DRUG DESIGN, DOCKING STUDIES AND SYNTHESIS OF CERTAIN COUMARIN DERIVATIVES AND EVALUATION OF THEIR α- AMYLASE INHIBITORY ACTIVITY

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of the Degree of
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IN
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Submitted by
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OCTOBER 2017

CERTIFICATE

This is to certify that the M.Pharm dissertation entitled **"Drug Design, Docking Studies and Synthesis of Certain Coumarin Derivatives and Evaluation of their α- Amylase Inhibitory Activity"** being submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai was carried out by **Ms. LEKHA.P** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision and guidance to my fullest satisfaction.

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This is to certify that the α- Amylase Inhibitory studies which was part of the dissertation entitled **"Drug Design, Docking Studies and Synthesis of Certain Coumarin Derivatives and Evaluation of their α-Amylase Inhibitory Activity"** was carried out by **Ms. Lekha.P** in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my direct supervision and co-guidance to my fullest satisfaction.

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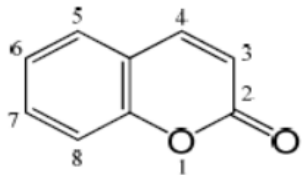
CONTENTS

INTRODUCTION

COUMARIN

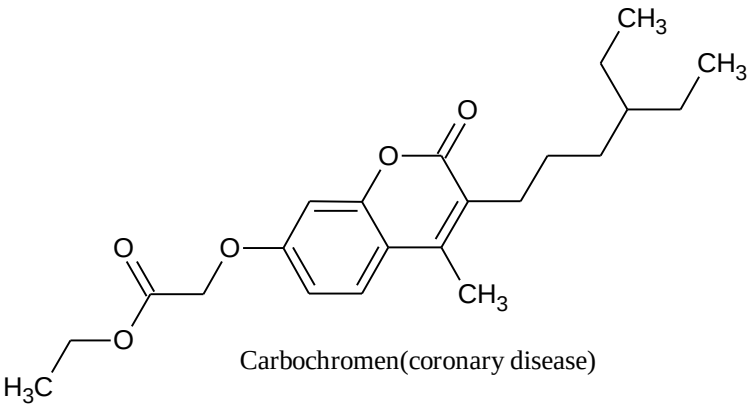
In Drug discovery, natural, synthetic and semisynthetic heterocyclic compounds play an important role in chemical biology. The heterocycles are mainly of the classes of alkaloids, flavones, isoflavones, chromans, chromones, coumarins and chromenes. It has been established that oxygen-containing heterocyclic compounds play an important role in designing new class of structural entities for medicinal applications. Among oxygen heterocyclic compounds, coumarin (2H-chromen-2-one or 2H-1-benzopyran-2-one) and its derivatives are significant because of their wide spectrum of biological activities.[1]



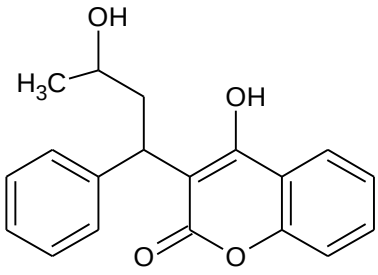
2H-chromen-2-one or 2H-1-benzopyran-2-one

The word coumarin derived from a French word *coumarou*. Coumarin is a large class of natural extract products, it considered to be the secondary metabolite in many higher plant species, particularly in leaves, seeds and roots[2]. They are found at high levels in some essential oils, particularly cinnamon bark oil (7,000 ppm), cassia leaf oil (up to 87,300 ppm) and lavender oil[3]. Coumarin is also found in fruits (e.g. bilberry, cloudberry), green tea and other foods such as chicory. Most of the coumarins occur in higher plants, with the richest sources being *Rutaceae* and *Umbelliferone*[4]. It was been found in the tonka bean, was isolated in 1820.*C*oumarin plays an important role in regulation of plant growth and metabolites. According to different substituents in the overring, the coumarins

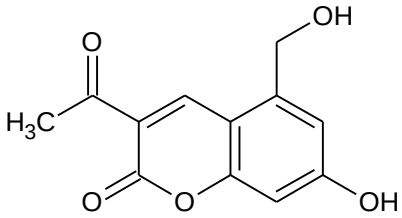
can be classified into simple coumarins, furano coumarins, pyrano coumarins and other coumarins, of which the furano coumarins and pyrano coumarins can be divided into linear type and angular type. Both natural and synthetic coumarin derivatives are considered to have a wide range of biological activity, such as anti-diabetic[5] , anti-inflammatory[6], anticancer[7,8], anti-coagulant[9], anti-oxidant[10], anti-HIV[11] and anti-bacterial[12,13], antiallergic, and antiproliferative activities[14]. Biological activity of coumarin has becoming an appealing point of studies owing to its different effects to diseases and less damage to normal cells[15]. Previous studies demonstrated that coumarin chalcone fibrates can down-regulate the total cholesterol (TC), phospholipids (PL), and triglycerides (TG), and regulate the levels of very low density lipoprotein(VLDL), low-density lipoprotein(LDL) and high density lipoprotein(HDL)[16]. Furthermore, 4-hydroxycoumarin dimers and Benzopyranocoumarin derivatives showed a powerful effect against HSV-1, HSV-2 and *vaccinia* virus at a nontoxic concentration[17]. Furthermore, the pharmacological properties as well as therapeutic applications of coumarins depend upon the pattern of substitution. Apart from their therapeutical activities, it is also an important analytical agent. Regarding their high fluorescence ability, they are widely used as optical whitening agents, brighteners, laser dyes and also as fluorescent probes in biology and medicine[18]. Phytoalexins, a biosynthetic hydroxylated derivative of coumarins, which are produced in carrots will respond to fungal infection and can be assumed to have antifungal activity. General antimicrobial activity was provided in Woodruff (*Galium odoratum*) extracts. When coumarin moiety is fused with other moieties, a synergistic effect occurs in biological activities. Such compounds are exploited as important scaffolds for drug development.Some of the coumarin derivatives are already booming in the market. Examples are given below in the figures



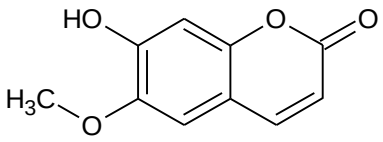
Carbochromen(coronary disease)



warfarin (anticoagulant)



Armillarisin A (antibiotic)



Scopoletin

Most common Biological Activity and Pharmacological Activity of Coumarins:

A. ANTI-INFLAMMATORY ACTIVITY

Osteoarthritis, Alzheimer's disease, Cancers are associated with chronic inflammation. A Coumarin derivative IMMLG5521 has the anti-inflammatory activity of rat lung edema, which exhibits its effects by inhibiting the TNF-α expression, increasing the VCAM-1 and ICAM-1 expression level[19].

B. ANTICOAGULANT ACTIVITY

Vitamin K plays an important role in blood coagulation. The structure of warfarin is similar to vitamin K and it has anticoagulant activity which act as the vitamin K antagonist. Initially Warfarin was used as the poison to exterminate rats and then it has been the drug of anticoagulant for almost 60 years. Vitamin K can prompt the Vitamin K dependent coagulation factors (II, VII, IX, X), Warfarin exerts its anticoagulant activity by inhibiting the formation of vitamin K dependent coagulation factors (II, VII, IX, X) in the liver microsomal[20].

C. ANTICANCER ACTIVITY

Malignancies have become the biggest threat to human health. The anticancer activity seems to be the most important properties of coumarins. A coumarins derivative (RKS262) synthesised could inhibit ovarian cancer cells proliferation.

D. 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[5].

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[21].

Type 2 Diabetes Mellitus results from interaction between genetic, environmental, behavioural factors[22,23], 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[24,25]. 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.

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[26,27].

ENZYMES[28]

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 reactions 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 enzyme since it begins its process by digesting the starch and breaks them into smaller pieces with two or three glucose units.

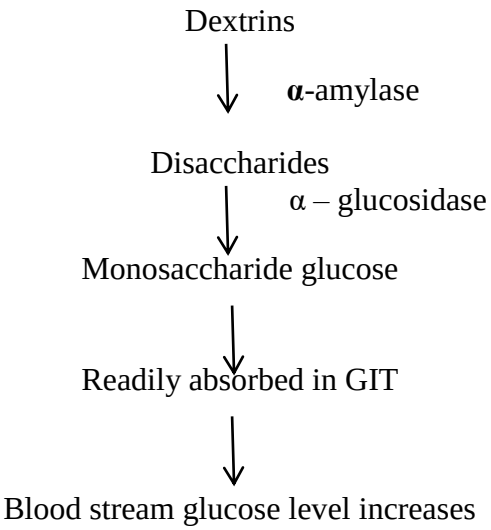
α – Amylase[29]

α- amylase is found in the saliva, pancreatic secretions, and in the gastrointestinal tract. It severs an obvious role in polysaccharide digestion. α-amylase determination has been recognized as an important role for the diagnosis of diabetes for many years because elevated levels of the enzyme are associated with liver and pancreatic disorders.

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.

Strach (insoluble)

↓ **α**-amylase



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 comes 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.

Enzyme inhibitors[30]

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 enhance 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 error. 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[31]

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.

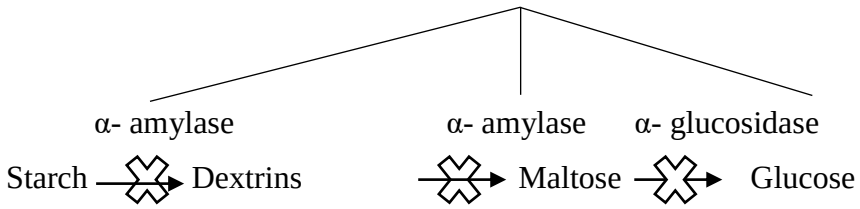
The inhibition of α- amylase is by.,

- ***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



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 compounds obtained from microbes.

Drug discovery[32-35]

Drug discovery and development is a research process where, a new chemical entity (synthetic or natural) is recognized and developed by designing and screening against a proper biochemical target. It is an innovative science where knowledge and technologies are incorporated to convert a chemical moiety into useful therapeutic drug. This system approaches the discovery in a trial and error method.

Drug discovery is an expensive and time consuming process. In this area a new drug may be developed or existing drugs may be modified with

- Same therapeutic activity with lesser toxic effects
- More therapeutic activity with lesser toxic effects

Drug discovery involves different phases and is a team effort which requires constant participation and heartful in, designing and experimental findings, development and marketing disciplines.

Drug design

Drug designing is otherwise known as rational drug design and it is a method of finding new medication based on the biological receptors and target molecules. It is also a process of developing a drug with high therapeutic index and specific action. It involves the identification of a compound that displays a biological profile and ends when the biological profile and chemical synthesis of the new chemical entity are optimized. Drug design includes the design of small molecules, which are having similarity in shape and charge to the biomolecular target to which the drugs will bind. Here drug is an organic small molecule which will either activate or inhibit the function of a protein.

Aim of drug design

- To improve potency
- To modify specificity of action
- To improve duration of action
- To reduce toxicity
- To effect ease of application or administration or handling
- To improve stability
- To reduce cost of production

Modern method of drug designing is done with the aid of computers and hence, the process is known as Computer Assisted/ Aided Drug Design (CADD).

It uses computational chemistry to study about the drugs and related biologically active molecules. The major aim is to find whether the given molecule bind to the target and causes pharmacological action or not.

The basic steps involved in CADD are:

- Hit identification using virtual screening
- Hit-to-lead optimization and selectivity
- Lead optimization of other pharmaceutical properties maintaining affinity

Drug design help to explain the following:

- The effects of biological compounds on the basis of chemical properties of the molecule involved.
- Different processes by which the drugs shows their pharmacological effects.
- How the drug react with the sites to produce a particular pharmacological response
- How the drugs are detoxicated , metabolized or eliminated by organism
- The relationship between chemical structure and biological response.

Types of Drug Design

- Ligand based drug design
- Structure based drug design
- De novo design
- Homology modelling

Ligand Based Drug Design (LBDD)

Ligand based method is used to know about inhibitors for the target receptors, in the absence of the structural information of the target. It is also

known as indirect drug design. By using structural or topological similarity or pharmacophoric similarity properties, biologically lead molecule is detected. There are several criterias for similarity comparisons such as structure as well as shape of individual fragment or electrostatic properties of the molecule. The generated lead molecules are ranked based on their similarity score or obtained by using different methods or algorithms.

Structure Based Drug Design (SBDD)

SBDD is used to create a homology model of target when the experimental structure is unavailable. It is also known as direct drug design. The ultimate goal of structure-based drug design is to develop a simple, robust process that starts with ahigh-resolution crystal structure of a validated biological macromolecular target and finally generates an easily synthesised, high-affinity small molecule with desirable pharmacological properties. It depends on the knowledge of 3D structure of the biological target that is obtained via methods such as X-ray crystallography, NMR spectroscopy or homology modelling. With the three dimensional structure of the target obtained from X-ray crystallography or NMR spectroscopy, one can begin the search for a ligand whose orientation and conformation is complimentary to the receptor structure.

Typically, the process involves

- Obtaining the structure of the target protein
- Identification of active site
- Virtual screening of a small molecule database
- Identification of potential ligands based on a chosen scoring function.

Drug Discovery methods[36,37]

1. Real screening
2. Virtual screening

Real screening

It include methods like high-through screening (HTS) which can experimentally check the activity of hundreds or thousands of compounds against the particular target limited time. Although, it is highly expensive, it provides real results which can be used for drug discovery.

Virtual screening

Virtual screening is a process for searching ligands i.e.; small biologically active molecules by means of computer assisted technique on the basis of biological structure. The crucial aim of virtual screening is the reduction of the enormous small organic molecules, to synthesize and screen against a particular target protein, to a manageable number of the compound as drug candidate.

Steps involved in drug design[35]

The drug design process may be categorized into following four distinct stages:

- **Selection and identification of the target**

Drug discovery process begins with the identification of a possible therapeutic target. The selected drug target must be a key molecule involved in a specific metabolic or cell signalling pathway that is known or believed to be related to a particular disease state.

Important drug targets include:

- Enzymes (inhibitor- reversible or irreversible)
- Receptors (agonist or antagonist)
- Nucleic acid intercalators or modifiers
- Ion channels (blockers or openers)
- Transporters (uptake inhibitors)

The 3D structure of the protein target is usually obtained by X-ray crystallography (crystal structures of different macromolecules are available from the research collaborator for structural bioinformatics (RCBS) protein database), Nuclear Magnetic Resonance (NMR) or homology modelling from a previously determined structure.

- **Search for lead (lead identification)**

Lead structures are ligands which are selected from a series of related compounds that exhibit suboptimal target binding affinity. After lead selection, they are tested for their activity towards a desired drug target.

a) De novo molecular design

This approach is used to design new structures by sequentially adding molecular fragments to a growing or by adding functionality to an appropriately sized molecular scaffold.

b) Database search methods

The lead molecule can be selected by screening structures found in various chemical database which contain an ocean of scaffolds.

c) Combinatorial methods

Combinatorial chemistry helps to create a large library of varied molecular structures by using a single scaffold and diverse array of reactants.

- **Lead optimization**

Lead optimization is a process in which lead compounds are altered to make them more effective and safer i.e., to achieve maximum affinity to the target

with improved bioavailability and low toxicity. By effective combination of two or more active moieties or by elimination or substitution of various groups, the properties of the lead compound can be modified.

Drug Likeness[38,39]

Drug likeness may be defined as a complex balance of various molecular properties and structural features which in turn determine whether a particular molecule is similar to the known drugs.

The concepts of drug likeness play an essential role in the transformation of a clinical candidate to a marketed product. These likeness properties can be found out from the molecular structure before synthesizing and testing a substance.

A drug should have both water and fat solubility because an orally administered drug has to go through the intestinal lining, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside of the cell. Partition coefficient known as log P is used to estimate solubility.

It has to be sufficiently water soluble because the drug is transported in aqueous media like blood and intercellular fluid. Solubility in water can be estimated from the number of hydrogen bond donors vs. alkyl side chain in the molecule.

Low water solubility leads to slow absorption and action. Too many hydrogen bond donors leads to low fat solubility, so that the drug cannot penetrate the cell wall reach the inside of the cell.

Smaller molecular weight is better, because diffusion is directly affected. 80% of traded drugs have molecular weights under 450 Dalton. Using a drug

likeness index leads to rejecting nonviable lead compounds before they are even synthesized. One of the traditional rules of thumb is Lipinski's Rule of five.

Lipinski's Rule of five

It is a rule of thumb which evaluates drug likeness and it determine whether a chemical compound can make an orally active drug in human. It may have specific pharmaceutical or biological properties that would make it a likely orally active drugs in humans. Most medications are relatively small lipophilic molecule. Based on this Christopher A Lipinski formulated this rule. The rule is important for drug development where a pharmacological active lead structure is optimized stepwise for increased activity and selectively as well as drug like properties as described by Lipinski's rule.

The Rule:

Lipinski's rule of five states that, in general, an orally active drug has:

1) Not more than 5 hydrogen bond donors (OH & NH group)
2) Not more than 10 hydrogen bond acceptors (N & O groups)
3) Molecular weight under 500g
4) Partition co efficient log P less than 5
5) Number of violations less than 5

It is to be noted that all the numbers are multiples of five, which is the basis for the rules name.

Improvements

- Partition coefficient Log P is -0.4 to 15.6 range
- Molecular refractivity from 40-130
- Molecular weight from 160-480

- No. of heavy atoms from 20 to 70
- Polar surface area must not be greater than 140 Å

Once the lead is being optimized, it is taken for the docking studies.

DOCKING[40,41]

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Preferred orientation helps to predict the strength of association of or binding affinity between two molecules. The associations with biological molecules such as proteins, nucleic acids, carbohydrates and lipids play an important role in signal transduction i.e. agonism or antagonism. So docking is a useful tool for predicting both the strength and type of signal produced.

Molecular docking may be defined as an optimization program, which would describe the 'best-fit' orientation of a ligand that binds to a particular protein of interest. The focus of molecular docking is to computationally stimulate the molecular recognition process. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.

A molecular docking calculation consists of the following steps:

- Optimization of the ligand geometry, calculation of pH-dependent partial charges, and identification of rotatable bonds.
- Calculation of electrostatic properties of the protein of interest and defining the ligand –binding region.
- Calculation of ligand-protein interaction by a scoring function that includes terms and equations that describe the intermolecular energies.

Docking produces plausible candidate structures. These candidates must be ranked by using scoring functions and to identify structures that are most likely to occur in nature.

Rigid-body docking and flexible docking

If the bond angles, torsion angles and bond lengths of the components are not modified at any stage of complex generation, then they are known as rigid body docking. A rigid-body docking is sufficiently good for most docking, when substantial change occurs within the components at rigid-body docking. Docking procedures which permit flexible docking procedures or conformational change, must intelligently select small subset of possible conformational changes for consideration.

Mechanics of docking

To perform a docking screen, the first requirement is a structure of interested protein. Usually the structure has been measured using a biophysical technique such as X-ray crystallography or NMR spectroscopy. The protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program is based on two components:

- **Search algorithm**

The search space includes all possible orientations and conformations of the protein paired with ligand. With present computing resources, it is impossible to exhaustively explore the search space; which involves enumerating all possible distortions of each molecule (molecules are dynamic and exist in an ensemble of conformational states) and all possible rotational and translational orientations of ligand relative to the protein at a given level of granularity. Most docking programs account for a flexible ligand, and several are attempting to model a flexible protein receptor. Each "snapshot" of the pair is referred to as a pose.

There are many conditions for sampling the search space. Here are some examples:

- Use a coarse-grained molecular dynamics simulation to propose energetically reasonable poses stimulation. (direct search-simplex method;

gradient-based search-steepest descent, Fletcher-Reeves method, Newton-Raphson method; least square methods-Marquardt method)

- Simulated annealing (Monte Carlo search of the parameter space)
- Use a "linear combination" multiple structures determined for the same protein to emulate receptor flexibility
- Use a genetic algorithm to "evolve" new poses that are successively more fragment-based construction.

➢ **Scoring function**

The scoring function takes a pose as input, returns a number indicating the likelihood that the pose represents the favorable binding interaction.

Most scoring functions are physics based molecular mechanics force fields that estimate the energy of the pose; a low (negative) energy indicates a stable system and thus likely for a binding interaction. It is an alternative approach to derive a statistical potential for interactions from a large database of protein-ligand complexes, such as the Protein Data Bank. This evaluates the fit of the pose according to this inferred potential.

There are a large number of structures from X-ray crystallography for complexes between proteins and high affinity ligands. It is comparatively fewer for low affinity ligands as the later complexes tend to be less stable and therefore more difficult to crystallize. Scoring function trained with this data can dock hits (ligands predicted to bind to the protein and actually does not, when placed together in a test tube).

Various softwares used for docking studies are:

- AutoDock 4.2
- Gold
- Vega

- Glide
- Flexidock
- Flex
- Fred
- Hint etc

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.

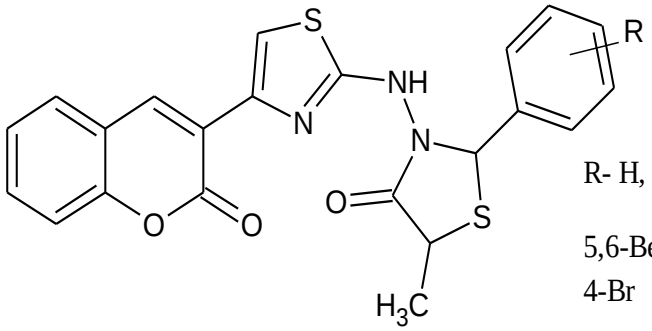
The aim of the present work was to synthesize new pyrazole and isoxazole derivatives containing coumarin moiety in order to explore the extent of their α-amylase inhibitory activity. The compounds were designed by *in silico* method using α- amylase as the target molecule.

LITERATURE REVIEW

COUMARINS

A. Coumarin as Antidiabetic agents

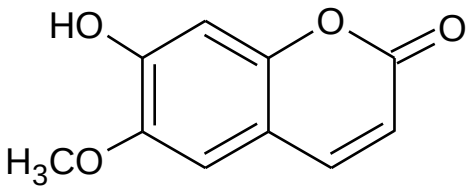
- **Deepthikini and Manjunathghate *et al.*, (2010),** performed a Synthesis on the series of 3-[5^{I}-Methyl – 2^{I} aryl- 3^{I} – (thiazol-2^{II}- yl amino) thiazolidin – 4^{I}-one] coumarin and screened for oral hypoglycemic activity in chemically induced Type 2 diabetic rates, to prove their promising activity[42].



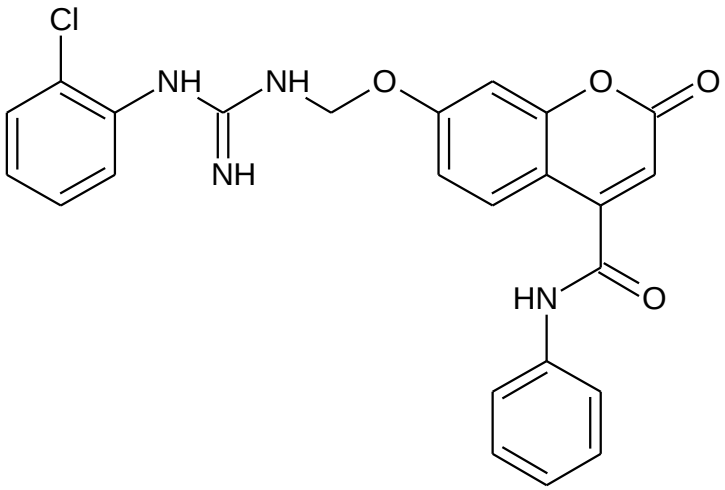
R- H, 4-CH_3, 4-Cl, 4-OH,

5,6-Benzo, 4-F, 4-NO_2, 2-Cl, 2-Br, 4-Br

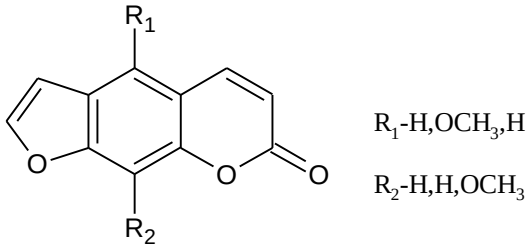
- **Anchal Verma *et al.*, (2013),** Performed an isolation of Scopoletin, a derivative of coumarins, 7-Hydroxy-6-methoxycoumarin was evaluated for the hypoglycemic and hypolipidemic activity in Wistar rats in streptozotocin induced diabetic rats[43].



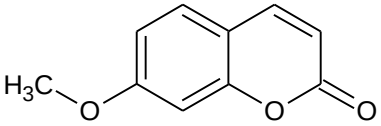
- **Vahan G. Prajapati *et al.*, (2013),** Performed an synthesis of Guanidine derivatives which is combined with 2H-chromene moiety and antidiabetic activity has been performed for guanidine derivatives which shows a promising activity[44].



- **Nagwa M. M. Shalaby *et al.*, (2014),** performed an isolation of furanocoumarins and in vitro evaluation of the antidiabetic activity of crude extract showed the most potent inhibiting power[45].



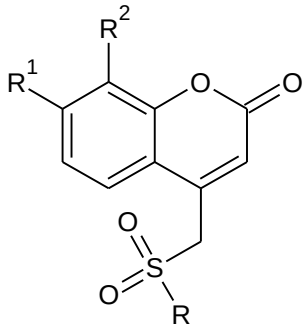
- **Andy Ramu and Veluchamy vijayakumar *et al.*, (2016),** Done a isolation of 7- methoxy coumarin from the bark of marine plant *Rhizophora mucronata* and screened for Invitro antidiabetic activity in chemically induced Wistar rats and male swis albino mice, which proved to coumarins having antidiabetic activity[46].



2. Coumarins with antimicrobial activity

a) Coumarins as antitubercular agents

- **Jeyachandran *et al.*, (2012),** performed a synthesis on the series of 4-aryl/ alkyl sulfonyl methyl coumarins and screened for in vitro antitubercular activity against Mycobacterium tuberculosis H37Rv (MTB) to prove their potential activity[47].

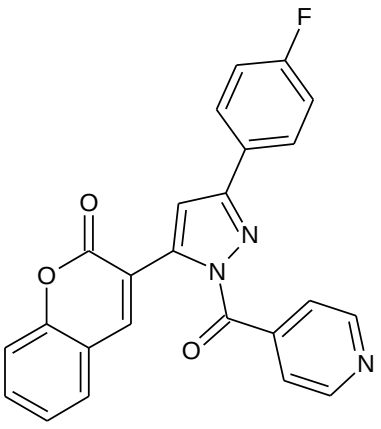


R-phenyl;p-Methylphenyl,p-chlorophenyl;
2-benzoxazolyl; 2-benzimidazolyl; cyclohexyl

R^1- H; OCH_3

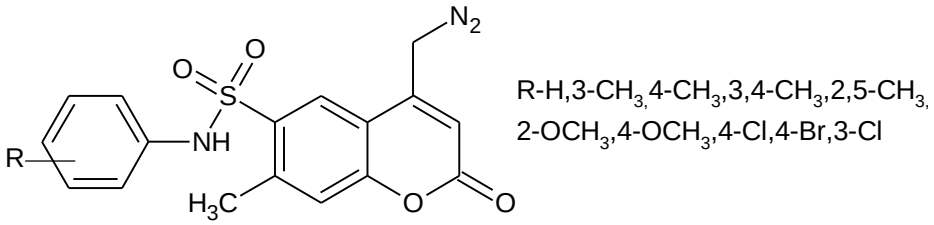
R^2- H; OCH_3

- **Kawate *et al.*,(2013),** investigated a series of coumarin derivatives conjugated with isoniazid and pyrazole moieties and evaluated their anti-TB activity against MTB H37Rv Using Resazurin MIC assay. The synthesised Compounds containing a4-fluoro group at C-3 phenyl ring of pyrazole ring showed promising antimycobacterial potential[48].

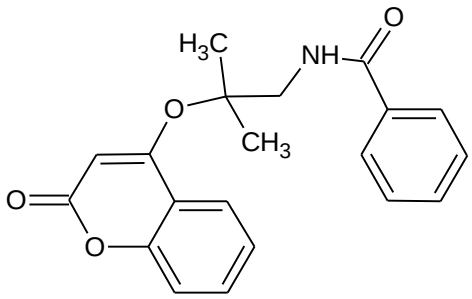


b) Coumarins with Antibacterial activity

- **Kulkarni *et al.*, (2010),** were synthesized some 4-azidomethyl-7-methylcoumarin-6-sulphonamides which showed very potent antibacterial activity[49].

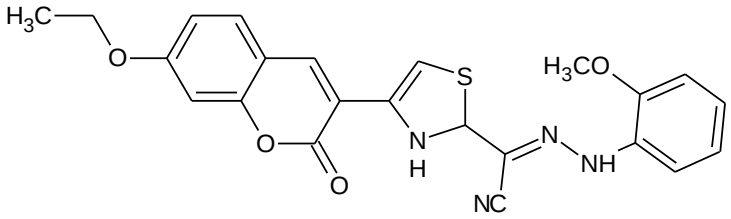


- **Lin *et al.*, (2012),** tested acyl coumarins, 4-hydroxy, and 7-Hydroxyl coumarins and coumaric amide dimers against *B. subtilis, S. aureus, E. coli and Pseudomonas aeruginosa* and Penicillin G potassium salt was used as a reference drug. The Compounds was the most potent compound out of the tested compounds against Bacillus subtilis[50]

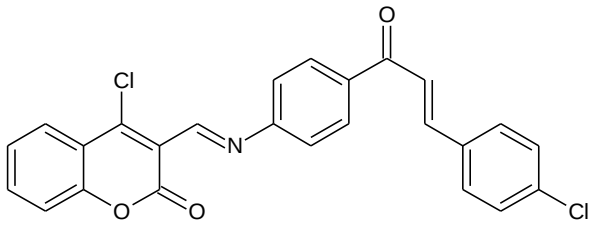


c) Coumarin as Antifungal agents

- **Mohamed *et al.*, (2012),** screened some 8-ethoxycoumarins for their *in-vitro* antimicrobial activities against two Gram negative B*ordetella bronchiseptica* and *E. coli and four Gram positive Bacillus pumilus, B. subtilis,S. aureus* and *Staphylococcus epidermidis* pathogenic bacteria and two fungi *Candida albicans* and *Saccharomyces cervesia.* Compound resulted in wide spectrum antimicrobial activity against all tested bacteria and fungi compared to ampicillin (25µg/ml) and mycostatin (25µg/ml) [51].

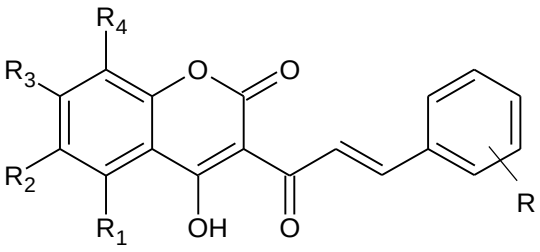


- **Kudale et al., (2012),** investigated a series of 3-(4-(4-(substituted phenyl)prop-1-ene-3-one) phenylimino) methyl)-4-chloro-2H-chromen-2-ones *in-vitro* against gram positive bacteria, *S. aureus, B. subtilis and S. epidermis* and *gram negative bacteria, E. coli, S. typhi* and *P. aeruginosa* and the antifungal activity was evaluated against *A. niger* and *Clostridium albicans* using amoxicillin and fluconazole as standard drugs for antibacterial and antifungal activities respectively. Compound was to be most active against all the tested organisms[52].



3. Coumarin as Antiviral agents

- **Trivedi *et al.*, (2007),** investigated a newly synthesized coumarinyl chalcone derivatives which were evaluated for their antiviral activity[49]. And shows potent activity[53].



