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Antiviral and Apoptosis Modulating Potential of Fluorinated Compounds

Krystyna Naumenko[1*], Golovan Anna[1], Baranova Galina[1], Shermolovych Yuriy[2], Zagorodnya Svitlana[1]

[1]Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Acad. Zabolotny str., 154, Kyiv, 03143, Ukraine
[2]Institute of organic chemistry, National Academy of Sciences of Ukraine, Murmanska 5 Str., Kyiv, Ukraine, 02660

***** Corresponding author: krystyn.naumenko@gmail.com

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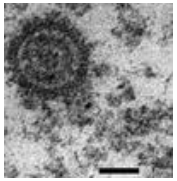
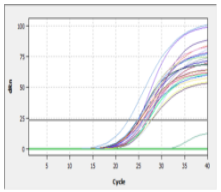


Graphical Abstract



Control cells	The cells+SBIO6 (125 µg/ml)	The cells+SBIO6 (250 µg/ml)
18,92%	57,24%	89,74%

Bioinformatic methods (PASS, IdTarget)

- Selected potential antiviral compounds and analyzed the possible target of action

Molecular-biological methods (PCR), virological

- Experimentally confirmed the antiviral activity

Microscopic methods (fluorescent)

- Characterized morphological features of the interaction of virus-cell and drugs

Flow cytometry

- Established of potential of apoptosis stimulating by compound

The basic idea for practical medicine is creating new drugs with antiviral activity, in particular against the Epstein-Barr virus.
Use of several methods enable us to define three classes of substances:
-substance with antiviral action;
-substance with antitumor activity in a system EBV-associated B-cell lymphoma;
-inducers of apoptosis.

Abstract:

In our laboratory, we focus on the design, synthesis, and evaluation of fluorinated compounds that could be used in the tretament of diseases caused by the Epstein-Barr virus (EBV) and virus vesicular stomatitis (VVS).

For this purpose, in a first step, we used the PASS software by which we could identify potential antiviral candidates among which a flurinated derivative of uracil (G27), a trifluoromethyl-substituted derivative of a thiosugar (SBIO6), a bisphosphonic acids (10s19) and several derivatives of alanin (10s20 - 10s28).

Our *in vitro* studies revealed an antiviral activity for a few compounds. Three compounds appeared to be effective against EBV: G27 (EC_{50} = 100 μg/ml), 10s20 (EC_{50} = μg/ml), and 10s25 (EC_{50} <62 μg/ml, replication of EBV was suppressed at 100 % at a concentration of 62 μg/ml). The ability to inhibit reproduction of VVS was showed for compound 10s19 (EC_{50} = 19 μg/ml) and for compound 10s24 (EC_{50} = 29 μg/ml). It was established that the index of selectivity for these compounds ranged from 10 to 100.

As Epstein-Barr virus (EBV) is the cause of several lymphoproliferative diseases, we studied the potency of G27 and SBIO6 compounds to make an apoptosis induction. Addition of G27 led to the observation of two peaks in the histogram. It was also established that by addition of SBIO6 the percentage of apoptotic cells was significantly increased when compared to the control and reached 70 - 90 percent.

