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REVIEW ARTICLE

An Overview of SARS-CoV-2 Potential Targets, Inhibitors, and Computational Insights to Enrich the Promising Treatment Strategies

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Abstract
The rapid spread of the SARS-CoV-2 virus has emphasized the urgent need for effective therapies to combat COVID-19. Investigating the potential targets, inhibitors, and in silico approaches pertinent to COVID-19 are of utmost need to develop novel therapeutic agents and reprofiling of existing FDA-approved drugs. This article reviews the viral enzymes and their counter receptors involved in the entry of SARS-CoV-2 into host cells, replication of genomic RNA, and controlling the host cell physiology. In addition, the study provides an overview of the computational techniques such as docking simulations, molecular dynamics, QSAR modeling, and homology modeling that have been used to find the FDA-approved drugs and other inhibitors against SARS-CoV-2. Furthermore, a comprehensive overview of virus-based and host-based druggable targets from a structural point of view, together with the reported therapeutic compounds against SARS-CoV-2 have also been presented. The current study offers future perspectives for research in the field of network pharmacology investigating the large unexplored molecular libraries. Overall, the present in-depth review aims to expedite the process of identifying and repurposing drugs for researchers involved in the field of COVID-19 drug discovery.

Introduction

The worldwide pandemic that began with the emergence of the novel coronavirus SARS-CoV-2 in late 2019 has deep and far-reaching effects on public health, society, and the economy [1, 2]. The virus has affected virtually every country, resulting in millions of deaths, hospitalizations, and long-term health complications [3]. The pandemic has also caused significant disruptions to daily life, including lockdowns, travel restrictions, and economic downturns [4]. The impact of the pandemic has underscored the urgent need to develop effective strategies to control the spread of the virus and mitigate its impact on human health [5]. SARS-CoV-2 is a highly infectious virus that primarily spreads through respiratory droplets and close contact with infected individuals [6]. The virus's ability to infect asymptomatic individuals has made it challenging to control its spread. Moreover, the emergence of new variants of the virus has complicated efforts to develop effective vaccines and treatments [7].

The pandemic has highlighted the importance of scientific research in combating infectious diseases and has created a pressing need for disease-fighting medications and vaccines [8]. Scientists worldwide have worked tirelessly to gain a better understanding of the virus's biology, transmission, and pathogenesis [9]. Such research has led to the development of effective vaccines and treatments that have saved countless lives [10]. However, there is still much to learn about SARS-CoV-2, and ongoing research is critical to developing better prevention and treatment strategies. One area of research that has gained increased attention in recent years is computational studies [11]. These studies utilize computer-based simulations to model the behavior of molecules and target proteins, allowing scientists to identify potential drug candidates and optimize their properties [12, 13]. Computational studies have played an essential role in developing effective treatments for various diseases, including cancer, HIV/AIDS, and hepatitis C [14]. These virtual approaches have accelerated the drug discovery process, as they allow researchers to rapidly screen large numbers of potential hit candidates and identify promising lead compounds for

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further testing [15]. In the context of COVID-19 research, in silico studies have played a critical role in the search for potential inhibitors and targets for SARS-CoV-2 [16]. Through these studies, researchers have been able to model and simulate the interactions between the promising targets and potential drug candidates [17].

Identification of a potential approach or therapy against COVID-19 is still under consideration. Therefore, gaining insights into SARS-CoV-2, its interacting partners, and functional domains is crucial for developing effective treatments. The article provides a comprehensive review on virus enzymes and their interacting host molecules that play the major role to enter, replicate its genomic material within the host and regulate host cell biology. Further, this review also summarizes the potential inhibitors that are being studied and identified to block these target molecules for the treatment of COVID-19. The deep insight of various computational approaches is also emphasized for repurposing existing drugs or designing new therapeutic options with special reference to SARS-CoV-2. By emphasizing the critical role of computational studies in combating the ongoing pandemic, this article aims to provide a valuable resource for scientists and pharmacologists worldwide.

Mechanisms of SARS-CoV-2 Entry into Host Cell

SARS-CoV-2 also known as β-coronavirus is an enveloped positive-strand RNA virus with an unusually large genome length of 29,881 bp of coronaviridae family [18].

The viral genome is structurally attached to Nucleocapsid (N) protein, and the double-layered lipid viral envelope is formed by the combination of Spike (S), Envelope (E), and Membrane (M) proteins (Fig. 1). The genome has 14 open reading frames (ORFs), which encode different structural proteins such as the S, E, M, and N proteins along with 16 non-structural proteins (NSP 1–16) as shown in Table 1 (Fig. 1).

The viral life cycle of SARS-CoV-2 is complex and involves several distinct steps, including viral entry, replication, assembly, and release [19]. To make effective drugs against COVID-19, it is necessary to have an in-depth knowledge of the targets of the virus and the receptors of the host cell. For this, the detailed mechanism of virus entry into the host cell, its growth and multiplication is given here (Fig. 2).

The entry of SARS-CoV-2 into host cells is a crucial step in the viral life cycle and is mediated by the virus's spike protein, which binds to the ACE2 receptor on the surface of the host cell [20]. The affinity of the spike protein for ACE2 is an important factor in the viral entry process [21]. The receptor-binding domain (RBD) in the S1 subunit of the spike protein recognizes and binds to the host cell's ACE2 receptor [22]. The S1 RBD of the spike protein binds to the ACE2 receptor with high affinity, allowing the virus to enter host cells even at low viral loads. This high affinity is thought to be a key factor in the high infectivity of SARS-CoV-2 [20, 23]. The fusion peptide and other sequences required for fusion of the viral membrane with the host cell membrane are found in the S2 subunit [24]. Once the virus is attached to the ACE2 receptor, the S2 subunit undergoes a conformational change that allows the virus to fuse its

