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

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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 (Aljehany & Allily, 2022). 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 (Oran *et al*. 2021). Several successful research tests (clinical trials) for the SARS-CoV-2 therapy are currently undergoing (Tam *et al*., 2022). 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)**.

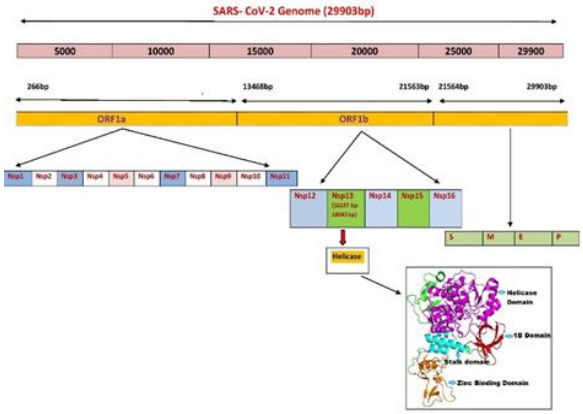

Figure 1. Genomic structure of SARS -CoV-2 highlighting the Helicase enzymes.

The viral genome contains two major open reading frames (ORF 1a and ORF 1b) from which the 16 numbers of nonstructural proteins were produced and the regions in which the four numbers of structural proteins are produced

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(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.

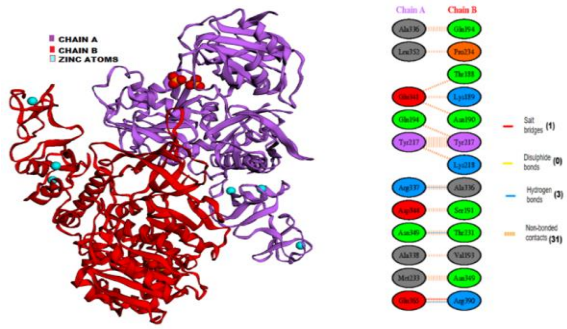
Structure and Function of NSP 13 Helicase Enzyme

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**.

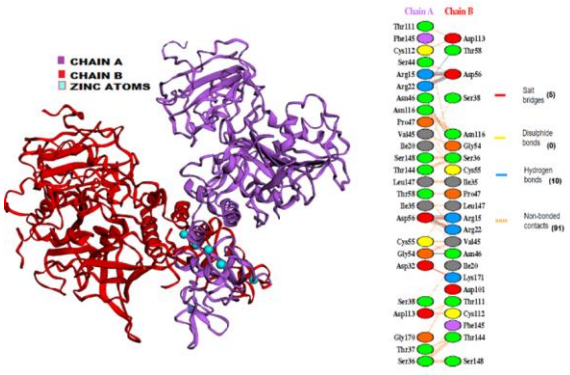
Table 1. Functional domains of SARS -CoV-2 helicase enzyme

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).



a)



b)

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).

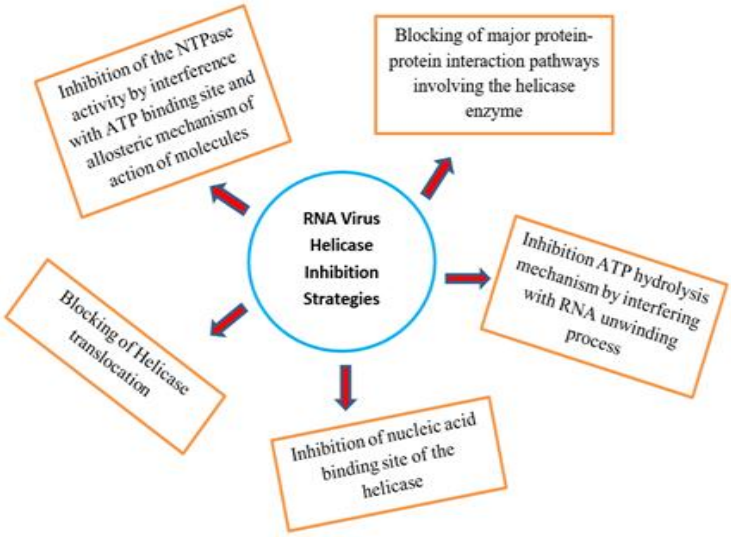


Figure 3. Strategies for inhibition of the viral RNA helicase enzyme by using potential drug molecules

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.

