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In vitro and *in silico* study of anti-influenza activity of 2-dioxopyrimidin-5-trifluoromethyl-tetrahydrothiophene with subsequent increase in its affinity for the target protein

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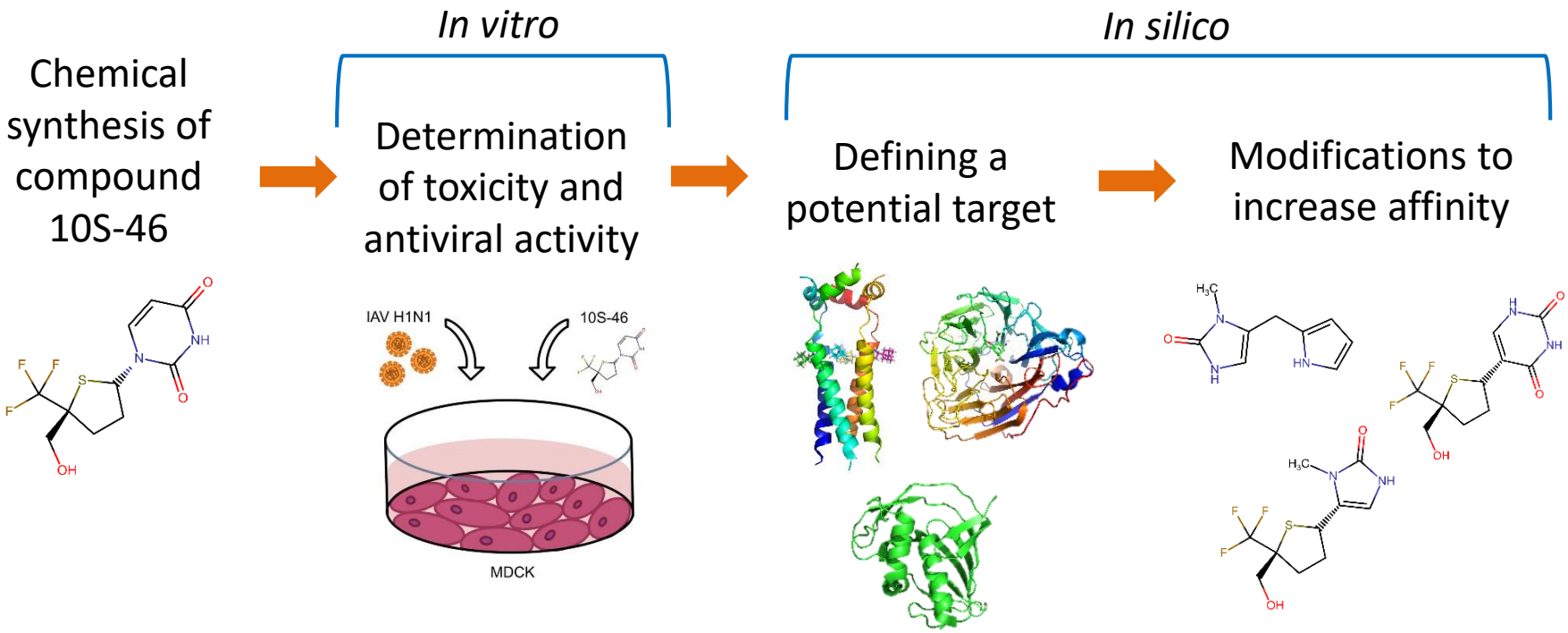
Graphical Abstract



Abstract:
Influenza viruses, which have the ability to mutate rapidly and cause outbreaks worldwide, remain poorly controlled. Today the development of new organofluorine compounds is one of the current areas of medical chemistry. The aim of our work is to determine the antiviral activity of 2-(2,4-dioxopyrimidin-1-yl)-5-hydroxymethyl-5-(trifluoromethyl)tetra-hydrothiophene (10S-46) against influenza virus in vitro, to define the probable target of its action with a further increase in affinity for the target by methods in silico.

