Chapter-1-Introduction

- Introduction.
- Literature Survey
- Development of COX-2 Inhibitors.
- Aims and Objectives.
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1.1 INTRODUCTION:-

Drugs are chemicals that prevent disease or assist in restoring health to diseased individuals; as such they play an indispensable role in modern medicine.

Medicinal chemistry is the branch of science that provides these drugs either through discovery or through design. In the last century, the classical drugs were primarily discovered either by alteration of natural substances or entirely by chemical synthesis. In the recent years, an ever-increasing understanding of pathophysiology of diseases has increasingly led to novel opportunities to deliberate design, synthesis and evaluation of candidate drug molecules.

Medicinal chemistry is a discipline firmly rooted in synthetic organic chemistry and has very close links to structural chemistry, computational chemistry, and molecular biology at the discovery interface, to structural biology, toxicology and pharmacology at the development interface and to medicine at clinical interface. Thus, medicinal chemistry has occupied the central position and will continue to play a crucial role in the new drug discovery process. What is the most commonly-taken drug today? It is an effective painkiller (analgesic-anti-inflammatory agent). It reduces fever and inflammation when the body gets overzealous in its defenses against infection and damage. The promise of effective anti-inflammatory drugs that do not cause gastrointestinal (GI) injury has been oft-repeated in the past 40 years, but not yet delivered on. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and edema by suppressing the formation of prostaglandins, by inhibiting the activity of the enzyme cyclooxygenase (COX-1) and (COX-2). However, prostaglandins are key mediators of several components of GI mucosal defense, so suppression of synthesis of prostaglandins (PGs) by NSAIDs greatly reduces the resistance of the mucosa to injury as well as interfering with repair processes. Selective COX-2 inhibitors were thought to be the solution to this conundrum, it is required that NSAIDs suppress prostaglandin synthesis at sites of inflammation and not in the GI tract. However, it is now clear that both COX-1 and COX-2 isoforms contribute to mucosal defense. Selective COX-2 inhibitors elicit less GI damage and bleeding than conventional NSAIDs, although the magnitude of this reduction
continues to be contested in the literature. As widely reported in the lay-press, the selective COX-2 inhibitors also cause significant adverse effects in the renal and cardiovascular systems, possibly more serious than those caused by conventional NSAIDs.

The global potential for NSAIDs, including the selective COX-2 inhibitors, exceeds $8 billion per year. The market for NSAIDs is expanding rapidly because of an aging population in developed countries and the associated increase in the prevalence of diseases like arthritis. Use of Aspirin is also increasing because of its utility in reducing the incidence of a number of common disorders including stroke, myocardial infarction, Alzheimer's disease and cancer. In the recent years, several novel approaches for reducing the GI toxicity of NSAIDs with promising results have been reported. These mainly involve structural modification of existing NSAIDs such that inhibition of COX is maintained, but other attributes are added that diminish GI (and other) toxicity, and in some cases boost efficacy and/or potency. In 1997, it was estimated that about 5-6% of world population in many regions have Rheumatoid arthritis (RA) while Osteoarthritis (OA) was claimed to affect about 10% of the world's population of which 50% were the elderly population

COX-2 inhibitors are implicated to possess a broad therapeutic spectrum besides anti-inflammatory, analgesic and to lesser extent antipyretic activities. For example inhibition of COX-2 can prevent growth of certain types of cancer, especially colon cancer. The expression of COX-2 in brain, kidney and bone marrow has made it an attractive therapeutic target for designing selective drugs for Alzheimer's disease, cancer etc. The efficacy of these drugs is proven to be better than that of traditional NSAIDs, with no or little side effects associated with traditional NSAIDs.

Inflammation- Since the ancient times, the mankind was under misery and suffering as his life was associated with many disorders and diseases like malaria, tuberculosis, arthritis, cancer, AIDS etc. Inflammation (an important symptom of arthritis) is one of the oldest diseases in medicinal literature. The history of arthritis highlights human struggle against a disease that dates from antiquity and is the
story of failures and successes of disaster and hope. Any living being has inevitably experienced certain unpleasant events at certain times in life. Inflammation may be considered to be one such unpleasant, at least at times, an unavoidable phenomenon.

Inflammatory diseases affect millions of people across the world leading to sufferings like economic loss and premature death as well as inflammatory lung diseases such as asthma, chronic obstructive pulmonary disorder (COPD) and other diseases include allergic rhinitis, rheumatoid arthritis, osteoarthritis, inflammatory bowel disease and psoriasis.

Billions of dollars are being spent by pharmaceutical and biotechnology companies to identify and develop innovative therapeutics to treat such diseases. Over the last few years despite intensive global research, cures for pain and inflammation with no toxicity have still not been found. Keeping in view the potential for potent and safer anti-inflammatory agents and in continuation of our efforts in search of bioactive molecules, it was thought of interest to design the novel New Chemical Entities (NCEs) containing heterocycle like substituted 1, 5 diaryl pyrazole and substituted 4,5-diaryl imidazole moieties.

What is inflammation?

Inflammation is part of the body’s natural defense system. It is a process whereby the body’s cells and natural chemicals protect us from physical damage and infection from foreign substances such as bacteria and viruses. White blood cells or leukocytes are the body’s major infection-fighting cells. The primary objective of inflammation is to isolate, localize and eradicate foreign substances and repair damaged tissues.

Types of Inflammation - Acute and Chronic

Acute inflammation is short-lasting, from a few minutes to a few days, and it may be caused by physical damage, heat, foreign substances, micro-organisms or other agents.

Chronic inflammation is an inflammatory response lasting for several weeks,
months or even years. It may arise as a result of acute inflammation if not treated at right time and often the two co-exist.

**Cardinal signs of Inflammation**

The four cardinal signs of acute inflammation were described in 1st Century AD by the Roman medical writer Aulus Cornelius Celsus.

1. **Redness (rubor):**
   An acutely inflamed tissue appears red, for example skin affected by sunburn, cellulitis due to bacterial infection or acute conjunctivitis. This is due to dilation of small blood vessels within and around the damaged area.

