DESIGN, DOCKING, SYNTHESIS OF CERTAIN FLAVONOIDS AND EVALUATION OF THEIR ANGIOTENSIN CONVERTING ENZYME INHIBITORY ACTIVITY

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CERTIFICATE

This is to certify that the M.Pharm dissertation entitled **"Design, Docking, Synthesis of Certain Flavonoids and Evaluation of their Angiotensin Converting Enzyme Inhibitory Activity"** being submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai was carried out by **Ms. DEVIKA GAYATRI.M** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision and guidance to my fullest satisfaction.

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

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INTRODUCTION

DRUG DISCOVERY [1-3,8]

Drug discovery and development is a research process that identifies a new chemical entity and brings out its capabilities by designing and screening proper biochemical targets. It is an innovative science in which both knowledge and technologies are incorporated to convert a chemical moiety into useful therapeutic drugs.

The discovery and development process of novel drugs generally takes a long time and it is recognized to be, risky and costly. It takes approximately 14 years to fulfil the typical drug discovery process and development cycle from concept to market and the cost ranges from 0.8 to 1.0 billion USD. Rapid developments in combinatorial chemistry and high-throughput screening technologies have provided an environment to expedite the drug discovery process by enabling huge libraries of compounds to be screened and synthesized in short time. Although the investment in new drug development has grown significantly in the past decades, the output is not directly proportional to the investment because of the low efficacy and high failure rate in drug discovery. Consequently, various approaches have been developed to shorten the research cycle and reduce the expense and risk of failure for drug discovery. Computer-aided drug design (CADD) is one of the most effective methods for reaching these goals.

CADD is a widely used term that represents computational tools and sources for the storage, management, analysis and modelling of compounds. It covers many aspects of drug discovery, including computer programs for designing compounds, tools for systematically assessing potential lead candidates and the development of digital repositories for studying chemical interactions. In the post genomic era, benefiting from the dramatic increase in bio-macromolecule and small molecule

information, computational tools can be applied to most aspects of drug discovery and development process, from target identification and validation to lead discovery and optimization; the tools can even be applied to preclinical trials, which greatly alters the pipeline for drug discovery and development. The use of computational tools could reduce the cost of drug development by 50%.

The efficiency, accuracy and speed of the computational methods largely depend on several technical aspects, including conformation generation and sampling, scoring functions, optimization algorithms, and molecular similarity calculations.

RATIONAL DRUG DESIGN [4,5]

The need for on-going development of new drug needs to emphasise in light of the current global situation of health and disease. Traditionally, the process of drug development has revolved around a screening approach, as nobody knows which compound or approach could serve as a drug or therapy. The short coming of traditional drug discovery; as well as the allure of a more deterministic approach to combating disease has led to the concept of "Rational Drug Design".

One cannot design a drug without knowing more about the disease or infectious process than the past. The first necessary step for "rational" design is the identification of a molecular target critical to a disease process or an infectious pathogen. Then the important prerequisite of "drug design" is the determination of the molecular structure of target, which makes sense of the word "rational". In fact the validity of "rational" or "structure based" drug discovery rests largely on a high resolution target structure of sufficient molecule detail to allow selectivity in the screening of compounds.

The concept of rational drug design simply lies in logical reasoning before designing any therapeutic agents. For example, to prepare any competitive inhibitor

of a particular target, the logic of predicting the structure is to simply design a molecule with similar structural features exhibited by the endogenous agents or by closely examining the active binding site. Close examination of the active sites gives many hints about the interacting amino acid residues, So it becomes simple to predict the nature and type of substituent and the favourable position in the molecule, which will favour better binding.

Aims of drug design

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

TYPES OF DRUG DESIGN [6-8]

- Ligand based drug design
- Structure based drug design

Ligand Based Drug Design (LBDD)

LBBD is also known as indirect drug design this method is used in the absence of the structural information of the target to know about inhibitors for the target receptor. Biologically active lead molecule is detected by using structural or topological similarity or pharmacophoric similarity properties. Several criteria are used for similarity comparisons such as structure as well as shape of individual fragment or electrostatic properties of the molecule. The generated lead molecules are ranked based on their similarity score or obtained by using different methods or algorithms.

Structure Based Drug Design (SBDD)

SBDD is 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 modelling. When the experimental structure is unavailable this method is used to create a homology model of the target. 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 [9,10]

1. Real screening
2. Virtual screening

Real Screening

Real screenings 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 highly expensive, it provides real results which can be used for drug discovery.

Virtual Screening

The application of computers and computational methods to select or prioritize molecules for experimental screening is known as virtual screening. Thus a large percentage of the proposed analogue can be eliminated, and those molecules with the highest probability to show biological activity can be selected. The time and cost associated with the production of libraries for screening can be considerably reduced by adopting this methodology.

STEPS INVOLVED IN DRUG DESIGN[7]

The drug design process may be categorized into the 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

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

Important drug targets include:

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

The 3D structure of the protein target is usually obtained by X-ray crystallography [crystal structures of different macromolecules can be obtained 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**

With the help of combinatorial chemistry we can 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. The properties of the lead compound can be modified by effective combination of two or more active moieties or by elimination or substitution of various groups.

ADMET Studies[11]

Nowadays, testing of drug metabolism, pharmacokinetics and toxicity are done before evaluating a compound in the clinic. The rate at which the biological screening data obtained has significantly increased and high-throughput screening (HTS) facilities are now widespread at large pharmaceutical companies. Combinatorial chemistry makes larger sequence of closely related libraries of chemicals using the same chemical reaction and appropriate reagents. Such libraries are run through the HTS to make hits and which further, more focussed, and then their series are designed in the next round.

As the capability for biological screening and chemical synthesis have dramatically increased, the demands for large quantities of early information on absorption, distribution, metabolism, excretion, toxicity (ADMET) (together called ADMET data) also have increased simultaneously. Various medium and high throughput *in vitro* ADMET screens are therefore now in use. Before the pharmaceutical development of molecules for the treatment of disease it is recommended that the pharmacokinetic properties of each compound should be considered prior to its selection as drug candidate.

Again, there is an increasing need for good tools for predicting these properties. Various software and servers such as Accerlys accord for excel are available for this purpose.

ADMET data to be predicted

A deeper understanding of the relationships between important ADMET parameters and molecular structures and properties has been used to develop *in silico* models that allow the early estimation of several ADMET properties. Among other important issues, we want to predict properties that provide information about dose size and dose frequency such as oral absorption, bioavailability, brain penetration, clearance and volume of distribution.

Need of ADMET data

- ADME/Tox properties are important parameters for the selection of drug candidates for development.
- The need for ADMET information starts with the design of new compounds.
- This information can influence the decision to proceed with synthesis either via traditional medicinal chemistry or combinatorial chemistry strategies. Obviously, computational approaches are the only option for getting this information at this stage.
- Drug candidate selection involving both pharmacological properties and ADME/Tox screening should lead to an enhanced probability of clinical success.
- This will inevitably save time and costs in drug discovery and development. ADMET properties can be influenced by various physico-chemical properties like

- Molecular weight
- Hydrogen donors
- Hydrogen acceptors
- Log P etc

DRUGLIKENESS [12,13]

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* behaviour 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[14,15]

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. Therefore, 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. Molecular docking aims to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.

A molecular docking calculation consists of the following steps:

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

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

Rigid-body docking and flexible docking

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

Mechanics of docking

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

- **Search algorithm**

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

- Use a coarse-grained molecular dynamics simulation to propose energetically reasonable poses stimulation. (direct search-simplex method; gradient-based search-steepest descent, Fletcher-Reeves method, Newton-Raphson method; least square methods-Marquardt method)
- Simulated annealing (Monte Carlo search of the parameter space)
- Use a "linear combination" multiple structures determined for the same protein to emulate receptor flexibility
- Use a genetic algorithm to "evolve" new poses that are successively more fragment-based construction.

➢ **Scoring function**

The scoring function takes a pose as input, returns a number indicating the likelihood that the pose represents the favourable 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 (ligand 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. It 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.

HYPERTENSION[16,17]

Hypertension is one of the most common worldwide diseases affecting humans. Because of the associated morbidity and mortality and the cost to society hypertension is an important public health challenge. Over the past several decades, extensive research, widespread patient education, and a concerted effort on the part of health care professionals have led to decreased mortality and morbidity rates from the multiple organ damage arising from years of untreated hypertension.

High blood pressure is the leading cause of strokes and a major risk factor for heart attacks, one of the most important aspects of preventive cardiology, and it is necessary to identify as many people who have the disease as possible and to take steps to lower the blood pressure before it causes damage to the blood vessels, heart, kidneys, eyes and other organs. It is a major health problem that challenges not only with health care professionals but also the pharmaceutical industries and drug regulatory agencies.

Hypertension can be defined as a sustained increase in BP (140/90mm hg), a criteria that characterises a group of patients whose risk of hypertension related cardiovascular disease is high enough to merit the medical attention. Actually, the risk of both critical and non-critical cardiovascular disease in adult is lowest with systolic BP less than or equal to 120 mm hg and diastolic BP less than or equal to 80 mm hg; these risk increase progressively with higher systolic and diastolic blood pressures. Acknowledgment of this continuously increases the risk that provides a simple definition of hypertension.

The risk of cardiovascular morbidity and mortality is directly correlated with blood pressure (BP) .Starting at a BP of 115/75 mm hg, risk of cardiovascular disease doubles with every 20/10 mm hg increase. Even patients with prehypertension have an increased risk of cardiovascular disease. Outcome trials have shown that antihypertensive drug therapy substantially reduces the risks of cardiovascular events and death.

TYPES OF HIGH BLOOD PRESSURES[18]

There are two main types of high blood pressure

Essential (primary) hypertension

- The main form of high blood pressure-accounts for around 85-95% of cases
- Has no single identifiable cause
- Potential causes include genetic and environmental factors

Secondary hypertension

- Rare form of high blood pressure.
- Caused by another medical condition or treatment.
- Causes include problems (renovascular hypertension), adrenal gland tumours, thyroid disease and narrowing of the aorta (the main artery that takes blood from the heart to the rest of the body).

Other types of high blood pressure include

- Isolated systolic hypertension- the systolic pressure (top number) is raised but the diastolic pressure is normal.
- Isolated diastolic hypertension-the diastolic pressure (bottom number) is raised but the systolic pressure is normal.
- White coat hypertension-where the blood pressure is raised due to the stress of a vsit to the doctor or nurse.

Category of hypertension

Blood Pressure Category	Systolic mm Hg (upper #)		Diastolic mm Hg (lower #)
Normal	less than 120	and	less than 80
Prehypertension	120 – 139	or	80 – 89
High Blood Pressure (Hypertension) Stage 1	140 – 159	or	90 – 99
High Blood Pressure (Hypertension) Stage 2	160 or higher	or	100 or higher
Hypertensive Crisis (Emergency care needed)	Higher than 180	or	Higher than 110

Fig 1: Category of Hypertension

Factors that affect high blood pressure [19,20]

There are risk factors that can contribute to developing high blood pressure. Some of these factors can be changed and some others cannot be changed.

Factors that **cannot be changed**

- Age
- Race
- Socioeconomic status
- Family history (heredity)
- Gender
- Body mass

Factors that can be changed

- Sodium (salt) sensitivity
- Alcohol use
- Birth control pills(oral contraceptive use)
- Obesity
- Life style and stress
- Medication

Anti-hypertensive therapy[21]

The aim of therapy is straightforward: reduction of blood pressure to within the normal range. There are three general approaches to the treatment of hypertension. The first involves the use of diuretics to reduce blood volume. The second employs drugs that interfere with the renin-angiotensin system, and the third is aimed at a drug-induced reduction in peripheral vascular resistance, cardiac output or both. A reduction in peripheral vascular resistance can be achieved directly by relaxing vascular smooth muscle with drugs known as vasodilators or indirectly by modifying the activity of the sympathetic nervous system.

Research in the field of hypertension has gained worldwide importance because of its high frequency and concomitant risks associated with cardiovascular diseases. It has been identified as leading risk factor for mortality and is ranked third as a cause of disability adjusted life-years.

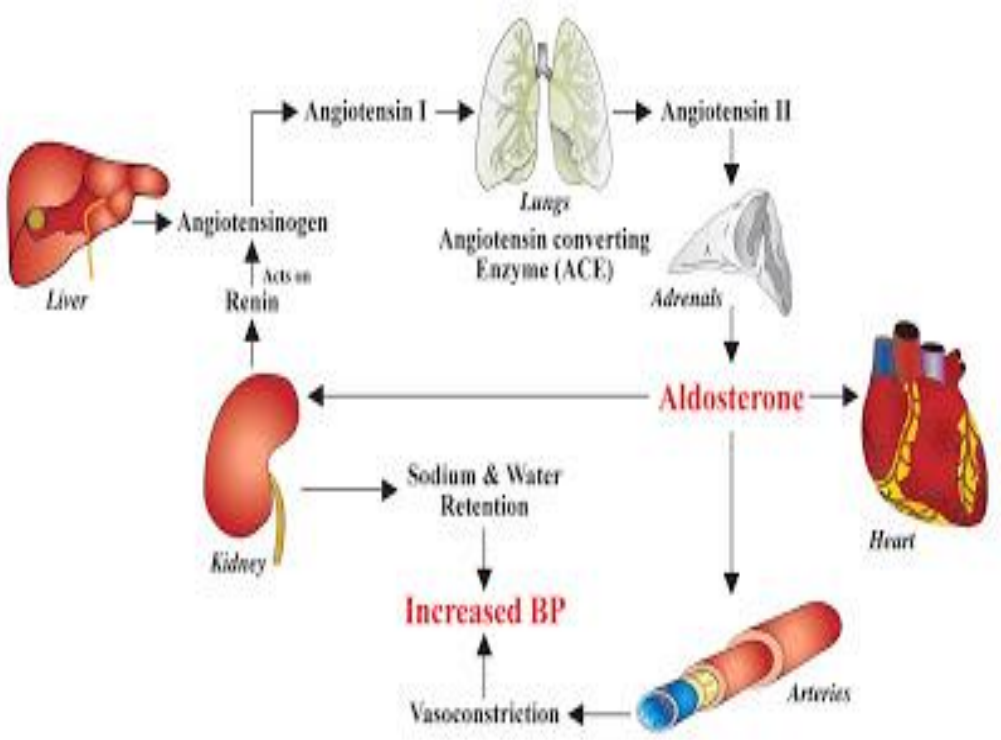
A pathophysiological view point, hypertension involves changes in at least one of the hemodynamic variables (cardiac output, arterial stiffness or peripheral resistance) that determine measurable blood pressure. Therefore each of these variables is a potential target for modulation. It is recognized that angiotensin converting enzyme (ACE) plays an important role as a regulatory sight in the renin angiotensin system (RAS) that control excessive activation and then hypertension.

RENIN ANGIOTENSIN ALDOSTERONE SYSTEM (RAAS)[21,22]

RAAS is a powerful system, regulating fluid-electrolyte balance and systemic blood pressure. Renin is a proteolytic enzyme synthesized, stored and secreted from the juxtaglomerular apparatus in the kidneys. The release of renin is triggered by a number of physiological stimuli, including PGI2, decreased Na+ concentration in the distal tubule, reduced arterial pressure, renal sympathetic nerve activation and stimulation of β1- receptors. Following secretion, renin acts upon the plasma protein angiotensinogen forming angiotensin I (ang I). Ang I has mild vasoconstrictor properties but not enough to cause significant functional changes. Ang I is further converted to angiotensin II (ang II) by ACE. Ang II is considered the main effector peptide of the renin-angiotensin system (RAAS). Binding of ang II to specific receptors activates a number of different events in various tissues and cell types. Four types of ang II receptors have been identified; AT_1-AT_4. Most of the cardiovascular effects are generated through the AT_1 receptor. Ang II plays an important role in blood pressure regulation by inducing arteriolar constriction, release of aldosterone from the adrenal medulla and enhanced secretion of vasopressin, resulting in increased reabsorption of Na+ and water in the kidneys. Ang II also induces several pathophysiological actions involved in the atherosclerotic process, such as Proliferation and migration of smooth muscle cells, generation of ROS, lipid peroxidation and formation of foam cells.

The degradation of ang II by a number of different enzymes occurs only seconds after its formation, giving rise to other angiotensin peptides. During the last decades, a number of new angiotensin peptides have been identified, and it has become evident that RAS is a more complex system than previously thought. Ang 2-8 acts through the ang II receptors $AT_1$and AT_2 and generates similar physiological effects as ang II. Ang 3-8 is involved in regulation of blood flow, neural development and learning and memory. Ang 1-7 is produced from the precursor Ang I and the conversion can be catalyzed by a number of

endopeptidases, includingneprilysin and prolyl-oligopeptidase. It is believed that ang 1-7 counteracts the effects caused by ang II. Ang 1-7 act through specificreceptors (Mas receptors), but have also been reported to activate the AT_2 receptor. Ang 1-7 have antihypertensive, antihypertpophic, antifibrotic and antitrombotic properties. In addition, ang 1-7 has been shown to be cardioprotective and it has been suggested that ang 1-7 has a role in the protective effect of ACE inhibitors (ACEi).



Angiotensin converting enzyme[23]

ACE is an octo enzyme and glycoprotein with an apparent molecular weight of 1,70,000. ACE present in humans contain 1277 amino acid residues and is found to have 2 homologous domains, each with a catalytic site and a Zn^{2+}binding region. ACE has a large amino terminal extracellular domain, a short carboxyl terminal intracellular domain, and a 17 amino acid lipophilic region that binds the octo

enzyme to the cell membrane. ACE may lose its C-terminal end and become dissolved in plasma as circulating ACE and is found mainly in the lungs due to their vast surface of vascular endothelium. ACE is also present in vascular tissues other than endothelium; such as smooth muscle cells the heart fibroblasts, the kidney, CNS, placenta and testis. ACE is a poly specific enzyme metabolizing angiotensin, angiotensin 1-7, enkephalins, substance P and luteinizing hormone-releasing hormone (LH-RH). ACE is responsible for at least 90% of the conversion of angiotensin I to angiotensin II in the blood, kidney, heart, lung and brain and at least 77% in the adrenal.

ANGIOTENSIN CONVERTING ENZYME INHIBITOR[24]

ACE inhibitors are considered as the most potent antihypertensive drugs and apart their major action, exhibit beneficial lateral effects in the prevention of cardiovascular disease in various classes of hypertensive patients. Additionally ACE inhibitors have been proven more effective than other hypertensive substances in reducing protein and urea and retarding the progression of renal damage in patients with various types of nephropathy.

MECHANISM OF ACTION OF ACE INHIBITORS[25]

All ACE inhibitors share the same mode of action, that is, elimination of the production of angiotensin ll inhibition of the degradation of bradykinin. Accordingly, all lead to suppression of angiotensin II with

1) Decrease of blood pressure.
2) Vasodilation varying in degree in various tissues (depending on the sensitivity of each organ's vascular tree to angiotensin) and leading to increase in cardiac output and redistribution of regional blood flows in favour of vital organs at the expense of the less-sensitive musculoskeletal tissues.

3) Partial suppression of aldosterone production by the adrenal zona glomerulosa.

4) Increase in circulating levels of plasma renin due to interruption of the negative feedback control exerted normally by angiotensin II on the release of renin.

5) Potentiation of the vasodilatory effect of bradykinin in sympathetic nervous system with diminished levels of plasma catecholamines as well as other hormones whose secretion is partly dependent upon the stimulus of angiotensin II.

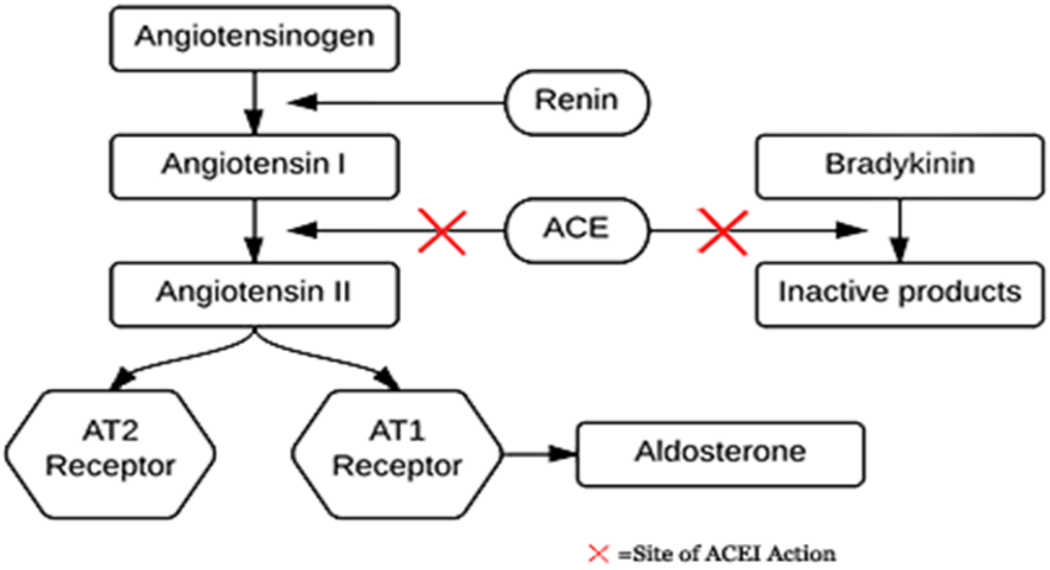


Fig 3: Mechanism of ACE inhibition

ENZYME ACTIVITY STUDY[26]

Angiotensin I is a decapeptide which is converted to the pressor of octapeptide, angiotensin II, in the presence halide-activated peptidase converted by hydrolytic removal of the C-terminal dipeptide, histidyl-leucine. The enzyme primarily responsible for the conversion *in vivo* of circulating angiotensin I to angiotensin II was localized in the lung. Existing biological methods for

determining the amount of angiotensin I converted to angiotensin II are inadequate for assay of the angiotensin converting enzyme in lung extracts or during the early stages of purification.

Angiotensin I is active to some extent in all biological assays *in vitro* and *in vivo* for angiotensin II; and "angiotensinases" present in all tissue homogenates destroy both the octapeptide, for this reason, assay that measured the amount of L-histidyl-[U-'4C]-L-leucine formed by the action of the angiotensin converting enzyme on synthetic [U-'4C-Leu'*], [Bes]-angiotensin I'O, and developed an automated ninhydrin method that measures the chloride ion-dependent increase in ninhydrin- reactive material upon incubation of the enzyme with synthetic-angiotensin I.

The possibility of using an assay for angiotensin II to determine the angiotensin converting enzyme activity. These assay methods, however, are still subjected to errors, due to hydrolysis of both substrates and products by other tissue peptidases. Previously reported the activity of angiotensin converting enzyme from sheep lungs.

The C-terminal protected tripeptide, hippuryl-L-histidyl-L-leucine(HHL), using a quantitative ninhydrin assay for detection of liberated hisitdylleucine. The histidyl leucine released from other protected tripeptide, Hippuryl-L-histidyl-L-leucine(HHL).

In vitro angiotensin converting enzyme inhibitory activity estimates the amount of hippuric acid and L-histidyl-L-leucine. Angiotensin converting enzyme converts the Hippuryl-L-histidyl-L-leucine (HHL) to hippuric acid and L-histidyl-L-leucine, and estimate the amount of hippuric acid and L-histidyl-L-leucine by spectrophotometry and spectroflourimetry.

FLAVONOIDS[27,28]

Flavonoids represent a diverse group of low molecular weight natural poly phenolic compounds. The term Flavonoid has been derived from the latin word flavus meaning yellow. Their presence in plant is responsible for various colours and combination of colours exhibited by roots, barks, heartwood, leaves, flowers, fruits and seeds. In plants, flavonoids occur in different forms, such as free aglycones, glycosides, and biflavonoids. Flavonoids function throughout the plant kingdom as UV protectants, attract insects for pollination, and antimicrobial compounds. Flavonoids and isoflavonoids may be of ecotoxicological importance because they are present in the heartwood of tree species used for wood pulp.

They are secondary metabolites characterised by flavan nucleus and C6-C8-C6 carbon-skeleton. These are group of structurally related compounds with a chromane-type skelton having phenyl substituent in C2-C3 position. The basic structural feature of flavonoid is 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C). The flavonoid biosynthesis starts with general phenyl propanoid pathway. It is a very complex process and involves series of reactions.

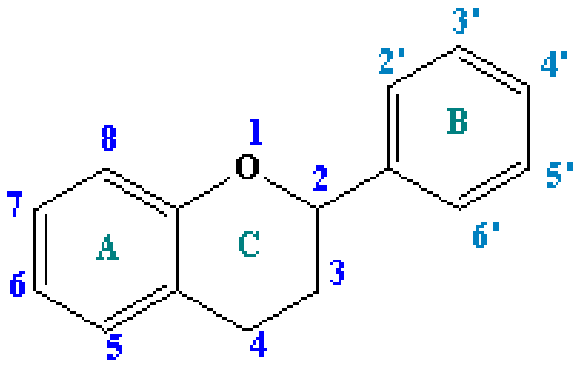


Fig 4: Structure of flavonoid nucleus

Flavonoids have aroused considerable interest because of their potential beneficial biochemical and antioxidant effects on human health. Most of the experimental results demonstrate that flavonoid compounds have several biological activities including radical scavenging, anti-inflammatory, anti-mutagenic, anti-cancer, anti-HIV and anti-allergic, anti-platelet and anti-oxidant activities. Flavonoids are also highly unstable compounds which undergo numerous enzymatic and chemical reactions during post-harvest food storage and processing thus adding to the complexity of plant polyphenol composition.

Flavonoids in ACE Inhibition[29,30]

ACE, a crucial enzyme in the regulation of the renin–angiotensin system, is a zinc-containing peptidyl dipeptide hydrolase. The active site of ACE is known to consist of three parts: a carboxylate binding functionality such as the guanidinium group of arginine, a pocket that accommodates a hydrophobic side chain of *C*-terminal amino acid residues and a zinc ion. The zinc ion coordinates to the carbonyl of the penultimate peptide bond of the substrate, whereby the carbonyl group becomes polarized and is subjected to a nucleophile attack. Therefore, some flavonoids were suggested to show *in vitro* activity via the generation of chelate complexes within the active centre of ACE. Free hydroxyl groups of phenolic compounds are also suggested to be important structural moieties to chelate the zinc ions, thus inactivating the ACE activity.

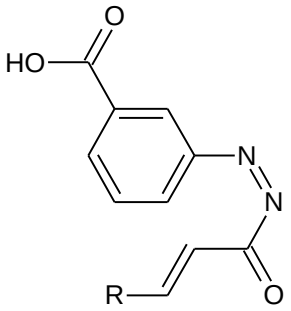
The aim of the present work was to synthesize new flavonoid derivatives in order to explore the extent of their angiotensin converting enzyme inhibiting activity. The compounds were designed by *in silico* method using 1o86 (ACE) as the target molecule.

LITERATURE REVIEW

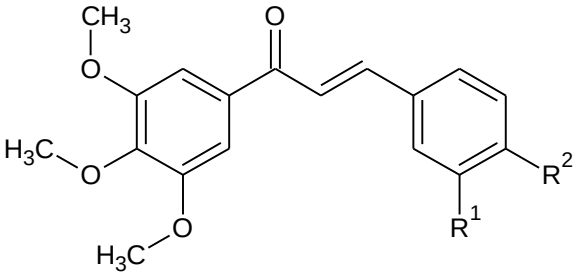
CHALCONES

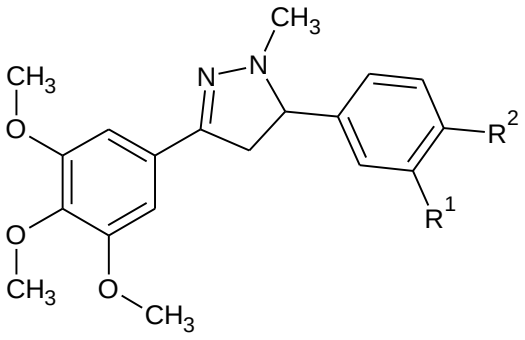
1) As Angiotensin Converting Enzyme inhibitors

- S.N.A Bukhari *et. al.*, [31] (2013) synthesized a series of chalcone analogues and used as precursor for the synthesis of novel series of pyrimidine and were then evaluated for their effect on ACE inhibition. Pyrimidine analogues were found to be more active than precursor chalcones.



- Macro Bonesi *et. al.*, [32] (2010) synthesized 9 chalcones and their 9 pyrazole derivatives and the synthesized compounds were tested for their angiotensin converting enzyme inhibiting activity.

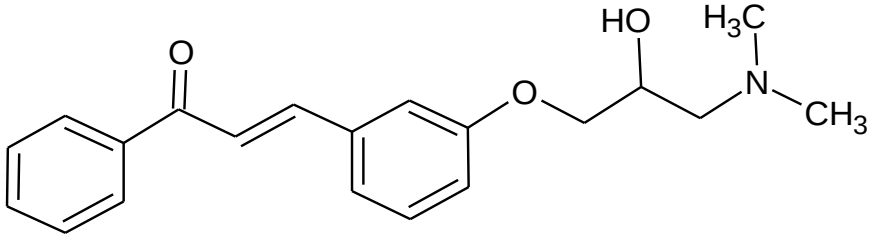




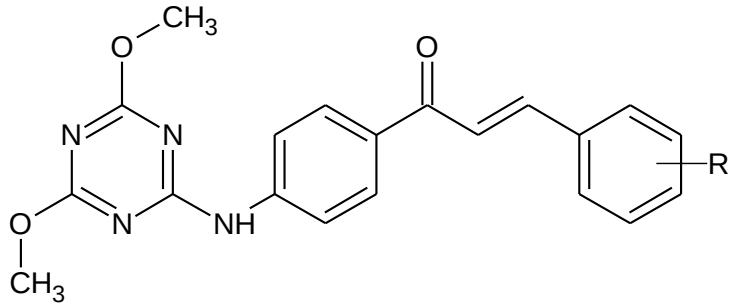
2) Antihyperglycemic activity of chalcones:

➢ Chentana B.Patil *et. al.,* [33] *(*2009*)* reported that chalcones have hypoglycemic activity. 3,4- dimethoxy chalcone displayed significant antihyperglycemic effect. Monomethoxy series showed blood glucose lowering activity. Compounds vicinally deoxygenated as dimethoxy and methylene di-oxy substituents showed the best antihyperglycemic activity when compared to the corresponding monomethoxy compounds. Compounds containing propanolamine chain at para position showed significant biological activity as compared to meta and ortho substituted compounds.

