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The Antiviral Activity of Trifluoromethylthiolane Derivatives †

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Abstract: The search of new antiviral agents is an important task today. The aim of this study was to elucidate the impact of trifluoromethylthiolane derivatives on herpetic and adenoviral infections. It was found that the 2-hydroxy-2-trifluoromethylthiolane significantly inhibited Herpes simplex virus type 1 (HSV-1) reproduction, reducing the virus titer obtained de novo. Such activity indicates that virus offspring are formed but virus particles are not complete and they are not able to cause an infection process. This is why compound may be a potential tool for the development of agents for the treatment of herpetic infections.

Keywords: antiviral potential; HSV-1; HAdV-5; cytotoxicity; trifluoromethylthiolane

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1. Introduction

In the world, there is a worsening of the epidemic situation, as well as an increase in economic losses from infectious diseases. Viruses occupy one of the key places among human infectious diseases. Against the background of viral infections, chronic pathology is more often exacerbated and manifest systemic, allergic and autoimmune diseases, as well as some types of malignant processes [1]. Adenoviruses and herpesviruses are characterized by the ability of latent persistence and reactivation under favorable conditions [2]. Also, these infections remain highly relevant in connection with circulation throughout the year [3]. The importance of diseases caused by adeno- and herpesviruses in organ transplantation is increasing, when latent viral infection, in the absence of specific etiotropic drugs, leads to a significant increase in the number of deaths [4]. The antigenic diversity of viruses inhibits the process of creating universal vaccines and causes the development of resistance to direct-acting antiviral drugs [5]. Despite significant achievements in the chemotherapy of viral infections, the problem is still open today [6]. This is due to the development of viral resistance to chemotherapy drugs, low bioavailability and effectiveness of specific drugs against latent forms of viral infections, as well as frequent relapses of the disease with long-term use of drugs [7,8].

It is known that analogs of nucleosides are widely used in antiviral therapy, because they imitate natural nucleosides and compete with thim, blocking virus replication process [9]. The introduction of fluorine atoms into analogs of nucleosides showed an increase in their biological activity, physicochemical properties, and metabolic stability [10–12]. Introduction of trifluoromethyl (CF_3), difluoromethyl (CF_2H), difluoromethylene (CF_2) or fluoromethylene (CHF) groups into the sugar moiety of nucleosides significantly increases its antiviral and antitumor properties [13]. For example, the introduction of fluorine into the sugar moiety of Emtricitabine accelerates its ability to blocking the synthesis of the HIV DNA and increases the half-life of the drug from the body compared to standard antiretroviral drugs [12]. The sugar moiety plays a key role in the interaction with target enzymes, so its fluorination leads to a change in its conformation and

functional properties. The sugar-fluorinated nucleosides exhibit antiviral properties against herpes viruses, hepatitis, influenza, various fevers, etc. [14]. In addition, it was found that the presence of fluorine contributes in nucleoside drugs to an increase in the stability of neighboring bonds and the whole molecule, which leads to a significant decrease in the susceptibility of the nucleoside to enzymatic cleavage of the glycosidic bond [11,15]. That is why the search for new effective etiotropic agents among fluorine-containing thiosugars is a rather promising direction in antiviral therapy. The aim of this study was to elucidate the impact of trifluoromethylthiolane derivatives on herpetic and adenoviral infections.

2. Materials and Methods

2.1. Structure of the Compounds

Compound 10S-52 (2-(hydroxymethyl)-2-(trifluoromethyl)thiolane) was obtained by the deacetylation of 2-(acetoxymethyl)-2-(trifluoromethyl)thiolane at room temperature. Compound SBIO-6 ((2RS,3RS,5RS)-3-hydroxy-2-(hydroxymethyl)-5-(trifluoromethyl)thiolane) was obtained by treatment of (2SR,3RS,5RS)-3-acetoxy-2-diacetoxymethyl-5-(trifluoromethyl)thiolane with sodium borohydride in isopropanol at room temperature. Both compounds were obtained as racemates. The structure of the compounds (Figure 1) was confirmed by the data of ^{1}H, ^{19}F, ^{13}C NMR spectra, as well as by chromatography-mass spectrometry and elemental analysis, the data are given in previously publications [16,17].

