Insight into the Hantaan virus RNA-dependent RNA polymerase inhibition using in-silico approaches

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Abstract

The Hantaan virus (HTN) is a member of the hantaviridae family. It is a segmented type, negative-strand virus (sNSVs). It causes hemorrhagic fever with renal syndrome, which includes fever, vascular hemorrhage, and renal failure. This illness is one of the most serious hemorrhagic diseases in the world, and it is a major public health concern due to its high mortality rate. The Hantaan virus RNA-dependent RNA polymerase complex (RdRp) is involved in viral RNA transcription and replication for the survival and transmission of this virus. Therefore, it is a primary target for antiviral drug development. Interference with the endonucleolytic “cap-snatching” reaction by the HTN virus RdRp endonuclease domain is a particularly appealing approach for drug discovery against this virus. This RdRp endonuclease domain of the HTN virus has a metal-dependent catalytic activity. We targeted this metal-dependent enzymatic activity to identify inhibitors that can bind and disrupt this endonuclease enzyme activity using in-silico approaches i.e., molecular docking, molecular dynamics simulation, predicted absorption, distribution, metabolism, excretion, toxicity (ADMET) and drug-likeness studies. The docking studies showed that peramivir, and ingavirin compounds can effectively bind with the manganese ions and engage with other active site residues of this protein. Molecular simulations also showed stable binding of these ligands with the active site of HTN RdRp. Simulation analysis showed that they were in constant contact with the active site manganese ions and amino acid residues of the HTN virus endonuclease domain. This study will help in better understanding the HTN and related viruses.

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Graphical abstract

Introduction

Hantaviruses are RNA viruses with segmented-negative strand genomes that belong to the Hantaviridae family of viruses in the genus Orthohantavirus and are placed in the Bunyavirales order [1]. Bats, shrews, moles, and rodents are among the mammalian host reservoirs of hantaviruses [2, 3]. There are several types of hantaviruses, some are non-pathogenic and some are pathogenic that cause serious illnesses in humans. These illnesses include hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) [4]. The transmission of these pathogenic viruses to their hosts can occur via different routes. These include transfer via aerosolization of rodent excretory waste [5], person-to-person transmission was also reported in Argentina in 2020 [6], and transmission through transfusion of infected blood is also possible [7].

Hantaviruses of different types are distributed across various regions worldwide; they can also be classified as Old World and New World Hantaviruses. The old world viruses such as the Hantaan virus, Seoul virus, and Dobrava-Belgrade virus causes HFRS diseases. The HCPS disease occurs due to New World hantaviruses such as the Sin Nombre virus (SNV), the New York-1 virus, and the Andes virus [8]. Proteinuria, hematuria, and acute kidney injury are all symptoms of HFRS disease, which has a mortality rate of up to 15% [9]. Dobrava, Hantaan, Seoul, and Puumala viruses are the most common serotypes that cause HFRS in Asia and Europe. The number of cases each year varies between 60,000 and 150,000 and is related to rodent populations in that region [10, 11]. The HCPS is a febrile disease marked by respiratory failure and diffuse interstitial edema caused by hantaviruses [12].

It is a major and direct cause of hemorrhagic fever with renal syndrome, characterized by fever, vascular hemorrhage, and renal failure. These conditions are collectively called hemorrhagic fever with renal syndrome (HFRS) [13]. This illness is among the most serious hemorrhagic diseases in the world, like Ebola hemorrhagic fever, and because of its high mortality rate, it is a major public health concern [14, 15]. The number of cases each year varies between 60,000 and 150,000 [10, 11]. HTN has a three-segment negative-stranded RNA genome and is enveloped. The viral RNA polymerase, two important membrane surface glycoproteins (G1 and G2), and the nucleocapsid protein (N) are encoded by the gene segments L, M, and S, respectively.
Hantavirus primarily infects and replicates inside the endothelial cells, kidney glomeruli, and epithelial cells of animals and humans [18]. In the entry to the host cells, the HTN virus attaches itself with the cell surface receptors integrins β-1, 2, and 3, and then its internalization occurs by clathrin-mediated endocytosis into the host cells, it is also reported that HTN when internalizing into the host cell also utilize the dynamin proteins of the host cells [19].

RNA-dependent RNA polymerase (RdRp) (250–280 kDa), is the most essential and major protein encoded by the L-gene of HTN and is the primary polymerase for Hantaan virus genome transcription and replication. HTN’s RdRp, in addition to transcriptase and replicase activities, has several other enzymatic activities. The RNA helicase function is among the extra activities of RdRp, on the other hand, it is known to lack the enzymatic activity needed for capping and proofreading [20]. Both positive and negative-strand RNA viruses have a structural polymerase domain that is conserved in RdRp, the same is the case with the HTN RdRp protein. The HTN RdRp core domain’s overall structure, which includes a right-handed fold, can be categorized into three sub-domains: thumb, finger, and palm domain. There are four motifs in the HTN RdRp A- to D-, and its polymerase function resides in the motif-C in the palm sub-domain [21].

A unique cap-snatching endonuclease domain has certain similarities to the influenza virus endonuclease domain [22]. The cap-snatching activity is essential for starting the RNA polymerase function of the RdRp. To begin the transcription of its vRNA, the HTN virus needs mRNA oligonucleotides, which it uses as a primer to initiate the process of viral transcription. This mRNA primer is provided by the cap-snatching domain, which snatches the mRNA caps from host cell mRNAs along with the endonuclease domain, which cleaves, and processes host mRNAs for viral use at the replication site. The resulting mRNA-capped primers of 10–15 bases are then used for viral mRNA transcription initialization [23]. The HTN virus polymerase snatches the mRNA caps from the host mRNA then cleaves it via its RdRp endonuclease domain and transfers it to the polymerase domain of RdRp. Other viruses i.e. Dengue virus use their capping enzyme to initiate the viral genome synthesis inside its host [24].

This RNA-directed RNA Polymerase-L protein cap-snatching endonuclease domain of the Hantaan virus performs the vital function of cap-snatching and cleaving the host mRNA by the endonuclease domain transferring it to the main RdRp domain to start the viral RNA transcription. It can be seen in Fig. 1 that the Hantaan virus endonuclease domain active site contains two divalent manganese metal ions which are catalytically active. One of the Mn$^{2+}$ ions is coordinated by His36, Asp97, Glu110, and Val111, and the second Mn$^{2+}$ ion is coordinated by Glu54 and Asp97 of the active site residues [23].

This endonuclease domain is thus a promising target for novel drug discovery against HTN viral infections. In this work, the endonuclease domain of the Hantaan virus was explored via various computational approaches. Screening of various compounds and anti-viral drugs was performed by utilizing molecular docking and molecular dynamics simulation. Similarly, other in-silico approaches were used to identify potential anti-viral molecules against this endonuclease domain which can effectively chelate the manganese metal ions and also interact with the active site residues. These antiviral molecules disrupt the association of these Mn$^{2+}$ metal ion within the catalytic active site residues of RdRp and inhibit its function completely.

