SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NAPHTHALENE- AND COUMARIN-BASED CHALCONES

by

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LIST OF ABBREVIATION

^{13}C-NMR	Carbon nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
4T1	Breast cancer cell
AcOH	Acetic acid
COSY	Correlation spectroscopy
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EtOH	Ethanol
FT-IR	Fourier transform infrared
FT-NMR	Fourier transform nuclear magnetic resonance
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
IC_{50}	Inhibitory concentration at 50%
K_2CO_3	Potassium carbonate
MTT	Dimethyl thiazolyl diphenyl tetrazolium
NIH-3T3	Fibroblast cell
$POCl_3$	Phosphorylchloride
SI	Selectivity Index

SINTESIS, PENCIRIAN DAN PENILAIAN BIOLOGI KALKON BERASASKAN NAFTALENA DAN KUMARIN

ABSTRAK

Kajian ini dimulakan dengan sintesis dan pencirian dua siri utama bahan pemula: pengalkilan naftaldehid (**2-8**) dan pengasetilan kumarin tertukar ganti (**9-11**). Bahan pemula yang diperoleh dikenalpasti melalui kaedah spektroskopi seperti inframerah transformasi Fourier (FTIR), resonans magnet nukleus (NMR) dan mikroanalisis CHN. Analisa pembelauan sinar-X hablur tunggal (XRD) juga dijalankan dengan hablur bagi sebatian **3**, **34**, dan **41** menghablur dalam sistem kristal triklinik. Lima siri kalkon kemudiannya disintesis melalui kaedah kondensasi Claisen-Schmidt dengan tiga siri diterbitkan daripada kumarin asetil tertukar ganti (**12-33**) dan dua siri yang lain diterbitkan daripada asetofenon tertukar ganti (**34-46**). Semua kalkon yang disintesis telah dicirikan dengan analisa unsur (CHN), spektroskopi FTIR , ^{1}H-NMR, ^{13}C-NMR, DEPT 135, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC dan ^{1}H-^{13}C HMBC. Kalkon **23**, **28**, **34**, dan **41** telah dipilih untuk analisis kristolografi. Kalkon yang disintesis telah dinilai bagi ciri farmakologi (anti-kanser) terhadap sel kanser payudara (4T1) dan sel fibroblas (NIH - 3T3) dengan cisplatin sebagai kawalan positif. Berdasarkan data yang diperoleh, kalkon yang mengandungi gelang kumarin menunjukkan kesitotoksi yang lebih kuat terhadap sel kanser payudara berbanding dengan kaklon yang mengandungi gelang fenil biasa. Sebatian **17** menunjukkan aktiviti perencatan tertinggi

terhadap titisan sel kanser 4T1 di kalangan kalkon yang disintesis dan lima kali ganda (IC_{50} = 3 ± 0.00) lebih aktif berbanding dengan cisplatin (IC_{50} = 16.5 ± 0.71). Kalkon **13**, **14**, **15**, **16**, **24** dan **31** juga menunjukkan aktiviti perencatan tinggi terhadap titisan sel kanser 4T1 dengan IC_{50} < 20.5 μM. Keputusan juga menunjukkan bahawa kalkon terfluorin (nilai IC_{50} antara 28-62 μM) mungkin menjadi ejen anti-kanser payudara yang lebih baik berbanding dengan kalkon tanpa fluorin (IC_{50} > 92.5 μM).

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NAPHTHALENE- AND COUMARIN-BASED CHALCONES

ABSTRACT

This study was started by the synthesis and characterization of two main series of starting materials: alkylation of naphthaldehydes (**2-8**) and acetylation of substituted coumarins (**9-11**). The starting materials thus obtained were elucidated by the spectroscopic methods such as fourier transform infrared (FTIR), nuclear magnetic resonance (NMR) and CHN microanalyses. Single crystal X-ray diffraction (XRD) analyses have also been carried out wherein the crystals of compound **3**, **34** and **41** crystallized in a triclinic crystal system. Five novel series of chalcones were subsequently synthesized by means of Claisen-Schmidt condensation wherein three series were derived from substituted acetyl coumarins (**12-33**) and the remaining two series was derived from substituted acetophenones (**34-46**). All the synthesized chalcones were characterized by elemental analysis (CHN), FTIR, ^{1}H-NMR, ^{13}C-NMR, DEPT 135, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC spectroscopy. Selected chalcones, **23**, **28**, **34**, and **41** were selected for crystallography analysis. The synthesized chalcones were evaluated for their pharmacological (anti-cancer) properties against breast cancer cell (4T1) and fibroblast cell (NIH-3T3) with cisplatin as positive control. Based on the data obtained, chalcones comprised of coumarin rings showed more potent

cytotoxicity against breast cancer cell compared to chalcones comprised of common phenyl rings. Compound **17** exhibited the highest inhibitory activity against 4T1 cancer cell line among the synthesized chalcones and five times (IC_{50} = 3±0.00) more active compared to cisplatin (IC_{50} = 16.5±0.71). Chalcones **13**, **14**, **15**, **16**, **24** and **31** also showed high inhibitory activities against 4T1 cancer cell line with IC_{50} < 20.5 μM. The results also indicated that fluorinated chalcones (IC_{50} value ranged from 28-62 μM) may serve as better anti-breast cancer agents than the chalcones without fluorine (IC_{50} > 92.5 μM).

CHAPTER 1: INTRODUCTION

1.1 Chalcone

Chalcones, a class of naturally occurring pigments which also known as benzelideneacetophenones are aromatic ketones that form the central core for a variety of important biological compounds (Rachmale *et al*., 2011). They are abundant in edible plants and are considered to be precursors for flavonoids biosynthesis in plants. Chalcones can also be synthesized in laboratory (Patil *et al*., 2009). Flavanoids consist mostly of antocynin, flavones and flanols, while chalcone, dihydroxychalcone and aurone are known as minor classes of flavanoids (Harborne, 1967).

Chemically, chalcones are open chain flavonoids in which the two aromatic rings are joined together by three carbons from the α,β-unsaturated carbonyl system (enone), which is a typical 1,4-conjugate addition (Michael addition) acceptor (Ko *et al*., 2003). The general chemical structure of a chalcone is depicted in Figure 1.1.

