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Research paper

Novel thioglycosyl analogs of glycosyltransferase substrates as antiviral compounds against classical swine fever virus and hepatitis C virus

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ABSTRACT

Hepatitis C virus (HCV) and classical swine fever virus (CSFV) are important pathogens for which new therapeutic approaches are in high demand. Herein, we report the synthesis of newly designed thioglycosyl analogs of glycosyltransferase substrates which were evaluated using cell-based assays for cytotoxicity and antiviral activity against both viruses. The antiviral activity of synthesized compounds against CSFV and HCV was confirmed using pseudo-plaque reduction assays where a significant arrest of viral growth was observed in the presence of selected compounds. We showed that compounds **13** and **14** exerted the most significant inhibitory effect on *in vitro* CSFV and HCV infections in the series. Glycoconjugates **13** and **14** not only inhibited both viral propagation with IC_{50} values in low micromolar range, but efficiently suppressed the production of viral proteins in a dose-dependent manner. In addition, studies using *in vitro* HCV infection and replication models have shown that both compounds are able to significantly reduce viral genomic replication. We demonstrated that compounds **13** and **14** showed a strong inhibition, up to 90% of replication which inscribe them in the promising alternative approach for the development of new *anti*-CSFV and *anti*-HCV drugs.

1. Introduction

The *Flaviviridae* family comprises many viruses associated with human and animal diseases of great impact on public health. The most well-known members of this family are important human pathogens including hepatitis C virus (HCV), dengue fever virus, yellow fever virus as well as viruses of veterinary importance causing economically important diseases like bovine viral diarrhea virus and classical swine fever virus (CSFV). In spite of effective vaccines and therapies for some of these pathogens, viral mutations, drug resistance or viral re-emergence generate problems for global control and eradication.

HCV, a member of the *Hepacivirus* genus and CSFV, a *Pestivirus* member, show high degree of homology in genomic organization,

Abbreviations: Ac, acetyl; Bz, benzoyl; BAIB, [bis(acetoxy)-iodo]benzene; CSFV, classical swine fever virus; DAAs, direct-acting antivirals; DMTMM, 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; DMSO, dimethyl sulfoxide; GTs, glycosyltransferases; HCV, hepatitis C virus; TBDMS, *tert*-butyldimethylsilyl; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy.

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replication and protein function. They are enveloped viruses with single-stranded RNA genomes encoding a single polyprotein that is cleaved into different structural and non-structural proteins by host and viral proteases. Due to the similarity of CSFV and HCV, the former was frequently used as a surrogate model to study the role of envelope glycoproteins of HCV [1,2].

CSFV causes an acute, highly infectious and economically damaging disease in swine and wild boars in many countries [3,4]. Classical swine fever (CSF) is a major threat to commercial pig production worldwide [5]. CSF outbreaks among wild boars present a constant threat for domestic pigs [6]. There is no treatment for classical swine fever, other than supportive care. Several vaccines against CSFV are currently available, however the most efficient vaccines are live attenuated ones developed over 50 years ago, which do not allow for differentiating between infected and vaccinated animals [7]. The virus is still widely circulating in endemic regions, therefore, the use of antiviral drugs could be a good control strategy to prevent transmission of the virus in case of an outbreak.

HCV is a major cause of liver diseases in human, like chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. So far, there is no vaccine available [8]. It is estimated that around 180 million people are chronically infected worldwide, which makes the virus one of the most serious burdens to public health. Since the introduction of PEGylated interferon alpha and Ribavirin as the standard drugs for clinical therapy of HCV in 1990, significant efforts have been made to discover new and more effective treatments. New potent HCV inhibitors belonging to direct-acting antivirals (DAAs) such as Boceprevir, Telaprevir, Sofosbuvir, Simeprevir, Daclatasvir, and Ledipasvir have been developed in recent years [9]. These DAAs directly target enzymatic functions of viral proteins such as NS3/4A proteases, NS5A or NS5B polymerase. New formulations based on combination of the new agents and the traditional HCV inhibitors as well as multiple DAAs and interferon-free therapies have improved the rates of sustained virologic response (SVR) and greatly reduced the side effects [10]. However, although the combination therapy based on Boceprevir or Telaprevir (inhibitors of NS3/4A protease) together with Ribavirin and PEGylated interferon alpha improves antiviral response, it is limited to hepatitis C genotype 1. Introduced in 2013 Sofosbuvir (NS5B polymerase inhibitor) is the first drug used in combination with Ribavirin in interferon-free therapy, however it is effective only for genotype 2 and 3 HCV patients. In 2013 another drug Simeprevir (protease inhibitor) has been approved by FDA in interferon-free regimen. Combination therapy containing two other NS5A inhibitors, Daclatasvir and Ledipasvir, show high success rates (even over 90%) depending on viral genotype [11]. Although a significant progress in *anti*-HCV therapy has been undoubtedly attained, many limitations still exist. Monotherapy with DAAs are associated with rapid emergence of drug-resistant viral mutants, therefore clinical application of DAAs is mostly limited to combination regimens which is associated with additional side effects, drug-drug interactions, high costs and availability. Thus, new therapeutic strategies consisting of new HCV inhibitors targeting different stages of HCV life cycle, with increased effectiveness and wider availability are still needed to overcome these limitations.

