



A hypothesis on designing strategy of effective RdRp inhibitors for the treatment of SARS-CoV-2

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Abstract

Vaccines are used as one of the major weapons for the eradication of pandemic. However, the rise of different variants of the SARS-CoV-2 virus is creating doubts regarding the end of the pandemic. Hence, there is an urgent need to develop more drug candidates which can be useful for the treatment of COVID-19. In the present research for the scientific hypothesis, emphasis was given on the direct antiviral therapy available for the treatment of COVID-19. In lieu of this, the available molecular targets which include Severe Acute Respiratory Syndrome Chymotrypsin-like Protease (SARS-3CL_{pro}), Papain-Like Cysteine Protease (PL_{pro}), and RNA-Dependent RNA Polymerase (RdRp) were explored. As per the current scientific reports and literature, among all the available molecular targets, RNA-Dependent RNA Polymerase (RdRp) was found to be a crucial molecular target for the treatment of COVID-19. Most of the inhibitors which are reported against this target consisted of the free amine group and carbonyl group which might be playing an important role in the binding interaction with the RdRp protein. Among all the reported RdRp inhibitors, remdesivir, favipiravir, and molnupiravir were found to be the most promising drugs against COVID-19. Overall, the structural features of this RNA-Dependent RNA Polymerase (RdRp) inhibitors proved the importance of pyrrolo-triazine and pyrimidine scaffolds. Previous computational models of these drug molecules indicated that substitution with the polar functional group, hydrogen bond donor, and electronegative atoms on these scaffolds may increase the activity against the RdRp protein. Hence, in line with the proposed hypothesis, in the present research work for the evaluation of the hypothesis, new molecules were designed from the pyrrolo-triazine and pyrimidine scaffolds. Further, molecular docking and MD simulation studies were performed with these designed molecules. All these designed molecules (DM-1, DM-2, and DM-3) showed the results as per the proposed hypothesis. Among all the designed molecules, DM-1 showed promising results against the RdRp protein of SARS-CoV-2. In the future, these structural features can be used for the development of new RdRp inhibitors with improved activity. Also, in the future lead compound DM-1 can be explored against the RdRp protein for the treatment of COVID-19.

Keywords COVID-19 · SARS-CoV-2 · RNA-Dependent RNA Polymerase (RdRp) · Remdesivir · MD Simulations

Introduction

COVID-19

WHO declared COVID-19 as a Global pandemic last year, which infected around 289 million people around the world until December 2021 and nearly 5 million people died due to this infection (<https://covid19.who.int/>). It is considered

as one of the biggest pandemics after the Spanish flu, affecting most of the population during 1918–1920. One of the biggest problems associated with COVID-19 is the high transmission rate from person to person through droplets or breath (Liu et al. 2020). Sometimes, asymptomatic patients are also carriers of the virus which make it difficult to contain the virus (Liu et al. 2020). Symptoms of COVID-19 patients vary from person to person, but the most common symptoms include fever, cough, fatigue, shortness of breath, and loss of smell and taste (Liang et al. 2020; Amin et al. 2021a; Tian et al. 2021a; Ma et al. 2021). Depending upon the immunity of an individual, severity varies from person to person. In severe cases, COVID-19 patients developed the complications like pneumonia, respiratory distress, blood

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clots, and organ-failure that consequently resulted in death (Liang et al. 2020; Amin et al. 2021a; Tian et al. 2021a; Ma et al. 2021). Through the continuous efforts of the scientific community, several vaccines have been developed. However, the mutation in the virus is causing breakthrough infections in fully vaccinated people, which is a cause of concern for the world.

Available WHO-approved vaccines and their limitations

To date, 10 vaccines have been approved by the WHO shown in Table 1 which include Pfizer-BioNTech-BNT162b2 (RNA-based Vaccine), Moderna-mRNA-1273-(RNA based Vaccine), Johnson & Johnson's Janssen Ad26.COV2.S-(Non Replicating Viral Vector-based vaccine), AstraZeneca/Oxford-AZD1222-(Non-Replicating Viral Vector-based vaccine), Serum Institute of India Covishield (Oxford/AstraZeneca formulation-(Non-Replicating Viral Vector- based vaccine), SinoPharm Beijing-BBIBP-CorV (Vero Cells based vaccine), Sinovac (CoronaVac vaccine), Novavax (NVX-CoV2373-protein subunit), Serum Institute of India COVOVAX (Novavax formulation-Protein Subunit) and Bharat Biotech Covaxin (<https://covid19.who.int/>). Vaccine discovery played an important role in the mitigation of COVID-19 in most parts of the world but unexpected mutation in the SARS-CoV-2 virus is still a big challenge for the scientific community (Ma et al. 2021; Plante et al. 2021; Chen et al. 2020a; Manuel and Timothy 2020; Tao et al. 2021; Harvey et al. 2021). Recently, the Omicron variant is haunting the world for the next outbreak as it caused some breakthrough infections in fully vaccinated people (<https://covid19.who.int/>). However, the deaths reported due to the Omicron variant are fewer, and it seems to be less lethal compared to the delta variant. It is well understood that the virus will continue to mutate which consequently may turn into a lethal variant in the future that may break the immunity developed by the vaccines. Hence, there are many uncertainties in the scientific community regarding the end of the current pandemic. Therefore, there is an urgent need to discover the effective SARS-CoV-2 inhibitors which may help to eradicate the pandemic from the world.

General features of SARS-CoV-2 virus and possible molecular targets

Coronavirus belongs to the family *Coronaviridae* that also has a subfamily *Coronavirinae* which contains four genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. SARS-CoV-2 mainly belongs to the *beta corona* virus family (Fu et al. 2021) which comprises of the other two deadlier viruses, i.e., Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East

Respiratory Syndrome Coronavirus (MERS-CoV). These viruses also created the havoc in the past. SARS-CoV-2 is a single-stranded RNA that has a genome of 27–32 kb which is larger than any other virus. It consists of four different types of structural protein, i.e., Spike Protein (S), Envelope Protein (E), Membrane Protein (M), Nucleocapsid Protein (N), and sixteen nonstructural proteins (NSP 1–16) (Wang et al. 2020). Among all the proteins, spike protein (S) plays an important role in the entry into the host cell through Angiotensin Converting Enzyme (ACE-2) as shown in Fig. 1 (Luan et al. 2020; Hussain et al. 2020). The Receptor Binding Domain (RBD) of the spike glycoprotein mediates the binding to the ACE-2 receptor; therefore, both ACE-2 and RBD are attractive targets for inhibition of the entry of the SARS-CoV-2 virus in the host cells. The advantage of targeting these receptors leads to the prevention of the spread of the infection in the body in the early stages. After the entry of the virus in the host cells, two viral polyproteins are produced with the aid of the host cell's ribosomes (Ghanbari et al. 2020). These polyproteins get converted into RNA-Dependent RNA Polymerase (RdRp) protein in the presence of the viral protease enzymes such as Severe Acute Respiratory Syndrome Chymotrypsin-like Protease (SARS-3CL^{pro}), and Papain-Like Cysteine Protease (PLpro) as shown in Fig. 1 (Mody et al. 2021; Rut et al. 2020; Gao et al. 2020). This non-structural RdRp protein is encoded with the viral genome which further produces the sub-genomic mRNA as shown in Fig. 1. This mRNA further produces the viral structural protein through the translation process as shown in Fig. 1. This viral protein along with genome RNA gets converted into the new virion. This new virion gets transported outside the cell through exocytosis. Therefore, inhibition of these enzymatic targets, i.e., RBD-ACE2 fusion, RdRp, SARS-3CL^{pro}, PLpro, plays a crucial role in ceasing the viral replication in the body.

Introduction to available treatment for COVID-19

In the early days of the COVID-19 pandemic, the genome sequence of the five SARS-CoV-2 patients from Wuhan revealed a similarity with the SARS-CoV-2 virus that forms the basis of the preliminary treatment of COVID-19 (Wang et al. 2020; Rossi et al. 2020; Abdelrahman et al. 2020; Maldonado et al. 2021; Fani et al. 2020). During this pandemic, many strategies were used which included the use of antibodies, IFNs, inhibitors of viral and host proteases, and host-directed therapies. A few of them were used as an experiment during the current pandemic. But these strategies were found to be ineffective against the delta strain of the SARS-CoV-2 virus (Fu et al. 2021; de Wit et al. 2016). Similarly, a few other drugs were repurposed for the treatment of the COVID-19 which include favipiravir, remdesivir, hydroxychloroquine, lopinavir, interferon, etc., which

Table 1 Vaccines available for COVID-19 and their disadvantages (As per WHO/CDC) updated on January 19, 2022 (<https://covid19.who.int/>)

