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Development of a database of RNA helicase inhibitors (VHIMDB) of pathogenic viruses and in silico screening for the potential drug molecules

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Abstract

The pathogenic RNA virus that infects human beings contains the RNA helicase enzyme, responsible for the replication of the viral genome. The enzyme is used as a suitable target against which the drug molecule acts. Therefore, the identification and proposal the novel compounds that can be targeted toward the helicase enzymes to stop the functioning of the enzyme is desirable. Although many viral helicase inhibitor molecules have been identified, still yet no unique database is available for these compounds. This research work envisages developing a curated database of RNA helicase inhibitors. The database contains in total of 353 entries that are computationally predicted and experimentally verified RNA helicase inhibitors. The database contains information like compound name, chemical properties, chemical format, and name of the target virus to which it acts against it with a user-friendly menu-driven search engine. Presently, the database is freely available at: https://vhimdb.rsatpathy.in/. Further, in silico screening of the whole database by drug-likeness and toxicity resulted in 14 potential drug molecules. The selected molecules were analyzed for their effectiveness in binding by using molecular docking score and interaction with the helicase enzymes of three categories of pathogenic viruses (SARS-CoV-2, SARS-CoV, and MERS-CoV).

Keywords: relational database, RNA helicase inhibitor, pathogenic virus, web development, molecular docking, interaction analysis, drug development

Introduction

A large number of diseases in the case of human beings are caused due to the infection of the RNA-based virus. The enhanced infection rate sometimes becomes pandemic and causes a huge loss in human society [1-2]. Also, the genomic evolutionary rate of these pathogenic viruses is rapid therefore creating resistance against the antiviral drugs. Different types of anti-viral drugs are available for the control of infection of pathogenic viruses based on their activity on the viral targets [3-4-5]. Interestingly, sometimes a single type of antiviral drug molecule can be used to control the pathogenic RNA viruses that usually block the genome replication. In this aspect, the RNA helicase enzyme is considered a suitable target upon which the inhibitor molecules can act [6-7]. The viral helicase enzyme is an essential enzyme that helps in the separation (post replication process) of nucleic acid strands by deriving the energy from the hydrolysis of the Adenosine Triphosphate (ATP) molecule [8]. The detailed structural studies of the helicase enzymes have been performed in the case of positive-sense single–stranded RNA viruses such as hepatitis C virus (HCV), severe acute respiratory syndrome coronavirus (SARS-CoV), Dengue virus, Japanese encephalitis virus (JEV), and West Nile virus (WNV) and so on [9-10-11]. Most recently their validity as effective antiviral drug targets has been confirmed in the case of novel coronavirus 2019 (Sars -CoV-2) [12-13-14]. Due to rapid progress in the viral assay development methods followed by high throughput screening (HTS) enables the researcher to discover the novel potential helicase inhibitor molecules [15-16-17]. Also, the major challenge encountered in understanding the interaction of these compounds with the viral helicase enzymes, so that they can be treated as drug molecules against the virus. Very few compounds with promising helicase inhibition activity

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have been identified [18-19]. However, more research work is essential to identify and establish the novel helicase inhibitor as a common drug. Current in vitro methods applied for novel viral helicase inhibitor identification is difficult and time-consuming [20-21]. Hence in this context, the in silico method has proven its effectiveness in finding novel RNA helicase inhibitors of pathogenic viruses as the method is performed with less time and low cost [22-23].

To avoid future pandemic situations caused by the pathogenic virus, searching for novel inhibitor molecules against the specific virus target is desirable. To date, there is no specific database of RNA helicase inhibitor molecules of the pathogenic virus. In this context, the present objective of this work is to develop a curated database of viral RNA helicase inhibitors and implementation of in silico methods to discover the novel inhibitor molecules by screening the database.

Materials and Methods

System configuration used
Processor: Intel® Core™ i3-10100 CPU@3.60GHz
Installed memory (RAM): 8.00 GB
System Type: 64-bit Operating System, Windows 10

Development of database

The information about the inhibitors and their activity towards RNA helicase was searched by the published literature and several search engine and other existing online resources. These data obtained from the journal article and other resources were curated manually and arranged with some other molecular information of the molecules for the database development (Figure 1). The architecture of the designed database was created based on a three-level system such as user interface (Client-side), application layer (Middle layer), and data storage system (Server-side). This was carried out by using the XAMPP tool (http://www.apachefriends.org/). XAMPP contains an integrated and cross-feature rich platform and is most commonly used for Web development. The acronym of the XAMPP tool is X (cross-platform), A (Apache server language), M (MySQL), P (PHP), and other P (PERL language) that facilitates the database creation [24]. MySQL was used to design the backend of the database, which is implemented in relational database management systems (RDBMS) throughout the world [25].

