

An Overview of Highly Efficient Prodrug Strategies in Design, Development, Bioactive Pathway and Recent Therapeutic Applications

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Chandravadivelu *et al.*: Prodrug Strategies and Recent Applications

A prodrug is a chemically inert drug precursor, which upon biotransformation liberates the pharmacologically active parent compound. It is also called proagent, latentiated drug, bio reversible derivative and congeners. There are several reasons to utilize prodrug strategy in drug design. The drug is insufficient in solubility, absorption, distribution, lack of target site-specificity, prolonged-release, toxicity, instability, poor acceptance and formulation issues can be made to prodrug. The concept of prodrug acts as a significant alternative to solve pharmaceutical and pharmacokinetic-related problems of drug molecules. Though a few studies have been concerned with some aspects of prodrugs, surprisingly there is no review on the complete information about prodrug and their recent applications. In the present study, an attempt had been made on the design, synthesis, development, bioactive pathway and latest research findings of newly synthesized prodrugs and their therapeutic uses.

Key words: Drug design, prodrug, synthesis and development, bioactive pathway, recent therapeutic applications

The word Prodrug was introduced by Adrien Albert in 1951 and the concept was identified by Harper in 1959^[1,2]. According to Harper, the word latentiated indicating to drugs that need bioactivation^[3]. This description is the most suitable even at present moment and is consistent with the International Union of Pure and Applied Chemistry (IUPAC) definition which states that a prodrug is a biologically inactive molecule that is altered into an energetic active drug by an enzymatic or chemical process^[4]. It is also called a bio-reversible derivative, latentiated drug, pro-agent and congeners etc.,^[5-7]. Many enzymes involving the activation of prodrugs are oxidoreductases like DT-diaphorase, β -glucuronidase, carboxylesterase and Cytochrome P450 (CYP450)^[8-10]. Pharmaceutical scientists are often facing serious formulation problems including inadequate oral absorption, poor solubility, instability, rapid metabolism, short half-life and toxicity^[11-13]. These problems lead to the growth of a comparatively great number of prodrugs. The healing power of the prodrug is enhanced by not only minimizing the unacceptable and harmful properties but also by maintaining the high selectivity on the targeted

site^[14,15]. In addition to that several prodrugs have gained massive experimental success in clinical studies. Therefore, the prodrug approach is quickly becoming a fundamental part of the drug discovery process. At present, 5 %-8 % of the therapeutic agents are categorized as prodrugs, and around 15 % of all new drug compounds are synthesized each year as prodrugs^[16]. Prodrugs have been utilized in a broad diversity of therapeutic areas including antiulcer^[17], analgesic^[18], anti-hypertension^[19], anti-influenza^[20], anti-biotics^[21], anticoagulant^[22], antifungal^[23], anticancer^[24], anti-inflammatory^[25] and anaesthetic agents^[26].

In recent years, many scientists were prepared different prodrug molecules through carrier-linked and chemical modification^[27,28]. But, there is no review concerning the complete information including the recent progress of prodrugs and their therapeutic activities. Therefore, the present work

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has been targeting a comprehensive summary of design, synthesis, development, bioactive pathway and recent therapeutic applications.

PREPARATION AND DEVELOPMENT OF PRODRUGS

Reason for prodrugs:

There are several reasons why one may wish to develop a prodrug strategy in drug design. Drugs having poor solubility, inappropriate absorption and improper distribution, lack of target site-specificity, prolonged release, reduced toxicity, instability, poor acceptance and formulation issues can be made to prodrug^[29-31].

Enhances the solubility:

Poor solubility and low dissolution rate of poorly water-soluble drugs in the aqueous gastrointestinal fluids often cause insufficient bioavailability. The major challenge with the design of oral dosage forms lies in their poor bioavailability. Consider an active drug that is insufficiently soluble in water, so that it cannot be injected in a small dose. The solubility of active drug molecules is enhanced by converting them into prodrugs of phosphate, ester, N-Mannich base and hydroxymethyl groups etc., a water-soluble group could be attached which could be metabolically released after drug administration. For example, methyldopa is a zwitter ion and although charged, its crystal structure causes it to be only poorly water-soluble. To formulate this drug for injection, it is converted into ethyl ester which may be formulated as hydrochloride salt (fig. 1).

Improve the absorption and distribution:

If the drug is not absorbed and transported to the

target site insufficient concentration it can be made more water-soluble or lipid soluble, depending on the desired site of action. Once absorption occurs or when the drug is at the appropriate site of action, the water or lipid soluble group is removed enzymatically. The low absorption of antiviral acyclovir is increased by orally active prodrug valacyclovir, the L-valyl ester of acyclovir which is rapidly hydrolysed by first-pass intestinal metabolism. The prodrug is converted into acyclovir in humans by the hydrolase enzyme present in the liver and gut.

