroxyxanthone is tabulated in Table 4.2.

Table 4.2: Summary of physical properties of 1,6-dihydroxyxanthone

Mass obtained, g	1.7713
Percentage yield, %	21.0
Physical appearance	Yellowish solid
Melting point	247^{o}C - 249^{o}C
R_f value on TLC	0.50 in dichloromethane:acetone (9:1)

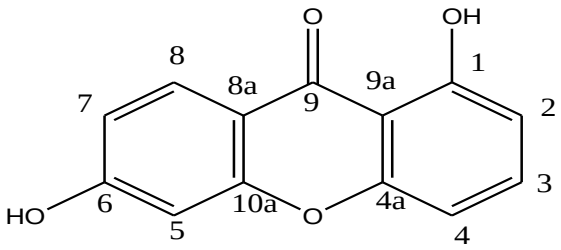
From the ^{1}H-NMR spectrum (Figure 4.2) of 1,6-dihydroxyxanthone, the peaks lying in the chemical shift (δ) range from 6.0 ppm to 8.0 ppm indicated the presence of aromatic protons. A signal at 12.74 ppm (1H, s, 1-OH) indicated the presence of a chelated hydroxyl proton. Besides, six aromatic proton signals at 6.66 ppm (1H, d, *J*=8.2 Hz), 7.52 ppm (1H, t, *J*=8.2 Hz), 6.83 ppm (1H, d, *J*=8.2 Hz), 6.71 ppm (1H, d, *J*=2.1 Hz), 6.81 ppm (1H, d, *J*=2.1 Hz), and 7.97 ppm (1H, d, *J*=8.2 Hz) were due to protons H-2, H-3, H-4, H-5, H-7, and H-8, respectively. The aromatic proton H-8 has a relatively higher chemical shift compared with others due to the reason of deshielding effect caused by its neighbouring carbonyl group.

The ^{13}C-NMR spectrum (Figure 4.3) of 1,6-dihydroxyxanthone revealed a resonance at 181.2 ppm, which was assigned to the highly deshielded carbonyl carbon at carbon position C-9. Besides, the presence of six carbon signals at 165.1 ppm, 161.6 ppm, 158.4 ppm, 156.3 ppm, 113.0 ppm, and 108,0 ppm were assigned to six quaternary carbons C-6, C-1, C-10a, C-4a, C-8a, and C-9a, respectively. The six protonated aromatic carbons at C-2, C-3, C-4, C-5, C-7, and C-8 gave resonance at 109.8 ppm, 136.0 ppm, 106.6 ppm, 101.8 ppm, 113.9 ppm, and 127.2 ppm, respectively.

In addition, the peaks at 161.6 ppm, 165.1 ppm, 158.4 ppm, and 156.3 ppm in the ^{13}C-NMR spectrum were characteristic signals for the aromatic carbons attached to electronegative oxygen atom, which correspond to carbon C-1, C-6, C-10a, and C-4a, respectively. Lastly, the peaks at 108.0 ppm and 113.0 ppm were assigned to the quaternary carbons at C-9a and C-8a, respectively. Both the ^{1}H-NMR and ^{13}C-NMR spectral data were in agreement with the proposed structure 1,6-dihydroxyxanthone having a molecular formula of $C_{13}H_8O_4$.

From the IR spectrum (Figure 4.4), the broad absorbance at 3476 cm^{-1} indicated the presence of -OH functional group while the peak at 1607 cm^{-1} showed the presence C=O functional group. These two characteristic bands in IR spectrum were in correspondence to the functional groups present in the compound and this further confirmed the compound to be 1,6-dihydroxyxanthone.

In the UV-Vis spectrum (Figure 4.5), it was shown that the isolated compound has UV absorption maxima at wavelengths of 230.8 nm, 290.8 nm, 305.98 nm and 358.49 nm. This indicated that the isolated compound was a highly conjugated compound which was in agreement with the proposed structure.



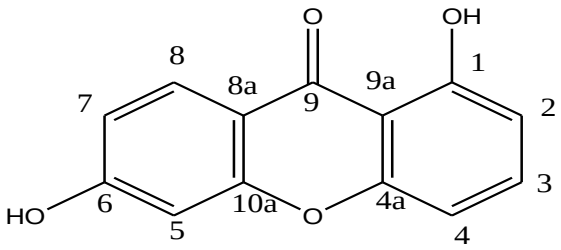
1,6-dihydroxyxanthone

Molecular formula: $C_{13}H_8O_4$

Molecular weight: 228.054 g mol^{-1}

Table 4.3: Summary of NMR spectral data of 1,6-dihydroxyxanthone

Position	**^{1}H δ (ppm)**	**^{13}C δ (ppm)**
1	-	161.6
2	6.66 (1H,d,*J*=8.2 Hz)	109.8
3	7.51(1H,t,*J*=8.2 Hz)	136.0
4	6.83 (1H,d,*J*=8.2 Hz)	106.6
4a	-	156.3
5	6.71 (1H,d,*J*=2.1 Hz)	101.8
6	-	165.1
7	6.81 (1H,dd,*J*=8.2, 2.1 Hz)	113.9
8	7.97 (1H,d,*J*=8.2 Hz)	127.2
8a	-	113.0
9	-	181.2
9a	-	108.0
10a	-	158.4
1-OH	12.74 (1H, s)	-



1,6-dihydroxyxanthone

Molecular formula: $C_{13}H_8O_4$

Molecular weight: 228.054 g mol^{-1}

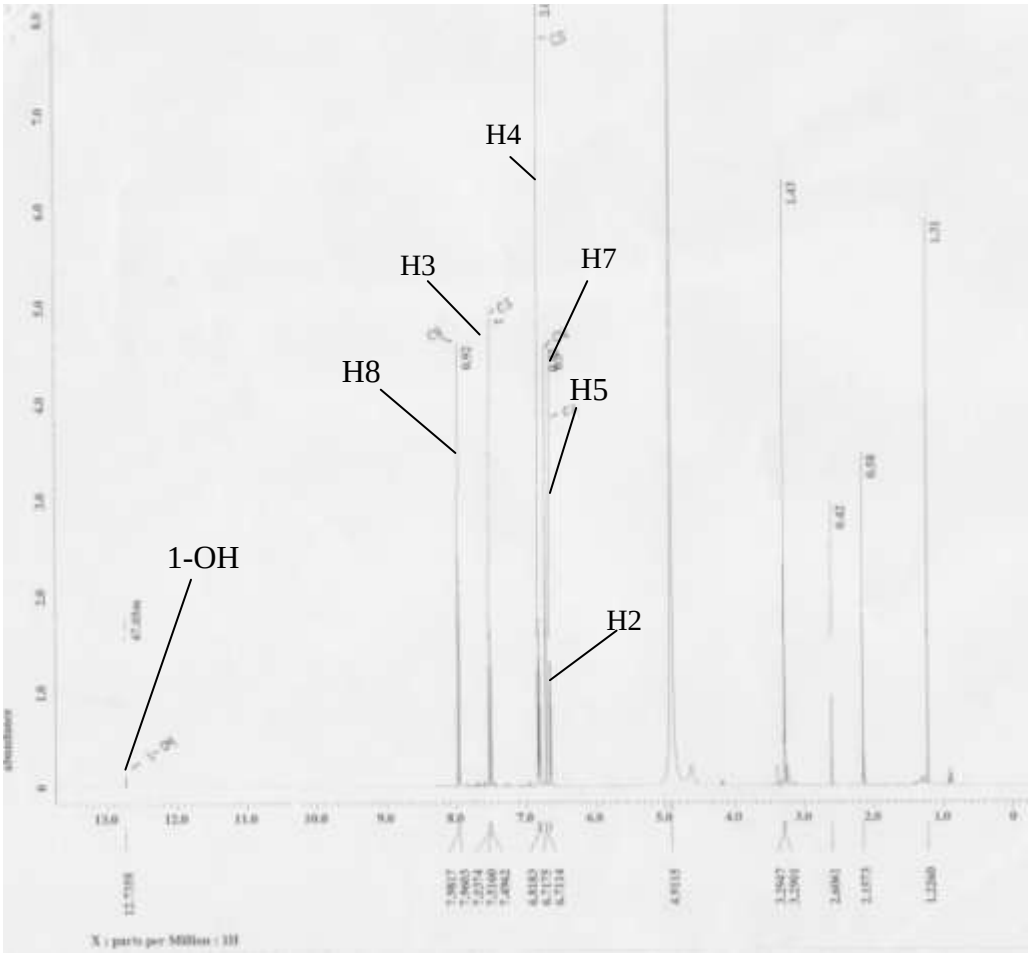
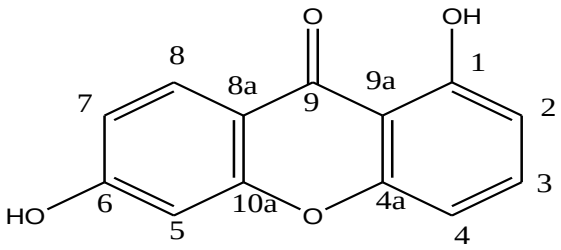


Figure 4.2: ^{1}H-NMR spectrum of 1,6-dihydroxyxanthone (100 MHz, methanol-d_4)



1,6-dihydroxyxanthone

Molecular formula: $C_{13}H_8O_4$

Molecular weight: 228.054 g mol^{-1}

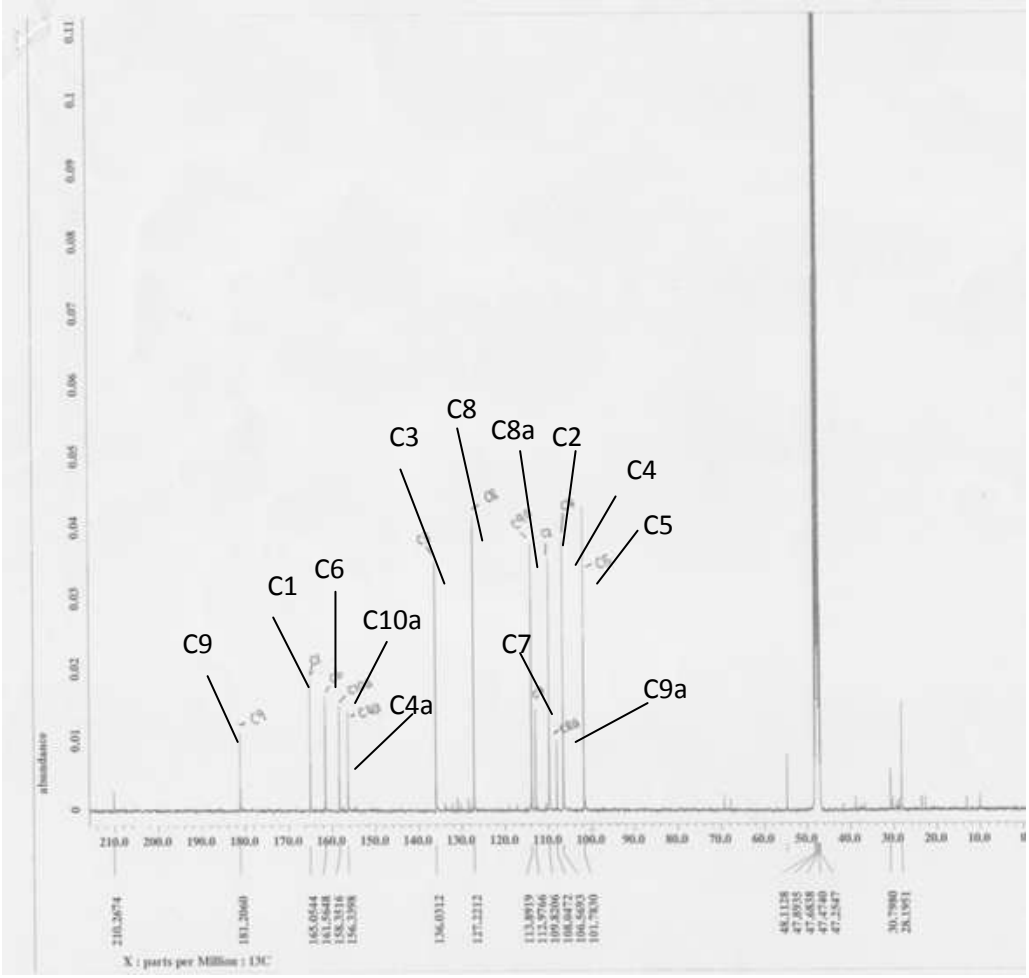
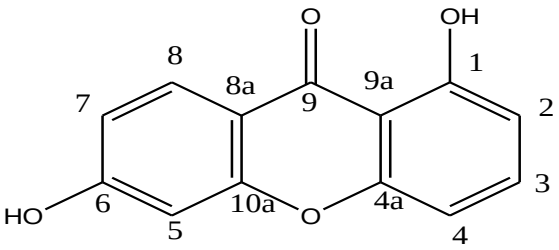


Figure 4.3: ^{13}C-NMR spectrum of 1,6-dihydroxyxanthone (400 MHz, methanol-d_4)



1,6-dihydroxyxanthone

Molecular formula: $C_{13}H_8O_4$

Molecular weight: 228.054 g mol^{-1}

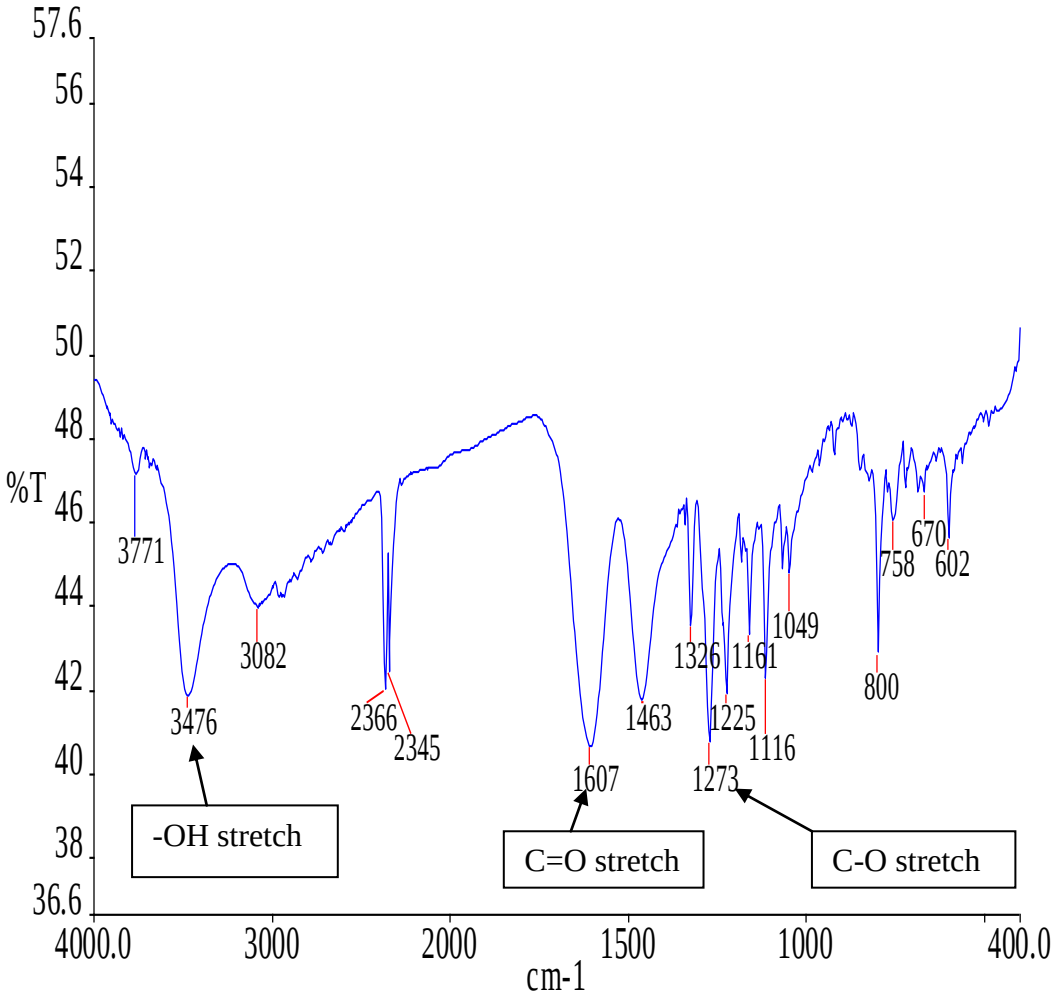
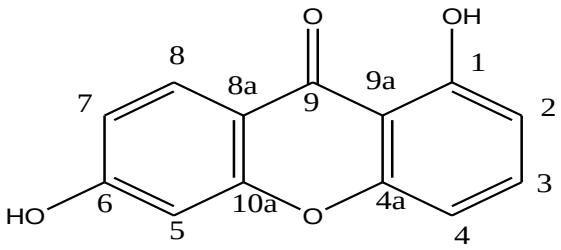