Further, HyperText Markup Language (HTML) was used to design the front-end part of the database. PHP programming scripts were used to connect various attributes of the database. PHP is a scripting language designed for the integration of server-side data with the network. The HTML code is rendered by a Web-based server with a specific PHP module which was applied for generating the resulting Web page [26]. Finally, a suitable domain name was chosen and the database was uploaded to the webserver for its free access throughout the world.

Screening of Database

The database containing compounds was screened further based on the drug-likeness and toxicity properties. The drug-likeness filter was implemented by using Lipinski's rule of five systems [27]. The compounds were selected based on zero violation of the rule. Further screening of the remaining compounds was performed by evaluating their toxicity profile using the ProTox-II server (https://tox-new.charite.de/protox_II/). ProTox-II is a sophisticated computational tool to predict the toxicity of compounds by the input of the compound information in SMILE format. This tool considers different pharmacophoric and molecular features of the input compounds and predicts different toxicity by using machine learning methods. The models for the prediction are generated based on the in vivo and in vitro experimental information. This server predicts six classes of toxicity, starting from 1 (highest toxicity) and 6 (lowest toxicity) [28]. In this work, those compounds showing toxicity levels 5 and 6 with the inactive prediction for the toxicity model were selected for further analysis.

Molecular docking study

The selected compounds were further used as ligands and subjected to molecular docking simulation. Molecular docking is a computational method to compute the ligand-receptor binding affinity along with the binding conformation of the ligand on the binding receptor. The docking program uses combinations of scoring and searching algorithms to perform the task [29-30]. The experimental structure of (receptor) helicase enzyme of SARS-CoV-2, SARS-CoV, and MERS-CoV was obtained from the Protein databank (PDB) available at https://www.rcsb.org/. The structures were further processed by removing water molecules and ligands by using the Pymol tool (https://pymol.org/). The ligand molecular structure was obtained from a PubChem database search (https://pubchem.ncbi.nlm.nih.gov/). The energy minimization of these ligands were carried out by Avogadro (version 0.80) software tool. The minimization process was performed by using the MMFF94 force field with steepest descent algorithm that run maximum 500 numbers of steps. For molecular docking methods, the Autodock Vina tool was used (https://vina.scripps.edu/). Autodock Vina is a popular tool and used worldwide for molecular docking study due to its accuracy and fast in nature [31]. The polar hydrogen and Kollman charges were added to the ligand PDB file and was converted to the corresponding PDBQT file. Similarly, the receptor PDB file format was also converted to PDBQT file format by using ADT tool. Further, the grid box was created by choosing the appropriate grid parameters such as centre and dimension values in x, y and z axis based on the size of the receptor molecule. Lamarckian Genetic Algorithm (LGA) was used as the searching algorithm in the molecular docking simulation process. The docking complexes were further analyzed to study the ligand-binding residues and positions by using the PLIP (Protein-ligand interaction profiler) server available at https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index. PLIP tool computes different types of

