"MOLECULAR DOCKING STUDY, SYNTHESIS AND ANTI INFLAMMATORY ACTIVITY OF 7-SUBSTITUTED COUMARIN SCHIFF BASE DERIVATIVES"

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CERTIFICATE

This is to certify that the dissertation work entitled "Molecular Docking Study, Synthesis and Anti-Inflammatory Activity of 7-Substituted Coumarin Schiff Base Derivatives submitted by Ms. Ira Ingrodiya with Regn. No. (11MPH404) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Chemistry" is a bonafide research work carried out by the candidate at the Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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I hereby declare that the dissertation entitled "Molecular Docking Study, Synthesis and Anti-Inflammatory Activity of 7-Substituted Coumarin Schiff Base Derivatives" is based on the original work carried out by me under the guidance of Dr. Manjunath Ghate, Director & Head, Department of Pharm. Chem., Co-Guided by Mr, Vivek K.Vyas, Assistant Professor , Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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CDH	Central Drug House
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DMF	Dimethyl formaminde
RNA	Ribonucleic acid
IL	Interleukin
PG	Prostaglandins
SEE	Standard error of estimate
SEM	Standard error of mean
LTs	Leukotrienes
H_1	Histamine
NASIDs	Nonsteroidal anti-inflammatory drugs
COX	Cyclooxygenase
kDa	Kilo Dalton
m.p	Melting point
Ile	Isoleucine
Val	Valinine
His	Histidine
Tyr	Tyrosine
Arg	Arginine
ppm	Parts per milioun

Abstract

Coumarins are an entity which is being synthesized in many of its derivative form from past few years. This entity is major source of interest for many of medicinal chemist to explore its various pharmacological potentials especially as anti oxidant, anti inflammatory, anti microbial , anti hepatitis, hepatoprotective, anti cancer, anti pyretic and analgesic activity. In present work, synthesized coumarin schiff base derivatives were screened for anti-inflammatory activity. Literature study of various research papers and other publications which provide detailed work and recent study on coumarin schiff base derivatives was taken into consideration. In the present study we have designed novel series of coumarin schiff base derivatives, docked in to active site of COX-2 with PDB ID [3NT1]. Based on the hydrogen bond interaction and docking score, MIP4 and MIP7 which shown good interaction. The target molecules were synthesized by coupling reaction of 7-(3-chloropropoxy)-4-methyl-2H-chromen-2-one with different substituted N-benzylpyridine-2-amine.They were further characterized by IR and ^{1}H NMR, MASS spectra. These newly formed Coumarin schiff base derivatives were screened for anti-inflammatory activity by carrageen induced rat paw edema model. Most of the compounds showed significant In-vivo anti-inflammatory activity. Comparing pharmacological activity and docking results, we conclude that heterocyclic derivatives linked with halogen at 7-position of coumarin schiff base seem to be potentially active drug.

Keywords: coumarin, schiff base, docking study, anti-inflammatory activity.

Chapter: 1
Introduction

Medicinal chemistry was previously defined by an IUPAC specialized commission. Medicinal chemistry concerns with the discovery, development, identification and interpretation of mode of action of biologically active compounds at the molecular level. It may involve synthesis of new compounds, investigation of the relationship between the structure of natural and synthetic compounds and their biological activities, their interactions with receptors of various kinds, including enzymes and DNA, determination of their absorption, transport and distribution properties and studies of the metabolic transformations of these compounds into other chemicals [1].

Medicinal chemistry covers three critical steps:

- **Discovery step:** consisting of the choice of the therapeutic target and the identification (or discovery) and production of the 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 biotechnical processes.
- **Optimization step:** that deals with the improvement of the lead structure. The optimization process take primarily into account the increase in potency, selectivity and toxicity. Its charecterics are the establishment and analysis of structure-activity relationship, is an ideal context to enable the understanding of the molecular mode of action.
- **Development step:** deals with the continuation of the improvement of the pharmacokinetic properties and the fine tuning of the pharmaceutical properties of the active substances in order to render them suitable for clinical use[1]

1.1. Coumarin ring

Coumarin (Figure 1 a) was first reported and isolated in the 1820's, recognized as the hay-like sweet aroma of the tonka bean[2]. Since then, more than 1000 derivatives have been investigated with naturally occurring coumarin derivatives which is isolated from 800 species of plant life. Most of the coumarin derivatives have at least one additional oxygen atom at one or more of the six other available positions, approximately 35 derivatives being oxygenated at the seventh position. For this reason, 7-hydroxy coumarin (Figure 1b), also known as umbelliferone, is often considered the "parent" of the more complex coumarins [2].

(a) (b)

Figure. 1.1. Structure and numbering scheme of (a) coumarins and (b) 7-hydroxycoumarin

Coumarin(1, 2-benzopyrone),the parent molecule of coumarin derivatives, is the simplest compound of a large class of naturally occurring phenolic substances made of fused benzene and α-pyrone rings [3]. Coumarins comprise a group of natural compounds found in a variety of plant sources. A lot of biological parameters should be evaluated for understanding the mechanisms by which these coumarins act. Coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances. In addition, these compounds are involved in the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis, as well as defense against infection. The coumarin have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities [4].

Schiff bases are aldehyde or ketone like compounds in which the carbonyl group is replaced by an imine or azomethine group. They are widely used for industrial purposes and also exhibit a broad range of biological activities. Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial ,antimalarial, antiproliferative, anti inflammatory, antiviral, and antipyretic properties [5].

1.2. Inflammation

Inflammation has been studied in an attempt to deal with it for thousands of years. Celsius (in 30 A.D.) described the four famous signs of inflammation,[6]

- Rubor
- Calor
- Tumor or redness
- Heat
- Pain and Swelling

That time extracts of willow leaves were used to relieve them. In 1860, Salicylic acid was chemically synthesized in Germany and its ready to supply for extended usage as an external antiseptic, antipyretic, and in the treatment of rheumatism, then aspirin was synthesized which is acting as a prodrug and its anti-inflammatory actions of were caused by liberation of salicylate. Many other aspirin like drugs are now available - the nonsteroid anti-inflammatory drugs (NSAIDs) [6].

More recently, inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality “or "the reaction to injury of the living microcirculation and related tissues. Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host.

While in the 19^{th} century, the contribution of inflammation to the body's defensive and healing process was recognised. The classical description of inflammation accounts for the visual changes seen.

(I) the sensation of heat was caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities, also resulting on the increased redness.

(II) The swelling (oedema) was the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue.

1.3. Pain and inflammatory mediators

Pain was occurring due to the direct effects of mediators, either from initial damage or by the inflammatory response itself. The loss of function refers to either simple loss of mobility in a joint, due to the oedema and pain, or to the replacement of functional cells with scar tissue [7]. Inflammation covers a host of pathophysiological events and means different things to different people acute or chronic, organ-specific such as asthma, reversible or irreversible but one thing is certain, there are many mediators available:

(1)Amines such as histamine and 5-hydroxytryptamine,

(2)Short peptides such as bradykinin,

(3)Long peptides such as interleukin-1 (IL-1),

(4) Lipids such as prostaglandins (PGs) and leukotrienes (LTs), enzymes released from migrating cells, complement, and so on [8].

1.3.1. Histamine

The release of histamine from mast cells during antigen antibody reactions is well known, as its involvement in the inflammatory response to skin injury. Also, there are increased numbers of mast cells in the rheumatoid synovium and in the asthmatic lung, which raised levels of histamine. When the first antihistamines were discovered in the 1940's, it was hoped that they would be potent anti-inflammatory agents and, indeed, they found a role in the treatment of hay fever and some cutaneous inflammation. But these H_1 antihistamines are ineffective in arthritis or asthma, so that histamine did not seem to play a major part in these conditions. After all suggests that histamine may, after all, play a role in allergic asthma [9].

1.3.2. Bradykinin

Small amounts of bradykinin cause pain, vasodilatation, and edema, all contributing to inflammation. Bradykinin-like immune reactivity was detected in rat pleural inflammatory exudates. Kinins are also present in nasal secretions after immunological challenge, and a kininogenase is released from human lung mast cells. Inhaled bradykinin causes broncho constriction in normal and asthmatic individuals, but not through release of PGs. Lack of effective antagonists makes it difficult to assess the extent of involvement of kinins in inflammation and asthma, but there is no evidence that inhibitors of the inactivation of bradykinin, such as captopril or enalopril, exacerbate these condition [10].