Figure 4.4: IR spectrum of 1,6-dihydroxyxanthone



1,6-dihydroxyxanthone

Molecular formula: $C_{13}H_8O_4$

Molecular weight: 228.054 g mol^{-1}

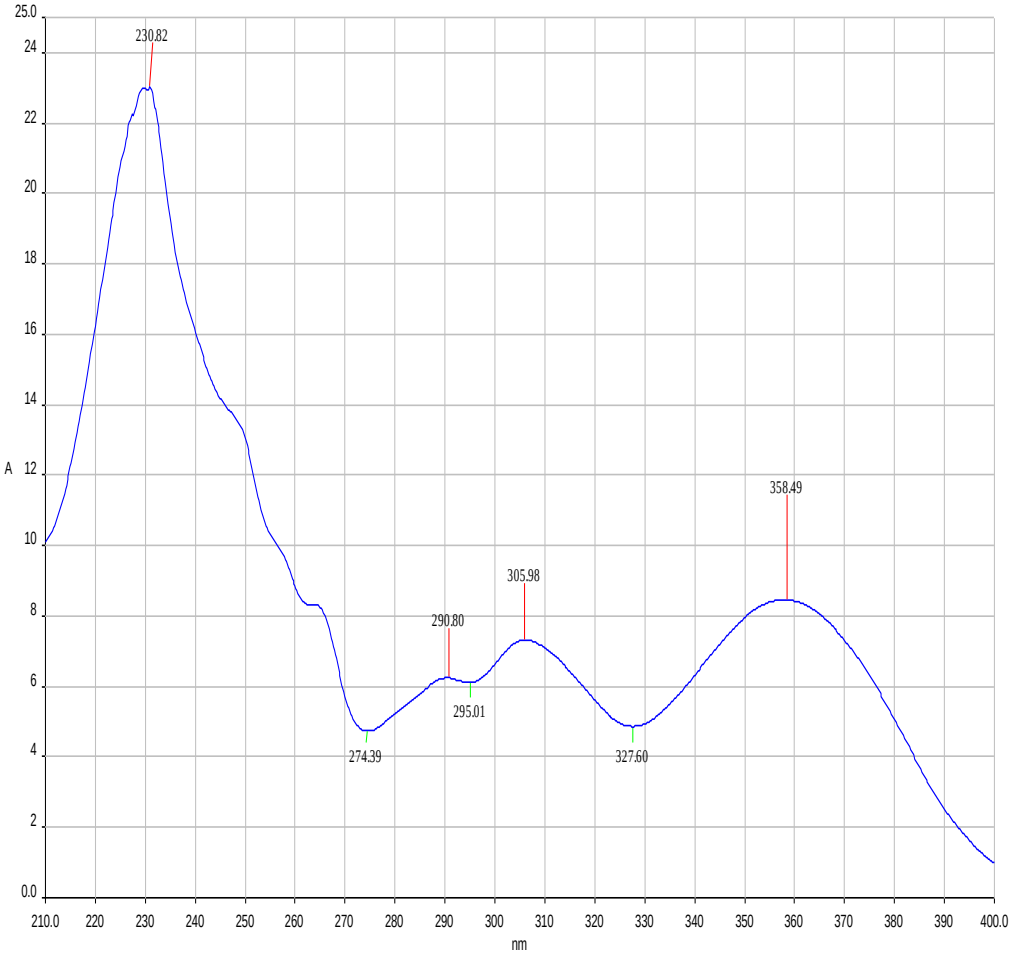


Figure 4.5: UV-Vis spectrum of 1,6-dihydroxyxanthone

4.2.2 Proposed Mechanism for Synthesis of 1,6-Dihydroxyxanthone

The outline of proposed mechanism to account for the formation of 1,6-dihydroxyxanthone is as follow:

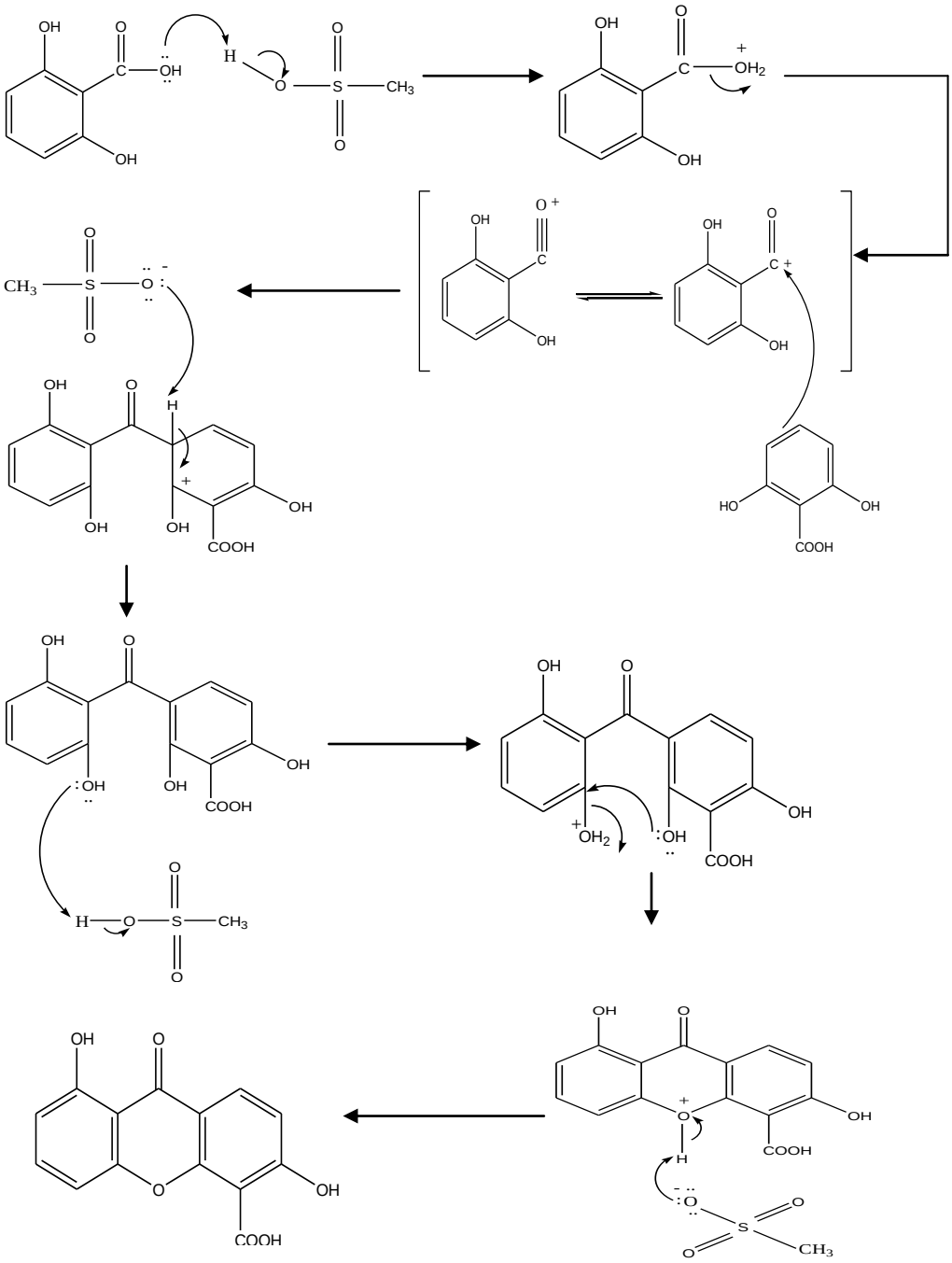
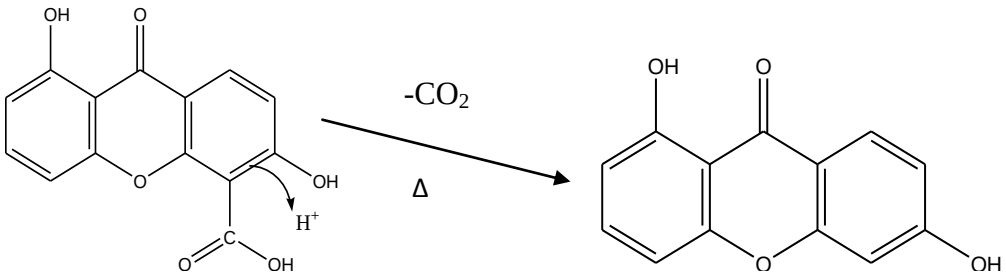


Figure 4.6: Reaction mechanism involved in synthesis of 1,6-dihydroxyxanthone

Decarboxylation:



Dehydrating agent:

$$P_4O_{10} + 6\ H_2O \longrightarrow 4\ H_3PO_4$$

Figure 4.7: Reaction mechanism involved in the synthesis of 1,6-dihydroxyxanthone (continued)

4.3 Prenylation of 1,6-Dihydroxyxanthone in Organic Medium

1-Hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one was a derivative compound of 1,6-dihydroxyxanthone. This compound was prepared from the reaction between 1,6-dihydroxyxanthone and prenyl bromide in the presence of potassium carbonate in the organic acetone medium. Potassium carbonate was used as a base and catalyst to accelerate the reaction speed. The total mass of pure 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one synthesized was 0.5471 g, which was 50.5% of the percentage yield. The overall equation of reaction is shown in Figure 4.8.

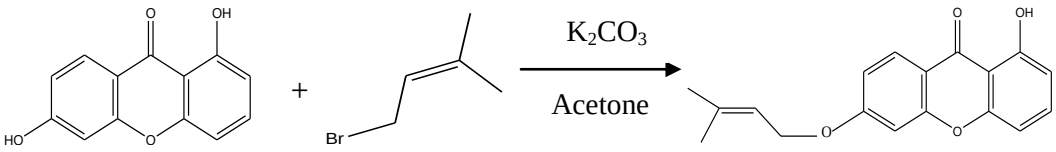


Figure 4.8: Equation of reaction for synthesis of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

4.3.1 Structure Elucidation of 1-Hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

1-Hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one was isolated in the form of yellowish solid with a melting point range of 123^{o}C to 125^{o}C. When the TLC was developed with solvent system of 70% hexane and 30% acetone, the collected fractions from 2 to 4 containing the compound gave a single spot with R_f value of

0.57. The summary of physical properties of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one is tabulated in Table 4.4.

Table 4.4: Summary of physical properties of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Mass obtained, g	0.5471
Percentage yield, %	46.1
Physical appearance	Yellowish solid
Melting point	123^{o}C to 125^{o}C
R_f value on TLC	0.57 in hexane: acetone (7:3)

^{1}H-NMR spectral data (Figure 4.9) indicated the compound to have a xanthone skeleton with one prenyl moiety. The presence of six carbon signals at δ 8.10 (1H, d, *J*=8.5 Hz), 7.65 (1H, t, *J*=8.5 Hz), 7.04 (1H, s), 7.03 (1H, d, *J*=8.5 Hz), 6.95 (1H, d, *J*=8.5 Hz), and 6.74 (1H, d, *J*=8.5 Hz) were due to the aromatic protons H-8, H-3, H-5, H-7, H-4, and H-2, respectively. A signal at 12.82 ppm (1H, s, 1-OH) indicated the presence of a chelated hydroxyl proton. A doublet signal at 4.76 ppm (2H, d, *J*=6.7 Hz) was assigned to proton H-11 and the low intensity peak at 5.51 ppm (1H, t, *J*=6.7 Hz) was assigned to proton H-12. An intense upfield singlet at 1.78 ppm, integrated for six protons, was attributed to the two groups of equivalent vinylic methyl protons H-14 and H-15.

From the ^{13}C-NMR spectrum (Figure 4.10), a signal at 181.3 ppm was assigned to the highly deshielded carbonyl carbon C-9. Besides, the carbon signals at 165.6 ppm, 162.0 ppm, 158.4 ppm, 156.5 ppm, 138.6 ppm, 113.9 ppm, and 108.4 ppm were assigned to seven quaternary carbons C-6, C-1, C-10a, C-4a, C-13, C-8a, C-9a, respectively. The protonated aromatic carbons C-3, C-8, C-12, C-7, C-2, C-4, and C-5 gave signals at 136.7 ppm, 127.1 ppm, 119.0 ppm, 114.5 ppm, 110.3 ppm, 106.8 ppm, and 100.9 ppm, respectively. Carbon signal at 65.8 ppm was assigned to methylene carbon C-11, and the other two signals at 25.0 ppm and 17.5 ppm were due to the methyl carbons C-14 and C-15, respectively. These were consistent with the structure of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one. The existence of the prenyl group in the compound was revealed by the characteristic carbon signals at 138.6 ppm (C-13), 119.0 ppm (C-12), 65.8 ppm (C-11), 25.0 ppm (C-14), and 17.5 ppm (C-15). Highly deshielded carbon signals at 165.6 ppm and 162.0 ppm were assigned to the oxygenated aromatic carbons C-6 and C-1, respectively.

The assignment of resonance in the ^{1}H-NMR and ^{13}C-NMR spectra of this compound was further confirmed by a 2D- NMR analysis. The verification of the structure of the proposed compound was accomplished through the combination of HMQC and HMBC spectral data.

HMQC spectrum (Figure 4.11) showed the direct ^{1}J coupling between proton and carbon. From the spectrum, the chemical shifts of the carbon signals which correlated to protons H-2 (6.74 ppm), H-3 (7.65 ppm), H-4 (6.95 ppm), H-5 (7.04 ppm), H-7 (7.03 ppm), H-8 (8.10 ppm), H-11 (4.76 ppm), H-12 (5.51 ppm), H-14 (1.78 ppm), and H-15 (1.78 ppm) were corresponded to 110.3 ppm (C-2), 136.7 ppm (C-3), 106.8 ppm (C-4), 100.9 ppm (C-5), 114.5 ppm (C-7), 127.1 ppm (C-8), 65.8 ppm (C-11), 119.0 ppm (C-12), 25.0 ppm (C-14), and 17.5 ppm (C-15), respectively

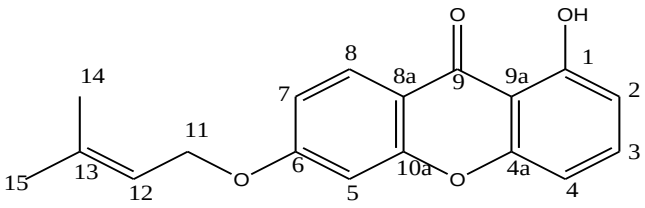
Quaternary carbons gave no correlation in HMQC spectrum but the assignment of their carbon positions in the structure can be accomplished through the long range coupling. HMBC analyses revealed the interaction of either ^{2}J or ^{3}J long range coupling between proton and its farther distance carbon. In the HMBC spectrum (Figure 4.12), it was observed that proton H-2 was ^{3}J coupling with carbon C-4. The assignment of this position was further confirmed by ^{3}J coupling of proton H-4 with carbon C-2. Besides, proton H-11 was ^{2}J coupling with carbon C-12 and ^{3}J coupling with carbon C-6 and C-13. From this ^{3}J long range heteronuclear connectivity between proton signals H-11 and carbon signal C-6 indicated that the prenyl group was attached to the oxygen atom that bonded to carbon C-6 position. On the other hand, the methyl protons H-14 and H-15 in prenyl group showed ^{2}J coupling with C-13 and ^{3}J coupling with C-12, indicating the presence of prenyl group in the structure. The aromatic proton H-3 was found to have ^{3}J coupling with C-1 and C-4a while proton H-5 was ^{2}J coupling with C-6 and C-10a, and ^{3}J

coupling with C-7. These correlations supported the assignment of xanthone nucleus in the structure proposed.

The combination of ^{1}H-NMR, ^{13}C-NMR and 2D- NMR spectra has proposed the structure of compound as 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one with molecular formula of $C_{18}H_{16}O_{4.}$

From the IR spectrum (Figure 4.13), a broad band at 3402 cm^{-1} was due to the presence of –OH functional groups in the compound. Other bands were observed at about 1617 cm^{-1} and 1280 cm^{-1} indicating the presence of C=O and C-O functional groups. Together, these data were found to be in correspondence to the characteristic of the proposed structure 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one.

From UV-Vis spectrum (Figure 4.14), UV absorption maxima at 238.71 nm, 306.98 nm, and 353.58 nm implied that the compound is a highly conjugated compound which was in agreement with the characteristic of the proposed 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one.



