Ivermectin as a promising RNA-dependent RNA polymerase inhibitor and a therapeutic drug against SARS-CoV2: Evidence from *in silico* studies

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Research Article

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Abstract

Purpose: COVID-19, caused by SARS-CoV2 virus is a contagious disease affecting millions of lives throughout the globe. Currently, there are no clinically approved drugs for SARS-CoV2 although some drugs are undergoing clinical trials. The present study investigates the binding property of ivermectin on four important drug targets, spike protein, RNA-dependent RNA polymerase, 3-chymotrypsin- and papain-like proteases of SARS-CoV2.

Methods: The 3D structure of ivermectin along with known antiviral drug lopinavir, simeprevir and four nucleotides ATP, GTP, CTP, and UTP were downloaded from PubChem database. Crystal structures of proteins were downloaded from PDB database. PDB files were converted into pdbqt file using AutoDock tools. After proper processing and grid formation, docking was carried out in AutoDock vina. Furthermore, the co-crystallized RNA and its binding interactions with RdRp were studied using various visualization tools including Discovery studio.

Results: Docking study showed that ivermectin is the best binding drug compared to lopinavir and simeprevir. The best binding interaction was found to be -9.7kcal/mol with RdRp suggesting potential inhibitor of the protein. Twenty-one amino acid residues of RdRp were found to interact with ivermectin including the catalytic residue Asp760. Furthermore, RNA-RdRp complex revealed that the catalytic active residues Ser759 and Asp760 of RdRp formed strong interactions with RNA chain. Binding of ivermectin in the active site of RdRp make clash with the nucleotides of RNA chain suggesting the possible inhibition of replication.

Conclusions: The present study suggests ivermectin as a potential inhibitor of RdRp which may be crucial to combat the SARS-CoV2.

Introduction

The pandemic of COVID-19 has disastrously affected human health and wealth since its emergence in 2019 from the Wuhan city of China. Severe Acute Respiratory Syndrome corona virus -2 (SARS-CoV2), a single-stranded positive-sense RNA virus belonging the family Coronaviridae is the causative organism of COVID-19 disease (Benvenuto et al. 2020). According to the latest WHO update (2:09 pm CEST, 22 August 2020), there have been 22,812,491 confirmed cases of COVID-19, including 795,132 deaths globally. Today, there are seven different species of human coronaviruse (HCoV) - 229E, NL63, OC43, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV2 (Benvenuto et al. 2020). Most of the HCoVs causes mild infections such as common cold while SARS, MERS, and SARS-CoV2 are highly pathogenic, and contagious causing severe respiratory problems and even death (Cui et al. 2019). Genomic and sequence homology studies emphasize on the common origin of all the HCoVs (from bats?) except HKU1 and HCoV-OC43 which are linked to rodents (Forni et al. 2017). The attack and pathogenicity of SARS-CoV2 progress with four stages – asymptomatic, moderate, extreme, and clinical which starts at the lower respiratory tract

followed by an invasion of pulmonary epithelial cells and hijacking the entire host cell machinery. The most common symptoms of COVID-19 include cough, fever, malaise, gastrointestinal symptoms, loss of smell, sore throat, heart failure, and acute kidney injury (Gandhi et al. 2020).

Currently, there is no clinically approved drug for SARS-CoV2. However, recent publications reveal that several medications such as ivermectin, hydroxychloroquine, and remdesivir are being used to reduce the virus load and improve disease symptoms (Caly et al. 2020; McKee et al. 2020; Wang et al. 2020). Researchers around the world are working diligently to develop effective drugs based on the pathogenicity and molecular details of the SARS CoV-2. Four key targets proteins such as spike protein (S-protein), virus mail protease (3Clpro), papain-like protease (Plpro), and RNA-dependent RNA polymerases (RdRp) are being explored by researchers around the world to develop medicines against COVID-19. The SARS-CoV S-protein is an important drug target which is a quaternary protein composed of two subunits - S1 subunit that contains a receptor-binding domain (RBD) which engages with the host cell receptor angiotensin-converting enzyme-2 and the S2 subunit that mediates fusion between the viral and host cell membranes (Du et al. 2009; Zhou et al. 2019). 3Clpro and Plpro are two important virus proteases that catalyse the processing of polyproteins pp1a and pp1ab leading to the formation of RdRp and other important non-structural proteins (nsp) (Anand et al. 2003; Chen et al., 2020). The RNA-dependent RNA polymerase, also known as nsp12 is the central component of CoV replication and transcription machinery, and therefore, it appears to be a primary target for the antiviral drug (Xu et al. 2003). Given the pivotal roles of S-protein, 3Clpro, PL-pro, and RdRp in the infection, replication, and generation of viral particle, these proteins are widely regarded as an important and attractive target for the rational design of anti-SARS-CoV2 drugs.

