Review

Current prodrug strategies for improving oral absorption of nucleoside analogues

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Abstract

Nucleoside analogues are first line chemotherapy in various severe diseases: AIDS (acquired immunodeficiency disease syndrome), cytomegalovirus infections, cancer, etc. However, many nucleoside analogues exhibit poor oral bioavailability because of their high polarity and low intestinal permeability. In order to get around this drawback, prodrugs have been utilized to improve lipophilicity by chemical modification of the parent drug. Alternatively, prodrugs targeting transporters present in the intestine have been applied to promote the transport of the nucleoside analogues. Valacyclovir and valganciclovir are two classic valine ester prodrugs transported by oligopeptide transporter 1. The ideal prodrug achieves delivery of a parent drug by attaching a non-toxic moiety that is stable during transport, but is readily degraded to the parent drug once at the target. This article presents advances of prodrug approaches for enhancing oral absorption of nucleoside analogues.

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1. Introduction

Nucleoside analogues are synthetic compounds that are structurally similar to natural nucleosides and, as such, are building blocks of nucleic acids. They act either as inhibitors of cellular and viral DNA and RNA polymerases or as chain terminators by incorporating into a growing DNA or RNA strand. Natural nucleosides are involved in almost all cellular processes and play a primary role in structural, energetic, regulatory and metabolic functions. Hence, many nucleoside analogues have cellular cytotoxicity with potency against bacteria, fungi, yeast, viruses and viral infections.
or neoplastic tissues, which is attributed to their biochemical mode action [1]. Currently, nucleoside analogues are supposed to be drugs that are given in first attention in many serious diseases such as acquired immunodeficiency disease syndrome (AIDS), hepatitis, cancer, herpes, smallpox, etc [2]. Of the approximately 40 antiviral drugs formally approved for use, half are nucleoside or nucleotide analogues [3]. Nucleoside drugs usually must be phosphorylated to the corresponding triphosphates by intracellular or viral kinases [4] in order to exert their pharmacological activity.

Transport of nucleoside analogues across the gastrointestinal tract is often mediated by passive diffusion or active transporters (Na\(^+\)-independent equilibrative transporters and Na\(^+\)-dependent concentrative transporters) [1]. However, their physicochemical properties are unsuitable for passive transcellular intestinal absorption. Meanwhile, nucleoside analogues are not natural substrates and show low affinity for nucleoside transporters. Hence, oral absorption of nucleoside analogues is often limited [5].

Variety of nucleoside analogues such as ganciclovir (GCV; marketed Cimevan\textsuperscript{®} and Virgan\textsuperscript{®}) (used for the treatment of cytomegalovirus retinitis) or cidofovir (CDV; marketed as Vistide\textsuperscript{®}) [6,7] are, however, not bioavailable after oral administration. Others like the anti-HIV nucleosides, although orally bioavailable, suffer from unfavourable pharmacokinetics [8–11] that necessitates the frequent oral administration to maintain therapeutic plasma level in human, also resulting in serious side effects such as bone marrow suppression (anaemia and neutropenia) [12], pancreatitis and peripheral neuropathy [13].

Since the polarity entails nucleosides with low permeability and bioavailability, increasing efforts in the literature are focussing on overcoming these difficulties with nucleotide prodrugs, an approach which improves the lipophilicity and eventually releases the parent nucleotide at a specific site. In a nucleotide prodrug, nucleoside analogues are usually covalently bonded to the carrier molecule via phosphoester bond, carboxylic ester bond, carbamate bond or amide bond. The sensitivity of these chemical bonds to enzymatic or chemical hydrolysis has a significant impact on the potency of nucleotide prodrugs. The article highlights the progresses of oral prodrug approaches for nucleoside analogues over the last two decades. Within this review, we have discussed several carboxylic ester prodrugs, monophosphate prodrugs and other prodrugs, with a special focus on rational prodrug design and their performances. It aims to provide some evidences for rational design of oral nucleotide prodrugs.

