1. INTRODUCTION

Inflammation is a tissue reaction to infection or foreign substance and it is a part of host defence mechanism. A number of different chemical mediators are released at the site of injury from various cell sources including neutrophils, basophils, mast cells, platelets, monocytes, macrophages and lymphocytes. The chemical mediators released include histamine, 5-hydroxytryptamine, plasmin, metabolic products of arachidonic acid cascade, leukotrienes, thromboxanes, platelet activating factor, lymphokines, lysosomal enzymes and components of complementary system. Inflammatory response occurs in three different phases namely: acute transient phase, delayed subacute phase, and chronic proliferative phase. In acute transient phase, local vasodilation and increased capillary permeability occurs, whereas in delayed subacute phase there is infiltration of leukocytes and phagocytes. Tissue degeneration and fibrosis occur in chronic proliferative phase. Each step is mediated by different mechanism. Inflammation leads to damage of cell membrane, which causes leukocytes to release lysosomal enzymes and cause series of events to occur. This process is called arachidonic acid cascade (Figure 1).
Inflammation is mediated by several families of mediators. Among these, the eicosanoids, families of lipid mediators produced through arachidonic acid metabolism, are among the most investigated family. Prostanoids are members of large group of bioactive oxygenated C18-C22 compounds that are derived from ω3 (n-3) and ω6 (n-6) polyunsaturated fatty acids. These include (Figure 1): (A) Prostanoids formed through cyclooxygenase pathway, (B) leucotrienes (LTs),3,4 lipoxins,5 hepoxillins6 and mono hydroxyl fatty acids7 produced via lipooxygenase pathway (C) epoxy and dihydroxy fatty acids produced via Cyt P450,8 and (D) isoprostanes,9,10 isoleukotrienes and other peroxidised fatty acid products that are formed non- enzymatically.11

1.1 CYCLOOXYGENASE PATHWAY

γ-Linolenic acid gets into the human body with food and then converted to arachidonic acid, which is accumulated in cell membrane phospholipids. Arachidonic acid, a 20-carbon tetraenoic fatty acid (C20: 4ω6), which acts as a precursor to the synthesis of PGs was discovered in 1964.12 Ten years later cyclooxygenase reaction through which arachidonic acid is enzymatically cyclised and is oxygenated to yield endoperoxide-containing PGG2 was identified.13,14 Ability of aspirin like drugs to suppress inflammation due to their ability to inhibit the production of PGs had already been discovered till that time.15,16 The COX enzyme (E.C. 1.14.99.1), also known as PGH synthase (PGHS) or prostaglandin endoperoxide synthase was identified as the major enzyme in the oxidative conversion of arachidonic acid to PGG2 and PGH2, with seminal vesicles of sheep, bovines being a rich enzyme source.13,17

COXs are a monotopic, membrane-bound, heme-dependent, bifunctional enzymes that are members of the mammalian heme-dependent peroxidase family which includes MPO and thyroid peroxidase.18 Role of COX activity in the production of PGs has been known since 1967.19 The biosynthesis of prostanoids involves a three step sequence:

(A) Stimulus initiated hydrolysis of arachidonate from glycerol phospholipids involve secretory phospholipase A2 (sPLA2), cytoplasmic phospholipase A2 (cPLA2), or both types of PLA2.20 Arachidonic acid can be released from phospholipids in two ways: (i) One step of PLA2 (Figure 2).
Figure 2: Outline of the structure of phospholipids and site of action of phospholipases-indicating a one-step process of release of arachidonate

(ii) Two steps involve phospholipase C (PLC) and then diacylglycerol lipase (DAG) (Figure 3).

Figure 3: Release of arachidonate from phospholipids by two-step process

B) Heme dependent bis-oxygenase or COX reaction converts arachidonic acid to PGG$_2$, a 9,11-endoperoxide-15-hydroperoxide product, by cyclizing and adding two molecules of O$_2$ to arachidonic acid. Subsequently peroxidase (POX) reaction reduces 15-hydroperoxide of PGG$_2$ to PGH$_2$ (Figure 4).
Figure 4: Cyclisation and peroxidation of arachidonic acid leading to formation of PGH$_2$

PGH$_2$ is a highly unstable endoperoxide, which functions as an intermediate substrate for biosynthesis of most biologically active end products PGD$_2$, PGE$_2$, PGF$_{2\alpha}$, PGI$_2$ and TXA$_2$ via specific isomerases (Figure 5).$^{22-25}$

All the different PGs have a characteristic physiological role to play in various tissues and human body systems, which are summed up in Table 1. PGs have variety of effects on blood vessels, nerve endings and cells involved in inflammation.

The resulting products then exist in the cell via a carrier mediated process$^{26}$ to activate prostanoid G protein-linked prostanoid receptors (GPCRs),$^{27-29}$ or in some cases may interact with nuclear receptors.$^{30}$ Similar to what occurs with PGH$_2$ downstream metabolizing enzyme (i.e. PG synthases and isomerases), prostanoid receptors are also cell/or tissue specific.
Figure 5: Synthesis of various PGs starting from PGH$_2$

There are at least 9 known PG receptors as well as several of their splice variants that belong to the subfamily of G-protein-coupled receptor (GPCR) super family of seven transmembrane spanning protein.$^{31}$ Four of the receptor subtypes bind PGE$_2$ (EP$_1$-EP$_4$), two bind PGD$_2$ (DP$_1$ and DP$_2$) and rest are single receptors for PGF$_{2\alpha}$, PGI$_2$, and TXA$_2$ (FP, IP and TP, respectively).$^{32,33}$ IP, DP, EP$_2$ and EP$_4$ receptors are coupled with the activation of G proteins and linked to increase in intracellular cAMP, whereas
EP₁, FP and TP signal through Gq mediated in intracellular concentration. Exceptionally, the EP₃ receptor is coupled to Gi and decreases cAMP formation. Interestingly, in addition to signaling through G-protein linked receptors, PGs generated from COX-2 located in the nuclear envelope may mediate signals preferentially through a nuclear pathway, whereas COX-1 derived PGs may mediate signals solely through cell surface receptors. This emphasizes the importance of COX-2 and nuclear receptors i.e. peroxisome proliferators-activating receptors (i.e. PPARs) in cell growth and survival.

<table>
<thead>
<tr>
<th>Tissue/organ</th>
<th>Mediators</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female reproductive organs</td>
<td>PGE₂, PGF₂₀</td>
<td>Uterine Contractions, Oxytocic Action</td>
</tr>
<tr>
<td>Male reproductive organs</td>
<td>PGE₂, PGF₂₀</td>
<td>Fertility</td>
</tr>
<tr>
<td></td>
<td>TXA₂, PGI₂</td>
<td>Thrombosis, Platelet aggregation</td>
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<td></td>
<td>TXA₂</td>
<td>Vascular permeability</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>PGE₂, PGI₂</td>
<td>Arterial vasodilation</td>
</tr>
<tr>
<td></td>
<td>TXA₂, PGF₂₀</td>
<td>Venous vasoconstriction</td>
</tr>
<tr>
<td></td>
<td>PGE₂, PGI₂</td>
<td>Potency of the fetal ductus arteriosus</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>PGE₂</td>
<td>Bronchodilation</td>
</tr>
<tr>
<td></td>
<td>PGF₂₀, TXA₂</td>
<td>Bronchoconstriction</td>
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<tr>
<td></td>
<td>PGE₂, PGI₂</td>
<td>Regulation of renal blood flow and glomerular filtration rate</td>
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<td>Renal system</td>
<td>PGE₂, PGI₂</td>
<td>Renin release</td>
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<td>PGE₂</td>
<td>Inhibition of hydroosmotic effects of ADH</td>
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<td>Gastrointestinal system</td>
<td>PGE₂, PGI₂</td>
<td>Cytoprotection</td>
</tr>
<tr>
<td>Immune system</td>
<td>PGE₂, PGI₂</td>
<td>Inhibiton of T and B lymphocyte activation and proliferation</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>PGD₂</td>
<td>Sleep</td>
</tr>
<tr>
<td></td>
<td>PGE₂, PGI₂</td>
<td>Pain</td>
</tr>
</tbody>
</table>

Table 1: Biological effects of various prostaglandins in various tissues and human body systems
1.1.1 COX-1 Vs COX-2: Discovery and Structure

In early 70's different groups reported that low concentration of aspirin and indomethacin inhibited the production of PGs suggesting for the first time, a biochemical mechanism of action for these drugs.\(^{15,16}\) A purified, constitutive COX enzyme (now called COX-1) was first isolated from seminal vesicles in 1973.\(^{34}\) For many years it was thought that COX was a single enzyme present constitutively in most cells. It was believed that inhibiting this enzyme would lead to decreased production of unwanted PGs (e.g. in inflamed tissue) as well as beneficial PGs produced in stomach, kidney, and elsewhere. The suggestion that an inducible COX (now called as COX-2) existed, arose from the studies in the field of inflammation and reproductive biology in rats in late 80's and early 90's. The existence of COX activity inhibited by acetaminophen in dog brain, but not in rabbit spleen was postulated in 1972.\(^{35}\) Similarly two catalytically distinct PG synthase activities were reported to be present in acetone powder extracts of sheep vesicular glands.\(^{18}\) Studies of autoinactivation rates of COX, inhibition of NSAIDs, and time course profiles of PGE\(_2\) and PGF\(_{2\alpha}\) synthesis showed that rabbit and mouse, but not rat brain, contained two forms of COX.\(^{36,37}\)

It was however, through the study of PG induction by mitogens and proinflammatory agents, as well as PG down-regulation by glucocorticoids, that the most proactive data regarding the potential of more than one COX were obtained.\(^{38}\) In 1985, it was observed that platelet-derived growth factor treatment of Swiss 3T3 cells resulted in an early (10 min.) and late (2-4 hr) peak induction of PG synthesis. Only the late peak was blocked by cycloheximide, leading to the conclusion that platelet-derived growth factor-stimulated PG synthesis occurred by "direct effects on the PG-synthesizing enzyme system, one involving a protein synthesis-independent mechanism and another that requires rapid translation of COX." These activities were indicative of an endogenous COX enzyme (COX-1) and an inducible enzyme (COX-2).\(^{39,40}\) In 1989, using a low-stringency northern blot hybridisation with an ovine seminal vesicle COX cDNA as a probe, a 4.0-kb mRNA was detected, in addition to known 2.8-kb mRNA encoding seminal vesicle COX.\(^{41}\) This 4.0-kb mRNA was inducible and parallel induction of enzyme activity. Thus, it was concluded that, "the larger mRNA may encode for a COX" encoded by a specific gene. In 1990, while studying lipopolysaccharide
(LPS) stimulation of monocytes, it was concluded that these cells may contain two pool of COX, each with a differential sensitivity to LPS or dexamethasone (DMS). During this time, using giant two-dimensional protein gel electrophoresis, proteins immunoreactive with COX-1 antibodies that were induced in v-src transformed cells, were detected. The evidence in these and other early studies was consistent with distinct inducible and constitutive COX isozymes encoded by separate genes but was also compatible with other explanations.

The answer to the mechanism of how COX enzyme activity rapidly increases PG induction in inflammation and other physiological contexts came from studies of cell division. In the late 1980s, different groups independently identified immediate-early genes in fibroblast-like cells activated by mitogens. Genes found in chicken and mice were activated by v-src oncogene, phorbol esters and serum. Swiss 3T3 cells were used to identify tetradecanoyl-13-phorbol acetate-inducible sequences (or IS gene), which were also induce by the mitogens. In 1991, each laboratory independently reported that one of their sequences encoded a new inducible COX enzyme. Also contributing to the identification of COX-2 in 1991, a partial predicted sequence of COX-2 from a murine cDNA was reported. The inducible enzyme cloned in these studies is now most frequently referred as COX-2 and the seminal vesicle form of the enzyme as COX-1.

Pure preparations of COX-1 enzyme, obtained from seminal vesicles were instrumental in elucidating the primary structure of this enzyme by molecular cloning. The N-terminus and the internal sequences following limited trypsin digestion of the sheep seminal enzyme were reported in 1987. It was shown in mid 70s that in sheep and bovine seminal vesicle enzyme preparations; aspirin acetylated COX enzyme. The new region of the active site residues and the determination of serine acetylated by aspirin were elucidated by sequencing 3H-aspirin labeled peptides of the sheep seminal vesicle enzyme. Finally the primary structure of COX-1 enzyme was elucidated by molecular cloning in late 80s. The tertiary and quaternary structures of COX-1 were elucidated in 1994.

The predicted amino acid sequences of COX-2 cloned in chicken and mammals showed it to possess approximately 60% amino acid identity with COX-1.
Crystallographic structure of COX-2 was elucidated during mid 90s showing striking similarity with COX-1. Structures of COX-1, COX-2, COX-3 and PICOX-1a genes are shown in Figure 6.

Outstanding reviews regarding structure, biology (cellular and molecular) and enzyme kinetics of cyclooxygenases have been written in the past decade. COX-1 and COX-2 are located in the lumen of nuclear envelop and endoplasmic reticulum and contains the following domains (Figure 7):

(A) **Amino acid terminal peptide**. Nascent COX-1 and COX-2 polypeptide are directed into lumen of the endoplasmic reticulum by amino-terminal signal peptides. These are hydrophobic in nature.
Figure 7: Crystallographic structures of ovine COX-1 (left, taken from Protein Data Bank file 1PRH) and murine COX-2 (right, taken from file COX 5 of Protein Data Bank) homodimers. Functional domains: Membrane binding domain (yellow); (B) Dimerization domain (green); (C) Catalytic domain (blue); and, (D) Heme (red). The open cleft of the peroxidase active site is observable at the top of each monomer.

(B) Dimerization domain- COX-1 and COX-2 dimers are held together via hydrophobic interactions, hydrogen bonding, and salt bridges between the dimerization domains of each monomer, immediately following amino terminal signal protein.

(C) Membrane binding domain- This forms the mouth of a narrow hydrophobic channel that is the cyclooxygenase active site. This has four amphipathic helices that allow COX dimers to float on the surface of lumen of endoplasmic reticulum/nuclear envelope.

(D) Catalytic domain- It is carboxy terminal to the membrane binding domain in COX primary structures, and comprises 80% (approximately 480) amino acids of the protein and contains two distinct enzymatic sites:

i. Peroxidase active site
ii. Cyclooxygenase active site

COX-1 and COX-2 are 63% identical and 77% similar at the amino acid level. But these have following main differences in their structure, origin and kinetics:

- COX-1 gene is 25 kb in size, is located on human chromosome 9q32-q33.3, contains 11 exons, and produces a 2.8-kb mRNA and a ~68 kDa protein containing 599 amino acids. COX-2 gene is an 8 kb in size, is located on human chromosome 1q25.2-q25.3, contains 10 exons, produces a 4.1-4.5 kb mRNA, and encodes a ~68 kDa protein containing 604 amino acid protein.
• Cyclooxygenase active site is preponderately hydrophobic in nature with two internal hydrophilic pockets I and II (Figure 8), both of which have valine in COX-2 and an isoleucine in COX-1 (positions 89, 434 and 523) at the opening of the pocket leading to the constriction of the side pocket of COX-1, making COX-2 active site larger and more accommodating in COX-2.72

• The difference in active site size and shape is due to three amino acid differences between COX-1 and COX-2: Ile 523 to Val 523 in the first shell of the active site, and Ile 434 to Val 434 and His 513 to Arg 513 in the surrounding second shell. The other major structural difference is in the position of helix D, the last of the four helices of the membrane binding domain, and as a consequence, Arg 120 is displaced.58,73 All these substitutions lead to larger and more flexible substrate channel in COX-2, and also making substrate binding site in COX-2 approximately 25% larger than COX-1 (394 Å Vs. 316 Å). Smaller size of valine at 523 of COX-2 allows the inhibitor access to a side pocket of the main substrate channel in COX-2, access that is denied sterically by the longer side chain of isoleucine in COX-1.

• There are 18 amino acids inserted 6 residues from C-terminus of COX-2 that are not present in COX-1. COX-1 is 17-amino acid longer in the N-terminal region than COX-2.59,60

• There are 8 amino acids, immediately following amino terminal signal peptide domain in COX-1. These are not found in COX-2, which might be possible for variants of COX-1 (COX-3 identified).74

• COX-2 gene appears to be relatively more concentrated within nuclear envelop and appears to be a primary response gene, playing proinflammatory role after induction of mitogens. COX-1 on the other hand is found to be attached only to the membranes of ER and exhibits the feature of a housekeeping gene.75-77

• COX-2 accept and oxygenates a wider range of fatty acids more efficiently as substrates than COX-1, such as eicosapentaenoic acid, γ-linolenic acid, α-linolenic acid and linolenic acid.59
Figure 8: Stereoview of environments at binding centres of (A) COX-1, and (B) COX-2.

- COX-2 acetylated by aspirin on Ser 530 still oxidises arachidonic acid but to 5S-hydroxy-6,8-trans-11,14-cis-eicosatetraenoic acid (15-HETE), whereas similarly acetylated COX-1 will not. 78–80

- COX-1 and COX-2 are labeled as to play physiological and pathophysiological roles, respectively, as most of the stimuli known to induce COX-2 are those associated with inflammation e.g. bacterial LPS, cytokines such as interleukin IL-1, IL-2 and TNFα. 81

1.1.2 COX-3 and other splice variants of cyclooxygenase:

The dual model of COX-1 and COX-2 can explain many issues concerning differences between non-selective NSAIDs and highly selective COX-2 inhibitors, but can not fully explain pharmacological actions of acetaminophen. Many actions of this drug resemble to that of COX-2 inhibitors (analgesic effects, antipyretic effects and a relative lack of GI toxicity). 82,83 However, it lacks antiinflammatory action, an important characteristic of both non-selective NSAIDs and COX-2 selective NSAIDs. 84,85 Moreover, in more than 50 years of clinical experience, this drug has shown no
appreciable anti-aggregatory or pro-aggregatory effects on platelets.\textsuperscript{69,82,84,86} For the first time in 1999, it was postulated that a third, acetaminophen sensitive isozyme of COX could be induced in a murine macrophage cell lines subjected to chronic COX inhibition with several NSAIDs. In 1999, a COX variant COX-2b (or COX-2\textsubscript{Vi}) was discovered although it was suggested that it might be considered a third COX (thought to be a splice variant of COX-2). Subsequently, the existence of an additional COX isozyme was suggested by others.\textsuperscript{83,87} In 2002, isolation of a splice-variant of COX-1 mRNA was reported to be present in high concentrations in the cerebral cortex and heart of dog, which was named as COX-3 (or COX-1\textsubscript{Vi}). It was later demonstrated that in transfected insects, canine COX-3 protein was expressed and selectively, but weakly inhibited by acetaminophen, whereas transfected murine COX-1 or COX-2 was not acetaminophen sensitive. In addition, other analgesic/antipyretic drugs that lacked significant antiinflammatory activity such as phenacetin and dipyrope, and classical NSAIDs with potent antiinflammatory activity also displaced more potent inhibition of COX-3 than other COX isoforms (Table 2).\textsuperscript{74}

<table>
<thead>
<tr>
<th>DRUG</th>
<th>COX-1</th>
<th>COX-2</th>
<th>COX-3</th>
</tr>
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<tbody>
<tr>
<td>Acetaminophen</td>
<td>&gt; 1000</td>
<td>&gt; 1000</td>
<td>460</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>&gt; 1000</td>
<td>&gt; 1000</td>
<td>688</td>
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<td>Antipyrine</td>
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<td>&gt; 1000</td>
<td>863</td>
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<td>Phenacetin</td>
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<td>&gt; 1000</td>
<td>102</td>
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<td>Dipyrope</td>
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<td>&gt; 1000</td>
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</tr>
<tr>
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<td>0.041</td>
<td>0.008</td>
</tr>
<tr>
<td>Caffeine (-ve control)</td>
<td>&gt; 1000</td>
<td>&gt; 1000</td>
<td>&gt; 1000</td>
</tr>
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</table>

Table 2: Dosages (\textmu g) of various analgesic/antipyretic drugs (IC\textsubscript{50} values) needed to reduce the activity of various COX isoforms by 50%.
**INTRODUCTION**

The messenger RNA that produced the COX-3 protein was derived from the same gene that coded for COX-1, except in COX-3 RNA, an intron made up of 90 nucleotides at or near the 5' end of the molecule was retained. The retention of this intron (which is normally cleaved prior to the final synthesis of the RNA) introduces the insertion of an additional 30 amino acids into the dog COX-3 molecule. It was then postulated that these extra amino acids would alter the folding and subsequent enzymatic properties of this newly discovered COX type (Figure 9).

