Synthesis of naproxen-antioxidant hybrids as gastro sparing NSAIDs.

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CERTIFICATE

This is to certify that the project report entitled **"Synthesis of naproxen-antioxidant hybrids as gastro sparing NSAIDs"** submitted by Ms. Shagun Sood and Ms. Ashima Thakur to the Department of Pharmacy, Jaypee University of Information Technology, Waknaghat (Solan), in partial fulfillment of the requirements for the award of the degree of Bachelor of Pharmacy, is a bonafide record of work out by them under my supervision. This work has not been partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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SHAGUN SOOD Shagun
ASHIMA THAKUR Ashima

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SUMMARY

Non steroidal anti-inflammatory drugs (NSAIDs) belong to one of the most widely prescribed therapeutic agents for treating various inflammation related disease. However, their usefulness is limited due to their gastrointestinal toxic side effects. The pharmacological activity of NSAIDs is related to their ability to inhibit the activity of the enzyme cyclooxygenases (COXs) involved in the biosynthesis of prostaglandin H_2 (PGH_2). It is now well known that COX exists in two isoforms, namely COX-I and COX-II, which are regulated differently. COX-I is constitutively expressed in stomach to provide cytoprotection of the gastrointestinal tract and COX-II plays a major role in prostaglandin biosynthesis in inflammatory cells. Since, most of the NSAIDs used clinically inhibit both isoforms, long term use of these agents results in appreciable gastric ulcer and there is enough evidence that inhibition of COX-I rather than that of COX-II underlies gastric ulcer formation. As a result, a number of selective COX-II inhibitors, including Celecoxib and Rofecoxib have been introduced for clinical use with exceptional anti-inflammatory properties and reduced gastric toxicity. But initial enthusiasm for selective COX-II inhibitors has faded due to emergence of serious side effects on long term use and the search for safer NSAIDs still continues. In this direction, we have synthesized naproxen-eugenol and naproxen-thymol hybrids. These hybrids have shown better anti-inflammatory activity than naproxen and less ulceration in comparison with naproxen.

CHAPTER -1

INTRODUCTION

Nonsteroidal anti-inflammatory 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) anti-inflammatory effect, ii) analgesic effect, and iii) antipyretic effect.[5,6] The anti-inflammatory 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 nociceptic 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 anti-inflammatory agents.

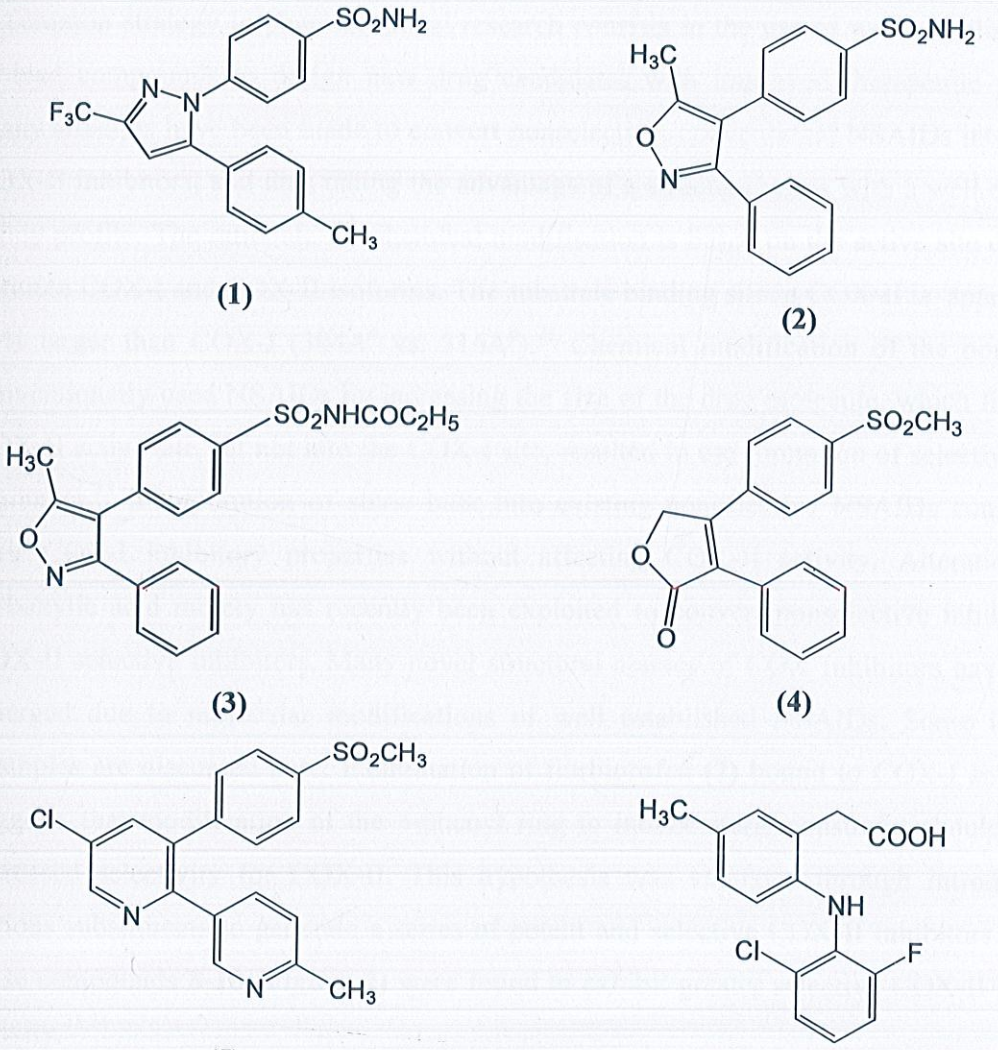
CHAPTER -2

REVIEW OF LITERATURE

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.[14] 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.[14-16] These side effects forced the drug companies to withdraw rofecoxib **(4)** and, soon afterwards, valdecoxib **(2)** from the market.[17-19] 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]



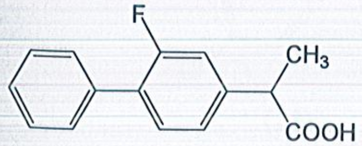
(1) (2) (3) (4) (5) (6)

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 (394A^o vs. 316A^o).[21] 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.[22] 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 flurbiprofen **(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.[23,24]



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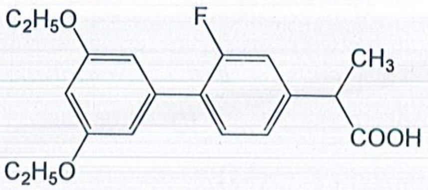

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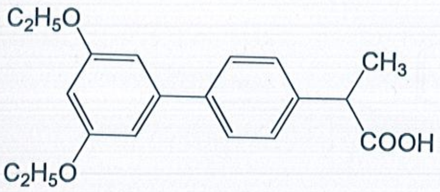

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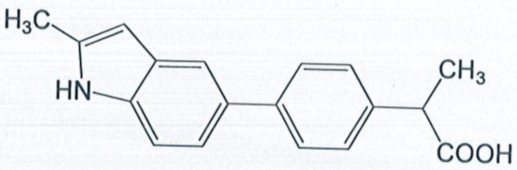
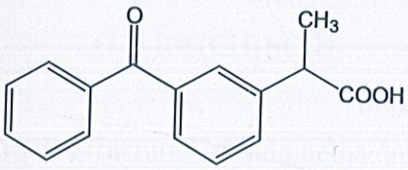


(10)

Figure 2: Structures of flurbiprofen (7) and its transformed selective COX-II inhibitors (8-10).