**Materials and methods**

**Retrieval of target protein**

The crystal structure of the Hantaan RdRp L-protein cap-snatching endonuclease domain was obtained from the RCSB protein data bank having the PDB ID number 5IZE [23]. The resolution of the structure was 1.70 Å. This cap-snatching endonuclease of RdRp was in dimeric form and the dimers were named ‘Biological Assembly-1’ and ‘Biological Assembly-2’ on the RCSB website, as both of the monomers of this protein were the same and had similar structure and functions so we selected the ‘Biological Assembly-1’ monomer and downloaded its protein PDB structure for our computational studies.
Ligands retrieval, selection, and preparation for computational studies

An in-depth literature study was performed for the selection and retrieval of inhibitory compounds that can target the endonuclease domain of the HTN RdRp enzyme. The HTN RdRp contains two Mn$^{+2}$ ions in its active site, which are vital for the catalytic activity of this endonuclease domain. The criteria for selecting the compounds used here was their potential for chelating and engaging the ions of the active site. Thus, these compounds can effectively engage and block the catalytic activity of the HTN RdRp enzyme. Previously, several compounds having metal chelating activity with different viruses’ target enzymes have been reported i.e. (Influenza virus and its endonuclease domain inhibitor ‘Xofluza’ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6336199). Based on these observations, we searched several scholarly online databases. The PubChem database SMILES search option was used to further explore the compounds. Compounds and their derivatives reported for their antiviral activity against several viruses present in the databases, were selected for docking studies. Several related synthesized compounds based on these antiviral compound structures were also retrieved from research articles. In addition, we also utilized several other metal-chelating compounds synthesized by various groups and described in the literature, which had the potential to target this type of viral protein targets. Based on these criteria, we carefully retrieved and selected the compounds and included them in our study. The selected compounds structures were drawn and prepared using the ChemDraw Ultra 12.0 software. The chemical structures of all the compounds used in this study can be seen in the (Supplementary Fig. 1).

Molecular docking studies and receptor preparation

Molecular-docking studies of our target protein HTN RdRp L-protein cap-snatching endonuclease domain were performed via the Molecular Operating Environment (MOE-2015.10) software [25]. First, the protein was prepared for molecular docking using the built-in ‘Structure Preparation’ module of the MOE-2015.10. Using this module, the missing atoms in the protein were added and the charges on the protein were corrected. The inter-chain broken bonds between the amino acids of the protein were also corrected. The charges and hydrogens were also added to the protein and then it was 3D-protonated. The active site of the HTN RdRp L-protein cap-snatching endonuclease domain was selected via the ‘Site-Finder’ module present in MOE. The amino acid residues of the active site of our protein and its two catalytic metal ions are listed in (Table 1). The parameters of docking via MOE 2015.10 during our study were set to:

1. Placement was set to ‘Triangular Matcher’
2. Refinement was set to “Rigid Receptor”
3. Scoring functions (both) were set to “London dG Scoring”
4. While poses during mol-docking were set to 20-poses per compound.

The structures of ligands were also energy minimized before molecular docking with the target protein [15, 26, 27].

ADMET and drug likeness in-silico studies

In-silico ADME and toxicity studies include the adsorption of the small molecules (drugs) from the gastrointestinal tract, its distribution and metabolism inside the living system, its excretion, and the drug toxicity prediction toward the organism, it can be determined via several computational tools [28]. We have utilized the ADMET-SAR online server, which can determine these ADMET properties for small molecules. Drug-like properties prediction of these top-performing small molecules, determined from the docking studies, were performed via the Swiss-ADME online server. Some drug-like parameters predicted by this server are the molecular weight and topological surface area (TPSA) parameters, if these values predicted are minimum and smaller for molecules, then these molecules have the potential to be developed as drugs. Other drug-likeness properties are the number of hydrogen bond acceptors and donors and the heteroatoms present in a molecule i.e. (sulfur, nitrogen, etc.) if these qualities are present in a molecule, then they are regarded to be more drug-like.

| S/N | Amino acid residues and manganese ions present in the active site | Residue number |
|-----|-------------------------------------------------|----------------|
| 1   | Asparagine                                      | 29             |
| 2   | Tyrosine                                        | 32             |
| 3   | Alanine                                         | 33             |
| 4   | Histidine                                       | 36             |
| 5   | Asparagine                                      | 37             |
| 6   | Glutamic acid                                   | 110            |
| 7   | Valine                                          | 111            |
| 8   | Threonine                                       | 112            |
| 9   | Valine                                          | 113            |
| 10  | Lysine                                          | 124            |
| 11  | 2 Mn$^{+2}$ (Manganese ions)                    | –              |
Molecular dynamics simulation

Schrodinger Desmond was used to performing molecular dynamics simulations for interaction analysis and evaluation. Maestro’s "System Builder" was used to configure all of the systems [29]. All three identified top hit complexes were energy minimized and placed in an orthorhombic box with a buffer distance of 10 Å. The solvated water-soaked MD-Simulation system was built using the Builder tool. In this experiment, the TIP3P model was used for solvation. The simulation systems were neutralized by adding an adequate amount of counter ions. The isoosmotic state was maintained by adding 0.10 M Sodium and Chloride ions to the simulation panel. A pre-defined equilibration procedure was followed before the simulation. The MD simulation was performed at an ambient pressure of about 1.013 bar and a temperature of 300 K for 100 ns [30, 31].

Results and discussion

Molecular docking

Those molecules, which performed well in the docking studies and had lower RMSD, suitable binding free energies (s-scores), and made the highest number of interactions with both the manganese ions and the amino acid residues of the active site of the HTN RdRp endonuclease domain will be discussed here in this section.

Interaction analysis of 2′-deoxy-2′-fluorocytidine

2′-Deoxy-2′-fluorocytidine is an analog of gemcitabine, which is an anti-cancer drug. 2′-Deoxy-2′-fluorocytidine is also reported to have antiviral activity against SARs-CoV-2 [32]. In our computational docking studies, this small molecule showed several interactions with the HTN virus RdRp endonuclease domain. It engaged and interacted with the manganese ions and the active site amino acids residues involved in the enzymatic activities and those amino acids, which coordinate these metal ions. Its docking s-score or binding affinity energy was − 9.21 kcal/mol. Seven interactions were noted for this compound with the protein's active site. The pyrimidine ring of this compound made two interactions with the active site amino acids. One interaction was hydrogen bonding with the Lys124 amino acid of the active site. The fluorinated ribose ring of this compound engaged in five interactions, the hydroxyl groups on this ribose ring made hydrogen bonds with the Lys127, Lys124, and one of the –OH also chelated and made contact with the manganese ion of the active site. Its second manganese ion also made metal contact with the ribose ring cyclic oxygen atom. Moreover, the Lys124 also engaged in H-bonding with this cyclic ring oxygen atom. The RMSD for this ligand-enzyme complex was 0.897 Å.