➢ Poonam Shukla *et. al.,* [34] (2006*)* synthesized a series of chalcone based aryl oxypropanolamines and tested their antihyperglycemic activity in SLM (Source Loaded Model) and STZ (Streptozotocin) rat models.

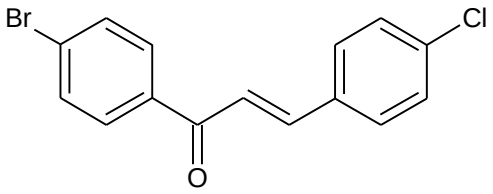


➢ R.S Shinde *et. al.,* [35] (2015) synthesised a series of S-triazine based chalcones and the synthesised compounds were studied for their antidiabetic and antioxidant activity.

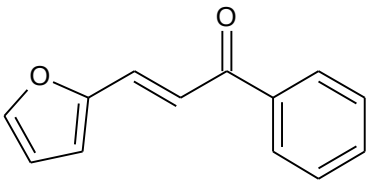


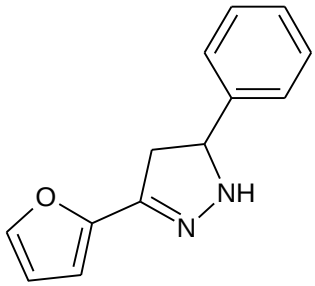
3) Chalcones As Antibacterial Agents:

- Y.Rajendra Prasad *et. al.,* [36](2008) synthesized 1-(4'-bromophenyl)-3-(4-chlorophenyl)-2-propene-1-one and tested antibacterial activity.

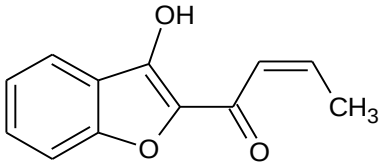


- Vishal.D.Joshi *et. al.,* [37](2012) synthesized some novel series of pyrazoline derivatives from chalcones. The newly synthesized pyrazoline derivatives were screened for anti-microbial activity.

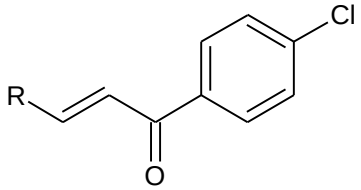




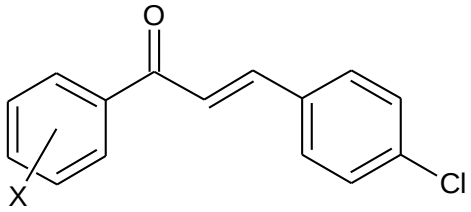
- P.M.Gurubasavaraja Swamy *et. al.,*[38] (2008) synthesized a series of 3-hydroxy benzofuran chalcones and the compounds were screened for antibacterial activity against Staphylococcus.



- Paramesh. M *et. al.,*[39] (2010) synthesized nine Chlorine containing chalcones and they were evaluated for antibacterial activity.

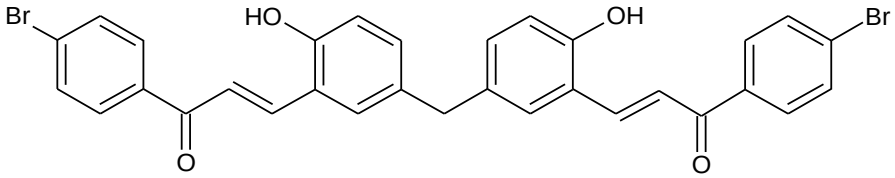


- Rajini Gupta *et. al.,*[40] (2010) synthesized 1-aryl-3-(4'-chlorophenyl)-prop-2-en-1-one and tested their antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.*

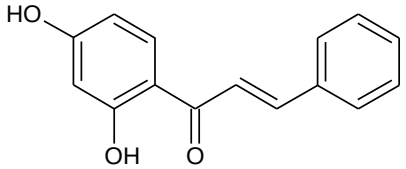


X = 4-H, Br, Cl, F, CH_3

- A. Nagaraj *et. al.,*[41]*(2008)* synthesized bis [3-[(E)-3(4-bromophenyl)-3-oxo-1-prop-enyl]-4-hydroxy phenyl] methane and tested its anti-bacterial activity.

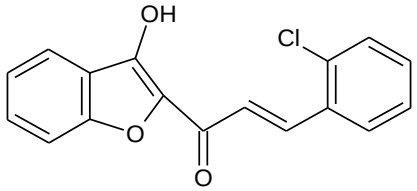


- Cristina M. Devia *et. al.,* [42]*(1998)* synthesized 2',4',2-trihydroxy chalcone and tested its antibacterial activity against *Staphylococcus aureus.*



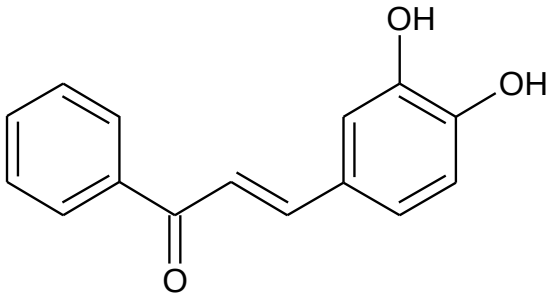
4) Chalcones as Antifungal Agents

- P.M. Gurubasavaraja swamy *et. al.,* [38](2008) synthesized 1-(3-hydroxy benzofuran-2-yl) -3-(4-chlorophenyl)-2-propene-1-one which possesses very good activity against the fungi *Aspergillus flavus.*

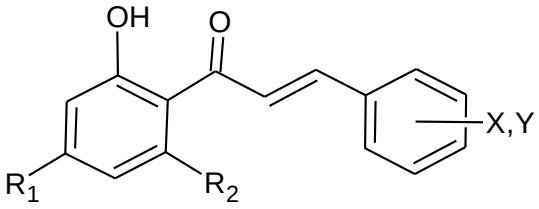


5) Chalcones as Anti-Oxidants

➢ Chentana B.Patil and S.K.Mahajan.,[33] (2009) synthesized 1-phenyl 3-(3,4-dihydroxy phenyl) propene-1-one which shows antioxidant activity. It was react with the radicals and they are readily converted to the phenoxy radicals due to the high reactivity of hydroxyl groups of chalcones.



➢ Anastasia Detsi *et. al.,* [43] (2009) synthesized 2'-hydroxy –chalcones and tested them for their anti-oxidant activity.



R_1 = H, OCH_3

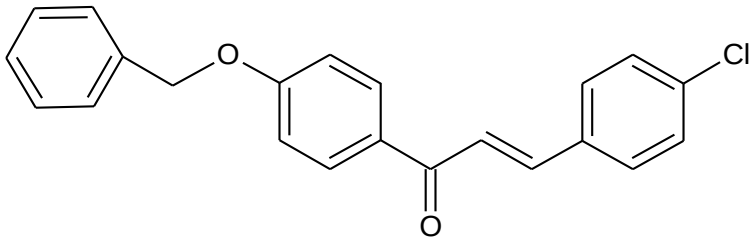
R_2 = H, OCH_3

X = OCH_3, CH_3, Cl

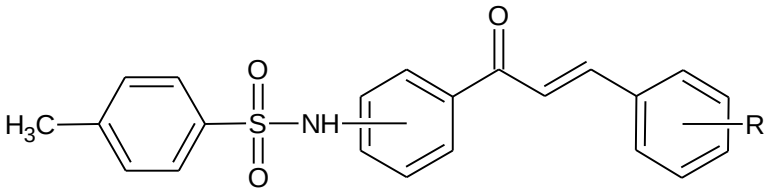
X,Y = OCH_3, OCH_2OCH_3, OH

6) Chalcones as Enzyme Inhibitors

➢ Franco chimenti *et. al.,*[44] (2009) synthesized 4'-benzyloxy chalcones and tested them *in vitro* for their ability to inhibit human monoamine oxidase A and B.

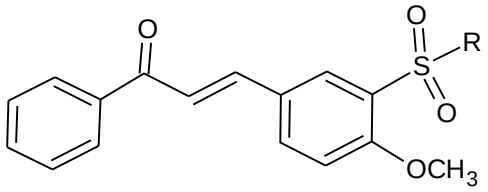


➢ Woo Duck Seo *et. al.,*[45] (2005) synthesized sulfonamide chalcones and their α-glucosidase inhibitory activities were investigated.



7) Antileishmanial activity of chalcones:

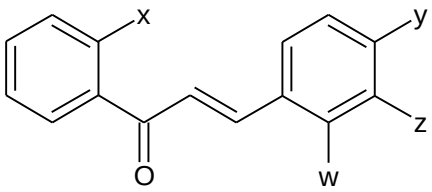
➢ Carla R. Andrighetti Frohner *et. al.,* [46] *(2008)* synthesized 4-methoxy chalcone derivatives and tested their potential antileishmanial activity.



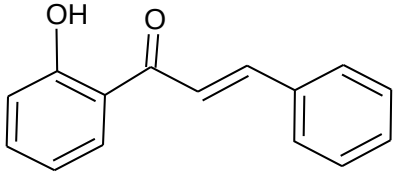
R= $N(CH_3)_2$, $N(CH_3)_2$.

8) Chalcones having anti-tumor activity:

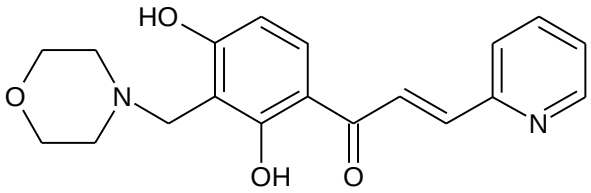
➢ Mauricio Cabrera *et. al.,* [47] (2006) synthesized chalcones and tested their anti-tumor activity.



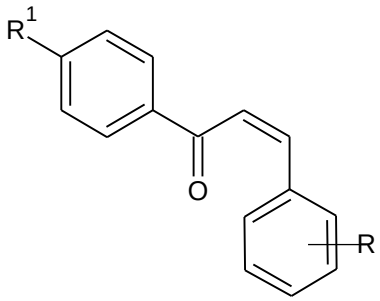
➢ Cesar Echeverria *et. al.,* [48] (2009) synthesized 2'-hydroxy chalcones and tested them for their anti-cancer activity.



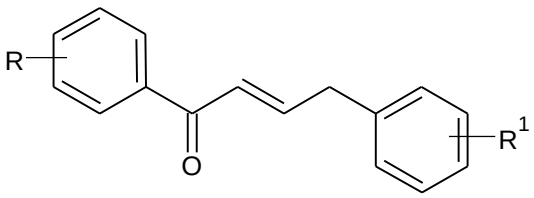
➢ M.Vijaya Bhaskar Reddy *et. al,.*[49]*(2008)* synthesized the mannich base of heterocyclic chalcone and tested its anticancer activity.



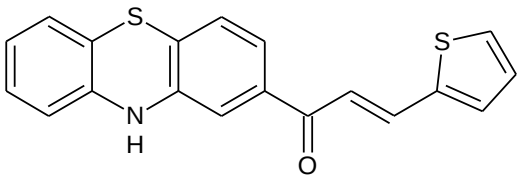
➢ Suvitha Syam *et. al.,* [50] (2012)synthesized several chalcones and their *in vitro* cytotoxicity against various human cell lines were evaluated.

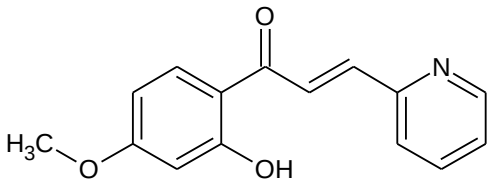


➢ Visakh Prabhakar *et.al.,* [51] (2014) designed and synthesized a set of mono substituted chalcone derivatives and evaluated them for potential cytotoxic activity against five human cells.



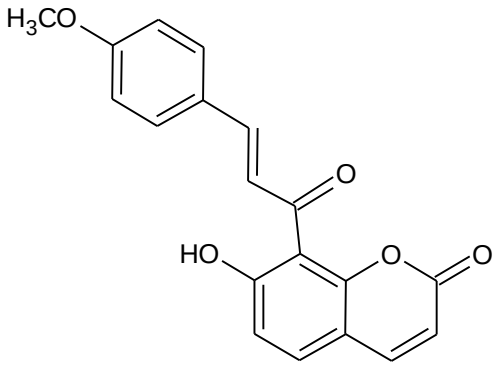
➢ Taran-Thanh-Dao *et. al.,* [52] (2015) synthesized a series of heterocyclic chalcones and were evaluated for their cytotoxicity on Rhabdomyosarcoma cell line.



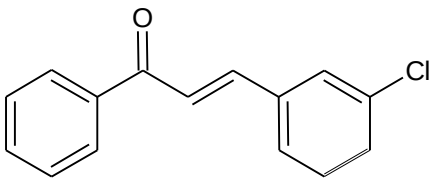


9) Anti-inflammatory activity of chalcones:

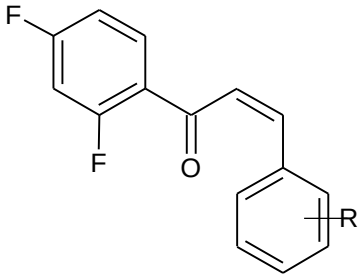
- M.S.Y Khan and Sandhya Bawa[53] (2001) synthesized 7-hydroxy-8-[(2*E*)-3-(4-methoxyphenyl)prop-2-enoyl]-2*H*-chromen-2-one and tested its anti-inflammatory activity.



- Mazin Nadhim Mousa *et. al.,* [54] (2016) synthesized some chlorinated chalcones derivatives and the synthesized compounds were tested for their anti-inflammatory activity and were showed comparable anti-inflammatory effect to Diclofenac sodium and less than dexamethasone.

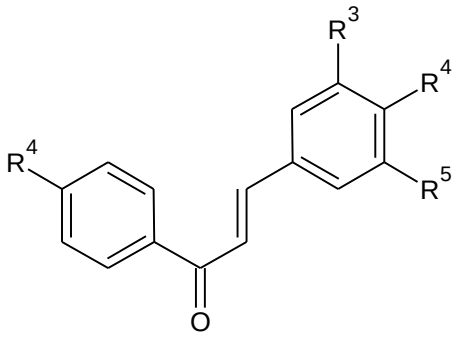


➢ Dhanaji H jadhav *et. al.,* [55] (2007) synthesized a series of twelve 2',4'-diflourinated chalcones and subjected to anti-inflammatory activity.



10) Antimalarial Activity of Chalcones:

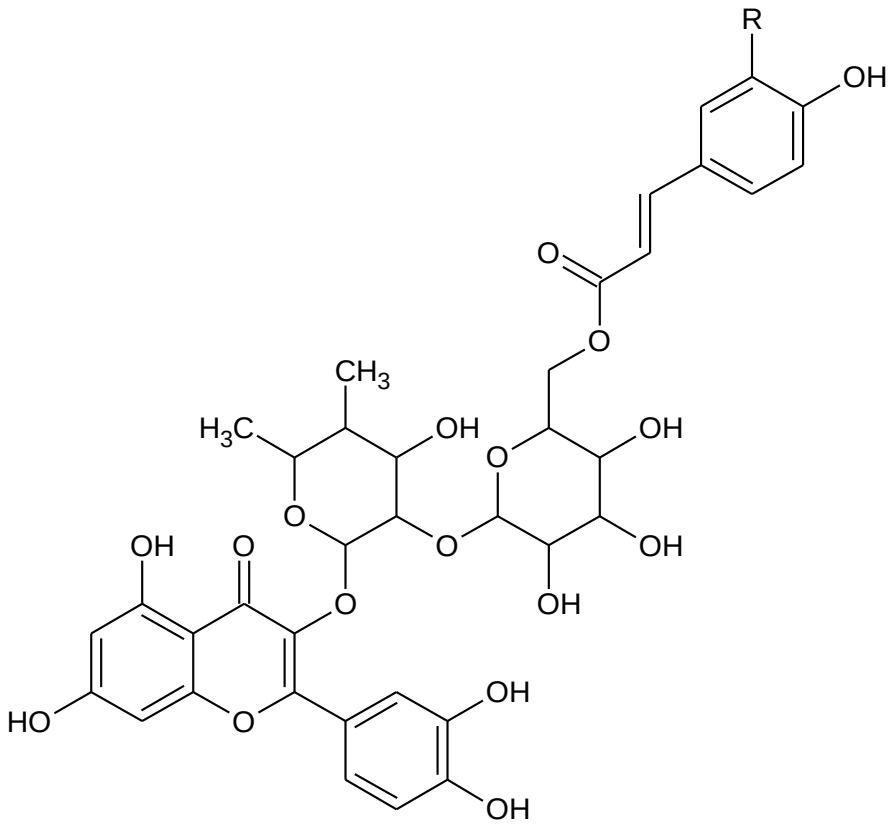
➢ Satish.K.awasthi *et. al.,* [56] (2008) synthesised several new chalcone analogues and evaluated as inhibitors of chloroquine sensitive Plasmodium falciparum strain of parasite.



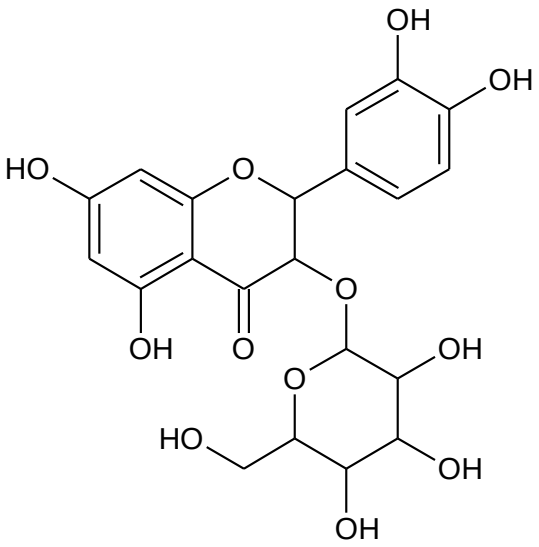
FLAVONOIDS

1) Angiotensin Converting Enzyme Inhibitory Activity of Flavonoids:

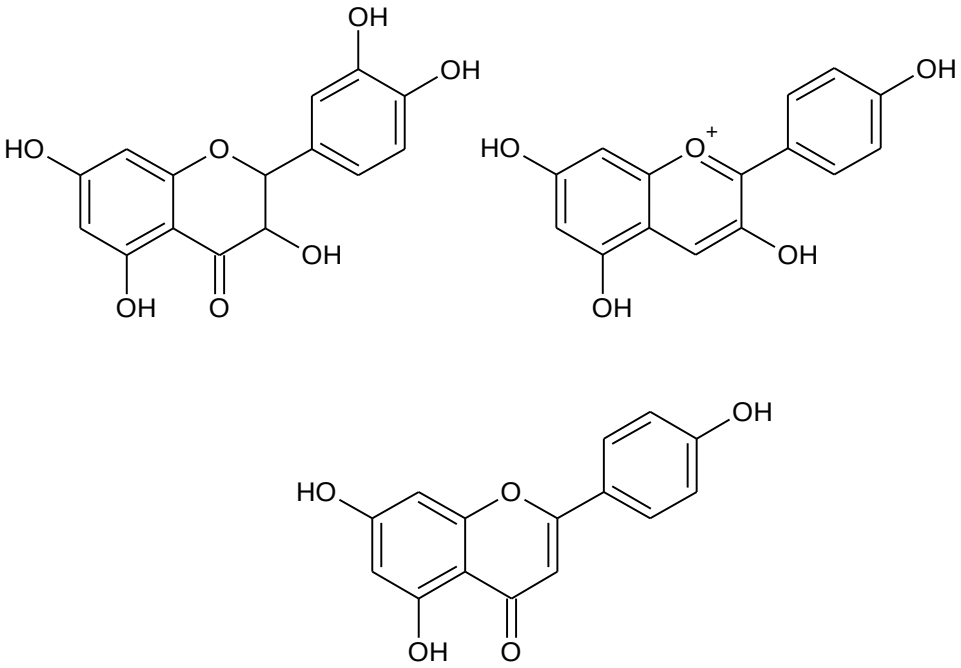
➢ Hyuncheol O.H *et. al.,* [57] (2004) by bioassay-guided fractionation of the EtOAc-soluble extract of Sedum sarmentosum afforded a new flavonoid, quercetin-3-O-α-(6’’’-caffeoylglucosyl-β-1,2-Rhamnoside) along with four known flavonoids. All the five compounds exhibited ACE inhibitory activity in a concentration dependent manner.



➢ Monica Rosa Loizzo *et. al.,* [58] (2006) extracted six flavonoids from the leaves of Alianthus excels and were analysed the in vitro hypotensive activity. All the flavonoids tested exhibited ACE inhibiyory activity and the most active compound was Kaempferol-3-O-β-galactopyranoside.

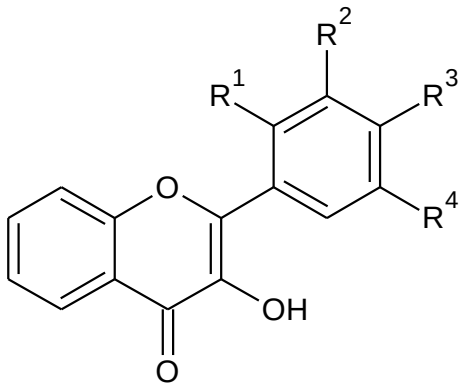


➢ B.W Nileeka Balasuriya and H.P Vasantha Rupasinghe[59] (2011) extracted apple skin rich in flavonoids and the major constituents of the extract and some of the selected metabolites were assessed for the ACE inhibitory property in vitro and all the flavonoids tested have a potential to inhibit ACE in vitro and the inhibitory property varies according to type of sugar moiety attached at C3 position.

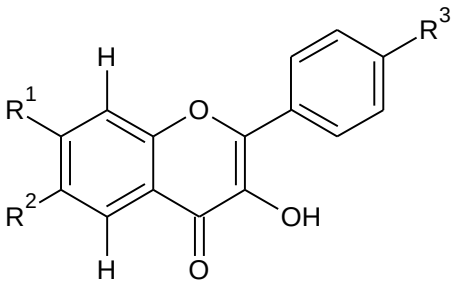


2) Anti diabetic activity of Flavonoids:

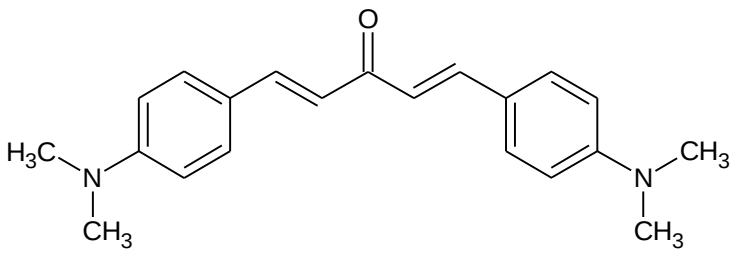
- Mkunthakumar *et. al.,* [60] synthesized a series of 2-(2,3,4 and 5 substituted phenyl)3-hydroxy-4H-chromen-4-one by reacting 2- hydroxyl acetophenone and various aromatic aldehydes and evaluated in vitro anti diabetic activity against streptozocin induced diabetic rats.



- Yogendra Nayak *et. al.,* [61] (2014) synthesized 3-hydroxy flavone analogues and the compounds are evaluated for their antidiabetic activity in high fructose fed insulin resistant rats.

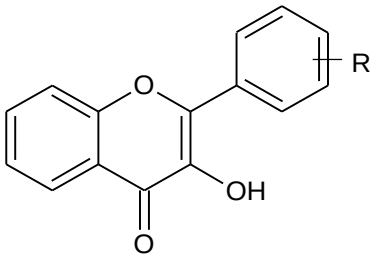


- Mahesh Vari Asogan *et. al.,* [62] (2016) synthesized bioactive flavonoid derivatives and were screened for their anti-diabetic activity.



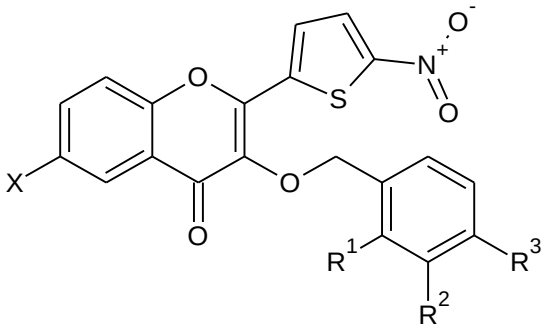
3) Anti oxidant activity of Flavonoids:

➢ Venkatachalam *et.al.*, [63] (2012) synthesized a series of newer flavones by Algar-Flynn-Oyamada method and were evaluated for in vitro antioxidant activity.

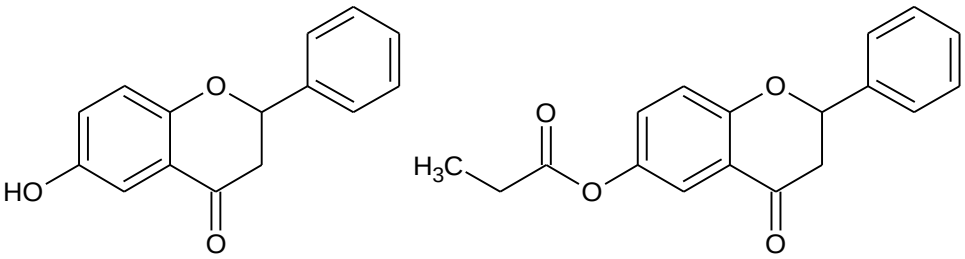


4) Anti cancer activity of Flavonoids:

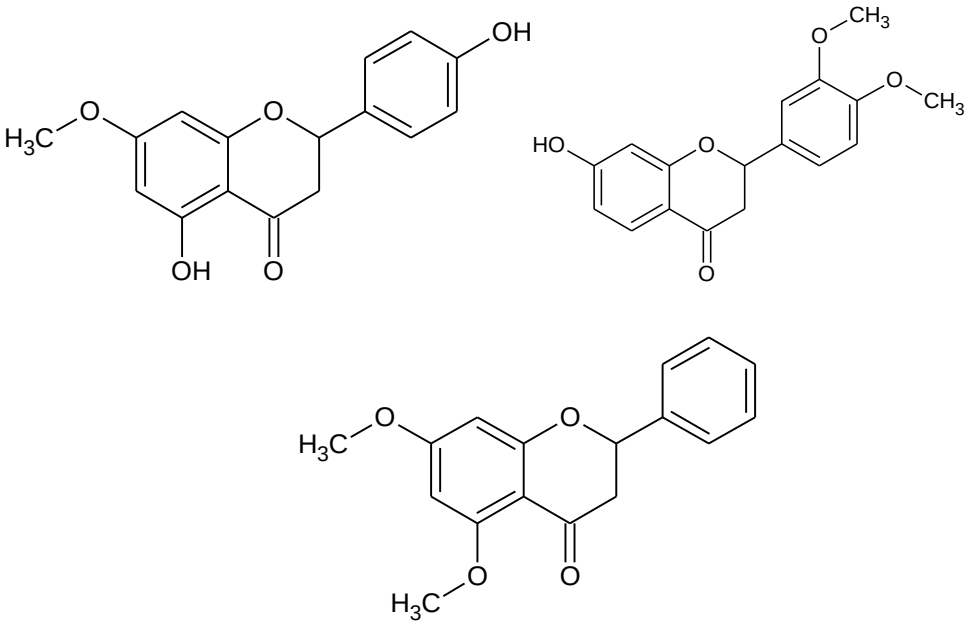
➢ Jayashree B.S *et.al.*, [64] (2012) synthesized some new substituted flavones and screened for in vitro anticancer activity.



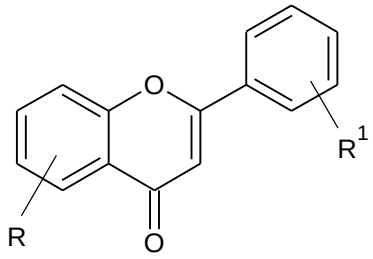
➢ Ewelina szliszka *et.al.*, [65] (2012) synthesized 6-hydroxy flavonone and its derivative 6-propionoxy flavonone and the compounds were screened for their anti-cancer activity.



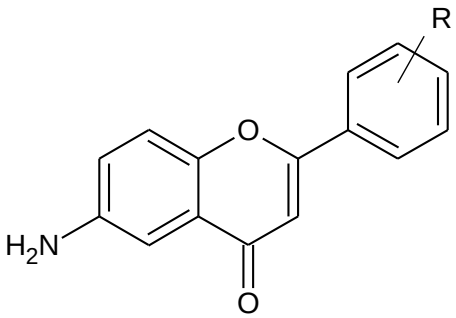
➢ K.Rajeshbabu *et. al.*, [66] (2016) synthesised various flavonoids under autoclave conditions and the compounds were screened for their anti-cancer activity.



