DRUG DESIGN, DOCKING STUDIES, SYNTHESIS AND *IN-VITRO* EVALUATION OF CERTAIN NOVEL ISOXAZOLE INCORPORATED COUMARIN DERIVATIVES AS POTENT α - AMYLASE INHIBITORS

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI- 600 032

In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

Branch-II – PHARMACEUTICAL CHEMISTRY

Submitted by

FAHIMA.S

Reg.no. 261915203

Under the guidance of

Dr. M. VIJAYABASKARAN, M.Pharm., Ph.D.,

PROFESSOR AND HEAD

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

J.K.K.NATTARAJA COLLEGE OF PHARMACY
KUMARAPALAYAM – 638183
TAMILNADU.
OCTOBER – 2021

EVALUATION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"DRUG DESIGN, DOCKING STUDIES, SYNTHESIS AND *IN-VITRO* EVALUATION OF CERTAIN NOVEL ISOXAZOLE INCORPORATED COUMARIN DERIVATIVES AS POTENT α - AMYLASE INHIBITORS"** submitted by the student bearing **Reg. No: 261915203** to "**The Tamil Nadu Dr. MGR Medical University – Chennai",** in partial fulfilment for the award of Degree of **Bachelor of Pharmacy** was evaluated by us during the examination held on …….

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"DRUG DESIGN, DOCKING STUDIES, SYNTHESIS AND *IN-VITRO* EVALUATION OF CERTAIN NOVEL ISOXAZOLE INCORPORATED COUMARIN DERIVATIVES AS POTENT α - AMYLASE INHIBITORS"** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University- Chennai"**, in partial fulfillment and requirement of university rules and regulations for the award of degree of **Master of Pharmacy** is a bonafide research work carried out by by the student bearing Reg. No: **261915203** during the academic year 2020 – 2021, under the guidance and direct supervision of **Dr. M. Vijayabaskaran, M. Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Komarapalayam.

Place : Komarapalayam

Date :

Dr. R. SAMBATHKUMAR, M. Pharm., PhD.,
Professor & Principal,
J. K. K. Nattraja college of Pharmacy,
Komarapalayam – 638183,
Tamil Nadu.

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"DRUG DESIGN, DOCKING STUDIES, SYNTHESIS AND *IN-VITRO* EVALUATION OF CERTAIN NOVEL ISOXAZOLE INCORPORATED COUMARIN DERIVATIVES AS POTENT α - AMYLASE INHIBITORS"** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University- Chennai"**, in partial fulfillment and requirement of university rules and regulations for the award of degree of **Master of Pharmacy** is a bonafide research work carried out by by the student bearing Reg. No: **261915203** during the academic year 2020 – 2021, under my guidance and direct supervision in the Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Komarapalayam.

Place : Komarapalayam
Date :

Dr. M. VIJAYABASKARAN, M. Pharm., PhD.,
Professor & Head,
Department of Pharmaceutical Chemistry,
J. K. K. Nattraja college of Pharmacy,
Komarapalayam – 638183,
Tamil Nadu.

DECLARATION

I do hereby declared that the dissertation entitled **"DRUG DESIGN, DOCKING STUDIES, SYNTHESIS AND *IN-VITRO* EVALUATION OF CERTAIN NOVEL ISOXAZOLE INCORPORATED COUMARIN DERIVATIVES AS POTENT α - AMYLASE INHIBITORS "** submitted to "The Tamil nadu Dr. M.G.R. Medical University- Chennai', in partial fulfilment and requirement of university rules and regulations to award the degree of Master of Pharmacy is a bonafide research work carried out by me during the academic year 2020-2021, under the guidance and supervision of Dr. M. Vijayabaskaran, M.Pharm., Ph.D., Professor, Department of Pharmaceutical chemistry, J.K.K. Nattraja College of Pharmacy, Komarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine at the best of my knowledge.

Place : Komarapalayam

Date :

Ms. FAHIMA.S

Reg.No. 261915203

Department of Pharmaceutical Chemistry

J. K. K. Nattraja College of pharmacy,

Komarapalayam – 638183,

Tamil Nadu.

ACKNOWLEDGEMENT

I acknowledge first of all, the almighty, for his goodness and grace, which have brought me this far successfully. Without his kindness and blessings, I could not have made this far.

I take this opportunity with pride and enormous gratification to express the deeply embedded feeling of thanks and gratefulness to all the persons who backed me directly or indirectly throughout the materialization of this research work. I am proud to dedicate our deep sense of gratitude to the founder, (Late) Thiru **J.K.K. Nattraja Chettiar**, providing me the historical institution to study.My sincere thanks and respectful regards to my reverent Chairperson **Smt. N. Sendamaraai, B.Com.,** Managing Director **Mr. S. Omm Sharravana, B.Com., LLB**., J.K. K. Nattraja Educational Institutions, Komarapalayam for their blessings, encouragement and support at all times.

It is most pleasant duty to thank for our beloved Principal, **Dr. R. Sambathkumar, M. Pharm., Ph.D.,** Principal, J. K. K. Nattraja College of Pharmacy, Komarapalayam. For ensuring all the facilities were made available to me for smooth running of the project and tremendous encouragement at each and every step of this dissertation work. Without his critical advice and deep- rooted knowledge, this work would not have been a reality.

My sincere thanks to my guide **Dr. M. Vijayabaskaran, M.Pharm, Ph.D,** Professor & Head, Department of Pharmaceutical Chemistry for his valuable suggestions.

I express whole hearted gratitude to Mrs. **P. Lekha, M.Pharm.,** Assistant Professor , Department of Pharmaceutical Chemistry for suggesting solution to problems faced by me and providing indispensable guidance, tremendous encouragement at each and every step of this dissertation work, without her critical advice and deep-rooted knowledge, this work would not have been a reality.

My special thanks to Mrs. **B. Vasuki M.Pharm.,** and **P. Devi M.Pharm.,** Assistant Professor, Department of Pharmaceutical Chemistry for her valuable

help during my project.

I greatly acknowledge the help rendered by Mr. Prabakaran, S.K Sugan, Lab Technician for their co-operation.

My special thanks to all the Technical and Non-Technical Staff Members of the institute for their precious assistance and help.

Last, but not the least, pay **tribute** to my endearing **PARENTS** for lifting me up till this phase of life, I sincerely thank them for their support they granted me with and also for their love, trust, patience and bearing all kinds of stress to make me what I am.

It is very difficult task to acknowledge the services to thank all those gentle people. So I would like to thank to my brothers and for those people who have helped me directly or indirectly to complete this project work successfully.

Ms. FAHIMA.S

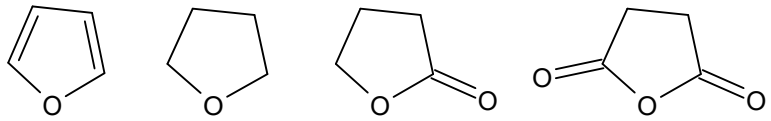
Reg. No. 261915203

CONTENTS

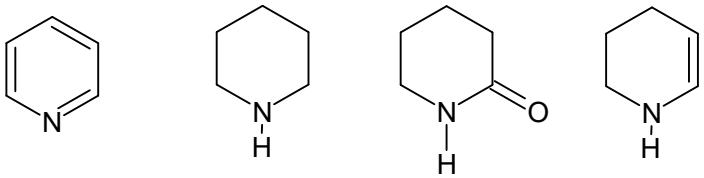
1. INTRODUCTION

1.1 HETEROCYLIC CHEMISTRY

Heterocyclic chemistry has dominated the field with decades of history and plays a primordial role in the synthesis of future drugs [1]. Development of organic chemistry contributes to the progress in multiple fields of science and majority of newly synthesized organic compounds contains at least one heterocyclic ring as a common feature. Thus, heterocyclic chemistry is an important tool in the search for new active substances with enormous potential applications [2]. Heterocyclic chemistry is the branch of chemistry dealing with the synthesis, properties, and applications of heterocycles and its derivatives are seen as a group that can be divided into two broad areas: aromatic and non-aromatic. The five-membered rings are shown in the first row, and the derivative 1 corresponds to the aromatic derivative, furan, while tetrahydrofuran (2), dihydrofuran-2-one (3), and dihydrofuran-2,5-dione (4) are not aromatic, and their reactivity would be not unlike that expected of an ether, an ester, or a carboxylic anhydride, respectively. The second row shows six-membered rings, initially in an aromatic form as pyridine (5), while piperidine (6), piperidin-2-one (7), and 1,2,3,4-tetrahydropyridine (8) are not aromatic; their reactivity would not be very different from that expected of an amine, amide, or enamine, respectively[3].



(1) (2) (3) (4)



(5) (6) (7) (8)

Heterocyclic compounds are a class of cyclic organic compounds containing heteroatoms, like nitrogen, sulfur, oxygen, etc., along with the carbon framework and

they possess diverse pharmacological activities and are employed in the treatment of a variety of diseases. In present days many therapeutic agents contain heterocyclic ring as the major structural component. Among these compounds, nitrogen-containing heterocyclic rings are distinctive not only because of their ease of synthesis but also due to their widespread distribution and biological profiles [4].

1.1.1. COUMARIN

Coumarin (1,2-benzopyrone or 2H-1-benzopyran-2-one) is a class of heterocyclic compounds containing a benzene ring structure and an α-pyrone moiety with multiple biological activities. Coumarin and their derivatives are nature occurring lactones frist derived from Tonka beans in 1820 and are widely available in plants as a heteroside or free form. They are polyphenolic compounds belonging a group of colourless and crystalline oxygenated heterocyclic compounds first isolated from the plant named Dipteryx odorata Willd (Fabaceae) known locally as "coumaroun" by Vogel in 1820.

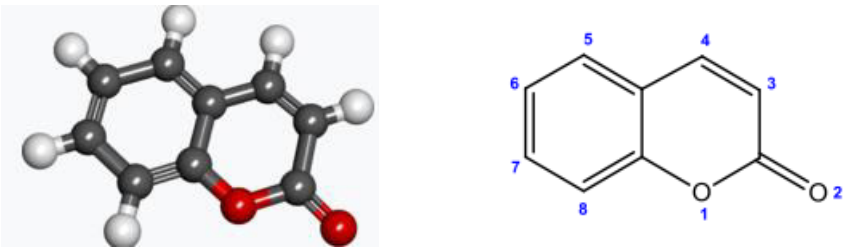


Figure.1: 2H-chromen-2-one or 2H-1-benzopyran-2-one

Molecular formula : $C_9H_6O_2$

Molecular weight : 146.145 $g \cdot mol^{-1}$

IUPAC : 2*H*-Chromen-2-one, 2*H*-1-Benzopyran-2-one

Other name : 1- Benzopyran-2-one

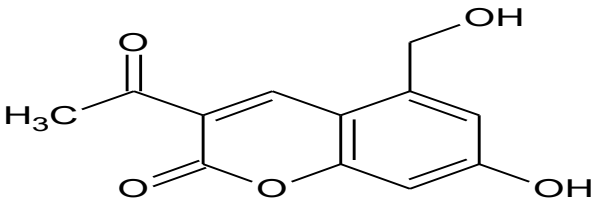
They have been employed as herbal medicines since early ages because they are oxygen containing heterocycles widely found in nature. More than 1300 coumarin derivatives have been identified, which are mainly obtained from the secondary metabolite in green plants, fungi and bacteria. This led to an incentive for researchers around the world to investigate the nature and identification of this molecule. Since

the reporting of the first synthetic route in 1882, this moiety has found its place in fabric conditioners, certain perfumes and in medicinal industry especially as anti-coagulants, viz. warfarin and dicoumarol [5].

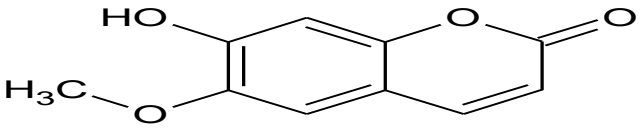
A total of 800 coumarin derivative compounds that naturally found were obtained from about 600 genera of 100 families to date and frequently found in the seeds, roots and leaves of many plant species belonging to families (especially Rutaceae and Apiaceae) in the Dicotyledonae class of the division of Spermatophyta. Although most natural coumarins are isolated from vascular plants, some coumarins such as novobiocin, coumermycin and aflatoxin are isolated from microbial sources [6]. These compounds have become indispensable structural units that are useful in medicinal chemistry, displaying anticancer, antioxidant, anti-plasmodial, anti-malarial, anti-rhinovirus, antifungal and antibacterial activity. Much research has been focused on the inhibition of bacterial growth by naturally occurring coumarins such as xanthoxin, herniarin, umbelliferone and scopoletin. Umbelliferone, scopoletin, and coumarin also exhibit good antifungal activity [7].

Coumarins derivatives with liquid crystalline and gel properties can be seen as new type of photo cross linkable materials for their practical applications. Coumarin derivatives have been used in material chemistry as optical materials, fluorescent whiteners, fluorescent tags, laser dyes, non-linear optical chromophores. Position of substituents and type of substituents on coumarin ring play very important role to overall optical properties of coumarin derivatives. Polymeric and non-polymeric coumarin derivatives have been reported for their liquid crystalline properties [8].

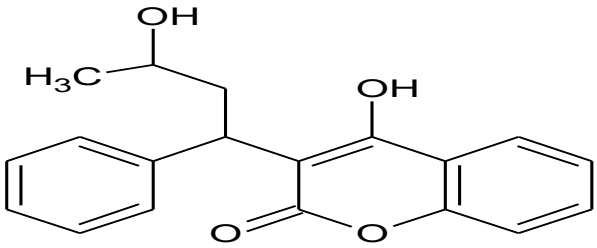
Coumarin and its compounds may be synthesized using various processes, such as Pechmann, Knoevenagel, Perkin, Friedel-Crafts, Reformatsky and Wittig reactions. Coumarin and its derivatives possess anticoagulant. Antimicrobial, antioxidant, anti-inflammatory, anticancer, anti-HIV, anti-tuberculosis, anti-influenza, anti-Alzheimer, antiviral, antihyperlipidemic, Antihypertensive, Anticonvulsant, Antiadipogenic, Cytochrome P450 Inhibiting, Neuroprotective, analgesic, antimalarial, antitumor, antipsychotics, anti-diabetic, xanthine oxidase inhibitors activities and are also used for the treatment of multiple sclerosis [9].



Armillarisin A (antibiotic)



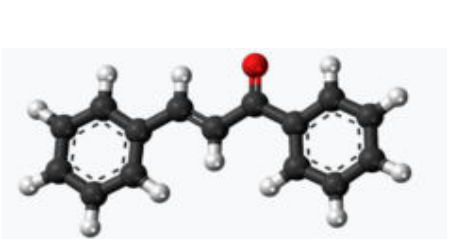
Scopoletin



warfarin (anticoagulant)

1.1.2. CHALCONE

Flavonoids and isoflavonoids are the major precursors in biosynthesis of chalcones as secondary metabolites in terrestrial plants. Chalcones (1,3-diaryl-2-propane-1-ones) is one of the main groups of natural products, commonly found in berries, herbs, spices, tea and soybased foods. Chemically chalcones comprise an open-chain flavonoid moiety, the fundamental form of which consists of two aromatic rings connected by three carbons, the β-unsaturated carbonyl system [10]. chalcones, the bichromophoric molecules separated by vinyl chains and the carbonyl group, are found be effective photosensitive materials, and exhibit promising nonlinear optical properties [11].



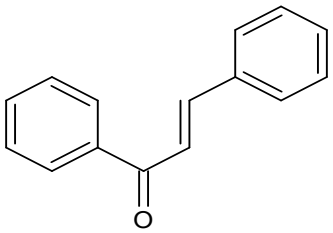


Figure.2: 1,3 – Diphenyl – 2 propane – 1- one

Molecular formula : $C_{15}H_{12}O$

Molecular weight : 208.260 $g \cdot mol^{-1}$

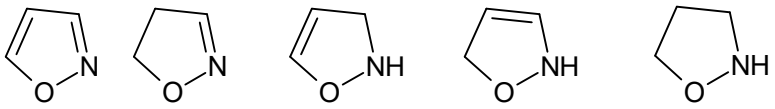
IUPAC : Chalcone , (2*E*)-1,3-Diphenylprop-2-en-1-one

Other names : Chalkone, Benzylideneacetophenone

Chalcones and its derivatives can be synthesized using several approaches, but the Claisen-Schmidt condensation always retains a high position. The best approach for chalcone synthesis is the traditional Claisen-Schmidt. Many renowned techniques include the Suzuki reaction, Friedel-Crafts acylation with cinnamoyl chloride, Allan-Robinson Condensation, Direct Cross-coupling Reaction, Microwave Irradiation, Julia-Kocienski Olefination, and Grinding Technique. Chalcones have been reported to possess many useful biological properties including antimicrobial, anti-inflammatory, anticancer, anti-HIV, antioxidant, Anticoagulant, Antituberculosis, antipsychotic and antimalarial activities. Many research groups reported the potential effects of coumarinchalcone hybrids like anti-microbial, anti-cancer, antimalarial, anti-oxidant, anti-tubercular, and anti-inflammatory. Even though the coumarin and chalcone and their derivatives obtained from natural origin, synthetic coumarin chalcone derivatives with structural modifications may be adapted for the development of potent biologically active molecules [10].

1.1.3. ISOXAZOLE

Isoxazole have condensed formula ($C_{15}H_{11}NO$) which is isomer of derivative of alpha pinene and also belong to class of ibotenic acid. All three atoms of this class are potentially reactive like chain of N-O-C [12]. Nitrogen containing heterocycles with an oxygen atom are considered as an important class of compounds in medicinal chemistry because of their diversified biological applications. The exploitation of a simple molecule with different functionalities for the synthesis of heterocycles is a worthwhile contribution in the chemistry of heterocycles. Isoxazole (1a) is a five membered heterocyclic compound containing oxygen and nitrogen atoms in the 1, 2 positions, its partially saturated analogs are called isoxazolines (1b-d) and completely saturated analog is isoxazolidine (1e).



1a 1b 1c 1d 1e

Two carbon-carbon double bonds contribute to the unsaturated property of the molecule. The structural features of isoxazole make it possible for multiple non-covalent interactions, especially hydrogen bonds (hydrogen bond acceptor N and O), pi-pi stack (unsaturated five-membered ring), and hydrophilic interactions (overall hydrophilic profile with CLogP = 0.121). There is a shared feature for the organic compounds developed in the recent decades that the majority of them may have included a heterocycle ring.1–3 The inclusion of isoxazole may contribute to the increased efficacy, decreased toxicity, and improved pharmacokinetics profiles. Successful applications of developing isoxazole compounds have resulted in multiple corresponding drugs in the market [13].

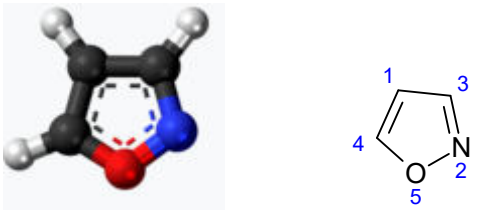


Figure.3: Isoxazole

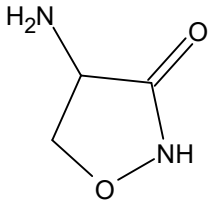
Molecular formula : C_3H_3NO

Molecular weight : 69.06 $g \cdot mol^{-1}$

IUPAC : 1, 2- oxazole

Isoxazoles are an important class of heterocycles, which are largely employed in the area of pharmaceuticals and therapeutics such as insecticidal, antibacterial, antibiotic, antitumour, antifungal, antituberculosis, anticancer and ulcerogenic. Isoxazole derivatives are used in the market as COX-2 inhibitor and anti-inflammatory drugs. Isoxazole derivatives such as sulfamethoxazole, sulfisoxazole, oxacillin, cycloserine and acivicin have been in commercial use for many years.

Cycloserine is the best-known antibiotic drug that possess antitubercular, antibacterial activities and in treatment of leprosy. Acivicin is an antitumour, antileishmania drug, while isoxaflutole is used as herbicidal drug [14].



Cycloserine

1.2. ANTIDIABETIC ACTIVITY

Coumarins are secondary metabolites found widely in plants and used mainly in anticoagulant and antithrombic therapy. Over the past two decades,literature related to the effects of coumarins and their derivatives on diabetes and its complications are reported. The search for new coumarins against diabetes and its complications, either isolated from traditional medicine or chemically synthesised, has been constantly expanding. The cellular and molecular mechanisms include protecting pancreatic beta cells from damage, improving abnormal insulin signalling, reducing oxidative stress/inflammation, activating AMP-activated protein kinase (AMPK), inhibiting α-amylase and α-glucosidases [15]

1.2.1. DIABETES MELLITUS

Diabetes Mellitus is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins. Etiology of Diabetes Mellitus includes defect in either insulin secretion or response or in both at some point in the course of disease. Mostly patients with diabetes mellitus have either Type 1 diabetes (which is immune-mediated or idiopathic) Type 2 Diabetes Mellitus (formerly known as non-insulin dependent Diabetes Mellitus) are the most common forms of Diabetes Mellitus which is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [16].

Type 2 Diabetes Mellitus results from interaction between genetic, environmental, behavioural factors [17, 18], and also includes gestational hormonal

environment, genetic defects, other infections, and even due to certain drugs. The worldwide prevalence of diabetes has continued to increase dramatically. Globally, as of 2011, an estimated 366 million people had Diabetes Mellitus, with Type 2 making up about 90% of the cases [19, 20]. The number of people with Type 2 Diabetes Mellitus is increasing in every country and 80% of the people living in low- and middle-income countries. The treatment goal of diabetic patients is to maintain near normal levels of glycemic control, in both fasting and post-prandial conditions.

Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The percentage of deaths attributable to high blood glucose or diabetes that occurs prior to age 70 were higher in low- and middle-income countries than in high-income countries (WHO,2016). The maximum number of diabetic patients was recorded in India followed by china and USA. If the current condition prevails and nothing much is done in future, then by the year 2030, number of individuals affected by diabetes in India would raise up to 79 million.

1.2.2. CLASSIFICATION OF DIABETES MELLITUS

The classification of Diabetes Mellitus was based on etiological factors of the diseases. The old and confusing terms of insulin-dependent (IDDM) or non-insulin-dependent (NIDDM) which were proposed by WHO in1980 and 1985 have disappeared and the terms of new classification system identifies four types of diabetes mellitus: Type 1, Type 2, gestational diabetes and Monogenic diabetes.

TYPE 1 DIABETES MELLITUS

Type 1 diabetes mellitus (juvenile diabetes) is characterized by beta cell destruction caused by an autoimmune process, usually leading to absolute insulin deficiency. Type 1 is usually characterized by the presence of anti–glutamic acid decarboxylase and islet cell or insulin antibodies which identify the autoimmune processes that lead to beta cell destruction. Eventually, all Type1 diabetic patients will require insulin therapy to maintain normoglycemia.

TYPE 2 DIABETES MELLITUS

The relative defects in insulin secretion or in the exhibit intra-abdominal (visceral) obesity, which is closely related peripheral action of the hormone in the occurrence of Type 2 diabetes. This is the most common form of diabetes mellitus and is highly associated with family history of diabetes, older age, obesity and lack of exercise. Type 2 diabetes comprises 80% to 90% of all cases of Diabetes mellitus. Most individuals with Type 2 diabetes will be having insulin resistance, hypertension and dyslipidemia (high triglyceride and low HDL-cholesterol levels; postprandial hyperlipidemia) often present in the individuals. It is more common in women, especially women with a history of gestational diabetes, and in Blacks, Hispanics and Native Americans.

GESTATIONAL DIABETES MELLITUS (GDM)

Gestational diabetes mellitus is an operational classification (rather than a pathophysiologic condition) in which women who develop diabetes mellitus during gestation. Women who develop Type 1 diabetes mellitus during pregnancy and women with undiagnosed asymptomatic Type 2 diabetes mellitus that is discovered during pregnancy are classified as Gestational Diabetes Mellitus (GDM). In most women who develop GDM; the disorder has its onset in the third trimester of pregnancy.

OTHER SPECIFIC TYPE (MONOGENIC DIABETES)

Types of diabetes mellitus of various known etiologies are grouped together to form the classifycation called “Other Specific Types”. This group includes persons with genetic defects of beta-cell function (this type of diabetes was formerly called MODY or maturity-onset diabetes in youth) or with defects of insulin action; persons with diseases of the exocrine pancreas, such as pancreatitis or cystic fibrosis; persons with dysfunction associated with other endocrinopathies (e.g. acromegaly); and persons with pancreatic dysfunction caused by drugs, chemicals or infections and they comprise less than 10% of Diabetes Mellitus cases.

Importance of α-Amylase Enzyme in the Body

In humans, the digestion of starch involves several stages. Initially, partial digestion by saliva results in the degradation of polymeric substrates into shorter oligomers. Later on in the gut these are further hydrolysed by pancreatic α-amylase

into maltose, maltotriose and small malto-oligosaccharides. The digestive enzyme (α-amylase) is responsible for hydrolysing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of α-amylase can lead to reduction in post-prandial hyperglycemia in diabetic condition.

Importance of α-Glucosidase Enzyme in the Body

α-glucosidase is a membrane bound enzyme located on the epithelium of the small intestine, catalysing the cleavage of disaccharides to form glucose. Inhibitors can retard the uptake of dietary carbohydrates and suppress post-prandial hyperglycemia. Therefore, inhibition of α-glucosidase could be one of the most effective approaches to control diabetes. Glucosidases are not only essential to carbohydrates digestion, but also vital for the processing of glycoprotein and glycolipids. This enzyme is a target for antiviral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion and infection. Glucosidase are also involved in a variety of metabolic disorders and carcinogenesis [21, 22].

1.3. ENZYMES [23]

Enzymes are proteins, macromolecules they catalyse the chemical reactions in biological systems. They are specific in nature.

There are three different types of enzymes in human body, they are

1) Metabolic enzymes

They are the spark of life, the energy of life, and the vitality of life. In human body every biochemical reaction that occurs are catalyst and regulated by enzymes only and making them essential to cellular functions and health.

2) Food enzymes

By consumption of supplemental enzyme products and through the raw foods we eat these food enzymes get into the body. Raw foods are the source of digestive enzymes when ingested. However raw food manifest only enough enzymes to digest the particular food, not enough to be stored in the body for the later use.

3) Digestive enzymes

Helps in digesting the food and the nutrients in the food are delivered to different parts of the body. The most important digestive enzymes are

a) Proteases (split proteins into their monomers, the amino acids),

b) Lipases (split fat into free acids and glycerol molecules),

c) Carbohydrases (split carbohydrates such as starch and sugar into sample sugars such as glucose) and d) Nucleases (split nucleic acid into nucleotides)

In which α-amylase is one of the digestive enzymes since it begins its process by digesting the starch and breaks them into smaller pieces with two or three glucose units.

1.3.1. Amylase

Amylases are enzymes, which hydrolyze starch molecules to give diverse products including dextrins, and progressively smaller polymers composed of glucose units. The α-amylase family comprises a group of enzymes with a variety of different specificities that all act on one type of substrate being glucose residues linked through an α-1-1, α-1-4, α-1-6, glycosidic bonds. Members of this family share a number of common characteristic properties.

Amylases can be divided into two categories,

endoamylases and

exoamylases.

Endoamylases catalyze hydrolysis in a random manner in the interior of the starch molecule producing linear and branched oligosaccharides of various chain lengths.

Exoamylases act from the non-reducing end successively resulting in short end products.

Amylases constitute a class of industrial enzymes having approximately 25% of the enzyme market. It is desirable that αamylases should be active at the high temperatures of gelatinization (100-110°C) and liquefaction (80-90°C) to economize processes, therefore there has been a need for more thermophilic and thermostable α-amylases. The spectrum of amylase application has widened in many other fields, such as clinical, medical, and analytical chemistries, as well as their wide spread application in starch sacccharification and in the textile, food, fermentation, paper, brewing and distilling industries. α-Amylases are universally distributed throughout the animal, plant and microbial kingdoms[24].

1.3.2. Types:

α-Amylase

The α-amylases are calcium metalloenzymes, completely unable to function in the absence of calcium., α-amylase breaks down long-chain carbohydrates by acting at random locations along the starch chain, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. α-amylase tends to be faster-acting than β-amylase because it can act anywhere on the substrate. In human physiology, both the salivary and pancreatic amylases are α-Amylases. Also found in plants (adequately), fungi (ascomycetes and basidiomycetes) and bacteria (Bacillus).

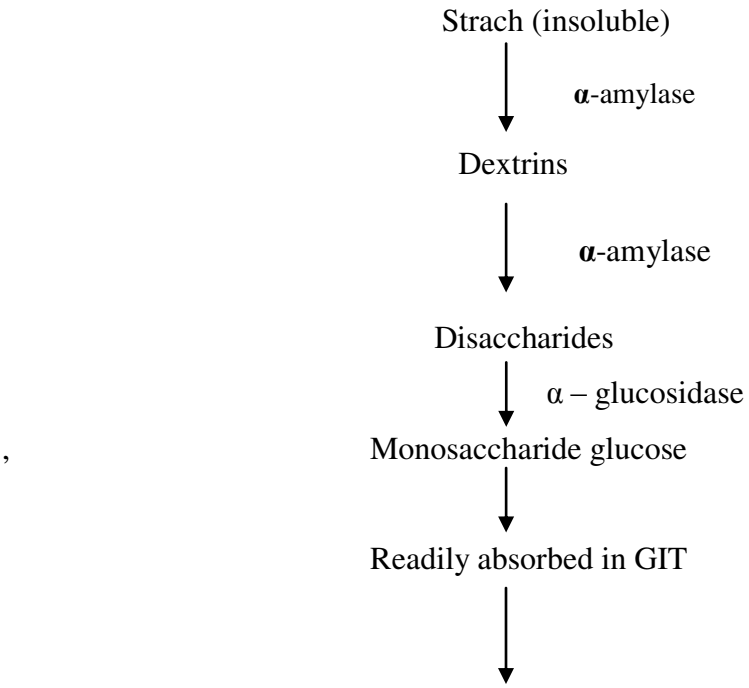
β-Amylase

β-amylase is another form of amylase synthesized by bacteria, fungi, and plants. β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, working from the non-reducing end, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. Both α-amylase and β-amylase are present in seeds; β-amylase is present in an inactive form prior to germination, whereas α-amylase and proteases appear once germination has begun. Animal tissues do not contain β-amylase.

γ-Amylase

γ-amylase cleaves α (1-6) glycosidic linkages, in addition to cleaving the last α(1-4)glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose. Unlike the other forms of amylase, γamylase is most efficient in acidic environments and has an optimum pH of 3[25].

The structure of starch consists of glucose polymers linked by α-1,4 and α-1,6 glycosidic bonds. α-amylase is an enzyme that catalyses the hydrolysis of starch into sugar. Amylase hydrolyse internal α-1,4- glucosidic linkage in starch. Largely at random, to produce dextrins and disaccharides.



,

First α-amylase degrade starch into dextrins and then to maltose by hydrolysing α-1,4 glucan bonds. In digestion, the primary role of α- amylase is to perform the first reaction of this process, generating dextrins that are subsequently hydrolysed by other enzymes. This will come under the classification of carbohydrates.

Starch

Starch is the most important dietary source for humans. High content of starch is found in cereals, roots, tubers etc. Starch is a homopolymer composed of D-Glucose units held by α-glycosidic bonds. It is known as glucosan or glucan. Starch consists of two polysaccharide components – water soluble amylaseand a water insoluble amylopectin. Chemically amylase is a long unbranched chain with 200-1000 D-glucose units held by α-1,4- glycosidic linkages.

Amylopectin, on the other hand, is a branched chain with α-1,6 glucosidic linkages at the branching points and α-1,4 linkage in the other place. Amylopectin molecule, which is composed of a few thousand glucose units, looks like a branched tree with 20-30 glucose units/ branch [26].

1.3.3. Enzyme inhibitors [27]

The molecules which bind to enzymes and decrease their activity are termed as Enzyme inhibitors. Many drugs are enzyme inhibitors, since it can block an enzyme activity and correct a metabolic imbalance. Some of the enzyme inhibitors are also herbicides and pesticides. Not all molecules that bind to enzymes are inhibitors. There are also enzyme activators that bind to the enzyme it enhances the enzymatic activity, while enzymes substrates bind and are converted to products in the normal catalytic cycle of the enzyme.

The binding of an inhibitor can stop a substrate from entering the enzymes's active site and/ or hinder the enzyme from catalysing its reaction. In the past the only way to discover these new inhibitors was by trial and 2iuerror. This brute force approach is still successful and has even been extended by combinatorial chemistry approaches that quickly produce large number of novel compounds and high-throughput screening technology to rapidly screen these huge chemical libraries for useful inhibitors.

α-Amylase Inhibitors [28]

The activity of α-amylase is reported to be inhibited incase of diabetes. Therefore, these α- amylase inhibitors are acting as antidiabetic drugs that work by preventing the digestion of carbohydrates.

The inhibition of α- amylase is by.,

- ***Metal chelators, organic acids and heavy inorganic metal ions:***

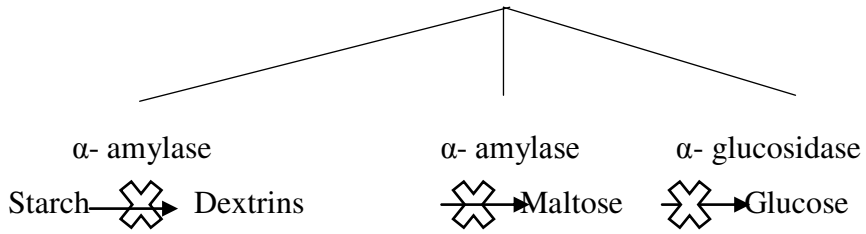
  All metal chelators are strong inhibitors of amylase as they are metalloenzymes. Eg. EDTA, of the organic acids, citric acid and oxalic acid is found to be the most potent inhibitor of amylase. Heavy metal ions such as Al^{3+}, Fe^{2+}, and Hg^{2+} are known to inhibit amylase at higher concentration.

- ***Crude plant extracts:*** A number of crude plants extracts have been reported to have α- amylase inhibitory activity by many researchers. Some of the plant species like Murraya koenigii and Ocimum tenuiflorum extracts of which are reported to have appreciable α- amylase inhibitory activity.

- ***Pure natural products:*** A synthetic pseudotetrasaccharide, Acarbose originally isolated from microorganisms, is an established inhibitor of both α- amylase and α- glucosidase.

ACARBOSE

Acarbose is an antidiabetic agent used for suppressing α- amylase enzyme. This inhibitor makes an environment in body, so that there is a delay in the breakdown of carbohydrate, and reduces the postprandial blood glucose levels.



Pharmaceutical significance of α- amylase inhibitors:

α- amylase inhibitors inhibit the digestion and the production of glucose from complex polysaccharides. These inhibitors have the potential to supress post prandial blood glucose level in diabetic patients. Acarbose which lower blood glucose by inhibiting α- amylase and α- glucosidases is currently used as an antidiabetic drug.

Tendamistat (produced by stretomyces tendae and stretomyces lividans) is an extracellular polypeptide containing 74 amino acids, which showed significant biological activity similar to α- amylase inhibitor and it has been shown to have significant application in the treatment of diabetes mellitus. Due to its resistance against most hydrolytic enzymes, tendamistat would be orally available for the treatment of diabetes mellitus. Adiposin-1 (isolated from Streptomyces calvus) inhibits human α- amylase, is another example of potential antidiabetic compound obtained from microbes.

1.4. DRUG DISCOVERY

Drug discovery is a multifaceted process, which involves identification of a drug chemical therapeutically useful in treating and management of a disease condition. Typically, researchers find out new drugs through new visions into a disease process that permit investigator to design a medicine to stopover or contrary the effects of the disease.

The process of drug discovery includes the identification of drug candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. When a molecule avails its satisfactory results in these investigations, it will commence the process of drug development subsequent to clinical trials.

Drug discovery and development is an expensive process due to the high budgets of R&D and clinical trials. It takes almost 12-15 years to develop a single new drug molecule from the time it is discovered when it is available in market for treating patients. The average cost for research and development for each efficacious drug is likely to be $900 million to $2 billion.

Stages of drug discovery and development include:

- Target identification
- Target validation
- lead identification
- lead optimization
- Product characterization
- Formulation and development
- Preclinical research
- Investigational New Drug
- Clinical trials
- New Drug Application
- Approval

Target Identification

- The first step in the discovery of a drug is identification of the biological origin of a disease, and the potential targets for intervention.

- Target identification starts with isolating the function of a possible therapeutic target (gene/nucleic acid/protein) and its role in the disease.
- Identification of the target is followed by characterization of the molecular mechanisms addressed by the target.
- An ideal target should be efficacious, safe, meet clinical and commercial requirements and be druggable ‘.
- The techniques used for target identification may be based on principles of molecular biology, biochemistry, genetics, biophysics, or other disciplines.

Approaches:

- Data mining using bioinformatics — identifying, selecting and prioritizing potential disease targets
- Genetic association — genetic polymorphism and connection with the disease
- Expression profile — changes in mRNA/protein levels
- Pathway and phenotypic analysis — In vitro cell-based mechanistic studies
- Functional screening — knockdown, knockout or using target specific tools.

Target Validation

Target validation is the process by which the expected molecular target – for example gene, protein or nucleic acid of a small molecule is certified. Target validation includes: determining the structure activity relationship (SAR) of analogs of the small molecule; generating a drug-resistant mutant of the presumed target; knockdown or over expression of the presumed target; and monitoring the known signaling systems downstream of the presumed target.

Target validation is the process of demonstrating the functional role of the identified target in the disease phenotype. Whilst the validation of a drug ‘s efficacy and toxicity in numerous disease-relevant cell models and animal models is extremely valuable – the ultimate test is whether the drug works in a clinical setting.

