CHAPTER 2

LITERATURE REVIEW

NSAIDs are widely used for treating various inflammatory diseases. The clinical value of many NSAIDs are limited due to their gastrointestinal toxicity, which range in both severity and frequency from relatively mild to more serious and potentially life threatening condition, such as GI ulceration and haemorrhage. It can be attributed to the direct local action on the gastric mucosa particularly of acidic NSAIDs and generalized systemic action that takes place after absorption of these agents. The development of prodrugs to temporarily mask the acidic group of NSAIDs has been considered as a promising means of reducing the GI toxicity due to the local action mechanism. Most prodrugs of NSAIDs have been prepared by derivatization of the carboxyl group and later the parent drug elicits the desired pharmacological response. The history and development, of conjugation with different promoieties, in designing new efficacious NSAID prodrugs is discussed briefly in the following section.

Few glycerol prodrugs of indomethacin have been synthesized and evaluated for anti inflammatory activity by rat paw carrageenan oedema assay. Most active compounds were also tested in rat adjuvant arthritis model and found to be essentially equivalent to indomethacin. On a molar basis the gastric irritating properties of these were seven to eight times less than indomethacin 62.

Butyl flufenamate, prodrug of flufenamic acid 63 and punaprofen, the pinacol ester of ibuprofen 64, launched in to market in 1984 in
Japan, are used topically for the treatment of eczema, contact seborrheic and atopic dermatitis. Fosfosal is a highly water soluble salicylic acid ester prodrug found useful in the treatment of musculoskeletal and arthritic pain. Piketoprofen (Fig 2.1), an amide derivative of ketoprofen, marketed in Spain in 1984, is used in the form of aerosol for treating topical inflammatory and painful musculoskeletal disorders.

![Fig 2.1 Piketoprofen- amide prodrug of ketoprofen](image)

Nabumeton is a non acidic NSAID, marketed in 1985 in Ireland, is a bioprecursur type of prodrug that gets converted into 6–methoxy–2-naphthyl acetic acid which is an active metabolite. Nabumeton (Fig 2.2) is reported to be effective in the treatment of rheumatoid and osteoarthritis.

![Fig 2.2 Nabumeton - a bioprecursur type of prodrug](image)

Osalazine sodium (Fig 2.3), a mutual prodrug, comprising two molecules of 5–amino salicylic acid, marketed in 1986 in Sweden and Netherlands, was found useful in the treatment of ulcerative colitis.
The pivalic ester prodrug of piroxicam which showed reduced gastric irritant activity was reported \(^{67}\). The time course of plasma levels of both the prodrug and the active principle were studied and a correlation of later with synovial fluid levels were made. Good gastric tolerance was achieved along with good clinical results.

The guaiacol ester of ibuprofen (Fig 2.4) with comparable anti-inflammatory activity but greater GI tolerance was found to be effective in ameliorating symptoms due to common cold and influenza \(^{68}\).

Rapid hydrolysis of benzoic acid ester of various substituted 2–hydroxyl acetamides in human plasma was observed and it could be largely attributed to the presence of cholin esterase. Results showed that it is possible to achieve ester derivatives with desired water solubility by retaining its ability to undergo the enzymatic hydrolysis \(^{69}\).

(N, N, N-trialkyl ammonium) alkyl ester and thio ester derivative of various NSAIDs were synthesized and evaluated for its pharmacological activities. The study revealed that the prodrugs
retained anti inflammatory characteristics of corresponding parent
drug, exhibited reduced gastrointestinal erosive properties and reduced
analgesic potencies.

The hydrolysis kinetics of glycolamide esters of indomethacin was
studied to assess the possibility of designing water soluble and solution
stable prodrug of indomethacin suitable for parenteral or ocular
administration. Indomethacin and its ester prodrug showed
maximum stability at pH 4.9 and 4.7 respectively. The shelf life was
observed to be almost two years for indomethacin but only 43 days for
ester prodrug. Very pronounced water catalyzed hydrolysis of ester
prodrug accounts for its poor water stability and limits its use as ready
to use formulation.

The pharmacological properties of LFP83, a prodrug of
flurbiprofen which is its active metabolite was studied and LPF83 showed
remarkable analgesic, antipyretic and anti inflammatory
activities. Its analgesic potency was more than that of FP, along with
rapid onset of action. In addition, LFP83 showed less ulcerogenicity
than FP in both single and consecutive (7 days) administration.

A series of glycolamide glycolate, acyloxy methyl, alkyl and aryl
esters of acetyl salicylic acid were synthesized and evaluated for
hydolysis and pharmacological profiles. Lipophilicity and water
solubility of the esters were also determined. The studies showed N, N–
disubstituted glycolamide as rapidly hydrolysable in human plasma.