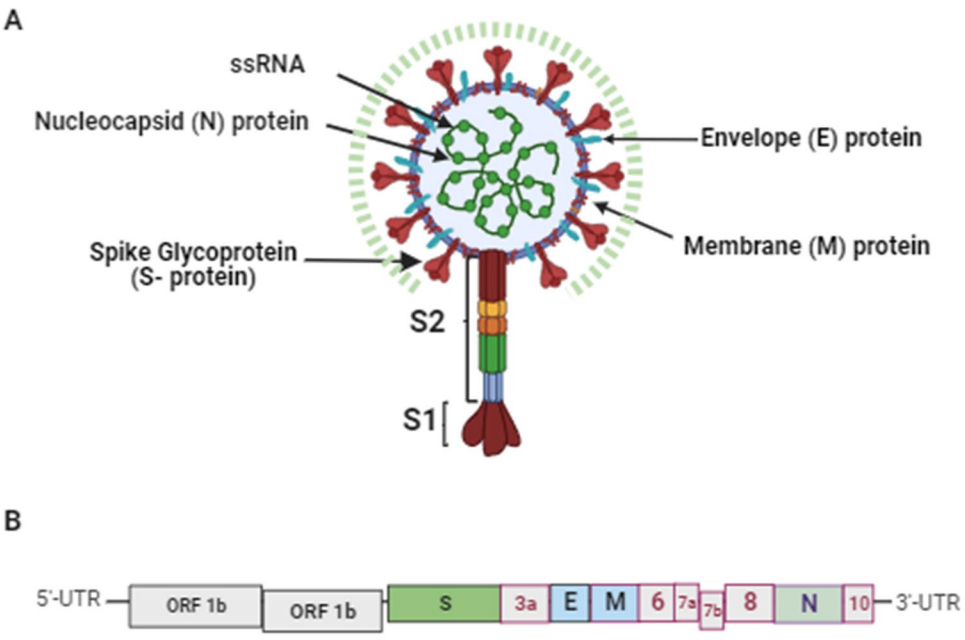

Fig. 1 Illustration of the structural composition and genomic organization within SARS-CoV-2. **A** An overview of structural features of SARS-CoV-2 and primary key proteins. **B** The genomic organization of SARS-CoV-2. (The Figure was created with https://www.biorender.com)

Table 1 Functional roles of structural and non-structural proteins, along with gene/ORF regions within SARS-CoV-2

S. no.	Protein type	Gene or ORF region	Function	References
Structural proteins (SP)-Gene				
1.	Spike (S)	S	Binds to Angiotensin-Converting Enzyme 2 (ACE2) receptor, heparan sulfate for viral entry and initiate the infection process	[50, 102]
2.	Envelope (E)	E	Maintains Virion structural integrity	[20, 42]
3.	Membrane(M)	M	Maintains Virion structural integrity and leads to the activation of β interferons (IFN-β) in cell lines	[20, 42]
4.	Nucleocapsid (N)	N	Contains viral genome, interferes with translation and cell cycle of the host cell and activator of Cyclooxygenase-2 (COX-2) enhancer	[42, 117]
Non-structural protein (NSP)-ORF region				
1.	NSP-1	ORF1a, ORF1b	RNA processing and replication, it plays specific roles in the interaction of the CoV with the innate immune response and suppresses apoptosis induction during the early stages of infection to promote viral growth	[118]
2.	NSP-2	ORF1a, ORF1b	Modulates the survival signaling pathway of the host cell and is also involved in disrupting intracellular host signaling	[119]
3.	NSP-3 (PLpro)	ORF1a, ORF1b	Facilitates mRNA transcript translation while suppressing host protein synthesis and possibly also separates the translated protein	[120]
4.	NSP-4 (MP1)	ORF1a, ORF1b	Membrane protein 1 plays an essential role in replication, contains assembly of the replicative structures, transmembrane domain 2 (TM2), and modifies ER membranes	[120]
5.	NSP-5 (3CL Pro)	ORF1a, ORF1b	Polyprotein replication, innate immune inhibitor and forms viroplasm-like structures (VLS)	[121]
6.	NSP-6	ORF1b	Presumptive transmembrane Domain, Forms autophagosome for immune modulatory protein degradation, nsp6 binds to TBK1 without affecting TBK1 phosphorylation, but the nsp6/TBK1 binding interaction decreases IRF3 phosphorylation, resulting in reduced IFN-β production	[120, 122]
7.	NSP-7	ORF1b	Increases the combination of NSP-12 and template-primer independent RNA polymerase activity	[122]
8.	NSP-8	ORF1b	Increases the combination of NSP-12 and template-primer RNA	[122]
9.	NSP-9	ORF1a, ORF1b	Single-stranded RNA (ss RNA) binding protein	[122]
10.	NSP-10	ORF1b	Cap methylation of viral mRNAs, work as a cofactor for both nsp16′s (2′O-methyltransferase) and nsp14's (N7-guaninemethyltransferase/exoribonuclease) activities	[123]
11.	NSP-11	ORF1b	Necessary for viral replication	[124]
12.	NSP-12 (RDRP)	ORF1a, ORF1b	RNA-dependent RNApolymerase (RdRp)	[57]
13.	NSP13 (Hel)	ORF1a, ORF1b	Helicase and RNATPase activity, RTPase activity hydrolyzes the RNA strand's at 5′ end, this protein Binds with ATP and the zinc-binding domain—required for replication and transcription of viral protein, nsp13 binds and inhibits TBK1 phosphorylation, resulting in decreased IRF3 activation and IFN-β production	[57]
14.	NSP-14 (ExoN)	ORF1a, ORF1b	Proofreading exoribonuclease activity and methyl transferase domain	[123, 125]
15.	NSP-15 (Nendo-U)	ORF1a, ORF1b	Mn (2+)-dependent Uridylate specific endoribonuclease activity.	[122]
16.	NSP-16 (2′-*O*-MT)	ORF1b	2′-*O*-ribose methyltransferasesmethylates the RNA strand at position 2′	[126]
Other ORF regions				
1.	–	ORF 3a	Ion channel protein-affected cytokine response.	[127]
2.	–	ORF 6	Inhibits antiviral interferon response, Nuclear Translocation IRF3 inhibitor.	[128]
3.	–	ORF 7a	Inhibits antiviral interferon response and STAT1phosphorylation.	
4.	–	ORF 7b	Inhibits antiviral interferon response, STAT1, and STAT2phosphorylation.	
5.	–	ORF 8	Inhibits antiviral interferon response.	

membrane with the host cell membrane [25]. This fusion step is critical for the release of the large viral genome into the host cell and the initiation of viral replication. The ACE2-mediated entry of SARS-CoV-2 into host cells is also affected by the distribution and differential expression levels of ACE2 receptors in different tissues and cells. The ACE2, and the protease enzymes of the host cell play important role for the cleavage and fusion of the viral spike protein

