early all therapeutic agents possess various physico chemical and biological properties, some desirable and others undesirable. In general, the pharmaceutical world is concerned with minimizing the number and magnitude of undesirable properties of a drug while retaining the desirable therapeutic activity.

Improvement of drug efficacy can be accomplished by biological, physical or chemical means. The biological approach entails varying the route of administration. Examples include the injectable route to optimize onset of action, maximize bioavailability and eliminate gastric irritation and acid catalysed drug degradation. Versatility is severely limited when utilizing the biological approach, because alternative routes of administration are frequently unavailable and are always less convenient than oral administration.

A great degree of flexibility of drug modification is offered by the physical approach, commonly referred to as dosage form design. The highest degree of flexibility in altering drug efficacy, however, is offered by the chemical approach.

Drug derivatization has been long recognized as an important means of producing better therapeutic agents. Bayer, as far back as in 1899, synthesised the drug aspirin in an attempt to improve the therapeutic activity of salicylic acid. Since that time, literally thousands of drug derivatives have been synthesized and tested. These drug derivatives can be broadly classified into two categories: irreversible or reversible. Irreversible derivatives or analogs are usually synthesised for the purpose of finding a similar, new, biologically active entity possessing increased potency, a broader spectrum of activity, or some other desirable property not possessed by the parent compound. A reversible drug derivative utilizes a chemical moiety of proven biological activity (the parent molecule) and seeks to deliver it to the site of action while overcoming some inherent drawback to the use of the parent compound.

In the case of the analog, precaution is taken as to what functional groups can be modified since indiscriminate modification may destroy all bioactivity. The reversible derivative can be modified at any functionality without undue concern for its involvement at the receptor level since it is reversible by definition. These facts eliminate the need to determine the bioactive centre(s) in the molecule and offer the chemist a greater number of chemical sites at which to modify.
In general, three approaches are followed in the search for new drug agents: (a) the general screening approach in which chemical substances from any source are tested for their effect against predetermined disease or disease state, (b) the chemical modification of existing drug substances whose biological effects are known, and (c) mimicking the nature by biochemical design, where a compound is made to exert an action in a manner similar to known biochemical substance. Any lead compounds obtained from these three approaches are usually further modified chemically to gain the biologically most potent representatives of series.

The present research work focuses on approach (b) the chemical reversible modification of some Non-steroidal anti-inflammatory drugs which, upon introduction to the appropriate biological system, revert back to the parent molecule by virtue of enzymatic and/or chemical liability.

Non steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications in the world. In 1988, it was estimated that 100 million prescriptions were written for NSAIDs annually in the United States alone\(^1\). The major limiting side effects of chronic NSAIDs use are gastrointestinal (GI) symptoms and complications. Prevalence studies have demonstrated that gastric or duodenal ulcers are present in 15-20% of patients taking NSAIDs chronically\(^3\). The incidences of significant gastrointestinal complications (bleeding, perforation, or gastric outlet obstruction) has been estimated to be 1-4% per year from population studies\(^5\). Even though the incidences of significant complications seem small, the large number of patients at risk make NSAID-induced GI complications a significant health hazard.

In addition to the increased morbidity and mortality, these GI complications contribute considerably to the cost of care. Database studies have suggested that the direct cost of treating GI symptoms and complications comprise 31-40% of the total cost of care for arthritis patients\(^6\) and that adverse event occurs in 25% of patients. Numerous perspective studies have identified a prevalence of 15-20% for gastric ulcers and 5-8% for duodenal ulcers after 12 weeks of therapy\(^9\).

It was in this regard that the present studies were undertaken to modify the existing NSAIDs with GI toxicities by the prodrug approach so as to alleviate these undesirable effects. These GI toxicities are associated in part with the presence of an acidic functionality in these agents. The carboxylic acid functionality commonly found in these agents is unionized in the highly acidic environment of the stomach. As a result, these agents are more lipophilic in nature and may pass into the cells of the stomach's
mucosa. The intracellular pH of these cells is more basic than that of the stomach lumen, and the NSAID becomes ionized. This results in the back flow of $\text{H}^+$ from the lumen into these cells, with concomitant cellular damage. This type of damage is preventable if the carboxylic acid function could be eliminated from these agents. However, this functional group is required for activity.

In this study of prodrug approach, the free acidic group was masked temporarily by a promoiety so as not to expose stomach’s mucosa to this free carboxylic group. The prodrug would then be absorbed intact into the systemic circulation and hydrolysed by various enzyme systems therein to the active drug thus minimising the direct insult. In these studies the prodrugs of ibuprofen, diclofenac, naproxen, mefenamic acid, indomethacin and probenecid were synthesised. These NSAIDs were modified by methods of glycosidation, amidation and synthesis of glycolamide ester derivatives. Their structures were elucidated by $^1\text{HNMR}$ spectrometry. Their ulcerogenic potential was determined and compared to the parent drugs. The anti-inflammatory and analgesic activities of these prodrugs were also determined to find out their efficiency in comparison to the parent compounds.
References

Prodrug Approach

Introduction

- The Concept
- The General Considerations
- The Chemical Basis and
- Applications
In 1958, Albert coined the term prodrug and used it to refer to a pharmacologically inactive compound that is transformed by the mammalian system into an active substance by either chemical or metabolic means. This included both compounds that are designed to undergo a transformation in order to yield an active substance and those that were discovered by serendipity to do so. These two situations were distinguished by Harper, who in 1959 introduced the term drug latentiation to refer to drugs specifically designed to require bioactivation.

These ideas have led to the development of a number of currently used drugs that have advantages over their non-prodrug counterparts. The type of prodrug that is to be produced depends upon the specific aspect of the drug’s action that requires improvement and the type of functionality that is present in the parent drug. Generally, prodrug approaches are undertaken to improve patient acceptability of the agent (i.e. reduce pain associated with administration), or alter metabolism, or alter elimination. The chemical nature of the prodrugs that can be prepared is somewhat limited, however, by the chemical nature of the active species.

Recently, the terms “hard drugs” and “soft drugs” have been introduced. Hard drugs are compounds designed to contain those structural characteristics necessary for pharmacological activity but in a form not susceptible to metabolic or chemical transformation. In this way, the production of any toxic metabolite is avoided, and there is an increased efficiency of action. Since the drug is not inactivated by metabolism, it may be less readily eliminated. On the other hand, soft drugs are the active compounds that, after exerting their pharmacological effect, are designed to undergo metabolic inactivation to give a non-toxic product.

THE BASIC CONCEPTS

The prodrug approach lies somewhere between the formulation and analog approaches with respect to both time and cost of development. The term prodrug as stated earlier is employed for that class of drug derivative that in vivo is hydrolysed or converted by other mechanisms to the parent compound. The important distinction between analogs and prodrugs is that the former is biologically active per se whereas the latter requires in vivo conversion to the parent drug for eliciting biological activity. The primary utility of analogs is to improve potency and to achieve specificity of action, whereas the prodrug is used to improve pharmaceutical or biological properties. The latter properties are collectively termed biopharmaceutical properties. Because it does not alter the primary structure of the parent drug, prodrug synthesis is usually much less difficult than analog synthesis and the probability of a prodrug being active is much greater than for an analog. In the case of the most common prodrugs, i.e., simple esters and amides, it might be
possible to speed up the development and registration process by using an abbreviated pre-clinical testing program.

One of the important applications of prodrugs is in improving oral absorption. The unique feature of the prodrug is that the physico-chemical properties of the resulting derivative can be carefully tailored by means of structural modifications of the promoiety. The intrinsic activity of the parent drug is assured through in vivo cleavage of the prodrug.

The prodrug approach has been successfully applied to a vide variety of drugs. It is most effective when an undesirable characteristic of the parent drug needs to be eliminated, especially if that characteristic can be related to a physical-chemical property such as melting point, boiling point, solubility, or partition coefficient. In these instances, the characteristic to be altered is related as quantitatively as to a physical chemical property. (For example, taste can be related to solubility for a series of drugs.) The physical-chemical property is then related to chemical structure (e.g., solubility and alkyl chain length). Finally, prodrugs are synthesised that have the desired physical chemical property and are, it is hoped, free of the undesirable biological property.

The design of prodrugs is subjected to a number of economic and biological constraints. Some of these are given below. The choice of the derivative or pro-moiety depends upon a number of factors such as:

1. The economics of synthesizing the prodrug should be such that the cost is not significantly higher than that of the parent drug. Single step prodrug synthesis using inexpensive pro-moieties is preferred.

2. The promoiety selected should yield a fragment, upon cleavage of the prodrug, that is free of toxicity or side effects and preferably free of biological activity. The choice of promoiety is also dependent on the nature of the parent drug. For example, the pro-moiety chosen for a parent drug with a dose of 1 mg may not be acceptable with another parent drug given at a higher dosage (such as 500mg) because of either the unacceptably high weight of an equivalent dose of the prodrug or the toxicity or other side effects of the promoiety at this high dose level.

3. The promoiety should be rapidly cleaved once the prodrug is absorbed in order to elicit a pharmacokinetic profile similar to that of the parent drug. A prodrug with both improved absorption and prolong action through chemical sustained release requires that the promoiety should be carefully tailored with respect to lability.

4. The intact drug should not produce any unusual drug distribution patterns that could lead to unfavourable tissue distribution.

In order for an orally administered drug to become available to its systemic receptor, it must first be dissolved in the gastrointestinal fluid and then transported across the gastrointestinal membrane, after which it can be picked up and distributed by the systemic circulation. For many drugs, poor dissolution and/or poor absorption limit availability to the receptor.
**Physico-chemical Considerations**

A. **Dissolution:**

The rate of dissolution of $R$ of a drug in an aqueous medium is known to be directly proportional to the solubility $S$ of the drug in the medium:

$$R = KS$$ \hspace{1cm} \text{Eq. 1}

Where $K$ is constant that reflects the surface area of the drug particles and the degree of agitation of the medium in the region of the drug particles.

B. **Transport:**

On the basis of straightforward diffusional model by Higuchi, Ho Stehle, Flyn and Yalkowsky\textsuperscript{5,6}, the rate of transport of a drug across a biological membrane adjacent to one or more aqueous compartment is:

$$F = \frac{\Delta C}{(Rm/PC) + Raq}$$ \hspace{1cm} \text{Eq. 2}

Where $\Delta C$ is the concentration across the membrane and aqueous region(s); $PC$ is the membrane-water partition co-efficient; and $Rm$ and $Raq$ are the resistances of the membrane and aqueous regions, respectively, to diffusion of the drug. These resistances depend primarily on the thickness and viscosity of the phases and only slightly on the structure of the drug.

If we assume that the concentration of the drug on the receptor side of the membrane is negligible, the term $\Delta C$ becomes simply the concentration of drug in donor compartment, i.e., the dose divided by the compartment volume.

Equation 2 predicts for any applied phase concentration, the transport rate will increase exponentially with increasing partition coefficient until $PC$ is sufficiently large such that $Rm >> Raq/PC$ and the curve levels off to a plateau. Increasing the applied phase concentration (i.e., the dose) results in a parallel line, as shown in Fig. 1. Whereas it is not possible, from the data available in the literature, to obtain a very accurate optimal value of $\log PC$ for intestinal absorption, it appears that a value of 2.0 (on the octanol-water scale) is appropriate. In other words, drugs that have $\log PC > 2.0$ are usually well absorbed from the gut. Provided of course, that they are completely soluble in the GI fluid at the dose given.

Many small polar compounds are known to be well absorbed. Since polar compounds would not be expected to pass rapidly through lipoidal membranes, it is postulated that they pass rapidly through pores in the membrane. The incorporation of the concept of aqueous pores would change Eq. 2 to:
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\[
F = \frac{\Delta C}{(Rm / PC) + Raq} + \frac{\Delta C}{Rp}
\]

Eq. 3

Where \( \text{Rp} \) is the total resistance of the aqueous pores. Because pore transport is dependent only on molecular size, it is independent of partition-coefficient. In general, the pore pathway is only important for low-molecular weight compounds.

C. Activity Limiting Phenomena and Solubility

There are situations in which activity decreases with increasing partition coefficient. Since this cannot be explained by simple transport theory, we must consider what other factors might be responsible for the decrease, and what roles these factors play in determining the shape of the overall structure-activity curves for a series of drugs.

These factors can be broadly classified as either biological or physical-chemical. The former would include such factors as receptor site fit, binding to inert receptors and uptake by lipoidal tissues. Since these factors do not generally affect the transport of a drug across its primary absorptive barrier, they will not be discussed here.

Physical chemical factors are those which alter the availability of the drug at the transport-limiting membrane and are thus an integral part of the structure transport picture. Since it is usually the free, unionized, monomeric drug that is most efficiently transported across biological barriers; any factor that limits the concentration of the unionized monomeric species will limit transport and decrease activity. The most important flux limiting, physical-chemical factor is solubility. Other factors, such as micellization or complexation also limit the concentration of free monomeric drug in solution although not to that much extent as for solubility.

The combined effect of aqueous solubility and membrane-water partition coefficient on absorption have been quantitatively described by Flynn and Yalkowsky\textsuperscript{5, 6}. By reasoning that the maximum value of the trans-membrane concentration differential is
limited to the solubility $S$ of the drug, they have shown that the maximum flux occurs from saturated donor phase solutions, i.e. suspensions and is equal to:

$$F_{\text{max}} = \frac{S}{\text{Ra} + \text{Rm} / \text{PC}}$$

Eq. 4

The effect of solubility limitation on transport are shown schematically by the descending line of Fig. 1. In their discussion of transport from equimolar and saturated solutions, Flynn and Yalkowsky\textsuperscript{5,6} have shown that flux can be dependent on concentration (or dose) and partition co-efficient, concentration alone, solubility alone or the product of solubility and partition coefficient.

For most homologous series, the partition coefficient increases threefold, while the solubility decreases by a factor of four for each methylene group, so that:

$$\log \text{PC} = \log \text{PC}_n + 0.6n$$

Eq. 5

and

$$\log S = \log S_n - 0.6n$$

Eq. 6

Because the observed biological activity of the members of a series of compounds can be dependent upon $PC$ and/or $S$, it would be a gross oversimplification to expect a single parameter to be sufficient to predict the complete structure-activity curve. It is, therefore, inappropriate to believe that there is a single optimum partition coefficient for maximum activity in a series of compounds.

D. Key Physical Parameters

Both solubility and partition coefficient are important factors in drug uptake after oral administration. Drugs that are too polar often exhibit poor transport properties, whereas those that are too non-polar frequently have low availabilities because of their poor solubility and dissolution characteristics. While designing of the efficient prodrugs, one must take cognizance of both these phenomena, otherwise gains in transport will be offset by losses in availability.

As just stated, a drug must first dissolve in the gastrointestinal fluid and then be transported across some portion of the gastrointestinal membrane. The first process, dissolution, is dependent upon the solubility in the gastric or intestinal fluid while the second process, transport, is dependent upon the drug’s gastrointestinal membrane-lumen partition coefficient as well as its solubility in the appropriate GI fluid.
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In spite of having reasonable *in vitro* systems for modeling solubility and partition coefficient, we are not able to assign, definitively, optimal values for these two parameters. Part of the reason for this is that bioavailability can be dependent on S and not PC, PC and not S, S and PC, or neither, depending on the experimental conditions of the study. On the basis of available data, we can say with a reasonable degree of certainty that drugs having octanol-water partition coefficients of 100 or more (Log PC ≥ 2) are well absorbed, provided that they are given in doses that do not exceed their solubility in the gastric medium. The problem with selecting an optimum solubility for good bioavailability is implicit in the preceding sentence, i.e., it depends on the dose. A solubility of 1mg/ml for a steroid derivative presents no problem, but a much greater solubility would be needed to assure the availability of tetracycline derivative.

In the absence of more definitive information, we can generally strive for prodrug solubility equal to or greater than that of the parent drug. This will ensure that efficiency will not be reduced as a result of the poor solubility equal to the parent drug dose divided by 100. Finally, if we do not have any dose for the parent drug, we can attempt to obtain an adequate solubility of 10μg/ml.

If we do not have any means of knowing the expected dose of the prodrug, we can apply the following factors:

1. Transport rate increases with partition coefficient until log PC ≥ 2 and then levels off.
2. Dissolution rates, as well as transport from saturated solutions, decrease as solubility decreases.
3. Solubility is frequently inversely proportional to partition coefficient.

From these factors we can reason that there is likely to be little benefit to transport from increasing log PC above 2, and that because this will likely reduce solubility, there is a possibility for loss of efficiency. This is believed to be the reason that for many series of compounds, maximum activity is observed when log PC ≥ 2.

Lien has shown that maximum absorption rate of several series of drugs in the GI tract of the rat occurs for derivatives or analogs with log PC near 2. This is supported by antibiotic behaviour where the main reason for poor oral absorption is attributed to low lipophilicity. Pore transport of the antibiotics should be negligible and absorption via the lipoidal regions of the intestinal tract should be the dominant pathway. Table I gives a listing of antibiotics and anti-bacterial agents grouped according to the isoamyl alcohol/water log PC as obtained from the solubility ratio. The compounds with log PC less than zero in general show poor oral absorption, whereas those with log PC above zero show good oral absorption in the human. Isoamyl alcohol/water log PC values approximate the n-octanol/water Log PC values, and the proposed cutoff point for intestinal absorption is thus at log P ≥ 0. The proposed lipophilicity cutoff point for
Tyrothricin, for example, is in the group with log $PC > 0$, and thus absorption is indicated; but in fact, absorption is poor in the human. Another apparent exception is oxytetracycline, which is placed in the list with log $PC < 0$, where absorption is not indicated. Oxytetracycline is absorbed upon oral administration in the human, although incompletely. Cycloserine is absorbed orally in spite of low isoamyl alcohol/water partition coefficient. However, this can be rationalized on the basis of aqueous pore transport due to low molecular weight.

Based on this analysis, adoption of log $PC = 2$ as the preferred lipophilicity for improving the absorption of drugs is reasonable. Again, this applies to large, bulky molecules, where the aqueous pore permeability coefficient is negligible. $N$-Hydroxy urea, with estimated n-octanol/water log $PC = -2.54$, would not be expected to be absorbed, on the basis of its hydrophilic nature. The compound, however, is well absorbed upon oral administration and this is attributed to facile aqueous pore transport resulting from its low molecular weight. 5-Fluorouracil, with an n-octanol/water log $PC$ of $-0.95$ is absorbed upon oral administration in humans in spite of its unfavorably low lipophilicity. Absorption presumably occurs via the aqueous pore pathway, because of the relatively small size of the drug.

**BIOLOGICAL CONSIDERATIONS**

**Enzymes**

The basis of rational design of biologically reversible drug derivatives is predicted on the ability of the host tissue or organism to regenerate the drug derivative to the bioactive parent molecular species. The manner in which it is frequently accomplished is through the mediation of an enzyme or enzyme system within the host. In this respect, Bender and Jencks discussed the mechanisms of enzyme catalysis. The enzyme(s) may be widely distributed throughout the host tissue (e.g., estrases) or localised in site specific tissue (e.g., amidase). Enzymes per se have been characterized chemically, but their efficiency and specificity have generally not been quantitatively determined in vivo.

Quantitative predictions of the effect of drug derivative enzymatic catalysis in vivo are difficult due to the variety and complexity of the enzyme systems involved. The
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liver, for example, is considered the most important organ for drug degradation, contains a complex, nonspecific variety of enzyme systems. The reactivity of these systems varies widely in individuals and largely depends on genetic and hormonal factors, sex age, etc., making absolute predictions impossible. Furthermore, in vitro enzyme hydrolysis studies can be misleading if not conducted with several animal species. For example, species specificity was demonstrated when hydrolysis rates of the reversible derivative 4-acetamidophenyl-1,2, 2, 2-trichloroethyl carbonate were compared using human and rat plasma. Hydrolysis proceeds about four times faster in rat than in human plasma, probably due to greater abundance of specific and/or nonspecific esterases present in rat plasma. This same phenomenon has been demonstrated for a series of lincomycin 2,7-dialkyl-carbonate esters.

Enzymes that are considered important to orally administered reversible derivatives are found in the gut, gut wall, blood and liver. The gut of several animal species, including humans, contains a variety of GI microflora whose enzymes systems are capable of hydrolysing a number of reversible drug linkages. Thus, the naturally occurring glycosides amygdalin and cyanasin are hydrolysed by gut microfloral enzymes to their aglycones amygdalin and cyanasin, respectively. β-Glucuronidases of similar microflora are capable of hydrolysing stilbestrol and indomethacin glucuronides excreted in the bile. The parent drug is then reabsorbed from the intestine. This hydrolytic activity thus serves as an important event in the enterohepatic circulation of these drug entities. Azo linkages are hydrolytically reduced by azoreductase enzymes present in gut microflora. The reversible drug derivative sulphachrysoidine is converted to parent bioactive species sulfanilamide by this mechanism.

Experiments in humans and germ free rats have shown that sulphasalazine (salicylazosulphapyridine), a drug used in the treatment of ulcerative colitis, is enzymatically reduced to 5-aminosalicylate and sulphapyridine in the GI tract. The former has been implicated for its direct effect on the colon. Other chemical linkages known to be hydrolysed by GI micro-organisms are carboxylic esters, sulphate and nitrate esters, amides and sulphamates.

Esterases

The distribution of esterases is ubiquitous and includes a wide assortment of vertebrate tissues and blood serum. Nonspecific esterases, as the name implies, hydrolyse a variety of ester types including aliphatic, aromatic and thiol esters and amino acid esters, as well as a number of aromatic amides such as acetanilide, phenacetin and lidocaine. Specific esterases include acetylcholinesterase, cholesterol esterase and possibly vitamin A esterase. Many bioreversible drug derivatives have been designed to take advantage of the catalytic activity of these esterases.
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Lipases, another subclass of hydrolases, can be considered as carboxyl esterases with one difference: they are unable to hydrolyse substrate ester derivatives that are fully dispersed (soluble) in water. There appears to be a minimum degree of molecular aggregation necessary for their hydrolytic activity\(^1\). Lipases have been shown to hydrolyse molecules in a micelle. Two classes of lipases are considered important for hydrolysis of reversible drug derivatives: tissue lipases and lipases found in the digestive tract due to discharge by specialised organs. The latter type is exemplified by pancreatic lipase. Good correlation was established between the hydrolysis rate of a micellar solution of tripropionin and lipase esterolytic activity on these solutions containing approximately 13 monomers per micelle.

The rapid hydrolysis of clindamycin palmitate given orally may be due to such hydrolytic activity since this drug exists as a micellar solution\(^2\). A similar lipase is also thought to hydrolyse micellar solutions of cholesterol and vitamin A esters. The fact that short chain fatty acid are not hydrolysed as readily as longer chain (C\(_{10}\) - C\(_{18}\)) esters may be due to the fact that short chain esters are, in general, water soluble whereas the longer chain esters tend to form micellar solutions. Another lipase of interest is the lipo-protein lipase. The liver contains at least three known lipases, one acidic lipase and two alkaline lipases\(^3\).

Another class of hydrolases that may be important in the hydrolysis of bioreversible drug derivatives is the proteolytic enzymes (peptidases) found in the duodenum and intestinal epithelium. The amino acid derivative hydrolysed by the endopeptidases can be represented by the general formula:

\[
\text{R} - \text{N} - \text{H} - \text{R}^{''}
\]

Those hydrolysed by carboxypeptidases can be represented by the general formulas:

\[
\text{R} - \text{N} - \text{H} - \text{C} - \text{COOH}
\]

\[
\text{R}^{''} - \text{O} - \text{C} - \text{COOH}
\]

The dotted lines represent the point of hydrolysis by the peptidase. Studies with glycyglycine indicate that hydrolysis occurs within the intestinal epithelial cell rather than at the microvilli surface\(^4\). Some of the better known endopeptidases include the chymotrypsins A, B and C. These enzymes catalyse the hydrolysis of amide substrates containing aromatic amino acids phenylalanine, tryptophan and tyrosine. Moreover esters of these derivatives are cleaved more rapidly than the amides. Chymotrypsin C differs from Chymotrypsins A and B in its ability to hydrolyse more rapidly the ester, amide and peptide bonds involving leucine. Trypsin, another peptidase found in the intestine, acts
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exclusively on peptide, amide and ester bonds formed with arginine and lysine. Carboxypeptidase A and B, exopeptidases found in the intestine, catalyse the hydrolysis of ester and peptide bonds of derivatives containing aliphatic side chains such as alanine, leucine, isoleucine and hippuryl-L-lysine. The abundance of proteolytic enzymes found in cancer cells led to the synthesis of glycine bis(2-chloroethyl)amide as a bioreversible form of nitrogen mustard which might selectively be hydrolysed in such cancer cells.

Alkaline Phosphatases

Alkaline phosphatases (orthophosphoric monoester phosphohydrolases) catalyse the hydrolysis of many alkyl and aryl monophosphate esters. This family of enzymes is localised mainly in kidney and intestinal mucosa. Ossifying cartilage is also a rich source of phosphatase and has an optimum activity at pH 8.4-9.4 similar to that found in blood plasma. These enzymes catalyse the hydrolysis of a wide variety of ester substrates, including phosphate monoesters, diesters and phosphoramidate.

Acid Phosphatases

Like alkaline phosphatase, the acid phosphatases or orthoester phosphohydrolases are widely distributed throughout nature. Their distribution in mammalian tissue differs somewhat from alkaline phosphatase, being found predominantly in red blood cells, plasma, prostatic tissue, spleen and liver.

The availability of acid phosphatases in red blood cells and plasma may account for the rapid hydrolysis of clindamycin phosphate to clindamycin when clindamycin phosphate is administered intramuscularly. Peak serum concentrations of clindamycin appear in less than 0.5 hr, reflecting rapid hydrolysis of the phosphate ester in the systemic circulation.

Biologically reversible antineoplastic drug, diethylstilbesterol diphosphate, was designed to take advantage of the high phosphatase activity in prostatic carcinoma tissue. Given as an intramuscular injection, the derivative was thought to diffuse from the injection site as the ester and circulate to the prostatic tissue where hydrolysis occurred, thus accumulating as diethylstilbesterol on this localised site.

Sulphatases

The sulphate ester hydrolysing are not necessarily limited to the catalytic hydrolysis of sulphate ester anions but exert their effects on sulphatophosphates (sulphatophosphatase, sulphohydrolases) and sulphamates (sulphamatases). The sulphatases can be subdivided into aryl, alkyl and steroid sulphatases. The function of the aryl and alkyl sulphatases is obscure, although they are found in liver and brain tissue. The steroid sulphatases, however, catalyse the hydrolysis of several types of steroid sulphate esters including estrone sulphate, androstenolone sulphate, etiocholanolone sulphate and cortisone-21-sulphate.
PRODRUGS: THEIR CHEMICAL BASIS

PRODRUGS OF CARBOXYLIC ACIDS AND ALCOHOLS

Prodrugs of agents that contain carboxylic acid or alcohol functionalities can often be prepared by conversion to an ester. This is the most commonly seen type of prodrug, due to the ease with which the ester can be hydrolyzed to give the active drug. Hydrolysis is normally accomplished by esterase enzymes present in plasma and other tissues capable of hydrolyzing a wide variety of ester linkages (Scheme 1). Included below are a number of the different types of esterase enzymes that prodrugs may utilize:

Scheme 1

Ester hydrolase
Lipase
Cholesterol esterase
Acetylcholinesterase
Carboxypeptidase
Cholinesterase

In addition to these agents, microflora present within the gut produce a wide variety of enzymes capable of hydrolyzing esters. It is also possible that chemical hydrolysis of the ester function may occur to some extent. An additional factor that has contributed to the popularity of esters as prodrugs is the ease with which they can be formed. If the drug molecule contains either an alcohol or carboxylic acid functionality, an ester prodrug may be easily synthesized. The carboxylic or alcohol promoiety can be chosen to provide a wide range of lipophilic or hydrophilic properties to the drug depending upon what is desired. Manipulation of the steric and electronic properties of the promoiety allows the rate and extent of hydrolysis to be controlled. This can be an important consideration when it is crucial that the active drug be revealed at the correct point in its movement through the biological system.

