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New class of hantaan virus inhibitors based on conjugation of the isoindole fragment to (+)-camphor or (−)-fenchone hydrazonesv

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ABSTRACT

This work presents the design and synthesis of camphor, fenchone, and norcamphor *N*-acylhydrazone derivatives as a new class of inhibitors of the Hantaan virus, which causes haemorrhagic fever with renal syndrome (HFRS). A cytopathic model was developed for testing chemotherapeutics against the Hantaan virus, strain 76–118. In addition, a study of the antiviral activity was carried out using a pseudoviral system. It was found that the hit compound possesses significant activity ($IC_{50} = 7.6 \pm 2$ μM) along with low toxicity ($CC_{50} > 1000$ μM). Using molecular docking procedures, the binding with Hantavirus nucleoprotein was evaluated and the correlation between the structure of the synthesised compounds and the antiviral activity was established.

Haemorrhagic fever with renal syndrome (HFRS) (synonyms: Haemorrhagic nephritis, Churilov's disease, epidemic nephritis, Far Eastern haemorrhagic fever, Korean haemorrhagic fever, Manchurian haemorrhagic fever, Scandinavian epidemic nephropathy, Tula fever) is an acute viral, natural-focal disease, characterised by systemic lesions of small vessels, haemorrhagic diathesis, haemodynamic disorders, and a peculiar lesion of kidneys with acute renal failure.[1] The HFRS pathogen belongs to the Orthohantaviruses, previously known as hantaviruses (family Hantaviridae, order Bunyavirales).[2] Over the past decades, Hantavirus diseases have been included in the range of topical and high-priority problems around the world, as one of the so-called emerging (unpredictable) infections, threatening complex epidemic situations.[3] This is due to the variability of the Hantavirus genome, and, therefore, is fraught with the emergence of new types and genetic variants in new regions of the world, with high virulence for humans. To date, >30 serologically and genetically different Hantaviruses are known. Two clinical forms of the Hantavirus infection in humans are described: HFRS caused by Hantaan, Seul, Puumala, Dobrava/Belgrade, Seoul, Amur; and Hantavirus pulmonary syndrome, first described in the USA in 1993, is caused by Sin-Nombre, Black Creek, New York, Bayou, Andes, and Laguna Negra Hantaviruses. The first clinical form of the disease (HFRS) is registered in Russia, and the circulation of 7 types, including 4 pathogenic Hantavirus types, is established. In the European region, the HFRS pathogen in the vast majority of cases is the Hantaan- and Puumala-type. HFRS is widespread mainly in China, the Republic of Korea and the Russian Federation, as well as in Sweden, Finland, and western and central Europe.[4] China has experienced approximately 90% of the cases worldwide over the last few decades.[5,6] From 2006 to 2015, >110,000 cases of HFRS were reported in China,[7] and from 2000 to 2018 , >130,000 cases were registered in the Russian Federation.[8] The hantavirus prototype strain, HTNV, was first isolated from the striped field mouse, *Apodemus agrarius*, in 1976.[9] The discovery of the etiological agent of HFRS in South Korea prompted research all over the world, resulting in the discovery of other HFRS-associated novel viruses in the Old World. Hantaviruses have since been discovered to circulate not only in Asia and Europe, but also in both the Americas and Africa.[10,11]

In addition to the serious epidemiological situation around the world, viruses causing HFRS can pose a threat as biological weapons. Depending on the severity of the disease they cause and their ability to spread, biological agents are classified by the Center for Disease Control

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and Prevention (CDC) into three categories: A–C. Category A agents include haemorrhagic fever viruses (some filoviruses, flavoruses, bunyaviruses and arenaviruses) and pox viruses, including Variola major (smallpox is natural). Viruses causing HFRS are considered to be potential weapons in bioterrorism attacks.[12]Fig 1.

