SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 4'- METHOXY-5,7-DIMETHOXYFLAVANONE & 2,4-(4-BUTYLPHENYL)-5,7-DIMETHOXYCHROMAN-4-ONE

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Final Year Project Report Submitted in
Partial Fulfilment of the Requirements for the
Degree of Bachelor (Hons.) Chemistry With Management
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This Final Year Report Project entitled **"4'-methoxy-5,7 -dimethoxyflavanone and 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one"** was submitted by Angellia Samantha anak Sibat, in partial fulfilment of the requirement for Degree of Bachelor of Science (Hons.) Chemistry with Management, in the Faculty of Applied Science, and was approved by

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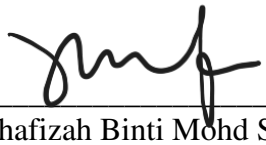

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Angellia Samantha anak Sibat

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LIST OF ABBREVIATIONS

AcOH	:	Acetic acid
CH_4O_3S	:	Methane Sulphonic acid
C_6H_{14}	:	Hexane
$C_7H_5NO_3$	:	3-nitrobenzaldehyde
C_8H_8O	:	4-methylbenzaldehyde
$C_{10}H_{12}O_4$	:	2-hydroxyacetophenone
DMSO	:	Dimethyl sulfoxide
EtOAc	:	Ethyl acetate
EtOH	:	Ethanol
HCl	:	Hydrochloric acid
H_2O	:	Distilled water
I^-	:	Iodide
FTIR	:	Fourier-transform infrared
GCMS	:	Gas Chromatography Mass Spectrometry
KOH	:	Potassium hydroxide
MBC	:	Minimum Bactericidal Concentration
MIC	:	Minimum Inhibitory Concentration
$MgSO_4$	:	Magnesium Sulphate
NaCl	:	Sodium chloride

NA	:	Nutrient agar
NB	:	Nutrient broth
NMR	:	Nuclear magnetic resonance
SiO_2	:	Silica
TLC	:	Thin layer chromatography
TMS	:	Tetramethyl silane
UV	:	Ultraviolet

LIST OF SYMBOLS

%	:	Percentage
°C	:	Degree Celsius
cm	:	Centimetre
g	:	Gram
L	:	Litre
mg	:	Milligram
mL	:	Millilitre
MHz	:	Megahertz
mm	:	Millimetre
mmol	:	Millimole
ppm	:	Parts per million
µg	:	Microgram

ABSTRACT

Plants are generally well-known for their benefits in becoming a natural resource to cure illnesses and diseases in human beings. Being secondary plant metabolites that are categorized as one of the largest classes, flavonoids can be effortlessly found in many different parts of the plants. The flavonoid has attracted massive attention for its involvement in antibacterial activities. Flavanone is well-known as the subclass of the flavonoid family and is also identified as dehydroflavones. It is one of the critical classes of flavonoids that can be widely found among citrus fruits. Hence, this study aims to synthesize 4' methoxy-5,7 -dimethoxy flavanone and 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one through the Claisen-Schmidt condensation, as well as investigate its antibacterial activity. The compound later was characterized by using Gas Chromatography Mass Spectrometry (GC-MS), Fourier-Transform Infrared Spectroscopy (FTIR), and Nuclear Magnetic Resonance (NMR). This research scope will focus on the synthesize and characterization of flavanone, its antibacterial activity, and the synthesis of flavanones from the corresponding chalconesThe MIC and MBC of the synthesized compounds were tested on *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA), and *Streptococcus pyrogens* (SP). It is found that by utilizing the methods of serial dilution method in order to determine the MIC and MBC. 4'-methoxy-5,7 -dimethoxyflavanone and 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one are unable to inhibit pathogenic bacterial.

ABSTRAK

Tumbuhan secara amnya dikenali dengan faedahnya dalam menjadi sumber semulajadi untuk menyembuhkan penyakit yang terdapat pada manusia. Menjadi metabolit tumbuhan sekunder yang dikategorikan sebagai salah satu daripada kelas terbesar, flavanoid boleh didapati dengan mudah pada pelbagai bahagian yang berbeza pada tumbuh-tumbuhan. Flavonoid telah menarik perhatian secara besar-besaran sehubungan dengan penglibatannya di dalam aktiviti antibakteria. Flavanon juga terkenal sebagai sub-kelas kepada flavonoid dan ianya juga dikenali sebagai dehidroflavon. Ianya juga tergolong dalam kelas flavonoid yang boleh didapati secara meluas dalam kalangan buah sitrus. Kajian ini akan menjalankan proses sintesis terhadap 4' metoksi-5,7-dimetoksiflavanon dan 2-(4-butilfenil)-5,7-dimetoksikroman-4-on menggunakan kaedah pemeluwapan "Claisen-Schmidt" sekaligus mengkaji aktiviti bakterianya. Seterusnya, sebatian flavanon tersebut telah dikenalpasti menggunakan Kromatografi Gas Spektroskopi Jisim (GC-MS)", "Spektroskopi Inframerah Fourier Transformasi (FTIR)", "Resonans Magnetic Nuklear (NMR)". Kajian ini akan menfokuskan terhadap sintesis dan pencirian flavanon, aktiviti antibakterianya serta sintesis flavanon daripada calkon yang sepadan. MIC dan MBC campuran yang telah disintesis telah diuji keatas *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Staphylococcis aureus* (SA) dan *Streptococcus pyrogens* (SP) melalui kaedah pencairan bersiri untuk menentukan kepekatan perencatan minimum dan kepekatan bakteria minimum. Telah ditemui bahawa, 4'-metoksi-5,7-dimetoksiflavanon and 2-(4-butilfenil)-5,7-dimetoksikroman-4-on tidak mampu menghalang beberapa bakteria yang digunakan.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

For over 30 centuries, human beings mainly depend on the existence of "drugs" to cure illnesses and diseases (Taylor, 2016). Additionally, the word "drug" originated from the French word, which is Drogue, which brought the meaning of "dry herb," and it is also suggested that drugs were initially taken out from the plants (Wadud et al., 2007). Back then, ancient people used natural resources such as plants or animal products to treat illnesses or cure diseases. Plants are well-known for their benefits in being used as medicine. In addition, both herbs and plants utilized by humans are notable for their richness in the source of phytonutrient compounds synthesized in the plants (Karak, 2019). Despite the abundance of natural resources becoming their natural remedies, plants were one of the natural resources that contributed the most (Yuan, et al., 2016). On the other hand, pharmaceuticals and other related fields are now actively inventing new medicines, vaccines, and antibiotics to cure illnesses and diseases. Furthermore, the invention of antibiotics increasingly extended the lifespan of humans by about 23 years, as well as changed modern medicine (Hutchings et al., 2019). However, even though scientists or pharmacists have invented and developed many new drugs and varieties of antibiotics, the viruses' resistance to the medications invented seems to improve and increase from time to time. Thus, researchers have attempted to develop and produce

synthetic and natural medicines. Furthermore, the World Health Organization (WHO) has highly recommended medicinal herbs as the best source among the various type of medicine (Nascimento et al., 2000). Flavonoids are generally known as hydroxylated phenolic substances (Figure 1.1) and are synthesized by plants as a reaction to microbial infection. Moreover, the flavonoid is categorized into an essential group of polyphenolic compounds that occurs naturally and it also has a vital role in the success of both ancient and modern medical treatments (Prithviraj, 2019). There is an increment of interest in utilizing natural remedies as cost-effective and trusted medicines for treating different types of illnesses or diseases (Fahmy et al., 2018). In accordance with this, the flavonoid is one of the examples of a natural substance that can be synthesized and later on utilized in modern medicines or remedies. Furthermore, flavonoids can be easily found in diverse parts of plants, and the flavonoid itself has attracted much attention for their potential in antibacterial activities (Yuan et al., 2021). Subsequently, the resistance to antimicrobials has severely jeopardized the health of human beings. Hence, new microbial agents are urgently needed to prevent any unwanted illnesses or diseases among human beings (Yuan et al., 2021). The characteristics of flavonoids that possess various valuable properties such as the activity of anti-allergic, anti-inflammatory, antimicrobial, antioxidant, cytotoxic antitumor, enzyme exhibition, estrogenic, and vascular action make it becoming the centre of attraction and a central subject in the medical research field (Cushnie & Lamb, 2005).

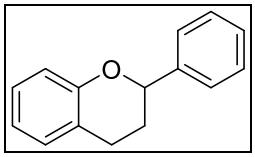


Figure 1.1 Basic Structure of Flavonoid

In general, flavanone is well-known as the subclass of the flavonoid family (Figure 1.2). Flavanone, also known as dehydroflavones, is a significant class of flavonoids that can be broadly found among citrus fruits (Dias et al., 2021). Furthermore, flavanones are correlated to numerous types of health benefits as they own the properties of free radical scavenging. In particular, citrus flavonoids generate stunning pharmacological effects such as blood lipid-lowering, antioxidant, cholesterol-lowering agents, and anti-inflammatory (Panchi et al., 2016). According to Karak (2019), Sabtu et al. (2019), and Gorniak et al. (2019), flavanones have been tested and proven to have an effective antimicrobial activity.

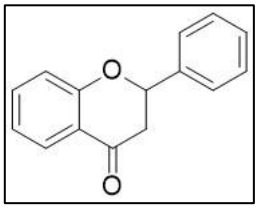


Figure 1.2 Basic structure of flavanone

Furthermore, subclasses of flavanones which is nobiletin, tangeretin and liquiritigenin have been claimed to be in charge of the antibacterial activity of certain medicinal plants (Xie et al., 2015). As shown in the basic structure of flavanone (Figure 1.2), there is a presence of the hydroxyl group in the structure of flavanones, and the number of the hydroxyl group was not significant. Still, the position of the two-hydroxyl group in the flavanones structure is crucial and effective in producing the antibacterial effect (Shamsudin et al., 2022). Chalcones can be found easily in some fruits, plants, and vegetables and belong to the significant flavonoid class (Figure 1.3). In addition, chalcones are the precursors of flavonoids and isoflavonoids (Ahmad et al., 2016). Rocha et al. (2019) also stated that chalcones act as the intermediates that their derivation in the amino acid phenylalanine. In addition, numerous amounts of research have been carried out on both natural and synthetic chalcones (Uchi et al., 2021). All of the focus on the research on flavanones are because chalcones have various biological activities such as anticancer, antioxidants, antibacterial, antileishmanial anti-allergic, anti-HIV, anti-inflammatory, anti-tuberculosis and antimicrobials (Rocha et al., 2021).

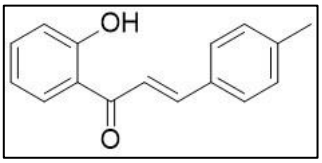


Figure 1.3 Basic structure of Chalcone

Furthermore, the properties of chalcones in terms of theoretical physicochemical and pharmacokinetic exhibits that chalcone does not gives out any severe risk of toxicity such as the mutation of the gene or cardiotoxicity, thus making the chalcones in becoming a good and acceptable active ingredient for

pharmacological (Rocha et al., 2021). Furthermore, Ammaji et al. (2021), mentioned that the -OH group at the aryl rings of the chalcones is an integral part of their antioxidant and anti-tubercular.

1.2 Problem statement

As years passed by, the demand for flavanones, especially in the pharmacological, have been increasing for the advantage and benefits it offers. Flavanones' benefits include antioxidant, anti-inflammatory, blood lipid-lowering, and cholesterol-lowering agents (Panchi et al., 2016). Researchers across a wide range of countries are actively experimenting with flavanone to continuously filter and improve results based on their findings of the new observations from past analysis and experiments. However, the time required for a plant to grow is exceptionally slow which affects its extraction procedure to be very time-consuming results in the difficulty for the flavanones to meet its high market demand. Thus, this research's main aim or target is to synthesize flavanones from chalcones through the use of Claisen Schmidt condensation by using acetophenone and benzaldehyde. Claisen Schmidt condensation is one of the best methods that can be used as it has short time for reaction and the materials are readily available as well as inexpensive (Mousavi, 2016)

1.3 Significance of study

Flavonoids and flavanones are very common for their various kind of pharmaceutical benefits that they can exert. For instance, flavonoids are remarkable for their advantages attributed to their high activity of antioxidants (Fahmy et al., 2018). A subclass of flavonoids called flavanones has numerous medicinal advantages as well. Panchi et al. (2016) stated that flavanones could have many pharmacological effects, including antioxidant, anti-inflammatory, blood lipid-lowering, and exerting the effects of cholesterol-lowering agents. With all the benefits that flavanones offer, this research is conducted to aim for the synthesize of flavanones from chalcone and to determine their antibacterial activity as well. In addition, data that has been gathered by the researchers before would be utilised in the research for the biomedical field to cure and treat illnesses and prevent the spread of any contagious diseases for the sake of the health and safety of the community.

1.4 Objectives of study

This research aims to;

1. To synthesize flavanone from corresponding chalcones using Claisen-Schmidt condensation.

2. To characterize chalcones and flavanones using Gas Chromatography Spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FTIR), and Nuclear Magnetic Resonance (NMR).

3. To study/determine the antibacterial activity of chalcones and flavanones using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) method.

CHAPTER 2

LITERATURE REVIEW

2.1 Preparation of Chalcones

In general, chalcones are aromatic compound with an unsaturated side chain and are abundantly present among various plants. Most importantly, they have numerous biological benefits that have become the main focus among researchers. For instance, chalcones offer natural advantages such as anticancer, antimalarial, anti-hyperglycaemic, and tyrosinases inhibitory (Adnan et al., 2020). Chalcones have a characteristic where they can naturally occur as a plant-derived polyphenolic compound. Nevertheless, they can also be modified synthetically into various derivatives by variations to the main preparation process, usually known as the reaction of Claisen-Schmidt condensation (Uchil et al., 2021). Furthermore, Claisen-Schmidt Condensation is the most desirable method for synthesizing chalcone derivatives as it uses a strong base or acid, either sodium and potassium hydroxide, dry HCl, and aluminium chloride (Sazegar et al., 2015).

2.1.1 Claisen-Schmidt Condensation

Chalcones are well-known for their production by the Claisen-Schmidt condensation between aldehydes and ketones (Kumar et al., 2010). The condensation of Claisen Schmidt is an aldol condensation that occurs between acetophenone and benzaldehyde (Uchil et al., 2021). The Claisen-Schmidt condensation is usually carried out in either acidic (Figure 2.1) or basic (Figure 2.2) media, which will be a homogenous condition (Raffee & Rahimi, 2013). Besides that, Raffee & Rahimi (2013) also mentioned that the condensation of Claisen-Schmidt is the most significant protocol in organic synthesis and medicinal chemistry among multicomponent reactions (MCRs). The process will involve two or more steps, which will be conducted without any segregation of any intermediates which also leads in time reduction, energy and raw materials can be thrifted.

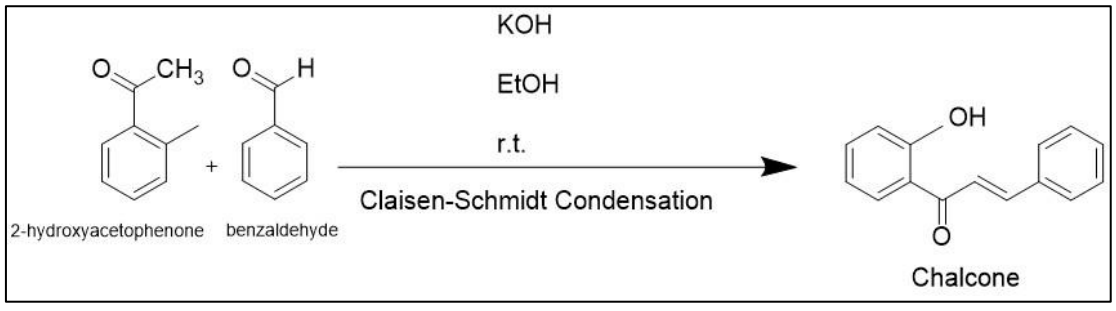


Figure 2.1 Claisen Schmidt reaction of chalcone catalyzed by a base

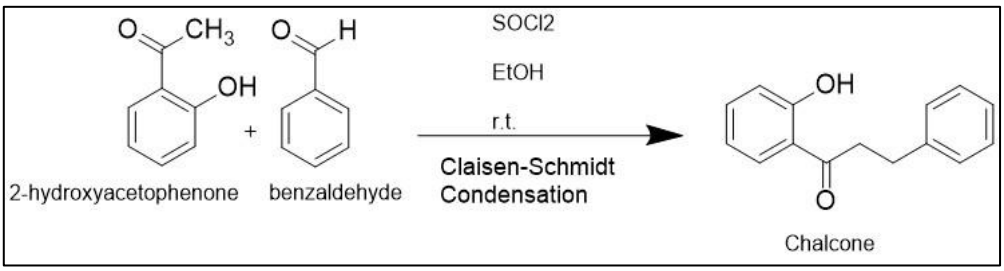


Figure 2.2 Claisen-Schmidt reaction of chalcone catalyzed by an acid

Besides that, H & Setyowati (2019) stated that the Claisen-Schmidt condensation could be conducted in two ways: the conventional method involves solvent and grinding, which does not include solvents. Compared with the traditional method, the grinding process is way better as it does not use any solvents that may become a hazard to human health as well as not environmentally friendly. For instance, acetonitrile, acids, dioxane, formaldehyde or tetrahydrofuran is an example of solvents that is not environmentally friendly (Capello et al., 2007). With that in mind, the technique of grinding is performed by grinding all of the reactants involved in a mortar so that there will be a collision between the reactants, which will eventually cause the energy of frictional from a local heat and result in the acceleration of chalcones formation (H & Setyowati, 2019).

