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Synthesis of Ester Derivatives of Resveratrol as Potential Anti-Cancer Drugs

A thesis
presented to
the faculty of the Department of Chemistry
East Tennessee State University

In partial fulfillment
of the requirements for the degree
Master of Science in Chemistry

by
Parasmani Pageni
August 2013

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Keywords: phytoalexin, polyphenols, cancer, resveratrol, stilbene, derivatives

ABSTRACT

Synthesis of Ester Derivatives of Resveratrol as Potential Anti-Cancer Drugs

by

Parasmani Pageni

Resveratrol is a naturally occurring phytoalexin of the stilbene family produced by various plants in response to stress, UV radiation, and fungal attack. It is primarily found in peanuts, berries, grape skin, and red wine. Resveratrol has been found to exhibit anti-cancer, anti-inflammatory, anti-aging, and anti-oxidant properties. Research indicates that diets enriched with resveratrol containing substances result in less incidence of cancer. Unfortunately, the low bioavailability and solubility has been a huge setback for its potential prospects. As a result, efforts have been made to synthesize derivatives of resveratrol with increased solubility and bioavailability. Three triester novel resveratrol derivatives 3, 4', 5-tri (benzoyloxy) stilbene, 3, 4', 5-tri (toluyloxy) stilbene and 3, 4', 5-tri (2''-butenoyloxy) stilbene have been synthesized by esterification process that can further be subjected for biological evaluation. Structures and purities of all newly synthesized derivatives were confirmed by ¹H, ¹³C NMR spectroscopy and infrared spectroscopy.

DEDICATION

In loving memory of my late grandmother, Chandra Kala Pageni

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
Fig	Figure
UV	Ultraviolet
R _f	Retention Factor
Hrs	Hours
Rpm	Rounds per minute
Temp	Temperature
M.p	Melting Point
B.p	Boiling Point
IR	Infrared
TMS	Tetramethylsilane
THF	Tetrahydrofuran
TEA	Triethyl Amine
Ph ₃ P	Triphenylphosphine
NMR	Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

With the advent of science and technology, there has been vast improvement in the field of health science. Medical conditions that were considered fatal and untreatable are no more threat for mankind. Just in US alone the average expectancy age has increased from 50 at early 1900s to almost 80 in 2000¹. This improvement is only possible with the discovery of new drugs and better medical facilities. A lot of these drugs actually have been discovered from the plant and natural sources after a long research². Among the compounds with a discovery origin to plants is resveratrol (RV). Resveratrol is a natural polyphenol that is primarily found in berries, peanuts, grape skin, and red wine. It is also a phytoalexin produced by plants in response to stress, injury, fungal attack, and UV radiation. It has been into media attention lately for its various health benefits. It has been found to exhibit anti-inflammatory, anti-cancer, anti-oxidant, anti-aging, antiobesity, and neuroprotective properties. As a phytoestrogen, it can provide cardiovascular protection. Its uses have also been linked with the metabolism of lipids, oxidation of low-density lipoproteins and aggregation of platelets³. Resveratrol was found to inhibit the initiation, promotion and progression stages of cancer displaying the chemo preventive effects⁴. Currently, resveratrol is in clinical phase II trials as anti-cancer drug for human colon cancer⁵. In vivo experiments conducted in rodents show evidence of positive effects in controlling stress and disease in rodent models⁶. However, the solubility and bioavailability of resveratrol is very low so higher concentrations should be administered to see any therapeutic effects in the body. To overcome the setback, efforts have been made to synthesize water soluble derivative. The new derivative with new increased solubility and bioavailability will be promising tools as

cancer chemo preventive agents, as well as cancer therapeutics in the prevention and treatment of cancer.

Cancer

Cancer is the second leading cause of deaths in western countries after heart disease. In US alone more than one million people are diagnosed with cancer annually and more than half result in death. Cancer is a medical condition where abnormal cells in body divide without any control. Cancer cells do not respond normally to the body's control mechanism, so they divide excessively and invade the neighboring tissues. If cancer cells are provided with the nutrition they need, they can divide indefinitely. An experiment was done to prove the immortality of cancer cell. HeLa cells, cancer cells taken from a woman named Henrietta Lacks, have been reproducing in culture since 1951⁶. Cancer starts when a normal cell in the body converts to a cancer cell through the process of transformation. A transformed cell is instantly detected by body's immune system. However, if it manages to evade, it may proliferate and form a mass of abnormal cells called tumor. If the abnormal cells remain at the original site, it will result in the less severe condition termed as benign tumor. Benign tumor can be completely removed by surgery and pose little threat. Malignant tumors have capability to enter the bloodstream invading other tissues and disrupting the normal function of various systems in the body. They are difficult to treat and may become life threatening. Cancer cells can spread to distant locations from the original site by a process called metastasis.

Types of Cancer

Cancers are named according to the type of tissue in which cancer originates i.e. histological type and contains suffix to represent the site⁶⁻⁸. Below are the five broad groups to classify the cancer.

1. Carcinomas consist of the cancer that originates at the cells that cover internal and external parts of body. It can further be divided into two major subtypes: adenocarcinoma, which develops in an organ or gland, and squamous cell carcinoma, which originates in the squamous epithelium. Most carcinomas affect organs or glands capable of secretion, such as the breasts, which produce milk, or the lungs, which secrete mucus, or colon or prostate or bladder.
2. Sarcoma refers to cancer that originates in supportive and connective tissues such as bones, tendons, cartilage, muscle, and fat. The most common sarcoma usually invades the bone creating a massive pain.
3. Lymphomas are cancers that initiate in the immune system tissues like lymph nodes.
4. Leukemias are cancers that begin in the bone marrow and often accumulate in the blood stream.
5. Adenomas are the cancers that arise in the glandular tissues. Cancer in glands like thyroid, pituitary, and adrenal falls into this category.

Causes of Cancer

Normal cells in the body follow a path of growth, division, and death. After a certain number of divisions a cell is programmed to die called apoptosis, and when the cell ceases to die cancer begins to form. Cancer cells do not experience programmatic death and instead continue to divide. This abnormal behavior of cells is the result of mutation in regulatory genes. Genes are the instructions that tell cells what to do and are made up of DNA. So, the cancer occurs when there are changes in the DNA of a cell. These changes may be either inherited genetically from parents or caused by environmental factors. The various factors that can cause the damage on DNA to lead cancer are listed below.

- Carcinogens: Any substance that is capable of developing cancer is known as a carcinogen. The carcinogen changes the structure of DNA resulting in a mutation that finally leads to cancer.

Commonly known carcinogens include asbestos, arsenic, benzene, bisphenolA (BPA), pesticides, dioxins, UV radiation, polybrominateddiphenylethers (PBDEs), tobacco smoke, and polycyclic aromatic hydrocarbons (PAHs)

- Age: The chances of developing cancer increase with age even though it can occur at any age.

A survey shows more than three out of five people who get cancer are over the age of 65, and more than a third are over 75⁷.

- Lifestyle: Some of the choices in life such as excessive drinking and smoking increase the risk of developing cancer.

- Nutrition: Diets of processed food and red meat are more likely to result in cancer than diets of fruits and vegetables. Poor diets choices that often lead to obesity have been linked to breast, womb, and kidney cancer.

- Immune System: People with poor immune systems are found to have developed certain forms of cancer like lymphomas.

- Viruses and Bacteria: These microbes can cause the damages to the cell making them more prone to cancer. Human Papilloma Virus (HPV) is linked with developing cancer on cervix, anal, or genital area.

Prevention of Cancer

Decades of research revealed that cancer is easier to prevent than to treat. There is not a single factor that leads to cancer. There are multiple things in our genes, lifestyle, and the peripheral environment that determine the risk of developing cancer. So, any step taken to

decrease the chances of developing cancer is called cancer prevention. The following measures can be taken to reduce the risk of cancer: no use of tobacco, healthy diet, physical activities to keep a healthy weight, less exposure to sun, immunization against viruses and bacteria, safe health practices, and regular medical checkup.

Treatment of Cancer

There options for cancer treatment is not limited to single rather a range of options are available. The best option is determined by various factors like age, history, and lifestyle. More often various types of treatment are combined to get the best possible result. The most popular types of cancer treatment are discussed below.

Surgery

Surgery is a common and oldest technique to treat or even help prevent cancer. The first use of this technique was employed by ancient Egyptians to remove breast tumors in 7th century. If the cancer is benign, it offers the biggest chance for cure as the tumor does not get a chance to spread around the body. However, if the cancer has metastasized, surgery won't be very beneficial. Some type of surgery is usually performed in most people with cancer. The symptoms of bowel obstruction or spinal cord compression can be controlled by surgery⁹.

Chemotherapy

Chemotherapy is the use of chemicals to kill the cancer cells or stop them from growing. The chemicals interferes the process of cell division by either damaging the RNA or DNA. Chemotherapy is primarily used to damage the DNA of the affected cancer cells, stop the cell from replicating by inhibiting by inhibiting the synthesis of new DNA strand, and stop the division of original cell into its daughter cells in mitotic phase of cell division. The drugs for chemotherapy are given in combination of two or three at the same time. These combinations are

called regimens. The purpose of regimens differs according to the stage of cancer. For instance, regimens lower the risk of cancer coming back in early stage breast cancer, while it makes cancer shrink or disappear in advanced stage. Chemo therapy not only kills the cancer cells but affects the healthy cells. Damage in the blood cells results in anemia, fatigue, and various kinds of infections. Mouth sores and diarrhea are caused by the damage in the cell at mucous membrane. Another common side effect of chemo therapy is the destruction of cells at hair roots and follicles that ultimately results in loss of hair. The cytotoxic chemotherapy drugs have different method of killing cancer cells. They are classified accordingly to following classes: alkylating agents, heavy metals, antimetabolites, anthracycline, antibiotics, epipodophyllotoxins, plant alkaloids, and antitumor antibiotics.

Radiation Therapy

In radiation therapy, high energy particles are used to damage the cancerous cells in the body. The common types of radiation used are X-rays, gamma rays, and charged particles. If the radiation is delivered by a machine outside the body, it is known as external-beam radiation therapy and if the radiation is delivered from radioactive material placed inside the body near cancer cells, it is called brachytherapy or internal radiation therapy. The radiation either damages DNA directly or it creates free radicals inside the cell that actually end up damaging the DNA. Once the DNA is damaged, cancer cells stop dividing and eventually die and will be eliminated by body's natural processes.

Targeted Therapy

Targeted therapy is a selective way of attacking the cancer cells with drugs or other substances. It is a newer type of treatment and is precise and does very little damage to normal cells. It interferes with the proliferation and spread of cancer cells. This form of therapy mainly

focus on proteins that are involved in cell signaling pathways regulating the basic cellular functions and activities like cell division, cell movement, cell responses, and cell death. When the signals to divide and grow uncontrollably are blocked, the progression of cancer is halted and may even induce death of the cancer cell through a process called apoptosis. Small-molecule drugs or monoclonal antibodies are the most common targeted therapies. It is convenient for small-molecule drugs to diffuse into the cell to act on the specific targets. Monoclonal antibodies are directed against the targets outside the cell because they cannot penetrate the plasma membrane. In case of chronic myeloid leukemia (CML), BCR-ABL gene is responsible to relay the signal regulating cell proliferation. Continuous proliferation of CML cells can be controlled by targeting BCR-ABL using targeted therapy. In contrast to traditional chemotherapy, targeted therapies are generally better tolerated, but they are associated with several adverse effects, such as acneiform rash, cardiac dysfunction, thrombosis, hypertension, and proteinuria¹⁰.

Immunotherapy

Immunotherapy is a new type of therapy to fight against various types of cancers where body uses own immune system to help fight cancer. The immune system is a group of cells and organs that work together to defend the body against foreign particles such as cancer cells, bacteria, and viruses. The defense mechanism is known as immune response where several kinds of cells like macrophages, lymphocytes, T lymphocytes, dendritic cells, and granulocytes go into action after the entry of foreign particles. These immune cells communicate with each other by a number of special protein molecules known as cytokines. Cytokines can be natural, recombinant, or synthetic. Some of the cytokines are interferons, interleukins, tumor necrosis, and colony-stimulating factors⁶⁻⁸. The idea of immunotherapy treatment involves giving larger amounts of proteins either by an injection or infusion so that the cells of immune system acts more

effectively to make tumor cells more prominent to the immune system. The active agents of immunotherapy are termed as immunomodulators. Flu, chills, fever, nausea, fatigue, and loss of appetite are some of the common side effects of this type of therapy.

Hyperthermia

Hyperthermia is a type of cancer treatment in which the temperature of tumor-loaded tissue is raised up to 113°F. The applied high temperature is capable of killing the cancer cells with minimal injury to normal cells. It is applied as an adjoining therapy with other established cancer treatments like radiotherapy and chemotherapy. With the use of this form of treatment, cancer cells become more sensitive to radiation that otherwise would resist radiation. Heat is applied by using microwave, radiofrequency, and ultrasound to a small specific area such as tumor in the case of local hyperthermia. Metastatic cancer that has spread throughout the body is treated by using whole-body hyperthermia by raising the body temperature to 107-108°F using thermal chambers¹¹.

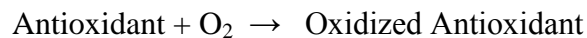
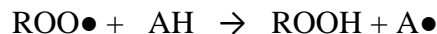
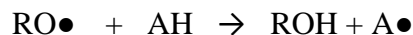
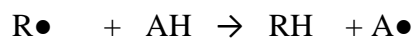
Antioxidants

In general sense, any substances that slow down or prevent the oxidation of other compounds are known as antioxidants. Chemically, antioxidants are compounds that have capability to act as an electron donor. This property is particularly important as they can stabilize or deactivate free radicals. A free radical is a species that contains one or more unpaired electrons and are electron-deficient. These are very reactive as they have unsatisfied electron valence pair and can be produced in both normal and pathological processes. Free radical represents reactive oxygen species (ROS) such as hydroxyl radical, the superoxide anion radical, hydrogen peroxide, nitric oxide radical, singlet oxygen, and various lipid peroxides. All of these species are capable to react with membrane lipids, nucleic acids, proteins, and enzymes resulting

in cellular damage. Aging, atherosclerosis, cancer, pulmonary dysfunction, Parkinson's disease, renal diseases, and neonatal lipoprotein oxidation are some of the effects of reactions initiated by free radicals. There are numerous pathways for the generation of ROS inside the body but following are the common ones.

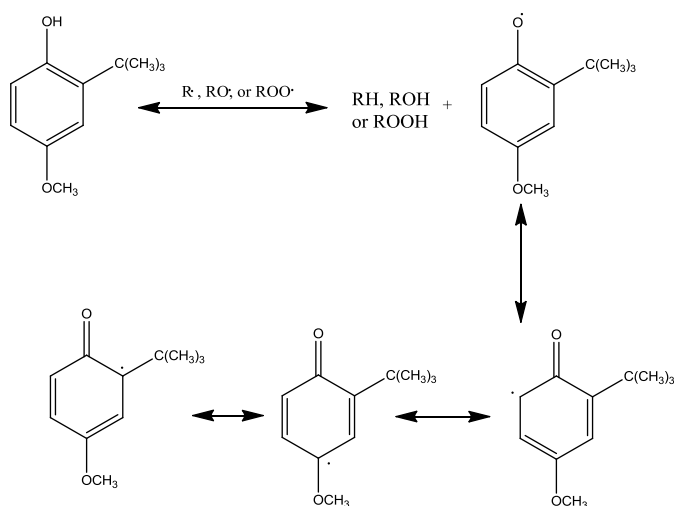
- Result of normal aerobic metabolism: Most of the oxygen in the human body gets consumed by the mitochondrial electron chain transport but some manage to escape to form free radicals.
- Consequence of oxidative burst from phagocytes in the process of defense mechanism against foreign particles including bacteria and viruses.
- Product of xenobiotic metabolism, detoxification, of toxic substances

Antioxidants terminate the chain reactions by removing free radical intermediate and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.



