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Synthetic Design Of Coumarin And 2H-Chromene-2-Thione Derivatives To Inhibit Carcinogenesis

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Synthetic Design of Coumarin and 2H-chromene-2-thione Derivatives to Inhibit Carcinogenesis

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Biographical Sketch

Rhashanda Haywood was born on July 6, 1987 in Spartanburg, SC. In 2009, she received her Bachelor's degree in Chemistry from Johnson C. Smith University in Charlotte, NC. After graduating from Johnson C. Smith University, she worked for Duke Energy at their McGuire Nuclear Station as a lab technician in the Central Fuels Laboratory. In the fall of 2010, she became a candidate in pursuit of a Master's Degree in Chemistry at North Carolina Agricultural and Technical State University. In the fall of 2011, she became a charter member for the Iron chapter of Iota Sigma Pi an Honors society for Women in Chemistry. She conducted research under the direction of Dr. Marion A. Franks on the Synthesis and chemopreventive activity of coumarin and 2H-Chromene-2-thione derivatives during her attendance at North Carolina A&T State University.

Dedication

I would like to dedicate this thesis to my intermediate family; to my parents Rosa Dunlap, and the late Albert Dunlap and my brother Donovan Haywood. I thank my parents for giving me life, and I thank my mother and brother for the continuous words of encouragement, for supporting me in what I am striving to accomplish, and for the unconditional love.

Acknowledgements

I would like to start by acknowledging God, because without his grace and mercy nothing I attempt to achieve would be possible. I would like to thank my advisor, Dr. Marion A. Franks, for all his guidance throughout my tenure at North Carolina Agricultural and Technical State University. Without his compassion and acts of kindness on my behalf, I don't know how I would have made it through. I would like to thank Ms. Carolyn Mayo for everything she has ever done for me. She has truly been a second mother to me. I would also like to thank Mr. Zadok Abrahaim, Carlos Crawford and Mr. James King, for aiding me in utilizing instrumentation for my compounds, and Ms. Joyce Njoroge and Mr. Charles Wilson, for helping me in providing the items needed to complete my research.

I thank my parents for giving me life, and I thank my mother and brother for the continuous words of encouragement, for supporting me in what I am striving to accomplish, and for the unconditional love. To my cousin Talisha Haywood, I thank her for making my transition to North Carolina A&T State University a smooth one. I thank my research group members, Davia McKoy, Brittany Nichols, Courtney Peace, Jasmine Fluker, Jonathan Moseley, Shaylon Johnson and past group members. I thank all of my other colleagues in the chemistry department for their encouragement, help, and support throughout my academic endeavors. I would also like to thank my committee for reviewing my thesis.

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List of Schemes

Nomenclature

^{13}C NMR Carbon ..... Carbon Nuclear Magnetic Resonance Spectroscopy

CH_2Cl_2 ..... Methylene Chloride

DI ..... De-Ionized Water

DMSO ..... Di-methyl Sulfoxide

EtOH ..... Ethanol

Et_3N ..... Tri-ethylamine

$InCl_3$ ..... Indium(III)Chloride

IR ..... Infrared Spectroscopy

Na_2SO_4 ..... Sodium Sulfate

THF ..... Tetrahydrofuran

TLC ..... Thin Layer Chromatography

TBDMSCl ..... Tertiarybutyldimethylchloro silane

$ZrCl_4$ ..... Zirconium(IV) Tetra Chloride

Abstract

The goal of this project was the synthesis of 4-substitued coumarins and 2H-chromene-2-thione derivatives that can be used to inhibit or retard the growth of cancer cells. According to the American Cancer Society's 2012 death statistics for prostate cancer, 28,170 men died from prostate cancer and there was 241,740 (29%) new cases of prostate cancer as well (Society, 2012). Few studies have been conducted on 2H-chromene-2-thiones and their ability to act as cancer chemopreventive or anticancer agents, therefore, we seek to synthesize a sequence of coumarins and 2H-chromene-2-thiones via the Von Pechmann reaction. We believe that the 2H-chromene-2-thiones will exhibit better chemopreventive activity then the analogous coumarins because of the presence of the softer sulfur atom. The syntheses of the coumarin derivatives in this study were accomplished using green chemistry. The indium chloride catalyzed Von Pechmann condensation required no solvent and formed coumarins efficiently. Upon synthesizing coumarins, they were converted into their 2H-chromene-2-thione analogs using Lawesson's reagent. Percent yields of the coumarin derivatives range from 40% to 80%. The 2H-chromene-2-thione percent yields range from 11% to 61%.

CHAPTER 1

Introduction

1.1 Cancer Statistics

According to the American Cancer Society, the number of new cases of cancer in 2011 was an estimated 1,596,670 (Society, 2011). This is an increase of 67,110 from 2010, and an increase of 117,320 from 2009. American Cancer Society's death statistics for Cancer shows that more than half a million individuals died from Cancer in 2011 (571,950) which is more than 1500 persons a day (Society, 2011). This is an overall increase of 12,070 deaths from Cancer in the past three years, (569,490 in 2010, and 562,340 in 2009)(Society, 2009, 2010).

1.1.1 Mechanism of cancer. Carcinogens are compounds that initiate tumor development and during the initiation phase (the first stage of carcinogenesis), the carcinogen interacts with DNA in the cell. Ultra-violet radiation, viruses, television waves, and microwaves; even down to the foods that we eat, and how they are prepared can allow for the development of cancerous tumors. (Alomirah et al., 2011) Carcinogenesis has four stages: Initiation, promotion, progression, and finally malignant conversion (Tannock, 2005).

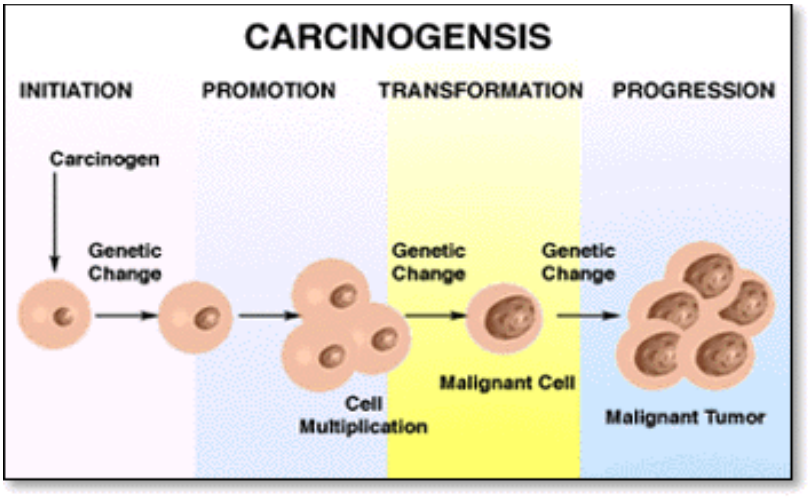


Figure 1. Mechanism of cancer (Tannock, 2005).

Figure one illustrates the mechanism of how cancer develops and causes damage to normal cells. During each stage of cancer, there is a genetic change that takes place. A carcinogen damages the DNA sequencing and creates a break in the DNA strand (Tannock, 2005). However, during initiation the mechanisms to allow cellular repair and apoptosis promotion are still functioning. During the promotion phase, in the cell cycle phase (M phase) the damaged genome is copied to a new daughter cell (Tannock, 2005). When an individual has prolonged exposure to the carcinogen, kinases (proteins) called promoters, are overexpressed. This increase in expression levels promotes the formation of tumors/lesions that are benign at this stage. Because the cancerous tumors have yet to progress to the final stage of carcinogenesis, apoptosis of the cell (programmed cell death) can occur and damage that occurred at this stage can be overturned. From the repeating of the first two stages, initiation and promotion, there is transformation of the tumor from benign to neoplastic. This is said to be progression, the third stage in carcinogenesis (Tannock, 2005). Malignant conversion is the final stage of cancer. In this stage, the cells divide and grow uncontrollably because of expression of the neoplastic phenotype. This process will allow for the development of malignant tumors (Tannock, 2005).

1.1.1.1 Tumor suppressor genes and oncogenes. During carcinogenesis oncogenes and tumor suppressor genes are altered. The difference between an oncogene and a tumor suppressor gene is that an oncogene is mutational activation of proteins that normally promote cell proliferation (Weinberg, 2007). A tumor suppressor gene is mutational inactivation of proteins that normally inhibits cell proliferation. During the cell cycle, if a cell is found to have any damage, the tumor suppressor genes normally repair the damage. However, if the damage is beyond repair the tumor suppressor genes will signal for the cell to undergo apoptosis. If there are mutations in the tumor suppressor genes, the signaling of the cells to undergo apoptosis will

not occur and the cells will continue to proliferate. An oncogene promotes cancer growth and transforms cells from a normal state to a cancerous state. Oncogenes are different from tumor suppressor genes with the loss of function such as unregulated activation of the oncogenes to promote a cell to proliferate even though it has mutations within itself (Weinberg, 2007). With the oncogene's gain of a function it can initiate cancer development. An example of a proto-oncogene that can undergo transformation into an oncogene is Src. Src is a kinase that phosphorylates substrates. When there is a mutation of Src it becomes v-src (viral-Src) and may cause proliferation of cells (Weinberg, 2007).

Tumor suppressors are defensive genes that restrict the growth of tumors. Cancer can develop if the tumor suppressor loses its function. An example of a tumor suppressor is Adenomatous Polyposis Coli (APC) and p53. Tumor suppressor gene, p53 serves as the "security" of the cell as it keeps everything in order. If p53 receives information about genetic damage or a metabolic disorder within a cell it may delay the cell going into the next phase of the cell cycle (Weinberg, 2007). If the damage is beyond repair, p53 can signal for the cell to undergo apoptosis. However if there is a mutation within p53 this disables the genes ability to function in its normal capacity. A mutation in p53 can bring about prostate cancer in men, and a mutation in APC can bring about colon cancer in 70% of sporadic cases of the disease (Weinberg, 2007). In terms of sporadic cases of cancer development, according to the multi-hit theory of cancer development Scientist Alfred Knudson hypothesized that cancer is brought about from a "series" of mutations and not a single mutation (Weinberg, 2007). In 1987, Knudson conducted a study on children with retinoblastoma to prove his hypothesis to be correct. Knudson's observations showed that there were two distinct categories of patients, patients who had family members with retinoblastoma (familial form), and the other category is

those who did not have a family history of retinoblastoma (sporadic form). Those who developed the sporadic form of retinoblastoma only developed a tumor in one eye. But the patients who developed the familial form developed a tumor in both eyes. If an individual has mutations in a specific gene, then this is probable cause for tumor development. Using the retinoblastoma case as an example, an individual had to inherit a mutated gene. Hence, mutations in both copies of a gene are needed for the retinoblastoma gene to initiate tumor development. However in the sporadic form, the tumor develops over a period of time, because only one gene mutation is inherited in the familial form. The multi-hit theory is also inclusive of the fact that cancer development is driven by not only the deactivation of tumor suppressor genes, but also the activation of proto-oncogenes.

1.1.1.1.1 The application of cancer chemoprevention. Cancer chemoprevention is the use of chemical compounds or dietary components either to protect against the initiation of carcinogenesis or stop the growth of a cancerous tumor. Figure 2 provides a schematic of the biological approach to preventing cancer. As previously stated in the multi hit theory of cancer development, before a carcinoma develops, there are a series of events that leads to this development. This development can be caused by abnormalities in several biological mechanisms. During carcinogenesis, there are several pathways that can lead to cancer. Figure 2 illustrates three of the numerous pathways to cancer formation, which are antiapoptosis, growth factors, and proangiogenesis. Ras, an oncogene, and Rb, a tumor suppressor gene, when altered can bring about cancer. EGF, VEGF, and BFGF are growth factors. When there is an imbalance in these growth factors, this mutation can accelerate the transformation of a normal cell into a cancerous cell (Tsao, Kim, & Hong, 2004). Therefore, cancer chemoprevention can be applied to inhibit or halt the abnormal signaling of tumor suppressor/oncogenes, growth factors as well as

other biological factors such as deregulation of enzymes (telomerase activity) and pathways (cyclooxygenase pathway) (Tsao et al., 2004). Figure 2 shows three different pathways of carcinogenesis and without treatment these pathways can lead to cancer. Figure 2 also shows the application of antiapoptotic and antiangiogenic compounds such as Farnesyltransferase inhibitors (to inhibit oncogene Ras activity), Retinoids (to stop the abnormal expression of tumor suppressor gene Rb), EGF, VGEF and COX-2 growth factor inhibitors to halt the formation of new blood vessels.

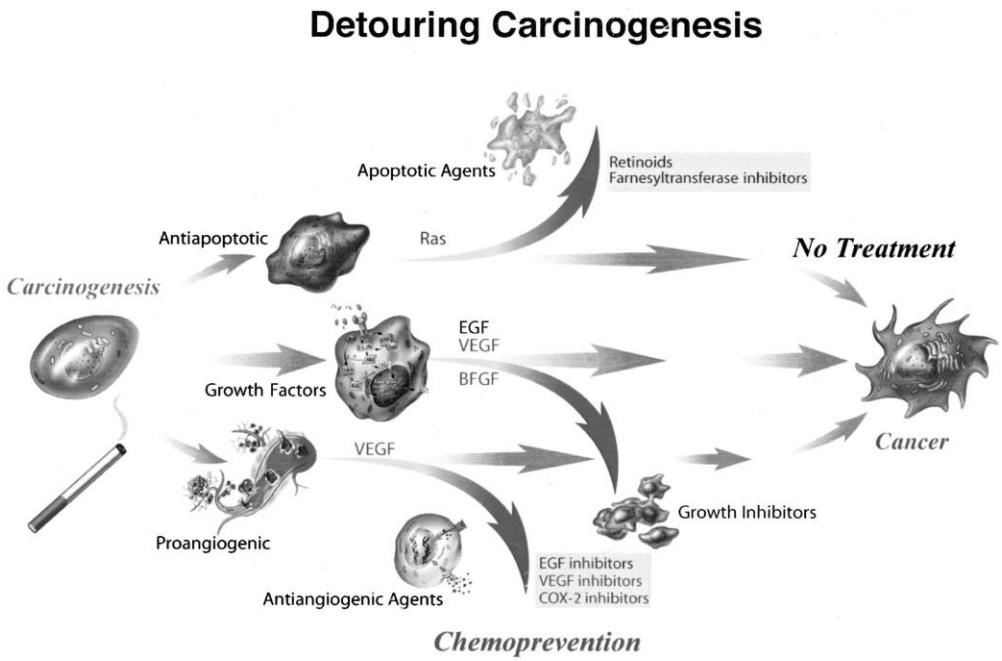


Figure 2. How cancer chemoprevention works.

Cancer chemoprevention is of importance to the field of cancer therapy and treatment because application of compounds found within nature and those that are similar in structure to natural products would be an appropriate alternative to conventional medicine used today for the treatment of cancer. Coumarin derivatives have been widely utilized in the synthesis of cancer chemoprevenetive agents (Subhas Bose, Rudradas, & Hari Babu, 2002). Some studies suggest that naturally occurring coumarins that are taken by mouth have the possibility to induce phase I

and II enzymes within the body (Kleiner et al., 2001). These coumarins can also induce GST (glutathione s-transferase) activity, a phase II enzyme. It was found that linear furanocoumarins when administered showed a lack in the inhibition of pentoxyresorufin ortho-dealkylase (PROD) activity in the liver of lab mice. However, isopimpinellin, a chemical isolated from linear furanocoumarins increased GST activity in the forestomach and the small intestine of the lab mice (Kleiner et al., 2001). Results from this study also suggested that isopimpinellin, induced phase I and II enzymes in several tissues within the body and some that are specific for polycyclic aromatic hydrocarbons (PAH) carcinogenesis (Kleiner et al., 2001). Thus coumarins are of significance in cancer chemoprevention and phase I and II enzyme induction.

CHAPTER 2

Literature Review

2.1 Naturally Occurring Coumarins

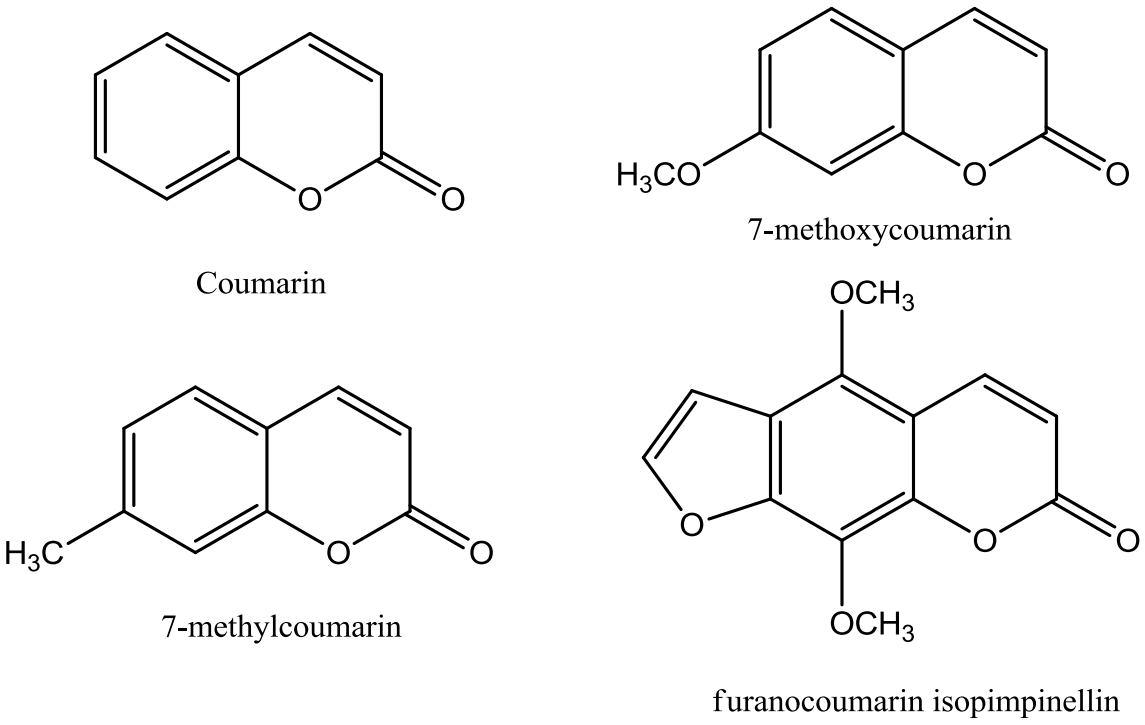


Figure 3. Structures of some naturally occurring coumarins.

2.1.1 Coumarins in foods. Coumarin, an oxygen heterocycle, has been found naturally in fruits and vegetables that we eat (Singh, Devi, Thokchom, & Sharma, 2010). Figure 3 shows the structures of some of the numerous coumarin derivatives that are found naturally. Coumarin, chemical name 1,2-benzopyrone are consumed by humans on a regular basis. In celery, the pulp and rind of limes is where furanocoumarin isopimpinellin is found. The coumarin derivative is found in fruits such as strawberries, grapefruits, apricots and cherries also. Cumin (used to make curry powder), cinnamon, parsnips, are used as flavorings in food preparation, are some of the numerous plant based foods that naturally contain coumarins (Kleiner et al., 2001). Coriander, cilantro in its leaf form, contains coumarin derivative 7-methylcoumarin.

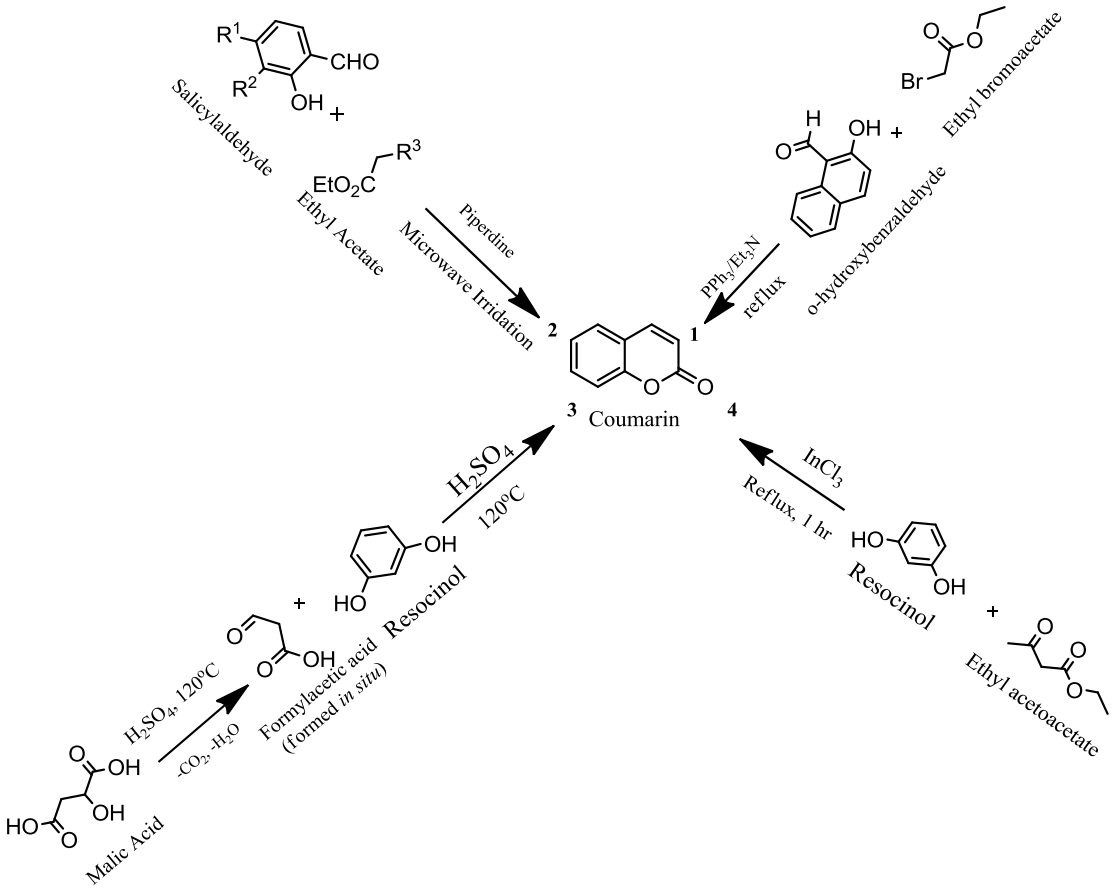
2.1.2 Coumarins in plants. Coumarins are also found naturally in plants as well. The plant sweet clover has a high content of coumarin which is responsible for the sweet aroma of the plant. Lavender, Tonka beans, and Licorice are other plants with high coumarin content as well (Goel, Prasad, Parmar, Ghosh, & Saini, 2007). Herniaria glabra, common name rupturewort, which is believed to have diuretic properties, contains the coumarin derivative 7-methoxycoumarin (Santamour Jr & Riedel, 1994). Not only are coumarins founds naturally in plants and foods, they possess medicinal properties as well. Some derivatives contain anti-oxidant, diuretic, anti-bacterial, and anti-hypertensive properties, while others have anti-HIV, and anti-tumor properties (Goel et al., 2007). From these foods naturally containing coumarins, they can be eaten to possibly reduce or minimize one's development of cancer.