![Crystallographic structures of proposed COX-3.](image)

Two other splice variants termed partial COX-1a (PCOX-1a) and COX-1b (PCOX-1b), with amino acids 119-337 deleted were also isolated. A 65 kDa protein in human aorta postulated to be COX-3 (COX-1V1) and ~53 and ~50 kDa protein postulated to be COX-1a (PCOX-1a) and COX-1b (PCOX-1b) at ~25%, respectively have been reported. PCOX-1a contains intron 1 but lacks exons 5-8 while PCOX-1b is identical to PCOX-1a except that it does not contain intron 1. The existence of a COX-1 splice variant, which includes intron 1, has also been reported in humans.

Enthusiasm mounted that the long sought after mechanism of acetaminophen's analgesic and antipyretic action had been discovered, in addition to a central mechanism for NSAID analgesics. Although many groups have published data regarding existence of COX-3, but there is great deal of dissimilarity in opinion among the scientific community regarding the COX-3 puzzle, some groups even claiming it to be a theory without experimental evidence.

**1.2 LIPOOXYGENASE PATHWAY**

Leukotrienes (LTs) are important family of eicosanoids lipid mediators derived from the metabolism of arachidonic acid and associated with asthma and allergic
reactions.\textsuperscript{111} In contrast to PGs, which are produced from arachidonic acid by the action of COX enzymes, LTs are made predominately by inflammatory cells like polymorphonuclear leukocytes,\textsuperscript{112} activated macrophages,\textsuperscript{113} and mast cells.\textsuperscript{114} LTs are lipid messengers that play central role in immune responses and tissue homeostasis. The term lipoxygenase currently designates enzymes 5-LOX, 8-LOX, 12-LOX and 15-LOX.\textsuperscript{115} 5-LO is the key enzyme in LT biosynthesis and is located in the nucleus in some cell types and in the cytosol of others.\textsuperscript{31} 5-LO is 72-80 kDa monomeric soluble protein containing one nonheme iron believed to be necessary for catalysis\textsuperscript{116} and possesses an NH$_2$-terminal domain that binds to calcium iron and is essential for nuclear membrane translocation.\textsuperscript{117} 5-LO requires Ca$^{2+}$ and is stimulated by adenosine triphosphate (ATP), phosphatidylcholine, lipids, and hydroperoxides to execute the LOX pathway (Figure 10).\textsuperscript{118}

Following cellular activation, 5-LOX translocates to the nuclear membrane where it is able to interact with an 18-kDa membrane-associated protein referred to as five-lipoxygenase-activating protein (FLAP). It is an arachidonic acid-binding protein whose function is to optimally present substrate to 5-LOX.\textsuperscript{119} In the resting cells, 5-LOX is either loosely associated with the cell membrane or free in the cytosol. When the enzyme is activated, it translocates to the nuclear envelope close to its substrate and to the FLAP, a 161 amino acid protein permanently localized in the perinuclear membrane.\textsuperscript{120} 5-LOX catalyse the reaction consisting of the regio- and enantioselective insertion of oxygen into C-5 position of arachidonic acid to form 5S-hydroperoxy-6,8-trans-11,14-cis-eicosatetraenoic acid (5-HPETE),\textsuperscript{121} which is an unstable metabolite. It is rapidly degraded either nonenzymatically or enzymatically by a peroxidase to the corresponding alcohol 5-HETE. LOX also catalyse the degradation of 5-HPETE to form epoxide LTA$_4$. The leukotriene can both be the substrate of the LTA$_4$ hydrolase to form leukotriene B$_4$ (LTB$_4$) and be metabolized by the glutathione-transferase enzyme (LTC$_4$ synthase) to provide the peptide-lipid LTC$_4$.\textsuperscript{122} This peptide leukotriene can be degraded by several specific enzymes in the order to form, in turn, LTD$_4$ and LTE$_4$. LTC$_4$, LTD$_4$ and LTE$_4$ are cysteinyll leukotrienes, constituting the biologically active mixture known as slow reacting substance of anaphylaxis (SRS-A).\textsuperscript{123} All the leukotrienes exert their biological actions by binding to specific receptors.
Only two receptors for LTB₄ called BLT₁ and BLT₂, which are seven-transmembrane receptors, have been cloned.³,¹²⁴ At least two receptors exist for cysteinyi leukotrienes, CysLT₁ and CysLT₂.³,¹²⁵ Synovial fluids from rheumatoid arthritic or gouty patients contain large number of neutrophils and elevated levels of LTB₄.¹²⁶,¹²⁷ Several data suggest that leukotrienes are closely involved in the gastric epithelial injury. Indeed these are the major arachidonic acid derivatives present in the gastric mucosa when the COX pathway is inhibited. These data suggest that the concurrent
administration of 5-LOX inhibitor could decrease the gastric toxicity commonly observed during NSAID therapy. Various inflammatory cells including leukocytes, eosinophils, macrophages, reticulocytes and mast cells produce leukotrienes from either endogenous or exogenous arachidonic acid in the presence of stimulants among which the chemotactic peptide F-met-leu-phe, platelet activating factor, and IgE directed antigens are the most common. Besides leukotrienes play vital role in allergic diseases, cancer proliferation, asthma etc. to name a few. These play a very vital role in various biological functions in human body. Major biological effects of various LTs are summarized in Table 2.

<table>
<thead>
<tr>
<th>Leukotriene Involved</th>
<th>Biological Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTB₄</td>
<td>Increased rolling and adhesion of leukocytes to endothelial cells.</td>
</tr>
<tr>
<td>LTB₄, LTD₄, LTE₄</td>
<td>Increased vascular permeability (the contraction of adjacent endothelial cells and opening of the tight junctions).</td>
</tr>
<tr>
<td>LTB₄</td>
<td>Stimulation of IL-5, IL-6 and IL-8 release from T-lymphocytes.</td>
</tr>
<tr>
<td>LTD₄</td>
<td>Stimulation of chemotaxis for neutrophils and to minor extent for other granulocytes, including eosinophils.</td>
</tr>
<tr>
<td>LTE₄</td>
<td>Stimulation of secretion of superoxide anion and release of different granular constituents from leukocytes.</td>
</tr>
<tr>
<td>LTD₄, LTE₄</td>
<td>Stimulation of chemotaxis for eosinophils in the airway mucosa</td>
</tr>
<tr>
<td>LTB₄, LTD₄, LTE₄</td>
<td>Increased muscular tone and mucous secretions of the bronchial tree.</td>
</tr>
<tr>
<td>LTB₄, LTD₄, LTE₄</td>
<td>Airway smooth muscle proliferation and remodeling.</td>
</tr>
</tbody>
</table>

Table 3: Major biological effects of leukotrienes

1.3 DRUGS FOR INFLAMMATION

Eicosanoids are involved in the control of many physiological processes and are among the most important mediators and modulators of the inflammatory reaction. The substances that inhibit the important signs of inflammation like local redness, swelling and pain are called as antiinflammatory agents. The nonsteroidal antiinflammatory drugs (NSAIDs) owe their action largely, if not entirely, due to the inhibition of the biosynthesis
of the eicosanoids. Antiinflammatory steroids inhibit the synthesis of both eicosanoids and platelet activating factors (PAF) (Figure 11).

**Figure 11:** Summary diagram of mediators derived from phospholipids and their action and the sites of action of antiinflammatory drugs

1.4 NONSTEROIDAL ANTIINFLAMMATORY DRUGS (NSAIDs)

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely used of all drugs because of their analgesic, antipyretic and antithrombogenic effects in addition to antiinflammatory activity. They provide symptomatic relief from pain and swelling. Most of the NSAIDs are available over the counter, therefore, these are often taken without prescription even for minor aches and pains. It has been reported that more than 35 million people worldwide take NSAIDs daily. NSAIDs can be broadly classified into: (A) Carboxylic acid and phenolic derivatives: aspirin (1), paracetamol (2).
Conventional NSAIDs inhibit both COX-1 and COX-2 but many tend to bind more tightly to COX-1. In contrast, COX-2 selective inhibitors have been designed to exhibit significantly higher selectivity towards COX-2 than COX-1. COX-2 inhibitors are weak time dependant inhibitors of COX-1 but potent time-dependent inhibitors of COX-2. COX-1 inhibitors can be further classified into following major classes (Figure 12) according to their structures, basic nuclei or groups present.

(A) Carboxylic acid and phenolic derivatives:

![Aspirin](image1)
![Paracetamol](image2)
![Salicylamide](image3)

(B) Acetic acids:

![Sulindac](image4)
![Indomethacin](image5)
![Diclofenac](image6)

Cont...
(C) Fenamic acids:

- Meclofenamic acid (9)
- Mefenamic acid (10)
- Flufenamic acid (11)

(D) Propionic acids:

- Ibuprofen (12)
- Naproxen (13)
- Flurbiprofen (14)

(E) Enolic acids:

- Fenoprofen (15)
- Ketoprofen (16)
Once having passed through the constriction site into the catalytic pocket, carboxyl-containing NSAIDs form a salt bridge between the carboxylate of the NSAID and the guanidinium moiety of Arg 120 in COX-1. The ionic bond formed, however, is stronger for competitively acting NSAIDs than for aspirin. Hydrophobic interactions between the aromatic ring(s) of NSAIDs and the hydrophobic amino acids lining the channel further stabilize binding. The sum of these interactions results in tight binding of many NSAIDs at the constriction point of the channel, where they totally block entry of arachidonic acid. Based on their models of inhibition of COX, traditional NSAIDs can be grouped into three classes:\(^\text{145}\)

1.4.1 Class I: Simple, competitive, reversible inhibitors that compete with arachidonic acid for binding to COX active site. Included in this class are: ibuprofen, piroxicam, sulindac sulfide, flufenamic acid, mefenamic acid and naproxen.\(^\text{145}\) These drugs have very rapid on and off rates and does not show time dependence. They inhibit COX activity essentially instantaneously after addition of the NSAID, and they readily wash out of the COX active site when the NSAID is removed from the environment of the enzyme.\(^\text{146}\)

1.4.2 Class II: Competitive, time dependent, reversible inhibitors that bind to the COX active site in the first phase, to form reversible enzyme inhibitor complexes, that if
retained for a sufficient time, because a non-covalent conformational change in the protein, associated with tighter binding. Included in this class are: indomethacin, flurbiprofen, meclofenamic acid and diclofenac. These require typically seconds to minutes to bind the COX active site. However, once bound, these drugs have low off-rates that may require hours for the NSAID to wash out of the active site. Time-dependent NSAIDs bind the COX active site first in a loose interaction and then in a productive tight complex. The rate-limiting step in drug binding is the formation of the tight binding conformation of the NSAID within the COX channel. Of particular importance to this second step in NSAID binding is the constriction point created by the hydrogen bonding network of Arg 120, Tyr 355, and Glu 524 and the proposed difficulty for some NSAIDs to traverse it.

1.4.3 Class III: Competitive, time dependent, irreversible inhibitors that form an enzyme complex after conformational changes in the protein. Included in this class is Aspirin. The crystallographic studies demonstrated the reason why this drug so efficiently acetylates Ser 530 of COX-1. Like other NSAIDs, aspirin diffuses into the COX active site through the mouth of the channel and traverses up the channel to the constriction point formed by Arg 120, Tyr 355, and Glu 524. At this point in the channel, the carboxyl of aspirin forms a weak ionic bond with the side chain of Arg 120, positioning aspirin only 5 Å below Ser 530 and in the correct orientation for transacetylation. Because the catalytic pocket of the channel is somewhat larger in COX-2 than in COX-1, orientation of aspirin for attack on Ser 530 is not as good, and transacetylation efficiency in COX-2 is reduced. This accounts for the 10- to 100-fold lowered sensitivity to aspirin of COX-2 in comparison to COX-1.

1.5 SIDE EFFECTS OF NSAIDs

NSAIDs are one of the most widely prescribed and used classes of drugs worldwide, with both prescription and over-the-counter formulations available in most countries. Numerous studies have determined that NSAIDs can have significant impact on the renal, cardiovascular and GI systems. They have shown to increase or exacerbate the risk of renal dysfunction; because they inhibit the intrarenal formation of vasodilatory PGs. They also exert a pressor effect, elevating blood pressure in both normotensive and hypotensive individuals, and antagonizing the blood-pressure-
lowering effect of anti-hypertensive agents.\textsuperscript{150,151} Since introduction of NSAIDs in the market lot of information has been collected and enormous literature has been published regarding their side effects. Although they affect renal and cardiovascular systems, but the most common, widely studied, reported and reviewed side effects are related to GIT. Occurrence of these GI events adds significantly to disease burden affecting both epidemiological and pharmaco economical part of the treatment. The greatest ill luck with NSAIDs is the fact that both the beneficial (antiinflammatory, analgesic and antipyretic) and side effects (GI) are mechanistically linked to their ability to inhibit PG synthesis. GI tract is a dynamic environment that serves primarily as an entry side to the body of nutrients, while restricting the uptake of harmful antigens, toxins and microbes. Pivotal to this role is the ability to respond appropriately to potentially damaging luminal agents, a process referred to as mucosal defence. Failure of mucosal defence can lead to sustain mucosal tissue injury and chronic inflammation, and in extreme cases, to systemic infection. The development of gastro-duodenal ulcers has been explained by the imbalance between the aggressive (e.g. hydrochloric acid, pepsin, gastrin, proteases, \textit{H. pylori}) and defensive (e.g. barrier mucus, bicarbonate, mucosal microcirculation) factors. Three main categories of GI toxicity are important to be considered: adverse symptoms (nausea, vomiting, abdominal pain); mucosal lesions; and serious complications (bleeding, perforation and obstruction). The first ever report of NSAID induced gastrotoxicity was reported in 1938.\textsuperscript{152}

1.5.1 Mechanism of mucus synthesis and defence

1.5.1.1 Bicarbonate secretion: The gastroduodenal epithelium is covered by an adherent mucus layer into which bicarbonate is secreted by surface epithelial cells. This mucus bicarbonate barrier is an important first line of defense against damage by gastric acid and pepsin, and it has been demonstrated in all species including humans. Prostaglandins of the E class are major factors that control bicarbonate secretion.\textsuperscript{153} Bicarbonate secretion and the mucus-bicarbonate layer in general, are adversely affected by ulcerogenic factors such as NSAIDs.\textsuperscript{154}

1.5.1.2 Mucus Secretion: Gastric mucus, a viscous gel that coats the entire gastric mucosa, is produced by and secreted from the surface epithelial cells. Topical prostaglandins, dimethyl PGE\textsubscript{2}, and PGF\textsubscript{2\alpha} and carbenoxolone have been observed to
increase mucus gel thickness. An unstirred layer of mucus, rich in bicarbonate, may offer significant protection against acid-peptic mucosal injury by neutralizing luminal acid and thereby providing the apical membrane of the surface epithelial cells with a near neutral pH milieu.\textsuperscript{155}

1.5.1.3 Hydrophobicity: Gastric mucosal surface hydrophobicity (GMSH) is an essential component of the mucosal defense system that is decreased by NSAIDs. A physiological decrease in GMSH with aging may contribute to the risk of ulcer development in the elderly, and may act simultaneously with \textit{H. pylori} and/or NSAIDs on gastric mucosal defense.\textsuperscript{156}

1.5.1.4 Back-diffusion of H\textsuperscript{+}: The removal of back-diffused H\textsuperscript{+} ions through the adaptation of microcirculatory flow represents a valid defense mechanism. The inability of the blood flow to contain H\textsuperscript{+} back-diffusion forms the basis for rapid-onset acute mucosal lesions; moreover, it probably contributes to the onset of chronic ulcer in certain areas already precariously supplied, because of the breakdown of the mucosal barrier or a further reduction in blood supply.\textsuperscript{157} Bicarbonate secretion from the surface epithelial cells in the duodenum is an active process depending on tissue metabolism and blood flow, it is regulated by humoral and neuronal factors as well as endogenous PGs. The duodenal mucosa is also able to respond to luminal acid by a significant rise in alkaline secretion, mediated mainly by PGs, and the impairment of this process is involved in the pathogenic mechanism of various duodenal ulcer models. The mechanism of mucosal protection by HCO\textsubscript{3}\textsuperscript{-} secretion is carried out in two ways: one is neutralizing luminal acid, and the other is establishing a pH gradient in the mucus gel with the aid of the physicochemical property of mucus. Although the majority of H\textsuperscript{+} is neutralized by secreted HCO\textsubscript{3}\textsuperscript{-} in the lumen and mucus gel, the ultimate mucosal protection is ensured by the removal of back-diffused H\textsuperscript{+} through intramucosal neutralization with HCO\textsubscript{3}\textsuperscript{-} and translocation by blood flow. Thus, HCO\textsubscript{3}\textsuperscript{-} secretion in collaboration with mucus plays an important role as the first line of defense (pre-epithelial barrier) in duodenal mucosal protection.\textsuperscript{158}
1.5.2 Mechanisms by which NSAIDs produce lesions in the GI tract

NSAIDs damage the gastroduodenal mucosa by direct toxic action, which depends on acid and occurs by means of prostaglandin inhibition. However, the gastroduodenal mucosa protects itself using different mechanisms which stop such attacks from making irreparable damage.

