Overview on the Recent Drugs Delivery Approaches

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Overview on the Recent Drugs Delivery Approaches

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Abstract

This review provides the reader a concise overview of the different biological barriers that hinder the delivery of therapeutic agents through membranes, such as intestinal mucosa, Brain Blood Barrier (BBB), and mediators of transport such as efflux transporters and etc., and the approaches for overcoming such barriers. The approaches discussed in this review include: utilizing natural occurring transporters to deliver drugs specifically to their targets, nucleoside analogues delivery, CYP-activated prodrugs that target drugs to the liver, modification of passive diffusion by efflux pumps, intestinal transporters such as PEPT1 and GLUT1, Carrier Mediated Transport (CMT) systems for transporting nutrients, vitamins or hormones into the central nervous system, tissue selective drug delivery, administration of an exogenous enzyme to reach the tumor site which is followed by systemic administration of non-toxic prodrugs (ADEPT, GDEPT and VDEPT), enzymes involve in the bioconversion of ester-based prodrugs for activation (hydrolysis) of prodrugs to their active forms, brain targeted Chemical Delivery Systems (CDS), amino acid prodrugs to improve oral bioavailability, sustained drug delivery and intravenous drug delivery.

In addition, Receptor-Mediated Transcytosis (RMT) for efficacious delivery of Nano particles through the intestinal mucosa and BBB, and the prodrug chemical approach based on intra molecularity to deliver anti-cancer drugs is discussed.

Keywords: Drug Delivery; Prodrugs; Tumor Targeting; Computational Approaches; Enzymes Targeting; Acyclovir; Codrugs; CYP-Activated Prodrugs

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Introduction

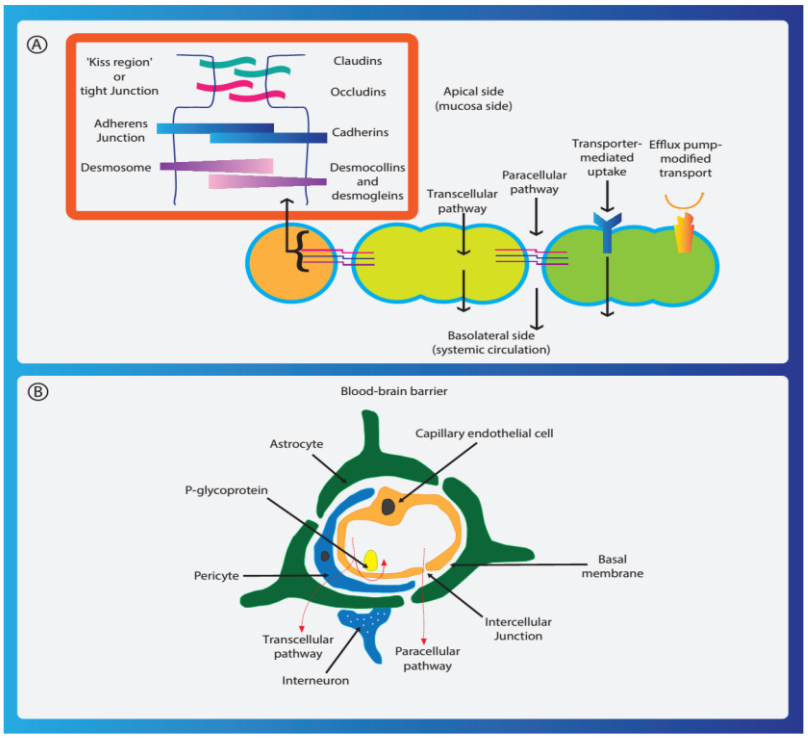
The presence of barriers such as intestinal mucosa and Blood Brain Barrier (BBB) is considered one of the major concerns when developing a drug since they pose a hurdle to the delivery of drugs to their active sites (targets). Strategies to overcome such barriers include altering the drug's physicochemical properties aiming to improve its absorption. Other strategies involve improving drug's transcellular delivery by blocking the efflux pumps or enhancing its paracellular transport via modulation of cell–cell adhesion in the intercellular junctions [1].

During the last two decades, many approaches and methods have been developed for facilitating the drug design and discovery phases. The majority of these methods were concentrated in finding new chemical entities that provide the most meaningful interaction with the desired receptors or enzymes and potentially to have minimal undesired interactions. However, this strategy is time consuming, costly and requires screening of thousands of molecules for biological activity of which a single compound might enter the drug market. One of the most attractive and promising method is the prodrug approach, in which the active drug molecule is masked by a promoiety to alter its undesired properties. The prodrug (predrug, proagent) is defined as a pharmacologically inactive moiety which is converted to an active form within the body. This term has been successfully used to alter the physicochemical, pharmacokinetic properties, (absorption, distribution, excretion and metabolism) of drugs and to decrease their associated toxicity. A prodrug must undergo chemical and/or enzymatic biotransformation in a controlled or predictable manner prior to exert its therapeutic activity. Basically, the use of the term prodrug implies a covalent link between an active drug and a promoiety. This strategy is

designed to overcome barriers through a chemical approach rather than a formulation approach [2, 3].

A schematic diagram shown in Figure 1 illustrates the intestinal mucosa barrier (Figure 1A) and brain blood barrier (BBB) (Figure 1B) that drugs generally penetrate through cell membranes (transcellular pathway) or in between the cells (paracellular pathway) [1, 4].

Figure 1: A schematic diagram illustrating the intestinal mucosa barrier and BBB.



Physical & metabolic barriers characteristics

The intestinal mucosa and BBB impose physical barriers that preclude the drug from reaching its site of action. As for BBB, the Brain Capillary Endothelial Cells (BCECs) are the most fundamental physical structure that composes this barrier. The BBB possesses some exceptional characteristics such as the existence of tight junctions, high metabolic activity of cells, inadequate vesicular transport (endocytosis) and an absence of fenestrations which in turn prohibit paracellular transport of the solute molecules [5].

Moreover, biochemical barriers such as enzymes and efflux pumps such as Multidrug-Resistant Proteins (MRP) and P-glycoproteins (P-gp), and many others ATP-dependent pumps have the potential to prohibit drug molecules from passing through biological barriers. Thus, it is expected that the determination of the substrate structural recognition will be quite difficult [1].

Enhancing the delivery of drug via transcellular pathways

Enhancing the passive diffusion of drugs through the transcellular pathway is a method to improve drug delivery through BBB and intestinal mucosa. The concept for passing transcellularly through the intestinal mucosa is partitioning of the drug molecules between two morphologically and functionally different cell membranes. Drugs molecules cross into the apical side, entering the intracellular space by influence of concentration gradient which is followed by molecules partitioning into the cell membranes at the basolateral side and from there they enter the systemic circulation [1, 6]

Drugs intended to enter the Central Nervous System (CNS) have to pass the BBB; the BBB contains tight-junctions which only permit the transport of lipophilic compounds to traverse it by diffusion or if the compound is a substrate for DBB carrier mediated transporters, also transcytosis takes place which can be absorptive mediated or receptor mediated trans cytosis [2].

Changing the drugs' physicochemical properties (transiently) in a way that favors membrane partitioning is among the methods used to improve the passive diffusion of drugs via transcellular pathway [7]. This can be achieved by developing a prodrug with a labile promoiety, that cleaved after passing biological barriers [8]. Drug's molecular size, charge, lipophilicity, hydrogen-bonding capability and solubility are the main factors determining the drug's permeation and its transport properties [7, 9]. However, in the case of peptides and proteins, the conformation and dynamic properties are important factors to be considered [1].

Nucleosides and approaches applied for drug delivery

Nucleoside analogues are entities consisting of a nucleic acid analogue and a sugar. They are commonly and widely used to treat viral infections and tumors. However, many nucleoside analogues have poor oral bioavailability due to their high polarity and low absorption through membranes (low permeability).

The poor absorption of many of nucleoside analogues is attributed to the large difference between the permeation of natural nucleosides and nucleoside analogues into cell membranes. Generally, natural nucleosides permeate cell membranes via active transport using Na^+-dependent

concentrative- and Na^+-independent equilibrated- transporters due to their significant hydrophilicity, whereas a passive diffusion or a combination of passive diffusion and nucleoside transporters is the pathway of the nucleoside analogues penetration into cells [4, 10]. Furthermore, it has been proposed that synthetic nucleoside analogues are not good substrates of nucleoside transporters, and since the capacity of these transporters is low, their saturation is reached at typical intestinal drug concentrations, the permeation of the synthetic nucleosides into cell membranes is therefore limited [4, 11].

Various chemical approaches have been applied to increase the nucleoside analogues lipophilicity and hence to enhance their oral absorption. Among these approaches: esterification, amidation, carbamation and synthesis of Prodrugs of Acyclic (ANP) and Cyclic (CNP) Nucleoside Phosphonates. In the ANP and CNP prodrugs the negative charges on the phosphonate groups are substituted with an alkyl group which results in enhancing the drug's permeation [4].

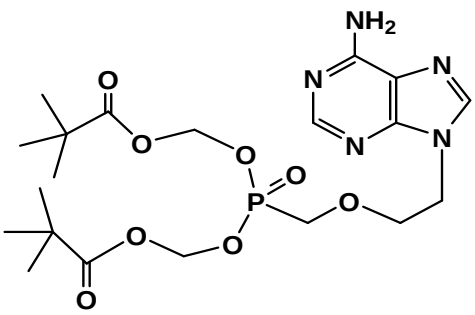
Prodrugs of Acyclic (ANP) and Cyclic Nucleoside Phosphonates (CNP)

Significant efforts have been made in the development of phosphonate-containing drugs for application in the treatment of various diseases. However, the free diacid phosphonates are ionized at physiological pH resulting in the formation of the corresponding dianion phosphonate drugs which have very poor oral bioavailability. To overcome this disadvantage a number of prodrugs for active phosphonate analogues have been developed. The concept of designing such prodrugs was to mask the free phosphonic group of the parent drug until being absorbed and reached the drug's target then the prodrug is allowed to undergo cleavage to release the active drug.

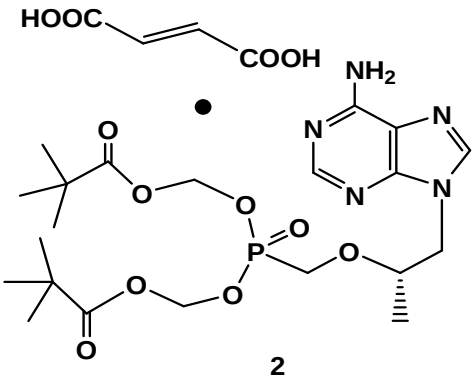
ANP and CNP analogues are converted into diphosphate analogues and inhibit viral DNA polymerase. Uniquely, ANP and CNP analogues do not rely on viral nucleoside kinases to initiate phosphorylation; they can employ their antiviral effect due to being monophosphorylated compounds. However, ANP and CNP analogues re converted to their active forms by the action of cellular enzymes that activate two phosphorylation processes. Thus, ANP and CNP analogues reduce the prevalence of virus drug resistance [4, 12, 13].

Several acyloxy ester and alkoxycarbonyl ester prodrugs of ANP analogues have been developed and among these, two prodrugs are currently in the market for the treatment of viral infections: the bis (pivaloyloxymethyl) ester of adefovir (**1** in Figure 2, bis-(POM)-PMEA; Hepsera®), approved in 2002 for the treatment of HBV infections and the diisopropyloxycarbonyloxymethyl ester of tenofovir fumarate (**2** in Figure 2, bis-(POC)-(*R*)-PMPA fumarate; Viread®) which was approved in 2001 for the treatment of HIV infections, and in 2008 for the treatment of chronic HBV.

Figure 2: Chemical structures of **1**-**3**.