Ivermectin is a widely used FDA approved broad-spectrum antihelmintic drug. It causes stimulation of gamma amino-butyric acid (GABA)-gated-Cl-channels, leading to hyperpolarization, and resulting in paralysis of helminth parasites (Canga et al. 2008). Furthermore, ivermectin is also known to act as inhibitor of importin-α/β-mediated nuclear import and suppress the replication of several RNA viruses, including HIV, chikungunya virus, and yellow fever virus (Lv et al. 2018). Recent studies have shown that ivermectin can inhibit the replication of SARS-CoV-2 in vitro (Sharun et al. 2020). The present study investigates the *in silico* binding affinity of ivermectin drug with S-protein, 3Clpro, PL-pro, and RdRp proteins of SARS-CoV2.

Materials And Methods

Ligand Selection and Preparation

The 3D structure of ivermectin (Drugbank ID: DB00602) along with two antiviral drugs simeprevir (DB06290), and lopinavir (DB01601) from Drugbank database (https://www.drugbank.ca/). Four nucleotides ATP (PubChem CID: 5957), GTP (CID: 135398633), CTP (CID: 6176), and UTP (CID: 6133) were downloaded from PubChem database. SDF files were converted into pdb file using PyMol

software. All the PDB files of the ligands were processed and finally converted into .pdbqt file using AutoDock tool (Trott and Olson 2010).

Collection and Preparation of Proteins

Three-dimensional structures of S-protein (RBD) (PDB ID: 7BZ5), 3Clpro (PDB ID: 6M2N), Plpro (PDB ID: 7JN2), and RdRp (PDB ID: 6XQB) were downloaded from PDB database. The protein structures were cleaned by removing the water and other hetatms. Polar hydrogen atoms and Kollman charges were added to the structure and finally converted into .pdbqt file format for docking using AutoDock Tools.

Molecular Docking

After the ligand drugs and the target enzymes were prepared docking was carried out in AutoDock Vina (Trott and Olson 2010). The grid box parameters for docking of all the four proteins are presented in table 1. The docking algorithm was carried out by keeping the default exhaustiveness at 8. After docking, the pose scoring the lowest binding energy (kcal/mol) was selected and visualize in Discovery Studio.

Table 1. Docking grid box parameters for AutoDock Vina docking.

Proteins	Active site amino acid residues	Center co-ordinates			Size co-ordinates		
		x	y	z	x	y	z
S-protein	Leu455, Phe486, Gln493, Ser 494, Asn501, Tyr505	-57.021	-34.024	14.358	42	74	58
3Clpro	His41, Thr45, Met49, Phe140, Asn142, Cys145, Asp187, Arg188, Gln189, Met165, His172, Glu166	-13.137	14.854	69.809	46	46	66
Plpro	Cys112, Leu163, Gly164, Asp165, Pro248, Pro249, Tyr269, Gln270, His273, Tyr274, Asp-287, Thr302	-37.547	22.916	-9.733	58	52	64
RdRp	Gly616, Trp617, Asp618, Tyr619, Leu758, Ser759, Asp760, Asp761, Ala762, Lys798, Cys799, Trp800, Glu811, Phe812, Cys813, Ser814.	93.080	81.814	97.063	58	48	48