Lead Identification

A chemical lead is defined as a synthetically stable, feasible, and drug like molecule active in primary and secondary assays with acceptable specificity, affinity and selectivity for the target receptor. This requires definition of the structure activity relationship as well as determination of synthetic feasibility and preliminary evidence of in vivo efficacy and target engagement.

Lead Optimization

Lead optimization is the process by which a drug candidate is designed after an initial lead compound is identified. The process involves iterative series of synthesis and characterization of a potential drug to build up a representation of in what way chemical structure and activity are related in terms of interactions with its targets and its metabolism.

In initial drug discovery, the resulting leads from hit-to-lead high throughput screening tests undergo lead optimization, to identify promising compounds. Potential leads are evaluated for a range of properties, including selectivity and binding mechanisms during lead optimization, as the final step in early stage drug discovery. The purpose of lead optimization is to maintain favorable properties in lead compounds, while improving on deficiencies in lead structure. In order to produce a pre-clinical drug candidate, the chemical structures of lead compounds (small molecules or biologics) need to be altered to improve target specificity and selectivity. Pharmacodynamic and pharmacokinetic parameters and toxicological properties are also evaluated. Labs must acquire data on the toxicity, efficacy, stability and bioavailability of leads, in order to accurately characterize the compound and establish the route of optimization [29].

1.4.1. Types of Drug Design

Computer Aided Drug Design (in silico) approaches have been widely employed in Lead Identification and Lead Optimization stages of drug development against various targets over the years. In comparison to traditional drug discovery methods rational drug design methods bring down the time and cost involved in drug development process. It can be used to identify/design new inhibitors de novo or for

optimization of absorption, distribution, metabolism, excretion and toxicity profile of identified molecules from various sources. Advances in computational techniques and hardware have facilitated the application of in silico methods in the discovery process.

Drug Design can be categorized as two types:

Structure based drug design (SBDD) and

Ligand based drug design (LBDD).

Structure Based Drug Design

SBDD is the approach where the structural information of the drug target is exploited for the development of its inhibitor. Receptor structure(s) is a prerequisite for this method. Most commonly the structure of the receptor is determined by experimental techniques such as X-ray crystallography or NMR. If the structure of the protein drug target is not available, protein structure can be predicted by computational methods like threading and homology modeling. Threading (also called as fold) is a modeling approach used to model proteins that do not have homologous proteins with known structure. During threading, a given amino acid sequence is searched for compatibility with the structures in a database of known folds. The structure of the query protein is built from these folds. Homology modeling (also called as comparative) is an approach that relies on a clear relationship or homology between the sequence of the target protein and at least one known structure.

De Novo Drug Design

De novo is a Latin expression meaning "from the beginning". Active site of drug targets when characterized from a structural point of view will shed light on its binding features. This information of active site composition and the orientation of various amino acids at the binding site can be used to design ligands specific to that particular target. Computational tools that can analyze protein active site and suggest potential compounds are extensively used for de novo design methods. Many promising approaches with the goal of ligand design have been reported. In his book chapter, Murcko provided a detailed analysis of computer aided ligand design methods and distinguished them as six major classes

i. Fragment location methods: To determine desirable locations of atoms or small fragments within the active site.
ii. Site point connection methods: To determine locations ("site points") and then place fragments within the active site so that those locations are occupied by suitable atoms.
iii. Fragment connection methods: Fragments are positioned and "linkers" or "scaffolds" are used to connect those fragments and hold them in a desirable orientation.
iv. Sequential buildup methods: Construct a ligand atom by atom, or fragment by fragment.
v. Whole molecule methods: Compounds are placed into active site in various conformations, assessing shape and/or electrostatic complementarity.
vi. Random connection methods: A special class of techniques combining some of the features of fragment connection and sequential buildup methods, along with bond disconnection strategies and ways to introduce randomness.

Ligand Based Drug Design

Ligand based drug design is an approach used in the absence of the receptor 3D information and it relies on knowledge of molecules that bind to the biological target of interest. 3D quantitative structure activity relationships (3D QSAR) and pharmacophore modeling are the most important and widely used tools in ligand-based drug design. They can provide predictive models suitable for lead identification and optimization [30].

In the absence of any structure information available for the therapeutic target, the alternative approach is LBDD. Unlike SBDD, LBDD does not require a priori knowledge of mechanisms of action and only needs structural information and bioactivity data for small molecules. The principle of LBDD is that structurally similar molecules are likely to have similar properties. An imperative step in LBDD is to retrieve and prepare small molecule libraries. Chemical structures are usually created, processed, and utilized as molecular graphs. A molecular graph is a

combination of nodes and edges in which atoms and bonds are represented as nodes and edges, respectively.

1.5. Docking

Docking is the computational determination of binding affinity between a protein structure and a ligand. This method involves proficient sampling of all possible poses of the ligand in the binding pocket of the target protein to ease optimal binding geometry, as measured by the defined scoring functions [31].

Molecular docking is a technique, used for predicting the best match between two molecules when they are bound to each other in order to generate a stable complex. This information about the preferred orientation can further be utilized for predicting the binding affinity or strength of association between two molecules with the help of scoring functions. The associations among biologically relevant molecules like nucleic acids, lipids, carbohydrates, and proteins plays the key role in signal transduction and this is further affected by relative orientation of the two molecules which are interacting together. Hence, docking is helpful in predicting the strength as well as the type of signal produced. It is utilized for determining how the small drug molecules binds to their target protein with which, the activity of these small molecules can be predicted further.

Binding of a small molecule ligand with an enzyme protein may either activate or inhibit the enzyme. In case when the protein is a receptor, this binding may either cause antagonism or agonism. Docking may be applied to:

- **Hit Identification** In combination with scoring function, docking can be used for quick screening of large databases of desirable drugs in-silico, for identifying the molecules that can bind to selected protein target (Virtual Screening).
- **Lead Optimization** With the help of docking, relative orientation of a ligand binding to the target protein can be predicted (both site and type of orientation) Which may help in designing the analogs with more potency and selectivity.

- **Bioremediation** Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes. Estimating the binding affinity.
- Searching for lead structures for protein targets [32].

1.5.1. Types of docking

The following are primarily applied method for docking

(1) Lock and Key\Rigid Docking-Both the receptor and ligand is maintained fixed and docking is executed.

(2) Induced fit\Flexible Docking-In induced fit docking both the ligand and the receptor are conformationally flexible. Every rotation the surface cell occupancy and energy are calculated; later the most optimum pose is selected.

1.5.2. Virtual Screening

Virtual screening (VS) is a computational approach for the discovery of new drugs that has successfully complemented High Throughput Screening (HTS) for hit detection. The objective is to use a computational approach for rapid cost-effective evaluation of large virtual databases of chemical compounds to find novel leads that can be synthesized and examined experimentally for their biological activity. Unlike HTS, VS does not rely on bruteforce search, and is instead based on starting information of the receptor under inspection or its active ligands. VS methods can be divided into two different categories, structure-based and ligandbased.

Structure-Based Virtual Screening

Structure-based virtual screening (SBVS) encompasses a variety of sequential computational phases, including target and database preparation, docking and post docking analysis and prioritization of compounds for biological testing. SBVS is employed in situations in which the 3D structure of the target protein is known. In the absence of structural information regarding the target, homology modeling can be employed to elucidate the structure of the target protein. Programs that utilize the

SBVS include GLIDE, FlexX and GOLD. SBVS involves explicit molecular docking of each ligand into the target binding site, producing a predicted binding mode for each compound, and measuring the quality of fit of the compound in the target binding site based on the fitness function. This is followed by ranking of compounds to select a small subset for investigation of biological activity.

Pharmacophore/Ligand Based Virtual Screening (PBVS/LBVS)

Pharmacophore based virtual screening (PBVS) uses a pharmacophore modeling approach to screen large databases to identify molecules of desired biological effects. To accomplish this, a query (pharmacophore model) that encodes the correct 3D organization of the required interaction pattern in the most likely manner is created. Different options are available for constructing a pharmacophore model (query) depending on the information available for the particular protein target. Examples of some programs that perform pharmacophore-based searches include UNITY, MACCS-3d, Catalyst, PHASE, and ROCS.

1.5.3. Approaches of Molecular Docking

For performing molecular docking, primarily two types of approaches are used.

Search algorithm

The algorithm should create an optimum number of configurations that admit by experimentation method determining binding modes. The following are the various algorithms applied for docking analysis such as Point complementary, Monte Carlo, Fragment-based, Genetic algorithms, Systematic searches, Distance geometry etc

Scoring Functions:

Scoring function is the most important component in structurebased drug design for evaluating the efficacy of ligands binding to their target proteins. The scoring function furnishes a mode to rank positioning of ligands proportional to some other. Ideally, the score should correspond directly to the binding affinity of the ligand for the protein, so that the best scoring ligands are the best binders. Scoring

functions can be empirical, knowledge based, or molecular mechanics based. Scoring is actually compiled of three different expressions applicable to docking and drug design:

1. Generated configurations ranking by the docking search.
2. Ranking different ligands against protein (virtual screening).
3. One or more ligands ranking against different proteins by their binding affinity (selectivity and specificity)

Scoring functions have been categorized into four different types:

1. Force-field or molecular mechanics-based scoring functions.
2. Empirical scoring functions.
3. Knowledge-based scoring functions.
4. Consensus scoring functions.

1.5.4. Major steps involved in mechanics of molecular docking

Molecular Docking is the process in which the intermolecular interaction between 2 molecules was studied in In-silico. In this process, the Macromolecule is the protein receptor. The micro molecule is the Ligand molecule which can be acted as an inhibitor. So, the Docking process involves the following steps:

Step I – preparation of protein:

Three-dimensional structure of the Protein should be retrieved from Protein data bank (PDB); afterward the retrieved structure should be pre-processed. This should admit removal of the water molecules from the cavity, stabilizing the charges, filling the missing residues, generation the side chains etc. according to the parameters available.

Step II – active site prediction:

After the preparation of protein, the active site of protein should be predicted. The receptor might possess lots of active sites merely the one of the concerns should

be picked out. Mostly the water molecules and hetero atoms are removed if present.

Step III – preparation of ligand:

Ligands can be retrieved from several databases such as ZINC, Pub Chem or can be sketched applying Chem sketch tool. While picking out the ligand, the LIPINSKY'S RULE OF 5 should be utilized. Lipinski rule of 5 assists in discerning amongst non-drug like and drug like candidates. It promises high chance of success or failure due to drug likeness for molecules abiding by with 2 or more than of the complying rules. For choice of a ligand allowing to the LIPINSKY'S RULE:

1. Less than five hydrogen bond donors
2. Less than ten hydrogen bond acceptors
3. Molecular mass less than 500 Da
4. High lipophilicity (expressed as LogP not over 5)
5. Molar refractivity should be between 40-130

Step IV- docking:

Ligand is docked against the protein and the interactions are analyzed. The scoring function gives score on the basis of best docked ligand complex is picked out [33].

Various softwares used for docking studies are:

- AutoDock 4.2
- Gold
- Vega
- Glide
- Flexidock
- Flex
- Fred
- Hint etc

1.6. Autodock 4.2

Autodock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Autodock uses *Monte Carlo method* and *simulated annealing* in combination with *genetic algorithm* for building the possible conformations. The genetic algorithm is used for global optimization. Autodock works in Linux platform. Cygwin is used as a user-friendly interface. The local search method is energy minimization and Amber "force field" model helps in the evaluation of binding positions compatible with several scoring functions based on the free energy. The atomic affinity grids can be visualized. This is helpful to guide organic synthetic chemists to design better binders.

Autodock consists of two main programs:

1. AutoGrid pre-calculates the grids.
2. AutoDock perform the docking of the ligand to a set of grids describing the target protein.

It also has got capabilities to visualize atomic affinity grids and its graphical user interface, thus to support the analysis of docking results. It has an advantage of getting free academic license, at the same time parallel computation is not supported.

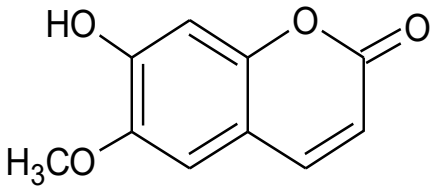
The aim of the present work was to synthesize new isoxazole derivatives containing coumarin moiety in order to explore the extent of their α- amylase inhibitory activity. The compounds were designed by *in silico* method using α-amylase as the target molecule.

2. REVIEW OF LITERATURE

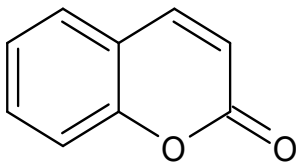
COUMARINS

1. COUMARIN AS ANTI - DIABETIC AGENTS

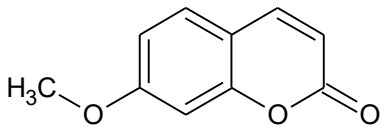
- **Anchal Verma *et al.,* (2013) [36],** Performed an isolation of Scopoletin, a derivative of coumarins, 7-Hydroxy-6-methoxycoumarin was evaluated for the hypoglycemic and hypolipidemic activity in Wistar rats in streptozotocin induced diabetic rats.



- **Leelavinothan Pari *et al.,* (2014) [35],** performed the hypolipidemic effect of coumarin on streptozotocin– nicotinamide induced type 2 diabetic rats. e. Streptozotocin–nicotinamide induced diabetic rats showed a significant increase in the levels of plasma and tissue (liver and kidney) lipids (total cholesterol, triglycerides, free fatty acids, phospholipids), LDL, VLDL and a significant decrease in the levels of HDL were observed. Conclusively, oral treatment of coumarin exhibited in a marked antihyperlipidemic effect against diabetes mellitus.

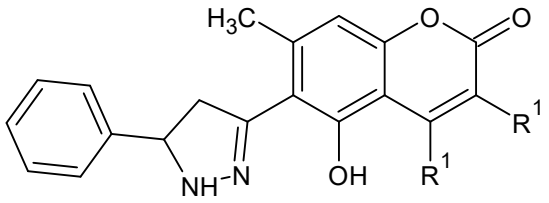


- **Andy Ramu and Veluchamy vijayakumar *et al.,* (2016) [37],** Done a isolation of 7- methoxy coumarin from the bark of marine plant *Rhizophora mucronata* and screened for Invitro antidiabetic activity in chemically induced Wistar rats and male swis albino mice, which proved to coumarins having antidiabetic activity.

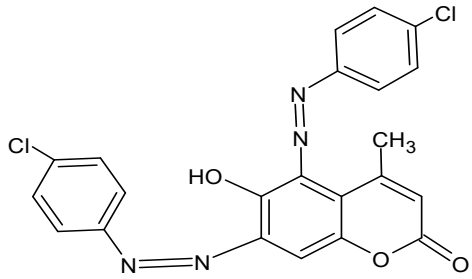


2. COUMARIN AS ANTI-CANCER AGENTS

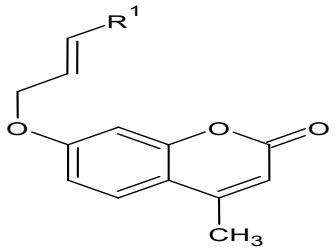
- **Yana Garazd *et al.*, (2016)** [38]**,** were synthesized a series of novel 6-pyrazolinylcoumarins. The synthetic procedure was based on the acetylation of hydroxycoumarins; Fries rearrangement and Claisen–Schmidt condensation and reported them as potential anti-Cancer agent.



- **Dalal M. Ibrahim *et al.*, (2016)** [39]**,** were synthesized and Biological Evaluation of 6-Hydroxy-4-Methyl5,7-(Bis-p-ChlorophenylAzo) Coumarin. characterized by CHN elemental analysis, FTIR, 1H-NMR-spectroscopy and mass-spectral data. Cytotoxic screening by MTT assay was carried out on the compound against breast cancer cells. These compounds are evaluated for their anti-Cancer activity.

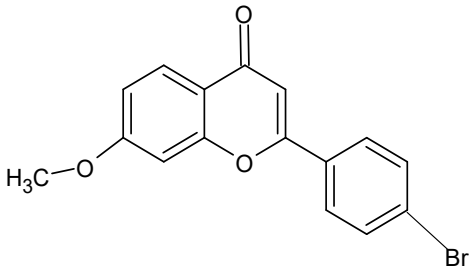


- **Vyshnavi Yelchuri *et al.*, (2016)** [40]**,** were synthesized a series of novel fatty substituted 4-methyl-2H-chromen-2-one (coumarins) via Cross Metathesis. Those compounds were evaluated for their anti-oxidant activities, while fatty substituted exhibited moderate anticancer activities against the four different cancer cell lines tested, namely DU145 (Prostate carcinoma cancer cell), HepG2 (Hepato cellular carcinoma cancer cell), SKOV3 (Ovarian cancer cell) and MDA-MB 231 (Human breast cancer cell). The study reveals that these substituted coumarins can be potential candidates in a number of food and pharmaceutical formulations.

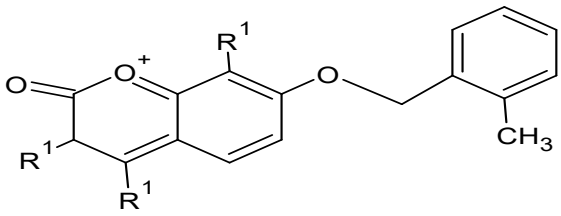


3. COUMARIN AS ANTI-FUNGAL AGENTS

- **Brown *et al.,* (2008)** [41]**,** Synthesized and reported 2-(4-bromophenyl)-7-methoxy4H-chromen-4-one as a more potent antifungal due to the electronegative halogen in the side chain by agar well diffusion method.

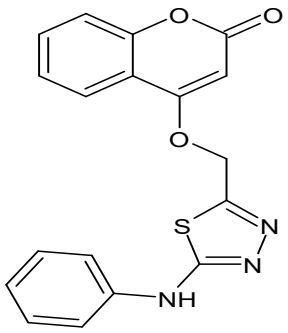


- **Ai-Ying Guan *et al.,* (2011)** [43]**,** were synthesized a series of coumarin derivatives (6-8) containing (E)-methyl 2-(methoxyimino)-2-phenylacetate, (E)-2-(methoxyimino)-N-methyl-2- phenylacetamide and methyl methoxy(phenyl)carbamate and reported them as a potential anti-fungalagents.

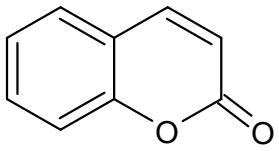


- **Ahmed A. Al-Amiery *et al.,* (2012)** [42]**,** have performed a newly synthesized coumarins 4-((5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)-methoxy)-2H-chromen-2-one and 4-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)-methoxy)- 2H-chromen-2-one were tested against selected types of fungi and showed significant activities. DFT calculations of the

synthesized coumarins were performed using molecular structures with optimized geometries. Those compounds were evaluated for their antifungal activity.

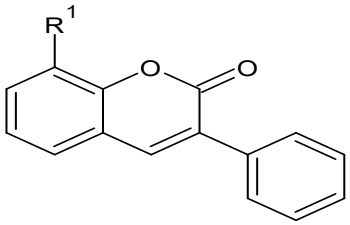


- **Rodrigo S. A. de Araújo *et al.,* (2012)** [45]**,** have reported that the Synthesis, Structure-Activity Relationships (SAR) and in Silico Studies of Coumarin Derivatives with Antifungal Activity. The study of twentyfour coumarin derivatives were screened in vitro for antifungal activity against strains of Aspergillus. Some of the compounds exhibited significant antifungal activity with MICs values ranging between 16 and 32 µg/mL. The structure-activity relationships (SAR) study demonstrated that O-substitutions are essential for antifungal activity. It also showed that the presence of a short aliphatic chain and/or electron withdrawing groups (NO2 and/or acetate) favor activity. These findings were OPEN ACCESS Int. J. Mol. Sci. 2013, 14 1294 confirmed using density functional theory (DFT), when calculating the LUMO density.



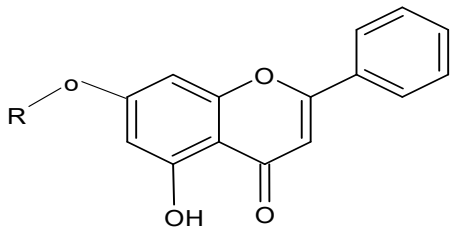
- **Yan Wei *et al.,* (2018)** [44]**,** were synthesized a series of 8-substituted coumarin derivatives and their structures were confirmed by FT-IR, 1 H-NMR, and MS (or HRMS). In activity screening, the synthesized compounds exhibited potent antifungal activity against 4 phytopathogenic fungi: Botrytis cinerea, Colletotrichum gloeosporioides, Fusarium oxysporum, and Valsa mali. Notably, 8-chloro coumarin and ethyl 8-

chloro-coumarin-3-carboxylate showed the strongest fungus inhibition with EC50 of 0.085 and 0.078mmol/L against V. mali. Using 3D-QSAR approaches and the information obtained will be very helpful for designing new derivatives with high antifungal activities.

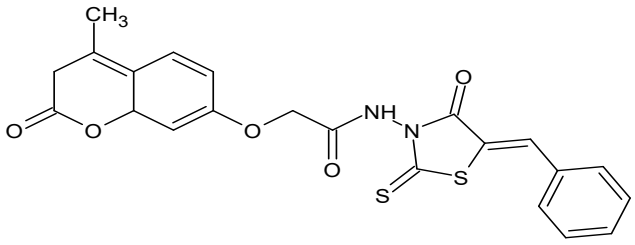


4. COUMARIN AS ANTI-BACTERIAL AGENTS

- **Suresh Babu *et al.*, (2006)** [46] **,** synthesized and reported Substituted 5-hydroxy-2- phenyl-7-ethoxy-4H-chromen-4-one (Fig 2.2) shows more potent anti-bacterial activity due to increase chain length by Serial dilution method.

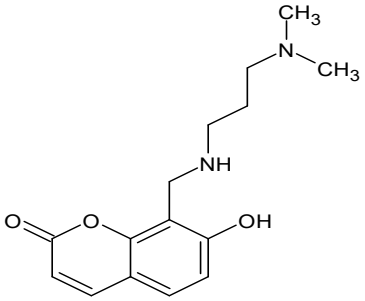


- **Nguyen Tien Cong *et al.*, (2014)** [47]**,** were synthesized 3-(4-methylcoumarin-7- yloxyacetylamino)-2-thioxo-1,3-thiazolidin-4-one. The condensation of compound 4 with different aromatic aldehydes afforded a series of 5-(arylidene)-3-(4-methylcoumarin-7-yloxyacetyl-amino)-2-thioxo-1,3-thiozolidin-4-one analogs and reported them as a potential anti-bacterial agent.

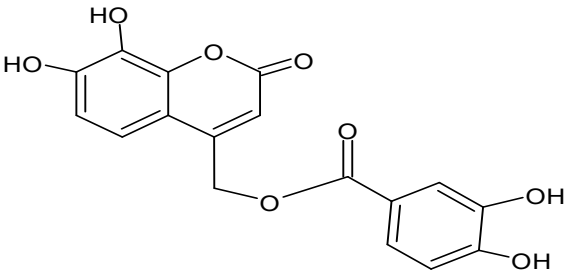


5. COUMARIN AS ANTI-OXIDANT AGENTS

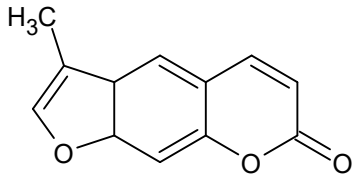
- **Vijaya Pawar *et al.,* (2011)** [49]**,** performed the synthesis of Novel transition metal Cu (II), Ni (II), Co (II) and Zn (II) ions complexes of bidentate Schiff base ligand obtained from 4-Methyl 7-hydroxy 8-formyl coumarin, and Dimethylamino propylene diamine. In order to evaluate the effect of metal ions upon chelation, the Schiff base and their metal complexes have been screened for antimicrobial activity. The transition metal complexes have shown enhanced antimicrobial activities as compared to Schiff base.



- **Karina Pe´rez-Cruz *et al.,* (2017)** [48]**,** were synthesized and antioxidant study of new polyphenolic hybrid-coumarins. . In this work, new hybrid compounds synthesis with a common coumarin scaffold and hydroxybenzoic acids is described. Their antioxidant capacity was evaluated against reactive oxygen species (ROS) using oxygen radical absorbance capacity-fluorescein (ORAC-FL), electron spin resonance (ESR) spin trapping, quenching of superoxide anion, cellular antioxidant activity (CAA) and a ferric reducing ability of plasma (FRAP assay).

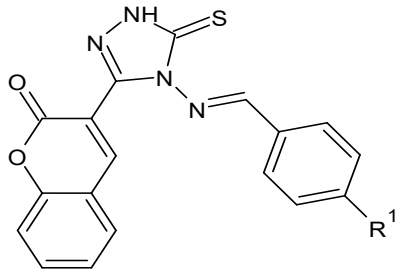


- **María del Pilar Olaya *et al.,* (2018)** [50]**,** have performed the synthesis of Coumarin analogue 3-methyl-7H-furo[3,2-g] chromen-7-one and these compounds exhibit the anti-0xidant and anti-parkinsonian activities.



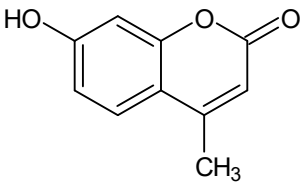
6. COUMARIN AS ANTI – CONVULSANT AGENTS

- **Mashooq A. bhat and Mohammed A. al-omar *et al.,* (2011)** [51]**,** were synthesized a A series of coumarin incorporated 1,2,4- triazole compounds (1-14) were evaluated for their possible anticonvulsant and neurotoxic properties, log P values, pharmacophoric mapping and three dimensional structure analysis. Compound (6) with para-fluoro substitution showed significant anticonvulsant activity.

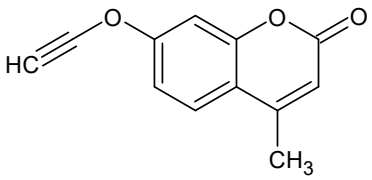


7. COUMARIN SYNTHESIZED USING DIFFERENT SOLVENTS

- **Souad Bouasla *et al.,* (2017)** [52]**,** performed a suitable methodology of synthesis of coumarin derivatives over heterogeneous solid acid catalysts in a free solvent media under microwave irradiation by pechmann reaction.



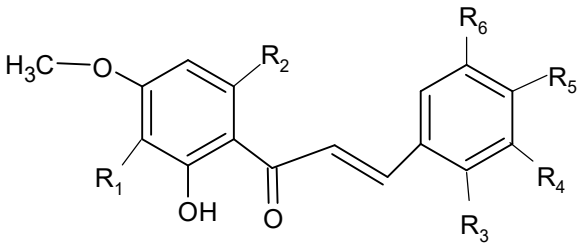
- **Zeydi *et al.,* (2020)** [5]**,** have reported that the synthesis of coumarins has received a lot of attention because of their highly useful biological and pharmacological properties. Both organic and medicinal chemists have considered it.The contents of the review have been classified based on coumarin ring co-groups. These facts have been reported and methods are carried out under both classical and non-classical situations, with a focus on green conditions, such as the use of green materials, a solvent, a catalyst, and other steps situations, with a focus on green conditions, such as the use of green materials, a solvent, a catalyst, and other steps.



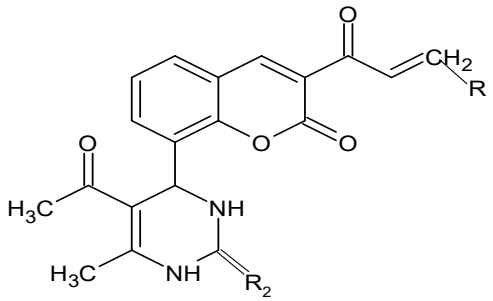
CHALCONES

1. CHALCONE AS ANTI – DIABETIC AGENTS

- **Aluru Rammohan *et al.,* (2019)** [53]**,** reported and performed the study, amino chalcones (3a-j) were synthesized and hydroxy chalcones were isolated from natural source such as Sophora interrupta, Clerodendrum phlomidis and Andrographis macrobotrys. In vivo studies were carried out with alloxan induced diabetic rats. And also docking studies were performed with aldose reductase, dipeptidyl peptidase, PPAR and glucosidase. Therefore, chalcones were implied as antidiabetic leads for in further studies and could be worthwhile for the development of new classes of effective antidiabetic agents.

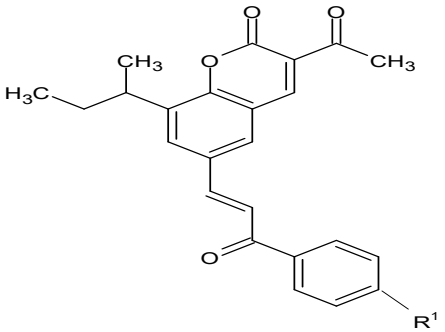


- **Sathish Kumar Konidala *et al.,* (2020)** [54]**,** have performed the four series of thirteen new coumarin-chalcone hybrids (DPCU 1–13, DPCT 1–13, DCCU 1–13 and DCCT 1–13) were designed and synthesized using Biginelli synthesis, Pechmann condensation, Acetylation, and ClaisenSchmidt reactions. Synthesized compounds were tested for insulin receptor in silico docking studies (PDB ID: 1IR3); DCCU 13 and DCCT 13 derivatives received the lowest docking score. Further detailed work could be required to determine the precise mode of action of the anti-diabetic behavior of hybrids.



2. CHALCONE AS ANTI – CANCER AGENTS

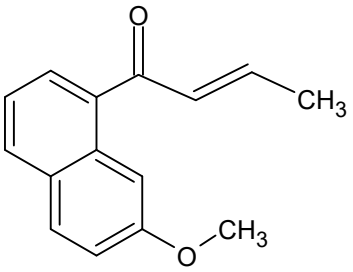
- **Koneni V. Sashidhara *et al.*, (2010)** [55]**,** performed a series of coumarin–chalcone hybrids have been synthesized and evaluated for their in vitro cytotoxicity against a panel of four human cancer cell lines and normal fibroblasts (NIH3T3). Among 21 compounds screened, three compounds (23, 25 and 26) showed IC50 range from 3.59 to 8.12 lM. Among those the compound 26 showed most prominent anticancer activity.



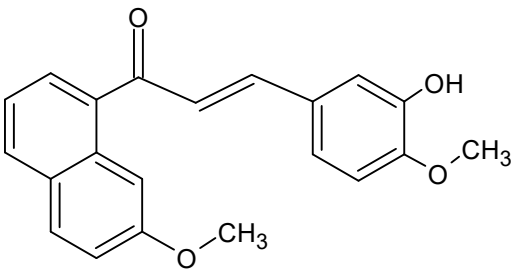
R^1 = 22 Cl

- **Guangcheng Wang *et al.*, (2019)** [56]**,** were synthesized, biological evaluation, and molecular modelling of new naphthalenechalcone derivatives as potential anticancer agents on MCF-7 breast cancer cells by targeting tubulin colchicine binding site. A series of naphthalene-chalcone derivatives (3a–3t) were prepared and evaluated as tubulin polymerisation inhibitor for the treatment of breast cancer. All compounds were evaluated for their antiproliferative activity against MCF-7 cell line. Molecular docking analysis suggested that 3a interact and bind at the colchicine

binding site of the tubulin. The most of compounds displayed potent antiproliferative activity.

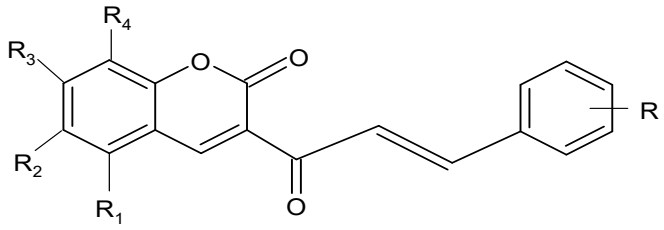


- **Heba M. Abosalim *et al.,* (2021)** [57], have performed the synthesis of Twenty derivatives of chalcones and their anticancer activities were estimated against both breast and liver cancer besides two human normal cell line. Docking study of all newly derivatives was achieved to decide the preeminent binding mode. Generally, the outcome of the docking study showed that 3h had better binding mode and reported them as potent anti-Cancer agent.



3. CHALCONE AS ANTI – VIRAL AGENTS

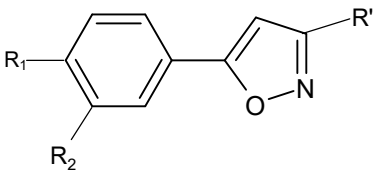
- **Jalpa C. Trived *et al.,* (2007)** [58]**,** performed the two closely structurally related coumarins, 4-hydroxy-8-isopropyl-5-methylcoumarin and 4-hydroxy-6-chloro-7-methylcoumarin were acylated at C-3 and further converted to the respective chalcones and two series of eighteen new compounds. Those compounds were evaluated for possible antiviral activity.



4. CHALCONE WITH ANTI – MICROBIAL ACTIVITY

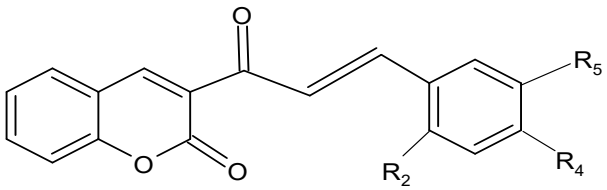
a) CHALCONE WITH ANTI – BACTERIAL, ANTI – FUNGAL ACTIVITY

- **R. Kalirajan *et al.*, (2009)** [59]**,** have synthesized the Some novel heterocyclic derivatives such as Thazines, Oxazines, Isoxazoles and Pyrazoles from various Chalcones. Biological evaluation of some heterocyclic derivatives of Chalcones. These compounds were screened for their Anti-inflammatory, Anti-Bacterial and Anti-fungal activities.



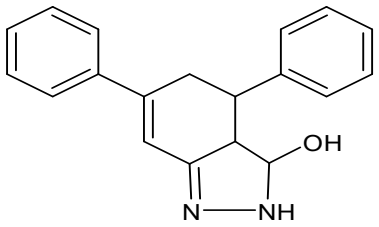
R_1	R_2	R'
OCH_3	H	C_6H_5
H	Cl	C_6H_5
H	NO_2	C_6H_5

- **Saleta Vazquez-Rodriguez *et al.*, (2010)** [60]**, performed** the synthesis of coumarin and chalcone hybrids using the Knoevenagel reaction as the key step. All the compounds are found to exhibit significant anti-bacterial activity.

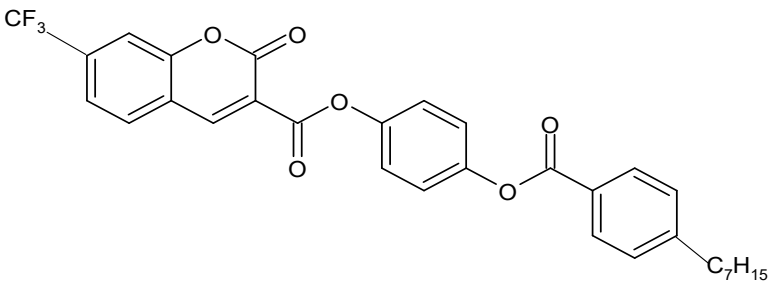


5. CHALCONE AS ANTI – OXIDANT AGENTS

- **Prafulla M Sable, Lata C Potey *et al.,* (2018)** [62]**,** have reported that the microwave assisted synthesis of biologically active Chalcone. Recently the microwave has become the useful nonconventional source for the organic synthesis. From decades the chalcone had been synthesizing by conventional way of heating which has taking a long time, 24 hrs to complete, so to improve the yield and to minimize the reaction time. The chalcone derivatives are synthesized using the microwave assisted methods which has diverse biological activity.

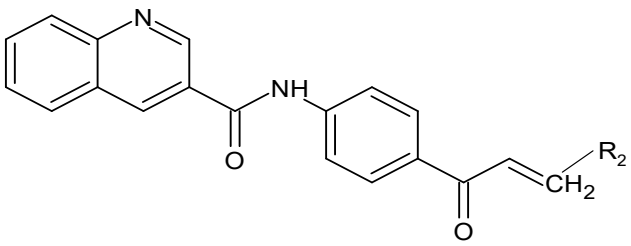


- **Sunil Dutt Durgapal *et al.,* (2019)** [63]**,** *have* reported that the Synthesis and mesomorphic properties of coumarin derivatives with chalcone and imine linkages along with terminal n-alkoxy chain. All the compounds were synthesized and characterized by combination of elemental analysis and standard spectroscopic methods. All compounds were screened under polarising optical microscope (POM) for liquid crystalline properties, thermogram of all compounds were studied using differential scanning calorimetry (DSC).

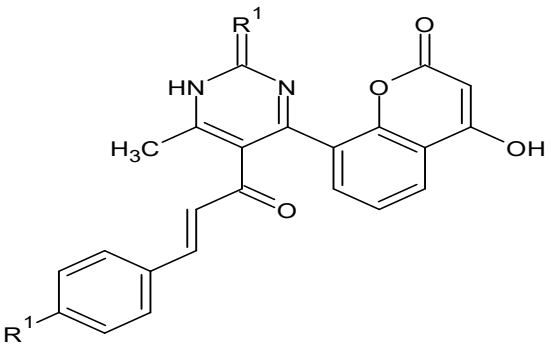


- **Efraín Polo *et al.,* (2019)** [61], performed the synthesis of the chalcone and bis-chalcone derivatives under sonication conditions via ClaisenSchmidt

condensation. These compounds were investigated using a computational methods and evaluation of their anti-oxidant properties.