1.3.3 Interleukin

IL-1 is a polypeptide produced by activated macrophages which mimics the symptoms of chronic inflammation. IL-1-like activity has been detected in synovial fluids from patients with rheumatoid arthritis, its actions include activation of lymphocytes and production of fever, the latter being mediated by release of PGE_2 [11]

1.3.4. Prostaglandins

In 1971 Vane discovered that aspirin and similar drugs which inhibit the biosynthesis of PGs, and proposed their mechanism of action. In other words, the pathological release of PGs that contributes to inflammation, fever, and pain which is inhibited by aspirin and other NSAIDs. The aspirin like drugs also share, a certain side effects, such as a propensity to irritate the stomach, nephrotoxicity in high concentrations, and interference with the birth process. It was suggested that these side effects resulted from the inhibition of the physiological release of a protective PG [12].

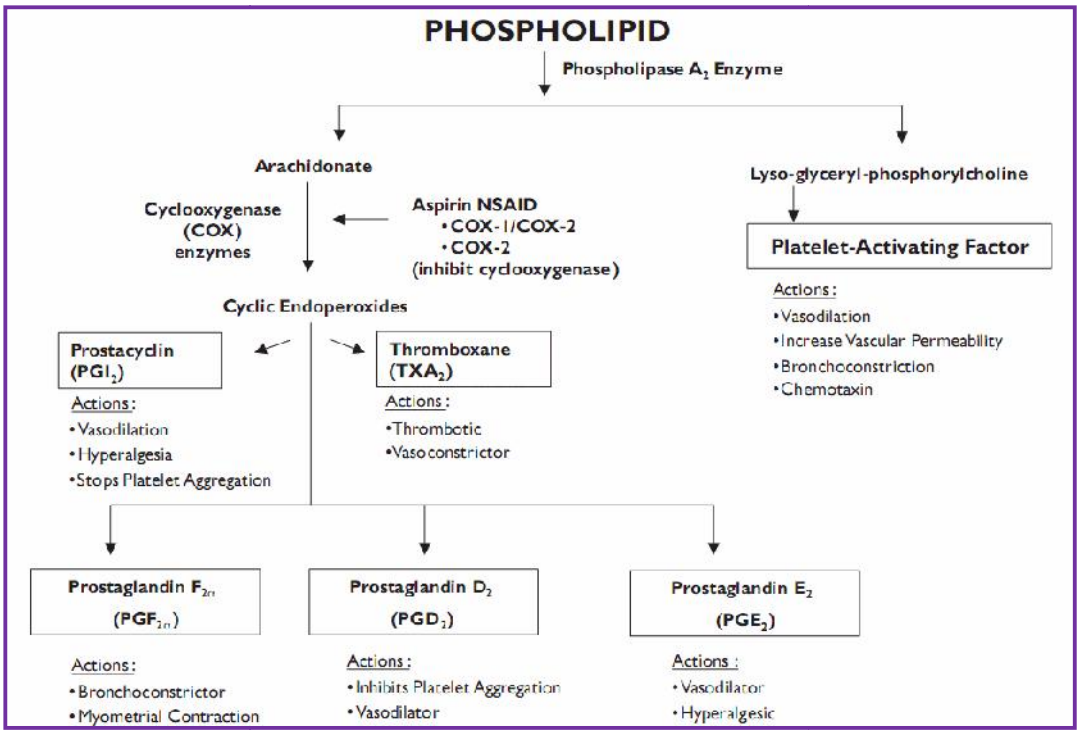

Figure 1.3 Biosynthesis of prostaglandins [12]

1.4. Cyclooxygenase

Cyclooxygenase (COX) was first purified in 1976 and cloned in 1988, use as a key enzyme in the synthesis of prostaglandins (PGs) from arachidonic acid[13]. An important advance in anti-inflammatory therapy was the discovery of two isoforms of COX (also known as synthetase prostaglandins)

- COX 1
- COX 2

More recently a third COX type has been described, called COX 3.5. Whereas COX 1 has 17 amino acids at the terminal amino section, COX 2 has 18 amino acids at the terminal carboxyl section. Although they are very similar in terms of their protein structure, these enzymes are coded by different genes. Genetically, COX 1 and COX 2 are approximately 60% homologous and their genes are located at chromosomes 9 and 1, respectively. COX 1 and COX 2 exhibit minor differences, which give distinct functions on them. COX 1 is present in almost all tissues (blood vessels, platelets, stomach, intestine, kidneys) and, for this reason, is defined as a constitutive enzyme. It is also associated with prostaglandin production and results in a variety of physiological effects, such as gastric protection, platelet aggregation, vascular homeostasis and maintenance of renal blood flow. In contrast, COX 2 is present at the site of inflammation, and because of this is defined as an inducible enzyme. It is primarily expressed by cells that are involved in the inflammatory process, such as macrophages, monocytes and synoviocytes. Nevertheless, COX 2 is induced by cytokines and other mediators at the site of inflammation. It is probably also expressed in the central nervous system and plays a role in central mediation of pain and fever. Expression of COX 2 can be suppressed by glucocorticoids, IL-4, IL-10 and IL-14 [14].

1.4.1. Structure of Cyclooxygenase enzyme

COX-1 and COX-2 are very similar in structure and almost identical in length, varying from 599 (human) to 602 (mice) amino acids for COX-1 and from 603 (mice) to 604 (human) for COX-2.Both isoforms possess molecular masses of 70 to 74 kDa and contain just over 600 amino acids, with an approximately 60% homology within the same species. Three amino acid differences result in a larger (about 20%) and more accessible channel, in COX-2.The exchange of a valinine (Val) at position of 523 in COX-2 for a relatively bulky isoleucine (Ile) residue in COX-1 at the same position of the active site of the enzyme.

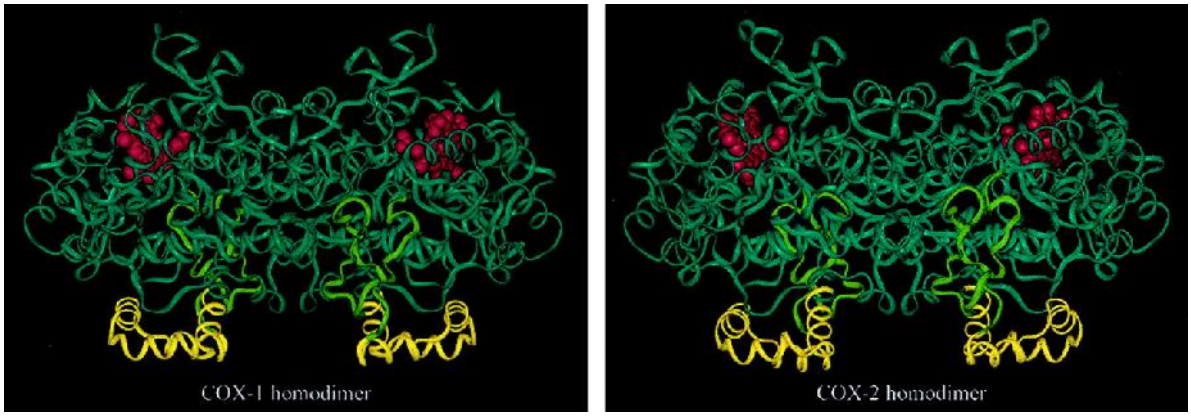

Figure. 1.4. Structure of Cyclooxygenase enzyme [15]

In addition, the exchange of Ile-434 for a valine in COX-2 allows a neighbouring residue phenylalanine-518 (Phe-518) to swing out of the way, increasing further access to the side cavity. There is another essential amino acid difference between the two isoforms, which does not alter the shape of the drug-binding site. but rather changes its chemical environment. Within the side pocket of COX-2 is an arginine in place of histidine-513 (His-513) in COX-1, which can interact with polar moieties. These differences between the COX active sites have major implications for the selectivity profile of inhibitors [15].

