Citation: Zaremba P.Yu., Zaremba A.A., Siry S.A., Zahorodnia S.D. Antiviral Activity of Low-Molecular-Weight Fluorinated Compounds Against Influenza A (H1N1) Virus. Microbiological Journal. 2024 (2). P. 51—64. https://doi.org/10.15407/microbiolj86.02.051

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ISSN 1028-0987. Microbiological Journal. 2024. (2)
Fluoride plays an important role in the development of medicine products since the first fluorine-containing drug appeared in the 1950s (Meanwell, 2018). The use of fluorine in drug development continues to grow, as does our knowledge and understanding of how to take full advantage of this element’s unique properties. The electronegativity, size, and lipophilicity of fluorine, together with electrostatic interactions, can strongly influence the chemical reactivity of the substance. Even one atom can completely change the biological properties of a compound. One of the main effects of fluorination is a change in the acid-base properties of a compound, which can greatly affect the binding affinity and bioavailability of a potential drug (Ismail & Ayoup, 2022). Fluorine also has the ability to increase lipophilicity (in particular CF₃, S—CF₃, and O—CF₃ radicals), which, together with the molecular size, affects the membrane permeability. The replacement of a hydrogen atom with fluorine also results in an increase in the metabolic stability of compounds (Cavaliere et al., 2017). All these effects are quite attractive in the perspective of drug development.

The number of successful fluorine containing drugs increases every decade. Among their wide variety, there are relatively few antivirals but they are quite effective. A significant number of the latter with different mechanisms of action are used in the antiretroviral therapy (Eggink et al., 2019; Amblard et al., 2022; Muller & Al Khalili, 2023) and in general for inhibiting the reproduction of RNA-viruses (Delang et al, 2018).

Also, the latest approved drug for the treatment of influenza A and B viruses is fluorine-containing baloxavir marboxil (commercial name «Xofluza»). It blocks the viral replication by inhibiting the initiation of the mRNA synthesis. However, despite the good results of clinical trials and clinical use, this drug is not approved for people under the age of 12 years (Dufrasne, 2021). Taking into account the fact that the complications of influenza infection are most severe in people belonging to risk groups, in particular children under 5 years of age (as well as pregnant women, the elderly, and immunocompromised individuals), and taking into account the rate at which influenza viruses acquire resistance to drugs, the search for new compounds continues constantly.

Therefore, the goal of this study was to investigate the activity of a group of low-molecular-weight fluorinated compounds against the influenza A (H1N1) virus and to determine the potential mechanism of their action using in silico methods.

Materials and Methods. Cell culture and virus. The experiments were conducted using MDCK cells (Madin-Darby canine kidney cells), which were provided by the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine.

Influenza virus A (H1N1) virus strain A/FM/1/47 was obtained from the L.V. Gromachevsky Institute of Epidemiology and Infectious Diseases of the NAS of Ukraine.

Studied compounds. Fluorinated low-molecular-weight compounds 10S-45, 10S-46, 10S-47, 10S-48, and 10S-49 were synthesized at the Institute of Organic Chemistry of the NAS of Ukraine. All the compounds, except for 10S-48,
are a mixture of diastereomers, their names are given using stereochemical descriptors according to R, S nomenclature.

- \(10S-45\): (2RS,5SR)-2-Hydroxy-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene and (2SR,5SR)-2-hydroxy-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene;
- \(10S-46\): (2RS,5SR)-2-(2,4-Dioxopyrimidine-1-yl)-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene and (2SR,5SR)-2-(2,4-dioxopyrimidine-1-yl)-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene;
- \(10S-47\): Bis(5-difluoromethyl-2-(tetrahydropyran-2-yl)-2H-triazole-4-yl)sulfone;
- \(10S-48\): (2RS,3RS,4RS)-2-(2,4-Dioxopyrimidine-1-yl)-3-hydroxy-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene and (2SR,3SR,5SR)-2,3-Dihydroxy-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene;
- \(10S-49\): (2SR,3SR,5SR)-2,3-Dihydroxy-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene and (2RS,3SR,5SR)-2,3-Dihydroxy-5-hydroxymethyl-5-(trifluoromethyl)tetrahydrothiophene.