The main route of infection is airborne dust, where the virus, contained in the biological excretions of rodents, in the form of an aerosol enters through the upper respiratory tract to the lungs, where the conditions for its reproduction are most favourable, and then is transferred with blood to other organs and tissues.[13] Infection is also possible through damaged skin, when exposed to excreta of infected rodents or to saliva when bitten by another human. Despite the severity of the disease and its widespread occurrence, there is no specific antiviral therapy at present. Treatment may be carried out with a wide range of drugs, including ribavirin.[14] Recent clinical studies (placebo-controlled, double-blind) have shown no efficacy of ribavirin in the treatment of HFRS.[15] It has been shown that Triazavirin, which has been approved in Russia as an anti-influenza agent, is active against the Hantavirus *in vitro*, but has not demonstrated high activity in animal models.[16] Arbidol is a broad-spectrum antiviral compound that has been shown to have an inhibitory effect on the influenza virus and hantavirus.[17] Arbidol inhibits TLR4 expression and iNOS production induced by HTNV infection in HUVECs, which represents one of the virus-sensing pattern-recognition receptors (PRRs) and its resulting inflammatory cytokines/chemokines.[18] The activity against Hantavirus of an analogue of ribavirin, agent ETAR containing a terminal alkyne bond in its triazole cycle, was shown. The dose-dependent effect was studied, and it was shown that this agent exhibits pronounced activity against Hantavirus *in vitro* and *in vivo*.[19] Compounds belonging to the purine nucleoside class have been synthesised and tested as inhibitors of Hantavirus infections, with compound FPI, containing fluorine in the aromatic ring, showing the greatest antiviral activity. In spite of the fact that the active concentration (EC_{50}) for compound FPI was 94 μM and SI was not >3, the authors of this work considered this compound as promising.[20] Using high-throughput flow cytometry, it has been shown that Antimycin is active against Hantavirus and binds directly to the viral particles.[21] Several 2-phenyl-benzotriazoles showed fairly potent inhibition of the Hantaan virus in a chemiluminescence focus reduction assay (C-FRA), showing EC_{50} between 4 and 5 μM, a ten-fold increase compared to ribavirin.[22]

The strategy of searching for new antiviral agents based on terpenic compounds is extremely promising.[23,24] The use of available natural substances as starting compounds allowed our team to discover new classes of agents with a wide range of antiviral activity. Thus, we discovered a new class of agents with high activity against the influenza virus, specifically imino derivatives based on camphor;[25,26] it was shown that borneol derivatives are effective inhibitors of the entry of Marburg and Ebola viruses;[27,28] and it was found that compounds containing a framework bicyclic fragment exhibit pronounced activity with orthopoxviruses.[29,30] The purpose of the presented work is synthesis and identification of new agents, based on natural compounds of the terpene series with specific activity to viruses causing HFRS.

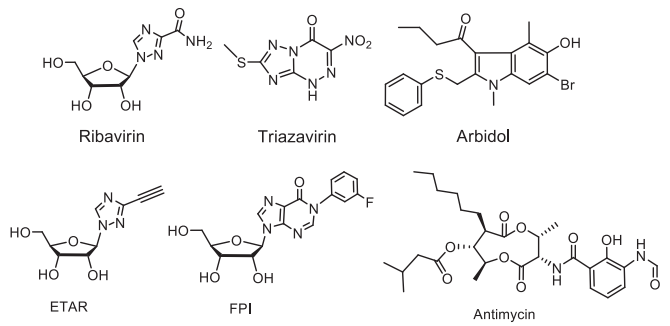


Fig. 1. Chemical structure of inhibitors of Hantavirus replication.

In the search for new virus inhibitors, we recently identified a series of various (+)-camphor *N*-acylhydrazone derivatives that are active against vaccinia and influenza viruses.[31] Among these, 6-methyl-1-oxo-2-phenyl-*N*'-[1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene]-1,2,3,6,7,7a-hexahydro-3a,6-epoxyisoindole-7-carbohydrazide, shown in Fig. 2, exhibited significant activity against both the smallpox virus and the influenza virus in the micromolar concentration range. As the surface protein structure of smallpox and influenza viruses differs significantly, we assume that this compound does not exhibit viral activity in the early stages of viral replication. Given the high importance of finding new agents with specific activity against Hantaan viruses, we have synthesised analogues of the specified *N*-acylhydrazone in this work. It was also of importance to find out the influence of both the structure of a natural fragment and the structure of an artificial epoxyisoindole scaffold. Thus, this work presents the synthesis and detailed study of the antiviral activity of 3a,6-epoxyisoindole derivatives, in which the four most important positions of the molecule vary; specifically, the C4═C5 double bond, the terpene fragment, substituents at the N-2 nitrogen atom and at the node C-6 carbon atom, as shown in Fig. 2.

