# SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NEW ENTITIES OF BIS INDOLE SUBSTITUTED COUMARIN DERIVATIVES

*Dissertation Submitted in partial fulfillment of the requirement for the award of the degree of*

**MASTER OF PHARMACY**

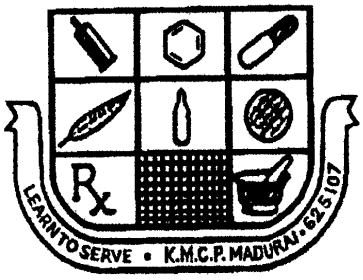
**IN**

**PHARMACEUTICAL CHEMISTRY**

**of**

**THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**

**CHENNAI**




**DEPARTMENT OF PHARMACEUTICAL CHEMISTRY**

**K.M.COLLEGE OF PHARMACY**

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**APRIL– 2012**

# *CERTIFICATE*

This is to certify that the dissertation entitled **"SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NEW ENTITIES OF BIS INDOLE SUBSTITUTED COUMARIN DERIVATIVES"** submitted by **Mr.M.JEGADHEESWARAN** to The Tamilnadu Dr.M.G.R.Medical University, Chennai, in partial fulfillment of the requirement for the award of **Master of Pharmacy** in Pharmaceutical chemistry at K.M. College of Pharmacy, Madurai. It is a bonafide work carried out by him under my guidance and supervision during the academic year 2011-2012.

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# INTRODUCTION [1-8]

The discipline of medicinal chemistry is committed to the discovery and development of new agents for treating diseases. Most of this activity is directed to new natural or synthetic organic compounds.

Inorganic compounds continue to be important in therapy, e.g., trace elements in nutritional therapy, antacids and radiopharmaceuticals but organic molecules with increasingly specific pharmacological activities are clearly dominant.

Development of organic compounds has grown beyond traditional synthetic methods. It now includes the exciting new field of biotechnology using the cell's biochemistry to synthesize new compounds.

Techniques ranging from recombinant DNA and site-directed mutagenesis to fusion of cell lines have greatly broadened the possibilities for new entities.

## Medicinal chemistry

Medicinal chemistry concerns the discovery, the development, the identification and the interpretation of the mode of action of biologically active compounds at the molecular level. But the interest of the medicinal chemistry is also concerned with the study, identification and synthesis of the metabolic products of drugs and related compounds.

## Developments and discoveries in medicinal chemistry

The pharmacist now dispenses modified human insulin's that provide more convenient dosing schedules, cell-stimulating factors that have changed the dosing regimens for chemotherapy, humanized monoclonal antibodies that target specific tissues and fused receptors that intercept immune cell-generated cytokines.

The process of establishing a new pharmaceutical is very difficult and involves the talents of people from variety of disciplines, including chemistry, biochemistry, molecular biology, physiology, pharmacology, pharmaceutics and medicine.

Medicinal chemistry itself is concerned mainly with the organic, analytical and biochemical aspects of this process but the chemist must interact productively with those in other disciplines. Thus, medicinal chemistry occupies a strategic position at the interface of chemistry and biology.

The earliest drug discoveries were made by random sampling of higher plants. Some of this sampling, although based on anecdotal evidence, led to the use of such crude plant drugs as opium, belladonna and ephedrine that have been important for centuries.

With the accidental discovery of penicillin came the screening of microorganisms and the large number of antibiotics from bacterial and fungal sources. Many of these antibiotics provided the prototypical structure that the medicinal chemist modified to obtain anti-bacterial drugs with better therapeutic profiles. With the changes in federal legislation reducing the efficacy requirement for “nutriceutical” the public increasingly is using so-called nontraditional or alternative medicinal that are sold over the counter, many outside of traditional pharmacy distribution channels.

Hundreds of thousands of new organic chemicals are prepared annually throughout the world and many of them are entered in to pharmacological screens to determine whether they have useful biological activity. This process of random screening has been considered inefficient, but it has resulted in the identification of new lead compounds whose structures have been optimized to produce clinical agents. Sometimes, a lead develops by careful observation of the pharmacological behavior of an existing drug.

The discovery that amantadine protects and treats early influenza A came from a general screen for antiviral agents. The use of amantadine in long-term care facilities showed that it also could be used to treat parkinsonian disorders. More recently, automated high-throughput screening systems utilizing cell culture systems with linked enzyme assays and receptor molecules derived from gene cloning have greatly increased the efficiency of random screening. It is now practical to screen enormous libraries of peptides and nucleic acids obtained from combinatorial chemistry procedures.

**Molecular design with target tissue**

Rational design, the opposite approach to high-volume screening, is also flourishing. Significant advances in x-ray crystallography and nuclear magnetic resonance have made it possible to obtain detailed representations of enzymes and other drug receptors.

The techniques of molecular graphics and computational chemistry have provided novel chemical structures that have led to new drugs with potent medicinal activities. Development of HIV protease inhibitors and angiotensin-converting enzyme (ACE) inhibitors came from an understanding of the geometry and chemical character of the respective enzyme's active site.

Even if the receptor structure is not known in detail, rational approaches based on the physiochemical properties of lead compounds can provide new drugs. For example, the development of cimetidine as an antinuclear drug involved a careful study of the changes in antagonism of $H_2$-histamine receptors induced by varying the physical properties of structures.

Medicinal chemistry is an interdisciplinary science covering a particularly wide domain situated at the interface of organic chemistry with life sciences such as biochemistry, pharmacology, molecular biology, immunology, pharmacokinetics and toxicology on one side and chemistry based disciplines such as physical chemistry, crystallography, spectroscopy and computer based information technologies on the other.

The knowledge of the molecular targets (enzymes, receptors, nucleic acids) has benefitted from the progress made in molecular biology, genetic engineering and structural biology. For an increasing number of targets the three dimensional structure and the precise location of the active site are known.

The design of new active substances is therefore more and more based on results obtained from ligand-receptor modeling studies. One can actually consider the existence of a molecular pharmacochemistry making a pair with molecular pharmacology.

**Principles of medicinal chemistry**

To provide an understanding of the principles of medicinal chemistry, it is necessary to consider the physiochemical properties used to develop new pharmacologically active compounds and their mechanism of action, the drug's metabolism including possible biological activities of the metabolites, the importance of stereochemistry in drug design, and the methods used to determine what " space " a drug occupies.

It is important for the pharmacist and the public to understand the rigor that is required for prescription only and FDA approved nonprescription products to be approved relative to the nontraditional products.

It also is important for all people in the health care field and the public to realize that whether these nontraditional products are effective as claimed or not many of the alternate medicines contain pharmacologically active agents that can potentiate or interfere with physician prescribed therapy.

**Objective of medicinal chemistry**

The objective of medicinal chemistry is design and production of compounds that can be used in medicine for the prevention, treatment and cure of human or animal disease. Thus medicinal chemistry is a part of pharmacology, this latter being taken in its etymological sense.

Medicinal chemistry also includes the study of already existing drugs, of their pharmacological properties and their structure activity relationships. An official definition of medicinal chemistry was given by an IUPAC specialized commission.

A certain number of terms more or less synonymous with medicinal chemistry are used: pharmacochemistry, molecular pharmacochemistry, drug design and selective toxicity. The French equivalent to medicinal chemistry is 'Chimie Therapeutique' and the german one is 'Arzneimittelforschung'

**Steps involved in medicinal chemistry**

Medicinal chemistry covers following three steps:

**A discovery step,** consisting of the choice of the therapeutic target (receptor, enzyme, transport group, cellular or in vivo model) and the identification (or discovery) and production of new active substances interacting with the selected target. Such compounds are usually called lead compounds; they can originate from synthetic organic chemistry, from natural sources or from biotechnological processes.

**An optimization step,** that deals with the improvement of the lead structure. The optimization process takes primarily into account the increase in potency, selectivity and toxicity. Its characteristics are the establishment and analysis of structure activity relationships, to enable the understanding of the molecular mode of action. However, an assessment of the pharmacokinetic parameters (ADME) and oral bioavailability is almost systematically practiced at an early stage of the development.

**A development step** is the continuation of the improvement of the pharmacokinetic and pharmaceutical properties (chemical formulation) of the active substances in order to render them suitable for clinical use. This chemical formulation process can consist in the preparation of better absorbed compounds of sustained release formulations of water-soluble derivatives or in the elimination of properties related to the patient's compliance

**Molecular and cellular pharmacology**

This is the study of pharmacological action of drug at the molecular or at the cellular level. The first objective is to identify the cellular levels of action. Three levels, important for drug activity can be distinguished:

*The plastic membrane* which is very rich in potential targets, notably in receptors. *The cytosol* with its enzymatic equipment and the organelle membranes with their particular ion transporters. *The nucleus* which notably responds to the steroid hormones to anti cancer drugs and to gene therapy.

**Systemic pharmacology**

The systemic pharmacology considers the effects of biologically active substances in integrated systems (cardiovascular, skeletal, central nervous, gastrointestinal, pulmonary, etc.). The experimentation is performed in intact animals or in isolated organs (isolated heart, isolated arteria, perfused kidney, etc.).

The main difficulty resides in the design of animal experimental models that are predictive of an activity in a human disease. As many pharmacological experiments are still performed on healthy animals or on disease-stimulating paradigms, their extrapolation to clinical situations is questionable.

The availability of transgenic mice, in which the genes of a human disease were introduced, represents an interesting progress. However in all animal models, intra and interspecific physiological variations account for rather imprecise results the margins being often as elevated as +/- 50 %.

**Clinical pharmacology**

Clinical pharmacology deals with the examination in humans of the effects of a new drug candidate. The tests are performed under the responsibility of the clinical pharmacologist who is generally a medical doctor and who has to report to an ethical committee.

Phase I tests take place in healthy volunteers. They aim to assess the level of dosing and tolerance (dose ranging) and to initiate metabolic studies in humans. Once the safety margin has been determined phase II, II and IV studies examine successively the beneficial effects in patients the possible side-effect, the comparison of the drug with reference drugs and the emergence of new therapeutic indications.

## CLASSIFICATION SYSTEMS OF MEDICINES

All attempts to establish clear-cut and well-balanced drug classifications lead to failures, due to the complex nature of the medicaments, which do not fit into simplified systems. The best way to illustrate how drugs are situated with regard to each other is to use several classification systems, based on different criteria.

### A. Theoretical classification

#### *Based on the origin of the drug*

Drug from natural origin can come from three sources. They can originate from minerals. Various simple inorganic substances are still in use in medicine: sulphur, iodine, phosphates and arsenates, calcium, sodium, magnesium, iron and bismuth salts etc. From the animal kingdom some hormones (e.g. insulin) and fish liver oils (vitamin A and E) were extracted for a long time. Biliary salts yield precursors for steroid hemi synthesis (corticoids, sexual hormones). However, the majority of natural compounds are produced by vegetals (alkaloids, cardiac glycosides, antibiotics, anti cancer drugs).

Drugs from synthetic origin relay the natural compounds in providing improved and or simplified synthetic analogues the production of which are not dependent on hazardous agricultural supplies.

In an intermediary position between natural and synthetic compounds are found the various fermentation products (vitamins, amino acids) and the products issued from genetic engineering (e.g. recombinant insulin).

#### *Based on the mode of action*

One can distinguish between medicaments which really treat the cause of the disease, medicaments which compensate for the deficiency of a given substance and medicaments which only aim to alleviate the symptoms of the disease.

The drugs acting on the casual agent of the disease are called etiological drugs and represent ‘true’ medicaments. Presently most of them belong to the class of chimiotherapeutic drugs, i.e. to compounds used to treat infections (antibacterial and

antiviral) and parasitic diseases. The principle of their activity resides in their selective toxicity: destroy the invader without destroying the host.

Logically can be added to this group a certain number of drugs used by healthy persons in a preventive way in order to protect them from a future illness (vaccinotherapy, aspirin and anticoagulants to prevent cardiac infarcts, vitamins and antioxidants against neurodegenerative disorders). Other drugs can temporarily modify a physiological process (steroidal contraceptives).

Substitutive drugs take the place of a missing substance: the lack can be due to dietary reasons (vitamin deficiency) or to a physiological disturbance (insulin in diabetes, estrogens in menopause). The substitutive treatment can cover a very short period (intravenous rehydration in case of hemorrhages and diarrhea), or can last the whole life (hormonal treatment in Addison's disease).

Symptomatic treatments are given in order to attenuate or to neutralize the disorders which result from a pathological state. They abolish general symptoms such as fever, pain or insomnia. However, their activity can be much more specific and targeted to a particular system: thus symptomatic drugs are available for cardiovascular for neuropsychiatric for respiratory for digestive diseases etc. As a rule, a symptomatic treatment is not supposed to cure the patient, but rather to render his all-day life more comfortable and to prolong his life. Antihypertensive drugs for example abolish, or at leaset diminish, the symptoms associated with arterial hypertension, but they play also a preventive role against the cardio-vascular complications of hypertension (notably myocardial infarct).

***Based on the nature of the illness***

The so-called physiological classification was adopted by the World Health Organization (WHO) in 1968. It classifies the drugs according to the body system on which they act (drugs affecting the central nervous system, the gastro-urinary tract, the musculoskeletal system, for example).

***Based on the chemical structure***

This classification is important to people involved in pharmaceutical research. An expert in peptide or prostaglandin chemistry will be primarily concerned with the various chemical manipulations that can be performed on these molecules and will rely on someone else to screen them for effects against the various illnesses susceptible to peptide or prostaglandin therapy. On the other hand, the chemical classification allows an excellent overview of all the congeners and analogues derived from an initial lead and thus facilitate structure-activity considerations.

**B. Practical classification**

In practice the most powerful and useful system developed so far is a compromise between the methods, known as the anatomical-therapeutic-chemical system (ATC). The system divides products in to 13 general groups according to the body system on which they act: A, alimentary system: B, blood and blood-forming organs, and so on. This is the usually followed by the name of the disease they cure and finally by a description of the chemical classes involved.