Improve the target site-specificity:

Specificity for a particular organ or tissue can be made, if there is a high concentration of uniqueness of enzymes, present at that site which can cleave the appropriate appendages from the prodrug and unmask the drug. Gamma Aminobutyric Acid (GABA) is too polar, to cross the blood-brain barrier, its effective lipophilic analogue of GABA (probabide) that crosses the blood-brain barrier, releases GABA inside the brain and shows anticonvulsant activity. In this example, the lipophilicity was increased, so that they could diffuse through the membranes.

Prolonged-release:

It may be desirable to have a steady low concentration of a drug released over a long period. The drug may be altered, so that it is metabolically converted to the active form slowly. For example, fluphenazine has a shorter duration of action (6-8 h), but prodrugs fluphenazine decanoate has duration of activity of about a month.

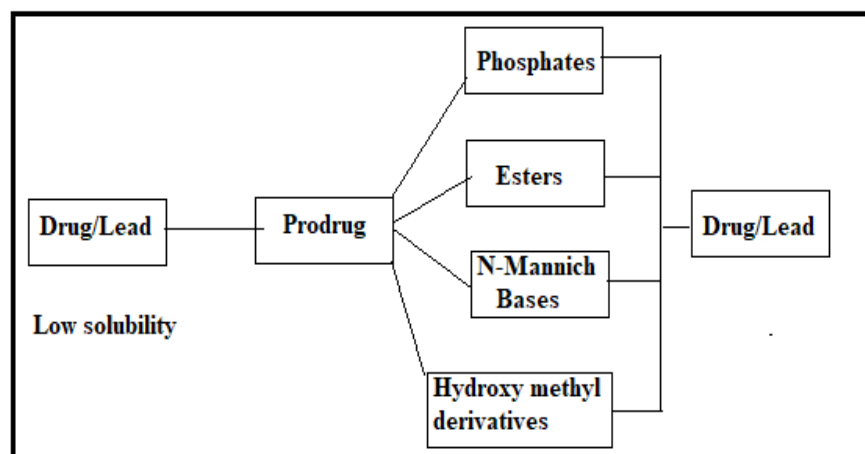


Fig. 1: Enhances the solubility of drug molecule by prodrug of phosphate, esters, N-Mannich bases, hydroxymethyl derivatives

Reduces the toxicity:

A drug may be toxic in its active form and would have a greater therapeutic index if it were administered in a nontoxic, inactive form that can be converted to the active form only at the site of action. Epinephrine is a drug used in the treatment of glaucoma. The drug has only limited local use because of poor penetration. But higher doses of it irritate the eyes and have undesirable cardiovascular effects. The ester prodrug dipivaloloyl epinephrine increases lipophilicity, which has better intraocular penetration and thus side effects are limited.

Improve the stability of the drug:

Many drugs are unstable and may either break down on prolonged storage or are degraded rapidly on administration. Several drugs may decompose in gastrointestinal tract when used orally or metabolized and rendered inactive, before when it reaches the site of action. Although enteric coatings may be used, it is also possible to utilize prodrug design to overcome this problem. The structure may be modified to block that metabolism until the drug is at the desired site. An antineoplastic drug azacytidine hydrolyzes readily in acidic pH, but the bisulphite prodrug is more stable.

Improve the patient acceptance:

An active drug may have an unpleasant taste or odour, produces gastric irritation, or causes pain when administered. The structure of the drug can be modified to alleviate these problems but once administered, the altered drug can be metabolized into the active drug. Several drugs (nicotinic acid, salicylic acid, kanamycin and diethylstilbesterol) cause irritation and damage to the gastric mucosa. The prodrug of the above-mentioned therapeutic

agents overcomes such problems of gastric distress.

Solving the formulation problems:

If the drug is a volatile liquid, it would be more desirable to prepare it in a solid form, so that it could be formulated as a tablet. An inactive solid derivative could be prepared which would be converted in the body to an active drug. Formaldehyde is a flammable, colourless gas which cannot be used directly as medicine so a stable hexamine is used. In acidic pH, hexamine is hydrolyzed to formaldehyde and ammonia which acts as a urinary antiseptic.

Ideal quality of prodrug:

A perfect prodrug must contain the following requirements. It is less effective or inactive compared to the parent compound. The pro-moiety must be cleared inside the body from prodrug. The pro-moiety should be harmless and excreted from the body^[32,33].