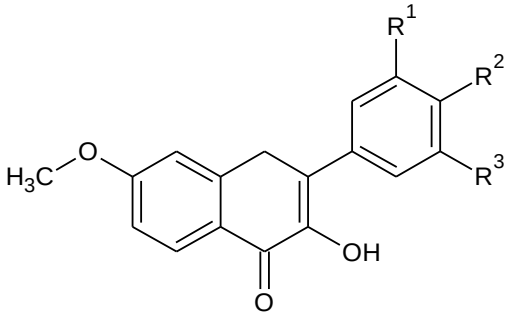
➢ Mariano Cardemas *et. al.,* [67] (2006) synthesised a series of flavonoids and was evaluated in vitro for their antitumor activity.



➢ Lalitha Simon *et. al.,* [68] (2015) synthesised a series of 6-Amino flavones and characterised by spectral technique .Their cytotoxic effects have been evaluated in vitro in relation to colon HCT116 and breast MCF7 cancer cell lines.

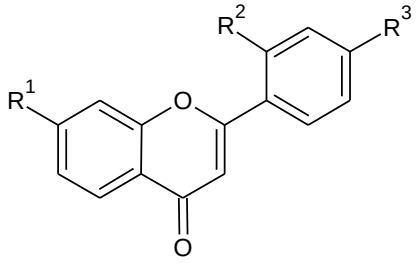


➢ Jing Zhang *et. al.,* [69] (2013) synthesised chalcones and 5-deoxy flavones and they were screened for their cytotoxicity against the cancer cell lines 0f MDA-MB-231, U251, BGC-823 and B16.

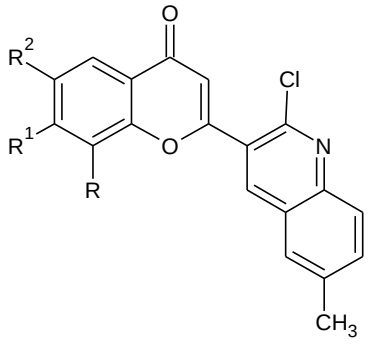


5) Anti-microbial activity of Flavonoids:

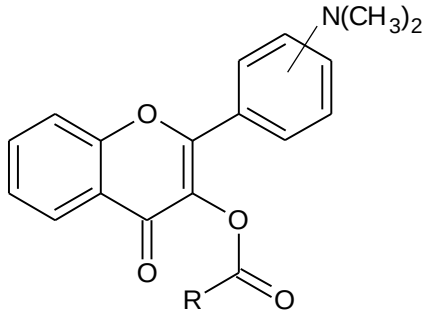
➢ Nazifi Saleh Ibrahim *et. al.,* [70] (2014) synthesised a series of flavonoids and screened for their antibacterial activity against Bacillus cereus, Enterococcus faecalis, Klebsiella pneumonia and Pseudomonas aeruginosa and antifungal activity against Aspergillus fumigatus, Candida glabrata. All Compounds exhibited activity against the entire tested micro organism.



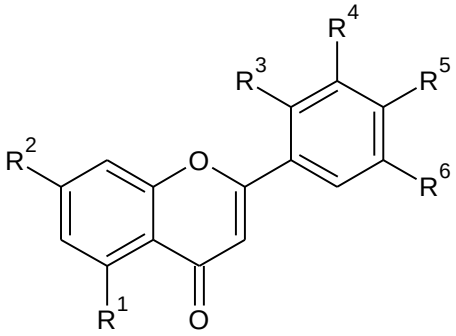
➢ S.S Mokle *et al.,* [71] (2010) synthesised a series of flavones and the compounds were evaluated for their antibacterial activity against Xanthomonas citri, Ervinia carotovara, Escherichia coli and Bacillus subtilis.



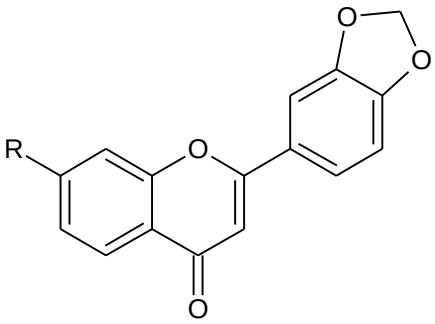
➢ Jayashree B.S *et. al.,* [72] (2008) synthesised different esters of 3-hydroxy flavones and the compounds were tested for their activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.



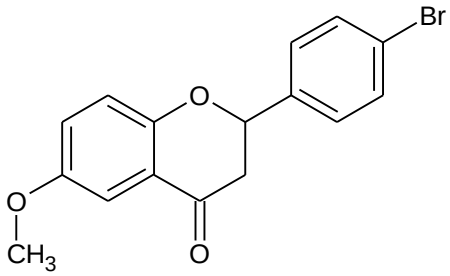
➢ Sohel mostahar *et. al.,* [73] (2007) synthesised a series of flavonoids and the synthesised compounds were tested for antibacterial, antifungal and cytotoxic activity.



➢ Sayed Alam [74] (2004) synthesised some derivatives of 2-phenyl chromen-4-one (flavone ring) and tested for antibacterial and antifungal activities along with their chalcone precurssors against four human pathogenic bacteria and five plant mould fungi.

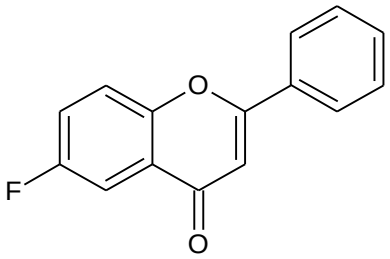


➢ Sherif B Abdel Ghani *et.al.,* [75] (2008) synthesised flavonoid derivatives and evaluated the compounds for their antifungal activity against Aspergillus niger and Fusarium oxysporium .



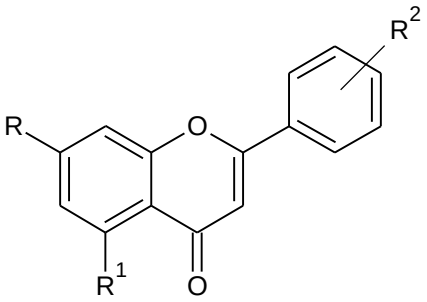
6) Anti-Rhinovirus activity of Flavonoids:

➢ Clinzia Conti *et. al.,* [76] (2005) synthesised fluro-substituted flavones and 2-styrylochromones and were tested for anti-rhinovirus activity. The antiviral potency was evaluated by a plaque reduction assay in HeLa cell cultures injected with rhinoviruses.

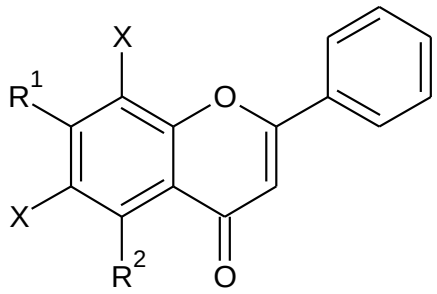


7) Anti-inflammatory activity of flavonoids:

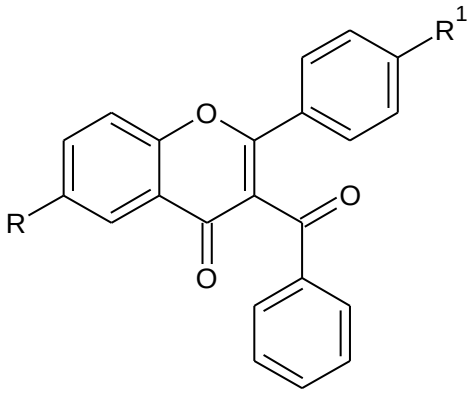
➢ Dharma Theja N *et. al.,* [77] (2001) synthesised a series of flavone derivatives and their biological activity was investigated using the model of carrageenan induced rat paw oedema.



➢ Tuong-Ha Do *et. al.,* [78] (2009) synthesised a series of flavonoids and screened for their anti-inflammatory activity.

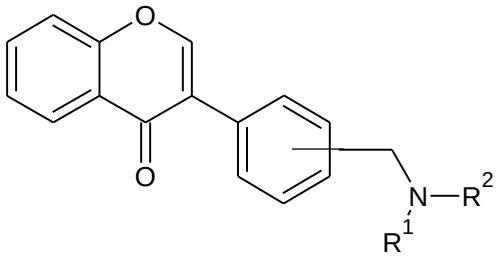


➢ Shrinivas .B.Patil [79] (2013) synthesised substituted 4-benzoyl flavone and the synthesised compounds were screened for their anti-inflammatory, anti-oxidant and anti-cancer activity. All compounds showed significant range of activity.



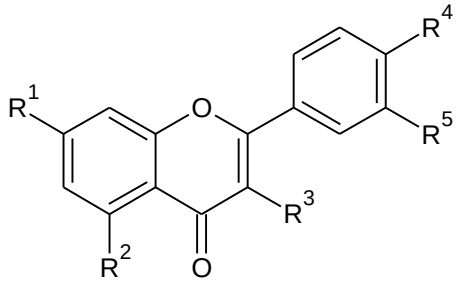
8) AChe inhibitory activity of Flavonoids:

➢ Rong Sheng *et. al.,* [80] (2009) designed and synthesised a new series of flavonoid derivative and evaluatedtheir activity as potent AChe inhibitors.



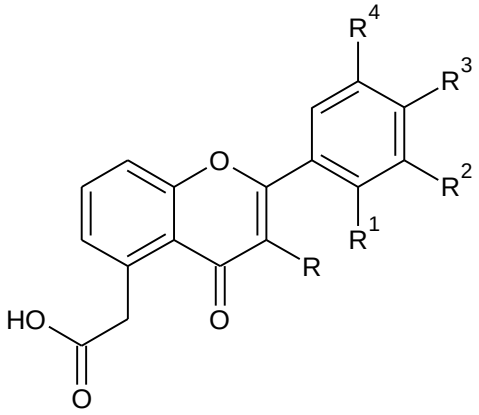
9) Peroxynitrite Scavenging activity of Flavonoids:

➢ C G M Heijnen *et. al.,* [81] (2001) synthesised a series of flavone for the evaluation of peroxynitrite scavenging activity.



10) Flavonoids as Amino peptidase N/CD13 inhibitors:

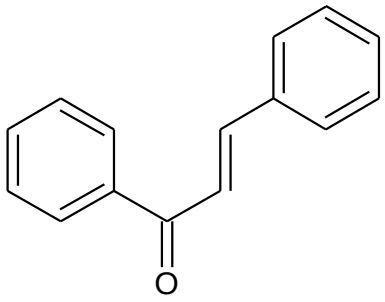
➢ Brigitte Bauvois *et. al.,* [82] (2003) synthesised a series of novel flavone -8-acetic acid derivatives and were evaluated for their APN/CD13 inhibitory activity.2' 3 di -nitro flavone -8-acetic acid proved to be the most efficient, which is two folds active than bestatin, the natural known inhibitor of APN/CD13.



CHEMISTRY

CHALCONE:[33]

Structure:

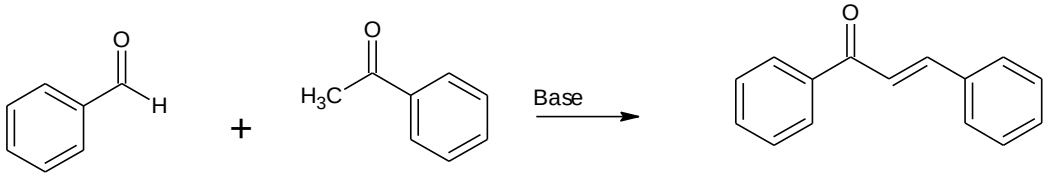


Chalcones are 1, 3-diphenyl-2-propene-1-ones,in which two aromatic rings are linked by a three carbon α,β-unsaturated carbonyl system. These are abundant in edible plants and are considered to be precursors of flavanoids and isoflavonoids.

Chalcones possess conjugated double bond and a completely delocalized π-electron system on both benzene rings. The complete delocalization of π electrons on both the benzene rings makes these compounds more susceptible in undergoing electron transfer reactions .Molecules possessing such systems have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions.

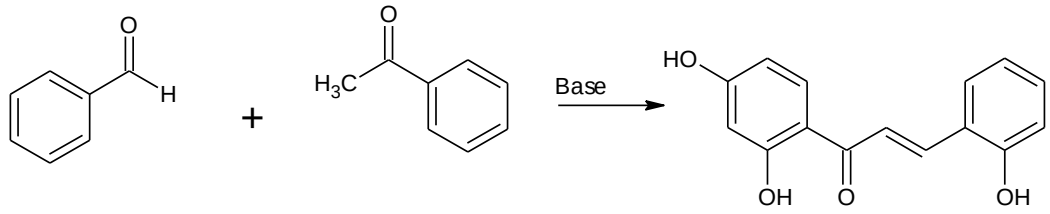
SYNTHESIS OF CHALCONES

Chalcone can be prepared by the reaction between a benzaldehyde and an acetophenone in presence of sodium hydroxide or potassium hydroxide as a catalyst.



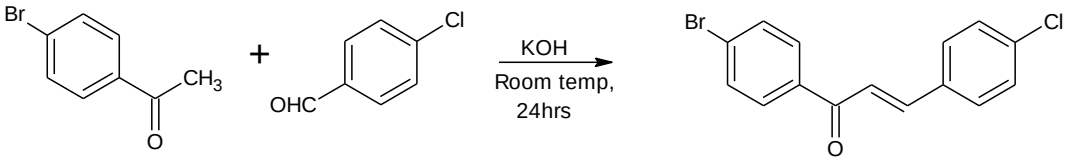
Synthesis of 2', 4', 2-trihydroxy chalcone:[83]

2', 4',2-trihydroxy chalcone was prepared by adding KOH to an equimolar solution of aldehyde and ketone solution in ethyl alcohol .



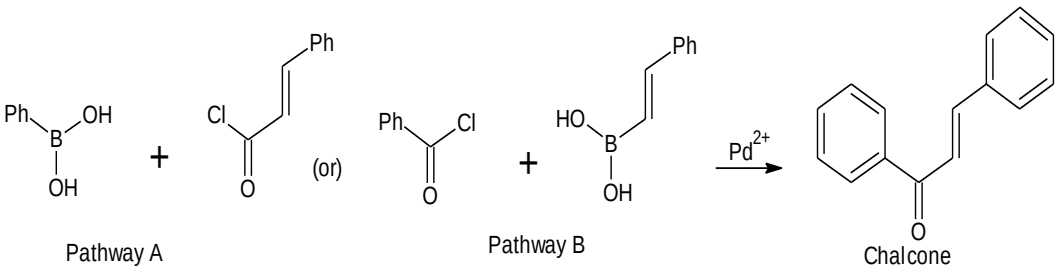
Synthesis of 1-(4'-bromophenyl)-3-(4-chlorophenyl)-2-propen-1-one:[36]

1-(4'-bromophenyl) -3- (4-chlorophenyl) -2- propen-1-one was synthesised by the reaction of 4'-bromo acetophenone and 4-chloro benzaldehyde in the presence of KOH .



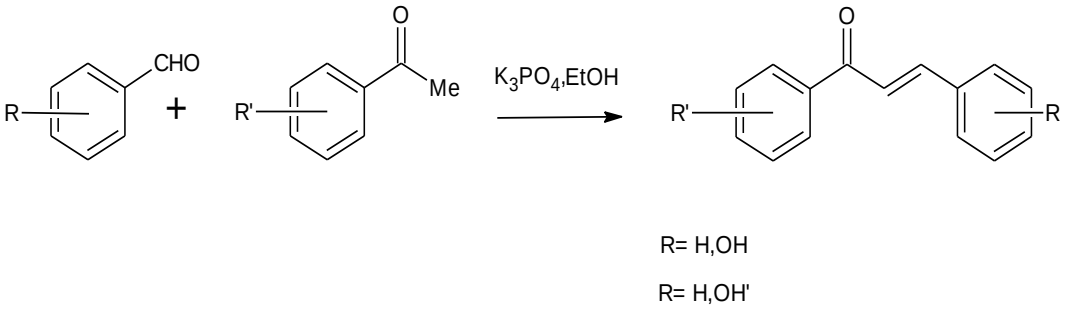
Synthesis of chalcones based on the Suzuki reaction:[84]

Synthesis of chalcones based on the Suzuki reaction either between cinnamoyl chlorides and phenyl boronic acids or between benzoyl chlorides and phenyl vinyl boronic acids are described.



Synthesis of chalcones in the presence of potassium phosphate:[85]

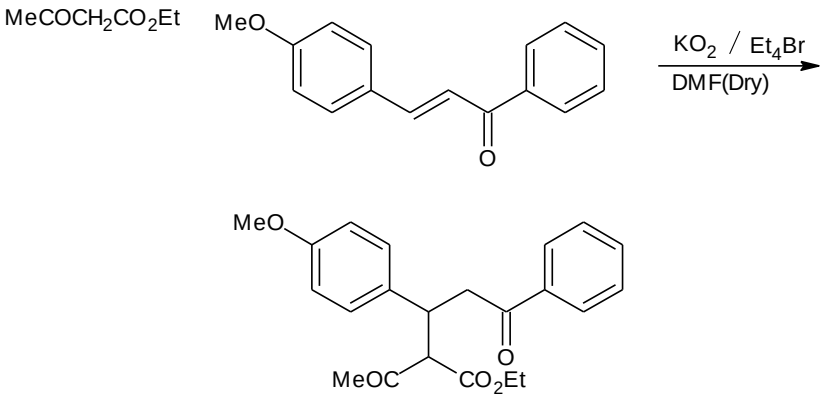
Chalcones are synthesised by the reaction of aldehydes and ketones from the presence of potassium phosphate.



REACTIONS OF CHALCONES

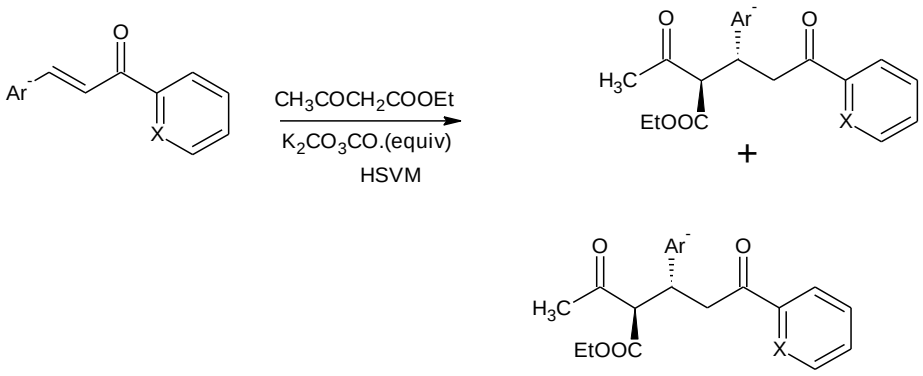
Formation of 3-(4-methoxyphenyl)ethyl 2-acetyl-5-oxo-3,5-diphenylpentanoate:[86]

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



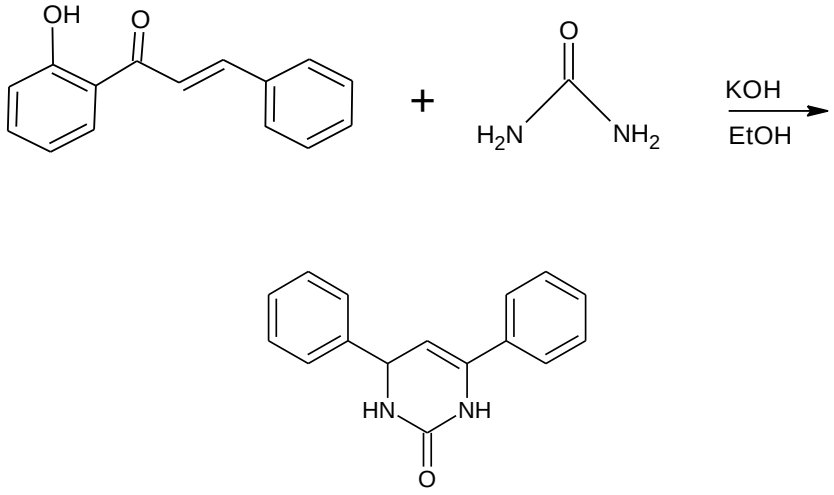
Mechanochemical reactions of chalcones:[87]

Chalcones react with ethylacetoacetate catalysed by K_2CO_3 under solvent free condition.



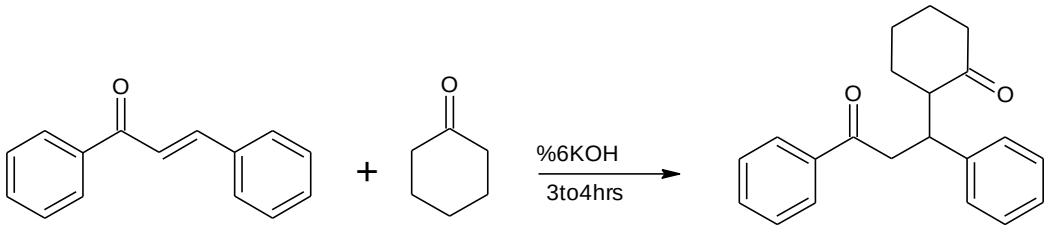
Formation of dihydropyrimidine under ultrasound irradiation:[88]

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



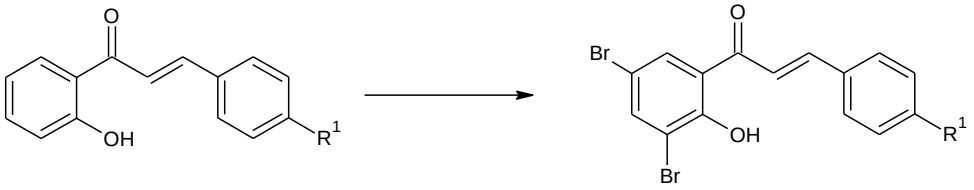
Formation of cyclohexanone derivative:[89]

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



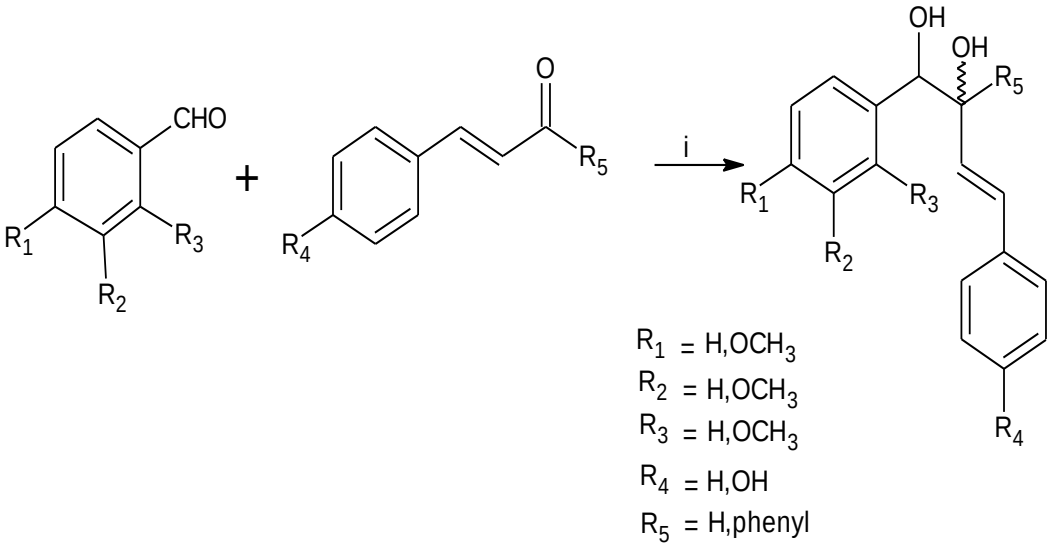
Reactions of chalcones with N-bromo succinamide:[90]

Chalcones react with N-bromo succinamide to form dibromo chalcones .



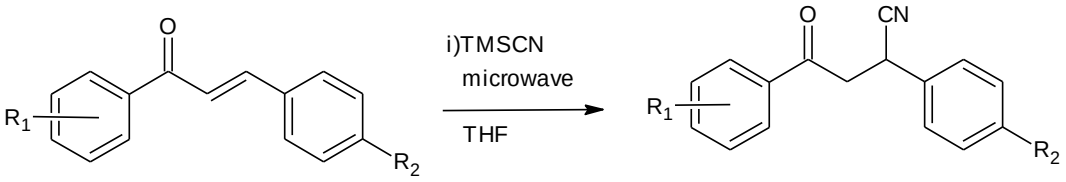
Formation of substituted but-3-ene-1,2-diol:[91]

Chalcones and aldehydes react together to form but-3-ene-1,2-diol in the presence of indium/indium trichloride in aqueous media.



Hydrocyanation of chalcones with trimethyl silyl cyanide:[92]

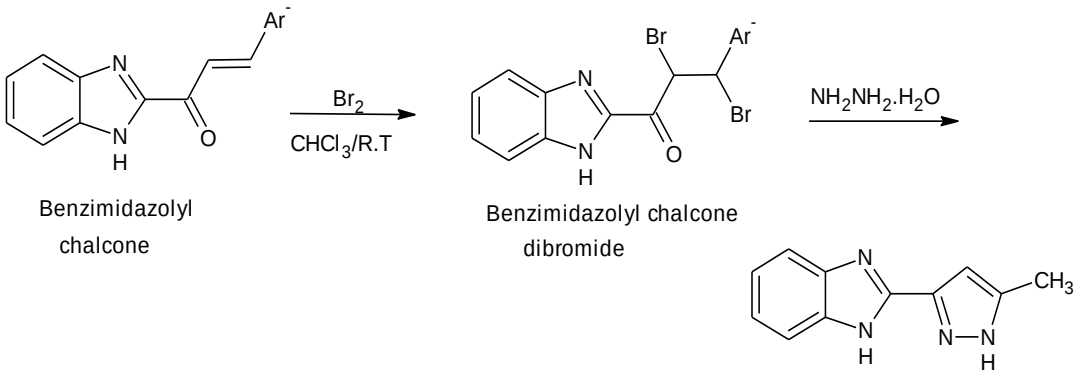
β-cyanoketones are formed by the reaction of chalcones with trimethyl silyl cyanide (TMSCN) .



R_1= H
R_2= OH

Formation of 3-benzimidazolyl-5-aryl-2-pyrazole:[93]

The reaction of benzimidazolyl chalcone with bromine in chloroform gave dibromochalcone which underwent condensation with hydrazine hydrate to form 3-benzimidazolyl-5-aryl-2-pyrazole .



Benzimidazolyl chalcone

Benzimidazolyl chalcone dibromide

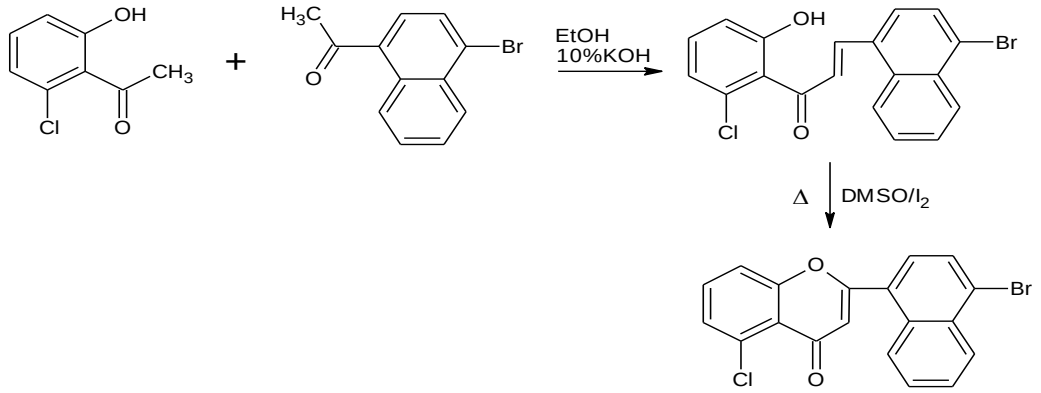
3-Benzimidazolyl-5-aryl-2-pyrazole

Preparation of 1-thiocarbamoyl-3,5-diphenyl-4,5-dihydro-1H-pyrazole:[94]

Chalcones condensed with thiosemicarbazide in ethanol and KOH under ultrasound irradiation to form 1-Thio carbomyl-3,5-diphenyl-4,5-dihydro-1H-pyrazole .

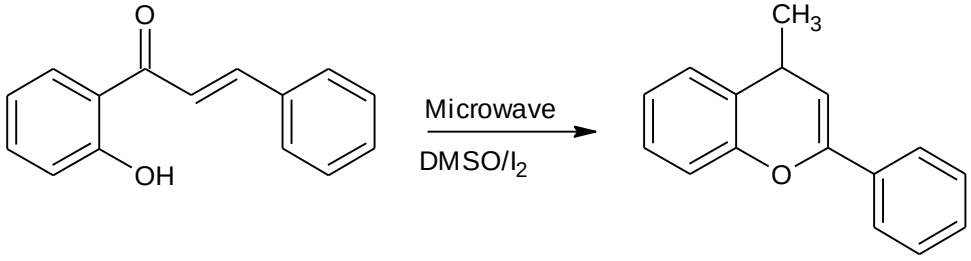
Formation of 2-(4-bromonaphthalene-1-yl)-6-chloro-chromen-4-one:[95]

It was formed by the reaction of chalcone with DMSO (dimethyl sulphoxide) and iodine.