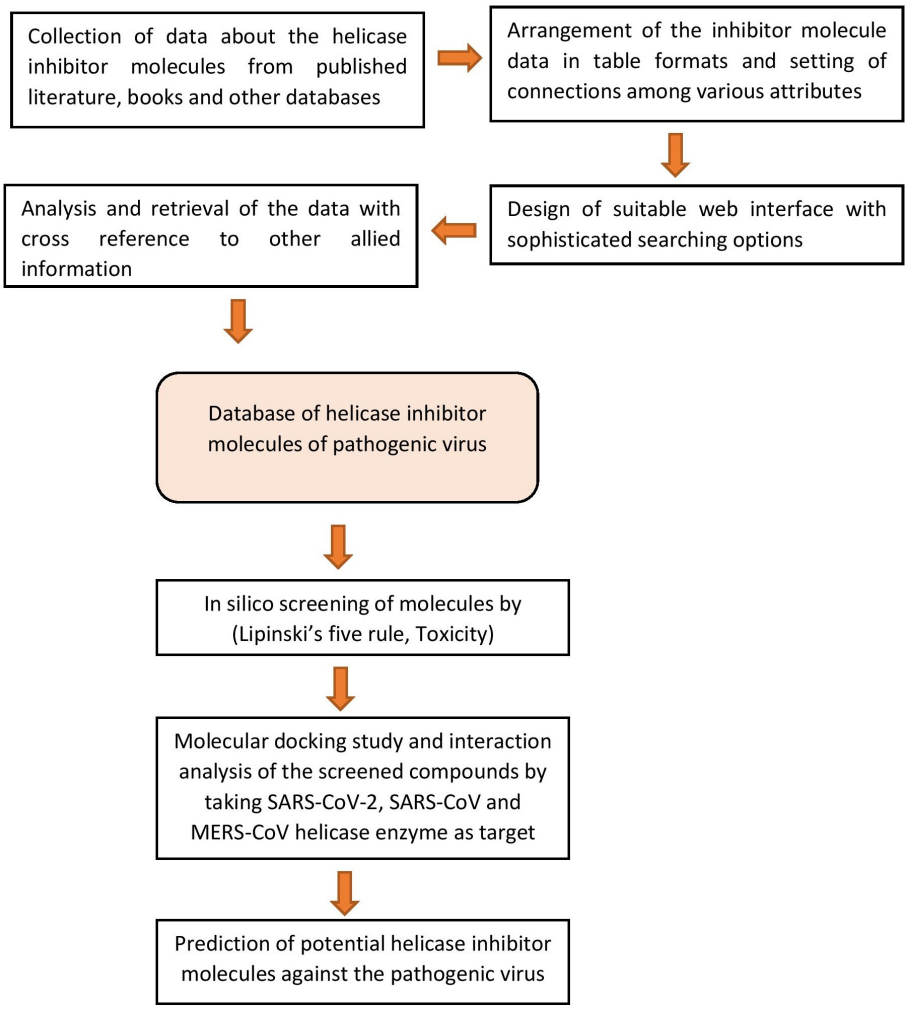


Figure 1. Schema for the design of RNA virus helicase inhibitor database and screening methods

interactions like hydrogen bonds, hydrophobic interaction, salt bridges π-stacking, and π-cation interactions between ligands and receptors by taking the PDB file as input [32]. The selected drug molecules were then searched in the drug bank database (https://go.drugbank.com) for their structures, compositions, and other allied properties for further analysis.

Results

The developed database was created and named VHIMDB which is available freely at https://vhimdb.rsatpathy.in/. To date there is no specific database is available that contains exclusive information about the RNA helicase inhibitor molecule of the pathogenic virus. Hence the present database is unique in this aspect.

The database contains several important information about the molecules such as name, molecular weight, log P, number of Hydrogen bond donors and acceptors, target virus against which it acts, as well as the bibliographic details about the helicase inhibition activity. Additionally, the PubChem link has been added as the external link attribute, and the molecular structure and the molecular formula in 2D format can also be obtained by using the structure and summary option that has been hyperlinked (Figure 1). The database can be explored in a user-friendly way, by searching the alphabet. The hyperlink features available in the database provide an advantage to users by saving the time required to search for other information in the databases. Additionally, the database allows the user to submit a new entry in the submit data button by filling out an online form. The information given by the user is to be verified and subsequently updated in the database. Presently, the database contains 353 numbers of potential RNA helicase inhibitor molecules that have been predicted to be effective against several pathogenic viruses (Figure 2). The database would be updated bi-annually by looking into the new information about the potential helicase inhibitor molecules from the published literature.

Screening and molecular docking study

All the 353 helicase inhibitor entries were subjected to analysis of Lipinski's rule of five to find out whether the drug molecules had zero violation of the rules. Out of 353 numbers, 159 molecules were selected from the primary screening. Further toxicity level screening of 159 inhibitor molecules by Protox II resulted in 14 numbers of potential inhibitor molecules those show