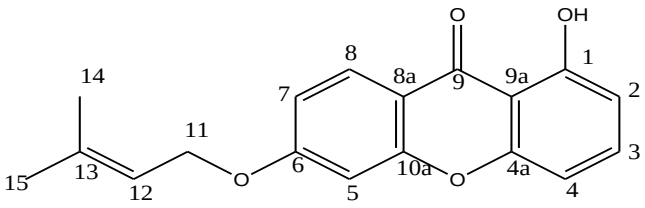
1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{18}H_{16}O_4$

Molecular weight: 296.168 g mol^{-1}

Table 4.5: Summary of NMR spectral data of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Position	^{1}H δ (ppm)	^{13}C δ (ppm)	HMBC
1	-	162.0	
2	6.74 (1H,d,*J*=8.5 Hz)	110.3	C-4(^{3}J)
3	7.65 (1H,t,*J*=8.5 Hz)	136.7	C-4a(^{3}J),1(^{3}J)
4	6.95 (1H,d,*J*=8.5 Hz)	106.8	C-2(^{3}J)
4a	-	156.5	
5	7.04 (1H,s)	100.9	C-7(^{3}J),10a(^{2}J),6(^{2}J)
6	-	165.6	
7	7.03 (1H,d,*J*=8.5 Hz)	114.5	
8	8.10 (1H,d,*J*=8.5 Hz)	127.1	C-10a(^{3}J),6(^{3}J)
8a	-	113.9	
9	-	181.3	
9a	-	108.4	
10a	-	158.4	
11	4.76 (2H,d,*J*=6.7 Hz)	65.8	C-12(^{2}J),13(^{3}J),6(^{3}J)
12	5.51 (1H,t,*J*=6.7 Hz)	119.0	
13	-	138.6	
14	1.78 (3H,s)	25.0	C-12(^{3}J),13(^{2}J),15(^{3}J)
15	1.78 (3H,s)	17.5	C-12(^{3}J),13(^{2}J),14(^{3}J)



1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{18}H_{16}O_4$

Molecular weight: 296.168 g mol^{-1}

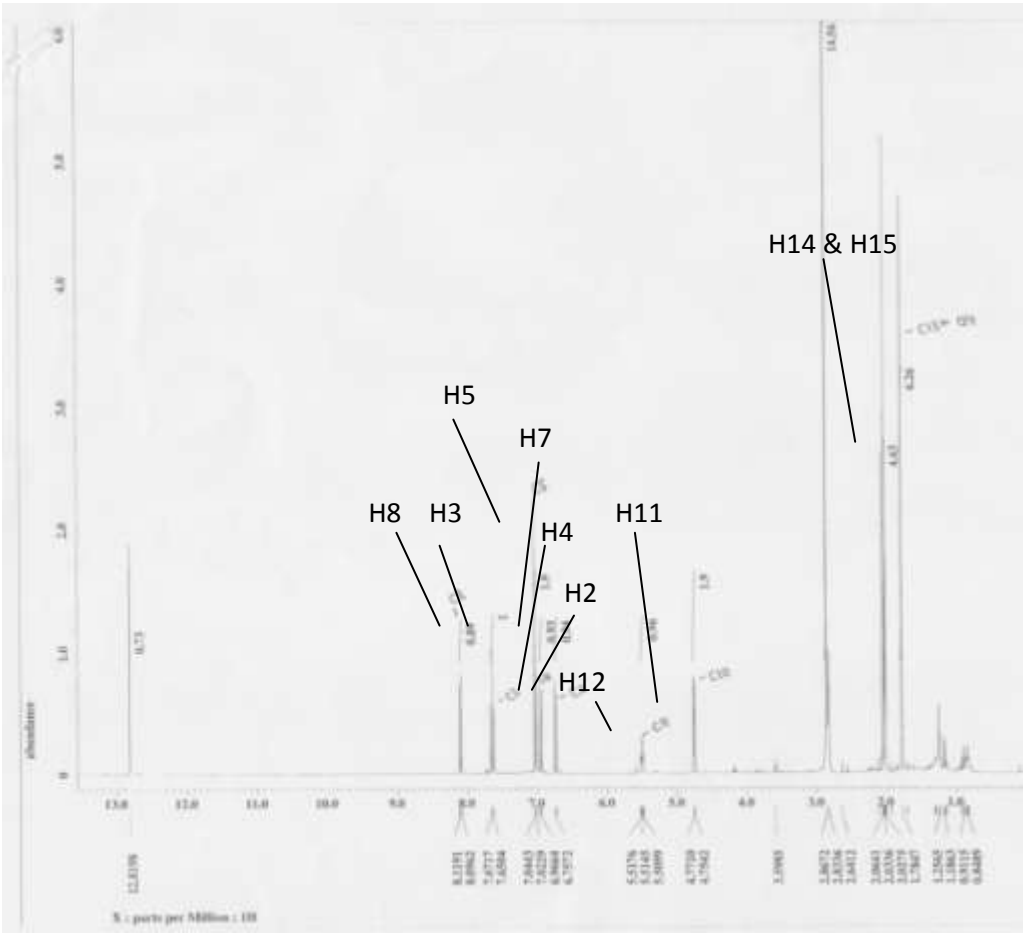
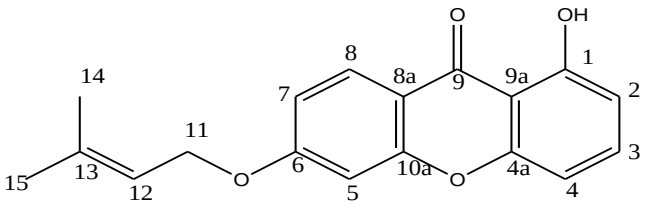


Figure 4.9: ^{1}H-NMR spectrum of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, acetone-d_6)



1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{18}H_{16}O_4$

Molecular weight: 296.168 g mol^{-1}

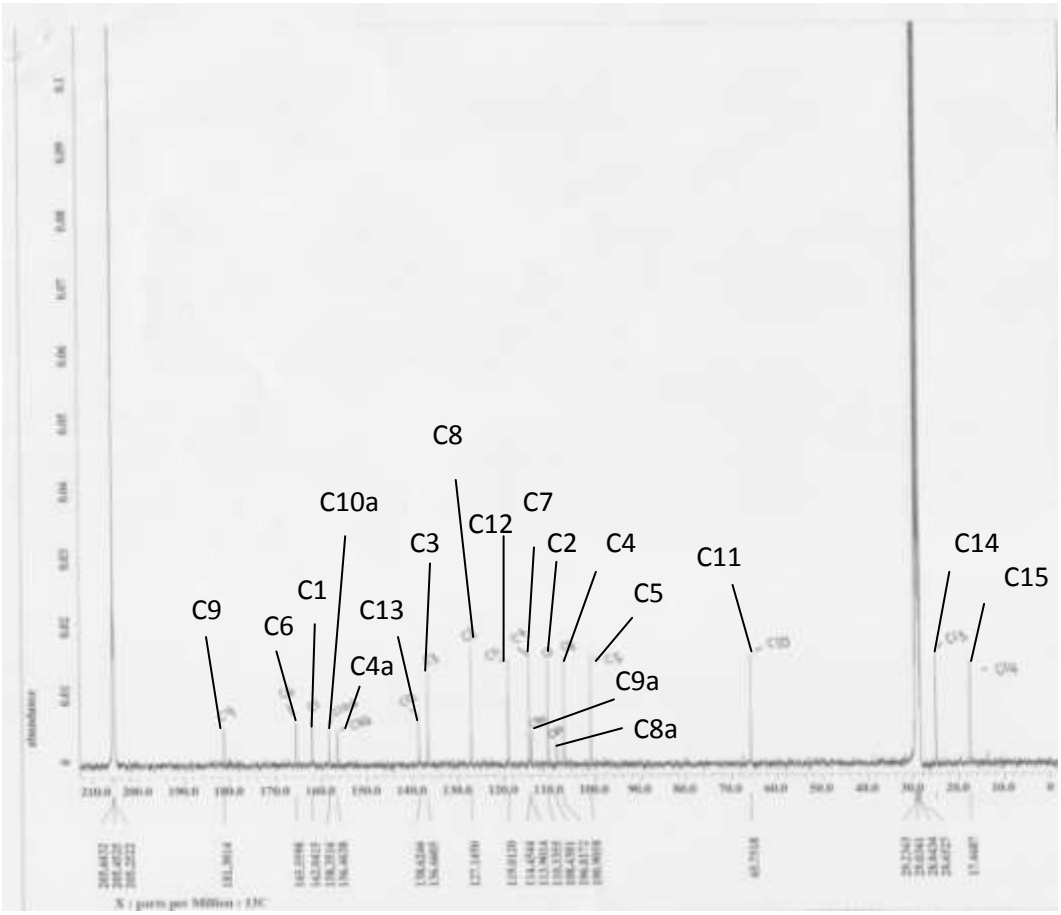
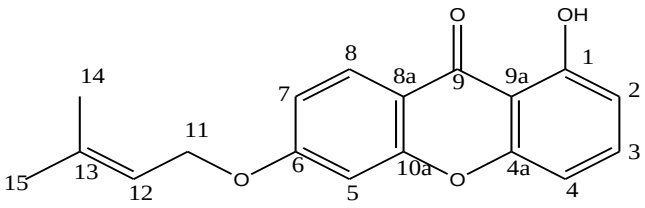


Figure 4.10: ^{13}C-NMR spectrum of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one (100 MHz, acetone-d_6)



1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{18}H_{16}O_4$

Molecular weight: 296.168 g mol^{-1}

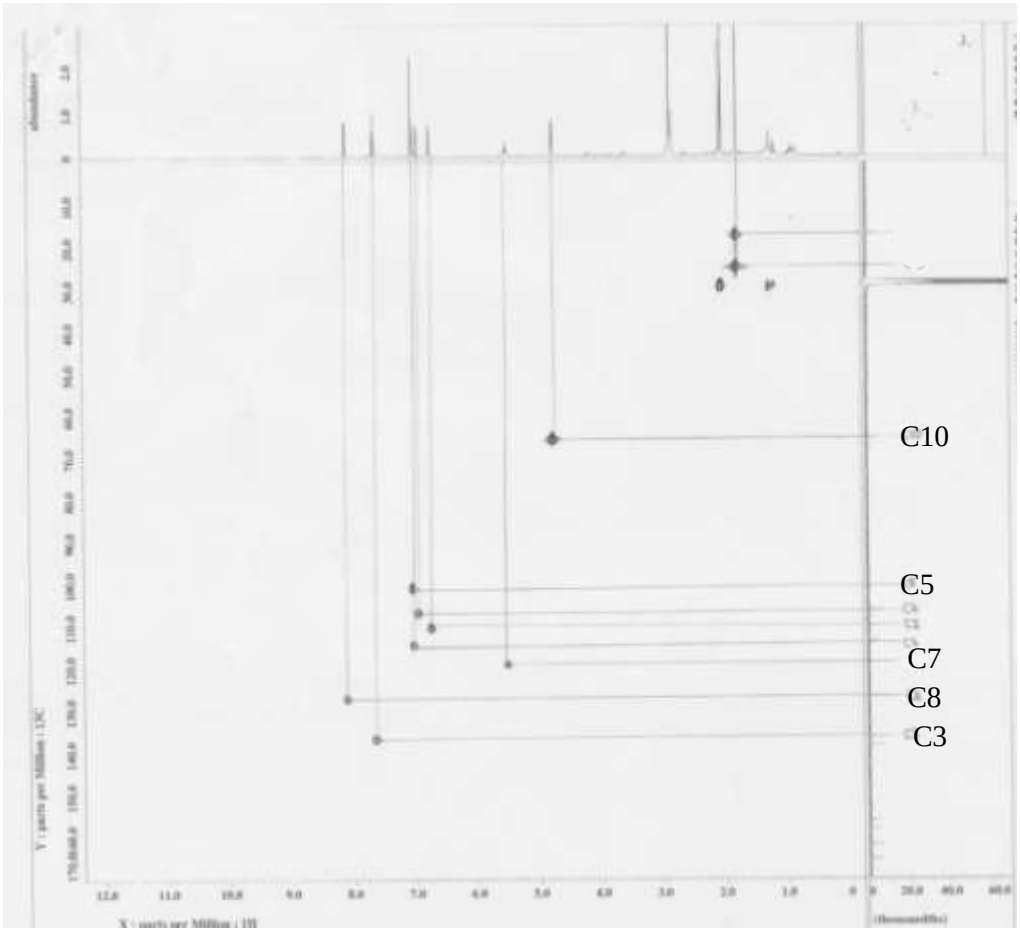
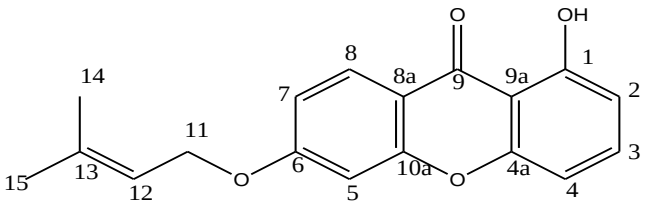


Figure 4.11: HMQC spectrum of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one



1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{18}H_{16}O_4$

Molecular weight: 296.168 g mol^{-1}

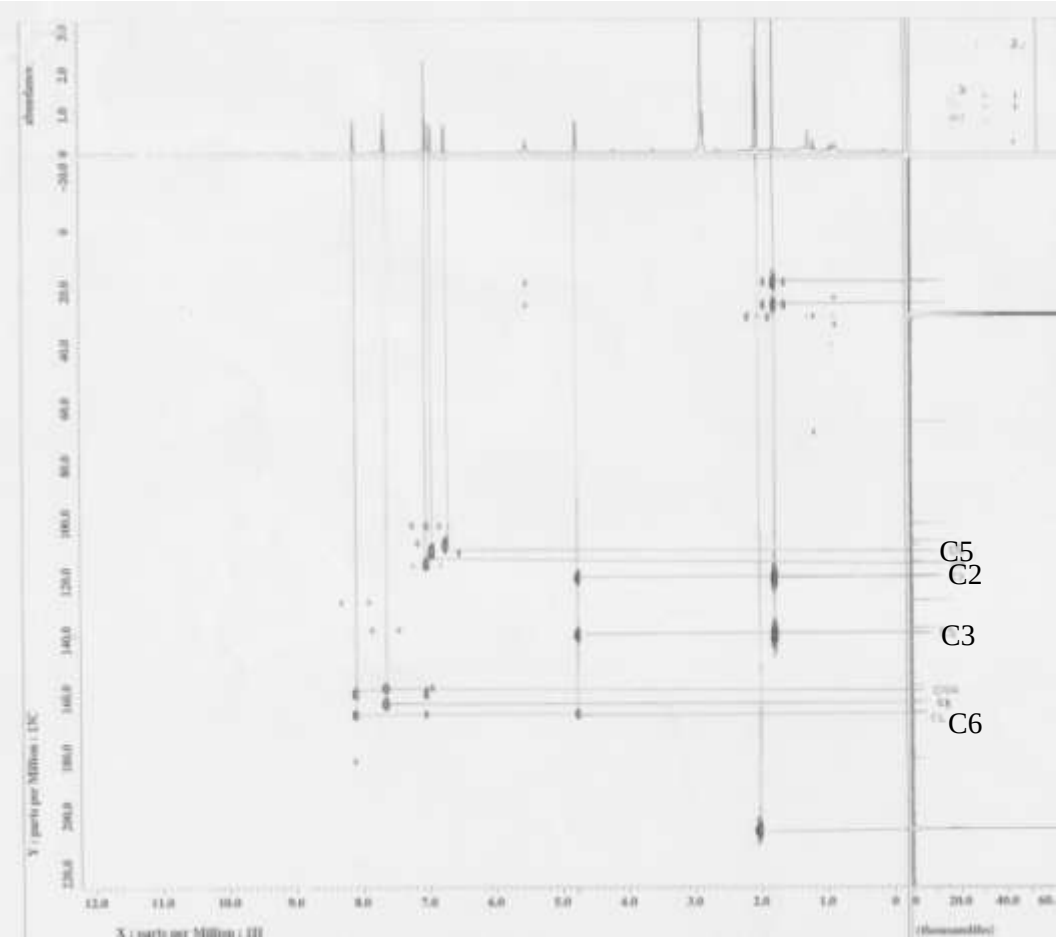
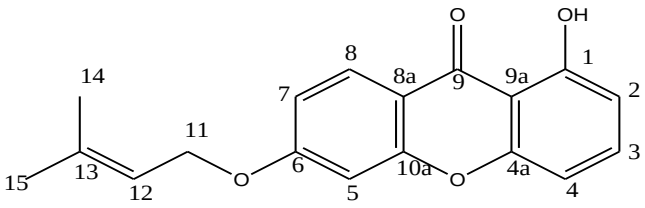


Figure 4.12: HMBC spectrum of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one