1.5.2.1 Topic Irritant Effect: These defense mechanisms form the so-called mucosal barrier which is made up of the surface epithelium, the surfactant and mucus. Most NSAIDs are weak acids which upon contact with the acid medium become somewhat dissociated and liposoluble. The clearest example of this is aspirin. This liposolubility makes its passage through the mucosal barrier easier, which as we have already explained, only allows liposoluble molecules to pass through. On the inside of the epithelium the pH changes and goes from acid to neutral, with a value of around 7.4. This makes the weak acid dissociate and it becomes hydrosoluble, and it is trapped inside the cells. This phenomenon is called NSAID-ionic trapping and at present is considered as one of the most fundamental mechanisms by which NSAIDs act on the digestive tract. NSAIDs can also disturb the gastric mucus hydrophobicity, whereby both pepsin and gastric acid can damage the surface epithelium. NSAIDs act on the gastroduodenal barrier locally by 3 main ways:144

a) On an epithelial level: they reduce the synthesis and the secretion of mucus and they make the proteolytic action of pepsin easier. This changes the viscosity of the mucus and its electric capacity, favoring the back diffusion of ions.

b) On an intraepithelial level: it causes epithelial denudation due to direct cellular damage, as ionized NSAIDs stay trapped inside the epithelium.

c) On a sub-epithelial level: thrombosis in the microcirculation and vasoconstriction of the arterioles of sub-mucosa.

1.5.2.2 Systemic Effect: The latter is the main reason why NSAIDs are taken; they are drugs which inhibit cyclooxygenase and subsequently inflammation. The problem arises because the prostaglandins in the digestive tract play a different role. Mucilaginose cells of the stomach secrete H$_2$CO$_3$ which together with the mucus has an important protective role. It seems that this H$_2$CO$_3$ breaks down into H$^+$ and HCO$_3^-$. This HCO$_3^-$ is
kept in the cell by the layer of liquid and mucus. This produces a pH of 2 on the outside and a pH of 7 inside. Bicarbonate arrives to the accessory cells through capillaries whose blood supply is stimulated by prostaglandin E₂. PGs of the series E and I have been described as having a direct specific inhibiting effect on histaminergic activation in isolated parietal cells. A specific receiver of PGE₂ has been identified in the membrane of these cells. Likewise, the PGs and their analogs, induce an increase in the mass of the mucosa cells, probably due to a rise in the proliferation and migration of cells. The mechanisms of NSAID associated GI injury can be broadly classified into:

1.5.2.2.1 Biochemical: Whilst many actions have been ascribed to NSAIDs to account for their ability to injure the gastrointestinal tract, the property that most centrally characterises all those that cause damage is an ability to inhibit prostaglandin synthesis. This may be enhanced in the stomach with NSAIDs that are weak acids since they ionize and become trapped in the mucosa achieving high local concentrations. This may both enhance their ability to inhibit prostaglandin synthesis and bring in to play other actions, including breaking the mucosal barrier by possibly non-PG mechanisms.

1.5.2.2.2 Pharmacological: A series of animal and human experiments in the 1980s established a range of prostaglandin dependent protective mechanisms and showed that these were abrogated by inhibition of prostaglandin synthesis with NSAIDs. Synthesis of prostaglandins is necessary for adequate perfusion of the gastroduodenal mucosa, secretion of bicarbonate by epithelial cells, secretion of mucus by the cells and the maintenance of an adequate thickness of mucus and maintenance of a neutral mucosa pH. There is some evidence that epithelial cells have intrinsic hydrophobicity and are thereby repellent to aqueous solutions of acid. Treatment with a number of NSAIDs including aspirin abrogates epithelial cell hydrophobicity.

1.5.2.2.3 Gastric Mucosal Adaptation: Several studies have shown that the gastric mucosa adapts to continued NSAID injury so that this becomes less marked. In the short term, stimulation of prostaglandin synthesis, increased nitric oxide release and protective enteric neuronal responses are important. In the longer term, epithelial metaplasia, increased expression of growth factors and their receptors and increased expression of COX-2 in response to injury, probably play an important part.
1.5.3 Process of ulcer healing

Ulcer is defined as a break in lining of the stomach that extends through the muscularis mucosae. Ulcer healing is an active and complicated process of filling the mucosal defect with proliferating and migrating epithelial cells and connective components, so as to reconstruct the mucosal architecture. Tissue repair is initiated with the aggregation of platelets, formation of a fibrin clot, and the release of growth factors from the activated coagulation pathways, injured cells, platelets, and extracellular matrix, followed by migration of inflammatory cells to the wound site. Thereafter, epithelial cells migrate over the damaged tissue. Critical to the repair process is the growth of new blood vessels. Angiogenesis occurs through the migration and division of endothelial cells, which gradually form tubes that will become the new blood vessels. Just as there are arrays of growth factors that will drive epithelial proliferation, there are numerous factors that regulate the process of angiogenesis. Endothelial cells and vascular smooth muscle cells produce some of these factors in the region of the injury. Other angiogenic factors are delivered to the site of injury via the platelets. Ulcer healing can be adversely affected by a number of factors, most notably the presence of acid in the lumen of the stomach and colonization of the ulcer bed by bacteria. The evidence suggesting that acid can delay ulcer healing consists mainly of studies demonstrating that ulcer healing is accelerated by anti-secretory agents, such as histamine H₂ receptor antagonists and proton pump inhibitors. The phenomenon of ‘cytoprotection’, was first introduced in 1979, which was an outcome of fascinating and unexpected findings that PGs can be crucial for maintenance of the gastric integrity. The discovery in the early 1990s of a second isoform of the key enzyme for PG synthesis, COX-2, led to a renewed interest in the role of prostaglandins in ulcer healing. While COX-1 appears to be the isoform that produces the majority of the prostaglandins in the normal stomach, it is the so-called ‘inducible’ isoform, COX-2, that appears to be the most important factor in the promotion of ulcer healing. PGs have to play a vital role in gastroprotection, gastric adaptation and ulcer healing. In a rat model of gastric ulcer, the ulcer bed was found to be rapidly colonized by a number of species of bacteria. Treatment with antibiotics accelerated ulcer healing. The bacteria were able to colonize the ulcer bed in part because of the relatively high pH in that region as compared to the pH of the luminal contents. Prostaglandins also appear to
play a key role in ulcer healing, as administration of prostaglandin analogs to patients with ulcers can accelerate their healing, while inhibition of endogenous prostaglandin synthesis, with NSAIDs, results in delayed ulcer healing. Indeed, NSAID administration can greatly reduce the effectiveness of H2 antagonists and proton pump inhibitors to promote ulcer healing.\textsuperscript{180}

1.6 STRATEGIES FOR DEVELOPMENT OF GASTROSPARING NSAIDs

It is clear that gastrointestinal intolerance and poor patient compliance are the major limitations of NSAIDs therapy and therefore, to design and develop gastric sparing NSAIDs is an important area of drug research. In this direction, a number of strategies have been adopted to search safer and effective NSAIDs with reduced gastrointestinal toxic side effects. These include:

1.6.A COX inhibitors as safer NSAIDs.

1.6.A.1 Selective COX-2 inhibitors as safer NSAIDs.

1.6.A.2 Transformation of conventional nonselective COX inhibitors to selective COX-2 inhibitors.

1.6.A.3 COX and 5-LOX dual inhibitors as safer NSAIDs.

1.6.B NSAIDs prodrugs/codrugs as gastrosparing therapeutic agents.

1.6.A.1 Selective COX-2 inhibitors as safer NSAIDs

The identification and characterization of an inducible form of COX-2 in inflammatory cells in the early 1990s started a race for the development of selective COX-2 inhibitors as safer NSAIDs devoid of ulcerogenic side effects. The concept of COX-2 selective inhibition is based on the differences of amino acids sequence existing between COX-1 and COX-2. The differences in the amino acid sequence between COX isoforms are responsible for the differences in the enzyme structures and especially in the access to the COX catalytic site. In comparison to COX-1 isoform, the active site of the COX-2 is larger. Based on this observation, medicinal chemists synthesized compounds suitable for interaction with the active site without inhibiting the COX-1 catalytic activity.\textsuperscript{61-66,\textsuperscript{181}} Due to the great expectation, these selective COX-2 inhibitors, known as coxibs, were rapidly introduced in the market and gained an impressive
success. The structures of six such marketed drugs are given in Figure 13. These include: celecoxib (20), valdecoxib (21), a water-soluble valdecoxib prodrug, paracoxib (22), rofecoxib (23), etoricoxib (24) and lumiracoxib (25). First three of these agents are sulphonamide derivatives. 23 and 24 are methylsulphones, whereas lumiracoxib (25) is a phenylacetic acid derivative. Celecoxib (20) and rofecoxib (23) were the first two coxibs approved by the FDA and belong to first generation of coxibs. Second generation includes: valdecoxib (21), paracoxib (22), etoricoxib (24) and lumiracoxib (25).

Figure 13: Marketed selective COX-2 inhibitors: celecoxib (20), valdecoxib (21), paracoxib (22), rofecoxib (23), etoricoxib (24), and lumiracoxib (25).

Sulphonamides derivatives may have the potential risk of allergic reactions. Additionally, differences in the molecule acidity may contribute to the drug tolerability profile, due to the direct irritant effect on the gastric mucosa. These selective COX-2
inhibitors were found to be devoid of GI ulcerogenic side effects. However, long term use of these agents revealed some potential limitations including ulcer exacerbation in high risk patients, delayed gastrointestinal ulcer healing, kidney toxicity, as well as cardiovascular side effects. These side effects forced the drug companies to withdraw rofecoxib (23) and, soon afterwards, valdecoxib (21) from the market. It was found out that COX-2 enzyme is not only inducible, but can also be constitutively expressed in a variety of noninflammatory tissues, including kidney, brain, neoplasms, bone, and cartilage. In the kidney, COX-2 mediated PGs are responsible for regulation of vascular tone, homeostasis of salt and water. Therefore, selective inhibition of either or both of the COX enzyme isoforms by NSAIDs or selective COX-2 inhibitors may result in renovascular adverse event.

In support of this fact, gastrointestinal outcomes research rofecoxib trial also reported the increased incidence of hypertension and fluid retention with rofecoxib (50 mg) treatment and subsequent increase in risk of myocardial infarction. Moreover, some studies demonstrated that selective COX-2 inhibitors, like conventional NSAIDs, cause comparable rates of edema and hypertension and may impair compensated renal function in the setting of congestive heart failure or volume depletion.

These findings raised serious concerns about the risk of thrombotic events during treatment with coxibs, and marking off the therapeutic benefits of selective COX-2 inhibition. Therefore, the initial enthusiasm of developing selective COX-2 inhibitors faded away and need for designing and developing safer NSAIDs, devoid of their ulcerogenic side effects still remains. In this direction, different strategies were developed.

1.6.A.2 Transformation of conventional nonselective COX inhibitors to selective COX-2 inhibitor

A common strategy in pharmaceutical research consists in the use of well established drugs as lead compounds to design new drug candidates with improved therapeutic properties. Many attempts have been made to convert nonselective, conventional NSAIDs into selective COX-2 inhibitors, and thus taking the advantage of a structural class with a well established safety profile. The rationale for chemical modification is based on the active site differences between COX-1 and COX-2.
isoforms. The substrate binding site in COX-2 is approximately 25% larger than COX-1 (394 Å Vs. 316 Å). Chemical modification of the nonselective, conventionally used NSAIDs by increasing the size of the drug molecule, which fits into the COX-2 active site but not into the COX-1 site, resulted in the formation of selective COX-2 inhibitors. Incorporation of steric bulk into existing nonselective NSAIDs could abolish their COX-1 inhibitory properties without affecting COX-2 activity. Alteration of the carboxylic acid moiety has recently been exploited to convert nonselective inhibitors into COX-2 selective inhibitors. Many novel structural classes of COX inhibitors have recently emerged due to molecular modifications of well established NSAIDs. Some illustrative examples are discussed here.

Examination of flurbiprofen (14) bound to COX-1 and COX-2 suggests that modification of the 4-phenyl ring to induce steric constraint should result in increased selectivity for COX-2. This hypothesis was validated through introduction of various substituents to generate a series of potent and selective COX-2 inhibitors. Three of these compounds 26-28 (Figure 14) were found to exhibit greater selective COX-2 inhibitory activity.

![Image of transformed flurbiprofen selective COX-2 inhibitors]

Similarly, novel selective COX-2 inhibitors have been designed and developed by transformation of nonselective ketoprofen (16). The strategy is based on combined use...
of pharmacophore of the diaryl NSAID and modeling of the 3D structure docked into the COX active site. The compound 29 of this series was found to be potent and selective COX-2 inhibitor.\textsuperscript{192}

Indomethacin (5) is one of the most potent nonselective NSAIDs. This agent has also been transformed into selective COX-2 inhibitors by systematic structural modification to increase the size (Figure 15).

![Chemical structures of indomethacin and its derivatives](image)

Figure 15: Transformed indomethacin selective COX-2 inhibitors (30-36).
In this direction 4-chlorobenzoyl group of indomethacin was replaced with a 2,4,6-trichlorobenzoyl group which resulted in the formation of compound 30, exhibiting reasonable COX-2 selectivity. Based on these results, Black et al. reviewed a number of indole acetic acid analogues and found that benzyl derivative 31 exhibited highly selective COX-2 activity. Using same strategy Kagutkar et al. also attempted to transform indomethacin to selective COX-2 inhibitors and taking the advantage of structural class with well established safety profiles. These investigators prepared ester derivatives (32 and 33) and amide derivatives (34-36) of indomethacin and found that large alkyl, arylalkyl and heterocyclic groups exhibited high activity and selectivity.

Aspirin (1) is the only NSAID that covalently modifies COX isoforms by acetylation of an active site serine residue. Although this drug acetylates both isoforms of COX, it is 10 to 100 times as potent against COX-1 as against COX-2. The antiinflammatory effects arise from acetylation of COX-2, whereas antithrombic and ulcerogenic effects result from acetylation of COX-1. Attempts have been made to transform this nonselective NSAID to selective COX-2 inhibitors by varying the length of the acyl group attached. In this direction, a series of acetoxybenzenes substituted at the ortho position with alkyl sulfides have been prepared. One such compound, o-(acetoxyphenyl)methyl sulfide (37) was identified with moderate inhibitory activity and selectivity for COX-2. Further, systematic variation at different parts of the molecule led to the synthesis of o-(acetoxyphenyl)hept-2-ynyl sulfide (38) having most potent COX-2 inhibitory activity.

Another NSAID studied for its transformation to selective COX-2 inhibitors was zomepirac (39), which is basically a COX-1 inhibitor. To achieve selective COX-2
inhibitory activity, the acetic acid group was replaced with other moieties such as pyridazinone ring or an N-acyl aminosulfonyl group to produce RS57067 (40) and RS1048934 (41) respectively.\(^{199,200}\)

Meclofenamic acid (9) has also been selected as lead for designing selective COX-2 inhibitors. A series of ester and amide derivatives of this NSAID have been prepared. It has been found that only amide derivatives (42-46) showed potent and selective COX-2 inhibitory activity. Based on SAR studies, it has been suggested that further optimization may be necessary to enhance selective COX-2 inhibitory activity.\(^{194,201}\)
This strategy of transforming nonselective NSAIDs to selective COX-2 inhibitors has also been successfully applied to diclofenac (6). Structure activity studies on series of diclofenac analogues indicated that methyl or chlorine substituents on the lower aniline ring in the ortho position are necessary to achieve potent COX inhibition. Various other compounds synthesized to resemble the parent drug diclofenac also showed very less activity. Chemical modifications of the carboxylic group of diclofenac gave compound 47 which exhibited selective COX-2 inhibitory activity. Other diclofenac derivatives with selective COX-2 inhibitory activity include derivative 48 having meta-alkyl substituents on the phenylacetic acid.191,202

![Chemical structures](image)

1.6.A.3 COX and 5-LOX dual inhibitors as safer NSAIDs

Leukotrienes (LTs) is an important family of eicosanoids lipid mediators derived from the metabolism of arachidonic acid and are the major arachidonic acid derivatives present in the gastric mucosa when the COX pathway is inhibited. These data suggest that the concurrent administration of 5-LOX inhibitor with NSAIDs could decrease the gastric toxicity commonly observed during NSAID therapy.128-130 Therefore recently, several approaches have been followed in order to develop dual COX / 5-LOX inhibitors. Dual inhibitors of both COX and LOX attract interest due to their ability to inhibit two key enzymes involved in the arachidonic acid metabolism. Currently, various classes of dual COX / 5-LOX inhibitors as safer NSAIDs have been described in the scientific literature.203 One such class of dual COX / 5-LOX inhibitors is that of di-tert-butylphenol derivatives. The general structure of these agents consists of 2,6-di-tert-butyl-1-hydroxybenzene substituted in fourth position, optimum for dual activity (Figure 16). The substituents are either five- or six- membered heterocycles or straight chains.
The phenol moiety confers on them antioxidant and free radical scavenging properties, which has been proposed to be relevant to their antiinflammatory activity with reduced ulcerogenicity activity.\textsuperscript{203}

Darbufelone (49) and S-2474 (50) belong to this class of compounds with selective COX-2 / 5-LOX inhibitory activity. In addition to its antiinflammatory efficacy, the latter agent showed cytokine modulating properties.\textsuperscript{204} It is currently being evaluated in clinical trials for arthritis.\textsuperscript{205} Another derivative, tebufelone (51) has been found to show a dual inhibitory potency against 5-LOX and COX. This agent has been extensively investigated and included in clinical trials as antipyretic agent.\textsuperscript{206}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figures/diagram.png}
\caption{Di-\textit{tert}-butyphenol class of dual COX / 5-LOX inhibitors.}
\end{figure}
In this clinical study, tebufelone has been found almost ten times more potent antipyretic than aspirin. Various investigations on different animal species have indicated that repeated doses of this agent for more than three weeks results in hepatic toxicity. An interesting observation has been made that tebufelone metabolized to dihydrodimethylbenzofuran derivative (52). Although this compound is not a phenol, it exhibited an anti-inflammatory activity equivalent to that of tebufelone (51) in the rat carrageenan induced paw edema and also showed hepatotoxicity. The hepatic toxicity of metabolite 52 and the parent molecule 51 was attributed to the terminal unsaturation of side chains.

Based on these observations, structural modifications were carried out to give different dihydrobenzofuran derivatives as COX-2 / 5-LOX inhibitors. For example, PGV-20229 (53) has been found to exhibit analgesic activity and excellent gastric safety in different in vivo tests.

Another class of COX-2 / 5-LOX dual inhibitors belong to thiophene derivatives. The lead compound RWJ-63556 (54) is a potent orally active COX-2 / 5-LOX inhibitor which is structurally related to the selective COX-2 inhibitor nimesulide (55). It has been found to produce significant anti-inflammatory activity in a canine model of carrageenan induced inflammation.

Pyrazoline derivatives, phenidone (56) and BW-755C (57) as antioxidant 5-LOX inhibitors appeared to be rather nonselective, inhibiting the COX isoforms.
Another dual COX / 5-LOX inhibitor is tepoxaline (58), which is a pyrazole containing hydroxamic acid. This agent is able to chelate the non-heme iron atom of 5-LOX and has undergone clinical evaluation for psoriasis and rheumatoid arthritis. Further, two chemical hybrid series (59a-b) of this potent dual inhibitor was synthesized. Unfortunately, neither improved on the duration of 5-LOX inhibitory activity.