When it is desirable to decrease water solubility, a nonpolar alcohol or carboxylic acid is chosen as the prodrug moiety. By decreasing the hydrophilicity of the compound, a number of benefits may be achieved, including increased absorption, decreased dissolution in the aqueous environment of the stomach, and prolongation of the duration
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of action. An example of increased absorption by the addition of a nonpolar carboxylic acid is seen with dipivefrin HCl (Scheme 2).

This is a prodrug form of epinephrine in which the catechol hydroxyl groups have been utilized in the formation of an ester linkage with pivalic acid. The increased lipophilicity relative to epinephrine allows the agent to move across the membrane of the eye easily when applied, achieving higher intraocular concentrations. Hydrolysis of the ester functions then occurs in the active form, epinephrine. By utilizing pivalic acid as the promoiety, the steric bulk around the scissile ester bond is increased, which slows the ester hydrolysis relative to less groups, yet still allows this reaction to proceed after the drug has crossed the membrane barriers of the eye. In addition to this benefit, the catechol system is somewhat susceptible to oxidation, and protection of the catechol as the diester prevents this oxidation and the resulting drug inactivation from occurring.

Decreasing the water solubility of a drug by the formation of a prodrug may have additional benefits beyond simply increasing absorption. A number of agents have an unpleasant taste when given orally. This results when the drug begins to dissolve in the mouth and then is capable of interacting with taste receptors. This can present a significant problem, especially in pediatric patients, and may lead to low compliance. A prodrug with reduced water solubility does not dissolve to any appreciable extent in the mouth and therefore does not interact with taste receptors. This approach has been utilized in the case of the antibacterial chloramphenicol, which produces a bitter taste when given as the parent drug (Scheme 3). The hydrophobic palmitate ester does not dissolve to any appreciable extent in the mouth, so there is little chance for interaction with taste receptors. The ester moiety is subsequently hydrolyzed in the gastrointestinal tract, and the agent is absorbed as chloramphenicol.

Listed below are a number of other agents that have been converted into ester prodrugs and other types of prodrugs in order to overcome an unpleasant taste:

Chloramphenicol palmitate
N-Acetyl sulfisoxazole
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N-Acetyl sulphamethoxypyridazine
Erythromycin estolate
Clindamycin palmitate
Troleandomycin

It should be mentioned that not all carboxylic esters are easily hydrolyzed *in vivo*. Steric inhibition around the ester, in some cases, prevents the prodrug from being hydrolyzed. This is seen in the β-lactams, in which it is often desirable to increase the hydrophobicity in the stomach, when acid catalyzed decomposition may occur. Simple esters of the carboxylic acid moiety, however, are not hydrolyzed *in vivo* to the active carboxylate (Scheme 4).

**Scheme 3**

A solution to this problem was to utilize the so-called double ester approach, in which an additional ester or carbonate function was incorporated into the R₂ substituent further removed from the heterocyclic nucleus\(^{28,29}\). Hydrolysis of such a function occurred readily, and the moiety was selected so that chemical hydrolysis of the second ester occurred quickly. This is seen in the cephalosporin cefpodoxime proxetil, where a carbonate function was utilized\(^{30}\) (Scheme 5). The carbonate is also susceptible to the
action of esterase enzymes, and the unstable product undergoes further reaction to give the active carboxylate. This approach is frequently used to improve the absorption or prevent dissolution in the stomach, and the subsequent acid-catalyzed decomposition of amino penicillins and second and third generation cephalosporins (cefpodoxime proxetil has been classified as both a second and third generation agent) so that these agents can be administered orally.

In order to increase the hydrophilicity of an agent, several different types of ester prodrugs have been utilized, including succinates, phosphates, and sulfonates. All are ionized at physiological pH and therefore increase the water solubility of the agents, making them more suitable for parenteral or oral administration where high water solubility is desirable (Scheme 6).

Succinate esters containing an ionizable carboxylate are useful when rapid in vivo hydrolysis of the ester functionality is required. The rapid hydrolysis is related to the intramolecular attack of the carboxylate on the ester linkage, which does not require the participation of enzymes. As a result, these agents are dissolved immediately prior to administration.
Phosphate esters of alcohols offer another method of increasing the water solubility of an agent. The phosphates are completely ionized at physiological pH and are generally hydrolyzed rapidly in vivo by phosphatase enzymes. Ionization of the phosphate function imparts high stability to these derivatives in solution, and solutions for administration can be stored for long periods of time without the hydrolysis of the phosphate. Such an approach has been utilized to produce clindamycin phosphate, which produces less pain in the injection site compared with clindamycin itself (Scheme 7). Pain after parenteral administration is associated with local irritation due to low aqueous solubility or highly acidic or basic solutions. In clindamycin phosphate, the reduction in pain is thought to be related to the increased water solubility of the agent.

Amines

Derivatization of amines to give amides has not been widely used as a prodrug strategy due to high chemical stability of the amide linkage and the lack of amidase enzymes necessary for hydrolysis. A more common approach has been to utilize Mannich bases as a prodrug form of the amines. Mannich bases result from the reaction of two amines with an aldehyde or ketone. As seen for hetacillin (Scheme 8), the effect of forming Mannich base is to lower the basicity of the amine and thereby increase lipophilicity and absorption.
react with formaldehyde and pyrrolidine to give the Mannich base rolitetracycline\textsuperscript{31} (Scheme 9). In this case the addition of the basic pyrrolidine nitrogen introduces an additional ionizable functionality and increases the water solubility of the parent drug. Hydrolysis of the Mannich base occurs completely and rapidly in aqueous media to give the active tetracycline.

\begin{center}
\begin{tikzpicture}

\node (Tetracycline) at (0,0) {\textbf{Tetracycline + Formaldehyde + Pyrrolidine}};
\node (Rolitetracycline) at (4,0) {\textbf{Rolitetracycline (Prodrug)}};

\draw[->] (Tetracycline) -- (Rolitetracycline);
\draw[->] (Tetracycline) -- (Rolitetracycline);
\end{tikzpicture}
\end{center}

\textbf{AZO LINKAGE}

Amines have occasionally been incorporated into an azo linkage for the purpose of producing a prodrug. In fact, it was an azo dye, prontosil that led to the discovery of the sulfonamides as the first antibacterials to be used to treat systemic infections\textsuperscript{32}. While prontosil itself was inactive \textit{in vitro}, it was active \textit{in vivo}, where it was converted by azo reductase enzymes in the gut to sulfanilamide, the active species.

\begin{center}
\begin{tikzpicture}

\node (Sulphasalazine) at (0,0) {\textbf{Sulphasalazine}};
\node (Aminosalicylic acid) at (4,0) {\textbf{Aminosalicylic acid}};
\node (Sulphapyridine) at (4,-2) {\textbf{Sulphapyridine}};

\draw[->] (Sulphasalazine) -- (Aminosalicylic acid);
\draw[->] (Sulphasalazine) -- (Sulphapyridine);
\end{tikzpicture}
\end{center}

Although prontosil is no longer used as an antibacterial, this type of linkage appears in sulfasalazine, which is used in the treatment of ulcerative colitis. The azo linkage is broken in the gut by the action of azo reductases produced by microflora. This releases the active agent, amino salicylic acid, which has an anti-inflammatory effect on the colon, and sulfapyridine (Scheme 10). The advantage of this prodrug approach is that cleavage of the azo linkage and generation of amino salicylic acid prior to absorption prevents the systemic absorption of the agent and helps concentrate the active agent at the site of action.
CARBONYL COMPOUNDS

A number of different functionalities have been evaluated as prodrug derivatives of carbonyl compounds, e.g., aldehydes and ketones, although this approach has not found wide clinical utility. These have generally involved derivatives in which the $sp^2$ hybridized carbonyl carbon is converted to a $sp^3$-hybridized carbon attached to two heteroatoms such as oxygen, nitrogen, or sulfur. Under hydrolysis conditions, these functionalities are reconverted to the carbonyl compounds. An example of this approach is methenamine\(^{32}\), which is shown below (Scheme 11).

Methenamine releases formaldehyde in the urine, which acts as an antibacterial agent by reacting with nucleophiles present in bacteria. The agent is administered in enteric coated capsules to protect the agent from premature hydrolysis in the acidic environment of the stomach. After dissolution of the enteric-coated capsules occurs in the intestines, the agent is absorbed and moves into the bloodstream, eventually ending up in the urine, where the acidic pH catalyzes the chemical hydrolysis to give formaldehyde. By utilizing a prodrug approach, the systemic release of formaldehyde is prevented and toxicity is reduced.

Other prodrug approaches have involved the use of oximes, imines, and enol esters, although these types of compounds have not been used clinically. There are a number of agents that contain imine and oxime linkages such as many of the third generation cephalosporins, e.g., cefotaxime and ceftriaxime, but these are not prodrugs.

Schiff's bases and oximes

These types of derivatives have found minimal use as prodrug forms. Saddamine\(^{34}\), a prodrug of salicylic acid is cleaved hydrolytically to benzylamine and salisaldehyde, the latter being oxidised rapidly in vivo to salicylic acid (Scheme 12).
Oxazolidines and Thiazolidines

Oxazolidine formation is a means of forming prodrugs of aldehydes or ketones, in which case \( \beta \)-amino alcohol component would act as a transport group. Oxazolidine prodrug of benzaldehyde is useful in the treatment of tumours\(^{35} \). In a search for improved topical anti-inflammatory steroids using prodrug approach, Bodor and co-workers\(^{36} \) have shown the applicability of thiazolidine derivatives (spirothiazolidines) of hydrocortisone and hydrocortisone-21-acetate were prepared.

This prodrug approach has also been extended to other steroids, such as testosterone and progesterone\(^{36} \). Thiazolidines derived from cystein have been proposed as prodrug form for various \( \alpha, \beta \)-unsaturated aldehydes (e.g. 4-Hydroxy pentenal and crotonal) with an anti-tumour activity, as a means of prolonging the action and decreasing the toxicity\(^{37} \) of these aldehydes.

Acetals and ketals

Such prodrug derivatives have found some utility for oral administration since they easily revert to the parent carbonyl-containing compound under acidic conditions. Cho et al\(^{38} \) prepared an ethylene ketal of prostaglandin E\(_2\) (dinoprostone) in order to improve the chemical stability of this drug. Experiments showed that oral administration of this ketal derivative was bioequivalent to the parent drug, thus indicating the ready cleavage of the ketal in stomach.

Enol esters

Enol esters may be quite useful as prodrugs for some drugs such as some steroids, anticoagulants and phenylbutazone. The enol ethers and esters may undergo ready hydrolysis with liberation of the free enol, which then reverts to the keto form almost instantaneously.
A. Use of Prodrugs to Overcome Pharmaceutical Barriers

The formulation of a new chemical entity with suspected therapeutic benefits requires that the drug be formulated into a delivery form that is chemically stable, free from taste and odour problems (particularly if it is for paediatric use or intended for parenteral administration), and the drug formulation must relatively be free of irritation on administration. For intravenous usage, the drug should have adequate water solubility and remain in solution for sufficient time to permit administration of the complete dose.

Masking Taste or Odour Problems

Chloramphenicol as previously described is an extremely bitter substance inhibiting its usage in paediatric formulations. Chloramphenicol palmitate, a sparingly soluble ester of chloramphenicol, is practically tasteless because of its low aqueous solubility. Since the interaction of a drug or prodrug with taste receptors requires the drug to be sufficiently soluble in saliva, by lowering the aqueous solubility to mask a taste problem, one runs the risk of creating a more serious problem, i.e. incomplete dissolution of the prodrug in the gastrointestinal tract, resulting in incomplete absorption. However, the commercially used form of chloramphenicol palmitate is efficiently hydrolysed to active chloramphenicol by the action of pancreatic lipase on solid chloramphenicol palmitate particles. Interestingly other polymorphs-different crystalline forms of chloramphenicol palmitate which are also tasteless, do not provide good plasma concentrations of chloramphenicol because of poor solubility and the fact that the solid-to-solution transition is not catalysed by lipase.

Odour is another aesthetic concern for some drugs. Such compounds are often volatile liquids, or solids with significant vapour pressure that makes them difficult to formulate. A classic example of this are the volatile mercaptans used as tuberculostatic agent and for the treatment of leprosy. Ethyl mercaptan has a boiling point of 25°C and a strong disagreeable odour. Diethyldithioisophthalate, a prodrug of ethylmercaptan, has a higher boiling point, is relatively odourless and has been used topically (as an enunction) for the systemic delivery of ethylmercaptan, which is generated from the prodrug by the action of systemic thioesterases.

Reduction of pain or Irritation at Injection Sites

Pain or irritation at an injection site may be caused by precipitation of the drug, by cell lysis due to hypo or hyperosmotic solutions, the properties of the drug itself, or the corrosive action of the drug at nerve endings. Some of these problems may relate to the vehicle composition or vehicle pH needed for formulation purposes.

Clindamycin hydrochloride, an antibiotic with an aqueous solubility of 3 mg/ml, produce a great deal of pain upon intramuscular injection while clindamycin 2-phosphate,
a prodrug of clindamycin with a solubility of >150 mg/ml, does not cause irritation or pain upon intramuscular administration. The prodrug is converted to clindamycin in vivo with a half-life of approximately 10 minutes by the action of phosphatase enzymes. The clinical advantage here is that an intramuscular clindamycin preparation may not have been commercially viable without the development of the phosphate prodrug.

A further example is provided by the anti-convulsant drug phenytoin (5.5-diphenylhydantoin which is formulated for intravenous or intramuscular use in 40% propylene glycol, 10% alcohol and 50% water with the pH adjusted to 12. At this pH, phenytoin is present in its anionic form (sodium phenytoin). These formulation conditions are necessary because phenytoin has an aqueous solubility of only 0.02 mg/ml and is weakly acidic (pKa 8.3). Therefore a high pH and the presence of co-solvents are necessary to provide a 50 mg/ml sodium phenytoin injection preparation suitable for intravenous or intramuscular usage. However, this formulation is very toxic after rapid intravenous injection due to possible precipitation of phenytoin in the vein as the pH adjusts to the physiological value of 7.4 and due to the fact that propylene glycol is a cardiac depressant. Similarly, on intramuscular injection, phenytoin precipitates at the injection site. Recently, Varia et al. evaluated a series of prodrugs of phenytoin that have superior solubility and potential therapeutic benefits over the current sodium phenytoin injectable form. The disodium salt of the phosphate ester of 3-hydroxymethylphenytoin was found to be pharmacologically inert, generated phenytoin rapidly and quantitatively in vivo in rats and dogs, and on intramuscular administration, gave no apparent irritation while rapidly releasing phenytoin. This prodrug is currently undergoing evaluation as a possible alternative to the use of sodium phenytoin.

**Alteration of Drug Solubility**

The prodrug approach can be used to increase or decrease the solubility of a drug depending on its ultimate use. One advantage of making a less soluble prodrug (to mask taste) and the possible problems it can create i.e. compromised bioavailability. There are numerous examples where solubility needs to be increased. The prime examples involve drugs whose solubility is so low that a solution dosage form for intravenous usage is not possible, for examples:

**Chloramphenicol and Corticosteroids:**

Whereas the palmitate ester of chloramphenicol has proved useful for oral formulation, a more water-soluble form, chloramphenicol sodium succinate, has been developed for parenteral administration. Chloramphenicol sodium succinate has no antibacterial activity, but is hydrolysed in the body to free chloramphenicol by the action of esterases. Steroids are another group of compounds having poor water solubility. Glucocorticoids such as betamethasone, prednisolone, methylprednisolone,
Hydrocortisone and dexamethasone are available as water-soluble disodium phosphate or sodium succinate prodrugs.

The succinate esters of corticosteroids are incompletely broken down to the parent steroid. Melby and St Cyr\(^4^5\) compared plasma concentrations of hydrocortisone, prednisolone and dexamethasone after intravenous and intramuscular administration of the phosphate and succinate esters. The areas under the 0 to 240-minute plasma concentration-time curves (AUCs) clearly demonstrated that hydrocortisone was more efficiently released from the phosphate ester (disodium salt). The rapid hydrolysis of the phosphate esters can probably be attributed to the abundance of phosphatase enzymes, while the poorer performance of the succinate esters, i.e. their inability to be rapidly and quantitatively cleaved by esterases.

Phenytoin:

More water-soluble prodrugs of phenytoin have recently been developed\(^4^3\) a, b, c, d. Phenytoin has a high melting point indicating strong crystal lattice energy, which on dissolution is not compensated for by the release of solvation forces. Therefore, phenytoin is very water-insoluble and can only be dissolved at a high pH, as sodium phenytoin, and with the help of co-solvents. Dramatic increase in aqueous solubility are seen with the disodium phosphate ester\(^4^3c,d\), which has also been determined to be the most desirable phenytoin prodrug based on chemical stability, as well as in vivo performance. Upon cleavage of the phosphate group, the 3-hydroxymethyl derivative is formed, which in the absence of formaldehyde rapidly loses formaldehyde (t\(^1/2\) <2 sec at 37°C and pH 7.4) to generate phenytoin.

\[
\begin{align*}
&\text{Phenytoin} \\
&\text{Solubility} \\
&(\text{Phenytoin} = 1) \\
&\text{R} = \\
&-\text{COCH}_2\text{NH}+(\text{CH}_3)_2\text{CH}_2\text{CO}_2^- \\
&-\text{CO}(\text{CH}_3)_2\text{NH}+(\text{C}_6\text{H}_5)_2\text{napsylate} \\
&-\text{COO}(\text{CH}_3)_2\text{NH}+(\text{CH}_3)_2\text{CH}_3\text{SO}_3 \\
&-\text{PO}_{32}\text{Na}_2^+ \\
&8610 \\
&39 \\
&4730 \\
&4500 
\end{align*}
\]

Sulindac:

Sulindac was developed primarily to reduce gastrointestinal toxicity and is 100 times more water-soluble than its sulphide derivative. At pH 7.4, the solubility of sulindac is 3.3 mg/ml while that of the sulphide is 0.03 mg/ml. Because of this greater solubility and the fact that it still maintains sufficient lipophilicity for gastrointestinal absorption (octanol: water partition coefficient at pH 7.2 is 1.52), sulindac is well absorbed after oral administration.
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Sulindac

Enhancement of chemical stability

A very important requirement of all drug products is that they must be chemically stable over a reasonable period. Except for products like vaccines, some cytotoxic agents, and other life saving products, a shelf life of at least two years is desirable. If a drug is chemically very unstable and the instability problem cannot be resolved by formulation means, it is sometimes possible to develop a prodrug with enhanced stability over the parent drug. This usually takes the form of chemical modification of the functional group responsible for the instability, or a change in the physical properties of the drug (via prodrug modification) resulting in the reduction of contact between the drug and the media in which it is unstable.

Aqueous sodium ampicillin is chemically very unstable in concentrated solution due to autoaminolysis, i.e. the side chain primary amino group of the ampicillin molecule is capable of attacking the β-lactam ring of a second ampicillin molecule to give various polymeric species. Hetcillin is a prodrug of ampicillin formed by its reaction with acetone. When hetcillin is diluted for intravenous infusion (< 20 mg/ml ampicillin equivalents) it readily dissociates to ampicillin and acetone. However, in concentrated solutions, the hetcillin only partially dissociates. Since the interaction of the side chain of ampicillin and acetone ties up the amino group, hetcillin does not undergo the autoaminolysis reaction and is a relatively stable prodrug form of ampicillin sodium. In the body, hetcillin readily dissociates to produce ampicillin.

B. Use of Prodrug to Overcome Pharmacokinetic Barriers

The absorption distribution, metabolism and excretion of a drug are all dynamic processes that are affected by the physicochemical properties of the drug. Variations in bioavailability, for example, can lead to variations in patient response to a drug and in animals can lead to ambiguous interpretation of the efficacy of the drug.

Enhancement of Oral Absorption

If a drug is very water insoluble, bioavailability after oral dosing is often dissolution rate limited. For agents that are highly polar in nature, it is often the transport...
of the drug across the gastrointestinal mucosal cell membranes that limits drug absorption. Since most drugs are absorbed by passive diffusion, a degree of lipophilicity is necessary for efficient absorption through the gastrointestinal barrier. For highly polar compounds, the administration of a less polar, more lipophilic prodrug may help promote gastrointestinal absorption. This approach has been successfully applied with various penicillin derivatives.

Ampicillin:

Ampicillin is highly polar and at the pH of the gastrointestinal tract is mostly present in a zwitter ionic form. Its bioavailability after oral dosing is only 20 to 60%. A number of less polar prodrugs of ampicillin have been prepared by esterifying the carboxyl group of ampicillin. Those currently receiving the most attention are bacampicillin, pivampicillin and talampicillin.

These ester prodrugs of ampicillin are efficiently absorbed and are cleaved by general esterase enzymes in the body, most often in the mucosal cells themselves, releasing ampicillin. In the case of bacampicillin, the products of the cleavage are acetaldehyde, carbon dioxide and ethanol. For ampicillin, it is formaldehyde and pivalic acid. The bioavailability characteristics of all these prodrugs are significantly superior to ampicillin itself. There is an approximate 2 to 5 fold increase in relative oral bioavailability when bacampicillin is administered. Simon et al demonstrated that ampicillin tissue concentrations following bacampicillin administration correlated with the increased blood concentrations of ampicillin.

Prevention of Presystemic Metabolism

Many drugs are efficiently absorbed from the gastrointestinal tract but undergo presystemic metabolism or inactivation before reaching the systemic circulation. The acid in the stomach, for example, partially degrades a number of drugs before they can be
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efficiently absorbed. The major problem associated with enzymatic metabolism is that orally administered drugs are exposed to the enzymes of the gastrointestinal lumen, the brush border, the mucosal cell and the liver, prior to reaching the systemic circulation.

Major classes of drugs that undergo marked presystemic metabolism are those containing phenolic moieties. Sulphation, glucuronidation and, to a minor extent, methylation all contribute to rapid inactivation of many of these drugs. For example only 4% of orally administered isoprenaline in intact form (isoproterenol) reaches the systemic circulation as compared with an equivalent intravenous dose. Similarly, conjugation of other phenolic molecules such as salicylamide, α-methyldopa, terbutaline and salbutamol also limits their systemic availability. Presystemic metabolism is not just limited to drugs with phenolic moieties. Oxidative N-and O-dealkylation ester cleavage and peptide degradation are other major cleavage routes.

Prolongation of Drug Action

For drugs that are rapidly cleared from the body frequent dosing with conventional dosage forms is required to maintain adequate plasma concentrations of the particular drug. This frequent dosing of short half-life drugs results in sharp peak-valley plasma concentration-time profiles, and consequently patient compliance is often poor. Sustained or prolonged release drug products will often overcome such problems. Traditionally formulation approaches are often quite successful but the combination of a prodrug and a suitable delivery system may allow for the sustained release of drugs for which a pure formulation approach may not work.

Sinkula has summarized the ways in which alterations in the physicochemical properties of drug, in the form of a prodrug, can be used to prolong or control drug action. They include alterations in:

1. The degree and rate of absorption.
2. The rate and extent of conversion of the prodrug to the active species.
3. The rate and extent of protein or tissue binding.
4. The degree of tissue or organ localization, distribution and subsequent release from such sites.

Testosterone:

Lipophilic prodrugs of testosterone such as its 17-propionate, 17-phenylacetate, and 17-cypionate esters are administered in an oil vehicle by deep intramuscular injection after which they are stored in fat depots and ultimately cleaved to testosterone.

Depot Antipsychotic Drugs

This same technology has been applied to various antipsychotic drugs. The depot forms of fluphenazine are its enanthate (heptanoate) and decanoate esters. These esters have greater lipophilicity than the parent compound, and when administered in sterile sesame
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oil, intramuscular injections exhibit a much longer duration of action, than fluphenazine hydrochloride.

Reduction in toxicity
One of the desired properties of all therapeutically active agents is that they have negligible or no toxicity associated with their clinical use. Sufficient examples of a reduction in toxicity due to prodrug administration exist so as to provide an impetus for this exciting, albeit difficult, area of prodrug research.

Aspirin and Sulindac:
Aspirin can be considered as a less corrosive prodrug form of salicylic acid. Similarly, sulindac, also mentioned earlier, is a prodrug of the corresponding sulphide. Sulindac itself as the sulphoxide form of the active sulphide moiety has little or no pharmacological activity but is much more water-soluble. Since the anti-inflammatory activity and gastrointestinal intolerance of many anti-inflammatory drugs seem to be mediated by their inhibition of prostaglandin synthetase and the ingested sulphoxide has little or no such activity, exposure of the gastrointestinal mucosa to the active substance is minimized. This may account for the reduced local gastrointestinal irritation caused by sulindac in comparison with certain other anti-inflammatory drugs such as aspirin, though such side effects are by no means absent. Sulindac is an excellent example of a clinically relevant prodrug with decreased toxicity and better absorption characteristics than its active drug.

BIOPRECURSOR PRODRUGS
Bioprecursor prodrugs do not contain a carrier or promoiety but rather contain latest functionality, which is metabolically or chemically transformed to the active drug molecule. The types of activation often involve oxidative activation, reductive activation, or phosphorylation. Of these, oxidation is commonly seen, because there are a number of endogenous enzymes that can carry out these transformations. Phosphorylation has been widely exploited in the development of antiviral agents, and many of the currently available agents depend upon this type of activation.

The abundance of oxidizing enzymes in the body has made this type of bioactivation a popular route. Isozymes of cytochrome P-450 are capable of oxidizing a wide variety of functionalities, generally to produce more polar compounds, which can be excreted directly or undergo Phase 2 conjugation reactions and subsequently undergo elimination. This occurs in a fairly predictable manner and therefore has been successfully exploited in prodrug approaches.

A good example of a prodrug that requires oxidative activation is the nonsteroidal anti-inflammatory drug (NSAID) nabumetone (Relafen). The use of NSAIDs produces irritation of the stomach, which in patients with pre-existing conditions or in patients
taking large amounts of NSAIDs for extended periods of time may be severe. This irritation is associated in part with the presence of an acidic functionality in these agents. The carboxylic acid functionality commonly found in these agents is unionized in the highly acidic environment of the stomach. As a result, these agents are more lipophilic in nature and may pass into the cells of the stomach’s mucosa. The intracellular pH of these cells is more basic than that of the stomach lumen, and the NSAID becomes ionized. This results in the back flow of $H^+$ from the lumen into these cells, with concomitant cellular damage. This type of damage could be prevented if the carboxylic acid function could be eliminated from these agents. However, this functional group is required for activity.

Nabumetone contains no acidic functionality and passes through the stomach without producing the irritation normally associated with this class of agents. Subsequent absorption occurs in the intestines, and metabolism in the liver produces the active compound as shown above. This approach however, did not completely eliminate the gastric irritation associated with this agent since this is due only in part to a direct effect on the stomach. Inhibition of the target enzyme, cyclooxygenase, while having an anti-inflammatory effect, also results in the increased release of gastric acid which irritates the stomach. So, while nabumetone exhibits reduced gastric irritation compared with other NSAIDs, this undesired effect was not completely eliminated by QA prodrug approach. Such an effect was also seen previously with the NSAID sulindac, which exhibited reduced gastrointestinal irritation, but this effect was not completely eliminated.

Reductive activation is occasionally seen a method of prodrug activation but generally is less common than oxidative activation due to lower number of reducing enzymes. One of the best known examples of reductive activation is the antineoplastic agent mitomycin C, which is used in treatment of bladder and lung cancer. Mitomycin C contains a quinone functionality that undergoes reduction to give a hydroquinone. This is important due to the differential effect of the quinone and hydroquinone on the electron pair of the nitrogen. While the quinone had an electron withdrawing effect on this electron pair, the hydroquinone has an electron releasing effect, which allows these electrons to participate in the expulsion of methoxide and subsequently, the loss of the carbonate to generate a reactive species, which may alkylate DNA.