In vitro studies were performed on MDCK culture, cytotoxicity was determined by the MTT assay. Antiviral activity (influenza A virus type H1N1) was determined by a post-exposure scheme with detection of the CPE using gentian violet. The 10S-46 target was searched using the simulation of molecular dynamics and molecular docking. An iterative approach was used to increase the affinity of the test compound for a pre-identified target.

The cytotoxic concentration (CC50) of the test compound was 530 µg/ml. Antiviral activity against H1N1 was recorded in the range of 60 – 70% at concentrations from 50 to 0.5 µg/ml. The probable target for 10S-46 was the Cap-binding domain (CBD) of H1N1 RdRp. The obtained derivatives 10S-46-m2, 10S-46-m16 and 10S-46-m18 in complex with the target protein showed greater stability compared to the 10S-46.

The presence of anti-influenza effect of the 10S-46 was established, a possible target of its action was identified, and three potentially more active compounds were developed, which indicates the prospects of their further study in vitro.

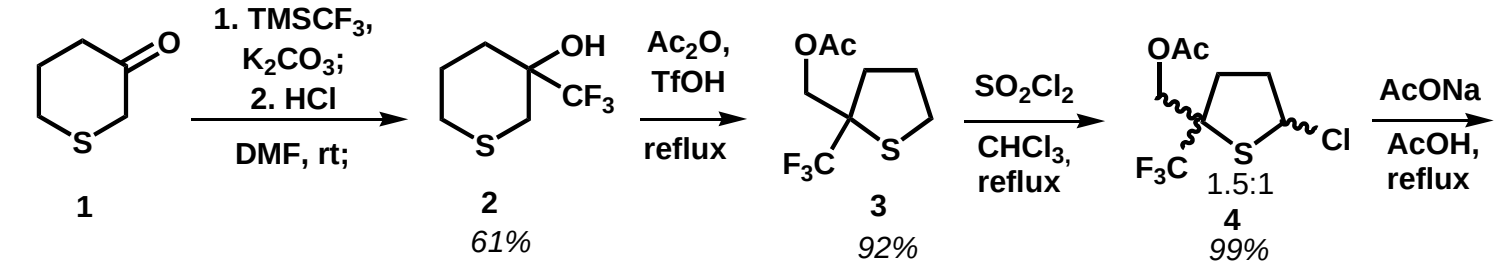
Keywords: antiviral activity; Gromacs; H1N1; molecular dynamics