A number of pyrrolizine derivatives have been found to possess dual inhibitory activity. Unlike most of the dual inhibitors described above, these agents are neither antioxidants nor iron chelators. One such compound, licofelone (60) has entered phase-III clinical trials for the treatment of osteoarthritis. In several animal models, this derivative has shown anti-inflammatory, analgesic and antiasthmatic effects. Although licofelone (60) is a COX / 5-LOX inhibitor, it does not cause any GI damage. However, the mechanism of gastric sparing action of this compound has not been fully elucidated.

A number of hydrazine derivatives have also been described as dual COX / 5-LOX inhibitors. One promising compound is CBS-1108 (61), which was evaluated in vivo in different animal models of inflammation. Topical administration of this agent was effective in inhibiting croton oil induced ear edema in rats. In the rat dorsal air pouch
edema model, it was found to be active and exhibited a dose dependent inhibition of leukocytes migration with an IC$_{50}$ of 35 µmol/kg.$^{206}$

A number of conventionally used NSAIDs, as well as selective COX-2 inhibitors have been structurally modified in an attempt to design and develop dual COX / 5-LOX inhibitors. For example, the carboxylic acid group of indomethacin was exchanged for N-hydroxyurea.$^{210}$ This group has the capability to chelate the non-heme iron of 5-LOX. Such two derivatives 62 and 63 have been prepared and found to inhibit not only 5-LOX but preferentially the inducible isoform COX-2.

Similarly, flufenamic acid (11) has been structurally modified by bioisosterically replacing the carboxylic acid group with tetrazole moiety (Figure 17).$^{211}$ The resulting compound 64 inhibited COX and to some extent 5-LOX. Other flufenamic acid transformed dual COX/5-LOX inhibitors, include 1,3,4-oxadiazole-2-thione (65) and 1,3,4-thiadiazole-2-thione (66). The thione function of these derivatives seems to play an important role in the 5-LOX inhibitory activity. Indeed, substitution of the carboxylate moiety with heterocycles having a carbonyl function led to inactive compounds.
Results with dual COX/5-LOX inhibitors seem to be promising. However, large number of clinical trials is required to evaluate safety and efficacy of these agents.

1.6.B NSAIDs prodrugs/codrugs as gastrosparing therapeutic agents.

Prodrug approach has been the most widely used technique for chemical modification of NSAIDs by the medicinal chemists all over the world. A large number of therapeutic medications have undesirable properties that may generate pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug application. The chemical approach using reversible derivatives such as prodrugs and soft drugs, can be useful in the optimization of the clinical application of a drug. Obviously, this approach can offer the highest flexibility and improve the drug efficacy. According to The International Union of Pure and Applied Chemistry (IUPAC), the term prodrug is defined as "any compound that undergoes biotransformation before exhibiting its pharmacological effects" \(^{212}\). Prodrugs can thus be viewed as drugs containing specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule.\(^{213}\)

The term "prodrugs" or "proagent" was first introduced by Albert in 1958 to signify pharmacologically inactive chemical derivatives that could be used to alter the physicochemical properties of drugs, in a temporary manner, to increase their usefulness or to decrease their toxicity.\(^{214}\) Researchers and scientists have used similar terms like “latentiated drugs”, “bioreversible derivatives”, and “congeners” for prodrugs in literature, but “prodrugs” is now the most commonly accepted term. Numerous

![Figure 17: Transformed flufenamic acid dual COX / 5-LOX inhibitors (64-66).]
Prodrugs have been designed and developed to overcome pharmaceutical and pharmacokinetic barriers in clinical drug application, such as low oral absorption, lack of site specificity, chemical instability, toxicity, and poor patient acceptance (bad taste, odor, pain at the site of application, etc.).

Prodrugs can be designed to target specific enzymes or carriers by considering enzyme-substrate specificity or carrier-substrate specificity in order to overcome various undesirable drug properties. The definition of the prodrug indicates that the protective group is covalently linked to the drug molecule.

Another similar term is “soft drug”, which according to IUPAC is “a compound that is degraded in vivo to predictable nontoxic and inactive metabolites, after having achieved its therapeutic role”. Nicholas Bodor was the first to introduce the idea of soft drugs in 1977. It should be an isosteric / isoelectronic analogue of the parent drug. They are strategically designed to undergo singular metabolic deactivation after they achieve their therapeutic roles. These kinds of compounds are ideal for producing specific action at the site of application without affecting the rest of the body. The idea of designing the soft drugs is based on the concept that we should build a fragment into the molecule that turns the new molecule into a compound that can function as a substrate for the metabolizing enzymes, but still has a sustained activity at its original target. There are a lot of important local sites where application of a drug can be achieved very easily, for example, eye, skin, major parts and compartments of the gastrointestinal tract, and lungs. Local application of a drug to these sites can easily be...
achieved, and then soft drugs can produce their desired pharmacological activity at the site of application. According to this concept, soft drugs have been divided into soft analogues, inactive metabolite-based soft drugs, active metabolite-based soft drugs and pro-soft drugs.

The rationale behind the prodrug strategy is to introduce lipophilicity and mask hydrogen bonding groups of an active compound by the addition of another moiety, most commonly an ester or amide. An ideal prodrug should exhibit the following properties:

(a) Weak (or no) activity against any pharmacological target
(b) Chemical stability across a pH range
(c) High aqueous solubility
(d) Good transcellular absorption
(e) Resistance to hydrolysis during the absorption phase
(f) Rapid and quantitative breakdown to yield high circulating concentrations of the active component post absorption.

1.6.B.1. Prodrugs of NSAIDs

The successes of prodrug design are many and several such agents have proved their therapeutic values. NSAIDs having carboxylic group is one of the most important class of therapeutic agents which is under extensive investigations for design and development of their prodrugs. Considerable attention has been focused on the development of bioreversible derivatives, such as prodrugs, to temporarily mask the acidic group of NSAIDs as a promising means of reducing or abolishing the GI toxicity due to the local action mechanism. Most prodrugs of NSAIDs have been prepared by derivatization of the carboxyl group. The esters have dominated prodrug research because they have the ideal characteristic of exhibiting reasonable in vitro chemical stability which allows them to be formulated with adequate shelf lives. In addition, by virtue of their ability to function as esterase substrates, esters are suitably labile, in vivo. With this aim different prodrugites have been taken into consideration to design
new efficacious NSAID prodrugs. In the following sections, various ester and amide derivatives of NSAIDs will be discussed.

Paris et al. prepared a series of 1,3-bisalkanoyl-2-(O-acetylsalicyloyl) glycerides, (triglycerides of aspirin) 67 having aspirin at 2 position of glycerol and fatty acids at 1 and 3 positions. The studies for presence of lesions in stomach showed that the derivatives in which fatty acids are of intermediate chain length (C4-C12) did not cause gastric lesions and had essentially all the systemic activity associated with aspirin.221

\[ (67) \]

Triglyceride derivatives of naproxen 68(a-b) and indomethacin 69(a-b) have been prepared. Comparison of the 2-glyceride of naproxen 68b with naproxen for gastric irritation, as determined by the minimum chronic dose producing occult blood in either faeces or urine in dog, gave a dose ratio of 3 in favour of the 2-glyceride 68b. The 2-glyceride 69b of indomethacin showed a 2.5 to 3.0 fold improvement in the therapeutic index.222,223

The methyl esters of salicylic acid, diflunisal, flufenamic acid, indomethacin, diclofenac and tolmetin were synthesized and found to be effective in reducing interaction of the irritant NSAIDs in the acidic milieu of the stomach with drug sensitive mucosal and parietal cells.224 Indomethacin farnesil, a prodrug of indomethacin has been reported to cause less gastric damage than indomethacin and loxoprofen due to its less potency for inhibiting the gastric mucosal prostaglandins.225,226
Schlegel and co-workers have reported the synthesis, and evaluation of antiinflammatory and anti-ulcer activity of several bulky amine analogues (70) of ketoprofen (16). These have been synthesized by replacement of carboxyl group of ketoprofen (16) with various bulky amines. It was found that activity was maintained on reduction of these analogues to corresponding alcohol (71) or methylene analogues (72), on conversion of the keto group to a primary amine (73) or oxime (74). Removal of the α-CH₃ group in some compounds (75) greatly reduced the antiinflammatory activity in the series.
Ester prodrugs (76-77) of ibuprofen (12) and naproxen (13) have been synthesized by reacting with paracetamol. Moreover some β-D-glucopyranosides (78-80) of aspirin (1), diclofenac (6) and ibuprofen (12) have also been synthesized and evaluated for their antiinflammatory and anti-ulcer activities.228
With the aim of designing potential NSAID prodrugs Caprariis et al. \textsuperscript{229} considered oligoethylene glycols as attractive promoities:

1) They are known to have good biocompatibility\textsuperscript{230}
2) They could give prodrugs with enhanced aqueous as well as lipid solubility compared to the parent drug so as to increase GI absorption\textsuperscript{231}
3) They cause enhanced residency period of NSAIDs into the system since they prevent enzymes from attacking the drug.\textsuperscript{232}

Five different oligoethylene ester derivatives \textsuperscript{(81)} of indomethacin were synthesized and evaluated. All the esters were found to be significantly less irritating to gastric mucosa than indomethacin after oral administration with better or similar antiinflammatory and analgesic activity.\textsuperscript{229}

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{H}_3\text{C} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\end{align*}
\]

Ethyl esters of flurbiprofen \textit{L}-arginine, flurbiprofen \textit{L}-lysine and flurbiprofen \textit{p}-guanidino-\textit{L}-phenylalanine were synthesized and evaluated for their availability as
prodrugs for flurbiprofen. They were found to release the parent drug in vitro upon enzymatic hydrolysis.

Succinamide esters and glycineamides of naproxen, ibuprofen, ketoprofen, aspirin, diclofenac and indomethacin have been synthesized by Singh et al. The succinamide esters retained their antiinflammatory property whereas the glycineamides exhibited lower activity as compared to those of the parent drugs. The glycineamides showed no hydrolysis at lower pH and in gastric fluid till 2 hours and had less GI toxicity than succinamide esters which exhibited complete hydrolysis within 15 minutes in the gastric fluid.

A series of glycolamides, glycolate, (acyloxy) methyl, alkyl and aryl esters of acetylsalicylic acid were synthesized and evaluated as potential prodrugs of aspirin. The N,N-disubstituted glycolamide esters were found to be rapidly hydrolysed in human plasma resulting in the formation of aspirin as well as the corresponding salicylate esters which in turn hydrolyzed rapidly to salicylic acid.

The kinetics of hydrolysis of glycolamide esters of indomethacin was studied to assess the possibility of designing a water-soluble and solution-stable prodrug of indomethacin suitable for parenteral or ocular administration.
prodrugs degraded both, at its ester group linkage and at the indole amide linkage of indomethacin, showing a pronounced water catalysed hydrolysis leading to the conclusion that design of an indomethacin ester prodrug with a stability allowing formulation of a ready-to-use aqueous solution may be difficult.

\[
\text{\textbf{(84(a-c))}}
\]

A series of novel \(\omega\)-(N,N,N-trialkylammonium)alkyl ester and thioester derivatives of eleven nonsteroidal antiinflammatory carboxylic acid agents (naproxen, ketorolac, indomethacin, ibuprofen, sulindac, ketoprofen, flufenamic acid, mefenamic acid, zomepirac, etodolac and tifurac) were prepared and evaluated for their antiinflammatory, analgesic and gastrointestinal erosive properties.\textsuperscript{237} The pharmacokinetics of ibuprofen ethylcarbonate and naproxen ethylcarbonate, two new prodrugs of ibuprofen and naproxen in dogs, was reported by Samara \textit{et al}.\textsuperscript{238}

The \textit{in vitro} skin permeabilities of ketorolac and its two ester analogs \textbf{85 (a-b)} as prodrugs through human cadaver skin were investigated.\textsuperscript{239} The \([N,N-\text{dimethylamino})\text{carbonyl}\) methyl ester \textbf{85a} appeared to be a better ester prodrug than the simple ethyl ester \textbf{85b} prodrug as it exhibited relatively higher skin flux and faster enzymatic hydrolysis in human serum to liberate the parent drug.

\[
\text{\textbf{(85(a-b))}}
\]

\[
\text{a) } R=\text{CH}_2\text{CON(CH}_3)_2 \quad \text{b) } R= \text{C}_2\text{H}_5
\]
Naproxen (13), diclofenac (6), flufenamic acid (11) and aspirin (1) have been modified into non-carboxylic $\Delta^2$-oxazoline derivatives (36-88, 89, 90 and 91, respectively), with an aim to mask the gastrotoxic carboxylic group.\(^{240}\)

\[
\begin{align*}
R = H, n = 1 & \quad \text{(86)} \\
R = CH_3, n = 1 & \quad \text{(87)} \\
R = H, n = 2 & \quad \text{(88)}
\end{align*}
\]

Morpholinoalkyl esters of naproxen and indomethacin were synthesized and evaluated \textit{in vitro} and \textit{in vivo} for their potential use as prodrugs for oral delivery.\(^{241}\) The prodrugs were found to be 30-36% more bioavailable orally than the parent drugs. A series of morpholinoalkyl ester prodrugs \textbf{92(a-c)} of diclofenac were synthesized by Tamara et al.\(^{242}\) and evaluated \textit{in vitro} and \textit{in vivo} for their potential use as prodrugs for oral delivery. All these esters were reported to exhibit a rapid bioconversion in rat plasma and were significantly less irritating to the gastric mucosa than the parent drug.

\[
\begin{align*}
\text{(92(a-c))} & \quad \text{a) } n=2, \text{ b) } n=3, \text{ c) } n=4
\end{align*}
\]
Many ester 93(a-d), 94(a-d) and amide 93(e-h), 94e prodrugs of ibuprofen and naproxen were synthesized and biologically evaluated by Shanbhag et al.\textsuperscript{243} The ulcerogenicity of the prodrugs 93a, 94a, 93h, and 94d was less than the respective parent drugs. All the prodrugs were found to be less active than the parent NSAIDs in their antiinflammatory efficacy.

\[ \begin{align*}
R = \\
a) & -O(CH_2)_2N(CH_3)_2 \cdot HCl \\
b) & -O(CH_2)_2N(C_6H_5)_2 \cdot HCl \\
c) & -\text{NH}(CH_2)_2N(C_6H_5)_2 \\
d) & -O(CH_2)_2N(CH_3)_2 \cdot HCl \\
e) & -\text{NH}(CH_2)_3N(C_6H_5)_2 \\
f) & -\text{NHCH}_2\text{COOH} \\
g) & -\text{NHCH}_2\text{COOH} \\
h) & -\text{NHCH}_2\text{COOH}
\end{align*} \]

Cyclodextrins (CyDs) are known to form inclusion complexes with various drug molecules wherein the complexes exist in equilibrium with the guest and host molecules in aqueous solution.\textsuperscript{244} However, such a situation is disadvantageous when drug targeting is to be attempted because the complex would dissociate before it reaches the target organ. This problem could be overcome by covalent binding of the drug to CyDs. CyDs are known to be capable of hardly being hydrolyzed and absorbed during passage through the stomach and small intestine. However, they are fermented into small saccharides by colonic microflora and thus get absorbed in the large intestine.\textsuperscript{245} This biodegradation property of CyDs has been exploited for site specific delivery of drugs to colon. Six 4-biphenylacetic acid prodrugs, coupled to alpha, beta and gamma-cyclodextrins through an ester or amide linkage, 6-O-[(4-biphenyl)acetyl]-\(\alpha/\beta/\gamma\)-cyclodextrins 95 (a-c) and 6-deoxy-6-[(4-biphenyl)acetyl]-\(\alpha/\beta/\gamma\)-cyclodextrins 95 (d-f) were prepared and investigated by Minami \textit{et al.}\textsuperscript{246} for their \textit{in vivo} drug release.
behaviour in rat gastrointestinal tracts after oral administration. The results suggested that this approach can provide a versatile means for constructions of not only colon-specific delivery systems but also delayed-release system for certain drugs. A study on biphenylacetic acid bound to β-cyclodextrin through an ester 95b or amide linkage 95e suggested the potential of the ester prodrug 95b for colon targeting.247

Synthesis, enantiomeric separation and evaluation of amide prodrugs (96-102) of some 2-arylpropionic acids, ibuprofen (12), naproxen (13), diclofenac (6) and ketorolac (8) has been reported.248 The compounds were prepared from the corresponding 2-arylpropionic acids and (R(-)-2-amino-1-butanol in the presence of N,N'-dicyclohexylcarbodiimide (DCC).
Abordo et al. carried the synthesis of 2-formylphenyl esters of indomethacin 102a, ketoprofen 103 ibuprofen 104 and aspirin 105a, together with two 6-substituted-2-formyl 105b,105c and two 2-acylphenyl aspirins 105d, 105e and 4-formylphenyl indomethacin 102b.249 The 2-formylphenyl esters 102a, 104, 103, 105a were found to be more potent as antiinflammatory agents than the parent compounds in the carrageenan-induced paw edema test. The n-butyl and n-octyl ester prodrugs of indomethacin did not show GIT and hepatic injury even after repeated oral administration in contrast to the severe irritating effect of the parent drug.250

\[
\text{102(a-b)}
\]

a) \( X = 2\text{-CHO} \)

b) \( X = 4\text{-CHO} \)

\[
\text{103}
\]

\( X = 3\text{-COPh} \)

\[
\text{104}
\]

\( X = 4\text{-CH}_2\text{-CH(CH}_3)_2 \)

\[
\text{105(a-e)}
\]

(a) \( X = \text{CHO}; Y,Z=H \)

(b) \( X = \text{CHO}; Y=\text{CH(CH}_3)_2; Z=H \)

(c) \( X, Y = \text{CHO},Z=\text{CH}_3 \)

(d) \( X = \text{COCF}_3; Y,Z=H \)

(e) \( X = \text{COCH}_3; Y,Z=H \)

Jung et al. reported a simple synthetic route for the preparation of amino acid conjugate of 5-aminosalicylic acid (5-ASA).251 In vitro and in vivo properties of 5-aminosalicylglycine (5-ASA-Gly) as a colon specific prodrug of 5-ASA were investigated using in rats as the test animals. Incubation of 5-ASA-Gly at 37°C with cecal and colonic contents released 65 % and 27 % of 5-ASA in 8 h, respectively. Free 5-ASA was not detected upon incubation of the conjugate with the homogenates of stomach or small intestine.
Various glycolamide ester prodrugs 106(a-l) of 6-MNA were synthesized and evaluated for the physicochemical properties, chemical stability and enzymatic hydrolysis in 80% human plasma. \(^{252}\) The chemically more stable disubstituted glycolamide esters 106(g-l) were more prone to enzymatic cleavage than the monosubstituted ones with half-lives ranging from 7s to 83s.

\[
\begin{array}{c|c|c}
R1 & R2 & R1 & R2 \\
\hline
a) H & H & b) H & CH_3 \\
\hline
c) H & CH_3 & d) H & CH(CH_3)_2 \\
\hline
e) H & (CH_3)_2 & f) H & C(CH_3)_3 \\
\hline
f) CH_3 & CH_3 & g) CH_3 & C_2H_5 \\
\hline
i) CH(CH_3)_2 & CH(CH_3)_2 & j) CH_3 & C_2H_5OH \\
\hline
k) CH_2CH_3 & CH_2CONH_2 & l) C_2H_5OH & C_2H_5OH \\
\end{array}
\]

(106(a-l))

Dhaneshwar and co-workers have reported the synthesis, in vitro and in vivo evaluation of a prodrug (107), in which diclofenac (6) has been conjugated with methyl ester of an amino acid, histidine. \(^{253}\) The prodrug was found to be stable in buffer of pH 1.2, but hydrolysed at buffer pH 7.4 and 80% human plasma (pH 7.4). The compound had significant analgesic and anti-ulcer activity.

\[
\begin{array}{c}
\text{(107)}
\end{array}
\]

Indomethacin (5) and naproxen (13) prodrugs (108-111 and 112-115, respectively) having 1-alkylazacycloalkan-2-ones as the alcohol portion of an ester prodrug of carboxylic acid functional group have been synthesized to enhance their topical delivery. \(^{254,255}\) 1-alkylazacycloalkan-2-ones have been used as promoieties as they have been used as permeation enhancers in formulation.
Bonina et al. evaluated two esters 116 (a-b), 1-ethylazacycloalkan-2-ones of indomethacin for their potential use as prodrugs for oral delivery. Evaluation indicated that the esters represented potentially useful indomethacin prodrugs for oral administration since they were found to be stable in aqueous solution as well as in simulated gastric fluid with a fast enzymatic hydrolysis in rat plasma. The antiinflammatory and analgesic activities of the parent drug were retained and the gastrointestinal irritation was notably inhibited by both the esters.