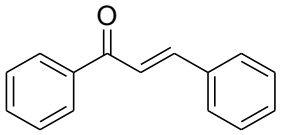


Figure 1.1: General structure of chalcone.

The term ‘chalcone’ also refers to the structurally simple and important plant secondary metabolite, which serves as defense mechanism in plants to counteract reactive oxygen species (ROS) to prevent damage by micro-organisms, insects or herbivores (Vaya *et al*. 1997). Chalcone strongly inhibits the polymerization of tubulin by binding to the colchicines-binding site (Lawrence *et al*., 2006).

A universal numbering system has been applied to distinguish the two aromatic rings of chalcones in which the two rings are labeled as A and B respectively. Figure 1.2 shows the chemical structure of 1-(4-hydroxyphenyl)-3-(2',4'-dihydroxyphenyl)-2-propen-1-one, with the corresponding atomic numbering (Bohm, 1998).

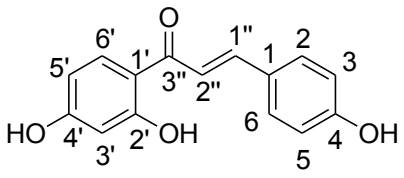


Figure 1.2: Chemical structure of 1-(4-hydroxyphenyl)-3-(2',4'-dihydroxyphenyl)-2-propen-1-one labeled with universal numbering system.

Chalcone is also acted as an important intermediate in biosynthesis of other classes of flavonoids in natural plants. Figure 1.3 shows chalcone acts as a main center core structure that undergoes different biosynthetic pathways to produce various classes of flavonoids such as flavanone (**I**), flavon (**II**), auron (**III**), flavonol (**IV**), antocianidin (**V**), dihydroflavonol (**VI**), dihydrochalcone (**VII**) and isoflavone (**VIII**) (Wong, 1976). The conversion of chalcone to other classes of flavonoids is catalyzed by certain plant enzymes. For an example, the metabolic pathway continues through a series of enzymatic modifications to yield **I** from chalcones, where research showed chalcone-flavanone isomerase was the enzyme that enhanced the biosynthesis (Swain, 1976).

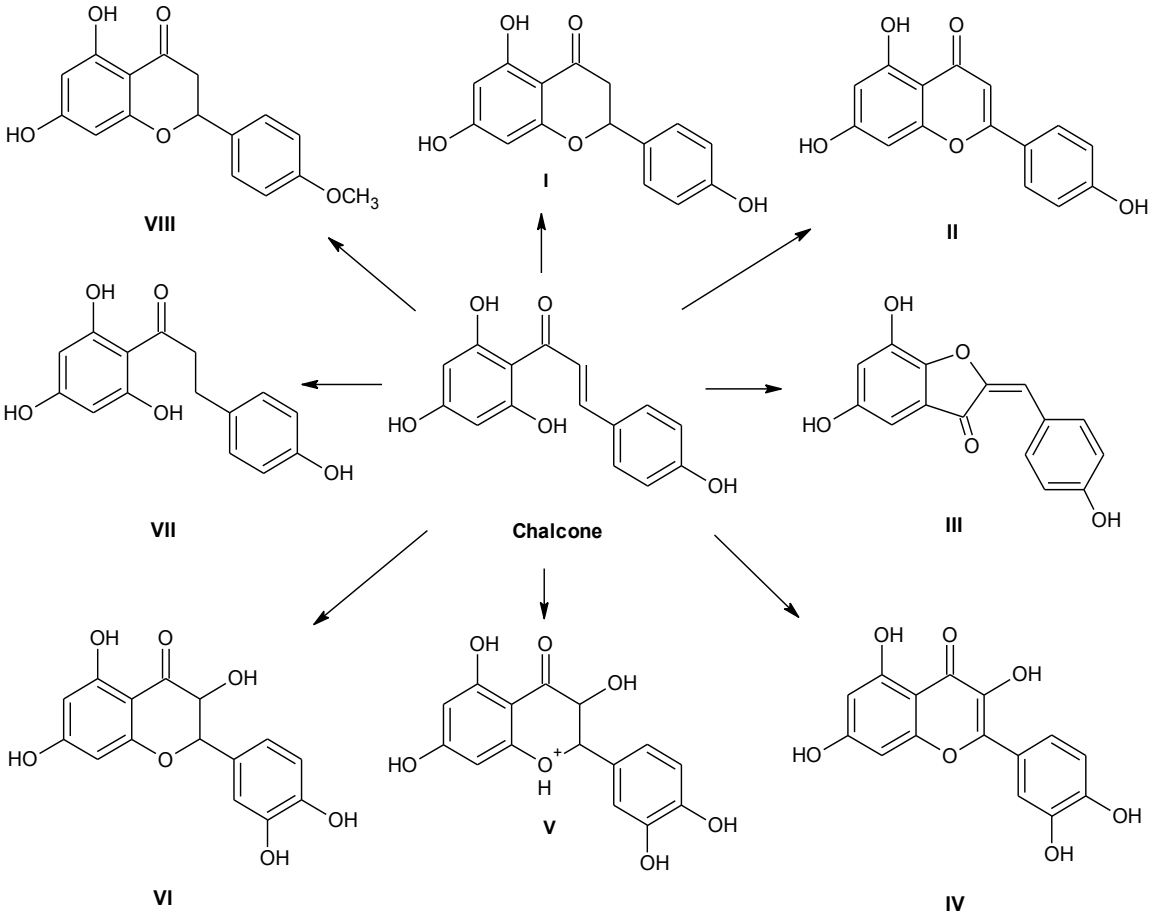


Figure 1.3: Chalcone as an intermediate in biosynthetic pathways leading to different classes of flavonoids.

In the recent years, synthesis of chalcones and their derivatives have attracted considerable attention due to their significant biological activities, sufficient fluorescence in the visible light range, large Stokes shifts and high quantum yields (Zhang *et al*., 2010). Chalcones have been reported to display an impressive array of biological activities such as antioxidant, antibacterial, anthelmintic, amoebicidal, antiulcer, antiviral, insecticidal, antiprotozoal, antileishmanial, antimalarial, antifungal, anti-inflammatory, anticancer, cytotoxic and immunosuppressive (Nowakowska, 2006; Trivedi *et al*., 2008; Srinivasan *et al*., 2009).