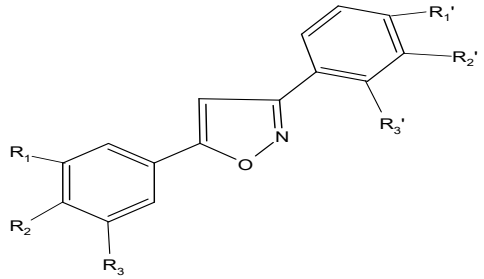
- **Sathish Kumar Konidala *et al.*, (2021)** [10]**,** were synthesized 26 coumarin clubbed chalcone hybrids and in-vitro antimicrobial and antioxidant screening of coumarin clubbed chalcone hybrids through molecular hybridization approach. The promising leads evolved through this investigation are important for the future development of novel and potential antioxidant compounds.



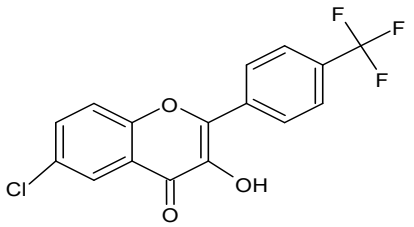
ISOXAZOLES

1. ISOXAZOLE AS ANTI-DIABETIC AGENTS

- **Lincy Joseph *et al.,* (2015)** [64]**,** reported and synthesized novel Isoxazole derivatives, characterize them and subject for screening antibacterial action and in vitro anti-diabetic activity. Those compounds exhibit a potent anti-diabetic agent.



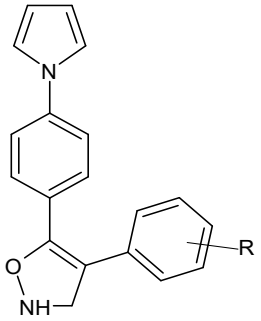
- **Faisal K. Algethami *et al.,* (2021)** [65]**,** reported and performed the condensation of a previously synthesized trifluoromethylated flavonol with different aryl nitrile oxides, affording 13 hybrid molecules indicated as trifluoromethylated flavonoid-based isoxazoles. Structure–Activity Relationship Analysis and Molecular docking studies has been done against the α-Amylase enzyme. Those synthesized compounds were evaluated for Antidiabetic and Anti-Obesity Activity.



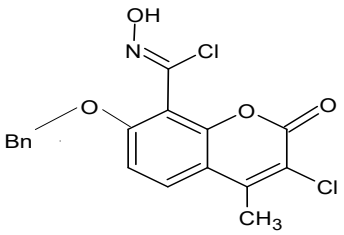
2. ISOXAZOLE AS ANTI – TUBERCULAR AND ANTI – BACTERIAL AGENTS

- **Shrinivas D. Joshi *et al.,* (2015)** [66]**,** reported and synthesized a series of 61 novel pyrrolyl derivatives bearing pyrazoline, isoxazole and phenyl thiourea moieties. Molecualr docking studies has been performed for the synthesized compounds against key enzymes involved in type II fatty acid

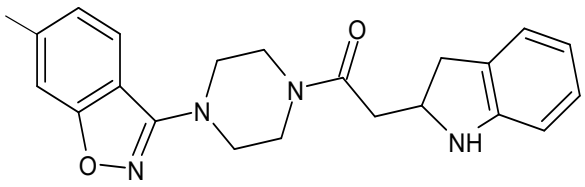
biosynthetic pathway of M. tuberculosis, an attractive target for designing novel antitubercular agent. Then it is evaluated for anti-tubercular and anti-bacterial activity.



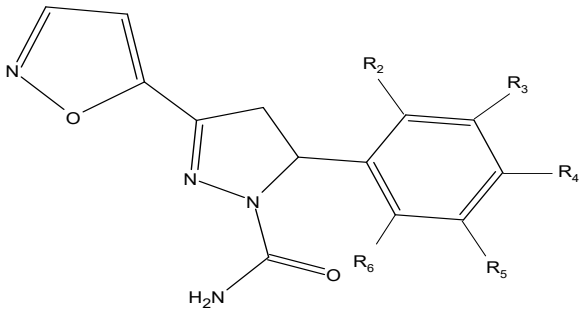
- **Garbapu Suresh *et al.,* (2016)** [67]**,** have reported that the Novel coumarin isoxazoline derivatives were designed and Synthesized. Those compounds are evaluated for antibacterial activity. The new compounds are highly efficient for antibacterial activity.



- **Kalaga Mahalakshmi Naidu *et al.,* (2016)** [68]**,** have perfomed the synthesis of thirty-eight novel 3-(4-((substituted-1H-1,2,3-triazol-4- yl) methyl) piperazin-1-yl/1,4-diazepan-1-yl) benzo[d]isoxazole and 1-(4-(benzo[d]isoxazol-3- yl) piperazin-1-yl/1,4-diazepan-1-yl)-2-(1H-indol-3-yl) substituted-1-one analogues. These compounds were characterized using various analytical techniques. All the synthesized compounds were docked to pantothenate synthetase enzyme site to know deferent binding interactions with the receptor. Those compounds are evaluated for in vitro antitubercular activity against Mycobacterium tuberculosis H37Rv strain.

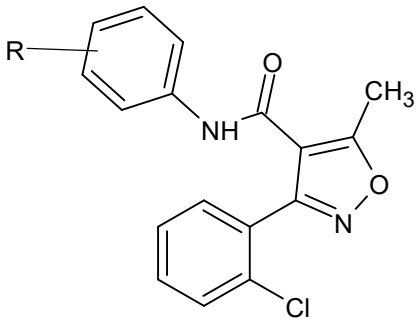


- **Kishor Palleapati *et al.,* (2018)** [69]**,** reported and synthesized a series of dihydropyrazole-1-carboxamides by the base-catalyzed condensation of isoxazolyl chalcones with semicarbazide. These compounds were screened for anti-tubercular activity.



3. ISOXAZOLE AS ANTI – CANCER AGENTS

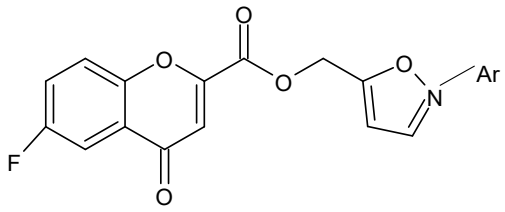
- **Ahmad M.Eid *et al.,* (2021)** [70]**,** reported and performed a series of isoxazole-carboxamide derivatives (2a–2g) were synthesised and evaluated for their cytotoxic activity against breast (MCF-7), cervical (HeLa), and liver (Hep3B) cancer cell lines and their antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Those compounds exhibit a potent Anticancer and Antioxidant Agents.



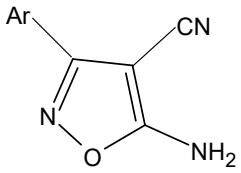
4. ISOXAZOLE AS ANTI – MICROBIAL AND ANTI – OXIDANT AGENTS

- **kumaraswamy battula *et al.,* (2016)** [71]**,** performed a series of novel (3-arylisoxazol-5-yl) methyl 6-fluoro-4-oxo-4H- -chromene-2-carboxylate derivatives (C1–C12) were synthesized by the Cu(I)- -catalyzed reaction of in

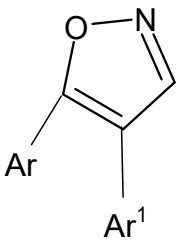
situ generated nitrile oxides with prop-2-ynyl 6-fluoro-4-oxo-4H-chromene-2-carboxylate. Then, those compounds were evaluated for as antioxidant and antimicrobial activity.



- **Hamid Beyzae *et al.*, (2018)** [72]**,** reported and performed the Green multicomponent synthesis of novel 5-amino-isoxazole-4-carbonitriles. Those synthesized compounds were evaluated for anti-microbial and anti-oxidant activity.

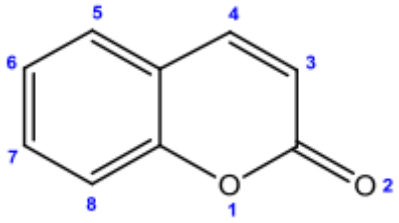


- **Pragi *et al.*, (2019)** [73]**,** reported a series of 4,5-disubstituted isoxazole derivatives of α,β-chalcone ditosylates which were synthesized by the reaction of α,β-chalcone ditosylates with hydroxylamine hydrochloride. Various α, β-chalcone ditosylates were prepared by the reaction of respective chalcones with hydroxyl (tosyloxy)iodobenzene. The above compounds were characterized and evaluated for anti-inflammatory and antioxidant properties.



3. CHEMISTRY

CHEMISTRY OF COUMARIN



COUMARIN

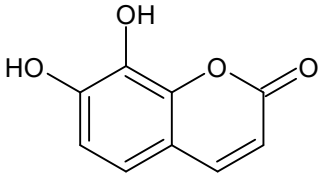
Coumarin (benzopyrones) is a compound containing two rings of six Members heterocycle rings with two oxygen atoms. Coumarin (1,2H-chromen-2-one or 2H-1-benzopyran-2- one) is a bicyclic heterocycle compound, consisting of benzene and 2-pyrone rings [74]. The pyran derivatives are ketonic compounds that in the form of α-pyron or γ-pyron. Secondary metabolites called benzo-α-pyrone (coumarin) and benzo-γ-pyrone (chromone) occur due to condensation of pyron derivatives with benzene in plants [6].

Coumarins are classified in four groups:

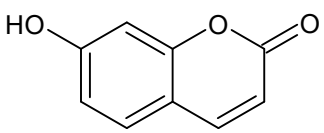
- simple coumarins,
- furanocoumarins,
- pyranocoumarins and
- pyrone-substituted coumarins.

Simple coumarins:

These are composed of hydroxylated, alkoxylated and alkylated derivatives of coumarin and their glycosides (e.g., Umbelliferone, skimmin, limettin, herniarin, esculetin, esculin, daphnetin and daphnin.



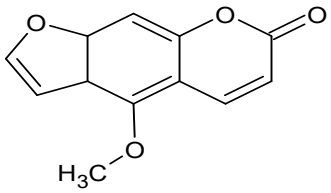
Daphnetin



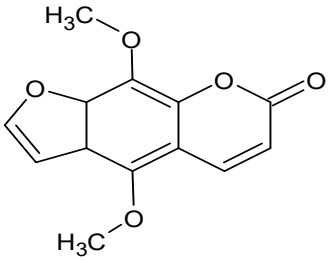
Umbelliferone

Furanocoumarins:

This group of coumarins consists of a furan ring fused with a coumarin. They are divided into two groups as C6/C7 (linear) type, C7/C8 (angular) type according to the attachment place of the furan ring. (e.g., psoralen, xanthotoxin, bergapten, imperatorin, isopimpinellin, anjelisin, isobergapten and pimpinellin.



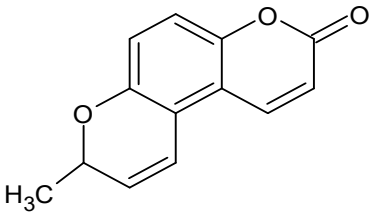
Bergapten



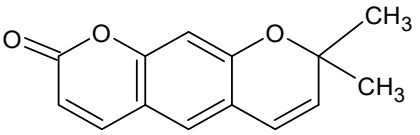
Isopimpinellin

Pyranocoumarins:

Six-membered pyran ring is fused with the benzene ring via C6-7 (linear) or C7–8 (angular) (e.g., visnadin, xanthyletin and seselin).



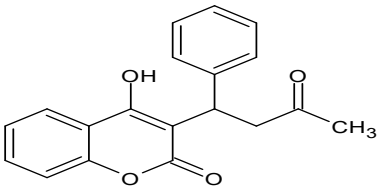
Seselin



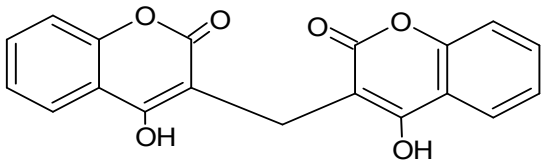
Xanthyletin

Pyrone-substituted coumarins:

These are classified in three groups: 4-Hydroxycoumarin (Novobiocin and Dicoumarol), 3-Phenylcoumarin (Coumestroln and Gravelliferone) and 3,4-Benzocoumarin (Aeternaryiol). 4-Hydroxycoumarins are not found in plants in free form. Warfarin, a synthetic compound, belongs to this group [6].



Warfarin



Dicoumarol

Physical data [75]

Synonym	:	1,2- Benzopyrone, 2H-1-Benzopyran-2-one
Chemical formula	:	$C_9H_6O_2$
Melting point	:	71° C
Boiling point	:	301° C
Density	:	0.935 g/cm^3
Dipole moment	:	4.51D
Solubility	:	Very soluble in boiling water

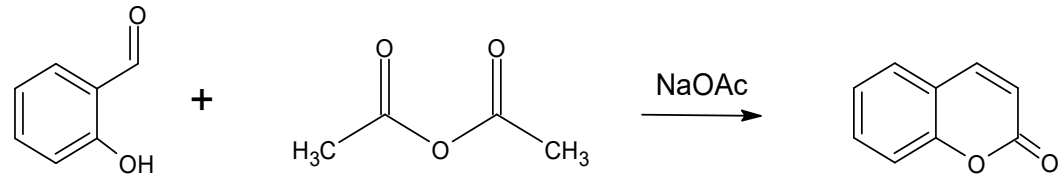
Coumarin in alkaline medium exhibits a green fluorescence in UV light. It has the characteristic odour that of vanilla beans and is used for the preparation of perfumes, soap and flavouring agents.

METHODS FOR THE SYNTHESIS OF COUMARIN DERIVATIVES [76,77]

Of the number of synthetic methods, there are a few which have yielded important results; there are several others whose applications are less general. All these methods center round the possibility of building up the pyrone ring on a suitable benzene derivative.

1. Perkin reaction

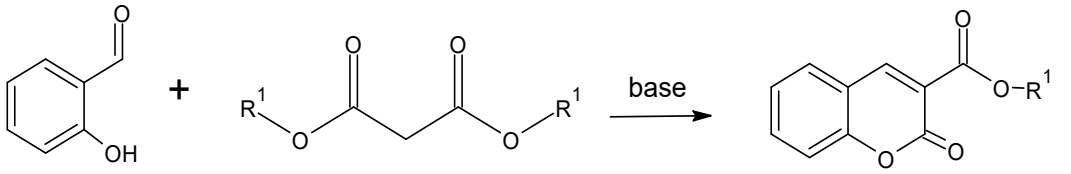
It involves in the formation of coumarins by aldol condensation of ortho-hydroxy benzaldehyde with acid anhydrides in the presence of an alkali salt of an acid.



0-hydroxy benzaldehyde acid anhydride coumarin

2. Knovenagel Reaction

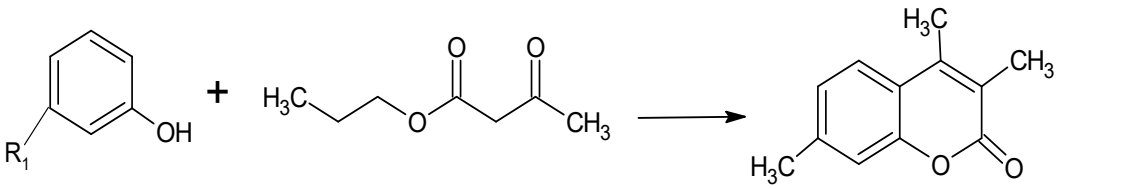
Condensation of ortho-hydroxy benzaldehydes with active methylene compounds (malanonitrile, diethyl malonate, ethyl malonate, ethyl acetoacetate, ethyl cyanoacetate, etc.,) in the presence of base (ammonia, amines, piperidine, pyridine) to form coumarins is known as knovenagel reaction.



o-hydroxy benzaldehyde active methylene compound 3-substituted coumarin

3. Pechmann Reaction

It involves in the condensation of phenols with β-ketoesters in the presence of an acid catalyst (concentrated sulphuric acid). This is the most widely used method for the synthesis of coumarins.

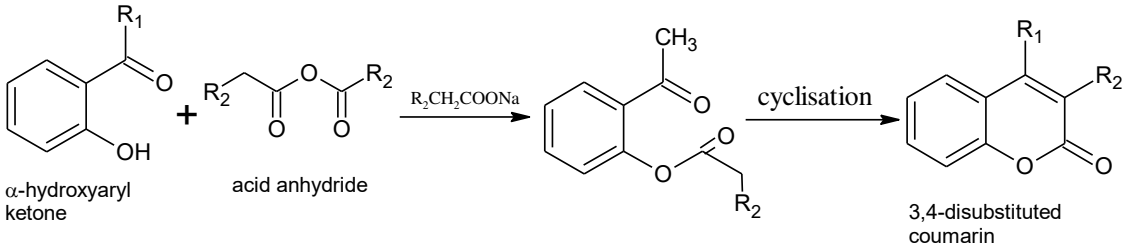


Substituted phenol beta- ketoester 3,4,7- trisubstituted coumarin

When acetoacetic esters and derivatives are used. The reaction is often referred as Pechmann-Duisberg.

4. Kostanecki-Robinson Reaction

The formation of coumarins, usually 3- and 4- substituted coumarins, by this reaction occurred by acylation of ortho–hydroxyaryl ketones with aliphatic acid anhydrides, followed by cyclisation.



Chemical Properties [78]

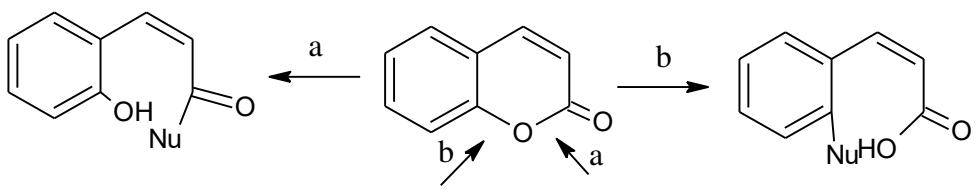
Reactivity of Coumarins

Coumarin and its derivatives are highly reactive because of the aliphatic moiety present in the coumarin, it is likely to undergo ring opening at the acyl centre. Carbon-6 on the aromatic ring can undergo electrophilic attack such as Friedel-Crafts acylation, sulphonation leading to the formation of 6-substituted derivatives. A methyl substituent on the coumarin nucleus may react differently depending on the position of attachment. Phenol group present in the C-7 position, easily undergo acylation, benzoylation and Friedel-Crafts reactions.

Reactions of coumarin nucleus

A. With Nucleophiles

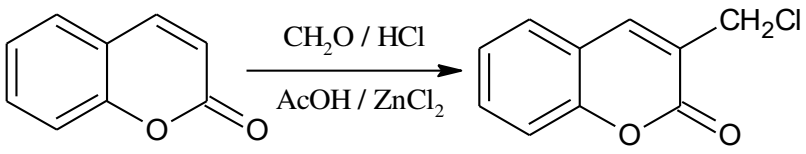
Several kinds of nucleophiles react with coumarins. Some of these reactions involve ring opening and occasionally, recyclisation into another ring. A nucleophile (Nu) which cleaves the ring, attacks and breaks one of the bonds of the ring oxygen atom as shown below.



B. With Electrophiles

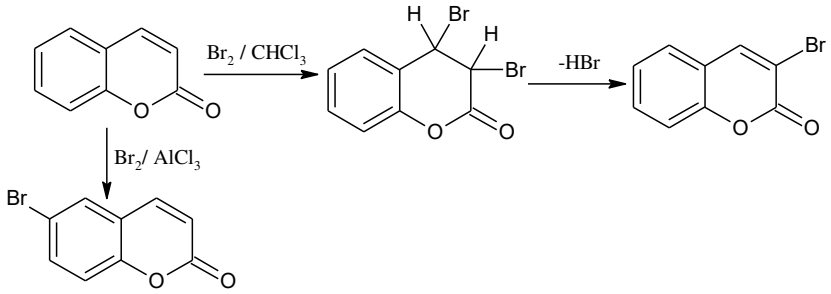
a. Chloromethylation

Chloromethylation occurs at C-3 position of coumarins by the reaction with formaldehyde in the presence of HCl or acetic acid and ZnCl2.



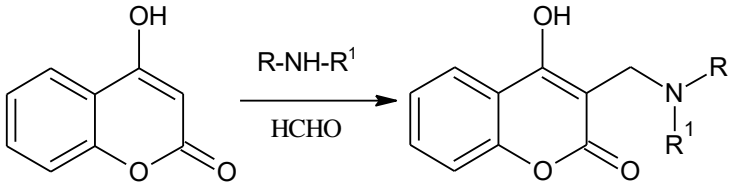
b. Bromination

Coumarin reacts with one molecule of bromine to form th3,4-dibromide which readily eliminates hydrogen bromide to form 3-bromocoumarin. Reaction with bromine, in the presence of excess of aluminium chloride, yields 6-bromocoumarin.

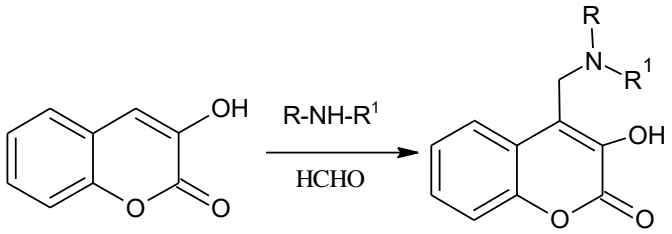


c. Mannich reaction [79]

Mannich reaction of 4-hydroxy coumarin with primary amines and formaldehyde resulted in the formation of 3-aminomethyl-4-hydroxycoumarins.



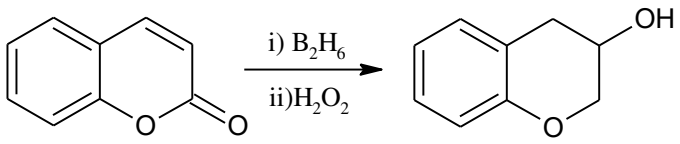
Similarly, Mannich reaction of 3-hydroxy coumarin with formaldehyde and primary or secondary amines resulted in 4-N, N-dialkylaminomethyl-3-hydroxy coumarins.



C. Reduction

a. Reduction with Diborane and Hydrogen peroxide

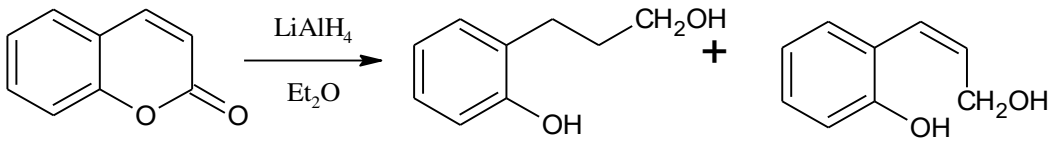
Reduction with diborane followed by hydrogen peroxide has two effects on coumarins: the carbonyl group is reduced to methylene and the elements of water are added across the 3,4-double bond in an anti-Markonikow manner but the overall yield is very low.



3,4-dihydro-h-chromone-3-ol

b. Reduction with Lithium aluminium hydride

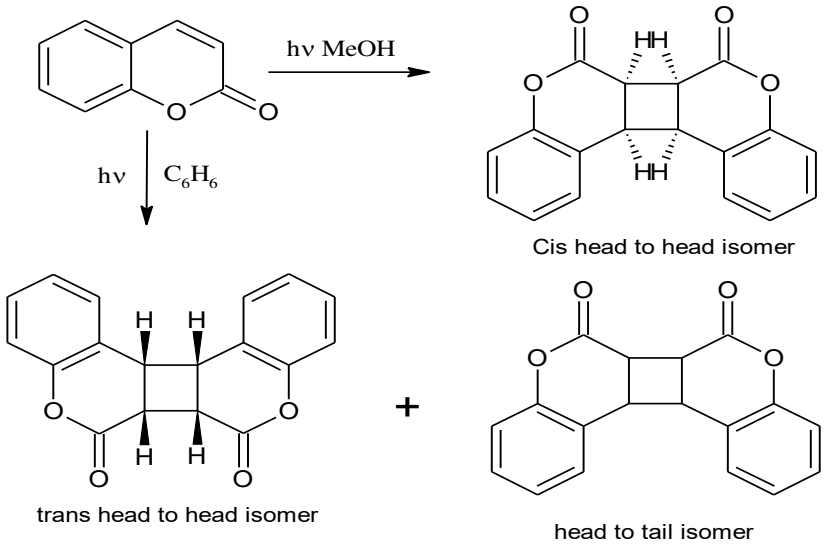
Hydride reagents can react either at carbonyl carbon or the conjugate position and therefore mixtures of two compounds are produced.



c. Photochemical Reactions [75]

The photo dimerisation of coumarin has been studied in several solvents and the nature of solvent has an effect on this complex reaction.

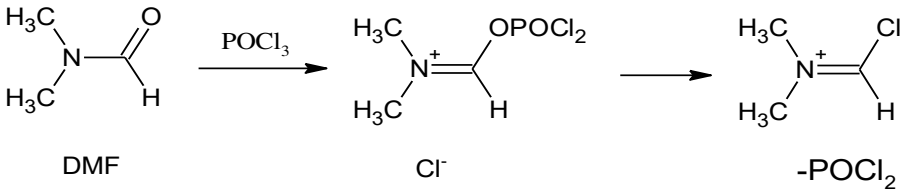
1. In a polar medium such as methanol, the only product formed is the cis head to isomer.
2. In a non-polar medium such as benzene or dioxane, trans head to head dimer is the main product of the reaction; small amounts of head to tail dimers are also formed in non-polar solvents.



d. Vilsmeier-Haack Reaction

The reaction of an N, N-disubstituted formamide, such as DMFor N-methyl formanilide, with acid chlorides, such as phosphoryl chloride or phosgene,leads to the formation of an 'adduct'. These adducts are usually referred to as the Vilsmeier reagent which is used in the formylation of electron rich aromatic compounds or olefins.

Formation of adduct:



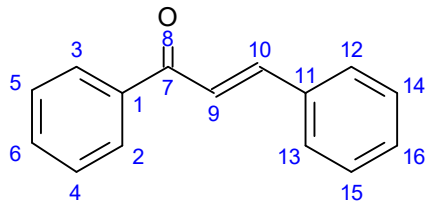
CHEMISTRY OF CHALCONES

CHALCONES

Chalcones, or 1,3-diphenyl-2-propen-1-ones, are one of the most important classes of flavonoids across the whole plant kingdom. Chalcones are open-chain precursors for biosynthesis of flavonoids and isoflavonoids and occur mainly as polyphenolic compounds whose colour changes from yellow to orange . They exist as either trans (E, 1) or cis (Z, 2) isomers having two aromatic rings that are joined by a three-carbon α,β-unsaturated carbonyl system.

CHEMISTRY

Chalcones are made up of a three carbon , -unsaturated carbonyl system.This is largely attributable to the electrophilic nature of the α,β-unsaturated carbonyl system. This moiety is capable of forming irreversible bonds with biological macromolecules, resulting in a number of toxic effects, such as allergenic reactions, carcinogenicity, and mutagenicity . On the other hand, this reactivity may be affected both by the decoration of the aromatic rings, and also, even more effectively, by α-X-substitution of the double bond of the enone system . Therefore, the design and synthesis of new analogs are particularly important for the future development of clinically useful chalcone derivatives.



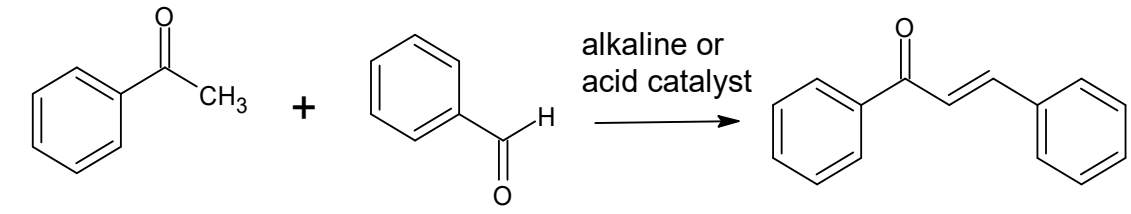
Synthesis of Chalcone Scaffolds [80]

Chalcones have a simple chemistry which enables a multiplicity of substitutions with easy synthesis. Currently, a variety of methods and schemes are available for the synthesis of chalcone derivatives. In each of these methods, the most important part is condensation of two aromatic systems (with nucleophilic and electrophilic groups) to yield the chalcone scaffold. Despite the multiplicity of

substitutions allowed, we describe below the reaction scheme using the standard scaffold of chalcones (1,3-diphenyl-2-propen-1-one)

1. Claisen-Schmidt Condensation

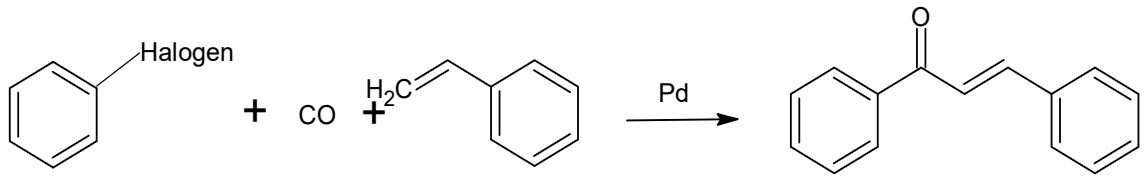
Claisen schmidt condensation is one of the most common method for synthesize chalcones. In this reaction, chalcones are formed by condensation of benzaldehyde and acetophenone derivatives.



Acetophenone Benzaldehyde Chalcone

2. Carbonylative Heck Coupling Reaction

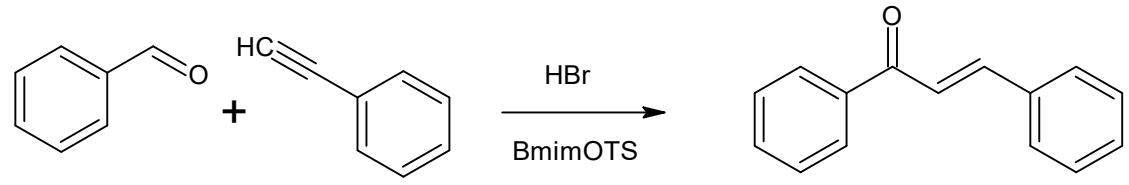
In this reaction, the carbonylative vinylation of phenyl halide with styrene in the presence of carbon monoxide and using palladium (Pd) as catalyst to form a chalcones.



Phenyl Halide Carbon Monoxide Styrene Chalcone

3. Coupling reaction

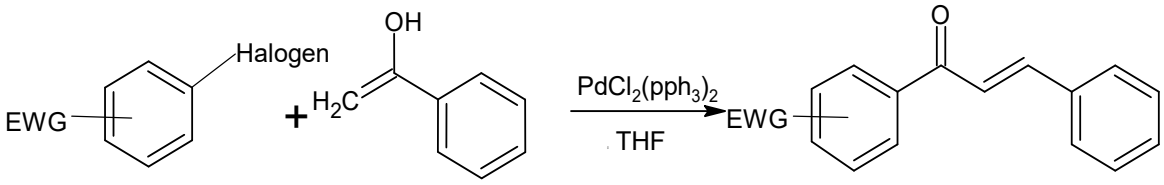
The coupling reaction between the benzaldehyde and phenylacetylene in the presence of HBr and ionic liquids, such as 1-butyl-3-methyl-1H-imidazolium 4-methylbenzenesulphonate (BmimOTs) for 12 hr at 100-degree celcius through the chalcone were synthesized.



Benzaldehyde Phenyl acetylene Chalcone

4. Sonogashira Isomerization Coupling

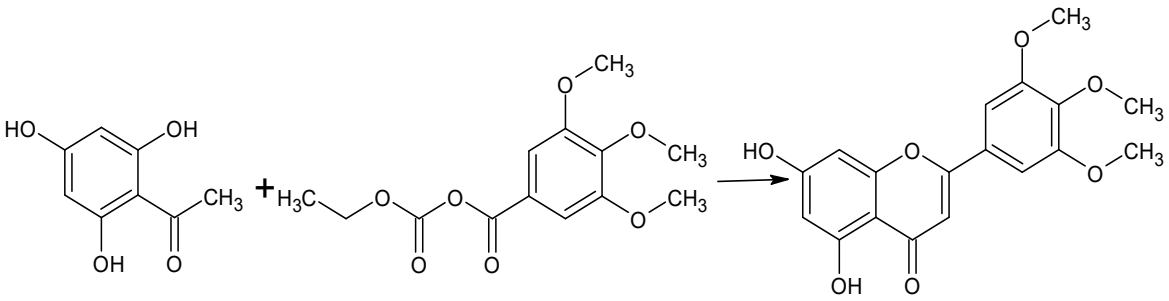
A reaction between the equimolar concentration of electron-deficient phenyl-halide and propargyl alcohol employing microwave irradiation and using PdCl2(PPh3)2 as catalyst and THF as solvent through the chalcone was synthesized.



Phenyl Halide Propargyl alcohol Chalcone

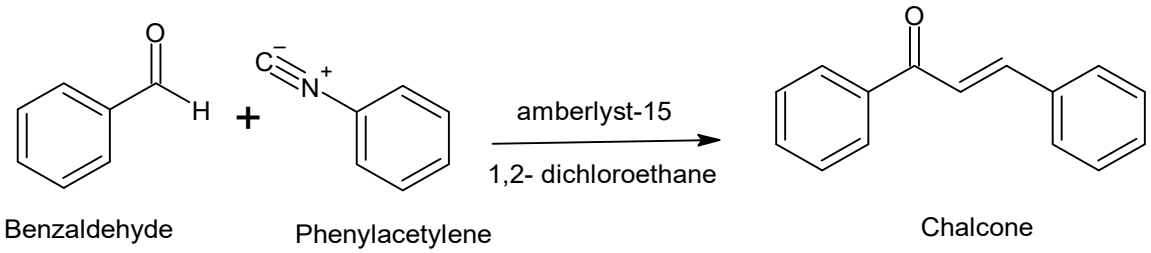
5. THE ALLAN-ROBINSON CONDENSATION

The Allan-Robinson Condensation is used mainly to synthesize flavones with chalcones as their precursors. The condensation of 2,4,6-trihydroxyacetophenone with aromatic anhydrides catalysed by the salt of the same acid will make corymbosin



6. Solid Acid Catalyst Mediated Reaction

Chalcones have also been synthesized by employing a heterogeneous solid acid catalyst. The reaction consists of the addition of an equimolar quantity of benzaldehyde and phenylacetylene in 1,2-dichloroethane solvent irradiated in a microwave and employing ion-exchange Molecules resin amberlyst-15 as heterogeneous solid acid catalyst.



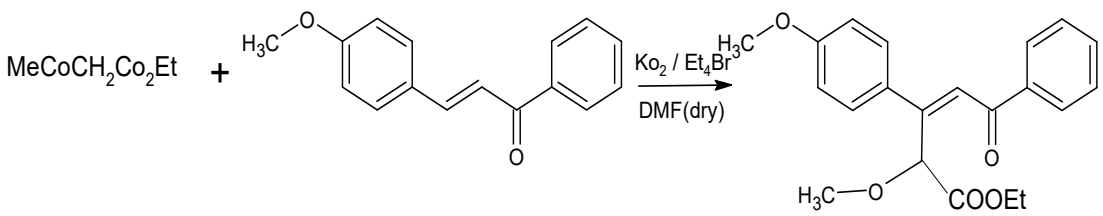
PROPERTIES AND TESTS OF CHALCONES

The Chalcones are generally coloured, usually yellow, orange, red or brown. They are comparatively more soluble than flavanones in ethanol and ethylacetate 2'-hydroxyChalcones dissolves in dilute alkali with an orange deep red colour.

Reactivity of Chalcones [82- 89]

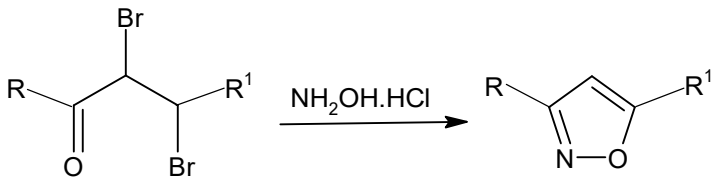
1) **Formation of 3-(4-methoxyphenyl) ethyl 2-acetyl-5-oxo-3,5-diphenylpentanoate:**

4-methoxy chalcone reacts with ethylacetoacetate in presence of potassium suproxide,tetraethylammonium bromide and dry DMF(dimethyl formamide) to form 3-(4-methoxyphenyl)ethyl 2-acetyl-5-oxo-3,5- diphenylpentanoate.



2) Reaction of hydroxylamine hydrochloride on D: E-dibromo – Chalcones

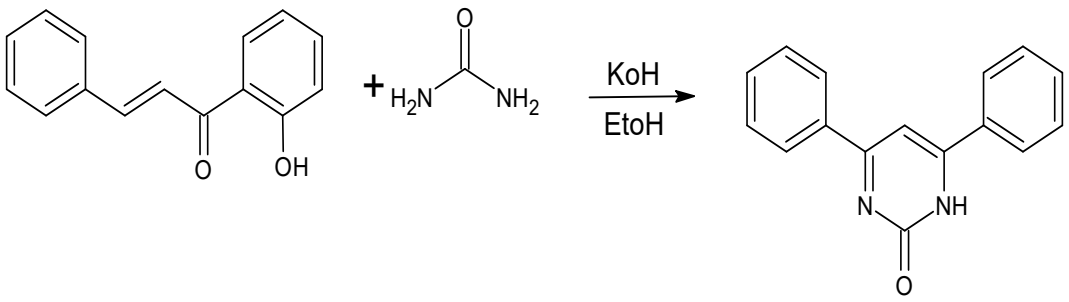
The alcoholic solution of D-E dibromochalcones when refluxed with molar proportion of hydroxylamine hydrochloride and aqueous potassium hydroxide, followed by acidification, yield that 2-isoxazole derivatives.



