J Biochem Technol (2023) 14(2): 66-74
https://doi.org/10.51847/Lp8N72wFgI
ISSN: 0974-2328

Recent Advances in the Development of Novel Inhibitors Targeting RNA Helicase Enzyme of SARS-CoV-2

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Received: 28 January 2023 / Received in revised form: 30 May 2023, Accepted: 04 June 2023, Published online: 20 June 2023

Abstract

The rapid spread and infection rate of novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) has created a worldwide pandemic since its origin in 2019. Another concern for improvement in controlling the infection is the non-availability of effective medications against the virus. So, these challenges generate the general scientific interest to focus on the development of novel drug molecules that can prevent viral propagation. The virus contains an RNA helicase enzyme known as nonstructural protein 13 (nsp 13), a critical viral replication regulator. Hence this enzyme can be used as a target so that the potential inhibitor molecules can be used to stop its function. Furthermore, the virus is related to other members of the Coronaviridae family, for which inhibitor molecules are available. Recently, the crystallographic structure of the nsp13 of SARS-CoV-2 has been resolved and is available in the protein data bank (PDB). Hence, this information provides the opportunity to apply several computational and experimental approaches to elucidate the enzyme's functional aspects and help to propose new inhibitor molecules. Several natural products, synthetic compounds, and previously proven effective compounds have been studied for their binding affinity and inhibition properties of the molecule. This review presents the basic idea about the genomic arrangement and structural and functional aspects of the RNA helicase enzyme of SARS-CoV-2. Also, the inhibition strategies of the enzyme by the inhibitor molecules along with challenges have been highlighted by narrating the recent literature.

Keywords: RNA helicase, Nonstructural protein 13, Novel inhibitor molecules, SARS-CoV-2, Drug repurposing, Drug target

Introduction

RNA viruses are usually more common and complex pathogens in terms of their genetic makeup, mutation frequency, and the modes of transmission to various host bodies to cause disease. Currently, there are about 180 species of RNA viruses have been recognized that can infect human beings (Andrei *et al*., 2015). RNA viruses have the potential to cross the species barrier between humans and other (animal and bird) hosts and are ultimately responsible for creating public health concerns (King *et al*., 2006; Woolhouse *et al*., 2013; Woolhouse & Adair, 2013). A recent challenge exists for the development of new therapeutics due to the epidemic outbreak of the novel coronavirus (SARS-CoV-2). The disease caused by the novel coronavirus is a highly contagious and infectious one, and it was first reported in Wuhan city of China, in December 2019 and further spread worldwide. The virus is closely related to the other members of the *coronaviridae* family, such as the severe acute respiratory syndrome (SARS) virus and middle-eastern respiratory syndrome (MERS) spotted by the genomic analysis (Tudoran *et al*., 2022). Presently, there is no effective mode of a drug therapy approach that is successful for treating the novel coronavirus virus. Several successful research tests (clinical trials) for the SARS-CoV-2 therapy are currently undergoing. Other emerging methods, such as repurposing the effective studied drugs, have been found as suitable alternatives to discover novel potential inhibitors against the virus (Omolo *et al*., 2020; Wu *et al*., 2020). The wild type of genomic constituent of the SARS-CoV-2 is positive-stranded RNA and contains 29903 base pairs **(Figure 1)**.

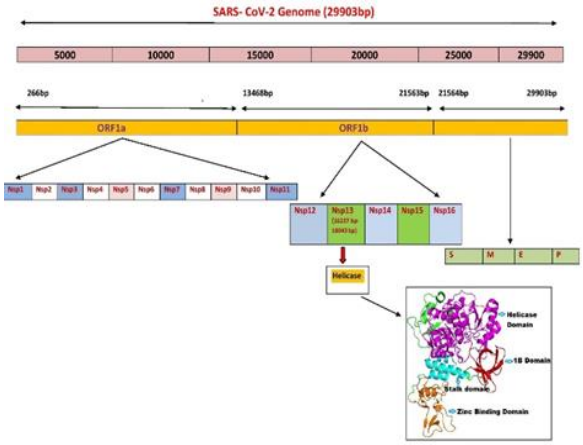


Figure 1. Genomic structure of SARS -CoV-2 highlighting the Helicase enzymes.