1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{18}H_{16}O_4$

Molecular weight: 296.168 g mol^{-1}

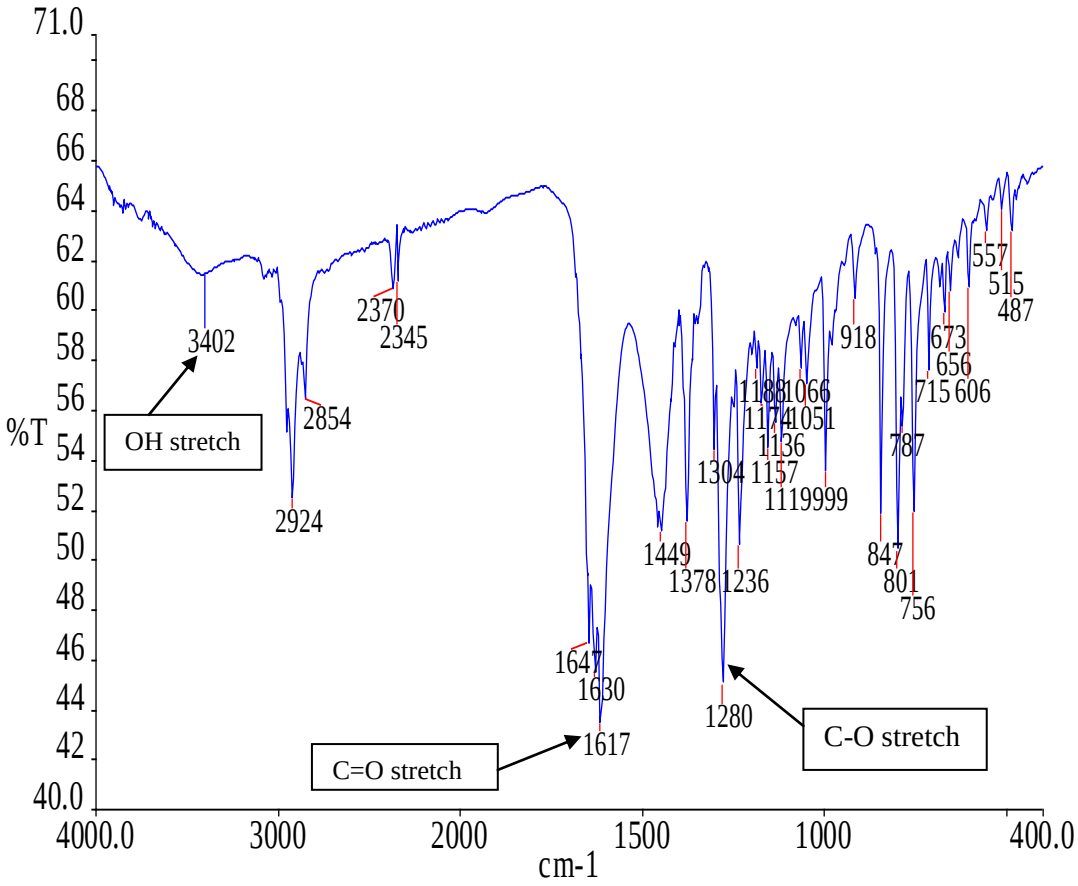
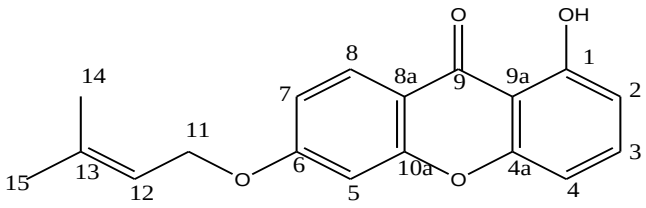


Figure 4.13: IR spectrum of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one



1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{18}H_{16}O_4$

Molecular weight: 296.168 g mol^{-1}

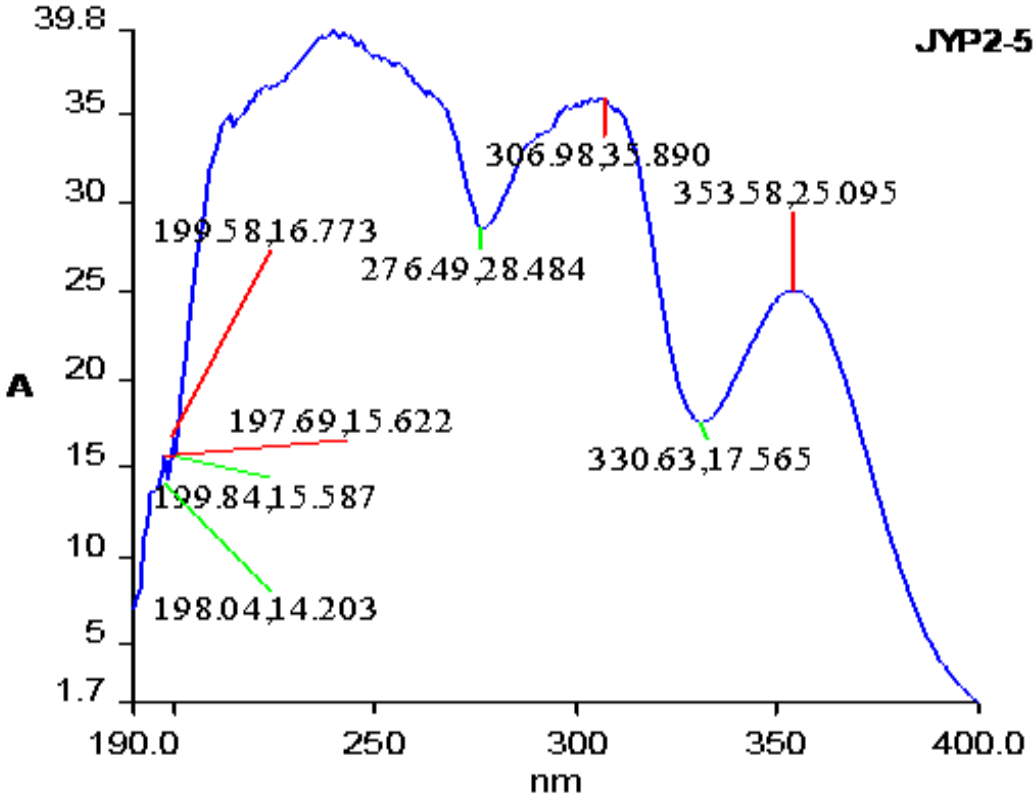


Figure 4.14: UV-Vis spectrum of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

4.3.2 Proposed Mechanism for Synthesis of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

The outline of proposed mechanism to account for the formation of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one is as follow:

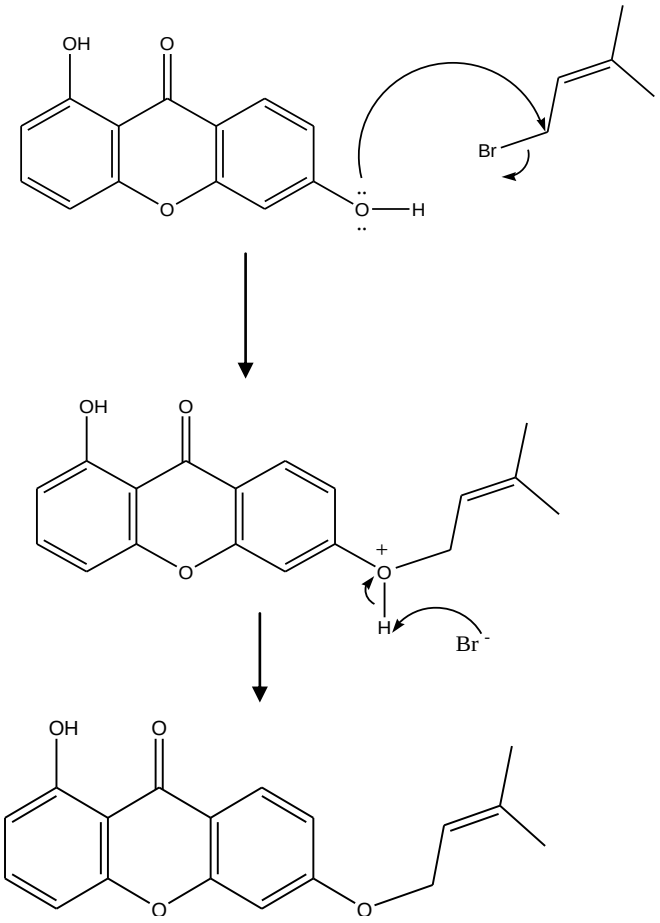
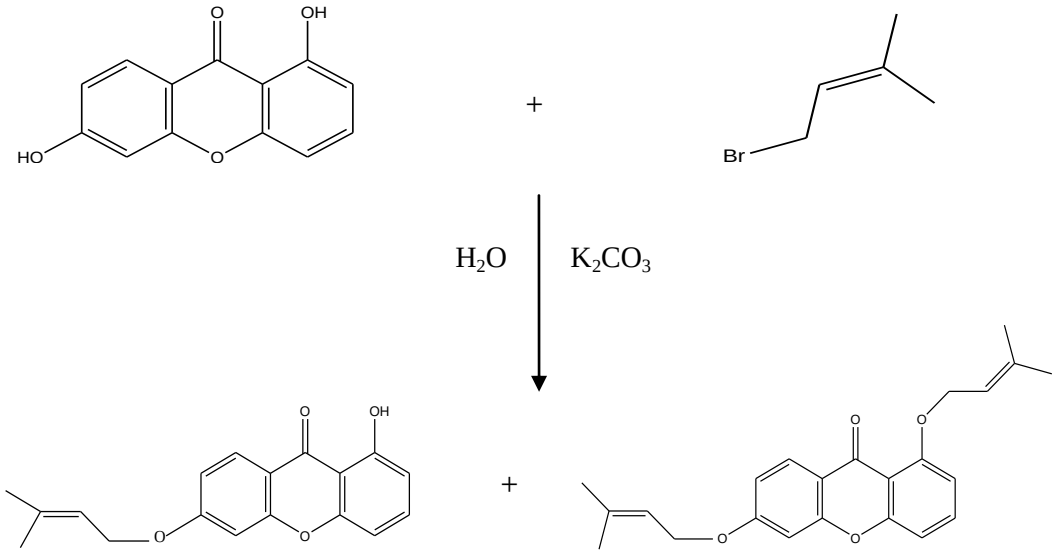


Figure 4.15: Reaction Mechanism involved in the synthesis of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

4.4 Prenylation of 1,6-Dihydroxyxanthone in Aqueous Medium

1-(3-Methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one was another derivatives compound from 1,6-dihydroxyxanthone. This compound was synthesized through the reaction between 1,6-dihydroxyxanthone and prenyl bromide in the presence of potassium carbonate in aqueous medium. Potassium carbonate acted as both a base and a strong catalyst to lower the free energy of activation so that the forward rate of reaction can be increased. The total mass of pure 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one isolated was 0.0800 g, which was 6.1% of the percentage yield.

Other than 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one, another major compound isolated from this synthesis was 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one which was identical to the compound produced in the former prenylation of 1,6-dihydroxyxanthone in organic acetone medium. The total mass of pure 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one obtained from the aqueous-medium based synthesis was 0.1847 g, or 17.3% of the percentage yield. When TLC plate was developed with solvent system of 85% hexane and 15% acetone, the single red spot gave a R_f value of 0.30. The overall equation of reaction is shown in Figure 4.16.



1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

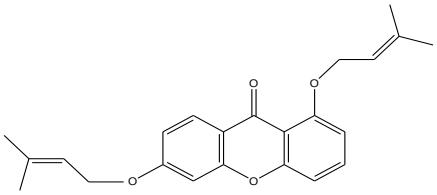
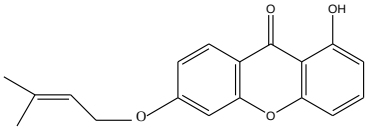
1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Figure 4.16: Equation of reaction for prenylation in aqueous medium

4.4.1 Structure Elucidation of 1-(3-Methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

1-(3-Methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one was isolated in the form of yellowish amorphous with a melting point range of 68^{o}C to 70^{o}C. When the TLC was developed with solvent system of 85% hexane and 15% acetone, the collected fractions from 4 to 7 gave a single blue spot with R_f value of 0.39. The summary of physical properties of both compounds 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one and 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one is tabulated in Table 4.6.

Table 4.6: Summary of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one and 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

		
Mass obtained, g	0.0800	0.1847
Percentage yield, %	6.1	17.3
Physical appearance	Yellowish amorphous	Yellowish solid
Melting point	68^{o}C to 70^{o}C	123^{o}C to 125^{o}C
R_f value on TLC	0.39 in hexane:acetone (85:15)	0.30 in hexane:acetone (85:15)

The ^{1}H-NMR analyses (Figure 4.17) of pure 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one revealed that two prenyl groups were substituted to the compound. The presence of two pairs of characteristic signals 4.47(2H,d,*J*=6.7 Hz) and 5.35(1H,t,*J*=6.7 Hz), 4.57(2H,d,*J*=6.7 Hz) and 5.48(1H,t,*J*=6.7 Hz) in the range from 4.0 ppm to 6.0 ppm signified the presence of two O-prenyl groups in structure of compound. The absence of signal in the range from δ 12.0 to 13.0 suggested the chelated hydroxyl group bonded to

carbon C-1 was reacted during the synthesis. This further confirmed that one of the prenyl groups was attached at the O-position of carbon C-1. The presence of six resonances at δ 8.03 (1H, d, *J*=9.2 Hz), 7.40 (1H, t, *J*=8.2 Hz), 6.86 (1H, d, *J*=8.2 Hz), 6.74 (1H, d, *J*=9.2 Hz), 6.67 (1H, d, *J*=2.4 Hz), and 6.65 (1H, d, *J*=8.2 Hz) were assigned to protons H-8, H-3, H-4, H-7, H-5, and H-2, respectively. The olefinic protons H-17 and H-12 in the prenyl groups gave two triplet signals at 5.36 ppm (1H, t, *J*=6.7Hz) and 5.48 ppm (1H, t, *J*=6.7 Hz), respectively. Two doublet signals at 4.58 ppm (2H, d, *J*=6.7 Hz) and 4.47 ppm (2H, d, *J*=6.7 Hz) were assigned to the benzylic methylene protons H-11 and H-16, respectively. The two intense singlet signals at 1.67 ppm and 1.63 ppm each integrated for six protons were respectively assigned to the methyl proton H-14&-15 and H-19&-20 in the two prenyl groups. The aromatic proton H-8 had a relatively higher chemical shift compared with others due to the deshielding effect by its neighbouring carbonyl group.

From ^{13}C-NMR spectrum (Figure 4.18) of this pure compound, a peak at 175.6 ppm was assigned to carbon C-9 which was highly deshielded carbonyl carbon. The presence of nine carbon signals at 175.6 ppm, 163.8 ppm, 159.9 ppm, 158.2 ppm, 156.7 ppm, 139.1 ppm, 137.4 ppm, 116.8ppm, and 112.7 ppm were assigned to quaternary carbon C-9, C-6, C-1, C-4a, C-10a, C-18, C-13, C-8a, and C-9a, respectively. Besides, another eight carbon signals at 134.2 ppm, 128.1 ppm, 119.6 ppm, 118.7 ppm, 113.3 ppm, 109.6 ppm, 107.0 ppm, and 100.2 ppm were assigned to methine carbons C-3, C-8, C-12, C-17, C-7, C-4, C-2, and C-5,

respectively. Two methylene carbons in both prenyl group gave two doublet signals at 66.4 ppm and 65.3 ppm which were assigned to carbon C-11 and C-16, respectively while the carbon signals at 25.7 ppm and 18.3 ppm were assigned to methyl carbons C-14&-15 and C-19&-20.

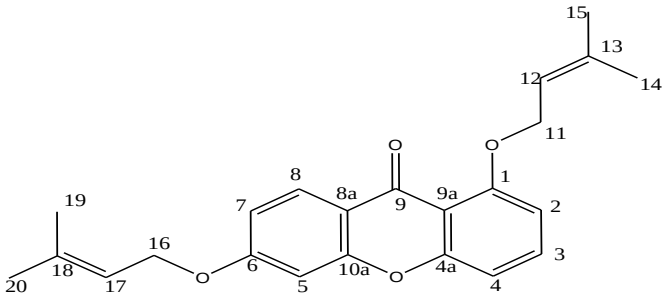
The assignment of chemical shifts in the ^{1}H-NMR and ^{13}C-NMR of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one was further confirmed by 2D-NMR spectroscopy such as HMQC and HMBC analyses.

From HMQC spectrum (Figure 4.19), the six aromatic methine carbon signals at 134.2 ppm, 128.1 ppm, 113.3 ppm, 109.6 ppm, 107.0 ppm and 100.2 ppm were correlated to the proton signals at 7.40 ppm, 8.03 ppm, 6.74 ppm, 6.86 ppm, 6.65 ppm, and 6.67 ppm, respectively. Besides, two methylene carbons at both prenyl group having signals at 66.4 ppm and 65.3 ppm were found to show connectivity to proton signals at 4.58 ppm and 4.47 ppm, respectively. The methine prenyl carbons at 119.6 ppm and 118.7 ppm were attached to proton signals at 5.48 ppm and 5.36 ppm, respectively. The two methyl carbons in both the prenyl side chain having signals at 25.7 ppm and 18.2 ppm was respectively connected to proton resonaces at 1.67 ppm and 1.63 ppm, respectively.

