INTRODUCTION
Most of the drugs, that are administered to the living organisms, are extensively transformed through different metabolic pathways. The metabolites formed are usually more polar and less lipophilic than the parent drug, thus increasing the possibility of renal excretion due to enhanced aqueous solubility. For the same reason, these metabolites are less active (often inactive) than the parent drug. Therefore, the usual net effect of metabolism may be considered to be of inactivation or detoxification. In the earlier literature on this subject, the term metabolism has been put more or less synonymous with detoxification.

However, the enzymes responsible for drug metabolism alter the substrates along their set mechanistic schemes and by doing so, sometimes produce metabolites which show activity and are called ‘active metabolites’. Whether the metabolite produced is active or inactive depends on the structure of the substrate, and not on the nature of the catalytic reaction.

The designation of an ‘active metabolite’ must be specific as to the experimental conditions because many factors including animal species, strain, sex, age, circardian rhythm, route of administration, dose, previous treatments and pathological conditions, may markedly influence the pathway and rate of drug metabolism. Any extrapolation to other experimental conditions and especially to human therapy must be cautious and validated.
by suitable data. The presence of an active metabolite should be suspected by the following observations:

1. Unusual lag time between intravenous administration of a drug and its effect.
2. Lack of *in vitro* effect for a compound that is active *in vivo*.
3. Presence of pharmacological activity when the drug is not present in the supposed target organ.
5. Alteration of pharmacological activity by treatment with other compounds known to stimulate or inhibit drug metabolising enzymes.

In 1959, Williams classified the reactions, that are catalysed by enzymes, into phase I and phase II reactions. Phase I reactions include oxidations, reductions and hydrolysis and these reactions transform the drug for conjugation by providing polar groups. In phase II, the metabolic products of phase I reactions or the parent drug itself, if it has a polar group, react with endogenous solubilising substrates like glucuronic acid, sulphate, glutathione and other molecules. Phase II metabolic pathways are generally considered deactivating, and a survey of a great number of active metabolites shows that they belong almost exclusively to phase I products. Active
metabolites can be divided into two classes\textsuperscript{5} : (1) stable metabolites and (2) chemically highly reactive metabolites.

Pharmacologically active stable drug metabolites can be formed both from active and inactive parent drugs. In the case of the former, two possibilities exist. Firstly, the active metabolite may differ from the parent drug in quantitative aspects such as dose-response and time-action characteristics; these metabolites are potentially beneficial at right concentrations in the body. Secondly, the active metabolite may differ from the parent drug qualitatively such that the spectrum of activity is altered. There are a number of reviews\textsuperscript{2,6-8} on active metabolites where many examples are described. Table I summarises some of the early recognised active metabolites.

Table I : Early recognised stable active metabolites.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Active Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacetin</td>
<td>Paracetamol</td>
</tr>
<tr>
<td>Proguanil</td>
<td>Cycloguanil</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Desmethylimipramine</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>Trichloroethanol</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Oxyphenylbutazone</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Prontosil</td>
<td>Sulphanilamide</td>
</tr>
</tbody>
</table>
Chemically highly reactive, electrophilic, biologically alkylating intermediate products with a very short half-life are formed in the course of metabolic conversions, particularly oxidation. These intermediates form covalent bonds with biological molecules and are responsible for toxicity. The reactive metabolites may result from a single enzymatic reaction and in some cases, several enzymatic and chemical reactions are involved to produce the ultimate toxic metabolite. These toxic metabolites may interact with cells in many different ways to produce serious consequences like mutagenesis, carcinogenesis, possibly accelerated aging, teratogenesis, allergic sensitisation, cell degeneration and necrosis, and photosensitisation. Some of the examples of chemically reactive metabolites are given in Table 2. Numerous examples of such reactive metabolites are described in an excellent review on this subject.

