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Review

The search for nucleoside/nucleotide analog inhibitors of dengue virus

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ABSTRACT

Nucleoside analogs represent the largest class of antiviral agents and have been actively pursued for potential therapy of dengue virus (DENV) infection. Early success in the treatment of human immunodeficiency virus (HIV) infection and the recent approval of sofosbuvir for chronic hepatitis C have provided proof of concept for this class of compounds in clinics. Here we review (i) nucleoside analogs with known anti-DENV activity; (ii) challenges of the nucleoside antiviral approach for dengue; and (iii) potential strategies to overcome these challenges. This article forms part of a symposium in Antiviral Research on flavivirus drug discovery.

Contents

1. Introduction

Dengue virus (DENV) non-structural protein 5 (NS5) possesses multiple enzymatic activities, including a guanylyltransferase (Bollati et al., 2009; Egloff et al., 2007; Issur et al., 2009), RNA cap methyltransferase (MTase) (Egloff et al., 2002; Ray et al., 2006; Zhou et al., 2007), internal RNA MTase (Dong et al., 2012), and RNA-dependent RNA polymerase (RdRp) (Ackermann and Padmanabhan, 2001; Lim et al., 2015; Zhao et al., 2015). Among these enzymatic activities, the RdRp plays a central role in viral replication and represents the most attractive target for antiviral research. The preference of targeting viral replication machinery has been clearly evident from the recent success of sofosbuvir in the treatment of chronic hepatitis C. For HIV therapy, even with the emergence of many new classes of inhibitors, nucleos(t)ide analogs remain the backbone of first-line treatments.

The structure of RdRp adopts the classical half-closed right hand configuration consisting of a palm, thumb, and finger domains. Flavivirus RdRp contains additional finger-loop motif connecting finger and thumb subdomains (Haudecoeur et al., 2013; Zhao et al., 2015). Two broad approaches have been pursued to inhibit RdRp for antiviral therapy: non-nucleoside and nucleos(t)ide inhibitors. Non-nucleoside inhibitors bind to an allosteric sites away from the active site and affect the enzymatic activity of RdRp (Niyomrattanakit et al., 2010). Non-nucleoside inhibitors could

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also inhibit viral replication through blocking the binding between RdRp and other viral replication components as well as host factors (Fraser et al., 2014). In the case of HCV, several allosteric sites (such as Palm I, Palm II, Thumb I and Thumb II sites) have been identified and explored for the development of non-nucleoside inhibitors (Beaulieu, 2009).

Nucleoside analogs are historically used for anti-cancer therapy through inhibiting cellular polymerases (Jordheim et al., 2013). The field of antiviral nucleoside development blossomed after the successful introduction in the HIV field. Unlike non-nucleoside inhibitors which directly bind to viral polymerase to exert their antiviral effect, nucleoside inhibitors have to be converted into their corresponding triphosphate forms inside cells by various host kinases (Stein and Moore, 2001). Even a small modification of the nucleoside structure could significantly affect the host kinases' activity on the nucleoside analogs because of the tight substrate specificity of some kinases (Golitsina et al., 2010). Since the substrate specificity of various kinases is not well defined, the kinases responsible for phosphorylating nucleoside analogs are often determined retrospectively.

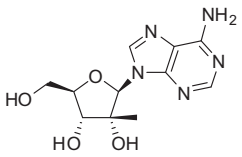
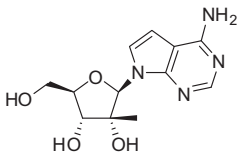
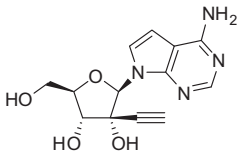
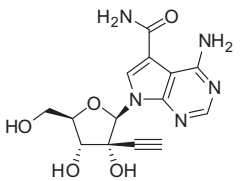
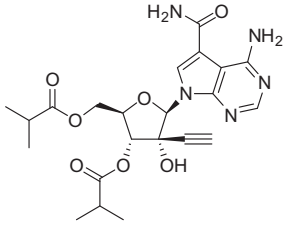
After conversion of nucleoside analogs into corresponding nucleoside triphosphates, they are incorporated into viral DNA/RNA (De Clercq, 2012). Such insertion of nucleoside often leads to inability of the viral polymerase to carry out further incorporation, leading to chain termination (Olsen et al., 2004). Therefore, two criteria are required for any nucleoside analogs to achieve antiviral activity: (i) efficient conversion to its triphosphate nucleotide inside cell; and (ii) inhibition of viral polymerase by the triphosphate nucleotide analog.

2. Known nucleoside inhibitors of DENV

Many nucleoside inhibitors of DENV were originated from HCV drug discovery due to the similarity between these two viruses, from different genera within the same family *Flaviviridae*. In particular, the 2′-*C*-methyl substitution was first reported to be active in HCV and it was soon found to have anit-DENV activity as well (Migliaccio et al., 2003). In addition, several other nucleoside analogs such as balapiravir and INX-08189 were active in both DENV and HCV (Yeo et al., 2015). It is of interest to note that all DENV-active nucleosides are potent inhibitors of HCV. However, the reverse is not true, as not all anti-HCV nucleoside had anti-dengue activity (Feng et al., 2014; Stuyver et al., 2006). Therefore, the chemical space for anti-DENV compounds seems to be smaller than the anti-HCV nucleosides. The reason behind this discrepancy is not well understood. Table 1 summarizes the nucleoside analogs with anti-DENV activities. Modifications of each of the four naturally occurring nucleoside types could lead to anti-DENV compounds.

In the case of adenosine analogs, 2′-*C*-methyladenosine was first reported to have anti-DENV activity, with an EC_{50} of 4 μM (Migliaccio et al., 2003). This compound was not suitable for drug

Table 1
Adenosine based Nucleoside inhibitors of dengue RNA dependent RNA polymerase. These adenosine based analogs do not need monophosphate prodrugs as they are efficiently converted to their corresponding monophosphate by host adenosine kinase.

Name	Nucleoside	Structure	Cellular activity (EC_{50}/CC_{50}) μM	*In vivo* mouse activity	References
2′-Methyl-adenosine	Adenosine		4/18	No	Migliaccio et al. (2003)
MK-0608	Adenosine		15/>320	Yes	Olsen et al. (2004)
NITD008	Adenosine		0.7/>100	Yes	Yin et al. (2009)
NITD449	Adenosine		1.62–6.99/>50	No	Chen et al. (2010b)
NITD203	Adenosine		0.1–0.71/>50	Yes	Chen et al. (2010b)