INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) are one of the most widely used class of drugs worldwide. These agents are used for the treatment of patients with rheumatoid arthritis and various other diseases associated with inflammation, fever and pain.\(^1\) The pharmacological activity of NSAIDs is related to their ability to inhibit the production of prostaglandins (PGs) from arachidonic acid by inhibiting the activity of enzyme cyclooxygenase (COX).\(^2,4\) NSAIDs exerts three major actions, mediated through the reduction of the production of PGs. These actions include: i) antiinflammatory effect, ii) analgesic effect, and iii) antipyretic effect.\(^5,6\) The antiinflammatory activity is due to the decrease in vasodilator PGs (PGE\(_2\), PGI\(_2\)), resulting in decreased vasodilation and, therefore reduced edema. The analgesic effect is due to decreased prostaglandin generation, resulting in decreased sensitization of nociceptive nerve endings to the inflammatory mediators including bradykinin and 5-hydroxytryptamine. Relief of headache is probably due to decreased prostaglandin mediated vasodilatation. The antipyretic activity is due to the inhibition of the synthesis of PGE\(_2\). The mediator triggers the hypothalamus to elevate body temperature by promoting the increase in heat generation and decrease in heat loss.

In spite of the usefulness of NSAIDs, their use is limited due to higher incidence of gastrointestinal (GI) damage, including gastric ulceration, perforation and their associated complications and these affect a large number of patients taking these drugs on long term basis. The incidence of clinically significant GI side effects due to NSAIDs is high (over 30%) and causes some patients to abandon NSAID therapy. NSAID related GI adverse side effects account for more than 70,000 hospitalizations and 7000 deaths annually in the United States. These GI side effects can be classified into three broad categories namely, i) nuisance symptoms such as heartburn, nausea, dyspepsia, and abdominal pain, ii) mucosal lesions such as ulcers, and iii) serious gastrointestinal complications, including perforated ulcers and catastrophic bleeding.\(^7\) The association between NSAIDs and gastrointestinal erosions and ulcers is well established. The relative
risk for experiencing serious adverse gastrointestinal events is approximately three times greater for NSAID users than for nonusers. Furthermore, patients with rheumatoid arthritis are nearly twice as likely as those with osteoarthritis to suffer a serious complication from NSAID treatment. Compared with rheumatoid arthritis, osteoarthritis is a milder disease and requires lower doses of NSAIDs, which may explain the lower risk for gastrointestinal complications in patients suffering from this disease.5,8,9

It has been reported that both therapeutic and side effects of NSAIDs are dependent on cyclooxygenase (COX) inhibition.10 The side effects are also mediated principally through the inhibition of PG synthesis in tissues where PGs are responsible for physiological homeostasis. This is a key element in NSAID gastropathy as prostaglandins maintain gastric mucosal blood flow and increase protective mucus as well as bicarbonate production. In early 1990s, two structurally related isoforms of cyclooxygenase (COX) have been identified namely, cyclooxygenase I (COX-I) and cyclooxygenase II (COX-II).11 COX-I is constitutive and provides cytoprotection in the GI tract, whereas COX-II is inducible which mediates inflammation. The mucosal integrity in normal GI tract is primarily maintained by PGs that are derived from COX-I and therefore, inhibition of COX-I rather than COX-II by NSAIDs is responsible for their ulcerogenic GI side effects.12,13 The systematic study of biosynthetic pathways leading to the generation of inflammatory mediators and the molecular interactions between mediators and receptors on target cells laid the groundwork for identifying safer antiinflammatory agents (vide infra).

Selective COX-II inhibitors as safer NSAIDs
The identification and characterization of an inducible form of COX-II in inflammatory cells in the early 1990s started a race for the development of selective COX-II inhibitors as safer NSAIDs devoid of ulcerogenic side effects. The concept of COX-II selective inhibition is based on the differences of amino acids sequence existing between COX-I and COX-II. 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-I isoform, the active site of the COX-II is larger. Based on this observation, medicinal chemists synthesized compounds suitable for interaction with the active site without inhibiting the COX-I catalytic activity. Due to the great expectation, these selective COX-II 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 1. These include, celecoxib (1), valdecoxib (2), a water-soluble valdecoxib prodrug, paracoxib (3), rofecoxib (4), etoricoxib (5), and lumiracoxib (6). First three of these agents are sulphonamide derivatives, 4 and 5 are methylsulphones, whereas lumiracoxib (6) is a phenylacetic acid derivative. Celecoxib (1), and rofecoxib (4) were the first two coxibs approved by the FDA and belong to first generation of coxibs. Second generation includes, valdecoxib (2), paracoxib (3), etoricoxib (5) and lumiracoxib (6). 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-II 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 (4) and, soon afterwards, valdecoxib (2) from the market. It was found out that COX-II 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-II 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-II inhibitors may result in renovascular adverse event. In support of this fact, rofecoxib gastrointestinal outcomes research 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-II 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.20

Figure 1: List of marketed selective COX-II inhibitors: celecoxib (1), valdecoxib (2), paracoxib (3), rofecoxib (4), etoricoxib (5), and lumiracoxib (6).

These findings raised serious concerns about the risk of thrombotic events during treatment with coxibs, and marking off the therapeutic benefits of selective
COX-II inhibition. Therefore, the initial enthusiasm of developing selective COX-II 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.

**Transformation of conventional nonselective COX-Inhibitors to selective COX-II 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-II 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-I and COX-II isoforms. The substrate binding site in COX-II is approximately 25% larger than COX-I (394Å vs. 316Å). Chemical modification of the nonselective, conventionally used NSAIDs by increasing the size of the drug molecule, which fits into the COX-II active site but not into the COX-I site, resulted in the formation of selective COX-II inhibitors. Incorporation of steric bulk into existing nonselective NSAIDs could abolish their COX-I inhibitory properties without affecting COX-II activity. Alteration of the carboxylic acid moiety has recently been exploited to convert nonselective inhibitors into COX-II 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 flurbipofen (7) bound to COX-I and COX-II suggests that modification of the 4-phenyl ring to induce steric constraint should result in increased selectivity for COX-II. This hypothesis was validated through introduction of various substituents to generate a series of potent and selective COX-II inhibitors. Three of these compounds 8-10 (Figure 2) were found to exhibit greater selective COX-II inhibitory activity.
Similarly, novel selective COX-II inhibitors have been designed and developed by transformation of nonselective ketoprofen (11). 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 12 of this series was found to be potent and selective COX-II inhibitor.  