An even simpler classification is usual among the medicinal chemist community, if distinguishes between four major classes of drugs:

***Agents acting on the central nervous system****: Psychotropic and neurological drugs*

In man, the central nervous system (CNS) comprises the brain and the spinal cord and it controls the thoughts, emotions, sensations and motor functions.

The psychotropic drugs such as

- Antidepressants,
- Antipsychotics,
- Anxiolytics
- Psychomimetics

The neurological drugs such as:

- Anticonvulsants
- Sedatives and hypnotics
- Analgesics
- Anti-parkinsonian drugs, etc.

***Pharmacodynamic agents***

The pharmocodynamic agents are drugs affecting the normal dynamic processes of the body and particularly the cardio-vascular domain. This group is composed of the anti-arrythmics, the anti-anginals, the vasodilators, the anti-hypertensives, the diuretics and the anti-thrombotics which all, directly or indirectly, concern the heart or the blood circulation. Traditional the anti-allergic drugs and the drugs acting on the gastrointestinal tract are also included in the class of pharmocodynamic agents.

***Chemotherapeutic agents***

Initially, the term chemotherapy referred to the treatment by means of drugs preventing selectivity the development of various kinds of infesting hosts: protozoa (amoebas, leishmania, hematozoa, treponema, trypanosoma), microbes, viruses and as rule all parasites which propagate infectious diseases. IN the same context of selective toxicity, the anti-cancer treatments also belong to the class of chemotherapeutic agents.

***Agents acting on metabolic diseases and endocrine functions***

This category of drugs is made up of a collection of agents which do not easily fit in the previous classes. It consists of the anti-inflammatory, the anti-arthritics, the anti-diabetics, and the hypolipidemic agents, the anorectics and most of the peptide and steroidal hormones.

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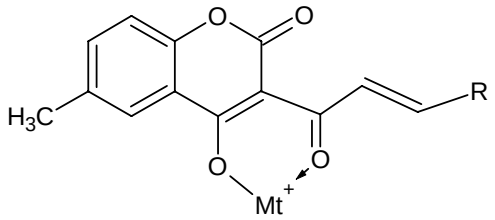
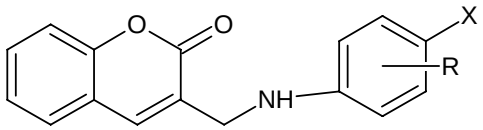
# LITERATURE REVIEW [9-38]

## COUMARIN DERIVATIVES

- **K. B. Vyas et al. [9] [2009]** have synthesised metal complexes of 3-[ {-(3',4'-di methoxy phenyl) }-prop-2-enoyl]-4-hydroxy-6-methyl-2H-chromene-2-one with Cu(II), Ni(II), Fe(II), Co(II) and Mn(II) which have been characterized using elemental analysis, IR spectra and conductivity measurements. These studies revealed that they are having octahedral geometry of the type [$ML_2(H_2O)_2$ ]. In vitro antimicrobial activity of all synthesized compounds and standard drugs have been evaluated against four strains of bacterial culture and one fungus, which includes two gram +ve bacterial culture and two gram -ve bacteria culture. The compounds show net enhancement in activity on coordination of metals with ligand but moderate activity as compared to standard drugs.

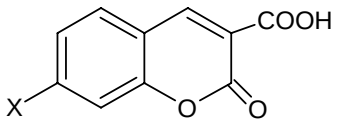



- **P. Selvam et al. [10] [2010]** have new synthesized new series of coumarin derivatives by condensation of ethyl acetoacetate and resorcinol. The chemical structures of the synthesized compounds were confirmed by means of IR, 1H-NMR and Mass spectral analysis. These compounds were screened for their Antioxidant, Analgesic and Anti-inflammatory activities.



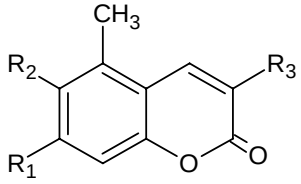
- **Parameswaran Manojkumar et al. [11] [2009]** have synthesized coumarinyl heterocycles elucidate the potential role of these compounds as antioxidants and cytotoxic agents against Dalton's lymphoma ascites tumour cells (DLA) and Ehrlich ascites carcinoma cells (EAC). The synthesis of coumarin derivatives containing pyrazole, pyrazolone, thiazolidin-4-one, 5-carboxymethyl-4-thiazolidinone and 3-acetyl-1,3,4-oxadiazole ring is reported. 4-Methylcoumarinyl- 7-oxyacetic acid hydrazide (1) reacted with arylazopropanes or hydrazono-3-oxobutyrate derivatives to form pyrazole (3a-c) and pyrazolone derivatives (5a-c). Heterocyclisation of Schiff's bases of 1 with thioglycolic acid, thiomalic acid or acetic anhydride afforded novel heterocyclic derivatives 4-thiazolidinones (7a-c), 5-carboxymethyl- 4-thiazolidinones (8a-c) and oxadiazoles (9a-c), respectively. Some of the compounds showed promising antioxidant activity in vitro and cytotoxic activity against DLA cells and EAC cells.

- **Shaabani et al. [12] [2009]** have synthesized coumarin derivatives in relatively high yields via Knoevenagel condensation reaction of an orthohydroxyaryl aldehyde and an activated β-dicarbonyl C-H acid in the presence of a recyclable ionic liquid 1,1,3,3-N,N,N',N'- tetramethylguanidinium trifluoroacetate under either classical heating conditions or using microwave irradiation. Application of microwave irradiation decreased the required time by a factor of about 200. The ionic liquid could be recycled several times without loss of efficiency with regards to the reaction times and yields.

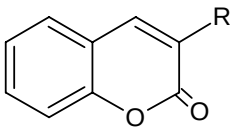



- **Hosanagara N. Harishkumar et al. [13] [2011]** have studied the influence of choline chloride/urea ionic liquid in solid phase on the Knoevenagel condensation. The active methylene compounds such as meldrum's acid, diethylmalonate, ethyl cyanoacetate, dimethylmalonate, were efficiently condensed with various salicylaldehydes in presence of choline chloride/urea

ionic liquid without using any solvents or additional catalyst. The reaction is remarkably facile because of the air and water stability of the catalyst, and needs no special precautions. The reactions were completed within 1hr with excellent yields (95%). The products formed were sufficiently pure, and can be easily recovered. The use of ionic liquid choline chloride/urea in solid phase offered several significant advantages such as low cost, greater selectivity and easy isolation of products.

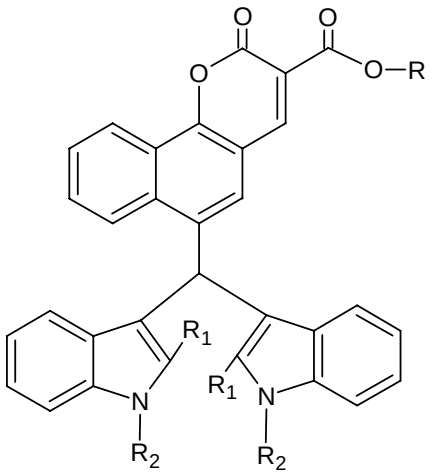



- **Afsheen Arshad et al. [14] [2011]** have synthesized two novel series of hydrazinyl thiazolyl coumarin derivatives and fully characterized by IR, 1H NMR, 13C NMR, elemental analysis and mass spectral data. The structures of some compounds were further confirmed by X-ray crystallography. All of these derivatives, 10aed and 15aeh, were screened in vitro for antimicrobial activity against various bacteria species including Mycobacterium tuberculosis and Candida albicans. The compounds 10c, 10d and 15e exhibited very good activities against all of the tested microbial strains.

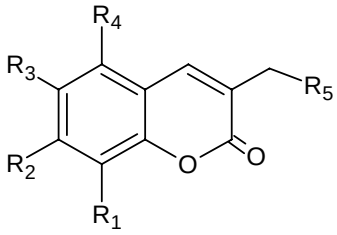



- **Koneni V. Sashidhara et al. [15] [2010]** have synthesized a series of novel coumarin bisindole heterocycles by following an uncommon method and evaluated for their antihyperlipidemic activity in hyperlipidemic hamster model. Among 12 compounds tested, the compound 5e showed potent antihyperlipidemic activity and was found to decrease the plasma triglyceride levels (TG) by 55%, total cholesterol (TC) by 20%, accompanied by an

increase in HDL-C/TC ratio by 42% in hyperlipidemic rats to a greater degree than some of the reference statins.

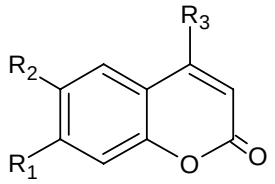



- **Andrea Behrenswertha et al. [16] [2009]** have synthesized 36 coumarin and 2H-chromene derivatives by applying a recently developed umpoled domino reaction using substituted salicylaldehyde and α,β-unsaturated aldehyde derivatives as starting compounds. In radioligand binding studies 5-substituted 3-benzylcoumarin derivatives showed affinity to cannabinoid $CB_1$ and $CB_2$ receptors and were identified as new lead structures. In further GTPγS binding studies selected compounds were shown to be antagonists or inverse agonists.



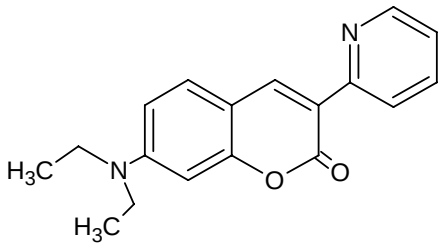

- **Hakkı Murat Bilgina et al. [17] [2009]** have evaluated the possible cytoprotective properties of coumarin and some coumarin derivatives against $CCl_4$ (carbon tetrachloride)-induced hepatotoxicity. Coumarin (1,2-benzopyrone) and coumarin derivatives esculetin (6,7-dihydroxycoumarin), scoparone (6,7-dimethoxycoumarin) and 4-methylumbelliferone (7-hyroxy-4-

methyl) were examined for their protective effect against $CCl_4$-induced hepatotoxicity in Male Sprague-Dawley rats. A single toxic dose of $CCl_4$ (1.25 ml kg−1, orally) produced liver damage in rats, seen histologically as centrilobular necrosis. Administration of $CCl_4$ increased serum enzyme levels of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). Pre-treatment of rats with esculetin (31.15 mg kg−1, orally) and scoparone (35 mg kg−1, orally) significantly prevented $CCl_4$-induced increase in serum enzymes, whereas 4-methylumbelliferone (35 mg kg−1) and coumarin (30 mg kg−1) had no effect against $CCl_4$-induced rise in serum enzymes. Morphological findings were consistent with the plasma transaminase observations. Among the coumarin analogs, esculetin, which possesses orthodihydroxy coumarins, showed the strongest protective effect against $CCl_4$-induced liver damage, followed by scoparone, 4-methylumbelliferone and coumarin, respectively. The results of this study indicate that the chemical structures of coumarins play an important role in the prevention of liver toxicity.

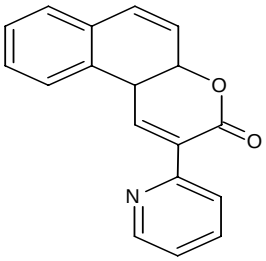



- **Tianzhi Yua et al. [18] [2009]** have synthesized two new coumarin derivatives, 7-(diethylamino)-3-(pyridin-2-yl) coumarin (DAPC) and 3-(pyridin-2-yl)-benzo[5,6] coumarin (PBC), and characterized by elemental analysis, 1H NMR, FT-IR and UV–visible absorption spectra. Their structures were determined by X-ray crystallography single crystal analysis. The fluorescence behaviours of the compounds in dichloromethane solutions were observed. The results show that the compound DAPC exhibits high fluorescence quantum yield (0.84) and they exhibit strong blue emissions under ultraviolet light excitation. The energy levels of the HOMO and LUMO of the compounds have been calculated with density functional theory (DFT) and

time-dependent density functional theory (TD-DFT) at B3LYP/6-31G(d) level.



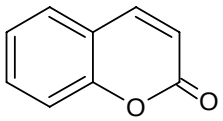

**(DAPC)**



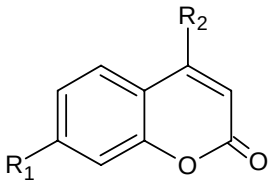

**(PBC)**

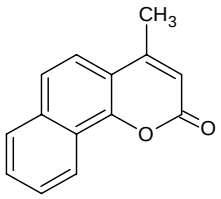
- **Kinza Aslam et al. [19] [2010]** have coumarin synthesized by Perkin reaction using salicylaldehyde, acetic acid and sodium acetate. Due to the misuse of acetic anhydride in narcotics synthesis, acetic acid was substituted for acetic anhydride in Perkin reaction. On the basis of this substitution a hypothesis was proposed that "acetic acid could be substituted as an acetylating agent in place of acetic anhydride in coumarin synthesis via Perkin reaction". In the present research project, salicylaldehyde was prepared from phenol, sodium hydroxide and chloroform for further procedure. Then four different coumarin samples were synthesized by changing the parameter of reactants proportions. From this parameter, we designed a trend of high product yield. Yields of Coumarin samples will lead towards either acception or rejection of the above proposed hypothesis. In the next step, these Coumarin samples were characterized by age yield (%), solubility and melting points. At last Antibacterial activities of all the four coumarin samples were evaluated against two bacterial strains; E.coli and S.aureus. As a consequence of all above, it was inferred that the yields of all coumarin samples obtained were low as compared to the yield obtained by the use of acetic anhydride in previous reports. This led to the rejection of proposed hypothesis. Among four Coumarin samples, sample-4 obtained by taking equal amounts of all the reactants had shown maximum yield, best characterization and excellent antibacterial activity. In spite of low yields obtained, the remarkable antibacterial activities of Coumarin samples

have enabled us to suggest coumarin as a strong antibacterial agent and it must be employed for further applications.