1.2 Problem Statement

There were several basic catalysts that have been applied in the synthesis of chalcone, such as sodium hydroxide, sodium methoxide, barium hydroxide, hydroapatite and lithium hydroxide. Even though these reactions provide advantages due to their Heterogenous nature. However, most of them require rather complicated neutralization process to obtain the synthesized compound. Recently, to improve the production-in synthesis of chalcones, piperidine has been tried. In this study, piperidine mediated chalcones formation without the neutralization requirement to obtain the solid yield. Hence, it is among the suitable candidate to be used as support in chalcone synthesis in respect to its unique properties.

1.3 Objectives of the research

In view of the important and uses of chalcones and their derivatives, this research project is aims to undertake an in-depth study on a few series of new chalcones.

The objectives of this project are:

1. To synthesize novel chalcones from inexpensive commercially available aromatic compounds.
2. To synthesize novel coumarin- and naphthalene-based chalcone derivatives with different alkyl chain length.
3. To elucidate all intermediary and targeted compounds using spectroscopic techniques (FT-IR, 1D-NMR, 2D-NMR), CHN elemental analysis and X-ray crystallography.
4. To evaluate the anti-cancer activity of the synthesized chalcones.

1.4 Scope of Study

The scope of this study is basically to synthesize and characterize the chalcones using Claisen-Schmidt condensation method and to determine the anti-cancer activity for the chalcone syntehsized. There are some significant tasks to be carried out in order to achieve the objective of this study.

I. In this study, the individual starting material, coumarin and naphthalene will react to synthesized chalcones.

II. The characterization of the result chalcones using fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), CHN microanalyses and single crystal X-ray diffraction (XRD).

III. Pharmacological (anti-cancer) properties of the synthesized chalcones will be evaluated through inhibition rate against breast cancer cell (4T1) and fibroblast cell (NIH-3T3) with cisplatin as positive control.

IV. Analyst the relationship of the alkyl group carbon chain lengths with the synthesized chalcones biological activity.

CHAPTER 2: LITERATURE REVIEW

2.1 Starting materials

For this research project, two naturally abundant compounds had been chosen as starting materials: coumarin and naphthalene. Both are valuable starting materials that represent a vast family of compounds which were naturally found in plants and are interesting bioactive targets for many organic medicinal chemists.

2.1.1 Coumarin

Coumarin; also known as 2H-1-benzopyran-2-one, is a fragrant organic chemical compound and naturally occurring polyphenolics distributed widely in plants and microorganisms. Vogel (1920) had isolated the first coumarin (Figure 2.1) from the fruit of *Dipteryx odorata Wild*. Coumarin can occur in two basic forms, either attached to sugar glucose or as a free molecule.

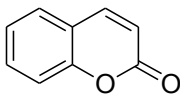


Figure 2.1. Structure of a coumarin.

Due to large number of natural products contain coumarin heterocyclic as nucleus, coumarins occupy an important position in natural and synthetic organic chemistry. They are widely used as additives in food, perfumes, photochemotherapeutic agents, insecticides and in optical brighteners (Fulchand *et al*., 2008). In particular, coumarin derivatives have been found to exhibit a variety of biological activities such as antitumor (Santana *et al*., 2000), antioxidant (Guiotto *et al*., 1995), anti-inflammatory

(Ploypradith *et al*., 2004), anticoagulant (Borges *et al*., 2005), antibacterial (Maria *et al*., 2013), anti-cancer (Riveiro *et al*., 2008; Belluti *et al*., 2010), anti-HIV (Zhao *et al*., 1997; Mahajan *et al*., 2009), and antiproliferative (Jung *et al*., 2009) properties. Among these properties, cytotoxic effects were most extensively examined. Neo-tanshinlactone (Figure 2.2), a coumarin containing compound, has shown significant inhibition activity against two ER+ human breast cancer cell lines, which has been associated with low toxicity and has raised considerable interest because of its potential beneficial effects on human health. Nevertheless, others coumarin-derivatives such as Geiparvarin, Umbeliferone, Xanthyletin and Seselin (Figure 2.2) have provoked great interest for their possible therapeutic uses (Sashidhara *et al*., 2010).

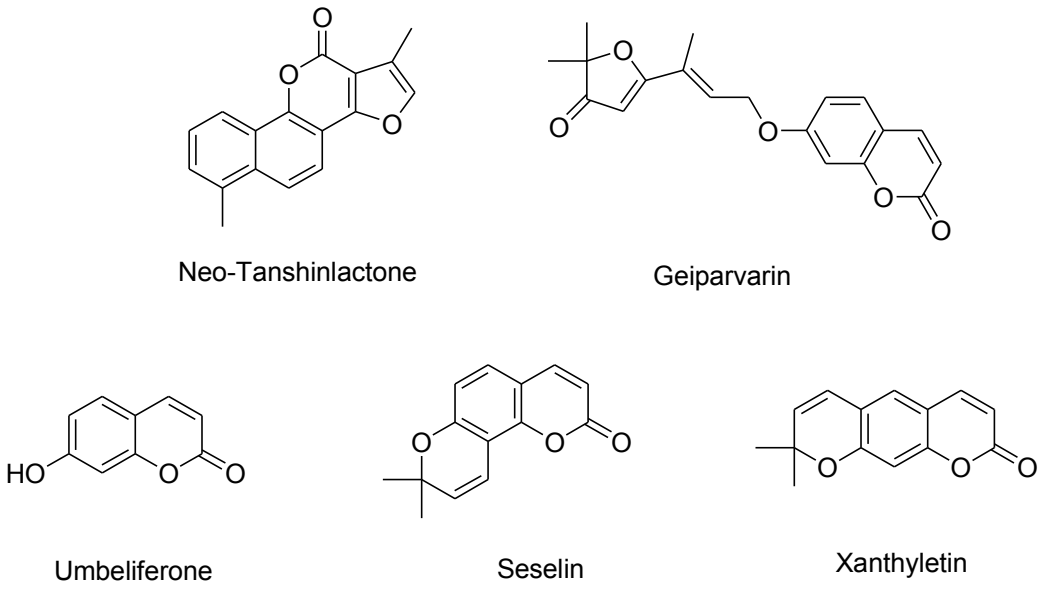


Figure 2.2. Chemical structures of some naturally occurring coumarins with potent anticancer activity.