Proteins protruding from the viral envelope of many viruses are usually highly glycosylated. Protein glycosylation is carried out by a family of glycosyltransferases (GTs), which catalyze the transfer of a sugar moiety from an activated nucleotide sugar donor to a hydroxyl or an amino groups of acceptor substrate [12,13]. GTs are involved in many fundamental biological processes and modulation of their activities by efficient inhibitors is a potential way for the control of certain cellular functions. A large number of potent natural as well as synthesized GT inhibitors based on 3D structures of several GTs have been identified. Most of synthesized GT inhibitors belong to donor substrate analogues, acceptor substrate analogues or bisubstrate analogues.

In case of metal-dependent GTs, the pyrophosphate moiety of the donor-type substrate interacts with bivalent cations such as Mn^{2+} or Mg^{2+} with coordination of two aspartate residues within a DXD motif in the enzyme active site. Numerous analogues of nucleosidediphosphate (NDP) sugars with modifications of the diphosphate bridge have been described. Some of them were obtained by varying the pyrophosphate moiety on phosphonate [14,15], methylenediphosphonate [16] or ethyl phosphonophosphate [17]. However, the main drawback of such compounds is their anionic character, which precludes their entry into cells through the phospholipid bilayer. Due to this fact, the promising strategy for *in vivo* biological applications is the preparation of GT inhibitors containing a neutral diphosphate surrogate [18,19].

Previously, a new kind of sugar nucleotide analogues, which were designed to act as GT inhibitors were reported [20]. When compared to the natural GT substrates, the anomeric oxygen atom was replaced in these structures by the sulfur in order to increase their resistance to enzymatic hydrolysis. Another change in the structure was the replacement of pyrophosphate bridge with a succinic linkage. The choice of such linker was based on the ability to coordinate divalent metal ions (Scheme 1) [21]. Based on the foregoing assumptions, glycoconjugates **8**–**10** where aminopyridyl 1-thioglycosides (derivatives of D-glucose **5** or D-galactose **6**) were connected to selectively protected uridine with succinic spacer **1** or **2** through the amide bond were prepared [20].

In this paper, we described the synthesis and evaluation of antiviral activity of novel thioglycosyl analogs of glycosyltransferase substrates against two viruses belonging to the *Flaviviridae* family – CSFV and HCV in cell culture system. To investigate the effect of the presence of the succinic linker on glycoconjugates biological activity, a series of new glycoconjugates (**11**–**17**) were synthesized based on the same structural fragments as in case of glycoconjugates **8**–**10** but omitting the succinic linker. For the propose of this study, glycoconjugates **8**–**10** were additionally prepared using improved method using microwave irradiation. Two promising compounds with novel properties were chosen for further development as lead hits. The broad spectrum activity exhibited by selected compounds against these two viruses suggests their suitability as potential inhibitors against infections caused by other viruses from the *Flaviviridae* family.

2. Results and discussion

2.1. Chemistry

Our preliminary studies on biological activity of compounds **8**–**10** indicated that some of them were able to inhibit cell proliferation of the classical swine fever virus. These results encouraged us to synthesize other glycoconjugates in which succinic fragment has been omitted and *N*-(6-mercaptopyridyn-3-yl)formamide part was the only substitute of diphosphate bridge (Scheme 2).

The effect of the presence and the type of protecting groups in both parts of glycoconjugate: in the sugar ring and in the uridine moiety was also examined. As protecting groups in the sugar ring acetyl, benzoyl and TBDMS groups were selected. Ribose in uridine moiety was protected using the isopropylidene group or more hydrophobic TBDMS groups. Ester type protecting groups were selected due to the possibility of their hydrolysis by enzymes present within the cells. These groups only increased the hydrophobicity of glycoconjugates and allowed them to penetrate into the cell. In turn, protecting groups in the uridine part were chosen not only to allow the regioselective synthesis of glycoconjugates but