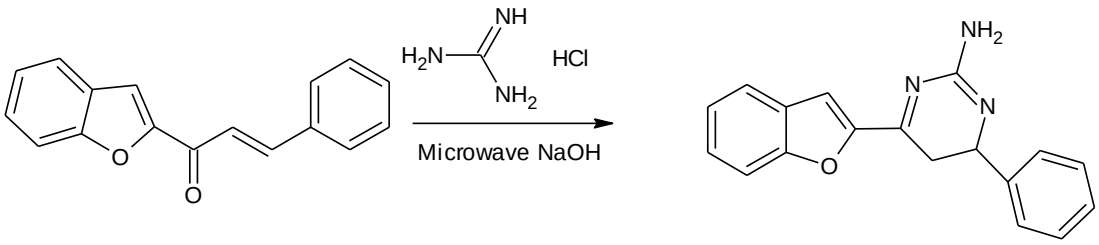
Formation of flavone from chalcone-2'-ol:[96]

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



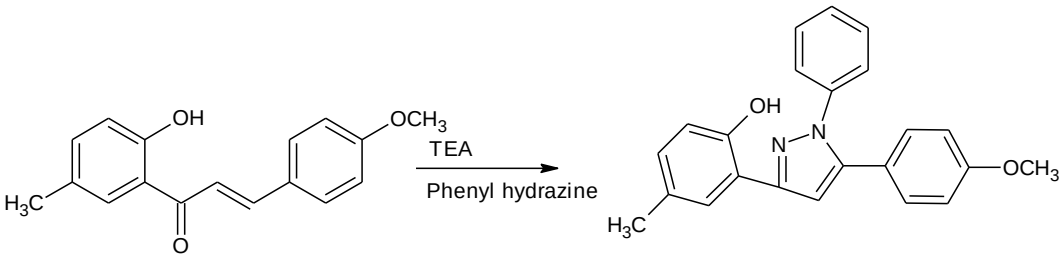
Formation of pyrimidine by microwave irradiation of chalcones:[97]

Benzofuro-3-aryl prop-2-en-1-one is react with guanidine hydrochloride by microwave irradiation to form 6-(1-benzofuran-2-yl)-4-phenyl-4,5-dihydropyrimidin-2-amine .



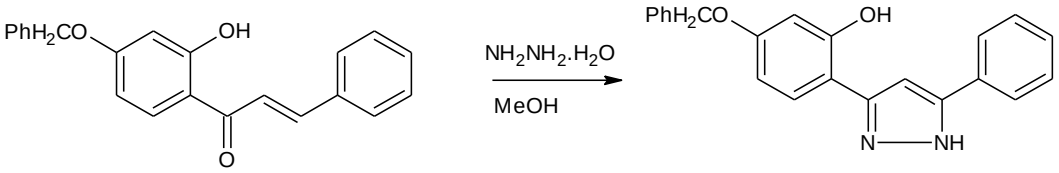
Formation of pyrazole from chalcone dibromide:[98]

Chalcone dibromide react with phenylhydrazine and TEA (Triethanolamine) to form pyrazole.



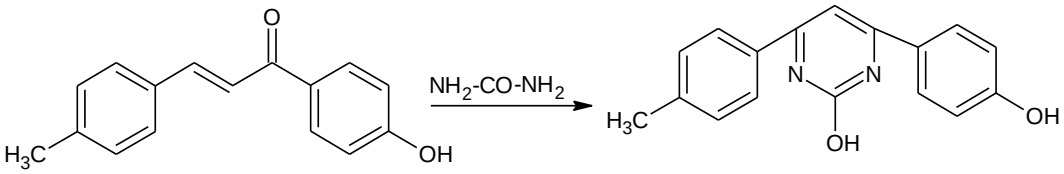
Formation of 3-(2'-hydroxy-4'-benzyloxy phenyl)-5-phenyl pyrazole from chalcone:[99]

Chalcone reacts with hydrazine hydrate and methanol to form 3-(2'-hydroxy-4'-benzyl oxy phenyl)-5-phenyl pyrazole.

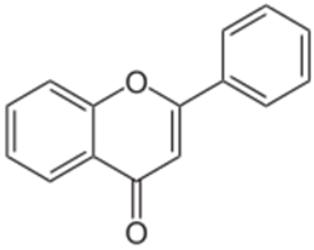


Formation of pyrimidine from chalcone:[100]

Chalcone reacts with urea to form pyrimidine.



FLAVONOIDS [101]



Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants. Their basic structure is a skeleton of Diphenyl propane, namely two benzene rings linked by a three carbon chain that forms a closed pyran ring. Therefore, their structure is also referred to as C_6-C_3-C. . They are synthesized by phenylpropanoid pathway. Chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization .According to the IUPAC nomenclature, they can be classified into:

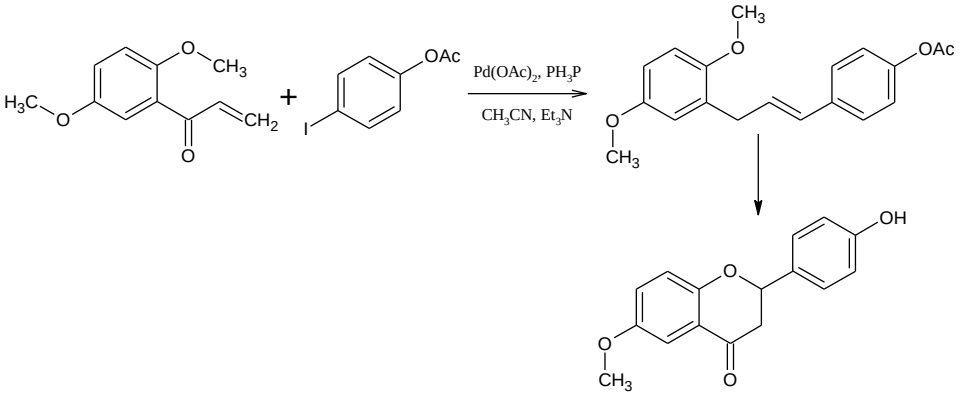
- isoflavonoids, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure
- neoflavonoids Flavonoids or bioflavonoids
- derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

Flavonoids have ability to induce human protective enzyme systems. The number of studies has suggested protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases.

SYNTHESIS OF FLAVONOIDS

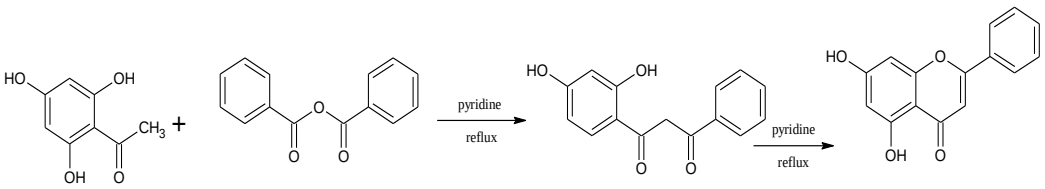
Synthesis of flavonoids via Heck reaction [102]

Synthesis of flavonoids can be achieved by using the Heck reaction. The key step involves the coupling of an aryl vinyl ketone with an aryl iodide. This procedure affords the flavonoid moiety in a single step.



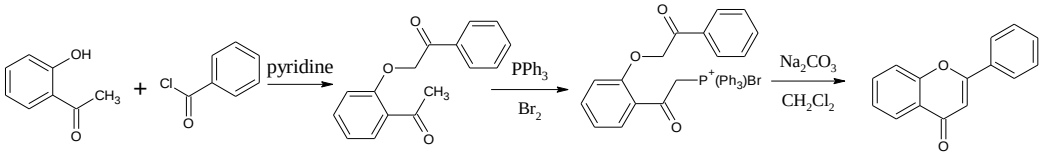
Allan-Robinson synthesis of flavonoids [103]

Allan-Robinson synthesis involves reaction of 2′-hydroxyacetophenone and benzoic acid anhydride in hot pyridine to give a 1, 3-diketone as the key intermediate and subsequent cyclisation in acidic medium gave the flavones.



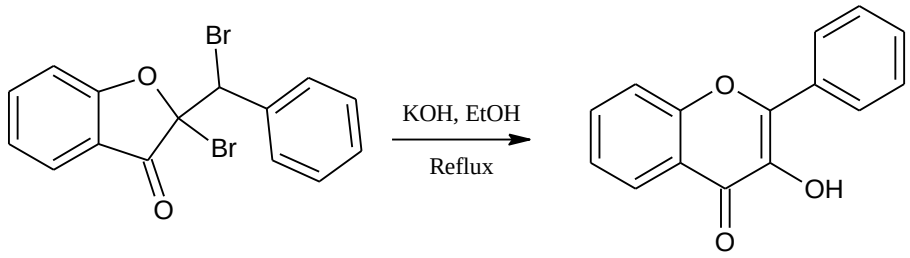
Synthesis of flavonoids via intra molecular Wittig reaction [103]

Acylation of the acetophenone with benzoyl chloride followed by reaction with bromine triphenylphosphine gives the corresponding phosphonium bromide, which then undergoes ring closure via intramolecular olefination of ester carbonyl group to afford the flavone.



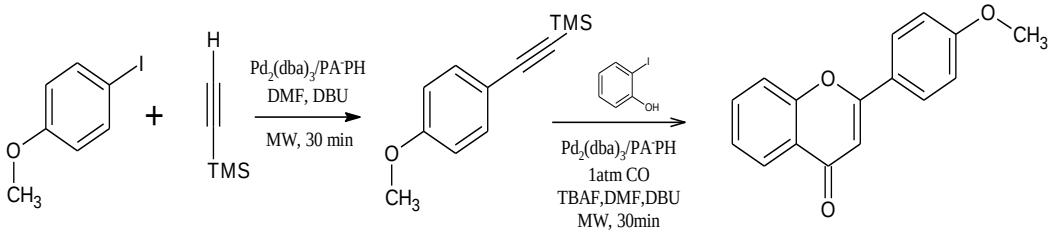
Auwers flavone synthesis [104]

The Auwers flavones synthesis involves treatment of a dibromoaurone with alcoholic alkali to give 3-hydroxyflavone.



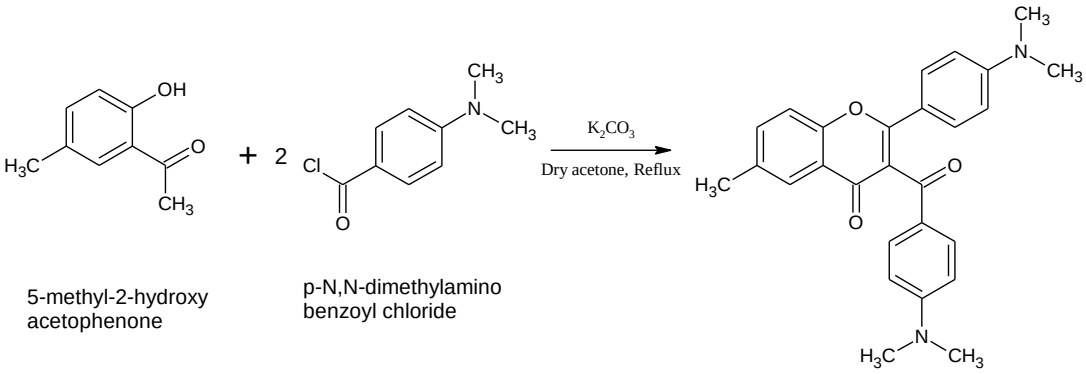
Synthesis of flavonoids by Sonogashira-Carbonylation-Annulation reaction [105]

This method involves the synthesis of flavones via the Pd catalyzed Sonogashira-Carbonylation-Annulation reaction between 2-iodophenols and terminal alkynes.



Synthesis of 3[4'-Dimethylamino] benzoyl-6-methyl-2[4'-dimethylamino] phenyl-2,3-dihydro- 4H-chromen-4-one, (substituted 3-benzoyl flavone) by modified Baker-Venkatraman Reaction [79]

Synthesis of 3[4'-Dimethylamino] benzoyl-6-methyl-2[4'-dimethylamino] phenyl-2,3-dihydro- 4H-chromen-4-one was synthesised by the reaction of 5-methyl-2-hydroxy acetophenone and p-N,N-dimethylamino benzoyl chloride in presence of K_2CO_3.

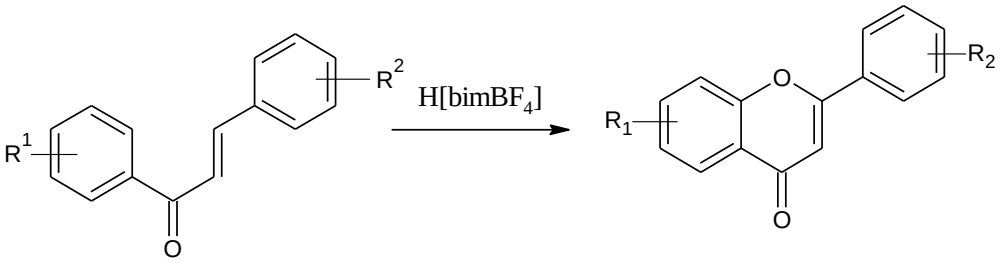


5-methyl-2-hydroxy acetophenone

p-N,N-dimethylamino benzoyl chloride

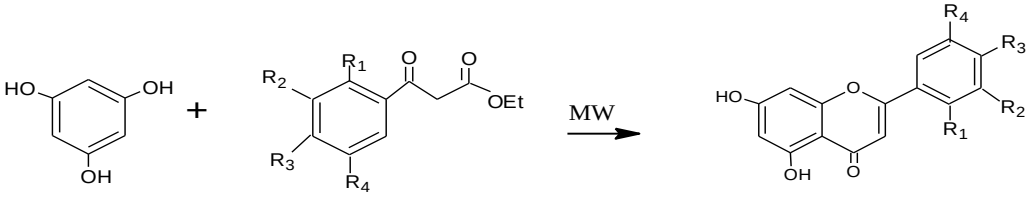
Synthesis of Flavonoid using ionic liquid catalyst [106]

Substituted α-β-unsaturated carbonyl compounds which are chalcones are converted to corresponding substituted 2-phenylchroman-4-one i.e. flavanone by grinding at room temperature using ionic liquid catalyst, H [bimBF4].



Solvent-Free Synthesis of Functionalized Flavones under Microwave Irradiation [107]

It involves the direct solvent-free synthesis of flavones is achieved by microwave irradiation of phloroglucinol and *β*-ketoesters. The reaction goes through a cycloaddition of an α-Oxo ketene intermediate followed by an uncatalyzed thermal Fries rearrangement.



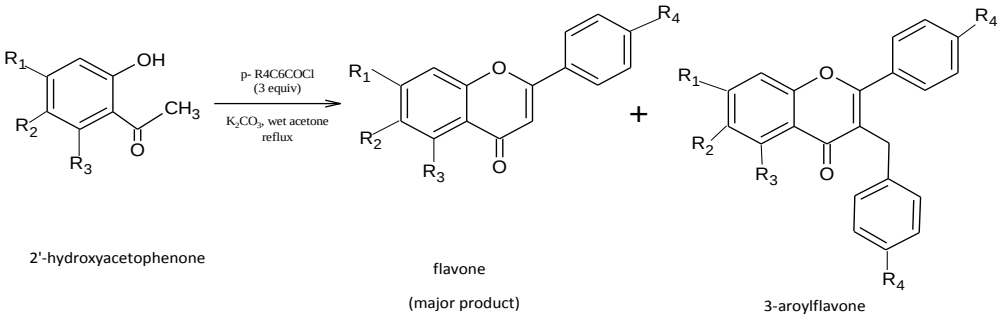
phloroglucinol

β-keto esters

flavone

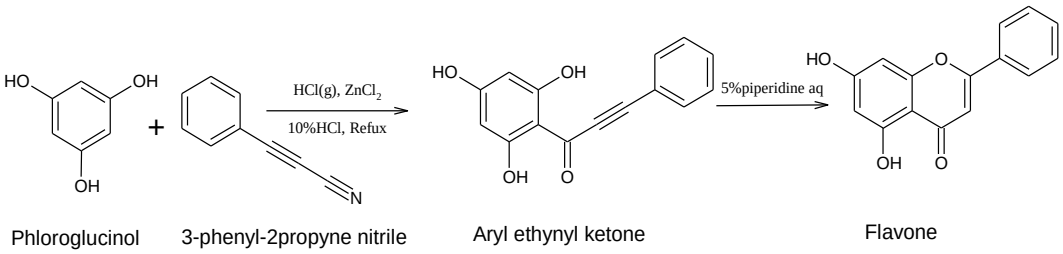
An efficient one-pot synthesis of flavones [108]

2'-hydroxy acetophenone was treated with 3 equivalents of aroyl chloride in wet K_2CO/acetone to afford a good yield of flavone and a smaller yield of 3-aroyl flavone. The reaction proceeds via triketone intermediate.



Synthesis of flavone via a Houben-Hoesch reaction [103]

Coupling of phloroglucinol and 3-phenyl-2-propynenitrile to afford an aryl ethynylketone, which was cyclised in aqueous piperidine to give flavone.



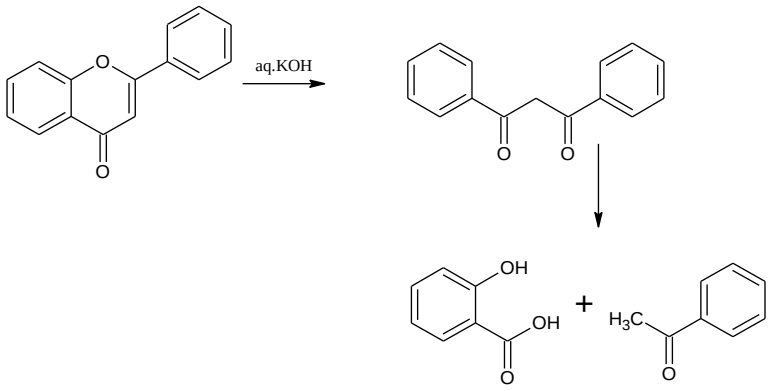
REACTIONS OF FLAVONOIDS

The chemistry of flavones is quite simple and a variety of reactions occur depending on the reagents used and the functional groups present. In general, they possess three functional groups viz. hydroxyl, carbonyl, and conjugated double bond; hence they give characteristic reactions of all three functional groups.

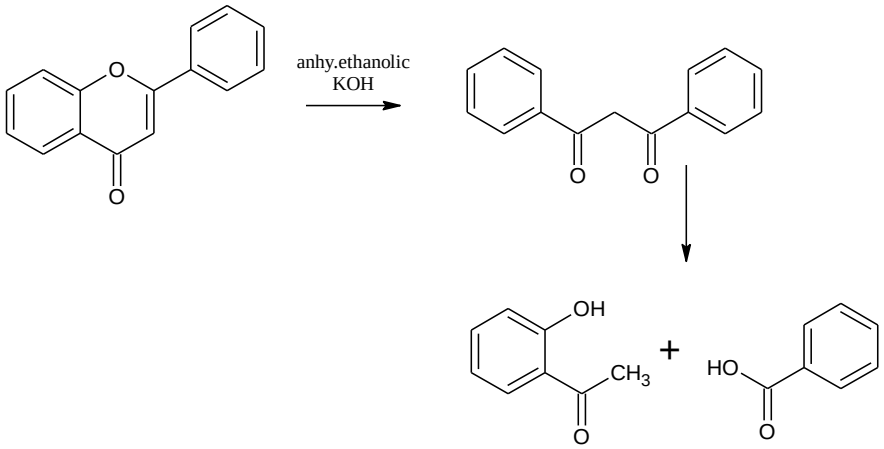
Degradation in presence of base[103]

The structure of flavones is determined mainly by identification of alkaline degradation products. Degradation by using anhydrous ethanolic KOH and aqueous KOH cleaves the molecules in two different ways.

a)



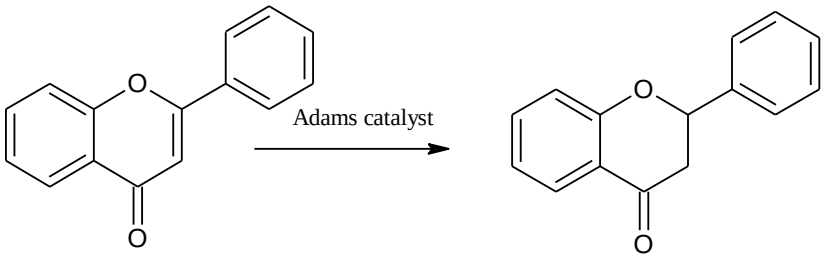
b)



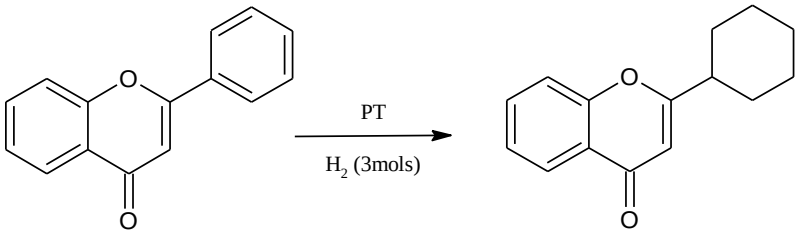
Reduction Reactions [103]

Catalytic Reduction

a) Flavones on reduction with Adam's catalyst, the 2,3-olefenic bond is reduced to yield flavonone.

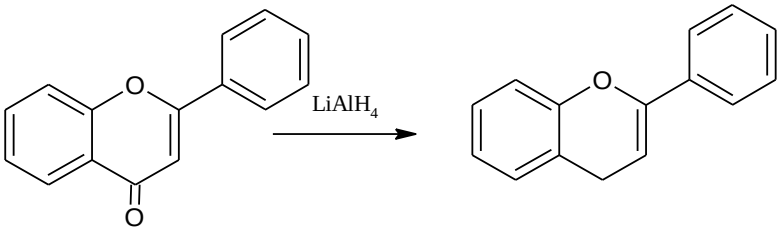


b) Structure of flavone is interrupted after the absorption of 3 mole of hydrogen in presence of platinum, resulting in the reduction of B ring.



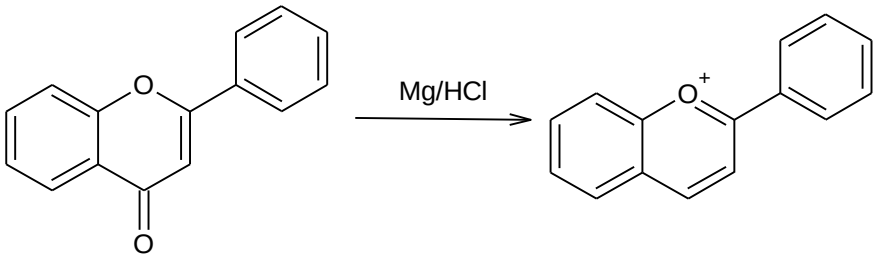
Reduction with Lithium Aluminium Hydride

Uncontrolled reduction of flavone will occur with Lithium Aluminium Hydride to yield flavenes.



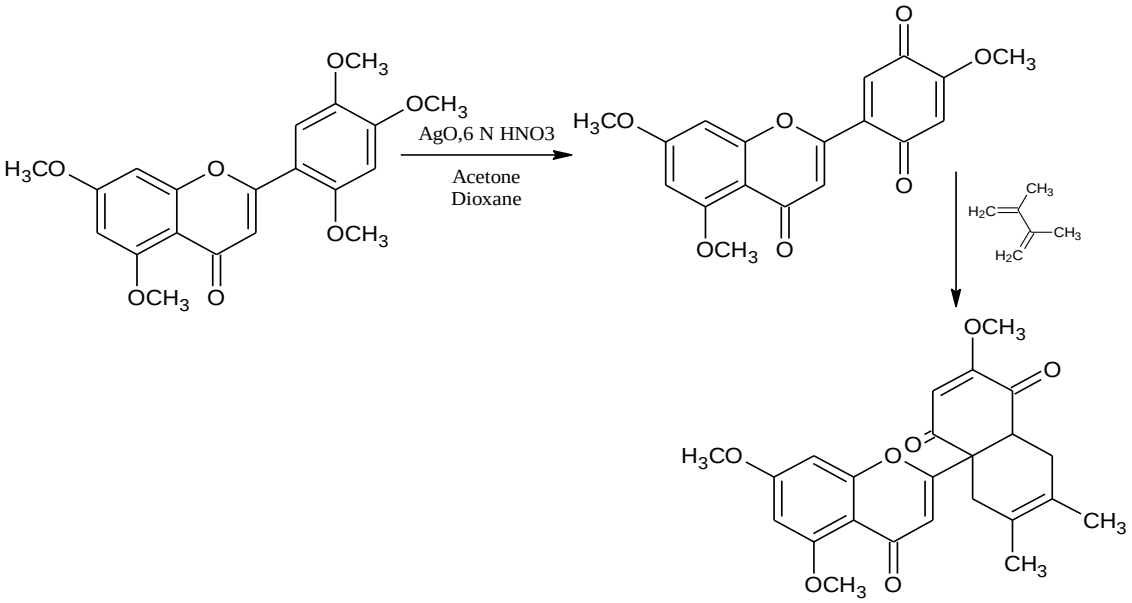
Shinoda test

In this keto group of the C ring is reduced with a shift in the bonds on that part of the ring, resulting in the formation of flavylium ion.



Oxidation reactions[103]

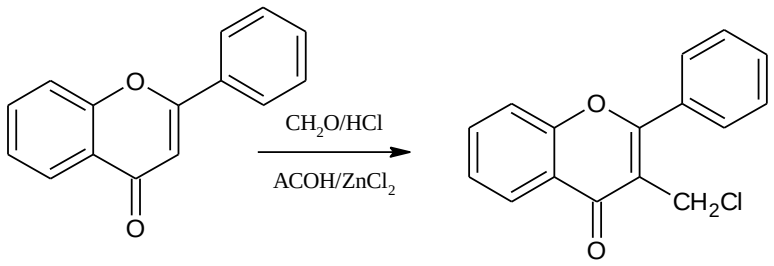
3',4',5,6',7-penta methoxy flavone is oxidised with silver 0xide and Nitric acid, only the methoxy group at 1,4 orientations are oxidised leading to the formation of a quinoline intermediate, which is a diel's alder adduct with butadiene.



Reaction with electrophiles

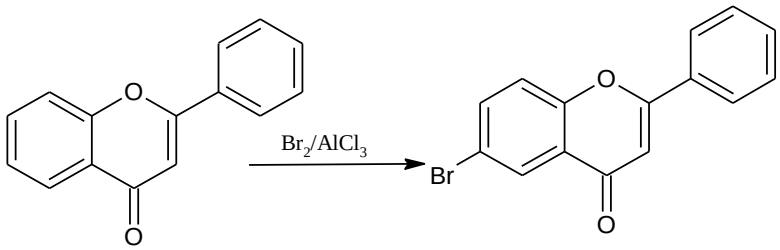
a) Chloromethylation

Chloromethylation occurs at C_3 position by the reaction with formaldehyde in the presence of HCl or Acetic acid with $ZnCl_2$.



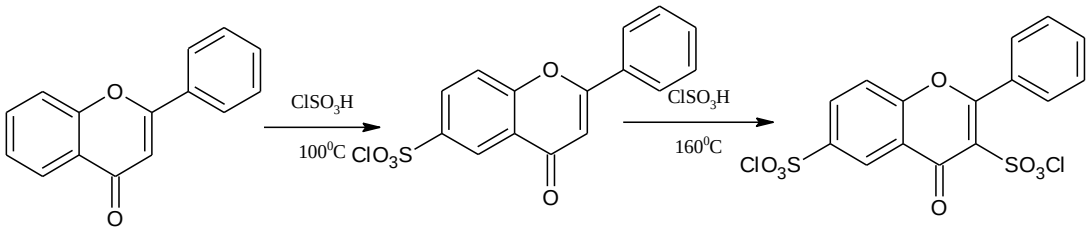
b) Bromination

Reaction with bromine in the presence of excess of $AlCl_3$ yields bromo flavone.



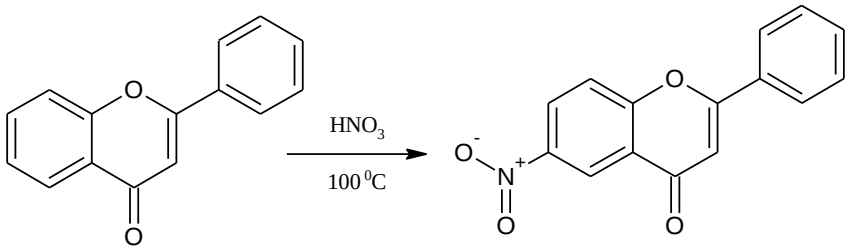
c) Sulphonation

Sulphonation of flavone with chlorosulphonic acid at 100^0C yields the 6-sulphonylderivative, but at 130-140^0C a second substituent is introduced to give 3,6-disulphonyl chloride.



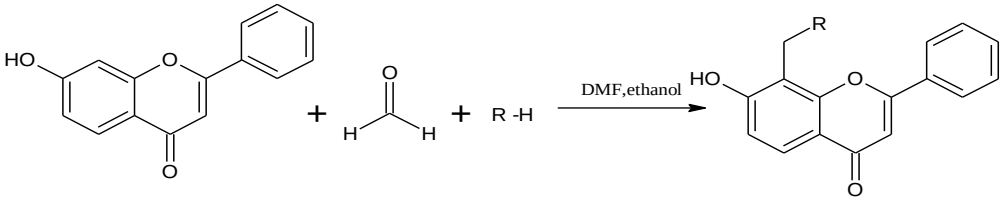
d) Nitration

Nitration occurs when flavone reacts with conc.HNO_3 at 100°C. It occurs mainly at C_6 position.



e) Mannich reaction

This is a multi-component condensation which involves an enolizable carbonyl compound, formaldehyde and a primary or secondary amine, resulting in the formation of a mannich base.