Rautio et al. synthesized and evaluated various aminoacyloxyalkyl esters of naproxen and naproxenoxyalkyl diesters of glutamic acid and aspartic acid as potential prodrugs of naproxen for transdermal delivery. These prodrugs were shown to have higher aqueous solubilities and similar lipid solubilities in terms of octanol-buffer
partition coefficients (log P) at pH 5.0, when compared with naproxen. Various aminoacyloxyalkyl esters, acyloxyalkylesters, and diacetylgluceryl ester prodrugs of ketoprofen and naproxen have been reported with potential for improving dermal delivery of the parent drugs.

Morpholinyl and piperazinylalkyl esters of naproxen have been reported as bioreversible topically administered dermal prodrugs of naproxen. A 4 to 9 fold enhancement of permeation was observed for 117d and 117b when compared to naproxen at pH 7.4 and a 4 fold better permeation was observed for 117b at pH 5.0. A novel 3-(N,N-diethylamino) propyl ester prodrug of indomethacin was found to be a potent antiinflammatory agent with lower ulcerogenicity in stomach.

Biphosphonates, a class of compounds structurally related to pyrophosphate, are clinically used to treat various bone disorders, including osteoporosis. Biphosphonates are known to have high affinity for hydroxyapatite (a major component of osseous tissue) and osseous tissues accumulate biphosphonates in high concentrations. Based on the concept of Osteotropic Drug Delivery System, disodium 2-(2,6-dichloroanilino)phenylacetoxyacetamino- methylene biphosphonate 118, a biphosphonic prodrug of diclofenac was synthesized and investigated for its potency and controlled delivery of diclofenac to the bones in rats. No side effect of gastrointestinal damage, typical of NSAIDs was observed for this prodrug 118. The bone specific delivery and sustained release properties of the prodrug could enhance the pharmacological effect of diclofenac for bone diseases, while simultaneously
preventing adverse GI effects and increasing the patient compliance by decrease in frequency of its administration.

A new polymerizable drug derivative of diclofenac sodium was synthesized and characterized by Chandrasekar et al.\textsuperscript{265} The \textit{in vitro} study showed that the drug release takes place predominantly at higher pH and in a sustained manner, as hypothesized, with complete drug absorption from the polymeric prodrug and a statistically significant decrease in ulcer scores was observed demonstrating its potential for site-specific and sustained delivery of diclofenac.

(118)

Polyoxyethylene esters of ketoprofen 119 (a-e), naproxen 120 (a-e) and diclofenac 121 (a-e) showed good stability in phosphate buffer (pH 7.4) and simulated gastric fluid (pH 2.0), and were readily hydrolyzed by human plasma. Antiinflammatory activity of the esters was found to be similar to the parent drugs although at higher doses, and good analgesic activity was exhibited with significantly reduced gastric irritation even at higher doses.\textsuperscript{266} These esters were also evaluated as dermal prodrugs.\textsuperscript{267} An appreciable and sustained \textit{in vivo} topical antiinflammatory activity was observed for the ester prodrugs in the erythema model in human volunteers.
Bonina and associates have also reported the synthesis, in vivo and in vitro evaluation of ketoprofen 1-alkylazacycloalkan-2-one esters (122-127) as dermal prodrugs. All the esters showed increased lipophilicity compared with the parent drug, good stability in phosphate buffer pH 7.4 and were readily hydrolysed by porcine esterase.

Glycolamide esters 128(a-c) of ibuprofen were also synthesized and studied for different physicochemical, pharmacological and toxicological properties. Khan and Khan have evaluated the glycolamide ester prodrugs of ibuprofen 129a, diclofenac 129b, naproxen 129c and indomethacin 129d for their GI toxicity in rats. Hydrazide derivatives of naproxen, diclofenac, ibuprofen and indomethacin were synthesized and evaluated biologically in rodent model.
Naproxen (13) and ibuprofen (12) have been modified to pyrazolone derivatives (130, 131, and 132), by condensing their hydrazide derivatives with β-keto esters. Further naproxen (13) and diclofenac (6) have been structurally modified to their oxadiazole analogues (133 and 134, respectively) by reacting their hydrazide derivatives with biphenyl acetic acid.272
Moriera and co-workers have reported the synthesis of tertiary N-acyloxymethylamide prodrugs (135,136) of ibuprofen (12) and naproxen (13).273
Phospholipids microemulsions have been suggested as a drug delivery system for hydrophobic compounds. Therefore, hydrophobicity was achieved by derivatising ibuprofen (12) and flufenamic acid (11) with cholesterol to get cholesteryl ibuprofen (137) and cholesteryl flufenamate (138), respectively.274

\[ \text{Ibuprofen p-D-glucopyranoside (139)} \]

Ibuprofen p-D-glucopyranoside (139) has been reported to possess superior antiinflammatory and analgesic activities over the parent drug with significantly less ulcerogenicity.275 Alkyl ester prodrugs of ibuprofen 140(a-l) have been reported by Bansal et al.276 with significant improvement in the oral delivery of ibuprofen in terms of reduced gastroucerogenicity and maintenance of pharmacological activity. These esters were also evaluated for their physicochemical properties and antiinflammatory activity in carrageenan induced rat paw edema by topical route.277 The benzyl ester prodrug 140m showed a significantly reduced gastric ulcerogenicity at equimolar doses with retention of antiinflammatory and analgesic activities.278
Hydrazone derivatives (141-150) of Ibuprofen (12) have been synthesized and screened for the anti-inflammatory activity. Compounds 141, 142, 146 and 149 were found to have better anti-inflammatory activity than the parent drug.

The formation of glycolamide esters has been utilized to mask the free carboxylic acid functional group in NSAIDs. Khatavakar and coworkers have reported the synthesis and pharmacological evaluation of glycolamide esters (151-161) of diclofenac (6) and mefenamic acid (10). All the compounds were found to be stable in aqueous buffer.
solution of pH 1.2 and showed rapid rate of hydrolysis at pH 7.4. All the prodrugs showed comparable antiinflammatory and lesser gastrotoxicity in comparison to the parent drugs.

Wang et al. have co-polymerized ibuprofen, ketoprofen and naproxen with 2-hydroxyethylmethacrylate (HEMA) with high methacrylate contents. The polymeric prodrug of ibuprofen retained the antiinflammatory potency of ibuprofen whereas the prodrugs of ketoprofen and naproxen displayed greater potency to inhibit acute inflammatory processes than the free drug.281

Mannich bases have been synthesized starting from conventional NSAIDs and they have been found to retain the activity of the drug and devoid of ulcerogenicity. Ibuprofen (12) has been modified into Mannich bases (162-169) and some of the derivatives were evaluated for analgesic, antiinflammatory and anti-ulcer activity. Prodrugs had better antiinflammatory activity and lesser gastrotoxicity in comparison to the ibuprofen (12).282
Besides synthesis, evaluation of physico-chemical properties of N-Mannich bases of ibuprofen (12) with morpholine and piperidine, as the amine component has been reported.\textsuperscript{283} N-hydroxymethyl derivatives of ibuprofen (12) have been synthesized by condensing 2(4-isobutylphenyl)propionamide with several active hydrogen containing compounds e.g. antipyrine, pyrrolidine, piperidine, phthalimide, morpholine, piperazine and hydrazine. The compounds synthesized (170-177) were found to have better antiinflammatory and gastroprotective activity than the parent drug.\textsuperscript{284}

With the aim of extending drug action and shielding the carboxylic acid group Shaaya et al. has reported the synthesis and \textit{in vivo} pharmacological evaluation of mixed anhydrides of ibuprofen with fatty acids of different chain length. The extended analgesic effect for over 24 hours in rodent model was found to be a function of fatty acid chain length.\textsuperscript{285}
Zovko and coworkers have reported synthesis and spectroscopic characterization of various amide prodrugs (ketoprofenamides, 178-186) of ketoprofen (16).\textsuperscript{286}

\begin{equation}
(178) \quad R = \text{OH} \quad (182) \quad R = \text{NHCH}_2\text{CH}_2\text{OH} \quad (186) \quad R = \text{HNCH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NH}_2
\end{equation}

\begin{equation}
(179) \quad R = \text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \quad (183) \quad R = \text{NHCH}_2\text{CH}_2\text{CH}_2\text{OH} \quad (184) \quad R = \text{N(CH}_2\text{CH}_3)_2
\end{equation}

\begin{equation}
(180) \quad R = \text{NHCH}_2\text{CH}_2\text{COOH} \quad (185) \quad R = \text{N(CH}_2\text{CH}_2\text{OH})_2
\end{equation}

Omar and coworkers have reported the synthesis and antiinflammatory activity of some 1,3,4-oxadiazole derivatives (187-190) of ibuprofen (12).\textsuperscript{287} Compound (188) was found to have better antiinflammatory and antiulcer activity than the parent drug. Omar has also reported some N-hydroxymethylphthalimide esters of ibuprofen, naproxen and aspirin, to be useful non-ulcerogenic prodrugs of acidic NSAIDs.\textsuperscript{288} Two additional analogous cyclic amides, N-hydroxymethylsuccinimides 191-194 and N-hydroxymethylisatins 195-198 were synthesized as alternate promoieties to N-hydroxymethylphthalimide. In contrast to the derivatives, the parent drugs treated groups were found to be ulcerogenic in stomach.\textsuperscript{289}

\begin{equation}
(187) \quad R = \text{CH}_2\text{CH}_3 \quad (189) \quad R = \text{-}
\end{equation}

\begin{equation}
(188) \quad R = \text{-} \quad (190) \quad R = \text{-}
\end{equation}

Omar has also reported some N-hydroxymethylphthalimide esters of ibuprofen, naproxen and aspirin, to be useful non-ulcerogenic prodrugs of acidic NSAIDs.\textsuperscript{288} Two additional analogous cyclic amides, N-hydroxymethylsuccinimides 191-194 and N-hydroxymethylisatins 195-198 were synthesized as alternate prodrugs.\textsuperscript{289}
promoieties to N-hydroxymethylphthalimide. In contrast to the derivatives, the parent drugs treated groups were found to be ulcerogenic in stomach.\(^{289}\)

\[
\begin{align*}
(191-194) & \quad R = \text{CH}_3, \text{CH}_2, \text{H}_2\text{CO} \\
(191), (195) & \quad (193), (197) \\
(192), (196) & \quad (194), (198)
\end{align*}
\]

Ibuprofen (12) has been modified into various heterocyclic amide derivatives\(^{290}\) having improved analgesic activity and lower ulcerogenic effects, as \(N-(\beta\text{-hydroxyethyl})\)-dl-2-(4'-isobutylphenyl)propionamide (aminoprofen, 199), an amide derivative of ibuprofen has been used for its topical antiinflammatory activity.

\[
\begin{align*}
(199)
\end{align*}
\]

Potential prodrugs of NSAIDs have been synthesized as agents for targeted drug delivery to the CNS, as recently NSAIDs have been proposed to prevent or to cure Alzheimer’s disease.\(^{291}\) The carboxylic group of diclofenac (6), ibuprofen (12), and ketoprofen (16) has been attached to 1,4-dihydro-1-methylpyridine-3-carboxylate moiety, which acts as a carrier, via an amino alcohol bridge. The prodrugs synthesised (200-211) have been found to be very good candidates for blood brain barrier (BBB) penetration.
INTRODUCTION

Khan and Akhter have reported the synthesis, hydrolysis and pharmacological evaluation of glyceride prodrugs of diclofenac (6). The prodrugs (212-213) were synthesized by reacting 1,2,3-trihydoxypropane-1,3-dipalmitate/stearate with acid chloride of diclofenac. In vitro studies showed that the prodrugs were resistant to hydrolysis at pH 3, 4 and 5, but rapidly hydrolysed at pH 7.4 as shown by HPLC analysis. In in vivo study, the concentration of diclofenac (6) released by the prodrugs was higher in animals administered with these prodrugs, as compared to animals treated with diclofenac (6). In addition, the prodrugs showed better antiinflammatory, analgesic and anti-ulcer activities than the parent drug.

Some new thiomer-diclofenac conjugates having thiolated and nonthiolated polyaspartamides as polymeric components have been synthesised and tested for their antiproliferative effects. These hydrophilic, bioadhesive, polymeric prodrugs enable inhibition of tumor cell growth with significantly lower doses of active substance. PAHMA-Dic i.e Poly[α,β-(n-2-aminoethyl-DL-aspartamide)]-poly[α,β-N-3-mercapto-2-
methoxycarbonyl-propyl-DL-aspartamide) copolymer diclofenac conjugate (214) showed considerable antitumor activity.

Metwally and co-workers have modified ibuprofen (12), flurbiprofen (14) and naproxen (13) into 1,2,4-triazoles derivatives (215-222, 223-230 and 231-239, respectively), and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles derivatives (240-241 and 246-248; 242-243 and 249-251; and 244-245, respectively). The compounds were found to possess comparable antiinflammatory and lesser gastrototoxicity in comparison to the parent drugs.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Ar</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>(215-222)</td>
<td>4-Isobutylyphenyl</td>
<td>H, 4-Cl, 3-Cl, 4-Br, 3-Br, 4-F, 4-CH₃ and 4-OCH₃, respectively.</td>
</tr>
<tr>
<td>(223-230)</td>
<td>2-Fluorobiphenyl</td>
<td>H, 4-Cl, 3-Cl, 4-Br, 3-Br, 4-F, 4-CH₃ and 4-OCH₃, respectively.</td>
</tr>
<tr>
<td>(231-239)</td>
<td>6-Methoxynaphthyl</td>
<td>H, 4-Cl, 3-Cl, 4-Br, 3-Br, 4-F, 3-F, 4-CH₃ and 4-OCH₃, respectively.</td>
</tr>
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</table>
Ester prodrugs of ibuprofen synthesized using α-methyl, ethyl and propyl glucopyranosides as promoietyes have been reported to undergo rapid cleavage inside the biological system and elicit a pharmacological profile quite similar to that of ibuprofen on oral administration, but, unlike the parent drug, they displayed reduced gastric ulceration.295,296

For reducing the gastrointestinal toxicity associated with ibuprofen, ester prodrugs with 1,2,3-trihydroxypropane-1,3-dipalmitate/ stearate were prepared and evaluated.297 Ibuprofen, naproxen and ketoprofen were linked to chondroitin sulfate (ChS) via a PEG 1000 as spacer. The ketoprofen-ChS conjugate was found to be susceptible to degradationin presence of esterases and chondroitinase with the liberation of ketoprofen and ChS.298

1.6.B.2 CODRUG CONCEPT

Generally, in a carrier linked prodrug, the carrier or promoiety used is inert and nontoxic. However, in certain cases, the prodrug comprises two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent. Such derivatives are termed as mutual prodrugs (Figure 19) and in the recent past,
there has been an increased interest in using mutual prodrug concept in prodrug design.\textsuperscript{299,300} A mutual prodrug can be a bipartate or tripartate prodrug. Recently, mutual prodrugs are referred as codrugs. The difference in prodrug and codrug approach is that an inactive promoiety is replaced with an active drug. The active drugs are coupled directly, or by means of a biolabile spacer to a single molecule, in which the drugs act as promoieties for each others. The biolabile bond or spacer is expected to hydrolyse after absorption, or in a target organ, and then release the active drugs. The basic requirement for the codrug approach is that the active drugs having functional groups suitable for linking them together and that this link can be severed \textit{in vivo}.\textsuperscript{301,302}

If two drugs are given separately, they are not necessarily absorbed simultaneously, or otherwise not transported to the site of action at the desired rate. Some of the advantages provided by codrugs include: improved absorption due to reduced polarity of the parent drug, quantitative and simultaneous release of parent drugs after absorption and possible synergistic pharmacological effects due to simultaneous release of the parent compounds.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{codrug.png}
\caption{The codrug concept.}
\end{figure}

Ideally, the active drugs of the codrug are directly coupled together; for example, by coupling a carboxylic acid group of one molecule to hydroxyl group of another to form single ester. The molecular weight remains relatively low and potentially harmful spacers are not released upon hydrolysis. Active drugs do not always have functional groups that are suitable for direct coupling, or the newly formed bond does not have desirable properties. When drugs are coupled together by a spacer, the chemical stability and the release of the parent drugs may be controlled by the structure of the spacer. The drawbacks of such structures are increased molecular weight and the possibility that the spacer may cause side-effects in the body.

The first codrug was in the clinical use long before the concept was introduced. Sulphasalazine (252) was synthesized in 1942 for the treatment of rheumatoid arthritis, where sulphapyridine was initially coupled to 5-aminosalicylic acid (5-ASA) by an azo-bond, which is cleaved by azo-reductases secreted by colonic microflora in the colon.\textsuperscript{303}
This releases the active agent 5-ASA in the colon, having antiinflammatory effect on the colon along with sulfapyridine. The advantage of this approach is that the cleavage of azo linkage and generation of 5-ASA prior to the absorption prevents its systemic absorption and helps it to concentrate at the active site.

