LATENTIATED PRODRUG APPROACH OF DRUGS: AN OVERVIEW

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Received: 29 Jul 2021, Revised and Accepted: 31 Aug 2021

ABSTRACT

Prodrugs, with their capability of declining the adverse events and elevating the bioavailability of certain drugs, have captured enormous attention throughout the world since the 20th century. The versatility of the prodrugs that are inert and after administration releasing the parent moiety for the desired effect has become a major criterion for the scientists to incorporate this to alleviate the undesired effects of a conventional drug. About 10% of the prevailing drugs are prodrugs and their usage is being amplified owing to its critical application in cancer therapy, toxicity alleviation, and specificity. The purpose of this review is to understand the prodrugs, strategies incorporated in designing the prodrugs, applications, their crucial benefits in targeted action at a specific site of the body, their advantageous effects in chemotherapy. Also, to be acknowledged with the ongoing clinical trials and researches on prodrugs and some notable marketed prodrugs in a depth manner.

Keywords: Prodrug, Carriers, Enzymes, Strategies, Specificity, Chemotherapy

INTRODUCTION

Adrian Albert coined the word ‘prodrug’ or ‘pro-agent’ in 1958, implying that this technique might temporarily adjust the properties of drugs in order to improve their effectiveness and/or to reduce concomitant toxicity. Prodrugs are bio-reversible derivatives of drug molecules that are used to circumvent some of the parent drug’s limitations. The name ‘prodrug’ refers to substances that exert their pharmacological or therapeutic action only after making some chemical modifications in their structures. There are plenty of prodrugs that are clinically useful. For instance, Hexamine (methenamine) and aspirin are prodrugs of formaldehyde and salicylic acid, respectively, and were formulated as early as the Prodrugs in Clinical Practice in the late nineteenth century. Prodrugs are a pharmaceutically inactive form of a drug that undergoes a transition in vivo to release the parent drug molecule. They are intended to address therapeutic and/or pharmacokinetic issues with the parent drug molecule that would otherwise limit the drug’s clinical usefulness [1]. Bio-precursor prodrugs are prodrugs that undergo structural alterations [2].

It’s easy to get confused between prodrugs, soft drugs (also known as ante drugs), and codrugs (also known as mutual drugs), but each category is structured with different purposes in mind. Soft drugs, unlike prodrugs, are pharmacologically active molecules that are quickly transformed to a less pharmacologically active or even fully inactive type once administered. Soft drug therapies are often used to target particular tissues (such as the eye, skin, or lungs) in order to reduce overall systemic exposure and Adverse Drug Reactions (ADRs) [3]. By modulating physicochemical properties that influence absorption or targeting particular enzymes or membrane transporters, the prodrug approach has been commonly used to enhance drug delivery to their site of action [4]. To be translated into the parent drug, most of the prodrugs currently in clinical use need enzymatic catalysis. The prodrugs that are intended to release the parent drug into the bloodstream after GI absorption comes under this category. These are especially ester derivatives of drugs with carboxyl or hydroxyl groups that are easily converted to the parent drug by hydrolysis with the help of the enzyme esterase [5]. Prodrugs are physiologically inactive analogues of parent drug that needs a chemical or enzymatic modification within the body to liberate the active drug and have better delivery than the parent moiety [6]. Typically, enzymatic or non-enzymatic cleavage occurs to cleave the pro-moiety bonds. Fig. 1 elucidates the concept of the prodrug. High chemical reactivity that hinders the regeneration of the parent drug occurs while incorporating the process of enzymatic cleavage. So, an unconventional approach of non-enzymatic cleavage has been emerged to regenerate the parent drug in which the prodrug activation is not induced by inter and intra-individual variability rather by the process of cyclization [7].

![Fig. 1: Schematic depiction of prodrug concept [1]](image-url)
Cahn and Hepp introduced acetanilide (as Antifebrin) into medical practice in 1867 as an antipyretic agent, and it was the first prodrug term for the first time in the middle of the twentieth century during experiments on changing the structure of chloramphenicol to overcome the antibiotic’s bitter taste and poor water solubility. Chloramphenicol sodium succinate, which has a better water solubility, and chloramphenicol palmitate, which is used in the form of a suspension in children, were the two prodrug forms of chloramphenicol formulated as a result of this research [8].

Prodrug classification

Prodrugs are divided into two categories:

(a.)’Carrier linked prodrugs’

Carrier-linked prodrugs involve a bio-reversible covalent linkage between the active molecule (the drug) and a carrier molecule (also known as a pro-the-moiety). Once in vivo, the parent drug and the carrier molecules are released due to the biotransformation of the carrier linked prodrug. The carrier should ideally be nonimmunogenic, simple to synthesize at a low cost, stable under prodrug administration conditions, and biodegrade to nonactive metabolites [9]. Whereas, in codeugs, two pharmacologically active agents that act a pro-moiety of each other bind together into a single molecule to form a prodrug. Sulphapyridine–5-aminosalicylic acid, indomethacin–paracetamol, and Levodopa (L-DOPA)–entacapone are some examples of co-drugs [10]. The prodrugs can be further divided into two classes based on the site of conversion into the pharmacologically active agent:

• Type I–Metabolized within cells. Especially, Type IA prodrugs metabolize at the cellular targets of their therapeutic actions. Acyclovir, cyclophosphamide, 5-fluouracil, L-DOPA, and zidovudine are some of the examples of Type IA prodrugs and Type IB prodrugs such as carbamazepine, captopril, molsidomine, primidone involve the metabolic tissue (liver) to get converted to the parent drug molecule. Some prodrugs, known as mixed-type prodrugs, belong to several classes.