Designing of prodrugs:

Based on the constitution, lipophilicity parameter, bio-active pathway and catalyst involved in bio-activation, the design of prodrugs is categorised into carrier-linked prodrugs and bioprecursors^[34,35]. The carrier-connected prodrugs are connected to a nontoxic moiety or carrier through a covalent bond to alter or relieve their unwanted properties^[36]. These prodrug molecules undergo enzymatic or chemical cleavage to discharge the active drug molecule^[37] (fig. 2). Bioprecursor does not have moiety or carrier but gives an active drug molecule upon biotransformation^[38]. It is changed to an active drug through oxidation, phosphorylation, reduction, nucleotide, glucuronic acid, decarboxylation and glutathione activation etc.,^[39-41] (fig. 3).

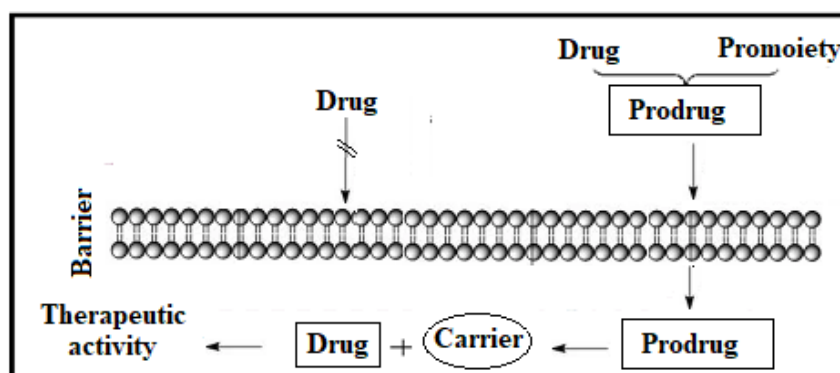


Fig. 2: Concept of carrier linked prodrug

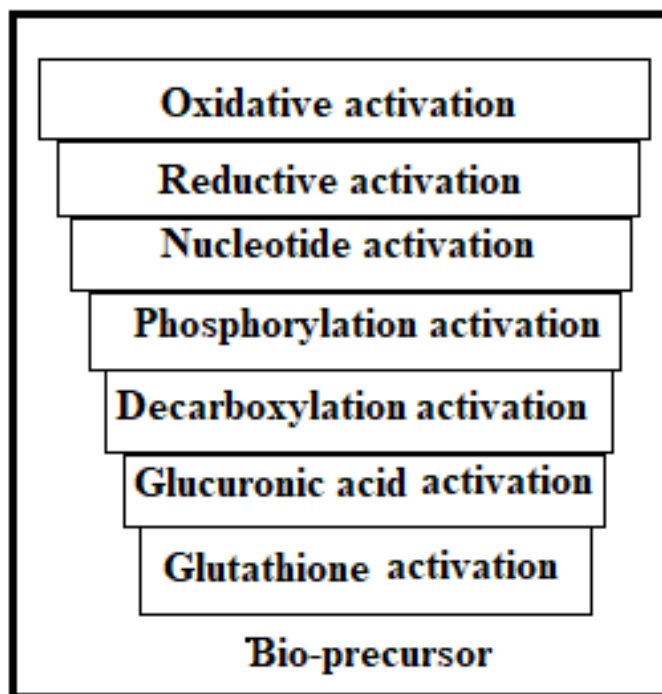


Fig. 3: Activation of bioprecursor by various methods

Preparation:

Carboxylic acid and alcohols: The drug-containing alcohol and carboxylic acid functionalities are converted to prodrug by esterification process. Carboxylic acid possessing drug molecules can be esterified with a variety of alcohols whereas; alcohols containing drugs can be acylated with aliphatic/aromatic acid. These esters can be easily hydrolysed by esterase enzymes such as acetylcholine esterase, lipase, cholesterol esterase, carboxypeptidase, etc., present in plasma and other tissues to furnish active drugs^[42].

Amines: Conversion of amines to give amide has not been commonly used as a prodrug, because of the strong stability of amide functionality and not having an amidase enzyme for metabolism in our body. A common method is to make Mannich bases as a prodrug form of amines. For example, hetacillin is a prodrug from ampicillin in which amine nitrogen and α -amino functionality have been permitted to interact with acetone to give a Mannich base^[43]. Amines are also converted to azo linkage prodrug rarely.

Carbonyl moiety: The compounds containing carbonyl functionalities such as ketone and aldehydes converted to prodrugs have not found wide clinical utility. These are converted into derivatives in which the sp^2 carbon of carbonyl

moiety is transformed to a sp^3 hybridized carbon linked to hetero atoms. These prodrugs are converted to carbonyl compounds by hydrolysis^[44,45].

