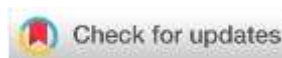
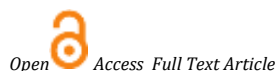


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Research Article

Structure-based Drug Design, ADME and Molecular Docking analyses of anti-viral agents against SARS-CoV-2 virus, Zika virus and Hepatitis C virus

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Abstract

Computer-aided drug design has been taking an increasing role in the field of modern drug discovery. These *in silico* computational methods are cost-effective, reduce the use of animal models in pharmacological research, and can be used to study pathogenic organisms without the need for any facilities. Based on the structure of known anti-viral agents, a total of 812 ligands have been designed. All ligands were screened for drug-likeness based on Lipinski rule of five. A database of ligands was constructed and *in silico* docking analyses were performed using MOE 2015.10 program against three selected viruses, viz., Zika virus, Hepatitis C virus and SARS-CoV-2 virus. Ligand 93 (-8.4469 kcal/mol) and ligand 123 (-8.3609 kcal/mol) were identified to be having higher docking scores as compared to the native ligand 6T8 (-8.2839 kcal/mol) and could be considered potential candidates for further studies in anti-viral drugs against Zika virus. Ligands 153 (-10.3108 kcal/mol), 63 (-9.9968 kcal/mol), 621 (-9.8700 kcal/mol), 31 (-9.5001 kcal/mol) and 779 (-9.3874 kcal/mol) were identified as the top five binding ligands, and have docking scores much higher than the reference native ligand K4J (-8.9037 kcal/mol). All these ligands can be potent candidates for anti-viral research against Hepatitis C virus. Ligand 798 (-8.0957 kcal/mol) and ligand 63 (-8.0778 kcal/mol) have higher docking scores as compared to the reference native ligand X77 (-8.0689 kcal/mol), they also interact with the catalytic dyad at the active site of the target protein and can be considered as possible candidates for studies in anti-viral drugs against SARS-CoV-2.

Keywords: Computer-aided drug design, ADME, Molecular Docking analyses, anti-viral agents, Sars-CoV-2 virus, Zika virus, Hepatitis C virus

INTRODUCTION

Zika virus is an arbovirus belonging to the genus flavivirus and are mainly transmitted by Aedes mosquitoes¹. In 2016 the virus rapidly spread throughout the American continent and its potential to cause congenital abnormalities in infants have raised serious concerns. This led to the World Health Organization declaring the ZIKV infection as a global health emergency on February 1, 2016². ZIKV RNA has been identified in foetal brain tissue and brains of microcephalic infants who died; amniotic fluid and placentas of pregnant mothers; and umbilical cord, cerebro-spinal fluid and meninges of newborns³. To date there are no existing therapeutic drugs or vaccines against ZIKV infections; most treatments only seek to address the symptoms⁴.

The Zika virus is composed of a positive sense, single strand RNA genome. It is an enveloped, icosahedral virus that is a member of the Spondweni clade. The Zika virus is a positive polarity RNA virus with a genomic size of about 11 kb. The single open reading frame sequence of its RNA genome encodes a polyprotein which constitutes the structural architecture of the virus. During the viral replication, the polyprotein is cleaved to produce three structural proteins involved in the viral particle assembly, namely the glycoprotein E (protein E), the capsid protein C (protein C), and the protein prM. Whereas seven non-structural proteins are responsible for the viral replication, assembly and evasion

from the host defense: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5⁵. Together, these proteins are the main targets for drugs and antibody recognition⁶.

Hepatitis C virus is a blood borne virus⁷ which have been identified in several domestic and wild mammals, the largest viral diversity being observed in bats and rodents⁸. It is currently thought that HCV chronically infects 170 million people worldwide, 3 % of the world's population, and creates a huge disease burden from chronic progressive liver disease⁷. Chronic HCV infection is an important infectious cause of death in the United States and a major contributor to morbidity and mortality from viral hepatitis globally⁹. Efforts toward development of vaccines were initiated after the discovery of HCV in 1989, but to date, no vaccine has been licensed¹⁰.

Hepatitis C virus (HCV) is a spherical, enveloped, positive-stranded, single-stranded RNA virus with a diameter of 40–80 nm. The nucleocapsid is surrounded by a host cell-derived lipid bilayer envelope in which envelope glycoproteins E1 and E2 are embedded. Core protein, E1 and E2 envelope glycoproteins are major protein components of the virion¹¹. The drug binds to the palm site II region of HCV RNA dependent RNA polymerase (NS5B) and inhibits the initiation step of the polymerase RNA replication cycle¹².

In late December 2019, an outbreak of a mysterious unidentified corona virus, currently named as SARS-CoV-2,

emerged from Wuhan, China, and resulted in a terrifying outbreak over the cities in China and expanded globally. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the ninth documented coronavirus that infects humans and the seventh identified in the last 20 years. All previous human coronaviruses have zoonotic origins, as have the vast majority of human viruses¹³. This virus has been identified as a global threat that is resulting to the outbreak of this mysterious viral pneumonia in humans with (SARS-CoV-2) severe acute respiratory syndrome¹⁴. Due to constant mutation taking place in the virus, most vaccines have become less effective and no specific anti-viral drug has been identified till date¹⁵.

The SARS-CoV-2 is an enveloped beta coronavirus with a positive single-strand RNA genome¹⁶ and possesses an Envelope (E) protein, Membrane (M), Nucleocapsid (N) protein and a “crown-like” spike (S) glycoprotein on their surface¹⁷. The Spike glycoprotein plays essential roles in virus attachment, fusion and entry into the host cell¹⁸. Due to constant mutations developing in the S1 and S2 subunits of the spike glycoprotein¹⁹, it is more practical to target the main protease (M^{pro}) whose active site is conserved at the catalytic site^{20,21}. SARS-CoV-2 M^{pro} is a homodimeric cysteine protease, with a catalytic dyad (cysteine and histidine) in its centre. This catalytic dyad of M^{pro} is located in a cleft between domain I and II^{22,23}. This substrate binding site which host the Cys-His catalytic dyad (HIS A:41 & CYS A:145), contains four sub-sites namely; S1', S1, S2 and S4¹⁶. Amino acids GLU A:166 and HIS A:163 are also considered as attractive residue at the active site for hydrogen bond formation²⁴.

In this study, we have designed 812 small molecule drugs based on three templates which were reported to have antiviral properties. Multiple derivatives of each template were obtained by assigning various functional groups. The molecules were then screened for druglikeness with Lipinski rule of five, and finally, docking analysis were performed with the three target proteins of ZIKV, HCV and SARS-CoV-2 viruses.