1



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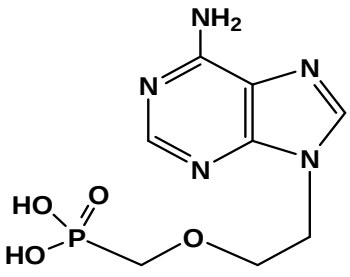


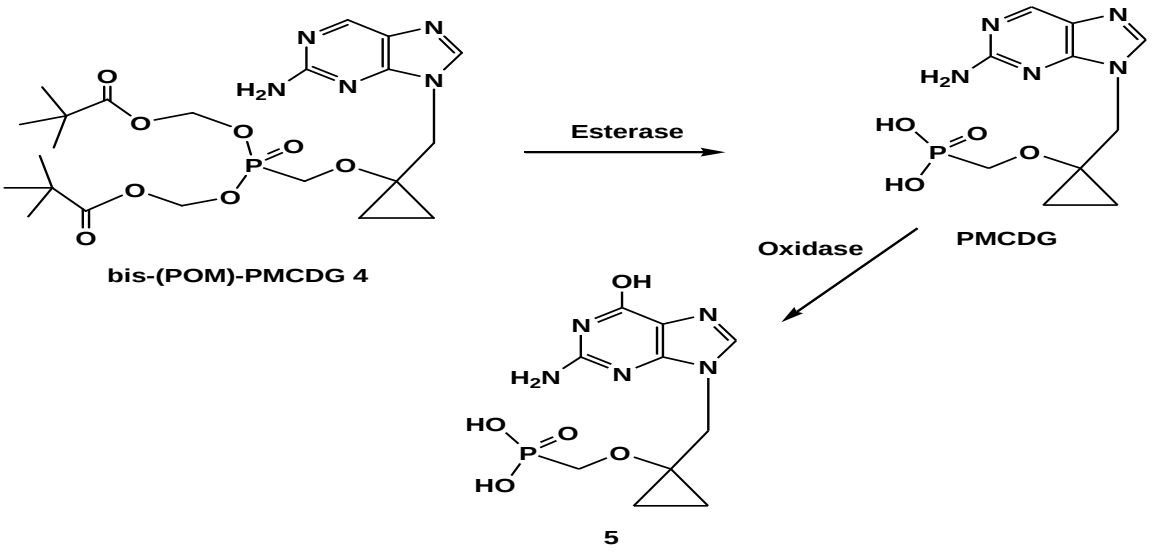
3

A bis(pivaloyloxymethyl) ester prodrug of adefovir, **1,** was designed and developed due to its favorable physicochemical properties. The prodrug showed enhanced lipophilicity and increased intestinal permeability compared to its active parent drug, adefovir (**3** in Figure 2). In addition, it demonstrated a rapid transformation to adefovir in vivo, which resulted in a significant increase in the oral bioavailability of the drug. Moreover, results demonstrated that the permeability of adefovir dipivoxil, **1,** in Caco-2 cell monolayers was 7.9×10^{-6} cm/s whereas that of adefovir was 1.0×10^{-6} cm/s. This increased permeability for **1** resulted in bioavailability of 36– 45% for 125–500 mg doses compared to less than 12% for adefovir, **3** [4, 14].

Prodrug **2** is a modification of **1** which offers the advantage of not generating pivalic acid during its bioconversion; **1** was found to be toxic during long-term use due to its decomposition products, formaldehyde and pivalic acid. The bis-(POC) motif was applied for the synthesis of bis-(POC)-(*R*)-PMPA, **2**, which was selected for clinical trials and was approved in the form of fumarate salt to treat of HIV. In addition, Bis-(POC)-(*R*)-PMPA fumarate, **2**, is currently in the market for use in combination with emtricitabine (Truvada®), and emtricitabine and efavirenz (Atripla®).

Subsequently, PMCDG dipivoxil (**4**, bis-(POM)-PMCDG in Figure 3) was synthesized to be used orally for HBV treatment and it is now in clinical trials. The prodrug **4** undergoes rapid esterase catalyzed-hydrolysis to its parent drug, PMCDG, in the liver and intestine. PMCDG is then degraded to a nucleotide analogue of guanosine monophosphate **5**, by aldehyde or xanthine oxidase (Figure 3). After phosphorylation to di- and triphosphate forms, the resulting entity inhibits viral replication upon incorporation into viral DNA ([4, 14]).

Figure 3: Metabolic pathway of prodrug **4** to **5**.

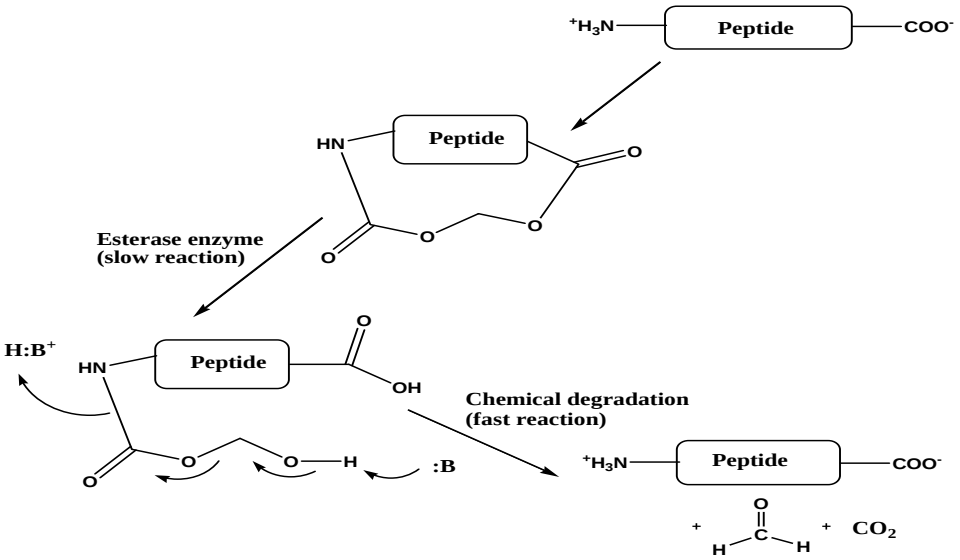


Prodrugs of Cyclic Peptides

Peptide drugs are associated with difficulty crossing the intestinal mucosa and BBB owing to their physicochemical properties that do not favor permeation into cell membranes via transcellular transport. They also restricted from crossing via paracellular route because of their large size which inhibits their passage through the tight junctions in this route.

To overcome this obstacle cyclic peptide prodrugs have been developed (Figure 4). The strategy was to modify the peptides' physicochemical properties such that it allows their permeation via transcellular passive diffusion. In contrast to the parent linear peptides, the cyclic peptide prodrugs showed less hydrogen-bonding capability, greater partition coefficients and reduced hydrodynamic radii. Yet, alongside the advantages, there are still some drawbacks associated with the cyclic peptide prodrugs. These disadvantages include recognition enhancement by efflux pumps and metabolism by cytochrome P450 (CP450), chemical instability of the cyclic promoiety in formulations and the risk of inter conversion of the cyclic prodrug to its acyclic active parent drug by enzymes before and during the diffusion across cellular barriers. This can lead to trapping of the drug inside the cells of the barrier and thus inhibiting the drug from crossing through the barrier [1].

Figure 4: A schematic diagram showing the conversion of the cyclic acyloxy alkoxy peptide prodrug to its parent peptide by esterase enzymes (slow reaction, few hours) followed by a fast chemical reaction (instantly, within few minutes).

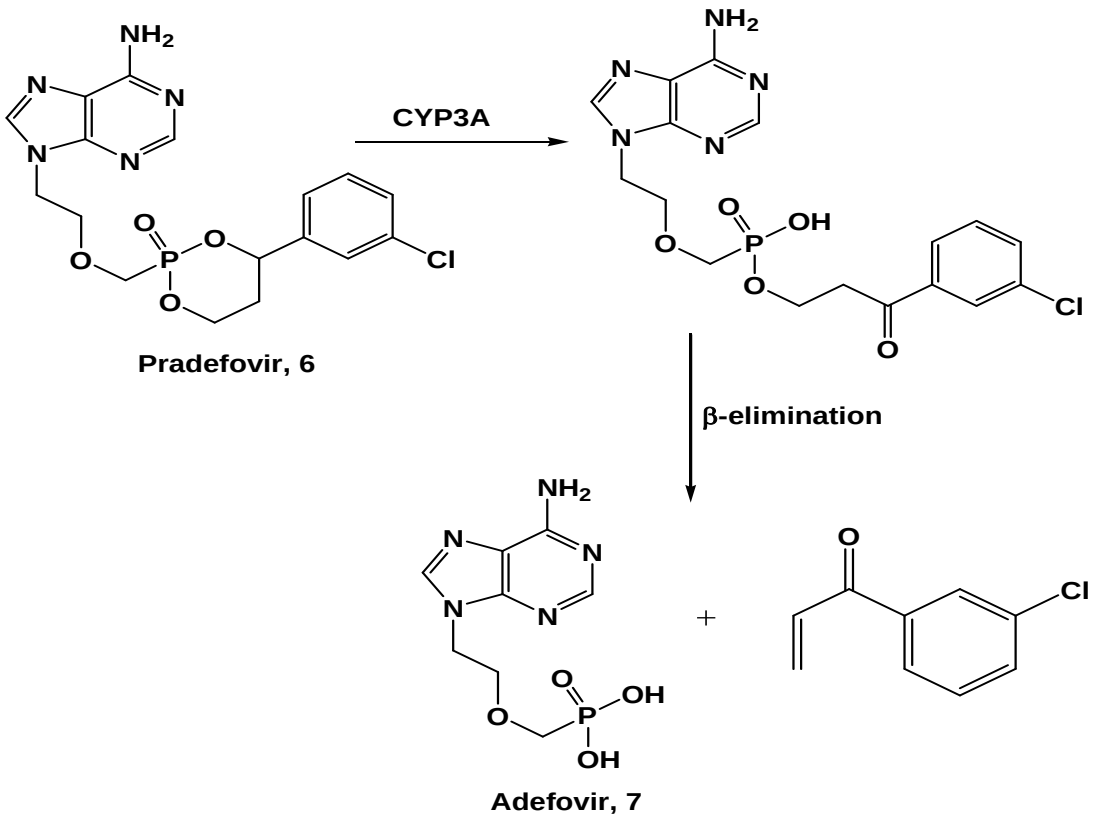


HepDirect Prodrugs

HepDirect prodrugs symbolize a novel class of CYP-activated prodrugs that target drugs to the liver. In this class, the drug is chemically modified to form a cyclic 1,3-propanyl ester prodrug of phosphonate or phosphate, which is pharmacologically inactive until it reaches the liver where it undergoes enzyme-catalyzed cleavage to release the active drug. In addition, HepDirect prodrugs are stable in the blood circulation and most of the body tissues except the liver owing to their resistant to cleavage by esterases.

As mentioned earlier, adefovir dipivoxil prodrug was developed to enhance the low oral bioavailability of adefovir; however, the prodrug exhibited dose-limiting nephrotoxicity. For this reason, pradefovir, a HepDirect prodrug of adefovir, **6**, was designed and developed. Studies demonstrate that upon reaching the liver, pradefovir **6**, is hydroxylated by CYP3A4 isozyme at the C4-methine position, followed by a rapid ring opening and β-elimination reaction to yield adefovir, **7**, and aryl vinyl ketone (Figure 5). [4, 15].

Figure 5: Metabolic pathway of pradefovir, **6** to adefovir, **7**.



Subsequently, adefovir is transformed by nucleotide kinases to adefovir diphosphate, whereas the aryl vinyl ketone forms a glutathione conjugate. In addition, studies revealed that in contrast to adefovir dipivoxil prodrug, pradefovir prodrug has good liver targeting properties and low risk of nephrotoxicity [4, 16, 17].

Modification of passive diffusion by efflux pumps or intracellular sequestration

Efflux pumps

As previously mentioned, existence of efflux pumps contributes to poor delivery of drugs through the intestinal mucosa and BBB. The proposed explanation for this phenomenon is that these pumps have the ability to distinguish and identify molecules with hydrophobic aromatic and/or tertiary amino groups and thus inhibit their permeation.

Using the X-ray structure of P-gp, various mechanisms of action of efflux pumps were proposed such as pore generation, hydrophobic vacuum cleaner and flippase mechanisms. Based on these proposals different methods were suggested and developed to avoid the activity of P-gp. Among these methods: drugs adjustment to prevent P-gp recognition, drugs with the potential to competitively inhibit P-gp and inhibitors that cause failure to P-gp function or completely shut it [1, 18].

The broadly known agents that have been recognized as competitive inhibitors of P-gp are verapamil, Cyclosporine A (CsA) and LY335979. Moreover, cyclosporine D (SDZ PCS833) has been used to improve intestinal mucosa delivery of some drugs due to its potential to inhibit P-gp without immunosuppressive activity that is associated with CsA. A number of pharmaceutical excipients such as surfactants (i.e., polysorbate 80, cremophore) and polymers (i.e., polyethylene glycols) have also been associated with P-gp inhibition and thus, drug transport enhancement [1, 19].