In this HMBC spectrum (Figure 4.20), the benzylic methylene proton H-11 (4.58 ppm) showed long-range heteronuclear connnectivities with an oxygenated aromatic carbon C-1 (159.9 ppm), quaternary carbon C-13 (137.4 ppm), and prenyl methine carbon C-12 (119.6 ppm) indicating the first prenyl group was attached to the xanthone skeleton through the oxygen atom bonded to carbon C-1. On the other hand, the benzylic methylene proton H-16 (4.47 ppm) showed ^{3}J coupling with oxygenated aromatic carbon C-6 (163.8 ppm), ^{3}J coupling with quaternary prenyl carbon C-18 (139.1 ppm), and ^{2}J coupling with methine prenyl carbon C-17 (118.7 ppm). The aromatic methine carbon C-8 showed correlation with oxygenated carbon C-6 (163.8 ppm), carbonyl carbon C-9 (175.6 ppm), and quaternary carbon C-10a (156.7 ppm) with ^{3}J couplings. These correlations again confirmed the presence of two units of prenyl side chain which were attached to O-position at carbons C1 and C6.

The IR spectrum (Figure 4.21) of the compound showed the absence of broad peak in the region 3600 - 3200 cm^{-1}, this implied that there was absence of –OH functional group in the molecule. In fact, this was expected in 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one as the proton in both the hydroxyl groups were substituted with prenyl groups. The intense peak at 1618 cm^{-1} was indication of the presence of C=O functional group in the compound.

Using UV-Vis spectrophotometry analysis, the isolated compound gave absorption maxima at 242.93 nm, 289.88 nm, and 339.39 nm (Figure 4.22), indicating that the isolated compound was highly conjugated and is agreement with the proposed structure 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one.



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

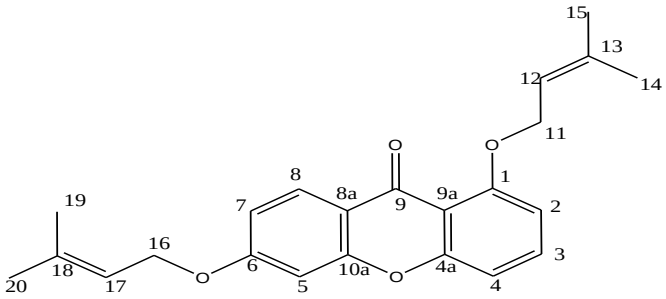
Molecular formula: $C_{23}H_{24}O_4$

Molecular weight: 364.192 g mol^{-1}

Table 4.7: Summary of NMR spectral data of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Position	^{1}H δ (ppm)	^{13}C δ (ppm)	HMBC
1	-	159.9	
2	6.65(1H,d,*J*=8.2 Hz)	107.0	C-4(^{3}J),9a(^{3}J)
3	7.40(1H,t,*J*=8.2 Hz)	134.2	C-1(^{3}J)
4	6.86(1H,d,*J*=8.2 Hz)	109.6	C-4a(^{2}J), 2(^{3}J)
4a	-	158.2	
5	6.67(1H,d, *J*=2.4 Hz)	100.2	C-6(^{2}J),10a(^{2}J),8a(^{3}J)
6	-	163.8	
7	6.74(1H,d,*J*=9.2 Hz)	113.3	C-8a(^{3}J), 5(^{3}J)
8	8.03(1H,d,*J*=9.2 Hz)	128.1	C-9(^{3}J),6(^{3}J),10a(^{3}J)
8a	-	116.8	
9	-	175.6	

9a	-	112.7	
10a	-	156.7	-
11	4.58(2H,d,*J*=6.7 Hz)	66.4	C-1(^3J),13(^3J),12(^2J)
12	5.48(1H,t,*J*=6.7 Hz)	119.6	
13	-	137.4	
14	1.63(3H,s)	18.3	C-12(^3J),13(^2J),15(^3J)
15	1.64(3H,s)	25.7	C-12(^3J),14(^3J)
16	4.47(2H,d,*J*=6.7 Hz)	65.3	C-6(^3J),18(^3J),17(^2J)
17	5.36(1H,t,*J*=6.7 Hz)	118.7	
18		139.1	-
19	1.63(3H,s)	18.3	C-17(^3J),18(^2J),20(^3J)
20	1.67(3H,s)	25.7	C-17(^3J),19(^3J),



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{23}H_{24}O_4$

Molecular weight: 364.192 g mol^{-1}

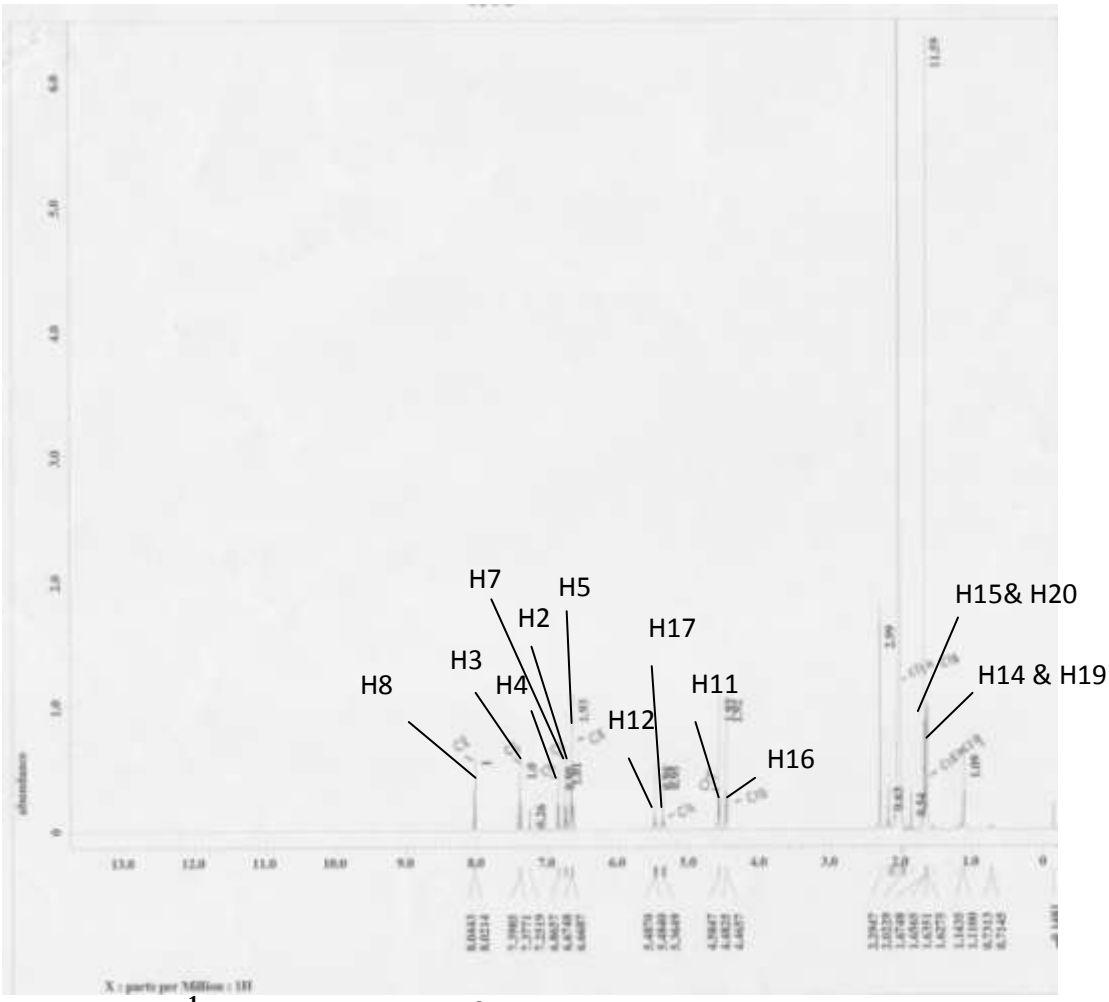
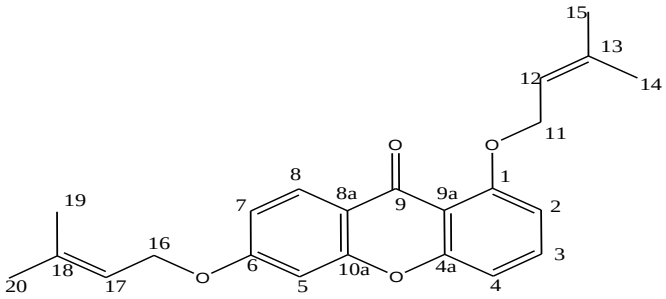


Figure 4.17: ^{1}H-NMR spectrum of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one (400 MHz, $CDCl_3$)



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{23}H_{24}O_4$

Molecular weight: 364.192 g mol^{-1}

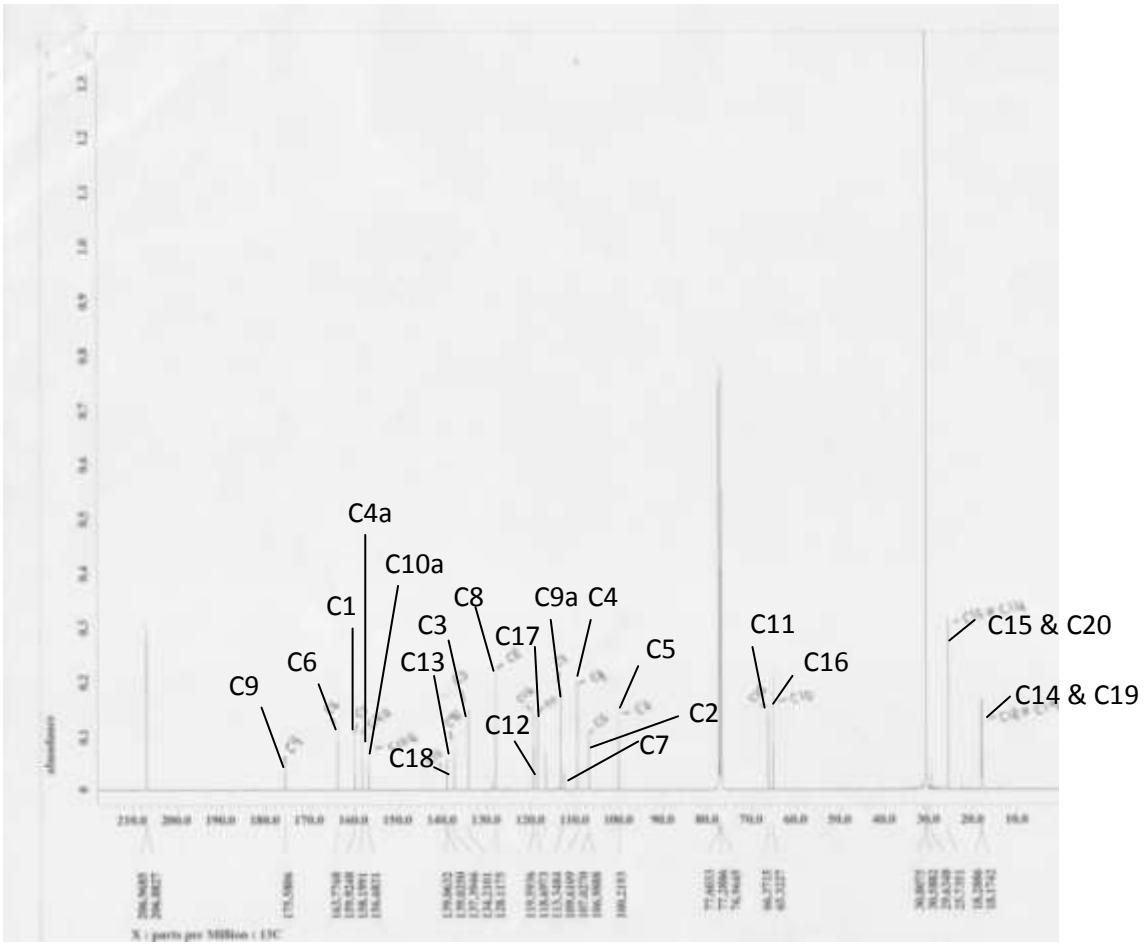
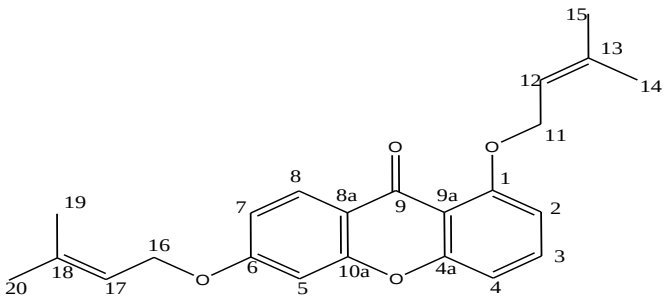


Figure 4.18: ^{13}C-NMR spectrum of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one (100 MHz, $CDCl_3$)



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{23}H_{24}O_4$

Molecular weight: 364.192 g mol^{-1}

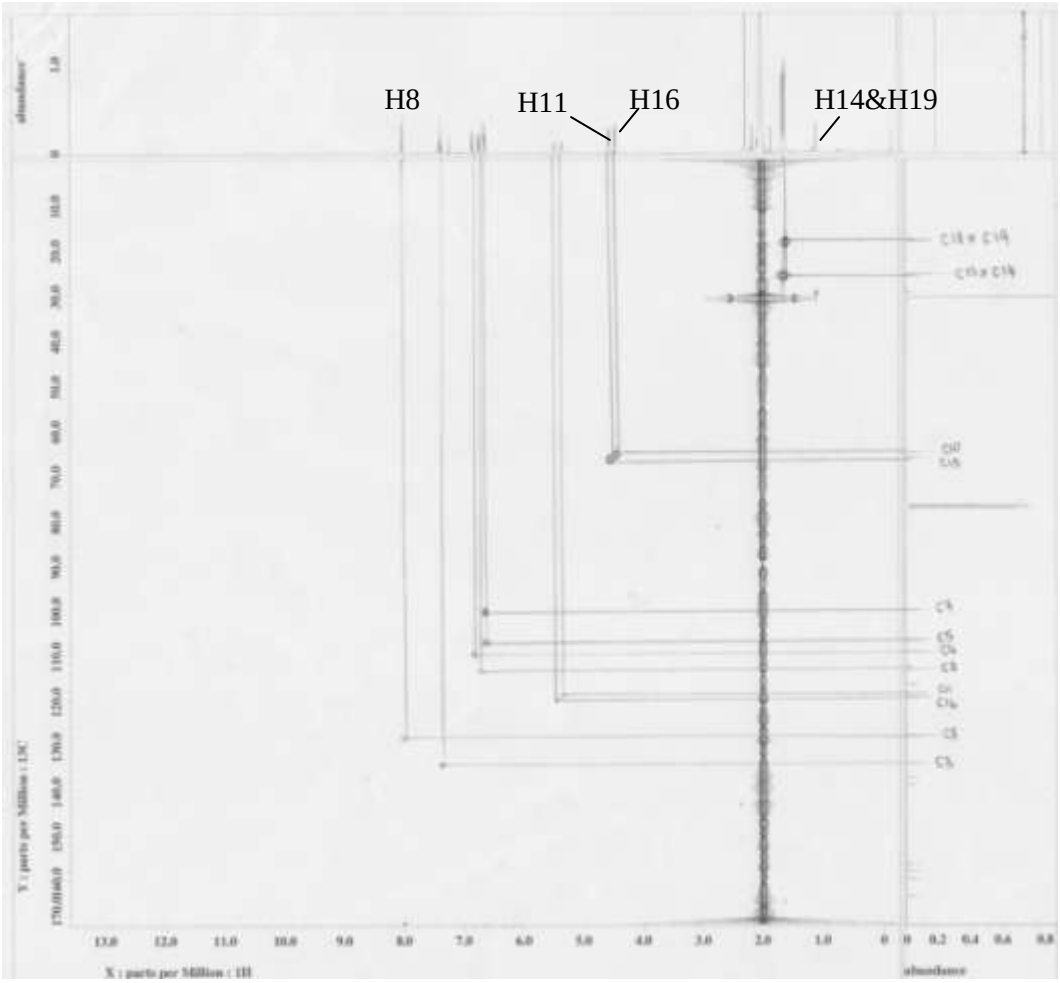
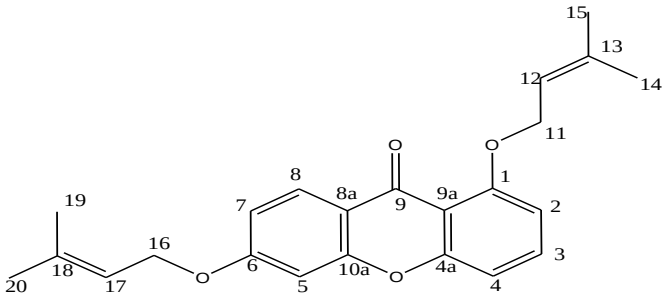


Figure 4.19: HMQC spectrum of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{23}H_{24}O_4$