Interaction analysis of peramivir

It is also an antiviral drug and is a 3-hydroxy monocarboxylic acid belonging to the cyclopentanols-cyclopentanone, acetamides, and guanidines family of compounds and is active against the influenza virus [33]. It showed seven interactions with the enzyme active site and chelated the Mn²⁺ ions. The hydroxyl group present on the cyclo-pentanol ring of this compound chelated the manganese ion and engaged in metal contact interaction with the acetamide carboxyl oxygen atom of peramivir, while the second manganese ion was also chelated by the same hydroxyl group which chelated the first manganese ion. This hydroxyl group made H-bond with the Glu110 amino acid of the active site. The carbon atom to which this acetamide moiety is attached also made an arene-H interaction with the His36. The carboxyl group on the cyclo-pentanol made two H-bonding interactions with the Lys124 and Lys127. The docking s-score observed was − 8.14 kcal/mol and the RMSD of this complex was 1.50 Å.

Interaction analysis of ingavirin

Ingavirin is also an antiviral drug medication used for various viral respiratory infections [34]. Its structure contains a carboxy group, an imidazole ring, and an ethaneamide group present in the middle of its chain structure. Ingavirin also showed best interactions with the HTN RdRp endonuclease domain's active site. It made interactions with the manganese ions and its amino acid residues and made six interactions with it. The ethaneamide’s group carbonyl oxygen of ingavirin chelated the manganese ions and interacted with them. The carbon atom to which ethaneamide is attached made an arene-H interaction with the His36 while the carboxyl group of ingavirin engaged in three interactions with the Glu110, Lys124, and Lys127 active site amino acids. Its docking s-score was − 8.74 kcal/mol and the RMSD of this complex was 1.60 Å.

Interaction analysis of 4-hydroxy-2-oxo-4-phenyl but-3-enoic acid

This compound is a carboxylic acid having a phenyl ring and a ketone group present in its structure. It also showed good chelating interactions with the manganese ions and hydrogen bonding with the active site amino acids of HTN virus RdRp endonuclease domain. Five bonding interactions were noted in the docking analysis of this compound. The
ketone (carbonyl) group oxygen atom chelated the manganese ions of the active site and made contact with them. This compound’s carboxyl group hydroxyl and carbonyl groups made three H-bonding interactions with the Glu110, Lys124, and Lys127 protein active site. Its docking s-score or binding affinity energy was $-8.13$ kcal/mol and the RMSD of the ligand–protein complex during docking was $1.91$ Å.

**Interaction analysis of pimodivir**

Pimodivir is also an antiviral drug and is active against the RdRp of the influenza A virus (IAV) [35]. Here, this docking study also effectively targeted the RdRp endonuclease domain of HTN and showed six interactions with this enzyme. Effective chelation of the manganese ion of the active site was noted and engaged the active site amino acids. This compound has a complex structure and contains several ring structures and functional groups. Only the carboxyl group chelated the single manganese ion and nitrogen atom present in this compound. This nitrogen was also engaged with the Glu54 amino acid of the active site and made a hydrogen bond with it. The pyrimidine ring of this compound made two arene-arene type interactions with the His36 amino acid, while the fluorene atom attached to this pyrimidine ring made a H-bond with the Lys124 amino acid. The docking s-score of this ligand was $-11.1$ kcal/mol and has an RMSD score of $1.25$ Å.

**Interaction analysis of raltegravir**

Raltegravir is another anti-viral drug that is used for the treatment of HIV infections. It targets the HIV integrase enzyme, a metalloprotein containing metal ions like the HTN RdRp endonuclease metal ions. HIV integrase is an essential enzyme for incorporating the viral genome of HIV into its host genome, and raltegravir inhibits this step of viral genome incorporation [36]. Similarly in our study favorable interactions of this compound were noted. Five interactions of raltegravir were noted, raltegravir interacted with both the manganese ions and the active site residues of the HTN RdRp endonuclease domain. The oxo-pyrimidine ring carbonyl oxygen interacted with the manganese ions and made an arene-arene type interaction between the pyrimidine ring with the His36 of the active site. The two carboxamide functional groups of raltegravir engaged with Asp37 and Val113 amino acids in hydrogen bonding. The docking s-score of raltegravir was $-9.09$ kcal/mol and its RMSD value was $1.50$ Å.

**Interaction analysis of baloxavir marboxil**

This compound is an influenza virus RdRp endonuclease inhibitor and is a potent drug used to treat influenza infections [37]. In this docking study, this drug made significant interactions and interacted with the manganese ions and an active site residue. Four interactions were observed, and several carbonyl groups present in this molecule chelated both the metal ions and made a hydrogen bond with the Lys124 amino acid of the active site. Its docking s-score was $-9.83$ kcal/mol. The RMSD of this complex was $1.53$ Å.

**Interaction analysis of N-[(5-hydroxy-4-oxopyran-2-yl) methyl] benzenesulfonamide**

This compound is also an inhibitor of the IAV RdRp endonuclease domain. This study also showed suitable interactions with the endonuclease of HTN. The oxo-pyran ring hydroxyl and carbonyl group made hydrogen bonding with the Glu123 and Val113, respectively. The sulfonamide group of this compound made four interactions, effectively engaging the manganese ions and two H-bonds with the Lys127 of the HTN RdRp endonuclease active site. Its docking s-score was $-8.41$ kcal/mol and has an RMSD value of $1.14$ Å.

**Interaction analysis of aureothricin**

Aureothricin is an antimicrobial compound isolated from a Streptomyces species. This small molecule had a docking s-score of $-6.09$ kcal/mol and an RMSD value of $1.05$ Å. The propanamide group in this molecule interacted with both manganese ions. At the same time, the oxodithiopyrrole ring also chelated one of the manganese ions and made a H-bond with the Lys124 active site amino acid of HTN RdRp endonuclease domain.

