Scientific Research Publishing

Pharmacology & Pharmacy, 2024, 15, 269-284
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

Exploring Ester Prodrugs: A Comprehensive Review of Approaches, Applications, and Methods

Guangyang Zhou

Faculty of Science, University of Sydney, Sydney, Australia
Email: noahzhousean@gmail.com

How to cite this paper: Zhou, G.Y. (2024) Exploring Ester Prodrugs: A Comprehensive Review of Approaches, Applications, and Methods. *Pharmacology & Pharmacy*, **15**, 269-284.
https://doi.org/10.4236/pp.2024.158016

Received: August 1, 2024
Accepted: August 24, 2024
Published: August 27, 2024

Abstract

The review provides an overview of the approaches, applications, and methods for ester prodrugs. Ester prodrugs are pharmacologically inactive compounds in their original form but become active drugs on biotransformation within the body, which offers advantages concerning the solubility, stability, and targeted delivery of the active drug. Several approaches of ester prodrugs have been reviewed in this review, including simple ester prodrugs, amino acid ester prodrugs, sugar ester prodrugs, lipid ester prodrugs, and polymeric ester prodrugs. This review incorporates *in vitro* and *in vivo* methods as well as the characterization of physical and chemical properties for ester prodrugs, cell culture systems, enzymatic assays, and animal models—all of these having a very important bearing on the evaluation of stability, bioavailability, and efficacy for ester prodrugs. While the benefits of using ester prodrugs are significant, there are also disadvantages like instability, poor or variable enzymatic hydrolysis, and toxicity from released promoieties or by-products. This review discusses solutions to the various limitations that include enhancing stability with ionizable promoieties and using physiologically-based pharmacokinetic modeling. The review also highlights the application of ester prodrugs in neurological disorders, such as Parkinson's disease, and the ongoing efforts to address the critical limitations in treatment efficacy. Future prodrug strategies are poised to advance significantly by harnessing diverse transport mechanisms across the blood-brain barrier and integrating nanotechnology.

Keywords

Ester Prodrugs, Solubility, Bioavailability, Stability, Ester Prodrug Approaches, Simple Ester Prodrugs, Amino Acid Ester Prodrugs, Sugar Ester Prodrugs, Lipid Ester Prodrugs, Polymeric Ester Prodrugs, Esterase-Responsive Nanoparticles, Hydrolysis, Cancer Treatment,

Cardiovascular Diseases, Neurological Disorders

1. Introduction

Prodrugs are pharmacologically inactive compounds, and after biotransformation in the body, they become active drugs [1]. They are designed with increased solubility that allows them to be absorbed and distributed through the human system [2]. Most prodrugs have improved chemical stability at storage and administration, are less readily degraded, and have a longer shelf-life compared to their active forms [2]. On top of that, prodrugs can be designed so that they are administered in a site-directed manner: they release active drugs only at the target site, thus maximizing efficacy and minimizing side effects [2] [3].

Ester prodrugs, in which the parent drug is coupled to an ester group, can be categorized by their respective functional groups. The nature of the various functional groups attached to the prodrug defines the time and location for release of the drug in the body [3]. Ester groups in prodrugs improve their lipophilicity, thus enhancing membrane permeability with the consequence of controlled release by enzymatic hydrolysis [3]. This leads to enhanced absorption, targeted delivery, and reduced toxicity of the drug. The problem is how to ensure predictable hydrolysis rates for the consistent activation of a drug in the face of inter-individual variability in enzyme activity [4]. A clear understanding of biochemical processes for conversion to active forms is necessary for designing appropriate prodrugs. Such knowledge is useful for the solution of common problems in poor solubility, low permeability, or fast metabolism of the parent drug [5].

This literature review is aimed at providing the audience with a critical overview of ester prodrug approaches and their applicability in medicine. In this literature review, various ester prodrug approaches will be discussed at the beginning: simple esters, amino acid esters, sugar esters, lipid ester prodrugs, and polymeric ester prodrugs. Furthermore, aside from the advantages and limitations, this review will be covering the applications of ester prodrugs in medicine, such as cancer treatments, cardiovascular diseases, neurological disorders, and lastly, *in vitro* and *in vivo* methods, followed by characterization of the physical and chemical properties of ester prodrugs.

2. Discussion

2.1. Ester Prodrug Approaches

Ester prodrug approaches refer to the different strategies to be employed in the design and development of prodrugs based on ester chemistry. Prodrugs are derivatives of drugs originally inactive or less active; however, upon entering into the organism, they are transformed into their active forms by any enzymatic or chemical process [6]. All these approaches aim to modify the pharmacokinetic and pharmacodynamic properties of the parent drug molecule by the addition of

ester functional groups [6]. Such pharmacokinetic properties include enhancement in solubility, stability, and bioavailability of molecules [6].

2.1.1. Simple Ester Prodrugs

Simple ester prodrugs are designed to be activated by esterases that exist in the body, hydrolyzing ester bonds and liberating the parent drug [7] [8]. Such prodrugs often have simple structures that are chemically stable and thus comparatively inexpensive to design and produce. An example of simple ester prodrugs is oseltamivir (**Figure 1**), an anti-influenza drug that manifests the effectiveness of ester-based prodrugs [8] [9].

Not all esters, however, are efficiently cleaved by *in vivo* esterases. The cleavage of some may be very slow, and poor cleavage rates can seriously limit this approach. Few years ago, enzyme-triggered prodrugs with spontaneously cleavable linkers were introduced to circumvent this limitation [8]. These prodrugs were designed for a more effective activation process, involving two steps: initial hydrolysis of the esters by esterases and the subsequent spontaneous linker cleavage [8]. This ensures a better release of the active drug in its active form and at the right time. Some simple ester prodrugs include examples such as Pivampicillin, which turns to ampicillin, and candesartan cilexetil, which converts to the angiotensin II receptor blocker candesartan [8]. The challenge remains in the enhancement of the permeability of the membrane and targeted delivery of the prodrug while having control over the release of the active drug with respect to time through enzymatic hydrolysis, and this will be helpful in improving absorption and reducing toxicity [8].