2. Prodrugs of nucleoside analogues

2.1. Carboxylicacidesters prodrugs

Carboxylicacidesters prodrug approach is widely used to improve oral absorption of nucleoside analogues, in which the hydroxyl group located at the side chain of nucleoside analogues is esterified with organic acid and vice versa. The carboxylicacidesters-type prodrugs usually possess significant enhancement in water-solubility, cell membrane permeability, enzyme stability and bioavailability, etc.

2.1.1. Acyclovir and its prodrugs

Acyclovir (ACV) belongs to BCS III class drugs and possesses activity against human herpes viruses. However, owing to its limited bioavailability (20%), ACV shows moderate antiviral efficacy after oral administration. Hence, it is necessary and feasible to design a prodrug for improving oral absorption of ACV.

Valacyclovir (VACV) is the valine ester prodrug of ACV targeting intestinal oligopeptide transporter 1 (PepT1) and has been proved to be safe and effective drug (Fig. 1). It has been the most successful prodrug targeting PepT1. PepT1 is a proton-coupled transporting protein and predominantly distributed in the small intestinal epithelial cells. It has become a striking prodrug-designing target recently, since some poorly absorbed drugs can be modified as peptidomimetic prodrugs targeting intestinal PepT1 to improve oral absorption of the parent drug. 3'-hydroxyl group of ACV was esterified with t-valine to prepare VACV. VACV has been reported to increase the oral bioavailability of ACV by 3- to 5-fold in humans. The C\textsubscript{max} values was increased significantly from 2.5 \(\mu\)M to 12.0 \(\mu\)M as well as the AUC \textsubscript{C\textsubscript{max}} values from 19.7 \(\mu\)M h to 49.7 \(\mu\)M h [14,15]. Enhanced oral absorption of ACV has been attributed to the hPepT1-mediated intestinal membrane translocation of prodrug VACV [16,17]. Recently, the enzyme responsible for hydrolytic activity toward VACV (hVACVase) has been identified and purified from Caco-2 cells. The high expression of hVACVase in the human intestine, kidney, and liver suggest an important role for hVACVase in the bioactivation of ACV in human tissues [18,19]. The peptide transport pathway was supposed to be more efficient than the amino acid transport system [20]. Therefore, several novel water-soluble dipeptide ester prodrugs of ACV were developed for improving ocular and oral absorption of ACV via targeting the peptide transporters [21].

The enzymatic stability and permeability of GVACV (Fig. 1) \(\left(t_{1/2} = 108.1 \pm 2.4\text{ min}, P_{app} = 2.99 \pm 0.59 \times 10^{-6}\text{ cm/s}\right)\) was comparable with that of VACV \(\left(t_{1/2} = 123.7 \pm 8.3\text{ min}, P_{app} = 3.01 \pm 0.21 \times 10^{-6}\text{ cm/s}\right)\) [22]. Interestingly, the oral bioavailability of ACV for GVACV (AUC\textsubscript{C\textsubscript{max}} = 416.1 \(\pm\) 140.9 \(\mu\)g min/ml) was approximately 2-fold higher than VACV (AUC\textsubscript{C\textsubscript{max}} = 208.4 \(\pm\) 41.2 \(\mu\)g min/ml), which may result from its higher enzymatic stability in Caco-2 cell homogenates than VACV [23]. Moreover, there was extensive metabolism by hepatic first pass-effect of the dipeptide prodrugs as evidenced by the higher levels of ACV observed in the portal vein than in jugular vein.

After the successful attempt of PepT1-targeted prodrug approach, the dipeptidylpeptidase IV (DPPIV/CD26) prodrug strategy was applied to ACV for improved water-solubility and oral bioavailability. DPPIV/CD26 belongs to a unique class of membrane-associated peptidases [24]. It is widely distributed on variety of cell membranes, such as various leucocyte cell subsets and several types of epithelial, endothelial, and fibroblast cells. Furthermore, a soluble form of the enzyme has been detected in cerebrospinal fluid and plasma at low amounts [25,26]. Depending on the site of attachment of the peptide promoiety, both peptide amide and ester prodrugs of ACV were prepared. The tetrapeptide amide prodrug 3 (Fig. 3) and the tripeptide ester prodrug 4 (Fig. 1) improved the water-solubility more than 17-fold and 9-fold, respectively,
compared to ACV (1.29 mg/ml). In contrast with valine ester prodrug of ACV, both the prodrugs were fully stable in PBS. Meanwhile, they could convert to VACV (for 4) or ACV (for 3) upon exposure to purified DPPIV/CD26 or human or bovine serum [27]. This result indicates that the DPPIV/CD26 prodrug approach could be useful for increasing the water-solubility of polar drugs and possibly oral absorption.