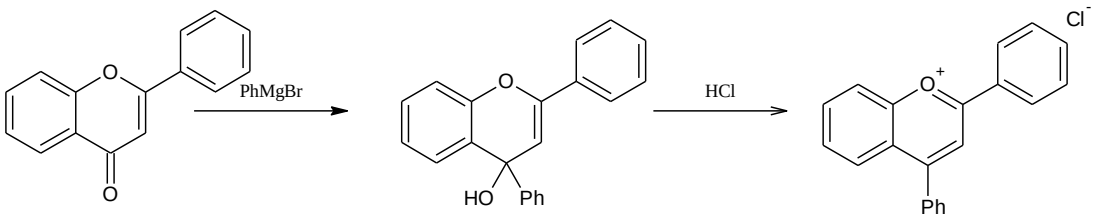


R- any 1^0 or 2^0 amino compound

Reaction with Carbon nucleophiles[109]

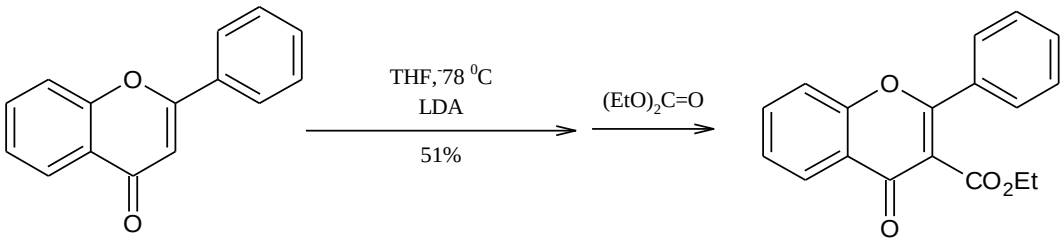
Reaction with Grignard reagent

Grignard reagent reacts with flavone at the carbonyl carbon resulting in flavonols and can be converted by acidinto the corresponding 4-substituted 1-benzopyrylium salt.



Reaction with Organometallic reagents

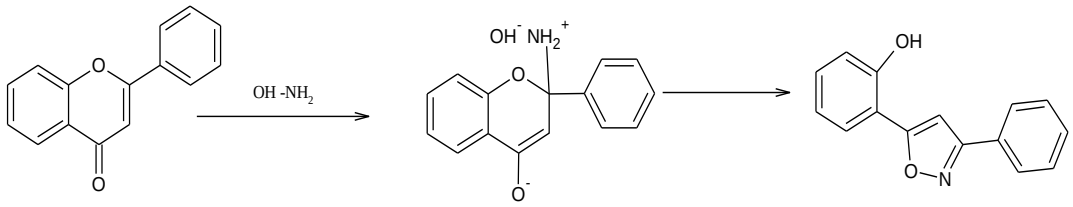
Flavone can be lithiated at C_3.



Addition/Condensation reactions [103]

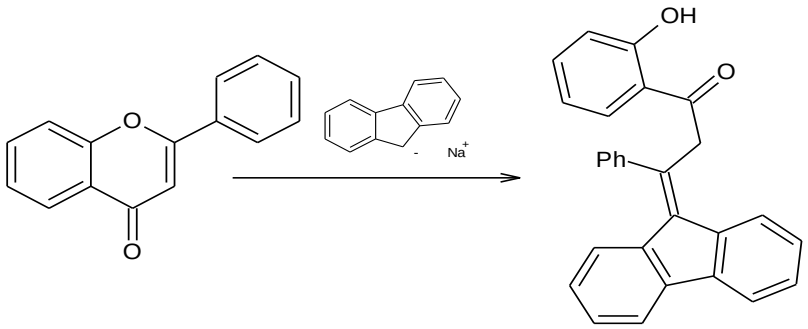
a) Reaction with hydroxylamine

Flavone on reaction with hydroxylamine yields phenyl-isoxazoles instead of expected oximes. This is due to the mesomeric effect of phenyl group as well as the existence of the non-canonical resonance form.



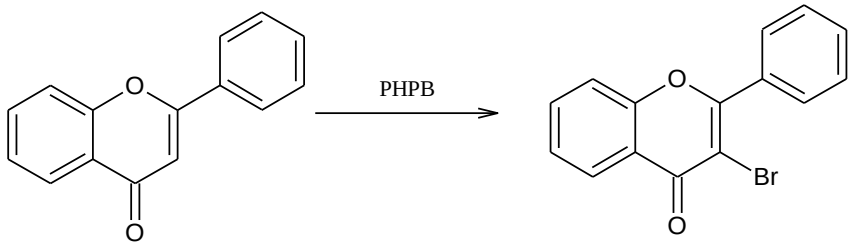
b) 1,4-dipolar addition to flavone

Flavone condenses with 9-fluoro enyl sodium to give the 1,4-addition product 2-hydroxy-β-fluorenylidene-β-phenyl-1-propiophenone.



Substitution Reaction

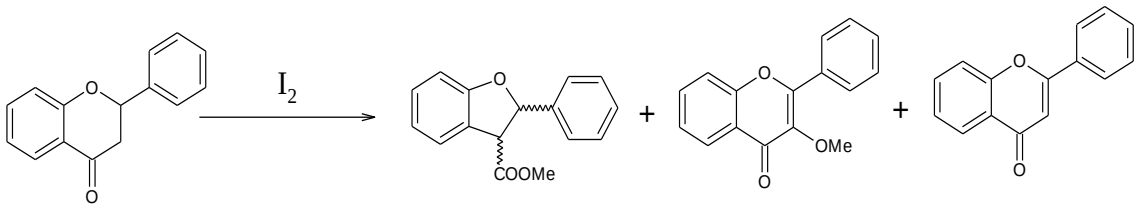
Flavone with reagents like pyridine hydrobromide per bromide (PHPB) without using mercury salts forms substituted product.



Ring Contraction Reactions [110]

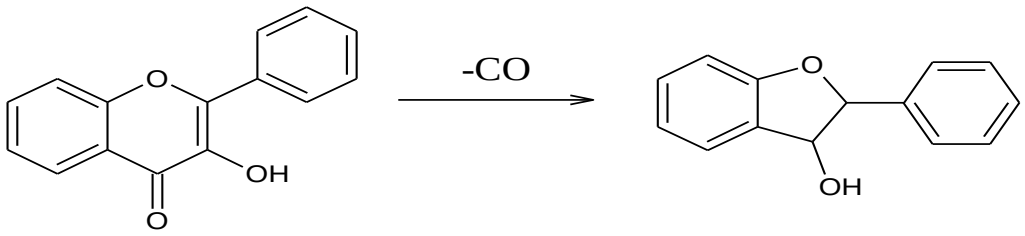
a) Reaction with iodine

Reaction of flavonone with Iodine results in the formation of a mixture of 3 products-dihydro benzofuran derivative,3-methoxy flavone and flavones.



b) Carbon monoxide elimination reaction

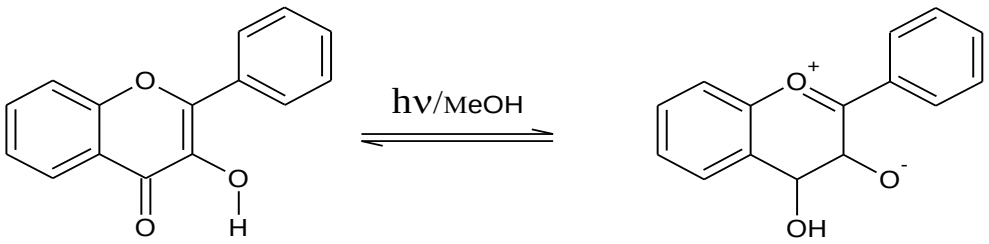
In some special conditions elimination of carbon monoxide from flavonoids will results in a ring contraction product.



Photochemical Reactions [111]

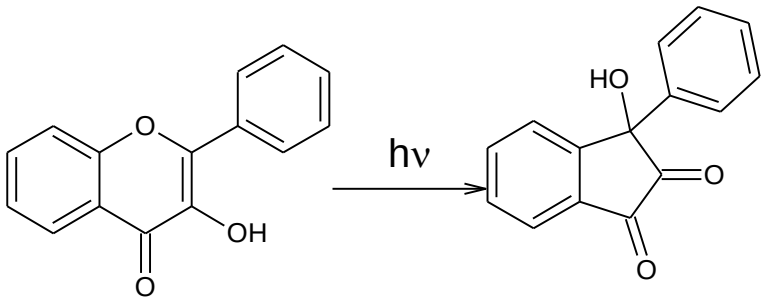
a) Photo tautomerization reaction

Flavonoids undergo photo tautomerism by the interconversion of isomeric forms in equilibrium by the migration of hydrogen atom.



b) Photo rearrangement Reaction

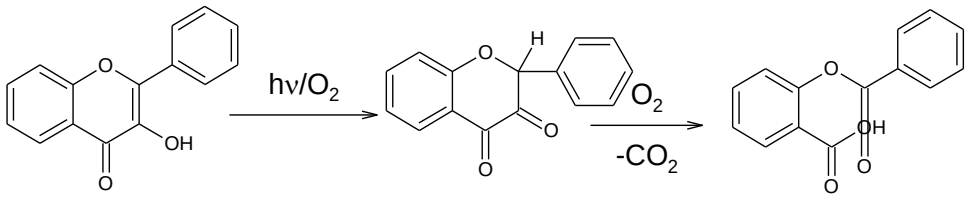
3-hydroxy flavone undergo photo rearrangement leading to the formation of indan-1,2-diones.



c) Photo oxidation Reaction

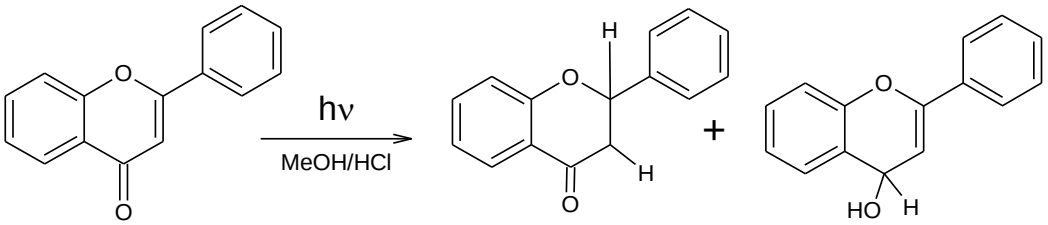
Photo oxidation of 3-hydroxy flavone has been found to yield mixture of two products two products through the reorganization and degradation.

H_2O



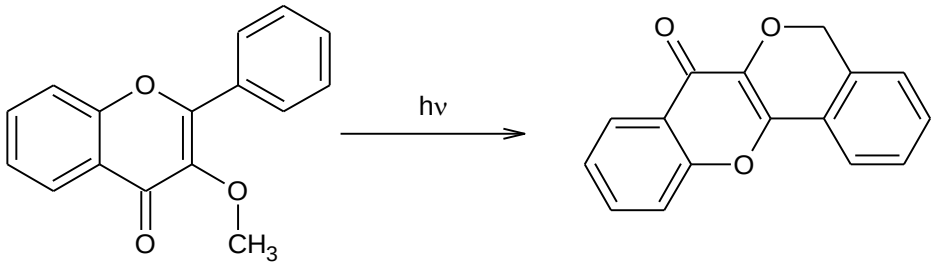
d) Photoreduction Reaction

Photochemical reduction of flavones results in the formation of two products which were obtained by the reduction of 2,3 double bond and >C=O group.



e) Photoinduced H- abstraction

Phototransformation of3-alkoxy flavones resulted in the formation of a tetracyclic compound instead of a ring contracted product,which could be ascribed from the intramolecular H-abstraction by photoexcited C=O group.



OBJECTIVE OF THE STUDY

High blood pressure or hypertension is a major cause of morbidity and mortality because of its association with coronary heart disease, cerebrovascular disease and renal disease. The extent of target organ involvement (i.e. heart, brain and kidneys) determines outcome. Worldwide, hypertension is estimated to cause 7.5 million deaths, about 12.8% of the total of all deaths and this also accounts for 57 million disability adjusted life years (DALYS) or 3.7% of total DALYS. The increasing prevalence of the condition is blamed on lifestyle and dietary factors such as physical inactivity, alcohol and tobacco use and a diet high in sodium.

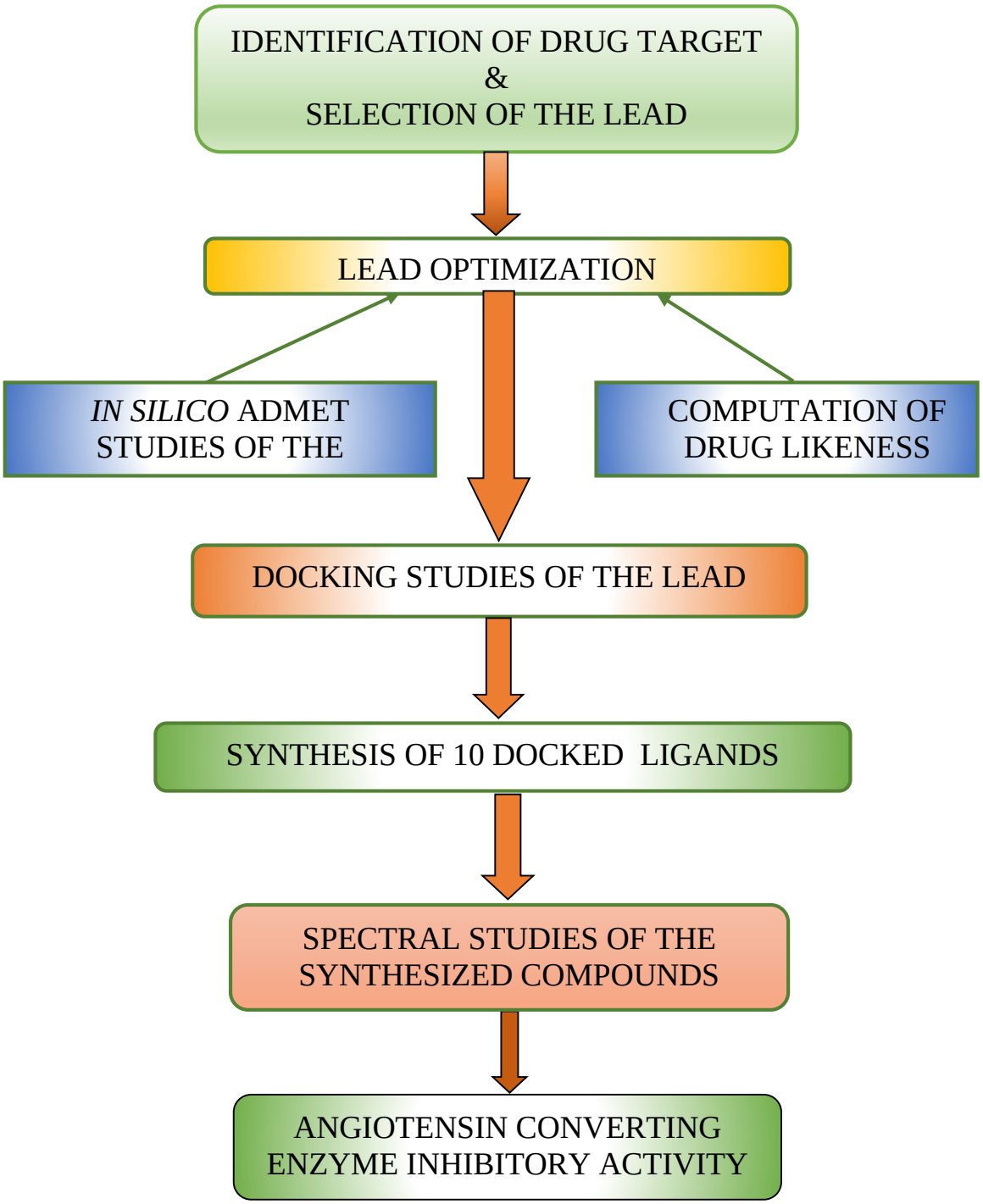
Angiotensin Converting Enzyme (ACE), the central component of the Renin-Angiotensin System (RAS) controls the blood pressure by regulating the volume of fluids in the body. It converts the hormone angiotensin 1 to the angiotensin ll. Therefore ACE indirectly increases blood pressure by causing blood vessels to constrict.

The inhibition of ACE is considered as one of the most effective therapeutic strategy for the treatment of Hypertension. Enzyme inhibitors are used as potent therapeutic agents for the treatment of various diseases. More than 100 drugs used worldwide are enzyme inhibitors. ACE inhibitors such as captopril, enalapril, fosinopril and ramepril currently available in the market; exert antihypertensive effect by competitively binding to the active site of ACE.

Flavonoids are fairly versatile compounds and are easy to synthesize. They are associated with a wide range of pharmacological properties including antimicrobial, antioxidant, anticancer, anti-inflammatory activities. In addition, literature review also revealed that flavonoids possess ACE inhibitory activity.

The present study focuses on the development of certain flavonoid derivatives in order to explore the extent of their antihypertensive property and to identify the correct conformations of ligands in the active site of protein and to predict the affinity of the ligands towards the protein. Drug discovery tools have been utilized in designing new chemical entities which are safe and effective without consuming much of the research hours.

PLAN OF WORK


IDENTIFICATION OF DRUG TARGET
&
SELECTION OF THE LEAD
LEAD OPTIMIZATION
IN SILICO ADMET
STUDIES OF THE
COMPUTATION OF
DRUG LIKENESS
DOCKING STUDIES OF THE LEAD
SYNTHESIS OF 10 DOCKED LIGANDS
SPECTRAL STUDIES OF THE
SYNTHESIZED COMPOUNDS
ANGIOTENSIN CONVERTING
ENZYME INHIBITORY ACTIVITY

EXPERIMENTAL SECTION

IN SILICO STUDIES

Softwares and Databases used

- Accerlys discovery studio viewer
- Molinspiration server
- 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 antihypertensive activity and the target selected for the work is **Angiotensin converting enzyme (1o86).**
- Based on various literature reviews and current researches, the active binding sites required for exhibiting antihypertensive activity was obtained.
- The pdb structure of the target enzyme is downloaded from RCSB protein data bank.

SELECTION OF LEAD

The lead compound flavonoid was selected based on several literature reviews. Many flavonoid derivatives were reported to have ACE inhibiting activity.

LEAD OPTIMIZATION

The lead moiety selected was optimized by substituting specific groups in order to enhance the safety and efficacy of the compounds. This process was performed by evaluating pharmacokinetic property of the compound. Toxicity studies are performed by the evaluation of drug likeness properties.

Evaluation of drug likeness properties[112]

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

Calculation of Lipinski's rule of five

1. Open the molinspiration home page.
2. Click calculation of molecular properties of drug likeness
3. Draw the structure of SB I in JME window.
4. Click calculate properties.
5. Save the properties

Rests of the compounds are observed in the same manner.

RESULTS AND DISCUSSION

Drug likeness scores of SB 1-5

Table 1. Drug likeness scores of SB 1-5 using Molinspiration

Sl. No	Compound Code	Log P	Molecular Weight	Hydrogen acceptors	Hydrogen donors	No. of Violations
1	SB1	3.78	403.39	7	3	0
2	SB2	4.20	432.39	9	2	0
3	SB3	4.05	415.40	7	2	0
4	SB4	4.94	421.84	6	2	0
5	SB5	3.60	433.42	8	3	0

Drug likeness scores of BF1-5

Table 2. Drug likeness scores of BF 1-5 using molinspiration

Sl. No	Compound code	Log P	Molecular Weight	Hydrogen acceptors	Hydrogen donors	No. of Violations
1	BF1	4.49	432.34	10	1	0
2	BF2	3.88	522.11	10	1	1
3	BF3	5.88	411.24	4	1	1
4	BF4	4.68	402.40	6	1	0
5	BF5	7.18	480.13	4	1	1

In vivo absorption abilities of the designed molecules were assessed by means of Lipinski's rule of five that predicts a compound administered orally will more likely to have a good absorption or permeation. Most of the designed compounds satisfy the rule; some shows violation. This indicates that all the ligands SB 1-5 and BF1-5 have good oral absorption.

DOCKING STUDIES FOR THE LEAD

Aim	:	To predict the bioactivity score of the ligand
Database	:	RCSB protein data bank
Protein selected	:	Angiotensin Converting Enzyme (1o86)

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

Steps involved in docking studies [113-115]

Running docking process 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
- Docked result from .dlg file
- Viewing docked confirmation
- Taking snapshots of the interactions.

STEP1:

Refinement of protein structure

Protein (1o86) downloaded from protein data bank and it was refined before docking. Refinement of downloaded protein involves the removal of water, hetero atoms and bound ligands if any.

The steps involved are

1. Open Accerlys discovery studio viewer.
2. File → open → Protein (downloaded from PDB).
3. View→ Hierarchy.
4. Click water molecules.
5. Select all water molecules.

6. Give right click and cut.
7. Select ligand, give right click and cut.
8. Save the molecule in desired area.

The 1o86.pdb is refined by the above method.

STEP 2:

Ligand File Format Conversion:

1. The ligands **SB 1-5** and **BF 1-5** are drawn in chemsketch software.
2. Tools→ Generate →SMILES notation [Simplified Molecular Input Line Entry System, which is a file format]
3. Save the SMILES in a word document.
4. Open the online smiles translator
5. Upload the SMILES.
6. By choosing the required file format we can save the file.

Here, we are saving it as .pdb format in Cygwin/usr/local/bin. The protein and ligand files which are prepared by above methods were used for docking.

STEP 3:

DOCKING

Docking was performed using AutoDock 4.2 and requires a refined protein and ligands in .pdbqt format.

Steps involved are as follows;

Conversion of refined protein to protein.pdb

Open Auto dock tool

- Open ⟶ Read molecule $\xrightarrow{\text{look in}}$ Refined Enzyme (1o86)
- Select ⟶ Select from string $\xrightarrow{\text{resdue}}$ Type(*HOH) ⟶ add ⟶ dismiss

- Edit ⟶ Hydrogen ⟶ Add ⟶ Ok
- Edit ⟶ Charges ⟶ Add Kollman Charges ⟶ Ok
- File ⟶ Save ⟶ Write PDB $\xrightarrow{\text{save}}$ Cygwin/usr/local/bin ⟶ Ok
- Edit ⟶ Delete all molecules ⟶ Continue.

Refined proteins were converted to pdb format and saved as1086.pdb in folder bin in Cygwin.

Conversion of PDB format of ligand into .pdbqt format

Algorithm:

- Ligand ⟶ Input ⟶ Open (**SB I.pdb**)
- Ligand ⟶ Torsion tree ⟶ Detect root
- Ligand ⟶ Torsion tree ⟶ Show root expansion
- Ligand ⟶ Torsion tree ⟶ Choose torsion
- Ligand ⟶ Torsion tree ⟶ Set no. of torsion
- Ligand ⟶ Torsion tree ⟶ Hide root expansion
- Ligand ⟶ Torsion tree ⟶ Show hide root marker
- Ligand ⟶ Output $\xrightarrow{\text{save}}$ Cygwin as (**SB I.pdbqt**) in Cygwin/usr/local/bin
- Edit ⟶ Delete all molecules ⟶ Continue

SB I.pdb was converted to **SB I.pdbqt**, which is the software acceptable form. Other ligands are done by the same method.

Autogrid Calculation and creating 'gpf' file

Algorithm:

- Grid $\xrightarrow{\text{click}}$ Macromolecule $\xrightarrow{\text{click}}$ Open $\xrightarrow{\textit{Cygwin}}$ protein.pdbqt(**1086.pdbqt**)
- Grid $\xrightarrow{\text{click}}$ Set map types $\xrightarrow{\text{click}}$ Open $\xrightarrow{\textit{Cygwin}}$ Ligand.pdbqt (**SB I.pdbqt**)

- Grid $\xrightarrow{\text{click}}$ Grid box $\xrightarrow{\text{set}}$ dimensions to 60 $\xrightarrow[\text{file}]{\text{click}}$ Close saving current.
- Grid $\xrightarrow{\text{click}}$ Output $\xrightarrow{\text{click}}$ Save as gpf $\longrightarrow$ **SB I.gpf** in cygwin/usr/local/bin

Autodock calculations and creating 'dpf' file

Algorithm:

- Docking $\xrightarrow{\text{click}}$ Macromolecules $\xrightarrow{\text{click}}$ Set rigid file name $\xrightarrow{\text{Open}}$ protein.pdbqt (**1o86.pdbqt**)
- Docking $\xrightarrow{\text{click}}$ ligand $\xrightarrow{\text{Open}}$ ligand.pdbqt (**SBI.pdbqt**)
- Docking $\xrightarrow{\text{click}}$ Search parameters $\xrightarrow{\text{click}}$ Genetic algorithm $\xrightarrow{\text{click}}$ Accept
- Docking $\xrightarrow{\text{click}}$ Docking parameters $\xrightarrow{\text{click}}$ Accept
- Docking $\xrightarrow{\text{click}}$ Output $\xrightarrow{\text{click}}$ Lamarckian GA(4.2) $\xrightarrow{\text{save}}$ **SB I. dpf** in cygwin/usr/local/bin

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

- Open Cygwin terminal

  cd C:

  cd Cygwin

  cd usr

  cd local

  cd bin

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

- **/autogrid4.exe –p SBI.gpf -l SBI.glg**

  The resulting file was obtained in .glg format

- **/autodock4.exe -p SBI.gpf -l SBI.dlg**

  The resulting file was obtained in .dlg format

STEP 4:

Viewing docking results

Algorithm

- ***To open dlg file***

- Analyze $\xrightarrow{\text{click}}$ Docking $\xrightarrow{\text{Open}}$ ligand.dlg (**SBI.pdbqt**)

- ***To obtain binding energy***

- Analyze $\xrightarrow{\text{click}}$ Confirmation $\xrightarrow{\text{click}}$ load

  Thus the binding energy of 10 docked confirmations of the ligands was obtained. Software automatically ranks the energy values in the order of increasing binding energy.

- ***Visualizing docked conformations & Obtaining snap shots of docked pose***

- Analyze → Conformations → Play (Note: & allows changing the ligand's color).
- Load **SBI.dlg** file.
- Choose the conformation.
- Analyze → Macromolecule → choose the **1o86.pdbqt.**
- Analyze → Conformations → Load [double click the desired conformation].
- Analyze → Dockings → Open.
- Analyze → Dockings →Show interactions.
- Protein and ligand interaction will be displayed. Zoom it and increase the contrast by holding right key & shift.
- File → save image → Cygwin/usr/local/bin as .jpeg

The steps above mentioned are done for all the 10 ligands (SB1-SB5 and BF1-BF5)

RESULTS AND DISCUSSIONS

The docking results of **ACE (1o86.pdb)** with the ligands **SB1-5, BF 1-5** and Standard drug **Lisinopril** are reported in the below tables. The best docked structures should have the binding energy lower to the standard. The binding sites are shown in the snapshots and the binding energy compared with the standard drug is given in the table 3 and 4.

Binding energies of SB 1-5 with ACE

Table 3: Binding energies of SB 1-5

Sl.No	Compound Code	Binding Energy Kcal/mol
1	SB 1	-9.39
2	SB 2	-12.29
3	SB 3	-9.47
4	SB 4	-9.56
5	SB 5	-6.49
6	Lisinopril (LIS)	-7.75

Binding energies of BF 1-5 with ACE

Table 4: Binding energies of BF 1-5

Sl.No	COMPOUND CODE	BINDING ENERGY Kcal/mol
1	BF 1	-5.06
2	BF2	-4.58
3	BF3	-8.11
4	BF4	-6.64
5	BF5	-8.63
6	Lisinopril (LIS)	-7.75

In the Schiff bases of flavonoid series (SB1-5), all the ligands except SB5 showed excellent binding interactions with the ACE. Among the derivatives SB2 had shown highest binding energy (-12.29 Kcal/mol) when compared to the standard drug Lisinopril (-7.75 Kcal/mol).The order of binding energy was found to be in the order SB2 (3-nitro) > SB4 (4-chloro)> SB3 (4-formyl) > SB1 (4-hydroxy) > SB5 (4-hydroxy-3-methoxy).

In the substituted benzoyl flavone series (BF1-5), the ligand BF5 (-8.63) showed highest interaction with the enzyme. The order of binding energy of these compounds was found to be BF5 (2,4-dichloro) > BF3 (2-chloro) > BF4 (3-methoxy) > BF1(3-nitro) > BF2 (3,4,5-trimethoxy).

The ligands SB2, SB4, SB3, SB1, BF5, BF3 shown best docked pose to enzyme (1o86).These ligands can be screened for the activity. Though some ligands showed inferior binding interactions, all the selected derivatives were planned to synthesize and to screen for the ACE inhibiting activity.

Binding of lisinopril with ACE

Lisinopril interacts with ACE at GLY 2000, GLU 384, LYS 511, ALA 354,PHE 512, HIS 353, GLU411,TYR 523,ARG 522 and the binding energy was found to be -7.75 Kc/mol.