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.[25]



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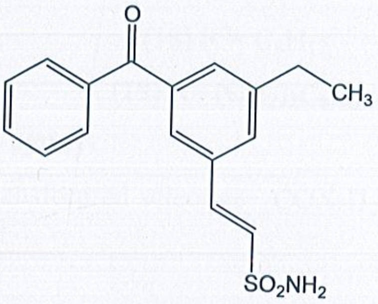

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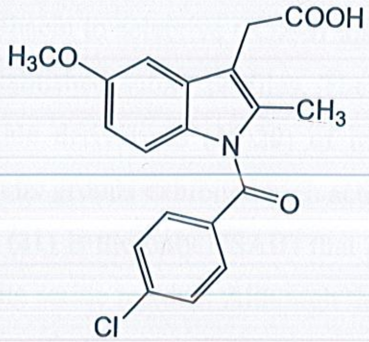
Indomethacin **(13)** is one of the most potent nonselective NSAIDs. This agent has also been transformed into selective COX-II inhibitors by systematic structural modification to increase the size (**Figure 3**).



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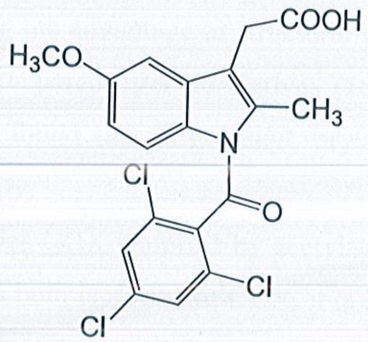

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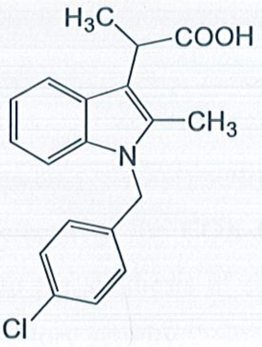

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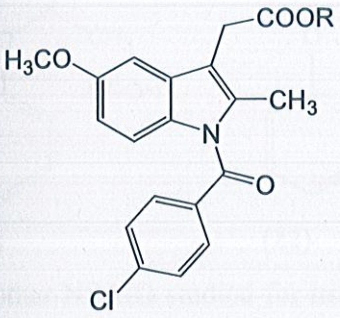
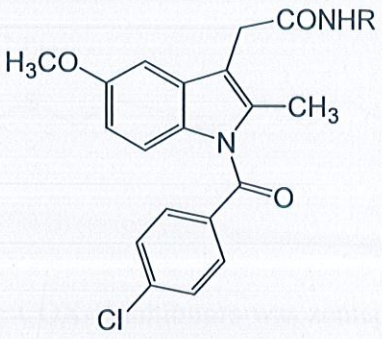




(16) R = C_7H_{15}

(17) R= $(CH_2)_2C_6H_5$

(18) R = C_8H_{17}

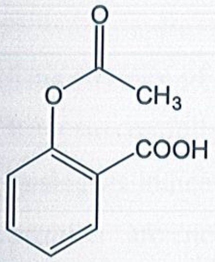
(19) R= $(CH_2)_2C_6H_5$

(20) R=5-chloro-3-pyridyl

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.[26] Based on these results, Black *et al* [26] 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* [27,28] 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 heteocyclic 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.[29,30] The anti-inflammatory 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*-(acetoxyphenyl)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*-(acetoxyphenyl)hept-2-ynyl sulfide **(23)** having most potent COX-II inhibitory activity.[31]



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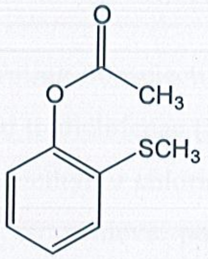

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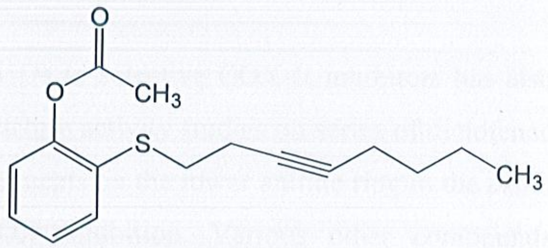
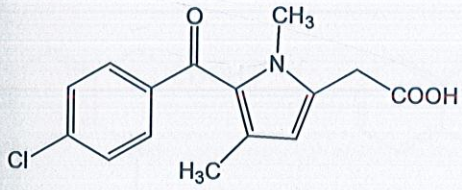


(23)

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)**.[32,33]



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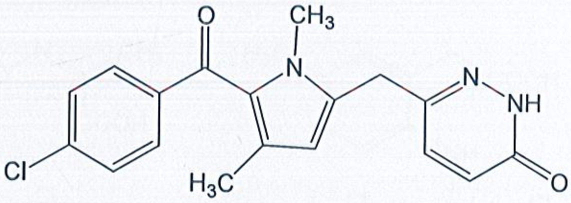

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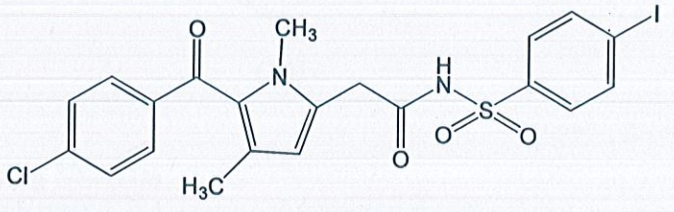
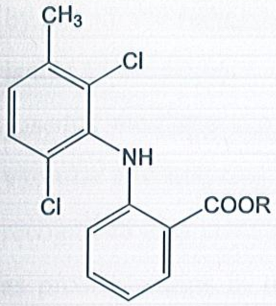


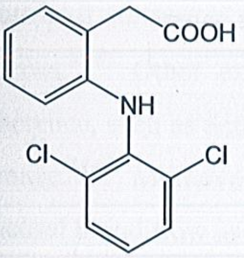
(26)

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.[27,34]



(27) R = H
(28) R = $NH(CH_2)_2\ CH_2Cl$
(29) R = $NH(CH_2)_2OC_6H_5$
(30) R = $NHOCH_2C_6H_5$
(31) R = $NHOCH_2C_6H_4(4\text{-}NO_2)$
(32) R = $NHCH_2COOCH_3$

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]



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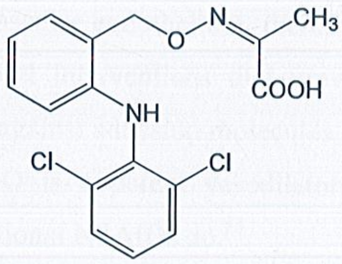

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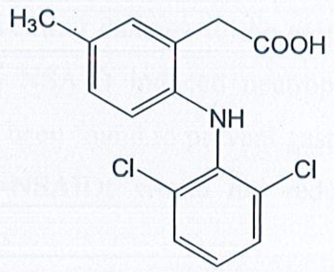