Scheme 1. Reaction of Oxidants with Radicals

There are two kinds of antioxidants: Natural and Synthetic antioxidants. The examples of natural antioxidants are tocopherols, nordihydroguaretic acid (NDGA), sesamol, and gossypol. Some of the synthetic antioxidants are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG), and tertiary butyl hydroquinone (TBHQ). Most of the antioxidants currently in use are monohydroxy or polyhydroxy phenol compounds with different ring substituents. They have low activation energy to donate hydrogen and the resulting antioxidant free radical does not initiate another free radical due to the delocalization of radical electron as shown in Scheme 2. The resulting antioxidant free radical is quite stable so does not undergo rapid oxidation.



Scheme 2. Stable Resonance Form of BHA

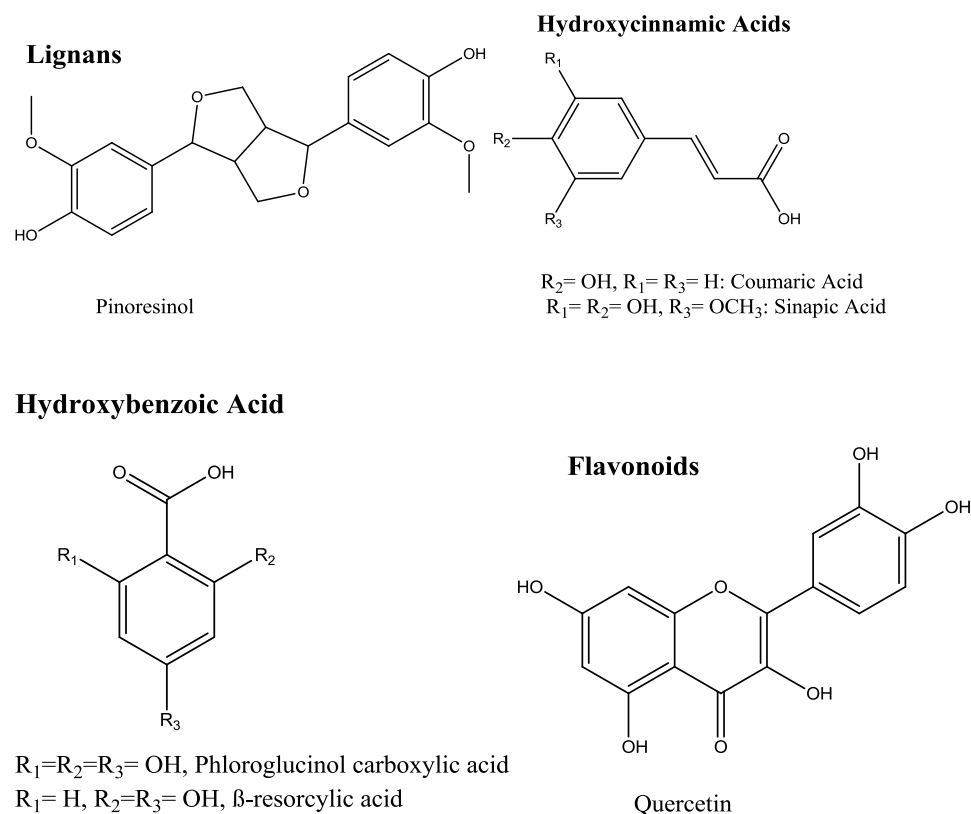
Natural Compounds with Health Benefits

The search for possible therapeutic drugs for cancer is ongoing on natural sources like plants, animals, marine organism, or microorganism. A recent report indicates that medicinal plants are used by 80% of people living in the rural areas of developing countries where the primary health care is inadequate¹⁴. Plants have always been used as sources of medicinal agents

since the beginning of mankind. The chemical and biological diversity found in nature has always been an indispensable resource for the development of novel anti-cancer compounds. The quest has been assisted by the improvement in technology like structure elucidation and organic synthesis. It is estimated that about 100 natural products have been included in treatment of various forms of tumor during the last five decades of research¹³. Plant derived anticancer agents represents just about one-fourth of the total treatment options at present. In US, nine compounds originating from plants have been approved for use in cancer therapy since 1961. The agents are vinblastine, vincristine, navelbine, etoposide, teniposide, taxol, taxotere, topotecan, and irinotecan. Another 11 agents are 10-hydroxycamptothecin, (-)-sophocarpine, monocrotaline, d-tetrandrine, lycobetaine, indirubin, colchicinamide, curcumol, curdione, gossypol, and homoharringtonine. For instance, vinblastine and vincristine, natural alkaloids found in *Catharanthus roseus* or *Vincarosea*, are used as drugs in the treatment of lymphoma and leukemia respectively. It has been found different plants contain different bioactive compounds that depend on the area, climate, and the mode of agriculture. The main challenge for the anti-cancer drug is the re-establishment of apoptosis and some plant products have been found to induce apoptosis. A significant progress has made using this particular capability of plant products. Vinca alkaloids that were isolated from *Catharanthus roseus* is an example of successful anticancer plant product. These compounds bind to tubulin and disrupt the mitotic assembly in course of cell division¹⁵. Another example is taxol, an extract from the bark of *Taxus brevifolia*. It has the cytotoxic capability that is mediated by the stabilization of microtubules¹⁶.

Polyphenols and Its Classification

Compounds that have more than one phenolic hydroxyl group attached to one or more benzene rings are known as polyphenol as shown in Fig 1. It is one of the most numerous and ubiquitous group of plant metabolites that can range from a simple phenolic molecule to highly polymerized compounds. The interests in polyphenols have recently surged because of the anti-cancer properties. The anti-cancer properties of polyphenols can be attributed to their ability to act as anti-oxidants, and also to their ability to interact with basic cellular mechanisms. Free radical scavenging and metal chelating are the modes of action to act as an antioxidant. The chain-breaking ability of polyphenols is attributed to the ease of donation of the phenolic H-atom to the attacking free radical. Fruits and vegetables are rich sources of polyphenols so the regular consumption of food with polyphenols significantly reduces the risk of various types of cancer.



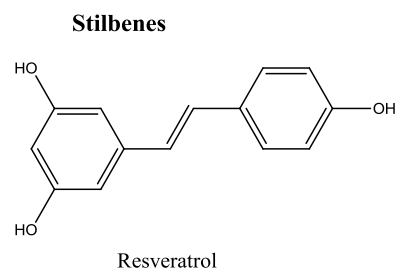
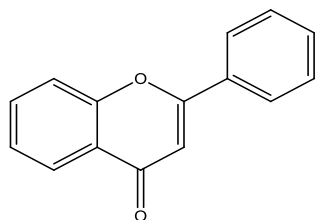


Fig 1. Classification of Polyphenols

Flavanoids as Anti-Cancer Compounds

Another class of natural compounds that show anti-cancer activity is flavanoids. The structure is shown in Fig 2. It is abundantly found in plants and vegetables and has been linked to reducing the risk of chronic diseases including cancer. At least 4000 distinct types have been found. Many flavonoids are found to have antioxidative activity, free-radical scavenging capacity, and coronary heart disease prevention along with anti-cancer activity. It is categorized as a chemo preventive substance that has been found to either delay or reverse the process of carcinogenesis. The mechanism involved during the course of action may be divided as blocking effects and suppressing effects. Flavanoids can regulate P-glycoprotein (Pgp), the MDR (multidrug-resistance) protein that plays a major role in overcoming the major hurdle, drug resistance¹⁷. Flavanoids have a major role in inhibiting various enzymes that are the targets in anticancer treatment, e.g. eukaryotic DNA topoisomerase I, Cox I and II, and estrogen 2- and 4-hydroxylases¹⁸.



FLavone Backbone Molecular Structure
2-phenyl-1,4-benzopyrone

Fig 2. Flavone Structure

Anthraquinones and Naphthaquinones as Anti-Cancer Compounds

Anthraquinone is a class of aromatic organic compound that contains two keto groups on the central ring as shown in Fig 3. Aloe emodin is one of the anthraquinones which inhibits cell growth in tumor of lung carcinoma, hepatoma, and leukemia. Aloe emodin particularly plays an important role in the regulation of cell-cycle progression and shows a high specificity for neuroectodermal tumor cells. Other anthraquinones like emodin and rhein were linked with G1/S cell-cycle arrest in hepatoma, glioma, and colon carcinoma cells¹⁹.

Naphthaquinone usually refers to organic compound derived from naphthalene. Juglone is the most studied naphthaquinone known for its anti-bacterial, anti-fungal, and anti-viral properties. Its ability to induce apoptosis in the tumor cell marks juglone as an anti-cancer compound.

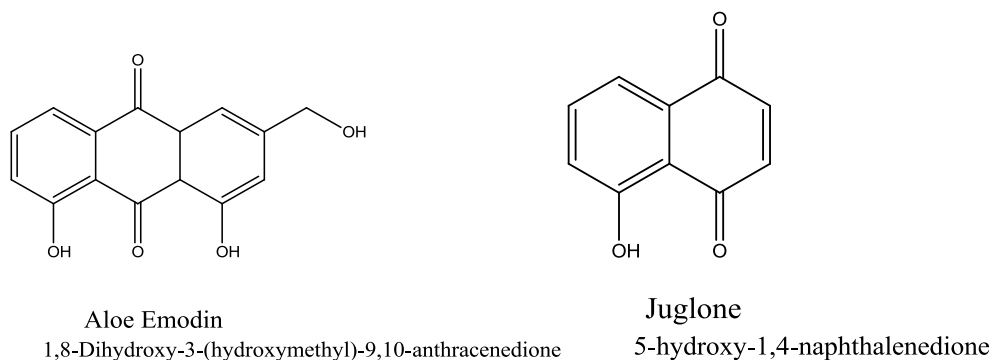


Fig 3. Structures of Anthraquinones and Naphthaquinones

Stilbene

The name stilbene was derived from a Greek word stilbos, which means shining. Stilbene is a highly conjugated compound and named 1, 2-diphenylethylene. There are two isomeric forms of stilbene shown in Fig 4: (E)-stilbene also known as trans-stilbene and (Z)-stilbene also known as cis-stilbene. The sterical interaction of phenyl rings forces them out of plane and prevents conjugation making the cis isomer thermodynamically less stable. (E)-stilbene has a

melting point of 125°C, while the melting point of Z isomer is around 6°C²⁰. Under the exposure to light, trans-stilbene can isomerizes to cis-stilbene using the features associated with absorption and fluorescent properties. During the process, the excitation of π - electrons of the conjugated ethenediyl group occurs to π^* orbitals. Thus, excited singlet state is governed by fluorescence from the S_1 state competing with isomerization. The process of photochromism, trans-cisphotoisomerization of stilbene derivatives, can be observed easily by a single steady-state fluorescence technique²⁰.

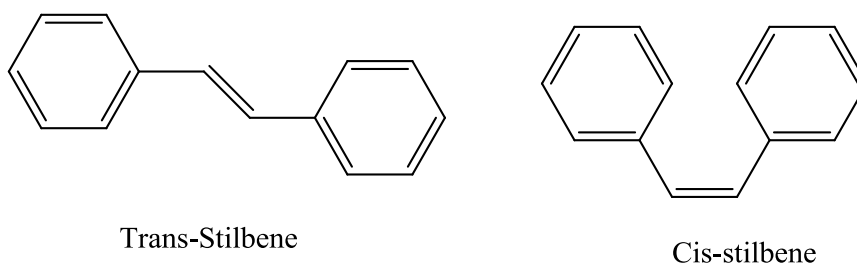


Fig 4. Structure of Cis and Trans Stilbene

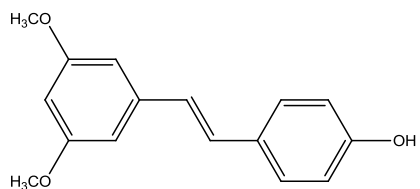
Derivatives of Stilbene

Synthesis of stilbene derivative is fairly simple as the derivatives are usually thermally and chemically stable. They exhibit absorption and fluorescence properties that can be monitored by various optical techniques. The derivatives of stilbene are primarily used for various industrial, photophysical, photo chemical, and biomedical research purposes. Stilbenoids, hydroxylated derivatives of stilbene, are the secondary products of heartwood formation of trees that can also act as phytoalexins²¹.

Pterostilbene

Researchers have found that pterostilbene (Fig 5) and resveratrol works in a synergistic fashion for various beneficial functions in the human body. For example, both the compounds activate one's "longevity genes" and pterostilbene assists in gene expression in a way that

increases the effects produced by resveratrol²¹⁻²³. Rimando and researchers at University of Illinois at Chicago made a huge discovery in 2002 about the anti-cancer properties after the tests in rats' mammary glands²².

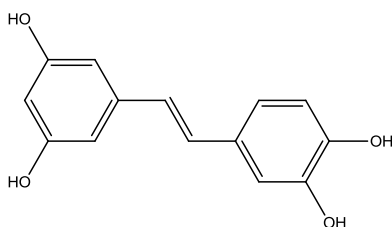


Pterostilbene
4-[(E)-2-(3,5-Dimethoxyphenyl)ethenyl]phenol

Fig 5. Structure of Pterostilbene

Piceatannol

Piceatannol is a naturally occurring hydroxylated analogue of resveratrol that shows wide spectrum of biological activity (Fig 6). Piceatannol has been found in grapes, passion fruit, white tea, and Japanese knotweed and has antioxidative and anticancer properties. In addition, piceatannol is found to be involved in the suppression of the activation of some transcription factors like NF-kappaB that plays a major role as a transcriptional regulator in response to the cellular stress caused by free radicals, UV radiation, cytokines and microbes²⁴. Piceatannol has been found to either delay or inhibit the process of adipogenesis indicating its use against obesity^{25, 26}.

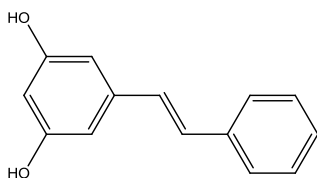


Piceatannol
3, 3', 4, 5'-trans-trihydroxystilbene

Fig 6. Structure of Piceatannol

Pinosylvin

Pinosylvin is another hydroxyl stilbene that lacks a hydroxyl group on the second ring as shown in Fig 7. The absence is accounted by its origin as a product of condensation of malonyl-CoA and cinnamoyl-CoA instead of p-coumaroyl CoA. Unlike phytoalexins, pinosylvin is produced before the infection and are called pre-infectious stilbenoid. Pinosylvin is found in the *Pinus* species. Pinosylvin has been found to exhibit anti-apoptotic effect²⁷ and a suitable candidate to act as a pro-angiogenic factor²⁷.