**Interaction analysis of compound ID = Schembl22890980**

This compound contains an oxo-triazatricyclic ring, a sulfon-nyloxy (−O−SH) group, and a dione (containing two ketone groups on its tricyclic ring). This molecule also exhibits good interactions with HTN RdRp endonuclease and engages in six interactions with its active site. The ketone carbonyl group oxygen atom interacted with one of the manganese metal ions and made a H-bond with the Lys124 amino acid. The second manganese ion was engaged by the oxygen of one of the cyclic rings and interacted with it. While the second ketone carbonyl group oxygen atom made a H-bond interaction with the Ala33 amino acid. This compound’s sulfon oxy (−O−SH) group also made a hydrogen bond interaction with the Val113 amino acid of HTN RdRp endonuclease active site. The docking s-score of this molecule was $-7.09$ kcal/mol and its RMSD value was $1.39$ Å.
Interaction analysis of (5Z)-3-benzyl-5-[(4-fluorophenyl) methylidene]-1-(methoxy) pyrazine-2, 6-dione

This compound is also a dione and is previously reported in the literature to be active against the endonuclease domain of IAV [38]. In our docking studies, this compound effectively made interactions with the manganese ions and made hydrogen bonds with the active site residues of this enzyme. The methoxy group oxygen present on the pyrazine ring of this compound interacted with the manganese ion of the active site, while the keto carbonyl group oxygen of this pyrazine ring also chelated both the manganese metal ions. This pyrazine ring also made an arene-cation type interaction with the Lys124 active site residue. While the fluorine substituted benzene group made an arene-H type of interaction with the Val113 amino acid of the HTN RdRp endonuclease active site. Its docking score was −7.30 kcal/mol, and the protein–ligand complex has an RMSD value of 2.41 Å.

Interaction analysis of compound with ID Schembl22813509

This compound contains an oxa-triazatricyclic ring and is a dione. This molecule also showed good interactions with HTN RdRp endonuclease and engaged in five interactions with its active site. The methoxy group on the ring structure chelated one of the manganese metal ions while the second metal ion of the active site interacted with the keto carbonyl oxygen atom, this oxygen atom also made a H-bond with the Lys124 of the active site. While the third heterocycle of this compound made an arene-arene type interaction with the His36. The ketone group present on this same ring also made a hydrogen bonding via its carbonyl oxygen atom with the Val1113 of the active site of the HTN RdRp endonuclease domain. The docking score of this molecule was −9.41 kcal/mol with an RMSD value of 1.25 Å.

Interaction analysis of compound with ID CHEMBL4435971

This compound is the same as the previously discussed one, but instead of two ketone carbonyl groups, one of them is replaced by a carboxyl group, and the rest of the structural features were the same. It is an HIV integrase enzyme inhibitor. In our studies, CHEMBL4435971 showed decent chelating activity against one of the manganese ions and interacted with it via its carbonyl oxygen and methoxy group oxygen atoms on the ring structure of this compound. The pyrazine ring of this compound engaged in arene-arene type interaction with the His36 amino acid. At the same time, ketone and carboxyl groups substituted on this ring also made two hydrogen bond interactions with the Val1113 and Lys124 active site residues. Its docking s-score was −10.40 kcal/mol with an RMSD value of 1.33 Å.

Interaction analysis of 3,4,5-Trihydroxy-N-[(Z)-(2-hydroxyphenyl) methylideneamino] benzamide

This compound belongs to the benzamide class of compounds. The carboxamide’s carbonyl oxygen of this compound effectively chelated the Mn$^{2+}$ ions of the active site of HTN RdRp endonuclease domain, while the phenyl ring is engaged in an arene-cation type of interaction with the Lys127 amino acid. The trihydroxy-phenyl rings of one hydroxyl group made a H-bonding with this enzyme’s Val1113 active site residue. This compound has a docking s-score of −11.73 kcal/mol with four interactions with its active site and has an RMSD value of 1.29 Å.

Interaction analysis of (Z)-4-[1-benzylsulfonyl-3-[(4-chlorophenyl) methyl] piperidin-3-yl]-2-hydroxy-4-oxobut-2-enoic acid

This compound is also an influenza A virus (IAV) endonuclease inhibitor. It is a carboxylic acid and has good chelating activity. The docking results showed five interactions with the RdRp endonuclease of HTN. The sulfonyl group oxygen atom chelated one of the manganese ions. This sulfonyl group and another oxygen atom also made a H-bond interaction with the Lys124 active site residue. The ketone functional group oxygen atom in this compound’s structure chelated the second Mn$^{2+}$ ion and an arene-hydrogen bonding interaction with the His36 of the benzene ring on which the sulfonyl group is substituted was noted. The docking s-score of this compound was-12.98 kcal/mol and has an RMSD value of 1.78 Å.

The docking poses obtained during the molecular docking of the compounds in the two-dimensional and 3-dimensional forms of these above-discussed compounds are listed in Supp. Figure 2, along with their chemical structures and their names. The three-dimensional images were drawn using Biovia DS software, while the 2-dimensional docking poses were obtained from the built-in MOE-2015-10 ligand interaction module. The chemical structures were prepared via the ChemDraw Ultra-12.0 software.

Almost half of all the compounds during the docking studies showed suitable interactions and chelating activities with the active site of the HTN RdRp endonuclease domain. They had good docking s-scores and low RMSD values. The molecular docking results of the 50-screened ligands against the HTN virus RdRp endonuclease domain are presented in Table 2.
Table 2 Molecular docking result of the main screened ligands against the HTN virus RdRp endonuclease domain

