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ANTIVIRAL ACTIVITY OF FLUORINATED HETEROCYCLIC COMPOUNDS

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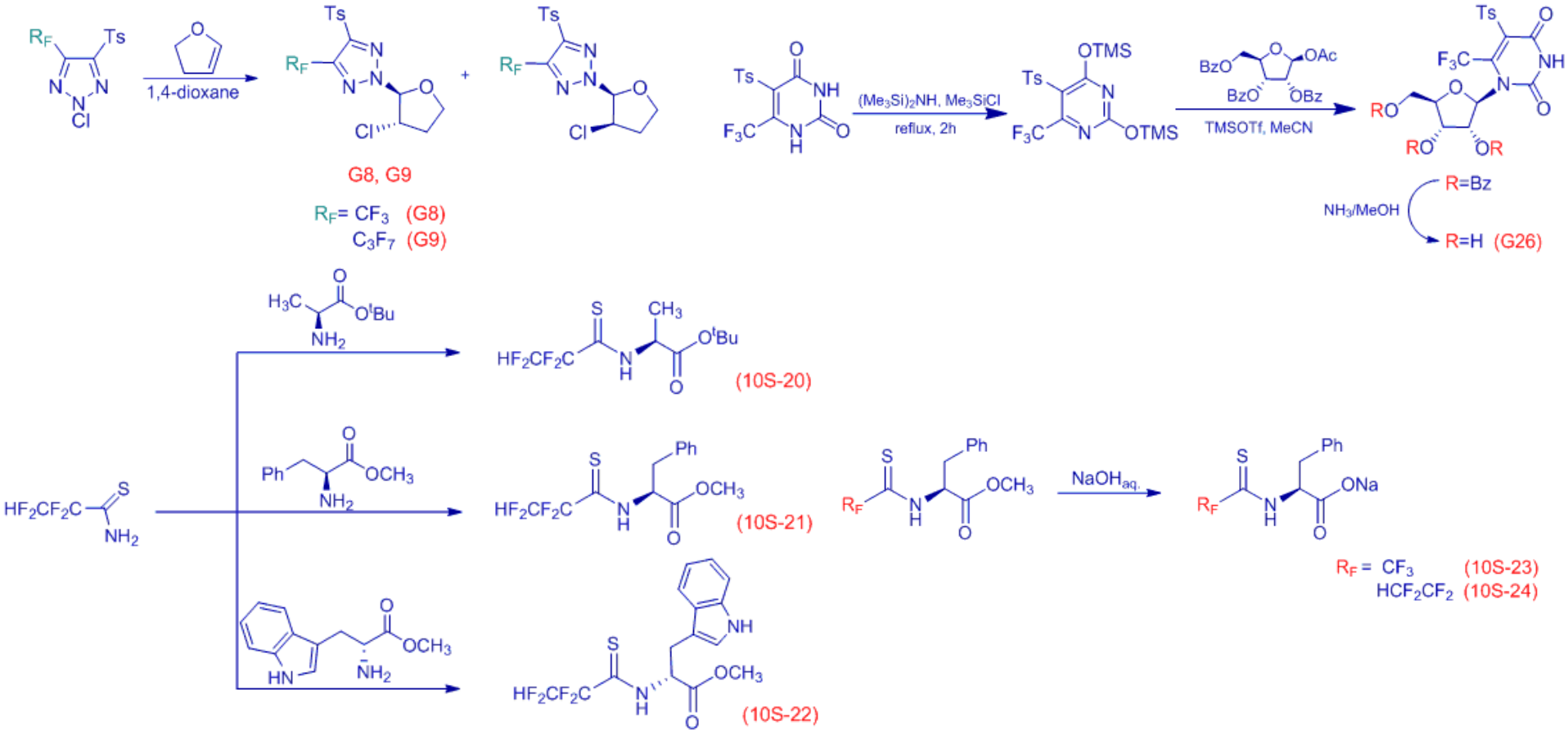
Graphical Abstract



Abstract: **Nucleoside analogues have a special place among the most effective antiviral drugs and the study of fluorinated nucleoside sugars has led to the development of novel promising chemotherapeutic agents. Our work is dedicated to the determination of cytotoxicity and antiviral activity towards HSV-1, HSV-2 and HAdV5 of such modified nucleosides.**

Human adenovirus (HAdV) are ubiquitous infectious DNA viruses possessing a broad spectrum of pathogenicity. More than 60 HAdV serotypes have been identified that are responsible for respiratory, gastrointestinal, and ocular diseases. HAdV are able to persist in humans for a long time in latent state and can be reactivated by various factors, especially grave problems they cause immunocompromised hosts by the development of generalized HAdV infection.