BIOACTIVE PATHWAY

Prodrug drug designing confirmed that they are boosting the biological activities of drug molecules through increasing physicochemical properties and target selectivity. The activation of prodrugs take place by many enzymes such as carboxylesterase, butylcholinesterase, acetylcholinesterase, paraoxonase, alkaline phosphatase, matrix metalloproteinase, β -glucuronidase, plasmin, carboxypeptidase, oxidoreductases, β -lactamase and β -galactosidase etc. Here, some of the important hydrolysis processes are mentioned with suitable examples.

Carboxylesterases:

Carboxylesterases are linked to serine hydrolase enzymes and strongly hydrolyze a wide range of carbamate, esters and amide-containing drug substances. In humans, there are two types of carboxylesterases (hCE1, hCE2) are lead the drug metabolism pathway. They act as an efficient barrier to toxic materials and make easy of their removal by changing them into highly polar substances (Table 1). Here, some of the prodrugs are metabolised by carboxylesterases as mentioned below^[46].

TABLE 1: BIOCONVERSION OF VARIOUS PRODRUGS BY CARBOXYLESTERASE ENZYME

Substrate	Name of enzyme	Hydrolysis product	Product activity
Capecitabine	hCE1	5'deoxy-5-fluorocytidine	Inactive
Cocaine	hCE1	benzoylecgonine	Inactive
Oseltamivir	hCE1	oseltamivir carboxylate	Active
Tenofovir disoproxil	hCE2	tenofovir	Active
Azilsartan medoxomil	hCE2	azilsartan	Active
Propranolol ester	hCE2	Propranolol	Active

Acetylcholinesterase (AChE):

AChE is an extrinsic membrane-bound enzyme that projects into the synapse. It can be able to quickly metabolised by acetylcholine at neuromuscular junctions and cholinergic synapses (Table 2). It is involved in the removal of ACh discharge from the presynaptic nerve process and is significant for protecting natural cholinergic action^[47].

Butyrylcholinesterase (BChE):

BChE is chiefly present in plasma as well as muscle, brain, kidney, retina, intestine and placenta. It is more in plasma, so often called serum cholinesterase. However, their biological role remains impossible to differentiate (Table 3). BChE can be metabolised into a huge number of prodrug-containing ester functional groups. It has a broader substrate specificity than acetylcholine esterase^[48].

Paraoxonase:

It is a member of hydrolases that comprise PON₁, PON₂, and PON₃. PON₁ is a chief esterase that can stimulate the hydrolysis of various organophosphates and arylesterase. PON₂ and PON₃ have 60 % chain similarity with PON₁, but, they contain very some degree of arylesterase behaviour (Table 4). Human PON₁ is produced in the liver and reaches into the blood. Whereas, PON₃ is synthesized in the liver and has little amount in the kidney tissues^[49].

Matrix metalloproteinase:

They are known as matrixes, zinc-dependent endopeptidase enzymes consisting of more than 25 associates categorised based on the specificity and structure of the molecule (Table 5). They participate in a significant function in the degradation of extracellular matrix substances such as collagen, fibronectin, laminin, elastin and a variety of proteins etc. They need proteolytic

enzymes to make their active forms^[50].

Alkaline Phosphatase (AP):

Alkaline phosphatase is a metalloenzyme with wide substrate specificity. It encourages the taking away of a phosphate moiety from various classes of compounds such as alkaloids, nucleotides and proteins. AP is present in a lot of tissues, with the maximum expression in the hepatic cells (Table 6). The degree of difference in the number of AP isozymes in bone and liver has been utilized as a diagnostic indicator for a variety of renal dysfunction and cancer diseases^[51].

 β -glucuronidase:

The β -glucuronidase is a lysosomal enzyme that acts a vital function in the metabolism of mucopolysaccharides, such as chondroitin sulfate, dermatan sulfate and heparan sulfate (Table 7). The action of β -glucuronidase is needed for the reforming of the extracellular matrix components and changes in the β -glucuronidase cause an atypical lysosomal storage disease described as mucopolysaccharidosis^[52].

LATEST RESEARCH ON PRODRUGS

Rubio-Ruiz *et al.*^[53] narrated that O-propargylation of the hydroxamate moiety of the strong Histone Deacetylases (HDAC) inhibitor panobinostat prodrug led to an immeasurable drop of its anti-tumour activity >500 fold (fig. 4). The authors also demonstrated that this prodrug is transformed into panobinostat in the existence of gold catalysts in cell culture and *in vitro*^[53].

Toublat *et al.*^[54] found that BChE might be believed as a putative target for the treatment of Alzheimer's Disease (AD). A pleiotropic drug recognized as a multi-target ligands is presently equipped to attempt AD. In this work, we prepared a pleiotropic carbamate 7 prodrug, which acts as a covalent inhibitor of BChE (fig. 5). *In silico*

docking and *in vitro* study results are parallel with the first *in vivo* results that show a guarantee in restoring functioning memory^[54].

