REVIEW

Recent updates in utilizing prodrugs in drug delivery (2013–2015)

Wajd Amly* and Rafik Karamanab

*Pharmaceutical Sciences Department, Faculty of Pharmacy, Al-Quds University, Jerusalem, Palestine, Israel; bDepartment of Sciences, University of Basilicata, Potenza, Italy

ABSTRACT

Introduction: Utilizing the prodrug approach as a method to overcome various pharmaceutical and pharmacokinetic barriers to drug delivery is significantly accelerating and achieving successes. In contrast to the older traditional prodrugs which suffer from decreased bioavailability and a high profile of side effects, due to activation at undesired sites, the targeted prodrug approach utilizes delivery systems to improve delivery for a wide range of therapeutics including anti-cancer, anti-bacterial and anti-inflammatory drugs.

Areas covered: Recent updates in utilization of prodrugs in drug delivery between 2013 and 2015 are discussed. Targeted prodrugs against cancer, solid tumors, microbial infections, inflammation and other diseases using advanced delivery systems such as theranostic approaches, siRNA, DOX immunoconjugate, C 60-ser carrier vector, biotinylated prodrug, human serum albumin (HSA) carrier and others are presented.

Expert opinion: Recent research efforts have been directed at developing targeted prodrugs to replace the classical prodrugs. The use of this approach has accelerated following the emergence of encouraging results from several studies on targeted prodrugs that have highlighted their higher efficiency and improved safety profiles. Targeted prodrug delivery is now considered more than a chemical modification method. It is an applicable and promising approach and, in the future, better knowledge and wide application of this approach may be attained which may pave the way for more forward-thinking and creative techniques.

1. Introduction

During the past 30 years, the pharmaceutical field has been experiencing considerable alterations in terms of eliminating or reducing drug drawbacks that stem from pharmacokinetic (PK) (absorption, distribution, excretion and metabolism), pharmaceutical and biological performance of marketed medicines.

Overcoming the unwanted PK, biological or organoleptic properties of some currently used drugs can be achieved through the development of new chemical molecules with the desired therapeutic profiles and safety. However, this process is time consuming and quite expensive that needs a precise screening of thousands of entities for pharmacological activity. Hence, the feasible and economical alternative way is to modify and improve the physicochemical properties of currently marketed drugs through exploring the prodrug approach.

The prodrug approach has become one of the most successful and utilized approaches that is used to solve various types of problems associated with the use of drugs. It has been used to overcome various physicochemical obstacles such as low oral absorption, acid sensitivity, poor permeability, lack of site specificity, insufficient chemical stability, poor solubility, toxicity, etc. [1-11].

Nowadays, a respected number of marketed drugs are prodrugs. Approximately 10% of all worldwide marketed drugs can be classified as prodrugs, and in 2008 alone, about a third of all approved drugs having small-molecular weights were prodrugs.

Prodrugs are bioreversible derivatives of drug molecules that generally inactive themselves but can be transformed into their active forms via chemical or enzyme-catalyzed reactions [1-11].

This review is devoted to the description of the recent updated in utilizing the prodrug approach in delivering a wide range of important drugs, such as anticancer, antibacterial, antiviral and anti-inflammatory, to different tissues and organs within the human body.

CONTACT Rafik Karaman dr_karaman@yahoo.com Faculty of Pharmacy, Department of Bioorganic & Pharmaceutical Chemistry, Al-Quds University, P.O. Box 20002, Jerusalem, Palestine, Israel

Supplemental data for this article can be accessed here.

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2. Targeted prodrugs used to deliver anticancer drugs

Cancer, an uncontrolled cell division of abnormal cells, can affect all types of cells in the body organs. Anticancer agents are not selective and result in many and severe side effects since normal cells are also affected by such agents.

Recently, the number of diagnosed patients with tumors has been escalating and the common treatments used to treat those patients are surgery, radiation and chemotherapy alone or in combination. Due to the absence of an ultimate cure and insufficient therapeutic activity of the currently marketed chemotherapeutic drugs, there is a pressing need for the health professionals and scientists to invoke potent and safe anticancer treatments with optimal therapeutic profiles.

During the past few years, scientists were focusing on modifications of currently available drugs into more effective regimens with better physicochemical properties by synthesizing prodrugs, especially targeted prodrugs that produce their therapeutic effects at the desired sites of action, which consequently lead to increased efficiency and reduced undesired side effects that are known to be associated with the administration of their parent cytotoxic agents.

Prodrugs targeting cancer cells take advantage of specific conditions available at these tumor cells such as hypoxia, existence and/or overexpression of certain enzymes, tumor-specific antigens and low extracellular pH [12,13].

2.1. Delivery approaches applied for paclitaxel

The microtubule inhibitor paclitaxel, taxol (1 in Figure 1), is a drug used for the treatment of various types of cancer, including breast, ovarian, lung and pancreatic. Its mechanism of action involves an interference with the normal breakdown of microtubules during cell division.

The use of paclitaxel is associated with two major drawbacks: a high nonspecific cytotoxicity and poor water solubility. Therefore, selecting the appropriate delivery vehicle for paclitaxel is crucially important.

Fullerene-based micelles have been developed as a drug delivery system (DDS) [14], and a number of water-soluble C60 derivatives have been suggested for various medical applications. Recently, the antioxidant, water-soluble, nontoxic transfection agent vector, fullerene was combined with paclitaxel to deliver a chemotherapeutic prodrug to cancer cell nuclei aiming to reduce the nonspecific toxicity associated with the use of the naked paclitaxel [15].

A study by Mackeyev et al. has shown that a fluorescently labeled biocompatible malonodiserinolamide-derivatized [60] fullerene (C60-ser) was able to escape endocytosis vesicles and enter into cancer cells along with serum proteins via multiple energy-dependent pathways with negligible cytotoxicity to normal cells. The study outcome demonstrates the great potential of using C60-ser as a delivery vehicle for various therapeutic agents with intranuclear activity such as siRNA, DNA plasmids, etc. Furthermore, tumor-specific antibodies can be utilized to attain targeted delivery for these vehicles as fullerenes are known for their strong affinity to antibodies to form immunoconjugate. An example of such entities is ZME-018, a designated antibody that targets the gp240 antigen (also known as the high-molecular-weight melanoma-associated antigen found on the surface of >80% of human melanoma cell lines and biopsy specimens) in order to achieve targeted fullerene immuno-therapy [14-16].

Two novel water-soluble paclitaxel conjugates with C60-ser (2 and 3 in Figure 1) that are anticipated to release paclitaxel under controlled conditions upon hydrolysis were designed in an attempt to treat cancer cells with carbon nanostructure-mediated radio frequency hyperthermia (including [60]fullerene). In vivo studies on compounds 2 and 3 revealed an equal or greater efficacy of the two conjugates over the current paclitaxel formulations, Cremophore EL and Abraxane® (Abraxis Bioscience, Los Angeles, CA, USA) [15,17].

C60-ser conjugated to other anticancer drugs is to be investigated in the future for establishing a proper class of prodrugs for a delivery through the C60-ser carrier vector.
2.2. Delivery approaches applied for doxorubicin and epirubicin

Doxorubicin (DOX) (4 in Figure 1) is an anthracycline antitumor antibiotic and like all other anthracyclines has various side effects with the most serious side effect being life-threatening heart damage. DOX is commonly used in the treatment of a variety of cancers, including hematological malignancies such as leukemia and lymphoma, many types of carcinoma, solid tumors and soft tissue sarcomas. It is generally used in combination chemotherapy with various chemotherapy regimens.
The mechanism of action of DOX is by intercalating DNA.

DOX is one of the anticancer drugs that received significant efforts for improving its clinical profile since it suffers from being readily distributed into almost all body tissues and intracellular compartments causing various toxic effects.

A number of strategies have been inspected for DOX’s efficient clinical profile. These strategies include combinations of DOX with other anticancer drugs, different methods of administration, addition of antioxidant nutrients and cardio protectors, development of liposomes and nanoparticles (NPs) and development of prodrugs [18].

The intracellular protease cathepsin B (Cat B) is a lysosomal cysteine protease found in perinuclear vesicles within normal cells and tissues and is highly upregulated in malignant tumors and premalignant lesions [19].

Based on this fact, DOX immunoconjugate that link DOX to a carcinoma-specific antibody through Cat B-cleavable oligopeptides have been advocated as targeting delivery system for DOX (Supplementary Figure S1).

Studies using this technique revealed that the release rate of DOX from the delivery system was found to be dependent on the length and structure of the spacer, where the optimal release was reached with tetrapeptide Gly-Phe-Leu-Gly. Furthermore, it was demonstrated that self-immolative spacer like para-aminobenzoyloxy carbonyl, which links the drug with the oligopeptide substrate, is crucially important to decrease or eliminate the steric interactions between the peptide substrate and Cat B since these interactions determine the rate by which DOX is released from the prodrug system (for this delivery system mechanism, see Supplementary Figure S1) [18,20].

Several studies have demonstrated that Cat B-cleavable DOX prodrugs are less toxic in vitro and more effective in vivo than DOX, and yet, additional studies on DOX prodrugs’ physicochemical properties, cytotoxicity and antitumor potency are required for obtaining DOX prodrugs with the desired clinical profile.

Another delivery system utilized to provide more efficacious DOX delivery system was advocated by Gou and coworkers [21]. They synthesized a novel DOX prodrug, polyethylene glycol (PEG)–DOX (5 in Figure 1), by conjugating a deprotonated DOX molecule (hydrophobic region) with a short-chain PEG (hydrophilic region) via a hydrazone linkage. In aqueous solutions, this amphiphilic prodrug was shown to be self-assembled into stable NPs, and in an acidic environment, the hydrazone bond cleaved and initiated a fast release of DOX [21].

The study found that the prodrug, PEG–DOX, showed an extremely high (~46 wt%) and stable drug loading due to the low molecular weight of its PEG chain, and the prodrug can bypass P-gp-mediated efflux of free DOX via the endocytosis pathway, allowing for a significant drug accumulation. In addition, PEG–DOX demonstrated an excellent in vivo and in vitro antitumor activity. This prodrug delivery system proved to be advantageous over other DOX delivery systems that include a high drug loading, a controlled drug release and a high in vivo safety. For instance, N-(2-hydroxypropyl)-methacrylamide (HPMA)–doxorubicin conjugate (PK1; FCE28068) and HPMA copolymer–doxorubicin–galactosamine conjugate (PK2; FCE28069), presently under clinical trials, were found to have a drug loading content of only ~8 wt% for PK1 and even lower for PK2 [21–23]. Further studies on this technique are currently underway.