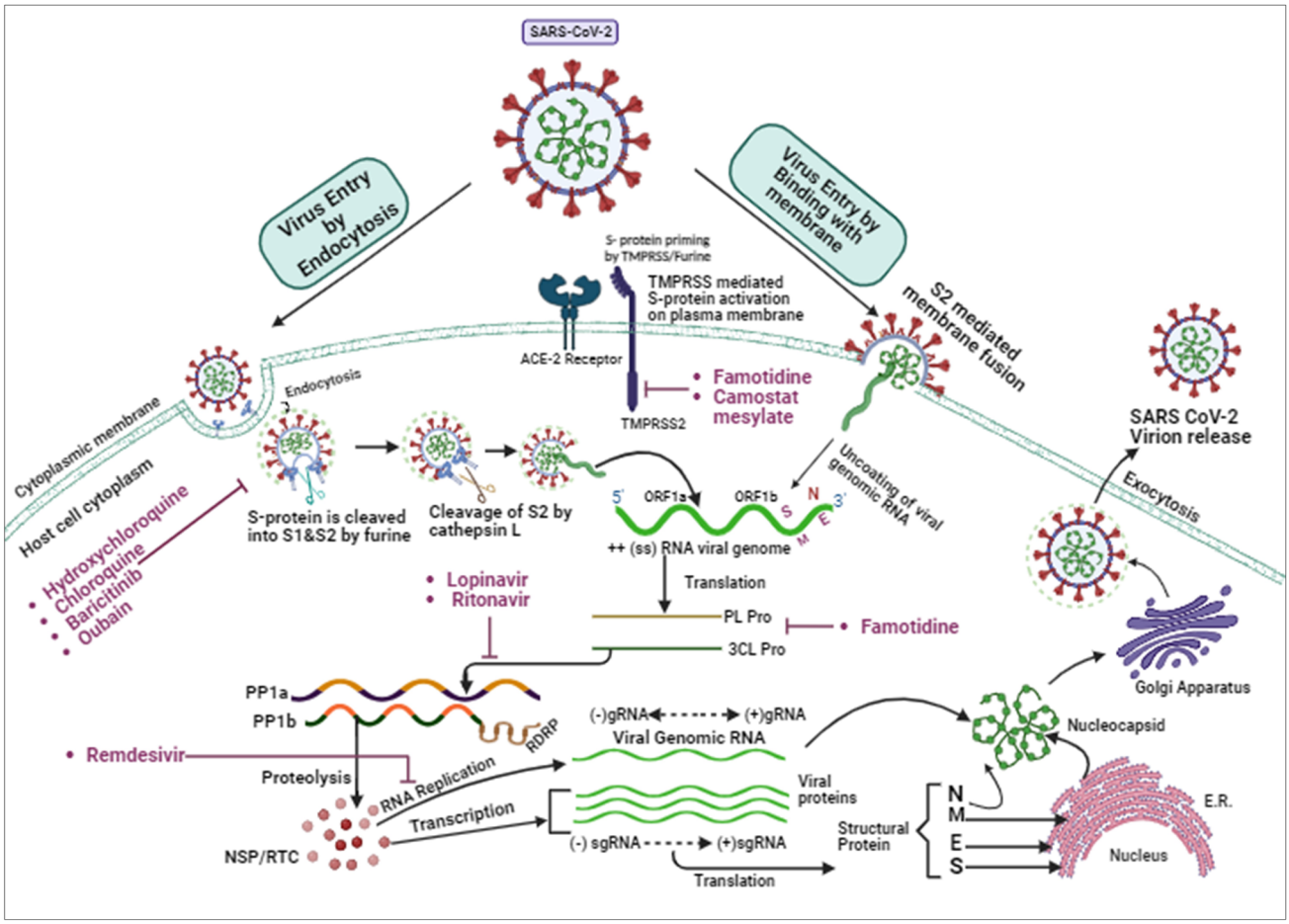

Fig. 2 SARS-CoV-2 infiltrates host cells through endocytosis or binding to the transmembrane angiotensin converting enzyme 2 (ACE2) receptors on cell surface. This triggers membrane fusion via host proteases, enabling viral entry, replication, maturation, and relentlessly efficient release to nearby cells. Note, the inhibitor arrows are pointing the activity of FDA-approved drugs against promising targets. (The Figure was created with https://www.biorender.com)

[26]. The location and transcript amount of ACE2 receptors, crucial in the viral entry process [27], vary across different tissues and cell types, including the lungs, heart, kidneys, and intestines [28]. The expression levels of ACE2 can also vary greatly depending on the individual [29]. These variations in expression levels of ACE2 may be correlated with the observed differences in COVID-19 severity among patients [30].

One of the important factor in the viral entry process is the proteases enzymes that cleave the viral spike protein and activate its fusion activity [31]. The spike protein of SARS-CoV-2 is cleaved by host cell proteases, including TMPRSS2 and cathepsin L (Fig. 2) [32]. The cleavage of the spike protein by these proteases is required for the fusion of the virus membrane with the host cell membrane [33].

In addition to ACE2-mediated entry, there is evidence to suggest that SARS-CoV-2 can enter cells through alternative pathways [20, 34]. One of these pathways is TMPRSS2-mediated entry, where the spike protein is cleaved by the host cell protease TMPRSS2 inducing the fusion activity of the spike protein and facilitating the fusion of the virus membrane with the host cell membrane [35] Another possible pathway is endocytosis, where the virus is taken up by the host cell in a vesicle [31]. The virus can then fuse with the endosomal membrane, releasing its genetic material into the cytoplasm (Fig. 2). There is also evidence to suggest that SARS-CoV-2 may be able to enter cells through a mechanism called membrane fusion without endocytosis, where the virus fuses directly with the host cell membrane.

Once the virus has entered the host cell, it begins to replicate and produce new viral particles. The viral genome is translated into viral proteins, which assemble into new viral particles. The new viral particles then leave the host cell through a process called budding, which involves the incorporation of viral proteins into the host cell membrane [36]. The virus can then spread to other nearby cells thereby infecting additional host cells, perpetuating the cycle of infection [37]. Given our understanding of these various

entry pathways for SARS-CoV-2, targeted drug therapy plays the crucial role for developing effective treatments for COVID-19 [38]. By targeting specific steps in these pathways, researchers can develop interventions that limit the viral entry and replication to spread the virus, and mitigate the impact of the COVID-19 pandemic [6, 39].