• Type II–Metabolized outside the cells. Particularly, Type IIA prodrugs are metabolized in GI’s milieu. Whereas the Type IIB are metabolized inside the circulatory system and/or other fluid compartments. Type IIC prodrugs are metabolized within the target cells, such as Antibody Directed Enzyme Prodrug Therapy (ADENPT) and Gene Directed Enzyme Prodrug Therapy (GDEPT) [8]. Mixed-type prodrugs are prodrugs that belong to more than one class [11]. Nearly half of the prodrugs that are currently available are activated by ubiquitous esterases such as acetycholinesterase, butyrylcholinesterases, carboxylesterases, and aryfeстерases, which are found all over the body [9].

Bio precursors v/s carrier prodrugs

The following conclusions were made after establishing a comparative balance sheet for the two prodrug approaches.

• A transient transport moiety is used to modulate the bioavailability of carrier prodrugs; however, no such linkage is inferred for bio precursors, which arise from a molecular alteration of the active principle itself.

• In the case of carrier prodrugs, the lipophilicity of the parent molecule is significantly altered, while it is remarkably unchanged in the case of bio precursors.

• For carrier prodrugs, the bioactivation mechanism is entirely hydrolytic; for bio precursors, it mostly incorporates redox systems.

• For carrier prodrugs, the catalysis that leads to the active principle is hydrolytic (either through general catalysis or through extra-hepatic enzymes). Whereas it appears to be limited to Phase I metabolizing enzymes for bio precursors. Esters and amides are the major groups of carrier-linked prodrugs; Phosphates, carbamates, carbonates, oximes, imines, and Nitrogen containing mannich bases (N-Mannich bases) are some of the other groups of carrier-linked prodrugs [8].

Prodrug modification strategies

To reduce the number of proposed candidates while increasing the explored space of physicochemical and pharmacokinetic properties, careful prodrug design is crucial. In rational prodrug design, the ability to predict target properties is critical [12]. Modifying an active drug’s Absorption, Distribution, Metabolism, and Excretion (ADME) properties necessitates a thorough understanding of the drug candidate’s physicochemical and biological nature. This involves a thorough assessment of drug-likeness, as well as the determination of ADME properties. Data obtained from in vitro and in vivo analysis and computational approaches such as Quantitative Structure-Activity Relationship (QSAR) and molecular docking can get in hand with the assessment of these properties. The overall objective is to design a well permeable drug (absorption) that is metabolically stable (metabolism) enough to reach its target efficiently (distribution) and that could eliminate in an appropriate time (elimination) [13].

Certain questions related to the issue with a drug/bioactive compound must be addressed before the rational design of a prodrug.

• What is the most suitable prodrug category that could be used to solve the problem?

• What are functional groups available in drugs/bioactive compounds for forming a labile linkage?

• Which carrier is the best to use (not for bio precursors)?

• Which linkage is the most appropriate to use?

• What are the enzymes and chemical processes involved in the release of a drug compound? [14].

Problems that need concern can be categorized into three categories based on their pharmaceutical, pharmacokinetic and pharmacodynamic nature [15]. Prodrugs can alter the parent drug’s tissue distribution, efficacy, and toxicity of the parent drug. So, the design consideration at the early stage of preclinical development is critical. When designing a prodrug structure, several important factors should be considered, including:

• Parent drug: What are the functional groups that are amenable to chemical prodrug derivatization in a parent drug?

• Pro-moiety: The disease state, dosage, and length of therapy should all be taken into account when selecting a pro-moiety.

• Parent molecule and prodrug: ADME and pharmacokinetic properties must be made clear.

• Degradation by-products: These factors contribute to the development of new degradation products by having an effect on chemical and physical stability [2].

The following are some examples of how prodrug design can be used:

(1) In enhancing the solubility, bioavailability, dissolution as it is the rate-limiting step for absorption

(2) In enhancing the membrane permeability since it has an effect on drug efficacy.

(3) In modifying the distribution profile before the predetermined effect [16].

Prodrugs for the treatment of diseases like myelodysplastic syndromes, Parkinson’s, malaria, hypertension, psoriasis, and osteoporosis were successfully synthesized. Enhanced dissolution, penetration, and bioavailability of the corresponding drugs were the results obtained in in vitro kinetic analysis [17]. Both drug and prodrug designs can be made by computational methods. Prodrug design is limited to resolving only pharmacokinetic issues relevant to a drug candidate, while drug design requires multi-step procedures to overcome challenges arising from pharmacodynamic and pharmacokinetic characteristics [18]. Since one can optimize all
of the ADMET properties while also extending the commercial life cycle of potential drug candidates, the prodrug approach is a very flexible technique for increasing the use of pharmacologically active compounds. Prodrugs usually have an apparent carrier or pro-moiety that is eliminated by enzymatic or chemical reaction(s), but certain prodrugs release their active drugs after molecular modification, which is certainly referred to as bio precursor prodrugs (e.g., after oxidation or reduction reaction). Where the target prodrug moiety cannot be connected directly to the parent molecule due to steric hindrance or functional properties, typical carrier-linked prodrugs have a synthetic handle, a spacer or linker, between the active drug and the pro-moiety. After the enzymatic or chemical decomposition of the prodrug bond between the pro-moiety and spacer, the spacers cleave conventionally [19]. Prior to the designing of prodrugs, two strategies must be considered.

**Solubility enhancing strategy**

When the parent drug’s low intestinal solubility or dissolution rate is a barrier to bioavailability but not hepatic metabolism, a solubility-enhancing strategy can be used:

- **Parent** is a Biopharmaceutical Classification System (BCS) Class II drug (low solubility and high permeability).
- **High dose/solubility ratio**, i.e., over a pH range of 6–7.5, the highest targeted parent drug dose will only dissolve in volumes>>250 ml.
- **Poor or moderate hepatic clearance** (Clh) in preclinical species.
- **Hepatic extraction ratio** (Eh)<0.5.
- **In vitro assays** showing great permeability
- **Fraction of dose absorbed** (Fa) after oral administration, measured, is low (<25%) [2].