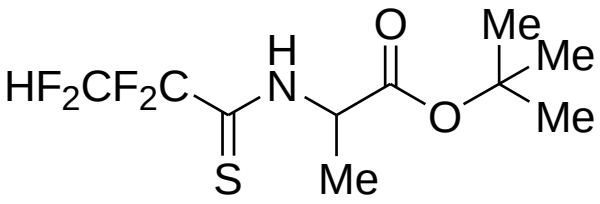
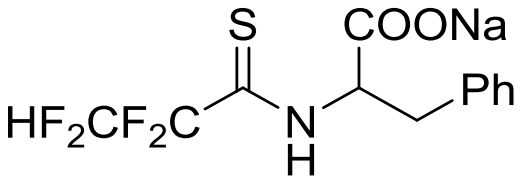
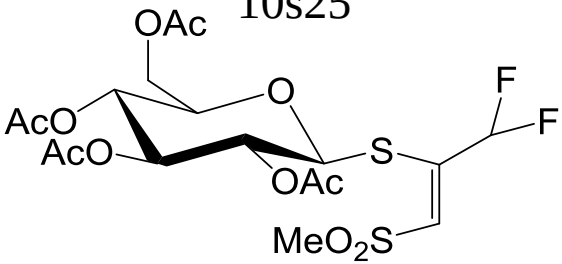
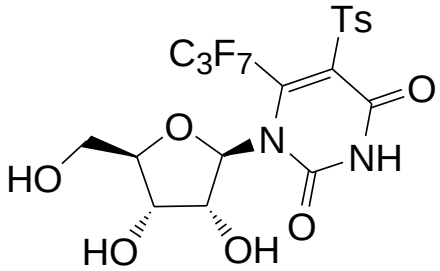
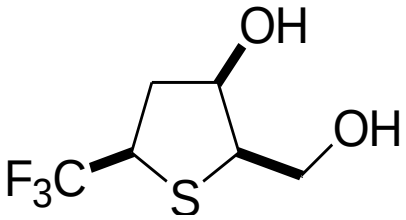
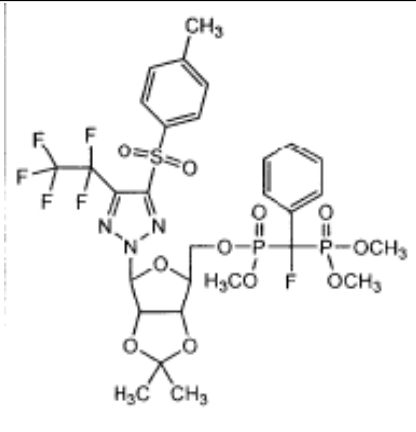
Introduction

Viral diseases occupy first place in clinical medicine. Therefore, the pharmaceutical companies focused on creating drugs against the viruses. Drugs created for today are not always effective due to a number of features of the viruses. Therefore, the search for new active molecules against viruses is going on.

Recently, much attention is paid to the nucleoside analogues, such as acyclovir and ganciclovir. But also it is important to create a non-nucleoside drugs based on natural fluorinated derivatives of bisphosphonic acids.

The models of Epstein-Barr virus (EBV) and virus of vesicular stomatitis (VVS) were used. EBV is a DNA-containing virus, which is characterized with an asymptomatic form of infection as well as a latent form, which may lead to cell transformation and to cancer (lymphoma, carcinoma). VVS – is a RNA-containing virus that causes stomatitis.

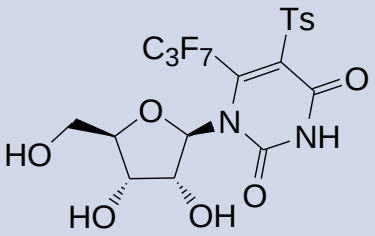
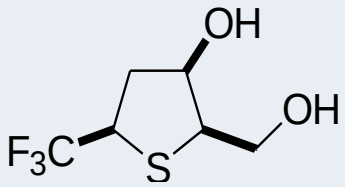
Chemical compounds

Tet-butyl ether N-(2,2,3,3-tetrafluoropropanethioyl) alanin	Sodium (2,2,3,3-tetrafluoropropanethioyl)-*L*-phenylalaninate	1-S-thio-(1-methylsulfonyl-2-difluoromethyl-vinyl)-2,3,4,6,-tetra-O-acetyl-*β*-D-glucopyranose
10s20 	10s24 	10s25 
fluorinated derivative of uracil	trifluoromethylsubstituted derivative of a thiosugar	derivative of bisphosphonic acids
G27 	SBIO6 	10s19 