On top of that, Claisen-Schmidt condensation is widely used as the reaction is quite simple and offers many other benefits such as being environmental friendly, the duration of the reaction is not time-

consuming, and the raw materials can be easily obtained as well (H & Setyowati, 2019). The reaction is quick and favourable with excellence in selectivity for the chalcones synthesis (Raffee & Rahimi, 2013).

2.2 Preparation of Flavanones

Most of the previous research has shown that flavanone is a natural compound that has an important role in the pharmaceutical industry for its ability to act as the antitumor and anti-inflammatory therapeutic agent (Kumar & Satbhaiya ,2021) in their journal article. Flavanone has attracted the attention of researchers for its biological benefits that might become an advantage in curing illnesses and diseases among living things. In addition, flavanones can be synthesized in various ways, such as the isomerization of 2-hydroxychalcones to flavanones (Scheme 2.4), synthesis of flavanones catalyzed by L-proline and synthesis of flavanones in sub-critical water.

2.2.1 The Isomerization of 2-hydroxychalcones to Flavanones

Ahmed et al. (2013) stated that 2-hydroxychalcones would produce flavanones without the enzyme chalcone isomerase; therefore, 2-hydroxychalcones are very commonly to be used in the synthesis of flavanones. Shareef et al. (2019) also mentioned that the Claisen-Schmidt condensation between 2-hydroxyacetophenone and substituted aromatic aldehydes would form a substituted 2-hydroxychalcones and afterward, on the process of isomerization, flavanones will be produced. In addition, the isomerization process of 2-hydroxychalcones to flavanone can be altered by either acid, base, thermal or photochemical conditions (Vimal et al., 2019). The method of isomerization of chalcones to flavanones is usually performed with either acid or base. As an illustration, methane sulphonic acid will be used to synthesize flavanone. Methane sulphonic acid is an efficient organic acid catalyst for the synthesis process as it makes the reaction time shorter and the substituted flavanones from 2-hydroxychalcones produce a higher yield (Kulkarni et al., 2012). Furthermore, various methods for the isomerization process of 2- hydroxychalcones often give either a moderate or poor yield, which is not a desirable result (Kulkarni et al., 2012).

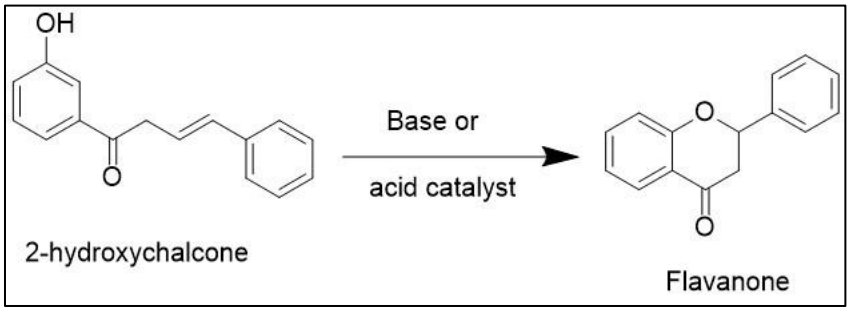


Figure 2.3 Isomerization of 2-hydroxychalcones to flavanone

2.2.2 Synthesis of Flavanones catalyzed by L-proline

Chandrasekar et al. (2006) have reported the discovery of the synthesis of flavanones that is catalyzed by L-proline in which the products are a mixture of both chalcone and flavanone. In addition, they also discovered the benefits of L-proline as a novel synthesis of flavanones for the condensation of aldol. In this process, benzaldehyde and the substituted 2- hydroxyacetophenones were used to complete the whole process in a single step (Figure 2.4). Afterwards, the mixture of benzaldehyde and the substituted 2-hydroxyacetophenones will be stirred at the temperature of 80ºC for a total of 18 hours in 0.02 M of dimethylformamide, DMF, along with the presence of 30% L-proline. The product will then be extracted with ether and washed with water. After that, through chromatography, flavanones and chalcones can be obtained at the ratio of 7:3. Other than that, Akcok & Cagir (2010) also stated that L-proline could catalyse both reactions of Michael addition

and Claisen-Schmidt at the same time. In addition, L-proline has dual functionality of acidic or basic, and catalyzed chemical transformations, which are homogenous to the catalysis of the enzyme (Sukanya et al., 2022). Furthermore, L-proline is known to have superior benefits over other catalysts as it has the characteristics of having a dual role as the catalyst and becoming a ligand. L-proline is also naturally occurring, readily available, inexpensive, and soluble in water (Bhattachrjee et al., 2017). L-proline is an efficient organocatalyst for the synthesis of flavanone.

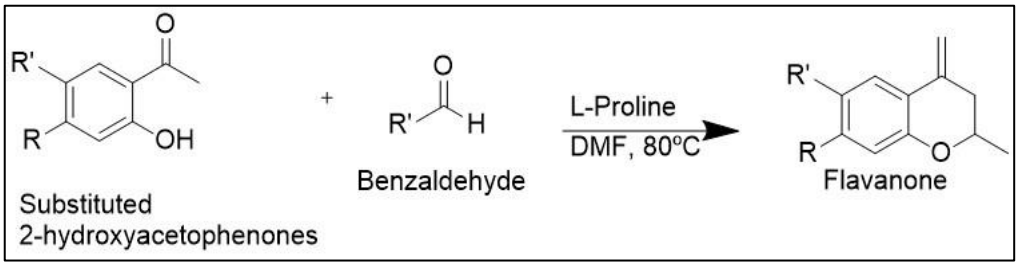


Figure 2.4 Synthesis of flavanones catalyzed by L-proline

2.2.3 Synthesis of Flavanones in Sub-critical water

According to Sirin et al (2013), several traditional methods are commonly practiced for the synthesis of flavanone, including the Baker-Venkataraman method and the Claisen-Schmidt condensation method. The Baker-Venkataraman method involves the conversion of 2-hydroxyacetophenones to benzoyl esters, followed by acid cyclization

to yield the ring system of flavone. The Claisen-Schmidt condensation method utilizes a synthetic route to synthesize the flavonoids from 2-hydroxyacetophenones and benzaldehyde, followed by the subsequent intramolecular Michael addition of the 2-hydroxychalcone intermediates, which will then be catalyzed either by acids or bases. Nevertheless, both methods anguish from the conditions that have harsh reactions. In addition, Lachos-Perez et al., (2018) also mentioned that flavanones from citrus fruits could be obtained by various extraction methods such as pressurised liquid extraction, ultrasound-assisted extraction, Soxhlet extraction, and microwave-assisted extraction. All the stated extraction methods involve organic solvents which can be volatile, expensive, flammable and, much worse, toxic (Cheng et al., 2021). As a result, a new clean and economical method has been developed for flavanone synthesis, which is the use of sub-critical water in the synthesis of flavanone. According to Zhao et al. (2020), subcritical water is a liquid with a temperature value between 100 to 374.2ºC. In a sub-critical region, the dielectric constant that is associated with the water polarity is reduced because of the breakdown of bonds between hydrogen and water molecules, which will then allow the extraction of either medium or compound that is non-polar such as flavonoids. Compared to regular water, sub-critical water can dissolve many organic compounds and catalyse reactions (Zhao et al., 2020). Furthermore, Gbashi et al. (2017) have mentioned that using the method

of sub-critical water is non-toxic, non-flammable and renewable as the extractant is water. Other than that, using sub-critical water is less time-consuming and much easier to perform as it has few steps to extraction compared to the traditional method. The carbonylation annulation reaction is the main highlight for the synthesis of flavanone in sub-critical water. As stated by Sirin et al. (2013), the process includes the reaction between benzaldehyde and 2-hydroxyacetophenone. The reaction will be carried out in one pot with subcritical water, serving as solvent and catalyst. The reagents will then be mixed in a stainless-steel reactor, conducted under N2 gas, followed by GC-MS analysis to display the flavanone synthesis in sub-critical water between benzaldehyde and 2-hydoxyacetophenone as demonstrated in Figure 2.5, where chalcone is used as an intermediary in the synthesis of flavanone.

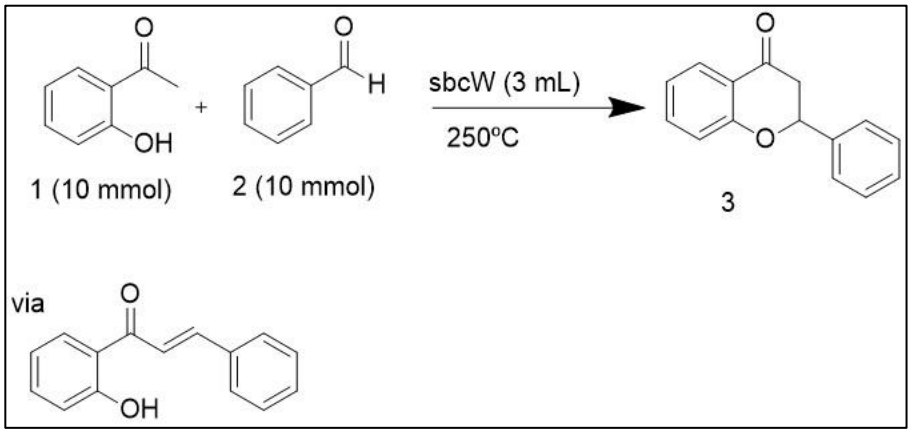
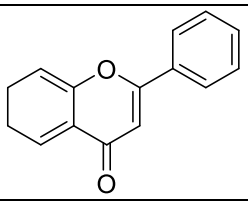
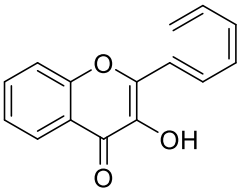
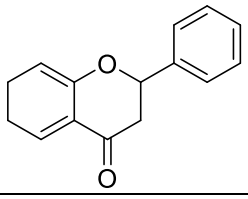
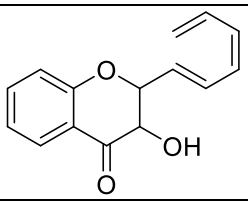


Figure 2.5 The carbonylation annulation reaction in sub-critical water
Source: (Sirin et al., 2013)

2.3 Flavanones Biological Activity

Flavonoids are a group of polyphenolic compounds that have health-related properties that can be broadly found in plants such as fruits, vegetables, cocoas, wines and teas (Bilbao et al. 2007). According to (Murti & Mishra, 2014), over 5000 naturally occurring flavonoids has been characterized by the diverse type of plants. Furthermore, they also stated that flavonoids have been sorted according to their chemical structure and are divided into subgroups, as shown in Table 2.1. As flavonoids possess interesting biological activity such as antimycobacterial, anti-lung cancer, antimicrobial, antibacterial, antifungal, anti-tuberculosis, antiproliferative, antiviral, antiarrhythmic, anti-inflammatory, antihypertensive and antioxidant, they have attracted attention among the researchers.

Table 2.1 Classification of flavonoids

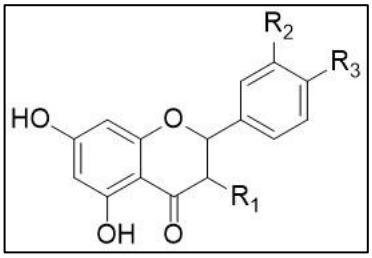
Group	Skeleton				Examples
	Description	Functional groups		Structural formula	
		3-Hydroxyl	2,3 - Dihydro		
Flavone	2-phenylchromen-4-one	**x**	**x**		Luteolin, Apigenin, Tangeritin,
Flavonol	3-hydroxy-2-phenylchromen-4-one	**/**	**x**		Quercetin, Kaempferol, Myricetin, Fisetin, Isohamnetin, Pachypodol, Rhamnazin
Flavanone	2,3-duhydro-2-phenylchromen-4-one	**x**	**/**		Hesperitin, Naringenin, Eriodictyol, Homoeriodictyol,
Flavanonol	3-hydroxy-2,3-diydro-2-phenylchromen-4-one	**/**	**/**		Taxifolin (Dihydroquercetin), Dihydrokaempferol

Source: (Murti & Mishra, 2014)

According to Barreca et al. (2017), flavanones are one of the main classes of flavonoids, and their structure is based on the generic flavonoids' structure. They also stated that there is a lack on a C2-C3 double bond, the presence of an atom for chiral carbon at the C2 position also the absence of substitution at the C3 position of the C ring characterized the flavanones' differences among the other classes of flavonoids that present in citrus, flavones as well as flavanols. As the chemical structure of flavanones has a chiral centre at C-2, the naturally occurring members are frequently optically active (Brahmachari, 2008). Besides that, Brahmachari (2008) also mentioned that flavanones are one of the most interesting flavonoid subclasses that naturally occur due to their pattern structure and potential in terms of biological and pharmacological. A study by Najmanova et al. ((2020) mentioned that flavanones are one of the significant parts of human diets, and most of them are closely related to citrus fruits such as limes, lemons, grapefruits and oranges. Some examples of flavanones in citrus fruits are hesperetin, naringenin and eriodictyol (Table 2.2), which are associated with biological activities such as anti-inflammatory activity, cardiovascular health and antioxidant (Szalay, 2015). Other than that, Kumar & Pandey (2013) has mentioned in their study that flavonoid also has the characteristics of scavenging free radicals. Free radicals are unstable molecules that form during a normal cell metabolism where chemical changes occur in a cell. Free radicals may build up in cells,

and they can cause damage to other cells, which can cause ageing and becoming a host of diseases (Villiness, 2017). Therefore, the antioxidant in flavonoids will act as a free radical scavenger and helps to enhance the immune system's defence in the human body and lower the risk of degenerative disease and cancer (Huy et al., 2008). The phenolic group in the flavanone structure, such as polyphenol, will scavenge the free radicals by transferring the H-atom. Therefore, the notorious effects will be decreased due to the oxidative stress (Meo et al., 2013). Therefore, flavanone is significant for human beings.

Table 2.2 Flavanones aglycones



	Compound	R_1	R_2	R_3
1	Hesperetin	H	OH	OMe
2	Naringenin	H	H	OH
3	Taxifolin	OH	OH	OH
4	Isosakurannetin	H	H	OMe
5	Eriodictyol	H	OH	OH

Source: (Gattuso et al., 2007)

2.4 Flavanones Antibacterial Activity

According to (Cushnie & Lamb, 2005), the resistance of bacteria and viruses toward antimicrobial and antibacterial agents has become a global problem as it causes many concerns about human health. Therefore, flavonoids have been increasingly being paid attention to for their antibacterial activity as well as included in one of the largest classes for plant secondary metabolites that can be found in various plant types (Yuan G et al., 2021). Based on the study by Xie et al. (2015), one of the flavonoids subclasses, flavanone, has the bond C ring, which is the saturated C3 and C4 bond that makes the nuclear skeleton of the flavanone non-planar (Scheme 2.7). By that, the substituents play an essential role as they significantly influence the antibacterial activity of flavanones.