2. **Heat (calor):**
   Increase in temperature is seen only in peripheral parts of the body, such as the skin. It is due to increased blood flow (hyperemia) through the region, resulting in vascular dilation and increased blood flow at the site of inflammation.

3. **Edema/Swelling (tumor):**
   Swelling results from edema, the accumulation of fluid in the extra cellular space as part of the fluid exudate, and to a much lesser extent, from the physical mass of the inflammatory cells migrating into the affected area.

4. **Pain (dolor):**
   For the patient, pain is one of the best known features of acute inflammation. It results partly from the stretching and distortion of tissues due to inflammatory edema in general and due to pus under pressure in an abscess cavity in particular.

5. **Amendment to Celsus's Cardinal Sign of Inflammation- Loss of Function (functio-laesa):**
   Loss of function, a well-known consequence of inflammation was added by Virchow (1821-1902) to the list of features drawn up by Celsus. Movement of an inflamed area is consciously and reflexly inhibited by pain, while severe swelling may physically immobilise the tissues.

**Systemic effects of acute inflammation**

`Design of New Chemical Entities as Potential Anti-Inflammatory Agents Using QSAR Approach.`
Apart from the local features of acute and chronic inflammation described above, an inflammatory focus produces systemic effects, these include.

a) **Pyrexia:** Polymorphs and macrophages produce compounds known as endogenous pyrogens which act on the hypothalamus to set the thermoregulatory mechanisms at a higher temperature. Release of endogenous pyrogens is stimulated by phagocytosis, endotoxins and immune complexes.

b) **Constitutional symptoms:** Constitutional symptoms include malaise, anorexia and nausea. Weight loss is common when there is an extensive chronic inflammation.

**Hematological changes**

a) **Increased erythrocyte sedimentation rate (ESR):** An increased erythrocyte sedimentation rate is a non-specific finding in many types of inflammation.

b) **Leukocytosis:** A Condition called Neutrophilia occurs in pyogenic infections and tissue destruction; eosinophilia in allergic disorders and parasitic infection; lymphocytosis in chronic infection (e.g. tuberculosis).

c) **Anaemia:** This may result from blood-loss in the inflammatory exudate (e.g. in ulcerative colitis), haemolysis (due to bacterial toxins), and the anaemia of chronic disorders' due to toxic depression of the bone marrow.

d) **Amyloidosis:** Long standing chronic inflammation (for example, in rheumatoid arthritis, tuberculosis and bronchiectasis), by elevating serum amyloid A protein (SAA), may cause amyloid to be deposited in various tissues resulting in secondary (reactive) amyloidosis.

**Role of Neutrophils in the cellular exudate**

Neutrophils have a life-span of only 13 days and must be constantly replaced. Most die locally, but some leave the site via the lymphatic circulation. Blood monocytes also arrive at the site and on leaving the blood vessels transform into macrophages becoming metabolically more active, motile and phagocytic.

Both neutrophils and macrophages may discharge their lysosomal enzymes into the extra cellular fluid by exocytosis, or the entire cell contents may be released.
when the cells die. Release of these enzymes assist in the digestion of the inflammatory exudate.

**Chemical Mediators of Acute Inflammation**

The spread of the acute inflammatory response following injury to a small area of tissue suggests that chemical substances are released from injured tissues, spreading outwards into uninjured areas. These chemicals, called endogenous chemical mediators cause vasodilatation, emigration of neutrophils, chemotaxis and increased vascular permeability.

**Various Chemical mediators released from cells:**

1. **Histamine:** - This is the best-known chemical mediator in acute inflammation.

2. **Lysosomal compounds:** - These are released from neutrophils and include cationic proteins, which may increase vascular permeability, and neutral proteases, which may activate complement system.

3. **Prostaglandins (PGs):** - PGs are one of the major mediators of inflammation. These are a group of long-chain fatty acids containing five membered cyclopentane ring and some unsaturations of oxidative functional groups like hydroxyl and/or keto, groups derived from arachidonic acid and synthesized by many cell types. Some prostaglandins potentiate the increase in vascular permeability caused by other compounds.

4. **Leukotrienes:** - These are also synthesized from arachidonic acid, especially in neutrophils, and appear to have vasoactive properties. SRS-A (Slow Reacting Substance of Anaphylaxis), involved in type I hypersensitivity, is a mixture of leukotrienes.

5. **5-hydroxytryptamine (Serotonin):** - It is present in high concentration in mast cells and platelets. It is a potent vasoconstrictor.

6. **Lymphokines:** - It is a family of chemical messengers released by lymphocytes. Apart from their major role in type IV hypersensitivity, lymphokines may also have vasoactive or chemotactic properties.

**Adverse Effects of NSAIDs**

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*Design of New Chemical Entities as Potential Anti-Inflammatory Agents Using QSAR Approach.*
The relationship between the risk of serious GI side effects and the use of nonselective NSAIDs is well-established. Side effects of NSAIDs vary from person to person. Common side effects to all NSAIDs are abdominal pain, diarrhoea, nausea, and fluid retention. These side effects are natural, expected and unavoidable selective COX-2 inhibitors such as Celecoxib are thought to reduce the risk of GI erosion and bleeding. NSAIDs have also been linked to adverse renal effects, again because of the antiprostaglandin effects.

Recent studies have established an increased risk of cardiovascular toxicity (stroke/ transient ischemic attack (TIAs), myocardial infarction, and symptomatic peripheral vascular diseases) from several COX-2 inhibitors and Rofecoxib was removed from the US market by its manufacturer and FDA requested the removal of Valdecoxib too. All NSAIDs elevate systolic blood pressure (median 5 mm Hg), which may be the probable cause of observed cardiovascular toxicity. Other side effects associated with the NSAIDs include peripheral edema, rashes, NSAID hypersensitivity and bronchospasm in patients with asthma and nasal polyps, blood dyscrasias.