Results

The present study investigates the binding affinity of ivermectin, a broad-spectrum antiparasitic FDA approved drug against four key enzymes of SARS-CoV2, the spike protein, 3Clpro, Plpro, and RdRp enzymes. Figure 1 showed the binding energies of ivermectin with all the four protein. Ivermectin showed the best and strongest binding affinity with RdRp (-9.7kcal/mol) followed by S-protein, Plpro, and 3Clpro. The reference antiviral drug simeprevir also showed strong affinity to all the proteins. Highest binding affinity was observed in Plpro. Lopinavir showed the weakest affinity among all the three drugs. To compare the affinity of nucleotides to the active site of RdRp enzymes, all the four nucleotides ATP, GTP, CTP, and UTP were also docked with the protein. All the four nucleotides showed weaker affinity to the enzyme compared to ivermectin. ATP showed the highest binding energy -8.1 kcal/mol followed by UTP (-8.0 kcal/mol), CTP (-7.8 kcal/mol), and lowest in GTP (-7.6 kcal/mol).

Fig. 2 showed the binding interactions of the ivermectin with the RdRp of SARS-CoV2 and Ramachandran plot of the amino acid residues. A total of 21 amino acid residues (Asn497, Arg555, Thr556, Val557, Leu576, Lys577, Ala580, Ile589, Gly590, Cys622, Asp623, Ser682, Gly683, Asp684, Ala685, Thr687, Ala688, Tyr689, Asn691, Leu758, and Ser759) were found to be interacting with the ivermectin. The ligand-binding site in the RdRp protein and the schematic view of the complex is shown in Fig. 2a. Five conventional H-bonds were seen between the ligand-protein complexes while majority of the binding interactions were found to be van der Waal's interaction (Fig. 2b,d). The H-bond donor and acceptor and the hydrophobicity property of the ligand surrounding amino acid residues were shown in Fig. 2e and 2f. Most of the surrounding amino acid residues showed hydrophilicity while eight residues showed hydrophobic property. Ramachandran plot study revealed that 11 amino acid residues (all glycine) of RdRp apoprotein were found to be distributed outside the allowed region of the plot.

The various amino acid residues interacting with the replicating RNA in the active site of RdRp enzyme is presented in Fig. 3. The co-crystallized structure of RNA-RdRp complex (Pdb ID: 6xqb) has been dissected to visualize the amino acids attaching to the dsRNA chain. A total of 41 amino acid residues were found to form the surrounding surface of the dsRNA (Fig. 3c). Out of 41 amino acid residues, 23 amino acids form interactions with the template RNA strand (3' – 5' strand, Fig. 3d) while 20 residues interact with the newly replicating strand (5'- 3' strand, Fig. e) of RNA. Two amino acids, Thr687 and Glu857 formed interactions with both the stands of dsRNA. Fig. 3f showed the H-bonding amino acid residues with the dsRNA strand. Out of 41 interacting residues, only 15 residues formed hydrogen bonding with the RNA chain. Ten amino acid residues formed eleven H-bonds with the template strand (3'-5' strand) while five amino acids form seven H-bonds with the complementary new strand of RNA. Asn497 of template strand form 2-H-bonds with the uracil (U2) nucleotide while Arg836 and Ser759 formed 2H-bonds each with cytosine (C7) and adenine (A9) nucleotides of new RNA strand (Fig. 3f). It is also observed that out of 21 amino acid residues of RdRp that are interacting with the ivermectin, 16 residues also made interactions with the dsRNA.

The interaction of ivermectin with active site amino acid and RNA strands is shown in Fig. 4. The RNA-ligand interactions and surrounding amino acids were shown in Fig. 4a and 4b. The co-crystallized dsRNA structure of RdRp and the best docking pose of ivermectin when fitted into the binding site five nucleotides – G1, U2, G3, and G4 from template strand (3' – 5' strand) and adenine-9 from newly synthesized RNA strand made unfavourable interactions with the ivermectin (Fig. 4c). Fig. 4d showed that 6 active site amino acid residues (out of 15) that were making *H-bonds* with dsRNA were also found to make interactions with the ligand.