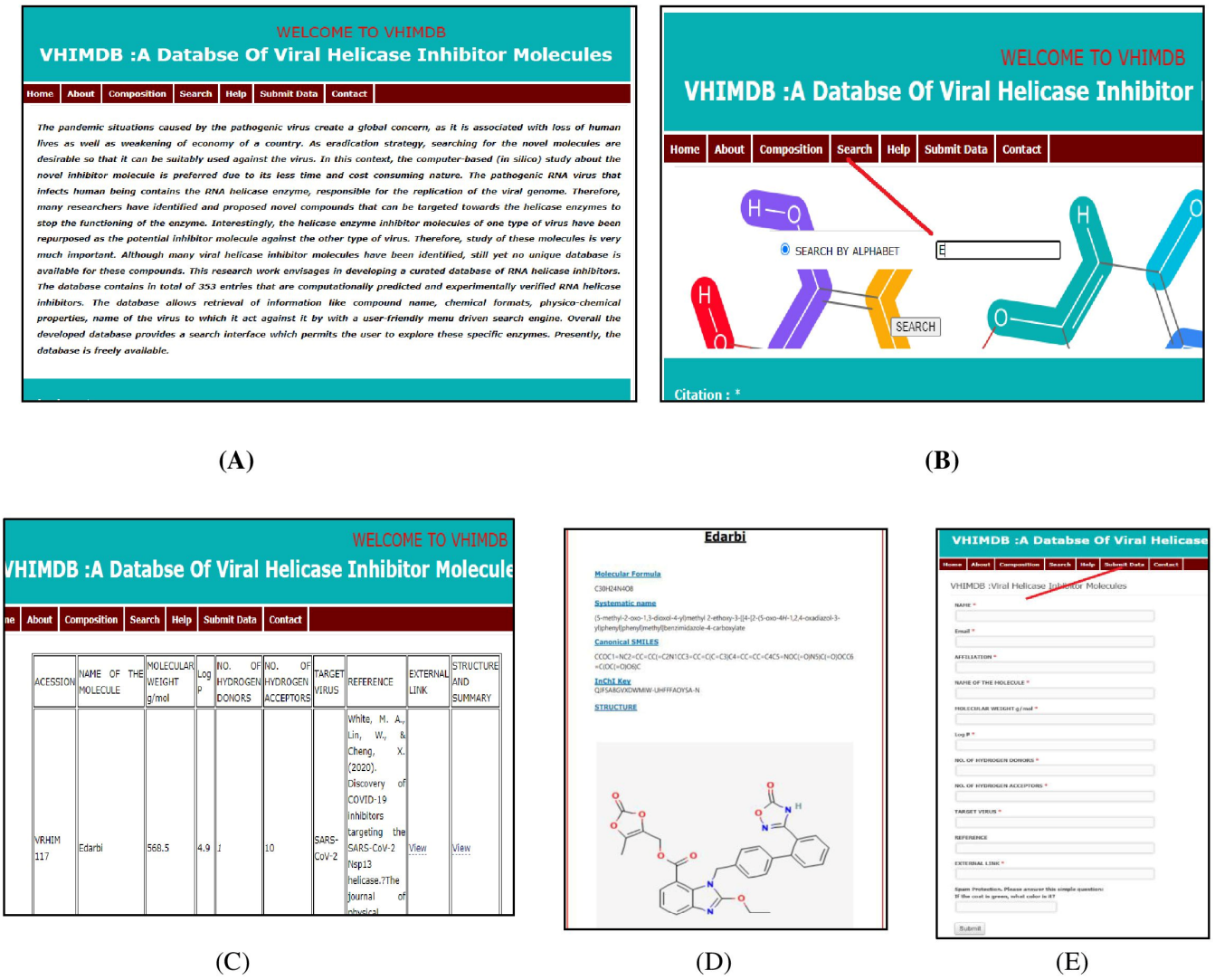


Figure 2. Snapshot of the database (VHIMDB): **A:** Home page **B:** Options of molecule search by alphabet **C:** Searching result of the molecule Edrabi (accession number 117) **D:** Detailed information of the molecule **E:** Data submission portal