Another technique that has been explored to inhibit P-gp and enhance drug delivery to the brain is dimer prodrug formation. This technique is based on the assumption that the ability of the dimer to occupy multiple binding sites on P-gp can been reached via blocking P-gp activity which concurrently enhances prodrug transport through the BBB. Examples for such technique are galantamine dimer, Gal-2 (Figure 6) and abcavir dimer, NBD-abcavir (Figure 7) prodrugs that have demonstrated higher binding affinities and lower the off-rate to P-gp receptors compared to their corresponding parent monomers, galantamine and abcavir monomers, respectively (Figures 6 and 7). NBD-abcavir was assembled by connecting two abcavir molecules with a disulfide bond promoiety and its uptake in rat brain micro vessel endothelial cells was shown to be greater than abcavir due to its success in inhibiting P-gp. Abcavir is released from the NBD-abcavir prodrug after disulfide cleavage which is followed by a promoiety cyclization [1, 20]. On the other hand, Gal-2 dimer has been shown to convert to galantamine monomer by esterase enzyme.

In addition, the codrug approach has been utilized to enhance drug delivery to the Central Nervous System (CNS) by chemically attaching a drug with efflux inhibitor such as P-gp inhibitor. For instance, entacapone; the catechol-O-methyltransferase inhibitor was coupled with L-dopa to increase the delivery of the latter into the brain. Despite its success, the codrug approach is not the optimal choice to treat chronic CNS diseases because of toxic metabolites that might be accumulated in the CNS if the efflux system is blocked [21, 22].

Figure 6: Schematic representation of the conversion of Gal-2 dimer prodrug to galantamine monomer by esterase.

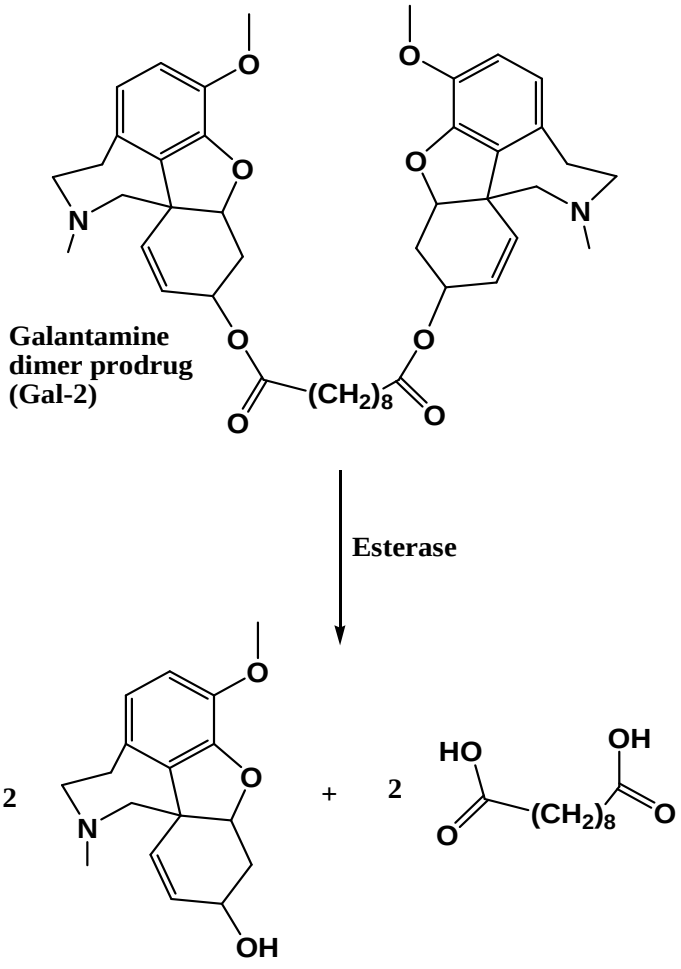
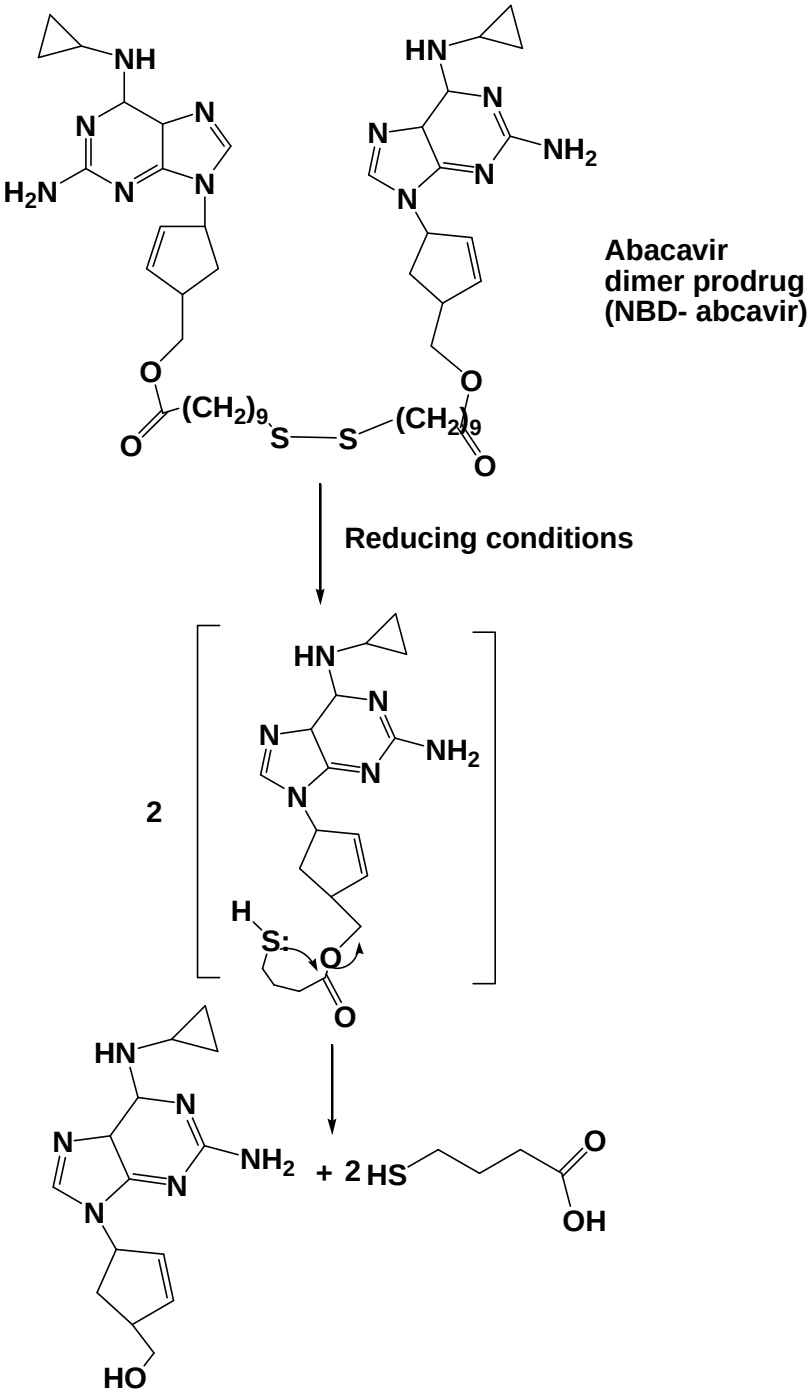
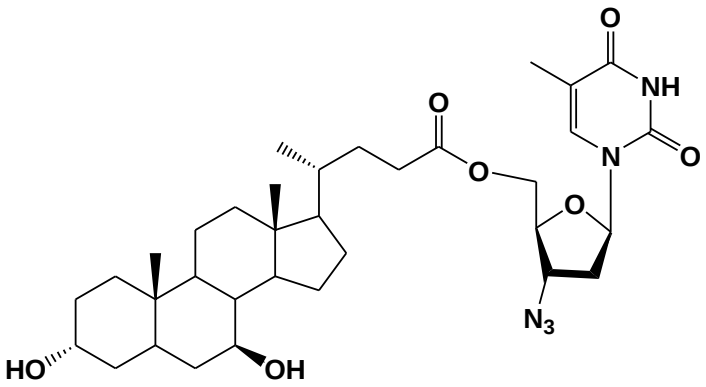


Figure 7: Schematic representation for NBD-abcavir dimer prodrug conversion to abcavir through the reduction of its disulfide bond.

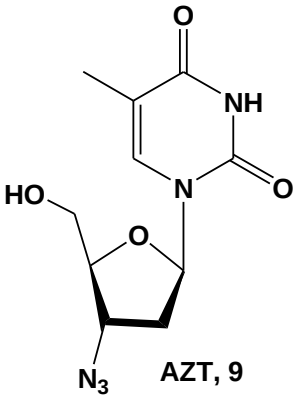


Another example is UDCA–AZT prodrug **8**, designed by Dalpiaz et al. in which zidovudine (AZT) **9**, was conjugated with Ursodeoxycholic Acid (UDCA), **10** and encapsulated in Poly (D, L-lactide-co-glicolide) (PLGA) nano-particulate system (Figure 8). Studies on UDCA–AZT have demonstrated three advantages to this technique: the ability of the prodrug to escape the active efflux transporter (AET) systems, controlling the release of the prodrug and enhancing the prodrug stability in physiologic fluids [23, 24].

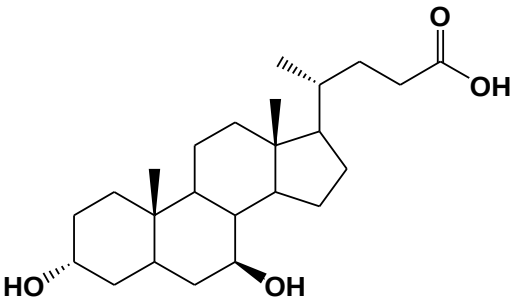
Figure 8: Chemical structures of UDCA– AZT prodrug, **8**, AZT, **9** and UDCA, **10**.



UDCA- AZT, 8



AZT, 9



UDCA, 10

Passive diffusion, intracellular sequestration and trapping

The sequestration mechanism has to be taken into consideration when evaluating drug transport across biological barriers. Trapping or sequestration of molecules in the intracellular space is one of the possible outcomes during the crossing biological barriers process. Intracellular space is over 50% inhabited by membrane-bound compartments such as endosomes and lysosomes, and it has been shown that many amine-containing drugs can be sequestered in the acidic compartment of the intracellular space due to the presence of more positively charged molecules than neutral molecules in the lysosomes (pH = 4.5), compared to the cytoplasmic domain (neutral pH). Therefore, the positively charged molecules are trapped in the lysosomes without being efficiently permeated into the membranes. Consequently, amine drugs have the tendency to accumulate in the lysosomes, despite being transported via receptor-mediated endocytosis [1, 25].

On the other hand, metabolism or transformation of certain drugs inside cells can be the cause of their trapping inside those cells. One of the common examples is Methotrexate (MTX) which enters into cells through transport receptors like membrane-associated folate binding protein (mFBP) and Reduced Folate receptor (RFC). After cellular uptake, MTX is converted into a highly negative charged polyglutamated MTX through polyglutamation of its glutamic acid. This results in molecules' trapping inside the cells and hence an inhibition of their permeation [1, 26, 27].

There are a limited number of proposed solutions for overcoming the sequestration of drugs. One solution includes a derivatization of the drug's amine group with a promoiety to provide a prodrug which is incapable to be protonated or positively charged, such that the prodrug molecule will not be protonated and sequestered when reached the lysosomes. However, this method is associated with a potential loss of the biological activity of the drug [1, 25, 28].