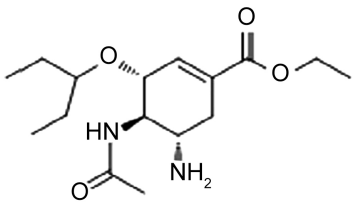

Figure 1. Chemical structure of oseltamivir [9].

2.1.2. Amino Acid Ester Prodrugs

Esterification of amino acids to the parent drug molecules could greatly enhance its solubility, stability, and cellular permeability [10]. Amino acid esters act as carriers for a drug across the blood-brain barrier (BBB), which involves targeting specific transporters or receptors and enables specific delivery [11]. By this approach, more precise targeting can be ensured, and it has a value in the drug design; however, the efficacy of this approach lies under the principle that improves drug transport [11].

One study was conducted on the human enzyme involved in hydrolysis of amino acid ester prodrugs of ketoprofen, along with that in rats and mice [12]. The objective of the study was to prepare a more stable ester prodrug of ketoprofen by attaching the ester bond to the meta-position of a phenylalanine residue

(Figure 2) [12]. Such modification will prevent the enzymatic hydrolysis of the ester bond. The choice rationale is that L-type amino acid transporter 1 will promote drug delivery into the brain and help bypass bioconversion problems in the rodent model [12]. However, the translational problem in humans from these animal models remains.

Another study used an amino acid ester prodrug for the poorly absorbed anti-cancer drug gemcitabine that is otherwise efficiently metabolized and has limited bioavailability through intestinal absorption [13]. In that respect, researchers designed the prodrug 5'-l-valyl-gemcitabine (V-Gem) to be absorbed by the intestinal peptide transporter PEPT1 to enhance absorption [13]. V-Gem was further engineered to make it resistant to cytidine deaminase, the enzyme responsible for the initial metabolism of gemcitabine [13]. Surprisingly, V-Gem was very fast cleared and did not improve system exposure to gemcitabine [13].

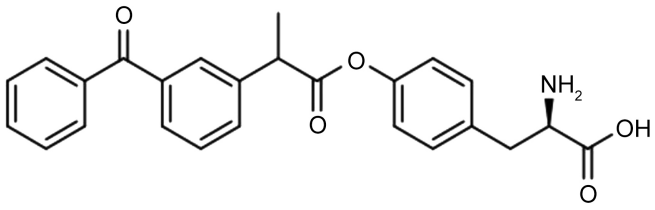

Figure 2. Chemical structure of ketoprofen L-tyrosine ester [12].

2.1.3. Sugar Ester Prodrugs

The sugar molecules, like glucose or galactose, may be connected to the parent drugs by the ester linkage to form sugar ester prodrugs accordingly [14]. This modification can enhance the water solubility and stability of the drug. This is because sugar ester prodrugs can make use of either the glucose transporters (GLUT1) for targeted delivery or become activated after metabolism by glycosidases [15]. One important advantage of sugar esters is the capacity of improving the solubility of otherwise hydrophobic drugs, thus making formulation easier and taste improvement to medication form [14]-[16].

One of the studies developed prodrugs for the treatment of Parkinson's disease by incorporating dopamine (DA) and levodopa (LD), consciously exploring the realm of the carrier-mediated prodrugs [14]. These prodrugs attach the drug to endogenous transporter substrates, such as sugars, in order to utilize active transport mechanisms and enhance penetration through the BBB [14]. Novel DA glycosyl-derivatives (Figure 3) were prepared by linking DA to sugars via various linkers (succinyl, carbamate, glycosidic, and ester bonds) in order to hijack the GLUT1 transportation pathway for better brain delivery [14]. DA-glycoconjugates 51 - 63, as an example, are attached via a succinyl linker [14]. This is a very important carrier-mediated approach in drug design, as it would help to enhance DA delivery into the central nervous system (CNS) [14]. Complications still arise, though, since treatment with LD in the long term chronically results in motor complications and dyskinesia due to fluctuating DA levels.