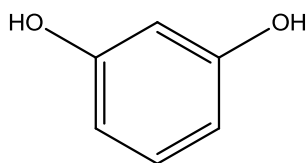


Pinosylvin
5-[(E)-2-Phenylethenyl]benzene-1,3-diol

Fig 7. Structure of Pinosylvin

Resveratrol

Resveratrol is a naturally occurring phytoalexin produced by plants in response to stress, UV radiation, and fungal attack. RV is found in grapes, wine, peanuts, blueberries, cranberries, mulberries, and jack fruit. RV has also been found in seeds, roots, vines, and stalk but the highest concentration is found on the skin which is 50-100 µg per gram. It is interesting to note that on average around 73 µg of RV is found in peanuts while 160 µg on an ounce of red wine. RV was first discovered in 1940 as an off-white powder from the extract of roots of white hellebore lily (*Veratrum grandiflorum*). It was considered a resorcinol (Fig 8) derivative coming from a *veratum* species so was named resveratrol. Dried roots of white hellebore and Japanese knotweed (*Polygonum cuspidatum*) were found to have RV and were used to treat various skin infections like athlete's foot.

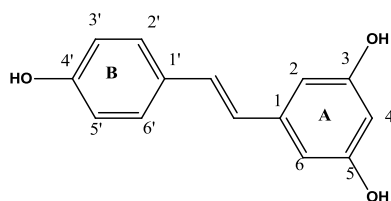


Resorcinol

Fig 8. Structure of Resorcinol

Nomenclature

According to IUPAC nomenclature, RV is named as trans-3, 4', 5-trihydroxystilbene (Fig 9). The structural formula and numbering of carbon is shown below. Some other names used for RV are 3, 4', 5-Stilbenetriol, (E)-5-(p-Hydroxystyryl) resorcinol and (E)-5-(4-hydroxystyryl) benzene-1, 3-diol.



Structural formula of RV
Trans-3,4',5-trihydroxystilbene

Fig 9. Structural Formula of RV

France has one of the lowest rates of cardiovascular disease (CVD) rates in the world³¹. According to a report by World Health Organization 2009 Mortality Database, the deaths from cardiovascular disease (CVD) for French population were 50 out of every 100,000, while it was 129 for American counterparts³¹. It was really surprising to see the low rates despite the intake of high cholesterol and saturated fats in French diet. It led to a phenomenon known as French Paradox that attributes the lower incidence of coronary diseases for regular consumption of red wine, a major source of RV. The term “French Paradox” was first coined by Serge Renaud in

1993 in an effort to explain the low rates of CVD in France on the basis of red wine consumption^{31,32}.

Chemical and Physical Properties

RV exists as white powder with slight yellow cast in two geometric isomers (Fig 10): cis (Z) or trans (E). The Z isomer is sterically hindered and less stable because the steric interactions forces the aromatic rings out of plane and prevent conjugation. The trans form can isomerize to give cis-form in presence of UV radiation by a process called photo isomerization.

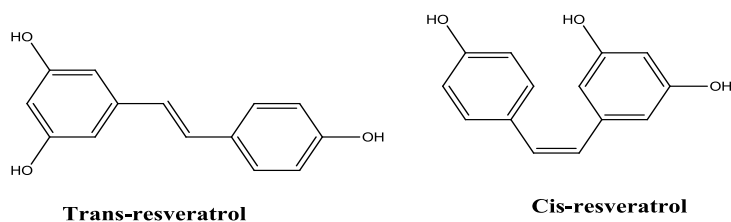
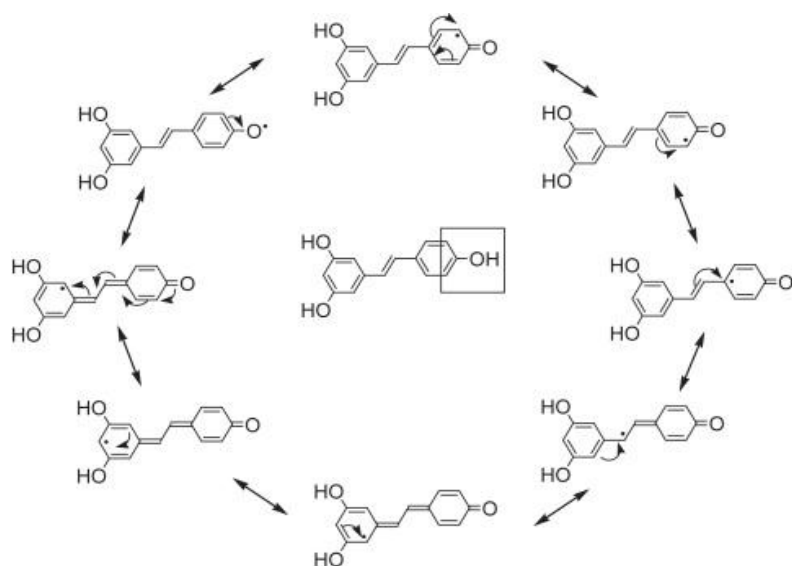


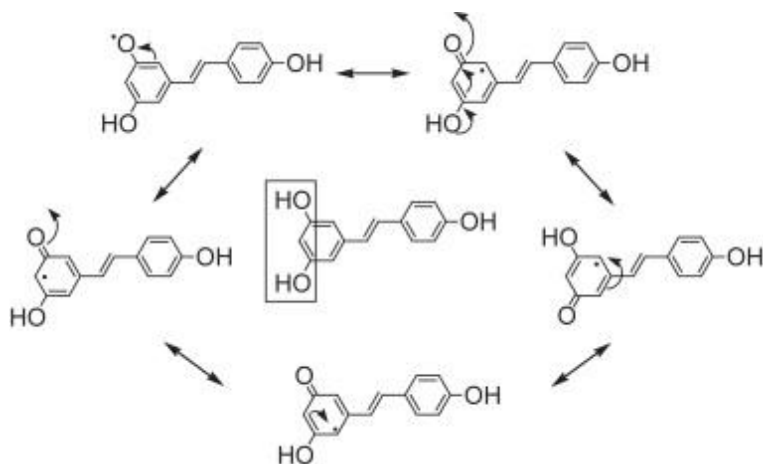
Fig 10: Structure of Cis-RV and Trans-RV

RV is a fat soluble compound and has very limited solubility in water. The solubility of RV in water is 0.03g/L while the solubility in ethanol and DMSO are 50g/L and 16g/L respectively. RV behaves in a similar manner to phenol when reacting with other chemical species as it a polyphenol. The stilbene skeleton is stable and the reactivity is associated with the hydroxyl functional group of phenol units. RV is a good radical scavenger through electron capture or proton donation to convert free radicals to more stable molecules. The hydroxyl group at 3 and 5-position (Fig 10) are equivalent because of the rotation of the sigma bond between carbon-1 and SP² hybridized carbon. The hydroxyl group at 4'-position (para) is more acidic than the ones at 3 and 5-position (meta). This is accounted by the stabilizing effect caused by the delocalization of electrons over a greater area resulting in more resonance structures. The formation

resonance structure for para and meta hydrogen abstraction is shown below in Scheme 3 and Scheme 4 respectively.



Scheme 3. Resonance Structures from Proton Abstraction at Para Position⁵⁰



Scheme 4. Resonance Structures from Proton Abstraction at Meta Position⁵⁰.

Biological Activities

RV has been reported to exhibit a wide range of biological and pharmacological properties that are beneficial for human health like anti-inflammatory³³⁻³⁵, antioxidant³³⁻³⁶, antiproliferative³⁴, proapoptotic³³, antiaging³⁴, cardioprotective³³⁻³⁶, anticancer³³⁻³⁶, neuroprotective^{34, 35}, and antiviral properties³³⁻³⁶. It has also been speculated RV can promote nitric oxide production³³⁻³⁶, inhibit platelet aggregation³³⁻³⁶, and increase high-density lipoprotein cholesterol to serve as a cardioprotective agent³³⁻³⁶. Recently, RV has been identified as a sirtuin-activating compound³³. Sirtuins are a conserved family of NAD⁺-dependent deacetylases (class III histone deacetylases). Like SIRT1, small-molecule activators of sirtuins including resveratrol extend lifespan in yeast and higher organisms³³. It has also been reported RV has potential inhibitory effects on cyclooxygenase³⁶, rat liver mitochondrial ATPase³⁶, human F1 ATP-ase³⁶, and tyrosinase³⁶. It has been that there is a close relationship between the process of aging and the onset of neurological disorders so an ideal compound to fight against the neurological disorder should have neuroprotective action and anti-aging effect. RV has both the properties and is a suitable candidate for the treatment of neurological disorders like Parkinson's and Alzheimer's³⁹. According to European Journal of Pharmacology, RV has important anti-inflammatory neuroprotective effects on animal models with Parkinson's disease. RV has been found to have a capability to prevent "free-radical-mediated damage of nerve cells"³⁹ that further assists in overcoming the damage caused by deep brain stimulation³⁹.

Resveratrol as a Cancer Drug

Hundreds of plant extracts were evaluated to find chemical agents that inhibit cancer. Resveratrol was discovered as a result of search for chemo preventive agents³⁷. Chemoprevention is the prevention of cancer by administering chemical agents that reduce the risk of carcinogenesis. Some of the examples of agents are nonsteroidal anti-inflammatory drugs

(NSAIDs) that include indomethacin, aspirin, piroxicam, and sulindac^{37,38}. The primary function of these agents is to inhibit cyclooxygenase (COX)³⁷. This inhibition is significant in course of chemoprevention as COX catalyzes the substances that induce tumor growth and suppress immune surveillance. RV is currently in clinical phase II trial as an anti-cancer drug for the cure of human colon cancer³⁶. In mice RV has been found to selectively suppress the transcriptional activation of acytochrome P-450 1A1 that further inhibits the formation of carcinogen-induced preneoplastic lesions. Some compounds turn into carcinogenic after they have been metabolized by enzymes like cytochrome P450. RV actually inhibits the function of certain cytochrome P450 which would be instrumental in reducing the exposure to the activated carcinogens. RV also stimulates apoptosis, cell cycle delay or a block in G₁→S₁ transition phase at cellular level³⁸.RV has been found not only to inhibit the proliferation of cancer cells but also induce apoptosis in various cancer cell lines. Another significant role played by RV against cancer is the inhibition of metalloproteinase, an enzyme that helps the cancerous cells to invade normal tissue.

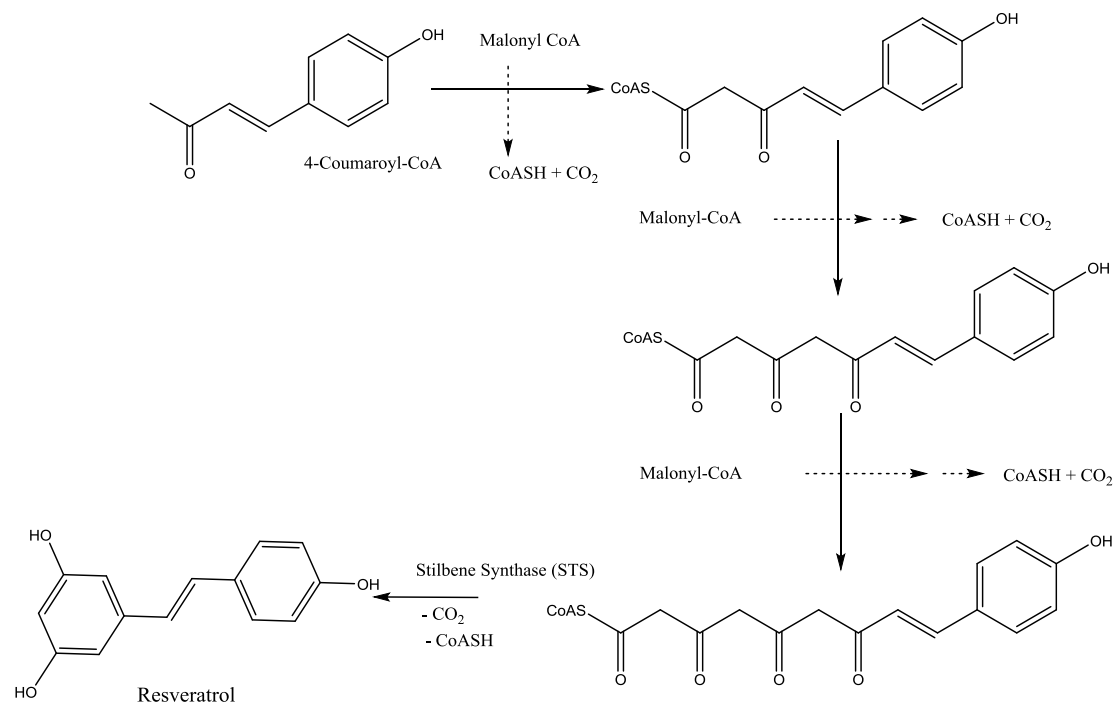
Pharmokinetics of Resveratrol

In spite of the therapeutic effects, pharmacokinetic properties of RV are not favorable. This is mainly because of poor bioavailability and rapid metabolization to glucuronide and sulfate conjugates. The plasma concentration of RV after the oral administration was found to be very low. In a study, RV was administered orally for 14 consecutive days at 50 or 150 mg/kg/day and plasma concentrations of RV and metabolites were measured using HPLC mass spectrometer system⁴⁰. RV was found to be approximately 20 percent bioavailable⁴¹. In rodents, RV was found to be absorbed mainly in duodenum and has the same 20 percent bioavailability⁴⁰. Majority of the absorbed RV was found in plasma as conjugated derivatives. The five distinct metabolites found were RV monosulfate, two isomeric forms of RV monoglucuronide,

dihydroresveratrolmonosulphate and dihydroresveratrol monoglucuronide^{40, 41}. The modification into the glucuronide and sulphate reduces the permeability and helps in excretion that has been found to reduce the bioavailability of RV. To enhance the pharmacokinetics, drug delivery systems need to be developed to protect and stabilize resveratrol and to enhance its bioavailability. Another method could be the administration of resveratrol through buccal cavity.

Biological Synthesis of Resveratrol

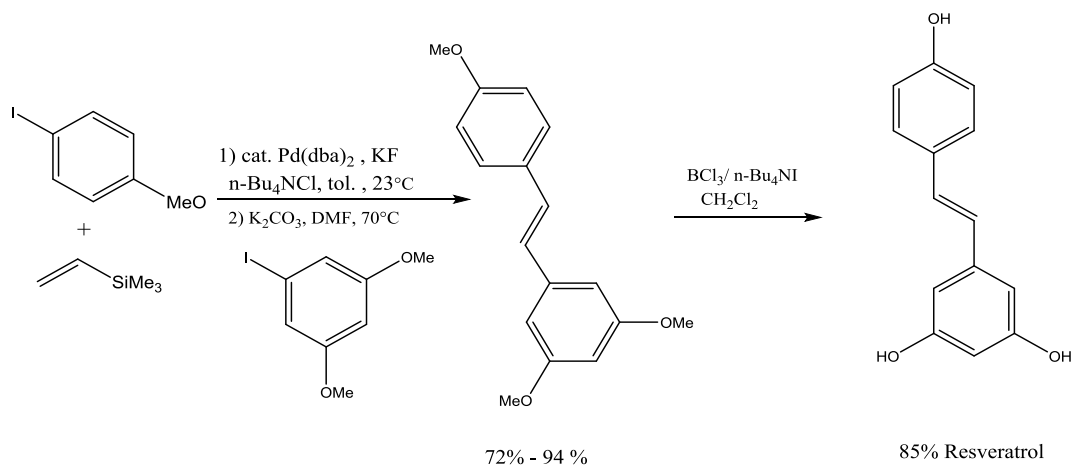
As discussed earlier, RV is produced as a phytoalexin by plants as a defense against pathogen attack. RV is synthesized from p-coumaroyl-CoA and three molecules of malonyl-CoA. Stilbene synthase is used as enzyme during the course of reaction and is not very specific because it has also capability to accept other CoA esters and aliphatic primers for polypeptide synthesis. The stilbene synthase is a condensing enzyme that uses three sequential condensation reactions with malonyl-CoA. The terminal carboxyl group is lost towards the end of the reaction as carbon dioxide. Chalcone is also synthesized in a similar fashion but uses different enzyme called chalcone synthase. The process is shown on Scheme 5 below.