2.1.2. Ganciclovir and valganciclovir

Ganciclovir is an acyclic guanosine analogue, which was first used intravenously to treat CMV infection in AIDS patients. To circumvent the inconvenience and risks associated with frequent intravenous administration, an oral formulation has been further developed, because of its low bioavailability (approximately 5%) [28].

Based on the model exemplified by valacyclovir, valganciclovir, an l-valyl-ester prodrug of ganciclovir (Fig. 1), has been synthesized. The bioavailability of orally administered valganciclovir rose up to 61%, almost 10 times higher than the parent drug [29]. Consequently, once-daily oral administration of 900 mg of valganciclovir was as effective as once-daily intravenous injection of 5 mg/kg of ganciclovir [30].

Fig. 1 – Names, chemical structures of the carboxylic acid esters prodrugs described throughout the report. The red parts stand for the parent drugs.
2.1.3. Didanosine and its prodrugs

Didanosine (5′-O-2′-3′-dideoxydidanosine, DDI) is the second anti-HIV drug approved by the FDA, which is well tolerated with chronic administration with rare and usually reversible toxicity [31]. However, DDI exhibits poor bioavailability (20–40%) [1], which necessitates the continuous infusion to maintain therapeutical plasma level in human. Many investigations had focused on the development of DDI prodrugs for improved oral absorption, but none of them were in routine clinical use [32–34]. Yan et al synthesized five peptidomimetic derivatives of DDI targeting PePT1. The 5′-O-L-valyl ester prodrug of DDI (5′-O-L-valyl-DDI) (Fig. 1) demonstrated the highest permeability in Caco-2 cell model and was selected as the optimal candidate for further studies. The oral absolute bioavailability of DDI was improved from 7.9% to 47.2% after 5′-valyl prodrug orally administered to rats at a dose of 15 mg/kg. It was reported that the prodrug could markedly improve DDI acidic stability in SGF, with the $t_{1/2}$ to be 36 min in SGF, while the parent drug could not be detected in the 2 min. The enhanced acidic stability was confirmed again by the coadministration with anti-acid agent in the in vivo oral pharmacokinetics. Antacid combination of DDI increased the oral bioavailability by 115.8%, while 5′-O-L-valyl-DDI (antacid combination) only increased by 30.1% [35].

2.1.4. Penciclovir and famciclovir

Penciclovir is an acyclic guanosine nucleoside analogues, which displays a similar spectrum of selectivity and antiviral
activity compared with acyclovir [36]. Due to its poor oral bioavailability (F<7%) [37], it is necessary to design an oral alternative of penciclovir. Famciclovir (Fig. 1) is a double prodrug containing acetyl diester and 6-deoxy promoietyes. It can be efficiently bioactivated to the parent drug via enzymatic deacetylation and oxidation after oral administration [38,39]. Famciclovir has been proved to be effective for human genital herpes infections and herpes zoster [40]. Clinical studies demonstrated the prodrug could be rapidly absorbed and the oral bioavailability of penciclovir rose up to 77% following a single dose of famciclovir [41]. In contrast, the acetyl diester of penciclovir did not show any enhancement in oral absorption compared to the parent drug [37]. Monocarbonate prodrugs of 6-deoxy penciclovir were also assessed in vivo with the hope of more efficiently converting the prodrug to the parent form. Slightly higher or comparable urinary recovery of penciclovir was observed with several monocarbonate prodrugs in mice and rats compared to famciclovir [42].