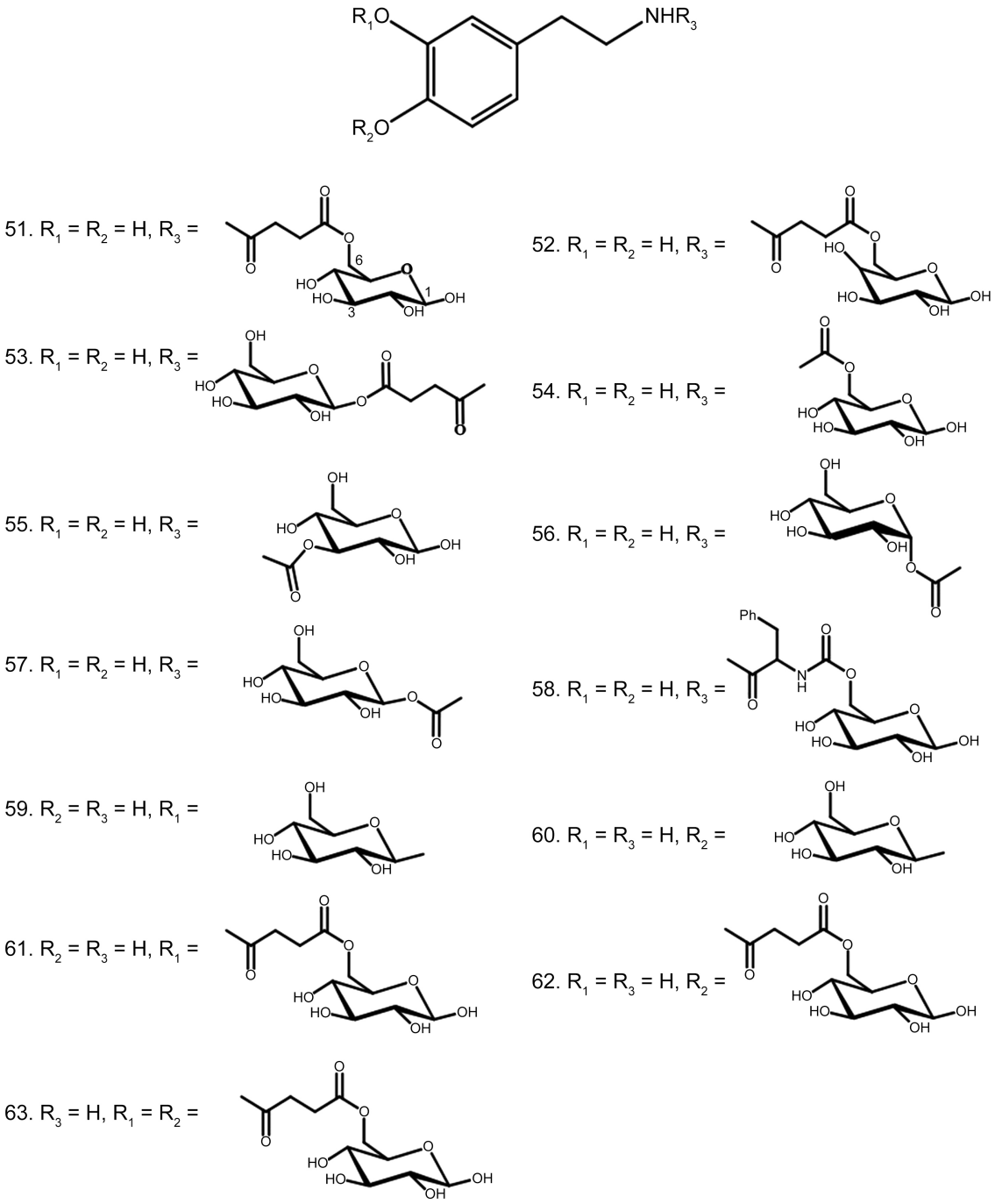

Figure 3. Chemical structure of DA glycosyl-derivatives 51 - 63 [14].

2.1.4. Lipid Ester Prodrugs

The drug molecules can be esterified with lipid molecules like fatty acids or glycerides. This modification makes the drug more lipophilic and thus more soluble, enabling diffusion through cellular membranes [17]. Attaching lipid moieties to a drug prolongs its absorption and distribution mainly in tissues rich in lipids [17]. Such prodrugs, by utilizing the advantages of lipids, increase activities of drugs needing intracellular uptake or those having poor solubility.

One study in designing prodrugs attached lipid moieties to drugs with the help of ester linkages [17]. This normally utilizes ester linkages to attach the drug to the free carboxylate group or at the ω-position of fatty acids [17]. While attachment to the carboxylate group is commonly practiced, attachment at the ω-position can enhance both albumin binding and cell membrane transport [17]. Plenty of examples exist where the lipid moieties improve the pharmacokinetics, enhance absorption, or even target delivery of a drug. These examples include conjugates with fatty acid-linked NSAIDs, ACE inhibitors, testosterone, and phospholipids (antiviral nucleoside analogues attached to the phosphate group) [17].

Another effort looked into lipid-based prodrug nanocarriers for cancer therapy, recognizing their advantages over traditional nanoparticle drug delivery systems [18]. In the approach, a drug is covalently conjugated to a lipid promoiety. Such modification enhances solubility and stability of the drug and promotes incorporation of the prodrug into the lipid-based nanocarriers [18]. One example is the lipid prodrug of the paclitaxel anticancer drug (**Figure 4**) [18].

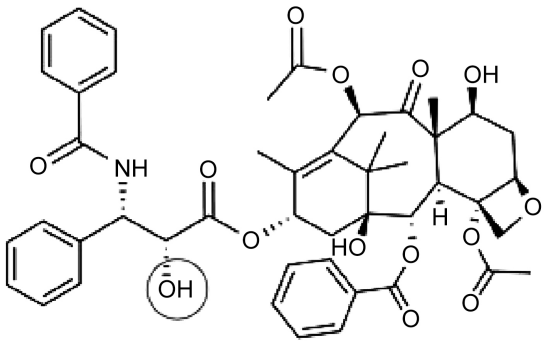

Figure 4. Chemical structure of paclitaxel [18].

2.1.5. Polymeric Ester Prodrugs

Polymeric ester prodrugs are designed by attachment of drug molecules to polymer chains via ester linkages, which provides a continuous release or targeted delivery of the active drug through the controlled hydrolysis of ester bonds [19]. Among the most notable advantages of this approach is modulation of the release rate of the drug. This has resulted in prolonged therapeutic activities that necessitate reduced dosing frequency [19]. The use of polymeric ester prodrugs can reduce dosing frequency, most especially in treatments where extended drug activities with increased compliance on the part of patients are required [19].

In one study, design and synthesis of four polymer prodrugs of TXA9, TXA9 being a newly isolated cardiac glycoside (**Figure 5**), which demonstrates highly potent antiproliferative action vs. human A549 lung cancer cells, was outlined [20]. Such an approach was rationalized by the very poor water solubility of TXA9 and the *in vivo* rapid metabolic rate that reduced its potential use as an anti-cancer agent [20]. The researchers enhanced the water solubility, pharmacokinetic behavior, and anti-tumor efficacy of TXA9 molecules by conjugating it with mPEG and TPGS carriers via carbonate ester and glycine linkers [20]. Previously, these

researchers have demonstrated that polymeric prodrugs (like the conjugation of doxorubicin-TPGS) are rather useful for enhancing solubilization and stability of drugs, in order to enhance therapeutic efficacy [20].