A similar drug delivery technique was proposed by Yuan et al., which is based on amphiphilic polymeric prodrug nanoassemblies to capture the free forms of therapeutic agents (Supplementary Figure S2). In this approach, the hydrophilic part is PEG (2k Da) with a targeting ligand attached to one of its end. As for the hydrophobic core, a hydrophobic drug was used that serves as the therapeutic agent and as the hydrophobic core of the prodrug/drug complex. An acid labile linker was used to link the drug with PEG for enhancing the conjugated drug’s retaining activity [13].

An example of such DDS is biotinylated-PEG-hydrazone-doxorubicin (BPD), which was synthesized as a model prodrug due to its amphiphilic structure that has the potential to be self-assembled into NPs in aqueous solution >42.8 mg/L (the critical aggregation concentration). As a result of this property, the prodrug moiety can be used to encapsulate any free drug. The BPD prodrug demonstrated good loading capacities for free DOX (35%) and camptothecin (CPT) (24%). Moreover, the prodrug/drug complexes have showed a very good cytotoxic effect on cancerous cells and their effects on normal cells were negligible. This strategy is promising for delivering multimolecular agents into cancerous cells [13].

Recently, considerable attention has been focused on (pseudo)polyrotaxanes prodrug micelles technique, which is based on noncovalent interactions between α-cyclodextrin (CD) and PEG. Studies on the interactions between those two entities proved the ability to induce a formation of nanoassemblies, which can be utilized as nanocarriers for bioactive molecules [24]. Distinct from other CD-based supramolecular polymers, α-CD has the
ability to thread onto PEG chains in a well-ordered and high-density manner, which allows for an incorporation of high amount of α-CD in the assembled complex. In addition, the assembly procedure takes place in water at room temperature, and there is no need for organic solvents during the fabrication process [25].

Accordingly, Wang et al. have developed stable pseudopolyrotaxane prodrug micelles with high drug content via a simple mixing of two hydrophilic segments ([α-CD-hydrazide-DOX HCl and poly(ethylene glycol)-β-poly(2-methacryloyloxyethyl phosphorylcholine)]) in an aqueous medium. DOX was conjugated to the CD rings by an acid-labile hydrazone bond (Supplementary Figure S3). Thus, following internalization inside cancer cells, DOX was released from the backbone at endo-/lysosomal pH, resulting in the inhibition of cancer cells proliferation [25].

In addition, the study revealed that replacement of some of the hydroxyl groups on the CD rings by other guest molecules did not show any disturbance to the interactions between CDs and PEG, and the formation of micelles was imminent. Furthermore, it was found that CD can be modified not only by DOX, but also by other various types of molecules. Besides, DOX content was found to be about 35% in the supramolecular prodrug micelles, a higher percentage compared with most of the polymer prodrug micelles. As for cellular uptake, it was confirmed that supramolecular prodrug micelles were able to deliver and release DOX into the nuclei of cancer cells and induce remarkable cytotoxicity to cells [25].

On the other hand, antibody-drug conjugate (ADC) is a promising method to incorporate toxic drugs to be delivered selectively to a tumor site. In this regard, Yamamoto and coworkers developed an anti-TF-NC-6300, consisting of epirubicin-incorporating micelles (NC-6300) conjugated with the F(ab')2 of anti-tissue factor (TF) monoclonal antibody (mAb). Each one of the anti-TF-NC-6300 molecules encapsulates around 600 epirubicin molecules and holds four molecules of F(ab')2 at the termini of PEG chains on the particle surface; therefore, the molecular ratio of epirubicin to F(ab')2 is around 150:1 [26].

Results demonstrated that the intracellular uptake of epirubicin was faster and greater in cells with high TF expression; human pancreatic cancer cell lines with BxPC3 and gastric cancer cell line (44As3) when treated with anti-TF-NC-6300 compared to NC-6300. As for tumor accumulation, it was higher for anti-TF-NC-6300 compared to NC-6300 regardless of the TF expression levels, and yet, anti-TF-NC-6300 localization was greater in tumor cells with high TF expression. In addition, the antitumor activity was superior in the cells treated with anti-TF-NC-6300, whereas the activities of both micelles (TF-NC-6300 and NC-6300) were similar in the human pancreatic cell line with low TF expression (SUIT2). These results indicated that enhancement of anti-TF-NC6300 antitumor activity may depend on the widespread intratumor distribution, selective intratumor localization and internalization of anti-TF-NC-6300 into high TF tumor cells [26].

2.3. Delivery approaches applied for gemcitabine

The in situ monitoring of the release of an active drug has great benefits especially if this can be achieved in a noninvasive manner. Recently, a theranostic (therapy and diagnostics) DDS that has targeting and reporting abilities was proposed.

Maiti and coworkers designed a cancer-targeting theranostic DDS composed of gemcitabine (GMC)-loaded coumarin moiety coupled with biotin as a targeting ligand. It is known that biotin or biotin conjugates are taken up by cancer cells. Conjugate 1 (Figure 2) contains the chemotherapeutic drug GMC, a disulfide linker that is cleaved to intracellular thiols, a coumarin moiety that improves the fluorescence intensity and biotin; the guiding molecule to tumor cells. Upon a cleavage of the disulfide bond, the resulting thiol undergoes an intramolecular nucleophilic substitution at the carbamate moiety to yield a five-membered ring thiolactone and GMC molecules (Figure 2) [27].

Furthermore, the study by Maiti et al. revealed that the disulfide bond of conjugate 1 undergoes a cleavage mediated by GSH in a dose-dependent manner, with no noteworthy interference from other biomolecules, leading to fluorescence enhancement. Moreover, compound conjugate 1 was found to be localized in the lysosome after receptor-mediated endocytosis and its transport was dominantly into biotin receptor-positive tumor cells, and no transport of conjugate 1 into biotin receptor-negative cells was observed. This DDS is a promising approach for specific tumor targeting drug delivery and cellular imaging [27].

2.4. Delivery approaches applied for platinum compounds

Another novel drug delivery strategy is the one used for loading platinum (Pt) prodrugs. It facilitates the delivery of Pt drugs utilizing a well-defined supramolecular system. Pt-based anticancer drugs are among the widely used anticancer classes, which include cisplatin, carboplatin and oxaliplatin.
Cisplatin prodrugs, Pt(IV) prodrugs, can be synthesized from oxidation reactions of the corresponding parent drugs, the active square-planar Pt(II) species. The oxidation products, Pt(IV) complexes, are more inert to ligand substitution than the Pt(II) complexes. For anticancer effect to be exerted, Pt(IV) prodrugs must be reduced intracellularly to their corresponding Pt(II) forms [28, 29].

Zheng et al. have innovated a delivery system based on the use of Pt(IV) prodrugs and a self-assembled metal-based complex. In this technique, Pt(IV) prodrugs are loaded into a hexanuclear Pt(II) cage complex via host-guest interactions. Cages or metal-based polyhedra are a group of complex inorganic moieties accessed through coordination-driven self-assembly that functions as a delivery vehicle for the cargo Pt(IV) prodrugs. Each cage contains four Pt(IV) building blocks. Consequently, this gives the nanoconstruct a high positive charge, which in turn facilitates the cellular uptake. Once inside the cell, the self-assembled supramolecular system releases cisplatin and destroys cancer cells [30]. Furthermore, the study has proved the effectiveness of the supramolecular complex against cancer cells [30].

In another study, Zheng et al. synthesized a series of Pt(IV) complexes to exploit the endogenous protein, human serum albumin (HSA), as a delivery device for cisplatin. HSA is the most abundant protein in the human blood. One of its vital roles in the body is to serve in the transportation of fatty acids in the blood. It is a good carrier for anticancer drugs due to its non-immunotoxicity, long circulation time in the blood and high tumor accumulation. In this study, the concept was to design compounds that mimic the amphiphilic structure of fatty acids. For this purpose, asymmetrically functionalized cisplatin prodrugs were developed in which one axial ligand is a succinate moiety and the other is an unbranched aliphatic carbamate (Figure 3). The assumption was that this chemical device will enable the association of compound 4 (Figure 3) with HSA. The most promising compound among all synthesized derivatives that is believed to have the highest interactions with HSA was 4e (Figure 3) [29].

The study has proven that 4e is much more stable in the blood than the previously explored Pt(II) and Pt(IV) compounds and that its ability to interact with HSA may confer this high stability. An increase in the chain length from C2 (4a in Figure 3) to C16 (4e in Figure 3) significantly enhances the cellular uptake. Following
cell entry, Pt(IV) prodrugs have shown to be activated by a reduction reaction mediated by glutathione or L-ascorbic acid (AA) leading to the release of cisplatin. Furthermore, in vitro study revealed that 5e possesses a magnificent anticancer activity in a variety of cancer cell lines, which reached 9–70-fold the activity of cisplatin. Moreover, 5e showed a higher half-life \( t_{1/2} \) than cisplatin. A strong noncovalent interaction between 5e and HSA provided the formation and isolation of a stable complex that serves as a delivery vehicle for 5e. The interaction with HSA protects 5e from the reducing environment found in the blood [29].

Xi and coworkers have developed HSA nanocarrier system for the delivery of Pt anticancer drugs into cancer cells. This system combines a photoactivatable Pt(IV) antitumor prodrug, which serves as a detector of the drug’s controlled release, and fluorescent light-up probe that evaluates the drug action and efficacy. This system is locally activated by light irradiation to liberate the active Pt species, which induces cell death. More importantly, the cytotoxicity induced by the light irradiation has the capacity to activate the apoptotic protease enzyme caspase 3, which in turn induces a cleavage of the recognition peptide moiety (DEVD) with a flanking fluorescent resonance energy transfer (FRET) pair that contains near-infrared, fluorophore Cy5 and quencher Qsy21 on the HSA nanocarrier surface. In addition, this kind of nanocarrier is distinguished for being able to allow for simultaneous

Figure 3. Synthetic routes for the preparation of 4a-e from cisplatin: (a) 30% \( \text{H}_2\text{O}_2 \), 50 °C; (b) succinic anhydride, anhydrous DMSO, RT and (c) \( \text{R}–\text{N}=\text{C}=\text{O} \), anhydrous DMF and RT, 6. (B) Photoactive Pt(IV) prodrug and peptide probe conjugated HSA nanocarrier for controlled drug activation and real-time imaging of activated apoptosis and (C) Pt(IV)-Probe@HSA Nanocarrier.
real-time imaging of the controlled drug release and thus an evaluation of the antitumor activities [31].