**Table 2: Examples of reactive metabolites**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reactive Metabolite</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacetin</td>
<td>N-Hydroxyphenacetin</td>
<td>Hepatocarcinogenesis</td>
</tr>
<tr>
<td></td>
<td>p-Ethoxyphenyl-hydroxylamine</td>
<td>Mutagenesis</td>
</tr>
<tr>
<td></td>
<td>p-Ethoxynitrosobenzene</td>
<td>Mutagenesis</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Epoxide metabolite</td>
<td>Teratogenesis</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>N-Acetyl-p-benzoquinone</td>
<td>Cell death</td>
</tr>
</tbody>
</table>
In recent years, with the ever increasing sophistication and sensitivity of bioanalytical techniques, the detection of active metabolites has become a routine process. The critical question is not whether an active metabolite is generated since it is not an uncommon situation. A more appropriate focus is whether the metabolite is contributory to the clinical situation and if so, to what extent? There are numerous examples in which active metabolites are generated, but they never significantly contribute to the drug response because their concentrations are too low. On the other hand, a metabolite having low potency may have clinical significance if it is formed in higher concentration. In this regard the relative disposition of parent and metabolite is critical, and pharmacokinetics of metabolite formation and elimination have recently received considerable attention.¹⁰⁻¹³.
ACTIVE METABOLITES OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS

Most antiinflammatory drugs are readily metabolised in the liver, kidney, intestine and other tissues, and excreted by both urinary and biliary routes. Common metabolic conversions are hydroxylation of aryl and alkyl groups, followed by glucuronide formation of carboxyl and hydroxyl groups. Oxidative demethylation and deacylation may also occur\textsuperscript{14}. Many times, the metabolites thus produced show antiinflammatory activity, and in some cases the active metabolite is completely responsible for the activity of the parent nonsteroidal antiinflammatory drug (NSAID).

Studies have indicated that intersubject variability in response to NSAIDs is a significant and clinically relevant phenomenon. This intersubject variability ranges from clear response to nonresponse to a particular NSAID\textsuperscript{15}. One of the reasons for this may be the complex and varied biotransformations in different individuals to produce active metabolites at different rates and in different concentrations. This problem may be reduced by selecting an alternate drug metabolism route, which is less prone to variability, to produce the same active metabolite. Hydrolytic enzyme systems produce less variability and therefore should be preferred.
In the following discussion active metabolites of various NSAIDs are described.

Sulindac (1) gets biotransformed into sulphone 2 by irreversible oxidation of sulfoxide group and to sulfide 3 by reversible reduction\(^{16}\) (Figure 1). Sulfide metabolite 3 is twice as potent as sulindac after oral administration. Sulindac was found to be inactive, when tested \textit{in vitro}, whereas sulfide metabolite was active.

\[ \text{Figure 1: Biotransformation of sulindac.} \]
These results suggest that sulindac’s activity is because of its active sulfide metabolite 3. The reversible interconversion, sulindac $\rightleftharpoons$ sulfide, and the different distributional and excretory properties of each, provide a theoretical basis for a long plasma half-life and prolonged effect of sulindac$^{17}$.

Metabolism of diclofenac (4) occurs via hydroxylation in each aromatic ring producing compounds 5 through 7; 5 being the main metabolite in humans$^{18}$. All these metabolites show antiinflammatory activity but they are much less active than diclofenac$^{19}$. 

\[ 
\text{HO-CH}_2\text{COOH} \quad \text{HO-CH}_2\text{COOH} \\
\text{Cl} \quad \text{Cl} \quad \text{Cl} \quad \text{Cl} \\
(4) \quad (5) \\

\text{HO-CH}_2\text{COOH} \quad \text{HO-CH}_2\text{COOH} \\
\text{Cl} \quad \text{Cl} \quad \text{Cl} \quad \text{Cl} \\
(6) \quad (7) \\

\]
The main metabolite of fentiazac (8) is a \( p \)-hydroxylated compound 9 which is formed rapidly. This compound is much less toxic than the parent molecule and possesses antiinflammatory activity with much higher therapeutic index than fentiazac\(^{20}\).