2.1.5. β-ν-2′-Deoxy-2′-fluoro-2′-C-methylcytidine and RG7128
β-ν-2′-Deoxy-2′-fluoro-2′-C-methylcytidine (PSI-6130), a potent inhibitor of HCV replication in Huh7 replicon assay, exhibits broad activity against HCV without obvious cytotoxicity [43]. It entered into clinical trials but showed only modest oral absorption and it was easily degraded to inactive uridine metabolite. In order to get around this drawback, Furman et al prepared a series of bis-isobutyryl ester prodrugs of PSI-6130. It was reported that RG7128 (mericitabine; Fig. 1) was the most advanced, simple nucleoside prodrug in clinical trials [44]. During Phase I study, RG7128 has shown efficacy when applied in patients infected with HCV genotypes 1, 2 and 3. RG7128 entered into Phase IIb clinical trials. Early reported data (a 12-week analysis) suggest an average 83% of complete early virological response for the 1000 mg b.i.d./SOC cohort [45]. RG7128 is expected to enter the market in the near future.

2.1.6. Cf1743 and its prodrugs
Bicyclic furanopyrimidine nucleoside analogues (BCNAs), a family of highly lipophilic antivirals, display high inhibitory potency and unusual selectivity against varicella zoster virus (VZV) [46]. The most potent anti-VZV prototype compound is the p-pentylphenyl BCNA analogue Cf1743, which displays activity against a broad range of VZV isolates and little or no detectable toxicity at micromolar concentrations [47]. However, the very poor aqueous solubility of Cf1743 gives low oral bioavailability (~14%) [48]. Formulation-based strategies were successful in enhancing the water-solubility of Cf1743 but did not have a remarkable impact on oral absorption [48]. To solve this problem, Alberto et al synthesized a series of prodrugs of Cf1743 (Fig. 1) targeting DPPIV/CD26. Alberto et al demonstrated the prodrugs efficiently released the parent BCNA drug upon selective conversion by purified DPPIV/CD26 and by soluble DPPIV/CD26 present in bovine, murine, and human serum. Additionally, the hydrolysis of the prodrugs in the presence of purified DPPIV/CD26, human, murine, and bovine serum was completely blocked, when treating with 2.5 μM vildagliptin (a specific inhibitor of DPPIV/CD26). Among the DPPIV/CD26-targeted prodrugs, several prodrugs showed significant increase in water-solubility (up to more than 1000-fold) compared to the poorly soluble parent drug. Those prodrugs have been reported to increase the oral bioavailability of Cf1743 by 7- to 5-fold in mice [49].

2.1.7. 3′-Fluoro – 2′, 3′-dideoxyguanosine and its valloyloxypropyl ester prodrug
GlaxoSmithKline Company developed 3′-fluoro-2′, 3′-dideoxyguanosine, which displays activity against HBV. It is phosphorylated by deoxyctydine kinase in vivo to form an active metabolite, 3′-fluoro-2′, 3′-dideoxyguanosine triphosphate. This analogue is then incorporated into DNA and prevents DNA reproduction of virus. However, it exhibits low oral bioavailability (only 10% ~ 20%). Hence, its 5′-O-(S)-2-L-valloyloxypropyl ester prodrug (Fig. 1) was synthesized to improve oral absorption of 3′-fluoro-2′, 3′-dideoxyguanosine. It was reported the oral bioavailability rose up to 50% [50].

2.2. Monophosphate prodrugs
For exerting antiviral activity, the majority of the nucleoside analogues are required to be phosphorylated by a kinase in vivo to form corresponding active metabolites, triphosphates. Since the rate-limiting step in the formation of triphosphate is conversion of nucleoside analogue to its monophosphate, monophosphate ester prodrugs of nucleoside analogues were designed in an attempt to circumvent the initial phosphorylation activation step. Acyclic nucleoside phosphate (ANP) is a new class of antiviral agents, which does not need initial phosphorylation by viral nucleoside kinases to exert their antiviral effect. Instead, the drugs undergo two phosphorylation reactions to their active forms by cellular enzymes [51]. Consequently, unlike other nucleoside analogues, ANP analogues do not readily lead to virus drug resistance. However, owing to poor intestinal membrane permeability of the charged molecules, many phosphate prodrugs are not suitable for oral absorption. Hence, prodrug derivatives to mask the ionized phosphate group of nucleosides have been exploited [52,53], but the strategy can be limited because of rapid hydrolysis of the phosphate esters in vivo. However, the problem can be resolved by attaching the phosphate group to acyclic nucleoside moiety through a stable P–C bond, which is resistant to esterase hydrolysis. Prodrugs of the acyclic nucleoside phosphonates (ANP) can mask the negative charges on the phosphate groups and thus improve cellular permeability.