Sr. no	Brand name/vector/platform	Age group	Disadvantages
	Pfizer-BioNTech -(BNT162b2)-RNA based Vaccine	People 12 years and older	Commonly reported symptoms (occurrence in descending order) were soreness, fatigue, myalgia, headache, chills, fever, joint pain, nausea, muscle spasm, sweating, dizziness, flushing, feelings of relief, brain fogging, anorexia, localized swelling, decreased sleep quality, itching, tingling, diarrhea, nasal stuffiness and palpitations
	Moderna-(mRNA-1273)-RNA based Vaccine	People 18 years and older	Not available for school going children; commonly reported side effects include pain Redness, swelling; individuals are susceptible to breakthrough infection specifically due to OMI-CRON variant
	Johnson & Johnson's Janssen (Ad26.COVS.S)-Non Replicating Viral Vector	People 18 years and older	Not available for school going children; commonly reported side effects include pain Redness, swelling; individuals are susceptible to breakthrough infection specifically due to OMI-CRON variant
	AstraZeneca/Oxford- (AZD1222)- Non Replicating Viral Vector	People 18 years and older	Not Available for School going Children; Commonly reported side effects include irritability in mood, 6 h after vaccines, some complained of myalgia, nausea, tenderness at the Injection site and feverish feeling. After 12 h, fever with chills are but serious cases of blood clots accompanied by low platelet counts (known as thrombosis with thrombocytopenia syndrome (TTS)) occurring 3–30 days after vaccination (WHO); Individuals are susceptible to Breakthrough Infection specifically due toOMICRON Variant
	Serum Institute of India Covishield (Oxford/AstraZeneca formulation)- Non Replicating Viral Vector	People 18 years and older	Not available for school going children; commonly reported side effects include irritability in mood, 6 h after vaccines, some complained of myalgia, nausea, tenderness at the Injection site and feverish feeling. After 12 h, fever with chills are but serious cases of blood clots accompanied by low platelet counts (known as thrombosis with thrombocytopenia syndrome (TTS)) occurring 3 to 30 days after vaccination (WHO); Individuals are susceptible to Breakthrough Infection specifically due to OMI-CRON Variant
	SinoPharm (Beijing- BBIBP-CorV (Vero Cells)	People 18 years and older	Not available for school going children; commonly reported side effects include Site pain and fatigue; Individuals are susceptible to breakthrough infection specifically due toOMICRON variant
	Sinovac (CoronaVac)	People 18 years and older	Fatigue, diarrhea, and muscle pain; individuals are susceptible to breakthrough infection specifically due toOMICRON variant
	Novavax (NVX-CoV2373)-Protein Subunit	People 18 years and older	Not available for school going children, breakthrough infection specifically due toOMICRON variant
	Serum Institute of India COVOVAX(Novavax formulation) Protein Subunit	People 18 years and older	Not available for school going children, breakthrough infection specifically due toOMICRON variant
	Bharat Biotech Covaxin	People 18 years and older	Not available for school going children, breakthrough infection specifically due toOMICRON variant

were found to be not that effective for the prevention of the mortality rate (Hassanipour et al. 2021; Bansal et al. 2021; Axfors et al. 2021; Cao et al. 2020). During the pandemic, the delta strain created a catastrophic effect on COVID-19

patients by inducing a cytokine storm that led to a sharp rise in the number of patients hospitalized. To overcome this problem, a few other therapies were utilized like monoclonal antibodies (bamlanivimab + etesevimab) (Dougan

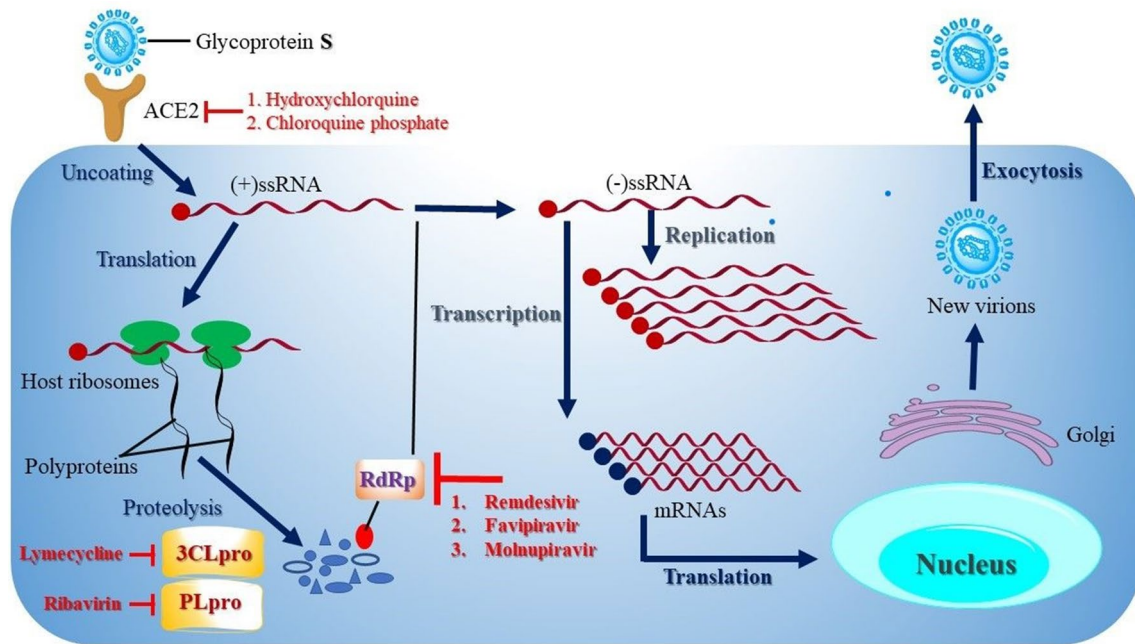


Fig. 1 Pathogenesis of COVID-19 along with the important molecular targets of the SARS-CoV-2

et al. 2021), Interleukin-1 Inhibitors (canakinumab) (Cremmer et al. 2021), Interleukin-6-Inhibitors (sarilumab) which have been effectively used for the treatment of COVID-19. All these therapies were found to be useful for the treatment of the hyperactivated immune response rather than against the virus.

Overall, many efforts are being put in by the scientific community to eradicate the COVID-19 pandemic from the world. In lieu of this, in the present work, we have tried to collect the possible molecular targets for the SARS-CoV-2 virus and their structural features with respect to the binding domain. Additionally, we enumerated various SARS-CoV-2 inhibitors to date to find the common features of these inhibitors. Based on the structural information of the various receptors of the SARS-CoV-2 virus and reported SARS-CoV-2 inhibitors, we have proposed the required features of the molecules that can be effective against SARS-CoV-2.

Severe acute respiratory syndrome (SARS) chymotrypsin-like Protease (SARS-3CL^{pro}) and its inhibitors

3CL^{pro} is a major replicating enzyme present inside the SARS-CoV-2 virus. It is responsible for the replication of the SARS-CoV-2 virus inside the host cells. 3CL^{pro} is responsible for the production of 16 non-structural proteins (NSPs). These NSPs are responsible for the replication, transcription, and virus recombination during the infection phase. Hence, inhibition of the 3CL^{pro} would be one of the

effective strategies for the antiviral therapy for COVID-19. The binding pocket of 3CL^{pro} is highly conserved with amino acids like glutamine, leucine, and methionine. 3CL^{pro} is responsible for the cleavage of SARS-CoV-2 polyprotein at the 11 sites which consequently results in the production of the NSPs. The 3CL^{pro} protein comprises three domains: domain-I, domain-II and domain-III. Among these domains, domain III has prime importance as it is involved in the dimerization and formation of an active 3CL^{pro} protease. The active site of the 3CL^{pro} lies between domain-I and domain-II. Recent studies of 3CL^{pro} indicated that it consisted of the His41 (part of domain-I) and Cys145 (part of domain-II) residues of S-glycoprotein which are the prime targets for the inhibition of the replication of the SARS-CoV-2 virus (Ferreira and Rabeh 2020).

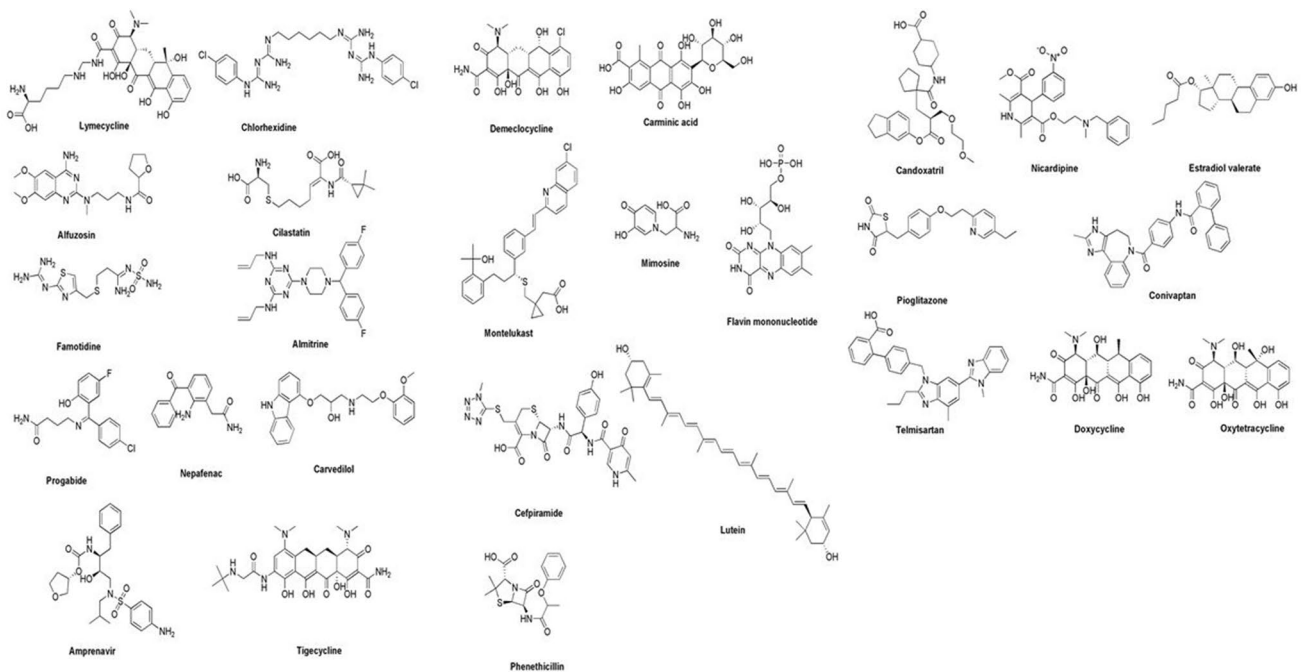
Earlier research on this target showed some promising drug candidates as shown in Table 2 (Konwar and Sarma 2021; Chen et al. 2005; Ghosh et al. 2007; Wu et al. 2020). Most of the drugs are polyphenolic as shown in (Fig. 2; Konwar and Sarma 2021; Chen et al. 2005; Ghosh et al. 2007; Wu et al. 2020) indicating the importance of the hydroxyl group required for the binding affinity with the 3CL^{pro}