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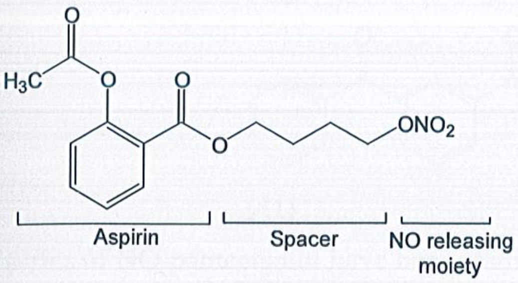
Nitric oxide releasing NSAIDs

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 anti-inflammatory 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.[36] NO is also a very potent inhibitor of neutrophil adherence to the vascular endothelium.[37] 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.[38-44] Moreover, NO suppresses the release of several inflammatory mediators from mast cells that are known to contribute to gastric ulceration, including platelet activating factor.[45,46] 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.[47-50] It is noteworthy that use of NO donors have been found to significantly reduce GI bleeding in patients, taking aspirin for cardiovascular indications.[51] 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.[52-55] Other experimental interventions that prevented NSAID induced neutrophil adherence, such as antibodies against adhesion molecules, have been found to prevent gastric damage.[39,43] Moreover, as NO is a potent vasodilator, NO-NSAIDs would not reduce mucosal blood flow as conventional NSAIDs do.[55]

Two distinct chemical classes, NO-NSAID and *S*NO-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.[56] 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.[57-60] In experimental models, NO-NSAIDs even protected gastric mucosa against damage induced by other deleterious stimuli and maintained gastric mucosal blood flow.[61-63] Ukawa *et al* [64] 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 anti-inflammatory and antinociceptive efficacy.[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)**.[66-68]



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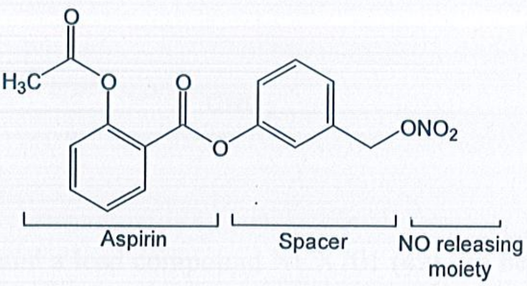
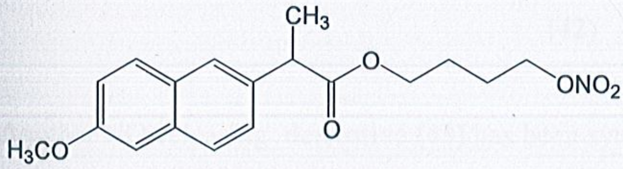


(37)

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.[69] However, it has been abandoned as one of its metabolite was found to be mutagenic.[70] NO-naproxen **(38)** is under phase-III clinical trials for treatment of osteoarthritis, acute and chronic pain.[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 anti-inflammatory effect.[52]



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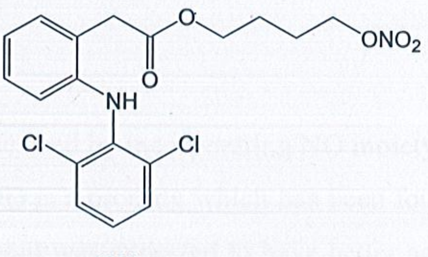
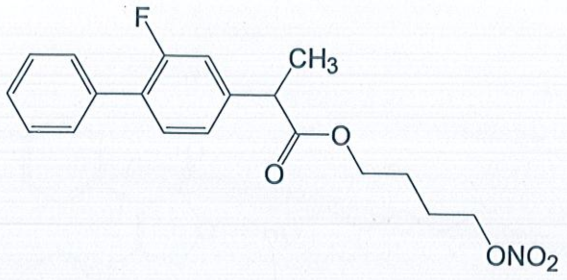


(39)

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 anti-inflammatory activity of the parent NSAID molecules with significantly reduced GI ulceration.[56,71]



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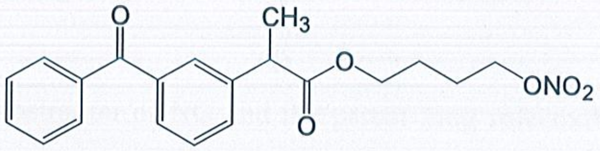
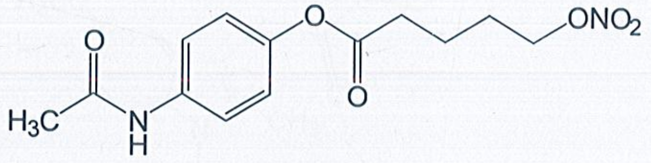


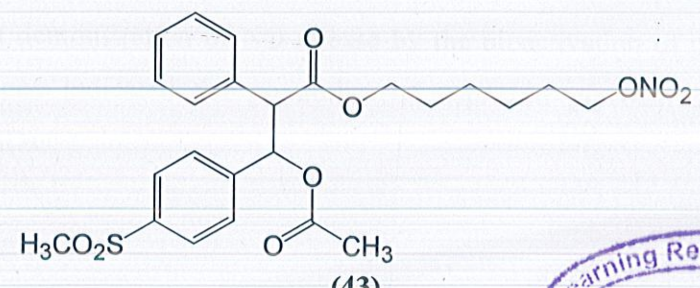
(41)

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 anti-inflammatory activity over a similar dose range. Moreover, NO-paracetamol was found to be safer than the parent drug on liver function.[72]



(42)

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.[73]



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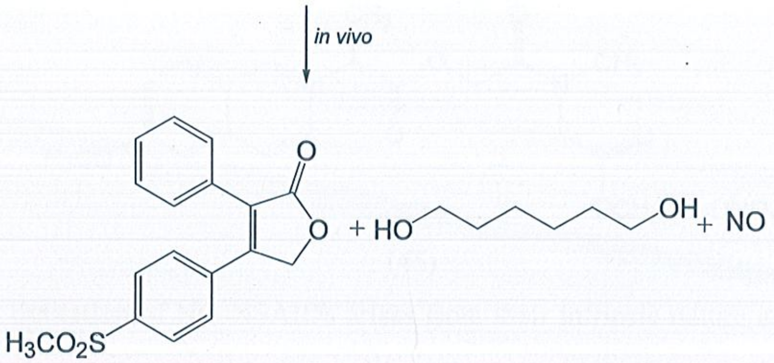
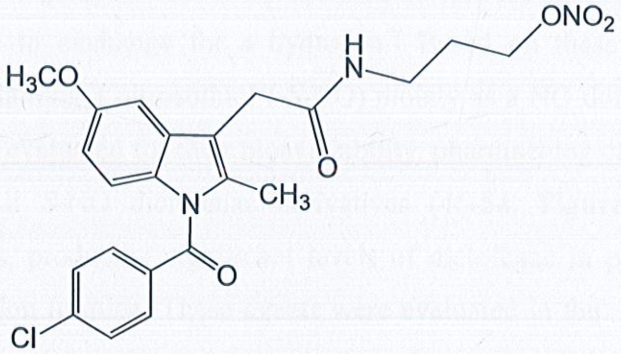


(4)

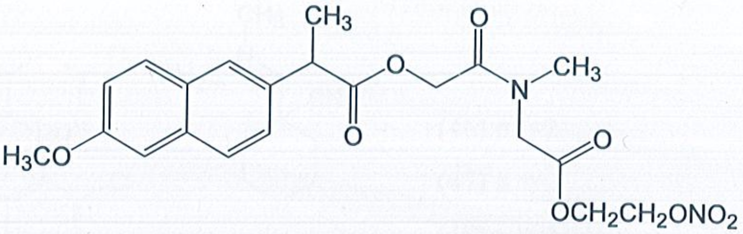
Figure 4: Schematic illustration of NO and the parent drug rofecoxib (4) release from the prodrug 43.