| S/N | Compound’s name                                                                 | Docking S-Score (Kcal/mol) | Interaction formed                                                                 | Interacting residues and Mn$^{2+}$ ions | RMSD  |
|-----|---------------------------------------------------------------------------------|-----------------------------|-----------------------------------------------------------------------------------|-----------------------------------------|-------|
| 1   | 2'-Deoxy-2'-fluorocytidine                                                        | −9.21                       | 7 Lys127, 3-interactions with Lys124 & both Mn + + ions                            |                                         | 0.89 Å|
| 2   | Peramivir                                                                        | −8.14                       | 7 His36, Glu110, Lys124, Lys127 & 3-interactions with both Mn + + ions             |                                         | 1.50 Å|
| 3   | Ingavirin                                                                        | −8.74                       | 6 His36, Glu110, Lys124, Lys127 & with both Mn + + ions                            |                                         | 1.60 Å|
| 4   | 4-hydroxy-2-oxo-4-phenyl but-3-enoic acid                                       | −8.13                       | 5 Glu110, Lys124, Lys127 & with both Mn + + ions                                  |                                         | 1.91 Å|
| 5   | Pimodivir                                                                       | −11.10                      | 6 His36, Glu54, Lys124, & with one Mn + + ion                                     |                                         | 1.25 Å|
| 6   | Raltegravir                                                                      | −9.09                       | 5 Asp29, His36, Asp37 & with both Mn + + ions                                     |                                         | 1.50 Å|
| 7   | Baloxavir Marboxil                                                               | −9.83                       | 4 Lys124 and 3 interactions with both the Mn + + ions                            |                                         | 1.53 Å|
| 8   | N-[(5-hydroxy-4-oxopyran-2-yl) methyl] benzene-sulfonamide                       | −8.41                       | 6 Val113, Glu123, Lys124, Lys127 & both Mn + + ions                              |                                         | 1.14 Å|
| 9   | Aureothricin                                                                     | −6.09                       | 4 Lys124 & 3-interactions with both the Mn + + ions                               |                                         | 1.05 Å|
| 10  | Schembl22890980Glu54,Glu123                                                       | −7.09                       | 6 Ala33, His36, Val113, Lys124 & both Mn + + ions                                |                                         | 1.39 Å|
| 11  | (S)-3-benzyl-5-[(4-fluorophenyl) methylidene]-1-(methoxy methoxy) pyrazine-2,6-dione | −7.30                       | 5 Val113, 3-interactions with Lys124, 1 with Lys127 & with 2 interactions both Mn + + ions |                                         | 2.41 Å|
| 12  | Schembl22813509                                                                  | −9.41                       | 5 His36, Val113, Lys124 & with both Mn + + ions                                  |                                         | 1.25 Å|
| 13  | CHEMBL4435971                                                                   | −10.40                      | 5 His36, Val113, Lys124 & one Mn + + ions                                         |                                         | 1.33 Å|
| 14  | 3,4,5-Trihydroxy-N-[(Z)-(2-hydroxyphenyl) methylideneaminamino] benzamide         | −11.73                      | 4 Val113, Lys127 & both Mn + + ions                                              |                                         | 1.29 Å|
| 15  | (Z)-4-[(benzylsulfonyl)-3-[(4-chlorophenyl)methyl] piperidin-3-yl]-2-hydroxy-4-oxobut-2-enoic acid | −12.98                      | 5 His36, Thr95, Lys124 ^ both Mn + + ions                                         |                                         | 1.78 Å|
| 16  | 2-(3,4-dihydroxyphenyl)-N-[2-(3,4-dihydroxyphenyl)ethyl]acetamide                | −14.10                      | 4 Glu54, Thr95, Thr112 and Glu123                                              |                                         | 1.36 Å|
| 17  | 3'-FdG                                                                           | −8.18                       | 5 Ala33, His36, Thr95 and one Mn + + ion                                         |                                         | 1.12 Å|
| 18  | 3-Fluoro-N-(2-piperidin-1-ylethyl)-5-(trifluoromethyl)benzamide                   | −7.86                       | 3 Lys124 and Both Mn + + ions                                                   |                                         | 1.62 Å|
| 19  | SICTEP                                                                           | −8.66                       | 6 Glu54, Thr112, Val113,Lys124 & one Mn + + ion                                 |                                         | 3.90 Å|
| 20  | (3Z)-3-[(4-fluorophenyl)methylene]-1-hydroxy-5-isobutyl-pyrazine-2,6-dione       | −6.98                       | 4 2 interactions with Lys124 one with Thr112 and one with Mn + + ion             |                                         | 2.01 Å|
| 21  | (E)-3-[(2-chlorophenyl)-N(1,2-dihydroxy-3-propynylindol-6-yl)prop-2-enamide      | −8.33                       | 3 Thr112 and 2 interactions with both Mn + + ions                                |                                         | 1.27 Å|
| 22  | (Z)-3-Hydroxy-1-phenyl-3-(1H-1,2,4-triazol-3-yl) prop-2-en-1-one                  | −7.61                       | 5 Lys124, Lys127 and with both Mn + + ions                                        |                                         | 2.37 Å|
| 23  | Baloxavir                                                                       | −10.47                      | 3 Lys127, and with both Mn + + ions                                              |                                         | 1.09 Å|
| 24  | BPR3P0128                                                                       | −8.72                       | 4 Glu110, Lys127, and with both Mn + + ions                                       |                                         | 1.09 Å|
| 25  | Elsulfavirine                                                                    | −7.92                       | 5 Lys124 and 3-interactions with both Mn + + ions                                |                                         | 2.14 Å|
| 26  | Favipiravir                                                                      | −6.27                       | 6 His36, Asp37, Thr112, Val113, Lys124 and one with Mn + + ion                   |                                         | 2.11 Å|
| 27  | Flutimide                                                                        | −7.34                       | 4 His36, Lys124 and 2 interactions with both Mn + + ions                          |                                         | 2.69 Å|
| 28  | L-708906                                                                        | −9.41                       | 4 Glu110, Lys124 and with both Mn + + ions                                       |                                         | 2.05 Å|
| 29  | PubChem CID = 9,931,569                                                          | −9.81                       | 6 Glu110, Lys124, Lys127 and with both Mn + + ions                               |                                         | 3.54 Å|
| 30  | SCHEMBL15863454                                                                  | −9.25                       | 3 Lys124 and with both Mn + + ions                                               |                                         | 1.49 Å|
| 31  | Schembl22813509                                                                  | −9.41                       | 5 His36, Val113, Lys124 and with both Mn + + ions                                 |                                         | 1.25 Å|
| 32  | Schembl22890980                                                                  | −7.09                       | 6 Ala33, His36, Val111, Val113, Lys124 and both Mn + + ions                      |                                         | 1.39 Å|
| 33  | PubChem CID = 6,479,291                                                          | −9.41                       | 3 Lys124, Lys27, Glu110                                                           |                                         | 1.17 Å|
Moreover, the top three performing compounds were subjected to further in-silico computational studies. Approaches like ADMET and drug-likeness studies were also performed to predict their bioavailability, toxicity, and excretion mechanisms in the living system by utilizing the ADMET-SAR server. While the drug-likeness studies of these three lead finds were performed via the SwissADME online server.

**ADME and toxicity in-silico prediction**

For the in-silico ADMET prediction study evaluation, the top lead hits identified through molecular docking studies were selected and admetSAR [39] server was used. All three lead compounds of our study had favorable Human intestinal absorption (HIA) and were listed as HIA +. The first hit compound 2′-deoxy-2′-fluorocytidine had the most enhanced HIA absorption values, followed by ingavirin and then peramivir. All three compounds were also BBB + and had good blood–brain barrier (BBB) absorption values. The same was the case in the blood–brain barrier (BBB) absorption of these three compounds. 2′-Deoxy-2′-fluorocytidine has more enhanced BBB values followed by ingavirin and then peramivir in the acceptable ranges. When the estimated values of P-glycoprotein (P-gp) discharge of these lead compounds were checked all of them were non-inhibitors of P-gp protein. Likewise, none of these compounds inhibited the renal-organic cation-transporter proteins and did not affect their drug secretion activity in the urine.