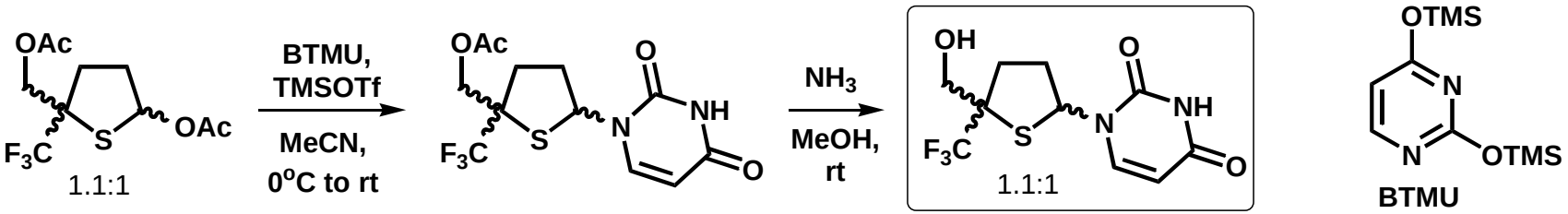
Introduction

Outbreaks, epidemics and pandemics of influenza have accompanied humanity for many years. Each of these events resulted in the deaths of hundreds of thousands of people. This is especially true for such highly aggressive strains as H1N1, H5N1 and H7N9. In an attempt to eliminate this threat, drugs have been developed - low molecular weight inhibitors of proteins such as neuraminidase, M2 and RdRp. However, mutations, which inevitably accompany the activity of any virus, lead over time to an increasing loss of sensitivity to existing drugs. In this case, the more actively we use existing inhibitors, the faster this process. Thus, the development of new antiviral drugs is a leading way to combat the spread of influenza, and especially its highly pathogenic strains. This is what our work is dedicated to. Here, we conduct primary *in vitro* studies aimed at detecting and evaluating the anti-influenza activity of the newly synthesized fluorine- and sulfur-containing nucleoside analog. With a further attempt to determine the potential target of its action and strengthen the affinity for it (target) by methods *in silico*.

Results and discussion

Chemical synthesis





Results and discussion

Determination of toxicity

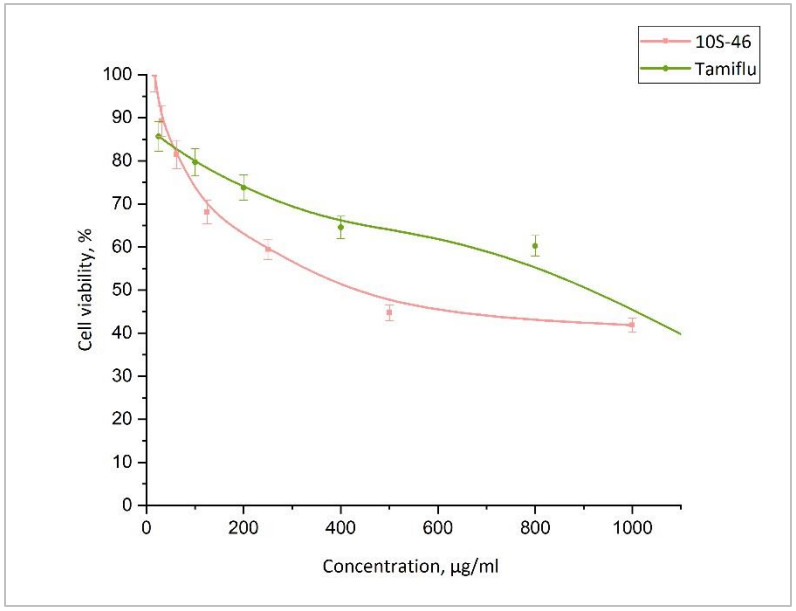


Fig. 1. Toxicity of 10S-46 and Tamiflu on MDCK cells

Tamiflu (oseltamivir phosphate) was used as a reference drug in the study.

To determine toxicity (MTT assay), the substances were incubated with cell culture for 48 hours. Based on the obtained results, the **CC50** indexes were calculated:

10S-46 – 529,6 µg/ml
Tamiflu – 826,2 µg/ml

Results and discussion

Antiviral activity

Studies of antiviral activity were performed according to the following scheme: introduction of test compounds into cell culture after pre-incubation with the virus, visualization with gentian violet.

The results show a relatively high activity of 10S-46 - up to 78% inhibition of influenza virus reproduction, as well as with the inverse dose-dependent effect.