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 anti-inflammatory agent devoid of GI toxicity *in vivo*.[74]



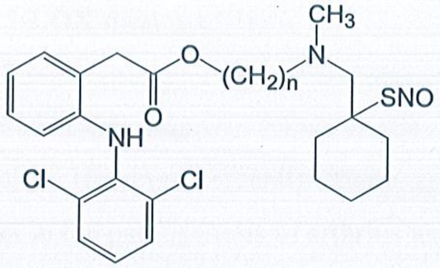
(44)

A series of glycolamide naproxen prodrugs containing a nitrate group as NO-donor moiety have been synthesized.[75] These compounds were evaluated for their anti-inflammatory 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.



(45)

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.[76] As an alternative, *S*-Nitrosothiols are considered as biological sources of NO. These agents release NO without undergoing any metabolic transformation.[77,78] 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.[9] 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 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 anti-inflammatory activity of the parent drug diclofenac, Additionally, these agents showed analgesic activity in mouse phenylbenzoquinone induced writhing test.[9] On similar lines, *S*NO-ibuprofen **(55)** and *S*NO-ketoprofen **(56)** have been synthesized. These derivatives have been reported to exhibit retention of analgesic and anti-inflammatory activities of the parent drug molecules with reduced gastrointestinal side effects.[56]

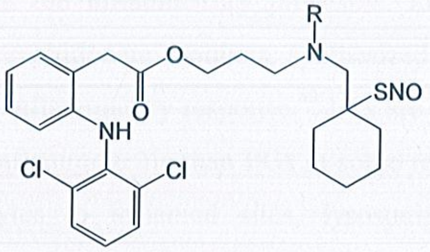


(46) n = 2

(47) n = 3

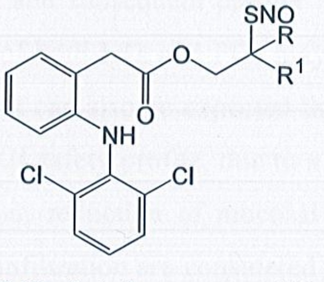
(48) n = 4

(49) n = 5



(50) R = $CH_2C_6H_5$

(51) R = CH_2CH_3

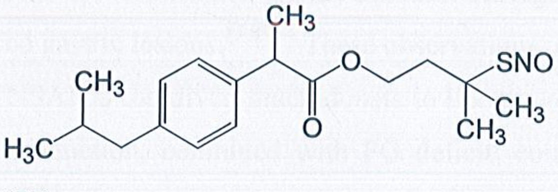


(52) R and R^1 = CH_3

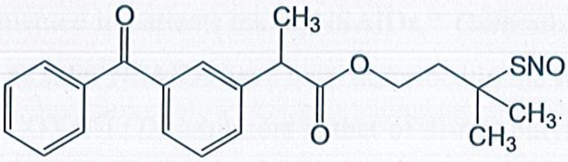
(53) R and R^1 = CH_2CH_3

(54) R and R^1 = $(CH_2)_5$

Figure 5: *S*NO-diclofenac esters (42-50).



(55)



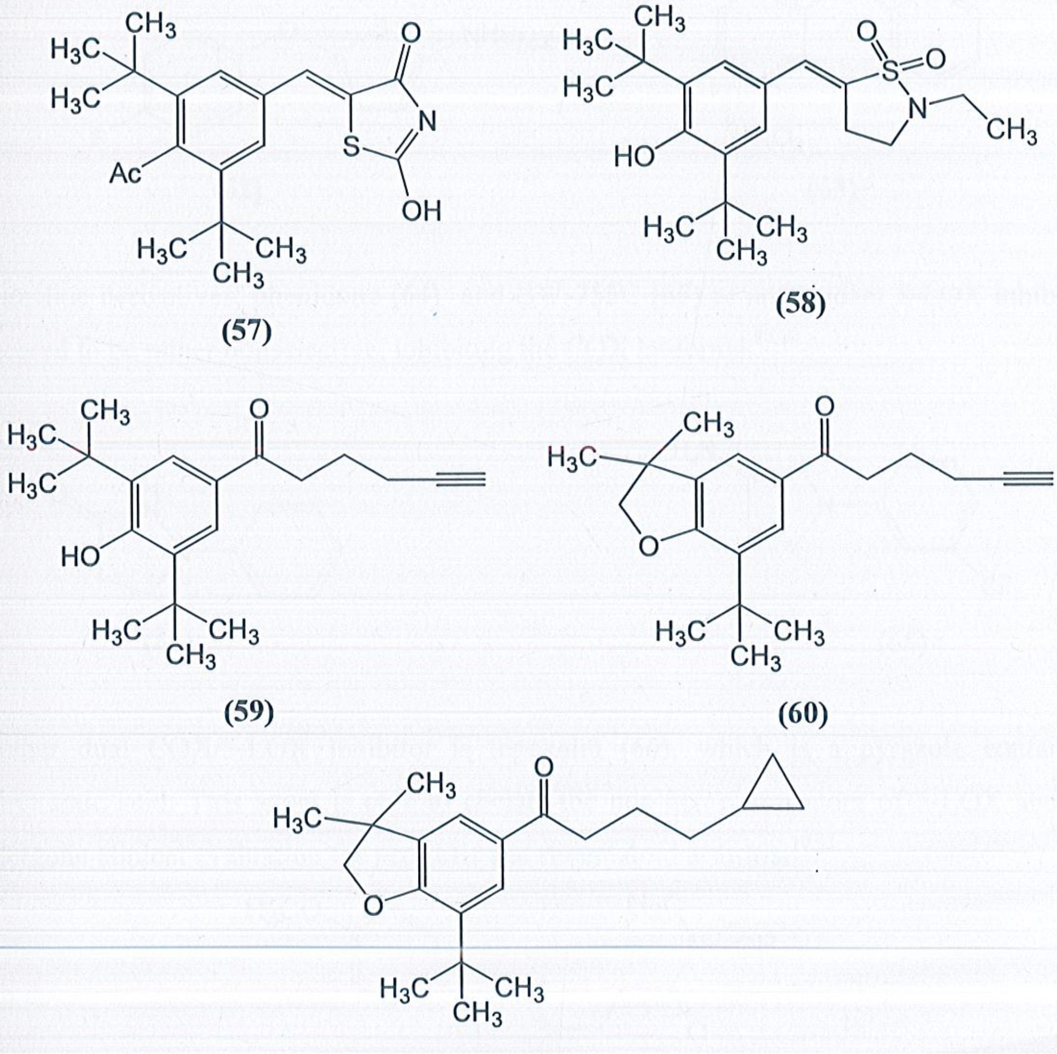
(56)