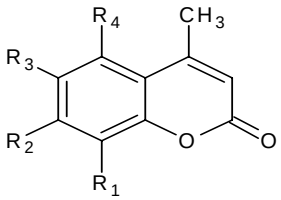


- **Hassan Valizadeh et al. [20] [2005]** have described the ability of titanium (IV) chloride as a catalyst to promote the Pechmann condensation reaction with a range of phenols and β-keto esters. The reaction was carried out by addition of $TiCl_4$ to a mixture of the phenol and the β-keto ester with thorough stirring in the absence of a solvent and represents an improvement on the classical Pechmann conditions. The yields of coumarins obtained via this novel protocol were significantly higher than those using the conventional method and the reaction duration was reduced to a few minutes or even a few seconds.

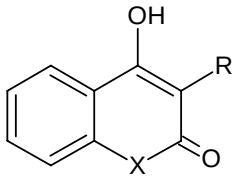




- **Rangappa S. Keri et al. [21] [2009]** have used Phosphotungstic acid (PTA) as an efficient catalyst for the von Pechmann condensation reaction of phenols and b-keto esters to synthesize coumarin under solvent-free conditions. This method was compared with those of the reactions in the different solvents and catalysts. The methodology presented offers significant improvements for the synthesis of coumarins with regard to yield of products, simplicity in operation and green aspects by avoiding toxic conventional catalysts and solvents.

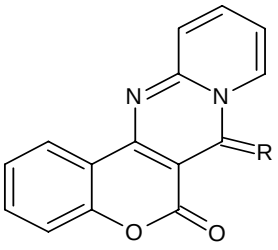


- **Jae-Chul Jung et al. [22] [2009]** have reviewed to summarize recent chemical syntheses and structural modifications of 4-hydroxycoumarin and its derivatives, of interest due to their characteristic conjugated molecular architecture and biological activities.

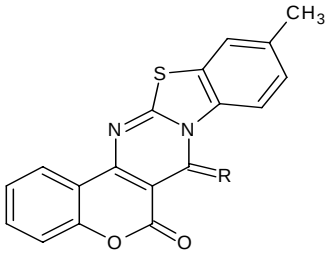



X=N & S

- **Stojadin V. Dekić et al. [23] [2007]** have studied the reactions of 4-chloro-2-oxo-2H-chromene-3-carbonitrile (1) with 4-methylpyridin- 2-ylamine (2) and 6-methoxy-benzothiazol-2-ylamine (3) in acetonitrile containing a catalytic amount of triethylamine which gave the new coumarin derivatives 7-imino-10-methyl-7H- 5-oxa-7a,12-diaza-benzo[a]anthracen-6-one (4) and 7-imino-10-methoxy-7H-5-oxa-12- thia-7a,13-diaza-indeno[1,2-b]phenanthren-6-one (5) in 52 and 39% yields, respectively. The novel compounds were subjected to acid hydrolysis giving the corresponding oxoderivatives 10-methyl-5-oxa-7a,12-diaza-benzo[a]anthracene-6,7-dione (6) and 10- methoxy-5-oxa-12-thia-7a,13-diaza-indeno[1,2-b]phenanthrene-6,7-dione (7) in 64 and 58% yields, respectively. The structural assignments of the synthesized compounds were based on elemental analyses, IR and proton NMR spectra.



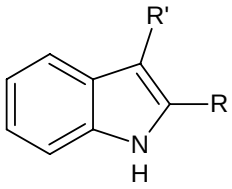

*R= O, NH*



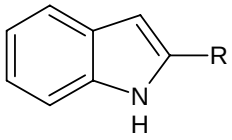

*R= O, NH*

**INDOLE DERVIVATIVES**

- **Feng Ping Yi et al. [24] [2007]** have performed Fischer indole cyclization of phenyl hydrazine and various ketones using carboxyl-functionalized ionic liquid, 1-carboxymethyl- 3-methylimidazolium tetra fluoro borate (abbreviated as [cmmim][BF4]) as catalyst. The yields of the target compounds were 80–92%, the purities were 96–98%. The catalyst could be recovered and reused for at least six times without significant loss in activity.

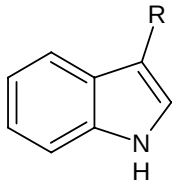



- **Huseyin Cavdar et al. [25] [2005]** have done regioselective alkylation at the 2-position of the indole nucleus by treating indole with 4,7-Dihydroindole through conjugate addition with a, β-unsaturated carbonyl compounds. The oxidation of the Michael adducts affords the corresponding 2-substituted indole derivatives which were characterized by spectroscopic methods.

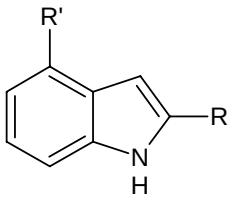



- **Mohamed A. A. Radwan et al. [26] [2007]** Treated 3-cyanoacetyl indole (1) with the diazonium salts of 3-phenyl-5-aminopyrazole and 2-aminobenzimidazole afforded the corresponding hydrazones (4) and (5). 3-Cyanoacetyl indole reacted with phenylisothiocyanate to give the corresponding thioacetanilide derivative (7). Treatment of (7) with hydrazonoyl chlorides afforded the corresponding 1,3,4-thiadiazole derivatives (8a–f) and (9). Also, the thioacetanilide reacted with a-haloketones to afford thiophene derivatives (10a,b) (tenidap analogues), or thiazolidin-4-one

derivative (11). The newly synthesized compounds were found to possess potential anti-inflammatory and analgesic activities.

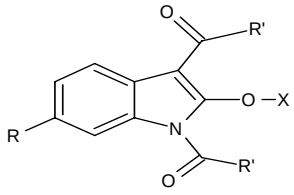



- **Yuichi Sugimoto et al. [27] [2006]** have discovered A novel series of 2-(1,2,4-oxadiazol-5-yl)-1H-indole derivatives as nociceptin/orphanin FQ (N/OFQ) receptor antagonists. Systematic modification of our original lead by changing the pendant functional groups, linker, heterocyclic core, and basic side chain revealed the structure–activity requirements for this novel template and resulted in the identification of more potent analog with improved potency as compared to the parent compound.

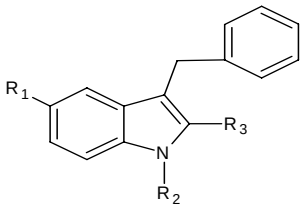



- **A Leonardi et al [28] [1994]** have done synthesis and pharmacological evaluation of a series of pyrrolidine analogues of thymoxamine allowed access to the basic SAR for the aromatic substitution pattern. Their results confirmed the relevance of the simultaneous presence of the hydroxy and methyl groups on the benzene ring and prompted to prepare the corresponding indole congener. The principle of the phenol-indol bioisosterism was confirmed by the results obtained. The introduction of the N-(2-methoxyphenyl) piperazine moiety instead of pyrrolidine changed the receptor affinity profile and introduced a good uroselectivity.

- **Mukund Jha et al. [29] [2011]** have developed the one-pot triacetylation of indolin-3-ones. They have devised a simple two-step reaction sequences to produce di- and mono-acetylated indoles from indolin-2-ones. The indolin-2-ones were first subjected to acetylation in the presence of acetic anhydride and a catalytic amount of N,N-dimethyl amino pyridine to give 2-acetoxy-1,3-diacetylindoles. Subsequently, an enzyme-assisted deacetylation resulted in the chemo selective deprotection of the acetoxy group to produce 1,3-diacetyl-2-hydroxyindoles. However, a chemical deacetylation of 2-acetoxy-1,3-diacetylinoles under mild basic or acidic conditions resulted in the formation of 3-acetyl-2-hydroxyindoles.

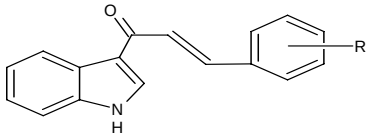



- **Chang Qing Shi et al. [30] [2007]** have designed A variety of indole derivatives then synthesized and preliminarily evaluated for their in vitro cytotoxic activity in the A431 and H460 cell lines. All the compounds examined conferred unusual potency in a tumor cell cytotoxicity assay. The findings showed the indole derivatives would be a promising candidate for the development of new anticancer agents.

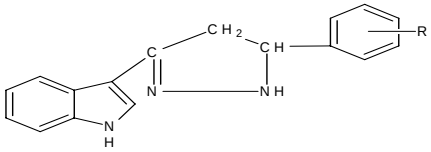



- **Preeti Rani et al. [31] [2004]** have synthesised Chalcones of indole (1–5) and their corresponding products; pyrazolines (6–10) and azo compounds (11–15) and evaluated for their anti-inflammatory activity against carrageenan induced oedema in albino rats at a dose of 50 mg kg–1 oral. The structure of

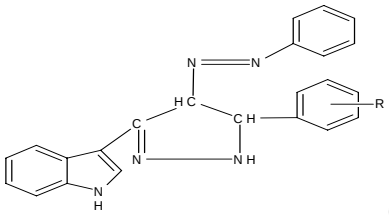
compounds was confirmed by IR, 1H-NMR and mass spectral data. All the compounds of this series showed promising anti-inflammatory activity. The most active compound of this series is 3-[1-acetyl-5-(p-hydroxyphenyl)-2-pyrazolin-3-yl] indole (7) was found to be most potent, which has shown higher percent of inhibition of oedema, lower ulcerogenic liability and acute toxicity than the standard drug phenylbutazone.



**(1–5)**

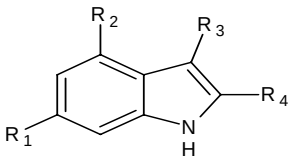


**(6–10)**



**(11–15)**

- **Matthias Nettekoven et al. [32] [2001]** have synthesised two indole derivative libraries. They have worked with 2-Acyl-3-amino-indoles (4) which could easily be accessed by treatment of the intermediates (3) with bases in a one-pot reaction sequence whereas the reaction of the isolated intermediates (5) (R3=aromatic-, heteroaromatic, or cycloalkyl) with acid chlorides yielded the novel indole derivatives (6). The products were purified by reversed phase column chromatography and obtained in multi-milligram quantities in acceptable yields.



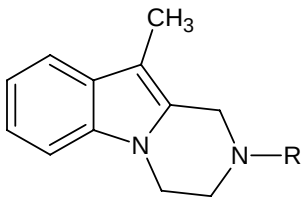
- **Mostafa Kiamehr et al. [33] [2009]** have synthesised pentacyclic indole derivatives which was achieved via domino Knoevenagel-hetero-Diels–Alder reactions of indolin-2-thiones and O-propargylated salicylaldehyde derivatives in $CH_3CN$ in the presence of 10 mol % of ZnO as a heterogeneous catalyst. The products are formed in good to excellent yields.

- **Michele Giampieri et al. [34] [2009]** have prepared Unsymmetrical methylene derivatives (5) following a known method, by reaction of the Mannich bases of 2-naphthols (4) with indoles. All synthesized compounds were tested against a wide panel of viruses, since previous work showed that Mannich bases on 7-hydroxycoumarin (1) and unsymmetrical methylene derivatives (2) were endowed with some antiviral activities. The symmetrical Mannich bases (4) were completely inactive, whereas the unsymmetrical methylene derivatives (5), although possessing a certain degree of toxicity, showed a significant activity against RSV. Some of compounds 5 showed a moderate antiviral activity against HIV-1, BVDV, YFV and CVB-2. The lack of activity of Mannich bases (4) demonstrates the crucial importance for antiviral activity of coumarin moiety present in Mannich bases (1).

- **Tetsuya Mochizuki et al. [35] [2010]** have designed Indole derivatives (3a) and (3b) of adenophostin A (2) in which the adenine of 2 was replaced with indole or 4-fluoroindole as potential inositol trisphosphate receptor ligands. These target compounds were successfully synthesized from the key disaccharide unit (6). Biological evaluation showed that 3b selectively activates IP3R1, a subtype of IP3 receptors.

- **Rakesh Kumar Tiwari et al. [36] [2006]** have synthesized A Series of substituted-10-methyl-1,2,3,4-tetrahydropyrazino [1,2-a] indoles derivatives and examined for their activity against pathogenic strains of Aspergillus fumigatus (ITCC 4517), Aspergillus flavus (ITCC 5192) Aspergillus niger (ITCC 5405) and Candida albicans (ITCC No 4718). All synthesized

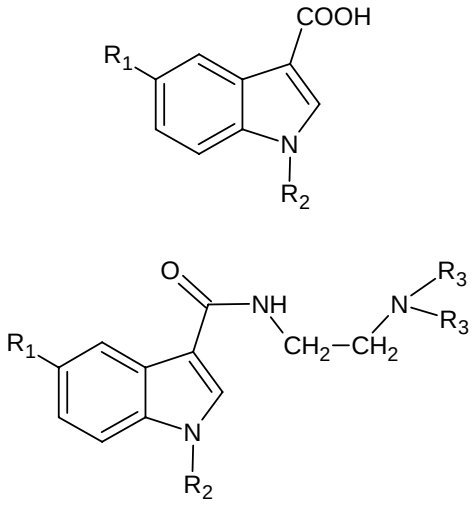
compounds showed mild to moderate activity, except for 2-substituted-10-methyl-1,2,3,4-tetrahydropyrazino [1,2-a] indoles (6a–d). The most active 1-(4-chlorophenyl)-10-methyl-1,2,3,4-tetrahydropyrazino [1,2-a] indole (4c) exhibited a MIC value of 5.85 lg/disc against A. fumigatus and 11.71 lg/disc against A. flavus and A. niger in disc diffusion assay. Anti-Aspergillus activity of active compound (4c) by micro broth dilution assay was found to be 15.62 lg/ml in case of Aspergillus fumigatus and 31.25 lg/ml with Aspergillus flavus and Aspergillus niger. The MIC90 value of the most active compound by percent germination inhibition assay was found to be 15.62 lg/ml against Aspergillus fumigatus. The MIC90 values of substituted-10-methyl-1,2,3,4-tetrahydropyrazino [1,2-a] indoles against C. albicans ranged from 15.62 to 250 lg/ml. The in vitro toxicity of the most active 1-(4-chlorophenyl)-10-methyl-1,2,3,4-tetrahydropyrazino[1,2-a]indole 4c was evaluated using haemolytic assay, in which the compound was found to be non-toxic to human erythrocytes up to a concentration of 312.50 lg/ml. The standard drug amphotericin B exhibited 100% lysis at a concentration of 37.5 lg/ml.