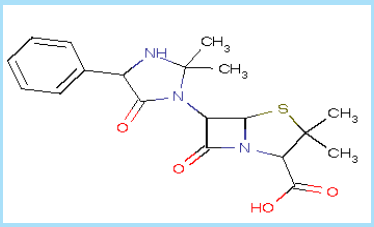
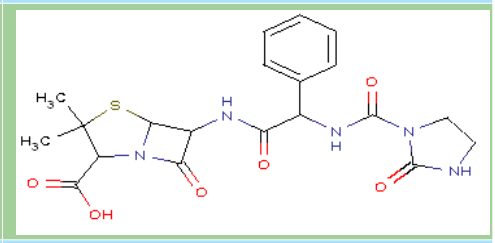
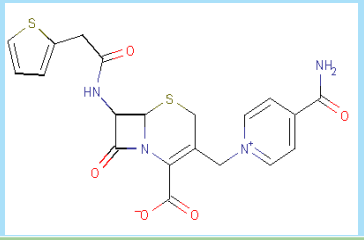
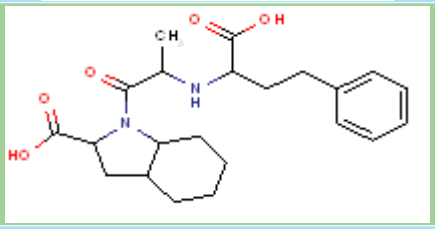
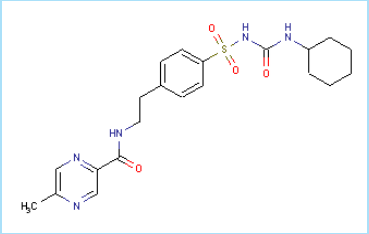
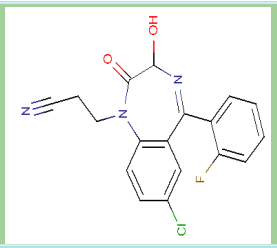
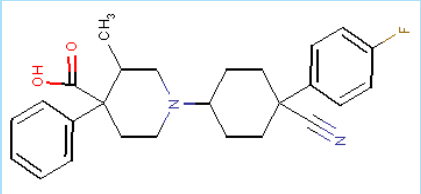
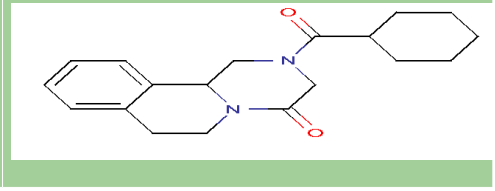
toxicity levels 5 and 6. These compounds were subsequently used as the ligands in a molecular docking study (Table 1).

The protein databank was searched for the crystallographic structures for the helicase enzyme of SARS-CoV-2, SARS-CoV, and MERS-CoV virus and was used as a receptor in the molecular docking study (Table 2).

After processing the ligand files suitable grid box was set for each of the receptors by using the ADT tool (https://ccsb.scripps.edu/mgltools/). The molecular docking was performed by the AutodockVina tool. The docking score and ligand-receptor interaction details were computed and given in the Supplementary material. Docking analysis was applied to the helicase protein of SARS-CoV-2 with the 14 selected drug molecules (Table 1). The docking simulation revealed that the highest docking score was obtained for canagliflozin drug molecule (-10.0 KJ/ Mole), and that shows 9 numbers of amino acid interaction to the SARS-CoV-2 helicase domain. However, the Maximum number of interactions (13 no.s) was observed in the helicase domain regions. No zinc-binding domain interactions were obtained. The molecule is also able to make 3 numbers of Hydrogen bonds with the Chain A of the helicase domain (Figure 4). In the case of SARS-CoV, canagliflozin drug molecule shows the highest docking score (-9.9 KJ/ Mole), and maximum interaction (11 no.s) was exhibited by the azlocillin drug molecule. Similarly, in the case of MERS-CoV, the molecule Amaryl shows the highest binding affinity (-8.5 KJ/ Mole)but the maximum number of interactions (11 no.s) were obtained for the 3 drug molecules such as hetacillin, cefalonium, and bentiromoide.

However B-chain interaction was obtained from the interaction analysis of hetacillin and Amaryl with the MERS-CoV helicase enzyme (Figure 3 and supplementary material). Overall,

Table 1. Showing the selected ligand molecules after screening by Lipinski's rule of five and toxicity features.

S.N	Accession Number	Name	IUPAC name	Structure
1	VR-HIM162	Hetacillin	(2S,5R,6R)-6-[(4R)-2,2-dimethyl-5-oxo-4-phenylimidazolidin-1-yl]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hepta-ne-2-carboxylic acid	
2	VRHIM31	Azlocillin	(2S,5R,6R)-3,3-dimethyl-7-oxo-6-[[(2R)-2-[(2-oxoimidazolidine-1-carbonyl)ami-no]-2-phenylacetyl]amino]-4-thia-1-azabi-cyclo[3.2.0]heptane-2-carboxylic acid	
3	VRHIM58	Cefalonium	(6R,7R)-3-[(4-carbamoylpyridin-1-i-um-1-yl)methyl]-8-oxo-7-[(2-thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylat	
4	VR-HIM329	Trandolap-rilat	(2S,3aR,7aS)-1-[(2S)-2-[[(1S)-1-car-boxy-3-phenylpropyl]amino]propanoy-l]-2,3,3a,4,5,6,7,7a-octahydroindole-2-car-boxylic acid	
5	VR-HIM156	Glipizide	N-[2-[4-(cyclohexylcarbamoylsulfamoyl) phenyl]ethyl]-5-methylpyrazine-2-carboxa-mide	
6	VRHIM78	Cinolaze-pam	3-[7-chloro-5-(2-fluorophenyl)-3-hyd-roxy-2-oxo-3H-1,4-benzodiazepin-1-yl] propanenitrile	
7	VR-HIM183	Levocabas-tine	(3S,4R)-1-[4-cyano-4-(4-fluorophenyl)cyclo-hexyl]-3-methyl-4-phenylpiperidine-4-car-boxylic acid	
8	VRHIM40	Biltricide	2-(cyclohexanecarbonyl)-3,6,7,11b-tetrahyd-ro-1H-pyrazino[2,1-a]isoquinolin-4-one	