Nabumetone, a novel NSAID with less cyclooxygenase inhibitor
activity has been converted to its active metabolites, which possess
more potent cyclooxygenase inhibitor activity by liver. Syntheses of rat
gastric prostaglandin as well as in vitro and in vivo comparative studies
were carried out on the effect of nabumetone and 6-methoxy-2-naphthyl acetic acid with indomethacin and naproxen respectively. The results showed inhibition of platelet TXA\(_2\) synthesis due to the administration of nabumetone \(^{74}\).

Piroxicam cinnamate is a longer active prodrug, and is found useful in once daily therapy for rheumatoid and osteoarthritis \(^{75}\). Aminoprofen, an amide prodrug of ibuprofen, marketed in 1990 in Spain, is a topical anti-arthritic drug with analgesic properties \(^{76}\).

The flurbiprofen complex of copper was prepared and characterized. The anti inflammatory and analgesic activities were performed \textit{in vivo} in rats. The study showed similar inhibitory effect for both copper-flurbiprofen complex and parent flurbiprofen \(^{77}\). However, the gastric irritation was found to be less for the prodrug than free flurbiprofen.

A range of ketoprofen-dextran ester prodrugs were prepared and administered orally in pigs. The bioavailability of ketoprofen after administration of prodrugs were determined and compared with that obtained from the administration of an equivalent dose of ketoprofen alone \(^{78}\). The plasma profile for the ketoprofen-dextran ester prodrugs demonstrated a characteristic time lag of 2 to 3 h and the average absorption fractions for the prodrugs vary from 67-100 \%.

The water soluble and water insoluble polymer derivatives of NSAIDs such as alclofenac, ketoprofen and ibuprofen were evaluated for their hydrolysis rate in simulated gastric juice with \([\alpha, \beta\text{-poly (N-hydroxyethyl)-DL-aspartamide (PHEA)}],\) a hydrophilic macromolecular
prodrug as carrier. Hydrazide derivatives of naproxen, diclofenac, ibuprofen and indomethacin were synthesized and evaluated biologically in rodent model.

The macromolecular prodrug of fenoprofen and probenacid using \( \alpha \beta \gamma \) poly (N–hydroxy ethyl–DL–aspartamide, a hydrophilic polymer and studied the release of drug in alkaline medium. The hydrolysis kinetics of various alkyl, glycolamide, amino ethyl and 2–(l–imidazolyl) ethyl ester of ibuprofen and flurbiprofen in 80 % human plasma using direct HPLC assay for the enantiomers for these acids were determined. The R–isomers undergo faster plasma catalyzed hydrolysis than the corresponding S–isomer.

The synthesis and evaluation of amide and ester derivative of ibuprofen and naproxen for anti inflammatory activity and GI toxicity were carried out. Some prodrugs exhibited significantly better reactivity toward hydrolysis and remaining exhibited considerably less irritation to the gastric mucosa in rats.

The amide prodrugs of indomethacin and diclofenac were synthesized by condensing methyl and ethyl ester of various amino acids. It was observed that during reaction with peripheral blood leukocyte, blood plasma or liver extract, the parent drugs were not regenerated from the amide substrate at \( 10^{-4} \) M. However the ester protection of some amino acid was hydrolyzed efficiently by leukocyte or liver enzyme. The condensation product of diclofenac and indomethacin with phenylalanine were observed to be less inhibiting than the free drug on prostaglandin F2 release from cultures fibroblasts.
The release process of ketoprofen from ketoprofen-dextran ester prodrugs was verified in pigs. The prodrug was given to three pigs at intervals of 12 h and in seven doses corresponding to 4 mg ketoprofen/Kg body weight. It was concluded on the basis of results that following administration of dextran prodrug, the plasma concentration curve and the dissolution profile are uniform with small inter individual variations.

The morpholinan alkyl ester prodrugs of indomethacin and naproxen were synthesized, evaluated and tested in rats, for pharmacokinetics, bioavailability, ulcerogenicity and solid state stability, for oral drug delivery. Results showed that the prodrugs were 30 to 60 % more bioavailable orally than parent drugs. In addition, they were found to exhibit irritation at a lesser amount to gastric mucosa than original drug during single dose administration.

In vivo and in vitro studies of morpholin alkyl prodrugs of diclofenac were also attempted for oral drug delivery. Results showed a 200 fold increase in solubility of prodrugs in SGF and phosphate buffer at pH 7.4 when compared with that of the parent drug. All the esters were reported to exhibit a rapid bioconversion in rat plasma and were significantly less irritating to the gastric mucosa than the parent drug.

The in vitro skin permeabilities of ketorolac and its two ester analogues ketorolac ethyl ester (KEE) and [N, N–dimethyl amino carbonyl] methyl esters (KDAE) through cadaver skin were investigated. The KDAE was observed to be a better ester prodrug
than KEE as it exhibited relatively higher skin flux and faster enzymatic hydrolysis in human serum to liberate the parent drug.