Discussion

SARS-CoV2 is a positive-sense single-stranded RNA virus. The entry of viral genome into the host body and its generation and invasion to the other cells is mediated by several proteins including spike proteins, 3chymotrypsin- and papain-like proteases. A polyprotein complex, RNA-dependent RNA-polymerase (RdRp) is a major protein in SARS-CoV2 that regulates viral replication (Yin et al. 2020). Inhibition of

replication may be an important strategy to combat SARS-CoV2 and COVID-19. RdRp protein, therefore, is an important and potential therapeutic target of antiviral drugs. Compounds that bind effectively with the active site of the RdRp and inhibit the enzyme's catalytic activity shall be an important aspect of controlling virus replication and generation. Several molecules and antiviral drugs have been screened by many researchers to find suitable molecule with high efficacy (Elfiky 2020; Babadaei et al. 2020; Touret et al. 2020). Ivermectin, a broad-spectrum antihelmintic drug has been recently established to be a potential inhibitor of SARS-CoV2 RdRp protein in *in vitro* study (Caly et al. 2020). It has also been opined that hydroxychloroquine and ivermectin may show effective result if administered simultaneously (Patri and Fabbrocini, 2020). Ivermectin is also known to inhibit DNA polymerase of pseudorabies virus (Lv et al. 2018). The present study revealed strong binding affinity of ivermectin to the RdRp protein. In association to surrounding key residues, Ser759, Aspertate760 and -761 forms the core catalytic site amino acid residues of RdRp in SARS-CoV2 (Yin et al. 2020). The present study observed that Ser759 form strong interaction with ivermectin and adenine-9 nucleotide as well. The study, however, did not find any interaction with catalytically active Asp760 and -761 residues of RdRp. Ornipressin, an FDA approved drug for vasoconstriction and liver cirrhosis, is reported to have strong interactions with eight amino acid residues of RdRp including the catalytic site residue Asp760 (Ahmad et al. 2020). In an *in silico* study, Yin et al. (2020) reported that remdesivir, a nucleoside analog used to inhibit the action of RNA polymerase in Ebola virus make phosphodiester bond with the 3'-OH group of the newly synthesized RNA strand as well as strong interaction with the Asp760 residue of SARS-CoV2 leading to the inhibition of replication. Ivermectin is not a nucleotide analog and therefore did not bind with the 3'OH- end of the leading strand. However, the study showed unfavourable interactions with the parental (template) RNA strand up to four nucleotides upstream of the replicating site. Binding of ivermectin to the active pocket of RdRp may prevent the catalytically favourable accommodation of the template RNA chain in the enzyme and hence inhibition of replication. The findings of the present study along with in vitro validation by earlier studies suggest the potentiality of ivermectin to be an effective inhibitor of SARS-CoV2 RNA-dependent RNA-polymerase.

Conclusion

COVID-19 pandemic has tremendously affected the health and wealth of global community. Speedy trial and approval of new drug is the only strategy to combat SARS-CoV2 along with social distancing. The present study revealed the structural inhibition of RdRp protein by ivermectin drug and therefore ivermectin may be a potential drug to combat COVID-19 and SARS-CoV2.

