REVIEW PAPER

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Recent advances in drug substance development – prodrug strategies for enhancing the bioavailability and potency of antiviral nucleosides

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ABSTRACT

Bioavailability is a prerequisite for drug activity. *In vivo* bioavailability (intestinal permeability), linked to drug substance solubility and drug product dissolution, became the basis of Gordon L. Amidon's Biopharmaceutical Classification System. One method of improving the drug substance's bioavailability is to modify its structure chemically, leading to increased lipophilicity and the ability to penetrate the phospholipid bilayer of the cell membrane. These modifications, known as prodrug strategies, involve derivatizing the drug substance by introducing substituents that reduce the hydrophilicity of the molecule. The present mini-review outlines the examples of Christopher McGuigan's prodrug strategies used to obtain antiviral nucleosides with enhanced bioavailability and activity. These strategies primarily involve forming and optimizing the structure of esters and amino acid esters, phosphoramidates, octadecyl phosphates, and *bis*-pivaloxymethyl phosphates. The review discusses the optimization of the phosphoramidate prodrug moiety of the SARS-CoV-2 antiviral nucleoside remdesivir in detail. It presents the resulting improvement in bioavailability and antiviral activity. Moreover, it focuses on the modern prodrug strategy as one of the major recent advances in drug substance development. This strategy effectively optimized physicochemical properties and improved the functional activity of the existing drug substances and drug substance candidates for the first time.

Introduction

Modern drug research has allowed the development of safe low-molecular compounds with high activity and selectivity [1]. However, several new compounds do not show the appropriate physicochemical properties [2] required for active drug substances. The high effectiveness of these substances *in vitro* is limited *in vivo* by their low solubility and bioavailability [3] and, thus, their poor

ability to penetrate the phospholipid bilayer of cell membranes. In addition to special pharmaceutical formulation techniques [4], the unfavorable physicochemical properties of these substances are currently eliminated through their chemical modifications [5]. These analogs are called prodrugs [6], as inactive derivatives of drug substances to be activated by endogenous enzymes. Until recently, the modifications have involved the conversion of drug substances into simple salts of inorganic or carboxylic acids [7], which improved solubility without affecting their activity.

The development of new drugs remarkably accelerated during the SARS-CoV-2 pandemic [8]. The search for new antiviral drugs has so far focused on derivatives of natural nucleosides [9] as potential inhibitors of replication [10] of the viral nucleic acids. As a result of the repositioning of these substances, synthetic nucleosides active also against the SARS-CoV-2 virus were rapidly obtained [11]. As highly polar compounds, however, they did not penetrate sufficiently cell membranes. It was, therefore, necessary to modify their structures to ensure adequate lipophilicity while maintaining antiviral activity.

New prodrug strategies, especially for polar substances with low lipophilicity, such as nucleosides, developed within the last three years, allowed not only a significant improvement in physicochemical properties but also a significant increase in their activity [12] for the first time. Therefore, the present mini-review focuses on the selected aspects of the modern prodrug strategy as one of the significant recent advances in drug substance development.

Amidon's Biopharmaceutics Classification System (BCS)

In his BCS concept (**Figure 1**), Gordon Amidon from the College of Pharmacy, the University of Michigan, proposed correlating *in vitro* drug product dissolution and *in vivo* bioavailability [13]. The correlation was based on recognizing that drug dissolution and gastrointestinal permeability (GI) are the fundamental parameters controlling the rate and extent of drug absorption. He used a transport model and human permeability to estimate *in vivo* drug absorption. BCS became widely accepted in the academic, industrial, and

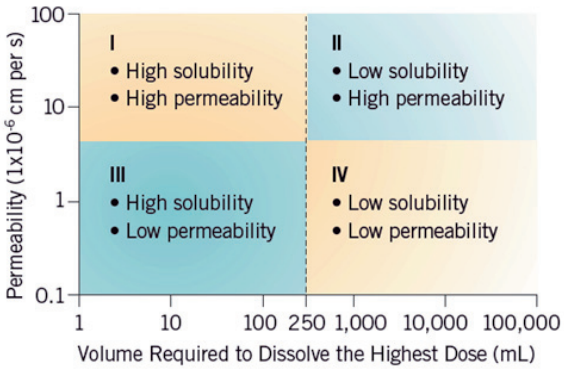

Figure 1. Amidon's Biopharmaceutics Classification System (BCS).