- **Rakesh Kumar Tiwari et al. [37] [2006]** have synthesized a series of substituted 1,2,3,4-tetrahydropyrazino [1,2-a] indole derivatives and tested against the Gram positive and Gram negative strains of bacteria namely Staphylococcus aureus (MTCCB 737), Salmonella typhi (MTCCB 733), Pseudomonas aeruginosa (MTCCB 741), Streptomyces thermonitrificans (MTCCB 1824) and Escherichia coli (MTCCB 1652). All synthesized compounds showed mild to moderate activity. Compounds (4d–f) were found to have potent activity against pathogenic bacteria used in the study. Their MIC ranged from 3.75 to 60 lg/disc. In vitro toxicity tests demonstrated that toxicity of (4d–f) was not significantly different than that of gentamycin.

However, at higher concentration (1000–4000 lg/ml) difference was highly significant.

- **Sandra Battaglia et al. [38] [1999]** have prepared a number of indole amide derivatives bearing a basic side chain, in which the indole ring replaces the isoster benzimidazole nucleus typical of some well-known antihistamines, and tested for their $H_1$-antihistaminic activity. The 1-benzyl-3-indolecarboxamides (32–42) showed antihistaminic ($H_1$) activity ($pA_2$ 6–8) the 3-indolylglyoxylylamides (7–16) and the 2-indolecarboxamides (48–56) showed little or no activity. Insertion of the basic side chain of the active 3-indolecarboxamide derivatives into a piperazine ring (compounds 57–59) led to a dramatic loss of activity. All the active compounds proved to be competitive antagonists, since the values of the regression slope were not statistically different from (1). The most active compounds, 32, 33, 38–41, were also tested both in vitro for their anticholinergic activity and in vivo for their ability to antagonize histamine-induced cutaneous vascular permeability in rats.



# AIM OF THE WORK

Coumarins are the compounds which posses a range of pharmacological activity like anti-HIV, antimicrobial, anticancer and anticoagulant. These pharmacological activities of coumarins have been revealed from literature review.

Literatures were also helpful to relate the coumarin and their novel derivatives called Bisindole substituted coumarins for their Antihyperlipidemic activity.

So the aim the present study could be divided as follows,

- ♣ Synthesis of some new chemical entities of bisindole substituted coumarins having different groups.
- ♣ Purification and characterization of the synthesised compounds based on their physical properties.
- ♣ Characterization of the compounds based on the spectral data to elucidate their nature.
- ♣ Evaluation of these compounds to check their Anti-hyperlipidemic potential.

# PLAN OF THE WORK

The various Bisindole substituted coumarin derivatives were decided to synthesis and to evaluate their Antihyperlipidemic activity.

The plan of the present study could be briefed as follows,

**SCHEME 1**

The scheme for the synthesis of various Bisindole substituted coumarin derivatives involves following steps.

**Step I**

This involves the synthesis of α-napthol dialdehyde **[II]** from α-napthol [**I]** in the presence of hexamine (Hexamethylene tetramine-HMTA) with trifluoro acetic acid (TFA) and 10% sulphuric acid. This type of reaction is known as Duff reaction.

**Step II**

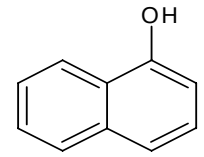
In this step, the compound obtained in the first step that is α-napthol dialdehyde **[II]** was treated with diethyl malonate in the presence of ethanol and piperidine to get a coumarinic adduct **[III]** with aldehyde functional group.

**Step III**

This step involves synthesis of different Bisindole substituted coumarin derivatives by treating various substituted indoles with coumarinic aldehyde compound **[III]** in the presence of Iodine and Acetonitrile.

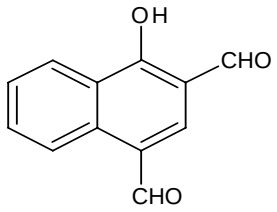
# SCHEME OF THE WORK

## General scheme



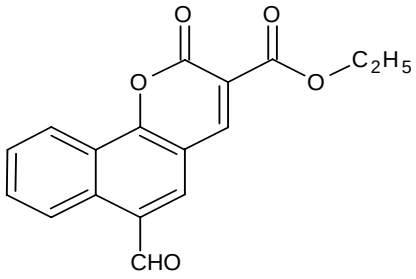
naphthalen-1-ol [I]

i) HMTA/TFA, 100 $^{o}$C, 3 hrs.
ii) 10 % $H_2SO_4$, 100 $^{o}$C, 2 hrs.



4-hydroxynaphthalene-1,3-dicarbaldehyde [II]

i) Diethyl malonate
ii) Ethanol, piperidine
iii) Reflux 30 mins



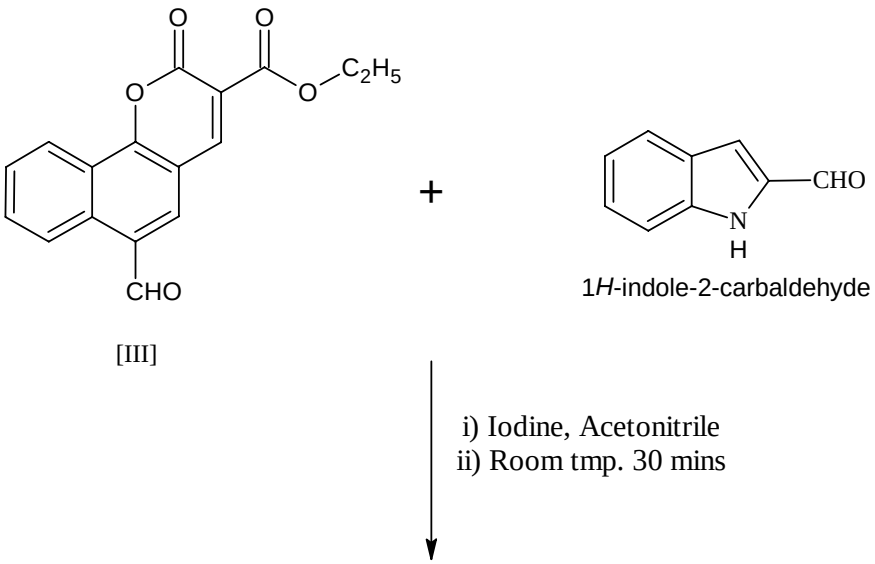
ethyl 6-formyl-2-oxo-2*H*-benzo[*h*]chromene-3-carboxylate [III]

Different substituted inole
i) Iodine
ii) Acetonitrile
Room temp. 30 mins

**Compound $S_1$-$S_6$**

## SCHEME FOR INDIVIDUAL SYNTHESIS

**Synthesis of Compound $S_1$**

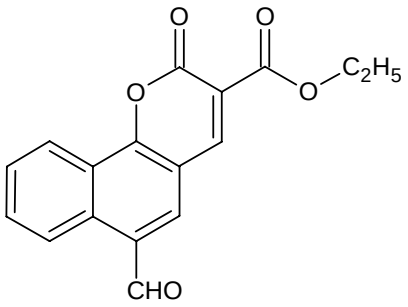



[III]

1*H*-indole-2-carbaldehyde

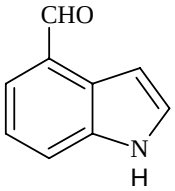
**Compound $S_1$**

**Synthesis of Compound $S_2$**



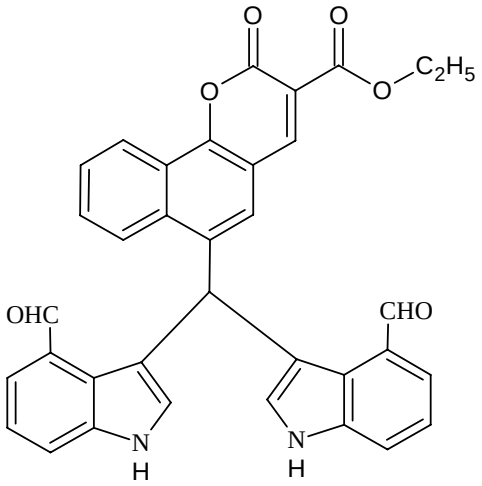

[III]

