Załącznik nr 3

Autoreferat w języku angielskim

Ewelina Król

Summary of the Professional Scientific Achievements

Development of innovative strategies to combat viral infections in humans using chemically synthesized inhibitors

Dr. Ewelina Król

Department of Recombinant Vaccines
Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk

Gdańsk 2019

1. Name: Ewelina Król

2. Awarded Diplomas and Degrees (Institution, Department/Faculty or any other Research Unit, Date of obtaining Academic Degree, title of the PhD thesis)

2011 Ph.D. in biological sciences, discipline: biochemistry, doctoral degree with honors, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Poland. Dissertation title: "Classical swine fever virus (CSFV) as a surrogate model to investigate new antiviral strategies against hepatitis C virus (HCV)". Promoter: prof. dr hab. Bogusław Szewczyk
The dissertation has been awarded with honors by the Council of Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk.

2004 M.Sc. in Biotechnology, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Poland. M.Sc. thesis title: "Cloning and expression of the truncated form of E2 glycoprotein of hepatitis C virus (HCV) produced in baculovirus expression system". Promoter: prof. dr hab. Bogusław Szewczyk.
Public presentation of the results of the thesis received an award of the Intercollegiate Faculty of Biotechnology.

3. Information on previous employment in Scientific Institutions

from
01.03.2012 Academic position: associate professor (adiunkt), Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Poland
(Maternity leave: 30.06.2014-28.06.2015)

2009 –2012 Research position, Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Poland, supported by the project entitled: POIG.01.01.02-14-007/08-00, „Innovative Technologies", „Centre of biotechnological drugs. Package of innovative biopharmaceuticals for people and animals therapy and prophylaxis"

2004- 2011 Ph.D. studies at Chemistry and Biochemistry, Faculty of Chemistry, University of Gdansk, Poland

4. Scientific Achievement, defined by Article 16, Clause 2 of the Act of March 14, 2003 on Academic Degrees and Titles as well as Degrees and Titles in the Arts (Journal of Laws 2017, item 1789)

A) Title of the scientific achievement

Development of innovative strategies to combat viral infections in humans using chemically synthesized inhibitors.

B) Research publications belonging to the Scientific Achievement:

The Scientific Achievement (a monothematic cycle) includes seven scientific publications (6 experimental publications and 1 review publication). **I am the corresponding author in all publications constituting the scientific achievement.**

IF – impact factor from the year of publication of the work; if IF was not present for the specific year, the IF of previous year was used, $IF_{5\text{-year}}$ – 5-year impact factor; MNiSW – journal scoring according to Ministry of Science and Higher Education; Lc – citation number (Web of Science (WoS); Google Scholar).

1. **Krol, E.*,** Wandzik, I., Gromadzka, B., Nidzworski, D., Rychlowska, M., Matlacz, M., Tyborowska, J., Szewczyk, B. (2013). Anti-influenza A virus activity of uridine derivatives of 2-deoxy sugars. Antiviral Research, 100, 90-97.
   IF = 3,434; $IF_{5\text{-year}}$ = 4,185; MNiSW = 35 pkt; Lc = WoS: 6, Google Scholar: 9

2. **Król, E. *,** Rychłowska, M., Szewczyk, B. (2014). Antivirals - current trends in fighting influenza. Acta Biochimica Polonica 61, 495–504.
   IF = 1,153; $IF_{5\text{-year}}$ = 1,541; MNiSW = 15 pkt; Lc = WoS: 28, Google Scholar: 38

3. **Krol, E.*,** Wandzik, I., Krejmer-Rabalska, M., Szewczyk, B. (2017). Biological evaluation of uridine derivatives of 2-deoxy sugars as potential antiviral compounds against influenza A virus. International Journal of Molecular Sciences, 18, 1700.
   IF = 3,687; $IF_{5\text{-year}}$ = 3,878; MNiSW = 30 pkt; Lc = WoS: 2, Google Scholar: 4

4. **Krol, E.*,** Wandzik, I., Pastuch-Gawolek, G., Szewczyk, B. (2018). Anti-hepatitis C virus activity of uridine derivatives of 2-deoxy sugars. Molecules, 23(7). pii: E1547
   IF = 3,098; $IF_{5\text{-year}}$ = 3,268; MNiSW = 30 pkt; Lc = WoS: 0, Google Scholar: 1

5. **Krol, E.*,** Wandzik, I., Brzuska, G., Eyer, L., Růžek, D., Szewczyk, B. (2019). Antiviral activity of uridine derivatives of 2-deoxy sugars against tick-borne encephalitis virus. Molecules, 24(6). pii: E1129.
   IF = 3,098; $IF_{5\text{-year}}$ = 3,268; MNiSW = 30 pkt; Lc = WoS: 0, Google Scholar: 0

6. Pastuch-Gawolek, G., Chaubey, B., Szewczyk, B., **Krol, E*.** (2017). Novel thioglycosyl analogs of glycosyltransferase substrates as antiviral compounds against classical swine fever virus and hepatitis C virus. European Journal of Medicinal Chemistry, 137, 247-262. **IF = 4,816; $IF_{5\text{-year}}$ = 4,527; MNiSW = 40 pkt; Lc = WoS: 6, Google Scholar: 6**

7. **Krol, E.*,** Pastuch-Gawolek, G., Chaubey, B., Brzuska, G., Erfurt, K., Szewczyk, B. (2018). Novel uridine glycoconjugates, derivatives of 4-aminophenyl 1-thioglycosides, as potential antiviral compounds. Molecules, 23(6). pii: E1435.
   IF = 3,098; $IF_{5\text{-year}}$ = 3,268; MNiSW = 30 pkt; Lc = WoS: 0, Google Scholar: 0

Summarized IF of publications belonging to the scientific achievement = 22,384
Summarized $IF_{5\text{-year}}$ of publications belonging to the scientific achievement = 23,935
Summarized value of the Ministerial Publication Points for publications belonging to the scientific achievement = 210

The publications described the results obtained during the implementation of 4 research projects in which I was the **Principal Investigator**:

1. Polish Ministry of Science and Higher Education IUVENTUS PLUS No. IP2010 020870; "Glycosylation inhibitors as new antiviral agents against different strains of Influenza virus type A".
2. Polish Ministry of Science and Higher Education IUVENTUS PLUS No. IP2011 027271; "Tunicamycin analogues and mimetics as new antiviral agents against different strains of Influenza virus type A"
3. Polish National Science Centre PRELUDIUM No. UMO-2011/03/N/NZ6/00059; "The influence of tunicamycin analogues and mimetics on hepatitis C virus (HCV) propagation"
4. Polish National Science Centre SONATA No. UMO-2015/19/D/NZ6/01717; "Tick-borne encephalitis virus – search for mechanisms useful in treatment and prophylaxis"

C) Description of the research aims and results of the above-mentioned Scientific Achievement along with a description of their potential applications

Introduction:

For centuries epidemics and pandemics have had a significant impact on human history. Currently, the World Health Organization (WHO) issues every year a report on new viral pathogens which pose a threat to public health. Emerging epidemics caused by new pathogens or more pathogenic strains of pre-existing viral pathogens call for the development of effective strategies for combating and preventing viral infections. Immunoprophylaxis with a specific vaccine is one of the most important elements of the combat against many infectious diseases. However, there are still many viruses for which no effective vaccine is available on the market. The use of effective antiviral drugs is then the only possible route to fight against infections caused by these viruses. It is fully understandable that the research aiming at the development of new treatments for viral infections are treated as a priority not only in Europe but in the whole world.

Many viral species contain a lipid envelope in which viral proteins are anchored. Envelope glycoproteins, as the most exposed structural elements of virions, are crucial for the viral life cycle. They participate in the assembly of infectious particles and play a role in viral entry, since they enable interaction with specific cell surface receptors and induce fusion between the viral envelope and the host cell membrane. Due to their important role in the viral life cycle, glycoproteins can also be an attractive target for antiviral therapies. Envelope proteins are usually highly N-glycosylated. The growth of sugar chains on a variety of biomacromolecules including glycolipids and glycoproteins in mammalian cells is mediated by

a large group of enzymes defined as glycosyltransferases (GTs) (Breton et al., 2012). These enzymes, which are present mostly in endoplasmic reticulum or Golgi apparatus, catalyze the transfer of a sugar moiety from an activated donor sugar (usually nucleotides) onto acceptors like saccharide, lipid or protein. The important role of proper N-glycosylation process in the maturation and survival of envelope glycoproteins was reported previously. N-glycosylation influences not only the correct folding and stability of many viral glycoproteins, but also has vital effects on their biological functions such as receptor binding, membrane fusion, and penetration into host cells (von Messling and Cattaneo, 2003; Shi and Elliott, 2004). The removal of N-oligosaccharides by glycosylation inhibitors very often leads to aggregation and protein retention in the endoplasmic reticulum. Misfolded proteins are usually translocated back to the cytosol for ER- associated degradation (Brodsky and McCracken, 1999; Parodi, 2000; Trombetta and Parodi, 2003). As a consequence, the inhibition of N-glycosylation process significantly affects the assembly and secretion of viral particles from infected cells. A significant decrease in the infectivity of the progeny viral particles was also observed due to the incorporation of non-functional glycoprotein complexes into the virions.

Due to the fact that GTs take part in glycosylation process, the modulation of their activities can be employed during the antiviral drug design. In recent years, intensive research on the design of new selective and effective GT inhibitors has been conducted. The design of the structure of GT inhibitors is generally based on similarity with their natural substrates (Gloster, 2012; Kajimoto and Node, 2009; Wang et al., 2003). The identification of potent inhibitors has been developing very rapidly during the last two decades since the 3D structure of several GTs were determined (Breton et al., 2006; Kikuchi and Narimatsu, 2006; Unligil and Rini, 2000). Designed inhibitors can be divided into donor analogs (source of transferred sugar residue), acceptor (target site of transferred sugar residue) or intermediate enzyme-substrate complex (Compain and Martin, 2001; Zou, 2005). Although many compounds have been designed and synthesized, only few of them exhibited significant activity against GTs.

The nucleoside antibiotic tunicamycin, produced by *Streptomyces lysosuperficus*, is one of the best known inhibitors of glycosyltransferases (Duksin and Mahoney, 1982; Elbein, 1987; Lehle and Tanner, 1976; Tkacz and Lampen, 1975). The significant antiviral activity of tunicamycin have been shown for many viruses (Nakamura and Compans, 1978; Pizer et al., 1980; Saito and Yamaguchi, 2000; Schwarz et al., 1976), however the therapeutic use of tunicamycin has been limited due to its toxicity in animals (Bourke and Carrigan, 1993; Kohsaka et al., 1985).

Thanks to scientific cooperation with the Silesian University of Technology in Gliwice and the Pharmaceutical Institute in Warsaw, I obtained a panel of several dozens of synthesized compounds designed as potential inhibitors of GTs enzymes. The designed compounds were based on analogies with donor substrates, which is UDP-sugar. A common structural motif of all compounds was the uridine fragment, which is responsible for binding at the active site of the enzyme. The most important discovery resulting from the results of my doctoral thesis, was the demonstration that uridine derivatives of 2-deoxy sugars (IW series) have significant antiviral activity against classical swine fever virus (CSFV), which is the causative agent of a highly contagious, economically damaging disease of swine and wild boars (Krol et al., 2010). These compounds can be treated as tunicamycin analogs, because they also contain glycosyl units as a substitute for diphosphate. Preliminary studies have also confirmed the efficacy of two selected compounds against hepatitis C virus (HCV). It was shown that the antiviral effect of two the most active compounds (IW3 and IW7) is attributed to the N-glycosylation inhibition at the late stage of glycan modification process characteristic for mammalian cells. Therefore, the research conducted as part of the doctoral thesis showed that glycosylation inhibition could be the basis for innovative therapeutic options in the treatment of viral infections in future. Most

of currently tested compounds belong to the inhibitors of specific viral proteins involved in various stages of the viral life cycle, which can induce the production of drug resistant viruses in a relatively short time. The use of glycosylation inhibitors that affect cellular proteins should contribute to the elimination of drug resistance.