![Figure 2: Structures of flurbiprofen (7) and its transformed selective COX-II inhibitors (8-10).](image)
Indomethacin (13) is one of the most potent nonselective NSAIDs. This agent has also been transformed into selective COX-II inhibitors by systematic structural modification to increase the size (Figure 3).

Figure 3: Structures of indomethacin (13) and its transformed selective COX-II inhibitors (14-20).
In this direction 4-chlorobenzoyl group of indomethacin was replaced with a 2,4,6-trichlorobenzoyl group which resulted in the formation of compound 14, exhibiting reasonable COX-II selectivity. Based on these results, Black et al. reviewed a number of indole acetic acid analogues and found that benzyl derivative 15 exhibited highly selective COX-II activity. Using same strategy Kalgutkar et al. also attempted to transform indomethacin to selective COX-II inhibitors and taking the advantage of structural class with well established safety profiles. These investigators prepared ester derivatives (16 and 17) and amide derivatives (18-20) of indomethacin and found that large alkyl, arylalkyl and hetecyclic groups exhibited high activity and selectivity.

Aspirin (21) 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-I as against COX-II. The antiinflammatory effects arise from acetylation of COX-II, whereas antithrombic and ulcerogenic effects result from acetylation of COX-I. Attempts have been made to transform this nonselective NSAID to selective COX-II inhibitors by varying the length of the acyl group attached. In this direction, a series of acetoxybenzenes substituted in the ortho position with alkylsulfides have been prepared. One such compound, \( o-(\text{acetoxyphenyl})\text{methyl sulfide (22) was identified with moderate inhibitory activity and selectivity for COX-II. Further, systematic variation at different parts of the molecule led to the synthesis of } o-(\text{acetoxyphenyl})\text{hept-2-ynyl sulfide (23) having most potent COX-II inhibitory activity.} \)
Another NSAID studied for its transformation to selective COX-II inhibitors was zomepirac (24), which is basically a COX-I inhibitor. To achieve selective COX-II inhibitory activity, the acetic acid group was replaced with other moieties such as pyridazinone ring or an N-acyl aminosulfonyl group to produce RS57067 (25) and RS1048934 (26).\textsuperscript{32,33}

![Chemical Structures]

Meclofenamic acid (27) has also been selected as lead for designing selective COX-II inhibitors. A series of ester and amide derivatives of this NSAID have been prepared. It has been found that only amide derivatives (28-32) showed potent and selective COX-II inhibitory activity. Based on SAR studies, it has been suggested that further optimization may be necessary to enhance selective COX-II inhibitory activity.\textsuperscript{27,34}

![Chemical Structures]
This strategy of transforming nonselective NSAIDs to selective COX-II inhibitors has also been successfully applied to diclofenac (33). 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 34 which exhibited selective COX-II inhibitory activity. Other diclofenac derivatives with selective COX-II inhibitory activity include derivative 35 having meta alkyl substituents on the phenylacetic acid.24,35

Nitric oxide releasing NSAIDs
A wane in the initial euphoria for COX-II inhibitors has emphasized that other strategies are required for the development of safer 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. NO is also a very potent inhibitor of neutrophil adherence to the vascular endothelium. 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. Moreover, NO suppresses the release of several inflammatory mediators from mast cells that are known to contribute to gastric ulceration, including platelet activating factor. 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. It is noteworthy that use of NO donors have been found to significantly reduce GI bleeding in patients, taking aspirin for cardiovascular indications. 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. Other experimental interventions that prevented NSAID induced neutrophil adherence, such as antibodies against adhesion molecules, have been found to prevent gastric damage. Moreover, as NO is a potent vasodilator, NO-NSAIDs would not reduce mucosal blood flow as conventional NSAIDs do.

Two distinct chemical classes, NO-NSAID and SNO-NSAID have been synthesized and biologically evaluated. In one such class, the nitrate (-ONO2) group as the NO donor is incorporated, whereas the other class consists of S-nitrosothiol (-S-NO) group.

NO-NSAIDs consist of a conventional NSAID esterified to a NO releasing moiety. 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-II inhibitor in equimolar dosages delayed the healing process. Apart from diminishing GI toxicity, NO-NSAIDs improve antiinflammatory and antinociceptive efficacy.\textsuperscript{65} NO-releasing analogues of several NSAIDs, such as aspirin, diclofenac, naproxen, ketoprofen, flurbiprofen have been synthesized. Various developed molecules have demonstrated their potential in both pharmacological tests and clinical trials. 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 (36) and NCX4016 (37).\textsuperscript{66-68}

NCX4215 (36) is under initial stages of development for cardiovascular diseases and cancer cell proliferation. NCX4016 (37) has demonstrable innovative properties for treatment of vascular disorders and cancer.\textsuperscript{69} However, it has been abandoned as one of its metabolite was found to be mutagenic.\textsuperscript{70} NO-naproxen (38) is under phase-III clinical trials for treatment of osteoarthritis, acute and chronic pain.\textsuperscript{57} Nitroxybutyl-diclofenac conjugate nitrofenac (39) has been reported to exhibit reduced GI side effects without alteration of the ability to suppress prostaglandin synthesis and exert antiinflammatory effect.\textsuperscript{52}
A number of other NO-NSAIDs have been synthesized and evaluated for their pharmacological activity. These include NO-flurbiprofen (40) and NO-ketoprofen (41). The compounds exhibited retention of antiinflammatory activity of the parent NSAID molecules with significantly reduced GI ulceration.56,71

A series of NO-paracetamol have been studied and a lead compound NCX701 (42) has been identified. In a model of nociception, 42 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.72

Another NO-releasing derivative (43) has been synthesized by incorporating NO moiety with selective COX-II inhibitor, rofecoxib (4). This CINOD is a prodrug which has been found to release rofecoxib and NO in vivo (Figure 4). This agent
was expected to have better activity and lesser side effects than the parent drug.\textsuperscript{73}

\[
\begin{align*}
\text{H}_3\text{C}O_2\text{S} & \quad \text{O} \\
\text{O} & \quad \text{ON}_2 \\
\text{O} & \quad \text{OCH}_3 \\
\text{H}_3\text{C}O_2\text{S} & \quad \text{O}
\end{align*}
\]

(43)

\[\text{in vivo}\]

\[
\begin{align*}
\text{C}_6\text{H}_{11} & \quad \text{O} \\
\text{O} & \quad \text{OH} \\
\text{H}_3\text{C}O_2\text{S} & \quad \text{O}
\end{align*}
\]

(4)

Figure 4: Schematic illustration of NO and the parent drug rofecoxib (4) release from the prodrug 43.

