DRUG DESIGN, DOCKING STUDIES AND SYNTHESIS OF CERTAIN COUMARINYL AZETIDIN-2-ONES AND EVALUATION OF THEIR ANTIMYCOBACTERIAL ACTIVITY

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
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Submitted by
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COIMBATORE – 641044

OCTOBER 2016

CERTIFICATE

This is to certify that the M.Pharm dissertation entitled **"Drug Design, Docking Studies and Synthesis of Certain Coumarinyl azetidin-2-ones and Evaluation of their Antimycobacterial Activity"** being submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai was carried out by **Ms. Arya Raveendran** 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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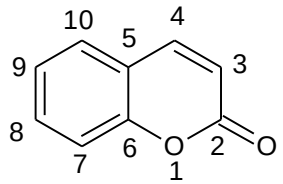
CONTENTS

INTRODUCTION

COUMARIN

Natural, synthetic and semisynthetic heterocyclic compounds play an important role in drug discovery and chemical biology. The heterocycles are mainly of the classes of alkaloids, flavones, isoflavones, chromans, chromones, coumarins and chromenes. Synthetic compounds of these classes show different biological activity. It has been established that oxygen-containing heterocyclic compounds play an important role in designing new class of structural entities for medicinal applications [1].

Among oxygen heterocyclic compounds, coumarin (*2H*-chromen-2-one or 2*H*-1-benzopyran-2-one) **(1)** and its derivatives are significant because of their wide spectrum of biological activities.



(1)

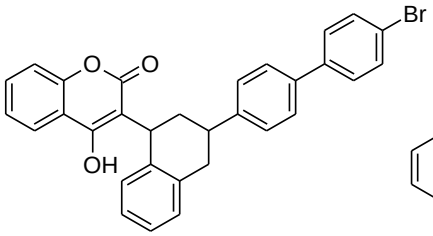
Coumarins owe their class name to 'coumarou', the vernacular name of the tonka bean, from which coumarin itself was isolated in 1820[2]. Coumarins comprise a very large class of compounds found throughout the plant kingdom. They are found at high levels in some essential oils, particularly *cinnamon* bark oil (7,000 ppm), *cassia* leaf oil (up to 87,300 ppm) and *lavender* oil. 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]. They belong to the family of lactones having 1-benzopyran-2-one system that can be isolated from plants as well as can be synthesized in the laboratory. Coumarin is a crystalline white powder with a hay-like, sweet aromatic creamy odor with certain nutty shadings, much used in

synthetic form as a fragrance chemical for perfumes and for fragranced soaps and detergents.

Coumarin is a versatile pharmacophore which exhibits a wide variety of biological activities like antibacterial and antimicrobial. Coumarins occupy a special role in nature. They belongs to the flavonoid class of plant secondary metabolite, which exhibit a variety of biological activities, associated with low toxicity and have achieved considerable interest due to their beneficial potential effects on human health[5]. Coumarin derivatives have been reported for their anticoagulant[6], anti-inflammatory[7], antimicrobial[8], anti HIV[9], antioxidant[10], antiallergic, anticancer[11] and antiproliferative activities. Furthermore, the pharmacological properties as well as therapeutic applications of coumarins depend upon the pattern of substitution.

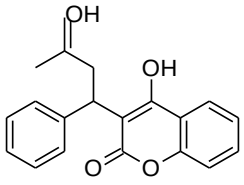
Apart from their therapeutical activities, it is also an important analytical agent. Regarding their high fluorescence ability, they are widely used as optical whitening agents, brighteners, laser dyes and also as fluorescent probes in biology and medicine[12]. Being synthetically important, their biosynthetic derivatives like, phytoalexins, which are hydroxylated derivatives of coumarins, produced in carrots in response to fungal infection and can be presumed to have antifungal activity. General antimicrobial activity was provided in Woodruff (*Galium odoratum*) extracts[13].

When coumarin ring is fused with other rings, a synergistic effect of biological activities of both the rings are obtained. Such compounds are exploited as important scaffolds for drug development. Various moieties, when combined with coumarin can produces same or different effects but with different potencies[14]. Some of the coumarin derivatives are already booming in the market[15]. Examples are given below in the figures **(2-13)**



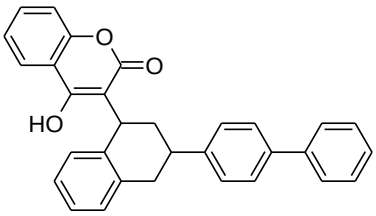
Brodifacoum (anticoagulant)

(2)



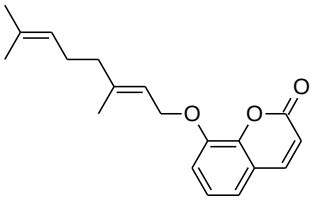
Warfarin (anticoagulant)

(3)



Difenacoum (anticoagulant)

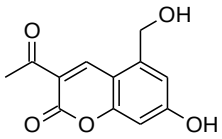
(4)



Auraptene

(chemopreventative agent)

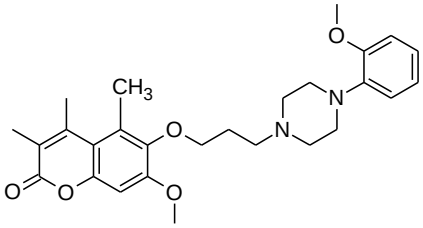
(5)



Armillarisin A

(antibiotic)

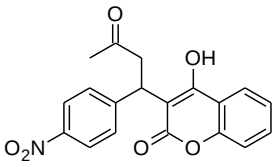
(6)



Ensaculin

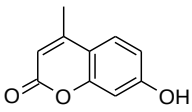
(NMDA antagonist and a 5HT1A agonist)

(7)



Acenocoumarol (anticoagulant)

(8)



Hymecromone

(choleretic and antispasmodic)

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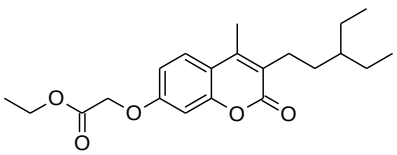
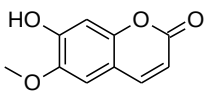


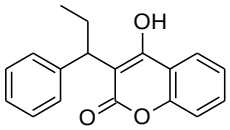
Carbochromen (coronary disease)

(10)



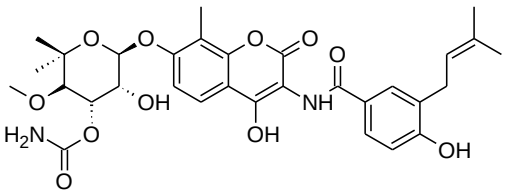
Scopoletin

(11)



Phenprocoumon (anticoagulant)

(12)



Novobiocin (antibiotic)

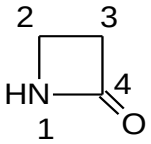
(13)

Therefore, the synthesis of coumarin and its derivatives have received an increasing attention to synthetic organic chemists and biologists.

AZETIDINONES

The discovery of penicillin by Sir Alexander Fleming in the year 1929 is considered as the beginning of the antibiotic era[16,17]. The widely cited definition of an antibiotic as a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other microorganisms was proposed by Waksman in 1942[18].

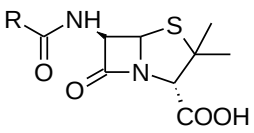
Azetidin-2-ones **(14)** are carbonyl derivatives of azetidine containing the carbonyl group at position 2, also called 2-azetidinone or β-lactam.



(14)

Though the ring system was known since 1907, but the investigation of their chemistry began from 1947 only [19]. Developments in the field of β-lactams during the last decades indicate that the only essential feature for antibacterial activity in β-lactam antibiotics is the presence of the β-lactam (2-azetidinone) ring.

Azetidinones are currently used for chemotherapy of bacterial infections. The selective inhibition during cell wall synthesis of bacteria is responsible for its unique and lethal antibacterial action[20]. The beta-lactam ring is part of the core structure of several antibiotic families, including penicillins**(15)**, cephalosporins**(16)**, carbapenems**(17)**, clavulanic acid**(18)**, sulbactams**(19)** and tazobactams**(20)** which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases.



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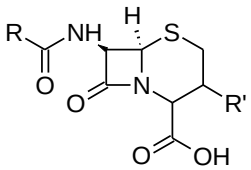

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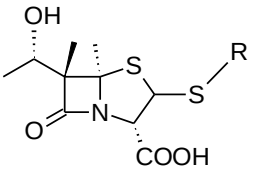

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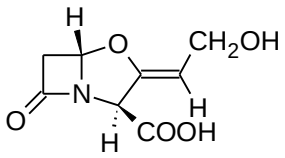

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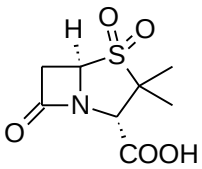

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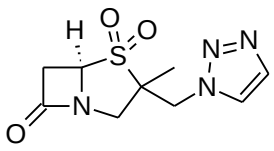


(20)

Nearly all of the antibiotics work by inhibiting bacterial cell wall biosynthesis. These molecules operate by forming a covalent adduct with membrane bound bacterial transpeptidases which are also known as penicillin binding proteins (PBPs) and prevent the biosynthesis of cell wall [21]. The biological activity of the β-lactam skeleton is generally believed to be associated with the chemical reactivity of the β-lactam ring as well as the substituents especially at the nitrogen of the β-lactam ring. The substituents at the N-1, C-3 and C-4 position may be varied [22].

A smaller population of bacteria, however, may survive as there is a possibility of becoming resistant against beta–lactam antibiotics. They achieve this by expressing one of many beta–lactamase genes. More than 1000 different β-lactamase enzymes have been documented in various species of bacteria [23]. These enzymes vary widely in their chemical structure and catalytic efficiencies. When bacterial populations have these resistant sub groups, treatment with beta–lactam can result in the resistant strain becoming more prevalent and therefore, more virulent[24].

The continuous increase in the activity spectrum of the β-lactam antibiotics and also the discovery of more types of microorganisms producing them can be attributed to the development of more sensitive screening techniques[25]. This has given impetus to increased interest in synthesis and evaluation of more and more derivatives of β-lactam. Furthermore, the β-lactam ring also serves as a synthon or versatile intermediates for the synthesis of aromatic amino acid derivatives, peptides, polyamines, polyamino alcohols, amino sugars and polyamino ethers, as well as, for many biologically important classes of organic compounds[26]. Due to

this, the investigation into the chemistry of these compounds continues to appeal the synthetic and medicinal organic chemists world over.

TUBERCULOSIS

Tuberculosis, one of the most common infections, is caused by *Mycobacterium tuberculosis.* According to the World Health Organization (WHO), nearly one third of the world's population has been exposed to the tuberculosis pathogen [27]. There are a number of known factors that make people more susceptible to tuberculosis infection worldwide, the most important of which is human immunodeficiency virus (HIV). The association of tuberculosis with HIV infection is so dramatic that in some cases, nearly two third of the patients diagnosed with the tuberculosis are also HIV-1 seropositive [28]. Smoking more than 20 cigarettes a day also increases the risk of tuberculosis by two- to four times [29].

Unfortunately, Tuberculosis treatment has not seen much progress as *Mycobacterium tuberculosis* (Mtb) is a stubborn pathogen [30]. The available treatment options are few depending on a relatively small set of chemotherapeutic agents, which includes the widely used front-line drugs like Isoniazid, Ethambutol, Rifampicin, and Pyrazinamide [31].

A number of anti-TB drugs are ineffective against TB because of the development of resistant strains. The limited effectiveness of current chemotherapy stems largely from the lengthy and complicated nature of first-line anti-TB drugs [32]. The most problematic issue with the current TB regimen is insufficient adherence to the treatment course, attributable to its length, complexity and adverse effects, led to difficult- and expensive to-treat multidrug-resistant tuberculosis (MDR-TB) [33]. Treatment for MDR-TB typically requires 18–24 months of combination therapy with second-line drugs which are less

efficacious, more toxic and expensive than the first-line drugs [34]. In few regions, almost 20% of MDR-TB cases were classified as extensively drug-resistant tuberculosis (XDR-TB). The treatment options for XDR-TB are very limited as XDR-TB bacilli are resistant not only to isoniazid and rifampicin, but also to fluoroquinolones and aminoglycosides [35]. More recently, another definition of XDR-TB as MDR-TB resistant to any fluoroquinolone and at least one of the second-line drugs (Capreomycin, Kanamycin and Amikacin) used in TB treatment [36]. There are serious adverse effects with most MDR-TB and XDR-TB drugs, such as nephrotoxicity and ototoxicity with aminoglycosides, hepatotoxicity with ethionamide and dysglycaemia with gatifloxacin. In few cases, XDR-TB has been shown aggressive form of TB, causing very high mortality.

The improvement in TB chemotherapy can be achieved by four primary goals:

(i) Shorten and simplify TB treatment

(ii) improve efficacy, safety and reduce long-lasting therapy

(iii) develop drugs for HIV-TB co-infection, which can be readily co-administered with antiretrovirals

(iv) shorten therapy of latent TB infection .

Moreover, to effectively treat and control MDR- and XDR-TB patients, physicians and national TB treatment programs require regimens based on safer, tolerable and efficacious drugs having new mechanisms of action [37].

The Global Alliance for Tuberculosis (GATB) drug development was established to address this need. Its top priority is the development of a new agent that will shorten the duration of chemotherapy from the current 6 – 8 months to two months or less. Also new drugs with activity against Mycobacterium drug-resistant tuberculosis and latent tuberculosis are needed [38].

WHO has recently launched its innovative "End TB Strategy"[39], supporting the TB elimination strategy and the vision of a TB-free world with zero death, disease and suffering due to TB. The new strategy clearly supports universal access to high-quality MDR-TB diagnosis and treatment. However, since the market launch of rifampicin in the early 1960s, no new anti-TB drug has been specifically developed until recently. The need for new drugs and regimens is obvious[40].

At present, the global TB development pipeline has nine candidates, but a key issue is how to develop them simultaneously in combination trials to identify the best candidate [41]. In this context, we try here to explore the ubiquitous heterocycle, coumarins based scaffold as promising antituberculars.

Coumarin: Structural requirements for anti-TB activity [15]

- From the published data, it is evident that coumarin nucleus substituted at all positions with varied substituents has produced potent anti-TB activity except the position 1 and 2.
- The 3rd position of coumarin may be unsubstituted or substituents may vary from alkyl to aryl and heterocyclic groups. Among them, coumarin with flourine, *n*-propyl and pyrazole substituents, demonstrated excellent anti-TB activity.
- Similarly, 4th position may be substituted with alkyl or bulky aryl/heteroaryl groups.
- Coumarins substituted with hydroxyl group, cyclohexyl, or bulky groups like naphthyl, benzoazepine with indole, benzoazepine with nitro groups at the 4th position enhances the anti-TB activity.
- Coumarins having substitutions at 3rd and 4th positions are found more because of the conjugation, and substituents may range from functional groups like halogens, alkene linker to heteroaryl groups.

- Coumarin with methyl group at the 5th position evinced much better activity almost like the derivatives having substitutions at 6th or 7th positions, which may range from functional groups like halogens, methyl pyridine and morpolino groups, enhances the activity.
- Finally, the coumarin derivatives with a methyl or methoxy substitution at 8th position show good anti-TB activity (Fig. **21**).

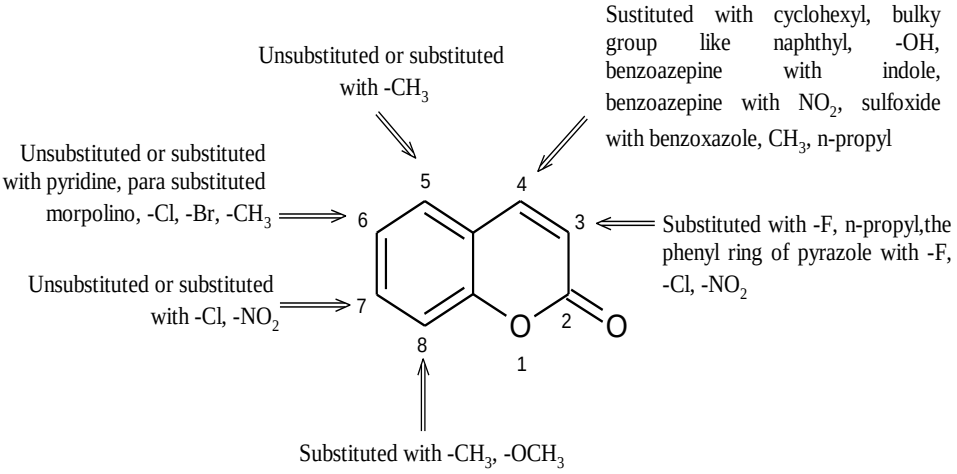


Fig. 21. Structural requirement around coumarin nucleus for anti-TB activity

Mycobacterium tuberculosis Cytochrome P450 lanosterol 14α-demethylase MT-CYP51

The cell envelope of *Mycobacterium tuberculosis* is unique and is associated with its pathogenicity[42]. Figure **22** shows a schematic representation of the structure of the cell envelope.

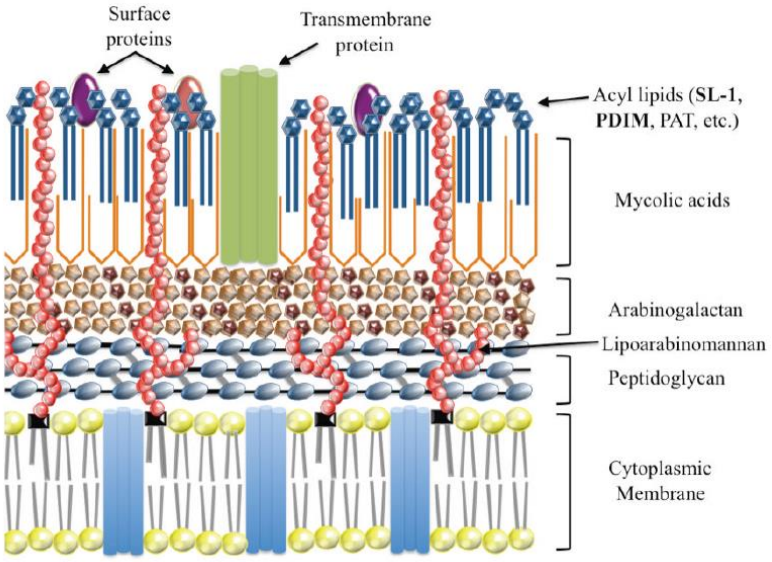


Figure 22. Schematic representation of the cell envelope of *Mtb*

Two of its most prominent features are the presence of: (*a*) arabinogalactan-mycolate covalently linked to the cell wall peptidoglycan *via* a phosphodiester bond located on the inner leaflet of the outer membrane (*b*) a free glycolipid called trehalose dimycolate (TDM) on the surface of the cells [43].

These particular structures provide a thick layer of lipid on the outer part of the cell that protects it against antibiotics, toxic chemicals and the host's immune system. Mycolic acids are the major constituents of this protective barrier and are essential for survival, virulence and antibiotic resistance [44]. Inhibitors of mycolic acid biosynthesis, such as isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA), are still in the frontline of antitubercular drugs.

The sequence of the *Mycobacterium tuberculosis* genome confirms the importance of lipid metabolism in this human pathogen. From the 4,411,529 base pairs, a very large proportion of the coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis [45]. The sequence of the genome

also revealed the existence of twenty putative cytochrome P450 enzymes, suggesting that at least some of these P450s could be involved in anabolism and/or catabolism of lipids. Of these, only CYP51B1 was functionally assigned based on its significant sequence similarity to eukaryotic CYP51 enzymes and its sterol 14α-demethylase catalytic activity.

A major target of antifungal azole compounds is the sterol demethylase (CYP51) and inhibition prevents synthesis of the membrane sterol ergosterol from lanosterol. This compromises membrane integrity and induces fungal cell lysis.

DRUG DESIGN [46-49]

Drug design is a process which 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 designing is otherwise known as rational drug design and it is a method of finding new medications based on the biological receptors and target molecules. It involves the designing of small molecules which is complementary to the biological receptor to which they bind and interact to cause the pharmacological actions. 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 actions or not. The basic steps involved in CADD are:

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

Types of Drug Design

- Ligand Based Drug Design
- Structure Based Drug Design

Ligand Based Drug Design (LBDD)

It is also known as indirect drug design. In the absence of the structural information of the target, ligand based method is used to know about inhibitors for the target receptor. Biologically active lead molecule is detected by using structural or topological similarity or pharmacophoric similarity properties. 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)

It is also known as direct drug design. 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 modeling. It is used to create a homology model of target when the experimental structure is unavailable. 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. This type of drug design requires the receptor's complete structural knowledge and the tools to design extremely specific molecule that interact with the receptor. Therefore, structures of the target molecules have to be decided and the exact ligand molecule need to be designed. Hence, any one of the following parameter should be available to start a structural based drug design:

- 3 dimensional structure of the target macromolecule
- 3 dimensional structure of a closely related analogue

DRUG DISCOVERY METHODS[50,51]

1. Real screening
2. Virtual screening

Real Screening

It include methods like high-throughput 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 the application of computers and computational methods to select or prioritize molecules for experimental screening. Thus a large percentage of the proposed analogs can be eliminated, and those molecules with the highest probability to show biological activity can be selected. By adopting this methodology, the time and cost associated with the production of libraries for screening can be considerably reduced.

STEPS INVOLVED IN DRUG DESIGN [49]

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

1. Selection and identification of the target.
2. Search for lead or lead identification.
3. Lead optimization.
4. Synthesis of new molecules.

1. 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 signaling 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 Collaboratory for Structural Bioinformatics (RCSB) Protein Database], Nuclear Magnetic Resonance (NMR) or homology modeling from a previously determined structure.

2. 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.

i) ***De novo* molecular design**

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

ii) **Database search methods**

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

iii) **Combinatorial methods**

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

3. 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.

DRUGLIKENESS [52,53]

Druglikeness 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.

In silico evaluation of druglikeness at an early stage involves the prediction of various ADMET (absorption, distribution, metabolism, excretion, toxicity) properties using computational approaches, i.e., it provides a preliminary prediction of the *in vivo* behavior of a molecule. Druglikeness score towards GPCR ligands , ion channel modulators, kinase inhibitors, nuclear receptor ligands and protease inhibitors may be evaluated online by using 'Molinspiration server'. The higher the value of the score, the more the probability that the particular molecule will be active.

LIPINSKI'S RULE OF FIVE

Christopher A. Lipinski (1997) proposed four parameters that define the 'druglikeness' of a potential drug candidate based on the observation that most medication drugs are relatively small and lipophilic molecules. Lipinski's Rule of Five can be applied to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. Lipinski's rule says that, in general, an orally active drug has not more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (sum of OHs and NHs)
- Not more than 10 hydrogen bond acceptors (sum of Ns and Os)
- Molecular weight not greater than 500 Daltons
- An octanol-water partition coefficient, log *P*, not greater than 5.

Improvements

To evaluate druglikeness in a better way, the rules have spawned many extensions by Ghose *et al.* in 1999.

- log *P* : -0.4 to +5.6
- Molecular refractivity : 40-130
- Molecular weight : 160-500
- Number of atoms : 20-70
- Polar surface area must not be greater than 140 Å

DOCKING[54,55]

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 musr 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
- 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:

- AutoGrid pre-calculates the grids.
- 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 azetidine-2-one derivatives containing coumarin moiety in order to explore the extent of their antitubercular activity. The compounds were designed by *in silico* method using MT-CYP51 as the target molecule.

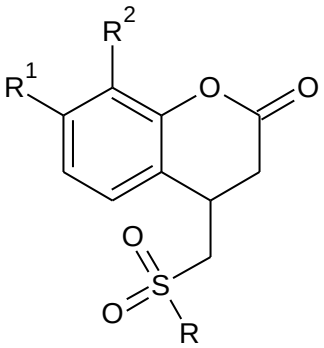
LITERATURE REVIEW

COUMARINS

1) Coumarins with antimicrobial activity

a) Coumarins as antitubercular agents

- Jeyachandran *et al.*,[56] (2012) synthesized a series of 4-aryl/ alkylsulfonylmethylcoumarins **(1)** and screened for in vitro antitubercular activity against Mycobacterium tuberculosis H37Rv (MTB) to prove their potential activity.



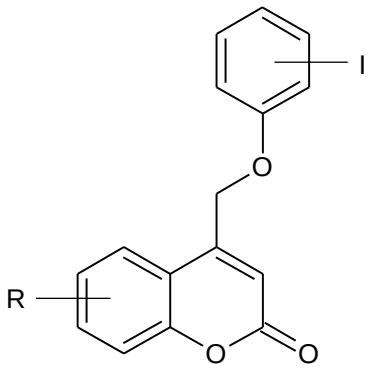
R – phenyl; p-methylphenyl, p-chloro phenyl; 2-benzoxazolyl; 2-benzimidazolyl; cyclohexyl

R^1 - H; OCH_3

R^2 – H; OCH_3

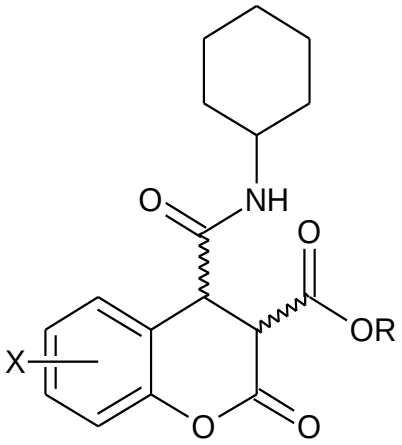
(1)

- Basanagouda *et al.*,[57] (2013) synthesized a series of new iodinated-4-aryloxymethylcoumarins **(2)** and were screened against two mycobacterial strains(*Mycobacterium tuberculosis* H37 RV and *Mycobacterium Phlei*).



(2)

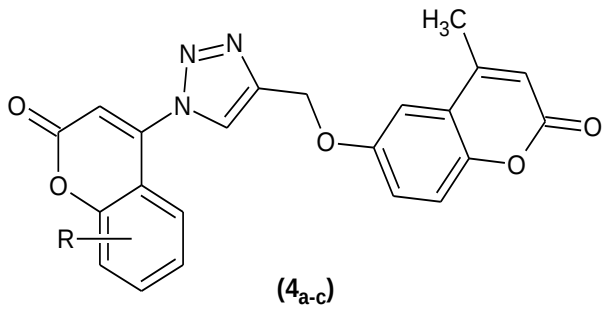
- Rezayan *et al.,*[58] (2012) reported synthesis of coumarin derivatives and their *in vitro* antimycobacterial activity evaluated by the broth microtiter dilution method against *M. bovis* and results were compared with first line anti-TB drug, ethambutol (EMB). Some of the synthesized compounds **(3_{a-c})** found active against *M. bovis*



a) X=H, R=Isobutyl
b) X=6-Br, R=Methyl
c) X=6-Br, R=4-Fluorobenzyl

(3_{a-c})

- Naik *et al.,*[59] (2012) described the synthesis of bischromenyltriazole hybrids and assessed against Mycobacterium tuberculosis. Compounds **(4_{a-c})** were found to be highly active as streptomycin.

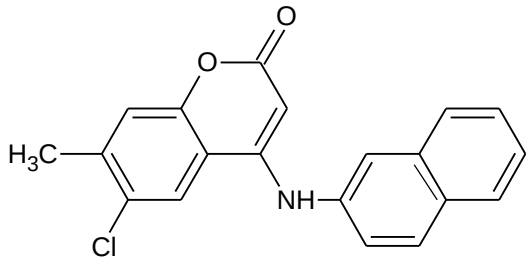


a) R=6-Cl
b) R=7-Cl
c) R=5,6-Benzo

(4_{a-c})

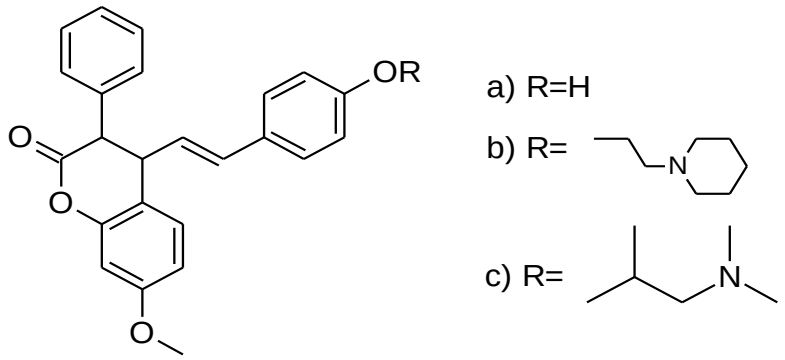
- Virsdoia *et al.,*[60] (2010) described the synthesis of 4-(arylamino) coumarin derivatives with various aromatic amines at the C_4 position of the coumarin under microwave condition. The compounds were evaluated for antimycobacterial activity against *MTB H37Rv*, and the results were

compared with standard drug Rifampin. Compound (**5)** was found to be the most potent candidate in this series.



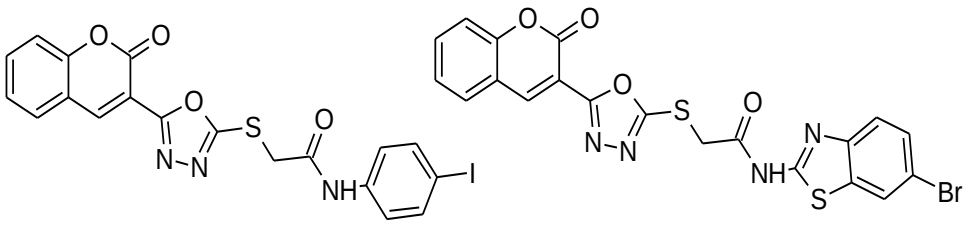
(5)

- Ahmad *et al.*,[61] (2013) synthesized a series of styrenylchromanone and their alkyl derivatives and reported for their anti-TB efficacy against *MTB H37RV* strain. Compounds **(6_{a-c})** were found active in the series.



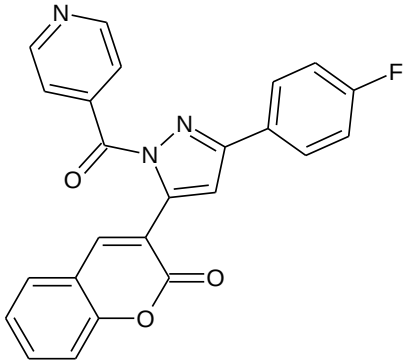
(6_{a-c})

- Patel *et al.*,[62] (2013) described the synthesis of coumarin-based 1,3,4-oxadiazolebenzothiazole acetamide derivatives and assessed their anti-TB activities against *MTB H37Rv* using BACTEC MGIT method. The halo substituents, particularly the compound bearing iodosubstitution on the phenyl acetamide moiety**(7_a)**, and bearing bromo substitution on the benzothiazolyl acetamide moiety linked to the 1,3,4-oxadiazole core *via* sulphur linkage**(7_b)** showed higher mycobacterial inhibitory efficacies.



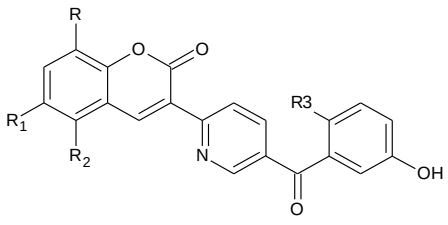
(7_a) **(7_b)**

- Kawate *et al.*,[63] (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. Compound **(8)** containing a4- fluoro group at C-3 phenyl ring of pyrazole ring showed promising antimycobacterial potential.



(8)

- Palkar *et al.*,[64] (2013) synthesized a series of substituted pyridyl coumarin derivatives using ammonium acetate as a catalyst by one-pot method and tested these for their potential *in vitro* anti-TB activity against *MTB H37Rv* strain. Compounds **(9a)** and **(9c)** were found to exhibit potent activity than the compound **(9b)**

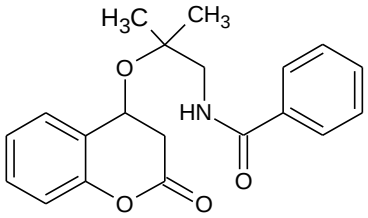


a) $R=R_1=R_2=H$, $R_3=CH_3$
b) $R=OCH_3$, $R_1=R_2=H$, $R_3=CH_3$
c) $R=H$, $R_1=Br$, $R_2=H$, $R_3=CH_3$

(9_{a-c})

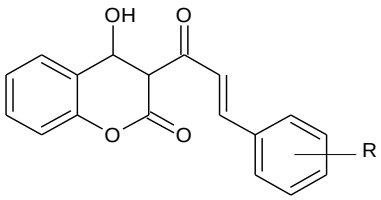
b) Antibacterial activity

- Lin *et al*.,[65] (2012) tested acyl coumarins, 4-hydroxy, and 7-hydroxycoumarins 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. Compound **(10)** was the most potent compound out of the tested compounds against *Bacillus subtilis*



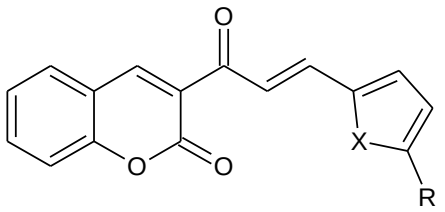
(10) .