Over the years, scientists had developed many strategic pathways for the synthesis of coumarins in laboratory. These include use of the Reformatsky, Wittig, Pechmann, Perkin, Claisen and Knoevenagel reactions. Knoevenagel reaction has been

applied in this project. The Knoevenagel reaction involves the condensation of benzaldehydes with activated methylene compounds and amine as a catalyst in organic solvents. Knoevenagel reaction method was more preferable compared to other methods because of its less severe reaction conditions (Knoevenagel *et al*., 1984).

2.1.2 Naphthalene

Naphthalene is the simplest member of polycyclic aromatic hydrocarbons which consists of two fused benzene rings (Price & Jayjock, 2008). Naphthalene (Figure 2.3) appears as white crystalline solid with a characteristic odor and readily sublimes at room temperature. This compound is easily generated during burning of wood or cigarette, grilling of meats, petroleum refining and distillation of coal tar (ADSTR, 2005).

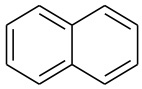


Figure 2.3. Structure of naphthalene.

Naphthalene has been largely used in manufacture as a chemical intermediate including moth repellents, plastics, wetting agent, lavatory scent discs, surfactant, dyes, resins, and soil fumigants (Brusick, 2008). Recent studies showed that naphthalene derivatives have attracted a great interest not only for their significant biological and photophysical properties (thermo- and photochromism, and non-linear optical behavior), but also as model compounds for assessing the nature of the hydrogen bonding (Martinez *et al*., 2011).

Naphthalene and its derivatives have been reported to possess a wide spectrum of biological activity such as anti-inflammatory (Huang *et al*., 2003; Jing *et al*., 2013),

anti-cancer (Andrea *et al*., 2012), anti-mycobacterial (Ram *et al*., 2010; Upadhayaya *et al*., 2010), anti-tubulin (Alvarez *et al*., 2007), antimicrobial (Yapici *et al.*, 2005), anti-proliferative (Sheila *et al*., 2013), anti-protozoal (Donald *et al*., 2009), and bactericidal activity (Misra & Kushwaha, 1977). Study conducted by Tanaka *et al*. (2009) shows that anti-inflammatory potency of naphthalene-chalcone hybrid has been associated with the increase in HO-1 expression (Tanaka et al., 2009). Additional to that, naphthalene has been shown as an ideal component of a fluorescent chemosensor due to its short fluorescence lifetime, low fluorescence quantum yield and ability to act as a donor as well as an acceptor (Sahana *et al*., 2011).

Zhou and his co-workers (2009) had extracted a naphthaldehyde derivative characterized as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-metho xy-3,4-dihydro-2-naphthaldehyde (Figure 2.4) from seeds of *Vitex negundo*, which proven to possess cytotoxic effect on breast, prostate, and ovarian cancer cells and induces apoptosis with cleavage in poly ADP ribose polymerase protein, up-regulation of Bax, and down-regulation of Bcl-2. Furthermore, Maioral *et al*. (2012) shown that synthetic chalcones, derived from 1-naphthaldehyde and 2-naphthaldehyde, possessed cytotoxic effect on human acute myeloid leukemia K562 cells and on human acute lymphoblastic leukemia Jurkat cells. From the experiment done by David (2004) also revealed that 2-hydroxy-1-naphthaldehyde derivatives showed higher activity and selectivity toward a range of neoplastic cells relative to normal cells.

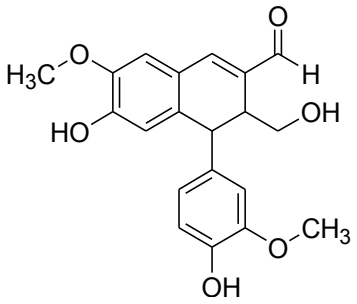


Figure 2.4. Chemical structure of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde extracted from seeds of Vitex Negundo.

2.2 Methods to synthesize chalcone

A chalcone can be prepared by Claisen-Schmidt condensation between a benzaldehyde and an acetophenone in the presence of base catalyst followed by dehydration to yield the corresponding product. It is also possible to synthesize chalcone under acidic conditions using perchloric acid and acetic acid (Siddiqui *et al*., 2008). Several other methods have been developed as well to synthesize chalcone, such as Heck reaction (Bianco *et al*., 2004), Hiyama coupling (Hatanaka & Hiyama, 1988), Negishi coupling (King *et al*., 1977), Stille reaction (Santos *et al*., 2007), Suzuki reaction (Suzuki, 1998) and so on.

2.2.1 Claisen-Schmidt reaction

Claisen-Schmidt reaction also known as aldol condensation has been the most convenient and widely used method for obtaining chalcones. It has been the most important enolate reactions of carbonyl compounds in which the acetophenone was reacted with benzaldehyde in the presence of alkali or acid as catalyst to form new carbon-carbon bonds. Condensation combined these two carbonyl compounds, often with the loss of small molecule such as water or an alcohol (Wade, 2006).

The condensation of the Claisen-Schmidt reaction can be carried out by using various basic catalysts such as sodium hydroxide (Alston & Albert, 2004), potassium hydroxide, sodium methoxide (Rojas *et al*., 2002), barium hydroxide (Krohn *et al*., 2002), hydroapatite and lithium hydroxide (Zahouily *et al*., 2003). It is also possible to synthesize chalcone under acidic condition by using silica-sulphuric acid (Zhang *et al*., 2003), dry hydrochloric acid, perchloric acid and acetic acid (Siddiqui *et al*., 2008).

Solhy and his co-workers found out that hydroxyapatite was a very efficient heterogeneous catalyst for the preparation of chalcone derivatives via Claisen–Schmidt condensation using microwave irradiation (Figure 2.5). The catalyst was easily recovered and efficiently re-used. Solhy had combined hydroxyapatite with water (act as co-catalyst) to produce various substituted chalcones with excellent yield of 95% (Solhy *et al*., 2010).

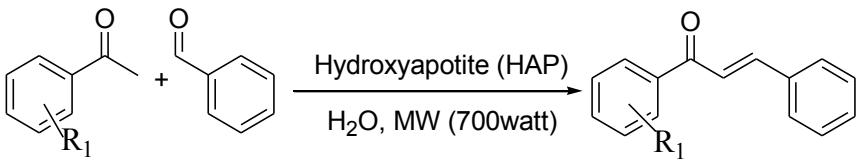


Figure 2.5: Synthesis of various substituted chalcones in the presence of water and hydroxyapatite as catalyst via Claisen–Schmidt condensation using microwave irradiation.