To exemplify, Carbamazepine (CBZ) is an efficient anticonvulsant drug that produces less sedation and cognitive impairment when compared with other anticonvulsants; nevertheless, its aqueous solubility is 120 g/ml forbidding the Intravenous (IV) [20]. To tackle this problem, CBZ prodrugs were synthesized with an enhanced solubility profile. A Cyclooxygenase-2 (COX-2) inhibitor prodrug of a drug named PC407 for parenteral administration was also developed using the sulphonamide group. The prodrug’s solubility increased dramatically, from 1.6 g/ml to 20.3 mg/ml. Prodrug exhibited in vivo analgesic activity and aqueous stability, making it a promising candidate as an injectable formulation for COX-2 inhibition [21]. As evidenced by numerous publications that show up to 400,000-fold increased solubility compared to the parent drug, the prodrug method has been an effective tool for enhancing water solubility. The type of linkage (e.g., ester, amide, carbamate, and phosphate) and the appropriate pro-moiety may decide the prodrug specificity, toxic effects, and ideal bioconversion profile [22].

**Permeability-enhancing strategy**

When the parent drug’s low intestinal solubility or dissolution rate is a barrier to bioavailability but not hepatic metabolism, a permeability-enhancing strategy can be used:

- **Parent belongs to Class III of BCS classification** (low permeability, high solubility).
- **Poor or average Clh in preclinical species**.
- **Eh<0.5**.
- **In vitro assays showing low permeability**
- **Fa<25%** [2].

Secondary pro-moiety which is cleaved by a dissimilar mechanism combines with the primary pro-moiety of the parent drug to form a so-called double prodrug which is an alternate way of preparing prodrugs. Double prodrug and bifunctional prodrugs are completely different and shouldn’t be confused since bifunctional prodrug involves the modification of prodrug at two functional groups. Another form which is named co-drugs exists, where two pharmacologically active drugs that act as a pro-moiety of each other are coupled together. Prodrugs that undergo metabolic bioconversion to the active parent drug by functionally efficient and diversity-resistant hydrolases namely the phosphatases, peptidases, and especially the carboxylesterases are the most common targets for prodrug design [9]. Determining the bioconversion rates, which is regulated by age, health conditions and gender factors, hydrolysis rate, pharmacological and toxicological effects are the key limitations in the design of the prodrug [23].

**Utilization of prodrugs**

The novel formulation of a chemical compound with the intended pharmacological benefits must be chemically stable and should be free from taste and odour problems [1]. In prodrug analysis, the three fundamental objectives stated by Testa are:

- **Pharmaceutical objective**: To enhance the solubility, chemical stability, and organoleptic properties of the active agent and to minimize the irritation and/or pain after local administration; and to solve problems associated with the active agent’s pharmaceutical technology.
- **Pharmacokinetic objective**: to enhance the absorption, tissue distribution, and reduce the pre-systemic metabolism of the active agent.
- **Pharmacodynamic objective**: to enhance the therapeutic index, reduce toxicity, and to design codrugs [24].

**Masking the taste**

Extremely bitter compounds like chloramphenicol are limited for administration to the pediatrics. So, chloramphenicol palmitate, a prodrug of chloramphenicol that possess low aqueous solubility, was formulated to counter the bitter taste [25]. Drug/prodrug must possess a satisfactory aqueous solubility to interact with the taste receptors by being solubilized in the saliva. Hence, the bitter taste of the drug was counteracted by decreasing the aqueous solubility. Table 1 lists some of the prodrugs that are designed to mask the taste.

**Table 1: List of some prodrugs used to mask the taste.**

| Parent drug | Pro-drug | References |
|-------------|----------|------------|
| Chloramphenicol | Palmitate | [25] |
| Clindamycin | Palmitate | [26] |
| Erythromycin | Ethyl succinate Ethyl carbonate | [1, 27] |
| Oleandomycin | Acyl ester and N-oxide | [28] |
| Lincomycin | Phosphate ester Carbonate ester | [26, 29, 30] |
| Sulphafurazole (sulfisoxazole) | N’-Acetyl | [31] |
| Dextroropropoxyphene | Napsylate | [31] |

**Toxicity reduction**

All the therapeutic agents are desired to possess negligible or no clinical toxicity. Prodrug designing has made it easier for researchers to achieve this no-toxicity level. An ample number of examples exist to support this development. One of which is Sulindac, a clinically pertinent prodrug with enhanced absorption and reduced Gastrointestinal (GI) toxicity [1].

**Prolongation of drug action**

Prodrugs can be used to control the release of an active drug by altering its aqueous solubility and dissolution properties that regulate the active drug’s release rate, absorption rate, and tissue distribution. Development of a number of subcutaneous or intramuscular sustained-release depot injections, which keep therapeutic plasma levels of a parent drug stable for weeks to
months has been an application of the prodrug concept. Prodrugs with a long duration of action may be due to sustained bioconversion after oral administration. Prodrug bonds, such as amides, are commonly used in sustained release strategies because they are relatively resistant to bioconversion. Lisinopril (Fergon) is a Levo lysine (L-lysine) amino acid amide prodrug of dextroamphetamine that has been on the market since 2007 [3]. To maintain adequate plasma concentrations of a drug that is rapidly cleared from the body, frequent dosing with conventional dosage forms is required. Patient compliance is also low due to the repeated dosing of short half-life medications, which results in sharp plasma concentration-time profiles. Such challenges are resolved by the sustained or prolonged release of a drug. Traditional formulation approaches are often efficient but are limited to the sustained release, which on the other hand, could be facilitated by the combination of prodrug with a suitable delivery system.