MATERIALS AND METHODS

Protein Selection and Preparation

The crystal structure of Zika Virus was downloaded from Protein Data Bank with a good X-ray diffraction resolution of 2.7 Å. The protein structure with the PDB ID 5LC0 was chosen

as the target protein. The NS2B-NS3 Protease of the structure of Zika virus is bound to an inhibitor 6T8²⁵. The crystal structure of Hepatitis C Virus was downloaded from Protein Data Bank with a good X-ray diffraction resolution of 2.3 Å. The protein structure with the PDB ID 6MVK was selected as the target protein. The structure of HCV NS5B 1b N316 is bound ligand K4J¹². The crystal structure of SARS-CoV-2 was downloaded from Protein Data Bank with a good X-Ray diffraction of 2.1 Å. The protein structure with the PDB ID 6W63 was chosen as the target protein. This structure of SARS-CoV- 19 main protease is bound to a potent broad spectrum non-covalent inhibitor X77 which has an imidazole ring. The desired protein was prepared with MOE 2015.10 software²⁶. Excess ligand and water molecules are removed to make computations easier and clear the binding pockets of possible water molecules that might distort the binding pose. The structural preparation for the protein was performed by structure correction and 3D protonation and energy minimization was done with AMBER12: EHT force field.

Ligand Selection and Preparation

A total of 812 Ligands were designed based on three templates (Figure. 1) and their structures and compounds numbers are given in supplementary table. 1. Ligand 1-32 were designed based on template 1 (Figure. 1A), where a series of N-((3-phenyl-1-(phenylsulfonyl)-1H-pyrazol-4-yl)methyl)anilines were synthesized and tested against a large panel of RNA and DNA viruses of public health significance²⁷. Ligands 33-212 were designed based on the work of M. Saudi and so-workers²⁸, where imidazole-4,5-dicarboxamides were synthesized and screened for their activity against dengue and yellow fever virus (Figure 1B). Finally, ligands 213-812 were designed based on template 3 (Figure 1C) which involves the synthesis and screening of benzo-imidazole compounds as broad spectrum antiviral agents²⁹.

In the given templates the various groups R, R1, R2 and R3 are replaced by the different desired substituents. Using 3D ChemDraw³⁰ tools the ligands were prepared. The structures were saved in SDF format which were then converted to MOL file by using Open Babel³¹ and using MOE 2015.10 Software, energy minimization was done with Amber12: EHT force field. Along with the reference ligand a database of all the ligands was prepared for docking with the target protein.

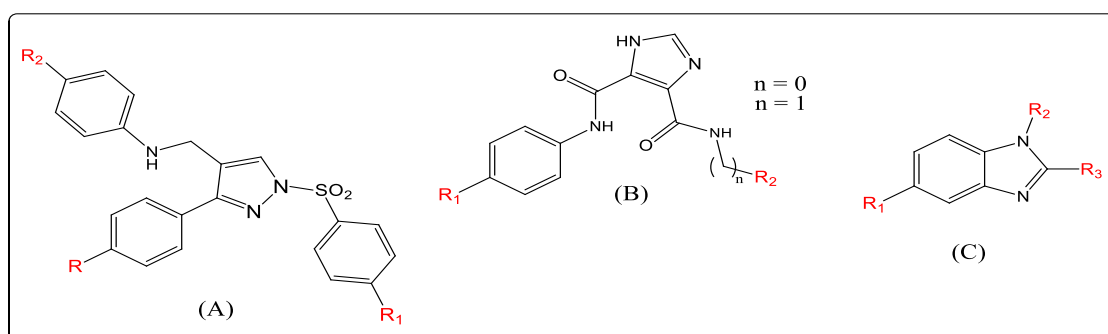


Figure 1: Templates used in the designing of 812 drugs (A) template 1 (B) template 2 (C) template 3

ADME Screening

ADME screening was performed with Lipinski Rule of Five³², using a freely accessible web-server at supercomputing facility for bioinformatics and computational biology, IIT Delhi³³. This rule predicts the various properties of the drugs such as, molecular mass, lipophilicity, total number of hydrogen bond donor, total number of hydrogen bond acceptors and molar

refractivity. The ligands which are saved in sdf format are feed into the server, with a pH value of 7.

Molecular docking analysis

By using the MOE 2015.10 Software, all the ligands that is present in the database were docked into the selected target proteins. In the docking analysis with ZIKV (PDB ID: 5LC0) the pocket atoms in the binding site of the non-covalent

native ligand 6T8 were selected as the docking site. In the case of HCV (PDB ID: 6MVK) the pocket atoms in the binding site of the non-covalent native ligand K4J were selected as the docking site. Similarly, the pocket atoms in the binding site of the non-covalent native ligand X77 were selected as the docking site for the SARS-CoV-2 main protease. For the docking studies a triangle matcher was used where London dG was used as the initial scoring methodology to generate 30 poses and induce fit receptor model was also used where GBVI/WSA dG was applied as the final scoring methodology to generate best 5 poses of the ligands. In all the docking analysis the native ligands of the selected proteins were considered as reference for comparing the docking scores.

RESULTS AND DISCUSSIONS

Screening for Drug likeness

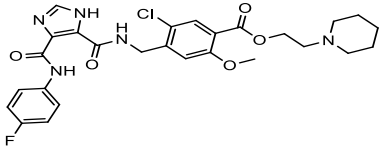
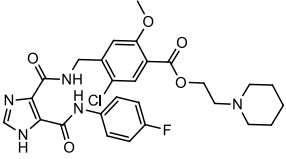
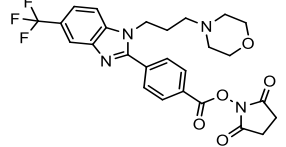
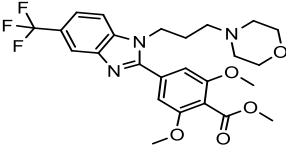
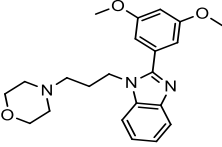
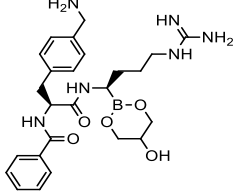
All 812 ligands were screened for drug-likeness based on Lipinski rule of five and the results are given in

supplementary table 2. Except a very few ligands, most ligands have molecular mass below 500 mg/mol. The number of hydrogen bond donors are below 5 in all the ligands. All ligands also have hydrogen bond acceptor in the range 0-10.