Thus, due to very promising preliminary results, in the publications describing the scientific achievement, I have attempted **to prove that glycosylation inhibition can be the new therapeutic option in the treatment of viral infections.** Human enveloped viruses, for which no antiviral therapies are available or viruses for which new drugs are highly needed to overcome the drug resistance, have been selected as research models. In my research influenza virus, hepatitis C virus and tick-borne encephalitis virus have been used.

Influenza virus, which belongs to *Orthomyxoviridae* family, cause severe respiratory illness in humans and animals. Each year about 3 to 5 million people is infected by the virus, which results in around 300,000–500,000 deaths annually (Fiore et al., 2008; Russell et al., 2008). Apart from human influenza, bird flu is also of great importance, since in the case of an outbreak within 72 hours the mortality in the herd reach 100% causing huge economic losses. Vaccines and antiviral drugs are currently two major options used to control influenza infections. Although it is obvious that vaccination is the best way of prevention, in case of influenza the composition of vaccines needs to be updated seasonally to account for antigenic changes of the viral glycoproteins. Moreover, variable effectiveness of vaccination is the result of high genetic variability of influenza virus caused by antigenic shift and antigenic drift of the viral genome. Antigenic mutation or reassortment can result in new highly virulent influenza strains which arise unexpectedly to cause new epidemics or worldwide pandemics (Nichol and Treanor, 2006).

Despite very intensive research for new antivirals, only a few compounds representing two classes of influenza virus inhibitors are currently available for clinical use: M2 ion-channel blockers (amantadine and rimantadine) (Hayden, 1997) and neuraminidase inhibitors (oseltamivir and zanamivir) (Monto et al., 1999; Nicholson et al., 2000). However, it has been shown that some available antiviral drugs are ineffective against specific viral strains; M2 inhibitors that prevent viral uncoating are not active against H5N1 strain (Li et al., 2004). What's more, the widespread use of antiviral drugs is associated with the emergence of drug-resistant viruses in a short period of time. In the USA, the number of oseltamivir-resistant H1N1 viruses quickly increased from 0.5% in 2006–2007 to 99% in the 2008–2009 season (Hurt et al., 2011; Memoli et al., 2011). In addition, influenza virus strains that are completely resistant to all available antiviral drugs have been discovered (Gubareva, 2004). The limitations of current drug therapy and the increasing emergence of multiple drug-resistant influenza strains calls for a new generation of broad-spectrum anti-influenza drugs with an alternative mode of action.

Two membrane glycoproteins of influenza virus: hemagglutinin (HA) and neuraminidase (NA) play a crucial role in the replication of the virus. Both NA and HA are highly glycosylated with 4 and 3–9 potential N-glycosylation sites, respectively (Schulze, 1997; Ward et al., 1983). Number and type of the attached oligosaccharides strongly depends on the virus subtype and strain (Inkster et al., 1993). It has been reported that N-linked oligosaccharides attached to the stalk region of HA are highly conserved, while those in other regions of the molecule vary considerably in structure and number among different influenza viruses, which suggests their important role in folding and homotrimeric structure (Wagner et al., 2000). Furthermore, it has been shown that the lack of HA-linked N-glycans inhibits viral replication in *in vitro* cell culture (Wagner et al., 2002).

Hepatitis C virus, which was discovered in 1989, is the major cause of liver diseases. It is estimated that around 180 million people are chronically infected globally, corresponding to 2% of the world's population. HCV is a major cause of chronic liver diseases such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. As the result, HCV is the main factor responsible for liver transplantation worldwide (Lavanchy, 2011).

Due to the lack of vaccine, until 2011, a combination of pegylated IFN alpha and ribavirin was used in the treatment of HCV-infected patients (Fried et al., 2002). The effectiveness of this therapy was relatively low; depending on the virus genotype, 40-80% of patients showed a sustained virological response (SVR). In recent years new anti-HCV drugs belonging to direct-acting antivirals (DAAs) (boceprevir, telaprevir, sofosbuvir, ledipasvir, daclatasvir, and simeprevir), inhibiting the non-structural proteins crucial for virus replication such as NS3/4A proteases, NS5A or NS5B polymerase have been registered. Despite high efficiency, the clinical usage of these drugs in monotherapies has been associated the emergence of rapid viral resistance (Barth, 2015; Pawlotsky, 2013). Nowadays, new formulations based on combination of different DAAs have been approved. Although, current drug combinations are well tolerated, their use is limited by drug-drug interactions and extremely high cost. 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.

HCV highly glycosylated envelope glycoproteins E1 and E2, abundant on the viral surface, form a heterodimer which plays an important role in viral entry, fusion, and secretion (Lindenbach and Rice, 2013). E1 and E2 have five to six and nine to eleven potential N-glycosylation sites, respectively. Their number depends on the virus genotype. The intensive research has been performed to define the role of N-glycans present on both HCV glycoproteins (Helle et al., 2010; Lavie et al., 2006; Meunier et al., 1999).

Tick-borne encephalitis virus (TBEV), a member of the *Flaviviridae* family, is a causative agent of tick-borne encephalitis (TBE). TBE is a disorder of the central nervous system which may lead to serious medical complications in humans, including meningitis, meningoencephalitis, or even death (Dumpis et al., 1999). Tick-borne encephalitis virus was isolated for the first time in 1937 in Soviet Union (Silber and Soloviev, 1946). TBEV is now present in at least 28 European countries, especially in Central and Eastern Europe, Scandinavia and parts of Asia (Donoso Mantke et al., 2011).

The incidence of TBE has markedly increased during the past 20 years (Donoso Mantke et al., 2011). TBEV is mainly endemic in Europe, Russia, and Asia, however, the virus extends its range outside endemic areas, where TBEV has never been found before, probably due to the climate changes (Steffen, 2016). Although vaccines against TBE based on inactivated viruses are available, the vaccination is not mandatory but only recommended for residents and tourists traveling to endemic areas. Vaccines are rarely used as a prevention tool, which results in more than 12,000 human cases reported annually globally (Ruzek et al., 2019). However, it is known that these data are estimates and may constitute only 30% of all TBEV infections, as they relate only to registered = hospitalized cases of TBE. Despite numerous strategies of research, currently there is no licensed therapeutic available for the treatment of TBEV infections. Patients diagnosed with TBE infection are usually treated to alleviate the symptoms, e.g., to reduce the inflammation and intracranial pressure by anti-inflammatory drugs. Therefore, the development of new effective antiviral compounds is highly demanded.

The TBEV envelope contains two viral proteins which play a major role in viral entry into the target cells: glycoprotein E and the small membrane protein prM/M. Both TBEV proteins possess at least one conserved N-glycosylation site (Rey et al., 1995). It has been proven that the loss of glycosylation of TBEV protein E affects the conformation of the protein,

consequently reducing the infectivity of secreted virions (Yoshii et al., 2013). It has been reported that the virus composed of glycoprotein E lacking the N-glycan chains was not infectious in a mouse model, confirming that glycosylation inhibition may be a new target for anti-TBEV compounds.

The main aim of my scientific achievement was to demonstrate that inhibition of the glycosylation process using designed compounds belonging to GTs inhibitors may constitute a novel antiviral approach to effectively combat viral infections. At the beginning of the studies there were almost no reports describing the synthesis and activity of compounds belonging to glycosylation inhibitors. Most of the published research was focused on the activity of inhibitors targeting viral proteins, e.g. protease and polymerase. Compounds that act on cellular processes should have a wide antiviral activity against many envelope viruses encoding glycoproteins involved in the first stage of life cycle. Therefore, IW3 and IW7 compounds, which belong to inhibitors of the late stages of the protein glycosylation process, were used in my research. Highly variable influenza virus was used as a model because despite the availability of seasonal vaccines and antiviral drugs, new, effective drugs are still needed [Publication 1]. The summary of drugs that are currently available to prevent influenza virus infection as well as several drugs that are currently in different stages of pre-clinical up to advanced clinical development have been presented in [Publication 2]. In the next part of my scientific achievement I have attempted to develop improved antiviral strategies against important viral pathogens based on new synthesized derivatives of IW3 and IW7 compounds, belonging to tunicamycin analogues and potentially targeting the N-glycosylation process. The conducted research was aimed at selecting compounds with significant antiviral activity against influenza virus, hepatitis C virus and tick-borne encephalitis virus [Publication 3, 4, and 5].

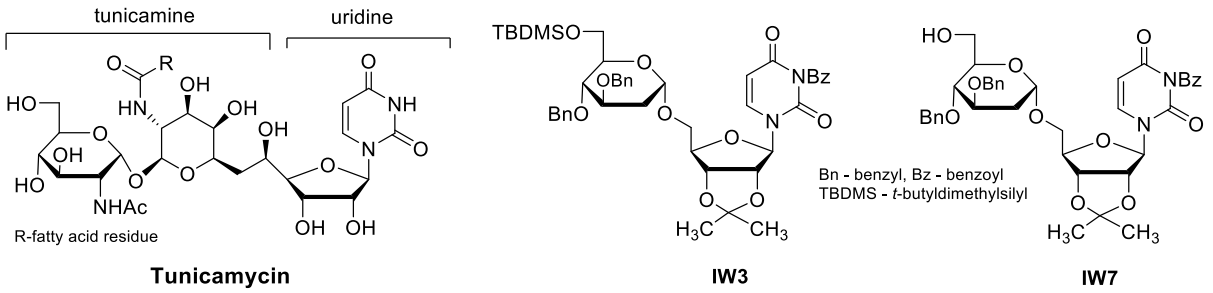
In parallel, I have tried to identify new antiviral compounds belonging to uridine glycoconjugates and to demonstrate their antiviral activity against viruses of the *Flaviviridae* family [Publication 6 and 7].

Detailed description of the publications included in the scientific achievement:

P.1. Krol, E.*, Wandzik, I., Gromadzka, B., Nidzworski, D., Rychlowska, M., Matlacz, M., Tyborowska, J., Szewczyk, B. (2013). Anti-influenza A virus activity of uridine derivatives of 2-deoxy sugars. Antiviral Research, 100, 90-97.

Aim of the study:

The main aim of the research was to study the *in vitro* antiviral activity of selected in previous research compounds IW3 and IW7 (belonging to uridine derivatives of 2-deoxy sugars) against influenza virus. The selected compounds possess uridine and a 2-deoxy-*O*-glycosidic unit (Scheme 1). They mimic the uridine diphosphate fragment, which is a part of the natural substrates of donor-type GTs. The observed antiviral effect of compounds against classical swine fever virus was attributed to the inhibition of N-glycosylation at the late stage of glycan modification process. The confirmation whether the antiviral mechanism of selected compounds consisting on the inhibition of protein glycosylation is universal was also the aim of this work. As part of this work, I also wanted to check the consequences of inhibiting the glycosylation process on the propagation of influenza virus in *in vitro* cell culture.



Scheme 1. Chemical structures of tunicamycin and the uridine derivatives of 2-deoxy sugars (IW3 and IW7).

Synthetic description of results:

To study the antiviral activity of compounds, the methodology for *in vitro* propagation of influenza viruses in Madin Darby canine kidney cells was introduced (Tree et al., 2001). The classical method for influenza virus propagation is to grow the virus in pathogen-free chicken embryos. However, this technique possesses serious drawbacks. One of them is the low titers of human influenza strains. What is more important, viruses obtained in pathogen-free chicken embryos differ from the original clinical isolates in the glycosylation profile of proteins, which makes them not suitable for studying the activity of glycosylation inhibitors (Meyer et al., 1993).