R=H,4-OH,4-OCH_3,3-OC_6H_5,2-NO_2

R_1=CH_3,CH_3,CH_3,CH_3,CH_3

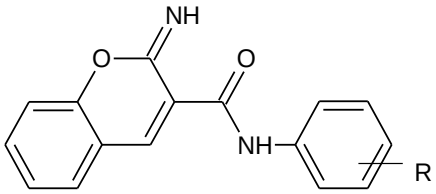
R_2=H,H,H,H,H

R_3=H,H,H,H,H

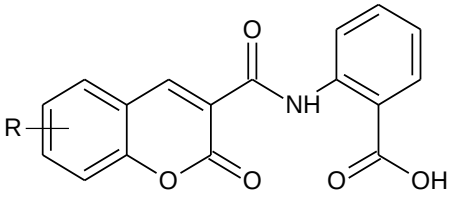
R_4=$CH(CH_3)_2$,$CH(CH_3)_2$,$CH(CH_3)_2$,$CH(CH_3)_2$, $CH(CH_3)_2$

4. Coumarins with Anti–inflammatory, Analgesic activity and Antipyretic agents

- **Bylov *et al.*, (1999),** evaluated a series of N-aryl substituted 2-imino -2H-1-benzopyran-3-carboxamides and 2-oxo-2H-1-benzopyran-3-carboxamides for anti-inflammatory activity in albino rats. The results were found to be comparable with piroxicam taken as the reference drug[54].

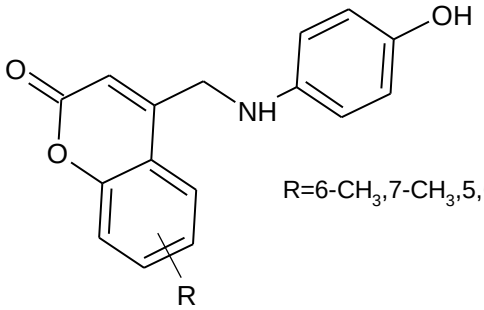


R=2-$COOCH_3$,4-$COOCH_3$



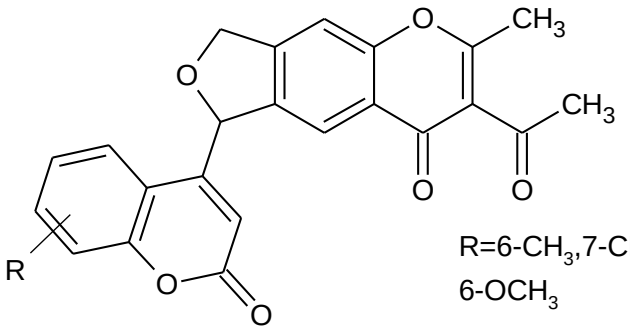
R=H,5-OCH_3,5-NO_2,5-Cl

- **Kalluraya *et al.*, (2001),** were synthesised 6-substituted-3-[4-(3-substituted pyrazolidine)hydrazine-4-thiazolyl] coumarins which exhibited analgesic activity[55].



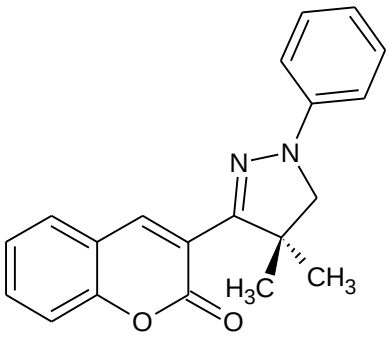
R=6-CH_3,7-CH_3,5,6-Benzo,6-Cl,7-Cl,6-OCH_3

- **Ghate and Kulkarni *et al.*, (2005),** have reported the synthesis of 4-(5'-acetyl-6'-hydroxy-3'-methyl benzofuran-2'-yl) coumarin and 6-acetyl-3,7-dimethyl-2-(coumarin-4'-yl)furo[3,2-g]chromen-5-one and established their good anti inflammatory and analgesic activity[56].

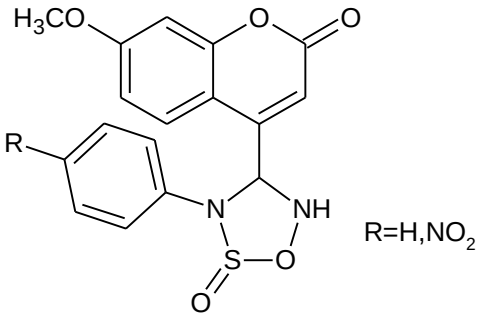


R=6-CH_3,7-CH_3,5,6-Benzo,7,8-benzo, 6-OCH_3

- **Khode *et al.*, (2009),** screened a series of 5-(substituted)aryl-3-(3coumarinyl)-1-phenyl-2-pyrazolines for their in vivo anti-inflammatory and analgesic activities. Some of the compounds exhibited significant analgesic activity and antipyretic activity with minimum ulcerogenic index[57].



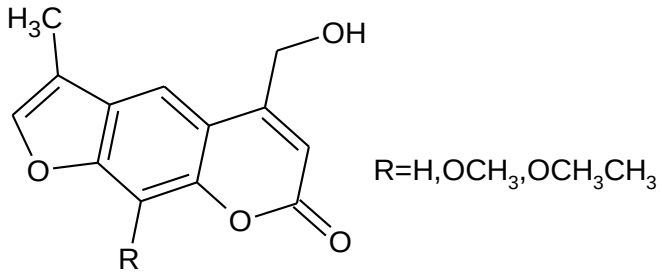
- **Bansal *et al.*, (2009),** were synthesised 7-methoxy-4-(3'-substituted 2'-oxo-1',2',3',5'-oxathiazol-4'-yl) coumarins which showed anti inflammatory activity[58].



R=H,NO_2

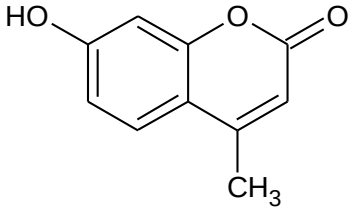
5. Coumarin as Vasorelaxant agents

- **Manuel Campose Toimil *et al.*, (2002),** were synthesised a new series coumarins and furocoumarins and evaluated their Vasorelaxant activity[59].

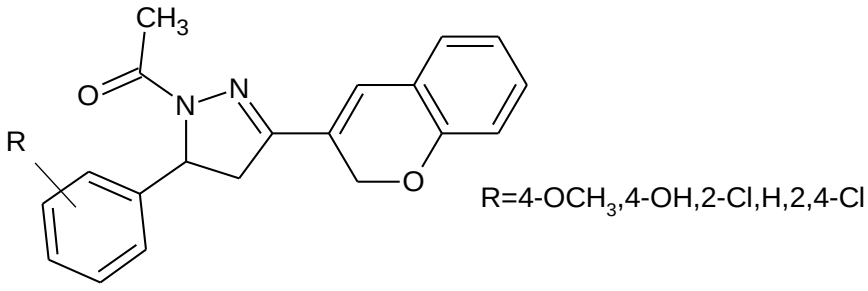


6. Coumarins with Anticancer activity

- **Bhattacharya *et al.*, (2009),** were synthesized 4-methyl-7-hydroxy coumarin and screened them for anticancer activity which have potential activity against DMBA-induced skin cancer in mice[60].

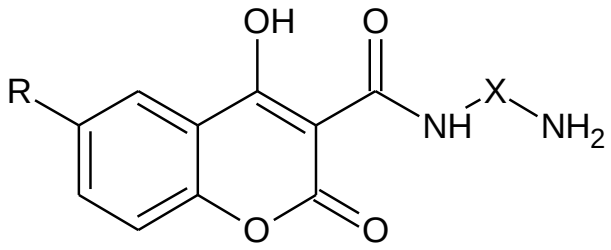


- **Xin-Hua liu *et al.*, (2010),** reported the synthesis of novel coumarin derivatives containing 4,5-dihydropyrazole moiety which exhibited potential antitumor activity[61].

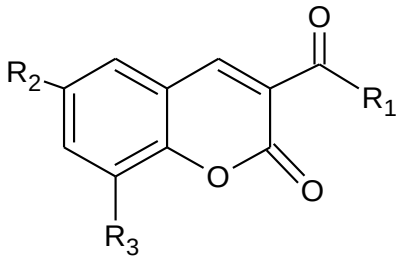


7. Coumarins with Antioxidant activity

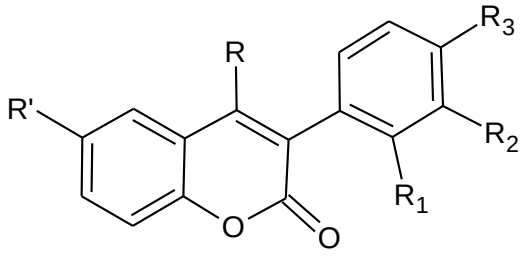
- **Melagraki *et al.*, (2009),** evaluated a series of coumarin-3-carboxamides for their in-vitro antioxidant activity and in-vivo anti-inflammatory activity. These derivatives were found to posses these activities[62].



- **Singh *et al.*, (2010),** tested 3-alkanoyl/aroyl/heteroaroyl-2H-chromene-2-thiones for their free radical scavenging capacity towards the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). These compounds exhibited profound antioxidant activity[63].

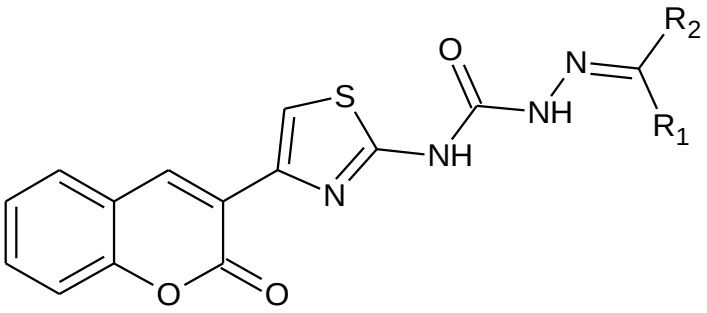


- **Roussaki *et al.*, (2010),** evaluated a series of coumarin analogues bearing a substituted phenyl ring on position 3 for their antioxidant activity by using two different antioxidant assays[64].



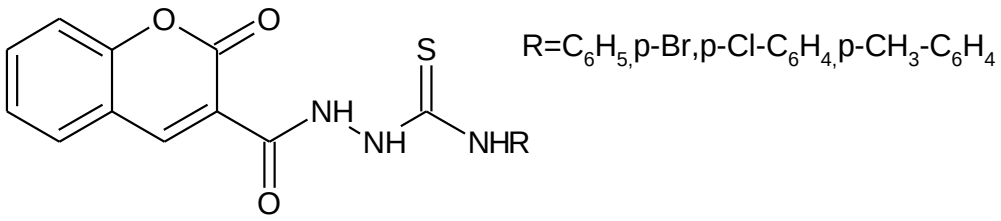
8. Coumarins with Antihyperlipidemic activity

- **Sashidhara *et al.*, (2010),** evaluated a series of coumarin bisindole heterocycles for antihyperlipidemic activity in hyperlipidemic hamster model.And showed a promising activity[65].

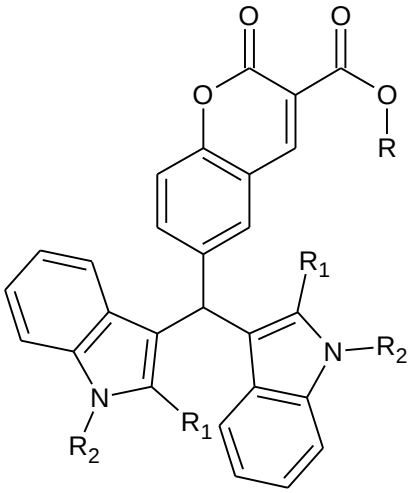


9. Coumarins with Anticonvulsant activity

- **Bhat *et al.*, (2006),** were synthesized a novel thio ureido derivatives of sulfonamides and thiosemicarbazido derivatives of coumarin and reported them as potential anti-Convulsant agent[66].



- **Siddiqui *et al.*, (2009),** tested some heteroaryl semicarbazones for their anticonvulsant activity using pentylenetetrazole (PTZ) induced seizure, maximal electroshock seizure (MES) and Neurotoxicity tests.The compounds having 3,4-Cl.C6H3, 2-OCH3.C6H4 and 4-Br.C6H4 exhibited significant anticonvulsant activity[67].



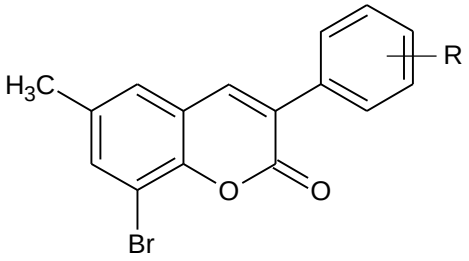
R-H,CH_3

R_1-CH_3, OH

R_2-H,CH_3

10. Coumarins with Antiparkinsonian activity

- **Matos *et al.*, (2009),** Evaluated a series of 8-bromo-6-methyl-3-phenylcoumarin derivatives as MAO-A and MAO-B inhibitors using R-(-)-deprenyl (selegiline) and Iproniazide as reference inhibitors[68].

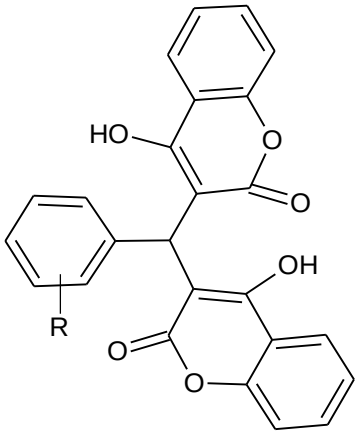


11. Coumarin with Anticoagulant activity

- **Abdelhafez *et al.*, (2010),** were synthesized 3-Pyridinyl, pyrimidinyl and pyrazolyl-4-hydroxycoumarin derivatives and a comparative in vivo (CT, PT determination) and in vitro (measurement of PIVKA-II levels) anticoagulant study with respect to warfarin showed that the synthesized compounds have different anticoagulant activities, the most prospective compounds were the 3-pyrazolyl-4-hydroxycoumarin derivatives[9].

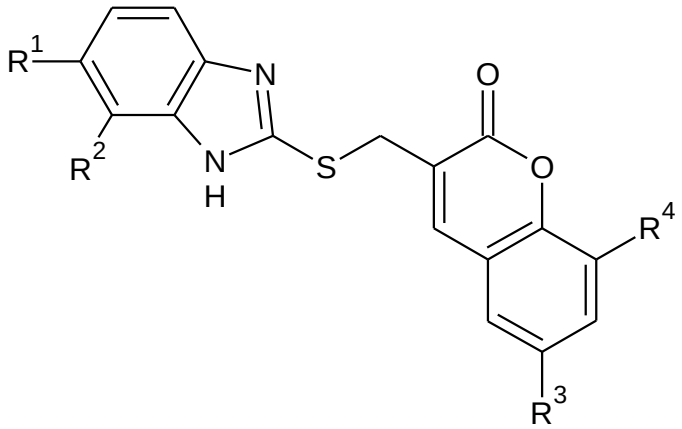
12. Coumarin with Anti HIV activity

- **Releva *et al.*, (2005),** were synthesized 3,3'-arylidene-bis-4-hydroxy coumarins and were shown to exhibit prominent anti HIV activity[69].



R=4-Br,4-OCH_3,2-OCH_3,2,4-diOCH_3, 2,3-diOCH_3

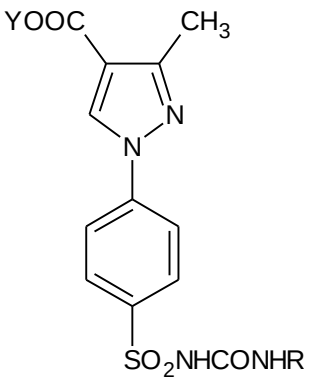
- **Hwa *et al.*, (2008),** were synthesized some new benzimidazole coumarin conjugates which exhibited anti hepatitis C antiviral activity[11].

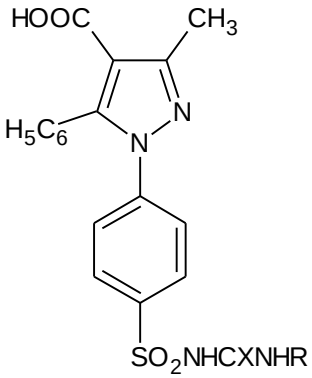


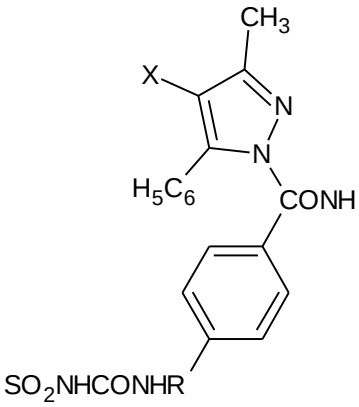
CHEMISTRY OF PYRAZOLE

1. Anti-Diabetic activity of pyrazoles

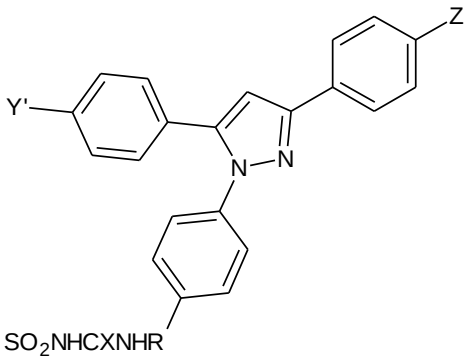
- **Raafat Soliman *et al.*, (1982),** Investgated for three series of 3,4,5-trisubstituted pyrazolesulfonylurea derivatives were prepared and evaluated as hypoglycemic agents. Preliminary biological testing revealed that the new compounds possess moderate hypoglycemic activity[70].



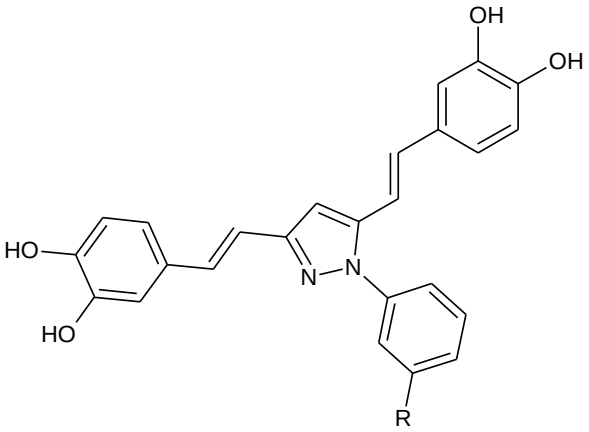




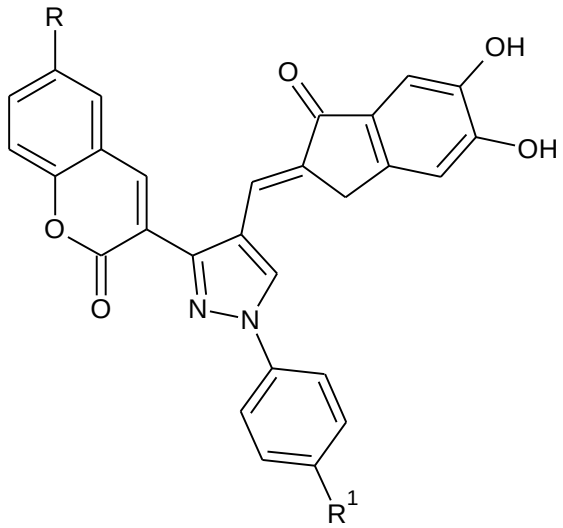
- **Hassan M. Faid-All *et al.*, (1987),** investigated a series of substituted p(3,5-diaryl-2-pyrazoline-l) benzenesulfonylurea and thiourea derivatives, along with their corresponding substituted p(3,5-diaryIpyrazole-l) benzenesulfonylurea and thiourea derivatives, were prepared for evaluation as hypoglycemic agents. Preliminary biological testing revealed that the new compounds possess potent hypoglycemic activity[71].



- **Honnalagere Ramesh Puneeth *et al.*, (2015),** Performed an Evaluation of *in-vitro* antidiabetic ability of curcumin pyrazole derivatives by the inhibition studies of the digestive enzymes, including alpha-amylase, rat intestinal alpha-glucosidase, and sucrose and showed a prominent inhibition of the enzymes[72]

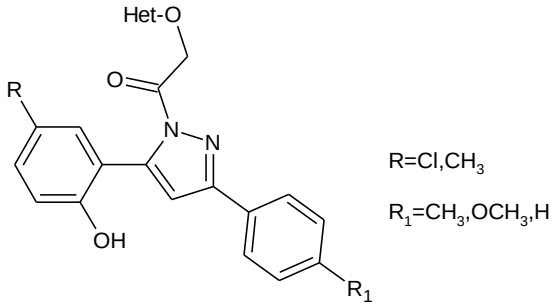


- **R.Kenchappa *et al.*, (2017),** done a synthesis of series of 6-substituted -3-(1-(4- substituted)-4- ((2)-95,6- dimethoxy-1-oxo-1H-inden-2(3H)-ylidene) methyl-1H-pyrazol-3-yl)- 2H-chromen-2-one derivatives and screened which posses potent anti oxidant and anti hyperglycemic agents, against chemically induced adult wistar rats[73].

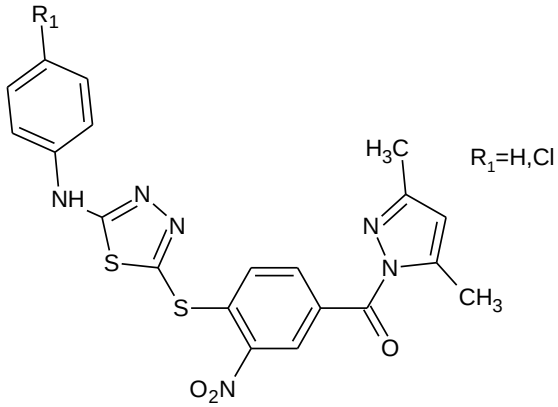


13. Antimicrobial activity of Pyrazoles

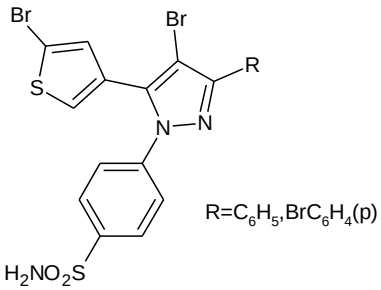
- **Bhawsar *et al.*, (1999),** were synthesised some new coumarino pyrazoles evaluated them for antimicrobial activity[74].



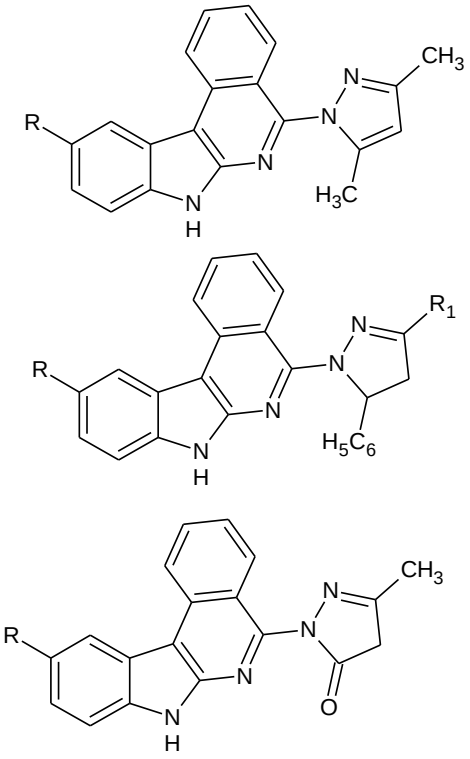
- **Sah *et al.*,(2002),** reported the synthesis of substituted pyrazoles bearing 2-aryl amino -5- mercepto-1,3,4- thiadiazole nuclei as possible antimicrobial agents[75].



- **Faidallah *et al.*, (2002),** were synthesised 3,5-disubstituted pyrazoles from chalcones. Their antimicrobial activities have been examined successfully[76].



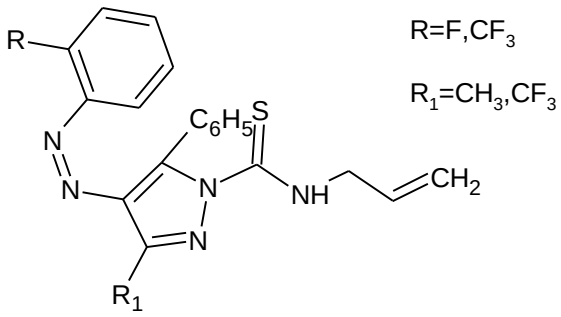
- **Hiremath *et al.*, (2002),** were synthesised substituted pyrazoles, pyrazolones and 3,5-disubstituted pyrazolines which exhibited antimicrobial activity[77].



R=Cl;

R_1=$C_6H_4OCH_3$(p)

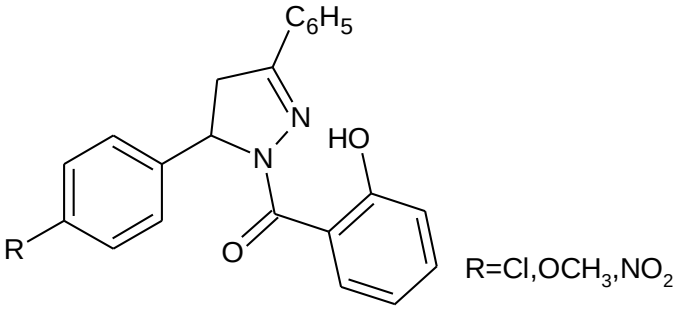
- **Garg *et al.*, (2002)**, were synthesised 1-thiocarbonyl 3-trifluoro methyl-5-phenyl-4(2-fluro phenyl azo) pyrazoles. These compounds showed significant antibacterial activity against S.*aureus,* S.*typhi* and E.*coli*[78].



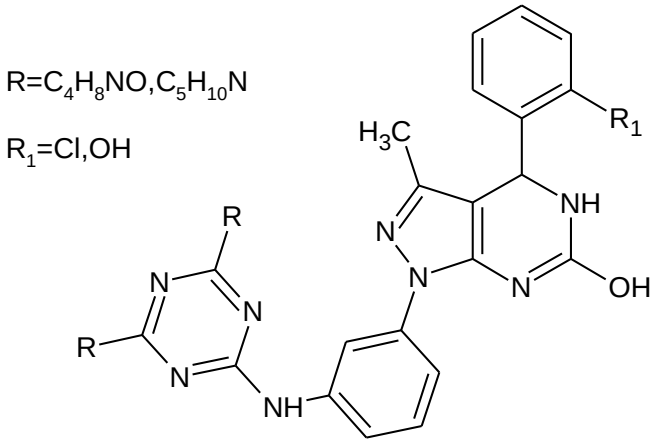
R=F,CF_3

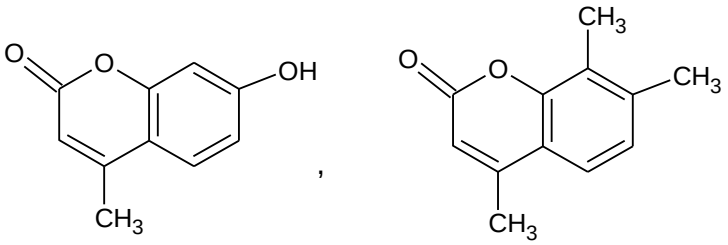
R_1=CH_3,CF_3

- **Garkwad *et al.*,(2004),** synthesised certain N-1-[2-hydroxy benzoyl]- 5 – substituted phenyl-3- phenyl 4,5- dihydro pyrazoles and screened them for antimicrobial activity[79].

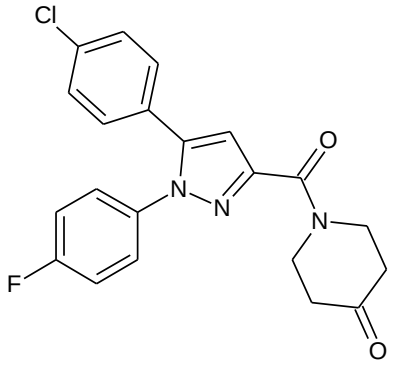


- **Mistry *et al.*, (2009),** succeeded in the synthesis of a series of pyrazolo (5,4-d) – pyrimidine derivatives which exhibited antimicrobial activity[80].



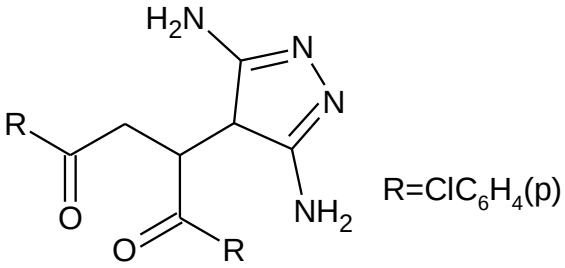
Het= 

- **Vijaykumar *et al.*, (2010),** synthesised novel 1,5 diaryl pyrazole derivatives which exhibited good antibacterial and antifungal activities[81].



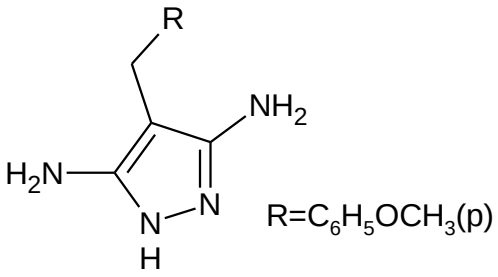
14. Antioxidant activity of pyrazoles

- **Padmaja *et al.*, (2009),** were synthesised substituted pyrazoles which possessed good antioxidant activity[82].



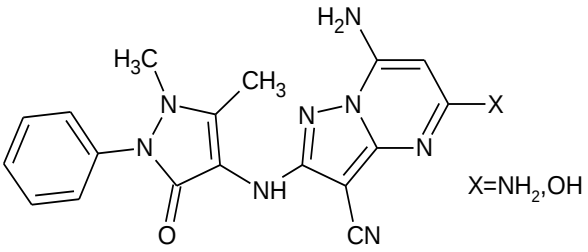
15. Antimalarial activity of pyrazoles

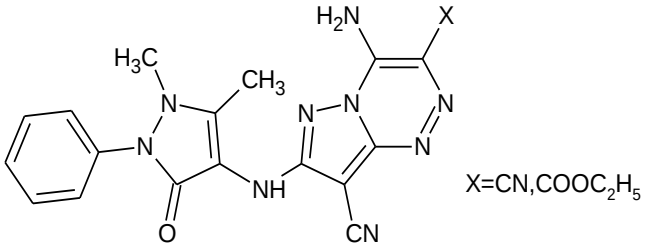
- **Vishnu *et al.*, (1994),** reported the synthesis of a few pyrazoles and these compounds were screened for anti malarial activity[83].



16. Antitumour activity of pyrazoles

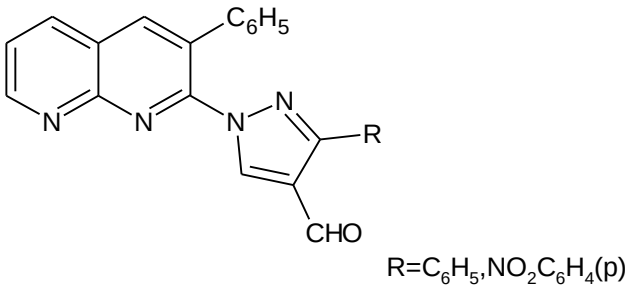
- **Fathalla *et al.*, (1998),** were synthesised 3-cyano-(p-antipyryl amino)-pyrazolo pyrimidines and pyrazolo triazines. Several biological activities have been established for pyrazolo derivatives. Various related compounds of this class have been found to be anti tumour and anti lukemic agents[84].