\[
\begin{align*}
\text{(8)} & \quad \text{Cl} & \quad \text{CH}_2\text{COOH} \\
\text{(9)} & \quad \text{Cl} & \quad \text{CH}_2\text{COOH} & \quad \text{OH}
\end{align*}
\]

Bermoprofen (10) gets metabolised to a number of metabolites in rats, monkeys and humans. The hydroxy metabolite 11 and carboxy metabolite 12 were synthesised, and these derivatives showed weak antiinflammatory activity in carrageenan-induced rat paw edema test\(^{21}\).

\[
\begin{align*}
\text{(10)} & \quad R=\text{CH}_3 \\
\text{(11)} & \quad R=\text{COOH} \\
\end{align*}
\]
The metabolism of phenylbutazone (13) produces two major metabolites, oxyphenylbutazone (14) and the side chain hydroxylated compound 15. Oxyphenylbutazone has a potency comparable to that of phenylbutazone but is slightly better tolerated (14).

!(13) R= H  
!(14) R= OH

Bumidazone (16) is rapidly converted in vivo to several metabolites, some of which have been identified as 2-hydroxy compound 17 and oxyphenylbutazone (14), a
metabolite of phenylbutazone. Direct evidence has been obtained for the conversion of bumidazone to phenylbutazone after oral administration to humans. Phenylbutazone and oxyphenylbutazone have been in use as independent antiinflammatory drugs.

\[
\begin{align*}
\text{(18)} & \quad \begin{array}{c}
N \quad \begin{array}{c}
\text{(CH}_2\text{)}_3\text{CH}_3 \\
\end{array} \\
\end{array} \\
\end{align*}
\]

Suxibuzone (18) gets converted to its active metabolites, phenylbutazone (13) and oxyphenylbutazone. Suxibuzone has no inhibitory activity in vitro against prostaglandin synthesis and therefore, its antiinflammatory activity is due to its metabolites, phenylbutazone and oxyphenylbutazone.

Fenbufen (19) gets metabolised mainly to γ-hydroxy-(1,1-biphenyl)-4-butanoic acid (GH-BPBA) (20) and 4-biphenylacetic acid (21) in human beings. It has been observed that the activity of fenbufen is due to these active metabolites. Recently, 4-biphenylacetic acid has also been introduced in therapy in the form of a gel for local application.
In humans furobufen (22) is rapidly converted to dibenzofuranacetic acid (23) which is an active metabolite, and is responsible for the activity of furobufen\textsuperscript{30,31}.

Similarly bucloxic acid (24) gets metabolised to two major metabolites 25 and 26 in man\textsuperscript{32}. 

\textsuperscript{12}
Fenbufen (19), furombufen (22) and bucloxic acid (24) are aroylpropionic acids, and Testa and Jenner have indicated the progressive degradation of side chain into arylacetic acids (Figure 2). For all these drugs, bioactivation takes place through a multistep process employing reductive, oxidative and hydration-dehydration sequences.

\[
\begin{align*}
\text{ArCOCH}_2\text{CH}_2\text{COOH} & \\
\downarrow & \\
\text{ArCHCH}_2\text{CH}_2\text{COOH} & \\
\downarrow & \\
\text{OH} & \\
\downarrow & \\
\text{ArCH=CHCH}_2\text{COOH} & \\
\downarrow & \\
\text{ArCH}_2\text{CHCH}_2\text{COOH} & \\
\downarrow & \\
\text{OH} & \\
\downarrow & \\
\text{ArCH}_2\text{COCH}_2\text{COOH} & \\
\downarrow & \\
\beta\text{-oxidation} & \\
\downarrow & \\
\text{ArCH}_2\text{COOH} & 
\end{align*}
\]

Figure 2: Progressive degradation of side chain of aroylpropionic acids into arylacetic acids.
In humans, meclofenamate (28) is converted mainly to the hydroxymethyl metabolite 29, a compound that retains some of the antiinflammatory activity of meclofenamate\textsuperscript{34}.