Reaction of Hydroxylamine hydrochloride

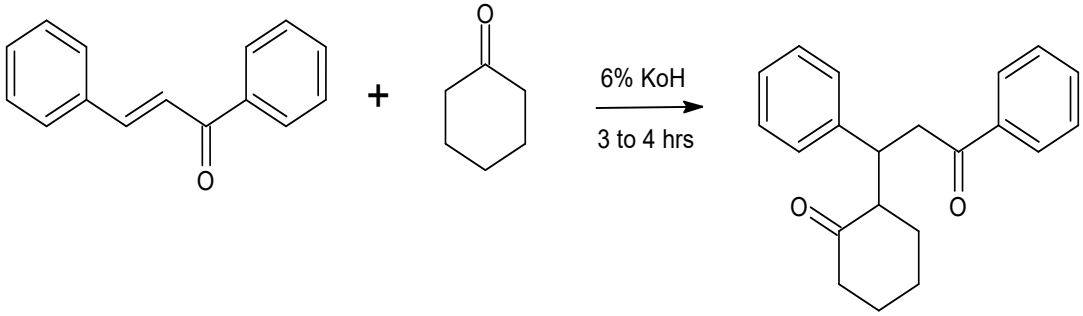
3) Formation of dihydropyrimidine under ultrasound irradiation:

Chalcones reacted with urea in presence of potassium hydroxide in ethanol to produce 4,6-(diphenyl)-3,4-dihydropyrimidine-2(1H)-one under ultrasound irradiation.



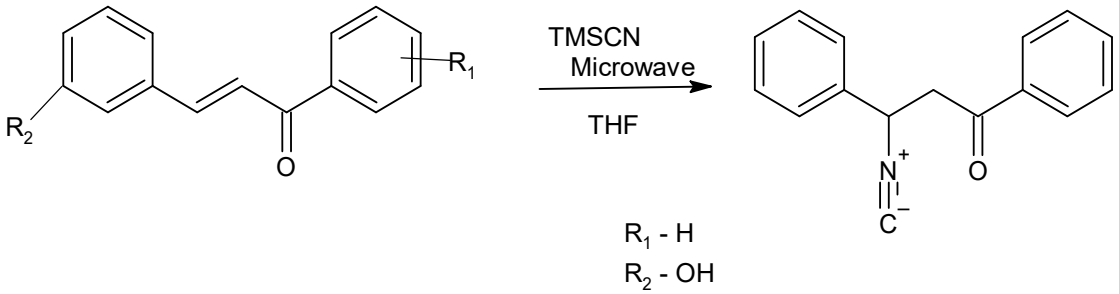
4) Formation of cyclohexanone derivative:

Chalcone react with cyclohexanone in presence of potassium hydroxide to form 2-(3-oxo-1,3-diphenylpropyl cyclohexanone.



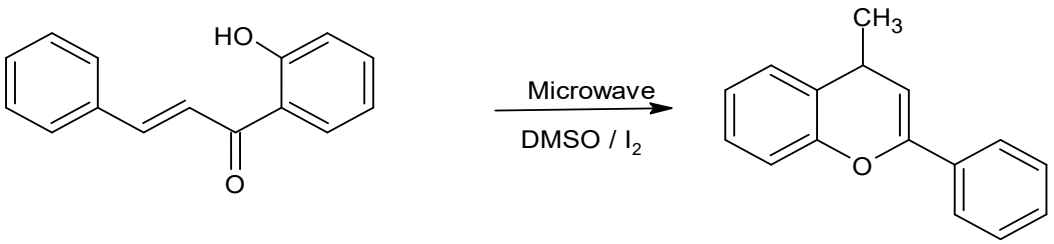
4) **Hydrocyanation of chalcones with trimethyl silyl cyanide:**

β-cyanoketones are formed by the reaction of chalcones with trimethyl silyl cyanide (TMSCN) .



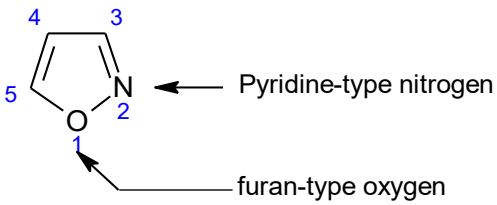
5) **Formation of flavone from chalcone-2'-ol:**

Chalcone react with DMSO (dimethyl sulphoxide) and Iodine under microwave condition to form flavones.



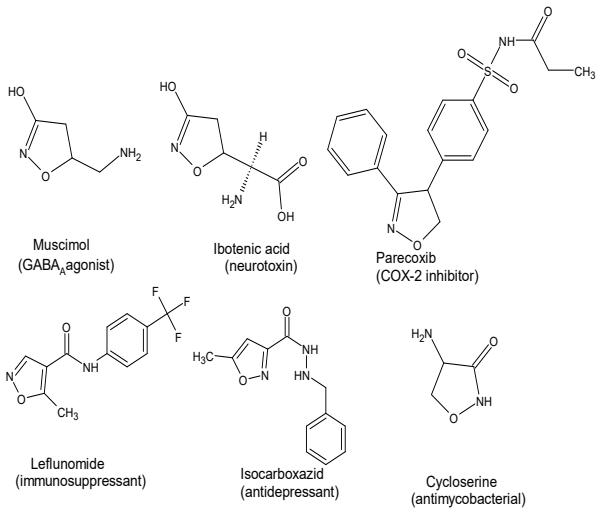
CHEMISTRY OF ISOXAZOLES [90]

Isoxazole is a five membered π-excessive heterocycle with oxygen (furan-type) and nitrogen (pyridine – type) at the positions-1and -2, but differs from oxazole by the presence of N-O bond. The partially reduced form of isoxazole (dihydroisoxazole or isoxazoline) exists in three isomeric forms, depending on the position of double bond. The position of double bond may be represented by prefix Δ (delta) with superscript. The completely reduced form of isoxazole is known as 2,3,4,5- tetrahydroisoxazole (isoxazolidine).



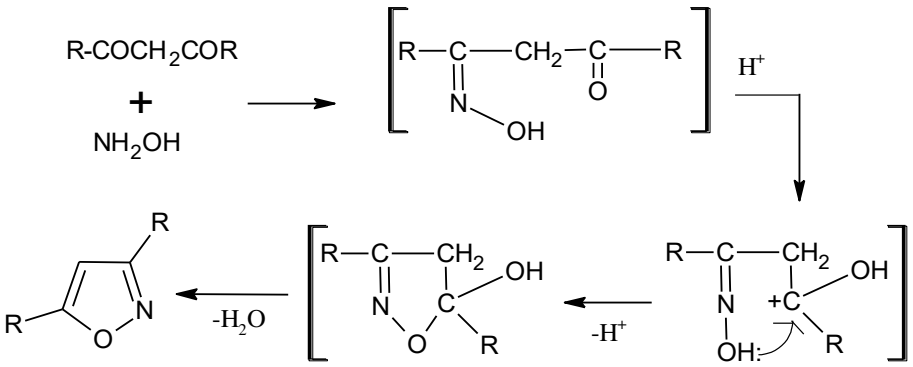
Isoxazole is a colourless liquid (b.p.= 95°C, D.M = 2.75 ± 0.01 D in benzene and 3.1 ± 0.03 D in dioxane) with strong pyridine like odour. The boiling point of isoxazole is although lower than of pyrazole and imidazole but higher than that of oxazole and furan. The higher boiling point of isoxazole is attributed to the greater intermolecular association in isoxazole molecules involving pyridine-type nitrogen and hydrogen at C-3.

The structures of biologically efficacious molecules containing an isoxazole scaffold are

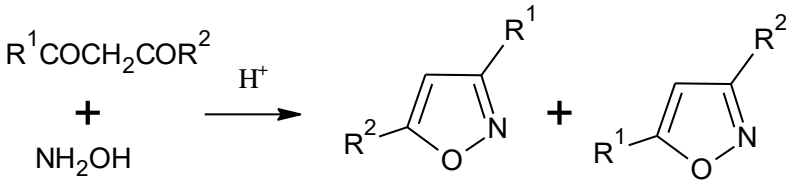


Synthesis

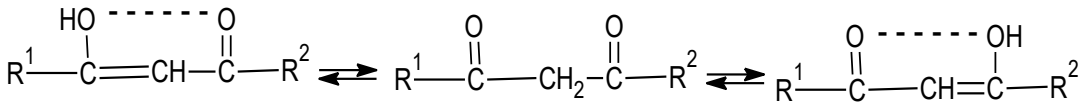
In the past few decades, many exciting advances in the synthesis and functionalization of isoxazoles have been reported. This five-membered ring system can be easily made by using a hydroxylamine, dipolar cycloadditions, or through adjacent heteroatom bond-forming strategies.



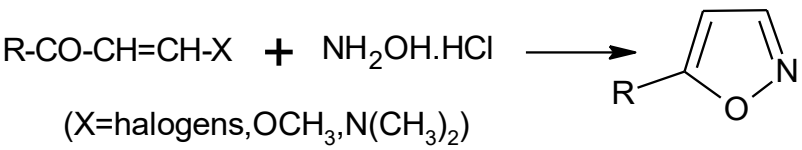
Scheme I

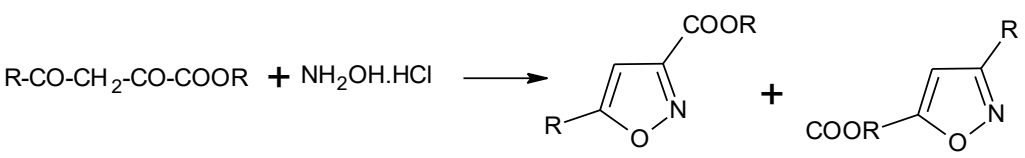


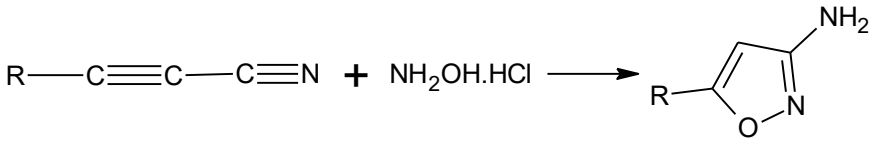
Enolization in β-diketones



This reaction has been extended to involve the reaction of hydroxylamine hydrochloride with C-C-C system of varying functional groups.







REACTIONS OF ISOXAZOLE

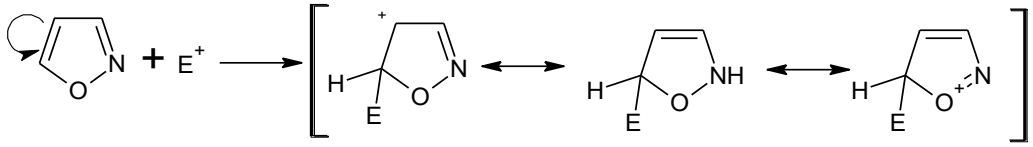
Reactivity

Isoxazole contains furan-type oxygen and pyridine-type nitrogen at the positions- and -2, it is therefore considered to exhibit characteristic reactions of furan and pyridine. But isoxazole undergoes electrophilic substitutions more readily than pyridine and less readily than furan because of combined effect of both the structural effects in isoxazole, (i) the electron-withdrawing effect of the pyridine-type nitrogen and (ii) the electron-releasing effect of the furan-type oxygen. As position-4 in isoxazole is with high electron density, electrophilic substitution, therefore, occurs at the position-4. The electron-releasing substitutents at the position-3 and/or C-5 exert activating effect, while the electron-withdrawing substitutents at C-3 and/or C-5 exert deactivating effect on the isoxazole nucleus at the position-4. The substitutent at the position-5 exerts activating or deactivating effect on the position-4 more strongly than the effect exerted by the substitutent if present at the position-3.

1. **Reaction with Electrophiles**

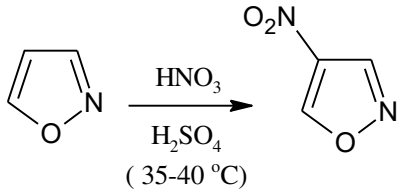
Electrophilic Attack at Carbon

Both the heteroatoms influence the electrophilic substitutions in isoxazole ring. The electron-withdrawinng nature of pyridine-type nitrogen retards the attack of electrophile, but the electron-releasing effect of the furan-type oxygen atom facilitates electrophilic attack in isoxazole nucleus.



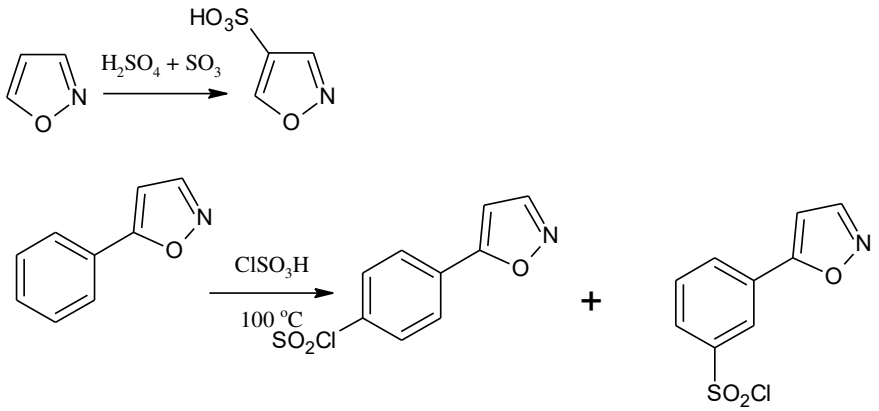
1. **Nitration**

Isoxazole is nitrated at the position-4 by the nitrating mixture of concentrated nitric and sulphuric acids under controlled conditions (35^0 C- 40°C).



2. Sulfonation

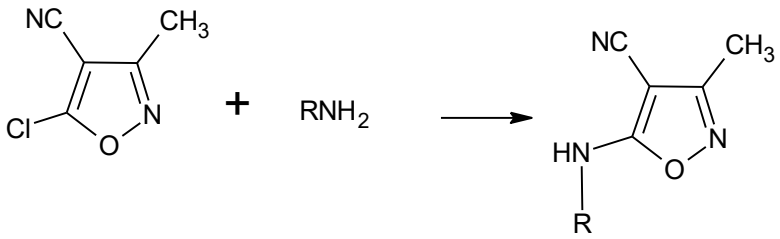
Isoxazole ring is resistant to sulfonation, however sulfonated with oleum under drastic conditions with the introduction of sulfonic acid group at the position-4. But 5-phenylisoxazole is sulfonated by chlorosulfonic acid with the sulfonation of only phenyl ring at the meta- and para- positions.



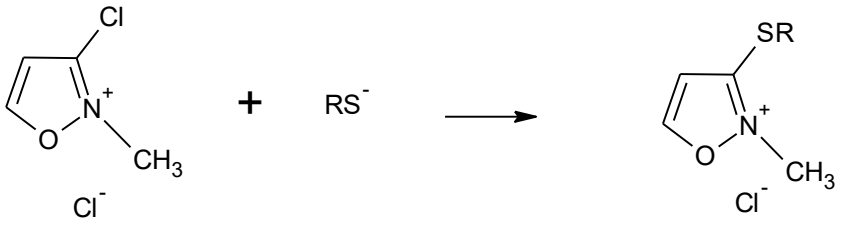
2. Reaction with Nucleophiles

a) Nucleophilic Displacement

Isoxazole with highest electron density at the position-4, it is therefore the preferred site for the electrophilic attack. The isoxazoles substituted with halogen atom at the position-4 will be less susceptible to nucleophilic substitution (SN^2) reactions. However, the halogen atom at the position-5 can be replaced if position-4 is substituted with the suitable activating substitutent.

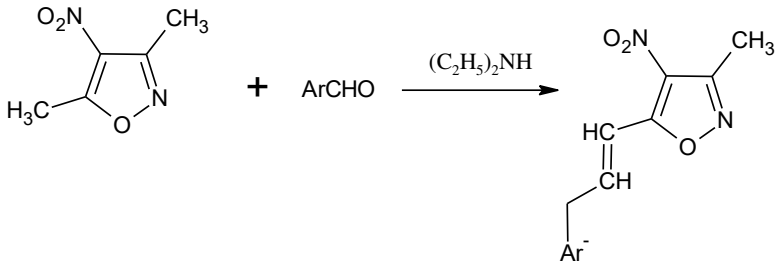


The halogen atoms at the 3- and 5- positons can be replaced by nucleophiles if activated by ring quaternization



b) Condensation Reactions

The methyl group at the position-5 will be more reactive than the methyl group at the position-3, if position-4 is substituted with an electron-withdrawing group. Thus, 5-methyl group with enhanced reactivity is easily condensed with aromatic aldehydes in the presence of diethylamine, but 3-methyl group remains intact.



4. PURPOSE AND PLAN OF WORK

PURPOSE OF THE STUDY

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the World. It is characterised by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an abosolute or relative lack of the hormone insulin. The number of people in the world with diabetes has increased dramatically over recent years. It is also predicted that by 2030, India, China, and the United States will have the largest number of people with diabetes. Currently treatment of diabetes, in addition to insulin supplement includes many oral hypoglycemic agents along with appropriate diet and exercise. The treatment goal of diabetic patients is to maintain near normal levels of glycemic control, in both fasting and post-prandial conditions.

Postprandial hyperglycemia has been proposed as an independent risk factor for diabetes mellitus. Therefore, control of postprandial hyperglycemia is suggested to be important in the treatment of diabetes. One of the effective methods to control diabetes is to inhibit the activity of α-amylase enzyme which is responsible for the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose, and glucose). This is contributed by α-amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals. As per literature review, it comes to know that coumarin, chalcone and isoxazole moiety possess good important anti- diabetic activity especially inhibit the α-amylase enzyme. This study is focussed to investigate the inhibitory potentials of the synthesised isoxazole incorporated coumarin derivatives on α-amylase, the key enzyme responsible for carbohydrate hydrolysis.

Despite an optimal use of available antidiabetic drugs (ADDs), many patients fail to experience therapeutic efficacy and others do so only at the expense of significant failure in reduction of elevated blood sugar level and toxic side effects. The limitations with the conventional ADDs highlighted the need for developing newer antidiabetic agents with less toxic and more effective drugs are required. Isoxazole are five membered ring system containing sulphur and oxygen atom with substituted phenyl ring, received a much attention of medicinal chemists due to their

potential biological activities. Substituent's' at C-4, 2 position of isoxazole moiety results in potent α- amylase inhibitory activity. Prompted by these reports, we aimed to prepare the following series of novel isoxazole incorporated coumarin derivatives as potent α-amylase inhibiting agents.

PLAN OF WORK

The steps involved in the present study are:

Designing the scheme of lead molecules by literature review

↓

Identification of the target site

↓

Lead molecule optimization by calculating drug likeness score

↓

Performing Molecular docking for the lead molecules with target protein using AUTODOCK software

↓

Synthesis of analogs of lead molecules

↓

Spectral studies of synthesized compounds

↓

Evaluation of *in vitro* α-amylase inhibitory activity

5. EXPERIMENTAL SECTION

DRUG DESIGN APPROACH

DOCKING [91-92]

Docking involves the fitting of a molecule into the target structure in a variety of positions, conformations and orientations. Molecular docking is used to predict the structure of intermolecular complex formed between two molecules. The small molecule called ligand usually interacts with protein's binding sites. Binding sites are areas of protein known to be active in forming of compounds. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. It also predicts the strength of the binding, the energy of the complex; the types of signal produced and calculate the binding affinity between two molecules using scoring functions.

TYPES OF DOCKING

Lock and key or rigid docking- In lock and key docking, both the internal geometry of the receptor and ligand is kept fixed and docking was performed.

Induced fit or flexible docking- An enumeration on the rotations of one of the molecules (usually smaller one) is performed. For every rotation the surface cell occupancy and energy are calculated; later the most optimum pose was selected.

α-Amylase as a target enzyme [93]

Amylase is an enzyme produced by salivary glands that digests starch molecules to give a breakdown product such as maltose, which in turn cleaves into two molecules of glucose. To continue digestion of incoming starch, the pancreatic duct sends a heavy amount of pancreatic amylase to the duodenum. Amylase serves both endocrine and exocrine functions. Among the different types of amylase enzymes, one major category of enzyme is pancreatic α-amylase. These are basically calcium metalloenzymes due to which calcium is an important cofactor in performing functions such as digestion of starch. Carbohydrates when consumed firstly should break down into smaller fragments such as monosaccharides, which would further absorb in the body. α-Amylase engages the catalysing of α-(1,4)- D-

glycosidic linkages present in starch to hydrolyse them into smaller fragments and other polymers of glucose. By performing the act of inhibiting α-amylase enzyme, it helps in reducing hyperglycaemia, obesity and problems such as overweight conditions. Salivary alpha amylase was first named as ptyalin, an agent presents in saliva for disintegration of starch content in food. As α-amylase is an important target for the treatment of diabetes mellitus and development of new drugs, scientists are showing their great interest in this enzyme. Currently, Acarbose and Miglitol are widely used; however, there are some side-effects such as flatulence, diarrhoea, bloating and abdominal discomfort.

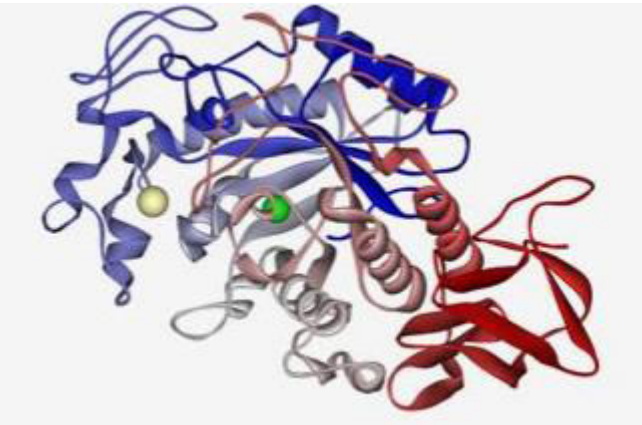


Fig.4: 3D Image of alpha amylase

IN SILICO STUDIES

Softwares and Databases used

- Accerlys discovery studio viewer
- Molinspiration server
- Accelrys accord for excel
- RCSB protein data bank
- Online SMILES translator
- Autodock 4.2 which combines
- Autodock tools
- Python molecule viewer 1.5.6
- Vision 1.5.6
- Cygwin 64

TARGET SELECTION

The present study was focused on Alpha amylase inhibitory assay. From the literature review and the current research on alpha amylase enzyme inhibitors, we have selected alpha amylase enzyme as the target for the present study. The pdb structure of Alpha amylase enzyme (1UA7) was downloaded from the RCSB protein data bank.

LEAD SELECTION

The lead Isoxazole incorporated derivatives of coumarin were selected based on several literature reviews.

TOXICITY STUDIES

Pharmacokinetic properties of the selected lead compounds were checked to ensure the safety and efficacy.

Toxicity studies are performed by two methods:

- Evaluation of drug likeness property
- Evaluation of ADME data

Evaluation of drug likeness properties [96]

For the better oral absorption of the ligands, the drug likeness scores are constructed by getting information about the solubility, diffusion, Log P, molecular weight etc. One of the ideal methods for this is using Lipinski's rule of five with the Molinspiration server.

Calculation of Lipinski's rule of five

1. Open the Molinspiration home page.
2. Click calculation of molecular properties and prediction of bioactivity.
3. Draw the structure of I_1 in JME window or paste the smile notation of the compound.
4. Then click calculate properties.

5. Save the properties.
6. JAVA program is required in the computer for the calculation of the properties.

Calculation of properties of the rest of the compounds is done in the same manner.

Table : 1 Drug likeness scores using Molinspiration

S.NO	Compound code	M Log p	Molecular weight	No. of H acceptors	No. of H donors	No. of violation
1	A_1	4.15	319.32	5	1	0
2	A_2	4.80	353.76	5	1	0
3	A_3	3.68	335.31	6	2	0
4	A_4	4.23	362.38	6	1	0
5	A_5	3.66	365.34	7	2	0
6	K_1	4.55	333,34	5	1	0
7	K_2	4.98	347.37	5	1	0
8	K_3	5.90	395.41	5	1	1
9	K_4	3.60	348.36	6	3	0
10	K_5	4.05	349.34	6	2	0
11	Acarbose	-5.51	645.61	19	14	3

In addition to ligand-protein complex modeling, in vivo absorption capabilities of the designed molecules were tentatively assessed by means of Lipinski's rule of five that predicts that a compound administered orally will more likely have a good absorption or permeation. All the compounds satisfy the rule which indicates that all the ligands $\mathbf{A_{1\text{-}5}}$ and $\mathbf{K_{1\text{-}5}}$ have good oral absorption.

DOCKING STUDIES FOR THE LEAD

Aim **:** To predict the bioactivity score of the ligands.

Database **:** RCSB protein data bank

Protein selected **:** Alpha amylase enzyme (1UA7)

Target proteins were downloaded from RCSB protein data bank and docking studies were performed.

Steps involved in docking studies [34, 94, 95]

- Docking process is done with AutoDock 4.2
- Conversion of refined enzyme into pdb format
- Conversion of pdb format of ligand into pdbqt format
- Preparation of grid box by setting grid parameters
- Docking process by setting docking parameters
- Saving the docked result as dlg file
- Viewing the docked conformation
- Taking snapshots of the interactions

STEP I:

Protein structure refinement

Alpha amylase enzyme was downloaded from RCSB Protein Data Bank (PDB) and the enzyme was refined before docking. The steps involved are:

- Open Accelrys discovery studio viewer.
- File → Open →Select the enzyme file downloaded from → RCSB PDB.
- Click View option and then click Hierarchy.
- Click water molecules.
- Click water molecule → select all water molecules → cut.
- Select ligand, which is unnecessary and cut.
- Save the molecule in a desired location

STEP II:

Ligand file format conversion

- The ligands which are desired are drawn in ChemSketch software.
- Tools→Click Generate → Click SMILES notation (Simplified Molecular Input Line Entry System, which is a file format).
- Save the SMILES in a word document.
- Open the online smiles translator cactus. nci.nih.gov/services/ translate
- Upload the SMILES.
- By choosing the required file format and save the file in a pdb format (e.g.:ligand.pdb).

Online smiles translator allows the user to convert SMILES format into PDB, MOL, SDF and smile text file format. Thus, the selected ligand molecule of canonical smile format was converted to pdb format.

The protein and ligand files which are prepared by above said procedures are taken for docking.

STEP III

- Docking with autodock 4.2
- Docking calculation in AutoDock was performed using the refined protein and the desired ligand in pdb format.

Preparation and running a docking programme

Preparing the protein

- Open autodock 4.2
- Open file → Click read molecule → Choose the particular → refined enzyme file.
- The elimination of the water is carried by the following steps.
- Press Select option
- Click Select → click select from string option
- Then write "*HOH*" in the Residue line & "*" in the atom line.

- Click Add → No new selection and then dismiss.
- Addition of hydrogens is done by,
- Press Edit option
- Click the Hydrogen
- Then click Add
- Choose all Hydrogen, No Bond Order, and 'yes' to renumbering click Ok.
- Next click → Edit option → click add the Kollmann Charges.
- Then save the enzyme molecule as 1ea1refined.pdb
- Select Edit →Delete → Delete all molecule

Preparing the ligand

- Confirm that all the hydrogens are added in the ligand.
- Toggle the Auto Dock Tools button.
- Open the Ligand → Click Input and choose the suitable ligand →file and finally open.
- The torsions are designed by following steps
- In the Ligand option select Torsion Tree
- Select Detect Root option
- Click Torsion Tree
- Then select the Choose Torsions option
- Amide bonds should NOT be active.
- After that click the Torsion Tree and select Set Number of Torsions
- Number of rotatable bonds is chosen.
- Finally Save the Ligand files by selecting the Output option (pdbqt file).
- Select Edit → Delete → Delete all molecule.
- Conversion of pdb files of protein into pdbqt file
- Select the Grid option and open the Macromolecule pdb file.
- Auto Dock adds the Charges and itself merges the Hydrogens.
- Save the object as pdbqt in desired area.
- AutoGrid Calculation and creating "gpf" file

- Open the grid and click Macromolecule option and choose the rigid protein then yes to preserve the existing charges.
- The Preparation of grid parameter file is carried out by,
- Open Grid
- Select the Set Map Types
- Choose Ligand
- Accept it.

Setting of grid properties,

- Open Grid
- Select the Grid box
- Set the proper Grid Dimensions (60.60.60)
- Adjust the Spacing
- Select the File and click Close Saving Current.
- Save the grid settings as gpf file in the input option (ligand.gpf).
- After running the grid file, the output automatically saves as 'glg' file

Auto Dock calculation and creating 'dpf' file:

- The rigid molecule specification is carried out by,
- Select the Docking option
- Click the Macromolecule
- Set Rigid File Name.
- The ligand specification is carried out by,
- Click the Docking option
- Select the ligand
- And then Accept it.
- In the next step, click Docking option and select Search Parameters in that click Genetic Algorithm and finally accept it.
- ClickDocking options → Select Docking Parameters → Choose the Defaults.
- Click Docking option→Select Output and adds Lamarckian Genetic algorithm (LGA).

- Save the docked settings as 'dpf' file in the input option (ligand.dpf)
- After running the docked file, the output automatically saves as 'dlg' file.

Programming of 'Auto Grid' and 'Auto Dock'execution:

1.Open Cygwin and typed as follows

- cd c:
- cd cygwin
- cd usr
- cd local
- cd bin

Program should list out the pdb, pdbqt, gpf and dpf files of an enzyme and ligand molecule.

2. Then type as: **./autogrid4.exe <space> -p <space>ligand.gpf –l <space>ligand.glg**

If a ligand gets into the spacing of the grid, then the execution of this command will be;

'Successful completion'.

3. Then type as: **./autodock4.exe<space> -p<space>ligand.dpf –l<space>ligang.dlg**

If the ligand binds to the amino acids through 10 different conformations, then the execution of this command will be;

'Successful completion'.

STEP IV

Viewing docking results

Reading the docking log file .dlg

- Toggle the AutoDock Tools button
- Click Analyze and Open Dockings.

- In the next step, click Analyze option and Conformations then Load.
- Double click on the conformation for to view it.

Visualizing docked conformations

- Click Analyze and Dockings then play.
- Load dlg file
- Choose the suitable conformations
- In the next step, click Analyze and Docking then Show Interactions.

Obtaining snap shots of docked pose

- Open the File and Read the Molecule
- Open Analyze → Click Dockings and Open dlg file
- Open Analyze → Click Macromolecule and Choose pdbqt file.
- Open Analyze → Click Conformations and Load
- Double click the desired conformation
- Click Analyze and Docking then Show Interactions.

Proteins and ligand interaction will be displayed. Zoom it and increase the contrast by holding right key and ctrl.

- Open File → Save image → cygwin/usr/local/bin as .png
- The above-mentioned steps involved in docking are done for all the 10 ligands.

This series include Isoxazole incorporated coumarin compounds with different Aromatic aldehydes **(A_1, A_3, A_4)** and Aromatic Ketones **(K_1, K_3, K_5)**

RESULT AND DISCUSSION

The new scheme have designed based on the review of literature. From that scheme, ten compounds were considered as ligand which undergo docking studies. The α-amylase enzyme was selected as the target protein obtained from the RCSB(Research Collaboratory for Structural Bioinformatics) protein data bank. Acarbose used as standard substance to inhibit the α-amylase enzyme. By Comparing the binding energies of all the ten compounds, six compounds shown similar binding affinity when compared with standard.

The docking results of α-amylase enzyme (1UA7.pdb) with the ligands A_{1-5}, K_{1-5} and Standard drug Acarbose are reported in the below table. The docked structures should have the similar binding energy compared with the standard. The binding sites are shown in the snapshots and the binding energy compared with the standard drug is given in the table

Table :2 Binding energies of all compounds with standard Acarbose

Code	**Binding Energy (Kcal/mol)**	**Code**	**Binding Energy (Kcal/mol)**
A1	**-23.25**	**K1**	**-25.44**
A2	-12.35	K2	-5.04
A3	**-21.56**	**K3**	**-23.27**
A4	**-19.66**	K4	-15.79
A5	-9.02	**K5**	**-19.22**
Acarbose		**-26.77**	

The ligands A_1, A_3, A_4 and K_1, K_3, K_5 shown good docked pose to enzyme (IUA7) when compared to the standard.These ligands can be screened for the activity. Though some ligands showed inferior binding interactions, they are not selected for

synthesis. The selected derivatives (A_1, A_3, A_4 and K_1, K_3, K_5) were planned to synthesize and to screen for the α-amylase Inhibitory activity.

Binding of Acarbose with α-amylase enzyme

Acarbose interacts with α-amylase enzyme at ASP212A, YS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A.Binding energy was found to be **-26.77 kcal/mol.**

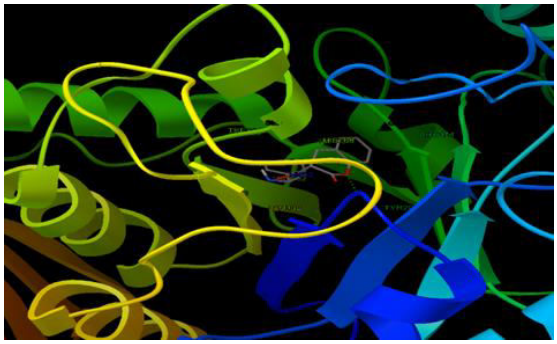


Figure 5. Snapshot of Acarbose binding with 1UA7

Binding of substituted isoxazole derivatives using aromatic aldehydes with α-amylase enzyme

A_1-A_5 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Snapshots of A1-A5 binding with 1UA7 were given in Figure 6- Figure 10.

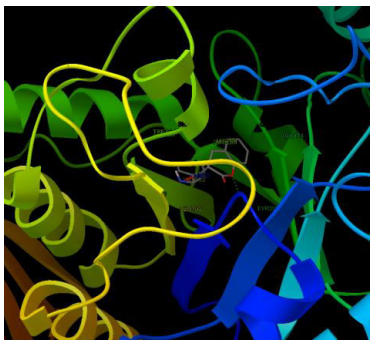


Figure 6. A_1 binding with 1UA7

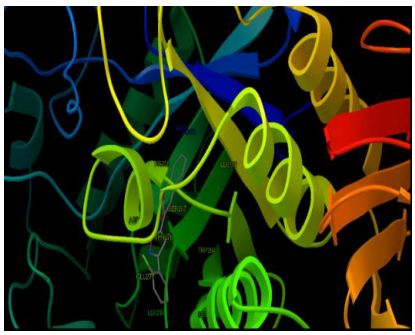


Figure 7. A_2 binding with 1UA7

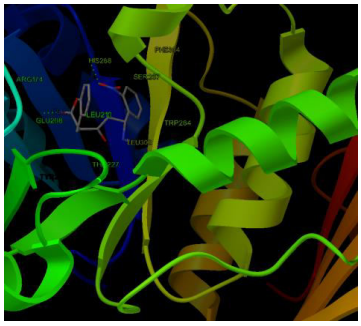


Figure 8. A_3 binding with 1UA7

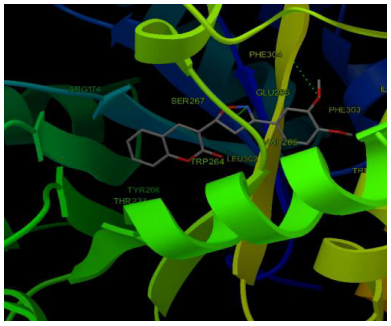


Figure 9. A_4 binding with 1UA7

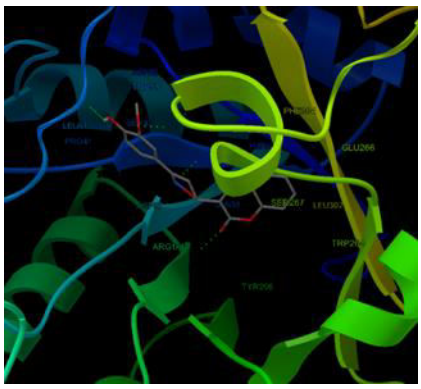


Figure 10. A_5 binding with 1UA7

Binding of Substituted isoxazole derivatives using aromatic ketones with α-amylase enzyme

K_1-K_5 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Snapshots of A1-A5 binding with 1UA7 were given in Figure 11- Figure 15.

