medium with addition of 10% bovine serum albumin under 5% CO_2 atmosphere until monolayer formed. Serial dilutions were prepared from the studied samples in MEM medium from 300 to 4 μg/mL. Dissolved samples were placed into plate wells and incubated for 48 h at 36°C. After that, the cells were washed 2 times for 5 min with phosphate salt buffer and the number of living cells was estimated by microtetrazolium test (MTT). For this purpose, 100 μL of 0.5 g/L solution of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (ICN BiochemicalsInc., Aurora, Ohio) on MEM medium was added into each well. The cells were incubated at 36°C under 5% CO_2 atmosphere for 2 h and washed for 5 min with phosphate salt buffer. The precipitate was dissolved in 100 μL DMSO per well and optical density was measured on a ThermoMultiskan FC plate reader at wavelength of 540 nm. Fifty percent cytotoxic dose (CC_{50}), i.e., concentration required for 50% death of cells, was determined for each compound.

Study of anti-influenza virus activity in vitro. 100 μL of the compounds dissolved in MEM with 1 μg/mL trypsin was added into wells with MDCK cells and the plates were incubated for 1 h at 36°C at 5% CO_2. Cells were infected with 100 μL of influenza virus A/Puerto Rico/8/34 (H1N1) (m.o.i. 0.01) for 1 h. Cells were washed twice with MEM and the fresh medium containing the compounds at the same concentrations was added. The plates were kept for 24 h at 36°C at 5% CO_2. The culture medium was used for the preparation of the series of 10-fold dilutions, fresh cells were infected with the dilutions, and the plates were incubated for 48 h at 36°C at 5% CO_2. After 48 h 100 μL of culture fluid was transferred into the wells of round-bottom plates. 100 μL per well of 1% suspension of chicken erythrocytes in saline was added and the results were checked after 40 min incubation at room temperature. The infectious titer of the virus was considered as a reciprocal to the maximum virus dilution that caused a complete erythrocytes agglutination. The decrease in the infectious titer of the virus indicated the antiviral activity of compounds. The data obtained were used for the calculation of the 50% effective concentration of the compound, or the substance concentration that caused the two-fold decrease in the virus titer (IC_{50}), and then the selectivity index was calculated, $SI=CC_{50}/IC_{50}$.

Molecular docking

All theoretical calculations (receptor and ligands preparations, molecular docking procedure and binding energies calculations) were carried out using the Small Molecule Drug Discovery Suite 2018-4, Schrödinger). The geometrical parameters of the proteins and ligands were subjected to restrained minimizations using the OPLS3e [34] force field. Docking was performed under the following conditions: a flexible ligand and protein (Glide docking protocol); standard prediction accuracy, the grid-box size of 15Å with the native being located at the center. Ligand binding energies and receptors were calculated using MM-GBSA technology.[34]

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

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Author Contribution Statement

Oleg I. Artyushin and Aleksandra A. Moiseeva carried out chemical transformation. Vladimir V. Zarubaev, Aleksandr V. Slita, Anastasiya V. Galochkina, and Anna A. Muryleva carried out biological assay. Sophia S. Borisevich carried out all theoretical calculations. Olga I. Yarovaya and Nariman F. Salakhutdinov carried out structure elucidation and prepared the manuscript. Valery K. Brel designed the experiments. All authors read and approved the manuscript.

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