Sinkula in 1978 summarised various methods for prolonged drug action by altering the physiochemical properties of the drug in the form of a prodrug. The made alterations were:

- The Absorption rate and degree
- The extent and rate of prodrug activation
- Protein or tissue binding process time and their extent
- The extent to which tissue or organs are localized, distributed, and then released from those centres.

The inability to rationally predict the impact of the prodrug structural alteration on the subsequent pharmacokinetics has generally prevented the production of prodrugs for the prolongation of therapeutic action [1].

Alteration of drug solubility

Poor aqueous solubility is a major issue that restricts the clinical use of many medications and drug molecules. The prodrug strategy is one of the many methods used to solve this flaw. Because of the ionic existence of the phosphate group, using esters and amides of phosphoric acid to improve a drug's aqueous solubility is a common practice. It's worth noting that phosphate derivatives have excellent chemical stability, sometimes surpassing that of the parent compound. Esterification of amino acids is another way to improve aqueous solubility [8]. To increase aqueous solubility, several prodrugs have been designed [20]. Even for prodrugs that are designed to promote membrane penetrability, stability, and other such therapeutic characteristics, optimal aqueous solubility is required to facilitate oral uptake and for parenteral.

Isavuconazonium sulphate, which was recently licensed, used a novel prodrug technique to increase aqueous solubility [3]. Depending on the drug's ultimate application, the prodrug strategy can be used to significantly alter the solubility of a particular compound. Although the palmitate ester of chloramphenicol has proven to be effective in oral formulations, chloramphenicol sodium succinate, a much more water-soluble type, that is converted into free chloramphenicol by esterases has been formulated for parenteral delivery [32]. Another type of compound with low water solubility is steroids. Sodium succinate water-soluble prodrugs are available for glucocorticoids such as betamethasone [1].

Reduction of pain at injection sites

Precipitation of drug due to cell lysis and corrosive function of the drug effects in pain and irritation. Clindamycin 2-phosphate, a prodrug of antibiotic clindamycin with a solubility of >150 mg/ml, triggers no irritation or pain upon Intramuscular (IM) administration, unlike the parent drug itself, which has the solubility of 3 mg/ml [33, 34]. Inside the body, Phosphatase enzymes convert the resulting prodrug, which has no inherent antibacterial function, into clindamycin with a half-life [1/2] of 10 min [35].

Enhancement of chemical stability

Chemical stability over a suitable period of time is a critical prerequisite for all drug products. If formulation methods can’t lend a hand in overcoming the stability problem, prodrug strategy comes into action. This involves the chemical modification of the functional groups that causes the instability and the physical modification that ensures less interaction between the drug and the unstable media [1]. For instance, auto-amo group of aqueous sodium ampicillin causes instability [36]. The reaction of ampicillin with acetone produces hetacillin, which is a prodrug of ampicillin and could easily dissociate into ampicillin and acetone when IV dilution 20 mg/ml is carried out. Yet, only the partial dissociation of hetacillin takes place while using concentrated solutions since auto amino is hindered due to the bond between ampicillin and acetone but could easily dissociate in vivo. This leads to the expected stability of the compound [1].

Enhancement of oral absorption

Bioavailability after oral administration is always dissolution rate-limited if a drug is more water-insoluble. The absorption rate of highly polar compounds is limited by GI mucosa. The degree of lipophilicity is necessary for the drugs that are absorbed by passive diffusion through the GI tract. Enhanced lipophilicity in the prodrug design of polar compounds is ideal for its GI absorption. Several penicillin derivatives use this strategy [1].

Improving metabolism

The following are the methods used to enhance drug metabolism

(i) Steric shields: Chemical and enzymatic degradation affect some functional groups more than others. Esters and amides, for instance, tend to hydrolyze more than carboxamides and oximes, where steric shields could prevent this by increasing their stability. Steric shields were created to prevent a nucleophile or a nucleophilic centre on an enzyme from approaching the susceptible group.

(ii) Electronic effects of bio-isosteres: Through this strategy, some labile functional groups are protected via electronic stabilization. For instance, a more stable ester functional group urethane is formed by the replacement of a methyl group with an amine group in an ester. The methyl and the amine group have the same size and valance but differ with the varying electronic properties as they can donate electrons to the carbonyl group through their inductive effect, lowering the electrophilicity of the carbonyl carbon and thus preventing hydrolysis by stabilizing it.

(iii) Stereo electronic modification: Labile groups are stabilized by the combination of steric hindrance and electronic stabilization.

(iv) Metabolic blockers: By the introduction of some polar group at some specific site, the metabolism of certain drugs takes place. For instance, replacing the hydrogen group with a methyl group at the 6th position of the mestrogel acetate, which is an oral contraceptive, metabolism of the drug gets prevented and thus extending the duration of action.

(v) Exclusion of susceptible metabolic groups: Some chemical molecules are labile to metabolic enzymes. For instance, a methyl group on an aromatic ring undergoes frequent oxidation to form carboxylic acid results in rapid elimination of the drug.

(vi) Group shifts: If the metabolically vulnerable group is not involved in essential binding interactions within the active site of the receptor or enzyme, it is possible to remove or replace it. Masking of the vulnerable group using prodrug or shifting the group is done if the said group is crucial. This method was used to create salbutamol from its analogue neurotransmitter, norepinephrine, in 1969. Catechol Ortho-methyl transferase (O-methyl transferase) metabolizes norepinephrine by methylating one of its phenolic groups.