Oseltamivir phosphate, trade name «Tamiflu» (F. Hoffmann-La Roche Ltd., Switzerland) was used as a reference drug.

**Cell culture cultivation.** MDCK cells were grown in a medium consisting of 46% DMEM (Sigma, USA), 46% RPMI-1640 (Sigma, USA) and 8% heat-inactivated fetal bovine serum or FBS (Sigma, USA) with the addition of a penicillin-streptomycin solution (100X, Biowest, France). The cells were subcultured every 48 hours with a multiplicity of 1:10, after a preliminary check of the integrity of the monolayer. Trypsin-EDTA 0.25% solution (Biowest, France) and Versene 0.02% solution (Biowest, France) were used to remove cells from the surface of the culture flask, and then resuspended in the medium. Cells were incubated in a thermostat at 37 °C in an atmosphere of 5% CO₂.

**Determination of cytotoxicity.** An MTT assay was performed to determine cytotoxicity. 100 μL of cell suspension (density 2.5—3 · 10⁵ cells/mL) was added into 96-well culture plates the day before. On the following day, the supernatant was removed from the wells, and 200 μL of compounds diluted in the growth medium was added. After incubation for 48 hours at 37 °C in an atmosphere of 5% CO₂, 20 μL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) dissolved in sterile phosphate buffer (PBS, pH = 7.2) at a concentration of 5 mg/mL was added. The plate was kept for 3—4 hours at 37 °C. Then, the supernatant was removed, and 150 μL of 96% ethanol was added. After dissolution, the optical density was measured on a Multiskan FC reader (Thermo Scientific, Waltham, MA, USA) at a wavelength of 538 nm. Based on the obtained data, CC50 indexes (cytotoxicity concentration 50%) were calculated.

**Determination of antiviral activity.** 100 μL of cell suspension (density 2.5—3 · 10⁵ cells/mL) was added in 96-well culture plates and left for 24 hours of incubation at 37 °C in an atmosphere of 5% CO₂. After that, the medium was removed, and cells were washed once with PBS (pH = 7.2). A suspension of influenza A (H1N1) virus (TCID₅₀/mL = 10⁸.₈₄) in a DMEM medium (Sigma, USA) was added in 40 μL per well and incubated for 60—90 min in a thermostat at 37 °C in an atmosphere of 5% CO₂. After the incubation, the supernatant was removed, and 200 μL of the test compounds diluted in 48% DMEM (Sigma, USA), 48% RPMI-1640 (Sigma, USA), 4% FBS and TPCK-treated trypsin 2 μg/mL (Sigma, USA) were added. The plate was left for incubation at 37 °C and 5% CO₂. After 48 h, the presence of CPE was checked using an inverted microscope. Next, the medium was removed, and 50 μL of 5 mg/mL crystal violet dye (neoFroxx, Germany) was added, then the plate was incubated on a shaker for 5 min and washed under running water. After washing, the plate was left to dry for 24 h at room temperature, then 150 μL of 96% ethanol was added. The optical density was measured at a wavelength of 538 nm on a Multiscan FC spectrophotometer (Thermo Scientific, Waltham, MA, USA). On the basis of the obtained results, the
indicator of antiviral activity and the EC50 index (half maximum effective concentration) were calculated. The indicator of antiviral activity of the compound (inhibition of the viral reproduction) was calculated according to the following formula:

\[
\text{Inhibition of the viral reproduction} = \frac{A - B}{C - B} \times 100\%
\]

where \(A\) is the optical density of the studied concentration of the compound, \(B\) is the optical density of the virus control, and \(C\) is the optical density of the cell control.