The target compounds for this study were prepared according to the general synthetic route depicted in Schemes 1 and 2, based on optically active D-(+)-camphor or L-(−)-fenchone. In the first stage of the work, camphor, fenchone and norcamphor hydrazones **1**, **2** and **3** were obtained by the reaction of the corresponding ketones with hydrazine hydrate in the presence of acetic acid (AcOH), according to a reported procedure.[32] In the second step, the condensation of hydrazones **1**–**3** with heterocyclic carboxylic acids was performed. Note that the synthesis of the carboxylic acids and compounds **4–7** and **10** was either described earlier or carried out using similar methods.[31,33,34,35,36]

As the result of this section of experiments, a wide range of diverse isoindole derivatives was obtained, including a) compounds **5–7**, which differ in substituents at the N-2 of the isoindole fragment; b) compounds **8–16**, containing an additional methyl group at the C-6 position; c) compounds **10–12**, which differ from each other by the presence or absence of the C4═C5 double bond, or the presence or absence of an additional 4,5-epoxy bridge; d) compounds **13** and **14**, bearing substituents in the *para*-position of the aromatic moiety; e) compounds **15** and **16**, which differ by the length of an aliphatic linker between the benzene ring and the isoindole nucleus; and f) compound **17**, possessing an aromatic isoindole fragment.

According NMR data, compounds **4** and **17** were obtained as a single isomer, while the other compounds **5–16** were a mixture of diastereomers. As the reaction occurs between the optically-pure hydrazone of camphor **1** and acid, which is a racemic mixture of isomers, a mixture of diastereomers is formed in a 1:1 ratio, as shown in Fig. 3. Attempts to separate these mixtures by preparative column chromatography failed, due to close retention factors. Therefore, in these cases, the diastereomer mixtures were used for subsequent biological tests.

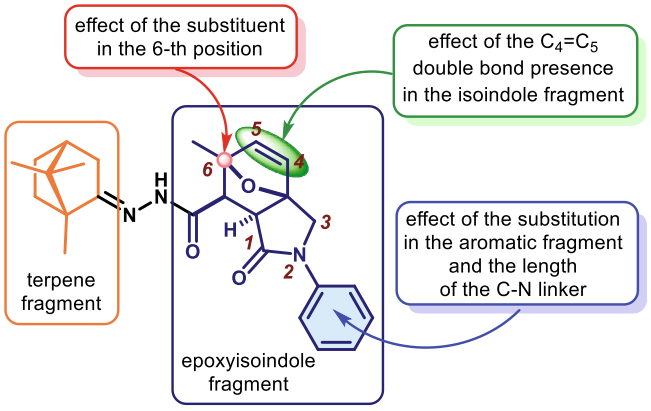
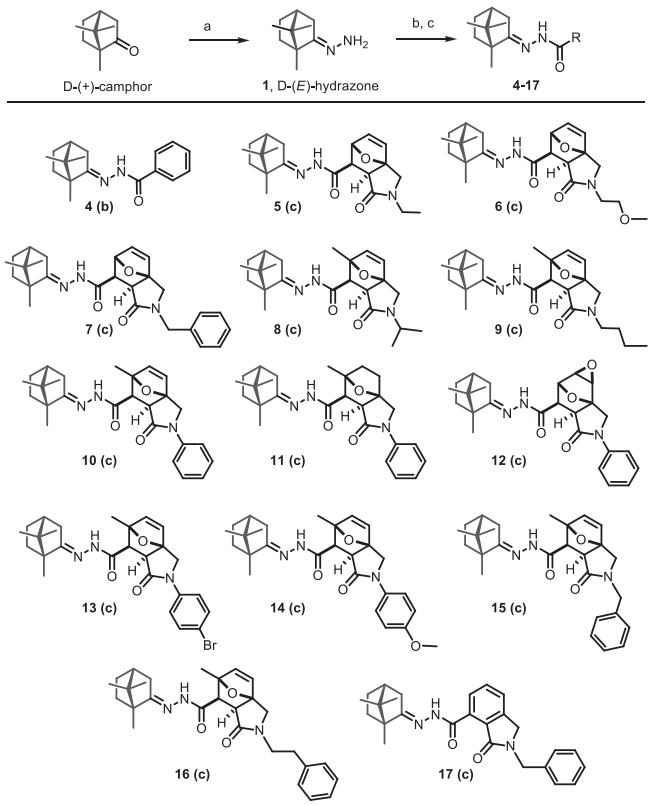
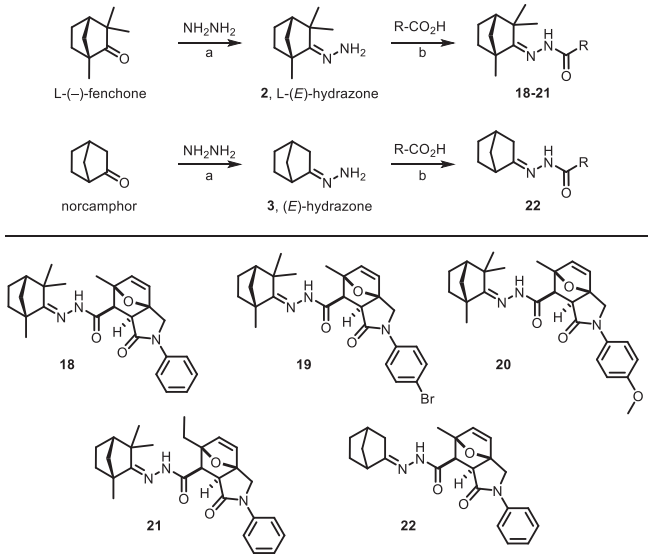