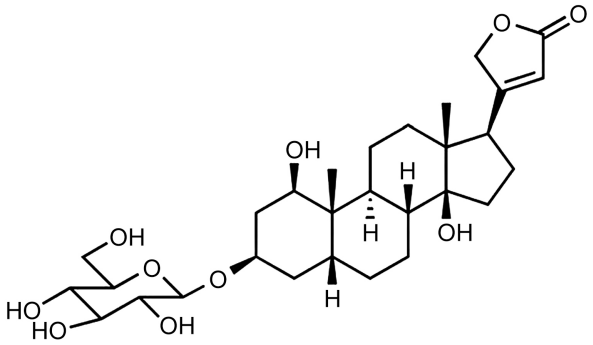

Figure 5. Chemical structure of TXA9 [20].

2.2. Pros and Cons of Ester Prodrug Approaches

2.2.1. Advantages

One notable advantage of ester prodrugs is their enhanced bioavailability [6]. Research has shown that esterification of carboxyl groups with aliphatic alcohols (ethyl ester prodrugs like enalapril) increases a molecule's lipophilicity, which facilitates its passive diffusion across cell membranes [6].

Ester prodrugs also offer benefits in drug targeting. They can be designed to remain active at the desired site for extended periods, as demonstrated with locally administered anti-inflammatory corticosteroids such as clobetasol propionate [6]. Another notable example is bambuterol—due to its increased lipophilicity and reduced hydrolysis rate—it allows for once-daily dosing [6]. Additionally, ester prodrugs can mask undesirable tastes, odors, or irritant properties of the parent drug [6].

One of the studies described a variety of benefits of ester prodrugs due to increased solubility achieved through the use of ionizable promoieties, for example, succinate, phosphate, or triazolium groups, which caused the drug's aqueous solubility to increase dramatically, thus enabling the parenteral administration of poorly soluble drugs [21]. Examples include prednisolone sodium succinate, fosfluconazole disodium, and isavuconazonium sulfate. Ester prodrugs also enhance bioavailability by being absorbed orally in drugs that are poorly absorbed either when taken or through the gastrointestinal tract; examples are cefuroxime axetil and cefpodoxime proxetil, as long as they remain stable within the gastrointestinal tract [21].

2.2.2. Limitations

Even with efforts made to enhance stability, challenges associated with ester prodrugs persist, including instability within the optimal pH range for ester stability and poor chemical stability in solution [21]. The second problem was that the rate of enzymatic hydrolysis might appreciably differ between species, and thus such

results from preclinical studies may be hard to interpret and extrapolate to humans [21]. For example, esterase activity in the gut of rodents is typically orders of magnitude greater than in humans, which can result in very low bioavailability in animal studies. Another problem might be that either the prodrug itself or the active drug—or the released promoieties/by-products—may be toxic [21].

To this regard, a recent article proposes some solutions to overcome these limitations: enhancing stability with ionizable promoieties, such as succinate, phosphate, or triazolium groups; reducing the variability of enzymatic hydrolysis *in vitro* by intestinal S9 data from animal and human models combined by using physiologically-based pharmacokinetic modeling; and careful assessment of the toxicity of released promoieties or by-products already at the stage of development [21]. This will be an important consideration in targeting the preferences of enzymes like CES1 for hydrolyzing esters having specific acyl sizes when designing prodrugs that remain less sensitive to unwanted enzymatic metabolism and related toxicity [22]. If these challenges are overcome, ester prodrugs can have very deep benefits in solubility enhancement, improved bioavailability, and setting up a platform for targeted delivery.

2.3. Ester Prodrug Applications in Medicine

2.3.1. Cancer Treatment

1) Gemcitabine

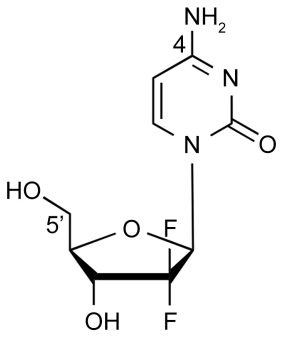

Figure 6. Chemical structure of gemcitabine [23].

There has been a development of gemcitabine prodrugs (**Figure 6**) in order to enhance their chemical stability and pharmacokinetic profile. Some strategically designed modifications increase the mechanisms of action (MoA) of gemcitabine prodrugs. They bypass the rapid deamination due to modifications in the 4-amino group with amide or carbamate derivatives, which generally enhance the stability of the drug [23]. These prodrugs, relying on ProTide technology, enhance cellular uptake and activation by masquerading the negative charges of the monophosphate form and hence improving membrane permeability, resulting in an intracellular release of active gemcitabine monophosphate [23]. Besides, they feature enhanced chemical stability, illustrated by the strength of NUC-1031 in human hepatocytes, liver microsomes, and serum [23]. However, the application of gemcitabine in

cancer treatment is limited by several inherent and acquired mechanisms of drug resistance. These include the inefficient conversion into its active forms, its rapid degradation by enzymes like CDA, and poor uptake by the cancerous cells [23]. These thus make this chemotherapeutic agent less effective and narrow the boundaries of its application in oncology.

2) Esterase-Responsive Nanoparticles

The MoA of esterase-responsive nanoparticles for cancer therapy is based on the fact that esterase is overexpressed in tumor cells and allows drug delivery [24]. Incorporated in the design is the placement of ester bonds that undergo quick hydrolysis due to the high activity esterase in the tumor cells, therefore releasing the encapsulated drugs [24]. Ester bonds, including phenyl hydroxyl esters, carbonic esters, and alkanoyloxymethyl esters, provide fast esterase responsiveness, although their stability is important to be balanced for effective delivery [24]. This technology delivers not only the chemotherapeutic drugs but also gene plasmids, photosensitizers, imaging agents; thus, it can be used for both therapy and imaging. Additionally, esterase-driven release can improve the accuracy of tumor imaging [24]. This can reduce side effects on normal tissues.