Using two influenza strains: pandemic human influenza A/H1N1 virus and avian influenza virus A/ostrich/Denmark/725/96 (H5N2) I have tested the potential antiviral activity of IW3 and IW7 compounds in *in vitro* cultures. Different influenza strains were selected for antiviral screening due to high variability of influenza strains as well as due to the fact that the number and type of the oligosaccharides attached to HA and NA strongly depends on the virus subtype and strain. Infection with influenza virus normally results in a severe cytopathic effect (CPE), therefore the antiviral property of IW3 and IW7 against influenza virus was investigated using CPE inhibition assay and plaque reduction assay. The results strongly indicated that IW3 and IW7 are highly active against influenza virus, because both compounds protected MDCK cells from cell death induced by influenza virus as well as caused a dose-dependent reduction in average size of plaques and their number in cell culture. Since the inhibition of the glycosylation process has a direct effect on the maturation of viral proteins, in the next step the influence of IW3 and IW7 inhibitors on synthesis of influenza proteins was examined. For both IW3 and IW7, a dose-dependent decrease of intracellular HA and NA glycoproteins was observed. Interestingly, at the highest doses of tested compounds the level of synthesis of both influenza proteins was below the level of detection. Moreover, nonglycosylated or underglycosylated species of HA or NA glycoproteins were also not detected. Tunicamycin is known as an endoplasmic reticulum (ER) stress inducer that triggers the accumulation of unfolded proteins in ER (Schröder, 2008). We hypothesized that uridine derivatives of 2-deoxy sugars which are structurally related to tunicamycin may possess similar mechanism of action. Hemagglutinin and neuraminidase without native oligosaccharide side chains due to IW3 and IW7 treatment, are most probably degraded by cellular protease(s) and cannot be detected in infected cells.

To corroborate the mechanism of anti-influenza virus activity, real-time PCR using SYBR Green dye technology was used to test the *in vitro* effect of the drugs on viral replication 8, 24 and 48 h p.i. No changes in the intracellular RNA level was detected upon short treatment (8 h) with IW3 and IW7, suggesting that both compounds do not affect virus replication during a single round of infection. However, analogous experiments using RNA extracted from the culture medium showed significant reduction in viral RNA indicating that the virus release from the inhibitor-treated cells is most probably impaired due to changes in glycosylation status of viral proteins. This was further confirmed by comparing the infectious virus titers from the same experiment, where significant loss of progeny viruses secreted from infected cells was observed for both compounds. Finally, we have shown that treatment with IW3 and IW7 for 24 or 48 h p.i., allowing for secondary infections, resulted in the reduction of both secreted and intracellular viral RNA levels, indicating that the loss of infectious virus progeny in sequential infection cycles affect also long-term intracellular accumulation of viral RNA. Taken together, these data confirmed our hypothesis that due to the changes in viral proteins glycosylation status, IW3 or IW7 interfere with the assembly and/or secretion of the mature virions after single round of infection which results further in the reduction of virus RNA in both infected cells and culture medium during secondary infections. The same results were observed for tunicamycin (Nakamura and Compans, 1978; Schwarz et al., 1976).

The importance of the results:

This study is the first one describing in detail the antiviral activity of synthesized compounds IW3 and IW7 belonging to uridine derivatives of 2-deoxy sugars (tunicamycin analogues) which inhibit the glycosylation process against influenza virus. So far only few scientific publication presented the influence of tunicamycin and castanospermine on maturation of influenza proteins (Elbein et al., 1984; Pan et al., 1987; Saito and Yamaguchi, 2000). The results confirmed that IW3 and IW7 compounds inhibiting the glycosylation process interfere with the assembly and / or secretion of progeny viruses after the first replication cycle of the virus, which directly reduces the viral progeny produced during secondary replication cycles. These findings support our idea that targeting glycan composition may be a promising therapeutic option for controlling viral infection. However, it was important that the compounds, despite their antiviral activity and much less toxicity than tunicamycin, did not show high selectivity indexes. The selectivity index characterizes the efficacy of compound and is defined as the ratio of the dose of the compound causing the cytotoxic effect to the dose of the compound needed to cause antiviral effect (CC_{50}/IC_{50}). Calculated SI values of IW3 and IW7 compounds were 8.83 and only 1.96, respectively. Nevertheless, the knowledge gained during the above studies was used to synthesize new compounds for which the antiviral activity is described in subsequent publications [Publication 3, 4 and 5].

P. 2. Król, E. *, Rychłowska, M., Szewczyk, B. (2014). Antivirals - current trends in fighting influenza. Acta Biochimica Polonica 61, 495–504.

Aim of the study:

The aim of this study was to collect the data and discuss the current state of the knowledge on currently available drugs for influenza treatment as well as to summarize some

new antiviral strategies that are now being tested at different stages of pre- clinical and clinical development.

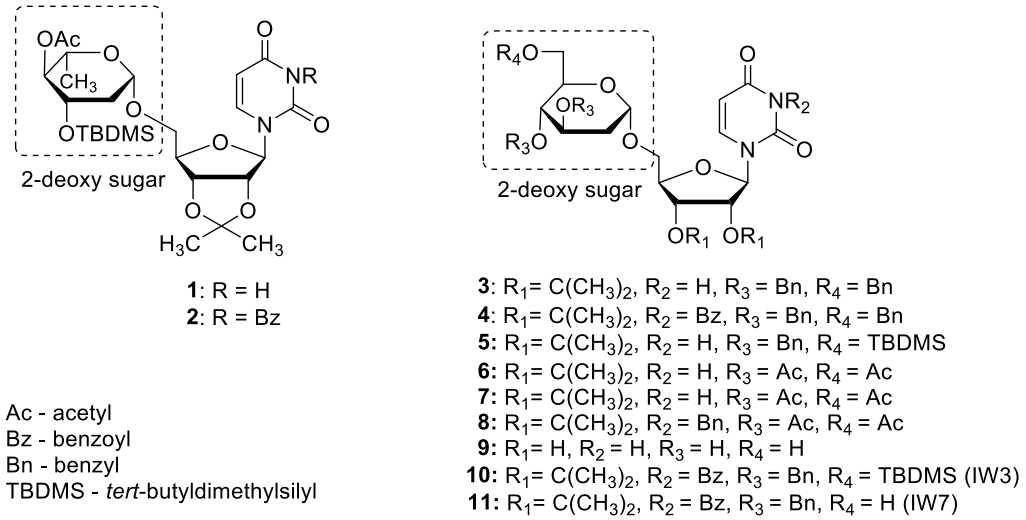
Synthetic description of results:

This publication was prepared for the special invitation of the Editor of Acta Biochimica Polonica. It summarizes drugs that are currently available to prevent influenza virus infection and describes recent developments in the field of new drugs against this virus. The paper presents antiviral strategies based on two types of antiviral drugs: M2 protein inhibitors (amantadine (Symmetrel®) and rimantadine (Flumadine®) and neuraminidase inhibitors (NA) (oseltamivir (Tamiflu®) and zanamivir (Relenza®). I discussed their mechanisms of action, resistance and lack of activity against certain strains of influenza as well as the effect of overuse of drugs on rapid emergence of drug-resistant variants which arise as a result of single amino acid substitutions in M2 and NA proteins. Next, the review describes new possible options in the treatment against this virus. I discussed new, most promising antiviral agents effective against a large variety of influenza strains which at present are at different stages of clinical trials. I discussed in detail the antiviral strategies targeting the viral proteins and/or the host-virus interaction. All available scientific studies using different inhibitors of viral proteins: hemagglutinin, M2 ion channel protein, neuraminidase and nucleoprotein were presented. The review also presents the collected data on possible strategies based on inhibition of host proteins: inhibitors of virus attachment or inhibitors of endocytosis and fusion. Moreover, combination therapies based on two or more different antivirals with potential for greater potency and clinical efficiency were also discussed.

P.3. Krol, E.*, Wandzik, I., Krejmer-Rabalska, M., Szewczyk, B. (2017). Biological evaluation of uridine derivatives of 2-deoxy sugars as potential antiviral compounds against influenza A virus. International Journal of Molecular Sciences, 18, 1700.

Aim of the study:

Thanks to the scientific cooperation with dr. hab. Ilona Wandzik, from the Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology at the Faculty of Chemistry of the Silesian University of Technology in Gliwice I have continued to examine the antiviral activity of novel uridine derivatives of 2-deoxy sugars. Based on the positive results using IW3 and IW7 inhibitors, the team of dr. Wandzik synthesized several new chemical compounds with various structural modifications either in the 2-deoxy sugar or uridine part (Scheme 2).



1: R = H
2: R = Bz

Ac - acetyl
Bz - benzoyl
Bn - benzyl
TBDMS - *tert*-butyldimethylsilyl

3: R_1= $C(CH_3)_2$, R_2 = H, R_3 = Bn, R_4 = Bn
4: R_1= $C(CH_3)_2$, R_2 = Bz, R_3 = Bn, R_4 = Bn
5: R_1= $C(CH_3)_2$, R_2 = H, R_3 = Bn, R_4 = TBDMS
6: R_1= $C(CH_3)_2$, R_2 = H, R_3 = Ac, R_4 = Ac
7: R_1= $C(CH_3)_2$, R_2 = H, R_3 = Ac, R_4 = Ac
8: R_1= $C(CH_3)_2$, R_2 = Bn, R_3 = Ac, R_4 = Ac
9: R_1= H, R_2 = H, R_3 = H, R_4 = H
10: R_1= $C(CH_3)_2$, R_2 = Bz, R_3 = Bn, R_4 = TBDMS (IW3)
11: R_1= $C(CH_3)_2$, R_2 = Bz, R_3 = Bn, R_4 = H (IW7)

Scheme 2. Chemical structures of synthesized compounds.

In this study, the antiviral activity of synthesized compounds against influenza virus was evaluated. An analysis of the influence of the introduced modifications on the activity of compounds was also carried out. The introduced modifications aimed at the improvement of selectivity indexes of the tested compounds in comparison to IW3 and IW7. For my studies I received compounds with various protecting groups in 2-deoxy sugar part as well as in the ribose part. In addition, different modifications were introduced to uracil nitrogen (N^3). In a series of compounds, totally deprotected compounds were also used for comparison.

Synthetic description of results:

Glycoproteins from different influenza strains can differ in the number of potential N-glycosylation sites, thus two different strains of influenza virus were used in the studies: human pandemic H1N1 strain and avian H5N2 strain. The results using CPE inhibition assay and plaque reduction assay showed that three synthesized compounds (2, 3, and 4) exhibit high antiviral activity against two strains of influenza virus. We showed that these compounds caused the dose-dependent reduction in virus propagation what was demonstrated in the reduction in average size and plaque number. Calculated IC_{50} values (*„inhibitory concentration 50%"*) ranged from 82 to 100 μM. The remaining compounds were less active.

Our comparative analysis of chemical structures of synthesized compounds revealed that the antiviral activity of tested compounds is enhanced by the addition of hydrophobic fragments, e.g. benzyl, benzoyl, tert-butyldimethylsilyl or isopropylidene groups. It can be assumed that the limiting step of antiviral activity is the ability to penetrate through the biological membranes. Compounds 2, 3 and 4, which were the most hydrophobic and were also the most active (IC_{50} in the range of 82-100 μM). The active compounds possessed different fragments of 2-deoxy sugars; 2-deoxy-ramnopyranose in the case of compound 2 and 2-deoxy-glucopyranose in other compounds. However, all of them had completely protected hydroxyl groups in the 2-deoxy sugar part: benzyl, tert-butyldimethylsilyl or acetyl groups in different combinations. Compound 5 was markedly more toxic than the others. Compounds 7-9 containing totally deprotected hydroxyl groups in 2-deoxy-sugar exhibited no activity (IC_{50} values ranged from 346 to 721 μM). Significant loss of activity was observed for compound 6

(IC_{50} = 575 μM) containing relatively polar per-O-acetylated 2-deoxy-glucose part, however the hydrophobicity was too low what probably affected the activity.

In the next step, to determine the mechanism of action, the influence of active compounds on different stages of viral infection was thoroughly examined. We showed that similarly to IW3 and IW7 also compounds 2-4 inhibited the propagation of influenza virus only when they are added to the cells after viral infection. The obtained results strongly suggested that these compounds target post-adsorption steps of influenza virus replication cycle. Moreover, the effect of compounds on viral glycoprotein synthesis demonstrated that both compounds caused a dose-dependent decrease in glycoprotein production, however the level of referenced host proteins is not affected. Similarly, to IW3 and IW7 compounds, the underglycosylated forms of proteins were not detected, suggesting that such incorrectly matured polypeptides are degraded very quickly in host cells.