Potential Targets for COVID-19

SARS-CoV-2 interacts with various host cell components to enter, replicate, and control the host cellular mechanism. Hence, all protein enzymes of the coronavirus and the involved host cell molecules are potentially druggable targets for the development of promising molecules against COVID-19. In principle, understanding the viral proteins and their interacting partners within host cell are essential to develop effective therapeutic strategies against the SARS-CoV-2 virus [40]. Herein, we have summarized the virus-based and host-based potential therapeutic targets in the search of therapeutic options to tackle with the SARS-CoV-2.

The virus's genome encodes for a range of structural and non-structural proteins that are essential for viral entry into host cell and replication, thus, these proteins represent promising targets in the search for drug-like molecules [41]. The viral genomic RNA begins with a 5′ un-translated region (UTR), then the ORF1a and ORF1b replication complexes followed by the four structural protein regions and 3′ un-translated region (UTR), ending with non-structural ORFs and a poly(A) tail. ORF1a contains 10 non-structural proteins and ORF1b contains16 non-structural proteins (Table 1). The combination of ORF1a and ORF1b codes pp1a (440–500 kDa) and pp1b (740–810 kDa) polyproteins that form the viral replication complex.

The present section is divided into two main categories, including SARS-CoV-2 virus-based proteins (Table 1) and host-based prominent receptors (Supporting Table 1) as targets for COVID-19. This section recapitulates the structural and non-structural proteins from virus and interacting receptors from host which are being promisingly targeted to design the novel drug candidates or for the repurposing of existing drugs against SARS-CoV-2 [42].

SARS-CoV-2 Components as Targets for COVID-19

Structural Proteins

Structural proteins are a category of molecular proteins that help organs and cells to maintain their molecular integrity, shape, and ability to withstand external stress [43].These proteins are involved in forming the physical structure of cells, tissues, and organs in living organisms. Overall, structural proteins are typically considered by their high tensile strength and rigidity, which is due to their unique molecular structure [44, 45]. Most structural proteins are composed of long, linear chains of amino acids that are cross-linked by strong covalent bonds called peptide bonds resisting mechanical stress [46].These amino acids are typically arranged in repeating patterns, forming a secondary structure such as an alpha-helix or beta-sheet [47]. These secondary structures then fold into a three-dimensional structure, which is stabilized by additional non-covalent interactions such as hydrogen bonds and van der Waals forces [48]. The most common structural proteins in living organisms, such as collagen, elastin, and keratin, not only serve structural roles but also have specific functional roles. For example, collagen provides strength, elasticity and involved in wound healing to tissues such as skin, bones, lungs, and cartilage [49].

In the context of SARS-CoV-2, the four key structural proteins are the Nucleocapsid protein (N), Spike (S), Envelope (E), and Membrane (M) proteins, which are essential for the virus's structural integrity and functional aspects [42]. The spike protein (S) is responsible for binding the virus to the host cell and initiating the infection process [50]. The S protein (spike protein) is the primary target for neutralizing antibodies against SARS-CoV-2. The envelope protein (E) and membrane protein (M) are involved in the assembly of the virus and are important for maintaining its structural integrity (Fig. 1). The nucleocapsid protein (N) is responsible for packaging the virus's genetic material (Fig. 1) [41]. Hence, with the extensive role of structural proteins to enter and multiplication of SARS-CoV-2 into the host cell, these molecules are the potential targets for the development of promising molecules against COVID-19. In principle, understanding the structural proteins is essential to develop effective therapeutic strategies to tackle SARS-CoV-2 infection.

Non-structural Proteins

Non-structural proteins are a group of proteins that are encoded by the single-stranded coronavirus (CoV) genome. These viral proteins are involved in various aspects of the viral life cycle and play the crucial role in viral replication and pathogenesis [41, 51]. In general, the non-structural proteins are essential for proliferation of SARS-CoV-2 and COVID-19. Open reading frames (ORFs) of viral genome encode the 16 different types of non-structural proteins (NSP 1–16) proteins. ORF1a contains 10 non-structural proteins and ORF1b contains 16 non-structural proteins (Table 1). The RNA-dependent RNA polymerase (RdRp), helicase (Hel), exonuclease (ExoN), MPro papain-like protease (PLpro), 3C-like protease (3CLpro), and ORF-8 protein (NS8 protein) are some of the key non-structural proteins

encoded by the CoV genome [19, 52]. Scientists have paid their attention to design lead molecules against SARS-CoV-2 by targeting these NSPs.

Mpro, also known as the main protease or 3CLpro, is a key enzyme in the replication of coronaviruses, including the SARS-CoV-2 virus responsible for the COVID-19 pandemic [53]. Mpro plays a crucial role in the cleavage of viral polyproteins at specific sites, which is required for the formation of new viral particles. RdRp (NSP12) or RNA-dependent RNA polymerase enzyme catalyzes the synthesis of the genomic RNA from a template of viral RNA and plays the key role in producing multiple copies of the viral RNA [54–56]. CoV2 contains a non-structural large viral polyprotein PLpro (Mpro papain-like protease/NSP3) that forms the replicase complex. This protein is essential for processing viral polyproteins, which are translated from the viral RNA. Mpro papain-like protease cleaves these polyproteins into individual functional viral proteins. Spike gly2/Receptor binding domain (RBD) is a clove-shaped kind of transmembrane protein (type I-TM) on the surface of the SARS-CoV-2 virus that allows it to enter human cells. It is the target of many vaccines and therapeutic antibodies. Helicase (Hel/NSP13)/NTPase is essential for the formation of the replicase complex, which is responsible for viral RNA replication [57]. NTPase/helicase plays a key role in the central viral dogma unwinding of double-stranded DNA to replicate viral RNA. The helicase enzyme of SARS-CoV-2 is part of superfamily I (SF1) that has a common core with two RecA-like motifs involving in ATP binding, nucleic acid binding, and unwinding. Exoribonuclease (ExoN) is involved in the subgenomic micro RNA (sgmRNA) recombination which is required for normal multiplication of coronavirus. This protein plays an important role in the fidelity of viral RNA replication and transcription, eliminating non-compatible nucleotides.

