ABSTRACT

Both Influenza A virus (IAV) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) belong to the group of RNA viruses. One, as well as the other viruses, are the trigger of pandemic and epidemic outbreaks in the world. Every year, the World Health Organization (WHO) records 3-5 million cases of severe flu and 290,000-650,000 deaths, and in the last 2 years of the pandemic, almost 652 million cases and over 6.5 million deaths have been reported to the WHO as caused by the Coronavirus disease 19 (COVID-19) pandemic. Both SARS-CoV-2 and IAVs attack the respiratory tract and cause similar symptoms. Due to the serious threat to public health posed by mentioned viruses, there is a need to look for new strategies to cure illnesses they cause and limit their spread.

The IAV genome consists of eight single-stranded, negative sense RNA segments. Each segment is a separate replication and transcription unit and encodes certain viral proteins. So far, it has been found that segments form complex secondary structures that contain conserved among virus strains motifs. It was also demonstrated that specific motifs play important roles in the viral replication cycle. Whereas SARS-CoV-2 has about 30 kb, 5′ capped positive sense, single-stranded RNA genome. The role of some structural motifs of the viral RNA has been confirmed experimentally. Because of its functional importance and due to the fact that both viruses' replication cycles are RNA-dependent with no DNA intermediate, RNA makes a good target for potential new antiviral strategies.

In this work, the search for small molecules was carried out as potential inhibitors of IAV and SARS-CoV-2. 1280 compounds Lopac library and 8960 compounds Enamine library were searched using high throughput screening analysis with fluorescent indicator displacement assay. Conserved RNA structural motifs of IAV and SARS-CoV-2 were used as targets in the assay. Fourteen compounds from the Lopac library and twenty-one compounds from the Enamine library that bind to the RNA motifs were selected. Their inhibitory characteristics against the IAV A/California/04/2009 (H1N1) were next checked in MDCK cells.

The results indicate that most of the tested compounds from both libraries have the potential to inhibit influenza virus propagation by targeting viral RNA. The most crucial reduction of the relative vRNA copy number from the Lopac SMs was achieved by the

influence of SML1545, B6311, and M4008 compounds. The mentioned SMs decreased the number of vRNA copies in the analyzed experiment by over 60%. Additionally, a significant reduction of viral titer was caused by SML2238. In the case of the Enamine library, three of the seven selected nontoxic compounds showed crucial inhibitory properties. Z154467994 showed the most significant inhibitory properties of all selected SMs. At 10 μM, it reduced the relative vRNA copy number by over 36 % compared to the untreated control. While Z134817028 reduced viral RNA copy number by over 27% in the highest concentration. Z215664726 at the highest concentration lowered the relative vRNA copy number compared to the control by 35% and greatly decreased the viral titer.

What is more, in order to check the binding affinity of promising ligands to selected vRNA motifs and their binding site, molecular docking was performed. For this purpose, the molecular dynamics simulation of RNA motifs was conducted first. In accordance with trajectory analysis, clustering was performed. On the basis of which tertiary structures of selected RNA motifs were chosen and used for molecular docking with compounds found in previous experiments.

The described studies showed that the conserved structural motifs of the viral RNA are natural and good targets for antiviral strategies. However, the appropriate selection of the sequence and structural context of the RNA target site is of great importance for the activity of the inhibitor. Different antiviral approaches have different advantages and limitations that must be considered for their effective application. Moreover, the obtained results can contribute to the creation of an experimental basis for the design of effective and specific therapeutic strategies involving small molecules.