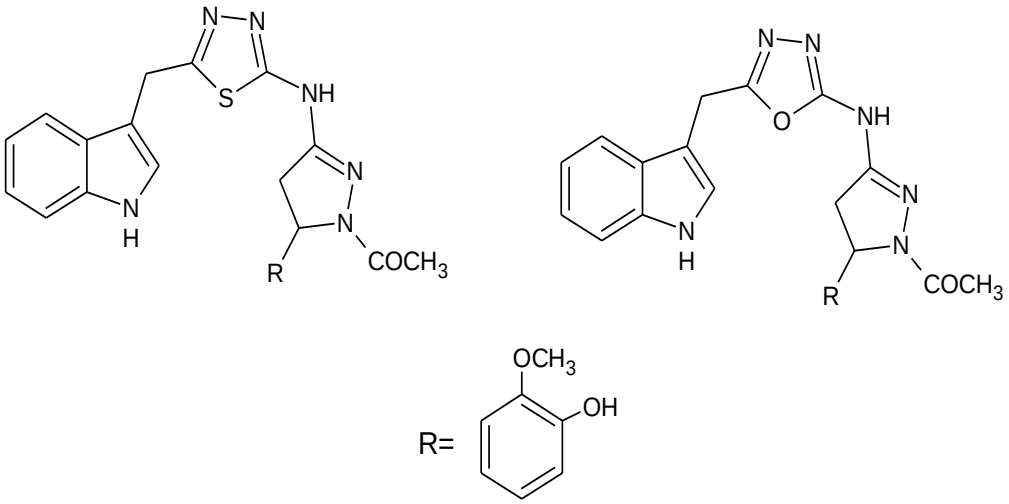


17. Anti inflammatory and Analgesic activity of pyrazoles

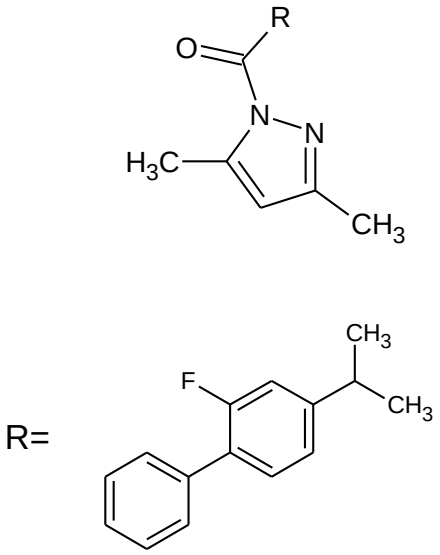
- **Rao *et al.*, (1996),** were synthesised 2-(3-aryl-4-formyl pyrazol-1-yl) 3-phenyl-1,8- napthyridines. It was reported that these compounds exhibit anti-inflammatory activities[85].



- **Sharma *et al.*, (2002),** were synthesised indolyl-thiadiazolyl- pyrazolines and indolyl- oxadiazolyl pyrazolines which showed anti-inflammatory activity[86].

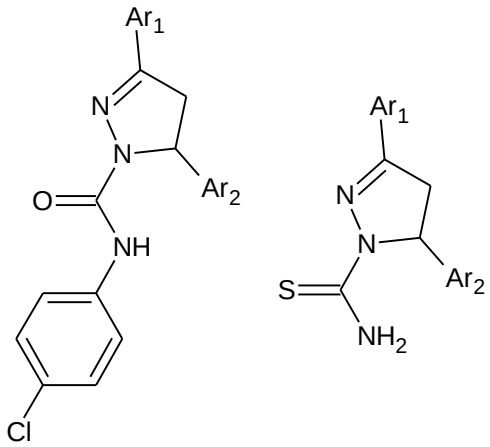


- **Amir *et al.*, (2005),** synthesised 3,5-dimethyl pyrazoles and evaluated their analgesic, ulcerogenic and anti inflammatory activities[87].



18. Anticonvulsant activity of Pyrazole

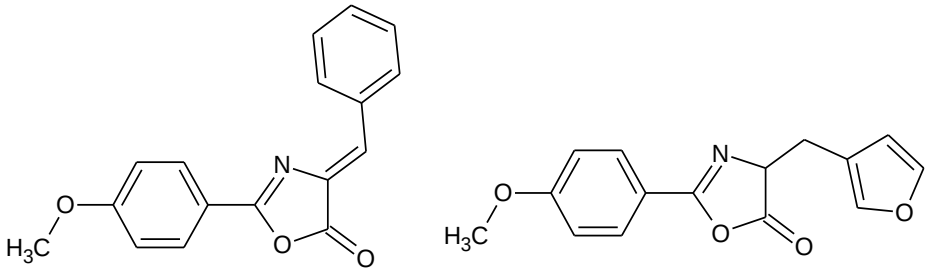
- **Nagihan Beyhan *et al.*, (2013),** Investigated 5-Disubstituted-4,5-dihydro-1H-pyrazole-1-carbothioamides and N-3,5-trisubstituted-4,5-dihydro-1H-pyrazole-1-carboxamides were synthesized. All compounds were tested for their anticonvulsant activity using pentylenetetrazole induced sei-zure (PTZ) and maximal electroshock seizure (MES) tests in mice which exhibited a potent activity in the tests[88].



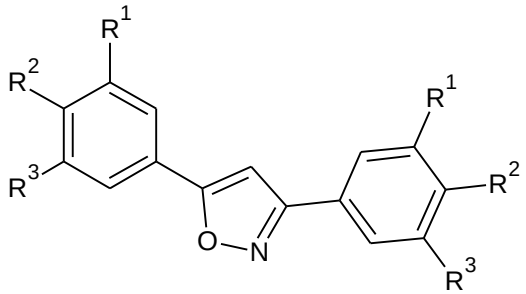
CHEMISTRY OF ISOXAZOLE

1. Antidiabetic activity of Isoxazole

- **G Mariappan *et al.*, (2011),** investigated a series of novel 4-arylidine 2-[4-methoxy phenyl] oxazol-5-one derivatives were synthesized and assayed in vivo to investigate their antidiabetic activities by streptozotocin-induced model in rat and shows prominent activity[89].

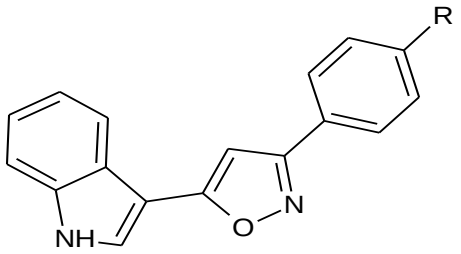


- **Lincy Joseph et al., (2016),** investigated a series of novel Isoxazoles derivatives. The prepared isoxazole compounds were subjected to in vitro anti-diabetic screening by yeast and enzymatic method. All compounds were screened for antibacterial action by disc diffusion method. 5-C and amine substituted phenyl ring at 3-C of isoxazole exhibited moderate anti-bacterial activity. In the anti-diabetic study halogenated or nitrated phenyl ring at 5-C and hydroxyl/amine substituted phenyl ring at 3-C of isoxazole exhibited anti-diabetic action[90].

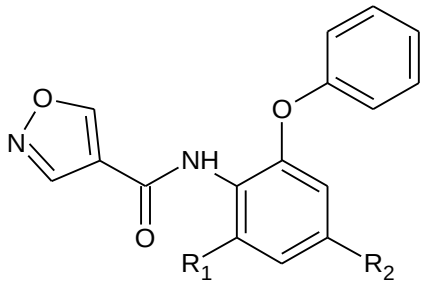


2. Antimicrobial and anti-inflammatory activity of Isoxazole

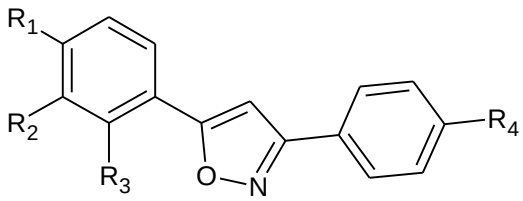
- **S. S. Panda et al., (2009),** investigation of Indolyl-isoxazoles which has been synthesised and the compounds were tested for the acute antiinflammatory activity and antibacterial activity using carrageenan-induced rat paw edema method and cup-plate method and the results exhibited good activity[91].



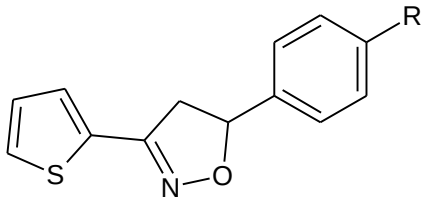
- **M. T. Shreenivas *et al.*, (2011),** investigated a series of isoxazole derivatives have been synthesized, The newly synthesized title compounds were screened for their in vitro antimicrobial activity. The compounds N-(2-chloro-5-nitrophenyl)-5-methylisoxazole-4-carboxamide and N-(4-cyano-3-(trifluoro-methyl)-phenyl)-5-methylisoxazole-4-carbox-amide are shown good antibacterial and anti fungal activity[92].



- **Sathish N.K *et al.*, (2011),** investigation of novel isoxazole derivatives were synthesized from various unstable Chalcones. The synthesized isoxazoles were evaluated for their anti-inflammatory activated and showed significant activity when compared to standard Diclofenac sodium[93].

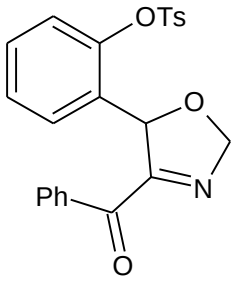


- **Kamala Chand Gautam *et al.*, (2013),** investigated a series of new isoxazole derivatives of thiophene were synthesised and the compounds were screened for antimicrobial activity by disc diffusion method. The result suggested that the four compounds were moderately to highly active[94].

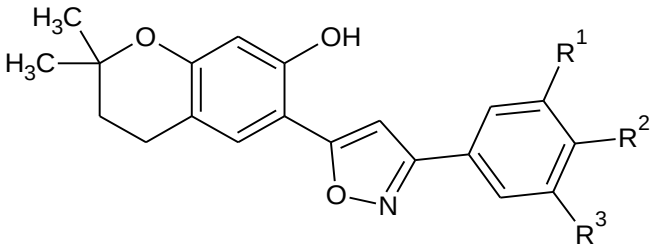


R-4C_8H_7O,3-$NO_2$4-NO_2,3-OCH_3,3-Cl,3-CH_3

- **Babasaheb V. Kendre *et al.*, (2015),** investigated a new series of pyrazole, isoxazole, benzoxazepine, benzothiazepine and benzodiazepine derivatives were prepared and all the synthesized compounds were evaluated and screened for their anti-bacterial, antifungal activities and anti-inflammatory activity. These derivatives showed good antibacterial activity and antifungal activity. Among the tested compounds for anti-inflammatory activity, the pyrazole derivatives have showed strong activity[95].

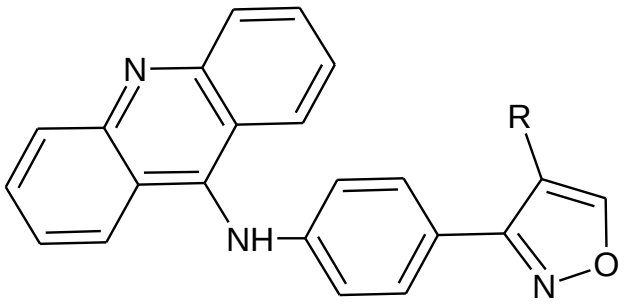


- **Gollapalli Naga Raju *et al.*, (2015),** Synthesis of 3-substituted phenyl-5-(2",2"-dimethyl, 7"-hydroxy chroman) isoxazoles. All the synthesized compounds were tested for their antibacterial and antifungal activity in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains and these compounds showed promising results against those strains[96].

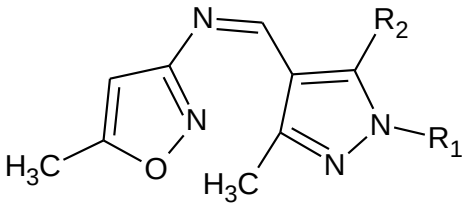


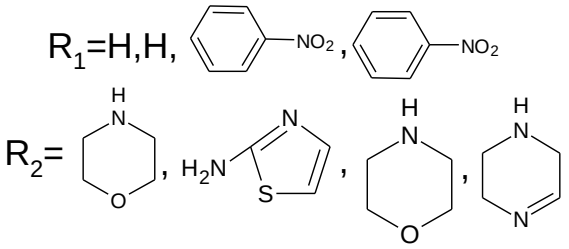
3. Anticancer activity of Isoxazole

- **R. Kalirajan *et al.*, (2012),** synthesised novel isoxazole-substituted 9-anilinoacridine derivatives. The compounds were confirmed by physical and analytical data and screened for in vitro antioxidant activity by DPPH method, reducing power assay and total antioxidant capacity method and the cytotoxic activity of the compounds was also studied in HEp-2 cell line. All the isoxazole-substituted compounds have significant activities[97].



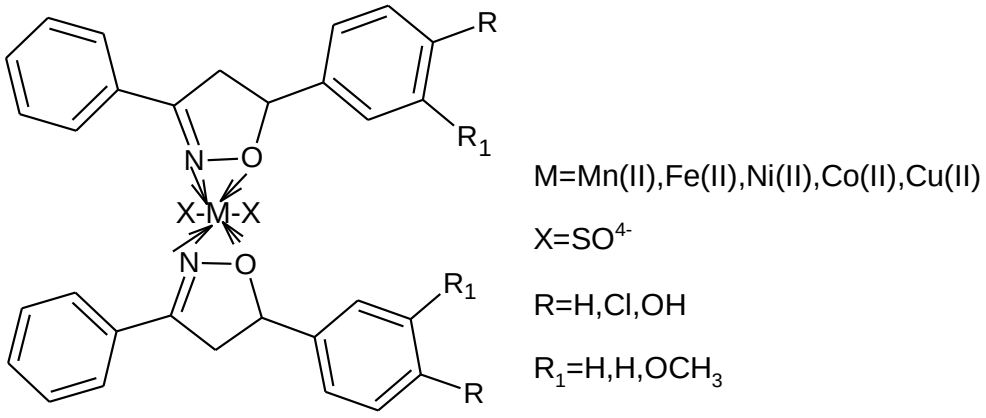
- **Sherifa M. Abu Bakr *et al.*, (2015),** investigated a series of 5-substituted pyrazole derivative with isoxazole ring system were synthesised and was evaluated against Panc-1 and Caco-2 cell lines. Most of the tested derivatives exhibited high cytotoxic potency against Panc-1 carcinoma cell lines, but moderate to weak activity was obtained against Caco-2 cell lines[98].





4. Antioxidant activity of isoxazole

- **KH. Kumar Naik *et al.*, (2013**), performed a synthesis of N/S/O functionalized Isoxazole ligands and their complexes ofMn(II), Fe(II),Ni(II),Co(II)and Cu(II) ions and evaluated for their antioxidant and antimicrobial activities which exhibited dominant antioxidant activity[99].



M=Mn(II),Fe(II),Ni(II),Co(II),Cu(II)

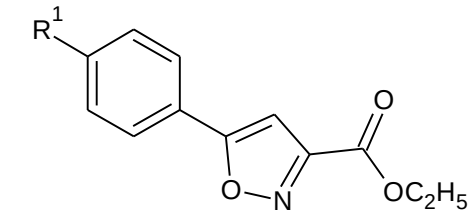
$X=SO^{4-}$

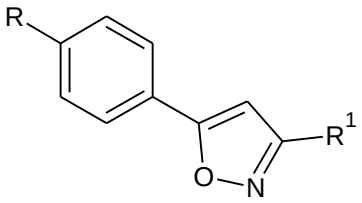
R=H,Cl,OH

R_1=H,H,OCH_3

5. Anti convulsant activity of Isoxazole

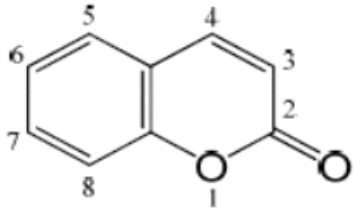
- **Udayan Banik *et al.*, (2013),** tested on substituted 3-isoxazole esters, 3-alkyl 5-aryl isoxazoles were synthesised and evaluated for in vitro anticonvulsant, by using cell lines anticancer and antioxidant activities and showed prominent activities in isoxazole derivatives[100].





CHEMISTRY

CHEMISTRY OF COUMARIN



INTRODUCTION

Coumarins, also known as benzopyrones, present in many plants, notably in high concentration in the tonka bean (*Dipteryx odorata)*, vanilla grass (*Anthoxanthum odoratum*) and sweet grass (*Hierochloe odorata*). Their presence has been detected in a few microorganisms asnd also in few animal sources.

Benzopyrones are of two types:

- 2*H*-benzopyran-2-ones (or) coumarins
- 4*H*-benzopyran-4-ones (or) chromones

Derivatives

Coumarins is the lactone of 4-hydroxy cinnamic acid. Coumarins and all its derivatives are considered as phenyl propanoids which are roughly classified as follows.

- Coumarins and simple derivatives: Hydroxy b) Methoxy c) Amino d) Bromo and chloro e) Thio and f) Carboxylic acid derivatives of coumasrins.
- Furano coumarins
- Pyrano coumarins

Coumarin itself was first isolated in 1820 from tonka bean and it was first synthesized by perkin from salicylaldehyde and acetic anhydride. The biosynthesis of coumarin in plants is via hydroxylation, glycolysis and cyclisation of cinnamic acid.

Physical data[101]

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.

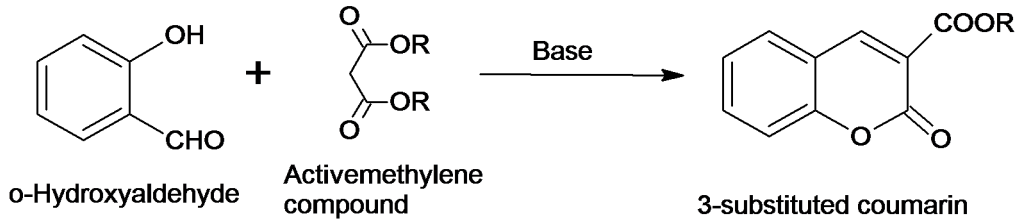
METHODS FOR THE SYNTHESIS OF COUMARIN DERIVATIVES[102,103]

Among the few methods which have significant important results; there are several other methods whose applications are less general. All these methods center round the possibility of building up the pyrone ring on a suitable benzene derivative**.**

1. Knovenagel Reaction

Condensation of ortho-hydroxy aldehydes or ketones with active methylene compounds (diethyl malonate, malanonitrile) in the presence of a base (ammonia or amines) to form coumarins is known as Knovenagel reaction.

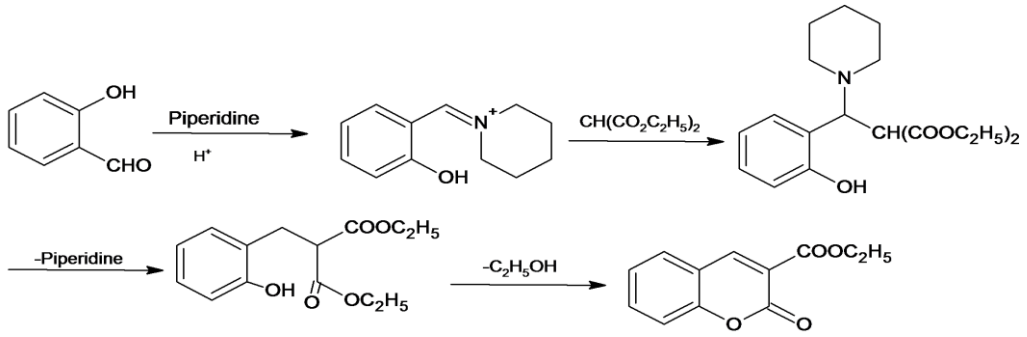
When malonic acid and piperidine are used, the reaction is called **Doebner modification.**



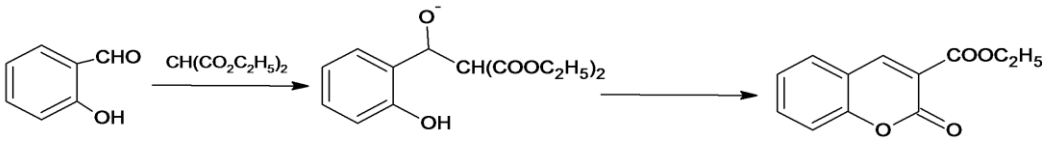
Mechanism

Two mechanism have been proposed for the Knoevengal reaction

- In one, the role of the amines is to form an imine or iminium salt which subsequently reacts with the enolate of the active methylene compound. Under normal circumstances, elimination of the amine would give the cinnamic acid derivative. However, when an O-hydroxy group is present in the aromatic aldehyde, intramolecular ring closure to the coumarin can occur.



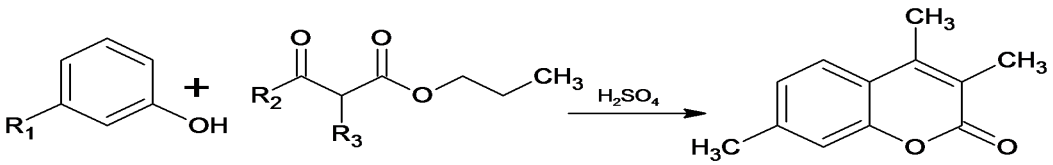
- In the second mechanism postulated, the carbanion derived from the active methylene compound by deprotonation by the amine is considered to attack the carbonyl group without further intervention by the base.



2. The Pechmann synthesis

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

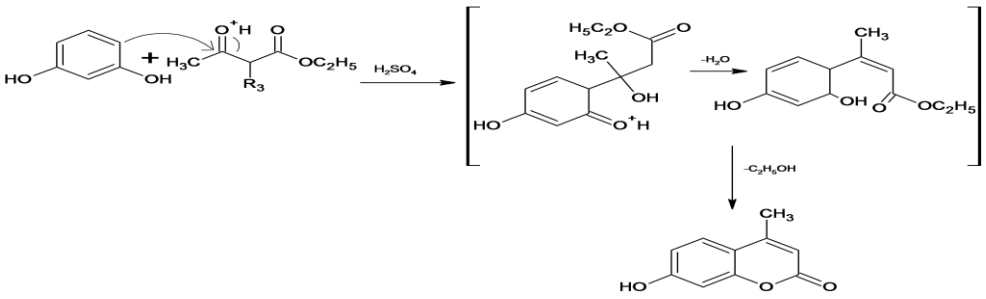
When acetoacetic ester and its derivatives are used, the reaction is referred as Pechmann - Duisberg reaction.



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

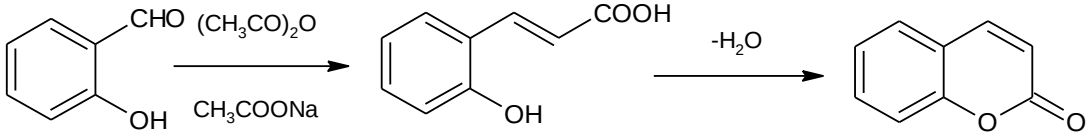
Mechanism

The Pechmann reaction is thought to proceed through the electrophilic aromatic substitution of resorcinol. The resulting β-hydroxy ester then undergoes dehydration and cyclisation to give coumarin.



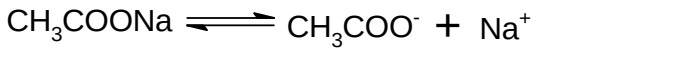
3. Perkin reaction

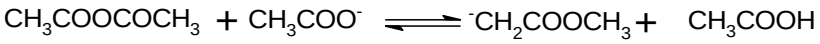
W.H. Perkin prepared coumarin by heating salicylaldehyde with acetic anhydride and anhydrous sodium acetate. 2-hydroxy cinnamic acid (coumarinic acid) was first formed as the intermediate which gets lactonised to form coumarin. Along with coumarin O-acetyl coumarinic acid is also produced.

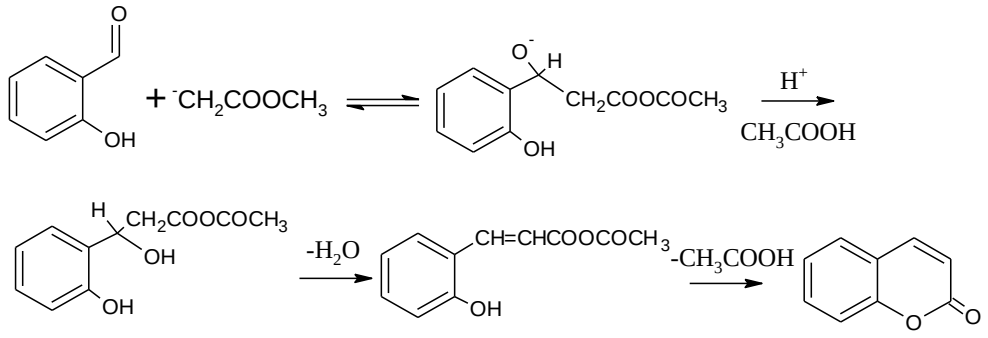


Mechanism

Carbonyl carbon of aldehyde reacts with carbanion to give an intermediate, which on cyclization gives coumarin. Sodium acetate functions as basic catalyst.

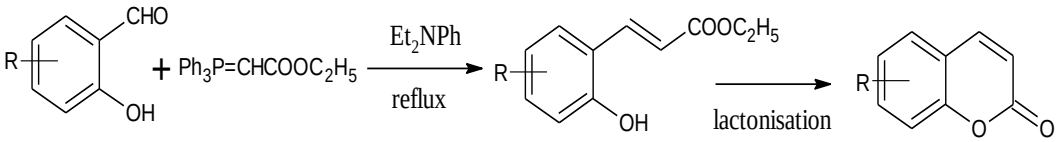






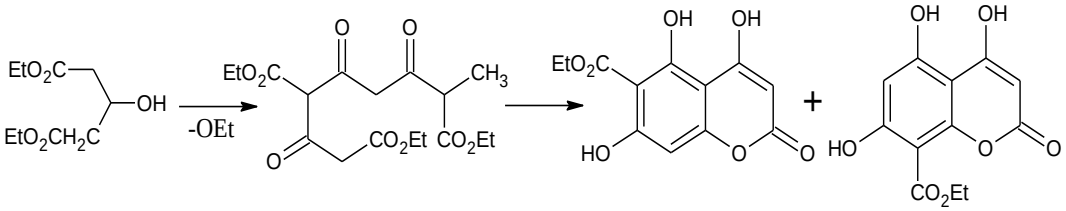
4. Wittig reaction

In this method, carbonyl compounds and phosphonium ylides react to form an alkene whose lactonisation will give coumarin derivatives.



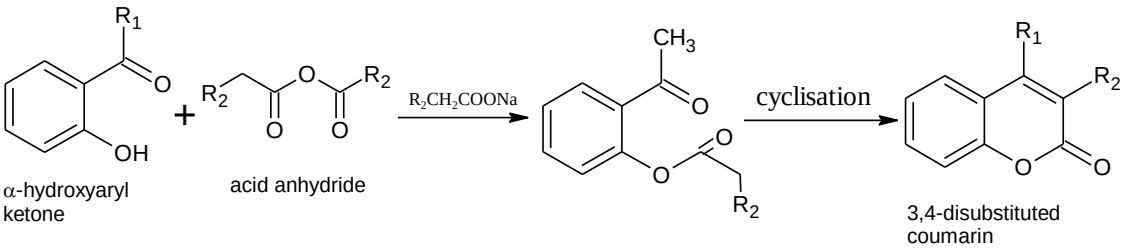
5. Claisen condensation

The base catalysed intermolecular condensation of 1,3-dicarbonyl compound can give rise to coumarins. For example, in the presence of sodium ethoxide, diethyl 3-oxopentanedionate (acetonedicarboxylate) affords 6-ethoxycarbonyl-4,5,7- trihydroxy coumarin through an initial claisen condensation followed by Dieckmann reaction.



6. Kostanecki – Robinson Reaction

In this reaction, coumarins (usually 3 and 4 substituted) are formed by acylation of o-hydroxy aryl ketones with aliphatic acid anhydrides followed by cyclisation.



α-hydroxyaryl ketone

acid anhydride

3,4-disubstituted coumarin

Chemical Properties[104]

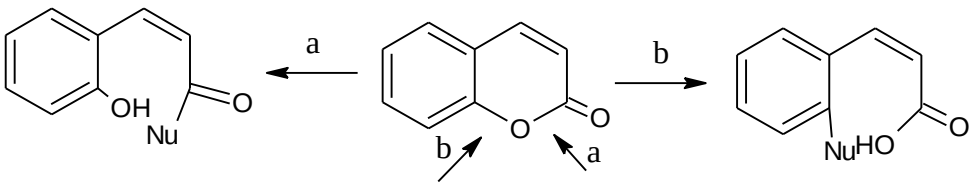
Reactivity of Coumarins

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

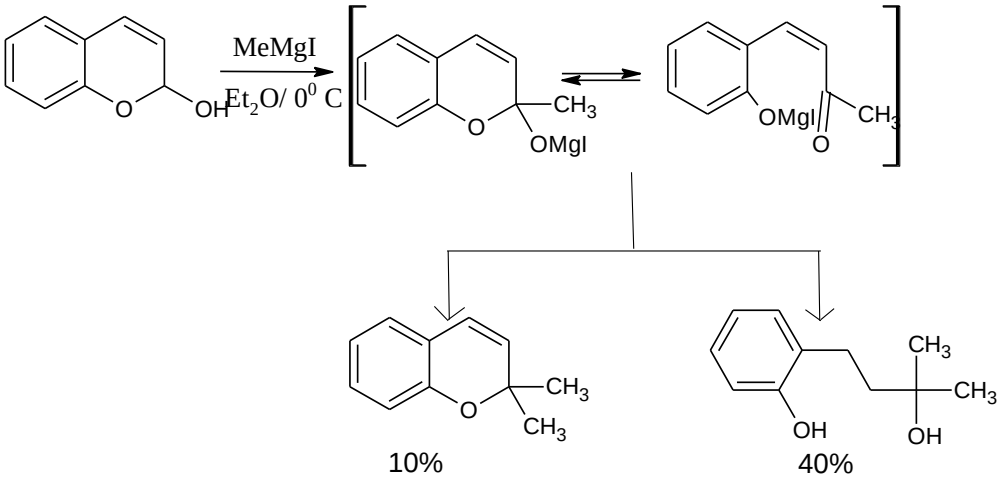
Several kinds of nucleophiles react with coumarins. Some of these reactions involve ring opening and occasionally, recyclisation into another ring. A nucleophile (Nu) which cleaves the ring, attacks and breaks one of the bonds of the ring oxygen atom as shown below.



1. With C-Nucleophiles

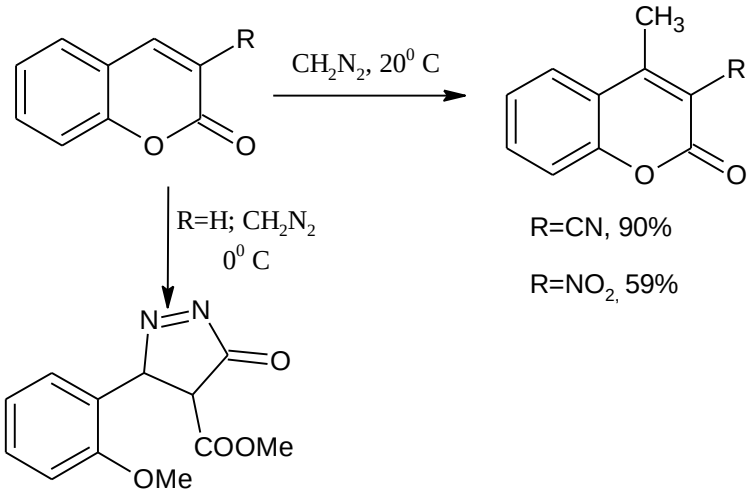
a. Grignard Reagents

Coumarins react with Grignard reagents, like esters, and give mixture of products,resulting from the ring opening of the initial carbonyl adduct.



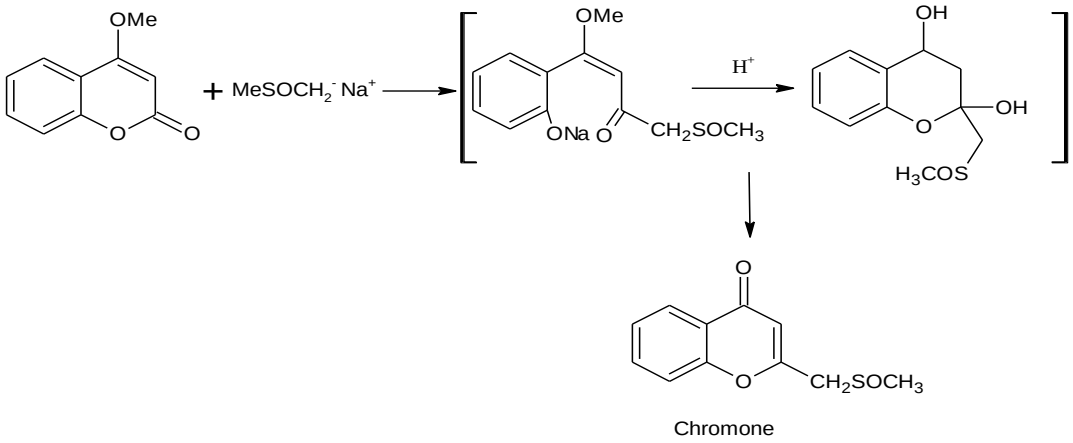
Diazomethane

On reaction with nucleophilic carbon of diazomethane, 3-cyano and 3-nitrocoumarin are readily converted into their 4-methyl homologues but coumarin is transformed into the pyrazolone.



b. Methylsulphinylmethide

Carbanions generated in situ by strong bases cleave pyran-2-one rings but acidification may result in the formation of a new compound.