For cancer therapy, esterase-responsive nanoparticles have numerous advantages, including the simple and cost-effective preparation of micelles or liposomes [24]. They have a high drug load capacity with facile functionalization when capped by esterase-responsive stoppers strengthened by the endosomal escape ability through the reverse carrier charge [24]. There is an accurate localization of inactive drugs or imaging reagents. The esterase-responsive nanoparticles, however, have a few points that are noticeable disadvantages: micelles or liposomes may induce the premature release of drugs; low drug loading of the drug-carrier conjugates and only a few drugs available for drug-drug conjugates; the poor biodegradability of esterase-responsive stoppers [24].

2.3.2. Other Indications

1) Ester Prodrugs in Cardiovascular Diseases

Ester prodrugs could become effective against the inhibition of COX-2, which is one of the very important enzymes involved in several cardiovascular diseases [25]. It has been suggested that flavonoids (**Figure 7**), in combination with active metabolites of ester prodrugs like nabumetone and nepafenac, could give rise to new inhibitors of COX-2 [25]. The ester bonds of such prodrugs are easily hydrolyzed *in vivo* and yield metabolites that show inhibitory activity on COX-2 [25].

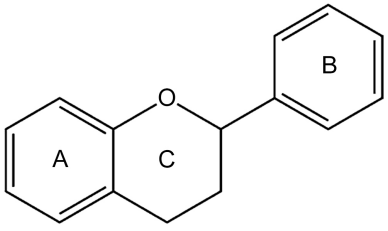

Figure 7. Chemical structure of flavonoid [25].

The phase I clinical trial involving the hydrogen sulfide (H_2S) prodrug SG1002 showed great promise in both healthy subjects and patients with heart failure, evidencing increased H_2S and nitric oxide (NO) bioavailability [26]. Doses of SG1002 were escalated to 200 mg, 400 mg, and 800 mg twice daily and demonstrated safety and tolerance in all groups [26]. It noticeably attenuated the elevation in brain natriuretic peptide levels of HF patients, thereby showing potential to be an agent in the treatment of cardiovascular diseases [26]. These findings justify additional studies using SG1002 in large clinical trials in order to establish its clinical efficacy and safety profile [26]. However, the small sample size of the trial mandates future studies of larger magnitude to fully ascertain its antioxidant benefits and therapeutic efficacy in the management of HF [26]. Mild gastrointestinal adverse events noted indicate that tolerability should continue to be assessed in future studies.

2) Ester Prodrugs in Neurological Disorders

Research in DA and LD prodrugs for Parkinson's disease shows an active field of investigation that addresses the critical limitations of treatment efficacy. While initial ester prodrugs did not significantly increase the duration or the potency of respective therapeutic activities, the newer developments of amide and dimeric amide prodrugs have improved the BBB penetration and pharmacokinetics [14]. Innovative approaches targeting mechanisms of glucose transporters and other carrier-mediated prodrugs aim to enhance drug delivery to the brain [14]. Despite the fact that so far no DA prodrug has already reached the market, recent developments in the intranasal delivery, together with pharmacokinetic enhancements, show a promising future of prodrug strategies in improving management of Parkinson's disease [14].

Prodrug strategies for CNS delivery will be very efficient in the future by utilizing various transport mechanisms across the BBB [27]. They will concentrate on enhanced passive diffusion through increased lipid solubility and carrier-mediated transport via some specific BBB transporters. They pursue a type of receptor-mediated transport for receptors such as transferrin and insulin receptors, and LDL receptors [27]. Further amplification in CNS drug delivery efficacy is promised by the integration of nanotechnology, such as self-assembling prodrug nanoparticles. This selective activation by CNS-specific enzymes or pathways within the brain is under the radar for future designs to optimize therapeutic outcomes while minimizing systemic side effects [27].

2.4. Methods

2.4.1. Commonly Used *in Vitro* Models for Evaluating Ester Prodrugs

1) Cell Culture Systems

Caco-2 cells are a line of cells obtained from human colon adenocarcinoma, which is cancer originating in the glandular tissue of the colon. The choice of the models was based on their advantage in improving predictability for oral absorption of ester prodrugs in humans [28]. Caco-2 cells represent the *in vitro* model from

human colonic adenocarcinoma, which is very well established in mimicking the human intestinal epithelium. This model may be used for estimating drug transport and metabolism across the intestinal barrier [28]. The rat intestine model enables the gaining of further information since the physiology of this animal model is close to the human gastrointestinal tract.

Hepatocytes represent very important *in vitro* models for the screening of ester prodrugs, such as tenofovir esters, due to their liver-specific functions and metabolic capabilities [29]. As liver-derived cells, they express enzymes liable for drug biotransformation, including those activating prodrugs into their active forms. In one research work, various studies on metabolic effects and antiviral activities against hepatitis B virus (HBV) were performed using hepatocytes as vehicles for different tenofovir ester prodrugs such as tenofovir (TFV) ester prodrugs (TDF, TAF, TMF) [29]. More specifically, primary rat hepatocytes and HBV-positive HepG2.2.15 cells, derived from human hepatocytes, were used to probe how these prodrugs impact HBV replication, metabolic pathways, and lipid profiles [29]. They looked into how these drugs affect hepatic metabolism and drive HBV inhibition through their activation within hepatocytes to the active metabolite TFV-DP.

2) Enzymatic Assays

Carboxylesterase enzymes are commonly used as *in vitro* models in the screening process for ester prodrugs because they hydrolyze ester-containing compounds, mimicking *in vivo* metabolic processes [30]. The enzymes, located in most tissues like the liver and intestine, are fundamental to the activation of prodrugs by enzymatically cleaving the ester bond, hence converting them into their respective active forms of drugs [30]. The activation process thus helps in estimating the stability, bioavailability, and hence efficacy of ester prodrugs in a biological system by researchers. The understanding of pharmacokinetic properties thus comes in very handy for the optimum design of drugs.