+



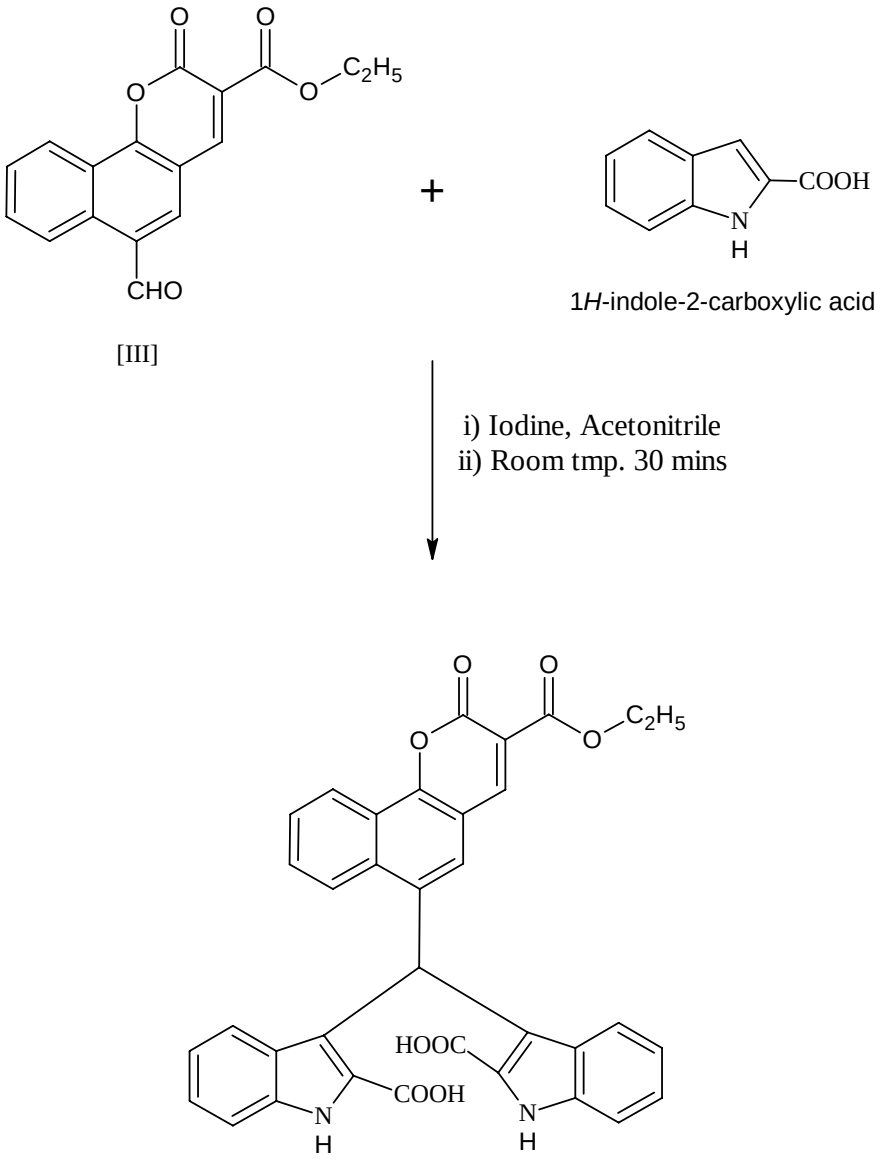

1*H*-indole-4-carbaldehyde

i) Iodine, Acetonitrile
ii) Room tmp. 30 mins



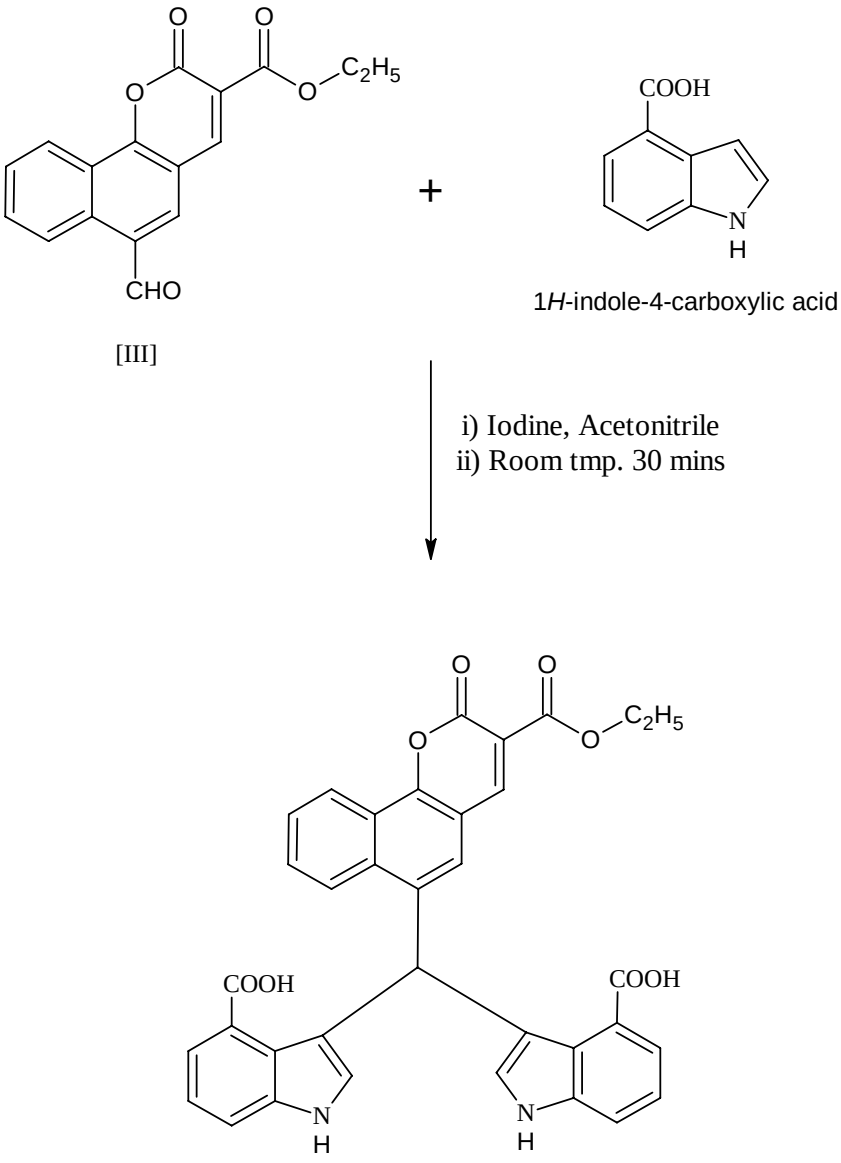

**Compound $S_2$**

**Synthesis of Compound $S_3$**



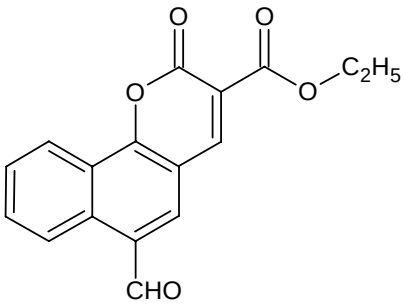

**Compound $S_3$**

**Synthesis of Compound $S_4$**



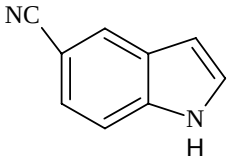

**Compound $S_4$**

**Synthesis of Compound $S_5$**



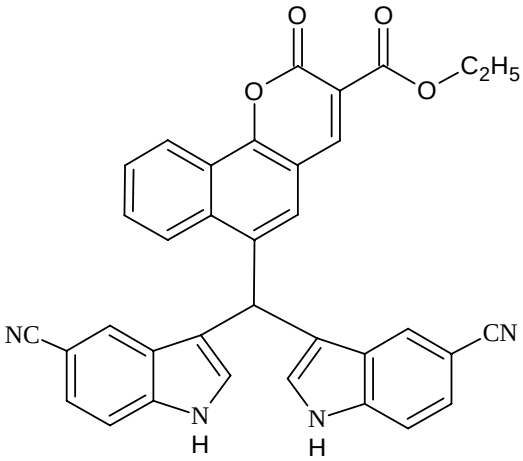

[III]

+



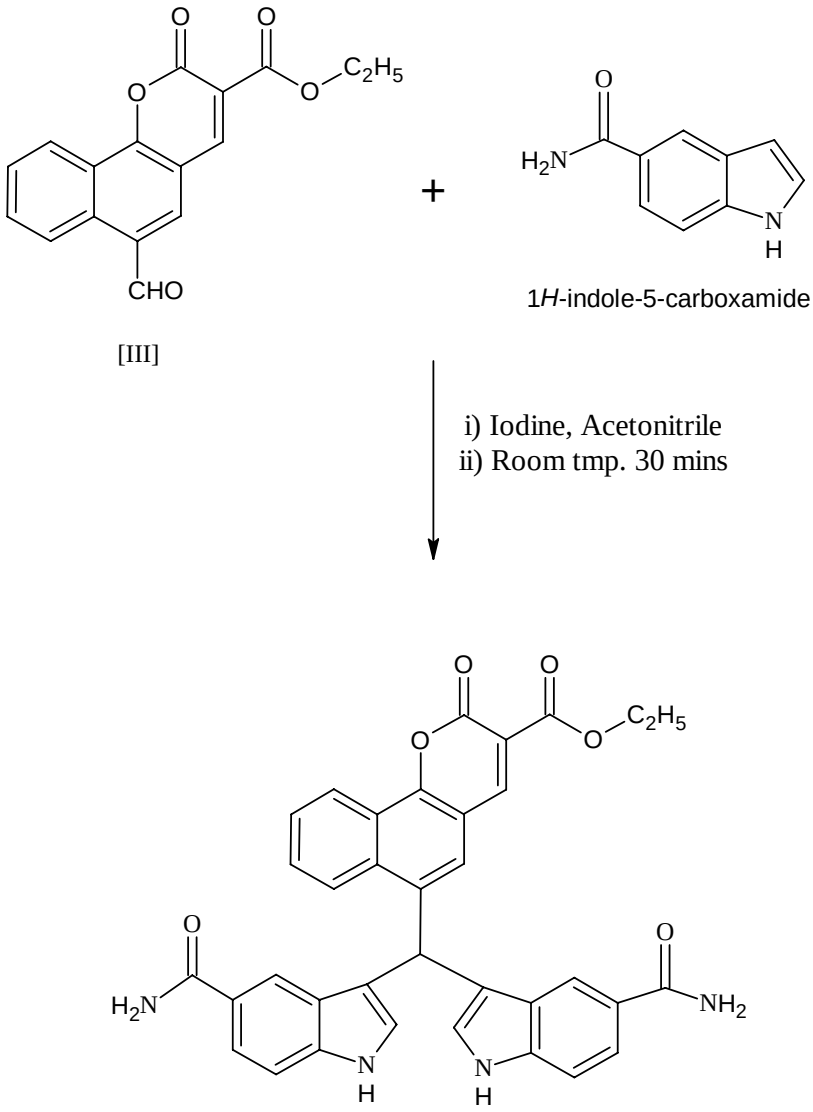

1*H*-indole-5-carbonitrile

i) Iodine, Acetonitrile
ii) Room tmp. 30 mins




**Compound $S_5$**

**Synthesis of Compound $S_6$**




**Compound $S_6$**

# EXPERIMENTAL WORK [39-44]

Experimental works of this thesis consists synthetic procedures, chemicals required, apparatus used and other laboratory works which are needed for this project.

**Apparatus and glass wares**

These include Round Bottom (RB) flask, beaker, conical flask, reflux condenser, funnel, pipette, measuring cylinder, glass rod, separating funnel, thermometer, etc.

**Instruments used**

- ♣ Magnetic stirrer, Melting point apparatus
- ♣ FT-IR instrument (Perkin Elmer)
- ♣ NMR instrument (Bruker)

**Chemicals used**

**Step I**

- ♣ α-napthol [I]
- ♣ Hexamine (Hexamethylene tetramine- HMTA)
- ♣ Trifluoro acetic acid
- ♣ 10% Sulphuric acid

**Step II**

- ♣ Napthol dialdehyde [II] obtained in step I
- ♣ Diethyl malonate
- ♣ Ethanol
- ♣ Piperidine

**Step III**

- ♣ Coumarinic compound [III] obtained in step II
- ♣ Different substituted indoles
- ♣ Iodine
- ♣ Acetonitrile

## SYNTHETIC PROCEDURE

### STEP I

14.4g of α-napthol **[I]** (0.1M) was weighed and taken in 500 ml RB flask. To this 14g of hexamine (0.1M) and 80 ml of trifluoro acetic acid were added. Then this RB was fixed under reflux condenser. Then it was refluxed on boiling water bath for 3 hours at 100° C.

After 3 hours of reflux, 10% sulphuric acid was to the reaction mixture. Then this was again refluxed under bath for 2 hours at 100° C the reddish brown napthol dialdehyde was formed in the reaction mixture. This was separated by extracting the mixture with ether. Then the ether was evaporated to obtain reddish brown colour napthol dialdehyde compound **[II].** The yield of this compound was 12g.

### STEP II

About 10g of (0.05M) of napthol dialdehyde **[II]** obtained in step I was weighed and taken in a 500 ml RB flask. To this 8 ml of diethyl malonate (0.05M), 5 ml ethanol and 5 ml of piperidine were added and shaked well. This reaction mixture was refluxed under water bath using reflux condenser for 30-40 minutes. Now the yellowish orange coumarinic adduct namely ethyl 6-formyl-2-oxo-2H-benzo[h]chromene-3-carboxaldehyde **[III]** was formed. This was then separated carefully by filtration using suction funnel. The product of this was recrystallized by using methanol. Yield was about 10g.

### STEP III

The coumarinic compound obtained in step III was decided to use for the synthesis six various Bisindole substituted coumarin derivatives. By treating the coumarinic adduct with different substituted indoles in the presence of acetonitrile and iodine. This mixture was shaked for 30 minutes to get product.

### Compounds and their codes

- Compound 1 [code-compound $S_1$]
- Compound 2 [code-compound $S_2$]
- Compound 3 [code-compound $S_3$]

- Compound 4 [code-compound $S_4$]
- Compound 5 [code-compound $S_5$]
- Compound 6 [code-compound $S_6$]

## PROCEDURE FOR INDIVIDUAL SYNTHESIS

### Synthesis of Compound $S_1$

About 1.4g (0.005M) of coumarinic adducts [III] which was obtained in step III weighed and taken in RB flask. To this 1.45g of (0.01M) Indole-2-carboxaldehyde was added. Then 0.63g of iodine and 20 ml of acetonitrile were added. This mixture was then shaked well for 30 minutes at room temperature. The reddish brown crystals of compound $S_1$ was separated by filtration. Recrystallization was done by using ethanol. The yield was about 1.2g.

### Synthesis of Compound $S_2$

1.4g (0.005M) of coumarinic adducts [III] was weighed and taken in RB flask. To this 1.45g of (0.01M) Indole-4-carboxaldehyde was added. Then 0.63g of iodine and 20 ml of acetonitrile were added. This mixture was then shaked well for 30 minutes at room temperature. The reddish powders of compound $S_2$ was separated by filtration. Recrystallization was done by using ethanol. The yield was about 0.86g.

### Synthesis of Compound $S_3$

About 1.4g (0.005M) compound [III] which was obtained in step III weighed and taken in RB flask. To this 1.61g of (0.01M) Indole-2-carboxylic acid was added. Then 0.63g of iodine and 20 ml of acetonitrile were added. This mixture was then shaked well for 30 minutes at room temperature. The orange colour compound $S_3$ was separated by filtration. Recrystallization was done by using chloroform. The yield was about 0.98g.

### Synthesis of Compound $S_4$

About 1.4g (0.005M) of coumarinic adducts [III] which was obtained in step III weighed and taken in RB flask. To this 1.61g of (0.01M) Indole-4-carboxaylic acid was added. Then 0.63g of iodine and 20 ml of acetonitrile were added. This mixture

was then shaked well for 30 minutes at room temperature. The brown crystals of compound $S_4$ was separated by filtration. Recrystallization was done by using chloroform. The yield was about 0.89g.

**Synthesis of Compound $S_5$**

About 1.4g (0.005M) of coumarinic compound [III] was weighed and taken in RB flask. To this 1.g of (0.01M) Indole-5-carbonitrile was added. Then 0.63g of iodine and 20 ml of acetonitrile were added. This mixture was then shaked well for 30 minutes at room temperature. The yellowish white crystals of compound $S_5$ was separated by filtration. Recrystallization was done by using methanol. The yield was about 0.74g.

**Synthesis of Compound $S_6$**

About 1.4g (0.005M) of coumarinic adducts [III] which was obtained in step III weighed and taken in RB flask. To this 1.45g of (0.01M) Indole-5-carboxamide was added. Then 0.63g of iodine and 20 ml of acetonitrile were added. This mixture was then shaked well for 30 minutes at room temperature. The pale yellow powders of compound $S_6$ was separated by filtration. Recrystallization was done by using ethyl acetate. The yield was about 1.3g.

**Purification of the compounds**

The synthesised compounds were purified by recrystallization using appropriate solvent like Methanol, Ethanol, Chloroform and Ethyl acetate.

**Identification compounds**

Identification of the products obtained in each steps and final compounds have done Thin Layer Chromatography (TLC) using appropriate solvent system as mobile phase and silica gel G as stationary phase.

**Preparation of TLC plates**

Silca gel G and water were measured at the ratio of 1g: 2.5ml and mixed together until to form slurry. This slurry of silica gel was poured evenly on the glass plate to make 1mm thickness. These plates were air dried and kept inside the hot air oven at the temperature of 100°C for 40 minutes. These were taken out prior to use.

**Calculation of $R_f$ values**

After every step of the reactions, the starting material and the reaction mixture were spotted on the TLC plates. Then this plate was air dried and kept in the mobile phase chamber. Now these spots were allowed to travel 1/4$^{th}$ of the plate height. Then plates were taken out and dried. The distance was measured and the $R_f$ values are calculated as follows.

$$Rf\ value = \frac{\text{distance travelled by solute}}{distance\ travelled\ by\ solvent}$$

Difference in the $R_f$ values of starting material and product (reaction mixture) indicated the completion of reaction. The $R_f$ values have also been determined for all individual synthesised compounds.

**Solubility**

The solubility of synthesised compounds was also checked by using different solvent like water, methanol, ethanol and DMSO.

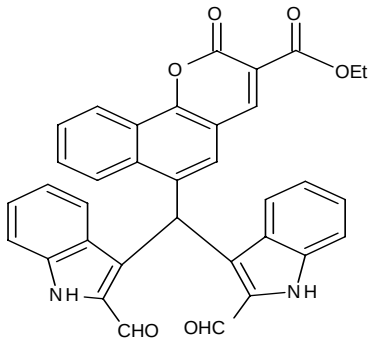
**Melting point determination**

The melting points of the synthesised compounds were checked using melting point apparatus. In this powder compounds were filled in the bottom closed capillary tube. These tubes were then kept into melting point apparatus in which the liquid paraffin was boiling. To this thermometer was also inserted. Now the temperature at where the compounds get started to melt was noted in thermometer. Thus the melting points of each compound were detected.