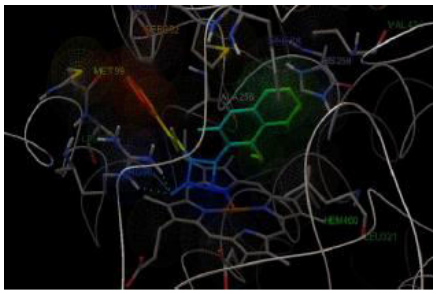


Figure 11. K_1 binding with 1UA7

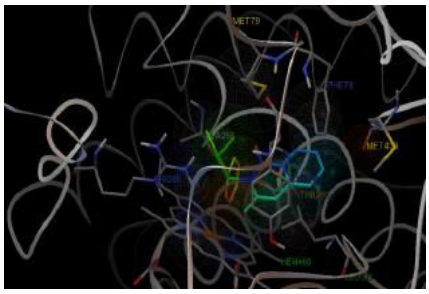


Figure 12. K_2 binding with 1UA7

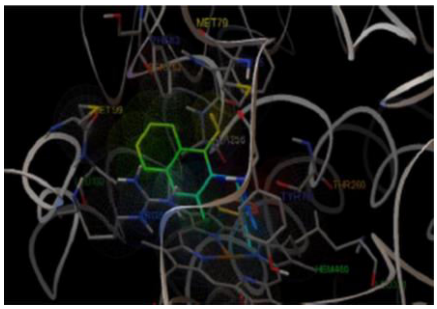


Figure 13. K_3 binding with 1UA7

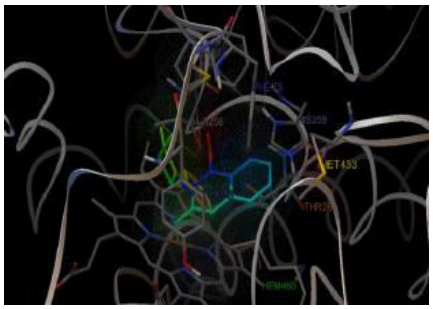


Figure 14. K_4 binding with 1UA7

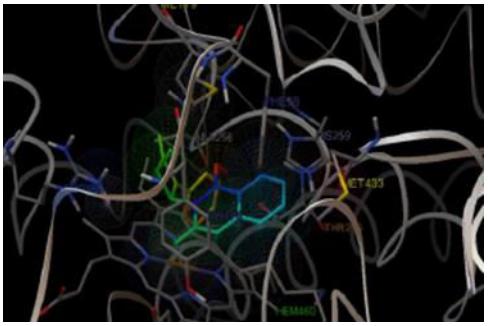


Figure 15. K_5 binding with 1UA7

SYNTHESIS

MATERIALS AND METHODS

Softwares

The Chemsketch tool (ACD Chemsketch 12 (i) (www. acdlabs.com/resources/freeware/chemsketch/) was used to draw molecules. The structures of the molecules were converted into a suitable f ile format (SMILES, mol and .pdb) Multiple tools and online serves such as Molinspiration (http://www.molinspiration.com//cgi-bin/properties)

Chemical and Reagents used

7-hydroxy-4 methyl coumarin, zinc chloride, glacial acetic acid, aromatic aldehyde [benzaldehyde, o-Hydroxy benzaldehyde, p-dimethylamino benzaldehyde,], aromatic ketone [acetophenone, p-hydroxy acetophenone, benzophenone], rectified spirit (ethanol), sodium hydroxide, hydroxylamine HCl, sodium acetate.

Apparatus used

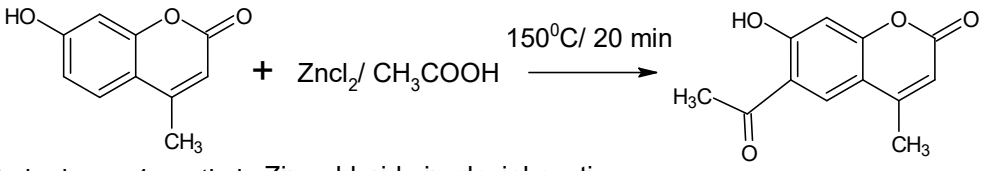
Beakers, conical flask, round bottom flask, test tubes, pipettes, glass rods, funnels, watch glass, magnetic stirrer and TLC plates.

Analytical work

- Melting points were determined taken in open capillaries on thomas hoover melting point apparatus and are uncorrected.
- Reactions were monitored by thin layer chromatography (TLC) on a precoated silica gel G plates using Iodine vapour as visualising agent.
- IR spectra is recorded on SHIMADZU FT\IR-140 spectrophotometer by KBr pellet technique in the department of pharmaceutical analysis, Nandha college of pharmacy, Erode.
- NMR spectra were recorded on BRUKER ULTRA SHIELDED NMR-400 MHz at VIT UNIVERSITY, Chennai.
- MASS spectra were recorded on HRMS at VIT UNIVERSITY, Chennai.

SCHEME I

Step-1:

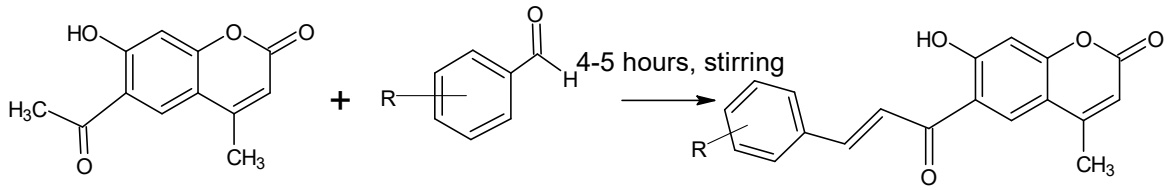


7 - hydroxy- 4- methyl Coumarin

Zinc chloride in glacial acetic acid

6-acetyl-7-hydroxy-4-methyl coumarin

Step-2:

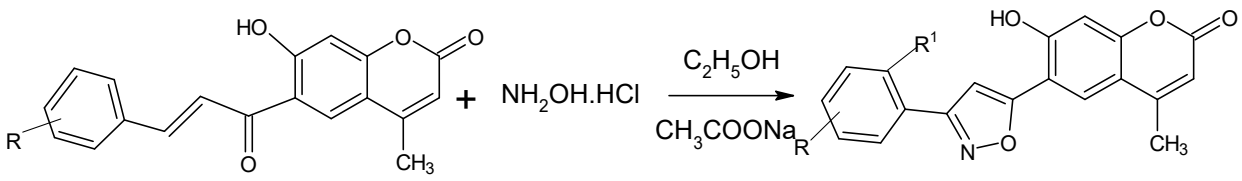


6-acetyl-7-hydroxy-4-methyl coumarin

Aromatic aldehyde

Chalcone

Step- 3:



Chalcone

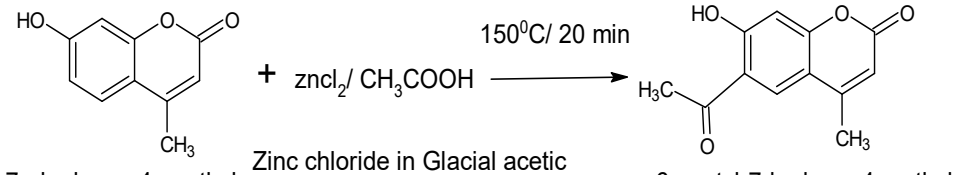
Hydroxylamine hydrochloride

A_1, A_3, A_4

COMPOUND CODE	R	R_1
A_1	**H**	**H**
A_2	4 - Cl	H
A_3	**2 - OH**	**H**
A_4	**4 - $N(CH_3)_2$**	**H**
A_5	OH	OCH_3

SCHEME II

Step-1:

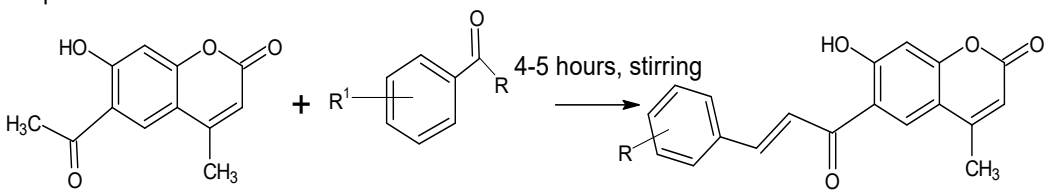


7 - hydroxy- 4- methyl coumarin

Zinc chloride in Glacial acetic acid

6-acetyl-7-hydroxy-4-methyl coumarin

Step-2:

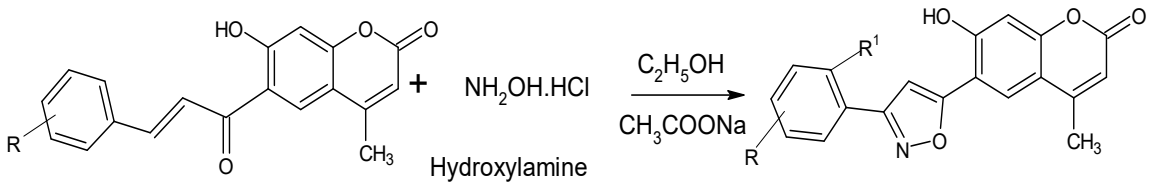


6-acetyl-7-hydroxy-4-methyl coumarin

Aromatic Ketone

Chalcone

Step-3:



Chalcone

Hydroxylamine hydrochloride

K_1, K_3, K_5

COMPOUND CODE	R	R_1
K_1	**H**	**CH_3**
K_2	CH_3	CH_3
K_3	**H**	**C_6H_5**
K_4	CH_3	NH_2
K_5	**OH**	**CH_3**

General Procedure

Step 1: Synthesis of 6-acetyl-7-hydroxy-4-methylcoumarin[97]

Powdered zinc chloride (13g) was dissolved in glacial acetic acid (12ml) by heating in beaker on a sand bath. Dry compound 7-hydroxy-4-methyl coumarin (10g) was added with stirring to the mixture at 1400^0C. Then the mixture is poured in crushed ice to obtained the 6-acetyl-7-hydroxy-4-methylcoumarin which was dried and recrystallized with ethanol.

Step 2: Synthesis of chalcone [97]

Equimolar quantities of substituted various aromatic aldehydes and 3-acetyl coumarin was taken in 250ml beaker, which was dissolved in 10ml of rectified spirit (ethanol). This mixture was stirred using mechanical stirrer at 20°-25°C. While stirring NaOH (40%) was added dropwise to this mixture for 30mins, the solution becomes turbid, and continued stirring for 4-5 hours by maintaining the temperature. After stirring has completed the reaction mixture was neutralised by using 0.2 N HCl, where by the precipitate occurs. It was filtered and the crude chalcone was dried in air and recrystallised with ethanol.

Step 3: Synthesis of Isoxazoles [59]

A mixture of chalcones (0.015 mole), hydroxylamine hydrochloride (0.015 mole) and sodium acetate (0.015mole) in 25ml of ethanol was refluxed for 6 hours, the mixture was concentrated and poured on 100gm of crushed ice. The residue thus obtained was filtered and recrystallised with methanol.

Figure.16: During Refluxing

PHYSICAL CHARACTERISATION DATA

1) Substituted Isoxazoles using aromatic aldehydes

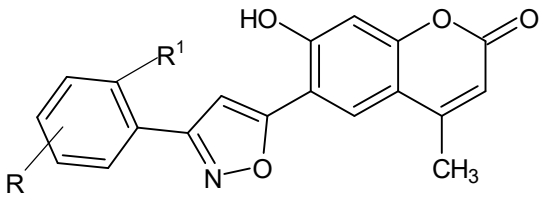


Table 3: Physical characterization of substituted Isoxazoles usimg aromatic aldehydes

Compound Code	R	R_1	Molecular formula	Molecular weight (g/mol)	% yield	Melting point	Rf value
A_1	H	H	$C_{19}H_{13}NO_4$	319.32	1.37%	175 - 176^{0}C	0.51
A_3	OH	H	$C_{19}H_{13}NO_5$	335.31	1.43%	165 - 167^{0}C	0.42
A_4	$N(CH_3)_2$	H	$C_{21}H_{18}N_2O_4$	362.37	5.30%	169 - 172^{0}C	0.37

Recrystallisation **:** Ethanol

Solvent system **:** Ethylacetate in hexane (1:10)

Visualizing agent **:** Iodine vapour

PHYSICAL CHARACTERISATION DATA

2) Substituted Isoxazoles using aromatic ketones

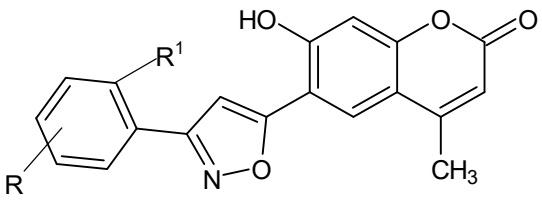


Table 4: Physical characterization of substituted Isoxazoles using aromatic ketones

Compound Code	R	R_1	Molecular formula	Molecular weight (g/mol)	% yield	Melting point	Rf value
K_1	H	CH_3	$C_{20}H_{15}NO_4$	333.33	65.57%	135 - 140^0 C	0.45
K_3	H	C_6H_5	$C_{25}H_{17}NO_4$	395.41	71.82%	155 - 160^0 C	0.32
K_5	OH	CH_3	$C_{20}H_{15}NO_5$	349.34	62.50%	160- 162^0 C	0.53

Recrystallisation **:** Ethanol

Solvent system **:**Ethylacetate in hexane (1:10)

Visualizing agent **:** Iodine vapour

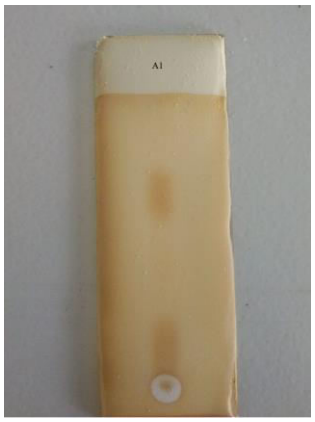
SPECTRA ANALYSIS

SPECTRAL ANALYSIS OF COMPOUNDS[117-119]

The structures of synthesized compounds were established on the basis of chemical datas IR, NMR and MASS spectra. The purity of all the compounds was established by single spot on TLC plates.

Compound code: A_1

Figure 17: Thin Layer Chromatography and Product image of A_1



Chemical name	7-hydroxy-4-methyl-6-(3-phenyl-1,2-oxazol-5-yl)-2*H*-1benzopyran-2-one
IR (KBr, v_{max} in cm^{-1})	3377.12(N-H),1694.35(coumarinylC=O), 1386.72(C=C), 1319.22(C-O), 1075.24(N-O), 3062.75(Aromatic C-H), 3334.69(Aromatic O-H)
^{1}H NMR spectral data (dmso-d_6, δ, ppm)	δ 1.241 (s, 1H isoxazole ring), δ 7.423 (s, 1H Ar-CH), δ 2.509 (s, 1H CH_3), δ 3.349(s, 1H NH)

Mass Spectral Data

Molecular weight of the compound: 321.24

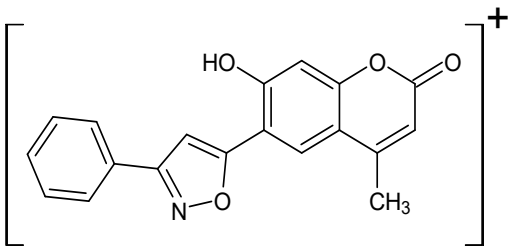
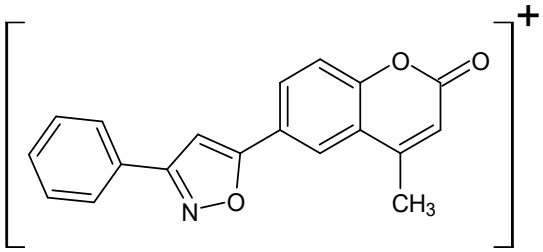
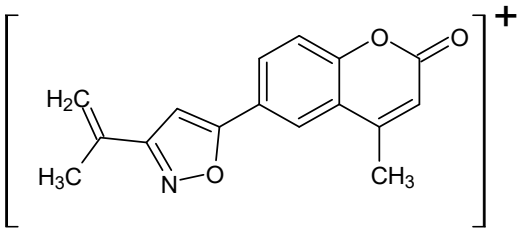
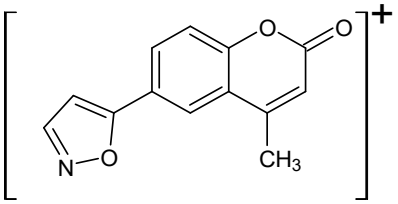
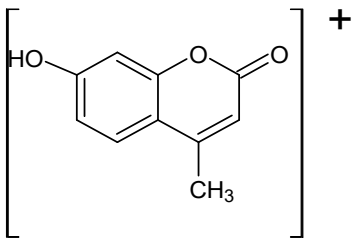
S/No	Fragments	m/z values
1		321.24
2		301.1419
3		265.08
4		227.12
5		174.12

Figure 18: IR Spectrum of A_1

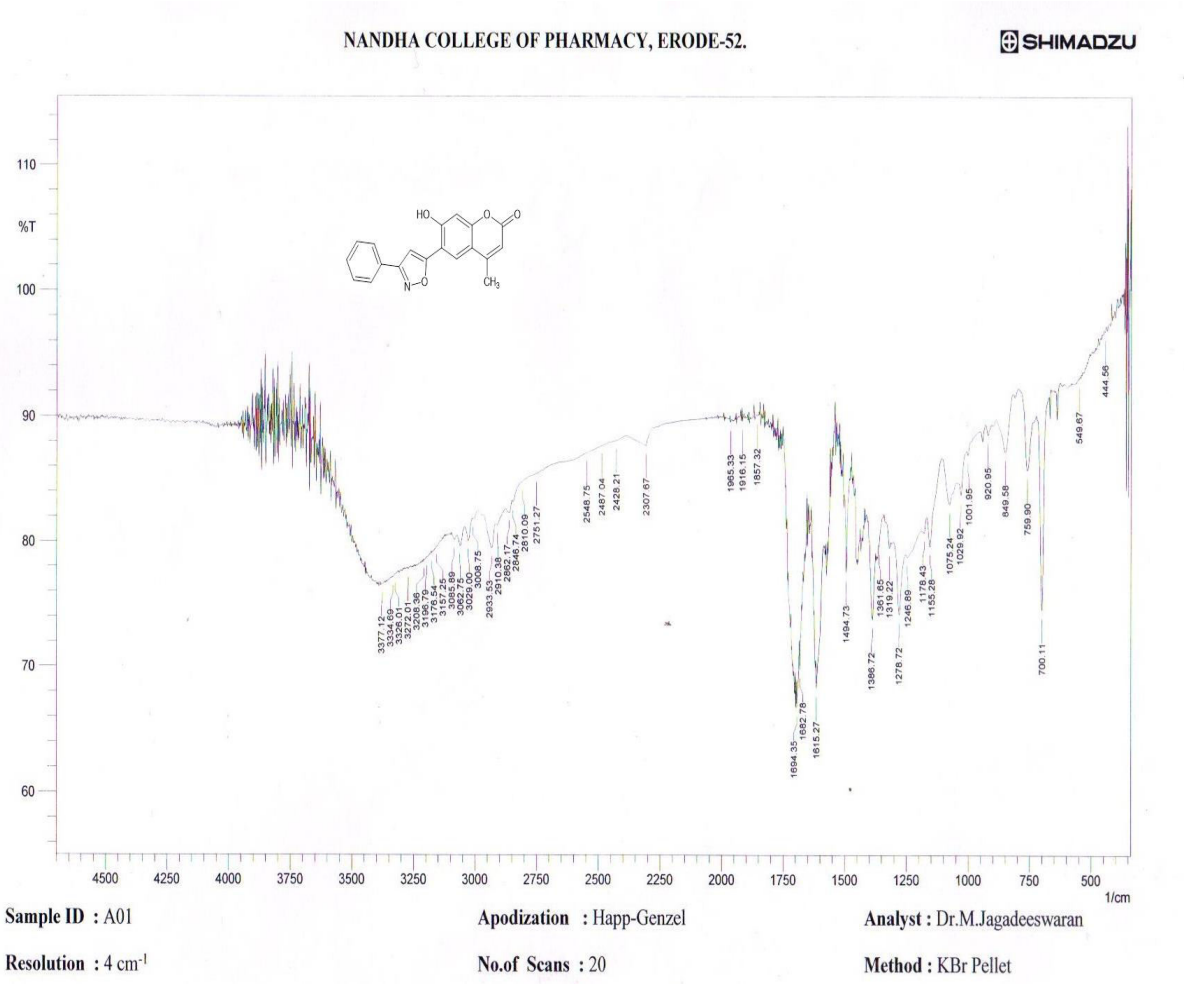


Figure 19: 1H NMR Spectrum of A_1

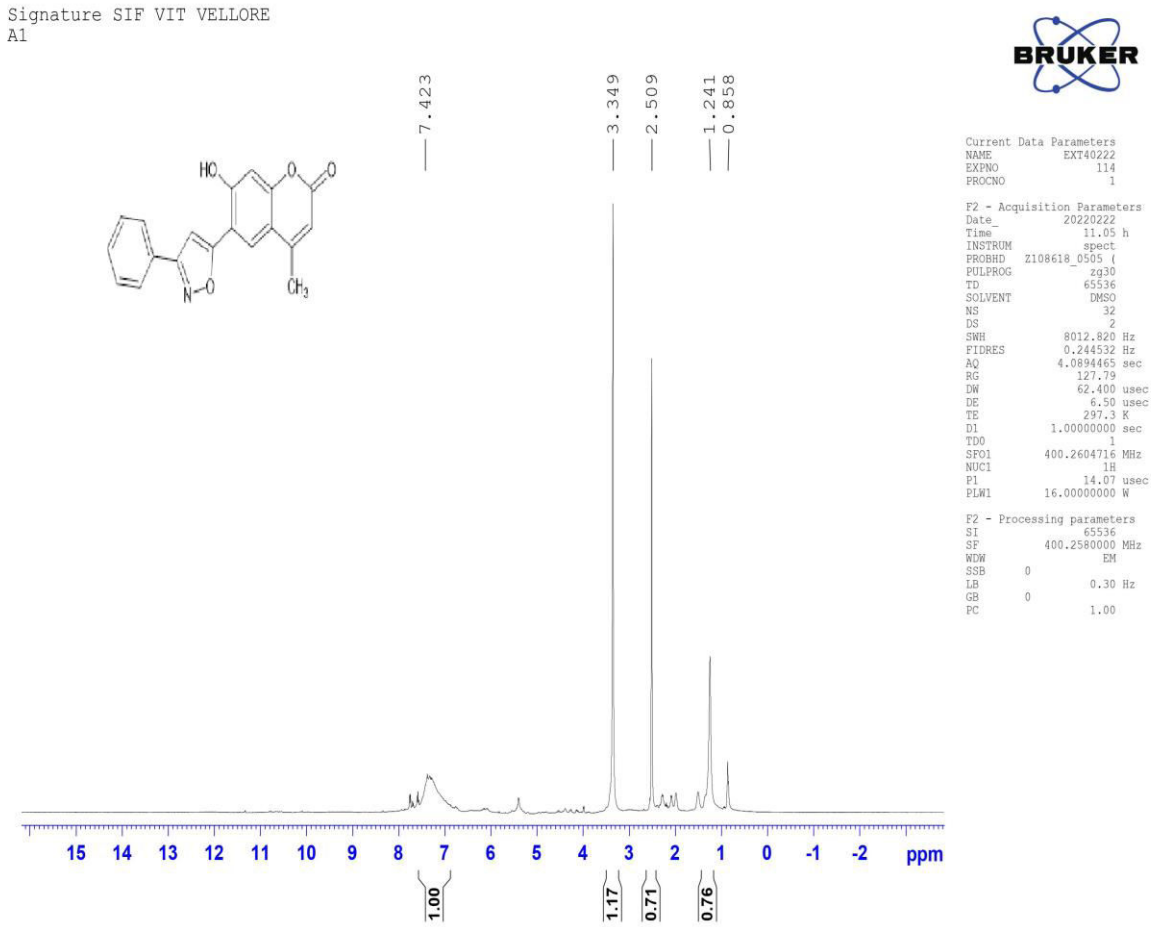
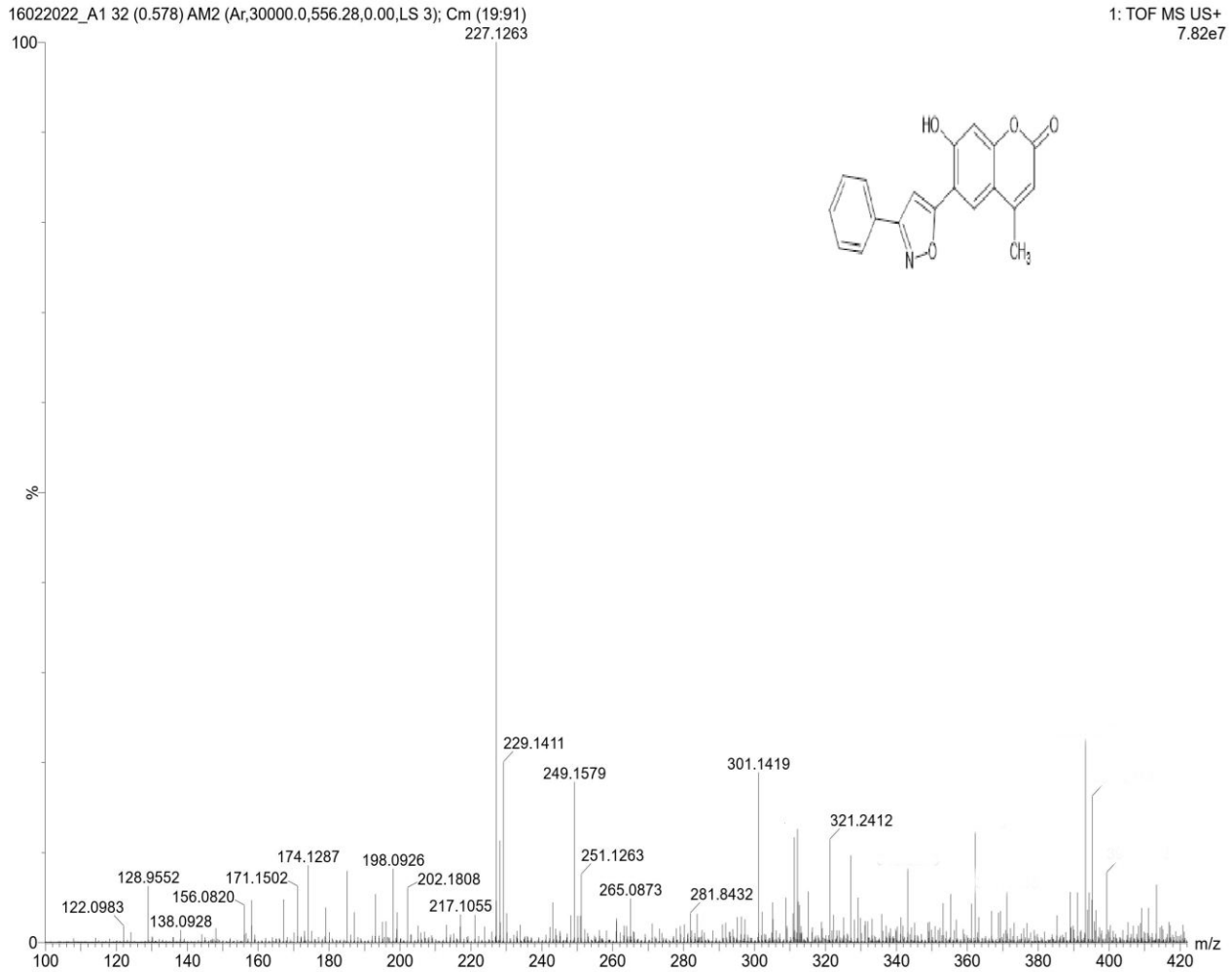


Figure 20: Mass Spectrum of A_1



Compound Code: A_3

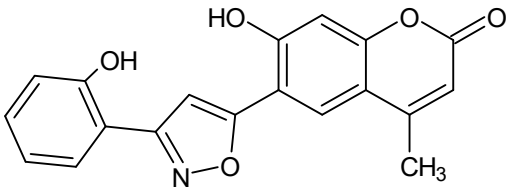


Figure 21: Thin Layer Chromatography and Product image of A_3

Chemical name	7-hydroxy-6-[3-(2-hydroxyphenyl)-1,2-oxazol-5-yl]-4-methyl-2*H*-1-benzopyran-2-one
IR (KBr, v_{max} in cm^{-1})	3440.77(N-H),3011.64(AromaticC-H), 1674.10 (coumarinyl C=O), 1394.44 (Aromatic C=C),1277.75 (C-O), 3461.02 (Aromatic O-H), 1076.21 (N-O)
^{1}H NMR spectral data (dmso-d_6, δ, ppm)	δ 1.225 (s, 1H isoxazole ring), δ 10.535 (s, 1H Ar-OH), δ 2.506 (s, 1H CH_3), δ 3.413(s, 1H NH), δ 6.108 (s, Ar-H), δ 6.697- 7.579 (m, 5H Ar-CH)

Mass Spectral Data

Molecular weight of the compound: 335.31

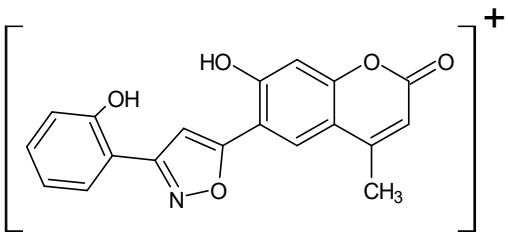
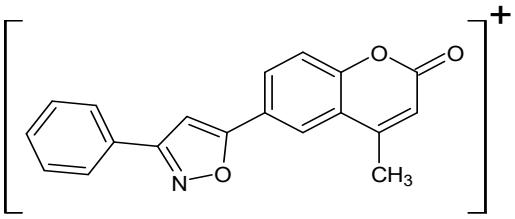
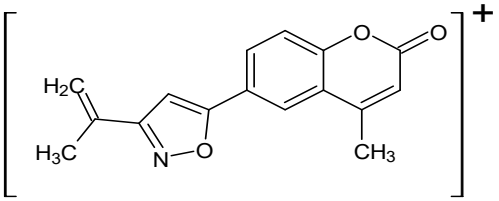
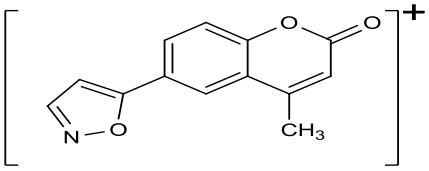
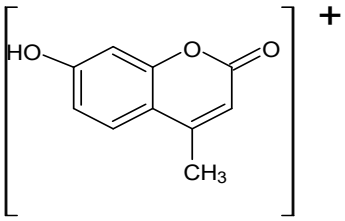
S/No	Fragments	m/z values
1		335.31
2		301.14
3		263.23
4		227.06
5		174.12

Figure 22: IR Spectrum of A_3

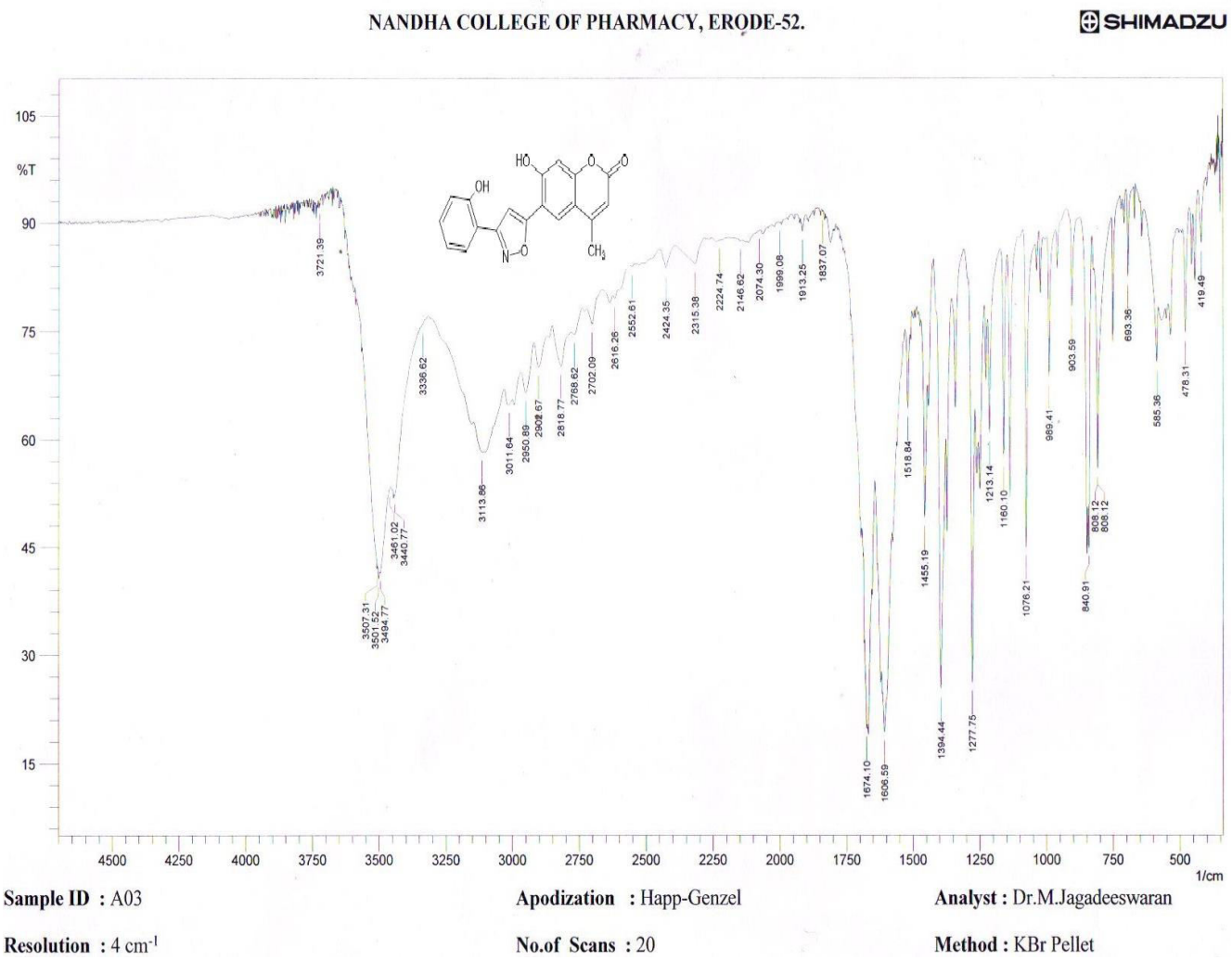


Figure 23: 1H NMR Spectrum of A_3

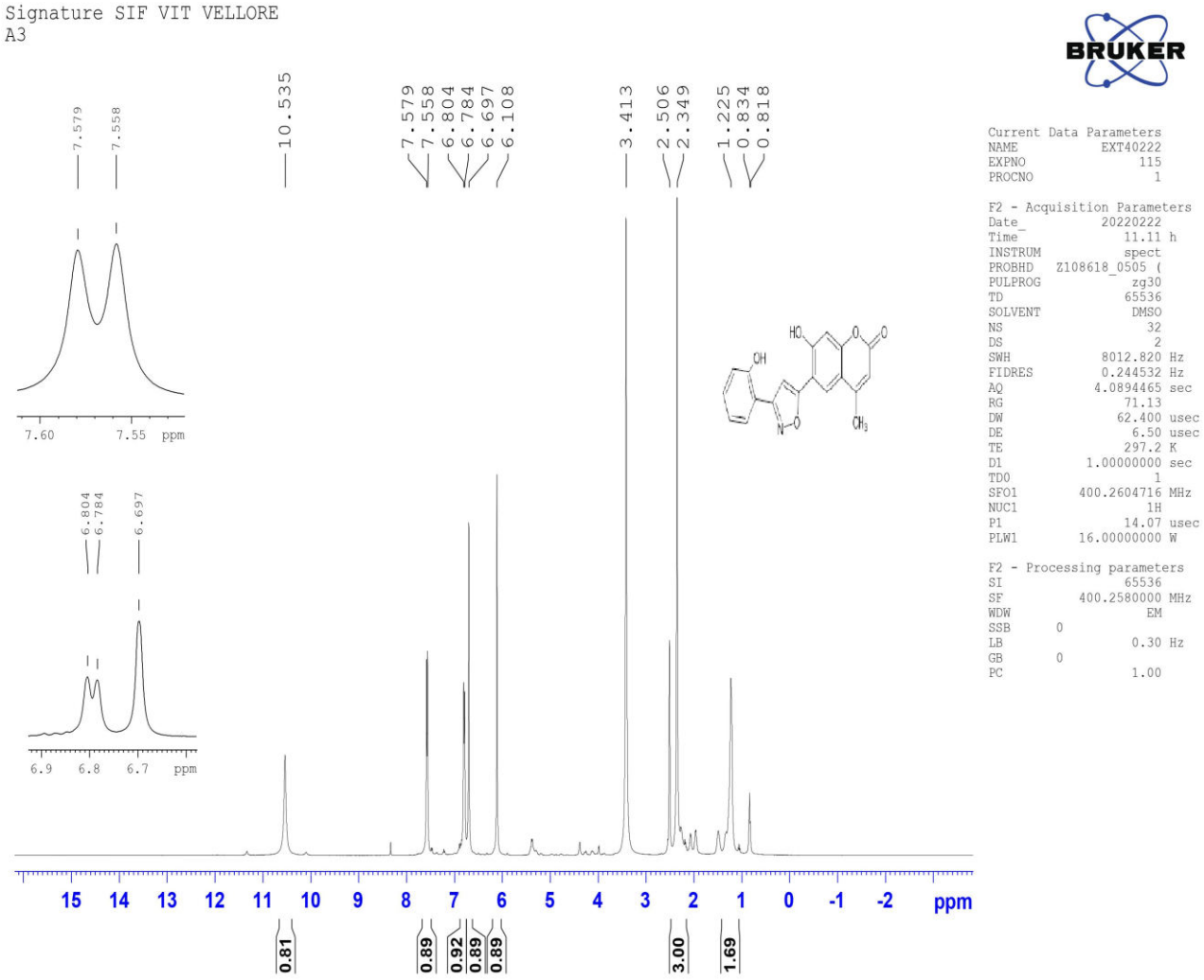
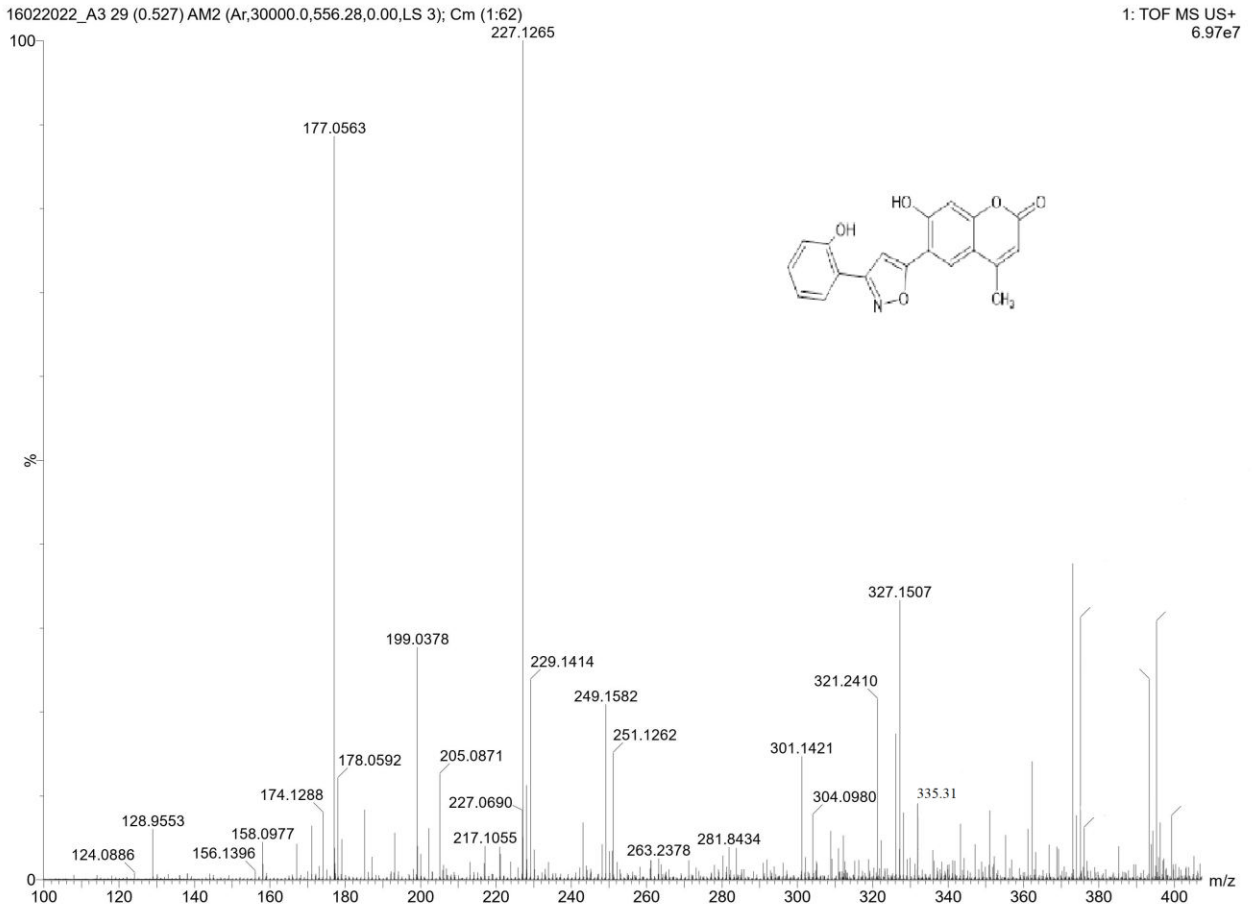