2.1.3 Coumarins for medicinal use. From the molding of the sweet clover plant by fungi such as penicillium, the coumarin derivative dicoumarol is formed (Kresge, Simoni, & Hill, 2005). In the 1920s, when cattle would eat the molded sweet clover, it caused them to bleed excessively due to dicoumarol's anti-coagulant properties (Antonella Tonna, 2004). In the 1940s, dicoumarol was used as an anti-coagulant drug to treat individuals with thrombosis which is the formation of blood clots in blood vessels that could potentially migrate to other parts of the body (Antonella Tonna, 2004). In 1954 dicoumarol was officially discontinued, as Warfarin (another coumarin derivative) was approved for use as an anti-coagulant drug which is currently still administered to treat thrombosis.

2.2 Synthesis of Coumarin Derivatives

As stated previously, coumarin derivatives have been widely utilized in the synthesis of cancer chemopreventive agents (Subhas Bose et al., 2002).There are numerous routes to synthesize the coumarin derivative. Scheme 1 illustrates a few synthetic routes to obtain the

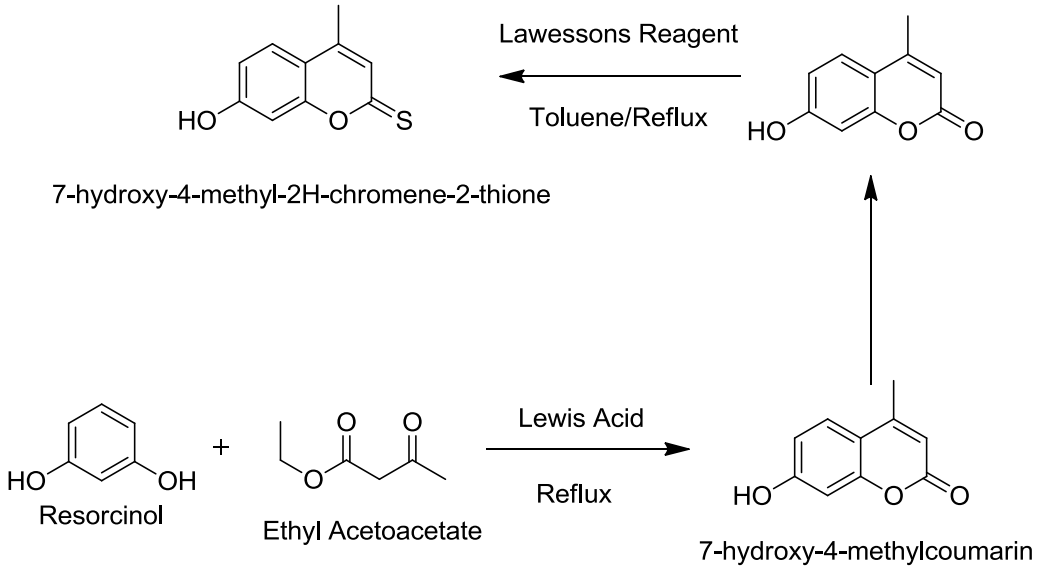
coumarin derivative. Each reaction listed undergoes condensation. The Witting reaction is shown in reaction 1. The reaction takes place via a one pot synthesis of ethyl bromoacetate and ortho-hydroxybenzaldehyde in the presence of triphenylphosphine. Triethylamine is used as the base in this synthesis. The reaction labeled 2 is the Knoevenagel condensation of salicylaldehyde with various derivatives of ethyl acetoacetate under microwave irradiation.



Scheme 1. Various synthetic routes to obtain coumarin derivatives.

In reaction 1, piperdine is utilized as the base. How the reaction proceeds is deprotonation from the piperdine allows for enol formation. The enol that is formed reacts with the aldehyde to have elimination of the aldol to obtain the coumarin molecule (Bogdal, 1998). In 1883, chemists, Hans Von Pechmann and Duisberg in were the first to synthesize 7-hydroxy-4-methylcoumarin

(β-methylumbelliferone) via condensation of the phenol and β- keto ester, resorcinol and ethyl acetoacetate. The Von Pechmann condensation can occur via numerous synthetic routes. The standard method for synthesizing coumarins via the Von Pechmann route is the reaction of phenol with ethyl acetoacetate in sulfuric acid. The overall mechanism is carried out under acidic conditions. Reaction scheme 3, in scheme 1 shows the reaction takes place via the condensation of Malic acid in the presence of sulfuric acid at 120^{o}C. Formylacetic acid is formed *in situ* (in solution). The formylacetic acid reacts with the resorcinol to yield the coumarin derivative.



Scheme 2. General synthesis of 7-hydroxy-4-methyl-2H-chromene-2-thione.

The studies of others have shown that the synthesis of 4-methyl coumarins via the Von Pechmann reaction proves to be a simple and capable way of carrying out the synthesis. A study conducted by D. Bose, A. Rudradas and M. Babu utilized Indium (III) chloride as a catalyst for the Von Pechmann condensation under solvent free conditions. They obtained excellent results with coumarin derivatives in high percent yields (90% yields or more in most cases) thus proving Indium Chloride's effectiveness in catalyzing the reaction (Subhas Bose et al., 2002). From their

studies and the excellent results they obtained, the Von Pechmann condensation utilizing Indium Chloride was chosen as the synthetic route to obtain the coumarin derivatives for this research.

Scheme 2 illustrates the synthetic steps taken to obtain the target molecules that are 7-hydroxy-4-methyl substituted 2H-chromene-2-thiones. These will be the necessary steps taken to obtain the target molecule within this research. For this particular study, ethyl acetoacetate was the β-keto ester of choice. Indium (III) chloride was used as a catalyst in this reaction due to its proven effectiveness as a Lewis acid catalyst. In this study, eight compounds were synthesized using various phenols and a β-keto ester.

2.3 Synthesis of 2H-Chromene-2-Thiones

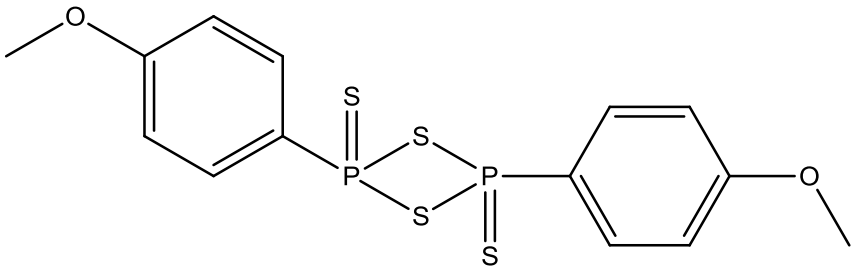
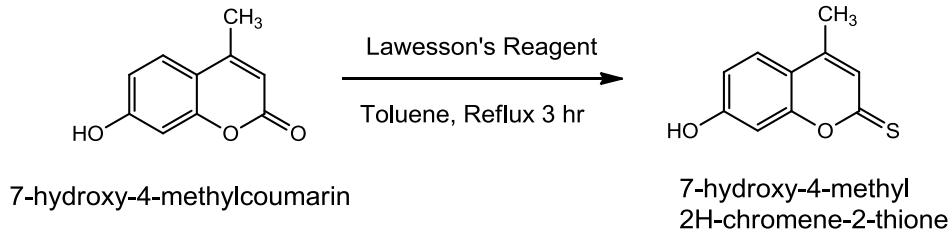


Figure 4. Structure of Lawesson's reagent.

2.3.1 Lawesson's reagent. Figure 4 shows the chemical structure of Lawesson's Reagent. Lawesson's Reagent is a potent yet mild thionating agent that has been widely utilized as it successfully transforms an oxygen atom into its respective thio analog (Z. Wang, 2010). It has also been used to carry out the transformation of carbon-oxygen single bonds into carbon-sulfur single bonds. Lawesson's Reagent has also been used for the cyclization of compounds that obtain at the least two oxygen atoms (Z. Wang, 2010). For this particular research 7-hydroxy-4-methyl derivatives are obtained for each synthesis due to ethyl acetoacetate being the β-keto ester of choice for the synthesis of the coumarins, and when converted into the 2H-chromene-2-thione derivative only the oxygen atom will be replaced. Preliminary studies suggest

that using Lawesson's reagent to carry out the conversion of 7-hydroxy-4-methyl coumarin to 7-hydroxy-4-methyl 2H-chromene-2-thione, is an easy and effective way of doing so (shown in scheme 1). This is due to the Lawesson's reagent's ability to easily substitute the oxygen atom for a sulfur atom (Levai & Jekoe, 2005).



Scheme 3. General synthesis of 2H-chromene-2-thione.

Table 1

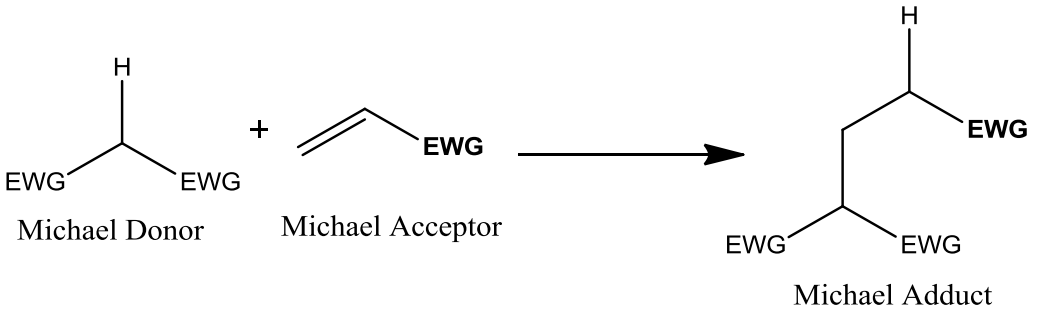
Different Classes of Cancer Chemopreventive Agents

Blocking Agents	Suppressing Agents
Anti-oxidants	Anti-inflammatory
Free Radical Scavengers	Protease inhibitors
Inducers of Glutathione S-transferase	Protein Kinases
Modifiers of mixed function oxidases	Ornithine Decarboxylase
	Inhibitors of prostaglandin synthesis

2.4 Michael Acceptors

Cancer chemopreventive agents are classified by the mechanism in which they inhibit carcinogenesis. According to studies conducted by Arnold and Wilkinson et.al, indicates there are three distinct categories that majority of cancer chemopreventives fall within; those are, agents that block the reactive metabolites from the target sites of cells, agents that suppress progression or promotion of neoplasia, and those that prevent metabolic activation of the carcinogen (Arnold, Wilkinson, Sharma, & Steele, 1995). There are nine classes of

chemopreventive agents; table 1 lists the nine classes. As seen in the table, some of these agents within this class are anti-oxidants, free radical scavengers, inducers of glutathione S-transferase, and anti-inflammatory. Some of the agents could aid in the inhibition of the binding and metabolism of carcinogens during the initiation phase, while others suppress the promotion phase of carcinogenesis.



Scheme 4. Michael adduct formation.

Michael acceptors are cancer chemopreventive agents that stop the progression of carcinogenesis. The mechanism the Michael acceptors begin with a formation of a covalent adduct of the Michael donor to the Michael acceptor (Dorai & Aggarwal, 2004). Scheme 4 illustrates the formation of the covalent Michael adduct. Biologically native Michael donors are compounds that are found within the body such as glutathione S-tranferase. We envisage that a nontoxic xenobiotic such as coumarin derivatives (Michael acceptors) can be introduced into the body and will begin a cascade of detoxification processes.

Coumarins are well known cancer chemopreventive agents and acts as an excellent Michael acceptor as it has proven capabilities of suppressing the progression of neoplasia in human lung adenocarcinoma cells (Goel et al., 2007). This occurs by allowing for the cells to undergo apoptosis. Figure 3 shows the structure of coumarin and its respective thio analog. As seen, the structures of the molecules are similar. The only variance is the oxygen atom is substituted for a sulfur atom. To improve the 1,4-addition, a sulfur atom is substituted at the 2

position of the coumarin due to the softness of sulfur. Softness in organic chemistry refers to an atom that has a small oxidation state (charge on the atom) and is strongly polar meaning its ability to share electrons with other atoms is high. Oxygen is a hard atom which means that it is smaller than sulfur and in conjunction with being very electronegative these factors are responsible for its hardness. Sulfur is twice the size of oxygen and very polar which allows Sulfur to share its electrons with other atoms. By making the substitution, the Michael acceptor is believed to be better at inhibiting carcinogenesis. Studies by chemists, Kumar and Singh et.al, proves that 2H-chromene-2-thione derivatives have better cancer chemopreventive activity than its analogous coumarin derivative. The 2H-chromene-2-thione derivatives studied showed free radical scavenging and anti-inflammatory properties.

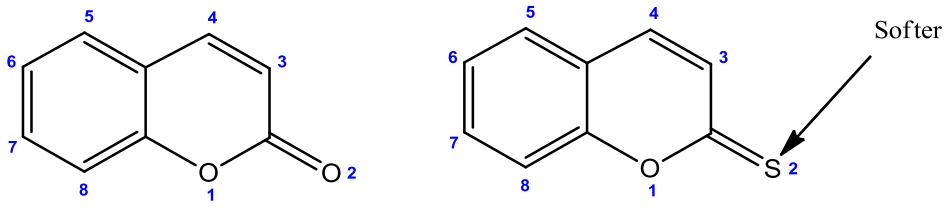


Figure 5. Structure of 2H-chromene-2-one and thione.

2.5 2H-Chromene-2-Thione Derivatives as Chemopreventive Agents

Few studies have been conducted on 2H-chromene-2-thione derivatives and their ability to act as cancer chemopreventive agents. However, via the research of Sharma, 2H-chromene-2-thione have proven to obtain excellent capabilities in being utilized as cancer chemopreventive compounds due to their potency (Kumar et al., 2005). 2H-chromene-2-thione derivatives and their ability to inhibit TNF-α induced ICAM-1 expression on human umbilical vein endothelial cells have been studied (Kumar et al., 2005). TNF-α is tumor necrosis factor a signaling protein that is responsible for inflammatory response in humans. This is why individuals with chronic disorders such as asthma, and rheumatoid arthritis experience episodes of inflammation. TNF-α

is responsible for the expression of Inter Cellular Adhesion Molecule-1 (ICAM-1) which is a protein expressed by human genes that are typically found in endothelial cells. ICAMs are molecules that are responsible for cell to cell binding to allow for transmigration of the leukocytes into the endothelial cells that are located within blood vessels. With the overexpression of ICAM-1, this can allow for infiltration of leukocytes across the blood vessels and allow for inflammation to occur. S. Kumar, and B. Singh during their study, concluded that 7,8-dibenzyloxy-4-methyl-2H-chromene-2-thione was superior over the 7,8-dibenzyloxy-4-methylcoumarin in the inhibition of TNF-α induced ICAM-1 expression (Kumar et al., 2005). The structure of the two chemical compounds may be an explanation as to why the 2H-chromene-2-thione worked better than the coumarin (Kumar et al., 2005).

Sulfur is of significance in this research because sulfur is one of the components in numerous organic compounds that have biological functions within the body. The compounds that were synthesized in this research will potentially induce enzymes found within the body. The thio group is a very important component in regards to biological functions. For example, glutathione, a peptide which contains a sulfur group, is involved in protein and DNA synthesis. Environmental and drug toxin metabolism and amino acid transports are some of the many functions of glutathione as well (Trudy McKee, 2011). Glutathione can also be used as a reducing agent by protection of cells from the vicious effects of oxidation. How this occurs is glutathione reacts with peroxides which are bi-products of O_2 metabolism (Trudy McKee, 2011). An example of this is, in red blood cells, hydrogen peroxide (H_2O_2) oxidizes the iron in hemoglobin to its ferric form Fe^{+3}. From this reaction a product methemoglobin, is produced. However, methemoglobin cannot attach itself to oxygen, therefore glutathione acts as a reducing agent by reducing hydrogen peroxide to protect against the formation of methemoglobin (Trudy

McKee, 2011). Biological important peptides, glutathione, oxytocin, cysteine, and vasopressin all have intra cellular antioxidant activity, and adulteration of these biothiols can be possible warning sign of AIDS and various cancers (Kim, Lee, Kwon, & Kim, 2011); hence, the synthesis of 2H-chromene-2-thione derivatives and determining their potency (their ability to aid the body in retarding the growth of cancer cells) is of great importance (Maresca et al., 2009).

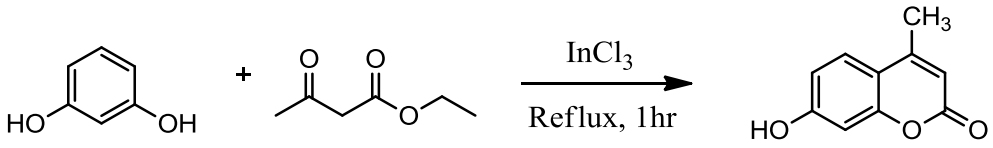
CHAPTER 3

Methodology

3.1 Experimental

3.1.1 Materials and methods. For this research, Indium (III) chloride, Lawesson's Reagent, Zirconium (IV) Chloride and various Resorcinol derivatives where purchased from Acros Chemicals. Ethyl acetoacetate was purchased from Sigma-Aldrich. DMSO ampoules (0.75mL) were purchased from Cambridge Isotope Lab Incorporated and the Ethanol used for recrystallization was purchased from Fisher. All reagents and solvents were used as purchased.

3.1.2 Characterization of compounds. For characterization purposes, ^{1}H and ^{13}C Nuclear Magnetic Resonance analysis were obtained using a 300 MHz NMR instrument from Varian and 400 MHz instrument from Varian used at the North Carolina Agricultural and Technical State University Joint School of Nanoscience and Nanoengineering. Preparation of the coumarin samples for NMR analysis was carried out using ampoules of Dimethyl Sulfoxide (0.75mL) to dissolve the coumarin. Each sample was dissolved with the DMSO and placed into sample tubes for NMR analysis. ^{1}H and ^{13}C NMR chemical shifts for the coumarin and 2H-chromene-2-thione are expected to vary in the ^{1}H and ^{13}C NMR. This variation will determine if the coumarin was converted into its thio analog.



Scheme 5. Synthesis of 7-hydroxy-4-methylcoumarin.

3.2 Synthesis of Coumarin Derivatives

3.2.1 Synthesis of 7-hydroxy-4-methylcoumarin (3). As shown in scheme 4, the synthesis of 7-hydroxy-4-methylcoumarin took place via a mixture of 1.103g of Resorcinol (1.0

mmol) and 1.304g of ethyl acetoacetate (1mmol) that refluxed for 1hr. in the presence of Indium Chloride as the catalyst. After the reflux, the reaction mixture was cooled to room temperature, and then washed with cold deionized water. After the water wash, the reaction mixture was filtered and re-crystallized with hot EtOH. After recrystallization, the product was filtered and washed with EtOH. In the final step, the product was placed into a storage vial and onto the high vacuum line for further drying overnight. Beige powder; 80% yield; m.p. 184^{o}C (Sharma, Janardhan Reddy, Sree Lakshmi, & Radha Krishna, 2005) ^{1}H NMR (301 MHz, DMSO-d_6) δ 10.53 (s, 1H), 7.58 (d, J = 8.7 Hz, 1H), 6.79 (d, J = 11.0 Hz, 1H), 6.69 (s, 1H), 6.12 (s, 1H), 2.35 (d, J = 1.0 Hz, 3H).

3.2.2 Synthesis of 7-hydroxy-4,8-dimethylcoumarin (4). The synthesis of 7-hydroxy-4,8 dimethylcoumarin took place via a mixture of 1.24g of Resorcinol (1.0 mmol) and 1.304g of Ethyl Aceto Acetate (1.0 mmol) that refluxed for 1hr. white powder; 84% yield; m.p. 253-255^{o}C (Sharma et al., 2005) ^{1}H NMR (301 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.54 – 7.36 (m, 2H), 6.86 (d, J = 9.4 Hz, 2H), 6.12 (s, 2H), 2.36 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (101 MHz, cd_3cn) δ 197.56, 163.24, 157.82, 146.33, 126.05, 115.20, 113.72, 100.36, 55.88, 29.90, 17.18.

3.2.3 Synthesis of 6-chloro-7-hydroxy-4-methylcoumarin (5). The synthesis of 6-chloro-7-hydroxy-4-methylcoumarin took place via a mixture of 1.45g of 4-Chloro Resorcinol (1.0 mmol) and 1.304g of ethyl acetoacetate (1.0 mmol) that refluxed for 1hr. Brown powder; 8% yield; m.p. 278^{o}-280^{o}C (Chakravarti & Ghosh, 1935) ^{1}H NMR (301 MHz, DMSO-d_6) δ 11.40 (s, 1H), 7.75 (s, 1H), 6.88 (s, 1H), 6.20 (s, 1H), 2.37 (s, 3H).

3.2.4 Synthesis of 6-n-dodecyl-7-hydroxy-4-methylcoumarin (6). The synthesis of 7-hydroxy-4-methylcoumarin took place via a mixture of 2.784g of 4-n-dodecyl Resorcinol (1.0 mmol) and 1.304g of ethyl acetoacetate (1.0 mmol) that refluxed for 1hr. Tan crystals; 84.8%

yield; m.p. 138^{o}C (Kansara & Shah, 1948); ^{1}H NMR (301 MHz, DMSO-d_6) δ 10.46 (s, 1H), 7.40 (s, 1H), 6.70 (s, 1H), 6.08 (d, *J* = 1.3 Hz, 1H), 2.34 (d, *J* = 1.2 Hz, 3H), 1.51 (p, *J* = 6.6 Hz, 2H), 1.22 (d, *J* = 12.1 Hz, 15H), 0.82 (t, *J* = 6.4 Hz, 3H).

3.2.5 Synthesis of 7,8-dihydroxy-4-methylcoumarin (7). The synthesis of 7,8-dihydroxy 4-methylcoumarin took place via a mixture of 1.261g of Pyrogallol (1.0 mmol) and 1.304g of ethyl acetoacetate (1.0 mmol) that refluxed for 1hr. purple crystals; 46.4% yield; m.p. 241-243^{o}C (Sharma et al., 2005) ^{1}H NMR (301 MHz, DMSO-d_6) δ 7.70 (s, 1H), 7.68 (s, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 6.95 (s, 1H), 6.21 (s, 1H), 3.86 (s, 5H), 2.40 (s, 3H).

3.2.6 Synthesis of 7-methoxy-4-methylcoumarin (8). The synthesis of 7-methoxy-4-methylcoumarin took place via a mixture of 1.241g of 3-methoxy phenol (1.0 mmol) and 1.304g of ethyl acetoacetate (1.0 mmol) that refluxed for 1hr. white powder; 33.3% yield; m.p. 159-160^{o}C (Shirini, Zolfigol, & Albadi, 2010); ^{1}H NMR (301 MHz, DMSO-d_6) δ 10.04 (s, 1H), 9.28 (s, 1H), 7.09 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 1H), 6.12 (s, 1H), 2.35 (s, 3H).