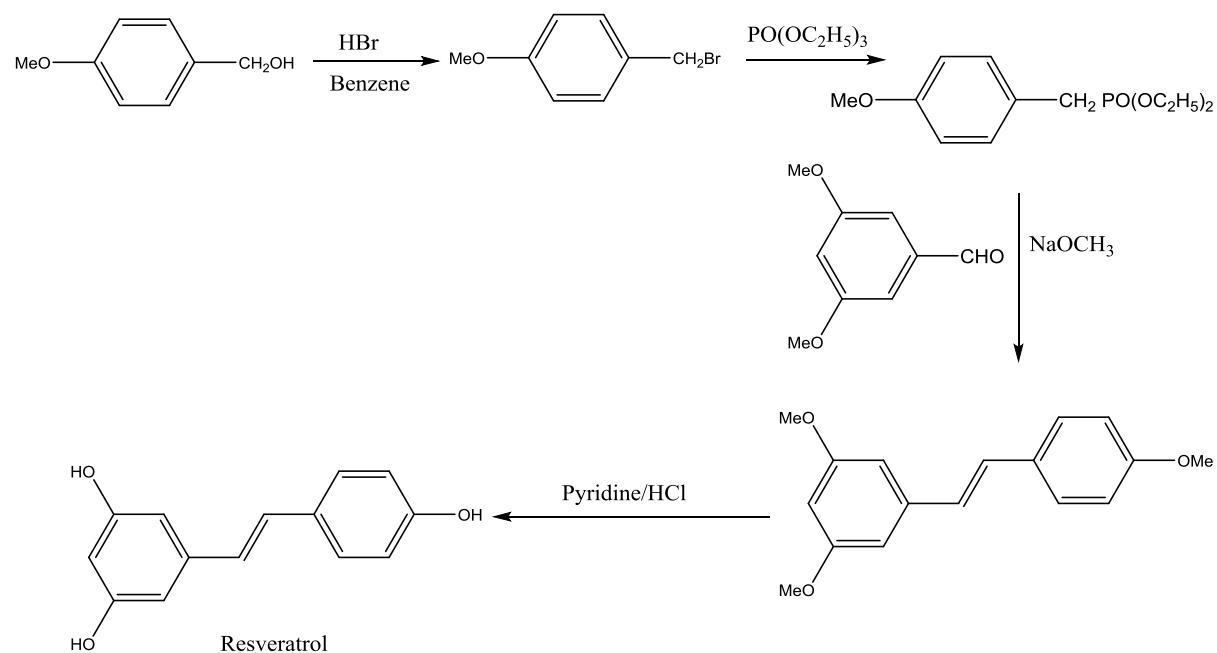
Scheme 5. Biosynthesis of Resveratrol

Chemical Synthesis of Resveratrol

It may not be quite feasible and economical to extract resveratrol from the natural sources all the time so various efforts have been made to synthesize in the laboratory. Some of the popular methods with a good yield are depicted below with reaction Scheme 6 and Scheme 7.



Scheme 6. Synthesis of RV Using Sequential Heck Reaction



Scheme 7. Resveratrol Synthesis by Mingfu Wang et al⁴⁶.

Derivatives of Resveratrol

Various attempts have been made to synthesize new derivatives of RV to improve its biological activities. Structure-activity is used to analyze information of various properties like anti-tumor by selective modification of stilbene scaffold of resveratrol. The substitution of hydroxyl groups of RV by methoxy groups has been found to significantly improve the cytotoxic activity. Various methoxylated analogs of RV have been made since and some of the examples are shown below in Fig 11. Tetramethoxy-trans-stilbene was found to be more powerful in inhibiting the growth of cancer cell lines like colon, prostate, and ovarian cancer cells³³.

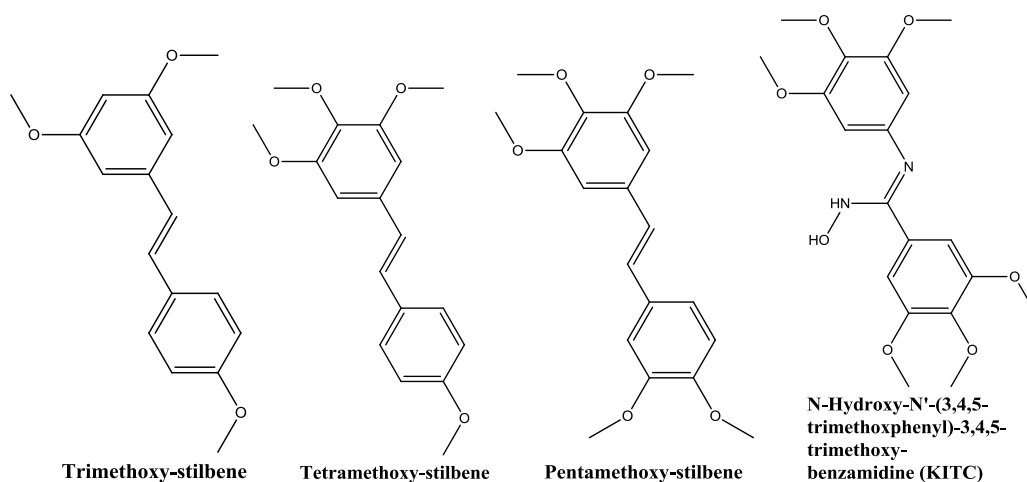


Fig 11.Methoxylated Derivatives of Resveratrol

Compounds formed by the introduction of hydroxyl groups in stilbene have shown more biological activities than RV. Both tetrahydroxy-trans-stilbene and hexahydroxy-trans-stilbene has been found to inhibit cell growth and induce apoptosis in higher extent than RV^{33, 47}. They have also been found to cause cell-cycle arrest at S or G2/M-phase. Some of the hydroxyl derivatives of RV are shown below^{47, 48} in Fig 12.

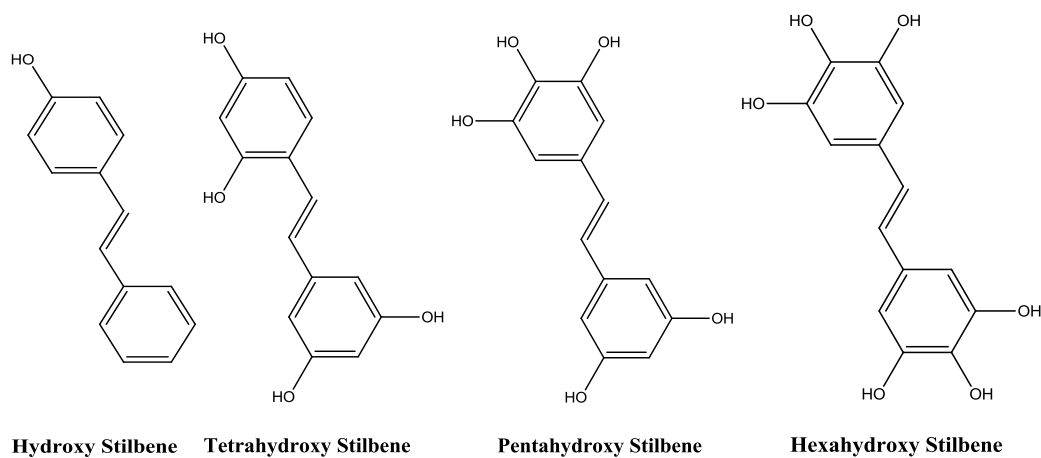


Fig 12.Hydroxylated Derivatives of Resveratrol

Acetylated derivatives of RV were found to enhance cellular uptake than the parent compound, RV. For example, resveratrol triacetate was found to be efficient in inducing cell-cycle arrest at S-phase and increases uracil mediated inhibition of proliferation. Another ester derivative, diallylresveratrol, was found to induce apoptosis and inhibit the transition from S to G2/M-phase of the cell cycle^{47, 48}. It also helped in the inhibition of the growth of colon cancer cells⁴⁸. Some of the acetylated derivatives are shown below in Fig 13.

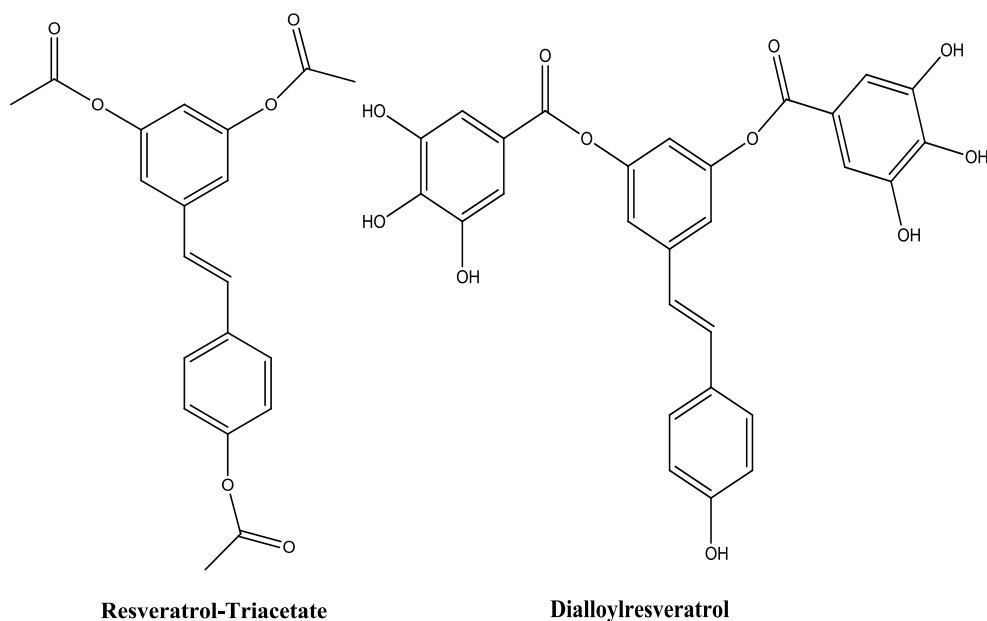


Fig 13. Miscellaneous Derivatives of Resveratrol

In 2008, Jiang synthesized two RV aliphatic acids and their esters in an attempt to overcome the limitations of RV. The derivatives showed better solubility in pure water and phosphate buffer (pH 7)³⁶. The binding affinity of new derivatives with human serum albumin was 41-fold improvement and was much better ligand³⁶. The derivatives are shown in Fig 14.

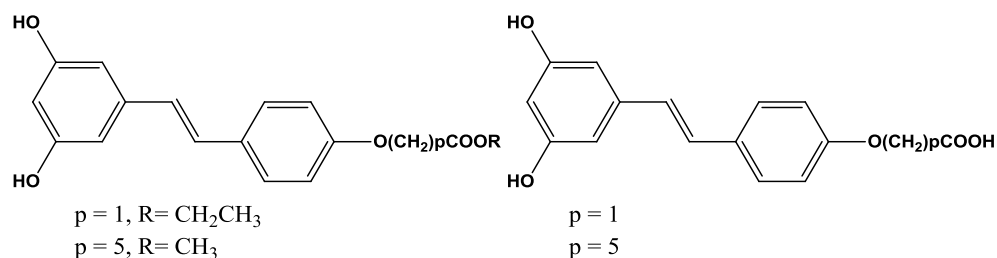


Fig 14. Derivatives of RV by Jiang

Drawbacks of RV

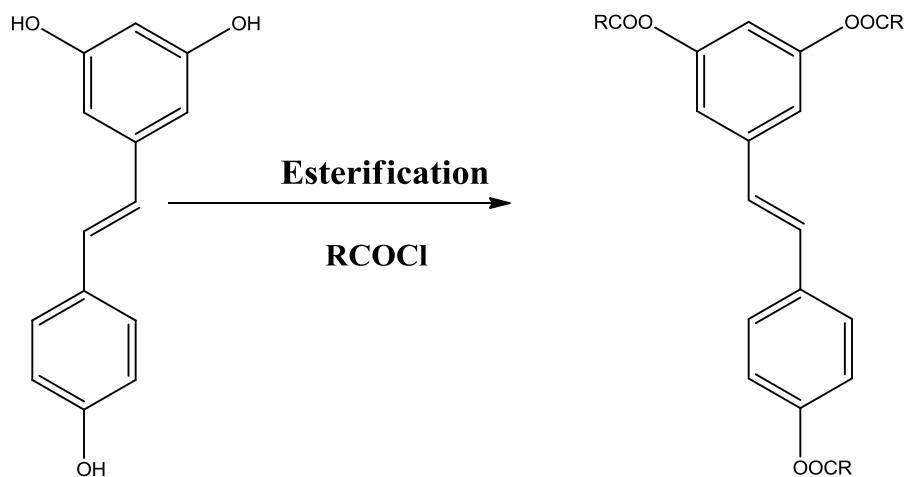
RV has been reported to have a lot of benefits to human health but the in vivo biological effects are strongly reduced by its low bioavailability and solubility. Its bioavailability is very limited due to high metabolism when subjected in plasma. This is a hurdle to the development of possible therapeutic applications. Most of the research is done in cultured cells that are exposed to unmetabolized RV and are of higher concentration than the one in blood plasma so it is also not quite sure if RV reaches the proposed site of action when administered orally. A study was conducted by giving a 25 mg oral dose RV to six healthy human but a trace amount of unchanged resveratrol (<5 ng/mL) was detected in plasma. Most of the oral dose was recovered in urine as metabolites⁴²⁻⁴⁵.

Objective of this Research

The main objective of this research was to synthesize new derivatives of RV to overcome its drawbacks i.e. low solubility and low bioavailability. The new derivatives are expected to show anti-cancer properties and thus be able to use as chemo preventive compounds. The initial plan was to synthesize triester derivatives and hydrolyze to form diester derivatives. However, the aim was changed and limited to the synthesis of triesters. Also, another purpose of the research was to carry out independent organic synthesis and structure elucidation of new derivatives by spectral analysis.