The importance of the results:

In summary, the data showed that three newly synthesized compounds (2, 3 and 4) belonging to the uridine derivatives of 2-deoxy sugars (analogs of IW3 and IW7 compounds) possess significant antiviral activity against two strains of influenza virus. It has been shown that targeting the maturation of influenza viral proteins by inhibiting the late step of N-glycosylation process may constitute the novel therapeutic option, different from currently available drugs. I have also shown that some modifications in chemical structures improved the selectivity indexes of synthesized compounds in comparison to previously tested compounds without increase in the cytoxicity. SI for compounds 2, 3 and 4 were higher than this for IW7 compound. Confirmation of the activity of uridine derivatives of 2-deoxy sugars allows for designing further compounds based on new structural modifications to obtain even more active inhibitors of influenza infection.

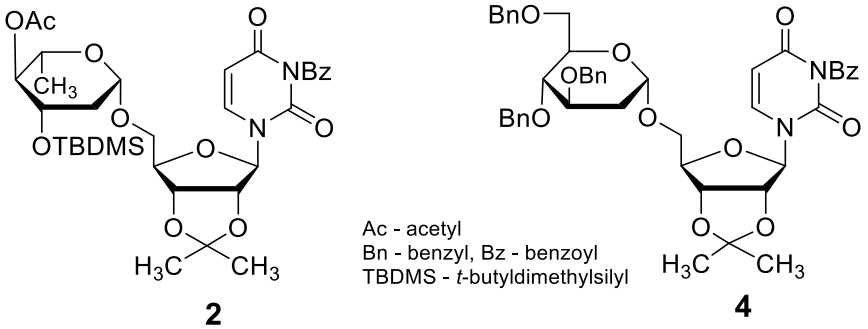
P.4. Krol, E.*, Wandzik, I., Pastuch-Gawolek, G., Szewczyk, B. (2018). Anti-hepatitis C virus activity of uridine derivatives of 2-deoxy sugars. Molecules, 23(7). pii: E1547

Aim of the study:

The current antiviral therapy against hepatitis C virus is based on combinations of direct-acting antivirals with different mode of actions, due to the emergence of rapid viral resistance in monotherapies. Although, current drug combinations are well tolerated, their use is limited by extremely high cost restricting the access to therapy which is low on a global scale (Barth, 2015; Rosenthal and Graham, 2016). In the beginning of the study, only one team was tested the antiviral activity of deoxynojirimycin (DNJ) derivatives, inhibitors of α-glucosidases which are present in endoplasmic reticulum as potential anti-HCV compounds (Chapel et al., 2006, 2007). Thus, the aim of the study was to check whether active compounds belonging to uridine derivatives of 2-deoxy sugars selected in previous studies (2, 3 and 4) exhibit the antiviral activity also against HCV. I analyzed the antiviral properties of these compounds using all available *in vitro* systems: cell culture-derived HCV (HCVcc), HCV pseudoparticles (HCVpp), and baculovirus expression system and replicon cell lines.

Synthetic description of results:

All synthesized uridine derivatives of 2-deoxy sugars described in Publication 3 were used to analyze the antiviral activity on HCV. I evaluated the anti-HCV activity of compounds using Huh-7.5 cell culture-derived HCV (HCVcc) (Lindenbach et al., 2005; Wakita et al., 2005). HCVcc pseudoplaque reduction assay was performed as the preliminary screening of compounds as HCV in comparison to influenza virus does not cause a cytopathic effect after infection. The results demonstrated that compounds 2 and 4 (Scheme 3) displayed high antiviral activity, however compound 3 active in anti-influenza studies did not show antiviral activity against HCV. Thanks to cooperation with Prof. Arvind Patel from University of Glasgow Centre for Virus Research, Glasgow the experiments using human hepatoma cell line Huh7-J20, which stably expresses EGFP fused in-frame to secretory alkaline phosphatase (SEAP) via a recognition sequence of the viral NS3/4A serine protease as a reporter system were conducted. The SEAP level in the culture medium of HCVcc infected cells directly correlates with the level of viral replication (Iro et al., 2009). Calculated IC_{50} values for the most active 2 and 4 compounds were 8,9 and 2,1 μM, respectively. Both compounds exhibited low cytotoxicity resulting in selectivity indexes of 12,7 and 67,6, respectively. Sofosbuvir, FDA-approved drug for anti-HCV therapy, an inhibitor of the NS5B RNA-dependent RNA polymerase, was used as a positive control (Lam et al., 2012; Sofia et al., 2010). This drug showed SI of 120.



Schemat 3. Chemical structures of uridine derivatives of 2-deoxy sugars (2 and 4).

By performing the experiments with stable cell line obtained from Prof. Patel Huh7-J17, which expresses monocistronic replicon encoding non-structural proteins, structural core protein and firefly luciferase (the levels of which directly correlate with virus RNA replication), I have proven that the antiviral activity of compounds 2 and 4 is not related to the inhibition of viral replication (Angus et al., 2012). The results with Sofosbuvir, the inhibitor of HCV replication, confirmed the usefulness of this cell line in the studies.

To test further the effect of compounds 2 and 4 on maturation of HCV viral glycoproteins, the surrogate retrovirus-based pseudoparticle system (HCVpp) was used (Bartosch et al., 2003; Hsu et al., 2003). We have shown that though compounds 2 and 4 do not change the production of HCVpp, they probably change their binding properties, which directly correlates with the reduction of interaction with Huh-7.5 cells. Surprisingly, my results demonstrated that the number of glycoproteins produced and incorporated to HCVpp was also not affected by tested compounds. Overall, our data have shown that the reduction of HCVpp infectivity was caused by the incorporation of modified forms of envelope glycoproteins into the pseudoparticles. These results are in agreement with previously-published data when testing the derivatives of deoxynojirimycin iminosugars as potential antiviral compounds (Chapel et al., 2007). In the comparative studies tunicamycin, an inhibitor of the first steps of glycosylation

process was used. In case of this compound the reduction of HCVpp infectivity was caused by impaired incorporation of glycoproteins into HCVpp.

The analysis of the activity of compounds clearly showed that HCVpp model may not be the optimum solution to study HCV the activity of compounds targeting the maturation of viral proteins. Previously, the differences in the glycosylation profiles of glycoproteins obtained in HCVpp and HCVcc systems have been shown (Vieyres et al., 2010). The differences in glycosylation may be due to the fact that the assembly of HCVcc occurs in ER-derived compartments, whereas the assembly of HCVpp molecules occurs in post-Golgi compartments (Gastaminza et al., 2008; Huang et al., 2007; Sandrin et al., 2005). This suggests that viral glycoproteins produced in HCVcc system undergo other post-translational modifications than those in HCVpp system.

The importance of the results:

In summary, we have demonstrated the significant antiviral activity of uridine derivatives of 2-deoxy sugars (compounds 2 and 4) against hepatitis C virus. SI for the most active compound 4 was 67, 6. The SI for Sofosbuvir was 120. Overall, our results suggested that inhibiting the glycosylation process might be a good target for new therapeutics against HCV, different form currently available drugs. These compounds could be used in future combination therapy. The current findings using hepatitis C virus have also confirmed potential broad-range antiviral activity of compounds 2 and 4, as they have been shown to be active against two untrelated viruses. Furthermore, it was very important and interesting to demonstrate that the HCVpp system is not the optimal system for studying the activity of compounds whose mechanism of action is associated with the maturation of viral surface glycoproteins.

P.5. Krol, E.*, Wandzik, I., Brzuska, G., Eyer, L., Růžek, D., Szewczyk, B. (2019). Antiviral activity of uridine derivatives of 2-deoxy sugars against tick-borne encephalitis virus. Molecules, 24(6). pii: E1129.

Aim of the study:

The aim of the work was to confirm finally the hypothesis concerning the inhibition of protein glycosylation by GTs inhibitors as potential innovative therapeutic method in the treatment of viral infections. The experiments were conducted using tick-borne encephalitis virus. At present there is no licensed drug therapy for TBEV infections. The studies were performed with all previously synthesized compounds belonging to the uridine derivatives of 2-deoxy sugars (compounds 1-9 and IW3 and IW7).

Synthetic description of results:

A method for propagation of TBEV virus in cell culture system (A549 lung cancer cells) was introduced to the laboratory in order to search for antiviral compounds against this virus. In this study, the antiviral screening was performed with two strains of TBEV; the highly virulent TBEV strain Hypr and the less virulent strain Neudoerfl. Due to the fact that TBEV is, similarly to influenza virus, a highly cytopathic virus, the preliminary screening of compounds

was performed using cytopathic effect inhibition assay and plaque reduction assay. The results demonstrated that compounds 2, 4, IW3 (10) and IW7 (11) were the most active and nearly completely inhibited the propagation of the virus in cell culture. Nearly complete inhibition of viral growth of both strains was observed after treatment with all four compounds. Compound 4 was the most active exhibiting the lowest dose needed for complete decrease in viral titer. Next, a detailed analysis of the effect of compounds on viral titer in cells treated with compounds over a wide range of concentrations and different time past infection was performed. Compounds 2, 4, IW3 and IW7 significantly reduced viral titers in a dose-dependent manner each day post-infection, indicating their stable activity. Compound 4 was the most active because the decrease in viral titer observed 3 days p.i. was the highest. When compound 4 was added to the cells no virus was detected. The calculated IC_{50} value for this compound was 1 4 μM. Due to the low cytoxicity of this compound, SI was 85. The calculated selectivity indices for compounds 2, 10, and 11 were 26,6, 18,6, and 13,8, respectively. The influence of selected uridine derivatives of 2-deoxy sugars on protein synthesis demonstrated that as in a case of other viruses, a dose-dependent decrease in the synthesis of proteins E and prM was observed. Less glycosylated or non-glycosylated forms of proteins E and prM were not detected indicating the very quick degradation of incorrectly matured proteins. The same results were observed for proteins of other viruses, e.g., HCV, CSFV, and influenza virus, in our previous studies.

The importance of the results:

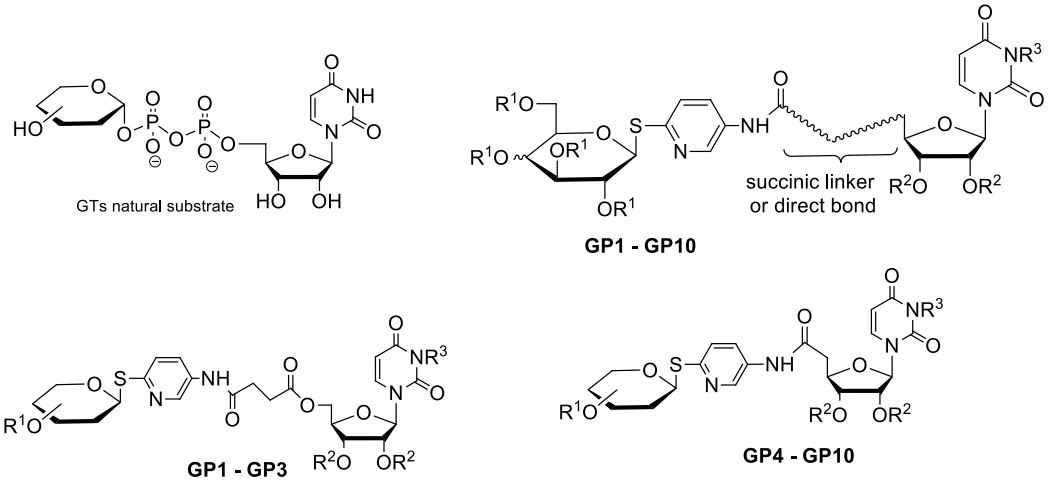
The presented results demonstrated that targeting the glycosylation process may be the basis for innovative antiviral therapy against tick-borne encephalitis virus. The results indicated that the selected compound 4 possesses high application potential, because in the presented studies it showed high selectivity index of 85. The presented results, together with our previous findings, confirm that the selected compounds possess a broad-range antiviral activity targeting the N-glycosylation process confirming their potential.