Yan *et al.*^[55] carried out that drugs holding a single P-N bond (phosphate and phosphonates) are often employed in prodrug design to increase cell permeability of these anionic functionalities. The P-N bond is hydrolysed by phosphoramidases to liberate the effective drug molecules *in vivo*.

Here author narrates a new mono-amidation approach to phosphonate-containing glycolysis inhibitors and demonstrates that a diverse panel of phosphoramidases may be quickly formed for *in vitro* screening. We illustrated that in contrast to the canonical benzylamine or L-alanine moieties which have earlier been reported as an efficacious prodrug, little and lengthy-chain aliphatic amines show better drug release efficacy for our phosphate inhibitors (fig. 6).

TABLE 2: BIOCONVERSION OF VARIOUS PRODRUGS BY ACETYLCHOLINESTERASE ENZYME

Substrate	Name of enzyme	Hydrolysis product	Product activity
Butyryl acyclovir	AChE	Acyclovir	Active
Lovastatin	AChE	Hydroxy acid form	Active
Temocapril	AChE	Temocapril diacid	Active
CPT-11	AChE	7-ethyl-10hydroxycamptothecin	Active
Dipivefrin	AChE	Epinephrin	Active

TABLE 3: BIOCONVERSION OF VARIOUS PRODRUGS BY BUTYRYLCHOLINESTERASE ENZYME

Substrate	Name of enzyme	Hydrolysis product	Product activity
Bambuterol	BChE	Terbutaline	Active
Isosorbide diaspirinate	BChE	Salicylic acid and isosorbide	Active
Methylprednisolone acetate	BChE	Methylprednisolone	Active
Dipivefrin hydrochloride	BChE	Epinephrin	Active
Propranolol ester	BChE	Propranolol	Active

TABLE 4: BIOCONVERSION OF VARIOUS PRODRUGS BY PARAOXONASE ENZYME

Substrate	Name of enzyme	Hydrolysis product	Product activity
Olmesartan medoxomil	hPON1	Olmesartan and diketone compounds	Active
Prulifoxacin	hPON1	Ulifoxacin	Active
Spironolactone	hPON1	7 α -thiomethylspironolactone and canrenone	Active
lovastatin	hPON1	beta-hydroxy acid metabolite	Active
mevastatin	hPON1	Lactone ring compound	Active

TABLE 5: BIOCONVERSION OF VARIOUS PRODRUGS BY MATRIX METALLOPROTEINASE ENZYME

Substrate	Name of enzyme	EC number	Product activity
Collagens (1,2, 3, 7, 8 and 10)	MMP-1	3.4.24.7	Active
Collagens (4, 5, 7 and 10)	MMP-2	3.4.24.24	Active
Collagens (3, 4, 5 and 9)	MMP-3	3.4.24.17	Active
Collagens (1, 2, 3, 5, 7, 8 and 10)	MMP-8	3.4.24.34	Active
Collagens (4, 5, 7, 10 and 14)	MMP-9	3.4.24.35	Active
Collagens (1, 2, 3, 4, 9, 10 and 14)	MMP-13	-	Active
Collagens (1, 2 and 3)	MMP-14	3.4.24.80	Active

TABLE 6: BIOCONVERSION OF VARIOUS PRODRUGS BY ALKALINE PHOSPHATASE ENZYME

Substrate	Name of enzyme	Hydrolysis product	Product activity
Amifosline(13, WR-2721)	Alkaline phosphatase	WR-1065	Active
125IQ2-P	Alkaline phosphatase	125IQ2-OH	Active
Fosfluconazole	Alkaline phosphatase	Fluconazole	Active
Fosphenytoin	Alkaline phosphatase	Phenytoin	Active
Mitomycin phosphate	Alkaline phosphatase	Mitomycin	Active

TABLE 7: BIOCONVERSION OF VARIOUS PRODRUGS BY β -glucuronidase ENZYME

Substrate	Name of enzyme	Hydrolysis product	Product activity
Glucuronide prodrugs of pacilitaxel	β -glucuronidase	Pacilitaxel	Active
Glucuronide prodrugs of 5-fluro uracil	β -glucuronidase	5-Fluro uracil	Active
Glucuronide prodrugs of 9-amino-camptotecin	β -glucuronidase	9-amino-camptotecin	Active
DOX-GA3	β -glucuronidase	Doxorubicin	Active
HMR1826	β -glucuronidase	Doxorubicin	Active