Sulfapyridine was selected as a carrier in this mutual prodrug design by taking into account its antibacterial activity, but even though sulfapyridine proved to be a good carrier for targeting 5-ASA to colon, it gave rise to many side effects resulting from its systemic toxicity. Therefore, even if according to definition, sulfasalazine is a mutual prodrug, due to disadvantages of its carrier, it cannot be referred to as a true mutual prodrug. This led to the development of interesting mutual prodrug of 5-ASA called olsalazine (253), which is actually a diammer of 5-ASA, where 5-ASA is linked through azo linkage to one more molecule of 5-ASA. When it reaches the large intestine, it is cleaved, releasing two molecules of 5-ASA for every molecule of olsalazine administered. This design overcomes the drawbacks of sulfasalazine, targets 5-ASA to colon, and fulfills all requirements of mutual prodrug too. Improvement in the bioavailability of 5-ASA is also achieved by this design. Clinical trials have been encouraging, although watery diarrhoea has emerged as a new and troublesome side effect, affecting 15% of the patients. It appears to be related to a combination of GIT transit and stimulation of small intestinal secretion.

The codrug approach has also been successfully utilized in antibiotic therapy. Antibiotics, such as ampicillin, are often administered with β-lactamase inhibitors; e.g. penicillanic acid sulfone, which prevent degradation of antibiotics by lactamase enzymes in the bacteria. However, to be effective, both of these drugs should have identical absorption rates, distribution and duration of action profiles to be in an appropriate balance at the site of infection. This usually will not occur despite simultaneous administration, partly due to different absorption profiles and incomplete...
absorption. Ampicillin-penicillanic acid sulfone (254) and mecillin-penicillanic acid sulfone codrugs were designed to overcome these problems. The carboxylic acid groups were employed for linking the two drugs, which resulted in chemically stable codrugs. Despite of relatively high molecular weight of the codrugs, they were absorbed almost completely from GI tract, due to an active uptake mechanism and increased lipophilicity. The parent drugs were rapidly and quantitatively released in a 1:1 ratio in human plasma, which is optimal for the desired antibiotic effect.

Probenecid is a drug that retards urinary excretion of ampicillin and its codrug was designed to prolong blood levels of ampicillin (255). The parent drugs were coupled with a methylene or ethylene spacer, which was expected to stabilize the codrug. A similar approach was applied in the design of codrugs of mecillinam coupled with benzylpenicillin, ampicillin, penicillin or amoxicillin in order to utilize the synergy often seen between these two classes of β-lactam antibiotics. These codrugs offer a broad spectrum antibiotic effect and are well absorbed from the small intestine.

Another example of a mutual prodrug is sultamicillin (256). In the design of sultamicillin, the irreversible β-lactamase inhibitor sulbactam has been combined chemically via ester linkage with ampicillin. This design is based on the rationale that as sulbactam, a β-lactamase inhibitor with very limited antibacterial activity in a physical
mixture with ampicillin, clearly enhances the activity of the latter against certain β-lactamase-producing bacteria, both in vitro and in vivo, the same phenomenon might hold true when these two drugs are linked chemically. Upon oral administration, sultamicillin is completely hydrolyzed to equimolar proportions of sulbactam and ampicillin, thereby acting as an efficient mutual prodrug.

The mutual prodrug effect produced by sultamicillin results from its having a more efficient oral absorption than the single agent does. Peak serum concentrations of ampicillin are achieved that are approximately 3.5-fold those obtained with an equivalent amount of oral ampicillin. Equimolar concentrations of sulbactam are also provided with both ampicillin and sulbactum, being widely distributed among various body fluids and tissues. The pharmacokinetic parameters of the two components are similar, both being eliminated primarily by renal excretion. Although the elimination half-lives of ampicillin and sulbactam are each approximately 1 hr, the high serum concentration achieved, coupled with their synergistic activity permit twice-daily dosing. One more important advantage presented by sultamicillin is that even though most β-lactamase-resistant antimicrobials must be given parenterally, sultamicillin is given by mouth. It has been found to be effective against skeletal infections in children, urinary infections in geriatric patients and uncomplicated gonorrhoea. β-Lactamase inhibitor sulbactam has also been linked to mecillinam. The mecillinam sulbactum mutual prodrug is well absorbed in man. This has been achievement in oral absorption since neither of its individual components is orally absorbed to any appreciable degree.
Similarly, olivanic acid – (Z)-2-isovaleramidobut-2-enoic acid codrugs ($R=\text{H or }\text{SO}_3\text{H}$) employ a methylene spacer for coupling these two drugs (258). Olivanic acids possess antibacterial activity, but are extensively metabolized by the renal dipeptidase inhibitor results in improved urinary recovery of the antibiotic. A codrug of these two drugs results in a six-fold urinary recovery of the antibiotic in mice. The antibacterial activity has, however, eight times less than the parent antibacterials, which is most probably caused by the slow release of parent drugs from the codrug.

Cephalosporins covalently bind to bacterial enzymes and open the β-lactam ring, accompanied by the liberation of 3'-substituent (Figure 20), which acts as a leaving group. In this case, the release mechanism of the parent drugs is closely related to that of bioprecursor prodrug because the activation is a consequence of an intermolecular reorganization that is initiated by specific enzyme(s), instead of a simple hydrolysis of an ester bond. This property has been applied in the design of dual action cephalosporins (DAC), which spontaneously release their 3'-substituent which has an antibacterial activity of its own. The antibacterial activity of cephalosporin-quinolone codrugs have also been evaluated. Ro-23-9424, for example was the most active and most thoroughly studied codrug in this series (Figure 20).
The antibacterial activity of cephalosporin-quinolone codrugs have also been evaluated. Ro-23-9424, for example was the most active and most thoroughly studied codrug in this series (Figure 20). It had excellent activity, both in vitro and in vivo, and is the first codrug from this group that has entered into clinical trials. Quinolones (e.g. ciprofloxacin) have also been coupled to cephalosporins through an ester quarternary nitrogen ester or carbamate linkages. These codrugs employ the same release mechanism presented in, and have been found to possess a broad spectrum of antibacterial activity. Their stability toward β-lactamase is high and the intestinal absorption is almost complete. Penems and carabapenems, structurally analogous to cephalosporins, can release quinolones by a similar mechanism. Additionally, they have good stability towards anaerobic organisms and β-lactam enzymes, and high oral activity.

A number of prodrugs with antibacterial activity have been reported which are composed of antiseptic phenols, antiinflammaory activity and polyethyleneglycol moieties attached by labile bonds to quinolone derivative oxolinic acid. Some of these derivatives have been found to be more soluble in water, exhibiting more sustained action and gave higher plasma and tissue concentration as compared to free drugs. Notable among these derivatives are 259 and 260.
Based on the property of cephalosporins to release 3'-substituted groups on cleavage of the β-lactam bond, a number of cephalosporins substituted at 3'-position with variety of oncolytic agents have been synthesized and evaluated as potential prodrugs for the treatment of solid tumors.\textsuperscript{325-329} These cephalosporins based prodrugs act as substrate for an antibody targeted enzyme. In this two step approach a monoclonal (MoAb) β-lactamase conjugate is utilized to deliver the enzyme to the antigen present on tumor cell surface and subsequent administration of the relatively nontoxic prodrug allows specific enzyme catalyzed release of the active cytotoxic agents at the tumor site as shown in Figure 20. The selection of β-lactamase enzyme has been done because of its narrow specificity for its substrate (β-lactam), as well as its good catalytic activity without metal ions or cofactors. Additionally, the enzyme is not endogenous to mammalian system and therefore, is subjected to minimal interference from inhibitors, substrate or endogenous enzyme systems.\textsuperscript{327}

Based on this principle, Alexander and coworkers\textsuperscript{325} synthesized a series of cephalosporin nitrogen mustard carbamates, for example 261, as potential prodrugs for interaction with a MOAb, β-lactamase tumor targeting system. These derivatives have been studied for their prodrug potential by their exposure to β-lactamase and found to
release free nitrogen mustards. This concept has been named as Antibody Directed Prodrug Therapy (ADEPT).\textsuperscript{325}

Another research group also reported cephalosporin phenylenediamine mustard (\textsuperscript{262}) as anticancer prodrug that has been found to be activated in a site specific manner by MoAb β-lactamase conjugates targeted to the tumor cells.\textsuperscript{326}

Using same principle, a number of cephalosporin-vinca alkaloid prodrugs have been designed and developed by Jungheim’s group.\textsuperscript{327,328} One such example is that of cephalosporin prodrug 263, substituted at 3-position with the potent cytotoxic agent desacetylvinblastine hydrazide. This derivative has been found to be an excellent substrate for the MoAb β-lactamase conjugate.

Similarly, cephalosporin-doxorubicin prodrug BMY-46633 (\textsuperscript{264}) has been synthesized which has been found to release the paret drug doxorubicin efficiently in the presence of MoAb β-lactamase conjugate, indicating the achievement of codrug principle.\textsuperscript{329}
An example of potential codrug which has structural and mechanistic similarity with DACs is compound 265. Acetylsalicylic acid was attached at 4-carboxylic group of cephalosporin cefuroxime through its carboxylic moiety to form double ester 265 as codrug. The attached acetylsalicylic acid moiety was thought to prolong the biological half life of cefuroxime due to its high protein binding capability. From the \textit{in vivo} studies, it was observed that the attached moiety substantially increased the binding of cefuroxime molecule to the plasma protein. The compound was, however found unstable at physiological pH.\textsuperscript{330}
The special ability of cephalosporins to cleave off the 3'-substituent has been suggested for use in selective tumor directed chemotherapy, essentially to decrease toxicity of anti-cancer agents (Figure 20). A codrug of ATRA (all-trans-β-retinoic acid) with butyric acid (BA) and codrugs of various cephalosporins have recently been reported with promising anti-cancer activity and low toxicity in rats.\textsuperscript{331}

One of the major problems in cancer treatment is drug resistance. If cancer is not totally eliminated by the administration of single anti-cancer drug, the cancer cells will most probably become resistance to it. If two anti-cancer drugs are administered as a codrug, the ability of a cancer to create resistance to both drugs is unlikely.

Dacarbazepine has been widely used in the treatment of malignant melanoma as a DNA methylating agent.\textsuperscript{332} In the cases of the dacarbazepine-ibuprofen abd dacarbazepine-butyric acid (BA) codrugs, the active drugs are coupled together by a methyl carbamate spacer (266). These codrugs are chemically stable \( t_{1/2} = 664 \text{ min (BA) } t_{1/2} = 9870 \text{ min (ibuprofen) at pH 7.7,} \) and rapidly release the parent drugs in human plasma \( (t_{1/2} = 15 \text{ min (BA) } t_{1/2} = 125 \text{ min (ibuprofen))}. \)

Butyric acid has antineoplastic activity for a wide variety of cells \textit{in vitro}.\textsuperscript{333,334} It is specifically known to inhibit histone deacetylase and its biological effects have been
attributed to this activity. However, butyric acid suffers from low potency \textit{in vivo} due to its rapid metabolism. The metabolism of butyric acid was markedly decreased when it was coupled to another anti-cancer agent; all-trans-retinoic acid (ATRA) by a methylene spacer (267). The chemical and enzymatic stability studies of the codrug are incomplete; only the shelf-life at -20°C was determined to be over 1 month. The \textit{in vitro} activity of the codrug is thought to be one the reasons for its increased activity, compared to the parent compounds.\textsuperscript{334}

\begin{align*}
\text{(267)}
\end{align*}

Another example of anti-cancer codrug is the combination of 5-fluorouracine (FU) and cytarabine (268). The systemic toxicity of the parent drugs were expected to decrease when the active pharmacophores were blocked by a biodegradable spacer, prior to the release of the parent drugs at the desired site of action. The secondary aim was to decrease the susceptibility of deactivating the primary amine of cytarabine by cytidine deaminase, and thus the spacer serves two functions. The absorption of the codrug was reported.\textsuperscript{300}

\begin{align*}
\text{(268)}
\end{align*}

Unsymmetrical polar disulfide prodrug of paclitaxel with captopril \textsuperscript{335} has been designed and synthesized as reductively activated codrug. It has been tested on L-2987 lung carcinoma cells, and \textit{in vivo} evaluation in mice has exhibited significant regressions and cures. Synthesis of mutual prodrugs, viz., 3-acyloxymethoxycarbonyl-1-
aryl-3-methyltriazenes associating the antitumour monoethyltriazenes with antiinflammatory NSAIDs as well as with the anticancer agent butyric acid has been reported.\textsuperscript{335}.

The simultaneous administration of the calcium-blocker nifedipine, together with propranolol type \( \beta \)-blockers (\( R = \text{formyl or benzyl} \)), results in a reduction of cardiac episodes and duration of painful ischemia.\textsuperscript{62} However, propranolol type \( \beta \)-blockers have low lipophilicity, and it was expected that the codrug 269 would have increased lipophilicity and, thus, synergistic biological activity of the hydrolysis products of the codrug. Surprisingly, the codrug itself had better Ca-blocking and \( \beta \)-blocking effects of the codrug were evaluated, but the release rate of the parent drugs from the codrug was not reported.\textsuperscript{336}

A U.S. Patent of codrug of amlodipine and atorvastatin (270) has been issued on May 18, 2004 [U.S. Patent No. 6,737,430] for the treatment of atherosclerosis, angina pectoris, combined hypertension, hyperlipidaemia and management of cardiac risk.\textsuperscript{337} Amlodipine is 1,4-dihydropyridine derivative of nifedipine. It is a second-generation calcium channel blocker, which has greater selectivity for vascular smooth muscle than myocardial tissue when compared to nifedipine. It is used in the treatment of chronic stable angina and management of mild to moderate essential hypertension, but it lacks the antihyperlipidaemic effect. On the other hand, atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase, an enzyme that catalyzes conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Its mechanism of LDL-lowering effect involves both reduction of VLDL concentration and induction of LDL receptor, leading to reduction in production of LDL or increased catabolism of LDL.
This lipid-lowering effect of atorvastatin indirectly helps to make the treatment and management of atherosclerosis, angina pectoris, hypertension and cardiac risk much easier than is possible independently by either amlodipine or atorvastatin. These two drugs are linked together by amide bond. Hydrolytic cleavage of this bond in vivo, releases the free drugs in the body. Ursodeoxycholic acid (UDCA) has been shown to dissolve gallstones by making bile unsaturated with cholesterol. N-acetylcysteine (NAC) and 2-mercaptopropionylglycine (MPG), on the other hand, lower the viscosity of bronchial and biliary mucus by reducing disulfide bonds in protein and dissolve macromolecular complex of mucine and bilirubine in the gallstone matrix. Using this rationale, mutual prodrugs of UDCA with NAC and MGP53 have been synthesized, which may potentially increase the efficacy of gallstone dissolution by combining both the mechanisms of action.

It is believed that an unstable arachidonic acid metabolite, thromboxane $A_2$ (TXA$_2$), may be involved in the pathogenesis of cardiovascular disease. Clinical trials of the TXA$_2$ receptor antagonist and TXA$_2$ synthase inhibitors have given promising results. However, the directly coupled ester codrug of these drugs did not show biological activity, most probably because of poor accessibility of the ester group for
hydrolysis. Therefore, the drugs were coupled by spacers (271). The kinetics of chemical and enzymatic hydrolyses was not studied in vitro prior to ex vivo studies. However, ex vivo antagonist or inhibitory activity was not observed after administration of these codrugs to dogs. The release of the active compounds could not be verified in vivo from these codrugs.338

A novel codrug 272, in which L-Dopa and entacapone are linked via a biodegradable carbamate spacer to form a single chemical entity, was synthesized and studied kinetically by Jukka Leppanen et al. This carbamate codrug provides adequate stability \([t_{1/2}]\) 12.1 h (pH 1.2); 1.4 h (pH 5.0); 1.1 h (pH 7.4)] against chemical hydrolysis but rapidly hydrolyzes to L-Dopa and entacapone in liver homogenate \((t_{1/2} = 7\) min; pH 7.4) at 37 °C, thus fulfilling the codrug criteria.339

A series of multifunctional codrugs (273-276), obtained by joining L-Dopa (LD) and dopamine (DA) with (R)-R-lipoic acid (LA), was synthesized and evaluated as potential codrugs with antioxidant and iron-chelating properties by Antonio Di Stefano et al. These multifunctional molecules were synthesized to overcome the pro-oxidant effect associated with LD therapy. The physicochemical properties, together with the chemical and enzymatic stabilities of synthesized compounds, were evaluated in order
to determine both their stability in aqueous medium and their sensitivity in undergoing enzymatic cleavage by rat and human plasma to regenerate the original drugs. The results indicate that codrugs show good stability toward G.I.T hydrolysis and release LD and DA in human plasma after enzymatic hydrolysis.\textsuperscript{340}

Further, a novel series of codrugs (277-280), in which L-dopa (LD) is linked covalently via an amide bond with glutathione (GSH), were synthesized and evaluated as potential anti-Parkinson agents with antioxidant properties by Antonio Di Stefano et al. These conjugates were characterized by evaluating solubility, chemical and enzymatic stabilities, and apparent partition coefficient (log $P$). The results suggest that compounds 277 and 280 could represent useful new anti-Parkinson agents devoid of the pro-oxidant effects associated with LD therapy and potentially able to restore the GSH depletion evidenced in the substantia nigra pars compacta (SNpc) of PD patients.\textsuperscript{341}

(273) $R=\text{COCH}_3$, (274) $R=\text{H}$

(275) $R=\text{COCH}_3$, (276) $R=\text{H}$

(277) $R=\text{Ac}$ ; $R'=\text{Me}$
(278) $R=\text{H}$ ; $R'=\text{H}$
A series of N-substituted-quinolinone-3-aminoamides and their hybrids containing the R-lipoic acid functionality were designed and synthesized by Anastasia Datsi et al as potential bifunctional agents combining antioxidant and antiinflammatory activity. The new compounds were evaluated for their antioxidant activity and for their ability to inhibit in vitro lipoxygenase as well as for their antiinflammatory activity in vivo. The result of the in vivo experiment reveals that all the synthesized quinolinone-LA hybrids (compounds 281-285) exhibit significantly higher activity than LA. The chemical stability of the compounds shows that they constitute new, promising, antiinflammatory agents as intact molecules and are not LA prodrugs. This provides an impetus for designing new antiinflammatory agents using the quinolinone-LA scaffold as the starting point.

1.6.B.3 Codrugs of NSAIDs

Another class of therapeutic agents where codrug approach has been utilized is nonsteroidal antiinflammatory drugs (NSAIDs). Despite the intensive research that has been aimed at the development of safer NSAIDs, their clinical usefulness is still restricted by their GI side effects like gastric irritation, ulceration, bleeding, and perforation, and in some cases may develop into life threatening conditions. Hence,
The codrug approach is used to develop safer NSAIDs devoid of their gastrointestinal side effects.