Phosphorylation is a common metabolic function of the body, which is used as a means of producing high-energy phosphodiester bonds such as those present in ATP and GTP. The body then typically uses these molecules to phosphorylate other molecules and, in the process of doing so, activates these molecules. The type of activation achieved is
dependent upon the molecule phosphorylated, but in many cases, phosphorylation introduces a leaving group, which can be displaced by an incoming nucleophile. This is seen for example in the synthesis of DNA and RNA, in which nucleotides are added to the 3’ end of a growing chain of DNA or RNA.

Phosphorylation is commonly required for the bioactivation of antiviral agents. The agents are commonly nucleosides, which must be converted to the nucleotides to have activity. Most often, antiviral agents disrupt the synthesis or function of DNA or RNA, and this is generally accomplished by conversion to the triphosphate. Since normal cells are also involved in the synthesis of DNA or RNA, compounds have been sought that would be converted to the triphosphates, the active form, in greater amounts in the infected cells than in normal cells. Therefore, nucleosides that have higher affinity for the viral kinase enzymes than the mammalian kinase enzymes are desirable and have greater selective toxicity.

This can be seen in the prodrug idoxuridine\textsuperscript{56}, which was the first agent to show clinical effectiveness against viruses. The nucleoside enters the cell, where it is phosphorylated. In virally infected cells, this phosphorylation is accomplished preferentially by viral thymidine kinase, the idoxuridine being a better substrate for the viral enzyme than for the corresponding mammalian enzyme.

Therefore, the drug is activated to a greater extent in the virally infected cells and achieves some level of selective toxicity, although this selectivity is rather low and significant toxicities to normal cells also occur. Once the drug has been phosphorylated to the triphosphate stage, it can inhibit DNA synthesis in a number of ways, including inhibition of viral DNA polymerase and incorporation into DNA, resulting in incorrect base pairing, which disrupts the ability of DNA to function as template for DNA and RNA synthesis.
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In addition to the selective toxicity mentioned, the prodrug approach offers the additional advantage of increased cell penetration. The prodrug can easily enter the cell via active transport mechanisms, whereas the active nucleotides are unable to utilize this process and are too polar to cross the membrane via passive diffusion.

CHEMICAL DELIVERY SYSTEMS

The knowledge gained from drug metabolism and prodrug studies may be utilized to target a drug to its site of action. Site-specific chemical delivery systems take advantage of higher levels of activity in a metabolic or chemical pathway at the target site. A prodrug form of the active drug is designed to serve as a substrate in that specific pathway, thus yielding a high concentration of active drug at the target site. Site-specific chemical delivery requires that the prodrug reach the target site and the enzymatic or chemical process exits at the target site for conversion of the prodrug to the active drug. Many factors are involved in the relative success of site-specific drug delivery, including extent of target organ perfusion, rate of conversion of prodrug to active drug in both target sites, and input/output rates of prodrug and drug from the target sites.

Site-specific chemical delivery systems involve the selective delivery of drug molecules to their site of action for increased therapeutic effectiveness and limited side effects. Other than chemical drug delivery, many carrier systems have been evaluated for drug delivery, including proteins, polysaccharides, liposomes, emulsions, cellular carries (erythrocytes and leukocytes), magnetic control targeting and implanted mechanical pumps. As the fate of drugs in the human body has become more clearly understood, research activity to improve the delivery of active drug to the target site has increased. The basic goal of these efforts is to protect the drug from the nonspecific biological environment and to protect the nonspecific biological environment from the drug in order to achieve some degree of site-specific drug delivery. Site-specific drug delivery has been extensively evaluated for the delivery of drugs with narrow therapeutic windows such as many of the anticancer drugs.

The site-specific delivery of the active drug via its prodrug counterpart requires that the prodrug be readily transported to the site of action and rapidly absorbed at the site. Upon arrival at the target site, the prodrug should be selectively converted to drug relative to its rate conversion at nontarget sites. Since high metabolic activity occurs in highly perfused tissues such as liver and kidney, delivery to these organs has a natural advantage. Unfortunately, prodrug delivery of active drug to other organs or tissues is disadvantageous for the same reasons. Furthermore, it is highly desirable that the active drug, once formed, should migrate from the target site at a slow rate. Based upon all these requirements it becomes quite clear that the site-specific delivery of a drug to the target by a prodrug chemical delivery system represents a far more complex undertaking than just the design of a prodrug to improve one aspect of its overall properties. Yet there are several excellent examples of site-specific chemical delivery systems in use in modern
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drug therapy. The target sites include cancer cells, the gastrointestinal tract, the kidney and urinary tract, bacterial cells, viral material, ocular tissue, and the blood-brain barrier.

The prodrug methenamine, already described, can be considered a site-specific chemical delivery system for the urinary tract antiseptic agent formaldehyde. The low pH of the urine promotes the hydrolysis of methenamine to formaldehyde, the active antibacterial agent. The rate of hydrolysis increases with an increase in acidity (decreased pH), and this can be promoted by administration of urinary pH-lowering agents or diet. The pH of the plasma is buffered to ~7.4 and the rate of hydrolysis is low, preventing systemic toxicity from formaldehyde. As mentioned previously, this compound is administered in enteric-coated tablets, which prevent dissolution and therefore premature hydrolysis in the highly acidic environment of the stomach.

A number of prodrugs for cancer chemotherapy have been designed to selectively deliver active drug to the tumor tissue based upon higher levels of activating enzyme in the tumor cell relative to normal tissue. Many enzymatic systems show higher levels of activity in tumor cells than in normal tissue; these enzyme systems are more active because of the higher activity in and near tumor cells. Derivatization of a drug molecule with an amino acid or peptide fragment has been used as a means of attempting to produce higher rates of drug incorporation into tumors as compared to surrounding normal tissue.

An interesting example of site-specific chemical delivery is an antiviral drug such as idoxuridine. Antiviral drugs serve as a substrate for phosphorylating enzymes found in viruses, and the phosphorylated species is incorporated into viral DNA, disrupting viral replication and thus producing the antiviral effect. These drugs do not undergo phosphorylation by mammalian cells, and thus the prodrug is specific for those sites at which it serves as a substrate for phosphorylation by mammalian cells, and thus the prodrug is specific for those sites at which it serves as a substrate for phosphorylation enzymes. One of the requirements for site-specific chemical delivery discussed earlier was the proper input/output ratios for prodrug and active drug species at the target. The relative physicochemical properties of prodrug and its phosphorylated derivative would suggest an appropriate input/output ratio for site specificity. The prodrug is readily able to penetrate into virus, and the increased polarity of the phosphorylated derivative would serve to retain that active species inside the virus. The increased polarity and viral retention of the active phosphorylated species likely reduce any human toxicity that might be associated with active species.

The amino acid drug L-Dopa can be considered a site-specific chemical delivery system delivering the drug dopamine to the brain. The brain has an active transport system that operates to incorporate L-amino acids into the central nervous systems. Once across the blood-brain barrier, the L-Dopa undergoes decarboxylation as shown below to yield the active metabolite, dopamine. Direct systemic administration of dopamine does not produce significant brain levels of the drug due to its facile metabolic degradation by

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oxidative deamination. However, dopamine formed on the inside of the blood-brain barrier is held there due to the poor membrane permeability of this drug. While some specificity for brain tissue is achieved by this delivery method, peripheral side effects of L-Dopa are the direct result of decarboxylation to dopamine in other organ systems. In this case, the enzyme-activating system is not localized at the target site, and its presence in other tissues and organs lead to the undesirable side effects.

![Chemical structure of L-Dopa and Dopamine](image)

Another example of the chemical delivery of a drug to the brain and nervous system is the prodrug form of 2-PAM (Pro-2-PAM), an important antidote for the phosphate and carbamate, acetylcholinesterase inhibitors used in insecticides and nerve gases. The polar properties of 2-PAM, a permanent cationic species, prevent this drug from being absorbed following oral administration and restrict the drug from access to the brain even after intravenous administration. Pro-2-PAM is a dihydropyridine derivative that undergoes metabolic and chemical oxidation to yield the active drug 2-PAM. The nonionic Pro-2-PAM can easily cross the blood-brain barrier, and the oxidation to 2-PAM within the brain essentially traps the active cationic drug species inside the brain. The oxidation of the dihydropyridine ring of PRO-2-PAM occurs throughout the mammalian system and not just in the brain, and the levels of the resulting 2-PAM are approximately the same in peripheral tissue as in the brain. However, intravenous administration of PRO-2-PAM yields brain levels of 2-PAM~10 times higher than those achieved by intravenous administration of the parent drug.

The delivery of drug across the blood-brain barrier has been a significant issue in the design of many therapeutic compounds. Only very lipophilic drugs are capable of crossing into the brain without the aid of some active uptake process such as that which operates to incorporate essential amino acids into the central nervous system. The facile oxidation of the dihydropyridine ring system has been extensively investigated as a general process for the chemical delivery of a number of drugs to the central nervous system. The approach has been described as a chemical delivery system and not just a prodrug designed to be able to penetrate the blood-brain barrier. This process is a multi
process procedure involving delivery of the drug dihydropyridine derivative to the brain via facile diffusion across the blood-brain barrier followed by oxidation to the quaternary pyridine cation, which is trapped in the brain. The drug is then released from the pyridine cation by a second metabolic/chemicals event. A number of functional groups can be added to the dihydropyridine to facilitate the derivatization of various functional groups found in central nervous system drug. Since many central nervous system drugs are amines, amides of dihydropyridine carboxylic acids are often prepared and these amides are used to deliver the drugs across the blood brain barrier into the brain. Additionally, these amide derivatives often serve to protect the amines from metabolic degradation before they reach the target site. Primary amines such as dopamine and norepinephrine are readily metabolized and degraded by oxidative deamination before reaching the central nervous system. The dihydropyridine derivative of a dopamine ester shown below has access to the central nervous system via passive absorption of the tertiary amine which upon oxidation restricts the resulting pyridinium amide to the brain. Amide hydrolysis then delivers the active form of the drug to or near its site of action. The amide hydrolysis step may be slower then the dihydropyridine oxidation step and thus a reservoir of pyridinium amide precursor may be available for conversion to the active drug species.

\[ \text{Dihydropyridine - prodrug} \rightarrow \text{Pyridinium ion intermediate} \]

\[ \text{Dopamine} \]

The use of prodrug concepts had been very successful in the delivery of active drug species to the human eye following local application. Lipophilic esters of epinephrine such as the dipivaloyl ester previously described (Scheme 2) show improved cornea penetration following direct application to the eye when compared to application of the more polar parent drug epinephrine. The esterases necessary for the hydrolysis of the prodrug are readily available in the eye and skin. The more polar drug species, epinephrine, is then localized within the lipophilic membrane barriers of the eye, and the drug remains available at the target site to produce its anti-glaucoma effects. The local
application of the prodrug species to the skin or eye allows metabolic processes to activate the drug without concern for competitive reactions at other tissues or sites of loss.

The delivery of drugs to the colon and lower gastrointestinal tract has been accomplished by taking advantage of the unique enzymatic processes found in colon bacteria. The glucosidase activity of these bacteria allows for the hydrolysis of glucoside derivatives of drugs in the colon and provides higher concentrations of active drug. A number of steroid drugs have shown increased effectiveness in the lower gastrointestinal tract following administration as their glucoside derivatives. The polar glucoside derivatives of the steroids are not well absorbed into the blood stream from the gastrointestinal tract and remain available to serve as substrates for the bacteria found primarily in the human colon.

The prodrug approach for the delivery of anticancer drugs to the site of action has been used in a number of cases in an effort to increase effectiveness and lower side effects. Several enzyme systems that shows higher activity in and near cancer cells have been evaluated for their ability to activate the prodrug species. In most cases, the enzyme activity level is simply higher near the faster-growing cancer cells, but the presence of the enzymes in normal tissue prevents the possibility of complete site specificity in these agents.

New prodrug Approaches

Future prodrug research, which is being pursued, involves a biochemical technique. Knowledge of the structure-reactivity requirements of a particular enzyme system (e.g. phenol sulpho-transferase) allows one to recognize that the promoiety could be placed at some other position in the drug molecule such that the prodrug is no longer a substrate due to either steric, electronic or other effects for the presystemic metabolizing enzyme, i.e. even though the functional group originally attacked is not directly masked' the physicochemical properties of the prodrug are such that it is not recognized as a substrate. The advantage of this approach may well be the use of promoieties attached to other parts of the molecule that could not be easily placed on the phenolic group directly. However, the cleavage of the promoiety should still be triggered after passage through the liver, otherwise sequential metabolism will occur.

It is clear from this brief discussion of site-specific drug delivery that in some cases the prodrug was in use before its mechanism of delivery and specificity were discovered. Thus, some compounds were discovered to represent site-specific drug delivery well after they were placed into therapeutic use. An evaluation of the properties of these agents has produced the framework for the design of other prodrugs having target sites in specific tissues. This process is really no different from the general drug discovery process, in which a unique substance is observed to have desirable pharmacological effects and studies of its properties lead to the design of better drugs.
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REFERENCES

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45. Melby, J. C. and St. Cyr, M., Metabolism 10 75 (1961).
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Recent Advances in NSAIDs Prodrug Design

Mahfouz et al. synthesized ester prodrugs of aspirin, ibuprofen, naproxen and indomethacin using N-Hydroxymethylsuccinimide and N-hydroxymethylisatin as promieties to reduce their gastrointestinal toxicity and improve bioavailability. In vivo ulcerogenicity studies using scanning electron microscopy on stomach specimens of rats treated with an oral dose for 4 days revealed that the synthesized ester prodrugs were significantly less irritating to gastric mucosa than the parent drugs.

Bandarage et al. synthesized a series of novel diclofenac esters containing a nitrosothiol (-S-NO) moiety as a NO donor functionality and evaluated in vivo for bioavailability, pharmacological activity, and gastric irritation. All S-NO-diclofenac derivatives acted as orally bioavailable prodrugs, producing significant levels of diclofenac in plasma within 15 min after oral administration to mice. At equimolar oral doses, S-NO-diclofenac derivatives displayed rat anti-inflammatory and analgesic activities comparable to those of diclofenac in the carrageenan-induced paw edema test and the mouse phenylbenzoquinone-induced writhing test, respectively. All tested S-NO-diclofenac derivatives were reported to be gastric-sparing in that they elicited markedly fewer stomach lesions as compared to the stomach lesions caused by a high equimolar dose of diclofenac in the rat.

A series of acyloxyalkyl esters of ketoprofen and naproxen were synthesized and investigated by Rautio et al. as topical prodrugs with the aim of improving the dermal delivery of the above mentioned drugs. In addition, some hydroxyalkyl esters of ketoprofen and naproxen were synthesized as possible intermediates of acyloxyalkyl prodrugs. They found that the prodrug with the highest aqueous solubility was the most effective prodrug to deliver naproxen through the skin.

Jung et al. prepared a new colon-specific prodrug of 5-aminosalicylic acid (5-ASA) and reported that the amount of 5-ASA liberated from incubation of the prodrug in cecal or colonic contents of rats was about 65% or 27% in 8 hrs, respectively, which indicated that the prodrug activation took place more readily in the rat cecum whose bacterial counts are high like human colon. Results from in vitro experiments suggested 5-ASA-Gly as a promising candidate of a colon-specific prodrug of 5-ASA.

The synthesis and study of a novel series of potential prodrugs of indomethacin, ketoprofen, ibuprofen and aspirin was carried out by Abordo et al. They prepared 2-formylphenyl esters of the NSAIDs, together with two 6-substituted 2-formyl and two 2-acylphenyl aspirins and 4-formylphenyl indomethacin. The 2-formylphenyl esters were reported to be more potent as anti-inflammatory agents than the parent compounds in the carrageenan-induced paw edema test.

Sartore et al. synthesised derivatives of Ibuprofen, in which the drug was bound by ester linkages to poly(ethylene glycols) (PEG 2000-I), monomethoxy poly(ethylene glycol)
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glycols) (PEG 1900-I), poly(N-vinyl pyrrolidinone) (PVP-I) and poly(N-acryloyl morpholine) (PACM-I) and tested for their pharmacokinetic properties after oral administration. It was reported that the two end-hydroxylated amphiphilic oligomers of polyvinylic structure, PACM and PVP, had a similar potential as promoieties for preparing oligomeric prodrugs.

Various aclyoxyethyl mefenamates were synthesized by Jilani et al. and were evaluated for potential application as prodrugs. Among the synthesized compounds, the beta-carboxypropionylethyl mefenamate and the pivaloyloxyethyl mefenamate were reported to have high stability against enzymatic and non enzymatic hydrolysis. Preliminary in vivo study showed that aclyoxyethyl mefenamate gave plasma concentration of mefenamic acid lower than that of control after oral administration.

Ogiso et al. synthesized the butyl and octyl ester prodrugs of indomethacin and evaluated for their ulcerogenic activity and hepatic injury after oral administration in rats. Ulcerogenic activity and hepatic injury, expressed by decreased hepatic microsomal enzyme activities, reportedly were hardly seen after repeated oral administration of the prodrugs, in contrast with the severely irritating effects of indomethacin alone.

The pharmacokinetics of ibuprofen diethylcarbonate and naproxen diethylcarbonate, two new diethylcarbonate prodrugs of ibuprofen and naproxen in dogs, was reported by Samara et al. The rationale for their development was that esterification of the carboxylic moiety of the parent compounds would suppress gastrototoxicity without adversely affecting their anti-inflammatory activity. In addition the biotransformation of the prodrugs to the parent compounds may be utilized to achieve rate and time controlled drug delivery of the active entities.

Tsunematsu et al. synthesized ethyl esters of flurbiprofen L-arginine (FP-Arg-OH), flurbiprofen L-lysine (FP-Lys-OH) and flurbiprofen p-guanidino-L-phenylalanine (FP-GPA-OH) and then examined the release of flurbiprofen enantiomers from these derivatives in the presence of trypsin (Tp), carboxypeptidase B (CPB) and carboxypeptidase Y (CPY) in order to evaluate their availability as prodrugs for flurbiprofen (FP).

Arakawa et al. evaluated indomethacin farnesil a prodrug of indomethacin in comparison with indomethacin and loxoprofen in rats to ascertain whether it caused less gastric mucosal damage than the two older drugs. Damage was evaluated in terms of the size of ulcers that formed after oral administration and the changes in concentrations of prostaglandins E2 and I2 in the mucosa. Indomethacin farnesil caused less damage than indomethacin and tended to cause less damage than loxoprofen. Indomethacin farnesil was reported to be less potent than indomethacin in inhibiting prostaglandin generation by gastric mucosa.

Indomethacin oligoethylene ester derivatives were synthesized by De Caprariis et al. and were evaluated for their anti-inflammatory, analgesic, and ulcerogenic activity.
after oral administration. Report revealed that esters showed an anti-inflammatory activity, determined as the percent inhibition of carrageenan-induced edema, similar to that of indomethacin, although at higher doses. From writhing test results, it was found that all the prodrugs exhibited better or similar analgesic activity compared to indomethacin. Esters were significantly less irritating to the gastric mucosa than indomethacin, after oral administration.

Tammara et al.\textsuperscript{13} synthesised morpholinoalkyl esters (HCl salts) of diclofenac and evaluated in vitro and in vivo for their potential use as prodrugs for oral delivery. Prodrugs were reported to be significantly less irritating to gastric mucosa than diclofenac following single and chronic oral administration in rats.

A prodrug of indomethacin, indomethacin octyl ester (IM-OE), was synthesized by Ogiso et al.\textsuperscript{14} and its pharmacokinetics was investigated in rat. Indomethacin rapidly appeared in plasma after iv administration of IM-OE and declined in a monoeponential manner, with a rapid decline and low plasma levels of IM-OE. The plasma concentrations of indomethacin after oral administration of IM-OE were much lower than those after oral administration of indomethacin.

Yamaguchi et al.\textsuperscript{15} newly synthesised a prodrug of 5-aminosalicylic acid (5-ASA), salicylazosulfanilic acid (SASA), which consists of sulphanilic acid linked to 5-ASA through an azo-linkage. Biopharmaceutical properties of SASA were evaluated in comparison with those of salicylazosulfapyridine (SASP) in rats.

Tammara et al.\textsuperscript{16} synthesised morpholinoalkyl esters (HCl salts) of naproxen and indomethacin and evaluated in vitro and in vivo for their potential use as prodrugs for oral delivery. The prodrugs were reportedly 30-36% more bioavailable orally than the parent drugs following a single equimolar solution dose in rats. These prodrugs were significantly less irritating to gastric mucosa than parent drugs following single-dose and chronic oral administration in rats.

Carty et al.\textsuperscript{17} evaluated ampiroxicam, a nonacidic ether carbonate prodrug of piroxicam, and demonstrated that, in contrast to piroxicam, ampiroxicam does not possess detectable prostaglandin synthesis inhibitory activity in vitro. Ampiroxicam, however, had similar in vivo potency to piroxicam in suppressing paw swelling in rat adjuvant arthritis.

Ueda et al.\textsuperscript{18} synthesised a novel indomethacin ester prodrug, 2-[N-[3-(3-piperidinomethyl) phenoxy)propyl] carbamoylmethylthio] ethyl 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate from a new histamine H2-receptor antagonist, N-[3-(3-piperidinomethyl) phenoxy) propyl]-2-(2-hydroxyethylthio) acetamide and indomethacin. The prodrug was shown to be essentially similar to indomethacin in its anti-inflammatory action and almost completely inhibited carrageenan-induced hind-paw edema in the rat at a very high dose of 230 mg/kg, which is comparable to that of 100 mg/kg of indomethacin, without producing gastric lesions.
Various novel morpholinyl- and methylpiperazinylacyloxyalkyl esters of naproxen were synthesized by Rautio et al. and evaluated in vitro for topical drug delivery as potential prodrugs and were shown to have improved topical delivery of naproxen.

Jung et al. reported a simple synthetic route for the preparation of amino acid conjugate of 5-aminosalicylic acid (5-ASA) and prepared 5-aminosalicyl-glycine (5-ASA-Gly) in good yield. In vitro and in vivo properties of 5-ASA-Gly as a colon-specific prodrug of 5-ASA were investigated using rats as the test animal. Incubation of 5-ASA-Gly with cecal or colonic contents at 37° released 5-ASA in 65 or 27% of the dose in 8 h, respectively. No 5-ASA was detected from the incubation of 5-ASA-Gly with the homogenates of stomach or small intestine.

The hydrolysis of tertiary amidomethyl ester prodrugs of carboxylic acids by rat liver homogenates was reported by Lee et al. Amidomethyl esters were rapidly and quantitatively converted to the corresponding acid and secondary amide.

Diacyl glyceryl ester derivatives of naproxen were synthesized by Thorsteinsson et al. and were tested for transdermal and dermal administration. The prodrugs were slowly hydrolyzed to naproxen inside the skin. The release of naproxen to the receptor compartment of diffusion cells showed that this type of prodrug could be used for controlled drug delivery.

Rautio et al. synthesized and evaluated various novel aminoacyloxyalkyl esters of naproxen and naproxenoxoalkyl diesters of glutamic acid and aspartic acid as potential dermal prodrugs of naproxen. The aminoacyloxyalkyl prodrugs were shown to have higher aqueous solubilities and similar lipid solubilities, in terms of octanol-buffer partition coefficients (log Papp) at pH 5.0, when compared with naproxen. The selected aminoacyloxyalkyl prodrugs possessed a higher flux across the skin than naproxen, with a maximum enhancement of 3-fold compared to naproxen.

In an attempt to obtain site-specific delivery of 5-ASA in the intestinal tract, Clerici et al. synthesised and determined the extent of absorption and metabolism of a number of novel 5-ASA derivatives, namely, (N-L-glutamyl)-amino-2-salicylic acid, (N-L-aspartyl)-amino-2-salicylic-acid, 5-amino salicyl-L-proline-L-leucine, and 5-(N-L-glutamyl)-aminosalicyl-L-proline-L-leucine, which are selectively cleaved by intestinal brush border aminopeptidase A and carboxypeptidases. The novel glutamate and aspartate derivatives behaved similarly to sulphasalazine, while administration of the proline-leucine derivative resulted in urinary and fecal recovery values intermediate with respect to those observed with 5-ASA and sulphasalazine. 5-(N-L-Glutamyl)-aminosalicyl-L-proline-L-leucine yielded the highest fecal recovery of 5-ASA and its N-acetyl derivative, indicating a more efficient delivery to the distal bowel.
Murtha et al. synthesized cholesteryl ibuprofen and cholesteryl flufenamate as hydrophobic compounds for phospholipid microemulsions. Cholesteryl ibuprofen was isolated as an amorphous, white solid with a melting range of 114-120°C. Cholesteryl flufenamate was isolated as a crystalline, white solid with a melting range of 145-148°C. Phospholipid microemulsions have been suggested as a drug-delivery system for hydrophobic compounds.
References

Discussion

• Synthesis
• Biological Evaluations and Results
Synthetic Work
The following section deals with the syntheses of reversible derivatives (prodrugs) of well known Non-Steroidal Anti-inflammatory Drugs (NSAIDs) such as ibuprofen, diclofenac, naproxen, mefenamic acid, indomethacin, probenecid and biphenyl acetic acid. These drugs were converted into the prodrugs by three different chemical methods viz. glucosidation, amidation and synthesis of glycolamide esters.

Glucosides of NSAIDs were synthesised by treating 2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl bromide with the sodium salts of appropriate NSAIDs using phase transfer catalyst in dichloromethane/water, two phase system by modified Koenig’s Knorr reaction. The products were purified by crystallization.

Amino acid conjugate prodrugs were synthesised by condensing acid chlorides of the above drugs with methyl esters of various amino acids using modified Schotten Bauman reaction. Acid chlorides were prepared by the method reported in Vogel’s Textbook of Practical Organic Chemistry. Amino acid methyl esters were prepared by the method of Ronald et al. The final products were purified by column chromatography.

On condensing 1, 6-hexanediamine with appropriate acid chlorides of the above anti-inflammatory drugs, the corresponding prodrugs were obtained.

Glycolamide esters of NSAIDs were prepared by reported method of Harkin’s which were purified by column chromatography.

The structures of all the above synthesised compounds were established by proton NMR spectrometry.

1. Igarashi K. Adv Carbohydr Chem Biochem 34 243 (1977)
3. Harkin’s E M US patent 3173900 (1965)
A. Synthesis of ester glucosides of ibuprofen, Diclofenac sodium, Biphenyl acetic acid, Indomethacin and Probenecid

Synthesis of β-D-glucopyranosyl derivatives of the above drugs was carried out through the following three steps:

i) Synthesis of β-D-glucose penta acetate (1)

ii) Synthesis of 2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) (2) from 1

iii) Condensation of 2 with different drugs

Synthesis of β-D-glucose penta acetate (1)

It was prepared by the standard procedure reported in Vogel's Text book of Practical Organic Chemistry. 4th Edn. ELBS, Longman (Pub.) London p455 (1989). It was obtained as TLC pure colourless needles, m.p. 130-132°.

\[
\text{Glucose} \xrightarrow{\text{Ac}_2\text{O/CH}_2\text{COONa}} \beta-D\text{-glucose pentaacetate (1)}
\]

Synthesis of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (2) from 1

It was prepared by the standard procedure reported in Vogel's Text book of Practical Organic Chemistry. 4th Edn. ELBS, Longman (Pub.) London p457 (1989). It was obtained as TLC pure colourless needles, m.p. 88-89°.

\[
1 \xrightarrow{\% \text{aq. NaOH/PTC}} 2
\]

Condensation of 2 with different NSAIDs having free carboxylic acid groups

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (3) of ibuprofen

To a cold solution of ibuprofen; 2 and tetrabutylammonium bromide in dichloromethane was added dropwise aq. sodium hydroxide solution and the reaction mixture stirred for 24
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hours. The reaction mixture after processing gave colourless needles, m.p. 110-112°. Its structure was elucidated by 1H NMR spectral data.