The diseases caused by Herpes Simplex virus are widely distributed. Treatment of these infections is the most significant medical problem. However, the appearance of resistant virus is current problem in the treatment of patients and deficiency in the antiviral preparations caused their toxicity. Therefore, it is very important to develop new antiviral drugs against this virus.

Keywords: Human adenovirus; Herpes Simplex virus; Fluorinated heterocyclic compounds.

Introduction

The studyi of fluorinated nucleosides became the basis for the development of promising chemotherapeutic agents with antitumor and antiviral effects. Based on the purine and pyrimidine nucleotide analogues and fluorinated heterocycle molecules a number of new generation drugs with anticancer effect was developed. Thus, fludarabine phosphate is an effective anticancer compound for the treatment of acute or chronic lymphocytic leukemia and non-Hodgkin's lymphomas.

The synthesis and implementation into clinical practice of 5-fluorouracil analogue was an extremely important achievement of modern medicinal chemistry. This compound is now widely used in the treatment of malignant tumors of various organs. It is known that 3'-deoxy-3'-fluoro-D-deoxyribonucleosides act as inhibitors of several DNA- and RNA-containing viruses. For example, 3'-deoxy-3'-fluoroadenosine inhibits the replication of different RNA-containing viruses including poliovirus, Coxsackie virus, Sindbis virus, and DNA-containing cowpox virus. Some pyrimidine ribonucleosides have antiviral activity against herpes simplex virus. For instance, 2'-deoxy-2'-fluorocytidine is a strong and selective inhibitor of HCV RNA polymerase.

Results and discussion

Methodology:

Cells and viruses. **Cell culture MDBK (bovine kidney), adenovirus serotype 5 (HAdV5), herpes simplex virus 1 and 2 (HSV-1/US, HSV-2/BH) were used.**

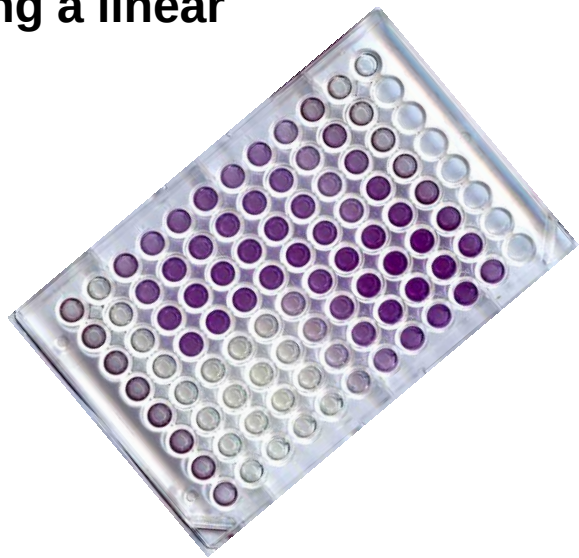
Tested substances: **analogs of nucleoside based on 5-tosyl-6-(trifluoromethyl)uracile (G26); 4-tosyl-5-(perfluoroalkyl)-1,2,3-triazoles (G8 and G9); polyfluoro-substituted thiopeptide analogs – namely t-butyl(tetrafluoropropanethioyl)-L-alaninate (10S-20), methyl (tetrafluoropropanethioyl)-L-phenylalaninate (10S-21) and methyl (tetrafluoropropanethioyl)-D-tryptophanate (10S-22), sodium (polyfluoroalkanethioyl)-L-phenylalaninate (10S-23 and 10S-24).**

The compounds were dissolved in dimethylsulfoxide (DMSO, Sigma, USA) to concentration of 2 mg/ml and stored at 4°C. Working twofold serial dilutions from 1 mg/ml to 8 µg/ml were prepared in the medium for cell culture (RPMI-1640, Sigma, USA) without serum immediately before use. Solutions were sterilized using syringe filtration through membrane filters with pore diameter of 0.22 microns (Sarstredt, Germany).