Docking analysis of Zika virus

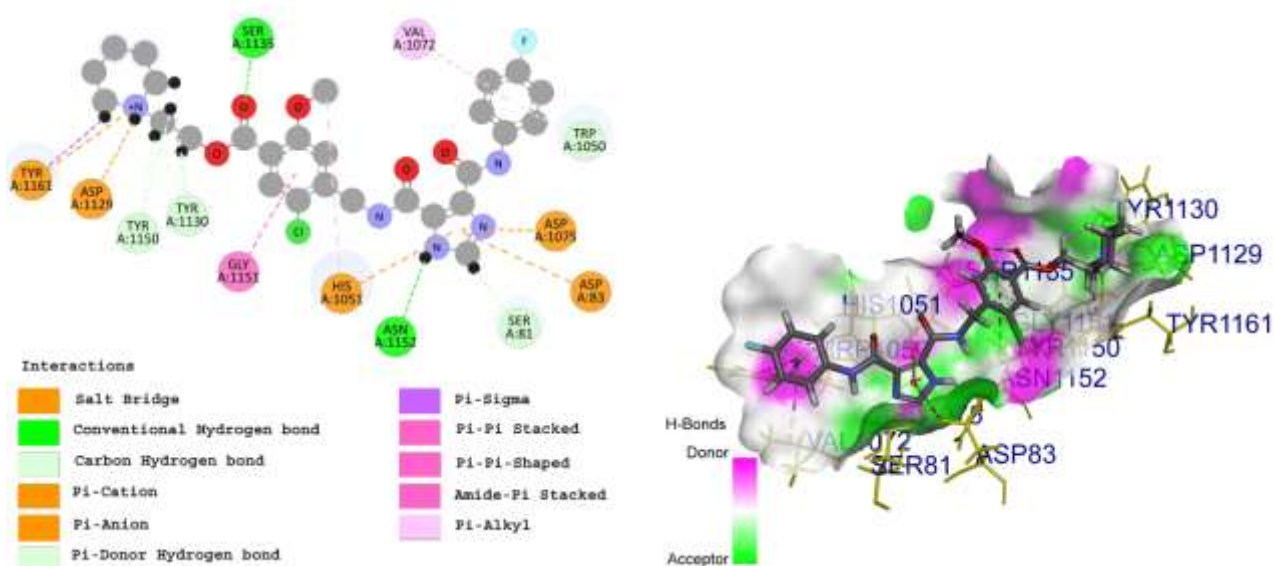
The top binding ligands together with the structure, binding scores and the interacting amino acids at the docking site are displayed in table. 1. Ligands 93 and 123 produced a binding scores of -8.4469 kcal/mol and -8.3609 kcal/mol, respectively, which are higher than the reference native ligand (-8.2839 kcal/mol). Among all the residuals amino acids at the docking site, TYR A: 1161, ASP A: 1129, HIS A: 1051, ASP A: 1075 and ASP A: 83 are the most common amino acids interacting with the ligands. All ligand also have molar refractivity within 40-130. A considerable amount of ligands have a slightly higher LogP due to the presence of aromatic rings in the templates and in the substituents.

Table 1: List of top docking ligands against ZIKV with the score and interacting amino acids

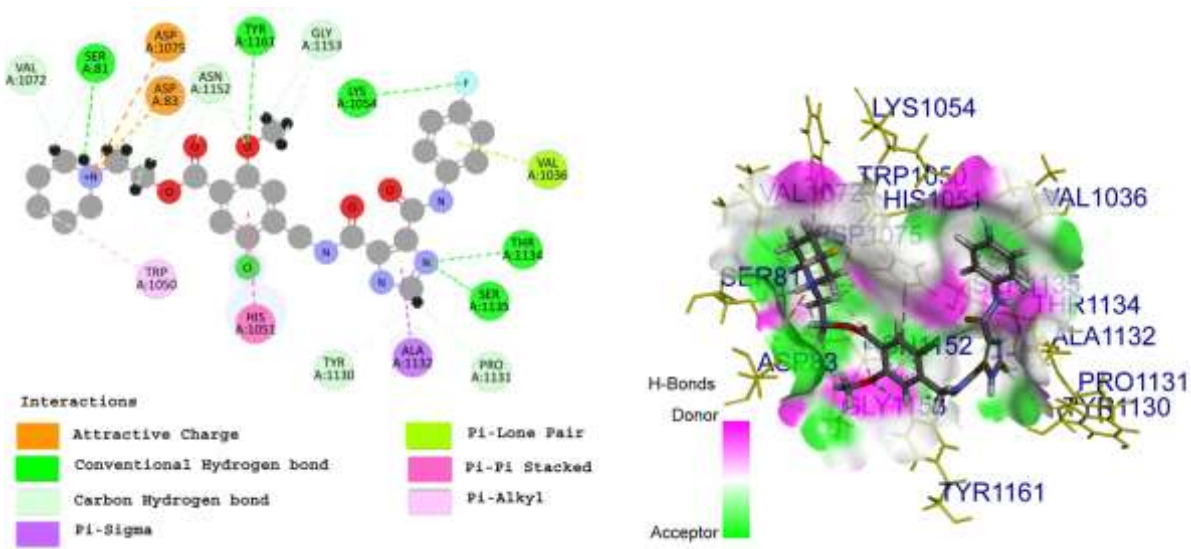
Ligand No.	Structure	Score kcal/mol	Interacting amino acids
93		-8.4469	Tyr A:1161, Asp A:1129, His A:1051, Asp A:1075, Asp A:83, Ser A:1135, Asn A:1152, Tyr A:1130, Trp A:1050, Ser A:81, Val A:1072
123		-8.3609	Asp A:1075, Asp A:83, Ser A:81, Tyr A:1161, Lys A:1054, Thr A:1134, Ser A:1135, Val A:1072, Asn A:1152, Gly A:1153, Tyr A:1130, Pro A:1131, Ala A:1132, Val A:1036, His A:1051, Trp A:1050
770		-7.8240	Asp A:83, His A:1051, Gly A:1153, Ser A:1135, Trp A:1050, Tyr A:1130, Pro A:1131, Tyr A:1161
778		-7.7758	Asp A:83, Asp A:1075, Val A:1155, Ser A:1135, Gly A:1133, Val A:1154, Ser A:81, Val A:1072, Asp A:1075, Asn A:1152, Gly A:1151, Val A:1036, Ala A:1132, Gly A:1153, His A:1051, Val A:1154)
479		-7.7342	Asp A:83, Asp A:1075, Asn A:1152, Gly A:1133, Ser A:1135, Ser A:81, Val A:1072, Gly A:1151, Pro A:1131, His A:1051
Reference: 6T8		-8.2839	Asp A:1075, Asp A:83, Asp A:79, Asp A:1129, Tyr A:1161, Ser A:81, Asn A:1152, Tyr A:1130, Tyr A:1150, His A:1051, Ser A:1135, Val A:1036