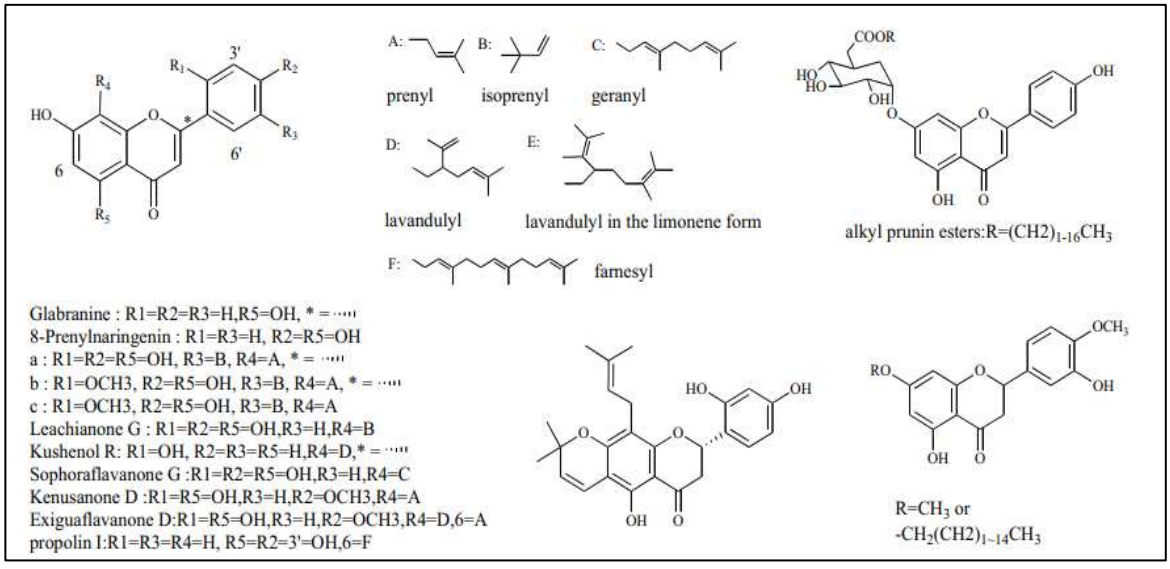


Figure 2.6 Structure of Antibacterial Flavanones
Source: (Xie et al., 2015)

Some examples of flavanones that have been reported as the activation agents for the antibacterial activity and effect (Table 2.3) of some the medicinal plants are liquiritigenin, nobiletin and tangeritin (Xie et al., 2015). As one of the subclasses of flavonoids in which flavonoids are also known as the natural phenolic compounds (Murtha et al., 2021), flavanone also contains phenolic compounds (Gorniak et al., 2013). The presence of the phenolic compounds displays significant antibacterial activity other than establishing the antioxidant activity (Bouarab-Chibane et al., 2019). In addition, Miklasinska-Majdanik et al., (2018) have mentioned in their study that the presence of the phenolic hydroxyl groups with high protein binding affinity might constrain any microbial enzymes and, at the same time, increase the affinity towards the cytoplasmic membrane which in the end enhancing the antibacterial activity

Table 2.3 Antibacterial Effect of Flavanone Compounds

Compounds	Source	Bacteria	Method	Activity	Ref.
7-Dihydroxy-2′-methoxy-3′,4′-methylenedioxyisoflavanone (76)	*Uraria picta*	*Staphylococcus aureus*	Microdilution titer	MIC: 12.5 μg/ml	(Rahman, Gibbons, & Gray, 2007)
Naringenin (77)	Pure	*Escherichia coli* *Bacillus subtilis*		Generation time: 25–39 (Activity: inhibition of nucleic acid synthesis)	(Ulanowska, Majchrzyk, Moskot, Jakóbkiewicz-Banecka, & Węgrzyn, 2007)
5,7-Dibenzyloxyflavanone (78)	*Helichrysum gymnocomum*	*Staphylococcus aureus*	Quick microplate method	MIC ≤125 μg/ml	(Drewes & van Vuuren, 2008)
3′-O-methyl-5′-hydroxydiplacone (79) 3′-O-methyl-5′-O-methyldiplacone (80) Mimulone (81), Diplacone (82)	*Paulowniatomentosa*	*Enterococcus faecalis* *Bacillus subtilis*	Broth microdilution method	MIC: 2 μg/ml	(Šmejkal et al., 2008)
Sophoraflavanone G (83)	*Sophora flavescens*	*Staphylococcus aureus*	Broth dilution method	MIC/MBC: 0.5/1 μg/ml	(Cha et al., 2009)
5,7-Dimethoxyflavanone-4′-O-b-D-glucopyranoside (84) 5,7,3′-Trihydroxy-flavano-ne-40-O-b-D-glucopyranoside (85) Naringenin-7-O-b-D-glucopyranoside (86)	*Retama raetam*	*Escherichia coli*	Microdilution broth methods	MIC: 7.5 μg/ml	(Orhan, Özçelik, Özgen, & Ergun, 2010)
Sophoraflavanone G (83) Kurarinol (89)	*Sophora flavescens*	*Staphylococcus aureus*	Microtiter dilution assay	MIC: 7.12–7.36 μg/ml IC_{50}: 107.7 ± 6.6 μM	(Oh et al., 2011)
Pinocembrin (87)	*Cryptocarya chinensis*	*Mycobacterium tuberculosis*		MIC: 3.5 μg/ml	(Chou, Chen, Peng, Cheng, & Chen, 2011)
Abyssione-V 4′-O-methyl ether (88)	*Erythrina caffra*	*Escherichia coli* *Staphylococcus aureus*	Microbroth dilution assay	MIC: 3.9–62 μg/ml	(Chukwujekwu et al., 2011)
7-Hydroxyflavanone (91)	*Zuccagnia punctata*	*Streptococcus pneumoniae*	Agar macrodilution method	MIC: 1,000 μg/ml	(Zampini et al., 2012)
5,7-Dihydroxyflavanone (Pinocembrin) (87)	*Combretum hereroense*	*Staphylococcus aureus*	Microtiter dilution assay	MIC: 12.5 μg/ml	(Katerere et al., 2012)
Lupinifolin (90)	*Mundulea sericea*	*Staphylococcus aureus*		MIQ: 0.5 μg	(Mazimba et al., 2012)
Ochnaflavone (92) Ochnaflavone 7-O-methyl ether (93)	*Ochna pretoriensis*	*P. aeruginosa* *S. aureus*		MIC 31.3, 62.5 μg/ml	(Makhafola, Samuel, Elgorashi, & Eloff, 2012)
Sophoraflavanone B (94)	Pure	*Staphylococcus aureus*	Broth microdilution method	MIC: 31.5 μm	(Mun et al., 2013)
6-8 Diprenyleriodictyc (95)	Pure	*Staphylococcus aureus*	Microbroth dilution method	MIC: 0.5 μg/ml Activity: depolarization of membrane	(Dzoyem et al., 2013)
Sophoraflavanone B (94)	*Desmodium caudatum*	*Staphylococcus aureus*	Checkerboard dilution test	MIC: 15.6 μg/ml	(Mun et al., 2014)
4′,7-Di-O-methylnaringenin (96)	*Macaranga trichocarpa*	*Escherichia coli* *Shigella dysenteriae*	Broth microdilution	MIC: 62.4–124.9 μg/ml	(Fareza, Syah, Mujahidin, Juliawaty, & Kurniasih, 2014)
Sophoraflavone G (83)	*Sophora alopecuroides*	*Staphylococcus epidermidis*	Microdilution method	MIC: 3.1 to 12.5 μg/ml	(Wan et al., 2015)
Liquiritigenin (97) Liquiritin (98)	Pure	*Escherichia coli*		IC_{50}: 198.6, 337.8 μg/ml	(Kong et al., 2015)
Mimulone (81)	*Paulownia tomentosa*	*Staphylococcus aureus*	Agar dilution method	MIC: 2/4.9 μg/ml/μM	(Navrátilová et al., 2016)
Pinocembrin (87) 7-O-Methyleriodictyol (99)	Pure	*Proteus mirabilis* *Staphylococcus aureus*	Microdilution method	MIC: 0.25–0.5 μg/ml	(Echeverría et al., 2017)

Source: (Farhadi et al., 2018)

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Chemicals

Below are the lists of chemicals used:

3-nitrobenzaldehyde

2-hydroxy-4, 6-dimethyoxyacetophenone

Glacial / Acetic acid

20% of potassium hydroxide, KOH

10% of hydrochloric acid, HCl

Dimethyl sulfoxide, DMSO

Magnesium Sulphate, $MgSO_4$

Sodium Chloride, NaCl

Methane Sulphonic acid, CH_4O_3S

Hexane

Ethyl acetate, EtOAc

Iodide solution, I^-

95% of ethanol, EtOH

3.1.2 Apparatus

Below are the lists of apparatus used:

Round bottom flask

Stopper

Magnetic bar

Hotplate and stirrer

Syringe 3 mL and 5 mL or micropipette

Test tubes

Beakers

3.1.3 Indicator

Below are the lists of indicators used:

Potassium manganate, $KMnO_4$ (powder)

Potassium carbonate, K_2CO_3

10% of sodium hydroxide, NaOH

Distilled water

3.2 Methods

3.2.1 Synthesis of 3'-nitro-2-hydroxy-4,6-dimethoxychalcone

For the synthesis of chalcone (Figure 3.1) 4.0 mL of KOH (20%) was added into a solution of 2-hydroxy-4,6-dimethoxyacetophenone (0.50 g, 2.57 mmol) in ethanol (25 mL) followed by addition of 4-bromobenzaldehyde (0.39 g, 2.57 mmol). The reaction mixture was stirred occasionally for 24 h at room temperature until completion of the reaction. Completion of the reaction was monitored by TLC. The reaction mixture was acidified with aqueous 10% HCl solution and then poured into crushed ice. The precipitate was filtered, washed with an excess of distilling water. The products were purified by recrystallization to give 3'-nitro-2-hydroxy-4,6-dimethoxy chalcone **(NC1)** as yellow crystals (0.71 g, 84.4%). All of the structures can be confirmed by using mass spectrometry and NMR spectra.

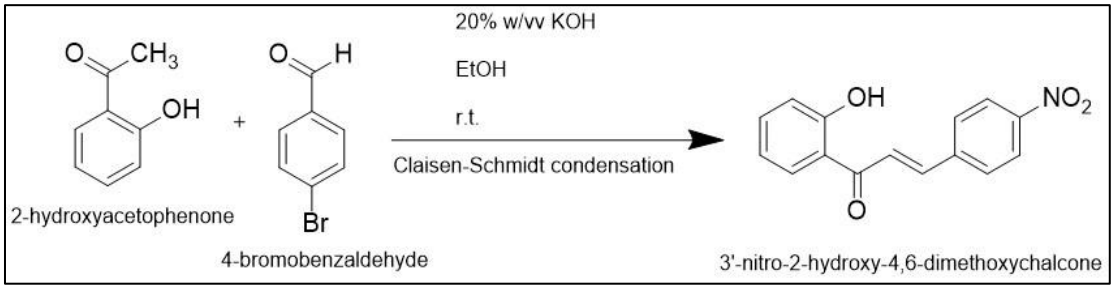


Figure 3.1 Synthesis of 3'-nitro-2-hydroxy-4,6-dimethoxychalcone

The analytical data: IR ν_{max} (ATR) cm^{-1}: 1634 (C=O), 1524 and 1320 (N-O), 1567 and 1435 (C=C aromatic), 1341 (C-O); ^1H NMR (400 MHz; $CDCl_3$): δ_H 3.84 (3H, *s*, $-OCH_3$), 3.93 (3H, *s*, $-OCH_3$), 5.97 (1H, *d*, *J* = 2.0 Hz, H-3), 6.11 (1H, *d*, *J* = 2.4 Hz,

H-5), 7.58 (1H, *t*, *J* = 7.6 Hz, H-5'), 7.73 (1H, *d, J* = 16.0 Hz, H-α), 7.85 (1H, *d*, *J*= 7.6 Hz, H-6'), 7.97 (1H, *d*, *J* = 15.6 Hz, H-4'), 8.20 (1H, *dd*, *J* = 7.2 and 8.0 Hz, H-β), 8.44 (1H, *d*, *J* = 2.0 Hz, H-2'); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 55.8 (-OCH_3, CH_3), 56.1 (-OCH_3, CH_3), 91.5 (C-5, C-H), 93.9 (C-3, C-H), 106.3 (C-1, C-4°), 122.3 (C-2', C-H), 124.2 (C-4', C-H), 129.9 (C-α, C-H), 130.6 (C-5', C-H), 134.3 (C-6', C-H), 137.5 (C-1', C-4°), 138.9 (C-β, C-H), 148.8 (C-3', C-4°), 162.6 (C-2, C-4°), 166.8 (C-6, C-4°), 168.6 (C-4, C-4°) and 191.4 (C=O, C-4°); M/S: M^+ 329.2508, $C_{17}H_{15}NO_6$, *m/z* 207.1, 181.2, 328.2, 208.3.

3.2.2 Synthesis of 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one

The process of synthesis of chalcone as shown in Figure 3.2, approximately 1.0 g of 4-butylbenzaldehyde will be mixed together with 2-hydroxyacetophenone. Afterwards, Claisen Schmidt condensation reaction will be conducted where the solution of the acetophenone and benzaldehyde will mix together with NaOH and the reaction will be stirred in a room temperature condition until the whole reaction are complete. Right after the reaction has been completed, the mixture will be placed into ice water in a beaker while a solution of HCl which is 10% will be utilised to neutralize the mixture. Afterwards, the mixture will be filtered to separate the precipitate and the chalcones obtained must be dried and be recrystallize using ethanol to get the compound of pure chalcones.

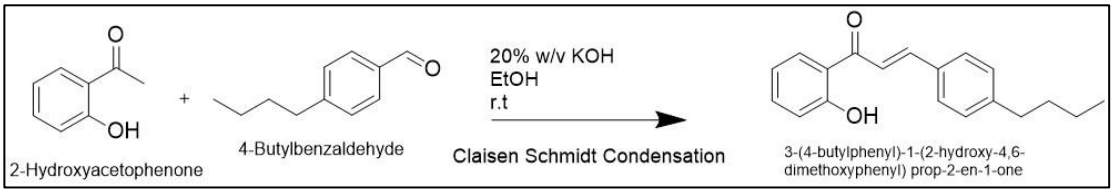


Figure 3.2 Synthesis 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one

The analytical data: IR v_{max} cm^{-1}: 2928 (OH), 1690 (C=O), 1637 and 1564 (C=C), 1463 (C-H). ^{1}H NMR (400 HMz ;$CDCl_3$): δ 12.29 (1H, s, OH), 0.91 (3H, s, H-10'), 1.35 (2H, s, H-9'), 1.60 (2H, s, H-8'), 2.64 (2H, s, H-7'), 6.97 (1H, s, H-3), 7.05 (1H, s, H-4, H-5) and 7.54 (1H, s, H-6'); ^{13}C NMR (400MHz: $CDCl_3$): δ 118.1 (C-3, C-H), 118.8 (C-1, C-H), 128.6 (C-5,C-H), 129.1 (C-3',C-5',C-H), 130.4 (C-6, C-H), 132.4 (C-1', C-H), 135.9 22 (C-2', C-6', C-H), 146.5 (C-α, C-H), 163.4 (C-2, C-H), 205.3 (C=O); M/S M^+ 280.37, $C_{19}H_{20}O_2$, m/z 280.15, 281.15, 282.15.

3.2.3 Synthesis of 3'-nitro-5,7-dimethoxyflavanone

For the process of synthesis of flavanone (Figure 3.3), the chalcone (0.70 g) was dissolved in glacial AcOH (10 mL). The solution was heated to reflux for 2–3 hours and using methane

sulphonic acid as a catalyst. EtoAc layer was ashed with brine and the organic layer $MgSO_4$. The product was purified by column chromatography performed on silica gel (230 – 400 mesh) using ethyl acetate and hexane (1:4) mixture as the mobile phase to give 3'-nitro-5,7-dimethoxyflavanone as white needle crystals.

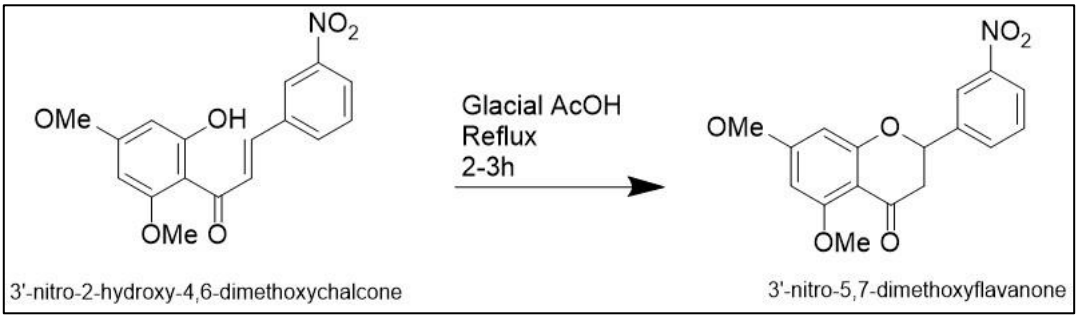


Figure 3.3 Synthesis of 3'-nitro-5,7-dimethoxyflavanone

The analytical data: IR ν_{max} (KBR) cm^{-1}: 3063 (N-H), 2930 and 2857 (C-H), 1663 (C=O), 1537 and 1357 (N-O), 1566 and 1464 (C=C aromatic), 1218 (C-O); ^1H NMR (400 MHz; $CDCl_3$): δ_H 2.85 (1H, *dd*, *J* = 16.8 and 3.6 Hz, H-3a), 2.96 (1H, *dd*, *J* = 16.4 and 3.6 Hz, H-3b), 3.84 (3H, *s*, -OCH_3), 3.89 (3H, *s*, -OCH_3), 5.51 (1H, *dd*, *J* = 12.8 and 3.6 Hz, H-2), 6.11 (1H, *d*, *J* = 2.0 Hz, H-8), 6.18 (1H, *d*, *J* = 2.4 Hz, H-6), 7.59 (1H, *t*, *J* = 8.0 Hz, H-5'), 7.75 (1H, *d*, *J* = 7.6 Hz, H-6'), 8.22 (1H, *ddd*, *J* =

8.4 and 1.2 Hz, H- 4') 8.38 (1H, *t*, *J* = 2.0 Hz, H-2'); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 45.5 (C-3, CH_2), 55.8 (C-7, -OCH_3), 56.3 (C-5, -OCH_3), 77.8 (C-2, C-H), 93.6 (C-6, C-H), 93.7 (C-8, C-H), 105.9 (C-4a, C-4°), 121.2 (C-4', C-H), 123.6 (C-2', C-H), 129.9 (C-5', C-H), 131.9 (C-6', C-H), 141.1 (C-1', C-4°), 148.6 (C-3', C-4°), 162.4 (C-5, C-4°) 164.4 (C-7, C-4°), 166.3 (C-8a, C-4°), and 188.0 (C=O, C-4°); M/S: M^+ 329.1848, $C_{17}H_{15}O_6N$ *m/z* 207.2, 181.1, 208.2, 102.1.