Inflammation	**Ulcerogenic potential**	**Inflammation**	**Ulcerogenic potential**
Pain	Alzheimer's disease.	Myocardial infection	Skin
Fever	Cancer	Stroke	Head and neck
Renal function	Colorectal	Diabetes	Breast
		Tissue repair	Pancreatic

Table.1.4 Known and potential processes involved with COX-2 up regulation.

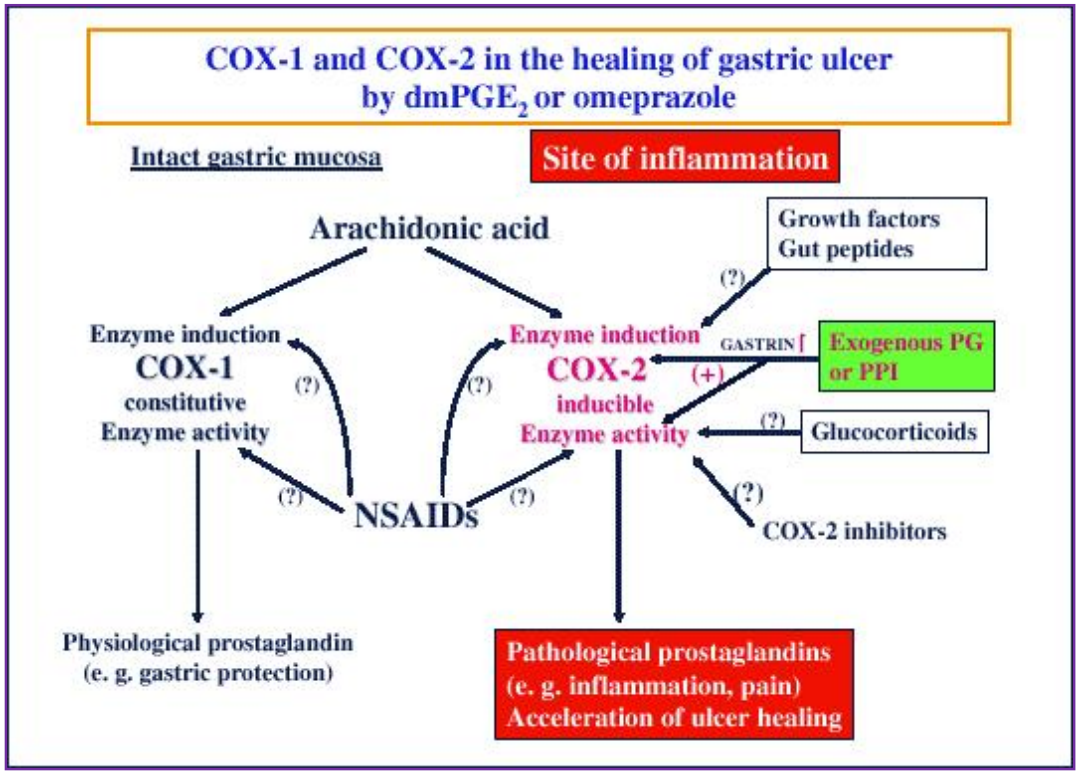

Fig.1.4.2 Schematic presentation of the actions of Cyclooxygenase (COX-1 and COX-2)[15]

1.5. Mechanism of action

The mechanism of action of NSAIDs consists in suppression of COX enzymes, resulting in reduction of the production of prostaglandins, thus controlling inflammation, pain and fever. There are some anti-inflammatories that selectively or specifically inhibit either COX 1 or COX 2. Only COX 1 inhibits thromboxane formation. COX 1 inhibition is associated with increased risk of gastrointestinal bleeding and damage. Selective and specific COX 2 inhibitors were developed in an attempt to reduce the incidence of adverse effects of COX 1 inhibition. These inhibitors include piroxicam, meloxicam, diclofenac, naproxen and nimesulide (first-generation selective COX 2 inhibitors), and celecoxib, etoricoxib, valdecoxib, parecoxib and lumiracoxib (second-generation, more specific, selective COX 2 inhibitors) [13].

1.6. Treatment

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in this capacity for the treatment of inflammation. NSAIDs such as ibuprofen, ketorolac, flurbiprofen, ketoprofen, diclofenac, aspirin, and aspirin derivatives are the traditional treatment for moderate inflammation and pain. NSAIDs can be broadly classified based on their chemical structure. NSAIDs within a group tend to have similar characteristics and tolerability. There is little difference in clinical efficacy among the NSAIDs when used at equivalent doses. Rather, differences among compounds are associated with dosing regimens (related to the compound & rsquo;s elimination half life), route of administration, and tolerability profile. NSAIDs can be classified based on their chemical structure or mechanism of action. Older NSAIDs were known long before their mechanism of action was elucidated and were for this reason classified by chemical structure or origin. Newer substances are more often classified by mechanism of action [16].

Salicylate	**Propionic acid derivative**	**Acetic acid derivatives**	**Enolic acid (Oxicam) derivatives**	**Fenamic acid derivatives (Fenamates)**	**Selective COX-2 inhibitors (Coxibs)**
Aspirin	Ibuprofen	Indomethacin	Piroxicam	Mefenamic acid	Celecoxib
Diflunisal	Dexibrupen	Tolmetin	Meloxicam	Meclofenamic acid	Rofecoxib
	Naproxen	Sulindac	Tenoxicam	Flufenamic acid	Valdecoxib
	Fenoprofen	Etodolac	Droxicam	Tolfenamic acid	Parecoxib

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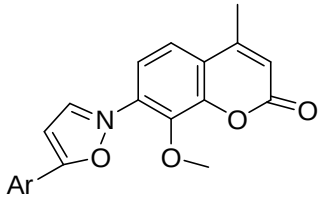
Chapter: 2
Literature review

Coumarin and their derivatives have attracted considerable attention due to their extensively biological activities such as antibacterial [1], antifungal, antiviral, anti tubercular, anti-malarial, anticoagulant, anti-inflammatory, anticancer [2], antioxidant [3], properties and so on. The plant extracts containing coumarin-related heterocycles, which were employed as herbal remedies in early days, have now been extensively studied for their biological activities. These investigations have revealed their potentials as versatile biodynamic agents. So far some coumarins, like, Warfarin, Acenocoumarol, Armillarisin-A, Hymecromone and Carbochromen have been approved for therapeutic purposes in clinic. More importantly, an increasing number of coumarin compounds have displayed great potency in the treatment of various types of diseases [4].

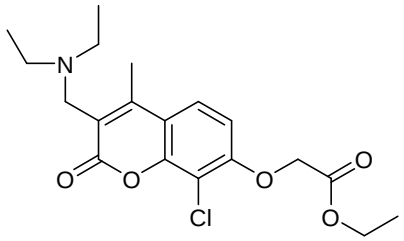
Schiff bases are the important compound owing to their wide range of biological activities and industrial application. They have been found to posses the pharmacological activities such as antimalarial [5], anticancer [6], antibacterial [7], antifungal [8], antitubercular, anti-inflammatory, antimicrobial and antiviral [4] etc. They also serve as a back bone for the synthesis of various heterocyclic compounds. The presence of azomethine and coumarin functional group is responsible for anti-inflammatory activity, which can be altered depending upon the type of substituent present on the aromatic rings. In view of these above biological importance of Schiff bases. We plan to synthesis of some novel coumarin analogs of schiff bases by schiff reaction [9].

2.1. Coumarin as an anti-inflammatory agent.

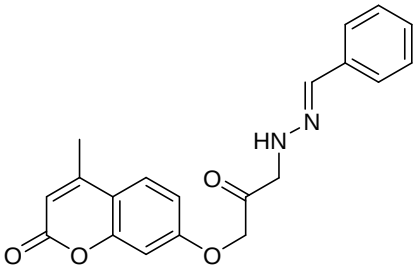
Gummudavelly S.*et al*. synthesised and evaluated 8-methoxy-4-methyl-7-(5-methyl isoxazol-3-aryl)-2H-chromen-2-one for anti-inflammatory activity [10].



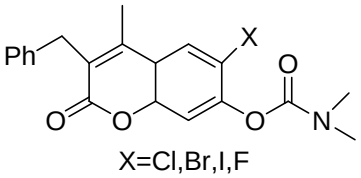
Cuzzocrea S.*et al*, Cloricromene a semi-synthetic coumarin derivative, was found to have anti-inflammatory activity [11].