Receptor-mediated transport

Receptor-mediated transport mechanisms have been explored for the purpose of delivering small molecules, peptides, proteins, Nano particles and liposomes through biological barriers. Transporters found in biological barriers such as iron (i.e., transferrin receptor), RFC, mFBP, amino acid, di-/tri-peptide, organic anions (e.g., OATP1A1, OATP1B3), organic cations (e.g., OCT1, OCTN2), Sodium-dependent Bile Acid (ASBT) family, sodium-dependent glucose (SGLT) family, Monocarboxylate (MCT) family and amino acid (PAT1) transporters have been exploited to carry drugs across biological barriers [1, 29, 30]. Among the intestinal transporters, Peptide Transporter 1 (PEPT1) has received the greatest attention for drugs transport due to its high prevalence throughout the entire small intestine, high capacity and varied substrate specificity. Moreover, both carriers PEPT1 and PEPT2 transport many β-lactam antibiotics, valacyclovir and other drugs and prodrugs due to their steric resemblance to di- and tripeptides [1, 2, 6].

Structure requirements for transport via PEPT1

Indirect structure-affinity relationships have been relied on for the design of new substrates for PEPT1. The following factors are crucial for the determination of a drug affinity to PEPT1: molecular size, stereochemistry, peptide bond, N- and C-terminals, and side chains. Bailey et al. have built a template for PEPT1 substrates which consists of four key binding sites: (1) a strong binding site for N-terminal (NH_3^+), (2) hydrogen bonding to the carbonyl group of the first peptide bond, (3) a carboxylate binding site, and (4) a hydrophobic pocket for the amino acid side chain (Figure 9). Moreover, it has been shown that PEPT1 is stereo selective with preference and higher affinity to L-amino acids and is more inclined toward peptides containing bulky aliphatic side chains [4, 31-33].

A combination of prodrug and receptor-mediated transport has been used to enhance the delivery of small drug molecules. For example, Zanamivir (Zan), **11**, has poor physicochemical properties and consequently very low oral bioavailability (≈2%). A conjugation of Zan with L-valine (L-Val) to provide Zan-L-Val prodrug, **12** (Figure 10) was the strategy to solve the drug's poor clinical profile. The conjugation was made via an ester-labile acyloxy promoiety and it was demonstrated that Zan-L-Val permeates into cells by using hPepT1 and as a result Zan bioavailability was enhanced [1, 2, 34-37].

Figure 9: Substrate template with four key structural elements for binding to PepT1.

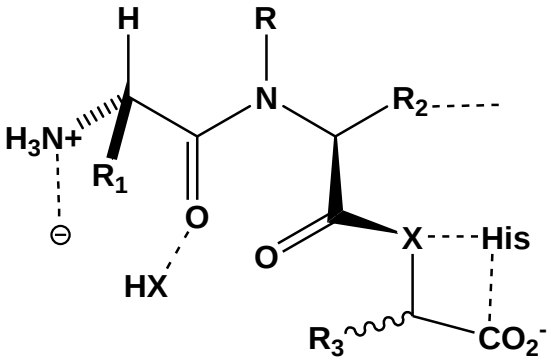
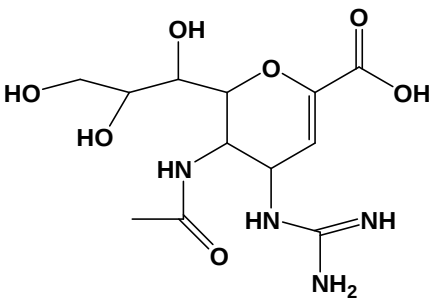
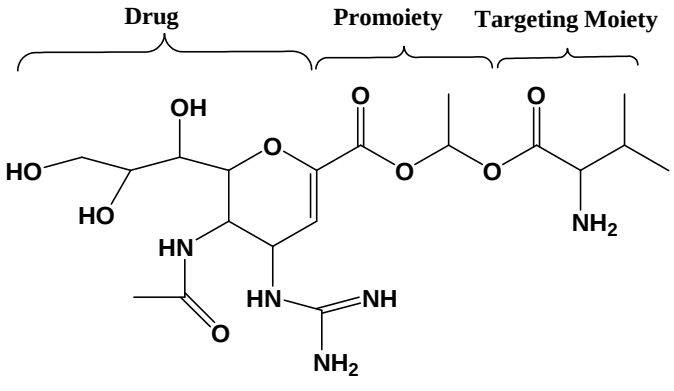


Figure 10: Chemical structures of Zanamivir, **11** and Zan-L-Val, **12**.



Zanavir (Zan), 11



Zan-L-Val, 12

On the other hand, Glucose Transporter 1 (GLUT1) is the most important glucose transporter present at the surface of Brain Capillary Endothelial Cells (BCECs), and therefore, it is one of the potential candidates as a target for BBB targeting and delivering drugs to the brain. Therefore, and in an attempt to enhance the CNS analgesic properties of nonglycosylated compounds, several opioid analogs have been glycosylated and their delivery to the CNS was studied. For instance, Bonina et al have demonstrated that glycosylated L-dopa and dopamine derivatives are efficient prodrugs to target GLUT1 and to enhance their parent drug's bioavailability [21, 38, 39].

Apical sodium dependent bile acid transporter (ASBT, SLC10A2)

The human apical sodium dependent bile acid transporter (SLC10A2) is an essential carrier protein expressed in the small intestine. It is a member of Solute Carrier family (SLC) of transporters. Knowing the structure and disposition of the bile acids might facilitate a precise prodrug design that targets human apical sodium dependent Bile acid Transporter (hASBT) [40].

Acyclovir valylchenodeoxycholate was designed to target hASBT. It owns a favorable affinity for hASBT that is equivalent to that of the native bile acid; cholic acid. Studies have demonstrated a two-fold enhancement in acyclovir bioavailability from 25% to 48% in rats upon using acyclovir valylchenodeoxycholate. The explanation for the less than expected increase in acyclovir bioavailability is attributed to the hydrolysis of the prodrug in the stomach and proximal intestine.

In addition, preliminary studies on drugs to be conjugated with bile acids showed that drugs can be attached to C-3 region of the steroidal ring through the C-24 carboxylate and C-17 region. However, results specified that C-24 conjugation boosts hASBT substrate affinity [40].

Transferrin Receptors

Transferrin Receptors (Tf-R) have been used in different aspects to deliver drugs through the BBB. More importantly, CRTIGPSVC-phages have been used as a new Tf-R-targeted technique which is composed of phage particles containing homing cyclic CRTIGPSVC peptides. These phage particles have the ability to use transferrin receptors indirectly to cross the BBB. For instance, apo-transferrin (apo-Tf) that imitates iron-bound transferrin Holo-Tf conformation is induced after the peptide on the phage interacts with apo-transferrin apo-Tf or iron-free Tf. Consequently, upon CRTIGPSVC peptide and transferrin binding, Transferrin Receptor (TfR) recognizes the phage-peptide/Tf complex and transports the phage-peptide across the BBB. Experiments have revealed a 100-fold particle brain deposition after intravenous (IV) administration of CRTIGPSVC-phages via the mouse tail vein compared to that of control phages without the peptide, demonstrating receptor-mediated transport. Based on this result it was concluded that CRTIGPSVC-phages are capable of delivering drugs to the brain. [1, 41, 42].

Sodium Dependent Multivitamin Transporter (SMVT)

SMVT is primarily responsible for the uptake of vitamins such as biotin, pantothenic acid and lipoate in epithelial cells; therefore, it has been used for the delivery of poorly permeable drugs through conjugating a drug to biotin which serves as the recognizable moiety for the drug transport [43, 44]. The apparent affinity constant (Km) values of SMVT substrates are in the low micro molar range, leading to saturation of the transporter and thus, limiting the drug's dose to be delivered via this carrier.

To overcome this problem, Vadlapudi et al. have explored the possibility of using lipid-raft based drug conjugates to maximize the amount of a drug to be transported. The approach was to produce synergistic effect by combining both lipid and transporter/receptor targeted delivery. The lipid raft enhances the interaction of the prodrug with membrane transporters/receptors possibly by facilitating the docking of the targeted ligand into the binding domain of the transporter/receptor protein [44].

Using this approach, different conjugates of Acyclovir (ACV) - biotinylated lipid prodrugs with various lipid rafts were synthesized and their ability to translocate across Caco-2 and MDCK-MDR1 cell lines by SMVT was assessed. Results demonstrated that the targeted lipid prodrugs of ACV have a higher affinity towards SMVT than ACV and their cellular accumulation is mainly mediated by SMVT as biotin uptake can be significantly inhibited [44].

Prodrugs and Carrier Mediated Transport (CMT)

CMT systems listed in Table 1 are responsible for transporting nutrients, vitamins and hormones into the central nervous system. They are highly stereospecific for their substrates, therefore, neuro active drugs are incapable of passing through these systems, which implies the use of the prodrug approach to overcome this obstacle [45].

The following subsections illustrate some examples on the use of these systems to deliver drugs to the CNS.

Table 1: CMT systems involved in the transport of nutrients into the CNS.

Carrier	Type	Representative substrate	Main expression in blood/ CNS barriers
Neutral amino acid	LAT 1	Phenylalanine	BBB
Hexose	GLUT 1	Glucose	BBB
Monocarboxylic acid	MCT 1	Lactic acid	BBB
Cationic amino acid	CAT 1	Arginine	BBB
Nucleoside	CNT 2	Adenosine	BBB
Ascorbic acid	SVCT 2	Vitamin C	Choroid Plexus

Neutral amino acid (LAT1)

LAT1 transporter is known to play a significant role in cell growth and proliferation; it is up regulated in malignant cells, which in turn has the potential to facilitate the intracellular accumulation of amino acid prodrugs in cancer cells.

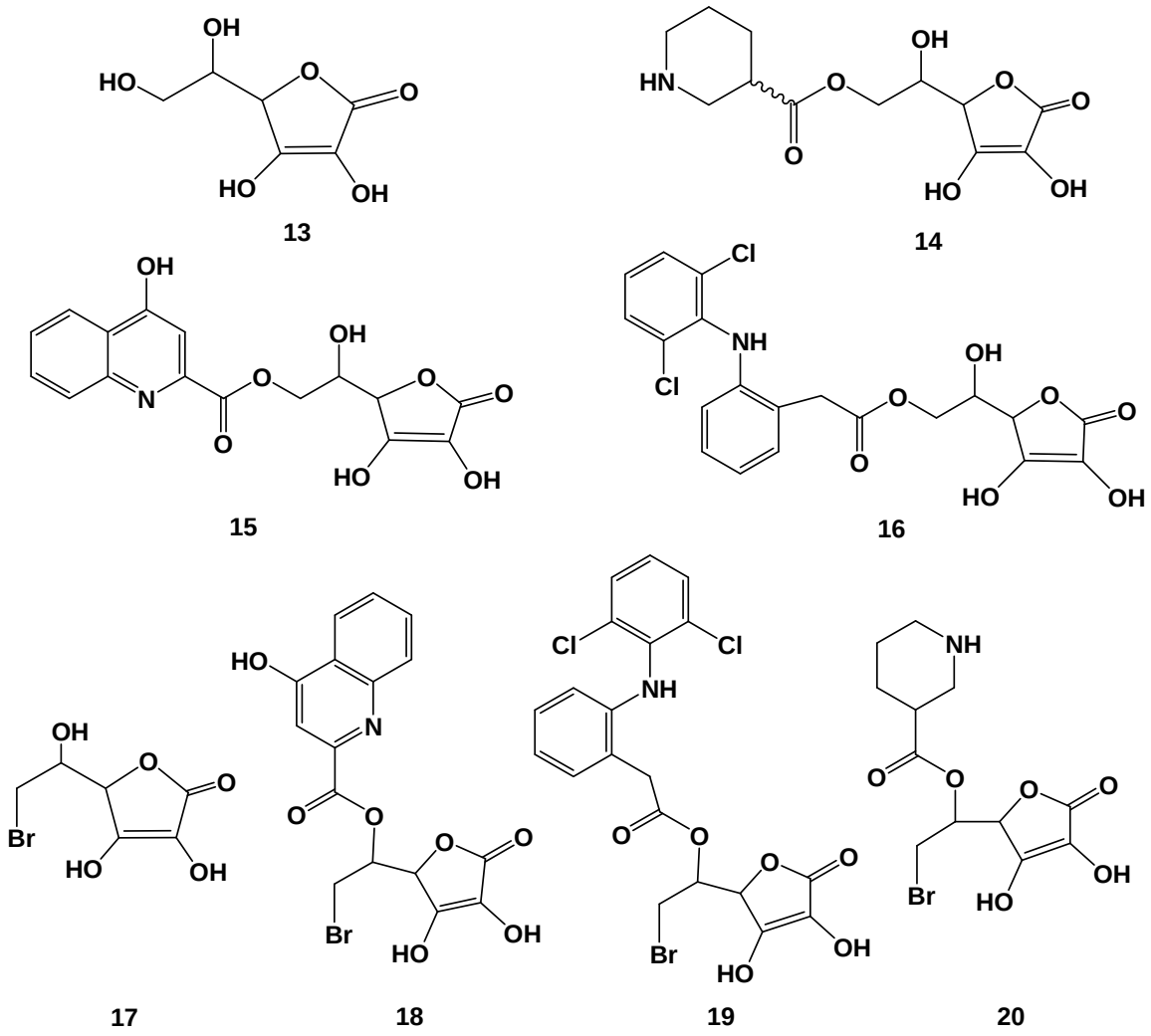
Alternatively, amino acid transporter targeted prodrug delivery might be a worthwhile strategy to be used to augment drug accumulation in P-gp overexpressing cells. This is based on a study which showed that P-gp mediated drug efflux could be circumvented by chemical modification of quinidine with neutral amino acids [46].