3.2.4 Synthesis of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one

In the process of synthesis for flavanone glacial acetic acid with a volume of 10 mL along with the 1.0 g of chalcone will be mixed together and heated to reflux for up to 2 – 3 hours. The layer of $MgSO_4$ and EtOAc will be washed by using brine with the target of drying the organic layer and the mixture of the solvent will be left to evaporate for them to dry up.

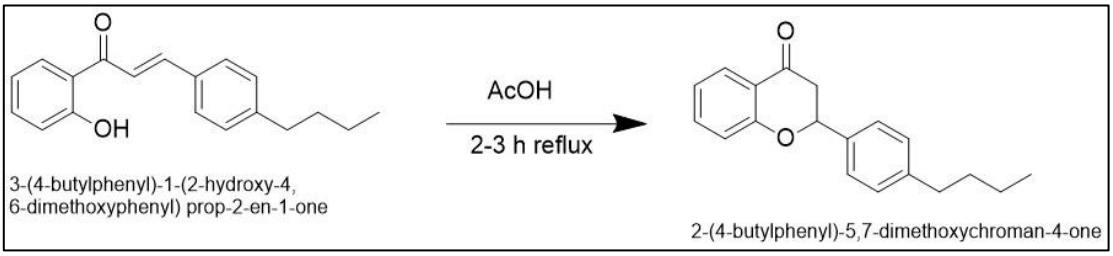


Figure 3.4 Synthesis of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one

The analytical data: IR_{vmax} cm^{-1} : 1638 (C=O), 1576 (C=C), 2928 (C-H), 1225 (C-O-C); ^{1}H NMR (400 MHz: $CDCl_3$): δ 5.48 (1H, d, H-β), 0.96 (3H, s, H-10'), 1.38 (2H, m, H-9'), 1.56 (2H, m, H-8'), 2.66 (2H, m, H-7'), 3.12 (1H, m, H-α), 7.08 (2H, m, H-3', H-5'), and 7.53 (1H, m, H-3). ^{13}C NMR (400MHz: CDCl3); δ 118.2 (C-2, C-H), 121.6 (C-4, C-H)), 127.8 (C-5, C-H), 128.9 (C-3', C-5', C-H), 135.9 (C-1', C), 143.8 (C-4', C-H), 161.7 (C-1, C), 192.3 (C=O); M/S M^+ 280.37, $C_{19}H_{20}O_2$, m/z 280.15, 281.15, 282.15.

3.2.3 Antibacterial Activity

To determine the antibacterial activity of flavanone, the method of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) will be used. Minimum Inhibitory Concentration (MIC) Minimum inhibitory concentrations (MIC) can be described as the lowest concentration of an antimicrobial that will restrain the growth of a visible microorganism after the subculture towards the antibiotic-free media. Furthermore, minimum inhibitory concentrations are usually used to classify antimicrobial efficacy for

different types of compounds by measuring the consequence of decreasing the concentration of either antibiotic over a controlled period. In addition, the minimum inhibitory concentration (MIC) guides the clinician to the vulnerability of the organism agent of antimicrobial, and it helps in assisting any treatment decisions (Microbiology, 2003). Besides that, the bacteria's visible growth inhibition after overnight incubation will also be determined by microdilution, which is the 96-well microplates against the bacteria such as *Clostridium difficile, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*. The first step for the process of minimum inhibitory concentration (MIC) is the nutrition broth (NB), pipette volume, micropipette, test tubes, beakers, and all needed apparatus must be in the autoclaved condition. Afterward, the crude extract was weighed and dissolved in 2 mL of DMSO (stock solution, 1800 µg / mL), which weighed approximately 3.6 mg. Next, the 24 hours broth cultures with the suspension need to be adjusted to 0.5 Mc Farland standard of turbidity, and it will be used to produce inocula of the strains of microbial. The sterile nutrition broth (NB) will be added to wells in rows B to H, weighing approximately 100 µg / mL. After that, add 100 µg / mL of the stock solution to rows A and B. The sample that is located at row B, along with the mixture of the nutrition broth (NB), will be transferred to the wells, which makes the two serial dilution of stock samples (1800, 900, 450, 225, 112.5,

56.25, 28.13 and 14.07 100 µg / mL) to be obtained. Microbial media preparation Nutrient broth (NB) The preparation of agar is done by dissolving the nutrient broth (NB) powder (8 g/L) in distilled water. Afterward, the mixture is mixed thoroughly, then autoclaved for 15 minutes at the temperature of 121 ºC. When the nutrient broth (NB) solution has reached the desired temperature, the media is poured into the bijou bottles at the height of 3/4. Bacterial strain Five bacteria, including *Clostridium difficile, Escherichia Coli, Staphylococcus aureus, and Streptococcus pyogenes* will be tested along with the sample. A loop that is sterilized will be used to strain the stock solution of bacteria to each of the bijou bottles that contain nutrient broth (NB), and it will be sealed with aseptically parafilm. Next, the bottle's lid that has been sealed altogether with the parafilm will undergo the process of incubation at the temperature of 37 ºC for 16 – 17 hours. In the meantime, the turbidity is measured and standardized to McFarland 0.5 standard. Nutrient agar (NA) For agar preparation, 20 g/L of nutrient agar (NA) powder is dissolved in distilled water and then mixed. Afterward, the mixture will be autoclaved for 15 minutes at 121 ºC. The media is poured aseptically into plates when the solution is heated sufficiently. McFarland Standard Solution Upon dissolving 0.51 mL of H_2SO_4 in 50 mL of nutrient broth (NB), approximately 1% of sulfuric acid, H_2SO_4, and barium chloride, $BaCl_2$ will be obtained. Furthermore, 0.5 g of $BaCl_2$

will be dissolved in 50 mL of nutrient broth (NB). In the test tube, 9.95 mL of H_2SO_4 and 0.05 mL of $BaCl_2$ will be mixed. These mixtures equal 150 x 105 colonies per unit of 5% McFarland Solution. Minimum Bactericidal Concentration (MBC) The minimum bactericidal concentration (MBC) is the method that uses the lowest concentration of an antibacterial agent to kill up to 99 percent of bacteria. In addition, minimum bactericidal concentration (MBC) is performed to identify the amount of organism that have survived by monitoring the growth activity of the bacteria, in which the evaluation is continued from the minimum inhibitory concentrations (MIC) results. The solution that is originally from the previous minimum inhibitory concentrations (MIC) test in the 96-well plate will be extracted and distributed over the plate containing alga by using a cotton swab that has been sterile. The plate will then be sealed and incubated at 37°C for 24 hours. When the alga placed on the plate is still clear during the observation, the bacteria have not grown. The MBC results would be identical to MIC, but the final result will soon reveal that the concentration in MBC is one level greater than MIC when the alga's condition is either cloudy or unclear. Microbial media preparation Nutrient agar (NA) Nutrient agar (NA) powder (20 g/L) will be dissolved in distilled water before it is autoclaved at the temperature of 121 ℃ for 15 minutes.

Afterward, the media is aseptically poured onto the Petri dish when the nutrient agar (NA) solution has reached the targeted temperature.

3.2.4 Spectroscopic Method

3.2.4.1 Gas Chromatography-Mass Spectrometry (GC-MS)

The molecular weight of compounds will be measured on a Perkin Elmer Clarus 680 spectrometer. Samples will be prepared in a 1.5 mL vials.

3.2.4.2 Infrared Spectra (IR)

The Fourier Transformed Infrared (FTIR) absorption spectra in the 4000 to 400 cm^{-1} will be recorded by the Perkin Elmer Frontier model and Varian 3100 Excalibur Series. The sample was added about 1 to 2% and grinded into a finer powder.

3.2.4.3 Nuclear Magnetic Resonance (NMR)

The NMR 1H spectra will be recorded by Bruker 400 spectrometer, which operates at approximately 400 MHz to measure ^{13}C NMR, the same instrument will be used, and it operates at approximately 100 MHz using tetramethyl silane (TMS) as an internal standard. For samples

weighing approximately 10 mg, deuterated solvents such as $CDCl_3$, DMSO-d and MeOH-d will be used. Broad signals (br s), singlets (s), doublets (d), triplets (t), doublets of doublets (dd) and multiplets (m) will be recorded as the multiplicity of the spectra. In addition, chemical shifts will be expressed in parts per million (ppm) relative to the solvent employed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Structure Elucidation of Chalcone and Flavanone

The removal of any undesirable impurities in the compound by using the method of column chromatography yield four pure compounds, 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one, 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one, 3'-nitro-2-hydroxy-4, 6 dimethoxy chalcone, 3'-nitro-5,7-dimethoxyflavanone. The compound of chalcone and flavanone obtained were characterized by using the instrument such as Gas Chromatography (GCMS), ^{13}C Nuclear Magnetic Resonance (^{13}C NMR), ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) and Fourier Transform Infrared Spectroscopy.

4.1.1 Characterization of 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one

The reaction 3-(4-butylphrenyl) benzaldehyde with 1-(2-hydroxy-4,6-dimethoxyphenylacetophenone successfully synthesized 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one. The structure of chalcone was confirmed by the spectral data (Figure 4.1) where the molecular ion peak is at m/z =280.1729 which as well indicating the formula of the compound which is $C_{19}H_{20}O_2$.

The APT ^{13}C NMR spectrum of chalcone (Figure 4.2) shows the presence of 21 signals attributed to 21 different carbons. The signal for alpha and beta carbon were detected at the value of 127.6 ppm and 145.1 ppm respectively. The spectrum also confirmed the presence of methoxy group and the signals for methyl carbon were observed at 55.8 ppm and 55.4 ppm. The spectrum of 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimetjoxyphenyl) prop-2-en-1-one also confirms the presence of three methyl butyl, nine quaternary carbons and nine methine carbons in this compound.

The ^{1}H NMR spectrum (Figure 4.3) displayed two peaks at 3.81 ppm and 3.90 ppm due to the presence of two methoxyl group. The other peak was at 0.89 ppm, 1.33 ppm, 1.56 ppm, 2.65 ppm, 6.30 ppm, 6.77

ppm, 7.6 ppm, 7.64 ppm, 8.06 ppm and peak at 16.47 which indicates the present of OH group.

On subjection to the IR spectroscopic analysis (Figure 4.4, absorption bands appeared at 1690.47 cm-^{1} due to the presence of the ketone carbonyl group (C=O), 1637.83 cm-1due to the aromatic C=C and at the position of 1463.37 cm^{-1} which is due to the C-H vibration of alkyl.

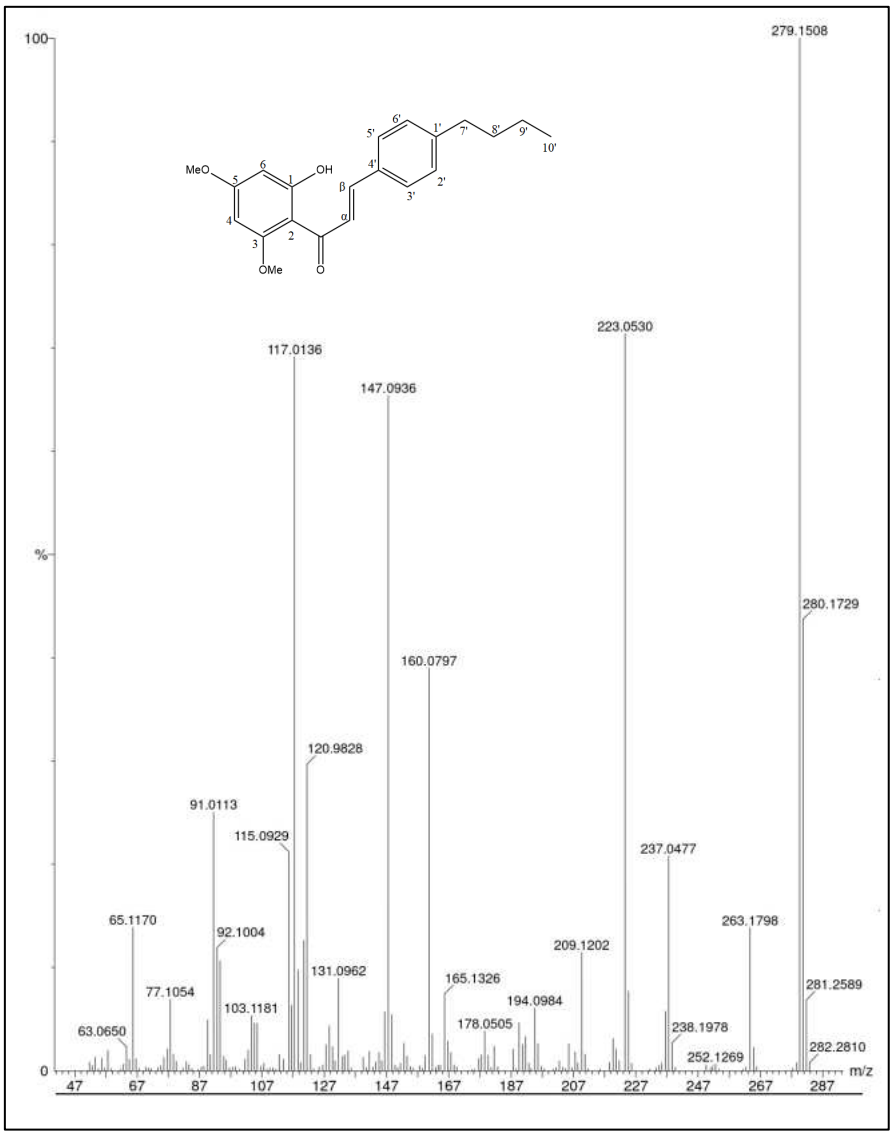


Figure 4.1 Mass Spectrum of 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one

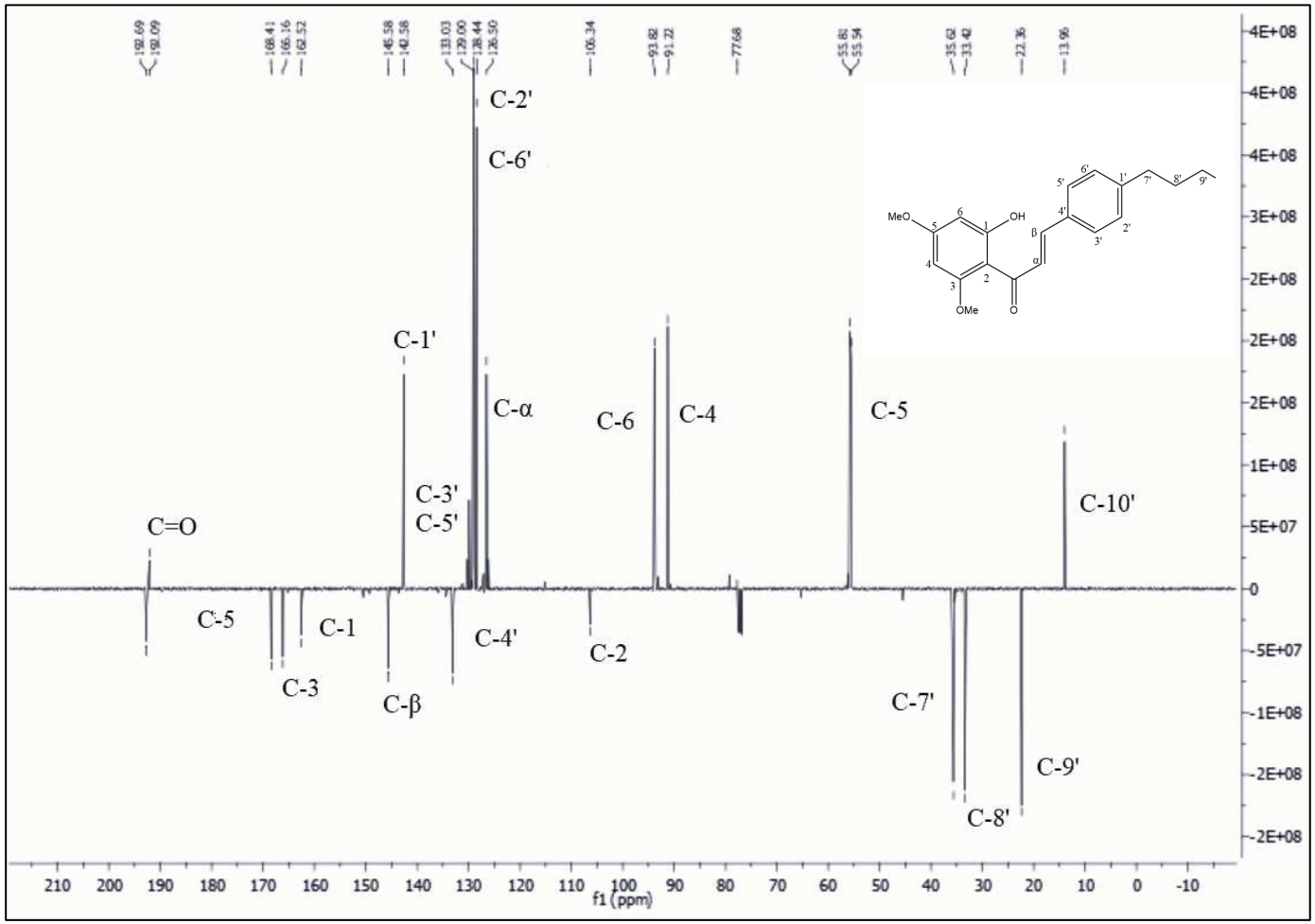


Figure 4.2 ^{13}C APT NMR of 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one