On similar lines, indomethacin (13) was modified to increase COX-II selectivity and enhance drug safety by covalent attachment of an organic nitrate moiety. This NO-Indomethacin (44) was found to be an effective and well tolerated antiinflammatory agent devoid of GI toxicity \textit{in vivo}.\textsuperscript{74}

\[
\begin{align*}
\text{H}_3\text{CO} & \quad \text{N} \\
\text{N} & \quad \text{ON}_2 \\
\text{N} & \quad \text{CH}_3 \\
\text{Cl} & \quad \text{O}
\end{align*}
\]

(44)
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 45 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.

The potential limitation of NO-NSAIDs 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. 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-transnitrosation reactions, by which NO group is effectively transferred from the S-nitrosothiol to the thiol of a target biomolecule in exchange for a hydrogen. 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 (46-54, Figure 5) acted as orally bioavailable prodrugs, producing significant levels of diclofenac in vivo.
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

Figure 5: SNO-diclofenac esters (42-50).

retention of the antiinflammatory activity of the parent drug diclofenac. Additionally, these agents showed analgesic activity in mouse phenylbenzoquinone induced writhing test. On similar lines, SNO-ibuprofen (55) and SNO-ketoprofen (56) 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.
COX and 5-LOX dual inhibitors
As discussed (vide supra), NO-NSAIDs show antiinflammatory activity devoid of ulcerogenicity. However, recently many studies have indicated the possibility of NO involvement in the pathogenesis of arthritis and subsequent tissue destruction.79 It is also well known that in addition to PGs, several other mediators of arachidonic acid metabolism are involved in the inflammatory processes. Leukotrienes (LTs) belong to the second main family of arachidonate products, synthesized via 5-lipoxygenase (5-LOX) and have a major role in the inflammatory response.80 LTs are extremely potent vasoactive compounds, which are more inflammogenic than PGs in some respects. LTB4, in particular, induces recruitment of leukocytes to inflamed sites, lysosomal release in neutrophils, adhesion molecule expression and subsequent plasma leakage.81,82 These findings have suggested that dual inhibition of both LTs and PGs may lead to enhanced and wider antiinflammatory activity. Moreover, it can also be expected that combined COX and LOX inhibition may originate an improved GI safety profile, due to a number of adverse effects of LTs in the GI mucosa.81,83 In particular, reduction of mucosal blood flow, leukocyte endothelial cell interaction and leukocyte infiltration are considered a prerequisite for NSAID induced gastropathy. On these lines, several studies have demonstrated that 5-LOX inhibitors or LT receptor antagonists exert protective effects on acute and chronic gastric mucosal damage in various ulcer models, including NSAID...

![Chemical structures](image-url)
induced gastric lesions.\textsuperscript{81,83,84} These observations, along with the possibility that COX Inhibition by NSAIDs can divert arachidonate to lipooxygenase pathway, led to the theory that excess LT production, combined with PG deficit, could contribute to NSAID induced mucosal damage.\textsuperscript{85} In this direction, elevated production of LTB\textsubscript{4} in the human stomach has been documented in patients taking NSAIDs.\textsuperscript{86} Currently, various classes of dual COX/5-LOX inhibitors as safer NSAIDs have been described in the scientific literature.\textsuperscript{87}

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 6). 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{87}

Darbufelone (57) and S-2474 (58) belong to this class of compounds with selective COX-II/5-LOX inhibitory activity. In addition to its antiinflammatory efficacy, the latter agent showed cytokine modulating properties.\textsuperscript{88} It is currently being evaluated in clinical trials for arthritis.\textsuperscript{89} Another derivative, tebufelone (59) 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{90} 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 (60). Although this compound is not a phenol, it exhibited an antiinflammatory activity equivalent to that of tebufelone (59) in the rat carrageenan induced paw edema and also showed hepatotoxicity.\textsuperscript{90,91} The hepatic toxicity of metabolite 60 and the parent molecule 59 was attributed to the terminal unsaturation of side chains.\textsuperscript{90}
Based on these observations, structural modifications were carried out to give different dihydrobenzofuran derivatives as COX-II/5-LOX inhibitors. For example, PGV-20229 (61) has been found to exhibit analgesic activity and excellent gastric safety in different in vivo tests.87,91

Another class of COX-II/5-LOX dual inhibitors belong to thiophene derivatives. The lead compound RWJ-63556 (62) is a potent orally active COX-II/5-LOX...
inhibitor which is structurally related to the selective COX-II inhibitor nimesulide (63). It has been found to produce significant antiinflammatory activity in a canine model of carrageenan induced inflammation.

Pyrazoline derivatives, phenidone (64) and BW-755C (65) as antioxidant 5-LOX inhibitors appeared to be rather nonselective, inhibiting the COX isoforms.

Another dual COX/5-LOX inhibitor is tepoxalin (66), 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.

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 (67) has entered phase-III clinical trials for the treatment of osteoarthritis. In several animal models, this derivative has shown antiinflammatory, analgesic and antiasthmatic effects. Although licofelone (67) is a COX-I/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.

![Chemical structure of licofelone (67)](image)

A number of hydrazone derivatives have also been described as dual COX/5-LOX inhibitors. One promising compound is CBS-1108 (68), 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.

![Chemical structure of CBS-1108 (68)](image)

A number of conventionally used NSAIDs, as well as selective COX-II 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. This group has the capability to chelate the nonheme iron of 5-LOX. Such two derivatives 69 and 70 have been prepared

![Chemical structure of derivatives 69 and 70](image)
and found to inhibit not only 5-LOX, but preferentially the inducible isoform COX-II.

Similarly, flufenamic acid (71) has been structurally modified by bioisosterically replacing the carboxylic acid group with tetrazole moiety (Figure 7).

![Figure 7: Structures of flufenamic acid (71) and its transformed dual COX/5-LOX inhibitors (72-74).](image-url)

Figure 7: Structures of flufenamic acid (71) and its transformed dual COX/5-LOX inhibitors (72-74).
The resulting compound 72 inhibited COX and to some extent 5-LOX. Other flufenamic acid transformed dual COX/5-LOX inhibitors, include 1,3,4-oxadiazole-2-thione (73) and 1,3,4-thiadiazole-2-thione (74). 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 are required to evaluate safety and efficacy of these agents.