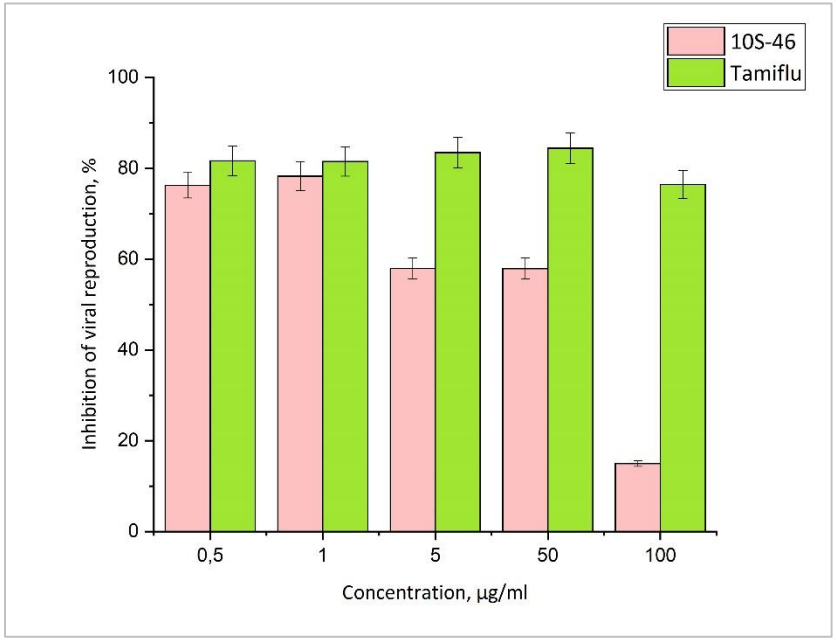
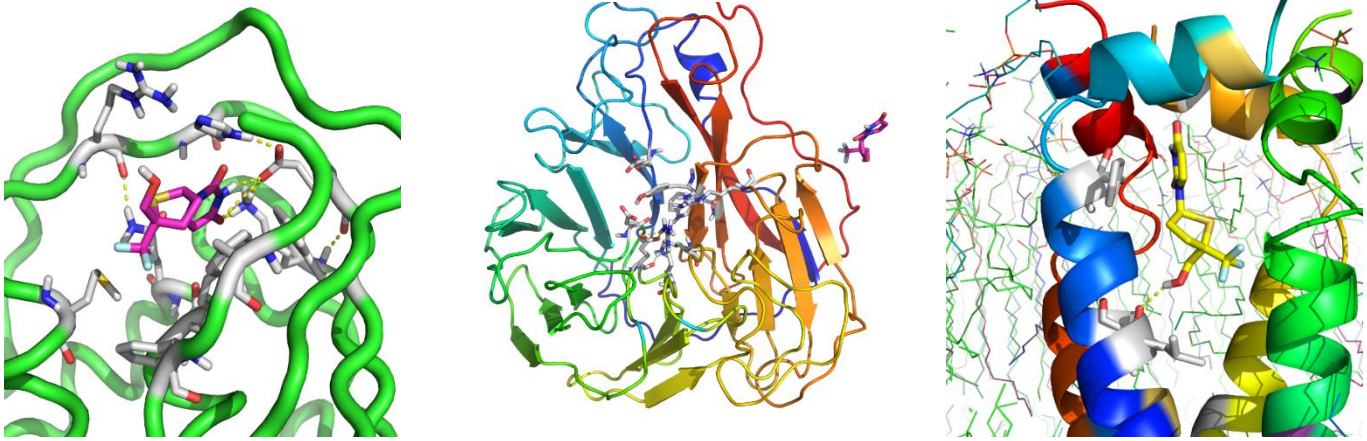


Fig. 2. Antiviral activity of 10S-46 against influenza A virus H1N1 compared to Tamiflu

Results and discussion

Defining a potential target for 10S-46



Two hydrogen bonds, multiple stacking

A

The ligand leaves the active center

B

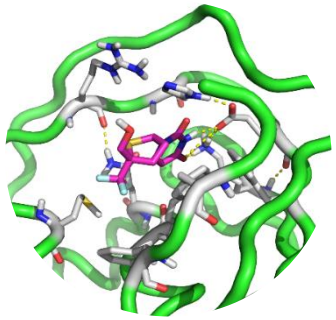
One hydrogen bond, one stacking

C

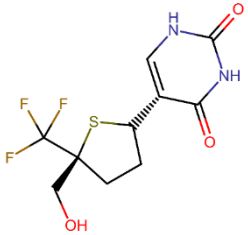
Fig. 3. Docking and simulation of molecular dynamics of 10S-46 with influenza virus proteins :
A - RdRp CBD (PDB ID: 4J2R); B - Neuraminidase (PDB ID: 3TI6); C - M2 (PDB ID: 2RLF)