The mechanism of the Claisen-Schmidt reaction was proposed by Nayak and Rout in 1970. Gasull *et al*. (2000) has re-analyzed the mechanism of Nayak and proposed a new mechanism that has been used in most of the modern organic textbooks. As illustrated in Figure 2.6, there are several steps involved in the formation of chalcone:

Step 1: A base removes an α proton to form an enolate ion.

Step 2: Nucleophilic addition of the enolate ion to a carbonyl group.

Step 3: A configurational equilibrium between *cis-s-cis* and *trans-s-trans* isomers.

Step 4: Protonation of alkoxide by water molecule to form a neutral intermediate.

Step 5: Intramolecular dehydration of the neutral intermediate to give the chalcone.

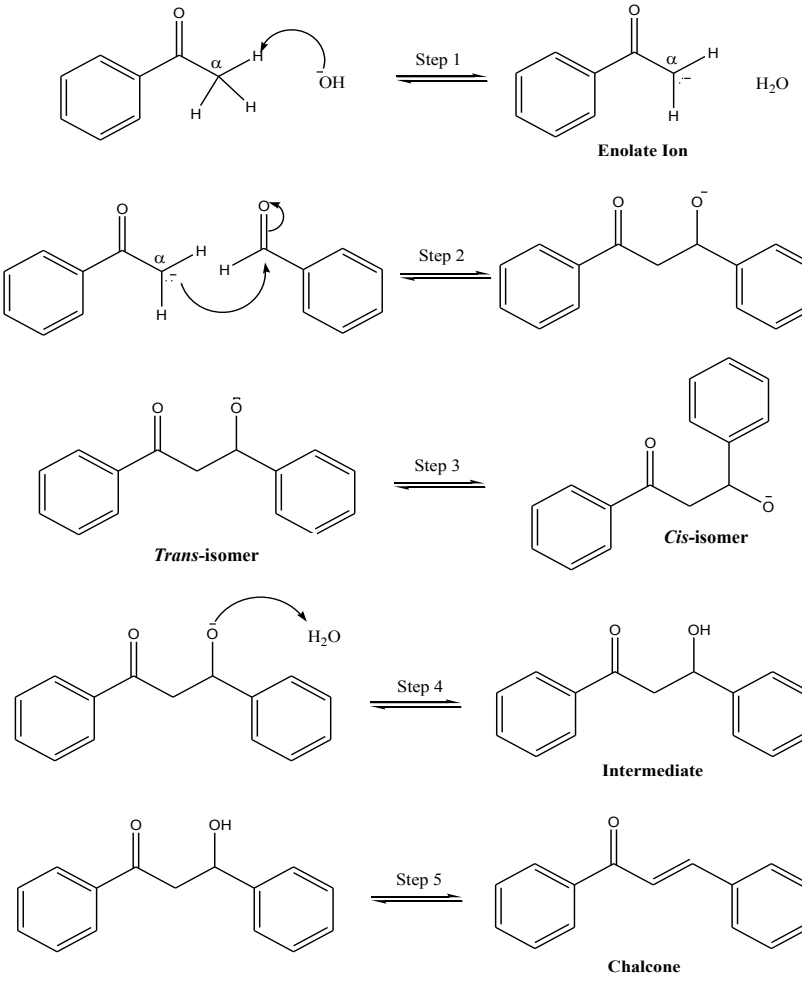


Figure 2.6: Proposed reaction mechanism for the chalcone formation under alkaline condensation in 65% EtOH-H_2O by Gasull *et al*. (2000).

2.2.2 Suzuki reaction

The Suzuki coupling reaction is the palladium-catalyzed cross coupling between organoboronic acid and aryl halides. Recent developments have extended the application of Suzuki reaction where the starting materials are not restricted to aryls, but include alkyls, alkenyls and alkynyls. Several reports have described the synthesis of polysubstituted chalcones based on the Suzuki reaction (Figure 2.7). The reaction has been performed using activated form of acid such as acyl chlorides, acid anhydrides or thio-esters coupling with phenylboronic acid (Eddarir et al., 2003).

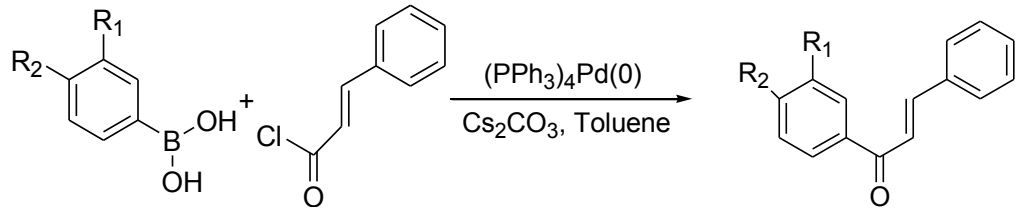


Figure 2.7. Synthesis of chalcones by Suzuki coupling between cinnamonyl chloride and phenylboronic acid.

The advantages of this reaction are the substituents on the acryl chloride or on the boronic acid did not affect the reaction mechanism, the selection of coupling site was quit specified and unaffected by the presence of water. Other than that, the Suzuki coupling is applicable in laboratories as well as in industrial processes due to the inorganic by-product of the reaction is non-toxic and easily removed from the reaction mixture (Suzuki, 1998).

2.2.3 Heck reaction

The Heck reaction (also called the Mizoroki-Heck reaction) is a palladium catalyzed chemical reaction involves the coupling between aryl halide and aryl alkene in the presence of base (Heck & Nolley, 1972). This reaction was originated by Tsutomu Mizoroki (1971) which described the reaction between iodobenzene and styrene to form stilbene with potassium acetate base and palladium chloride catalyst (Figure 2.8).

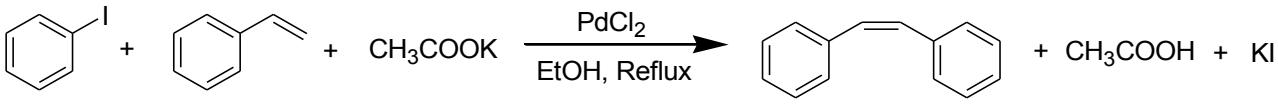


Figure 2.8. The reaction scheme by Tsutomo Mizoroki (1971).