**Preparation of input data for molecular dynamics simulation.** The three-dimensional structure of the RdRp PB2 Cap binding domain (CBD) was obtained from the Protein Data Bank (PDB ID: 4J2R) (Zardecki et al, 2022). The initial preparation was carried out using PyMOL 2.3 (Schrödinger & DeLano, 2020) and involved the removal of small molecules and the second CBD monomer.

The input structural data of the ligands were obtained in the SDF format. RDKit 2022.9.5 (Landrum, 2016) was used to generate all possible stereoisomers of all compounds except for 10S-48, with the additional permission to analyze all stereocenters and remove overlapping structures. Open Babel 3.1 (O’Boyle et al., 2011) was used for conversion to the SMILES, PDB, and MOL2 formats.

**Molecular docking.** The prepared ligands and PB2 CBD monomer were used for flexible molecular docking. MGLTools 1.5.7 and AutoDock Vina 1.2.3 were used for docking (Eberhardt et al., 2021). The grid box encompassed the m7GTP binding site and was \(40 \times 40 \times 40\) Å with the center at \(19.817 \times -36.662 \times -29.562\) (XYZ). F323, F325, F330, R332, H357, E361, F363, K376, F404, Q406, and M431 were considered flexible. Exhaustiveness was equal to 32.

**Molecular dynamics simulation.** One optical isomer of each of the substances selected on the basis of the interaction energy (10S-48 had a known three-dimensional structure) was investigated within the framework of a simulation experiment in a complex with PB2 CBD. The actual molecular dynamics simulation was performed using the GROMACS 2020.6 software package (Abraham, 2015) in the CHARMM27 force field (Vanommeslaeghe et al., 2010) and the TIP3P three-point water model (Pekka & Lennart, 2001). The ligand was parameterized using the free SwissParam online server (Zoete et al., 2011). In the process of building the complete system, the CBD/ligand complex was placed in a box in the form of a truncated octahedron, which was filled with a clear solvent with a physiological concentration of NaCl (0.156 M). The distance between the complex and the box walls was kept at 14 Å. Periodic boundary conditions were used. Electrostatic energy was calculated using the PME (Particle-Mesh Ewald) method. Coulomb and van der Waals interactions had a limiting distance of 1.2 nm. Energy was minimized using the steepest descent algorithm to a system energy value of less than 1000 kJ/mol/nm (50,000 steps). Equilibration was carried out in two consecutive phases, each with a length of 100 ps. First, there was equalization of temperature (300 K), then of pressure (1 atm). Direct simulation of molecular dynamics was carried out for 100 ns, with a time step of 2 fs.

**Statistical data processing.** Results obtained in silico were analyzed using standard software provided by GROMACS (trjconv, rms) and visualized using XMGRACE (Turner, 2005) and PyMOL 2.3.

For each compound concentration, in vitro experiments were performed three times in three independent replicates. Statistical data processing was performed in Microsoft Excel 2010 and Origin 2018 programs. According to the standard approaches, statistical errors (standard deviation) were calculated, and confidence intervals were determined (for all arrays \(p \geq 0.05\)).

**Results. Cytotoxicity.** For all compounds, including the reference drug, toxicity was determined in the concentration range from 1000 to 15 μg/mL. Oseltamivir phosphate at the studied
concentrations was not toxic to MDCK, it did not cause a decrease in cell viability by less than 80% compared to the control (the optical density of the cell control was taken as 100%).

A dose-dependent action was established for the fluorinated compounds: the cell viability decreased with increasing concentration. The calculated CC50 indicators of compounds 10S-45, 10S-46, 10S-47, and 10S-48 were 537.08 ± 26.85 μg/mL, 529.57 ± 26.48 μg/mL, 471.36 ± 23.57 μg/mL, and 577.50 ± 28.87 μg/mL, respectively. Substance 10S-49 turned out to be almost 2 times less toxic compared to the rest, its CC50 equaled 1156.67 ± 57.83 μg/mL.

**Antiviral activity.** Compound 10S-49 showed no antiviral activity against influenza A virus at all concentrations ranging from 0.5 μg/mL to 100 μg/mL. The results of the determination of the antiviral activity for the remaining compounds are presented in Fig. 1.