The viral genome contains two major open reading frames (ORF 1a and ORF 1b) from which the 16 numbers of nonstructural

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proteins were produced and the regions in which the four numbers of structural proteins are produced (https://www.ncbi.nlm.nih.gov/sars-cov-2/). The major functions of the structural proteins are to facilitate the virus's entry into the host cell. The nonstructural proteins play a significant role in viral molecular processes such as replication, transcription, and assembly (Singh *et al*., 2021; Yadav *et al*., 2021). Among different types of nonstructural proteins, the nonstructural protein 13 is also known as (RNA) Helicases **(Figure 1)**. This helicase's functional importance is that it acts as the motor protein that helps in the winding and unwinding activity of the RNA genome in the replication process by consuming adenosine triphosphate (ATP). Due to its vital role in replication, the enzyme is considered an important drug target for developing new therapeutic molecules to combat novel coronavirus infections (Mahajan & Marcus, 2021). Another essential nature of the enzyme is it is very much conserved and subjected to little mutational effect; hence can be considered for the development of stable inhibitor molecules. Developing novel therapeutic drugs has been extensively studied for other viruses (Ghosh & Basu, 2008; Steimer & Klostermeier, 2012; Kim *et al*., 2021). The therapeutic approaches have been developed for several viral pathogens such as Dengue, West Nile, and Japanese encephalitis virus, including SARS-CoV (severe acute respiratory syndrome-Coronavirus), including SARS-CoV-2 by considering the viral RNA helicases as a suitable target (Crumpacker & Schaffer, 2002; Kleymann *et al*., 2002; Frick, 2003). The review aims to highlight the structural and functional aspects of the SARS-CoV-2 viral helicase enzyme and its inhibitors by reviewing the published literature.

Structure and Function of NSP 13 Helicase Enzyme

Nsp13 of SARS CoV-2 is a dimeric and multifunctional protein and is categorized as a superfamily 1 type of helicase. The enzyme generally contains an N-terminal metal-binding domain known as Zn binding domain (ZBD) and a conserved helicase domain in the C-terminal region. The molecular weight of the nsp13 protein of SARS-CoV-2 is 66.85 KDa and has a chain length of 601 amino acids. The domain structure is shown in **Figure 1**, and functional aspects are presented in **Table 1**.

Table 1. Functional domains of SARS -CoV-2 helicase enzyme

S. N	Functional domain	Function
1	ZINC Binding domain (ZBD)	Interaction with other nonstructural proteins like nsp 12 and nsp 8
2	Stalk domain	Forms a rigid and direct connection between the ZBD and helicase domain and is essential for the unwinding of double-stranded RNA
3	1B domain	Binds to the 3′ ends of the single-stranded RNA and remains attached to the Stalk domain
4	Helicase domain (Rec 1A and Rec 2A)	Rec1A: Provides the surface to the RNA-binding tunnel of helicase enzyme by the formation of hydrogen-bond with the ribose sugar and bases. Rec2A: Dynamics of the Rec2A from the stalk domain enhance the space, which facilitates the accommodation of binding of the 5' end of the single-stranded RNA

Nsp13 is strongly conserved among SARS -Co V2 and consists of five domains that fold in a triangular pyramid shape (Hao *et al*., 2017; Chen *et al*., 2020; Littler *et al*., 2020; Kangarshahi *et al*., 2021; Mickolajczyk *et al*., 2021). The first experimental SARS-CoV-2 helicase structure was solved by Newman *et al*., with 1.94 Angstrom resolution and available in the protein databank (PDB ID:6ZSL). It showed almost identical to the SARS-CoV helicase structure (PDB ID: 6JYT), however, both the dimeric structures differ significantly in the protein-protein interactions among their chains, represented in **Figure 2** (Jia *et al*., 2019; Newman *et al*., 2021). The availability of this structural information creates the opportunity to develop structure-based drug design by searching for the potential compounds against the enzyme (Davidescu *et al*., 2022). Additionally, the information about the conformational change of the domains and their precise role upon binding of the substrate with the helicase enzyme has been established **(Table 2)**. The viral replication mechanism of SARS-CoV-2 has been similar in the case of SARS-CoV and other related viral species (Khosla *et al*., 2021). For this reason, nsp13 of SARS-CoV-2 has been suggested as a preferred target for developing new antiviral drugs (Berta *et al*., 2021).