Cytotoxicity **of the compounds was determined by MTT-test or neutral red dye (NR) according to standard protocol. Most studies of cytotoxic compounds are carried out using the MTT-test. This assay is based on the transformation of mitochondrial enzymes of live cells yellow tetrazolium salt (MTT) to a purple formazan dye. Color formation is read on a microplate spectrophotometer.**

The NR assay is based on the incorporation of the supravital dye, NR, into the lysosomes of viable cells after their incubation with toxic chemicals.

The concentrations of substances that inhibit 50% of cell viability compared to control cells (CC_{50}) were measured using a linear regression method in Microsoft Excel 10.

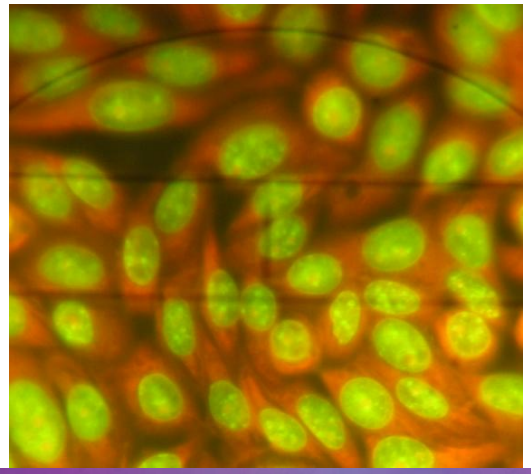


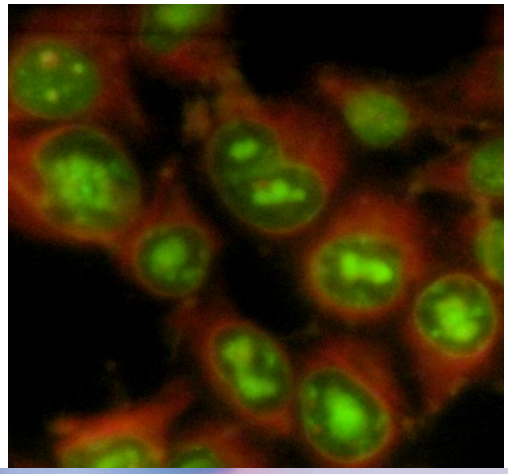
Antiviral effect **was determined using PCR, MTT-test and cytomorphological method. Cytomorphological method was used to identify infected cells containing specific virus inclusion. Cells were treated with the compounds in the growth medium after virus adsorption at non-toxic concentrations.**

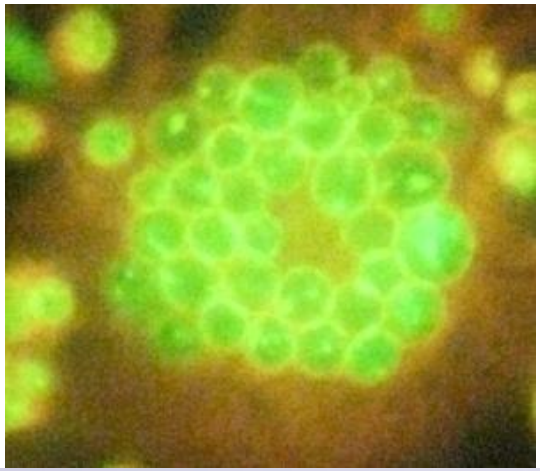
The half maximal effective concentration (EC_{50}) was estimated as the concentration of the compound which induced to 50% of its maximal effectiveness that was observed.