Chromone

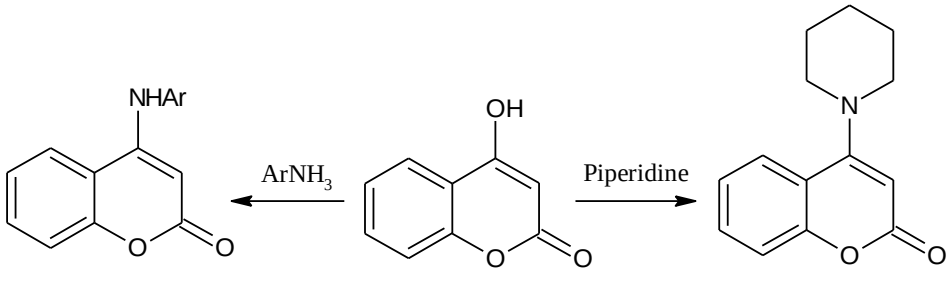
2. With N-Nucleophiles

a. Aliphatic Primary and Secondary Amines:

Coumarins do not react with ammonia and amines to produce α-quinolones, even under forced conditions. The reaction is not favourable since it involves a non-aromatic intermediate.

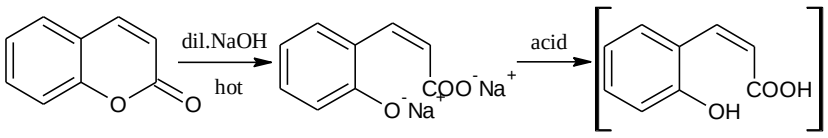
b. Aromatic Primary and Secondary amines:

Coumarins react with primary aromatic and secondary cyclic amines to yield substitution products such as 4-arylamino and 4-piperidino coumarin.



3. With O-Nucleophiles Reaction with alkali

The reaction of coumarins with hydroxides involves initial addition to the carbonyl carbon, followed by opening of the lactone ring to give yellow solutions of the salts of the corresponding cis-cinnamic acids (coumarinic acids).

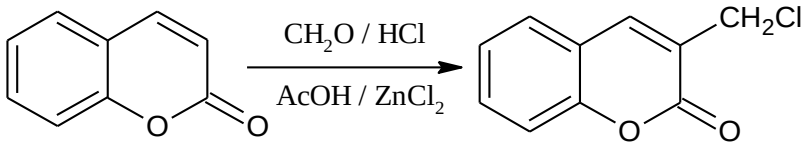


2-Hydroxy-cis-cinnamic acid

B. With Electrophiles

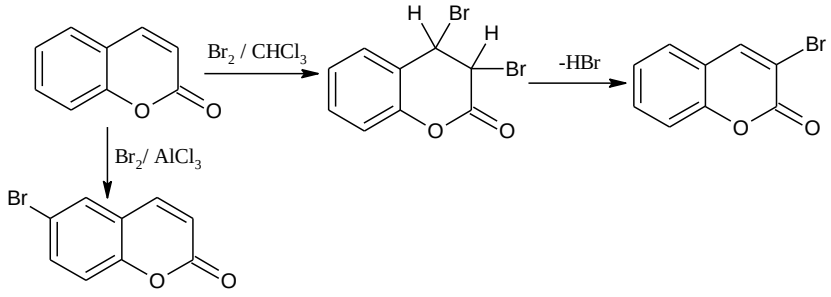
a. Chloromethylation

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



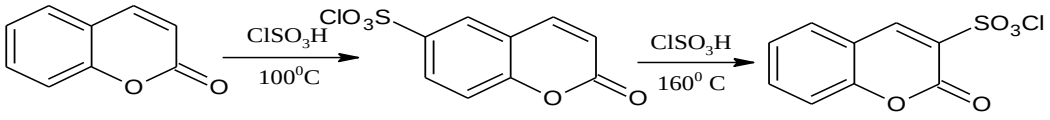
b. Bromination

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.



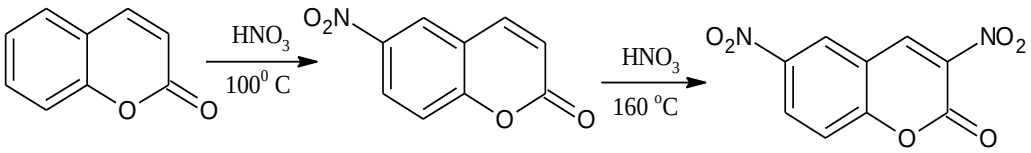
c. Sulphonation

Sulphonation of coumarin with chlorosulphonic acid at 100°C yields the 6-sulphonyl derivative, but at 130-140°C, a second substituent is introduced to give 3,6-disulphonyl chloride.



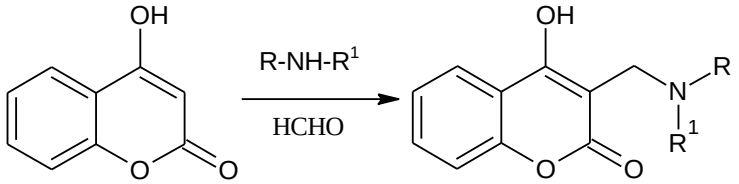
d. Nitration

Nitration occurs mainly at C-6 position and under vigorous conditions, substitution occurs at C-3 position also.

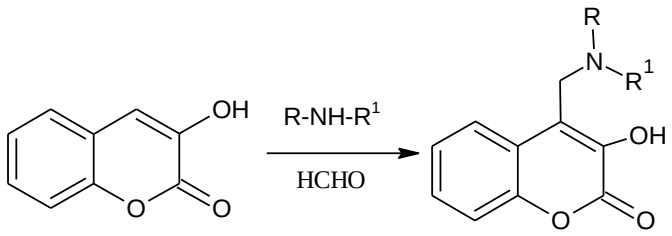


e. Mannich reaction[105]

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



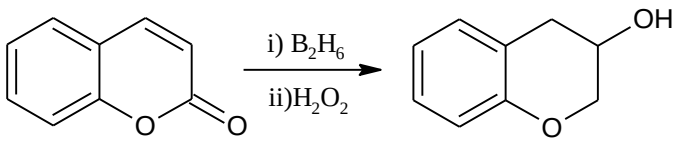
Similarly, Mannich reaction of 3-hydroxy coumarin with formaldehyde and primary or secondary amines resulted in 4-N,N-dialkylaminomethyl-3-hydroxy coumarins.



C. Reduction

a. Reduction with Diborane and Hydrogen peroxide

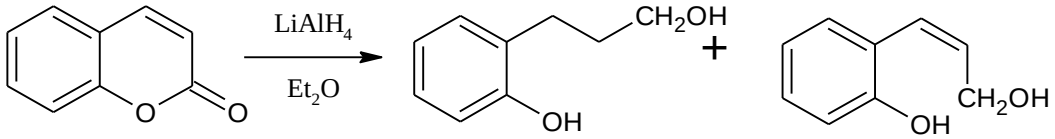
Reduction with diborane followed by hydrogen peroxide has two effects on coumarins: the carbonyl group is reduced to methylene and the elements of water are added across the 3,4-double bond in an anti-Markonikow manner but the overall yield is very low.



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

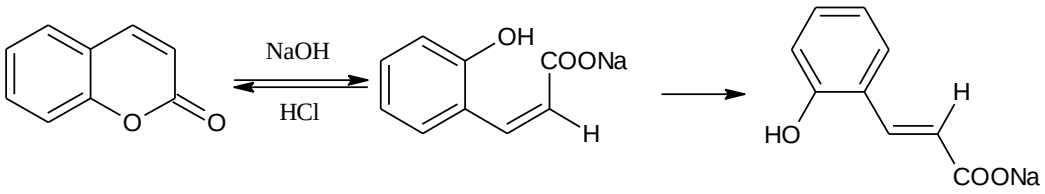
b. Reduction with Lithium aluminium hydride

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



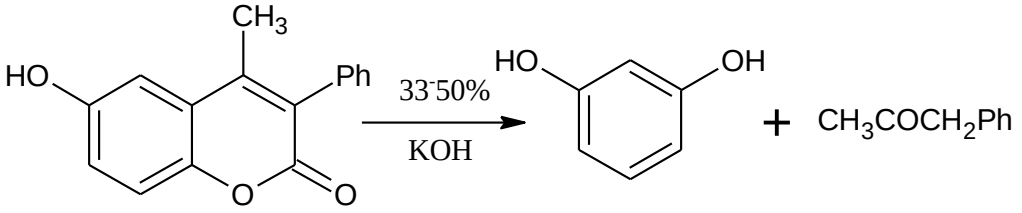
D. Hydrolytic Reaction

Coumarins hardly undergo hydrolysis by alkali to form coumarinic acids which are cis in form and can be converted to trans form by prolonged treatment with alkali.



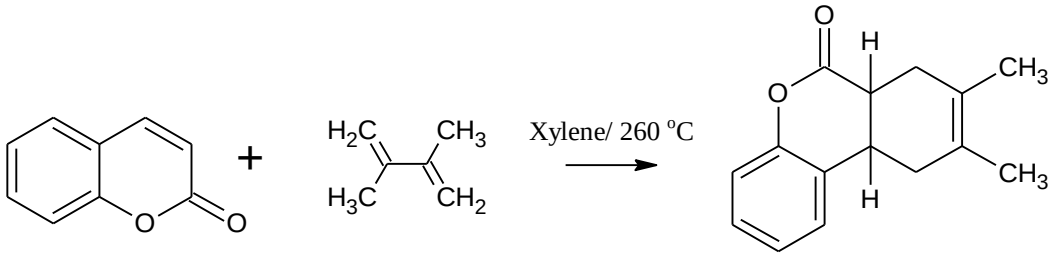
On the other hand, substituted coumarins give a mixture of a phenol and a

ketone as a result of hydrolysis with alkali.



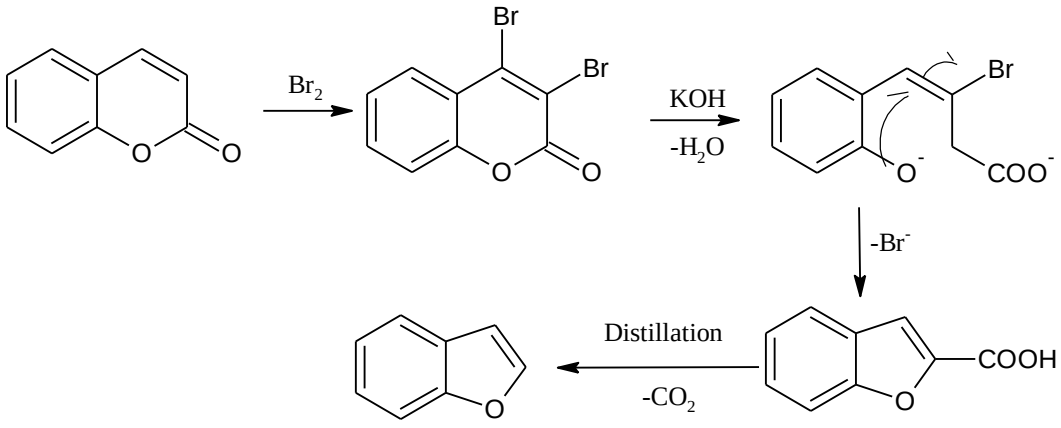
E. Diels Alder Reaction

Coumarins serve as dienophiles in Diels Alder reaction, but only under relatively strong conditions.



F. Ring Contraction Reaction

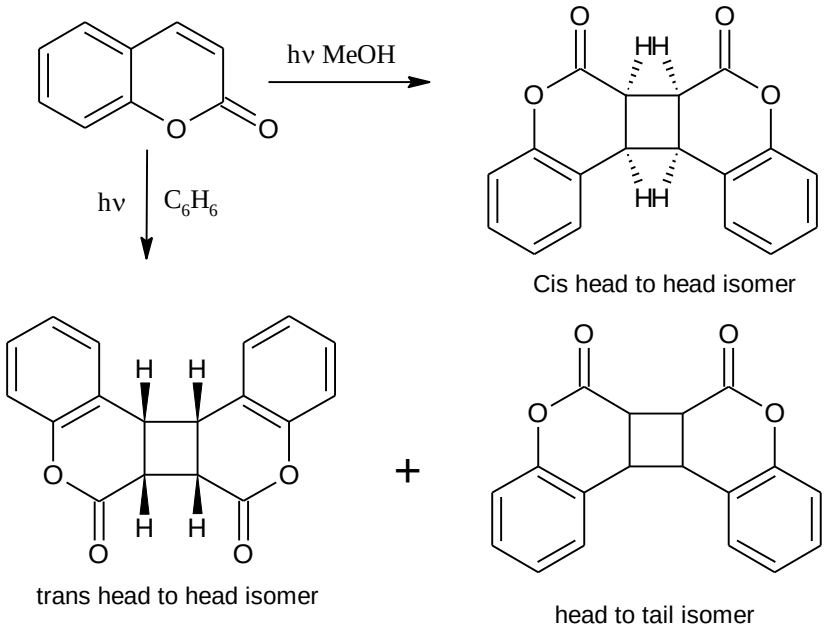
The reaction is a type of Perkin rearrangement reaction. The reaction proceeds with the bromination of coumarins. The resultant dibromocoumarin on Alkaline degradation gives coumarilic acid which is decarboxylated to benzofuran.



G. Photochemical Reactions[101]

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

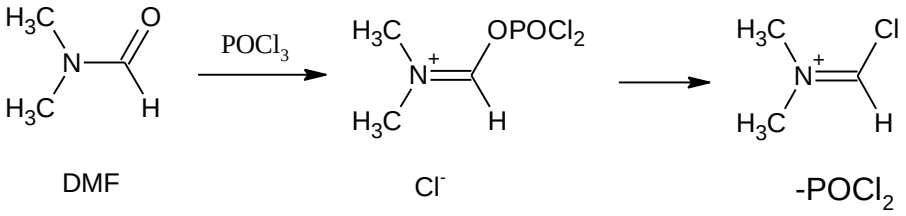
1. In a polar medium such as methanol, the only product formed is the cis head to head isomer.
2. In a non-polar medium such as benzene or dioxane, trans head to head dimer is the main product of the reaction; small amounts of head to tail dimers are also formed in non-polar solvents.



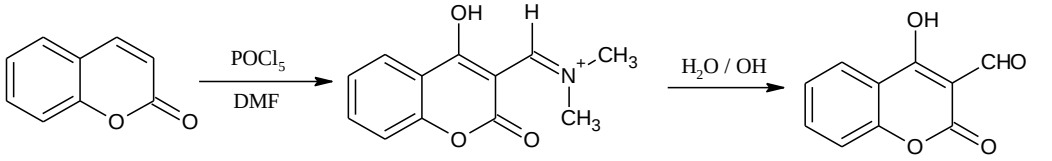
H. Vilsmeier-Haack Reaction

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:



Vilsmeier-Haack Reaction in 4-hydroxy coumarin:



3-formyl-4-hydroxycoumarin

CHEMISTRY OF PYRAZOLE[106-109]

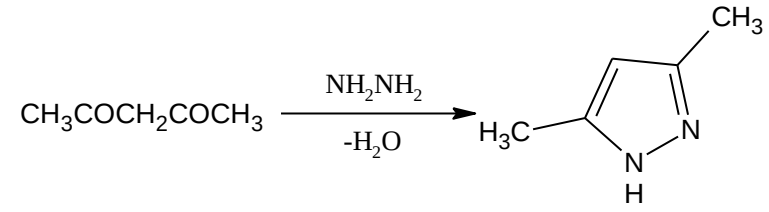
Pyrazole was first described by Buchner who discovered it during the decomposition of pyrazole 3,4,5- tricarboxylic acid. Pyrazole is a colourless solid with a melting point of 70°C and is soluble in water. It possesses a penetrating pleasant smell. Pyrazole has a high boiling point of about 187° C.

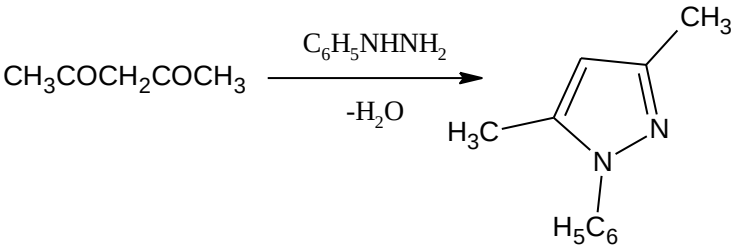
Synthetic methods

The pyrazoles can be synthesised by the following general methods.

1. From Dicarbonyl Compounds

The most straight forward method of pyrazole synthesis involves a reaction between a 1,3-[dicarbonyl compound and hydrazine or its derivatives. A simple pyrazole is obtained with a 1,3-dicarbonyl compound such as acetylacetone on treatment with hydrazine or phenyl hydrazine.

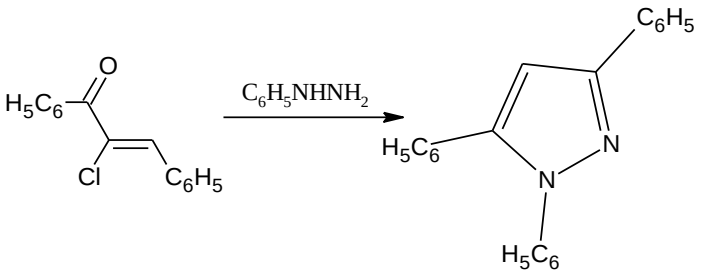




The reaction probably proceeds via the formation of monohydrazone which cyclizes under the experimental conditions. The principal drawback of this method is that unsymmetrical dicarbonyl compounds or a substituted hydrazine generally gives an isomeric mixture of two products.

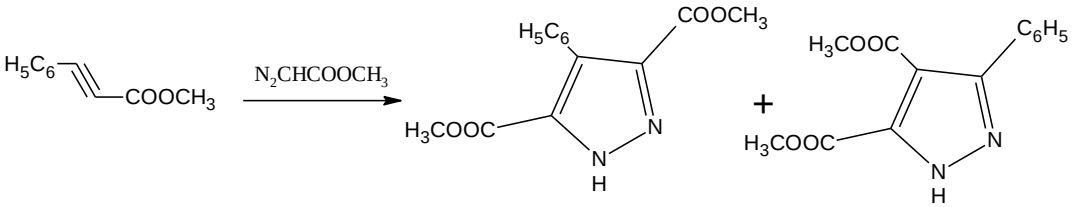
2. From α,β- Ethylene Carbonyl Compounds

This consists of a reaction between α,β- ethylene carbonyl derivative and hydrazine. The former must contain an easily replaceable group at the β-position. This is illustrated in the following equation.



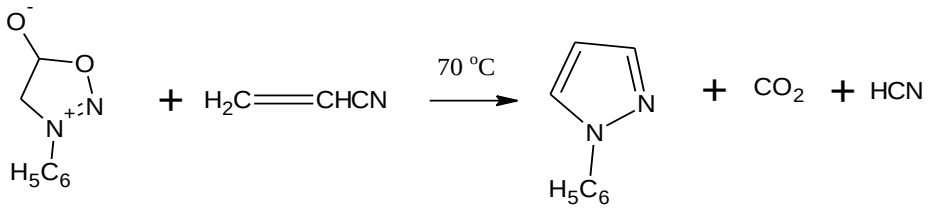
3. From 1,3-Dipolar Addition

A diazo compound adds to an acetylenic derivative which has its triple bond activated by an electron-withdrawing substituent. The reaction is usually carried out in a suitable solvent at room temperature. Diazomethane or methyl or ethyl diazoacetate is commonly employed. Thus methyl diazoacetate and methyl phenyl propiolate yield the following isomeric pyrazoles in equal amounts.



From Other Ring Systems

Various heterocyclic compounds transform to pyrazoles under appropriate conditions. Syndone, for instance, and acrylonitrile result in pyrazole formation. The cyanopyrazoline formed as intermediate is immediately converted to pyrazole.

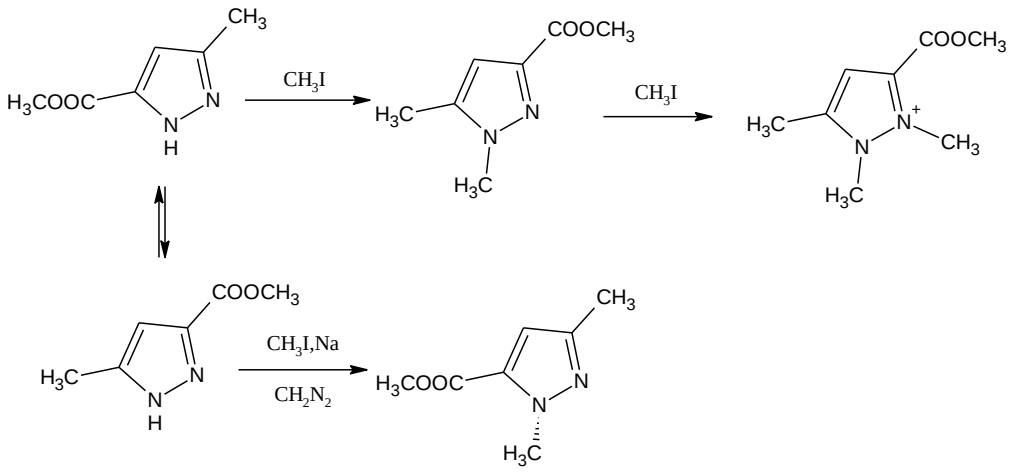


CHEMICAL REACTIONS

Pyrazole is very stable and inert. In acid medium it exists as cation. Pyrazole are known to form coordinate complexes with several metal ions.

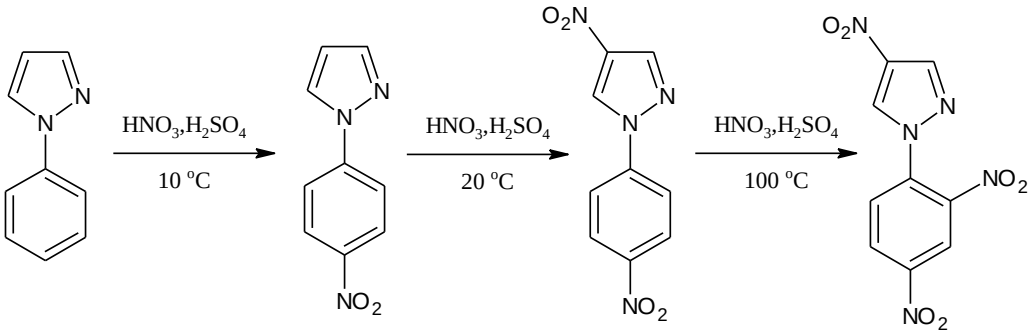
1. Alkylation

The alkylation of the free NH group of pyrazoles proceeds with alkylating agents such as alkyl halides, diazomethane or dimethylsulphate. Substituted pyrazoles undergo alkylation to give a mixture of two isomeric products. Excess of alkylating agent causes quarternization.



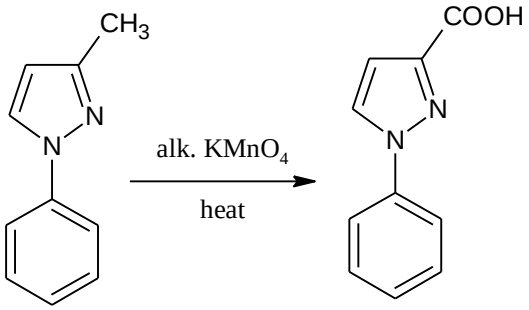
2. Electrophilic Substitution

Pyrazoles are subject to electrophilic substitution and the attack takes place at position 4. The electrophilic attack on pyrazole takes place readily in neutral or basis media. Chlorination (SO_2Cl_2 or free Cl_2) of pyrazole yields 4-Chloropyrazoles. Bromination (Br_2/ dioxane) similarly occurs at position 4. Nitration (HNO_3/ H_2SO_4) and sulfonation also occurs at position 4, but under more severe conditions the reactiontakes place on the pyrazolium cation. Nitration of 1-phenylpyrazole can be carried out in such a manner so as to obtain any one of the three successive nitration products.

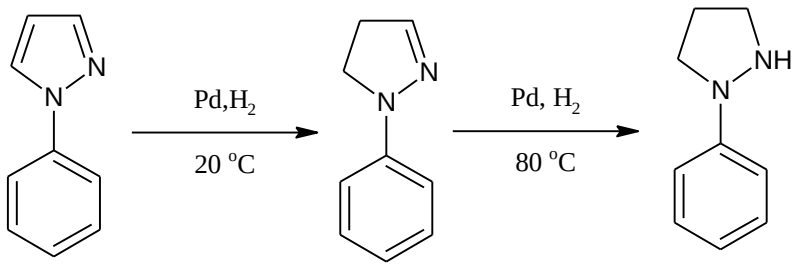


3. Reaction with Oxidizing and Reducing Agents

The pyrazole ring is remarkably stable to the action of oxidizing agents but the side chain may be oxidized to the carboxylic function. The oxidation proceeds well in the presence of alkaline permanaganate. Pyrazole and its derivatives have been reduced under a variety of conditions. Thus with Na/C_2H_5OH, 2-pyrazoline is obtained.

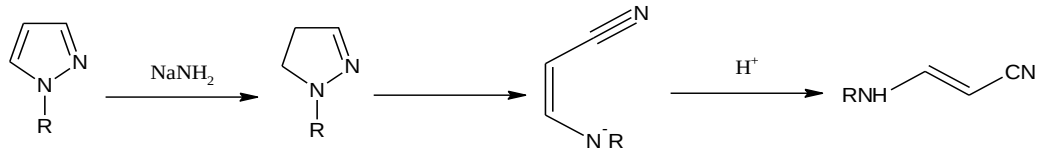


The catalytic reduction of 1- phenylpyrazole yields both phenylpyrazoline and 1-phenylpyrazolidine.



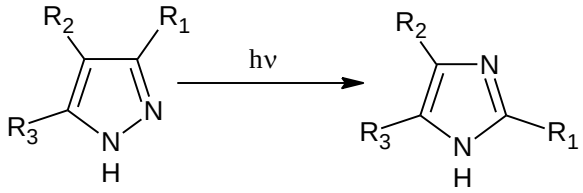
4. Reaction with Nucleophilies

Halogens attached to a pyrazole nucleus are exceptionally inert and thus will not undergo displacement under the usual reaction condition. In 1-phenylpyrazole, the halogen is most reactive in the 5-positions, less so in 4-position and least in the 3-position. The presence of electron-withdrawing groups markedly assists nucleophilic displacement of halogens. Pyrazole quarternary salts are particularly reactive in nucleophilic reactions and in these compounds 3 and 5 halogen substitutents can readily be replaced by a variety of nucleophiles. Direct amination of pyrazole with sodalime has not been observed, but it rather causes ring opening and metallation with n-butyllithium takes place at the 5-position. Pyrazoles exist partly as anions and thus react with electrophiles as phenols and undergo diazo coupling, nitrosation and Mannich reaction.



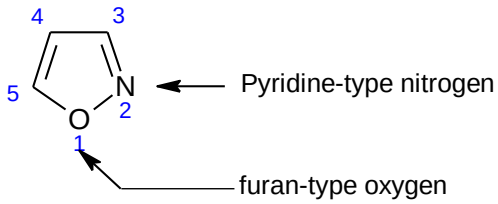
5. Photochemical Reactions

The most important photochemical reaction of pyrazole is its conversion to imidazole.



CHEMISTRY OF ISOXAZOLE[110]

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).

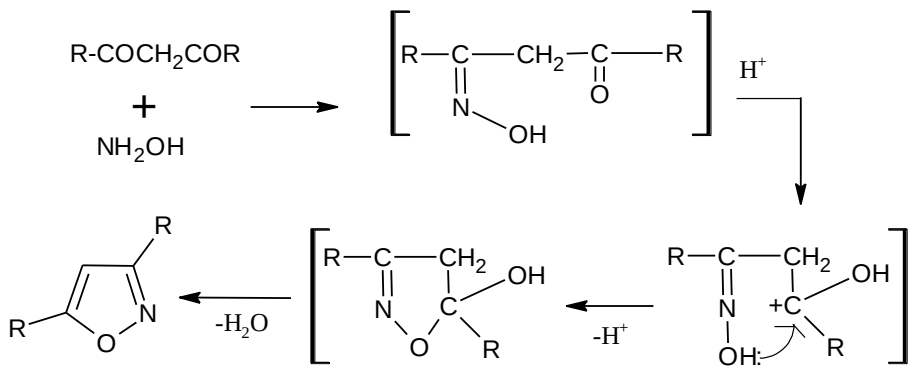


Isoxazole is a colourless liquid (b.p.= 95°C, D.M = 2.75 ± 0.01 D in benzene and 3.1 ± 0.03 D in dioxane) with strong pyridine like odour. The boiling point of isoxazole is although lower than of pyrazole and imidazole but higher than that of oxazole and furan. The higher boiling point of isoxazole is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

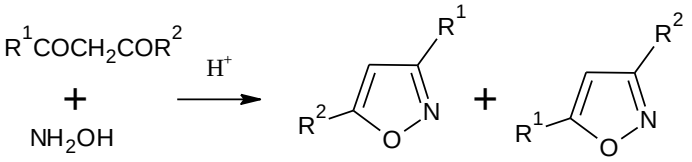
Synthesis

1. Reaction of β-Diketones with Hydroxylamine

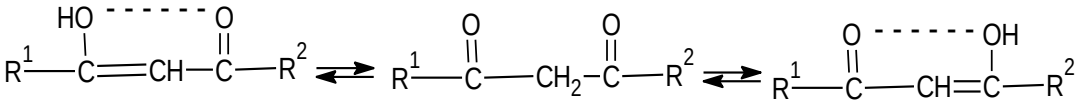
This is the most general and widely used method which involves condensation- cyclisation (3+2 cyclisation) of β-diketones with hydroxylamine in the presence of an acid. The reaction proceeds via the monoxime intermediate which subsequently on cyclizative-dehydration leads to the formation of isoxazoles.