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References

Abidi, S. H., Almansour, N. M., Amerzhanov, D., Allemailem, K. S., Rafaqat, W., Ibrahim, M. A. A., la Fleur, P., Lukac, M., & Ali, S. (2021). Repurposing potential of posaconazole and grazoprevir as inhibitors of SARS-CoV-2 helicase. *Scientific Reports*, *11*(1), 10290. doi:10.1038/s41598-021-89724-0

Aljabali, A. A., Tambuwala, M. M., Barh, D., & Lundstrom, K. (2022). Therapeutic Challenges in COVID. In *COVID-19: From Bench to Bedside* (pp. 1-9). Boca Raton; CRC Press.

Aljehany, B. M., & Allily, R. K. (2022). Impact of Covid-19 Quarantine on Life Style Changes, In the Western Saudi Arabia: A Cross-Sectional Study. *Journal of Organizational Behavior Research*, *7*(1), 182-197.

Andrei, C. S. E. P., Vaida, L., Bungau, S., & Todor, B. I. (2015). Clinical and Biological Correlations in Toxoplasma gondii Infection in HIV Immune Suppressed Persons. *Iranian Journal of Public Health*, *44*(7), 1012.

Appelberg, S., Gupta, S., Svensson Akusjärvi, S., Ambikan, A. T., Mikaeloff, F., Saccon, E., Végvári, Á, Benfeitas, R., Sperk, M., Ståhlberg, M., et al. (2020). Dysregulation in Akt/mTOR/HIF-1 signaling identified by proteo-transcriptomics of SARS-CoV-2 infected cells. *Emerging Microbes and Infections*, *9*(1), 1748-1760. doi:10.1080/22221751.2020.1799723

Basavannacharya, C., & Vasudevan, S. G. (2014). Suramin inhibits the helicase activity of the NS3 protein of the dengue virus in a fluorescence-based high throughput assay format. *Biochemical and Biophysical Research*

Communications, *453*(3), 539-544. doi:10.1016/j.bbrc.2014.09.113

Berta, D., Badaoui, M., Martino, S. A., Buigues, P. J., Pisliakov, A. V., Elghobashi-Meinhardt, N., Wells, G., Harris, S. A., Frezza, E., & Rosta, E. (2021). Modeling the active SARS-CoV-2 helicase complex as a basis for structure-based inhibitor design. *Chemical Science*, *12*(40), 13492-13505. doi:10.1039/d1sc02775a

Borowski, P., Deinert, J., Schalinski, S., Bretner, M., Ginalski, K., Kulikowski, T., & Shugar, D. (2003). Halogenated benzimidazoles and benzotriazoles as inhibitors of the NTPase/helicase activities of hepatitis C and related viruses. *European Journal of Biochemistry*, *270*(8), 1645-1653. doi:10.1046/j.1432-1033.2003.03540.x

Borowski, P., Mueller, O., Niebuhr, A., Kalitzky, M., Hwang, L. H., Schmitz, H., Siwecka, M. A., & Kulikowsk, T. (2000). ATP-binding domain of NTPase/helicase as a target for hepatitis C antiviral therapy. *Acta Biochimica Polonica*, *47*(1), 173-180. doi:10.18388/abp.2000_4075

Briguglio, I., Piras, S., Corona, P., & Carta, A. (2011). Inhibition of RNA helicases of ssRNA+ virus belonging to Flaviviridae, Coronaviridae, and Picornaviridae families. *International Journal of Medicinal Chemistry*, *2011*, 213135. doi:10.1155/2011/213135

Chen, J., Ali, F., Khan, I., & Zhu, Y. Z. (2021). Recent progress in the development of potential drugs against SARS-CoV-2. *Current Research in Pharmacology and Drug Discovery*, *2*, 100057. doi:10.1016/j.crphar.2021.100057

Chen, J., Malone, B., Llewellyn, E., Grasso, M., Shelton, P. M. M., Olinares, P. D. B., Maruthi, K., Eng, E. T., Vatandaslar, H., Chait, B. T., et al. (2020). Structural basis for helicase-polymerase coupling in the SARS-CoV-2 replication-transcription complex. *Cell*, *182*(6), 1560-1573. doi:10.1016/j.cell.2020.07.033