The NMR spectrum of the compound showed two doublets located at δ7.08 and δ7.17 arising from p-substituted phenyl ring. In the aliphatic region, there could be seen a doublet arising from two CH₃ groups of the isopropyl system at δ0.8 while one doublet at δ1.49 arising from secondary methyl group, a multiplet at δ1.81 arising from the CH group of the isopropyl system was also present. The CH group attached to secondary methyl group was seen as a multiplet at δ3.55. The CH₂ group (benzylic) appeared as a doublet at δ2.42. These data accounted for protons of ibuprofen moiety. The CH₃ protons of the four acetoxyl groups of glucose moiety appeared as four singlets at δ2.0, δ2.01, δ2.06 and 2.09 while the sugar proton H5 appeared as a multiplet at δ3.8. The sugar protons H6 and H6’ appeared as two doublets at δ4.05 and δ4.26. The rest of the protons H2, H3, H4 could be seen as a multiplet at δ5.1 while H1 appeared as a doublet at δ5.68. These data are completely in agreement with the structure assigned to the compound.

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (4) of diclofenac sodium

To a stirring mixture of 2 and tetra butyl ammonium bromide in dichloromethane was added dropwise a solution of diclofenac sodium in water and stirred for 24 hours. The reaction mixture on usual work up gave colourless needle shaped crystals, m.p. 146-148°.

The NMR spectrum of the compound clearly indicated the formation of the glucoside derivative. In the aliphatic region, the signals could be analysed for the sugar moiety. There were four singlets arising from the four acetoxyl groups at δ1.77, δ1.99, δ2.03 and δ2.08. The H6 protons of the sugar moiety arising from CH₂ group appeared as two doublets located at δ6.49 and δ6.28. The protons H2, H3 and H4 of the sugar molecule appeared as a multiplet centered at δ5.19 while the sugar proton H1 appeared as a doublet centered at δ5.7. The proton H5 of the sugar moiety could be seen as a multiplet located at δ3.85. The benzylic CH₂ protons could be seen as a multiplet centered at δ3.86. In the aromatic region, the proton H3’ appeared as a multiplet centered at δ6.5 while the protons H4’ and H5’ could be seen as a multiplet centered at δ6.96 and the proton H6’ appeared as a doublet centered at δ7.17. The protons from the dichlorophenyl ring could be seen at δ7.12 as a multiplet and at δ7.3 as a doublet arising from H4'; and H3, H5 respectively. These data satisfactorily explained the structure assigned to the compound.
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Synthesis of tetraacetyl β-D-glucopyranosyl derivative (5) of biphenyl acetic acid
Biphenyl acetic acid was condensed with 2 in presence of tetrabutylammonium bromide and aq. sodium hydroxide to give light green needle shaped crystals, m.p. 144-146°.

![Chemical Structure](image)

The NMR spectrum of the compound showed the presence of four acetoxyl functions as four singlets located at δ1.75, δ1.99, 2.02 and 82.09. In the aromatic region, there could be seen a doublet at δ7.3 which could arise from the aromatic protons ortho to methylene group. The proton H4 of the phenyl group was present as a triplet at δ7.34. The protons H3 and H5 of the phenyl ring could be seen as a triplet located at δ7.43 while the protons H2, H6 and the two protons meta to the methylene group appeared as a multiplet centered at δ7.55. These assignments are satisfactory for the protons of the biphenyl system. The benzylic methylene group appeared as a singlet at δ3.70 while the protons H5; H6; H2; H3; H4; and H1 appeared as multiplet at δ3.86; at δ4.14, at δ4.3; as a multiplet at δ5.15 and as a doublet at δ5.71 respectively. These data are satisfactory for the sugar protons.

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (6) of indomethacin
To a stirring mixture of 2 and tetra butyl ammonium bromide in dichloromethane was added dropwise a solution of indomethacin in sodium bicarbonate and stirred for 24 hours. The reaction mixture on usual work up gave colourless needle shaped crystals, m.p. 210-12°.

![Chemical Structure](image)

The NMR spectrum of tetraacetyl glucoside derivative showed a singlet for the methyl group at δ1.56 while the four acetoxyl groups appeared as four singlets located at δ1.97, 82.02, 82.08 and 82.3 each integrating for three protons. The OCH3 protons
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appeared as a singlet at 63.84 while the CH₂ group could be located as a singlet at 63.77. In the aromatic region, there were two doublets located at 67.49 and 67.7, which could arise from p-substituted phenyl ring. Protons H4 and H7 appeared as a multiplet centered at 66.9 while H6 could be seen as a double doublet at 66.67. These assignments perfectly explain the aglycone part of the glucoside while the protons from the sugar part besides the protons of the four acetoxyl groups explained above appeared as a multiplet at 64.27 arising from the protons of the CH₂ group. The proton H5 was located as a multiplet at 63.8. The protons H2, H3 and H4 could be seen as a multiplet located at 65.11 while the proton H6 was located as two doublets at 64.15 and 64.3. The proton H1 appeared as a doublet located at 65.66. These data satisfactorily explain the above structure assigned to the glucoside derivative.

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (7) of probenecid
To a cold solution of probenecid, 2 and tetrabutylammonium bromide in dichloromethane was added dropwise aq. sodium hydroxide solution and stirred for 24 hours. The reaction mixture when processed gave colourless needles, m.p. 128-130°.

The NMR spectrum of the compound by the presence of four singlets at 62.0, 62.05, 62.06 and 62.08 arising from the four acetoxyl groups coupled by two doublets located at 67.8 and 68.1 arising from the p-substituted phenyl ring suggested successful formation of the glucoside derivative. The other sugar protons could be picked up at 63.85 as a multiplet arising from H5, the two double doublets located at 64.12 and 64.34 arising from H6 and a multiplet located at 65.3 arising from H2, H3 and H4 of the glucose system and a doublet at 65.92 arising from H1 of the glucose moiety. The CH₃ group, CH₂ group and the CH₂-N group of the probenecid molecule appeared as a triplet at 60.87, as a hextet at 61.56 and as a triplet at 63.1 respectively. These data satisfactorily supported the above structure assigned to the glucoside tetra acetate derivative.

Synthesis of β-D-glucopyranosyl derivative (8) of 3
A solution of 3 in 0.5% sodium methoxide was kept at room temperature for one hour, neutralised with H⁺ exchange resin and concentrated in vacuum. The semisolid mass so
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obtained was crystallised from absolute ethanol to give colourless crystals, m.p. 162-163°. Its structure was established on the basis of ¹H NMR spectrum.

The NMR spectrum did not show the presence of the acetoxyl groups by signals around δ2.0. However, the other features of both the aglycone part and the sugar part were present in the spectrum. It showed the presence of two methyl groups of the isopropyl system by a doublet located at δ0.83 and the presence of one secondary CH₃ group by a singlet at δ1.52. The CH proton of isopropyl group was present as a multiplet at δ1.77 while the CH proton attached to the secondary methyl group could be seen as a multiplet around δ3.5. The sugar protons could be seen as a multiplet around δ3.45 and δ5.0 while the benzylic methylene group was present as a doublet at δ2.3 and the protons of p-substituted phenyl ring were located as two doublets centered at δ6.9 and δ7.15. These data satisfactorily explained the structure assigned to the compound as the glucoside of ibuprofen.

Attempted synthesis of β-D-glucopyranosyl derivative (9) of 4

Compound 4 was treated with sodium methoxide in methanol as described above and processed. The resultant solid was crystallised from absolute ethanol to give colourless crystalline compound that showed TLC behaviour and melting point identical to diclofenac and was soluble in 2% NaHCO₃ solution with effervescence suggesting that the reaction did not proceed as desired.

Attempted synthesis of β-D-glucopyranosyl derivative (10) of 6

Acetylated glucoside of indomethacin (6) was treated with 0.5% sodium methoxide. The reaction mixture on processing gave a colourless crystalline compound. It showed TLC pattern and melting point identical to indomethacin, also it dissolved in 2% NaHCO₃ with effervescence indicating unsuccessful results.
Synthesis of β-D-glucopyranosyl derivative (11) of 7
Probenecid tetraacetyl glucoside (7) on treatment with 0.5% sodium methoxide gave a solid mass, which was crystallised from absolute ethanol to give colourless crystalline compound. m.p 140-142°.

The NMR spectrum of the compound showed the presence of two methyl groups by a triplet at δ0.79 and a hextet at δ1.52 arising from the middle CH₂ group of the probenecid system while the protons of methylene group attached to N-atom along with the two protons of the CH₂ group of the sugar moiety appeared as a multiplet at δ3.0. The rest of the protons i.e. H1 to H5 alongwith the protons of the four hydroxyl groups of sugar moiety could be seen as a multiplet centered at δ4.38. The protons of the p-substituted phenyl ring appeared as two doublets at δ7.92 and δ8.13. Absence of any signals around δ2.0 confirmed the deacetylation of the hydroxyl groups of sugar moiety. These data are in complete accord with the structure proposed to the compound.
B Synthesis of Prodrugs of NSAIDs by condensing their free carboxylic acid group with different L-amino acids

The synthesis was carried out through the following steps:

i) Synthesis of methyl ester hydrochlorides of α-amino acids (12-19)
ii) Synthesis of acid chlorides (20-25) of NSAIDs
iii) Synthesis of amide derivatives (26-54) by condensing the above methyl ester derivatives and acid chlorides.

Synthesis of methyl ester HCl (12) of L-alanine
It was prepared by the standard procedure to give a colourless crystalline compound, m.p. 208-210°.

Synthesis of methyl ester HCl (13) of L-phenylalanine
It was prepared in a similar way to give the methyl ester derivative, m.p. 162-64°.

Synthesis of methyl ester HCl (14) of L-leucine
It was obtained as a white crystalline compound, m.p. 144-46°.

Synthesis of methyl ester HCl (15) of L-isoleucine
It was obtained as a colourless crystalline compound, m.p. 110-12°.
Synthesis of methyl ester HCl (16) of L-valine
It was obtained as a colourless crystalline compound, m.p. 164-66°.

Synthesis of methyl ester HCl (17) of L-tyrosine
The methyl ester HCl derivative was obtained as a colourless crystalline compound, m.p. 190-92°.

Synthesis of methyl ester HCl (18) of DL-serine
The required ester HCl was obtained as a colourless crystalline compound, m.p. 218-20°.

Synthesis of methyl ester HCl (19) of L-tryptophan
L-tryptophan methyl ester HCl was obtained as a colourless crystalline compound, m.p. 220-22°.
Synthesis of acid chlorides (20-25)

All these compounds were prepared by following the standard procedure given in ‘A TextBook of Practical Organic Chemistry’ by Arthur I. Vogel, 3rd Edn. 1964, p 367

Synthesis of naproxen acid chloride (20) from naproxen

It was obtained as a viscous residue, which was TLC pure and showed different TLC behavior when compared with the starting material and was used as such for further reactions.

\[
\begin{align*}
\text{Naproxen} & \xrightarrow{\text{SOCl}_2} \text{Naproxen acid chloride (20)} \\
\end{align*}
\]

Synthesis of mefenamic acid chloride (21) from mefenamic acid

It was obtained as a yellow solid mass which was TLC pure and showed different TLC behavior in comparison to the starting material and was used as such for further reactions.

\[
\begin{align*}
\text{Mefenamic acid} & \xrightarrow{\text{SOCl}_2} \text{Mefenamic acid chloride (21)} \\
\end{align*}
\]

Synthesis of ibuprofen acid chloride (22) from ibuprofen

It was obtained as a viscous residue, which was TLC pure and showed different TLC behavior in comparison to the starting material and was used as such for further reactions.

\[
\begin{align*}
\text{Ibuprofen} & \xrightarrow{\text{SOCl}_2} \text{Ibuprofen acid chloride (22)} \\
\end{align*}
\]

Synthesis of diclofenac acid chloride (23) from diclofenac

It was obtained as a TLC pure red coloured solid mass which showed different TLC behavior in comparison with the starting material and was used as such for further reactions.
Synthesis of indomethacin acid chloride (24) from indomethacin
It was obtained as a viscous mass, which was TLC pure and showed a different TLC behaviour in comparison with indomethacin and was used as such for further reactions.

Synthesis of probenecid acid chloride (25) from probenecid
It was obtained as a viscous mass, which was TLC pure and showed a different TLC behaviour in comparison with the starting material and was used as such for further reactions.

iii Synthesis of amide derivatives (26-54) by condensing methyl esters and acid chlorides

Synthesis of L-alanine methyl ester amide (26) of naproxen
Naproxen acid chloride was condensed with L-alanine methyl ester and purified by column chromatography to give TLC pure colourless needles, m.p. 86-88°.

The NMR spectrum of the compound showed two doublets located at δ1.29 and δ1.6. The former may be due to methyl group of L-alanine while the second one may be due to secondary methyl group of naproxen system. The CH proton of the naproxen moiety appeared as a quartet at δ3.7 while the CH proton of the alanine moiety could be
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seen as a quartet at $\delta 4.5$. The CONH proton was located as a broad singlet at $\delta 5.9$. The protons of methoxyl group appeared as a singlet at $\delta 3.9$, while the protons of methyl ester were located as a singlet at $\delta 3.65$. The protons of the naphthalene ring could be seen as a multiplet at $\delta 7.17$ accounting for $H_5$ and $H_7$, at $\delta 7.37$ as a double doublet arising from $H_3$ and at $\delta 7.71$ as a multiplet arising from $H_1$, $H_4$ and $H_8$. These data are in complete accord with the structure assigned to the compound.

Synthesis of L-phenylalanine methyl ester amide (27) of naproxen

It was synthesised by condensing naproxen acid chloride with L-phenylalanine methyl ester. After purification from column chromatography it gave TLC pure colourless needles, m.p. 92-94°

The NMR spectrum of the compound showed a doublet located at $\delta 1.53$ arising from secondary methyl group of naproxen moiety and CH proton attached to it appeared as a quartet at $\delta 4.80$. The CH proton of phenylalanine appeared as a multiplet at $\delta 4.16$ while the protons of OCH$_3$ group and COOCH$_3$ group appeared as two singlets at $\delta 3.93$ and $\delta 3.69$ respectively. The NH proton was located at $\delta 5.78$. The two protons of phenyl ring ortho to CH$_2$ group appeared as a doublet at $\delta 6.58$ while the remaining three protons of phenyl ring appeared as a triplet at $\delta 8.66$. The protons $H_5$, $H_7$ appeared as a multiplet at $\delta 7.16$; $H_3$ as a double doublet at $\delta 7.3$; and $H_1$, $H_4$ and $H_8$ as a multiplet at $\delta 7.69$. These data are satisfactory for the structure assigned to the compound.

Synthesis of L-leucine methyl ester amide (28) of naproxen

L-leucine methyl ester was condensed with naproxen acid chloride and purified by column chromatography to give TLC pure colourless needles, m.p. 106-108°.

The NMR spectrum of the compound showed a multiplet at $\delta 0.8$ arising from the isopropyl group of the L-leucine system. The CH$_2$ group could be seen as a multiplet at $\delta 1.50$. There was a doublet at $\delta 1.59$ arising from secondary methyl group of naproxen moiety. A multiplet at $\delta 3.7$ could arise from the CH group attached to secondary methyl
group of naproxen system. There was a multiplet at δ1.4 arising from the CH of isopropyl system. There was another multiplet at δ4.6, which could arise from CH group attached to methyl ester function. The protons of the methoxyl group and COOCH₃ group were located as two singlets at δ3.9 and δ3.62 respectively. The signal due to NH proton could be observed as a singlet at δ5.72 while in the aromatic region, there could be seen a multiplet at δ7.14 from H5, H7; a double doublet at δ7.37 from H3 and another multiplet at δ7.72 accounting for H1, H4 and H8 protons. These data are satisfactory for the structure assigned to the compound.

Synthesis of L-isoleucine methyl ester amide (29) of naproxen
It was synthesised by condensing naproxen acid chloride with L-isoleucine methyl ester HCl to give TLC pure needle-shaped colourless crystals, m.p. 108-110°.

The NMR spectrum of the compound showed two peaks, one as a doublet at δ0.8 for secondary methyl group and the second one as a triplet at δ0.84 arising from the primary methyl group of isoleucine system. There was a multiplet at δ1.0 arising from the CH₂ group of the ethyl moiety. A doublet at δ1.59 could be due to the secondary methyl group attached to naphthalene ring. The CH proton attached to secondary methyl group of naphthalene moiety may be seen as a quartet located at δ3.76 while the CH proton attached to secondary methyl group of amino acid moiety was located at δ1.82 and the CH proton attached to methyl ester function was located at δ4.56. The protons of methyl ester group and methoxyl function appeared as singlets at δ3.6 and δ3.9 respectively. The CONH proton gave a signal at δ5.96. In the aromatic region, the protons ortho to methoxyl group i.e. HD and HE appeared as a multiplet at δ7.13 while the proton HB could be seen as a double doublet at δ7.37 and the protons HA, HC and HF appeared as a multiplet at δ7.7. These data are in accord with the structure assigned to the compound.

Synthesis of L-tyrosine methyl ester amide (30) of naproxen
Naproxen acid chloride was condensed with L-tyrosine methyl ester HCl. After purification by column chromatography it was obtained as TLC pure needle-shaped colourless crystals, m.p. 128-30°.

The NMR spectrum of the compound showed a doublet at δ1.57 arising from the secondary methyl group. The protons of the benzylic CH₂ group appeared as a multiplet...
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at δ2.89. It showed a singlet at δ3.6 arising from the methyl ester group and to it was attached a multiplet at δ3.7 which could arise from the CH proton attached to secondary methyl group. There was another singlet located at δ3.9, which could be due to the protons of the methoxyl group. The CH proton attached to the methyl ester group could be seen as a quartet at δ4.75. The CONH proton appeared as a doublet at δ5.84. In the aromatic region, there were two doublets forming an $A_2B_2$ pattern arising from the p-substituted phenyl ring located at δ6.47 and δ6.57. The protons H3, H5 and H7 of naphthalene moiety appeared as a multiplet at δ7.17 while the protons H1, H4 and H8 appeared as a multiplet at δ7.67. These data are in complete accord with the structure assigned to the compound.

Synthesis of L-valine methyl ester amide (31) of naproxen

Condensation of naproxen acid chloride with L-valine methyl ester HCl followed by column chromatography gave TLC pure colourless needles, m.p. 116-18°.

The NMR spectrum of the compound showed two doublets located at δ0.74 and δ0.85 arising from the two methyl groups of the isopropyl system. There was a doublet located at δ1.61, which could arise from the secondary methyl group. There was a multiplet at δ2.03 arising from the CH proton of the isopropyl system. There were two singlets at δ3.6 and δ3.9, which could arise from the protons of methyl ester function and methoxyl group respectively. There was a multiplet at δ3.75 arising from CH proton attached to secondary methyl group. The CH proton attached to COOCH$_3$ group appeared as a multiplet at δ4.5 while the proton of the amide function could be seen as a doublet at δ5.85. The protons of naphthalene system namely H5 appeared as a meta coupled doublet at δ7.12, the proton H7 appeared as a double doublet at δ7.17, the proton H3 could be seen as a double doublet centered at δ7.38 and the protons H1, H4 and H8 appeared as a
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Synthesis of DL-serine methyl ester amide (32) of naproxen
DL-Serine methyl ester HCl on condensation with acid chloride of naproxen followed by column chromatography gave TLC pure needle-shaped colourless crystals, m.p. 132-34°C.

The NMR spectrum of the compound showed a doublet at δ1.5, which could arise from secondary methyl group of naproxen system. The methyl protons of COOCH₃ function and methoxyl group merged together to give a singlet at δ3.89. The CH proton attached to the secondary methyl group appeared as a multiplet at δ3.7 while the CH proton attached to COOCH₃ function could be seen as a multiplet at δ4.62. The NH proton appeared as a doublet at δ6.5. The CH₂ proton gave a signal at δ3.95. In the aromatic region, the protons ortho to methoxyl group i.e. H5 and H7 appeared as a multiplet at δ7.1 while the proton H3 appeared as a double doublet at δ7.5 and protons H1, H4 and H8 could be located as a multiplet at δ7.67. These data are satisfactory for the structure assigned to the compound.

Synthesis of L-tryptophan methyl ester amide (33) of naproxen
Naproxen acid chloride was condensed with L-tryptophan methyl ester HCl as above and after purification by column chromatography gave TLC pure needle-shaped colourless crystals, m.p. 160-62°C.

The NMR spectrum of the compound showed a doublet located at δ1.5 which could arise from methyl group of naproxen moiety. The protons of the methoxyl group appeared as a singlet at δ3.9 while the CH₂ protons of the tryptophan system appeared as a multiplet centered at δ3.6. The protons of methyl ester function appeared as a singlet at δ3.59. The CH proton of the naproxen system appeared as a quartet at δ3.8 and CH proton of the tryptophan system was located as a multiplet at δ4.9. The proton NH of
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The indole ring appeared as a singlet at 66.53. The other four protons of the indole ring appeared as a multiplet centered at 67.12. The proton of the CONH group appeared as a broad singlet at 67.94. The lone proton of the indole ring adjacent to NH group appeared as a singlet at 67.50. The protons of the naphthalene ring i.e. H5 and H7 appeared as a multiplet at 67.25 while the proton H3 of the naphthalene ring appeared as a double doublet at 67.4 and protons H1, H4 and H8 appeared as a multiplet at 67.6. These data are in complete accord with the structure assigned to the compound.

Synthesis of L-alanine methyl ester amide (34) of mefenamic acid
It was synthesised by condensing mefenamic acid chloride with L-alanine methyl ester HCl in presence of triethylamine followed by column chromatography to give a TLC pure brown coloured compound, m.p. 32-34°.

The NMR spectrum of the compound showed a doublet integrating for three protons arising from the methyl group of amino acid moiety located at 81.2. The other two methyl groups were located at 82.09 and 82.4. The protons of COOCH3 group were located as a singlet at 83.79. The CH proton merged with the signal of the COOCH3 group. In the aromatic region there was a multiplet integrating for two protons centered at 86.63, which could arise from the protons ortho and para to NH group in the xylene ring and the proton meta to NH group could be located at 87.0. The protons of the other ring i.e. para to CONH group appeared as a multiplet at 87.2 while the proton ortho to CONH group could be seen as double doublet centered at 87.95 and the remaining two protons of this ring appeared as a multiplet centered at 87.03. The proton of CONH group was located as a singlet at 89.0. These data support the structure assigned to the compound.

Synthesis of L-phenylalanine methyl ester amide (35) of mefenamic acid
L-Phenylalanine methyl ester HCl and mefenamic acid chloride were condensed followed by column chromatography to give a TLC pure brown coloured low melting compound, m.p. 32-34°.

The NMR spectrum of the compound showed two singlets located at 82.1 and 82.2, which could arise from the two methyl groups. There was a multiplet at 83.15, which could arise from protons of benzyllic CH2 group, a singlet at 83.69 could be due to
the protons of the methyl ester function. The CH proton attached to COOCH₃ group appeared as a multiplet located at δ4.9. The CONH proton appeared as a broad singlet at δ8.9. The aromatic protons namely HE appeared as a double doublet δ6.5; HF appeared as a triplet at δ6.6 while the proton HG appeared as a double doublet located at δ6.77 and the proton HD appeared as a double doublet at δ6.9. The rest of the protons i.e. HC, HB and HA appeared along with the protons of the phenyl group of phenylalanine as a multiplet between δ6.96 to δ7.23. These data are in agreement with the structure assigned to the compound.

**Synthesis of L-leucine methyl ester amide (36) of mefenamic acid**

It was synthesised by condensing mefenamic acid chloride with L-leucine methyl ester HCl followed by column chromatographic purification to give a TLC pure brown coloured compound, m.p. 42-44°.

The NMR spectrum of the compound showed a multiplet arising from two methyl groups of the isopropyl system of the amino acid located at δ0.9. The CH₂ group of the amino acid moiety was located as a multiplet at δ1.59. The CH group of isopropyl system merged with the protons of the CH₂ group at δ1.59. The CH proton attached to ester function appeared as a multiplet at δ4.7 while the CONH proton could be seen as a broad singlet at δ9.0. There could be seen a singlet at δ3.7 arising from ester function while the two methyl groups of o-xylene ring were located at δ2.1 and δ2.24. In the aromatic region, the proton HE could be seen as double doublet centered at δ6.42 while proton HB was located as a multiplet at δ6.65 and the proton HG was seen as a double doublet located at δ6.86. The protons HD and HF were located as multiplets centered at δ6.8 and
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δ6.96 respectively while the proton HC was located as a multiplet at δ7.18. The proton HA was located as double doublet at δ7.4. These data are satisfactory for the structure assigned to the above compound.

Synthesis of L-isoleucine methyl ester amide (37) of mefenamic acid

L-Isoleucine methyl ester HCl and mefenamic acid chloride were condensed as above and subjected to column chromatography to give a TLC pure brown coloured compound, m.p. 46-48°.

![Diagram of L-isoleucine methyl ester amide (37) of mefenamic acid]

The NMR spectrum of the compound showed a closely packed triplet and a doublet arising from primary methyl group and secondary methyl group of isoleucine moiety centered at δ0.87. The protons of the methylene group and the CH group attached to secondary methyl group appeared as a multiplet located at δ1.1 and δ1.8 respectively. The CH proton attached to methyl ester function could be picked up at δ4.7 as a multiplet while the protons of methyl ester group appeared as a singlet at δ3.67. The CONH proton appeared as a singlet at δ8.9. These data accounted for all the protons of the amino acid molecule. The two methyl groups of mefenamic acid moiety appeared as two singlets at δ2.08 and δ2.21. In the aromatic region, the proton HB appeared as a multiplet at δ6.6, while the proton HE appeared as double doublet centered at δ6.77. The proton HG also appeared as a double doublet centered at δ6.84. The proton HF appeared as a doublet of triplet at δ6.9 while the proton HD appeared as a double doublet at δ7.04. The proton HC could be picked up as a doublet of triplet located at δ7.1. The proton HA was located as double doublet most down field at δ7.4. These data are in agreement with the structure assigned to the compound.

Synthesis of L-valine methyl ester amide (38) of mefenamic acid

It was synthesised by following the same procedure as described above by condensing mefenamic acid chloride with L-valine methyl ester HCl. On column chromatography, it give a TLC pure brown coloured compound, m.p. 36-38°.

The NMR spectrum of the compound showed a multiplet at δ0.88 arising from the protons of two methyl groups of isopropyl moiety. The CH proton of the isopropyl group could be seen as a multiplet located δ2.1. The protons of the two methyl groups of
mefenamic acid appeared as two singlets at $\delta 2.03$ and $\delta 2.18$. The protons of methyl ester

\begin{center}
\includegraphics[width=0.5\textwidth]{structure1.png}
\end{center}

function could be seen as a singlet at $\delta 3.67$ while the CH proton attached to it appeared as a multiplet centered at $\delta 4.64$. The CONH proton was seen as a singlet at $\delta 8.9$. The proton HA could be picked up as double doublet located at $\delta 7.39$ while the rest of the aromatic protons of mefenamic acid appeared as multiplet located between $\delta 6.5$ and $\delta 7.29$. These data are in accord with the structure assigned to the compound.

**Synthesis of L-tyrosine methyl ester amide (39) of mefenamic acid**

Mefenamic acid and L-tyrosine methyl ester HCl were condensed in presence of triethylamine followed by column chromatographic purification to give a TLC pure yellow coloured compound, m.p. 34-36°.

\begin{center}
\includegraphics[width=0.5\textwidth]{structure2.png}
\end{center}

The NMR spectrum of the compound showed the presence of two methyl groups by two singlets located at $\delta 2.08$ and $\delta 2.23$. The protons of COOCH$_3$ group appeared as a singlet at $\delta 3.70$. The CH proton attached to the ester function could be seen as a multiplet at $\delta 4.9$ while the CH$_2$ protons appeared as a multiplet centered at $\delta 3.1$. The proton from amide group appeared as a broad singlet at $\delta 8.9$. The protons from $p$-substituted phenyl ring appeared as two doublets centered at $\delta 6.6$ and $\delta 6.9$. The aromatic protons of mefenamic acid could be seen as a complex multiplet from $\delta 6.5$ to $\delta 7.3$. The proton HA could be seen most downfield as a double doublet at $\delta 7.29$. These data are satisfactory for the structure assigned to the compound.