P.6. Pastuch-Gawolek, G., Chaubey, B., Szewczyk, B., **Krol, E*.** (2017). Novel thioglycosyl analogs of glycosyltransferase substrates as antiviral compounds against classical swine fever virus and hepatitis C virus. European Journal of Medicinal Chemistry, 137, 247-262.

Aim of the study:

Thanks to the scientific cooperation with dr. hab. Gabriela Pastuch-Gawołek from the Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology at the Faculty of Chemistry of the Silesian University of Technology in Gliwice new series of glycoconjugates containing uridine fragment as potential glycosyltransferases inhibitors have been synthesized (the compounds have been marked GP1-10 according to their synthesis) (Scheme 4). In these glycoconjugates (5-amino-2-pyridyl) 1-thioglycosides are linked directly to the uridine derivative or using succinic spacer linked. The modification of the natural diphosphate linker, present in the UDP-sugar structure, was aimed at changing its anionic character and facilitating penetration into cells through biological membranes. Therefore, in the synthesized compounds, the diphosphate bridge was replaced with a linker containing a pyridyl ring connected to succinic acid attached by in the C-5' position of uridine (GP1-3 compounds). In addition, a series of compounds (GP4-10) in which the diphosphate bridge was replaced with a linker containing a pyridyl ring linked directly with the oxidized uridine derivative in the C-5 'position

were synthesized. To increase the resistance of synthesized glycoconjugates to enzymatic hydrolysis, in both series of compounds, the anomeric oxygen atom in D-galactose or D-glucose was replaced by the sulfur. Moreover, such selected glycoconjugate structures allowed to determine whether the succinic acid fragment significantly influences the antiviral activity of the designed compounds.



R^1: acetyl, benzoyl, TBDMS or H, R^2: isopropylidene, TBDMS or H, R^3: H or benzoyl

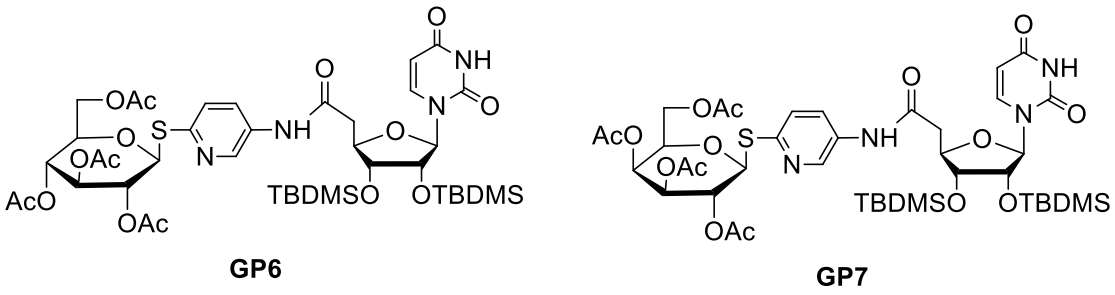
Scheme 4. Natural GT substrates and general structure of tested glycoconjugates used in the studies.

The main aim of the study was to analyze the antiviral activity of a series of synthesized glycoconjugates using human hepatitis C virus. Antiviral activity of compounds was also carried out using animal classical swine fever virus to determine whether the synthesized compounds are characterized by a broad spectrum of activity against viruses belonging to the *Flaviviridae* family. The analysis of the introduced modifications on the antiviral activity of compounds was also carried out. 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 examined. For my studies I used compounds where acetyl, benzoyl and TBDMS groups were used as protecting groups in the sugar ring. These groups were introduced to increasee the hydrophobicity of glycoconjugates and allowed them to penetrate into the cell. The protecting groups in uridine moiety (isopropylidene group and TBDMS) were selected to improve the hydrophobicity of the products and stability within the cell.

Synthetic description of results:

In the primary screening we evaluated the *in vitro* antiviral activities of all synthesized glycoconjugates using classical swine fever virus. CSFV causes an acute, highly infectious and economically damaging disease in swine and wild boars in many countries (Artois et al., 2002; Laddomada, 2000; Edwards et al., 2000). After determining the cytotoxicity of the compounds for swine kidney cells (SK6), I tested the antiviral activity of a series of compounds using the previously optimized pseudoplaque reduction assay. CSFV replication does not result in cytopathic effect, therefore it is not possible to observe directly the foci of viral growth, which normally, in case of cytopathogenic viruses, are visible as viral plaques (Laude, 1987). Among

all tested compounds, some compounds were found to inhibit CSFV replication. These compounds had IC_{50} values < 50 μM showing selectivity indexes (SI), defined as the CC_{50}/IC_{50} ratio, from 3,5 to 28,7. Two compounds (GP6 and GP7) (Scheme 5) that contained a fragment of acetylated (5-amino-2-pyridyl) 1-thioglycoside linked directly with the protected TBDMS groups with uracil-5'-carboxylic acid proved to be the most effective of all tested compounds.



Scheme 5. Chemical structures of glycoconjugates (GP6 and GP7).

All synthesized compounds were also evaluated for anti-HCV activity in HCV cell culture system (HCVcc) in human hepatoma Huh-7.5 cells, with Sofosbuvir, an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, as a positive control. The analysis showed that, like in case of CSFV, two compounds (GP6 and GP7) inhibited the HCV virus propagation in a dose-dependent manner in Huh-7.5 cells and showed IC_{50} values of 7 μM which led to selectivity index of 19,3 and 24,7, respectively.

The analysis of antiviral activity of all synthesized compounds revealed that the sugar moiety type (D-glucose or D-galactose) does not significantly affect the biological activity. In addition, the omission of the succinic linker and the direct connection of acetylated (5-amino-2-pyridyl) 1-thioglycosides and 2',3'-di-*O*-tert-butyldimethylsilyluridine-5'-carboxylic acid improved the antiviral activity of the compounds, as evidenced by results for GP6 and GP7 compounds. The structure analysis also demonstrated the important role of the protecting groups present in synthesized glycoconjugates. The protecting groups for two the most active compounds were: acetyl groups in the sugar part and TBDMS group in the uridine fragment. Changing of the protecting groups in both parts of the glycoconjugates significantly reduced the activity of the compounds against two tested viruses. In addition, compounds that were partially or fully deprotected did not exhibit *in vitro* antiviral activity.

The mechanism of action of the synthesized compounds was not known. The compounds were synthesized as potential glycosyltransferase inhibitors, therefore, the effect of selected compounds on the glycoprotein synthesis of HCV and CSFV was investigated. Both compounds reduced CSFV and HCV protein production in a dose-dependent manner. Using previously described Huh7-J20 stable cell line, I also conducted the experiments to determine the effect of compounds on the different stages of the viral life cycle. The designed experiments made it possible to hypothesize that both compounds can affect the replication of HCV in host cells, which was quite an unexpected result. This hypothesis was supported by the results obtained for Sofosbuvir, which is a licensed drug targeting viral replication. To confirm this hypothesis, the experiments using Huh7-J17 stable cell line, which constitutively harbors the subgenomic HCV RNA were conducted. The obtained results confirmed the previous observation that both compounds highly inhibited viral RNA replication in low doses. Half

maximal inhibitory concentration (IC_{50}) values for compound 13 and 14 were 4.005 μM and 3.741 μM, respectively.

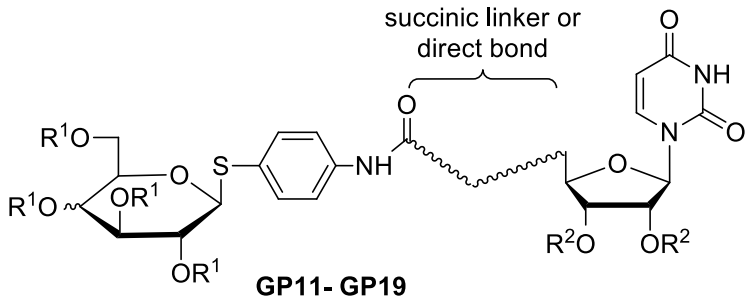
The importance of the results:

The presented results demonstrate for the first time that compounds belonging to the uridine glycoconjugates containing the pyridyl ring in the linker structure and the amide moiety show significant antiviral activity, which is probably related to the inhibition of the viral RNA replication process. Two compounds with significant antiviral activity against hepatitis C virus and classical swine fever virus have been selected. Our experiments determined which modifications have a significant effect on antiviral activity of compounds and should be taken into account in the synthesis of new, potentially more active derivatives. It was surprising but on the other hand extremely interesting that compounds have been synthesized as potential inhibitors of glycosyltransferases involved in the protein glycosylation process. Preliminary analysis using molecular modeling showed that these compounds are too large to bind in the active center of these enzymes. It is therefore probable that these compounds are non-competitive inhibitors. Research to confirm their exact mechanism of action is currently in progress.

P.7. Krol, E.*, Pastuch-Gawolek, G., Chaubey, B., Brzuska, G., Erfurt, K., Szewczyk, B. (2018). Novel uridine glycoconjugates, derivatives of 4-aminophenyl 1-thioglycosides, as potential antiviral compounds. Molecules, 23(6). pii: E1435.

Aim of the study:

Due to interesting results with compounds derived from (5-amino-2-pyridyl) 1-thioglycosides and selectively protected uridine, dr hab. Gabriela Pastuch-Gawołek synthesized a new group of compounds belonging to thioglycosyl analogs of glycosyltransferases substrates, in which the linker structure was modified by replacing the aromatic nitrogen atom in the pyridyl ring with a carbon atom (compounds GP11-19) (Scheme 6). A detailed description of the activity of new compounds against classical swine fever virus and hepatitis C virus as well as the mechanism of action of active compounds was adopted as a scientific goal.

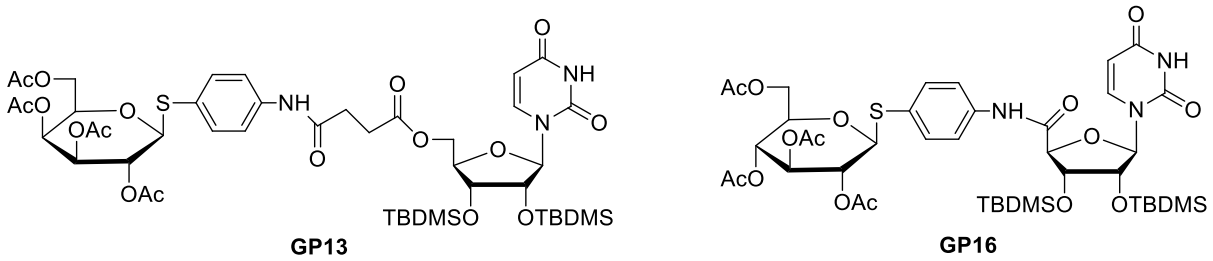


R^1: acetyl or H, R^2: isopropylidene, TBDMS or H

Scheme 6. Diagram showing the structures of synthesized compounds.

Synthetic description of results:

After determining the cytotoxicity of the compounds, I performed a preliminary analysis of antiviral activity of the compounds against classical swine fever virus using the viral pseudoplaque reduction test. Among tested compounds, GP13 and GP16 appeared to be the most active against CSFV (Scheme 7). These results were confirmed by using cell-culture-infectious HCV (HCVcc) using Sofosbuvir as a positive control. IC_{50} values for GP13 and GP16 were 4,9 and 13,5 μM, which led to SI of 52,4 and 20,0, respectively. The structural analysis of a whole series of compounds showed that substitution of the aromatic nitrogen atom in the linker by the carbon atom adversely affects the antiviral activity. Compounds containing carbon in the aromatic ring were less active and more toxic. In addition, the succinic linker appears to be in this case a moiety affecting the activity of the compounds. Probably, in the absence of an aromatic nitrogen atom in the linker structure, the fragment derived from succinic acid is responsible for the increase of antiviral activity. In the case of anti-HCV activity studies, GP13 compound containing the succinic linker was the most active of all previously tested glycoconjugates.



Scheme 7. Diagram showing the structures of active compounds.