2.2.1. Adefovir and its prodrugs
Adefovir is an acyclic analogues of deoxyadenosine and it displays low oral bioavailability as other ANP analogues due to limited intestinal permeability of the anionic phosphate moiety [54,55]. Hence, various prodrugs of adefovir were designed to mask the charged phosphate groups and improve oral absorption of adefovir. It was reported that simple alkyl di-esters or amides prodrugs of adefovir failed to efficiently convert to adefovir in vivo [56]. Additionally, monoesters showed poor oral bioavailability probably because of the unmasked ionized phosphate groups. In this situation, a bis(pivaloyloxymethyl) ester prodrug of
adeefovir (adeefovir dipivoxil) (Fig. 2) was prepared. The prodrug possesses increased lipophilicity and greater intestinal permeability than adeefovir. Furthermore, it is rapidly converted to the parent drug in vivo and leads to observably higher oral absorption. According to the published data, the oral bioavailability of adeefovir dipivoxil in humans rose up to 3- and 3.8-fold of free adeefovir at dose of 125–500 mg [57,58]. Another study with a dose of 10 mg adeefovir dipivoxil showed approximately 4-fold increase of oral bioavailability of adeefovir [59]. Adeefovir dipivoxil is currently licensed for a standard treatment for chronic hepatitis B, particularly in patients with a lamivudine-resistant HBV infection.

2.2.2. Cidofovir and its prodrugs
Cidofovir (HPMPC), an acyclic nucleoside phosphonate, is a potent and selective inhibitor of viral DNA synthesis licensed for the treatment of cytomegalovirus retinitis in AIDS patients [60]. Cidofovir itself is a prodrug, which should be transformed to activated triphosphate in vivo. However, due to its polarity of the phosphate group and limited intestinal membrane permeability, HPMPC exhibits low oral bioavailability of <5% and it must be administered by intravenous infusion [7,54]. Meanwhile, cidofovir displays dose-limiting nephrotoxicity due to high concentration in the kidney, which promotes the development of safe cyclic analogue of cidofovir. Cyclic cidofovir (Fig. 2) is chemically stable and can be converted to cidofovir in vivo by a cellular cyclic CMP phosphodiesterase. Compared to intravenous cidofovir, the prodrug has similar antiviral activity but lower potential for nephrotoxicity in humans [61]. The oral bioavailability of cyclic cidofovir is also limited, because it can only be absorbed through cell pinocytosis in the gut. To improve oral bioavailability of cidofovir and cyclic cidofovir, ether lipid ester prodrugs were synthesized. These prodrugs were designed to use the lysophosphatidylcholine (LPC) uptake pathway in the small intestine and achieve high oral availability. Esterification of the phosphonate with several alkoxyalkanols showed remarkable improvement in oral bioavailability in mice (88–97%) [62–64]. The lipid prodrugs were absorbed intact and converted slowly to the parent drug in tissues. Enhanced antiviral activities of these prodrugs were observed both in vitro and in vivo [65,66]. The improvement in activity was attributed to the increased cellular uptake of cidofovir and intracellular levels of cidofovir diphosphate [67]. After that attempt, another prodrug Val-Ser-cyclic HPMPC was synthesized by Amidon group, which was a promising peptide prodrug targeting puromycin-sensitive aminopeptidase and had been shown to improve the permeability and bioavailability of HPMPC in rodent models. The prodrug was initially activated by puromycin-sensitive aminopeptidase to remove the L-valine residue. Subsequent chemical hydrolysis resulted in the generation of cyclic HPMPC [68,69].