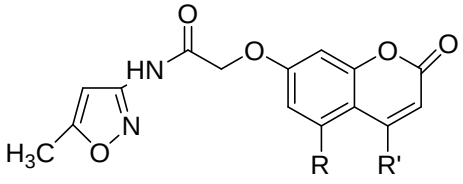
Deepak P. *et al*, synthesized and evaluated (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid hydrazide showed significant anti-inflammatory activity [13].



Bharatnagar A *et al*, synthesized coumarin based carbamates and evaluated for anti-inflammatory activity.C6- position of compound ring system was found to have the most influence on TNF-α inhibitory activity [14].

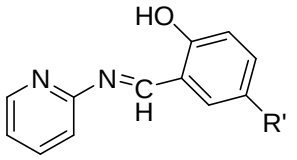


Rajanarendar *et al.* synthesized new isoxazolyl coumarins by pechmann condensation of isoxazolyl phenols with β-ketoester, dipyridine cobalt chloride is used as a catalyst. The method is simple, cost-effective and at ambient temperature gives good yield.

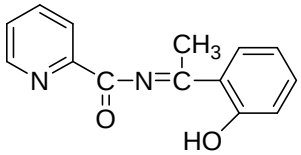


2.2. Schiff base as an anti-inflammatory agent.

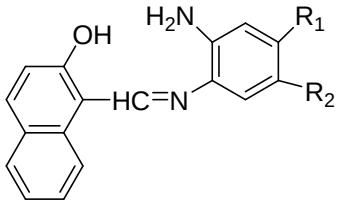
Dueke C.U *et al*. synthesized (E)-2-((pyridin-2-ylimino) methyl) benzene-1,4-diol and evaluated for anti-inflammatory and antibacterial activity [15].



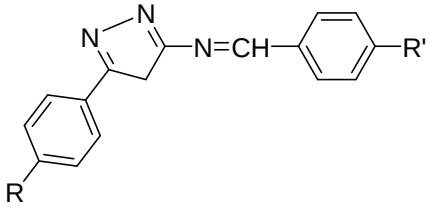
Mishra *et al*. reported new bidentate or tridentate Schiff bases and their Vo (II) and Co (II) complexes some of the complexes have been screened for their antimicrobial activity on different species of pathogenic bacteria/fungi, *E. coli, S. aureus, S. fecalis, A. niger, T. Polysporum* [16].



Sondhi *et al*. presented a series of N-(acridin-9-yl)-4- (benzo[d] imidazole/oxazol-2-yl) benzamides and their evaluation as anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibitions. Compounds 30e, 30f and 31 showed good anti-inflammatory and analgesic activity [17].

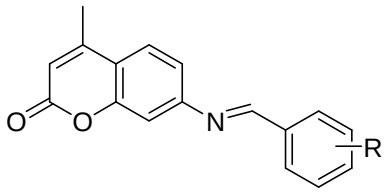


Pandey *et al*, Synthesized Some Schiff Bases of 2-amino-5-aryl-1, 3, 4-thiadiazole derivatives with different aromatic aldehyde were reported and evaluated for their analgesic, anti-inflammatory, and anti-bacterial (*Staphylococcus aureus* and *E. coli*) and antitubercular activity (Mycobacterium tuberculosis)[18].

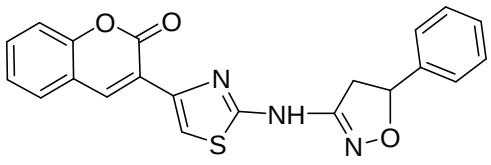


2.3. Coumarin schiff base nucleus.

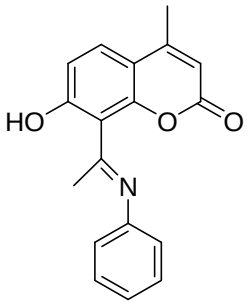
Ronad *et al*. prepared a series of 7-(2-substituted phenyl thiazolidinyl)-benzopyran-2-one derivatives of Schiff bases and evaluated for anti-bacterial and anti-fungal activities against various bacterial and fungal strains. The result showed that compound 46d, 46f exhibited good anti-bacterial and anti-fungal activity [19].



Desai J. T. *et.al.* Synthesized 3-(2-(5-phenyl-4,5-dihydroisoxazol-3-ylamino)thiazol-4-yl)-2H-chromen-2-one and evaluated for anti-inflammatory ,antifungal ,anti HIV, anti microbial activity [20].



Balaji P. N. *et al*, synthesized (E)-7-hydroxy-4-methyl-8-(1-(phenyl imino)ethyl)-2H chromen-2-one and evaluated for antimicrobial activity.[21]



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Chapter: 3
Aim & Objective

The ever growing resistance to anti inflammatory or pathogenic organism leads to continuous screening and development of new biological effective substance of natural or synthetic origin.

Heterocyclic compounds play a very important role in biological system. Many heterocyclic compound of natural origin with Useful medicinal properties have served as lead compound in designing synthetic drug.

3.1. Rationale for designing the target molecule

Coumarin and their derivatives are reported to exhibit wide spectrum of biological activities like anti-bacterial, anti-inflammatory, analgesic, anticoagulant, antiviral and HIV protease inhibitor activity etc.

A synthesis of coumarin and their derivatives has attracted considerable attention from organic and medicinal chemist from many years as large number of natural products contains this heterocyclic nucleus. Member of this group display a broad range of application fragrance pharmaceuticals and agro chemicals.

There are excellent monograph and review articles describing the structure, synthesis and properties of coumarin.

So, in view of all observation, we though it is to undertake the synthesis and biologically evaluation of some new coumarin derivatives, which are substituted at 7^{th} position.

Pechmen synthesis involves synthesis of 7-hydroxy 4-methyl coumarin from phenols and β-keto esters. It reacted with further 1-bromo -3 chloro propane to get the coumarin derivatives. Schiff base derivatives have been synthesized by 2-amino pyridine react with different aldehyde.

We can get useful derivatives of this coumarin ring to get a new drug with high potency and minimum side effects.

A number of substituted coumarin are known for their biological importance like anti microbial, anti-inflammatory, analgesic, anticoagulant, antiviral, antipsychotic activity.

Coumarin schiff base derivatives have been synthesized by schiff base derivative react with coumarin derivative react with different substituted aldehyde and to carry out their biological activity.

The synthesized compounds have been confirmed by IR, NMR, and MASS spectral data, screened for anti-inflammatory activity by standard methods.

All synthesized compound have shown significant anti-inflammatory activity.

3.2. Objective of present research work

The objectives of present research work can be summarized as:

- To study the modification of coumarin at –C7 position.
- To perform molecular docking study for coumarin schiff base derivative as anti-inflammatory agent.
- Identification of active site amino acid residues, which are responsible for receptor binding for potent activity.
- To devise the synthetic scheme for the designed molecules.
- Characterize the synthesized compounds physical and spectral analysis.
- Evaluation of anti-inflammatory activity of synthesized coumarin schiff base derivatives.

3.3. BIBLIOGRAPHY

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Chapter: 4
Docking study

4.1. Molecular docking study

Surflex–Dock module of SYBYL was used for molecular docking. The X-ray crystallographic structures of COX- 2 solved at 1.73 Å complex with ligand [PDB: 3NT1] was taken from protein data bank (PDB) and modified for docking calculations [1]. Co-crystallized ligand was removed from the structure, water molecules were removed, Hydrogen atoms were added and side chains were fixed during protein preparation. Protein structure minimization was performed by applying Tripos force field and partial atomic charges were calculated by Gasteiger-Huckel method. Twenty conformers were generated for each molecule.