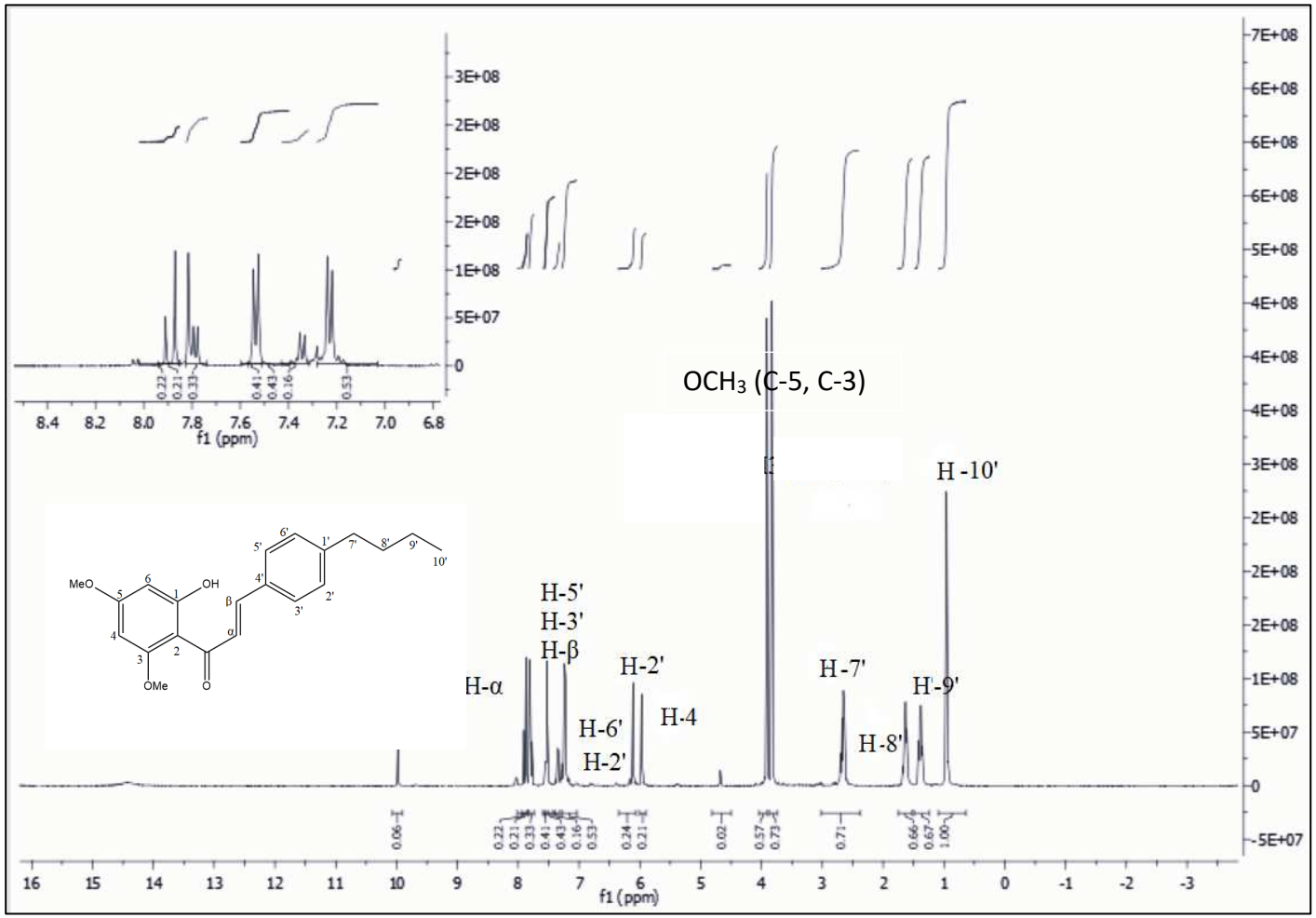


Figure 4.3 [1]H NMR of 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one

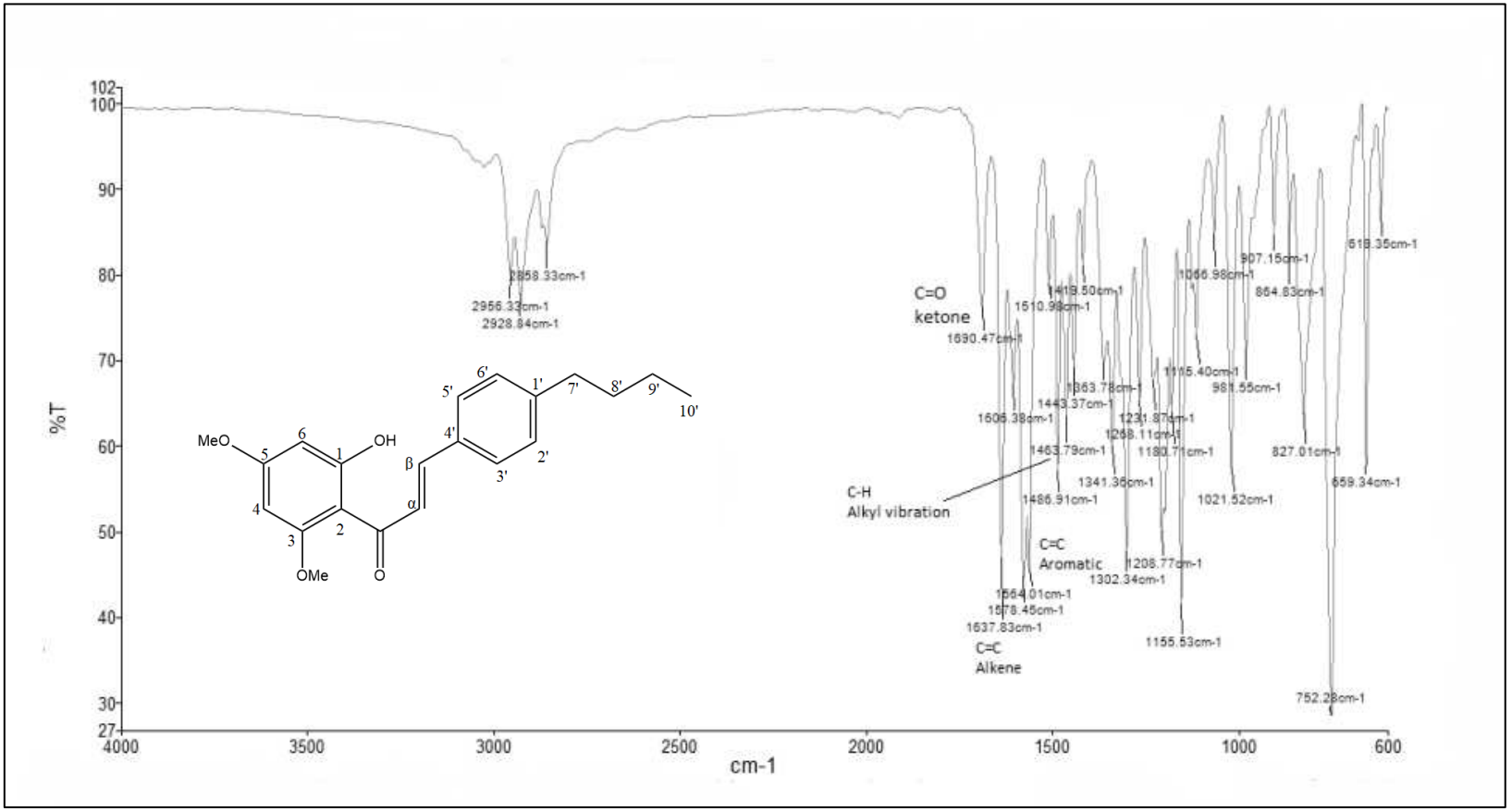


Figure 4.4 IR Spectrum of 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one

4.1.2 Characterization of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one

Compound of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one was successfully synthesized as bright yellow crystals. The (Figure 4.5) shows the mass spectrum of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one in which indicates that this compound has the formula of $C_{17}H_{15}O_4$. as the molecular ion peak is at m/z = 280.15.

Flavanone has the IR absorptions (Figure 4.6) characteristics of carbonyl group of ketones (1638 cm^{-1}), aromatic C=C (1567 cm^{-1}) and stretching of C-H alkyl (2928 cm^{-1}) functionalities as well as at 1225 cm^{-1} (C-O-C).

The ^{1}H NMR spectrum (Figure 4.7) of forming compound of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one shows two peaks at 3.81 ppm and 3.90 ppm due to the presence of two methoxyl group. The other peaks were at 0.89 ppm, 1.33 pm, 1.56 ppm, 2.65 ppm, 3.38 ppm, 3.13 ppm, 3.81 ppm, 3.9 ppm, 5.51 ppm, 6.31 ppm, 7.08 ppm and 7.28 ppm.

The The APT ^{13}C NMR spectrum of flavanone (Figure 4.8) showed the presence of 17 signals attributed to 17 different carbons. The signals for

methoxy carbons were observed at 55.8 ppm. The spectrum of 3'-nitro-2-hydroxy-4,6 dimethoxychalcone also confirmed the presence of three methyl carbons, seven quaternary carbons and seven methine carbons in this compound.

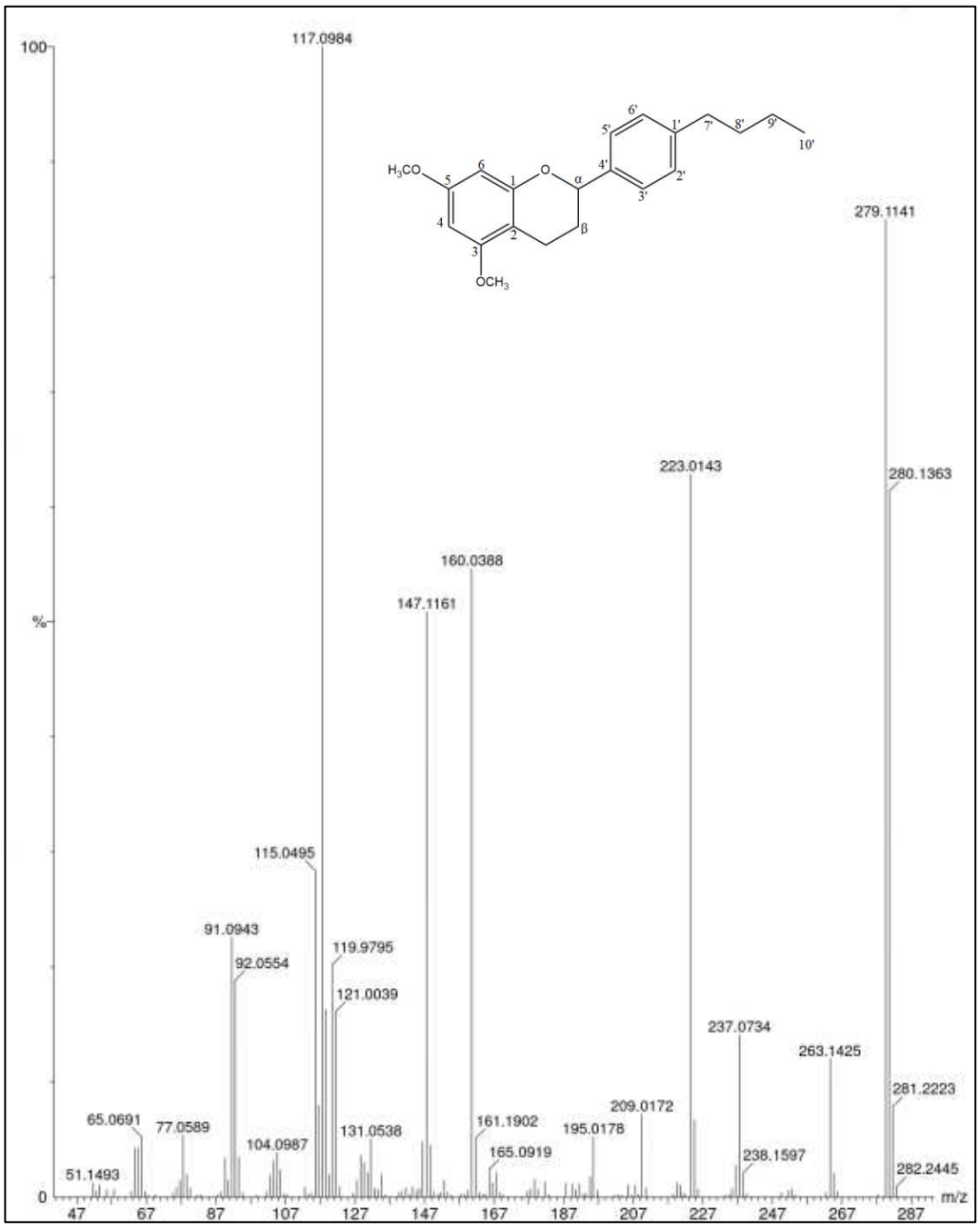


Figure 4.5 Mass Spectrum of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one

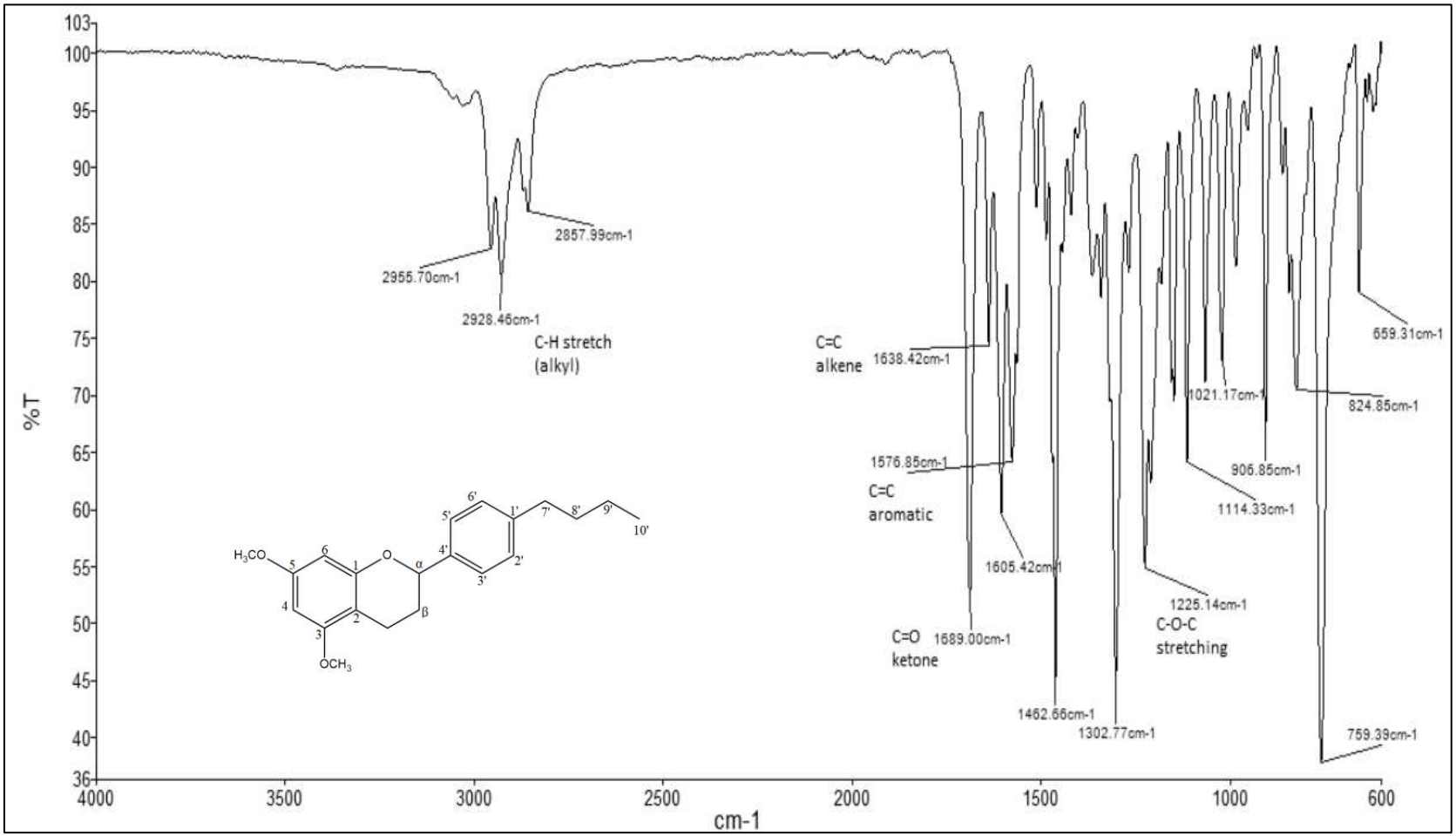


Figure 4.6 IR Spectrum for 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one