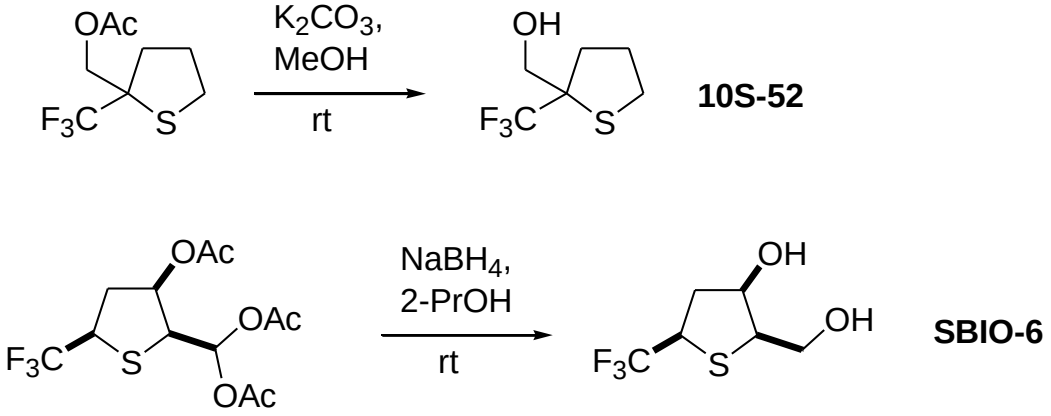


Figure 1. Structure and schemes of compounds synthesis.

2.2. Virus and Cells

BHK-21 cells (Syrian hamster kidney) and Hep-2 cells (human laryngeal carcinoma) were obtained from Cell Bank of the Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine (Ukraine). Cells were cultivated in growth medium consisting of 45% Dulbecco's Modified Eagle's Medium (DMEM, Sigma, USA), 45% RPMI-1640 Medium (Sigma, USA) supplemented with 10% fetal bovine serum (FBS, Sigma, USA) and 1% antibiotic solution (Sigma, USA).

BHK-21 and Hep-2 cells were infected, respectively, with HAdV-2 (human adenovirus type 2, obtained from Institute of Microbiology, Budapest Medical University (Hungary)) and HSV-1 (herpes simplex virus type 1, obtained from the Institute of Antiviral Chemotherapy of the Center of Clinical and Theoretical Medicine (Germany)) and incubated for observing of 100% cytopathic effect (CPE). Then infectivity of the viruses was estimated (HSV-1—5.1 $\log_{10}$ TCD_{50}/mL and HAdV-2—5.8 $\log_{10}$ TCD_{50}/mL). The viral suspension containing the supernatant was aliquoted into sterile cryovials and stored at −80 °C until use.

2.3. Cytotoxicity Assays

Effect of the compounds on BHK-21 and Hep-2 cells was determined using a tetrazolium-based colorimetric (MTT) assay as described previously [18]. Briefly, cells were incubated with compounds at concentration of 47–1510 μg/mL for 72 h, then in a medium was added 20 μL of a 5 mg/mL solution of MTT (Sigma, USA). After 3–4 h' incubation, 150 μL of 96% ethanol was added to the cells. The plates were detected using an automatic plate reader Multiskan FC (Thermo Scientific, USA) with a 538 nm test wavelength. The viability (%) of the treated cells was defined as the percentage of absorbance compared to control untreated cells (100% viability). A reduction in viability corresponds to a likelihood of increased cytotoxicity. Percentage of cell viability after exposure to the silver nanoparticle solution was calculated by the following formula [19]:

$$\% \text{ cell viability} = A/B \times 100$$

where A is the mean optical density of the studied samples at a certain concentration, and B is the mean optical density of the control cell samples.

The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve and the mean CC_{50} (±S.D.) value of compound was calculated from three independent experiments.