4.1.1 Molecular docking analysis

To study the binding modes of the synthesized compounds with the COX-2 enzyme, we performed molecular docking experiments of 10 molecules into the ligand binding site of the enzyme. Surflex-Dock uses an empirically derived scoring function that is based on the binding affinities of protein–ligand complexes and on their X-ray structures. Surflex–Dock docks ligands automatically into a receptor's ligand binding site using a protomol based method and an empirically derived scoring function. Protomol represents interaction of ligand with binding site of protein.[2] The protomol is a unique and important factor of the docking algorithm and is a computational representation of assumed ligands that interact with the binding site. Surflex–Dock's scoring function contains hydrophobic, polar, repulsive, entropic, and solvation terms. Co-crystal structure of human COX-2 was retrieved from the protein data bank. After running Surflex– Dock, the scores of active docked conformers were ranked in a molecular spread sheet. We selected the best total score conformers and speculated regarding the detailed binding patterns in the cavity. Surflex dock results contain 3 information, total score which is the total Surflex-Dock score expressed as -log (K_d) to represent binding affinities which include hydrophobic, polar, repulsive, entropic and solvation [3]. Crash value is the degree of inappropriate penetration by the ligand into the protein and of interpenetration between ligand atoms (self-clash) that are separated by rotatable bonds. Crash scores close to 0 are favourable. Polar value is contribution of the hydrogen bonding and salt bridge interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds [4].

4.1.2. Binding pose of compound in COX-2

Docking result suggests that MIP4 and MIP7 have highest docking score of 6.32 and 6.30 shown in Table 4.1. The overall binding of synthesized compounds and in COX-2 is illustrated in Fig. 4.1. Docking result shows that four hydrogen bonds are formed between ligand (MIP4) and protein (COX-2) (Fig. 4.1a).

Table 4 1. Designed compounds with their Docking scores

Compound	R	Docking score
SC-558	-	5.50
MIP1	H	
MIP2	4-Cl	
MIP3	3-Cl	
MIP4	4-OCH_3	
MIP5	4-Br	
MIP6	3-Br	
MIP7	4-F	
MIP8	4-NO_2	
MIP9	3,4- diOCH_3	
MIP10	2-NH_2,3,4-diCH_3	

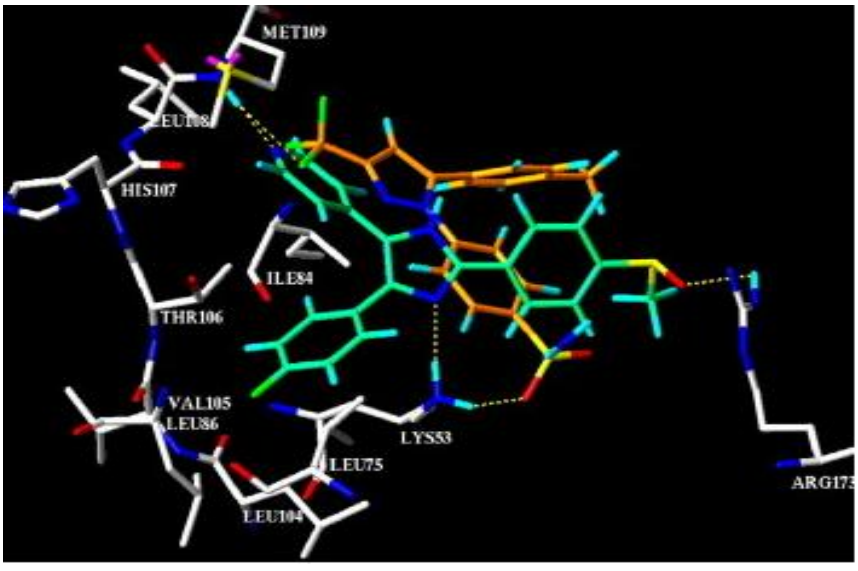

Fig. 4.1 .Binding mode of Celecoxib and SC-558 into its binding site of COX-2

Figure 4.1 shown binding interaction of standard (SC-558) with COX-2. It forms total three hydrogen bonds with different amino acid residues of the target enzyme. (F of CF_3.....Arg120, H of NH_2.....Ser353 and O of SO_2 ..... His90). Arg120, Ser353, His90 are the essential amino acids to confirm the appropriate binding of inhibitor in the active site pocket of arachidonic acid binding site along with Leu352, Ser530, Tyr355 and Tyr385.

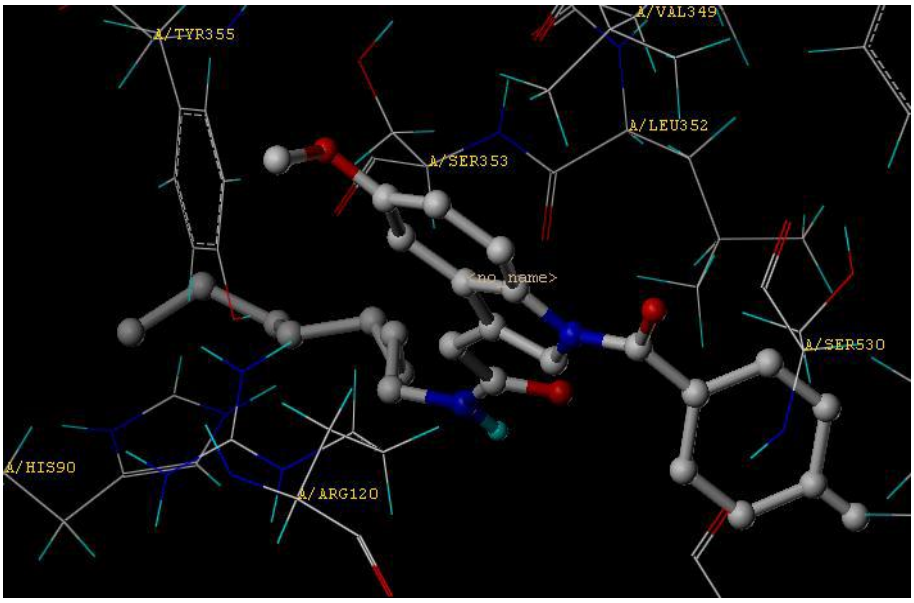

Figure 4.2. MIP 4 forms total three hydrogen bonds with the target enzyme

MIP 4 which is 2-methoxy substituted coumarin schiff base derivative was found to have good hydrogen bond interactions with COX-2.Total three hydrogen bonds with the target enzyme, (O of CO.....Arg120 and Tyr355, O of OCH3.....Ser530). Since all these amino acids were reported to be actively involved in confirming proper binding of inhibitor in the binding pocket of COX-2, MIP 4 can be a good inhibitor of the COX-2.

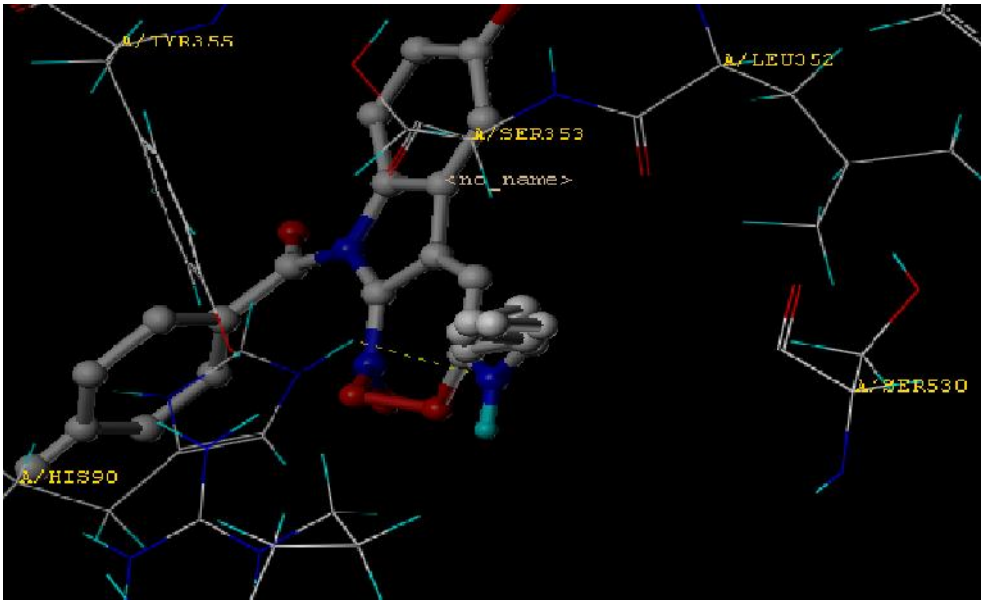

Figure. 4.3.Hydrogen bonding interactions of MIP 7

MIP 7 has high score compound having total score of 6.34. It forms total three hydrogen bonds with the target enzyme,(O of CO.....Tyr355 and two with Arg120).since MIP7 has good score and number of hydrogens are also greater than the standard.