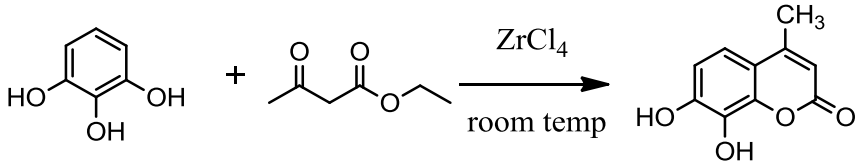
3.2.7 Synthesis of 5,7-dihydroxy-4-methylcoumarin (9). The synthesis of 5,7-dihydroxy 4-methylcoumarin took place via a mixture of 1.261g of Phloroglucinol (1.0 mmol) and 1.304g of ethyl acetoacetate (1.0 mmol) that refluxed for 1hr. Orange powder; 66% yield; m.p. 280-281^{o}C (Sharma et al., 2005); ^{1}H NMR (301 MHz, DMSO-d_6) ^{1}H NMR (301 MHz, DMSO-d_6) δ 10.52 (s, 1H), 10.29 (s, 1H), 6.25 (d, *J* = 2.3 Hz, 1H), 6.16 (d, *J* = 2.3 Hz, 1H), 5.84 (d, *J* = 1.2 Hz, 1H), 2.48 (s, 3H).

3.2.8 Synthesis of 5-hydroxy-4,7-dimethylcoumarin (10). As shown in scheme 10, the synthesis of 5-hydroxy-4,7-dimethylcoumarin took place via a mixture of 1.065g of orcinol (1.0 mmol) and 1.116g of ethyl acetoacetate (1.0 mmol) that refluxed for 1hr. white powder; 76%

yield m.p. 259-260°C (Y. Wang, Xu, Tian, Li, & Wang, 2009) ^{1}H NMR (301 MHz, DMSO-d_6) δ 6.62 (s, 1H), 6.57 (s, 1H), 6.05 (s, 1H), 2.54 (s, 3H), 2.27 (s, 3H).

3.2.9 Synthesis of 7-methoxy-4-methylcoumarin (11). As shown in scheme 11, the synthesis of 7-methoxy-4-methylcoumarin took place via a mixture of 1.241g of 3-methoxy phenol (1.0 mmol) and 1.304g of ethyl acetoacetate (1.0 mmol) that refluxed for 1hr.Ivory powder; 40% yield; m.p. 159-160°C (Shirini et al., 2010) ^{1}H NMR (301 MHz, DMSO-d_6) δ 6.98 (s, 1H), 6.20 (s, 1H), 3.85 (s, 3H), 2.39 (d, J = 1.3 Hz, 3H).

3.3 Synthesis of Coumarin Derivatives with $ZrCl_4$



Scheme 6. Synthesis of 7,8-dihydroxy-4-methylcoumarin.

3.3.1 Synthesis of 7,8-dihydroxy-4-methylcoumarin (12). Into a 50mL round bottom flask 1.26g of pyrogallol, 1.30g of ethyl acetoacetate was allowed to react in the presence of 0.233g of $ZrCl_4$. The reaction took place at room temperature and stirred for 10 minutes. After 10 minutes, the reaction mixture was quenched with cold DI water, washed (3x with cold DI water) and filtered via vacuum filtration. After the filtration, the product was placed into a storage vial, and placed onto the hot vacuum for drying overnight. Purple powder ; 21% yield; m.p. 241-243°C (Sharma et al., 2005) ^{1}H NMR (301 MHz, Methanol-d_4) δ 4.82 – 0.93 (m, 1H).

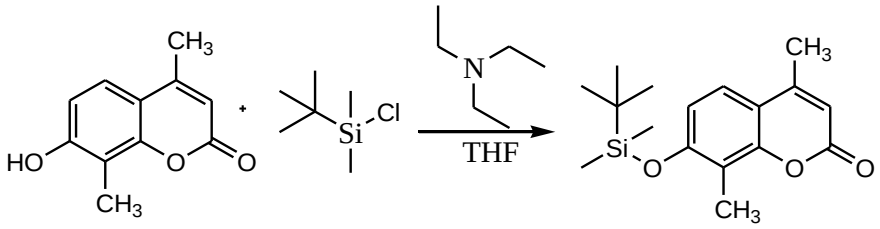
3.3.2 Synthesis of 7-hydroxy-4-methylcoumarin (13). (1.10g) of Resorcinol, (1.30g) ethyl acetoacetate, (0.145g) $ZrCl_4$. White powder ; 35% yield. m.p. 184°C (Sharma et al., 2005) ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H), 7.05 (d, J = 8.5 Hz, 1H), 6.46 (d, J = 2.5 Hz, 1H), 6.20 (s, 1H), 1.64 (s, 3H).

3.3.3 Synthesis of 5-hydroxy-4,7-dimethylcoumarin (14). (1.24g) of Orcinol, (1.30g) ethyl acetoacetate, (0.232g) $ZrCl_4$. 13% yield; m.p.259-260^oC (Sharma et al., 2005); ^1H NMR (301 MHz, DMSO-d_6) δ 10.54 (s, 1H), 6.62 (s, 1H), 6.56 (s, 1H), 6.06 (s, 1H), 2.28 (s, 3H), 2.09 (s, 3H).

3.3.4 Synthesis of 6-chloro-7-hydroxy-4-methylcoumarin (15). (2.89g) of 4-chloro resorcinol, (2.60g) ethyl acetoacetate, (0.931g) $ZrCl_4$. Beige solid; 11% yield; m.p. 278-280^oC (Chakravarti & Ghosh, 1935) ^1H NMR (301 MHz, Pyridine-d_5) δ 9.03 (s, 1H), 7.96 (d, J = 42.4 Hz, 1H), 7.52 (s, 1H), 6.49 (s, 1H).

3.3.5 Synthesis of 7-hydroxy-4-methylcoumarin (16). (1.10g) of Resorcinol, (1.30g) ethyl acetoacetate, (0.145g) $ZrCl_4$. Purple crystals; 68% yield; m.p. 184^oC (Sharma et al., 2005); ^1H NMR (301 MHz, DMSO-d_6) δ 10.52 (s, 1H), 7.65 – 7.53 (m, 1H), 6.70 (t, J = 1.8 Hz, 1H), 6.11 (d, J = 1.8 Hz, 1H), 2.43 – 2.28 (m, 3H).

3.4 Synthesis of TBDMS Coumarin Derivatives



Scheme 7. Synthesis of 7-TBDMS-4,8-dimethylcoumarin.

3.4.1 Synthesis of 7-tbdms-4,8-dimethylcoumarin (17). A solution of 7-hydroxy-4,8-dimethylcoumarin (0.5g, 1.0eq) was treated with TBDMSCl (1.1eq) and Et_3N (1.0eq) in THF (20mL).The reaction was stirred at room temperature until the starting material was no longer in solution (~18hrs). The reaction was monitored via TLC to determine if the reaction was complete. The reaction was quenched with H_2O (40mL) and extracted with ethyl acetate (3x 15mL). The organic layers were combined and washed with H_2O (2x 20mL), and dried with

Na_2SO_4. The product was filtered and concentrated under vacuum to give a residue that was purified by silica gel column chromatography eluting with 20% ethyl acetate/n-hexane (v/v). white powder; 68% yield; ^{1}H NMR (301 MHz, DMSO-d_6) δ 10.47 (dd, J = 15.8, 9.1 Hz, 3H), 7.62 – 7.42 (m, 3H), 6.90 (q, J = 8.6, 7.5 Hz, 3H), 6.29 – 6.09 (m, 3H), 5.44 – 5.23 (m, 2H), 3.61 – 3.41 (m, 7H), 3.02 (s, 1H), 2.96 – 2.76 (m, 1H), 2.62 (s, 3H), 2.45 – 2.34 (m, 8H), 2.31 – 2.12 (m, 9H), 1.11 – 0.81 (m, 29H), 0.21 – 0.04 (m, 2H).

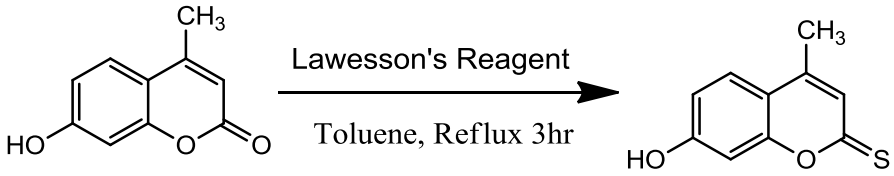
3.4.2 Synthesis of 5,7-(tbdms)$_2$-4-methylcoumarin (18). 5,7-dihydroxy-4-methylcoumarin 0.808g, (1.0eq) was treated with 0.696g TBDMSCl (1.1eq) and 0.425g Et_3N (1.0eq) in THF (20mL).The reaction was stirred at room temperature until the starting material was no longer in solution (~18hrs). Ivory powder; 21% yield ^{1}H NMR (301 MHz, DMSO-d_6) δ 6.23 (s, 1H), 6.14 (s, 1H), 5.83 (s, 1H), 1.21 (s, 2H).

3.4.3 Synthesis of 5-tbdms-4,7-dimethylcoumarin (19). 5-hydroxy-4,7-dimethylcoumarin 0.752g, (1.0eq) was treated with TBDMSCl 0.447g (1.1eq) and 0.274g Et_3N (1.0eq) in THF (20mL).The reaction was stirred at room temperature until the starting material was no longer in solution (~18hrs). 0.31% yield; ^{1}H NMR (301 MHz, Acetonitrile-d_3) δ 6.67 (s, 1H), 6.56 (s, 1H), 5.98 (d, J = 1.5 Hz, 1H), 2.56 (d, J = 6.4 Hz, 3H), 2.32 (d, J = 6.0 Hz, 3H), 1.00 (d, J = 1.4 Hz, 2H), 0.36 (s, 1H).

3.4.4 Synthesis of 6-n-dodecyl-7-tbdms-4-methylcoumarin (20). 6-n-dodecyl- 7-hydroxy-4-methylcoumarin 2.0g, (1.0eq) was treated with 0.965g TBDMSCl (1.1eq) and 0.370g Et_3N (1.0eq) in THF (25mL).The reaction was stirred at room temperature until the starting material was no longer in solution (~18hrs). Tan solid; 47% yield; ^{1}H NMR (301 MHz, DMSO-d_6) δ 7.41 (s, 1H), 6.74 (d, J = 13.2 Hz, 1H), 6.09 (s, 1H), 2.37 (d, J = 7.9 Hz, 3H), 1.53 (s, 3H), 1.25 (d, J = 13.5 Hz, 19H), 0.99 (s, 2H), 0.94 – 0.71 (m, 5H), 0.28 (s, 1H), -0.05 (s, 1H).

3.4.5 Synthesis of 7,8-(tbdms)$_2$-4-methylcoumarin (21). 7,8-dihydroxy-4-methylcoumarin 1.980g (1.0eq) was treated with TBDMSCl (1.1eq) and 1.016g Et_3N (1.0eq) in THF (20mL).The reaction was stirred at room temperature until the starting material was no longer in solution (~18hrs). White powder; 15% yield; ^{1}H NMR (301 MHz, DMSO-d_6) δ 6.91 (d, J = 8.5 Hz, 1H), 6.63 (d, J = 8.4 Hz, 1H), 5.95 (s, 2H), 2.33 (s, 3H), 2.18 (s, 6H), 0.84 (s, 6H).

3.5 Synthesis of 2H-Chromene-2-Thione Derivatives



Scheme 8. Synthesis of 7-hydroxy-4-methyl-2H-chromene-2-thione.

3.5.1 Synthesis of 7-hydroxy-4-methyl-2h-chromene-2-thione (3a). As shown in scheme 12, the synthesis of 7-hydroxy-4-methyl-2H-chromene-2-thione will take place via a mixture of 7-methoxy-4-methylcoumarin (5.0 mmol) and Lawesson's Reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. After the reflux, the reaction mixture was evaporated under reduced pressure to collect the product. The solid product was re-crystallized from methanol to give 7-hydroxy-4-methyl-2H-chromene-2-thione. Brown solid crystals. 11% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 7.72 (s, 1H), 7.06 (s, 1H), 6.93 (s, 1H), 6.88 (s, 1H), 2.35 (s, 3H). IR (KBr): ν 3183, 2366, 1619, 1595, 1546, 1443, 1397, 1304, 1265, 1215, 1156, 1099, 1070 cm $^{-1}$

3.5.2 Synthesis of 7-hydroxy-4,8-dimethyl-2h-chromene-2-thione (4a). The synthesis of 7-hydroxy-4,8-dimethyl-2H-chromene-2-thione will take place via a mixture of 7-hydroxy-4,8 dimethylcoumarin (5.0 mmol) and Lawesson's Reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. Dark green solid; 54% yield; m.p. 205^{o}C ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.67 (dt, J = 8.7, 0.4 Hz, 1H), 7.30 – 6.60 (m, 3H), 3.89

(s, 3H), 2.33 (d, *J* = 1.1 Hz, 4H). ^{13}C NMR (101 MHz, cd_3cn) δ 197.56, 163.24, 157.82, 146.33, 126.05, 115.20, 113.72, 100.36, 55.88, 29.90, 17.18. IR (KBr): ν 1632, 1599, 1546, 1513, 1447, 1387, 1298, 1245, 1208, 1149, 1096 cm^{-1}

3.5.3 Synthesis of 7-tbdms-4,8-dimethyl-2h-chromene-2-thione (4b). The synthesis of Synthesis of 7-tertbutyldimethylsilyloxy-4,8-dimethyl-2H-chromene-2-Thione will take place via a mixture of 7-tertbutyldimethylsilyloxy-4,8-dimethylcoumarin (5.0 mmol) and Lawesson's Reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. Yellow powder; 33% yield; m.p. 243^{o}C; ^{1}H NMR (301 MHz, DMSO-d_6) δ 7.59 (dd, *J* = 13.8, 6.6 Hz, 1H), 7.21 (d, *J* = 21.9 Hz, 1H), 6.99 (s, 1H), 2.43 – 2.02 (m, 3H), 0.85 (d, *J* = 6.7 Hz, 1H), 0.13 (s, 1H), -0.05 (s, 1H). IR (KBr): ν 1615, 1595, 1556, 1500, 1443, 1387, 1278, 1156, 1106, 1020, 755 cm^{-1}

3.5.4 Synthesis of 6-chloro-7-hydroxy-4-methyl-2h-chromene-2-thione (5a). The synthesis of 6-chloro-7-hydroxy-4-methyl-2H-chromene-2-thione will take place via a mixture of 6-chloro-7-hydroxy-4-methylcoumarin (5.0 mmol) and Lawesson's Reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. An azeotrope was done using ethanol to remove the solvent from the reaction mixture. Brown powder. 46% yield. m.p. 243^{o}C; ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.67 (s, 1H), 7.00 (s, 1H), 6.56 (s, 0H), 5.01 (s, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 197.02, 157.76, 155.88, 144.23, 127.12, 113.81, 112.07, 108.66, 55.12, 22.38.

3.5.5 Synthesis of 6-n-dodecyl-7-hydroxy-4-methyl-2h-chromene-2-thione (6a). As shown in scheme 14, the synthesis of 6-n-dodecyl-7-hydroxy-4-methyl-2H-chromene-2-thione will take place via a mixture of 6-n-dodecyl-7-hydroxy-4-methylcoumarin (5.0 mmol) and Lawesson's reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry)

toluene for 3 hours. Yellow powder 31% yield; m.p. 299^{o}C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.55 (s, 1H), 7.03 (t, *J* = 1.1 Hz, 1H), 6.90 (s, 1H), 2.63 – 2.56 (m, 2H), 2.36 (d, *J* = 1.1 Hz, 3H), 1.34 – 1.18 (m, 16H), 0.90 – 0.81 (m, 3H). ^{13}C NMR (101 MHz, cdcl$_3$) δ 197.25, 158.62, 156.35, 128.92, 125.99, 125.27, 115.01, 111.69, 102.54, 31.90, 30.93, 29.99, 29.65, 29.62, 29.59, 29.48, 29.47, 29.33, 22.67, 18.20, 14.10. IR (KBr): ν 2915, 1625, 1559, 1390, 1265, 1215 cm $^{-1}$

3.5.6 Synthesis of 7,8-dihydroxy-4-methyl-2h-chromene-2-thione (7a). As shown in scheme 15, the synthesis of 7,8-dihydroxy-4-methyl-2H-chromene-2-thione thiocoumarin will take place via a mixture of 7,8-dihydroxy-4-methylcoumarin (5.0 mmol) and Lawesson's Reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. Yellow solid; 21% yield; m.p. 230-235^{o}C ^{1}H NMR (DMSO-d$_6$ + CDCl3, 300 MHz): d 2.34 (3H, s, CH3), 6.95 (1H, d, J = 8.6Hz, C-6H), 7.02 (1H, s, C-3H), 7.09 (1H, d, J = 8.6 Hz, C-5H), 8.83 and 8.94 (2H, 2s, 1H each, 2 · OH); ^{13}C NMR (DMSOd$_6$+ CDCl$_3$, 75.5 MHz): d 20.68 (CH$_3$), 116.42 (C-6), 117.72 (C-10), 118.07 (C-5), 118.31 (C-3), 134.23 (C-4), 148.55 and 148.93 (C-7 and C-8), 151.96 (C-9) and 199.31 (C-2). IR (KBr): 3312 (OH), 2925, 2855, 1561, 1434, 1366, 1254 and 1095 cm^{-1} (Kumar et al., 2005)

3.5.7 Synthesis of 7-methoxy-4-methyl-2h-chromene-2-thione (8a). As shown in scheme 16, the synthesis of 7-methoxy-4-methyl-2H-chromene-2-thione will take place via a mixture of 7-methoxy-4-methyl coumarin (5.0 mmol) and Lawesson's Reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. An azeotrope was done using hexanes to extract the product from the reaction mixture. Brown crystals; 61% yield; m.p. 183^{o}C; ^{1}H NMR (400 MHz, Acetonitrile-*d*$_3$) δ 7.74 – 7.63 (m, 1H), 7.04 (q, *J* = 1.1 Hz, 1H), 7.02 (d, *J* = 0.4 Hz, 0H), 7.01 (d, *J* = 2.5 Hz, 0H), 6.99 (d, *J* = 2.5 Hz, 0H), 3.90 (s, 3H), 2.34 (d, *J* = 1.1 Hz, 3H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, cd$_3$cn) δ 202.17, 197.60, 163.26,

157.85, 146.36, 126.28, 115.22, 113.73, 100.38, 55.89, 17.17. IR (KBr): ν 1625, 1599, 1546, 1443, 1387, 1294, 1245, 1208, 1146, 1070, 841 cm^{-1}

3.5.8 Synthesis of 5,7-dihydroxy-4-methyl-2h-chromene-2-thione (9a). As seen in scheme 17, the synthesis of 5,7-dihydroxy-4-methyl-2H-chromene-2-thione will take place via a mixture of 5,7-dihydroxy-4-methyl coumarin (5.0 mmol) and Lawesson's reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.84 – 7.61 (m, 1H), 7.10 – 6.94 (m, 1H), 5.63 (s, 1H). ^{13}C NMR (101 MHz, cd_3cn) δ 206.66, 162.71, 160.37, 157.24, 156.91, 133.08, 132.42, 113.65, 109.85, 23.00.

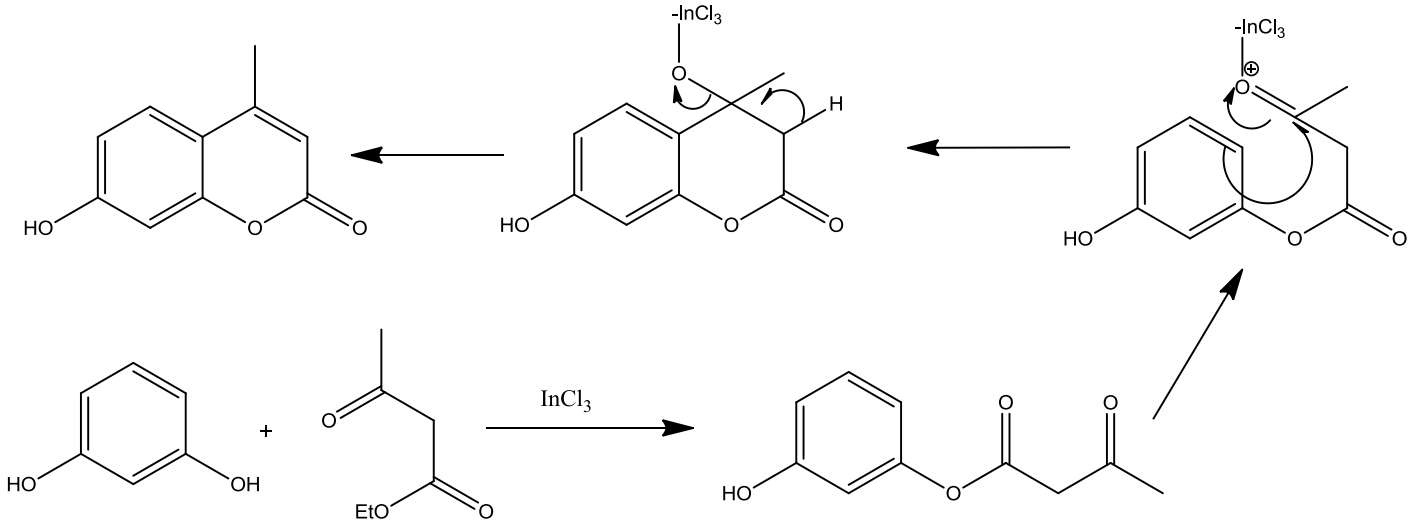
3.5.9 Synthesis of 5-hydroxy-4,7-dimethyl-2h-chromene-2-thione (10a). The synthesis of 5-hydroxy-4,7-dimethyl-2H-chromene-2-thione will take place via a mixture of 6-chloro-7 hydroxy-4-methyl coumarin (5.0 mmol) and Lawesson's Reagent (6.0 mmol) refluxed in ~30mL (less in some cases) of hot anhydrous (dry) toluene for 3 hours. Brown solid; 13% yield; m.p. 249-250^{o}C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 (d, *J* = 38.5 Hz, 1H), 6.78 (s, 1H), 6.64 (s, 1H), 6.47 (s, 1H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 200.96, 162.49, 161.98, 153.01, 149.10, 131.62, 113.82, 112.74, 27.94, 26.46. IR (KBr): ν 1625, 1586, 1539, 1506, 1443, 1278, 1338, 1156, 1132, 828 cm^{-1}

CHAPTER 4

Results and Discussion

4.1 Discussion

To improve the effectiveness of coumarin as a therapeutic compound for the treatment of cancer, a sulfur atom was substituted at the 2 position on the coumarin. To increase the potency of the compound (meaning to increase the compounds ability to enhance the activity of mechanisms already within the body) a sulfur atom was substituted for this reason also. Each coumarin and 2H-chromene-2-thione derivatives were synthesized successfully. To determine if indeed the coumarin and 2H-chromene-2-thione derivatives were synthesized, the NMR instrument was used to characterize each compound synthesized.