Papain-like cysteine protease (PLpro) and its inhibitors

Basically, the PLpro protein of SARS-CoV-2 consists of two domains, i.e., the N-terminal UBL domain and the C-terminal USP domain, where all the process of regulation takes

Table 2 Molecular targets of SARS-COV 2 virus and promising molecules against the SARS-COV-2 (Chen et al. 2005; Lan et al. 2020; Pachetti et al. 2020)

Sr. no	Name of the targets	Potential molecules acting on the target
	SARS-3CL-pro	Lymecycline, Chlorhexidine, Alfuzosin, Cilastatin, Famotidine, Almitrine, Progabide, Nepafenac, Carvedilol, Amprenavir, Tigecycline, Demeclocycline, Montelukast, Carminic acid, Mimosine, Flavin mononucleotide, Lutein, Cefpiramide, Phenethicillin, Candoxatril, Nicardipine, Estradiol valerate, Pioglitazone, Conivaptan, Telmisartan, Doxycycline, Oxytetracycline
	PL-pro	Ribavirin, Valganciclovir, b-Thymidine, Aspartame, Oxprenolol, Doxycycline, Acetophenazine, Iopromide, Riboflavin, Reproterol, Chloramphenicol, Chlorphenesin, carbamate, Levodropropizine, Cefamandole, Floxuridine, Tigecycline, Pemetrexed, Glutathione, Hesperetin, Ademetionine, Masoprocol, Isotretinoin
	ACE2	NCT04311177, NCT04312009, NCT04338009, and NCT04394117, Chloroquine Phosphate
	RdRp	Remdesivir, Favipiravir, Valganciclovir, Chlorhexidine, Ceftibuten, Fenoterol, Fludarabine, Itraconazole, Cefuroxime, Atovaquone, Chenodeoxycholic acid, Cromolyn, Pancuronium, bromide, Cortisone, Tibolone, Novobiocin, Silybin, Idarubicin, Bromocriptine, Diphenoxylate, Benzylpenicilloyl G, Dabigatran etexilate

**Fig. 2** Severe Acute Respiratory Syndrome (SARS) chymotrypsin-like protease (SARS-3CL^{pro}) inhibitors

place. PL^{pro} of the SARS-CoV-2 virus is cysteine proteases which are responsible for protein maturation, dysregulating host inflammation response and impairing the host type I interferon antiviral immune responses. All these changes take place due to the interferon-stimulated gene (ISG15) modification. Therefore, the binding cleft of the PL^{pro} protein which comprises ISG15 is considered a hotspot for antiviral therapy (Shin et al. 2020).

The recent protein structure of PL^{pro} complexed with the GRL-0617 revealed that the side chains of the Glu269 and Tyr268 revealed the polar interaction with the co-crystallized ligand and is responsible for the stabilization of the protein–ligand complex. Carbonyl oxygen and the free amine group of the ligand played an important role in

this polar binding interaction with the protein. Additionally, naphthalene of the inbound ligand showed binding interactions with the Tyr264 and Tyr268. Overall, this complex structure represented the importance of the carbonyl oxygen and hydrophobic ring like naphthalene for the appropriate binding interactions of any ligand with this target protein (Fu et al. 2021; Wu et al. 2020; Shin et al. 2020; Verma et al. 2021; Amin et al. 2021b; Mirza et al. 2020; Bhati 2020; Swaim et al. 2021; Rajpoot et al. 2021). Most of the PL^{pro} inhibitors shown in Fig. 3 (Fu et al. 2021; Wu et al. 2020; Shin et al. 2020; Verma et al. 2021; Amin et al. 2021b; Mirza et al. 2020; Bhati 2020; Swaim et al. 2021; Rajpoot et al. 2021) bears the free amine group

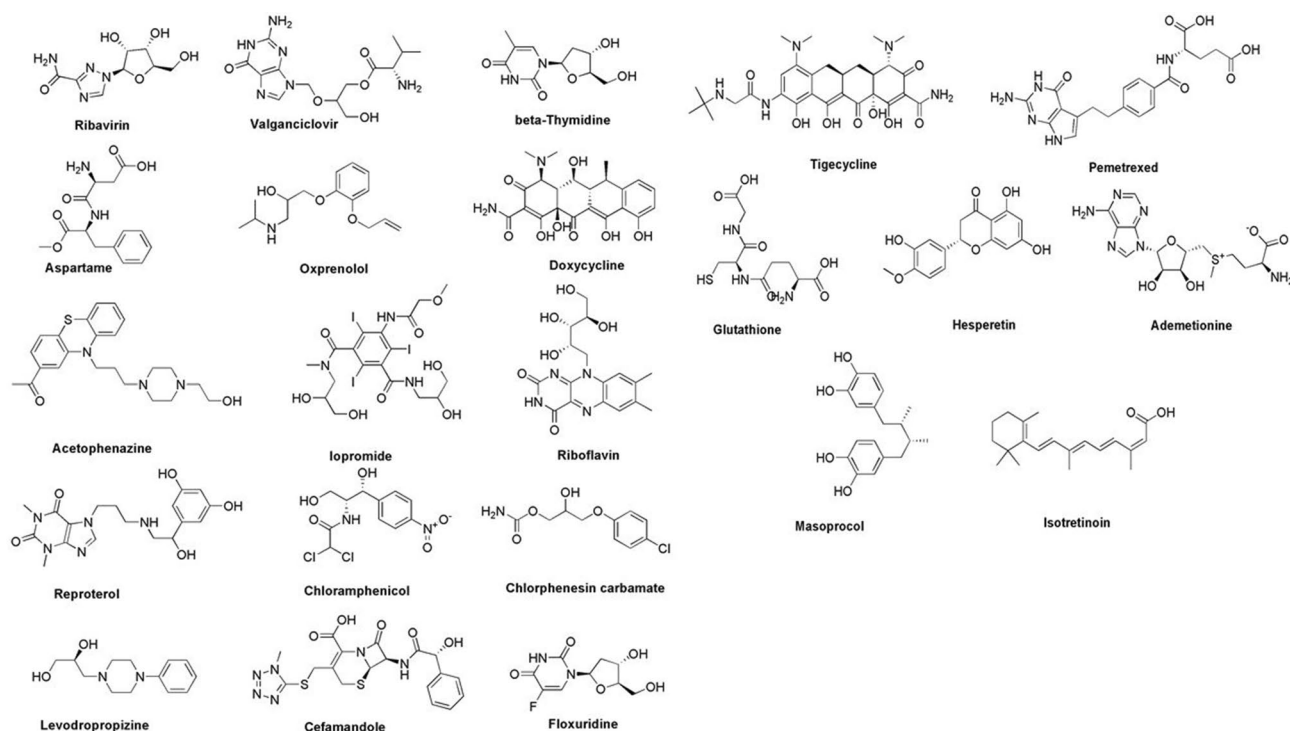


Fig. 3 Papain-like cysteine protease (PLpro) inhibitors

along with the carbonyl group which might be playing an important role in the inhibition if the PLpro protein.

Angiotensin converting enzyme (ACE2) and its inhibitors

ACE2 is one of the most attractive targets for the SARS-CoV-2 virus (South et al. 2020; Arno et al. 2020; Li et al. 2020; Lan et al. 2020). This receptor is usually present in the lower respiratory system of the human body; hence, this virus is responsible for more damage in the lower respiratory tract (South et al. 2020; Arno et al. 2020; Li et al. 2020; Lan et al. 2020). S-glycoprotein of the SARS-CoV-2 interacts with the ACE2 receptor which aids the entry of the virus inside the host cell and further replication takes place (Lan et al. 2020; Duan et al. 2020). This S-glycoprotein present in the spikes of SARS-CoV-2 virus is considered to be the protective envelope of the virus. This S-glycoprotein comprises two subunits, i.e., S1 and S2. The S1 subunit identifies the receptor in the host and attaches itself to the receptor. This attachment of the S1 subunit with the host receptor takes place between the Receptor Binding Domain (RBD) of the S1 subunit of the viral protein and the peptidase domain (PD) of the ACE2 receptor. On the other hand, S2 is responsible for the fusion process between the virus and host receptor.

Once this S1-RBD attaches itself to the receptor of the host cell, host proteases cleave the S-glycoprotein which resulted in the shedding of the S1 subunit. The previous finding suggested that the amino acid residues of S1-RBD (Thr333-Gly526) interact with the amino acid residues of the ACE2 receptor (Ser19-Asp615) which is responsible for the entry of the virus in the host cells (Singh et al. 2021a).

In the initial period of the COVID-19 pandemic, anti-malarial drugs like hydroxychloroquine and chloroquine phosphate were found to be effective and taken for prophylactic measure by healthcare professionals (Chen et al. 2020b). The administration of these antimalarial drugs is based on a possible hypothesis that these drugs may act as fusion inhibitors of the S-RBD and ACE2. Later on, the scientific community proved that this hypothesis was wrong. Also, the inhibition of ACE-2 may create other pharmacological problems like vasoconstriction, inflammation as ACE-2 is involved in the Renin Angiotensin Aldosterone System (RAAS) pathway and consequently reducing the harmful effect of vasoconstrictor, anti-inflammatory peptide, and angiotensin 2. Its inhibition may disturb the RASS pathway which may create other severe health issues (Behl et al. 2020).

Despite this, there are certain molecules as shown in Table 2 (McKee et al. 2020) sent for the clinical trials meant for ACE2 inhibition.