Fig. 2. Design strategy for new heterocycle-containing *N*-acylhydrazones.



Scheme 1. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$ (4 eq.), CH_3CO_2H (1 eq.), EtOH, reflux 4 h; (b) Et_3N (1.2 eq.), carboxylic acid chloride (1 eq.), $CHCl_3$; (c) carboxylic acid (1 eq.), EtO_2CCl/ Et_3N, $CHCl_3$.



Scheme 2. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$ (4 eq.), CH_3CO_2H (1 eq.), EtOH, reflux 4 h; (b) carboxylic acid (1 eq.), EtO_2CCl/ Et_3N, $CHCl_3$.

The structures of compounds **8**, **9**, and **11–17** were elucidated using the combination of 1D and 2D methods of NMR spectroscopy (COSY, TOCSY, NOESY, HSQC, HMBC, and HSQC-COSY). It should be noted here that the ^{1}H NMR spectra of the derived *N*-acylhydrazones were complex. For example, in DMSO-d_6 at room temperature, solutions of **5–16** represented mixtures of four isomers, two of which (**a** and **b**, see Fig. 3) were diastereomers and the other two of which (**c** and **d**) were rotamers on the amide fragment. The (*E*)-configuration of the hydrazone

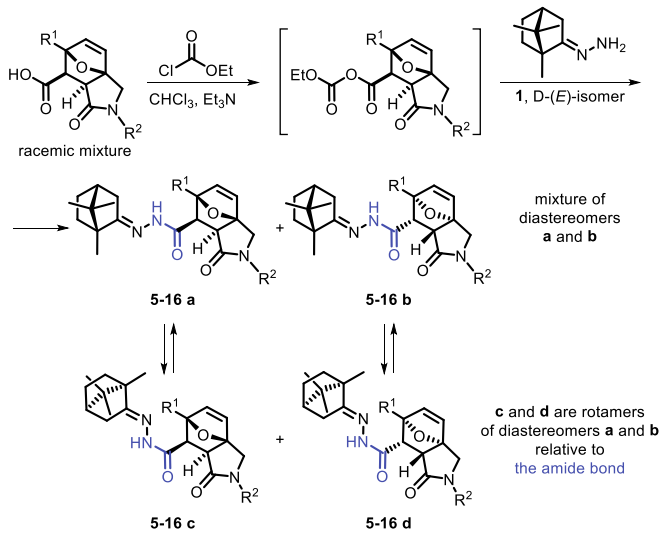


Fig. 3. Chemical structure of diastereomers 5–16.

fragment of all four isomers was established based on 2D NOESY experiments.