Scheme 9. Mechanism of Von Pechmann condensation.

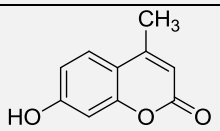
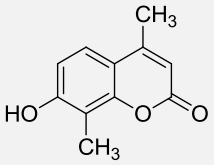
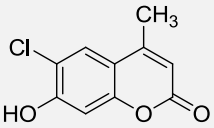
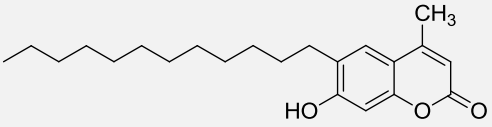
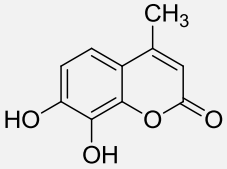
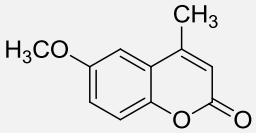
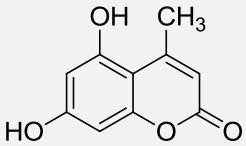
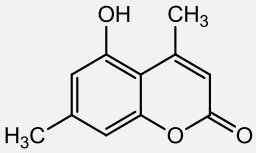
4.1.1 Mechanism of the von pechmann condensation. The Von Pechmann condensation takes place via the condensation of a β-keto ester and a phenol. Derivatives of Resorcinol were utilized as the phenol in this research. A strong Brøsted-Lowry Acid or a Lewis Acid is required to provide the acidic conditions to carry out the transformation of the coumarin

derivatives. Scheme 8 illustrates the mechanism for the coumarin formation. The reaction proceeds via the transesterification and keto-enol tautomerisation of the resorcinol and ethyl aceto acetate. A Michael 1,4 addition will take place to lay the frame work for the coumarin structure. The Michael addition takes place via a conjugate 1, 4 addition of a resonance stabilized carbanion (which is the Michael donor) to an activated α,β-unsaturated compound (Michael acceptor) (William H. Brown, 2005). The carbon-carbon double bond situated at the four position is the Michael acceptor. Rearomatisation follows the Michael addition and a loss of water gives the coumarin structure.

4.1.2 Comparison of lewis acid catalysts. The Von Pechmann condensation utilized in this research used green chemistry via a one pot synthesis that required no solvent. No solvent is required for the reaction to take place however, ethyl aceto acetate is a liquid reagent which creates a homogenous mixture with the catalyst and resorcinol and the reaction can proceed as followed. In this particular research, $InCl_3$ was chosen as the Lewis Acid to carry out the formation of the coumarin derivatives due to its effectiveness as a catalyst, obtaining the coumarin derivatives in 80-90% percent yields. $InCl_3$ although an excellent catalyst for the Von Pechmann condensation it is expensive selling at $75 for 10g of the catalyst. $ZrCl_4$, however is inexpensive selling at $39 for 10g. In this research, strategies to efficiently synthesize the coumarin derivatives were investigated. To allow for a more green synthesis of the coumarin derivatives $ZrCl_4$ was explored as a the Lewis Acid in the Von Pechmann reaction as it has proven capability to effectively carry out the transformation of coumarin derivatives in a solvent free ten minute reaction at room temperature (Sharma et al., 2005). For this reason, another set of coumarin derivatives in the research utilized $ZrCl_4$ as the catalyst. Table 1 displays the coumarin derivatives synthesized utilizing $InCl_3$ and $ZrCl_4$ as the catalyst.

Table 2

Percent yields of $InCl_3$ and $ZrCl_4$ Catalyzed procedure

Compound	$InCl_3$ Percent Yield (%)	$ZrCl_4$ Percent Yield (%)
	80	68
	84	___
	13	11
	85	___
	46	21
	33	___
	66	___
	76	13

Shown in table are the percent yields obtained for the respective coumarin derivatives synthesized. The coumarin derivatives synthesized using $InCl_3$ that contain hydroxyl substituents all had semi relatively high yields (with the exception of 7,8-dihydroxy-4-methylcoumarin in 46% percent yield). The 6-chloro-7-hydroxy-4-methylcoumarin resulted in a very low yield; this could possibly be due to the solubility of the reagent 4-chloro resorcinol. Table 2 also shows the

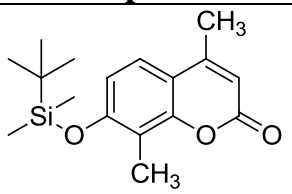
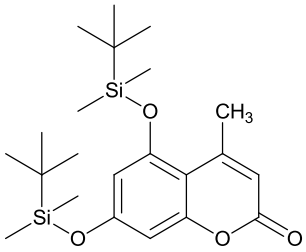
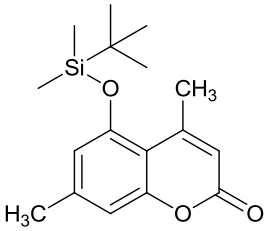
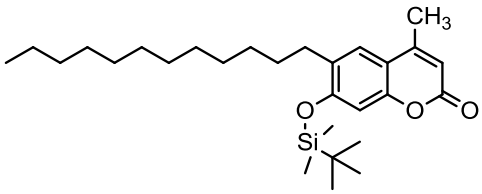
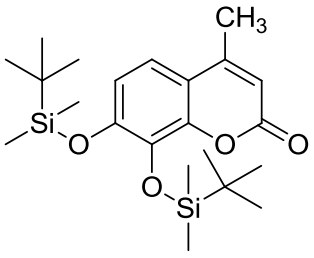
percent yields of the coumarins obtained via the $ZrCl_4$ procedure. Although the yields were fair, from the NMR spectra it proved that several coumarin derivatives were not synthesized, due to impurities within the spectra. For the coumarin derivative 7,8-dihydroxy-4-methylcoumarin a suitable NMR solvent could not be obtained to perform NMR analysis. Both DMSO and CH_2Cl_2 were utilized as the solvent for NMR analysis of the compound but attempts were unsuccessful. It was proposed that possibly the hydroxyl groups being adjacent to each other chelated with the Zirconium in $ZrCl_4$.

4.2 Discussion of TBDMS Coumarin Derivatives

4.2.1 Tertbutyldimethylsilyloxy (tbdms) coumarin derivatives. One method of synthesizing the 2H-chromene-2-thione derivatives successfully involved a protection step of the hydroxyl substituents attached to the coumarin atom using t-butyldimethylchlorosilane, triethyl amine in THF, to minimize the reactivity of the oxygen atoms. The percent yields of the protected coumarin derivatives range from 68%-15% respectively. Table 3 shows the experimental results obtained from the protection of the hydroxyl substituents on the coumarin molecules. The trend shown in the table is the coumarin derivatives that contained multiple hydroxyl substituents had lower percent yields than the coumarin derivatives that contained only one hydroxyl substituent with the exception of 6-n-dodecyl-7-TBDMS-4-methylcoumarin which obtained the highest percent yield (74%). The 12 carbon chain is, therefore steric hindrance is is relatively minimized in comparison to branched chain structures. In the 7,8-di-TBDMS-4-methylcoumarin, the percent yield is lower due to steric hindrance from the branching of both substituents that are adjacent to each other. The percent yield of 5-TBDMS-4,7-dimethylcoumarin was extremely low due insoluble precipitate formation during the work up steps to obtain the coumarin derivative.

Table 3

Percent Yields of the Protected Coumarins

Compounds	Percent Yields (%)
	68
	21
	0.31
	74
	15

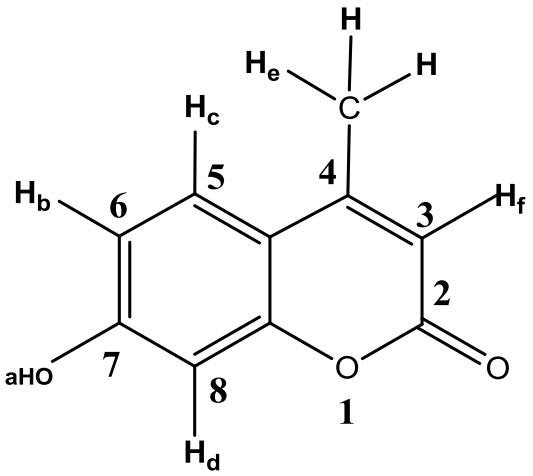


Figure 6. Expected NMR chemical shifts.

4.3 Expected Coumarin NMR Spectra

For the ^{1}H NMR spectra of the coumarin derivatives a singlet peak around 2.3 ppm is expected. This is indicative of methyl protons bound to the allylic carbon on the coumarin molecule. This is a key characteristic of the 4 substituted coumarin derivatives synthesized in this research. In the aromatic region of the ^{1}H NMR spectra two sets of doublets and two singlet peaks are expected from the ^{1}H NMR spectra. The protons responsible for these chemical shifts are the adjacent protons on the aromatic ring (Carbon 5 and 6 in figure 5) and the hydrogen attached to the carbon at the eighth position on the aromatic ring (carbon 8 in figure 5). The vinylic hydrogen (carbon 3 in figure 5) is responsible for the other singlet peak at 6.1ppm. Since various resorcinol's were used in the formation of the coumarins also, it is expected that there will be hydroxyl peaks in the NMR spectra around 10.0-11.0 ppm.

4.3.1 Expected thione spectra. With the 2H-chromene-2-thione derivatives, similar spectra to the coumarin derivatives is expected for these derivatives as well. However, there will be a chemical shift in the protons of the 2H-chromene-2-thione derivatives due to the sulfur atom attachment. From the variance in the ^{1}H and ^{13}C NMR Spectra of coumarin and the 2H-

chromene-2-thione derivatives, it will be determined if the 2H-chromen-2-thione derivatives were synthesized.

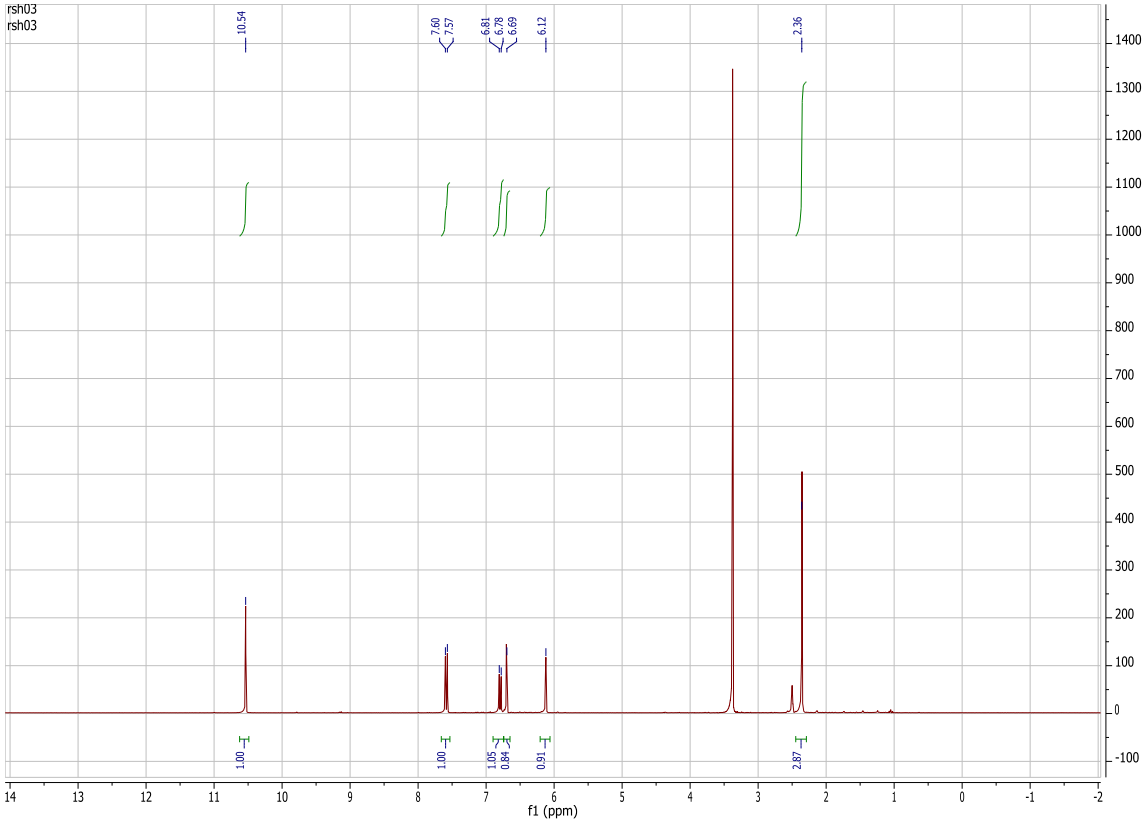


Figure 7. [1]H NMR spectra of 7-hydroxy-4-methylcoumarin.

4.3.2 Discussion of the 7-hydroxy-4-methylcoumarin spectra. The formation of coumarin **(3)** is shown in scheme 3. This reaction took place via the Von Pechmann condensation of equimolar amounts of ethyl aceto acetate and resorcinol. Figure 6 represents the NMR spectra obtained from 7-hydroxy 4-methylcoumarin. The coumarin was obtained in 80% yield. From the [1]H NMR spectra it shows that the desired coumarin was indeed synthesized. In the aromatic region, there are two sets of doublet peaks at 6.74ppm and 7.57ppm indicative of the neighboring hydrogen's on the aromatic ring. Also in the aromatic region there is a singlet peak at 6.11δ which is the chemical shift for the proton on the aromatic ring at the eighth carbon position

(shown in figure 5). The chemical shift at 10.5δ is the hydroxyl proton. The singlet peak at 6.0 is the chemical shift of the vinylic proton (the hydrogen attached to the alkene carbon). This proton is expected to undergo the most change of all the protons on the coumarin molecule due to the proton being adjacent to the sulfur atom in the thione derivatives.

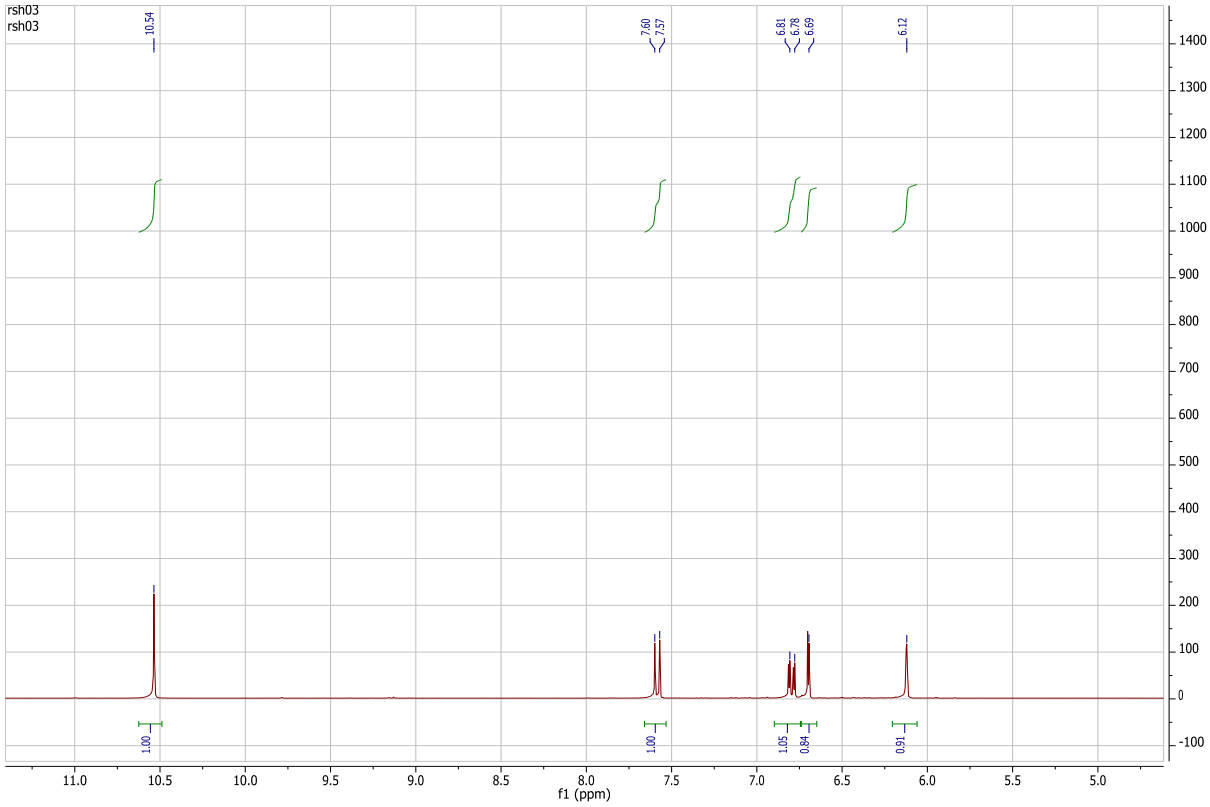


Figure 8. [1]H NMR spectra of the aromatic region of 7-hydroxy-4-methylcoumarin.

Also in the aromatic region as shown in figure 8, there is a singlet peak at 6.11δ which is the chemical shift for the proton on the aromatic ring at the eighth carbon position (shown in figure 5). The chemical shift at 10.5ppm is the hydroxyl proton.

The singlet peak at 6.0 is the chemical shift of the vinylic proton (the hydrogen attached to the alkene carbon). These protons are known to have a shift in this region. It is only when they are attached/adjacent to an electronegative atom that they experience a chemical shift. Therefore,

the vinylic proton is expected to undergo the most change of all the protons on the coumarin molecule due to the proton being adjacent to the sulfur atom in the thione derivatives.

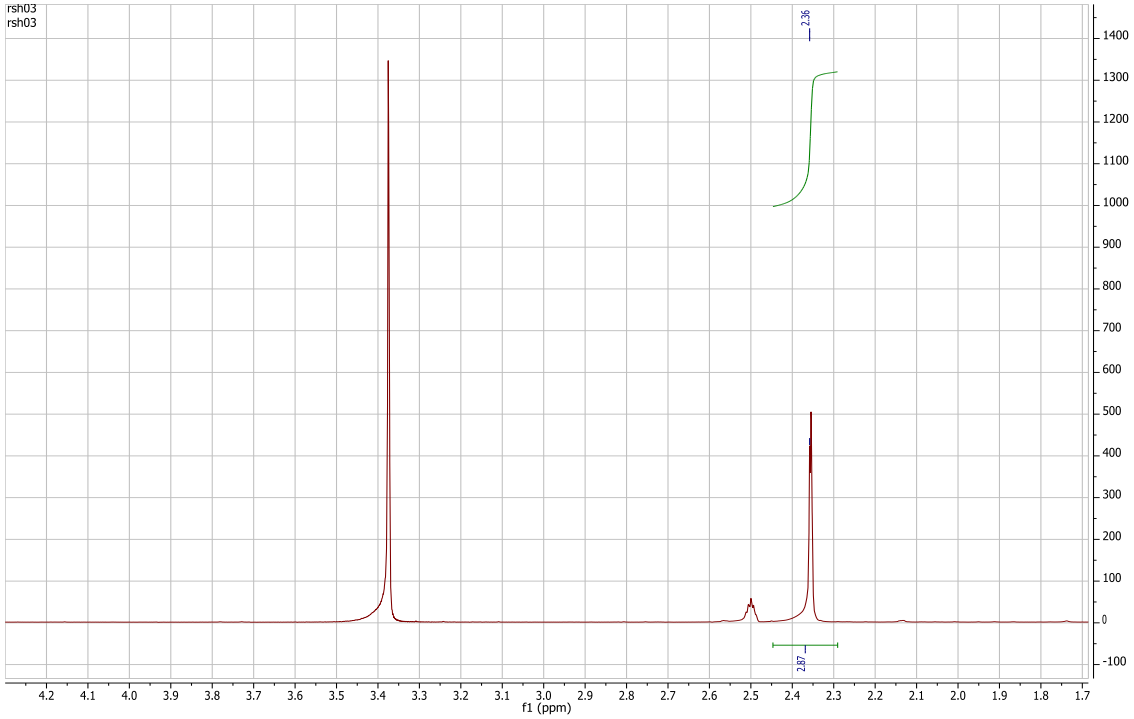


Figure 9. [1]H NMR spectra of the allylic hydrogen chemical shift of 7-hydroxy-4-methylcoumarin.

The methyl protons attached to the allylic carbon have a chemical shift at 2.34ppm. This chemical at 2.34ppm will be a singlet peak due to no neighboring hydrogens adjacent to the allylic protons. Also, the allylic proton shift is a key characteristic in the compounds synthesized in the research due to ethyl acetoacetate being the β-keto ester utilized in this research. If another β-keto ester derivative where used, there would be a difference in spectra due to a different atom being attached at the alkene position. The NMR solvent that was used for the NMR analysis was DMSO, which is responsible for the chemical shifts at 2.5ppm. The broad chemical shifts at 3.3ppm represent waters of hydration.

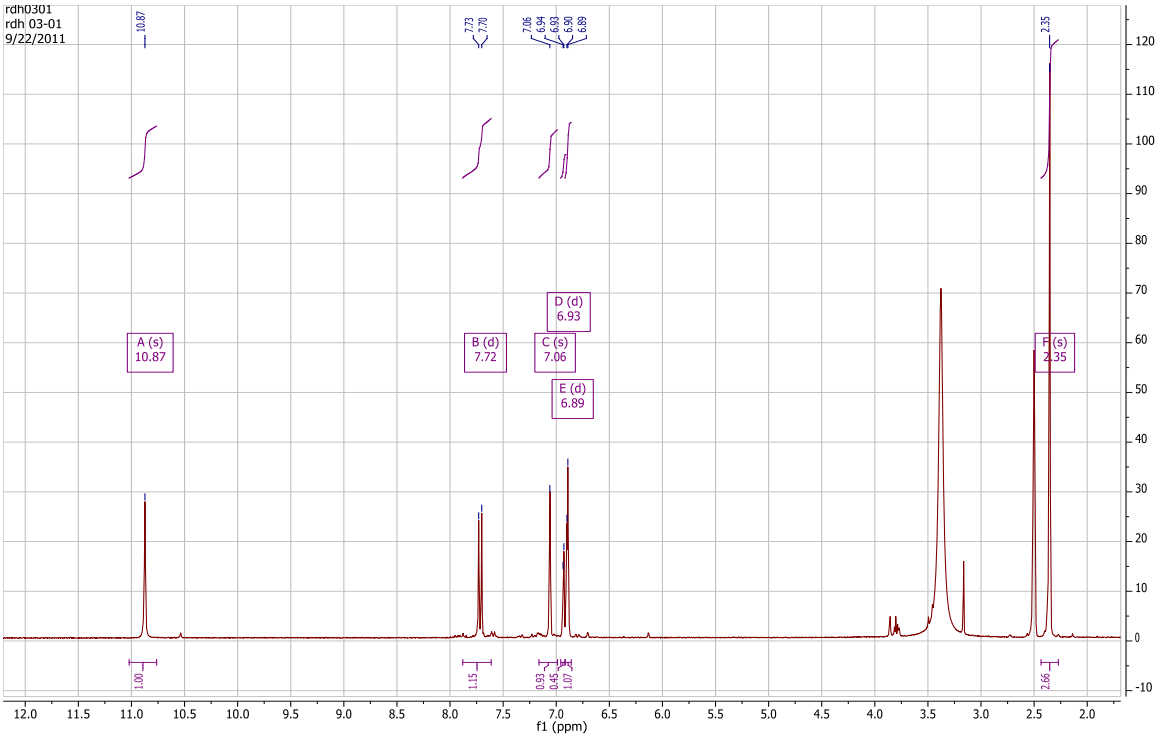


Figure 10. ^{1}H NMR spectra of 7-hydroxy-4-methyl-2H-chromene-2-thione.