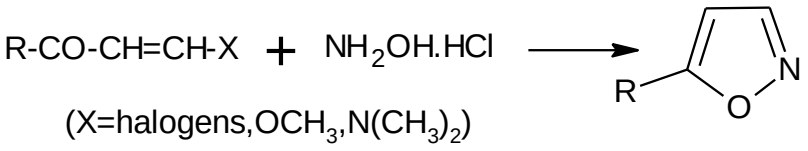
Scheme I

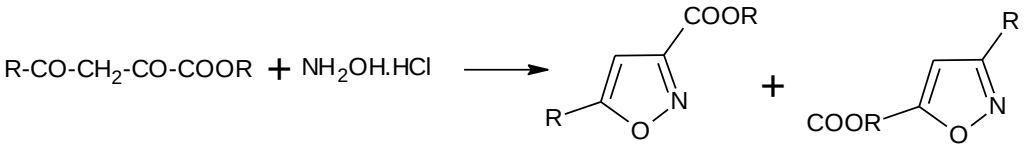


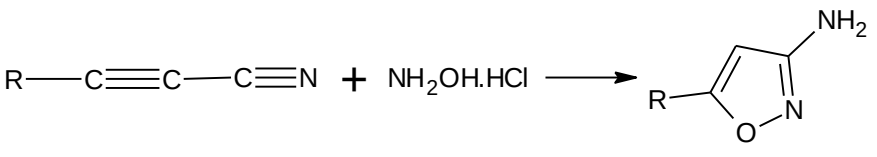
Enolization in β-diketones



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

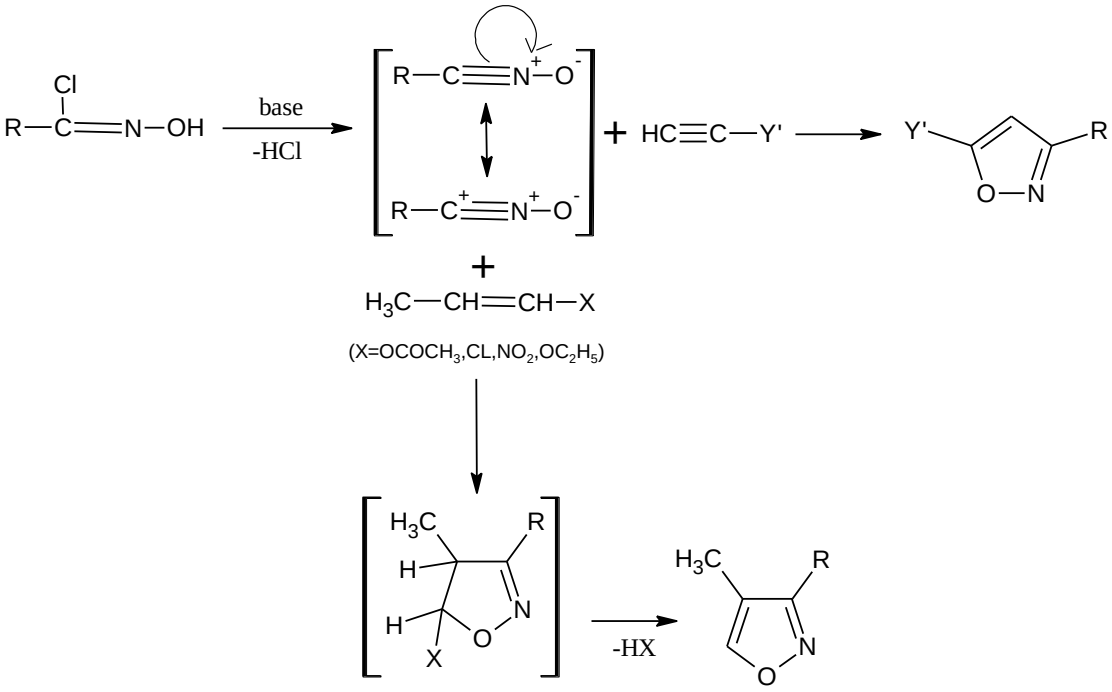






2. Reaction of Nitrile N-Oxides with Alkenes and Alkynes

The reaction of nitrile oxides, generated in situ by treating chloroximes with a base (triethylamine), with alkenes and alkynes results in isoxazoles via 1,3-dipolar cycloaddition.



REACTIONS OF ISOXAZOLE

Reactivity

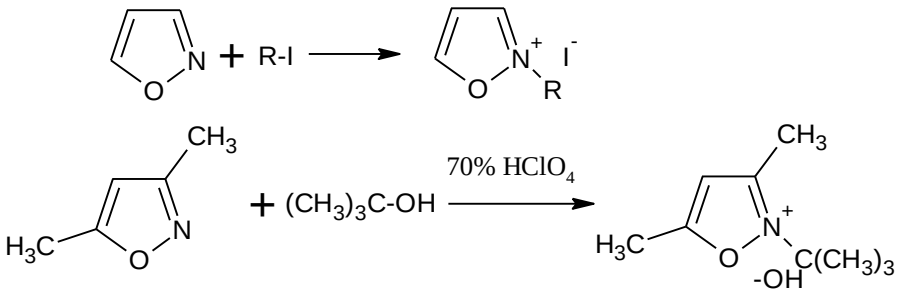
Isoxazole contains furan-type oxygen and pyridine-type nitrogen at the positions- and -2, it is therefore considered to exhibit characteristic reactions of furan and pyridine. But isoxazole undergoes electrophilic substitutions more readily than pyridine and less readily than furan because of combined 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

a) Electrophilic attack at Nitrogen

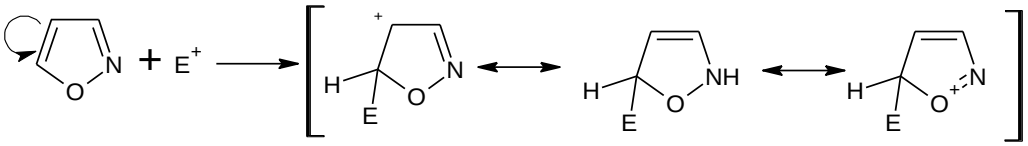
N-Alkylation

Isoxazole, although least basic among the azoles, undergoes N-alkylation when treated with alkyl iodides or sulfates with the formation of quaternary azolium salts. However, the isoxazolium salts with bulky N-substitutents are obtained by treating isoxazoles with an alcohol in the presence of perchloric acid.



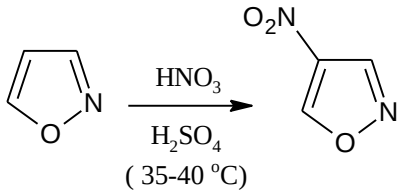
b) Electrophilic Attack at Carbon

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.



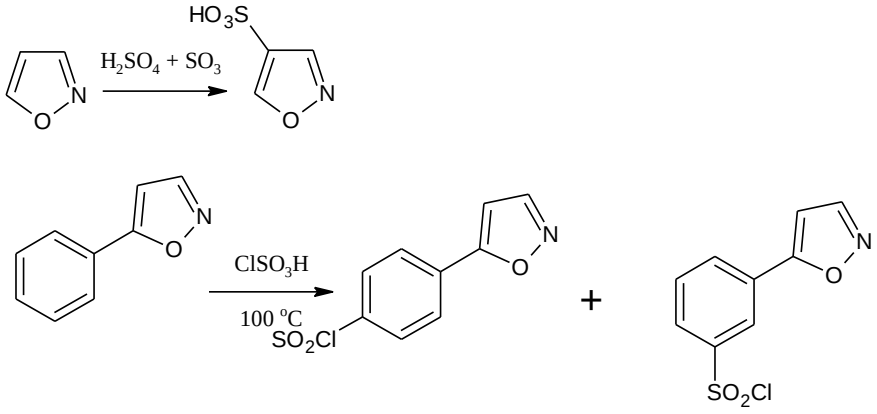
2. Nitration

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).



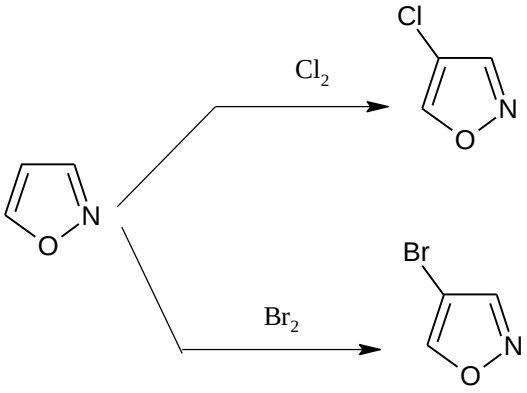
3. Sulfonation

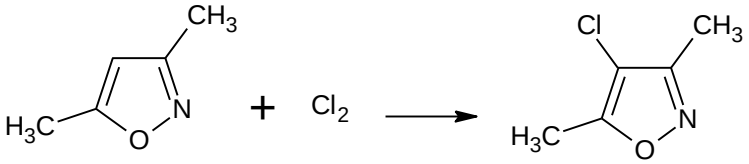
Isoxazole ring is resistant to sulfonation, however sulfonated with oleum under drastic conditions with the introduction of sulfonic acid group at the position-4. But 5-phenylisoxazole is sulfonated by chlorosulfonic acid with the sulfonation of only phenyl ring at the meta- and para- positions.



4. Halogenation

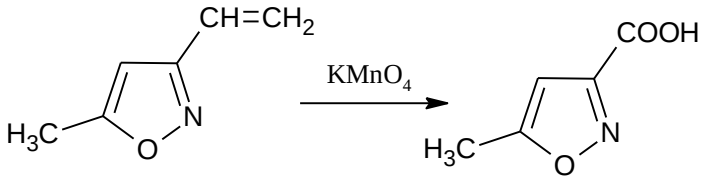
Isoxazoles undergo halogenation with chlorine or bromine at the position-4 with the formation of the corresponding 4-chloro- or 4-bromo- isoxazoles.

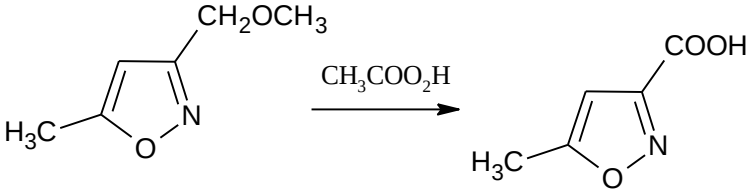




5. Oxidation

Isoxazoles are stable oxidizing agents, but unsaturated side chain and the oxygenated functional groups are oxidised to their corresponding acids.

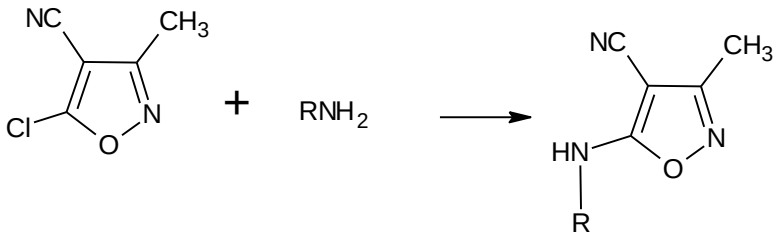




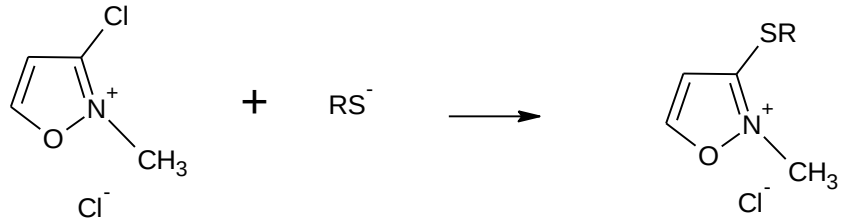
6. Reaction with Nucleophiles

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.

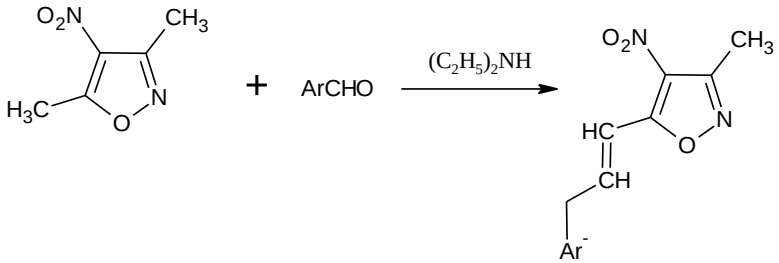


The halogen atoms at the 3- and 5- positons can be replaced by nucleophiles if activated by ring quaternization



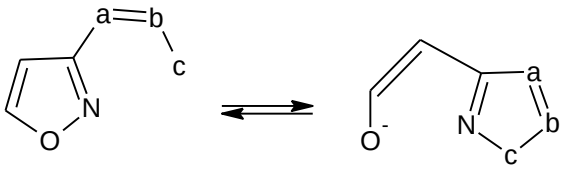
b) Condensation Reactions

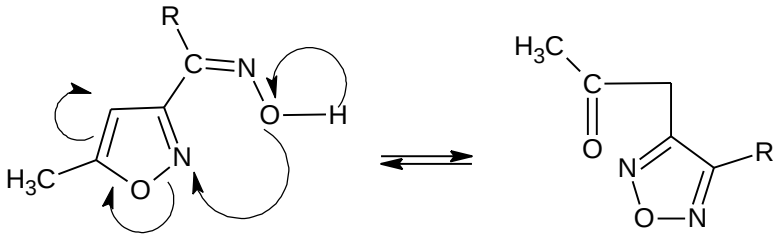
The methyl group at the position-5 will be more reactive than the methyl group at the position-3, if position-4 is substituted with an electron-withdrawing group. Thus, 5-methyl group with enhanced reactivity is easily condensed with aromatic aldehydes in the presence of diethylamine, but 3-methyl group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



c) Rearrangement

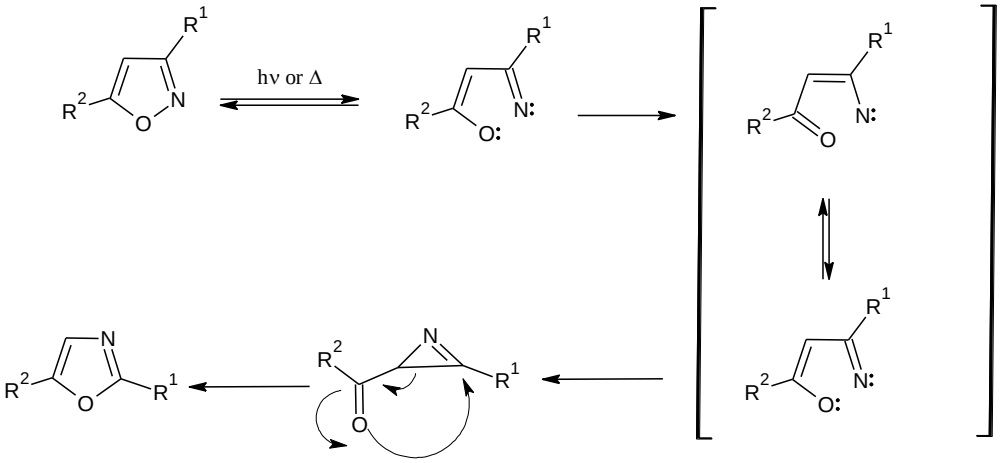
Isoxazoles (also other heterocycles with N-O bond), substituted with suitable side chains of three atoms (hydrazone, oximine and imidine) at carbon α- to the pyridine-type nitrogen, undergoe special type of thermal and base catalysed rearrangement, known as Boulton-Katrizky rearrangement, by following generalised mechanism





d) Photochemical and Thermal Reactions

Isoxazoles are photochemically or thermally transformed into oxazoles via 2H-azirine intermediate.



PURPOSE AND PLAN OF WORK

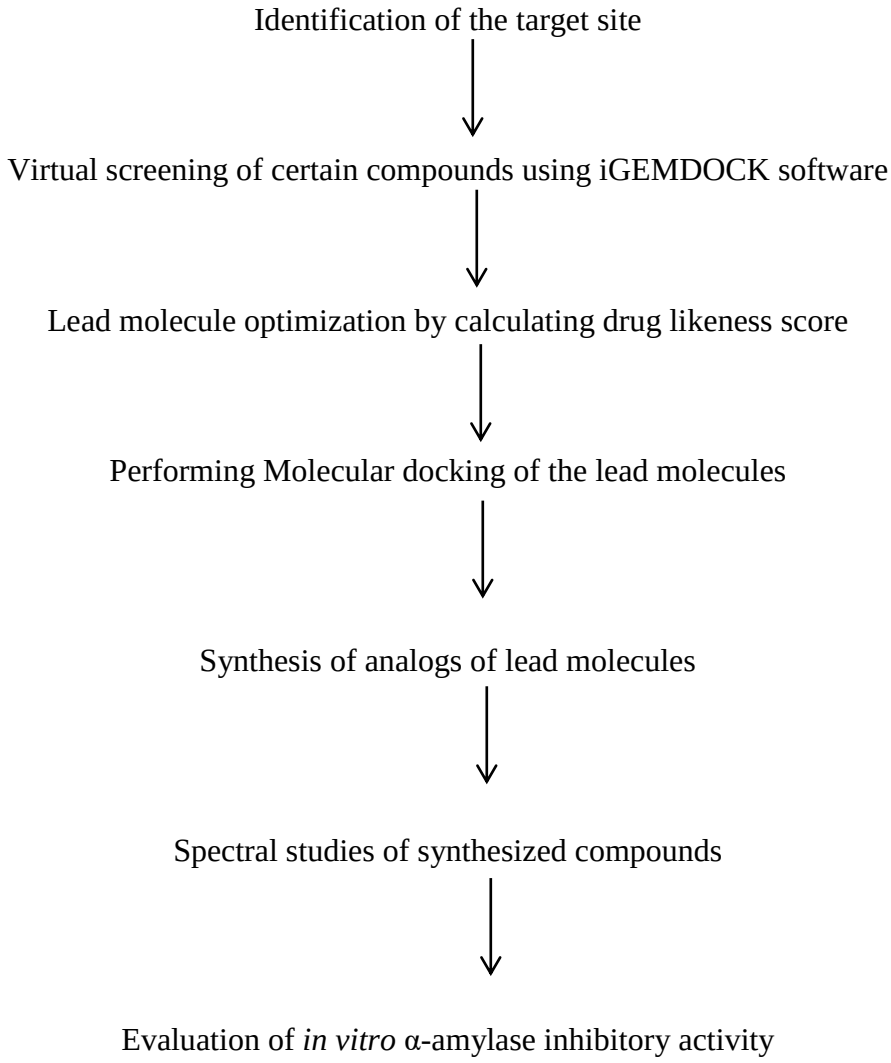
PURPOSE OF THE STUDY

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 method 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. This study is focussed to investigate the inhibitory potentials of the synthesised coumarin derivatives on α-amylase, the key enzyme responsible for carbohydrate hydrolysis.

PLAN OF WORK

The steps involved in the present study are:



EXPERIMENTAL SECTION

DRUG DESIGN APPROACH

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 for the 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 Pyrazole derivatives and Isoxazole derivatives of coumarin were selected based on several literature reviews.

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 [41,111,112]

- 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 save 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 $\rightarrow$ Click Dockings and Open dlg file
- Open Analyze $\rightarrow$ Click Macromolecule and Choose pdbqt file.
- Open Analyze $\rightarrow$ 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 50 ligands.

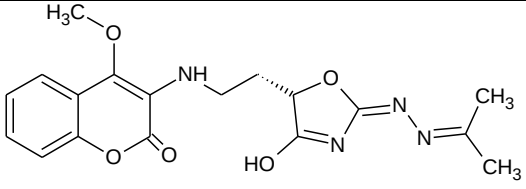
RESULTS AND DISCUSSION

The docking results of **α-amylase (1UA7.pdb)** with the 50 similar structural ligands are reported in the **Table 1 and Table 2.** The best docked structures should have lower binding energies. The binding sites and the active sites are shown in the snapshots.

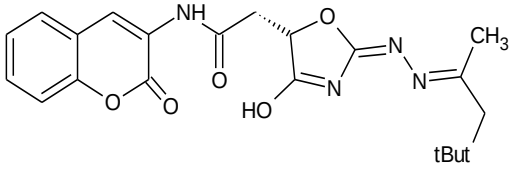
Fig 1: snapshots of 50 ligands

Table 1: Binding energies of 1-25

Sl. No	Compound code	Binding energy k/cal
1	Zinc_18249417	-90.53
2	Zinc_20031600	-83.49
3	Zinc_9365179	-75.59
4	Zinc_19166762	-84.51
5	Zinc_1700294	-94.06
6	Zinc_841803	-71.13
7	Zinc_3901268	-71.31
8	Zinc_19799526	-82.5
9	Zinc_982962	-102.76
10	Zinc_19989886	-82.58
11	Zinc_2258599	-96.1
12	Zinc_19990070	-79.21
13	Zinc_5556455	-95.45
14	Zinc_19990034	-95.57
15	Zinc_1240782	-94.08
16	Zinc_19794473	-76.95
17	Zinc_5286115	-95.45
18	Zinc_984053	-95.57
19	Zinc_5519407	-94.08
20	Zinc_2758246	-76.95
21	**Zinc_8575396**	**-104.43**
22	**Zinc_12378847**	**-108.44**
23	Zinc_6182368	-90.93
24	Zinc_6219168	-94.9
25	Zinc_19318821	-93.13



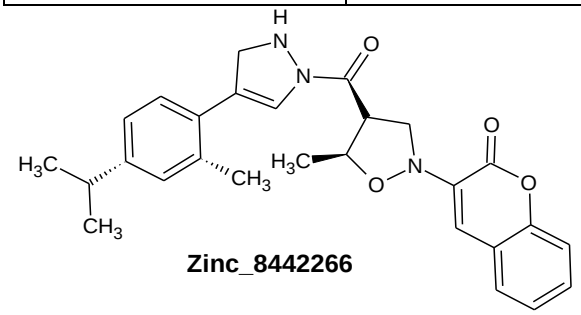
Zinc_12378847



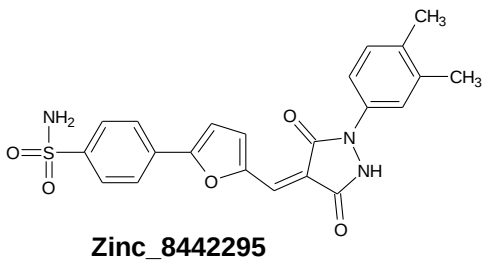
Zinc_8575396

Table 1: Binding energies of 25-50

Sl. No	Compound code	Binding energy k/cal
26	Zinc_2063189	-66.85
27	Zinc_18141403	-79.99
28	Zinc_634140	-68.29
29	Zinc_8442294	-67.43
30	Zinc_8442293	-59.04
31	Zinc_8442288	-55.47
32	Zinc_651582	-62.16
33	Zinc_8442281	-55.46
34	Zinc_730699	-83.88
35	Zinc_ 8442279	-69.79
36	Zinc_8442278	-77.97
37	Zinc_8442277	-71.89
38	Zinc_8442276	-62.55
39	Zinc_8442275	-78.22
40	Zinc_8442273	-60.38
41	Zinc_8442272	-86.63
42	Zinc_8442271	-73.91
43	Zinc_8442270	-88.19
44	Zinc_8442268	-60.29
45	**Zinc_8442295**	**-93.49**
46	Zinc_634138	-83.9
47	Zinc_1019824	-78.77
48	**Zinc_8442266**	**-88.9**
49	Zinc_8442267	-81.93
50	Zinc_8442265	-76.15



Zinc_8442266



Zinc_8442295

The binding energy of all the 50 compounds selected and the lowest binding energies were found to be Table 1 **-104.43, -108.44 and** Table 2 **-93.49, -88.9** which possess coumarin moiety. So that, in this project, we are only concerned coumarins as lead molecule. In the series of 50 ligands, the binding energy of 22(table 1) (-108.44) and 20 (table) (-93.49) is the lowest binding energy. Hence, total of Ten compounds with similar structure as that of **Zinc_12378847** and **Zinc_8442266** is selected, to which Isoxazole and pyrazole moieties were attached and forms a new series of coumarins derivatives and carried to further studies. Therefore, this series include Isoxazole compounds $\mathbf{I_{1\text{-}5}}$ and Pyrazole compounds $\mathbf{T_{1\text{-}5}}$ with coumarin ring system.

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 **-16.67 kcal/mol.**

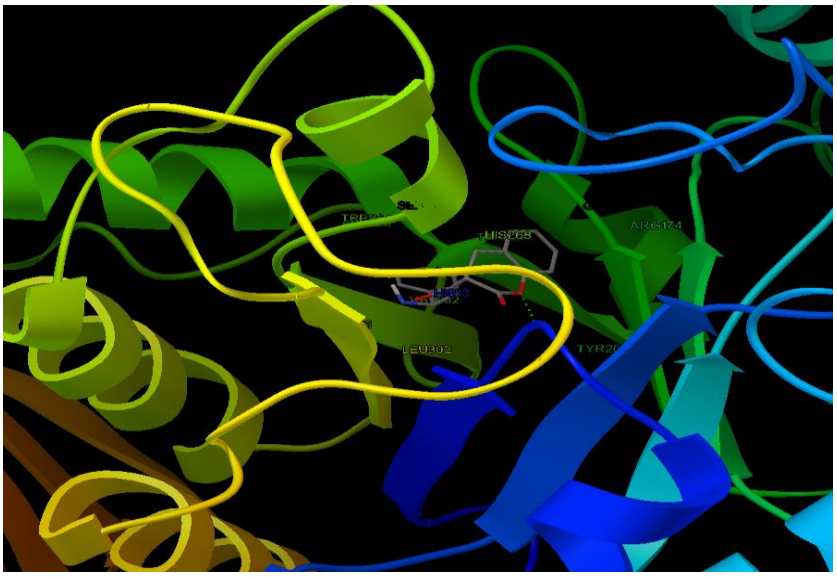


Figure 2.Snapshot of I1binding with 1UA7

Binding of Isoxazole compounds with α-amylase enzyme

I_1

I_1 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-11.2kcal/mol.**

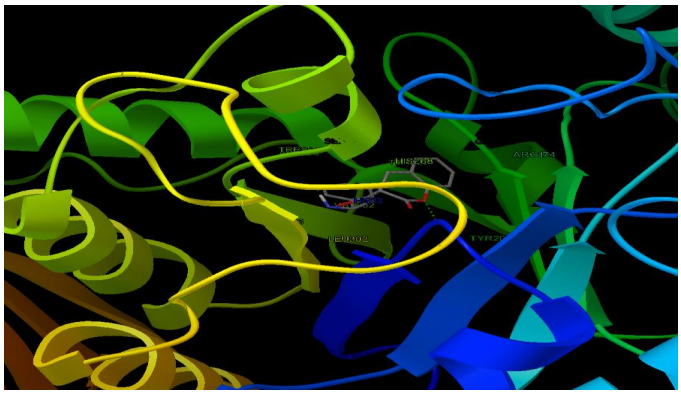


Figure 3.Snapshot of I_1 binding with 1UA7

I_2

I_2 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-5.89 kcal/mol.**

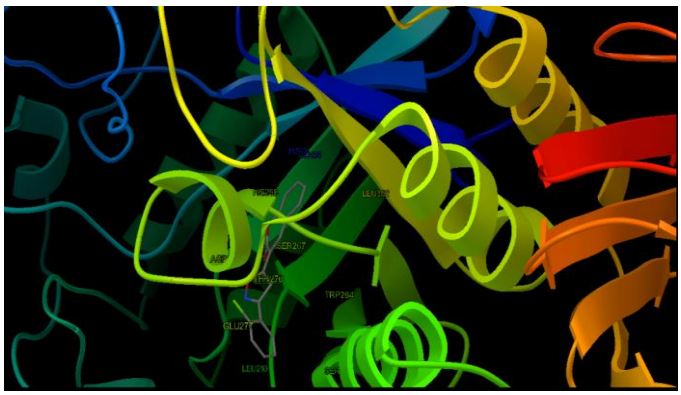


Figure 4.Snapshot of I_2 binding with 1UA7

I_3

I3 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-7.32kcal/mol**.

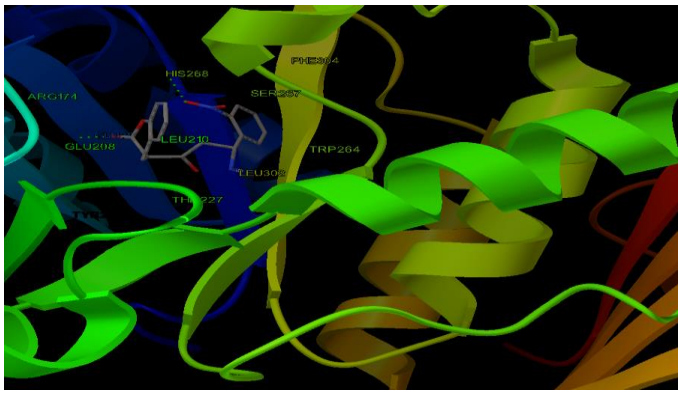


Figure 5. Snapshot of I_3 binding with 1UA7

I_4

I4 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-12.15 kcal/mol.**

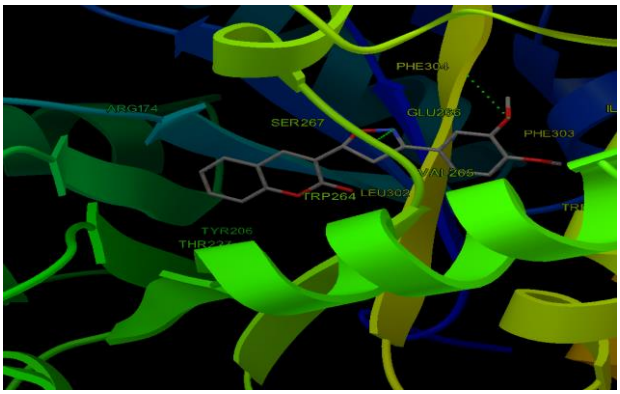


Figure 6.Snapshot of I_4 binding with 1UA7

I_5

I5 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-8.83 kcal/mol.**

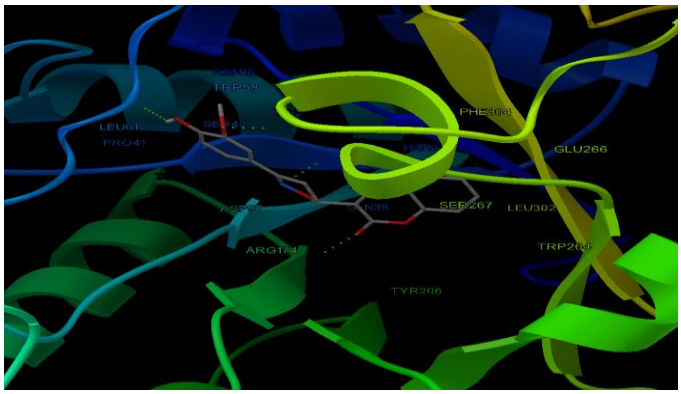


Figure 7.Snapshot of I_5 binding with 1UA7

Binding of Pyrazole compounds with α-amylase enzyme

T_1

T1 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-14.08 kcal/mol.**

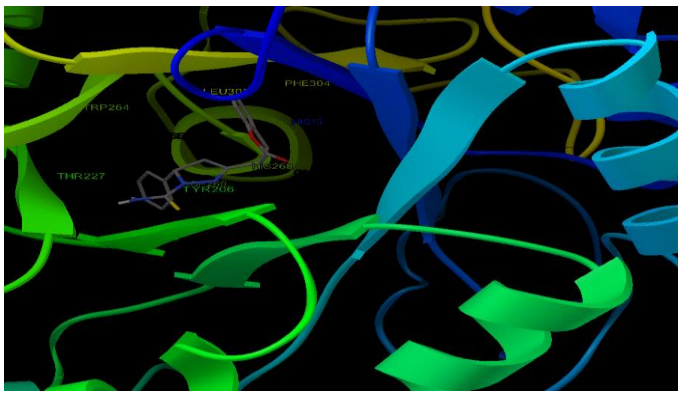


Figure 8.Snapshot of T_1 binding with 1UA7

T_2

T_2 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-9.14 kcal/mol**

Figure 9.Snapshot of T_2 binding with 1UA7

T_3

T_3 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-12.15 kcal/mol**

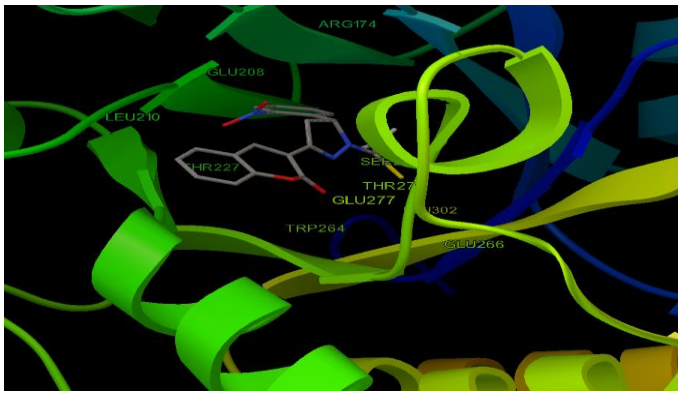


Figure 10. Snapshot of T_3 binding with 1UA7

T_4

T_4 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-6.00 kcal/mol.**

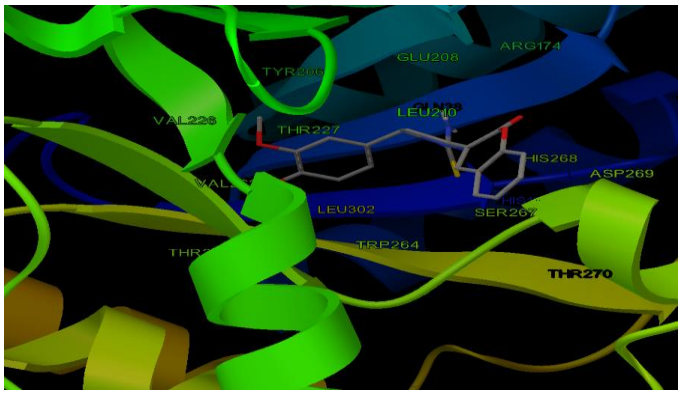


Figure 11. Snapshot of T_4 binding with 1UA7

T_5

T_5 interact with α-amylase enzyme at ASP212A, LYS179A, ASN273A, TYR59A, GLn63A, Gln126A, Leu142A, HIS180A, and GLu208A. Binding energy was found to be **-5.87 kcal/mol.**

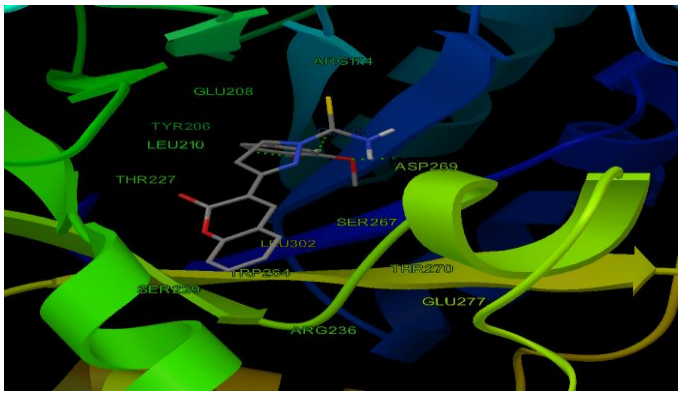


Figure 12 . Snapshot of T_5 binding with 1UA7

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[113]

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 method 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.