For instance, Patel et al. have conducted a study in which the possibility of overcoming P-gp mediated cellular efflux of quinidine was inspected via exploring the influence of modified neutral amino acids. L-isoleucine ester prodrug of quinidine (Ile-quinidine) was made and ^{14}C-erythromycin was selected as a model substrate to study quinidine and Ile-quinidine interactions with P-gp. The results showed a significant improvement in the rate of ^{14}C-erythromycin uptake in the presence of quinidine, whereas it remained fairly constant in the case of Ile- quinidine. Moreover, it was shown that quinidine has greater substrate affinity toward P-gp than Ile-quinidine and the latter was recognizable by multiple amino acid transporters such as LAT1, LAT2 and cationic amino acid transporter, as revealed from competitive inhibition studies [46, 47].

Prodrugs and the SVCT 2 system

A class of Na^+ dependent transporters named sodium-vitamin C co-transporters (SVCT) is responsible for the intake of vitamin C (ascorbic acid, **13**, Figure 11) in the body compartments of mammals. SVCT1 allows for the absorption of vitamin C from the intestine and its recovery by the kidneys, whereas SVCT2 is expressed by the retinal pigment epithelium and neuroepithelial cells of the choroid plexus and therefore it is responsible for the vitamin accumulation in the eyes and brain. Based on this, Pavan and coworkers have considered SVCT2 as a candidate for transporting neuroactive drugs into the CNS. For this purpose, they synthesized conjugates of vitamin C with neuroactive drugs and investigated their uptake to the CNS. The selected model compounds were nipecotic, kynurenic and diclophenamic acids, due to their inability to reach the brain from the bloodstream but still, they have the ability to induce therapeutic effect against various CNS pathologies. The conjugates of the selected compounds with vitamin C, **14-16,** are shown in Figure 11. Human retinal pigment epithelium (HRPE) cells were chosen as the cell line because they selectively express the SVCT2 transporter. The study on compounds **14-16** revealed that nipecotic and kynurenic acids have the potential to interact with SVCT2 only as ascorbate conjugates, in which they behave as competitive inhibitors of vitamin C. Furthermore, the Br-ascorbate, **17**, conjugates; **18-20** (Figure 11) showed a similar behavior where nipecotic and kynurenic acids have the ability to interact with SVCT2 on the same binding site of vitamin C. On the other hand, diclofenamic acid behaved as a noncompetitive inhibitor. Based on these results the authors concluded that a conjugation with vitamin C induced further improvement of the drug affinity towards SVCT2 [45, 48-50].

Figure 11: Chemical structures of vitamin C, **13**, its Br-ascorbate and their conjugates with nipecotic acid (Nipec), kynurenic acid (Kynur) and diclophenamic acid (**14-20**).

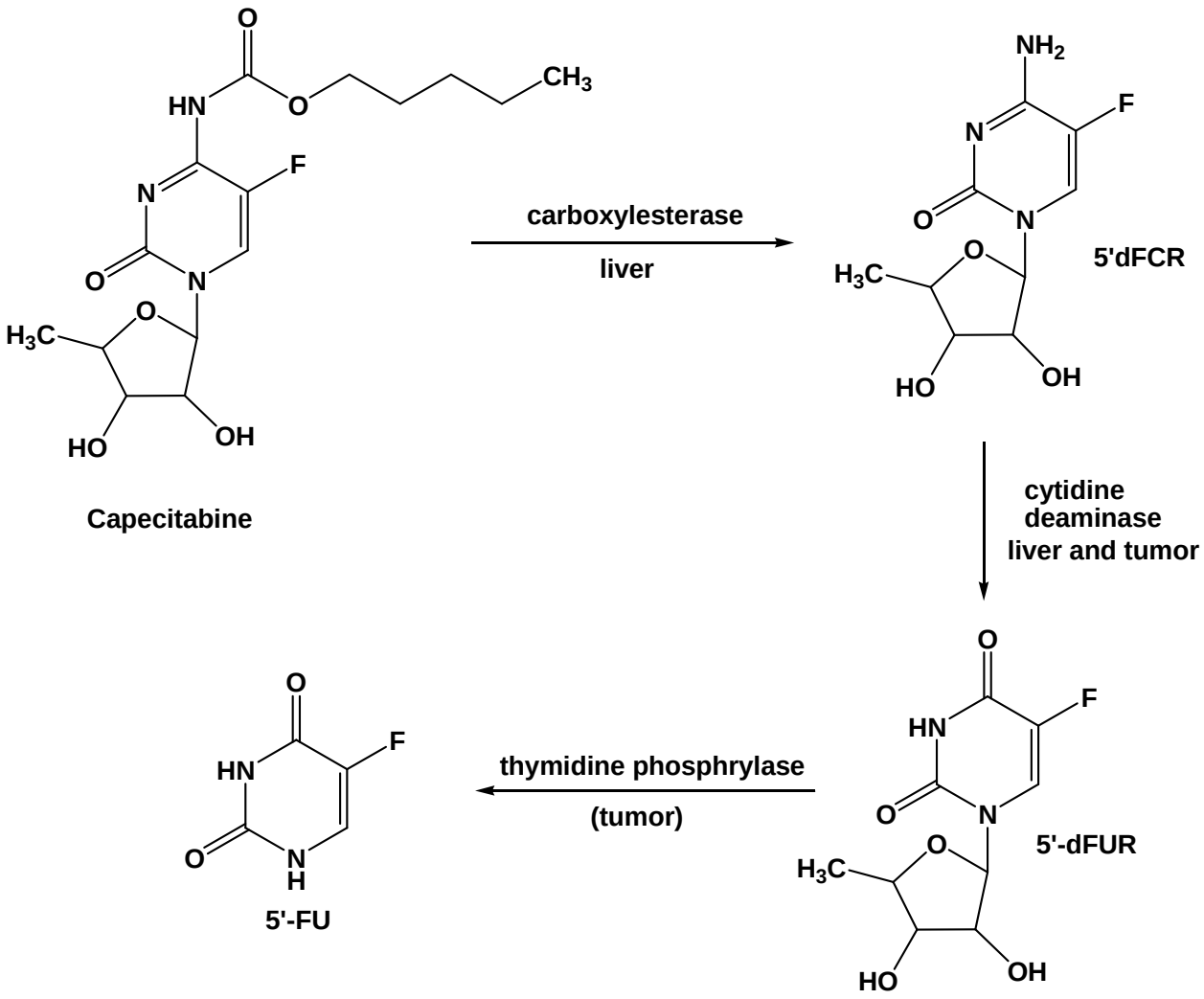


Tissue and tumor targeting

Due to sever cytotoxic effects of most antitumor and antiviral agents, tissue selective drug delivery has been proposed. It has a significant potential to enhance the safety and efficacy profiles of drugs. For this purpose, different prodrug approaches have been investigated such as prodrugs activated by enzymes, preferentially localized in target tissues, specific transportation of prodrugs to the target tissues via specific transporters, and conjugates of drugs and molecules selectively bound to the target tissues [4, 51].

Capecitabine is a triple prodrug of 5-fluorouracil (5-FU). It is rapidly and extensively absorbed and over 95% of its dose is recovered in urine after oral administration. Following oral absorption, capecitabine is converted in the liver to 5'-deoxy-5-fluorocytidine (5'-dFCR) by the action of carboxylesterase, then to 5'-deoxy-5-fluoro-uridine (5'-dFUR) by the action of cytidine deaminase in the liver and tumor tissues, and finally to 5'-FU by the action of thymidine phosphorylase (dThdPase) in tumor cells (Figure 12). Capecitabine lessen the toxicity of the gastrointestinal tract that occurs with 5'-dFUR because it passes intact through gastrointestinal tract and is converted to 5'-FU in tumor tissues [4].

Figure 12: Three-step enzymatic conversion of capecitabine to 5-FU.



Other methodologies involve an administration of an exogenous enzyme to reach the tumor site, followed by systemic administration of non-toxic prodrug that selectively converted into the active drug. The most common approaches are Antibody-Directed Enzyme Prodrug Therapy (ADEPT), Gene-Directed Enzyme Prodrug Therapy (GDEPT) and Viral-Directed Enzyme Prodrug Therapy (VDEPT). ADEPT is a two-step approach in which an antibody- drug Activating Enzyme Conjugate (AEC) is given first and targeted to the tumor and accumulates predominantly at the tumor cells that have the desired tumor associated antigen, allowed to be localized and to clear the unbounded conjugate from the plasma, then, in the second step, a nontoxic prodrug is injected systemically and is converted to its corresponding active form by the localized enzyme. On the other hand, the concept in VDEPT and GDEPT approaches is based on packaging the gene encoding for the prodrug-activating exogenous enzyme either in a non-viral- (GDEPT) or viral- vector (VDEPT), which is then delivered to the tumor tissue where the target enzyme is expressed for selective transformation of prodrugs designed to be activated by this enzyme (Table 2) [52-55].

Table 2: Examples of ADEPT, VDEPT and GDEPT in cancer therapy.

1- Examples of ADEPT in Cancer Therapy

Enzymes	Antibodies	Prodrugs	Model systems
Carboxy-peptidase G2	Anti-CEA antibody	CMDA	Xenograft of human colon carcinoma
Human β- glucuronidase	Humanized CEA-specific binding region	Anthracyclin prodrugs	Murine L 1210 tumor cell Line
Human β -glucuronidase	Single-chain anti-CD20 antibody	Doxorubicin	Fused protein

2- Examples of VDEPT in Cancer Therapy

Viral vectors Model	Enzymes delivered	Prodrugs	Model systems
EBV	Nitroreductase (NTR)	CB1954	EBV-positive B-cell lines
Retrovirus	Yeast cytosine deaminase	5-FC	Murine squamous carcinoma cells and YCD-expressing tumors
Retrovirus	Human CYP & P450 reductase	CPA & IFA	Gliosarcoma cells and in vivo tumor model
Adenovirus	Human carboxylesterase	Irinotecan	Human lung adenocarcinoma cell lines & nude mice tumor model

3- Examples of GDEPT in Cancer Therapy

Enzymes	Prodrugs	Model systems
Cytosine deaminase	5-FC	Murine fibroblast cells
Thymidine kinase	GCV	Cisplatin-resistant human ovarian carcinoma cells
Carboxypeptidase	MTX-alpha-peptide	Cos-1 cells

CEA, carcinoembryonic antigen; CMDA, 4-[(2-chloroethyl)(2-mesyloxyethyl) amino] benzoyl-L-glutamic acid; 5-FC, 5-fluorocytosine;GCV, ganciclovir; 5-FU, 5'-fluorouracil; 5'-DFUR, 5'-deoxy-5-fluorouridine; MTX, methotrexate; CB1954, 5-(aziridin-1-yl)-2,4-dinitrobenzamide; CPA, cyclophosphamide; EBV, Epstein-Barr virus; IFA, ifosfamide; HSV, herpes simplex virus; NTR, nitroreductase.