Non-structural proteins (NSP)

Apart from the structural protein, the virus also comprises non-structural proteins (NSPs), i.e., NSP1 to NSP10 and NSP12 to NSP16. These proteins are encoded by the genes and are located in the 5' -region of viral RNA. NSP1 is an N terminal product of the viral replicase and it comprises 180 amino acid residues. The major function of the NSP1 is to degrade the host mRNA (Yadav et al. 2021; Singh et al. 2022). NSP2 is also the N-terminal product of the viral protein which consists of 638 amino acids. It binds to prohibitin 1 and prohibitin 2. NSP3 is a papain-like proteinase which consists of the 1945 amino acid residues. This protein is responsible for the release of the NSP1, NSP2, and NSP3. NSP4 comprises 500 amino acids and is involved in the viral replication. NSP5 is also known as proteinase which consist of 306 amino acid residues. The major role of this protein involves cleaving the protein at multiple sites which consequently results in the formation of the intermediate non-structural protein. NSP6 is the Putative transmembrane domain that is responsible for the formation of ER-derived autophagosomes. NSP7 and NSP8 are two important non-structural proteins that play a vital role in the RdRp activity in the SARS-CoV-2. NSP7 comprises 83 amino acid residues. Mainly, it forms the complex with the NSP8 and NSP12 and induces the RNA polymerase activity of the NSP8. NSP8 is involved in the formation of the heterodimer with the NSP8 and 12. The major role of NSP 9 to 11 in SARS-CoV-2 is not yet clear. NSP12 and 13 are involved in viral replication. Both these proteins comprise 932 amino acid residues. NSP14 with the 527 amino acid residues is involved in the N7-guanine methyltransferase activity. Similarly, NSP15 which comprises of 346 amino acid residues is responsible for the Mn(2+)-dependent endoribonuclease activity.

Among all the non-structural proteins, NSP16 plays a vital role in the reproduction of the CoV RNA genome. This NSP16 consists of 301 amino acid residues, among which Phe6947, Asp6912, Leu6898, Cys6913, Met6929, Gly6871, Asp6897, Asn6899, Asp6928, Tyr6845, Asn6841, and Gly6871 are parts of the binding pocket of the NSP16 protein. Inhibition of the NSP16 would be advantageous for inhibiting the SARS-CoV-2 virus.

RNA-dependent RNA polymerase (RdRp) and its inhibitors

Until now, and since the pandemic, this target is one of the most important target meant for the treatment of the SARS-CoV-2 infection (Elfiky 2020; Gao et al. 2020;

Pachetti et al. 2020; Aftab et al. 2020; Khater et al. 2021). RNA-Dependent RNA Polymerase is an enzyme responsible for RNA synthesis, and hence, it plays an important role in viral replication. In the current pandemic, many antiviral drugs like remdesivir and favipiravir were repurposed against the SARS-CoV-2 virus (Tian et al. 2021b). One of the interesting things observed in the RdRp inhibitor is that most of these inhibitors consisted of the free amine group along with the carbonyl group shown in Fig. 4 (Tian et al. 2021b). Few of these inhibitors showed binding interactions with the key amino acids of the RdRp protein as shown in Table 3 (Sada et al. 2020; Nimgampalle et al. 2021; Poustforoosh et al. 2021; Alexpandi et al. 2020). In addition to this synthetic molecule, lots of natural molecules are also screened against the RdRp protein as shown in Table 3. Examples of these natural molecules include 7-hydroxyaloin-B of *Aloe vera*; Isoquercetin and sonimocinolide of *Azadirachta indica*; which showed good binding affinity with the RdRp protein (Kushwaha et al. 2021). Interestingly, as per the Piplani et.al study (Piplani et al. 2021), digoxin showed a good binding affinity and interaction with the RdRp protein. It showed key binding interactions with the Ser682, Arg553, Arg624, and Cys622 of the RdRp protein. Similarly, Singh et.al reported curcumin and diacetylcurcumin as promising drug candidates as an RdRp inhibitor for the mitigation of COVID-19 (Singh et al. 2021b). During the pandemic, the plants which were explored more were *Swertia chirayita*, *Tinospora cordifolia*, and *Withania somnifera*. Koulgi et. al in their study (Koulgi et al. 2021) reported important phytoconstituents of these plants which include swertia-punisiide, cordifolide A, sitoindoside IX, and amarogentin. Glycosidic moiety of these phytoconstituents formed the key binding interactions with the polar and charged amino acids of the RdRp protein which includes Arg553, Arg555, Asp618, Asp760, Asp761, Glu811, and Ser814. Similarly, Singh et.al (Singh et al. 2021c) showed the importance of polyphenolic compounds for the treatment of COVID-19. He reported a few polyphenolic phytoconstituents which include Epigallocatechin Gallate (EGCG), theaflavin (TF1), theaflavin-3'-O-gallate (TF2a), theaflavin-3'-galate (TF2b), theaflavin 3,3'-digallate (TF3), hesperidin, quercetageitin, and myricetin. These phytoconstituents revealed excellent binding affinity with the RdRp protein.

RdRp protein also known as Non-Structural Protein 12 (NSP12) consists of two subunits NSP7 and NSP8 which have an important role in the replication of the SARS-CoV-2 virus (Kirchdoerfer and Ward 2019; Velthuis et al. 2012; Sun 2020; Bhalla et al. 2021). A schematic representation of this RdRp protein (PDB ID:7D4F) is shown in Fig. 5. In addition to this, RdRp protein consists of a few other important domains. These include the polymerase domain (Ser367 to Phe920), and nidovirus-specific N-terminal expansion

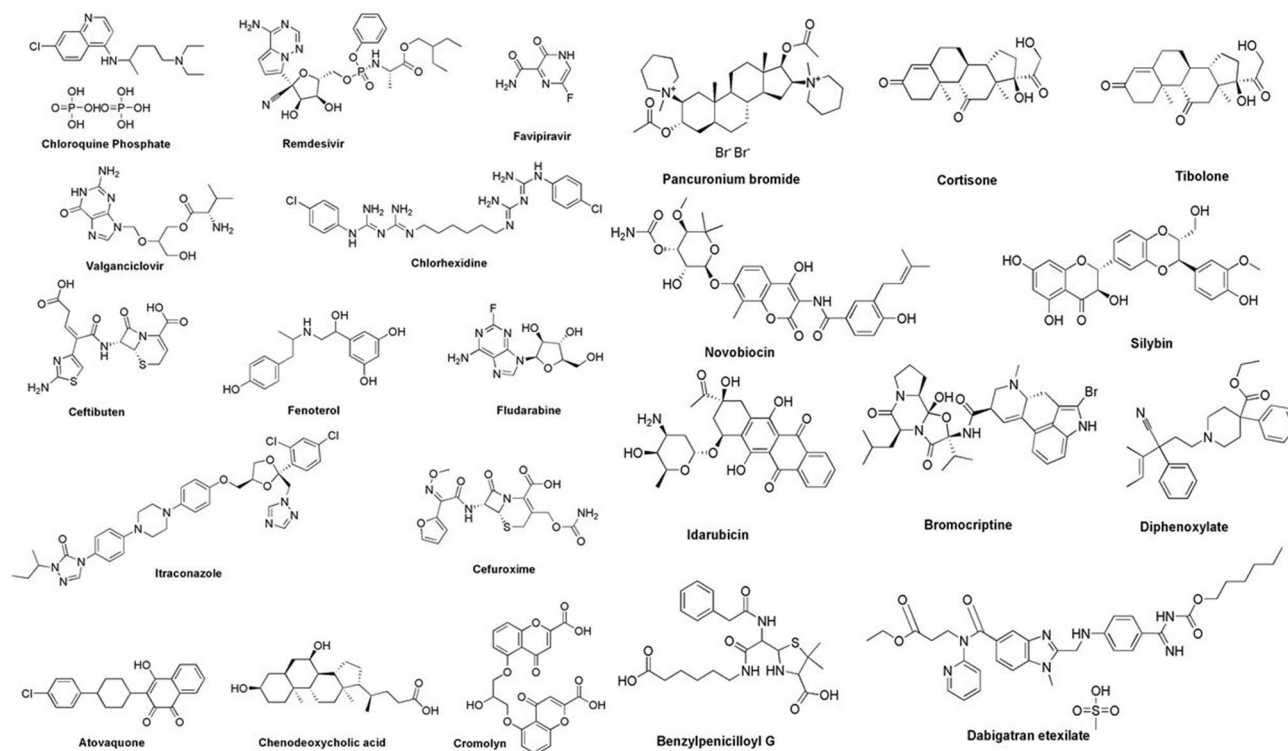


Fig. 4 RNA-dependent RNA polymerase (RdRp) inhibitors

domain (NiRAN domain) which consists of the amino acids from Asp60 to Arg249. RdRp protein also consists of an interface domain that joins the polymerase and NiRAN domains together. This interface domain consists of amino acids ranging from Ala250 to Arg365. NiRAN domain is responsible for the SARS-CoV-2 viral replication (Venkataraman et al. 2018; Bhardwaj et al. 2021a; Yin et al. 2021).

Apart from this, RdRp also has a few other domains which include the palm domain (Tyr582-Pro620 and Tyr680-Gln815), the finger domain (Leu366-Ala581 and Lys621-Gly679), and the thumb domain. The palm domain of the RdRp protein consists of the catalytic aspartate and RNA Recognizing motif which are responsible for the catalysis. The finger domain is responsible for the proper geometry of the active site of the RdRp protein. The thumb domain is required for nucleic acid synthesis. The N-terminal hairpin which has an amino acid residue from Asp29 to Lys50 is responsible for the stability of the RdRp structure.