In the metabolism evaluation studies, it was discovered that none of the three lead compounds affected the activity of the cytochrome-P450 enzymes and were listed as non-inhibitors for this class of enzymes. The non-inhibitor of cytochrome p450 enzymes denotes that these compounds do not interfere with the biotransformation of compounds metabolized by the cytochrome p450 class of enzymes. Furthermore, the AMES toxicity profile revealed that these three compounds were non-mutagen and would not cause or induce congenital fetal defects in pregnancies. In the carcinogenicity profile studies of these compounds, it was found that they were all non-carcinogenic. In oral toxicity in-silico evaluation studies, all lead compounds were listed in category-III of toxic compounds. This category contains compounds with LD_{50} concentrations of more than 500 mg/kilogram but less than 5000 mg/kilogram they are lower toxic compounds. In contrast, the most poisonous compounds are placed in Category-I according to the United States Environmental Protection Agency (US-EPA). Lethal dosage or, in short LD_{50} in-silico toxicity (Acute-toxicity) profile in Rat-Model of these compounds showed that LD_{50} value for 2′-deoxy-2′-fluorocytidine was 2.0673 mol/kg. In

### Table 2 (continued)

| S/N | Compound’s name | Docking S-Score (Kcal/mol) | Interaction formed | Interacting residues and Mn^{2+} ions | RMSD |
|-----|-----------------|-----------------------------|-------------------|---------------------------------------|------|
| 34  | PubChem CID = 136,254,250 | −9.71 | 3 | Lys124, His36 and with one Mn + + ion | 1.73 Å |
| 35  | Q27454867 | −13.79 | 1 | Glu10 | 0.89 Å |
| 36  | Ro-32–7804 | −11.02 | 2 | Lys124, His36 | 1.23 Å |
| 37  | S-1360 | −8.74 | 4 | His36 and one Mn + + ion | 2.59 Å |
| 38  | SCHEM BL15456183 | −9.99 | 2 | Lys127 and & with Mn + + ion | 1.15 Å |
| 39  | STK445921 | −11.55 | 2 | With both Mn + + ions | 3.70 Å |
| 40  | N-(3,4-Dihydroxyphenyl)-2-(3,4-dihydroxyphenyl)acetamide | −13.68 | 2 | Val112 & one Mn + + ion | 1.37 Å |
| 41  | Efavirenz | −8.13 | 2 | His36 and Lys124 | 0.72 Å |
| 42  | Doravirine | −10.47 | 3 | His36, Lys124, Lys127 | 1.70 Å |
| 43  | Chembi4452342 | −9.31 | 2 | Tyr32, Asp29 | 1.06 Å |
| 44  | BMY-27709 | −8.97 | 3 | His36, Thr112 & one Mn + + ion | 1.41 Å |
| 45  | 6-(4-Fluorophenyl)-5-[4-(1 h-Tetrazol-5-Yl)phenyl]pyridine-2,3-Diol | −8.69 | 1 | With one Mn + + ion only | 2.23 Å |
| 46  | 2-(4-bromophenyl)-6-chloroquinoline-4-carboxylic acid | −10.37 | 3 | Thr112, Glu110, Lys124 & an Mn + + ion | 1.97 Å |
| 47  | 4,4,4-trifluoro-3-hydroxy-3-(2-methyl-1,2,4-triazol-3-yl)-1-phenylbutan-1-one | −7.61 | 2 | His36, Lys124 | 3.10 Å |
| 48  | 3-[(3-benzoyl-1,2-dihydroxyindol-6-yl)carbamoylamino]benzoic acid | −11.54 | 2 | Glu123 & one Mn + + ion | 1.63 Å |
| 49  | 2-hydroxy-4H-isoquinoline-1,3-dione | −7.08 | 2 | His36 & Val113 | 3.11 Å |
| 50  | 2′-FdG | −9.27 | 5 | Val113, Glu123, Lys124, Lys127 and with one Mn^{2+} ion | 2.26 Å |
comparison, the LD50 concentrations for ingavirin and peramivir were 1.9207 mol/kg and 2.5219 mol/kg, respectively. These are high LD50 values, which means that these three compounds will be well tolerated by the living system when administered to its bodies. All of the predicted ADMET properties are listed in Table 3.

**Drug-likeness properties**

The three top lead compounds were further subjected to drug-likeness through *in-silico* prediction studies to check if these compounds have the potential to be used as drugs against the HTN virus. These studies were performed via the SwissADME server [40] which predicts the drug-likeness properties of small molecules by checking various chemical and structural features of these compounds i.e. Topological surface area (TPSA) which can be defined as the number of surface polar atoms in a molecule. An increase in surface polar atoms of a compound causes the TPSA value to increase, and substances with higher TPSA values are better p-glycoprotein substrates, meaning they are discharged from cells at higher rates and have lower membrane permeability. So the lower a compound TPSA value is, the more drug-like it will be. The recommended TPSA value is either equal to or less than 140 Å². Another drug-like substance feature is lower molecular weight; most drugs have a smaller molecular weight, which increases their absorption. As a result, most drugs are created with the smallest possible molecular weights. But with, slightly higher TPSA and molecular weights can also be regarded as drug-like as several medications have been approved by the FDA which do not fully satisfy Lipinski’s drug rule [41], but higher TPSA and molecular weights cause the drugs to have lower absorption from the GI-tract and has poor pharmacokinetic properties [42]. The TPSA values for 2′-deoxy-2′-fluorocytidine; ingavirin and peramivir were 110.60 Å², 95.08 Å², and 151.03 Å², respectively. While their molecular weights were 245.21 g/mol, 225.24 g/mol, 328.41 g/mol, and 281.28 g/mol, respectively. All of the compounds had acceptable TPSA and molecular weight values, good solubility in water, and good GI-tract absorption in the tolerable range. All four of these compounds satisfied and completely followed the Lipinski drug-likeness rule. All the other Lipinski’s drug rule parameters of our top lead compounds are given in Table 4.

Moreover, other physicochemical properties on which drug-likeness of a compound depends include the instauration in compounds, size, its lipophilicity, insolubility, polarity, and flexibility (Number of rotatable bonds in a compound) are also have been given in the Radar diagrams (Fig. 2a–c) for each of our top lead compounds.

Moreover, further analysis of pharmacokinetics of these compounds showed that they had bioavailability scores of 0.55. Normally, a drug candidate requires a bioavailability score of at least 0.10 to be taken into consideration for further drug evaluation studies. These compounds also had good water solubility properties as predicted by swissADME web server [40].

| Compound          | Mol-weight (g/mol) | Lipophilicity (MLogP) | H-bond donors | H-bond acceptors | TPSA       | Rule-violations |
|-------------------|--------------------|-----------------------|---------------|------------------|------------|----------------|
| 2′-Deoxy-2′-fluorocytidine | 245.21             | 0.75                  | 3             | 6                | 110.60 Å² | None           |
| Peramivir         | 328.41             | 1.04                  | 5             | 5                | 151.03 Å² | None           |
| Ingavirin         | 245.21             | 0.77                  | 3             | 4                | 95.08 Å²  | None           |
The colored space in the radar diagrams (Fig. 2a–c) below represents the appropriate physicochemical space for a drug-like compound's oral bioavailability.