Chen, T., Fei, C. Y., Chen, Y. P., Sargsyan, K., Chang, C. P., Yuan, H. S., & Lim, C. (2021). Synergistic inhibition of SARS-CoV-2 replication using disulfiram/ebselen and remdesivir. *ACS Pharmacology and Translational Science*, *4*(2), 898-907. doi:10.1021/acsptsci.1c00022

Corona, A., Wycisk, K., Talarico, C., Manelfi, C., Milia, J., Cannalire, R., Esposito, F., Gribbon, P., Zaliani, A., Iaconis, D., et al. (2022). Natural compounds inhibit SARS-CoV-2 nsp13 unwinding and ATPase enzyme activities. *ACS Pharmacology and Translational Science*, *5*(4), 226-239. doi:10.1021/acsptsci.1c00253

Crumpacker, C. S., & Schaffer, P. A. (2002). New anti-HSV therapeutics target the helicase–primase complex. *Nature Medicine*, *8*(4), 327-328. doi:10.1038/nm0402-327

Das, G., Ghosh, S., Garg, S., Ghosh, S., Jana, A., Samat, R., Mukherjee, N., Roy, R., & Ghosh, S. (2020). An overview of key potential therapeutic strategies for combat in the COVID-19 battle. *RSC Advances*, *10*(47), 28243-28266. doi:10.1039/D0RA05434H

Davidescu, L., Ursol, G., Korzh, O., Deshmukh, V., Kuryk, L., Nortje, M. M., Godlevska, O., Devouassoux, G., Khodosh, E., Israel, E., et al. (2022). Efficacy and safety of masitinib in corticosteroid-dependent severe asthma: a randomized placebo-controlled trial. *Journal of Asthma and Allergy*, 737-747.

Dumitru, M., Berghi, O. N., Taciuc, I. A., Vrinceanu, D., Manole, F., & Costache, A. (2022). Could Artificial Intelligence Prevent Intraoperative Anaphylaxis? Reference Review and Proof of Concept. *Medicina*, *58*(11), 1530.

El Hassab, M. A., Eldehna, W. M., Al-Rashood, S. T., Alharbi, A., Eskandrani, R. O., Alkahtani, H. M., Elkaeed, E. B., & Abou-Seri, S. M. (2022). Multi-stage structure-based virtual screening approach towards identification of potential SARS-CoV-2 NSP13 helicase inhibitors. J*ournal of Enzyme Inhibition and Medicinal Chemistry*, *37*(1), 563-572. doi:10.1080/14756366.2021.2022659

El-Sayed, A. F., Mohammed, A. T., Hamed, W., & Abdelmalek, S. (2021). Repurposing of available antiviral drugs against SARS-CoV-2 by targeting crucial replication machinery proteins: A molecular docking study. *Egyptian Pharmaceutical Journal*, *20*(4), 371.

Frick, D. N. (2003). Helicases as antiviral drug targets. *Drug News and Perspectives*, *16*(6), 355-362. doi:10.1358/dnp.2003.16.6.829307

Frick, D. N. (2007). The hepatitis C virus NS3 protein: A model RNA helicase and potential drug target. *Current Issues in Molecular Biology*, *9*(1), 1-20.

Frick, D. N., & Lam, A. M. I. (2006). Understanding helicases as a means of virus control. *Current Pharmaceutical Design*, *12*(11), 1315-1338. doi:10.2174/138161206776361147

Ghosh, D., & Basu, A. (2008). Present perspectives on flaviviral chemotherapy. *Drug Discovery Today*, *13*(13-14), 619-624. doi:10.1016/j.drudis.2008.04.001

Hao, W., Wojdyla, J. A., Zhao, R., Han, R., Das, R., Zlatev, I., Manoharan, M., Wang, M., & Cui, S. (2017). Crystal structure of Middle East respiratory syndrome coronavirus helicase. *PLOS Pathogens*, *13*(6), e1006474. doi:10.1371/journal.ppat.1006474

Hosseini, M., Chen, W., Xiao, D., & Wang, C. (2021). Computational molecular docking and virtual screening revealed promising SARS-CoV-2 drugs. *Precision Clinical Medicine*, *4*(1), 1-16. doi:10.1093/pcmedi/pbab001