Liver microsomes containing cytochrome P450 enzymes are commonly used *in vitro* models for assessing ester prodrugs due to their ability to replicate human liver cells under metabolic conditions [31]. These enzymes, in which cytochrome P450 is included, participate in significant catalyzes of drug metabolism and are implicated in reactions similar to *in vivo* metabolism. More specifically, most prodrugs are metabolized into their active forms by cytochrome P450 enzymes through oxidative reactions and account for a large part of drug metabolism in the liver [31]. Thus, with the liver microsomes, one will be able to study ester prodrugs' metabolism and activation in the liver and hence understand their bioavailability for improved therapeutic efficacy and reduced toxicity.

Another standard tool in assessing ester prodrugs is plasma stability assays (*in vitro* model) because they can provide information on the metabolic stability of compounds in human plasma. The plasma incubation process involves incubating the prodrug in plasma samples and observing the degradation with time, which may indicate susceptibility to enzymatic hydrolysis by plasma esterases [32]. Thus,

a study of the rate of conversion of the prodrug into the active form or parent drug will be a good predictor of its efficacy and *in vivo* pharmacokinetic behavior—both important in choosing and optimizing the prodrug for further development. For instance, curcumin diethyl dissuccinate (CDD), an ester prodrug of curcumin, in dog and human plasmas had shown equal plasma stability and metabolizing enzyme activities but quite distinct from those detected in the rat plasma itself [32]. This confirms that species-specific metabolism studies are very imperative during the preclinical drug development process.

2.4.2. Methods Employed in *In Vivo* Studies for the Evaluation of Ester Prodrugs

1) Animal Models

Rodents, such as rats and mice, are used in the testing of ester prodrugs because they are relatively cheap, easily handled, and physiologically close enough to humans [33]. Such animal models would be used to undertake larger-scale exploratory studies of efficacy and safety. Rodents are used for studying drug absorption, distribution, metabolism, and elimination, and the relationship between drug concentration and biological effect is researched [33]. Blood sampling, tissue collection methods, and imaging techniques provide opportunities for measurement of drug levels and responses and, thus, the performance of detailed pharmacokinetic and pharmacodynamic studies [33]. Such concerns should be related to the proper choice of the animal model, with mice being used for the study of genetic pathways and rats for detailed behavioral analysis. Other factors that need to be taken into account include age, sex, and genetic background [33]. While rodent *in vivo* data are a very valuable basis for understanding drug metabolism, distribution, and possible toxic effects, the translation of findings such as these into clinical success will require careful interpretation, comprehensive validation through clinical trials, and consideration of human-specific factors to help ensure safety and relevance of prodrugs for use in humans [33].

Non-human primates (NHPs) are used *in vivo* studies for ester prodrug evaluation since they have physiological and metabolic features similar to humans; thus, data from them would be most predictive of human clinical results [34]. The most commonly used species are cynomolgus and rhesus monkeys [34]. The studies involve oral and intravenous administration in a bid to determine bioavailability, systemic clearance, and distribution using frequently collected blood samples in their detailed pharmacokinetic profiling [34]. These techniques include the LC-MS/MS quantitation of prodrug and metabolites and the enzyme assays for esterase activity [34].

2) Dosing Regimens

In single-dose studies, researchers will administer a single dose of the ester prodrug to perform preliminary pharmacokinetic/pharmacodynamic evaluations [34] [35]. They will collect blood samples several times to estimate the concentrations of the prodrug and its active metabolites, which provides insights into the ADME profiles [35]. On the other hand, in multiple-dose studies, researchers will

administer the prodrug repeatedly [35]. This allows researchers to assess the accumulation of the prodrug, its long-term toxicity potential and efficacy [35].

2.5. Characterization of Physical and Chemical Properties

Researchers typically use ultraviolet, infrared, and nuclear magnetic resonance spectroscopy to determine the chemical structure and purity of prodrugs [36]. They can also use mass spectrometry to determine the molecular weight of the prodrug [36]. On top of that, to evaluate the prodrug's purity, chromatography techniques (high-performance liquid chromatography and gas chromatography) are in place for use [36].

Detailed characterization of agents helps to identify as well as eliminate impurities which may lead to toxicities [36]. Therapeutic outcomes can hence be kept consistent with chemical stability and purity maintained, hence minimizing the degradation-induced risks [36]. The latter also calls for proper characterization of agents in respect of their regulatory needs since, in most cases, the regulatory bodies base their decision on the physical and chemical properties of drugs.

3. Summary

This literature review allows for the overall view of ester prodrug approaches and their medical applications, together with associated methods. Ester prodrugs designed for conversion into active forms within the organism offer several advantages, such as improved physicochemical properties and improved stability, which enable targeted drug delivery. The review presents various strategies of ester prodrugs, including simple esters, amino acid esters, sugar esters, lipid esters, and polymeric esters, together with design rationales, advantages, and limitations, along with examples. Simple ester prodrugs make use of esterase activity for the release of the drug, but some of them are now facing certain cleavage at a slow pace. The problem brought about the development of enzyme-triggered prodrugs involving spontaneously cleavable linkers for effective release. Amino acid ester prodrugs are capable of improving the solubility, stability, and cell permeability of drugs while being directed to some specific transporters or receptors for improved delivery. In turn, polymeric ester prodrugs present controlled release and targeting potential via the sustained hydrolysis of ester bonds, which offers improved efficacy. Such prodrugs also enhance bioavailability by increasing the lipophilicity and permeability of the parent drug and, at the same time, mask undesirable properties. However, instability within the optimum pH ranges, poor chemical stability of the solution, and variability across species regarding its enzymatic hydrolysis rates are some issues that could complicate the translation of preclinical findings.

It also details related applications in cancer, cardiovascular disease, and neurology-related disorders and *in vitro* and *in vivo* methods for evaluation, along with physical and chemical characterization. Among others, the most explored

ester prodrugs are the gemcitabine derivatives in the field of cancer therapy, investigating especially the mechanisms of resistance and trying to improve the drug-delivery approaches. Most of the *in vitro* metabolic stability and activation studies are done with enzymatic assays and plasma stability tests. To do this, species-specific differences in esterase activity and drug metabolism should be known to translate preclinical findings into human clinical outcomes.