- Zavrsnik *et al*.,[66] (2011) tested a series of 3-cynnamoyl-4-hydroxycoumarins **(11)** on bacteria *P. aeruginosa*, *E.oli*, *Salmonellatyphimurium*, *Bordatella bronchiseptica*, *B. subtilis* and *S. aureus*. The compounds having halogens showed the highest antimicrobial activity. Compounds having 4-Br and 4-Cl were found to be the most effective against *B. subtilis*. Compound having 4-Cl was found to be the most effective against *S. aureus.*



(11)

- Ajani *et al*.,[67] (2010) synthesised 3-[3-(s-aryl and s-heteroaromatic)acryloyl]-2*H*-chromen-2-one derivatives **(12)** and were screened for antibacterial activity against five gram positive bacteria (*Bacillus anthracis*, *B. Stearothermophilus*, *B. Subtilis*, *B. cereus* & *S. aureus*) and five gram negative bacteria (*E. coli*, *Klebsiella pneumonia*, *P. aeruginosa*, *P. fluorescence* & *Shigella dysenteriae*).

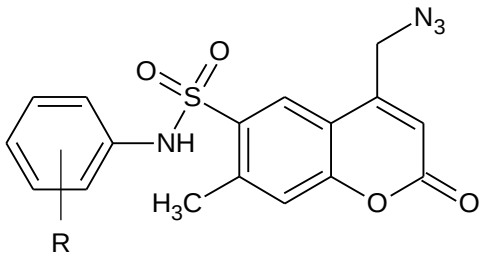


X=O, NH

R=H, NO_2, Cl, CH_3, C_2H_5

(12)

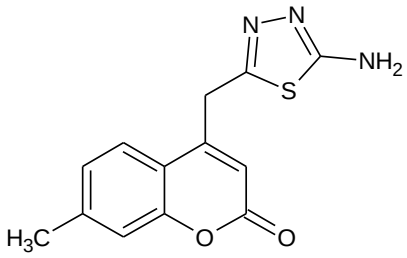
- Kulkarni *et al.*,[68] (2010) synthesized some 4-azidomethyl-7-methylcoumarin-6-sulphonamides**(13)** which showed very good antibacterial activity.



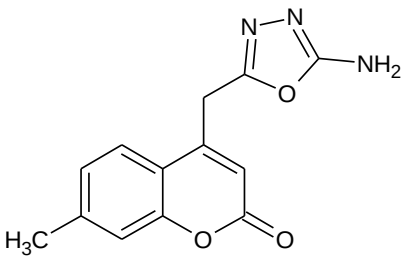
R=H, 3-CH_3, 4-CH_3, 3,4-CH_3, 2,4-CH_3, 2-OCH_3, 4-OCH_3, 4-Cl, 4-Br, 3-Cl, 4-CH_3

(13)

- Mashelkar *et al*.,[69] (2006) evaluated some 4-substituted coumarins in vitro against Gram positive *S. aureus* and Gram negative *Salmonella typhi*. Ampicillin and trimethoprim were used as standard drugs. Two compounds **(14** and **15)** were showed significant antibacterial activity against *S. aureus* and *S. typhi.*



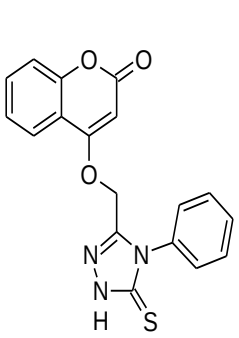
(14)



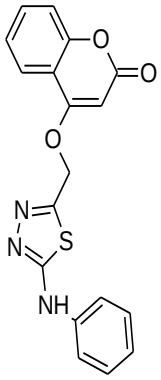
(15)

c) Antibacterial and antifungal activity

➢ Behrami *et al*.,[7] (2012) evaluated 4-[(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)-methoxy]-2H chromen-2-one for their antifungal and antibacterial activity. Two compounds **(16** and **17)** showed good antifungal activity against *Aspergillus niger* and *Candida albicans.* Compound (**18**) showed a significant antibacterial effect against *S. aureus, E. coli* and *B. cereus*



(16)



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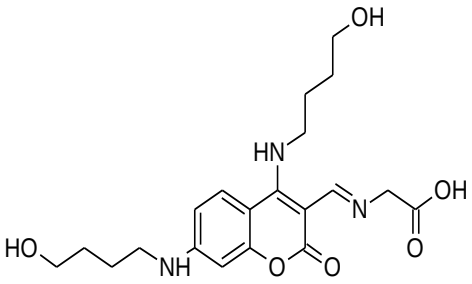
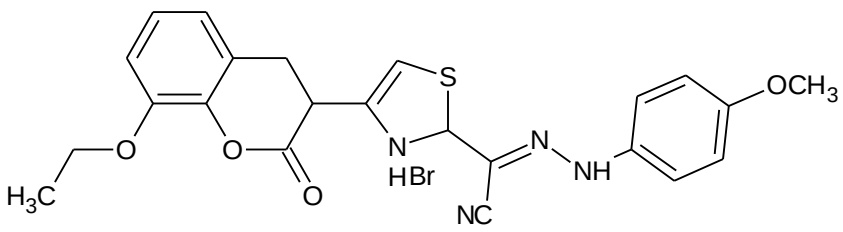


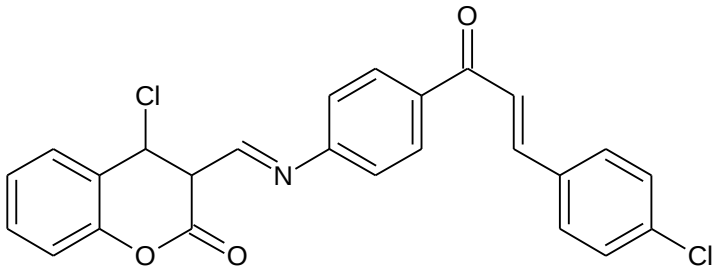
(18)

➢ Mohamed *et al*.,[70] (2012) screened some 8-ethoxycoumarins for their in-vitro antimicrobial activities against two Gram negative *Bordetella 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 **(19)** resulted in wide spectrum antimicrobial activity against all tested bacteria and fungi compared to ampicillin (25µg/ml) and mycostatin (25µg/ml).



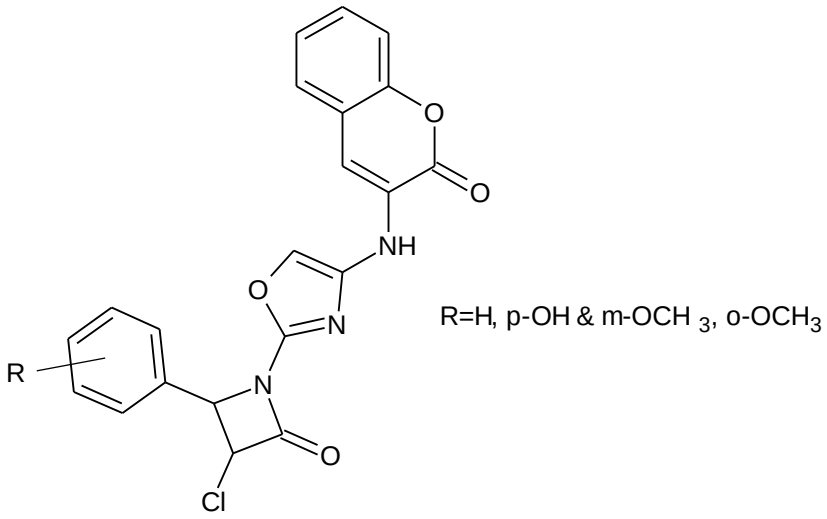
(19)

➢ Kudale *et al.*,[71] (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 **(20)** was found to be most active against all the tested organisms.



(20)

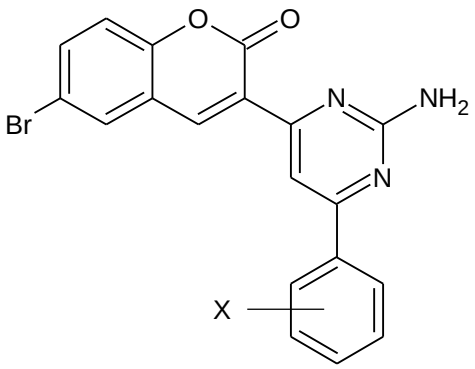
➢ Kumar *et al.*,[72] (2010) synthesized 3-[(2'-substituted benzylidine aminothiazol-4'-yl)amino]coumarins and 3-[(2'-substituted benzylidine amino oxazol-4'-yl)amino]coumarins**(21)** and screened them for antibacterial and antifungal activities.



(21)

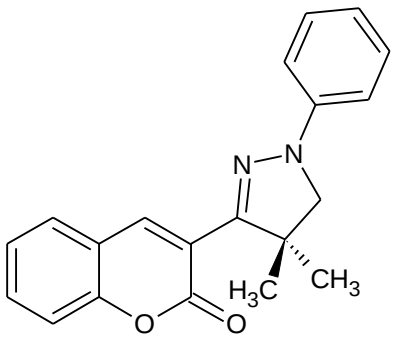
2. Coumarins with Anti–inflammatory and Analgesic activity

➢ Gupta *et al*.,[73] (2010) tested a series of 3-(2-amino-6- pyrimidin-4-yl)-6-bromo-2H chromen-2-ones **(22)** for their analgesic activity . Compound having 2,6-dichloro phenyl was found to be most promising analgesic agent devoid of ulcerogenic effects.



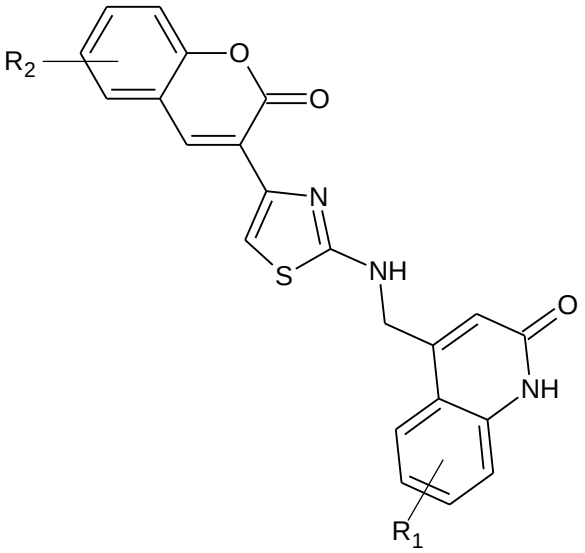
(22)

➢ Khode *et al*.,[8] (2009) screened a series of 5-(substituted)aryl-3-(3coumarinyl)-1-phenyl-2-pyrazolines **(23**) for their in vivo anti-inflammatory and analgesic activities. Some of the compounds exhibited significant analgesic activity and antipyretic activity with minimum ulcerogenic index.



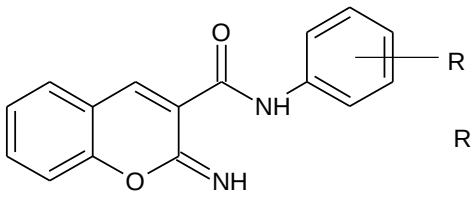
(23)

➢ Kalkhambkar *et al*.,[74] (2007) evaluated triheterocyclic thiazoles containing coumarin and carbostyril(1-azacoumarin) **(24)** for their analgesic and anti-inflammatory activities. The 7-chloro substitution in carbostyril and 6,8-dibromo substitution in the coumarin ring improved anti-inflammatory activity.



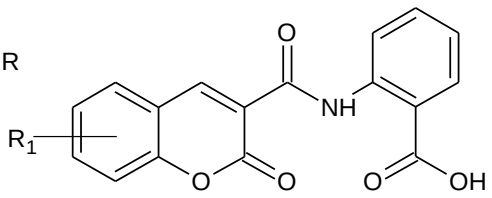
(24)

➢ Bylov *et al.*,[75] (1999) evaluated a series of N-aryl substituted 2-imino -2*H*-1-benzopyran-3-carboxamides (2**5**) and 2-oxo-2*H*-1-benzopyran-3-carboxamides (**26**) for anti-inflammatory activity in albino rats. The results were found to be comparable with piroxicam taken as the reference drug.



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

(25)

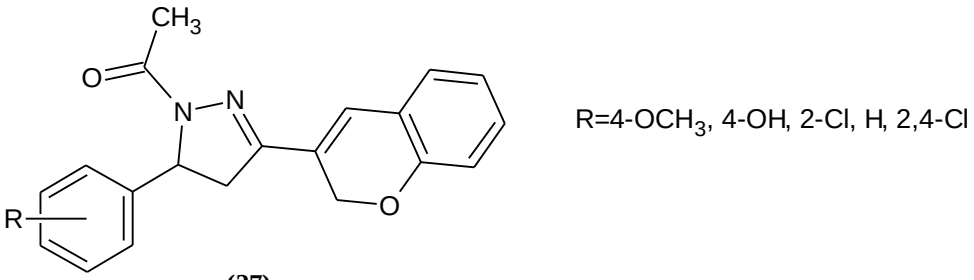


R=H, 5-OCH_3, 5-NO_2, 5-Cl

(26)

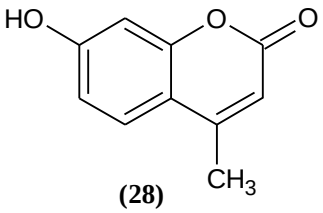
3. Coumarins with Anticancer activity

➢ Xin-Hua liu *et al*.,[11] (2010) reported the synthesis of novel coumarin derivatives containing 4,5-dihydropyrazole moiety **(27)** which exhibited potential antitumor activity.



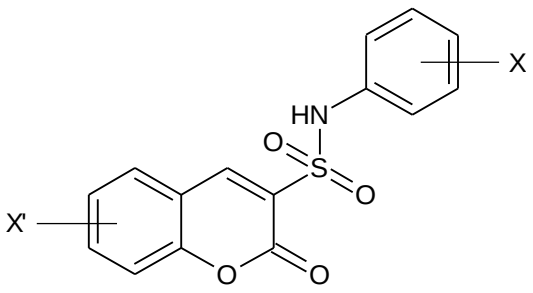
(27)

➢ Bhattacharya *et al*.,[76] (2009) synthesized 4-methyl-7-hydroxy coumarin **(28)** and screened them for anticancer potential against DMBA-induced skin cancer in mice.



(28)

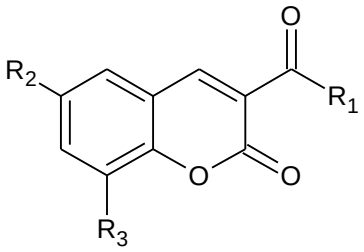
➢ Reddy *et al*.,[77] (2004) evaluated the effect of coumarin 3-(N-aryl) sulphonamides **(29)** on the growth of human tumor cells in culture using androgen receptor negative prostate (DU145), colorectal (DLD-1), non-small cell lung carcinoma (H157), estrogen receptor negative breast (BT20), and chronic myeloid leukemia (K562) cell lines.



(29)

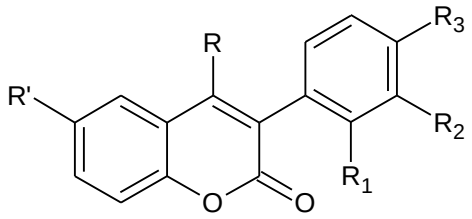
4. Coumarins with Antioxidant activity

➢ Singh *et al*.,[10] (2010) tested 3-alkanoyl/aroyl/heteroaroyl-2H-chromene-2-thiones **(30)** for their free radical scavenging capacity towards the stable free radical 2,2-diphenyl-1- picrylhydrazyl (DPPH). These compounds exhibited profound antioxidant activity.



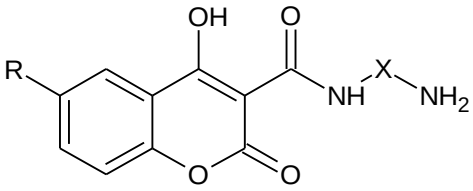
(30)

➢ Roussaki *et al*.,[78] (2010) evaluated a series of coumarin analogues **(31)** bearing a substituted phenyl ring on position 3 for their antioxidant activity by using two different antioxidant assays.



(31)

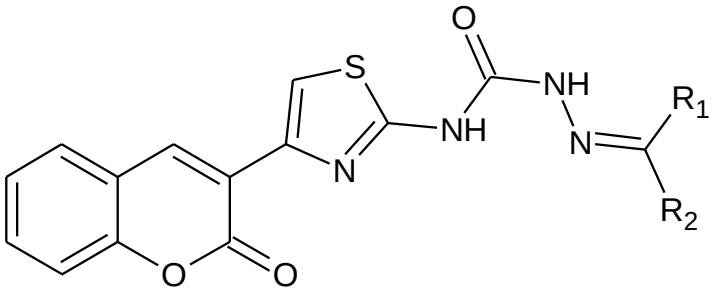
➢ Melagraki *et al*.,[79] (2009) evaluated a series of coumarin-3-carboxamides **(32)** for their in-vitro antioxidant activity and in-vivo anti-inflammatory activity. These derivatives were found to possess these activities.



(32)

5. Coumarins with Antihyperlipidemic activity

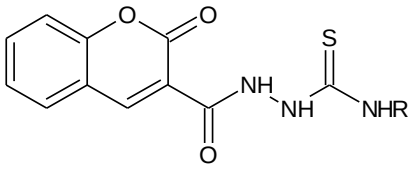
➢ Sashidhara *et al*.,[80] (2010) evaluated a series of coumarin bisindole heterocycles **(33)** for antihyperlipidemic activity in hyperlipidemic hamster model.



(33)

6. Coumarins with Anticonvulsant activity

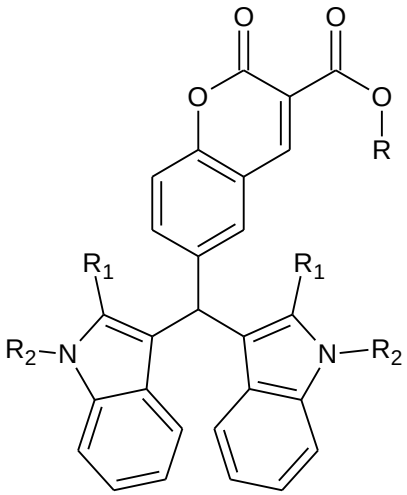
➢ Bhat *et al.*,[81] (2006) synthesized novel thio ureido derivatives of sulfonamides and thiosemicarbazido**(34)** derivatives of coumarin and reported them as potential anti-convulsant agent.



(34)

R=C_6H_5, p-Br-C_6H_4, p-Cl-C_6H_4, p-CH_3-C_6H_4

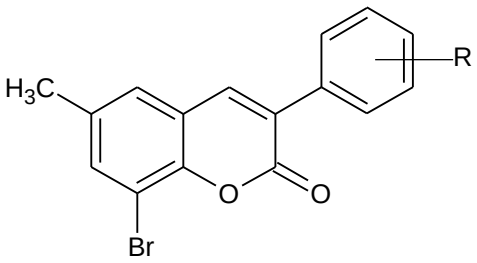
➢ Siddiqui *et al*.,[82] (2009) tested some heteroaryl semicarbazones **(35)** for their anticonvulsant activity using pentylenetetrazole (PTZ) induced seizure, maximal electroshock seizure (MES) and Neurotoxicity tests. The compounds having 3,4-Cl.C6H3, 2-OCH3.C6H4 and 4-Br.C6H4 exhibited significant anticonvulsant activity.



(35)

7. Coumarins with Antiparkinsonian activity

➢ Matos *et al*.,[83] (2009) evaluated a series of 8-bromo-6-methyl-3-phenylcoumarin derivatives **(36)** as MAO-A and MAO-B inhibitors using R-(-)-deprenyl (selegiline) and Iproniazide as reference inhibitors.



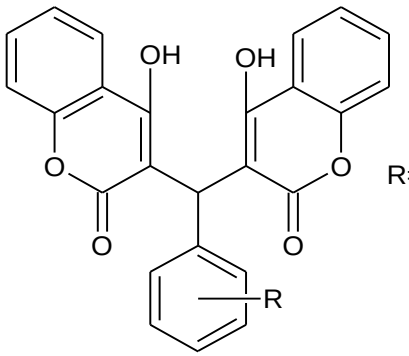
(36)

8. Coumarin with Anticoagulant activity

➢ Abdelhafez *et al*.,[6] (2010) 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[6].

9. Coumarin with Anti HIV activity

➢ Releva *et al.*,[9] (2005) synthesized 3,3'-arylidene-bis-4-hydroxy coumarins**(37)** and were shown to exhibit good anti HIV activity.



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

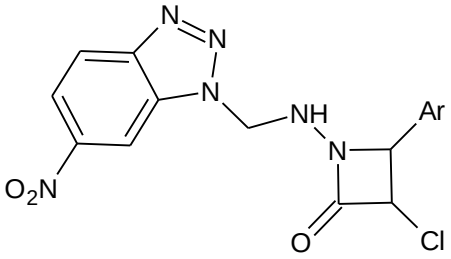
(37)

AZETIDINONES

1. Azetidinones with Antimicrobial activity

a) Antitubercular activity

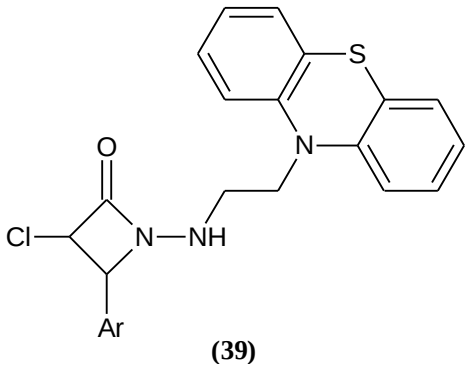
➢ Samadhiya *et al.*,[84] synthesized a novel series of 3-chloro-1-{[2-(6-nitro-1H-indazol-1-yl) ethyl] amino}-4-(substituted-phenyl)- 2-azetidinones**(38)** and evaluated them for antitubercular activity against M. tuberculosis (H37Rv strain).



Ar=Ph, 4-ClPh, 3-ClPh, 2-ClPh, 4-BrPh, 3-BrPh, 2-BrPh, 4-NO_2Ph, 3-NO_2Ph

(38)

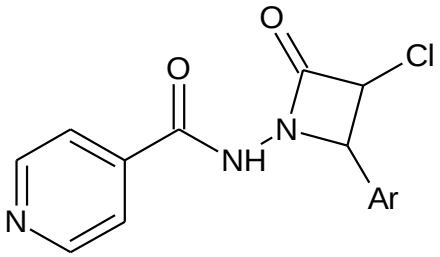
➢ Sharma Ritu *et al.*,[85] (2011) synthesized of N-[2-(10H-phenothiazinyl)ethyl]-4-(phenyl)-3-chloro-2-oxo-1-iminoazetidine**(39)**. All synthesized compounds were evaluated for their antibacterial, antifungal and antitubercular activity which displayed acceptable results.



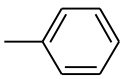
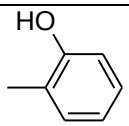
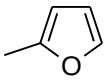
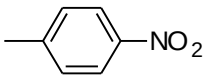
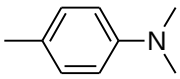
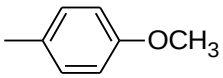
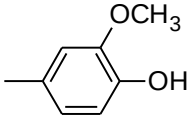
(39)

➢ Jaju *et al.*,[86] (2009) synthesized a novel series of 14 new isonicotinyl hydrazide derivatives **(40_{a-g})**, containing a 2-azetidinone nucleus. All the title compounds were tested for their in-vitro antimycobacterial activity against Mycobacterium tuberculosis H37Rv using Alamar-Blue susceptibility test. Some

of the compounds displayed an encouraging antimycobacterial activity profile.

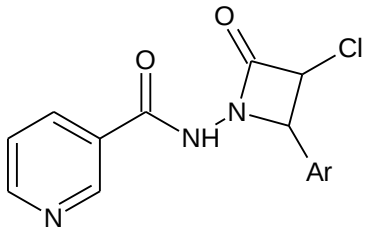


(40_{a-g})

Compound	Aryl /Het.
a	
b	
c	
d	
e	
f	
g	

- Ramalakshmi *et al.*,[87] (2008) synthesized a novel series of 4 - aryl – 3 -

chloro – 1 – nicotinamido – 2 –azetidinones**(41)** and were screened for anticonvulsant and antimycobacterial activities. Antimycobacterial activity was screened using standard Strain H37RV and two Human Strains (Human strain-I and Human strain-II) isolated from patients suffering from pulmonary tuberculosis.

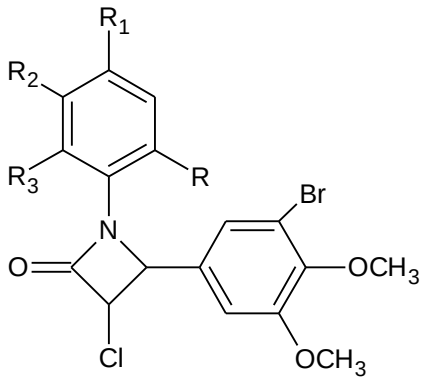


(41)

Ar	**Ar**
2-Hydroxy phenyl	3-Hydroxy phenyl
4-Hydroxy phenyl	3.4-Dihydroxy phenyl
4-Methyl phenyl	4-Methoxy phenyl
3,4,5-Trimethoxy phenyl	4-Hydroxy-3-methoxy phenyl
4-Dimethylamino phenyl	4-Nitro phenyl
3-Nitro phenyl	4-Chloro phenyl
2-Chloro phenyl	Cinnamyl

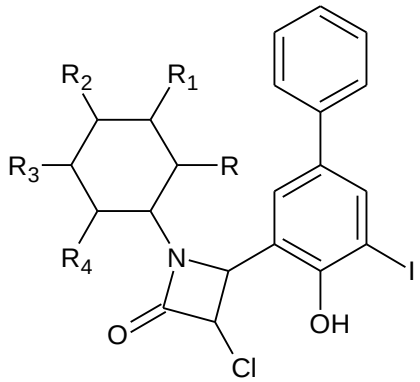
b) Antibacterial and Antifungal

➢ Chavan *et al.*,[88] (2013) synthesized several 2-azetidinones**(42)** from halo-substituted Schiff bases using conventional as well as microwave technique. All compounds were screened for antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus*. Most of the titled compounds show potent activity.



(42)

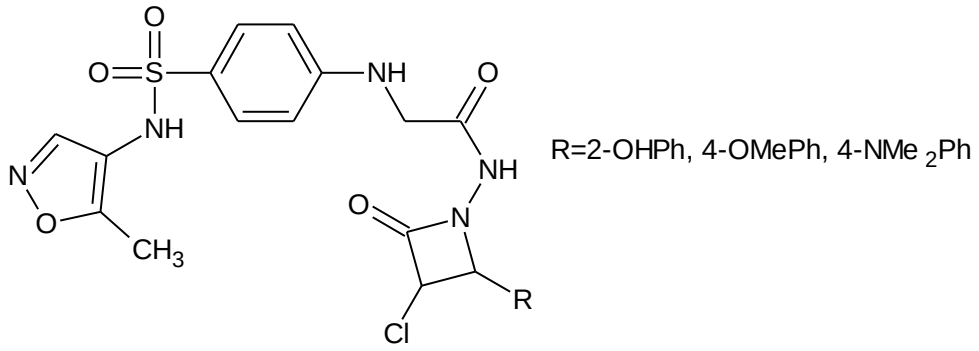
➢ Junne *et al.*,[89] (2012) prepared a series of 3-chloro-4-(4 - hydroxy - 5 - iodobiphenyl - 3 - yl) -1 (substitutedphenyl) azetidin-2-one**(43$_{a-h}$)** derivatives and screened for antibacterial activity against *Xanthomonas citri, E. coli, Erwinia carotovora* and *B. subtilis* using penicillin G as standard antibiotic for comparison.



(43$_{a-h}$)

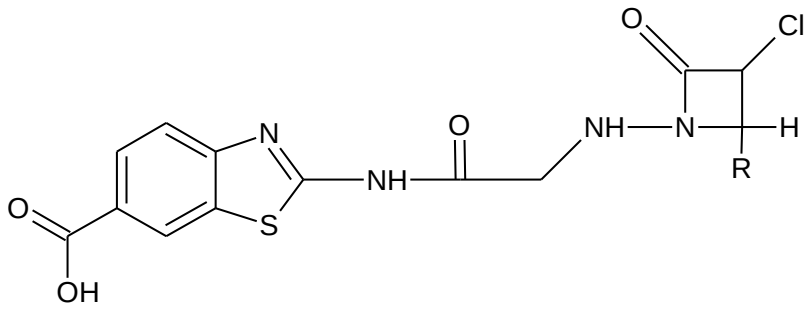
Comp	R	R_1	R_2	R_3	R_4	Comp	R	R_1	R_2	R_3	R_4
a	H	H	NO_2	H	I	e	I	H	Me	H	I
b	H	H	I	H	NO_2	f	I	H	NO_2	H	I
c	H	H	I	H	Cl	g	Me	I	H	NO_2	H
d	H	H	Cl	H	I	h	Cl	H	I	H	Cl

➢ I.K. Bhat *et al*.,[90] (2011) have prepared a series of N-[3-chloro-4-(4-substitutedphenyl)-2-oxoazetin-1-yl]-2-(N'-5-methyl-3-isoxazolyl) sulphonamides **(44)** and were tested for their antibacterial and antifungal activities against four different bacterial cultures, viz., *S. aureus, E. coli, P. aeruginosa and B. subtilis* and one fungal culture; *C. albicans*. Some of the synthesized compounds showed remarkable antibacterial and antifungal activities as comparable to the standard drugs.



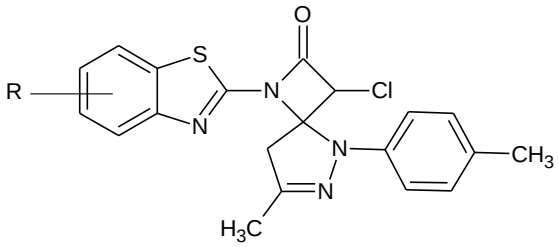
(44)

➢ Ameya A. Chavan, and Nandini R. Pai[91] (2007) reported synthesis of 2-{2-[3-chloro-2-(aryl)-4-oxoazetidin-1-ylamino]-acetylamino} benzothiazole-6-carboxylic acids**(45)** and were screened for their anti-bacterial activity against *S.aureus, B.subtilus, P. aeruginosa* and *E.coli.*



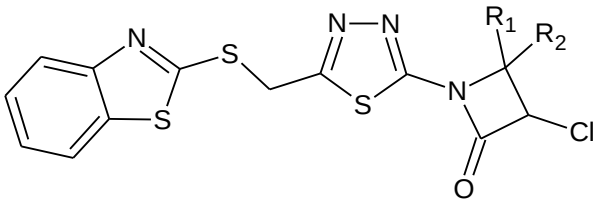
(45)

➢ Mistry and Desai[92] (2005) synthesized a series of compounds 4-[spiro-{4''-methylphenyl)-3'-methyl}-pyrazole]-3-chloro-1-(substituted benzothiazole)-2-azetidinones**(46)** and were screened for their anti-bacterial activity against *S.aureus, B.subtilus, S.typhi* and *E.coli.*



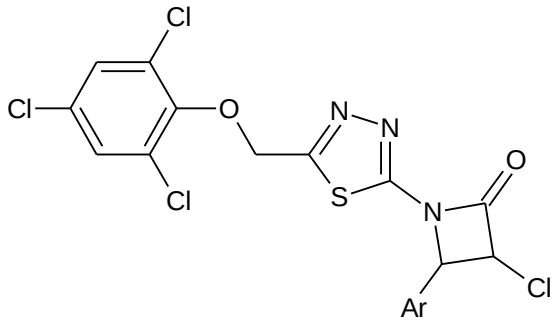
(46)

➢ Srivastava *et al.,*[93] (2004) synthesized 1-[5'-{(2-benzothiazolylthio)methyl}-1,3,4-thiadiazol-2-yl]-4-(substitutedphenyl)-3-chloro-2-oxoazetidine**(47)** as antimicrobial and anthelmintic agents.



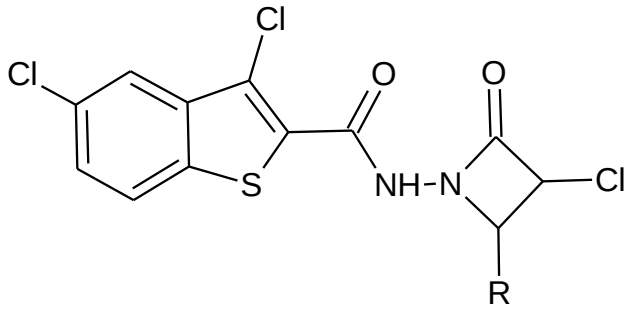
(47)

➢ Desai *et al.,*[94] (2004) synthesized 4-substituted phenyl-1-[2-(2,4,6-trichlorophenoxymethyl)-1,3,4-thiadiazol-5-yl]3-chloro-2-azetidinone **(48)** and screened for their antibacterial activity.