4.3.3 Discussion of the thione spectra. As seen in the NMR spectra of 7 hydroxy-4-methyl 2H-chromene-2-thione, there is a change in the chemical shifts. The allylic and phenolic chemical shifts in the ^{1}H NMR of the thione derivative did not vary much from the ^{1}H NMR spectra of the coumarin derivative. The chemical shift that underwent the most change was the vinylic hydrogen as it has shifted from 6.0-6.1ppm where it comes in on the coumarin molecule to 7.0-7.05ppm in the ^{1}H NMR of the 2H-chromene-2-thione derivative. This indicates that the sulfur atom being substituted at the carbonyl, has affected this proton shift. However, in order to validate this claim, ^{13}C and infrared spectroscopy will be the determining factors for the prescence of the C=S bond, and the absence of the carbonyl stretch in the infrared spectra.

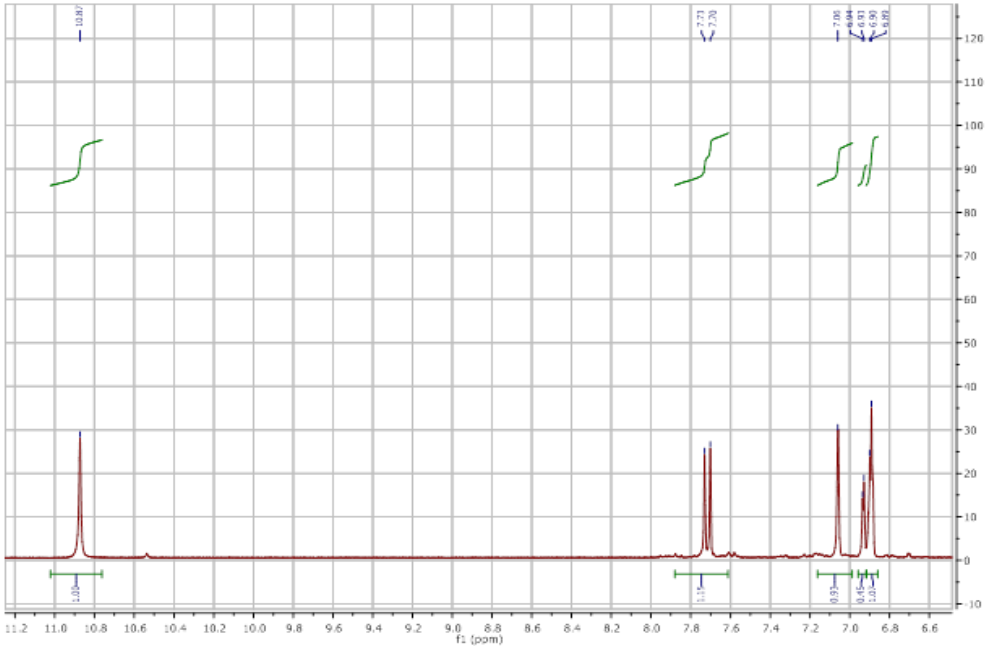


Figure 11. Aromatic region of the ^{1}H NMR spectra of 7-hydroxy-4-methyl-2H-chromene-2-thione.

Figure 11 is the aromatic region of the NMR spectra. The aromatic hydrogens of the coumarins typically have a chemical shift around 7.0-8.0ppm, however in the chromene-2-thione derivative; it appears that the aromatic and vinylic hydrogen's have switched chemical shifts. This may possibly be due to the sulfur atom at the 2-one position.

There are three sets of doublets and two singlets. The pairs of doublets in the aromatic region of the ^{1}H NMR spectrum indicates that the hydrogen's adjacent to each other on the aromatic ring are coupling each other. However from the *J* coupling value proved that this was inconclusive as no *J* coupling value was calculated for both hydrogens (see experimental 3.5.1). The doublet at 6.8ppm is from the lone hydrogen that has no neighboring hydrogen's. It is possible that the hydrogen is being coupled as well via "W" coupling. In order to determine that this is occurring, there is to be observed a *J* coupling value of 2-3 Hz. The "W' coupling theory would prove to be inconclusive as no *J* coupling value was obtained for the aromatic hydrogen.

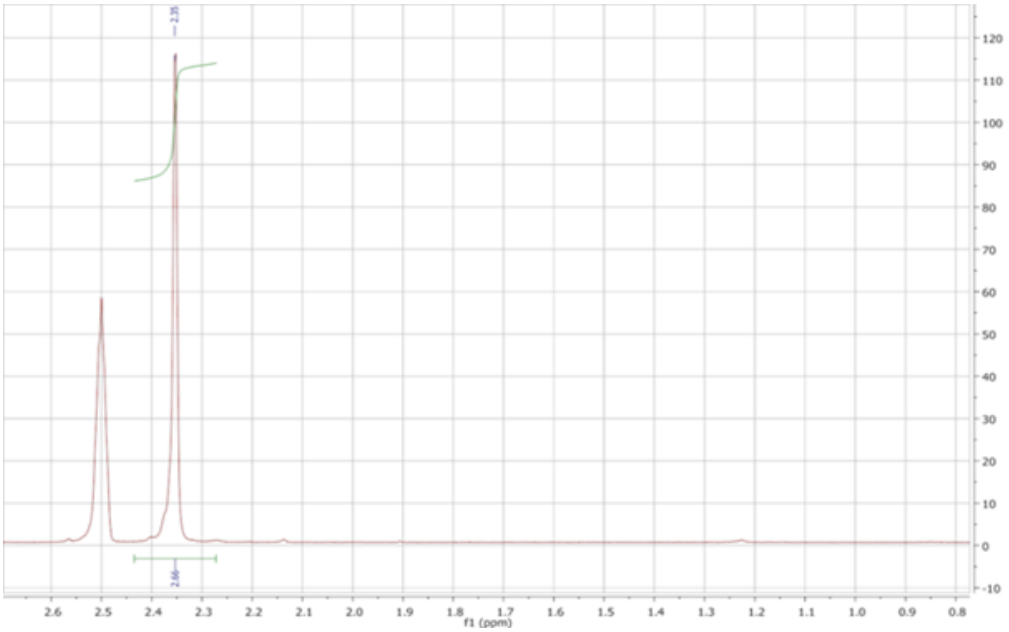


Figure 12. ^{1}H NMR spectra of the allylic hydrogen chemical shift.

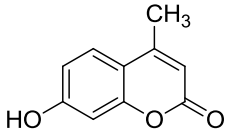
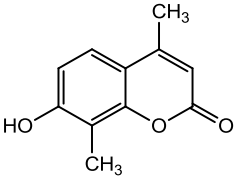
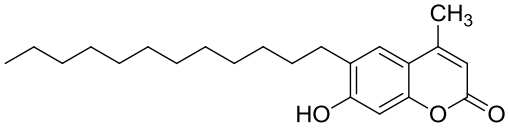
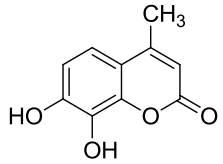
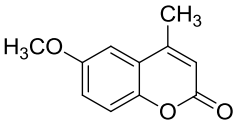
Figure 11 is the aromatic region of the NMR spectra. There are three sets of doublets and two singlets. The pairs of doublets in the aromatic region of the ^{1}H NMR spectrum indicates that the hydrogen's adjacent to each other on the aromatic ring are coupling each other. The lone hydrogen that has no neighboring hydrogen's is being coupled as well via "W" coupling. The aromatic hydrogens of the coumarins typically have a chemical shift around 7.0-8.0ppm, however in the chromene-2-thione derivative; it appears that the aromatic and vinylic hydrogen's have switched chemical shifts.

Figure 12 illustrates the region of the spectra that is called the "up field" indicative of the shielding of hydrogens. The vinylic protons on the coumarin molecule have a shift in this region typically around 2.3ppm from the three methyl protons. This peak always shows up as a singlet in this region, however as shown in Figure 9, there is a doublet indicating that the vinylic hydrogen is coupling with other hydrogens on the chromene-2-thione molecule. Table 4 shows the variance in the chemical shifts of the vinlyic, allylic, and phenolic protons in the ^{1}H NMR spectra. From the data table it is seen that there isn't much change in each chemical shift of the

coumarin derivatives, possibly a few points difference. However, in the 2H-chromene-2-thione derivatives, there is a drastic change in the chemical shifts particularly the vinylic hydrogen, shifting from 6.0ppm to approximately 7.0-7.1ppm and in some cases the phenolic chemical shifts (at 10-10.8ppm). The allylic protons on both the coumarin and 2H-chromene-2-thione derivative underwent the least amount of change as it didn't experience a change in chemical shift in the thione derivative or the coumarin. From the change in the chemical shifts of vinylic protons one can possibly conclude that the carbonyl molecule was transformed into its thio analog. ^{13}C NMR and infrared spectroscopy will be the ultimate factor to determine if the thione derivative was synthesized.

Table 4

Vinylic, Allylic and Phenolic ^{1}H Chemical Shifts

Compound	Vinyllic ^{1}H shift (ppm)	Allylic ^{1}H Shift (ppm)	Phenolic ^{1}H Shift (ppm)
	O = 6.10 S = 7.02	O = 2.35 S = 2.35	O = 10.52 S = 12.39
	O = 6.12 S = 8.05	O = 2.15 S = 2.33	O = 10.41
	O = 6.08 S = 7.08	O = 2.34 S = 5.62	O = 10.46 S = 11.76
	O = 6.20 S = 7.09	O = 2.38 S = 2.34	O = 7.70, 7.68 S = 8.83; 8.94
	O = 6.10 S = 7.23	O = 2.33 S = 2.09	

4.3.4 Discussion of the carbon 13 spectra of coumarin. As stated previously, to determine if the thio analogs have successfully been synthesized, the determining factor will be the ^{13}C NMR spectra. According to the referenced literature (indicated by the asterisk in the table) for the allylic, vinylic, and carbonyl chemical shifts will fall within range of 18-24, 110-112 and 155-162ppm (Karimi & Zareyee, 2008). Table 5 provides a quantitative analysis of the chemical shifts of the various carbons on the coumarin molecule and the environments they are in. The allylic, vinylic, and carbonyl shifts of the coumarin were compared to the thio analogs. As seen from the table there wasn't a drastic change in the allylic, and vinylic carbons of the coumarin molecule and thio derivatives. However, the chemical shift of the carbonyl carbon has shifted downfield in most of the thio derivatives (with the exception of 6-chloro 7-hydroxy 4-methyl 2H-chromene-2-thione) from approximately 160ppm to 197 and further downfield at 206ppm in some thio derivatives. However, the chemical shift of the carbonyl carbon has shifted downfield in most of the thio derivatives (with the exception of 6-chloro 7-hydroxy 4-methyl 2H-chromene-2-thione) from approximately 160ppm to 197 and further downfield at 206ppm in some thio derivatives.

When compared to referenced literature for the thio analogs, spectral data of the thio analogs synthesized in this research correlate to the chemical shifts of the new carbon-sulfur double bond that is formed during the transformation of the coumarin derivatives. From the experimental analysis of S. Kumar, B. Singh, and N. Kalra et al. the carbon-sulfur double bond is expected to fall within the range of 195-200ppm respectively (Kumar et al., 2005). Table 5 shows the chemical shifts of the thio derivatives all have a carbonyl shift of approximately 195-200ppm; whereas in the coumarin derivatives the chemical shift was 155-162ppm. With this

significant change in the chemical shift of the carbonyl carbon the conclusion can be drawn that the 2H-chromene-2-thione derivatives were successfully synthesized.

From the ^{13}C spectra of 6-chloro-7-hydroxy 4-methylcoumarin it seemed as though the transformation of the coumarin derivative into the 2H-chromene-2-thione derivative did not take place due to the chemical shift of the carbonyl carbon (157.76ppm) compared to the coumarin carbonyl shift of 160.8ppm. This could revert back to the factor of the solubility of the reagent 4-chloro resorcinol. Infrared spectroscopy would validate this statement with the absence of the C=S band in the spectra.

Table 5

Vinylic, Allylic and Carbonyl Carbon ^{13}C Chemical Shifts

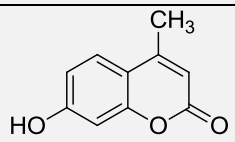
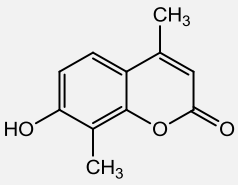
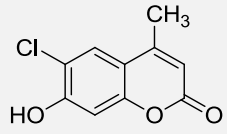
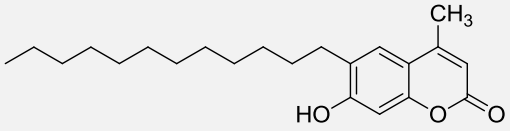
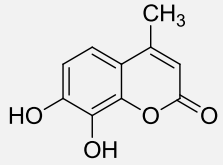
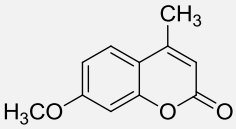
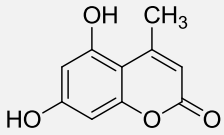
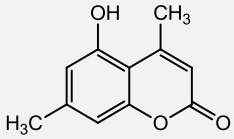
Compound	Allylic ^{13}C shift (ppm)	Vinylic ^{13}C Shift (ppm)	Carbonyl ^{13}C Shift (ppm)
	O = 18.48* S = 19.50	O = 113.24* S = 105.60	O = 161.56* S = 195.90
	O = 18.57* S = 17.19	O = 112.36* S = 100.36	O = 160.83* S = 197.55
	O = 19.40* S = 20.64	O = 112.50* S = 112.46	O = 160.80* S = 157.76
	O = 19.40* S = 22.67	O = 112.50* S = 102.54	O = 160.80* S = 197.25

Table 5

Cont.

Compound	Allylic ^{13}C shift (ppm)	Vinylic ^{13}C Shift (ppm)	Carbonyl ^{13}C Shift (ppm)
	O = 18.66* S = 20.68	O = 112.55* S = 116.42	O = 160.64* S = 199.31*
	O = 18.58* S = 17.18	O = 112.55* S = 100.39	O = 162.81* S = 206.46
	O = 23.87* S = 26.32	O = 109.25* S = 154.35	O = 155.41* S = 206.67
	O = 23.70* S = 26.32	O = 112.50* S = 112.74	O = 160.8* S = 200.96

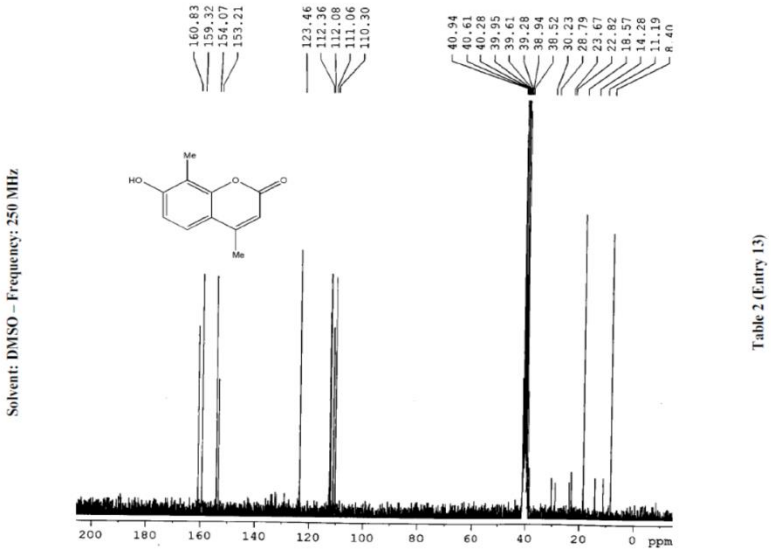


Figure 13. ^{13}C NMR spectra of 7-hydroxy-4,8-dimethylcoumarin (Karimi & Zareyee, 2008).

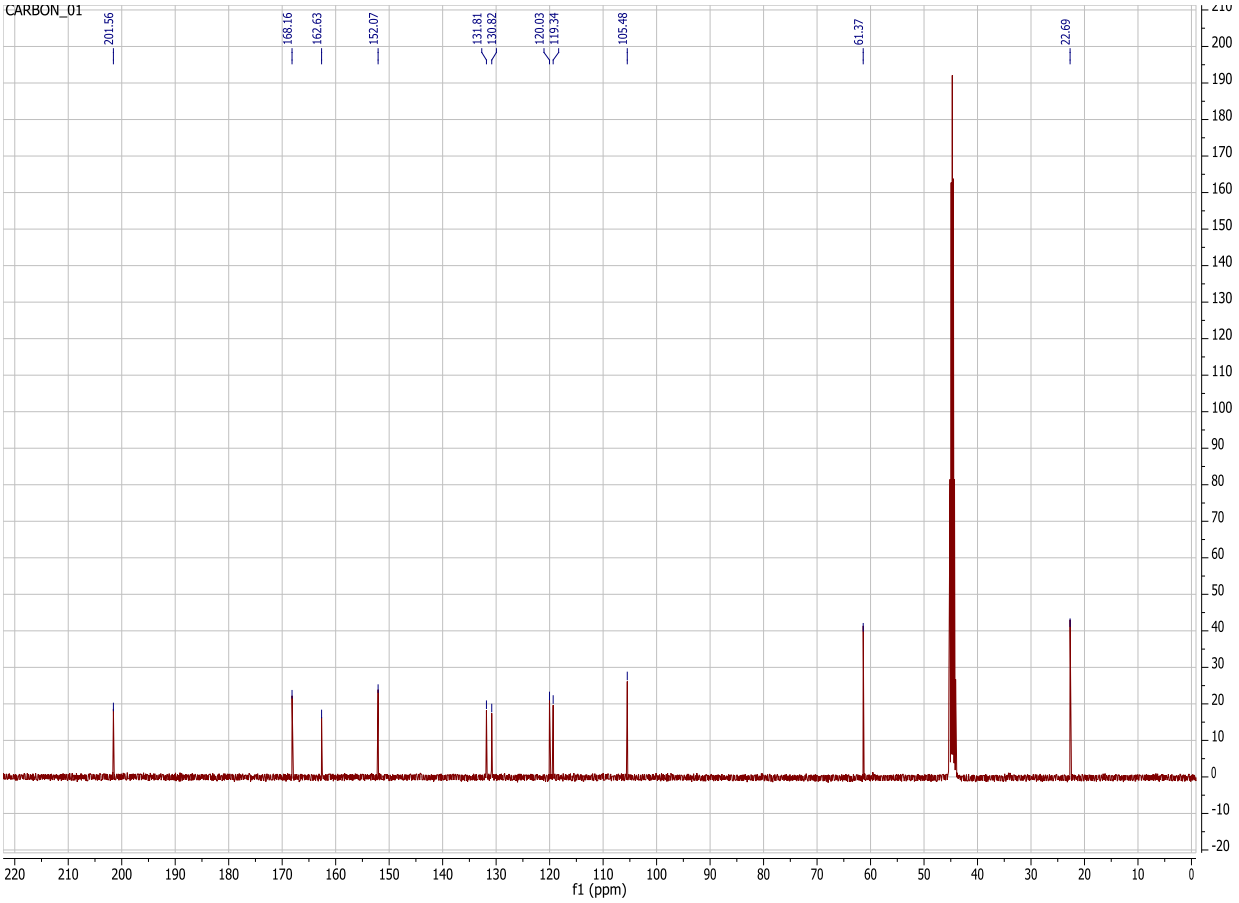


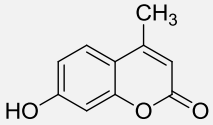
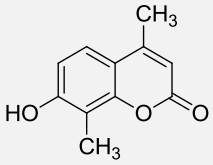
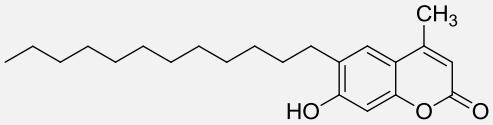
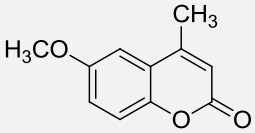
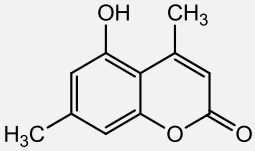
Figure 14. ^{13}C NMR spectra of 7-hydroxy-4,8-dimethyl-2H-chromene-2-thione.

4.3.5 Discussion of the thione carbon 13 spectra. To provide an illustration of Table 5, figure 13 is the ^{13}C spectra of 7-hydroxy-4-methylcoumarin (Karimi & Zareyee, 2008). The peaks that are of interest in this research are the allylic vinylic and phenolic carbon peaks. Variance in the chemical shifts of these three peaks of in coumarin and thione derivatives were observed. The carbonyl carbon on the coumarin molecule is expected to undergo the most change as a sulfur atom is being substituted for an oxygen atom. Shown in the spectra, the allylic, vinylic, and carbonyl chemical shifts are 18.57, 110.30 (the alkene carbon closest to the oxygen atom) and 160.83ppm. Figure 14 is the ^{13}C spectra of 7-hydroxy-4-methyl-2H-chromene-2-thione. As seen in the spectra, the chemical shift of the carbonyl carbon underwent

the most change shifting from 160.83 to 201.56ppm. From the quantitative analysis shown in table 5, and the spectral data shown, it has been proven that the 2H-chromene-2-thione derivatives were synthesized.