In the design of this nanocarrier technique, photoactivatable Pt(IV) complex (the prodrug moiety) was linked to the amino groups contained in the HSA moiety and a peptide sequence that can be activated by an enzyme, such as Cy5-acp-CGDEVDAK-Qsy21, was flanked with a FRET pair in order to obtain the desired real-time apoptosis imaging. The study demonstrated that the ideal Pt(IV) prodrug loading was 4.1% on the surface of HSA nanocarrier [31].

In conclusion, this multifunctional protein-based nanocarrier is a smart promising strategy that reduces the incidence of side effects as it controls the localized activation of Pt(IV) prodrug and permits a drug mechanism imaging.

Another interesting ligand-mediated DDS was proposed by Miura et al., which is based on using Pt anticancer drug-incorporating polymeric micelle (PM) with cyclic Arg-Gly-Asp (cRGD) ligand molecules. Their study aimed to adapt intravital confocal laser scanning microscopy technique to long circulating cRGD-linked nanocarriers in order to study the penetration pathway and derived therapeutic activity of cRGD-mediated DDS. This delivery system is made from long-circulating, cyclic RGD-linked PMs that combine the parent complex of the potent anticancer drug oxaliplatin (1,2-diaminocyclohexane)platinum(II) (DACHPt) via a metal complex formation-driven self-assembly to long circulating cRGD-linked nanocarriers. This delivery system was prepared by using the cRGD-mediated DDS. The results were promising as the micelle showed rapid tumor accumulation and high permeability from vessels into the tumor parenchyma. It also was proposed that the selective and accelerated accumulation achieved by cRGD/m into tumors occurred via an active internalization pathway, possibly transcytosis [32-35].

Another tumor-targeted delivery of cytotoxic drugs is antibody-mediated therapies including ADCs. This system is based on clinical evidence that highly toxic anticancer agents should be conjugated to a mAb in order to administer a reasonable amount of ADC to patients without compromising the affinity of the mAb. Till now, reported antibody-conjugated micelles (immunomicelles) for specific delivery of drugs in cancer therapy are few, and yet, no immunomicelle has proceeded to clinical evaluation. Therefore, in an attempt to achieve selective Pt drugs targeting to pancreatic tumors with high potential for clinical translation, Ahn and coworkers have made antibody fragment-installed PMs incorporating an active complex of oxaliplatin, (1,2- diaminocyclohexane)platinum(II) (DACHPt) (DACHPt/m) via maleimide-thiol conjugation in one-to-one ratio. Anti-TF antibody (clone 1849), which can target TF overexpressed on the surface of cancer cells, was chosen. The study revealed >15-fold increase in cellular binding of Fab′-installed Pt-loaded micelles (anti-TF Fab′-DACHPt/m) within 1 h and fast cellular internalization due to antigen recognition ability of anti-TF Fab′. In addition, in vivo, Fab′-installed micelles were able to considerably suppress the growth of pancreatic tumor xenografts for >40 days, which is even better than the parent drug itself. These results indicate the potential of Fab′-installed PMs for efficient drug delivery to solid tumors with enhanced safety profile [36-39].

### 2.5. Delivery approaches applied for SN-38

SN-38 is the active metabolite of irinotecan (CPT-11), an analogue of CPT, a topoisomerase I inhibitor and is formed through hydrolysis of irinotecan by carboxylesterases and metabolized via glucuronidation by UGT1A1. It is about 1000 times more potent than its parent drug, irinotecan.

Recently, Wang et al. have concluded that suitable molecular engineering could facilitate the incorporation of SN-38 into favorable copolymer-based delivery platforms and thus enhance its antitumor effect [40,41].

To accomplish this goal, it was necessary to obtain a system containing SN-38 linked to a lipophilic tail, which upon exposure to physiological environment induces a self-assembly system that is able to effectively release the parent drug SN-38 inside the tumor cells. To test this notion, novel SN-38 derivatives were made in which the 10 (or 20)-hydroxyl groups were functionalized by different hydrophobic moieties (Figure 4) [40].

The study revealed that derivatives in which the 10-hydroxyl group was linked to saturated fatty acids with long and short alkyl chains or alkyl silyl groups have shown immediate precipitation and no formation of transparent solutions was observed upon their injection into water. Furthermore, prodrugs that were modified with polyunsaturated fatty acids – docosahexaenoic acid (6 in Figure 4), linoleic acid (LA) (7 in Figure 4) and α-linolenic acid (8 in Figure 4) – have shown better ability to assemble than prodrugs modified with saturated alkyl chains due to structural flexibility and intermolecular π–π stacking, which contribute drastically to stabilization of their solutions. On the other hand, prodrugs designed with Boc- protection amino acid (9 and 10 in Figure 4) were water soluble and readily co-assembled with amphiphilic block copolymers such as PEG methyl ether-block-
poly(L-lactide) NPs. Unlike the 10-hydroxyl derivatives, prodrugs in which the 20-hydroxyl group was shielded by lipophilic tails (saturated or unsaturated fatty acids) did not form assemblies and resulted in large precipitates. This instability might be due to the strong polarity of the unshielded phenolate groups contained in SN-38. The combined results revealed that the 20-hydroxyl group in SN-38 has a vital role, as a weakly polar moiety, in stabilizing assemblies [40,41].

As for cytotoxicity, prodrugs 6–10 showed superior cytotoxicity than CPT-11, but comparable to that of SN-38 due to delayed release of the active SN-38 from the delivery system. Overall, these supramolecular nanoparticles (SNPs) can induce efficient early and late apoptosis in a large population of cells after 2 days [40].

Furthermore, it was demonstrated that prodrugs modified with flexible polyunsaturated fatty acids (7 and 8) have increased the drug presence in the blood and the structural stability of SNPs have a significant effect on the drug’s PK. More importantly, SNPs 6–8 arrested the colon tumor (HT-29 tumors) progression with the more drastic tumor growth inhibition seen with prodrug 7, in contrast to CPT-11, which only reduced the volume of tumor by 38% [40].

In another study with SNPs, tumor-specific ligands were integrated onto the surfaces of the nanoplatforms by co-assembly in an attempt to improve the potential of these nanoassemblies as a practical therapeutic modality. The recently discovered cyclic peptide ligand, iRGD (CRGDK/RGPD/EC), was used to achieve specific

Figure 4. Rational molecular engineering of SN-38 to induce self-assembly in aqueous media for in vivo drug delivery along with the chemical structures of prodrugs 6–10.
SNPs delivery to tumor sites. In this study, iRGD motif with a reactive cysteine residue was conjugated to hydrophobic LA via maleimide–thiol coupling. The resulting targetable iSNP 7 (SNP 7 with iRGD) outperformed prodrg 7 (SNP 7) in reducing tumor regression. Overall, incorporation of the tumor-specific ligand has a great potential in enhancing the performance of SN-38-based nanoprodrugs [40,42].

### 2.6. Anticancer prodrugs used in gene-directed enzyme prodrug therapy (GDEPT)

Gene-directed enzyme prodrug therapy (GDEPT) is considered one of the important strategies for the treatment of cancer. It is based on the delivery of a suicide gene that is considered as being a cancer treatment without affecting normal cells or tissues.

GDEPT is a promising strategy that aims to limit the systemic toxicity and improve the selectivity of chemotherapy use through the expression of a gene that encodes an enzyme that converts nontoxic prodrug into an activated cytotoxic agent.

In the treatment for cancer chemotherapy using GDEPT, there are two steps: (i) a gene of a foreign enzyme is delivered to a tumor by a vector and the gene is then transcribed into mRNA, which is later translated into the enzyme inside the tumor cell; and (ii) a prodrug is then administered into the human body, which is selectively activated inside the tumor site. The implementation of a prodrug strategy has an improvement in the physicochemical and PK properties of the prodrug system over that of its parent drug.

A key feature of GDEPT is the bystander effect, the ability of the activated prodrug to be transported out of the transfected cells to enter nontransfected cells [43–49].

Several enzyme/prodrug systems have been investigated in the last two decades and are shown in Table 1 [45,50].

For instance, SV-TK gene transfection into tumor cells, followed by treatment with the prodrug ganciclovir (GCV), was studied. The expression of HSV-TK gene leads to the synthesis of viral thymidine kinases, which in turn convert GCV into GCV monophosphate and then into a toxic triphosphate form by cellular kinases. In addition, in vivo antitumor activity of the HSV-TK/GCV system has been confirmed in different animal tumor models such as glioma, leukemia, bladder cancer, liver cancer, colon adenocarcinoma and oral cancer, and a number of clinical trials against different types of cancers are being investigated. Nevertheless, various obstacles were faced by this delivery system, and this includes the small number of cells dividing at any given time, which prevents efficient viral vector transfection and thus prodrug activation, high diffusibility of GCV and insolubility and low diffusibility of GCV’s active metabolite [45,51–60].

A few other substrates have been studied preclinically and clinically. For example, acyclovir is the most frequently used alternative prodrug with HSV-TK gene and one study showed an equal or higher cell killing efficacy and bystander effect with acyclovir versus GCV. Moreover, a recent phase IB study performed by Chiocca et al. suggested that HSV-TK along with valacyclovir can be administered safely alongside surgery and accelerated radiation in newly diagnosed malignant gliomas patients with enhanced survival rates [45,61,62].

Another example is bacterial type I nitroreductase enzymes that activate nitroaromatic prodrugs have a great potential and application for the use in GDEPT. Promising agents for GDEPT are dinitrobenzamide mustards (DNBMs) and nitro- chloromethylbenzindoles (nitro-CBIs). *Escherichia coli* nitroreductase NfsB (NfsB-Ec) has the ability to reduce nitro-CBIs in an oxygen-independent manner to produce highly cytotoxic metabolites that alkylate the N3 of adenine in the minor groove of DNA. A study that was conducted on the nitro-CBI prodrg (nitro-CBI-5-[(dimethylamino)ethoxy]indole (nitro-CBI-DEI)) showed a poor affinity of this prodrug to NfsB-Ec compared to that of DNBMs. Based on this negative result, Green et al. have screened other genes for more active nitroreductases to reduce nitro-CBI-DEI [63,64].