Compounds 10S-45, 10S-46, and 10S-48 sufficiently inhibit the influenza virus reproduction in MDCK cells. At the same time, there is a dependence: as the concentration of the substance decreases, its antiviral activity increases. Thus, 10S-45 at 100 μg/mL inhibits the viral reproduction by 29.80 ± 1.49%, and at 0.5 μg/mL — by 66.93 ± 3.34%; 10S-46 — at 100 μg/mL by 14.96 ± 0.75% and at 0.5 μg/mL — by 70.25 ± 3.51%. Compound 10S-48 suppresses the viral reproduction at 100 μg/mL by 12.30 ± 0.61% and at 0.5 μg/mL by 67.86 ± 3.39%.

Substance 10S-47 at the tested concentrations showed a relatively stable inhibition of the influenza virus reproduction in the range of 63—69%, with a peak of 76.31 ± 3.81% at 5 μg/mL. Such efficacy is similar to the results for the reference drug, which also demonstrated a relatively similar percentage of inhibition of the viral reproduction — about 80%, with a peak of 84.43 ± 4.22% at 50 μg/mL.

**Calculation of the selectivity index.** The selectivity index (SI) of a compound is a widely used parameter that makes it possible to evaluate the effectiveness of a drug in inhibiting viral reproduction in vitro. It is the ratio between toxicity (CC50) and antiviral activity (EC50).

For the compounds that showed antiviral activity against the influenza A virus, the above ratio was calculated (Table 1).

Compound 10S-48 had the highest SI value (>25), while 10S-47 - the lowest (>8) among the tested fluorinated compounds. But compared to the SI of oseltamivir (>1250 for H1N1), these values are expectedly low (Fujiwara et al., 2002).
Table 1. The selectivity index of the tested compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>10S-45</th>
<th>10S-46</th>
<th>10S-47</th>
<th>10S-48</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50, μg/mL</td>
<td>51.49±2.57</td>
<td>50.96±2.55</td>
<td>57.76±2.88</td>
<td>22.48±1.12</td>
</tr>
<tr>
<td>SI (CC50/ EC50)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;8</td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

Table 2. Stereoisomers of the studied compounds and their affinity to the active site of CBD RdRp (semi-energetic)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structural formula</th>
<th>Affinity (kcal/mol)</th>
<th>Compounds</th>
<th>Structural formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>10S-45</td>
<td><img src="image1" alt="Structural formula" /></td>
<td>-4.882</td>
<td>10S-46</td>
<td><img src="image2" alt="Structural formula" /></td>
</tr>
<tr>
<td></td>
<td><img src="image3" alt="Structural formula" /></td>
<td>-5.027</td>
<td></td>
<td><img src="image4" alt="Structural formula" /></td>
</tr>
<tr>
<td></td>
<td><img src="image5" alt="Structural formula" /></td>
<td></td>
<td></td>
<td><img src="image6" alt="Structural formula" /></td>
</tr>
</tbody>
</table>
**Generation of stereoisomers.** For biologically active substances obtained as a mixture of diastereoisomers (10S-45, 10S-46, and 10S-47), all their possible spatial forms were generated (Table 2).

As seen, all compounds have two chiral centers. Structures 10S-45 and 10S-46 contain a tetrahydrothiophene ring in positions 2 and 5, of which there are stereocenters. Accordingly, 4 stereoisomers were generated for these compounds. Substance 10S-47 differs from the abovementioned ones due to the presence of two stereocenters not in one aliphatic ring but in two