Results and discussion

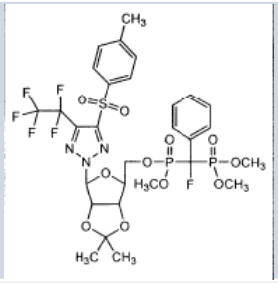
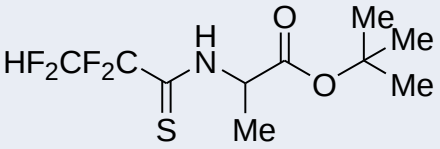
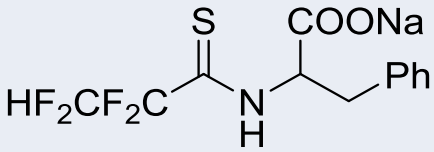
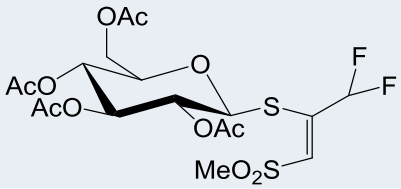
Formulation of potential drugs is a long and costly process. Therefore, it is expedient to use the computer forecasting at the first phase of this work. For this purpose the software PASS can be used. PASS provides a possibility for simultaneous prediction of many types of biological activities based on the structure of compounds and its likelihood to other compounds of which is set by the ratio Pa ("to be active")/Pi ("to be inactive"). We use it to analyze a number of new synthesized compounds and, as a result, several substances were selected for further examination. These are the derivative of fluorinated uracil (G27), the trifluoromethyl substituted derivative of thiosugar (SBIO6), a bisphosphonic acids (10s19) and several derivatives of alanin (10s20 - 10s28).

Predicted biological activities according to PASS

Substences	Structure	Pa	Pi	Predicted biological activities
G27		0,412	0,097	Antineoplastic (non-Hodgkin's lymphoma)
		0,372	0,049	Antiviral
		0,303	0,043	Antileukemic
SBIO6		0,816	0,033	CYP2C12 substrate
		0,689	0,004	Antiviral
		0,424	0,040	Caspase 8 stimulant
		0,330	0,012	Antineoplastic (carcinoma)

PASS prediction revealed an antiviral activity for these compounds. It had also predicted that each of these compounds may possess an antineoplastic activity.

Predicted biological activities according to PASS

Substences	Structure	Pa	Pi	Predicted biological activities
10s19		0,203	0,155	Immunomodulator
		0,160	0,158	Antiviral
		0,104	0,091	Antineoplastic antimetabolite
10s20		0,294	0,005	Antiviral
		0,228	0,082	Antineoplastic (brain cancer)
10s24		0,176	0,038	Antineoplastic antimetabolite
		0,126	0,061	Antiviral
10s25		0,408	0,082	Antiviral
		0,370	0,116	Antineoplastic

According to PASS these compounds may possess anticancer and antiviral activities.

Molecular modeling techniques have been playing an increasingly crucial role in the search for new drugs and their optimization, in every area of drug design.

Modern antiherpetic drugs are aimed at blocking/inhibiting the replication of viral DNA. The compound G27 is acyclic nucleoside (fluorinated derivative of uracil) and may have similar mechanism of action.

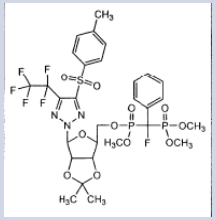
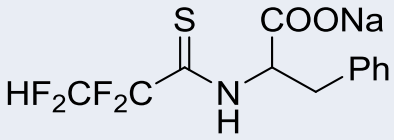
Because compounds 10s20, 10s24 and 10s25 include an alanin moiety, they can integrate proteins and disrupt their structure and functions. They could interact with viral proteins and on blockage of certain stages of EBV reproduction.”

The compound SBIO6 is also of interest since there is a high possibility that it is a substrate for the cytochrome c (CYP2H substrate) and might be stimulant for caspase 8. Thus, this compound may stimulate apoptosis.