Synthetic Design

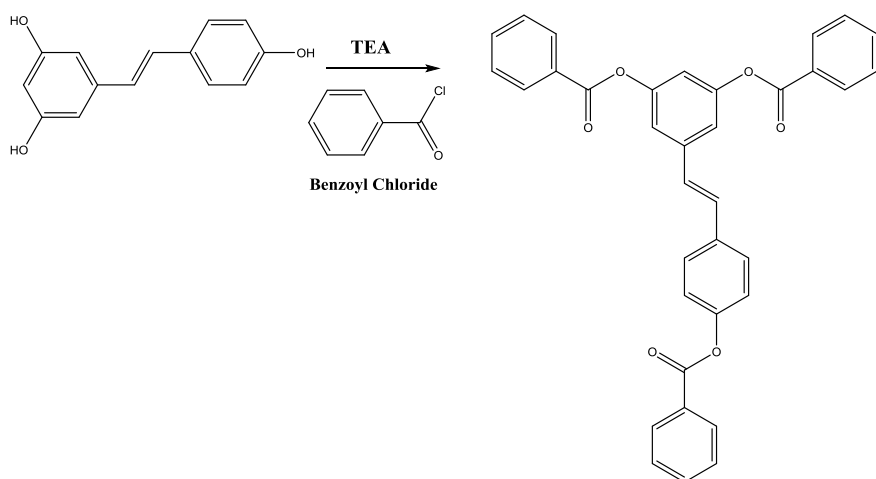
The objective of the research was inspired by the derivatives reported by Jiang³⁶. Jiang reported the synthesis of new RV aliphatic acids and esters that was found to be much better ligand for human serum albumin than RV³⁶. The design for this research was to add both aliphatic and aromatic chain in all hydroxyl groups through an esterification reaction resulting triesters and is shown in Scheme 8.



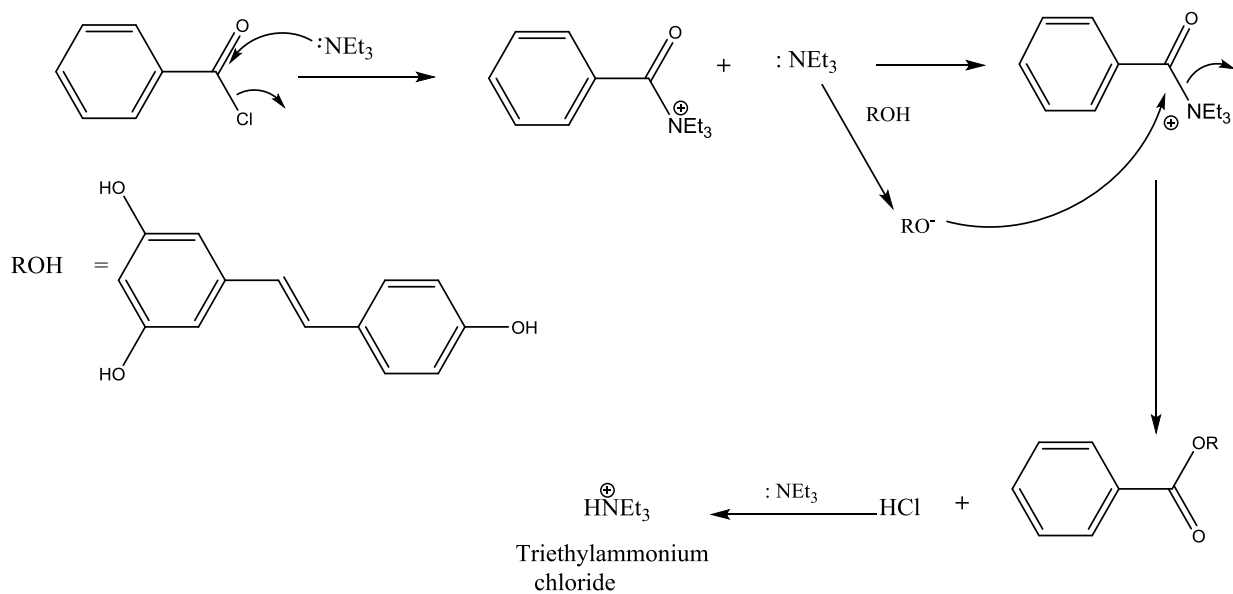
Scheme 8. Synthesis of Resveratrol Triester by Esterification

The proposed synthetic pathways for the compounds 1, 2, and 3 have been shown below respectively in Scheme 9, Scheme 11, and Scheme 12. Scheme 10 shows the reaction mechanism involved.

Proposed Synthetic Pathway for Synthesis of 3, 4', 5-tri (benzoyloxy) stilbene

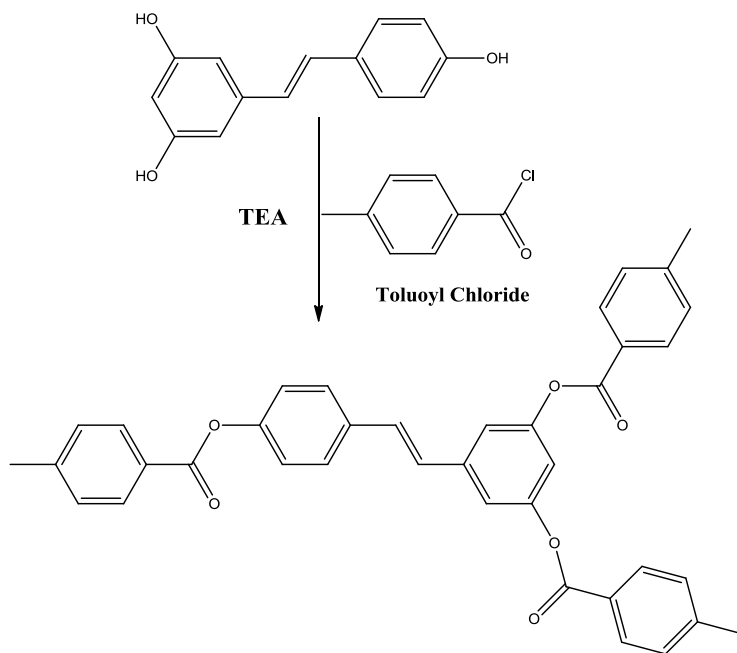


Scheme9: Synthesis of 3, 4', 5- (benzoyloxy)stilbene



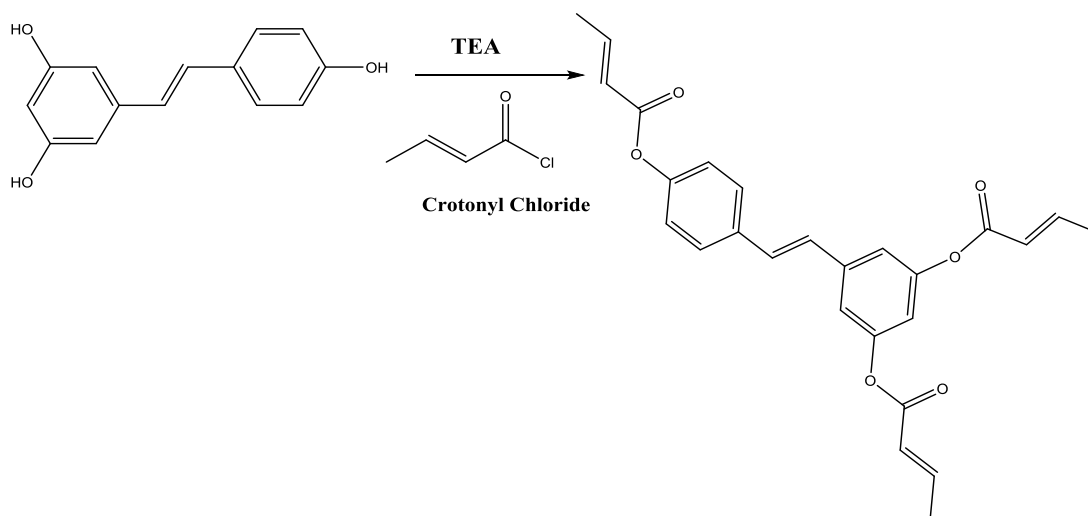
Scheme 10. Mechanism of Esterification of Resveratrol

Proposed Synthetic Pathway for Synthesis of 3, 4', 5- tri (toluyloxy) stilbene



Scheme 11: Synthesis of 3, 4', 5- tri(toluyloxy)stilbene

Proposed Synthetic Pathway for Synthesis of 3, 4', 5- tri (2''- butenoyloxy) Stilbene

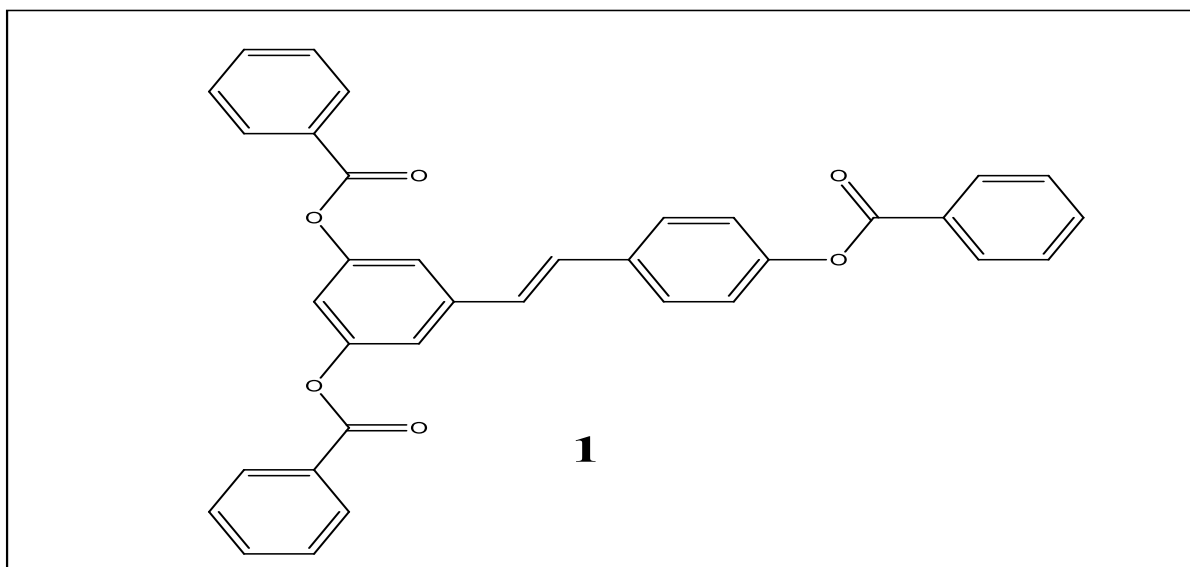


Scheme 12: Synthesis of 3, 4', 5 –tri (2''-butenoyloxy) Stilbene

CHAPTER 2

RESULTS AND DISCUSSION

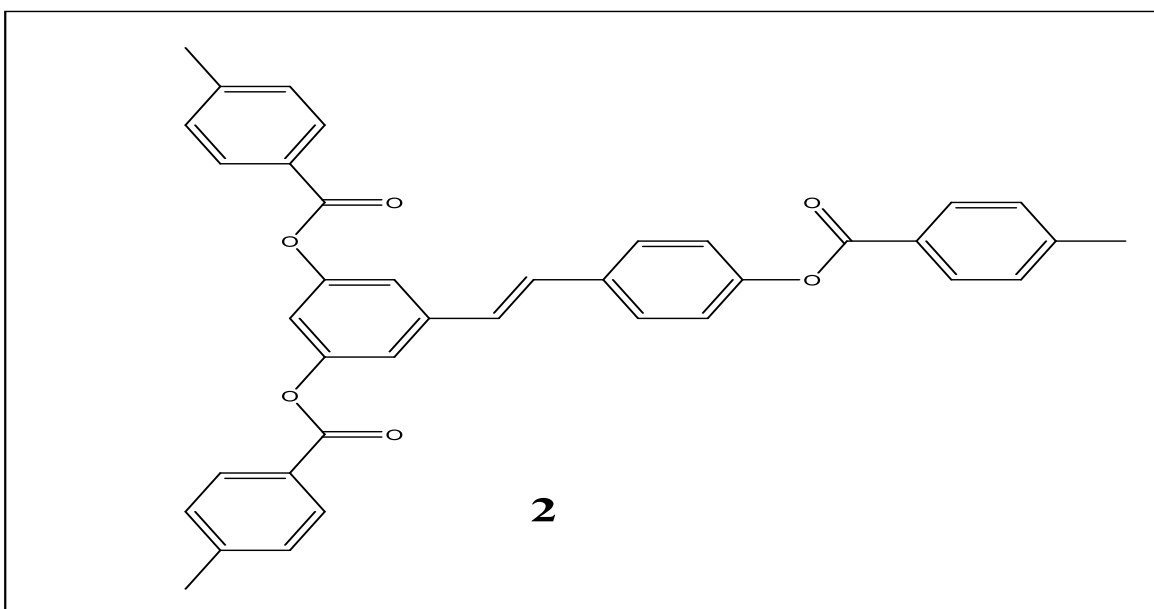
Synthesis of 3, 4', 5- tri (benzoyloxy) stilbene



A high yield of approximately 90% of compound **1** was obtained as white solid after the purification using gradient column chromatography. The melting point of the compound **1** was found to be 158.2°C - 160°C and the R_f value was 0.65. The compound has not been synthesized before and can be subjected for further biological evaluation or can be used as a precursor to make diesters. Triethyl amine (TEA) was used as a base that also acts as a nucleophile to attack the carbonyl carbon of benzoyl chloride. This resulted in the formation of a salt that acted as an electrophile to attack the hydroxyl group in RV. The hydroxyl group at para position (4') got attacked because of acidity as shown in scheme 2. During the course of reaction, hydrogen chloride was produced that was removed by the reaction with TEA forming a salt, triethylammonium chloride. Removal of hydrogen chloride was essential for the reaction to proceed further. The integration of peaks in NMR spectrum was carried out on the basis of

aromatic hydrogen versus non- aromatic hydrogen. The ratio of aromatic to non-aromatic hydrogen was 22:2 that could be obtained by adding the integration values. The peaks on the higher field were due to solvents and moisture. Attempts to dry the sample were made, but the peaks were still present. There were all together 19 different carbons were confirmed by the 19 peaks at ^{13}C spectrum. The IR spectrum was used to confirm the presence of carbonyl groups between $1735\text{-}1750\text{ cm}^{-1}$ as indicated on the spectrum. There was no stretch to indicate the presence of hydroxyl group around the region of $3200\text{-}3500\text{ cm}^{-1}$. All these results confirmed the formation of triester which is compound **1**.