<table>
<thead>
<tr>
<th>Affinity (kcal/mol)</th>
<th>Compound</th>
<th>Structural formula</th>
<th>Affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-6.811</td>
<td>10S-47</td>
<td><img src="image" alt="Structural formula of 10S-47" /></td>
<td>-8.448</td>
</tr>
<tr>
<td>-7.205</td>
<td></td>
<td><img src="image" alt="Structural formula of Compound" /></td>
<td>-9.461</td>
</tr>
<tr>
<td>-8.265</td>
<td></td>
<td><img src="image" alt="Structural formula of Compound" /></td>
<td>-10.01</td>
</tr>
<tr>
<td>-6.722</td>
<td>10S-48</td>
<td><img src="image" alt="Structural formula of 10S-48" /></td>
<td>-8.155</td>
</tr>
</tbody>
</table>

Flexible molecular docking
tetrahydropyran rings. Actually, the stereocenter itself is in the second position of the ring. So, 10S-47 has only three structural forms.

**Molecular docking.** The obtained 12 structures were docked to the active site of CBD RdRp. Their calculated affinity ranges from −4.882 kcal/mol to −10.01 kcal/mol (Table 2). Moreover, substance 10S-45 has the lowest calculated affinity, and 10S-47 has the highest. Compounds 10S-46 and 10S-48 are close by this parameter. As for 10S-46, it is in the range from −6.722 to −8.265 kcal/mol, and for the single 10S-48 stereoisomer, it equals −8.155 kcal/mol. At the same time, the calculated affinity of the most active form of 10S-46 is close (slightly higher) to that of 10S-48.

**Molecular dynamics simulation of the obtained complexes.** Selected on the basis of the calculated affinity, the only stereoisomer of each of the compounds provided in an undetermined isomeric form (10S-45, 10S-46, and 10S-47), as well as 10S-48, were investigated using the classical method of molecular dynamics simulation.

All compounds, except for 10S-45, were within the active site of CBD by the end of the simulation (Fig. 2). Substance 10S-45 showed no tendency to form orderly interactions with CBD. As early as 10 nanoseconds (ns) into the simulation, it left the docking zone and by the end of the experiment demonstrated disordered thermal movements.

**Fig. 2.** The studied compounds in a complex with CBD RdRp. Yellow dashed lines fragments indicate hydrogen bonds

**Fig. 3.** Root-mean-square deviation for substances 10S-45, 10S-46, 10S-47, and 10S-48 in a complex with CBD RdRp
Compound 10S-47, along with its stable location within the active center of the receptor protein, underwent quite significant conformational changes during the simulation period. They, however, concerned almost entirely only one of the two fluorinated 2-(oxan-2-yl)triazole residues, which interacted directly with the apparent solvent. Another residue, like the sulfonyl group, directly interacted with amino acids F323, R355, H357, F363, F404, Q406, and M431 through stacking and hydrophobic interactions. Orientational interactions, such as hydrogen and ionic bonds, between ligand and receptor were not observed within the scope of this simulation experiment.

Compound 10S-48 at the beginning of the simulation, within 1—9 ns, kept its original position acquired at the stage of molecular docking. Its complex with CBD was characterized by hydrogen bonds between the uracil ring, H357, and K376. Moreover, H357 acted both as a hydrogen bond donor (amine-K376/4O-uracil/amide-H357) and as an acceptor (3N-uracil/imidazole-H357). Hydroxyl groups of the tetrahydrothiophene ring did not form any hydrogen bonds. In addition, there were multiple stacking interactions between the ligand uracil and F404/Q406, as well as the sulfur atom in the tetrahydrothiophene ring, F323 and F325. After the initial period, 10S-48 underwent some significant conformational rearrangements. As a result, the interaction of the ligand with K376 was completely lost. Instead, hydrogen bonds with Q406 and R332 appeared. They, as well as the interaction with H357, were present in single frames (resolution = 1 ns) of the trajectory. At the same time, no significant difference in the stacking interaction pattern was observed.