With this in-depth knowledge about important role of different structural and non-structural proteins in viral entry and replication in host cell, researchers would be able to design medicines and vaccines against SARS-CoV-2 more efficiently.

Host Cell Receptors as Targets for COVID-19

As discussed above, SARS-CoV-2 viral proteins and enzymes interact with various host cell receptors to enter, replicate, and control the cellular mechanism in COVID-19 patient. Several human corona viruses (HCoVs) functional receptors play a critical role in viral replication in an infected host cell. Hence, along with the CoV protein enzymes, interacting host cell partners are also promising drug targets for the development of effective medications against COVID-19. Herein, an effort has been made to review host-based potential therapeutic targets collected from several studies and listed in Supporting Table 1. These host cell receptors are studied and targeted to develop novel drug candidates or repurposing the existing drug to combat COVID-19. Biomedical researchers are working extensively using computational methods to find effective antiviral agents against various human corona viruses (HCoVs). Scientists continuously simulate small and large molecules with different PDB IDs of various virus-based and host-based targets to analyze hotspot residues and their interactions to discover novel and potent inhibitors against SARS-CoV-2 as well as to study the binding of interacting amino acid site residues of protein with any ligand molecule (Supporting Table 2) [58].

Inhibitors of SARS-CoV-2 Targets

Following the detailed discussion about druggable targets, potential therapeutic agents targeting either the viral proteins or interacting partners in host cell could be effective inhibiting the viral replication [20]. The rapid emergence of the pandemic left the scientists with limited time to design new drug molecules. As of now, there is no specific approved therapy for treating COVID-19. Concerning the emergence of pandemic and time-consuming traditional drug designing process, reprofiling existing drugs have become the most practiced and effective strategy. Therefore, different categories of drugs are being repurposed in different in vitro and in vivo studies to combat SARS-CoV-2 [59]. In this regard, the World Health Organisation (WHO) has preapproved the repositioning of Remdesivir, Hydroxy-chloroquine, and Ribavirin as a temporary therapy to cure COVID-19 patients. Nevertheless, additional medications are also being re-profiled via if they pass clinical trials and demonstrate efficacy in in silico, in vitro and in vivo research.

Ongoing clinical trials offer a range of therapeutic alternatives including various Food and Drug Administration (FDA)-approved drugs, convalescent plasma therapy, cell therapy, monoclonal antibodies, and immunoglobulin therapy. The current section summarized a comprehensive description about the available potential therapeutics against SARS-CoV-2 to assist researchers in their current and future endeavours to treat COVID-19 patients. Table 2 lists the many kind of drugs that have been reiterated for the treatment of COVID-19 including anti-inflammatory, antimalarial drugs as well as numerous other prescribed drugs (Table 2).

FDA-Approved Drugs

Several FDA-approved drugs have shown significant potential to inhibit the SARS-CoV-2 activity. These drugs have been repurposed from their original indication and are being used in clinical trials to treat COVID-19 [60]. In this regard,

Table 2 Therapeutic options and their mode of action on repurposing against SARS-CoV-2

S. no.	Drugs	Related disease	Action of the drug on SARS-CoV-2 targets	References
Antiviral drugs				
1.	Favipiravir (FPV)	Anti-influenza	RNA-dependent RNA polymerase inhibitor	[129]
2.	Remdesivir (GS-5734)	Anti-ebola	RNA-dependent RNA polymerase inhibitor	[61]
3.	Hydroxychloroquine	Anti-malarial, anti-autoimmune agent	Virus-cell membrane fusion blocker, replication inhibitor	[65]
4.	Arbidol Hydrochloride (Umifenovir)	Anti-influenza, anti-arboviruses	Virus-cell membrane fusion inhibitor	[130]
5.	APN01 (Soluble recombinant human ACE2)	Undergone phase II trial for ARDS (acute respiratory distress syndrome)	Virus-cell membrane fusion inhibitor	[131]
6.	Ribavirin	Anti-influenza	RNA-dependent RNA polymerase inhibitor	[68]
7.	Galidesivir	Anti-ebola	Viral RNA polymerase inhibitor, Premature termination of RNA transcription inhibitor	[73]
8.	Pegylated interferon with ribavirin	Anti-HCV (Hepatitis C virus), anti-HIV	Replication inhibitor	[67]
9.	Cepharanthine (CEP)	Anti-viral, anti-inflammatory, anti-oxidative, immunomodulating and anti-parasitic	Viral replication inhibitor, suppresses nuclear factor-kappa B (NF-κβ) activation	[132]
10.	Sofosbuvir/daclatasvir	Anti-HCV	Nucleoside analog/NS5A inhibitor	[75]
11.	Tenofovir	Anti-HIV	nucleotide analog reverse transcriptase inhibitor	[77]
12.	Molnupiravir	Anti-influenza	RNA mutagenesis inhibitor	[133]
13.	Danoprevir	Anti-HCV	NS3/4A protease inhibitor (Inhibiting the cleavage of HIV- encoded Gag-Pol polyproteins in the virus-infected cells to prevent the formation of mature infected virus cells)	[134]
14.	Nafamostat	Anti-influenza, anti-acute pancreatitis and anti-disseminated intravascular coagulation (DIC)	Viral replication inhibitor, Serine protease inhibitor (Inhibiting SARS-CoV-2 S-protein -mediated virus entry into the host epithelial cell)	[135]
15.	Lopinavir/Ritonavir (LPV/r)	Anti-HIV	Viral protease inhibitor	[69]
16.	Ivermectin	Anti-parasitic agent, anti-HIV	Viral protease inhibitor	[136]
Anti-inflammatory drugs				
1.	Tocilizumab	Anti-inflammation	Interleukin-6 inhibitors (Class-I Anti-IL-6 receptor monoclonal antibodies)	[71]
2.	Sarilumab	Anti-inflammation	Interleukin-6 inhibitors (Class-I Anti-IL-6 receptor monoclonal antibodies)	[68]
3.	Siltuximab	Anti-inflammation (undergone phase III clinical trial by FDA)	Interleukin-6 inhibitors (Class-II anti-IL-6 monoclonal antibodies)	[68]
4.	Ruxolitinib	Anti-inflammation, anti-myelofibrosis	Cytokine release inhibitors/Oral Janus kinases (JAK) inhibitor	[68]
5.	Baricitinib	Anti-inflammation, anti-rheumatic	Cytokine release inhibitors/JAK inhibitor	[68]
6.	Ruxolitinib	Anti-rheumatoid arthritis	JAK inhibitor	[137]
7.	Tofacitinib	Anti-rheumatoid arthritis	JAK inhibitor	[138]
8.	Imatinib	Anti-cancer	JAK inhibitor	[139]
9.	Fluvoxamine	Anti-depressant	Agonist for the sigma-1 receptor	[140]
10.	Methylprednisolone	Anti-inflammation, anti-immune system disorders	Proinflammatory cytokine production inhibitor	[141]
11.	Budesonide	Anti-asthmatic	Proinflammatory cytokine production inhibitor	[142]