Most prominent serious side effects are gastrointestinal ulcers and bleeding and less frequently, kidney and liver damage. New understanding of how NSAIDs work, has finally shown why certain nonsteroidal anti-inflammatory drugs have more serious side effects than others and has stimulated development of new NSAIDs that will greatly reduce the potential of developing dangerous side effects.

1.2- Cyclooxygenase-2 : An Overview 

This enzyme actually has two different active sites, collectively termed prostaglandin synthase. On the opposite side, it has an entirely separate peroxidase site, which is needed to activate the heme groups that participate in the cyclooxygenase reaction. The enzyme complex is a dimer of identical subunits; so altogether, there are two cyclooxygenase active sites and two peroxidase active sites in close proximity. Each subunit has a small carbon-rich knob pointing downward. These knobs anchor the complex to the membrane of the endoplasmic reticulum. The cyclooxygenase active site is buried deep within...
Figure 1.2.1: NSAID binding sites of COX-1 and COX-2.

Figure 1.2.2: Celecoxib, being COX-2 selective unable to fit in to hydrophilic side pocket of COX-1 Enzyme.
the polar sulphonamide side chain tightly bind to hydrophilic “side pocket”

Figure 1.2.3: Celecoxib, being COX-2 selective fits perfectly in to hydrophilic side pocket of COX-2 Enzyme,

the protein and is reachable by a tunnel that opens out in the middle of the knob. The comparison of the binding pocket of COX-1 and COX-2 is shown in (Fig. 1.2.1). This acts like a funnel, guiding arachidonic acid out of the membrane and into the enzyme for processing. In the structure shown here, a drug Celecoxib is unable to fit in to the hydrophilic pocket of COX-1 (Fig. 1.2.2) and fits properly in the hydrophilic pocket of COX-2 (Fig. 1.2.3) blocking the active site in both subunits. The heme groups are also shown above the drug in each subunit.

Cyclo-oxygenase-2 and its Physiological Role\textsuperscript{17-20}: Cyclooxygenases are critical enzymes in the biosynthetic pathways of many bioactive compounds originating from arachidonic acid, including prostaglandins, thromboxanes, and prostacyclins.

Together with the Lipooxygenase, cyclooxygenase (COX) enzymes play a key role in inflammation, pain and other biologic processes. COX exists in three isoforms COX-1, COX-2 and recently reported, COX-3. Specifically targeting these enzymes has been a major goal of potent NSAID design for the past 2
decades. The discovery of two separate COX isoforms, COX-1 and COX-2, led to the hypothesis that the therapeutic and conversely, adverse effects of NSAIDs lay in the specific distribution and function of each isoenzyme. Inhibition of COX-1, the enzyme involved in the synthesis of prostaglandins responsible for integrity of the gastrointestinal (GI) mucosa, would lead to GI damage, while COX-2-selective inhibition should specifically alleviate inflammation.

The most important role of COX-2 in inflammation is that it is involved in producing prostaglandins, a major mediator of inflammatory response. COX-1 is stimulated constitutively while COX-2 is stimulated only as a part of an immune response.

Other modes of actions include:
1. Antioxidant and free radical scavenging properties.
2. Immunosuppressive action.
3. Inhibition of neutrophil activation and function.

**Role of COX-3 isoform**

Simmons and co-authors discovered COX-3 in 2002 and analyzed this new isozyme's relation to Acetaminophen (Paracetamol), arguably the most widely used analgesic drug in the world. The authors postulated that inhibition of COX-3 could represent a primary central mechanism by which these drugs decrease pain and possibly fever. The clinical ramifications and knowledge of COX isozymes are therefore rapidly expanding and could perhaps offer significant hope for future treatments of pain, inflammation and fever.

**PROPERTIES OF COX-1 AND COX-2 ENZYMES:**

Recent studies have documented that there are actually two cyclooxygenase isoenzymes (COX-1 and COX-2) that share 62% homology at the message and protein level. Despite the close relationship of the cDNAs for COX-1 and COX-2, the mRNA for these two isozymes differ greatly with respect to size, 2.8-3.0 Kb and 4.0 Kb for COX-1 and COX-2 respectively. Unlike COX-1, mRNA
transcript for COX-2 enzyme is quickly degradative and the promoter region contains many transcriptional factors which can be upregulated by proinflammatory cytokines. COX-1, a constitutive enzyme located in most tissues, for example, the platelets, endothelium, stomach, kidney, smooth muscles and lumen of endoplasmic reticulum and performs a housekeeping function to synthesize PGs with normal cell regulatory activity.

A comparison of cyclooxygenase activities is summarized in Table 1.2.1

Table 1.2.1 - Physiological Roles of COX-isoforms

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Expression</th>
<th>Function</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX 1</td>
<td>Constitutively</td>
<td>Organ pain, platelet function, stomach protection</td>
<td>NSAIDs including Aspirin</td>
</tr>
<tr>
<td>COX 2</td>
<td>Induced by growth factors, neurotransmitters, inflammatory cytokines, oxidative stress, injury. Constitutively in brain, kidney</td>
<td>Inducible COX2 inflammation, pain, fever. Constitutive COX2 synaptic plasticity</td>
<td>NSAIDs, COX-2 inhibitors including Celecoxib which has few GI problems associated with its use</td>
</tr>
<tr>
<td>COX 3</td>
<td>Constitutively, high in brain, heart</td>
<td>pain pathways, not inflammation pathways</td>
<td>Acetaminophen (no GI problems, good antipyretic, fever reducer), some NSAIDs</td>
</tr>
</tbody>
</table>

COX-1 is a membrane bound haemo and glycoprotein with a molecular weight of 71 KDa with 599 amino acid residues. The protein contains both the cyclooxygenase and endoperoxidase activities required to form PGG₂ and PGH₂ respectively. COX-2 is an immediate early gene product, with a molecular weight
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of 70 KDa with 604 amino acid residues. It is expressed, as inducible form, considerably after exposure to inflammatory mediators like fibroblasts, cytokines etc. Levels of COX-2 protein increase in parallel with over production of prostaglandins in many cells and tissues in chronic inflammation\textsuperscript{25}.