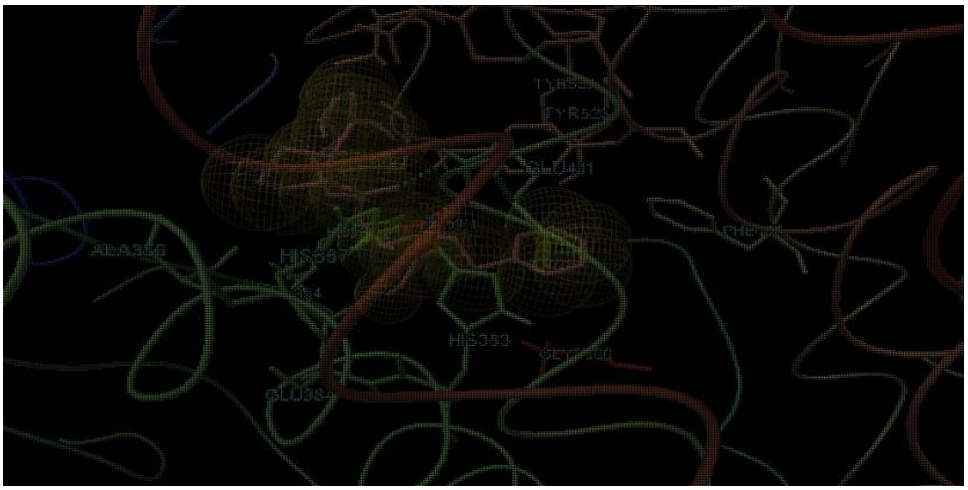


Fig 5: Snapshot of lisinopril binding with 1o86

Binding of SB1-5 with ACE

SB1

SB1 interacts with ACE at ALA 354,GLY 2000,SER 255 and HIS353 and the binding energy was found to be -9.39Kcal/mol

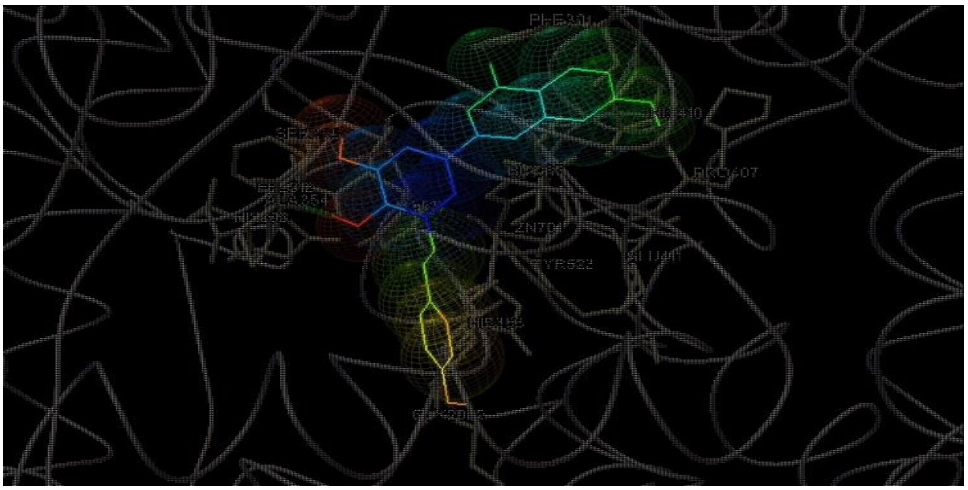


Fig 6: Snapshot of SB1 binding with 1o86

SB2

SB2 interacts with GLY 2000, HIS 353 ,SER 355,TYR 523 and ALA 354 and the binding energy was found to be -12.29Kcal/mol.

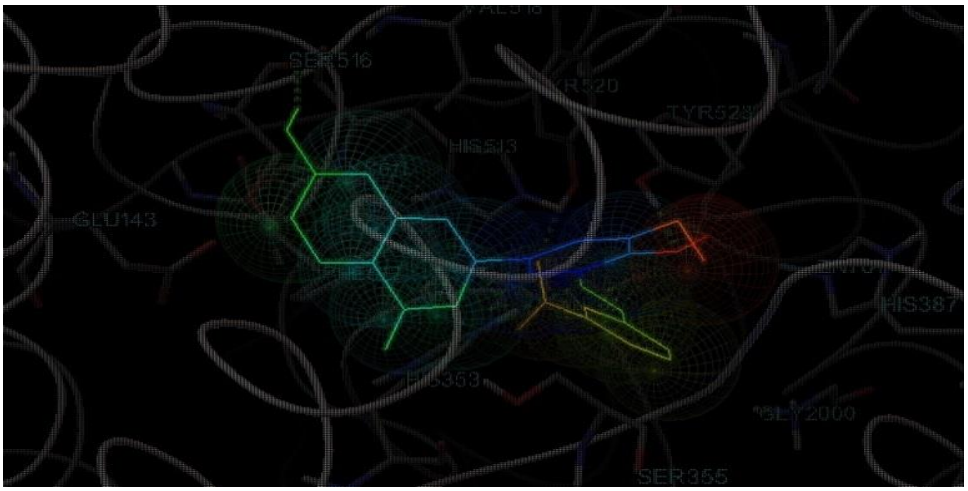


Fig 7: Snapshot of SB2 binding with 1o86

SB3

SB3 binds with ACE at ALA 354,GLY 2000,TYR 523 and SER 355 and the binding energy was found to be -9.47Kcal/mol.

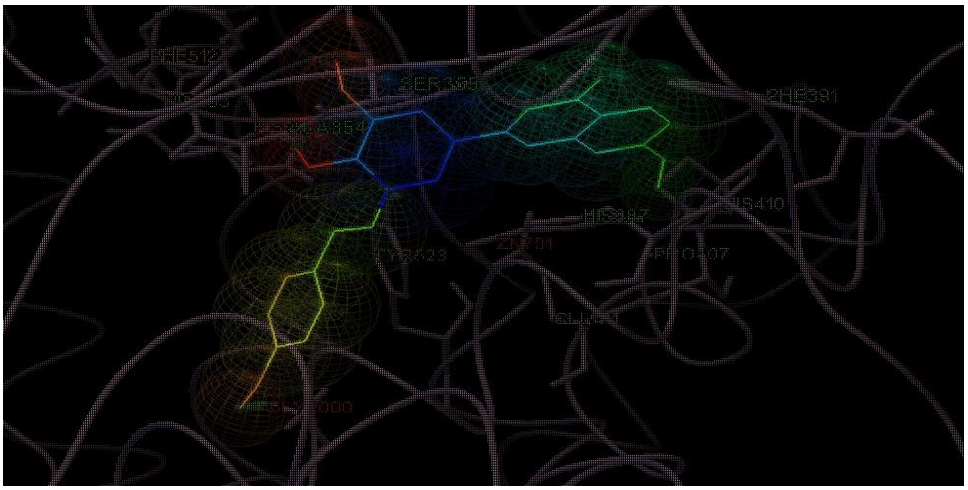


Fig 8: Snapshot of SB3 Binding with 1o86

SB4

SB4 interacts with ACE at GLY2000,ALA 354, HIS 353, PHE 512,TYR 523 and SER 355 and the binding energy was found to be -9.56Kcal/mol.

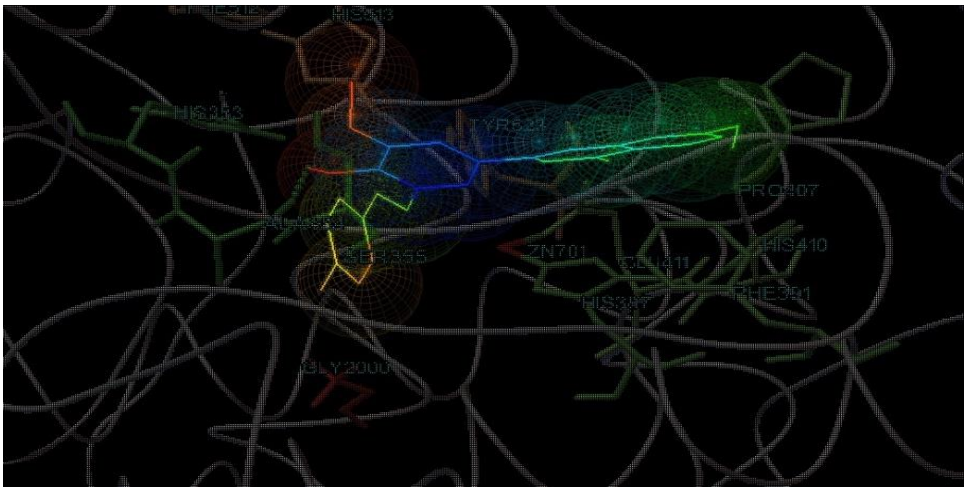


Fig 9: Snapshot of SB4 binding with 1o86

SB5

SB5 interacts with ACE at GLY 2000, ALA 354, TYR 523,PHE 512, GLU 411 and LYS 511 and the binding energy was found to be -6.49Kcal/mol.

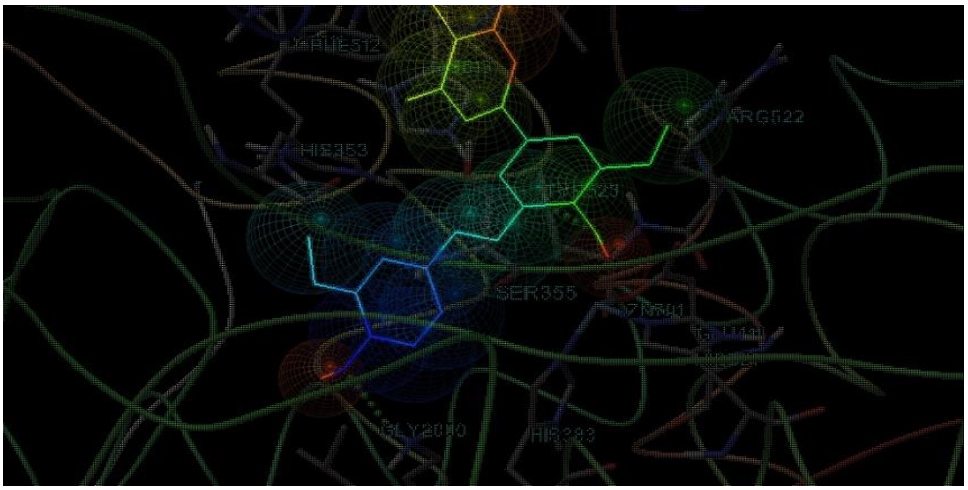


Fig 10: Snapshot of SB5 binding with 1o86

Binding of BF1-5 with ACE

BF1

BF1 interacts with ACE at GLY 2000,TYR 523, HIS 353 and ALA 354 and the binding energy was found to be -5.06Kcal/mol.

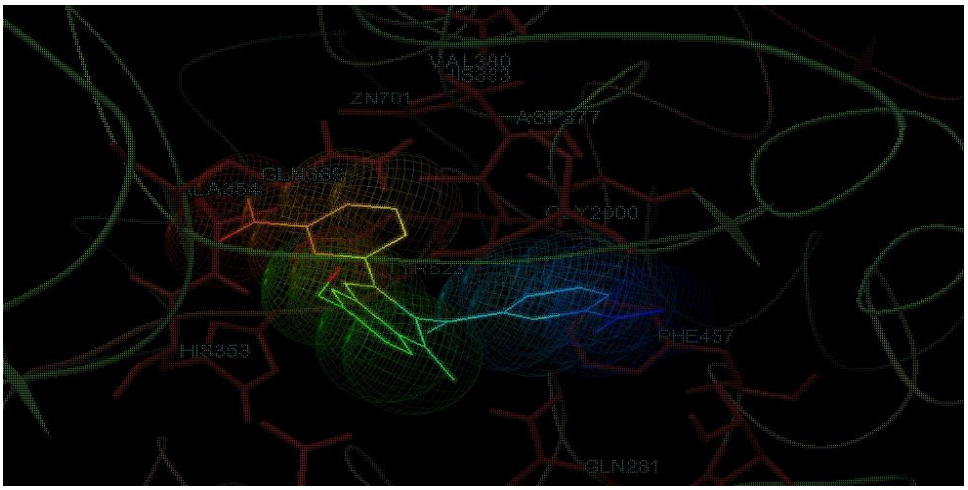


Fig 11: Snapshot of BF1 binding with 1o86

BF2

BF2 interacts with ACE at HIS 353, ALA 354, GLU 384, TYR 523, VAL 518 and ARG 522 and the binding energy was found to be -4.58 Kcal/mol.

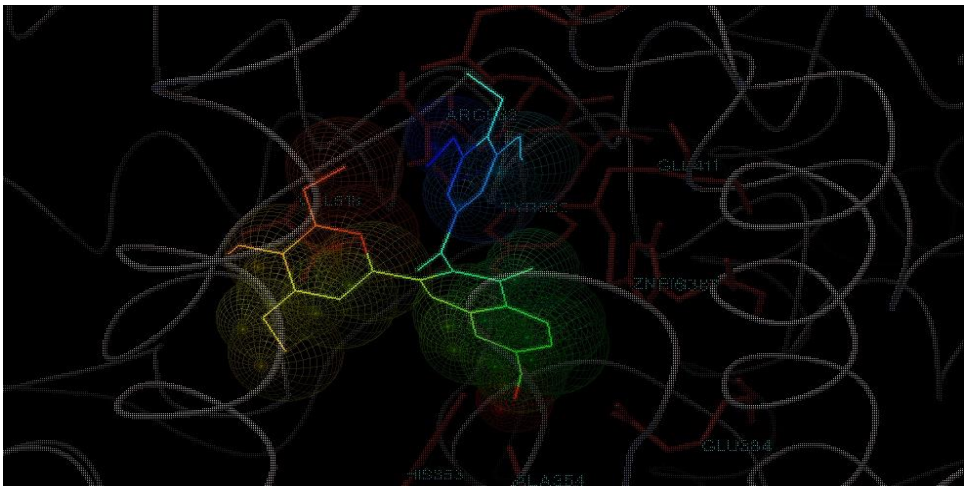


Fig 12: Snapshot of BF2 binding with 1o86

BF3

BF3 interacts with ACE at HIS 353, ALA 354, TYR 523, GLY 2000 and GLU 384 and the binding energy was found to be -8.11Kcal/mol.

Fig 13: Snapshot of BF3 binding with 1o86

BF4

BF4 interacts with ACE at ALA 354, HIS 383, ALA 354 and GLU 384 and the binding energy was found to be -6.64

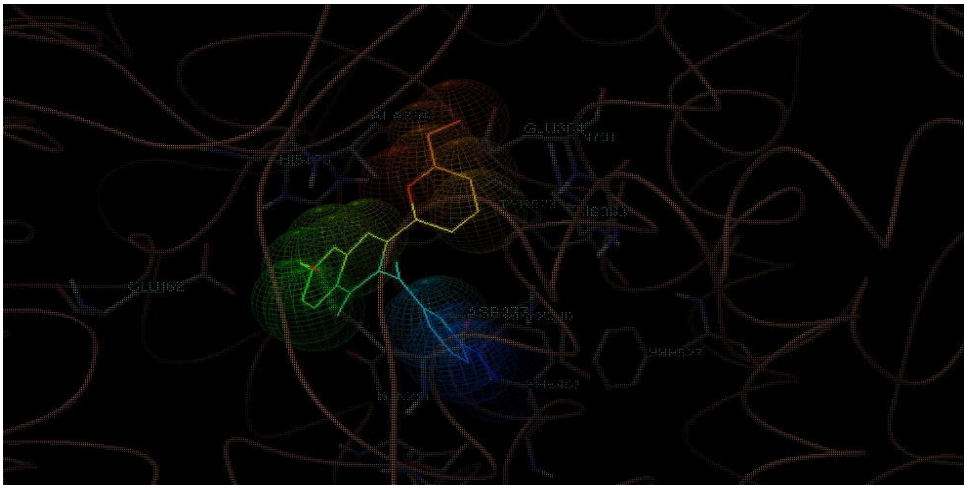


Fig 14: Snapshot of BF4 binding with 1o86

BF5

BF5 reacts with ACE at GLU 384, GLY2000, TYR 523 and HIS 353 and the binding energy was found to be -8.63Kcal/mol.

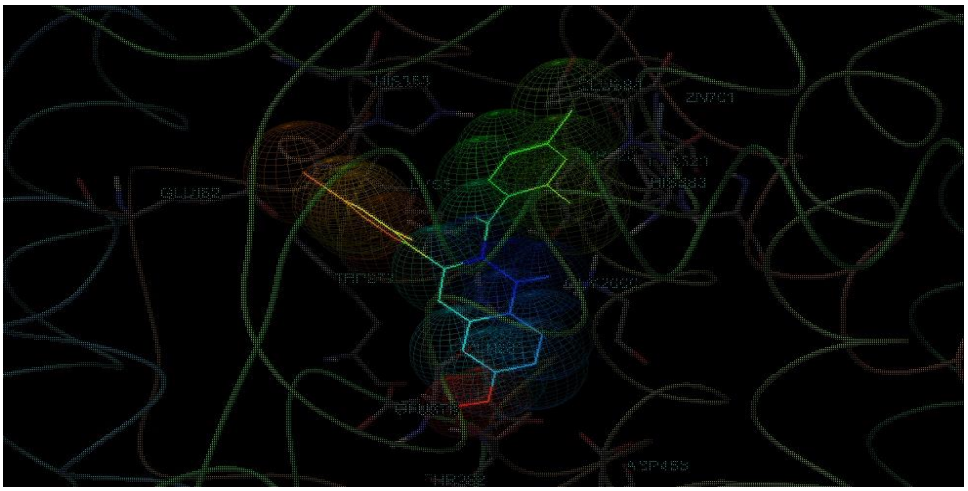


Fig 15: Snapshot of BF5 binding with 1o86

SYNTHESIS

REAGENTS AND CHEMICALS USED

2, 4-dihydroxy acetophenone, 5-nitrovanillin, p-hydroxy benzaldehyde, m-nitro benzaldehyde, terephthaldehyde, p-chloro benzaldehyde, m-nitro benzoic acid, 3,4,5-trimethoxy benzoic acid, 3-methoxy benzoic acid, 2-chloro benzoic acid, 2,4-dichloro benzoicacid, Thionyl chloride, DMSO, Ethanol, Methanol dichloromethane, Dimethyl formamide, Sodium hydroxide, Potassium carbonate, Iodine, Hydrochloric acid, Borate buffer, Potassium chloride, Alkaline copper sulphate reagent, Cyanuric chloride, HEPES buffer, EDTA, Hippuryl Histidyl Leucine solution.

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

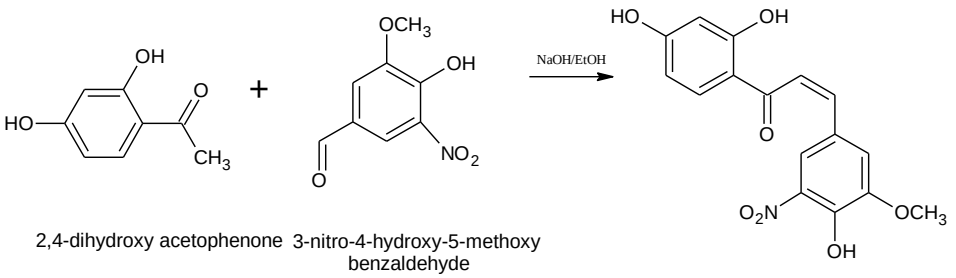
APPARATUS USED

Beakers, test tubes, glass rods, mechanical stirrer, reflux condenser, thermometer, round bottom flask, separating funnels, pipettes

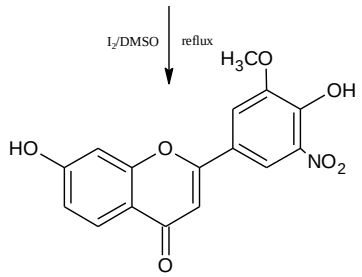
ANALYTICAL WORKS

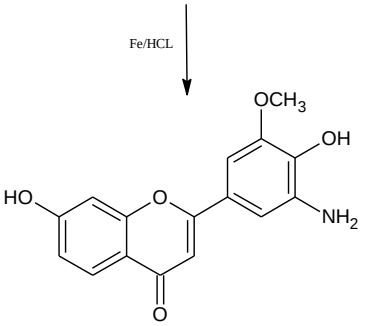
- Melting point was determined by using melting point apparatus MR-VIS, visual melting range apparatus, LABINDIA and uncorrected.
- Reactions were monitored by thin layer chromatography (TLC) on a pre-coated silica gel G plated using Iodine vapor as visualizing agent.
- UV spectra were recorded on JASCO 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 400MHz NMR spectrometer at SAIF, CUSAT, Cochin.
- Mass spectra were recorded on Shimadzu, LC-class 2010 EV at SAIF, MG University, Kottayam.

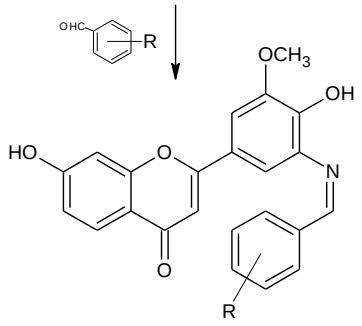
SCHEME 1[116-118]



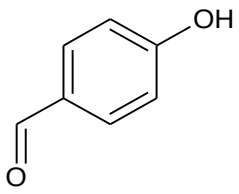
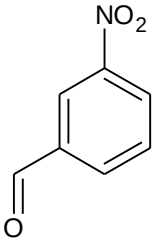
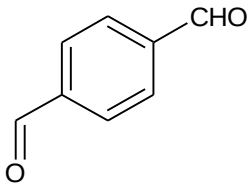
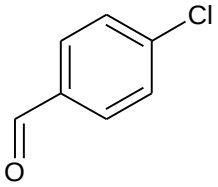
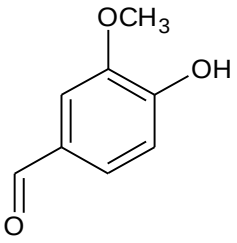
2,4-dihydroxy acetophenone 3-nitro-4-hydroxy-5-methoxy benzaldehyde







Schiff base of flavonoid

COMPOUND	SUBSTITUTION(R)
SB1	
SB2	
SB3	
SB4	
SB5	

Procedure for scheme 1

Step 1: Synthesis of Chalcones

A mixture of 2,4-dihydroxy acetophenone (1mmole) and 3-methoxy-4-hydroxy-5-nitro benzaldehyde (1mmole) was stirred in ethanol and added NaOH (30%,10ml) drop wise to the mixture. The reaction temperature was maintained between 20-25 ^{o}C. After vigorous stirring for 4-5 hours the reaction mixture was neutralized with 0.2N HCl. The precipitate formed was filtered and recrystallized from ethanol.

Step 2: Synthesis of Flavonoids from Chalcones

Iodine (0.1) was added to the solution of chalcone (1mmole) in DMSO (20ml) and refluxed for 1 hour. The mixture was cooled to room temperature and added excess of water into it. The product formed was filtered and dried.

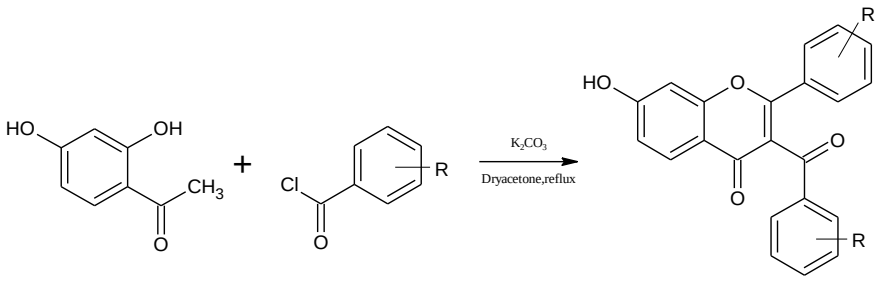
Step 3: Reduction of Nitro group:

Flavonoids (1g) along with a pinch of zinc dust was taken in a round bottom flask, add conc.HCl (5ml) and ethanol (25ml) and the mixture is refluxed for 1 hour. After cooling to room temperature it is neutralised with 0.5molar Na0H.The gelatinous precipitate formed was filtered, dried and recrystallized with ethanol.

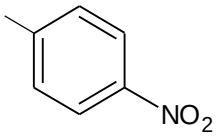
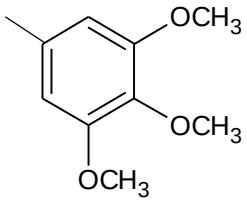
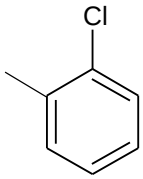
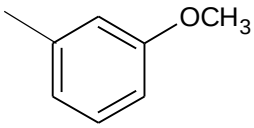
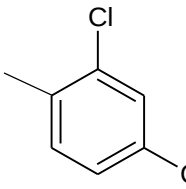
Step4: Synthesis of Schiff bases from flavonoids- (2-(3-amino-4-hydroxy-5-methoxyphenyl)-7-hydroxy-4*H*-chromen-4-one):

Equimolar quantities of amino flavonoid and substituted aldehyde were dissolved in a mixture of methanol and conc.HCl and refluxed for 2 hours. It was then cooled and poured into crushed ice.The product formed was obtained by filtration and recrystallized from chloroform.

SCHEME 2[79]



2,4-dihydroxy acetophenone substituted benzoyl chloride substituted benzoyl flavones

COMPOUNDS	SUBSTITUTION(R)
BF1	
BF2	
BF3	
BF4	
BF5	

Step1: Synthesis of substituted acid chlorides

Substituted benzoic acid (5g) was dissolved in dichloromethane. Added a few drops of DMF as catalyst for the reaction. Thionyl chloride (2.4g) was slowly added and the reaction is stirred for 2 hours. The reaction mixture was then evaporated; the acid chloride formed can be used without any purification.

Step2: Synthesis of substituted benzoyl flavones

To a stirred solution of 2,4-dihydroxy acetophenone (0.3mole) in dry acetone added anhydrous K_2CO_3 and stirred at room temperature for 10 minutes. Added substituted benzoyl chloride (0.9moles) in small portions to the reaction mixture and allowed to stir for about half an hour. The reaction mixture was then refluxed for 24 hours. After refluxing the solvent was evaporated and the residue cooled to room temperature and acidified with dil.HCl. The product was dried and recrystallized from dichloromethane.

PHYSICAL CHARACTERISATION DATA

1. Substituted Schiff bases of flavonoids

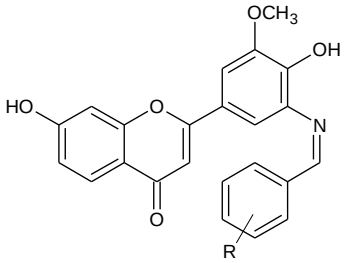
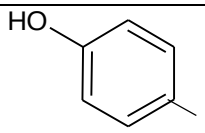
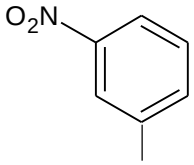
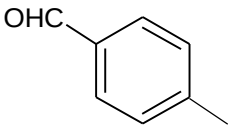
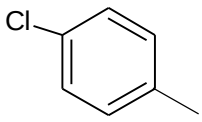
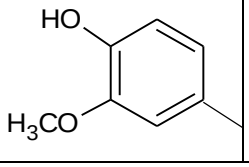


Table 5: Physical characterisation of substituted Schiff bases of 2,4'di hydroxy-3'methoxy-flavonoids.

Compound code	R	Molecular formula	Molecular weight (g/mol)	Percentage yield(%)	Melting point	Rf value
SB1		$C_{23}H_{17}NO_8$	403.38	69%	107-109	0.45
SB2		$C_{23}H_{16}N_2O_7$	432.38	76%	117-118	0.67
SB3		$C_{24}H_{17}NO_6$	415.39	73%	126-127	0.78
SB4		$C_{23}H_{19}NO_7$	421.82	65%	180-182	0.59
SB5		$C_{24}H_{19}NO_7$	433.41	72%	135-136	0.47

Recrystallization : Chloroform
Solvent system : Chloroform: water (7:3)
Visualizing agent : Iodine vapour

2. Physical characterisation of Substituted 3-benzoyl flavones

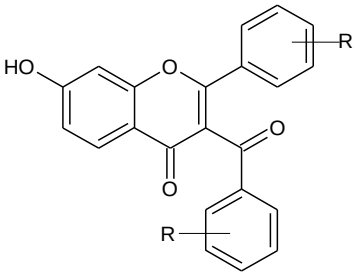
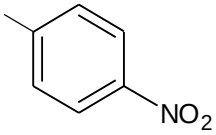
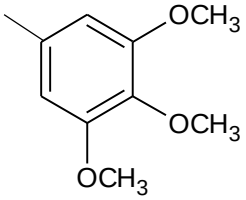
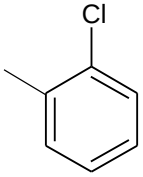
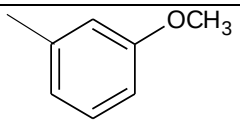
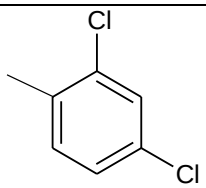


Table 6: Physical characterisation of 3-substituted Benzoyl flavones

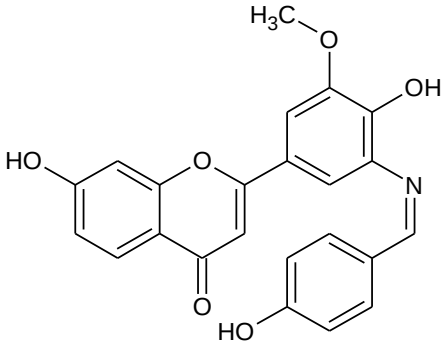
Compound code	R	Molecular formula	Molecular weight (g/mol)	Percentage yield (%)	Melting point	Rf value
BF1		$C_{22}H_{12}N_2O_8$	432.33	81%	108-109	0.57
BF2		$C_{28}H_{26}O_{10}$	522.50	79%	222-223	0.56
BF3		$C_{22}H_{12}Cl_2O_4$	411.23	74%	110-111	0.78
BF4		$C_{24}H_{18}O_4$	402.39	77%	135-136	0.39
BF5		$C_{22}H_{10}Cl_4O_4$	480.12	84%	128-129	0.42

Recrystallization : Dichloromethane
Solvent system : Chloroform: Water (4:5)
Visualizing agent : Iodine vapour

SPECTRAL ANALYSIS OF COMPOUNDS

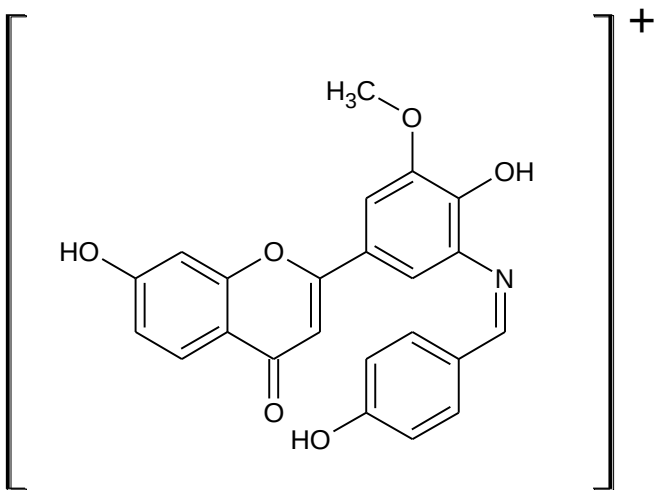
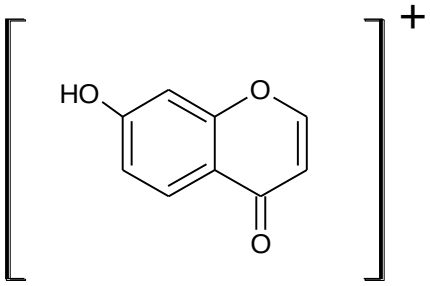
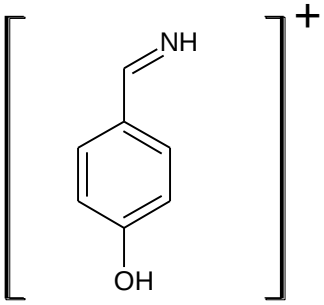
The structures of synthesized compounds during the present investigation were established on the basis of chemical data IR, UV, NMR, and MASS spectral data. The purity of the compounds was established by single spot on TLC plates.