### Table 1. Major GDEPT systems.

| Enzyme                  | Prodrug         | Active drug     | Mechanism of action                                                                 |
|-------------------------|-----------------|-----------------|-------------------------------------------------------------------------------------|
| Thymidine kinase        | Ganciclovir     | Ganciclovir     | Inhibit DNA polymerase, GT incorporate into replicating cell DNA leading to replication failure and cell death |
| Cytosine deaminase      | 5-Fluorocytosine| 5-FU            | Inhibition of thymidylate synthase; 5-FU forms complex with DNA and RNA leading to inhibition of protein synthesis and DNA breakdown |
| Cytochrome P450 4-OH    | Cyclophosphamide| 4-OH cyclophosphamide | The 4-OH derivatives decompose to phosphoramid mustard which generates a highly electrophilic aziridinium species that forms DNA crosslinks |
| Nitroreductase 4-OH     | CB1954          | N-Acetoxy derivatives | Induce rapid cell death by forming interstrand DNA crosslinks |

S-FU: 5-fluorouracil; GDEPT: gene-directed enzyme prodrug therapy.
A scanning of candidate genes in the *E. coli* reporter strain SOS-R2 identified *E. coli* NfsA and *Pseudomonas aeruginosa* NfsB (NfsB-Pa) as more effective activators for nitro-CBI-DEI than the *E. coli* NfsB. In addition, the studies revealed that cells transfected with *P. aeruginosa* NfsB were more sensitive to nitro-CBI-DEI than cells transfected with NfsB-Ec. The level of bystander killing and the superiority of NfsB_Pa over NfsB_Ec demonstrated that the combination of NfsB-Pa/nitro-CBI-DEI is to be further evaluated in preclinical GDEPT models [64].

In summary, GDEPT approach has accomplished considerable progress in the last two decades in which various enzyme/prodrug systems showed effectiveness in preclinical and clinical trials. Substantial work still needs to be dedicated for achieving better cancer treatment option.

### 2.7. Prodrugs targeted against specific enzymes and/or cell compartment

#### 2.7.1. Prodrugs targeting certain enzymes

Lung cancer is a common type of cancer with 85% accounting for non-small cell lung cancer (NSCLC) and a survival rate of only 15%. It has the highest rate of mortality in both male and female populations in the US [65,66].

Classical, old cytotoxic chemotherapy is associated with very severe side effects and high rate of morbidity among patients; therefore, the necessity to find more selective drugs becomes an important issue. This has led to the development of drugs that is more selective to specific targets such as gefitinib; however, its effectiveness has been seen in only a limited number of patients, and its target might become resistant upon prolonged treatment. Therefore, new therapeutic strategies are crucially needed [65].

Among the new agents to treat lung tumors is β-lapachone (β-lap), which is effective against a wide range of cancer cells through *p53*-, cell cycle- and caspase- independent mechanisms. It is mainly reliant on expression of NAD(P)H:quinone oxidoreductase 1 (NQO1, a.k.a. DT-diaphorase, xip3, E.C.1.6.5.2), a two-electron oxidoreductase that detoxifies quinones after environmental exposure [65,67].

Expression of NQO1 is found to be 5–100 times higher in types of tumors that show high levels of reactive oxygen species (ROS) than in normal tissues. More importantly, it was found that NQO1 is up to 100 times overexpressed in ~90% NSCLC and pancreatic cancers, and up to 10 times in ~60% of prostate and breast cancers [65,68].

What is unique about β-lap and distinguishes it from all other quinone drugs is that it undergoes a futile redox cycle, which is specifically catalyzed by NQO1 that produces ROS. For every mole of β-lap, >60 mol of NAD(P)H is consumed and >120 mol of H$_2$O$_2$ is generated within <2 min. This high level of H$_2$O$_2$ contributes to DNA single-strand breaks, hyperactivation of poly(ADP-ribose) polymerase-1, loss of NAD$^+$ and ATP pools, and ultimately a unique pattern of cell death referred to as ‘programmed necrosis’ [65,69].

Cancer cells that overexpressed NQO1 are specifically prone to death, whereas normal cells and tissues with low endogenous levels of this enzyme are secured. On the other hand, β-lap has a poor PK profile, low water solubility and its use is associated with methemoglobinemia. Using hydroxypropyl β-cyclodextrin (HPβCD) to formulate β-lap (ARQ501) has showed an ~400-fold increase in solubility, but the rapid clearance of the drug (t$_{1/2}$, β = 24 min) alongside the hemolysis induced by HPβCD carrier and β-lap-induced methemoglobinemia, prohibited the drug from entering clinical trials [65,70].

Different approaches for β-lap delivery have been developed, but they were associated with various problems such as low drug loading efficiencies and scale-up problems. To enhance the stability of β-lap nanotherapeutics and the loading content, Ma et al. have generated a novel prodrug micelle strategy using diester derivatives of β-lap: β-lap-dC3 and β-lap-dC6 (dC3 and dC6). β-lap prodrugs with carbonic ester side chains located at the 1,2-ortho keto positions of β-lap were made and loaded into PEG-β-lap micelles. Among the strategies that were developed, the micelles of dC3 and dC6 demonstrated high apparent drug solubility, physical stability, the ability for reconstitution after lyophilization and easy scale-up formulations [65,71].

#### 2.7.2. Delivery approaches based on using siRNA

A single therapeutic mechanism has failed to effectively treat cancer, and long-term use of any anticancer agent might lead to drug resistance and tumor relapse. In some cases, relapse can lead to development of phenotypically distinct and more virulent tumors. Cisplatin and other DNA adducts forming chemotherapeutics cause DNA damage; yet, cellular pathways such as DNA repair pathways that correct the damage and trans lesion DNA synthesis (TLS) are activated, which in turn helps in tolerating the DNA damage. The Rev1/ Rev3L/Rev7-dependent error-prone TLS pathway has...
been shown to exhibit a significant role in cisplatin-induced mutations that allow tumor cells to adapt or repair the damage, which eventually leads to existence of acquired chemoresistance. Recent studies on cancer cell models revealed the possibility of inhibiting drug-induced mutagenesis via knocking down Rev1 or Rev3L to suppress error-prone TLS activity in mammalian cells. This eventually prevents relapse of tumors and keeps them sensitive to subsequent treatment [72–75].

siRNA can be used to target and silence nearly any gene of interest. It is a specific and potent gene expression regulator and holds a great promise for cancer therapy. A construction of certain siRNA to target genes encoding proteins that are involved in DNA repair and the acquisition of multidrug resistance are among the strategies considered [72,75]. The possibility of combining chemotherapeutics with siRNA using various nanocarrier platforms has been explored. However, for obtaining the finest synergistic effects, the drug and siRNA have to be temporally co-localized within the tumor cells. Therefore, nanocarrier platforms that are capable of concurrently delivering siRNA and anticancer drugs to the same tumor cells are considered as a promising nanomedicine approach for improved cancer therapy [76].

Xu et al. invoked an integrated nanodelivery system that has the ability to simultaneously deliver cisplatin prodrugs along with siRNAs against REV1 and REV3L to enhance chemosensitivity of tumors. In their study, poly(lactide-coglycolide), poly(lactic-co-glycolic acid (PLGA)-PEG NPs were formulated as G0-C14 NPs, cationic lipid-like molecules that encompass three components: an aqueous inner core, a cationic and hydrophobic layer composed of PLGA and G0-C14, and a hydrophilic PEG corona. To efficiently hold and bind siRNA groups via electrostatic interaction, the G0-C14 moiety was synthesized with a cationic head and flexible hydrophobic tails for self-assembly with PLGA-PEG to serve as Pt(IV)-prodrug encapsulating NPs. In this approach, a unique Pt(IV) precursor compound, c.c.t [Pt(NH3)2Cl2(O2C(CH2)8CH3)2] (11 in Supplementary Figure S4A), was made to promote the release of a lethal dose of cisplatin upon intracellular reduction. In vitro and in vivo trials revealed that the siRNA-containing NPs have the potential to efficiently lower the expression levels of target genes (reporter and both TLS genes) without any toxicity. Additionally, the drug showed an ability to cause silence target genes for at least three days after an administration of a single dose [72].

This study demonstrates a promising nanomedicine approach for cancer therapy, and this platform could be modified to be tested with other cancer models.

Hypoxic areas are far away from blood vessels and have enhanced efflux transports; therefore, siRNA delivery to such areas is challenging. Yet, it is one of the promising strategies in cancer treatment. In this aspect, another siRNA-based approach was conducted by Perche et al. and was the first example of hypoxia-induced siRNA uptake and silence using a PEG 2000-azobenzenepolyethyleneimine 1.8 kDa-1,2-dioleyl-sn-glycero-3-phosphoethanolamine (PAPD)-based nanocarrier, wherein hypoxia sensitivity and specificity are guaranteed by the hypoxia-responsive bioreductive linker; azobenzene [77,78].

The effectiveness of the azobenzene linkage in delivering siRNA was assessed by connecting its tail to PEG2000, as a hydrophilic block and for imparting stability in circulation, and its head to polyethyleneimine (PEI) 1.8 kDa-DOPE conjugate, used for siRNA complexation and initiation of micellar NP formation, to obtain PAPD PEI-DOPE conjugate (Supplementary Figure S4B). It was predicted that siRNA will be condensed into NPs with a PEG layer being protected from nuclease attack and thus providing a stability to the system in physiological fluids (Supplementary Figure S4C). The mechanism commences by deshielding of PEG groups from hypoxia-induced siRNA uptake and silence using a PEG 2000-azobenzenepolyethyleneimine 1.8 kDa-1,2-dioleyl-sn-glycero-3-phosphoethanolamine (PAPD)-based nanocarrier, wherein hypoxia sensitivity and specificity are guaranteed by the hypoxia-responsive bioreductive linker; azobenzene [77,78].

The other promising method to achieve systemic delivery of siRNA into solid tumors proposed by Oe et al. is the polyion complex (PIC) micelle. It is assembled using PEG block copolymers and a polycation that is served as the binding segment for siRNA. PIC micelles formation is facilitated by initiating charge neutralization between siRNA and the polycationic segment of the block copolymer in aqueous solution. Herein, nonionic and hydrophilic PEG shell is encircling the siRNA-loaded PIC core and the overall core–shell structure provides colloidal stability and minimizes the nonspecific interactions with charged biomacromolecules [80–83].

Disulfide crosslinks were among the techniques investigated to further increase micelle stability for in vivo delivery as they impart reversible stability to the micelle core once cleaved inside the cell due to elevated glutathione concentration. Moreover, to achieve further stabilization for the crosslinked siRNA micelle structure and thus improved tumor accumulation; cholesterol-conjugated siRNA (CholsiRNA) was used for this purpose. The assumption is
that hydrophobized siRNAs are expected to prevent micelle disruption and leakage of siRNA. As a result, thiolated block copolymer and Chol-siRNA combination construct a stable, yet reversible siRNA delivery platform [80–84].