Further studies will be required in the near future to investigate new modifications of functional groups, combination therapies, and more importantly, to apply transgenic animal models that express human-specific enzymes or transporters of metabolic pathways relevant to humans.

Acknowledgements

I would like to extend my sincere gratitude to my best friend Matthew, who got my back as always. Thank you for being here for me like a handprint on my heart, and for our friendship hug ring engraved with "always with you".

So much of me is made of what I learned from you. Because I knew you, I have been changed for good.

I really hope that we will meet up, like what you mentioned in my birthday letter, on a day when the sun shines not brightly, but meekly, and it is drizzling.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Choudhary, D., Goykar, H., Kalyane, D., Sreeharsha, N. and Tekade, R.K. (2020) Prodrug Design for Improving the Biopharmaceutical Properties of Therapeutic Drugs. In: Tekade, R.K., Ed., *The Future of Pharmaceutical Product Development and Research*, Elsevier, 179-226. https://doi.org/10.1016/b978-0-12-814455-8.00006-2

[2] Dubey, S. and Valecha, V. (2014) Prodrugs: A Review. *World Journal of Pharmaceutical Research*, **3**, 277-297.

[3] Rautio, J., Kumpulainen, H., Heimbach, T., Oliyai, R., Oh, D., Järvinen, T., *et al.* (2008) Prodrugs: Design and Clinical Applications. *Nature Reviews Drug Discovery*, **7**, 255-270. https://doi.org/10.1038/nrd2468

[4] Zawilska, J.B., Wojcieszak, J. and Olejniczak, A.B. (2013) Prodrugs: A Challenge for the Drug Development. *Pharmacological Reports*, **65**, 1-14. https://doi.org/10.1016/s1734-1140(13)70959-9

[5] Testa, B. (2004) Prodrug Research: Futile or Fertile? *Biochemical Pharmacology*, **68**, 2097-2106. https://doi.org/10.1016/j.bcp.2004.07.005

[6] Hajnal, K., Gabriel, H., Aura, R., Erzsébet, V. and Blanka, S.S. (2016) Prodrug Strategy in Drug Development. *Acta Medica Marisiensis*, **62**, 356-362. https://doi.org/10.1515/amma-2016-0032

[7] Perez, C., Daniel, K.B. and Cohen, S.M. (2013) Evaluating Prodrug Strategies for Esterase-triggered Release of Alcohols. *ChemMedChem*, **8**, 1662-1667. https://doi.org/10.1002/cmdc.201300255

[8] Hamada, Y. (2017) Recent Progress in Prodrug Design Strategies Based on Generally

Applicable Modifications. *Bioorganic & Medicinal Chemistry Letters*, **27**, 1627-1632. https://doi.org/10.1016/j.bmcl.2017.02.075

[9] Wang, Z., Cheng, L.P., Zhang, X.H., Pang, W., Li, L. and Zhao, J.L. (2017) Design, Synthesis and Biological Evaluation of Novel Oseltamivir Derivatives as Potent Neuraminidase Inhibitors. *Bioorganic & Medicinal Chemistry Letters*, **27**, 5429-5435. https://doi.org/10.1016/j.bmcl.2017.11.003

[10] Vig, B.S., Huttunen, K.M., Laine, K. and Rautio, J. (2013) Amino Acids as Promoieties in Prodrug Design and Development. *Advanced Drug Delivery Reviews*, **65**, 1370-1385. https://doi.org/10.1016/j.addr.2012.10.001

[11] Huttunen, K.M. (2024) Improving Drug Delivery to the Brain: The Prodrug Approach. *Expert Opinion on Drug Delivery*, **21**, 683-693. https://doi.org/10.1080/17425247.2024.2355180

[12] Huttunen, K.M. (2018) Identification of Human, Rat and Mouse Hydrolyzing Enzymes Bioconverting Amino Acid Ester Prodrug of Ketoprofen. *Bioorganic Chemistry*, **81**, 494-503. https://doi.org/10.1016/j.bioorg.2018.09.018

[13] Thompson, B.R., Shi, J., Zhu, H. and Smith, D.E. (2020) Pharmacokinetics of Gemcitabine and Its Amino Acid Ester Prodrug Following Intravenous and Oral Administrations in Mice. *Biochemical Pharmacology*, **180**, Article ID: 114127. https://doi.org/10.1016/j.bcp.2020.114127

[14] Haddad, F., Sawalha, M., Khawaja, Y., Najjar, A. and Karaman, R. (2017) Dopamine and Levodopa Prodrugs for the Treatment of Parkinson's Disease. *Molecules*, **23**, Article 40. https://doi.org/10.3390/molecules23010040

[15] Shimoda, K., Hamada, H. and Hamada, H. (2008) Chemo-Enzymatic Synthesis of Ester-Linked Taxol-Oligosaccharide Conjugates as Potential Prodrugs. *Tetrahedron Letters*, **49**, 601-604. https://doi.org/10.1016/j.tetlet.2007.11.156

[16] Zheng, Y., Zheng, M., Ma, Z., Xin, B., Guo, R. and Xu, X. (2015) Sugar Fatty Acid Esters. In: Ahmad, M.U. and Xu, X.B., Eds., *Polar Lipids*, Elsevier, 215-243. https://doi.org/10.1016/b978-1-63067-044-3.50012-1

[17] Zaro, J.L. (2014) Lipid-Based Drug Carriers for Prodrugs to Enhance Drug Delivery. *The AAPS Journal*, **17**, 83-92. https://doi.org/10.1208/s12248-014-9670-z

[18] Mura, S., Bui, D.T., Couvreur, P. and Nicolas, J. (2015) Lipid Prodrug Nanocarriers in Cancer Therapy. *Journal of Controlled Release*, **208**, 25-41. https://doi.org/10.1016/j.jconrel.2015.01.021