Among all the antivirals acting against the RdRp, the remdesivir monophosphate was found to be the most effective. A recent electron microscopical study of a complex of remdesivir monophosphate and RdRp revealed the importance of the pyrrolo-triazine scaffold of the remdesivir (Bravo et al. 2021). RdRp protein consequently delays the replication of the viral protein. However, this interaction is not enough to completely stop the viral replication. Hence,

there is the scope for suitable structural modification of remdesivir which could lead to the discovery of a powerful RdRp inhibitor. In addition to this, remdesivir showed many side effects such as anemia, infusion site reactions, increased liver enzymes, cutaneous rash, kidney injuries, and hypotension. Hence, its use has to be restricted in critically comorbid patients (Nabati and Parsaee 2022). Similar to the remdesivir, one more drug was found to be effective against the SARS-CoV-2, i.e., molnupiravir showed crucial binding interaction with the RdRp protein (Yip et al. 2022).

Hypothesis

Currently, there are various therapies available against SARS-CoV-2 which include both natural and synthetic sources that act against the above-mentioned targets of the SARS-CoV-2 virus. Singh et al. in their studies reported various bioactive compounds from tea as potential SARS-CoV-2 nonstructural protein 16 inhibitors. In their study, they found theaflavin as a potential lead candidate as an NSP-16 inhibitor where theaflavin forms a hydrogen bond with the key amino acids, i.e., Asp6928, Asp6897, Lys6844, Asp6931, Met6929. This study proved the importance of polyphenolic compounds in the treatment of SARS-CoV-2. Hence, during the designing of NSP16 inhibitors, the

Table 3 Few reported synthetic and natural RdRp inhibitors with their crucial binding interaction with the RdRp protein

Sr. no	Name of the drugs	Key amino acid involved in binding interactions
Synthetic RdRp inhibitors		
1	Favipiravir	Arg473, Thr600, Ser602, Thr607, Asn611, Ser679
2	Remdesivir	Thr677, Asn688, Ser756, Asp757
3	Molnupiravir	Lys345, Arg555, Ala688, Leu758, Ser759, Asp761
4	Idarubicin	Arg555, Lys545, Tyr546, Ala547
7	Elvitegravir	Tyr455, Ala554, Tyr619, Pro620, Cys622, Thr556, Thr680, Ser681, Ser682, Thr687, Asn691, Ser759, Lys621
8	Oxolinic acid	Thy456, Ser682, Arg624, Lys545, Arg553, and Arg555
9	Hydroxychloroquine	Asn52 and Asn209
Natural RdRp inhibitors		
10	7-hydroxyaloin-B	Asp618, Tyr619, Lys621, Asp760, Lys798, Glu811
11	Isoquercetin	Trp617, Asp618, Tyr619, Asp760, Lys798, Pro620, Lys621, Cys622, Cys799, Trp800, Gly616, Ala762, Asp761, Glu811, Ser814, Ser759
12	Sonimocinolide	Tyr619, Lys621, Asp761, Ser814, Asp618, Lys798, Asp623, Cys622, Pro620, Leu758, Asp760, Ser759, Cys813, Ala762, Phe812, Glu811, Trp617, Gly616, Trp800
13	Digoxin	Ser682, Arg553, Arg624 and Cys622
14	Curcumin	Lys545, Arg553, Ser759, Ser682, Arg555, Ala688, and Val557
15	Diacetylcurcumin	Thr680, Asn691, Thr687, Lys545, Arg555, Asp623, Val557, Asp761
16	Swertiapuniside, Cordifolide A, Sitoindoside IX, Amarogentin	Arg553, Arg555, Asp618, Asp760, Asp761, Glu811, Ser814
17	Epigallocatechin Gallate (EGCG)	Asp623, Tyr 619, Lys 621 Ser 795, Cys 622, Asp 618, Met794, Pro 620, Lys 798
18	Theaflavin (TF1)	Trp 617, Asp 761, Asp 760, Tyr 619, Arg 553, Lys 621, Pro 620, Phe 793, Asp 164, Ser795, Lys 798, Asp 618
19	Theaflavin-3'-O-gallate (TF2a)	Cys 813, Phe 812, Asp 761, Asp 760, Asp 618, Lys798, Lys 551, Ala550, Ser 549, Lys 621, Tyr 619, Trp 800, Trp 617, E811
20	Theaflavin-3'-gallate (TF2b)	Lys 551, Tyr 619, Asp 760, Lys 798, Trp 617, Trp800, Asp 761, Phe 812, Cys 813, Glu811, Asp 618, Ser 549, Ala550
21	Theaflavin 3,3'-digallate (TF3)	Trp 617, Lys551, Ser 549, Asp 623, Arg 836, Ser 814, Glu811, Phe 812, Cys 813, Asp 761, Asp 618, Ser 759, Tyr 619, Cys622, Arg 553, Lys 621, Asp 760
22	Hesperidin	Tyr619, Asp 618, Lys 798, Ser 795, Met794, Pro 793, Asp 164, Val166, Pro 620, Lys621, Asp 623, Arg 555, Tyr455
23	Quercetagenin	Arg 553, Lys 545, Lys 621, Asp 623, Cys 622, Asp 760, Pro620, Tyr 619
24	Myricetin	Trp 617, Trp 800, Asp 760, Glu811, Lys 798, Asp 618, Tyr 619, Cys 622, Asp 761, Phe 812

incorporation of the hydroxyl (OH) group in the structure can play a crucial role in NSP-16 inhibition (Singh et al. 2022).

Similarly, Singh et.al. in their other in silico study reported Dicaffeoylquinic acid and Diacetylcurcumin as potential inhibitors of the Receptor Binding Domain (RBD)-S protein of the SARS-CoV-2 virus which is mainly involved in the fusion process with the ACE-2 receptor of the host cells. Dicaffeoylquinic acid is mainly involved in the amino acid interactions with the Lys417, Gln493, Tyr489, Phe456, Tyr473, and Glu484 of S-RBD. Inhibition of these residues prevents the entry of the virus into the host cell as these amino acid residues are involved in the interaction with the ACE-2 protein of the host cell. Here also, the hydroxyl group

(OH) played an important role in the binding interaction with the target protein (Singh et al. 2021a).

Bhardwaj et.al in their study reported the acridinedione analogs as potential SARS-CoV-2 main protease inhibitors (Mpro or 3CLpro). In their study, they found these analogues better than saquinavir, which is a well-known antiviral drug. This study proved the importance of the carbonyl group (C=O) which can play a crucial role in the designing of 3CLpro inhibitors (Bhardwaj et al. 2021b).

Among all the molecular targets available for the inhibition of the SARS-CoV-2 virus, the RNA-Dependent RNA Polymerase (RdRp) has evolved as an important and crucial target for the treatment of COVID-19. Drugs like remdesivir, favipiravir and molnupiravir were used against these targets,

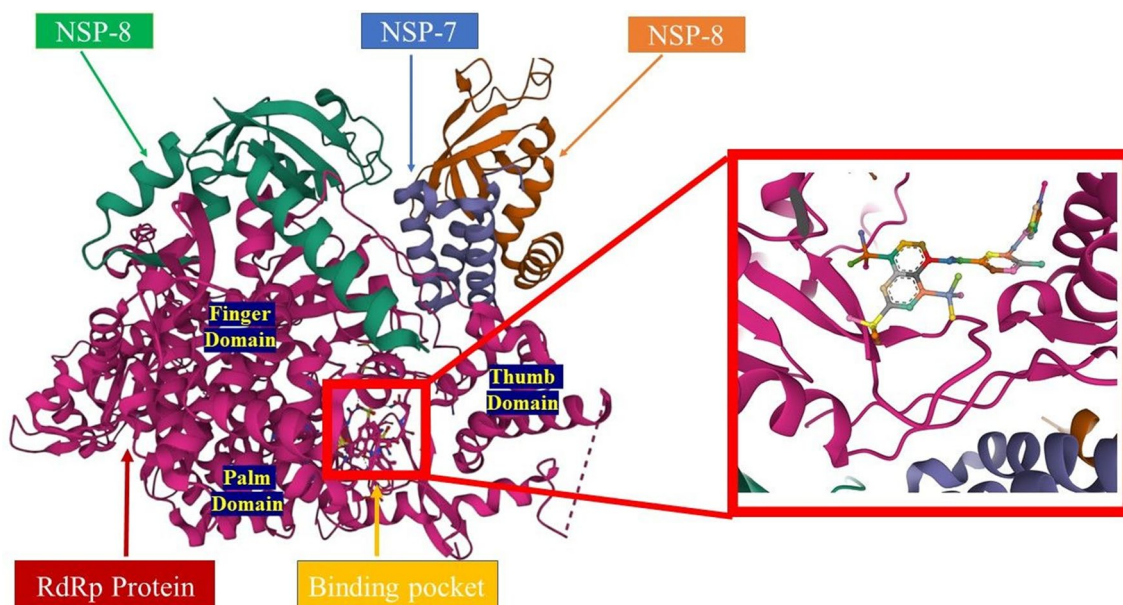


Fig. 5 Schematic representation of RdRp protein (PDB ID:7D4F) with its important structural domains, where orange and green color helix represent the NSP8 protein, violet-red color helix represents RdRp protein and purple color helix represents NSP7 protein

and they have shown some promising outcomes during this pandemic.

