**Modified Nucleosides “(ProTide)” as Potential Anti-HCV Therapeutics**

***N.A. Elsharif***\****, O. Dakeel***

Department of Chemistry, Faculty of Science, University of Benghazi, Benghazi, Libya

***Abstract***

*Hepatitis C virus (HCV) is among the most common causes of cirrhosis and chronic liver disease worldwide. As a result, many researchers are interested in designing and synthesis of a clinical treatment for HCV. Nucleoside monophosphates and monophosphonates play such an important role for treatments of incurable diseases such as hepatitis. For example, 2'-C-methyladenosine and 2'-C-methyl guanosine have shown activities against HCV in the replicon assay as well as against several members of the flavivirus family. However, their development as drug molecules has been hindered by the inherent poor drug-like properties of the monophosphate and monophosphonate groups. These groups have low bioavailability due to the inefficient cellular uptake, poor in vivo stability and poor intracellular metabolism; the latter drawback being most relevant to monophosphates than monophosphonates. These limitations can be addressed by using por Tide strategy, which is able to help the nucleoside monophosphate delivered inside the cell. In this review, we have discussed the different keys monophosphate and monophosphonate nucleoside prodrugs that has entered clinical development. In addition, the role of the ProTide technology is highlighted for the success in the discovery of nucleoside therapeutics.*

***Keywords:*** *Hepatitis C****,*** *antiviral****,*** *nucleoside****,*** *ProTide****,*** *monophosphates*

\****Author for Correspondence*** E-mail: [nada.elsharif@gmail.com](mailto:nada.elsharif@gmail.com)

**INTRODUCTION**

**Hepatitis**

Hepatitis is a viral infection that has emerged as a major public health problem throughout the world affecting several hundreds of millions of people [1]. Viral hepatitis is a cause of considerable morbidity and mortality in the human population, both for acute (lasting less than six months) or chronic (lasting more than six months), such as hepatitis B, C and D, chronic active hepatitis and cirrhosis [2]. It can destroy the liver tissue, spread from person to person, weaken the body's immune system, cause liver cancer (hepatitis B and C), and lead to death [3]. The increasing number of publications and patents devoted to Hepatitis C virus (HCV)-related research shows the attempts of researchers to design an active antiviral agent against hepatitis.

***Hepatitis C Virus Infection***

HCV is an RNA virus that belongs to the family Flaviviridae [4]. It has been discovered in 1989 by Choo *et al*. [5]. Infection with HCV has become a serious public health problem worldwide because it leads to chronic liver disease worldwide as well as the primary indication for liver transplantation [1]. In fact, more people are expected to die from HCV than acquired immunodeficiency syndrome (AIDS) in the near future [6]. The World Health Organization (WHO) estimates that about 3% of the world’s population, i.e., 170 million people, are currently infected with HCV, which is four times as many as those infected with human immunodeficiency virus (HIV) [7]. A new study in 2015 demonstrated that the Central, East Asia, North Africa, and the Middle East have the highest prevalence of HCV infection [8]. In the fact, with the high risk of HCV infection, there is no vaccine available for preventing infection. Furthermore, many of current conventional treatments such as ribavirin and pegylated interferon are unsatisfactory.

***Therapy of HCV***

The current therapy for HCV, such as, pegylated interferon and ribavirin (Figure 1) [9], showed nonspecific inhibition of HCV with limited efficacy in at least half of the patients [10, 11]. Moreover, pegylated interferon and ribavirin provide limited sustained virologic response (SVR) rates and can produce various undesirable side effects ranging from flu-like symptoms to severe adverse effects, including anemia, cardiovascular events, and psychiatric problems such as suicidal ideation [12]. In contrast, some nucleosides such as 2'-C-methyladenosine (Figure 2b) and 2'-C-methyl guanosine (Figure 2c) are more active against HCV with the absence of detectable cytotoxicity [13]. 2'-C methylguanosine triphosphate has been known as a potent inhibitor of HCV RNA polymerase for some time, but the parent nucleoside is only moderately active due to poor intracellular phosphorylation [14]. Consequently, more effective therapies with better tolerability profiles are urgently needed. The application of phosphoramidate ProTide technology has been used to bypass the rate-limiting initial phosphorylation of this nucleoside.