2.4. Antiviral Assay

HSV-1 or HAdV-2 virus suspension was added to BHK-21 or Hep-2 cells, respectively, after adsorption (1–2 h), any unadsorbed virus was aspirated and 200 μL of the compounds-containing medium (15–503 μg/mL) was added to each well, and incubated at 37 °C and 5% CO_2 for 2–3 days until the appearance of 100% cytopathic effect in the virus control [20]. Then, the virus-containing material was taken for further study of the virus titer using the TCID method [21]. The decrease of the virus infectious titer after treatment with the compounds was determined by the formula:

Reduction of infectious titer = virus titer in the control − virus titer in the experiment

A decrease of the virus infectious titer by 2 $\log_{10}$ or more, compared to the control, indicates a pronounced activity of the compound against the virus, by ≥1.5 $\log_{10}$—a moderate effect.

2.5. Statistical Analysis

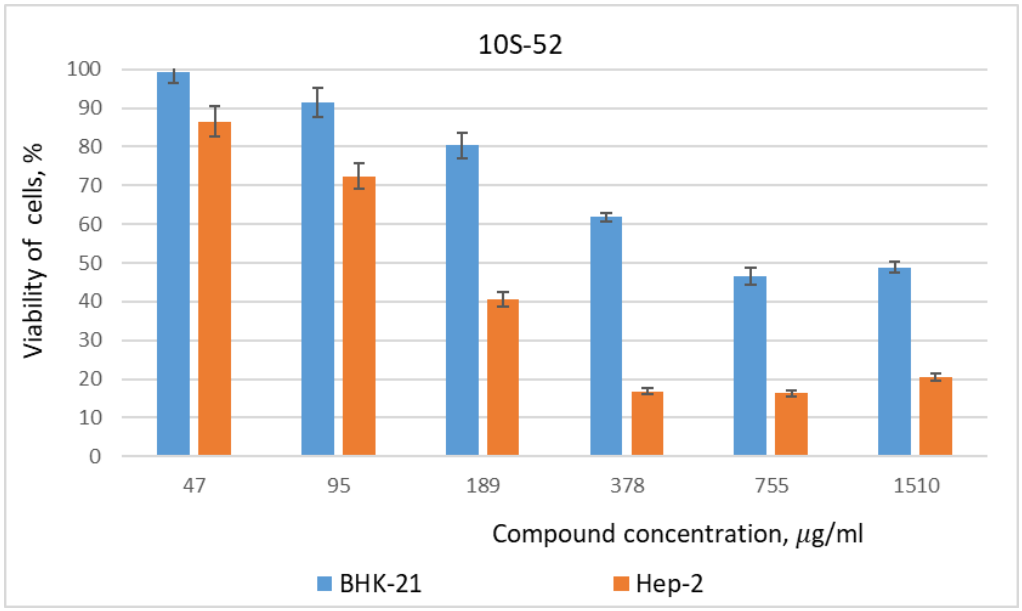
The data from all cytotoxicity and antiviral experiments were expressed as the arithmetic mean ± standard deviation (SD) and were statistically analyzed by MS Excel. A p value lower than 0.05 was considered statistically significant.