Table 2 (continued)

S. no.	Drugs	Related disease	Action of the drug on SARS-CoV-2 targets	References
12.	Artesunate	Anti-malarial	Inhibitor for NF-κβ-coronavirus effect and chloroquine like endocytosis	[143]
13.	Type I interferons	Anti-multiple sclerosis	Balances the expression of pro- and anti-inflammatory agents	[144]
Other common drugs				
1.	Telmisartan	Anti-hypertension	Angiotensin receptor blocker	
2.	Nitazoxanide	Anti-parasitic	Pyruvate: ferredoxin/flavodoxin oxidoreductase cycle inhibitor	[145]
3.	Niclosamide	Anti-parasitic	Prevention of viral entry by altering endosomal pH, Autophagy inhibitors to prevent viral replication	[146]
4.	Bromhexine	Mucolytic	TMPRSS2 protease inhibitor	[147]
5.	Dornase alfa	Anti-cysticfibrosis	Recombinant human deoxyribonuclease I, Degrades extracellular DNA	[148]
6.	Dexmedetomidine	Sedation, analgesic, adjunct for anesthesia	Selective α-2 adrenoceptor agonist,	[149]
7.	Fluoxetine and Fluvoxamine	Anti-depressant	Selective serotonin reuptake inhibitors (SSRI-s)	[150]

few examples such as Remdesivir, Lopinavir/ritonavir, and chloroquine/hydroxychloroquine are being discussed here as a time being therapy that has preapproved by World Health Organization.

Remdesivir

Remdesivir is an antiviral drug that has shown promise in inhibiting the replication of several RNA viruses, including SARS-CoV-2 [61]. The researchers have found potent activity of remdesivir against SARS-CoV-2 in silico, in vitro, as well as in animal models of MERS and SARS [62, 63]. In May 2020, the US Food and Drug Administration (FDA) granted emergency use authorization (EUA) for remdesivir for the treatment of hospitalized COVID-19 patients [64]. This authorization was based on data from a clinical trial conducted by the National Institute of Allergy and Infectious Diseases (NIAID), which showed that remdesivir reduced the median time to recovery in hospitalized COVID-19 patients from 11 to 15 days [64]. Overall, remdesivir remains an important therapeutic option for the treatment of COVID-19, particularly in hospitalized patients.

Hydroxychloroquine

Hydroxychloroquine is an antimalarial drug that has been repurposed as a potential treatment for COVID-19, caused by SARS-CoV-2 [65]. The drug works by altering the pH of intracellular compartments, leading to inhibition of viral entry into cells and reduced replication of the virus [66]. Several in vitro investigations have shown that hydroxychloroquine has antiviral activity against SARS-CoV-2, which has led to interest in using the drug as a potential treatment for COVID-19 [65]. In addition, the drug and its combination with azithromycin has been associated with several adverse effects, including cardiac arrhythmias and gastrointestinal symptoms [65].

Ribavirin

Another antiviral drug is ribavirin that has been used to treat a range of viral infections, including respiratory syncytial virus (RSV), hepatitis C, and viral hemorrhagic fevers [67]. It is a broad-spectrum antiviral drug that works by inhibiting viral RNA synthesis, and has shown in vitro activity against SARS-CoV-2 [68]. In the context of COVID-19, ribavirin has been evaluated in combination with other drugs such as interferon and lopinavir/ritonavir [69]. However, due to its potential side effects and lack of efficacy in COVID-19 patients, ribavirin is not a recommended treatment option for COVID-19 [69].

Other Potential Inhibitors

Several other potential therapeutic agents have also shown promising effects in inhibiting SARS-CoV-2 replication in vitro and in preclinical investigations. These agents are currently being evaluated in clinical trials for their safety and efficacy in treating COVID-19 [70].

Tocilizumab

Tocilizumab is a monoclonal antibody that targets the interleukin-6 (IL-6) receptor, which is involved in the body's immune response to infection and inflammation [71]. It is a FDA-approved medication for the treatment of rheumatoid arthritis and other autoimmune diseases. Tocilizumab has also been investigated as a potential therapeutic agent for COVID-19 due to its ability to reduce inflammation and cytokine release syndrome (CRS), a severe immune overreaction that can occur in some patients with COVID-19 [72]. Several clinical trials have evaluated the safety and efficacy of tocilizumab in the treatment of COVID-19. Some studies have reported that tocilizumab can reduce the risk of mechanical ventilation or death in COVID-19 patients with severe symptoms [71]. For example, a randomized controlled trial demonstrated that tocilizumab significantly reduced the risk of death and the need for mechanical ventilation in hospitalized COVID-19 patients with severe respiratory disease [71]. However, there are also concerns about the potential side effects of tocilizumab, including an increased risk of serious infections, liver toxicity, and a reduction in white blood cell count [71]. Therefore, the use of tocilizumab in COVID-19 patients needs to be carefully evaluated on a case-by-case basis. The drug is currently not approved for the treatment of COVID-19, but it has received emergency use authorization from the FDA in some circumstances.

