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Synthesis and antiviral activity of a novel glycosyl sulfoxide against classical swine fever virus

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ABSTRACT

A novel compound—2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2′,3′,6′-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl-(5-nitro-2-pyridyl) sulfoxide—designated GP6 was synthesized and assayed for cytotoxicity and in vitro antiviral properties against classical swine fever virus (CSFV) in this study. We showed that the examined compound effectively arrested CSFV growth in swine kidney cells (SK6) at a 50% inhibitory concentration (IC_{50}) of 5 ± 0.12 μg/ml without significant toxicity for mammalian cells. Moreover, GP6 reduced the viral E2 and E^{rns} glycoproteins expression in a dose-dependent manner. We have excluded the possibility that the inhibitor acts at the replication step of virus life cycle as assessed by monitoring of RNA level in cells and culture medium of SK6 cells after single round of infection as a function of GP6 treatment. Using recombinant E^{rns} and E2 proteins of classical swine fever virus produced in baculovirus expression system we have demonstrated that GP6 did not influence glycoprotein production and maturation in insect cells. In contrast to mammalian glycosylation pathway, insect cells support only the ER-dependent early steps of this process. Therefore, we concluded that the late steps of glycosylation process are probably the main targets of GP6. Due to the observed antiviral effect accompanied by low cytotoxicity, this inhibitor represents potential candidate for the development of antiviral agents for anti-flavivirus therapy. Further experiments are needed for investigating whether this compound can be used as a safe antiviral agent against other viruses from unrelated groups.

1. Introduction

Classical swine fever virus (CSFV), belongs to the family of Flaviviridae, genus Pestivirus.[1] CSFV is the causative agent of classical swine fever (CSF) and can cause an acute, highly infectious and economically damaging disease in swine and wild boars.[2,3] Several vaccines against CSF have been developed. These are live attenuated vaccines, subunit marker vaccines or DNA vaccines. Each of these vaccines have some disadvantages. Vaccination with conventional vaccines does not allow for discrimination between vaccinated and infected pigs and is banned in the European Union, others are less potent and more expensive.[4–6] CSF is present in many parts of the world, however since the 1980s, it is eradicated from the domestic pig population in most countries of the European Union. Nevertheless, the virus is still circulating in some populations of wild boars and the pig population is continuously at risk for virus introduction. Present outbreaks are controlled by culling of infected animals or those in contact with infected herds and the restriction of animal movements which are very costly and ethically questionable. Therefore, new strategies have to be implemented to control CSF. Few drugs against CSFV infections have been developed recently.[7–9] The use of antiviral agents could be a good control strategy to prevent transmission of the virus in case of an outbreak.

CSFV is an enveloped virus with a single-stranded positive sense RNA genome of 12.5 kb that contains a single open reading frame

Abbreviations: CC_{50}, concentration of the compound required to reduce cell viability by 50%; CSF, classical swine fever; CSFV, classical swine fever virus; Ct, cycle threshold; ER, endoplasmic reticulum; GTs, glycosyltransferases; IC_{50}, concentration of the compound required to reduce virus plaque formation by 50%; IPMA, immunoperoxidase monolayer assay; MOI, multiplicity of infection; PRV, pseudorabies virus; Sf9, *Spodoptera frugiperda* insect cell line; SD, standard deviations; S.I., selectivity index; SK6, swine kidney cells.

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encoding a polyprotein of approximately 4000 amino acids. The polyprotein is processed into mature structural and non-structural proteins by host and viral proteases. Structural components of the virion include the capsid (C) protein and three envelope glycoproteins: E^{rns}, E1 and E2.[10] E^{rns} and E2 glycoproteins are present in virions as disulfide-linked homodimers: an E^{rns} homodimer with a size of about 97 kDa and an E2 homodimer with a size of about 100 kDa. E2 is also present as a heterodimer with E1 (75 kDa).[11] Both E1 and E2 are type I transmembrane proteins with an N-terminal ectodomain and a C-terminal anchor.[10] E^{rns} lacks a typical membrane anchor. Apart from being a virion protein, E^{rns} is secreted from virus-infected cells as a soluble protein.[11,12] Moreover, E^{rns} has RNase activity, which is the unique feature for a viral surface protein, and is classified as a member of RNase T2 family.[13,14] E2 glycoprotein is essential for virus attachment and entry into target cells as well as for cell tropism.[15,16] It is the major immunodominant protein inducing the production of neutralizing antibodies and protection against lethal challenge.[17] Both E^{rns} and E2 glycoproteins are highly glycosylated with 7 and 6 glycosylation sites, respectively.[18,19]

N-glycans of enveloped virus glycoproteins have been shown to be important not only for the correct folding and stability, but also for various functions such as host cell receptor binding, membrane fusion, penetration into host cells.[16,20] Lack of N-glycan chains can lead to protein misfolding causing aggregation and protein retention in the endoplasmic reticulum or proteasome degradation.[21,22] It has been observed that changes in the glycosylation patterns of viral glycoproteins can influence infectivity, virulence and host immune response.

Glycosyltransferases (GTs) are a large class of enzymes involved in the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates, such as glycoproteins and glycolipids.[23] GTs catalyze glycosidic bond formation by the transfer of a saccharide, typically a monosaccharide, from an activated nucleotide sugar donor to an acceptor substrate.[24] GTs are involved in many fundamental biological processes and modulation of their activities by efficient inhibitors has potential for the control of certain cellular functions. Since the 3D structures of several GTs have been proposed, a large number of potent inhibitors have been identified. Structures of these compounds are based on analogies between donor substrates, acceptor substrates as well as transition state.