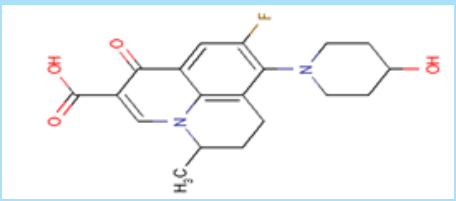
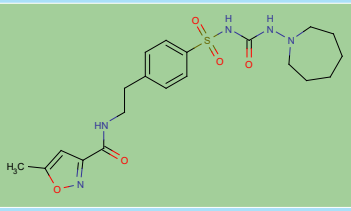
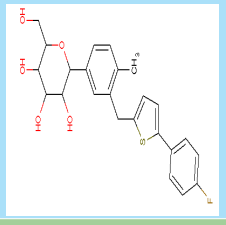
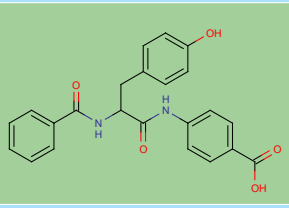
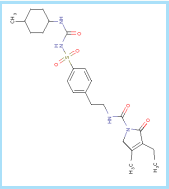
9	VR-HIM215	Nadifloxacin	7-fluoro-8-(4-hydroxypiperidin-1-yl)-12-methyl-4-oxo-1-azatricyclo[7.3.1.05,13]trideca-2,5,7,9(13)-tetraene-3-carboxylic acid	
10	VR-HIM158	Glisoxepide	N-[2-[4-(azepan-1-ylcarbamoylsulfamoyl) phenyl]ethyl]-5-methyl-1,2-oxazole-3-carboxamide	
11	VRHIM52	Canagliflozin	(2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl) thiophen-2-yl]methyl]-4-methylphenyl]-6-(hydroxymethyl)oxane-3,4,5-triol	
12	VRHIM36	Bentiromide	4-[[(2S)-2-benzamido-3-(4-hydroxyphenyl) propanoyl]amino]benzoic acid	
13	VRHIM11	Amaryl	4-ethyl-3-methyl-N-[2-[4-[(4-methylcyclohexyl)carbamoylsulfamoyl]phenyl]ethyl]-5-oxo-2H-pyrrole-1-carboxamide	
14	VR-HIM159	Retinoyl beta-glucuronide	(2S,3S,4S,5R,6S)-6-[(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl) nona-2,4,6,8-tetraenoyl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid	

Table 2. Receptor structure details considered for molecular docking study.

S.N	PDB ID	Molecule Details	Viral species	Resolution (Å)	Chain and number of amino acid	References
1	7NIO	Crystal structure of the SARS-CoV-2 helicase	SARS-CoV-2	2.20	603 (dimeric)	[33]
2	6JYT	Crystal structure of the SARS-CoV helicase	SARS-CoV	2.80	603 (dimeric)	[34]
3	5WWP	Crystal structure of MERS-CoV helicase	MERS-CoV	3.00	600 (dimeric)	[35]

the drug molecule bentiromide shows the major interaction in all types of receptors chosen in this study (Figure 3). The drug molecule Canagliflozin is an established drug that is frequently used as the inhibitor for sodium-glucose co-transporter 2 (SGLT2) and is mainly applied to diabetes and cardiovascular disease management [36]. Since these diseases are associated with the severity of SARS-CoV-2, hence can be effectively used against SARS-CoV-2 by preventing the patients from complications like multiple organ failure [37-38]. Several derivatives of the Glucuronide analogue such as Baicalin, acyl glucuronide was observed as potential proteases enzyme inhibitor of SARS-CoV-2 by the molecular docking study [39-40]. Similarly, Wu et al. studied the effect of Chrysin-7-O-β-glucuronide from the source *Scutellaria baicalensis* as the potential protease in-