Galidesivir

Galidesivir is an antiviral drug that belongs to a class of drugs called nucleoside analogs and acts by inhibiting the replication of RNA viruses [73]. Galidesivir was originally developed to treat the Ebola virus and has shown promising results in preclinical studies against a broad range of RNA viruses, including SARS-CoV-2 [73]. In vitro studies have shown that galidesivir has potent activity against SARS-CoV-2, and this drug has also demonstrated efficacy in animal models of COVID-19. Galidesivir has been shown to reduce viral load and lung damage in hamsters infected with SARS-CoV-2. Clinical trials of galidesivir for the treatment of COVID-19 are ongoing, with results from phase 1 and 2 studies indicating that the drug is safe and well-tolerated. A phase 3 trial of galidesivir in hospitalized COVID-19 patients is currently underway [73]. Galidesivir's mechanism of action and broad-spectrum antiviral activity make it a promising candidate for the treatment of COVID-19. However, further studies are needed to evaluate its efficacy and safety in larger clinical trials [60, 74].

Sofosbuvir

Sofosbuvir is a direct-acting antiviral drug that has been used to treat chronic hepatitis C infection. It is a nucleotide analog inhibitor that acts by blocking the replication of the hepatitis C virus (HCV) by targeting the NS5B RNA polymerase enzyme, which is essential for HCV replication [75]. More recently, sofosbuvir has shown potential in inhibiting SARS-CoV-2 replication in vitro, and some clinical trials have suggested its potential benefits in treating COVID-19 [76]. In vitro studies have shown that sofosbuvir can inhibit the replication of SARS-CoV-2 by interfering with the virus's RNA-dependent RNA polymerase (RdRp) [76]. Several clinical trials have evaluated the safety and efficacy of sofosbuvir in combination with other drugs such as daclatasvir, ribavirin, and interferon in treating COVID-19 patients [75]. A randomized controlled trial conducted and reaveled that the combination of sofosbuvir and daclatasvir, along with standard care, was associated with a significant reduction in mortality and hospital stay in COVID-19 patients compared to standard care alone [76]. Another study evaluated the efficacy of sofosbuvir and ribavirin in treating COVID-19 patients with moderate to severe disease [75]. The study revealed that the combination of sofosbuvir and ribavirin was associated with a significant reduction in the time to clinical recovery and viral clearance. Sofosbuvir is generally well-tolerated, but some of its side effects include headache, fatigue, and gastrointestinal disturbances. It is also expensive and may not be widely available in some regions, which could limit its use as a potential therapeutic agent for COVID-19 [75]. Therefore, further clinical trials are needed to establish the safety and efficacy of sofosbuvir in treating COVID-19.

Tenofovir

Tenofovir is a nucleotide analog reverse transcriptase inhibitor that is commonly used as an antiviral drug to treat human immunodeficiency virus (HIV) infection. It works by inhibiting the reverse transcriptase enzyme, which is essential for viral replication [77]. Tenofovir has also been investigated for its potential to inhibit the replication of other viruses, including SARS-CoV-2. In vitro studies have shown that tenofovir can effectively inhibit SARS-CoV-2 replication [78]. Clinical trials have been conducted to evaluate the safety and efficacy of tenofovir in the treatment of COVID-19. In one randomized, controlled trial, patients with mild to moderate COVID-19 were treated with a combination of tenofovir and interferon alfa-2b. The study revealed that the combination therapy resulted in a shorter time to clinical recovery, a shorter duration of hospitalization, and a higher rate of viral clearance compared to standard of care [79]. Another study investigated the use of tenofovir disoproxil

fumarate (TDF) as pre-exposure prophylaxis (PrEP) for SARS-CoV-2 in healthcare workers [78]. The study found that TDF treatment was associated with a lower risk of SARS-CoV-2 infection in healthcare workers compared to those who did not receive TDF, while Tenofovir shows good agreement as a potential therapeutic agent for COVID-19, however, further research is needed to fully evaluate its safety and efficacy. Additionally, it is important to note that tenofovir is not currently FDA-approved for the treatment of COVID-19, and it should only be used under the supervision of a healthcare provider [78, 80].

In Silico Approaches to Combat SARS-CoV-2

In silico approaches to drug discovery involve the application of computational methods of screening and optimize the potential therapeutic agents [81, 82]. These methods encompass a range of techniques that leverage our understanding of the biological systems involved in the virus–host interaction, as well as our knowledge of the physicochemical properties of small molecules [17]. In recent years, researchers have used a combination of in vitro and in silico studies to investigate efficacy and the pharmacokinetic properties of lead molecules. In vitro studies involve experiments conducted in a laboratory setting, such as cell culture experiments or biochemical assays, whereas in silico studies involve computational methods, such as hierarchical virtual screening of small and large molecules or inhibitors, homology modeling, molecular docking, pharmacokinetic (ADMET) prediction, QSAR study and molecular dynamics simulations [83, 84]. Virtual screening involves the use of computer algorithms to search large databases of compounds to identify potential drug candidates that are likely to bind to specific viral proteins. There are two main types of virtual screening: ligand-based and structure-based. Ligand-based virtual screening uses the known structure of a ligand that binds to the target protein as a template to search for compounds that have similar structures and are therefore likely to bind to the target protein. On the other hand, structure-based virtual screening uses the 3-D structure of the target protein to search for compounds that can bind to specific regions of the protein.

A recent survey of COVID-19 drug discovery studies published in the Journal of Medicinal Chemistry found that the majority of studies (54%) used a combination of in vitro and in silico methods, whereas 28% of studies used only in silico methods and 18% of studies used only in vitro methods [85, 86]. Computational tools have been used to extract and analyze the structural information about pharmacophores and drug–receptor complexes. Some of the commonly used computational tools, program codes, and web servers are listed in Supporting Table 3.

In the context of SARS-CoV-2 drug discovery, virtual screening has been employed greatly to identify potential drug candidates that can bind to the druggable targets (discussed in section "Potential Targets for COVID-19"). Once potential lead compounds are identified, they can be further tested and optimized for their effectiveness and safety in treating COVID-19. One advantage of virtual screening is that it can rapidly identify potential drug candidates, reducing the time and cost required for drug discovery. However, it is important to note that virtual screening is a computational prediction and further experimental studies are needed to validate the effectiveness and safety of the identified compounds [14]. Looking an important role of in silico approaches using computational tools in the drug discovery, herein, we discussed the commonly used computational methods by researchers for curing from Covid-19 to reduce the failures in clinical trials and speed-up the drug design process.