Table 6

Variance of the C=S Peaks

Compound	C=S Stretch cm^{-1}
	1218 cm^{-1}
	1242 cm^{-1}
	1215 cm^{-1}
	1208 cm^{-1}
	1238 cm^{-1}

4.4 Discussion of the IR Spectra

4.4.1 Infrared spectra interpretation. In order to validate the ^{13}C NMR spectra of the thione derivatives, Infrared Spectroscopy was utilized to determine the presence of the C=S stretch and the absence of the C=O (carbonyl) stretch. The referenced wavelength of the carbonyl stretch in a pyrone molecule is around 1715 and 1775cm^{-1} (William H. Brown, 2005). Two bands are expected in this region. According to NIST (National Institute of Standards and Technology) chemistry web book for vibrational energy, the C=S stretch is to show up in around

1275-1030cm^{-1}. The IR spectra of the thione derivatives synthesized in this research shows there is the absence of the carbonyl stretch around 1715 and 1775 cm^{-1}. Table 6 quantitatively shows the C=S stretch of the thione derivatives. Each thione derivative within the chart has a stretch within 1275-1030cm-1; all were closer to the latter end (average wavelength 1225 cm-1).

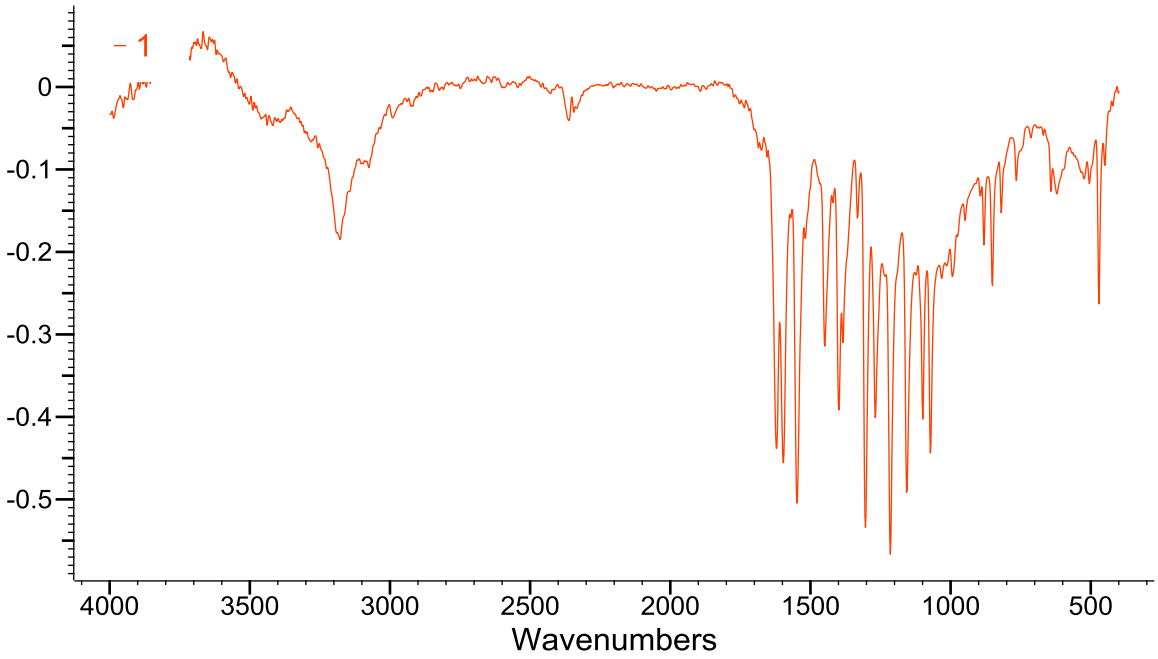


Figure 15. IR spectra of 7-hydroxy-4-methyl-2H-chromene-2-thione.

Figure 15 illustrates the IR spectra of 7-hydroxy-4-methyl-2H-chromene-2-thione. As seen, there is a strong peak at 1218 cm-1 which is indicative of the C=S stretch. Also seen in the IR spectra, there is the absence of peaks in the region for pyrone carbonyls. From the validation of the IR spectra it has been proven that the thione derivatives were synthesized with success.

CHAPTER 5

Discussion and Future Research

From the NMR and IR analysis, it was proven that the 2H-Chromeme-2-Thione derivatives were indeed synthesized. The key factor in this determination was the vinylic hydrogen chemical shift in the ^{1}H NMR, the chemical shift of the carbonyl carbon in the ^{13}C spectra and the absence of the carbonyl stretch in the lactone molecule. As stated, the referenced shift for the vinylic hydrogen of the coumarin will have a chemical shift of 6.1ppm, for the carbonyl carbon, a chemical shift of approximately 160ppm in the ^{13}C NMR and the IR stretch for the carbonyl in the lactone will have 2 bands around 1715 and 1775 cm^{-1}. For each 2H-chromene-2-thione synthesized there was a singlet peak around 7.0-7.1ppm and in the ^{13}C NMR spectra, there was a chemical shift of approximately 197-200ppm for the carbonyl carbon. For the IR spectra, there was an absence of the lactone bands and a strong peak around 1225 cm^{-1} for wan indication that the C=S bond is present in the molecule. Future recommendations for this research are to improve upon the synthesis of the 2H-Chromeme-2-Thione derivatives and increase the percent yields. The protective procedure of the coumarins to be converted into the 2H-chromene-2-thione derivatives seems to be the best strategy to successfully obtain the 2H-chromene-2-thione-derivatives. Although the $ZrCl_4$ catalyzed Von Pechmann condensation requires no solvent and is carried out at room temperature, to obtain better percent yields and compounds without impurities heat will be introduced during the reaction to synthesize the coumarins with ease. Also, other resorcinol derivatives are currently being investigated to be used in the synthesis of more coumarin derivatives. Recently, 2-Nitro resorcinol was investigated to determine if the 2H-chromene-2-thione derivative of 2-Nitro resorcinol would make for a cancer chemopreventive agent of interest. Procedures to synthesize the coumarin derivatives

containing multiple hydroxyl substituents could also be investigated. Other findings has concluded that 7,8-dihydroxy-4-methyl-2H-chromene-2-thione derivatives were more potent at inhibiting TNFα ICAM-1 expression then their respective coumarin analogs. This is said to be due to the fact that the hydroxyl groups can readily be converted into the quinone form (Kumar et al., 2005). Therefore synthesizing 2H-chromene-2-thione derivatives with multiple hydroxyl substituents may provide an excellent candidate as a cancer chemopreventive. The 2H-chromene-2-thione-derivatives synthesized in this study will be sent to the NIH DTP National Institute of Health Developmental Therapeutics Program to be tested against 60 cancer cell lines to determine if the thione derivatives inhibit or retard cancer activity in vitro. Optimistically, by having the thio substituent attached to the coumarin molecule will have a greater effect on the anticancer activity of the coumarin derivatives.

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Appendix A

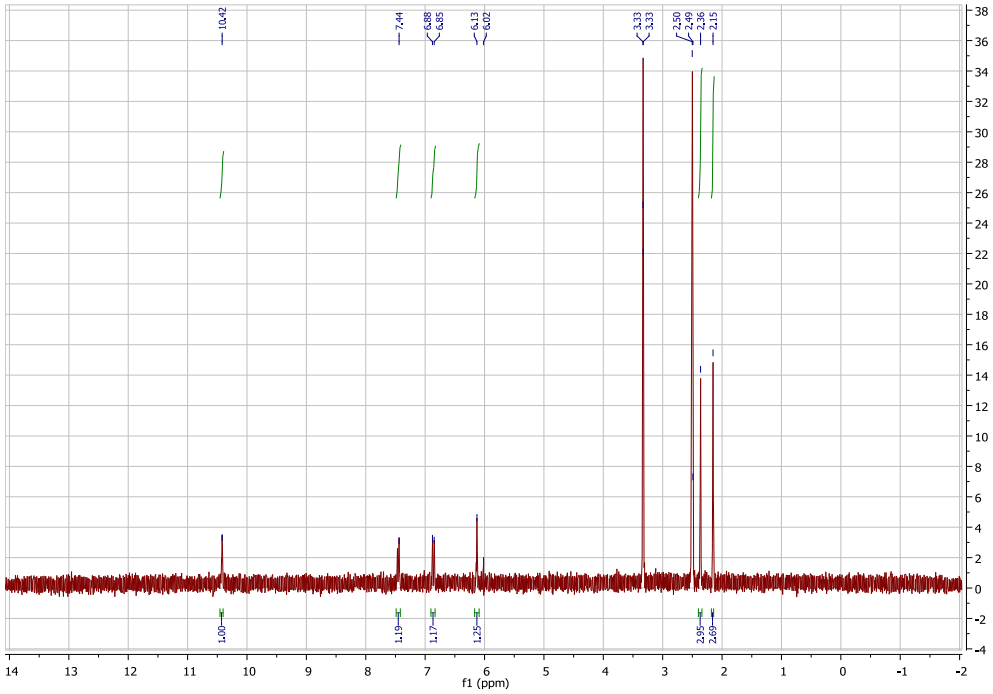

Figure. [1]H NMR spectra of 7-hydroxy-4,8 –dimethylcoumarin.

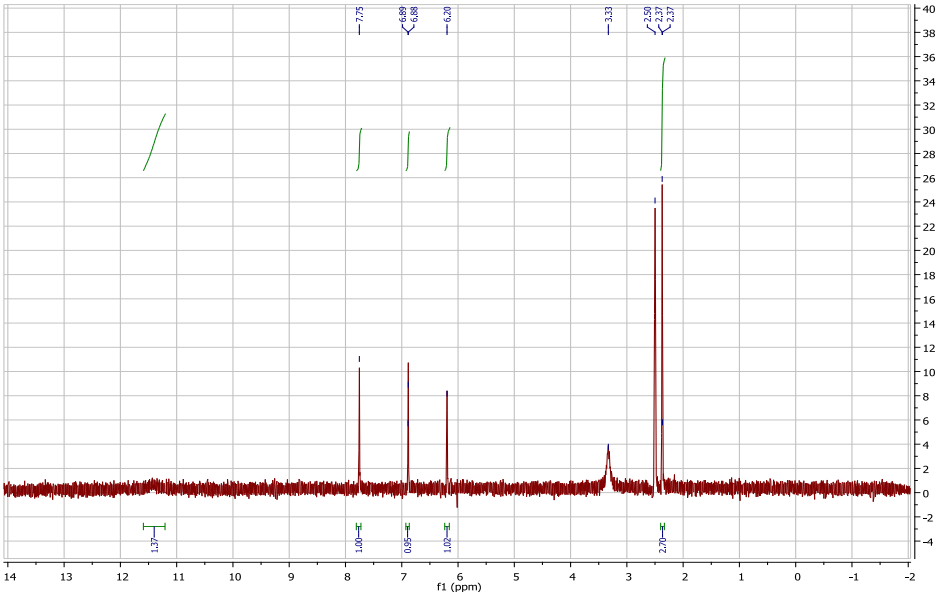

Figure [1]H NMR spectra of 6-chloro-7-hydroxy-4-methylcoumarin.

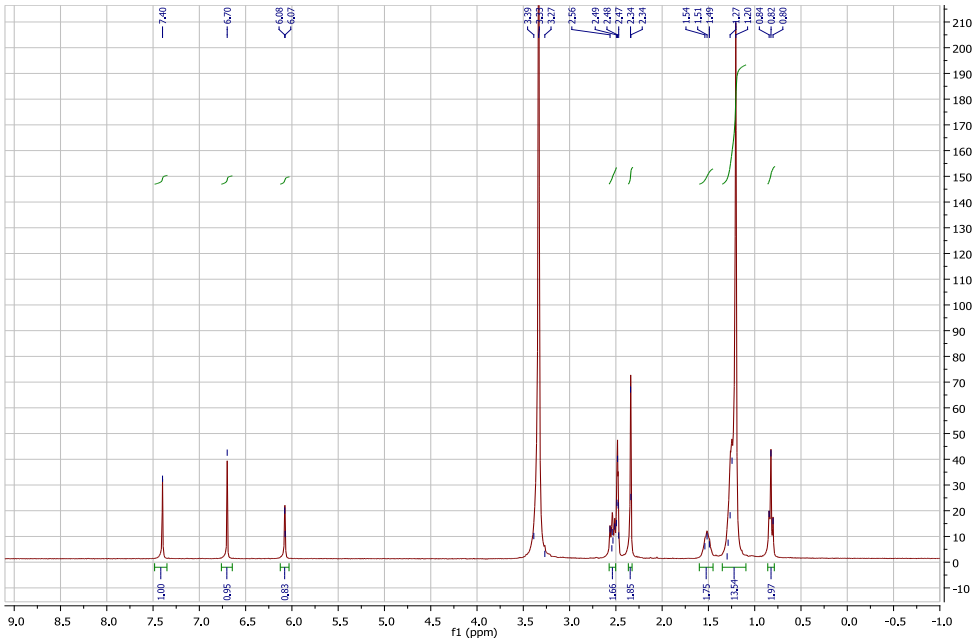

Figure. ^{1}H NMR spectra of 6-n-dodecyl-7-hydroxy-4-methylcoumarin.

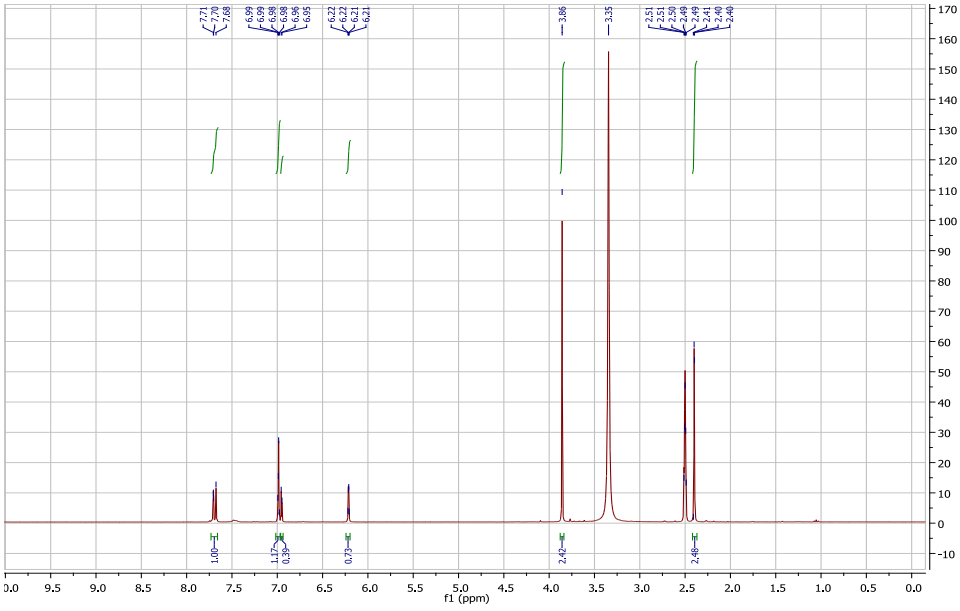

Figure. ^{1}H NMR spectra of 7,8-dihydroxy-4-methylcoumarin.

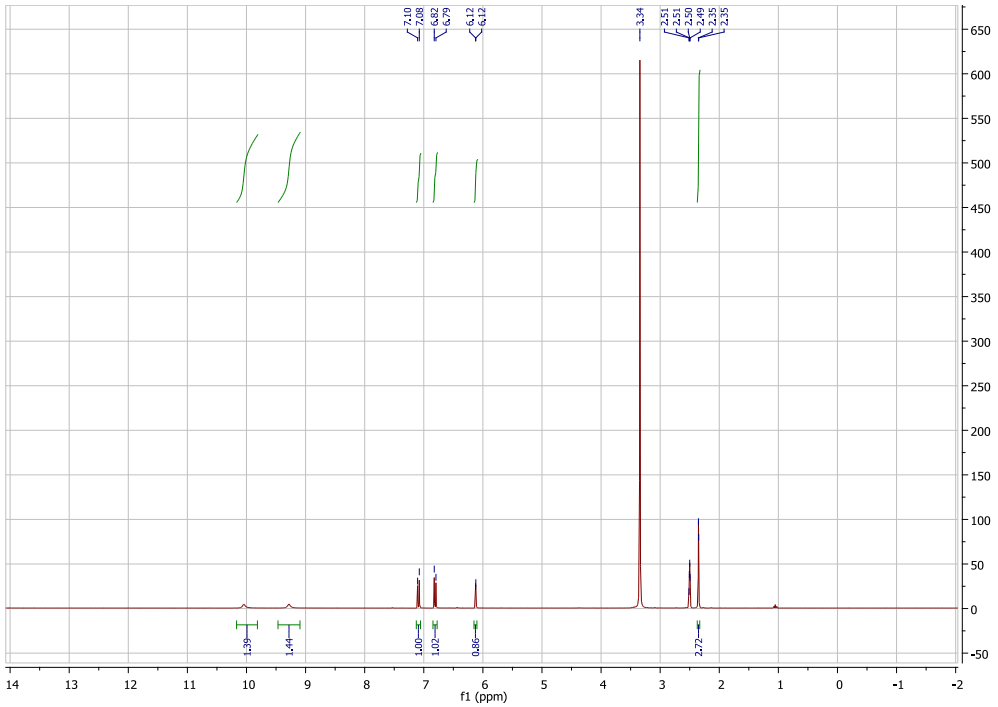

Figure. [1]H NMR spectra of 7-methoxy-4-methylcoumarin.

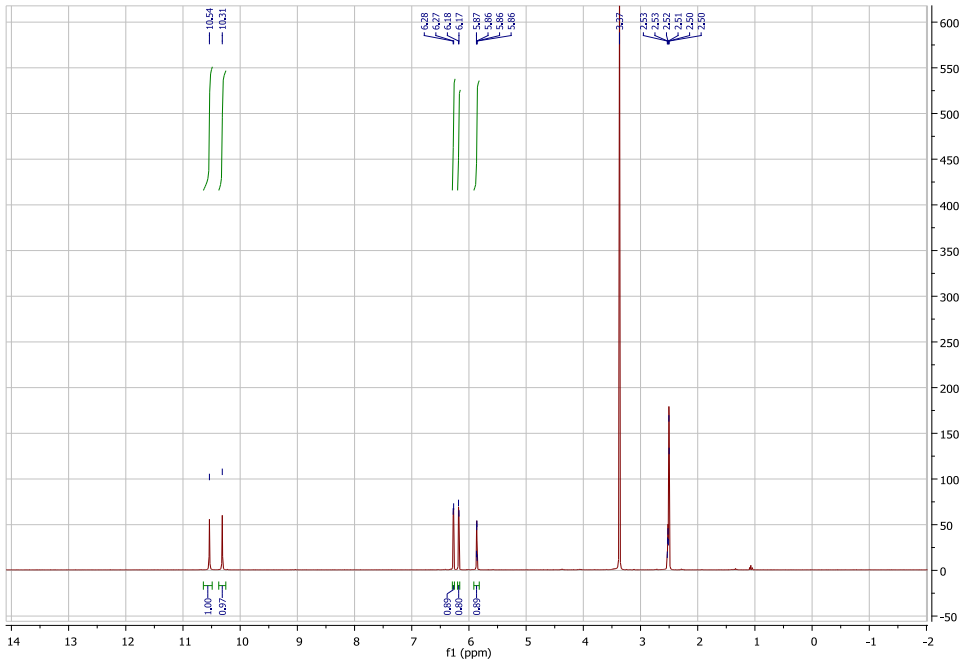

Figure. [1]H NMR spectra of 5,7-dihydroxy-4-methylcoumarin.

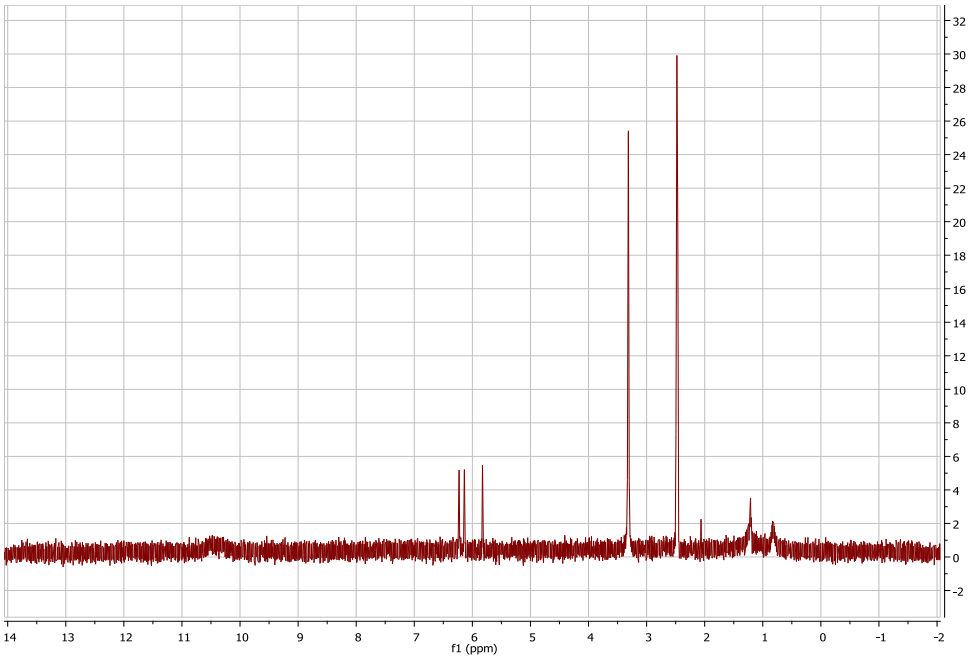

Figure. ^{1}H NMR spectra of 5,7-ditertbutyldimethylsilyloxy-4-methylcoumarin.

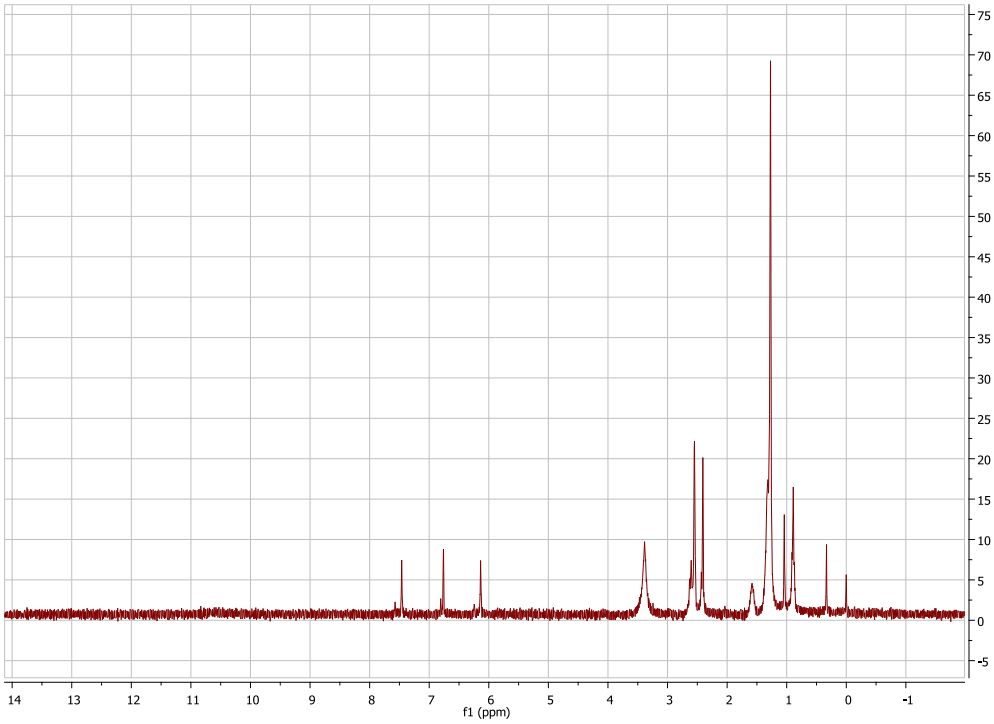

Figure. ^{1}H NMR spectra of 5-tertbutyldimethylsilyloxy-4,7-dimethylcoumarin.