The two-dimensional and three-dimensional representations of the interactions of the top two binding ligands with the reference ligand are shown in figure 2. Interactions of ligand 93 with the target protein is stabilized by two conventional hydrogen bond interactions with SER A:1135 and ASN A:1152. In the case of ligand 123, there are five conventional hydrogen

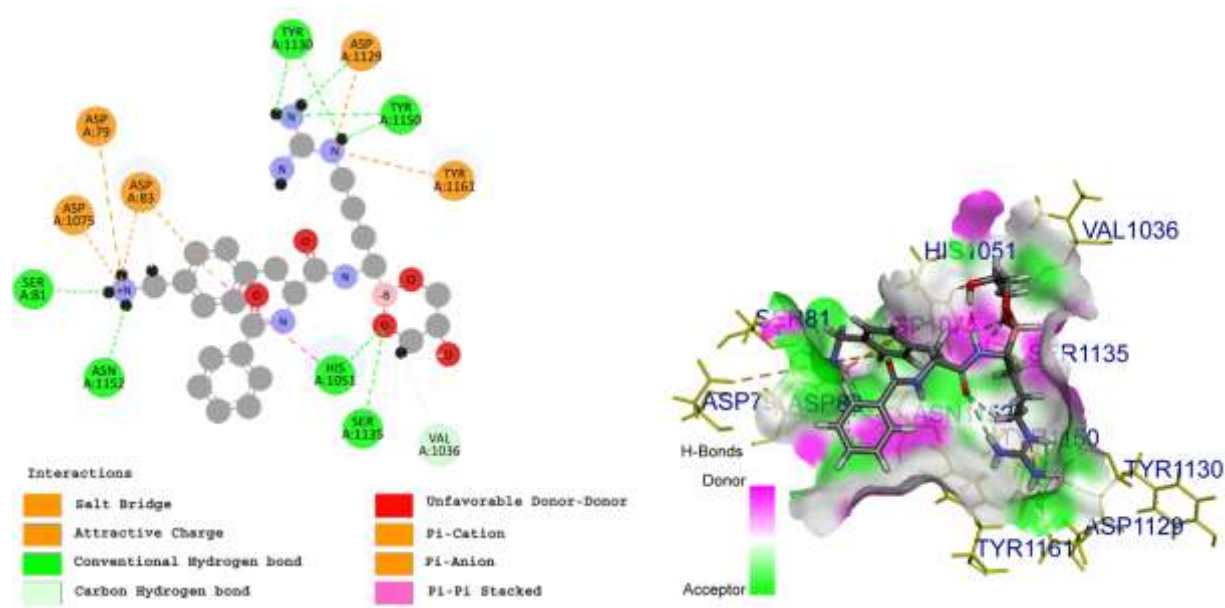
bonding interactions with SER A:81, TYR A:1161, LYS A:1054, THR A:1134 and SER A:1135. In the interactions of the reference native ligand with the protein six conventional hydrogen bonding interactions are involved with SER A:81, ASN A:1152, HIS A:1051, SER A:1135, TYR A:1150 and TYR A:1130.



Ligand 93



Ligand 123



Reference Ligand 6T8

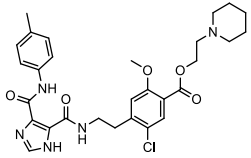
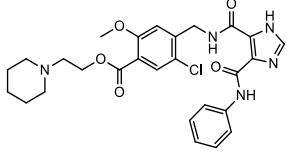
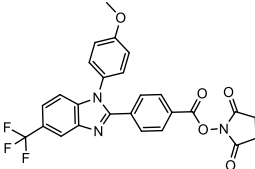
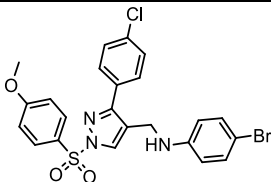
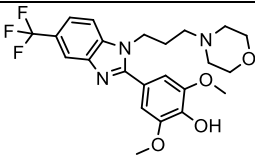
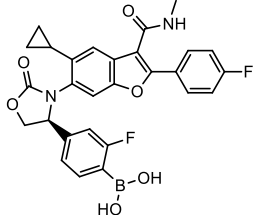
Figure 2: 2D and 3D (hydrogen bond surface) images of the interactions of top binding ligands with Zika virus (PDB ID: 5LC0

Docking analysis of Hepatitis C virus

The list of the top five binding ligands and the details of the binding scores and interacting amino acids are given in table 2. All the top binding ligands exhibits higher docking scores with respect to the reference ligand. Ligand 153 has an

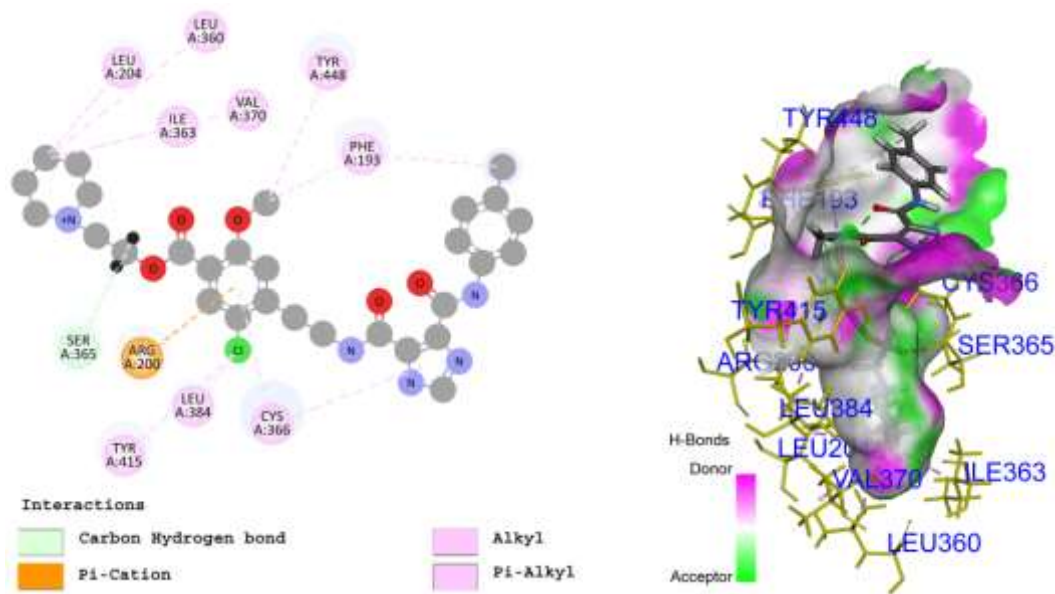
exceptionally high docking score of -10.3108 kcal/mol, which can be considered as a good interaction. Arg A:200, Tyr A:448, Leu A:384, Cys A:366 and Ser A:365 are the most common amino acids interacting with the ligands at the binding cite.