The reaction was further modified by Heck to eliminate two major difficulties found in Mizoroki reaction: difficulty in obtaining the required organomercury (lead or tin compounds) and the problem of working with thick slurries of salts, particularly if the reaction is carried out catalytically in palladium (Heck & Nolley, 1972). The improved reaction differs in lack of solvent, selection of catalyst and base used (Figure 2.9).

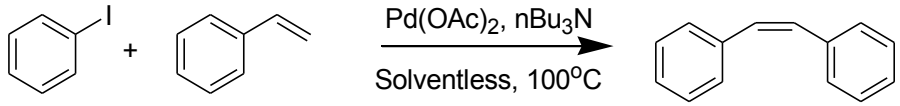


Figure 2.9. The reaction scheme by Richard F. Heck (1972).

Over the last few years, chalcones had been synthesized using the Heck coupling reaction. Palladium-catalyzed Heck coupling reaction between the aryl vinyl ketone with the aryl iodide will yield the corresponding chalcone (Figure 2.10) (Bianco *et al*., 2004). This procedure affords the desired chalcone in yields that are

always higher than those using conventional methods such as Claisen-Schmidt condensation. It is possible to design various novel chalcone derivatives by monitoring the aryl halides or aryl vinyl ketones (Bianco *et al*., 2003; Reichwald *et al*., 2008).

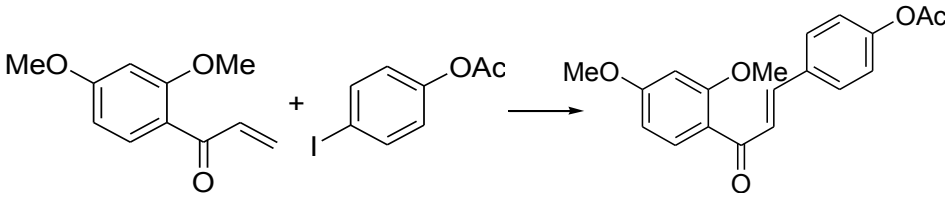


Figure 2.10. Chalcone synthesis by Heck coupling reaction.

2.3 Bioactivities of chalcones

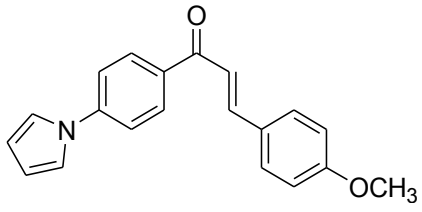
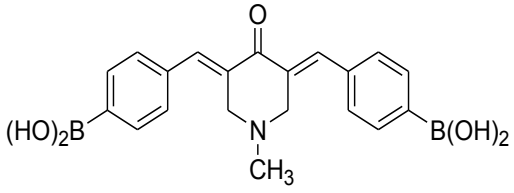
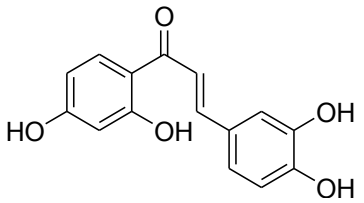
Cancer (also known as a malignant neoplasm) is a broad group of various diseases that involving uncontrolled, rapid and pathological proliferation of unregulated cell growth. Being the second leading cause of death in developing as well as advanced countries, it had increased the pressing need for new anticancer agents with high potency, less toxicity in non-cancerous cells, and unique targets of action (Chari *et al*., 1992).

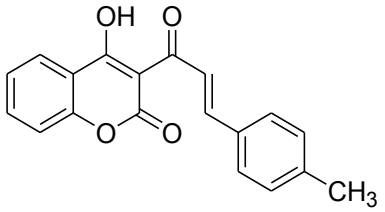
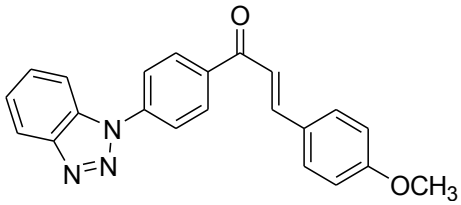
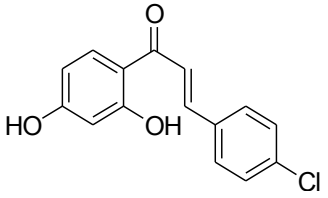
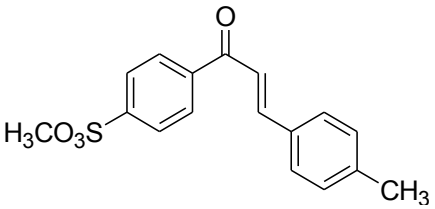
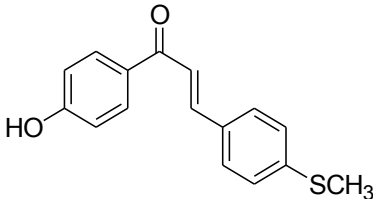
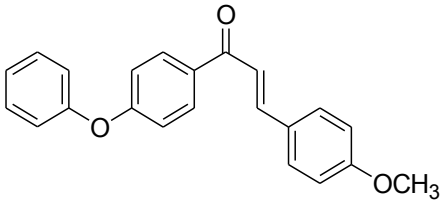
Chemotherapy had been the most common treatment for diseases especially by killing micro-organisms such as prokaryotes, eukaryotes and cancerous cells. The development of novel and efficient drugs by molecular manipulation of promising lead compounds is still a major line of approach. Following this aim hybrid molecules were designed from two natural occurring compounds: naphthalene and coumarin. Naphthalene and coumarin derivatives have well known pharmacological activities such as antimicrobial (Yapici *et al*., 2005), anti-tubulin (Huang *et al*., 2003), anti-mycobacterial (Upadhayaya *et al*., 2010), antitumor, anti-inflammatory,

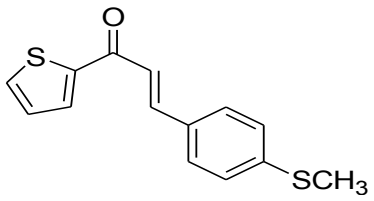
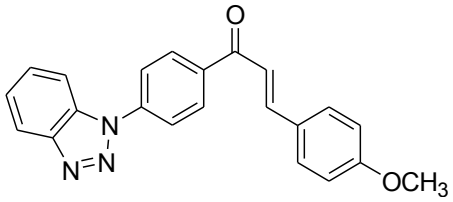
anti-HIV (Nohemi *et al*., 2009), antithrombotic (Alvarez *et al*., 2007), cardio protectors or enzymatic inhibitors (Park & Jang, 1995).