COMPOUND CODE: SB1



Chemical name	7-hydroxy-2-{4-hydroxy-3-[(*Z*)-(4-hydroxybenzylidene)amino]-5-methoxyphenyl}-4*H*-chromen-4-one
UV Spectrum	Solvent used : methanol λ max : 284nm
IR (KBR, V_{max} in cm^{-1})	3201.26(aromatic OH), 1217.83(cyclic ester-C-O-C), 1669.09 (C=N), 1779.01(C=O), 1602.56 (C=C), 2878.24 (C-H)
^{1}HNMR spectral data	2.732 (s, 3H, Ar-OCH_3) , 6.245-6.388 (s, 3H, Ar-OH) , 7.736-8.612 (m, 9H).

Mass Spectral Data

Sl No:	Fragments	m/z value
1		**403**
2		**162**
3		**121**

UV SPECTRUM

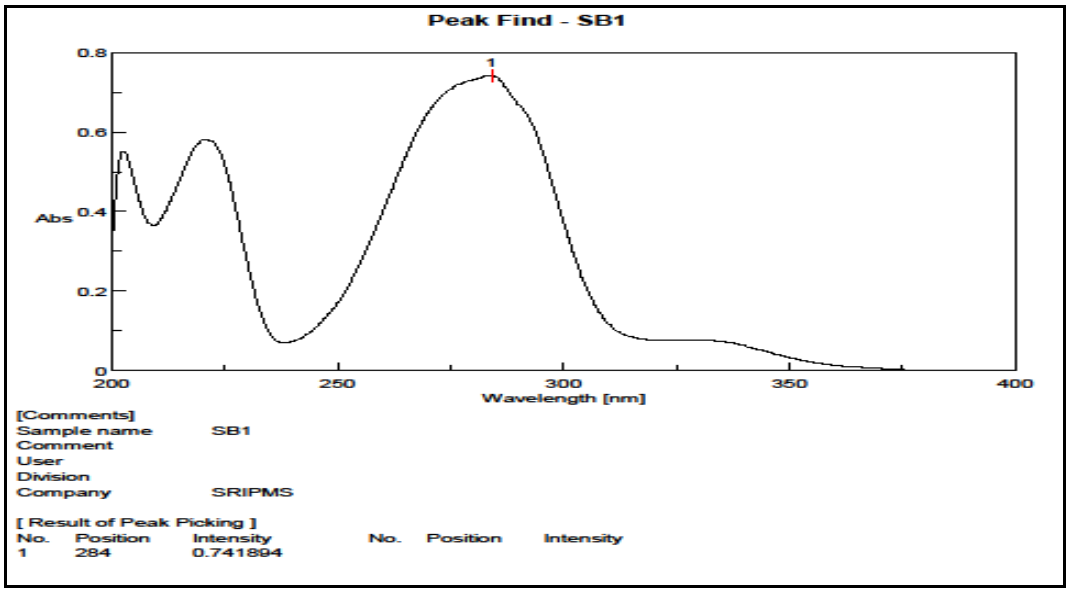


Fig:16 UV Spectrum of compound SB1

IR SPECTRUM

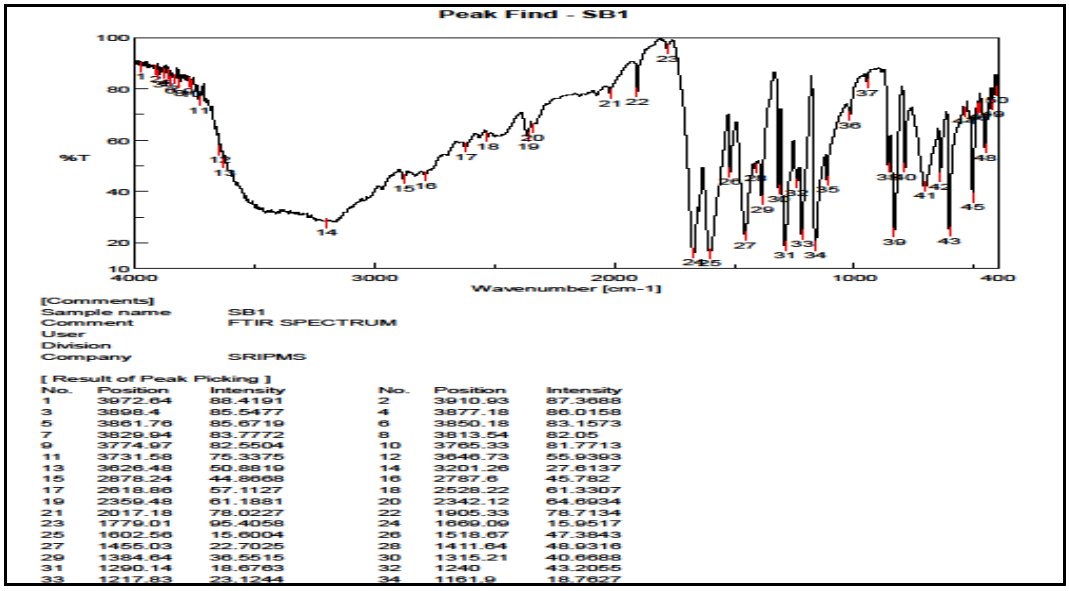


Fig:17 IR Spectrum of Compound SB1

^{1}H NMR SPECRUM

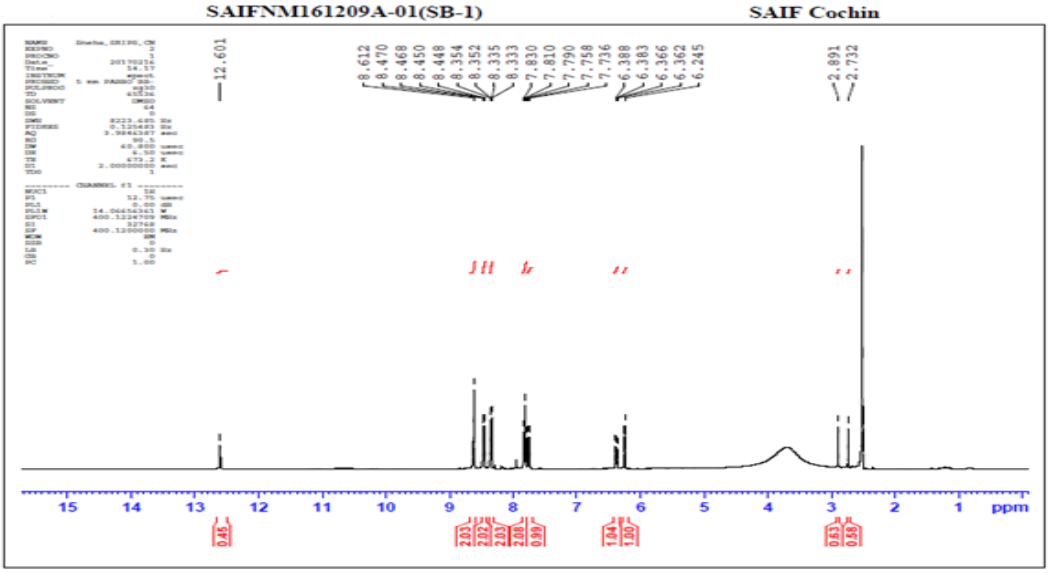


fig 18: ^{1}HNMR Spectrum of compound SB1

MASS SPECTRUM

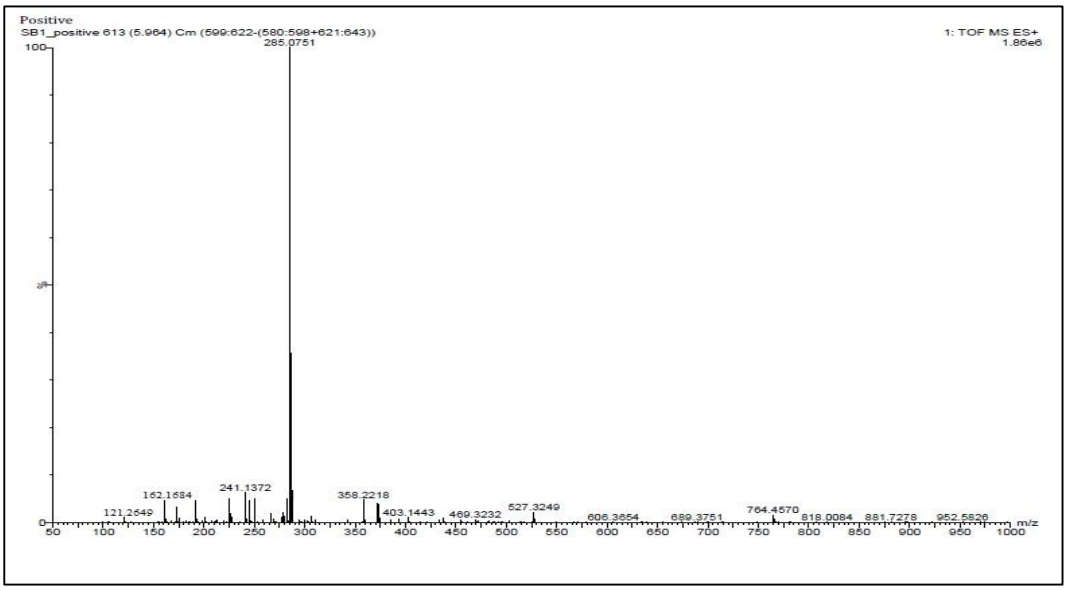
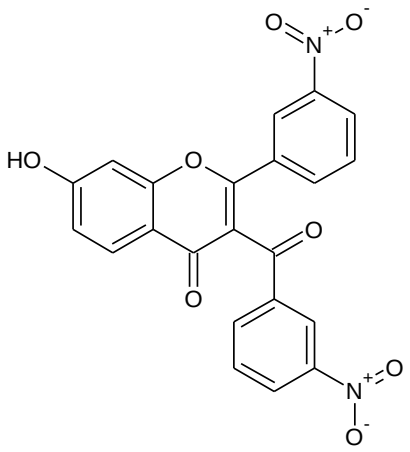


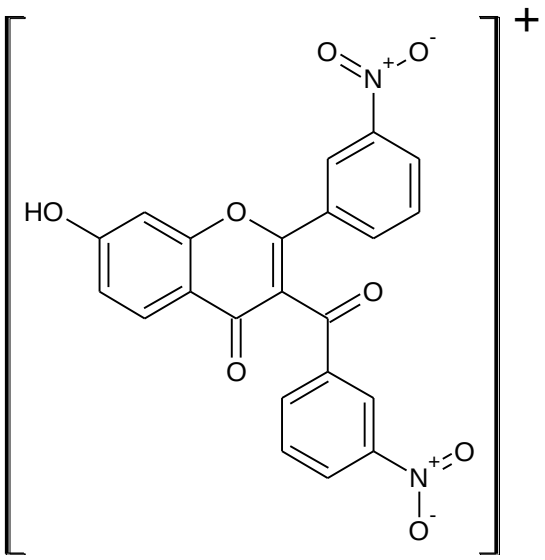
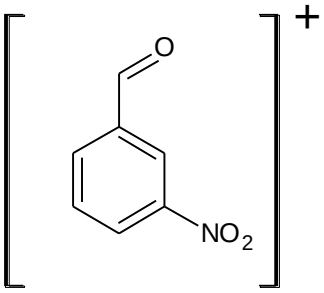
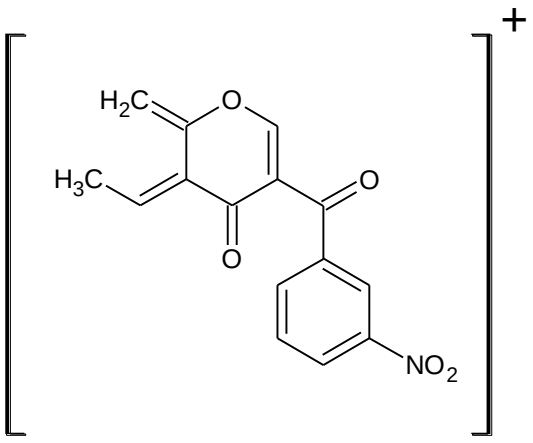
Fig 19: Mass spectrum of compound SB1

COMPOUND CODE: BF1



Chemical name	7-hydroxy-3-(3-nitrobenzoyl)-2-(3-nitrophenyl)-4*H*-chromen-4-one
UV Spectrum	Solvent : dichloromethane λmax : 270nm
IR (KBR, V_{max} in cm^{-1})	3404.81(aromatic OH), 1840.72 (aromatic C=O), 1591.95(NO_2) , 1618.95 (C=C) , 1706.69 (aliphatic C=O) ,3087.48 (C-H)
^{1}HNMR Spectral data	6.792 (s, 1H, Ar-OH) 6.928-8.350 (m, 11 H, Ar-H & CH=C)

Mass Spectral Data

Sl No:	Fragments	m/z value
1		432
2		151
3		285

UV SPECTRUM

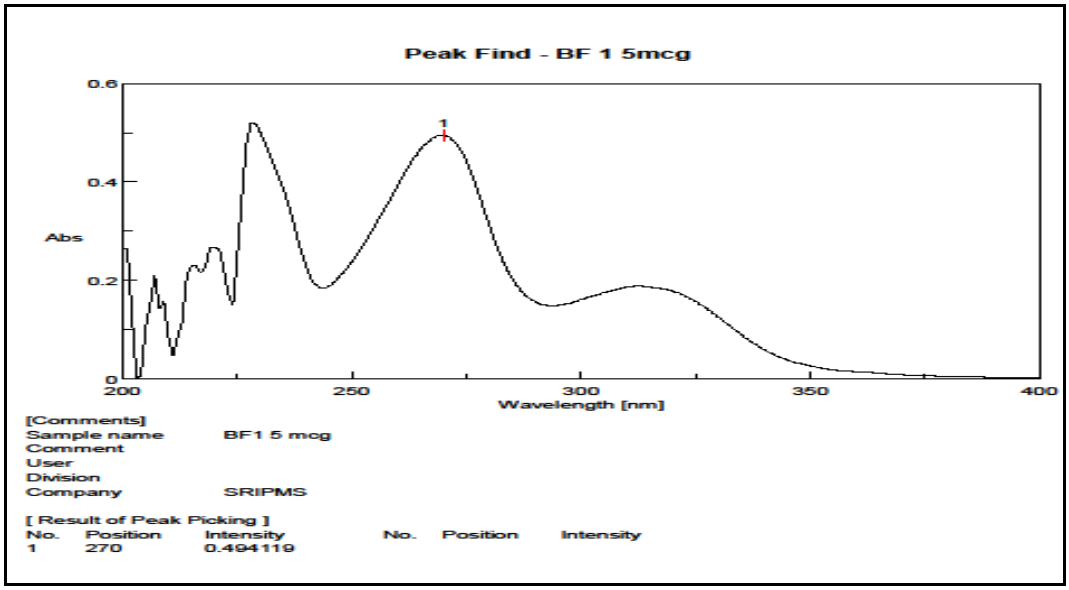
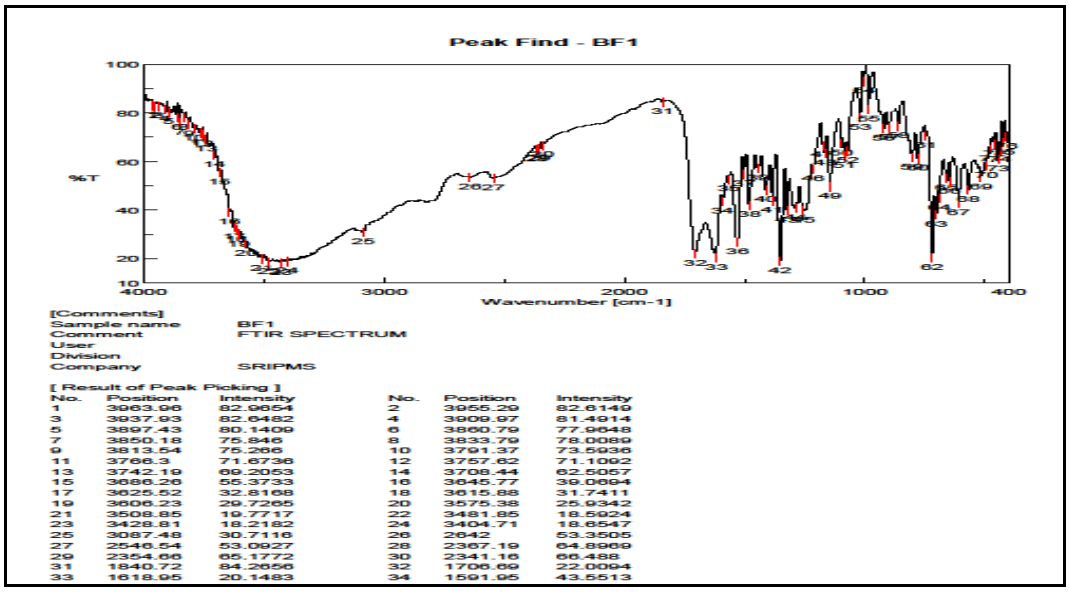


Fig 20: UV spectrum of compound BF1

IR SPECTRUM



No.	Position	Intensity	No.	Position	Intensity
1	3963.96	82.9854	2	3955.29	82.6149
3	3937.93	82.6462	4	3909.97	81.4614
5	3897.43	80.1409	6	3860.79	77.9648
7	3850.18	75.846	8	3833.79	78.0089
9	3813.54	75.288	10	3791.37	73.5039
11	3766.3	71.6738	12	3757.62	71.1092
13	3742.19	69.2053	14	3708.44	62.5057
15	3686.26	55.3733	16	3645.77	39.0694
17	3625.52	32.8168	18	3615.88	31.7411
19	3606.23	29.7265	20	3575.38	25.9342
21	3508.85	19.7717	22	3481.85	18.5924
23	3428.81	18.2182	24	3404.71	18.6547
25	3087.48	30.7116	26	2642	53.3505
27	2548.54	53.0927	28	2367.19	64.8969
29	2354.66	65.1772	30	2341.16	66.488
31	1840.72	84.2656	32	1706.69	22.0094
33	1618.95	20.1483	34	1591.95	43.5513

Fig 21: IR spectrum of compound BF1

^{1}HNMR SPECTRA

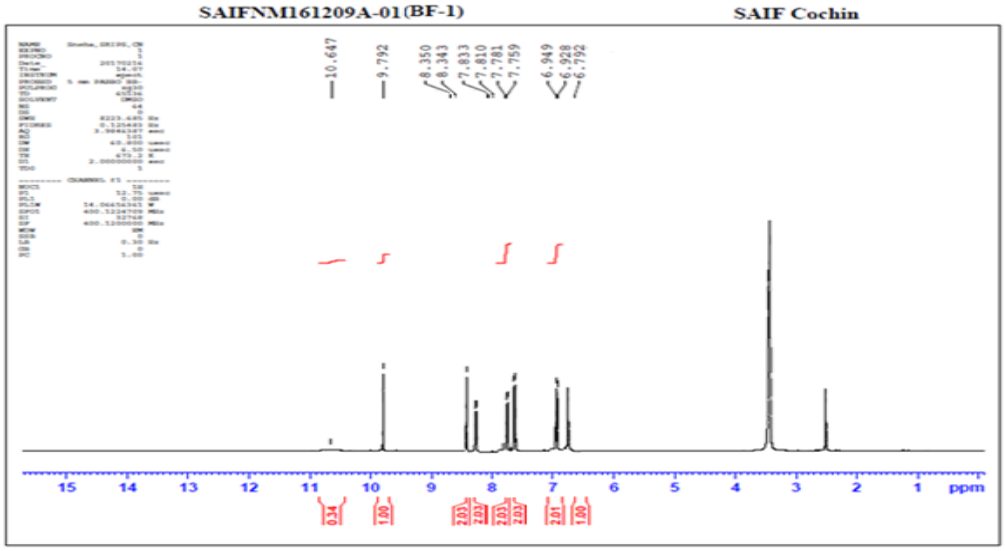


Fig 22: ^{1}HNMR spectrum of compound BF1

MASS SPECTRA

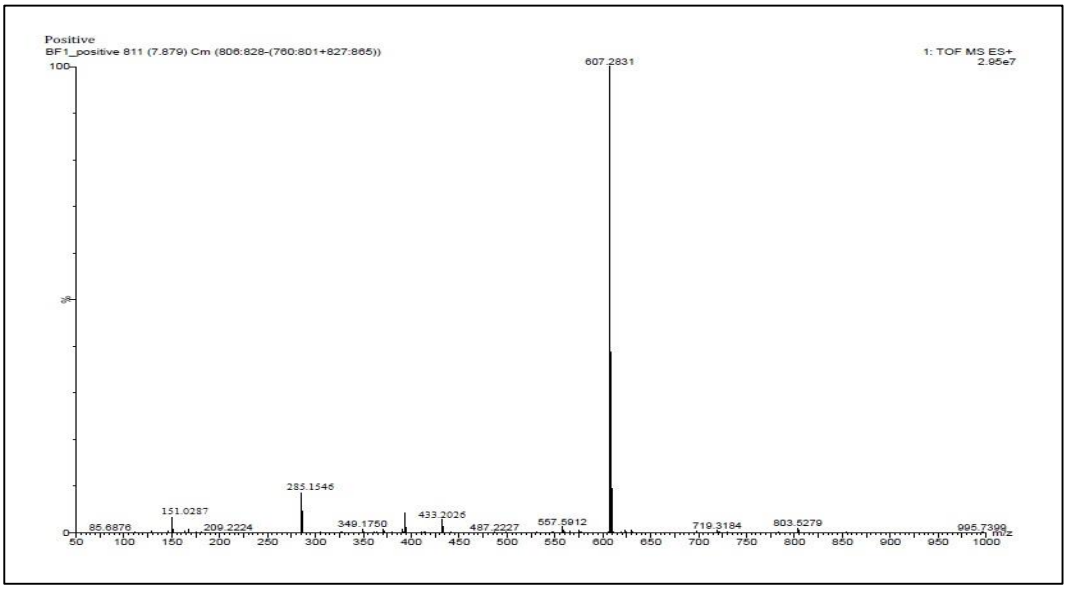


Fig 23: Mass spectrum of compound BF1

Extraction of angiotensin converting enzyme from sheep lung extract[119,120]

Sheep lungs was collected from slaughterhouse and one gram of sheep lung tissue was diced and homogenised in 10 ml of the ice cold 100 mM borate buffer (pH-8.3) containing 50 mM KCl using a homogenizer at 4°C. The homogenate was centrifuged at 8000g at 4°C for half an hour, and the supernatant was collected. Remove any molecular impurities if present and stored at 4°C. This supernatant was used as the source of ACE.

Estimation of protein content by Lowry method [121]

Different dilutions of Bovine serum albumin (0.2-1µg/ml) were prepared from stock BSA solution (1mg/ml) in a test tube. The final volume in each test tube was adjusted to 5ml with distilled water. From these dilutions, pipette out 0.2ml protein solution to different test tubes and add 2ml of alkaline copper sulphate reagent (analytical reagent) and mix well. The solution was incubated for 10 minutes at room temperature. Then add 0.2 ml of Folin Ciocalteau solution to each test tube and incubated for 30 minutes. The colorimeter was set to zero with blank and the optical density was measured at 450 nm. Plot the absorbance against protein concentration to get a standard calibration curve. Check the concentration of unknown samples and determine the concentration of unknown from the standard curve.

Estimation of ACE from sheep lung extract [119]

ACE inhibitory activity was measured by using Hippuryl-L-histidyl-L-leucine (HHL) as substrate. This reaction mixture contained 0.2ml of 5mM HHL prepared in 200mM borate buffer (pH 8.3) with 1000 mM KCl. The reaction was initiated by the addition of different concentration of lung extract and then incubated at 37°C foe 30 minutes. The reaction was stopped by addition of 2ml of HEPES buffer and 1ml of 136 mM cyanuric chloride in 1,4-dioxan and yellow colour developed was measured at 405nm.Various concentration of hippuric acid (which is formed by the reaction of ACE on HHL) were used for the preparation of standard graph.

***In vitro* angiotensin converting enzyme inhibitory activity [119]**

Colorimetric Method

The reaction mixture contained 5mM HHL prepared in 200mM Borate buffer (pH8.3), containing 1000mM KCl 10µl of different concentration of unknown drug sample (50-800µg), lung extract (50µl)and distilled water in a volume of 1ml. The reaction mixtures were incubated at 37ºC for 30 minutes. The reaction was stopped by adding 2ml of HEPES buffer (pH 9), which contain 2.5mM EDTA. Finally add 1ml of 136mM cyanuric chloride in 1,4- dioxan was added to the reaction mixture and shake vigorously for 15 seconds. The absorbance of yellow colour developed was measured at 405nm. Lisinopril was used as the standard drug for comparison with the assay system.

RESULTS

Estimation of sheep lungs extract (ACE)

Estimation of sheep lung extract was used to estimate the protein and plotting the calibration curve of ACE.