Compound 10S-46 also maintained its initial position after the start of the simulation and up to 5 ns. Its interaction pattern consisted of hydrogen bonds between the aromatic uracil ring and amino acids H357, E361, and K376 and hydrophobic stacking interactions of both the tetrahydrothiophene ring and the ligand uracil with amino acids H357, F404, and Q406. After this short initial period, a loss of all the orientational contacts listed above was observed, although the hydrophobic and stacking bonds remained intact. No long-term hydrogen bonds were observed up to 42 ns of the experiment. Starting from 43 ns of simulation, 10S-46 stabilization was observed with the restoration of orientational interactions between the aromatic ring of uracil and amino acids H357 and K376. Moreover, H357 acted both as a donor and an acceptor of hydrogen bonds, similar to the experiment with 10S-48.

The root-mean-square deviation (RMSD) of the investigated compounds in a complex with CBD generally corresponds to their trajectory (Fig. 3). In particular, a rapid growth of the RMSD of compound 10S-45 is observed from the first nanoseconds. In the first 5 ns of the simulation, it reaches a value of above 10 Å and continues to increase. Smaller, but also significant, aberrations are observed in the case of substance 10S-47. Several conformational rearrangements of this compound can be clearly distinguished in periods of 1—3 ns, 4—35 ns, 36—81 ns, and 82—100 ns. Moreover, the largest RMSD is observed in the period between the second and third conformational forms. Subsequently, the RMSD gradually decreases to a value of 4 Å at the end of the simulation.

The RMSD of substances 10S-46 and 10S-48 changes similarly over time. In particular, in both cases, it ranges from 1 to 3 Å. Also, there is some short initial period of the very low RMSD followed by its jump and stabilization at a new level. However, there are significant differences. In particular, 10S-46 undergoes primary rearrangements 5 ns earlier than 10S-48. Besides, this compound is characterized by a short negative RMSD jump and, accordingly, another period of conformational rearrangements. The RMSD falls and stabilizes within 2.5 Å at the level of 42 ns of the simulation. In general, the range of the RMSD is significantly higher in 10S-48 compared to 10S-46.
Discussion. An important factor in the process of developing any drug is its safety. A candidate substance must exhibit antiviral activity at concentrations that do not exhibit a toxic effect on cells. The fluorinated compounds investigated in this work correspond to the above-mentioned parameter: the calculated CC50 indices are significantly higher than the EC50 parameters.

The selectivity index allows for evaluating the efficiency of inhibition of the viral reproduction by a substance. The higher its value, the more effective and safer the drug will be in vivo. The SI values obtained in vitro are a reference point in the search for active compounds for the further development of drugs. Since in general, those with SI ≥10 are called active substances, the studied compounds 10S-45, 10S-46, and 10S-48 can also be considered active against the influenza A (H1N1) virus type and promising for further improvement. Based on the results of determining the antiviral activity, they effectively enough inhibit the influenza A virus reproduction in MDCK cells. At the same time, a dependency is observed: as the concentration of the substance decreases, its inhibitory activity increases. Such a dependence can be related to many factors. First of all, it is influenced by the direct target of a compound and with which metabolic pathways of the cell it can interact. One of the explanations for this effect may be the presence of specific ATP-binding cassette transporters (so-called ABC transporters) in MDCK cells. This is a superfamily of proteins that transport various substrates across cell membranes using the energy of ATP hydrolysis. They have been shown to play a role in the development of multiple drug resistance. In particular, three subfamilies of these proteins cause cell resistance to anticancer drugs (Gottesman et al., 2002). Thus, based on the research data, it can be assumed that compounds 10S-45, 10S-46, and 10S-48 at certain concentrations activate the work of ABC transporters, which in turn export excess molecules of the substance from the cell.

Unlike the above-mentioned compounds, substance 10S-47 has a slightly different pattern of activity, without a dose-dependent effect. We assume that in the studied range of concentrations, its maximum effect on the influenza virus reproduction is expressed. However, despite the high antiviral activity, 10S-47 is more toxic compared to the rest of the compounds and, accordingly, has a lower SI. Considering its high potential, it can be put in a row with other investigated substances, especially taking into account the perspective of molecular modifications.