Targeting by prodrugs

Brain targeted Chemical Delivery Systems (CDS) have been developed to deliver drugs to the CNS in a specific and sustained manner [45, 56]. This approach is based on chemical and/or enzymatic multistep conversion of the lipophilic prodrug moiety to its active drug. The mechanism of these CDS is based on a dihydropyridine pyridinium salt equilibrium type redox molecular carrier, similarly to the endogenous nicotinamide adenine dinucleotide ($NADH/NAD^{+)}$ coenzyme system. In this CDS, the lipophilic carrier, dihydropyridine, is attached to the drug for enhancing the drug's BBB penetration before its conversion by enzymatic oxidation to a water soluble quaternary pyridinium salt. Upon intravenous (IV) administration, the dihydropyridine form is promptly distributed throughout the body as well as the CNS and is oxidized to impermeable pyridinium

salt. The polar, oxidized prodrug (Drug-T^+) is stocked behind the lipoidal BBB and remains locked-in in the CNS, whereas the ionic species formed peripherally are eliminated rapidly. The Drug-T^+ conjugate remained in the CNS undergoes enzymatic hydrolysis that releases the active drug slowly and in a sustained manner, whereas the small carrier molecule, pyridinium salt, is actively transported out of the CNS [45]. Figures 13 and 14 illustrate the application of this approach in delivering dopamine to the CNS.

Figure 13: Drug delivery into CNS by molecular packaging and sequential metabolism. The drug is packaged by covalently attached lipophilic groups including a lipoidal Lpf and a 1, 4- dihydropyridine "Targetor" (T) that undergoes enzymatic oxidation and turns to a ionic, membrane-impermeable pyridinium moiety (T^+). After distribution in the body and CNS by crossing the BBB, the CDS is converted to ionic compounds retained in the brain tissues, but ionic conjugates produced in the rest of the body are easily eliminated. The membrane impermeable conjugates "locked" into the brain undergo sequential metabolism and yield the drug in the CNS. A spacer (S) function controls the enzymatic rate of the drug's release.

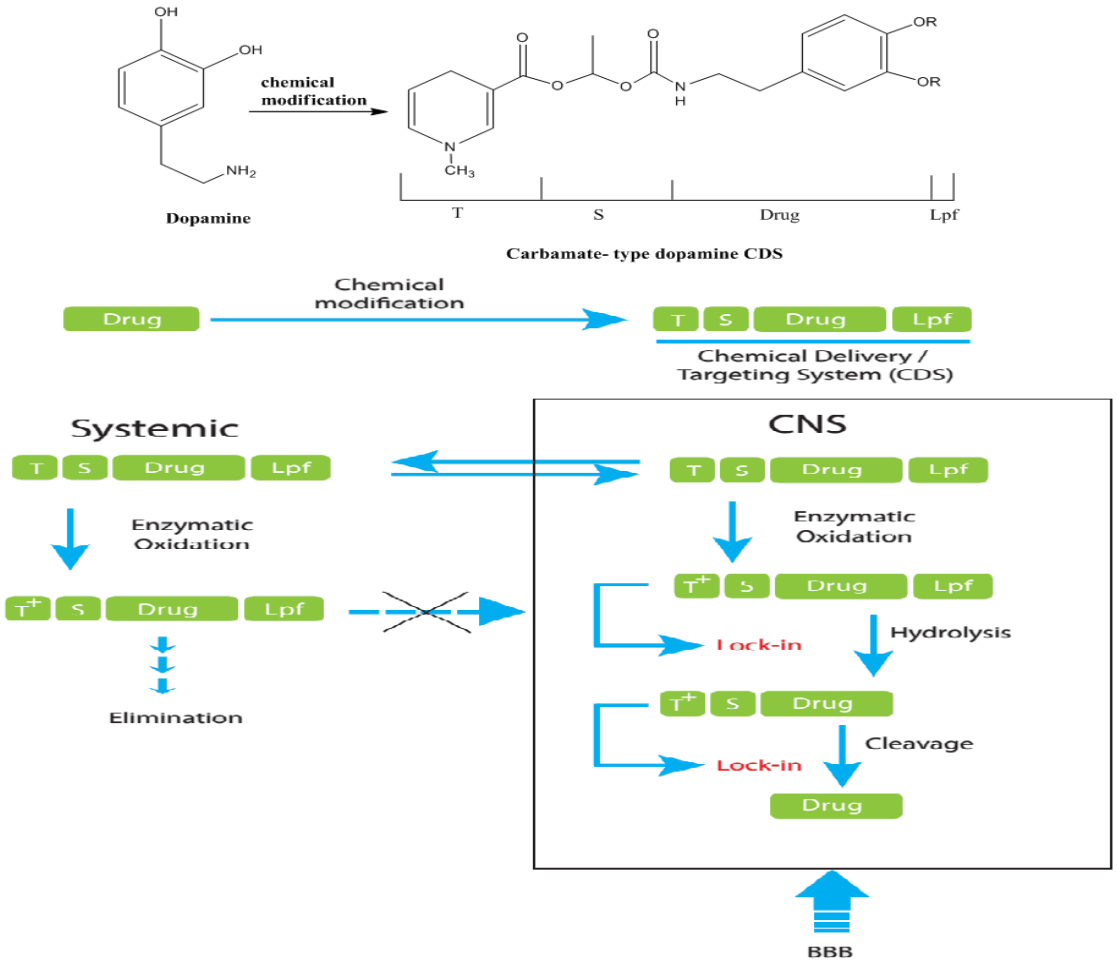
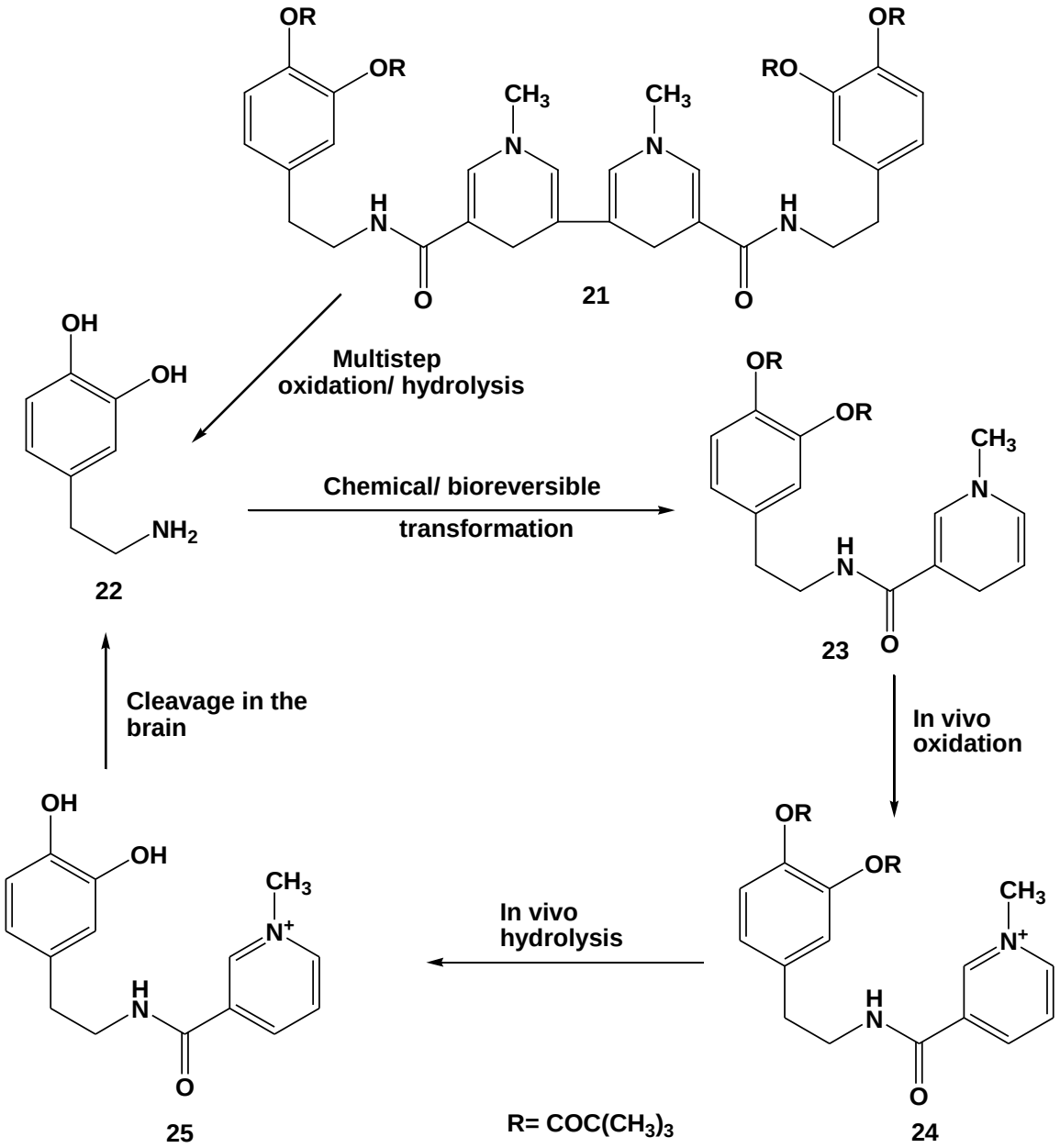


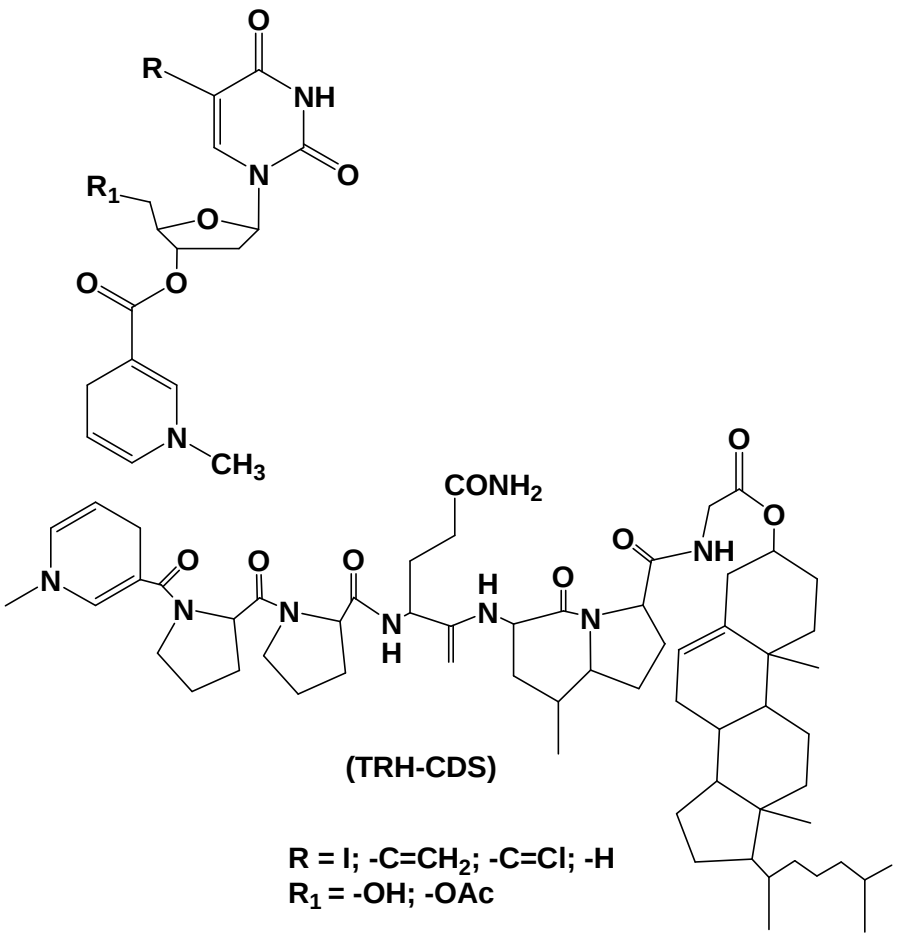
Figure 14: Regeneration of dopamine (**22**) from its amide-type of CDSs (**21** and **23**) in the CNS.



Perioli et al. have designed a prodrug backbone for non-steroidal anti-inflammatory agents such as diclofenac, ibuprofen, ketoprofen, tiaprofenic acid and tolmetin to treat Alzheimer disease, in which the drug is attached to 1,4-dihydropyridine via an amino alcohol bridge and have studies their DBB penetration using the BBB VolSurf model developed by Crivori et al. The study revealed that among all tested compounds ibuprofen and diclofenac derivatives were the best candidates for BBB penetration, and the latter was found to correlate well with the log P of the tested prodrugs [5, 45, 57, 58].