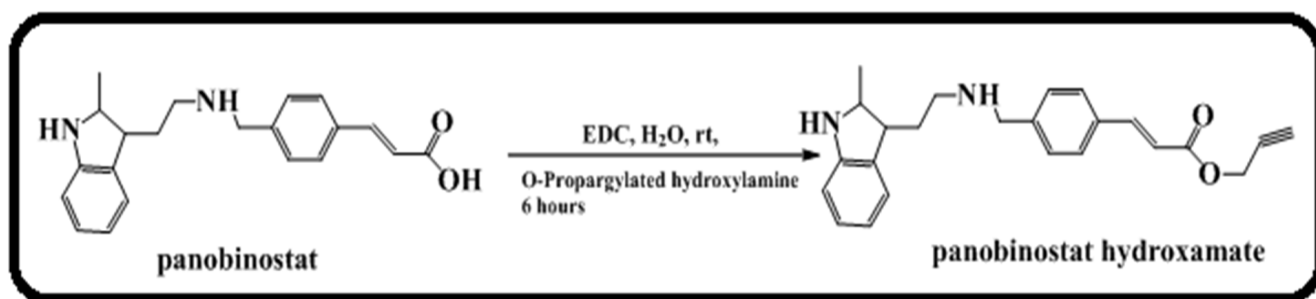


Fig. 4: Synthesis of panobinostat prodrug

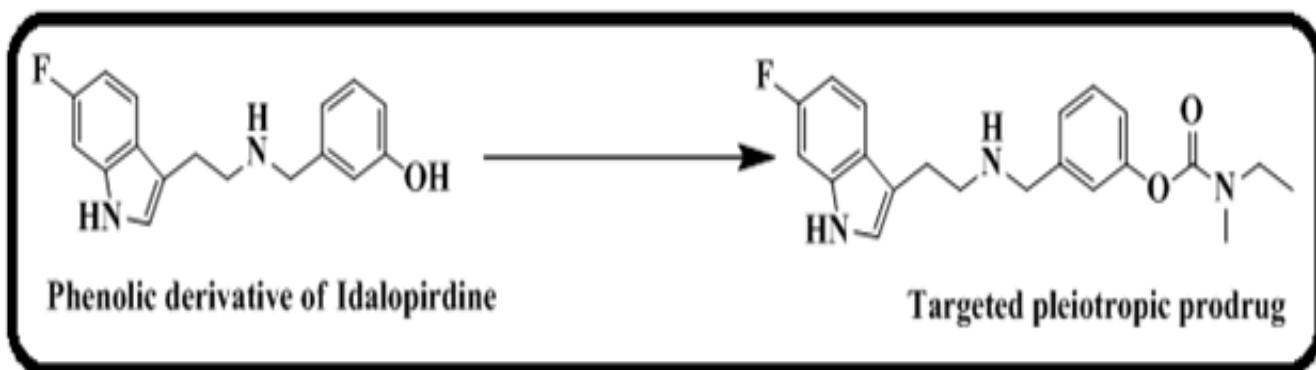


Fig. 5: Synthesis of pleiotropic prodrug

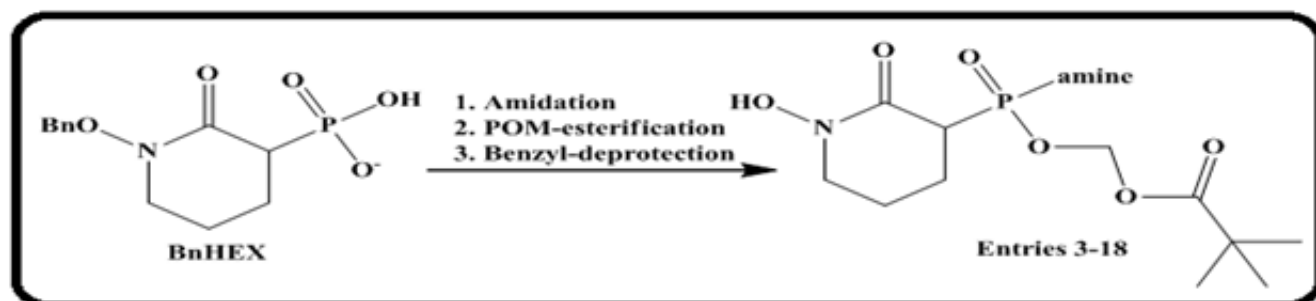


Fig. 6: Synthesis of phosphate and phosphonate prodrug

Thiabaud *et al.*^[56] examined gadolinium (III) texaphyrinplatinum (IV) conjugates are highly effective antiproliferative properties *in vitro* against mouse and human cell cancer lines. Compared to the current platinum clinical standard, a lead Gd (III) texaphyrin-Pt (IV) prodrug rising from this progress effort was found to be more effective in subcutaneous route in mouse models involving both cell-derived xenografts and platinum-resistant patient-derived xenografts. Based on the present study, we conclude that mellotexaphyrin-Pt conjugates may have considerable potential as anticancer agents.