Declarations

Acknowledgment

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Authors' contributions: NA

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Figures

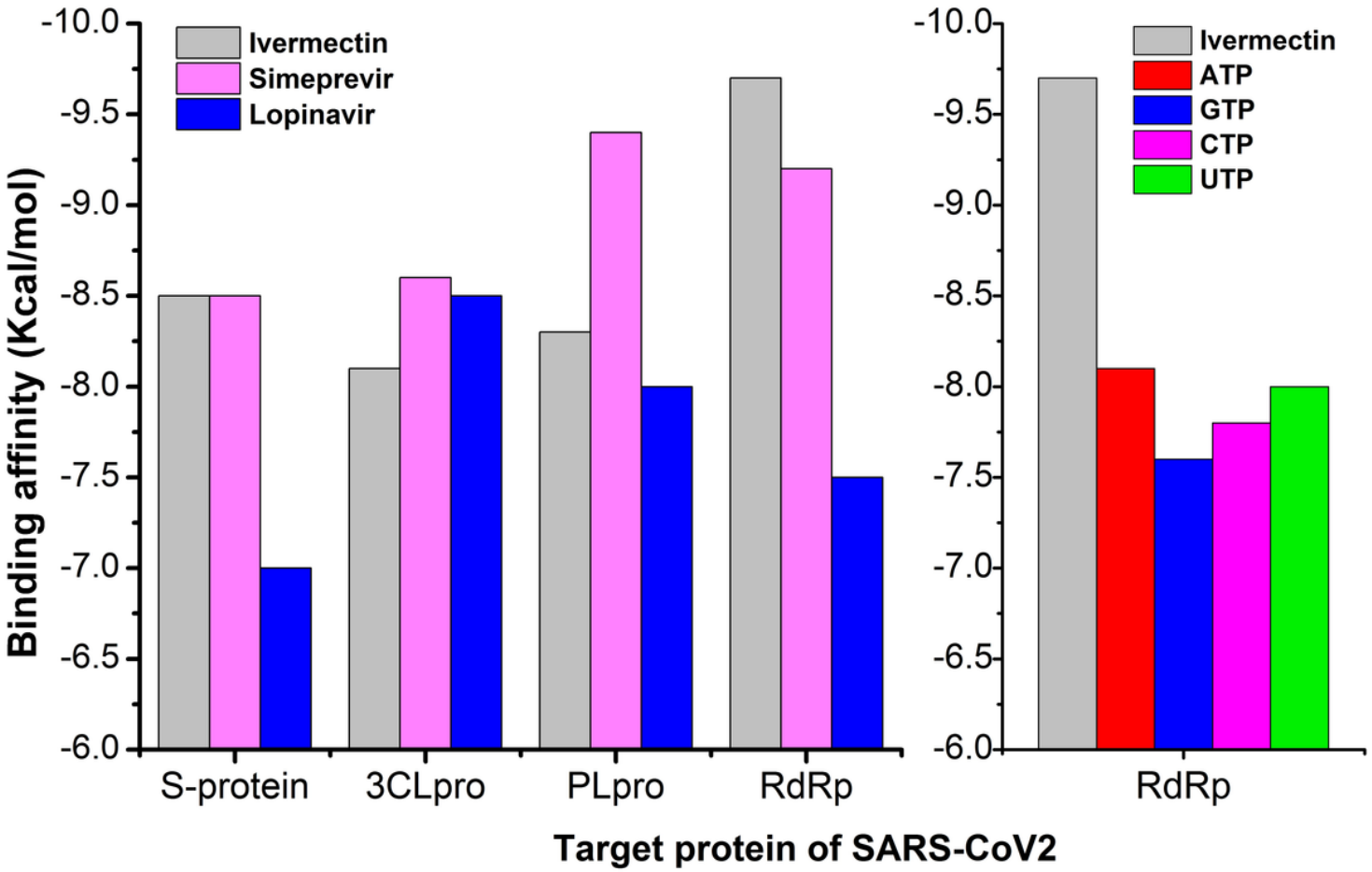

Figure 1

Docking energy and binding affinities of drug wit spike protein, 3chymotrypsin-like protease (3Clpro), papain-like protease (Plpro), and RNA-dependent RNA polymerase (RdRp).