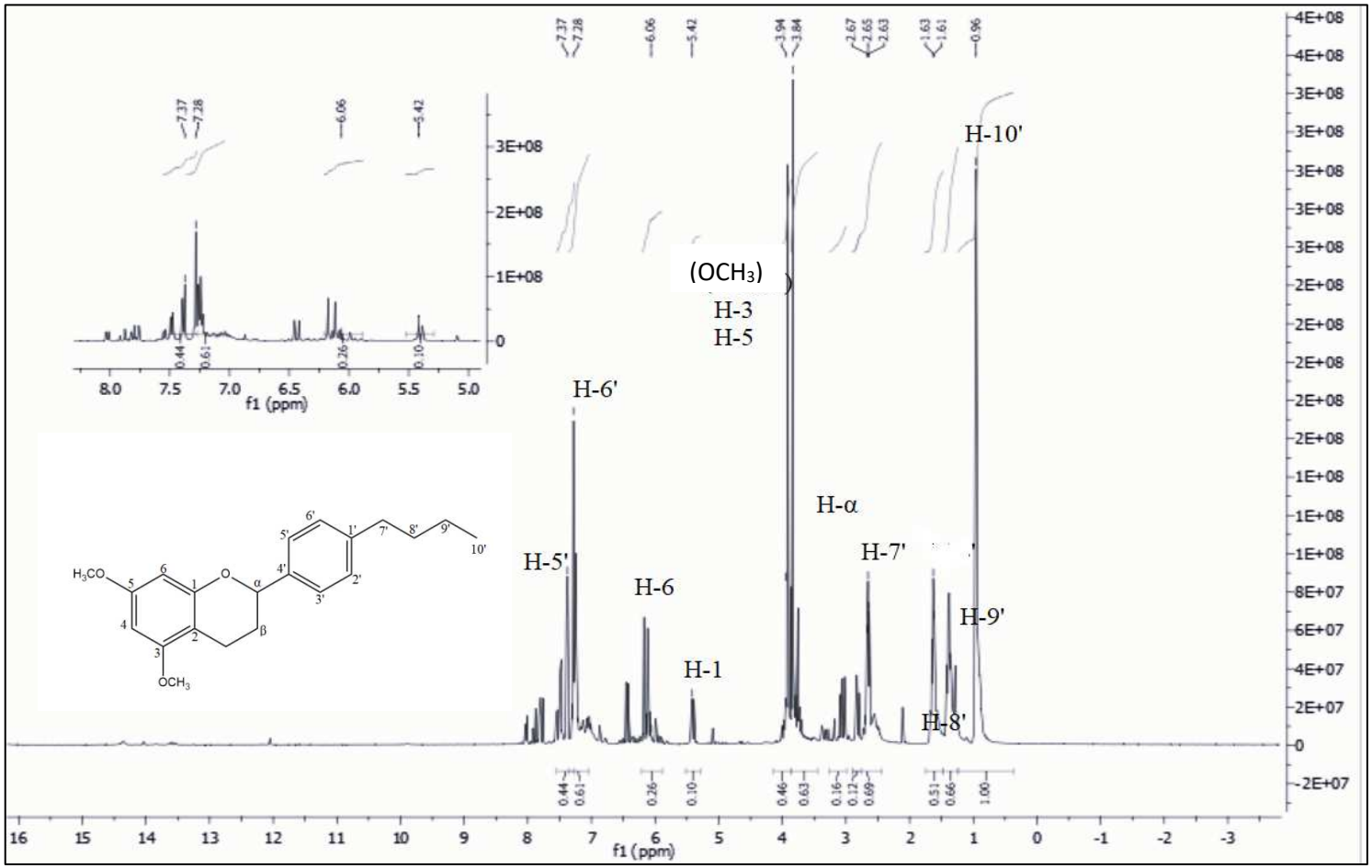


Figure 4.7 ^{1}H NMR of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one

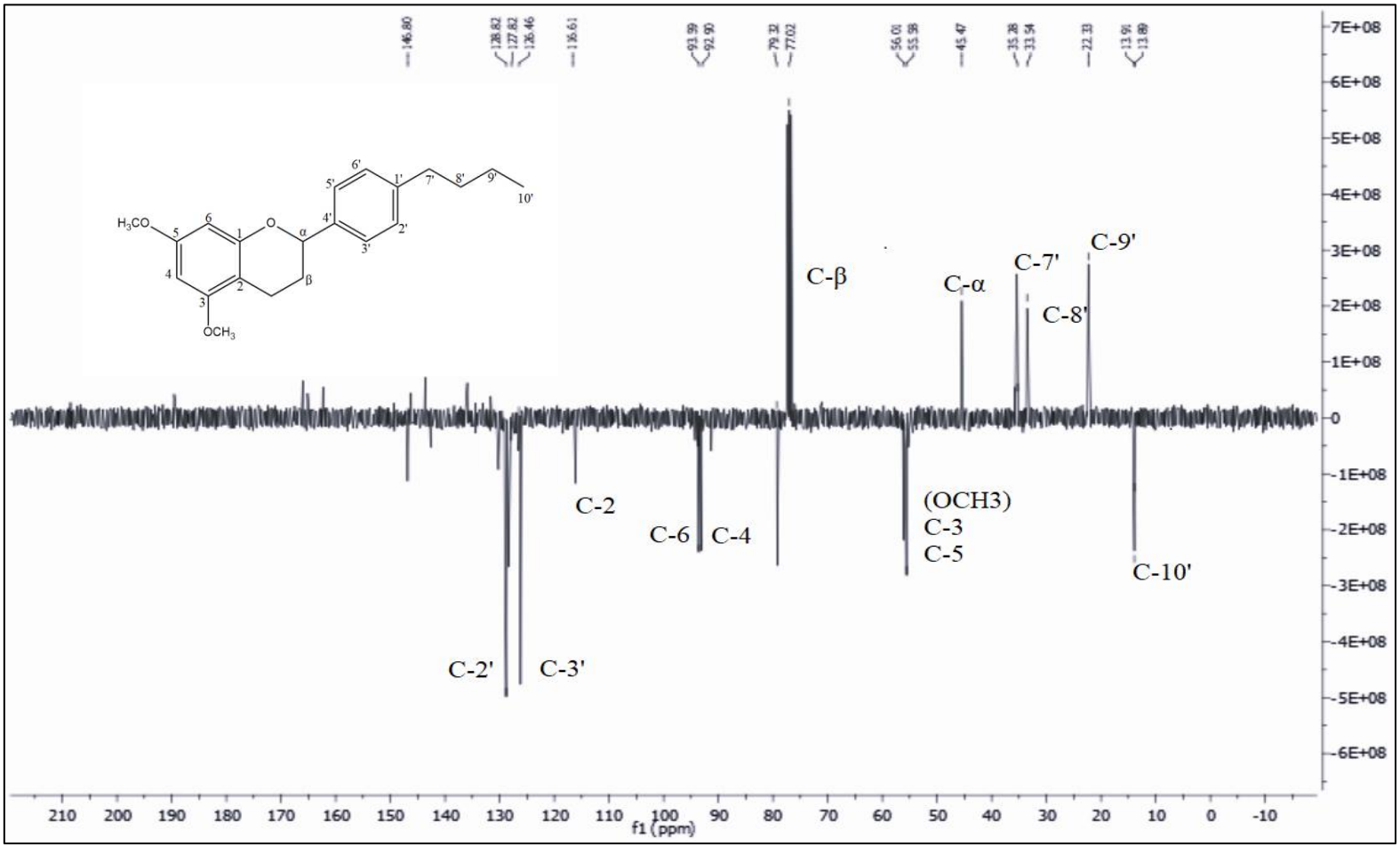


Figure 4. 8 ^{13}C APT NMR of 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one

4.1.3 Characterization of 3'-nitro-2-hydroxy-4, 6-dimethoxy chalcone

The reaction between 2-hydroxy-4,6-dimethoxychalcone with 3'-nitrobenzaldehyde in ethanol produces 3'-nitro-2-hydroxy-4,6-dimethoxychalcone in an excellent yield which is 84.4%. 3'-nitro-2-hydroxy-4,6-dimethoxychalcone was obtained in the crystal form with a yellow color. The MS spectrum (Figure 4.9) of 3'-nitro-2-hydroxy-4,6- dimethoxychalcone showed a molecular ion peak at *m/z* 329.2508 which has the molecular formula of $C_{17}H_{13}NO_6$. The structure of chalcone was confirmed by the spectral data.

The APT ^{13}C NMR spectrum of chalcone (Figure 4.10) showed the presence of 17 signals attributed to 17 different carbons. The signals for methyl carbons were observed at 55.8 ppm and 56.1 ppm. The spectrum of 3'nitro-2-hydroxy-4,6 dimethoxychalcone also confirmed the presence of two methyl carbons, seven quaternary carbons and eight methine carbons in this compound.

The ^{1}H NMR spectrum (Figure 4.11) of 3'nitro-2-hydroxy-4,6 dimethoxychalcone displayed two singlet due to signals for methoxyl groups at 3.804 ppm and 3.93 ppm integrated for three protons each. The meta coupled protons of the A-ring appeared at 5.97 ppm (1H, *d*, *J*

= 2.0 Hz, H-3) and 6.11 ppm (1H, *d, J* = 2.4 Hz, H-5). The four aromatic protons of the B-ring were observed at 7.58 ppm (1H, t, *J* = 7.6 Hz, H-5'), 7.85 ppm (1H, *d* = 7.6 Hz, H-6'), 7.97 (1H, *d, J* = 15.6 Hz, H-4') and 8.44 (1H, *d, J* = 2.0 Hz, H-2'). The signals for a chalcone moiety appeared as two doublets at 7.73 ppm (1H, *d, J* = 16.0 Hz, H-α) and 8.20 (1H, *d, J* = 7.2 and 8.0 Hz, H-β).

Chalcone has the IR absorptions (Figure 4.12) characteristics of carbonyl (1634 cm^{-1}), N-O stretching (1524 and 1320 cm^{-1}), aromatic C=C (1567 and 1435 cm^{-1}) and C-O (1341 cm^{-1}) functionalities. There was no IR absorption band for hydroxyl (-OH) group at C-2 position.