Cytomorphological features of virus infection in MDBK cells (acridine orange staining)

uninfected cells **+ HAdV-5** **+ HSV-2/BH (HSV-1/US)**





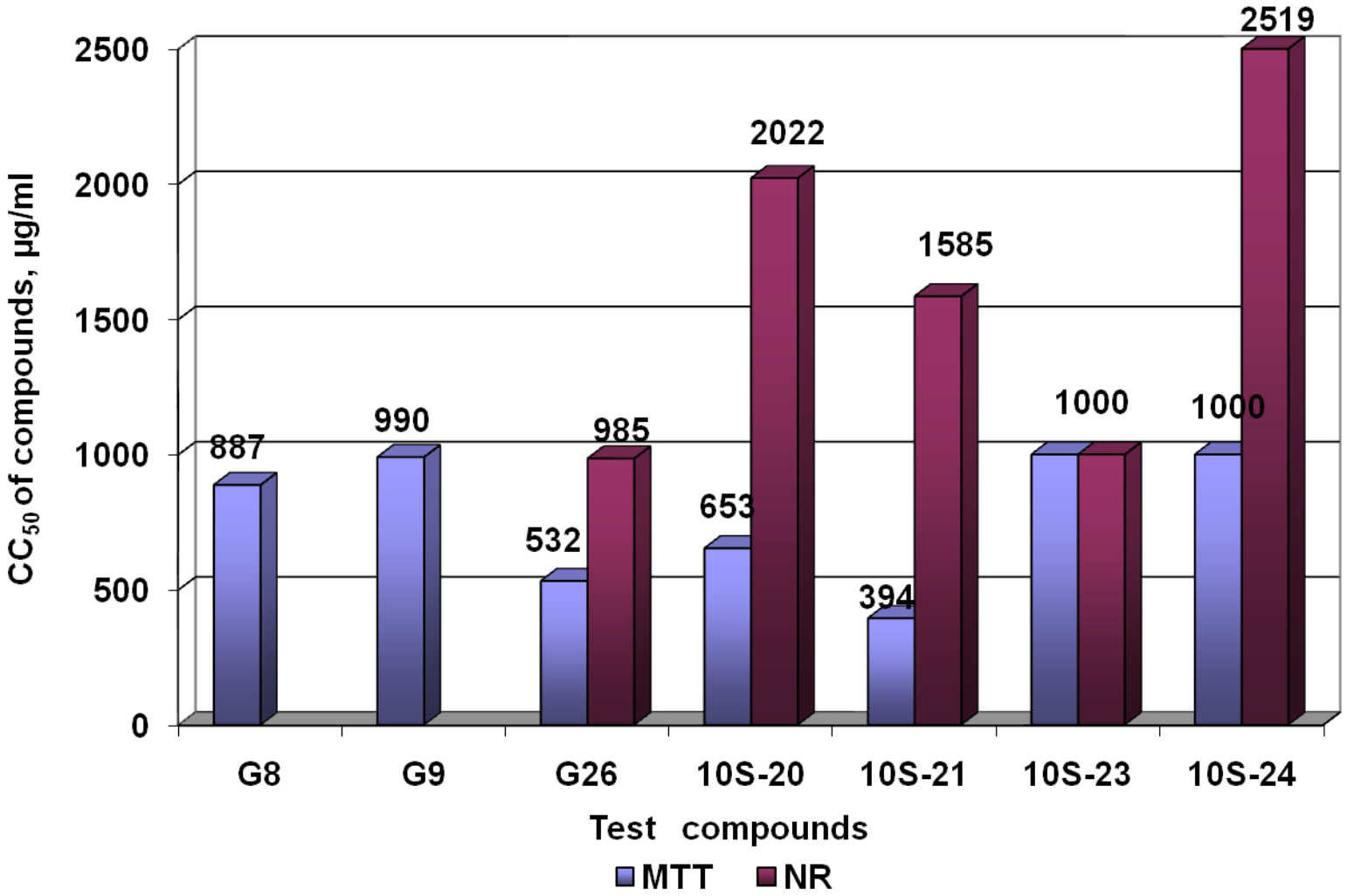


According to the MTT test results, CC_{50} values of compounds G8 and G9 were 887 and 990 µg/ml, respectively. Other compounds demonstrated the following CC_{50} values, G26: 532 µg/ml (MTT) and 985 µg/ml (NR); 10S-22: 290 µg/ml (MTT) and 554 µg/ml (NR).