regulatory areas [14]. The major challenge is now moving a drug substance from the BCS Class IV of low solubility and low permeability to at least the BCS Class II of low solubility but high permeability. One of the chemical approaches to achieve this aim is briefly discussed below.

McGuigan's ProTide strategy for nucleoside prodrugs

ProTide procedure, developed by Christopher McGuigan from the School of Pharmacy of the University of Cardiff (**Figure 2**), is a prodrug approach for efficient intracellular delivery of synthetic nucleoside monophosphates and monophosphonates [15]. In this approach, the hydroxyl groups of the monophosphate or monophosphonates are masked by the aromatic group and the ester group of the amino acid [16]. The

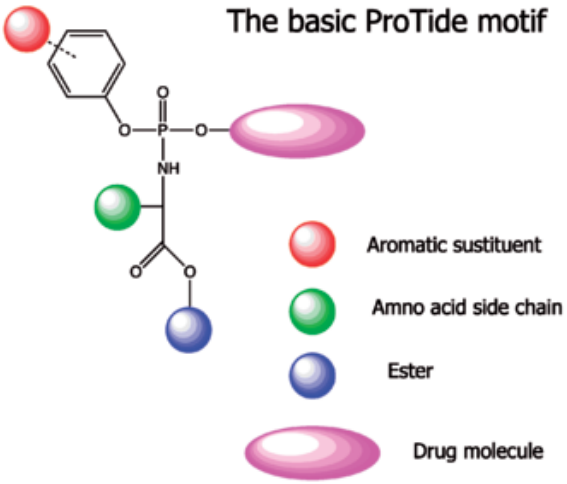

Figure 2. McGuigan's ProTide strategy for nucleoside prodrugs.

masking is designed to increase the lipophilicity of the synthetic nucleoside to enhance its penetration through the phospholipid double layer of the cell membrane. The masking groups are enzymatically cleaved inside the cells to release free nucleoside monophosphates and monophosphonates. The procedure represents the culminating achievement of medicinal chemistry research in the past few decades. The nucleoside drug substances must be converted by endogenous enzymes into the respective triphosphates for further incorporation into the viral nucleic acids. However, the first phosphorylation step is very slow, resulting in a low potency of the drug substance [17]. Protected phosphoramidates already introduce a phosphate moiety into the structure of a nucleoside to avoid the slow step. Other groups attached to the phosphate neutralize the two negative charges of the phosphate group, thus facilitating its permeability through cell membranes and oral bioavailability. Removal of the masking groups exposes the nucleotide phosphate to the next two phosphorylation. Since its discovery, the technology has been widely used in drug research. Most recently, it has led to the discovery of two FDA-approved ProTide antiviral drug substances, vis., sofosbuvir [18], and tenofovir alafenamide [19] (**Figure 3**). ***Sofosbuvir*** is a defective substrate of NS5B protein, inhibiting the viral RNA polymerase. It is orally used against the hepatitis C virus (HCV). ***Tenofovir alafenamide***, a PROTIDE analog of tenofovir, is an orally active hepatitis B virus (HBV) nucleotide reverse transcriptase inhibitor for therapy in HIV/AIDS and chronic hepatitis B. It is more antivirally active and better distributed into lymphoid tissues than the previously developed tenofovir disoproxil. ***Remdesivir*** is a phosphoramidate analog of a derivative of adenosine nucleoside [20]. It is a PROTIDE product readily diffusing into the cell. There, it is converted into monophosphate by CES1 and CTSA esterases and HINT1 phosphoramidase. As other nucleoside phosphoramidates, it is further phosphorylated to the respective triphosphate by nucleoside-phosphate kinases. *In vitro*, remdesivir penetrates the cell in its original form of phosporamidate. However, the pharmacokinetic analysis revealed premature serum hydrolysis of remdesivir *in vivo*, contributing to its relatively low antiviral potency. ***Dioxolanyl brivuridine phosphoramidates*** (DBPs) are derivatives of antiviral brivudine [21], an analog of