The natural bicyclic ketone (−)-fenchone differs from camphor by the location of the *gem*-dimethyl groups. In order to study the effect of the natural ketone type on biological activity, we synthesised *N*-acyl hydrazones **18–21** on the basis of fenchone hydrazone **2** (Scheme 2). Among these substances, compounds **18–20** have different substituents in the aromatic fragment, and compound **21** has an ethyl substituent in the 6th position of the epoxyisoindole fragment. Substances **18–21** are also a mixture of diastereomers. In addition, compound **22** was synthesised from a racemic mixture of norcamphor hydrazone **3**, and, hence, has a mixture of 4 stereoisomers.

To study the biological activity of synthesised substances against the Hantaan virus, we developed a cytopathic model. Hantaviruses do not adapt to reproduction in laboratory animals as well as they do in cell cultures, which likely explains over thirty s of unsuccessful attempts to isolate the HFRS pathogen, whose viral nature has already been proven. The laboratory model of hantavirus infection is only known for certain types of hantaviruses.[37] The possibility of using cell cultures to cultivate hantaviruses was actively studied after the successful adaptation of the Hantaan virus, isolated in laboratory mice, to human lung carcinoma cell culture, A549.[38] Later, various properties of hantaviruses, including the pathogenesis of hantavirus infections and the development of inactivated vaccines, were used as primary and transferable cell lines.

Despite numerous studies, the selection of a cell culture suitable for the successful cultivation of HFRS viruses remains a current problem in virology. Difficulties in the cultivation of hantaviruses are due to the fact that under normal cultivation conditions they do not have a visible cytopathic effect on cell culture, and they are accumulated in low titres even with long incubation periods. Difficulties in selecting animal models and cell cultures to visualise the results of exposure to hantaviruses make it highly difficult to find antiviral drugs. The methods described earlier are difficult and time-consuming.

Initially, 3 strains of HFRS virus deposited in the State Collection of viral and rickettsiosis agents of the State Research Center of Virology and Biotechnology VECTOR – Hantaan 76-118, Amur AP 94-415 and FE HTN P-98-87 – were used. Viruses were cultivated in Vero cell culture for between 14 and 36 d. The level of virus reproduction was assessed using real-time quantitative PCR analysis, with a set of primers to the S segment of the nucleocapse. The results obtained were confirmed using ELISA using components of the VektoKhanta-IgG (Vector-Best CJSC) and GLPS Diagnosticum of the culture, polyvalent for the indirect method of immunofluorescence (Chumakov Immunofluorescence Department of the Russian Academy of Medical Sciences).

We have assessed the possibility of using an MTT test to indicate the replication of hantaviruses. The Hantaan 76-118 virus strain was used for this, for which the results were more visible, i.e., the difference between the optical density values in wells with infected and non-infected cells after colouration was the largest for this strain. We prepared successive ten-fold dilutions of the virus, starting with 0.1 of the original concentration. The 96-well culture plates were infected with the prepared virus dilutions, incubated at 37 °C for 10 and 14 d, and then dyed with MTT. The results were recorded on an automatic tablet photometer at a wavelength of 540 nm and data was processed using the SOFTmax PRO 4.0 program. The data obtained indicates that a replication of the Hantaan 76-188 virus can be recorded as early as 10 d post-infection. In this work, we also studied the biological activity of the synthesised compounds, relating to the Hantaan virus.

In order to understand at what stage of the virus's life cycle chemotherapeutic agents are active, a time of addition test is often used. However, in the study of hantavirus, this method is extremely difficult to apply due to the length of the experiment. Thus, we have additionally studied the action of the synthesised compounds on a pseudovirus system with Hantaan virus Gn glycoprotein on its surface.

The results of biological testing are presented in Table 1. We selected two broad-spectrum antiviral drugs for reference – Ribavirin and Triazavirin. Our research revealed Triazavirin's activity in relation to the Hantaan virus and showed that Ribavirin does not exhibit any activity at all, being quite toxic under experimental conditions.