2.2.3. Tenofovir and its prodrugs
Tenofovir is structurally similar to adeefovir with an extra methyl side chain. As other ANP analogues, poor oral bioavailability of tenofovir (10%) was anticipated in humans. Hence, prodrugs were developed for enhanced oral delivery. Previous clinical experience with adeefovir dipivoxil showed that pivalic acid discharged from the prodrug formed conjugates with carnitine leading to decreased serum carnitine levels [58]. To solve this problem, a series of prodrugs with alkoxycarbonyloxyethyl groups was designed but with a weak absorption because of hydrolysis before membrane. Meanwhile, carbamate prodrugs also failed to improve bioavailability of tenofovir, probably because of their high enzymatic stability [70]. Based on stability, solubility, and enhanced oral absorption (1.5-fold of tenofovir in dogs), bis(1-isopropoxyloxyethylmethyloxymethyl) ester of tenofovir (tenofovir disoproxil) (Fig. 2) was chosen for further study [55,71]. Furthermore, Caco-2 cell transport of the prodrug was increased from 0.1% to 2.7% [55]. The oral bioavailability of tenofovir was 25% after oral administration of tenofovir disoproxil fumarate at dose of 300 mg in human and rose up to 39% with food [72]. Moreover, tenofovir disoproxil fumarate showed a broad spectrum of antibacterial activities compared to the parent drug and could be applied for the treatment of drug-resistant HIV and HBV infections.

2.2.4. Abacavir and its prodrugs
McGuigan has investigated a series of pronucleotides such as alkoxy phosphoramidates, phosphorodiamidates, diaryl triesters and aryloxy phosphoramidates. However, the aryloxy phosphoramidates (‘ProTides’) were proved to be the most successful pronucleotides, in which an amino acid ester promoiety was attached to the drug (as an aryl monophosphate or phosphonate) via a P–N bond. The ProTides approach has been applied to many nucleoside analogues, such as 3’-azidothymidine [73], abacavir [74] and tenofovir [75]. However, it is difficult to obtain optimal antiviral activity of each pronucleotide, the fine-tuning of each element (amino acid ester, and aryl moiety) is required.

Arloxy phosphoramidates were expected to release the nucleoside monophosphate intracellularly via both chemical and enzymatic mechanisms. The first step is cleavage of the amino acid ester by a carboxyesterase. Subsequently, nucleophilic attack at the phosphorus by the carboxyl group releases the aryloxy group, forming the amino acyl metabolite (AAM). Finally, the amino acid moiety is removed by a phosphoramidase to release the nucleoside monophosphate and an amino acid [76].

The pharmacokinetics and oral bioavailability of abacavir phosphoramidates (Fig. 2) were investigated [77]. It was found that the pronucleotide was rapidly converted to AAM with a half-life of several minutes, after oral administration of the abacavir methylalaninyl-phosphoramidate. Total exposure to the pronucleotide and its active metabolites was reported to approach that estimated for abacavir at a similar dose, resulting in an overall bioavailability of 50%.

2.3. Amide-type prodrugs

2.3.1. Gemcitabine and its prodrugs
Nucleoside analogues are usually poorly active after oral administration, because of their limited intestinal permeability and rapid metabolism to inactive metabolite in the gut or due to their high first pass metabolism. For example, Gemcitabine (dFdC) is an important anticancer drug that has been licensed for the treatment of non-small cell lung, breast cancer and pancreatic bladder [78]. Gemcitabine is extensively
metabolized to 2’,2’-difluoro-2’-deoxyuridine (dFdU) by cytidine deaminase [79] which abound in blood, liver and gut. This has limited dFdC use to the parenteral route in clinical.