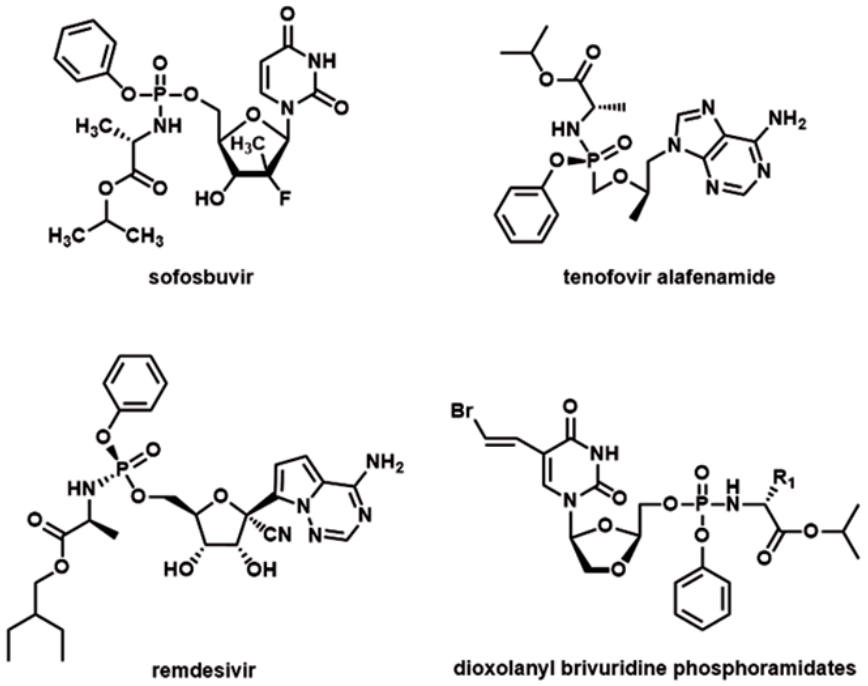

Figure 3. ProTide antiviral drug substances.

thymidine nucleoside. Its active metabolite, brivudine triphosphate, is incorporated into the viral DNA, inhibiting viral DNA polymerases. Brivudine is used orally against herpes zoster. The bromovinyl hydroxymethyl dioxolanyl analog of brivudine, **L-BHDU** is significantly active against the varicella-zoster virus. It was further structurally optimized by introducing various prodrug moieties.

Current phosphoramidate prodrug strategy

Unfortunately, **L-BHDU** is insufficiently lipophilic to penetrate nerve cells. Thus the very recent optimization of the nucleoside prodrug structures [21] included not only phosphoramidates of bromovinyl hydroxymethyl dioxolanyl uracil (L-BHDU, dioxolanyl analog of brivuridine) but also long-chain phospholipids and phosphate esters (**Figure 4**). *In vitro*, most of these prodrugs exhibited significant anti-varicella zoster virus (VZV) activity. Monophosphate ester prodrugs (POM-L-BHDU-MP and POC-L-BHDU-MP) and long-chain phospholipids (octadecyloxyethyl ODE-L-BHDU-MP) were antivirally active similarly as the starting L-BHDU. However, pharmacokinetics revealed that monophosphate ester prodrugs (POM-L-BHDU-MP) exhibited a 2.2-fold increase in oral absorption/availability compared to the parent L-BHDU. Long-chain monophosphate phospholipids (ODE-L-BHDU-MP) and monophosphate ester prodrugs (POM-L-BHDU-MP and POCL-BHDU-MP) are potentially antivirally active. Therefore, they are examined as novel anti-VZV agents. Current studies of these monophosphate prodrugs are directed to clinically relevant systems to select potential clinical candidates.