Another example of CDS approach which is considered as a challenging approach due to a rapid inactivation of peptides by ubiquitous peptidases has been successfully used to deliver enkephalin, thyrotropin-releasing hormone (TRH) and kyotorphin analogues to the brain. This successful delivery system overcomes three different delivery obstacles: (1) enhances passive transport by increasing the drug's lipophilicity, (2) guarantees enzymatic stability to premature degradation and (3) uses the "lock-in" mechanism to provide targeting [45, 59, 60]. A demonstration of this system to deliver TRH is illustrated in Figure 15.

Figure 15: Chemical structure of TRH-CDS.



Amino acid prodrugs for improved oral drug delivery

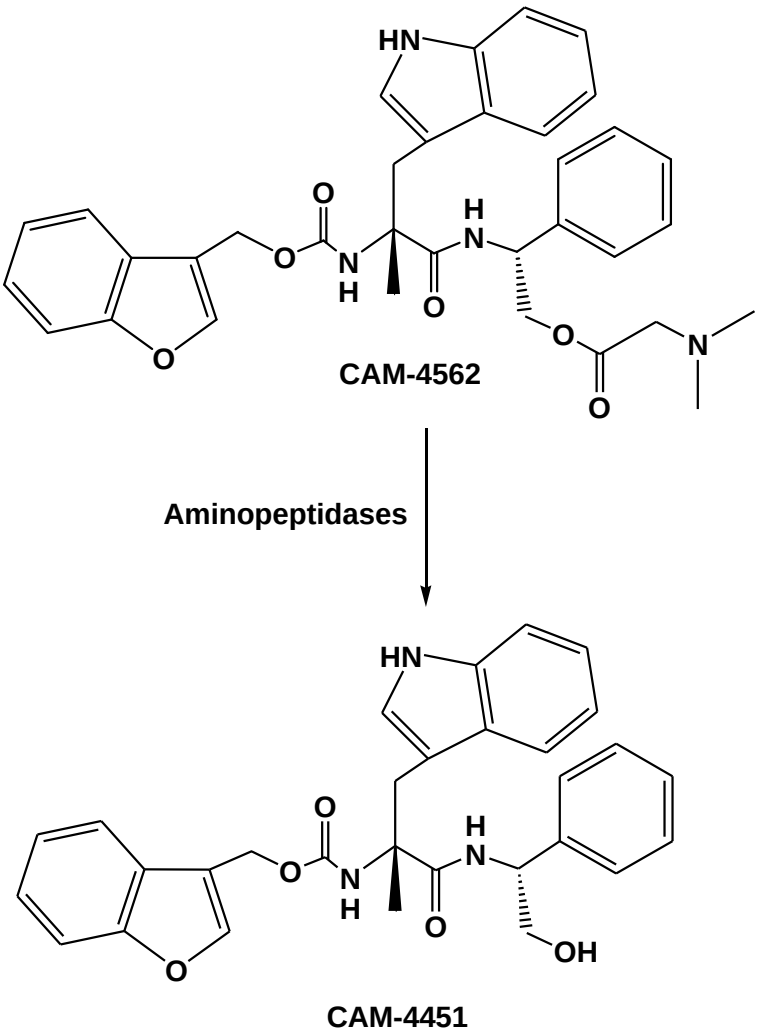
Amino acids are considered as an excellent promoieties to increase the aqueous solubility of parent drugs. In addition, the salt forms of amino acid prodrugs increase the drug's dissolution rate.

Amino acid prodrugs have been utilized to improve oral bioavailability, sustained drug delivery, intravenous drug delivery, target drugs to their site of action and improve enzymatic stability [61].

Poor aqueous solubility is a common problem among therapeutics, thus, the prodrug approach was utilized to improve their aqueous solubility and hence it's oral delivery. For instance, CAM-4562 is a selective nonpeptide neurokinin receptor antagonist (NK1). It is a dimethyl glycine ester of CAM-4451 and used as an antidepressant, antiemetic and anxiolytic agent.

CAM-4451 has a poor oral bioavailability due to its very low aqueous solubility (<2 µg/ml). In an attempt to enhance its solubility, dimethyl glycine prodrug, CAM-4562 was synthesized. Studies on this prodrug demonstrated a 1500-fold greater solubility (3 mg/ml) and 3-fold better oral bioavailability (39%) than its parent drug. In addition, the study revealed a selective hydrolysis of dimethyl glycine prodrug to its parent drug by aminopeptidases, at the brush border membrane of the gastrointestinal tract [61, 62] (Figure 16).

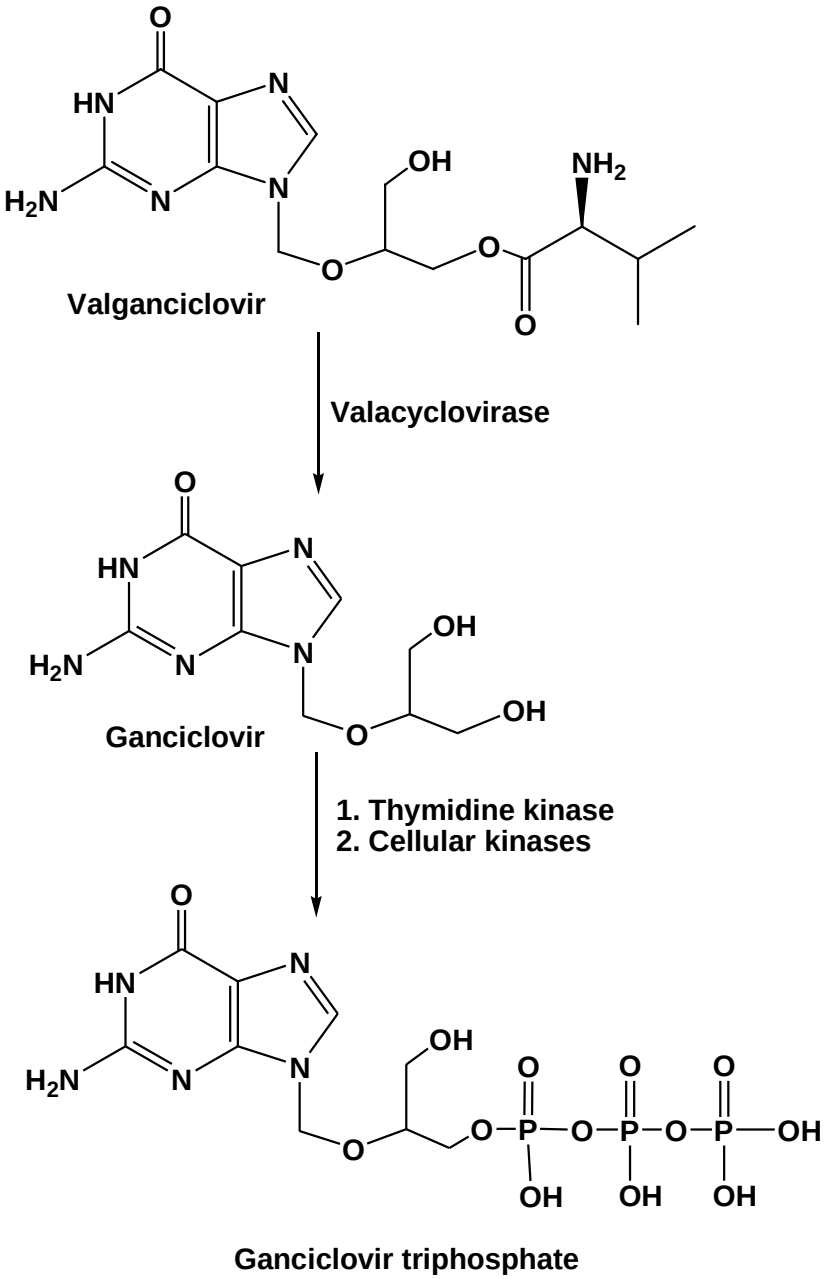
Figure 16: Bioconversion of amino acid ester prodrug CAM-4562 to its parent drug, CAM-4451, by amino peptidases.



Another example of amino acid prodrugs is valacyclovir (Valtrex®). It was the pioneer of L-valyl ester prodrugs, which achieved 3–5-times higher oral bioavailability (>60%) than its parent drug, acyclovir (10–20%) [61, 63].

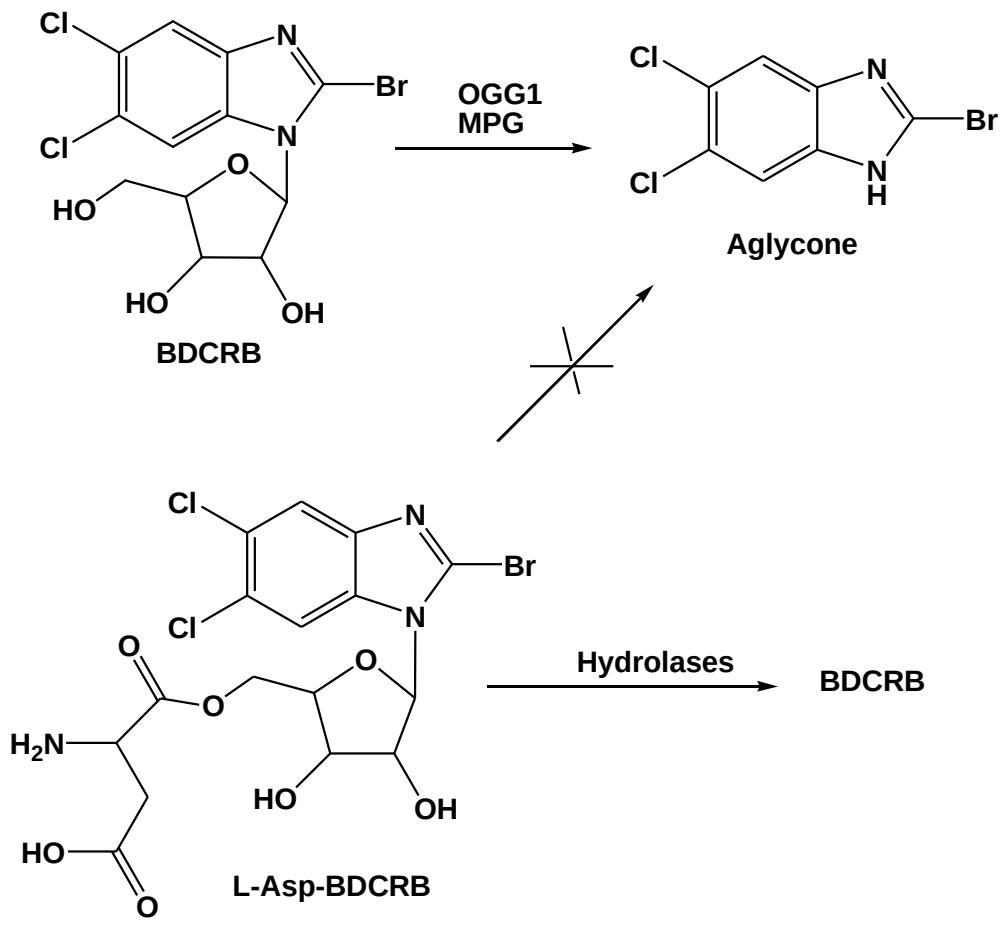
Shortly after valacyclovir discovery, valganciclovir (Valcyte®) was designed. Similar to valcyclovir, valganciclovir is bioactivated by valacyclovirase and absorbed by the PepT1 and amino acid transporters ($ATB^{0,+}$) (Figure 17). Currently, there are several other L-valyl ester prodrugs, including nucleoside analogues levovirin valinate, valopicitabine, and valtorcitabine, that are under clinical investigation [61, 64].

Figure 17: Bioconversion of valganciclovir to its parent drug by valacyclovirase and to its corresponding triphosphates firstly by thymidine kinase and secondly by cellular kinases.



In addition, the amino acid prodrug approach was exploited for the improvement of metabolic stability of nucleoside drugs. For example, the potent and selective inhibitor of human cytomegalovirus (HCMV) 2-bromo-5,6-dichloro-1- (β-D ribofuranosyl) benzimidazole (BDCRB) has no clinical utility because of its rapid in vivo metabolism. The two enzymes, 8-oxoguanine DNA glycosylase (OGG1) and N-methyl purine DNA glycosylase (MPG), are responsible for the cleavage of the N-glycosidic bond of BCDRB, rendering the molecule inactive (Figure 18). Therefore, amino acid ester prodrugs of BDCRB (L-Asp-BDCRB) were synthesized to enhance the metabolic stability, in vitro potency and systemic exposure of the drug [61, 65].