Molecular weight: 364.192 g mol^{-1}

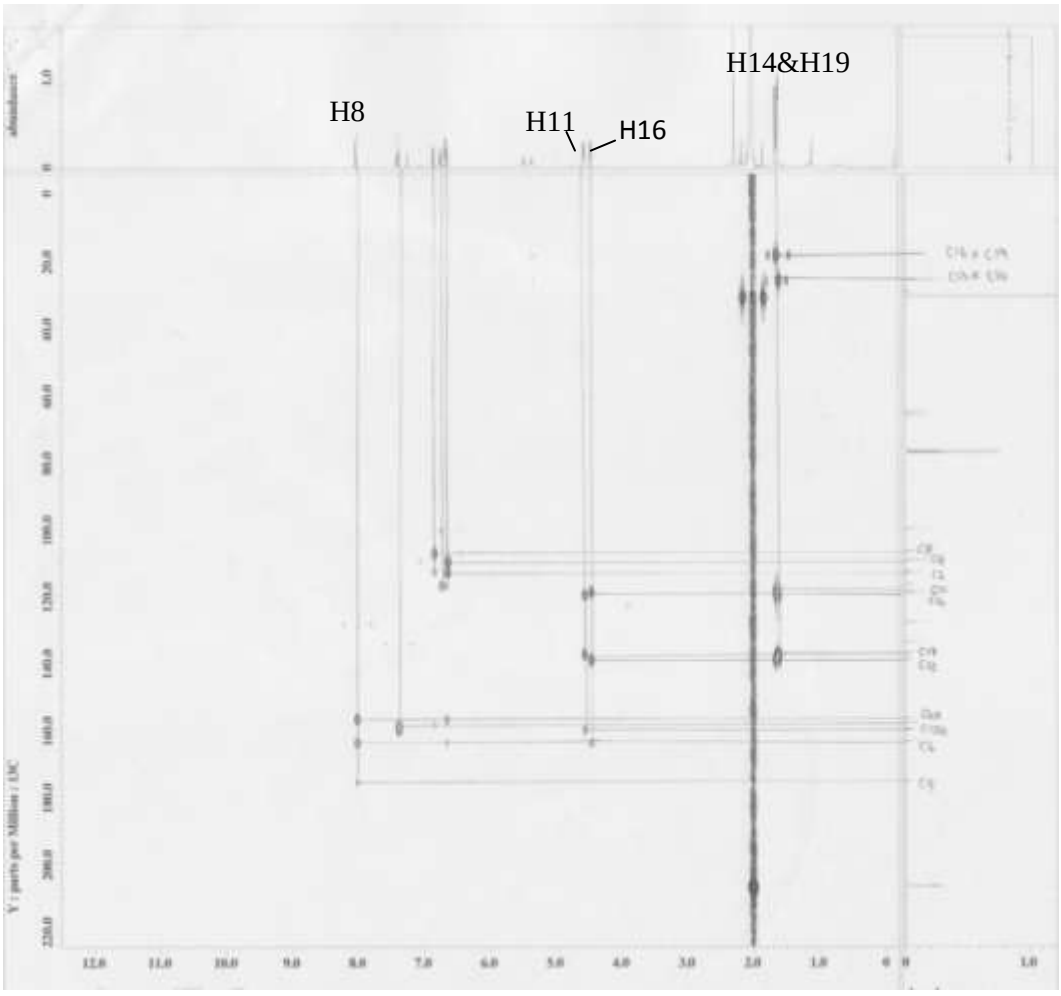
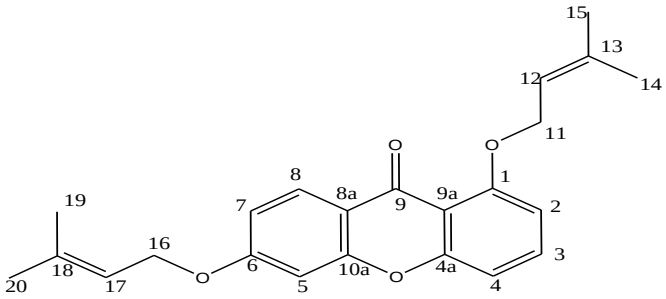


Figure 4.20: HMBC spectrum of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{23}H_{24}O_4$

Molecular weight: 364.192 g mol^{-1}

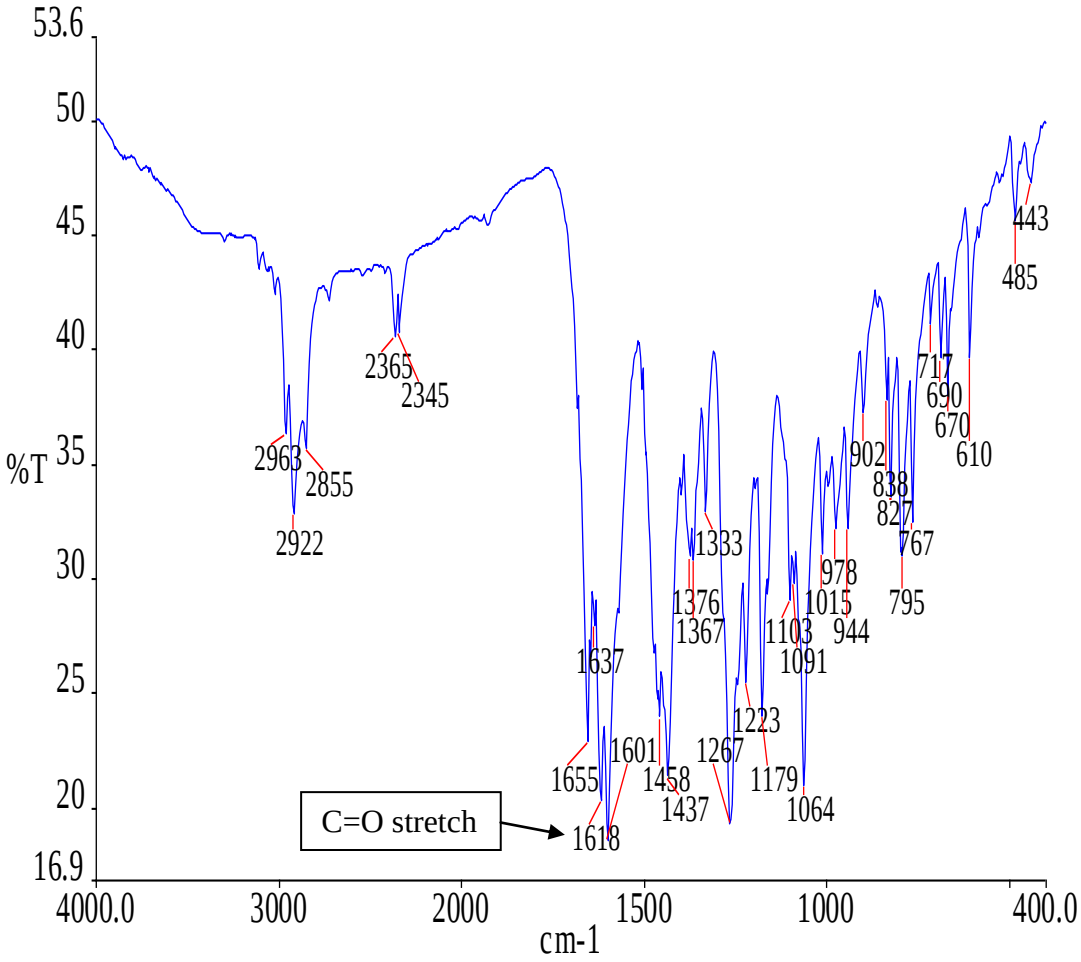
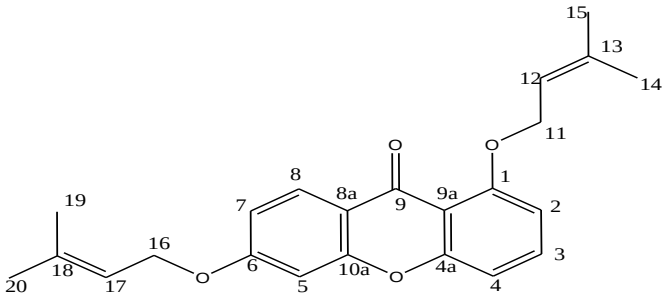


Figure 4.21: IR spectrum of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one



1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

Molecular formula: $C_{23}H_{24}O_4$

Molecular weight: 364.192 g mol^{-1}

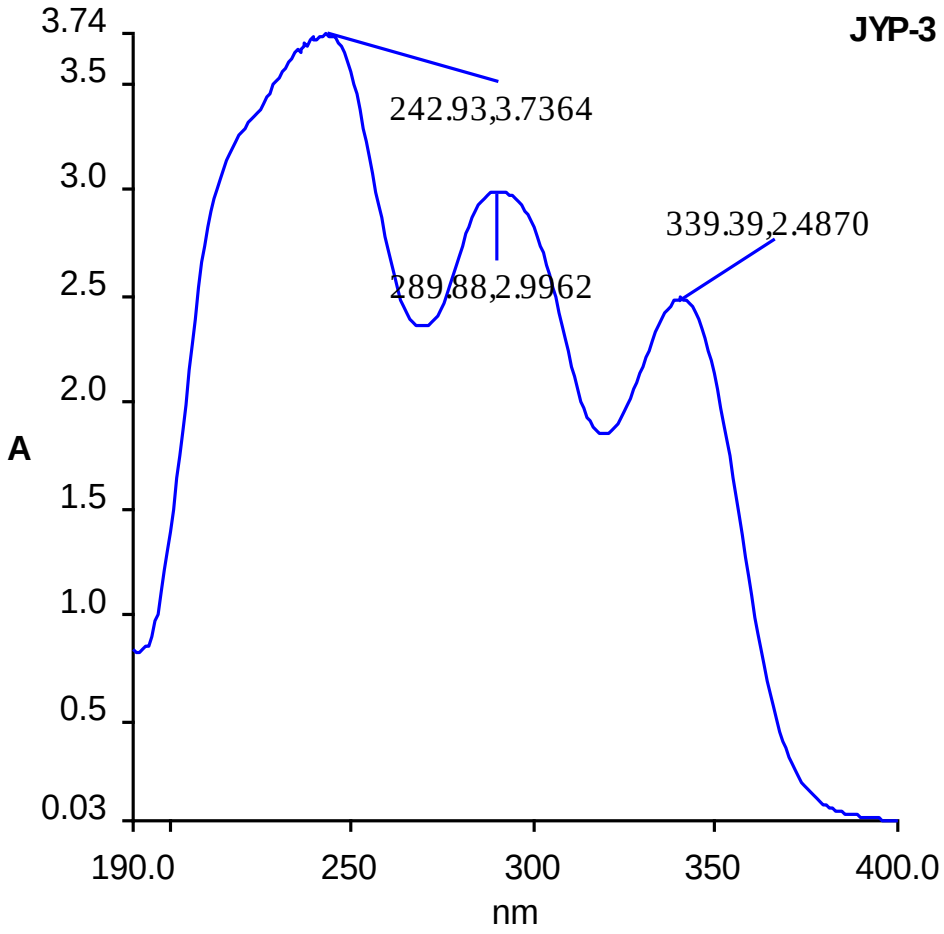


Figure 4.22: UV-Vis spectrum of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

4.4.2 Proposed Mechanism for Synthesis of 1-(3-Methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

The outline of proposed mechanism to account for the formation of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one is as follow:

Dissociation of potassium carbonate in aqueous:

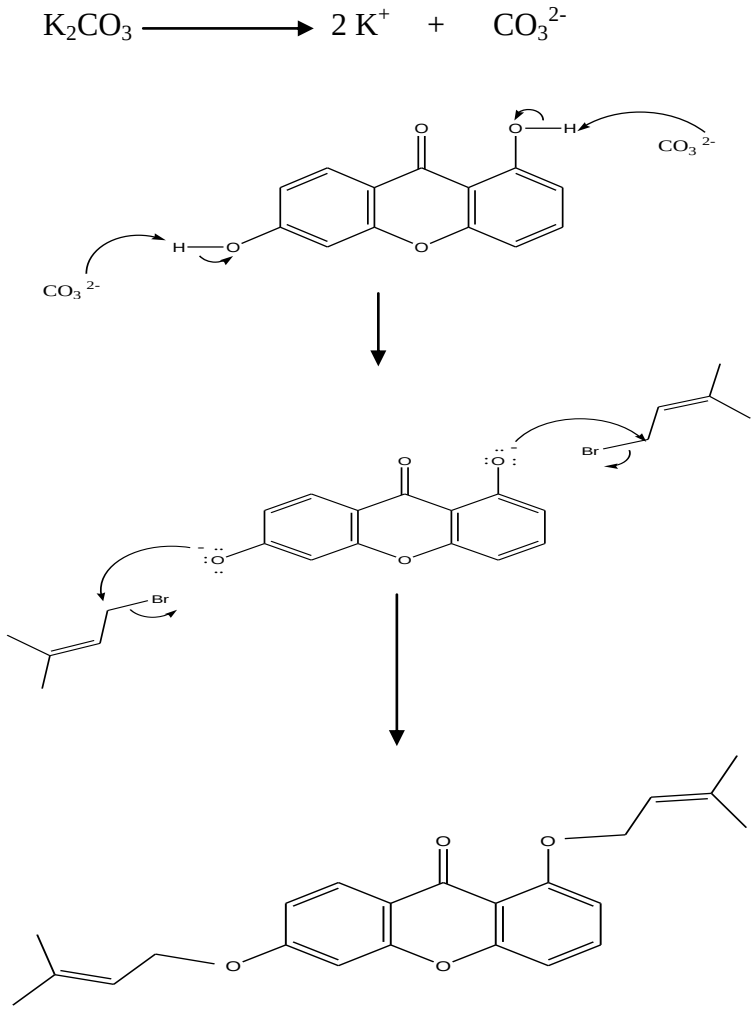


Figure 4.23: Reaction mechanism involved in the synthesis of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one

4.5 Bioassay

The xanthone block, 1,6-dihydroxyxanthone and its derivatives, 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one were synthesized and tested for their cytotoxic activities toward HeLa and MDA-MB-231 cancer cell lines. The cytotoxicity of xanthone block and its derivatives were evaluated according to the cell viability percentage of HeLa and MDA-MB-231 cancer cells at various concentrations by using MTT method. Structure-activity relationships can be established from the analysis of the IC_{50} values of xanthone block and its derivatives. The value of half maximal inhibitory concentration, IC_{50} for each compound was obtained through the graph of cell viability against concentration of drug and these values were summarized in Table 4.8.

Cell viability is a determination of either living or dead cell, based on a total cell sample. In this research, cell viability measurement by MTT method was used to determine the effectiveness of a particular drug due to its toxicity towards the cancer cell line. In fact, cell viability is low when an extremely effective drug is treated with it, which means the cell survival is very low. The half maximal inhibitory concentration, IC_{50} is a measurement of the effectiveness of a drug in inhibiting biological function of a cell. This quantitative measurement gives the concentration of a particular drug required to inhibit the biological process of

given cancer cells by half. Low value of IC_{50} indicates the drug is very effective as low concentration of drug is sufficient to inhibit cell growth.

Table 4.8: Cytotoxicity of xanthone and its derivatives against HeLa and MDA-MB-231 cancer cell lines

Compound	Inhibitory concentration, IC_{50} (μg / ml)	
	HeLa cancer cell line	MDA-MB-231 cancer cell line
1,6-dihydroxyxanthone	7.0	> 50
1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one	> 50	> 50
1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one	> 50	>50

The first observation from Table 4.8 is that the HeLa cancer cell line was found to be highly susceptible towards 1,6-dihydroxyxanthone with IC_{50} value of 7.0 μg / ml. Meanwhile, both the derivatives of xanthone, 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one gave no significant inhibitory activity towards HeLa cancer cell line. This result revealed that the cytotoxic activity was depend on the presence of hydroxyl group in the xanthone. Inhibitory activity was also reported to show significant dependence on the number of hydroxyl group (Liu *et al*.,

2006). For 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one, the hydrogen atom of hydroxyl group on the ring was replaced by the prenyl group during etherification which resulted in a significant decrease in cytotoxic activity. In addition, Liu *et al* also stated that, in order to achieve significant inhibitory activities, there must be three or more hydroxyl group attached to the xanthone ring. This was well consistent with the result of this project where the prenylated xanthones with less hydroxyl substituents were shown to have greatly reduced cytotoxic activity.

Besides the screening on HeLa cancer cells, the three compounds were also subjected to cytotoxic assay against MDA-MB-231 cancer cell line. However, all the tested compounds gave insignificant inhibitory activity against the cell line with IC_{50} value > 50 μg/mL. This result suggested that the disubstitued prenyl group on the oxygen atom of the xanthone backbone was not essential for eliciting inhibitory activities toward MDA-MB-231 cell lines. Therefore, further researches need to be carry out in order to have better understanding on how 1,6-dihydroxyxanthone exerts its cytotoxic activity on Hela cancer cell line.