(vii) Ring variation: Altering the rings enhances the metabolic stability since some of the ring systems can be amendable to metabolism. For instance, Stability is enhanced while replacing the imidazole ring with a 1,2,4-triazole ring [13].

To enhance permeability

Increasing lipophilicity in order to improve passive transcellular absorption while maintaining a standard level of solubility becomes the primary goal of prodrug derivatization of polar compounds [39]. When the parent drug's low intestinal permeability is a significant barrier to bioavailability, a permeability-enhancing strategy can be used [40].
Selective delivery nature of prodrugs

To resolve various undesirable drug properties, prodrugs can be engineered to target particular enzymes or carriers by including enzyme or carrier-substrate specificity. Targeted-prodrug design necessitates a thorough understanding of specific enzymes or carrier systems and their characteristics [39]. Drug aiming for specific tissues, locations, enzymes, and other factors accounts for a significant portion of drug-related experiments around the world. One strategy being vigorously sought is the use of prodrugs to achieve site-specificity with minimal side effects [1, 4]. For a site-specific delivery, the configuration of at least 3 factors needs to be made by using the prodrug strategy.

- The prodrug uptake must be rapid and the perfusion rate must be effectively minimal for the easy targeted action.
- The cleavage of prodrugs to the active drug must be performed once at the site of action.
- The retention of the active drug must be performed by the tissues after the generation of the active drug [41, 42].

The targeted drug release depends on the enzymes present in the target organ. After a thorough understanding of the cleavage produced by that specific enzyme, the design of the corresponding prodrug is altered [43]. Site-selectivity is the most prominent feature that a prodrug could offer that can be accomplished in four distinct ways: by passive supplementation of the drug into the organ, transporter facilitated delivery, selective enzymatic metabolism, antigen targeting [44].

Prodrugs for CNS delivery

The Blood-brain barrier (BBB) which is formed by endothelial cells, has become a permeability factor for Central Nervous system (CNS) acting drugs. It maintains the chemical environment suitable for brain function and facilitates the transport of nutrients but prevents the entry of blood-borne and neurotoxic drugs into the brain [8]. Also, the CNS neurotransmitters and neuromodulators are restricted to reach the circulatory system [45]. Having known the transport mechanisms and enzyme activity at BBB, significant improvement in CNS-specific delivery could be achieved. For instance, levodopa is a prodrug of dopamine. It is converted back to dopamine and exhibits its pharmacodynamic activities since dopamine is a hydrophilic molecule [2]. Enhancing the lipophilicity of the parent drug has conventionally been used to increase CNS drug concentration. The prodrug must easily reach the tissue of the brain, must easily convert back to the parent drug, must be more site-specific, and the parent drug should have prolonged retention within the tissue of the brain for this strategy to be effective [46]. To ease the transportation of drugs into CNS, three methodologies have been used:

a) Involving lipidization of molecules to boost passive diffusion.

b) Improving carrier or receptor-mediated transport via BBB

c) Lowering the drug’s active efflux from the brain into the blood. To achieve the intended therapeutic effect in CNS, the bioconversion of prodrug should be extended in peripheral tissues but must be rapid in the tissues of the brain, especially the bioconversion must be limited to specific brain areas due to its anatomical complexity [47, 48].

Prodrugs that can use carried-mediated transport pathways make for interesting targets in drug development. Levodopa, with its L-type amino acid transporter 1, is one best example of this since it crosses the BBB and could treat Parkinson’s, unlike the hydrophilic dopamine. Despite this, more developed carrier-mediated prodrugs have been designed to facilitate intestinal absorption after oral administration. Intestinal peptide transporter 1 lends a hand in improving the oral absorption of numerous amino acid prodrugs [3].

Prodrugs for hepatic delivery

The liver, which is the major metabolizing organ, may have enormous potential for organ-specific delivery of drugs since it contains a wide range of liver-specific metabolizing enzymes sufficient for prodrug activation [51]. Hep-Direct prodrugs are a new type of Cytochrome P (CYP)-activated prodrug that is designed to target the liver [52]. Hepatic-direct prodrugs get arrested in blood and other tissues except for the liver due to resistant activity towards the esterase cleavage. Certain drugs could benefit from this prodrug strategy, which could increase their efficacy and safety. Adefovir dipivoxil, a prodrug of adefovir, is developed to enhance the oral bioavailability. Yet, adefovir dipivoxil caused nephrotoxicity. As a result, a hep direct prodrug pradefovir, designed to overcome that nephrotoxicity and to enhance the anti-hepatitis B virus (HBV) action. The CYP3A4 isozyme hydroxylates pradefovir at the Carbon (4)-methylene site in the liver, then enacts a rapid ring opening followed by a b-helminic response to produce adefovir and vinyl ketone [53]. Nucleotide kinases transform adefovir to adefovir diphosphate, whereas the aryl vinyl ketone generates a glutathione conjugate. Preclinical studies which involved rats and monkeys show that the pradefovir produced a substantial increase in adefovir and its mono- and diphosphates exposure in the liver compared to the kidney, implying that pradefovir has strong liver targeting properties and may have a lower risk of nephrotoxicity [54]. Pradefovir mesylate, adefovir that is being used for hepatitis B therapy. Pradefovir is oxidised primarily in the liver’s hepatocytes and is catalysed by CYP450 enzyme. Pradefovir has shown a better effectiveness with low systemic adefovir levels in Phase II trials in hepatitis B patients, which contributes to the affirmations for liver targeting [2]. Pradefovirs became the most effective redirect prodrug for hepatic delivery of adefovir. The application of hepatic-directed prodrugs includes the designing of cystalabine 50-monophosphate and lamivudine for liver tumour and HBV, respectively [55]. Chronic HBV, Hepatitis-C virus (HCV) and hepatocellular carcinoma patients have been promisingly benefited by the Hep-Direct prodrugs [53].