**Synthesis of DL-serine methyl ester amide (40) of mefenamic acid**

Condensation of mefenamic acid chloride with DL-serine methyl ester HCl gave a TLC pure brown coloured compound, m.p. 46-48°.
The NMR spectrum of the compound showed the two CH3 groups as two singlets at 82.05 and 82.23 arising from mefenamic acid system. In the aromatic region, there could be seen four multiplets centered at 86.6, 86.8, 87.0 and 87.47 integrating for one proton, two protons, three protons and one proton which accounted for all the seven aromatic protons of the mefenamic acid moiety. The last multiplet may be due to the proton ortho to the carbonyl function in mefenamic acid system. The CH2 and CH protons of the amino acid moiety could be located as multiplet at 83.9 and 84.7 respectively. The peak due to the protons of the CH3 group of methyl ester function appeared as a singlet at 3.74. The proton due to amide group appeared as broad singlet at 89.0. These data are in agreement with the structure assigned to the compound.

Synthesis of L-tryptophan methyl ester amide (41) of mefenamic acid
A TLC pure brown coloured compound was obtained by the condensation of mefenamic acid chloride and L-tryptophan methyl ester HCl in presence of triethylamine. On column chromatographic purification, it gave a TLC pure compound. m.p. 52-54°.

The NMR spectrum of the compound showed two singlets located at 82.1 and 82.3, which could arise from two methyl groups. There was a singlet at 83.7, which accounted for protons of COOCH3 group. The CH group attached to the ester function could be seen as a multiplet at 85.1. The CH2 group attached to indole moiety appeared as a multiplet at 83.4. The proton from NH group of indole ring appeared at 88.1 while the amide group proton could be picked up at 89.0. The proton HA was located as a double doublet at 87.55 while the proton HB was seen as doublet of triplet at 86.56. The rest of the aromatic protons appeared as a multiplet located between 86.7 to 87.3. These data are in complete accord with the structure assigned to the compound.
Attempted synthesis of L-alanine methyl ester amide (42) of ibuprofen
Condensation of ibuprofen acid chloride and L-alanine methyl ester HCl in cold aq. potassium carbonate gave a TLC pure colourless compound. The NMR spectral data of the compound indicated that the reaction did not proceed in the desired direction to give the expected amide derivative.

Attempted synthesis of L-phenylalanine methyl ester amide (43) of ibuprofen
The NMR spectral data of compound obtained by the condensation of ibuprofen acid chloride and L-phenylalanine methyl ester HCl in presence of cold aq. potassium carbonate showed that the reaction did not proceed in desired direction to give the expected amide derivative.

Synthesis of L-leucine methyl ester amide (44) of ibuprofen
Condensation of ibuprofen acid chloride with L-leucine methyl ester HCl gave a product, which was subjected to column chromatographic purification to give a TLC pure colourless crystalline compound, m.p. 42-44°. Its structure was elucidated by ¹H NMR spectral data.

The NMR spectrum of the compound showed the presence of four methyl groups arising from the isopropyl system of the amino acid moiety and ibuprofen moiety located as a multiplet located at δ0.81. The CH protons of the two isopropyl systems could be seen as two multiplets at δ1.72 and δ1.8. The secondary methyl group of ibuprofen moiety and the CH₂ group of amino acid system were located as a multiplet at δ1.50. The methylene protons of benzylic system appeared as a doublet at δ2.4 while the CH proton attached to secondary methyl group could be seen as a multiplet at δ3.55. The protons of
the methyl ester function appeared as a singlet at 83.68 while the CH proton attached to COOCH$_3$ group was located as a multiplet at 84.57. The CONH proton appeared as a broad signal at 85.71. The protons of the p-substituted phenyl ring appeared as two doublets located at 87.12 and 87.22. These data are satisfactory for the structure assigned to the compound.

Synthesis of L-isoleucine methyl ester amide (45) of ibuprofen

It was synthesised by condensing ibuprofen acid chloride with L-isoleucine methyl ester HCl followed by purification by column chromatography to give TLC pure colourless crystals, m.p. 48-50°.

The NMR spectrum of the compound showed a multiplet centered at 80.82, which accounted for the protons of two methyl groups of isopropyl system and one primary methyl group of the amino acid. There could be seen a multiplet at 81.7 which could arise from the CH proton of isopropyl group. There was a multiplet at 81.89, which could be due to another methine proton attached to secondary methyl group of isoleucine system. There could be seen a multiplet at 83.51 which may arise from the CH group attached to secondary methyl group. There was a doublet centered at 81.52 arising from the two secondary methyl groups. The CH$_2$ protons of the ethyl function appeared as a multiplet at 81.1. The protons of the methyl ester function appeared as a singlet at 83.7 while the CH proton attached to the ester group could be seen as a multiplet at 84.53. The protons of the benzylic methylene group appeared as a doublet at 82.45 and the protons of the p-substituted phenyl ring were located as two doublets at 87.1 and 87.2. These data are satisfactory for the structure assigned to the compound.

Synthesis of L-tyrosine methyl ester amide (46) of ibuprofen

Ibuprofen acid chloride was condensed with L-tyrosine methyl ester HCl. Column chromatographic purification gave TLC pure needle-shaped colourless crystals, m.p. 40-42°.

The NMR spectrum of the compound showed the presence of a multiplet at 80.82, which could arise from the protons of the two methyl groups of isopropyl system. There was a doublet at 81.4 arising from protons of secondary methyl group and CH proton adjacent to it appeared as a multiplet at 83.52. The CH proton of the isopropyl system appeared as a multiplet at 81.7. The methylene protons of two benzylic systems appeared
as multiplets at 82.4 and 82.71. The CH proton attached to methyl ester function could be seen as a multiplet centered at 84.7. The protons of methyl ester group appeared as a singlet at 83.62. The protons of the p-substituted phenyl ring of tyrosine system appeared as two doublets at 86.58 and 86.9 while the protons of p-substituted phenyl ring appeared as a singlet at 7.07. These data are in complete accord with the structure assigned to the compound.

Synthesis of L-valine methyl ester amide (47) of ibuprofen
Condensation of ibuprofen acid chloride with L-valine methyl ester HCl gave a compound which was purified by column chromatography to give a TLC pure colourless compound, m.p. 42-44°.

The NMR spectrum of the compound showed a multiplet located at 80.7 which could arise from the protons of the four methyl groups of the two isopropyl systems. The secondary methyl group appeared as a doublet at 81.4. The two CH protons of two isopropyl systems appeared as two multiplets at 81.79 and 81.9 while the CH proton attached to ester function could be seen as a multiplet at 84.4. The protons of methyl ester were located as a singlet at 83.62 and the CONH proton appeared as a broad singlet at 85.7. The CH proton attached to secondary methyl group was located at 83.55. The benzylic methylene group was located as a multiplet at 82.3 while the protons of p-substituted phenyl ring appeared as two doublets located at 87.0 and 87.14. These data are in complete accord with the structure assigned to the compound.

Synthesis of DL-serine methyl ester amide (48) of ibuprofen
It was synthesised by condensing ibuprofen acid chloride with DL-serine methyl ester HCl. On column chromatography it gave a TLC pure colourless compound, m.p. 32-34°.

The NMR spectrum of the compound showed a multiplet at 80.9, which could arise from the protons of the two methyl groups of isopropyl system. There was a
multiplet at δ1.8, which could be due to the CH proton of the isopropyl group. A multiplet at δ3.5 may be due to CH proton adjacent to secondary methyl group. There was another multiplet at δ3.9, which may arise from the CH₂ group attached to the hydroxyl group. The protons of the benzylic CH₂ group appeared as a multiplet at δ2.47. There was a doublet at δ1.5, which may arise from the protons of the secondary methyl group. A singlet at δ3.7 may be due to the protons of the methyl ester function. The CH proton adjacent to ester group appeared as a multiplet at δ4.68. The aromatic protons of the p-substituted phenyl ring appeared as a multiplet at δ7.14. These data are satisfactory for the structure assigned to the compound.

**Synthesis of L-tryptophan methyl ester amide (49) of ibuprofen**

Condensation of ibuprofen acid chloride with L-tryptophan methyl ester HCl in presence of cold aq. potassium carbonate gave TLC pure needle-shaped colourless crystals, m.p. 92-94°.

The NMR spectrum of the compound showed a multiplet arising from two methyl groups of isopropyl system located at δ0.8. The protons of secondary methyl group and methylene group attached to indole moiety appeared as a multiplet at δ1.39. There was a multiplet at δ1.8, which may arise from methine proton of isopropyl system. The methylene protons attached to the benzene ring appeared as a multiplet at δ2.37. The CH proton attached to the secondary methyl group merged with protons of methyl ester function at δ3.5. The methyl ester function appeared as a singlet at δ3.5. The methine proton attached to the ester function gave a signal at δ4.8. The proton H₂ of indole system gave a signal at δ7.25. The CONH proton was located at δ8.18 while the NH proton of indole ring was located at δ6.6. The protons of the p-substituted phenyl ring appeared as a multiplet centered at δ6.9 and the aromatic protons of indole moiety appeared as a multiplet centered at δ7.22. These data are satisfactory for the structure assigned the compound.
Synthesis of L-leucine methyl ester amide (50) of diclofenac
L-leucine methyl ester HCl in presence of triethylamine was condensed with acid chloride of diclofenac, which on column chromatographic purification gave a red coloured compound, m.p. 116-18°.

The NMR spectrum of the compound showed a multiplet at 60.86 arising from the protons of the isopropyl group of the amino acid. There was a multiplet at 61.5 arising from the CH proton of isopropyl system and neighbourly CH₂ group. The CH proton adjacent to methyl ester group appeared as a multiplet centered at 64.5. There were two singlets located at 63.66 and 63.71, the former may arise from the protons of benzylic methylene group and the latter may arise from the protons of methyl group of methyl ester function. The proton H₆' appeared as a doublet at 66.32 while the H₄' and H₅' protons appeared as doublets of triplets at 67.02 and 67.13 respectively. The proton H₃' could be seen as double doublet located at 67.32. The proton H₄ was seen as a doublet of triplet at 67.28 and the protons H₃ and H₅ appeared as a doublet at 67.43. These data are in complete accord with the structure assigned to the compound.

Synthesis of L-valine methyl ester amide (51) of diclofenac
Diclofenac acid chloride and L-valine methyl ester HCl were condensed in presence of triethylamine. The condensation product was purified by column chromatography to give a TLC pure amide derivative, m. p. 106-108°.

The NMR spectrum of the compound showed a multiplet for two methyl groups at 60.8 arising from isopropyl system. The CH proton of isopropyl group appeared as a multiplet at 62.0 while the CH proton attached to the methyl ester function could be seen as a multiplet at 64.3. The protons of the methyl ester group appeared as a singlet at 63.7.
while the CH$_2$ protons of the diclofenac system appeared as a singlet at 83.6. The aromatic protons of diclofenac moiety namely, H3' could be seen as double doublet at 86.3, the proton H4' could be seen as a multiplet at 86.8, H4 appeared as doublet of triplet located at 87.0 while proton H5' was located as a multiplet at 87.12. The proton H6' also appeared as a double doublet located at 87.24 while the two protons ortho to chlorine atoms i.e. H3 and H5 appeared as a doublet centered at 87.42. These data are satisfactory for the structure assigned to the compound.

**Synthesis of L-tryptophan methyl ester amide (52) of diclofenac**

It was synthesised by condensing diclofenac acid chloride with L-tryptophan methyl ester HCl in presence of triethylamine. The resulting product was column chromatographed to give a TLC pure yellow coloured compound, m.p. 130-32°.

![Chemical structure](image)

The NMR spectrum of the compound showed the presence of a methoxyl group by a singlet at 83.71 arising from the ester function. There was a singlet at 83.6 integrating for two protons, which could arise from benzylic CH$_2$ group of diclofenac. There was another closely packed singlet at 83.71 merging with the singlet of methyl ester function, which could be due to the CH$_2$ group of tryptophan moiety. There was a multiplet at 84.2 arising from the CH group of tryptophan moiety. The CH and NH proton of the indole system could be located as a singlet at 87.3 and 86.2 respectively. The protons of the benzene ring of tryptophan appeared as a multiplet centered at 87.27 while the proton ortho to the NH group in diclofenac moiety appeared as a double doublet at 86.32 and the remaining three protons appeared as a multiplet centered at 87.07. The three protons of dichlorobenzene ring appeared as a multiplet at 87.43. These data are satisfactory for the structure assigned to the compound.

**Synthesis of L-tryptophan methyl ester amide (53) of indomethacin**

Condensation of L-tryptophan methyl ester HCl with indomethacin acid chloride gave yellow coloured crystalline compound, m.p. 58-60°.

In the NMR spectrum of the compound, the methyl group of indomethacin was located as a singlet at 81.6. The methoxyl function and COOCH$_3$ group were located as singlets at 83.8 and 83.65 respectively. The protons of the CH$_2$ group of indomethacin moiety appeared as a singlet at 83.58. The CH$_2$ protons of the tryptophan moiety appeared
Discussion

as a multiplet at δ3.14. The CH proton attached to ester function appeared as a multiplet at δ4.8 while the proton of CONH group appeared as a broad singlet at δ7.91. The protons of p-chlorobenzoyl ring appeared as two doublets located at δ7.3 and δ7.4 forming an A₂B₂ pattern. The NH and CH protons of indole ring appeared as a broad singlet at δ6.26 and as a singlet at δ7.25. The protons HB and HA appeared as a doublet of triplet at δ6.84 and as a doublet at δ6.87 respectively. The protons HC and HD appeared as a multiplet at δ7.19 while the protons HF, HE and HG appeared as multiplets at δ6.9, δ7.0 and δ7.65 respectively. These data are in complete accord with the structure assigned to the compound.

Synthesis of L-tryptophan methyl ester amide (54) of probenecid
It was synthesised by condensing probenecid acid chloride with L-tryptophan methyl ester HCl in presence of triethylamine to give a TLC pure yellow coloured compound, m.p. 36-38°.

The NMR spectrum of the compound showed the presence of two methyl groups by a triplet centered at δ0.8. The middle CH₂ group of probenecid moiety could be seen as a hextet located at δ1.52 while the two CH₂ groups attached to the N-atom appeared as a triplet centered at δ3.05. The CH₂ group attached to the indole moiety appeared as a triplet located at δ3.41. The methyl ester function appeared as a singlet located at δ3.72.
Discussion

and CH proton adjacent to it appeared as a singlet at δ4.6. The CH group of tryptophan adjacent to methyl ester group appeared as a multiplet centered at δ5.09. The protons of p-substituted phenyl ring appeared as a singlet at δ7.73. The aromatic protons of the indole ring were located at δ7.04 (HC) as a doublet of triplet; at δ7.15 (HB) as a doublet of triplet; as a double doublet at δ7.3 (HD); as a double doublet at δ7.49 (HA) and the indole ring hydrogen and NH gave a signal at δ6.98 and δ6.3. These data are in accord with the structure assigned to the compound.
Synthesis of amide derivatives (prodrugs) of NSAIDs by condensing with 1,6-hexanediamine
These derivatives were synthesised in order to enhance the lipophilic character and study their biological activity.

Synthesis of the prodrug (55) of naproxen and 1,6-hexanediamine
Naproxen was condensed with 1,6-hexanediamine in cold in presence of phosphorus oxychloride to give a TLC pure colourless crystalline amide derivative, m.p. 56-58°.

\[
\text{Naproxen} + \text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \rightarrow \text{POCl}_3/\text{Pyridine} \rightarrow 55
\]

The symmetrical nature of the spectrum suggested that it's a diamide derivative. In the NMR spectrum there was a singlet for methoxyl group located at δ3.9. The secondary methyl groups of naproxen moiety appeared as a doublet located at δ1.5 while the CH proton appeared as a quartet located at δ3.5. In the aromatic region, the protons H5 and H7 appeared as a multiplet located at δ7.03 while the proton H3 appeared as a double doublet located at δ7.27 and the protons H1, H4 and H8 appeared together as a multiplet centered at δ7.61. In the aliphatic region, the γ protons and β protons of the n-hexane system appeared as two multiplets centered at δ1.0 and δ1.18 while the α protons i.e. the two N-CH₂ protons appeared as a multiplet at δ3.0. These data are satisfactory for the structure assigned to the compound.

Attempted synthesis of the prodrug (56) of mefenamic acid and 1,6-hexanediamine
It was synthesised by condensing mefenamic acid chloride and 1, 6-hexanediamine in presence of triethylamine to give TLC pure red coloured crystalline compound, m.p. 92-94°.

The NMR spectrum of the compound showed two singlets arising from two CH₃ groups at δ2.11 and δ2.23. There could be seen five multiplets located at δ0.8 integrating for two protons, at δ1.18 integrating for four protons, at δ1.3 integrating for two protons, at δ1.5 integrating for two protons and at δ3.35 integrating for two protons. The multiplet integrating for four protons located at δ1.18 could arise from the four protons of the two
Discussion

middle methylene groups of the hexane moiety and the last multiplet at δ3.35 could arise from -CH₂ group attached to N-atom. The rest of the three multiplets may arise from the three methylene groups of the hexane system. The aromatic protons appeared as a multiplet between δ6.5 and δ7.4. The data indicated that it is only a monoamide derivative (56a) of mefenamic acid and 1, 6-hexanediamine.

Synthesis of the prodrug (57) of ibuprofen and 1,6-hexanediamine
It was synthesised by condensing ibuprofen with 1,6-hexanediamine in presence of phosphorus oxychloride in cold conditions to give the diamide derivative, m.p. 118-20° and was characterised on the basis of ¹HNMR spectral data. It gave TLC pure colourless crystalline compound.

The NMR spectrum of the compound showed that it is a diamide derivative having ibuprofen moiety at both the ends. There was a doublet integrating for four methyl groups of the isopropyl moiety of the ibuprofen system located at δ0.89. The CH proton of the isopropyl system appeared as a multiplet at δ1.84 and the CH₂ protons attached to the benzene ring appeared as a doublet located at δ2.45. The two secondary methyl groups appeared as a doublet located at δ1.5. The CH proton attached to secondary
Discussion

The methyl group appeared as a quartet located at 83.5, while the protons of the $p$-substituted phenyl ring appeared as two doublets forming an $A_2B_2$ pattern and centered at 87.09 and 87.23. The $\alpha$ protons of the two methylene groups attached to nitrogen of the hexane system ($\alpha$ protons) appeared as a multiplet located at 83.1 while the $\gamma$ and $\beta$ protons also appeared as two multiplets at 81.1 and 81.3. These data satisfactorily explain the above diamide structure assigned to the prodrug.

Attempted synthesis of the prodrug (58) of diclofenac and 1,6-hexanediamine

Diclofenac acid chloride in presence of triethylamine was condensed with 1, 6-hexanediamine. It gave TLC pure red coloured crystalline compound of m.p. 92-93°.

\[
\begin{align*}
\text{Cl} & \quad \text{H} \quad \text{H} \\
\text{Cl} & \quad \text{H} \quad \text{H} \\
\text{Cl} & \quad \text{H} \quad \text{H} \\
\text{Cl} & \quad \text{H} \quad \text{H} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \\
1,6-\text{Hexanediame}
\end{align*}
\]

\[
\begin{align*}
\text{Pyridine} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH} \\
\text{Cl} \\
\text{Cl} \\
\text{Cl} \\
\end{align*}
\]

The NMR spectrum of the compound indicated that the reaction did not take place with 1, 6-hexanediamine to give the expected diamide but instead gave the methyl ester derivative of diclofenac.
D. Synthesis of glycolamide esters of naproxen, ibuprofen, diclofenac, mefenamic acid and indomethacin

N, N-Disubstituted 2-chloroacetamides (59) and (60) required for the synthesis of glycolamide esters of different NSAIDs were prepared by the method reported in literature.

\[
\begin{align*}
R &= CH_2CH_3 \quad (59) \\
R &= CH(CH_3)_2 \quad (60)
\end{align*}
\]

Synthesis of glycolamide ester (61) from 59 and naproxen

It was synthesised by treating naproxen with 59 to give the expected glycolamide ester derivative. It was purified by column chromatography to give a TLC pure yellow compound, m.p. 52-54°.

The NMR spectrum of the compound showed a singlet for methoxyl group at 63.79. The other protons due to naphthalene moiety of naproxen were present at 67.0 arising from H5 and H7 and at 67.33 as a multiplet arising from H3 and at 67.60 as a multiplet arising from H1, H4 and H8. The secondary methyl group of naproxen moiety appeared as a doublet at 51.52 and the hydrogen atom attached to this methyl function could be seen as a quartet at 53.91. In the aliphatic region, there could be seen the protons due to the glycolamide moiety. The protons due to the two methyl functions appeared as a multiplet at 51.03 while the protons due to two N-CH₂ groups appeared at 53.05 and 53.27 as multiplets. The protons of the -OCH₂CO- group appeared as two doublets at 54.44 and 54.69. These data are in complete accord with the structure assigned to the compound.

Synthesis of glycolamide ester (62) from 60 and naproxen

Naproxen on condensation with 60 gave the expected glycolamide derivative. Repeated attempts to crystallize from different solvents failed to give a crystalline compound. On purification by column chromatography it gave a TLC pure brown coloured semisolid compound.
The NMR spectrum of the compound showed a singlet at δ3.9 arising from the OCH$_3$ group while in the aromatic region there could be seen protons located at three positions as multiplets centered at δ7.12, δ7.43 and δ7.7 arising from H5, H7; from H3 and from H1, H4, H8 of the naphthalene ring. The protons due to the secondary methyl group merged with the protons of methyl groups of the isopropyl system and were located as a doublet at δ1.5 while the two CH protons of isopropyl system appeared as two multiplets at δ4.0 and δ4.5, however, the CH proton of the secondary methyl group merged with the protons of the OCH$_3$ group at δ3.9. The protons of the –OCH$_2$CO$^-$ group could be located as a multiplet at δ4.6. These data are satisfactory for the structure assigned to the compound.

**Synthesis of glycolamide ester (63) from 59 and ibuprofen**

Ibuprofen was treated with 59 in presence of triethylamine and sodium iodide. A semi-solid mass so obtained was treated with different solvents in an attempt to crystallize it. Finally by column chromatography a TLC pure yellow semisolid compound was obtained.

The NMR spectrum of the compound showed a doublet from the protons of the two methyl groups of isopropyl system at δ0.9. There were two triplets at δ1.12 and δ1.13, which could arise from the protons of the two methyl groups of the N-diethyl moiety. There was a doublet at δ1.5, which could be due to the protons of the secondary methyl group. There was a multiplet at δ1.83, which may be due to the CH proton of the isopropyl system. The benzylic methylene group could be seen as a doublet at δ2.4. The
methylene group of glycolamide moiety appeared as two doublets located at $\delta 4.6$ and $\delta 4.7$. The two CH$_2$ groups of the two N-diethyl groups appeared as two quartets located at $\delta 3.19$ and $\delta 3.35$. The CH proton attached to secondary methyl group was located as a quartet at $\delta 3.83$ while the protons of p-substituted phenyl ring appeared as two doublets located at $\delta 7.07$ and $\delta 7.2$. These data are satisfactory for the structure assigned to the compound.

**Synthesis of glycolamide ester (64) from 60 and ibuprofen**

Ibuprofen and 60 on condensation gave the expected glycolamide ester. Repeated attempts to crystallize from different solvents failed to give a solid. Finally it was chromatographed on a column of silica gel to give a TLC pure brown coloured semisolid compound.

The NMR spectrum of the compound showed a doublet at $\delta 0.8$ arising from the two CH$_3$ groups of isopropyl system. The CH proton of the isopropyl group of ibuprofen appeared as a multiplet at $\delta 1.7$. The benzylic CH$_2$ group appeared as a doublet located at $\delta 2.35$. The two doublets in aromatic region forming an A$_2$B$_2$ pattern at $\delta 7.0$ and $\delta 7.1$ could arise from the p-substituted phenyl ring. The secondary methyl group of the ibuprofen moiety appeared as a doublet at $\delta 1.45$ while the CH$_3$-CH proton appeared as a quartet located at $\delta 3.79$. These data accounted for the protons of the ibuprofen system. The protons of the glycolamide moiety appeared as two multiplets located at $\delta 1.1$ and $\delta 1.3$ arising from the four methyl groups of the di-isopropyl amino group. The two N-CH protons appeared as two multiplets at $\delta 3.39$ and $\delta 3.5$ while the protons of the OCH$_2$CO group appeared as two doublets located at $\delta 4.4$ and $\delta 4.6$. These data are in complete accord with the structure assigned to the glycolamide ester derivative.

**Synthesis of glycol amide ester (65) from 59 and diclofenac**

Diclofenac in presence of triethylamine and sodium iodide was condensed with 59 to give a TLC pure yellow compound, m.p. 90-92°.

The NMR spectrum of the compound showed two triplets located at $\delta 1.12$ and $\delta 1.17$ arising from the two methyl groups of the N-diethyl moiety. The two CH$_2$ groups of
ethyl molecule appeared as two quartets located at 63.2 and 63.7. The benzylic CH$_2$ group could be seen as a singlet located at 63.9 while the protons of the OCH$_2$CO group appeared as a singlet at 64.77. The aromatic protons of diclofenac molecule could be

\[
\text{Diclofenac} \xrightarrow{\text{Et$_3$N/NaI}} \quad 66
\]

analysed as below. The proton HC appeared as a double doublet located at 66.5, the NH proton could be seen as a singlet at 66.86, while HD and HE appeared as a multiplet centered at 66.96. The proton HB appeared as a doublet of triplet at 67.1 and the proton HF appeared as a double doublet at 67.25. The two protons HA and HA appeared as a doublet at 67.31. These data are satisfactory for the structure assigned to the compound.

**Synthesis of glycolamide ester (66) from 60 and diclofenac**

Diclofenac in presence of triethylamine and sodium iodide was condensed with 60 to give a TLC pure yellow crystalline compound, m.p. 118-20$^\circ$.

The NMR spectrum of the compound showed two multiplets located at 81.2 and 81.3 arising from the four methyl groups of isopropyl system. The benzylic CH$_2$ group appeared as a singlet at 83.9 while another methylene group of OCH$_2$CO function appeared as a singlet at 84.73. The two CH groups of the N-diisopropyl system appeared as two multiplets located at 83.48 and 83.7. In the aromatic region, the proton HC appeared as a double doublet located at 86.52, NH group appeared as a singlet at 86.87, the protons HD and HE appeared as a multiplet centered at 86.96 while the proton HB appeared as a doublet of triplet at 87.11. The proton HF appeared as a double doublet at 87.2 and the two HA protons appeared as a doublet at 87.32. These data are satisfactory for the structure assigned to the compound.
Discussion

Synthesis of glycolamide ester (67) from 59 and mefenamic acid

Mefenamic acid and 59 in ethyl acetate were treated with triethylamine and sodium iodide to give a brown coloured semisolid mass. Repeated attempts to crystallize from different solvents failed to give a crystalline compound. Finally by column chromatography a TLC pure brown coloured semisolid compound was obtained.

The NMR spectrum of the compound showed two triplets located at δ1.06 and δ1.14 arising from the two methyl groups of the N-diethyl moiety. The two CH₂ groups of the ethyl molecule appeared as two quartets located at δ3.18 and δ3.33. The CH₂ group attached to NC=O group appeared as a singlet at δ4.89, while the NH group was present as a broad singlet at δ9.01. There could be seen from mefenamic acid moiety, two methyl groups as singlets at δ2.08 and δ2.24. The aromatic protons HE and HG appeared as a multiplet at δ6.5, the proton HF appeared as a doublet of triplet at δ6.9 while the protons HD and HB appeared as a multiplet at δ7.04 and the proton HC could be seen as a multiplet at δ7.20. The proton HA was seen as a multiplet at δ7.98. These data are satisfactory for the structure assigned to the compound.