***Fig. 1:*** *Structure of Ribavirin.*

**NUCLEOSIDES AS ANTIVIRALS**

Nucleosides play such an important role for the treatment of incurable diseases such as hepatitis, HIV, and cancer. In order to be effective, all nucleosides require metabolic activation in their target cell to the bioactive phosphate “nucleotide” form [14]. Several families of nucleoside analogues (Figure 2 ) have emerged with apparent selectivity for HCV [10]. However, this metabolic activation is often not very efficient, and thus the therapeutic potential for these nucleosides is often quite limited [15]. Also, preformation of phosphate does not offer any advantage on account of poor permeation of the phosphate through the cell membrane. The nucleoside monophosphate analogues are unstable in biological media where they are highly susceptible to dephosphorylation. They also show poor membrane permeation because of the associated negative charges at physiological pH. This barrier must be overcome in order to produce a marketable drug. One avenue of investigation has used phosphate prodrugs which become known as the “ProTide” approach. One of the ProTide approaches which has proved particularly effective to date is the use of aryloxy phosphoramidates (Figure 3) [14].



***Fig. 2:*** *Some Anti-HCV Nucleosides.*



***Fig. 3:*** *Aryloxy Phosphoramidate.*

**PHOSPHORAMIDATE (ProTide) PRODRUGS**

Pronucleotides "ProTide" technology has been discovered by the McGuigan's team at Cardiff University in 1996 [15]. It is a prodrug strategy that involves masking of nucleoside phosphate and phosphonate groups by an aryl motif and an amino acid ester, respectively. These technologies have been used to overcome the limitations of the nucleoside monophosphate and monophosphonate therapeutics as mentioned earlier. Indeed, this technology has inspired the discovery of numerous ProTide entities that has progressed to clinical trials such as cancer, HIV and hepatitis C treatments, and much more are currently undergoing (pre)clinical development. The basic structure of a phosphoramidate motif is shown in Figure 4 [15]. The concept of pronucleotide (protide) analogue is a strategy that masks the charges of the nucleoside analogue monophosphates so that they penetrate the membrane and then selectively release the nucleoside analogue monophosphate inside the cell shown in Figure 5.



***Fig. 4:*** *A General ProTide Structure.*



***Fig. 5:*** *General Pronucleotide (ProTide) Concept (NA: nucleoside analogue).*



***Fig. 6:*** *New Antihepatitis C Virus (HCV) Therapeutics Continues as the Current Treatment.*

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| ***Fig. 7:*** *Some Nucleoside ProTides Entering Clinical Trials.* | |

**ProTides Nucleoside Against HCV**

The Phosphoramidate (ProTide) approach led to the successful development of numerous of nucleoside phosphonate analogues, that are approved for clinical use against HCV such as, cidofovir (Figure 6A) [16], tenofovir (Figure 6B) [16], sofosbuvir (Figure 6C) [17] which is now used to treat patients with HCV [18],and tenofovir alafenamide (Figure 6D) [19–21].

Additionally, this particular prodrug approach was adopted to discover numerous of other nucleoside ProTides entering clinical trials (Figure 7) [22].

***NUC-1031*** (Figure 7A) [23] is an aryloxy triester phosphoramidate (ProTide) of the anticancer drug gemcitabine.

***NUC-3373*** (Figure 7B), showed excellent preclinical properties [14]. McGuigan and co-workers reported the discovery in 2011 and highlighted its advantageous properties in terms of tumors reduction.

***GS-5734*** (Figure 7C) [24] is currently the only C-nucleoside-based ProTide undergoing clinical trials following the termination of clinical development of the initial anti-HCV [22].