Compound 10S-23 showed CC_{50} value of >1000 µg/ml (MTT, NR). CC_{50} values obtained using NR were higher than the previous results for the compounds 10S-20, 10S-21, 10S-24 (2022, 1585, and 2519 µg/ml, respectively). CC_{50} values assessed via MTT test for these compounds were lower – 653, 394 and >1000µg/ml, respectively.

The NR assay has been found to be more sensitive than the MTT assay. Comparing the effects on cell viability estimated by two methods it was suggested that compounds have different influences on cell compartments. These compounds showed significant inhibitory effect on the functioning of the mitochondria with increased levels of lysosomal activity, indicating the activation of cell death.

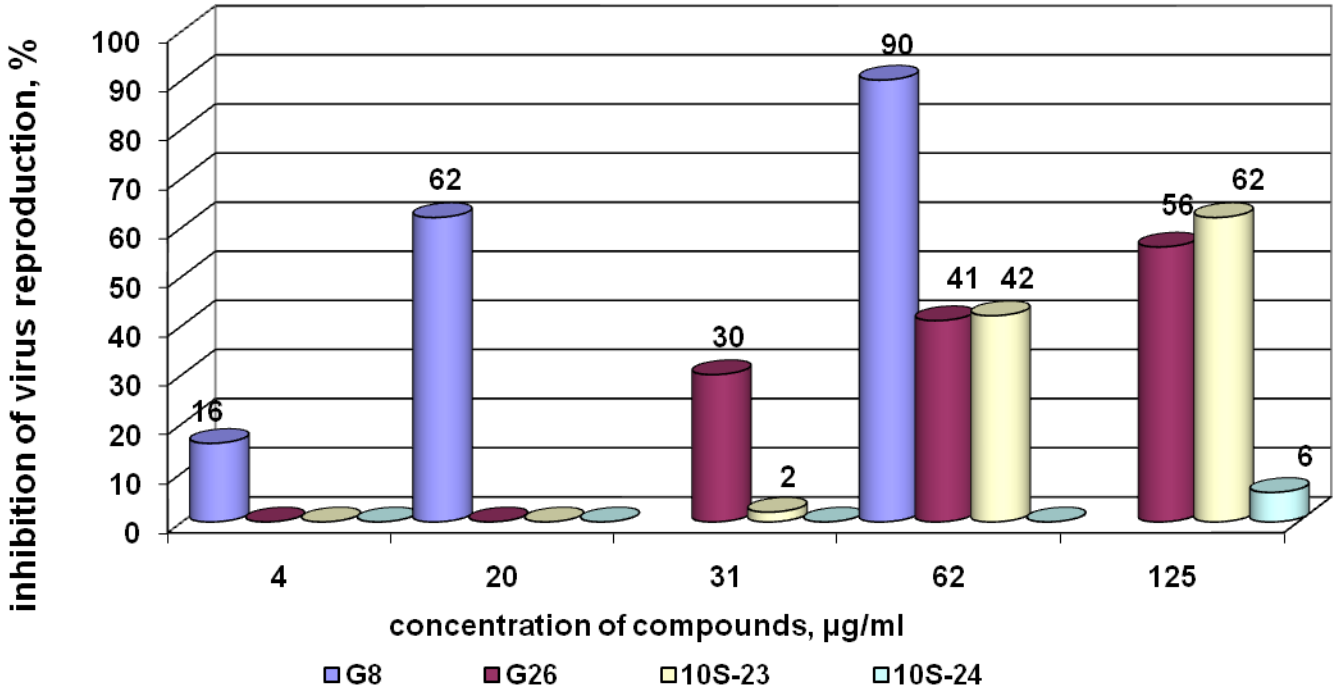
Cytotoxicity (CC_{50}) of the compounds was determined by MTT-test or neutral red dye (NR)