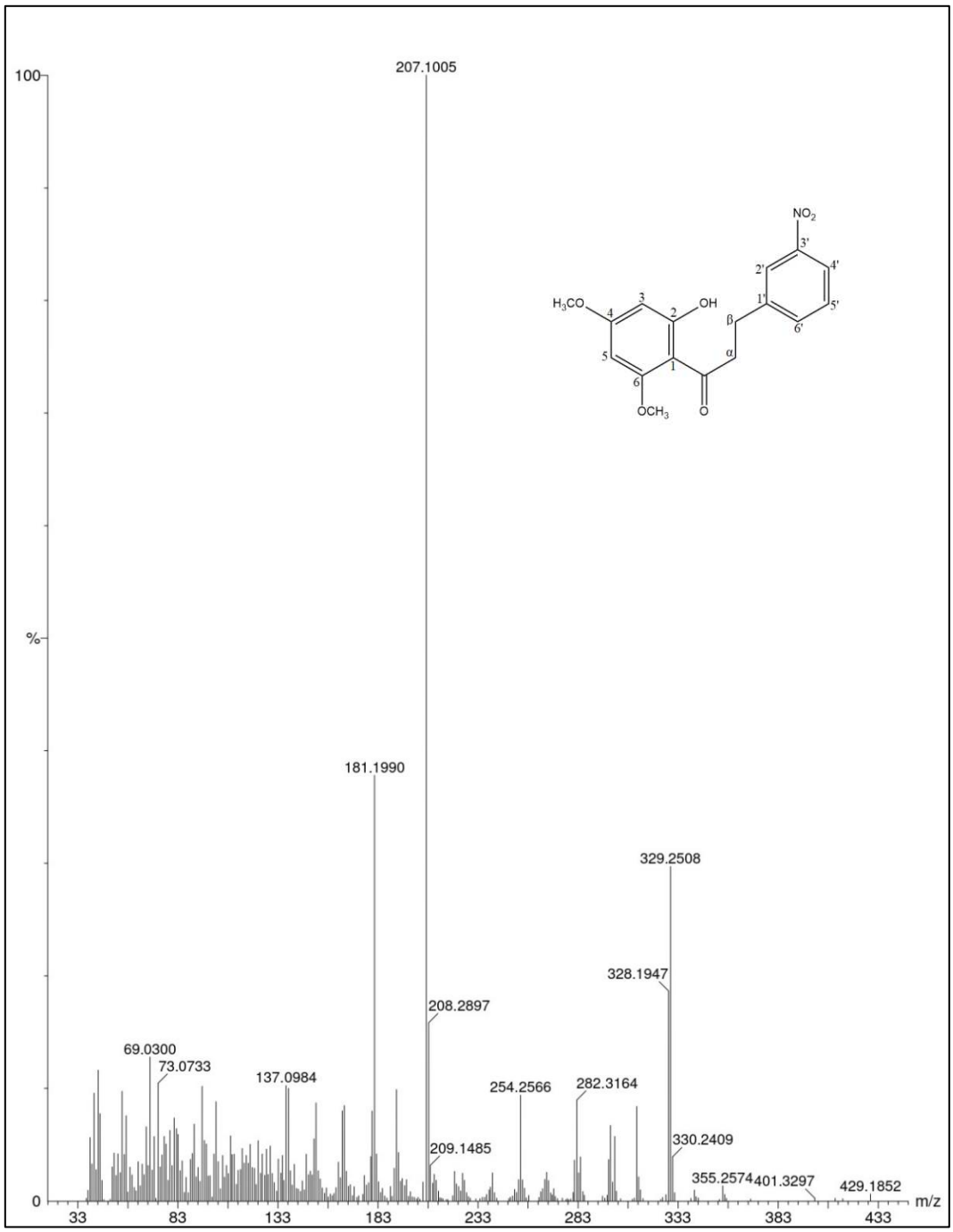


Figure 4.9 Mass Spectrum of 3'-nitro-2-hydroxy-4, 6 dimethoxy chalcone

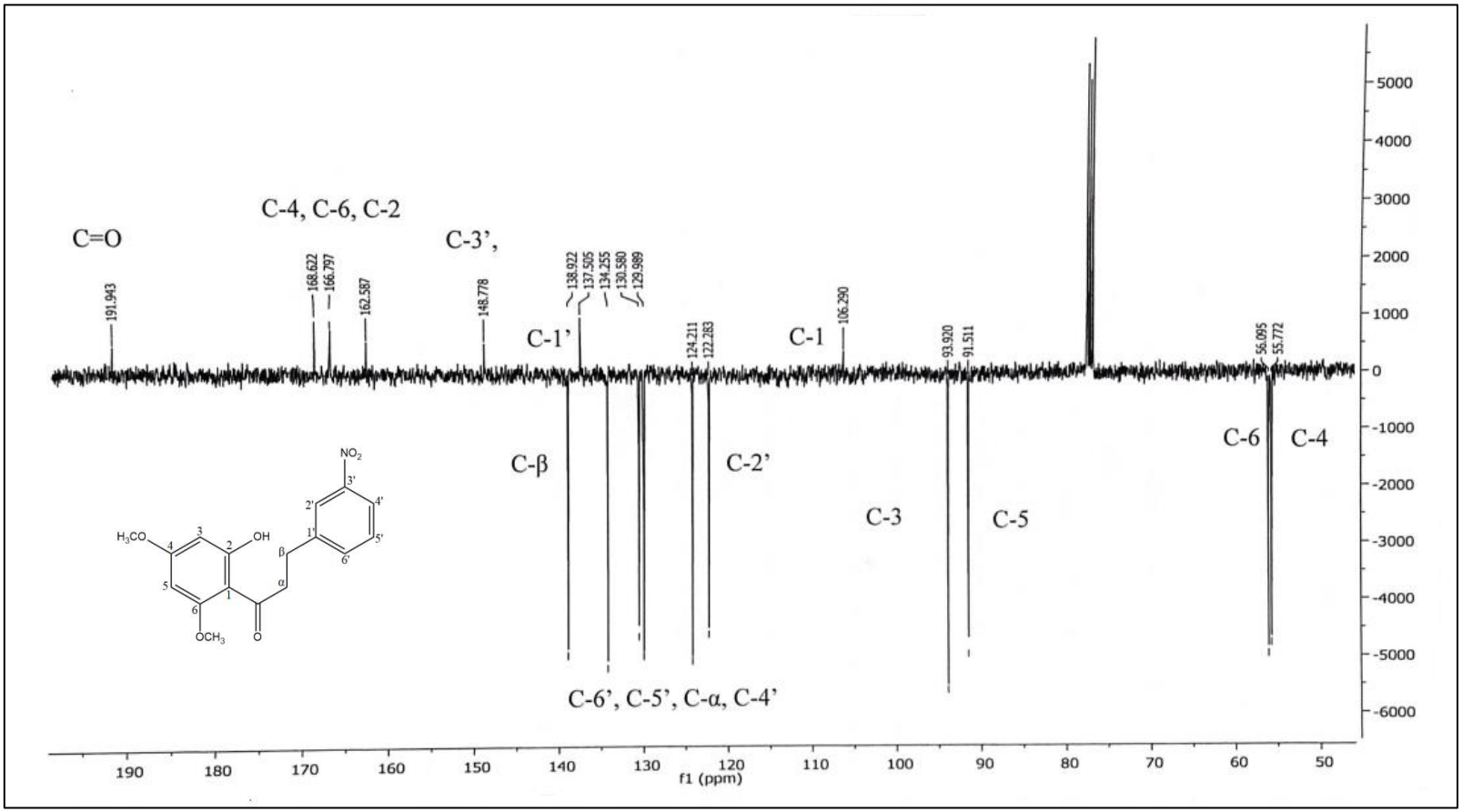


Figure 4.10 ^{13}C APT NMR 3'-nitro-2-hydroxy-4, 6 dimethoxy chalcone

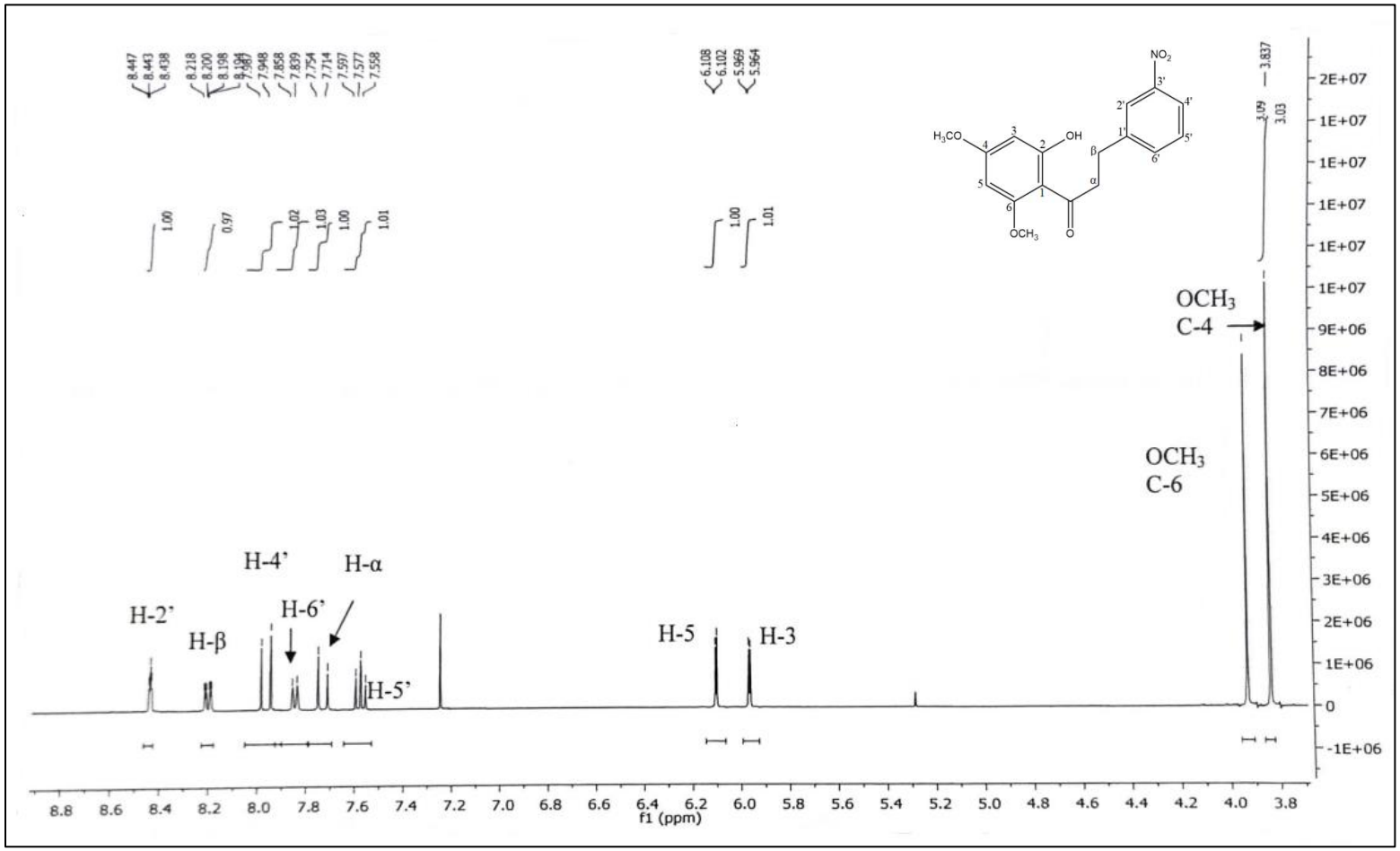


Figure 4.11 [1]H NMR 3'-nitro-2-hydroxy-4, 6 dimethoxy chalcone

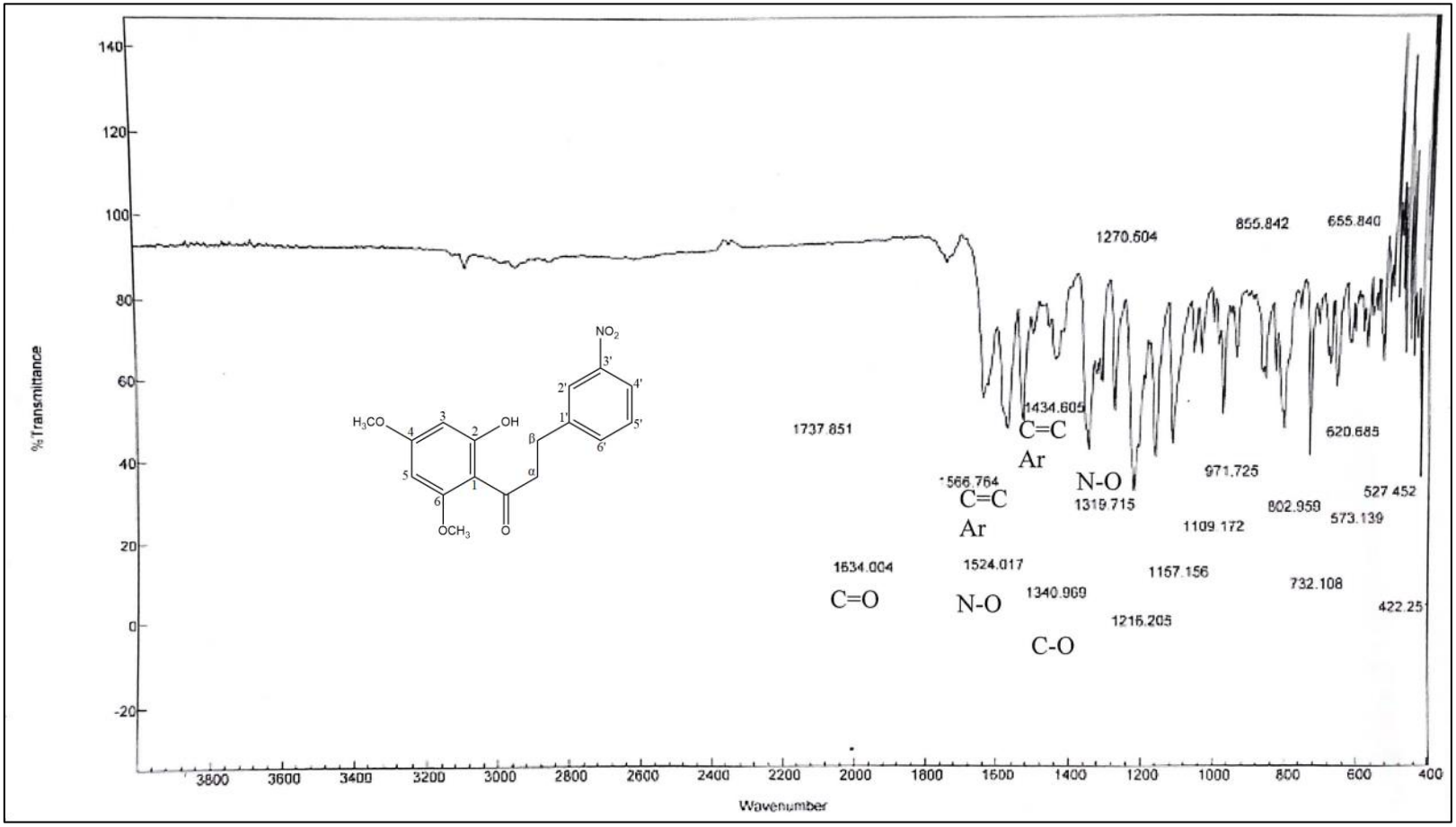


Figure 4.12 IR Spectrum of 3'-nitro-2-hydroxy-4, 6 dimethoxy chalcone

4.1.4 Characterization of 3'-nitro-5,7-dimethoxyflavanone

Compound of 3'-nitro-5,7-dimethoxyflavanone was successfully synthesized as white needle crystals. The percentage yield was 31.4%. The 3'-nitro-5,7-dimethoxyflavanone mass spectrum (Figure 4.13) showed the molecular ion peak at *m/z* 329.1848 indicating that this compound had the formula of $C_{17}H_{15}O_6N$.

The ^{13}C APT NMR spectrum of flavanone (Figure 4.14) showed the presence of 17 signals attributed to 17 different carbons. The signals for methoxy carbons were observed at 55.8 ppm and 56.3 ppm. The spectrum also confirmed the presence of two methyl carbons, one methylene, seven quaternary carbons and seven methine carbons in this compound.

The ^{1}H NMR spectrum (Figure 4.15) of 3'-nitro-5,7-dimethoxyflavanone displayed two singlets at 3.84 ppm (3H) and 3.89 ppm (3H) due to the methoxyl groups at C-5 and C-7 respectively. Two doublets of doublets of one proton each at 2.85 ppm (*dd, J* = 16.8 and 3.6 Hz) and 2.96 (*dd, J* = 16.4 and 3.6 Hz) attributed to H-2. The *meta* coupled protons of the A-ring appeared at 6.11 ppm (1H, *d, J* = 2.0 Hz, H-8) and 6.18 ppm (1H, *d, J* = 2.4 Hz, H-6). The four aromatic protons

of the β – ring was observed at 7.59 ppm (1H, *t, J* = 8.0 Hz, H-5'), 7.75 ppm (1H, *d, J* = 7.6 Hz, H-6'), 8.22 (1H, *ddd, J* = 8.4 and 1.2 Hz, H-4') and 8.38 (1H, *t, J* = 2.0 Hz, H – 2") respectively.

The IR spectrum (Figure 4.16) shows the absorptions band at 2930 cm^{-1} and 2857 cm^{-1} due to the C-H stretching, 1663 cm^{-1} (C=O), 1566 cm^{-1} and 1464 cm^{-1} due to aromatic C=C stretching, 1537 cm^{-1} and 1357 cm^{-1} are due to N-O stretching and 1218 cm^{-1} are due to (C-O).