The hybrid molecules, chalcones, precursors of open chain flavonoids and isoflavonoids are present in edible plants, and have displayed a broad spectrum of pharmacological activity. Changes in their structure have offered a high degree of diversity that have proven useful for the development of new medicinal agents having improved potency and lesser toxicity (Rahman, 2011). The chemotherapeutic effects of various chalcones and their derivatives against a wide variety of microorganisms and diseases are given in Table 2.1.

Table 2.1: Biological applications of chalcones and their derivatives.

Compounds	**Biological application**	**Reference**
	Inhibitors of malaria parasite (Chloroquine-sensitive *P. falciparum*)	Awasthi *et al*.
	Anti-cancer activity	Achanta *et al*.
	Anti-inflammatory	Yadav *et al.*

	Anti-bacterial (Against Gram +ve bacteria, *Staphylococcus aureus*)	Hamdi *et al*.
	Anti-filarial (Setaria cervi using GST enzyme as a drug target)	Awasthi *et al*.
	Monoamine Oxidase (MAOs) inhibitory	Chimenti *et al*.
	Cyclooxygenase (COX) inhibitory	Zarghi *et al*.
	Anti-oxidant	Vasil'ev *et al*.
	Anti-convulsant	Kaushik *et al*.

	Anti-fungal	Bag *et al*.
	Anti-filarial	Awasthi *et al*.

In 2000s, a broad series of various chalcones derivatives had been synthesized and evaluated for their pharmacological activities. Achanta *et al.* (2006) had reported the anticancer and the mechanism of action of eight boronic chalcones derivatives. From the test result, boronic chalcones shown potent growth inhibition activity in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and colony formation assay with IC_{50} values of <1.5 μM. Mechanistic studies showed that boronic chalcones induced significant cytotoxic effect in cancer cells by inhibitied the chymotrypsin like activity of the 20S proteasome *in vitro*.

Romagnoli *et al*. (2008) had synthesized a series of chalcone-like agents, in which the α,β unsaturated bond of the enone system is embedded within a thiophene ring were evaluated for antiproliferative activity and inhibition of tubulin assembly and colchicine binding to tubulin. The replacement of the double bond with a thiophene maintained antiproliferative activity (IC_{50} < 2 μM) and electron-releasing group (ERG) caused only minor changes in antiproliferative activity. The synthesized compounds were found to inhibit the growth of several cancer cell lines

at nanomolar to low micromolar concentrations and some compounds (IC_{50}, 0.8 μM) having twice the potency of antitubulin agent CA-4 (IC_{50}, 1.4 μM).

Echeverria *et al.* (2009) had reported the relationships between the structural characteristic of synthetic chalcones and their antitumoral activity toward hepatocellular carcinoma cells (HepG2). From the calculated reactivity indexes and the adiabatic electron affinities, it shown that the absence of methoxyl substituents has the major structure reactivity pattern along the series. Due to this, the chalcone 1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one was found to be possessed the most active structure-activity between the chalcone structure and the apoptosis in HepG2 cells.

Recently, another series of chalcones were synthesized and evaluated by Llango *et al*. (2010) for their *in vitro* cytotoxic activity by microculture Tetrazolium Test Assay method using two breast cancer cell lines MCF-7 and T47D. The synthesized chalcones shown significant cytotoxicity against both of the cell lines and values lied between 52-89 μM. Due to the presence of nitro group in the compound, *N*-(4-hydroxy-3-(3-(4-nitrophenyl)acryloyl)phenyl)acetamide showed better activity than other compounds.

Szliszka *et al.* (2010) had synthesized five chalcones that hybrided with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Results showed that all the five tested chalcones markedly augmented TRAIL-mediated apoptosis and cytotoxicity in prostate cancer cells and confirmed the significant role of chalcones in chemoprevention of prostate cancer. Their study suggested that chalcones help anticancer immune defense in which endogenous TRAIL takes part.

CHAPTER 3: EXPERIMENTAL

3.1 Chemicals

All the chemicals were used as received without further purification. Table 3.1 summarizes the chemicals used in this study.

Table 3.1: List of chemicals used in this study.

Chemicals	Source
1-Bromoethane	Merck
1-Bromobutane	Fluka
1-Bromohexane	Merck
1-Bromooctane	Merck
1-Bromodecane	Merck
1-Bromododecane	Merck
1-Bromotetradecane	Merck
2-Hydroxyacetophenone	Merck
2-Hydroxybenzaldehyde	Merck
2-Hydroxynaphthaldehyde	Fluka
3-Acetylcoumarin	Sigma-Aldrich
4-Fluoro-2-hydroxyacetophenone, 98%	Sigma-Aldrich
4-Hydroxycoumarin	Merck
Acetic acid (glacial)	Merck
Acetone	QRec
Deuterated chloroform, $CDCl_3$, with TMS (0.03 vol.%), deuteration degree min. 99.8%	Merck
Diethyl ether	QRec
Dimethylformamide (DMF)	QRec
Ethanol, 99.97%	QRec
Ethyl acetoacetate	Merck
Petroleum ether	QRec
Piperidine	Sigma-Aldrich
Phosphorylchloride	Merck
Potassium carbonate anhydrous	R & M Chemical
Sodium carbonate	Merck

3.2 Instruments

The instrumentation used for the qualitative characterization of synthesized starting materials and chalcone derivatives are listed below:

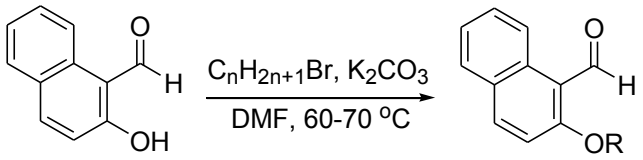
- Thin Layer Chromatography (TLC) analyses were carried out using aluminium-backed silica gel 60 F_{254} plates. Visualization was done under UV light and by using iodine spotting.
- CHN microanalyses were carried out on a Perkin Elmer 2400 LS Series CHNS/O analyzer at the School of Chemical Sciences, University Sains Malaysia (USM).
- Fourier Transform Infrared (FT-IR) data were recorded using a Perkin Elmer 2000-FTIR spectrophotometer at the School of Chemical Sciences, USM.
- 1D and 2D Fourier Transform Nuclear Magnetic Resonance (FT-NMR) spectroscopy performed using a Bruker AC 500 MHz at the School of Chemical Sciences, USM.
- X-ray crystallographic analyses were done using either Bruker SMART Apex II or Apex II Duo CCD diffractometers at the School of Physical Sciences, USM.