RNA-dependent RNA polymerase is the key protein complex of influenza virus essential for the replication of the viral genome. In addition to the replicative function, RdRp is also responsible for the implementation of genetic information, in particular, for the transcription process, which is initiated by the phenomenon called «cap-snatching». It consists in using RNA polymerase II of the host as an adapter specific to the host mRNAs and its own additional functionality of RdRp - cap binding domain (CBD) and RNA endonuclease domain (Pagadala et al., 2020). After binding m7GpppNm CBD, the endonuclease domain cleaves the host mRNA at the level of 10—15 nucleotides from the cap. Subsequently, this short fragment is used as a primer for the amplification of viral RNA.

Currently, three main classes of anti-influenza drugs targeting RdRp are considered promising and are actively being developed: inhibitors of RNA polymerase activity, inhibitors of cap-snatching, and inhibitors of the polymerase subunits interaction with each other (Pagadala et al., 2020). In the case of inhibition of polymerase activity, the majority of candidates and approved drugs have an irreversible mechanism of action (Wu et al., 2017). Instead, for the PPI (protein-protein interface) inhibition, substances on average have a significant size to mimic the protein surface as best as possible (Massari et al., 2021). Since most of the substances investigated in this work are not of sufficient size, and it is impos-
sible to simulate chemical reactions within the classical molecular dynamics simulation, CBD RdRp was chosen as the most likely target. The influence on this target likely mediates their above-described antiviral activity.

Most of the compounds presented were obtained as a mixture of diastereoisomers, so the first step in identifying their potential target was the generation of all their available stereoisomeric forms. 11 of their optical isomers were obtained from 3 initial structures. All obtained structures, as well as the single form of 10S-48, were used for semi-flexible docking with CBD as a receptor. Most of the amino acids that directly form the cap-binding center of the domain were considered flexible.

Among all structures, compound 10S-47 had the best calculated affinity, and 10S-45 had the worst, which is obviously related to the difference in their sizes and, accordingly, the surfaces of interaction with CBD. Substances 10S-46 and 10S-48 had similar calculated affinities. The best isomer of 10S-46 was characterized as even slightly better in this parameter. In turn, this is a consequence of their structural and optical similarity. These compounds are distinguished only by the presence of an additional hydroxyl group in the tetrahydrothiophene ring in 10S-48.

To understand the stability level of the complexes of the studied compounds with CBD RdRp, each of the substances was investigated within the framework of the classical molecular dynamics simulation method. For this, each compound was involved in a single stereoisomeric form in the conformation obtained after molecular docking. The latter also applied to amino acids in the receptor composition, which at the previous stage of the study were considered mobile.

During the simulation experiment, compound 10S-45 showed extremely low affinity for the target protein. It did not form any ordered interactions with CBD and left its cap-binding center already in the first nanoseconds of the simulation. Correspondingly, the RMSD of this compound is significantly beyond the optimal range for the predicted small-molecule effectors.

The next in terms of stability can be considered the 10S-47/CBD complex. During the molecular dynamics simulation of this complex, significant changes were observed in the position of the ligand, especially of its part that was in direct contact with the solvent. This was reflected in the RMSD level: sharp conformational transitions of this compound can be clearly observed along with its gradual stabilization near the end of the simulation. In addition, we did not observe stable orientational bonds between receptor and ligand, which indicates the low selectivity of such an interaction.

As an example of a more stable but also non-selective interaction, the 10S-48/CBD complex can be considered. The initial high structural features of this associate is quickly interrupted by the loss of hydrogen bonds between receptor and ligand. This is manifested in the growth of RMSD from 1 to 3 Å. The compound remains in this position until the end of the simulation. At the same time, the range of its RMSD is quite significant, which indicates in favor of significant oscillatory movements of 10S-48 around one position. This is consistent with the loss of all hydrogen bonds at the beginning of the simulation.