3. Results and Discussion

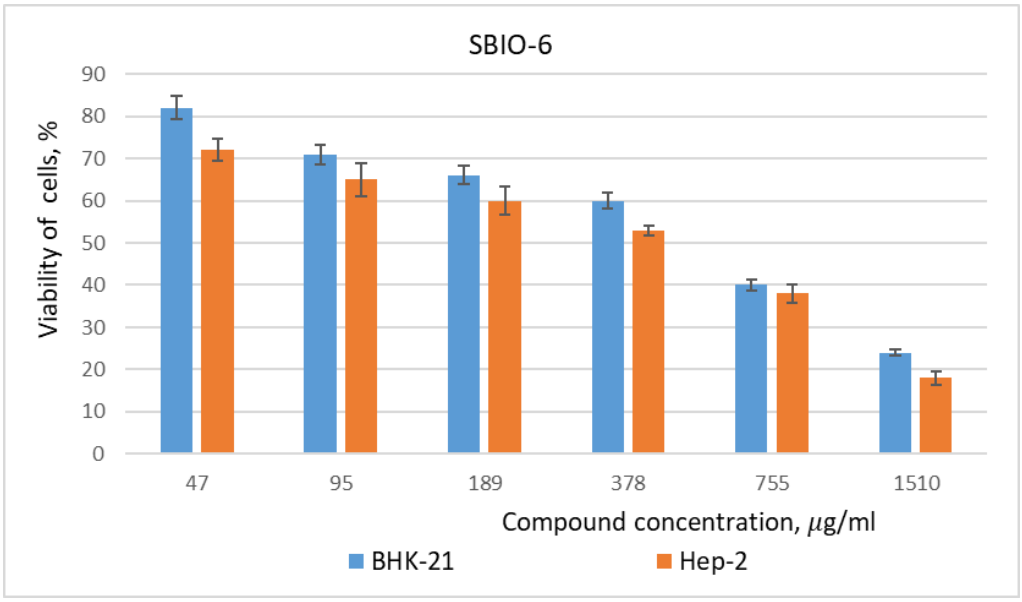
3.1. Cell Viability

The presence of the cytotoxic effect of the compounds was checked by a metabolic test that evaluates the normal functioning and mitochondrial activity of cells under the influence of one or another factor. The percentage of viability of BHK-21 and Hep-2 cells was determined for each compounds dilutions. It was found that both investigated compounds were less toxic for the BHK-21 cells culture (Figure 2a,b), because only at the maximal used concentrations (755–1510 μg/mL) they reduced cell viability by 51–76%. With the use of the compounds 10S-52 and SBIO-6 in lower concentrations, the mitochondrial activity of BHK-21 cells was within 60–99%. For the culture of Hep-2 cells, the SBIO-6 compound showed a similar influence obtained on BHK-21 cells. At the higher concentrations SBIO-6 reduced cell viability by 60–82%, while with a decrease in the concentration of the compound, the mitochondrial activity of the cells increased up to 72% (Figure 2b). However, compound 10S-52 showed a significant toxic effect on the vital activity of Hep-2 cells. Thus, at a concentration of 189–1510 μg/mL, it reduced the mitochondrial activity

of Hep-2 cells by 59–84%, and only with a decreasing of its concentration, cell viability increased and reached 75–86% (Figure 2a). Using the Microsoft Excel linear regression program, the concentration of compounds that inhibited cell viability by 50% (CC_{50}) compared to the cell control was calculated. The CC_{50} value of compound 10S-52 for BHK-21 and Hep-2 cell cultures was 627 and 161 μg/mL, respectively, while for SBIO-6 it was 670 and 516 μg/mL, respectively.



(**a**)



(**b**)

Figure 2. Influence of trifluoromethylthiolane derivatives on viability and mitochondrial activity of cells. Hep-2 and BHK-21 cells growth after 72 h exposure with difference concentrations of the compounds were monitored by colorimetric MTT assay. Control untreated cells—100% viability. Values represent as the mean ± S.D. for three independent experiments. Statistically significant difference between the growth inhibition effect was $p < 0.05$.

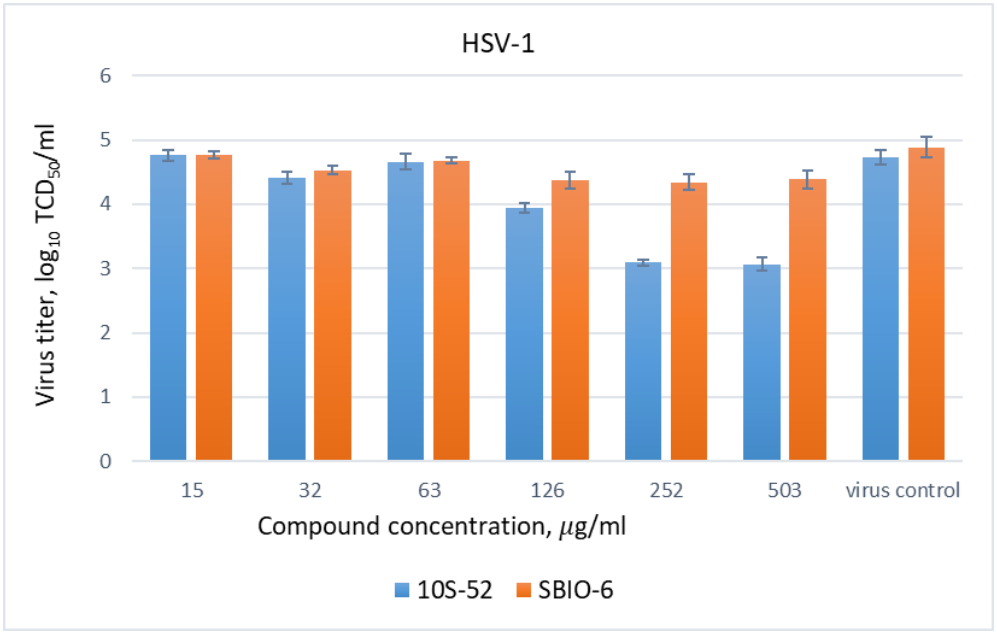
Mloston showed that the introduction of a fluorine atom, fluoroalkyl or fluoroaloxy substituents (F, CF_3 or OCF_3) enhances the cytotoxic properties of compounds on cancer cell cultures [22]. So, the newly synthesized 10S-52 compound demonstrated various