**NSAIDs and antioxidants**

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. 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, \(^{98-100}\) NSAIDs \(^{101-105}\) and of *Helicobacter pylori* is mediated by ROS (vide infra). \(^{106,107}\)

A free radical may be defined as a molecule carrying an unpaired electron, which makes it extremely reactive and ready to acquire an electron in any way possible. In the process of acquiring an electron, the free radical will attach itself to another molecule, thereby modifying it biochemically. However, as free radicals acquire an electron from the other molecules, they either convert these molecules into other free radicals, or break down or alter their chemical structure. These attacks by free radicals are capable of causing cells to lose their structure, function and eventually destroy them. Thus, free radicals are capable of damaging virtually any biomolecule, including proteins, sugars, fatty acids and nucleic acids. \(^{108,109}\)

Free radicals damage to long lived biomolecules such as collagen, elastin, DNA,
polysaccharides, lipids that make up the membranes of cells and organelles, blood vessel walls and lipofuscins is thought to be a major contributor to cell death.\textsuperscript{110} The most common free radicals include superoxide (O$_2^-$), hydroxyl (‘OH), alkoxyl, (RO’), peroxyl (ROO’) and nitric oxide (NO’). Other non-free radical molecules, such as singlet oxygen (’O$_2$), hydrogen peroxide (H$_2$O$_2$), and hypochlorous acid (HOCl), are similar but not real free radicals. Together, the free radicals and free radical mimics are called reactive oxygen species (ROS).\textsuperscript{108} Free radicals have extremely short half-lives ranging from nanosecond to seconds. The shortest half life is only one nanosecond (10$^{-9}$ sec) for hydroxyl radical (‘OH) and the longest half life is 1-10 seconds for nitric oxide radical (NO’).\textsuperscript{111} The half life dictates the intrinsic properties of the damaging effects of the free radicals, whether they can travel far enough to reach other cellular compartments or just attack the most nearby molecules. The further they can travel, the broader the range of molecules and organelles they can damage.\textsuperscript{108}

A wide range of major diseases closely related to free radical damage, such as cancer, heart/artery disease, essential hypertension, Alzheimer’s Disease, cataracts, diabetes, Parkinson’s disease, arthritis and inflammatory disease, aging, gastrointestinal inflammation and gastric ulcers are now believed to be caused in part or entirely by free radical damage.\textsuperscript{112-114} There are more than six primary sources of free radicals formed endogenously within living organisms; i) the major source of free radicals and oxidants is through the respiratory generation of ATP using oxygen, ii) peroxisomal oxidation of fatty acids, which generates H$_2$O$_2$ as a byproduct, iii) cytochrome P450 enzymes, iv) chronic inflammatory cells which use a mixture of oxidants to overcome infection by phagocytosis, v) other enzymes capable of generating oxidants under normal or pathological condition, and vi) various biomolecules including thiols, hydroquinones, flavins, catecholamines, pterin and hemoglobin, may spontaneously auto-oxidize and produce superoxide radicals. Many exogenous sources, such as environmental radiation (sunlight), polluted urban air, cigarette smoke, iron and copper salts, some phenolic compounds found in many plant foods, and various drugs could contribute to free radical production.\textsuperscript{108,113 115,116}
The most relevant free radicals in biological systems are radical derivatives of oxygen. Reduction of oxygen by the transfer of a single electron to it, will generate the superoxide free radical anion (O$_2^-$) and two electron reduction of oxygen would yield hydrogen peroxide (H$_2$O$_2$). Hydrogen peroxide is often produced in biological systems via the generation of superoxide (O$_2^-$). Two superoxide (O$_2^-$) molecules can react together to form H$_2$O$_2$ and oxygen. Free radical reactants generate nonradical products and the phenomenon is known as dismutation. It can take place spontaneously or can be catalyzed by the enzyme superoxide dismutase. Hydrogen peroxide is a nonradical ROS, which plays an important role in free radical biochemistry because of its break down to reactive hydroxyl radical (·OH), particularly in the presence of transition metal ions. This reaction is often referred to as the iron catalysed Haber-Weiss reaction. The noncatalysed Haber-Weiss reaction is the direct reaction of superoxide (O$_2^-$) with hydrogen peroxide (H$_2$O$_2$). The spontaneous reaction is not common in biological systems due to low steady state concentrations of the reactants. Ferrous (Fe$^{2+}$) iron and cuprous (Cu$^+$) copper are more reactive with H$_2$O$_2$ than their oxidised counterparts, ferric (Fe$^{3+}$) and cupric (Cu$^{2+}$), respectively. The autoxidation of reduced transition metals can also produce superoxide (Figure 8).

\[
\begin{align*}
O_2^- + e^- & \rightarrow O_2^- \\
O_2^- + 2e^- + 2H^+ & \rightarrow H_2O_2 \\
2O_2^- + 2H^+ & \rightarrow H_2O_2 + O_2 \\
H_2O_2 + Fe^{3+} & \rightarrow \cdotOH + OH^- + Fe^{2+} \\
O_2^- + H_2O_2 & \rightarrow \cdotOH + OH^- + O_2 \\
O_2^- + Fe^{3+} & \rightarrow Fe^{2+} + O_2^- \\
O_2^- + Cu^{2+} & \rightarrow Cu^+ + O_2 \\
Fe^{2+} + O_2 & \rightarrow Fe^{3+} + O_2^- \\
Cu^+ + O_2 & \rightarrow Cu^{2+} + O_2^- 
\end{align*}
\]

Figure 8: Generation of various reactive oxygen species (ROS).

Thus, the reactions of the transition metal ions with oxygen can be considered reversible redox reactions and are extremely relevant in the promotion of free
radical reactions.\textsuperscript{117,118} ROS can attack vital cell components like polyunsaturated fatty acids, proteins, and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross linking, inhibition of protein synthesis, DNA damage and ultimately resulting in cell death. Lipid peroxidation mediated by ROS is an important cause of damage to the cell membrane.\textsuperscript{119} Free oxygen radicals, such as superoxide anion and its active compounds, initiate lipid peroxidation. Xanthine oxidase is an enzyme responsible for the production of free oxygen radicals and is believed to be an important cause of cell membrane destruction and cell damage. Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs), which are readily attacked by oxidising radicals. The oxidative destruction of PUFAs, known as lipid peroxidation, is particularly damaging because it proceeds as a self-perpetuating chain reaction as described in Figure 9.\textsuperscript{118}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{peroxidation.png}
\caption{Illustration of peroxidation reaction involving fatty acids.}
\end{figure}

The presence of double bond adjacent to a methylene group makes the methylene C–H bonds of polyunsaturated fatty acid (PUFA) weaker and therefore the hydrogen becomes more prone to abstraction. The peroxidation is initiated by hydroxyl ('OH), alkoxy radicals (RO'), and peroxy radicals (ROO'),
resulting in initiating the lipid peroxidation. This can lead to a self perpetuating process since peroxy radicals (ROO') are both reaction initiators as well as the products of lipid peroxidation. Lipid peroxy radicals react with other lipids, proteins, and nucleic acids, propagating thereby the transfer of electrons and bringing about the oxidation of substrates. Cell membranes, which are structurally made up of large amounts of PUFA, are highly susceptible to oxidative attack and, consequently, changes in membrane fluidity, permeability, and cellular metabolic functions result.