Herein, Oe et al. employed a functional block copolymer comprising PEG segment that was synthesized to contain a targeting ligand, cationic charges and free thiol groups. The copolymer was installed with cRGD peptide as the tumor-targeting hydrophilic block that binds specifically to αvβ3 and αvβ5 integrins overexpressed on several cancer cells and poly-L-lysine (PLL) segment modified with dithiobispropionimidate as the cationic block [80–82,85].

Results proved a stronger association force of Chol-siRNA PICs that can overcome electrostatic repulsion. Moreover, the vital role of 1-(3-mercaptopropyl)amidine (MPA) moieties for the uniform micelle formation between Chol-siRNA and PEG-PLL(MPA) was established and it was explained by the stronger intermolecular interactions allowed with each MPA moiety and/or siRNA through hydrogen bonding, dipole–dipole interactions or Van der Waals force that is directed to provide a more stable and uniform construction of micelle. Additionally, the Chol modification of siRNA allowed PIC micelles production at wider mixing ratios, enhanced the micelle structure stability and thus the blood circulation property of the micelles. Moreover, cRGD ligand targeting ability was verified by the enhancement in cellular uptake in vitro and tumor accumulation in vivo [80].

This nanof ormulation is a very promising tumor environment-responsive modality for cancer targeting and siRNA delivery that can be further investigated to optimize and enhance silencing activity [80–87].

3. Targeted prodrugs used for antiviral and antibacterial drugs delivery

Infectious diseases like herpes simplex, hepatitis and human immunodeficiency are affecting a large number of populations worldwide. They are widely used, and yet, some unfavorable physicochemical properties and low bioavailability made them as an excellent potential candidate for prodrug strategy [88,89]. The following sections discuss some examples and attempts conducted to enhance the clinical profiles for some antimicrobials.

3.1. Prodrugs against hepatitis C

Ribavirin (RBV) is a guanosine analogue that is used to treat hepatitis C, a disease that mainly affects the liver. Moreover, new studies proposed the possibility of using RBV to treat rhinovirus and acute myeloid leukemia. Nevertheless, its use is restricted by its dose-dependent hemolytic anemia, which occurs as a result of its accumulation in the anucleate red blood cells (RBCs), where it undergoes irreversible phosphorylation that results in competitive ATP depletion and exerts oxidative damage to the cell membrane. Ultimately, this contributes to dose lowering in 20% of the treated patients or treatment dissolution in 5% of the treated patients. Hence, finding a liver-specific drug delivery approach has the potential to solve this drawback [90–92].

Consequently, an RBV prodrug that exclusively targets the liver has been explored. In this approach, the intention was to target the human sodium taurocholate co-transporting polypeptide (NTCP). NTCP is a sodium-dependent bile acid transporter predominantly expressed at the basolateral membrane of hepatocytes and is responsible for transporting >80% of the conjugated bile acids from the portal blood into liver. Accordingly, bile acid conjugates were proposed as a potential candidate to serve as prodrugs for achieving liver-specific drug delivery [90].

Dong et al. have designed some novel bile acid RBV prodrugs in an attempt to minimize RBV’s hemolytic anemia. The bile acid RBV prodrugs were conjugated via an amino acid linker. Following in vitro evaluation, RBV prodrug conjugated to glycochenodeoxycholic acid (GCDCA) via L-valine linker (RBV–L-Val–GCDCA) was chosen to be examined for in vivo assessment, where it showed a good substrate affinity to NTCP and attained liver-specific delivery and low accumulation of RBV in nontarget organs. In addition, it demonstrated a favorable drug release profile over its parent drug when exposed to mouse liver S9 fraction [90].

RBV is actively transported into RBCs by equilibrative nucleoside transporter 1, and its coupling with bulky bile acids hinders its uptake into RBC. Additionally, the presence of a negative charge in RBV–L-Val–GCDCA structure has shown to decrease the prodrug’s passive permeability into RBC and hence its accumulation (1%) [90,93].

Other approaches were used to optimize PK of RBV. Among these approaches is the use of macromolecular prodrugs (MPs) [94]. Using the chemistry of self-immo lative linkers (SILs), thiol-free prodrug with disulfide reshuffling as a mechanism for drug release was utilized by Ruiz-Sanchis et al., who designed and synthesized a methacrylate monomer containing a disulfide linkage conjugated to a carbonate with a functionality of RBV via an SIL. Upon thiol trigger, the designed moiety releases the drug via the formation of a cyclic thiocarbonate (Figure 5A). A series of polymers were made from copolymerization of the synthesized monomer
Figure 5. (A) Mechanism of RBV release through disulfide reshuffling under thiol trigger. (B) Chemical structure of ciprofloxacin prodrug. (C) Maleimides of the prodrug or fluorescein were linked to a biotinylation reagent via a glutathione coupler with conjugation at the sulfhydryl (arrow). (D) Biotinylated compounds were targeted to the cell wall (CW) of biofilm cells via streptavidin (StAv) in a sequence of reactions. B-Ab = biotinylated secondary Ab; B-prodrug = biotinylated prodrug; B-fluor = fluorescent analogue. (E) Amide prodrugs of ciprofloxacin: ciprofloxacyl-glycine methyl ester (Cipro-Gly-OMe) and ciprofloxacyl-glycine (Cipro-Gly) and (F) Chemical structure of prodrug 12.
with methacrylic acid through reversible addition-fragmentation chain transfer mechanism, RAFT [94].

Ruiz-Sanchis’s study revealed that the synthesized MPs were stable in serum, whereas they were extremely responsive to intracellular trigger for releasing the drug upon cell entry. They also demonstrated equivalent efficacy to the parent drug and a less toxic profile. They were capable of prohibiting viral genome expression to the same extent as RBV.

This novel approach can be utilized for other nucleoside analogues used to treat various therapies (antiviral, anticancer, etc.) in order to achieve safer and more effective therapy.

3.2. Prodrugs of ciprofloxacin against oral biofilms

Periodontitis is a chronic inflammatory condition induced by oral biofilms, and aggregatibacter actinomycetemcomitans (Aa), a major risk factor for localized aggressive periodontitis. Periodontitis is treated using the DNA gyrase inhibitor ciprofloxacin. Controlled and sustained release devices were used to achieve a lethal dose to Aa. However, several grams of ciprofloxacin over the dosing period are needed to achieve full recovery from the disease. Therefore, attempts were made to develop ciprofloxacin prodrug that fulfills the requirements needed to achieve an optimal clinical profile.

The targeted cleavable prodrug approach has the potential to specifically release the drug at the site of infection. This optimizes the pathogens killing and minimizes the amount of drug.

Biotinylated prodrug and biotinylated fluorescent analogue of ciprofloxacin were synthesized by coupling a commercially available biotinylation reagent to maleimides of either the prodrug or fluorescein analogue via glutathione (Figure 5B). Studies on ciprofloxacin prodrug shown in Figure 5B revealed that the prodrug’s methylene-O-diester undergoes a cleavage at an appreciable rate under mild basic conditions with first-order kinetics [95].

For Aa biofilms targeting, ciprofloxacin prodrug was biotinylated via an intervening glutathione linker (Figure 5C). A fluorescent analogue was synthesized to assist in measuring the ciprofloxacin prodrug concentration that reaches the biofilms through the biotin-streptavidin couple. The Aa-specific targeting reagent used was a monoclonal antibody (Aa-mAb) 325AA2 against Aa, isotype mlgG2b. The targeting scheme is shown in Figure 5D.

An amide prodrug of ciprofloxacin (ciprofloxacyl-glycine methyl ester (Cipro-Gly-OMe)), in which the methyl ester of glycine (Gly-OMe) was chosen to be the amide promoiety, was designed and synthesized by Sun (Figure 5E) [96].

The synthesized prodrug showed a much higher solubility in physiological pH than its parent drug, ciprofloxacin. Although Cipro-Gly-OMe (Figure 5E) may have a higher intestinal permeability at neutral pH than its parent drug, yet, there was a loss in the pharmacological effect due to the extreme stability of the Cipro-Gly moiety, and its inefficient conversion to ciprofloxacin.

Despite the failure of Cipro-Gly-OMe, still, the prodrug approach presented by Sun has the potential to improve the solubility and permeability of zwitterions compounds [96].

4. Brain-targeted prodrugs

Treatment of central nervous system (CNS) diseases is considered one of the major challenges encountered by the medical community. The main obstacle that hinders the drug delivery to CNS is the blood–brain barrier that allows only certain molecules to permeate.

On the other hand, chronic intake of nonsteroidal anti-inflammatory drugs such as ibuprofen was suggested by various reports to reduce the risk or even delay the onset of CNS diseases [97,98]. Due to this, ibuprofen received a great attention as a promising drug for CNS diseases.

For ibuprofen to achieve the intended therapeutic effect, it has to reach the CNS in high concentrations, yet, it has a poor permeability, thus larger doses are required, which can cause severe side effects [98].

Zhao and coworkers have invoked a delivery system for ibuprofen (12 in Figure 9F), which is composed of two ibuprofen molecules, one is connected through its carboxyl group to the C-6 position of AA and the other linked to a spacer that incorporates both ibuprofen and AA molecules through its terminals as shown in Figure 9F with the expectation that this delivery system will be capable of functioning as a ‘lock-in’ system. This would increase the concentration of the active drug (ibuprofen) inside the brain as this strategy changes the behavior of prodrugs from bidirectional to unidirectional system [98].

The study with this system revealed that after IV (intravenous) administration the concentration of ibuprofen reaching the brain was significantly higher than that with the naked ibuprofen upon using the same dose. The authors concluded that GLUT and SVCT2 have recognized AA moiety and efficiently transported the prodrug into the brain, where it was locked through reduction and cyclization processes [98].
An illustration of the mechanism by which this novel drug delivery to the CNS releases ibuprofen from its prodrug is depicted in Supplementary Figure S5.

5. Summary and conclusion

Targeted prodrugs delivery systems are rising rapidly as a method to overcome low bioavailability and poor PK properties of a mosaic of marketed drugs aiming to achieve drugs with better bioavailability and less undesired side effects. In this article, we have described a respected number of novel DDS. Among these systems are theranostic approaches, siRNA, DOX immunoconjugate, C60-ser carrier vector, biotinylated prodrug, HSA carrier and other new vehicles for the delivery of some important drugs. The studied drugs include anticancer agents such as DOX, GMC, paclitaxel and cis-platinum, antimicrobial agents such as RBV and ciprofloxacin, anti-inflammatory agents such as ibuprofen, etc.