3.3 Synthesis

The starting materials, aldehydes and ketones as well as the targeted chalcones were synthesized and purified at normal atmospheric pressure by conventional methods unless stated otherwise.

3.3.1 Preparation of 2-alkyloxy-1-naphthaldehydes, 2-8

2-Alkyloxy-1-naphthaldehydes were synthesized according to the synthetic scheme below (Figure 3.1).



1 **2-8**

n= 2, 4, 6, 8, 10, 12 and 14

R = C_2H_5, C_4H_9, C_6H_{13}, C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$

Figure 3.1: Synthetic scheme for the formation of 2-alkyloxy-1-naphthaldehyde, **2-8**.

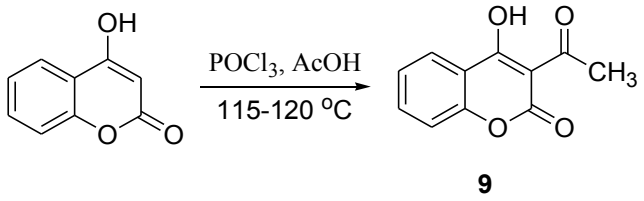
2-Hydroxy-1-naphthaldehyde (2.00 g, 11.62 mmol) was mixed with 1.5 equiv. of the corresponding bromoalkane in *N,N*-dimethylformamide (20.0 mL), which served as a solvent. Three equiv. of potassium carbonate anhydrous was then added to the solution and the mixture was refluxed for 12 hours. The mixture was cooled to room temperature and poured into large amount of crushed ice with stirring. The resulting precipitate was filtered and washed with cold distilled water. The precipitate was air-dried and recrystallized from ethanol in cool condition. The scientific names and the respective R group for **2-8** are tabulated in Table 3.2. The condensed spectroscopy data for **2-8** can be found in Appendix I.

Table 3.2: Scientific name and respective R group of **2-8**.

Compound	R group	Scientific name
2	C_2H_5	2-ethoxy-1-naphthaldehyde
3	C_4H_9	2-butoxy-1-naphthaldehyde
4	C_6H_{13}	2-hexoxy-1-naphthaldehyde
5	C_8H_{17}	2-octoxy-1-naphthaldehyde
6	$C_{10}H_{21}$	2-decoxy-1-naphthaldehyde
7	$C_{12}H_{25}$	2-dodecoxy-1-naphthaldehyde
8	$C_{14}H_{29}$	2-tetradecoxy-1-naphthaldehyde

3.3.2 Preparation of 3-acetyl-4-hydroxycoumarin, 9

3-Acetyl-4-hydroxycoumarin was synthesized according to the synthetic scheme below (Figure 3.2).



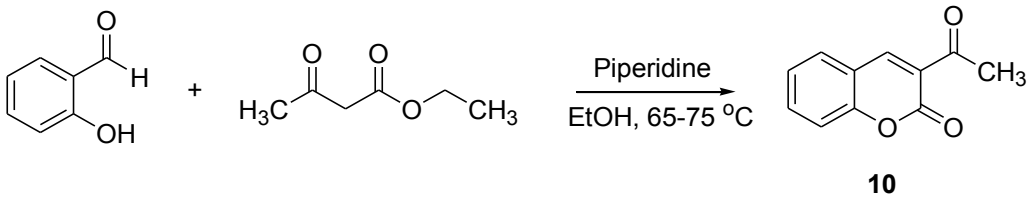
9

Figure 3.2: Synthetic scheme for the formation of 3-acetyl-4-hydroxycoumarin, **9**.

4-Hydroxycoumarin (3.00 g, 18.60 mmol) was added to acetic acid (16.00 mL), which served as a reactant and solvent. Phosphorylchloride (5.60 mL) was then added to the solution and the mixture was refluxed for 12 hours (solution color changed from yellow to intense red). The mixture was cooled to room temperature and poured into large amount of crushed ice with stirring. The resulting precipitate was filtered and washed with cold distilled water. The precipitate was air-dried and recrystallized from ethanol.

3.3.3 Preparation of 3-acetylcoumarin, 10

3-Acetylcoumarin was synthesized according to the synthetic scheme below (Figure 3.3).



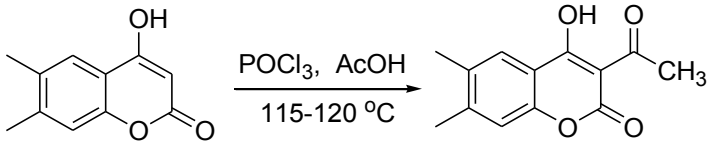
10

Figure 3.3: Synthetic scheme for the formation of 3-acetylcoumarin, **10**.

Salicylaldehyde (2.70 mL, 25.00 mmol), ethyl acetoacetate (3.20 mL, 25.00 mmol) and ethanol (50.00 mL) were all added into a 250 mL round-bottom flask. A catalytic amount of piperidine (0.50 mL) was added dropwise and the reaction mixture was refluxed at 65-75 ^{o}C for 4 hours. The mixture was cooled to room temperature. The resulting precipitate was filtered and washed with small portion of cold ethanol. The precipitate was air-dried and recrystallized from ethanol/chloroform mixture.

3.3.4 Preparation of 3-acetyl-6,7-dimethyl-4-hydroxycoumarin, 11

3-Acetyl-6,7-dimethyl-4-hydroxycoumarin was synthesized according to the synthetic scheme below (Figure 3.4).



11

Figure 3.4: Synthetic scheme for the formation of 3-acetyl-6,7-dimethyl-4-hydroxycoumarin, **11**.