Iftikhar, H., Ali, H. N., Farooq, S., Naveed, H., & Shahzad-Ul-Hussan, S. (2020). Identification of potential inhibitors of three key enzymes of SARS-CoV2 using a computational approach. *Computers in Biology and Medicine*, *122*, 103848. doi:10.1016/j.compbiomed.2020.103848

J. Woolhouse, M. E., Adair, K., & Brierley, L. (2013). RNA viruses: a case study of the biology of emerging infectious diseases. *Microbiology Spectrum*, *1*(1), 10-1128. doi:10.1128/microbiolspec.OH-0001-2012

Jia, Z., Yan, L., Ren, Z., Wu, L., Wang, J., Guo, J., Zheng, L., Ming, Z., Zhang, L., Lou, Z., et al. (2019). Delicate structural coordination of the severe acute respiratory syndrome coronavirus Nsp13 upon ATP hydrolysis. *Nucleic Acids Research*, *47*(12), 6538-6550. doi:10.1093/nar/gkz409

Kangarshahi, Z. T., Lak, S., Ghadam, M., Motamed, N., Sardari, S., & Rahimi, S. (2021). The proteins of sars-cov-2 and their functions. *Military Medical Science Letters*, *90*(4), 172-190. doi:10.31482/mmsl.2021.018

Khater, S., & Das, G. (2020). Repurposing ivermectin to inhibit

the activity of SARS CoV2 helicase: Possible implications for COVID-19 therapeutics.

Khosla, D., Zaheer, S., Madan, R., Goyal, S., Kumar, N., & Kapoor, R. (2021). Tele-oncology in cancer care during COVID-19 pandemic-expanding role in the developing world. *Clinical Cancer Investigation Journal*, *10*(4), 182-185.

Kim, J., Park, S. J., Park, J., Shin, H., Jang, Y. S., Woo, J. S., & Min, D. H. (2021). Identification of a direct-acting antiviral agent targeting RNA helicase via a graphene oxide nano biosensor. *ACS Applied Materials and Interfaces*, *13*(22), 25715-25726. doi:10.1021/acsami.1c04641

King, D. A., Peckham, C., Waage, J. K., Brownlie, J., & Woolhouse, M. E. (2006). Epidemiology. Infectious diseases: Preparing for the future. *Science*, *313*(5792), 1392-1393. doi:10.1126/science.1129134

Kleymann, G., Fischer, R., Betz, U. A., Hendrix, M., Bender, W., Schneider, U., Handke, G., Eckenberg, P., Hewlett, G., Pevzner, V., et al. (2002). New helicase-primase inhibitors as drug candidates for the treatment of herpes simplex disease. *Nature Medicine*, *8*(4), 392-398. doi:10.1038/nm0402-392

Ko, C., Lee, S., Windisch, M. P., & Ryu, W. S. (2014). DDX3 DEAD-box RNA helicase is a host factor that restricts hepatitis B virus replication at the transcriptional level. *Journal of Virology*, *88*(23), 13689-13698. doi:10.1128/JVI.02035-14

Li, K., Frankowski, K. J., Hanson, A. M., Ndjomou, J., Shanahan, M. A., Mukherjee, S., Kolli, R., Shadrick, W. R., Sweeney, N. L., Belon, C. A., et al. (2013). Hepatitis C virus NS3 helicase inhibitor discovery. *Probe Reports from the NIH Molecular Libraries Program [Internet]*.

Littler, D. R., MacLachlan, B. J., Watson, G. M., Vivian, J. P., & Gully, B. S. (2020). A pocket guide on how to structure SARS-CoV-2 drugs and therapies. *Biochemical Society Transactions*, *48*(6), 2625-2641. doi:10.1042/BST20200396

Ma, M., Yang, Y., Wu, L., Zhou, L., Shi, Y., Han, J., Xu, Z., & Zhu, W. (2022). Conserved protein targets for developing pan-coronavirus drugs based on sequence and 3D structure similarity analyses. *Computers in Biology and Medicine*, *145*, 105455. doi:10.1016/j.compbiomed.2022.105455

Maga, G., Gemma, S., Fattorusso, C., Locatelli, G. A., Butini, S., Persico, M., Kukreja, G., Romano, M. P., Chiasserini, L., Savini, L., et al. (2005). Specific targeting of hepatitis C virus NS3 RNA helicase. Discovery of the potent and selective competitive nucleotide-mimicking inhibitor QU663. *Biochemistry*, *44*(28), 9637-9644. doi:10.1021/bi047437u

Mahajan, R., & Marcus, S. (2021). Low-dose radiation therapy for COVID-19 pneumonia. *Clinical Cancer Investigation Journal*, *10*(1), 1-4.