Oligoethylene ester prodrugs of indomethacin were synthesized and evaluated for chemical and enzymatic stability, anti inflammatory, analgesic and ulcerogenic activities in rats and mice. All prodrugs demonstrated good chemical stability and were readily hydrolyzed by human plasma. Better and similar anti inflammatory and analgesic activities along with significantly less gastric irrigation were observed in prodrugs.

The effect of substitutes on the physicochemical properties, such as aqueous solubility, octanol–water partition coefficient and hydrolysis kinetics in aqueous buffer and human plasma, of a series of synthesized alkyl ester prodrug of ibuprofen were studied and concluded that it is possible to get ibuprofen prodrug that is able to achieve their objectives without compromising on therapeutic activities.

Phospholipid microemulsions were suggested as a drug delivery system for hydrophobic compounds. The cholesteryl ibuprofen and cholesteryl flufenamic acid derivatives were synthesized and prepared emulsion of prodrug and phospholipid using various concentrations of prodrug and lipids. The results indicated that a molar ratio of 75:25 and a total lipid concentration of 60 mg/ml consistently gave microemulsions with a mean size of 100–150 nm.

The protein binding studies of the enantiomers of the non–opiatic analgesic ketorolac using plasma and serum albumin at physiological
pH and temperatures were carried out. Tritium labeled ketorolac was synthesized in order to detect the very low levels of unbound enantiomers in protein solution. HPLC column afforded labeled enantiomers of high activity. The in vitro use of (R)– and (S) ketorolac enabled reproducible radiometric detection of enantiomers.

Ethyl ester flurbiprofen based amino acid prodrugs were synthesized and subsequent release of enantiomers of flurbiprofen, in presence of trypsin and carboxy peptidase, was carried out to assess their use as prodrugs for flurbiprofen. No significant differences between the kinetic parameters for two diastereomers were observed suggesting that the orientation differences between (S)– FP and (R) FP diastereomers does not have any effect on the tryptic hydrolysis of the ester bond.

The synthesis of indomethacin polyoxyethylene esters as indomethacin dermal prodrug was reported. The esters possess good water solubility, rapid chemical and enzymatic hydrolysis. It was observed that the rate of chemical and enzymatic hydrolysis was not significantly affected by the length of the polyoxy ethylene chain used as a promoiety.

The pharmacokinetics of diethyl carbonate ester prodrugs of ibuprofen and naproxen was evaluated in dog plasma, SGF and SIF at 37°C. Significant difference was found between the rate of absorption of prodrug and their respective parent compounds. It was concluded that these prodrugs did not offer any pharmacokinetic advantage over the parent compound and were unstable in the gastrointestinal tract.
The histidine conjugate of diclofenac from diclofenac acid chloride and histidine ester hydrochloride by modified Schotten Baumann reaction was carried out. Compounds were physicochemically characterized and studied for rate of hydrolysis in phosphate buffer (pH 7.4) and 80 % plasma (pH 7.4). Hydrolysis study indicated rapid hydrolysis following first order kinetics. The compound showed less anti inflammatory tendencies in comparison to diclofenac 96.

To reduce the gastrointestinal irritation caused by indomethacin (IM), butyl and octyl ester prodrugs of (IM) were synthesized. The kinetics of hydrolysis of prodrugs was examined to characterize the tissue or organ capable of hydrolyzing the ester bonds. Diminished plasma levels of IM was observed after administration of IM–OE and IM–BE when compared with indomethacin administered alone. Hydrolysis of prodrugs was adequately described by first order kinetics. Ulcerogenicity and hepatic injury were markedly seen after repeated oral administration of prodrugs in contrast with the severely irritating effects of indomethacin alone 97.

The in vivo and in vitro stereo selective hydrolysis characteristics of mutual prodrug FP–PPA, which is a conjugate of flurbiprofen, with the histamine H2–antagonist piperidinyl methyl phenoxypropyl-2-hydroxy ethyl thio acetamide (PPA) to reduce gastrointestinal lesions induced by flurbiprofen were studied 98. The prodrug was also compared with flurbiprofen methyl ester (rac-flurbiprofen–Me) and FP ethylene glycol ester (rac-flurbiprofen–EG) and it was found that the
rac–flurbiprofen derivatives hydrolyzed preferentially to the (+) S–isomer in plasma and to the (-) R–isomer in liver.

Nalidixic acid amide of amino acid esters as prodrugs were attempted to overcome the drawbacks of nalidixic acid. It was observed that prodrugs were more stable in buffer (pH 1.2 and pH 7.4) and in 80 % plasma. The plasma protein binding potency was studied in vitro and revealed a decrease in the percentage bound in case of glycine and alanine derivatives and increase in the percentage bound of phenylalanine leucine and isoleucine derivatives.