In conclusion, unlike the old classical/traditional prodrugs which suffer from inefficient bioavailability and a high profile of side effects due to activation at sites other than the desired site/s, the new targeted prodrugs approach that utilizes sophisticated systems based on studies on the molecular and cellular levels rely on organic bioorganic, computational and inorganic chemistry, biochemistry and biological engineering data have proven to be more efficient with less unwanted side effects.

Although a large number of the drug delivery techniques described in this article still need to be further investigated, some of them have succeeded in improving the clinical profiles of a number of crucially needed medications and others have a good potential to provide drugs with optimum therapeutic outcomes.

6. Expert opinion

Traditional chemical prodrugs approach aims to improve the physicochemical properties of marketed drugs such as water solubility and permeation by covalently attaching these entities directly or through a spacer to a labile chemical moiety. The history of prodrugs made via this approach indicates that most of them suffer from nonspecific activation at sites other than the desired active site, resulting in related toxicities and poor bioavailability.

The molecular revolution in the recent three decades and the increasing knowledge on the mode and action of enzymes and transporters along with advances in molecular and cellular biology and better understanding of the chemistry of many organic reactions that can be effectively utilized to enable the development of novel prodrugs have created a new era of prodrugs ‘targeted prodrugs’. Scientists have switched their way of making classical prodrugs into designing prodrugs for specific targeting of certain enzymes and transporters. A broad and comprehensive understanding of molecular and cellular factors such as membrane influx and efflux transporters, cellular protein expression and distribution has led to new innovations in drug delivery, which have succeeded in increasing bioavailability and reducing toxicity of a number of medications.

In the recent years, various techniques were advocated to deliver drugs to their targets including modified NPs, immunoconjugate, self-assembled metal complexes, HSA as carrier, the use of theranostic approach (therapy and diagnosis), which allows for possible drug monitoring during the drug’s treatment period, new chemical devices that target certain enzymes and repair systems such as inhibiting the DNA repair system solely in cancer cells, GDEPT and gene silencing method using siRNA. These techniques have proved their capability and ability to successfully deliver very important drugs such as anticancer, antimicrobial and anti-inflammatory agents selectively to their targets with no toxicity and undesired side effects.

Moreover, other DDS that are not described in this article due to limited space have been made and utilized to deliver a variety of drugs. Among these systems are hypoxia activated prodrugs or bioreductive prodrugs that are activated only under hypoxic conditions, which showed very promising results for the delivery of tirapazamine, anthraquinone (AQ4N), etc. [99–111]. In addition, mitochondria-targeted prodrugs have been developed to target molecules that possess a therapeutic potential to mitochondria. Among those are mitochondria-targeted antioxidant prodrugs such as N-alkyl 3-aminoxypropanoic acids, 2-(1,3-dithiolan-2-yl)acetic acid and 2-(1,3-dithian-2-yl)acetic acid [112,113]. Further, cytochrome P450 activated prodrugs such as cyclic phosphate prodrugs of 5-aminosalicylic acid (ASA) have been made and demonstrated selective transport inside the enterocytes where they transformed to 5-ASA by intestinal alkaline phosphatase [114–116].

The computational approach using different computational methods provided an important tool for the design of linkers to be linked to drugs with inefficient bioavailability to assemble chemical devices (prodrugs) based on intramolecularity with a potential to release their parent drugs in a programmable manner with improved bioavailability. Based on such findings, a respected number of innovative prodrugs such as those of aza-nucleosides [117], atovaquone [118,119], dopamine [120], aciclovir
An excellent review on prodrugs design and clinical applications. Nat Rev Drug Discov. 2008;7(3):255–270. doi:10.1038/nrd2468.

7. Fattash B, Karaman R. Chemical approaches used in prodrugs design. In: Karaman R, editor. Prodrugs design – a new era. Hauppauge (NY): Nova Science Publishers; 2014. p. 103–138.

8. Karaman R. Using prodrugs to optimize drug candidates. Expert Opin Drug. Discov. 2014;9(12):1405–1419. doi:10.1517/17460441.2014.954545.

**An excellent review on targeted prodrugs.**

9. Karaman R, editor. Prodrugs design based on inter- and intramolecular processes. In: Karaman R, editor. Prodrugs design – a new era. Hauppauge (NY): Nova Science Publishers; 2014. p. 1–76.

10. Han H-K, Amidon GL. Targeted prodrug design to optimize drug delivery. AAPS PharmSci. 2000;2:48–58. doi:10.1208/px020106.

**An excellent overview of the modern targeted approach to prodrug design.**

11. Dahan A, Zimmermann EM, Ben-Shabat S. Modern prodrug design for targeted oral drug delivery. Molecules. 2014;19(10):16489–16505. doi:10.3390/molecules191016489.

12. Bader M, Thawabteh A, Karaman R. Targeted prodrugs. In: Karaman R, editor. Prodrugs design – a new era. Hauppauge (NY): Nova Science Publishers; 2014. p. 139–176.

13. Yuan Z, Yi X, Zhang J, et al. A prodrug nanoassembly entrapping drugs as a tumor-targeted delivery system. Chem Commun. 2013;49:801–803. doi:10.1039/c2cc37798e.

14. Kellermann M, Bauer W, Hirsch A, et al. The first account of a structurally persistent micelle. Angew Chem Int Ed Engl. 2004;43:2959–2962. doi:10.1002/anie.200335510.

15. Mackeyev Y, Raof M, Cisneros B, et al. Toward paclitaxel–[60]fullerene immunoconjugates as a targeted prodrug against cancer. Nanosystems. 2014;1:67–75.

**An excellent paper on paclitaxel–[60]fullerene immunoconjugate prodrugs.**

16. Ashcroft JM, Tsyboulski DA, Hartman KB, et al. Fullerene (C60) immunoconjugates: interaction of water-soluble C60 derivatives with the murine anti-gp240 melanoma antibody. Chem Commun. 2006;3004–3006. doi:10.1039/b601717g.

17. Gannon CJ, Cherukuri P, Yakobson BI, et al. Carbon nanotube-enhanced thermal destruction of cancer cells in a noninvasive radiofrequency field. Cancer. 2007;110:2654–2665. doi:10.1002/cncr.23155.

18. Zhong Y-J, Shao L-H, Li Y. Cathepsin B-cleavable doxorubicin prodrugs for targeted cancer therapy (Review). Int J Oncol. 2013;42:373–383. doi:10.3892/ijo.2012.1754.

**An excellent review on cathepsin B-cleavable doxorubicin prodrugs.**
An excellent paper on novel self-assembling doxorubicin prodrugs.

Duncan R. Polymer conjugates as anticancer nanomedicines. Nat Rev Cancer. 2006;6:688–701. doi:10.1038/nrc1958.

An excellent paper on (pseudo)polyrotaxanes prodrug.  

Wang Y, Wang H, Chen Y, et al. Biomimetic pseudopolyrotaxane prodrug micelles with high drug content for intracellular delivery. Chem Commun. 2013;49:7123–7125. doi:10.1039/c3cc43687j.

An excellent paper on novel doxorubicin prodrugs.

Wang Y, Wang H, Chen Y, et al. Biomimetic pseudopolyrotaxane prodrug micelles with high drug content for intracellular delivery. Chem Commun. 2013;49:7123–7125. doi:10.1039/c3cc43687j.

An excellent paper on thermoasistant drug delivery systems technique.

Wexselblatt E, Gibson D. What do we know about the reduction of Pt(IV) pro-drugs? J Inorg Biochem. 2012;117:220–229. doi:10.1016/j.jinorgbio.2012.06.013.

An excellent paper on human serum albumin platinum (Pt(IV)) prodrugs.

Zheng Y-R, Suntharalingam K, Johnstone TC, et al. Encapsulation of Pt(IV) prodrugs within a Pt(II) cage for drug delivery. J Am Chem Soc. 2014;136:8790–8798. doi:10.1021/ja5038269.

A cyclic RGD-linked polymeric micelles for targeted delivery of platinum anticancer drugs to glioblastoma through the blood-brain tumor barrier. ACS Nano. 2013;7:8583–8592. doi:10.1021/nn402662d.

Cabrál H, Matsumoto Y, Mizuno K, et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. Nat Nanotechnol. 2011;6:815–823. doi:10.1038/nnano.2011.166.

Cabrál H, Nishiyama N, Okazaki S, et al. Preparation and biological properties of dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt)-loaded polymeric micelles. J Control Release. 2005;101:223–232. doi:10.1016/j.jconrel.2004.08.022.

Cabrál H, Nishiyama N, Kataoka K. Optimization of (1,2-diaminocyclohexane)platinum(ii)-loaded polymeric micelles directed to improved tumor targeting and enhanced antitumour activity. J Control Release. 2007;121:146–155. doi:10.1016/j.jconrel.2007.05.024.

Ahn J, Miura Y, Yamada N, et al. Antibody fragment-conjugated polymeric micelles incorporating platinum drugs for targeted therapy of pancreatic cancer. Biomaterials. 2015;39:23–30. doi:10.1016/j.biomaterials.2014.10.069.

Gao Z, Lukyanov AN, Chakilam AR, et al. PEG-PE/phosphatidylcholine mixed immunomicelles specifically deliver encapsulated taxol to tumor cells of different origin and promote their efficient killing. J Drug Target. 2003;11:87–92. doi:10.1080/1061186031000138623.

Torchilin VP, Lukyanov AN, Gao Z, et al. Immunomicelles: targeted pharmaceutical carriers for poorly soluble drugs. Proc Natl Acad Sci U S A. 2003;100:6039–6044. doi:10.1073/pnas.0314281100.

Li W, Zhao H, Qian W, et al. Chemotherapy for gastric cancer by finely tailoring anti-Her2 anchored dual targeting immunomicelles. Biomaterials. 2012;33:5349–5362. doi:10.1016/j.biomaterials.2012.04.016.

Wang T, Xie H, Wang J, et al. Self-assembling prodrugs by precise programming of molecular structures that contribute distinct stability, pharmacokinetics, and anti-tumor efficacy. Adv Funct Mater. 2013;25:4956–4965. doi:10.1002/adfm.201302531.

An excellent paper on self-assembling prodrugs.