COX-1 is the only isoform in the normal gastric mucosa and platelets and is responsible primarily for the biosynthesis of eicosanoids involved in gastrointestinal mucosal cytoprotection and the maintenance of platelet function.

COX-2, on the other hand, is involved in many physiologic responses, but mainly in the amplification of inflammation and pain. Since COX-2 over expression is observed in diseases like Alzheimer's and colorectal cancer, selective COX-2 inhibitors may have useful therapeutic benefits in such conditions\textsuperscript{26}.

Structure of COX protein consists of three different domains, the N-terminal epidermal growth factor domain, a membrane binding motif and a C-terminal catalytic domain that contains the COX and peroxidase active sites. Both isoforms consists of a cassette of 17 amino acids and 18 amino acids sequence near the N-terminal of COX-1 and COX-2 respectively.

**STRUCTURE OF COX-1 AND COX-2 ENZYMES:-**

X-ray crystallography of 3D structures of COX-1 and COX-2 enzymes as well as complexes with NSAIDs has provided insight onto the mechanism of action\textsuperscript{27}. COX-1 and COX-2 are very similar enzymes consisting of a long narrow channel with a hairpin bend at the end and both are membrane associated. Arachidonic acid released from damaged membranes adjacent to the opening of the enzyme channel, mostly hydrophobic, is sucked in and twisted around the hairpin bend and subjected to chemical reactions, resulting in the formation of the cyclopentane ring of PGs. Supporting evidence is strongest from the work on COX-2 selective inhibitors; mutation of Isoleucine 523 to Valine in the COX-1 protein allows COX-2 selective inhibitors to bind and inhibit PGH\textsubscript{2} formation without altering the \( k_m \) for arachidonic acid. The reverse mutant of COX-2 in which Valine 523 is exchanged for Isoleucine in the active site of COX-2 as against the bulkier Isoleucine in COX-1 gave better access to the inhibitor in case of former.\textsuperscript{28}
PHYSIOLOGICAL AND PATHOLOGICAL FUNCTIONS OF COX-1 AND COX-2:

1. The Stomach:

   In most species, including humans, cytoprotective PGs in the stomach are synthesized by COX-1, although small quantities of COX-2 are also expressed constitutively. It has always been assumed that the cytoprotective role of PGs (e.g. prostacyclin; PGI2) in the stomach is largely due to their vasodilating properties, enhancing mucosal blood flow.

2. The Kidney:

   Maintenance of normal kidney function is dependent of PGs both in animal models of disease states and in patients with congestive heart failure, liver cirrhosis, or renal insufficiency.

3. Genitourinary system:

   Raj S. Pruthi et al have reported the role of COX-2 activity and the potential clinical usefulness of COX-2 specific inhibitors to urological oncology and discussed the outcomes of the molecular mechanisms and clinical effects of COX-2 function and suggested that COX-2 specific inhibitors may serve as antitumor drugs with therapeutic and chemo preventive roles for urological cancers.

4. Gastrointestinal tract:

   Cyclooxygenase enzyme is reported to be involved in the reduction of gastric acid production, stimulate gastric fluid secretion, increase secretion of viscous mucosa and exert a direct vasodilator action on gastric mucosa.

5. Central Nervous System:

   Cyclooxygenase-2 and Alzheimer’s Disease:

   Cyclooxygenase-2 enzyme is reported to be involved in various nervous system disorders which include Alzheimer’s disease.

6. Blood Vessel’s:

   Cyclooxygenase enzymes are also expressed in blood vessels. For e.g. Protein expression of angiogenic factor during gastric ulcer healing.
7. Smooth Muscles\textsuperscript{36}: Enhanced expression of cyclooxygenase-2 enzyme is thought to be involved in the relaxation of smooth muscles of bronchial tissue.

8. Cyclooxygenase-2 and Cancer\textsuperscript{37}: Cox-2 have been detected in gastric and breast tumors.

- Cyclooxygenase-2: As a target for cancer chemoprevention\textsuperscript{38}: COX-2 inhibitors are considered as attractive candidates for the chemoprevention.

- The role of cyclooxygenase-2 (COX-2) in breast cancer\textsuperscript{39}: COX-2 enzymes are also expressed in the breast cancer; that is they are involved in the pathogenesis of breast cancer.

- Cyclooxygenase-2 Selective Inhibitors For Prostate Cancer Chemoprevention\textsuperscript{40}: COX-2 inhibitors are considered as attractive candidates for the chemoprevention.

- COX-2 represents potential targets for the prevention/treatment of colorectal cancer\textsuperscript{41}: COX-2 inhibitors also play a promising role in the prevention of colorectal cancer.

- Cyclooxygenase and colon cancer\textsuperscript{42}: It is also reported that COX-2 expression in intestinal epithelial cells increases resistance to apoptosis, promotes tumor angiogenesis, and enhances invasion and metastasis.

- Cyclooxygenase 2: A pharmacological target for the prevention of cancer\textsuperscript{43}: Cyclooxygenase 2 (COX-2) an inducible form of the enzyme, is a potential pharmacological target to prevent cancer.

Cox-2 inhibitors (Coxibs) and Cardiovascular Actions\textsuperscript{44-46}: COX-2 inhibitors lack anti-platelet activity, coxibs are suited for the provision of cardiovascular prophylaxis and in patients at risk of myocardial infarction.

Cyclooxygenase inhibition and thrombogenicity\textsuperscript{47}: COX-2 inhibitor-treated patients with diseases that predispose to thrombosis should be monitored carefully for such type of complications.

9. Endothelial Cells\textsuperscript{48}: 
Cyclooxygenase-2 has been demonstrated in human umbilical vein endothelial cells after induction by IL-1α and phorbol ester. During inflammation, the increased permeability of the vascular endothelium is caused by retraction of endothelial cells leading to exudation and migration of phagocytic cells.

10. **Gestation and Parturition** - Both COX-1 and COX-2 are expressed in the uterine epithelium at different times and may be involved in implantation of the ovum and angiogenic processes of placenta formation.