RESULTS AND DISCUSSION

S.NO	Compound code	M Log p	Molecular weight	No. of H acceptors	No. of H donors	No. of violation
1	I_1	3.35	291.31	4	1	0
2	I_2	3.98	329.27	4	1	0
3	I_3	3.43	336.30	7	0	0
4	I_4	2.99	351.36	6	1	0
5	I_5	3.36	335.31	6	1	0
6	T_1	3.21	341.42	5	3	0
7	T_2	3.84	383.86	5	3	0
8	T_3	3.29	394.41	8	2	0
9	T_4	2.86	409.47	7	3	0
10	T_5	2.55	395.44	7	4	0

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 $\mathbf{I_{1\text{-}5}}$ and $\mathbf{T_{1\text{-}5}}$ have good oral absorption.

SYNTHESIS

MATERIALS AND METHODS

Chemical and Reagents used

Salicylaldehyde, ethylacetoacetate, piperdine, aromatic aldehyde [benzaldehyde, chloro benzaldehyde, nitro benzaldehyde, veratraldehyde, vanillin], rectified spirit (ethanol), sodium hydroxide, hydroxylamine HCl, sodium acetate.

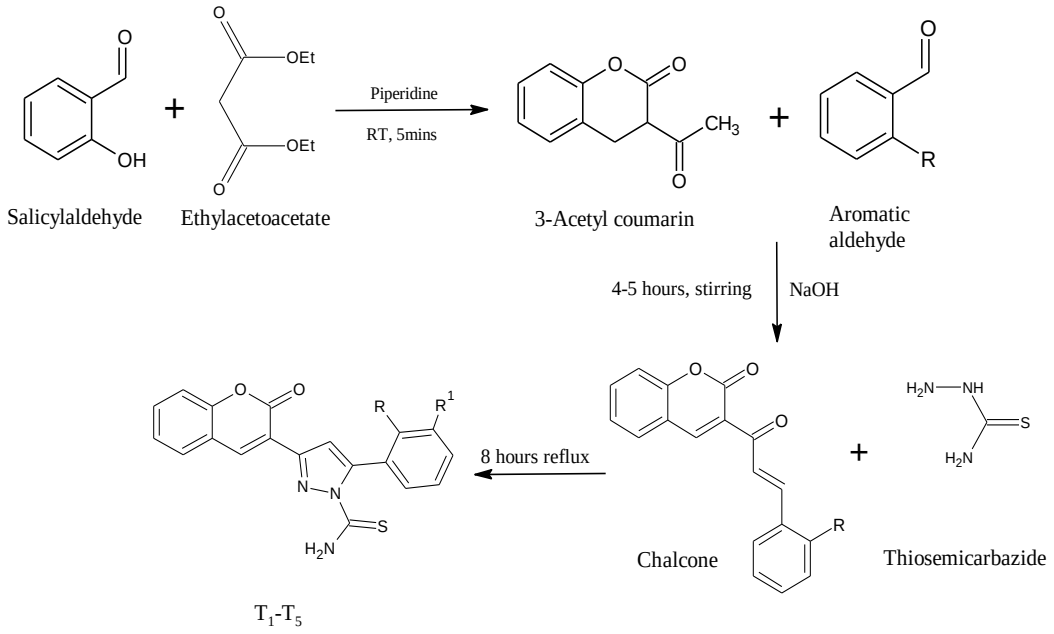
Apparatus used

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

Analytical work

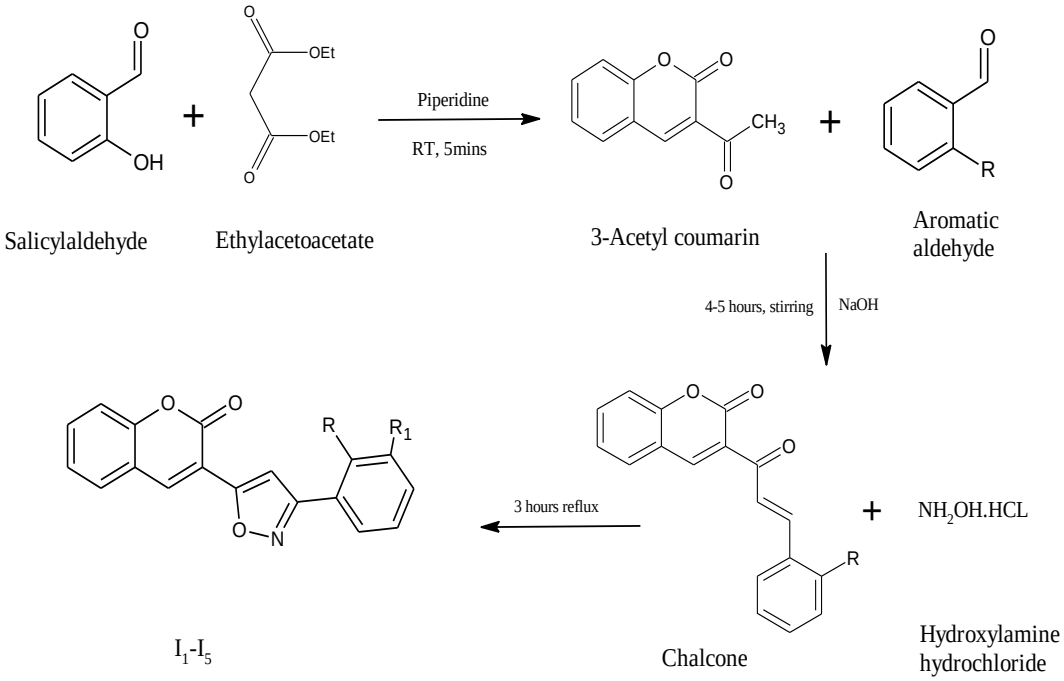
- Melting points were determined by using melting point apparatus MR-VIS, Visual Melting Range Apparatus LABINDIA and were uncorrected in the department of pharmaceutical chemistry, college of pharmacy SRIMPS, Coimbatore.
- Reactions were monitored by thin layer chromatography (TLC) on a precoated silica gel G plates using Iodine vapour as visualising agent.
- The compounds purity are recorded on JASCO V-530 UV\V is spectrophotometer in the department of pharmaceutical analysis college of pharmacy SRIPMS, Coimbatore.
- IR spectra is recorded on JASCO FT\IR-140 spectrometer in the department of pharmaceutical analysis college of pharmacy SRIPMS, Coimbatore.
- PMR spectra were recorded on BRUKER ULTRA SHIELDED NMR-400 MHz at VIT UNIVERSITY, Chennai.
- MASS spectra were recorded on GCMS at VIT UNIVERSITY, Chennai.

SCHEME I



Compound code	R	R^1
T_1	H	H
T_2	Cl	H
T_3	NO_2	H
T_4	OCH_3	OCH_3
T_5	OH	OCH_3

SCHEME II



Compound	R	R_1
I_1	H	H
I_2	Cl	H
I_3	NO_2	H
I_4	OCH_3	OCH_3
I_5	OH	OCH_3

Procedure

Step 1: Synthesis of 3-acetyl coumarin[114]

A mixture of salicylaldehyde (0.204 mole) and ethylacetoacetate (0.255 mole) was cooled and maintained at 0°-5°C, piperidine was added dropwise to the mixture while stirring (Doebner modification). The reaction mixture was left overnight, resulting the formation of a yellow coloured solid, the solid thus obtained was filtered and recrystallized with ethanol to give 3-acetyl coumarin as fine yellow needles.

Step 2: Synthesis of chalcone[115]

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 (30%) 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 Pyrazoles[116]

A mixture of chalcones (3 mmole), and thiosemicarbazide (14mmole) was refluxed in ethanol (50ml) containing 0.5ml of concentrated hydrochloric acid for 8 hours and poured on 100gm of crushed ice. The residue thus obtained was filtered and recrystallised with methanol.

Synthesis of Isoxazoles[116]

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.

PHYSICAL CHARACTERISATION DATA

1) Substituted Isoxazoles

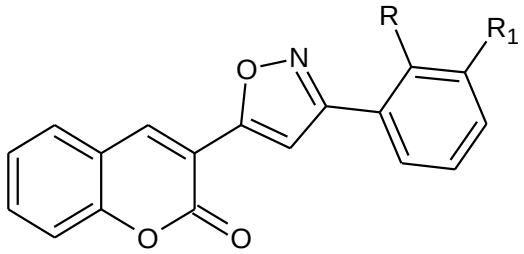


Table 3: Physical characterization of substituted Isoxazoles

Compound code	R	R_1	Molecular formula	Molecular weight (g/mol)	% yield	Melting point	Rf value
I_1	H	H	$C_{18}H_{13}NO_3$	291.300	58.25%	174-175^0 C	0.57
I_2	Cl	H	$C_{18}H_{12}ClNO_3$	329.27	88%	115-117^0 C	0.60
I_3	NO_2	H	$C_{18}H_{12}N_2O_5$	336.298	70%	167-179^0 C	0.39
I_4	OCH_3	OCH_3	$C_{20}H_{17}NO_5$	351.352	55%	121-123^0 C	0.51
I_5	OH	OCH_3	$C_{19}H_{15}NO_5$	337.326	57.9%	167-179^0 C	0.32

Recrystallisation **:**Methanol

Solvent system **:**10% Ethylacetate in hexane

Visualizing agent **:**Iodine vapour

2) Substituted Pyrazoles

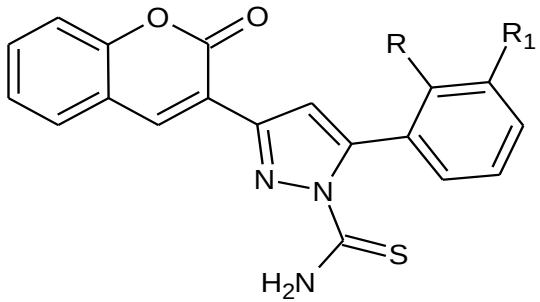


Table 4: Physical characterization of substituted Pyrazoles

Compound code	R	R_1	Molecular formula	Molecular weight (g/mol)	% yield	Melting point	Rf value
T_1	H	H	$C_{19}H_{15}N_3O_2S$	341.406	67%	177^0 C	0.56
T_2	Cl	H	$C_{19}H_{14}ClN_3O_2S$	383.851	76%	172^0 C	0.42
T_3	NO_2	H	$C_{19}H_{14}N_4O_4S$	394.403	87%	188^0 C	0.66
T_4	OCH_3	OCH_3	$C_{21}H_{19}N_3O_4S$	409.458	46%	166^0 C	0.43
T_5	OH	OCH_3	$C_{20}H_{17}N_3O_4S$	35.431	57%	169^0 C	0.77

Recrystallisation **:**Methanol

Solvent system **:**10% Ethylacetate in hexane

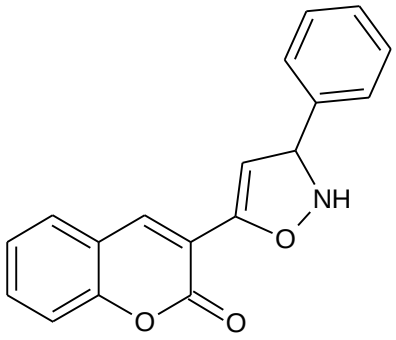
Visualizing agent **:**Iodine vapour

SPECTRA ANALYSIS

SPECTRAL ANALYSIS OF COMPOUNDS[117-119]

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

Compound code: I_1



Chemical name	3-(3-phenyl-2,3-dihydro-1,2-oxazol-5-yl)-2*H*-chromen-2-one
UV Spectrum	Solvent used: Methanolλ_{max} : 276
IR (KBr, v_{max} in cm^{-1})	3433(N-H),1635.34(coumarinylC=O), 1384.04(C=C), 1099.23(C-O), 1041.37 (N-O), 697.14 (Aromatic C-H)

Figure 13:UV Spectrum of I_1

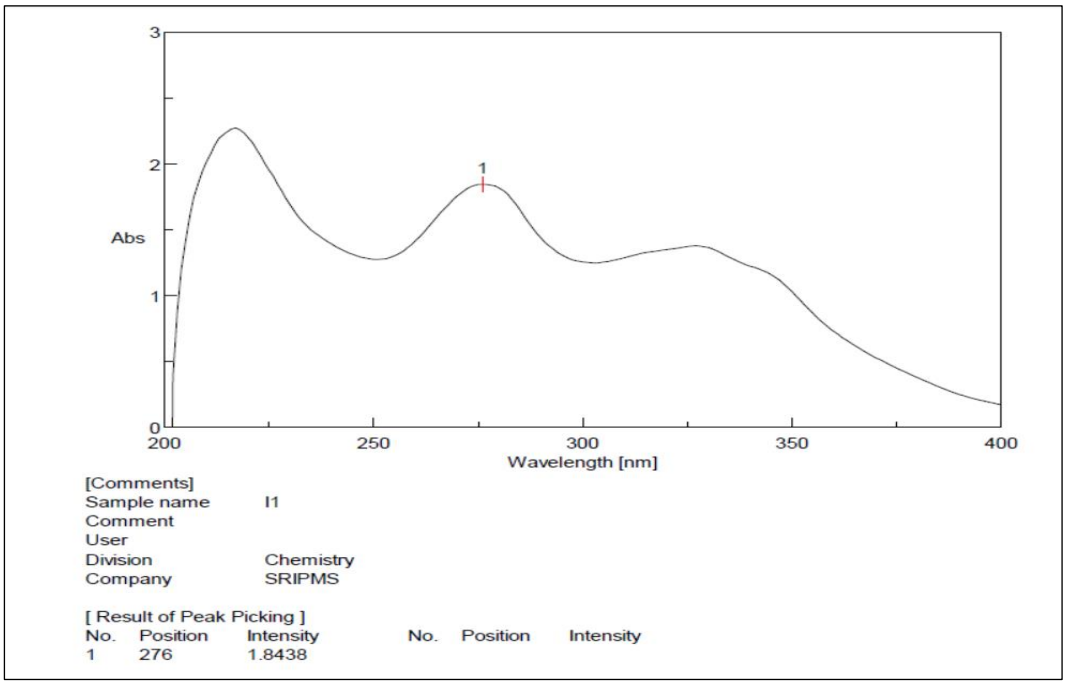
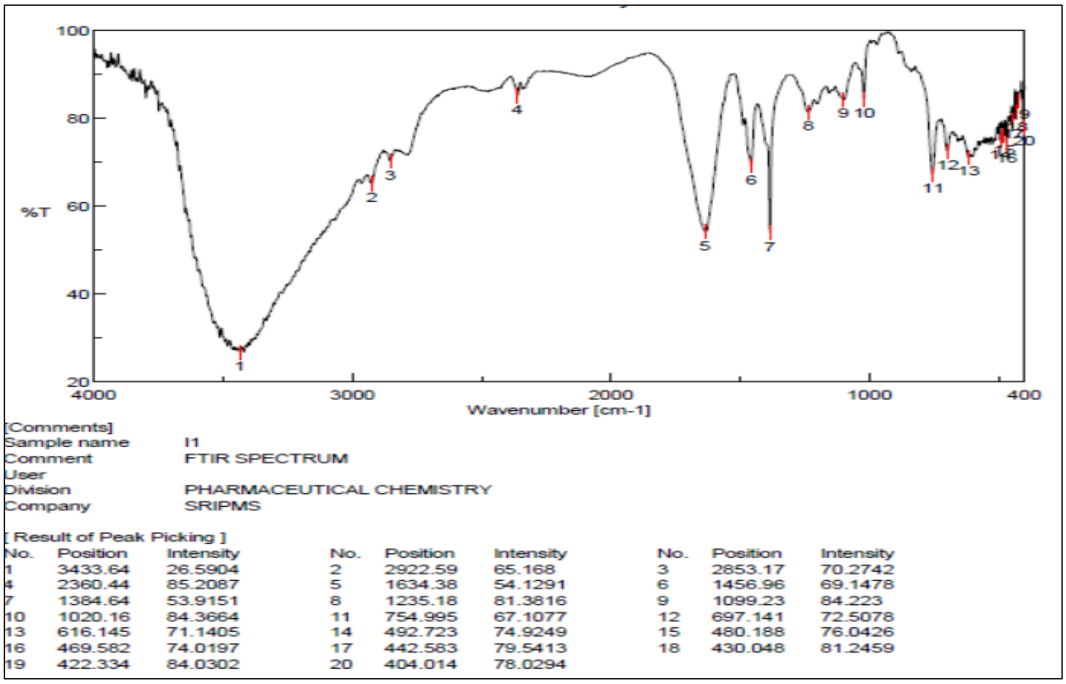
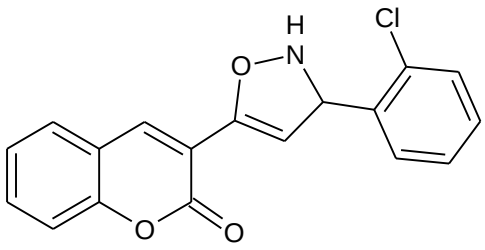


Figure 14: IR Spectrum of I_1



Compound Code: I_2



Chemical name	3-[3-(2-chlorophenyl)-2,3-dihydro-1,2-oxazol-5-yl]-2*H*-chromen-2-one
UV Spectrum	Solvent used: Methanol λ_{max} : 275
IR (KBr, v_{max} in cm^{-1})	3339.89(N-H),2357.55(Aromatic C-H),1635.38 (coumarinyl C=O), 1488.78 (Aromatic C=C),1384.64 (C-O),754.995 (C-Cl),
^{1}H NMR spectral data	δ 1.22 (s, 2H isoxazole ring), δ 6.96 (s, 1H NH), δ 7.470- 8.04 (m, 8H ArH), δ 8.57 (s, 1H coumarin (CH)),

Mass Spectral Data

Molecular weight of the compound: 329

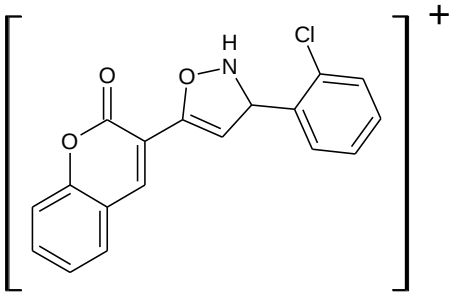
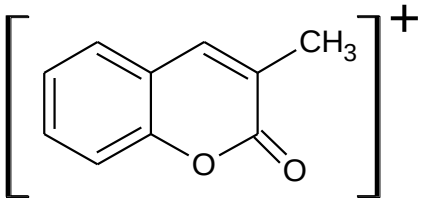
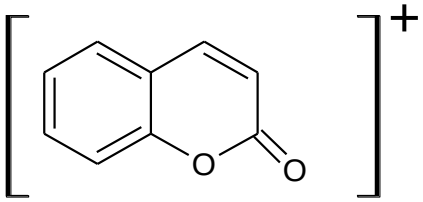
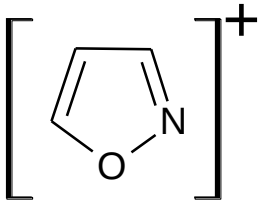
S/No	Fragments	m/z values
1		329.27
2		167
3		149
4		71.09

Figure 15: UV Spectrum of I_2

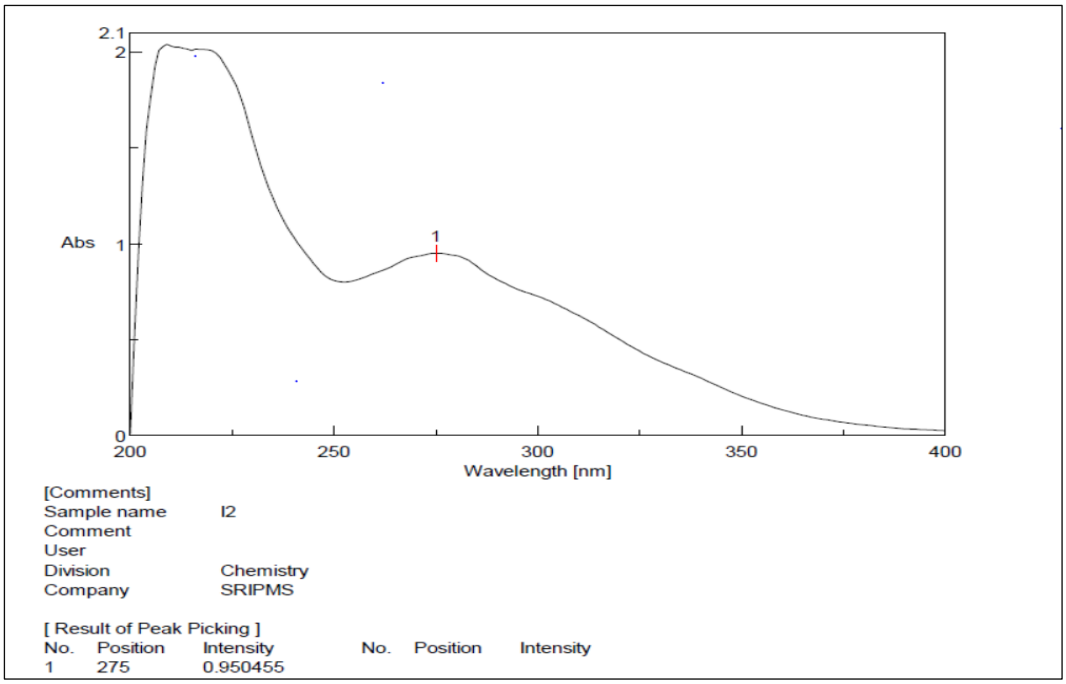


Figure 16: IR Spectrum of I_2

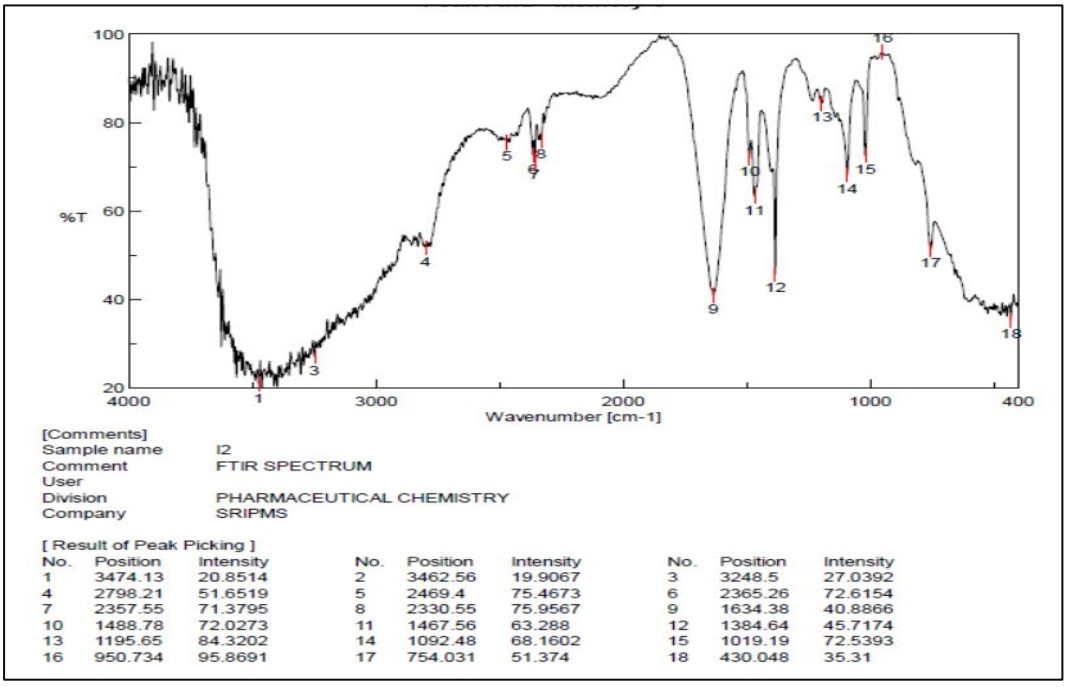


Figure 17: Mass Spectrum of I_2

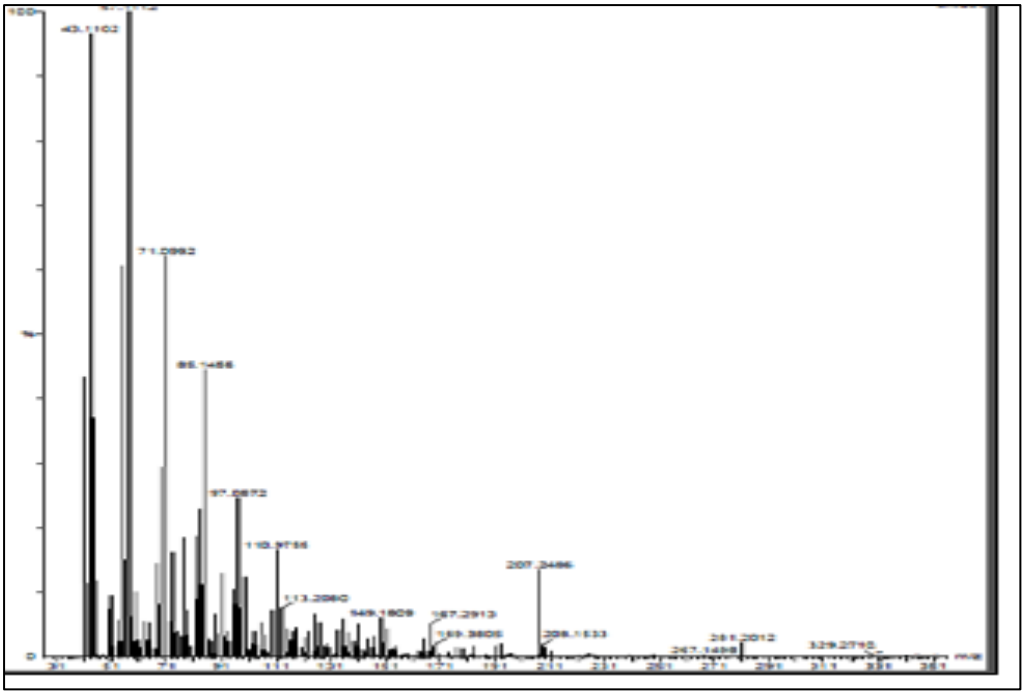
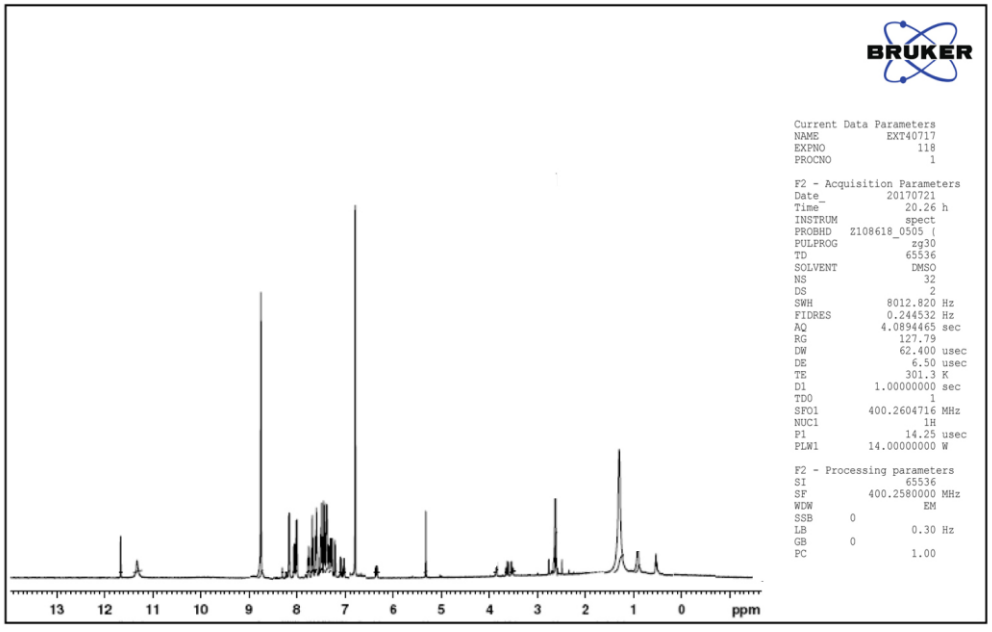
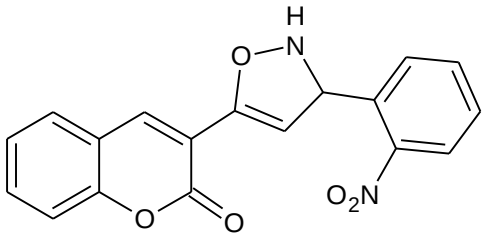


Figure 18: NMR Spectrum of I_2



Compound Code: I_3



Chemical name	3-[3-(2-nitrophenyl)-4,5-dihydro-1,2-oxazol-5-yl]-2*H*-chromen-2-one
UV Spectrum	Solvent used: Methanol λ_{max} : 264
IR (KBr, v_{max} in cm^{-1})	3443.28(NH), 1634.38 (coumarinyl C=O), 1529.27(C-O),1457.92(C=C), 1384.29(CNO_2),687.498 (Aromatic C-H)