***Stampidine***(Figure 7D) [25] and thymectacin (Figure 7E) [26], both are anti-HIV ProTide clinical candidates.

**The Hypothesis**

As we have mentioned earlier, nucleoside analogues are structurally different from natural nucleosides. The phosphorylation that is utilized to generate bioactive monophosphate in the cell has often limited efficiency (Figure 8A) [27]. These limitations can be addressed by using ProTide strategy that delivers the phosphorylated metabolites of these nucleoside analogues rather than relying on active transport, the kinase-dependent phosphorylation steps, and hence achieve better potency. However, as unmodified agents, nucleoside monophosphates (Figure 8B) are unstable in biological media, are highly susceptible to dephosphorylation and also show poor membrane permeation because of the associated negative charges at physiological pH [22]. This barrier must be overcome in order to achieve a marketable drug [28]. The discovered ProTide technology that masks the charges of the nucleoside analogue monophosphates, have shown success in delivering the nucleoside into the cell, where they are converted into their active species (Figure 8C). A phosphoramidate ProTide generates lipophilic prodrugs of the monophosphate of the nucleoside. Modification of the ester and amino acid moieties lead to make the ProTide nucleotide 500 times more potent than the parent nucleoside [13].

***Fig. 8:*** *(A) A General Representation of the Intracellular Activation of Nucleoside Analogues; (B) Nucleoside Monophosphates and Monophosphonates; (C) Masked Nucleoside Monophosphates and Monophosphonates.*



***Fig. 9:*** *Mechanism of ProTides in vivo Breakdown to Release the Nucleoside Analogue Monophosphate or Monophosphonate.*

**Mechanism of ProTide Compounds’ Action**

The ProTide technology, discovered by the McGuigan team at Cardiff University, is a strategy that masks the charges of the nucleoside analogue monophosphates by an aryl motif and an amino acid ester group; so that, they pass through the membrane (Figure 9). After that, two enzymatic activation steps remove the masks intracellularly to release the nucleoside monophosphate. The first enzyme, esterases cleave the ester motif of the ProTide (ester hydrolysis) [29]. Under physiological pH (< 7.4), the negatively charged carboxyl group carries out a nucleophilic attack on the phosphate or phosphonate group. As a result, the aryl motif leaves and forms a highly unstable five-membered ring; with the speed of this process being dependent on the structure of the analogue [29]. A water molecule attacks the released nucleoside and opens up the heterocyclic ring to give a phosphoramidate metabolite. A second enzyme known as phosphoramidase-type enzyme mediates the cleavage of the P-N bond of this metabolite leading to release of the nucleoside analogue monophosphate or monophosphonate (Figure 9) [30].

**CONCLUSION**

This review was focused on the (ProTide) phosphate prodrug technologies that are utilized to discover the nucleotide HCV therapeutics. ProTide technology, discovered by the McGuigan team at Cardiff University in the early 1990s, is one of the best areas of improving drugs. One of these technologies, known as the phosphoramidate ProTide method was described in this review. These technologies aimed for intracellular delivery of nucleoside monophosphates into cells have proved to be effective in improving the therapeutic potential of antiviral and anticancer nucleosides. They embarked on designing phosphoramidate-based ProTides following the concept that HCV protease might cleave a suitable oligopeptide from the phosphate moiety of a blocked nucleotide phosphoramidate. The ProTide approach has been well investigated and established as a viable method for the intracellular delivery of monophosphate nucleoside analogues. This approach has been proven to improve the antiviral and anticancer profiles of many nucleoside analogues. Clearly, the phosphoramidate triester pronucleotide technology has proved effectively as a tool for drug discovery, and numerous of phosphoramidate triester-based drugs are undergoing clinical trials so far. In addition, phosphoramidate-based compounds may be explored as treatments for a wide range of diseases. For example, a new antiviral drug Sofosbuvir (GS-7977), with the chemical name L-Alanine, N-[[P(S),2′R]-2′-deoxy-2′-fluoro-2′-methyl-P-phenyl-5′-uridylyl]-, 1-methyl-ethyl ester, is a new prodrug, that shows a high potent activity against all HCV genotypes.

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