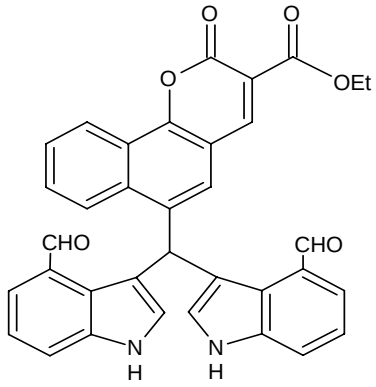
## CHARACTERIZATION OF COMPOUNDS

## STRUCTURES

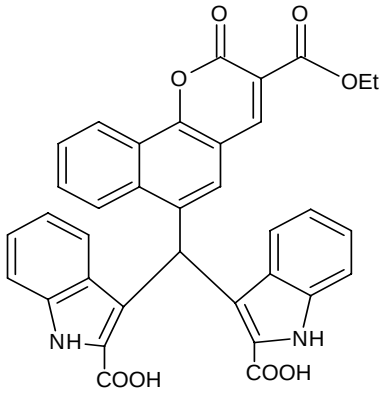
### Compound $S_1$



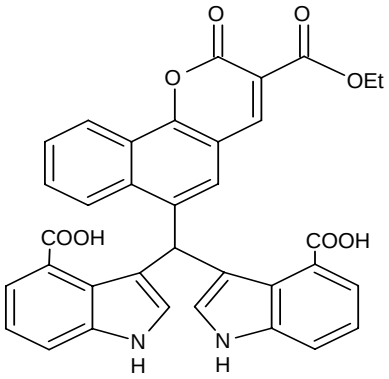

### Compound $S_2$



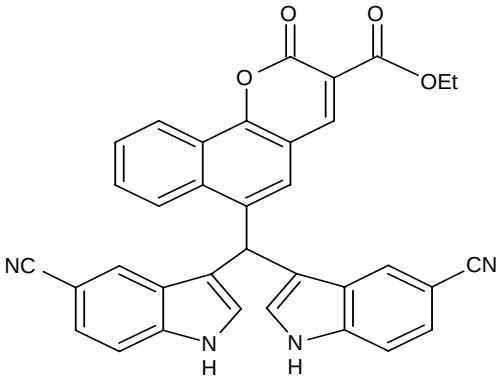

### Compound $S_3$



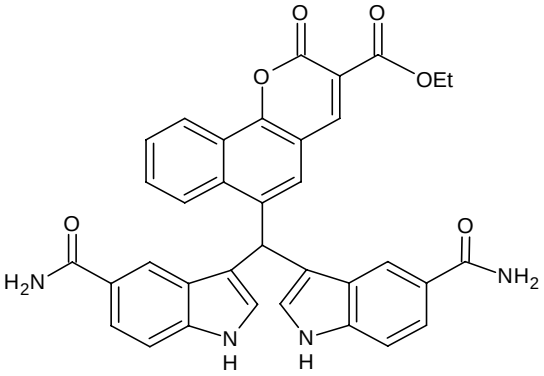
**Compound $S_4$**




**Compound $S_5$**




**Compound $S_6$**



## PHYSICAL CHARECTERS

### 1. Molecular formula, molecular weight and yield of the compounds

**TABLE NO.1**

**Data for Molecular formula, molecular weight and yield %**

| S.No | Compound names | Molecular formula | Molecular weight | Percentage yield |
|---|---|---|---|---|
| 1 | $S_1$ | $C_{35}\ H_{24}\ N_2\ O_6$ | 568 | 42.56% |
| 2 | $S_2$ | $C_{35}\ H_{24}\ N_2\ O_6$ | 568 | 28.37% |
| 3 | $S_3$ | $C_{35}\ H_{24}\ N_2\ O_8$ | 600 | 32.90% |
| 4 | $S_4$ | $C_{35}\ H_{24}\ N_2\ O_8$ | 600 | 29.88% |
| 5 | $S_5$ | $C_{35}\ H_{22}\ N_4\ O_4$ | 562 | 26.52% |
| 6 | $S_6$ | $C_{35}\ H_{26}\ N_4\ O_6$ | 598 | 43.80% |

**Percentage yield calculation**

$$Percentage\ yield = \frac{Practical\ yield\ yield}{Theoritical\ yield} \times 100$$

$$Theoritical\ yield = mole\ value \times molecular\ weight\ of\ product$$

**2. Colour, appearance and melting points of the compounds**

**TABLE NO. 2**

**Data for Colour, Appearance and Melting Point**

| S. No | Compound name | Colour | Appearance state | Melting point °C |
|---|---|---|---|---|
| 1 | $S_1$ | Reddish brown | Crystalline powder | 182-184 |
| 2 | $S_2$ | Red | Powder | 193-195 |
| 3 | $S_3$ | Orange | Course powder | 247-249 |
| 4 | $S_4$ | Brown | Powder | 234-236 |
| 5 | $S_5$ | Yellowish white | Course powder | 176-178 |
| 6 | $S_6$ | Pale yellow | Course powder | 212-215 |

**Instrument for melting point detection**

- ♣ Instrument used : Melting point apparatus
- ♣ Model : Veego – VMP (Silicon oil bath)
- ♣ Method : Open capillary method

**3. Solubility of compounds in different solvents**

**TABLE NO. 3**

**Data for the Solubility of Compounds**

| S. No | Compound name | Solubility in solvents | | | |
|---|---|---|---|---|---|
| | | Water | Methanol | Ethanol | chloroform |
| 1 | $S_1$ | - | + | - + | - + |
| 2 | $S_2$ | - | + | - + | - + |
| 3 | $S_3$ | - | - + | - + | + |
| 4 | $S_4$ | - | - + | - + | + |
| 5 | $S_5$ | - | + | + | - + |
| 6 | $S_6$ | - | + | + | - + |

- Indicates insoluble
+ Indicates soluble
-+ Indicates partially soluble

## 4. TLC parameters

The $R_f$ value of synthesised compounds have been found out using appropriate solvents system.

Adsorbent or stationary phase : Silica gel G

Developing Solvent or Mobile phase : Ethanol and Chloroform

Detecting agent : Iodine vapour

Colour of the spots : Yellow [for $S_1$ $S_5$ $S_6$]

Brown [for $S_2$ $S_3$ $S_4$]

**TABLE NO. 4**

**Data showing $R_f$ values of compounds**

| S.No | Compound | Adsorbent | Mobile phase & ratio | $R_f$ value |
|---|---|---|---|---|
| 1 | $S_1$ | Silica gel G | Ethanol : chloroform 6 : 4 | 0.68 |
| 2 | $S_2$ | | Ethanol : chloroform 8 : 2 | 0.73 |
| 3 | $S_3$ | | Chloroform : benzene 7 : 3 | 0.62 |
| 4 | $S_4$ | | Chloroform : benzene 6 : 4 | 0.79 |
| 5 | $S_5$ | | Ethanol : hexane 5 : 5 | 0.70 |
| 6 | $S_6$ | | Ethanol : hexane 3 : 7 | 0.59 |

## INTERPRETATION OF SPECTRA

**Compound $S_1$**

**IR Interpretation**

Instrument : FT-IR instrument

Name : Perkin Elmer

Method : KBr pellet

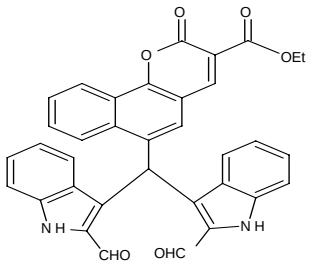



**TABLE NO. 5**

**IR Spectral data for Compound $S_1$**

| S. No | Frequency $cm^{-1}$ | Groups assigned |
|---|---|---|
| 1 | 3747.89 | Due to N-H stretching |
| 2 | 2929.97 | Due to C-H stretching (aromatic) |
| 3 | 2366.96 | Due to C-H stretching |
| 4 | 1637.36 | Due to C-O stretching (aldehyde) |
| 5 | 1297.90 | Due to C-O stretching |
| 6 | 998.60 | Due to C-H bending (opposite) |
| 7 | 834.76 | Due to N-H bending (opposite) |

**$^1$H NMR interpretation**

Instrument : $^1$H NMR spectrophotometer

Name : Bruker

Solvent used : MeOD

**TABLE NO. 6**

**1H NMR spectral data for compound $S_1$**

| S. No | Signal value (δ ppm) | Group assigned |
|---|---|---|
| 1 | 3.3042 | Singlet, CH proton adjacent to -OCOAr |
| 2 | 3.3753-5.1848 | Singlet, CH proton adjacent to -OCOAr |
| 4 | 6.7659-7.1884 | Singlet, aromatic protons |
| 5 | 7.4044-7.9429 | Singlet, aromatic protons |
| 6 | 8.4811-8.5722 | Singlet, NH protons |

**Compound $S_2$**

**Structure**

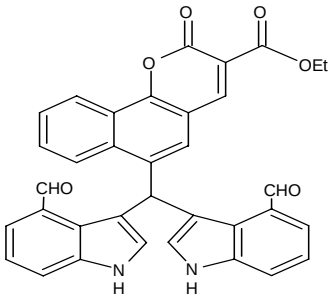



**TABLE NO. 7**

**IR Spectral data for Compound $S_2$**

| S. No | Frequency $cm^{-1}$ | Groups assigned |
|---|---|---|
| 1 | 3293.13 | Due to N-H stretching |
| 2 | 2923.53 | Due to C-H stretching (asymmetric) |
| 3 | 2850.90 | Due to C-H stretching (symmetric) |
| 4 | 2365.67 | Due to C-H stretching |
| 5 | 1464.46 | Due to C-H bending ($sp^3$) |
| 6 | 1123.53 | Due to C-O stretching |
| 7 | 1064.05 | Due to C-O stretching |

## $^1$H NMR interpretation

Instrument : $^1$H NMR spectrophotometer

Name : Bruker

Solvent used : MeOD

**TABLE NO. 8**

**$^1$H NMR spectral data for compound $S_2$**

| S. No | Signal value (δ ppm | Group assigned |
|---|---|---|
| 1 | 3.3842-5.1748 | Singlet, CH proton adjacent to -OCOAr |
| 2 | 6.7959-7.4241 | Singlet, aromatic protons |
| 3 | 7.8589-7.9429 | Singlet, aromatic protons |
| 4 | 8.4233-8.5234 | Singlet, NH protons |

**Compound $S_3$**

**Structure**

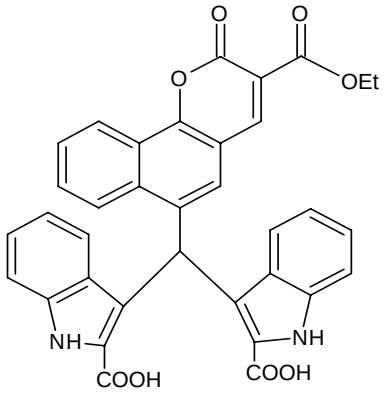



**TABLE NO. 9**

**IR Spectral data for Compound $S_3$**

| S. No | Frequency $cm^{-1}$ | Groups assigned |
|---|---|---|
| 1 | 3757.72 | Due to N-H stretching |
| 2 | 3431.72 | Due to N-H stretching |
| 3 | 2956.52 | Due to C-H stretching (asymmetric) |
| 4 | 2870.59 | Due to C-H stretching (symmetric) |
| 5 | 1635.49 | Due to C-O stretching |
| 6 | 1592.89 | Due to C=C stretching (aromatic) |
| 7 | 1431.93 | Due to C-H bending (opposite) |
| 8 | 955.29 | Due to C-H bending (aromatic) |
| 9 | 848.5 | Due to N-H bending |

**$^1$H NMR interpretation**

Instrument : $^1$H NMR spectrophotometer

Name : Bruker

Solvent used : MeOD

**TABLE NO. 10**

**$^1$H NMR spectral data for compound $S_3$**

| S. No | Signal value (δ ppm | Group assigned |
|---|---|---|
| 1 | 1.3015- 1.4284 | Singlet $CH_3$ proton |
| 2 | 1.5602-1.7295 | Singlet $CH_3$ proton |
| 3 | 3.3029-3.6429 | Singlet CH proton adjacent to -OCOAr |
| 4 | 4.8556 | Singlet CH proton adjacent to -OCOAr |
| 5 | 7.0506-7.3049 | Multiplet, aromatic proton |
| 6 | 7.5228-7.5431 | Multiplet, aromatic proton |

**Compound $S_4$**

**Structure**

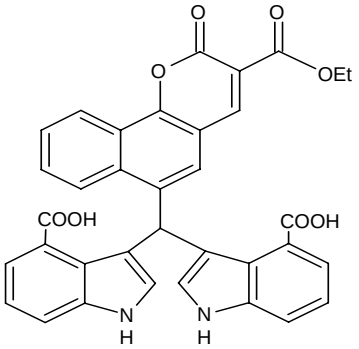



**TABLE NO. 11**

**IR Spectral data for Compound $S_4$**

| S. No | Frequency $cm^{-1}$ | Groups assigned |
|---|---|---|
| 1 | 3754.13 | Due to N-H stretching |
| 2 | 3446.54 | Due to N-H stretching |
| 3 | 2923.97 | Due to C-H stretching (asymmetric) |
| 4 | 2854.14 | Due to C-H stretching (symmetric) |
| 5 | 1627.14 | Due to C-O stretching |
| 6 | 1440.51 | Due to C-H bending |
| 7 | 1030.76 | Due to C-O stretching |
| 8 | 748.58 | Due to N-H bending |

**$^1$H NMR interpretation**

Instrument : $^1$H NMR spectrophotometer

Name : Bruker

Solvent used : MeOD

**TABLE NO. 12**

**$^1$H NMR spectral data for compound $S_4$**

| S. No | Signal value (δ ppm | Group assigned |
|---|---|---|
| 1 | 1.3147-1.711 | Singlet, $CH_3$ proton |
| 2 | 3.3029-3.6354 | Singlet, CH proton |
| 3 | 7.0321-7.5225 | Singlet, aromatic proton |
| 4 | 8.4811-8.5788 | Singlet, NH proton |