Hughes *et al.*^[57] discussed the safety and pharmacokinetic study of the antiviral agent adefovir dipivoxil in children infected with Human Immunodeficiency Virus (HIV). It is a powerful inhibitor of retroviruses including HIV. Two dosages of the adefovir dipivoxil were assessed in a phase I study with children having HIV. Here, a total of fourteen children were divided into different groups ranging from 6 to 18 y. Eight patients were administered 1.5 mg of adefovir dipivoxil per kg of body weight and 6 patients received 3 mg per kg respectively. No significant adverse events were encountered (fig. 7).

Chang *et al.*^[58] demonstrated phenanthridinium derivatives of prodrug activation by gold. It is an upcoming technique in targeted drug delivery through metal. We introduced this approach based on the activation of a phenanthridinium-based prodrug through hydroamination under *in vivo* conditions. To build the prodrug compatible with the body, a gold Artificial Metalloenzyme (ArM) is made up of human serum albumin and triggers prodrug activation rather than the free gold metal complex (fig. 8).

Mohammed *et al.*^[59] found a new prodrug

Methotrexate (MTX) with chitosan and studied its bioactivity evaluation. The work intended to make an anti-colon prodrug loading into a biopolymer. Chitosan was obtained from the scales of local fish by using a standard protocol. The MTX was then loaded into chitosan to prepare chitosan-methotrexate conjugates as colon cancer prodrugs. There are 3 types of cancer cells such as MCF-7, MDA-MB-453 and MDA-MB-231 breast cancer cell lines were committed to investigating the antitumour activity of CS-MTX by using tetrazolium assay. The results pointed out that the survival possibility of human breast tumour cell lines was reduced and proved that CS-MTX was a lesser amount toxic than the MTX mother drug.

Rana *et al.*^[60] investigated Symbiotic Prodrugs (SymProDs) that targeting of CDK and NFκB. To test this design, we synthesised SymProDs using NFκB inhibitors and CDK inhibitors. Each drug acts as a pro-moiety of the each other and eliminates the adverse effect. The mentioned prodrug is 200-fold less active than CDK inhibitors in the *in vitro* method. Against ovarian cancer cell lines study revealed that energy trends of the SymProDs mirrored those of the single treatments suggesting their dissociation in cells. Our outcomes suggest that prodrugs offer a productive path for progressing the compounds and can be engaged as dual-targeting drugs.

Siegel *et al.*^[61] designed and synthesized a phosphoramidate prodrug for the treatment of emerging Ebola Viral (EBOV) disease. A focused screening and optimization of lead molecules effort identified 4b (GS-5734) with antiEBOV activity (fig. 9). It has been tested in a non-human-primate EBOV challenge model. The result suggested that there is a 100 % survival of infected animals after dosing of 4b (GS-5734) with 10 mg/kg, once daily.