Figure 24: Mass Spectrum of A_3



Compound Code: A_4

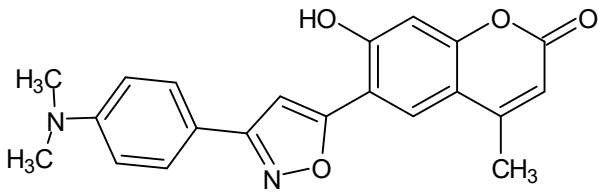


Figure 25: Thin Layer Chromatography of A_4

Chemical name	6-{3-[4-(dimethylamino)phenyl]-1,2-oxazol-5-yl}-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one
IR (KBr, v_{max} in cm^{-1})	3493.81(N-H), 1670.24 (coumarinyl C=O), 1277.75(C-O),1395.40 (C=C), 1075.24 (N-O),3015.50 (Aromatic C-H), 3444.63 (Aromatic O-H)
^{1}H NMR spectral data (dmso-d_6, δ, ppm)	δ 1.228 (s, 2H isoxazole ring), δ 10.526 (s, 1H Ar-OH), δ 2.498 (s, 4H CH_3), δ 3.355(s, 1H NH), δ 6.130(s, Ar-H), δ 6.704- 7.606 (s, 8H Ar-CH)

Mass Spectral Data

Molecular weight of the compound: 362.24

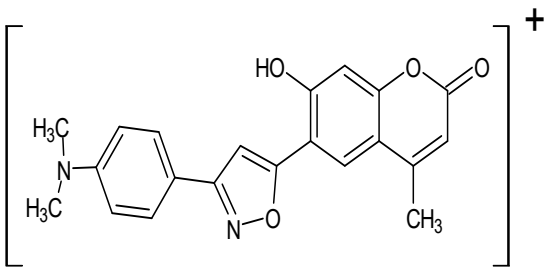
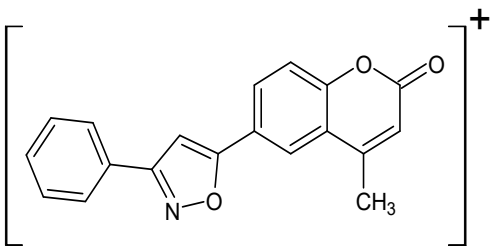
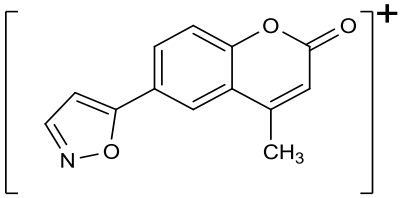
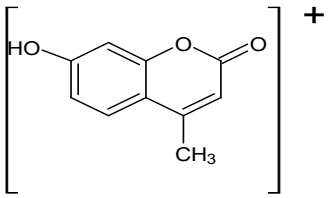
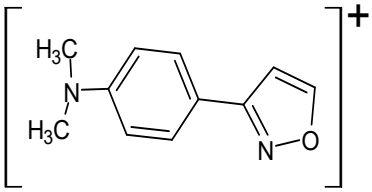
S/No	Fragments	m/z values
1		362.24
2		301.14
3		227.12
4		174.12
5		188.09

Figure 26: IR Spectrum of A_4

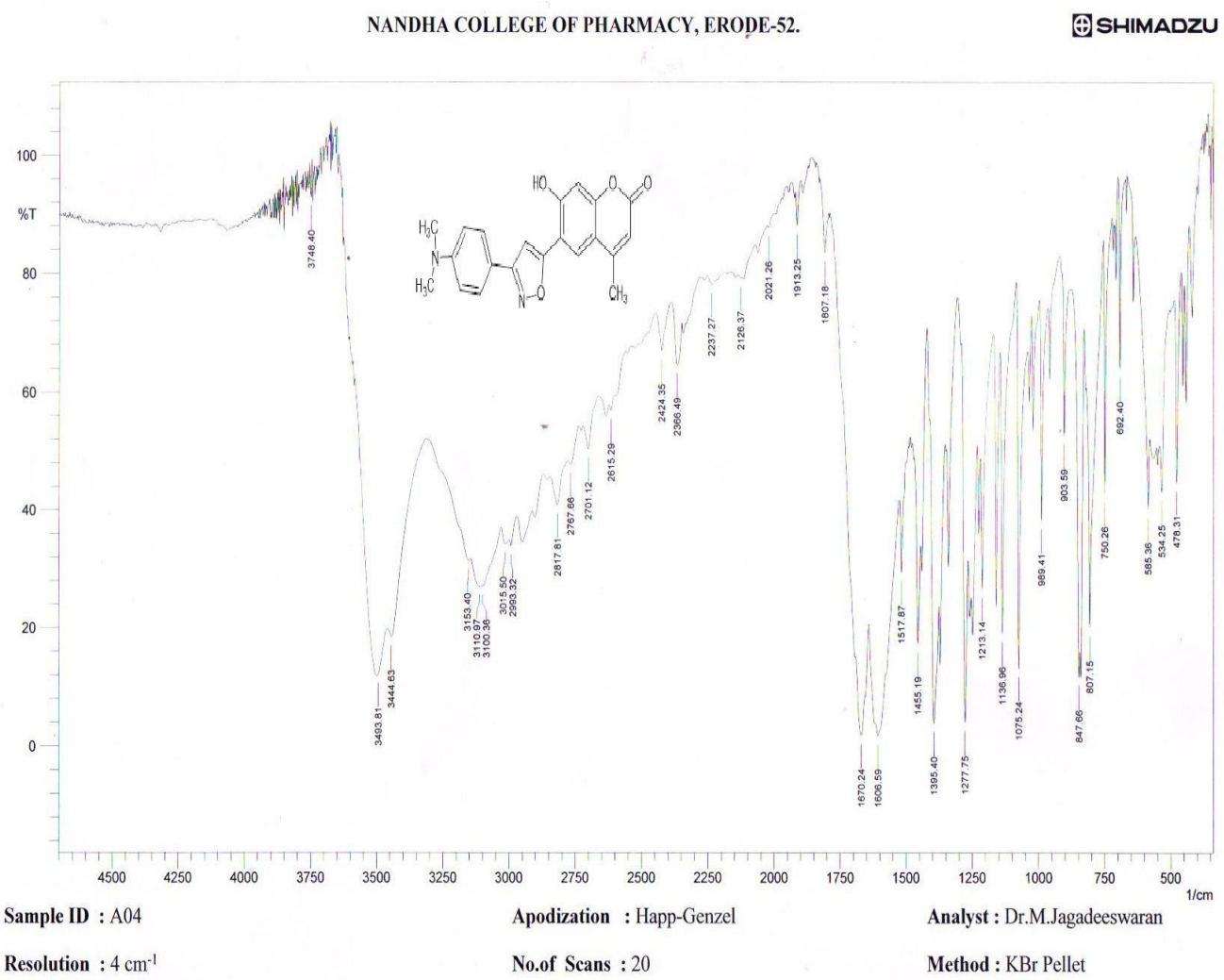


Figure 27: 1H NMR Spectrum of A_4

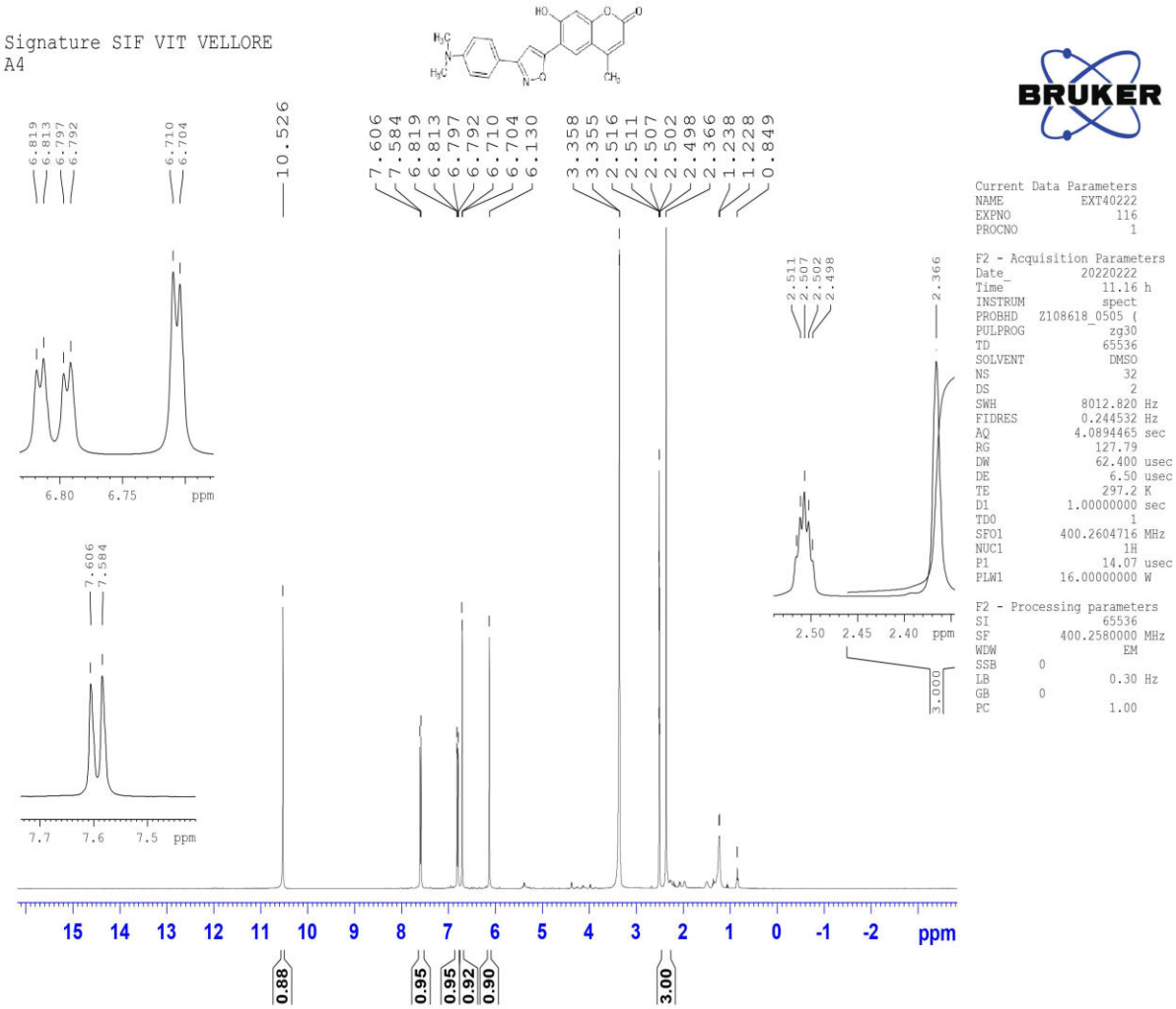
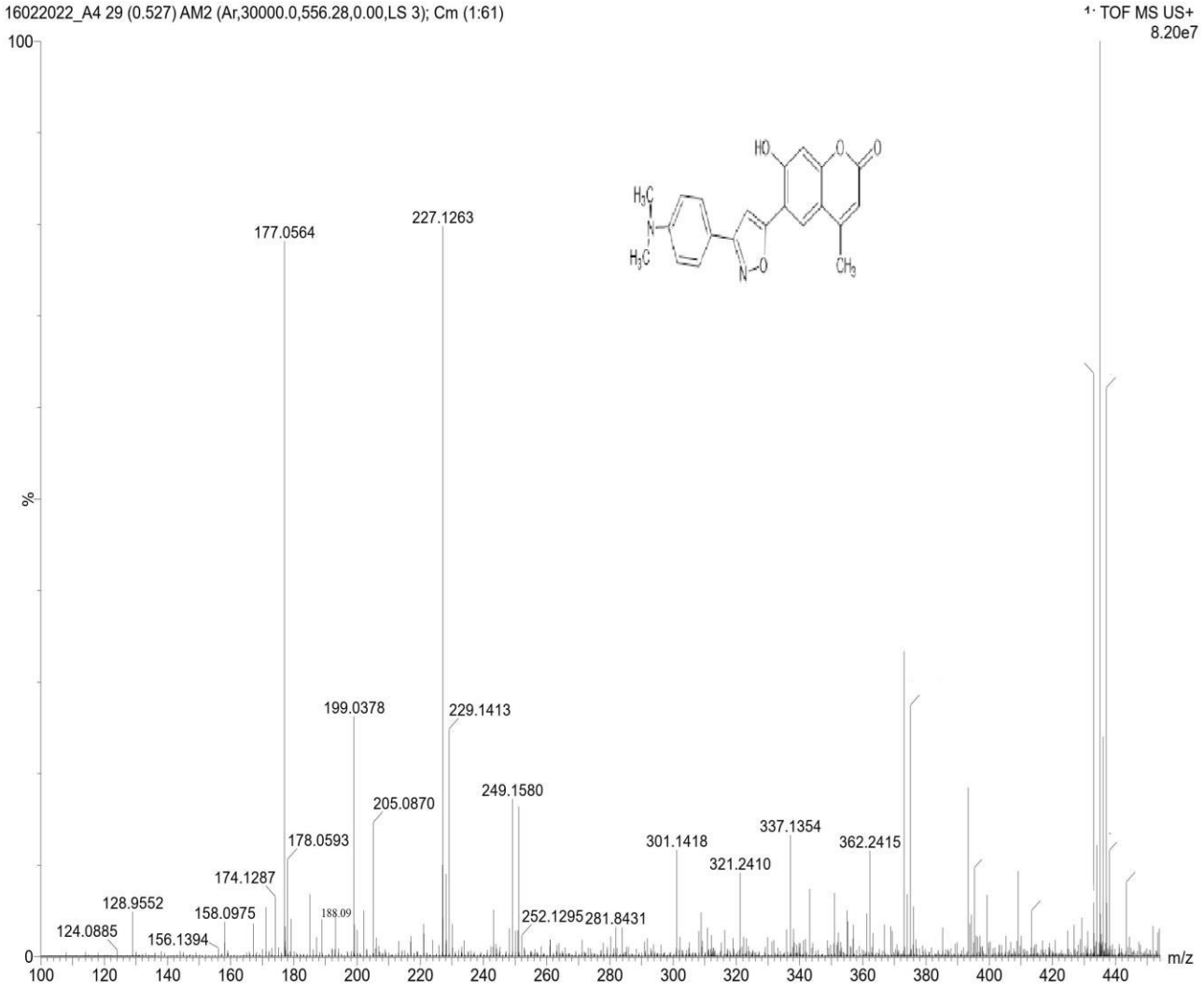


Figure 28: Mass Spectrum of A_4



Compound Code: K_1

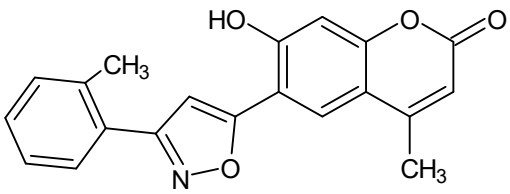
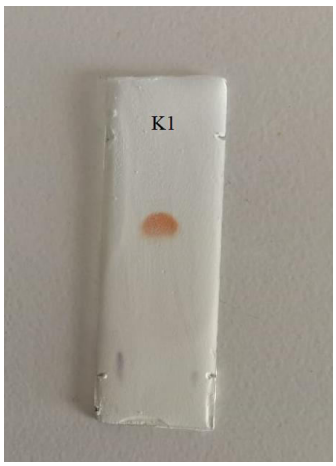


Figure 29: Thin Layer Chromatography and Product image of K_1



Chemical name	7-hydroxy-4-methyl-6-[3-(2-methylphenyl)-1,2-oxazol-5-yl]-2*H*-1-benzopyran-2-one
IR (KBr, v_{max} in cm^{-1})	1670.24 (coumarinyl C=O), 1248.82(C-N),3325.05(Aromatic O-H), 1277.75(C-O), 3010.67 (Aromatic C-H), 1394.44(C=C), 3444.63 (N-H), 1075.24 (N-O)
^{1}H NMR spectral data (dmso-d_6, δ, ppm)	δ 1.225 (s, 1H isoxazole ring), δ 10.528 (s, 1H Ar-OH), δ 2.509 (s, 1H CH_3), δ 3.422(s, 1H NH), δ 6.109 (s, Ar-H), δ 6.696 – 7.579 (s, 5H Ar-CH)

Mass Spectral Data

Molecular weight of the compound: 333.33

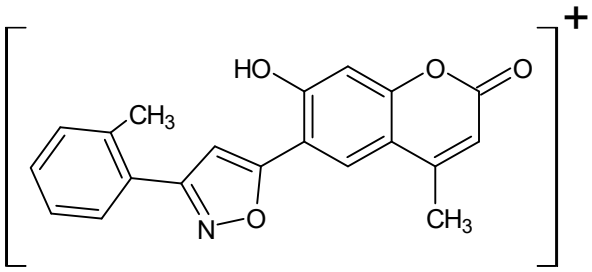
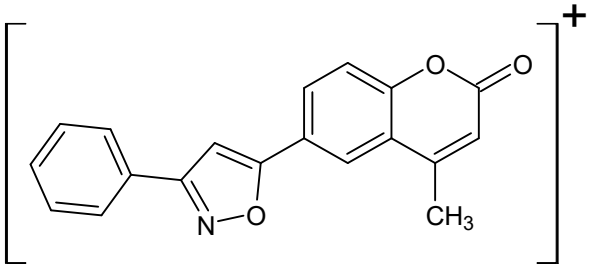
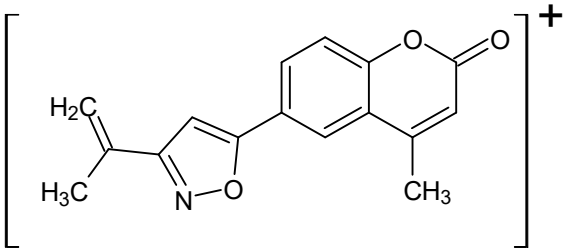
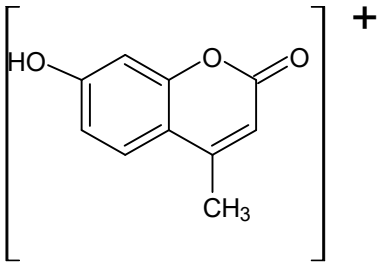
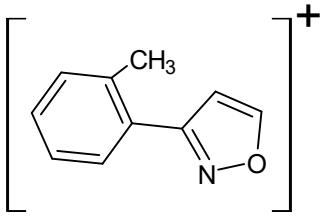
S/No	Fragments	m/z values
1		333.33
2		301.14
3		263.23
4		174.12
5		158.09

Figure 30: IR Spectrum of K_1

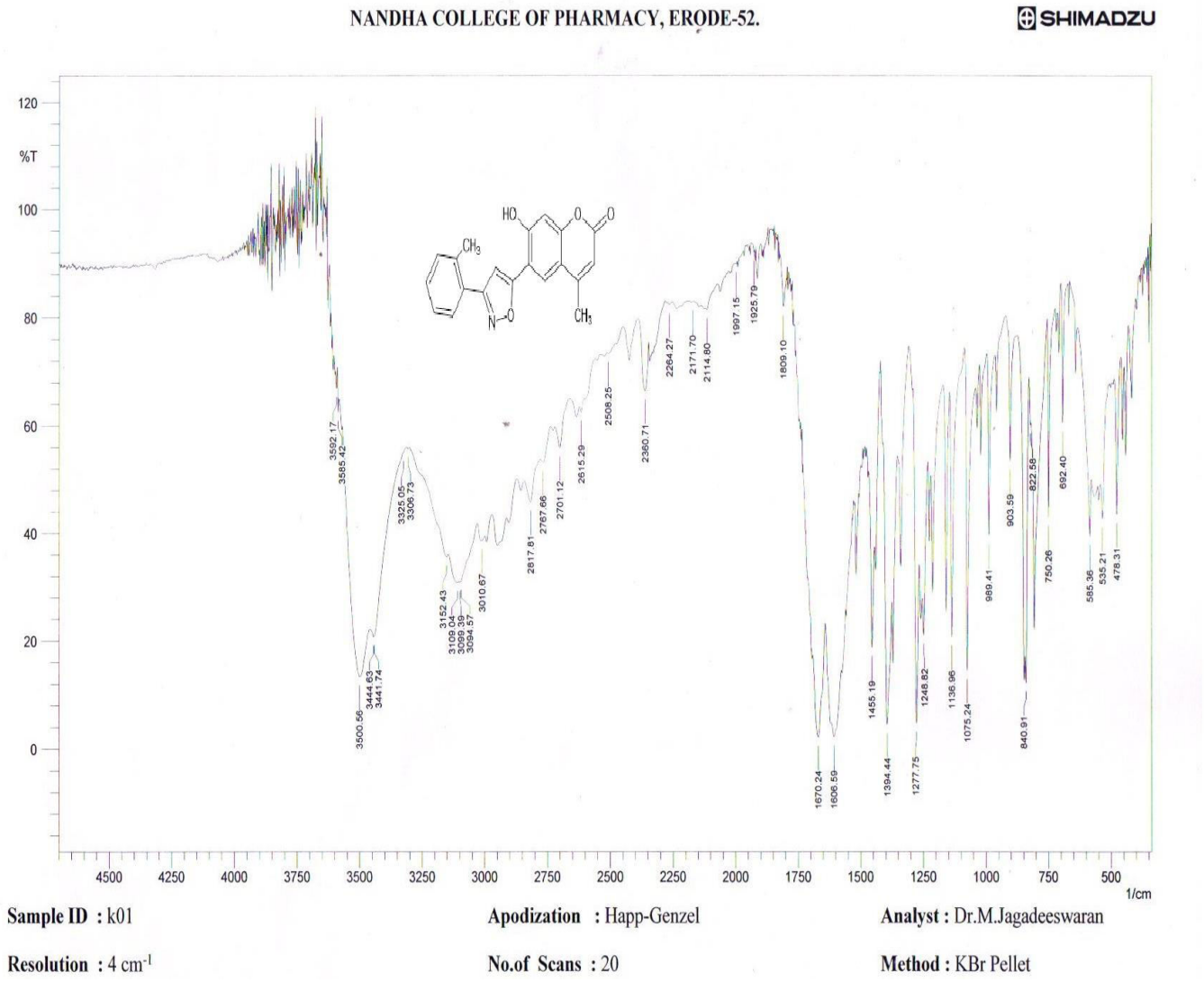


Figure 31: 1H NMR Spectrum of K_1

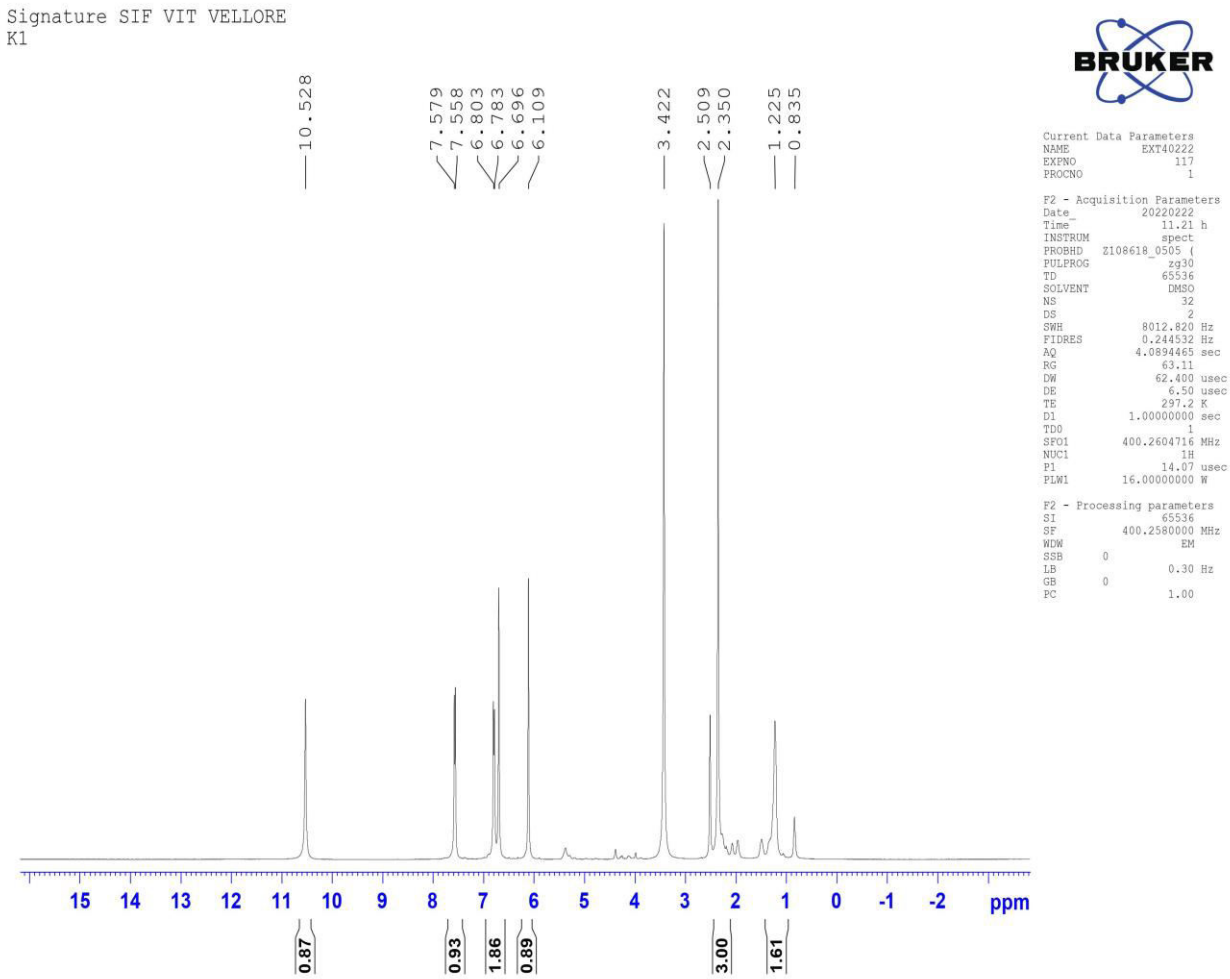
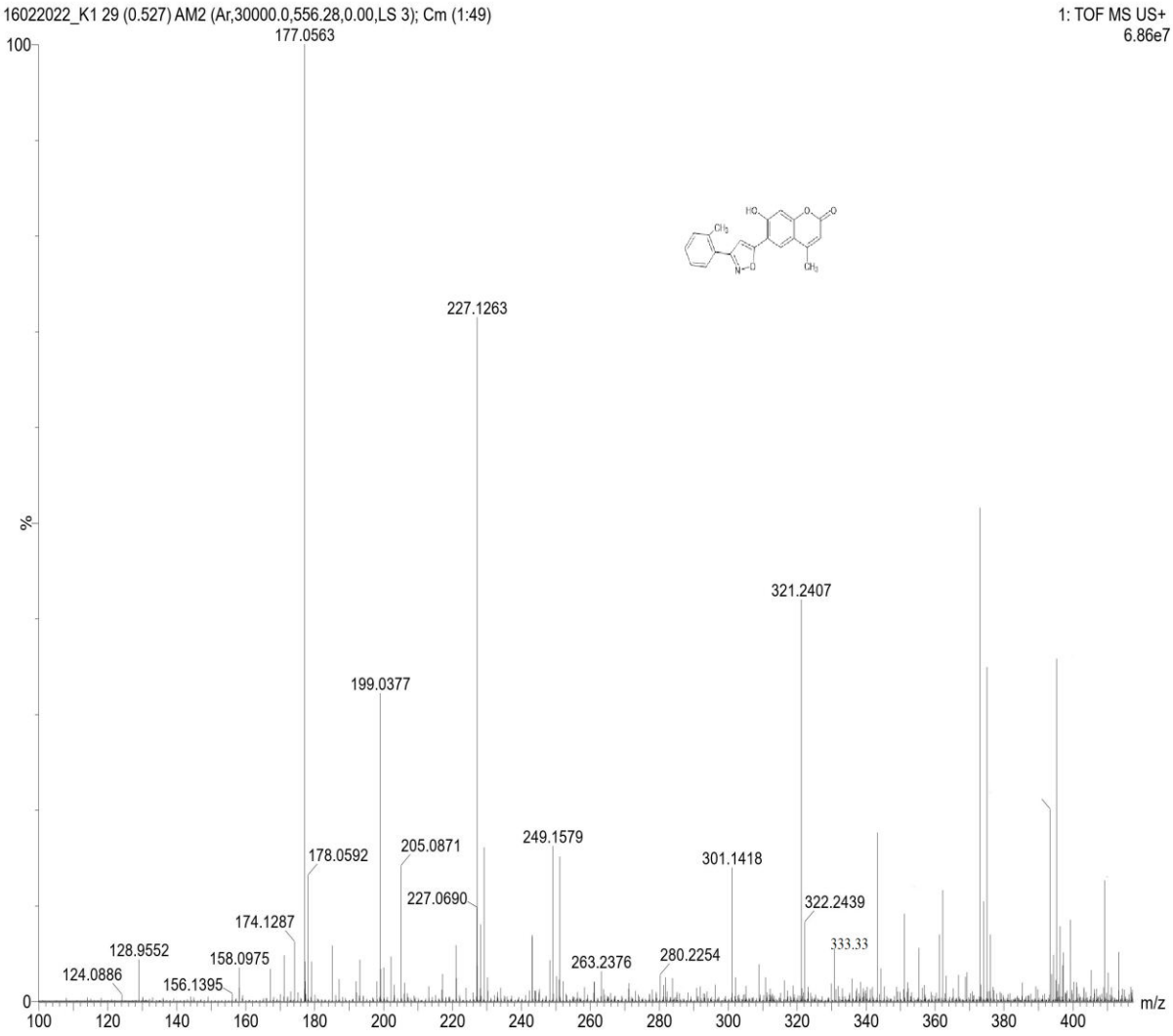


Figure 32: Mass Spectrum of K_1



Compound Code: K_3

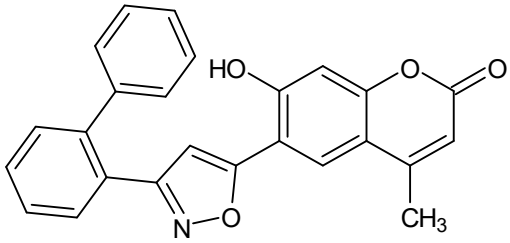


Figure 33: Thin Layer Chromatography of K_3

Chemical name	6-[3-([1,1'-biphenyl]-2-yl)-1,2-oxazol-5-yl]-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one
IR (KBr, v_{max} in cm^{-1})	3440.77 (N-H),1670.24 (coumarinyl C=O), 1277.75 (C-O), 1213.14(C-N), 1140.69(Ar-O-H),3097.47 (C-H), 1395.40 (C=C)
^{1}H NMR spectral data (dmso-d_6, δ, ppm)	δ 10.535 (s, 1H Ar-OH), δ 2.513 (s, 3H CH_3), δ 3.353 (s, 1H NH), δ 6.136 (s, Ar-H), δ 7.376-7.470 (s, 11H Ar-CH)

Mass Spectral Data

Molecular weight of the compound: 393.29

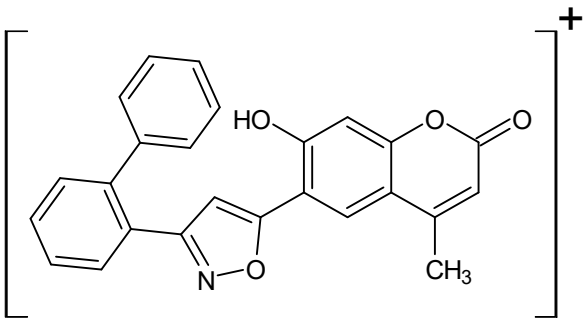
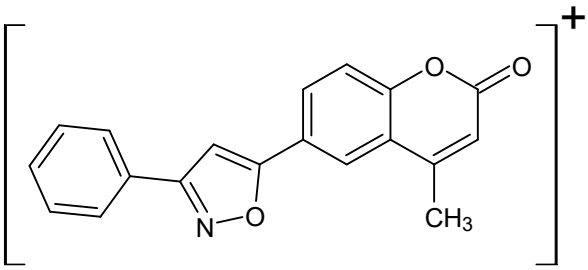
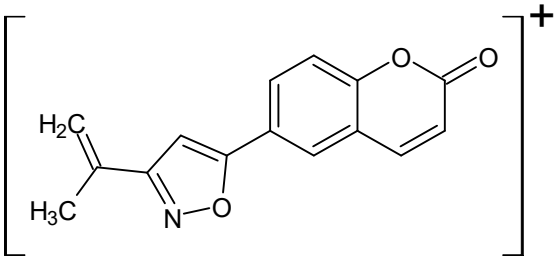
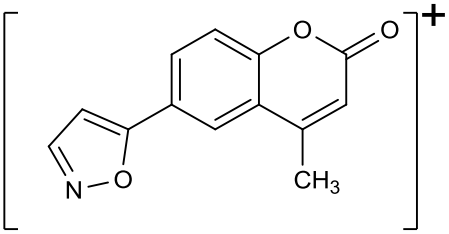
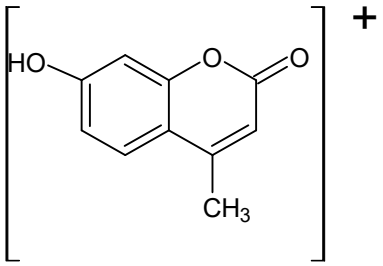
S/No	Fragments	m/z values
1		393.29
2		301.12
3		249.15
4		227.06
5		174.12