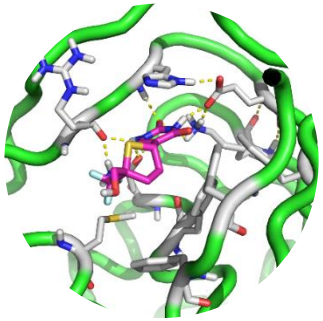
Results and discussion

Potentially bioactive derivatives of 10S-46

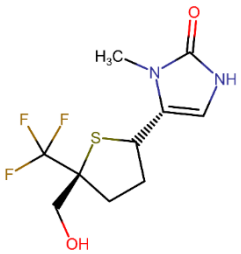


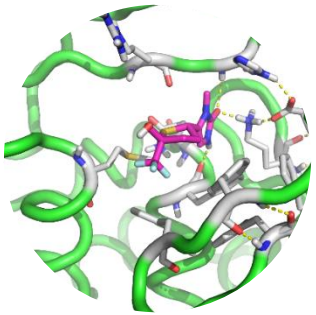
10S-46



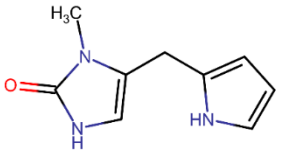


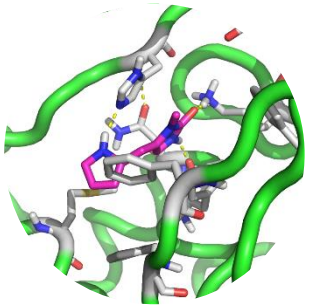
10S-46-m2





10S-46-m16



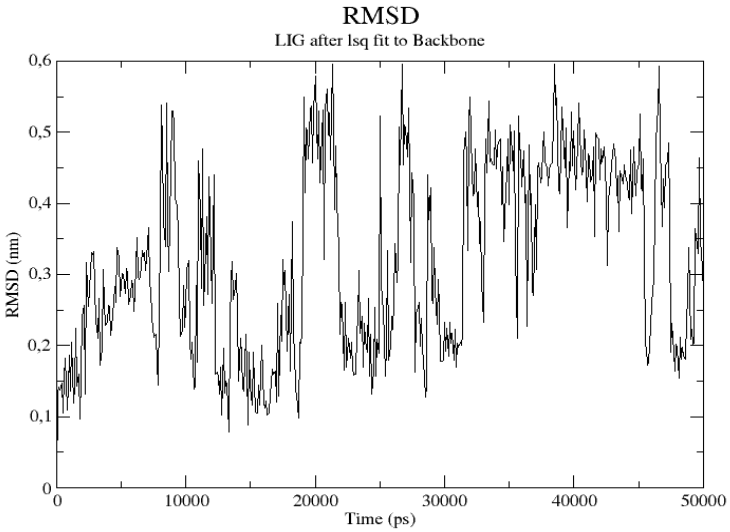


10S-46-m18

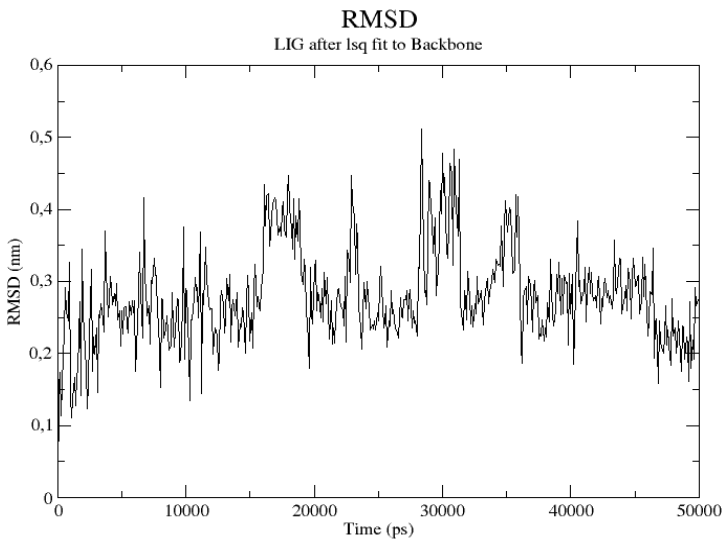
Iterative approach and simulation of molecular dynamics