[19] Khandare, J. and Minko, T. (2006) Polymer-Drug Conjugates: Progress in Polymeric Prodrugs. *Progress in Polymer Science*, **31**, 359-397. https://doi.org/10.1016/j.progpolymsci.2005.09.004

[20] Li, Y., Ye, C., Cai, C., Zhao, M., Han, N., Liu, Z., *et al.* (2020) Design and Synthesis of Polymer Prodrugs for Improving Water-Solubility, Pharmacokinetic Behavior and Antitumor Efficacy of Txa9. *Pharmaceutical Research*, **37**, Article No. 66. https://doi.org/10.1007/s11095-020-02789-w

[21] Rautio, J., Meanwell, N.A., Di, L. and Hageman, M.J. (2018) The Expanding Role of Prodrugs in Contemporary Drug Design and Development. *Nature Reviews Drug Discovery*, **17**, 559-587. https://doi.org/10.1038/nrd.2018.46

[22] Lesniewska-Kowiel, M.A. and Muszalska, I. (2017) Strategies in the Designing of Prodrugs, Taking into Account the Antiviral and Anticancer Compounds. *European Journal of Medicinal Chemistry*, **129**, 53-71. https://doi.org/10.1016/j.ejmech.2017.02.011

[23] Miao, H., Chen, X. and Luan, Y. (2020) Small Molecular Gemcitabine Prodrugs for

Cancer Therapy. *Current Medicinal Chemistry*, **27**, 5562-5582. https://doi.org/10.2174/0929867326666190816230650

[24] Dong, H., Pang, L., Cong, H., Shen, Y. and Yu, B. (2019) Application and Design of Esterase-Responsive Nanoparticles for Cancer Therapy. *Drug Delivery*, **26**, 416-432. https://doi.org/10.1080/10717544.2019.1588424

[25] Chen, W., Zhong, Y., Feng, N., Guo, Z., Wang, S. and Xing, D. (2021) New Horizons in the Roles and Associations of COX-2 and Novel Natural Inhibitors in Cardiovascular Diseases. *Molecular Medicine*, **27**, Article No. 123. https://doi.org/10.1186/s10020-021-00358-4

[26] Polhemus, D.J., Li, Z., Pattillo, C.B., Gojon, G., Gojon, G., Giordano, T., *et al.* (2015) A Novel Hydrogen Sulfide Prodrug, sg1002, Promotes Hydrogen Sulfide and Nitric Oxide Bioavailability in Heart Failure Patients. *Cardiovascular Therapeutics*, **33**, 216-226. https://doi.org/10.1111/1755-5922.12128

[27] Xia, X., Zhou, Y. and Gao, H. (2021) Prodrug Strategy for Enhanced Therapy of Central Nervous System Disease. *Chemical Communications*, **57**, 8842-8855. https://doi.org/10.1039/d1cc02940a

[28] He, X., Sugawara, M., Takekuma, Y. and Miyazaki, K. (2004) Absorption of Ester Prodrugs in Caco-2 and Rat Intestine Models. *Antimicrobial Agents and Chemotherapy*, **48**, 2604-2609. https://doi.org/10.1128/aac.48.7.2604-2609.2004

[29] Hong, X., Cai, Z., Zhou, F., Jin, X., Wang, G., Ouyang, B., *et al.* (2022) Improved Pharmacokinetics of Tenofovir Ester Prodrugs Strengthened the Inhibition of HBV Replication and the Rebalance of Hepatocellular Metabolism in Preclinical Models. *Frontiers in Pharmacology*, **13**, Article 9329374. https://doi.org/10.3389/fphar.2022.932934

[30] Hosokawa, M. (2008) Structure and Catalytic Properties of Carboxylesterase Isozymes Involved in Metabolic Activation of Prodrugs. *Molecules*, **13**, 412-431. https://doi.org/10.3390/molecules13020412

[31] Sun, H. (2012) Capture Hydrolysis Signals in the Microsomal Stability Assay: Molecular Mechanisms of the Alkyl Ester Drug and Prodrug Metabolism. *Bioorganic & Medicinal Chemistry Letters*, **22**, 989-995. https://doi.org/10.1016/j.bmcl.2011.12.005

[32] Ratnatilaka Na Bhuket, P., Jithavech, P., Ongpipattanakul, B. and Rojsitthisak, P. (2019) Interspecies Differences in Stability Kinetics and Plasma Esterases Involved in Hydrolytic Activation of Curcumin Diethyl Disuccinate, a Prodrug of Curcumin. *RSC Advances*, **9**, 4626-4634. https://doi.org/10.1039/c8ra08594c

[33] Vandamme, T. (2014) Use of Rodents as Models of Human Diseases. *Journal of Pharmacy and Bioallied Sciences*, **6**, 2-9. https://doi.org/10.4103/0975-7406.124301

[34] Padilla, A.M., Wang, W., Akama, T., Carter, D.S., Easom, E., Freund, Y., *et al.* (2022) Discovery of an Orally Active Benzoxaborole Prodrug Effective in the Treatment of Chagas Disease in Non-Human Primates. *Nature Microbiology*, **7**, 1536-1546. https://doi.org/10.1038/s41564-022-01211-y

[35] Chen, L., Zhou, L., Huang, J., Wang, Y., Yang, G., Tan, Z., *et al.* (2018) Single- and Multiple-Dose Trials to Determine the Pharmacokinetics, Safety, Tolerability, and Sex Effect of Oral Ginsenoside Compound K in Healthy Chinese Volunteers. *Frontiers in Pharmacology*, **8**, Article 965. https://doi.org/10.3389/fphar.2017.00965

[36] Cavallaro, G., Licciardi, M., Caliceti, P., Salmaso, S. and Giammona, G. (2004) Synthesis, Physico-Chemical and Biological Characterization of a Paclitaxel Macromolecular Prodrug. *European Journal of Pharmaceutics and Biopharmaceutics*, **58**, 151-159. https://doi.org/10.1016/j.ejpb.2004.02.012