The only substance that can be considered a CBD inhibitor with a relatively high probability is 10S-46. This compound up to 42 ns of simulation had similar dynamics of interaction with the receptor to that of 10S-48. Also present is the loss of all orientational interactions at the beginning of the simulation. However, after a relatively long period of conformational instability, 10S-46 restores interaction with amino acids H357 and K376. This is reflected in the drop in the RMSD to the level of 2.5 Å, which is not the optimal value characteristic for completely stable compounds and indicates a change in the position of the studied compound relative to its initial position. However, the stability of the hydrogen bonds and the low RMSD range suggest a stable interaction of 10S-46 with CBD in this new conformation.
Conclusions. Four out of five investigated fluorinated compounds (except for 10S-49) show the ability to inhibit the reproduction of the influenza A (H1N1) virus in MDCK cell culture with the inhibition efficiency close to that of the reference drug. Based on this, the possible mechanism of the influence of the studied compounds was checked using molecular dynamics simulation, in particular, the ability of the substances to interact with the cap binding domain of the PB2 RdRp subunit. Having summarized the results obtained in silico, we can conclude that substances 10S-47 and 10S-48 have some inhibitory effect on the cap-snatching process, but this impact is probably not specific to this target alone. Compound 10S-45 has an extremely low probability of exhibiting an inhibitory effect on CBD RdRp. Instead, compound 10S-46 is most likely an inhibitor of the cap-binding activity of RdRp. Although, probably, its complex with the target protein will differ from the one calculated at the level of molecular docking.

Thus, the studied fluorinated compounds 10S-45, 10S-47, 10S-48, and especially 10S-46, in view of their moderate toxicity and pronounced activity against the influenza virus, are good candidates for further research on the role of antiviral drugs.


Conflict of interest. The authors declare that there are no conflicts of interest.

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Antiviral Activity of Low-Molecular-Weight Fluorinated Compounds Against Influenza A (H1N1) Virus


Received 17.07.2023
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ПРОТИВІРУСНА АКТИВНІСТЬ НИЗЬКОМОЛЕКУЛЯРНИХ
ФТОРОВАНЬ ФТОРАМИ ФТОРОВАНИХ СПОЛУК ІЗ ВІРУСУ ГРИПУ ТИПУ А (H1N1)

Вірус грипу типу A займає стійке положення у щорічних спалахах ГРВІ під час осінньо-зимового періоду. Противірівневі лікарські засоби при грипоподібній інфекції допомагають значно полегшити перебіг хвороби та запобігти появи ускладень, проте досі не існує ефективного препарату, до якого не був би резистентним жоден з циркулюючих штамів. Тому пошук нових ефективних препаратів проти вірусу грипу типу A проводиться постійно, зокрема і серед фторвмісних органічних сполук, зважаючи на особливі властивості фтору.

Мета. Дослідити активність групи низькомолекулярних фторованих сполук відносно вірусу грипу типу A (H1N1) та визначити потенційний механізм їхньої дії за допомогою методів in silico.

Методи. У роботі досліджували 5 фторованих сполук, позначені як 10S-45, 10S-46, 10S-47, 10S-48 та 10S-49. Експерименти in vitro проводили з використанням культури клітин MDCK, штаму A/FM/1/47 вірусу грипу типу A (H1N1) та озельтамівіру фосфату у якості референс-препарату. Цитотоксичний вплив на культуру клітин вимірювали за допомогою MTT-тесту. Антивірусний ефект досліджували через пост-експозиційний інкубаційний тримання сполук з клітинами за допомогою лізенії фіолетовим. Оцінку можливості взаємодії сполук з кеп-зв'язуючим доменом (CBD) субодиниці РВ2 РНК-залежної РНК-полімерази вірусу проводили за допомогою симуляції молекулярної динаміки.


Висновки. Усі досліджувані сполуки (крім 10S-49) демонструють антивірусну активність in vitro відносно вірусу грипу типу A (H1N1). За результатами аналізу їх взаємодії з CBD PB2 in silico речовина 10S-46 з високою ймовірністю може бути інгібітором саме кеп-зв'язуючої активності RdRp.

Ключові слова: антивірусні агенти, фторовані сполуки, віруси грипу, молекулярна динаміка.