(48)

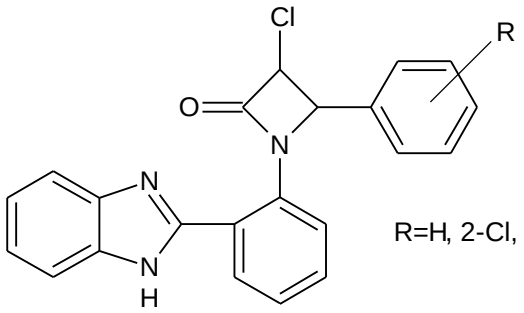
➢ Thakar *et al.,*[95] (2003) have synthesized 4-Aryl-3-chloro-1-(3',5'-dichloro-2'benzo(b) thio phenoylamino)-2-azetidinones**(49)** and evaluated their antimicrobial activity against *E.coli, S.aureus, P.vulgaris* and *A.niger.*



(49)

2. Azetidinones with Analgesic and Antiinflammatory activity

➢ Chhajed *et al.,*[96] (2012) synthesized a series of 1-(2-(1H-benzimidazol-2-yl) phenyl) – 3 – chloro – 4 - (un/substituted -phenyl) azetidin-2-ones**(50)** and screened them for analgesic and anti-inflammatory activities on acetic acid induced writhing in mice and carrageenan induced paw edema in rats

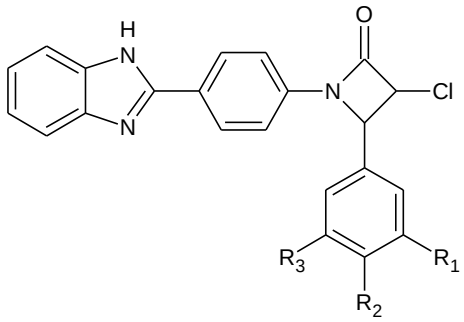


R=H, 2-Cl, 4-Cl,4-F, 2-OH, 4-OH, 2-NO_2, 4-OMe

(50)

.

➢ Shanmugapandiyan *et al.,*[97] (2010) prepared series of 2-[4-(azetidin-2-one)-3-Chloro-4-phenyl]-1H-Phenylbenzimidazoles**(51_{a-h})** and the compounds were screened for antibacterial, antifungal, analgesic activity by writhing reflex method and anti-inflammatory activity by carrageenan induced paw edema method.

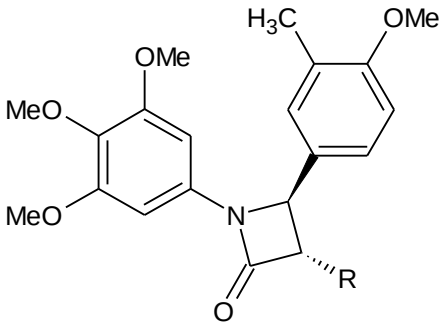


(51_{a-h})

Comp. No.	R_1	R_2	R_3
a	-H	-H	-H
b	-H	-Cl	-H
c	-H	-OH	-H
d	-H	$-CH_3$	-H
e	-H	$-N(CH_3)_2$	-H
f	-H	$-OCH_3$	-H
g	$-OCH_3$	$-CH_3$	-H
h	$-OCH_3$	$-OCH_3$	$-OCH_3$

3. Azetidinones with Anticancer activity

➢ O'Boyle *et al.*,[98] (2011) have synthesized a series of azetidin-2-ones**(52)** substituted at positions 1, 3 and 4 of the azetidinone ring scaffold via the Staüdinger reaction and evaluated for antiproliferative, cytotoxic and tubulin-binding activity.

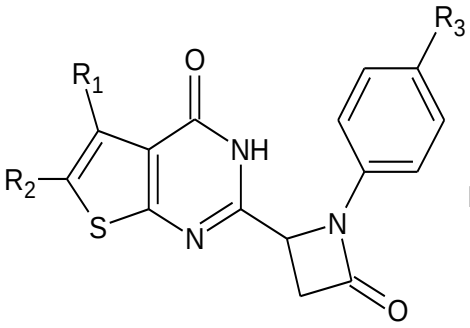


R=2-Thienyl, 3-Thienyl

(52)

4. Azetidinones with Antihyperlipidemic activity

➢ Jain *et al*.,[99] (2013) have synthesized a series of novel 2-[1 (substituted phenyl) - 4-oxo-azetidin-2-yl]-5, 6-disubstitutedthieno [2, 3-d] pyrimidin-4(3H)-ones**(53)** and were evaluated for their lipid lowering activity in Wistar albino rats. Some of them showed significant lipid lowering effects.

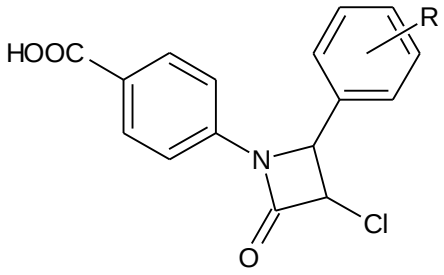


R_1,R_2=aryl, cycloalkyl, H etc; R_3=alkyl, halo etc

(53)

5. Azetidinone with Anticonvulsant activity

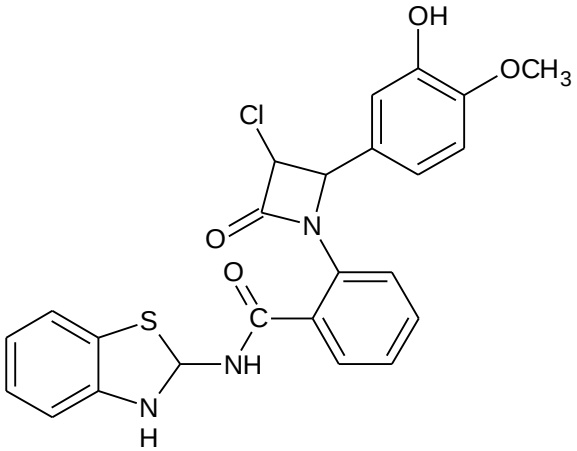
➢ Pratap Y Pawar *et al*.,[100] (2012) synthesized 10 derivatives of 2-azetidinones**(54)** and were evaluated for in vitro anticonvulsant activity. Most of the compounds exhibited mild to moderate anticonvulsant activity.



(54)

➢ Vijaya Kumar MM J *et al*.,[101] (2009) synthesized novel N-Substituted-3-

Chloro-2- Azetdinones**(55)** as potential anticonvulsant agents. These newly synthesized heterocyclics showed promising anticonvulsant activity.

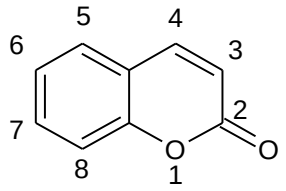


(55)

CHEMISTRY

CHEMISTRY OF COUMARINS

Coumarins are naturally occurring oxygen heterocycles. They contain a benzo-α-pyrone (2*H*-1-benzopyran-2-one) nucleus as the main structural unit [1].



2H-1- benzopyran-2-one

Coumarins are classified on the basis of their chemical structure into the following types:

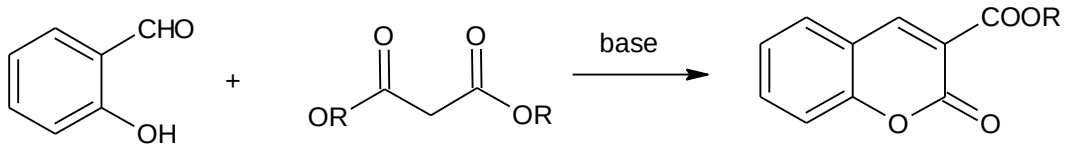
- Simple coumarins: Coumarin and its hydroxylated, alkoxylated, alkylated derivatives and their glycosides (scopoletin umbelliferone)
- Furanocoumarins: Coumarins containing a furan ring fused to the benzopyrone nucleus including dihydrofurocoumarins, (psoralen, bergapten)
- Pyranocoumarins: six membered ring analogues of furocoumarins (Xanthyletin)
- Coumarins substituted in the pyrone ring: 3 or 4 substituted coumarins like 4-hydroxycoumarins (V), 4-phenylcoumarins (VI) and 3, 4-benzocoumarins (VII).
- Isocoumarins: lactones of benzenecarboxylic acids with a hydroxylated side chain unsaturated in the β-position, isomeric with coumarins.

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

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

1. Knovenagel Reaction

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

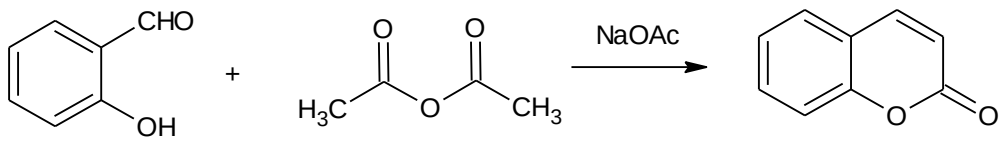


o-hydroxy aldehyde active methylene compound 3-substituted coumarin

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

2. Perkin reaction

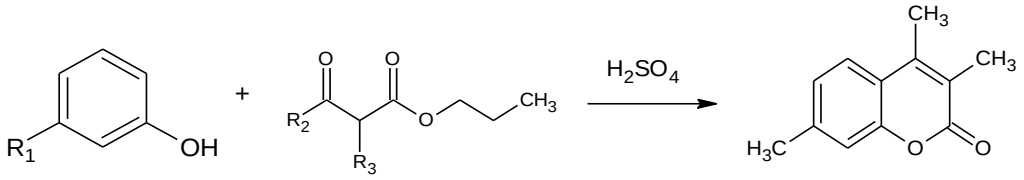
It involves the formation of coumarins by aldol condensation of an aromatic ortho-hydroxy benzaldehyde with acid anhydrides in the presence of an alkali salt of an acid.



o-hydroxy aldehyde acid anhydride coumarin

3. Pechmann Reaction

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.

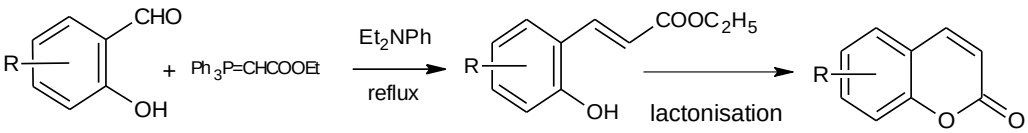


substituted phenol β-keto ester 3,4,7-trisubstituted coumarin

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

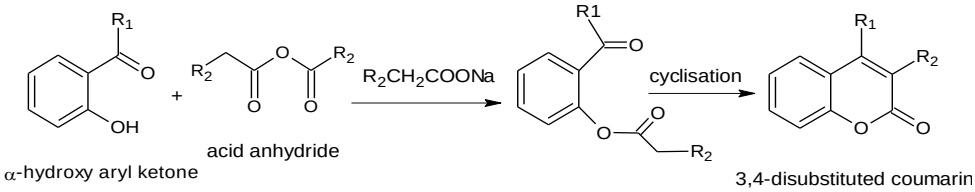
4. Wittig reaction

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



5. Kostanecki – Robinson Reaction

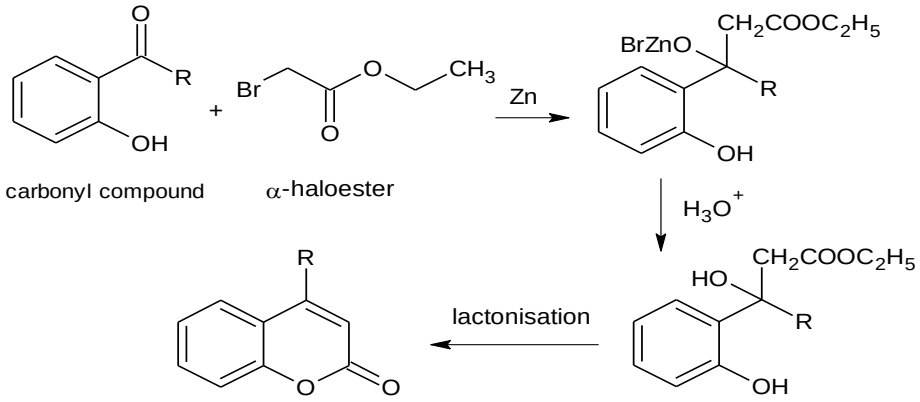
In this reaction, coumarins (usually 3 and 4 substituted) are formed by acylation of o-hydroxy aryl ketones with aliphatic acid anhydrides followed by cyclisation.



α-hydroxy aryl ketone acid anhydride 3,4-disubstituted coumarin

6. Reformatsky Reaction

In this, coumarins are obtained by the lactonisation of the β-hydroxy ester formed from the reaction between aldehydes or ketones with organozinc derivatives of α-haloesters.



PROPERTIES OF COUMARINS[104]

I) Physical Properties

Synonym : 1,2-Benzopyrone, 2H-1-Benzopran-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 : Slightly soluble in water and ethanol

Soluble in chloroform, diethyl ether, pyridine

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

II) Chemical Properties[105]

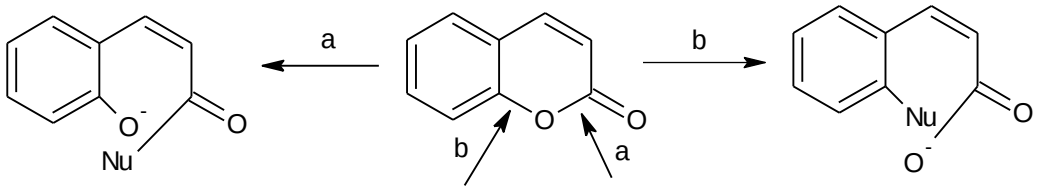
Reactivity of Coumarins

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

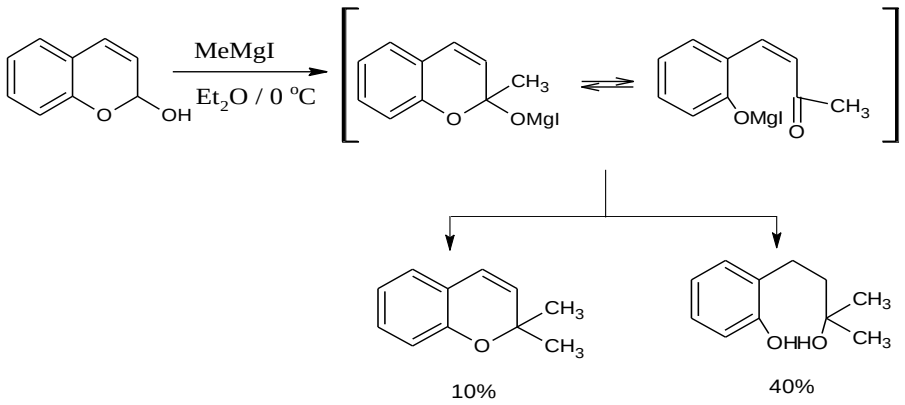
Several kinds of nucleophiles react with coumarins. Some of these reactions involve ring opening and occasionally, recyclisation into another ring. A nucleophile (Nu) which cleaves the ring, attacks and breaks one of the bonds of the ring oxygen atom as shown below.



1. With C-Nucleophiles

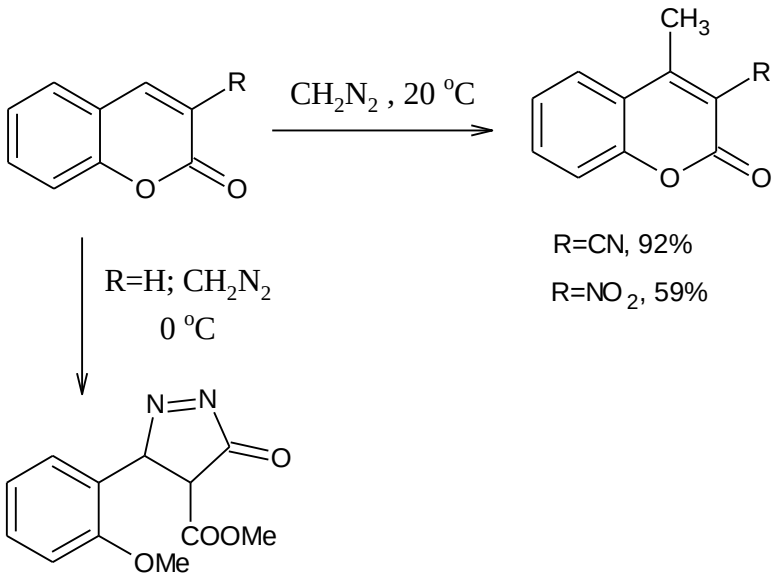
a. Grignard Reagents

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



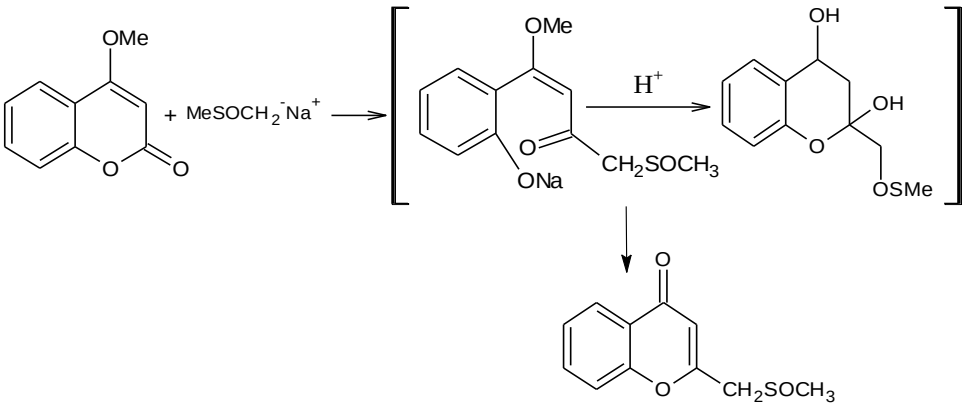
b. Diazomethane

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.



c. 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

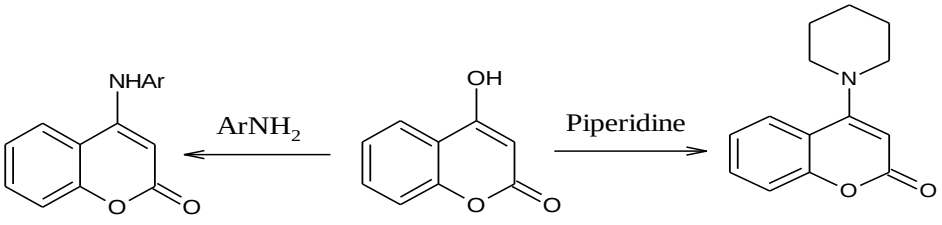
2. With N-Nucleophiles

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

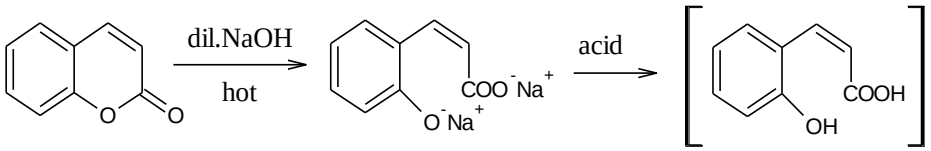
Coumarins react with primary aromatic and secondary cyclic amines to yield substitution products such as 4-arylamino and 4-piperidino coumarin.



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).

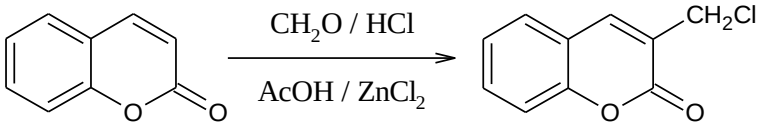


2-Hydroxy-*cis*-cinnamic acid

B. With Electrophiles

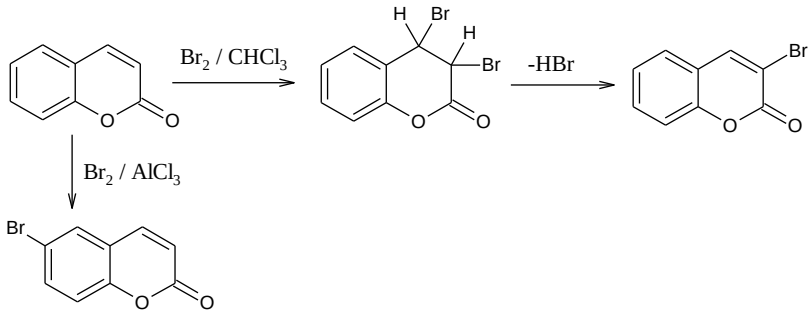
a. Chloromethylation

Chloromethylation occurs at C-3 position of coumarins by the reaction with formaldehyde in the presence of HCl or acetic acid and $ZnCl_2$.



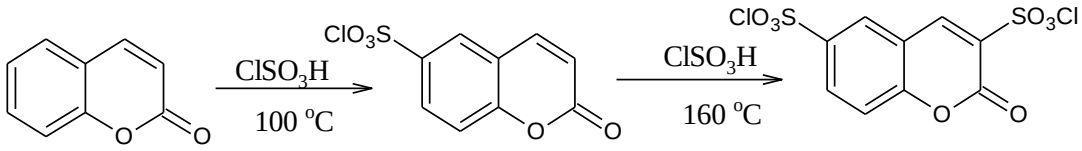
b. Bromination

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.



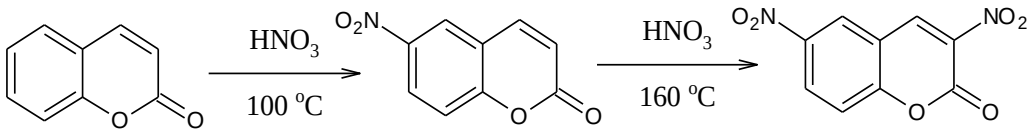
c. Sulphonation

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



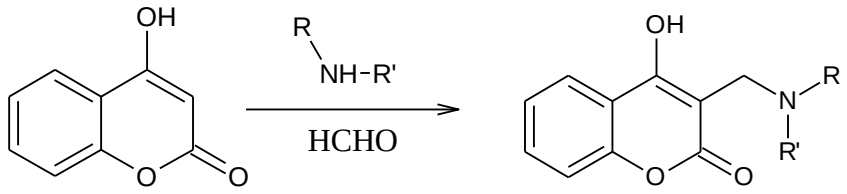
d. Nitration

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

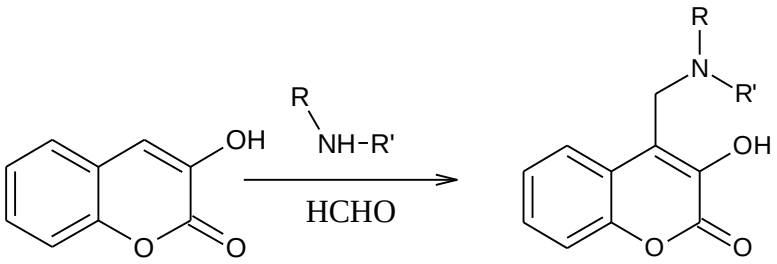


e. Mannich reaction [106]

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



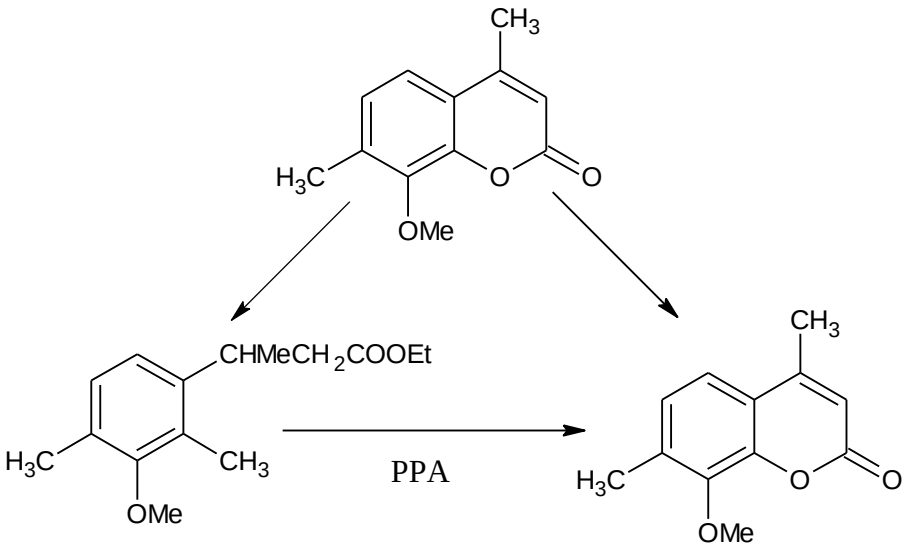
Similarly, Mannich reaction of 3-hydroxy coumarin with formaldehyde and primary or secondary amines resulted in 4-N,N-dialkylaminomethyl-3-hydroxy coumarins.



C. Reduction

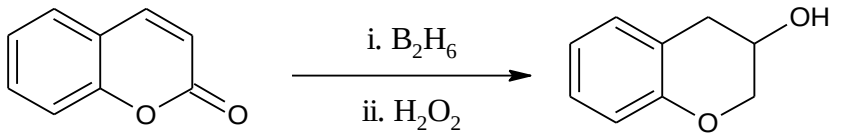
a. Catalytic redction

Catalytic reduction of coumarin under suitable conditions results in the formation of either 3.4-dihydroxy coumarin or the open ring product which can be cyclised efficiently.



b. Reduction with Diborane and Hydrogen peroxide

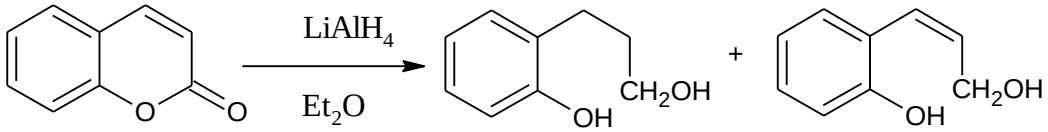
Reduction with diborane followed by hydrogen peroxide has two effects on coumarins: the carbonyl group is reduced to methylene and the elements of water are added across the 3,4-double bond in an anti-Markonikow manner but the overall yield is very low.



3,4-dihydro-*H*-chromen-3-ol

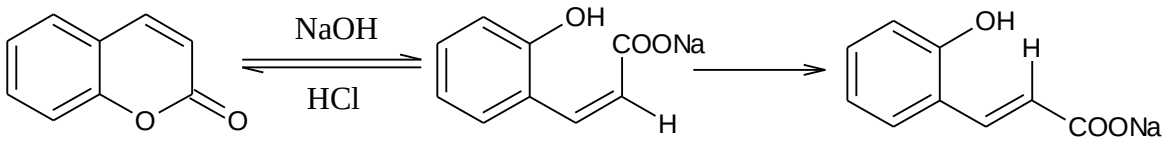
c. Reduction with Lithium aluminium hydride

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

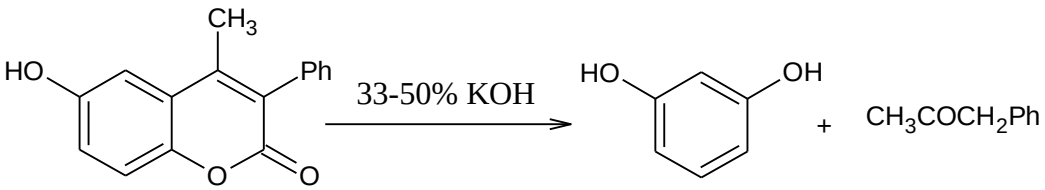


D. Hydrolytic Reaction

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.

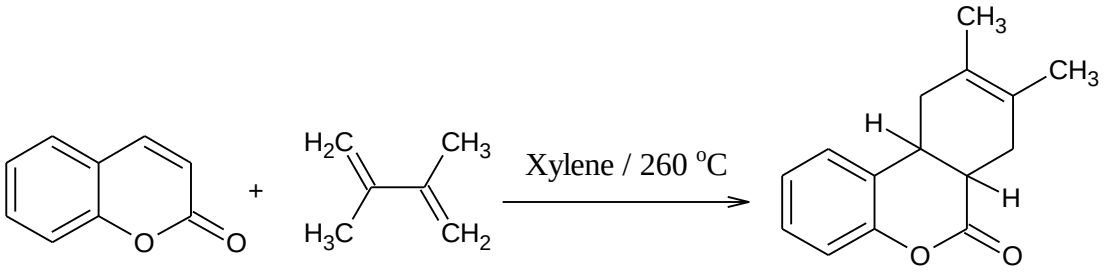


On the other hand, substituted coumarins give a mixture of a phenol and a ketone as a result of hydrolysis with alkali.



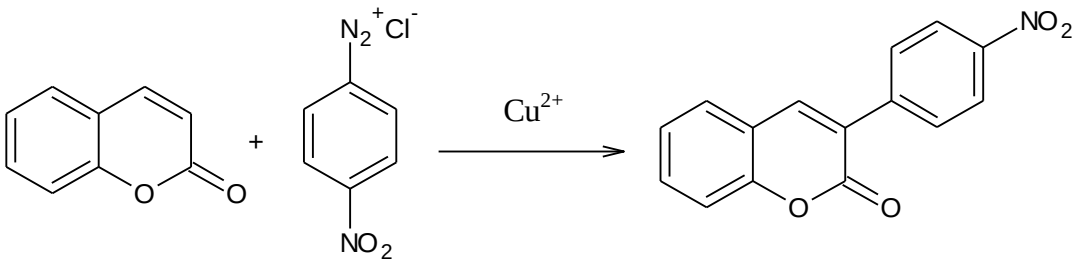
E. Diels Alder Reaction

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



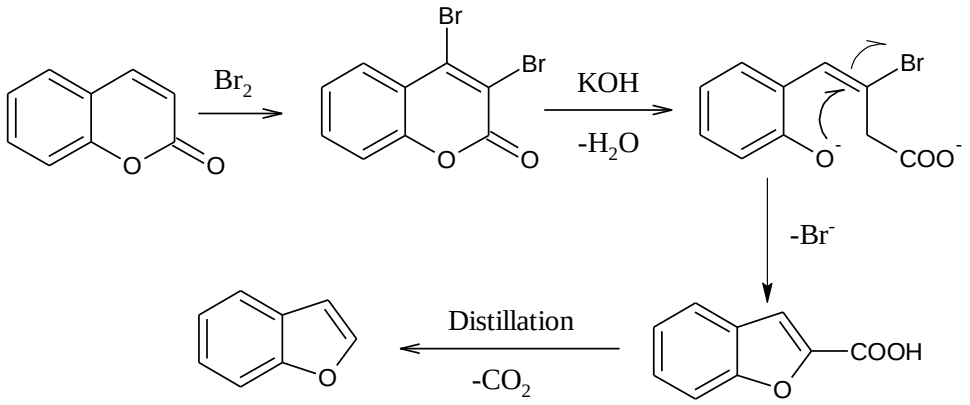
F. Reactions with Radicals

The C-3 position of coumarin is most susceptible to attack by radicals. Coumarin is arylated at C-3 by treatment with 4-nitrobenzenediazonium chloride under Meerwein reaction conditions. Variation of solvent and pH showed that acetone with a buffered pH of 2-4 gave the best results.



G. Ring Contraction Reaction

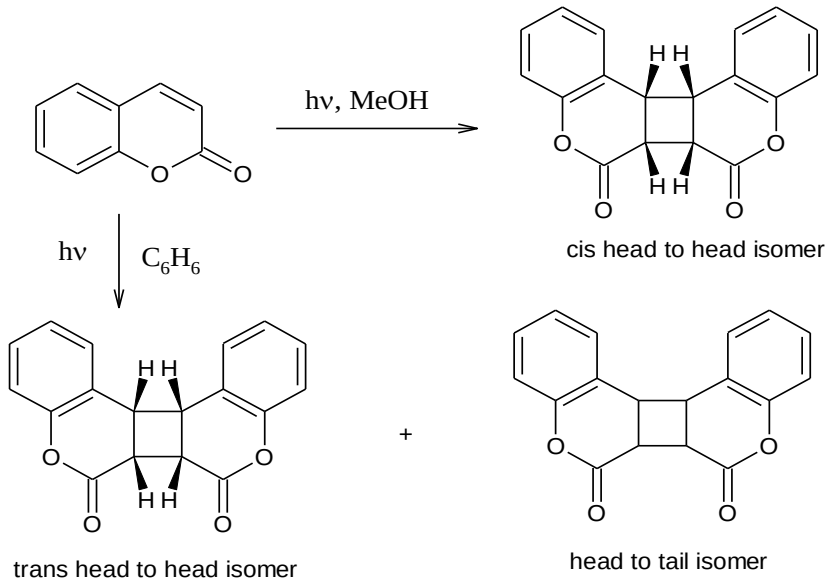
The reaction is a type of Perkin rearrangement reaction. The reaction proceeds with the bromination of coumarins. The resultant dibromocoumarin on alkaline degradation gives coumarilic acid which is decarboxylated to benzofuran.