Figure 34: IR Spectrum of K_3

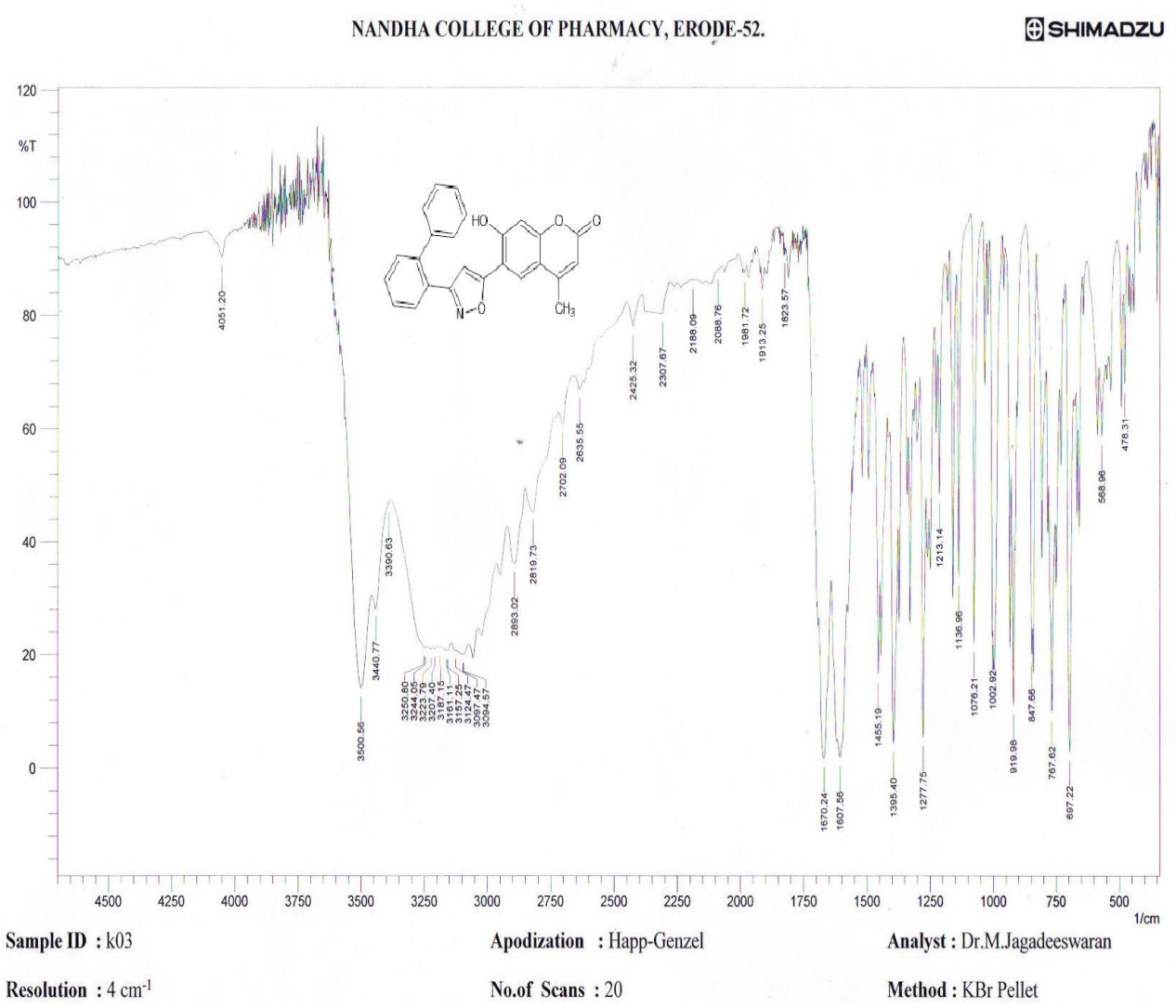


Figure 35: 1H NMR Spectrum of K_3

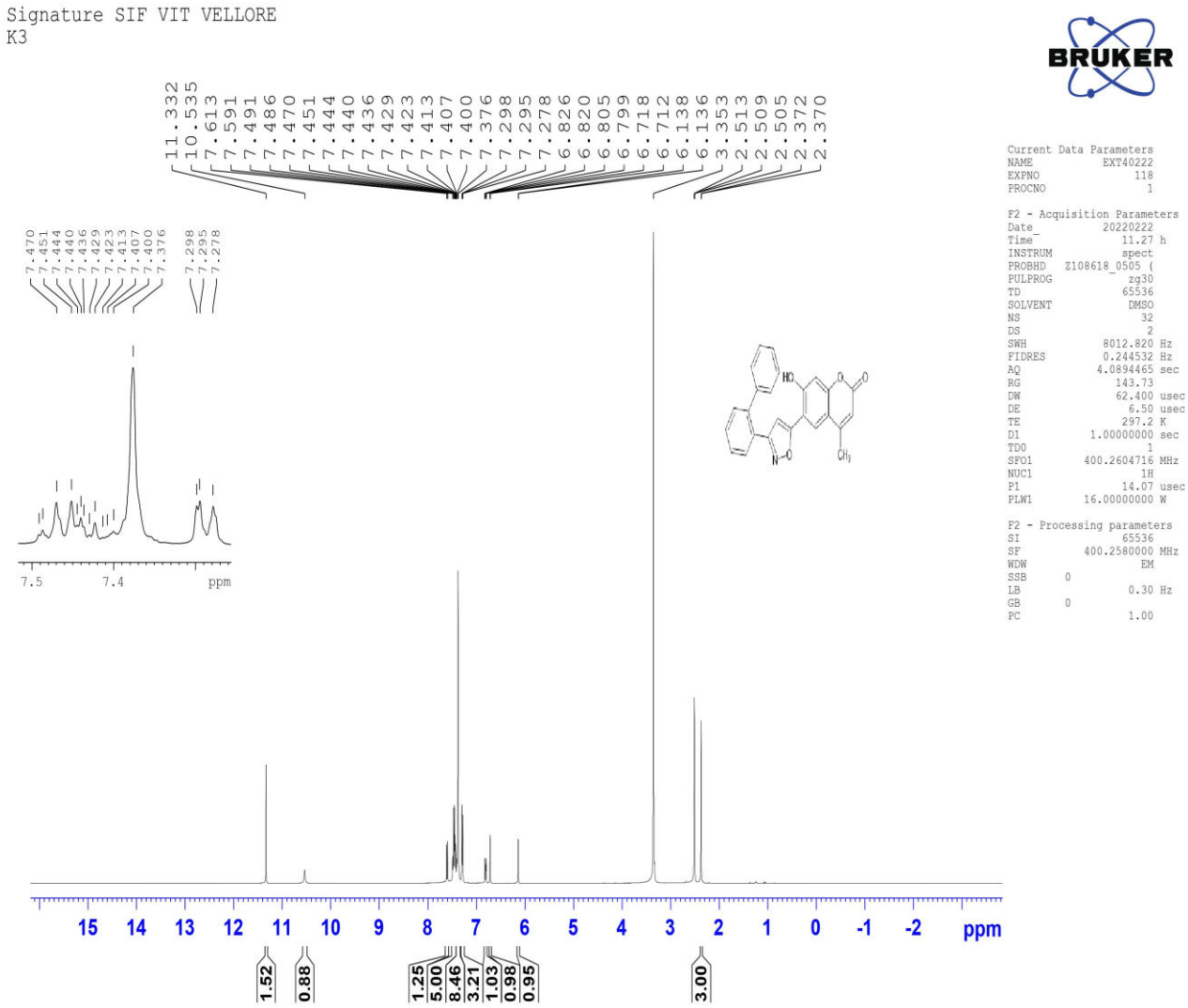
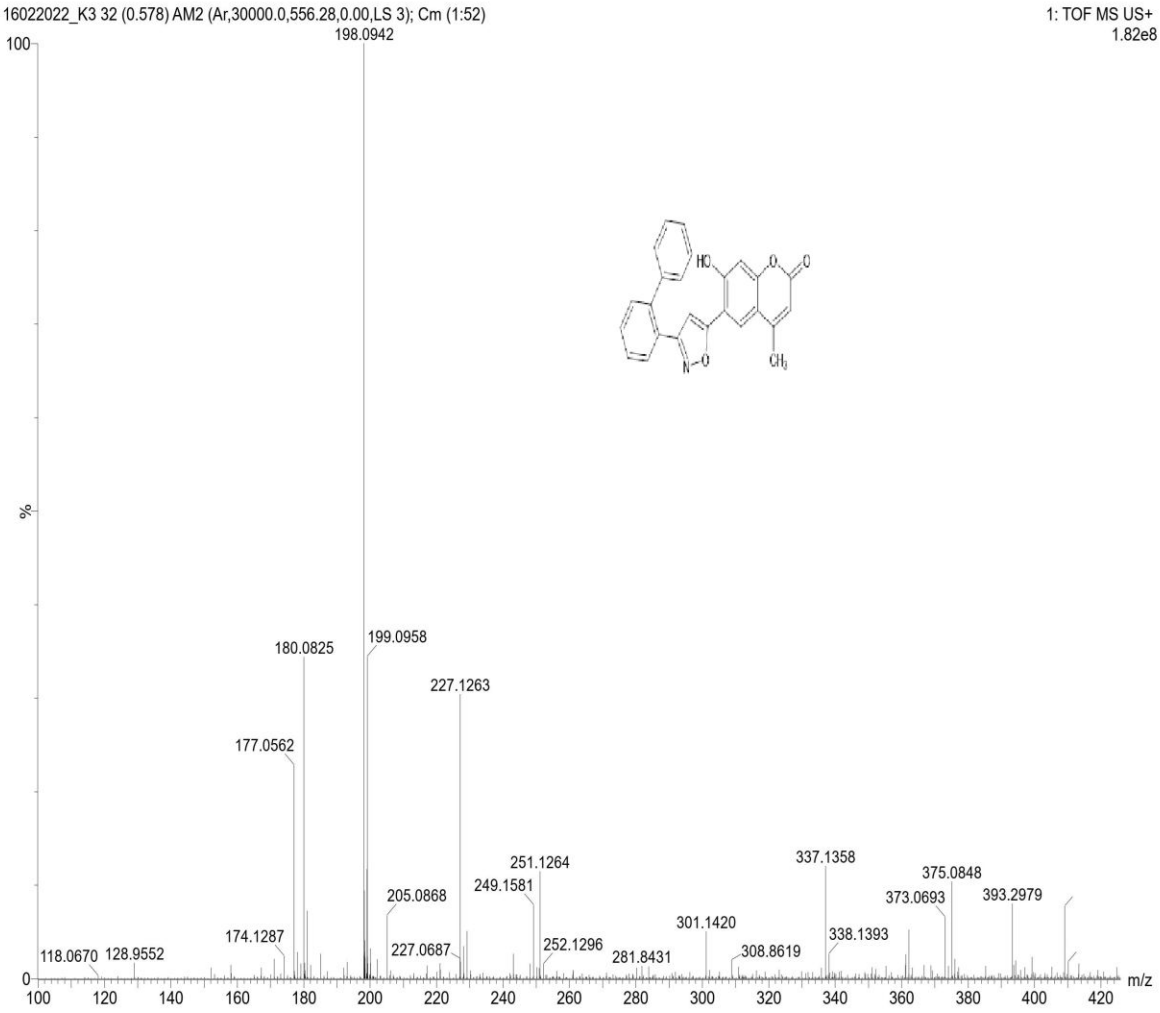


Figure 36: Mass Spectrum of K_3



Compound Code: K_5

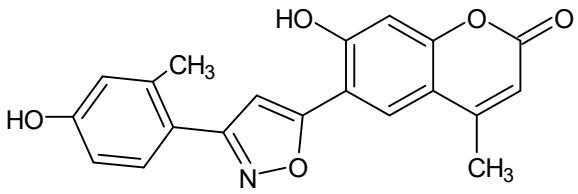
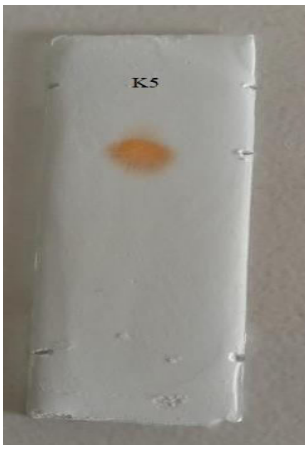


Figure 37: Thin Layer Chromatography of K_5



Chemical name	7-hydroxy-6-[3-(4-hydroxy-2-methylphenyl)-1,2-oxazol-5-yl]-4-methyl-2*H*-1-benzopyran-2-one
IR (KBr, v_{max} in cm^{-1})	3495.74(N-H), 3325.05(Ar-OH), 1669.28 (coumarinyl C=O), 1394.44(AromaticC=C), 1213.14(C-N), 3016.46 (C-H), 1276.79 (C-O), 1075.24 (N-O)
^{1}H NMR Spectral data (dmso-d_6, δ, ppm)	δ 1.221 (s, 1H isoxazole ring), δ 10.533 (s, 1H Ar-OH), δ 2.506 (s, 1H CH_3), δ 3.417 (s, 1H NH), δ 6.108 (s, Ar-H), δ 6.696 – 7.578 (s, 5H Ar-CH)

Mass Spectral data

Molecular weight of the compound: 349.34

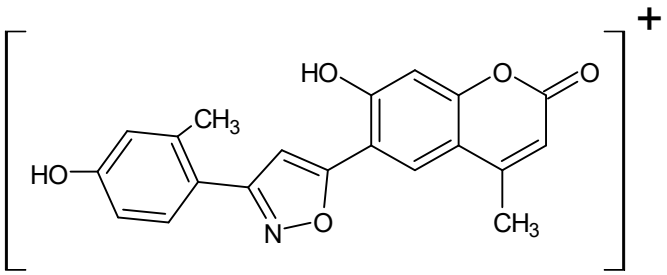
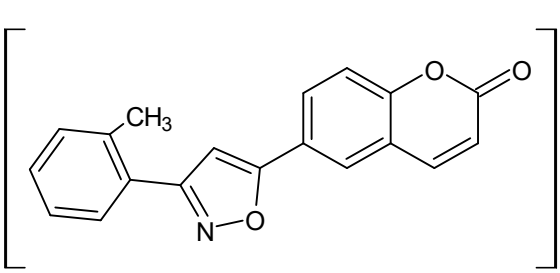
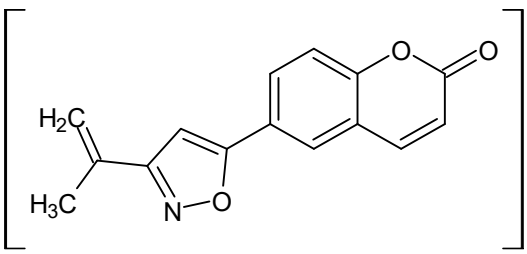
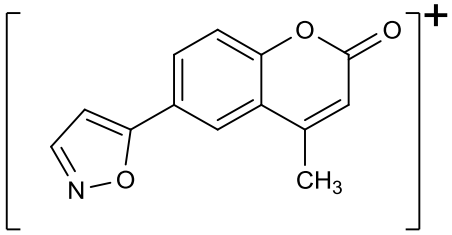
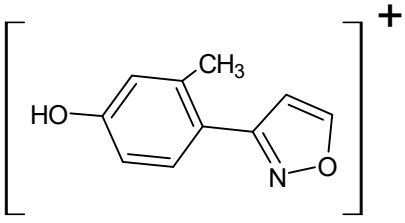
S/NO	Fragments	m/z values
1		349.34
2		301.14
3		249.14
4		227.06
5		174.12

Figure 38: IR Spectrum of K_5

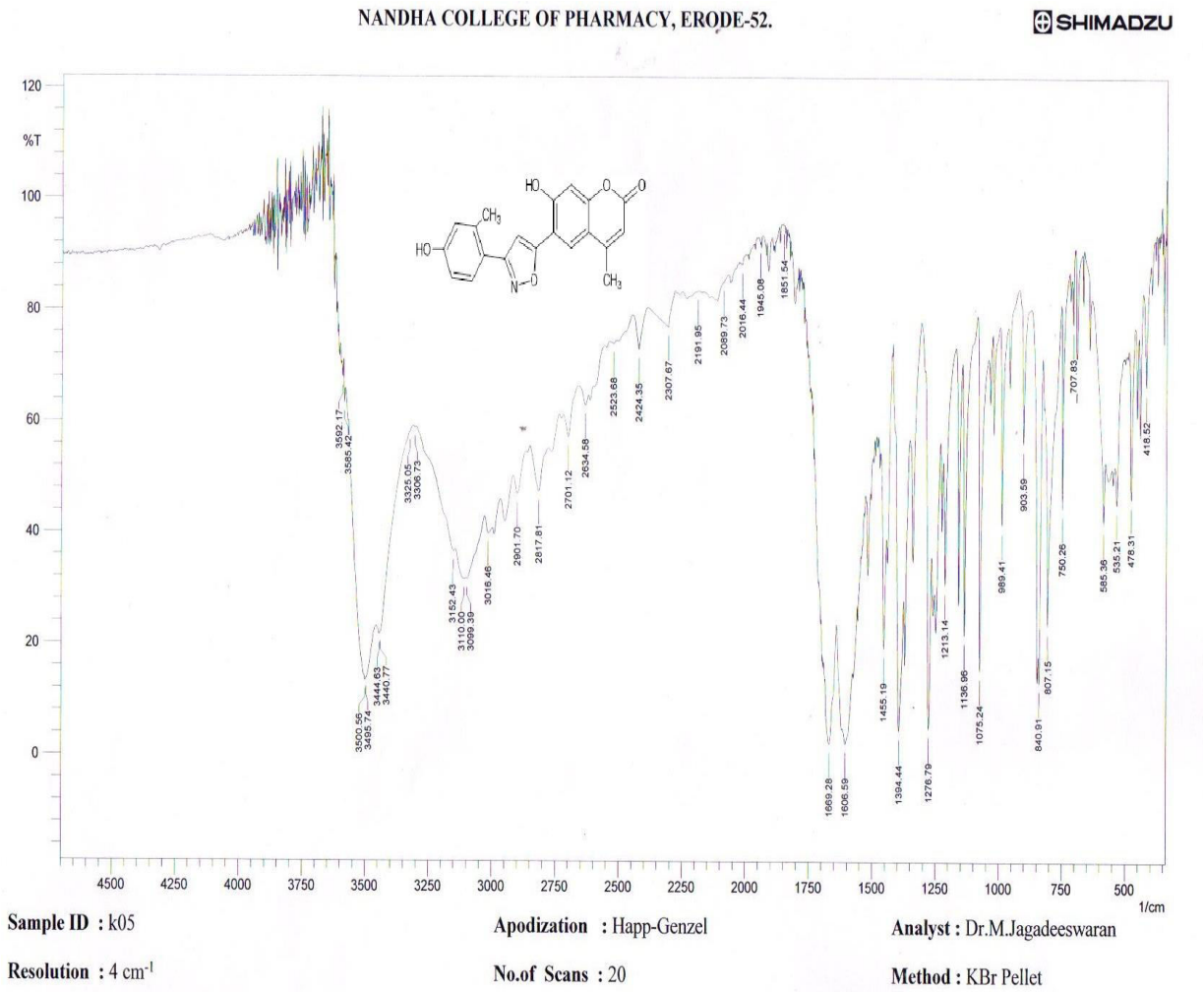


Figure 39: 1H NMR Spectrum of K_5

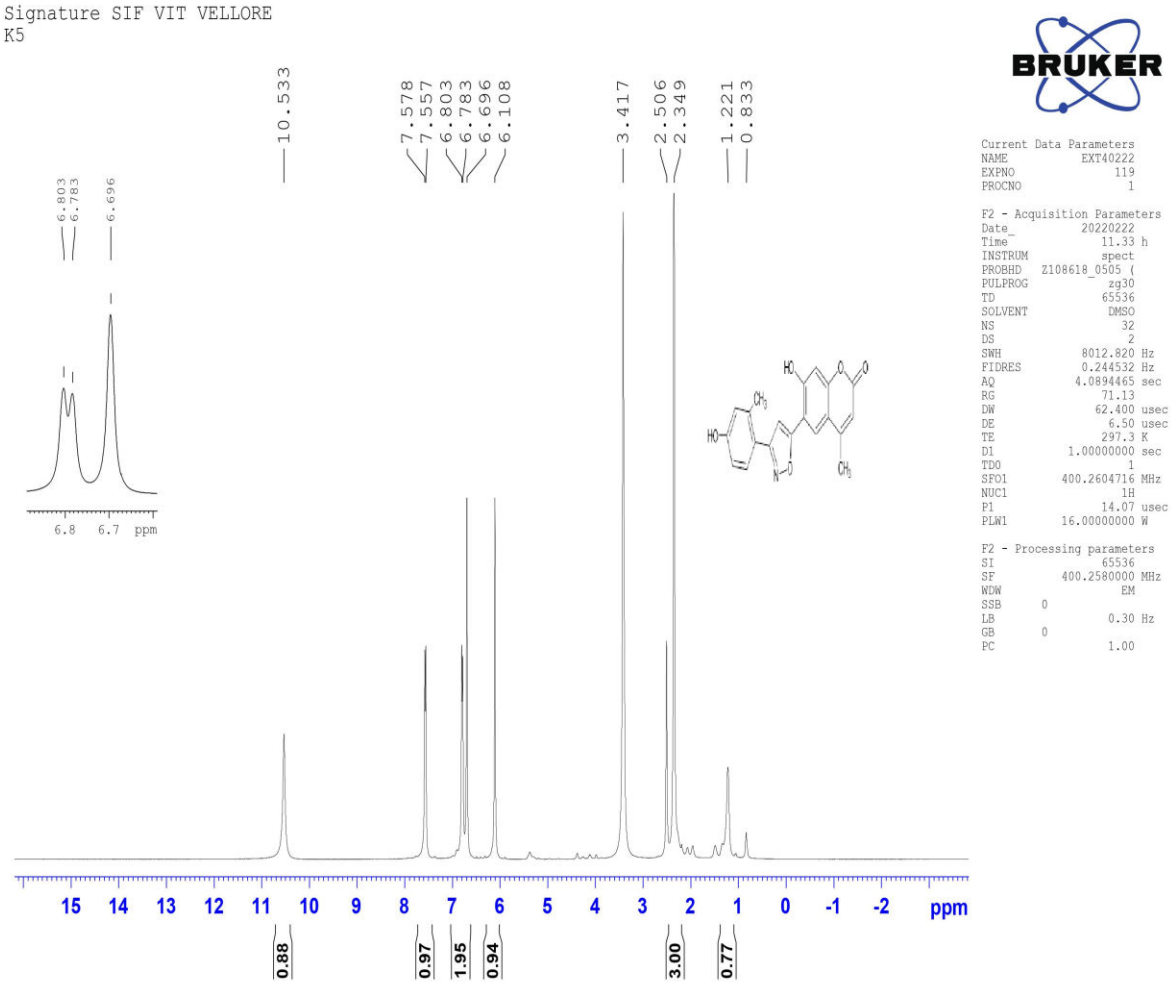
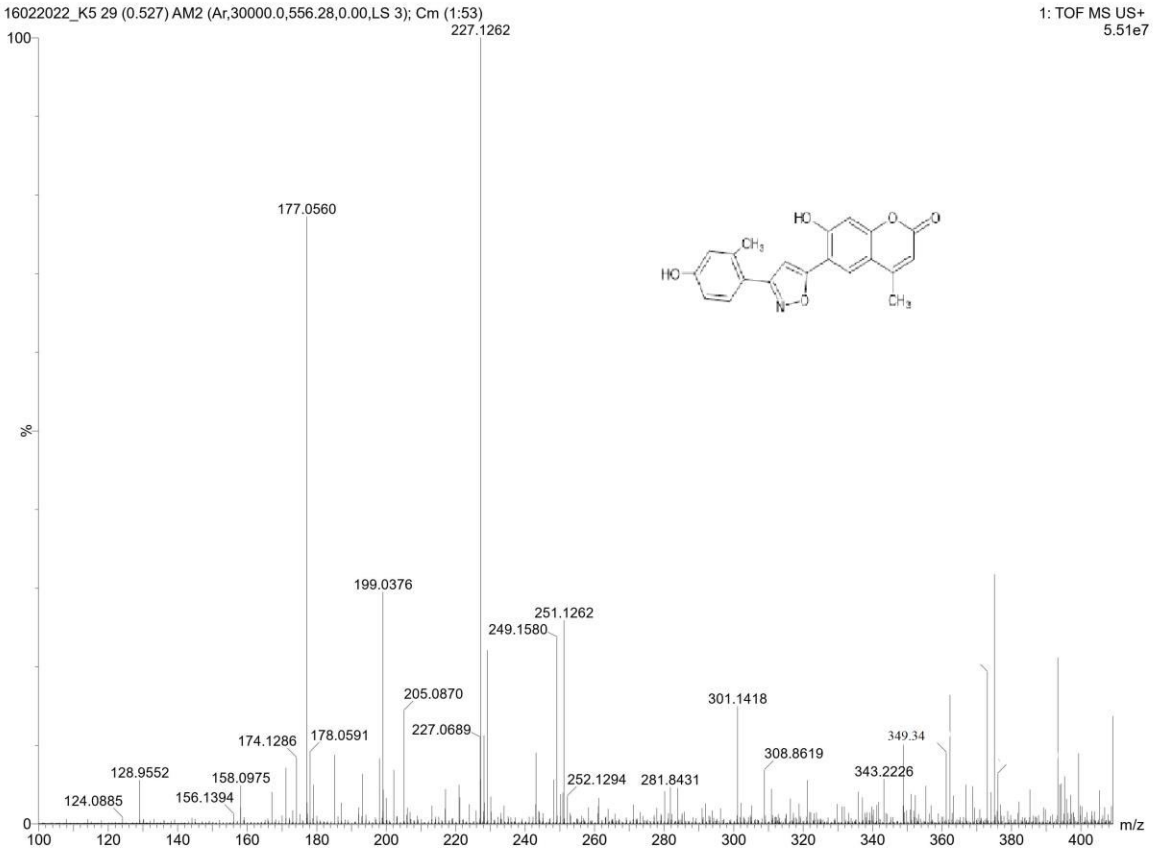


Figure 40: Mass Spectrum of K_5



ENZYME INHIBITION STUDIES

DRUGS AND CHEMICALS

Acarbose (SLCF5122, Sigma), α-amylase (BCBZ9021, Sigma-Aldrich, USA), Glucose assay kits (Agappe Diagnostics, Kerala), 3, 5-dinitro salicyclic acid (HiMedia, Mumbai) and potato starch and maltose (Lobachemie,Mumbai), Potassium Sodium tartrate (sigma) were purchased for the study. All the other chemicals used in the study were of analytical grade and were of commercial grade and obtained from respective manufacturers.

IN VITRO ANTIDIABETIC STUDIES

In vitro anti-diabetic potential of the synthesized coumarin derivatives were studied by performing the enzyme inhibition assay using carbohydrate digestive enzymes i.e., α-amylase.

IN VITRO INHIBITION OF α- AMYLASE

The study was carried out with porcine pancreatic α-amylase with starch as substrate. Acarbose was selected as the standard drug for comparison of results and coumarin derivatives dissolved in water.

PRINCIPLE [102]

α-amylase digests the starch in reaction mixture to yield maltose. The maltose produced would reduce the 3, 5-dinitrosalicyclic acid in the colouring agent to 3 amino 5-nitrosalicyclicacid. The reaction mixture produced a colour change from orange to red. The intensity of red colour will be directly proportional to the amount of maltose produced. When an enzyme inhibitor is present in reaction mixture digestion of starch, production of maltose and intensity of red colour produced will be less.

METHOD

Outline of the method

An inhibitory activity of test substance α- amylase at 1000 to 62.5µg/mL performed by biochemical method.

PREPARATION OF REAGENTS [103]

Preparation of Phosphate Buffer

Phosphate buffer (20 mM) of pH 6.9 (prepared with sodium phosphate monobasic and sodium chloride).

Preparation of test substance

Test substance was prepared at 1000µg/mL concentration by dissolving 2mg of test material in 2mL of Sodium phosphate buffer. Further solution was serially diluted to 1000 to 62.5 µg/mL to obtain lower concentration.

Preparation of Dinitro salicylic acid

Dissolve 1.6g NaOH in 100mL distilled water to make 2M NaOH. To the above solution, add 30g of Sodium potassium tartarate and dissolve. Add 1g of Dinitro salicylic acid to this solution and sonicate. Preserve final Dinitro salicylic acid solution in the amber bottle.

Preparation of standard

In 2mL of sodium phosphate buffer, 2mg of Acarbose was dissolved to obtain solutions of 2000 µg/mL which is serially diluted to get lower concentration of 62.5, 125, 250, 500 and 1000µg/mL

Preparation of α- amylase enzyme

Working solution:

Weighed 5mg of α- amylase enzyme dissolved in 10mL of Sodium phosphate buffer that gives to 0.5 mg/ml of α- amylase stock

Preparation of 1% Starch solution

In 10mL of water, 0.1g of starch was dissolved.

PROCEDURE [103-104]

In the test and test blank 250µl of different concentration of test substance solution was taken and 250µL of α- Amylase was added to the test and control whereas in place of amylase 500µl of sodium phosphate buffer was added to the test blank and control blank. For control and control blank, 500µL of sodium phosphate buffer was taken in the place of test. The reaction mixture was incubated for 10 minutes at 25˚c. After incubation, 250µL of starch solution was added to test and control tubes. Whereas in test blank and control blank 500 µL of sodium phosphate

buffer was taken in place of starch solution. Reaction mixture was incubated at 25˚C for 10minutes. After incubation 500µl of Dinitro salicylic acid was added to all the reaction mixture. All the tubes were kept in boiling water bath for 5 minutes and cooled to room temperature. Then reaction mixture was diluted with 5ml distilled water. Transfer 0.1mL of reaction mixture to the tube. Same procedure was repeated for standard by replacing the test sample with standard. Test and control were performed in triplicates and test blank and control blank were conducted in singlet. An inhibitory activity was determined by the brick red color released due to maltose from starch at 540 nm. The α- amylase inhibition was expressed as percentage of inhibition and the IC_{50} values determined by linear regression plots with varying concentration of synthesized coumarin against percentage inhibition.

CALCULATION OF PERCENTAGE OF INHIBITION:

$$\text{PERCENTAGE INHIBITION} = \left(\frac{C-T}{C} \times 100\right)$$

STATISTICAL ANALYSIS

All the analyses were carried out in triplicates and the results were expressed in mean ± SD.

Evaluation of α-amylase inhibitory activity

Ten compounds are newly designed, from that six compounds were synthesised and screened for *in vitro* α-amylase inhibitory activity at 62.50, 125, 250, 500, 1000 µg/ml concentration. Acarbose was used as the standard drug in the same concentration. A graded increase in the percentage of inhibition was observed with increase in concentration. In this study six compounds were synthesised in that 3 compounds belongs to Isoxazole derivatives by using different aromatic aldehydes in which IC_{50} of compound- A_1 (805.65 µg/ml) and other 3 compounds belong to isoxazole derivatives by using different aromatic ketones in which IC_{50} of compounds-K_1 (605.13 µg/ml) showed percentage of inhibition closer to that of standard (Acarbose- 77.32 µg/ml). The IC_{50} values of synthesised compounds were found by plotting a graph of percentage inhibition verus concentration in µg/ml. The values were compared with that of standard.

Among **A_1, A_3, A_4** series of isoxazoles derivatives of coumarin using Aromatic aldehyde, **A_1** (benzaldehyde) showed good percentage of inhibition at all concentration (62.50 µg/ml- 1000µg/ml). The IC_{50} values for these compounds were found to be 805.65 µg/ml respectively which is close to IC_{50} value of acarbose (77.32 µg/ml).

A_3 (2-hydroxy benzaldehyde) showed moderate α-amylase inhibitory activity at all concentrations. The IC_{50} value for this compound found to be 860.62 µg/ml.

Other compound **A_4** (p- dimethyl amino) exhibited the least α-amylase inhibitory activity at all concentrations with IC_{50} values 905.12 µg/ml respectively.

Among **K_1**, **K_3**, **K_5** of isoxazoles derivatives of coumarin using Aromatic ketones, **K_1** (acetophenone) showed good percentage of inhibition at all concentration (62.50 µg/ml-1000 µg/ml). The IC_{50} values for these compounds were found to be 605.13 µg/ml which is close to IC_{50} value of acarbose (77.32 µg/ml).

K_3 (benzophenone showed moderate α-amylase inhibitory activity at all concentrations. The IC_{50} value for this compound found to be 640.03 µg/ml.

Other compound **K_5** (p-hydroxy acetophenone)) exhibited the least α-amylase inhibitory activity at all concentrations with IC_{50} values 660.01 µg/ml respectively.

PERCENTAGE OF α-AMYLASE INHIBITORY POTENTIAL OF SYNTHESISED COUMARIN DERIVATIVES

IN VITRO α-AMYLASE INHIBITORY ACTIVITY

Compound code	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	IC_{50} µg/ml
A_1	20.33	29.35	38.68	45.69	52.74	**805.65**
A_3	20.50	29.42	35.23	43.12	51.57	860.62
A_4	21.21	29.50	32.42	42.41	50.53	905.12
K_1	19.64	29.40	38.38	47.71	58.59	**605.13**
K_3	21.53	32.13	40.41	49.31	60.11	640.03
K_5	20.12	31.21	39.41	48.63	59.61	660.01
Standard(Acarbose)	40	58	67	71	80	77.32

Figure 41. Standard curve

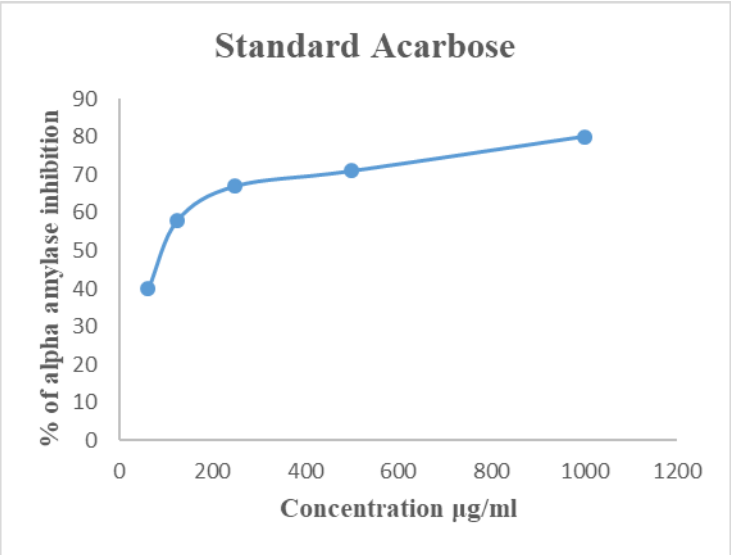
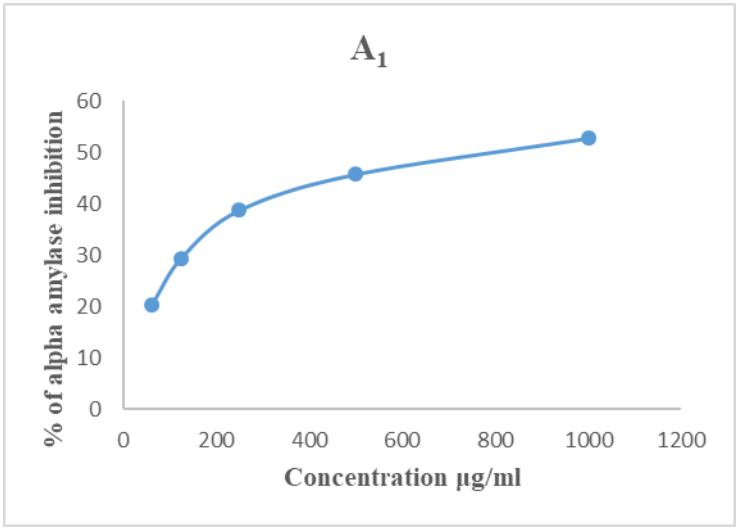
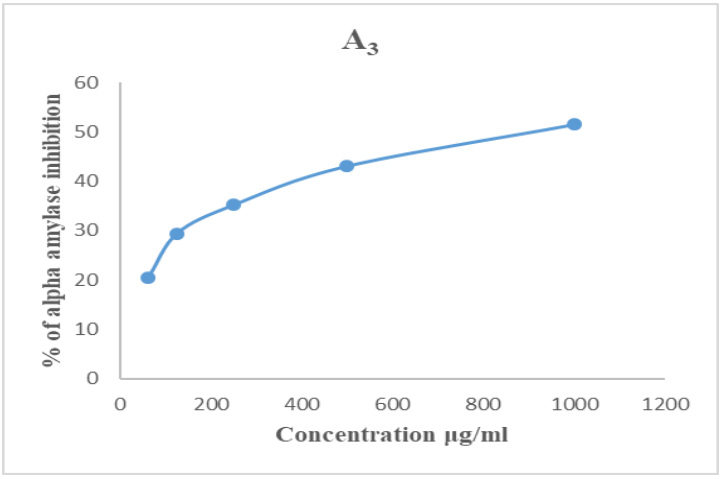