It has been reported that free radicals are involved in gastrototoxicity. The pathogenesis of gastric mucosal lesions has been studied in rats using water immersion restraint stress and burn shock models. It has been found that gastric ulceration is associated with the increased products of lipid peroxidation as remnants of the destructive effects of free radicals. Recent studies have shown that stress induced gastric ulcers are associated with increased lipid peroxidation and depletion of endogenous glutathione. This is due to the increased formation of $O_2^-$, activation of superoxide dismutase (SOD) and inactivation of gastric peroxidase (GPO). The generation of $O_2^-$ leads to increased $H_2O_2$ levels and subsequently resulting in 'OH generation, a radical well capable of inducing antioxidant depletion and tissue destruction (Figure 10). During stress related gastric ulceration, the cytoprotective enzyme prostaglandin synthetase (PGS) was also found to be inactivated, leading to a reduced defense system against the oxidative reactants. Lipid peroxidation caused by 'OH is increased in ethanol induced gastric ulceration, indomethacin, ischemia reperfusion, water immersion or burn shock. Dimethyl sulphoxide, a specific 'OH scavenger has been found to reduce gastric mucosal injury produced by ischemia, stress or ethanol ingestion, indicating a critical role of 'OH in mucosal damage. Stress causes both sympathetic and parasympathetic stimulation of the stomach, which induces increased motility and muscular contraction, leading to vascular compression and mucosal

27
Sympathetic stimulation also causes direct arteriolar vasoconstriction and therefore greatly reduces blood flow to the stomach leading to hypoxia and near or actual ischemia. The ischemic condition increases the leakage of $O_2^-$ from the mitochondrial electron transport chain and facilitates the availability of redox active copper or iron. Increased $O_2^-$ production leads to...
elevated levels of H$_2$O$_2$, which in collaboration with O$_2^-$ generates 'OH via the metal catalyzed Haber-Weiss reaction. The in vivo production of 'OH from O$_2^-$ and H$_2$O$_2$ requires the presence of a redox active transitional metal such as iron or copper.\textsuperscript{131} The involvement of metal ions in 'OH mediated lipid peroxidation and mucosal injury was prevented by the administration of nontoxic metal ion chelator, desferrioxamine (DFO).

Interestingly gastric peroxidase (GPO), a major antioxidant enzyme in the gastric mucosa,\textsuperscript{121} is inactivated during stress and antioxidants such as GSH (glutathione), vitamin E and the metal ion chelator, not only prevent gastric ulceration and lipid peroxidation but also preserve GPO activity.\textsuperscript{121} Ingestion of ethanol is the predisposing cause of acute gastric erosions in the human.\textsuperscript{132} Ethanol lowers the concentration of nonprotein sulphhydrils, especially GSH,\textsuperscript{133} thereby exerting an ulcerogenic effect by increasing oxygen based reactant formation. There is enough evidence to indicate the role of ROS in NSAIDs induced ulcers.\textsuperscript{134} In this direction, studies have been carried out to investigate the role of various antioxidants in prevention of peptic ulcers.

The role of antioxidants, especially vitamins C (75) and E (76), in the prevention of NSAID induced gastric injury is relatively little studied, and large outcome studies are missing.\textsuperscript{135} Both vitamins C and E seem to play an important role in the preservation of gastric mucosal integrity. However, it has been demonstrated that aspirin generates ROS which significantly contribute to gastric mucosal damage in humans, probably by initiating lipid peroxidation.\textsuperscript{135-137} On the other hand, mRNA expression and activity of protective antioxidant enzymes like superoxide dismutase and glutathione peroxidase in the stomach, as well as intragastric vitamin C levels were impaired by aspirin. Coadministration of vitamin C and NSAID due to scavenging of ROS, significantly attenuated gastric damage. Vitamin C is actively secreted into the gastric lumen of healthy subjects, and its concentrations are decreased in patients with gastroduodenal diseases, such as peptic ulcer, gastric malignancy\textsuperscript{138,139} or H. pylori associated gastritis.\textsuperscript{140} However, the underlying molecular mechanisms are not fully understood.
It has been recently reported that gastroprotective effects of vitamin C in humans are partly mediated by heme-oxygenase-1 (HO-I). This enzyme is an ubiquitous and crucial tissue protective enzyme with vasodilative, antiinflammatory and antioxidant properties. Its pathway and functions are illustrated in Figure 11.

**Figure 11: Pathway of heme-oxygenase.**

Effects/Function

- ROS increase
- Co-factor for enzymatic reactions
- Iron homeostasis
- Others....

- Cellular messenger
- Vasodilation
- Effects on hemoproteins
- Decreased NOS activity
- Platelet aggregation
- Decreases others....

- NADPH + H^+ → NADP^+ + H_2O
- Biliverdin reductase
- Biliverdin
- Bilirubin-potent antioxidant properties
- NADPH + H^+ → NADP^+
In the stomach, HO-I counteracts the two major mechanisms of NSAID induced gastric injury, i) disturbance of gastric microcirculation and ii) free radical release. Vitamin C was identified as a potential nonstressful inducer of HO-I in the stomach. However, till today, there are only very limited data about this enzyme in the stomach. It has been shown that healing of gastric ulcers in rats is paralleled by an upregulation of HO-I. Further studies are needed to examine the role of HO-I in the stomach in vivo. Vitamin E (76) is a lipid soluble antioxidant that interacts with oxygen and lipid radicals, and prevents the propagation of free radical lipid peroxidation. Some studies have shown its protective effect against gastric mucosal injury induced by ischemia reperfusion and other insults. In addition, it has been demonstrated that it has a protective effect against NSAIDs induced gastric mucosal injury. Effect of vitamin E on aspirin induced gastric mucosal injury was investigated in rats, and it has been found that vitamin E protected gastric mucosal injury by inhibiting lipid peroxidation and accumulation of activated neutrophils.