Similarly amide prodrugs of ibuprofen, naproxen, diclofenac and ketorolac prepared from the corresponding 2–aryl propionic acids and R–(-)–2-amino–l–butanol in the presence of N, N′-dicyclo hexyl carbodiimide (DCC). The prodrugs prepared in the study showed significant analgesic activity.

The pharmacokinetic profile of triethylene glycol indomethacin ester (TIE), an indomethacin was synthesized and in vitro enzymatic hydrolysis studies showed that TIE was quantitatively recovered in to indomethacin at a very fast rate. TIE oral administration to rats gave lower but relatively constant indomethacin levels for 24 h observation period. Both the prodrug and the drug were able to inhibit the inflammation of carrageenan induced paw oedema.

In order to minimize sodium diclofenac side effect and to increase its therapeutic efficiency, diclofenac polymer prodrug was conjugated. The hydrolysis of polymer drug conjugate was carried out in cellophane membrane dialysis bags containing aqueous buffer solutions (pH 8) at
37°C. Compounds were found useful as polymeric prodrug and it was found that polyvinyl chloroacetate is an appropriate carrier for release of drug in human condition 102.

The chemical stability, enzymatic hydrolysis, anti inflammatory and analgesic activity and GI toxicity of 1-ethyl azacycloalkane-2-one indomethacin esters were studied 103. The esters are found stable in pH 7.4 buffer and simulated gastric fluid but showed rapid hydrolysis rate in plasma due to plasma esterase. Esters were found less irritating to gastric mucosa and showed good analgesic activity in the mouse acetic acid induced writhing assay.

In another study, terpenoid esters of indomethacin for topical use were prepared. Chemical and enzymatic stability, solubility, lipophilicity, stability of aqueous formulation of prodrugs and in vitro profile to inhibit methyl nicotinate induced skin erythema was investigated. All prodrugs showed high lipophilicity, poor water solubility in hydrochloric acid medium and rapid enzymatic cleavage 104.

Hydrolysis study performed on acryloyl and methacryl oxy ethyl ester and amide prodrugs of ibuprofen and indomethacin using di functional spacer group between the drug and acryl moiety showed that the drug was released by hydrolysis of ester or amide bonds between the drug and spacer group 105.

Various acyloxy ethyl mefenamate were synthesized and hydrolysis kinetics was studied in pH 1.2, pH 7.4 and human plasma at 37°C 106. Among the synthesized compounds, β carboxy propionyl
ethyl mefenamate and pivaloyl oxyethyl mefenamate showed high stability in aqueous and in enzymatic and non-enzymatic hydrolysis.

The hydroxyl ethyl ester of diclofenac and mefenamic acid were conjugated and evaluated for stability in aqueous buffer solution of pH 7.4, 1 N HCl and in human plasma. The hydrolytic degradation of diclofenac ester in aqueous buffer solution was slow ($t_{1/2} > 22$ h) while rapid enzymatic hydrolysis occurred in the plasma ($t_{1/2} - 12$ h). Mefenamic acid ester showed a relatively higher stability in buffer solution ($t_{1/2} > 38$ h at pH 10) as well as in the plasma ($t_{1/2} - 7.28$ h) compared with the diclofenac ester. It was concluded that mefenamic hydroxyl ethyl ester would not be considered as prodrug.

Synthesis and evaluation of acyloxyalkyl ester and hydroxyl alkyl esters of ketoprofen and naproxen as topical prodrug was carried out with an aim to improve the dermal delivery of drugs. All prodrugs were found more lipophilic than parent drug. Hydrolysis study showed that prodrugs were stable in aqueous solutions of pH (7.4) but hydrolyzed in human plasma and human skin homogenates.

The synthesis and characterization of 2-formylphenyl and 2-acetyl phenyl esters of indomethacin, ketoprofen, ibuprofen and aspirin showed that all compounds, except 2-acetyl phenyl aspirin, act as prodrugs. Study also proved 2-formyl phenyl esters as more potent anti-inflammatory agent than the parent compounds.

Reports showed N-hydroxy methyl phthalimide esters of ibuprofen, naproxen and aspirin to be useful non-ulcerogenic prodrugs of acidic NSAIDs.
Various aminoacyloxy alkyl esters of naproxen and naproxenoxyalkyl diesters of glutamic acid and aspartic acid were synthesized and their use as potential prodrugs for transdermal delivery was evaluated. The prodrugs were shown to have higher aqueous solubilities and similar lipid solubilities in terms of octanol-buffer partition coefficients (log $P$) at pH 5, when compared with naproxen $^{111}$. The diacyl glyceryl ester prodrug of naproxen has been reported with potential for improving dermal delivery of the parent drug $^{112}$.

Two additional analogous cyclic amides, N-hydroxy methyl succinimides and N-hydroxy methyl isatins were synthesized as alternate promoieties to N-hydroxy methyl phthalimide and found that the parent drugs treated groups as more ulcerogenic in stomach than the prodrugs $^{113}$.