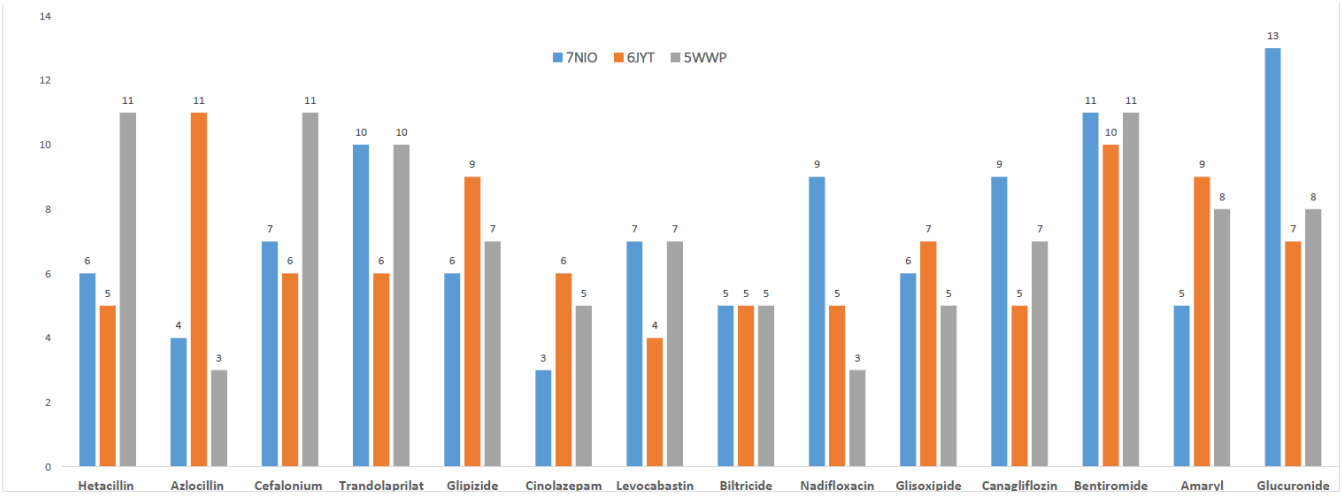


Figure 3. Showing total numbers of interactions of the selected drug molecules with the helicase enzyme of the selected 3 receptor molecules

hibitor against SARS-CoV-2 [41]. Lau et al. studied the derivatives of several glucuronide derivatives as the effective receptor binding motif (RBM) of the RBD of SARS-CoV-2 by molecular docking followed by MM/GBSA analysis [42]. Azlocillin (DrugBank Accession: DB01061) is a semisynthetic ampicillin-derived compound and is used for the treatment of bacterial and viral infections. Recent computational methods by several researchers have proposed azlocillin as a potential spike protein inhibitor molecule against SARS-CoV-2. A [41,43-44]. Bentiromide (DrugBank Accession: DB00522) is a dipeptide molecule that is used for evaluating pancreatic exocrine function. It is typically administered orally. Although the molecule shows ACE inhibitor activity, however recent study repurposed the molecules as a potential binder and inhibitor of the PLpro enzyme of SARS-CoV-2 by implementing the computational analysis [45-46]. Hetacillin (DrugBank Accession: DB00739) is also a penicillin beta-lactam antibiotic used in the treatment of bacterial infections by inhibiting cell wall synthesis. The molecule also shows TMPRSS2 (serine protease act as Spike (S) protein) inhibitor activity to prevent CoVs from host molecules entering by interaction with ACE2 and Spike protein [41,47-48]. A comparative molecular docking study of phytochemicals with several known drug molecules such as hetacillin shows potential inhibitors of SARS-CoV-2 spike protein receptor [49]. Cefalonium (DrugBank Accession: DB11385) is also known as cephalosporins and chemically it consists of a 1,2-thiazine fused to a 2-azetidinone that forms to form a oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid or any derivative of this type. Amaryl is the brand name of glimepiride (DrugBank Accession: DB00222), a drug used for the treatment of type 2 diabetes mellitus (T2DM). Ugwueze reviewed the role of angiotensin-converting enzyme (ACE) and dipeptidyl peptidase in SARS-CoV-2 infection and diabetes. Based on the immunogenic mechanisms the effect of diabetes on the recovery of SARS-CoV-2 patients was studied and the relationship between the two has been established. For example, some of the anti-diabetic drugs like dapagliflozin and linagliptin were found effective against the SARS-CoV-2 infection [50]. White et al. in their computational screening study, selected the above compounds to predict them as potential helicase inhibitors of SARS-CoV-2 [16]. However, none of the above selected compounds have been reported as potential helicase inhibitors in current studies. In our in silico analysis, these compounds show good affinity and interaction with the helicase enzymes of SARS-CoV-2, SARS-CoV, and MERS-CoV.