Melatonin (77), a pineal secretory product was recently found to be a potent antioxidant and free radical scavenger, especially hydroxyl radical (·OH) and peroxyl radical (ROO·). In in vitro and in vivo experiments, melatonin has been found to protect tissues against oxidant damage induced by various free radical generating agents. It has been observed that pretreatment with melatonin reduced indomethacin induced gastric damage and plasma malondialdehyde (MDA) level. MDA is a major aldehyde resulting from the peroxidation of biological membrane PUFA. The results showed that in multistress conditions, the intensity of gastric damage and the plasma MDA levels are high and melatonin reduces the negative effect of lipid peroxidation and cell damage due to its antioxidant activity.
The antiulcer effect of melatonin on gastric lesions caused by piroxicam (78) has been studied to investigate the mechanism of action of this agent. Melatonin dose dependently reduced indomethacin (13) and piroxicam (78) induced gastric damage with more than 90% inhibition at a dose of 60 mg/kg. Increased lipid peroxidation, augmented protein oxidation and decreased glutathione content of the gastric tissue following piroxicam treatment indicated the possible involvement of oxidative stress in this NSAID induced gastropathy. Pretreatment of rats with melatonin prevented these changes. Oral administration of piroxicam (78) to rats caused a threefold increase in the tissue levels of hydroxyl radical (·OH) generation, a change significantly attenuated by melatonin. An advantage of melatonin as an antioxidant lies in the fact that it is amphiphilic. Thus, it can readily reach all subcellular compartments to scavenge the reactants generated during oxidative stress at their sites of generation. Its potent antioxidant activities and the absence of any demonstrated toxicity at even pharmacological concentrations may predictably make this small indole an important therapeutic molecule in gastroprotection. This study therefore, showed melatonin’s gastrophreptive ability against piroxicam induced gastric damage. Based on these results, it has been proposed that coadministration of melatonin and NSAIDs may result in safer therapy.

Polaprezinc (79) is a novel antiulcer agent developed in Japan which is chemically a chelate compound that consists of L-carnosine and zinc. L-carnosine and its related compounds are present in high concentrations (1-20 mM) in the muscle and brain of mammals including humans and these agents have been shown to have antioxidant activities, both in vivo and in vitro.
Earlier studies have shown that the zinc ion exerts an antioxidant effect through the protection of sulfhydryl groups against oxidation and through the inhibition of the production of ROS by the transition metals. Thus, both zinc ions and L-carnosine may contribute to the antioxidant property of polaprezinc.\textsuperscript{150,151}

\[
\begin{align*}
\text{(79)}
\end{align*}
\]

It has been shown that polaprezinc (79) protected the gastric mucosa against experimental ulceration and accelerated the healing of gastric ulcers in rats as well as in humans. This property may be due to the stimulation of mucus production, antioxidant activity and the maintenance of gastric mucosal barrier, although the precise mechanisms remain obscure.

Allopurinol (80), vitamin E and selenium have been used separately as well as in combination against gastric mucosal damage caused by ROS in cold restraint stress model. It has been reported that stress causes a rapid decrease in glutathione levels in gastric mucosa. It was also reported that vitamin E has an antioxidant effect by scavenging free radicals resulting in reduced gastric

\[
\begin{align*}
\text{(80)}
\end{align*}
\]
mucosal damage. Effects of allopurinol were examined on gastric mucosal injury in cold restraint stress and it was observed that this xanthine oxidase inhibitor exhibited protective effects on gastric mucosa. In view of lipid peroxidation, it was observed that MDA levels decreased significantly. Selenium is an important element for metabolic functions and is found in various tissues and is a cofactor of glutathione peroxidase, an enzyme which detoxifies 'OH. Therefore, selenium protects ROS mediated damage by activating this enzyme. When these agents namely, allopurinol, vitamin E and selenium were studied in combination, it was found that they decrease gastric damage. Based on these results, it may be concluded that prevention of gastric mucosal damage induced by cold restraint stress and free radicals mediated lipid peroxidation can be prevented by various exogenous agents, including moderate doses of vitamin E, allopurinol and selenium.152

The role of L-arginine (81) in ibuprofen (82) induced oxidative stress in gastric mucosa has been studied. Oral administration of ibuprofen produced severe damage on gastric mucosa, which was more severe after 6h test period, and accompanied by a significant increment in myeloperoxidase (MPO) activity. Simultaneous treatment with equimolar doses of L-arginine orally, as well as intraperitoneally considerably reduced the number and intensity of lesions, and at the same time the maximum protection was also observed during 6h. In addition, L-arginine inhibited the ibuprofen induced lipid peroxidation and xanthine oxidase enhancement. These findings suggested that i) L-arginine protective effect on gastric mucosa against ibuprofen induced mucosal lesions could be explained by a local effect and also might be due to the systemic action
of the amino acid, ii) the active oxygen species derived both from xanthine oxidase and activated neutrophils, could play an important role in the pathogenesis of gastric injury induced by ibuprofen, iii) L-arginine exhibited a protective effect against ibuprofen induced mucosal damage, probably through the inhibition of oxidative stress derived via xanthine oxidase.\textsuperscript{153}

In clinical settings, proton pump inhibitors have been found to be effective in preventing and healing NSAID induced gastroduodenal lesions (Figure 12). The role of acid suppression effect of omeprazole (83) on gastroprotection against some necrotizing agents including ethanol, acidified aspirin, hypertonic saline, 0.6 M HCl, has been studied and it has been found that acid inhibition plays no significant role on the gastroprotective effect of omeprazole. Moreover, omeprazole neither stimulated prostaglandin biosynthesis nor increased bicarbonate secretion to offer gastroprotection. Thus, omeprazole exerted its antiulcer activity through some other mechanism that has not been explored yet. Using animal models of stress and indomethacin induced gastric lesions and pylorus ligation induced acid secretion, it has been reported that the gastroprotective effect of omeprazole is not mediated through its acid inhibitory

![Chemical structures of proton pump inhibitors](image)

Figure 12: List of proton pump inhibitors namely, omeprazole (83), lansoprazole (84) and pantoprazole (85).
Further evidence has been presented to show that endogenous \( {\cdot} \text{OH} \) plays one of the major roles in gastric lesions and that omeprazole acts as a potent antioxidant to scavenge the endogenous \( {\cdot} \text{OH} \), thereby preventing the oxidative damage by increased lipid peroxidation and protein oxidation. Moreover, it offered an antiapoptotic effect by blocking DNA fragmentation during ulceration. Evidence has also been presented to show that omeprazole (83) or lansoprazole (84) blocked \( {\cdot} \text{OH} \) induced oxidative damage of DNA by scavenging \( {\cdot} \text{OH} \) in vitro. Analysis of the major oxidation product of lansoprazole (84) indicated that this antioxidant activity was due to scavenging of \( {\cdot} \text{OH} \) to form an oxygenated product that is assigned to lansoprazole sulfone. The studies thus provided new insights on the mechanism of the antiulcer effect of proton pump inhibitors.\(^{137}\)

The mechanisms of protection afforded by pantoprazole (85) against gastric injury in rats induced by different NSAIDs, like ketoprofen and indomethacin was investigated. Two possible mechanisms have been suggested to explain the protective effects of pantoprazole against NSAID induced mucosal oxidative damage. Pantoprazole may directly scavenge ROS and/or interfere with the oxidative metabolism arising from activation of polymorphonuclear cells. It was also reported that pantoprazole directly protected native LDLs from copper induced oxidation. These results supported the earlier findings whereby omeprazole was shown to significantly scavenge hypochlorous acid and inhibited iron and copper driven oxidative reactions \textit{in vitro}. It has also been observed that proton pump inhibitors can significantly counteract the oxidative stress associated with polymorphonuclear cell activation.