Malone, B., Urakova, N., Snijder, E. J., & Campbell, E. A. (2022). Structures and functions of coronavirus replication–transcription complexes and their relevance for SARS-CoV-2 drug design. Nature Reviews. *Molecular Cell Biology*, *23*(1), 21-39. doi:10.1038/s41580-021-00432-z

Mastrangelo, E., Pezzullo, M., De Burghgraeve, T., Kaptein, S., Pastorino, B., Dallmeier, K., de Lamballerie, X., Neyts, J., Hanson, A. M., Frick, D. N., et al. (2012). Ivermectin is a potent inhibitor of Flavivirus replication specifically targeting NS3 helicase activity: New prospects for an old drug. *Journal of Antimicrobial Chemotherapy*, *67*(8), 1884-1894. doi:10.1093/jac/dks147

Mickolajczyk, K. J., Shelton, P. M. M., Grasso, M., Cao, X., Warrington, S. E., Aher, A., Liu, S., & Kapoor, T. M. (2021). Force-dependent stimulation of RNA unwinding by SARS-CoV-2 nsp13 helicase. *Biophysical Journal*, *120*(6), 1020-1030. doi:10.1016/j.bpj.2020.11.2276

Mirza, M. U., & Froeyen, M. (2020). Structural elucidation of SARS-CoV-2 vital proteins: Computational methods reveal potential drug candidates against main protease, Nsp12 polymerase, and Nsp13 helicase. *Journal of Pharmaceutical Analysis*, *10*(4), 320-328. doi:10.1016/j.jpha.2020.04.008

Nandi, R., Bhowmik, D., Srivastava, R., Prakash, A., & Kumar, D. (2022). Discovering potential inhibitors against SARS-CoV-2 by targeting Nsp13 Helicase. *Journal of Biomolecular Structure and Dynamics*, *40*(22), 12062-12074. doi:10.1080/07391102.2021.1970024

Newman, J. A., Douangamath, A., Yadzani, S., Yosaatmadja, Y., Aimon, A., Brandão-Neto, J., Dunnett, L., Gorrie-Stone, T., Skyner, R., Fearon, D., et al. (2021). Structure, mechanism, and crystallographic fragment screening of the SARS-CoV-2 NSP13 helicase. *Nature Communications*, *12*(1), 4848. doi:10.1038/s41467-021-25166-6

Omolo, C. A., Soni, N., Fasiku, V. O., Mackraj, I., & Govender, T. (2020). Update on therapeutic approaches and emerging therapies for SARS-CoV-2 virus. *European Journal of Pharmacology*, *883*, 173348. doi:10.1016/j.ejphar.2020.173348

Oran, I. B., Yilmaz, S., & Erol, M. (2021). The Impacts of Artificial Intelligence and Covid-19 on Brics and Performance Analysis of South-African Republic. *Journal of Organizational Behavior Research*, *6*(2), 89-99. doi:10.51847/cLL8rdINmJ.

Pandey, A., Nikam, A. N., Shreya, A. B., Mutalik, S. P., Gopalan, D., Kulkarni, S., Padya, B. S., Fernandes, G., Mutalik, S., & Prassl, R. (2020). Potential therapeutic targets for combating SARS-CoV-2: Drug repurposing, clinical trials, and recent advancements. *Life Sciences*, *256*, 117883. doi:10.1016/j.lfs.2020.117883

Pathak, A., Gupta, A., Rathore, A., Sud, R., Swamy, S. S., Pandaya, T., Dubey, A. P., & Guleria, B. (2021). Immunotherapy during COVID-19 pandemic: An experience at a tertiary care center in India. *Clinical Cancer Investigation Journal*, *10*(1), 22-28.