11. **Synovial tissues**: Both COX isoforms are present in synovial tissues in patients with rheumatoid arthritis and osteoarthritis, up regulation of COX-2 by IL-1 has been reported in human chondrocytes and osteoblasts.

12. **Lungs**: Cyclooxygenase-2 may be induced in the lungs either locally in pulmonary structures (airway epithelium, airway smooth muscle, lung macrophages and activated leucocytes) after airway damage or as part of a systemic response to cytokines. Endogenous PGE₂ may have a bronchoprotective function such as modulation of airway and vascular tone, inflammatory cell activity and recruitment, cytokine release, mucus secretion, and cholinergic and sensory nerve function.

13. **Vascular smooth muscle**: Induction of COX-2 has been demonstrated in vitro in arterial smooth muscle cells treated with platelet-derived growth factor.

14. **Action of COX-2 on Connective tissue**: Cyclooxygenase-2 are thought to be involved in many stress responses and its activity can produce oxidative damage.

15. **COX-2 In Bone**: Both COX-1 and COX-2 have been thought to be expressed in bone cells.

16. **COX-2 and Pain**: COX-2 enzyme is expressed extensively in the pain disorders.

A. **The Role of Rofecoxib, a Cyclooxygenase-2-Specific Inhibitor, for the Treatment of Non-Cancer Pain**: The selective cyclooxygenase-2 inhibitors produce less gastrointestinal lesions as compared to COX-1 inhibitors.
B. Role of COX-2 Inhibitors in the Evolution of Acute Pain Management:-
COX-2 inhibitors have role in central sensitization, also offers peripheral relieving benefits of NSAIDS;

CONCLUSION

In summary, the identification of COX-2, few years ago has been followed by an unprecedented period of discovery and drug development. An awareness of the existence of two COX isoforms has led to potential novel insights into disease pathogenesis (Arthritis, Alzheimer's disease, Cancer) and the regulation of normal physiology (brain, kidney). The preliminary in-vivo experience with COX-2 selective inhibitors has provided evidence for "proof of concept" for the COX-1/COX-2 hypothesis, namely that the selective inhibition of COX-2 derived prostaglandins is sufficient to inhibit inflammation and is nonulcerogenic. It may well be said that we have moved closer to the "better aspirin" envisioned by Sir John Vane for the treatment of degenerative and inflammatory arthritis.

Cyclooxygenase-2 enzyme has proved to be very useful target for number of therapeutic purposes.

Finally, COX-2 selective agents thus promise to provide significant advantage to patients with chronic diseases such as rheumatoid arthritis and osteoarthritis.

1.3 DEVELOPMENT OF COX-2 INHIBITORS

The discovery of a second cyclooxygenase (COX-2) enzyme, 10 years ago is changing the multimillion-dollar market for non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of Alzheimers disease, Rheumatic and Osteoarthritis, Cancer, Kidney and Osteoporosis.

COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells (monocytes/macrophages) and in central nervous system. These observations suggest that COX-1 and COX-2 serve different physiological and pathological functions. Classical NSAIDs inhibit both COX-1 and COX-2 to varying extent.
The differential tissue distribution of COX-1 and COX-2 provides a rationale for the development of selective COX-2 inhibitors as anti-inflammatory and analgesic agent that lack the GI and hematologic liabilities exhibited by currently marketed NSAIDs. This hypothesis has been validated in animal models and has led to the marketing of two diaryl heterocycles, Celecoxib (Celebrex ®) and Rofecoxib (Vioxx ®) as selective COX-2 inhibitors.

These two drugs, developed by new screening methods using enzyme selectivity, have had spectacular launches in many countries as 'selective' or 'specific' COX-2 inhibitors. Others, such as Meloxicam (Mobic), Etodolac (Lodine), and Nimesulide (Aulin) were developed by classic pharmacological testing before COX-2 was discovered.

J.G. Lombardino®^" reported discovery and synthesis of a series of substituted diaryl imidazoles with anti-inflammatory activity. The most potent agent from this series for a non-steroidal anti-inflammatory was 4,5-Bis(p-methoxyphenyl)-2-(trifluoromethyl) imidazole which was named as Flumizole.

E.H. Wiseman et al®° reported potent anti-inflammatory activity profile of Flumizole and biopharmaceutical developments such as optimization of drug absorption during pharmacological and toxicological investigations.

Peter Gund and T.Y. Shen® studied conformational analysis of anti-inflammatory aryl acetic acids to hypothesize a detailed model for the active site of cyclooxygenase unit of the PG synthetase enzyme. This mode rationalizes the structure-activity relationships of enzyme substrates and inhibitors and appears to be in agreement with biochemical studies of the enzyme.

Cherkofsky, S.C.® reported synthesis of non-acidic non-steroidal anti-inflammatory activity of a series of 4, 5-diaryl-2-(substituted thio)-1H-imidazoles
and their sulfoxides. Tiflamizole of this series was found to represent a second-
generation class with the potential of producing less gastric mucosal damage as a
result of their lack of acidic properties.

Tiflamizole was found to be 8 times as potent as Indomethacin in the rat
adjuvant induced arthritis assay.

\[
\begin{array}{c}
\text{Tiflamizole} \\
\includegraphics[width=0.5\textwidth]{tiflamizole.png}
\end{array}
\]

Atkinson, D.C. et al \(^6^2\) reported synthesis, and anti-inflammatory activity of
a series of substituted (2-phenoxyphenyl) acetic acids. Screening in the adjuvant
arthritis test showed that halogen substitution in the phenoxy ring enhanced
activity with minimum ulcerogeneity, low toxicity and wide therapeutic range.

\[
\begin{array}{c}
n = 0, 1, 2, R = \text{CF}_2\text{CH}_2\text{F}, \text{CH}_2\text{CF}_3 \\
X = Y = 4-\text{F}, 4-\text{OCH}_3, 4-\text{Cl}
\end{array}
\]

Sharpe, T.R. et al.\(^6^3\) have reported preparation and anti-arthritis and
analgesic activity of a series of 4,5-Diaryl-2-(substituted thio)-1H-imidazoles and
their sulfoxides and sulfones.

\[
\begin{array}{c}
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\end{array}
\]

\[
X = Y = 4-\text{F}, 4-\text{OCH}_3, 4-\text{Cl}, n = 0, 1, 2, R = -\text{CH}_2\text{CH}_2\text{F}, -\text{CF}_2\text{CF}_3, -\text{CF}_2\text{CH}_2\text{F}
\]

Design of New Chemical Entities as Potential Anti-Inflammatory Agents Using
QSAR Approach.
Several analogues were found to be more potent than Indomethacin and Phenylbutazone in the rat adjuvant induced arthritis assay.