Prodrugs for colonic drug delivery

Various anti-inflammatory agents and other drug molecules are intended for colon specific delivery to treat Inflammatory bowel disease (IBD) with the help of this prodrug strategy. Coating with biodegradable and pH sensitive polymers, and the formation of biodegradable complexes are just a few of the options for this type of delivery. All these methodologies tend to reduce the release and absorption in the stomach thereby making the effective delivery to the colon [56]. Amino salicylates, immunosuppressants and corticosteroids are the only medications used to treat IBD. When making prodrugs, the carrier of choice is determined by the functional group on the drug compound that can be conjugated with the carrier [41, 57]. As colon-targeted drug delivery compounds, various forms of prodrugs linked to different carriers have been prepared and evaluated. Absorption and stability in the upper GI tract, hydrolysis selectivity, and the concentration of the drug regenerated in the colon are among the parameters assessed. Some of the conjugates tested for colon-specific delivery include amino-acid, glucuronide, glycoside, azo, cyclodextrin and dextran. A prodrug is generally active as a colon drug carrier if it is hydrophilic and rigid to reduce absorption from the upper GI tract, and is converted into a more lipophilic compound in the colon for absorption [56].

Prodrugs for transdermal drug delivery

Amino acid and ester prodrugs have sparked interest in the field of transdermal drug delivery science and are the widely used prodrugs [58]. One of the prodrug applications in transdermal delivery is that the absorption rate of the drug can be enhanced by the modification of the skin permeability through physical and chemical alterations, which are achieved by manipulating the interaction between the drug-skin and drug vehicle [59]. If the parent drug has a low affinity for the skin, a prodrug strategy comes in handy to enhance the drug permeation and is readily converted back to the parent drug inside the skin with the aid of certain metabolic processes and enzymes. This drug’s partitioning behavior can be enhanced by modifying it chemically to develop a hydrophilic or lipophilic prodrug, depending on the vehicle used [60]. Amino acids have been frequently utilized as pro-moieties in designing prodrugs since they are safe on in vivo and are readily ionizable [61]. Ester prodrugs are designed by utilizing a carboxylic acid group with an alcohol group, whereas amide prodrugs are designed by combining an amine and a
carboxylic acid group [58]. Transdermal phenol delivery has significant clinical benefits, particularly in the fields of narcotic analgesics and in therapies involving hormone replacement [62].

**Prodrugs for tissue targeting**

The majority of antitumor and antiviral drugs are cytotoxic and have a variety of side effects. Tissue-specific drug delivery has a lot of promise in terms of both safety and efficacy. To target tissue delivery, multiple prodrug methods have been studied, including:

(a) Enzymatic activation of the prodrug with the aid of enzymes localized at the target site.

(b) Tissue-specific transporters deliver prodrugs to their target tissues.

(c) Drug and molecule conjugates that are uniquely coupled to target tissues [53].

**Prodrugs in cancer therapy**

Chemotherapy is a fundamental cancer treatment. Chemotherapy for different cancers has made significant strides over the past few decades. Most of the anticancer drugs currently on the market work by inhibiting cell proliferation or by stopping the cell cycle at a specific phase. Since oncstatic drugs have low selectivity, they affect both neoplastic and rapidly proliferating normal cells, including bone marrow, epithelia, gut, lymphatic cells, hair follicles, and gametes. Anticancer drugs' ineffectiveness and long-term use are hampered by their lack of specificity and related toxicity. As a result, it's unsurprising that there's a serious need to boost their specificity. Prodrug design is one of the most promising approaches to overcome this problem. An anticancer prodrug should be delivered to cancer cells, where recombinant enzymes can be administered to tumour cells with the help of antibodies or genes to broaden the spectrum of tumour cells to produce the therapeutic activity. Both enzymes and prodrugs must follow certain criteria in order for this technique to be clinically effective. The enzymes can be either nonhuman or human proteins that are only produced at low levels in normal tissues [74]. The conjugated prodrug remains inactive until it reaches the tumour site and once reached, it undergoes transformation and binds to the surface of tumour cells, releasing carrier molecules to restore its efficacy. Drug conjugates can thus be thought of as tumour-activated prodrugs (TAPs). While most of the typical prodrugs are transformed to parent drugs by hydrolysis, restoring TAP activity should preferably depend on interaction with antigens and receptors expressed on the cancer cells [75]. Exogenous enzymes can be administered to tumour cells with the help of antibodies or genes to broaden the spectrum of tumour cells to produce the therapeutic activity [76]. Onco-static drug precursors are used in enzyme activated prodrug strategies (ADEPT, GDEPT). Mitomycin C phosphate, Cyclophosphamide ifosfamide, and Etoposide phosphate are few examples of enzyme-activated prodrugs [77, 78].

**Recent approaches and proposals**

Since 1993, the number of drug patents has risen exponentially (by over 2000% in 2002) with patents for cancer treatment accounting for 37% of all patents [79]. Prodrugs make up about 1% of all medicines marketed in the world today [8]. Prodrugs are increasingly being used to enhance drug characteristics, and their influence and advancement are projected to increase [80]. The Food and Drug Administration (FDA) approved 30 prodrugs from 2008 to 2017 are listed in table 2.