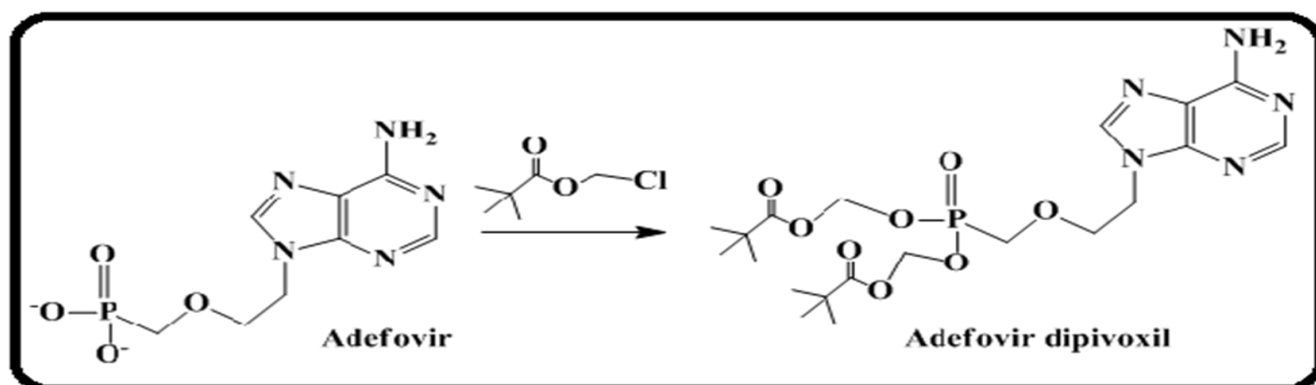


Fig. 7: Synthesis of adefovir dipivoxil prodrug

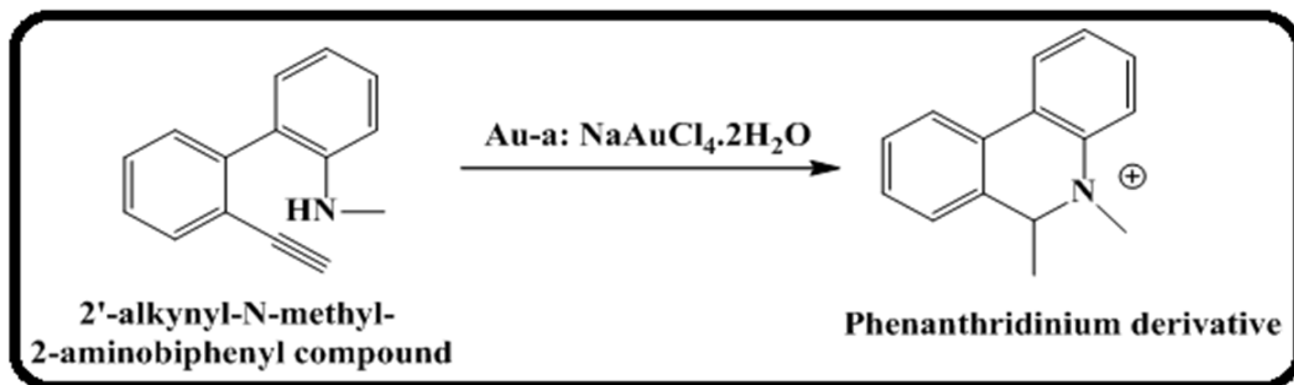


Fig. 8: Synthesis of phenanthridinium derivative prodrug

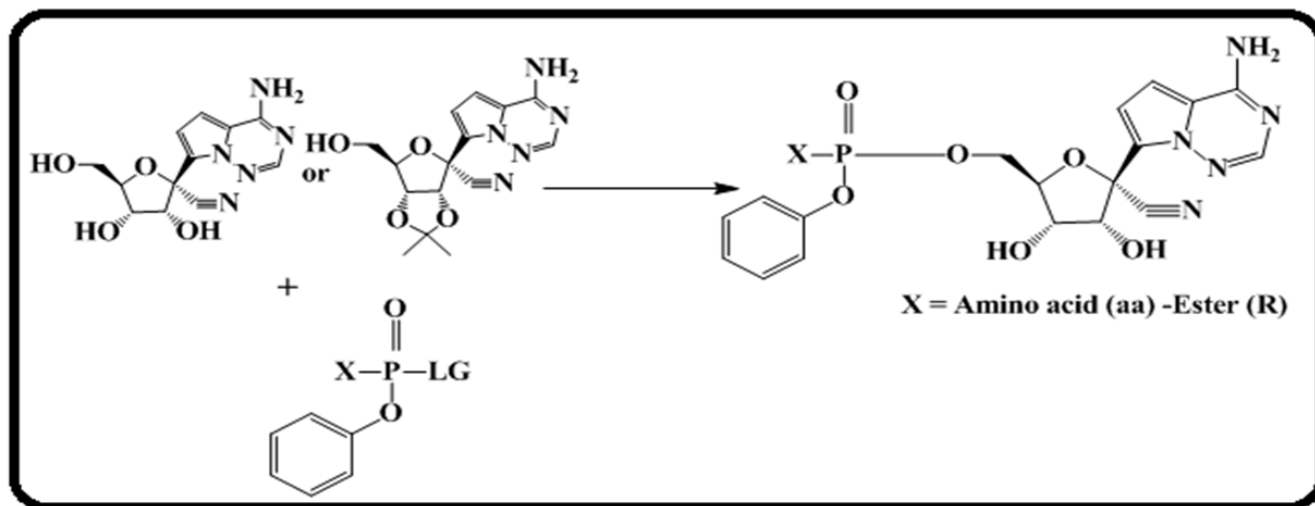


Fig. 9: Synthesis of a phosphoramidate prodrug

CONCLUSION

The concept of prodrug has found several useful therapeutic applications and acts as a significant alternative to solve pharmaceutical and pharmacokinetic-related problems of drug molecules. The growing proportion of prodrugs indicates the significant role of the prodrug in modern medicine. These prodrugs are activated by enzymes or/and chemical reactions. To the best of our knowledge, this review has entirely analyzed the complete information of prodrugs in all aspects such as design, synthesis, development, bioactive pathway and their recent therapeutic applications. It is the right time to consider the prodrug due to the wide variety of therapeutic applications including to treat of neglected diseases.

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Conflict of interest

The authors declare no competing interests.

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