Wilkerson, W.W. et al., synthesized, studied QSAR and biologically evaluated a series of 2-substituted- and 2,3-disubstituted-4-(4-fluorophenyl)-5-[4-(methylsulfonyl) phenyl]-1H-pyrroles. The most active compounds were the 2-halo derivatives in the order of chloro>bromo>iodo.

\[ R^1 = R^2 = R^3 = \text{H}, \quad R^1 = \text{CH}_3, \quad R^2 = \text{SCN}, \quad R^3 = \text{H}, \quad R^1 = \text{H}, \quad R^2 = \text{COCF}_3, \quad R^3 = \text{H} \]

QSAR studies suggested that the activity could be correlated with the molar refractivity and the inductive field effect of the 2-substituent and the lipophilicity of the 3-substituent.

Talley J.J. et al. patented synthesis of a series of substituted pyrazolyl benzenesulfonamides for the use of inflammation and inflammation related disorders.

\[ R^1 = \text{SO}_2\text{NH}_2, \quad R^2 = \text{CF}_3, \quad R^3 = \text{H}, \quad R^4 = \text{Cl Ph} \]
\[ R^2 = \text{CF}_2\text{H}, \quad R^3 = \text{H}, \quad R^4 = \text{Cyclopentyl} \]
\[ R^2 = \text{CF}_2\text{CF}_3, \quad R^3 = \text{H}, \quad R^4 = \text{4-OCH}_3\text{-2-Napthyl} \]
Penning T.D. et al. have reported the discovery of a series of sulfonamide-containing 1,5-diarylpyrazole and evaluated for their ability to inhibit cyclooxygenase-2 in-vitro and in-vivo.

\[
\begin{align*}
R^1 &= \text{H}, R^2 = \text{CF}_3, R^1 = \text{4}-\text{F}, R^2 = \text{CF}_3, R^1 = \text{4}-\text{CONH}_2, R^2 = \text{CHF}_2, \\
R^1 &= \text{4}-\text{OCH}_3, R^2 = \text{CHF}_2
\end{align*}
\]

Extensive structure activity relationship was studied and number of potent and selective inhibitors of COX-2 were identified. 4-methyl, trifluromethyl derivative SC-58635, Celecoxib was successively passed phase III clinical trials and launched in market for the treatment of rheumatoid arthritis and osteoarthritis.

Khanna, I.K. et al. have reported synthesis of a series of 1,2-diarylimidazoles as potent, cyclooxygenase-2 selective and orally active anti-inflammatory agents.

\[
\begin{align*}
R &= R^1 = \text{CH}_3, R = \text{CH}_3, R^1 = \text{SO}_2\text{CH}_3, R = \text{NH}_2, R^1 = \text{4}-\text{F}, R = \text{NH}_2, R^1 = \text{3,4-F}_2
\end{align*}
\]

Detailed SAR studies on the different portions of the molecule indicate that their potency, selectivity, and in-vivo profile are greatly influenced by the substitution pattern.

Desiraju, G.R., et al. have performed three-dimensional quantitative structure activity relationship (3D-QSAR) studies of some 1, 5-diaryl pyrazoles: Analogue based design of selective cyclooxygenase-2 inhibitors. Three different

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analogue-based rational drug design methods comparative molecular field analysis (CoMFA) with partial least squares (PLS) fit, Molecular Field Analysis (MFA) and Receptor Surface Analysis (RSA) with genetic function algorithms (GFA) have been used in the optimization of COX-2 selective inhibitors design.

Talley, J.J. et al\textsuperscript{69} have reported synthesis of a potent and selective inhibitor of COX-2, 4-[5-methyl-3-phenylisoxazol-4-yl]-benzene sulfonamide, Valdecoxib.

![Valdecoxib](image1)

Valdecoxib is a highly selective and potent inhibitor of COX-2 in human whole blood and against recombinant human enzyme. An active metabolite was also found to be a COX-2 selective inhibitor.

Khanna, I.K. et al\textsuperscript{70} have reported synthesis of a series of heteroaryl modified 1, 2-diaryl imidazoles and found to be potent and highly selective inhibitors of human COX-2.

![SAR study](image2)

Het=3-pyridyl, R=CH\textsubscript{3}, Het=5-CH\textsubscript{3}, 2-thiazolyl, R=NH\textsubscript{2}

SAR studies and anti-inflammatory properties are discussed. 3-pyridyl derivative exhibited excellent activity in acute and chronic models of inflammation.

Slee, D.H. et al\textsuperscript{71} reported development of potent non-carbohydrate imidazole-based small molecule selection inhibitors with anti-inflammatory activity by high-throughput screening using a ELISA-based assay system. The approach is essential for lead optimization and gives more predictive results in terms of
identifying compounds with in-vivo activity.

R=R²=H, R¹=-C₆H₁₃, R=R²=-H, R¹=-C₆H₄C₇H₁₅, R=R¹=-C₆H₁₃, R²=H

Habeeb A.G. et al\textsuperscript{72} reported design and synthesis of Celecoxib and Rofecoxib analogues as selective cyclooxygenase-2 inhibitors in which the respective SO₂NH₂ and SO₂CH₃ hydrogen-bonding pharmacophores were replaced by a dipolar azido bioisosteric substituent.