Remdesivir is one of the broad-spectrum antivirals, invented in the year 2014–2016 used for the treatment of the Ebola pandemic spread in West Africa. It consisted of

the pyrrolo-triazine, a carboxylic ester, a nitrile scaffold, a phosphoramidate ester, and aromatic amine which is considered as a prodrug. Further, this prodrug is converted into the active metabolite GS-441524 which acts as an ATP competitive inhibitor. The research study carried out by Nguyen et.al

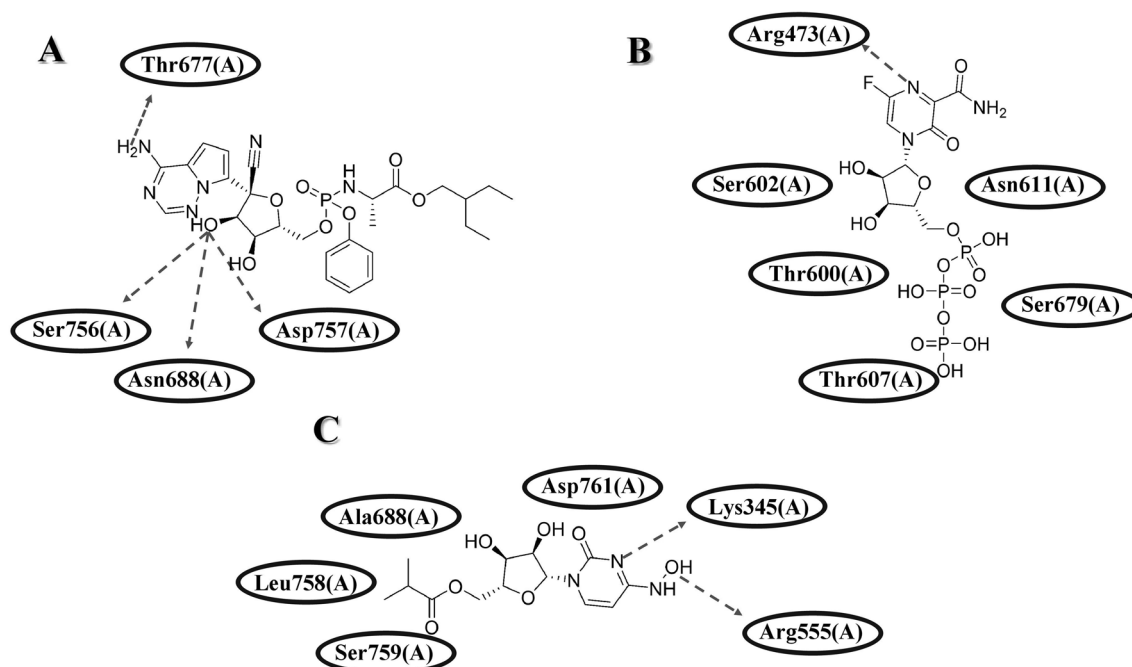


Fig. 6 Binding interaction of **A** remdesivir with the RdRp protein (Khater et al. 2021) **B** favipiravir with the RdRp protein (Sada et al. 2020) and **C** molnupiravir with the RdRp protein (Nimgampalle et al. 2021)

of the remdesivir–RdRp complex indicated that $-NH_2$ of pyrrolo-triazine of remdesivir active metabolite is an essential part of the binding interactions with the crucial amino acid of A chain which includes Thr677 as shown in Fig. 6A. The hydroxyl (OH) group of the furan ring of the remdesivir indicated the binding interactions with the Asp757, Asn688, and Ser756 as Fig. 6A (Nguyen et al. 2020; Khan et al. 2021).

Similar to remdesivir, favipiravir was discovered for the treatment of the influenza virus as a prodrug. It consists of pyrazine carboximide as a core scaffold which is converted into triphosphate which is an active form of the drug that consequently exerts its biological effect against the RdRp. As per the study conducted by Sada et al., after the conversion into triphosphate, the phosphate group (P=O) plays an important role in the binding interactions with the crucial amino acids of the RdRp protein which includes binding interactions with the Ser602, Thr600, Thr607, Asn611, Ser679, and Arg 473 as shown in Fig. 6B (Sada et al. 2020).

Molnupiravir is another category of prodrug which exerts RdRp inhibitory activity after the conversion into triphosphate. It consists of a pyrimidine scaffold where the hydroxyl group showed important binding interaction with the Arg555 and Lys545 as shown in Fig. 6C (Wang et al. 2021) and is one of the important parameter for the inhibition of the RdRp. In addition to this, the nitrogen of the pyrimidine ring indicated the important binding interaction with the target protein.

Overall, the summary of all the scientific studies on RdRp inhibitors, to date, indicated the importance of the triazine and pyrimidine scaffolds as shown in Fig. 7A–F. The important features of these drugs include the electronegative

nitrogen atom of the aromatic ring system. In addition to this, free amine and hydroxyl groups also play an important role in the binding process with the RdRp protein.

In line with these facts, we have proposed the designing strategy as shown in Fig. 7A–F for the novel RdRp inhibitors which can be used against the SARS-CoV-2. Many docking-based analysis of the remdesivir–RdRp complex suggested that free amine group of triazine–pyrrole complex is surrounded by the charged and polar amino acids as shown in Fig. 6A. This computational model indicated the importance of polar functional groups at this position of the scaffold as shown in Fig. 7A. Therefore, this position of the scaffold can be explored for substitution with the various electronegative atoms and hydrogen bond donors groups as shown in Fig. 7D. Similarly, remdesivir acts as a prodrug which get converted to monophosphate and then consequently exerts its effect against the RdRp. This conversion takes place at the pyrrole ring of the remdesivir. Therefore, this position is favorable for the electronegative atoms and the polar functional groups as shown in Fig. 7D. If the intention is to design the prodrug, then this pyrrole ring should not be modified as monophosphate attaches itself to the pyrrole ring of the remdesivir and get converted into active metabolite.

Similar to the remdesivir, previous research related to the favipiravir–RdRp complex indicated that the amide functional group of the favipiravir is surrounded by the polar and charged amino acids as shown in Fig. 6B. Therefore, this position of the pyrimidine scaffold of the favipiravir is favorable to the polar functional groups as shown in Fig. 7B and E. Further to this, $-NH$ of the pyrimidine scaffold as shown in Fig. 7E is important for the conversion of the favipiravir into the favipiravir phosphate which is an active form

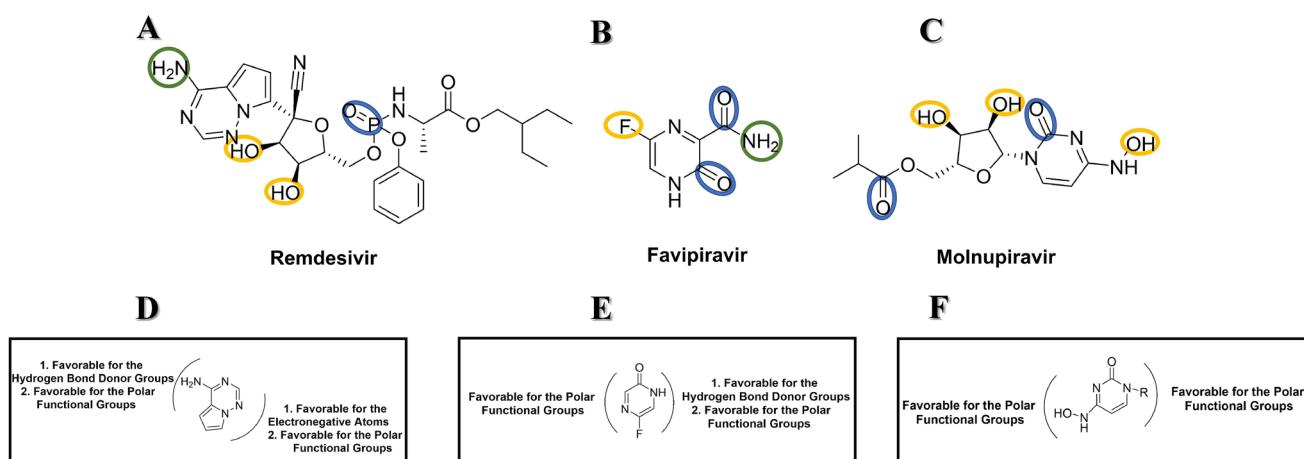


Fig. 7 **A** Amino, hydroxyl, phosphate and carbonyl groups of pyrrolo-triazine of remdesivir. **B** Amino, fluorine and carbonyl groups of pyrimidine scaffold of favipiravir and **C** amino, hydroxyl and carbonyl groups of pyrimidine scaffold of molnupiravir are essential part biological activity against the RdRp protein. **D** Essential features of

the pyrrolo-triazine scaffold of remdesivir required for the binding affinity with the RdRp protein. **E** Essential features of the pyrimidine scaffold of favipiravir required for the binding affinity with the RdRp protein. **F** Essential features of the pyrimidine scaffold of molnupiravir required for the binding affinity with the RdRp protein

of the drug. This position of the scaffold is favorable for the polar functional group as the previous research findings revealed that this position is surrounded by the polar and charged amino acids.

Recently, new drug molecule like molnupiravir showed some excellent results in the clinical trials. It also consists of the pyrimidine scaffold as shown in Fig. 7C and F. Previous computational model of this molecule in complex with the RdRp as shown in Fig. 6C indicated that it is also surrounded by the polar and charged amino acids. Hence, the modification with the polar functional groups on the pyrimidine scaffold may give favorable results against the RdRp protein.

In a nutshell, our hypothesis suggested that these scaffolds along with the required substitutions can be explored for the development of novel RdRp inhibitors which can be helpful in the treatment of COVID-19.

Evaluations of hypothesis

Overall, in this work, we enumerated the important targets of SARS-CoV-2 along with their inhibitors. Additionally, we proposed important chemical scaffolds and groups which are responsible for SARS-CoV-2 inhibition. Among all the molecular targets, RdRp is one of the choices for the development of the new lead compound which can be used for the treatment of COVID-19. Hence, more emphasis was given on the RdRp protein. In lieu of this, to prove the importance of our suggested hypothesis, we have designed a few

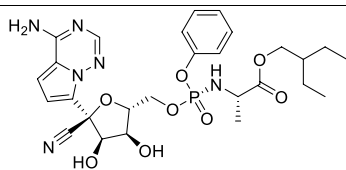
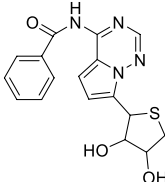
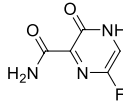
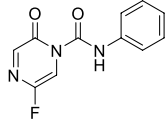
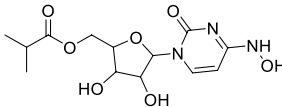
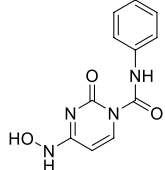
molecules against the RdRp protein and their molecular docking and dynamics study was performed.