H. Photochemical Reactions [104]

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

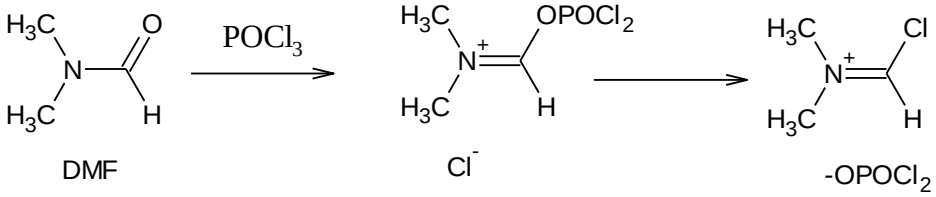
1. In a polar medium such as methanol, the only product formed is the cis head to head isomer.
2. In a non-polar medium such as benzene or dioxane, trans head to head dimer is the main product of the reaction; small amounts of head to tail dimers are also formed in non-polar solvents.



I. Vilsmeier-Haack Reaction

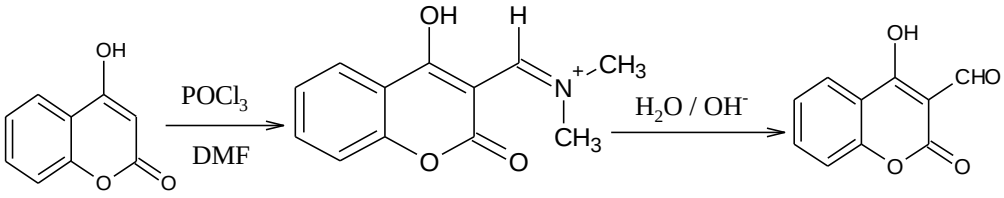
The reaction of an N,N-disubstituted formamide, such as DMF or N-methyl formanilide, with acid chlorides, such as phosphoryl chloride or phosgene, leads to the formation of an 'adduct'. These adducts are usually referred to as the Vilsmeier reagent which is used in the formylation of electron rich aromatic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

Formation of adduct:



DMF

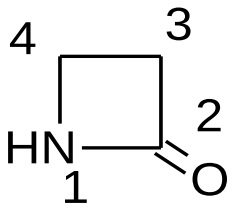
Vilsmeier-Haack Reaction in 4-hydroxy coumarin:



3-formyl-4-hydroxy coumarin

CHEMISTRY OF AZETIDINONES [107-109]

The parent heterocyclic ring of azetidinones is azetidine.Azetidine is a 4 membered heterocyclic ring system with nitrogen as hetero atom. 2-Azetidinones are also knownas β-lactams and it is one of the most common heterocyclic rings found in antibiotics. 2-Azetidinones consists of a carbonyl group on the second position.



Properties of Azetidin-2-ones

Azetidin-2-ones are moisture sensitive, colourless solids with melting point in the range of 73-74 °C. X-ray crystallographic studies of a number of monocyclic azetidin-2-ones indicate that the ring is essentially planar with the nitrogen atom slightly out of the mean plane of its substituents except where steric factors enforce greater deviations from planarity.

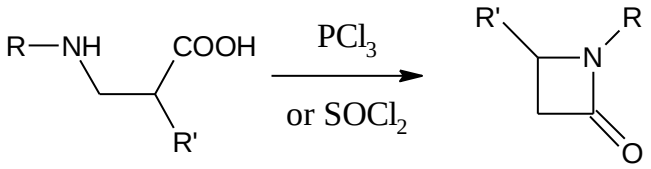
In normal amides, C=O shows a distance of 1.32Å, but azetidinones shows a distance of 1.38. The increased distance is the reason for angle strain. The IR spectra of monocyclic β-lactams have absorption maxima in the region of 1730-1760 cm^{-1} while the fused 2 and 3 cepham systems show IR maxima in the region of 1772-1784 cm^{-1}.

METHODS FOR THE SYNTHESIS OF AZETIDINONES

1. From non-heterocyclic precursors by closure of one bond:

i) Formation of N-C(2) bond:

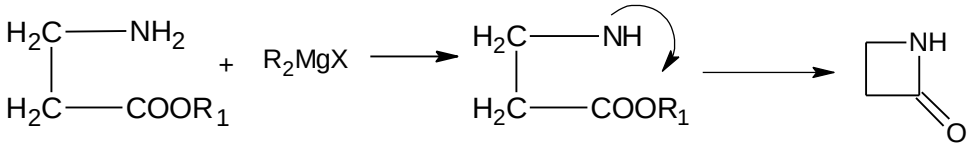
The thermal cyclization of β-amino acids to β-lactams fails because of β-elimination resulting in deamination, although there is a report of preparation of the parent compound.



1,4-disubstituted β-lactam

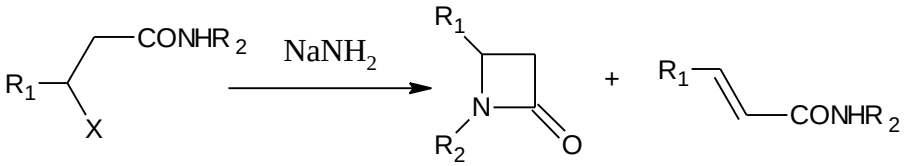
ii) From β-amino acid esters:

Ring closure between β-amino ester of Grignard reagent is generally used to produce β-lactam. The yield is about 50-70%. Further reaction of the Grignard reagent with the carboxyl group is prevented because of the hindered nature of the Grignard reagent.



iii) Formation of N-C(4) bond

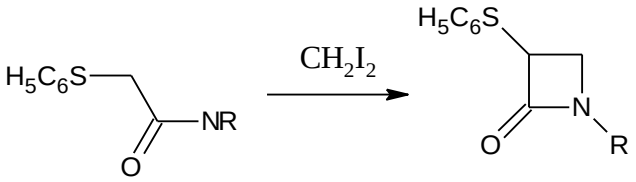
A number of examples of cyclization of N-substituted 3-halopropanamides in the presence of strong base like $NaNH_2$ or KNH_2 to give β-lactams have been reported.



2. From non-heterocyclic precursors by closure of two bonds

i) Formation from [3+1] fragments

Formation of the β-lactam by the reaction of the dianion with methylene diiodide provides an example of a [3+1] type of ring closure.



ii) Formation from [2+2] fragments

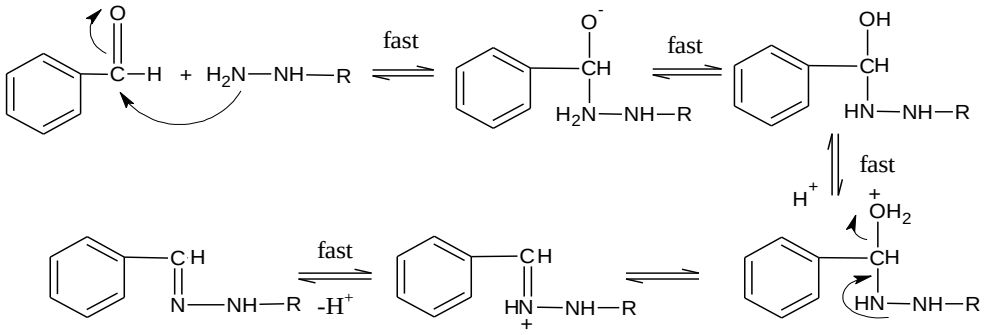
The reaction is also called as ketene-imine cycloaddition was reported by Staudinger as a smooth well documented route for β-lactam derivatives. Ketenes, on reaction with imines through a non-photochemical 2+2 cycloaddition ,will yield β-lactam.

Mechanism

STEP-1

Schiff's base formation

The mechanism for imine formation is essentially a two-step process. The first is the addition of the nucleophilic amine to the partially positive carbonyl carbon, followed by the loss of a proton by the oxygen. Next is the protonation of the OH group, which then can be lost as water in an elimination reaction.

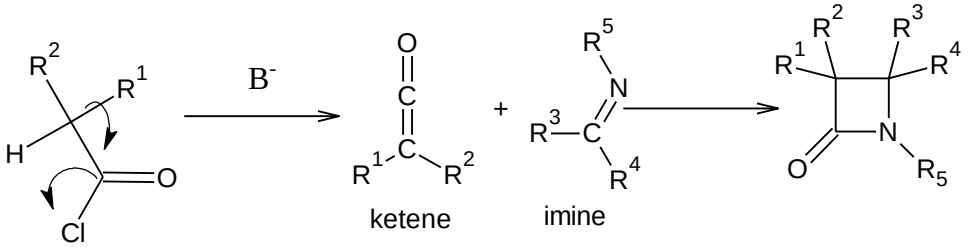


STEP-2

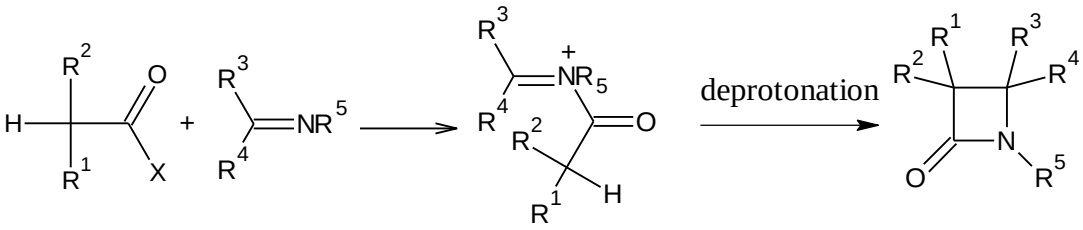
Azeitidinone formation

The mechanism involved in a particular case may well depend on the reactants and the timing of mixing.

One possible mechanism involve prior formation of a ketene from acid chlorides in the presence of bases such as triethylamine, which interacts with imines resulting in cycloaddition reaction.

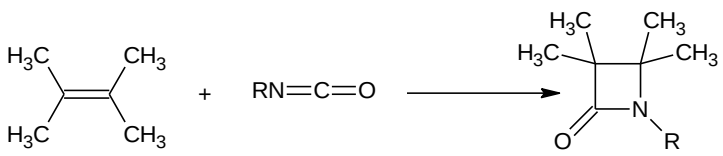


Another mechanism is also proposed that involves the interaction of the imine with the acid chloride to give an immonium ion. This is then cyclized by deprotonation under the influence of the base.



3. Formation of N-C(4) and C(2)-C(3) bonds:

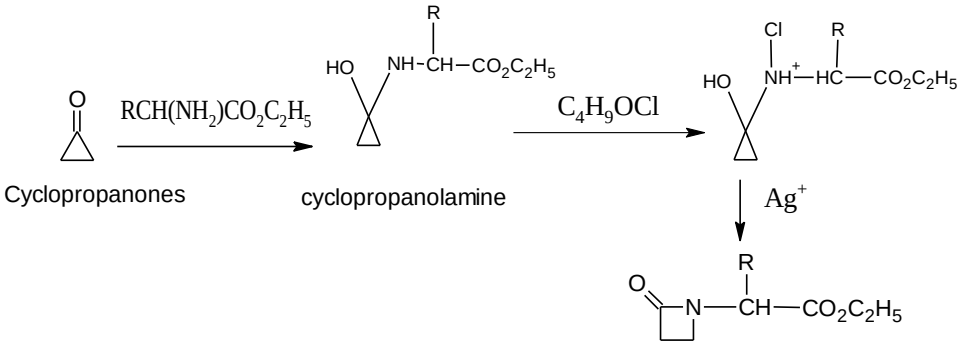
The [2+2] cycloaddition of isocyanates to alkenes provides one of the best routes to β-lactams.



4. Miscellaneous routes to azetidin-2-ones

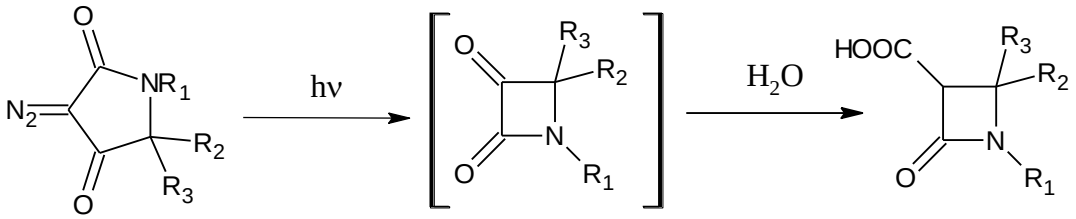
i) Ring expansion

Ring expansion reaction of cyclopropanones leads to β-lactams via the cyclopropanolamine.



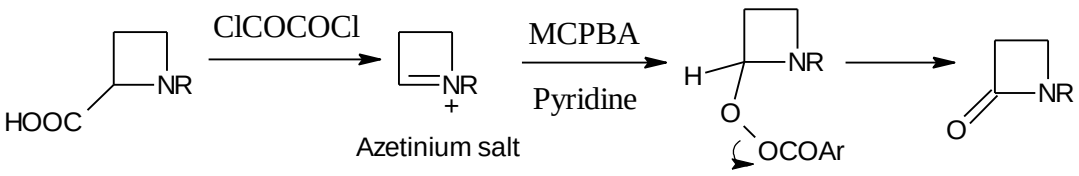
ii) Ring contractions

Several ring contraction routes to β-lactams have been developed. One of the most important is the Wolf rearrangement of 3-diazopyrrolidine -2,4-diones to give 3-carboxy azetidin-2-ones.



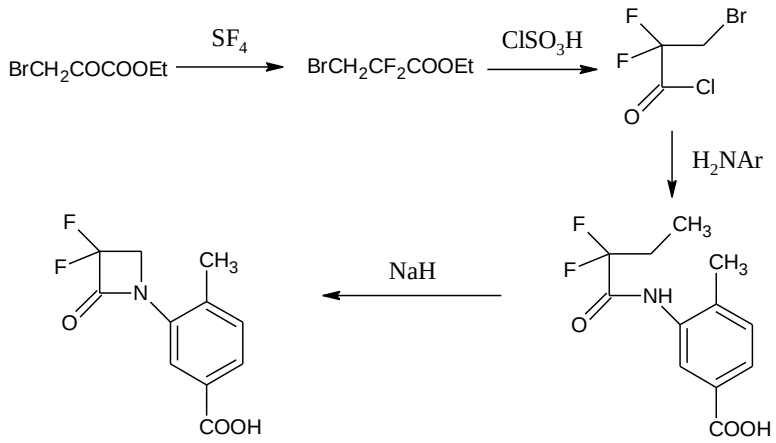
iii) From Azetidines

Azetidin-2-carboxylic acid is treated with oxalyl chloride to give the azetinium salt, followed by the reaction with MCPBA and pyridine.



iv) Wasserman Cyclisation

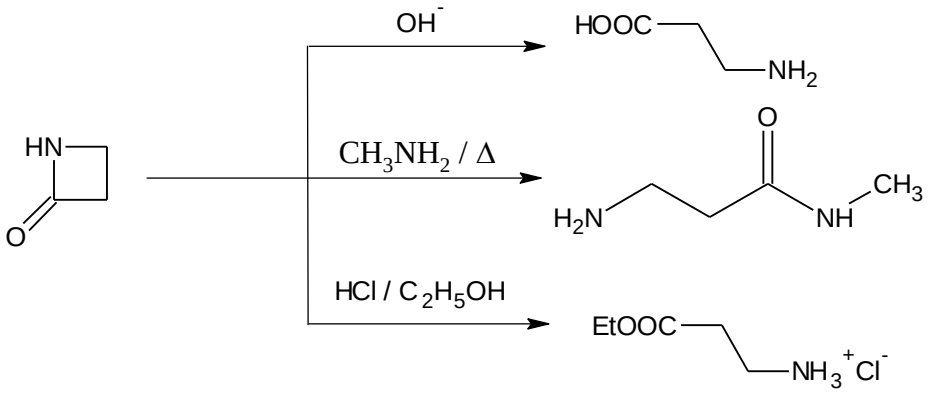
A halogen β- to the carbonyl would increase the IR absorption of the C=O, one of the criteria of the reactivity of the β-lactam. β-Bromopropionamide derivative was prepared, which can be cyclized by wasserman procedure using sodium hydride to give the N-(3-carboxy-6-methylphenyl)-3-difluoro-2-azetidinone.



REACTIONS OF AZETIDINONES

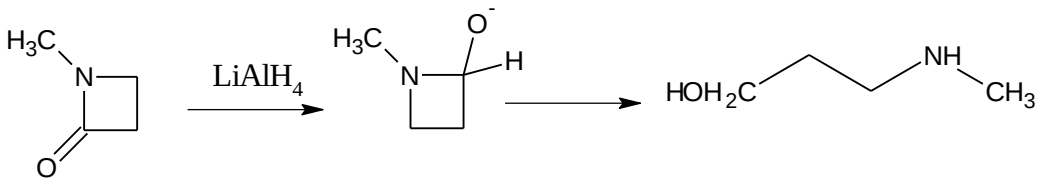
1) Ring opening reactions

β-aminopropionic acid is obtained from β-lactam in the presence of a base. The β-lactam ring is also susceptible to the action of alcoholic hydrochloric acid.



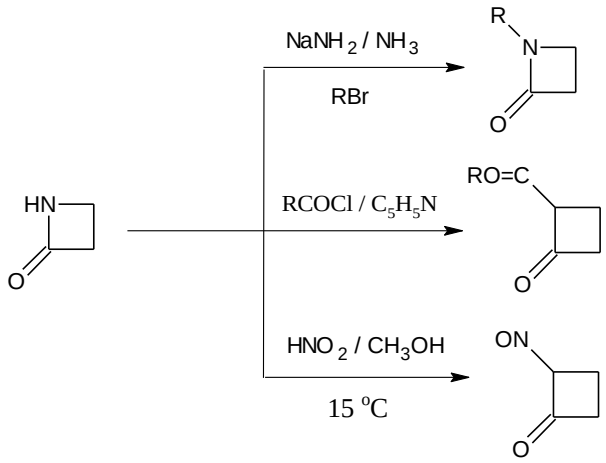
2) Reduction

Azetidinones on reduction with $LiAlH_4$ undergoes cleavage of the ring to form aminoalcohol.



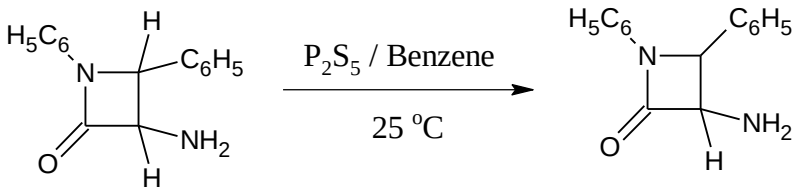
3) Formation of N-Derivatives

The N-substituted azetidin-2-ones undergo facile N-substitution to yield products which can be further manipulated to give derivatives of β-lactams.



4) Reaction with phosphorus pentasulphide

β-lactams are converted to thiolactams on reacting with P_2S_5 in benzene at 25°C.



PURPOSE OF THE STUDY

Tuberculosis (TB) is the most common cause of the death from infectious disease world-wide, which affects mainly the poorest countries of the world. Cell wall of *Mycobacterium tuberculosis* includes peptidoglycans and complex lipids (mycolic acids) which are significant determinant of its virulence. Novel antitubercular drugs are urgently needed because TB remains a global health priority [110].

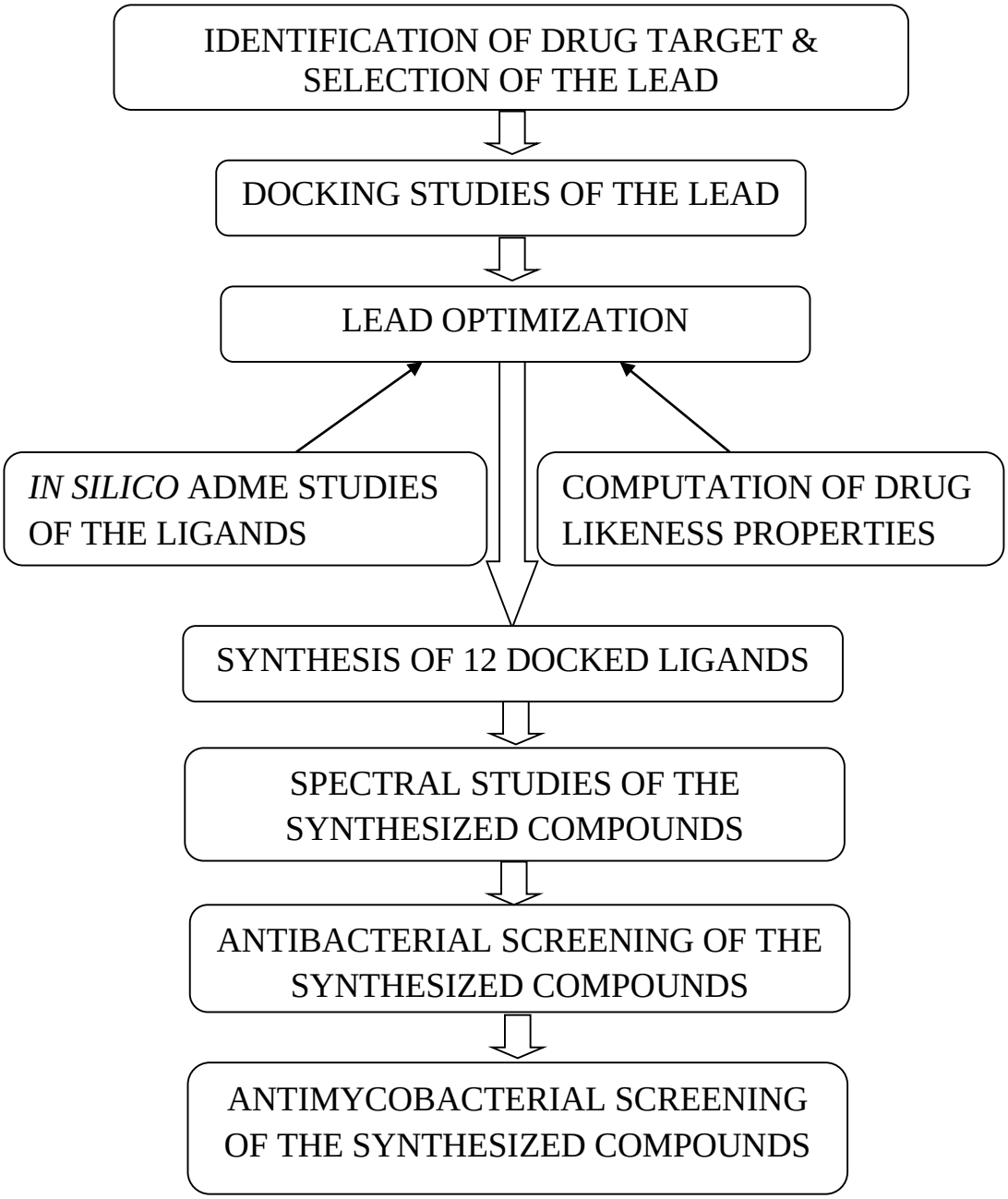
Coumarin compounds as medicinal drugs have been increasingly attracting special interest due to their potential outstanding contributions in the prevention and treatment of diseases, and the related researches and developments have received an increasing attention to synthetic organic chemists [110]. A great deal of effort has been made directly or indirectly towards the discovery and development of coumarin-based antitubercular drugs and some excellent achievements have been acquired.

Azetidinone is an exciting pharmaceutical fragment in drug discovery. The strong activity of the famous antibiotics such as penicillins, cephalosporins, thienamycins, nocardicins, aztreonams as well as carbapenems is attributed to the presence of the 2 -azetidinone ring [110].

Mycobacterium tuberculosis genome has high number of cytochrome P450 enzymes and parallel studies indicated that cytochrome P450 inhibiting azole drugs has potent antitubercular activity. Recent research explains potential drug target on *Mycobacterium tuberculosis* P450 and provides evidence for roles of selected P450 isoforms in host lipid and sterol or steroid transformations [111].

Drug discovery tools help in designing new molecular entities which are safe and effective without consuming much of the research hours. The purpose of the present work was to design and synthesize new azetidine-2-one derivatives containing coumarin moiety in order to explore the extent of their antitubercular activity. The compounds were designed by *in silico* method using MT-CYP51 as the target molecule.

PLAN OF WORK


IDENTIFICATION OF DRUG TARGET & SELECTION OF THE LEAD
DOCKING STUDIES OF THE LEAD
LEAD OPTIMIZATION
IN SILICO ADME STUDIES OF THE LIGANDS
COMPUTATION OF DRUG LIKENESS PROPERTIES
SYNTHESIS OF 12 DOCKED LIGANDS
SPECTRAL STUDIES OF THE SYNTHESIZED COMPOUNDS
ANTIBACTERIAL SCREENING OF THE SYNTHESIZED COMPOUNDS
ANTIMYCOBACTERIAL SCREENING OF THE SYNTHESIZED COMPOUNDS

EXPERIMENTAL SECTION

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 antimycobacterial activity. From the literature review and the current research on CYP51 inhibitors, we have selected cytochrome P450 lanosterol 14α-demethylase in *Mycobacterium tuberculosis* as the target for the present study. The pdb structure of MT-CYP51 was downloaded from the RCSB protein data bank.

LEAD SELECTION

The lead azetidin-2-one, imidazolidin-2-one, oxazolidin-2-one and thiazolidin-2-one 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 : MT-CYP51 (1EA1)

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

Steps involved in docking studies [55,112,113]

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

MT-CYP51 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 →Cick 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 Hydrogens
  - 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→ Click Dockings and Open dlg file
- Open Analyze→ Click Macromolecule and Choose pdbqt file.
- Open Analyze→ Click Conformations and Load
- Double click the desired conformation
- Click Analyze and Docking then Show Interactions.

  Proteins and ligand interaction will be displayed. Zoom it and increase the contrast by holding right key and ctrl.
- Open File→Save image→cygwin/usr/local/bin as .png

  The above mentioned steps involved in docking are done for all the 150 ligands (az1-42, im1-36, ox1-36 and th1-36)

RESULTS AND DISCUSSION

The docking results of **MT-CYP51 (1EA1.pdb)** with the ligands **az1-42, im1-36, ox1-36 and th1-36** and standard (fluconazole) are reported in the **Tables 1-4.** The best docked structures should have the binding energy lower to the standard. The binding sites and the active sites are shown in the snapshots.

Table 1. Binding energies of az1-42

Sl. No.	Compound code	Binding energy (Kcal/mol)	Sl.No.	Compound code	Binding energy (Kcal/mol)
1	az1	-10.51	22	az22	-10.5
2	az2	-10.8	23	az23	-10.36
3	az3	-10.34	24	az24	-10.45
4	az4	-10.7	25	az25	-10.4
5	az5	-10.6	26	az26	-11.15
6	az6	-10.47	27	az27	-8.94
7	az7	-10.2	28	az28	-8.75
8	az8	-9.54	29	az29	-7.46
9	az9	-9.94	30	az30	-11.22
10	az10	-10.32	31	az31	-8.15
11	az11	-11.15	32	az32	-8.32
12	az12	-9.47	33	az33	-9.27
13	az13	-10.77	34	az34	-8.73
14	az14	-10.67	35	az35	-8.44
15	az15	-11.02	36	az36	-8.44
16	az16	-11.03	37	**az37**	**-11.28**
17	az17	-11.03	38	**az38**	**-11.24**
18	az18	-10.97	39	az39	-10.43
19	az19	-10.83	40	az40	-9.99
20	az20	-10.77	41	az41	-9.88
21	az21	-8.62	42	az42	-10.01
Fluconazole					-6.67

Table 2. Binding energies of im1-36

Sl.No.	Compound code	Binding energy (Kcal/mol)	Sl.No.	Compound code	Binding energy (Kcal/mol)
1	im1	-11.19	19	im19	-9.96
2	im2	-11.08	20	im20	-10.84
3	im3	-10.9	21	im21	-10.85
4	im4	-10.52	22	im22	-11.21
5	im5	-10.79	23	im23	-11.05
6	im6	-10.72	24	im24	-9.08
7	im7	-10.73	25	im25	-9.17
8	im8	-9.47	26	im26	-8.72
9	im9	-10.31	27	im27	-8.73
10	im10	-10.5	28	im28	-8.72
11	im11	-11.05	29	im29	-9.48
12	im12	-10.31	30	im30	-9.89
13	im13	-10.56	31	im31	-9.77
14	im14	-11.14	32	im32	-8.96
15	im15	-11.13	33	im33	-10.14
16	im16	-10.94	34	im34	-8.54
17	im17	-11.24	35	im35	-10.37
18	im18	-9.79	36	im36	-10.11
Fluconazole					-6.67

Table3. Binding energies of ox1-36

Sl.No.	Compound code	Binding energy (Kcal/mol)	Sl.No.	Compound code	Binding energy (Kcal/mol)
1	ox1	-11.2	19	ox19	-11.25
2	ox2	-10.76	20	ox20	-11.17
3	ox3	-10.96	21	ox21	-10.23
4	ox4	-10.97	22	ox22	-11.24
5	ox5	-11.08	23	ox23	-10.99
6	ox6	-11.23	24	ox24	-11.2
7	ox7	-11.2	25	ox25	-11.25
8	ox8	-10.34	26	ox26	-10.79
9	ox9	-11.15	27	ox27	-11.21
10	ox10	-11.16	28	ox28	-11.18
11	ox11	-11.27	29	ox29	-8.9
12	ox12	-10.59	30	ox30	-11.16
13	ox13	-10.59	31	ox31	-9.29
14	ox14	-11.24	32	ox32	-9.11
15	ox15	-11.27	33	ox33	-8.49
16	ox16	-10.02	34	ox34	-8.3
17	ox17	-11.23	35	ox35	-8.36
18	ox18	-10.37	36	ox36	-9.59
Fluconazole					-6.67

Table 4. Binding energies of th1-36

Sl.No.	Compound code	Binding energy (Kcal/mol)	Sl.No.	Compound code	Binding energy (Kcal/mol)
1	th1	-10.91	19	th19	-11.26
2	th2	-10.83	20	th20	-11.23
3	th3	-10.93	21	th21	-11.25
4	th4	-10.66	22	th22	-10.43
5	th5	-10.58	23	th23	-10.65
6	th6	-11.05	24	th24	-11.25
7	th7	-10.83	25	th25	-10.87
8	th8	-10.29	26	th26	-11.23
9	th9	-9.98	27	th27	-11.25
10	th10	-9.97	28	th28	-10.76
11	th11	-11	29	th29	-11.11
12	th12	-10.19	30	th30	-11.26
13	th13	-10.7	31	th31	-9.32
14	th14	-10.89	32	th32	-9.69
15	th15	-11.02	33	th33	-10.67
16	th16	-11.13	34	th34	-8.46
17	th17	-11.27	35	th35	-8.45
18	th18	-11.13	36	th36	-9.75
Fluconazole					-6.67

The binding energy of all the 150 compounds selected were found to be lower than that of the standard (fluconazole). In this project, we are only concerned with the azetidinone series (az1-36) of compounds. In the series, the binding energy of az38 (-11.28) is the lowest. Hence, twelve compounds with similar structure as that of az38 is selected for further studies. This series include az31-42 and for ease, we rename them as **N_{1-6} and P_{1-6}.**

Binding of fluconazole with MT-CYP51

Fluconazole interacts with MT-CYP51 at ARG 96, ALA 256, MET 99, TYR 76, THR 260 and HEM 460. Binding energy was found to be -6.67 kcal/mol.

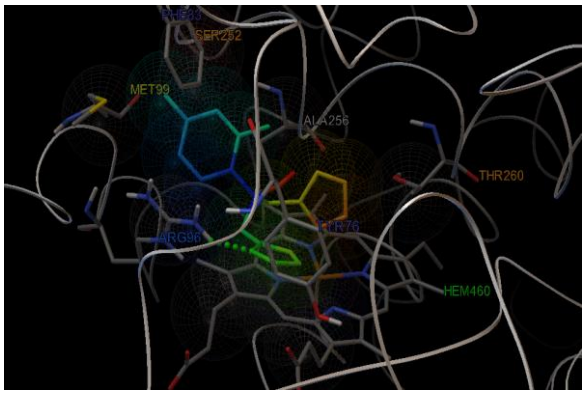


Figure 1.Snapshot of fluconazole binding with 1EA1

Binding of az31-42 with MT-CYP51

az31 (N_1)

N_1 interact with MT-CYP51 at ARG 96, ALA 256, MET 99 and HEM 460. Binding energy was found to be -8.15 kcal/mol.

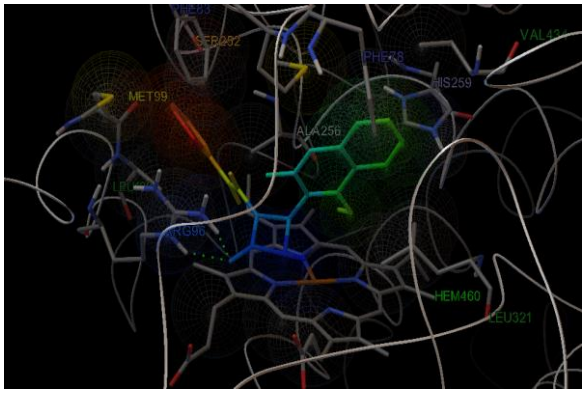


Figure 2.Snapshot of N_1 binding with 1EA1

az32 (N_2)

N_2 interact with MT-CYP51 at ARG96, ALA 256, THR 260 and HEM 460. Binding energy was found to be -8.32 kcal/mol.