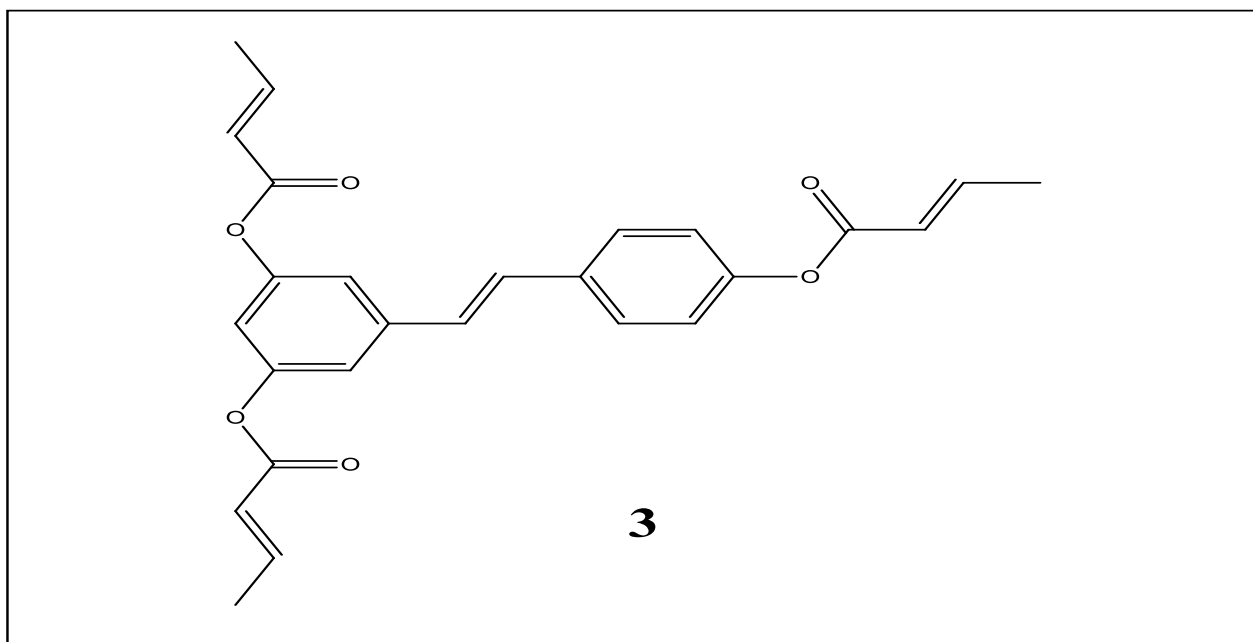
Synthesis of 3, 4', 5- tri (toluyloxy) stilbene



Compound **2** was obtained as a white solid after the purification using gradient column chromatography. The compound had a melting point of $156.5\text{-}157.2^\circ\text{C}$ and R_f of 0.7. It is a novel triester derivative of RV which can be used either to form diester by hydrolyzing the para position substituent using a hydrolytic enzyme or it can be subjected for biological evaluation to test its bioavailability. The NMR spectrum was integrated separately for aromatic and non-

aromatic hydrogen. The methyl H's of toluyl group was made a reference by normalizing it and integrating other hydrogen present in the compound. NMR spectrum still indicates the presence of some impurities so ^{13}C spectrum was not taken for this compound. The IR spectrum was used just to confirm the introduction of ester group. There was no indication for any peaks for hydroxyl group around $3200\text{-}3500\text{ cm}^{-1}$, which means the esterification reaction was carried out successfully.

Synthesis of 3, 4', 5- tri (2''-butenoyloxy) stilbene



Compound **3** was obtained as brown dense oil with a moderately high yield of around 75%. Repeated attempts to purify the compound using column chromatography with various solvent systems were made but were not successful. The TLC indicated the presence of multiple products. It was a possibility for the double bond in ester chain to have different configuration (cis and trans) that led to multiple products with similar polarity causing difficulty in separation. The crotonyl chloride used was a mixture of both cis and trans isomer. It was a novel derivative

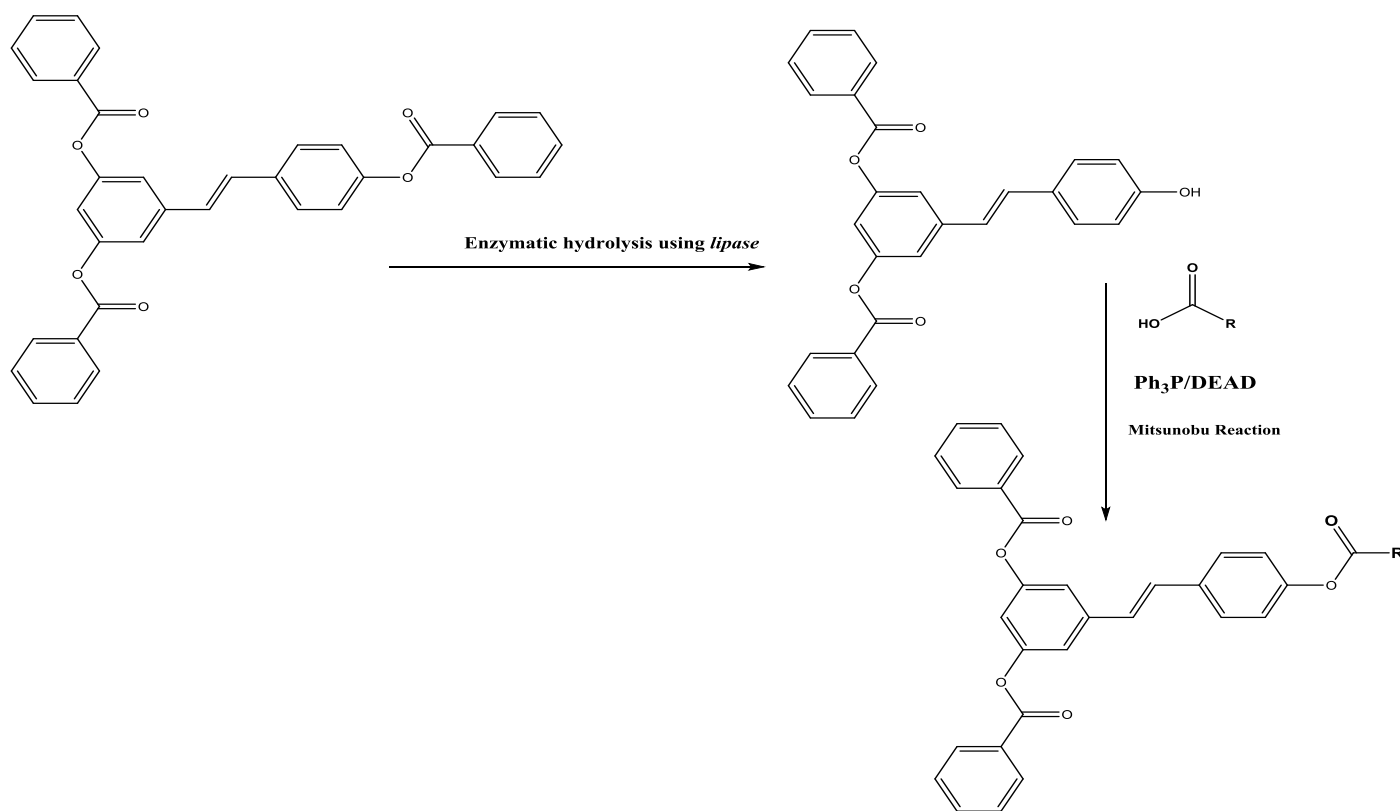
of resveratrol that could be subjected for biological evaluation after further purification. Even though the NMR spectrum showed some impurities, most of peaks for the hydrogen were accounted. There were three different kinds of hydrogen in the ester chain that is shown on the ^1H NMR spectrum. The farthest peak at the high field represents the hydrogens at methyl group of the ester chain and then the alkene hydrogens. All the peaks are not accounted for, which means there were some impurities. Various attempts to purify the compound were not successful. As a result, the compound was not pure to the publishable standard. IR spectrum was used to make sure the hydroxyl groups were converted to the ester group by the esterification reaction. The three carbonyl groups are represented by the peaks at 1734 cm^{-1} and there are not any peaks corresponding to hydroxyl group. These entire spectrums confirmed the formation of compound **3** and indicated some impurities.

Conclusions

The objectives of the research were fulfilled with the synthesis of new RV triester (**1**, **2**, & **3**) derivatives. All of these compounds are the new derivatives of RV that has not been synthesized before. As per the standards of Journal of America Chemical Society, compound **1** is publishable while **2** and **3** are not pure enough. The initial goal was to synthesize diesters by hydrolyzing the ester chain at para position, but the objective was changed during the course of research. Chemical esterification of phenolic compound was the primary synthetic route that was used during the course of research. With this route, there were formation of many side products that cost time, energy, and chemicals for purification. Also, it was not easy to separate the impurities, the case with compound **3**. It is primarily because of unselective nature of reaction. Various attempts were made to make the reaction selective and to increase yield. The purity of

the product was confirmed using ^1H and ^{13}C NMR. The confirmation of introduction of carbonyl group and the disappearance of hydroxyl group in the novel derivatives were confirmed by IR.

All the compounds are new so can be subjected to biological evaluation. A similar triester derivative of RV has been synthesized and reported to have much better bioavailability than RV. Various future works can be carried out on these new derivatives. As has been investigated before⁴⁹, similar compounds like 1, 2, & 3 have biological potential as anti-cancer agents with increased solubility and bioavailability. Collaborations with Dr. Deling Yin have used RV derivatives in the past to pursue pharmaceutical applications. It is anticipated that compound 1-3 will also be investigated along these lines⁴⁹. The triester derivatives can be used as a precursor for synthesizing new derivatives (Scheme 13).



Scheme 13. Future Reactions of the New Triester

The enzymatic hydrolysis can be carried out using an enzyme lipase like *C. Antarctica* that will selectively hydrolyze the ester group at 4' position. This position is less sterically hindered than the 3' and 5' position. The next step after the enzymatic hydrolysis can be the conversion of alcohol group into a variety of functional groups through Mitsunobu reaction. Oye Mitsunobu discovered the reaction that uses triphenylphosphine and an azodicarboxylate like diethyl azodicarboxylate (DEAD). The reaction proceeds with inversion of configuration on the alcohol carbon. The order of addition of reagents is crucial, typically the alcohol, acidic nucleophile, triphenyl phosphine are dissolved in THF, then DEAD added slowly to the mixture and stirred for about 12 h. Various acid and ester derivatives have shown increased biological activities and better solubility so the prospects and potential of compounds 1, 2, &3 to make other derivatives are huge.

CHAPTER 3

EXPERIMENTAL

General Methods

All the reagents used during the course of the experiment except resveratrol (RV) and crotonyl chloride were purchased from Sigma (St Louis, MO, USA) and used without further purification. RV and crotonyl chloride were purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan) and used as supplied. Proton (^1H) and Carbon-13 (^{13}C) NMR spectra were taken from JOEL-NMR Eclipse spectrometer operating at 400 MHz and 100 MHz for proton and carbon nuclei respectively. Chemical shifts were recorded as delta values in parts per million (ppm) relative to TMS. The multiplicity of the signals was documented as: s, singlet; d, doublet; m, multiplet. CDCl_3 and CD_3COCD_3 were used as solvents for NMR unless otherwise stated. The infrared spectrum was obtained using a Shimadzu FTIR Presctige-21. Silica gel for gravity column filtration was used as a stationary phase, while different mixtures of solvents were used as eluents to carry out the column chromatography. Thin Layer Chromatography (TLC) was conducted with UV active silica gel plates using suitable solvent mixtures that were then viewed under UV lamp using short wave. All mass measurements were taken using Ohaus Adventure Pro AV114 scale unless otherwise stated. Melting points of the compounds were recorded using Thermo Scientific 9100 instrument.

Experimental Procedures

Synthesis of 3, 4', 5-tri (benzoyloxy) stilbene, 1

In a 50 mL round bottom flask, resveratrol (0.73 g, 3.18 mmol) was dissolved in 20 mL acetone. Benzoyl chloride (2.32 mL, 20 mmol), and triethyl amine (2.79 mL, 20 mmol) were added and the mixture was stirred for 48 hours under nitrogen. A TLC was done every 12 hours to observe the progress of the reaction and to confirm the formation of minimal side products. The mixture was acidified using 2N HCl (pH < 5). The resulting mixture was extracted by ethyl acetate (2×25 mL), washed with saturated sodium bicarbonate solution (2×10mL) to remove excess acid, and dried over anhydrous magnesium sulfate. The crude solution was concentrated and purified by column chromatography (30:70 v/v, acetone:hexane). Compound 1 {C₃₅H₂₅O₆} (1.55 g, 90%), M.P = 149.6 – 151°C, R_f value = 0.65 was obtained as white solid. ¹H NMR (400 MHz, CDCl₃, δ, ppm), 7.3 (d, 2H), 7-8.2 (m, 22H, Ar-H), ¹³C NMR (100 MHz, CDCl₃, δ, ppm), 114.8, 117.2, 122.6, 127.9, 128.9, 129.3, 130, 130.2, 130.23, 130.5, 130.7, 134, 134.6, 135.5, 139.9, 151, 153.3, 164.9, 165.2. IR (cm⁻¹) 1732.08 (C=O stretch), 1701.22, 1593.2, 1585.49, 1504.48, 1494.83.

Synthesis of 3,4',5-tri(toluyloxy) Stilbene, 2

To a one-neck 50 mL round bottom flask were added resveratrol (0.7 g, 3.1 mmol), acetone (20 mL), toluoyl chloride (2.64 mL, 20 mmol), and triethyl amine (2.79 mL, 20 mmol). The mixture was stirred at room temperature under nitrogen for 48 hours. A TLC was done every 12 hours to check the progression of the reaction and the formation of products. The mixture was acidified using 2N HCl (pH<5). A mixture was obtained that was extracted in the organic layer by means of ethyl acetate (2× 20 mL) and washed with saturated sodium

bicarbonate solution ($2 \times 10\text{mL}$) to remove excess acid. Anhydrous magnesium sulfate was used to absorb the moisture and dry the product. Gravity filtration was used to remove the magnesium salt from the solution. The reaction mixture was concentrated and purified using column chromatography (40:60 v/v, acetone: hexane) to give compound 2 $\{\text{C}_{35}\text{H}_{30}\text{O}_6\}$ (1.430g, 79%). M. P= 156.5-157.2°C. R_f value = 0.7 (Acetone/ Hexane, 30:70, V/V), ^1H NMR (400 MHz, CDCl_3 , δ , ppm), 7-8.2 (m, 19H, Ar-H), 7.5(d, 2H), 2.5 (s, 9H) IR (cm^{-1}) 2922.16, 2877.79, 2852.72, 1735.93 (C=O stretch), 1714.72, 1693.5, 1683.86.

Synthesis of 3, 4', 5-tri (2''-butenoyloxy) Stilbene, 3

To a one-neck 50 mL round bottom flask were added resveratrol (0.726 g, 3.18 mmol), acetone (20 mL), crotonyl chloride (1.92 mL, 20 mmol), and triethyl amine (2.79 mL, 20 mmol). The mixture was stirred under nitrogen atmosphere for 48 hours with TLC performed every 12 hours. A TLC was done to check the progression of reaction and to check the number of products being formed during the course of the reaction. The mixture was acidified using 2N HCl keeping pH less than 5. Ethyl acetate ($2 \times 20\text{ mL}$) was used to extract the crude product that was then washed with saturated sodium bicarbonate to remove any leftover acid. The organic layer was dried using anhydrous magnesium sulfate. The magnesium salt was filtered using gravity and purified with column chromatography (10-40% acetone to hexane in volume) to give compound 3 $\{\text{C}_{26}\text{H}_{24}\text{O}_6\}$ (1.04 g, 75%). R_f value = 0.60 (Acetone/ Hexane, 30:70, V/V), ^1H NMR (400 MHz, CDCl_3 , δ , ppm), 6.8-7.5 (stilbene, 9H), 6-6.1 (d, 2H, vinylic), 1.9 (s, 9H, CH_3). IR (cm^{-1}) 2927.94, 2926.01, 1734.01 (C=O stretch), 1676.14, 1654.92, 1625.99, 1608.63, 1602.85, 1598.99, 1591.27.