Figure 19: UV Spectrum of I_3

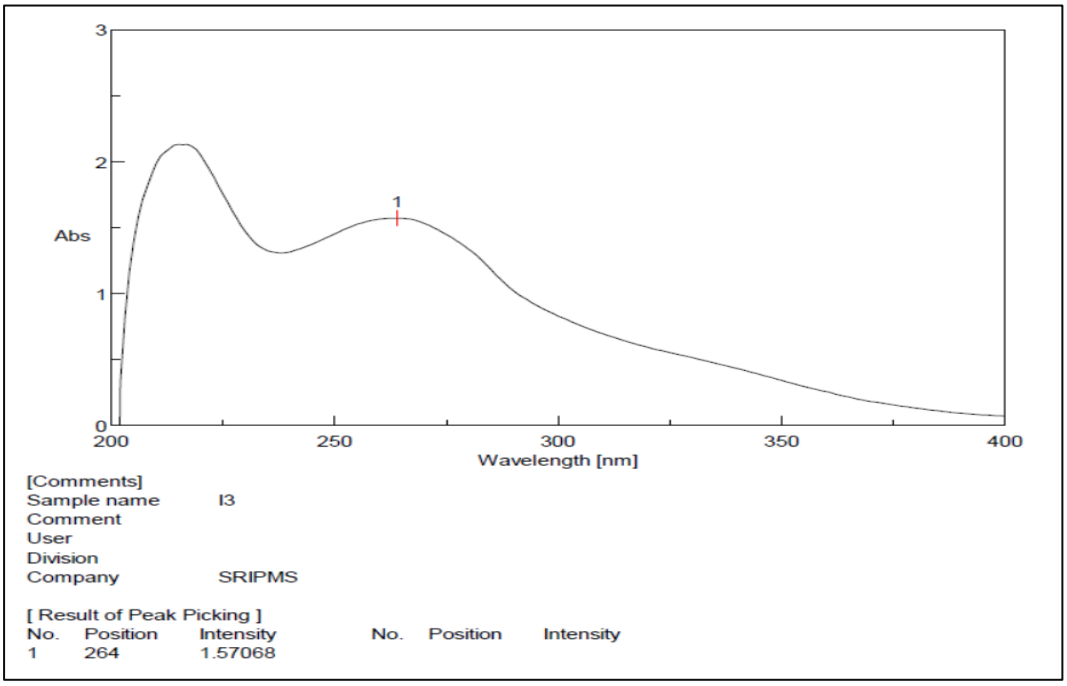
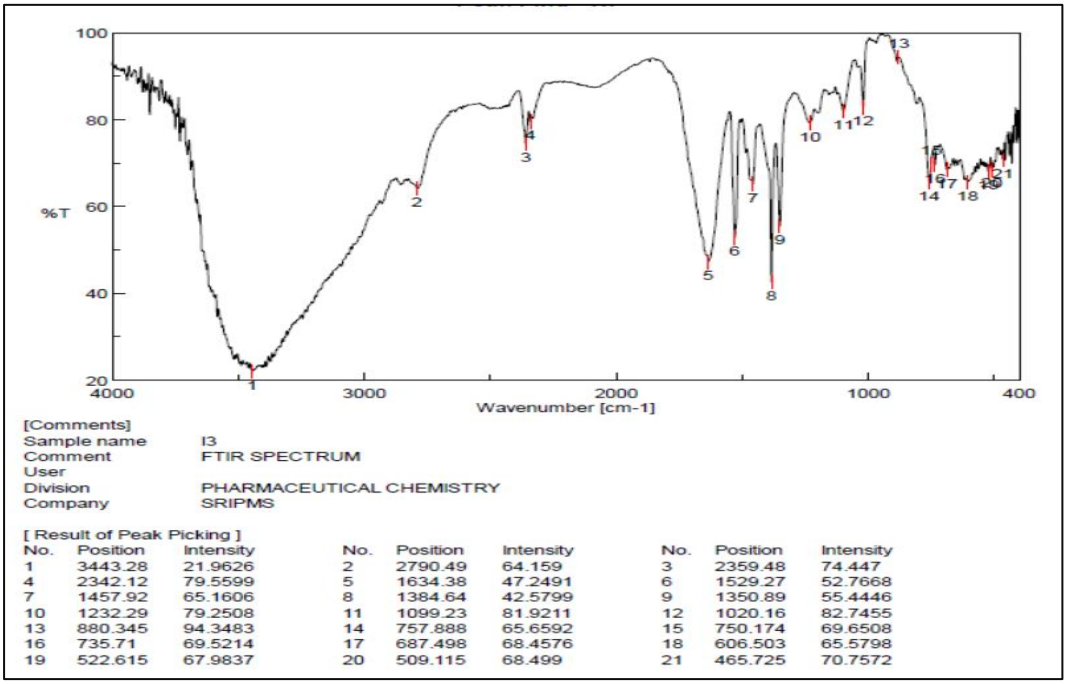
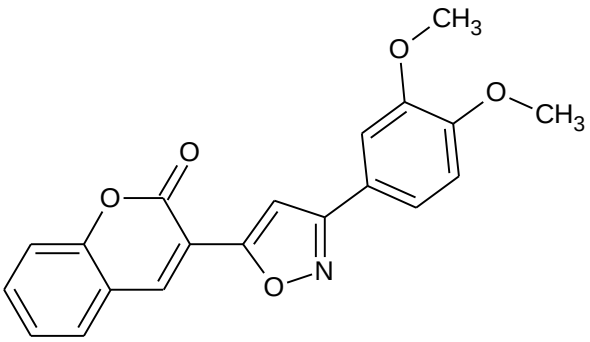


Figure 20: IR Spectrum of I_3



Compound Code: I_4



Chemical name	3-[3-(3,4-dimethoxyphenyl)-2,5-dihydro-1,2-oxazol-5-yl]-2*H*-chromen-2-one
UV Spectrum	Solvent used: Methanol λ_{max} : 276
IR (KBr, v_{max} in cm^{-1})	1630.52 (coumarinyl C=O), 1264.11 (C=N),1140(C-OCH_3), 1514.81 (C-O), 808.992 (Aromatic C-H),756.923(C=C)

Figure 21: UV Spectrum of I_4

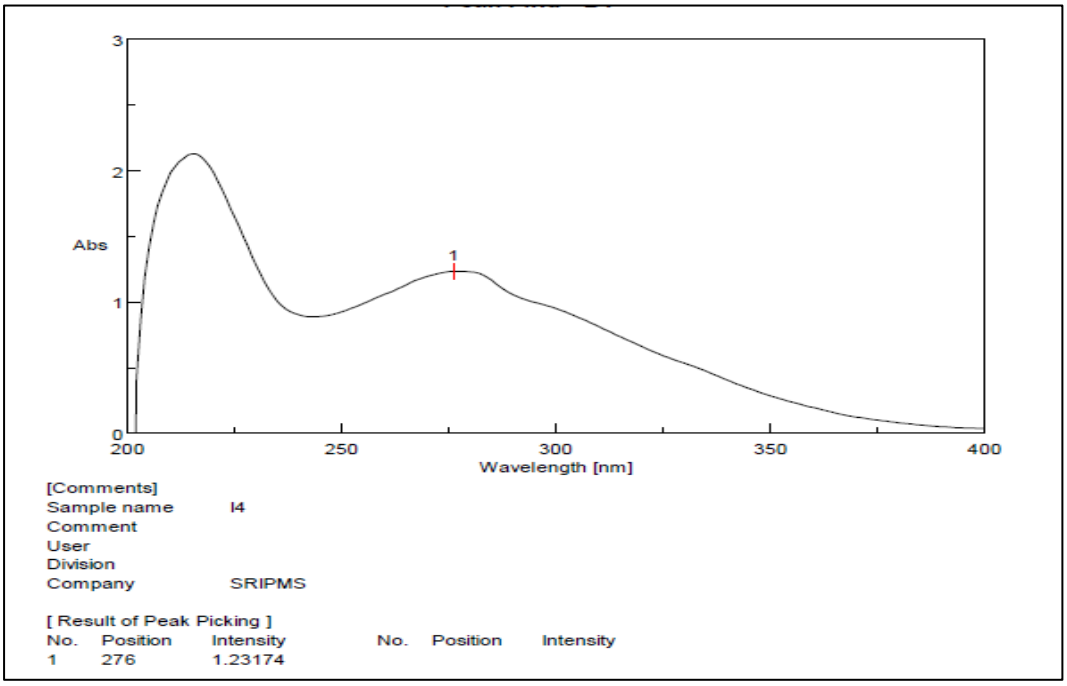
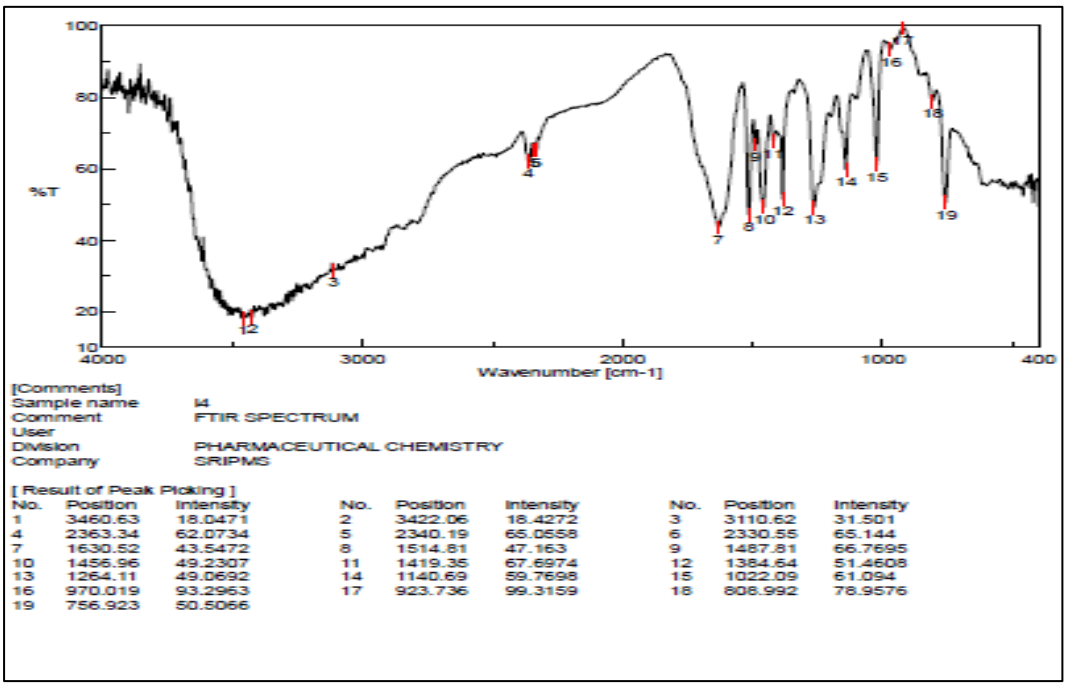
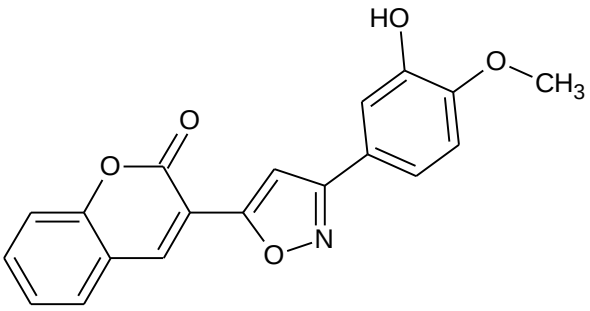


Figure 22: IR Spectrum of I_4

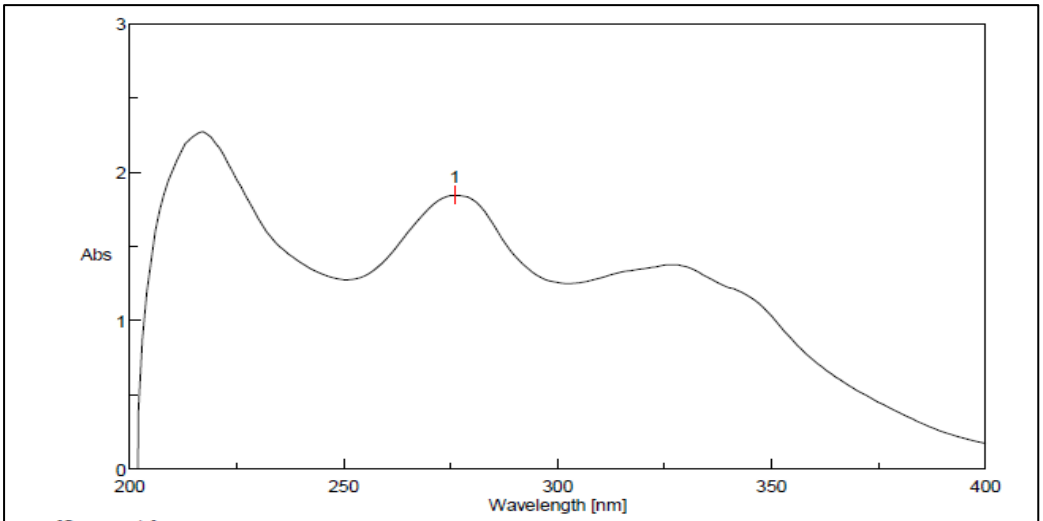


Compound Code: I_5



Chemical name	3-[3-(3-hydroxy-4-methoxyphenyl)-1,2-oxazol-5-yl]-2*H*-chromen-2-one
UV Spectrum	Solvent used: Methanol λ_{max} : 276
IR (KBr, v_{max} in cm^{-1})	3064.33 (Aromatic C-H),1634.38 (coumarinyl C=O), 1486.85 (C-O),1231.33 (C=N),1140.69(C-OCH_3),719.318 (C-H bend)

Figure 23: UV Spectrum of I_5

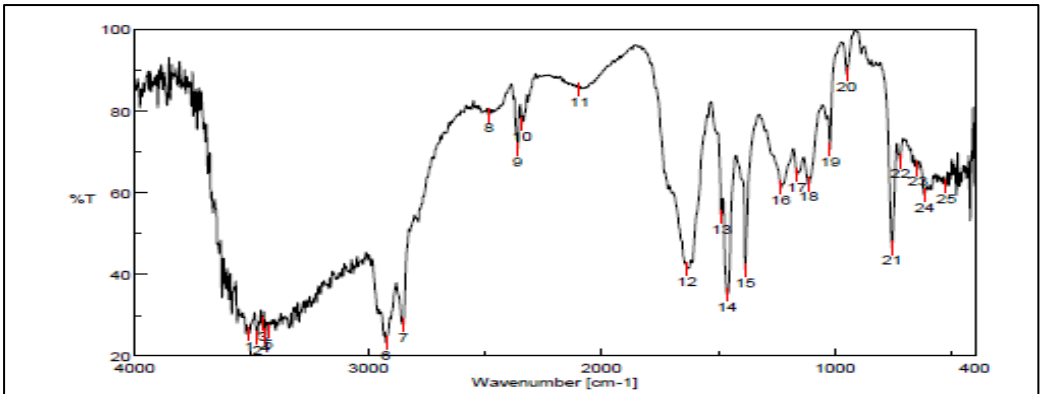


[Comments]
Sample name I5
Comment
User
Division Chemistry
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	276	1.8438						

Figure 24: IR Spectrum of I_5

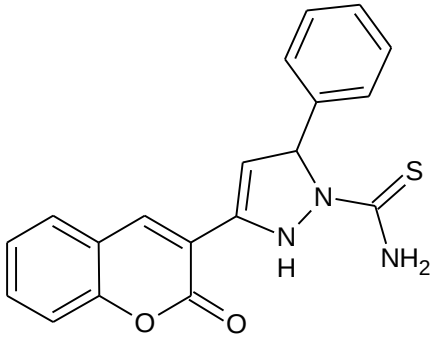


[Comments]
Sample name I5
Comment FTIR SPECTRUM
User
Division PHARMACEUTICAL CHEMISTRY
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3507.88	25.5386	2	3474.13	24.587	3	3451.96	27.9917
4	3440.39	24.9113	5	3425.92	26.1714	6	2923.56	23.2724
7	2849.31	27.541	8	2481.94	78.9755	9	2359.48	70.8034
10	2342.12	76.8702	11	2095.28	85.4639	12	1634.38	41.2376
13	1486.85	54.1047	14	1461.78	35.0848	15	1383.68	41.1425
16	1231.33	61.1896	17	1161.9	64.46	18	1113.69	62.1173
19	1020.16	70.7144	20	947.842	89.0223	21	754.031	46.5328
22	719.318	67.7751	23	645.072	65.8211	24	616.145	59.4447
25	523.579	61.5999						

Compound Code: T_1



Chemical name	3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide
UV Spectrum	Solvent used: Methanol λ_{max} : 276
IR (KBr, v_{max} in cm^{-1})	3436.53 (NH_2), 3209.22 (C-NH), 1608.34 (coumarinyl C=O), 1488.78(AromaticC=C), 1384.64 (C=S), 1270.86 (C-N), 884.20(C-H bend)
^{1}H NMR Spectral data	δ 6.84 (s, 2H pyrazole ring), δ 6.71, 6.88, (s, 2H NH), δ 9.04 (s, 1H coumarin (CH)), δ 7.25-8.02 (m, 9H ArH), 6.86 (s, 1H =CH)

Mass Spectral data

Molecular weight of the compound: 341

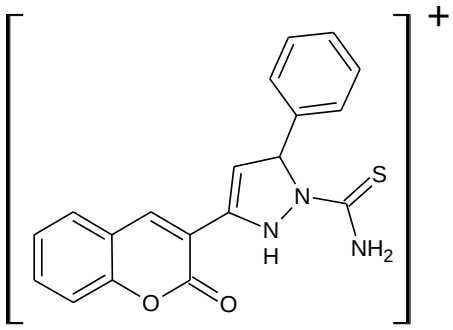
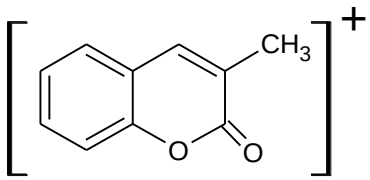
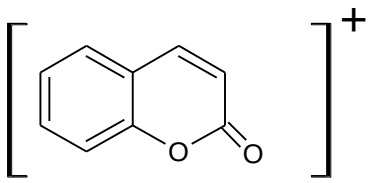
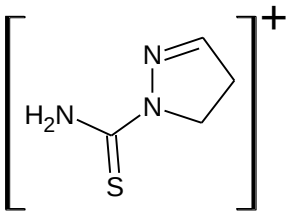
S/NO	Fragments	m/z values
1		341
2		167
3		147
4		133

Figure 25: UV Spectrum of T_1

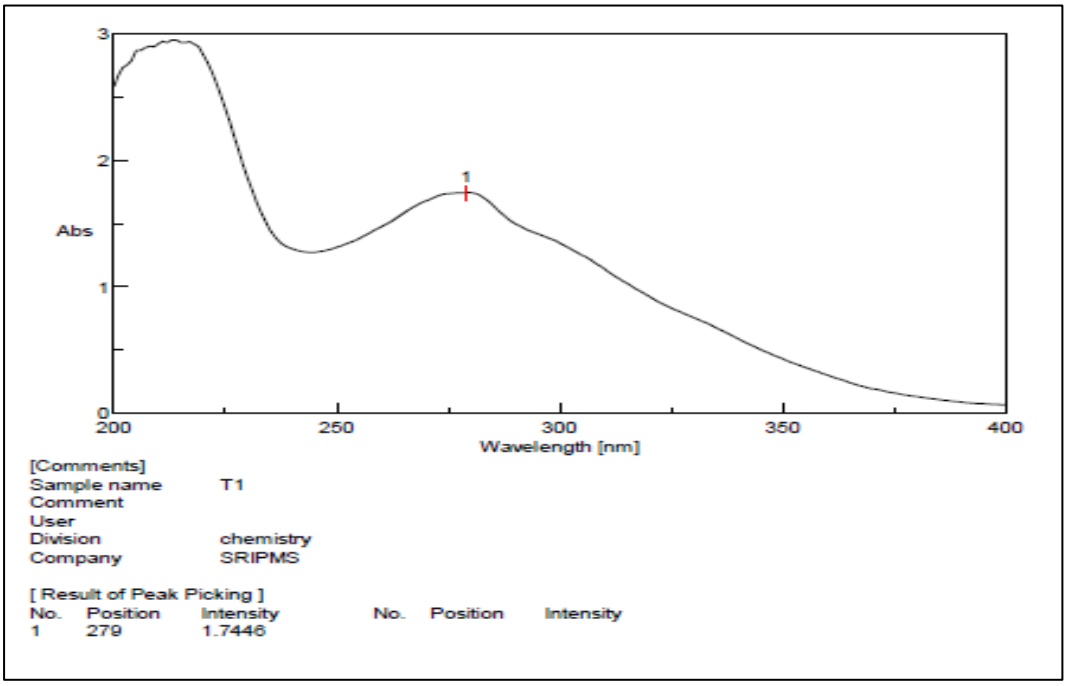


Figure 26: IR Spectrum of T_1

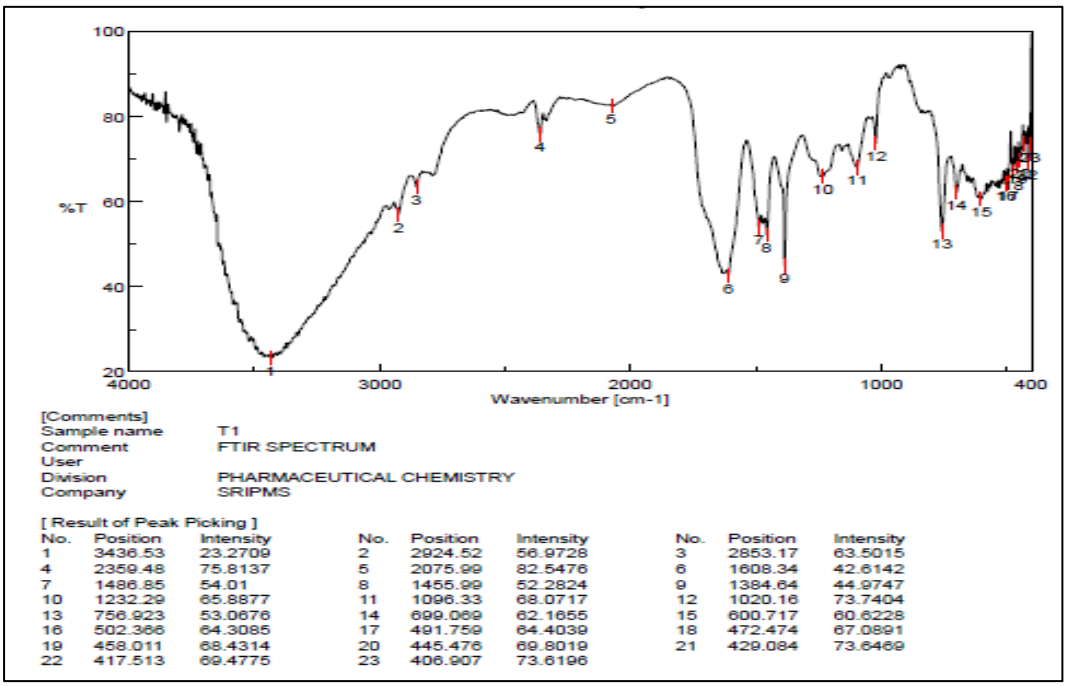


Figure 27: Mass Spectrum of T_1

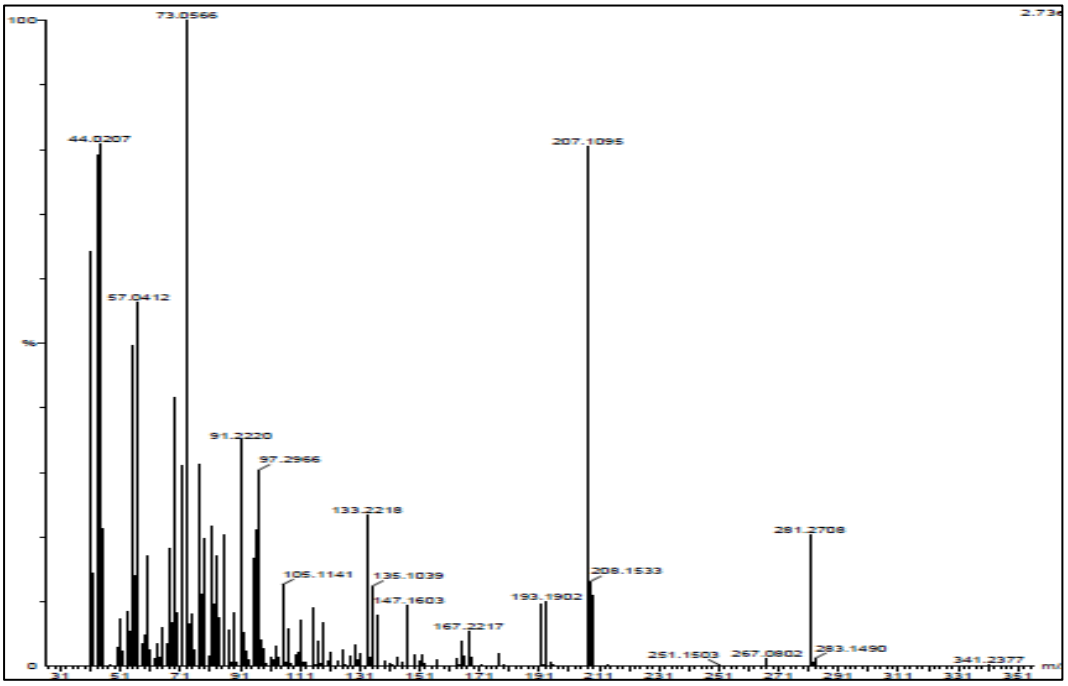
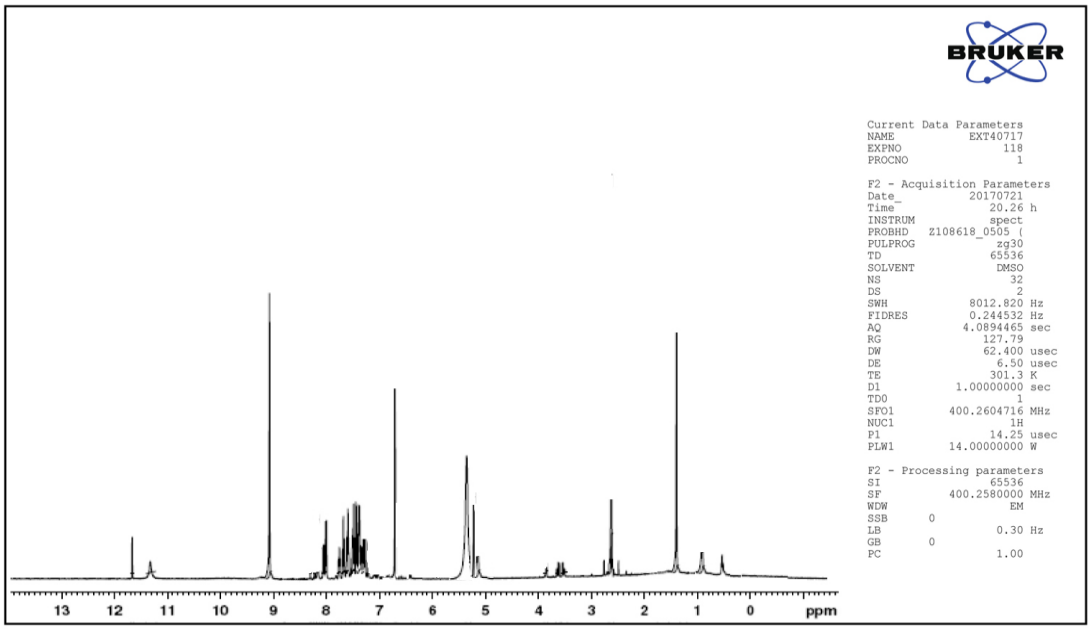
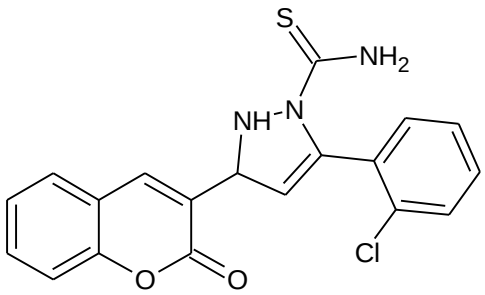


Figure 28: NMR Spectrum of T_1



Compound Code: T_2



Chemical name	5-(2-chlorophenyl)-3-(2-oxo-3,4-dihydro-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazole-1-carbothioamide
UV Spectrum	Solvent used: Methanol λ_{max} : 275
IR (KBr, v_{max} in cm^{-1})	3450.03(NH_2), 3334.32(C-NH), 1625.7(coumarinylC=O),1176(C=S),1488.78 (AromaticC=C),884.20(Aromatic C-H), 759.81(C-Cl)

Figure 29: UV Spectrum of T_2

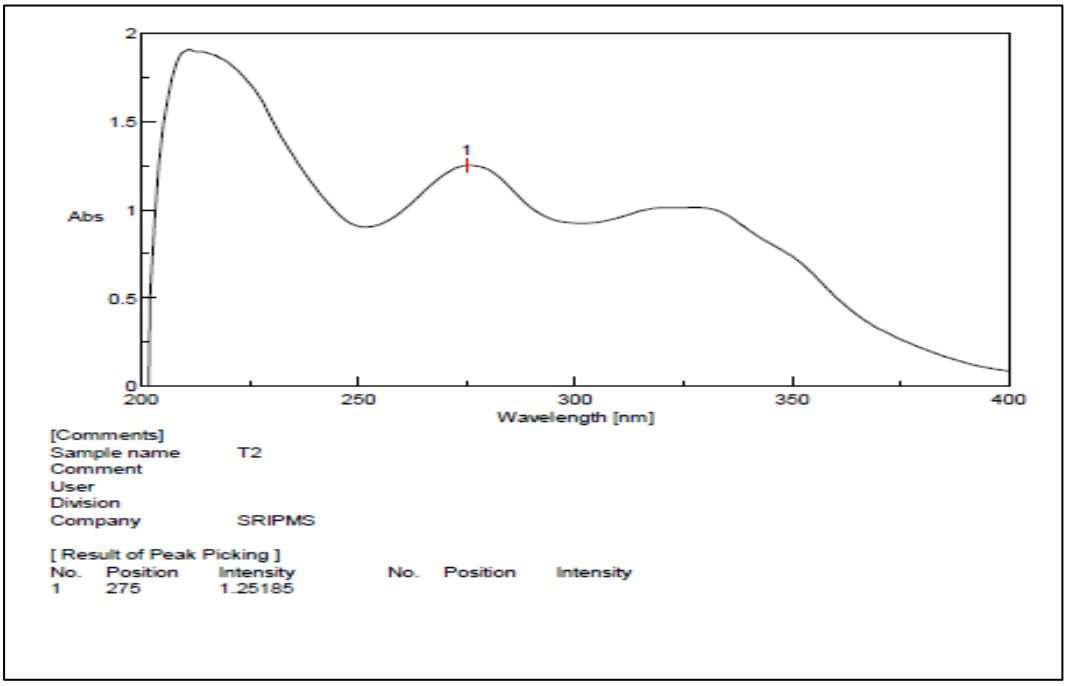
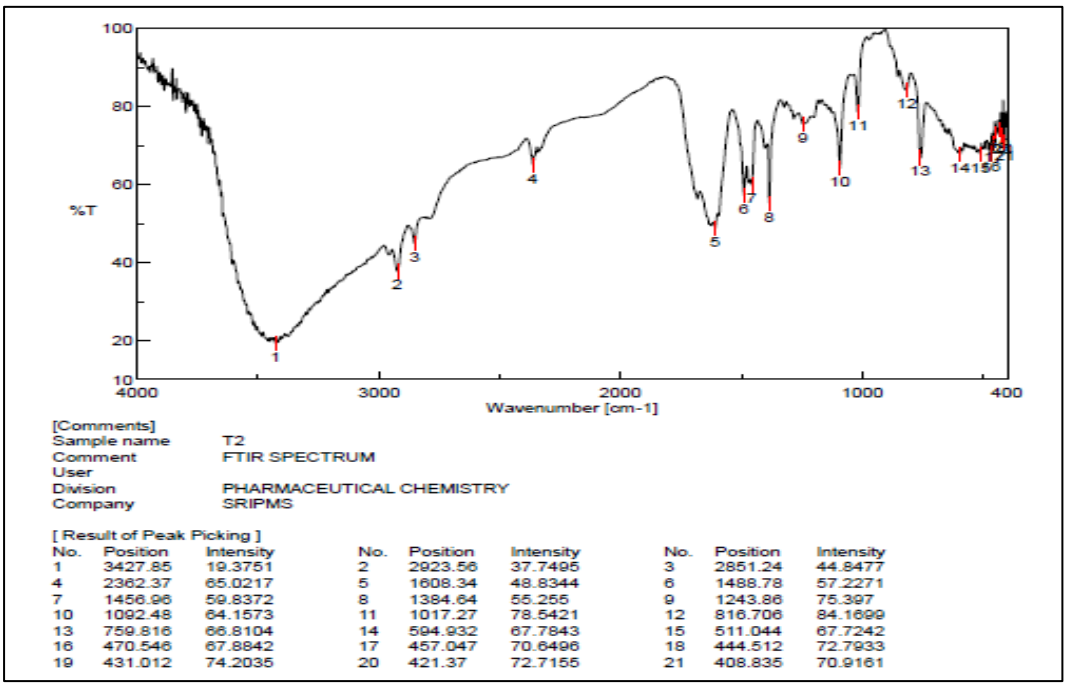
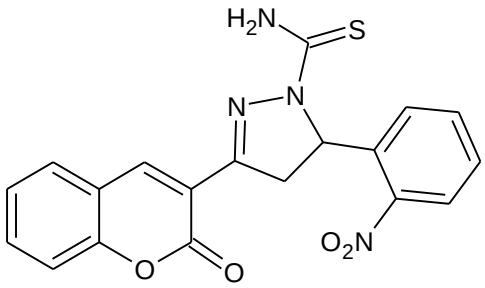