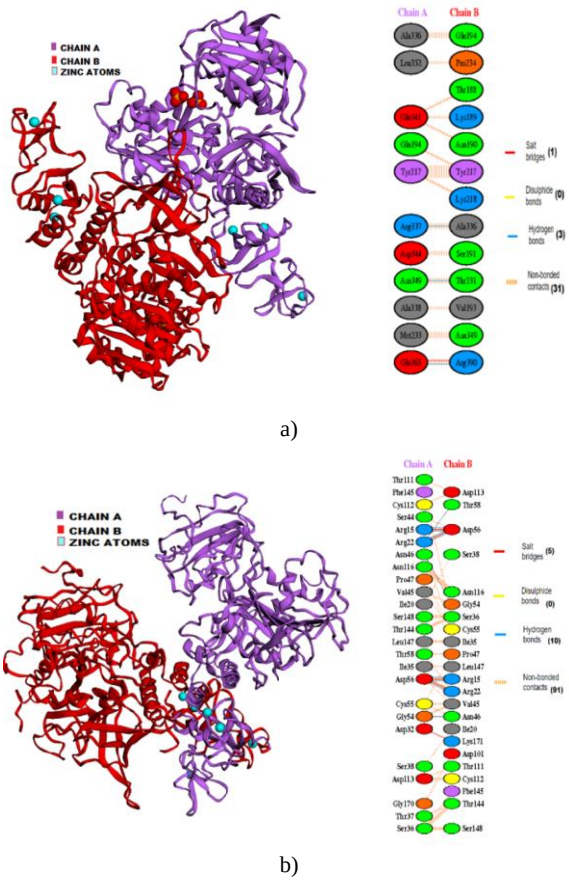


Figure 2. 3D structure of helicase enzyme. a) SARS -CoV-2 (PDB ID: 6ZSL) and b) SARS -CoV (PDB ID: 6JYT).

Dimeric interaction of the proteins has been computed by the PDBSUM server available at www. https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum

Table 2. Effective drug molecules against pathogenic viruses other than SARS-CoV-2

S. N	Target virus (Other than SARS-COV-2	Name of the compound	References
1	West Nile virus (WNV)	Aminophenylbenzimidazole	(Borowski *et al*., 2003)
		Benzothiazole	(Ujjinamatada *et al*., 2005)
		Manoalide	
		Tetrachlorobenzotriazole (TCBT)	(Frick & Lam, 2006)
2	Human papillomavirus (HPV)	Doxycycline	(Ko *et al*., 2014)
3	Hepatitis C virus (HCV)	Epigallocatechin-3-Gallate (EGCG)	(Li *et al*., 2013)
		Fpa-124	(Shadrick *et al*., 2013)
		Myricetin	(Frick, 2007)
		Pyrrolone	(Borowski *et al*., 2000)
		Quercetin	(Maga *et al*., 2005)
		Scutellarein	
		Trifluoperazine	
4	Severe acute respiratory syndrome coronavirus (SARS -CoV)	Ivermectin	(Khater & Das, 2020)
5	Human immunodeficiency virus (*HIV*)	Micafungin (MCFG)	(Yedavalli *et al*., 2008)
6	Dengue virus (*DENV*)	Suramin	(Basavannacharya & Vasudevan, 2014)
		Tetrabromobenzotriazole (TBBT)	(Briguglio *et al*., 2011)
		Tropolones	(Mastrangelo *et al*., 2012)
7	Herpes simplex virus (HSV)	Pritelivir	(Uhlig *et al*., 2021)

Discovery of Drug Molecules Against SARS-CoV-2 Helicase

Recently, attempts have been made to discover and develop novel potential antiviral agents by taking the SARS -CoV-2 helicase enzymes as the target. However, a more in-depth study related to helicase structure is needed to discover specific drug molecules with enhanced binding activities. The drug-binding sites of the helicase enzymes have been identified, and this can be taken as the strategy to develop the inhibitor against the Sar -CoV-2 **(Figure 3)**. Some of the compounds like tenofovir, disoproxil, and lamivudine have been predicted as effective replication inhibitors for the treatment of SARS-COV-2 (Das *et al*., 2020; Pandey *et al*., 2020; Wondmkun & Mohammed, 2020).