Further to analyze the mechanism of antiviral activity of two selected compounds the experiments with Huh7-J20 stable cell line were performed. It has been proven that, as with the previous series of glycoconjugates, the antiviral mechanism of these compounds is probably also associated with inhibition of the replication process. The effects of compounds on the early stages of the HCV life cycle have not been observed. The proposed research hypothesis was confirmed using the Huh7-J17 stable line containing the incomplete genome of HCV. The analysis showed that the tested compounds, despite the fact that they were designed as potential inhibitors of glycosyltransferases, inhibit the activity of enzymes participating in the replication cycle of the virus.

The importance of the results:

The obtained results confirmed that uridine glycoconjugates containing the amidophenyl fragment in the linker structure possess significant antiviral activity against human HCV and animal CSFV. As a consequence of the research, two compounds (GP13 and GP16) with strong antiviral activity were selected. The modifications made in the previously selected compounds yielded a compound that possessed significantly higher selectivity index. Previously, the SI for the most active GP7 was 24,7. The introduced modifications resulted in obtaining a compound GP13 with twice higher SI of 52,4. The SI value of Sofosbuvir currently used as a component of anti-HCV therapy is around 120. The GP13 compound was also nearly

8-times less toxic than Sofosbuvir. Structural analysis can be used to design new, urgently needed inhibitors with high selectivity indexes.

Summary of the most important achievements

The most important achievements of the presented monothematic cycle include:

1. ***Development of innovative strategy to combat viral infections in humans using chemically synthesized glycosylation inhibitors.***

   As part of the study, I have shown a significant antiviral activity of glycosylation inhibitors belonging to uridine derivatives of 2-deoxy sugars against influenza virus, hepatitis C virus and tick-borne encephalitis virus. The analysis of the structure and activity of all synthesized compounds allowed for selection of protecting groups present that significantly affect antiviral activity. I selected compounds which are characterized by favourable selectivity indexes which makes possible to use them as components of monotherapies or combination therapies in future. Selected compounds have been tested using the above-mentioned viral models, but their wide spectrum of activity clearly indicates that they can be an effective therapeutic option against other important enveloped viruses. The studies that have been carried out as part of this work have been limited to *in vitro* experiments but may be the basis for further clinical trials.

2. ***Development of new antiviral strategy against hepatitis C virus using uridine glycoconjugates targeting RNA replication.***

   As part of the habilitation achievement, I have also selected compounds belonging to uridine glycoconjugates with a different mechanism of action than inhibiting the glycosylation process. The significant antiviral activity of uridine glycoconjugates against the hepatitis C virus is probably related to the inhibition of viral RNA replication. Currently, the work to confirm their exact antiviral mechanism is in progress. Selected compounds were characterized by significantly high values of the selectivity indexes, which indicates their high application potential. Knowledge obtained as part of structure and activity analysis can be used for designing new, potentially more active antiviral compounds.

3. ***Introduction of a new research topic and a new scientific methodology to the Department of Recombinant Vaccines at Intercollegiate Faculty of Biotechnology UG and MUG***

   The research carried out at the Department of Recombinant Vaccines concerned mainly on the development of effective recombinant vaccines against viral pathogens. I have introduced the research topic related to the study of antiviral activity of compounds to the Department. Thanks to scientific cooperation with numerous national and foreign centers, I have introduced many research methods to the Department of Recombinant Vaccines. I have created and optimized a scientific methodology that opens up perspectives for new research with viral pathogens.

Scientific plans:

In 2016, I received funds from National Science Center SONATA Program for the project entitled: "Tick-borne encephalitis virus – search for mechanisms useful in treatment and prophylaxis". During realization of this project, a few compounds with significant antiviral activity against tick-borne encephalitis virus have been selected [P.5]. Due to these promising results, in collaboration with dr. hab. Ilona Wandzik and dr. hab. Gabriela Pastuch-Gawołek from Silesian University of Technology in Gliwice, I plan to analyze the antiviral activity of other synthesized compounds e.g. compounds belonging to uridine glycoconjugates containing a 1,2,3-triazole ring in the linker structure. In addition, as part of this cooperation, I plan to submit a research project within OPUS NCN program for the synthesis and testing of the activity of new, designed and synthesized compounds against TBEV.

To continue the research related to the tick-borne encephalitis virus, I have also started the cooperation with prof. Theodore Hupp from Experimental Cancer Research in Edinburgh, currently the head of the International Centre for Cancer Vaccine Science, which was established at the University of Gdańsk as part of the International Research Agenda program. This cooperation aims to understand the role of transmembrane proteins induced by interferon 1, 2 and 3 (interferon-induced transmembrane protein) in the course of infection caused by the tick-borne encephalitis virus.

Continuing my interest in searching for new, innovative methods of prevention and combating dangerous viral diseases, including TBEV, at the end of 2019, I plan to submit to the National Science Center another project related to the TBEV virus under DAINA program for Polish-Lithuanian cooperation research projects. Due to the fact that another possible route of TBEV infection in humans is the consumption of unpasteurized milk or dairy products from infected goats, sheep and cows, the project will attempt to develop a potential vaccine for animals based on TBEV virus-like particles. I have already started the cooperation with Prof. Arunasa Stankevicius from Lithuanian University of Health Sciences, Kaunas, Lihue. The group of Dr. Daniel Ružek from Veterinary Research Institute, Brno, Czech Republic will be also involved.

From 2017 I am a principal investigator of another project funded by The National Center for Research and Development project LEADER untitled: "Anti-Zika vaccine - innovative methods for antigen construction". This project is focussed on the production of potential vaccine against Zika virus based on virus-like particles. Due to the methodology introduced to the laboratory as part of this grant, I would like to continue the research related to Zika virus. Recent reports indicate that in the case of infection with the Zika virus, previous infection with another flavivirus, in particular Dengue virus, may contribute to the strengthening the severity of infection, which is related to the presence of cross-reactive antibodies directed against the highly conserved flavivirus fusion loop of E protein. Therefore, it is necessary to produce virus-like particles with modified antigenic properties, which may possibly limit the production of cross-reactive antibodies. I started the cooperation with Dr. Simone Fonseca from the Laboratory of Immunoregulation, Institute of Tropical Pathology and Public Health, Federal University of Goias, Goiania-Goias-Brazil and Dr. Daniel Ružek, from Veterinary Research Institute, Brno, Czech Republic. The project under National Science Center SONATA-BIS Program entitled "Understanding the mechanism of antibody-dependent enhancement of infection (ADE) in related, highly pathogenic flaviviruses to develop new methods for diagnosis and prevention" is being prepared and will be submitted in the middle of 2019.

5. Presentation of other scientific and research achievements.

(The detailed list of all my research publications (IF, Ministerial Publication Points and number of citations per article), is present in the Attachment no. 4 together with the list of all my other achievements)

Research and scientific achievements before a doctoral degree.

I started my scientific career when, after graduating with a university class with a biological-chemical profile in First High School in Gdynia, I obtained an index to study at the Intercollegiate Faculty of Biotechnology of University of Gdańsk and MUG without exams. On my second year I started an individual laboratory practice under the supervision of Prof. Bogusław Szewczyk at the Department of Recombinant Vaccines. I concentrated my efforts on the development of methods for the efficient production of hepatitis C glycoproteins, which was a part of EU project under 5 th Framework Program ”Structural and functional studies of hepatitis C virus glycoproteins: identification of targets for antiviral therapy; European Network for Hepatitis C Virus Envelope Glycoprotein Research (ENHCV)”. The topic related to structural proteins of the hepatitis C virus was also the basis of my master's thesis which I continued under the supervision of Prof. Szewczyk. My M.Sc. thesis has been highly evaluated and awarded the prize during 12th International Students’ Scientific Conference for Students and Young Doctors.

After graduating in 2004 I started a new research project in the framework of Doctoral Studies at the Faculty of Chemistry of the University of Gdańsk under the direct supervision of Prof. Bogusław Szewczyk. This Ph.D. project was a part of a bigger project KBN 2 P04B 012 26 (2004-2007) funded by Polish Ministry of Science and Higher Education realized in cooperation with Prof. Grzegorz Grynkiewicz from Pharmaceutical Institute in Warsaw and Prof. Wiesław Szeja from Silesian University of Technology. The aim of my Ph.D. project was to study the effect of new synthesized in Poland tunicamycin analogues and mimetics on classical swine fever virus, which was used for many years as a surrogate model for hepatitis C virus due to the lack of infectious HCV virus for *in vitro* studies. In December 2011 I was awarded a Ph.D. degree with honors. During the study I have evaluated for the first time the antiviral properties and the mechanism of action of tunicamycin analogues and mimetics against classical swine fever virus and hepatitis C virus. It should be emphasized that experiments using the HCVcc infection system were pioneering studies in Poland. Designed, synthesized and tested compounds during my Ph.D. studies were the basis for two patents field in Polish Patent Office (PL211936-B1 oraz PL212067-B1). I am a co-author of both of them. The results of this work have been published in international journals (Krol et al., 2010; Reszka et al., 2010) and presented on a large number of national and international conferences. A few times, I was granted awards for the best abstracts allowing for the free participation in conferences. Moreover, in 2012 I was granted an award for young researchers in the field of biological and medical sciences by Gdańsk Scientific Society and the Mayor of Gdańsk for my Ph.D. thesis. After joining the Gdańsk Scientific Society in 2012, I also became a member of the Young Researchers' Club, in which I am now the Vice-chairman. The purpose of the Club's activity is integration and cooperation of young scientists from all universities in Gdańsk. With the support of the GSS Authorities, the "Gdańsk Young Science" project has been developed and the online platform is currently under preparation.

Research and scientific achievements obtained after a doctoral degree.

Apart from my Ph.D. project I was also involved in other research project realized at the Department of Recombinant Vaccines, aimed construction and implementation of vector vaccines against different viral pathogens. I have participated in the NCBR project „Innovative Technologies", „Centre of biotechnological drugs. Package of innovative biopharmaceuticals for human and animals therapy and prophylaxis" financed by POIG (2008-2015) which was focused on the development of a vaccine against avian influenza. This project was carried out by a consortium which included, Department of Recombinant Vaccines, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Institute of Biotechnology and Antibiotics, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences and Department of Poultry Diseases, National Veterinary Research Institute Pulawy. The project was successfully completed with a DNA vaccine with high efficacy *in vivo*, which in the near future may have a commercial application. The results of the project have been published in international journals (Stachyra et al., 2014, Szewczyk et al., 2014).

To continue the extremely interesting studies which were started as a part of the doctoral thesis, but at the same time to combine the experience gained as part of the POIG project related to the influenza virus in 2010, I submitted the project to the Ministry of Science and Higher Education under the Iuventus Plus program. In 2011 I received my own funds for antiviral activity studies of compounds against influenza virus. After my Ph.D. defense I was the winner of the second edition of "Iuventus Plus" Program and received funds for other antiviral compounds against influenza virus in the next two years. The results of work published under these two grants are the basis of my scientific achievement described above, but the methodology introduced to the Department of Recombinant Vaccines, among others the method of influenza virus propagation in cell culture allowed me to participate in other research projects. In searching for new antiviral compounds against influenza virus I also cooperated with Prof. Grzegorz Grynkiewicz from the Pharmaceutical Institute in Warsaw. The goal was to search for new, more effective analogues of existing drugs, mainly neuraminidase inhibitor (oseltamivir), currently used in the treatment of influenza virus infection (Adamska et al., 2012). In addition, I have propagated influenza viruses used to analyze the effectiveness of a universal immunosensor for detection of influenza virus in human throat swabs, which was carried out as part of the VENTURES project funded by the Foundation for Polish Science headed by Dr. Dawid Nidzworski. The results were presented by Nidzworski et al., 2014, in the prestigious journal Biosensors and Bioelectronics.
The published papers constituting my scientific achievement as well as those presented above obtained the team award in 2015 from the Rector of the University of Gdańsk.

In 2012 I obtained financing for another project funded by the Polish National Science Centre PRELUDIUM 2 program. The purpose of this project was to study the antiviral activity of synthesized compounds against hepatitis C virus. To elucidate the molecular mechanism of the antiviral effect of tested compounds all available *in vitro* systems for HCV propagation were used. The data obtained during performance of this project can be used for designing new therapies that could be beneficial for patients infected with HCV. During the duration of the project I established a scientific cooperation with Prof. Arvind Patel from the University of Glasgow Center for Virus Research from Scotland. The results obtained during this project have been published and constitute my scientific achievement; two other papers have also been published (Krol et al., 2014, Chmielewska et al., 2015).