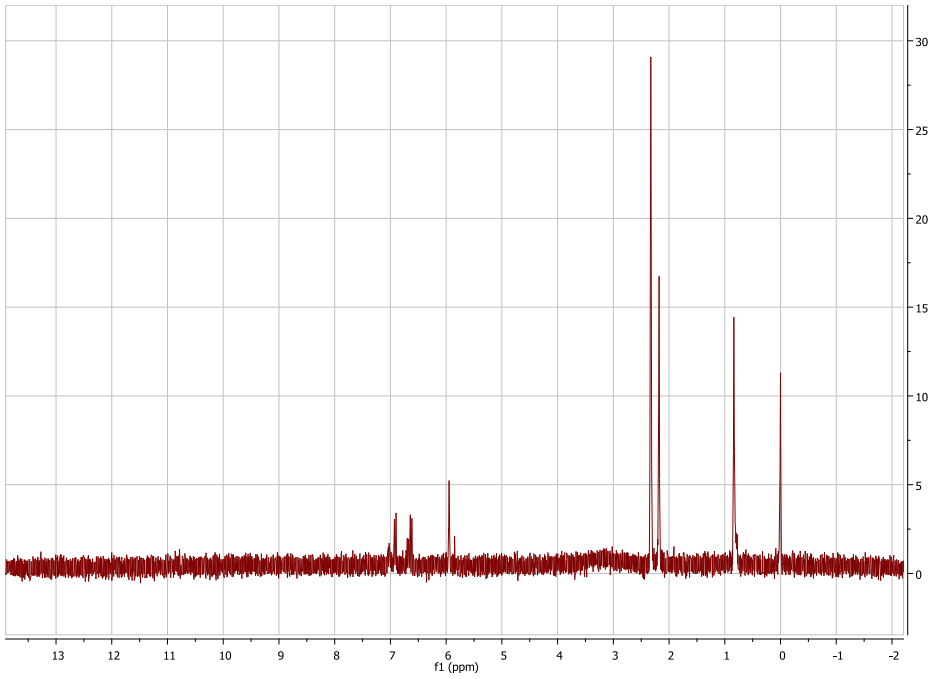

Figure. ^{1}H NMR spectra of 7,8-ditertbutyldimethylsilyloxy-4-methylcoumarin.

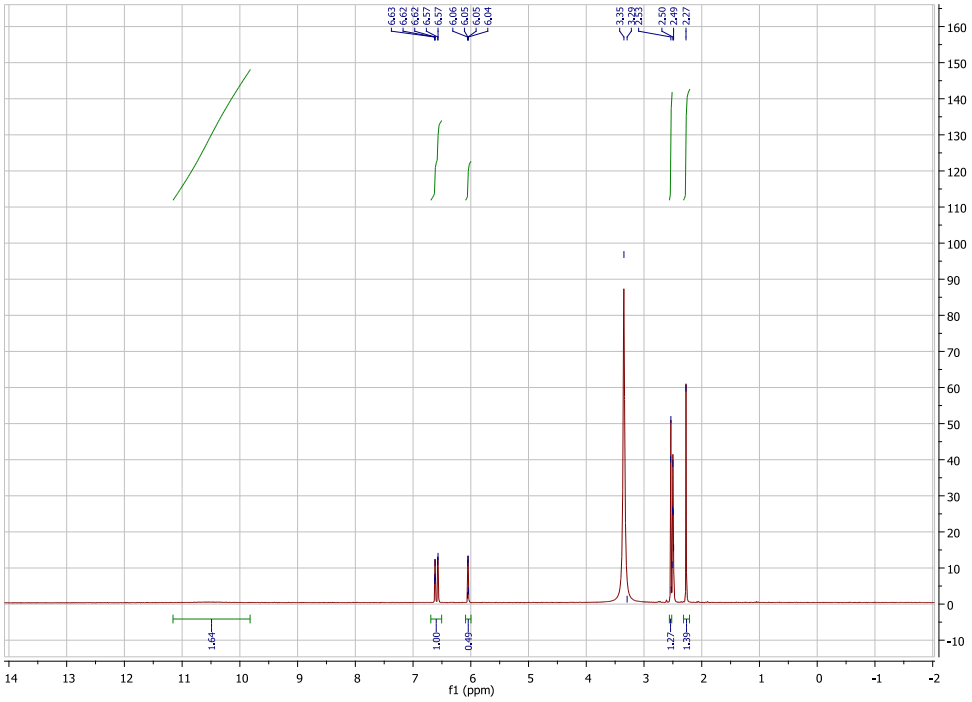

Figure. ^{1}H NMR spectra of 5-hydroxy-4,7-dimethylcoumarin.

Appendix B

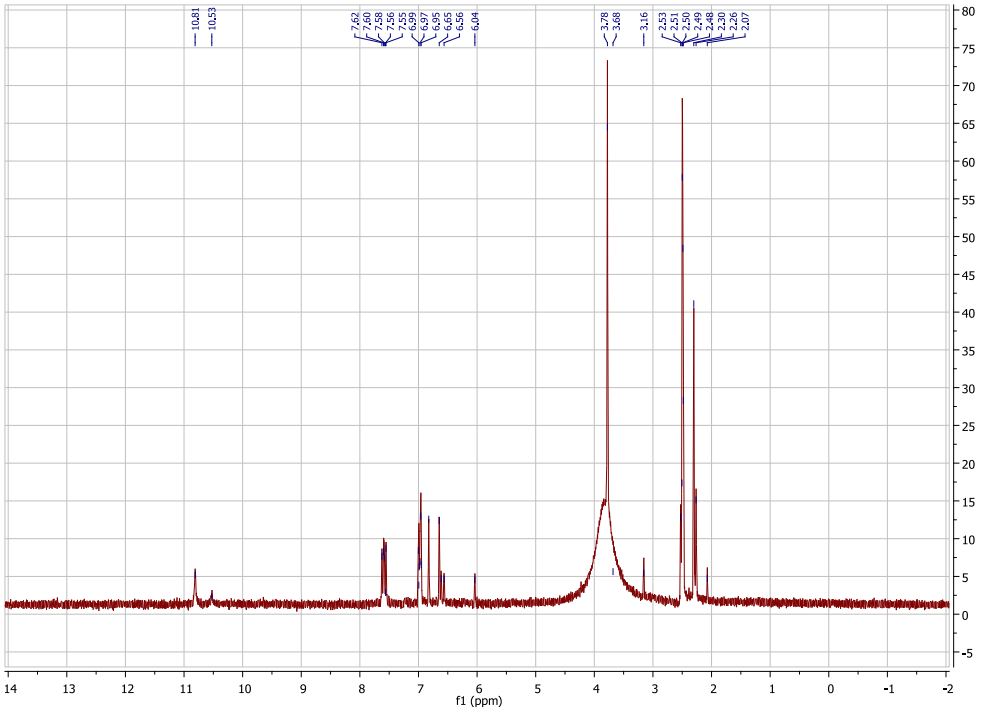

Figure. [1]H NMR spectra of 7-tertbutyldimethylsilyloxy-4,8-dimethyl-2H-chromene-2-thione.

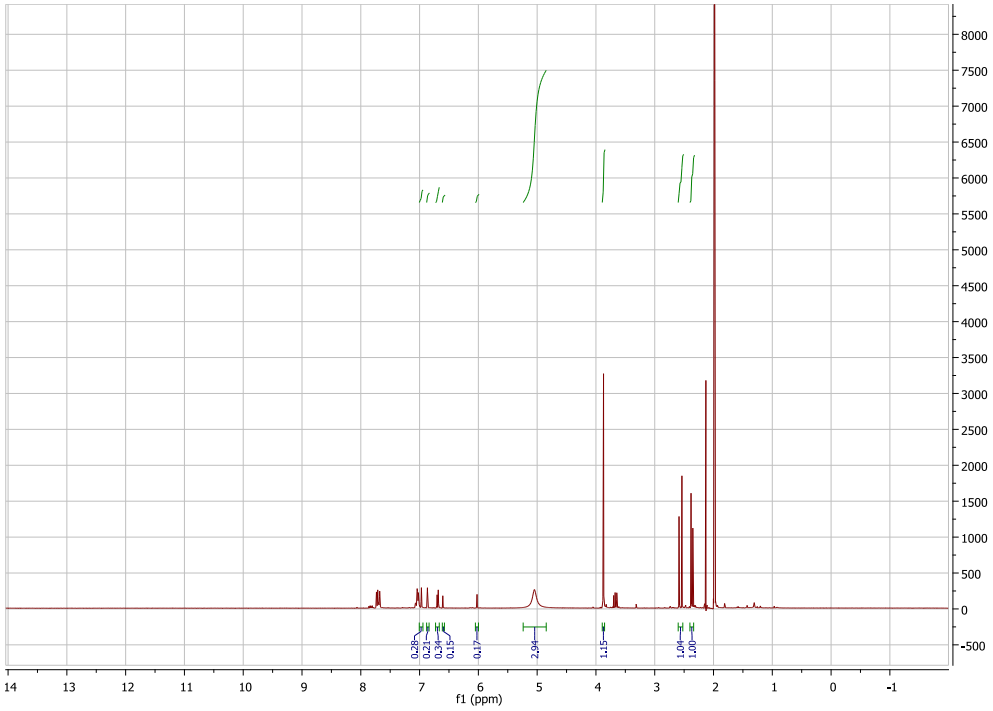

Figure. [1]H NMR spectra of 6-chloro-7-hydroxy-4-methyl-2H-chromene-2-thione.

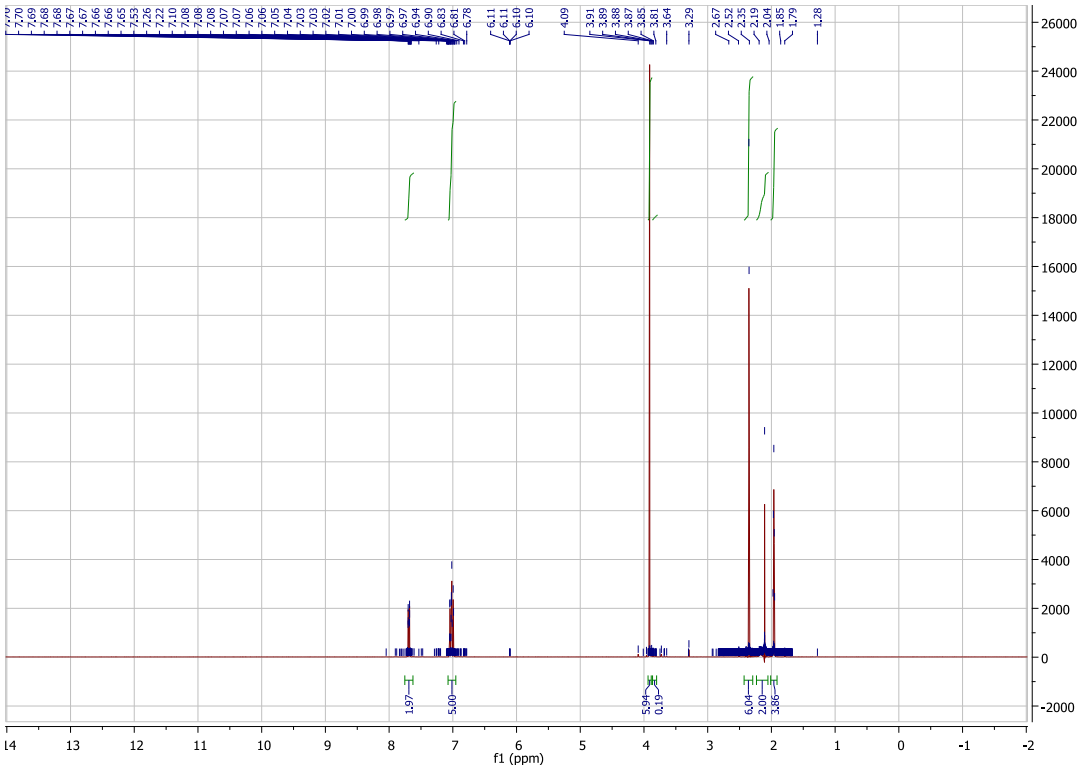

Figure. ^{1}H NMR spectra 7-hydroxy-4,8-dimethyl-2H-chromene-2-thione.

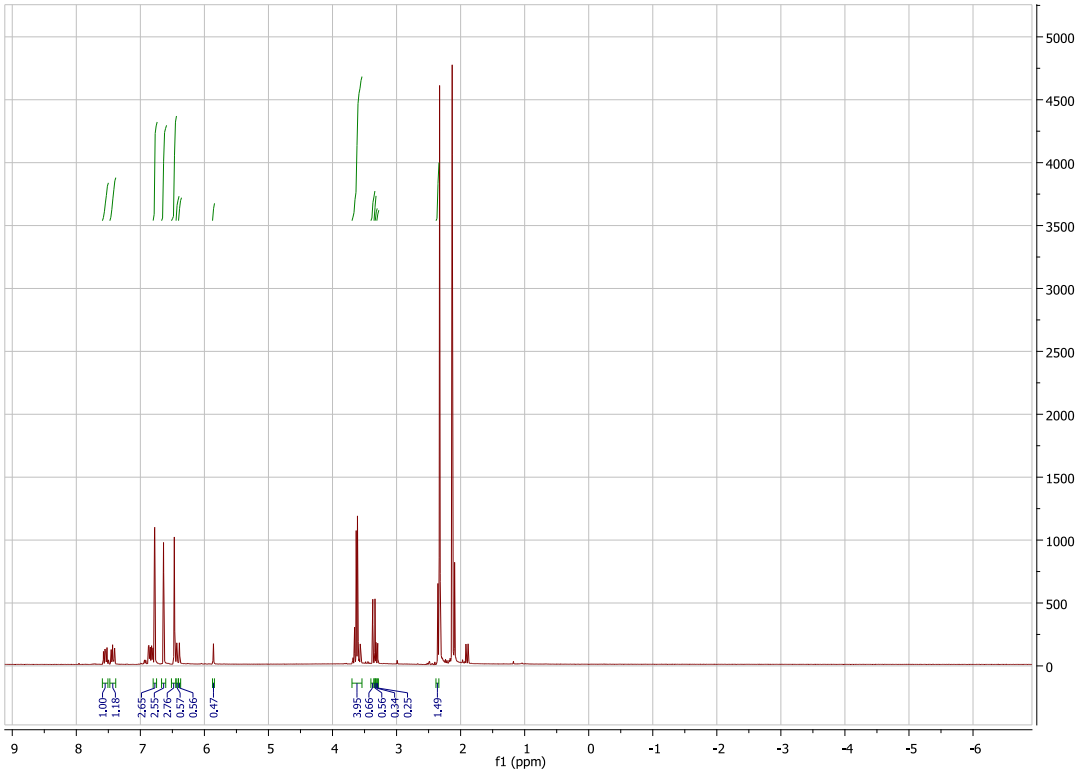

Figure. ^{1}H NMR spectra of 5-hydroxy-4,7-dimethyl-2H-chromene-2-thione.

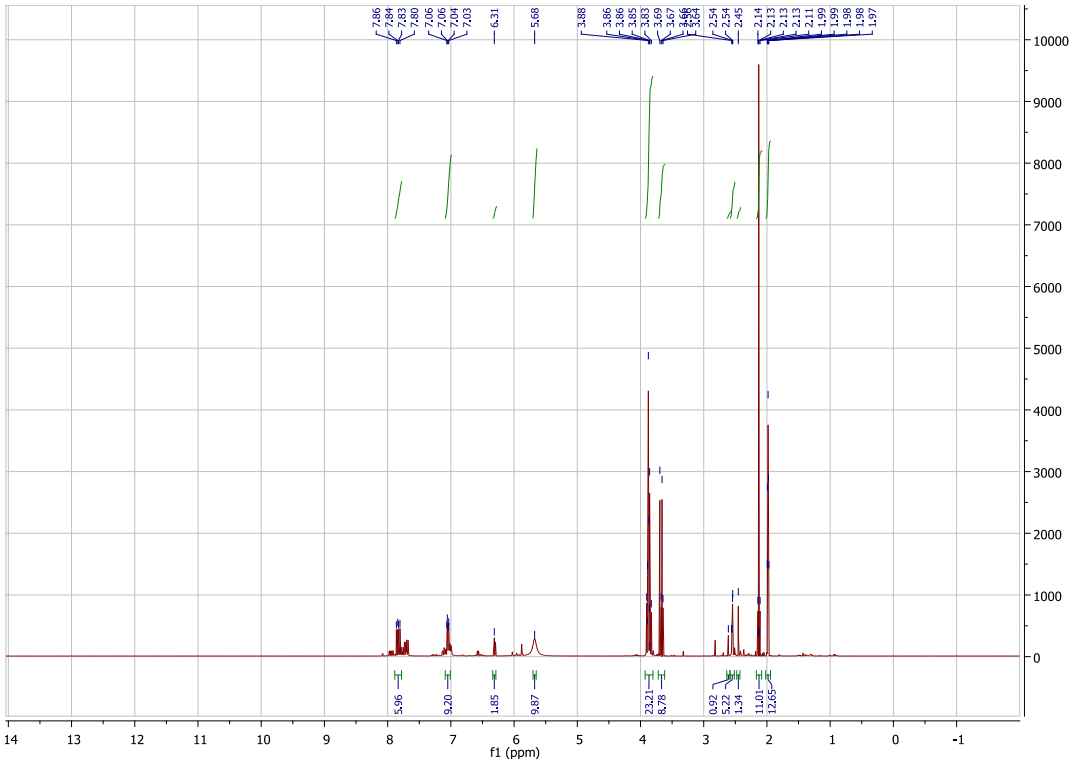

Figure. ^{1}H NMR spectra of 7,8-dihydroxy-4-methyl-2H-chromene-2-thione.

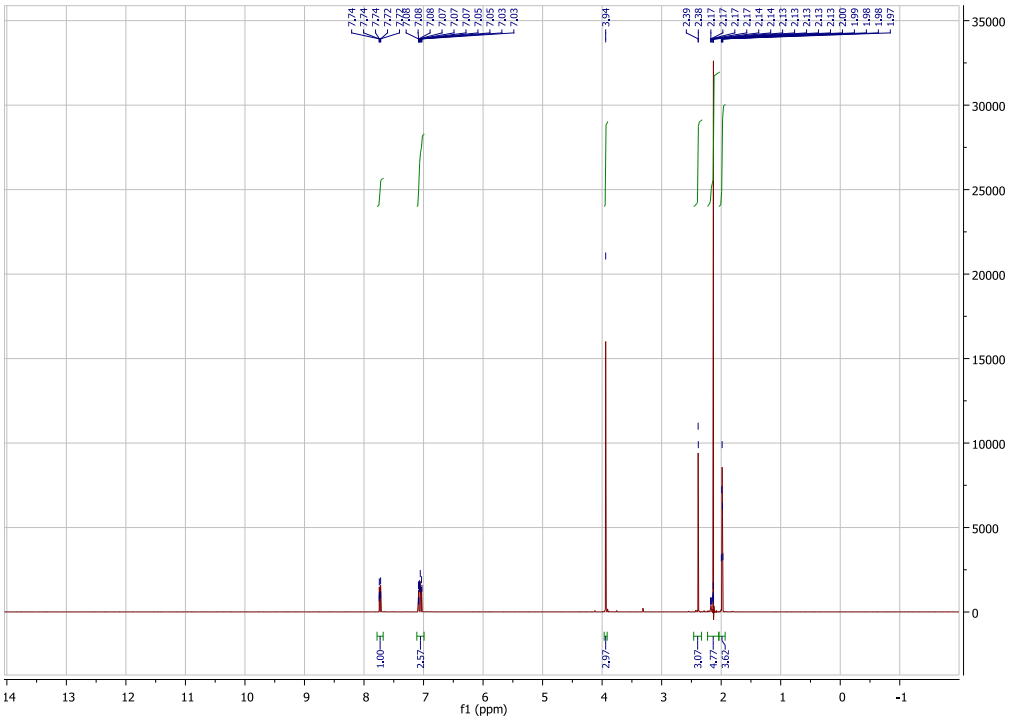

Figure. ^{1}H NMR spectra of 7-methoxy-4-methyl-2H-chromene-2-thione.

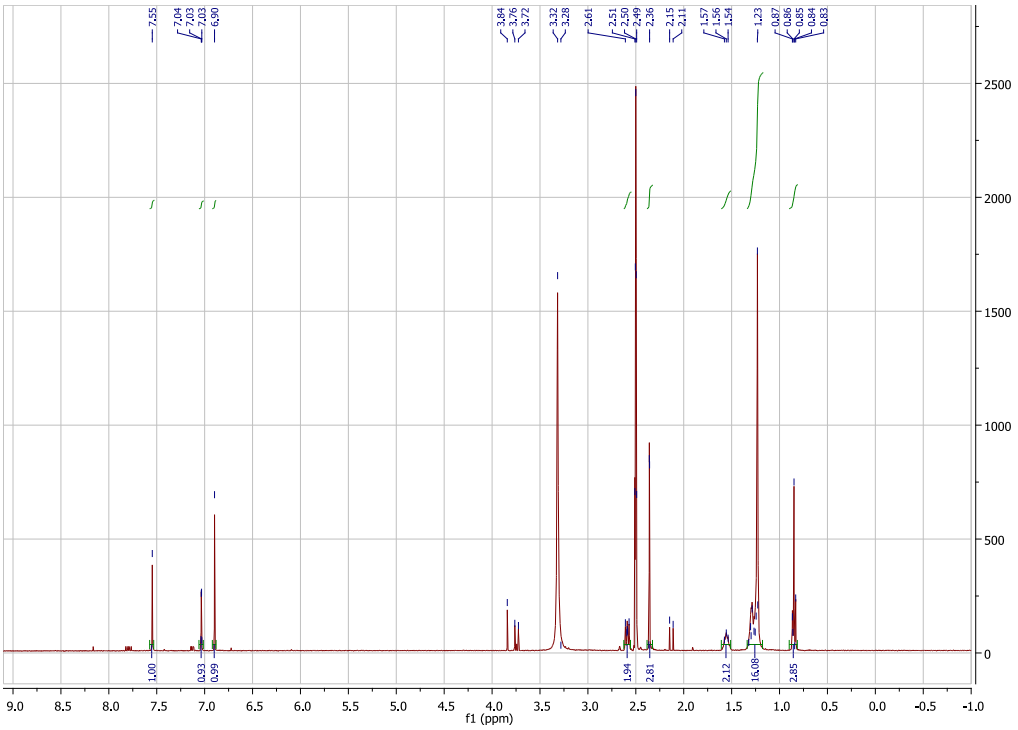

Figure. [1]H NMR spectra of 6-n-dodecyl-7-hydroxy-4-methyl-2H-chromene-2-thione.