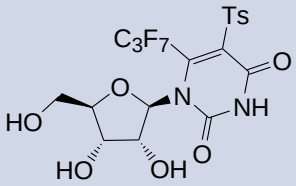
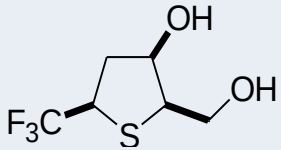
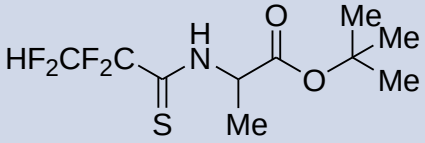
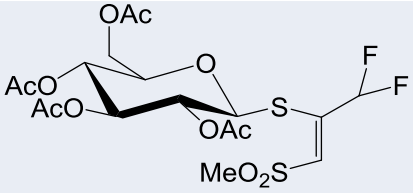
Computer modeling helps to conduct fast screening and determine possibly way further study.

The cytotoxicity and antiviral activity of the compounds was studied with MTTassay and real time PCR, respectively. The ability to inhibit reproduction of VVS was marked for two compounds: 10s19 and 10s24. The EC50 were 19 μg/ml and 29 μg/ml respectively.

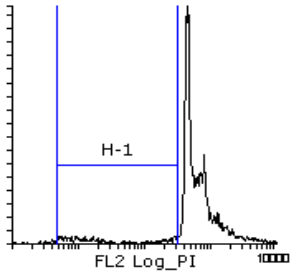
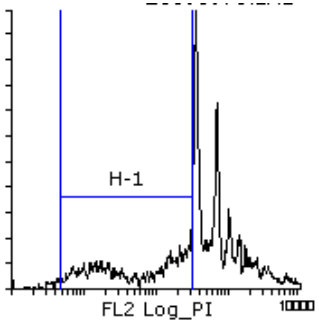
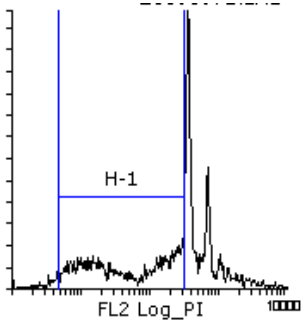
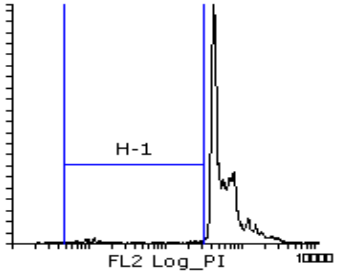
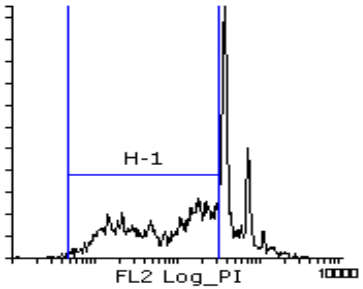
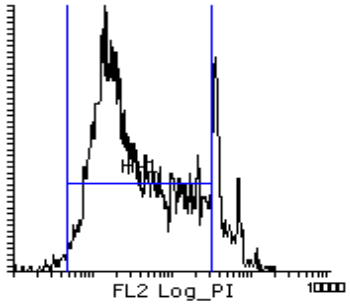
Inhibition of reproduction of VVS

Substances	Structure	CC_{50}, μg/ml	EC_{50}, μg/ml	SI
10s19		240	19	13
10s24		1700	29	57