**Compound $S_5$**

**Structure**

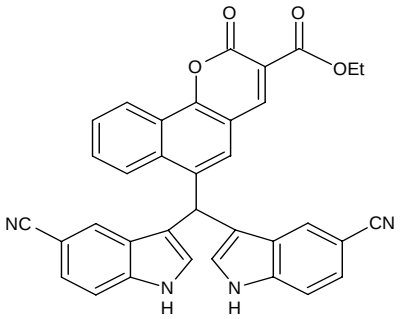



**TABLE NO. 13**

**IR Spectral data for Compound $S_5$**

| S. No | Frequency $cm^{-1}$ | Groups assigned |
|---|---|---|
| 1 | 3755.66 | Due to N-H stretching |
| 2 | 3428.21 | Due to N-H stretching |
| 3 | 2924.67 | Due to C-H stretching (asymmetric) |
| 4 | 2854.92 | Due to C-H stretching (symmetric) |
| 5 | 1599.70 | Due to C-O stretching |
| 6 | 1439.67 | Due to C-H bending |
| 7 | 1025.54 | Due to C-O stretching |
| 8 | 747.38 | Due to N-H bending |

**$^1$H NMR interpretation**

Instrument : $^1$H NMR spectrophotometer

Name : Bruker

Solvent used : MeOD

**TABLE NO. 14**

**$^1$H NMR spectral data for compound $S_5$**

| S. No | Signal value (δ ppm | Group assigned |
|---|---|---|
| 1 | 1.3825-1.7349 | Singlet, $CH_3$ proton |
| 2 | 2.2711-2.3817 | Singlet $CH_2$ proton |
| 3 | 3.2978-4.8834 | Singlet CH proton adjacent to –OCOAr |
| 4 | 7.0531-7.5424 | Singlet, aromatic proton |

**Compound $S_6$**

**Structure**

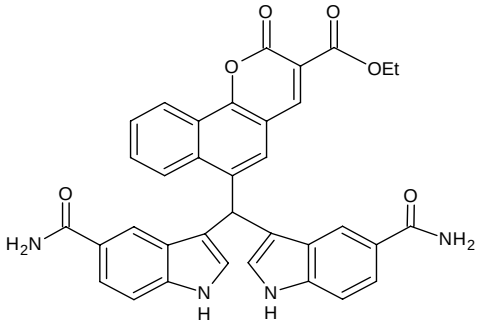



**TABLE NO. 15**

**IR Spectral data for Compound $S_6$**

| S. No | Frequency $cm^{-1}$ | Groups assigned |
|---|---|---|
| 1 | 3755.09 | Due to N-H stretching |
| 2 | 3447.24 | Due to N-H stretching |
| 3 | 2922.59 | Due to C-H stretching (asymmetric) |
| 4 | 2853.66 | Due to C-H stretching (symmetric) |
| 5 | 1728.17 | Due to C-O stretching |
| 6 | 1562.09 | Due to N-H bending (amide) |
| 7 | 1436.24 | Due to C-H stretching |
| 8 | 1030.92 | Due to C-N stretching |
| 9 | 934.97 | Due to C-H bending (opposite) |
| 10 | 745.27 | Due to N-H bending |

**$^1$H NMR interpretation**

Instrument : $^1$H NMR spectrophotometer

Name : Bruker

Solvent used : $CDCl_3$

**TABLE NO. 16**

**$^1$H NMR spectral data for compound $S_6$**

| S. No | Signal value (δ ppm | Group assigned |
|---|---|---|
| 1 | 1.3914-2.3634 | Singlet, $CH_3$ proton |
| 2 | 3.3020-3.339 | Singlet CH proton adjacent to –OCOA |
| 3 | 7.2617-7.5424 | Singlet, aromatic proton |

## HYPOLIPIDEMIC ACTIVITY OF VARIOUS SYNTHETIC DRUGS IN HYPERLIPIDEMIC MODELS OF WISTAR ALBINO RATS [45-53]

### INTRODUCTION:

Many people with diabetes have conditions called “risk factor” that contribute to atherosclerosis and its complications. These include high blood pressure, excess weight and high blood glucose levels. Dyslipidemia further raises risk of atherosclerosis in people with diabetes. Dyslipidemia affects people with type 2 diabetes more often than those with type 1 diabetes. The most common Dyslipidemia in diabetes is the combination of high triglycerides and low HDL levels. People with diabetes may also have elevated LDL cholesterol.

Among the drugs available to treat Dyslipidemia, statins are often the first choice for lowering total and LDL cholesterol levels, other drugs that lowers cholesterol include cholesterol-adsorption blockers, bile acids, sequestrants, and nicotinic acids. These may be used in combination, if a single drug is not effective in reaching target levels. Fibrates and extended release niacin may be used to lower triglycerides (or) raise HDL cholesterol levels [1].

Hyperglycemia and Dyslipidemia are significant and independent risk factors for the vascular complications and suggested to cause cardio vascular pathological changes in diabetic states through the following molecular mechanism, formation and accumulation of advanced glycation products, increased oxidative stress, activation of proteinkinase C pathway, increased activity of hexosamine pathway and vascular inflammation and the impairment of insulin action in the vascular tissues [2]. In the present study an attempt has been made to screen the various synthetic drugs for the Hypolipidemic activity.

The present investigation is undertaken to study the effect of various synthetic drugs on changes in Total cholesterol, Triglycerides, HDL, LDL, VLDL, AI, and LDL/HDL.

**Materials and methods**

**Animals**

Male albino strains of wistar rats were obtained from central animal house, K.M.College of pharmacy, Madurai. The animals were given standard rodent diet and water ad libitum throughout the study. The rats used in the present study were maintained in accordance with guidelines of the national institute of nutrition, Indian council for medical research, Hyderabad, India and study approved by Institutional animal ethical committee.

**Materials:**

- Various synthetic drugs such as S1 to S6
- Cholesterol extra pure for feeding purpose was obtained from S.D fine-chem. limited, Mumbai, India. Coconut oil was used as a vehicle for cholesterol feeding.
- *Atorvastatin* was obtained from Micro labs, Bangalore, India.

**Experimental procedure:**

All the animals were weighed and divided into nine groups each of six animals.

Group I : Normal control.

Group II : Cholesterol control. Fed cholesterol at a dose of 400mg/kg body weight for 30 days.

Group III : fed cholesterol as in group II and Atorvastatin 1mg/kg body weight from days 15 to day 30.[3]

Group IV : fed cholesterol as in group II and synthetic compound S1 at a dose of 50mg/kg body weight from days 15 to day 30.

Group V : fed cholesterol as in group II and synthetic compound S2 at a dose of 150mg/kg body weight from days 15 to day 30.

Group VI : fed cholesterol as in group II and synthetic compound S3 at a dose of 50mg/kg body weight from days 15 to day 30.

Group VII : fed cholesterol as in group II and synthetic compound S4 at a dose of 50mg/kg body weight from days 15 to day 30.

Group VIII : fed cholesterol as in group II and synthetic compound S5 at a dose of 50mg/kg body weight from days 15 to day 30.

Group IX : fed cholesterol as in group II and synthetic compound S6 at a dose of 50mg/kg body weight from days 15 to day 30.

At the end of 30 days all the rats were sacrificed, blood was collected, allowed to clot and serum was obtained by centrifugation. The serum samples were used for various biochemical procedures.

**Biochemical analysis:**

The serum was analyzed for total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), by using standard protocol methods. (Auto Analyzer)

**Atherogenic index (AI) and LDL-C/HDL-C ratio**

- The AI was calculated by the following formula
- AI = (total cholesterol – HDL-C)/HDL-C
- LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C Levels

**Statistical analysis**

- All the values were expressed as mean ± SEM.
- Data was analyzed by one way analysis of variance (ANOVA) followed by Newman keul's multiple range tests.
- P values <0.05 were considered as statistically significant.

**Table 1: Effect of various synthetic drugs on Lipid Profile**

| GROUPS | Total cholesterol (Mg/dl) | Tri glycerides (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | AI | LDL/ HDL |
|---|---|---|---|---|---|---|---|
| Normal Control | 52.60 ± 2.75 | 57.10± 2.89 | 26.40 ± 1.16 | 14.30 ± 0.70 | 31.88 ± 1.58 | 0.99 ± 1.37 | 0.54 |
| Cholesterol Control | 115.40 ± 4.50**(a) | 160.4 ± 5.68**(a) | 11.90 ± 0.65**(a) | 31.95 ± 1.86**(a) | 13.10 ± 0.70**(a) | 8.69 ± 5.92**(a) | 2.68 |
| Standard Control | 73.65± 3.52**(b) | 80.8 ± 3.78**(b) | 21.8 ± 1.06**(b) | 21.01 ± 1.04**(b) | 26.90 ± 0.92**(b) | 2.37 ± 2.32**(b) | 0.96 |
| Treatment control (S1) | 90.80 ± 3.75**(b) | 118.42 ± 4.90**(b) | 18.2 ± 0.90**(b) | 25.23 ± 1.58**(b) | 19.90 ± 0.45**(b) | 3.98 ± 3.16**(b) | 1.38 |
| Treatment control (S2) | 88.90 ± 2.94**(b) | 94.3 ± 3.77**(b) | 20.22 ± 1.20**(b) | 22.08 ± 1.12**(b) | 21.20 ± 0.88**(b) | 3.39 ± 1.45**(b) | 1.09 |

**Table 2: Effect of various synthetic drugs on Lipid Profile**

| GROUPS | Total cholesterol (Mg/dl) | Tri glycerides (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | AI | LDL/ HDL |
|---|---|---|---|---|---|---|---|
| Treatment control (S3) | 85.83 ± 2.82**(b) | 96.4 ± 3.80**(b) | 19.32 ± 1.30**(b) | 20.15 ± 1.72**(b) | 23.30 ± 0.96**(b) | 3.44 ± 1.16**(b) | 1.04 |
| Treatment control (S4) | 83.80 ± 2.65**(b) | 98.6 ± 3.92**(b) | 21.30 ± 1.42**(b) | 20.35 ± 1.62**(b) | 22.35 ± 0.86**(b) | 3.13 ± 2.93**(b) | 0.95 |
| Treatment control (S5) | 85.90 ± 2.90**(b) | 92.0 ± 3.07**(b) | 20.32 ± 1.08**(b) | 21.42 ± 1.82**(b) | 23.32 ± 0.98**(b) | 3.22 ± 1.68**(b) | 1.05 |
| Treatment control (S6) | 87.80 ± 2.98**(b) | 94.8 ± 3.12**(b) | 21.55 ± 1.35**(b) | 22.42 ± 1.72**(b) | 22.86 ± 0.95**(b) | 3.07 ± 1.20**(b) | 1.04 |

- Values are expressed as Mean ± SEM.
- Values were find out by using ONE WAY ANOVA followed by Newman Keul's multiple range tests.
- ** (a) values were significantly different from normal control at P< 0.01.
- ** (b) Values were significantly different from hyperlidemic control at P< 0.01.

## RESULTS

**Table 1 and 2** shows the levels of Cholesterol, Triglycerides, HDL, LDL and VLDL of control and experiment rats respectively. Serum of hyperlipidemic rats showed significantly increased levels of Cholesterol, Triglycerides, LDL - C and low HDL – C, when compared with normal rats. In rats treated with various synthetic drugs and Atorvastatin there was significant decrease in the content cholesterol, TGs, LDL – C, and VLDL and increases HDL – C, when compared with cholesterol control rats.

### Atherogenic index (AI) and LDL – C / HDL – C ratio:

**Table 1 and 2** shows the changes of Atherogenic Index and LDL – C / HDL – C ratio in control and treated rats. It appears clear form these results that the cholesterol induction significantly affects the cardio vascular risk markers.

Indeed, AI was statistically increased in cholesterol control group 90% compared with the values found in their normal control group.

Besides there were significant further increase of LDL – C / HDL – C ratio in cholesterol control group compared to normal control group.

Promising results in lowering of AI by various synthetic drugs that is bis indole substituted coumarin derivatives was found in Table 1 and 2. The various synthetic drugs showed an improvement of the cardio vascular risk level by decrease of AI in the treated groups when compared to the cholesterol control group.

The ratio of LDL – C to HDL – C is also a protective indicator of cardio vascular disease incidence. The cholesterol induction produced a significant increase of this marker. In contrast, elevated ratio in treated group and Atorvastatin group returned to basal value when the data were compared in the same period to the data found for cholesterol rats. **(Table 1 and 2)**

# RESULTS AND DISCUSSION

This work started with synthesis of novel bisindole substituted coumarin derivatives by conventional method. By this method the compounds have been synthesised.

- Compound 1 [code-compound $S_1$]
- Compound 2 [code-compound $S_2$]
- Compound 3 [code-compound $S_3$]
- Compound 4 [code-compound $S_4$]
- Compound 5 [code-compound $S_5$]
- Compound 6 [code-compound $S_6$]

## Characteristics of Compounds

The synthesised compounds were subjected to check their characters like melting point, solubility, $R_f$ value and spectral data.

## Melting point

Melting points these compounds were determined by using melting point apparatus. Melting points of individual compounds were checked in each step and differ from the parent compound which conformed that reactions were completed and product has formed.

## $R_f$ value determination

The difference in the $R_f$ values of individual compounds shown that products were formed and these compounds were different compound.

## IR interpretation

An interpretation of IR spectra of individual compounds showed their respective functional groups. Presence of expected peaks proved that reactions have been completed.

**$^{1}$H NMR data**

Interpretation of the $^{1}$H NMR data showed that the synthesised compounds have specific nature of protons in their structure.

**ANTI-HYPERLIPIDEMIC ACTIVITY**

The reduction of plasma total cholesterol was associated with a decrease in its LDL fraction which is a major, potentially modifiable risk factor of cardio vascular disease and the target of drug. Many suggest that the cholesterol lowering activity of this product appears to be due to the enhancement of LDL – C catabolism through hepatic receptors [4].

In addition various synthetic drugs showed protective action which is reported to have a preventive function against atherogenesis since an independent inverse relationship between blood HDL – C levels and cardio vascular risk incidence is reported [5].