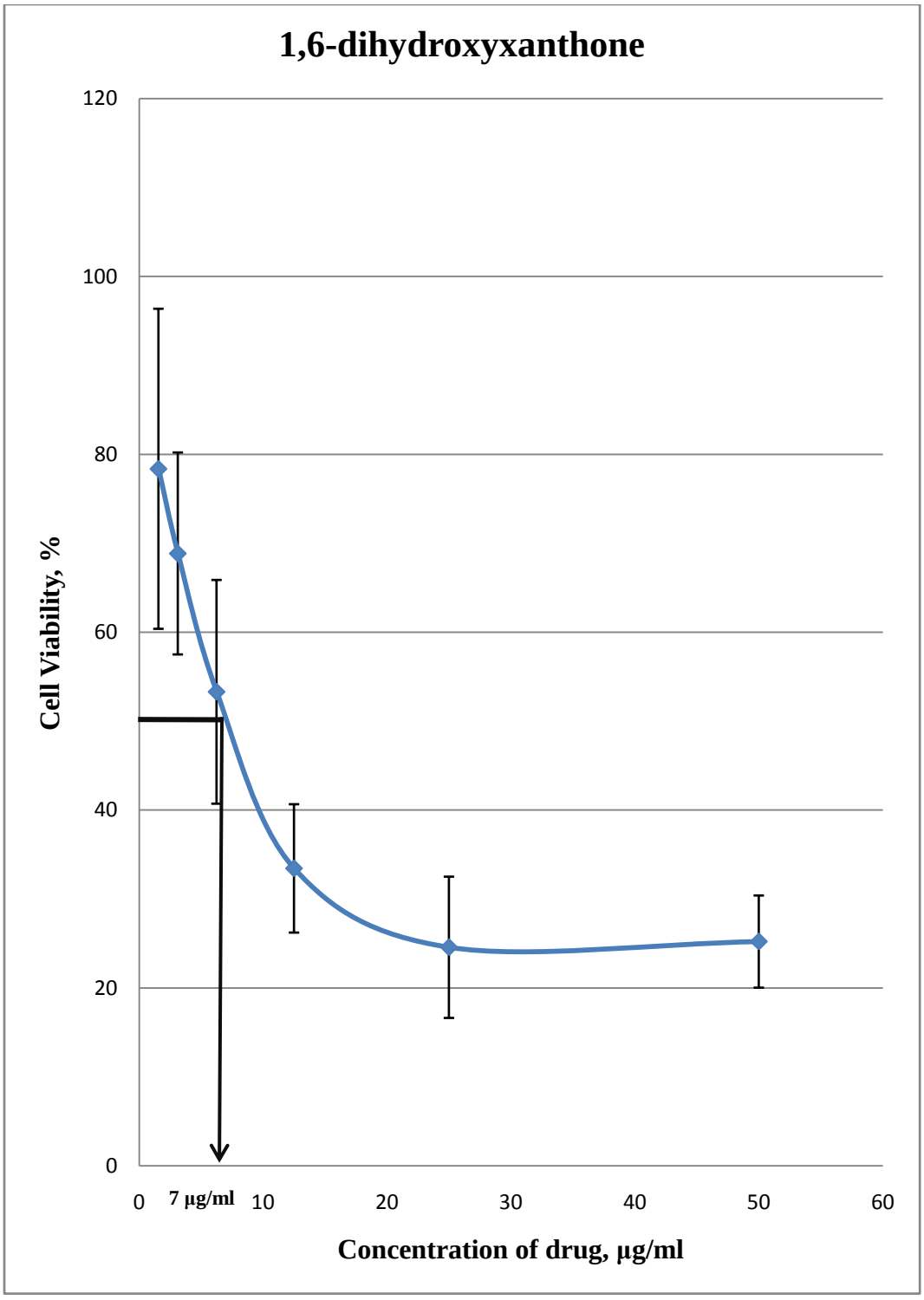


Figure 4.24: Graph of cell viability against concentration of 1,6-dihydroxyxanthone (HeLa cancer cell line)

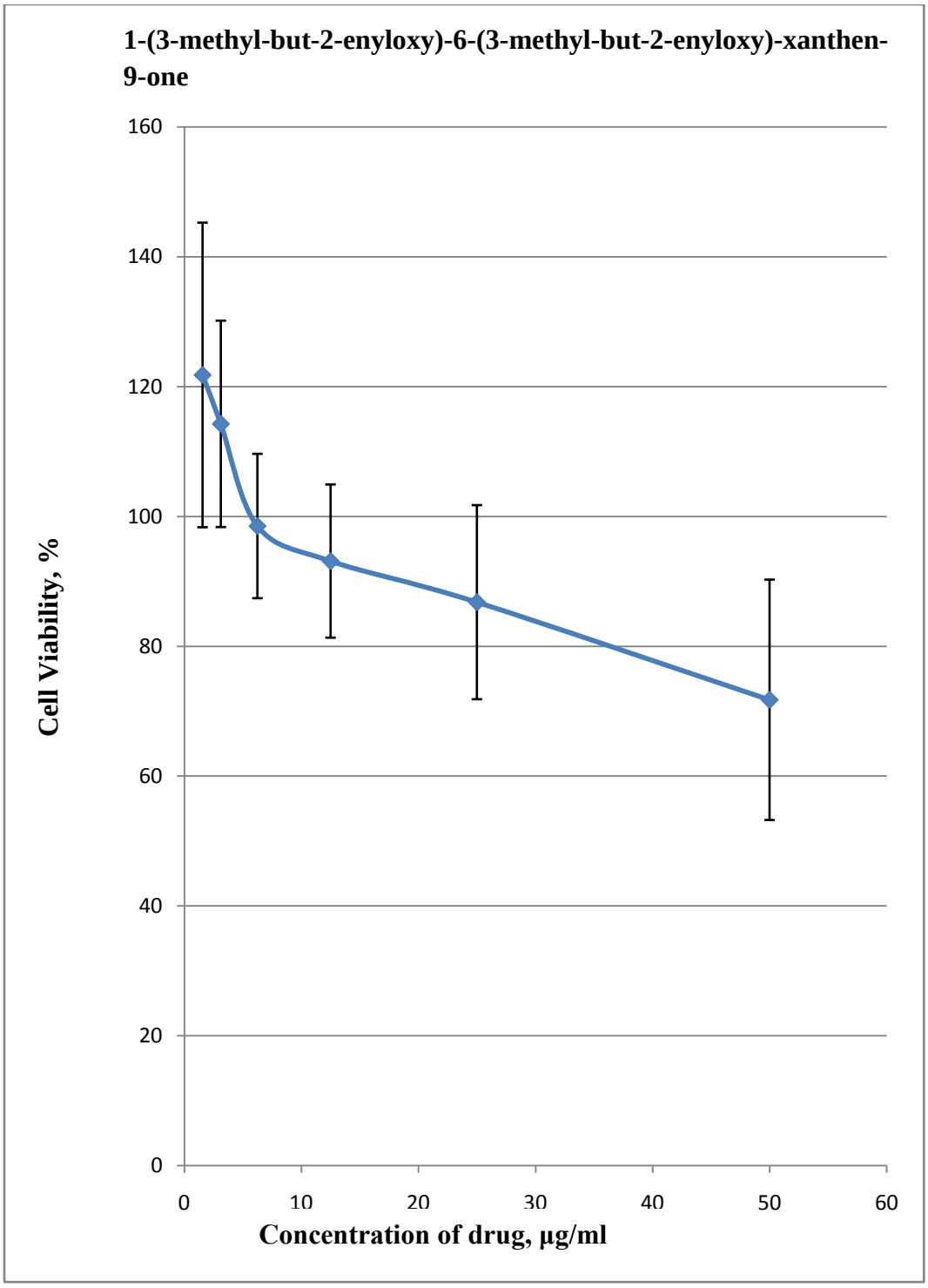


Figure 4.25: Graph of cell viability against concentration of 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one (MDA-MB-231 cancer cell line)

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

A total of three pure compounds have been successfully synthesized, isolated, and characterized in this study, namely 1,6-dihydroxyxanthone, 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one, and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one. The structures of these pure compounds were established through ^{1}H-NMR, ^{13}C-NMR and mass spectrometry analyses. The structure elucidation of 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one, and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one was further confirmed by the application of 2D- NMR spectroscopy including HMQC and HMBC analyses.

The bioassay test was carried out to xanthone block and its derivatives toward HeLa and MDA-MB-231 cancer cell lines. The half maximal inhibitory concentration, IC_{50} for each compound was determined through the graph plotted. The xanthone block, 1,6-dihydroxyxanthone, was found to have moderate inhibitory activity towards HeLa cancer cell line with IC_{50} value of 7.0 μg/ml but

was found to be not active against MDA-MB-231 cell line with IC_{50} value of more than 50 μg/mL. Meanwhile, 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one and 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one were found to have insignificant inhibitory activity towards MDA-MB-231 and HeLa cancer cell line with IC_{50} value of more than 50 μg/ml.

5.2 Recommendations for Further Studies

In this project, one xanthone block and two prenylated xanthones had been successfully been synthesized. However, each of these compounds has its own characteristic in term of bioactivities due to the different substituent groups. It is assumed that combination of different substituent groups at various positions can give rise to unique characteristics. For future studies, it is sugested to synthesize more diverse xanthones and then followed by extensive study for their desirable characteristic in different areas such as anti-tumour and anti-fungi. The potential benefits of these substituted xanthones are very promising especially in the medical area and also in the industries. For instance, further synthesis such as cyclization and dehydrogenation can be carried out to chemically modified xanthone derivatives to become potential drug leads for treatment of cancer diseases.

Besides the gravity column chromatography, other more advanced chromatographic can be used in this project, it is recommended the use of flash column chromatography and high performance liquid chromatography to effect a better separation to the crude product in future studies.

From the results of bioassay, it was suggested that a further study is needed to understand the mechanism of action involved in the cells to induce apoptosis for 1,6-dihydroxyxanthone which gave a moderate activity against Hela cancer cell line in the preliminary screening. Lastly, other biological activities such as anti-malaria, anti-inflammatory, anti-fungal, and anti-bacterial activities should be also explored in further researches so that the full potential of the xanthone compounds in medical use can be discovered and utilized completely.

REFERENCES

Abou-Shoer M., Boettner F.E., Chang CJ. (1988). Potent Inhibitory Effect by Furanoxanthones. Phytochemistry 27:2795-800.

Afzal M and Al-Hassan J. M. (1980). Naturally Occuring Pentaoxygenrated, Hexaoxygenated, and Dimeric Xanthones. *Heterocycles*, 14, 1173-1177.

Bennett G. J. and Lee H. H. (1989). Xanthones from *Guttiferae*. *Phytochemistry*, 28, 967-998.

Birch AJ., Baldas J., Hlubucek JR., Simpson TJ., Westerman PW. (1976). Acetate Polymalonic Route of Biosynthesis Xanthones. *J. Chem. Soc. Perkin Trans* no. 8, 898-904.

Boonnak N., Karalai C., Chantrapromma S., Ponglimanont C., Fun H. K., Akkharawit K. O., Surat L. (2006). Bioactive Prenylated Xanthones and Anthraquinones frm *Cratoxylum Formosum ssp. Pruniflorum. Tetrahedron*, 62, 8850-8859.

Burling E. D., Jefferson A., Scheinmann F. (1965). Unified Synthesis of Caged for *Carcinia* Natural Products Based on Site-selective Claisen/Diels – Alder Claisen Rearrangement. *Tetrahedron*, 21, 2653-2669.

Castanheiro R. A. P., Pinto M. M. M., Cravo S. M. M., Pinto D. C. G. A., Silva A. M. S., Kijjoa A. (2009). Improved Methodologies for Synthesis of Prenylated Xanthones by Microwave Irradation and Combination of Heterogeneous Catalysis (K10 clay) with Microwave Irradiation. *Tetrahedron*, 65, 3848-3857.

Castanheiro R. A. P., Pinto M. M. M., Silva A. M. S., Cravo S. M. M., Gales L. Damas A. M., Nazareth N., Nascimento M. S. J., Eaton G. (2007). Dihydroxyxanthones Prenylated Derivatives: Synthesis, Structure Elucidation and Growth Inhibitory Activity on Human Tumor Cell Lines with Improvement of Selectivity for MCF-7. *Bioor. Med. Chem.*, 15,6080-6088.

Castanheiro R. A. P., Silve A. M. S., Campos N. A. N., Nascimento M. S. J., Pinto M. M. M. (2009). Antitumor Activity of Some Prenylated Xanthones. *Pharmaceuticals*, 2, 33-43.

Chaivisuthangkura A., Malaikaew Y., Chaovanalikit A., Haratrungtawee A., Panseeta P., Ratananukul P., Suksamrarn S. (2009). Prenylated Xanthone Composition of *Carcinia* Mangostana Fruit Hull. *Chromatographia*, 69, 315-318.

Chaverri J. P., Noemi C. R., Marisol O. I., Jazmin M. P. R. (2008). Medicinal Properties of Mangosteen (*Carcinia Mangostana*). *Food and Chemical Toxicology*, 46, 3227-3239.

Diderot N. T., Silvere N., Etienne T. (2006). Xanthones as Therapeutic Agents:Chemistry and Pharmacology. *Leads Molecules from Natural Products*, 273-298.

Ding L., Liu B., Lin L. Q., Zhou Q. Y., Hou Q., Li J., Zhang Q. (2009). Anti proliferation, Cell Cycle Arrest and Apoptosis Induced by A Natural Xanthone from *Gentianopsis Paludosa Ma*, in Human Promyelocytic Leukemia Cell Line HL-60 Cells. *Toxicology in Vitro*, 23, 408-417.

Ellis R. C., Whalley W. B., Ball K. (1967). Phenolic Constituents of *Gentiana Lutae. Chem. Comm.* 1, 803.

Epifano F., Genovese S., Menghini L., Curini M. (2007). Chemistry and Pharmacology of Oxyprenylated Secondary Plant Metabolites. *Phytochemistry*, 68, 939.

Fowler C. J. and Ross S. B. (1984). Selective Inhibitors of Monoamine Oxidase A and B: Biochemical, Pharmacological, and Clinical Properties. *Med. Res. Rev.*, 4, 323-358.

Fujita M. and Inoue T. (1980). Biosynthesis of Mangiferin in *Anemarrhena Asphodeloides*, The Origin of Xanthone Nucleus. *Chem. Pharm. Bull.*, 28, 2476-2481.

Giri R., Goodell J. R., Xing C., Benoit A., Kaur H., Hiasa H., Ferguson D. M. (2010). Synthesis and Cancer Cell Cytotoxicity of Substituted Xanthenes. *Bioorg. Med. Chem.*, 18, 1456-1463.

Gottlieb O. R. (1968). Biogenetics Proposals Regarding Aucuparins and Xanthones. *Phytochemistry*, 7, 411-421.

Groger D., Erge D., Franck B., Ohnsorge U., Flasch H., Huper F. (1968). Mycotoxin from Mold Fungi. *Chem. Ber.* 101, 1970-1978.

Gupta P. and Lewis J. R. (1971). Xanthones in Cell Suspension Cultures of Two *Centaurium* Species. *J. Chem. Soc.*, 4, 629-631.

Grover P. K., Shah G. D., Shah R. C. (1955). Xanthones Part IV. *J. Chem. Soc.* 3982.

Han Q. B., Yang N. Y., Tian H. L., Qiao C. F., Song J. Z., Chang D. C., Chen S. L., Luo K. Q., Xu H. X. (2008). Xanthones with Growth Inhibition Against HeLa Cells from *Carcinia Xipshuanbannaensis. Phytochemistry*, 69, 2187-2192.

Hay A. E., Helesbeux J. J., Duval O., Labaied M., Grellier P., Richomme P. (2004). Antimalarial Xantones from *Calophyllum Caledonicum* and *Carcinia Vieillardii*. *Life Sciences*, 75, 3077-3085

Herringson TP., Altin JG. (2010). Increasing the antitumor efficacy of doxorubicin-loaded liposomes with peptides anchored via a chelator lipid. *J. Drug Target.*

Ho L. K., Yu H. J., Ho C. T., Don M. J. (2001). Synthesis of Naturally Occuring Rubilactone, Mollugin, and Dihydromollugin of *Rubia Cordifolia. J. Med. Chem.*, 48, 77-79.

Huang L., Lei T., Lin C., Kuang X., Chen H., Zhou H. (2009). Blumeaxanthene II, a Novel from *Blumea Riparia* DC. *Fitoterapia*, 4.

Isaka M. (2001). Phomoxanthones A and B, Novel Xanthone Dimers from the Endophytic Fungus *Phomopsis* Species. *J. Nat. Prod.,* 64, 1015-1018.

Jain Y., Ghosal S., Parra M. (1974). Furano and Pyrano Derivatives from O-Prenylated Xanthones. *J. Nat. Pro.* 50:301-4

Jiang D. J., Dai Z., Li Y. J. (2004). Pharmacological Effects of Xanthones as Cardiovascular Protective Agents. *Cardiovasc. Drug. Rev.*, 22, 91-102.

Kang W. Y., Ji Z. Q., Wang J. M. (2009). A New Xanthone from the Roots of *Securidaca Inappendiculata*. *Chem. Papers*, 63, 102-104.