Anti HAdV5 activity of the compounds

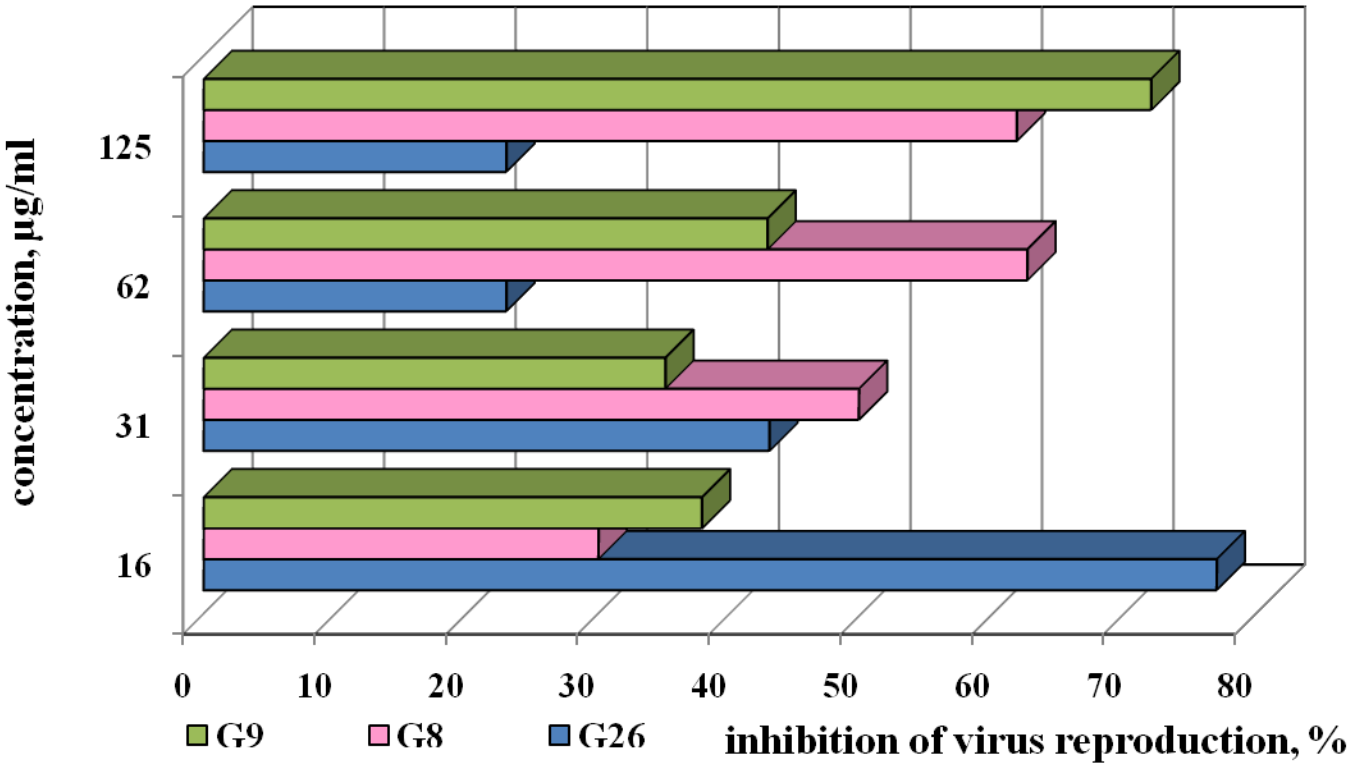
Inhibition of HAdV5 reproduction was demonstrated for three compounds G8, G26 and 10S-23. PCR revealed inhibition of virus reproduction (13%) for the compound G8 at a concentration 62 μg/ml, 95-100% for G26 at concentrations 31-62 μg/ml and 31% for 10S-23 at a concentration 36 μg/ml. :

The results of cytomorphologycal method showed the following values of EC_{50} for the compounds: 16, 120 and 90 μg/ml, respectively.