COX and 5-LOX dual inhibitors

As discussed (*vide supra*), NO-NSAIDs show anti-inflammatory 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. LTB_4, 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 anti-inflammatory 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 induced gastric lesions.[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.[85] In this direction, elevated production of LTB_4 in the human stomach has been documented in patients taking NSAIDs.[86] Currently, various classes of dual COX/5-LOX inhibitors as safer NSAIDs have been described in the scientific literature.[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 anti-inflammatory activity with reduced ulcerogenicity activity.[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 anti-inflammatory efficacy, the latter agent showed cytokine modulating properties.[88] It is currently being evaluated in clinical trials for arthritis.[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.[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 anti-inflammatory activity equivalent to that of tebufelone **(59)** in the rat carrageenan induced paw edema and also showed hepatoxicity.[90,91] The hepatic toxicity of metabolite **60** and the parent molecule **59** was attributed to the terminal unsaturation of side chains.[90]

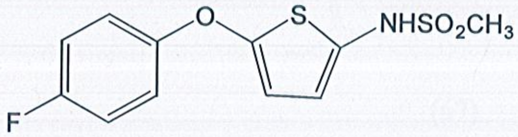


(57) **(58)** **(59)** **(60)** **(61)**

Figure 6: Structures of di-*tert*-butylphenol class of dual COX/5-LOX inhibitors

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 belongs 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)**. [87] It has been found to produce significant anti-inflammatory activity in a canine model of carrageenan induced inflammation.



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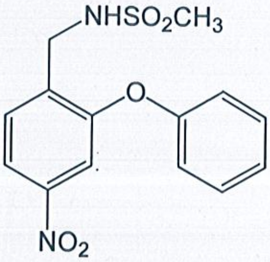
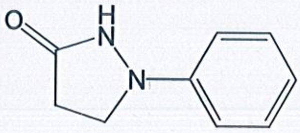


(63)

Pyrazoline derivatives, phenidone **(64)** and BW-755C **(65)** as antioxidant 5-LOX inhibitors appeared to be rather nonselective, inhibiting the COX Isoforms. [87,90]



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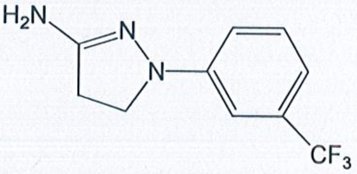
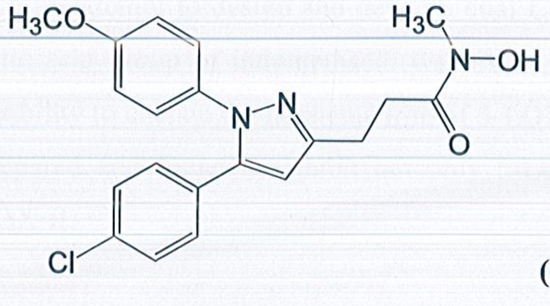


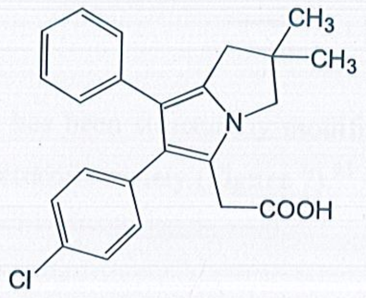
(65)

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. [79,92]



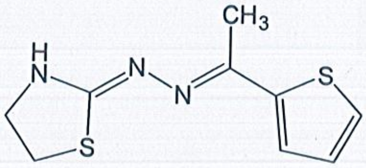
(66)

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.[87,93] In several animal models, this derivative has shown anti-inflammatory, 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.



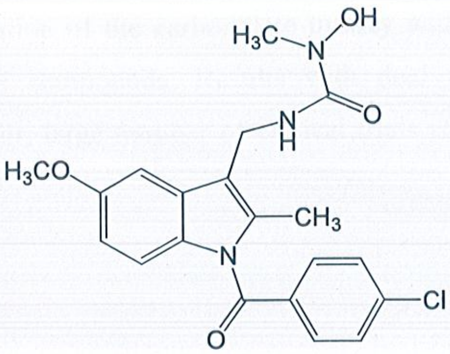
(67)

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.[90]



(68)

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.[94] This group has the capability to chelate the nonheme iron of 5-LOX. Such two derivatives **69** and **70** have been prepared and found to inhibit not only 5-LOX, but preferentially the inducible isoform COX-II.



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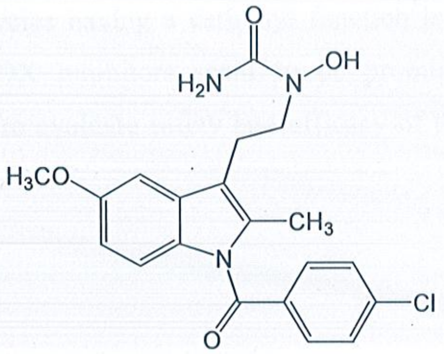
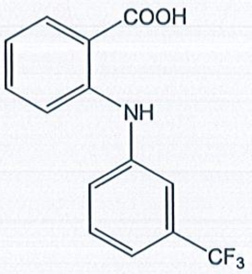


(70)

Similarly, flufenamic acid **(71)** has been structurally modified by bioisosterically replacing the carboxylic acid group with tetrazole moiety **(Figure 7).**[95]



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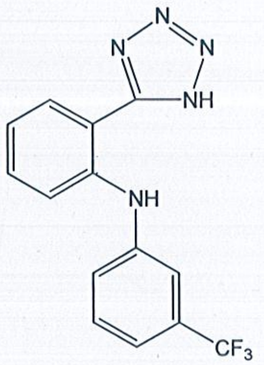

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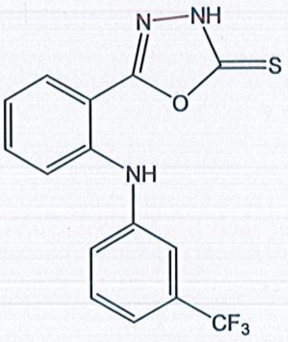

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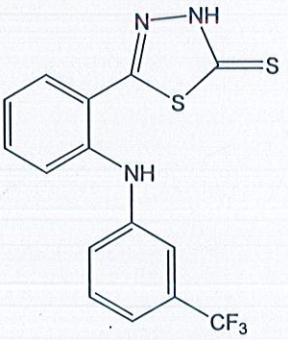


(74)

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 is required to evaluate safety and efficacy of these agents.

CHAPTER-3

RESEARCH ENVISAGED

NSAIDs are one of the most widely used classes 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] However, the major limitation of NSAID therapy is the higher incidence of 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 pharmacological activity of NSAIDs is related to their ability to inhibit the production of PGs from arachidonic acid by inhibiting the activity of the enzyme COX. Various conventional NSAIDs in clinical use have been shown to inhibit COX, leading to a marked reduction in PG synthesis. Now, it is well known that COX exists in two isoforms namely COX-I and COX-II.[2,11] 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] These observations stimulated an intense and competitive race to design and develop selective COX-II inhibitors as safer NSAIDs devoid of their GI side effects and number of such derivatives such as celecoxib, rofecoxib etc., collectively named as coxibs, have been introduced in the market for clinical use. However, long term use of these selective COX-II inhibitors revealed some potential limitations including ulcer exacerbation in high risk patients, delayed gastrointestinal ulcer healing, kidney toxicity, as well as cardiovascular side effects.[14-16] These observations indicate that safety of these agents is questionable on their long term use and recently, some of the coxibs have been withdrawn from the market. Therefore, the initial enthusiasm of developing selective COX-II inhibitors has faded away and need for designing and developing safer NSAIDs devoid of their ulcerogenic side effects still remains.