Results and discussion

Stability of the complex - CBD with the designed compound



A

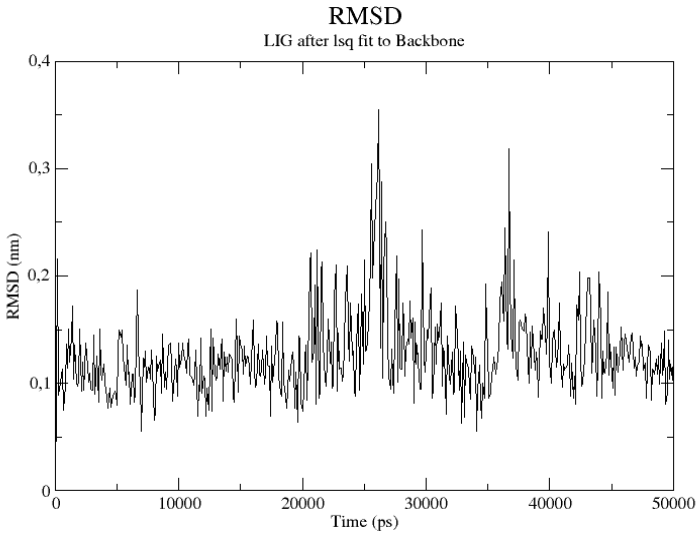


B

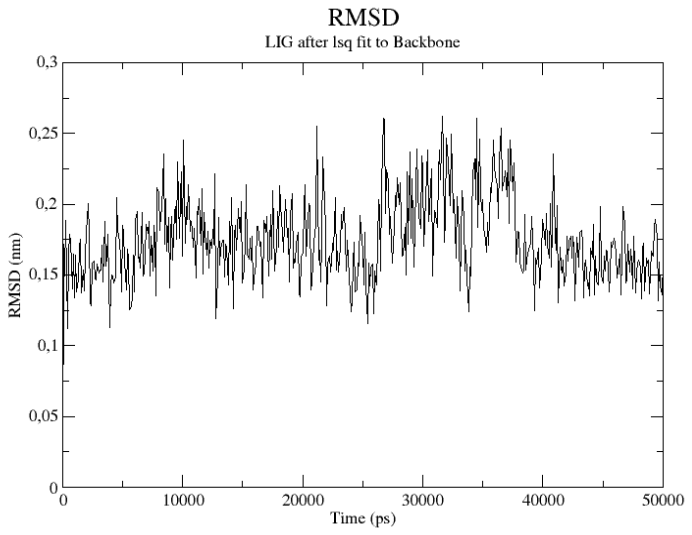
Fig. 4. The root-mean-square deviation of compounds in complex with CBD:
A – original 10S-46; B – derivative 10S-46-m2

Results and discussion

Stability of the complex - CBD with the designed compound



A



B

Fig. 5. The root-mean-square deviation of compounds in complex with CBD:
A – derivative 10S-46-m16; B – derivative 10S-46-m18

Conclusions

The newly synthesized compound 10S-46 *in vitro* has a relatively low toxicity and significant antiviral activity against influenza A virus H1N1. In addition, it has an inverse dose-dependent effect, understanding of which requires further research.

In silico, this substance is able to interact with the M2 ion channel and the Cap-binding domain of RdRp. However, binding to an active center of CBD involves the formation of more orienting interactions. Three derivatives designed on the basis of 10S-46 during the simulation experiment are characterized by greater stability in combination with CBD RdRp and can potentially show greater activity in inhibiting this viral enzyme.

Therefore, we believe that 10S-46 and its derivatives have a high anti-influenza potential and need further comprehensive research.