A series of acyloxy methyl derivative of the NH acidic drugs and carboxylic acid drugs were prepared to find out the effect of varying nature of drug on the rate of in vitro hydrolysis catalyzed by procaine liver esterase and human plasma $^{114}$. The derivatives followed first order kinetics in both enzyme systems. The NH acidic derivatives showed a rapid hydrolysis than the carboxylic acid derivatives in both the systems.

Twelve new non–proteinogenic amino acid conjugates of diclofenac were examined for various pharmacological properties. The conjugates were found non ulcerogenic as well as were found to retain generalized anti–phlogistic activity $^{115}$.
The anti inflammatory and antioxidant properties of derivatives of indomethacin, diclofenac, tolfenamic acid and ibuprofen with cystamine, a polar antioxidant, were studied. Their effect on protection against rat hepatic microsomal lipid peroxides and interaction with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were analyzed. Results revealed all prodrugs as potent antioxidant and found to reduce lipid peroxidation very significantly with IC\textsubscript{50} values ranging from 55 to 510 mm. They also interacted approximately 90% with DPPH at equimolar concentrations.

Melatonin, an antioxidant was reported to show protective effects in indomethacin induced gastric injury by virtue of its radical scavenging activity.

The amide derivatives of diclofenac, ibuprofen and indomethacin were synthesized with a well known antioxidant cysteamine. The prodrugs exhibited good anti inflammatory and antioxidant activities and a significant reduction in ulcerogenicity.

In vitro and in vivo properties of 5-aminosalicylglycine (5-ASA-Gly) as a colon specific prodrug of 5-ASA were investigated in rats. In the study, free 5-ASA was not detected upon incubation of the conjugate with the homogenates of stomach or small intestine.

Glycolamide esters of ibuprofen were synthesized and evaluated for various physicochemical, pharmacological and toxicological properties and found that the prodrugs are better in action than the parent drug.
The *in vitro* study of polymerizable drug derivative of diclofenac sodium showed that the drug release takes place predominantly at a higher pH and in a sustained manner with complete drug absorption from the polymeric prodrug. Also a significant decrease in ulcerogenicity was observed revealing its potential for site-specific and sustained delivery of diclofenac.\(^{121}\)

Alkyl ester prodrugs of ibuprofen have been reported with significant improvement in the oral delivery of ibuprofen. The prodrugs were evaluated for physicochemical properties and pharmacological activities and found to exhibit enhanced anti inflammatory activity in carrageenan induced rat paw oedema by topical route and reduced gastro ulcerogenicity.\(^{122}\)

*In vitro* and *in vivo* evaluation of polyoxyethylene of ketoprofen, naproxen and diclofenac as dermal prodrugs were attempted.\(^{123}\) An appreciable and sustained *in vivo* topical anti inflammatory activity was observed for the ester prodrugs in the erythema model in human volunteers.

In the study dealing with oligoethylene ester derivatives of ketoprofen, naproxen and diclofenac, the prodrugs showed good stability in phosphate buffer (pH 7.4) and simulated gastric fluid (pH 2), and were readily hydrolyzed by human plasma. Anti inflammatory activity of the esters was found to be similar to the parent drugs and good analgesic activity was exhibited with significantly reduced gastric irritation even at higher doses.\(^{124}\)
Glucosamine, an amino sugar is conjugated with flurbiprofen to mask COOH group temporarily. Glucosamine hydrochloride and sulphate are being used as anti arthritic agents as well as a nutritional supplement in conditions like joint ache, stiffness severely restricted movements and serious pains. These prodrugs have additional advantage of producing non-toxic, nutrient by product, glucosamine on cleavage, which shows the synergistic effect. Glucosamine is also used in wound healing and gastric disorders\textsuperscript{125}.

The glycolamide ester prodrugs of ibuprofen, diclofenac, naproxen and indomethacin were synthesized and evaluated for their GI toxicity in rats\textsuperscript{126}. The results showed better pharmacological response by the prodrugs.

Mutual prodrugs of naproxen-propyphenazone were synthesized for improving the therapeutic properties by preventing the various gastrointestinal toxicities. Esterification of naproxen with different alkyl esters and thio-esters led to prodrugs with retained anti inflammatory activity and exhibited reduced erosive properties and analgesic potency. But esterification with ethyl piperazine showed that analgesic activity was preserved whereas anti inflammatory activity was generally reduced. Propyphenazone is converted to its active metabolite, 3-hydroxy methyl propyphenazone, which actually gives the analgesic effect\textsuperscript{127}.

The NSAIDs such as ibupofen, ketoprofen and naproxen have been co-polymerized with 2-hydroxyethyl methacrylate having high methacrylate content\textsuperscript{128}. The polymeric prodrug of ibuprofen was found
to retain the anti inflammatory activity while the prodrugs of ketoprofen and naproxen showed greater potency to inhibit acute inflammatory activity than their corresponding parent drugs.