Figure 18: BDCRB N-glycosidic bond cleavage by 8-oxoguanine DNA glycosylase (OGG1) and N-methyl purine DNA glycosylase (MPG) and bio-evasion by enhanced stability via conversion to L-Asp-BDCRB prodrug.



The use of computational approaches in modern prodrugs design

Nowadays the use of computational methods by chemists and biochemists to compute energies, geometries and physicochemical properties for drug and prodrug molecules has received a great attention. Various quantum mechanics methods such as ab initio, semi-empirical and Density Functional Theory (DFT) and molecular mechanics are considered very useful tools that offer structure-energy calculations for the prediction of potential drugs and prodrugs alike [2].

The above mentioned modern computational methods have been utilized by us for the design of innovative prodrugs for commonly used drugs containing hydroxyl, phenol, or amine groups. For this purpose, we have computationally researched the mechanisms of a number of enzyme models that have been advocated to understand enzyme catalysis [66-87] and were exploited by us for the design of some novel prodrug promoieties [3, 88-110]. It is well known that the classic prodrug approach is focused on altering various physiochemical properties. On the other hand, the modern computational approach utilized by us, considers a design of linkers (promoieties) to be covalently linked to the active forms of drugs and upon reaching a physiologic environment (the target) undergo a programmed (controlled) intraconversion to non-toxic linker and the active parent drug without being activated by metabolic enzymes. Furthermore, since the promoieties of the prodrugs are small molecules, it is feasible that the prodrugs themselves will possess considerable therapeutic activity prior to their intra-conversion to the corresponding active parent drugs.

Using, DFT, ab initio, semi-empirical and molecular mechanics methods, a number of enzyme models were investigated in an attempt to determine the factors affecting the rate-limiting step and exert a significant role in overriding the process rate. The enzyme models (intramolecular processes) that have been researched are: (1) proton transfer between two oxygens and proton transfer between nitrogen and

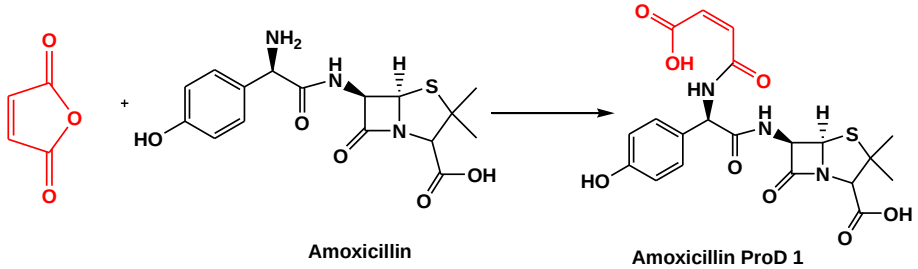
oxygen in Kirby's acetals [111-119] (2) acid-catalyzed hydrolysis in N-alkylmaleamic acids [111-119] (3) proton transfer between two oxygens in Menger's rigid systems [120-124] (4) acid-catalyzed lactonization of hydroxyl-acids as explored by Cohen and Menger [120-126] and (5) SN2-based catalyzed cyclization as studied by Beaumont Webster, Gardner, Dack, Bruice, Pandit, Karaman and Mandolini and Galli [127-131]. For the prodrug approach to be successful it is necessary that the prodrug undergoes inter- or intraconversion at the target site [127, 132]. The main problem in using the classical prodrug approach is the great difficulty in predicting the prodrug's conversion rate, and thus the therapeutic profile of the administered prodrug [133, 134] .

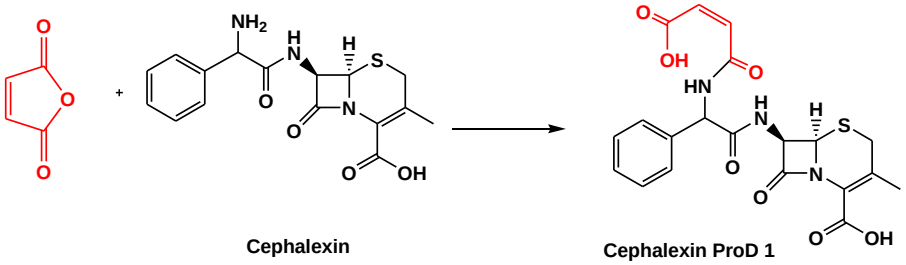
On the other hand, using our approach utilizing intramolecularity (enzyme models) has a potential to provide an efficient chemical device to be used as a prodrug promoiety that can be linked to an active drug to yield a prodrug entity which can chemically, and not enzymatically, undergo cleavage to release the active parent drug in a controlled fashion.

For instance, exploring the mechanism of Kirby's acetals [111-119] led to a design of novel aza-nucleosides prodrugs to treat myelodysplastic syndromes [89] and statins such as simvastatin and atorvastatin to reduce cholesterol levels in the blood circulation [88]. In these examples, the prodrug promoiety was directly attached to the hydroxyl group in the parent drug such that the prodrug will chemically cleave upon reaching the target such as stomach, intestine, and/or blood circulation, with rates that are solely determined by the chemical features of the therapeutically inactive promoiety (Kirby's acetal). Further, acid-catalyzed N-alkylmaleamic acids (Kirby's enzyme model) [111-118] was researched to be utilized as promoieties in the design of tranexamic acid prodrugs to treat bleeding conditions [84], acyclovir prodrugs as antiviral for the treatment of Herpes Simplex [135] and atovaquone prodrugs for the treatment of malaria [93, 136-138]. Menger's intramolecular proton transfer in Kemp acid enzyme model [120-124] was also studied and exploited for the design of dopamine prodrugs to treat Parkinson's disease [139]. Moreover, alkyl fumarates such as monomethyl and dimethyl fumarate prodrugs for the treatment of psoriasis were also designed and developed [95].

This novel prodrugs approach was also applied for the design of bitterless prodrugs for commonly used drugs having bitter sensation such as the pain killer agent, paracetamol, the anti-hypertensive drug atenolol, the decongestant agent, phenylephrine, the anti-inflammatory drugs, diclofenac and mefenamic acid and the antibacterials cefuroxime, amoxicillin and cephalexin [91, 96-98, 101-104]. The role of the linker in the above mentioned prodrugs is to block the amine (for the antibacterial agents) or hydroxyl groups (in paracetamol, atenolol and etc.) which are believed to be responsible for the drug bitter taste of the parent drug. The only chemical difference between the designed antibacterials prodrugs and their parent drugs is that the free amine group in the parent drug is replaced with an amide group (Figure 19). Replacing the amine with an amide group eliminates the capability of the antibacterial to interact with the bitter taste receptor, thus masking the bitter sensation of the parent antibacterial drug.

Figure 19: Chemical structures of amoxicillin and cephalexin and their prodrugs, amoxicillin ProD 1 and cephalexin ProD 1, respectively.





Peptides

Peptides have received a great attention as drug candidates due to their significant importance to efficiently and specifically cure many CNS diseases. Various studies have demonstrated that peptides penetration into the brain cannot be simply predicted by single molecular properties or net charges. Therefore, more comprehensive models were proposed in order to evaluate possible BBB permeability of peptides. For instance, Giralt and coworkers were able to construct a Genetic Algorithm (GA)-based model where nine physiochemical parameters including log P, conformation and aromaticity were considered to model the penetration of BBB by randomly generated peptides. From this study it was concluded that BBB permeability is difficult to be predicted using simple rules of small molecules and it requires combined approaches. Recently, the light has been spotted on a family of short peptides known as Cell Penetration Peptides (CPPs) as potential peptide-based delivery vectors. Some efforts were made to predict sequences that have the potential to penetrate into biological barriers, especially BBB. Recently a set of descriptors called Z-scaled was used to model the BBB permeability. It derived from a lot of physiochemical parameters for each amino acid. The study revealed that bulk property values ($z\sum/n$) have a good predictive ability. The shortcoming of these descriptors is overlooking the peptides sequence because values of $z\sum/n$ are similar for a set of scrambles analogues. [5, 140, 141].

Paracellular pathways

Paracellular pathways of the intestinal mucosa and BBB share some similarities. Via this pathway, small hydrophilic molecules and ions can passively pierce, whereas large molecules can't pass due to the presence of tight junctions. Moreover, peptides and proteins can't effectively pass through these pathways, thus making it difficult for their delivery to the brain or for oral absorption. Modulation of the intercellular junctions to increase their porosity and permit the passage of large hydrophilic molecules (of certain size to prevent toxins entrance) was one of the experienced techniques to improve the delivery of large hydrophilic molecules. Beforehand, a success in opening the intercellular junctions of the BBB to deliver anticancer drugs to patients with brain tumors has been reached using a hypertonic solution of mannitol)[1, 142-144].

Nano particles to improve drug delivery across biological barriers

Nano particles were among the strategies investigated to improve delivery of drugs across the intestinal mucosa and the BBB. It has been demonstrated that Nano particles with peptide ligands are able to pass the intestinal mucosa using receptor-mediated transport. Therefore, Receptor-Mediated Transcytosis (RMT) was used for efficacious delivery of Nano particles through the intestinal mucosa and BBB. Particles decorated with large molecules such as apolipoprotein, ligands to Transferrin (Tf) receptors (i.e., Tf and anti-Tf-receptor antibodies), and antibodies to insulin receptors were used and achieved a successful delivery of Nano particles across BBB [1, 145, 146].

Despite the fact that Nano particles can be internalized via pinocytosis or absorptive-mediated transcytosis (AMP), these processes are not efficient due to the low amount of transported Nano particles. Besides, Nano particles can be taken up by other cells non-selectively in the case of absence of targeting molecules [1, 145].

On the other hand, Nano particles do not have the ability to cross via the paracellular pathway because of their size. Small particles (<200 nm) pass via clathrin-mediated endocytosis and large particles (500 nm) via caveolae-mediated endocytosis. Yet, endocytosis is accompanied with its shortcomings that were mentioned previously. The most promising particles for delivering anticancer agents through BBB to treat brain tumors are modified liposomes [1, 145, 147].

Summary and Conclusion

Delivery of therapeutics to their target sites is considered crucially important to the drug discovery and development sector. Various approaches and tarnishes have been invoked and used in order to improve the delivery of a variety of drugs. Among the utilized methods is the use of the natural occurring transporters to enhance the passive diffusion of drugs through transcellular pathways and to modify efflux pumps to avoid P-gp activity. Nucleoside analogues were exploited to improve drug's delivery and CYP-activated prodrugs, called HepDirect, were utilized to target drugs to the liver, in which they remain inactive until being activated by specific enzymes in the liver.

Moreover, receptor-mediated transport mechanisms have been explored for the purpose of delivering small molecules, peptides, proteins, nanoparticles and liposomes through biological barriers. These include PEPT1, GLUT1, hASBT and Transferrin Receptors (Tf-R). In addition, Carrier Mediated Transport (CMT) systems such as neutral amino acid (LAT1), hexose (GLUT1), monocarboxylic acid (MCT 1), Cationic Amino Acid (CAT 1), nucleoside (CNT 2) and ascorbic acid (SVCT 2) were heavily studied.

As for cancer drugs, tissue selective drug delivery is the most exploited approach in which prodrugs are activated by enzymes preferentially localized in target tissues, and specific transportation of prodrugs to the target tissues is accomplished via specific transporters, conjugates of drugs and molecules selectively bound to the target tissues. Other methods which were investigated to deliver anti-cancer drugs include ADEPT, GDEPT and VDEPT.

Likewise, enzymes involve in the bioconversion of ester-based prodrugs were also used for the activation (hydrolysis) of prodrugs to their corresponding active therapeutic agents.

Amino acid prodrugs have been investigated to improve drug delivery and other associated drugs' pharmaceutical issues.

The combined results emerged from the studies described in this review emphasize the importance of the prodrug approach and the use of transporters in enhancing drug delivery and overcoming obstacles stem from physiological barriers or pharmaceutical issues.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Graphical Abstract

Conversion of the cyclic peptide prodrug to the parent peptide by the action of esterase enzymes