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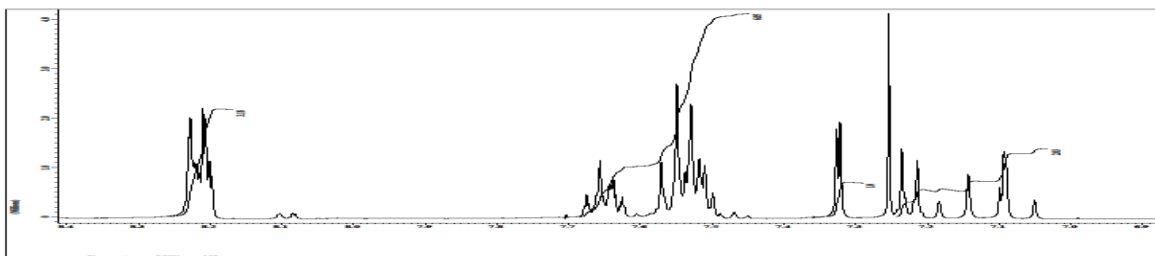
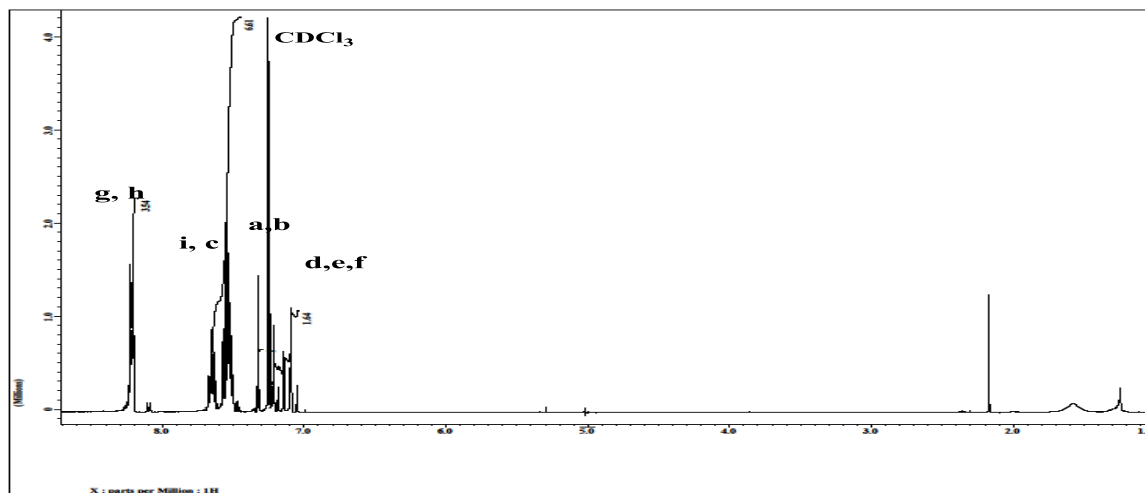
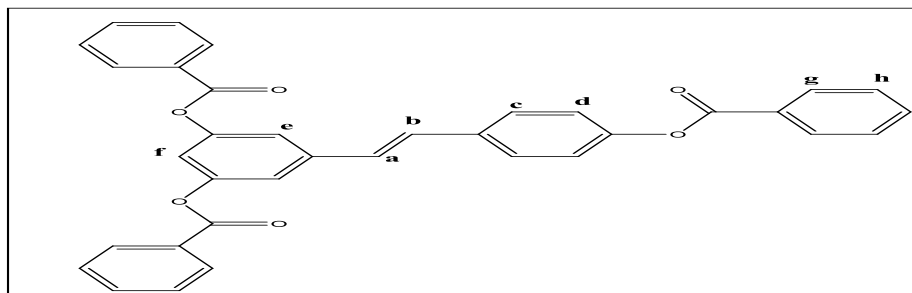
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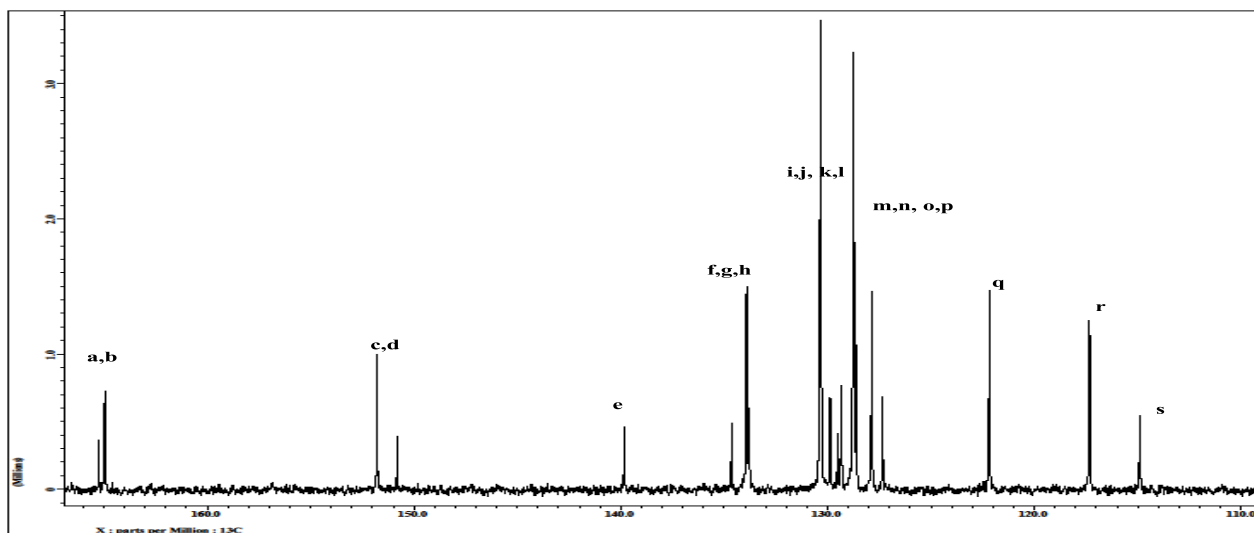
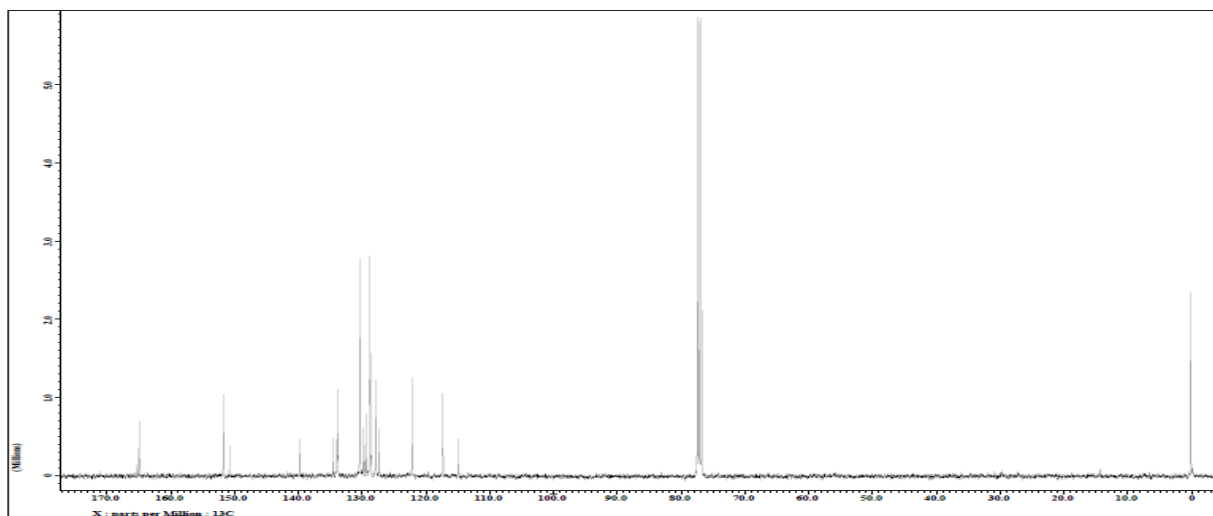
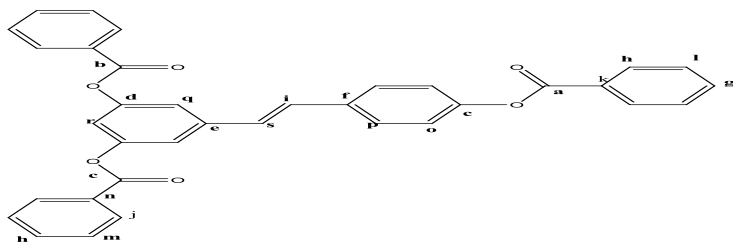
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APPENDICES

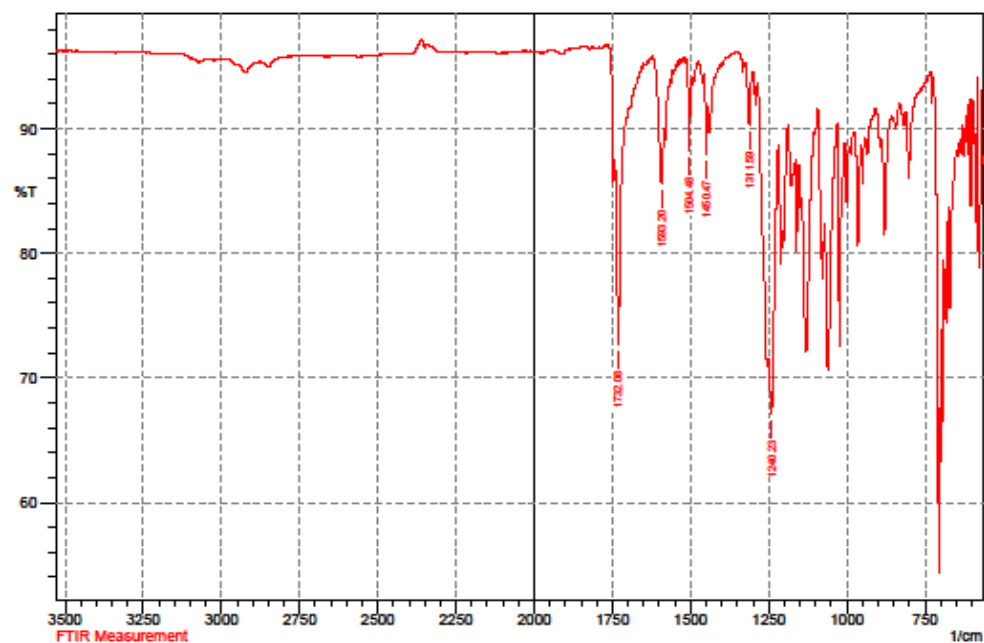
Appendix A: ^1H NMR spectrum of compound 1 in CDCl_3



Appendix B: ^{13}C NMR spectrum of compound 1 in CCl_3D

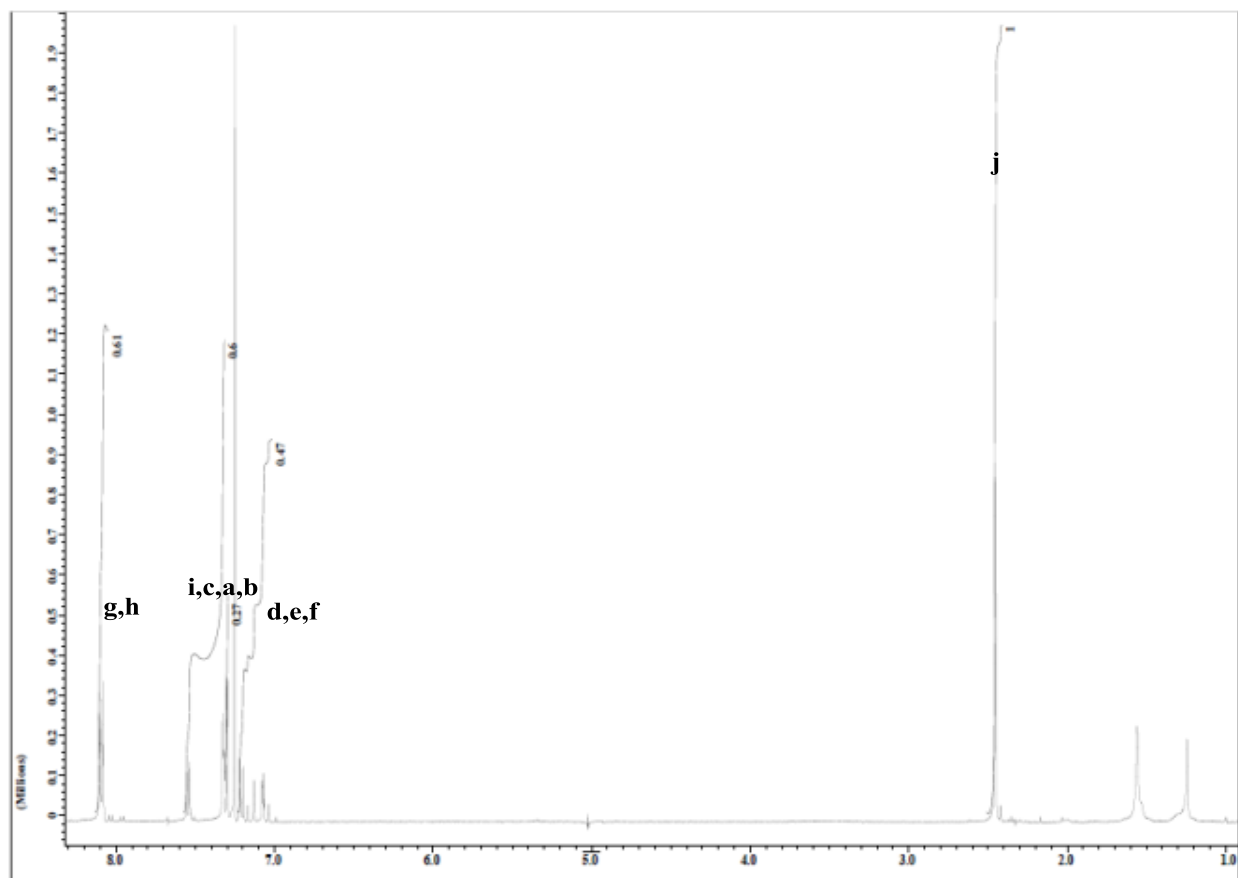
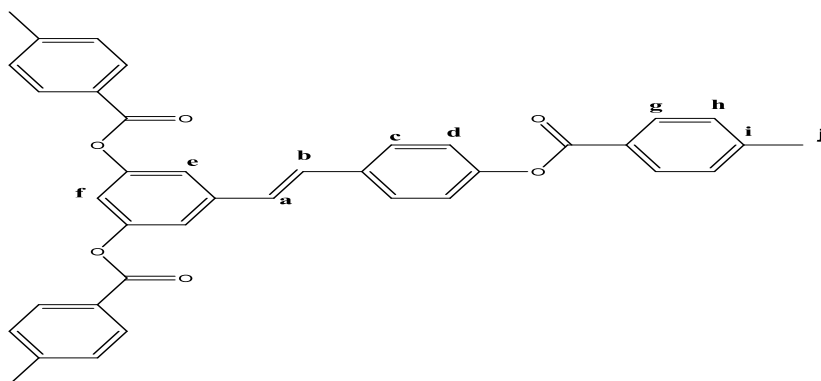