Synthesis of glycolamide ester (68) from 60 and mefenamic acid

A solution of mefenamic acid and 60 in ethyl acetate in presence of triethylamine was refluxed was refluxed on a water bath and the reaction mixture on processing gave a semi-solid mass. In spite of best efforts it could not be crystallised. Finally it was purified by column chromatography to give a TLC pure yellow semisolid product.
The NMR spectrum of the compound showed two multiplets located at δ1.14 and δ1.3, which could arise from the four methyl groups of the di-isopropyl moiety while the two methine groups appeared as a multiplet at δ3.6. The two methyl groups of the mefenamic acid system appeared as two singlets at δ2.1 and δ2.2. The protons of the CH₂ group appeared as a singlet at δ4.6. In the aromatic region, there could be seen multiplets located at δ6.5, δ6.9, δ7.05, δ7.1 and δ7.98 which could arise from the protons EG; F; DB; C and A respectively. The NH proton could be seen as a broad singlet at δ8.9. These data satisfactorily explained the structure assigned to the compound.

Synthesis of glycolamide ester (69) from 59 and indomethacin
Indomethacin and 59 in ethyl acetate in presence of triethylamine and sodium iodide were refluxed and the reaction mixture on processing gave a semi-solid, which was crystallised from alcohol to give a TLC pure yellow coloured compound, m.p. 112-14°C.

The NMR spectrum of the compound showed a multiplet at δ1.1, which could be due to the protons of the two methyl groups of the N-diethyl moiety. There were present two quartets at δ3.2 and δ3.4 which may arise from the two CH₂ groups of N-(CH₂CH₃)₂ system. There was present a singlet for methyl group at δ2.4 from the indomethacin molecule. There was a singlet for one OCH₃ group at δ3.83 and another singlet closely packed with it at δ3.81 arising from the CH₂ group attached to indole moiety. There was present another singlet at δ4.7, which may arise from the protons of CH₂ group of glycolamide moiety. In the aromatic region, there could be seen two doublets located at δ7.43 and δ7.63 which could arise from the p-chlorobenzoyl ring. The proton H₄ of the indole system appeared as a doublet at δ7.02 and the proton H₇ could also be located as a doublet at δ6.85. The proton H₆ appeared as a double doublet at δ6.64. These data are satisfactory for the structure assigned to the compound.

Synthesis of glycolamide ester (70) from 60 and indomethacin
Indomethacin was condensed with 60 under the same conditions as above. A semisolid mass so obtained was crystallised from ethanol to give TLC pure yellow crystalline compound, m.p. 142-44°C.
The NMR spectrum of the compound showed two multiplets located at δ1.2 and δ1.4, which may arise from four methyl groups of di-isopropyl system. There was a singlet at δ2.39 arising from methyl group of indole moiety and another singlet at δ3.87 for OCH$_3$ group. The two N-CH protons appeared as two multiplets at δ3.48 and δ3.67. There was present a very closely spaced singlet at δ3.85, which may arise from CH$_2$ group of indole moiety. Another singlet at δ4.71 may arise from the CH$_2$ group attached to NC=O group. The protons of p-substituted phenyl ring appeared as two doublets at δ7.47 and δ7.66 while the protons HA, HB and HC appeared as a meta coupled doublet at δ7.07, at δ6.6 as double doublet and at δ6.8 as an ortho coupled doublet. These data are in favour of the structure assigned to the compound.
Discussion

Biological Evaluation

Gastrointestinal (GI) toxicity is a major limiting factor in the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Because of the widespread use of these medications, the morbidity and costs associated with GI complications of NSAID use are significant. On the other hand, the costs of providing prophylactic cotherapy to all patients to prevent NSAID induced ulcers and bleeding are prohibitive. Attempts to reduce complication rates have focused on cotherapy, but it leads to higher treatment costs. It is a well known fact that the free carboxylic group present in these NSAIDs is one of the reasons for these GI complications and also this group is essential for activity.

In the present research work, prodrug approach has been used to prepare reversible derivatives of well known NSAIDs such as ibuprofen, Diclofenac sodium, naproxen, mefenamic acid and indomethacin with an aim to reduce GI toxicity associated with the free carboxylic group. This group has been temporarily masked with various promoieties, which after absorption into systemic circulation will yield the parent drug through enzymatic hydrolysis. These drugs were tested for their GI toxicity, anti-inflammatory activity and analgesic activity in animal models. The results are highly encouraging as the GI toxicity was markedly reduced and also some prodrugs showed better analgesic and anti-inflammatory activity than their parent moieties.
Gastrointestinal Toxicity Studies

Wistar rats of either sex were used for the study. Synthesised prodrugs were analysed for their ulcerogenic potential. All the test compounds under investigation produced less degree of necrosis of gastric tissue. The gastric mucosa was examined by means of a magnifying glass. The gastric mucosal damage was graded according to the following score:

<table>
<thead>
<tr>
<th>Ulcers</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ulcers</td>
<td>0.0</td>
</tr>
<tr>
<td>Less than 2 ulcers</td>
<td>1.0</td>
</tr>
<tr>
<td>2 to 5 ulcers</td>
<td>2.0</td>
</tr>
<tr>
<td>5 to 10 ulcers</td>
<td>3.0</td>
</tr>
<tr>
<td>More than 10 ulcers</td>
<td>4.0</td>
</tr>
</tbody>
</table>

The parent drugs (standards) were found to be most ulcerogenic. The prodrugs showed much less ulceration in comparison to their parent drugs.

Compound 8, a glucoside derivative of ibuprofen, gave good results. It gave an average of less than one ulcer per rat in its group. All other glucoside derivatives caused 50-60% less ulceration than their parent drugs.

Amino acid conjugate prodrugs of naproxen gave very encouraging results. In comparison to naproxen they reduced GI toxicity by 60-85%.

Amino acid conjugate prodrugs of mefenamic acid reduced ulceration associated with mefenamic acid by 80-85%.

Amino acid conjugate prodrugs of ibuprofen showed 65-85% less ulcers as compared to ibuprofen.

1, 6-Hexanediamine derivatives showed much less gastric damage than their parent drugs.

Glycolamide ester prodrugs also showed good gastrointestinal tolerance. They were able to reduce the ulceration associated with their parent compounds by 70 to 85%.

The results have been given in the following tables (1-6) and charts (1-6).

In the light of the above findings, it may be concluded that the prodrugs synthesised above are better candidates as compared to their respective parent drugs in terms of their gastrointestinal toxicity.
**TABLE 1** Gastrointestinal Toxicity of Glucoside Prodrugs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>No. of Ulcers</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>4.5</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>5.33</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>4.8</td>
<td>2.0</td>
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<tr>
<td>4</td>
<td>6</td>
<td>6.4</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>Ibuprofen</td>
<td>9.16</td>
<td>3.0</td>
</tr>
<tr>
<td>7</td>
<td>Diclofenac sodium</td>
<td>7.16</td>
<td>3.0</td>
</tr>
<tr>
<td>8</td>
<td>Biphenyl acetic acid</td>
<td>12.33</td>
<td>4.0</td>
</tr>
</tbody>
</table>

**TABLE 2** Gastrointestinal Toxicity of Amino Acid Conjugate Prodrugs of Naproxen

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>No. of Ulcers</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>2.83</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>1.83</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
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<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>1.66</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>2.83</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>2.66</td>
<td>2.0</td>
</tr>
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<td>7</td>
<td>32</td>
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<td>3.50</td>
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</tr>
<tr>
<td>9</td>
<td>Naproxen</td>
<td>9.66</td>
<td>3.0</td>
</tr>
</tbody>
</table>
**Discussion**

**Chart 2** Gastrointestinal Toxicity of Amino Acid conjugate Prodrugs of Naproxen

**TABLE 3** Gastrointestinal Toxicity of Amino Acid Conjugate Prodrugs of Mefenamic acid

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>No. of Ulcers</th>
<th>Average Score</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>2.5</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>2.5</td>
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**Chart 3** Gastrointestinal Toxicity of Amino Acid Conjugate Prodrugs of Mefenamic Acid
Discussion

TABLE 4 Gastrointestinal Toxicity of Amino Acid Conjugate Prodrugs of Ibuprofen

<table>
<thead>
<tr>
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<th>Score</th>
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Chart 4 Gastrointestinal Toxicity of Amino Acid Conjugate Prodrugs of Ibuprofen

TABLE 5 Gastrointestinal Toxicity of Amide Prodrugs with 1, 6-hexanedianeamine

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<th>Score</th>
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</thead>
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<td>Indomethacin</td>
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<td>9</td>
<td>Probenecid</td>
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<td>10</td>
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<td>Ibuprofen</td>
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TABLE 6 Gastrointestinal Toxicity of Glycolamide ester Prodrugs

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<th>No. of Ulcers</th>
<th>Score</th>
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<tr>
<td>15</td>
<td>Indomethacin</td>
<td>11.33</td>
<td>4.0</td>
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</table>

Chart 5 Gastrointestinal Toxicity of Amide Prodrugs with 1, 6-hexanediarnine

Chart 6 Gastrointestinal Toxicity of Glycolamide ester Prodrugs
Discussion

Anti-inflammatory activity

Anti-inflammatory activity was done by carrageenan induced edema method in hind paw of rats. The test compounds were administered orally 30 minutes prior to subcutaneous injection of carrageenan in hind paw of rats. Percentage inhibition in paw volume was calculated at 1, 2, and 3 hours with respect to carrageenan control. Maximum activity was observed at 3 hours.

Glucosidic prodrugs 3, 4, 6, 7 and 8 showed improved activity with respect to their parent drugs. Prodrugs 3, 6 and 7 showed almost 10% increase in activity while prodrug 8 showed 21% improvement in activity (see table 7 and chart 7).

Amino acid prodrug conjugates of naproxen inhibited edema to the same level as that of naproxen. There was no appreciable increase or decrease in activity for these derivatives (see table 8 and chart 8).

Amino acid prodrug conjugates of mefenamic acid i.e. 35, 37 and 39 showed better activity than mefenamic acid (see table 9 and chart 9).

Amino acid prodrug conjugates of ibuprofen gave better protection than ibuprofen. Compound 49 i.e. L-tryptophan methyl ester amide of ibuprofen gave 86.7% protection, an increase of 26% in activity over ibuprofen (see table 10 and chart 10).

Tryptophan prodrug conjugates of Diclofenac sodium, indomethacin and probenecid, i.e. compounds 52, 53 and 54 respectively, showed improved activity with respect to their parent drugs (see table 11 and chart 11).

Diamide derivatives (55-57) with 1, 6-hexanediamine were found to be less active than their parent compounds. They showed a decrease of 8-19% in activity with respect to their parent compounds (see table 11 and chart 11).

Glycolamide prodrugs of Naproxen i.e. compounds 61 and 62 gave 10% increase in activity while ibuprofen glycolamide prodrugs 63 and 64 showed no appreciable gain or loss in activity. Other glycolamide derivatives (65-70) of Diclofenac sodium, mefenamic acid and indomethacin also gave same protection as by their parent drugs (see table 12 and chart 12).
TABLE 7 Percentage Inhibition by Glucoside Prodrugs against Carrageenan Induced Rat Paw Edema

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>% Inhibition</th>
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<td></td>
<td></td>
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<td>3</td>
<td>17.3</td>
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<tr>
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<td>4</td>
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<td>25.6</td>
</tr>
<tr>
<td>7</td>
<td>Ibuprofen</td>
<td>28.5</td>
</tr>
<tr>
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<td>Diclofenac sodium</td>
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<td>Probenecid</td>
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</tr>
<tr>
<td>11</td>
<td>Indomethacin</td>
<td>19.3</td>
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</table>

Chart 7 Percentage Inhibition By Glucoside Prodrugs Against Carrageenan Induced Rat Paw Edema
### TABLE 8
Percentage Inhibition by Amino Acid Prodrug conjugates of Naproxen against Carrageenan Induced Rat Paw Edema

<table>
<thead>
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<th>2 hrs.</th>
<th>3 hrs.</th>
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</thead>
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<td>Naproxen</td>
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<td>48.6</td>
<td>50.1</td>
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### Chart 8
Percentage Inhibition by Amino Acid Prodrug conjugates of Naproxen Against Carrageenan Induced Rat Paw Edema
## Discussion

**TABLE 9** Percentage Inhibition by Amino Acid Prodrug conjugates of Mefenamic Acid against Carrageenan induced Rat Paw Edema

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**Chart 9** Percentage Inhibition By Amino acid Prodrug conjugates of Mefenamic acid against Carrageenan induced Rat Paw Edema

![Chart 9 Percentage Inhibition By Amino acid Prodrug conjugates of Mefenamic acid against Carrageenan induced Rat Paw Edema](chart_image)
### TABLE 10 Percentage Inhibition by Amino Acid Prodrug conjugates of Ibuprofen against Carrageenan Induced Rat Paw Edema

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<tr>
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### Chart 10 Percentage Inhibition By Amino Acid Prodrug conjugates of Ibuprofen against Carrageenan Induced Rat Paw Edema

![Chart showing percentage inhibition by amino acid prodrug conjugates of ibuprofen against carrageenan induced rat paw edema]
TABLE 11 Percentage Inhibition by Amide Prodrug derivatives with Hexanediamine against Carrageenan induced Rat Paw Edema

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</thead>
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</tr>
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</table>

Chart 11 Percentage Inhibition by Amide Prodrugs with 1, 6-hexanediamine against Carrageenan induced Rat Paw Edema
### TABLE 6 Percentage Inhibition by Glycolamide ester Prodrugs against Carrageenan Induced Rat Paw Edema

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### Chart 12 Percentage Inhibition by Glycolamide ester Prodrugs against Carrageenan Induced Rat Paw Edema

![Chart 12 Percentage Inhibition by Glycolamide ester Prodrugs against Carrageenan Induced Rat Paw Edema](chart12.png)

- 1 hr
- 2 hrs
- 3 hrs
Analgesic Activity
Analgesic activity was done by acetic acid induced writhing method in albino mice. A 1% v/v solution of acetic acid was used as writhing inducing agent. Test compounds were administered orally 3 hours prior to acetic acid injection. Numbers of writhings in control and test compounds were counted and compared. Analgesic activity was measured as percent decrease in writhings in comparison to control.

Glucoside prodrugs provided similar protection with respect to their parent compounds indicating no loss of activity. However, compound 8 gave 89.4% protection, an increase of 31% activity in comparison to ibuprofen (see table 13 and charts 13 & 14).

Amino acid conjugate prodrugs of naproxen i.e. compounds 28, 29, 30 and 33 showed improved activity while other compounds did not show any appreciable loss of activity (see table 14 and charts 15 & 16).

Amino acid conjugate prodrugs of mefenamic acid showed similar results as shown by naproxen derivatives. Compounds 34, 39, 40 and 41 showed better activity than mefenamic acid. Compound 41, a derivative of DL-serine methyl ester, in particular gave 61.1% protection in comparison to mefenamic acid which gave 51.1% protection (see table 15 and charts 17 & 18).

Amino acid conjugate prodrugs of ibuprofen showed good activity in comparison to ibuprofen. Compound 44, a derivative of L-leucine methyl ester, in particular showed 14.3% increase in activity (see table 16 and charts 19 & 20).

L-Tryptophan derivatives, i.e. compounds 52 and 53 gave better protection than their parent drugs, Diclofenac sodium and indomethacin respectively (see table 17 and charts 21 & 22).

1, 6-Hexanediamine derivatives, 55, 56 and 57 showed an appreciable loss of activity. Compounds 55 and 56 showed 10% and compound 57 showed 14% loss of activity (see table 17 and charts 21 & 22).

All glycolamide derivatives showed improved activity with respect to their parent compounds. Compounds 61 and 62 gave 8% and 10% more protection than the parent drug naproxen. Compounds 63 and 64 were found to be 6% and 8% more active than ibuprofen while mefenamic acid glycolamide derivatives, 67 and 68 gave 8% and 16% more protection than mefenamic acid (see table 18 and charts 23 & 24).
### TABLE 13 Percentage Protection by Glucoside Prodrugs in Mice against Acetic Acid Induced Writhings

<table>
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<th>Treatment</th>
<th>No. of Writhings</th>
<th>% Protection</th>
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<tr>
<td>9.</td>
<td>Biphenyl Acetic Acid</td>
<td>16</td>
<td>42.8</td>
</tr>
<tr>
<td>10.</td>
<td>Probenecid</td>
<td>19</td>
<td>32.1</td>
</tr>
<tr>
<td>11.</td>
<td>Control</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

![Graph showing the number of writhings in mice treated with glucoside prodrugs](image1)

![Graph showing the percentage protection by glucoside prodrugs in mice against acetic acid induced writhings](image2)
TABLE 14 Percentage Protection by Amino Acid conjugate Prodrugs of Naproxen in Mice against Acetic Acid Induced Writings

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>No. Of Writings (average)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>11.16</td>
<td>51.4</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>11.16</td>
<td>51.4</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>10.34</td>
<td>55.04</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>10.00</td>
<td>56.5</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>10.00</td>
<td>56.5</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>11.66</td>
<td>49.3</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>11.50</td>
<td>50.0</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>11.33</td>
<td>50.7</td>
</tr>
<tr>
<td>9</td>
<td>Naproxen</td>
<td>11.00</td>
<td>52.1</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>23.00</td>
<td></td>
</tr>
</tbody>
</table>

Chart 15 Number of Writings in Mice Treated With Amino Acid conjugate Prodrugs of Naproxen

Chart 16 Percentage Protection by Amino Acid Methyl Ester Amide Prodrugs Of Naproxen in Mice Against Acetic Acid Induced Writings
TABLE 16  Percentage Protection by Amino Acid conjugate Prodrugs of Mefenamic Acid in Mice against Acetic Acid induced Writhings

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>No. of Writhings Average</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>11.83</td>
<td>58.2</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>14.00</td>
<td>50.5</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>13.83</td>
<td>51.1</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>14.33</td>
<td>49.4</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>14.16</td>
<td>50.0</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>11.41</td>
<td>59.7</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>11.00</td>
<td>61.1</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>12.83</td>
<td>54.7</td>
</tr>
<tr>
<td>9</td>
<td>Mefenamic Acid</td>
<td>13.83</td>
<td>51.1</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>28.33</td>
<td></td>
</tr>
</tbody>
</table>

Chart 17  No. of Writhings in Mice Treated With Amino Acid conjugate Prodrugs of Mefenamic Acid

Chart 18  Percentage Protection by Amino Acid conjugate Prodrugs of Mefenamic Acid in Mice against Acetic Acid induced Writhings
### TABLE 16 Percentage Protection by Amino Acid conjugate Prodrugs of Ibuprofen in Mice against Acetic Acid Induced Writhings

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>No. of Writhings Average</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>6.67</td>
<td>72.5</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>7.33</td>
<td>69.8</td>
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<tr>
<td>3</td>
<td>46</td>
<td>12.83</td>
<td>47.2</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>10.49</td>
<td>56.8</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>10.66</td>
<td>56.1</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>8.99</td>
<td>63.0</td>
</tr>
<tr>
<td>7</td>
<td>Ibuprofen</td>
<td>10.17</td>
<td>58.2</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>24.33</td>
<td></td>
</tr>
</tbody>
</table>

**Chart 19** Number of Writhings in Mice Treated With Amino Acid conjugate Prodrugs of Ibuprofen

**Chart 22** Percentage Protection by Amino Acid conjugate Prodrugs of Ibuprofen in Mice against Acetic Acid Induced Writhings
TABLE 17 Percentage Protection by Amide Prodrugs with 1, 6-hexanediamine in Mice against Acetic Acid Induced Writhings

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Writhings Average</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>6.17</td>
<td>76.5</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>6.50</td>
<td>75.3</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>11.99</td>
<td>54.4</td>
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<td>4</td>
<td>55</td>
<td>15.99</td>
<td>39.2</td>
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<td>5</td>
<td>56</td>
<td>15.99</td>
<td>39.2</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>14.67</td>
<td>44.2</td>
</tr>
<tr>
<td>7</td>
<td>Diclofenac sodium</td>
<td>9.16</td>
<td>65.2</td>
</tr>
<tr>
<td>8</td>
<td>Indomethacin</td>
<td>13.33</td>
<td>49.3</td>
</tr>
<tr>
<td>9</td>
<td>Probenecid</td>
<td>17.83</td>
<td>32.2</td>
</tr>
<tr>
<td>10</td>
<td>Naproxen</td>
<td>12.66</td>
<td>51.8</td>
</tr>
<tr>
<td>11</td>
<td>Mefenamic acid</td>
<td>12.83</td>
<td>51.2</td>
</tr>
<tr>
<td>12</td>
<td>Ibuprofen</td>
<td>11.00</td>
<td>58.2</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>26.33</td>
<td></td>
</tr>
</tbody>
</table>

Chart 21 Number Of Writhings in Mice Treated With Amide Prodrugs with 1, 6-hexanediamine

Chart 22 Percentage Protection by Amide Prodrugs with 1, 6-hexanediamine in mice against acetic acid induced Writhings
TABLE 18 Percentage Protection by Glycolamide ester Prodrugs in Mice against Acetic Acid Induced Writings

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>No. of Writings</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>11.66</td>
<td>60.2</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
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<td>57.9</td>
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<td>3</td>
<td>Naproxen</td>
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<td>50.0</td>
</tr>
<tr>
<td>4</td>
<td>63</td>
<td>10.49</td>
<td>64.2</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>9.83</td>
<td>66.4</td>
</tr>
<tr>
<td>6</td>
<td>Ibuprofen</td>
<td>10.99</td>
<td>62.5</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>8.16</td>
<td>72.1</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>8.99</td>
<td>69.3</td>
</tr>
<tr>
<td>9</td>
<td>Diclofenac sodium</td>
<td>9.99</td>
<td>65.9</td>
</tr>
<tr>
<td>10</td>
<td>67</td>
<td>11.83</td>
<td>59.6</td>
</tr>
<tr>
<td>11</td>
<td>68</td>
<td>9.66</td>
<td>67.0</td>
</tr>
<tr>
<td>12</td>
<td>Mefenamic acid</td>
<td>14.33</td>
<td>51.1</td>
</tr>
<tr>
<td>13</td>
<td>69</td>
<td>10.16</td>
<td>65.3</td>
</tr>
<tr>
<td>14</td>
<td>70</td>
<td>10.83</td>
<td>63.0</td>
</tr>
<tr>
<td>15</td>
<td>Indomethacin</td>
<td>11.83</td>
<td>59.6</td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>29.33</td>
<td></td>
</tr>
</tbody>
</table>

Chart 23 Number Of Writings in Mice Treated With Glycolamide ester Prodrugs

Chart 24 Percentage Protection by Glycolamide ester Prodrugs in Mice against Acetic Acid Induced Writings
In vivo Hydrolysis of Compound 8

Hydrolysis studies of prodrug 8 of ibuprofen were performed in rabbits. The plasma levels of ibuprofen (standard) and prodrug were analysed by HPLC. Ibuprofen was given in a dose of 60 mg/kg body weight and the prodrug was given in a dose equivalent to 60 mg/kg body weight dose of ibuprofen. The drugs were administered by oral route. Blood samples were drawn simultaneously for the standard and prodrug and processed under identical conditions. The graphs were plotted for concentration Vs time of ibuprofen and the prodrug. Chart 1 indicates the plasma levels of ibuprofen in both the animals given ibuprofen and prodrug 8. It can be seen from the graph that $T_{\text{max}}$ is same for both the curves but $C_{\text{max}}$ for ibuprofen is more in case of rabbit II, which was given the prodrug. Also there is a parallel shift in the curve indicating larger area under curve (AUC) and thus indicating higher bioavailability for ibuprofen from prodrug.

Chart 2 indicates the levels of prodrug 8 in rabbit plasma. $C_{\text{max}}$ is seen at 2 hours and a sudden disappearance at 3 hours indicating rapid hydrolysis in plasma to ibuprofen.
Discussion

Chart 1: Plasma Levels of Ibuprofen in Rabbits Given Ibuprofen and Compound 8

Chart 2: Plasma Levels of Prodrug 8 in Rabbit II Given Prodrug 8
Experimental

- Synthesis and
- Biological Evaluations
MATERIALS AND METHODS

The reagents / chemicals / solvents used during the course of these studies were obtained from E. Merck (India), S.D Fine and CDH Laboratories and were of Laboratory grade. All the solvents were purified by distillation before use.

Silica gel G used for Thin Layer Chromatography was of E. Merck brand. Original Stahl Q applicator designed by Desaga (Germany) was used for preparing TLC plates. Iodine chamber and U.V lamps were used for visualization of TLC spots. The solvent systems used for TLC were TEF (Toluene: Ethylacetate: Formic acid: 5:4:1), BM (Benzene: Methanol::7:3), BA (Benzene:Acetone::8:2) and PTEa (Petrol: Toluene:Ethyl acetate:: 5:4:3). Whatman filter paper (No.1, England) was used for filtration (vacuum or ordinary). For column chromatography silica gel for column chromatography from E. Merck was used. Compounds were eluted by petroleum ether/benzene mixture with increasing concentration of benzene. The resulting compounds were then crystallized by conventional solvents.
A. Synthesis of ester glucosides of Ibuprofen, Diclofenac sodium, Biphenyl acetic acid, indomethacin and probenecid

Synthesis of β-D-glucopyranosyl derivatives of aryl acetic acid drugs was carried out through the following steps:

i) Synthesis of β-D-glucose penta acetate (1)

ii) Synthesis of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (2) from 1

iii) Condensation of 2 with different drugs

Synthesis of β-D-glucose penta acetate (1)

It was prepared by the reported standard procedure mentioned in discussion section. m.p. 130-32°; yield 92% (Rf 0.39, PTEA).

Synthesis of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (2) from 1

It was prepared by the reported standard procedure mentioned in discussion section. m.p. 88-89°; yield 85%.

Condensation of 2 with different NSAIDs having free carboxylic acid groups

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (3) of ibuprofen

To an ice cold solution of ibuprofen (3mmol; 0.62g) in dichloromethane (20ml) were added 2 (4mmol; 1.64g) and tetra butyl ammonium bromide (2mmol; 0.65g) with stirring. Aqueous sodium hydroxide solution (5%; 10ml) was added to the reaction mixture dropwise and stirring continued for 24 hours. After completion of the reaction, dichloromethane layer was separated, washed with water, 5% aqueous sodium bicarbonate solution and again with water, dried over anhydrous sodium sulphate and filtered to remove the inorganic salt. The solvent was removed from the filtrate under reduced pressure and a viscous residue so obtained was crystallized from methanol to give colourless needles (yield, 65%; 1.1g), m.p. 110-112° (Rf 0.50, PTEA).

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (4) of diclofenac sodium

A solution of diclofenac sodium (3mmol; 0.95g) in double distilled water (10ml) was added dropwise to an ice cold stirring mixture of 2 (4mmol; 1.64g) and tetra butyl ammonium bromide (2mmol; 0.644g) in dichloromethane while stirring. Stirring was continued for 24 hours. After completion of the reaction, dichloromethane layer was separated, washed with water, 5% aqueous sodium bicarbonate solution and again with water, dried over anhydrous sodium sulphate and filtered. The solvent was removed from the filtrate under reduced pressure to give a viscous residue which was crystallized from methanol. It gave colourless needles (yield, 69%; 1.35g) m.p. 146-148° (Rf 0.47, PTEA).
Experimental

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (5) of biphenyl acetic acid
To a solution of biphenyl acetic acid (3mmol; 0.63g) in dichloromethane (20ml) were added 2 (4mmol; 1.64g) and tetra butyl ammonium bromide (2mmol; 0.644g) with stirring. Aqueous sodium hydroxide solution (5%, 10ml) was added to the reaction mixture dropwise and stirring continued for 24 hours. The reaction mixture was processed as described above and a viscous residue so obtained was crystallized from methanol to give light green needles (yield, 73%; 1.16g) m.p. 144-146° (Rf 0.41, PTEA).