\[
\begin{align*}
(28) & \quad \text{COOH} \quad \text{Cl} \\
& \quad \text{R}^1=\text{H}, \text{R}^2=\text{CH}_2\text{OH}, \text{R}^3=\text{H} \\
(29) & \quad \text{COOH} \quad \text{Cl} \\
& \quad \text{R}^1=\text{H}, \text{R}^2=\text{CH}_3, \text{R}^3=\text{CH}_2\text{OH}
\end{align*}
\]

Tolfenamic acid (30) gets metabolised to a number of metabolites, notable among these are hydroxylated derivatives 31, 32 and 33 and oxidised form 34; 31 being the major product\textsuperscript{35}. Metabolites 31 and 32 showed acute toxicities in rats and mice and much weaker antiinflammatory activity than tolfenamic acid in carrageenan rat paw edema test\textsuperscript{36}.
The pharmacological activities of various metabolites of 6-chloro-5-cyclohexylindan-1-carboxylic acid, TAI-284 (35), were investigated and compared to the parent drug and phenylbutazone\textsuperscript{37}. The metabolite 36 was found to have almost equivalent antiinflammatory activity to that of the parent drug, whereas it showed more analgesic activity. The metabolites 37 and 38 showed about half the antiinflammatory activity and 25 to 30% the analgesic activity.
activity of 35, however, degree of ulcerogenicity was considerably lower. Metabolites 39 and 40 showed the weakest activity. Antipyretic activity of all the metabolites was lower.

Piroxicam (41), a relatively new NSAID, has been reported to give various metabolites, a number of these have been tested for their biological activity38. All of these were found to be less potent than piroxicam but 42 and 43 approach the potency of phenylbutazone.

![Chemical structures](image.png)

Two primary metabolites of oxaprozin (44) in humans have been identified as 4-((p-hydroxyphenyl)-5-phenyl-2-oxazolepropionic acid (45) and 5-((p-hydroxyphenyl)-5-phenyl-
2-oxazolepropionic acid (46). Both 45 and 46 have been found to be equally potent to oxaprozin as antiinflammatory agents in animal models. However, less than 5% of an administered oxaprozin dose is found in human plasma in the form of these metabolites, their contribution to the clinical activity is probably minimal.

Nabumetone (47) is a new, nonacidic NSAID. It undergoes extensive first-pass metabolism after absorption producing a range of metabolites. One of the major metabolites, 6-methoxy-2-naphthylacetic acid (48) is responsible for the antiinflammatory activity of nabumetone.

Loxoprofen sodium (49) is an antiinflammatory/analgesic agent useful in the management of rheumatoid
arthritis and related disorders. It is converted in vivo to the corresponding trans-hydroxycyclopentane analog (50), through which it appears to be acting.\textsuperscript{42}

The above account on active metabolites highlights the importance of metabolism studies in providing better understanding of drug's mode of action, toxicity and interactions with other drugs. Now a days, metabolism studies are carried out as a requirement of drug legislation to evaluate the safety of drugs. The information gained from such studies can also be profitably utilised for rational design and development of new drugs, and therefore, in recent years metabolism has become a matter of great interest in drug research. Numerous concepts and methods have evolved which make use of metabolic knowledge in rational drug design. The following discussion will demonstrate the contributions made by the active metabolites in the development of new drugs.

**ACTIVE METABOLITES AS INDEPENDENT DRUGS**

The discovery that the activity of a drug is due to a metabolite sometimes leads to introduction of the metabolite itself into the therapy. The reasons for this may be either the metabolite is less toxic or has fewer side effects than the parent drug, or generally the metabolite reduces the variability of the clinical response within the population due to different metabolising powers of individuals.
Arsphenamine (51) was used for the treatment of syphilis, and it was shown that this drug owed its activity to the metabolite oxophenarsine (52). Oxophenarsine was introduced as independent drug and replaced arsphenamine in therapy since it was less toxic at the effective dose.\textsuperscript{43}