Sulfhydryl radicals (\( \cdot \text{SH} \)) play a significant part in mechanisms involved in the defense of tissues against oxidative injury. Indeed, there is enough evidence to suggest that sulfhydryl donor drugs can afford protection against gastric mucosal injuries elicited by various necrotizing agents, stress, or ischemia. Adequate levels of sulfhydryl compounds also appear to be important for the prevention of NSAID induced gastropathy. This is supported by previous reports, which showed that mucosal depletion of sulfhydryl radicals contributed to the
pathogenesis of gastric lesions evoked by several NSAIDs. Moreover glutathione (GSH) levels were significantly decreased in mucosal biopsy obtained from patients with NSAID induced gastric bleeding. Consistent with these findings, in another study, animals were treated with ketoprofen, indomethacin, piroxicam, and marked reduction in mucosal GSH levels was observed. Further, it was found that this reduction in GSH levels could be reversed by pretreatment with pantoprazole. These findings indicated the antioxidant properties of pantoprazole, and preservation of mucosal sulfhydryl compounds from the excessive scavenging activity required to counteract NSAID induced oxidation. Therefore, it is conceivable that an increased bioavailability of endogenous sulfhydryls plays a significant role in the prevention of NSAID induced gastropathy by pantoprazole.154

Synthetic quinolinone derivative, rebamipide (86) is an effective antioxidant and antiulcer agent. This agent mediates its pharmacological activity mainly by increasing endogenous prostaglandin synthesis and ROS scavenging. The structural characteristics of rebamipide important for scavenging the hydroxyl radical ('OH) include 3,4-double bond and the 2-oxo functionality of the quinolinone moiety and the carbonyl part of the amido group in conjunction with a p-chlorobenzyl function.134

\[ \text{(86)} \]

Recently various SOD-mimetic complexes have been studied, based on the evidence that ROS was involved in the pathogenesis of gastric mucosal injury. One such agent is copper (II) aspirinate which has been found to show antiulcer activity because of its potent antioxidant action.155
These studies have provided enough evidence to explain the involvement of ROS in the NSAIDs induced gastric mucosal injury. Based on these observations it has been suggested that different antioxidants can be used to prevent NSAIDs induced gastric ulcers.

**Plants as source of antiulcer remedies**

Spices and herbs are recognized as sources of natural antioxidants that can protect from oxidative stress and thus play an important role in the prevention of ROS mediated diseases. A number of medicinal plants are used in the treatment and prevention of peptic ulcers, some of these are listed in Table 1.

**Table 1: List of plants with antiulcer activity**

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Plant part</th>
<th>Ulcer-model</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alpinia galanga</em></td>
<td>Rhizome</td>
<td>Stress, pylorus-ligated, ethanol, HCl</td>
<td>157</td>
</tr>
<tr>
<td><em>Amphipitygium adstringens</em></td>
<td>Stem bark</td>
<td>Ethanol</td>
<td>158</td>
</tr>
<tr>
<td><em>Angeissus latifolia</em></td>
<td>Root</td>
<td>Ethanol</td>
<td>159</td>
</tr>
<tr>
<td><em>Asparagus racemosus</em></td>
<td>Bark</td>
<td>Aspirin, stress, pylorus-ligated, ethanol</td>
<td>160</td>
</tr>
<tr>
<td><em>Astronium unundueva</em></td>
<td>Bark</td>
<td>Aspirin, stress, histamine</td>
<td>163</td>
</tr>
<tr>
<td><em>Atractylodes lancea</em></td>
<td>Rhizome</td>
<td>HCl</td>
<td>164</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>Leaves, bark</td>
<td>Stress, ethanol, indomethacin</td>
<td>165</td>
</tr>
<tr>
<td><em>Baccharis triptera</em></td>
<td>Small branches</td>
<td>Pylorus-ligated, stress, indomethacin</td>
<td>166</td>
</tr>
<tr>
<td><em>Bauhinia racemosa</em></td>
<td>Flower buds</td>
<td>Aspirin</td>
<td>167</td>
</tr>
<tr>
<td><em>Bryophyllum pinnatum</em></td>
<td>Leaves</td>
<td>Aspirin, indomethacin, serotonin, reserpine, stress, ethanol, pyrrolus-ligated, acetic acid</td>
<td>168</td>
</tr>
<tr>
<td><em>Caesalpinia ferrea</em></td>
<td>Stem</td>
<td>Acetic acid</td>
<td>169</td>
</tr>
<tr>
<td><em>Calendula officinalis</em></td>
<td>Rhizome</td>
<td>Caffeine-arsenic, butadione, pyrrolus-ligated</td>
<td>170</td>
</tr>
<tr>
<td><em>Calliandra portocicilens</em></td>
<td>Leaves</td>
<td>Stress, pyrrolus-ligated, E. coli</td>
<td>171</td>
</tr>
<tr>
<td><em>Camellia sinesis</em></td>
<td>Leaves</td>
<td>Stress, ethanol, asprin, indomethacin, reserpine, histamine, serotonin</td>
<td>172</td>
</tr>
<tr>
<td><em>Cassia nigrans</em></td>
<td>Leaves</td>
<td>Aspirin, pyrrolus-ligated</td>
<td>173, 172, 173</td>
</tr>
<tr>
<td><em>Cistus incanus</em></td>
<td>Aerial part</td>
<td>HCl, ethanol, reserpine, serotonin</td>
<td>174</td>
</tr>
<tr>
<td><em>Curcuma longa</em></td>
<td>Rhizome</td>
<td>Pylorus-ligated, cold-restraint stress, indomethacin, reserpine, histamine, serotonin</td>
<td>175</td>
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<tr>
<td><em>Diodia sarmentosa</em></td>
<td>Whole plant</td>
<td>Aspirin, pyrrolus-ligated</td>
<td>173</td>
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<tr>
<td><em>Emblca officinalis</em></td>
<td>Fruit</td>
<td>Indomethacin</td>
<td>176</td>
</tr>
<tr>
<td><em>Entandrophragma utile</em></td>
<td>Bark</td>
<td>Ethanol</td>
<td>177</td>
</tr>
<tr>
<td><em>Eremomastax speciosa</em></td>
<td>Leaves</td>
<td>Ethanol, HCl, pyrrolus-ligated</td>
<td>178</td>
</tr>
<tr>
<td><em>Ficus exasperata</em></td>
<td>Leaves</td>
<td>Aspirin, pyrrolus-ligated, Ethanol</td>
<td>173</td>
</tr>
<tr>
<td><em>Garcinia indica</em></td>
<td>Fruit</td>
<td>Water immersion stress, indomethacin</td>
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<tr>
<td><em>Glycyrrhiza glabra</em></td>
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<td>Acetic acid, pyrrolus ligated</td>
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<tr>
<td><em>Kochia scoparia</em></td>
<td>Fruit</td>
<td>Ethanol, indomethacin</td>
<td>164</td>
</tr>
<tr>
<td><em>Laurus nobilis</em></td>
<td>Seeds</td>
<td>Ethanol</td>
<td>181</td>
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</table>