**MD simulations analysis**

**Simulation of free HTN RdRp endonuclease**

To better analyze the interactions and dynamics of the HTN RdRp endonuclease domain and the resultant top hit complexes from the molecular docking studies, the free protein and all the three top complexes were simulated for 100 ns using the Schrodinger Desmond MD simulation package. The MD simulation of the free HTN RdRp showed an initial root mean square deviation of 3 Å, which increases to 5.4 Å during 30 ns simulation time. Then there is a drop to 4.0 Å, beyond which it increases again and from 50 to 100 ns it is around 5.4 Å. Thus, in half of the simulation time from 50 to 100 ns, it is in equilibrium with minor fluctuations (Fig. 3). The root means square fluctuation showed that the initial 20 amino acids and residues number from 80 to 90 have the highest fluctuation, while the rest of the amino acids in the HTN RdRp endonuclease are quite rigid (Supplementary Fig. 3).

![Fig. 2](image1.png)  
**Fig. 2** a Physicochemical properties radar diagram of 2’-Deoxy-2’-fluorocytidine. b Physicochemical properties radar diagram of Peramivir. c Physicochemical properties radar diagram of ingavirin

![Fig. 3](image2.png)  
**Fig. 3** C-α root mean square deviation of the HTN RdRp endonuclease over a 100 ns simulation time
Mn\(^{2+}\) ions with the Asp40, Glu110, Asp97, and Glu54 coordinating these ions. Ingavirin also interacted with the other active site residues which are the HTN endonuclease active site via ionic interactions and ingavirin effectively interacted with the manganese ions of the HTN virus endonuclease domain Active site can be seen interacting with ingavirin along the 100 ns simulation trajectory.

**Simulation of HTN RdRp endonuclease in complex with ingavirin**

As previously observed in the molecular docking studies, the top identified hits effectively interacted with the Mn\(^{2+}\) ions and other active site residues of the HTN virus endonuclease domain, the same was the case with the MD simulation interaction studies, in which these ligands showed effective interactions with the target protein active site during the 100 ns MD simulation time. These interactions occurred significantly along the simulation trajectories of all the simulated complexes. The RMSD of the protein in complex with the ligand showed a different behavior because of complexation with the ligand during the 100 ns simulation time (Fig. 4a). The RMSD of protein is in continuous fluctuation from an initial 2.5 Å to rise at 30, 40, and 80 ns simulation time window. Around 45 ns it even jumped to 7.5 Å, but at the end of simulation time, the RMSD of the protein was 5.5 Å. In Fig. 4a, the maroon color represents the RMSD of the ingavirin ligand inside the protein's binding pocket. It can be observed that the RMSD of the ligand also fluctuated during the simulation time due to the making and breaking of various contacts between the ligand and protein. The average RMSD of the ligand is in the range of 4–5 Å (Fig. 4a). The RMSF of the C-α of the amino acids of the protein in complex with ligands showed quite a different fluctuation from that of the free protein (Supp. Figure 3). In the complex form with ingavirin, the initial 20 amino acids of the HTN RdRp showed fluctuation up to 9 Å, however, the region from amino acid 80–90 have lower fluctuation. This lower motion in these residues is an indirect effect of various residues in contact with the ingavirin (Fig. 4a right panel).

It can be observed from the MD simulation of the ingavirin in complex with the HTN RdRp complex (Fig. 4b), that ingavirin effectively interacted with the manganese ions of the HTN endonuclease active site via ionic interactions and also interacted with the other active site residues which are coordinating these ions. Ingavirin also interacted via these Mn\(^{2+}\) ions with the Asp40, Glu110, Asp97, and Glu54 amino acids of the active site.

Moreover, the heterocyclic ring of ingavirin, which contains nitrogen atoms also made a hydrogen bond with the Asp37 of the HTN virus endonuclease domain. Furthermore, these ionic interactions involving Mn\(^{2+}\) ions have a likelihood of appearing above 60 percent during the 100 ns MD simulation. It can be inferred from the MD simulation results, that it appears that ingavirin carbonyl and hydroxyl groups are required for interaction with the HTN viral endonuclease domain. Throughout the simulation, the interaction fraction of ingavirin + HTN endonuclease domain contacts can be seen in Fig. 4c, ionic interactions between the active site of the endonuclease domain of the HTN virus are the major type of interactions. Asp40, Glu54, Asp97, and Glu110 can be seen that they are engaged in significant ionic interactions with the manganese ions of the active site. The contacts across water molecule bridges are particularly noteworthy, such as the water-assisted ionic contact to Asp40, Glu54, Asp97, and Glu110. In Fig. 4d, the intensity of the interacting amino acids of the HTN virus endonuclease domain can be seen along the 100 ns simulation trajectory, which suggests that ingavirin, was in stable association during the MD simulation with the target protein. Supplementary Fig. 4 showed the variation in various surface area parameters, the radius of gyration, and RMSD of the protein in complex with ingavirin during the 100 ns simulation.

**Simulation of HTN RdRp endonuclease in complex with peramivir**

Peramivir and HTN RdRp endonuclease domain complex was also simulated for 100 ns via Schrödinger Desmond MD simulation software. Figure 5a (Left panel) shows the RMSD of the RdRp endonuclease (blue color). It can be observed that the RMSD of the protein rises from 1.8 to 5.6 Å during the 10 ns simulation time and then it becomes constant with minor fluctuation till 40 ns. The RMSD of the protein rises from 4 Å at 40 ns to 70 ns and then it fluctuates in the range of 4.8 Å till the end of simulation time. The RMSD (maroon color) of the ligand present in the protein–ligand complex has an initial value of 1.6 Å that rises a little bit and is in the average value of 2.4 Å till 40 ns but from 40 ns it showed a sudden rise to 4 Å average till 60 ns. A further rise was observed with an average value of 13-14 Å from 70 to 100 ns simulation time window. This higher RMSD showed the formation of fewer contacts between the protein and the ligand (peramivir). The right-hand side of Fig. 5a showed the root mean square fluctuation of the C-alpha of the amino acid residues of the HTN RdRp endonuclease in complex with peramivir. The initial 5–20 amino acids showed a maximum RMSF value of 4.8 Å. While the protein region from amino acids 80–90 showed a root mean square fluctuation of 3.2 Å while the rest of the amino acids in the protein are quite rigid during the simulation time.