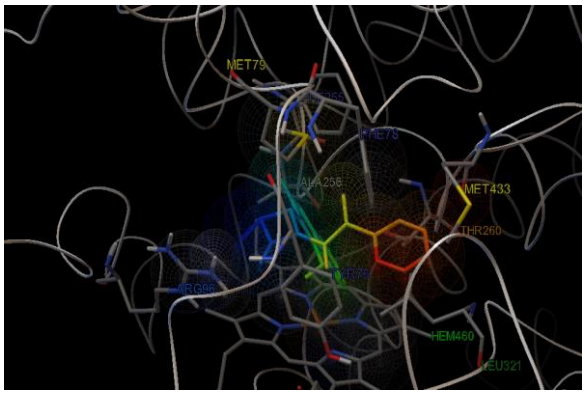


Figure 3.Snapshot of N_2 binding with 1EA1

az33 (N_3)

N_2 interact with MT-CYP51 at ARG96, ALA 256, TYR 76 and HEM 460. Binding energy was found to be -9.27 kcal/mol.

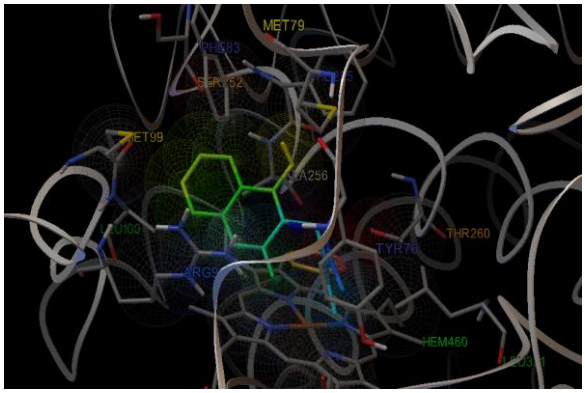


Figure 4.Snapshot of N_3 binding with 1EA1

az34 (N_4)

N_4 interact with MT-CYP51 at ALA 256, TYR 76, THR 260 and HEM 460. Binding energy was found to be -8.73 kcal/mol.

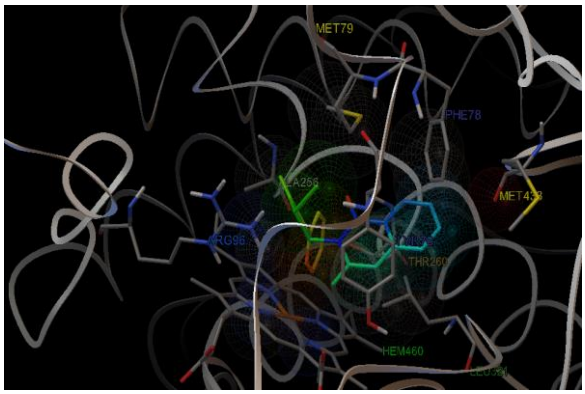


Figure 5.Snapshot of N_4 binding with 1EA1

az35 (N_5)

N_5 interact with MT-CYP51 at ALA 256, TYR 76 and HEM 460. Binding energy was found to be -8.44 kcal/mol.

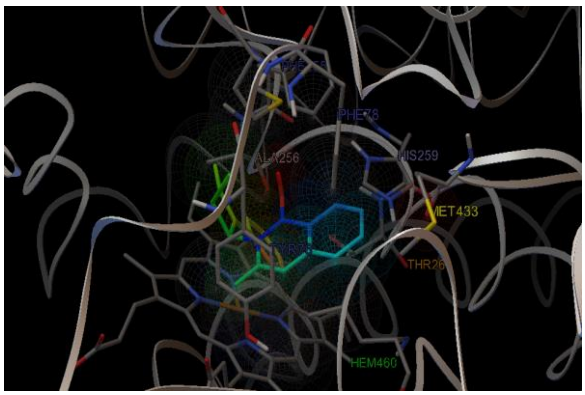


Figure 6.Snapshot of N_5 binding with 1EA1

az36 (N_6)

N_6 interact with MT-CYP51 at ALA 256, TYR 76 and HEM 460. Binding energy was found to be -8.44 kcal/mol.

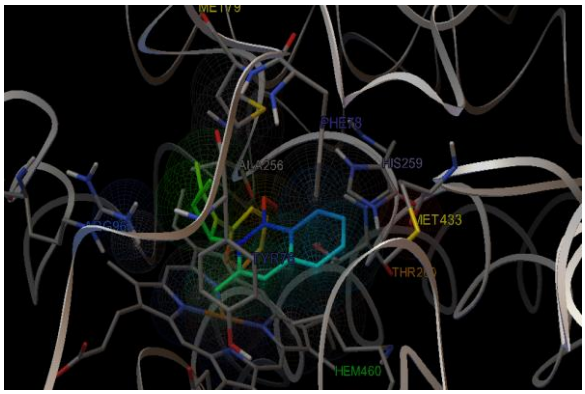


Figure 7.Snapshot of N_6 binding with 1EA1

az37 (P_1)

P_1 interact with MT-CYP51 at ALA 256, TYR 76, THR 260 and HEM 460. Binding energy was found to be -11.28 kcal/mol.

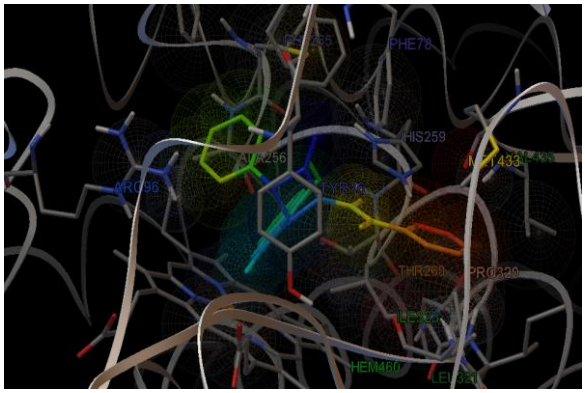


Figure 8.Snapshot of P_1 binding with 1EA1

az38 (P_2)

P_2 interact with MT-CYP51 at ALA 256, TYR 76, THR 260 and HEM 460. Binding energy was found to be -11.24 kcal/mol.

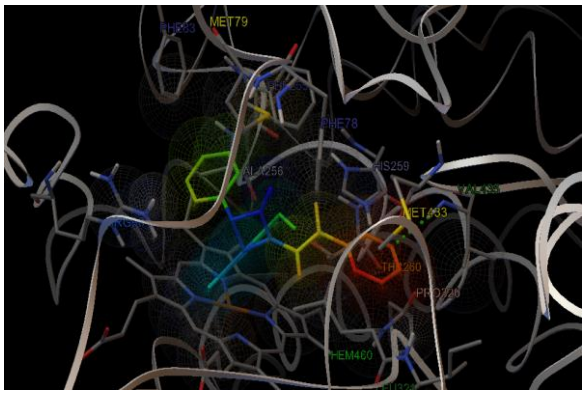


Figure 9.Snapshot of P_2 binding with 1EA1

az39 (P_3)

P_3 interact with MT-CYP51 at ALA 256, TYR 76, ARG96, THR 260 and HEM 460. Binding energy was found to be -10.43 kcal/mol.

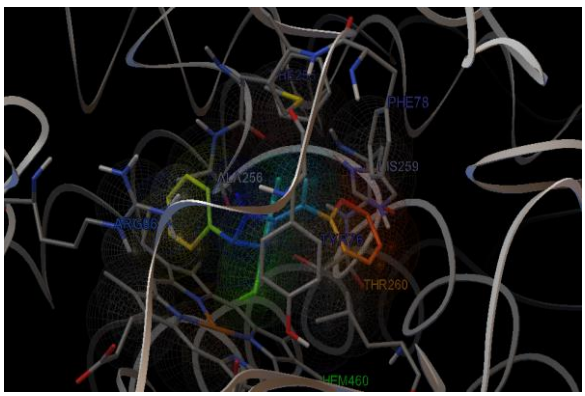


Figure 10.Snapshot of P_3 binding with 1EA1

az40 (P_4)

P_4 interact with MT-CYP51 at ALA 256, TYR 76, THR 260 and HEM 460. Binding energy was found to be -9.99 kcal/mol.

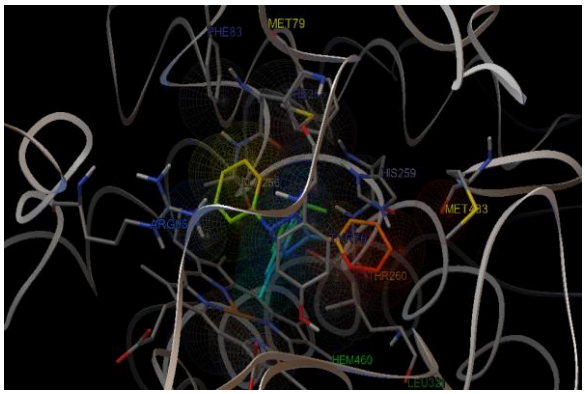


Figure 11.Snapshot of P_4 binding with 1EA1

az41 (P_5)

P_5 interact with MT-CYP51 at ALA 256, TYR 76, THR 260 and HEM 460. Binding energy was found to be -9.88 kcal/mol.

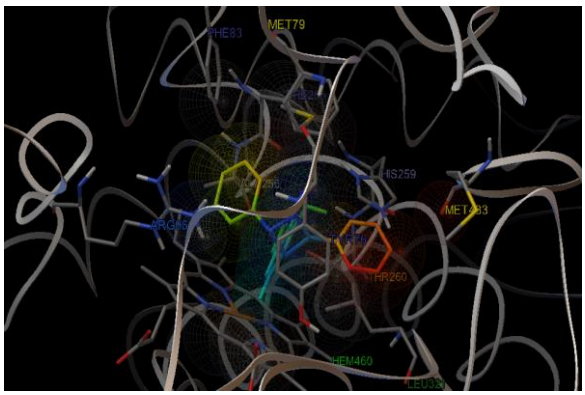


Figure 12.Snapshot of P_5 binding with 1EA1

az42 (P_6)

P_5 interact with MT-CYP51 at ALA 256, TYR 76, THR 260 and HEM 460. Binding energy was found to be -10.01 kcal/mol.

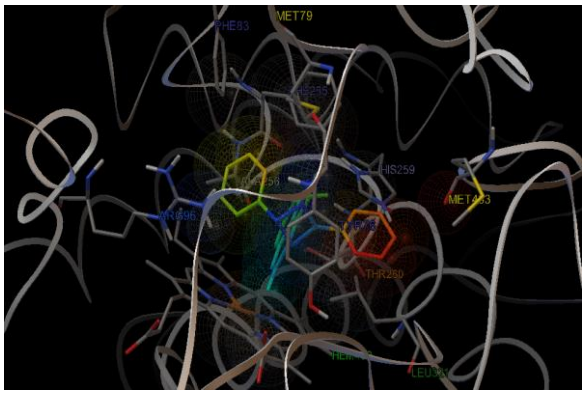


Figure 13.Snapshot of P_6 binding with 1EA1

TOXICITY STUDIES

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

Toxicity studies are performed by two methods:

- Evaluation of drug likeness property
- Evaluation of ADME data

Evaluation of drug likeness properties [114]

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 N_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

Table 5: Drug likeness scores of N_{1-6} and P_{1-6} using molinspiration server

Sl.No.	Compound code	mLogP	MW	No. of H acceptors	No. of H donors	No. of violations
1	N_1	-0.10	385.76	8	2	0
2	N_2	0.74	370.75	7	1	0
3	N_3	2.21	342.74	6	1	0
4	N_4	1.82	342.74	6	1	0
5	N_5	1.82	343.73	7	1	0
6	N_6	-0.04	385.76	8	2	0
7	P_1	1.38	427.42	8	2	0
8	P_2	2.21	412.40	7	1	0
9	P_3	3.69	384.39	6	1	0
10	P_4	3.30	384.39	6	1	0
11	P_5	3.30	385.38	7	1	0
12	P_6	1.43	427.42	8	2	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 N_{1-6} and P_{1-6} have good oral absorption.

IN *SILICO* ADME STUDIES OF THE DOCKED LIGAND

ADME studies are performed to evaluate the absorption, distribution, metabolism of the selected ligands using Accord for Excel server.

Procedure

2 D structures were directly introduced into **Accord for Excel** for carrying out ADME studies by using **Edit Chemistry** module of software. Then the structure is subjected to **Function** module and the data for descriptors like blood brain barrier penetration (BBB), human intestinal absorption (HIA), plasma protein binding, aqueous solubility and hepatotoxicity were calculated.

Table 6: ADME data of N_{1-6} and P_{1-6}

Comp. code	FPSA	AQ. SOL. LOG.	AQ. SOL. LOG. LEV.	BBB. DIST. T2	BBB. LOG.	BBB. LOG. LEV.	CYP2D6	CYP2D6. PROB	FST. ALOGP98	HEPATOTOX	HEPATOTOX. PROB	HIA. FABS. LEV	HIA. FABS. T2	PROT. BIND. LEV	PROT. BIND. LEV. LOG
N_1	109.0717	-2.87356	3	8.407494	-1.6156	3	0	0.267327	0.8537	1	0.807947	0	3.226168	2	0
N_2	96.2615	-3.6258	3	5.779164	-1.06074	3	0	0.405941	1.993101	1	0.774834	0	1.635727	2	0
N_3	78.9607	-3.69516	3	2.561484	-0.74286	3	1	0.514851	2.1359	1	0.860927	0	0.367197	2	0
N_4	78.9607	-3.79516	3	2.561484	-0.90949	3	0	0.425743	1.5968	1	0.860927	0	0.460503	2	0
N_5	90.2217	-3.2166	3	4.30963	-1.12077	3	0	0.425743	1.4897	1	0.934437	0	1.109506	2	0
N_6	109.0717	-2.87364	3	8.407494	-1.6156	3	0	0.29703	0.8537	1	0.940397	0	3.226168	2	0
P_1	1099.0717	-3.58	3	8.917111	-1.32435	3	1	0.643564	1.7960022	1	0.940397	0	3.117621	1	0
P_2	96.2615	-4.45063	2	7.29044	-0.76948	3	1	0.693069	2.9354	1	0.940397	0	2.469873	0	0
P_3	78.9607	-4.56566	2	3.555211	-0.45161	2	1	0.712871	3.07820	1	0.92053	0	0.991146	2	0
P_4	78.9607	-4.09475	2	2.831851	-0.61823	3	1	0.782178	0.539101	1	0.874172	0	0.511625	2	0
P_5	90.2217	-4.0871	2	4.898544	-0.82951	3	1	0.534653	2.432	1	0.92053	0	1.282406	2	0
P_6	109.0717	-3.58008	3	8.91711	-1.32435	3	1	0.643564	1.796001	1	0.913907	0	3.11762	1	0

SYNTHESIS

MATERIALS AND METHODS

Reagents and chemicals used:

4-Hydroxy coumarin, Phosphorous oxychloride, Dimethyl formamide, soniazid, Nicotinamide, 2-Amino pyridine, 4-Amino pyridine, 2-Amino pyrimidine, Nicotinic hydrazide, Ethanol, Dioxane, Triethylamine, Chloroacetyl chloride, Phenylacetyl chloride

All the reagents and chemicals were procured from Sigma Aldrich High media and Lobachem. All the compounds procured were purified and dried, whenever necessary before use, following standard methods.

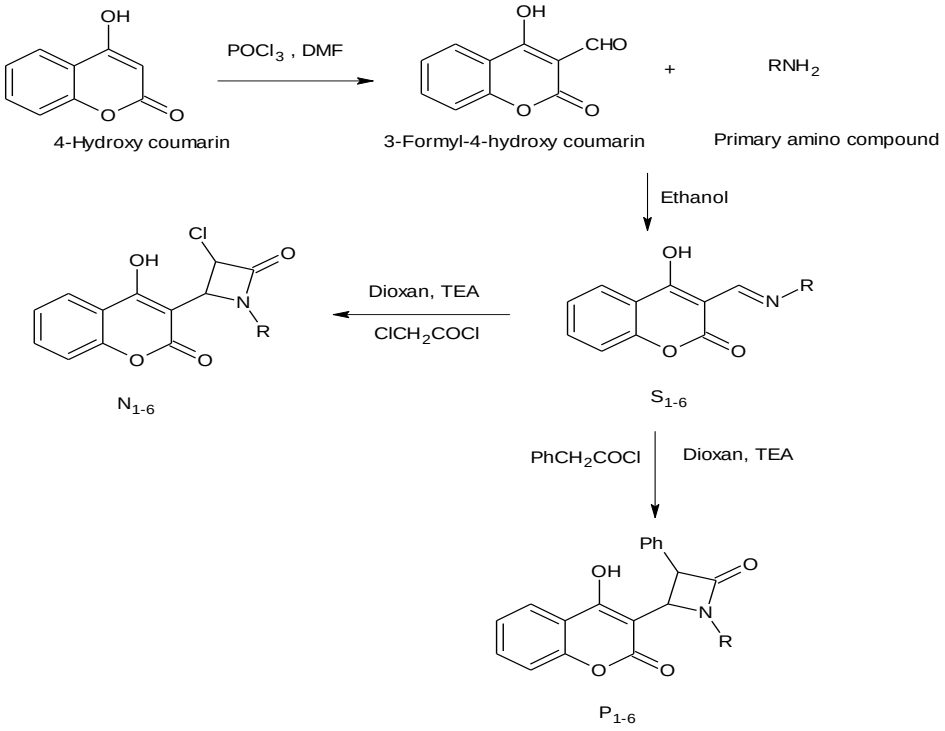
Apparatus used:

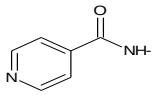
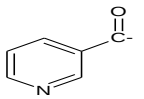
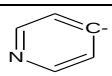
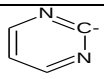
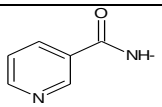
Beakers, test tubes, glass rods, mechanical stirrer, thermometer, round bottom flask, reflux condenser, conical flasks, separating funnel, dropping funnel and pipettes.

Analytical work:

- Melting point was determined by using melting point apparatus MR-VIS, visual melting range apparatus, LABINDIA and uncorrected.
- Reactions were monitored by thin layer chromatography (TLC) on a pre-coated silica gel G plated using Iodine vapor as visualizing agent.
- UV spectra were recorded on JASCO V-530 UV-VIS spectrometer in the department of Pharmaceutical analysis, College of Pharmacy, SRIPMS, Coimbatore.
- IR spectra were recorded on JASCO FTIR-420 series in the department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore.
- NMR spectra were recorded on Bruker AVANCE III 500MHz NMR spectrometer at SAIF, IIT, Madras.
- Mass spectra were recorded on JEOL GCMATE II GC-MS spectrometer at SAIF, IIT, Madras.

SCHEME



Compound code	R
S_1, N_1 , P_1	
S_2, N_2 , P_2	
S_3, N_3 , P_3	
S_4, N_4 ,P_4	
S_5, N_5 ,P_5	
S_6, N_6 , P_6	

Procedure

Step 1: Synthesis of 4-hydroxy-3-formyl coumarin [115]

The compound was prepared from 4-hydroxycoumarin under Vilsmeier conditions ($POCl_3$/DMF). An amount of 9.72 g (0.6 mol) of 4-hydroxycoumarin was dissolved in DMF (46.2 ml). In this mixture, at -5°C, $POCl_3$ (0.18 mol) was added slowly. The reaction mixture was stirred at 60°C for 1hr. This mixture was kept overnight at 0°C. The precipitate was hydrolyzed by boiling with 5% Sodium carbonate solution. The yellow product obtained was filtered and recrystallized from ethanol.

Step 2: Synthesis of Schiff base from formyl coumarin (S_{1-6}) [116]

Equimolar quantities of 4-hydroxy-3-formyl coumarin and primary amino compound (RNH_2) was dissolved in ethanol and refluxed for 2hrs. It was then cooled and poured into crushed ice. The product formed was obtained by filtration and recrystallised from ethanol.

Step 3: [117]

Synthesis of Chlorine substituted Azetidinones (N_{1-6})

A mixture of 0.01 mol Schiff bases (S_{1-6}) and triethyl amine (0.025 mol) was dissolved in dioxane (10 ml) and stirred. To this well stirred solution, chloroacetyl chloride (0.025mol) was added drop by drop for a period of 30 min at 0-5°C. The reaction mixture was further stirred for 6hrs. It was then kept at room temperature for 48hrs.The reaction mixture is poured into crushed ice and the resultant product (N_{1-6}) was filtered and washed with water, dried and recrystallized from DMSO.

Synthesis of Phenyl substituted Azetidinones (P_{1-6})

A mixture of 0.01 mol schiff bases (S_{1-6}) and triethyl amine (0.025 mol) was dissolved in dioxane (10 ml) and stirred. To this well stirred solution, phenylacetyl chloride (0.025mol) was added drop by drop for a period of 30 min at 0-5°C. The reaction mixture was further stirred for 6hrs. It was then kept at room temperature for 48hrs.The reaction mixture is poured into crushed ice and the resultant product (N_{1-6}) was filtered and washed with water, dried and recrystallized from DMSO.

PHYSICAL CHARACTERISATION DATA

1) Substituted 3-Chloro azetidin-2-ones

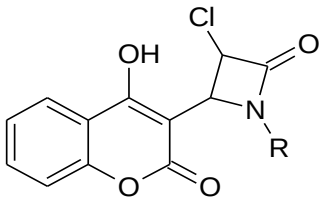
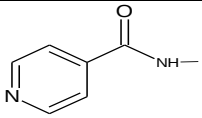
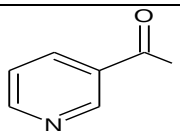
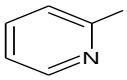
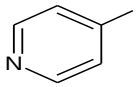
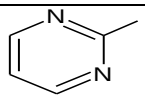
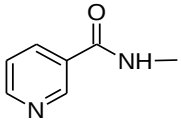


Table 7: Physical characterization of substituted 3-Chloro azetidin-2-ones

Comp. code	R	Molecular formula	Molecular weight (g/mol)	% Yield	Melting point	Rf value
N_1		$C_{18}H_{12}ClN_3O_5$	385.75	77	252-253	0.57
N_2		$C_{18}H_{11}ClN_2O_5$	370.74	77	167-168	0.66
N_3		$C_{17}H_{11}ClN_2O_4$	342.73	78	66-67	0.55
N_4		$C_{17}H_{11}ClN_2O_4$	342.73	74	134-35	0.43
N_5		$C_{16}H_{10}ClN_3O_4$	343.72	73	108-109	0.41
N_6		$C_{18}H_{12}ClN_3O_5$	385.75	75	236-237	0.77

Recrystallisation : Dimethyl sulfoxide

Solvent system : Chloroform : Water (5.5:4.5)

Visualizing agent : Iodine vapour

2) Substituted 3-Phenyl azetidin-2-ones

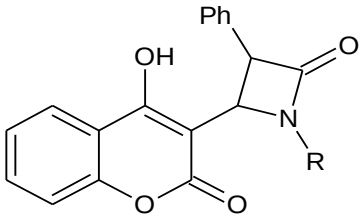
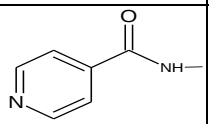
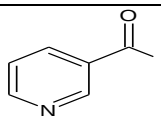
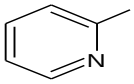
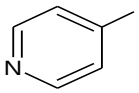
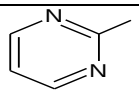
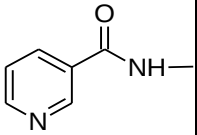


Table 8: Physical characterization of substituted 3- Phenyl azetidin-2-ones

Comp. code	R	Molecular formula	Molecular weight (g/mol)	% Yield	Melting point	Rf value
P_1		$C_{24}H_{17}N_3O_5$	427.40	75	162-164	0.32
P_2		$C_{24}H_{16}N_2O_5$	412.39	76	220-221	0.51
P_3		$C_{23}H_{16}N_2O_4$	384.38	75	85-86	0.78
P_4		$C_{23}H_{16}N_2O_4$	384.38	74	140-141	0.60
P_5		$C_{22}H_{15}N_3O_4$	385.37	74	212-213	0.39
P_6		$C_{24}H_{17}N_3O_5$	427.40	75	218-219	0.70

Recrystallisation : Dimethyl sulfoxide

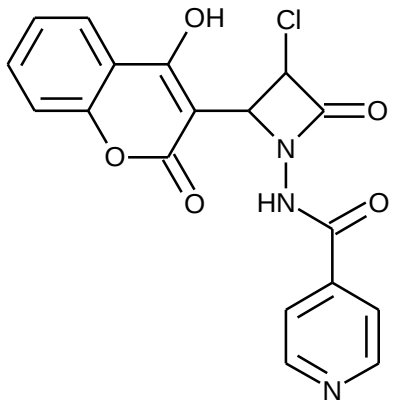
Solvent system : Chloroform : Water (3:7)

Visualizing agent : Iodine vapour

SPECTRAL ANALYSIS OF COMPOUNDS [118-120]

The structures of synthesized compounds during the present investigation were established on the basis of chemical data IR, UV, NMR and MASS spectral data. The purity of the compounds was established by single spot on TLC plates.

COMPOUND CODE : N_1



Chemical name	3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridine-4-carboxamido)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 273nm
IR (KBr, ν_{max} in cm^{-1})	3433.64 (Alcoholic O-H), 1727.91 (lactam C=O), 1671.02 (Carbonyl C=O), 1608.34 (Amide C=O), 1553.38 (Aromatic C=C), 1185.04 (C-N), 887.095 (C-Cl)
^{1}H NMR spectral data	8.297 (bs, 1H, N-H), 5.313 (d, 1H, Cl-CH-CO), 7.321-7.763(m, 8H, Aromatic H and Pyridyl H)

Mass Spectral Data

Molecular weight of the compound : 385

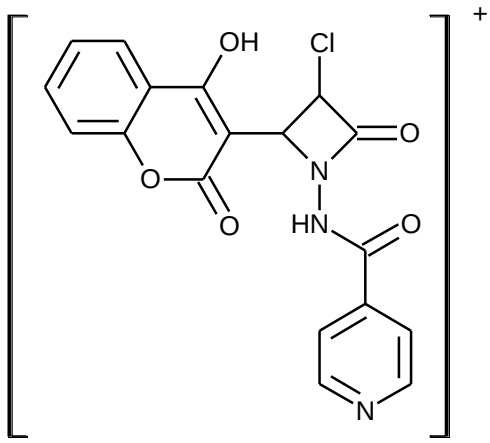
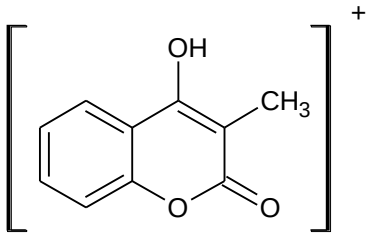
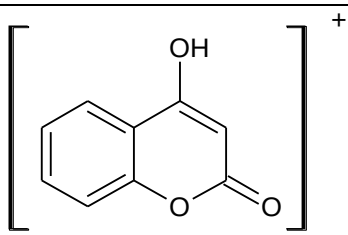
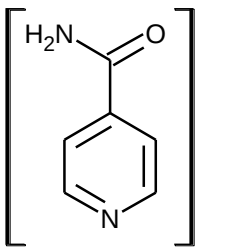
Sl. No.	Fragments	m/z values
1		385
2		176
3		162
4		121

Figure 14:UV Spectrum of N_1

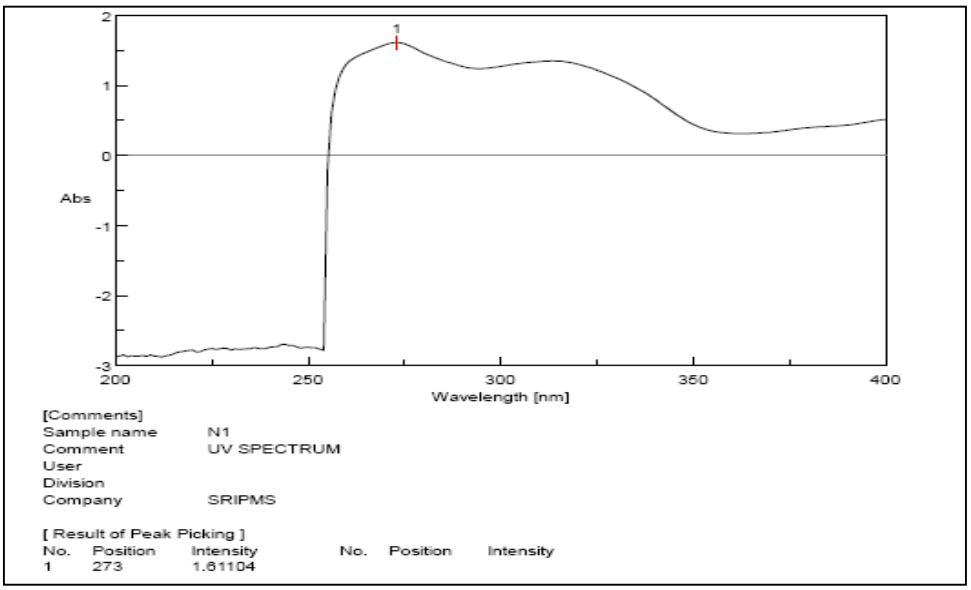


Figure 15:IR Spectrum of N_1

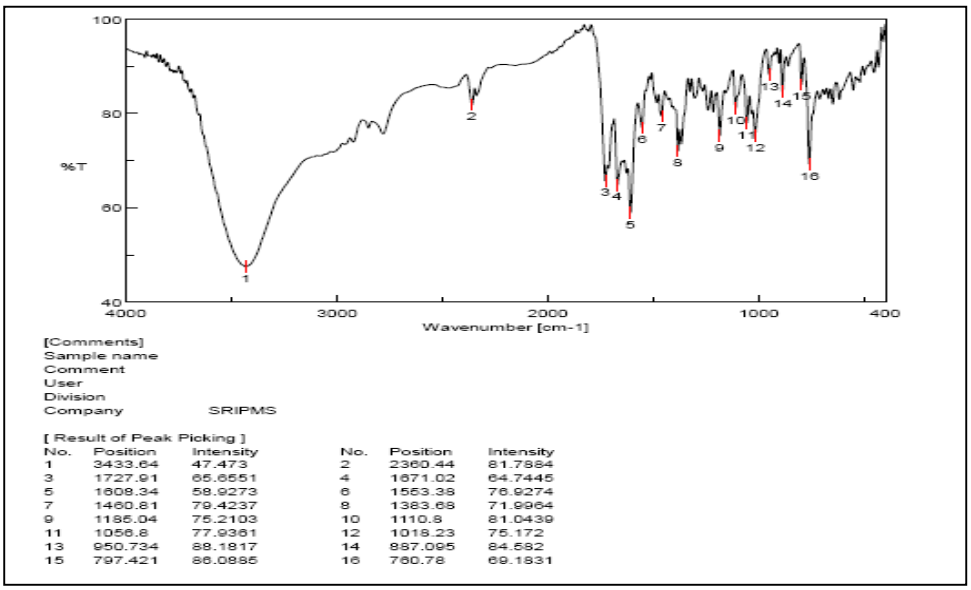


Figure 16: NMR Spectrum of N_1

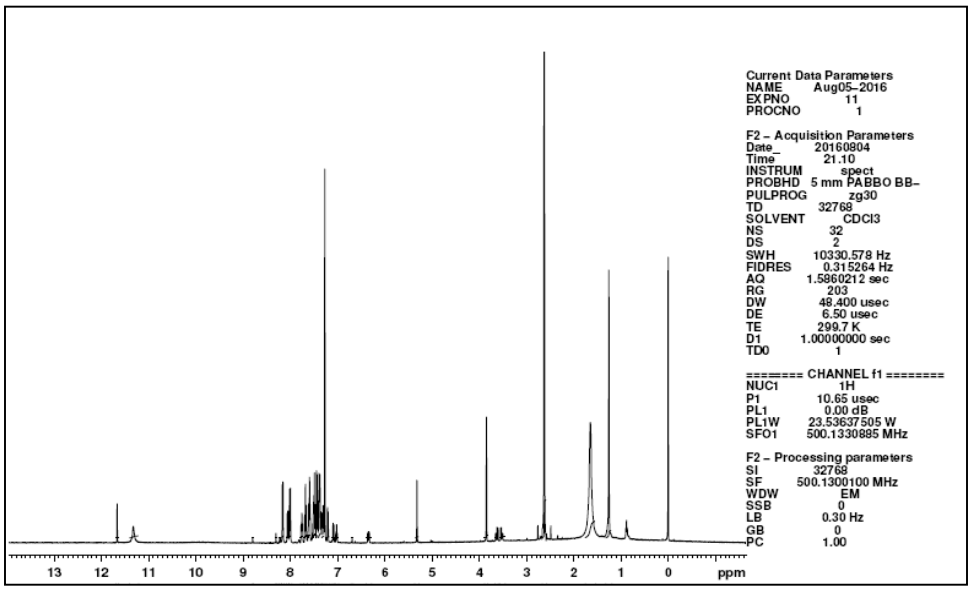
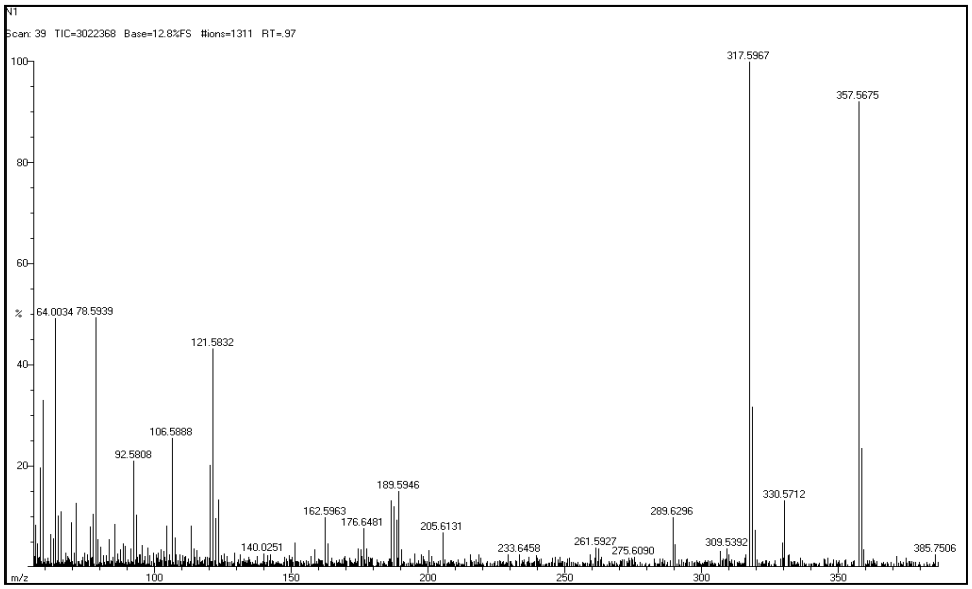
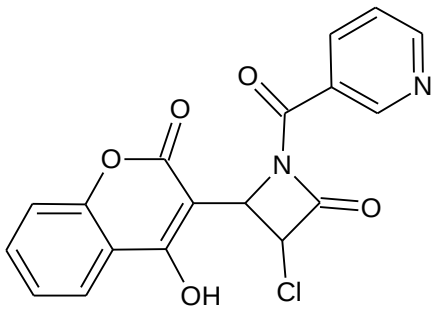