The discovery of the azo dye prontosil (53) marked the advent of present day sulphonamide therapy. Prontosil is inactive against microorganisms in vitro but active in vivo. The finding that prontosil was a precursor and that the active form was a metabolite, p-aminobenzenesulphonamide (54), directed research away from dyes and towards modifications of 54 leading to a wide range of therapeutically superior sulphonamides now available for treatment.\textsuperscript{43}

\begin{center}
\begin{align*}
\text{(51)} & \quad \text{(52)} \\
\text{(53)} & \quad \text{(54)} \\
\text{(55)} & \quad \text{(56)}
\end{align*}
\end{center}

Phenacetin (55) gets metabolised to paracetamol (56)\textsuperscript{44} which was introduced as an independent drug. The
main advantage of paracetamol is that it does not cause methemoglobinemia in children and renal dysfunction as noticed with the use of phenacetin. However, extensive hepatic necrosis may occur when overdoses are ingested, since the normal biotransformation pathway is then saturated and a highly reactive metabolite is formed which reacts irreversibly to hepatic tissue\textsuperscript{45}.

Phenylbutazone (13) gets metabolised to oxyphenylbutazone (14) which is responsible for the antiinflammatory activity of the parent\textsuperscript{46}. Therefore, oxyphenylbutazone was introduced as an independent drug\textsuperscript{14}.

\[
\text{CH}_2\text{CH}_2\text{NH}_2 \quad \text{CH}_3
\]

\[
\text{N} \quad \text{CH}_3
\]

(57) \quad (58)

Desipramine (57) has become an established antidepressant following its detection as a metabolite formed by oxidative N-dealkylation of imipramine (58)\textsuperscript{47}. Desipramine is a selective blocker of noradrenaline uptake, whereas imipramine blocks the uptake of both serotonin and noradrenaline. Biotransformation of desipramine is less complex than that of imipramine\textsuperscript{48}. Imipramine is converted
to other primary metabolites including pharmacologically active ones, such as the products of N-oxidation and C-hydroxylation. Apart from a different neurobiochemical profile, desipramine offers favourable metabolic properties.

Biotransformation of diazepam (59) leads to the formation of three active compounds namely, temazepam (60), nordazepam (61) and oxazepam (62) (Figure 3), and all of

![Diagram of diazepam biotransformation](image)

**Figure 3**: Biotransformation of diazepam (59) to temazepam (60), nordazepam (61) and oxazepam (62) resulting from C-hydroxylation and N-demethylation.
these have been developed as independent tranquillising agents\textsuperscript{49}. The metabolising reactions involve $N$-demethylation and $C$-hydroxylation. Such reactions are known to be sensitive towards enzyme induction or inhibition caused by other xenobiotics. Among these three metabolites, oxazepam (62) has presumably found the widest acceptance. Its biotransformation proceeds in one step by direct $O$-glucuronidation which is less sensitive than oxidative pathways. The half-life of oxazepam is shorter than that of diazepam.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.3\textwidth]{molecule1.png}};
\node (b) at (4,0) {\includegraphics[width=0.3\textwidth]{molecule2.png}};
\end{tikzpicture}
\end{center}

Mesoridazine (63), the side chain sulfoxide metabolite of thioridazine (64), may account for a significant amount of therapeutic activity of thioridazine in man and has been introduced into clinical practice as an antipsychotic drug\textsuperscript{50,51}. 

22
Cholecalciferol (65) is hydroxylated at position 25 in liver resulting in a small increase in activity of the first step metabolite. The main activation takes place in renal mitochondria where it is further hydroxylated to form 1,25-dihydroxy metabolite (66)\textsuperscript{52}. This derivative has been reported to increase calcium and phosphate uptake from the intestines and developed as an independent drug to be used in the treatment of hypocalcemia in patients undergoing chronic renal dialysis.