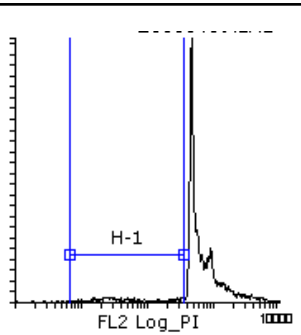
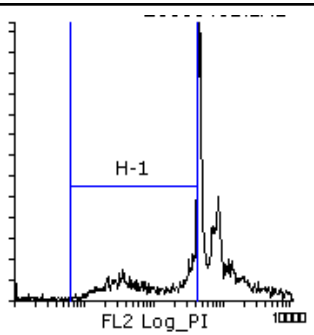
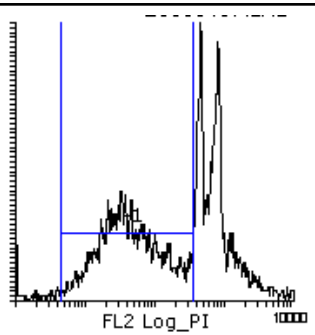
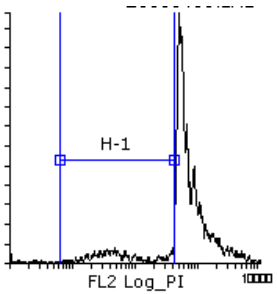
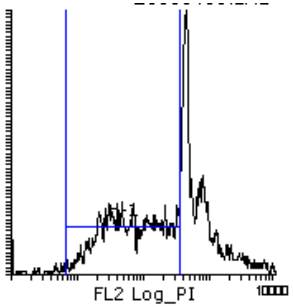
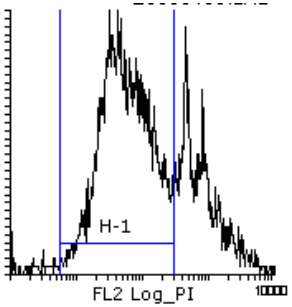
Three compounds were effective against EBV and their EC_{50} were 1 μg/ml for 10s20, <62 μg/ml for 10s25 (a 100% suppression of EBV replication was detected at this concentration) and for the G27 it was 100 μg/ml. The selective indices (SI) for these compounds were in the range 3 -100.

Substances	Structura	CC_{50}, μg/ml	EC_{50}, μg/ml	SI
G27		500	100	5
SBIO6		400	66	6
10s20		100	1	3
10s25		100	100	31

Epstein-Barr virus is the cause of several lymphoproliferative diseases and that increases the need for new effective drugs. We studied the ability of compound G27 to induce apoptosis. Apoptotic cells were detected with flow cytometry. Under action of G27, two peaks in the cytometry histograms were observed that may indicate a disruption of cell cycle by this compound.

48 h			
	Cell control (9%)	Cell culture + G27 250 µg/ml (32%)	Cell culture + G27 500 µg/ml (41%)
72 h			
	Cell control (6%)	Cell culture + G27 250 µg/ml (60%)	Cell culture + G27 500 µg/ml (90%)

Treatment of tumor cells with apoptosis modulators is an effective procedure against most cancer disease. We studied the potency of SBIO6 to cause an apoptosis. It was established that at 125 µg/ml of SBIO6 the percentage of apoptosis cell exceeds control values and was 42% after 48 h exposure. It was established that the percentage of apoptotic cells significantly increased, in comparison with the control, and reached 70-90 percent under the action of an increased concentration.

48 h			
	Cell control (10%)	Cell culture + SBIO6 125 µg/ml (32%)	Cell culture + SBIO6 250 µg/ml (52%)
72 h			
	Cell control (14%)	Cell culture + SBIO6 125 µg/ml (51%)	Cell culture + SBIO6 250 µg/ml (69%)

Conclusions

According to PASS predictions, some of the selected compounds could possess anticancer and antiviral activities. That may be due to cytochrome c/caspase induced apoptosis.

We determined the cytotoxic effect of compounds G27, SBIO6, 10s19-10s25. Values of CC_{50} for these compounds ranged from 100 to 1700 μg/ml. Analysis of antiviral activity of the compounds demonstrated high level of activity against EBV and VVS.

Compounds G27 and SBIO6 had a significant impact on the growth of transformed cells. It can be assumed that the destruction of cells infected with virus occurs through apoptosis.

Obtained data allow considering these compounds as perspective antiviral and antitumor agents.

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