Table 2: List of top docking ligands against HCV with the score and interacting amino acids

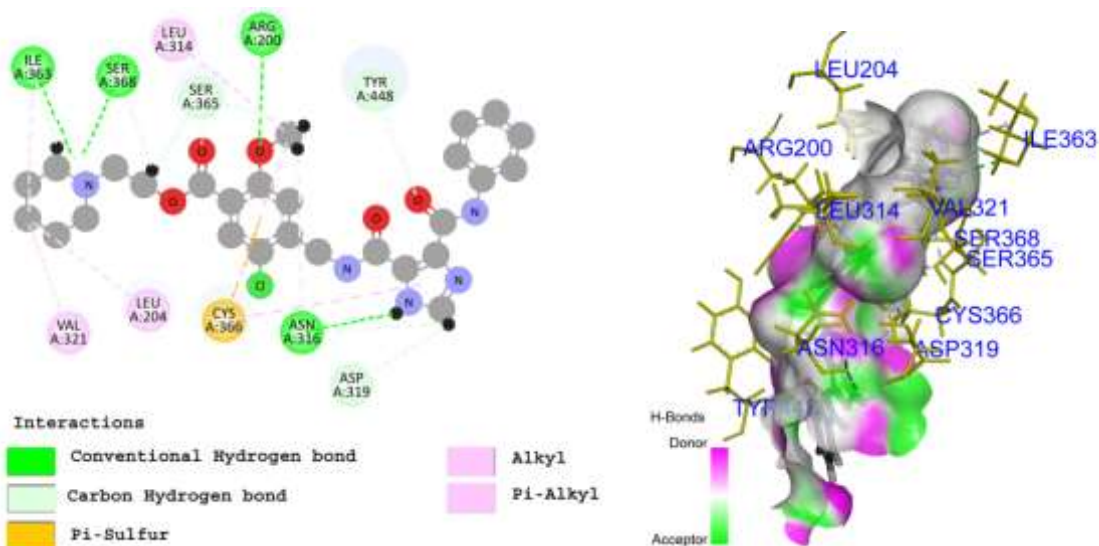
Ligand Number	Structure	Score kcal/mol	Interacting amino acids
153		-10.3108	Ser A:365, Arg A:200, Leu A:204, Leu A:360, TyrA:448, Ile A:363, Val A:370, Phe A:193, LeuA:384, Cys A:366, Tyr A:415
63		-9.9968	Ile A:363, Ser A:368, Arg A:200, Asn A:316, Ser A:365, Tyr A:448, Asp A:319, Cys A:366, Leu A:314, Leu A:204, Val A:321
621		-9.8700	Tyr A:448, Gly A:449, Met A:414, Leu A:384, Ser A:368, Ser A:365, Asn A:369, Ile A:447, Gln A:446, Arg A:200, Cys A:366, Tyr A:415, Pro A:197
31		-9.5001	Asn A:316, Arg A:200, Ile A:363, Leu A:360, Tyr A:448, Met A:414, Pro A:197, Tyr A:415, Leu A:384, Cys A:366, Leu A:204, Val A:321, Val A:370
779		-9.3874	Asn A:316, Tyr A:448, Ser A:365, Leu A:314, Met A:414, Arg A:200, Cys A:366, Phe A:193
Reference K4J		-8.9037	Tyr A:448, Ser A:368, Arg A:394, Asn A:411, Ile A:447, Arg A:200, Ser A:365, Leu A:384, Cys A:366, Phe A:193

The two-dimensional and three-dimensional representations of the interactions of the top binding ligands with the reference ligand are shown in figure 3. The three-dimensional representation also includes Hydrogen-bonds surface. Interactions with ligand 153 is being stabilized with a couple of alky and pi-alkyl interactions, together with a carbon hydrogen bond with SER. A:365. Interactions with ligand 63 is stabilized with four hydrogen bonding interactions with ILE A:363, SER A:368, ARG A:200 and ASN A:316. Interaction with

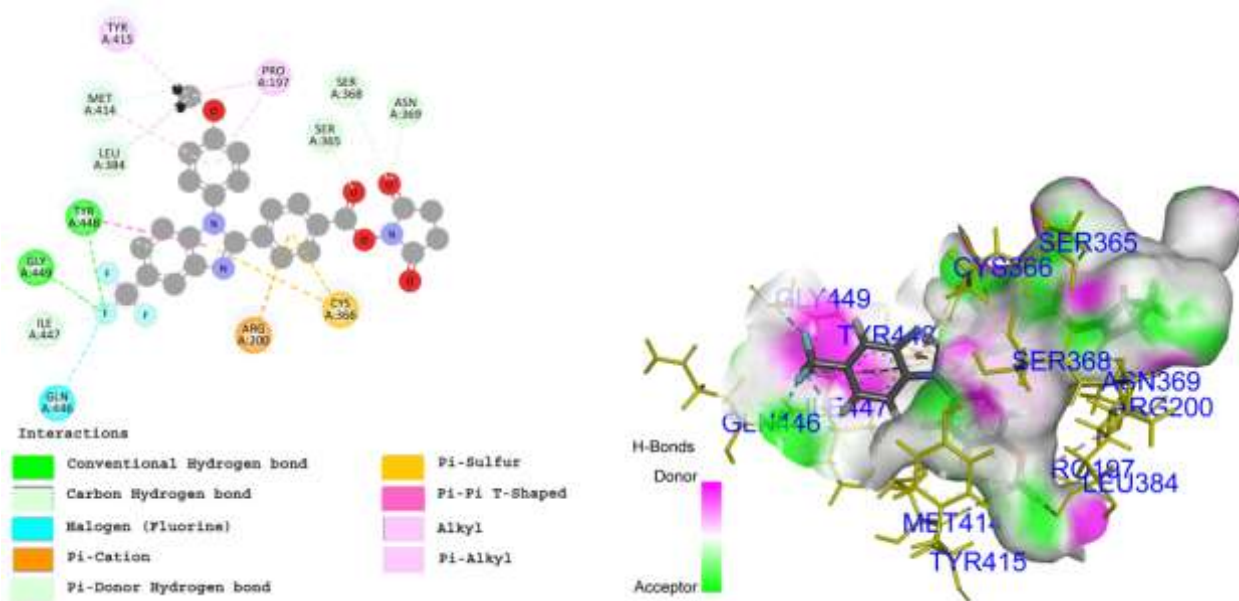
ligand 621 has two conventional hydrogen bonding interactions with GLY A:449 and TYR A:448. Interaction with ligand 31 also has two conventional hydrogen interactions with ASN A:316 and ARG A:200. Ligand 779 has a conventional hydrogen bond interaction with ASN A:316 but also has unfavourable positive-positive interaction with ARG A:200. The reference ligand is stabilized with a number of interactions including a conventional hydrogen interaction with TYR A:448.