The lipoprotein called “good cholesterol” facilitates the mobilization of triglycerides and cholesterol from plasma to liver where it is catabolised and eliminated in the form of bile acids. The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT) and inhibition of Hepatic Triglyceride Lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through enterohepatic tissues [6].

It is also recently reported that triglycerides plays a key role in the regulation of lipoprotein interaction to maintain normal lipid metabolism. Indeed, the elevated plasma TG levels were associated with an increased incidence of coronary artery disease.

Moreover these higher plasma TG levels have been attributed mainly to increase population of small, dense LDL deposits which are very atherogenic and enhanced cholesteryl ester mass transfer from apolipoprotein B containing lipoproteins (VLDL and LDL)[7]. TG has also been proposed to be major determinant of cholesteryl esterification, its transfer and HDL remodeling in human plasma [8].

various synthetic drugs significantly suppress the elevated blood concentration of TGs. This result suggests that the product is able to restore, at least partially, the catabolism of TG. The underlying the mechanism of this activity is not elucidated by the present study. However, as hypothesized by many works with other plants, the restoration of catabolic mechanism of TGs would be due to an increased stimulation of the lipolytic activity of Plasma Lipoprotein Lipase (LPL) [9].

Administration of various synthetic drugs provides a beneficial action on rat lipid metabolism with regard to the reduction of AI. Infact, the AI was decreased in all treated groups.It is also desirable to have higher plasma HDL and lower LDL – C to prevent atherogenesis, since there is a positive correlation between an increased LDL – C / HDL – C ratio and the development of atherosclerosis. Again, the administration of various synthetic drugs significantly suppress the higher values of LDL – C / HDL – C ratio showing the beneficial effect of this plant in preventing atherosclerosis incidence.

## CONCLUSION

### SYNTHESIS

From the physical characteristics and spectral data of compound $S_1$ – $S_6$ showed that the synthesised compounds were our expected substituted compounds. So these were selected to study their anti-hyperlipidemic potential.

So it was concluded that synthetic reactions were completed and yielded our final compounds.

### PHARMACOLOGICAL STUDY

Hyperlipidemia is considered to be major risk factor for the premature atherosclerosis and essentially the cholesterol in atherosclerotic plaque is derived from that of circulatory cholesterol. The antihyperlipidemic effect of various synthetic drugs of bisindole substituted coumarin could be considered as a possible therapeutic value.

# BIBLIOGRAPHY


1. Camille George Wermuth. The practce of medicinal chemistry. 2nd ed. Woburn: Elsevier; 2004. p. 29-41.
2. John H Black and John M Baeale. Text book of organic medicinal and pharmaceutical chemistry. 11th ed. London: Lippincott William and Wilkinson; 1998. p. 1-5.
3. David A Williams, Thomas and Lemke. Priciples of medicinal chemistry. 5th ed. Philadelphia: Lippincoot William and Wilkinson; 2004. p. 1-9.
4. Wolft, Manfred E. Burger's medicinal chemistry and drug design. 5th ed. London; 1995. p. 1-7.
5. Richard B Silverman. The organic chemistry of drug design and drug action. 2nd ed. California: Elsevier; 2004. p. 1-5.
6. Ashutoskar. Medicinal chemistry. 3rd ed. Lucknow: New Age International; 2005. p. 1-25.
7. SS Kadam, KR Mahadik and KG Bothara. Priciples of medicinal chemistry. 14th ed. Pune: Nirali Prakashan; 2005. p. 1-20.
8. Povil krogsgaard, Tommy liljefors and Ulf Madsen. Text book of drug design and discovery. 3rd ed. London: Taylor and Francis; 2002. p. 1-10.
9. K. B. Vyasa, K. S. Nimavata, G. R. Janib, M. V. Hathic. Synthesis and antimicrobial activity of coumarin derivatives metal complexes: An in vitro evaluation. Orbital The Elecronic Journal of Chemistry. 2009; 1(2): p. 183-92.
10. P. Selvam, N. Ramlakshmi, G. Uma, S. Arun Kumar and A. Umamaheswari. Synthesis, Characterisation and Biological Evaluation of Novel Coumarin Derivatives. Rasayan J. Chem. 2010; 3(2): p. 275-80.
11. Parameswaran Manoj Kumar, Thengungal Kochupappy Ravi and Gopalakrishnan Subbuchettiar. Synthesis of coumarin heterocyclic derivatives with antioxidant activity and in vitro cytotoxic activity against tumour cells. Acta Pharm. 2009; 59: p. 159-70.

12. A. Shaabani, R. Ghadari, A. Rahmati and A.H. Rezayan. Coumarin Synthesis via Knoevenagel Condensation Reaction in 1,1,3,3-N,N,N',N'-Tetramethylguanidinium Trifluoroacetate Ionic Liquid. J. Iran. Chem. Soc. 2009; 6(4): p. 710-14.

13. Hosanagara N. Harishkumar, Kittappa M. Mahadevan , Hittalumane, C. Kiran Kumar and Nayak D. Satyanarayan. A facile, choline chloride/urea catalyzed solid phase synthesis of coumarins via Knoevenagel condensation. Org. Commun. 2011; 4(2): p. 26-32.

14. Afsheen Arshad a, Hasnah Osman , Mark C. Bagleyb, Chan Kit Lam , Suriyati Mohamad and Anis Safirah Mohd Zahariluddin. Synthesis and antimicrobial properties of some new thiazolyl coumarin derivatives. European Journal of Medicinal Chemistry. 2011; 46: p. 3788-94.

15. Koneni V. Sashidhara, Abdhesh Kumar, Manoj Kumar, Anuj Srivastava and Anju Puri. Synthesis and antihyperlipidemic activity of novel coumarin bisindole derivatives. Bioorganic & Medicinal Chemistry Letters. 2010; 20: p. 6504-7.

16. Andrea Behrenswertha, Nicole Volzb, Jakob Torangb, Sonja Hinza, Stefan Braseb and Christa E.Müllera. Synthesis and pharmacological evaluation of coumarin derivatives as cannabinoid receptor antagonists and inverse agonists. Bioorganic & Medicinal Chemistry. 2009; 17(7): p. 2842-51.

17. Hakk Murat Bilgina, Mukadder Atmacaa, Basra Deniz Obaya, Selver Ozekincib, Ezel Taşdemirc and Aydın Ketanid. Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. Experimental and Toxicologic Pathology. 2011; 63(4): p. 325-30.

18. Tianzhi Yua, Sidan Yanga, Yuling Zhaob, Hui Zhanga, Xiaoqian Hanb, Duowang Fana et al. Synthesis, crystal structures and fluorescence properties of 3-(2-

pyridyl)coumarin derivatives. Journal of Photochemistry and Photobiology A: Chemistry. 2010; 214(1): p. 92-99.

19. Kinza Aslam, M. Kaleem Khosa, Nazish Jahan and Sofia Nosheen. Synthesis and applications of coumarin. Pak. J. Pharm. Sci. 2010; 23(4): p. 449-54.

20. Hassan Valizadeha and Abbas Shockravi. An efficient procedure for the synthesis of coumarin derivatives using TiCl4 as catalyst under solvent-free conditions. Tetrahedron Letters. 2005; 46: p. 3501-3.

21. Rangappa S. Keri, Kallappa M. Hosamani and Harisha R. Seetharama Reddy. A Solvent-Free Synthesis of Coumarins Using Phosphotungstic Acid as Catalyst. Catal Lett. 2009; 131: p. 321-7.

22. Jae-Chul Jung and Oee-Sook Park. Synthetic Approaches and Biological Activities of 4-Hydroxycoumarin Derivatives. Molecules. 2009; 14: p. 4790-803.

23. Stojadin V. Dekic, Vidoslav S. Dekic, Branimirka Vucic, Biljana R. Dekic and Milan S. Dekic. Synthesis Of New Condensed Coumarin Derivatives. Facta Universitatis. 2007; 5(1): p. 85-88.

24. Feng Ping Yi, Hai Yang Sun, Xian Hua Pan, Yuan Xu and Ji Zong Li. Synthesis of Fischer indole derivatives using carboxyl-functionalized ionic liquid as an efficient and recyclable catalyst. Chinese Chemical Letters. 2009; 20: p. 275-78.

25. Huseyin Cavdar and Nurullah Saracoglu. A new approach for the synthesis of 2-substituted indole derivatives via Michael type adducts. Tetrahedron. 2005; 61: p. 2401-5.

26. Mohamed A. A. Radwan, Eman A. Ragab, Nermien M. Sabry and Siham M. El-Shenawy. Synthesis and biological evaluation of new 3-substituted indole derivatives as potential anti-inflammatory and analgesic agents. Bioorganic & Medicinal Chemistry. 2007; 15: p. 3832-41.

27. Yuichi Sugimoto, Atsushi Shimizu, Tetsuya Kato, Atsushi Satoh, Satoshi Ozaki, Hisashi Ohta et al. Design, synthesis, and biological evaluation of indole derivatives as novel nociceptin/orphanin FQ (N/OFQ) receptor antagonists. Bioorganic & Medicinal Chemistry Letters. 2006; 16: p. 3569-73.

28. A Leonardi, C Riva, C De Toma, C Boi, R Pennini and G Sironi. Synthesis and pharmacological evaluation of new indole derivatives structurally related to thymoxamine. Eur J Med Chem. 1994; 29: p. 551-9.

29. Mukund Jha, Ting-Yi Chou and Brian Blunt. General synthesis of mono-, di-, and tri-acetylated indoles from indolin-2-ones. Tetrahedron. 2011; 67: p. 982-99.

30. Chang Qing Shi, Zhang Qin Liu, Wen Qing Lin and Yuan Wei Chen. Synthesis and preliminary cytotoxic evaluation of substituted indoles as potential anticancer agents. Chinese Chemical Letters. 2007; 18: p. 899-901.

31. Preeti Rani,V.K. Srivastava and Ashok Kumar. Synthesis and antiinflammatory activity of heterocyclic indole derivatives. European Journal of Medicinal Chemistry. 2004; 39: p. 449-52.

32. Matthias Nettekoven. Combinatorial Synthesis of Libraries of Indole Derivatives. Bioorganic & Medicinal Chemistry Letters. 2001; 11: p. 2169-71.

33. Mostafa Kiamehr and Firouz Matloubi Moghaddam,. An efficient ZnO-catalyzed synthesis of novel indole-annulated thiopyrano-chromene derivatives via Domino Knoevenagel-hetero-Diels–Alder reaction. Tetrahedron Letters. 2009; 50: p. 6723-7.

34. Michele Giampieri, Alessandro Balbi, Mauro Mazzei, Paolo La Colla, Cristina Ibba and Roberta Loddo. Antiviral activity of indole derivatives. Antiviral Research. 2009; 83: p. 179-85.

35. Tetsuya Mochizuki a, Akihiko Tanimura, Akihiro Nezu, Mika Ito, Hiroshi Abe Yoshihiro Ito et al. Design and synthesis of indole derivatives of adenophostin A. A entry into subtype-selective IP3 receptor ligands. Tetrahedron Letters. 2010; 51: p. 977-9.

36. Rakesh Kumar Tiwari, Akhilesh K. Verma, Anil K. Chhillar, Devender Singh, Jaspal Singh, V. Kasi Sankar et al. Synthesis and antifungal activity of substituted-10-methyl-1,2,3,4-tetrahydropyrazino[1,2-a]indoles. Bioorganic & Medicinal Chemistry. 2006; 14: p. 2747–52.

37. Rakesh Kumar Tiwari, Devender Singh, Jaspal Singh, Vibha Yadav, Ajay K. Pathak and Rajesh Dabur. Synthesis and antibacterial activity of substituted 1,2,3,4-tetrahydropyrazino [1,2-a] indoles. Bioorganic & Medicinal Chemistry Letters. 2006; 16: p. 413-16.

38. Sandra Battaglia, Enrico Boldrini, Federico Da Settimo, Giulio Dondio, Concettina La Motta, Anna Maria Marini. Indole amide derivatives: synthesis, structure–activity relationships and molecular modelling studies of a new series of histamine H1-receptor antagonists. Eur. J. Med. Chem. 1999; 34: p. 93-105.

39. Douglas A. Skoog, F.James, Holler, Nieman. Infrared spectrometry. In Principles of Instrumental Analysis. Australia: Thomson brooks/cole; 2003. p. 381-428.

40. Elbs, William Kemp. IR and NMR. In Organic spectroscopy.; 1979.

41. K. Peach, M.V. Treacay. NMR spectroscopy. In Modern methods of plant analysis.; 2001. p. 462-474.

42. Kaur, H. NMR. In Spectroscopy. Meerut: Pragati prakashan; 2010. p. 322-414.

43. R.M. Silverstein, G. Clayton Bassler, Terence.C. Mossill. NMR Spectroscopy. In Spectrometric identification of organic compounds. New York: John Wiley and sons. INC; 2000. p. 91-226.

44. Sharma, Y.R. Infrared and NMR. In Elementary organic spectroscopy. Newdelhi: S.Chand and company ltd; 2004. p. 69-155, 182-255.

45. D.J. Ecobichon. The basis of toxicology testing. 2nd ed. In. New York: CRC Press; 1997.

46. A.E. Ahire and K.S. Laddha. Indian drugs. 2005; 42(8): p. 545-46.

47. C.Vijaya, M.Ramanathan and B.Suresh. Ind. J. Expt. Bio. 2009; 47: p. 182-5.

48. F.Khanna and R. Rizvi Chandar. J. of Ethnopharmacology. 2002; 82: p. 19-22.

49. L.Anila and NR. Vijayalaksmi. J of Ethnopharmacology. 2002; 79: p. 81-9.

50. M.A.Austin and J.E. Hokenson. current opinion in lipidology. 1984; 5: p. 395 – 403.

51. M.Guerin Le Goff. Thrombosis and Vascular Bio. 2001; 21: p. 282-9.

52. T.Murakai and R. Longhli. Thrombosis and Vascular Bio. 1995; 15: p. 1828-91.

53. C.Perez and J.R Canal. Phytotherapy Res. 1999; 13: p. 188-91.