Kappe C. O and Stadler A. (2005). Microwaves in Organic and Medicinal Chemistry. *Weinheim,* 25, 9-55.

Kupchan S.M., Streelman D. R., Sneden A. T. (1980). Two Furanoxnathones from *Mammea Acuminata*. *J. Nat. Prod.* 43, 296-301.

Lembege M. V., Moreau S., Larrouture S., Montaudon D., Robert J., Nuhrich A. (2008). Synthesis and Antiproliferative Activity of Aryl- and Heteroaryl-hydrazones Derived from Xanthone Carbaldehydes. *European Journal of Medicinal Chemitry*, 43, 1336-1343.

Lewis JR. (1963). Phenol Oxidative Coupling. *Proc. Chem. Soc.* 363:373-9.

Likhitwitayawuid K., Phadungcharoen T., Krungkrai J. (1998). Antimalaria Xanthones from *Carcinia* Gowa Plant. *Med.,* 64, 70-72.

Lin C. N., Chung M. I., Liou S. J., Lee T. H., Wang J. P. (1996). A Summary of 31 Mangosteen Studies. *J. Pharma. Pharmacol*., 48, 532-538.

Lin C. N., Hsieh H. K., Liou S. J., Ko H. H., Lin H. C., Chung M. I., Ko F. N., Liu H. W., Teng C. M. (1996). A Summary of 31 Mangosteen Studies. *J. Pharma. Pharmacol*., 48, 887-890.

Lin C. M. (1996). Xanthone Derivatives as Potential Anti-cancer Drugs. *J. Pharm Pharmacol.*, 48, 539-544.

Liu Y., Zou L., Ma L., Chen W. H., Wang B., Xu Z. L. (2006). Synthesis and Pharmacological Activities of Xanthone Derivatives as α-Glucosidase Inhibitors. *Bioorg. Med. Chem.*, 14, 5683-5690.

Liou S.S. (1993). γ-Pyrone Compounds as Potential Anti-cancer Drugs. *J. Pharm. Pharmacol*., 45, 791-794.

Locksley H. D. and Murray I. G. (1970). Chemosystematic Implications of Xanthones in *Bonnetia* and *Archytaea. J. Chem. Soc.,* 2, 392-398.

McMaster WJ., Scott AI., Trippett S. (1960). Biosynthesis Pathway of Xanthones: Acetate Polymalonic Route. *J. Chem. Soc*. 5(894): 4628-31.

Mandal S., Das PC., Joshi PC. (1992). Xanthones from Natural Resources. *J. Indian. Chem. Soc*., 69:611.

Mehta G., Shah S. R., Venkateswarlu Y. (1994). Total Synthesis of Xanthone Antibiotics. *Tetrahedron*, 50, 11729-11742.

Micheal S., Kostanecki Y. (1892). Xanthone in Synthesis: Reactivity via Polyphenol and Salicylic acid. *Tetrahedron*, 20, 1082-1195.

Na Y. H. (2009). Recent Cancer Drug Development with Xanthone Structures. *Journal of Pharmacy and Pharmacology*, 61, 707-712.

Oger J. M., Morel C., Helesbeux J. J., Litaudon M., Seraphin D., Dartigeulongue C., Larcher G., Richomme P., Duval O. (2003). First 2-hydroxy-3-methylbut-3-enyl Substituted Xanthones Isolated from Plants: Structure Elucidation, Synthesis, and Antifungal Activity. *Natural Product Research*, 17 (3), 195-199.

Oliver Kappe C. (2004). Microwave Synthesis. *Angew. Chem. Int. Ed.*, 43, 6250.

Oliver Kappe C. and Dallinger D. (2006). One-Pot Multicomponent Preparation of Tetrahydropyrazoloquinolinones. *Nat. Rev. Drug Discov.,* 5, 51.

Patel G. N. and Trivedi K. N. (1988). Abnormal Claisen Rearrangement. *J. Indian. Chem. Soc.,* 65, 192-193.

Pedro M., Cerqueira F., Sousana M. E., Nascimento M. S. J., Pinto M. (2002). 1,7-Dihydroxy-2,3,4-trimethoxy-9Hxanthen-9-one monohydrate from Halenia Elliptica. *Bioorg. Med. Chem.*, 10, 3275-3730.

Pedro M., Cerqueira F., Sousa M. E., Nascimento M. S. J., Pinto M. (2002). Xanthones as Inhibitors of Growth of Human Cancer Cell Lines and Their on the Proliferation of Human Lymphocytes In Vitro. *Bioorg. Med. Chem.,* 10, 3725-3730.

Pickert M and Frahm A. W. (1998). Synthesis and Assignment of $^{1}H/^{13}C$ NMR Chemical Shifts. *Arch. Pharm. Med. Chem.,* 331, 177-192.

Pinto D. C. G., Fuzzati N., Pazmino X. C., Hostettmann K. (1994). New Xanthones from *Calophyllum Caledonicum. Phytochemistry,* 7, 875-877.

Pinto M. M. M., Sousa M. E., Nascimento M. S. J. (2005). 1-hydroxy-3-(3-methylbut-2-enloxy) xanthone. *Curr. Med. Chem.* 12, 2517.

Roberts J.C. (1961). The Taxonomic Significance of C-glycosylxanthones in Flowering Plants. *Chem. Rev.* 61, 591-605.

Sandulache A., Silva A. M. S., Cavaleiro J. A. S. (2001). Diels-Alder Reactions of chromone-3-carboxaldehydes with *Ortho*-benzoquinodimethane, New Synthesis of benzo [*b*]xanthones. *Tetrahedron,* 58, 105-114.

Sordat M., Braca A., Politi M. (1992). Assays of Xanthone: Colon Cancer Cell Culture. *European Journal of Medicinal Chemistry,* 27, 809-821.

Sousa E., Paiva A., Nazareth N., Gales L., Damas A. M., Nascimento M. S. J., Pinto M. (2009). Bromoalkoxyxanthones as Promising Antitumor Agents: Synthesis, Crystal Structure and Effect on Human Tumor Cell Lines. *European Journal of Medicinal Chemistry*, 44, 3830-3835.

Sultanbawa M.U.S. (1980). Xanthonoids of Tropical Plants. *Tetrahedron*, 36, 1465-1506.

Tanaka N. and Takaishi Y. (2006). Xanthones from *Hypericum Chinese*. *Phytochemistry*, 67, 2146-2151.

Tanaka N., Kashiwada Y., Kim S. Y., Sekiya M., Ikeshiro Y., Takaishi Y. (2009). Xanthones from *Hypericum Chinese* and Their Cytotoxicity Evaluation. *Phytochemistry*, 70, 1456-1461.

Taur J.S., Desjardins C.S., Schuck E.L., Wong Y.N. (2010). Interactions between the chemotherapeutic agent eribulin mesylate (E7389) and P-glycoprotein in CF-1 abcb1a-deficient mice and Caco-2 cells. *Xenobiotica.*

Ulmann F. and Pauchaud L. (1906). Xanthones in *Hypericum*: Synthesis and Biological Activities. *Ann.*, 350, 108-115.

Vennetier P. (2009). Natural Product of Xanthones. *J. Nat. Prod.* 2009, 63, 1537

Vieira L. M. M. and Kijjoa A. (2005). Naturally Occuring Xanthones: Recent Development. *Current Medicinal Chemistry*, 12, 2413-2446.

Woo S. (2007). Synthesis of New Xanthone Analogues and Their Biological Activity Test – Cytotoxicity, Topoisomerase II Inhibition, and DNA Cross-linking Study. *Bioorg. Med. Chem. Lett.*, 17, 1163-1166.

Wu C., Ye H., Bai W., Li Q., Guo D., Lv G., Yan H., Wang X.. (2010) New Potential Anticancer Agent of Carborane Derivatives: Selective Cellular Interaction and Activity of Ferrocene-substituted Dithio-o-carborane Conjugates. *Bioconjug Chem.*

Yazaki K., Sasaki K., Tsurumaru Y. (2009). Prenylation of Aromatic Compounds, A Key Diversification of Plant Secondary Metabolites. *Phytochemistry,* 70, 1739-1745.

Yoshimi N., Matsunaga K., Katayama M., Yamada Y., Kuno T., Qiao Z., Hara A., Yamahara J., Mori H. (2001). The Inhibitory Effects of Mangiferin, A Naturally Ocurring Glucosylxanthone in Bowel Carcinogenesis of Male F344 Rats. *Cancer Lett.,* 163, 163-170.

Zhong D., Liao D. F., Jiang D. J., Deng H. W., Li Y. J. (2004). 3,4,5,6-tetrahydroxyxanthone Prevents Vascular Endothelial Cell Apoptosis Induced by High Glucose. *Arch. Pharmacol,* 370, 314-319.

Zhou J. and Larock R. C. (2007). Synthesis of Xanthones, Thioxanthones and Acridones by the Coupling of Arynes and Substituted Benzoates. *J. Org. Chem.,* 72 (2), 583-588.

Book:

Farnworth N. R. and Bunyapraphatsara N. (1992). Thai Medicinal Plant: Recommended for Primary Health Care System. Prachachon Company, Bangkok.

APPENDICES

APPENDIX 1

The following tables summarise the triplicate results of absorbance analysis for 1,6-dihydroxyxanthone towards HeLa cancer cell line.

First trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	0.302	0.292	0.348	27.61
25	0.291	0.280	0.339	26.30
12.5	0.252	0.286	0.343	25.12
6.25	0.385	0.442	0.418	40.01
3.125	0.489	0.540	0.610	56.13
1.563	0.555	0.564	0.565	57.97
Control	0.916	0.877	0.918	
Blank	0.088	0.084	0.095	

Second trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	0.298	0.317	0.35	19.27
25	0.260	0.294	0.304	15.90
12.5	0.415	0.524	0.598	37.25
6.25	0.694	0.802	0.601	54.85
3.125	0.900	0.885	0.872	72.46
1.563	1.168	0.98	0.913	85.16
Control	1.225	1.149	1.159	
Blank	0.108	0.116	0.128	

Third trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	0.355	0.358	0.371	28.76
25	0.384	0.412	0.364	31.50
12.5	0.453	0.402	0.484	37.95
6.25	0.781	0.707	0.602	65.03
3.125	0.787	0.855	0.807	77.97
1.563	1.041	0.980	0.817	91.99
Control	1.196	0.991	0.873	
Blank	0.095	0.091	0.100	

APPENDIX 2

The following tables summarise the triplicate result of absorbance analysis for 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one towards HeLa cancer cell line.

First trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.51	1.284	1.282	95.12
25	1.412	1.27	1.249	91.40
12.5	1.186	1.411	1.191	87.74
6.25	1.281	1.458	1.541	100.35
3.125	1.156	1.554	1.513	98.89
1.563	1.379	1.358	1.341	95.17
Control	1.673	1.254	1.339	
Blank	0.110	0.125	0.132	

Second trial:

Concentration (µg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.335	1.274	1.22	109.30
25	1.435	1.475	1.347	122.76
12.5	1.459	1.466	1.356	123.51
6.25	1.419	1.409	1.243	116.91
3.125	1.61	1.491	1.422	131.12
1.563	1.459	1.442	1.396	124.01
Control	1.225	1.149	1.159	
Blank	0.108	0.116	0.128	

Third trial:

Concentration (µg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.461	1.350	1.350	128.41
25	1.444	1.304	1.250	122.91
12.5	1.341	1.232	1.260	117.35
6.25	1.470	1.239	1.251	121.63
3.125	1.424	1.311	1.129	118.40
1.563	1.264	1.296	1.425	122.48
Control	1.084	1.111	1.123	
Blank	0.112	0.116	0.123	

APPENDIX 3

The following tables summarise the triplicate results of absorbance analysis for 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one towards HeLa cancer cell line.

First trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.26	1.205	1.275	106.50
25	1.352	1.275	1.519	119.27
12.5	1.325	1.414	1.326	116.72
6.25	1.252	1.239	1.318	108.67
3.125	1.104	1.257	1.312	104.40
1.563	1.179	1.311	1.143	103.14
Control	1.225	1.149	1.159	
Blank	0.108	0.116	0.128	

Second trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.472	1.282	1.459	100.38
25	1.873	1.651	1.466	120.41
12.5	1.229	1.927	2.052	126.03
6.25	1.391	1.42	1.289	97.47
3.125	1.598	1.674	1.185	106.67
1.563	1.071	0.931	1.255	75.74
Control	1.590	1.191	1.417	
Blank	0.108	0.110	0.100	

Third trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.583	1.418	1.322	122.55
25	1.229	1.289	1.296	106.91
12.5	1.34	1.265	1.37	111.86
6.25	1.259	1.076	1.149	96.77
3.125	0.973	0.97	1.056	81.86
1.563	1.166	1.001	1.098	90.04
Control	1.246	1.137	1.206	
Blank	0.113	0.103	0.119	

APPENDIX 4

The following tables summarise the triplicate results of absorbance analysis for 1,6-dihydroxyxanthone towards MDA-MB-231 cancer cell line.

First trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.281	1.768	1.487	80.35
25	2.474	2.02	2.39	124.58
12.5	2.332	2.413	2.314	127.87
6.25	2.291	1.784	1.75	104.63
3.125	2.014	1.781	1.708	98.56
1.563	2.958	2.248	1.464	120.55
Control	2.098	1.817	1.664	
Blank	0.094	0.084	0.092	

Second trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	2.651	3.198	2.7	171.08
25	2.756	3.138	2.684	171.68
12.5	2.657	2.414	2.149	143.39
6.25	1.863	1.887	1.955	111.83
3.125	1.624	1.616	2.269	107.75
1.563	1.723	1.399	1.928	98.18
Control	2.142	1.581	1.414	
Blank	0.109	0.111	0.117	

Third trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	2.26	2.434	2.225	140.00
25	2.212	2.384	2.562	145.07
12.5	2.452	2.829	2.664	161.75
6.25	2.11	2.135	2.188	129.70
3.125	2.059	2.058	1.958	122.12
1.563	1.965	2.026	1.699	113.96
Control	2.099	1.474	1.458	
Blank	0.102	0.100	0.110	

APPENDIX 5

The following tables summarise the triplicate result of absorbance analysis for 1-hydroxy-6-(3-methyl-but-2-enyloxy)-xanthen-9-one towards MDA-MB-231 cancer cell line.

First trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	3.032	3.348	3.294	202.05
25	3.08	3.214	3.209	198.31
12.5	2.904	2.543	2.763	169.99
6.25	2.67	3.175	2.509	173.14
3.125	2.501	2.694	2.051	148.88
1.563	1.792	1.865	1.999	114.06
Control	1.768	1.668	1.578	
Blank	0.145	0.126	0.177	

Second trial:

Concentration (µg/mL)	Raw Data			Cell Viability
	1	2	3	
50	3.13	3.203	3.184	178.16
25	2.645	2.539	2.557	143.69
12.5	2.289	2.355	2.797	137.86
6.25	2.129	2.412	2.705	134.08
3.125	2.054	2.272	2.182	119.75
1.563	2.075	1.92	2.179	113.27
Control	2.244	1.691	1.555	
Blank	0.114	0.116	0.108	

Third trial:

Concentration (µg/mL)	Raw Data			Cell Viability
	1	2	3	
50	3.107	2.698	3.334	132.88
25	2.922	2.427	2.865	118.84
12.5	2.248	2.138	2.74	102.32
6.25	2.184	2.378	1.843	91.37
3.125	2.066	1.92	2.378	90.75
1.563	2.794	2.433	3.186	121.86
Control	2.508	2.331	2.134	
Blank	0.126	0.126	0.134	

APPENDIX 6

The following tables summarise the triplicate results of absorbance analysis for 1-(3-methyl-but-2-enyloxy)-6-(3-methyl-but-2-enyloxy)-xanthen-9-one towards MDA-MB-231 cancer cell line.

First trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.976	0.887	1.507	85.89
25	1.674	1.52	1.946	102.75
12.5	1.386	1.759	2.135	105.82
6.25	1.784	1.795	1.538	102.25
3.125	2.199	1.753	2.156	123.95
1.563	2.744	2.191	2.132	144.96
Control	1.768	1.668	1.578	
Blank	0.145	0.126	0.177	

Second trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.651	1.093	1.284	78.56
25	1.669	1.333	1.304	84.479
12.5	1.463	1.498	1.252	82.49
6.25	2.546	1.505	1.327	107.30
3.125	2.944	1.579	1.588	122.90
1.563	2.747	1.741	1.598	122.37
Control	1.789	1.691	1.555	
Blank	0.114	0.116	0.108	

Third trial:

Concentration (μg/mL)	Raw Data			Cell Viability
	1	2	3	
50	1.953	1.614	1.712	50.79
25	2.196	2.633	2.614	73.13
12.5	2.814	3.362	3.002	91.04
6.25	3.219	3.17	2.302	86.01
3.125	3.334	3.133	3.183	95.91
1.563	3.393	3.312	3.152	98.04
Control	3.252	3.417	3.377	
Blank	0.117	0.124	0.117	