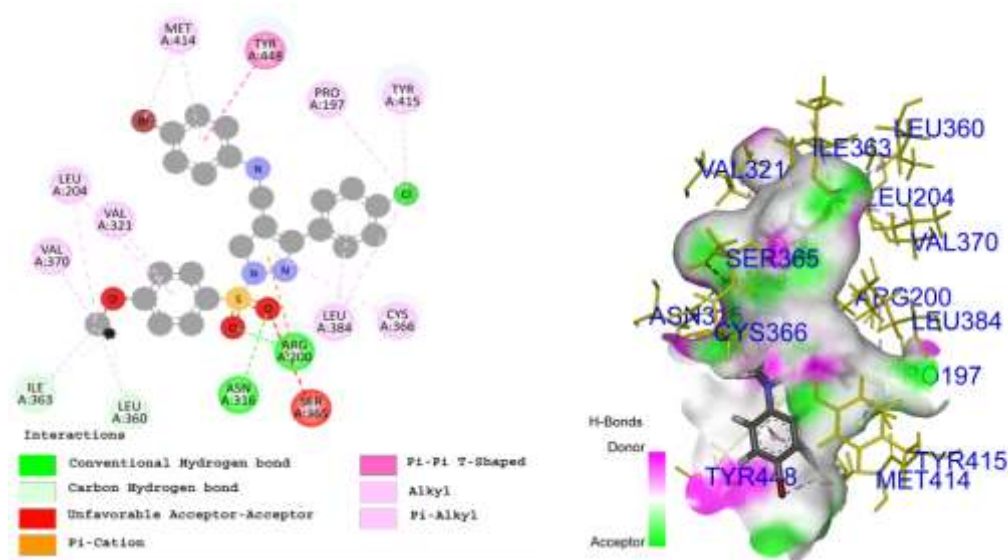
Ligand 153



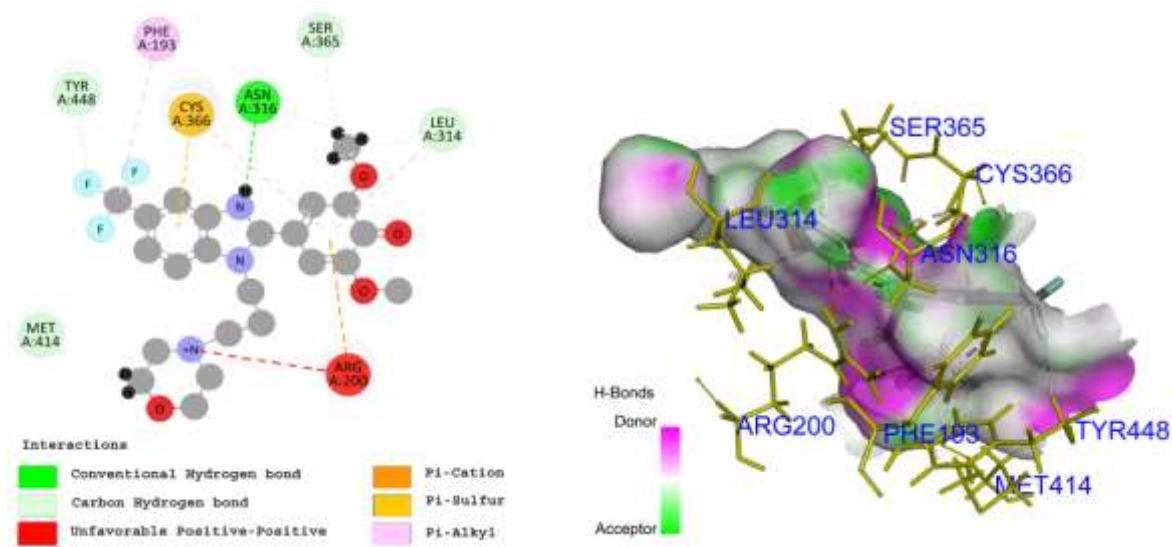
Ligand 63



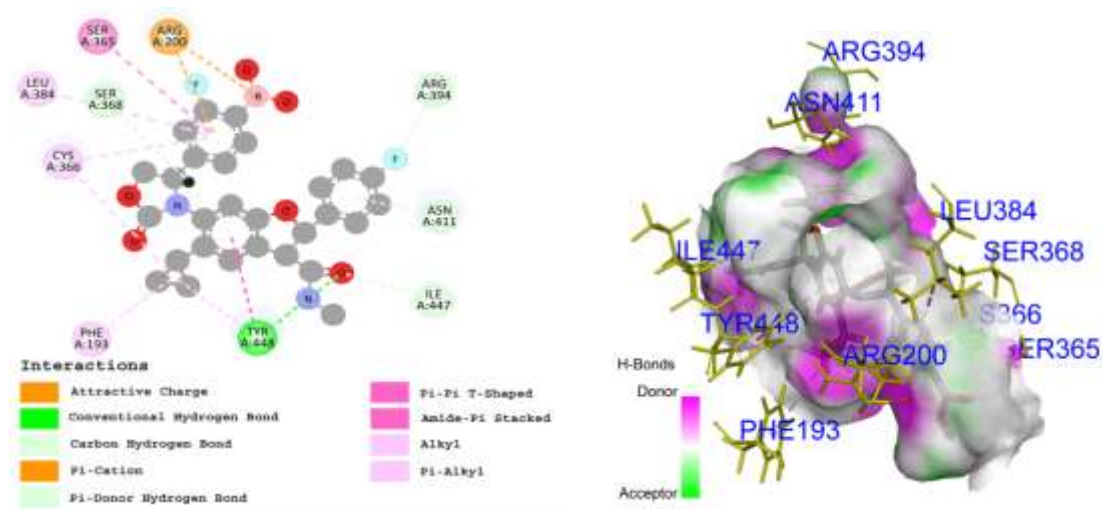
Ligand 621



Ligand 31



Ligand 779



Reference: K4J

Figure 3: 2D and 3D (hydrogen bond surface) images of the interactions of top binding ligands with Hepatitis C virus (PDB ID: 6MVK)

Docking analysis of SARS-CoV-2 virus

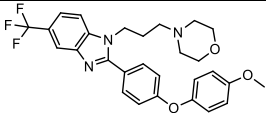
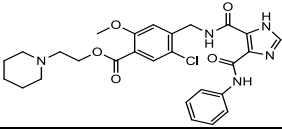
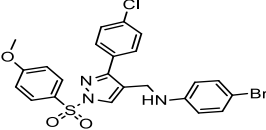
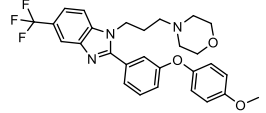
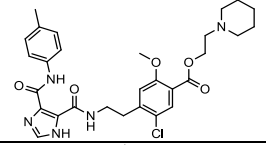
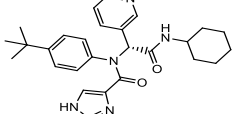
The top binding ligands with their structure, binding scores and interacting amino acid details are listed in table 3.

Among the residual amino acids at the docking cite, GLU A: 166, MET A: 49 and HIS A: 41 are the most common amino acids. The top two binding ligands with binding scores of -8.0957 kcal/mol and -8.0778 kcal/mol, respectively, have

higher binding scores compared to the reference native ligand, which has a binding score of -8.0689 kcal/mol. It is evident that all the top binding ligand together with the reference ligand binds to either or both amino acids of the