Figure 42. Test compound curve of A series





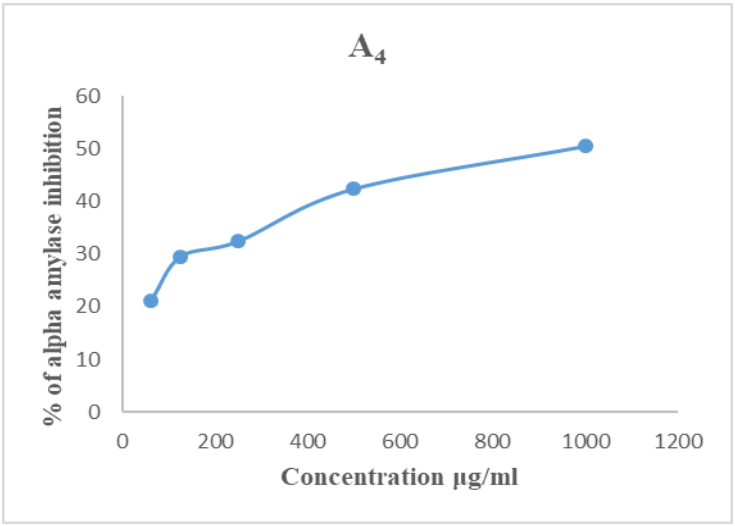
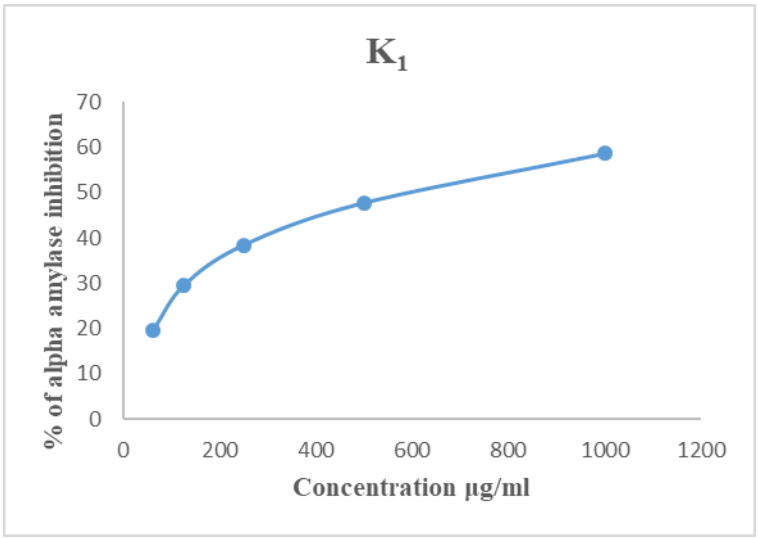
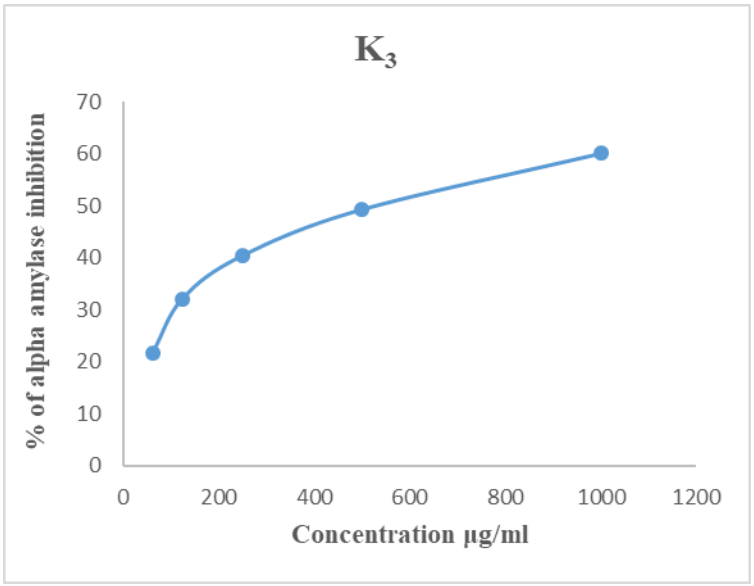


Figure 43. Test compound curve of K series





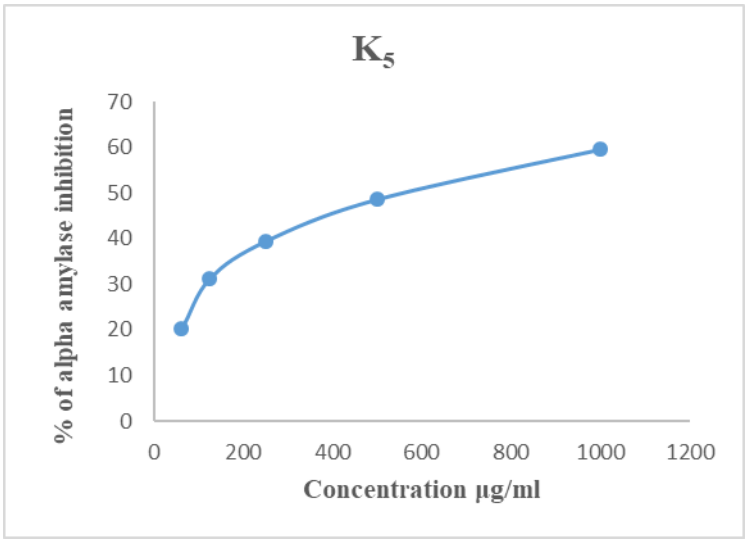
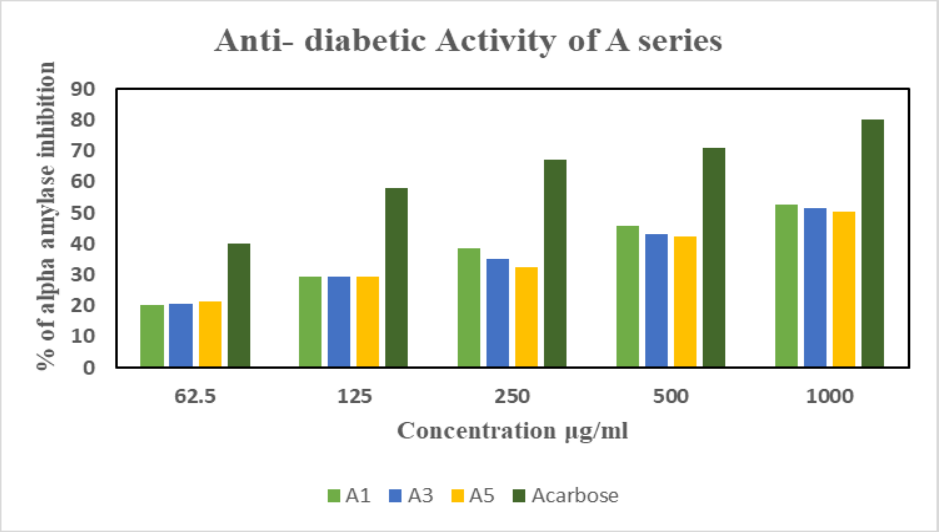
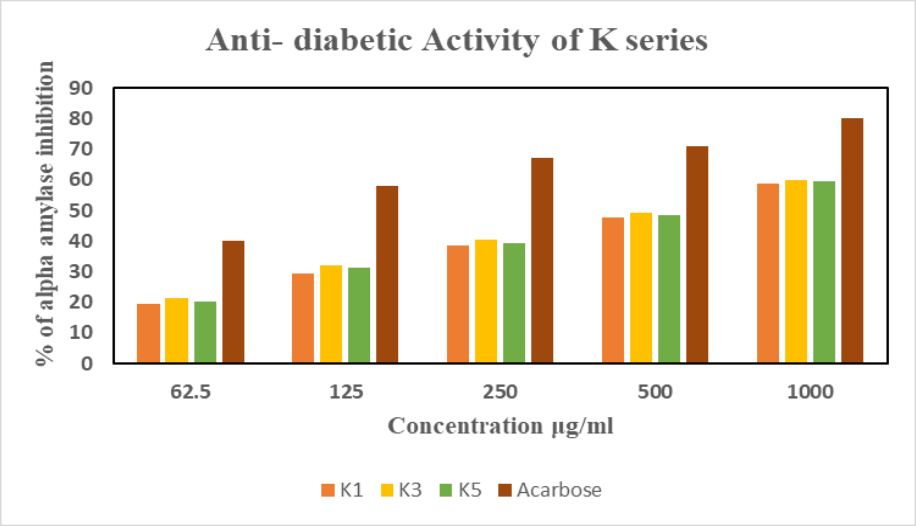


Figure 44. Overall graphical representation of all compounds





The overall graphical representation of *in-vitro* alpha amylase inhibitory activity of synthesized compounds.

Among the test compounds, compound 7-hydroxy-4-methyl-6-(3-phenyl-1,2-oxazol-5-yl)-2*H*-1-benzopyran-2-one (A_1) and compound 7-hydroxy-4-methyl-6-[3-(2-methylphenyl)-1,2-oxazol-5-yl]-2*H*-1-benzopyran-2-one (K_1) was found to be the most active agent which gives good α-amylase inhibition.

6. SUMMARY AND CONCLUSION

SUMMARY

The present work was focused on the designing and synthesis of novel isoxazole derivatives incorporated with coumarin moiety having α-amylase enzyme inhibitory activity. For this, following approach has been adopted.

PHASE I: LITERATURE REVIEW

Literature survey showed that coumarin is a drug like scaffold and is a core skeleton for the active sites involved in enzyme inhibiton in Type 2 diabetes. It also revealed that isoxazole possess enzyme inhibition for Type 2 diabetes.

PHASE II: DRUG DESIGN APPROACH

It involves the following stages:

Stage 1: Identification of target

α-amylase was selected as the target enzyme as its inhibition will prevent carbohydrate hydrolysis. The target enzyme (1UA7) was downloaded from RCBs Protein Databank.

Stage 2: Lead optimization

Lead optimisation was done by computation of drug likness score. Isoxazole derivatives of coumarin were the desired compounds with good molecular properties and bioactivity score, ie., the compounds **A_1**, **A_3**, **A_4** and **K_1**, **K_3**, **K_5** showed good scores.

PHASE III: SYNTHESIS AND PHYSICAL CHARACTERIZATION

A) Synthesis of the designed compounds

In this work, ten new compounds are designed in which Six different isoxazole derivatives were synthesized by using three aromatic aldehydes and three aromatic ketones with coumarin moiety. The first step involved the synthesis of 6-acetyl 7- hydroxy 4- methyl coumarin by acetylation. Chalcones were prepared from 6- acetyl 7- hydroxy 4-methyl coumarin by using different aromatic aldehydes and aromatic ketones. Finally, the chalcones were reacted with hydroxylamine

hydrochloride to form isoxazoles.

B) Physical characterization

Melting point of all the newly synthesised compounds was determined by capillary tube method. R_f values were determined by fixing various suitable solvent system on precoated silicagel- G plates.

PHASE IV: SPECTRAL STUDIES

The structure of the synthesised compounds was established by using IR, ^1H NMR, and Mass spectral data.

PHASE V: EVALUATION OF BIOLOGICAL ACTIVITIES

Evaluation of α-amylase inhibitory activity

All the newly sysnthesised compounds were screened for *in vitro* α-amylase inhibitory activity. All compounds showed significant activity in inhibition of the α-amylase enzyme. Comparatively A_1 and K_1 showed good % of inhibition activity, while A_3, A_4, K_3, K_5 showed moderate activity.

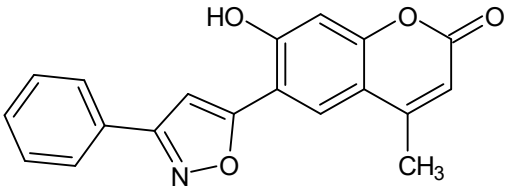
CONCLUSION

- The present study establishes that computational tools help in minimizing the tedious process of drug discovery and delivers new drug candidate more quickly.
- α-amylase enzyme was selected as target and virtual screening made selection of lead compounds easier and coumarin was selected as lead molecule.
- From among the ten docked molecules, six molecules with good Binding affinity were chosen for further laboratory synthesis. Drug likeness was predicted insilico before proceeding for synthesis.
- Compounds A_1, A_3, A_4 and K_1, K_3, K_5 were found to have significant binding score against target enzyme α-amylase. compared to standard drug Acarbose. The selected derivatives were planned for synthesis.
- The proposed Six compounds of isoxazole derivatives with coumarin ring system were synthesised in good yield using the developed

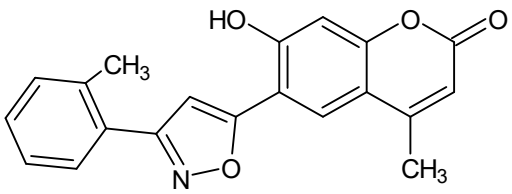
schemes.

- All the reactions were monitored by TLC one spot technique and the structures of the synthesised compounds were confirmed by IR, ^{1}H NMR, Mass spectra.
- Compounds A_1, K_1 and K_3 exhibited maximum α-amylase inhibitory activity.
- Among the synthesized compounds, A_1 and K_1 can be taken for further studies as the lead molecule and acute toxicity studies are to be done on these promising compounds.

Structure of Lead Molecules identified



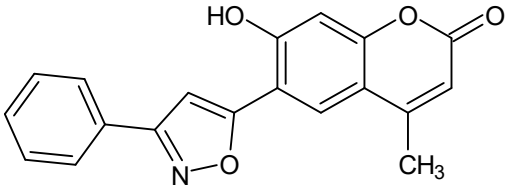
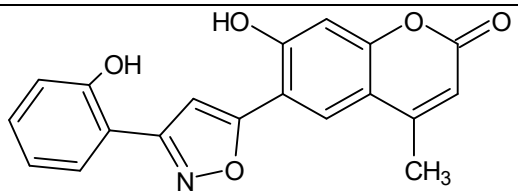
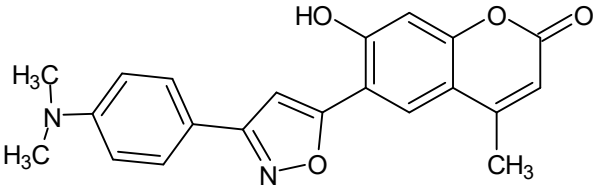
A_1



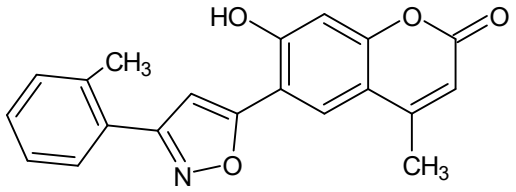
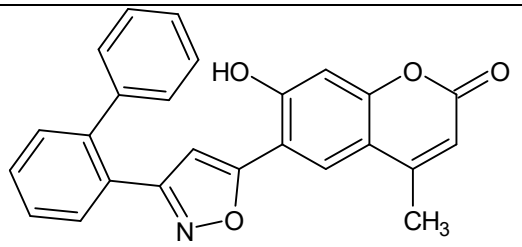
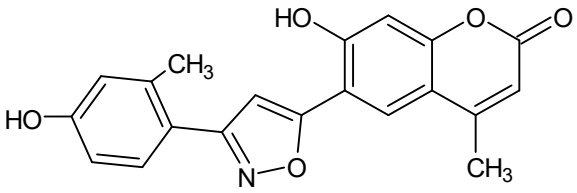
K_1

7. LIST OF NEWLY SYNTHESIZED COMPOUND

SUBSTITUTED ISOXAZOLE DERIVATIVES USING AROMATIC ALDEHYDES

Compound Code	IUPAC name	Structure
A_1	7-hydroxy-4-methyl-6-(3-phenyl-1,2-oxazol-5-yl)-2*H*-1benzopyran-2-one	
A_3	7-hydroxy-6-[3-(2-hydroxyphenyl)-1,2-oxazol-5-yl]-4-methyl-2*H*-1-benzopyran-2-one	
A_4	6-{3-[4-(dimethylamino)phenyl]-1,2-oxazol-5-yl}-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one	

SUBSTITUTED ISOXAZOLE DERIVATIVES USING AROMATIC KETONES

Compound Code	IUPAC name	Structure
K_1	7-hydroxy-4-methyl-6-[3-(2-methylphenyl)-1,2-oxazol-5-yl]-2*H*-1-benzopyran-2-one	
K_3	6-[3-([1,1'-biphenyl]-2-yl)-1,2-oxazol-5-yl]-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one	
K_5	7-hydroxy-6-[3-(4-hydroxy-2-methylphenyl)-1,2-oxazol-5-yl]-4-methyl-2*H*-1-benzopyran-2-one	

8. BIBLIOGRAPHY

1. Basappa VC, Kameshwar VH, Kumara K, Achutha DK, Krishnappagowda LN, Kariyappa AK. Design and synthesis of coumarin-triazole hybrids: biocompatible anti-diabetic agents, in silico molecular docking and ADME screening. Heliyon. 2020; 6(10):05290.
2. Sysak A, Obmińska-Mrukowicz B. Isoxazole ring as a useful scaffold in a search for new therapeutic agents. European journal of medicinal chemistry. 2017; 137:292-309.
3. Alvárez- Builla J, Barluenga J. Heterocyclic compounds: An introduction. Modern Heterocyclic Chemistry. 2011; 1-9.
4. Shaik, A., Bhandare, R.R., Palleapati, K., Nissankararao, S., Kancharlapalli, V. and Shaik, S. Antimicrobial, antioxidant, and anticancer activities of some novel isoxazole ring containing chalcone and dihydropyrazole derivatives. Molecules, 2020; *25*(5), p.1047
5. Zeydi MM, Kalantarian SJ, Kazeminejad Z. Overview on developed synthesis procedures of coumarin heterocycles. Journal of the Iranian Chemical Society. 2020; 1-64.
6. Küpeli Akkol E, Genç Y, Karpuz B, Sobarzo-Sánchez E, Capasso R. Coumarins and coumarin-related compounds in pharmacotherapy of cancer. Cancers. 2020; 12(7):1959.
7. Tiwari SV, Seijas JA, Vazquez-Tato MP, Sarkate AP, Karnik KS, Nikalje AP. Facile synthesis of novel coumarin derivatives, antimicrobial analysis, enzyme assay, docking study, ADMET prediction and toxicity study. Molecules. 2017 ; 22(7):1172.
8. Zhang RR, Liu J, Zhang Y, Hou MQ, Zhang MZ, Zhou F, Zhang WH. Microwave-assisted synthesis and antifungal activity of novel coumarin derivatives: Pyrano [3, 2-c] chromene-2, 5-diones. European journal of medicinal chemistry. 2016 ;116:76-83.
9. Durgapal SD. Studies in Synthesis and Applications of Chromene Derivatives (Doctoral dissertation, Maharaja Sayajirao University of Baroda (India)). 2020; 10-12.
10. Konidala SK, Kotra V, Danduga RC, Kola PK, Bhandare RR, Shaik AB. Design, multistep synthesis and in-vitro antimicrobial and antioxidant screening of coumarin clubbed chalcone hybrids through molecular hybridization approach. Arabian Journal of Chemistry. 2021;14(6):103154.

11. Sun YF, Cui YP. The synthesis, characterization and properties of coumarin-based chromophores containing a chalcone moiety. Dyes and Pigments. 2008; 78(1):65-76.
12. Hussain Z, Shoaib M, Ali U, Ramzan H, Ali M, Tariq A, Naqash M. Theoretical study of isoxazoles and their derivatives for evaluating its pharmaceutical properties with density functional theory. J Comput Chem Mol Model. 2020; 4(3):415-26.
13. Zhu J, Mo J, Lin HZ, Chen Y, Sun HP. The recent progress of isoxazole in medicinal chemistry. Bioorganic & medicinal chemistry. 2018; 26(12):3065-75.
14. Kumar KA, Jayaroopa P. Isoxazoles: molecules with potential medicinal properties. Int J Pharm Chem Biol Sci. 2013; 3:294-304.
15. Li H, Yao Y, Li L. Coumarins as Potential Antidiabetic Agents. Journal of Pharmacy and Pharmacology. 2017; 1-12.
16. Maitra A, Abbas AK. Endocrine system. Robbins, Cotran. Pathologic Basis of disease (7^{th}edtn). Saunders, Philadelphia, Diabetes: Estimates for the Year 2000 and Projections for 2030. Diabetes care. 2005; 27: 1047-53, 1156-1226.
17. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. Diabetes care. 2006; 27:1040-53.
18. WHO Expert Committee on Definition Diagnosis and classification of Diabetes Mellitus and its Complications, Geneva.1999; 1-59.
19. Jump up. Williams Textbook of endocrinology (12^{th}edtn). Elsevier/ Saunders, Philadelphia, USA. 2007; 1371-1435.
20. Chen L, Magliano DJ, Zimmet PZ. The Worldwide Epidemiology of Type2 Diabetes Mellitus: Present and Future Perspectives. Nature Reviews Endocrinology. 2012; 8:228-236.
21. Sheikh JHI, Tsujiyama MT, Ashabul IM, Rajat SB, Hitoshi. A Total Phenolic Content, Anti-oxidative, anti-amylase, anti-glucosidase and anti-histamine release activities of Bangladeshi fruits. Food Science Technology Research. 2008; 14: 261-68.
22. Dennis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS. Beta 1-6 branching of Asn-linked of oligosaccharides is directly associated with metastasis. Science. 1987; 236:582-85.
23. Satyanarayana U, Chukrapani U. Biochemistry, (3^{rd} edtn), Uppla Author Publisher, A P. 2006: 20-21
24. Reddy NS, Nimmagadda A, Rao KS. An overview of the microbial α-amylase family. African journal of biotechnology. 2003; 2(12):645-8.

25. Singh SU, Sharma VI, Soni ML, Das SH. Biotechnological applications of industrially important amylase enzyme. International Journal of Pharma and Bio Sciences. 2011; 2(1):486.
26. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α-amylase: a biotechnological prespective. Process Biochemistry. 2003; 38:1599-1616.
27. Copeland RA, Harpel MR, Tommino PJ. Targeting enzyme inhibitors in drug discovery. Expert Opinion on therapeutic targets, 2007; 7: 967-978.
28. Muralikrishna G, Nirmala M. Cereal α-amylase an overview. Carbohydrate polymers. 2005; 60:163-173.
29. Deore AB, Dhumane JR, Wagh R, Sonawane R. The stages of drug discovery and development process. Asian Journal of Pharmaceutical Research and Development. 2019; 7(6):62-7.
30. Aparoy P, Kumar Reddy K, Reddanna P. Structure and ligand based drug design strategies in the development of novel 5-LOX inhibitors. Current medicinal chemistry. 2012; 19(22):3763-78.
31. Hassan Baig M, Ahmad K, Roy S, Mohammad Ashraf J, Adil M, Haris Siddiqui M, Khan S, Amjad Kamal M, Provazník I, Choi I. Computer aided drug design: success and limitations. Current pharmaceutical design. 2016; 22(5):572-81.
32. Kumar S, Purohit D, Pandey P. Molecular docking and its application towards modern drug discovery. World J. Pharm. Pharm. Sci. 2019; 6:691-6.
33. Chaudhary KK, Mishra N. A review on molecular docking: novel tool for drug discovery. databases. 2016; 3(4):1029.
34. Autodock.scripps.edu/-united states
35. Pari L, Rajarajeswari N, Saravanan S, Rathinam A. Antihyperlipidemic effect of coumarin in experimental type 2 diabetic rats. Biomedicine & Preventive Nutrition. 2014; 4(2):171-6.
36. Anchal V, Priyanka D, Disha K, Shailendra PK. Hypoglycemic and Hypolipidemic Activity of Scopoletin (Coumarin Derivative) in Streptozotocin Induced Diabetic Rats. International Journal of Pharmaceutical Sciences Review and Research. 2013; 22(1):79-83.
37. Ramu A, Vijayakumar V. Antidiabetic Activity of 7-Hydroxy Coumarin Bark of Marine Plant *Rhizophora mucronata.* Global Advanced Research Journal of Medicinal Plants. 2016; 4(1): 001-006.

38. Garazd Y, Garazd M, Lesyk R. Synthesis and evaluation of anticancer activity of 6-pyrazolinylcoumarin derivatives. Saudi Pharmaceutical Journal. 2017; 25(2):214-23.
39. Ibrahim DM, Jumal J, Harun FW. Synthesis and biological evaluation of 6-hydroxy-4-methyl-5, 7-(bis-p-chlorophenylazo) coumarin. American Journal of Applied Sciences. 2015; 11-95.
40. Yelchuri V, RBN P, Karuna MS, Poornachandra Y, Kumar CG. Synthesis of Novel Fatty Substituted 4-methyl-2HChromen-2-one via Cross Metathesis: Potential Antioxidants and Chemotherapeutic Agents. Journal of oleo science. 2016; 65(12):1023-31.
41. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Journal of antimicrobial chemotherapy. 2008; 61(6):1295-301.
42. Al-Amiery AA, Kadhum AA, Mohamad AB. Antifungal activities of new coumarins. Molecules. 2012; 17(5):5713-23.
43. Guan AY, Liu CL, Li ML, Zhang MX, Zhang H. Synthesis and bioactivity of novel coumarin derivatives. Natural product communications. 2011; 6(12):1912-32.
44. Yoon JA, Lim C, Han YT. Preliminary study on novel expedient synthesis of 5-azaisocoumarins by transition metal-catalyzed cycloisomerization. Frontiers in chemistry. 2020:772.
45. De Araújo RS, Guerra FQ, Lima DO, De Simone CA, Tavares JF, Scotti L, Scotti MT, De Aquino TM, De Moura RO, Mendonça FJ, Barbosa-Filho JM. Synthesis, structure-activity relationships (SAR) and in silico studies of coumarin derivatives with antifungal activity. International Journal of Molecular Sciences. 2013; 14(1):1293-309.
46. K. Suresh Babu, T. Hari Babu, P. V. Srinivas, K. Hara Kishore, U. S. N. Murthy and J. Madhusudana Rao et al, Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agent J.Bioorg. Med. Chem. Lett. 2006; 16:221–224.
47. Cong NT, Nhan HT, Van Hung L, Thang TD, Kuo PC. Synthesis and antibacterial activity of analogs of 5-arylidene-3-(4-methylcoumarin-7-yloxyacetylamino)-2-thioxo-1, 3-thiazoli-din-4-one. Molecules. 2014; 19(9):13577-86.
48. Pérez-Cruz K, Moncada-Basualto M, Morales-Valenzuela J, Barriga-González G, Navarrete-Encina P, Núñez-Vergara L, Squella JA, Olea-Azar C. Synthesis and

antioxidant study of new polyphenolic hybrid-coumarins. Arabian journal of chemistry. 2018; 11(4):525-37.

49. Pawar V, Chavan SV, Yamgar RS, Atram RG, Thorat BR, Bisht S, Sawant SS. Synthesis and characterization of novel transition metal complexes of 4-methyl-7-hydroxy 8-formyl coumarin and their biological activities. Asian Journal of Research in Chemistry. 2011; 4(8):1238-42.
50. Olaya MD, Vergel NE, López JL, Viña MD, Guerrero MF. Coumarin analogue 3-methyl-7H-furo [3, 2-g] chromen-7-one as a possible antiparkinsonian agent. Biomédica. 2019; 39(3):491-501.
51. Bhat MA, Al-Omar MA. Coumarin incorporated triazoles: a new class of anticonvulsants. Acta Pol Pharm. 2011; 68(6):889-95.
52. Bouasla S, Amaro-Gahete J, Esquivel D, López MI, Jiménez-Sanchidrián C, Teguiche M, Romero-Salguero FJ. Coumarin derivatives solvent-free synthesis under microwave irradiation over heterogeneous solid catalysts. Molecules. 2017; 22(12):2072.
53. Rammohan A, Bhaskar BV, Venkateswarlu N, Gu W, Zyryanov GV. Design, synthesis, docking and biological evaluation of chalcones as promising antidiabetic agents. Bioorganic chemistry. 2020; 95:103527.
54. Konidala SK, Kotra V, Danduga RC, Kola PK. Coumarin-chalcone hybrids targeting insulin receptor: Design, synthesis, anti-diabetic activity, and molecular docking. Bioorganic chemistry. 2020; 104:104207.
55. Singh N, Sarkar J, Sashidhara KV, Ali S, Sinha S. Anti-tumour activity of a novel coumarin–chalcone hybrid is mediated through intrinsic apoptotic pathway by inducing PUMA and altering Bax/Bcl-2 ratio. Apoptosis. 2014; 19(6):1017-28.
56. Wang G, Liu W, Gong Z, Huang Y, Li Y, Peng Z. Synthesis, biological evaluation, and molecular modelling of new naphthalene-chalcone derivatives as potential anticancer agents on MCF-7 breast cancer cells by targeting tubulin colchicine binding site. Journal of enzyme inhibition and medicinal chemistry. 2020; 35(1):139-44.
57. Abosalim HM, Nael MA, El- Moselhy TF. Design, synthesis and molecular docking of chalcone derivatives as potential anticancer agents. ChemistrySelect. 2021; 6(4):888-95.

58. Trivedi JC, Bariwal JB, Upadhyay KD, Naliapara YT, Joshi SK, Pannecouque CC, De Clercq E, Shah AK. Improved and rapid synthesis of new coumarinyl chalcone derivatives and their antiviral activity. Tetrahedron Letters. 2007; 48(48):8472-4.
59. Kalirajan R, Sivakumar SU, Jubie S, Gowramma B, Suresh B. Synthesis and biological evaluation of some heterocyclic derivatives of chalcones. International J. of Chem Tech Research. 2009; 1(1):27-34.
60. Vazquez-Rodriguez S, Figueroa-Guíñez R, Matos MJ, Santana L, Uriarte E, Lapier M, Maya JD, Olea-Azar C. Synthesis of coumarin–chalcone hybrids and evaluation of their antioxidant and trypanocidal properties. MedChemComm. 2013; 4(6):993-1000.
61. Polo E, Ibarra-Arellano N, Prent-Peñaloza L, Morales-Bayuelo A, Henao J, Galdámez A, Gutiérrez M. Ultrasound-assisted synthesis of novel chalcone, heterochalcone and bis-chalcone derivatives and the evaluation of their antioxidant properties and as acetylcholinesterase inhibitors. Bioorganic chemistry. 2019; 90:103034.
62. Sable PM, Potey LC. Microwave Assisted Synthesis of Chalcone and Biological Activity. Scholars Research Library. 2018; 10(4):68-78.
63. Durgapal SD, Soni R, Soman SS, Prajapati AK. Synthesis and mesomorphic properties of coumarin derivatives with chalcone and imine linkages. Journal of Molecular Liquids. 2020; 297:111920.
64. Joseph L, George M. Anti-bacterial and in vitro Anti-diabetic Potential of Novel Isoxazole Derivatives. Journal of Pharmaceutical Research International. 2016:1-7.
65. Algethami FK, Saidi I, Abdelhamid HN, Elamin MR, Abdulkhair BY, Chrouda A, Ben Jannet H. Trifluoromethylated Flavonoid-Based Isoxazoles as Antidiabetic and Anti-Obesity Agents: Synthesis, In Vitro α-Amylase Inhibitory Activity, Molecular Docking and Structure–Activity Relationship Analysis. Molecules. 2021; 26(17):5214.
66. Joshi SD, Dixit SR, Kirankumar MN, Aminabhavi TM, Raju KV, Narayan R, Lherbet C, Yang KS. Synthesis, antimycobacterial screening and ligand-based molecular docking studies on novel pyrrole derivatives bearing pyrazoline, isoxazole and phenyl thiourea moieties. European journal of medicinal chemistry. 2016; 107:133-52.
67. Suresh G, Venkata Nadh R, Srinivasu N, Kaushal K. Novel coumarin isoxazoline derivatives: Synthesis and study of antibacterial activities. Synthetic Communications. 2016; 46(24):1972-80.
68. Naidu KM, Srinivasarao S, Agnieszka N, Ewa AK, Kumar MM, Sekhar KV. Seeking potent anti-tubercular agents: Design, synthesis, anti-tubercular activity and docking

study of various ((triazoles/indole)-piperazin-1-yl/1, 4-diazepan-1-yl) benzo [d] isoxazole derivatives. Bioorganic & medicinal chemistry letters. 2016 ; 26(9):2245-50.

69. Palleapati K, Kancharlapalli VR, Shaik AB. Synthesis, characterization and antitubercular evaluation of some new isoxazole appended 1-carboxamido-4, 5-dihydro-1H-pyrazoles. J. Res. Pharm. 2019; 23(2):156-63.
70. Eid AM, Hawash M, Amer J, Jarrar A, Qadri S, Alnimer I, Sharaf A, Zalmoot R, Hammoudie O, Hameedi S, Mousa A. Synthesis and biological evaluation of novel isoxazole-amide analogues as anticancer and antioxidant agents. BioMed Research International. 2021; 20-21.
71. Battula K, Narsimha S, Nagavelli VR, Srinivasa RM. Synthesis and biological evaluation of (3-arylisoxazol-5-yl) methyl 6-fluoro-4-oxo-4H-chromene-2-carboxylates as antioxidant and antimicrobial agents. Journal of the Serbian Chemical Society. 2017; 82(1):1-2.
72. Beyzaei H, Deljoo MK, Aryan R, Ghasemi B, Zahedi MM, Moghaddam-Manesh M. Green multicomponent synthesis, antimicrobial and antioxidant evaluation of novel 5-amino-isoxazole-4-carbonitriles. Chemistry Central Journal. 2018; 12(1):1-8.
73. Dua JS, Singh J. Chalcone Ditosylates as Potent Precursor for Synthesis of Some 4, 5-Disubstituted Isoxazoles with Antioxidant and Anti-inflammatory Activities. Asian Journal of Chemistry. 2019; 31(8):1847-50.
74. Kurt BZ, Gazioglu I, Sonmez F, Kucukislamoglu M. Synthesis, antioxidant and anticholinesterase activities of novel coumarylthiazole derivatives. Bioorganic chemistry. 2015; 59:80-90.
75. Hammond GS, Stout CA, Lamola AA. Mechanisms of photochemical reactions in solution. XXV. The photo dimerization of coumarin. Journal of the American chemical Society. 1964; 86(15):3103-6.
76. Borges F, Roleria F, Milhazes N, Santana L, Uriarte E. Simple coumarins and analogues in Medicinal Chemistry: Occurrence, Synthesis and biological activity. Current Medicinal Chemistry. 2005; 12(8):887-916.
77. Joule JA, Mills K. Heterocyclic Chemistry. Jhon Wiley & Sons. 2008.
78. Rodd EH, Sainbury M. Rodd's Chemistry of Carbon Compounds: A Modern Comprehensive Treatise: Second Supplement to the (2nd Edtn). Elsevier; 1995.
79. Robertson DN, Link KP. Studies on 4-hydroxy coumarins. XII. 3-Substituted-aminomethyl-4-hydroxy coumarins derivatives by Mannich Reaction. Journal of the American Chemical Society. 1953; 75(8): 1883-5.

80. Gomes MN, Muratov EN, Pereira M, Peixoto JC, Rosseto LP, Cravo PV, Andrade CH, Neves BJ. Chalcone derivatives: promising starting points for drug design. Molecules. 2017; 22(8):1210.
81. P.S. Satpati and J.P. Trivedi, (1960) Curr. Sci. (India), 29, 429 (1960).
82. Dr. Rajarshi N Patel, Keten S. Patel, Jiten C. Patel; Chemistry of Chalcone Synthesis and its derivatives; LAMBERT academic publishing; 2016: 9-11.
83. Raghvendra Singh Raghuvan Shi and Krishna Nand Singh.tetraethyl ammonium superoxide induced Michael addition of active methylene compounds to chalcones. Indian Journal of Chemistry. August 2009; 48B: 1161-1163.
84. Ze Zhang, Ya Wei Dong, Guan Wu Wang. Highly efficient mechanochemical reactions of 1,3- Dicarbomnyl compounds with Chalcones and Azachalcones catalysed by Potassium Carbonate. Chemistry Letters. 2004; 33:61-64.
85. Javad Safaei-Ghomi, Mohammad Ali Ghasemzadeh. An efficient root to the synthesis of Pyrimidine -2-ones under ultrasound Irradiation. Digest Journal of Nanomaterials and Biostructures.2002; 5(2): 303-306.
86. Mustafa ceylan, Hayreddin gezegen. Preparation of 1,5-Diketones by Addition ofCyclohexanone to Chalcones under Solvent-free PhaseTransfer Catalyst Condition. Turk J Chem 2008; 32: 55 – 61.
87. Hirokazu lida,Tatsuya Moromizato, Hiroshi Hamana and Kiyoshi Matsumoto. Tetrahedron Letters., March 2007; 48 (11): 2037-2039.
88. M J Menezes, S Manjerkar,V Pai,R E Patre & S G Tilve. A Facile Microwave assisted Synthesis of Flavones. Indian Journal of Chemistry. September 2009; 48B: 1311-1314.
89. P N Balaji , M Sai Sreevani and P Harini. Antimicrobial activity of some novel synthesized heterocyclic compounds from substituted chalcones Journal of Chemical and Pharmaceutical Research . 2010; 2(4): 754- 758.
90. Gupta RR, Kumar M, Gupta V. Text book of Heterocyclic Chemistry-II. 450-457.
91. Burger's Medicinal Chemistry, Sixth Edition, Vol: 1-8
92. Sajujoy,Parvathy S Nair, Ramkumar Hariharan,M.Radhakrishna Pillai, Detailed comparison of protein-ligand docking efficiency of GOLD ,a commercial package and Argus lab,a licensable freeware Insilico biology 2006; 6(6):601-5.
93. Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. Alpha- amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. Chemical Biology & Drug Design. 2021; 98(4):539-60.
94. http://www.python.org/

95. http://www.python.org/download/
96. http://www.molinspiration.com/cgi-bin/properties
97. Sai Chandra J. Design and pharmacological studies of new coumarin derived chalcone synthesized compounds. World Journal of Pharmaceutical Research. 2018; 7(7):1157-1166.
98. Silversteinm MR, Webster FX. Spectroscopic identification of organic compounds. (6^{th} edtn). Jhon Wiley & Sons, Inc 2005; 71-83.
99. Sharma YR. Elementary organic spectroscopy: Principles and chemical applications. Chand S & Company Ltd. 2007; 128-130.
100. Spirtovic-Halilovic S, Salihovic M, Trifunovic S, Roca S, Veljovic E, Osmanovic A, Vinkovic M, Zavrsnik D. Density functional theory: H- and 13 C-NMR spectra of some Coumarin derivatives. Journal of Serbian Chemical Society. 2014; 79(11):1405-11.
101. Vogel HG. Drug Discovery and Evaluation: Pharmacological Assay, (2^{nd} edtn), Spinger-Verlag, New york. 2003; 1042
102. Jabir KV, Jayalakshmi B, Hashim KM. *In-vitro* anti diabetic studies and phytochemical evaluation of Heracleum candolleanum. Asian Journal of Plant Science Research. 2014; 4(4):31-36.
103. Makarand A, Amisha V, Alice V, Yusuf K. Synthesis and evaluation of chalcone derivatives for its alpha amylase inhibitory activity. Organic Chemitry: An Indian Journal. 2014; 10(5):192-204.
104. Kazeem, M., Adamson, J. and Ogunwande, I. Modes of Inhibition of α-Amylase and α-Glucosidase by Aqueous Extract of Morinda lucidaBenth Leaf.2013; 1-6.