4.2. Result

From above docking results it was understood that Oxygen atom of the carbonyl group of all designed molecules is actively involved in hydrogen bond interaction of these molecules with the COX-2. Thus presence of Oxygen atom is necessary for good inhibitory activity. Above docking results also conclude that only total docking score solely cannot be considered as a parameter to evaluate the inhibitors for their binding affinity but hydrogen bonding interactions are equally important as they ensures correct binding of the inhibitor to the enzyme which is important for inhibitor to exert its activity. From above studies it was found that Arg120, Tyr355, His90, Ser530, Leu352, and Ser353 are the amino acids residue important for appropriate binding of the inhibitor into binding pocket of the enzyme.

4.3. BIBLIOGRAPHY

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Chapter: 5
Synthesis & Characterization

5.1 Materials & Method.

Step 1: Synthesis of 7-hydroxy-4-methyl-2H-chromen-2-one

Step 2: Synthesis of 7-(3-chloropropoxy)-4-methylchromen-2-one

Step 3: Synthesis of N-benzylidenepyridin-2-amine

Step 4: Synthesis of N-benzylpyridin-2-amine.

Step 5: Synthesis of 7-(3-(benzyl(pyridin-2-yl)amino)propoxy)-4-methyl-2H-chromen-2-one

Step 1:

Reaction

General method of synthesis:

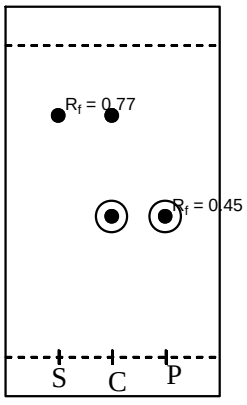
General synthesis of coumarin involves a interaction of a phenol with a β-ketoester in the presence of an acid condensing agent via pechmann reaction. Concentrated sulphuric acid is generally used as a condensing agent for phenols and β-ketoester, although phenol itself reacts better in the presence of aluminium chloride. The mechanism of the reaction is thought to involve the intial formation of a β-hydroxy ester, which then cyclise and dehydrates to yield the coumarin.

Reaction mechanism:

Requirements:

Procedure:

TLC: Solvent system:60%: Hexane :Ethyl acetate(4:6)(S= starting material ,C=co spot ,P=product)



Step 2:

Synthesis of 7-(3-chloropropoxy)-4-methylchromen-2-one

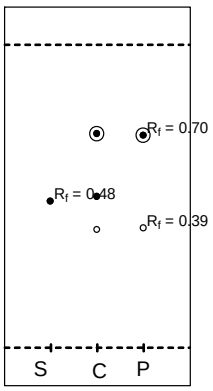
Reaction:

General protocol and discussion:

Requirements:

Procedure:

TLC: Solvent system:60%: Hexane :Ethyl acetate(4:6)(S= starting material ,C=co spot ,P=product)



Step 3:

Synthesis of N-benzylidenepyridin-2-amine.

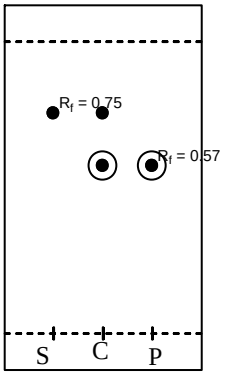
Reaction:

General protocol and discussion:

Requirements:

Procedure:

TLC: Solvent system: 30%: Methanol :Chloroform (3:7)(S= starting material ,C=co spot ,P=product)



Step 4:

Synthesis of N-benzylpyridin-2-amine.

Reaction:

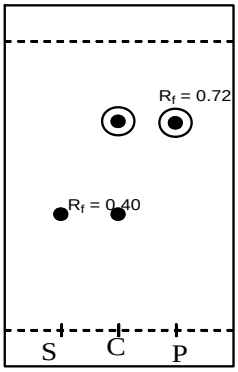
General protocol and discussion:

Reduction of Schiff base was carry out by simple reducing agent active metal like zinc, magnesium, sodium, aluminium etc. There were Zn/HCl was used as a reducing agent in this method, it gives evolution of hydrogen gas so reaction was carrying out very carefully.

Requirements:

Procedure:

TLC: Solvent system: 40%: Methanol :Chloroform(4:6)(S= starting material ,C=co spot ,P=product)



Step 5:

Synthesis of 7-(3-(benzyl(pyridin-2-yl)amino)propoxy)-4-methyl-2H-chromen-2-one.

Reaction:

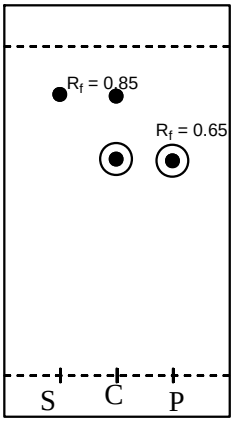
General protocol and discussion:

Column chromatography:

Requirements:

Procedure:

TLC: Solvent system:60%: Hexane :Ethyl acetate(6:4)(S= starting material ,C=co spot ,P=product)



5.2. Outline of synthetic protocol

For the synthesis of coumarin schiff base derivatives, the most important step is coupling of coumarin alkane chain with reducing Schiff base derivative. Initially the 7-hydroxy-4-methyl-2H-chromen-2-one is likely formed via pechmen reaction between resorcinol and ethyl acetoacetate. Alternatively, the synthesis of coumarin schiff base derivatives was synthesized by reacting 7-(3-chloropropoxy)-4-methylchromen-2-one with excess of reducing Schiff base derivative in presence of anhydrous K_2CO_3 under microwave condition.

The synthesis was carried out in 2 steps:

Step 1: synthesis of intermediates

Step 2: synthesis of final compounds

5.2.1 Synthesis of Intermediate

I. Synthesis of intermediate 1

Reaction

(21) (22) (23)

Requirements:

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

II. Synthesis of Intermediate 2

Reaction:

(23) (24) (25)

Requirements:

Procedure:

.

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

III. Synthesis of Intermediate 3

5.2.2.1.1 Synthesis of N-benzylidenepyridin-2-amine.(28)

Reaction:

(26) (27) (28)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system
1.	N-benzylidenepyridin-2-amine.	188-190	90	0.69	Methanol:chloroform(3:7)

5.2.2.1.2 Synthesis of N-benzylpyridin-2-amine.(29)

Reaction:

(28) (29)

Requirements:

Assembly: Round bottom flask (500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	**IUPAC name Code No.**	**Melting point(^{0}C)**	**% yield**	**R_f value**	**Solvent system**

5.2.2.2.1 Synthesis of N-(4-chlorobenzylidene) pyridin-2-amine. (31)

Reaction:

(26) (30) (31)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.2.2 Synthesis of N-(4-chlorobenzyl) pyridine-2-amine.(32

Reaction:

(31) (32)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.3.1 Synthesis of N-(3-chlorobenzylidene) pyridin-2-amine.(34)

Reaction:

(26) (33) (34)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.3.2 Synthesis of N-(3-chlorobenzyl) pyridine-2-amine.(35)

Reaction:

(34) (35)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

.

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.4.1 Synthesis of N-(4-methoxybenzylidene) pyridin-2-amine. (37)

Reaction:

(26) (36) (37)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	**IUPAC name Code No.**	**Melting point(^{0}C)**	**% yield**	**R_f value**	**Solvent system**

5.2.2.4.2 Synthesis of N-(4-methoxybenzyl) pyridin-2-amine.(38)

Reaction:

(37) (38)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.5.1 Synthesis of N-(4-bromobenzylidene) pyridin-2-amine.(40)

Reaction:

(26) (39) (40)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.5.2. Synthesis of N-(4-bromobenzyl) pyridin-2-amine. (41)

Reaction:

(40) (41)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	**IUPAC name Code No.**	**Melting point(^{0}C)**	**% yield**	**R_f value**	**Solvent system**

5.2.2.6.1 Synthesis of N-(3-bromobenzylidene) pyridin-2-amine.(43)

Reaction:

(26) (42) (43)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.5.2. Synthesis of N-(3-bromobenzyl) pyridin-2-amine. (44)

Reaction:

(43) (44)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.7.1. Synthesis of N-(4-fluorobenzylidene) pyridin-2-amine (46)

Reaction:

(26) (45) (46)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer , magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.7.2. Synthesis of N-(4-fluorobenzyl) pyridin-2-amine.(47)

Reaction:

(46) (47)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	**IUPAC name Code No.**	**Melting point(^{0}C)**	**% yield**	**R_f value**	**Solvent system**

5.2.2.8.1 Synthesis of N-(4-nitrobenzylidene) pyridin-2-amine(49)

Reaction:

(26) (48) (49)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.8 .2. Synthesis of N-(4-nitrobenzyl) pyridin-2-amine. (50)

Reaction:

(49) (50)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

.