Review of literature reveals that involvement of various ROS is responsible for the formation of gastric ulcers associated with long term NSAIDs use.[96-98] Based on these observations, it has been suggested that concomitant use of an antioxidant and NSAID may decrease the risk of gastrointestinal toxicity and make the therapy safer. 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 site of action. On these lines, a number of NSAID-antioxidant mutual prodrugs devoid of their ulcerogenic side effects have been reported. These observations indicated that there is merit to carry out studies in this area of drug research for the design and development of NSAID-antioxidant mutual prodrugs as safer therapeutic agents. For this purpose, well known and widely used NSAID naproxen has been selected.

CHAPTER-4

MATERIAL AND METHODS

The reaction was monitored and purity of all compounds was established by single spot on the Merck percoated silica gel TLC plates. Iodine vapor was used for detection. The solvent system used was petroleum ether (60-80 °C): ethyl acetate (4:1). Melting points were determined on an Indosati digital melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin Elmer-spectrum RX-IFTIR, using potassium bromide pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AVANCE II 400 MHz spectrometer using $CDCl_3$ as solvent, and tetramethylsilane was used as internal standard. Mass spectra were obtained with Micromass 70-VSE mass spectrometer at 70 eV using electronionization (EI). Elemental analysis of compounds was within ± 0.04% of the theoretical values. All solvents were freshly distilled and dried prior to use according to standard procedures.

Synthesis of eugenol and thymol substituted naproxen esters

Synthesis of acetic 2-(6-methoxynaphthalen-2-yl)propanoic anhydride

The 400mg naproxen (1.732 mM) and 124.09µL acetyl chloride (136.35mg, 1.737 mM) were refluxed for 12 hr in 20ml dichloromethane in presence of 140.19µL pyridine (137.39 mg, 1.737 mM). The reaction was monitored by thin layer chromatography (TLC silica gel 60 F254, Merck, Germany) in solvent system petroleum ether (60-80 °C): ethyl acetate – 4:1. Reaction mixture was washed with 5% hydrochloric acid solution (15ml×3) and organic solvent was removed under reduced pressure to give 351mg anhydride product (1.28 mM, 74.20% w/w). White powder, M.P. 84-85 °C.

Synthesis of 4-allyl-2-methoxyphenyl 2-(2-methoxynaphthalen-6-yl)propanoate and 2-isopropyl-5-methylpheny2-(methoxynaphthalen-6-yl)propanoate

The 272.30mg anhydride of naproxen (1.00 mM), 153.88 µL eugenol (164.2 mg, 1.00 mM)/ 150.22mg thymol (1.00mM), 122.17mg 4-(dimethylamino)pyridine (1.00mM) were refluxed in 20ml pyridine at 80 °C for 24 hr to give ester products. The reaction was monitored by using TLC (solvent system petroleum ether (60-80 ºC): ethyl acetate– 4:1). Reaction mixtures were washed with 5% hydrochloric acid solution (15 ml×3). Washed products were extracted with dichloromethane and organic solvent was removed under reduced pressure to give final products, which were loaded for column chromatography, mobile phase petroleum ether (60-80 °C): ethyl acetate– 4:1, silica gel 100-200 mesh (Loba Chemie, Mumbai) to give final purified products.

4-Allyl-2-methoxyphenyl 2-(2-methoxynaphthalen-6-yl)propanoate

Crystallized from absolute ethanol to give white crystals, yield 41%, mp 99-100°C. Anal. calcd. for $C_{24}H_{24}O_4$ (376.17): % C, 76.57; H, 6.43. IR (ν_{max}, KBr, cm^{-1}): 3059.67 (C=CH-Ar stretch), 3008.91 ($CH=CH_2$ stretch), 2974.99, 2936.95, 2841.81(Ar C–H stretch), 1750.22 (ester, C=O), 1604.83, 1508.33 (C=C stretch), 1163.20, 1147.37, 1031.40, 1000.40 (C-O-C stretch). ^{1}H NMR ($CDCl_3$, δ ppm): 7.7922 (1H, d, J=1.00, Ar-H of nap), 7.7328 (2H, m, Ar-H of nap), 7.5209 (1H, dd, J= 8.56 & 1.8, Ar-H of nap), 7.1591 (1H, d, J= 2.52, Ar-H of nap), 7.1347 (1H, s, Ar-H of nap), 6.8324 (1H, d, J= 7.92, C-6-H), 6.7062 (2H, m, C-3-H & C-5-H), 5.9226 (1H, m, 1H, C-9-H), 4.1287 (1H, q, J=7.16, C-2′), 3.9197 (3H, s, C-7-H), 3.6580 (3H, s, C-13), 3.3375 (2H, d, J= 6.64, 2H, C-8-H), 1.6870 (3H, d, J= 7.08, C-2′). ^{13}C NMR ($CDCl_3$, δ ppm): 172.90 (C-1′), 157.64 (C-2), 150.95 (C-8′), 138.87 (C-9), 138.20 (C-1), 137.11 (C-4), 135.47 (C-3′), 133.75 (C-6′), 129.34 (C-11′), 128.98 (C-10′), 127.06 (C-4′), 126.51 (C-13′), 126.25 (C-5′), 122.33 (C-6), 120.62 (C-5), 118.94 (C-9′), 116.08 (C-10), 112.79 (C-3), 105.61 (C-7′), 55.73 (C-7), 55.33 (C-13′), 45.35 (C-8), 40.06 (C-2′), 18.84 (C-2″). ESI-MS (*m/z*): M$^+$, 376.08.

2-Isopropyl-5-methylpheny2-(methoxynaphthalen-6-yl)propanoate

Crystallized from absolute ethanol to give white crystals, yield 39%, mp 91-92°C. Anal. calcd. for $C_{24}H_{26}O_3$ (362.19): % C, 76.57; H, 6.43. IR (ν_{max}, KBr, cm^{-1}): 3028.48, 2962.38, 2927.35, 2871.14, 2731.60 (C–H stretch), 1733.18 (ester, C=O), 1618.98, 1583.88, 1517.13, 1507.2, 1457.69, 1420.02 (C=C, stretch), 1227.94, 1153.07 (C-O-C, stretch). ^{1}H NMR ($CDCl_3$, δ ppm): 8.5299 (1H, d, J=8.52, Ar-H of nap), 8.1863 (1H, d, J=9.00, Ar-H of nap), 8.0867 (1H, dd, J= 8.48 & 1.856, Ar-H of nap), 7.7086 (1H, s, Ar-H of nap), 7.6435 (1H, s, Ar-H of nap), 7.4594 (1H, d, J=7.88, Ar-H of nap), 4.6841 (1H, q, J= 7.192, C-2′-H), 4.3198 (3H, s, C-13′-H), 3.814 (1H, sept, C-7-H), 2.7039 (3H, T, J=23.68, C-10-H), 1.7844 (3H, d, J=7.2, C-8-H), 1.7433 (3H, d, J=8.2, C-9-H), 1.4849 (3H, s, C-2′-H), ^{13}C NMR ($CDCl_3$, δ ppm): 172.46 (C-1′), 158.09 (C-8′), 148.32 (C-1), 137.26 (C-2), 135.26 (C-5), 134.57 (C-3′), 132.43 (C-6′), 130.04 (C-11′), 129.77 (C-10′), 128.09 (C-4′), 127.09 (C-13 127.04 (C-3), 126.89 (C-5′), 126.73 (C-4), 119.58 (C-6), 116.88 (C-9′), 106.45 (C-7′), 55.63 (C-13′), 46.35 (C-1′), 26.93 (C-7), 23.52 (C-10), 23.30 (C-8), 21.27 (C-9), 18.38 (C-2″). ESI-MS (*m/z*): M^+, 362.17.