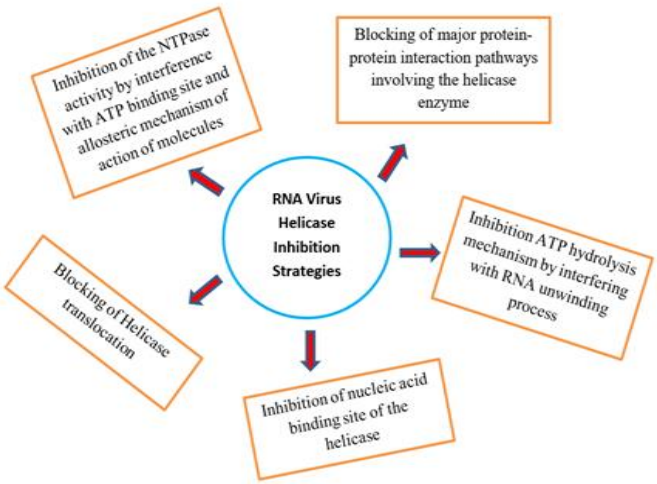


Figure 3. Strategies for inhibition of the viral RNA helicase enzyme by using potential drug molecules

To develop the possible inhibitors against SARS-CoV-2 RNA helicase, it is essential to know its conserveness among other related virus. Several researchers have conducted research to identify the potential inhibitor molecules against other pathogenic viruses **(Table 2)**. Since most of the RNA helicases are conserved in nature, already available effective drug molecules can be suitable for repurposing against the SARS -CoV-2 nsp 13 target (Ma *et al*., 2022). Several published articles are also available that focus on the essential function of the RNA helicase in viral genome replication. also, the interaction of nsp13 with other nsps is responsible for the signaling pathway for the replication and RNA synthesis initiation (Appelberg *et al*., 2020; Xia *et al*., 2020; Yan *et al*., 2020; Yuen *et al*., 2020). Researchers have performed experimental and computational approaches to predict the effective SARS -CoV-2 helicase inhibitors. Mirza and Froeyen 2020 analyzed the structural aspects of nbsp 13. They used computational techniques such as screening, pharmacological property evaluation, and molecular dynamics simulation to evaluate the effective compounds against the nbsp 13 of SARS-CoV-2 (Mirza & Froeyen, 2020). Satpathy, in 2020, studied the effectiveness of the drug remdesivir molecule as a potential nsp13 inhibitor by using molecular docking methods (Satpathy, 2020). Iftikhar *et al*. analyzed the key binding sites of the nsp13 helicase of SARS-CoV-2 with nucleotide. Further molecular modeling techniques such as 3D structure prediction and molecular docking showed that the anti-parasitic drug molecule such as *meclonazepam* and *oxiphenisatin* could bind to the key residues, hence can interfere with RNA helicase enzyme activity (Iftikhar *et al*., 2020). White *et al*., in 2020, screened about 970,000 chemical compounds based on their binding to the ATP-binding site of the SARS-CoV-2 helicase enzyme by using computational methods. A molecular docking study revealed that the compounds likeCepharanthine, Cefoperazone, Dihydroergotamine, Cefpiramide, Ergoloid, Dihydroergocristine, Ergotamine, Netupitant, Dpnh, Lifitegrast, Nilotinib, and Tubocurarin show the effective ATP site binding activity (White *et al*., 2020). Shu *et al.* analyzed that the Bismuth salt can be considered the key inhibitory agent of the NTPase and helicase activities of the SARS-CoV-2 helicase enzyme (Shu *et al*., 2020). El-Sayed *et al.* repurposed the effective drugs of SARS-CoV, such as Ledipasvir and Galidesivir could be effective against the helicase of novel coronavirus disease 2019 (El-Sayed *et al*., 2021). Wu *et al*. in 2021 used computational screening methods to predict the effectiveness of the drugs like lymecycline, itraconazole, saquinavir, dabigatran, and carnosic acid as potential helicase inhibitors (Wu *et al*., 2020). Yuan *et al.* investigated the inhibition of the unwinding effect of SARS-CoV-2 helicase (nsp13) by using the drug molecule clofazimine during the replication process (Yuan *et al*., 2021). Nandi *et al*. screened the effect of a series of nucleoside analogs against the SARS-CoV-2 helicase protein using molecular docking and molecular dynamics simulation methods. After the selection of the best inhibitor, further, the pharmacokinetic study revealed that the molecules cordycepin and pritelivir show a good inhibitory effect against the nsp13 helicase protein (Nandi *et al*., 2022). The computational analysis was performed by Hosseini *et al*. to investigate the multiple targeting of drug molecules against the SARS-CoV-2 proteins. A molecular docking study