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.9.1 Synthesis of N-(3,4-dimethoxybenzylidene)pyridin-2-amine(52)

Reaction:

(26) (51) (52)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	**IUPAC name Code No.**	**Melting point(^{0}C)**	**% yield**	**R_f value**	**Solvent system**

5.2.2.9.2.Synthesis of N-(3, 4-methoxybenzyl) pyridin-2-amine. (54)

Reaction:

(53) (54)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	**IUPAC name Code No.**	**Melting point(^{0}C)**	**% yield**	**R_f value**	**Solvent system**

5.2.2.10.1 Synthesis of N-(2-amino-3,4-dimethylbenzylidene)pyridin-2-amine(56)

Reaction:

(26) (55) (56)

Requirements:

Assembly: Beaker (250 ml), magnetic stirrer, magnetic stirring bar.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.2.2.10.2. Synthesis of N-(2-amino,3,4-methoxybenzyl)pyridin-2-amine. (57)

Reaction:

(56) (57)

Requirements:

Assembly: Round bottom flask(500 ml), beaker(250 ml) ,Funnel, magnetic stirrer, magnetic stirring bath.

Procedure:

Sr no.	IUPAC name Code No.	Melting point(^{0}C)	% yield	R_f value	Solvent system

5.3 Synthesis of final compounds

5.3.1. Synthesis of 7-(3-(benzyl (pyridin-2-yl)amino)propoxy)-4-methyl-2H-chromen-2-one derivatives.

I. Synthesis of MIP1

Reaction:

(25) (29) (MIP1)

Requirements:

Procedure:

.

II. Synthesis of MIP2

(25) (32) (MIP2)

Requirements:

Procedure: Same as given in 5.3.1.1

III. Synthesis of MIP3

(25) (35) (MIP3)

Requirements:

Procedure: same as given in 5.3.1.1

IV. Synthesis of MIP4:

(25) (38) (MIP4)

Requirements:

Procedure: same as given in 5.3.1.1

V. Synthesis of MIP5:

(25) (41) (MIP5)

Requirements:

Procedure: same as given in 5.3.1.1.

VI. Synthesis of MIP6

(25) (43) (MIP6)

Requirements:

Procedure: same as given in 5.3.1.1

VII. Synthesis of MIP7

(25) (47) (MIP7)

Requirements:

Procedure: same as given in 5.3.1.1

VIII. Synthesis of MIP8

(25) (50) (MIP 8)

Requirements:

Procedure: same as given in 5.3.1.1

XI. Synthesis of MIP 9

(25) (54) (MIP 9)

Requirements:

Procedure: same as given in 5.3.1.1

X. Synthesis of MIP10.

(25) (57) (MIP10)

Requirements:

Procedure: same as given in 5.3.1.1

5.4. Physical characterization & spectral analysis.

The synthesized compounds were characterized by TLC and Melting point. Precoated thin layer chromatography (TLC) plates and appropriate solvent system were used to monitor the completion of reaction.

Table: 5.4: Physical Characterization Of Synthesized Compounds.

Sr no.	IUPAC name	Compound code	Molecular weight (g/mol)	R_f value	Melting point (^{0}C)	% Yield
1.		MIP1				
2.		MIP2				
3.		MIP3				
4.		MIP4				
5.		MIP5				
6.		MIP6				
7.		MIP7				
8.		MIP8				
9.		MIP9				
10.		MIP10				

5.5. Spectral analysis of designed molecule

5.5.1 Infrared spectra of MIP1

Figure. 5.1 Infrared spectrum of MIP1

No.	Wave number(cm^{-1})	Functional group

Table. 5.5. IR spectrum of MIP1

5.5.2. Mass spectra of MIP1

Figure. 5.2 Mass spectrum of MIP1

5.5.3. Infrared spectra of MIP2

Figure. 5.4. Infrared spectrum of MIP2

Table. 5.3. Infrared spectrum of MIP2

No.	Wave number(cm^{-1})	Functional group

5.5.4. Mass spectra of MIP2

Figure 5.5 Mass spectra of MIP2

5.5.5. Nuclear magnetic resonance spectra of MIP2

Figure 5.6 NMR spectra of MIP2

5.6. BIBLIOGRAPHY

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Chapter: 6
Biological Evaluation

Pharmacological evaluation was necessary for the therapeutic indication of newly synthesized chemical entities due to the emergence of synthetic chemistry. It helps in the determining as well as validating the particular therapeutic indication for which any compounds have been designed and synthesized. In the present work, pharmacological evaluation is performed by studying the anti-inflammatory activity on animals.

6.1 Anti-inflammatory activity

Inflammation has different phases: In first phase vascular permeability is increased, resulting in exudation of fluid from blood into interstital fluid. In second phase infiltration of leukocytes from blood into tissue and in third phase the granuloma is formed. Accordingly, the anti-inflammatory tests have to be divided into those measuring acute inflammation, chronic inflammation and acute toxicity study. In some cases, the screening is directed to test compounds for local application.

- Acute anti-inflammatory model (Paw edema induced by Carrageen).
- Chronic anti-inflammatory activity (Cotton pellets-induced granuloma test)
- Acute Toxicity study

The various methods implicated for the measurement of anti-inflammatory activity in-vivo regarding the acute and sub acute phases are as follows.

- Ultraviolet erythema in guinea pigs
- Vascular permeability
- Inhibition of leukocyte adhesion on rat mesenteric venules
- Oxazolone and croton oil induced ear edema in mice and rats
- Rat paw edema: Carrageenan, formalin, prostaglandin etc. induced paw edema.
- Granuloma pouch technique
- Cotton wool granuloma
- Sponge implantation technique

From the above mentioned methods for anti-inflammatory activity, one of the most commonly employed techniques is Carrageenan, induced rat paw edema method.

6.1.1 Experimental Protocol

The experimental protocol is approved by institutional animal ethics committee as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social justice and Empowerment, Government of India under protocol number **IP/PCEM/MPH/12-13/022.**

6.1.2 Requirements

6.1.2.1 Chemicals

Sr No	Name of chemical	Supplier's name	Quantity required
1	Carrageenan	Sigma-Aldrich	0.1 g
2	Carboxy methyl Cellulose (CMC)	CDH	0.5 g
3	Ether	CDH	25 mL
4	Diclofenac Sodium	Sigma-Aldrich	0.1 g

6.1.2.2 Instrument

Plethysmometer (PTH707, Medicaid) was required to measure the paw edema volume by water displacement at different predetermined time intervals. The instrument was properly calibrated before performing the experiment using standard calibrated probe number.

6.1.2.3 Animals

Wistar rats, weighing around 200–400 g were used in the present study and were maintained in colony cages at 25±2°C and relative humidity of 45-55% under a 12 hrs light and dark cycle. They were fed standard animal food. They were starved overnight before experiment.

6.1.2.4 Test compounds

The test compounds and standard drug were administered in the form of suspension of 0.5% W/V sodium carboxy methyl cellulose (CMC) as vehicle by the oral route of administration. Diclofenac sodium as dose of 50 mg/kg of body weight was administered orally as reference of drug comparison. The test compounds were administered orally at the same dose of standard of 50 mg/kg of body weight.

6.1.3 Screening method

Overnight starved wistar rats of 200-400 g were divided into four groups of two animals in each group for the evaluation of anti-inflammatory activity.