Design and molecular docking study

As suggested in the hypothesis, triazine and pyrimidine scaffolds were found to be promising scaffolds for the designing of RdRp inhibitors. In line with this, based upon the available data and a proposed hypothesis, the modification was carried out in the triazine and pyrimidine scaffolds of remdesivir, favipiravir, and molnupiravir, which resulted in the Designed Molecule-1 (DM-1), Designed Molecule-2 (DM-2), Designed Molecule-3 (DM-3), respectively, as shown in Table 4. While designing the molecules against the RdRp protein, modifications carried out at the free amine groups of the standard drugs showed that it may be beneficial for the reduction in the toxicity which may occur due to the free amine groups.

Further docking study of these designed molecules along with the standard drugs which include remdesivir, favipiravir, and molnupiravir was carried out with the aid of AutoDock Vina as shown in Fig. 8A–F. All the standard drugs and designed molecules were converted into the pdbqt file with the aid of Open Babel software. RdRp protein (PDB ID: 7D4F) was taken from the protein data bank. Further, the protein was prepared by adding polar hydrogens and Kollman Charges. A Grid box was generated surrounding the binding pocket of the RdRp protein and converted into the pdbqt file. After, the completion of the molecular docking study, the docking interactions were analyzed with the

Table 4 Designed molecules and their binding affinity with the RdRp protein

Standard Drugs	Binding affinity (Kcal/Mol)	Designed Molecules as Per the Hypothesis	Binding affinity (Kcal/Mol)
 Remdesivir	-7.8	 DM-1	-7.9
 Favipiravir	-5.8	 DM-2	-7.1
 Molnupiravir	-6.7	 DM-3	-7.8

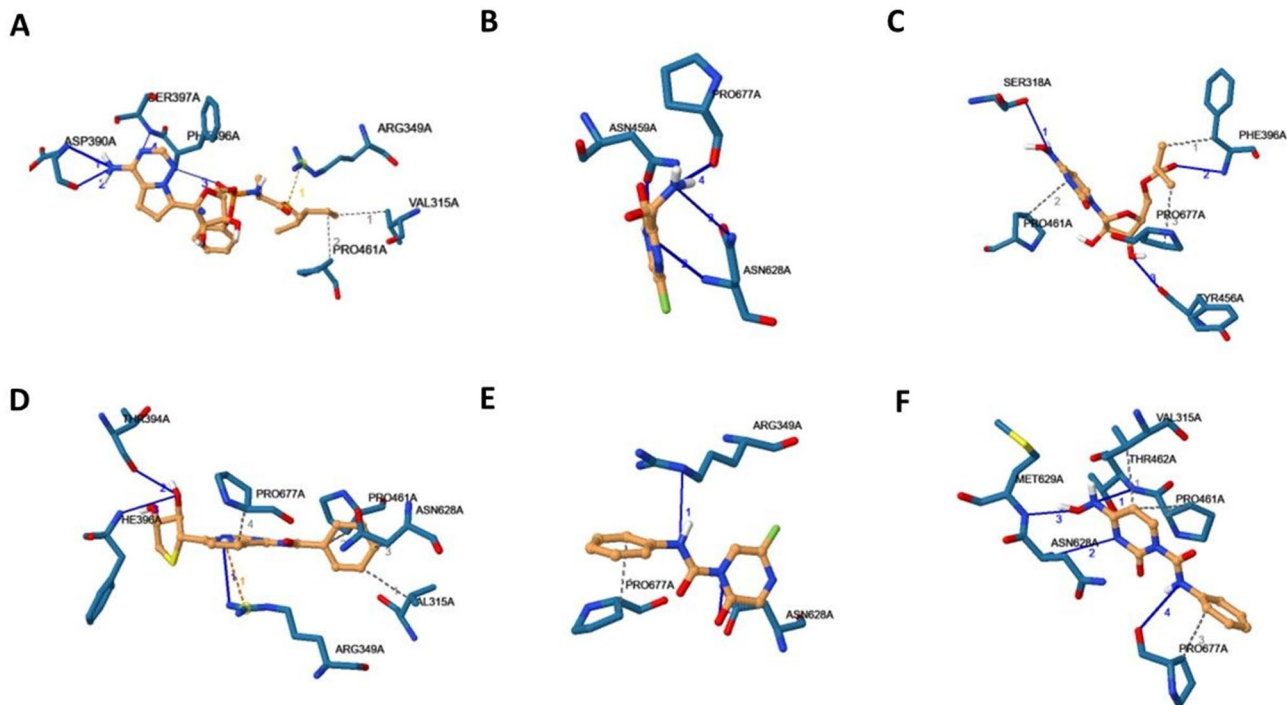


Fig. 8 Molecular Docking Study was carried out against the RdRp Protein (PDB ID:7D4F) for the evaluation of the hypothesis, where the designed molecules showed similar binding interactions as that of the standard drug molnupiravir. **A** Remdesivir showed hydrogen bond interactions (solid blue color lines), hydrophobic interactions (green color dotted lines), salt bridge (yellow color dotted line) with the RdRp protein. **B** Favipiravir showed hydrogen bond interactions (solid blue color lines) with the RdRp protein. **C** Molnupiravir showed hydrogen bond interactions (solid blue color lines) and

hydrophobic interactions (green color dotted lines) with the RdRp protein. **D** DM-1 showed hydrogen bond interactions (solid blue color lines), π -cation interaction (orange color dotted lines) and hydrophobic interactions (green color dotted lines) with the RdRp protein. **E** DM-2 showed hydrogen bond interactions (solid blue color lines) and hydrophobic interactions (green color dotted lines) with the RdRp protein. **F** DM-3 showed hydrogen bond interactions (solid blue color lines) and hydrophobic interactions (green color dotted lines) with the RdRp protein

help of Pymol and a web-based protein–ligand interaction profiler.

The DM-1 designed from the triazine scaffold of the remdesivir showed a similar docking score of -7.9 kcal/Mol compared to the remdesivir docking score of -7.8 kcal/Mol. Also, DM-1 showed binding interactions with the Pro461A and Val315A which is similar to remdesivir. It validates the effectiveness of the designed molecule against the RdRp protein.

The DM-2 which is designed from the pyrimidine scaffold of the favipiravir showed an excellent docking score of -7.1 kcal/Mol compared to the favipiravir docking score of -5.8 kcal/Mol. Also, this DM-2 indicated the binding interaction with the Pro677A and Asn628A which is similar to the binding interactions of the favipiravir that validates the proper binding orientation of the DM-2.

The DM-3 is also designed from the pyrimidine scaffold of molnupiravir indicated a docking score of -7.1 kcal/Mol which is good as compared to the molnupiravir which showed a docking score of -6.7 kcal/Mol. Further, DM-3 showed binding interactions with the Pro461A and Pro677A

similar to molnupiravir which validated the potency of the DM-3.

Molecular dynamics simulation study

Further, for the support of the docking analysis, an MD simulation study was performed with the aid of the DESMOND module of DE Shaw Research. Solvation of the docked complex was carried out with the help implicit solvent model in a cubic box of 10 \AA spacing. The neutralization of the solvated system was carried out with the help of the counter ions. The salt concentration was maintained at 0.15 M . All the systems were set up for 50 ns at 300 K temperature and 1.013 atmospheric pressure (Pandey et al. 2021). As DM-1 showed good binding affinity, i.e., -7.9 kcal/mol as compared to the standard drug, it was selected for this analysis. DM-1 which is present in the binding cavity of the RdRp protein taken for the MD simulation study for 50 ns is shown in Fig. 9A. After the completion of the MD simulation study, the RMSD value protein–ligand complex revealed that both the protein and ligand achieved their equilibrium at 10 ns

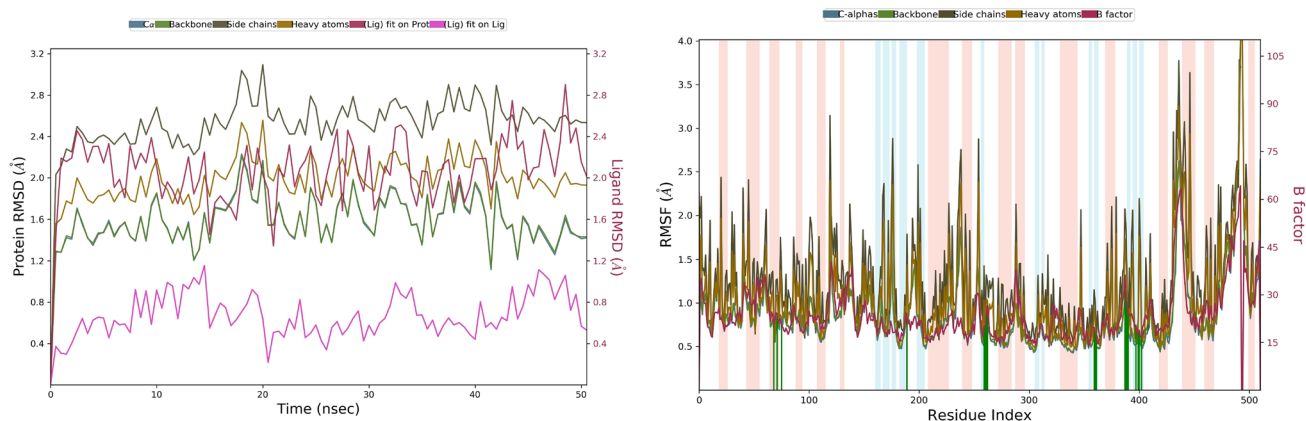


Fig. 9 **A** RMSD plot of RdRp protein with the Lead compound (DM-1) during 50 ns MD simulation time. **B** RMSF profiles of RdRp protein with the Lead compound (DM-1) during 50 ns MD simulation

and remain stabilized for the remaining 40 ns as shown in Fig. 9A. The RMSD value for the receptor remains within the 3.2 Å. Interestingly, the lower RMSD value of docked molecule (DM-1) within the 1.2 Å shown in pink color in Fig. 9A validates the stability of the complex and proved that the docked ligand does not diffuse away from its actual binding pocket.