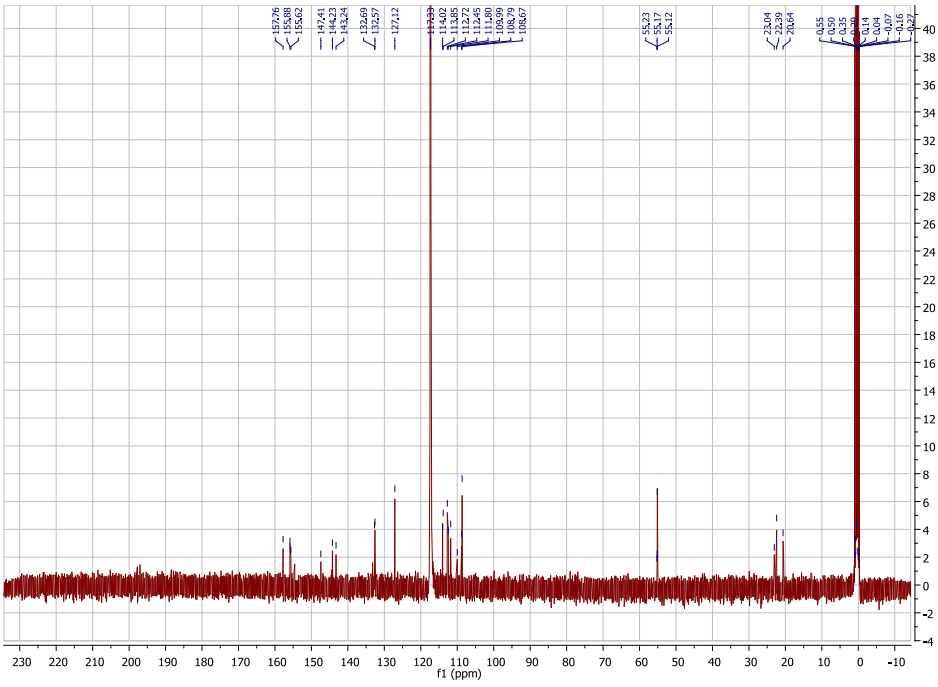

Figure. [13]C NMR spectra of 6-chloro-7-hydroxy-4-methyl-2H-chromene-2-thione.

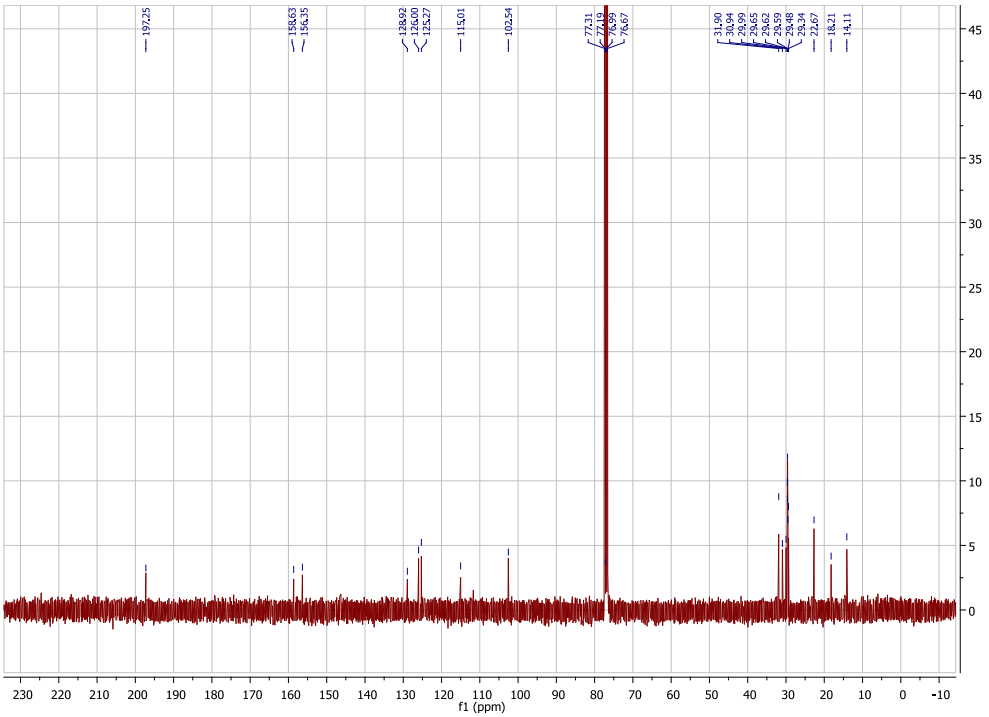

Figure. ^{13}C NMR spectra of 6-n-dodecyl-7-hydroxy-4-methyl-2H-chromene-2-thione.

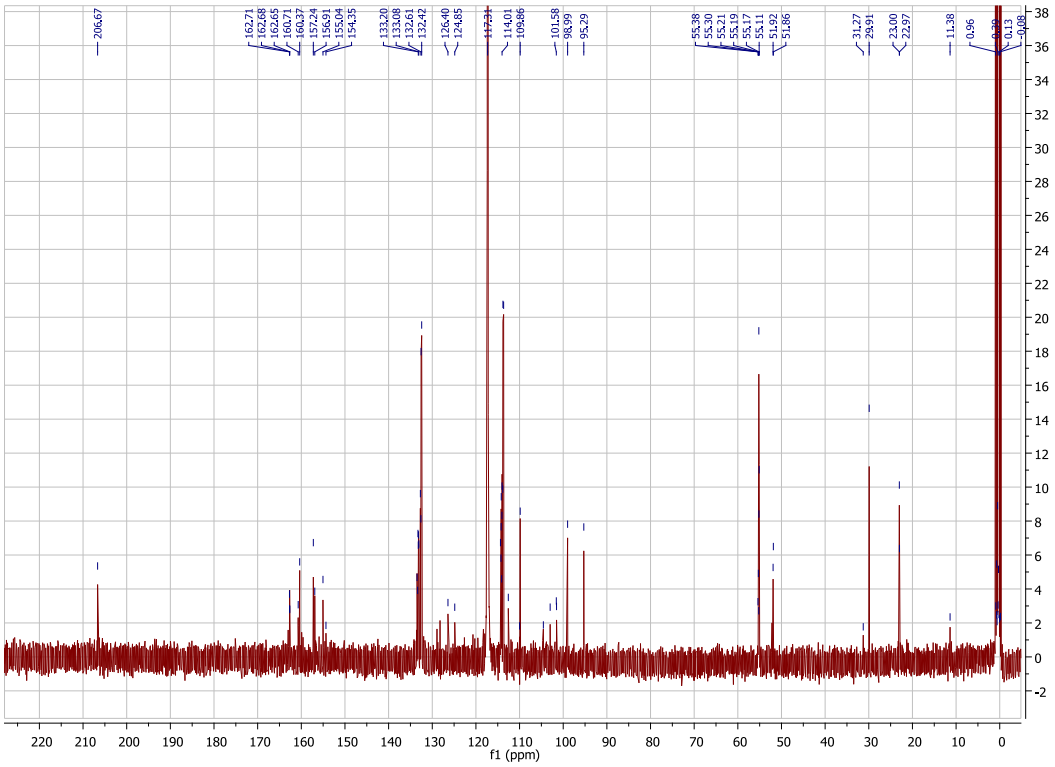

Figure. ^{13}C NMR spectra of 5,7-dihydroxy-4-methyl-2H-chromene-2-thione.

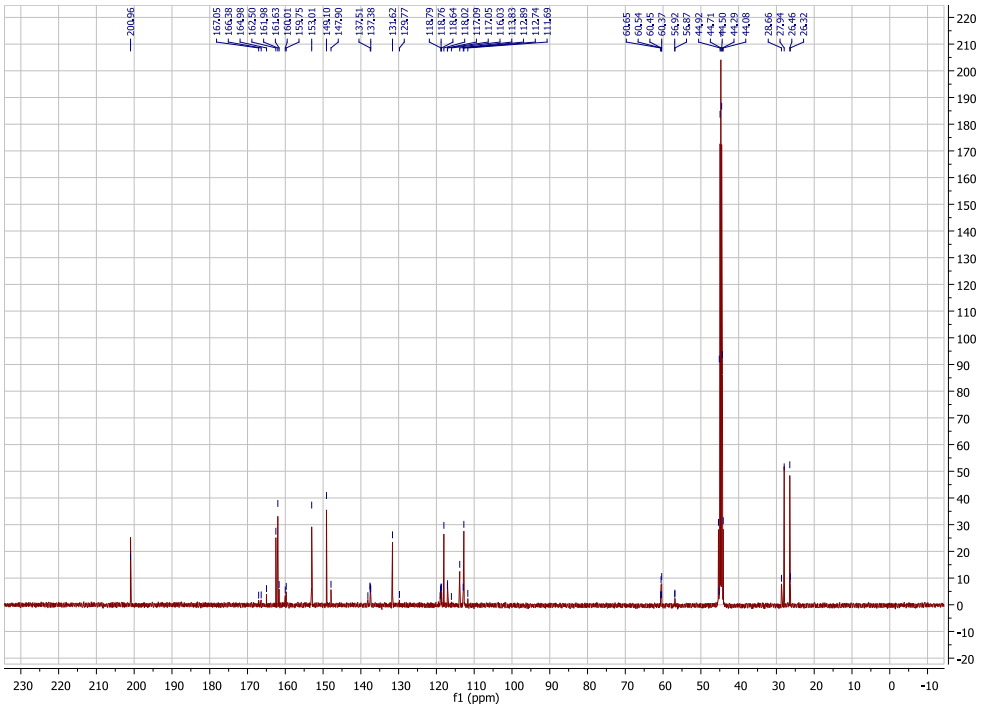

Figure. ^{13}C NMR spectra of 5-hydroxy-4,7-dimethyl-2H-chromene-2-thione.

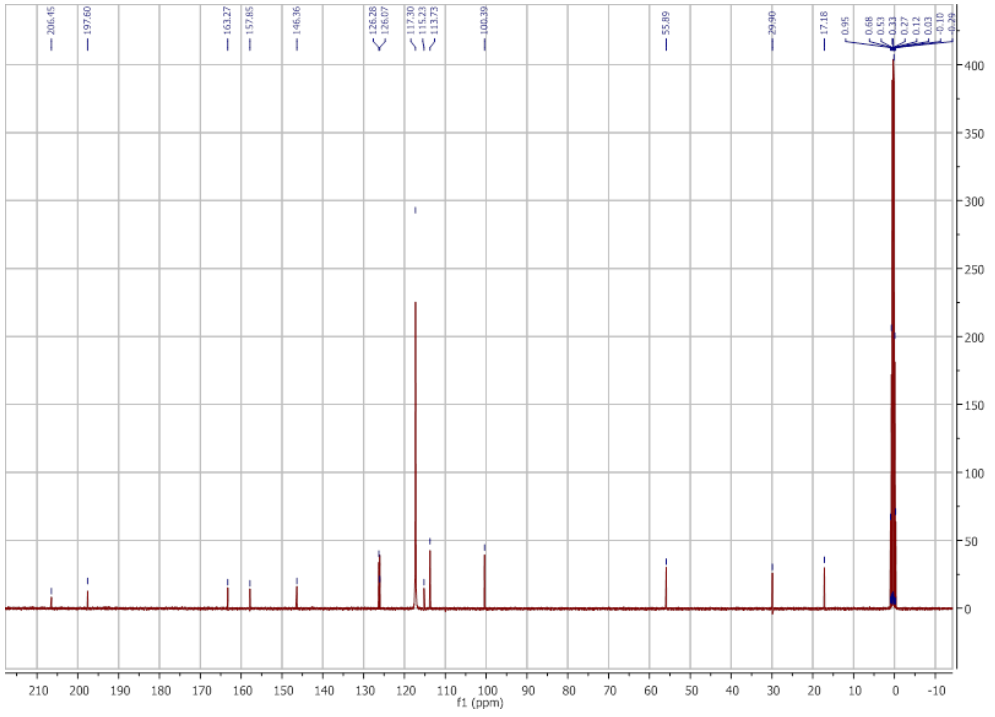

Figure. ^{13}C NMR spectra of 7-methoxy-4-methyl-2H-chromene-2-thione.

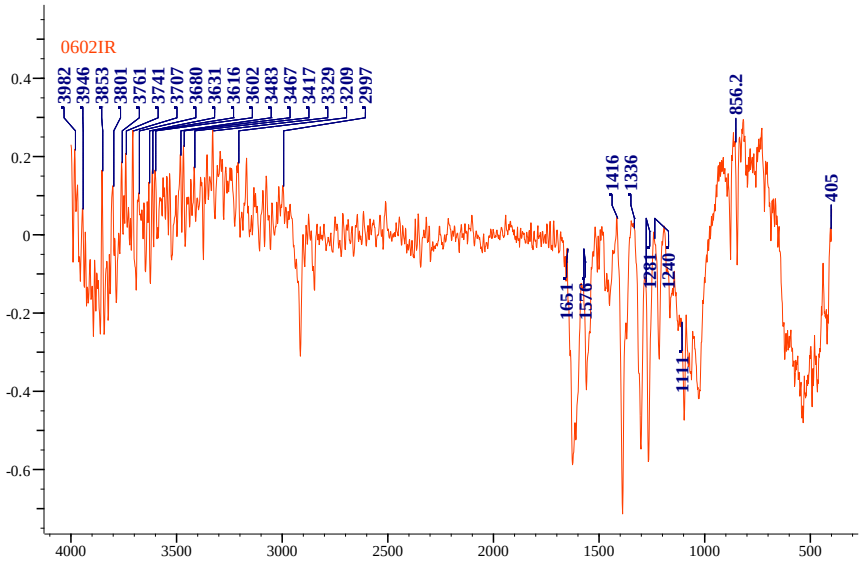

Figure. IR spectra of 6-n-dodecyl-7-hydroxy-4-methyl-2H-chromene-2-thione.

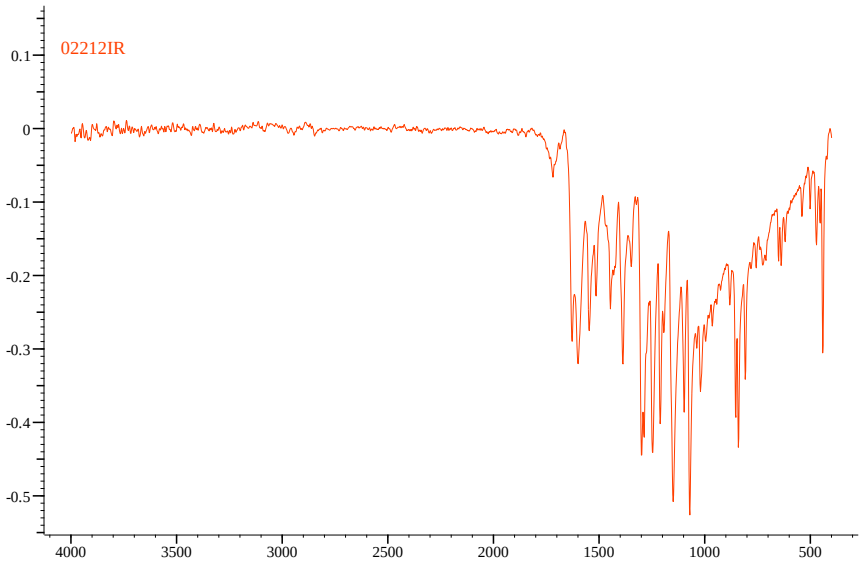

Figure. IR spectra of 7-methoxy-4-methyl-2H-chromene-2-thione.

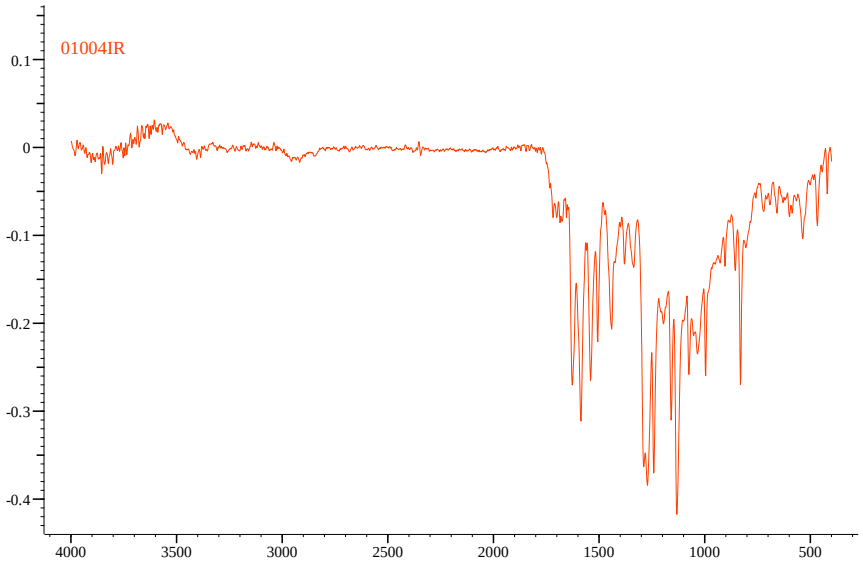

Figure. IR spectra of 5-hydroxy-4,7-dimethyl-2H-chromene-2-thione.

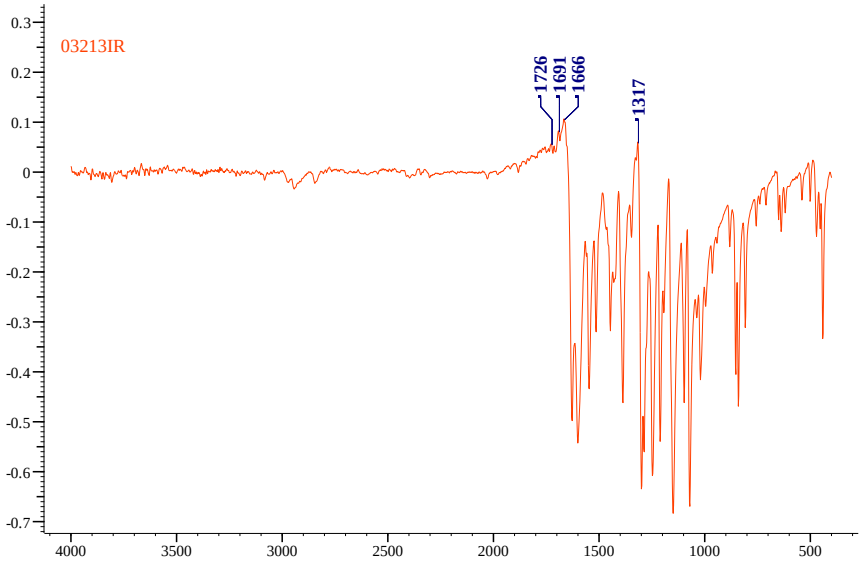

Figure. IR spectra of 7-hydroxy-4,8-dimethyl-2H-chromene-2-thione.

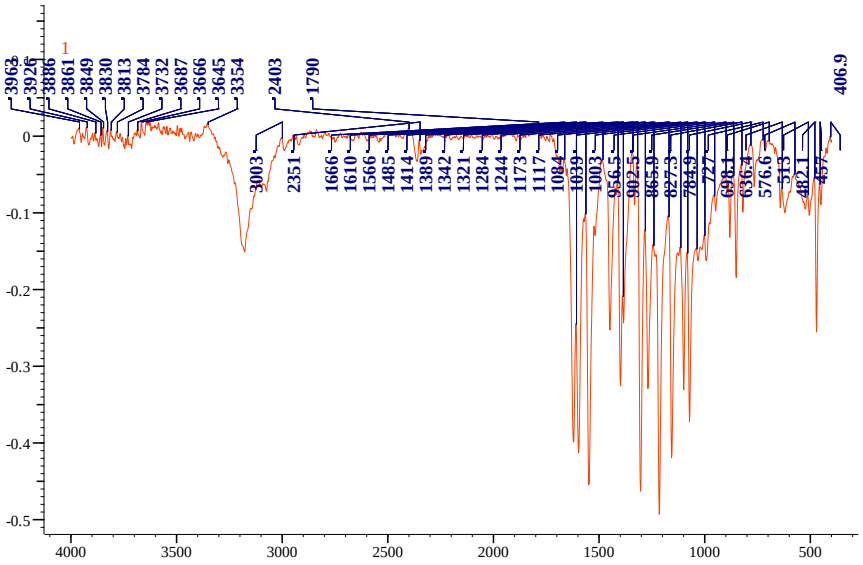

Figure. IR spectra of 7-tertbutyldimethylsiloxy-4,8-dimethyl-2H-chromene-2-thione.

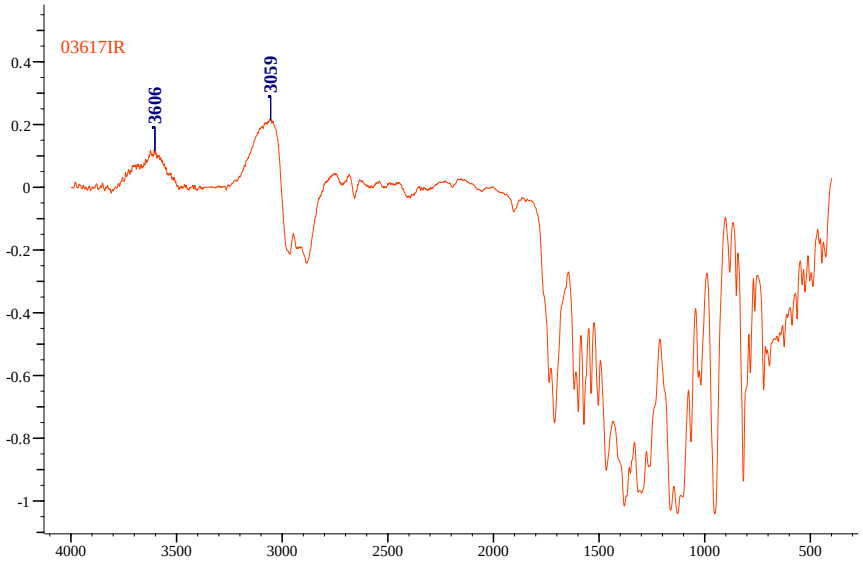

Figure. IR spectra of 7,8-dihydroxy-4-methyl-2H-chromene-2-thione.