Perez-Lemus, G. R., Menéndez, C. A., Alvarado, W., Byléhn, F., & de Pablo, J. J. (2022). Toward wide-spectrum antivirals against coronaviruses: Molecular characterization of SARS-CoV-2 NSP13 helicase inhibitors. *Science Advances*, *8*(1), eabj4526. doi:10.1126/sciadv.abj4526

Raubenolt, B. A., Islam, N. N., Summa, C. M., & Rick, S. W. (2022). Molecular dynamics simulations of the flexibility and inhibition of SARS-CoV-2 NSP 13 helicase. *Journal of Molecular Graphics and Modelling*, *112*, 108122.

doi:10.1016/j.jmgm.2022.108122

Saidijam, M., Khaksarimehr, N., Rezaei-Tavirani, M., & Taherkhani, A. (2021). Bioinformatics prediction of potential inhibitors for the SARS-CoV-2 NTPase/helicase using molecular docking and dynamics simulation from organic phenolic compounds. *Journal of Cellular and Molecular Anesthesia, 6*(3), 222-239.

Satpathy, R. (2020). In silico modeling and docking study of potential helicase (nonstructural proteins) inhibitors of novel coronavirus 2019 (severe acute respiratory syndrome coronavirus 2). *Biomedical and Biotechnology Research Journal, 4*(4), 330. doi:10.4103/bbrj.bbrj_149_20

Shadrick, W. R., Ndjomou, J., Kolli, R., Mukherjee, S., Hanson, A. M., & Frick, D. N. (2013). Discovering new medicines targeting helicases: Challenges and recent progress. *Journal of Biomolecular Screening, 18*(7), 761-781. doi:10.1177/1087057113482586

Shu, T., Huang, M., Wu, D., Ren, Y., Zhang, X., Han, Y., Mu, J., Wang, R., Qiu, Y., Zhang, D. Y., et al. (2020). SARS-coronavirus-2 Nsp13 possesses NTPase and RNA helicase activities that can be inhibited by bismuth salts. *Virologica Sinica, 35*(3), 321-329. doi:10.1007/s12250-020-00242-1

Siminea, N., Popescu, V., Sanchez Martin, J. A., Florea, D., Gavril, G., Gheorghe, A. M., Iţcuş, C., Kanhaiya, K., Pacioglu, O., Popa, L. L., et al. (2022). Network analytics for drug repurposing in COVID-19. *Briefings in Bioinformatics, 23*(1), bbab490. doi:10.1093/bib/bbab490

Singh, A. K., Singh, A., & Dubey, A. K. (2021). Repurposed therapeutic strategies towards COVID-19 potential targets based on genomics and protein structure remodeling. In *Biotechnology to Combat COVID, 19*. IntechOpen.

Spratt, A. N., Gallazzi, F., Quinn, T. P., Lorson, C. L., Sönnerborg, A., & Singh, K. (2021). Coronavirus helicases: Attractive and unique targets of antiviral drug-development and therapeutic patents. *Expert Opinion on Therapeutic Patents, 31*(4), 339-350. doi:10.1080/13543776.2021.1884224

Steimer, L., & Klostermeier, D. (2012). RNA helicases in infection and disease. *RNA Biology, 9*(6), 751-771. doi:10.4161/rna.20090

Tam, L. T., An, H. T. T., Linh, T. K., Nhung, L. T. H., Ha, T. N. V., Huy, P. Q., & Luc, P. T. (2022). Value Co-Creation Activities of Students on The Covid-19 Pandemic: Empirical Evidence from Economics Students in Vietnam. *Journal of Organizational Behavior Research, 7*(2), 214-228.

Tudoran, C., Velimirovici, D. E., Berceanu-Vaduva, D. M., Rada, M., Voiţă-Mekeres, F., & Tudoran, M. (2022). Increased susceptibility for thromboembolic events versus high bleeding risk associated with COVID-19. *Microorganisms, 10*(9), 1738.