Animals

Male Wistar rats weighing 150–210 g were housed in colony room 12/12 hr light/dark cycle at 21 ± 2°C and had free access to water and food.

In vivo anti-inflammatory activity

The anti-inflammatory activity was carried out by xylene induced rat ear edema model. The Wister rats (n= 6) weighing 150-210g were randomly divided into 4 groups. The rats were fasted overnight. The group -1 was treated with vehicle, group-2 was treated with naproxen and group -3 and 4 were orally treated with naproxen- euginol and naproxen-thymol hybrids respectively. 0.03 ml of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. Two hours after xylene application, rats were killed and both ears were removed. Circular sections were taken, using a cork borer with a diameter of 7 mm, and weighed. The increase in weight caused by the irritant was measured by subtracting the weight of the untreated left ear section from that of the treated right ear sections.

Ulcerogenicity studies:

The Wister rats (n= 6) weighing 150-210g were randomly divided into 4 groups. The rats were fasted overnight. The group -1 was treated with vehicle, group-2 was treated with naproxen and group -3 and 4 were orally treated with naproxen- euginol and naproxen-thymol hybrids respectively. The animals were administered the parent drug as well as the mutual drug according to their groups. Animals were sacrificed 8 hours after the treatment. The stomach was removed and opened along the greater curvature, washed with saline and observed for ulcers. The gastric contents were collected in tubes for further studies. The comparison of the ulcers could be done by looking at the ulcers induced by the parent drug. The tubes containing the gastric content were analyzed using a pH meter. To further confirm this, the gastric content was titrated against N/10 NaOH solution with phenolphthalein as the indicator and the end point was noted.

Statistical analysis

Data are expressed as means ± SEM. Statistical comparisons were performed by one-way analysis of variance followed by Tukey's test. experimental values were statistically analysed using the unpaired Student's *t*-test. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

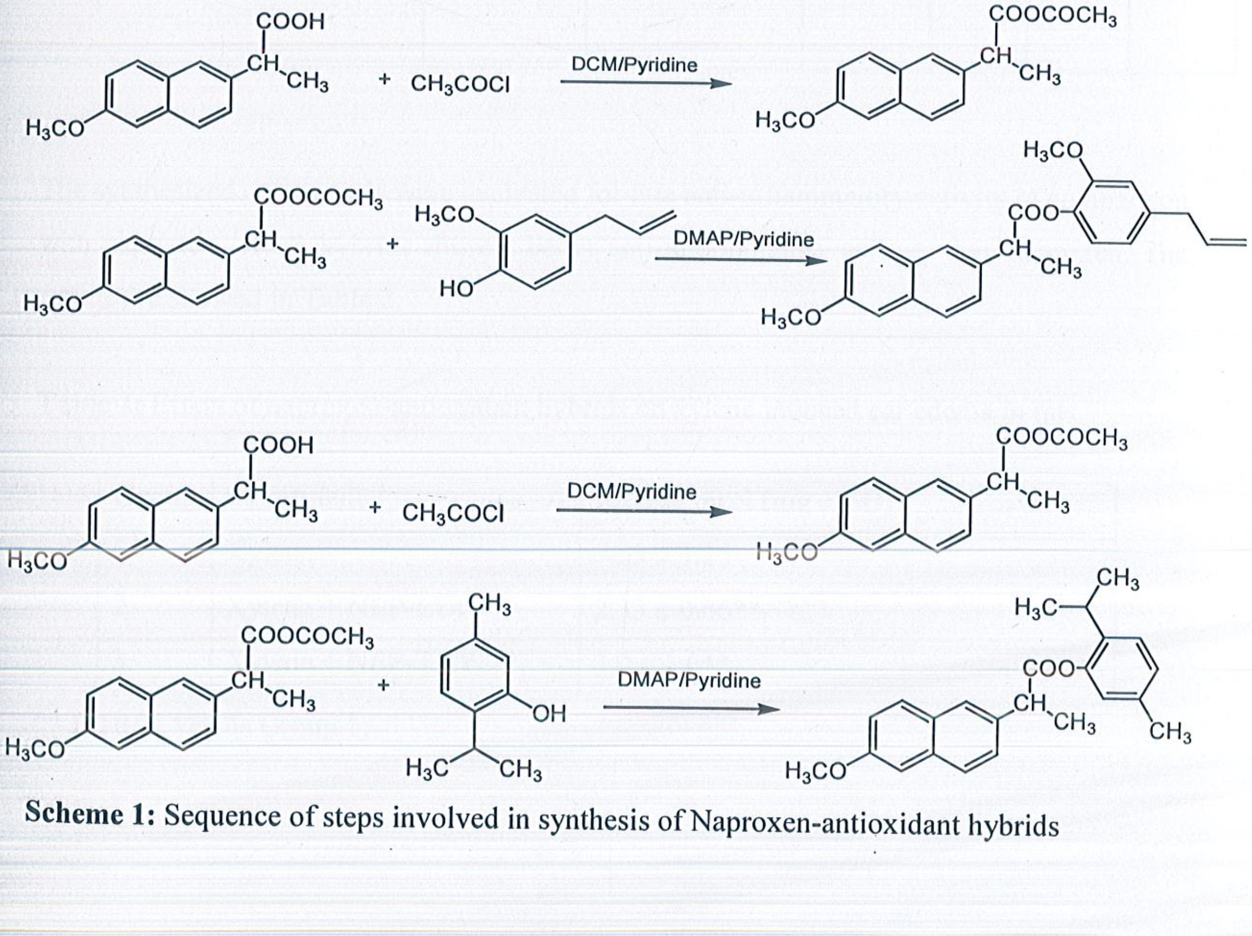
The work carried out in the present study is discussed under the following headings:

- Synthesis of naproxen –antioxidants hybrids
- Anti inflammatory and ulcerogenic activity evaluation

Synthesis of naproxen –antioxidants hybrids

Derivatisation of -COOH group of NSAIDs through esterification has been considered as a general strategy for the design and development of prodrugs of this class of therapeutic agents.[230-232] Since the ulcerogenic side effects of NSAIDs are thought to be mediated through ROS, conjugation of NSAIDs with phytophenols having antioxidant properties is one of the recent strategies for developing gastrosparing safer NSAIDs.[217-219,225] In the present study, the most commonly used NSAID, naproxen has been conjugated with various antioxidant (eugenol and thymol). These derivatives are expected to be absorbed in an inactive form through the GIT and cleaved to release the parent drug molecule and antioxidant moiety, which may prevent the NSAID induced gastric ulceration through their antioxidant properties by quenching ROS.