Wang H, Xie H, Wu J, et al. Structure-based rational design of prodrugs to enable their combination with polymeric nanoparticle delivery platforms for enhanced antitumor efficacy. Angew Chem Int Ed Engl. 2014;53:11532–11537. doi:10.1002/anie.201406685.

An excellent paper on polymeric nanoparticle delivery for cancer therapy.

Zhong Y, Peng F, Deng C, et al. Ligand-directed active tumor-targeting polymeric nanoparticles for cancer chemotherapy. Biomacromolecules. 2014;15:1955–1969. doi:10.1021/bm5003009.

An excellent paper on polymeric nanoparticle delivery for cancer therapy.

Zawińska JB, Wojcieszak J, Olejniczak AB. Prodrugs: a challenge for the drug development. Pharmacol Rep. 2013;65:1–14.

Denny WA. Tumor-activated prodrugs—a new approach to cancer therapy. Cancer Invest. 2004;22:604–619.
45. Zhang J, Kale V, Chen M. Gene-directed enzyme prodrug therapy. Aaps J. 2015;17:102–110. doi:10.1208/s12248-014-9675-7.

46. Both GW. Recent progress in gene-directed enzyme prodrug therapy: an emerging cancer treatment. Curr Opin Mol Ther. 2009;11:421–432.

47. Lo H-W, Day C-P, Hung M-C. Cancer-specific gene therapy. Adv Genet. 2005;54:235–255. doi:10.1016/S0065-2660(05)54010-0.

48. Saukkonen K, Hemminki A. Tissue-specific promoters for cancer gene therapy. Expert Opin Biol Ther. 2004;4:683–696. doi:10.1517/14712598.4.5.683.

49. Maitland NJ, Stanbridge LJ, Dussupt V. Targeting gene therapy for prostate cancer. Curr Pharm Des. 2004;10:531–555.

50. Greco O, Dachs GU. Gene directed enzyme/prodrug therapy of cancer: historical appraisal and future prospectives. J Cell Physiol. 2001;187:22–36. doi:10.1002/jcp.1074652200199999999999+:AID-JCP1060>3.0.CO;2-H.

51. Staquicini FI, Ozawa MG, Moya CA, et al. Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma. J Clin Invest. 2011;121:161–173. doi:10.1172/JCI44798.

52. Bondanza A, Hambach L, Aghai Z, et al. IL-7 receptor expression identifies suicide gene-modified allospecific CD8+ T cells capable of self-renewal and differentiation into antileukemia effectors. Blood. 2011;117:6469–6478. doi:10.1182/blood-2010-11-320366.

53. Tang W, He Y, Zhou S, et al. A novel Bifidobacterium infantis-mediated TK/GCV suicide gene therapy system exhibits antitumor activity in a rat model of bladder cancer. J Exp Clin Cancer Res. 2009;28:155. doi:10.1186/1756-9966-28-121.

54. Kakinoki K, Nakamoto Y, Kagaya T, et al. Prevention of intrahepatic metastases of liver cancer by suicide gene therapy and chemokine ligand 2/monocyte chemoattractant protein-1 delivery in mice. J Gene Med. 2010;12:1002–1013. doi:10.1002/jgm.1528.

55. Chen L-S, Wang M, Ou W-C, et al. Efficient gene transfer using the human JC virus-like particle that inhibits human colon adenocarcinoma growth in a nude mouse model. Gene Ther. 2010;17:1033–1041. doi:10.1038/gt.2010.50.

56. Ambade AV, Joshi GV, Mulherkar R. Effect of suicide gene therapy in combination with immunotherapy on antitumor immune response & tumour regression in a xenograft mouse model for head & neck squamous cell carcinoma. Indian J Med Res. 2010;132:415–422.

57. Greish K, Frandsen J, Scharff S, et al. Silkworm elastinlike protein polymers improve the efficacy of adenovirus thymidine kinase enzyme prodrug therapy of head and neck tumors. J Gene Med. 2010;12:572–579. doi:10.1002/jgm.1469.

58. Rainov NG. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther. 2000;11:2389–2401. doi:10.1089/104303400750038499.

59. Xu F, Li S, Li X-L, et al. Phase I and biodistribution study of recombinant adenovirus vector-mediated herpes simplex virus thymidine kinase gene and ganciclovir administration in patients with head and neck cancer and other malignant tumors. Cancer Gene Ther. 2009;16:723–730. doi:10.1038/cgt.2009.19.

60. Freytag SO, Movsas B, Aref I, et al. Phase I trial of replication-competent adenovirus-mediated suicide gene therapy combined with IMRT for prostate cancer. Mol Ther. 2007;15:1016–1023. doi:10.1038/mt. sj.6300120.

61. Tong XW, Engenhoven DG, Kaufman RH, et al. Improvement of gene therapy for ovarian cancer by using acyclovir instead of ganciclovir in adenovirus mediated thymidine kinase gene therapy. Anticancer Res. 1998;18:713–718.

62. Sangro B, Mazzolini G, Ruiz M, et al. A phase I clinical trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma. Cancer Gene Ther. 2010;17:837–843. doi:10.1038/cgt.2010.40.

63. Wilson WR, Stribbing SM, Pruijn FB, et al. Nitro-chloromethylbenzindolines: hypoxia-activated prodrugs of potent adenine N3 DNA minor groove alkylators. Mol Cancer Ther. 2009;8:2903–2913. doi:10.1158/1535-7163.MCT-09-0571.

• An excellent paper on hypoxia-activated prodrugs.

64. Green LK, Syddall SP, Carlin KM, et al. Pseudomonas aeruginosa NfsB and nitro-CBI-DEI—a promising enzyme/prodrug combination for gene directed enzyme prodrug therapy. Mol Cancer. 2013;12:58–63. doi:10.1186/1476-4598-12-58.

• An excellent paper on gene-directed enzyme prodrug therapy.

65. Ma X, Huang X, Moore Z, et al. Esterase-activatable beta-lapachone prodrug micelles for NQO1-targeted lung cancer therapy. J Control Release. 2015;200:201–211. doi:10.1016/j.jconrel.2014.12.027.

• An excellent paper on prodrug micelles for NAD(P)H:quinone oxidoreductase 1-targeted lung cancer therapy.

66. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63:11–30. doi:10.3322/caac.21166.

67. Ross D, Siegel D. NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase), functions and pharmacogenetics. Methods Enzymol. 2004;382:115–144. doi:10.1016/S0076-6879(04)82008-1.

68. Belinsky M, Jaiswal AK. NAD(P)H:quinone oxidoreductase 1 (DT-diaphorase) expression in normal and tumor tissues. Cancer Metastasis Rev. 1993;12:103–117.

69. Bey EA, Reinicke KE, Srougi MC, et al. Catalase abrogates beta-lapachone-induced PARP1 hyperactivation-directed programmed necrosis in NQO1-positive breast cancers. Mol Cancer Ther. 2013;12:2110–2120. doi:10.1158/1535-7163.MCT-12-0962.

70. Blanco E, Bey EA, Khemtong C, et al. Beta-lapachone micellar nanotherapeutics for non-small cell lung cancer therapy. Cancer Res. 2010;70:3896–3904. doi:10.1158/0008-5472.CAN-09-3995.

71. Ma X, Huang X, Huang G, et al. Prodrug strategy to achieve lyophilizable, high drug loading micelle formulations through diester derivatives of β-Lapachone. Adv Healthc Mater. 2014;3:1210–1216. doi:10.1002/adhm.201300590.

• An excellent paper on high drug loading micelle formulations technique.
72. Xu X, Xie K, Zhang X-Q, et al. Enhancing tumor cell response to chemotherapy through nanoparticle-mediated co-delivery of siRNA and cisplatin prodrug. Proc Natl Acad Sci U S A. 2013;110:18638–18643. doi:10.1073/pnas.1303958110.

**An excellent paper on chemotherapy through nanoparticle-mediated co-delivery of siRNA and cisplatin prodrug.**

73. Woodcock J, Griffin JP, Behrman RE. Development of novel combination therapies. N Engl J Med. 2011;364:985–987. doi:10.1056/NEJMp1101548.

74. Zhao Y, Bierumptpfel C, Gregory MT, et al. Structural basis of human DNA polymerase eta-mediated chemoresistance to cisplatin. Proc Natl Acad Sci U S A. 2012;109:7269–7274. doi:10.1073/pnas.1202681109.

75. Xie K, Doles J, Hemann MT, et al. Error-prone translesion synthesis mediates acquired chemoresistance. Proc Natl Acad Sci U S A. 2010;107:20792–20797. doi:10.1073/pnas.1011412107.

76. Xiong X-B, Lavasanifar A. Traceable multifunctional micellar nanocarriers for cancer-targeted co-delivery of MDR-1 siRNA and doxorubicin. ACS Nano. 2011;5:5202–5213. doi:10.1021/nn103707o.

77. Perche F, Biswas S, Wang T, et al. Hypoxia-targeted siRNA delivery. Angew Chem Int Ed Engl. 2014;53:3362–3366. doi:10.1002/anie.201308368.

**An excellent paper on hypoxia-targeted siRNA delivery.**

78. Kiyose K, Hanaoka K, Oushiki D, et al. Hypoxia-sensitive fluorescent probes for in vivo real-time fluorescence imaging of acute ischemia. J Am Chem Soc. 2010;132:15846–15848. doi:10.1021/ja105937q.

79. Navarro G, Sawant RR, Biswas S, et al. P-glycoprotein silencing with siRNA delivered by DOPE-modified PEI overcomes doxorubicin resistance in breast cancer cells. Nanomedicine (Lond). 2012;7:65–78. doi:10.2217/nmm.11.93.

80. Oe Y, Christie RJ, Naito M, et al. Actively-targeted polyn complex micelles stabilized by cholesterol and disulfide cross-linking for systemic delivery of siRNA to solid tumors. Biomaterials. 2014;35:7887–7895. doi:10.1016/j.biomaterials.2014.05.041.

81. Christie RJ, Matsumoto Y, Miyata K, et al. Targeted polymeric micelles for siRNA treatment of experimental cancer by intravenous injection. ACS Nano. 2012;6:5174–5189. doi:10.1021/nn300942b.

82. Kim HJ, Ishii T, Zheng M, et al. Multifunctional polyn complex micelle featuring enhanced stability, targetability, and endosome escapability for systemic siRNA delivery to subcutaneous model of lung cancer. Drug Deliv Transl Res. 2014;4:50–60. doi:10.1007/s13346-013-0175-6.