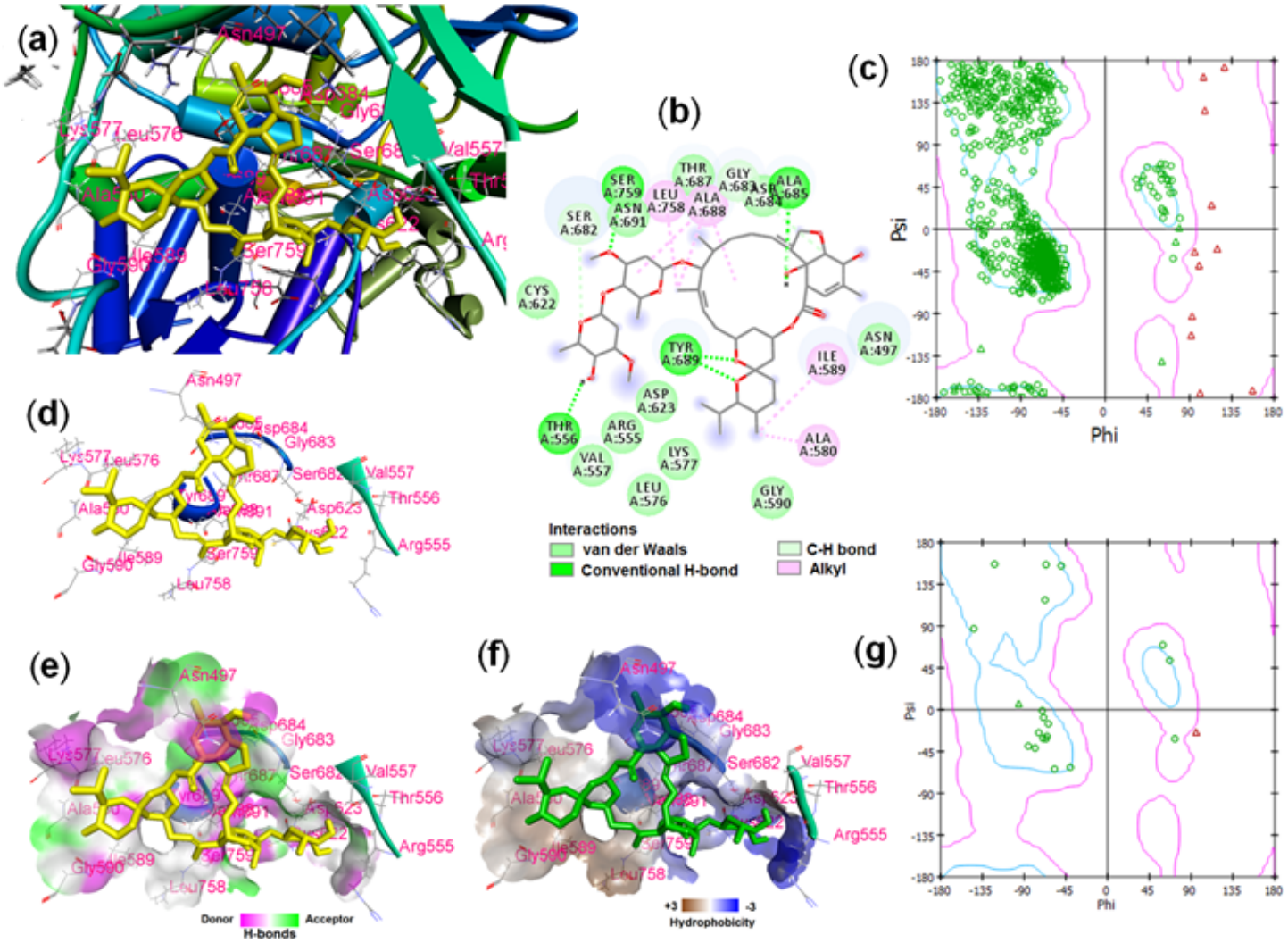

Figure 2

Binding interactions of SARS-CoV2 RNA-dependent RNA polymerase and ivermectin. (a) RdRp-ligand complex schematic, (b) 2D display of RdRp-ligand interactions, (c) Ramachandran plot of PLpro, (d) amino acid residues surrounding the ligand, (e) H-bond property of binding pocket, (f) Hydrophobicity profile of binding pocket, and (g) Ramachandran plot of ligand-interacting amino acid residues.