The premature hydrolysis of ***remdesivir*** resulted in limited efficacy and low accumulation in the lungs as the target tissue, encouraging further structure optimization of the remdesivir ProTide moiety. The structure of both substituents at the phosphate group was optimized using various aryl groups and long lipid chain substituted at the amino acid protecting group [22]. The lead analog **MMT5–14** (drawn below) resulting from this study showed reduced premature hydrolysis in plasma. That resulted in a 2- to 7-fold higher antiviral activity in four variants of SARS-CoV-2. The optimized MMT5–14 was 3- to 8-fold more stable than remdesivir in the plasma and liver microsome. It showed 200- to 300-fold increased prodrug concentration in the plasma and lungs, 5-fold enhanced lung accumulation of the active metabolite (remdesivir-TP), and 4- to 25-fold increased intracellular uptake and activation in lung epithelial cells. The optimized analog is a new potential antiviral drug to treat COVID-19 patients with severe symptoms.

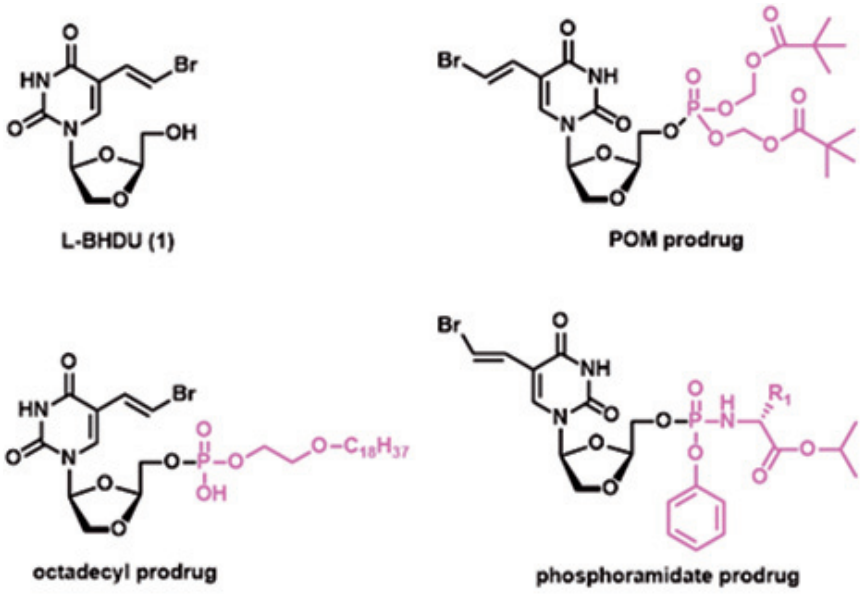

Figure 4. Nucleoside prodrug structures.

Summary and future directions

The prodrug strategy for nucleosides (PROTIDE) uses an inactive derivative (***phosphoramidate***) to ensure oral bioavailability and improved pharmacokinetics of a drug substance. The first two phosphoramidate nucleosides (sofosbuvir and tenofovir) have already been introduced to antiviral therapy. Optimization of the phosphoramidate moiety of remdesivir resulted in a more hydrolysis-resistant analog showing much higher antiviral activity, fundamentally increased prodrug concentration in the plasma and lungs, and enhanced accumulation of the active metabolite in the target tissue. The optimized analog is considered a new potential antiviral drug to treat COVID-19 patients with severe symptoms. Not only phosphoramidates but also other monophosphate esters were successfully introduced as prodrugs of therapeutic nucleosides and showed improved pharmacokinetics and antiviral potency. Considering the current progress in this field, prodrug strategy is among the most important achievements in drug substance development. The development of a new drug substance might not be sometimes necessary, as the prodrug strategy already improves the physicochemical properties, plasma stability, functional activity, and target tissue concentration.

Footnote

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Conflict of interest statement
The authors declare no conflict of interest.

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