The papers from my scientific achievement as well as those presented related to influenza virus and hepatitis C virus obtained Rector's individual awards in 2013 and 2018 and additionally, I received one year scholarship for best young researchers at the University of Gdansk.

Due to the fact that my research projects are highly applicable with large commercial potential, in 2012, I became a laureate of the prestigious program of Ministry of Science and Higher Education "TOP500 Innovators: Science-Management-Commercialization", and I completed a two-month internship at Stanford University in Silicon Valley in the USA, where I gained the knowledge about commercialization, cooperation between science and business, management of research. I also had the opportunity to learn about the research carried out at this prestigious, one of the best in the world university during my internships in the labs. Since 2013, I am also a member of the TOP500 Innovators Association, which was established to create an interdisciplinary platform of cooperation between representatives of scientific communities and representatives of technology transfer centers in Poland, using experience gained at the best universities in the world, including at Stanford University or the University of California, Berkeley.

To continue the antiviral activity studies, I established new scientific cooperation with dr. Daniel Růžek from the Veterinary Research Institute in the Czech Republic. In 2016 I became a leader of SONATA project funded by Polish National Science Centre related to the search for new methods of treatment and prevention of infection with tick-borne encephalitis virus. In 2016, I also completed my research stay in the laboratory of Dr. Růžek, where I have learned many new techniques related to tick-borne encephalitis virus, a pathogen of the third class of biological safety. One paper belonging to my scientific achievement have been already published, two others are in preparation.

In 2012-2016 I was also actively involved in two research grants funded by The National Centre for Research, Applied Research Program which aimed at construction of a vector vaccine against Newcastle disease in chickens (PBS1/B8/2/2012) as well as innovative production of antigens as safe vaccines for humans against main groups of influenza viruses (PBS2/A7/14/2014). Participation in these two grants allowed me to gain very valuable experience in working on the development of vaccines. In 2016 I became the PI of another project LIDER funded by The National Centre for Research and Development. The aim of the LIDER project is to develop a vaccine against the Zika virus based on virus-like particles. The development of potential vaccines based on virus-like particles against many viral pathogens transmitted by mosquitoes, among others Zika virus is a very important issue of public health. This is demonstrated by my work published in the prestigious journal Trends in Biotechnology (IF 13,578) (Krol et al., 2019). Due to very satisfactory *in vivo* results with a mouse model for the potential vaccine developed by us, the proposed vaccine solution will be submitted for protection to the Patent Office in Poland, and then a patent application under the PCT procedure will be prepared. In addition, two papers describing these results are in preparation and will be sent for review in the near future.

Scientific achievement:

- **The total number of publications: 19** (before doctoral degree: 4, after doctoral degree: 15; the first author of 9 papers; corresponding author of 9 papers)
- **Scientific achievement: 7 papers** (the first author of 6 papers; corresponding author of 7 papers) IF = **22,384**, $IF_{5\text{-year}}$ = **23,935**; MNiSW = **210**
- **The total number of publications excluding scientific achievement: 12** (the first author of 3 papers; corresponding author of 2 papers) IF = **35,291**; $IF_{5\text{-year}}$ = **38,617**; MNiSW = **255**
  - before doctoral degree: 4 papers, IF = 5,673; $IF_{5\text{-year}}$ = 5,726; MNiSW = 50
  - after doctoral degree: 8 papers, IF = 29,618; $IF_{5\text{-year}}$ = 32,891; MNiSW = 205

- **The overall impact factor journals**, by year of publication: **57,675**; $IF_{5\text{-year}}$ = **62,552** (papers before doctoral degree: IF = 5,673; papers after doctoral degree: IF = 52,002).
- **The total number of MNiSW points**: **465** (works before doctoral degree: MNiSW = 50; works after doctoral degree: MNiSW = 415).
- **Number of citations (Web of Science): 130, (Google Scholar): 249**
- **Hirsch Index: Web of Science: 6; Google Scholar: 7**
- The total number of conference reports: **30** (17 before doctoral degree; 13 after doctoral degree); 21 from international conferences and 9 from national conferences.
- **The number of granted patents: 2**

In parallel with the scientific activity I also conduct educational activities complementary to the profile of my research. I teach in the form of lectures and laboratory exercises for students at IFB UG and MUG (detailed list in **Appendix 5**). I am now a promoter of 5 master's theses, a promoter of 2 students' bachelor projects and co-promoter in 2 doctoral thesis (**Appendix 5**). I am actively involved in the activities for promotion of the Faculty and supporting its development. I am the Chairwoman of the Team responsible for organization of promotional and educational events at the Intercollegiate Faculty of Biotechnology UG and MUG. I am also a member of the Senate of the University of Gdańsk and the Faculty Council of the Intercollegiate Faculty of Biotechnology of UG and MUG.

Literatures:

1. Angus, A.G.N., Loquet, A., Stack, S.J., Dalrymple, D., Gatherer, D., Penin, F., and Patel, A.H. (2012). Conserved Glycine 33 Residue in Flexible Domain I of Hepatitis C Virus Core Protein Is Critical for Virus Infectivity. J Virol *86*, 679–690.
2. Artois, M., Depner, K.R., Guberti, V., Hars, J., Rossi, S., and Rutili, D. (2002). Classical swine fever (hog cholera) in wild boar in Europe. Rev. - Off. Int. Epizoot. *21*, 287–303.
3. Barth, H. (2015). Hepatitis C virus: Is it time to say goodbye yet? Perspectives and challenges for the next decade. World J Hepatol *7*, 725–737.
4. Bartosch, B., Dubuisson, J., and Cosset, F.-L. (2003). Infectious Hepatitis C Virus Pseudo-particles Containing Functional E1–E2 Envelope Protein Complexes. J Exp Med *197*, 633–642.
5. Bourke, C.A., and Carrigan, M.J. (1993). Experimental tunicamycin toxicity in cattle, sheep and pigs.

Aust. Vet. J. *70*, 188–189.
6. Breton, C., Snajdrová, L., Jeanneau, C., Koca, J., and Imberty, A. (2006). Structures and mechanisms of glycosyltransferases. Glycobiology *16*, 29R-37R.
7. Breton, C., Fournel-Gigleux, S., and Palcic, M.M. (2012). Recent structures, evolution and mechanisms of glycosyltransferases. Curr. Opin. Struct. Biol. *22*, 540–549.
8. Brodsky, J.L., and McCracken, A.A. (1999). ER protein quality control and proteasome-mediated protein degradation. Semin. Cell Dev. Biol. *10*, 507–513.
9. Chapel, C., Garcia, C., Roingeard, P., Zitzmann, N., Dubuisson, J., Dwek, R.A., Trépo, C., Zoulim, F., and Durantel, D. (2006). Antiviral effect of alpha-glucosidase inhibitors on viral morphogenesis and binding properties of hepatitis C virus-like particles. J. Gen. Virol. *87*, 861–871.
10. Chapel, C., Garcia, C., Bartosch, B., Roingeard, P., Zitzmann, N., Cosset, F.-L., Dubuisson, J., Dwek, R.A., Trépo, C., Zoulim, F., et al. (2007). Reduction of the infectivity of hepatitis C virus pseudoparticles by incorporation of misfolded glycoproteins induced by glucosidase inhibitors. Journal of General Virology *88*, 1133–1143.
11. Compain, P., and Martin, O.R. (2001). Carbohydrate mimetics-based glycosyltransferase inhibitors. Bioorg. Med. Chem. *9*, 3077–3092.
12. Donoso Mantke, O., Escadafal, C., Niedrig, M., Pfeffer, M., and Working Group For Tick-Borne Encephalitis Virus, C. (2011). Tick-borne encephalitis in Europe, 2007 to 2009. Euro Surveill. *16*.
13. Duksin, D., and Mahoney, W.C. (1982). Relationship of the structure and biological activity of the natural homologues of tunicamycin. J. Biol. Chem. *257*, 3105–3109.
14. Dumpis, U., Crook, D., and Oksi, J. (1999). Tick-borne encephalitis. Clin. Infect. Dis. *28*, 882–890.
15. Edwards, S., Fukusho, A., Lefèvre, P.C., Lipowski, A., Pejsak, Z., Roehe, P., and Westergaard, J. (2000). Classical swine fever: the global situation. Vet. Microbiol. *73*, 103–119.
16. Elbein, A.D. (1987). Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. Annu. Rev. Biochem. *56*, 497–534.
17. Elbein, A.D., Legler, G., Tlusty, A., McDowell, W., and Schwarz, R. (1984). The effect of deoxymannojirimycin on the processing of the influenza viral glycoproteins. Arch. Biochem. Biophys. *235*, 579–588.
18. Fiore, A.E., Shay, D.K., Broder, K., Iskander, J.K., Uyeki, T.M., Mootrey, G., Bresee, J.S., Cox, N.S., Centers for Disease Control and Prevention (CDC), and Advisory Committee on Immunization Practices (ACIP) (2008). Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. MMWR Recomm Rep *57*, 1–60.
19. Fried, M.W., Shiffman, M.L., Reddy, K.R., Smith, C., Marinos, G., Gonçales, F.L., Häussinger, D., Diago, M., Carosi, G., Dhumeaux, D., et al. (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N. Engl. J. Med. *347*, 975–982.
20. Gastaminza, P., Cheng, G., Wieland, S., Zhong, J., Liao, W., and Chisari, F.V. (2008). Cellular Determinants of Hepatitis C Virus Assembly, Maturation, Degradation, and Secretion. J Virol *82*, 2120–2129.
21. Gloster, T.M. (2012). Development of inhibitors as research tools for carbohydrate-processing enzymes. Biochem. Soc. Trans. *40*, 913–928.
22. Gubareva, L.V. (2004). Molecular mechanisms of influenza virus resistance to neuraminidase inhibitors. Virus Res. *103*, 199–203.
23. Hayden, F.G. (1997). Antivirals for pandemic influenza. J. Infect. Dis. *176 Suppl 1*, S56-61.
24. Helle, F., Vieyres, G., Elkrief, L., Popescu, C.-I., Wychowski, C., Descamps, V., Castelain, S., Roingeard, P., Duverlie, G., and Dubuisson, J. (2010). Role of N-Linked Glycans in the Functions of Hepatitis C Virus Envelope Proteins Incorporated into Infectious Virions. J Virol *84*, 11905–11915.
25. Hsu, M., Zhang, J., Flint, M., Logvinoff, C., Cheng-Mayer, C., Rice, C.M., and McKeating, J.A. (2003). Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. Proc Natl Acad Sci U S A *100*, 7271–7276.
26. Huang, H., Sun, F., Owen, D.M., Li, W., Chen, Y., Gale, M., and Ye, J. (2007). Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. Proc Natl Acad Sci U S A *104*, 5848–5853.
27. Hurt, A.C., Deng, Y.M., Ernest, J., Caldwell, N., Leang, L., Iannello, P., Komadina, N., Shaw, R., Smith, D., Dwyer, D.E., et al. (2011). Oseltamivir-resistant influenza viruses circulating during the first