Glycosyl sulfoxides have been found to possess a wide application in the field of complex oligosaccharides synthesis as powerful glycosyl donors.[25] Many chiral sulfoxides were reported to exhibit broad spectrum biological activities including inhibition of many different enzymes.[26–28] According to the literature phenyl sulfinyl β-D-galactopyranosides were cleaved in the diastereoselective manner in the presence of β-galactosidase from *Escherichia coli*. It could be explained in such a way that only one diastereoisomer orients its sulfinyl oxygen toward the activating amino acid of enzyme whilst second diastereoisomer behaves as inhibitor.[29] On the other hand, heterocyclic sulfoxides and sulfones proved to be 10–100 fold more potent inhibitor of fatty acid amide hydrolase (FAAH) than corresponding sulfides.[28] Also polyhydroxylated cyclohexenyl sulfoxides demonstrated weak abilities to the inhibition of α-D-glucosidase from Brewers yeast and β-D-glucosidase from sweet almonds.[27]

On the basis of these data we decided to focus our attention on sulfoxides derivatives of 5-nitro-2-pyridyl 1-thioglycosides as potential GTs inhibitors. Most of GTs are usually metal ion dependent, where manganese is the most typical metal found in active sites.[30,31] We expected, that heteroaryl sulfoxides, due to the presence of free electron pairs on sulfinyl oxygen and heterocyclic nitrogen, could favourably interact with Mn^{2+} similarly to natural GTs substrates.

Therefore, a number of glycosyl sufoxides containing different sugar moieties (D-glucose, D-galactose or lactose with ester or ether protecting groups) and varying aromatic substituents at sulfur atom were synthesized in order to evaluate their potential biological activity. Here, we describe the synthesis and the in vitro antiviral evaluation of a lead compound—designated GP6, which was found to have significant antiviral activity and the lowest toxicity out of all synthesized compounds. We further investigated the potential mechanism of action by which GP6 exerts its anti-CSFV activity. Further experiments are needed for investigating whether this compound can be used as a safe antiviral agent against other viruses from Flaviviridae family and other viral families.

2. Materials and methods

2.1. Chemistry

2.1.1. General methods

Melting points were determined on a SRS OptiMelt melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P2000 polarimeter using sodium lamp (589 nm) at room temperature. Mass spectra were recorded with a WATERS LCT Premier XE system (high resolution mass spectrometer with TOF analyzer) using electrospray-ionization (ESI) technique. FT-IR spectra were recorded using ATR method with a Thermo Scientific NICOLET 6700 spectrophotometer. UV–vis spectra were recorded with a JASCO V-650 spectrophotometer with the use of 1.0 cm quartz cell and methyl alcohol as a solvent. NMR spectra were recorded on an Agilent spectrometer (400 MHz). Deuterochloroform 98.8% (code 209561000) of isotopic purity (ACROS) with 0.03 v/v% TMS as internal standard was used as a solvent. Column chromatography was performed on silica gel 60 (Merck; 70–230 mesh, code 1.07734.5000) column. All compounds were routinely checked by TLC by using aluminium-baked silica gel plates (Merck TLC Silica gel 60 F_{254}, code 1.05554.0001). Developed plates were visualized by UV light and by charring after spraying with 10% H_2SO_4 in EtOH. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions, extractions and column chromatography involved the use of rotary evaporator (Heidolph, Germany) operating at a reduced pressure (ca. 40 Torr). Organic solutions were dried over anhydrous magnesium sulfate (POCH Gliwice, Poland).

2.1.2. 2″,3″,4″,6″-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2′,3′,6′-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl-(5-nitro-2-pyridyl) sulfoxide GP6

To a solution of (5-nitro-2-pyridyl) per-*O*-acetyl-1-thio-β-D-lactoside (1.4 mmol) in a dry dichloromethane (30 mL) cooled down to 0 °C the *m*-CPBA (1.4 mmol) was added. The reaction mixture was stirred at 0 °C and was monitored by TLC. The reaction was stopped without full conversion of 1-thioglycoside in order to limiting creating the sulfone. The reaction mixture was diluted with CH_2Cl_2 and washed with water (3 × 15 mL). The organic layer was dried over anhydrous $MgSO_4$. The solid was filtered off and the filtrate was concentrated to give crude product which was purified by a column chromatography (toluene/AcOEt 20:1 to 2:1 [v/v]) to give diastereoisomeric mixture of sulfoxides GP6 (yield 59% or 68% when calculated on utilized 1-thioglycoside). The CPBA oxidation of the sulfide substrate gave approximately equal amounts of epimeric sulfoxides and no attempt was made to control its stereoselectivity. Small amount (approximately 15 mg) of each diastereoisomer was isolated by repeated column chromatography and characterized separately.

Less polar, dextrorotatory sulfoxide had: mp 93–98 °C (with decomposition); $[\alpha]_D^{20} = 7.5^\circ$ ($CHCl_3$, $c = 0.4$); IR (ATR method) ν 1743 (C=O), 1361 (NO_2), 1213 (C–O), 1043 (S=O) cm^{-1}; UV–vis (MeOH) λ_{max} 204.8, 237.5 and 291 nm; ^1H NMR ($CDCl_3$, 400 MHz)