83. Miyata K, Nishiyama N, Kataoka K. Rational design of smart supramolecular assemblies for gene delivery: chemical challenges in the creation of artificial viruses. Chem Soc Rev. 2012;41:2562–2574. doi:10.1039/c1cs15258k.

84. Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature. 2004;432:173–178. doi:10.1038/nature03121.

85. Xiong J-P, Stehle T, Zhang R, et al. Crystal structure of the extracellular segment of integrin alpha Vbeta3 in complex with an Arg-Gly-Asp ligand. Science. 2002;296:151–155. doi:10.1126/science.1069040.

86. Pittella F, Cabral H, Maeda Y, et al. Systemic siRNA delivery to a spontaneous pancreatic tumor model in transgenic mice by PEylated calcium phosphate hybrid micelles. J Control Release. 2014;178:18–24. doi:10.1016/j.jconrel.2014.01.008.

87. Kim HJ, Miyata K, Nomoto T, et al. siRNA delivery from triblock copolymer micelles with spatially-ordered compartments of PEG shell, siRNA-loaded intermediate layer, and hydrophobic core. Biomaterials. 2014;35:4548–4556. doi:10.1016/j.biomaterials.2014.02.016.

88. Karaman R, Dajani KK, Qtait A, et al. Prodrugs of acyclovir - a computational approach. Chem Biol Drug Des. 2012;79:819–834. doi:10.1111/j.1747-0285.2012.01335.x.

89. Karaman R, Al-Kurd S, Yaghmour R, et al. Antibacterial activity of novel prodrugs of amoxicillin and cephalixin. World J Pharm Res. 2015;4(9):334–360.

90. Dong Z, Li Q, Guo D, et al. Synthesis and evaluation of bile acid-ribavirin conjugates as prodrugs to target the liver. J Pharm Sci. 2015;104:2864–2876. doi:10.1002/jps.24375.

**An excellent paper on bile acid-ribavirin conjugates.**

91. Shiffman ML. What future for ribavirin? Liver Int. 2009;29:68–73. doi:10.1111/j.1478-3231.2008.01936.x.

92. De Franceschi L, Fattovich G, Turrini F, et al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. Hepatology. 2000;31:997–1004. doi:10.1053/he.2000.5789.

93. Endres CJ, Moss AM, Govindarajan R, et al. The role of nucleoside transporters in the erythrocyte disposition and oral absorption of ribavirin in the wild-type and equilibrative nucleoside transporter 1/-/- mice. J Pharmacol Exp Ther. 2009;331:287–296. doi:10.1124/jpet.109.153130.

94. Ruiz-Sanchis P, Wohl BM, Smith AAA, et al. Highly active macromolecular prodrugs inhibit expression of the hepatitis C virus genome in the host cells. Adv Healthc Mater. 2015;4:65–68. doi:10.1002/adhm.201400307.

95. Reeves BD, Young M, Grieco PA, et al. Aggregatibacter actinomycetemcomitans biofilm killing by a targeted ciprofloxacin prodrug. Biofouling. 2013;29:1005–1014. doi:10.1080/08972014.2013.823541.

**An excellent paper on targeted ciprofloxacin prodrugs.**

96. Sun K. Improved targeting and biopharmaceutical properties of prodrugs of anti-infective agents [doctoral dissertation]. Ann Arbor (MI): University of Michigan; 2013.

97. Weggen S, Rogers M, Eriksen J. NSAIDs: small molecules for prevention of Alzheimer’s disease or precursors for future drug development? Trends Pharmacol Sci. 2007;28:536–543. doi:10.1016/j.tips.2007.09.004.

98. Zhao Y, Qu B, Wu X, et al. Design, synthesis and biological evaluation of brain targeting l-ascorbic acid prodrugs of ibuprofen with “lock-in” function. Eur J Med Chem. 2014;82:314–323. doi:10.1016/j.ejmech.2014.05.072.

**An excellent paper on brain targeting l-ascorbic acid prodrugs of ibuprofen with ‘lock-in’ function.**
pharmacokinetic/pharmacodynamic modeling. Front Oncol. 2013;3:314. doi:10.3389/fonc.2013.00314.

• An excellent paper on hypoxia-activated prodrugs.

101. Singh Y, Palombo M, Sinko PJ. Recent trends in targeted anticancer prodrug and conjugate design. Curr Med Chem. 2008;15:1802–1826.

102. Patterson AV, Saunders MP, Chinnie EC, et al. Enzymology of tirapazamine metabolism: a review. Anticancer Drug Des. 1998;13:541–573.

103. Hicks KO, Siim BG, Jaiswal JK, et al. Pharmacokinetic/pharmacodynamic modeling identifies SN30000 and SN29751 as tirapazamine analogues with improved tissue penetration and hypoxic cell killing in tumors. Clin Cancer Res. 2010;16:4946–4957. doi:10.1158/1078-0432.CCR-10-1439.

104. Karnthaler-Benbakka C, Groza D, Kryeziu K, et al. Tumor-targeting of EGFR inhibitors by hypoxia-mediated activation. Angew Chem Int Ed Engl. 2014;53:12930–12935. doi:10.1002/anie.201403936.

• An excellent paper on tumor targeting of epidermal growth factor receptor (EGFR) inhibitors.

105. Heffern MC, Yamamoto N, Holbrook RJ, et al. Cobalt derivatives as promising therapeutic agents. Curr Opin Chem Biol. 2013;17:189–196. doi:10.1016/j.cbpa.2012.11.019.

106. Cazares-Korner C, Pires IM, Swallow ID, et al. CH-01 is a hypoxia-activated prodrug that sensitizes cells to hypoxia/reoxygenation through inhibition of Chk1 and Aurora A. ACS Chem Biol. 2013;8:1451–1459. doi:10.1021/cb4001537.

107. Sorensen CS, Syljuasen RG. Safeguarding genome integrity: the checkpoint kinases ATR, CHK1 and WEE1 restrain CDK activity during normal DNA replication. Nucleic Acids Res. 2012;40:477–486. doi:10.1093/nar/gkr697.

108. Lindquist KE, Cran JD, Kordic K, et al. Selective radiosensitization of hypoxic cells using BCCA621C: a novel hypoxia activated prodrug targeting DNA-dependent protein kinase. Tumor Microenviron Ther. 2013;1:46–55. doi:10.2478/tumor-2013-0003.

• An excellent paper on novel hypoxia-activated prodrug targeting DNA-dependent protein kinase.

109. Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochem J. 2009;417:639–650. doi:10.1042/BJ20080413.

110. Bouquet F, Ousset M, Biard D, et al. A DNA-dependent stress response involving DNA-PK occurs in hypoxic cells and contributes to cellular adaptation to hypoxia. J Cell Sci. 2011;124:1943–1951. doi:10.1242/jcs.078030.

111. Kumareswaran R, Ludkovski O, Meng A, et al. Chronic hypoxia compromises repair of DNA double-strand breaks to drive genetic instability. J Cell Sci. 2012;125:189–199. doi:10.1242/jcs.092262.

112. Brookes PS, Yoon Y, Robotham JL, et al. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am J Physiol Cell Physiol. 2004;287:C817–C833. doi:10.1152/ajpcell.00139.2004.

113. Anders MW, James LR, Shey-Shing S Mitochondria-targeted antioxidant prodrugs and methods of use. U.S. Patent Application 12/094,618, filed. 2006 Nov 20.

114. Pelkonen O, Maenpaa J, Taavitsainen P, et al. Inhibition and induction of human cytochrome P450 (CYP) enzymes. Xenobiota. 1998;28:1203–1253. doi:10.1080/0099498259823886.

115. Huttunen KM, Mahonen N, Leppanen J, et al. Novel cyclic phosphate prodrug approach for cytochrome P450-activated drugs containing an alcohol functionality. Pharm Res. 2007;24:679–687. doi:10.1007/s11095-006-9187-y.

116. Huttunen KM, Tani N, Juvenon RO, et al. Design, synthesis, and evaluation of novel cyclic phosphates of 5-aminosalicylic acid as cytochrome p450-activated prodrugs. Mol Pharm. 2013;10:532–537. doi:10.1021/mp300330v.

• An excellent paper on cyclic phosphates of 5-aminosalicylic acid as cytochrome p450-activated prodrugs.

117. Karaman R. Prodrugs ofaza nucleosides based on proton transfer reactions. J Comput Aided Mol Des. 2010;24:961–970. doi:10.1007/s10822-010-9389-6.

118. Karaman R, Fattash B, Mecca G, et al. Computationally designed atovaquone prodrugs based on Bruce’s enzyme model. Curr Comput Aided Drug Des. 2014;10:15–27.

119. Karaman R, Hallak H. Anti-malarial pro-drugs- a computational aided design. Chem Biol Drug Des. 2010;76:350–360. doi:10.1111/j.1747-0285.2010.01018.x.

120. Karaman R. Computational aided design for dopamine prodrugs based on novel chemical approach. Chem Biol Drug Des. 2011;78:853–863. doi:10.1111/j.1747-0285.2011.01208.x.

121. Karaman R, Dajani KK, Hallak H. Computer-assisted design for atenolol prodrugs for the use in aqueous formulations. J Mol Model. 2012;18:1523–1540. doi:10.1007/s00894-011-1180-7.

122. Karaman R, Qtait A, Dajani KK, et al. Design and synthesis of an aqueous stable atenolol prodrug. ScientificWorldJournal. 2014;2014(2014):Article ID 248651, 13 p. doi:10.1155/2014/248651.

123. Karaman R, Amly W, Scrano L, et al. Computationally designed prodrugs of statins based on Kirby’s enzyme model. J Mol Model. 2013;19:3969–3982. doi:10.1007/s00894-013-1929-2.

124. Hejaz H, Karaman R, Khamis M. Computer-assisted design for paracetamol masking bitter taste prodrugs. J Mol Model. 2012;18:103–114. doi:10.1007/s00894-011-1040-5.

125. Karaman R, Karaman D, Ziaideh I. Computationally designed phenylephrine prodrugs- a model for enhancing bioavailability. J Mol Phys. 2013;111:3249–3264. doi:10.1080/00268976.2013.779395.

126. Karaman R, Ghareeb H, Dajani KK, et al. Design, synthesis and in-vitro kinetic study of tranexamic acid prodrugs for the treatment of bleeding conditions. J Mol Aided Comput Sci. 2013;27:615–635. doi:10.1007/s10822-013-9666-2.

• An excellent paper on tranexamic prodrugs based on intramolecularly.