In this work, a curated database of RNA helicase of the pathogenic virus has been developed and named VHIMDB which is available freely on the web. The database was developed with the relational scheme and currently contains 353 numbers helicase inhibitor molecules. At present no database containing RNA helicase inhibitor data of pathogenic viruses is available. Hence this database will act as a unique resource by offering a

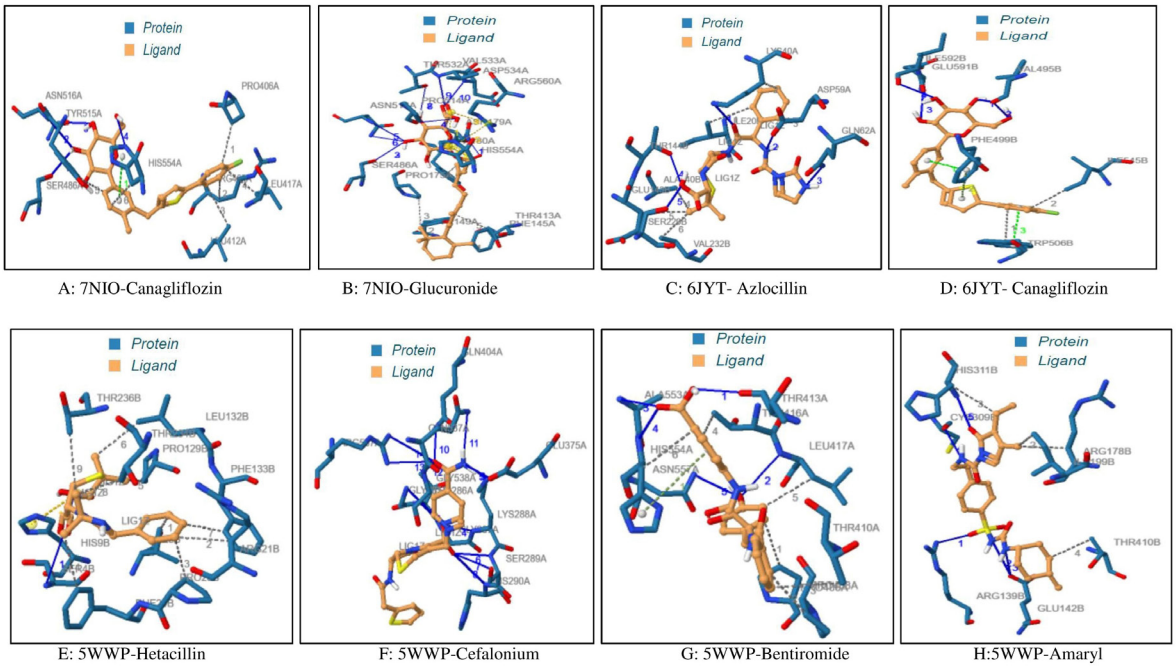


Figure 4. Showing the interaction details of the selected ligand-receptor complexes

common platform for scientific communities working in this emerging area. In a subsequent study, all the inhibitors were screened based on drug-like properties and toxicity prediction. The analysis resulted in 14 numbers of potential inhibitor molecules. Further, a molecular docking study of these selected compounds was performed by selecting the 3D structure of the helicase enzyme of the virus SARS-CoV-2, SARS-CoV, and MERS-CoV as a receptor. Additionally, receptor-ligand interaction analysis revealed that drug molecules like Canagliflozin and Glucuronide derivatives (Retinoyl beta-glucuronide) were obtained as a potential inhibitor against SARS-CoV-2, Azlocillin, and Canagliflozin as the good helicase inhibitor of SARS-CoV and Hetacillin, Cefalonium, Bentiromide and Amaryl as an inhibitor of MERS-CoV. The drug molecule bentiromide shows more or less equipotent properties against all types of receptors selected. So, these molecules can be considered as promising candidates for the virus helicase enzyme inhibition. Further extensive molecular dynamics simulation study followed by in vitro analysis is required to evaluate their effectiveness.

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