**Table 2: FDA approved prodrugs in the period 2008 to 2017**

| No | Prodrug | Mechanism of action | Prodrug strategy and improved property | Approval date | Reference |
|----|---------|---------------------|---------------------------------------|--------------|-----------|
| 1  | Fosaprepitant (Emed, fosaprepitant dimeglumine) | Prevention of chemotherapy-induced nausea and vomiting (SIP antagonist) | Improved solubility allowing intravenous administration | 25 Jan 2008 | [81] |
| 2  | Fesoterodine (Tovia, fesoterodine fumarate) | Overactive bladder (competitive α3B-adrenoceptor antagonist) | Reduced interpatient pharmacokinetic variability | 31 Oct 2008 | [82] |
| 3  | Fospropofol (Lusedra, fospropofol disodium) | Anaesthetic (sedative–hypnotic agent) | Aquous solubility property of propofol is enhanced from 150 μg ml⁻¹ to ~500 mg ml⁻¹ | 12 Dec 2008 | [83, 84] |
| 4  | Prasugrel (Effient, prasugrel hydrochloride) | Reduction of thrombotic and cardiovascular events (inhibitor of the platelet P2Y12 receptor that is Bioprecursor prodrug that releases a thiolactone by ester hydrolysis and undergoes further metabolic | 7 Oct 2009 | [85] |
| No | Prodrug                                      | Mechanism of action                                      | Prodrug strategy and improved property                                                                 | Approval date | Reference |
|----|---------------------------------------------|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------|--------------|-----------|
| 5  | Romidepsin (Istodax)                        | Activated by Adenosine diphosphate (ADP)                 | Activation to a form that sulfonylates the platelet P2Y12 receptor                                      | 5 Nov 2009   | [87]      |
| 6  | fingolimod (Gilenya, fingolimol hydrochloride) | Multiple sclerosis. Sphingosine-1-phosphate (S1P) agonist. | Hydroxyl form is a more lipophilic prodrug that enables permeation                                        | 21 Sept 2010 | [87]      |
| 7  | Dabigatran etexilate (Pradaxa, dabigatran etexilate mesylate) | Thrombomodulin (direct inhibitor of thrombin)            | Permeation through increased lipophilicity                                                              | 19 Oct 2010  | [88]      |
| 8  | Ceftaroline fosamil (Tcefaro, ceftaroline fosamil monoacetate monohydrate) | Pneumonia or skin or skin structure infections (antibacterial; inhibits cell wall synthesis) | Improved solubility (from 2.3 mg ml⁻¹ to 100 mg ml⁻¹) thus allowing intravenous administration | 29 Oct 2010  | [89]      |
| 9  | Azilsartan medoxomil (Edarbi, azilsartan medoxomil monohydrate) | Hypertension (angiotensin II antagonist)                  | The absolute bioavailability of azilsartan after oral intake of the prodrug tablet is ~58%              | 25 Feb 2011  | [90]      |
| 10 | Gabapentin enacarbil (Horizant)              | Restless leg syndrome or postherpetic neuralgia. Gamma-aminobutyric acid (GABA) and calcium-channel modulator. | Increased permeation via gastrointestinal transporters                                                  | 6 Apr 2011   | [91]      |
| 11 | Abiraterone acetate (Zytiga)                | CYP1A1 inhibitor                                         | Rapid dissolution and subsequent prodrug hydrolysis may generate an intraluminal supersaturated solution of abiraterone, leading to better absorption | 28 Apr 2011  | [92]      |
| 12 | Tafluprost (Zioptan)                        | Intraocular hypertension (prostaglandin analogue)        | Ocular permeation through increased lipophilicity                                                      | 10 Feb 2012; | [93, 94]  |
|    |                                             |                                                          | first approval in Germany on 1 Mar 2008                                                                |              |           |
| 13 | Dimethylfumarate (Teckflera)                | Multiple sclerosis                                        | Increased permeation, probably through increased lipophilicity • Marketed in Germany since the mid-1990s for the treatment of psoriasis | 27 Mar 2013  | [95]      |
| 14 | Eslicarbazepine acetate (Aptiom)            | Epilepsy (anticonvulsant; voltage-gated sodium-channel blocker) | Changes the metabolic profile to reduce potential drug interactions, have a more favourable safety profile Improved membrane permeation and targeting to the liver | 8 Nov 2013   | [96, 97]  |
| 15 | Sofosbuvir (Sovaldi and others)             | HCV infection (nucleotide analogue; HCV NS5B polymerase inhibitor) | Improved membrane permeation                                                                            | 6 Dec 2013   | [98]      |
| 16 | Drosidopa (Northera)                        | Neurogenic orthostatic hypotension or Parkinson disease-off-label (dopamine precursor) | Improved brain permeability267 • Marketed in Japan since 1989                                         | 18 Feb 2014  | [99]      |
| 17 | Tedizolid phosphate (Silvextro)             | Acute bacterial skin infections (oxazolidinone of antibacterial class; binds 50S ribosomal subunit)       | Improved solubility                                                                                     | 20 Jun 2014  | [100]     |
| 18 | Isavuconazonium (Cresemba, isavuconazonium sulfate) | Invasive aspergillosis or invasive mucormycosis (azole antifungal; inhibits synthesis of ergosterol, a cell wall component) | Improved solubility                                                                                  | 6 Mar 2015   | [101]     |
| 19 | Sabolicrul sodium (part of Entresto, which contains anionic forms of sabolicrul and valsartan, sodium cations and water) | Heart failure (neprilysin inhibitor)                     | Likely improved permeability through increased lipophilicity                                           | 7 Jul 2015   | [102]     |
| 20 | Uridine triacetate (Xuriden)                | Hereditary orotic aciduria (pyrimidine analogue for uridine replacement)                               | Improved membrane permeability through increased lipophilicity                                         | 4 Sept 2015  | [103]     |
| 21 | Aripiprazole lauroxil (Aristada)            | Schizophrenia (antipsychotic; partially owing to inhibition of the D2 receptor)                      | Sustained release and prolonged duration of action following intramuscular administration               | 6 Oct 2015   | [104]     |
| 22 | Tenofovir alafenamide (Gemoya and other combinations, tenofovir alafenamide hemifumarate) | Human immune deficiency virus (HIV)-1 infection (HIV-1 nucleoside analogue reverse-transcriptase inhibitor) | Increased permeability through increased lipophilicity and enhanced intracellular trafficking247,248 • Prodrug targets T cells for HIV-1 but is also cleaved in the liver and thus is also used for HBV infection | 5 Nov 2015   | [105, 106]|
| 23 | Ixazomib citrate (Ninlaro)                 | Multiple myeloma (reversible proteasome inhibitor)                                                   | Likely increased stability of oxidatively unstable boronic acid                                        | 20 Nov 2015  | [107]     |
| 24 | Selexipag (Uptravi)                         | Pulmonary hypertension (non-                           | Prolonged duration of action                                                                           | 22 Dec 2015   | [108, 109]|
While approximately three prodrugs were approved each year during the ten-year period from 2008 to 2017, it is noteworthy to note that drugs accounted for 17% of New chemical entity (NCE) approvals (30% biologics) and the remaining 70 percent of NCEs were prodrugs [5].