Contd.
Table 1 Contd.

<table>
<thead>
<tr>
<th>Botanical name</th>
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<th>Ulcer-model</th>
<th>Ref.</th>
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<td>Bark</td>
<td>(Clinical study)</td>
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<td>184</td>
</tr>
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<td>Microgramma squamulosa</td>
<td>Rhizome</td>
<td>Stress, ethanol, HCl, acetic acid</td>
<td>185</td>
</tr>
<tr>
<td>Mikania cordata</td>
<td>Root</td>
<td>Stress, ethanol, aspirin, phenylbutazone, pylorus-ligated</td>
<td>186</td>
</tr>
<tr>
<td>Moringa pterygosperma</td>
<td>Flower buds</td>
<td>Aspirin</td>
<td>167</td>
</tr>
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<td>187</td>
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<td>189, 190</td>
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<td>Ethanol</td>
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<td>171, 193</td>
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<td>Indomethacin</td>
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<td>Veronica kotschyanana</td>
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<td>Veronica officinalis</td>
<td>Aerial parts</td>
<td>Indomethacin, reserpine</td>
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<td>Viburnum dilatatum</td>
<td>Seeds</td>
<td>Stress</td>
<td>208</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Root</td>
<td>Indomethacin, stress</td>
<td>162</td>
</tr>
<tr>
<td>Zingiber officinalis</td>
<td>Root</td>
<td>HCl, ethanol</td>
<td>209</td>
</tr>
</tbody>
</table>
Extracts of various medicinal plants have shown promising results in the treatment of gastric ulcers. It has been reported that antiulcerogenic activities of these extracts are due to the presence of antioxidant compounds. Their activities may also be influenced by other organic and inorganic compounds such as coumarins, phenolic acids and antioxidant micronutrients, e.g., Cu, Mn, Zn. Plant phenolics belong to recent popular phytochemicals, with potential beneficial effects on human health. Experimental studies have shown that several of the commonly used spices are effective in prevention of NSAID related gastric mucosal injuries in experimental animals.

**NSAID-antioxidant mutual prodrugs**

Based on the observation that NSAID 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 mutual prodrugs, which are designed with improved physicochemical properties and at the same time release the parent molecules at the desired site of action.

Prodrug approach has been used as an strategy to prevent the gastrointestinal side effects of various NSAIDs having carboxylic functions. In this strategy the -COOH group has been masked by derivatization. However, recently NSAID-antioxidant mutual prodrugs have attracted attention of many medicinal chemists and number of such derivatives have been reported.

In this direction, a series of amide derivatives (87-93, Figure 13) of different conventionally used NSAIDs with L-cysteine ethyl ester have been reported. The sulfhydryl (-SH) group of the latter moiety is likely to confer antioxidant properties to the novel compounds.
Figure 13: Structures of various NSAID-L-cysteine ethyl ester mutual prodrugs (87-93).

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.

Similarly, a number of NSAID–cysteamine conjugates (94-97, Figure 14) have also been reported.\textsuperscript{218,219} 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 \textit{in vitro} lipid peroxidation.

![Figure 14: Structures of NSAID cysteamine mutual prodrugs namely, diclofenac-cysteamine (94), tolfenamic acid-cysteamine (95) indomethacin- cysteamine (96) and ibuprofen-cysteamine (97).](image)

Similarly, ibuprofen has been conjugated with number of curcuminoids. For example, ibuprofen-dehydrozingerone derivative (98) was studied for pharmacological evaluation and found to exhibit strong antiinflammatory activity without any gastrointestinal toxicity in animal studies.\textsuperscript{220}
Similarly, mefenamic-guaiacol ester (99) 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.221

4-Biphenylacetic acid (BPA,100) is one of the active metabolites of NSAID fenbufen (101). 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 mutual prodrugs have been prepared. For this purpose, different naturally occurring antioxidants have been conjugated through its
-COOH group, directly (102-105) as well as through spacer (106-109).\textsuperscript{222} The structures of these agents have been shown in Figure 15.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Figure 15: Structures of BPA-antioxidant mutual prodrug.}
\end{figure}

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 (110) has been found to exhibit significant antiinflammatory activity with significantly reduced ulcerogenic side effects.\textsuperscript{223}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structure110.png}
\caption{110}
\end{figure}

Ibuprofen has also been conjugated through its -COOH group with the -OH group of various naturally occurring phytophenols, including QTME to give ibuprofen-QTME mutual prodrug (111).\textsuperscript{224}
Other flavonoids including naringenin and hesperetin have also been conjugated to give ibuprofen-antioxidant mutual prodrugs (112 and 113 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.224

A series of indomethacin phenolic antioxidants conjugates (114-121) have been synthesized with the objective of reducing ulcerogenic potential of...
indomethacin. The structures of the target compounds are shown in Figure 16.

Figure 16: Structures of indomethacin-antioxidant conjugates (114-121).
It was found that all conjugates were very potent antioxidants \textit{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.

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 17).\textsuperscript{226} In these agents, a NSAID and cysteamine or cysteine ethyl ester have been chemically attached to a proline, 4-hydroxy-proline or piperolic acid moiety. The compounds (122-128) were found to retain antiinflammatory and antioxidant activities, with hypocholesterolemic and a greatly reduced gastrointestinal toxic action.

\textbf{Figure 17:} List of indomethacin and naproxen-antioxidant conjugates (122-128).

It has been proposed that these novel compounds may find useful applications in slowing the progression or delaying the onset of neurodegenerative diseases.