Naproxen, probenecid, diclofenac, ibuprofen and indomethacin were converted to hydrazide derivatives which were further condensed with keto esters to give pyrazolone derivatives. The hydrazide derivatives of probenecid and diclofenac were also reacted with biphenyl acetic acid, an active metabolite of fenbufen. The prodrugs exhibited comparable anti inflammatory activity and analgesia when evaluated in rodent models.\textsuperscript{129}

The nitric oxide (NO) donating groups exhibited some useful properties like local protective actions including mucosal vasodilatation and prevention of neutrophil adhesion in both gastric and intestinal microcirculation and preserved the mucosal cell integrity. It has been tested in various animal models and proved the improved pharmacological activities of the prodrugs.\textsuperscript{130, 131} NCX-530, an NO releasing derivative of indomethacin, has been reported to decrease gastric motility, increased mucosal blood flow and caused a marked inhibition of PGE2 formation in intact and ulcerated gastric mucosa.\textsuperscript{132} Studies carried out revealed that conjugation of NO-NSAIDs could be effective in a variety of diseases including cardiovascular, rheumatological, lung disease and cancer.\textsuperscript{133-135}

NO-naproxen, prodrug of naproxen, reduced the GI toxicity of naproxen and was proved by their efficient anti inflammatory and analgesic activities.\textsuperscript{136} In another study, the anti inflammatory and
antiplatelet properties of NO donor esters of aspirin were evaluated. Aspirin was conjugated with furoxan moieties through an ester linkage, and had the ability to release NO. The prodrugs showed an improved anti inflammatory activity devoid of acute gastro toxicity, mainly due to their ester nature, and an antiplatelet activity due to their ability to release NO 137.

Various authors have evaluated the efficacy, potency and spectrum of activity of prodrugs of NSAIDs such as naproxen, ibuprofen, flurbiprofen, ketoprofen and aspirin by coupling to NO-donating moieties 138-140. All the prodrugs exhibited better result than the parent drugs. Ibuprofen esterified with NO donor moiety abolished the GI irritation and significantly reduced thinning with no alteration in levels of diaphorase 141.

A novel group of hybrid NO-NSAIDs possessing 1-(pyrrolidin-1-yl) diazen-1-ium-1,2-diolate or 1-(N,N-dimethyl amino) diazen-1-ium-1,2-diolate moiety attached through methylene spacer to the carboxylic acid group of aspirin, ibuprofen and indomethacin were reported. The prodrugs showed better in vivo anti inflammatory activities than the parent drugs 142. A series of NO-donating N-substituted glycolamides of naproxen were shown to posses anti inflammatory activity in rat carrageenan paw oedema model 143.

For reducing the gastrointestinal toxicity associated with ibuprofen, ester prodrugs with 1,2,3-trihydroxy propane-1,3-dipalmitate/stearate were prepared and evaluated 144. Ester prodrugs of ibuprofen synthesized using methyl, ethyl and propyl gluco pyranosides
as promoieties have been reported to undergo rapid cleavage inside the biological system and elicit a pharmacological profile quite similar to that of ibuprofen on oral administration. But, unlike the parent drug, they displayed reduced gastric ulceration.

Ibuprofen, naproxen and ketoprofen were linked to chondroitin sulfate (ChS) via a PEG 1000 as spacer. The ketoprofen-ChS conjugate was found to be susceptible to degradation in presence of esterases and chondroitinase with the liberation of ketoprofen and ChS.

Indomethacin was coupled with amino alcohols having structural resemblance with amino ethanol ester class of anti-cholinergics. The N,N-disubstituted amino alcohol esters were specifically designed to possess a terminal tertiary nitrogen atom with an ethylene bridge between the terminal nitrogen and carbonyl group of bulky ester, which are also the structural features of anti-cholinergics. Gastric toxicity was reduced considerably not only because of blockage of acidic group but also due to the cessation of gastric acid secretion caused by local inhibition. Hydrolysis of such entities leads to the formation anti-cholinergic molecules in intact form.

Ester derivatives of aspirin, ibuprofen and indomethacin with 2-acetoxy methyl-1-[N-(2-hydroxy ethyl-N-methyl amino] diazenium diolate were synthesized as NO-releasing prodrugs. The derivatives did not exhibit in vitro COX inhibitory activity against cyclooxygenase isozymes but significantly decreased carrageenan induced rat paw oedema showing an enhanced in vivo anti inflammatory activity relative
to the parent drugs. All derivatives showed less ulcerogenicity than the
parent drugs \(^{148}\).

Ester prodrugs of ibuprofen, indomethacin, ketoprofen, naproxen, diclofenac and aspirin with (4-thiocarbamoyl phenol, 5-[4-hydroxy phenyl]-1, 2-dithiole-3-thione) have been synthesized and analyzed. It was reported that these moieties release hydrogen sulphide which exerts anti inflammatory and analgesic activity. Results of the study showed significantly less gastric irritancy of the prodrugs than the NSAIDs alone \(^{149, 150}\).