established that the protease inhibitor molecule natamycin is also a potential SARS-CoV-2 helicase enzyme (Hosseini *et al*., 2021). Molecular docking simulation and molecular dynamics simulation study by Saidijam *et al*. predicted that the molecules such as Amentoflavone, theaflavin 3'-gallate, and procyanidin can be the potential SARS-CoV-2 helicase inhibitors (Saidijam *et al*., 2021). Spratt *et al*. suggested the use of some patented key drug molecules such as SSYA10-001, aryl keto acids, dihydroxy chromones, adamantane-derived bananas, natural flavonoids, acrylamide derivatives can be used as SARS-CoV-2 helicase inhibitors. This fact was supported by the computation of the IC50 value of these molecules obtained from the experimental SARS-CoV-2 helicase assay (Spratt *et al*., 2021). Zeng *et al*. screened a library of 5000 pharmaceutically important compounds for nsp13 inhibitors of the virus by using fluorescence resonance energy transfer (FRET) techniques. From the *in vitro* study, they have identified that molecules such as FPA-124 and several suramin-related compounds were able to decrease the growth of SARS-CoV-2 in Vero E6 cell culture (Zeng *et al*., 2021). Abidi *et al*. studied the possibility of repurposing the drug molecules such as posaconazole and grazoprevir as the key inhibitors of the helicase enzyme of SARS-CoV-2 (Abidi *et al*., 2021). Chen *et al*. used virtual screening methods and identified that some of the approved drugs, such as lymecycline, itraconazole, saquinavir, dabigatran, and carnosic acid could be used as helicase inhibitors of the SARS-CoV-2 helicase (Chen *et al*., 2021). Chen *et al*. studied the effect of the molecules such as disulfiram and ebselen were able to inhibit SARS-CoV-2 nsp13 ATPase activity (Chen *et al*., 2021). Perez-Lemus *et al*. suggested that the molecules like bananin, SSYA10-001, and chromone-4c can be used for blocking the ATPase activity of the nsp13 helicase after their binding (Perez-Lemus *et al*., 2022). Pharmacophore modeling and screening of the ZINC database by El Hassab *et al*. suggested that the best-hit compound FWM-1 is a potential nsp13 helicase inhibitor (El Hassab *et al*., 2022). Protein-ligand docking followed by interaction analysis of the complex and molecular dynamics simulation work by Vivek-Ananth *et al*. predicted that, that the phytochemicals such as Picrasidine M, Epiexcelsin, Isorhoeadine, Euphorbetin, and Picrasidine N can be used as effective SARS-CoV-2 helicase inhibitors (Vivek-Ananth *et al*., 2022). Recently, Raubenolt *et al*. studied the dynamics of the binding site of the nsp 1 protein structures by extensive molecular dynamics simulation analysis. In this research work, they identified that four numbers of potential drug-binding pockets are available between the 1A and 2A regions of the helicase domain. Also, they predicted three numbers of allosteric binding sites between the ZBD-stalk, stalk-1B, and 1A-2A domains, which was not previously reported in the case of helicase enzyme. Therefore, this information can be used further to discover novel inhibitor molecules targeting these locations (Raubenolt *et al*., 2022). Corona *et al.* implemented an integrated mode of study by using virtual screening and molecular dynamics simulation methods to identify the key binding sites. Further, the binding mode was predicted by determining the favorable molecular interaction of ligand molecules with the nsp 13 protein. In the study, it was identified that the molecules such as myricetin, quercetin, kaempferol, and flavanone are the potential SARS-CoV-2 inhibitor that inhibits the nsp13 enzyme by interfering with the unwinding activity by non-competitive inhibition activity. Also, the researchers reported that natural