The data presented in Table 1 on the activity of synthesised compounds against the Hantaan virus showed that compounds **8–12**, **14–16**, **18** and **20–22** exhibited specific antiviral activity, while substance **18**, which we synthesised on the basis of fenchone hydrazine **2**, proved to be much more effective than the reference drug. It should be noted that a similar compound **10**, which has the camphor core, possesses comparable activity, but its toxicity is significantly higher. The analysis of the results led us to conclude that the presence of the isoindole fragment attached to the nodal carbon atom is necessary for the target activity to occur. For example, substances **5–7**, which have no substituents in the 6th position of the isoindole fragment, are completely inactive against Hantaan viruses.

Among compounds **10**, **11** and **12**, which differ from each other in the presence/absence of the double bond in the C4-C5 position of the isoindole fragment or the presence of the additional C4-C5 epoxy bridge, compound **10** was the most active, with comparable toxicity. Comparison of the biological properties of compounds based on camphor hydrazone **10**, **13**, **14**, and compounds based on fenchone hydrazone **18**, **19**, **20**, leads to the conclusion that the introduction of substituents in the *para*-position of the *N*-phenyl moiety reduces the activity and increases the toxicity of substances. An increase in the length of the linker between the aromatic fragment and the N-2 nitrogen atom in camphor-based compounds **10**, **15** and **16** leads to a complete loss of activity. Compound **17**, which has an aromatic isoindole fragment, does not exhibit any activity. Compound **22**, which we synthesised on the basis of norcamphor hydrazone **3**, being active against the Hantaan virus, is a diastereomeric mixture which is very poorly soluble in water, DMSO, $CHCl_3$, EtOH and other organic solvents, which makes it difficult to work with. Therefore, compound **18** exhibits the highest activity and lowest toxicity from the entire chemical library.

To study the mechanism of antiviral activity of the compounds, we tested them using a pseudovirus system. Previously, the receipt and use of such a system was described for the Puumala virus.[39,40] In the first stage, HEK293T cell culture was transfected with a plasmid containing the gene Gn-Gc of the Hantaan virus strain 76–118. After 48 h, VSV-ΔG-G was added to the cells. After another 48 h, a supernatant containing pseudoviruses was collected. In the second stage, different concentrations of substances were added to a suspension of pseudoviruses (500,000 RLU), incubated for 1 h, and then a HEK293T cell suspension was added. After 48 h, the luminescence level was measured. The results presented in Table 1 show that the substances we describe are virtually inactive against VSV-ΔG-Gn-Gc pseudoviruses.

Table 1
Antiviral activity of compounds 4–22 against virus Hantaan 76–118 in MDCK cells and recombinant vesicular stomatitis virus (rVSV-ΔG) particles, pseudotyped with Gn-Gc, in HEK293T cells.

Compound	CC_{50} (μM)[a] Vero	IC_{50} (μM)[b] Hantaan 76–118	SI[c]	CC_{50} (μM)[a] HEK293T	IC_{50} (μM)[b] rVSV-ΔG-Gn-Gc	SI[c] rVSV-ΔG-Gn-Gc
4	>150	NA[d]	–	1095 ± 46	780	–
5	250 ± 17	NA	–	1072	790	–
6	250 ± 18	NA	–	1029	>800	–
7	250 ± 12	NA	–	860	750	–
8	250 ± 15	92 ± 5	3	>2500	468	5
9	246 ± 15	26 ± 3	9	NT[e]	NT	
10	142 ± 13	14 ± 3	10	448	96	4
11	130 ± 10	20 ± 3	6	1452	375	3
12	195 ± 12	37 ± 5	5	1039	195	5
13	78 ± 10	NA	–	175	120	–
14	120 ± 8	19 ± 3	6	NT	NT	
15	245 ± 12	33 ± 6	7	335	147	2
16	195 ± 17	42 ± 7	4	216	69	3
17	>300	NA	–	NT	NT	–
18	1064 ± 26	7.6 ± 2	140	1180	431	3
19	80 ± 10	NA	–	78	65	–
20	292 ± 16	30 ± 4	9	152	74	2
21	120 ± 7	40 ± 6	3	NT	NT	–
22	269 ± 17	28 ± 7	9	NT	NT	–
Ribavirin	20 ± 3	NA	–	NT	NT	–
Triazavirin	368 ± 18	14 ± 3	26	1513	408	4