Ten prodrugs of ketorolac were synthesized by amidation with ethyl esters of amino acids glycine, L-phenyl alanine, L-tryptophan, L-valine, L-isoleucine, L-alanine, L-leucine, L-glutamic acid, L-aspartic acid and alanine. Marked reduction in ulcer index and comparable analgesic, anti inflammatory activities were obtained in all cases as compared to ketorolac \(^{151}\).

The ketorolac dextran (KD) conjugates were synthesized and characterized to improve the aqueous solubility and to reduce the gastrointestinal side effects of ketorolac \(^{152}\). The ester bond in ketorolac dextran conjugates were confirmed by various spectral studies. In vitro hydrolysis studies were performed in various aqueous buffers (pH 1.2, 7.4 and 9) and in human plasma (pH 7.4). At pH 9, a higher rate of ketorolac release from KD was observed as compared to aqueous buffer of pH 7.4 and human plasma. The conjugates followef first order kinetics pattern. The various pharmacological screening carried out in mice and rats showed better analgesic activity, markable anti
inflammatory activity and reduced ulcerogenicity of the conjugates than ketorolac.

Simple ester prodrugs of NSAIDs like ibuprofen, flurbiprofen, ketoprofen with ethanol and isopropyl alcohol have been synthesized and evaluated. This kind of system can easily undergo enzymatic hydrolysis by the action of esterase present abundantly in the small intestine; hence stomach’s mucosa is not exposed to the free carboxylic group. Similarly, simple amide prodrugs of ibuprofen, flurbiprofen, ketoprofen have also been reported, wherein simple amines were used to form amide bond with the carboxylic acid. These are more stable in stomach as amidases that bring upon the hydrolysis of amide bond are present only in intestine $^{153, 154}$.

Polymeric drugs are polymer-conjugated drugs, polymeric micelles and liposomal drugs or solid phase depot formulations of various agents. Polymeric drugs can target selectively, solid tumors by exploiting abnormalities of tumour vasculature, extensive production of vascular permeability factors stimulating extravasation within tumor tissues and lack of lymphatic drainage. Dextrans are polysaccharide polymeric carriers devoid of selective transport properties and may serve as one of the most promising carrier candidates for a wide variety of therapeutic agents like hormones, iron and methotrexate. Bone targeting by conjugation of drugs with bis phosphates has shown promise in enhancing their effects in bones and reducing adverse drug reactions. Tetracycline–conjugated estradiol and oligopeptide–
conjugated estradiol are reported as novel bone–specific drug carriers with high affinity for hydroxyl apatite crystals \textsuperscript{155}.

In \textit{vivo} studies on the polymeric micro and nanoparticles revealed that their particle characteristics are very useful in controlling drug behavior. Recently, research based on the combination of the concepts of polymeric prodrugs and micro or nanoparticles have been reported. Polymeric prodrugs enable drugs to be released at a certain controlled rate based on the features of the macromolecule drug linkage. Various reports proved that the micro and nanoparticles can control \textit{in vivo} behavior based on their size, surface charge and surface structure \textsuperscript{156}.

To control the rate of release of methyl prednisolone (MP) in lysosomes, a novel dextran-MP prodrug having peptide linkages were synthesized and characterized \textsuperscript{157}. Methyl prednisolone succinate was attached to dextran 25 kDa using linkers with 1–5 Gly-residues. The drug release of prodrugs in various buffers, blood, liver lysosomes, and various lysosomal proteinases were carried out. The study revealed that the novel dextran-MP prodrug can be successfully applied for the controlled delivery of MP in lysosomes.

In another study on the treatment of ulcerative colitis, dextran–budesonide conjugates were attempted as colon specific prodrugs \textsuperscript{158}. The previous reports showed budesonide as a potent glucocorticoid for the treatment of inflammatory bowel diseases. Dextran–budesonide conjugates were prepared with different molecular weights of dextran in the presence of dimethyl amino pyridine (DMAP) using succinate spacer. The prodrugs were subjected to various spectral studies and
physicochemical characterization. Drug release characteristics of the conjugates were also studied in the presence of the luminal contents of different segments of the rat GI tract. The study revealed the promising use of dextran–budesonide conjugates for treating various anti-inflammatory bowel diseases.

Diclofenac was conjugated with natural antioxidants like vanillin, sesamol, umbelliferone using glycolic acid spacer (-OCH$_2$COO-) and found that diclofenac-antioxidant mutual prodrugs are better and safer than diclofenac 159.