The secretolytic bromhexine (67) is, to a small extent, subject to demethylation and hydroxylation\textsuperscript{53}. One of these biotransformation products, ambroxol (68), shows stronger secretolytic activity and is free from weak bronchoconstriction caused by bromhexin.
Zoxazolamine (69) is metabolised in man to chloroxazone (70)\textsuperscript{54}. This metabolite has been found to possess muscle relaxant activity and has been subsequently introduced into clinical use.

\begin{align*}
\text{(69)} & \quad \text{(70)} \\
\end{align*}

The antimalarial drug paludrine (71) is metabolised by ring closure to active dihydrotriazine (72) which is now used as the insoluble embonate salt, and a single intramuscular injection of a suspension of this drug in an oily base provides protection against malarial infection for several months\textsuperscript{43}.

\begin{align*}
\text{(71)} \\
\text{(72)} \\
\end{align*}
The once used hypnotic, chloral hydrate (73) is metabolised to trichloroethanol (74) and its glucuronide\textsuperscript{55}. The depressant effect of this drug was found to be due to the formation of trichloroethanol. This knowledge led to the use of trichloroethanol acid phosphate in place of chloral hydrate because of the latter’s unpalatable taste and gastrointestinal irritation.

\[
\begin{align*}
    \text{Cl}_3\text{CCH(OH)}_2 & \quad \text{Cl}_3\text{CCH}_2\text{OH} \\
    \text{(73)} & \quad \text{(74)}
\end{align*}
\]

Enalapril (75), as enalapril maleate, was introduced for the first time in 1984 as an angiotensin converting enzyme (ACE) inhibitor for the treatment of hypertension and congestive heart failure\textsuperscript{56}. Its active metabolite enalaprilat (76) was marketed subsequently, as an injectable ACE inhibitor, for the treatment of accelerated hypertension and hypertension emergencies\textsuperscript{57}. Enalaprilat is more
effective in reducing blood pressure in elderly patients than in the young, despite the fact that renin activity is lower in old age.

Fisalamine (77) is an intestinal metabolite of sulphasalazine (78) useful in the treatment of ulcerative colitis and to a lesser degree in the management of Crohn's disease. Administered as a suppository, it appears to lack the hypersensitivity type side effects of sulphasalazine\textsuperscript{57}.

![Chemical structures](image)

Biphenylacetic acid (21) is the active metabolite of the NSAID, fenbufen (19). It has been introduced in the form of a gel for topical application to joints\textsuperscript{58}. It is useful in symptomatic relief of articular inflammation and pain.
Clospipramine hydrochloride (79) is a metabolite of clozapramine (80) reportedly useful in the treatment of schizophrenia. In schizophrenic patients, its clinical efficacy and overall safety rating were not significantly different from clozapramine. However, at a lower dosage, clospipramine hydrochloride was more effective than clozapramine.

Acitretin (81) is the free acid form of etretinate (82) useful in the treatment of severe psoriasis and other disorders of keratinisation. Although, the two compounds have virtually the same efficacy and teratogenic side effects, acitretin is advantageous for child bearing woman, as its shorter half-life reduces the necessary contraception period from two years to only one month after treatment ceases.
The main goal of introducing the active metabolite as such in therapy is not to obtain a second pharmacon in addition to the existing one, rather it is often possible to have a product showing less pharmacokinetic variability, less complex biotransformation and better therapeutic performance. Inspite of these additional advantages, the number of active metabolites introduced in therapy is very less as compared to their number detected during biotransformation studies on drugs. This has been attributed to two main considerations.

The first is purely economical consideration\textsuperscript{49,53}. The entire process from the synthesis of a new chemical entity to its approval as a drug takes 10 to 15 years. Roughly, one third of this time is needed for preclinical work. Therefore, metabolism studies in man can only be initiated after considerable investments have already been made. At this stage it is impossible to replace the trial drug by an active metabolite without undue delay in development and increase in cost. It is essential that potentially active metabolites be recognised as early as possible. Suitable in vitro models with human organ preparations offer a way out\textsuperscript{62}. In addition, attempts should be made to predict biotransformation based on historic knowledge. Therefore, putative metabolites can be synthesised and tested at an early stage. Computer assisted
metabolic predictions\textsuperscript{63,64} provide further help in predicting the metabolites.