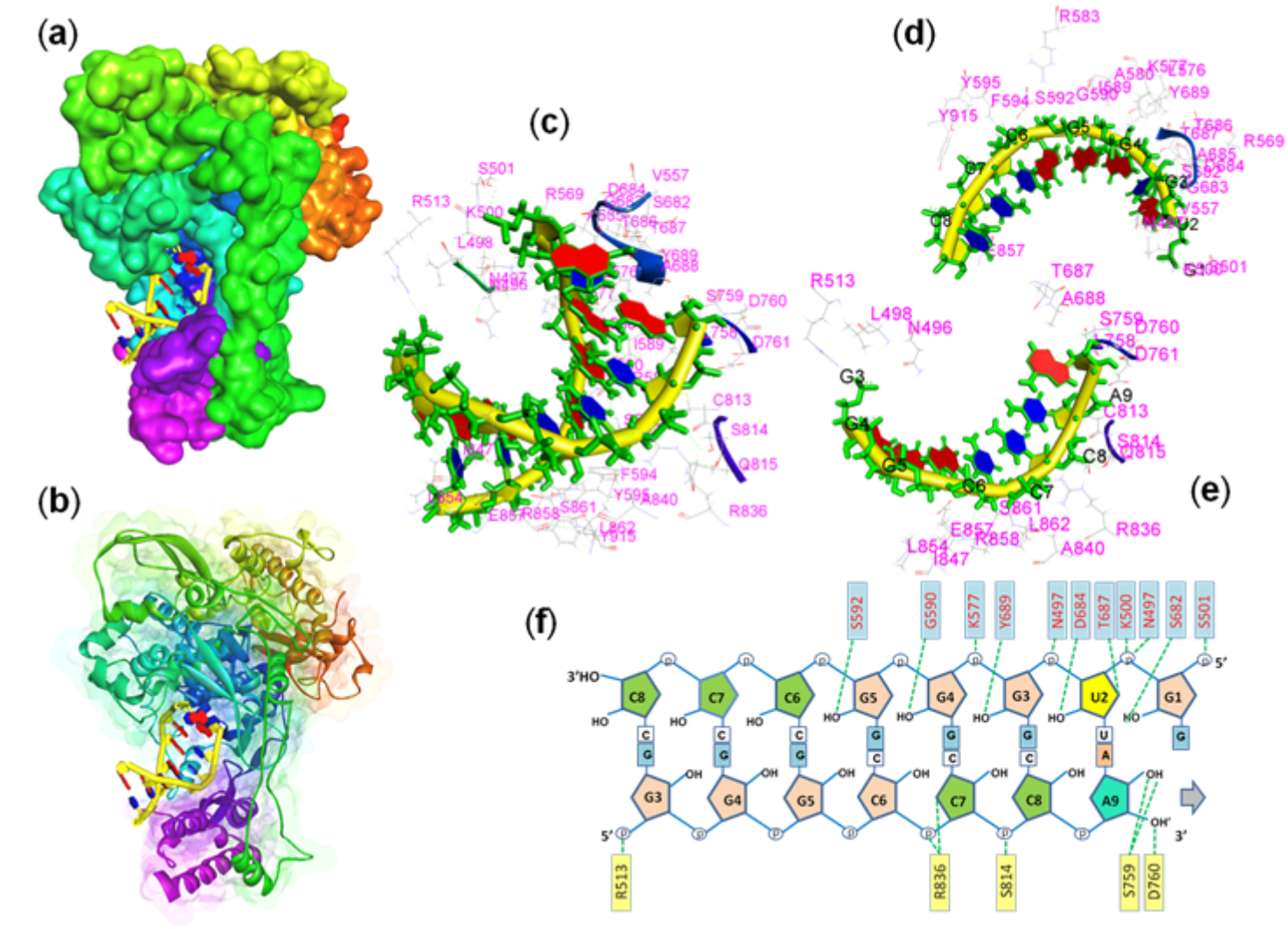

Figure 3

Binding interactions of RNA and RNA-dependent RNA polymerase. (a & b) surface view of RNA-RdRp complex, (c) RNA and surrounding amino acid residues, (d) 3'-5' RNA strand with surrounding amino acids, (e) 5'-3'-strand with surrounding amino acids and (f) amino acid residues forming H-bond with both the strand of dsRNA.