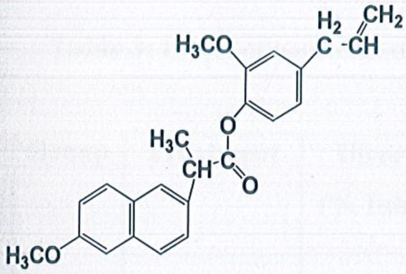
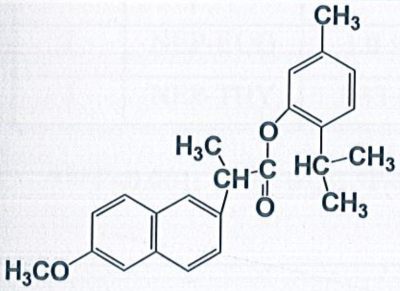
The synthesis of naproxen-antioxidant hybrids were synthesized as shown in Scheme 1.



Scheme 1: Sequence of steps involved in synthesis of Naproxen-antioxidant hybrids

The physical data of naproxen-antioxidants are described in Table 1.

Table-1: Physical properties of eugenol and thymol - naproxen hybrids

Structure	Mol. Formula	Chemical Name	Mol. Weight	M.P. (°C)	% Yield	R_F Value
	$C_{24}H_{24}O_4$	4-allyl-2-methoxyphenyl 2-(2-methoxy naphthalen-6-yl)propanoate	376.17	99-100	41	0.46
	$C_{24}H_{26}O_3$	2-isopropyl-5-methylpheny2-(methoxy naphthalen-6-yl)propanoate	362.19	91-92	39	0.49

The synthesized compounds were evaluated for thie anti-inflammatory activity in comparison with naproxen. These hybrids showed better anti-inflammatory activity than naproxen. The results are showed in Table 2.

Table 2: Effect of naproxen-antioxidant hybrids on xylene induced ear edema in rats

Group	Treatment	Edema level (mg ± SD)
1	Xylene	7.33 ± 1.15
2	Xylene + NEP-EUG	2.33 ± 0.66**
3	Xylene + NEP-THY	3.0 ± 1.0**

** P< 0.01 versus Group 1.

These hybrids also showed marked decrease in ulceration in comparison to naproxen. This effect may be attributed to antioxidant promoiety attached with naproxen and masked –COOH group in naproxen. The gastric volume also decreased significantly in naproxen-antioxidant hybrid groups. The gastric pH increased significantly and there was decrease in total acidity of gastric content in naproxen-antioxidant hybrid groups in comparison to naproxen. The results are shown in Table 3.

Table 3: Effect of naproxen-antioxidant hybrids on ulcers and gastric content

Group	Treatment	Ulcer Index (% Inhibition)	Gastric Volume (ml/100 g body weight)	Gastric pH	Total acidity (MEq/L)
1	Naproxen	3.16 ± 0.57	2.38 ± 0.14	2.47 ± 0.12	117.66 ± 22.36
2	NEP-EUG	1.0 ± 0.50*	1.23 ± 0.03***	5.5 ± 0.10***	63.66 ± 5.68**
3	NEP-THY	1.33 ± 0.76*	1.32 ± 0.02***	5.27 0.11***	78.33 ± 6.65*

***P<0.001; ** P< 0.01; *P<0.05 versus Group 1.

The naproxen-antioxidant hybrids showed marked decrease in ulcer index in comparison with naproxen. The macroscopy of stomach is shown in Figure 8 & 9.

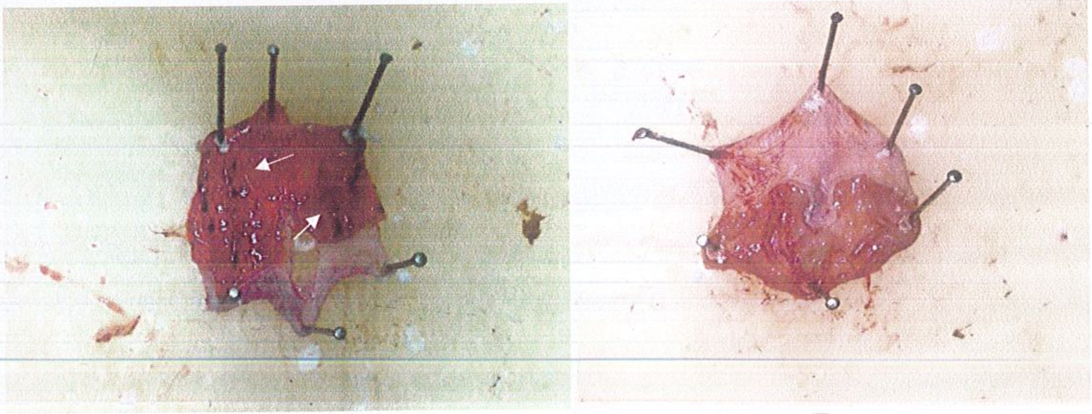
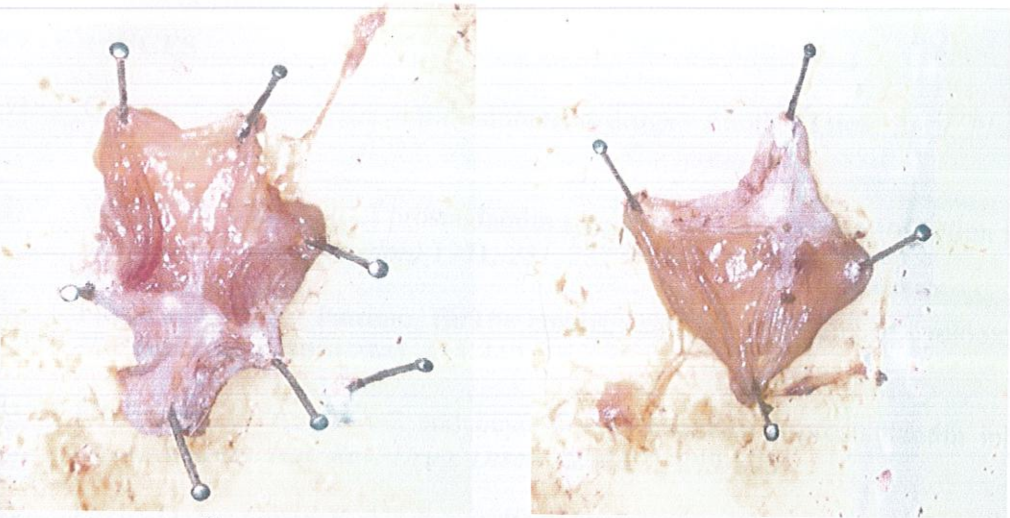

A B

Figure 8: A) Marked ulceration in Naproxen group shown as black spots; B) Control group with no ulceration



C D

Figure 9: C) No marked ulceration in naproxen-eugenol hybrid; D) No significant ulceration in naproxen-thymol hybrid

The results approve the hypothesis that conjugation of antioxidant with NSAIDs will decrease the ulcerogenicity associated with use of NSAIDs. Further, physicochemical studies/ stability studies of these hybrids are required to ensure their bio-availability and stability in plasma.

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