Benorylate (286) is a codrug of aspirin and paracetamol, linked through ester linkage, which claims to have decreased gastric irritancy with synergistic analgesic action. Glycine amide conjugate of tolmetin (287) as codrugs was reported to have less ulcerogenicity with better antiinflammatory/analgesic action than its parent drug, tolmetin. Mutual prodrugs of tolmetin with paracetamol 288, and of aspirin with salicylamide 289 have been evaluated with the aim of abolishing the gastrointestinal toxicity of these drugs. Similarly, a cyclic paracetamol acetylsalicylic acid ester codrug, paracetasal (290) has been reported, which undergoes enzymatic hydrolysis to release the parent drugs, acetylsalicylic acid and paracetamol and had reduced gastric irritancy.
Codrugs of 4-biphenylacetic acid have been synthesized. These are paracetamol and salicylamide (291, 292) derivative of 4-biphenylacetic acid, which have been found to retain the antiinflammatory activity of the parent drug.348

\[
\begin{align*}
&\text{Paracetamol} \\
&\text{Salicylamide}
\end{align*}
\]

The drug conjugate 293 of flurbiprofen with a histamine $H_2$ receptor antagonists, $N\text{-}[3\text{-}(3\text{-}(1\text{-piperidinomethyl)phenoxy})\text{propyl}]\text{-}2\text{-}(2\text{-hydroxyethylthio})$ acetamide was synthesized and investigated by Imai and coworkers for obtaining reduction in gastric damage.349

A significant reduction in gastric toxicity in comparison to an equivalent dose of flurbiprofen and methyl ester of flurbiprofen was observed with rapid plasma catalysed hydrolysis suggesting that the drug complex of flurbiprofen with $H_2$ antagonist is superior to simple ester or plain drug in its therapeutic profile. A new term has been introduced for mutual prodrug called chimera drug.350

\[
\begin{align*}
&\text{Flurbiprofen} \\
&\text{H2 Antagonist}
\end{align*}
\]
The ester prodrug 2-[N-[3-(3-(1-piperidinomethyl)phenoxy)propyl]carbamoyl methylthio]ethyl-1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate 294 of an H₂-antagonist and indomethacin was shown to be essentially similar to indomethacin in its antiinflammatory potency. Codrug almost completely inhibited carrageenan-induced hind-paw edema and had low ulcerogenicity that resulted in twenty-fold improvement in the ratio of anti-edema activity to ulcerogenicity.351

Chlorzoxazone [5-chloro-2 (3H)-benzoxazolone] is a centrally active muscle relaxant, while acetaminophen (N-acetyl-p-aminophenol) exhibits analgesic properties. Owing to their synergistic effects, these two drugs can be prescribed together.352 Combinations of various oxazolidinones (skeletal muscle relaxants and analgesics agents) with acetaminophen (paracetamol) were observed to be therapeutically superior to their individual components, and their combination in a single codrug was expected to offer therapeutic advances.353 Using this rationale, a codrug of chlorzoxazone and acetaminophen (295) has been designed, and its synthesis and kinetics have been reported.354 Similarly codrugs of acetaminophen with metaxalone (296) and mephenoxalone (297) have been synthesized by Vigroux et al.355 These codrugs proved to be therapeutically superior than their individual components i.e. better muscle relaxant and analgesic combination.

![Figure 21](image)

The carbamate ester of acetaminophen and various oxazolidinones (chlorzoxazone and metaxalone) release the parent drugs in vivo (Figure 21). Release of the open form of the oxazolidone resulted in an unstable isocyanate, which
spontaneously cyclized to chlorzoxazone-acetaminophen codrug was chemically unstable ($t_{1/2} = 7.1 \text{ s}$; pH 7.4) and released the parent drugs quantitatively in human plasma, but at slower rate ($t_{1/2} = 66 \text{ s}$). The metaxalone-acetaminophen codrug released the parent drugs in human plasma at the same rate ($t_{1/2} = 45 \text{ s}$) as the chlorzoxazone-acetaminophen codrug, but was more stable towards chemical hydrolysis ($t_{1/2} = 416 \text{ s}$; pH 7.4). Enzymatic release of the parent drugs was rapid and quantitative, which suggests the usefulness of the carbamate function for the codrug design, although the chemical stability of these codrugs was generally low.353

![Figure 21: The release mechanism of parent drugs from oxazolidinone-acetaminophen and metaxalone-acetaminophen codrugs.](image)

Codrugs of chlorzoxazone with NSAIDs; ibuprofen, naproxen and diclofenac (298-300) have been synthesized and evaluated with the aim of minimizing the gastrointestinal toxicity of NSAIDs and improving pharmacokinetic properties of both chlorzoxazone and NSAIDs while maintain the useful antiinflammatory and skeleton muscle relaxation activities.356

![Diagram of codrugs 298 and 299](image)
Glycine methyl ester conjugate of ketoprofen (301)\textsuperscript{357}, histidine methyl ester conjugate of diclofenac (302)\textsuperscript{355}, and various conjugates of flurbiprofen (303a-d) with amino acid like L-tryptophan (a), L-histidine (b), phenylalanine (c) and alanine (d) as codrugs\textsuperscript{359} were reported to have less ulcerogenicity with better antiinflammatory/analgesic action than their parent drugs.
Codrugs of ibuprofen with paracetamol (304) and salicylamide (305) have been reported with better lipophilicity and reduced gastric irritancy than the parent drug.\textsuperscript{360}

![Codrugs](image)

Fadl and Omar have reported the mutual prodrug of paracetamol and some acidic NSAIDs with faster rates of release of the corresponding NSAIDs ($t_{1/2} = 15-385$ min) and paracetamol (1-140 min).\textsuperscript{361} A significant improvement in latency of pain threshold in mice has been observed up to 4 h after p.o. administration of 0.02 mmol/kg of the prodrugs, when compared to the corresponding physical mixtures. Synthesis of mutual prodrugs, viz., 3-acyloxymethoxy carbonyl-1-aryl-3-methyltriazenes associating the antitumor mono ethyltriazenes with antiinflammatory NSAIDs as well as with the anticancer agent butyric acid has been reported.\textsuperscript{362,363}

With the aim of improving the therapeutic index through prevention of gastrointestinal irritation and bleeding, naproxen–propyphenazone codrug (306) was synthesized as mutual prodrug.\textsuperscript{364}

![Codrug](image)

With the goal of combining high antipyretic activity of paracetamol into commonly used NSAIDs, seven different NSAIDs were chemically combined with $p$-aminophenol to yield the $p$-amidophenol derivatives.\textsuperscript{365} These were acetylated at the phenolic hydroxyl group to yield corresponding acetate derivatives for evaluating the impact of
blocked phenolic hydroxy group on the biological activity of these derivatives. Only the $p$-amidophenol derivatives showed improved antipyretic activity over paracetamol with retention of antiinflammatory activity and no ulcerogenicity.

Codrug of indomethacin with paracetamol (307) have been reported by prakash and group with better lipophilicity. Codrug was found to dissociate in the intestinal pH because of less partition coefficient in octanol phosphate buffer. These result indicates that codrug may be devoid of GIT irritation due to its unionized form in stomach and ionized form in intestine and may have synergic action due to its parent drugs.

Codrugs of ketoprofen (308a), ibuprofen (308b), diclofenac (308c) and flurbiprofen (308d) with an antiarthritic nutraceutical D-glucosamine have been reported with reduced gastrointestinal ulcerogenicity, better analgesic/antiinflammatory effects and additional antiarthritic activity. Glucosamine is used as an antiarthritic drug and nutritional supplement in conditions like joint ache, stiffness, severely restricted movements and serious pain. It acts as an essential substrate for the biosynthesis of glucosaminoglycans and the hyaluronic acid backbone needed for formation of proteoglycans found in the structural matrix of joints. NSAIDs are used for the symptomatic treatment of inflammation associated with arthritis but are unable to remove the underlying cause of the disease. Their prolonged use results in GI side effects. When tested in Fruend’s adjuvant-induced arthritis assay, these codrugs have shown antiarthritic activity, which was lacking in the parent drugs with comparable antiinflammatory activity and lowered ulcerogenicity.
Another codrug of 5-ASA has been reported, where 5-ASA is conjugated with ursodeoxycholic acid (UDCA). UDCA is the bacterial product of chenodeoxycholic acid and has application in gallstone dissolution and treatment of cholestatic liver diseases. Recent studies have also shown that UDCA may be beneficial in colonic polyp reduction. It has been shown that UDCA-5ASA codrug (309) is poorly absorbed from intestine and is targeted to colon, where it is partially hydrolyzed to UDCA and 5-ASA. While a portion of UDCA-5-ASA escapes bacterial cleavage, part of the UDCA is absorbed from the colon, enters enterohepatic circulation, is converted into taurine conjugate by hepatic enzymes and is secreted into the bile. It is postulated that both 5-ASA and UDCA may exhibit their antiinflammatory and cytoprotective effects in colon as well as liver. UDCA has also shown to inhibit polyp formation in experimental rats. As patients with ulcerative colitis are at a greater risk of primary sclerosing cholangitis (PSC) and as UDCA has been reported to be beneficial in PSC, the enterohepatic
circulation of UDCA generated in colon may be cytoprotective to the hepatocyte in these patients.\textsuperscript{373,374}

\begin{align*}
\text{UDCA} & \quad \text{L-Tyrosine} \\
\text{H} & \quad \text{CONH} \\
\text{H}_2\text{C} & \quad \text{COOH}
\end{align*}

\textbf{(309)}

Similarly, another codrug of 5-ASA (310) has been reported by Dhaneshwar et al, where 5-ASA is conjugated with \textit{L}-tyrosine for targeted drug delivery to the inflamed gut tissue in inflammatory bowel disease. \textit{L}-Tyrosine was chosen as promoiety due to its marked antiinflammatory activity. Being natural component of our body, it would be nontoxic and free from any side effect. Codrug showed comparable mitigating effect as that of sulfasalazine on colitis in rats.\textsuperscript{375}

\begin{align*}
\text{HO} & \quad \text{NaO} \\
\text{HOOC} & \quad \text{N} = \text{N} \\
\text{NH} & \quad \text{HO}
\end{align*}

\textbf{(310)}

In recent years, it has been well established that generation of reactive oxygen species (ROS) plays a decisive role in the ulceration of GI. ROS generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cell may contribute to gastric mucosal damage.\textsuperscript{376-384} NSAIDs affect a variety of enzyme systems, resulting in an increased ROS concentration within the cell, with irreversible damage to proteins, nucleoproteins, and DNA. There is enough experimental and clinical evidence indicating that the ulcerogenic capacity of ethanol, NSAIDs and of \textit{Helicobacter pylori} is mediated by ROS (\textit{vide infra}).\textsuperscript{385}

Based on the observation that NSAIDs induced ulcerogenic side effects are mediated via the involvement of free radicals, it has been suggested that
coadministration of antioxidants and NSAIDs in formulated dosage form may possibly decrease the risk of NSAIDs induced gastrointestinal side effects. However, there is an added advantage in giving such agents in the form of a single chemical entity. Such hybrid molecules consisting of two different therapeutic agents having complementary pharmacological activities are named as codrugs, which are designed with improved physicochemical properties and at the same time release the parent molecules at the desired site of action. However, recently NSAID-antioxidant codrugs have attracted attention of many medicinal chemists and number of such derivatives has been reported.

Cioli et al. have investigated the toxicological and pharmacological profile of ibuprofen-guaiacol ester 311. The gastrointestinal toxicity, behavioral disorders and acute toxicity of the ester was much reduced in comparison to ibuprofen. The ester and the parent drug at equimolar doses, were equally effective in edema and fever. The ester was found to be better tolerated than its parent drug because of its peculiar pharmacokinetics i.e. the slow release of the parent drug, which reduced its local and general toxicity.

MED-15, nonacidic NSAID, in which tolmetin is linked to glycine through an amide bond, whereas glycine moiety in turn is linked to guaiacol with ester bond is reported by Amico Roxas et al. Oral administration of amtolmetin guaiacol codrug (312) i.e. MED-15 showed marked antiinflammatory activity similar to tolmetin with reduced gastric ulceration. It also possesses high antiaggregatory activity comparable to tolmetin. Hence it is recommendable in the treatment of inflammatory and thromboembolic conditions where long term therapy is required.
A number of NSAID-cysteamine conjugates (313-316, Figure 22) have also been reported. These agents are non-acidic and have significant antioxidant activities attributed to cysteamine moiety and reported to exhibit antiinflammatory activity similar to or higher than the parent NSAIDs with significant reduction in ulcerogenicity. All the synthesized compounds offered significant protection against in vitro lipid peroxidation.

Figure 22: Structures of NSAID cysteamine mutual prodrugs namely diclofenac-cysteamine (313), tolfenamic acid-cysteamine (314) indomethacin-cysteamine (315) and ibuprofen-cysteamine (316).

A series of phenolic antioxidants have been synthesized and conjugated with naproxen through ester and amide linkages to give naproxen - antioxidant conjugates (317-318) to be used in ocular surgery. These ester and amide derivatives are designed
as codrugs to deliver both naproxen (NSAID) and an antioxidant to the wound environment following metabolism. Unexpectedly, these derivatives were found to be unique as they possess both antioxidant and antiproliferative activity.\textsuperscript{395}

Similarly, ibuprofen has been conjugated with number of curcuminoids. For example, ibuprofen-dehydrozingerone derivative (319) was studied for pharmacological evaluation and found to exhibit strong antiinflammatory activity without any gastrointestinal toxicity in animal studies.\textsuperscript{396}

Similarly, mefenamic-guaiacol ester (320) was found to be stable at wide pH range from 1-10. It has been observed that chemical and enzymatic hydrolysis of this agent is delayed significantly. This derivative exhibited retention of antiinflammatory activity of the parent drug, with increased gastrointestinal tolerance.\textsuperscript{397}
Ascorbic acid - diclofenac conjugate (321) and 6-amino-ascorbic acid-diclofenac conjugate (322) are another examples of NSAID–antioxidant codrugs with enhanced lipophilicity so that it could permeate through blood brain barrier.\textsuperscript{398,399}

Vitamin C conjugate of acetylsalicylic acid as codrug was reported by Konturek and coworkers, to have less ulcerogenicity with better antiinflammatory/analgesic action than their parent drug due to the attenuation of oxidative stress and proinflammatory cytokines.\textsuperscript{400} A series of amide derivatives (323-329, Figure 23) of different conventionally used NSAIDs with L-cysteine ethyl ester have been reported.\textsuperscript{401} The sulfhydryl (-SH) group of the latter moiety is likely to confer antioxidant properties to the novel compounds. This molecular modification has resulted in the formation of compounds with antioxidant and free radical scavenging properties. These derivatives exhibited enhanced antiinflammatory activity with drastically reduced gastrointestinal toxicity.
Figure 23: Structures of various NSAID-L-cysteine ethyl ester codrugs (323-329).

4-Biphenylacetic acid (BPA, 330) is one of the active metabolites of NSAID fenbufen (331). This metabolite has been marketed as gel for local application in treatment of inflammation and pain. However, on oral administration BPA has been found to exhibit ulcerogenic side effects.
To extend the therapeutic use of this potential NSAID, a number of BPA-antioxidant codrugs have been prepared. For this purpose, different naturally occurring antioxidants have been conjugated through its −COOH group, directly (332-335) as well as through spacer (336-339). Similarly a number of diclofenac-antioxidant codrugs (340-343) have been prepared. (Figure 24)

Figure 24: Structures of BPA-antioxidant and diclofenac-antioxidant codrug.

Codrugs of ibuprofen with eugenol i.e. ibuprofen-eugenol ester (344), have been evaluated with the aim of reducing the gastrointestinal toxicity of the ibuprofen while retaining its antiinflammatory activity. Ibuprofen-eugenol ester (344) is highly lipophillic compound hence, its microemulsion formulation for parenteral delivery has been prepared by Zhao and coworkers.
A series of indomethacin phenolic antioxidants conjugates (345-352) have been synthesized with the objective of reducing ulcerogenic potential of indomethacin, as shown in Figure 25.

It was found that all conjugates, 347-352, were very potent antioxidants in vitro. However, these agents showed little inhibition against croton oil induced mouse ear...

---

Figure 25: Structures of indomethacin-antioxidant conjugates (347-352).

It was found that all conjugates, 347-352, were very potent antioxidants in vitro. However, these agents showed little inhibition against croton oil induced mouse ear...
swelling which may be due to the stability of the conjugates, resulting in not releasing this parent NSAID indomethacin, \textit{in vivo} quantitatively.

Flavonoids including naringenin and hesperetin have also been conjugated with ibuprofen to give ibuprofen-antioxidant mutual prodrugs (353 and 354, respectively). These derivatives have been found to exhibit retention of the antiinflammatory activity of the parent NSAID and at the same time found to be devoid of GI ulcerogenic side effects. This may be due to the combined effect of the antioxidant properties of the promoieties and the masking of the free carboxylic group of parent NSAID, ibuprofen.407

\[
\begin{align*}
&\text{CH}_3 \\
&\text{H}_3 \text{C} \\
&\text{CH}_3 \\
&\text{O} \\
&\text{O} \\
&\text{OH} \\
&\text{OH}
\end{align*}
\]

(353)

Inflammation and oxidative stress are involved in the pathobiochemistry of neurodegenerative diseases. A number of epidemiological studies have shown a lower incidence of Alzheimer's disease (AD) when NSAIDs are taken on a regular basis. However, chronic use of NSAIDs in such conditions is seriously limited by their GI toxicity. A series of novel molecules have been designed and synthesized with a residue of a classical NSAID and an antioxidant moiety (Figure 26).408 In these agents, a NSAID and cysteamine or cysteine ethyl ester have been chemically attached to a proline, 4-hydroxy-proline or pipecolinic acid moiety. The compounds (355-361) were found to retain antiinflammatory and antioxidant activities, with hypocholesterolemic and a greatly reduced gastrointestinal toxic action. It has been proposed that these novel
compounds may find useful applications in slowing the progression or delaying the onset of neurodegenerative diseases.

![Chemical structures](image)

**Figure 26: List of indomethacin and naproxen-antioxidant conjugates (355-361).**

A novel group of alpha-lipoic acid-containing hydrophobic prodrugs of nonsteroidal antiinflammatory drugs (NSAIDs) was synthesized and transformed into nanometer-sized prodrugs (nanoprodrugs). Three NSAIDs, indomethacin, ibuprofen and naproxen were linked to alpha-lipoic acid via tetraethylene glycol through hydrolytically degradable ester bonds to form codrugs (362-367). Novel stimuli-responsive antioxidant nanoprodrugs of NSAIDs were prepared by spontaneous emulsification of hydrophobic derivatives of NSAIDs. Despite the highly hydrophobic nature of the derivatives, NSAIDs were readily hydrolyzed enzymatically from the nanoprodrugs, and the hydrolysis was accelerated when the nanoprodrugs were destabilized upon ROS scavenging. The unique interaction between the oxidative destabilization and enzyme reactivity characterizes this novel family of ROS-sensitive anti-inflammatory nanoprodrugs. The nanoprodrugs may be used as anti-inflammatory and antioxidant drug delivery vehicles. Whenever the drug combination is favorable to the treatment of diseases, the antioxidant and anti-inflammatory properties of the nanoprodrugs may increase the therapeutic effect of the delivered drugs and reduce ROS-related adverse effects. Notably, the design and synthesis of water-insoluble...
hydrophobic prodrugs and their preparation into nanoprodrugs may create a new paradigm in the prodrug strategy.