[a] CC_{50} – 50% cytotoxic concentration; the concentration resulting in death of 50% of cells; M ± I95, n = 3.
[b] IC_{50} – 50% inhibitory concentration; the concentration leading to 50% inhibition of virus replication; M ± I_{95}, n = 3.
[c] SI – selectivity index, ratio CC_{50}/IC_{50}.
[d] NA – not active.
[e] *NT* – not tested.

It should be noted that the toxicity of the compounds was tested on the HEK293T cell line, with a residence time of 72 h, whereas residence time is up to 10 d with the live virus, which does not allow direct comparison of these results.

The results presented are consistent with the fact that we have previously shown marked activity against the smallpox virus and influenza virus for compound **10**. It appears that this class of compounds may not be an inhibitor of virus entry, but an inhibitor of intracellular replication. Hantavirus nucleoprotein (N) may then be considered as a potential biological target. This protein N is involved in the processes of virus transcription and replication, and, therefore, represents an attractive target for therapeutic effects. The nucleoprotein structure describes a highly conservative RNA binding site.[41]. It has been shown that N protein binds to the host mRNA cap and protects the 5′ end of this molecule from degradation, which is later trapped and used as a viral RdRp primer

during transcription initiation.[42] Protein N also seems to be responsible for switching the host's broadcast machine for the preferred broadcast of viral transcripts. Moreover, nucleoprotein N has been shown to delay the induction of cell apoptosis and facilitate the transport and localisation of viral ribonucleoproteins (RNP).[43] Presumably, binding small molecules in a given location may inhibit the binding of viral RNA and/or oligomerisation of the protein, and therefore block replication of the virus. Assessment of the affinity of the studied compounds to the described site binding was carried out using molecular modelling methods.

The crystalline structure of nucleoprotein (PDB code 5FSG)[41] was downloaded from the Protein Data Bank database.[44] Model protein structures were prepared using the Schrodinger Protein Preparation Wizard tool: hydrogen atoms were added and minimised; missing amino acid side chains were added; bond multiplicities were restored; solvent molecules were removed; and the entire structure was restrained and optimised in the OPLS3e force field[45] at physiological pH values (7.0 ± 0.2). The geometric parameters of potential ligands were also optimised, taking into account all permissible conformations. All biological experiments were conducted for a mixture of diastereomers, although calculations were made for each isomer individually.

The binding site of nucleoprotein binding with viral RNA was considered as a potential binding site for the studied compounds. The active site contains a number of functional amino acid residues: Ser180, Asn183, Ser186, Ser187 and Thr 194 line the deduced RNA binding grove, and could form hydrogen bridges; Lys189, Arg197, and Arg199 can form salt bridges;[41] and Arg339, Arg367 and Arg368 neutralise the charge of the RNA phosphate group (Fig. 4).

Molecular docking procedures were performed using Schrodinger Suite (Release 2018-4) software. Compounds **8–12, 14–16, 18** and **20–22** were docked using the forced ligand positioning protocol (IFD), with the following conditions: flexible protein and ligand; grid matrix size of 15 Å; and amino acids (within a radius of 5 Å from the ligand) restrained and optimised, taking into account the influence of the ligand. Docking solutions were ranked by evaluating the following calculation parameters: docking score (based on GlideScore minus penalties); ligand efficiency (LE, which takes into account atomic distribution of scoring function); and parameter of model energy value (Emodel), including GlideScore value, energy unrelated interactions and the parameters of the energy spent on the formation of the laying of the compound in the binding site. According to the results of molecular docking, all the compounds may bind in the place of binding viral RNA with N and form a number of intermolecular interactions with the amino acid residues described above (Fig. 5A).