Figure 17: Mass spectrum of N_1



COMPOUND CODE : N_2



Chemical name	3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridin-3-ylcarbonyl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 274nm
IR (KBr, ν_{max} in cm^{-1})	3428.81 (Alcoholic O-H), 1714.41 (lactam C=O), 1649.8 (C=O), 1608.34 (Amide C=O), 1555.31 (Aromatic C=C), 1185.04 (C-N), 888.059 (C-Cl)
^{1}H NMR spectral data	5.319 (d, 1H, Cl-CH-CO), 4.969 (d, 1H, -N-CH-C-Cl), 7.269-8.156(m, 8H, Aromatic H and Pyridyl H)

Mass Spectral Data

Molecular weight of the compound : 370

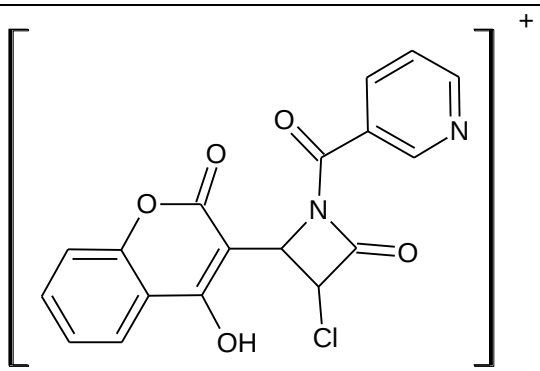
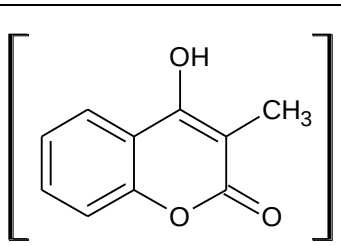
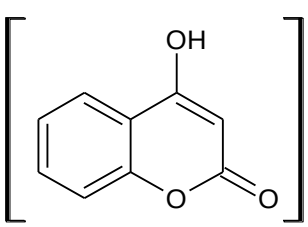
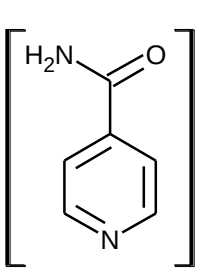
Sl. No.	Fragments	m/z values
1		370
2		176
3		162
4		121

Figure 18: UV spectrum of N_2

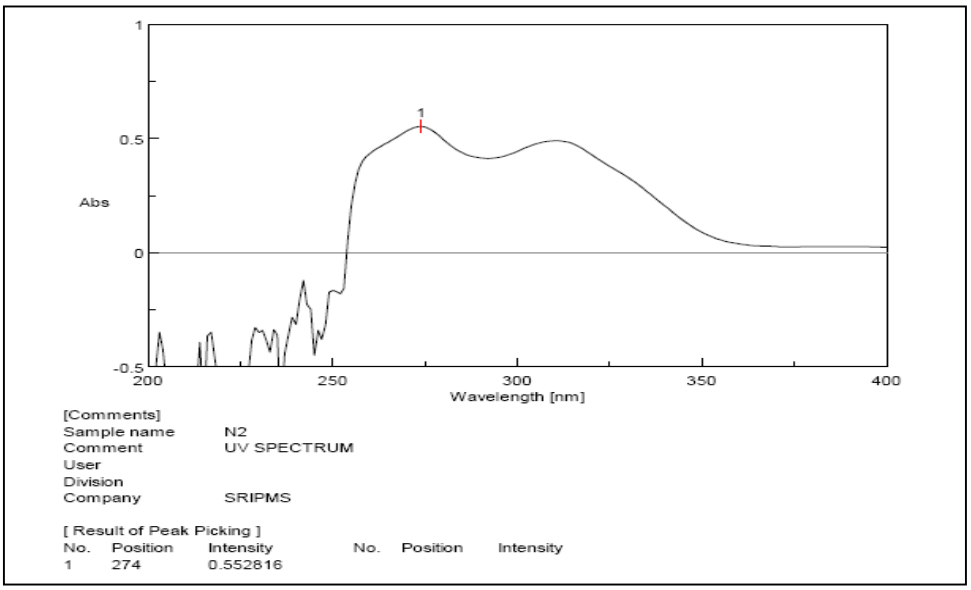


Figure 19: IR Spectrum of N_2

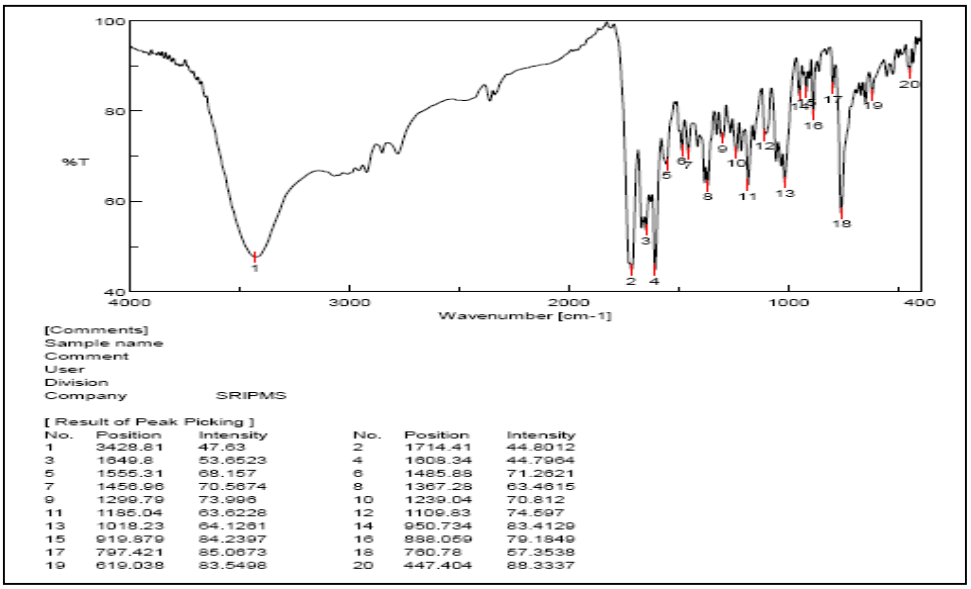


Figure 20: NMR Spectrum of N_2

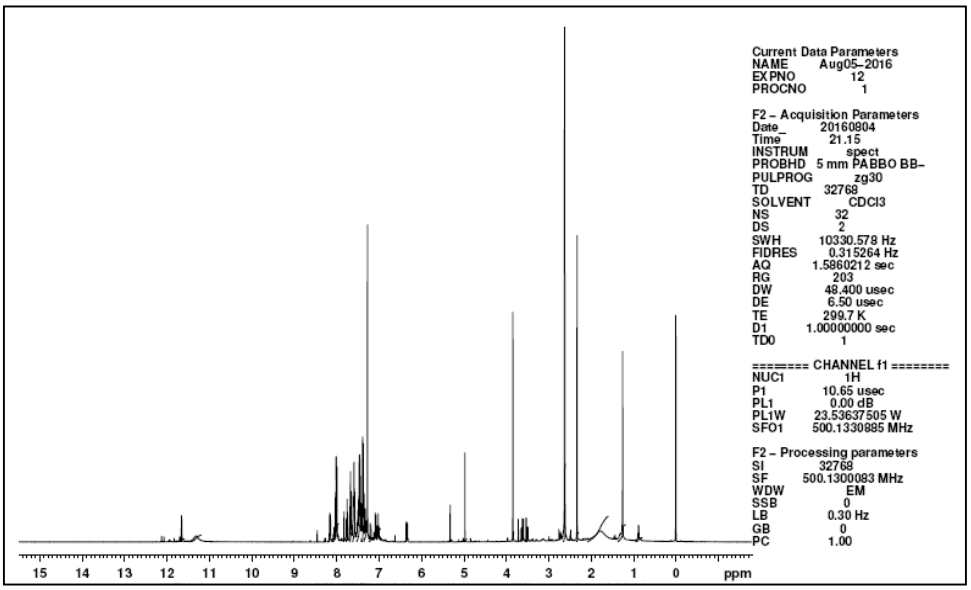
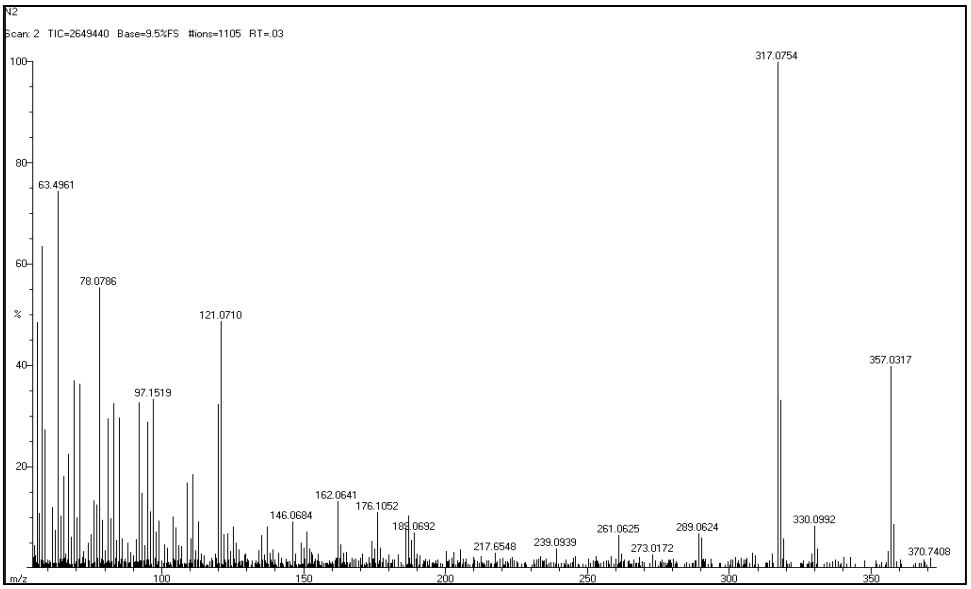
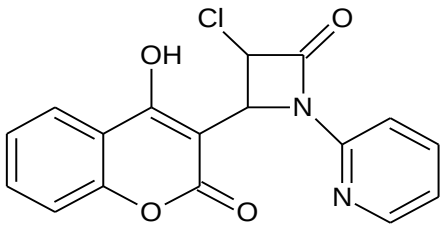


Figure 21: Mass Spectrum of N_2



COMPOUND CODE : N_3



Chemical name	3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridin-2-yl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 269nm
IR (KBr, v_{max} in cm^{-1})	3426.89 (Alcoholic O-H), 1724.05 (lactam C=O), 1663.3(C=O), 1530.24 (Aromatic C=C), 1326.79 (Aromatic C-N), 1179.26 (C-N), 888.059 (C-Cl)

Figure 22: UV Spectrum of N_3

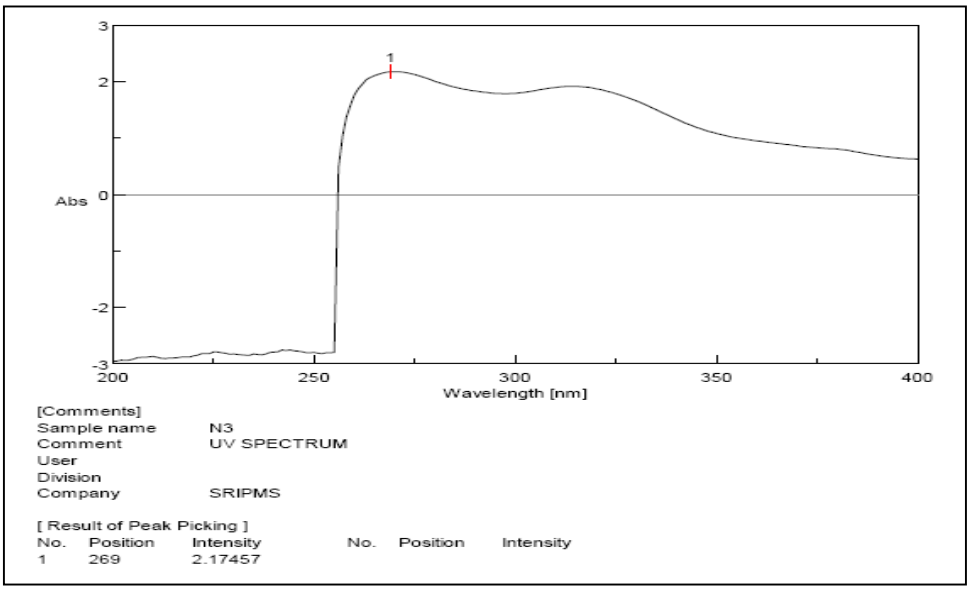
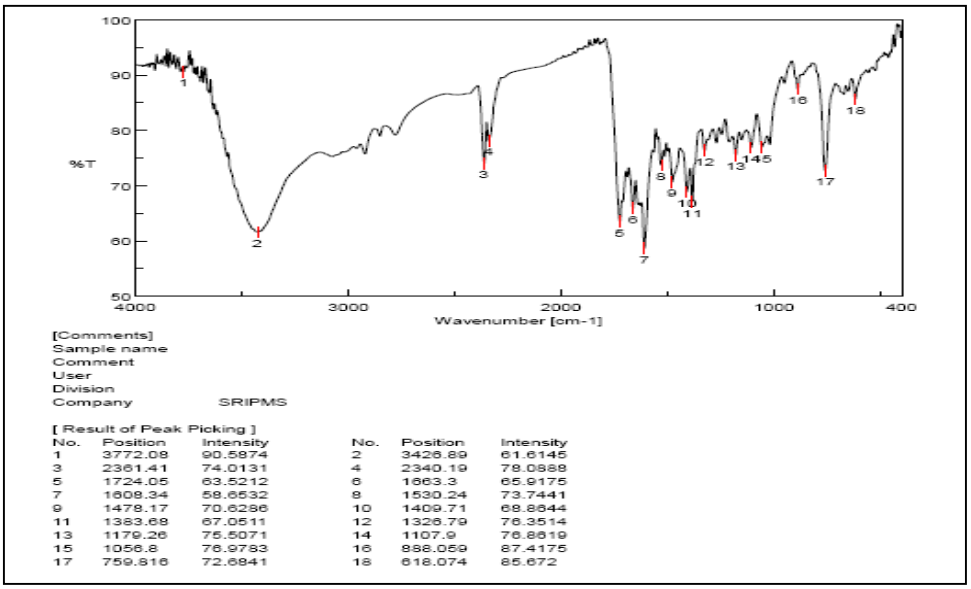
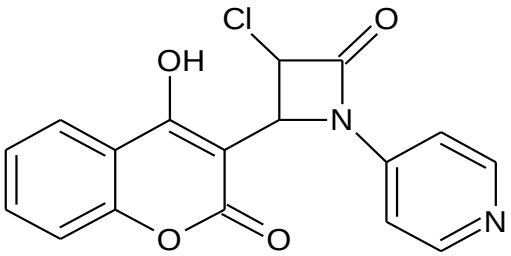


Figure 23: IR Spectrum of N_3



COMPOUND CODE : N_4



Chemical name	3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridin-4-yl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 274nm
IR (KBr, v_{max} in cm^{-1})	3432.67 (Alcoholic O-H), 1728.87 (lactam C=O), 1671.98 (C=O), 1554.34 (Aromatic C=C), 1307.5 (Aromatic C-N), 1185.04 (C-N), 888.059 (C-Cl)

Figure 24: UV Spectrum of N_4

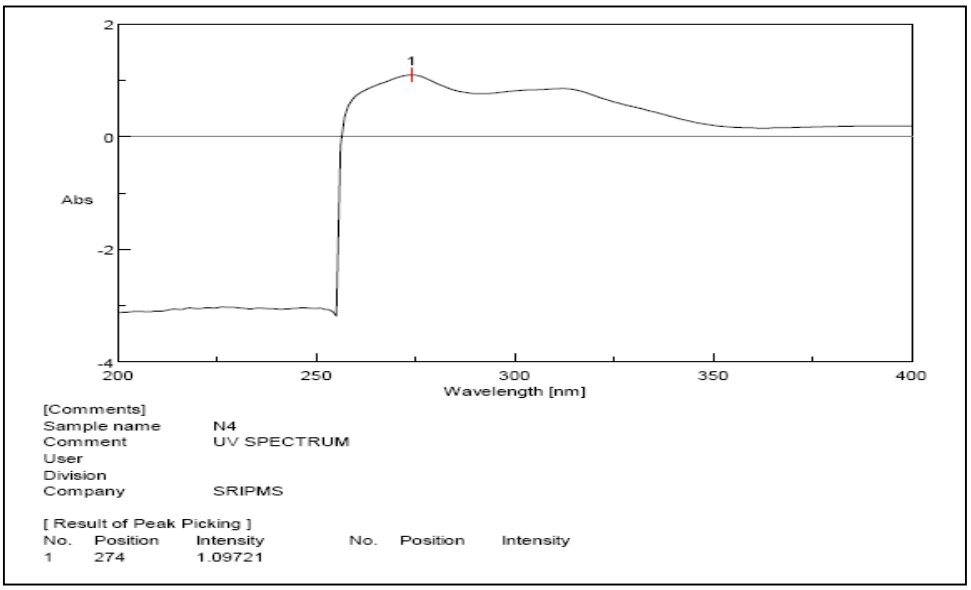
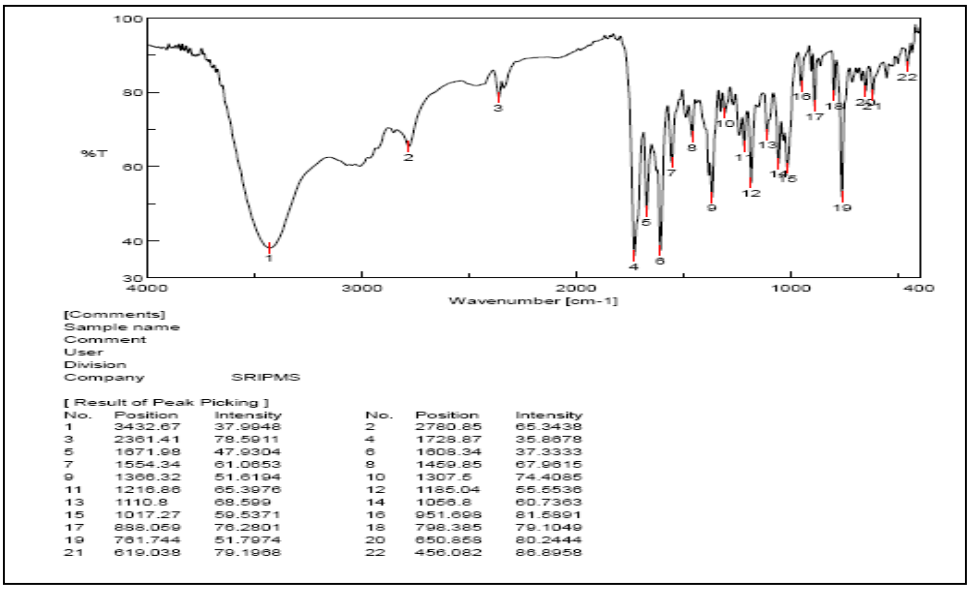
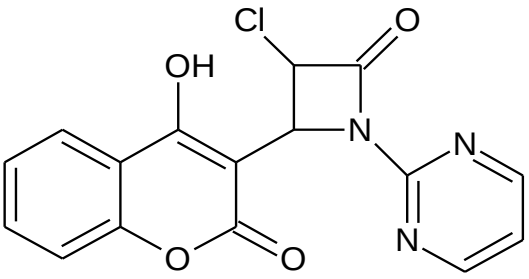


Figure 25: IR Spectrum of N_4



COMPOUND CODE : N_5



Chemical name	3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyrimidin-2-yl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 272nm
IR (KBr, v_{max} in cm^{-1})	3479.92 (Alcoholic O-H), 1730.8 (lactam C=O), 1661.37 (C=O), 1542.77 (Aromatic C=C), 1306.54 (Aromatic C-N), 1193.72 (C-N), 888.059 (C-Cl)

Figure 26: UV Spectrum of N_5

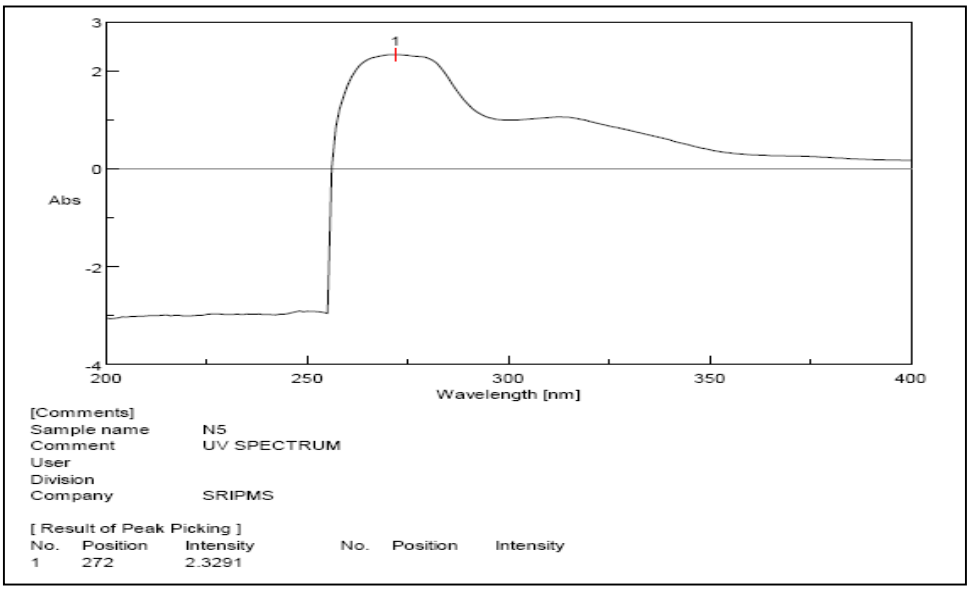
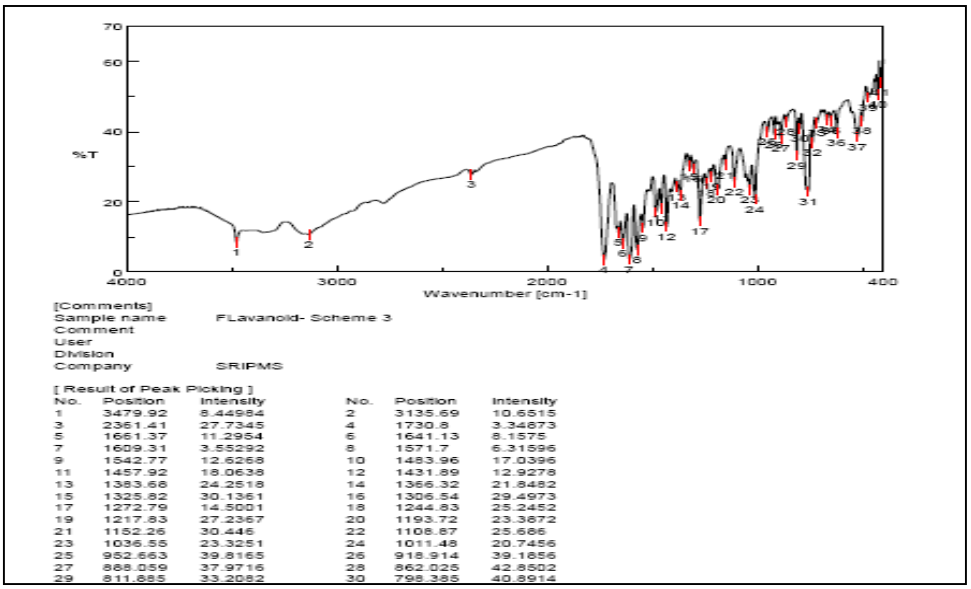
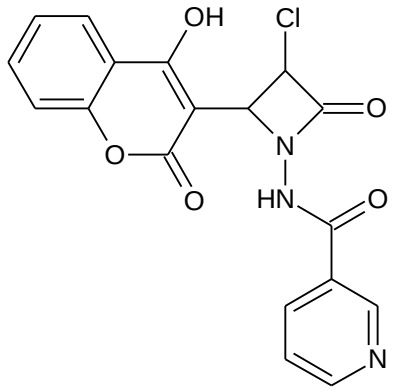


Figure 27: IR Spectrum of N_5



COMPOUND CODE : N_6



Chemical name	3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridine-3-carboxamido)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 273nm
IR (KBr, v_{max} in cm^{-1})	3425.92 (Alcoholic O-H), 1729.83 (lactam C=O), 1672.95 (C=O), 1556.27 (Aromatic C=C), 1185.04 (C-N), 889.023 (C-Cl)

Figure 28: UV Spectrum of N_6

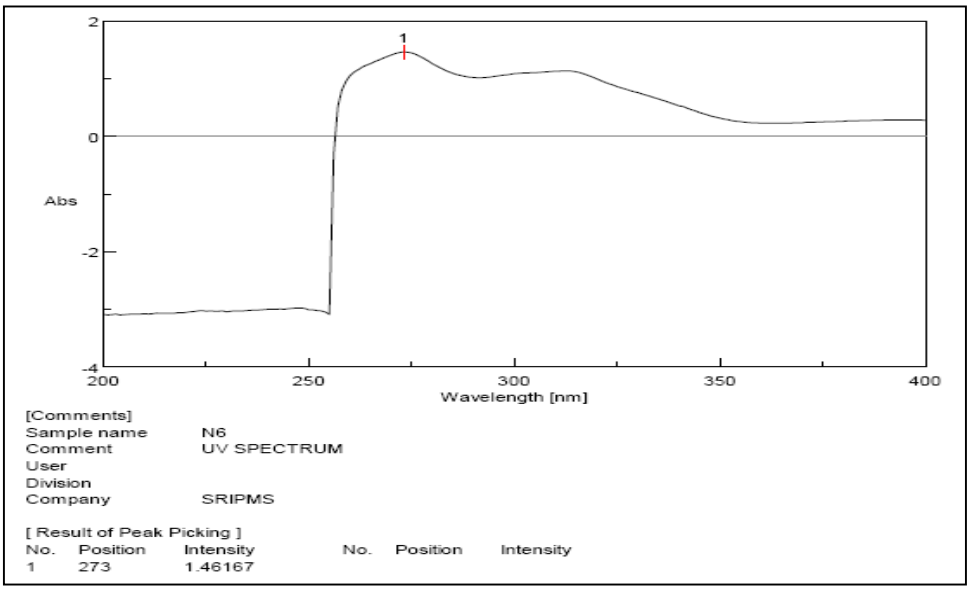
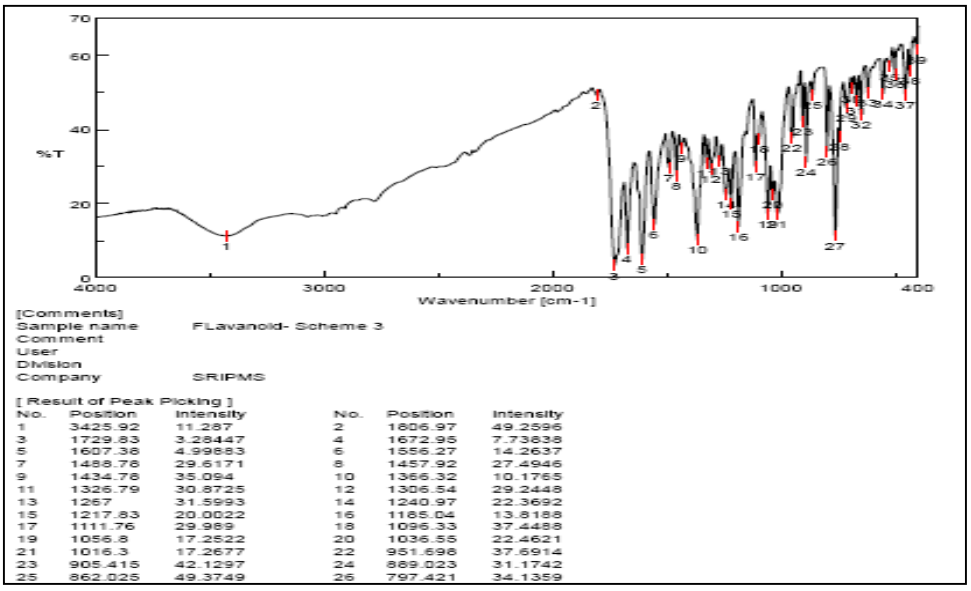
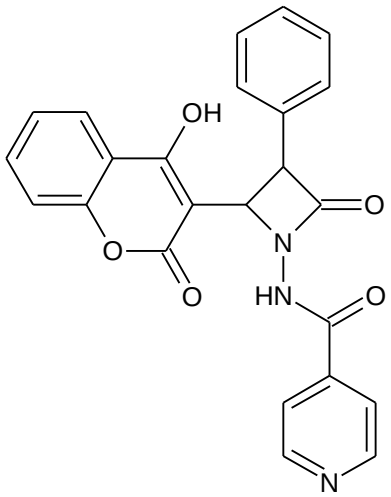


Figure 29: IR Spectrum of N_6



COMPOUND CODE : P_1



Chemical name	4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridine-4-carboxamido)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 273nm
IR (KBr, v_{max} in cm^{-1})	3448.1 (Alcoholic O-H), 1721.16 (lactam C=O), 1634.38 (C=O), 1607.38 (Amide C=O), 1555.31 (Aromatic C=C), 1185.04 (C-N),
^{1}H NMR spectral data	5.325 (d, 1H, Cl-CH-CO), 7.266 (bs, 1H, N-H), 7.419-8.161(m, 13H, Aromatic H and Pyridyl H)

Mass Spectral Data

Molecular weight of the compound : 427

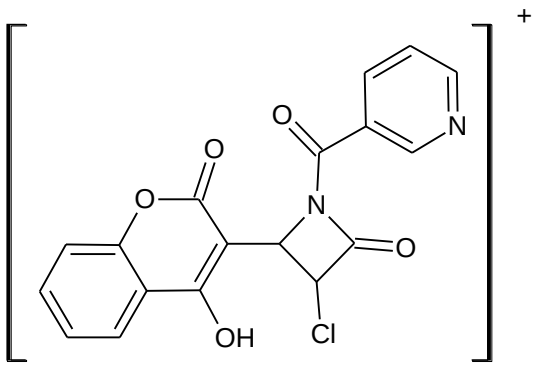
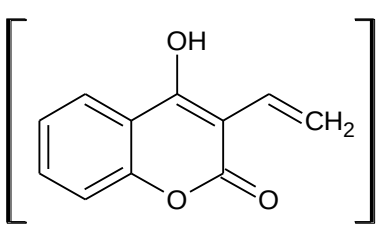
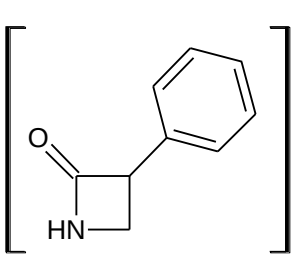
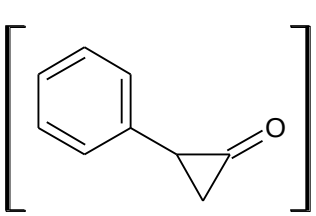
Sl. No.	Fragments	m/z values
1		427
2		187
3		147
4		131