(362)

(363)

(364)

(365)
The BPA has also been linked successfully with flavonoids in 1:1 ratio to give NSAID-antioxidant mutual prodrug. For example quercetin tetramethyl ether-BPA (QTME-BPA) conjugate (368) has been found to exhibit significant anti-inflammatory activity with significantly reduced ulcerogenic side effects.410

Another example of NSAIDS codrug is NO-NSAIDs. Recently, nitric oxide (NO) releasing NSAIDs (NO-NSAIDs) have been developed by incorporating a NO moiety onto a well established NSAID using various chemical spacers. Generally, these compounds maintain the antiinflammatory properties of the parent compounds while showing enhanced tolerability and a wider range of pharmacological activities. Specifically, NO-NSAIDs are characterized by a dramatic reduction in GI side effects in comparison with the parent drug molecules. This is due to the protective effects of NO on gastric mucosa. Nitric oxide (NO) is an endogenous gaseous mediator that is involved in a wide variety of physiological processes, including vascular and nonvascular smooth muscle relaxation and neurotransmission. It has also been
recognized as a critical mediator of GI mucosal defenses, exerting many of the same actions as prostaglandins in the GI tract. Like prostaglandins, NO modulates mucosal blood flow, mucus and bicarbonate secretion, and repair mucosal injury.\(^\text{411}\) NO is also a very potent inhibitor of neutrophil adherence to the vascular endothelium.\(^\text{412}\) This observation was critical to the development of NO-NSAIDs, since it had been discovered in the early 1990s that adherence of neutrophils to the vascular endothelium in the gastric microcirculation was a critical event in the pathogenesis of NSAID induced gastric damage.\(^\text{413-419}\) Moreover, NO suppresses the release of several inflammatory mediators from mast cells that are known to contribute to gastric ulceration, including platelet-activating factor.\(^\text{420,421}\) It is not surprising that due to these effects, NO donors have been reported to exhibit reduction in the severity of gastric injury in experimental models and can accelerate healing of experimental gastric ulcers.\(^\text{422-425}\) It is noteworthy that use of NO donors have been found to significantly reduce GI bleeding in patients, taking aspirin for cardiovascular indications.\(^\text{426}\) The development of NO-NSAIDs was based on the fact that slow release of NO would suppress NSAID induced neutrophil adherence to the vascular endothelium, thereby preventing damage to the gastric mucosa.\(^\text{427-430}\) Other experimental interventions that prevented NSAID induced neutrophil adherence, such as antibodies against adhesion molecules, have been found to prevent gastric damage.\(^\text{414-418}\) Moreover, as NO is a potent vasodilator, NO-NSAIDs would not reduce mucosal blood flow as conventional NSAIDs do.\(^\text{430}\)

Two distinct chemical classes, NO-NSAID and SNO-NSAID have been synthesized and biologically evaluated. In one such class, the nitrate (-ONO\(_2\)) group as the NO donor is incorporated, whereas the other class consists of S-nitrosothiol (-S-NO) group.\(^\text{431}\)

NO-NSAIDs consist of a conventional NSAID esterified to a NO releasing moiety. The general structural features of NO–NSAIDs enable a large number of variations within the linking spacer and the NO-donating moiety. Owing to the ease of formation of these nitrate esters, several derivatives could be prepared for a given spacer. Till date, a significant amount of work on NO–NSAIDs and other related compounds has been reported.\(^\text{432}\)
These so-called "NO-NSAIDs" (also known as COX inhibiting nitric oxide donors, CINODS) have been claimed to have comparable or superior anti-inflammatory and analgesic activities in acute and chronic inflammation model in rat while sparing the gastrointestinal tract and kidney of injury. Interestingly, NO-NSAIDs have been shown to accelerate the healing of pre-existing gastric ulcers and restore renal function and structure in rats when subjected to renal ablation. Many studies in animals impressively demonstrated the ability of NO-NSAIDs to spare GI mucosa in acute and chronic administration. In experimental models, NO-NSAIDs even protected gastric mucosa against damage induced by other deleterious stimuli and maintained gastric mucosal blood flow. Ukawa et al. showed that healing of gastric ulcers was not impaired by NO-NSAID whereas the parent substance as well as a selective COX-2 inhibitor in equimolar dosages delayed the healing process. A NO-NSAID consists of three parts; the parent NSAID, NO releasing moiety and the spacer used for synthesis as exemplified by two NO-aspirins, NCX4215 (369) and NCX4016 (370).

3-(Nitroxyethyl)phenyl2-acetoxybenzoate, NCX4016 (370) has demonstrable innovative properties for treatment of vascular disorders and cancer. However, it has been abandoned as one of its metabolite was found to be mutagenic. NCX-4016, a stable compound otherwise, requires enzymatic hydrolysis to liberate NO at a constant rate. Following intragastric administration of NCX-4016, levels of NO are elevated both in gastric contents and plasma. NCX-4016 was shown to possess greater anti-inflammatory and analgesic activities than aspirin. It also exhibited antithrombotic activity in several platelet dependent and independent animal models. 4-Nitroxybutyl 2-acetoxybenzoate, NCX4215 (369) is under initial stages of development for cardiovascular diseases and cancer cell proliferation. NCX-4215 did not produce macroscopically visible histological damages in the rat stomach when
administered up to 300 mg/kg, whereas 100 mg/kg aspirin produced widespread hemorrhagic damage.\textsuperscript{454,455} These protective effects were also seen in the stomach of aged rats treated with NCX-4016 \cite{112}. NCX-4016 produced an equipotent inhibition of mucosal PGE$_2$ generation in the stomach when compared with aspirin.\textsuperscript{456-457}

The hypothesis that nitroaspirins could positively modulate changes in gastrointestinal damage was verified by testing the ability of NCX-4016 to prevent gastric damage in a rat model of shock.\textsuperscript{458} Oral administration of NCX-4016 indicated the lack of gastric toxicity of NCX-4016, but not of aspirin, in the stomach of diabetic rats.\textsuperscript{459} To improve upon efficacy of aspirin in hypertensive patients, Glimer \textit{et al.} have reported synthesis and evaluation of isosorbide mononitrate derivatives of aspirin.\textsuperscript{460} Isosorbide-5-mono-nitrate-2-aspirinate (ISMNA) was found to be stable enough in hydrolysis studies to be absorbed intact from GIT and liberate nitric oxide in plasma to support GI mucosal integrity and augment aspirin’s antiplatelet effects.

A range of standard NSAIDs, like naproxen, ibuprofen, flurbiprofen, ketoprofen and aspirin, have been coupled to NO-donating moieties and their actions extensively explored in a variety of experimental models over the past ten years demonstrating the efficacy, potency and spectrum of activity.\textsuperscript{461,462}

Beneficial properties of NO-donating groups have been characterized in several animal models of upper and lower GI damage\textsuperscript{463} by exerting local protective actions including mucosal vasodilatation and prevention of neutrophil adhesion in both the gastric and intestinal microcirculation and maintaining mucosal cell integrity.\textsuperscript{464} NO-naproxen (371) is under phase-III clinical trials for treatment of osteoarthritis, acute and chronic pain.\textsuperscript{436} Clinical studies on NO-naproxen, coded as AZD3582, supported the experimental findings demonstrating its effective antiinflammatory and analgesic actions.\textsuperscript{463-466} NO-donating prodrug of naproxen, NMI-1182 and AZD3582, are reported to produce significantly lesser gastric lesions after oral administration than naproxen.\textsuperscript{467} Nitroxybutyl-diclofenac conjugate nitrofenac (372) has been reported to exhibit reduced GI side effects without alteration of the ability to suppress prostaglandin synthesis and exert antiinflammatory effect.\textsuperscript{427}
NO-flurbiprofen (373) and NO-ketoprofen (374) have been synthesized and evaluated for their pharmacological activity. The compounds exhibited retention of antiinflammatory activity of the parent NSAID molecules with significantly reduced GI ulceration.\textsuperscript{431,468}

A series of NO-paracetamol have been studied and a lead compound NCX701 (375) has been identified. In a model of nociception, 375 administered orally was considerably more potent than paracetamol. Thus, compared with paracetamol, NO-paracetamol not only showed greater antinociceptive activity in both rat and mouse but also exhibited antiinflammatory activity over a similar dose range. Moreover, NO-paracetamol was found to be safer than the parent drug on liver function.\textsuperscript{469}
Another NO-releasing derivative (376) has been synthesized by incorporating NO moiety with selective COX-2 inhibitor, rofecoxib (377). This CINOD is a prodrug which has been found to release rofecoxib and NO *in vivo* (Figure 27). This agent was expected to have better activity and lesser side effects than the parent drug.\textsuperscript{470}

![Chemical structure of 376](image)

**Figure 27**: Schematic illustration of NO and the parent drug rofecoxib (377) release from the prodrug 376.

On similar lines, indomethacin was modified to increase COX-2 selectivity and enhance drug safety by covalent attachment of an organic nitrate moiety. This NO-Indomethacin (378) was found to be an effective and well tolerated antiinflammatory agent devoid of GI toxicity *in vivo*.\textsuperscript{471} NCX-530, an NO releasing derivative of indomethacin has been reported to decrease gastric motility, increased mucosal blood flow and caused a marked inhibition of PGE\textsubscript{2} formation in intact and ulcerated gastric mucosa.\textsuperscript{472}
Cena and coworkers reported a new series of NSAIDs in which aspirin was joined through an ester linkage to furoxan moieties, having the ability to release NO. All the products described presented a trend towards antiinflammatory activity devoid of acute gastrototoxicity, principally due to their ester nature, and an antiplatelet activity due to their ability to release NO. But, they did not behave as aspirin prodrugs in human serum.\textsuperscript{473}

A novel group of hybrid NO-NSAIDs possessing a 1- (pyrrolidin-1-yl)diazen-1-iium-1,2-diolate or 1-(N,N-dimethylamino) diazen-1-iium-1,2-diolate moiety attached via methylene spacer to the carboxylic acid group of the traditional NSAIDs aspirin, ibuprofen, and indomethacin were reported by Carlos group. These prodrugs showed equipotent antiinflammatory activities \textit{in vivo} to that of the parent drugs aspirin, ibuprofen, and indomethacin.\textsuperscript{474} Ester derivatives of aspirin, ibuprofen and indomethacin with O-(2)-acetoxymethyl 1-[N-(2-hydroxyethyl-N-methylamino]diazenium diolate \textbf{379-381} were synthesized as NO-releasing prodrugs.\textsuperscript{475} The derivatives did not exhibit \textit{in vitro} COX inhibitory activity against COX-1 and COX-2 isozymes but significantly decreased carrageenan induced rat paw edema showing an enhanced \textit{in vivo} antiinflammatory activity relative to the parent NSAIDs. The \textit{in vivo} ulcer index (Ul) assay showed that aspirin derivative (Ul = 0.8), ibuprofen derivatives (Ul = 0) and indomethacin derivatives (Ul = 1.3) were significantly less ulcerogenic when compared to the parent drugs, aspirin (Ul = 57), ibuprofen (Ul = 46) and indomethacin (Ul = 34) at equimolar doses.
A series of glycolamide naproxen prodrugs containing a nitrate group as NO-donor moiety have been synthesized. These compounds were evaluated for their antiinflammatory activity, naproxen release, and gastric tolerance. Out of these, compound 382 was found to exhibit excellent demonstration of NO release by the bioactivation of glycolamide nitrates. These observations indicated the possibility of naproxen glycolamide nitrates as safer alternative NSAIDs.

At the University Institute of Pharmaceutical Sciences, Panjab University, a number of NO-NSAIDs have been synthesized. Paracetamol (2), a proven liver toxic and having no antiinflammatory activity of its own has been converted to more active and lesser toxic NO releasing compounds 383 and 384. These derivatives showed better analgesic, antiinflammatory, biochemical (SGOT, ALP levels) and histopathologic
profile. Besides these derivatives have shown to inhibit COX-2 *in vitro* and release NO *in vivo*.\(^{478}\)

\[
\begin{align*}
\text{Ibuprofen esterified with NO donor moiety abolished GI irritation and significantly reduced thinning with no alteration in levels of diaphorase.}^{479} \text{ Data from several laboratories indicate that NO-NSAIIDs could be effective in a variety of diseases including cardiovascular, rheumatological, lung and Alzheimer’s diseases, and cancer.}^{480-482}
\end{align*}
\]

The potential limitation of NO-NSAIIDs arises from their intrinsic nature as indirect sources of NO. Organic nitrates require metabolic conversion like, enzyme mediated reductive catabolism in order to produce NO under physiological conditions. Furthermore, tolerance issues may restrict the therapeutic applicability and efficacy of organic nitrates. As an alternative, S-nitrosothiols are considered as biological sources of NO. These agents release NO without undergoing any metabolic transformation.\(^{483,484}\) Although, nitrosothiol transformation to NO is not completely understood, transition metal dependent redox processes and enzyme catalyzed decompositions likely predominate biological pathways for NO release *in vivo*. Furthermore, S-nitrosothiols can directly modulate cell physiology without generating NO as the effector molecule. This is possible through S-*trans*-nitrosation reactions, by which NO group is effectively transferred from the S-nitrosothiol to the thiol of a target biomolecule in exchange for a hydrogen.\(^{485}\)

Based on these facts, various novel diclofenac esters containing a nitrosothiol (-S-NO) moiety as a NO donor functionality have been synthesized and evaluated for their bioavailability, pharmacological activity, and gastric irritation *in vivo*. All S-NO diclofenac derivatives (385-394, Figure 28) acted as orally bioavailable prodrugs, producing significant levels of diclofenac in plasma within 15 min after oral administration to mice. These agents were evaluated in the carrageenan induced rat
paw edema test and found to exhibit retention of the antiinflammatory activity of the parent drug diclofenac. Additionally, these agents showed analgesic activity in mouse phenylbenzoquinone induced writhing test.  

\[ (385-389) \ n = 2-6 \]

\[ (390) \ R = \text{CH}_2\text{C}_6\text{H}_5 \]

\[ (391) \ R = \text{CH}_2\text{CH}_3 \]

\[ (392) \ R \text{ and } R' = \text{CH}_3 \]

\[ (393) \ R \text{ and } R' = \text{CH}_2\text{CH}_3 \]

\[ (394) \ R \text{ and } R' = (\text{CH}_2)_5 \]

Figure 28: SNO-diclofenac esters (383-394).

On similar lines, SNO-ibuprofen (395) and SNO-ketoprofen (396) have been synthesized. These derivatives have been reported to exhibit retention of analgesic and antiinflammatory activities of the parent drug molecules with reduced gastrointestinal side effects.  

\[ (395) \]

\[ (396) \]
INTRODUCTION

Hydrogen sulphide was observed to exert antiinflammatory and analgesic activity. It is also reported to be a vasodilator and suppressor of leukocyte adherence to vascularendothelium. Fiorucci group have reported that inhibition of hydrogen sulfide generation contributes to gastric injury caused by antiinflammatory nonsteroidal drugs. Based on these findings various ester derivatives of clinically used NSAIDs namely ibuprofen, naproxen, diclofenac, indomethacin, ketoprofen and aspirin with various hydrogen sulphide releasing moieties (4-thiocarbamoylphenol, 5-[4-hydroxyphenyl]-1,2-dithiole-3-thione) have been reported. These derivatives have been reported to show significantly less gastric injury than the NSAID alone.

Reduced mucosal prostaglandin (PG) levels, increased gastric acidity and increased gastric motility are reported to be important causes for the NSAIDs induced gastropathy. The increased gastric motility leads to a reduced mucosal blood flow, hypoxia and destruction of the mucous bicarbonate barrier, which prevents back diffusion of pepsin and hydrogen ions from lumen into the mucosal layer. Microcirculation in gastroduodenal mucosa supplies energy and oxygen to mucosal cells, removes hydrogen ions, waste products, and transports bicarbonate to the surface of the gastric epithelium. This way, the mucosal blood flow plays a very crucial role in supporting the defense mechanism of mucosa. Based on these reports an attempt was made to incorporate anticholinergic activity into the basic molecules of conventional NSAIDs (flurbiprofen, biphenylacetic acid, naproxen, 6-methoxynaphthylacetic acid, diclofenac, aspirin and ketorolac) by derivatizing them into N,N-disubstituted aminoalcohol esters. These derivatives were designed specifically to resemble the aminoalcohol ester class of anticholinergics. An entirely new pharmacodynamic property was incorporated into the original NSAIDs molecules with the anticipation that besides preventing local GI irritation by temporarily blocking carboxyl group present in the NSAIDs, the introduction of anticholinergic activity in the intact esters would further aid in reducing the GI toxicity by (i) decreasing gastric acid secretion and (ii) decreasing gastric motility to maintain optimal mucosal blood flow. Most of the aminoalcohol esters were found to undergo fast enzymatic cleavage in 80 % human plasma and possessed antiinflammatory activity comparable to the respective parent drugs in carrageenan induced rat paw edema model. A significant reduction in ulcerogenic potency in comparison to the parent drugs with a slightly higher
antiinflammatory potency suggests that majority of these candidates have an improved therapeutic profile over their parent drugs.

Although the codrug approach is a promising drug design method, only a limited number of codrugs are currently available in the market. This is due to several reasons. First, there is only a limited number of drugs that would gain synergy from the codrug approach; e.g. they do not have same target site. Secondly, the increased molecular weight of the codrug may hinder oral absorption of these compounds. Thirdly, only a few drugs have a similar dose on a molar bases. Forth, the optimal physicochemical properties are often hard to combine into a codrug, i.e. the "rule of five" is often broken (Lipinsky et al.). Finally, codrugs often require a few extra synthetic steps and the use of protective groups, which makes synthesis more complicated, and therefore, higher manufacturing costs may eventually override the therapeutic benefit of a codrug.

In spite of extensive efforts in the direction of separation of therapeutic effect of NSAIDs from their GI toxicity, the search for an ideal prodrug with a superior therapeutic advantage for clinical use still remains unmet. Further, research is needed to design and identify prodrugs, which would be appropriate for clinical use in terms of stability, metabolism, toxicology and side effects. Instead of synthesizing new compounds which is a time consuming and too costly an affair, the designing of derivatives of existing clinically used NSAIDs is definitely an interesting and promising area of research. Moreover, as the metabolic profile of the liberated parent drug (after cleavage of the derivative in the body) would be already known, it could be advantageous to design derivatives of parent NSAIDs.

Synthesis of codrugs of NSAIDs is not only an effective way of overcoming the GI toxicity but could also be used for combining other pharmacological properties or incorporating a chemical moiety for an added beneficial effect like development of NO-NSAIDs, conjugation with H$_2$ receptor antagonist or an analgesic agent and incorporating anticholinergic activity for reducing gastric acid secretion.