A molecular modeling (docking) study showed that azido substituent of these two analogues was inserted deep into the secondary pocket of human COX-2 binding site where it undergoes electrostatic interaction with Arg₅₁₃. Both analogues are most potent and selective inhibitors of COX-2.

Chavatte, Philippe et al\textsuperscript{73} reported three-dimensional quantitative structure activity relationships of an extensive series of 305 varied diarylheterocyclic derivatives of cyclooxygenase-2 inhibitors using Comparative Molecular Field Analysis. Five statistically significant models were obtained. CoMFA confirms the great accuracy of compounds of COX-2 enzymes and offers important structural insight into designing novel selective COX-2 inhibitors.

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Introduction

Shrikhande, A.A. et al\textsuperscript{74} patented heterocyclic compounds, diaryl pyrazoles, for their COX-2 inhibition.

\[ \text{All derivatives were found to be COX-2 specific without any undesirable side effects.} \]

Liu, Hong et al\textsuperscript{75} reported inhibitory mode of 1,5-diaryl pyrazole derivatives against cyclooxygenase-2 and cyclooxygenase-1 using molecular docking and 3D QSAR analyses. Structure based investigations and 3D QSAR provided possible guidelines and accurate activity predictions for novel inhibitor design.

Desiraju, G.R. et al\textsuperscript{76} reported computer-aided design of selective COX-2 inhibitors: Comparative Molecular Field Analysis, Comparative Molecular Similarity Indices Analysis and docking studies of some 1,2-diaryl imidazole derivatives.

A 3D QSAR equation was obtained by multiple regression analysis which brings important structural insights to aid the design of selective COX-2 inhibitors.

Sarathy, K.P. et al\textsuperscript{77} reported QSAR study by Fujita-Ban model of some substituted α, β-Diaryl five-membered heterocycles as COX-1/ COX-2 inhibitors. The results are indicative of selective inhibition of COX-2 by diarylimidazoles than diaryloxazolones and diarylpyrazoles.

Md. Jashim Uddin et al\textsuperscript{78} have reported the a group of Celecoxib analogues in which the para-SO\textsubscript{2}NH\textsubscript{2} substituent on the N-phenyl ring was replaced by a para-sulfonylazido (-SO\textsubscript{2}N\textsubscript{3}), or a meta-SO\textsubscript{2}N\textsubscript{3}, substituent were designed for evaluation as selective cyclooxygenase-2 (COX-2) inhibitors. A molecular modeling (docking) study showed that the SO\textsubscript{2}N\textsubscript{3} group inserts deep inside the secondary pocket of the COX-2 binding site. The -SO\textsubscript{2}N\textsubscript{3} moiety undergoes a dual H-bonding interaction via one of its -SO\textsubscript{2} oxygen-atoms, and an electrostatic (ion-ion) interaction via the terminal azido (N\textsubscript{3}) nitrogen-atom, to the guanidino NH\textsubscript{2} of
Arg513 in the secondary pocket of COX-2. These observations indicate that an appropriately positioned \(-\text{SO}_2\text{N}_3\) moiety is a novel alternative bioisostere to the traditional \(-\text{SO}_2\text{NH}_2\) and \(-\text{SO}_2\text{Me}\) pharmacophores present in selective COX-2 inhibitors that are only capable of H-bonding interactions with the COX-2 isozyme.

Susana Sañchez-Fidalgo et al\(^7\) have reported that the effects of Rofecoxib, a selective cyclooxygenase-2 inhibitor, and Ibuprofen, a nonselective cyclooxygenase inhibitor, on the evolution of acetic-acid-induced gastric ulcers in rats, evaluating growth factor expression, the angiogenic process, cell proliferation and cell apoptosis. No changes in VEGF expression were detected.

Results also showed that proliferation and apoptosis were increased in control ulcerated animals. Rofecoxib reduced significantly both processes. These findings demonstrate that a reduction of bFGF expression and an antiangiogenic action, as well as proliferation/apoptosis inhibition, are some of the mechanisms possibly implicated in the delay in ulcer healing seen after the administration of the highly selective COX-2 inhibitor Rofecoxib.

Yasuyoshi Miyata et al\(^8\) reported that expression of cyclooxygenase-2 and Ep\(_4\) receptor in transitional cell carcinoma of the upper urinary tract: prostaglandin E2, produced by cyclooxygenase (COX)-2, affects the behavior of tumor cells possibly through 1 of the prostaglandin E\(_2\) receptors, the EP\(_4\) receptor (EP\(_4\)R). The relationship between tumor development and EP\(_4\)R in transitional cell carcinoma of the upper urinary tract (TCC-UUT) has not been fully understood.

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S. Revathi et al\textsuperscript{81} have reported that the development of dual inhibitors and their role in preventing a drift of arachidonic acid metabolism towards the other pathway, leading to potential side effects.

Subas M. Sakya, et al\textsuperscript{82} have described, Structure–activity relationship (SAR) studies of novel 2-[3-trifluoromethyl-5-alkyl (thio) ether pyrazo-1-yl]-5-methanesulfonyl pyridine derivatives for canine COX enzymes.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Canine COX-2 selective leads}
\end{figure}

Subas M. Sakya\textsuperscript{83} have reported the structure–activity relationship toward canine COX-1 and COX-2 in vitro whole blood activity of 4-hydrogen versus 4-cyano substituted 5-aryl or 5-heteroatom substituted N-phenyl versus N-2-pyridyl sulfone pyrazoles. The differences between the pairs of compounds with the 4-nitrile pyrazole derivatives having substantially improved in vitro activity are also highlighted, for both COX-2 and COX-1 and concluded that this difference in activity may be due to the contribution of the hydrogen bond of the 4-cyano group using molecular modeling studies. In addition, the derived model suggested a potential contribution from hydrogen bonding of the pyridyl nitrogen to Tyr 355 for the increased activity over the phenyl sulfone analogs.
Latifeh Navidpour et al.\textsuperscript{84} have reported 1-aryl-5-(4-methylsulfonylphenyl)imidazoles, possessing C-2 alkylthio (SMe or SET) substituents, as selective cyclooxygenase-2 (COX-2) inhibitors with binding mode at COX-2 active site and in vivo anti-inflammatory activity.