Figure 30: UV Spectrum of P_1

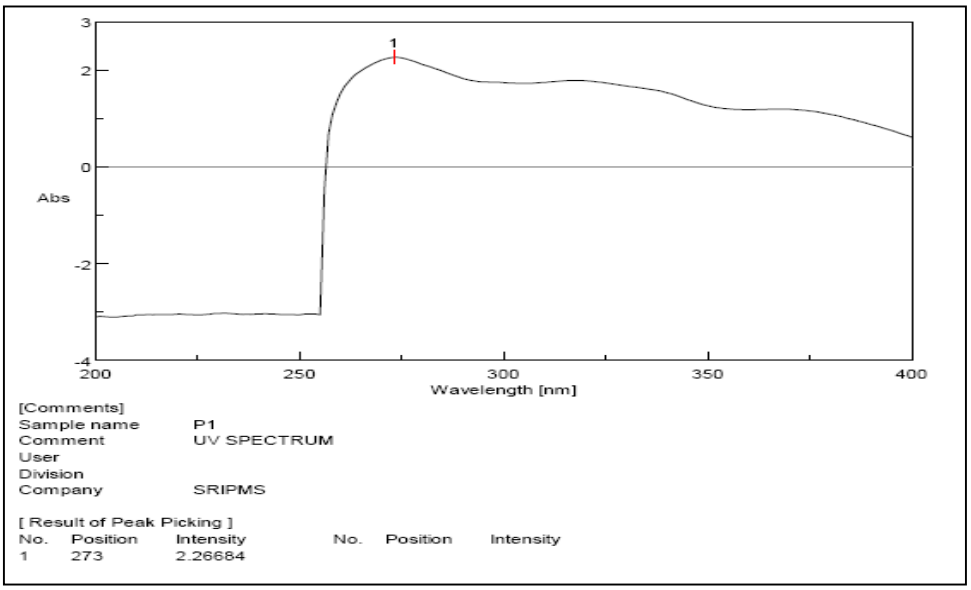


Figure 31: IR Spectrum of P_1

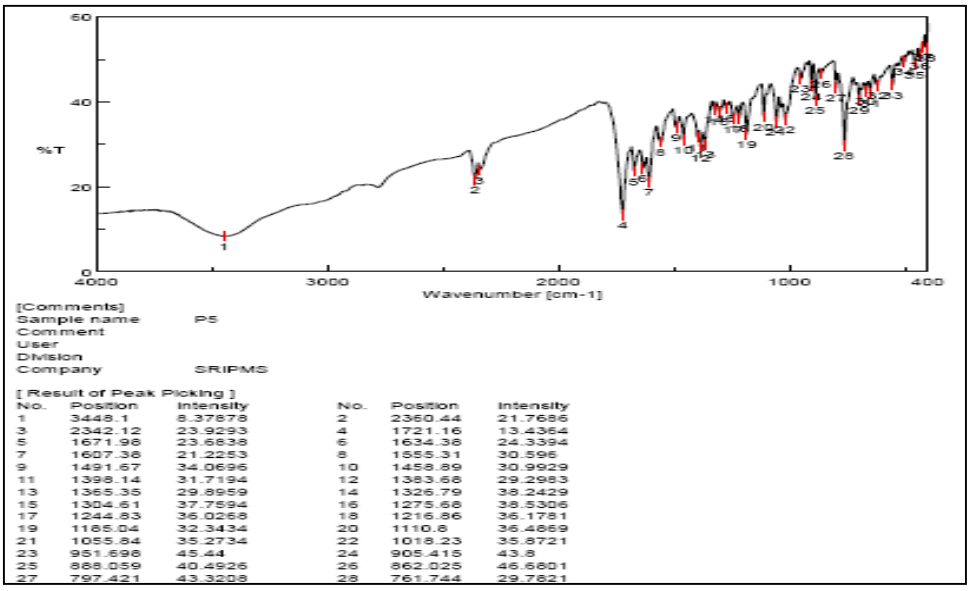


Figure 32: NMR Spectrum of P_1

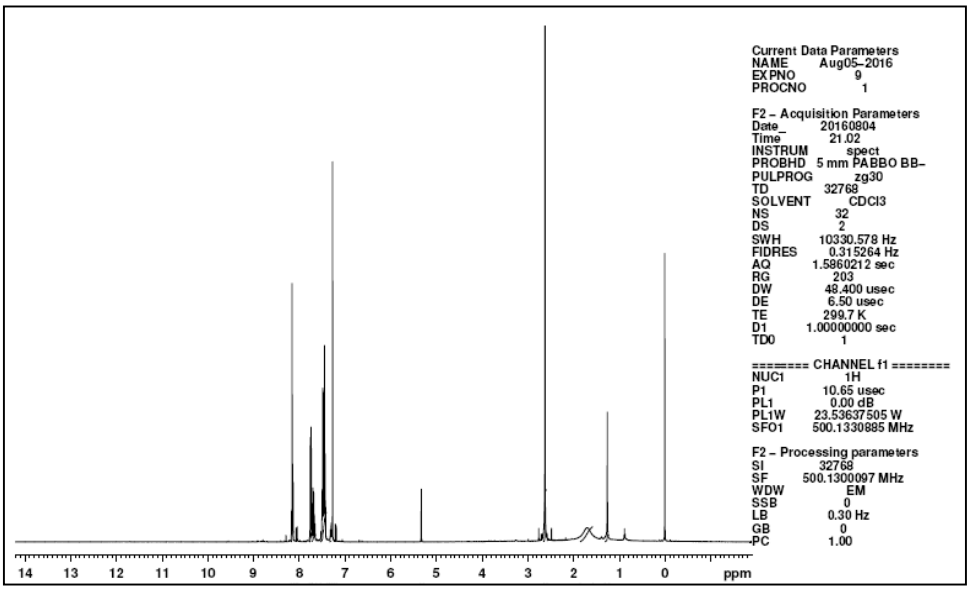
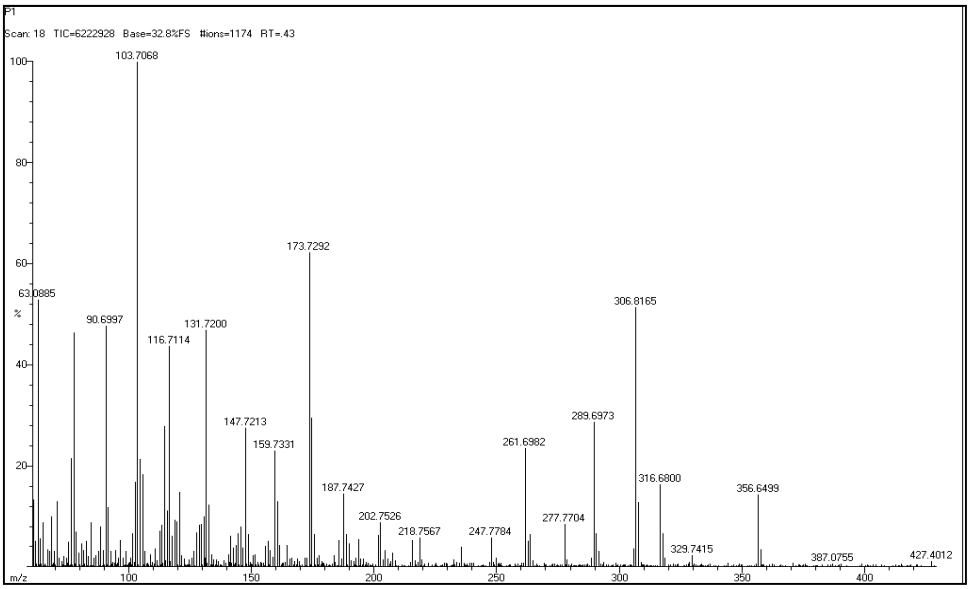
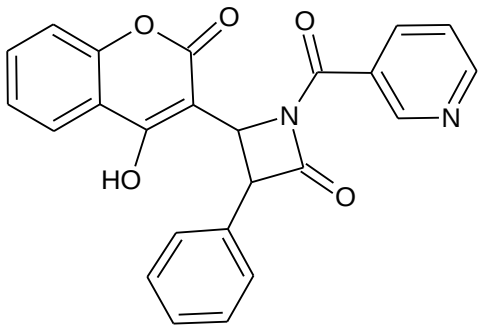


Figure 33: Mass Spectrum of P_1



COMPOUND CODE : P_2



Chemical name	4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridin-3-ylcarbonyl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 273nm
IR (KBr, ν_{max} in cm^{-1})	3414.35 (Alcoholic O-H), 1729.83 (lactam C=O), 1644.02 (C=O), 1607.38 (Amide C=O), 1555.31 (Aromatic C=C), 1186.97 (C-N)

Mass Spectral Data

Molecular weight of the compound : 412

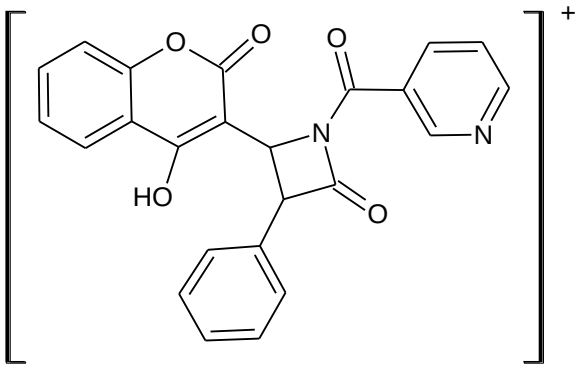
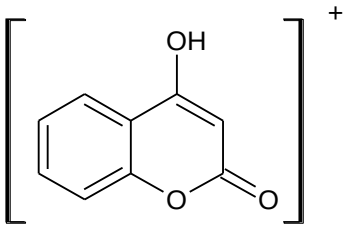
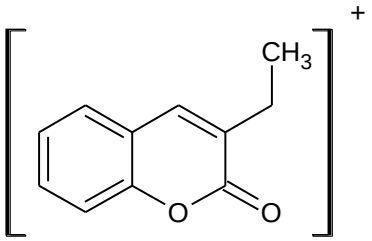
Sl. No.	Fragments	m/z values
1		412
2		162
3		174

Figure 34: UV Spectrum of P_2

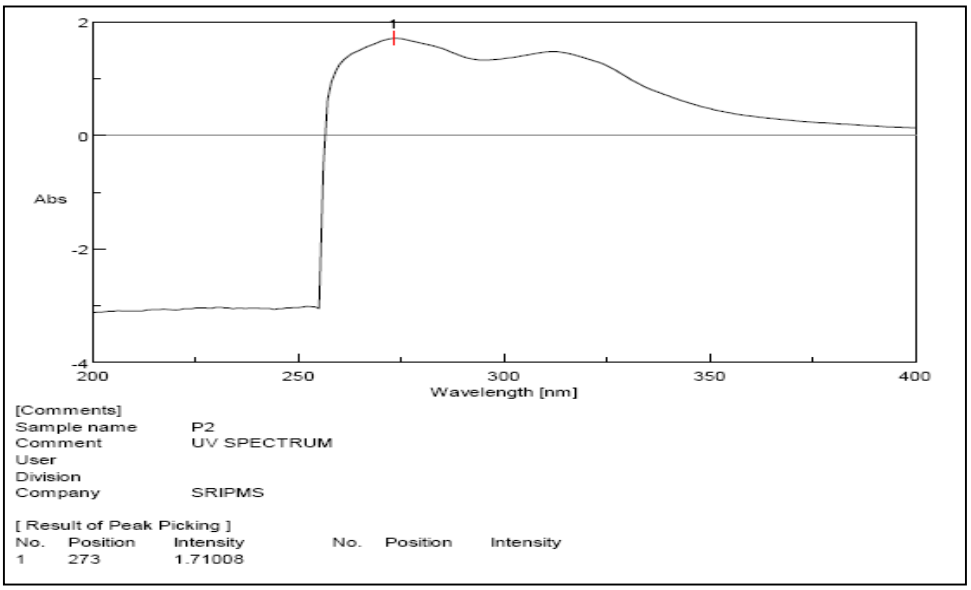


Figure 35: IR Spectrum of P_2

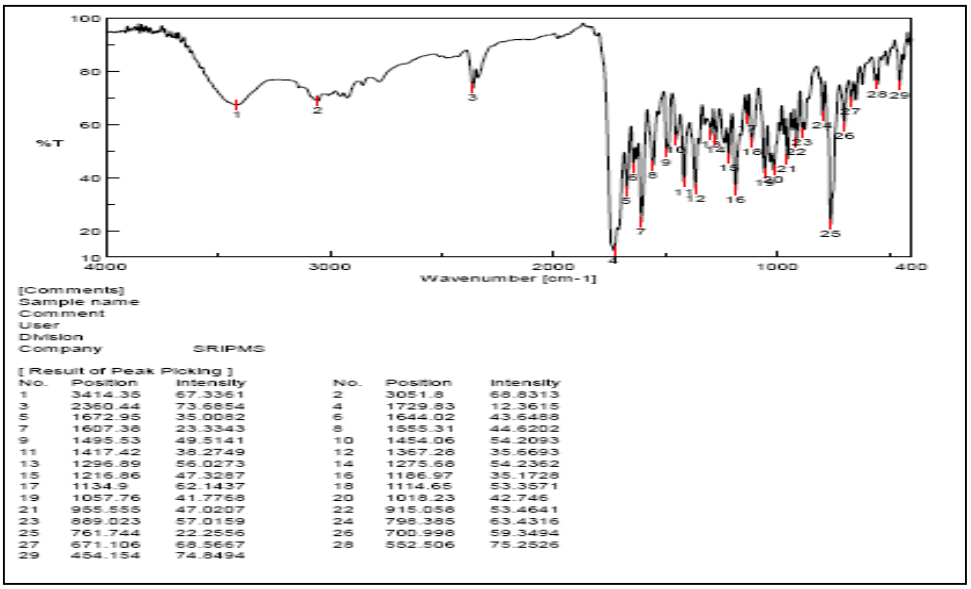
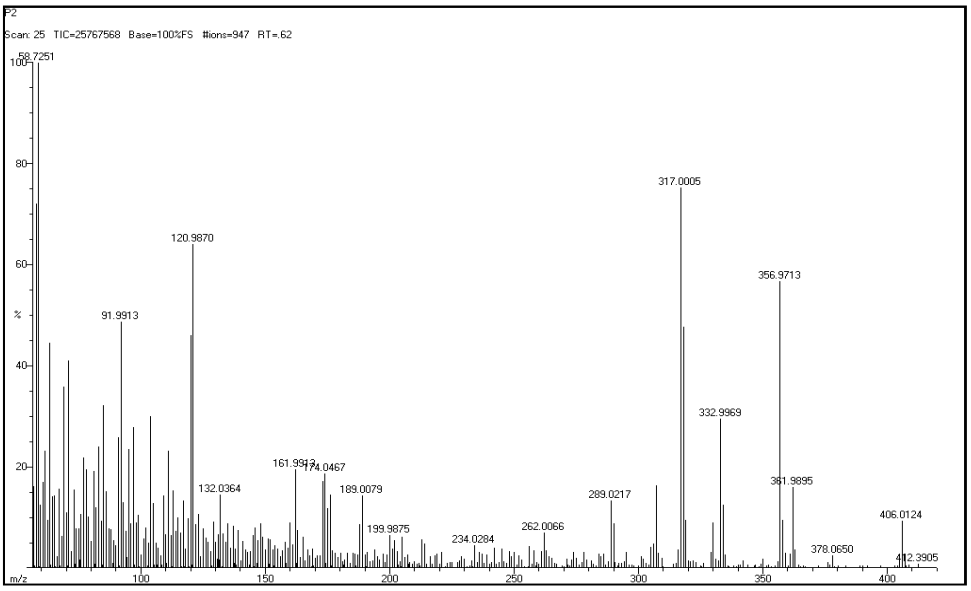
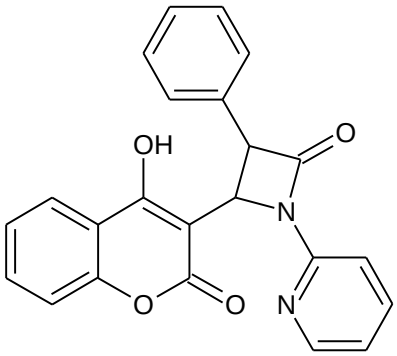


Figure 36: Mass Spectrum of P_2



COMPOUND CODE : P_3



Chemical name	4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridin-2-yl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 261nm
IR (KBr, ν_{max} in cm^{-1})	3428.81 (Alcoholic O-H), 1726.94 (lactam C=O), 1637.27 (C=O), 1567.84 (Aromatic C=C), 1182.15 (C-N)

Figure 37: UV Spectrum of P_3

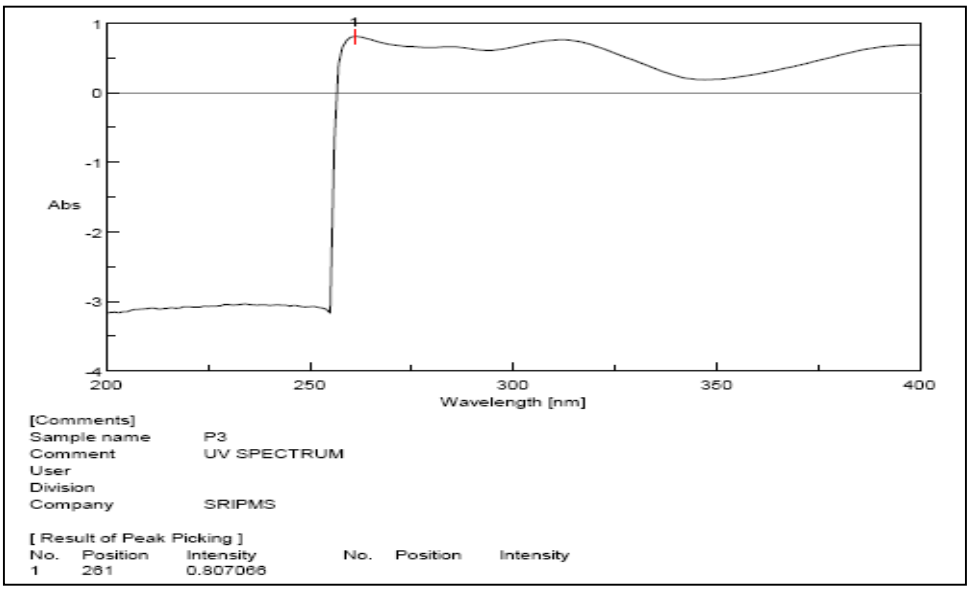
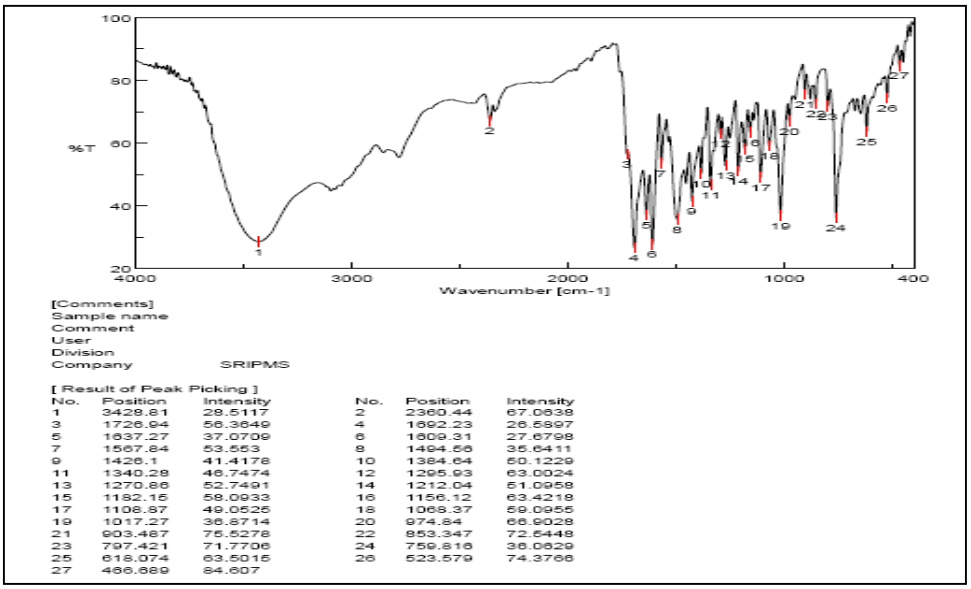
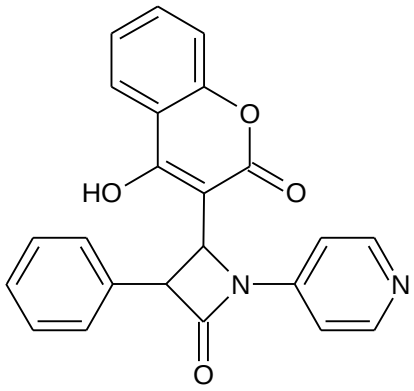


Figure 38: IR Spectrum of P_3



COMPOUND CODE : P_4



Chemical name	4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridin-4-yl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 273nm
IR (KBr, v_{max} in cm^{-1})	3439.42 (Alcoholic O-H), 1730.8 (lactam C=O), 1671.02 (C=O), 1556.27 (Aromatic C=C), 1186.01 (C-N)

Figure 39: UV Spectrum of P_4

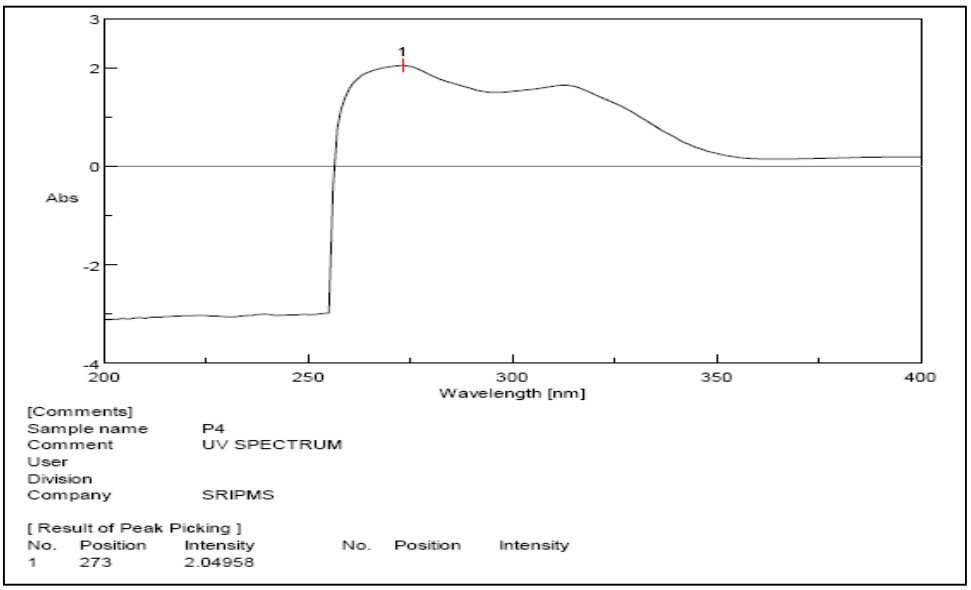
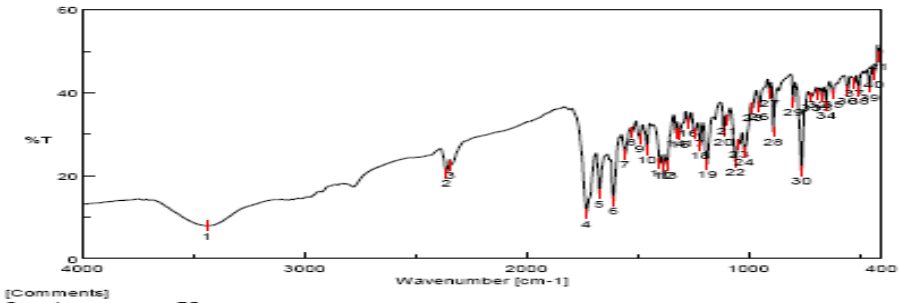


Figure 40: IR Spectrum of P_4

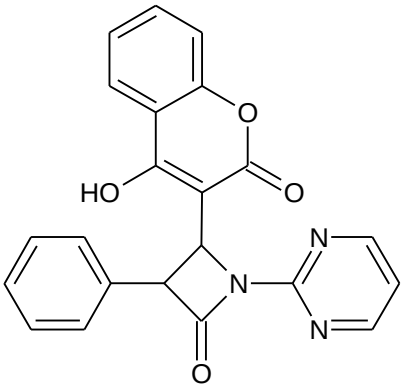


[Comments]
Sample name P5
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3439.42	7.90695	2	2360.44	20.6597
3	2341.16	22.5041	4	1730.8	10.829
5	1671.02	15.5372	6	1608.34	13.93
7	1556.27	25.0692	8	1523.49	30.5241
9	1489.74	28.8951	10	1457.92	26.1571
11	1400.07	22.9536	12	1384.64	22.4272
13	1366.32	22.4334	14	1327.75	30.278
15	1309.43	30.0511	16	1270.86	32.7237
17	1244.83	30.3396	18	1216.86	27.2475
19	1186.01	22.759	20	1111.76	30.6536
21	1095.37	33.3096	22	1058.73	23.2444
23	1041.37	27.5766	24	1018.23	25.6069
25	980.625	36.3547	26	951.696	36.6037
27	905.415	39.8593	28	868.059	30.5906
29	797.421	37.8092	30	760.78	21.2251

COMPOUND CODE : P_5



Chemical name	4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyrimidin-2-yl)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 274nm
IR (KBr, ν_{max} in cm^{-1})	3450.99 (Alcoholic O-H), 1729.83 (lactam C=O), 1629.55 (C=O), 1556.27 (Aromatic C=C), 1186.01 (C-N)

Figure 41: UV Spectrum of P_5

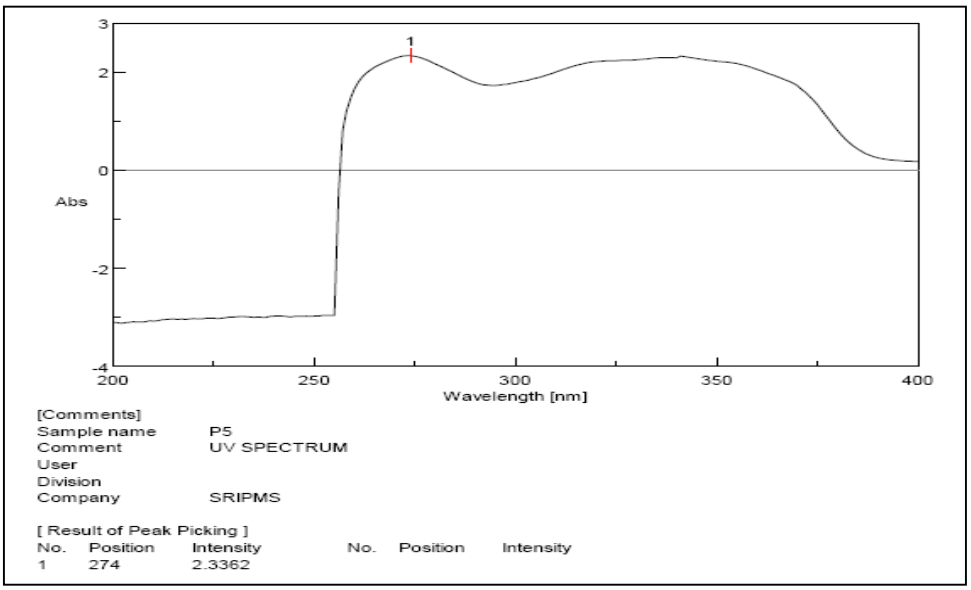
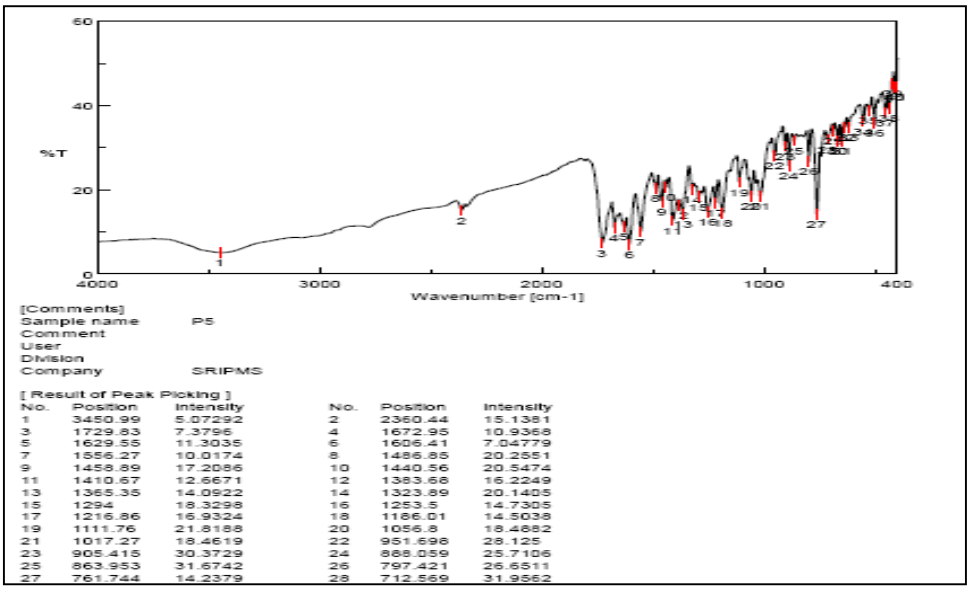
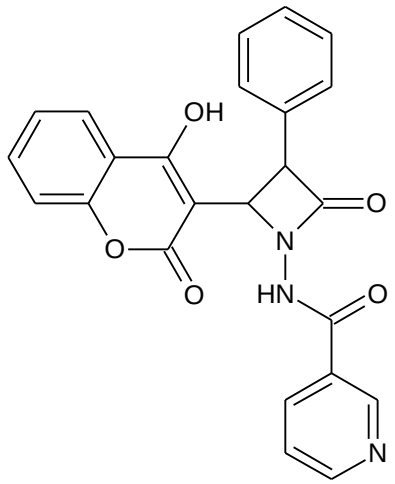


Figure 42: IR Spectrum of P_5



COMPOUND CODE : P_6



Chemical name	4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridine-3-carboxamido)azetidin-2-one
UV Spectrum	Solvent used : DMSO λ_{max} : 268nm
IR (KBr, ν_{max} in cm^{-1})	3372.89 (Alcoholic O-H), 1729.83 (lactam C=O), 1638.23 (C=O), 1610.27 (Amide C=O), 1565.92 (Aromatic C=C), 1185.04 (C-N)

Figure 43: UV Spectrum of P_6

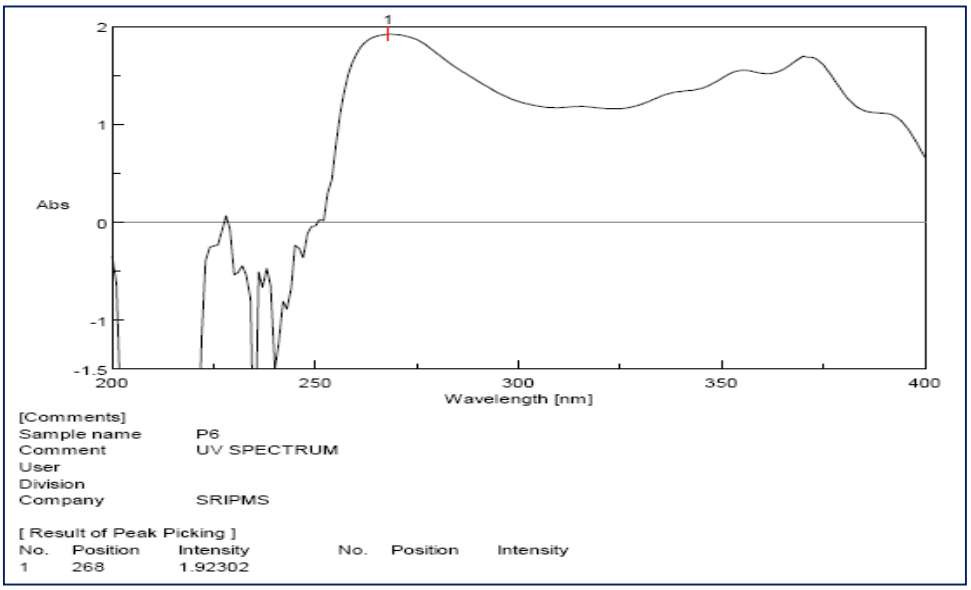
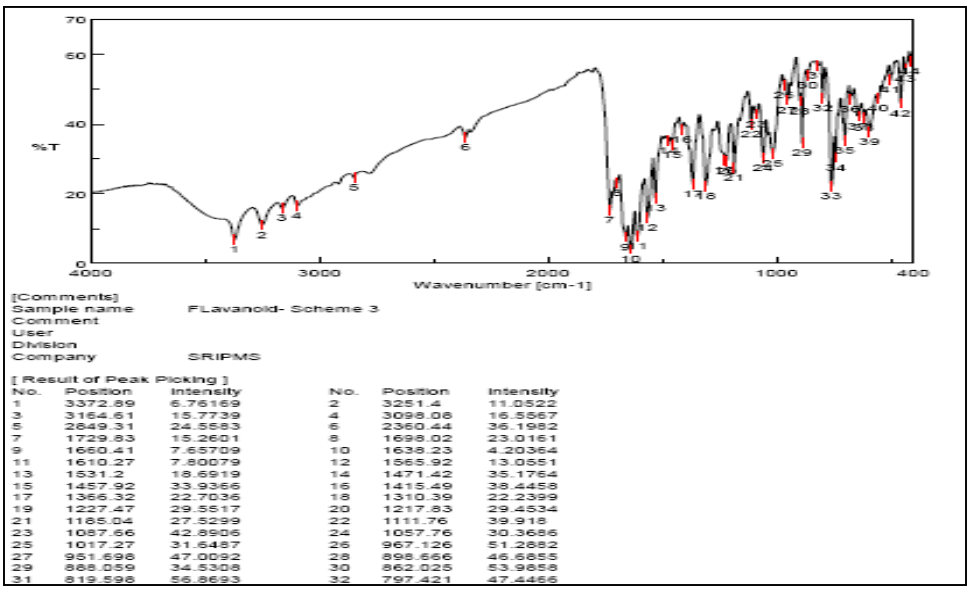


Figure 44: IR Spectrum of P_6



ANTIMICROBIAL STUDIES

ANTIBACTERIAL SCREENING [121]

Apparatus and chemicals required

Sterile swab	:	Hi Media
Non-absorbent cotton	:	Rama Raju Surgical cotton Ltd.
Conical flask	:	Borosil
Test tubes	:	Borosil
Petri dishes	:	SD Fine-Chem Ltd.
Micropipettes	:	VARI pipettes (Hi-Tab Lab)
Autoclave	:	Universal Autoclave
Laminar Air Flow unit	:	CLEAN AIR Instruments Inc.
Microtips	:	Tarsons

The antibacterial screening was carried out in the Pharmaceutical Biotechnology laboratory, College of Pharmacy, SRIPMS, Coimbatore.