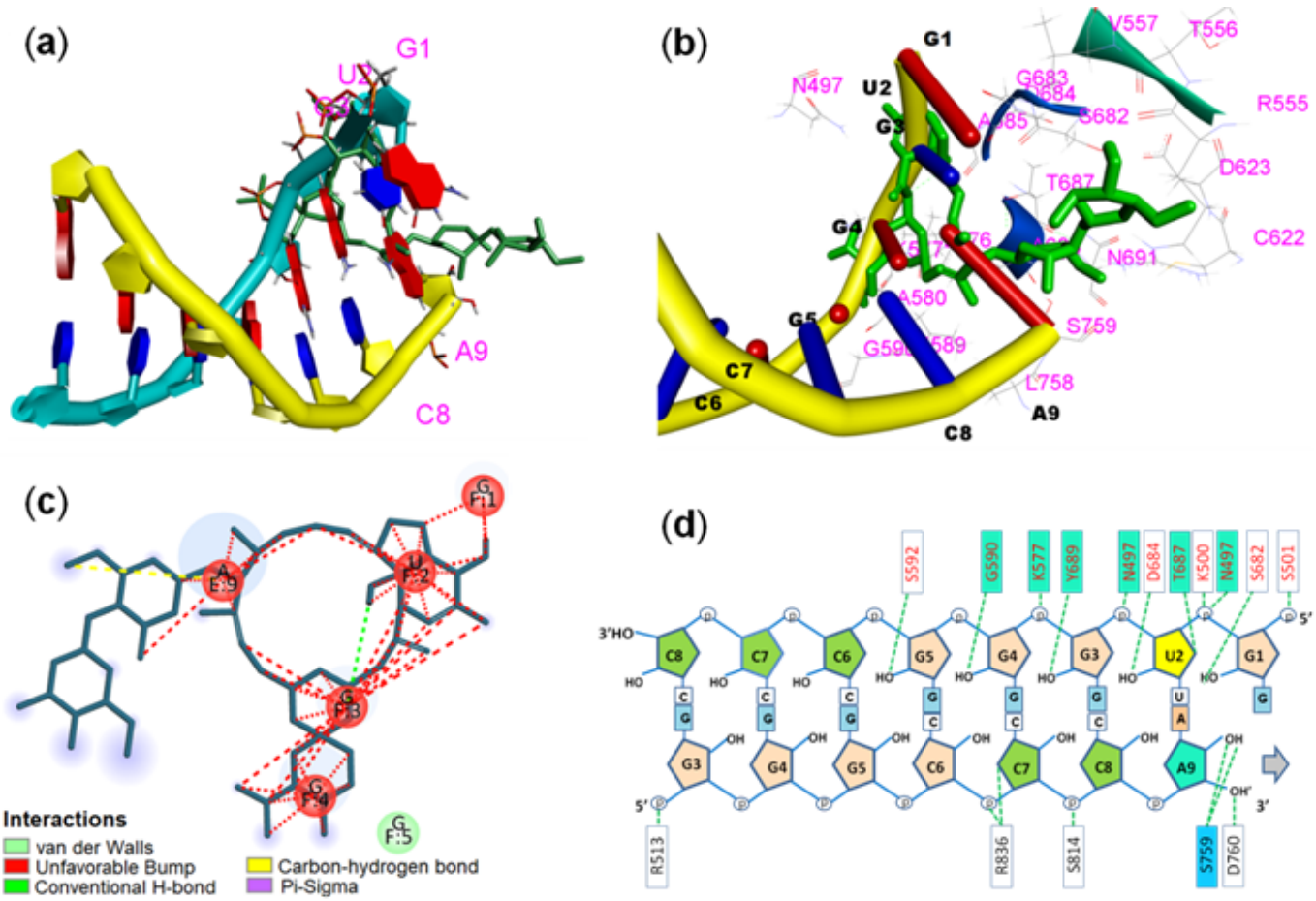

Figure 4

Interactions between RNA, RdRp, and ivermectin. (a) rode and ring view of dsRNA with attached ivermectin, (b) rode and ladder view of dsRNA with attached ivermectin and interacting amino acid residues of RdRp, (c) 2D display of ivermectin and nucleotide interactions, and (d) amino acid residues forming H-bond with RNA chain and ivermectin (cyan/blue coloured boxes).