Estimation of protein in lung extract (ACE)

Table 7: Absorbance of Bovine serum albumin (BSA)

Concentration (µg/ml)	Absorbance at 450nm			Average absorbance
	Abs1	Abs2	Abs3	
0.2	0.1256	0.1430	0.1524	0.1403
0.4	0.2132	0.2038	0.1987	0.2052
0.6	0.2432	0.2469	0.2571	0.2499
0.8	0.3178	0.3253	0.3215	0.3213
1	0.3855	0.3253	0.3802	0.3810

Table 8: Absorbance of lungs extracts (ACE)

Volume of lung extract (µl)	Absorbance at 450nm			Average
	Abs1	Abs2	Abs3	
10	0.1721	0.1734	0.1854	0.1779
20	0.2956	0.3158	0.2478	0.2816

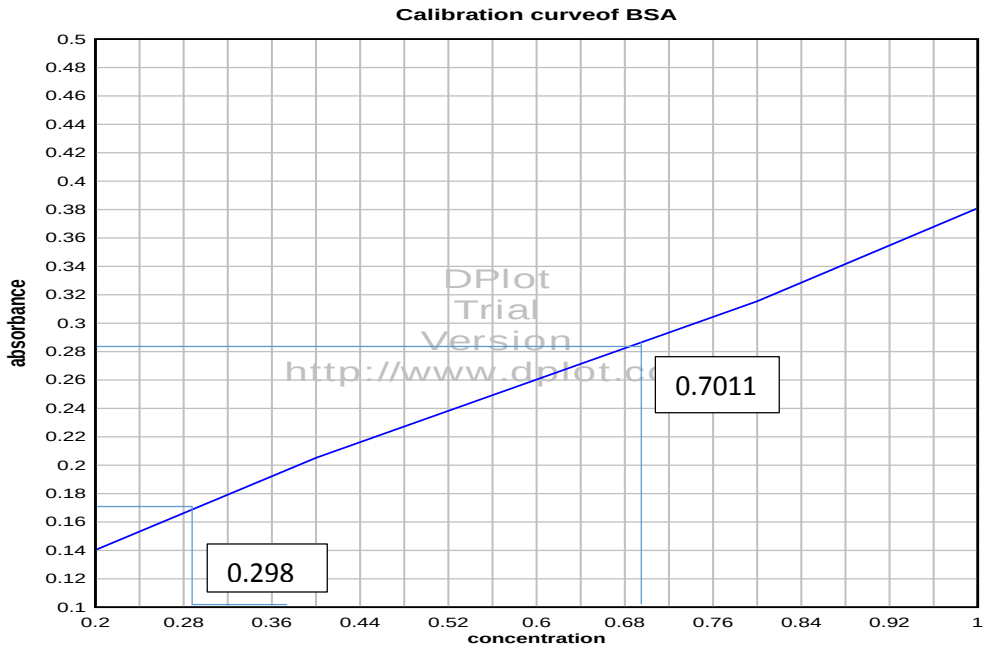


Fig 24: Standard curve for protein estimation

Table 9: Interpolation of lungs extract

Volume of lung extract (µl)	Interpolating value	In 100 µl	In 1 ml	Average
10	0.2987	2.987	29.87	49.99 µg/ml
20	0.7011	7.011	70.11	

=49.99 x10=499.90µg/ml

Protein content of lung extract (ACE) is **499.90µg/ml**

Estimation of ACE from sheep lung extract

Table 10: Absorbance of Hippuric Acid

Concentration of hippuric acid (µM)	Absorbance at 405nm			Average absorbance
	Abs1	Abs2	Abs3	
0.2	0.4239	0.4250	0.4147	0.4212
0.4	0.4771	0.4640	0.4846	0.4752
0.6	0.5324	0.5275	0.5441	0.5346
0.8	0.5897	0.5979	0.5820	0.5898
1	0.6496	0.6509	0.6494	0.6880
1.2	0.7094	0.7126	0.7081	0.7100
1.4	0.7881	0.7763	0.7659	0.7767

Table 11: Absorbance of sheep lungs extract (ACE)

Volume of lung extract (µl)	Abs1	Abs2	Abs3	Average absorbance	Interpolating value(µM)	For 1µM (µl)	Average(µl)
100	0.2760	0.2850	0.2842	0.2817	-	-	405.323
200	0.3921	0.3946	0.3897	0.3921	-	-	
300	0.5182	0.5298	0.5104	0.5614	0.6945	431.965	
400	0.6413	0.6459	0.6471	0.6447	0.9671	413.607	
500	0.7612	0.7624	0.7598	0.7611	1.3499	370.397	

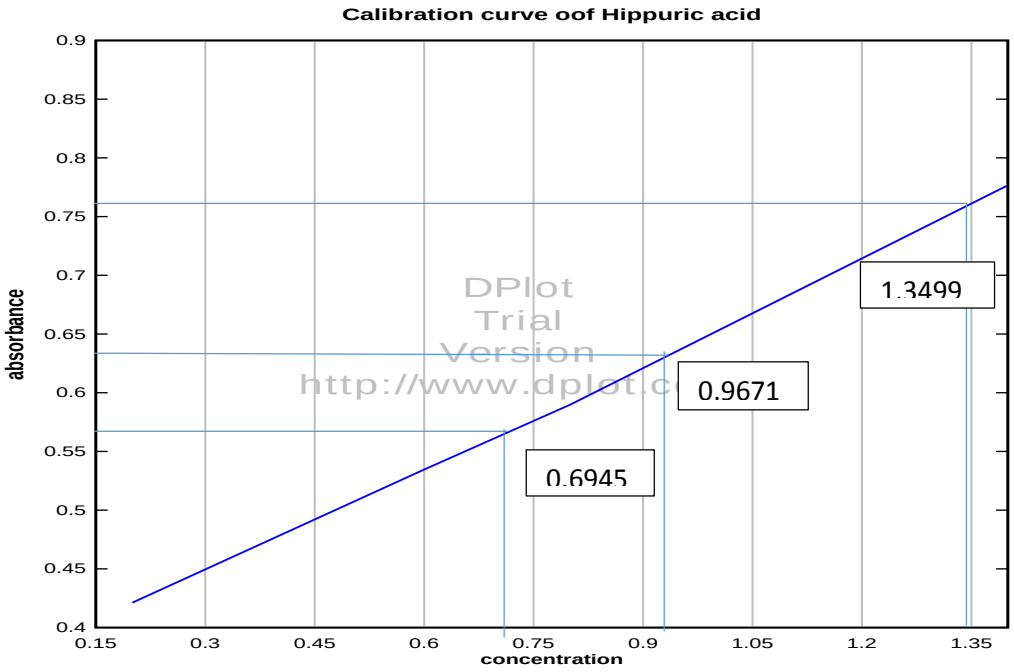


Fig 25:Calibration curve of Hippuric Acid

1Unit of ACE =**405.323µl** of Sheep lung Extract

***In vitro* Angiotensin Converting Enzyme Inhibitory Activity**

Table 12: *In vitro* ACE inhibitory activity of standard drug (Lisinopril)

Concentration of Lisisnopril (µg/ml)	**Percentage inhibition**
10	31.13±28
20	50.48±25
40	65.12±04
80	80.08±36
160	90.95±28
IC_{50}	**19.85±24**

ACE inhibitory activity of synthetic compounds

Table 13: ***In Vitro*** **ACE inhibitory activity of synthetic compounds SB 1-5**

Concentration (µg/ml)	**Percentage inhibition(%) of ACE**				
	SB1	**SB2**	**SB3**	**SB4**	**SB5**
50	32.90±24	37.22±34	27.39±29	31.71±17	23.41±39
100	48.88±14	60.17±39	40.78±20	53.10±45	44.01±31
200	63.97±52	71.86±17	58.87±30	61.83±46	57.92±25
400	79.86±15	84.06±18	76.87±37	78.33±17	71.94±11
800	89.28±17	91.38±34	93.92±24	86.26±67	84.81±09
IC_{50}	**110.12±54**	**81.54±26**	**162.32±33**	**95.64±09**	**149.97±18**

All the determinations were carried out in triplicate and the values are expressed as a mean ± SEM

Table 14: *In Vitro* ACE inhibitory activity of synthetic compounds BF 1-5

Concentration (µg/ml)	Percentage inhibition (%) of ACE				
	BF1	**BF2**	**BF3**	**BF4**	**BF5**
50	24.94±19	17.19±23	46.58±35	22.38±22	46.52±37
100	30.07±31	30.16±37	57.41±47	37.81±11	59.34±37
200	45.15±65	43.13±35	76.03±09	57.13±36	73.61±29
400	64.59±52	54.76±09	82.60±57	66.49±21	80.08±36
800	73.89±24	65.99±57	88.05±05	78.85±18	90.58±28
IC_{50}	**259.65±42**	**325.36±17**	**70.21±35**	**165.52±22**	**73.89±24**

All the determinations were carried out in triplicate and the values are expressed as a mean ± SEM.

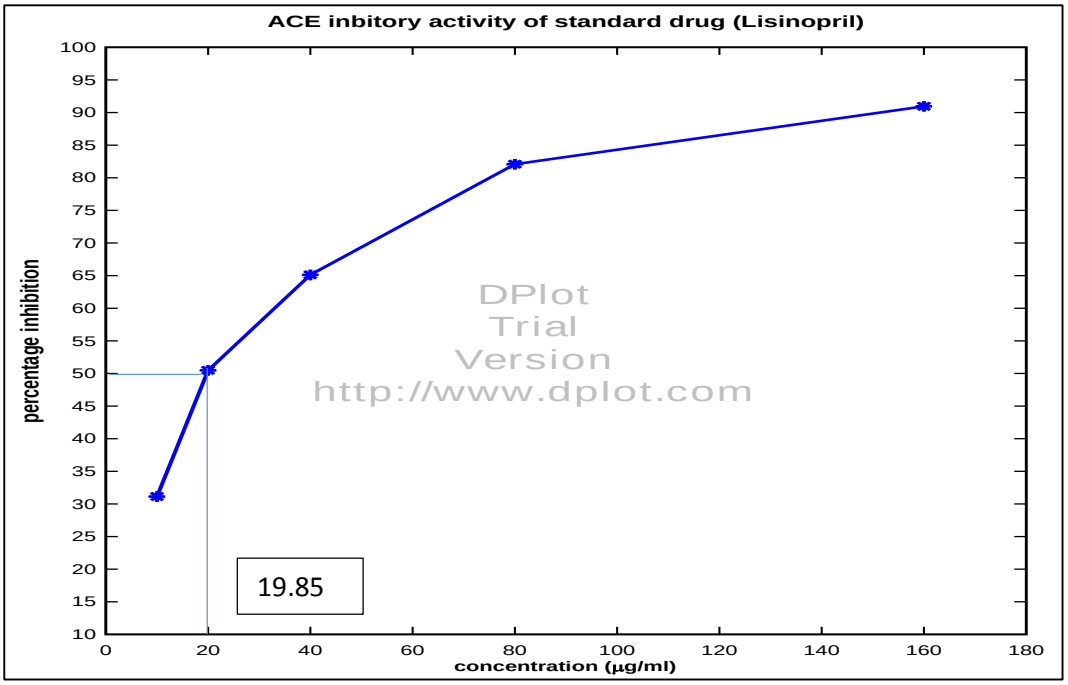


Fig 26:ACE Inhibitory Activity of Lisinopril

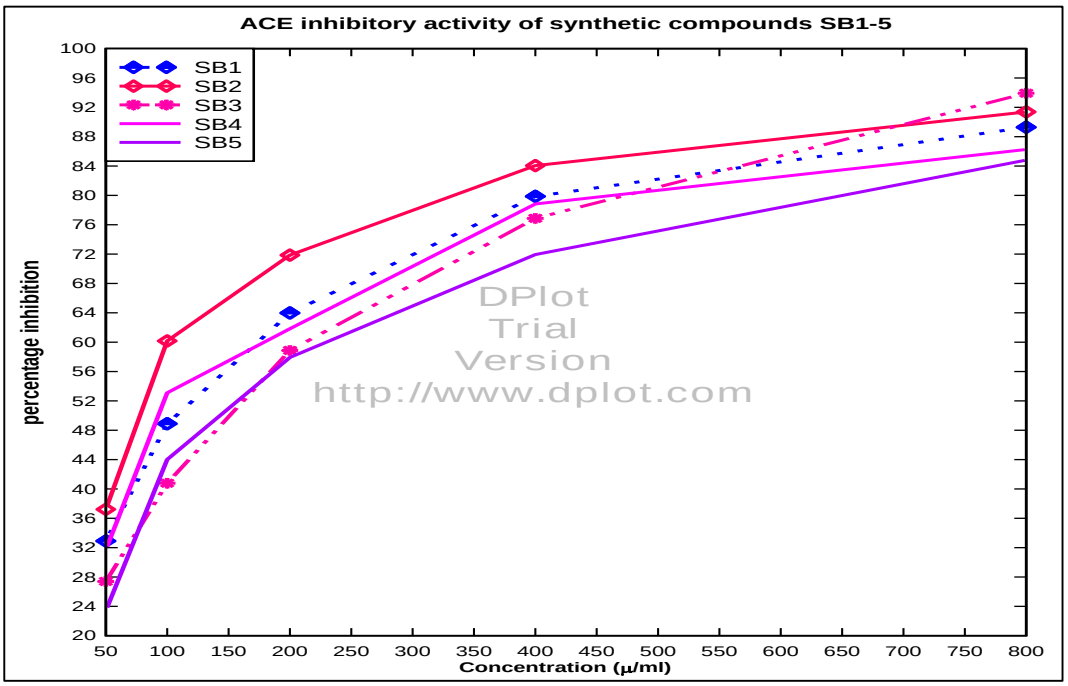


Fig 27: ACE Inhibitory Activity of Synthetic compounds SB1-SB5

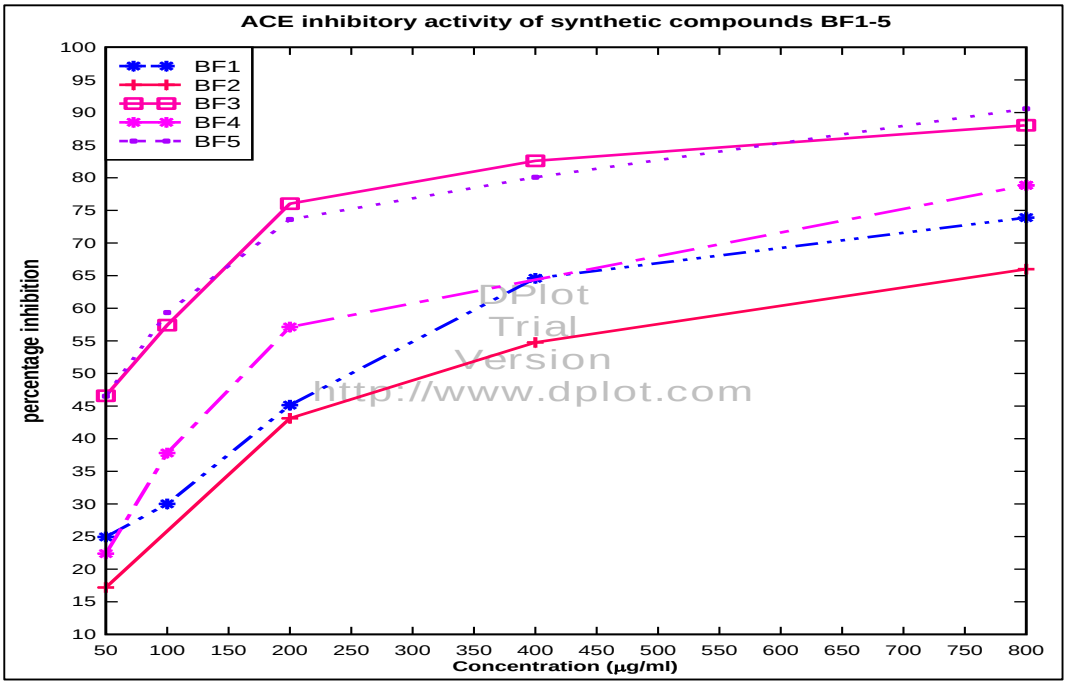


Fig 28: ACE Inhibitory Activity of Synthetic compounds BF1-BF5

ACE inhibitory activity of synthetic flavonoids.

Hypertension is one of the major chronic diseases. Angiotensin converting enzyme (ACE) is one of the major regulators of blood pressure and inhibition of this enzyme will leads to lowering of blood pressure. ACE is a membrane bound enzyme present on the luminal surface of vascular endothelium and therefore remains in close contact with -circulation. It is particularly abundant in lung which has a vast surface area of vascular endothelium. Therefore the lung serves as major organ for the production of circulating angiotensin II. In this study the ACE enzyme was extracted from sheep lungs and used for in vitro study. There are different *in vitro* methods for the determination of ACE inhibitory activity. The assay is based on the hydrolysis of hippuryl-L-histidyl-leucine (HHL) by ACE. The amount of hippuric acid formed in reaction is determined by measuring the absorbance at 405 nm. The difference of absorbance in the absence and presence of inhibitor is proportional to the inhibitory activity of tested sample.

In this assay, estimation of protein content and estimation of ACE in the lung extract was done. Protein estimation was done by using Lowry method. Different concentrations of (BSA) bovine serum albumin (0.2 to 1 μg/ml) were used for the preparation of the standard graph and the estimation of protein in different volumes of lung extract was obtained by interpolating the average absorbance in the calibration curve of BSA. About 10 ml of the lung extract contains 499.90 μg/ml of protein (Table no.9)

Estimation of ACE in the sheep lung extract was done by using HHL as substrate. The standard curve was plotted by taking concentration of hippuric acid in X-axis and absorbance on Y-axis. The amount of ACE was estimated by interpolating the average absorbance value of different volumes of sheep lung extract in the standard curve. From the table it was found that 1 unit of ACE is contained in 405.323 μl of sheep lung extract.

The Schiff bases of flavonoids (SB1-5) and 3-subtituted benzoyl flavones (BF1-5) were tested for their *in vitro* angiotensin converting enzyme activity at various concentrations ranging from 50μg/ml to 800μg/ml. A dose dependent increase in percentage inhibition was noticed for all the compounds. The IC_{50} values were obtained by plotting a standard graph with percentage inhibition versus concentration in μg/ml. The values obtained were compared with that of standard drug lisinopril.

The standard drug lisinopril exhibited 31.13% inhibition of ACE at a concentration of 10 μg/ml. An increase in inhibition to 90.95% was observed at 160μg/ml and the IC_{50} value was found to be 19.85μg/ml. A dose dependent increase in percentage inhibition was observed for all tested concentration.

The Schiff bases of flavonoids (SB1-5) were found to inhibit ACE and produced 32.90%, 37.22%, 27.39%, 31.71%, 23.41% inhibition respectively at a concentration of 50μg/ml and the percentage inhibition was increased with an increase in concentration and reached to 89.28%, 91.38%, 93.92%, 86.26% and

84.81% respectively at 800µg/ml. The IC_{50} values of the synthesized compounds were found to be 110.12 µg/ml, 81.54 µg/ml, 112.32 µg/ml, 95.64 µg/ml and 149.97 µg/ml. The order of potency of the compounds were found to be SB2>SB4>SB1>SB5>SB3. From the results obtained with Schiff bases of flavonoids it can be confirmed that SB2 and SB4 produced excellent ACE activity since these two compounds could inhibit the ACE enzyme at lower concentration compared to other compounds used in the study.

The results of 3-subtituted derivatives of benzoyl flavones (BF1 to BF5) similarly exhibited ACE inhibition of 24.94%, 17.19%, 46.58%, 22.35%, and 46.52% respectively at 50 µg/ml and at 800 µg/ml the percentage inhibition increased to 73.89%, 65.99%, 88.05%, 78.85%, 90.58% respectively. IC_{50} values obtained for these compounds were found to be 259.65 µg/ml, 325.36 µg/ml, 70.21 µg/ml, 165.52 µg/ml and 73.89 µg/ml. The order of potency of benzoyl flavone derivatives was found to be BF3> BF5> BF4> BF1>BF2. Among the five compounds synthesized in 3-subtituted benzoyl flavones derivatives BF3 and BF5 exhibited better ACE inhibition. The obtained results when compared with standard lisinopril are much lesser in terms of percentage inhibition and this can be overcome if the compounds purity is enhanced.

All the synthesized derivatives exhibited moderate potency to inhibit ACE. The compounds BF3, BF5 and SB2 and SB4 offered comparatively highest inhibitory activity. Compound BF2 showed the lowest potency with an IC_{50} value 325.36 µg/ml.

ACE inhibitory ability of all the synthesized compounds was found to be less than that of the standard drug. Activity of compounds largely depends on the substitutions on the nucleus. Introduction of an electron withdrawing group results in increased activity. Electron donating substituents resulted in decreased activity. Substitution on meta position of aromatic ring resulted in decreased activity, while substitution on ortho and para positions resulted in an increased of ACE inhibitory activity.

SUMMARY AND CONCLUSION

SUMMARY

The present study is focussed on the designing and synthesis of some novel flavonoid derivatives and evaluation of their possible Angiotensin converting enzyme inhibitory activity. For this the following approaches has been adopted.

Phase 1: Literature review

Literature review provides a solid background to the back one's investigation. It plays a critical role in analysing the existing literature. Literatures reported flavonoids as a good lead for ACE inhibition.

Phase 2: Drug design approach

It involves the following steps

1) Identification of drug target

ACE was selected as the target enzyme for the antihypertensive activity.

2) Lead identification

The lead molecule flavonoid was selected based on literature reviews. Various naturally occurring flavonoids were reported to have Angiotensin converting enzyme inhibiting activity.

3) Lead optimization

Lead optimization is an operationally diverse stage in which the chemical structure of compounds is modified to improve the specificity and selectivity. Lead optimization was done by observing the computational drug likeness properties. All the 10 compounds possessed good drug likeness score and good oral bioavailability. Hence were eligible for further study.

4) Molecular docking studies

Molecular docking study was done by using Autodock4.2. The target enzyme ACE was downloaded from RCSB protein data bank (pdb ID: 1o86). The ligands were subjected to docking and most of the compounds showed binding energy to the enzyme higher than that of standard drug (Lisinopril) used. The ligands SB2, SB4, SB3, SB1, BF5, BF3 were showing best docked to1o86.

Phase 3: Synthesis

In this work, 10 new compounds were synthesized. Two schemes were developed for the synthesis of compounds. Resacetophenone was used as the starting material for both schemes.

In the first scheme resacetophenone reacted with nitro substituted vanillin to form chalcone in presence of ethanol and sodium hydroxide. This formed chalcone was subjected to cyclisation in presence of iodine and DMSO. The product formed was nitro substituted flavonoid and was further reduced to convert the nitro group into amino group. Finally Schiff bases were prepared from this amino flavonoid by reaction with various substituted benzaldehydes.

In the second scheme various substituted benzoyl chlorides were prepared from corresponding benzoic acids and treated with resacetophenone which was refluxed for 24 hours to obtain desired products (substituted benzoyl flavones).

Phase 4: Physical characterisation

Melting points and Rf value of all the compounds are found out.

Phase 5: Spectral characterisation

The structures of the synthesized compounds are established based on the UV, IR, ^{1}HNMR and Mass spectral data.

Phase 6: Evaluation of *in vitro* Angiotensin converting enzyme inhibitory activity.

All the newly synthesized ligands were screened for their angiotensin converting enzyme inhibitory activity by using modified Cushman and cheung method. All compounds showed ACE inhibitory activity. The order of potency of synthesized compounds against ACE were

Scheme 1: SB2> SB4> SB1> SB5> SB3

Scheme 2: BF3> BF5> BF4> BF1> BF2

The synthesized compounds exhibited moderate ACE inhibitory activity. It was observed that nature, position and size of the substituent plays a crucial role in the determination of inhibitory activity of the compound. The compounds BF3, BF5, SB2 and SB4 were found to be more potent in inhibiting the ACE. BF3 was found to be more potent with an IC_{50} value of 71.25µg/ml. This class of drug prevents the formation of Angiotensin I to Angiotensin II by inhibiting ACE, which will results in the lowering of blood pressure and can be used in the treatment of hypertension.

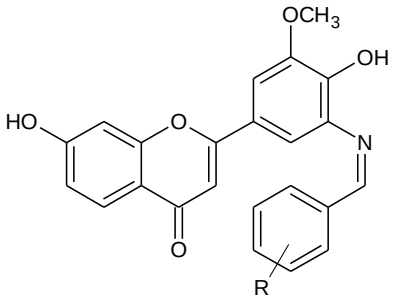
CONCLUSION

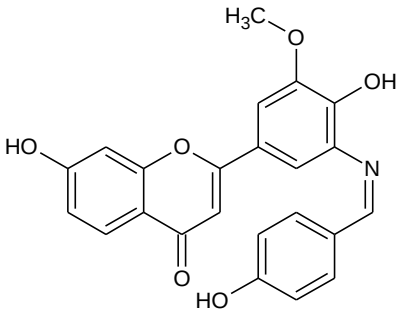
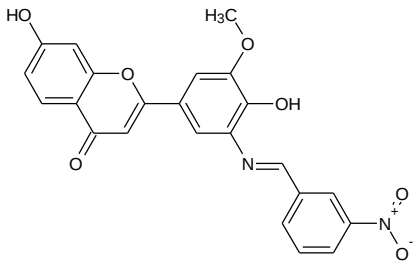
- The basic aim of the present work is to identify the correct conformation of ligands in the active site of enzyme and also to predict their affinity towards the enzyme.
- Computational drug designing approach helps in minimizing the wearisome process of drug discovery and delivers a new drug candidate more rapidly.
- The drug likeness proved the compounds to be orally bioactive
- Docking results established the possibility of flavonoid moiety to possess Angiotensin converting enzyme inhibitory activity.
- The 10 derivatives were synthesized based on the schemes which are previously fixed and it led to good yields.
- Physical characteristics of the compounds were confirmed by melting point and Rf value.
- Structure of the compounds were finally confirmed by UV, IR ^{1}HNMR and Mass spectral studies.
- The synthesized compounds were subjected to the *in vitro* ACE inhibitory activity.
- Derivatives of the flavonoids exhibited moderate activity against ACE. These leads to the inference that all the 10 newly synthesized compounds possess angiotensin converting enzyme inhibitory activity. Novel structure based drug designing methods helped to screen various compounds for a specific activity within a short period of time.

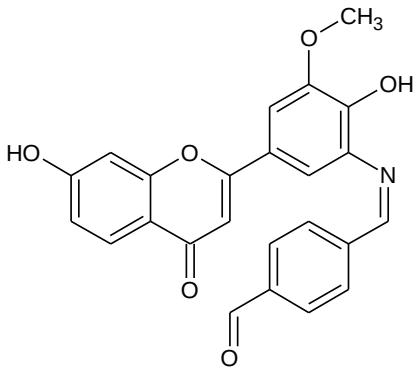
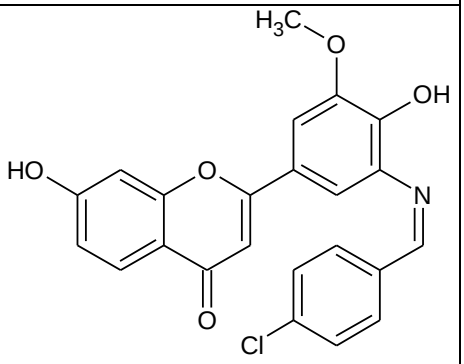
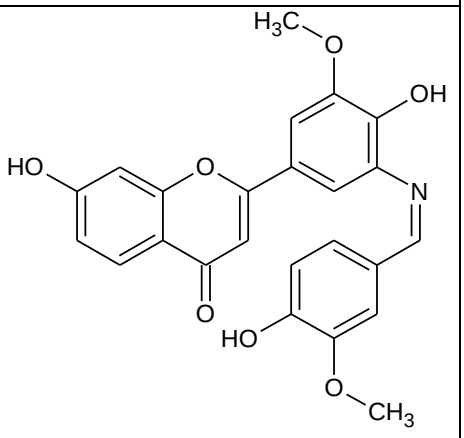
Further *in vivo* studies with these tested synthetic flavonoids have to be carried out to corroborate the result of *in vitro* activity. The present work could be considered as a propitious step for finding the prominence of flavonoid moiety in the treatment of hypertension by inhibiting the key enzyme ACE.

LIST OF NEWLY SYNTHESIZED COMPOUNDS

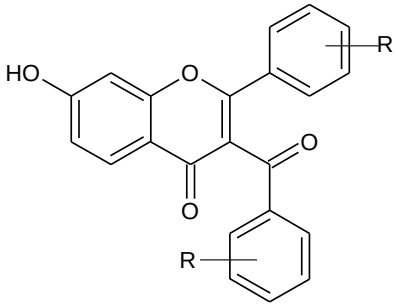
Scheme 1

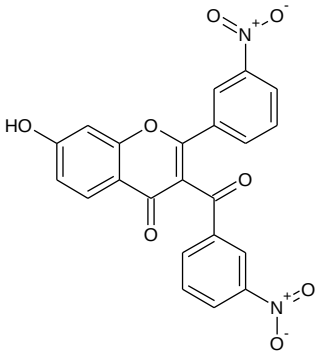
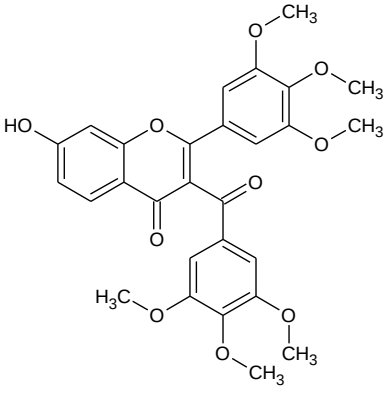


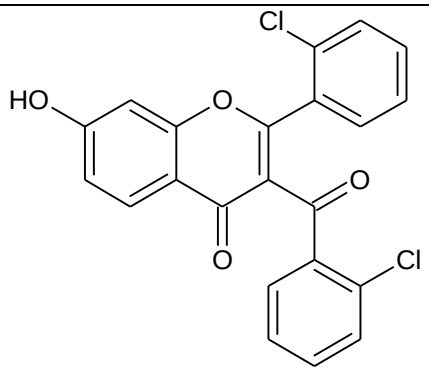
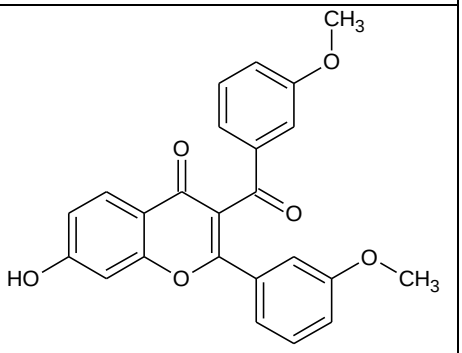
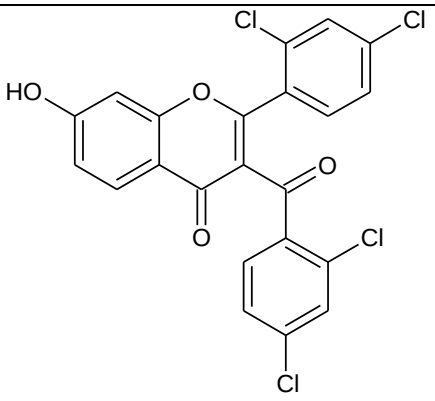
Sl No.	Compound code	Chemical name	Structure
1	**SB1**	7-hydroxy-2-{4-hydroxy-3-[(*Z*)-(4-hydroxybenzylidene)amino]-5-methoxyphenyl}-4*H*-chromen-4-one	
2	**SB2**	7-hydroxy-2-{4-hydroxy-3-methoxy-5-[(*E*)-(3-nitrobenzylidene)amino]phenyl}-4*H*-chromen-4-one	

3	**SB3**	4-[(*Z*)-{[2-hydroxy-5-(7-hydroxy-4-oxo-4*H*-chromen-2-yl)-3-methoxyphenyl]imino}methyl]benzaldehyde	
4	**SB4**	2-{3-[(*Z*)-(4-chlorobenzylidene)amino]-4-hydroxy-5-methoxyphenyl}-7-hydroxy-4*H*-chromen-4-one	
5	**SB5**	7-hydroxy-2-{4-hydroxy-3-[(*Z*)-(4-hydroxy-3-methoxybenzylidene)amino]-5-methoxyphenyl}-4*H*-chromen-4-one	

Scheme 2



Sl no:	Compound code	Chemical name	Structure
1	BF1	7-hydroxy-3-(3-nitrobenzoyl)-2-(3-nitrophenyl)-4*H*-chromen-4-one	
2	BF2	7-hydroxy-3-(3,4,5-trimethoxy benzoyl)-2-(3,4,5-trimethoxyphenyl)-4-*H*-chromen-4-one	

3	BF3	3-(2-chlorobenzoyl)-2-(2-chlorophenyl)-7-hydroxy-4*H*-chromen-4-one	
4	BF4	7-hydroxy-3-(3-methoxybenzoyl)-2-(3-methoxyphenyl)-4*H*-chromen-4-one	
5	BF5	3-(2,4-dichlorobenzoyl)-2-(2,4-dichlorophenyl)-7-hydroxy-4*H*-chromen-4-one	

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