The second reason because of which the active metabolites are not introduced as such is that the active metabolite may not be having optimum physicochemical properties desired in a drug. One such example is that of allopurinol (83). The half-life of allopurinol is estimated to be about only 40 min. It is rapidly transformed into its main metabolite oxipurinol (84), which has been found to possess a half-life of 14 h after oral administration. Although oxipurinol itself inhibits xanthine oxidase, it could not become an independent drug because of its poor absorption from the intestine\textsuperscript{53}.

In these circumstances, prodrug approach may be useful for optimising physicochemical properties of the active metabolites for their delivery.
approach is that the physicochemical properties can be tailored by changing the structure of the promoiety, and the intrinsic activity of the parent drug is assured through in vivo cleavage of the prodrug.

Figure 4: Schematic illustration of carrier linked prodrug concept
A well designed prodrug should satisfy the following criteria:\textsuperscript{3,76}:

1. The linkage between the parent drug molecule and promoiety is usually a covalent bond.
2. The prodrug is inactive or less active than the parent compound.
3. The chemical linkage between the parent compound and promoiety must be bioreversible in nature.
4. The prodrug, as well as, the \textit{in vivo} released promoiety must be nontoxic in nature.
5. However, the prodrug should be sufficiently stable to allow its formulation into an appropriate dosage form.

The prodrug approach has been successfully applied to a wide variety of drugs and the objectives achieved through the use of this approach can be listed as follows:

1. Enhanced bioavailability.
2. Enhanced site specificity.
3. Improved drug formulation.
4. Improved patient acceptance and compliance.
5. Decreased side effects and toxicity.
6. Prolonged duration of action.
7. Improved chemical stability.
8. Marketing considerations and "me-too" drugs.

An illustrative example of prodrug design taking into account these criteria is that of ampicillin prodrugs to improve the oral bioavailability of this commonly used \(\beta\)-lactam antibiotic. Ampicillin (85) is highly polar in nature and is present in a zwitterionic form at the pH of
gastrointestinal tract and therefore, possesses low lipophilicity. It is absorbed to an extent of 30-40% following oral administration, and the unabsorbed drug destroys intestinal flora resulting in toxic side effects. A number of prodrugs with increased lipophilicity have been prepared by esterifying the free carboxylic group of ampicillin; notable among these are pivampicillin (86)\(^7\), bacampicillin (87)\(^8\) and talampicillin (88)\(^1,8^2\). These prodrugs are almost completely absorbed on oral administration, and cleaved by non-specific esterases of the body resulting in the release of the parent drug ampicillin. The products of cleavage are formaldehyde and pivalic acid for pivampicillin, and acetaldehyde, ethanol.

\[
\begin{align*}
\text{(85)} & \quad R = \text{H} \\
\text{(86)} & \quad R = \text{CH}_2\text{OOCC(CH}_3\text{)}_3 \\
\text{(87)} & \quad R = \text{CH(CH}_3\text{)}\text{OCOOC}_2\text{H}_5 \\
\text{(88)} & \quad R = \text{CH}_2\text{OOC\text{C}O}_2
\end{align*}
\]
and carbon dioxide in case of bacampicillin. These three latter compounds are the natural metabolites in human body. This may explain the better tolerance of bacampicillin as compared to pivampicillin.

A major problem in the general application of prodrug approach is the limited possibilities available for making bioreversible derivatives because drug molecules do not possess apparently readily derivatisable functional groups. It is relatively easier to design prodrugs of chemical compounds possessing hydroxyl (OH) and carboxyl (COOH) groups. It is interesting to note that in the biotransformation of drugs into their active metabolites, new functional groups are created which can be used as synthetic handles for the chemical modifications to prepare prodrugs of these metabolites.

The successes of prodrug design are many and several drugs are now used clinically in the form of prodrugs. This approach is mostly applied to improve known and currently marketed drugs for solving problems of drug delivery, which otherwise cannot be handled by conventional means. However, for this approach to be more useful, it is imperative that it should become an integral part of basic drug design. In this direction, modifications of active metabolites using the exciting prodrug approach may contribute to the development of new improved and safer drugs.