cytotoxicity levels towards the tested normal (BHK-21) and cancer (Hep-2) cell lines. It's cytotoxicity for Hep-2 was significantly (4×) higher in comparison with the BHK-21 cell line, indicating that 2-hydroxy-2-trifluoromethylthiolane could be a great candidate for consideration in future cancer therapy.

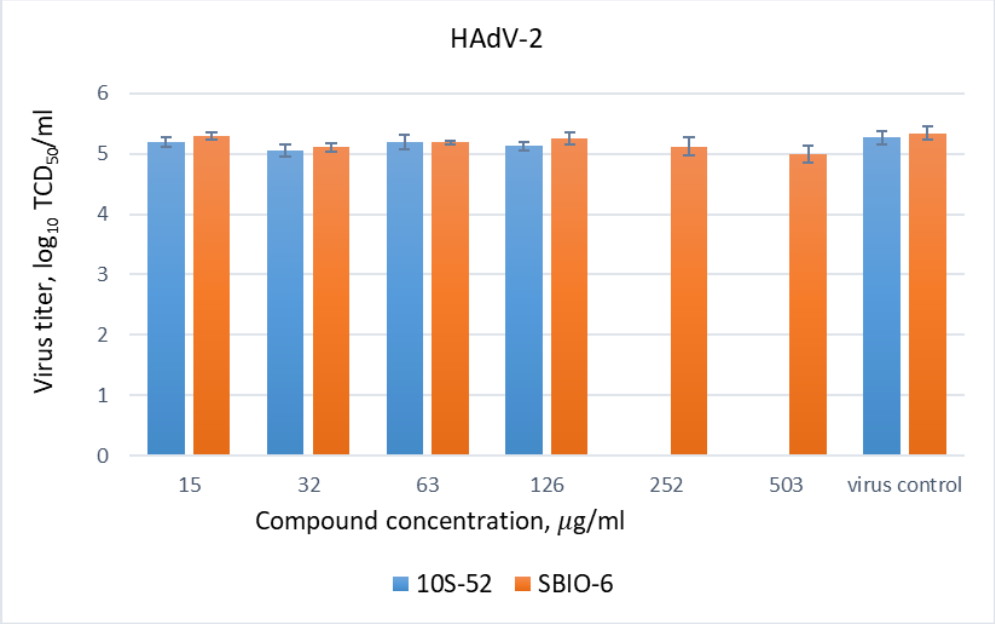
3.2. Influence Trifluoromethylthiolane Derivatives on Human Viruses' Reproduction