The docking score values range from −7.2 to −4.1 kcal/mol (Fig. 5B). There is a trend, namely that the compounds characterised by low IC_{50} values, measured by us using the cytopathic model for the Hantaan virus, show better affinity to the binding site than connections with low antiviral activity. The statistical dependence of pIC_{50} and the docking score is observed. To find a statistical relationship between the values that characterise the antiviral activity of the compounds and the affinity to the selected binding site, regression analysis was performed using the method of least squares. The dependence of pIC_{50} (decimal logarithm of half-maximal inhibition concentration) values and the weighted average value of the docking score was plotted. The contribution of each diastereomer was taken into account. The dependence between these parameters can be considered as evidence of the correct choice of a biological target.

It was shown that different diastereomers fold slightly differently in the binding site. For example, one diastereomer of the lead-compound **18a** is located in the site binding with the formation of hydrogen bridges with amino acids Arg144, Gln147 and Thr148, and another (**18b**) forms hydrogen bridges with Thr194 and Gly196 (Fig. 5C). At the same time, the energy parameters of diastereomers binding do not differ significantly (detailed data are presented in Supplementary Material).

As a result of our research, we have synthesised a small library of compounds based on hydrazones of frame ketones, namely

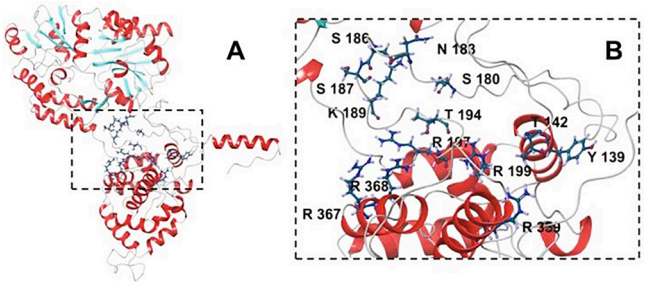


Fig. 4. A) Secondary protein structure (PDB code 5FSG); B) functional amino acids located at the binding site of potential inhibitors.

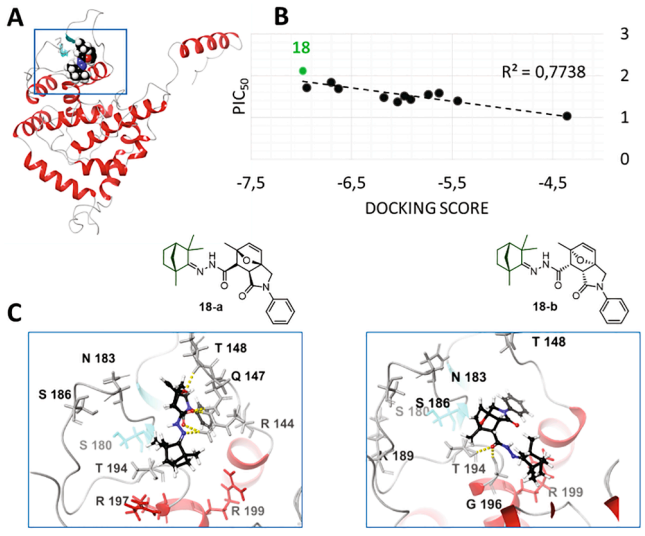


Fig. 5. Molecular docking results: A) the intended binding location of potential nucleoprotein inhibitors; B) regression analysis of the results of biological experiments and theoretical calculations; C) the location of two lead-compound 18 diastereomers in the inhibitor binding site (hydrogen bridges are shown by the yellow dotted line).

(+)-camphor, (−)-fenchon and norcamphor, with all compounds fully characterised using a set of physical and chemical methods. A cytopathic model suitable for testing chemical compounds against the Hantaan virus, strain 76-118, has been developed, and a study of the activity of compounds **4–22** against this virus and with the pseudovirus system used has been carried out. It has been shown that the agents we describe have a pronounced activity against the Hantaan virus, and are not active in the pseudovirus system. A synthesised compound **18**, based on hydrazone-(−)-fenchon with a fragment of epoxyisoidol, is the agent with the highest activity and has low toxicity. The relationship between the structure of the synthesised agents and their antiviral activity has been studied. Based on a combination of biological experiments and molecular modelling data, it can be assumed that the compounds under study may be associated with the site of binding RNA of nucleoprotein N Hantan virus. Such competitive binding of small molecules may lead to blockages in the replication process of viral infection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127926.

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