Therefore, several oral prodrugs of gemcitabine were designed by coupling acyl chains covalently to the 4-amino group of gemcitabine, in which the 4-amino group was modified with fatty acids [80] or acyclic isoprenoid chain of squalene (GemSq) [81] (Fig. 3). These lipophilic derivatives of gemcitabine were found to have a slower metabolism in plasma and higher cytotoxicity than gemcitabine. Due to their lipophilicity, these derivatives are also expected to have increased oral bioavailability compared to gemcitabine. This latest strategy (enhancing lipophilicity) undergoes further development.

LY2334737 is another amide prodrug of gemcitabine (valproic acyl prodrug). Early preclinical studies have shown that LY2334737 is more stable to hydrolysis and leads to enhanced bioavailability by blocking the site of deamination to its uridine metabolite. This can lead to prolonged systemic exposure of gemcitabine compared to both IV and oral administration of gemcitabine. Subsequent pharmacokinetic study proved that the prodrug was absorbed mostly intact across the intestinal membrane and then delivered to systemic circulation. The hydrolysis of LY2334737 was relatively slow, leading to sustained release of gemcitabine in vivo [82]. Phase I study of oral LY2334737 in Japanese patients with advanced solid tumours demonstrated LY2334737 was tolerated by Japanese patients up to 30 mg/day. The toxicities observed at the 40 mg dose may require the development of alternative dosing schedules [83].

2.3.2. Carbovir and abacavir
Carbovir is a carbocyclic nucleoside analogue with anti-HIV activity. It was abolished as a drug candidate because of poor oral absorption in rats (26%) and monkeys (23%), poor brain penetration, and kidney and cardiac toxicities [84]. Abacavir (Fig. 3) was a 6-modified carbovir analogues, which is more lipophilic than carbovir with a log P value of 1.22 for abacavir versus −0.62 for carbovir, leading to higher CNS penetration and oral bioavailability (76–100% in animals and 83% in human) [85]. After oral administration, abacavir is first phosphorylated to abacavir monophosphate in vivo by adenosine phosphotransferase and then converted to carbovir monophosphate by a cytosolic deaminase, finally transformed to carbovir triphosphate [86]. The unique activation pathway enabled abacavir to overcome the deficiencies of carbovir.

2.3.3. Ribavirin and viramidine
Viramidine (Fig. 3), a liver-targeting amidine prodrug of ribavirin, is designed to circumvent haemolytic anaemia caused by the parent drug.

Probably due to the positive charge on viramidine molecule, the uptake of viramidine in red blood cells is less than ribavirin, eventually resulting in a reduction in haematological toxicity [87]. The oral bioavailability of viramidine was 66.1%, 43.9% and 61.7% in human, monkey and rat, respectively [88,89]. Viramidine is activated to ribavirin in the liver by adenosine deaminase and exhibited a higher liver-to-erythrocyte drug ratio, which suggesting better liver-targeting properties. Furthermore, both preclinical and clinical studies showed superior safety profiles of viramidine compared to the parent drug [90,91]. Currently, the prodrug entered in Phase III studies in patients with chronic hepatitis C infection.

3. Conclusion
Nucleotide analogues play an essential role in the treatment of cancer and viruses. Since the rate-limiting step in the formation of triphosphate is conversion of nucleoside analogues to its monophosphate, monophosphate ester prodrugs of nucleoside analogues were designed in an attempt to circumvent the initial phosphorylation activation step. However, both nucleoside analogues and monophosphate ester prodrugs of nucleoside analogues are polar molecules and have limited membrane permeability. Hence, traverse of intestinal epithelial membrane is often limited. Over the past decade, several creative prodrug strategies have been utilized to overcome these limitations. The examples described in this review illustrate the significant research efforts done to improve the oral bioavailability of nucleoside analogues. Traditional prodrug approaches by enhancing lipophilicity have been applied to improve passive diffusion. Prodrugs targeted to PepT1 have been found very useful for enhancing oral absorption of polar drugs. PepT1 has become a promising target since they are highly expressed in the intestine with high capacity and diverse substrate specificity. Advances in prodrug design have improved the value of nucleoside compounds as anticancer and antiviral agents. The examples described in this article further prove that prodrug approach is an effective strategy for improving oral absorption of nucleoside analogues.

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