Figure 30: IR Spectrum of T_2



Compound Code: T_3



Chemical name	5-(2-nitrophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide
UV Spectrum	Solvent used: Methanol λ_{max} : 271
IR (KBr, v_{max} in cm^{-1})	3445.21(NH_2),1636.3(coumarinylC=O), 1524.45(C=N), 1470.46(C=S), 1384.64(C-NO_2) ,1350.89(C-N), 615.18(aromatic C-H)

Figure 31: UV Spectrum of T_3

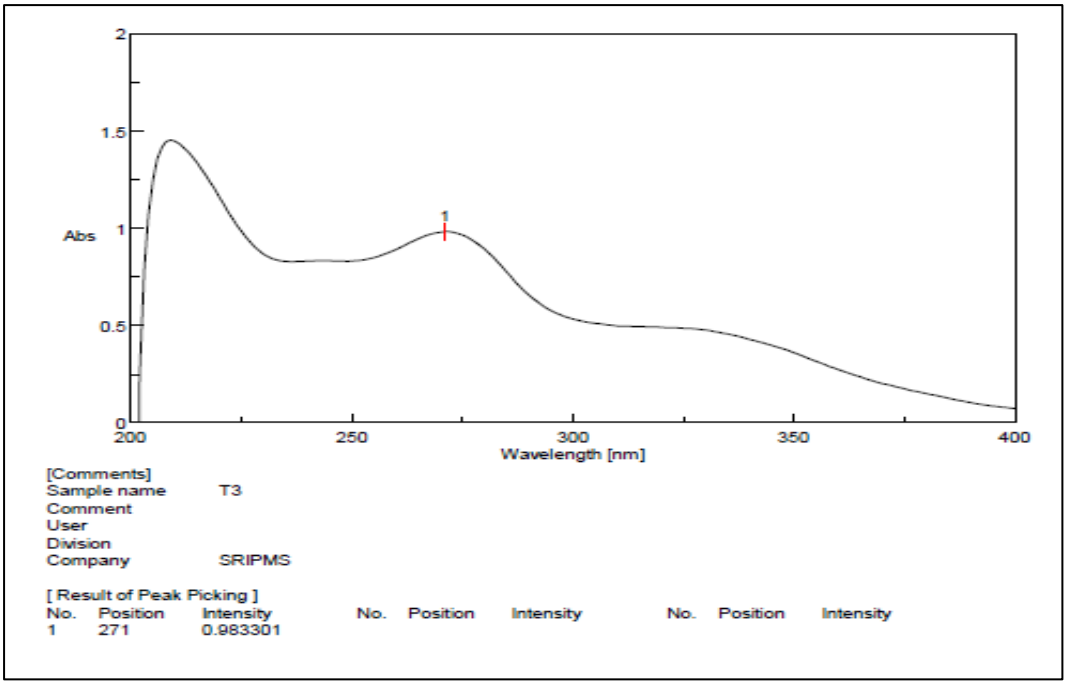
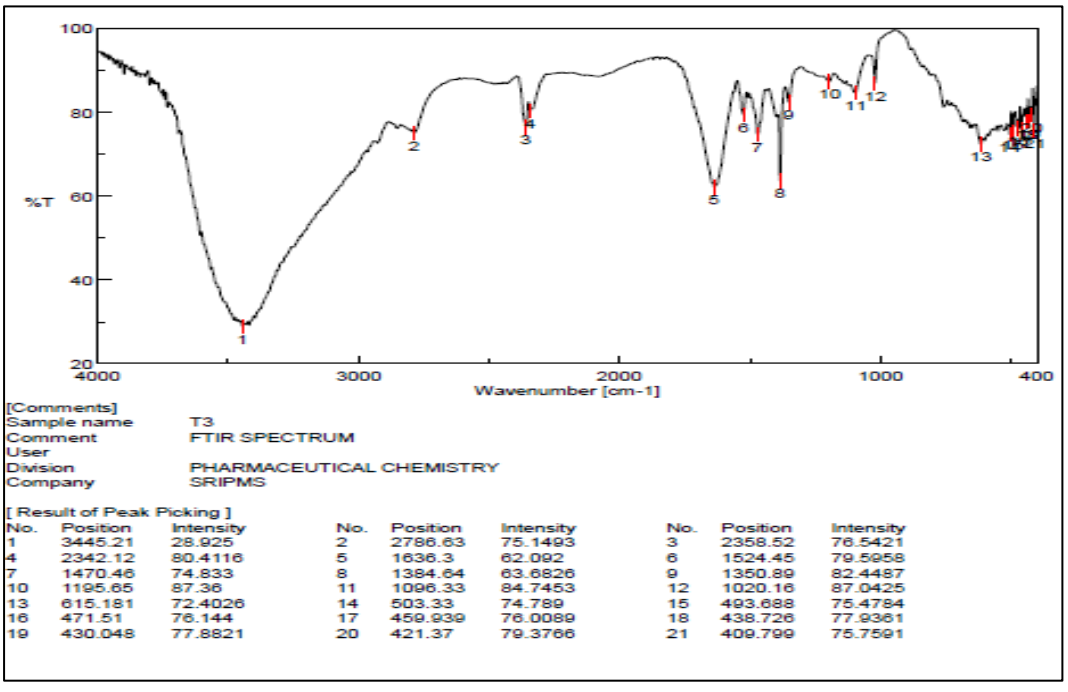
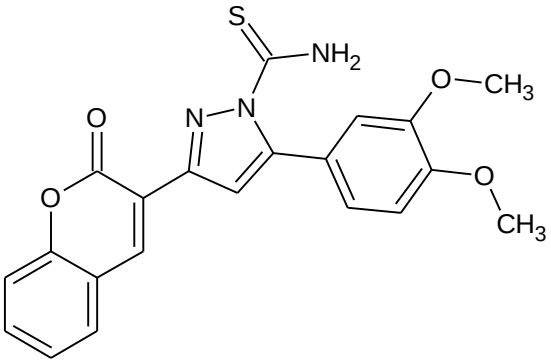


Figure 32: IR Spectrum of T_3



Compound Code: T_4



Chemical name	5-(3,4-dimethoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazole-1-carbothioamide
UV Spectrum	Solvent used: Methanolλ_{max} : 276
IR (KBr, v_{max} in cm^{-1})	3478.95(NH_2),1605.45(coumarinylC=O), 1509.99(C=N) ,1455.99(C-N), ,1383.68(C=S),1195.65(C-OCH_3), 811.88(C-H)

Figure 33: UV Spectrum of T_4

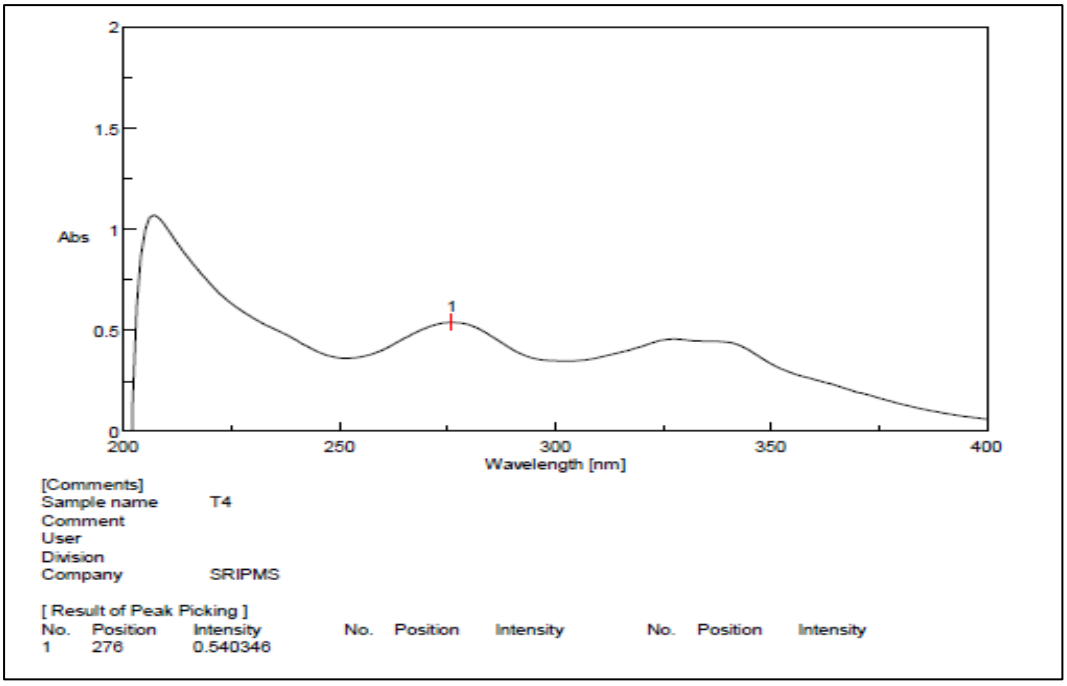
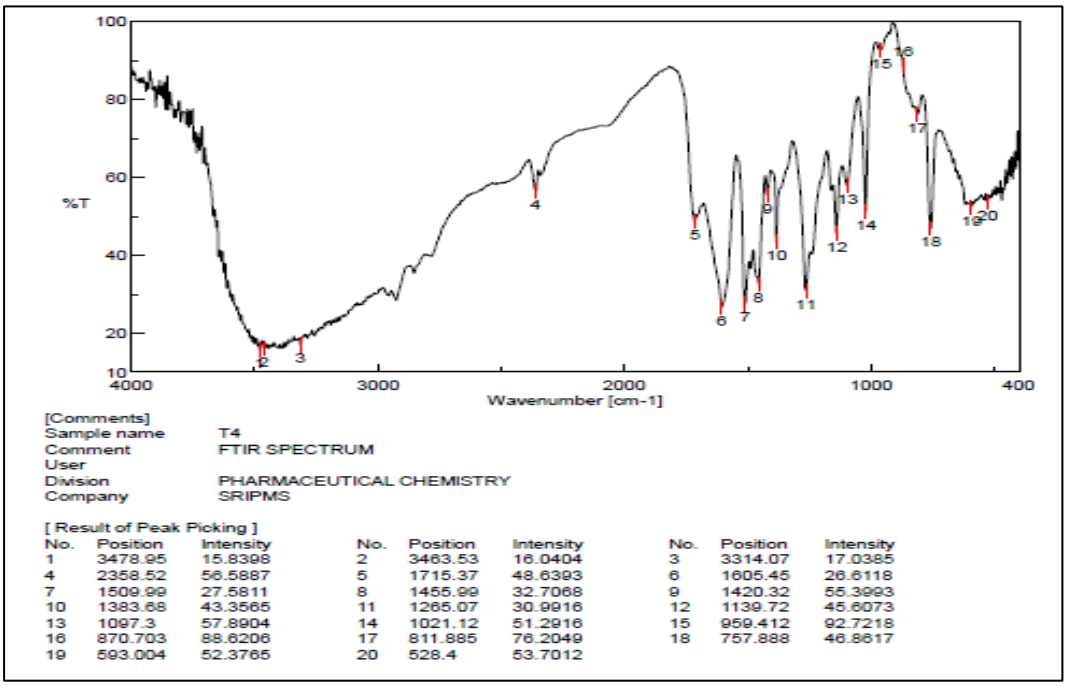
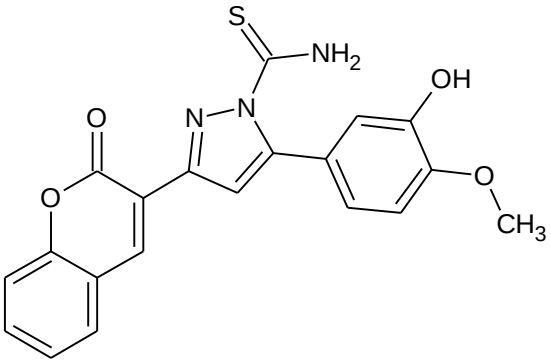


Figure 34: IR Spectrum of T_4

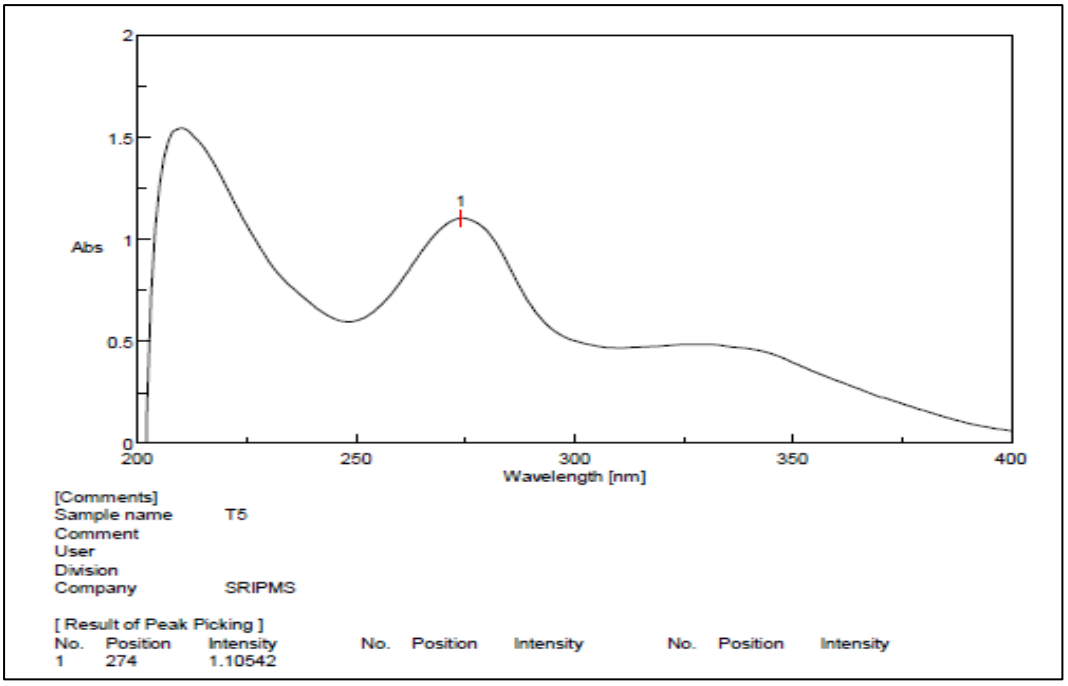


Compound Code: T_5



Chemical name	5-(3-hydroxy-4-methoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazole-1-carbothioamide
UV Spectrum	Solvent used: Methanol λ_{max} : 276
IR (KBr, v_{max} in cm^{-1})	3353.6(NH_2),3379.64(AromaticC-H), 1607.38(coumarinylC=O), 1487.81(C=N),1455.03(C-N), 1383.68(C=S),1101.15(C-OCH_3)

Figure 35: UV Spectrum of T_5

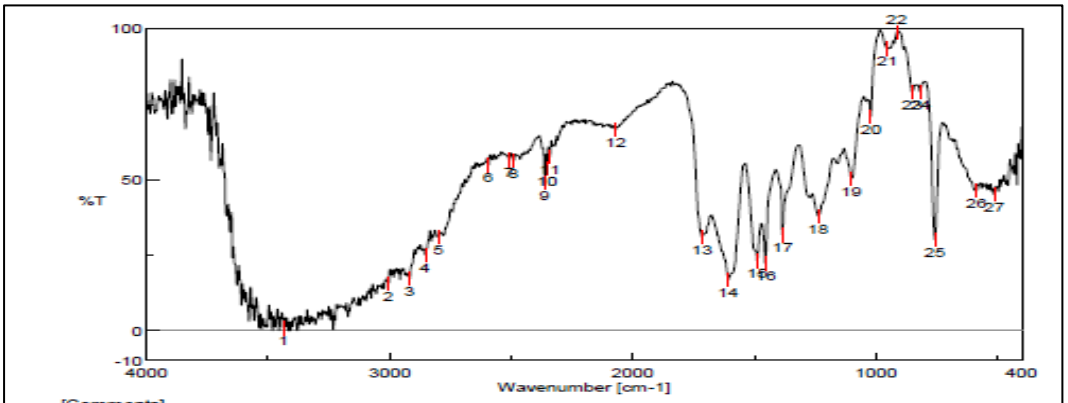


[Comments]
Sample name T5
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	274	1.10542						

Figure 36: IR Spectrum of T_5



[Comments]
Sample name T5
Comment FTIR SPECTRUM
User
Division PHARMACEUTICAL CHEMISTRY
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3436.53	0.764955	2	3004.55	15.615	3	2917.77	17.1375
4	2853.17	25.0404	5	2799.17	30.8875	6	2593.79	54.822
7	2507.97	56.4544	8	2489.65	56.4215	9	2362.37	48.9621
10	2353.69	53.716	11	2341.16	57.5608	12	2070.21	66.5345
13	1715.37	31.0256	14	1607.38	16.6946	15	1487.81	23.0729
16	1455.03	22.1682	17	1383.68	31.6877	18	1238.08	37.9347
19	1101.15	50.435	20	1020.16	70.9774	21	952.663	93.3746
22	914.093	98.6866	23	851.418	79.108	24	817.67	79.2001
25	755.959	30.0368	26	586.254	46.2661	27	513.936	45.0929

ENZYME INHIBITION STUDIES

DRUGS AND CHEMICALS

Acarbose (Bicon Ltd, Banglore), porcine pancreatic α-amylase (Sigma-Aldrich, USA), Glucose assay kits (Agappe Diagnostics, Kerala) 3, 5-dinitro salicyclic acid (HiMedia, Mumbai) and potato starch and maltose (Lobachemie,Mumbai) 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[120]

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.

PREPARATION OF REAGENTS[121]

Preparation of Phosphate Buffer

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

Preparation of Starch Solution

Starch solution (1.0%) prepared with phosphate buffer by boiling.

Preparation of Coloring Reagents

Colouring reagent is prepared by slowly adding sodium potassium tartarate solution [prepared in the ratio 12 g of solid dissolved in 8 ml of 2M sodium hydroxide] to 20 ml of 96 mM 3,5-dinitrosalicyclic acid (prepared in distilled water) and then diluting the mixture to 40 ml with distilled water.

Preparation of enzyme solution

Enzyme solution, alpha amylase (0.5 mg/ml) prepared with phosphate buffer pH 6.9.

PROCEDURE[122-124]

From 1 mg/ml stock solution different concentration (5-500 µg/ml) of coumarin derivatives were prepared by adding few drops of dimethylsulfoxide and volume made up with water. About 500 µl of α-amylase (0.5 mg/ml) was added and was incubated for 10 minutes at room temperature. Then added 500µl of 1.0% starch solution and incubated for another 10 minutes. After that 1 ml of the coloring reagent was added to the reaction mixture and heated in a boiling water bath for 5 minutes. After cooling, 10 ml of distilled water was added for dilution. To measure the absorbance of the colored extracts, blank was prepared for each set of concentration of test sample by replacing the enzyme solution with buffer. Control incubations representing 100% enzyme activity was prepared by replacing

the test drug with water. The absorbance was then measured 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.

RESULT AND DISCUSSION

Evaluation of α-amylase inhibitory activity

All the newly designed and synthesised compounds were screened for *in vitro* α-amylase inhibitory activity at 5, 10, 25, 50, 100, 200, 400, 500μg/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 Ten compounds were synthesised in that 5 compounds belongs to Isoxazole derivatives in which IC_{50} of compound-I_4(28 μg/ml) and other 5 compounds belongs to Pyrazole derivatives in which IC_{50} of compounds-T_2 (35 μg/ml) and T_3(25 μg/ml) showed percentage of inhibition closer to that of standard(Acarbose-12 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 $\mathbf{I_{1\text{-}5}}$ series of isoxazoles derivatives of coumarin, $\mathbf{I_4}$ showed good percentage of inhibition at all concentration (5 μg/ml-500 μg). The IC_{50} values for these compounds were found to be 28 μg/ml respectively which is close to IC_{50} value of acarbose (10 μg/ml). $\mathbf{I_1}$ and $\mathbf{I_5}$ showed moderate α-amylase inhibitory activity at all concentrations. The IC_{50} value for this compound found to be 49 μg/ml and 50 μg/ml. Other compounds $\mathbf{I_2}$ and $\mathbf{I_3}$ exhibited the least α-amylase inhibitory activity at all concentrations with IC_{50} values 210 μg/ml and 110 μg/ml respectively.

Among $\mathbf{T_{1\text{-}5}}$ series of pyrazoles derivatives of coumarin, $\mathbf{T_1}$ and $\mathbf{T_3}$ showed good percentage of inhibition at all concentration (5 μg/ml-500 μg/ml). The IC_{50} values for these compounds were found to be 24 μg/ml and 25 μg/ml respectively which is close to IC_{50} value of acarbose (25 μg/ml). $\mathbf{T_2}$ showed moderate α-amylase inhibitory activity at all concentrations. The IC_{50} value for this compound found to be 35 μg/ml. Other compounds $\mathbf{T_4}$ and $\mathbf{T_5}$ exhibited the least α-amylase inhibitory activity at all concentrations with IC_{50} values 75 μg/ml and 150 μg/ml respectively.

PERCENTAGE OF α-AMYLASE INHIBITORY POTENTIAL OF SYNTHESISED COUMARIN DERIVATIVES

TABLE 5: IN VITRO α-AMYLASE INHIBITORY ACTIVITY

Compound code	5 µg/ml	10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	500 µg/ml	IC_{50} µg/ml
I_1	30.69	40.46	42.79	51.16	57.67	60.46	71.16	79.06	49
I_2	9.01	16.86	22.13	31.96	39.89	48.23	59.20	67.20	210
I_3	19.16	28.83	35.75	42.75	49.85	58.75	73.58	79.00	110
I_4	29.16	35.16	44.83	56.25	64.16	67.33	78.75	81.58	28
I_5	29.41	36.37	40.47	51.37	59.70	65.37	75.64	79.60	50
T_1	34.00	42.50	55.30	61.00	64.00	71.00	79.00	82.03	24
T_2	24.54	35.00	46.81	49.09	65.00	75.00	85.00	88.00	35
T_3	33.75	41.25	54.50	60.41	68.75	77.50	80.16	85.40	25
T_4	19.58	32.91	37.30	45.33	54.66	65.50	76.25	79.50	75
T_5	21.96	27.84	30.98	40.39	46.60	52.54	56.47	66.80	150
Standard (Acarbose)	27.28	46.17	52.05	63.34	73.11	79.24	82.21	94.01	12

SUMMARY AND CONCLUSION

SUMMARY

The present work was focused on the designing and synthesis of novel isoxazole and pyrazole 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, pyrazole posses 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: Virtual screening

Virtual screening was done by **iGEMDOCK v.2 software**. More than 500 compounds from the pubchem and zinc database were screened from which 50 lead compounds were selected. From this, based on the bioactivity score, Coumarin was selected as the lead molecule

Stage 3: Lead optimization

Lead optimisation was done by computation of drug likness score. Isoxazole and Pyrazole derivatives of coumarin were the desired compounds with good molecular properties and bioactivity score, ie., the compounds **I_{1-5} and T_{1-5},** showed good scores.

PHASE III: SYNTHESIS AND PHYSICAL CHARACTERIZATION

A) Synthesis of the designed compounds

In this work, ten new compounds were synthesized in which five different isoxazole and five different pyrazole derivatives with coumarin moiety. The first step involved the synthesis of 3- acetyl coumarin by Knovenagel reaction. Chalcones were prepared from 3- acetyl coumarin and different aromatic aldehydes. Finally, the chalcones were reacted with hydroxylamine hydrochloride and thiosemicarbazide to form isoxazoles and pyrazoles.

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 UV, IR, ^{1}H NMR, and Mass spectral data.

PHASE V: EVALUATION OF BIOLOGICAL ACTIVITIES

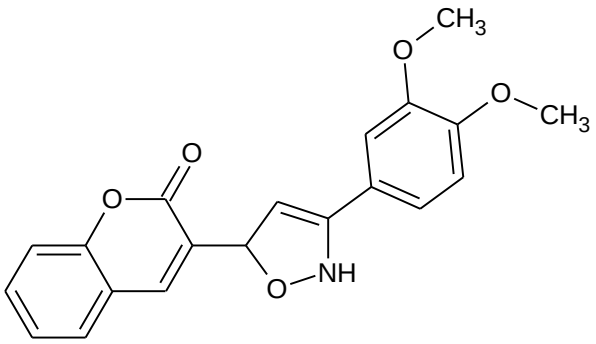
Evaluation of α-amylase inhibitory activity

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 **I_4, T_1** and **T_3** showed good % of inhibition activity at lower concentrations (20-50μg/ml), while I_1, I_5, T_2, T_4 showed moderate activity. (50-200μg/ml)

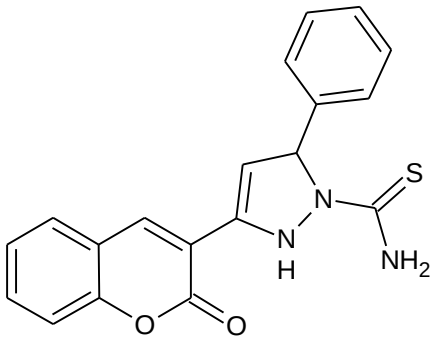
CONCLUSION

- 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.
- The drug likeness score established the compounds to be pharmacokinetically active.
- The proposed ten compounds of isoxazole and pyrazole 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 UV, IR, ^{1}H NMR, Mass spectra.
- Compounds I_4, T_1 and T_3 exhibited maximum α-amylase inhibitory activity.
- Among the synthesized compounds, I_4 and T_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



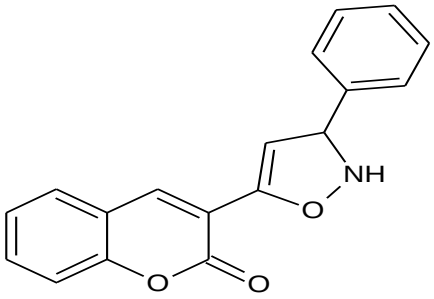
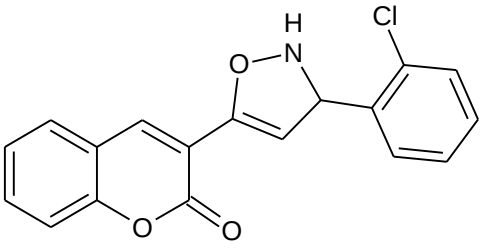
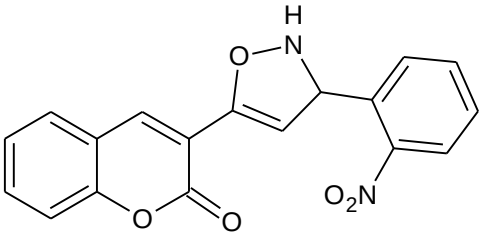
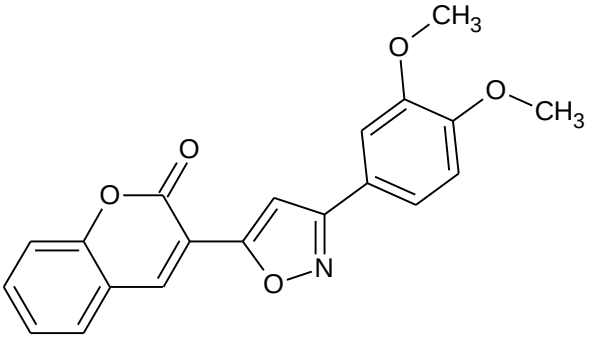
I_4

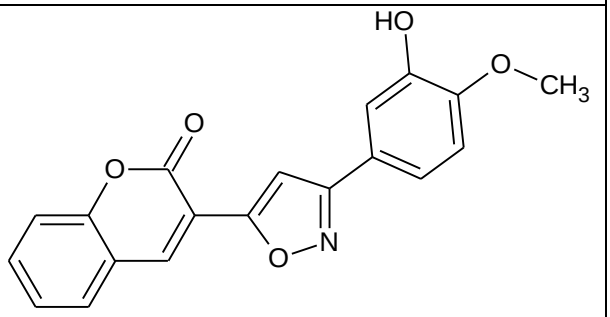


T_1

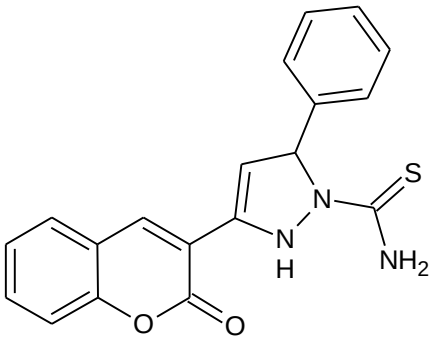
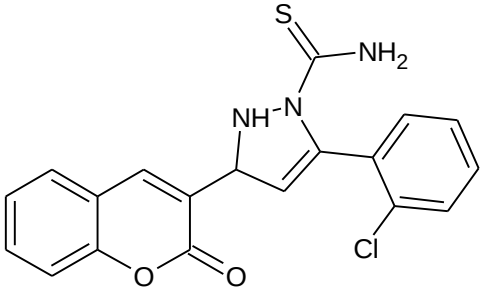
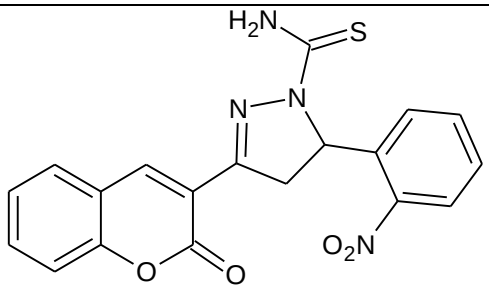
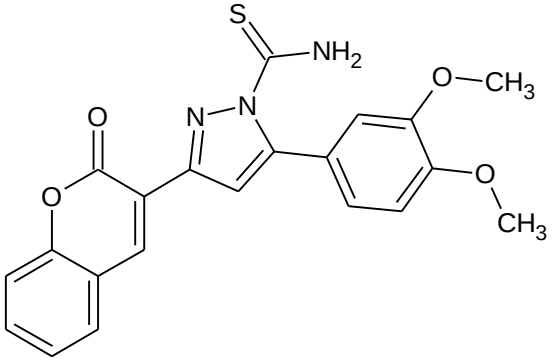
LIST OF NEWLY SYNTHESIZED COMPOUND

SUBSTITUTED ISOXAZOLE DERIVATIVES

Compound code	IUPAC name	Structure
I_1	3-(3-phenyl-2,3-dihydro-1,2-oxazol-5-yl)-2*H*-chromen-2-one	
I_2	3-[3-(2-chlorophenyl)-2,3-dihydro-1,2-oxazol-5-yl]-2*H*-chromen-2-one	
I_3	3-[3-(2-nitrophenyl)-4,5-dihydro-1,2-oxazol-5-yl]-2*H*-chromen-2-one	
I_4	3-[3-(3,4-dimethoxyphenyl)-2,5-dihydro-1,2-oxazol-5-yl]-2*H*-chromen-2-one	

Compound code	IUPAC name	Structure
I_5	3-[3-(3-hydroxy-4-methoxyphenyl)-1,2-oxazol-5-yl]-2*H*-chromen-2-one	

SUBSTITUTED PYRAZOLE DERIVATIVES

Compond code	IUPAC name	Structure
T_1	3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide	
T_2	5-(2-chlorophenyl)-3-(2-oxo-3,4-dihydro-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazole-1-carbothioamide	
T_3	5-(2-nitrophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide	
T_4	5-(3,4-dimethoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazole-1-carbothioamide	

Compond code	IUPAC name	Structure
T_5	5-(3-hydroxy-4-methoxyphenyl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazole-1-carbothioamide