Uhlig, N., Donner, A. K., Gege, C., Lange, F., Kleymann, G., & Grunwald, T. (2021). Helicase primase inhibitors (HPIs) are efficacious for the therapy of human herpes simplex virus (HSV) disease in an infection mouse model. *Antiviral Research, 195*, 105190. doi:10.1016/j.antiviral.2021.105190

Ujjinamatada, R. K., Agasimundin, Y. S., Zhang, P., Hosmane, R. S., Schuessler, R., Borowski, P., Kalicharran, K., & Fattom, A. (2005). A novel imidazole nucleoside containing a diaminodihydro-S-triazine as a substituent: Inhibitory activity against the West Nile virus NTPase/helicase. *Nucleosides, Nucleotides, and Nucleic Acids, 24*(10-12), 1775-1788. doi:10.1080/15257770500267063

Vivek-Ananth, R. P., Krishnaswamy, S., & Samal, A. (2022). Potential phytochemical inhibitors of SARS-CoV-2 helicase Nsp13: A molecular docking and dynamic simulation study. *Molecular Diversity, 26*(1), 429-442. doi:10.1007/s11030-021-10251-1

White, M. A., Lin, W., & Cheng, X. (2020). Discovery of COVID-19 inhibitors targeting the SARS-CoV-2 Nsp13 helicase. *Journal of Physical Chemistry Letters, 11*(21), 9144-9151. doi:10.1021/acs.jpclett.0c02421

Wondmkun, Y. T., & Mohammed, O. A. (2020). A review of novel drug targets and future directions for COVID-19 treatment. *Biologics: Targets and Therapy, 14*, 77-82. doi:10.2147/BTT.S266487

Woolhouse, M. E. J., & Adair, K. (2013). The diversity of human RNA viruses. *Future Virology, 8*(2), 159-171. doi:10.2217/fvl.12.129

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., Wang, Q., Xu, Y., Li, M., Li, X., et al. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica. B, 10*(5), 766-788. doi:10.1016/j.apsb.2020.02.008

Wu, D., Wu, T., Liu, Q., & Yang, Z. (2020). The SARS-CoV-2 outbreak: What we know. *International Journal of Infectious Diseases, 94*, 44-48. doi:10.1016/j.ijid.2020.03.004

Xia, H., Cao, Z., Xie, X., Zhang, X., Chen, J. Y., Wang, H., Menachery, V. D., Rajsbaum, R., & Shi, P. Y. (2020). Evasion of type I interferon by SARS-CoV-2. *Cell Reports, 33*(1), 108234. doi:10.1016/j.celrep.2020.108234

Yadav, R., Chaudhary, J. K., Jain, N., Chaudhary, P. K., Khanra, S., Dhamija, P., Sharma, A., Kumar, A., & Handu, S. (2021). Role of structural and non-structural proteins and therapeutic targets of SARS-CoV-2 for COVID-19. *Cells, 10*(4), 821. doi:10.3390/cells10040821

Yan, L., Zhang, Y., Ge, J., Zheng, L., Gao, Y., Wang, T., Jia, Z., Wang, H., Huang, Y., Li, M., et al. (2020). Architecture of a SARS-CoV-2 mini replication and transcription complex. *Nature Communications, 11*(1), 5874. doi:10.1038/s41467-020-19770-1

Yedavalli, V. S., Zhang, N., Cai, H., Zhang, P., Starost, M. F., Hosmane, R. S., & Jeang, K. T. (2008). Ring-expanded nucleoside analogs inhibit RNA helicase and intracellular human immunodeficiency virus type 1 replication. *Journal of Medicinal Chemistry, 51*(16), 5043-5051. doi:10.1021/jm800332m

Yuan, S., Yin, X., Meng, X., Chan, J. F. W., Ye, Z. W., Riva, L., Pache, L., Chan, C. C., Lai, P. M., Chan, C. C., et al. (2021). Clofazimine broadly inhibits coronaviruses including SARS-CoV-2. *Nature, 593*(7859), 418-423. doi:10.1038/s41586-021-03431-4

Yuen, C. K., Lam, J. Y., Wong, W. M., Mak, L. F., Wang, X., Chu,

H., Cai, J. P., Jin, D. Y., To, K. K., Chan, J. F., et al. (2020). SARS-CoV-2 nsp13, nsp14, nsp15, and orf6 function as potent interferon antagonists. *Emerging Microbes and Infections*, *9*(1), 1418-1428. doi:10.1080/22221751.2020.1780953

Zeng, J., Weissmann, F., Bertolin, A. P., Posse, V., Canal, B., Ulferts, R., Wu, M., Harvey, R., Hussain, S., Milligan, J. C., et al. (2021). Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of nsp13 helicase. *Biochemical Journal*, *478*(13), 2405-2423. doi:10.1042/BCJ20210201