Media used

Mueller Hinton agar which is gelled with 2% agar (bacteriological grade) is used as the medium for the antibacterial screening.

The Mueller Hinton contain the following ingredients:-

Casein enzymic hydrosylate	:	17.5 g/l
Beef infusion	:	300 g/l
Soluble starch	:	1.5 g/l
Agar	:	17.00 g/l
Final pH at 25°C	:	7.4(±0.2)

Media preparation and sterilization

The ingredients were dissolved in distilled water with the aid of heat and the pH was adjusted to 7.4(±0.2) by using dilute acid or alkali.

30-35 ml of Muller Hinton agar was transferred to Petri plates and sealed. The media is autoclaved at a pressure of 15 psi (121°C) for not less than 15 minutes.

Microorganisms used

- *Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2063, *Escherichia coli* NCIM 2918 and *Pseudomonas aeruginosa* NCIM 2036 were procured from National Chemical Laboratory, Pune and stored in the Pharmaceutical Biotechnology laboratory, College of pharmacy, SRIPMS, Coimbatore.
- The strains were confirmed for their purity and identity by Gram's staining method and their characteristic biochemical reactions.
- The selected strains were preserved by sub culturing them periodically on nutrient agar slants and storing them under frozen conditions.
- For antimicrobial study, fresh 24 hr broth cultures were used after the standardization of the culture.

Drugs used	:	N_{1-6}, P_{1-6}(500µg/ml)
Standard drugs	:	Ciprofloxacin (5µg/ml)
Solvent	:	Dimethyl sulfoxide

WORKING CONDITIONS IN LABORATORY

The entire work was done by using horizontal laminar flow hood so as to provide aseptic conditions. Before commencement of the work, air sampling was carried out using a sterile nutrient agar plate and exposing it to the environment inside the hood. After incubation, it was checked for the growth of microorganism and absence of growth confirmed aseptic working condition.

INOCULAM STANDARDIZATION

- All organisms were grown overnight (24 hours) at 37°C on nutrient agar and harvested during the stationary phase.
- Active cultures for experiments were prepared by transforming a loopful of cells from the stock culture to the test tubes containing Mueller Hinton broth, incubated for 24 hours at 37°C.
- Inoculum was standardized by matching the turbidity of the culture to 0.5 McFarland standards. The standard was produced by mixing 0.05 ml of 1% $BaCl_2$ with 9.95 ml of 1% H_2SO_4 [122].
- If the turbidity of the culture matches that of the McFarland standard, the culture inoculating suspension has approximately 1.5×10^8CFU/ml of bacteria.

ANTI- BACTERIAL SCREENING BY KIRBY-BAUER METHOD [123]

Mueller Hinton agar plates were prepared aseptically to get a thickness of 5-6mm. The plates were allowed to solidify and inverted to prevent condensate falling on the agar surface. The plates were dried at 37°C before inoculation. The organisms were inoculated as per the following method in the plates prepared earlier:

- The sterile swab was dipped in the previously standardized inoculum and excess of inoculums was removed by pressing and rotating the swab firmly against the sides of the culture tube above the level of liquid.
- The swab was streaked all over the surface of the medium three times, rotating the plates through an angle of 60°C after each application.
- Finally the swab was pressed round the edges of the agar surface.
- The inoculation medium was allowed to dry at room temperature, with the lid closed.

The drug was poured in the wells, which are made with the help of a borer. And the measured quantity of the drug is poured with the help of the micro-pipette. Nearly

50µl of the solution is poured into the wells. The plates were kept in the refrigerator for 1 hour to facilitate the diffusion of the drugs. Plates were prepared in triplicate and they were then incubated for 18-24 hours at 37°C. After the incubation, the diameter of the zone of inhibition around the drugs were measured and compared with that of the standard. All the synthesized compounds were tested for antibacterial activity against Gram positive and Gram negative bacteria. Saturated solutions of the compounds were first studied and the compounds with zones of inhibition greater than 15mm were taken for quantitative studies.

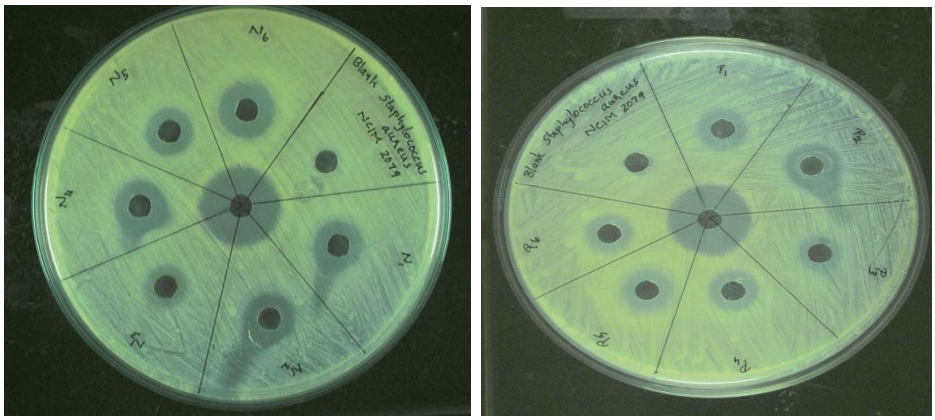
RESULTS AND DISCUSSION

The zone of inhibition of eighteen synthesized compounds is shown in the Fig. 46 and 47 and their diameters were compared with that of the standard ciprofloxacin in Table 7 and 8.

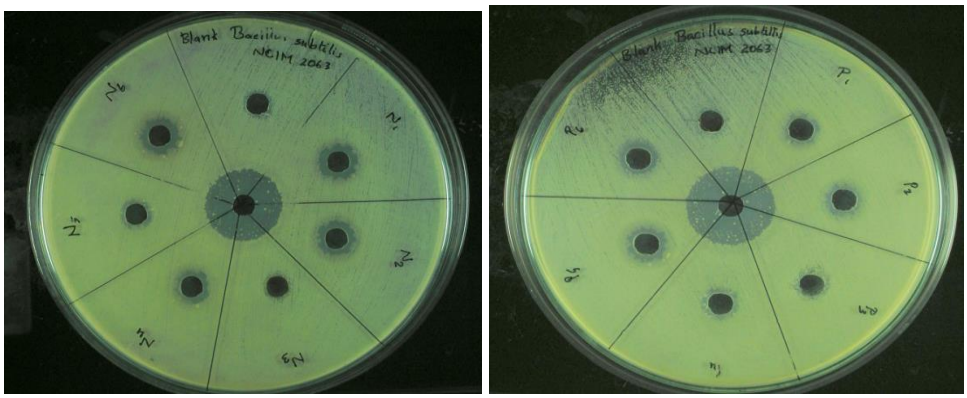
Table 7: Antibacterial Screening of test compounds against Gram positive bacteria

Sl.No.	Compound code	Diameter of Zone of Inhibition (mm)		Report	
		***Staphylococcus aureus* NCIM 2079**	***Bacillus subtilis* NCIM 2063**	***Staphylococcus aureus* NCIM 2079**	***Bacillus subtilis* NCIM 2063**
1	N_1	13	11	Moderately Sensitive	Resistant
2	N_2	11	11	Resistant	Resistant
3	N_3	11	10	Resistant	Resistant
4	N_4	16	12	Moderately Sensitive	Moderately Sensitive
5	N_5	14	11	Moderately Sensitive	Resistant
6	N_6	11	13	Resistant	Moderately Sensitive
7	P_1	13	11	Moderately Sensitive	Resistant
8	P_2	14	11	Moderately Sensitive	Resistant
9	P_3	11	11	Resistant	Resistant
10	P_4	14	11	Moderately Sensitive	Resistant
11	P_5	14	12	Moderately Sensitive	Moderately Sensitive
12	P_6	14	12	Moderately Sensitive	Moderately Sensitive
13	Std. Ciprofloxacin	26	27	Sensitive	Sensitive

Figure 45: Zone of Inhibition of N_{1-6} and P_{1-6} against Gram positive bacteria



Zone of inhibition against *Staphylococcus aureus* NCIM 2079



Zone of inhibition against *Bacillus subtilis* NCIM 2063

Table 8: Antibacterial Screening of test compounds against Gram negative bacteria

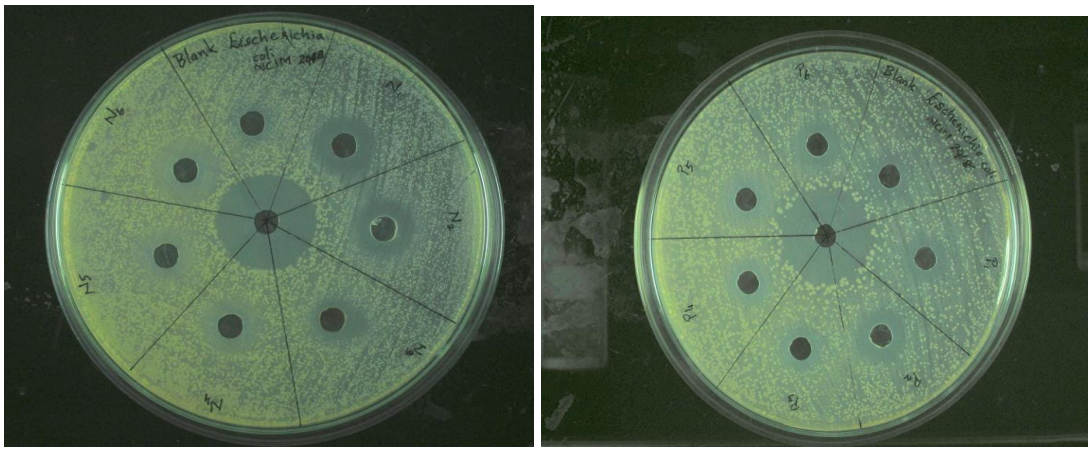
Sl. No.	Compound code	Diameter of Zone of Inhibition (mm)		Report	
		Escherichia coli NCIM 2911	*Pseudomonas aeruginosa* NCIM 2036	*Escherichia coli* NCIM 2911	*Pseudomonas aeruginosa* NCIM 2036
1	N_1	15	12	Moderately Sensitive	Moderately Sensitive
2	N_2	11	11	Resistant	Resistant
3	N_3	13	10	Moderately Sensitive	Resistant
4	N_4	15	14	Moderately Sensitive	Moderately Sensitive
5	N_5	13	13	Moderately Sensitive	Moderately Sensitive
6	N_6	11	10	Resistant	Resistant
7	P_1	14	14	Moderately Sensitive	Moderately Sensitive
8	P_2	16	17	Moderately Sensitive	Moderately Sensitive
9	P_3	11	12	Resistant	Moderately Sensitive
10	P_4	14	14	Moderately Sensitive	Moderately Sensitive
11	P_5	14	17	Moderately Sensitive	Moderately Sensitive
12	P_6	15	14	Moderately Sensitive	Moderately Sensitive
13	Std. Ciprofloxacin	32	29	Sensitive	Sensitive

Sensitive: Diameter of zone of inhibition ≥ 18mm

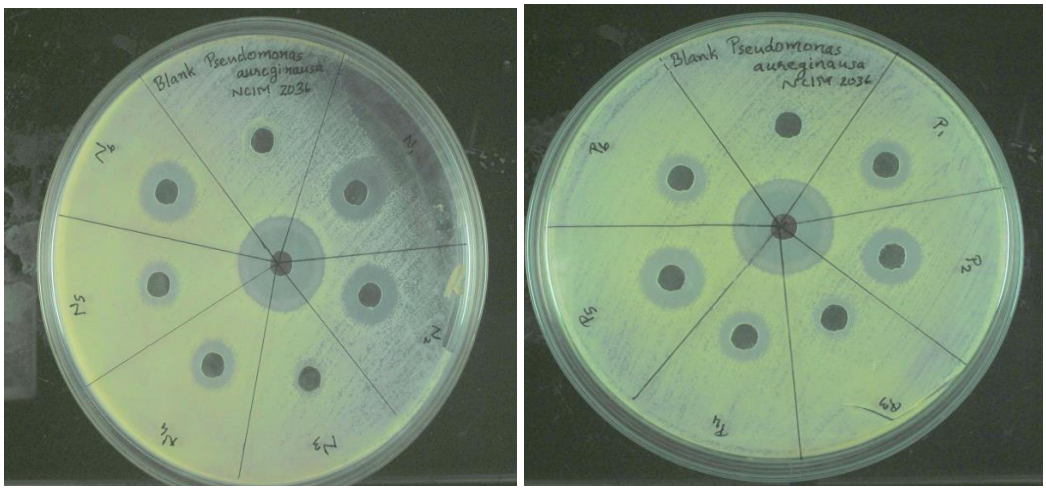
Moderately sensitive: Diameter of zone of inhibition 12 to 18mm

Resistant: Diameter of zone of inhibition < 12

Figure 46: Zone of Inhibition of N_{1-6} and P_{1-6} against Gram negative bacteria



Zone of inhibition against *Escherichia coli* NCIM 2911



Zone of inhibition against Pseudomonas aeruginosa NCIM 2036

ANTIMYCOBACTERIAL STUDIES [124]

Conventional agar diffusion technique for susceptibility tests, which rely on the size of zone of inhibition surrounding a drug containing disc are not suitable for the slowly growing Mycobacterium species because the drug diffuses throughout the medium before the organism has the chance to grow. So, the following principles are recognized for the antimycobacterial screening.

- The composition of medium should have a minimal effect on drug inactivation. So, Middle Brook 7H9 broth base is used.
- Drug containing medium should be stored in a refrigerator shielded from light and kept in plastic tubes tightly closed in order to protect them from evaporation.
- Homogenization of inoculum is essential to eliminate large clumps of cells.

SUSCEPTIBILITY TESTING

This is done by two methods:

- Direct method
- Indirect method

Direct method

This was done if acid-fast bacilli are seen on the smear of the concentrated clinical specimen. Dilutions are made and inoculated.

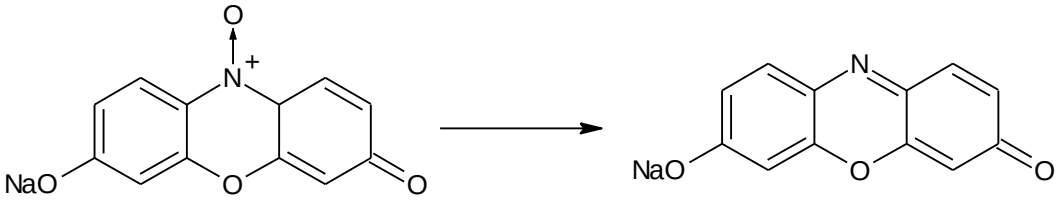
Indirect method

In this method, bacterial mass is suspended in Middle Brook 7H9 broth containing these or four small sterile glass beads. Mixture is placed on a vortex mixer and precautions are taken to prevent aerosol production. Tube is allowed to stand for 15 min. The stock suspensions are diluted and 0.1 ml is inoculated into control and drug containing media.

The antimycobacterial screening was carried out in Maratha Mandal NGH Institute of Dental Sciences and Research Center, Belgaum.

ANTITUBERCULAR SCREENING BY MICROPLATE ALAMAR BLUE ASSAY (MABA)

Alamar Blue is a cell viability assay reagent which contains the cell permeable, non-toxic and weakly fluorescent blue indicator dye called resazurin. It is an oxidation-reduction indicator used for the screening of cell growth, particularly in various cell toxicity studies. The dye changes its colour from blue to pink and becomes fluorescent, when reduced to resorufin by oxidoreductases within viable cells.



Resazurin (oxidized) Resorufin (reduced)

Alamar Blue is used for the screening of antitubercular activity. Since Mycobacterium is an aerobic organism, its presence of growth turns Alamar Blue to pink colour. Hence, pink colour indicates the presence of growth (no antitubercular activity) and blue colour indicates the absence of growth (inhibitory activity of agents tested).

Procedure [125]

1) The antimycobacterial activity of compounds were assessed against *M. tuberculosis* using Microplate Alamar Blue Assay (MABA).

2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

3) To the outer perimeter of sterile 96 wells plates, 200µl of sterile deionized water was added to minimize evaporation of medium in the test wells during incubation.

4) The 96 wells plates received 100 µl of the Middlebrook 7H9 broth (inoculated with *Mycobacterium tuberculosis* of H37RV Strain) and serial dilution of compounds were made directly on plate.

5) The final drug concentrations tested were 100 to 0.2 µg/ml.

6) Plates were covered and sealed with parafilm and incubated at 37ºC for five days.

7) After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

9) The MIC was defined as the lowest drug concentration which prevented the color change from blue to pink.

RESULTS AND DISCUSSION

The synthesized compounds were evaluated for *in-vitro* antimycobacterial activity by Alamar Blue assay method and the results are shown in table 9.

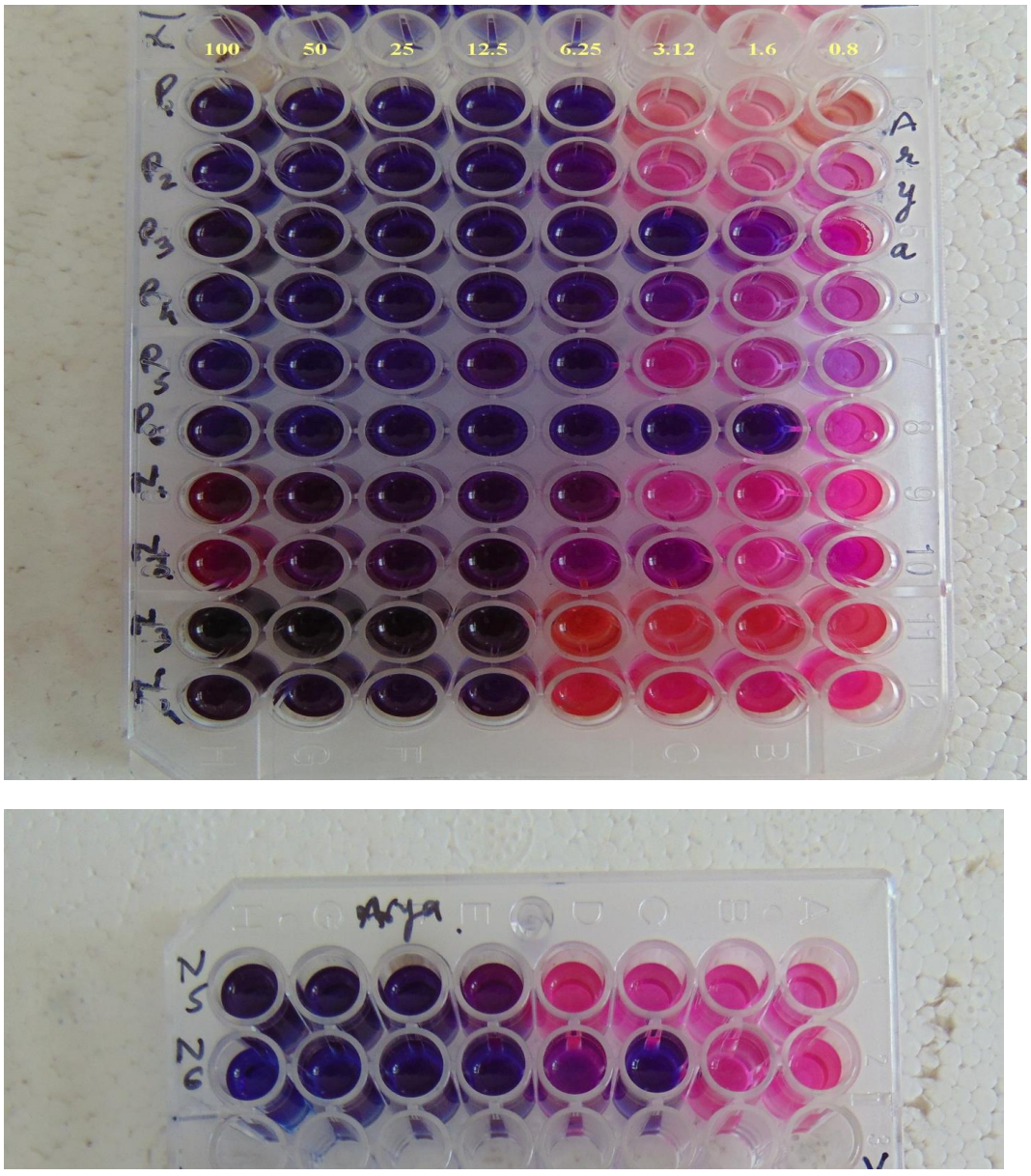
Table 9: Antimycobacterial activity of N_{1-6} and P_{1-6}

Sl. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	N_1	S	S	S	S	S	R	R	R
2	N_2	S	S	S	S	S	S	R	R
3	N_3	S	S	S	S	R	R	R	R
4	N_4	S	S	S	S	S	R	R	R
5	N_5	S	S	S	S	R	R	R	R
6	N_6	S	S	S	S	S	S	R	R
7	P_1	S	S	S	S	S	R	R	R
8	P_2	S	S	S	S	S	R	R	R
9	P_3	S	S	S	S	S	S	S	R
10	P_4	S	S	S	S	S	S	R	R
11	P_5	S	S	S	S	S	R	R	R
12	P_6	S	S	S	S	S	S	S	R
13	Pyrazinamide (standard)	S	S	S	S	S	S	R	R
14	Streptomycin (standard)	S	S	S	S	S	R	R	R
15	Ciprofloxacin (standard)	S	S	S	S	S	S	R	R

S- Sensitive

R- Resistant

Figure 47: Antimycobacterial screening of N_{1-6} and P_{1-6} using Alamar Blue Assay method



SUMMARY AND CONCLUSION

SUMMARY

The present work was focused on the designing of novel azetidin-2-one derivatives with coumarin moiety having antitubercular activity. For this, following approach has been adopted.

PHASE I: DRUG DESIGN APPROACH

It involves the following stages:

Stage 1: Identification of target

Cytochrome P450 lanosterol 14α-demethylase in *Mycobacterium tuberculosis* (MT-CYP51) was selected as the target enzyme as its inhibition will prevent synthesis of the membrane lipids. This compromises membrane integrity and induces Mycobacterial cell lysis.

Stage 2: Lead Identification

The lead coumarin was selected based on several literature reviews. 150 compounds including a series of azetidinone, imidazolidinone, thiazolidinone and oxazolidinone derivatives of coumarin were subjected to molecular docking studies. All the compounds were found to have binding energy lesser than that of the standard fluconazole. A series of twelve azetidinone (az31-az42) derivatives were selected from these compounds on the basis of their binding energy. **az37 (P_1)** and **az38 (P_2)** was found to have the least binding energy of **-11.28** and **-11.24,** respectively.

Stage 3: Lead optimization

Safety and efficacy of the lead molecules were evaluated by observing the *in silico* ADME studies and computation of drug like properties. Twelve ligands (**N_{1-6}** and **P_{1-6}**) were subjected to *in silico* lead optimization. Oral bioavailability was evaluated was evaluated by using Molinspiration server and ADMET data were obtained from Accelry's Accord for Excel. All the compounds possessed good bioavailability and permeability.

PHASE II: SYNTHESIS AND PHYSICAL CHARACTERIZATION

A) Synthesis of the designed compounds

In this work, twelve new compounds were synthesized. The first step involved the synthesis of 4-hydroxy-3-formyl coumarin by Vilsmeier-Haack Reaction. Schiff bases were prepared from these aldehydes using various primary amino compounds. Finally, the Schiff bases were cyclized to form azetidin-2-ones.

B) Physical characterization

Melting point and Rf values of all the synthesized compounds were found out.

PHASE III: SPECTRAL STUDIES

The structures of the synthesized compounds were established on the basis of UV, IR, ^{1}H NMR and MASS spectral data.

PHASE IV: EVALUATION OF BIOLOGICAL ACTIVITIES

A) Antimycobacterial activity

Antitubercular screening of the synthesized compounds were done by Alamar Blue assay method in Middlebrook 7H9 broth against *Mycobacterium tuberculosis* H37RV strain (ATCC No. 27294). All newly synthesized compounds **N_{1-6}** and **P_{1-6}** showed antimycobacterial activity by inhibiting the growth at concentration of 12.5µg/ml. In the chlorine substituted series of

azetidinones, **N_2** (nicotinamide) and **N_6** (nicotinic hydrazide) showed minimum inhibition at a concentration of 3.12µg/ml. **N_1** (isoniazid) and **N_4** (4-amino pyridine) showed minimum inhibition at 6.25µg/ml. In the series of phenyl substituted azetidinones, **P_3** (2-amino pyridine) and **P_6** (nicotinic hydrazide) had shown promising activity with MIC of 1.6µg/ml. **P_4** showed MIC at 3.12µg/ml.

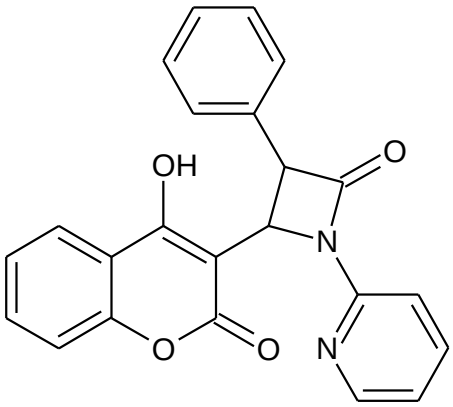
B) Antibacterial activity

The synthesized compounds were screened for antibacterial activity against both gram positive (*Staphylococcus aureus* NCIM 2079 and *Bacillus subtilis* NCIM 2063) and gram negative (*Escherichia coli* NCIM 2911 and *Pseudomonas aeruginosa* NCIM 2036) organisms by Kirby-Bauer method. All the compounds were found to be moderately to weakly active against bacteria. Comparatively, **N_4** and **N_6** were found to have better activity against Gram positive bacteria. **N_1**, **N_2**, **N_6**, **P_2**, **P_5** and **P_6** were found to have better activity against Gram negative bacteria.

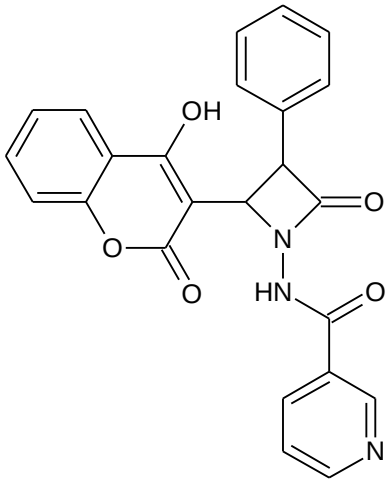
CONCLUSION

- The present study establishes that computational tools help in minimizing the tedious process of drug discovery and delivers new drug candidate more quickly.
- Cytochrome P450 lanosterol 14α-demethylase in *Mycobacterium tuberculosis* (MT-CYP51) was selected as the target and coumarin as the lead, which was optimized based on drug likeness score.
- The proposed twelve compounds were synthesized and their structures were established based on spectral data. The compounds were evaluated for antibacterial and antimycobacterial activity.
- The synthesized compounds showed poor to moderate antibacterial activity.
- Five synthesized derivatives (N_2, N_6, P_3, P_4 and P_6) showed equipotent antitubercular activity compared to the standards Pyrazinamide and Ciprofloxacin at concentration of 3.12µg/ml. Two synthesized derivatives (P_3 and P_6) exhibited higher level of antitubercular activity even at a low concentration of 1.6µg/ml.
- Among the synthesized compounds, 3-phenyl substituted azetidin-2-ones showed promising antimycobacterial activity.
- Among the synthesized compounds, P_3 and P_6 can be taken as the lead molecule and acute toxicity studies are to be done on these promising compounds.

Structure of Lead Molecules identified



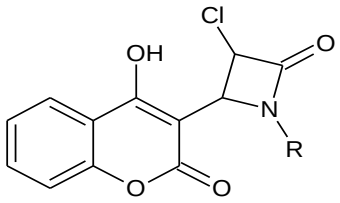
P_3

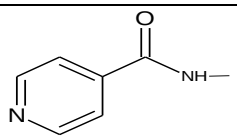
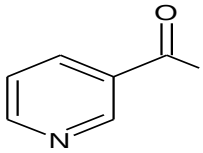
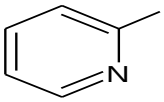
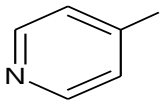
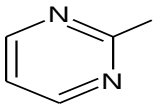
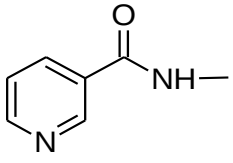


P_6

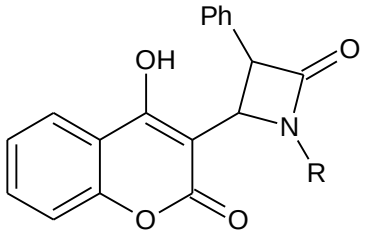
LIST OF NEWLY SYNTHESIZED COMPOUNDS

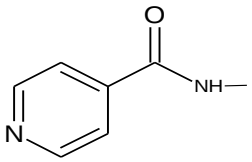
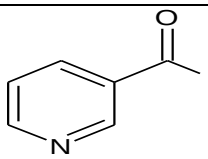
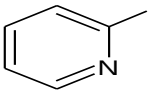
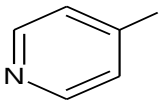
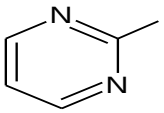
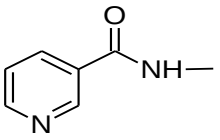
CHLORINE SUBSTITUTED AZETIDINONE DERIVATIVES



Compound code	R	Compound name
N_1		3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridine-4-carboxamido)azetidin-2-one
N_2		3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridin-3-ylcarbonyl)azetidin-2-one
N_3		3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridin-2-yl)azetidin-2-one
N_4		3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridin-4-yl)azetidin-2-one
N_5		3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyrimidin-2-yl)azetidin-2-one
N_6		3-chloro-4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-1-(pyridine-3-carboxamido)azetidin-2-one

PHENYL SUBSTITUTED AZETIDINONE DERIVATIVES



Compound code	R	Compound name
P_1		4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridine-4-carboxamido)azetidin-2-one
P_2		4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridin-3-ylcarbonyl)azetidin-2-one
P_3		4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridin-2-yl)azetidin-2-one
P_4		4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridin-4-yl)azetidin-2-one
P_5		4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyrimidin-2-yl)azetidin-2-one
P_6		4-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(pyridine-3-carboxamido)azetidin-2-one

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