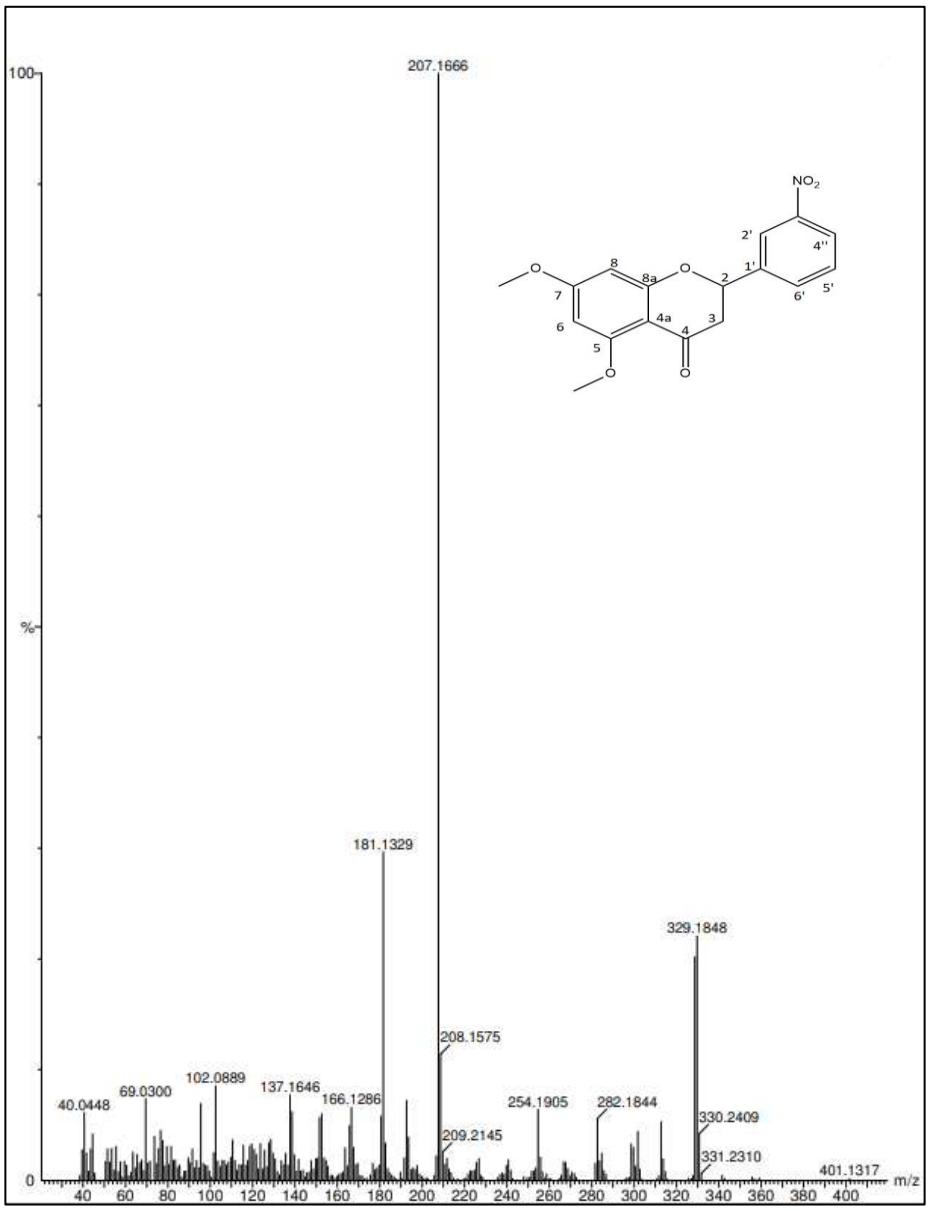


Figure 4.13 Mass Spectrum of 3'-nitro-5,7-dimethoxyflavanone

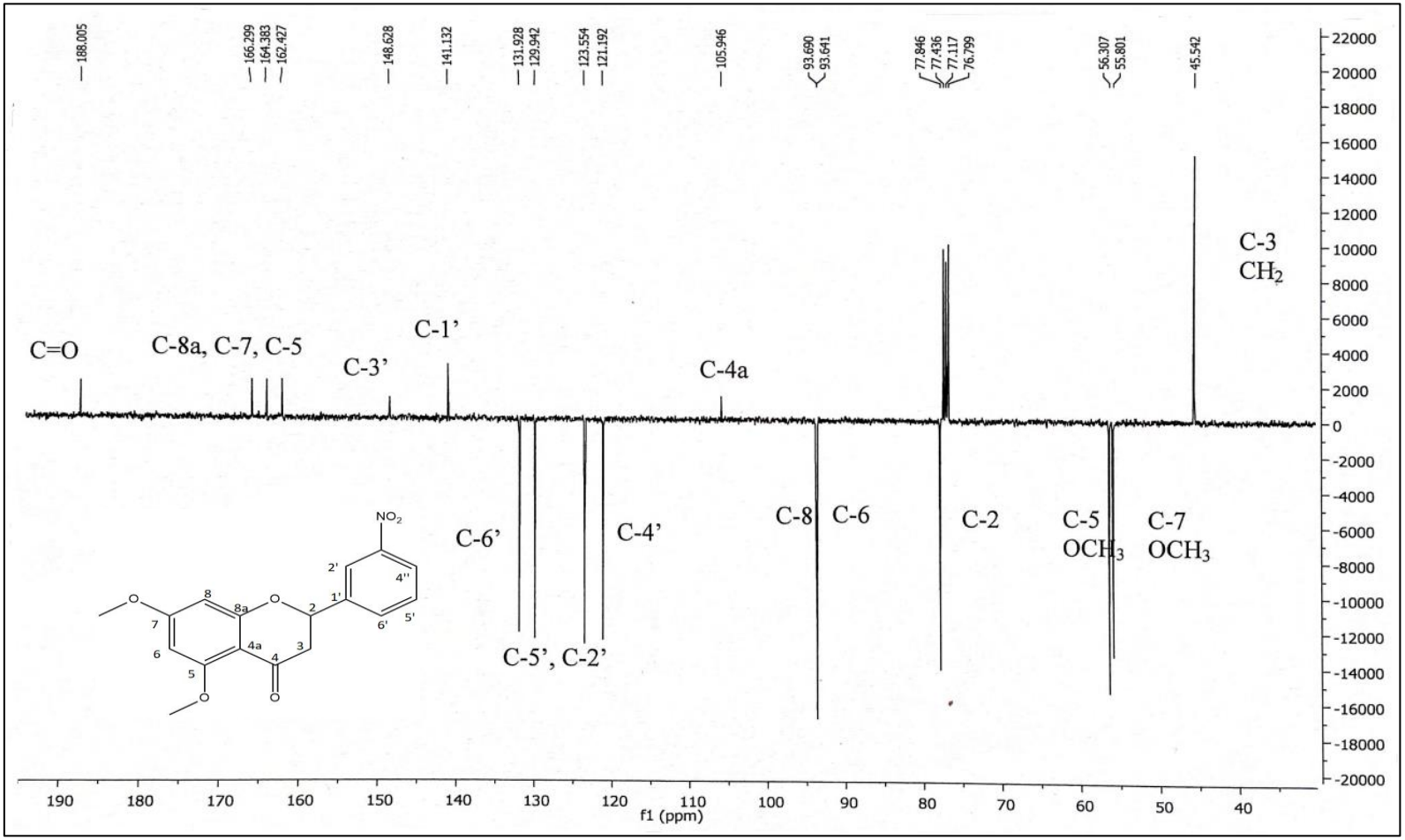


Figure 4.14 ^{13}C APT NMR of 3'-nitro-5,7-dimethoxyflavanone

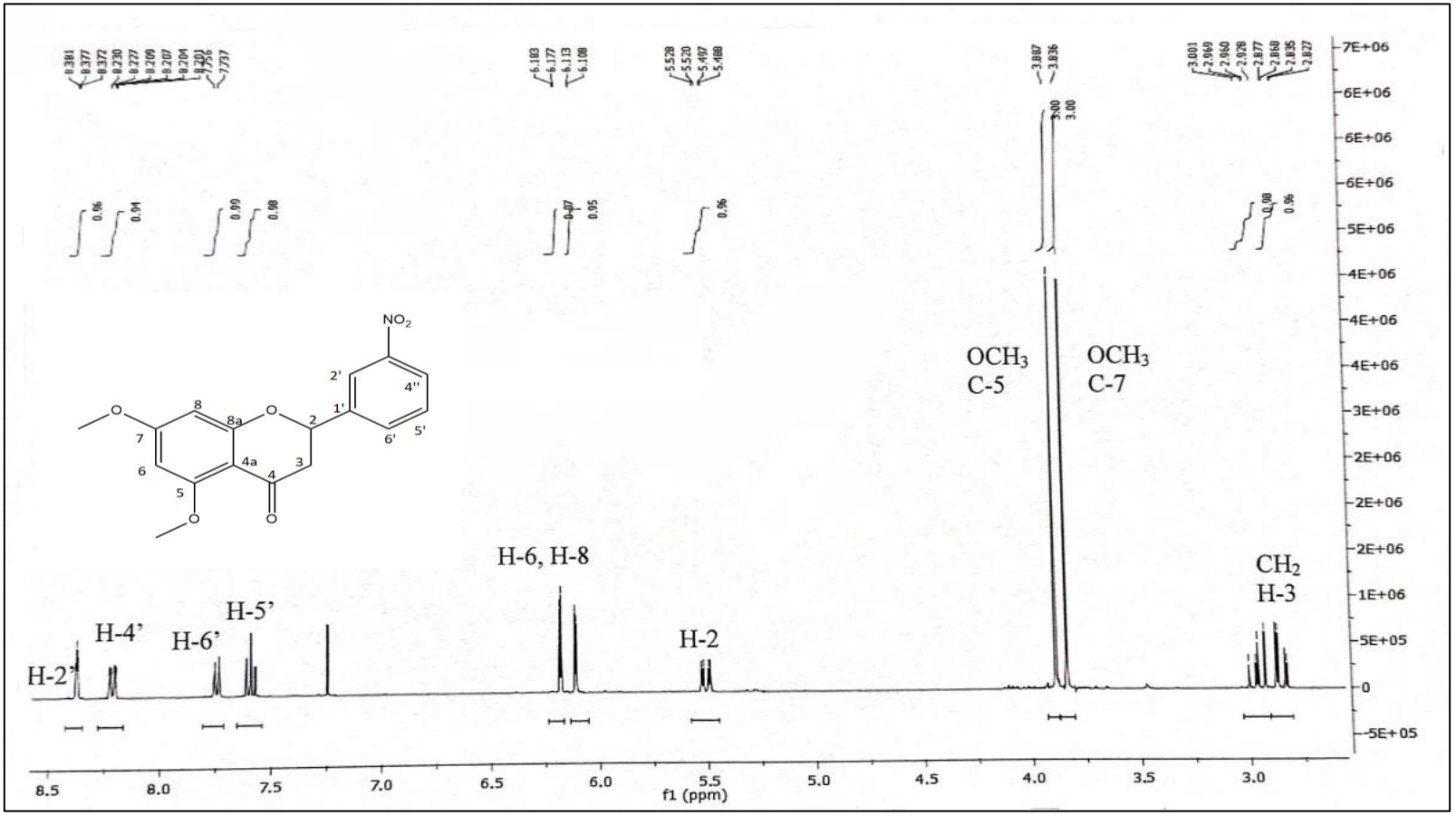


Figure 4.15 [1]H NMR APT NMR of 3'-nitro-5,7-dimethoxyflavanone

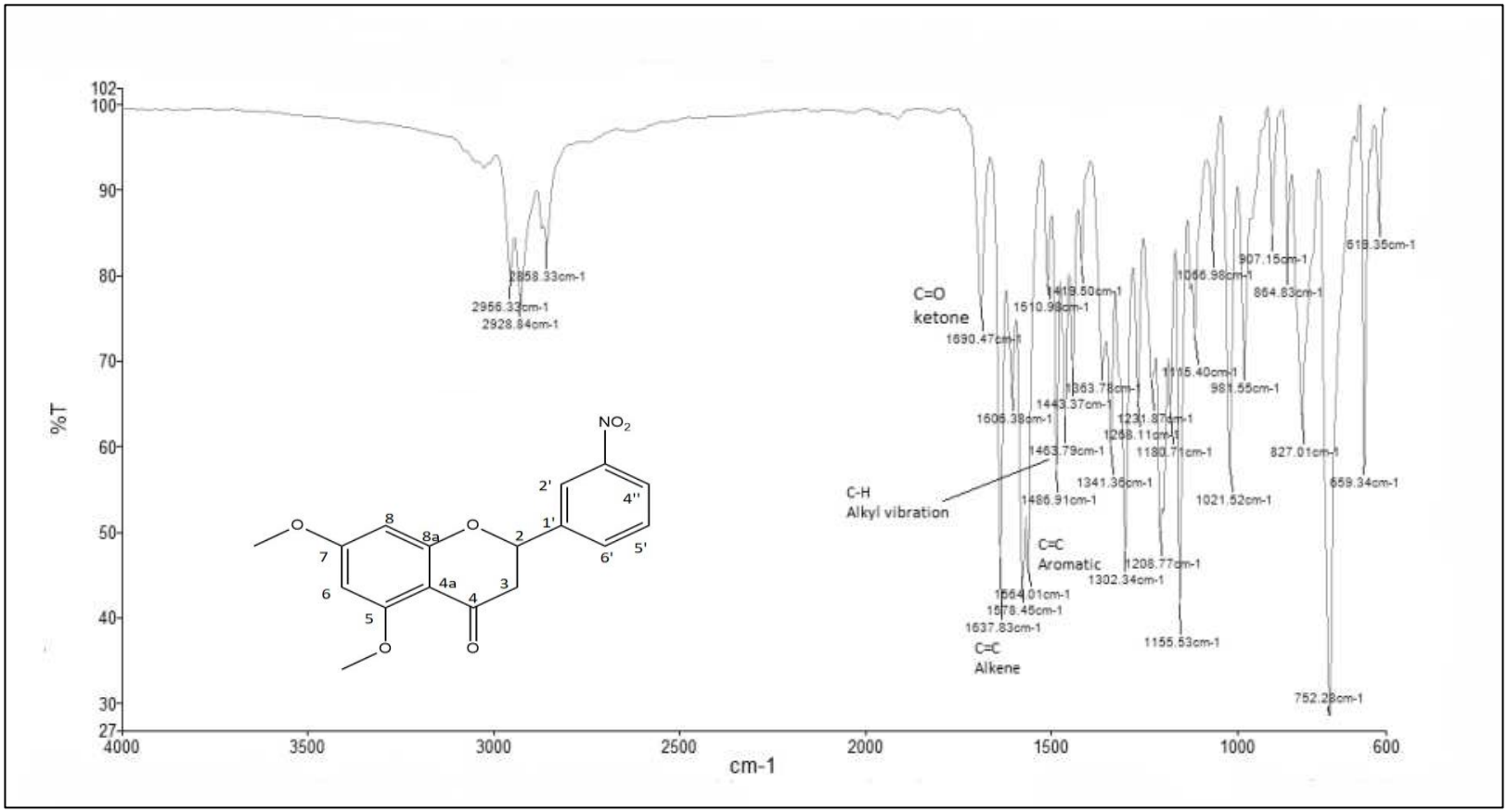


Figure 4.16 IR Spectrum of 3'-nitro-5,7-dimethoxyflavanone

4.2 Biological activities

Flavanone is classified as one of the classes of the family of flavonoid. In addition, flavonoid also possessed a characteristic of having various kind of biological activities such as being the anticancer, antifungal, anti-inflammatory and anti-oxidant. All of these biological activities have a very high demands in the industries of both medical and pharmaceutical. In this study, the antibacterial of flavanone were studied.

4.2.2 Antibacterial activities

The evaluation of the antibacterial activity has been carried out on 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one and 3'-nitro-5,7-dimethoxyflavanone. The assay was carried out by the method of micro-dilution for the determination of minimum inhibitory (MIC) and minimum bacterial concentration (MBC)

2-(4-butylphenyl)-5,7-dimethoxychroman-4-one and 3'-nitro-5,7-dimethoxyflavanone were tested against four pathogenic bacteria which is *Staphylococcus aureus* (SA), *Streptococcus pyrenes* (SP), *Pseudomonas aeruginosa* (PA) and *Escherichia coli (*EC). According to Weijian et al. (2022), all of the bacterial pathogens that is included in this study were those that are capable of producing numerous kinds of infection and diseases such as meningitis, pneumonia, and sepsis.

Based on the result of the antibacterial assays against the four bacteria used, it showed that 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one and 3'-nitro-5,7-dimethoxyflavanone is a weak inhibitor towards the bacteria used which is *Staphylococcus aureus* (SA), *Streptococcus pyrenes* (SP), *Pseudomonas aeruginosa* (PA) and *Escherichia coli (*EC). All of the bacteria are being compared to streptomycin sulphate. According to Wallace et al. (1979), streptomycin is classified as an antibiotic that is active in fighting against a huge number of both gram positive and grams negative bacteria.

Table 4.1 Inhibitory Concentrations of MIC and MBC Assays for Synthesized Compounds

Compound	EC		SA		SP		PA	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
2-(4-butylphenyl)-5,7-dimethoxychroman-4-one	1800	1800	1800	1800	1800	1800	1800	1800
3'-nitro-5,7-dimethoxyflavanone	1800	1800	1800	1800	1800	1800	1800	1800
Streptomycin suplhate	14.1	14.1	14.1	14.1	14.1	14.1	14.1	14.1

Note: EC-*Escherichia coli*, SA-*Staphylococcus aureus*, SP- *Streptococcus pyrenes*, PA- *Pseudomonas aeruginosa* (unit in µg/mL)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Synthesis of Chalcone and Flavanone

2-(4-butylphenyl)-5,7-dimethoxychroman-4-one and 3'-nitro-5,7-dimethoxyflavanone has been synthesized and their antibacterial activity were determined using MIC and MBC. The synthesis of these two compounds was successfully accomplished through two – step reaction which is the Claisen Schmidt condensation and isomerization of 2-hydroxychalcones to flavanone and the goal of this study also have been achieved as well. The first step to the synthesis of the compounds was synthesizing the chalcone through the process of Claisen-Schmidt condensation where acetophenone and benzaldehyde were utilized in the synthesis to produce chalcone which is 3-(4-butylphenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) prop-2-en-1-one and 3'-nitro-2-hydroxy-4, 6 dimethoxy chalcone. For the second step which is basically the last step of the synthesis, flavanone was synthesized by using the cyclization of chalcone. Both compounds of chalcones and flavanones were characterized by using Gas Chromatography Mass Spectroscopy

(GCMS), Fourier Transform Infrared (FTIR), ^{1}H Nuclear Magnetic Resonance (NMR), and ^{13}C Nuclear Magnetic Resonance.

5.2 Bioactivity Studies

In this study, the antibacterial activity was performed by doing the method of micro-dilution to determine the minimum inhibitory concentration and minimum bactericidal concentration. There are four pathogenic bacterial that were used in this study which is *S. aureus* (SA), *S. pyrenes* (SP), *P. aeruginosa* (PA) and *E. coli (*EC).

Based on the result of the study, 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one and 3'-nitro-5,7-dimethoxyflavanone was not able to hinder the growth of the four species of the pathogenic bacterial, *(EC), (SA), (SP)* and *(PA)* in all the concentration for both of the MIC and MBC. Therefore, this relatively shows that 2-(4-butylphenyl)-5,7-dimethoxychroman-4-one and 3'-nitro-5,7-dimethoxyflavanone are weak inhibitors as well as inactive towards all pathogenic bacteria used when being compared to the streptomycin sulphate.

5.3 Recommendation

Based on the research study that has been conducted on chalcone and flavanone, both of these compounds can be synthetically obtained. Besides that, it is also frequently utilized as the approved starting material for the process of synthesizing the other compound of polycyclic aromatic. Other than that, both chalcones and flavanone also possessed the beneficial potential of pharmacological with various kind of activities of biological such as anticancer, antioxidant and anti-inflammatory. Thus, studying and analyzing the compound of chalcone and flavanone can be helpful in fighting diseases that negatively affect the health quality of human beings. Apart from that, iodine is often used as the catalyst in the synthesis of flavanone and it is one of the toxic compounds as it is hazardous. Therefore, it is preferable to use less hazardous catalysts along with the presence of air under the condition of solvent – free which in the end also produces a better result of yield compared to the method of I_2-DMSO.

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CURRICULUM VITAE

A. Personal profile

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B. Hobbies and interests

I enjoy dancing and reading autobiography. I like to travel to a new place to enjoy the nature and fresh air. I also like to listen to pop and soft music.

C. Academic qualifications

Degree	Area	Institution	Year Awarded
B. Sc. (Hons)	Chemistry with management	Universiti Teknologi MARA, Sarawak	Current
Diploma	Science	Universiti Teknologi MARA, Sarawak	2019
SPM	Science	Maktab Rendah Sains MARA, Mukah	2016
PT3	Science	Sekolah Menengah Kebangsaan Bandar Bintulu, Bintulu	2014

D. Related experience

Post	Place	Year
Committee member	BCHEM Association, University Teknologi MARA, Sarawak	2021 -2022
Event Helper	Kuching Community Hall (Kuching Jazz Festival)	2018

E. Awards

Type	Name of Award / awarding organization	Year
Certificate	Undergraduates Colloquium Faculty of Applied Sciences (as committee member)	July 2022
Certificate	BCHEM Association, University Teknologi MARA, Sarawak (Saving our planet)	Nov 2021
Certificate	BCHEM Association, University Teknologi MARA, Sarawak (Monochrome Picasso Art Painting Contest)	Oct 2022

F. Work Experience

Type	Name of Award / awarding organization	Year
Admin Clerk	Danrie Enterprise Sdn Bhd. (Shipping Company)	2022