The analysis of antiHSV-2/BH activity of compounds

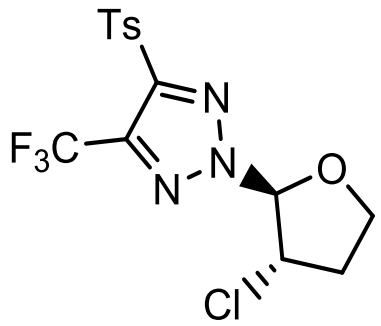
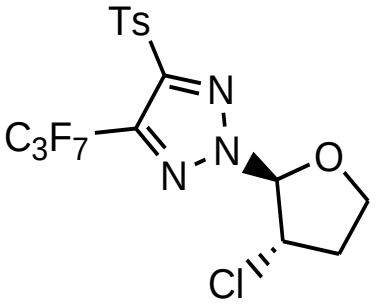
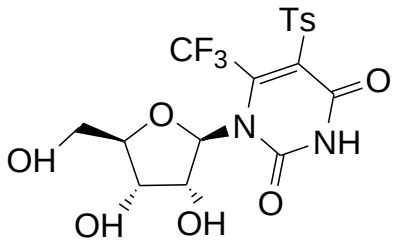
Compounds G8, G9, G26 suppressed HSV-2/BH reproduction by 50% at the concentrations of 56, 71 and 44 μg/ml, respectively.



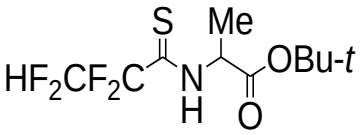
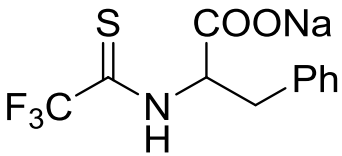
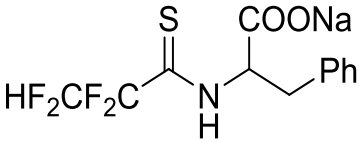
Using MTT test it was shown that compounds G8, G9, 10S-20, 10S-23 and 10S-24 suppressed HSV-1/US reproduction by 50% at concentrations of 50, 59, 62, 71, 35 μg/ml, respectively. Compounds 10S-21 and 10S-22 had lower efficiency and suppressed virus reproduction by 37% and 22%, respectively.

Therefore, from the 8 tested compounds 6 compounds showed antiviral action: two compounds G8 and 10S-23 inhibited the reproduction of all viruses with different efficiency; the compound G9 was active only in relation to both types of herpes; G26 suppressed reproduction of HSV-2/BH and HAdV5. The compounds 10S-20 and 10S-24 were active only in relation to reproduction HSV-1/US.

Biological activity of compounds

Test substances		Formula	CC_{50}(μg/ml)	EC_{50}(μg/ml)	SI
G8	2-(*trans*-3-Chlorotetrahydrofuran-2-yl)-4-tosyl-5-(trifluoromethyl)-2*H*-1,2,3-triazole		887 (MTT)	16 for HAdV5 56 for HSV-2 50 for HSV-1	55 16 18
G9	2-(*trans*-3-Chlorotetrahydrofuran-2-yl)-4-tosyl-5-(heptafluoropropyl)-2*H*-1,2,3-triazole		990 (MTT)	71 for HSV-2 59 for HSV-1	14 17
G26	5-tosyl-6-(trifluoromethyl) uracile derivatives		532 (MTT) or 985 (NR)	120 for HAdV 44 for HSV-2	4 or 8 12 or 22

Biological activity of compounds

Tested substances		Formula	CC_{50}(μg/ml)	EC_{50}(μg/ml)	SI
10S-20	**polyfluoroalkyl-substituted thiopeptide analogs – namely *t*-butyl (tetrafluoropro-panethioyl)-L-alaninate**		**653 (MTT) or 2022 (NR)**	**62 for HSV-1**	**11 or 33**
10S-23	**Sodium(2,2,2-trifluoropropanethioyl)-*L*-phenylalaninate**		**>1000 (MTT, NR)**	**90 for HAdV 71 for HSV-1**	**11 14**
10S-24	**Sodium (2,2,3,3-tetrafluoropropanethioyl)-*L*-phenylalaninate**		**>1000 (MTT) or 2519 (NR)**	**35 for HSV-1**	**29 or 72**

Conclusions

According to the results obtained in the present study:
- antiviral activity of substances submitted to the post-treatments was demonstrated;
- the absence of antiviral activity of substances submitted to the pre-treatments, during absorption and penetration of the virus was shown;
- the absence of virucidal activity of substances was shown.

These data suggest that the substances may be active on the latter stages of virus reproduction.

The results are evidence of promising compounds for further in-depth study on models *in vitro* and *in vivo.*