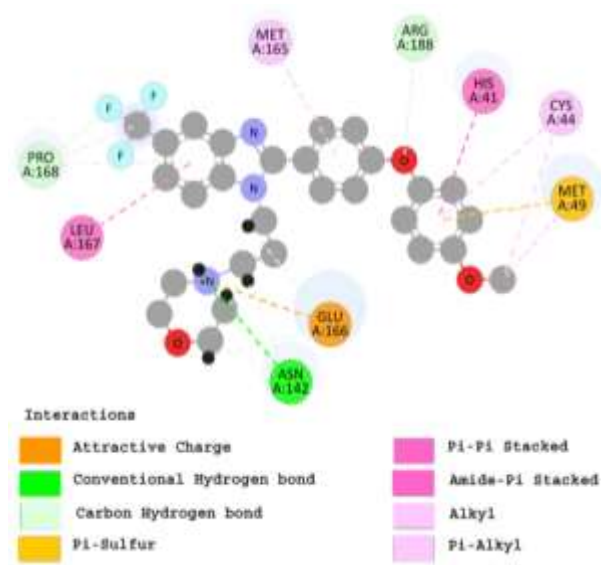
catalytic dyad (His A:41 and Cys A:145), which points to their possible inhibitory function against SARS-CoV-2 main protease.

Table 3: List of top docking ligands against SARS-CoV-2 with the score and interacting amino acids

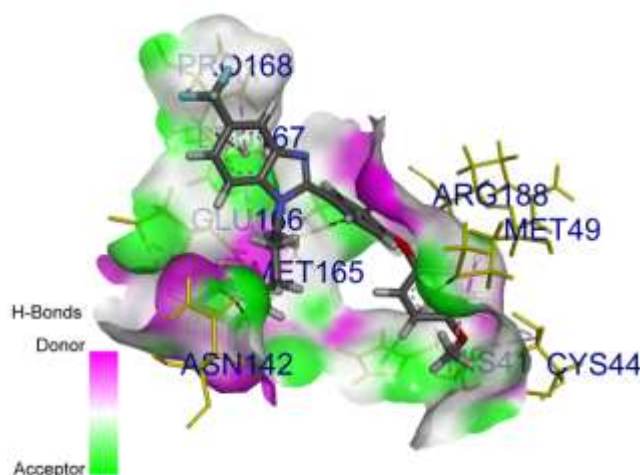
Ligand Number	Structure	Score (kcal/mol)	Interacting amino acids
798		-8.0957	Glu A:166, Asn A:142, Pro A:168, Arg A:188, Glu A:166, Met A:49, Leu A:167, His A:41, Met A:165, Cys A:44
63		-8.0778	Asp A:187, Glu A:166, Met A:49, His A:41, His A:164, Cys A:145, Cys A:44
31		-8.0439	Gln A:189, Met A:165, Ser A:144, Glu A:166, Met A:49, Cys A:145, His A:41, Arg A:188, His A:172, Pro A:168
797		-8.0325	Glu A:166, His A:163, Ser A:144, Leu A:167, Asn A:142, His A:41, Leu A:141, Met A:165, Met A:49, Cys A:44,
153		-8.0212	Glu A:166, His A:164, Asp A:187, Cys A:145, His A:41, His A:163, Met A:49, Cys A:44
Reference X77		-8.0689	Cys A:145, Glu A:166, Asp A:187, Met A:165, Met A:49