compounds such as flavonoids could be used as selective inhibitors of SARS-CoV-2 nps13 helicase (Corona *et al.*, 2022).

Challenges and Future Aspects

Repurposing existing drug molecules against the SARS-CoV-2 helicase enzyme can be a crucial and rapid method to predict novel antiviral compounds against the virus. The current approach to the drug screening process against SARS-CoV-2 is mainly based on computer-aided drug design (CADD) or a few previously approved antiviral drugs against wild-type SARS-CoV-2. However, the methods may encounter several limitations. For example, the CADD method is primarily used to screen drug molecules for a single or multiple virus target without considering the whole life cycle of the virus. This process may result in missing a large number of potential drug molecules. Also, few compounds are known for which the clinical knowledge of inhibitory activity against SARS-CoV-2 nsp 13 is available (Pathak *et al*., 2021). Hence, a combined approach of high-throughput screening along with a suitable infection model is necessary to accelerate the drug discovery study against the SARS-CoV-2 helicase enzyme (Aljabali *et al*., 2022; Malone *et al*., 2022; Siminea *et al*., 2022).

However, the research opportunities and challenges in this aspect are presented below:

- Most of the predicted compounds are based on a computational approach, but the inhibitory concentration in the *in vitro* and *in vivo* assay methods shows a large variation in the concentration range.
- The pharmacokinetics data obtained from the compounds may not be correlated with the *in vivo* parameters.
- The recently reported structure of SARS-CoV-2 helicase can be studied for its association with other nsps like nsp12, nsp7, and nsp8. Also, the structural information from other CoVs can be thoroughly studied to discover the potential binding site related to function. So this will lead to the discovery of a novel target of the helicase enzyme for which the drug molecule can be developed (Dumitru *et al*., 2022).
- The role of dimerization of the helicase enzyme is less understood; more study regarding the structural approach is necessary to understand the function and dynamics.
- Although many effective drug molecules have been screened and predicted by several researchers, there is no such unique database of *Viral RNA helicase inhibitors* available to date. So, the information on the drug molecule, along with the other structural information for the scientific community, might be useful to repurpose novel compounds against any pathogenic virus.

Conclusion

The infection caused by SARS-CoV-2 has resulted in pandemic situations throughout the globe, hence a great concern for public health. However, no such proper therapeutic methods are available to fight against the virus. The continuous mutation in the genome is also another challenge to developing drugs against the virus. However, nsps like nbsp 13 are more conserved and play a crucial role in the viral replication cycle by combination with other nonstructural proteins. Hence, the enzyme can be used as an attractive drug target. The high-resolution crystallographic structure of nsp 13 helicase is recently available, providing the opportunity to develop suitable nsp 13 helicase inhibitors of SARS -CoV-2. Several researchers have predicted many of these potential compounds using computational and experimental methods. In this review, the structural and functional aspects of the nbsp 13 of SARS-CoV-2 have been presented. The potential compounds against the nsp 13 helicase enzyme of SARS-CoV-2 have been listed by reviewing the recent literature. Further, we present the challenges and research opportunities associated with discovering inhibitor molecules against nsp 13 of the virus. The information available in the manuscript may be used to carry out further research in this emerging area.

Acknowledgments: This research was supported by the OURIIP- SEED FUND grant, sponsored by Odisha state higher education Council, Government of Odisha, India (OURIIP Seed fund -2020/06-Biotechnology).

Conflict of interest: None

Financial support: Financial support by the OURIIP- SEED FUND grant, sponsored by Odisha state higher education Council, Government of Odisha, India (OURIIP Seed fund -2020/06-Biotechnology).

Ethics statement: None

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