![Structure of 1-aryl-5-(4-methylsulfonylphenyl)imidazoles](image)

**Aims and Objectives:**

The Non Steroidal Anti-inflammatory agents are being used since very long ago for their therapeutic activities for the treatment of various types of inflammatory disorders like osteoarthritis, rheumatoid arthritis involving moderate to severe pain and inflammatory symptoms.

**Need of present Research Work:**

The traditional NSAIDS are suffering from following drawbacks;

Severe Gastrointestinal toxicities, which include;

i) Gastric ulcers, bleeding, erosion of gastric epithelial layer.

ii) Destruction of gastrointestinal epithelial morphology.

Different Approaches used for development of safer and potent anti-inflammatory agents
Currently used NSAIDs.

NSAIDs can be classified as,

1) Acidic NSAIDS.

2) Non-acidic NSAIDS.

1) Acidic NSAIDS

1. Salicylic Acid derivative

\[
\text{Aspirin} \quad \begin{array}{c}
\text{COOH} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{C} - \text{CH}_3
\end{array}
\]

2. Indole Acidic Acid derivatives

\[
\text{Indomethacin} \quad \begin{array}{c}
\text{H}_3\text{CO} \\
\text{N} \\
\text{CH}_3 \\
\text{C} - \text{O} \\
\text{Cl}
\end{array}
\]

3. 3,5-Pyrazolidine-dione derivatives

\[
\text{Phenyl butazone} \quad \begin{array}{c}
\text{N} \\
\text{C} - \text{H}_3
\end{array}
\]

4. N-Aryl Anthranilic Acid derivatives

\[
\text{Mefenamic acid} \quad \begin{array}{c}
\text{NH} \\
\text{C} - \text{H}_3
\end{array}
\]

5. Phenyl Acetic acid derivatives

\[
\text{Diclofenac} \quad \begin{array}{c}
\text{CH}_2\text{COOH} \\
\text{C} - \text{N} \\
\text{Cl} \\
\text{Cl} \\
\text{Cl}
\end{array}
\]

6. Phenyl Propionic Acid derivatives

\[
\text{Ibuprofen} \quad \begin{array}{c}
\text{HOOC} \\
\text{C} - \text{CH}_2\text{CH}_3
\end{array}
\]

Adverse effects of currently used Non acidic NSAIDS:

i) Cardiovascular toxicity;

ii) CNS toxicity;

iii) GI toxicity;

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iv) Renal toxicity;
v) Others.

Major Goals of the present Research Work:-

Taking the findings of a thorough literature survey of NSAIDS right from 1960 to 2004, (recent happenings in the period 2004 to 2007 are also studied thoroughly). We have focused on following major objectives,

i) To optimize the pharmacophore required for selective COX 2 inhibition in order to develop safer and potent analgesic anti-inflammatory agents, which exhibit following characteristic features;

a) The designed NCEs are nonulcerogenic to gastrointestinal tract,
b) The designed NCEs fulfill the pharmacokinetic requirements or behave like a drug molecule,
c) The designed NCEs are potent anti-inflammatory agents.
d) The designed NCEs have structural features required for selective binding with active site of cyclooxygenase-2 (COX-2) enzyme.

Plan of Work:-

In order to implement the goals as discussed in aims and objectives section, the present research work was planned on the following lines.

i) Literature Survey:-

a) Literature survey of development of safer, nontoxic NSAIDS,
b) Literature survey of development of cyclooxygenase-2 enzyme, major focus was on,
   - Various actions catalyzed by COX-2 enzymes,
   - Structural details of COX-2 enzymes,
   - Research work reported for exploration of COX-2 for development of selective COX-2 inhibitors as safer NSAIDS,
   - QSAR studies
Molecular docking/Studies
-Selective COX-2 inhibitory assays
c) Literature survey of diaryl-pyrazole pharmacophore containing compounds as selective COX-2 inhibitors
d) Literature survey of diaryl imidazoles as selective COX-2 inhibitory compounds.
e) Literature Survey of contribution of molecular modeling studies including QSAR for development of selective COX-2 inhibitors.

II] Dry Lab Work (Theoretical Work) included;
a) 2D QSAR studies of two selective series of compounds
b) 3D QSAR studies of two selective series of compounds using
   I) Comparative Molecular Field Analysis (CoMFA) Studies
   II) kNN molecular field Analysis using various methods to develop the best possible 3D QSAR models for both series of compounds
III) Design of New Chemical Entities using results obtained by different 2D and 3D QSAR studies
IV) Molecular Docking studies of the designed, synthesized and pharmacologically tested and nonulcerogenic compounds.

III] Wet Lab Work:-
To verify the findings of dry lab work, the wet lab work was attempted.
a) Design of NCES and then design of synthetic steps/ methods for NCES
b) Structural characterization of synthesized compounds using following physicochemical studies
   I) Physical constant (M.P/ B.P) determination.
   II) Reaction monitoring using Thin Layer Chromatographic (TLC) Studies.
iii) Elemental Analysis-
   Qualitative Elemental Analytical Studies
   Quantitative Elemental Analytical Studies.
iv) FT IR Spectral studies
v) ¹H NMR Spectral Studies
vi) Mass spectral studies.

IV) Pharmacological Screening of synthesized NCES from both 1,5 diaryl pyrazole and 4,5 diaryl imidazole series of compounds,

Following pharmacological screenings were anticipated;
i) Evaluation of Anti-inflammatory activity using Carragenan induced Rat Paw Edema Model.
ii) Evaluation of Analgesic activity using Acetic acid induced Writhing model.
iii) Evaluation of potential for ulcerogenicity of most active/potent, synthesized NCES from both series.
iv) Histopathological studies of stomach specimen subjected to ulcerogenicity studies.
v) Finally to present Summary and Conclusions derived out of research undertaken.
vi) Future Scope.
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Chapter 1

Introduction