Fluorination of compounds is one of the strategies for creating of antiviral drugs [23]. Incorporation of the fluorine atom into compounds can impact their solubility and lipophilicity, and affect their biological potency. The presence of hydrogen or hydroxyl in the C2′fragment of the nucleotide molecule is a unique difference between DNA and RNA, which increases the interest of scientists in compounds that have modifications in this position other than hydrogen or hydroxyl [13]. Accordingly, the study of biological properties (antimicrobial, antiviral, antitumor, etc.) of the C2′fluorinated nucleosides remains relevant. Compounds with such modifications, for example, 2-deoxy-2-fluorocytidine, 2-deoxy-2-fluoro-5-methyl-1-β-darabinosyluracil, 2-fluorinated-2,3-dideoxyadenosines, a β-D-2′- de-oxy-2′-α-fluoro-2′-β-C-methyluridine, shown excellent activities against many types of RNA and DNA viruses (HIV, influenza viruses, hepatitis B and C viruses, herpesviruses, coronaviruses etc.) [14,24–26]. It was shown that them inhibit viral RNA and DNA replication in cell cultures. Obtained date encourage pharmaceutical scientists to create their analogues bearing different functional groups.

That's why antiviral effectivity of a few newly synthesized 2′-fluorinated nucleosides were analyzed. Antiviral activity of fluorine-containing thiolans in non-toxic concentrations added to cells after adsorption of HSV-1 and HAdV-2 was studied by inhibition of the cytopathogenic effect of viruses on cells. The decrease in the titer of adenovirus and herpes virus synthesized de novo under the action of 10S-52 and SBIO-6 was calculated (Figure 3a,b). It was found that compound 10S-52 at the highest used concentrations (252 and 503 μg/mL) reduced the infectious titer of the herpes virus to 2 $\log_{10}$, indicating its moderate antiherpetic efficiency (Figure 3a). Instead, the SBIO-6 compound, regardless of the used concentration, is significantly reduced the titer of HSV-1 (up to 0.8 $\log_{10}$). 10S-52 and SBIO-6 compounds did not show anti-adenovirus activity, because of their useas the value of the virus titers was similar to the values of the control virus (Figure 3b).



(**a**)



(**b**)

Figure 3. Antiviral effect of trifluoromethylthiolane derivatives against HSV-1 (**a**) and HAdV-2 (**b**). The antiviral activity of the compounds 10S-52 and SBIO-6 was investigated using yield reduction assay. Values represent the mean ± S.D. for three independent experiments, $p < 0.05$.

One of the important advantages of the fluorine atom is its high electronegativity. Which, on the one hand, leads to an improvement in oral bioavailability of the drugs (adsorption and metabolism), and on the other hand, affects on the increase in the acidity of vicinal hydrogens. For example, the presence of a trifluoromethyl group in the structure of the drug molecule contributes to the formation of hydrogen bonds necessary for blocking of reverse transcriptase or DNA polymerases [27]. Therefore, it can be assumed that such anti-herpetic activity of 2-(hydroxymethyl)-2-(trifluoromethyl)thiolane may also be associated with blocing of viral replicative process. Moreover even if the viral offspring are formed, virus particles are not complete and they are not able to cause infection process.

4. Conclusions

The cytotoxic and antiviral effects of trifluoromethylthiolane derivatives were analyzed. It was found that changes in the cell viability were dose dependent and more pronounced with an increase in compounds concentration. Moreover, the viability results were dependent on the type of used cells. The 50% cytotoxic concentration (CC_{50} value) of 10S-52 and SBIO-6 for BHK-21 cells was 627 and 670 μg/mL, respectively. But for Hep-2 cells 10S-52 was significantly toxic, and it CC_{50} value was 161 μg/mL. Such a low cytotoxicity index of compound 10S-52 may indicate its antitumor properties. It was found that among the investigated compounds only 10S-52 (2-hydroxy-2-trifluoromethylthiolane) significantly inhibited HSV-1 reproduction. In concentrations 252–503 μg/mL its reduced the herpes virus obtained de novo by 1.7 $\log_{10}$. Whereas for adenoviral infection such effect was not detected, as the decreasing of virus infectious titer did not exceed 0.2 $\log_{10}$. Obtained date indicate that 2-hydroxy-2-trifluoromethylthiolane may be a potential antiherpetic agents, but further research are required to determine its anti-HSV mechanism of action.

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visualization, O.P.; supervision, Y.S.; project administration, S.Z.; funding acquisition, Y.S. and S.Z. All authors have read and agreed to the published version of the manuscript.

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