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (6) of indomethacin
A solution of indomethacin (3mmol; 1.0g) in aqueous sodium bicarbonate solution (5%; 10ml) was added dropwise to an ice cold stirring mixture of 2 (4mmol; 1.64g) and tetra butyl ammonium bromide (2mmol; 0.644g) in dichloromethane. Stirring was continued for 24 hours. The reaction mixture on processing gave a viscous residue which was crystallized from methanol to give colourless needles (yield, 80%; 1.8g) m.p. 210-12° (Rf 0.41, PTEA).

Synthesis of tetraacetyl β-D-glucopyranosyl derivative (7) of probenecid
To an ice cold solution of probenecid (3mmol; 0.85g) in dichloromethane (20ml) were added 2 (4mmol; 1.64g) and tetra butyl ammonium bromide (2mmol; 0.644g) with stirring. Aqueous sodium hydroxide solution (5%, 10ml) was added to the reaction mixture dropwise and stirring continued for 24 hours. Finally the reaction mixture was worked up as usual to give a viscous residue which was crystallized from methanol. It gave colourless needles (yield, 60%; 1.1g) m.p. 128-130° (Rf 0.39, PTEA).

Synthesis of β-D-glucopyranosyl derivative (8) of 3
In a 50ml conical flask protected with CaCl2 guard tube was placed a solution of 3 (3.0g) in dry methanol (30ml) and to it was added 5ml of 0.5% methanolic solution of sodium methoxide. The contents were kept at room temperature for one hour. Sufficient H+ ion exchange resin was then added to neutralize the solution. Acetate ions were removed by OH-ion exchange resin (0.5g). The solution was filtered and evaporated under reduced pressure. A semi-solid mass so obtained was triturated with absolute ethanol when it solidified. The resultant solid was recrystallised from absolute ethanol to give colourless a crystalline compound. m.p. 162-63° (yield, 58%; 1.2g).

Attempted synthesis of β-D-glucopyranosyl derivative (9) of 4
In a 50ml conical flask was placed a solution of 4 (3.0g) in dry methanol (30ml) and to it was added 5ml of 0.5% methanolic solution of sodium methoxide. For anhydrous conditions the mouth of the conical flask was guarded with calcium chloride tube and the contents left at room temperature for one hour. The reaction mixture was then processed.
Experimental

as mentioned above to give a semi-solid mass, which was triturated with absolute ethanol to cause solidification. The resultant solid was crystallised from absolute ethanol to give a colourless crystalline compound that showed TLC behavior and melting point identical to diclofenac and dissolved in 2% NaHCO₃ solution with effervescence suggesting the reaction did not proceed as desired.

**Attempted synthesis of β-D-glucopyranosyl derivative (10) of 6**
In a 50ml conical flask was placed a solution of 6 (3.0g) in dry methanol (30ml) and to it was added 5ml of 0.5% methanolic solution of sodium methoxide. The mouth of the conical flask was closed with calcium chloride guard tube and the reaction mixture kept at room temperature for one hour. The rest of the procedure was the same as described above when a colourless compound identical with indomethacin ($R_f$ 0.35, m.p. 160-62°, mixed m.p. 162-64°) was obtained indicating unsuccessful reaction.

**Synthesis of β-D-glucopyranosyl derivative (11) of 7**
In a 50ml conical flask was placed a solution of 7 (3.0g) in dry methanol (30ml) and to it was added 5ml of 0.5% methanolic solution of sodium methoxide. By following the same procedure described above a solid mass was obtained which was crystallised from absolute ethanol to give a colourless crystalline compound m.p. 140-42° (yield, 27%; 0.6g).

**Synthesis of Prodrugs of NSAIDs by condensing their free carboxylic acid group with different L-amino acids**
The synthesis was carried out through the following steps:

i) Synthesis of methyl ester hydrochlorides of amino acids (12-19)

ii) Synthesis of acid chlorides (20-25) of NSAIDs and

i) Synthesis of amide derivatives (26-54) by condensing the above methyl ester derivatives and acid chlorides.

**Synthesis of methyl ester hydrochlorides (12), (13), (14), (15), (16), (17), (18) and (19) of L-alanine, L-phenylalanine, L-leucine, L-isoleucine, L-valine, L-tyrosine, DL-serine and L-tryptophan**
These were synthesised by following the standard procedure of Ronald et al. The physical data of the above amino acid methyl ester hydrochlorides are summarised in the following table.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Amino acid methyl ester.HCl</th>
<th>m.p.</th>
<th>Yield (%/g)</th>
<th>Rf value/BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-alanine methyl ester.HCl</td>
<td>208-10°</td>
<td>83/1.15</td>
<td>0.74</td>
</tr>
<tr>
<td>2</td>
<td>L-phenylalanine methyl ester.HCl</td>
<td>162-64°</td>
<td>89/1.91</td>
<td>0.69</td>
</tr>
</tbody>
</table>
Experimental

<table>
<thead>
<tr>
<th></th>
<th>Compound</th>
<th>Melting Point</th>
<th>Density</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>L-leucine methyl ester.HCl</td>
<td>144-46°</td>
<td>93/1.7</td>
<td>0.79</td>
</tr>
<tr>
<td>4</td>
<td>L-isoleucine methyl ester.HCl</td>
<td>110-12°</td>
<td>89/1.6</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>L-valine methyl ester.HCl</td>
<td>164-66°</td>
<td>81/1.35</td>
<td>0.62</td>
</tr>
<tr>
<td>6</td>
<td>L-tyrosine methyl ester.HCl</td>
<td>190-92°</td>
<td>83/1.9</td>
<td>0.74</td>
</tr>
<tr>
<td>7</td>
<td>DL-serine methyl ester.HCl</td>
<td>218-20°</td>
<td>85/1.27</td>
<td>0.66</td>
</tr>
<tr>
<td>8</td>
<td>L-tryptophan methyl ester.HCl</td>
<td>220-22°</td>
<td>88/2.3g</td>
<td>0.57</td>
</tr>
</tbody>
</table>

ii Synthesis of acid chlorides (20-25)

Synthesis of naproxen acid chloride (20) from naproxen
Thionyl chloride (5.5mmol; 0.4ml) was added to the solution of naproxen (5mmol; 1.15g) in dry chloroform (25ml) and refluxed on a water-bath for 4 hours. Excess of thionyl chloride was removed by distilling under reduced pressure. A viscous residue so obtained was TLC pure and showed different TLC behavior in comparison to the starting material and was used as such for further studies.

Synthesis of mefenamic acid chloride (21) from mefenamic acid
Thionyl chloride (5.5mmol; 0.4ml) was added to the solution of mefenamic acid (5mmol; 1.20g) in dry chloroform (25ml) and refluxed on a water-bath for 4 hours. It was prepared as above to give a viscous residue which was TLC pure and showed different TLC behavior in comparison with the starting material and was used as such for further reaction.

Synthesis of ibuprofen acid chloride (22) from ibuprofen
Thionyl chloride (5.5mmol; 0.4ml) was added to the solution of ibuprofen (5mmol; 1.03g) in dry chloroform (25ml) and refluxed on a water-bath for 4 hours. On further processing as above a viscous residue was obtained which was TLC pure and showed different TLC behavior in comparison to the starting material and was used as such for further studies.

Synthesis of diclofenac acid chloride (23) from diclofenac
Thionyl chloride (5.5mmol; 0.4ml) was added to the solution of diclofenac (5mmol; 1.48g) in dry chloroform (25ml) and refluxed on a water-bath for 4 hours. On usual workup a viscous mass was obtained which was TLC pure and showed different TLC behavior in comparison with the starting material and was used as such for further reaction.

Synthesis of indomethacin acid chloride (24) from indomethacin
Thionyl chloride (5.5mmol; 0.4ml) was added to the solution of indomethacin (5mmol; 1.8g) in dry chloroform (25ml) and refluxed on a water-bath for 4 hours. Excess of
Experimental

Thionyl chloride was removed by distilling under reduced pressure. A viscous residue so obtained was TLC pure and showed different TLC behavior in comparison with the starting material and was used as such for further studies.

Synthesis of probenecid acid chloride (25) from probenecid
Thionyl chloride (5.5mmol; 0.4ml) was added to the solution of probenecid (5mmol; 1.42g) in dry chloroform (25ml) and refluxed on a water-bath for 4 hours. Usual workup of the reaction mixture gave a TLC pure viscous residue. It showed different TLC behavior in comparison with the starting material and was used as such for further studies.

iii Synthesis of amide derivatives (26-54) by condensing methyl esters and acid chlorides

Synthesis of L-alanine methyl ester amide (26) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-alanine methyl ester hydrochloride (5.25mmol; 0.73g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. A semi-solid mass, which separated out, was filtered, washed with cold solution of aqueous sodium hydroxide (0.5%; 25ml) and again with water, dried and purified on a column of silica gel. Crystallization from methanol gave TLC pure colourless needles (yield, 70%; 1.1g), m.p. 86-88° (Rf 0.72 BA).

Synthesis of L-phenylalanine methyl ester amide (27) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-phenylalanine methyl ester hydrochloride (5.25mmol; 1.13g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. A semi-solid mass, which separated out, was filtered, washed with cold solution of aqueous sodium hydroxide (0.5%; 25ml) and again with water, dried and purified by column chromatography. Crystallization from methanol gave TLC pure colourless needles (yield, 64%; 1.25g), m.p. 92-94° (Rf 0.70 BA).

Synthesis of L-leucine methyl ester amide (28) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-leucine methyl ester hydrochloride (5.25mmol; 0.95g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. On usual processing as given above followed by purification on a column of silica gel and crystallization from methanol it gave TLC pure needles (yield, 79%; 1.4g), m.p. 106-108° (Rf 0.82 BA) were obtained.
Experimental

Synthesis of L-isoleucine methyl ester amide (29) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-isoleucine methyl ester hydrochloride (5.25mmol; 0.95g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Usual processing followed by column chromatography on a column of silica gel and crystallization from methanol gave TLC pure needle-shaped colourless crystals (yield, 82%; 1.45g), m.p. 108-110° (Rf 0.84 BA).

Synthesis of L-tyrosine methyl ester amide (30) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-tyrosine methyl ester hydrochloride (5.25mmol; 1.2g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. On usual workup and column chromatography followed by crystallization from methanol TLC pure needle-shaped colourless crystals (yield, 79%; 1.6g), m.p. 128-30° (Rf 0.53 BA) were obtained.

Synthesis of L-valine methyl ester amide (31) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-valine methyl ester hydrochloride (5.25mmol; 0.86g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Usual processing of the reaction mixture and column chromatography followed by crystallization from methanol gave TLC pure colourless needles (70.5%; 1.2g), m.p. 116-18° (Rf 0.82 BA).

Synthesis of DL-serine methyl ester amide (32) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added DL-serine methyl ester hydrochloride (5.25mmol; 0.8g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Usual processing followed by column chromatography on a column of silica gel and crystallization from methanol gave TLC pure needle-shaped colourless crystals (yield, 66%; 1.1g), m.p. 132-34° (Rf 0.642 BA).

Synthesis of L-tryptophan methyl ester amide (33) of naproxen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-tryptophan methyl ester hydrochloride (5.25mmol; 1.07g) and stirred for 30 minutes. Naproxen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Usual processing of the reaction mixture and column chromatography followed by crystallization from methanol gave TLC pure needle-shaped colourless crystals (yield, 69%; 1.47g), m.p. 160-62° (Rf 0.58 BA).
Experimental

Synthesis of L-alanine methyl ester amide (34) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) were added L-alanine methyl ester hydrochloride (5.25mmol; 0.73g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The reaction mixture was filtered to remove precipitated triethylamine hydrochloride. The organic solvent was washed with 2% hydrochloric acid, 5% aq. sodium bicarbonate solution and water, dried over anhydrous sodium sulphate and concentrated in vacuum. A semi-solid mass so obtained was purified on a column of silica gel followed by crystallization from petroleum ether gave TLC pure brown coloured compound (yield, 52.4%; 0.85g), m.p. 32-34° (Rf 0.71 BA).

Synthesis of L-phenylalanine methyl ester amide (35) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) were added L-phenylalanine methyl ester hydrochloride (5.25mmol; 1.12g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The reaction mixture was filtered to remove precipitated triethylamine hydrochloride. The organic solvent was washed with 2% hydrochloric acid, 5% aq. sodium bicarbonate and water, dried over anhydrous sodium sulphate and concentrated in vacuum. A semi-solid mass so obtained was chromatographed on a column of silica gel and crystallised from petroleum ether to give TLC pure brown coloured compound (yield, 65%; 1.3g), m.p. 32-34° (Rf 0.80 BA).

Synthesis of L-leucine methyl ester amide (36) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) was added L-leucine methyl ester hydrochloride (5.25mmol; 0.95g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The reaction mixture was filtered to remove precipitated triethylamine hydrochloride. Usual workup and purification by column chromatography followed by crystallization from petroleum ether gave a TLC pure brown coloured compound (yield, 52%; 0.95g), m.p. 42-44° (Rf 0.73 BA).

Synthesis of L-isoleucine methyl ester amide (37) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) were added L-isoleucine methyl ester hydrochloride (5.25mmol; 1.07g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The reaction mixture was filtered to remove precipitated triethylamine hydrochloride. Usual workup of the reaction gave a semi-solid mass which was purified by column chromatography and crystallised from petroleum ether to give a TLC pure brown coloured compound (yield, 63%; 1.15g), m.p. 46-48° (Rf 0.89 BA).
Experimental

Synthesis of L-valine methyl ester amide (38) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) were added L-valine methyl ester hydrochloride (5.25mmol; 0.88g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The reaction mixture was filtered to remove precipitated triethylamine hydrochloride. On usual processing of the contents a semi-solid mass was obtained which was column chromatographed and crystallised from petroleum ether to give a TLC pure brown coloured compound (yield, 51%; 0.9g), m.p. 36-38° (Rf 0.73 BA).

Synthesis of L-tyrosine methyl ester amide (39) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) was added L-tyrosine methyl ester hydrochloride (5.25mmol; 1.2g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The reaction mixture was filtered to remove precipitated triethylamine hydrochloride. The contents were then processed as usual to give a semi-solid mass which was purified by column chromatography and crystallised from petroleum ether to give a TLC pure yellow coloured compound (yield, 41%; 0.85g), m.p. 34-36° (Rf 0.57 BA).

Synthesis of DL-serine methyl ester amide (40) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) was added DL-serine methyl ester hydrochloride (5.25mmol; 0.81g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The reaction mixture was filtered to remove precipitated triethylamine hydrochloride. The contents on usual workup gave a semi-solid mass which was purified by column chromatography and crystallisation from petroleum ether to give a brown coloured compound (yield, 45%; 0.8g), m.p. 46-48° (Rf 0.46 BA).

Synthesis of L-tryptophan methyl ester amide (41) of mefenamic acid
To a solution of mefenamic acid chloride in dry chloroform (25ml) was added L-tryptophan methyl ester hydrochloride (5.25mmol; 1.32g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The processing of the reaction mixture gave a semi-solid mass which was column chromatographed and crystallised from petroleum ether to give a TLC pure brown coloured compound (yield, 54%; 1.2g), m.p. 52-54° (Rf 0.67 BA).

Attempted synthesis of L-alanine methyl ester amide (42) of ibuprofen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-alanine methyl ester hydrochloride (5.25mmol; 0.73g) and stirred for 30 minutes. Ibuprofen acid chloride was then added to the above reaction mixture and stirred
vigorously on a magnetic stirrer for 2 hours. On processing the contents in the usual way followed by crystallization ibuprofen was obtained back indicating unsuccessful results.

**Attempted synthesis of L-phenylalanine methyl ester amide (43) of ibuprofen**

To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-phenylalanine methyl ester hydrochloride (5.25mmol; 1.13g) and stirred for 30 minutes. Ibuprofen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Usual processing of the reaction mixture gave back ibuprofen indicating that the reaction did not take place as desired.

**Synthesis of L-leucine methyl ester amide (44) of ibuprofen**

To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-leucine methyl ester hydrochloride (5.25mmol; 0.95g) and stirred for 30 minutes. Ibuprofen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Usual workup of the contents followed by column chromatography and crystallization from methanol gave a TLC pure colourless crystalline compound (yield, 54%; 0.9g), m.p. 42-44° (Rf 0.65 BA).

**Synthesis of L-isoleucine methyl ester amide (45) of ibuprofen**

To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-isoleucine methyl ester hydrochloride (5.25mmol; 0.95g) and stirred for 30 minutes. Ibuprofen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Finally on usual processing followed by column chromatography and crystallized from methanol gave TLC pure colourless crystals (yield, 45%; 0.75g), m.p. 48-50° (Rf 0.84 BA).

**Synthesis of L-tyrosine methyl ester amide (46) of ibuprofen**

To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-tyrosine methyl ester hydrochloride (5.25mmol; 1.2g) and stirred for 30 minutes. Ibuprofen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. Further processing of the reaction mixture as usual followed by column chromatography and crystallization from methanol gave TLC pure needles (yield, 58%; 1.1g), m.p. 40-42° (Rf 0.61 BA).

**Synthesis of L-valine methyl ester amide (47) of ibuprofen**

To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-valine methyl ester hydrochloride (5.25mmol; 0.86g) and stirred for 30 minutes. Ibuprofen acid chloride was then added to the above reaction mixture and stirred vigorously on a magnetic stirrer for 2 hours. On further processing the contents in the usual way followed
Experimental

by column chromatography and crystallization from methanol gave a TLC pure
colourless compound (yield, 53%; 0.85g), m.p. 42-44° (Rf 0.70 BA).

Synthesis of DL-serine methyl ester amide (48) of ibuprofen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added DL-
serine methyl ester hydrochloride (5.25mmol; 0.8g) and stirred for 30 minutes. Ibuprofen
acid chloride was then added to the above reaction mixture and stirred vigorously on a
magnetic stirrer for 2 hours. On usual processing and crystallization from methanol a
TLC pure colourless compound (yield, 56%; 0.85g), m.p. 32-34° (Rf 0.82 BA) was
obtained.

Synthesis of L-tryptophan methyl ester amide (49) of ibuprofen
To an ice-cold aqueous solution of potassium carbonate (10%; 25ml) was added L-
tryptophan methyl ester hydrochloride (5.25mmol; 1.32g) and stirred for 30 minutes.
Ibuprofen acid chloride was then added to the above reaction mixture and stirred
vigorously on a magnetic stirrer for 2 hours. Usual workup of the contents, column
chromatography and crystallization from methanol gave TLC pure needle-shaped
colourless crystals (yield, 80%; 1.6g). m.p. 92-94° (Rf 0.65 BA).

Synthesis of L-leucine methyl ester amide (50) of diclofenac
To a solution of diclofenac acid chloride in dry chloroform (25ml) were added L-leucine
methyl ester hydrochloride (5.25mmol; 0.95g) and triethylamine (5.5mmol; 0.76ml) and
the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The
reaction mixture was filtered to remove precipitated triethylamine hydrochloride. Usual
workup of the filtrate gave a semi-solid mass which on column chromatographic
purification and crystallization from petroleum ether gave a red coloured crystalline
compound (yield, 43%; 1.2g). m.p. 116-18° (Rf 0.81 BA).

Synthesis of L-valine methyl ester amide (51) of diclofenac
To a solution of diclofenac acid chloride in dry chloroform (25ml) were added L-valine
methyl ester hydrochloride (5.25mmol; 0.86g) and triethylamine (5.5mmol; 0.76ml) and
the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. The
reaction mixture was filtered to remove precipitated triethylamine hydrochloride. Usual
workup of the filtrate followed by column chromatography and crystallization from
petroleum ether gave a pink coloured crystalline compound (yield, 52%; 1.3g). m.p. 106-
08° (Rf 0.83 BA).

Synthesis of L-tryptophan methyl ester amide (52) of diclofenac
To a solution of diclofenac acid chloride in dry chloroform (25ml) were added L-
tryptophan methyl ester hydrochloride (5.25mmol; 1.32g) and triethylamine (5.5mmol;
Experimental

0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. Processing of the contents as described in parallel cases followed by column chromatography and crystallization from petroleum ether gave a yellow coloured crystalline compound (yield, 48%; 1.2g), m.p. 130-32° (Rf 0.78 BA).

Synthesis of L-tryptophan methyl ester amide (53) of indomethacin
To a solution of indomethacin acid chloride in dry chloroform (25ml) was added L-tryptophan methyl ester hydrochloride (5.25mmol; 1.07g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. A parallel workup of the contents as described in similar cases gave a semi-solid mass which was purified by column chromatography and crystallization from methanol to give a yellow coloured crystalline compound (yield, 55%; 1.6g), m.p. 58-60° (Rf 0.63).

Synthesis of L-tryptophan methyl ester amide (54) of probenecid
To a solution of 25 in dry chloroform (25ml) was added 19 (5.25mmol; 1.07g) and triethylamine (5.5mmol; 0.76ml) and the reaction mixture shaken on a wrist action mechanical shaker for 12 hours. It was worked up as usual to give a semi-solid mass which was column chromatographed and crystallized from methanol to give a yellow coloured crystalline compound (yield, 75%; 1.8g), m.p. 36-38° (Rf 0.68 BA).

Synthesis of amide derivatives (prodrugs) of NSAIDs by condensing with 1,6-hexanediamine

Synthesis of the prodrug (55) of naproxen and 1,6-hexanediamine
To an ice-cold solution of naproxen (10mmol; 2.31g) and 1,6-hexanediamine (5mmol; 0.58g) in dry pyridine was added phosphorus oxychloride dropwise with stirring and stirring was continued for another 3 hours. The reaction temperature was maintained below 5° by keeping the reaction flask in an ice-bath. After completion of the reaction, the contents were poured onto crushed ice. A semi-solid mass so obtained was washed well with water, 2% sodium bicarbonate solution and again with water, dried and crystallized from methanol. It gave TLC pure brown coloured crystalline compound (yield, 76%; 2.1g) m.p.56-58° (Rf 0.45 BA).

Attempted synthesis of the prodrug (56) of mefenamic and 1,6-hexanediamine
To an ice-cold solution of mefenamic acid chloride and 1,6-hexanediamine (5mmol; 0.58g) in dry chloroform was added triethylamine (11mmol; 1.57ml) stirred for 3 hours. The temperature was maintained below 5° in an ice-bath. After completion of the reaction, the contents were filtered to remove precipitated triethylamine hydrochloride. Chloroform was removed from the filtrate under reduced pressure and the residue was
Experimental

washed with 2% HCl, 2% sodium bicarbonate solution followed by washing with water. It was then dried and crystallised from methanol to give a TLC pure pink coloured crystalline compound (yield, 58%; 1.65g), m.p.92-94° (Rf0.66). The $^1$HNMR spectrum of the compound showed it to be a monoamide derivative.

Synthesis of the prodrug (57) of ibuprofen and 1,6-hexanediamine

To an ice-cold solution of ibuprofen (10mmol; 2.06g) and 1,6-hexanediamine (5mmol; 0.58g) in dry pyridine was added phosphorus oxychloride dropwise while stirring. Stirring was continued for another 3 hours while maintaining the temperature below 5° in an ice-bath. After completion of the reaction, the contents were poured onto crushed ice. A semi-solid mass so obtained was washed well with water, 2% sodium bicarbonate solution and again with water. It was dried and crystallised from methanol to give a TLC pure colourless crystalline compound (yield, 85%; 2.1g) m.p. 118-120° (Rf0.50 BA).

Attempted synthesis of the prodrug (58) of diclofenac and 1,6-hexanediamine

To a stirring ice-cold solution of diclofenac acid chloride and 1,6-hexanediamine (5mmol; 0.58g) in dry chloroform was added triethylamine (11mmol; 1.57ml) and stirring was continued for 3 hours. Temperature was maintained constantly below 5° in an ice-bath. After completion of the reaction, the contents were filtered to remove precipitated triethylamine hydrochloride. Chloroform was removed from the filtrate under reduced pressure and a residue so obtained was washed with 2% HCl, 2% sodium bicarbonate solution and with water. It was dried and crystallised from methanol to give a TLC pure red coloured crystalline compound which showed a similar TLC pattern and melting point identical with diclofenac. This indicated that the reaction did not proceed as desired.

D. Synthesis of glycolamide esters of naproxen, ibuprofen, diclofenac, mefenamic acid and indomethacin

N, N-Di-substituted-2-chloroacetamides (59) and (60) required for the synthesis of glycolamide esters of different NSAIDs were prepared by the reported method mentioned in the discussion section.

Synthesis of glycolamide ester (61) from 59 and naproxen

To a solution of naproxen (0.01 mol; 2.31g) in ethyl acetate (40ml) were added triethylamine (0.011mol; 1.53ml), sodium iodide (0.001 mol; 0.15g) and N, N-diethyl-2-chloroacetamide (0.01mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% sodium thiosulphate solution, 2% sodium bicarbonate and water, dried and ethyl acetate removed under reduced pressure. A semi-solid mass so obtained was purified by column chromatography and crystallised
Experimental

from alcohol to give a TLC pure yellow coloured compound (yield, 82%; 2.8g), m.p. 52-54° (Rf/0.67 BA).

Synthesis of glycolamide ester (62) from 60 and naproxen
To a solution of naproxen (0.01 mol; 2.31g) in ethyl acetate (40ml) were added triethylamine (0.011mol; 1.53ml), sodium iodide (0.001mol; 0.15g) and N, N-di-isopropyl-2-chloroacetamide (0.01mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% sodium thiosulphate solution, 2% sodium bicarbonate and water, dried and ethyl acetate removed under reduced pressure. A semi-solid mass so obtained was column chromatographed which afforded a TLC pure semi-solid compound. Repeated attempts to crystallize from different solvents failed to give a solid but a brown coloured semi-solid compound was obtained (yield, 67%; 2.5g) (Rf/0.69 BA).

Synthesis of glycolamide ester (63) from 59 and ibuprofen
To a solution of ibuprofen (0.01mol; 2.06g) in ethyl acetate (40ml) were added triethylamine (0.011mol; 1.53ml), sodium iodide (0.001mol; 0.15g) and N, N-diethyl-2-chloroacetamide (0.01mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was worked up as above. A semi-solid mass so obtained was purified by column chromatography to give a TLC pure yellow coloured semi-solid compound. Attempts to crystallize from different solvents failed to give a solid compound. (yield, 81%; 2.6g) (Rf/0.73 BA).

Synthesis of glycolamide ester (64) from 60 and ibuprofen
To a solution of ibuprofen (0.01mol; 2.06g) in ethyl acetate (40ml) were added triethylamine (0.011mol; 1.53ml), sodium iodide (0.001mol; 0.15g) and N, N-di-isopropyl-2-chloroacetamide (0.01mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate on usual workup and column chromatography gave a TLC pure semi-solid compound which could not be crystallized in spite of best efforts. (yield, 80%; 2.8g) (Rf/0.79 BA).

Synthesis of glycolamide ester (65) from 59 and diclofenac
To a solution of diclofenac (0.01mol; 2.96g) in ethyl acetate (40ml) were added triethylamine (0.011mol; 1.53ml), sodium iodide (0.001mol; 0.15g) and N, N-diethyl-2-chloroacetamide (0.01mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% sodium thiosulphate solution, 2% sodium bicarbonate and water, dried and ethyl acetate removed under reduced pressure. A semi-solid so obtained was crystallised from ethanol to give a TLC pure yellow coloured compound (yield, 85.7%; 3.5g). m.p. 90-92° (Rf/0.75).
Experimental

Synthesis of glycolamide ester (66) from 60 and diclofenac

To a solution of diclofenac (0.01 mol) in ethyl acetate (40 ml) were added triethylamine (0.011 ml), sodium iodide (0.001 mol; 0.15 g) and N, N-di-isopropyl-2-chloroacetamide (0.01 mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% solution of sodium thiosulphate, 2% sodium bicarbonate and water, dried and ethyl acetate removed under reduced pressure. A semi-solid so obtained was crystallised from ethanol to give a TLC pure yellow coloured crystalline compound (yield, 87%; 3.8 g), m.p. 118-20° (Rf 0.78).