In the peramivir and HTN RdRp complex MD simulation assessments, Peramivir efficiently interacted with the manganese ions of the HTN endonuclease active site via...
ion interactions, as well as with the other active site residues that are coordinating these ions, as shown in Fig. 5b. Both of the manganese ions of the active site made strong interactions with the peramivir ligand. This ligand’s carboxyl moiety mostly interacted with one of the Mn$^{+2}$ ions of this target protein. The hydroxyl and the carbonyl groups of this carboxyl group engaged this Mn$^{++}$ ion individually, the hydroxyl –OH group was in contact with the Mn$^{+2}$ ion over 80% of the 100 ns simulation time. In contrast, the carbonyl of the carboxyl group interacted with this Mn$^{+2}$ ion of the active site for over 40 percent of the simulation time. This carboxyl group also interacted with the Glu54 and Asp97 amino acids of the active site via the Mn$^{+2}$ ion (Fig. 5b). The acetamide group carbonyl oxygen atom of peramivir interacted with the second manganese ion of the active site. This same acetamide’s group carbonyl oxygen atom also interacted with the Glu110 and Asp110 residues of the active site of the HTN RdRp endonuclease domain.

The interaction fraction diagram of this complex during the 100 ns simulation can be seen in Fig. 5c that there were significant and continuous interactions of the ligand peramivir with the manganese ions and other active site residues of the HTN RdRp endonuclease domain. Interactions of various types’ mostly ionic interactions of our ligand peramivir, can be seen with the Glu54, Thr95, Asp97, and Glu110 of the HTN RdRp endonuclease domain. Contacts like across water molecule bridges, such as peramivir water-assisted ionic contact to Asp40, Glu54, Asp97, Thr95, and Glu110 active site residues of the HTN RdRp endonuclease domain, are particularly meaningful interactions. The interactions made by peramivir with the active site during the 100 ns simulation trajectory were significant interactions and the ligand was in contact with the target protein during the MD simulation, indicating that the association of peramivir with the target protein was stable associations. Supplementary Fig. 5 showed the variation in various surface area parameters, the radius of gyration, and RMSD of the protein in complex with peramivir during the 100 ns simulation.

The MD simulation of our study’s third identified lead compound was 2’-Deoxy-2’-fluorocytidine. Although this compound showed significant interactions with the target protein during the molecular docking studies, however, when this ligand–protein complex was simulated for 100 ns time, it was noted that the association of this ligand was not very stable in the simulation and interacted very few times with the target protein during the MD simulation studies. Although in this computational study, we consider the HTN RdRp endonuclease alone and performed docking and simulation with small molecule inhibitors. However, in physiological conditions, different nucleocapsid, nucleoproteins, and RNA molecules are in continuous contact with the RdRp of the Hantaan virus [43]. These proteins mostly protect and stabilize this polymerase, exert allosteric effects on the enzyme, and enhance the catalytic activity by providing different reacting molecules to RdRp [44]. Further during the synthesis of viral RNA activity, this multitask enzyme is in continuous dynamic condition and its catalytic site is transiently available to the drug inhibitors that mimic the nucleotide molecules of the producing RNA molecules.

The compounds identified in this study have previously been shown to have broad-spectrum antiviral activities against multiple viruses. For example, this study identified ingavirin, a lead compound, possesses inhibitory activity against human metapneumovirus (hMPV). The hMPV is a respiratory virus and cause upper and lower respiratory tract infections. Studies have shown that ingavirin can effectively block the replication of this virus at 50 to 500 μg/mL concentrations [45]. Studies on Parainfluenza virus (PI) which is also another respiratory pathogen, showed that ingavirin could effectively inhibit this virus and protect the bronchial epithelium and exerts its inhibitory activity by decreasing the cytopathogenic effect caused by the PI virus [46]. Similarly, research investigations on human adenovirus (type-5) which is also a pathogenic respiratory virus, showed that ingavirin could also inhibit this virus by disturbing the normal morphogenesis of this virus during infection [47], along with these viruses ingavirin is also a drug of choice against multiple Influenza virus (IAV) variants and it is an approved drug for the infections caused by IAV [34, 48]. Researchers have also proposed Ingavirin and its derivatives that may possess antiviral activity against SARS-CoV-2 [49, 50].

2’-Deoxy-2’-fluorocytidine, the other identified lead in the molecular docking studies, has also been reported to be active against Crimean-Congo hemorrhagic fever virus (CCHFV) which as the name suggests, also causes hemorrhagic fever in humans, research studies had shown that 2’-Deoxy-2’-fluorocytidine can more potently inhibit the activity of this virus than ribavirin which is also a potent broad-spectrum antiviral [51]. Several derivatives of this compound have also been reported to be active against HCV by targeting its RdRp enzyme [52]. Moreover, peramivir is also a drug of choice against the influenza virus A and B (IAV) and has been reported in the literature to have broad spectrum activity against several of the virulent strains of IAV (H1N1, H5N1) [53, 54].

Computer-aided drug design and discovery (CADD) techniques like the one we have used in this study have been previously successful in identifying potent inhibitory
compounds against several target proteins in various viral diseases [31, 55–57]. So taking into account the past literature studies which showed full antiviral activity of these top identified compounds against multiple human pathogenic viruses, it can be inferred from this study that further experimental cell-based in vitro and in vivo investigations on these compounds against the HTN virus can help identify the potency of these compounds in combating the hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) caused by HTN virus.

Conclusions

Small molecular weight compounds were screened for their inhibitory action against the Hantaan virus RdRp endonuclease domain by utilizing different in-silico techniques. Molecular docking provides the initial information about the top hit compounds. These top hit identified molecules in complex with the target enzyme were then subjected to molecular dynamics simulations. Two identified top hit compounds showed stable binding during the MD simulations. The ADMET analysis of Peramivir, 2'-Deoxy-2'-fluorocytidine, and Ingavirin showed that these compounds have suitable human intestinal absorption (HIA) values and are easily available when taken orally. They were also non-inhibitors of the cytochrome p450 class of enzymes and non-mutagenic, and non-carcinogenic. The drug-likeness studies of these compounds also showed that these compounds have acceptable molecular weights and have a fair number of hydrogen bonds (both acceptor and donor type H-Bonds). These compounds have lower TPSA values, resulting in better drug absorption and suitable bioavailability scores of 0.55, and also fully satisfied Lipinski’s and GSK drug rules. These molecules showed strong binding interactions with the manganese ions and the active site residues. We recommend peramivir and ingavirin for future in vitro and in vivo investigations against the HTN virus because these compounds possess broad-spectrum antiviral activity against multiple human pathogenic viruses. These identified lead compounds can also be repurposed and used against other related viruses if further in vitro and in vivo studies are pursued.

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Authors contribution SF: performed the docking, ADMET study, and analysis of the simulation, methodology, validation, and visualization under the supervision of SLB. They also wrote the manuscript. MS and MA: performed the simulation and its methodology.

Data availability Data related to this research will be provided upon request.

Declarations

Conflict of interest All the authors declare that they have no competing interest.

Ethical approval The authors have compliance with all the ethical standards for this research work.

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