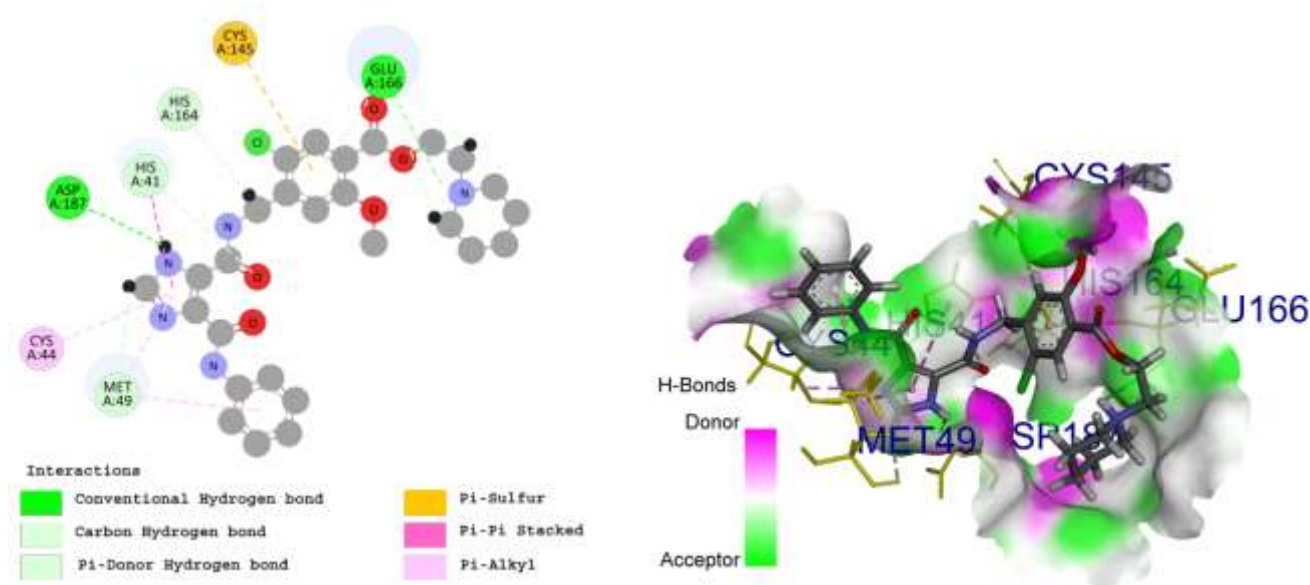
The two-dimensional and three-dimensional representations of the interactions of the top two binding ligands with the reference ligand are shown in figure 4. Among other interactions, the interaction with ligand 798 is stabilized by conventional hydrogen bond interactions with ASN A:142, and carbon hydrogen bond with PRO A:168 and ARG A:188. The ligand interacts with HIS A:41, a residue of the catalytic dyad,

and with GLU A:166, which is considered as an attractive residue at the active site. Ligand 63 interacts with both the catalytic dyad, and also with GLU A:166. The reference native ligand interacts with both the catalytic dyad and with the amino acids (GLU A:166 & HIS A:163) considered as attractive residues.

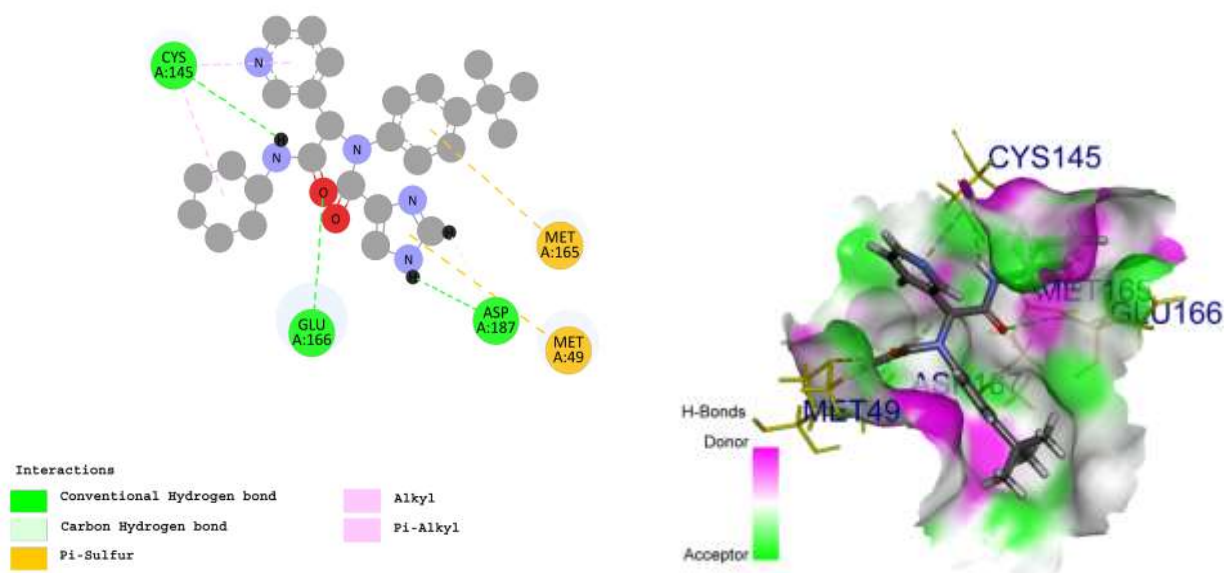


Ligand 798





Ligand 63



Reference X77

Figure 4: 2D and 3D (hydrogen bond surface) images of the interactions of top binding ligands with SARS-CoV-2 virus (PDB ID: 6W63)

CONCLUSION

In summary, a total of 812 ligands have been designed based on structures of known anti-viral agents. All ligands were screened for drug-likeness based on Lipinski rule of five. A database of ligand was constructed and *in silico* docking analyses were performed against three selected viruses, viz., Zika virus, Hepatitis C virus and SARS-CoV-2 virus. The docking analyses against Zika virus identified ligand 93 (-8.4469 kcal/mol) and ligand 123 (-8.3609 kcal/mol) to be having higher docking scores as compared to the native ligand 6T8, and could be considered as potential candidates for further studies in anti-viral drugs against Zika virus. The docking analyses against Hepatitis C virus identified ligands 153 (-10.3108 kcal/mol), 63 (-9.9968 kcal/mol), 621 (-9.8700 kcal/mol), 31 (-9.5001 kcal/mol) and 779 (-9.3874 kcal/mol) as the top five binding ligands, and have docking scores much higher than the reference native ligand K4J (-8.9037 kcal/mol). All these ligands can be potent candidates for anti-viral research against Hepatitis C virus. The docking analyses against SARS-CoV-2 identifies ligand 798 (-8.0957 kcal/mol) and ligand 63 (-8.0778 kcal/mol) with higher docking scores

as compared to the reference native ligand X77 (-8.0689 kcal/mol). Ligands 798 and 63 also interacts with the catalytic dyad at the active site of the target protein, and can be considered as possible candidates for studies in anti-viral drugs against SARS-CoV-2.

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Conflict of Interest

The authors declare that there is no conflict of interest

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