Combinatorial chemistry and high throughput screening make it possible to find a new chemical entity with high therapeutic efficacy [116]. These techniques too have limitations due to the undesired physicochemical properties and to surpass this, chemical alterations or new formulation strategies need to be developed to promote the efficacy of the compound [80]. Gorrod had cited four mechanisms causing toxicity in a review of 'potential hazards of the prodrug approach.'

1. A non-parent drug-mediated toxic metabolite formation.
2. During the prodrug activation phase, a vital constituent, for instance, glutathione is consumed. Hepatic cells are protected by l-cysteine since it is needed for glutathione biosynthesis.
3. An "inert" process takes place while a toxic derivative is being formed from a transport moiety.
4. Discharge of pharmacokinetic modifiers [43].

Numerous diseases, whether infectious or caused by normal physiological abnormalities, have benefited from the prodrug approach. Prodrug strategy which is a rational drug design alternative makes a contribution to the recent advancements in the biotech field. Prolongation of action and toxicity reduction has had alternative makes a contribution to the recent advancements in the approach. Prodrug strategy which is a rational drug design necessary [118, 119]. Although directing the prodrug to a targeted site is difficult in this strategy, it is an effective tool for improving physicochemical and/or biopharmaceutical properties to overcome the in vivo limitations of a drug. Modifying the drug kinetics, achieving extended drug release, lowering toxicity, are some of the possible goals of this approach [120].

### The traditional approach of prodrugs

To enhance the permeability of lipophilic groups and the solubility of the parent drug, the involvement of covalent bond formation between the parent and the hydrophilic functional groups is necessary [118, 119]. Although directing the prodrug to a targeted site is difficult in this strategy, it is an effective tool for improving physicochemical and/or biopharmaceutical properties to overcome the in vivo limitations of a drug. Modifying the drug kinetics, achieving extended drug release, lowering toxicity, are some of the possible goals of this approach [120].

### The molecular revolution in biomedicine

It is a modern strategy in designing the prodrugs where Cellular parameters such as influx and efflux of transporters, protein expressions are considered to enhance the targeting properties of a drug [121–123]. Specific targeting of the enzymes and transporters include the binding of carrier and parent drug. Manipulating the mechanism and site of release becomes a major advantage and a challenge in designing the prodrugs which can be optimized by several computational methods [124, 125]. A research group has developed and tested a novel phospholipid-based prodrug approach [126]. Currently, multiple clinical trials are being performed to assess the effectiveness of prodrugs for various therapeutic purposes. Some of those are listed in table 3. Some of the few notable examples include a study that is ongoing to assess the potency of the anticancer prodrug named NUC-1031, which was developed using Proticid technology, in patients with severe biliary tract cancer [127]. The AKR1C3-activated prodrug OBI-3424 is a prodrug currently being tested in patients with unmanageable T-cell acute lymphoblastic leukaemia [128].

### Table 3: Some ongoing clinical trials for prodrugs

| Prodrug                  | Therapeutic uses                                               | Reference |
|--------------------------|----------------------------------------------------------------|-----------|
| Tenofovir alafenamide    | HIV/AIDS and chronic hepatitis B                               | [129]     |
| Anticancer prodrug 'NUC-1031' | Severe biliary tract cancer                                    | [127, 130]|
| AKR1C3 activated 'OBI-3424' | Relapse acute lymphoblastic leukaemia, hepatocellular carcinoma, and castrate-resistant prostate cancer | [80, 128] |
| Baloxavir marboxil        | Influenza                                                       | [131]     |
| Remdesivir               | Coronavirus disease 2019 (covid-19) in adults and adolescents with pneumonia requiring supplemental oxygen | [132]     |
CONCLUSION
In addition to the beneficiary aspects of prodrugs in reducing ADR, it also plays a role in the target specificity of certain drugs. Multiple studies have shown that prodrugs can positively impact the pharmacokinetic and pharmacodynamics of a drug in many ways, including the targeted delivery, prolongation period, and therapeutic index of specific drugs. Also, the prescription of prodrugs has been widely regarded as the first priority drugs in case of chemotherapy. Hence, prodrug formulations have been considered as one of the vital fields in recent times.

ACKNOWLEDGMENT
The authors are very much thankful to the Crescent School of Pharmacy, B. S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, India for providing essential services to carry out the study.

FUNDING
Nil

AUTHORS CONTRIBUTIONS
All authors have contributed equally.

CONFLICT OF INTERESTS
We know of no conflict of interest associated with this publication.

REFERENCES
We know of no conflict of interest associated with this publication.

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