Dextran was oxidized by sodium periodate, and then free amino group of lamotrigine was coupled with oxidized dextran in alkali medium to form Schiff’s base. The structure of conjugate was confirmed by UV, IR and NMR spectroscopy. In vitro hydrolysis study showed a negligible release of lamotrigine from the conjugate at acidic pH 1.2, but faster release was observed in alkaline pH 9.0 than pH 7.4. Synthesized conjugates were screened for preliminary anticonvulsant activity in rats and showed a comparable activity than lamotrigine. The histological studies of liver parenchymal cells in control and lamotrigine-dextran conjugate groups were similar and showed no abnormalities whereas, the parent lamotrigine treated group showed focal scattered coagulative necrosis. The results of study concluded that the prodrug would be a safer and suitable drug for the treatment of partial and generalized epilepsy without hepatotoxic side effect 160.

The colon targeted polymeric prodrugs of celecoxib were synthesized with dextran having various molecular weight using
succinic acid as linker between the drug and dextran \textsuperscript{161}. The maximum degree of substitution was observed for dextran conjugate. The results of the \textit{in vitro} hydrolysis studies carried out in simulated colonic fluid (SCF) at pH 6.8 and in simulated intestinal fluid (SIF) at pH 7.4 suggested the successful use of dextran as a polymeric carrier for colon targeting of celecoxib.

\textbf{2.1 RESEARCH ENVISAGED}

In the recent years a large number of NSAIDs have been introduced into clinical practice and with more drugs of this class at various stages of development, this number will increase. But gastrointestinal side effects constitute the most frequent of all the adverse reactions of NSAIDs and often these reactions lead to GIT ulceration and haemorrhage.

Literature survey reveals that many efforts had been made to synthesize amino acid ester, glycolamide ester, and amide prodrugs using various amines. But few attempts were made to develop amide prodrugs using amino acids. With this background, in the present research three well recognized NSAIDs viz. Aceclofenac (AC), Mefenamic acid (MA) and Dexibuprofen (Dex) were selected which suffer with the gastrointestinal side effects. The aim was to synthesize amide prodrugs of the well known NSAIDs using various amino acids. With this approach, it is expected to get non-toxic prodrugs with minimal GIT disturbance but maintaining the useful physicochemical and pharmacological properties.
The salient features of the usefulness of conjugation of amino acids with NSAIDs are:

- Aminoacids are normal dietary constituent and they are non-toxic in moderate doses as compared to other promoieties.
- Amino acids have healing effect on gastric lesions produced by NSAIDs.
- A drug with free carboxyl group can be derivatized into corresponding esters or amide of amino acids, so as to alter the physical properties of a parent drug with one of more of the hydrolase enzymes serving as the in vivo reconversion site (s).
- Being a nutritional substance, the use of amino acids as a derivatizing group might also permit more specific targeting site for enzymes involved in the terminal phase of digestion.
- Many amino acids possess marked anti inflammatory activity against carrageenan induced hind paw oedema in rats.
- By using different types of amino acids viz. non - polar, polar, acidic and basic, the drug molecule can be made more or less polar, or more or less soluble in a given solvent.

Amides are derivatives of amine and carboxyl functionalities of a molecule. In prodrug design, amides have been used only to a limited extent owing to their relatively high enzymatic stability in vivo. An amide bond is usually hydrolyzed by ubiquitous carboxyl esterases, peptidases or proteases. Amides are often designed for enhanced oral absorption by synthesizing substrates of specific intestinal uptake transporters. The use of amide prodrugs to provisionally hide the acidic
group of NSAIDs was reported to provide better lipophilicity, reduced
gastric irritancy than the parent drug, improved therapeutic index
through prevention of GI irritation and bleeding, improved anti
inflammatory activity and reduced GI erosive properties.

2.2 OBJECTIVES

The major objectives of the present research are as follows.

1. To synthesize amide prodrugs of NSAIDs with amino acids.
   (a) Aceclofenac with L-histidine, L-alanine, L-thyrosine and
glycine
   (b) Mefenamic acid with L-histidine, L-tryptophan, L-thyrosine
and glycine
   (c) Dexibuprofen with L-tryptophan, L-phenylalanine, glycine and
L-tyrosine

2. To study the physicochemical properties of the synthesized
prodrugs.

3. To characterize the synthesized prodrugs by elemental and
spectral analysis (IR, $^1H$ NMR, $^{13}C$ NMR and Mass spectroscopy).

4. To determine the partition coefficient of the synthesized prodrugs
in octanol/acidic buffer (pH 1.2) and in octanol/phosphate buffer
(pH 7.4).

5. To perform protein binding studies of the synthesized prodrugs in
phosphate buffered saline (PBS) (pH 7.4).

6. To carry out hydrolysis study of synthesized prodrugs in
simulated gastric fluid (pH 1.2), simulated intestinal fluid (pH 7.4),
rat fecal matter (pH 7.4) and 80 % human plasma (pH 7.4).
7. To screen the synthesized prodrugs for anti inflammatory activity, analgesic activity, ulcerogenic activity and histopathology.