Appendix C: IR spectrum of compound 1

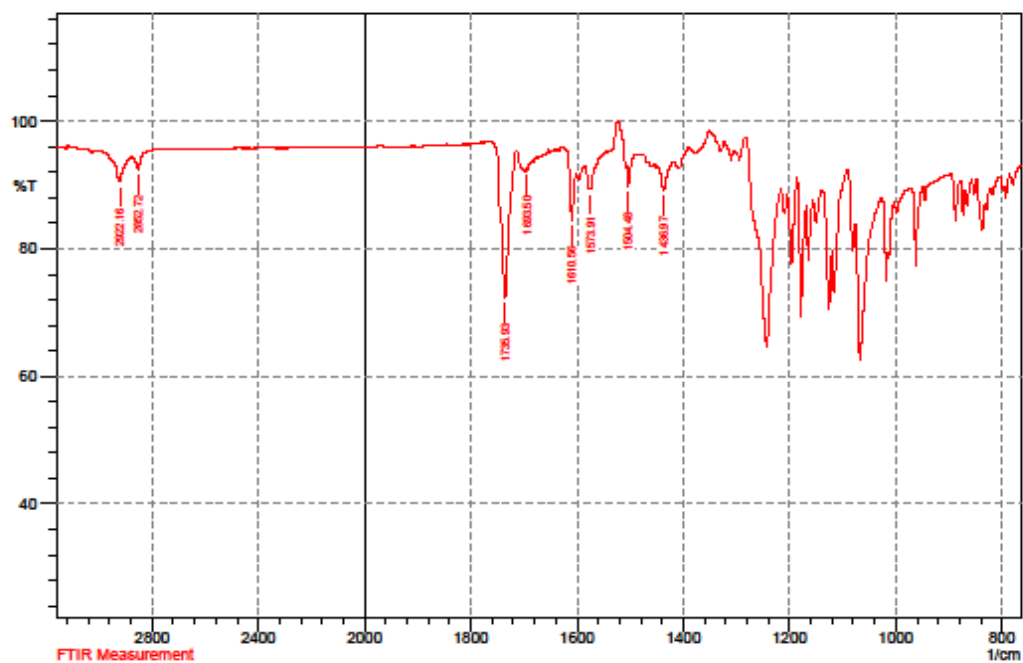


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1240.23	67.09	10.46	1251.8	1219.01	4.01	0.77
2	1311.59	90.28	4.6	1327.03	1300.02	0.82	0.21
3	1450.47	87.95	5.14	1456.26	1444.68	0.48	0.12
4	1504.48	88.19	6.7	1514.12	1494.83	0.62	0.18
5	1593.2	85.59	5.56	1624.06	1585.49	1.53	0.29
6	1732.08	72.65	15.78	1741.72	1701.22	2.97	1

Appendix D: ^1H NMR Spectrum of Compound 2 in CDCl_3

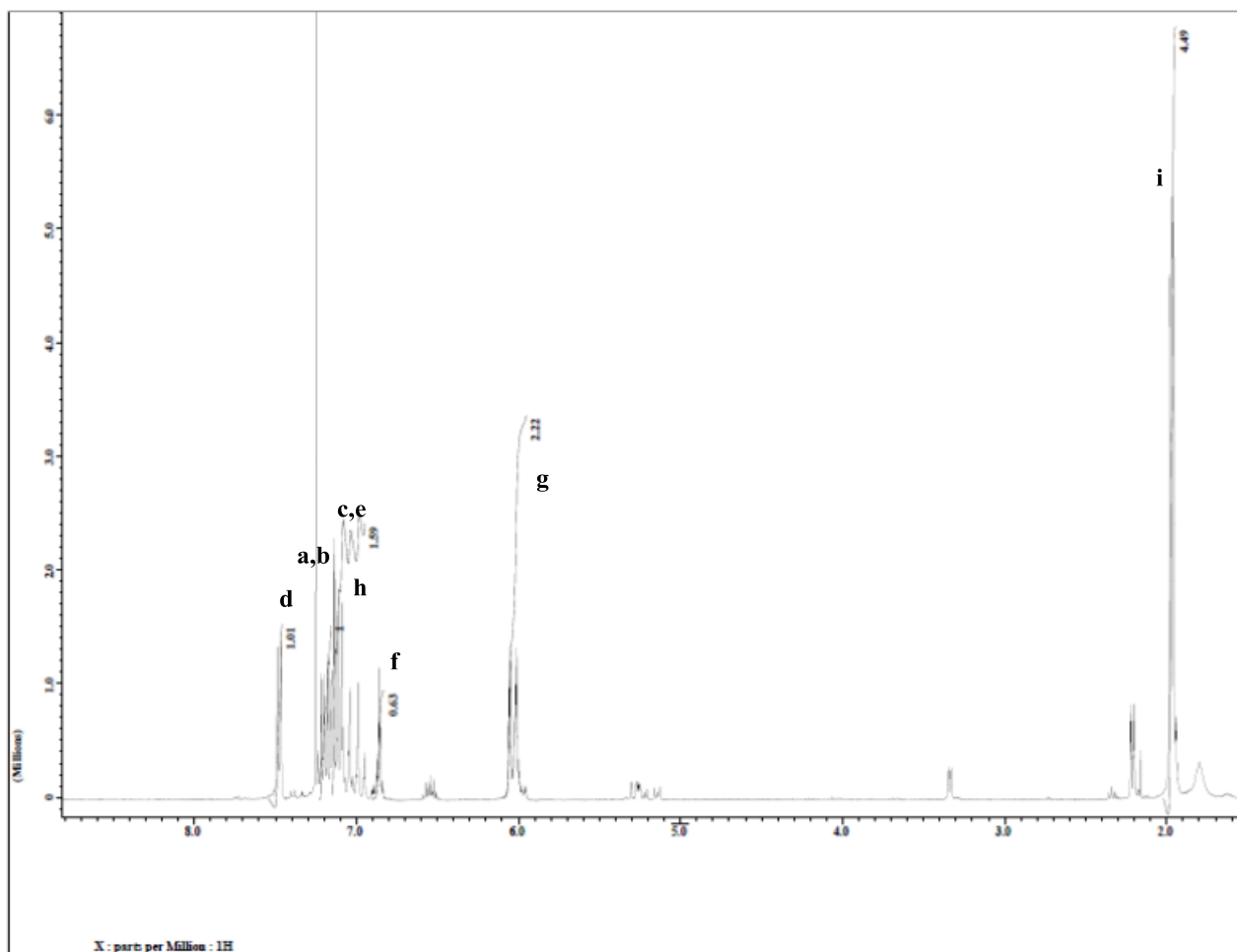
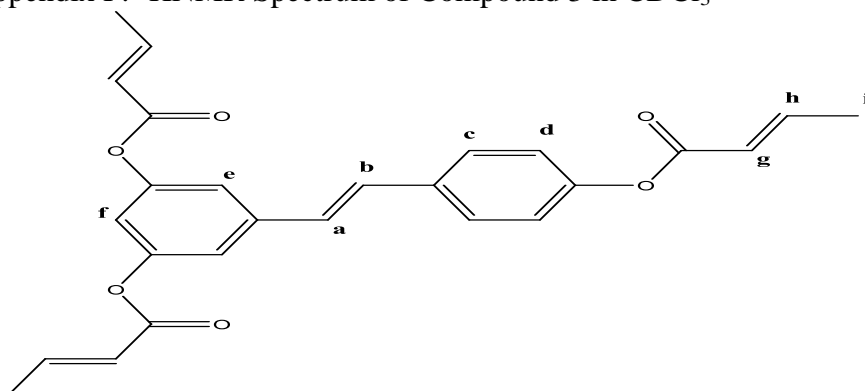


Appendix E. IR Spectrum of Compound 2

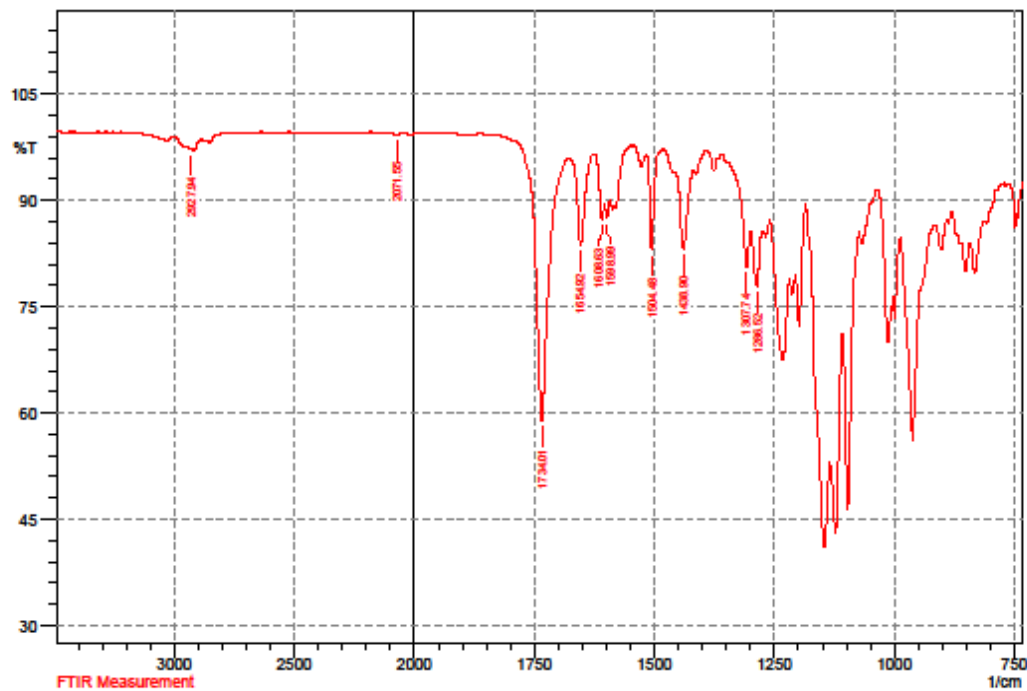


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1436.97	89.14	4.01	1448.54	1419.61	1.09	0.21
2	1504.48	89.78	3.32	1506.41	1490.97	0.51	0.08
3	1573.91	89.31	4.18	1585.49	1560.41	0.96	0.23
4	1610.56	84.53	8.71	1627.92	1600.92	0.99	0.25
5	1693.5	92.42	0.33	1695.43	1683.86	0.36	0.01
6	1735.93	72.41	23.63	1759.08	1714.72	2.71	1.93
7	2852.72	92.47	2.35	2877.79	2812.21	1.7	0.22
8	2922.16	90.54	3.51	2951.09	2877.79	2.38	0.45

Appendix F: ^1H NMR Spectrum of Compound 3 in CDCl_3

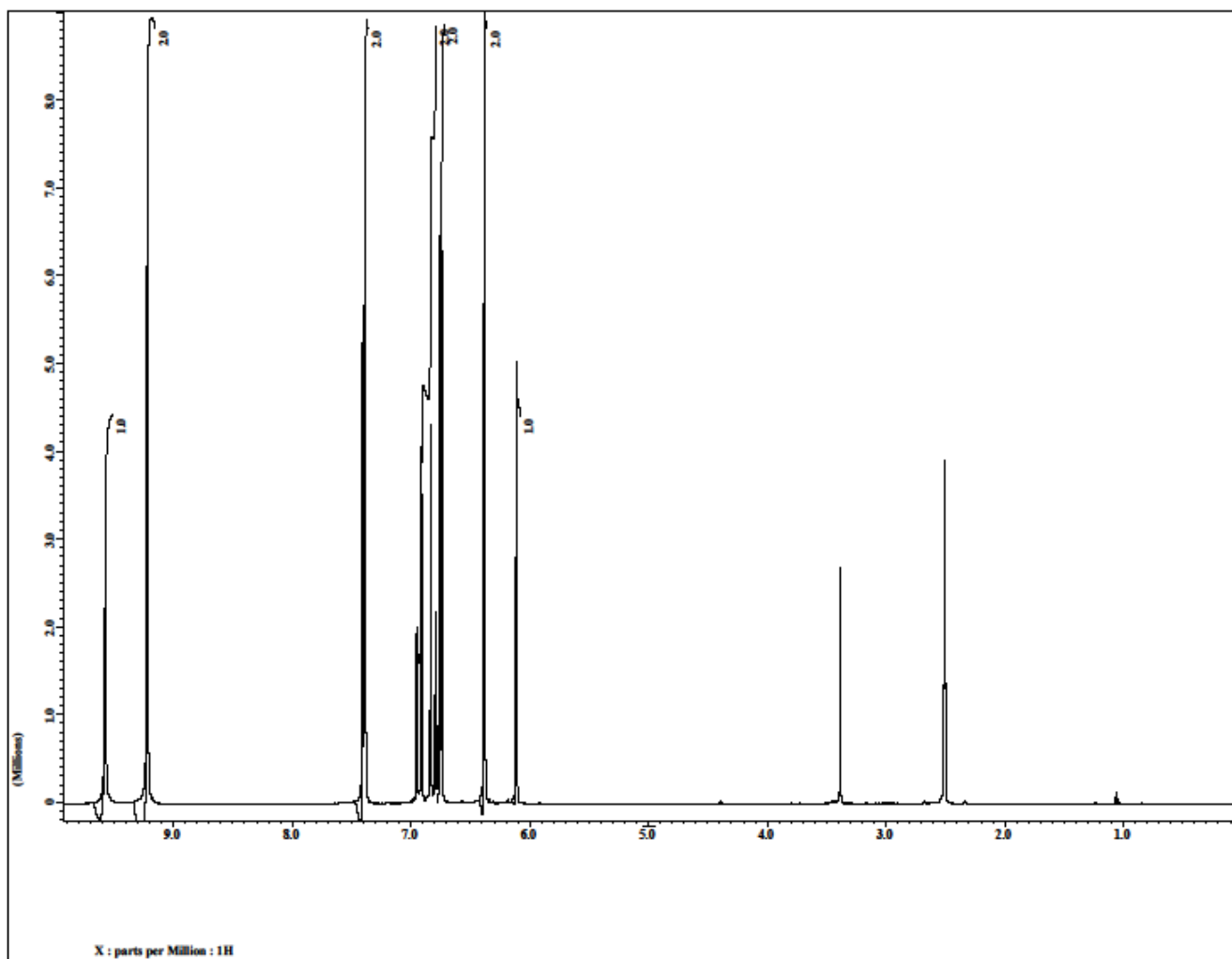


Appendix G: IR Spectrum of Compound 3



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1286.52	77.93	7.67	1300.02	1273.02	2.29	0.47
2	1307.74	80.65	6.53	1346.31	1300.02	2.23	0.18
3	1438.9	83.23	10.98	1458.18	1419.61	1.86	0.86
4	1504.48	82.99	13.75	1517.98	1485.19	1.08	0.61
5	1598.99	87.57	2.09	1602.85	1591.27	0.6	0.05
6	1608.63	87.29	3.97	1625.99	1602.85	0.85	0.11
7	1654.92	83.69	12.52	1676.14	1625.99	1.85	1.02
8	1734.01	58.93	38.18	1791.87	1683.86	7.24	5.91
9	2071.55	99.33	0.01	2073.48	2069.62	0.01	0
10	2927.94	97.27	0.01	2929.87	2926.01	0.05	0

Appendix H: ^1H NMR Spectrum of RV in DMSO



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