Synthesis of glycolamide ester (67) from 69 and mfenamic acid

To a solution of mfenamic acid (0.011 mol; 2.41 g) in ethyl acetate (40 ml) were added triethylamine (0.011 mol; 1.53 ml), sodium iodide (0.001 mol; 0.15 g) and N, N-diethyl-2-chloroacetamide (0.01 mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% sodium thiosulphate solution, 2% sodium bicarbonate and water. It was dried and ethyl acetate was removed under reduced pressure. Repeated attempts to crystallize from different solvents failed to give a solid mass but finally by column chromatography a TLC pure brown coloured semi-solid compound (yield, 65%; 2.3 g), (Rf 0.76 BA) was obtained.

Synthesis of glycolamide ester (68) from 60 and mfenamic acid

To a solution of mfenamic acid (0.01 mol; 2.41 g) in ethyl acetate (40 ml) were added triethylamine (0.011 mol; 1.53 ml), sodium iodide (0.001 mol; 0.15 g) and N, N-di-isopropyl-2-chloroacetamide (0.01 mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% solution of sodium thiosulphate, 2% sodium bicarbonate and water. It was dried and ethyl acetate was removed under reduced pressure. A semi-solid so obtained was treated with different solvents in an attempt to crystallize but finally by column chromatography a TLC pure yellow coloured semi-solid compound (yield, 76%; 2.9 g), (Rf 0.75 BA) was obtained.

Synthesis of glycolamide ester (69) from 59 and indomethacin

To a solution of indomethacin (0.01 mol; 3.58 g) in ethyl acetate (40 ml) were added triethylamine (0.011 mol), sodium iodide (0.001 mol; 0.15 g) and N, N-diethyl-2-chloroacetamide (0.01 mol). The reaction mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% sodium thiosulphate solution, 2% sodium bicarbonate and water. It was dried and ethyl acetate was removed under reduced pressure. A semi-solid mass so obtained was crystallised from ethanol to give a TLC pure yellow coloured compound (yield 81%; 3.8 g), m.p. 112-114° (Rf 0.63 BA).

Synthesis of glycolamide ester (70) from 60 and indomethacin

To a solution of indomethacin (0.01 mol; 3.58 g) in ethyl acetate (40 ml) were added triethylamine (0.011 mol), sodium iodide (0.001 mol; 0.15 g) and N, N-di-isopropyl-2-
Experimental chloroacetamide (0.01mol). The mixture was refluxed for three hours on a water-bath, cooled and filtered. The filtrate was washed with 2% sodium thiosulphate solution, 2% sodium bicarbonate and water. It was dried and ethyl acetate was removed under reduced pressure. A semi-solid mass so obtained was crystallised from ethanol to give TLC pure yellow coloured compound (yield 84%; 4.2g), m.p. 142-144° ($R_f$ 0.75).
BIOLOGICAL EVALUATIONS

A. Gastrointestinal Toxicity

REQUIREMENTS

Animals: The gastrointestinal toxicity (GI) was determined by the method of Wilhemi et al. These studies were carried out on healthy male Wistar rats weighing between 150-200gms. Animals were divided in groups with six animals in each group. They were fed on standard pellet diet (Hindustan Lever Ltd.).

Standard drugs: All drug suspensions were prepared in 0.5% carboxymethyl cellulose (CMC).

a) Ibuprofen: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of ibuprofen was prepared and administered orally 0.5ml/100g body weight of the animal.

b) Diclofenac sodium: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of diclofenac sodium was prepared and administered orally 0.5ml/100g body weight of the animal.

c) Biphenyl acetic acid: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of biphenyl acetic acid was prepared and administered orally 0.5ml/100g body weight of the animal.

d) Probenecid: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of probenecid was prepared and administered orally 0.5ml/100g body weight of the animal.

e) Naproxen: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of naproxen was prepared and administered orally 0.5ml/100g body weight of the animal.

f) Mefenamic acid: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of mefenamic acid was prepared and administered orally 0.5ml/100g body weight of the animal.

g) Indomethacin: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of indomethacin was prepared and administered orally 0.5ml/100g body weight of the animal.

Test Compounds: All suspensions of test compounds were prepared in 0.5% carboxymethyl cellulose (CMC) as a suspending agent. The doses of all the prodrugs were equivalent to the dose of 20mg/kg bodyweight of their respective parent drugs. All the test compounds were given by oral route.
Experimental Procedure: The animals were divided in groups with six animals in each group. Control group was given only 0.5% CMC suspension. Test compounds and standard drugs were administered orally once in a day for 10 days. The animals were fasted for 8 hrs prior to dosing and for 4 hrs post dosing. Food was available at all other times, free access to water was provided throughout the experiment. Four hours after the last dose, the animals were sacrificed using chloroform. The abdomen was opened at the midline and the stomach and the first 3 cm of the duodenum were extracted. The stomach was opened along the larger curvature and washed with distilled water. The mucus was wiped off and the numbers of ulcers were examined by means of a magnifying glass. All ulcers were counted and recorded as average number of ulcers per animal and assessed as score given below. The results are given in the discussion section.

<table>
<thead>
<tr>
<th>Ulcers</th>
<th>Score</th>
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<tr>
<td>No ulcers</td>
<td>0.0</td>
</tr>
<tr>
<td>Less than 2 ulcers</td>
<td>1.0</td>
</tr>
<tr>
<td>2 to 5 ulcers</td>
<td>2.0</td>
</tr>
<tr>
<td>5 to 10 ulcers</td>
<td>3.0</td>
</tr>
<tr>
<td>More than 10 ulcers</td>
<td>4.0</td>
</tr>
</tbody>
</table>
B. Anti Inflammatory Activity

Inflammation is a part of the host defense mechanism. It is a tissue reaction to infection, irritation or foreign substance. There are several tissue factors or mechanisms that are known to involve in the inflammatory reactions such as release of histamine, bradykinin and prostaglandins. The development of anti-inflammatory agents in recent years have contributed a lot in not only overcoming the human sufferings such as arthritis but also have helped in understanding the tissue mechanisms of inflammation.

PRINCIPLE

The inflammatory reaction is readily produced in rats in the form of paw edema with the help of irritants/inflammagens. Substances such as carrageenan, formalin, histamine, bradykinin, 5- HT, mustard and egg white when injected in the dorsum of the foot in rats produce acute edema within a few minutes of the injection. Carrageenan-induced paw edema is the most commonly used in experimental pharmacology. Carrageenan is a sulphated polysaccharide obtained from the seaweed *Chondrus crispus* and by causing the release of histamine, 5HT, bradykinin and prostaglandins, it produces inflammation and edema.

REQUIREMENTS

Anti-inflammatory activity was carried out by the method of Winter *et al*.

**Animals:** The studies were carried out on healthy wistar rats of either sex weighing between 150-200gms. Each group comprised of five animals and was housed in polypropylene cages. They were fed on standard pellet diet (Hindustan lever Ltd.) and fasted for 12 hours before starting the experiment.

**Standard drugs:** All drug suspensions were prepared in 0.5% carboxymethyl cellulose (CMC).

- **Carrageenan:** A 1% w/v suspension was prepared using CMC (carboxymethyl cellulose) as a suspending agent.
- **Ibuprofen:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of ibuprofen was prepared and administered orally 0.5ml/100g body weight of the animal.
- **Diclofenac sodium:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of diclofenac sodium was prepared and administered orally 0.5ml/100g body weight of the animal.
- **Biphenyl acetic acid:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of biphenyl acetic acid was prepared and administered orally 0.5ml/100g body weight of the animal.
Experimental

1) **Probenecid**: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of probenecid was prepared and administered orally 0.5ml/100g body weight of the animal.

m) **Naproxen**: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of naproxen was prepared and administered orally 0.5ml/100g body weight of the animal.

n) **Mefenamic acid**: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of mefenamic acid was prepared and administered orally 0.5ml/100g body weight of the animal.

o) **Indomethacin**: The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of indomethacin was prepared and administered orally 0.5ml/100g body weight of the animal.

Test Compounds: All suspensions of test compounds were prepared in 0.5% carboxymethyl cellulose (CMC) as a suspending agent. The doses of all the prodrugs were equivalent to the dose of 20mg/kg bodyweight of their respective parent drugs. All the test compounds were given by oral route.

**EQUIPMENT:**

*Plethysmograph*

It is a simple apparatus containing mercury. The displacement of mercury due to the dipping of the paw can be directly read on the scale attached to the mercury column or adjusting the mercury level in the arm B to the original level by moving arm B up down and noticing the volume required to bring the level in arms equal.

Alternatively, a third arm C is fused in such a way that the mercury displaced by dipping of the paw is allowed to rise in the arm C until the level of mercury is same in A and B. The flow of mercury can be regulated by stop cock. This modification can thus be effectively employed.

**PROCEDURE:**

1. The animals were weighed and marked.
2. A mark was made with the help of a permanent marker on the left hind paw near tibia tarsus junction to ensure constant paw volume.
3. The mercury arms A and B were adjusted to the same level. Mercury levels in arm C was set at zero. Initial paw volume of left hind paw of rats divided in various groups was noted by dipping the paw in arm A up to the predetermined mark. This raised the level of mercury in arm B. Stopcock (1) was closed to keep the level raised, stopcock
(2) then opened and mercury level was allowed to rise in arm C till the mercury level in arm A and B were same. The mercury level in arm C was noted down.

4. The animals were divided in groups of five animals each. The groups comprised of:
   i. Control
   ii. Positive control (carrageenan)
   iii. Standards
   iv. Test (test drugs)

5. Carrageenan suspension (0.1ml; 1%) in CMC was injected subcutaneously into the subplantar region of the left hind paw of the rats after 30 minutes of the administration of test drugs and standards. The control group was given only 0.5% CMC.

6. Hind paw volume was recorded by plethysmograph at 0, 1, 2 and 3 hours after carrageenan injection. The percent inhibition in edema was then calculated by the formula given below. The results are given in the discussion section.

**CALCULATIONS:**

\[
\text{\% inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where

- \( V_c \) is the volume of carrageenan control
- \( V_t \) is the volume for test compounds at time ‘t’
C. Analgesic activity

**Acetic Acid Induced Writhing Method**: 

**Principle**

Analgesia is defined as a state of reduced awareness to pain, and analgesics are substances which decrease pain sensation by increasing threshold to painful stimuli. The commonly used analgesics are aspirin, ibuprofen, diclofenac, naproxen etc.

Chemicals can provoke painful reaction in animals. Intraperitoneal injection of phenylquinone, bradykinin, acetic acid produces pain reaction, which is characterised by a writhing response. Constriction of abdomen, turning of trunk and extension of hind legs are taken as reaction to chemically induced pain. Analgesics both narcotic and non-narcotic type inhibit writhing responses.

** REQUIREMENTS**

**Animals:** The studies were carried out on healthy mice of either sex weighing between 25-30gms. Animals were grouped with each group comprising of six animals. They were fed on standard pellet diet (Hindustan lever Ltd.) and fasted for 12 hours before starting the experiment.

**Standard drugs:** All drug suspensions were prepared in 0.5% carboxy methylcellulose (CMC). Acetic acid 1% v/v was taken as writhing inducing agent and injected intraperitoneally 1ml/100g bodyweight of the animal.

p) **Ibuprofen:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of ibuprofen was prepared and administered orally 0.5ml/100g body weight of the animal.

q) **Diclofenac sodium:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of diclofenac sodium was prepared and administered orally 0.5ml/100g body weight of the animal.

r) **Biphenyl acetic acid:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of biphenyl acetic acid was prepared and administered orally 0.5ml/100g body weight of the animal.

s) **Probenecid:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of probenecid was prepared and administered orally 0.5ml/100g body weight of the animal.

**t) Naproxen:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of naproxen was prepared and administered orally 0.5ml/100g body weight of the animal.

**u) Mefenamic acid:** The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of mefenamic acid was prepared and administered orally 0.5ml/100g body weight of the animal.
v) *Indomethacin:* The dose administered was 20 mg/kg of bodyweight. Stock solution containing 4mg/ml of indomethacin was prepared and administered orally 0.5ml/100g body weight of the animal.

**Test Compounds:** All suspensions of test compounds were prepared in 0.5% carboxymethyl cellulose (CMC) as a suspending agent. The doses of all the prodrugs were equivalent to the dose of 20mg/kg bodyweight of their respective parent drugs. All the test compounds were given by oral route.

**Method:** Animals were weighed and marked. Control group was given only 0.5% CMC suspension. Test compounds and standards were given orally 3 hours before the intraperitoneal administration of 1%/v acetic acid. Numbers of writhings in each animal were noted for 10 minutes after injecting acetic acid. The percentage protection was calculated by the formula given below. The results are given in the discussion section as average number of writhings per mouse and as percentage protection.

\[
\% \text{ Protection} = 100 - \frac{\text{No. of Writhings in Test}}{\text{No. of writhings in Control}} \times 100
\]
IN VIVO HYDROLYSIS OF COMPOUND 8

The determination of in vivo hydrolytic profile of the compound 8 was planned and rabbits were chosen as the animal model due to their easy handling and by the fact that sufficient quantity of blood sample can be collected repeatedly. A reverse phase HPLC procedure was used for the quantitative determination of prodrug 8 and parent compound ibuprofen. HPLC studies were performed on a Shimadzu instrument. A reverse phase µBondapak C18 column was used for the analysis. The mobile phase consisted of acetonitrile and 0.1 M acetic acid of pH 4.9. The flow rate was 1.0ml/min and the column effluent was monitored at 220nm.

DETERMINATION OF IBUPROFEN AND COMPOUND 8 IN PLASMA OF RABBIT

Preparation of mobile phase:
Acetonitrile HPLC grade (Merck) and water HPLC grade (SDfme) were degassed and filtered through 0.2 µm Whatman filter prior to use.
Preparation of 0.1 M acetic acid: 2.86 ml of AR grade acetic acid was added to water and volume made upto 500 ml to give 0.1 M acetic acid.

Acetonitrile and 0.1 M acetic acid were mixed in the ratio of 55:45 to give mobile phase of pH 4.9.

Preparation of calibration curves in plasma:
Plasma (drug free) was obtained from the blood of rabbit by centrifugation at 4000 rpm for 30 minutes. Plasma was stored in deep freezer and used when required.

Standard solutions:
All the standard solutions of ibuprofen were prepared in mobile phase and all the standard solutions of compound 8 were prepared in double distilled water.

Calibration curve of ibuprofen:
Ibuprofen (25.4mg) was dissolved in 25ml of mobile phase to give a concentration of 2.54mg/ml and was labeled as stock solution No. 1(SS1). 4.0 ml of SS1 was diluted to 10ml to give concentration of 1016 µg/ml. This solution was labeled as stock solution No. 2 (SS2). From this solution various stock concentrations were prepared.
Table 1  Preparation of Standard stock solution No. 3 (SS3) for ibuprofen

<table>
<thead>
<tr>
<th>Volume of stock solution No. 2 (ml)</th>
<th>Volume of diluent added (ml)</th>
<th>Final stock conc. (SS3) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>1.6</td>
<td>203.4</td>
</tr>
<tr>
<td>0.6</td>
<td>1.4</td>
<td>305.1</td>
</tr>
<tr>
<td>0.8</td>
<td>1.2</td>
<td>406.8</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>508.5</td>
</tr>
<tr>
<td>1.2</td>
<td>0.8</td>
<td>610.5</td>
</tr>
<tr>
<td>1.4</td>
<td>0.6</td>
<td>711.9</td>
</tr>
<tr>
<td>1.6</td>
<td>0.4</td>
<td>813.6</td>
</tr>
</tbody>
</table>

Spiking of plasma:
For standard curve, plasma aliquots (drug free; 475µL) were taken in 5 ml centrifugation tubes (PET) and spiked with stock solution No. 3 (25µL) to yield concentrations of 10-40 µg/ml. Acetonitrile (1.5ml) was added to each test tube to precipitate plasma proteins. The tubes were vortexed for 2 minutes and then centrifuged for 20 minutes at 4000 rpm. The supernatant was separated and 10µL injected directly into the injector.

Table 2  Preparation of final concentrations of ibuprofen in plasma

<table>
<thead>
<tr>
<th>Volume of plasma taken (µL)</th>
<th>Spiking volume (SS3 µL)</th>
<th>Final concentration of spiked plasma (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>475</td>
<td>25</td>
<td>10.17</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>15.25</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>20.34</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>25.42</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>30.52</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>35.59</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>40.68</td>
</tr>
</tbody>
</table>

Table 3  Details of concentration Vs area under peak and peak height for calibration curve of ibuprofen

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Final concentration of spiked plasma (µg/ml)</th>
<th>Area under curve (AUC)</th>
<th>Peak height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.17</td>
<td>46885</td>
<td>1805</td>
</tr>
<tr>
<td>2</td>
<td>15.25</td>
<td>71093</td>
<td>2694</td>
</tr>
<tr>
<td>3</td>
<td>20.34</td>
<td>92729</td>
<td>3556</td>
</tr>
<tr>
<td>4</td>
<td>25.42</td>
<td>111825</td>
<td>4303</td>
</tr>
<tr>
<td>5</td>
<td>30.52</td>
<td>138245</td>
<td>5349</td>
</tr>
<tr>
<td>6</td>
<td>35.59</td>
<td>163099</td>
<td>6273</td>
</tr>
</tbody>
</table>
Preparation of calibration curve of compound 8

Compound 8 (47.8 mg) was dissolved in 10ml of double distilled water to give a concentration of 4.78 mg/ml and was labeled as stock solution No. 1 (SS1). 4.0 ml of SS1 was diluted to 10ml to give concentration of 187.79 μg/ml. This solution was labeled as stock solution No. 2 (SS2). From this solution various stock concentrations were prepared.

Table 4 Preparation of standard stock solutions (SS3) of compound 8

<table>
<thead>
<tr>
<th>Volume of stock solution No. 2 (ml)</th>
<th>Volume of diluent added (ml)</th>
<th>Final stock conc. (SS3) (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>1.6</td>
<td>375</td>
</tr>
<tr>
<td>0.8</td>
<td>1.2</td>
<td>751.91</td>
</tr>
<tr>
<td>1.2</td>
<td>0.8</td>
<td>1127.86</td>
</tr>
<tr>
<td>1.6</td>
<td>0.4</td>
<td>1503.82</td>
</tr>
</tbody>
</table>

Spiking of plasma:

For standard curve, plasma aliquots (drug free) (475μL) were taken in 5 ml PET centrifugation tubes and spiked with stock solution No. 3 to yield concentrations of 10-40 μg/ml. Acetonitrile (1.5ml) was added to each test tube to precipitate plasma proteins. The tubes were vortexed for 2 minutes and then centrifuged for 20 minutes at 4000 rpm. The supernatant was separated and 10μL injected directly into the injector.

Table 5 Preparation of final concentrations of compound 8 in plasma

<table>
<thead>
<tr>
<th>Volume of plasma taken (μL)</th>
<th>Spiking volume (SS3, μL)</th>
<th>Final concentration of 7 in spiked plasma (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>475</td>
<td>25</td>
<td>18.79</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>37.59</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>56.39</td>
</tr>
<tr>
<td>475</td>
<td>25</td>
<td>75.19</td>
</tr>
</tbody>
</table>
Details of concentration Vs area under peak and peak height for calibration curve of prodrug 8

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration of spiked plasma (µg/ml)</th>
<th>Area under curve (AUC)</th>
<th>Peak height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.79</td>
<td>45260</td>
<td>1750</td>
</tr>
<tr>
<td>2</td>
<td>37.59</td>
<td>84953</td>
<td>3335</td>
</tr>
<tr>
<td>3</td>
<td>56.39</td>
<td>135761</td>
<td>5301</td>
</tr>
<tr>
<td>4</td>
<td>75.19</td>
<td>182073</td>
<td>7053</td>
</tr>
</tbody>
</table>

Methodology:

Animals: Healthy rabbits weighing 2-3 kg were chosen for the study.

Drugs: Ibuprofen 60 mg/kg
       Prodrug 8 112.42 mg/kg equivalent to 60 mg/kg of ibuprofen

Procedure:
1. The animals were marked as I and II.
2. The animals were fasted overnight, weighed and doses calculated.
3. The drug suspensions were given orally with the help of oral feeding needle.
4. Animal I was given ibuprofen and animal II was given prodrug 8.
5. Blood samples were withdrawn from the marginal ear vein at predose, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 and 8.0 hours.
6. Plasma was separated by centrifugation at 4000 rpm and stored in deep freezer.
7. In a 5 ml centrifugal tube 0.5 ml of plasma was taken and to it was added 1.5 ml of acetonitrile and vortexed for 2 minutes. The precipitated proteins were separated by centrifugation at 4000 rpm for 20 minutes. 10 µL of the supernatant was injected.
8. The results were tabulated and Tₘₐₓ, Cₘₐₓ, T₁/₂, etc calculated.

HPLC parameters

The following parameters were set for estimation of drugs.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Shimadzu HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical column</td>
<td>µ bondapak C₁₈ (30cm) (reverse phase)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile: 0.1 M acetic acid: 55:45</td>
</tr>
<tr>
<td>Protein precipitant</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>UV detection</td>
<td>220 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1mL/min</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10µL</td>
</tr>
</tbody>
</table>
### Table 7: Plasma levels of ibuprofen in animal I given ibuprofen

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time (hrs.)</th>
<th>AUC</th>
<th>Peak height</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>52303</td>
<td>1969</td>
<td>11.5</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>100634</td>
<td>3877</td>
<td>22.3</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>124750</td>
<td>4869</td>
<td>27.8</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>91935</td>
<td>3533</td>
<td>21.0</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>68647</td>
<td>2684</td>
<td>15.0</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
<td>38183</td>
<td>1497</td>
<td>8.5</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>27601</td>
<td>1087</td>
<td>5.5</td>
</tr>
<tr>
<td>8</td>
<td>6.0</td>
<td>16849</td>
<td>724</td>
<td>3.0</td>
</tr>
<tr>
<td>9</td>
<td>8.0</td>
<td>7403</td>
<td>360</td>
<td>0.98</td>
</tr>
</tbody>
</table>

### Table 8: Plasma level of ibuprofen in animal II given compound 8

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time (hrs.)</th>
<th>AUC</th>
<th>Peak height</th>
<th>Ibufrofen (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>91795</td>
<td>3646</td>
<td>20.5</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>122797</td>
<td>4856</td>
<td>27.5</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>146888</td>
<td>5747</td>
<td>34.0</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>129297</td>
<td>5060</td>
<td>26.5</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>96079</td>
<td>3777</td>
<td>21.5</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
<td>76083</td>
<td>3117</td>
<td>17.5</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>49157</td>
<td>1959</td>
<td>11.5</td>
</tr>
<tr>
<td>8</td>
<td>6.0</td>
<td>39994</td>
<td>1618</td>
<td>8.0</td>
</tr>
<tr>
<td>9</td>
<td>8.0</td>
<td>23578</td>
<td>983</td>
<td>5.5</td>
</tr>
</tbody>
</table>

### Table 9: Plasma level of compound 8 in animal II

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time (hrs.)</th>
<th>AUC</th>
<th>Peak height</th>
<th>Prodrug (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>45229</td>
<td>1545</td>
<td>18.77</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>45266</td>
<td>1572</td>
<td>18.79</td>
</tr>
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<td>4</td>
<td>2.0</td>
<td>118778</td>
<td>4904</td>
<td>49.31</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>12136</td>
<td>548</td>
<td>5.03</td>
</tr>
<tr>
<td>6</td>
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<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
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<tr>
<td>8</td>
<td>6.0</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>9</td>
<td>8.0</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Fig 1 Chromatogram showing plasma levels of prodrug 8 (Rt=5.52 min.) and ibuprofen (Rt=8.76 min.) at 2.0 hrs. time interval in rabbit given prodrug 8.

Retrieval time (Rt) of prodrug 8 = 5.52 min.

Retrieval time (Rt) of ibuprofen = 8.76 min.
**LD$_{50}$ evaluation**  
The LD$_{50}$ value was determined by Miller and Trainter method$^5$.  

**Animals**  
The study was carried out on Swiss albino mice (either sex) weighing between 20-25gm. The mice were grouped with eight animals in each group ($n = 8$) and maintained under standard laboratory conditions with natural light dark cycle. They were fed on standard pellet diet and water *ad libitum*. Mice were acclimatized to their environment for one week prior to experimentation.

**Test compound, standard dose and route of administration**  
Prodrug 8 was administered by oral route in mice in ascending and widely spaced doses of 250 mg/kg, 500 mg/kg, 750 mg/kg, 1000mg/kg, 1100mg/kg and 1250mg/kg in 0.5% CMC (Carboxymethyl Cellulose) suspension.

**Experimental Procedure**  
The animals were fasted overnight and divided in groups with six animals in each group. Test compound 8 was given by oral route and the animals observed for 48 hrs for death. The percentage mortality values were calculated and converted to probit values by reading the corresponding probit units from the probit table. Graph was plotted between probit values against log doses and LD$_{50}$ value was read as the dose, which corresponded to probit 5.

**RESULT**  
The LD$_{50}$ value of prodrug 8 in mice by oral route was found to be 1047 mg/kg (reported for ibuprofen, 1050 mg/kg in mice by oral route$^6$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg</th>
<th>Log Dose</th>
<th>Dead/Total</th>
<th>%Dead</th>
<th>%dead Corrected*</th>
<th>Probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>2.39</td>
<td>0/8</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>2.69</td>
<td>0/8</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>750</td>
<td>2.87</td>
<td>0</td>
<td>0</td>
<td>0.325</td>
<td>3.38</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>3.00</td>
<td>3/8</td>
<td>37.5</td>
<td>37.5</td>
<td>5.00</td>
</tr>
<tr>
<td>5</td>
<td>1100</td>
<td>3.04</td>
<td>6/8</td>
<td>75</td>
<td>75</td>
<td>5.68</td>
</tr>
<tr>
<td>6</td>
<td>1250</td>
<td>3.09</td>
<td>8/8</td>
<td>100</td>
<td>96.8</td>
<td>6.88</td>
</tr>
</tbody>
</table>

* Correction for 0% dead = 100(0.25/n)  
And for 100% dead = 100(n-0.25/n)
Experimental

Determination of Partition co-efficient Of Compound 8

Partition Coefficient (P) was determined by the conventional shake flask method. Partition coefficient of a drug between water and n-octanol fairly gives conclusive idea about its onset and duration of action hence affecting biological activity. It is defined as a ratio of concentration of a particular substance in a lipophilic solvent and a hydrophilic solvent.

\[
P = \frac{C_1}{C_2}
\]

Where \(C_1\) is the concentration in the lipophilic solvent and \(C_2\) is the concentration in water.

Procedure:

Preparation of standard curve in water: 5 mg of prodrug 8 was dissolved in 50 ml of water to give stock concentration of 100\(\mu\)g/ml. Dilutions were made in the range of 10 to 100\(\mu\)g/ml concentrations. The absorbances of the solutions were determined on LambdaBio-20 UV spectrophotometer at 220 nm with water as blank.

Partition coefficient measurements: In a 100 ml volumetric flask, compound 8 was dissolved in water presaturated with n-octanol to give a concentration of 80\(\mu\)g/ml. The above solution was poured in a 250 ml conical flask containing 100 ml of n-octanol presaturated with water. The above flask was shaken gently on a sideways mechanical shaker at room temperature of 25±2\(^\circ\)C. Samples from aqueous layer were withdrawn at 0, 1, 2, 4, 6, 12 and 24 hours. The absorbances were taken and concentrations were calculated from standard curve. It showed that the equilibrium between the two phases was achieved in 4 hours. The concentration of prodrug 8 in n-octanol was found out by subtracting its concentration in water from the initial concentration of 80\(\mu\)g/ml. The relative concentrations of prodrug 8 in n-octanol and water were found out to be 12 \(\mu\)g/ml and 68 \(\mu\)g/ml respectively. The partition coefficient (P) was found to be 0.176 (Log P = -0.75).
References