Further, for accessing the local changes in the protein chains during the MD simulation study, root mean square fluctuation (RMSF) was determined as shown in Fig. 9B. It

indicated that no major conformational changes occurred in the RdRp protein. After docking, the RMSF value remained within the 2 Å indicated in green bars as shown in Fig. 9B. It validated the hypothesis proving that the docked complex of RdRp protein remained more stable without major fluctuation.

Similar to the protein, ligand RMSD value as shown in Fig. 10A remains within the 1.2 Å indicating the conformation of the docked ligand (DM-1) does not change so much with reference to the parent conformation of the ligand

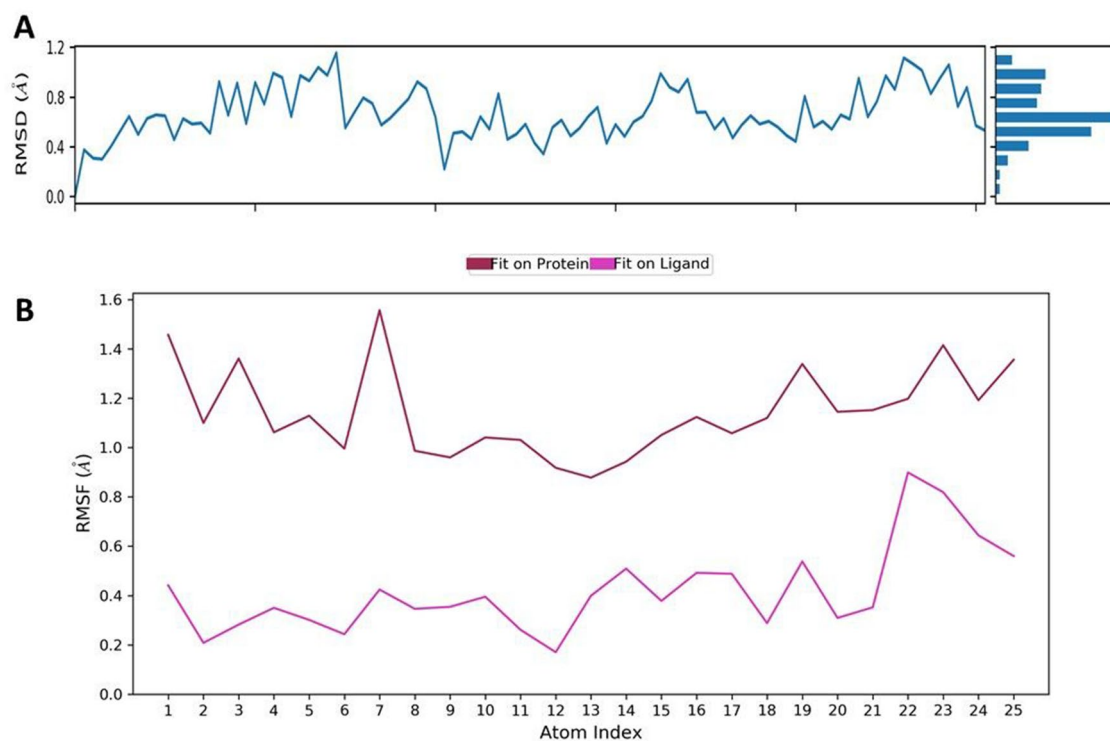


Fig. 10 **A** RMSD and **B** RMSF plot of the docked ligand DM-1

(DM-2). Similar, to the RMSD, the RMSF value of DM-1 lies within the 1.5 Å as shown in Fig. 10B which proved that each atom of the ligand does not deviate so much from its original position. The ligand torsion profile during the simulation is shown in Fig. 11A–F which consists of the radial plots which indicate the flexible position of the docked ligand (DM-1) as shown in Fig. 11G. This flexible point of a ligand can further be considered for the modifications to get the novel RdRp inhibitors. In the case of DM-1, dots are more scattered in Figs. 11C and E. Figure 11C corresponds to the 5th position of the triazine scaffold as shown in Fig. 11G with the same color coding indicating that this position is flexible for further modification to get the best binding fit in the RdRp protein. Figure 11E corresponds to the hydroxyl group of the tetrahydrothiophene ring shown in Fig. 11G with the same color coding. This position can be modified further to get the best binding mode of the ligand in the binding pocket of the RdRp protein.

Conclusion

In this work, efforts were made to explore the available treatments and protective vaccines for COVID-19. Overall, the current scenario indicated that the available vaccines played an important role in slowing down the pandemic

in the world, although certain mutations in the virus are creating hindrances in the vaccine effectiveness. The rise of different strains across the world is creating challenges for the scientific community. Based on the previously published literature, it is well understood that the RdRp is one of the important molecular targets for drug development against SARS-CoV-2. All the published data to date related to the RdRp protein revealed some key amino acids of the binding pocket of the RdRp which consisted a combination of polar amino acids along with some hydrophobic amino acids. Based on this, we have proposed various hypotheses for designing novel potent RdRp inhibitors which will be beneficial for the treatment of SARS-CoV-2. All the given hypotheses were evaluated by the molecular docking-assisted molecular dynamics simulation study.

Overall, as per the evaluation of the hypothesis, designed analogues showed a good binding affinity with the target protein RdRp compared to the standard drugs which validate the accuracy of the proposed hypothesis. Among all the designed molecules, DM-1 showed promising results in the MD simulation-assisted molecular docking study against the RdRp protein. In the future, this lead compound can be explored for the inhibition of the RdRp protein which will be beneficial for the treatment of SARS-CoV-2.

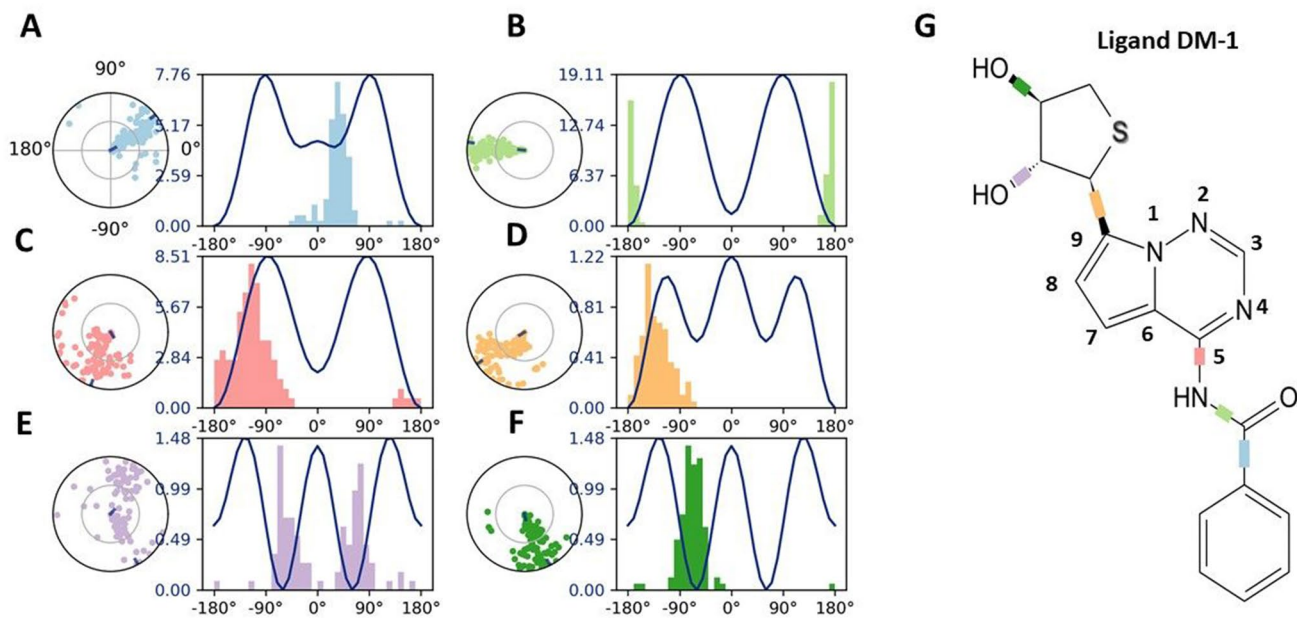


Fig. 11 Ligand torsion profile of compound DM-1, where **A** radial plot corresponds to the carbonyl group of the phenyl ring of DM-1 indicated in the cyan color. **B** Radial plot corresponds to the (-CONH) group of DM-1 indicated in light green color. **C** Radial plot corresponds to the 5th position of the triazine scaffold indicated in the peach color. **D** Radial plot corresponds to the 9th position of

the triazine scaffold indicated in orange color. **E** Radial plot corresponds to -OH group of the tetrahydrothiophene of the DM-1 ligand indicated in the purple color. **F** Radial plot corresponds to -OH group of the tetrahydrothiophene of the DM-1 ligand indicated in the dark green color. **G** Docked ligand (DM-1) with different color codes corresponds to the radial plots

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Availability of data and material (Code availability) All data analyzed during this study are included in this published article. Also, the data analyzed during the current study are available from the corresponding author on reasonable request as per the journal guidelines.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent statement/ethical approval Not required.

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