Group 1	Control
Group 2	Standard (Diclofenac Sodium 50 mg/kg)
Group 3	MIP1
Group 4	MIP2

6.1.3.1 Procedure

Method describe by Northover and Subramanian (1961) in which 0.05 ml of 0.1% Formalin in Normal Saline was injected in the subcutaneous tissue of the planter surface of right hind paw produced sub maximal degree of swelling. The paw volume was measured at 0, 0.5, 1, 2, 3, 4 and 5 hour after injection of Carrageenan. The volume of the inflamed paw was measured by standard volumetric technique, using a calibrated Plethysmometer. The paw was immersed up to the tibiotarsic articulation (marked with ink) in a cylinder filled with mercury. The increased level, consequent on the increase of the mercury meniscus, was measured from the increase of dyed ethanol in a glass tube connected to the surface of the calibrated glass tube. The increase in volume of the paw was calculated by subtracting the initial volume from the volume obtained after formalin administration and expressed as paw volume increase over time (ml ± SD) and effect (percent of negative control) for each rat and each group was obtained as follows.

Percentage inhibition = $[(V_o - V_t) / V_o] \times 100$

Where, Vo = Volume of the paw of control at any time‘t’.

Vt = Volume of the paw of drug treated at any time‘t’

Table 6.1 Effect of test compounds and Diclofenac sodium on Carrageenan induced rat paw edema

Drugs	Dose (mg/ mL)	Before Carrageenan	Mean paw edema Volume ± SE			
			1 hour	2 hours	3 hours	6 hours
Control	-					
Std (Diclofenac Sodium)	30					
Std (Diclofenac Sodium)	60					
MIP1	30					
MIP1	60					
MIP2	30					
MIP2	60					

The results given above are mean ± S.E.M.; no. of animals used (n=2) * P value of < 0.05 was considered significant in comparison to control.

Table 6.2 % inhibition of test compounds and Diclofenac sodium on Carrageenan induced rat paw edema

Drugs	Dose (mg/mL)		% Inhibition							
			1 hour		2 hours		3 hours		6 hours	
MIP1	30	60								
MIP2	30	60								
Std (Diclofenac Sodium)	30	60								

The results given above are mean ± S.E.M.; no. of animals used (n=2) * P value of < 0.05 was considered significant in comparison to control.

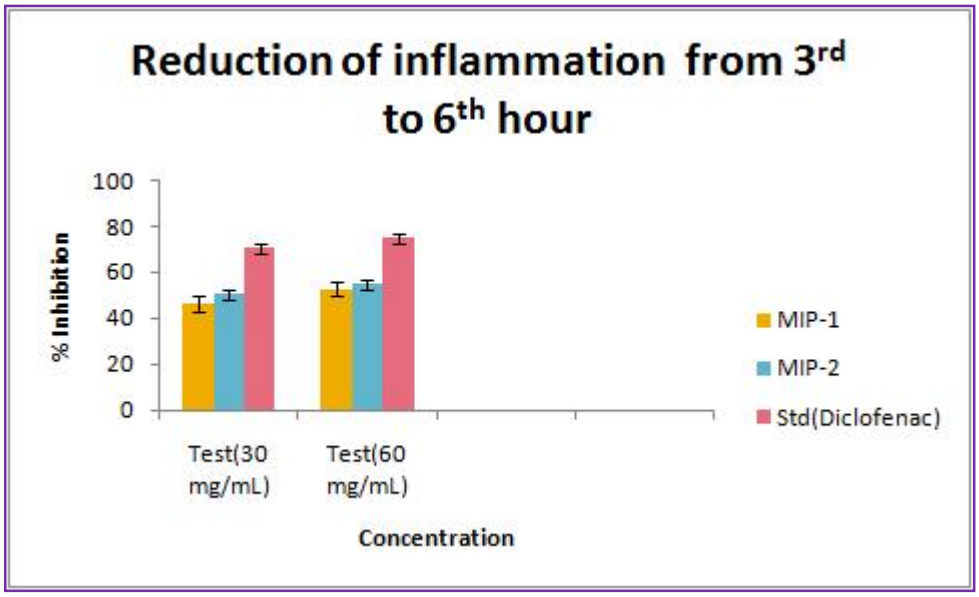

Figure 6.1 Graphical representation of Anti-inflammatory activity

6.1.4 Result

Two compounds MIP1 and MIP2 were selected for the anti-inflammatory activity and both the compounds show good anti-inflammatory activity.

6.2. BIBLIOGRAPHY

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Chapter: 7
Result and Discussion

To pre-asses anti-inflammatory behaviour of designed coumarin schiff base derivatives, automated docking studies were carried out and scoring functions, their binding affinities and orientation of these compounds at the active site of the COX- II enzyme were found out. We performed molecular docking experiments of 10 molecules into the ligand binding site of the enzyme. Docking result suggests that MIP4 and MIP7 have highest docking score of 6.32 and 6.30, and MIP1, MIP2, MIP8 shown good docking score. From docking results it was concluded that Oxygen atom of the carbonyl group of all designed molecules is actively involved in hydrogen bond interaction of these molecules with the COX-2. Thus presence of Oxygen atom is necessary for good inhibitory activity.

However only total docking score solely cannot be considered as a parameter to evaluate the inhibitors for their binding affinity but hydrogen bonding interactions are equally important as they ensures correct binding of the inhibitor to the enzyme which is important for inhibitor to exert its activity. some amino acids residues like Arg120, Tyr355, His90, Ser530, Leu352, and Ser353 are important for appropriate binding of the inhibitor into binding pocket of the enzyme.

Coumarin is a simple molecule and many of its derivatives have been known for more than a century. Coumarin and coumarin-related compounds have proved for many years to have significant therapeutic potential. They come from a wide variety of natural sources and new coumarin derivatives are being discovered or synthesized on a regular basis. It is evident from the research described that coumarin schiff base derivatives are a plentiful source of potential drugs candidate in relation to its safety and efficacy. New coumarin schiff derivatives have been synthesized using conventional and microwave heating methodology and characterized. The advantages in the use of microwave methodology are shorter reaction times, higher yields and simplified work up procedures for the point of purification of the prepared compound

The synthetic protocol for the target molecules involved 5 steps reaction. Initially the 7-hydroxy-4- methyl -2H-chromen-2-one is likely formed via Pechmann reaction between resorcinol and ethyl acetoacetate. In the second step 7-hydroxy 4-methyl coumarin reacted with 1-bromo 3-chloro propane to form coumarin halo compound. In the third step schiff base was synthesized by using 2-amino pyridine react with

different aldehyde and further reduction was carried out in fourth step, so, reduced schiff base derivative coupled with coumarin halo compound by using K_2CO_3 under Microwave conditions.

All the reactions were monitored by TLC, structures and purity of compounds were characterized by physical constant and spectral studies by FTIR, 1H-NMR and Mass spectroscopy. In the FTIR spectra of compound showed the expected bands for the characteristic groups which are present in the compounds such as C-H stretching bands at around 2900-3000 cm^{-1} and C=O stretching bands at around 1750 cm^{-1} and C=N stretching at 1600-1500 cm^{-1}.

Two compounds MIP1 and MIP2 were assayed *In-vivo* for their anti-inflammatory activity by carrageen induced rat paw edema method; it showed significant anti inflammatory activity.

The obtained result revealed that the nature of substituent on the coumarin schiff base ring may have a considerable impact on the anti-inflammatory activity. This clearly indicate that anti-inflammatory activity increases by modification of heterocyclic ring linked with halogen in 7-position of coumarin schiff base nucleus.

Chapter: 8
Conclusion

Coumarin is a simple molecule and many of its derivatives have been known for more than a century. Coumarin and coumarin-related compounds have proved for many years to have significant therapeutic potential. A series of ten compounds of coumarin schiff base derivatives were successfully synthesized by microwave condition. All the compounds were characterized by physical, spectral analysis and tested for their *In-vivo* anti inflammatory activity. On the basis of given data for synthesized compounds, we say that MIP1 and MIP2 were giving good docking score and showed significant anti-inflammatory activity by carrageenan induced rat paw edema method. Comparing biological activity and docking results, we conclude that heterocyclic derivatives linked with halogen at 7-position of coumarin schiff base molecule to be potentially active drug