year of the influenza A(H1N1) 2009 pandemic in the Asia-Pacific region, March 2009 to March 2010. Euro Surveill. *16*.
28. Inkster, M.D., Hinshaw, V.S., and Schulze, I.T. (1993). The hemagglutinins of duck and human H1 influenza viruses differ in sequence conservation and in glycosylation. J. Virol. *67*, 7436–7443.
29. Iro, M., Witteveldt, J., Angus, A.G.N., Woerz, I., Kaul, A., Bartenschlager, R., and Patel, A.H. (2009). A reporter cell line for rapid and sensitive evaluation of hepatitis C virus infectivity and replication. Antiviral Research *83*, 148–155.
30. Kajimoto, T., and Node, M. (2009). Synthesis of Glycosyltransferase Inhibitors. Synthesis *2009*, 3179–3210.
31. Kikuchi, N., and Narimatsu, H. (2006). Bioinformatics for comprehensive finding and analysis of glycosyltransferases. Biochim. Biophys. Acta *1760*, 578–583.
32. Kohsaka, S., Mita, K., Matsuyama, M., Mizuno, M., and Tsukada, Y. (1985). Impaired development of rat cerebellum induced by neonatal injection of the glycoprotein synthesis inhibitor, tunicamycin. J. Neurochem. *44*, 406–410.
33. Krol, E., Wandzik, I., Szeja, W., Grynkiewicz, G., and Szewczyk, B. (2010). In vitro antiviral activity of some uridine derivatives of 2-deoxy sugars against classical swine fever virus. Antiviral Research *86*, 154–162.
34. Laddomada, A. (2000). Incidence and control of CSF in wild boar in Europe. Vet. Microbiol. *73*, 121–130.
35. Lam, A.M., Espiritu, C., Bansal, S., Micolochick Steuer, H.M., Niu, C., Zennou, V., Keilman, M., Zhu, Y., Lan, S., Otto, M.J., et al. (2012). Genotype and Subtype Profiling of PSI-7977 as a Nucleotide Inhibitor of Hepatitis C Virus. Antimicrob Agents Chemother *56*, 3359–3368.
36. Laude, H. (1987). Hog cholera virus: art and facts. Ann. Rech. Vet. *18*, 127–138.
Lavanchy, D. (2011). Evolving epidemiology of hepatitis C virus. Clin. Microbiol. Infect. *17*, 107–115.
37. Lavie, M., Goffard, A., and Dubuisson, J. (2006). HCV Glycoproteins: Assembly of a Functional E1–E2 Heterodimer. In Hepatitis C Viruses: Genomes and Molecular Biology, S.-L. Tan, ed. (Norfolk (UK): Horizon Bioscience), p.
38. Lehle, L., and Tanner, W. (1976). The specific site of tunicamycin inhibition in the formation of dolichol-bound N-acetylglucosamine derivatives. FEBS Lett. *72*, 167–170.
39. Li, K.S., Guan, Y., Wang, J., Smith, G.J.D., Xu, K.M., Duan, L., Rahardjo, A.P., Puthavathana, P., Buranathai, C., Nguyen, T.D., et al. (2004). Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature *430*, 209–213.
40. Lindenbach, B.D., and Rice, C.M. (2013). The ins and outs of hepatitis C virus entry and assembly. Nat Rev Microbiol *11*, 688–700.
41. Lindenbach, B.D., Evans, M.J., Syder, A.J., Wölk, B., Tellinghuisen, T.L., Liu, C.C., Maruyama, T., Hynes, R.O., Burton, D.R., McKeating, J.A., et al. (2005). Complete Replication of Hepatitis C Virus in Cell Culture. Science *309*, 623–626.
42. Matrosovich, M., Zhou, N., Kawaoka, Y., and Webster, R. (1999). The surface glycoproteins of H5 influenza viruses isolated from humans, chickens, and wild aquatic birds have distinguishable properties. J. Virol. *73*, 1146–1155.
43. Memoli, M.J., Davis, A.S., Proudfoot, K., Chertow, D.S., Hrabal, R.J., Bristol, T., and Taubenberger, J.K. (2011). Multidrug-resistant 2009 pandemic influenza A(H1N1) viruses maintain fitness and transmissibility in ferrets. J. Infect. Dis. *203*, 348–357.
44. von Messling, V., and Cattaneo, R. (2003). N-linked glycans with similar location in the fusion protein head modulate paramyxovirus fusion. J. Virol. *77*, 10202–10212.
45. Meunier, J.C., Fournillier, A., Choukhi, A., Cahour, A., Cocquerel, L., Dubuisson, J., and Wychowski, C. (1999). Analysis of the glycosylation sites of hepatitis C virus (HCV) glycoprotein E1 and the influence of E1 glycans on the formation of the HCV glycoprotein complex. Journal of General Virology *80*, 887–896.
46. Meyer, W.J., Wood, J.M., Major, D., Robertson, J.S., Webster, R.G., and Katz, J.M. (1993). Influence of host cell-mediated variation on the international surveillance of influenza A (H3N2) viruses. Virology *196*, 130–137.
47. Monto, A.S., Fleming, D.M., Henry, D., de Groot, R., Makela, M., Klein, T., Elliott, M., Keene, O.N., and Man, C.Y. (1999). Efficacy and safety of the neuraminidase inhibitor zanamivirin the

treatment of influenza A and B virus infections. J. Infect. Dis. *180*, 254–261.
Nakamura, K., and Compans, R.W. (1978). Effects of glucosamine, 2-deoxyglucose, and tunicamycin on glycosylation, sulfation, and assembly of influenza viral proteins. Virology *84*, 303–319.
48. Nichol, K.L., and Treanor, J.J. (2006). Vaccines for seasonal and pandemic influenza. J. Infect. Dis. *194 Suppl 2*, S111-118.
49. Nicholson, K.G., Aoki, F.Y., Osterhaus, A.D., Trottier, S., Carewicz, O., Mercier, C.H., Rode, A., Kinnersley, N., and Ward, P. (2000). Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. Lancet *355*, 1845–1850.
50. Pan, Y.T., Hori, H., and Elbein, A.D. (1987). The effect of glycoprotein-processing inhibitors on the secretion of glycoproteins by Madin-Darby canine kidney cells. Biochem. Cell Biol. *65*, 345–353.
51. Parodi, A.J. (2000). Protein glucosylation and its role in protein folding. Annu. Rev. Biochem. *69*, 69–93.
52. Pawlotsky, J.-M. (2013). Chapter Five - Hepatitis C Virus: Standard-of-Care Treatment. In Advances in Pharmacology, E. De Clercq, ed. (Academic Press), pp. 169–215.
53. Pizer, L.I., Cohen, G.H., and Eisenberg, R.J. (1980). Effect of tunicamycin on herpes simplex virus glycoproteins and infectious virus production. J. Virol. *34*, 142–153.
54. Rey, F.A., Heinz, F.X., Mandl, C., Kunz, C., and Harrison, S.C. (1995). The envelope glycoprotein from tick-borne encephalitis virus at 2 A resolution. Nature *375*, 291–298.
55. Roberts, N.A. (2001). Treatment of influenza with neuraminidase inhibitors: virological implications. Philos. Trans. R. Soc. Lond., B, Biol. Sci. *356*, 1895–1897.
56. Rosenthal, E.S., and Graham, C.S. (2016). Price and affordability of direct-acting antiviral regimens for hepatitis C virus in the United States. Infect Agent Cancer *11*.
57. Russell, C.A., Jones, T.C., Barr, I.G., Cox, N.J., Garten, R.J., Gregory, V., Gust, I.D., Hampson, A.W., Hay, A.J., Hurt, A.C., et al. (2008). Influenza vaccine strain selection and recent studies on the global migration of seasonal influenza viruses. Vaccine *26 Suppl 4*, D31-34.
58. Ruzek, D., Županc, T.A., Borde, J., Chrdle, A., Eyer, L., Karganova, G., Kholodilov, I., Knap, N., Kozlovskaya, L., Matveev, A., et al. (2019). Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. Antiviral Res.
59. Saito, T., and Yamaguchi, I. (2000). Effect of glycosylation and glucose trimming inhibitors on the influenza A virus glycoproteins. J. Vet. Med. Sci. *62*, 575–581.
60. Sandrin, V., Boulanger, P., Penin, F., Granier, C., Cosset, F.-L., and Bartosch, B. (2005). Assembly of functional hepatitis C virus glycoproteins on infectious pseudoparticles occurs intracellularly and requires concomitant incorporation of E1 and E2 glycoproteins. Journal of General Virology *86*, 3189–3199.
61. Schröder, M. (2008). Endoplasmic reticulum stress responses. Cell. Mol. Life Sci. *65*, 862–894.
62. Schulze, I.T. (1997). Effects of glycosylation on the properties and functions of influenza virus hemagglutinin. J. Infect. Dis. *176 Suppl 1*, S24-28.
63. Schwarz, R.T., Rohrschneider, J.M., and Schmidt, M.F. (1976). Suppression of glycoprotein formation of Semliki Forest, influenza, and avian sarcoma virus by tunicamycin. J. Virol. *19*, 782–791.
64. Shi, X., and Elliott, R.M. (2004). Analysis of N-linked glycosylation of hantaan virus glycoproteins and the role of oligosaccharide side chains in protein folding and intracellular trafficking. J. Virol. *78*, 5414–5422.
65. Silber, L.A., and Soloviev, V.D. (1946). Far Eastern tick-borne spring-summer (spring) encephalitis. Am Rev Sov Med 1–80.
66. Sofia, M.J., Bao, D., Chang, W., Du, J., Nagarathnam, D., Rachakonda, S., Reddy, P.G., Ross, B.S., Wang, P., Zhang, H.-R., et al. (2010). Discovery of a β-d-2′-Deoxy-2′-α-fluoro-2′-β-C-methyluridine Nucleotide Prodrug (PSI-7977) for the Treatment of Hepatitis C Virus. J. Med. Chem. *53*, 7202–7218.
67. Steffen, R. (2016). Epidemiology of tick-borne encephalitis (TBE) in international travellers to Western/Central Europe and conclusions on vaccination recommendations. J Travel Med *23*.
68. Tkacz, J.S., and Lampen, O. (1975). Tunicamycin inhibition of polyisoprenyl N-acetylglucosaminyl pyrophosphate formation in calf-liver microsomes. Biochem. Biophys. Res. Commun. *65*, 248–257.
69. Tree, J.A., Richardson, C., Fooks, A.R., Clegg, J.C., and Looby, D. (2001). Comparison of large-scale mammalian cell culture systems with egg culture for the production of influenza virus A vaccine

strains. Vaccine *19*, 3444–3450.
70. Trombetta, E.S., and Parodi, A.J. (2003). Quality control and protein folding in the secretory pathway. Annu. Rev. Cell Dev. Biol. *19*, 649–676.
71. Unligil, U.M., and Rini, J.M. (2000). Glycosyltransferase structure and mechanism. Curr. Opin. Struct. Biol. *10*, 510–517.
72. Vieyres, G., Thomas, X., Descamps, V., Duverlie, G., Patel, A.H., and Dubuisson, J. (2010). Characterization of the Envelope Glycoproteins Associated with Infectious Hepatitis C Virus. J Virol *84*, 10159–10168.
73. Wagner, R., Wolff, T., Herwig, A., Pleschka, S., and Klenk, H.D. (2000). Interdependence of hemagglutinin glycosylation and neuraminidase as regulators of influenza virus growth: a study by reverse genetics. J. Virol. *74*, 6316–6323.
74. Wagner, R., Heuer, D., Wolff, T., Herwig, A., and Klenk, H.-D. (2002). N-Glycans attached to the stem domain of haemagglutinin efficiently regulate influenza A virus replication. J. Gen. Virol. *83*, 601–609.
75. Wakita, T., Pietschmann, T., Kato, T., Date, T., Miyamoto, M., Zhao, Z., Murthy, K., Habermann, A., Kräusslich, H.-G., Mizokami, M., et al. (2005). Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med *11*, 791–796.
76. Wang, X., Zhang, L.-H., and Ye, X.-S. (2003). Recent development in the design of sialyltransferase inhibitors. Med Res Rev *23*, 32–47.
77. Ward, C.W., Murray, J.M., Roxburgh, C.M., and Jackson, D.C. (1983). Chemical and antigenic characterization of the carbohydrate side chains of an Asian (N2) influenza virus neuraminidases. Virology *126*, 370–375.
78. Yoshii, K., Yanagihara, N., Ishizuka, M., Sakai, M., and Kariwa, H. (2013). N-linked glycan in tick-borne encephalitis virus envelope protein affects viral secretion in mammalian cells, but not in tick cells. J. Gen. Virol. *94*, 2249–2258.
79. Zou, W. (2005). C-glycosides and aza-C-glycosides as potential glycosidase and glycosyltransferase inhibitors. Curr Top Med Chem *5*, 1363–1391.

Król Ewelina