Molecular Docking Simulations

Molecular docking simulation is a computational technique used to predict the binding affinity of small molecules to protein targets. These molecular simulations have played a crucial role in the drug discovery process for SARS-CoV-2, the virus responsible for COVID-19 [87–90]. By using docking simulations, researchers can identify potential drug candidates that can bind to viral proteins and inhibit their activity, ultimately preventing the virus from replicating and spreading. Docking simulations have been used to identify small molecules that can bind to specific regions of the spike protein and prevent it from binding to the human ACE2 receptor [91]. These studies have also identified small molecules that prevent proteases from functioning properly [92, 93]. Overall, docking studies can help to identify potential drug candidates for SARS-CoV-2 by predicting their binding affinity to viral proteins. However, it is important to note that these predictions need to be validated through further experimental studies to confirm their effectiveness and safety [94].

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations are a computational technique used to study the behavior of molecules over small fraction of time (nano seconds). By simulating the movements and vibrations of the atoms in the protein and drug, researchers can determine the strength and stability of their interactions, as well as identify any potential sites for optimization [95]. These simulations can be used to investigate the interactions between a drug and its target protein, providing valuable insights for drug discovery. Using MD simulations, researchers can study how drug molecules interact with the

SARS-CoV-2 determining the potential targets at an atomic level. Keretsu & co-workers have used MD simulations to study the interaction of the 19 antiviral drugs including remdesivir with the MProtease (3CL Pro protein) [62]. They observed the good binding affinity of saquinavir and three other investigational drugs aclarubicin, TMC-310911, and faldaprevir. MD simulations can also be used to screen large libraries of potential drug compounds for their ability to bind to the spike protein. By simulating the binding affinity and stability of thousands of compounds, researchers became able to identify promising candidates for further testing and development. Overall, MD simulations offer a powerful tool for drug discovery in the fight against SARS-CoV-2. By providing insights into the atomic-level interactions between drugs and their target proteins, these simulations has accelerate the discovery of effective therapeutics and helped to combat the COVID-19 pandemic [96].

Quantitative Structure–Activity Relationship (QSAR) Modeling

Quantitative structure–activity relationship (QSAR) modeling is a computational approach used to reveal the associated biological activity of a compound with its chemical structure [97, 98]. This technique has been widely used in drug discovery and development to identify compounds with specific biological activities and optimize their structures. In the case of SARS-CoV-2, researchers have applied QSAR modeling to identify potential drug candidates that can target the virus [99]. QSAR modeling involves building a mathematical relationship between the chemical structure of a compound and its biological activity against the virus. This relationship can then be used to predict the biological activity of new compounds that have not yet been synthesized or tested. To build a QSAR model for SARS-CoV-2 drug discovery, researchers first collect a dataset of compounds with known biological activities against the virus. This dataset is typically a combination of experimental data and in silico calculations. The dataset should also include information on the chemical structures of the compounds, such as their molecular weight, size, shape, and functional groups. Once the QSAR model is built, it can be used to screen large databases of compounds to identify potential lead candidates. These candidates can then be synthesized and tested in vitro and in vivo to confirm their biological activity and optimize their chemical structure. By predicting the biological activity of compounds based on their chemical structure, QSAR modeling can accelerate the discovery of effective therapeutics and help to combat the COVID-19 pandemic [99, 100]. Overall, QSAR modeling offers a powerful tool for drug discovery in the fight against SARS-CoV-2.

Homology Modeling

Homology modeling is a comparative computational modeling to predict the three-dimensional structure of a protein that has not been experimentally determined [99]. This technique relies on the assumption that the structure of a protein is more conserved than its sequence across evolution. Therefore, if the sequence of a target protein is known, and a homologous protein with a similar sequence and known structure exists, the structure of the target protein can be predicted by aligning the target sequence with the template sequence and transferring the template structure to the target sequence [101].

Several research groups have used homology modeling, comparative modeling (CM), template-based modeling (TBM), and template-free modeling (TFM) (ab initio or de novo modeling methods), to predict the structure of Mpro and screen a library of compounds for potential inhibitors against SARS-CoV-2 [102–104]. Homology modeling has been used extensively to study the structure of the virus's proteins, including the spike protein, main protease (Mpro), RNA-dependent RNA polymerase (RdRp), and papain-like protease (PLpro). As we have discussed in section "Potential Targets for COVID-19", these proteins are essential for the virus's replication and are considered important targets for drug development against COVID-19. Modeling studies of RdRp protein showed functional consequences for RdRp targeting potential therapeutics by investigating the alteration in the stability of RdRp protein by mutations [105, 106]. Another study by Dai & coworkers using homology modeling revealed the structure of RdRp and designed drugs that could potentially bind to the active site of the enzyme [107]. In some other studies the researchers used homology modeling to predict the structure of the spike protein receptor-binding domain (RBD) and design neutralizing antibodies that could bind to the RBD and block the virus's entry into cell [108–110]. Overall, homology modeling is a valuable tool for predicting the structure of COVID-19 targets and designing drugs that could potentially block the virus's replication. Combined with other in vitro and in silico methods, homology modeling has played a critical role in the development of potential COVID-19 therapeutics.

Using the above theoretical approaches, scientists have paid their attention for new lead molecules as well as repurposing the existing drugs for the medication of Covid-19. Remdesivir, an antiviral medication created initially to treat infections caused by the Ebola virus, is a possible inhibitor discovered through in silico research. In silico studies have shown that it has potent activity against SARS-CoV-2 by inhibiting the virus's ability to replicate. Clinical studies have demonstrated its effectiveness in treating COVID-19, and governing organizations in many nations have authorized its usage in emergency situations [64]. In the search for

potential inhibitors of SARS-CoV-2, another illustration was hydroxychloroquine, an antimalarial drug that gained widespread attention. In silico studies suggested that hydroxychloroquine could potentially interfere with the virus's ability to enter human cells. In addition to hydroxychloroquine and remdesivir, many other approved drugs such as antiviral drugs, natural products, and small molecules have been investigated as potential inhibitors of SARS-CoV-2 through in silico studies [63]. Number of flavonoids and alkaloids from natural sources have also been identified as potential hit molecules to inhibit Mpro activity [111, 112]. In silico studies have identified several compounds that can bind to RdRp and inhibit its activity, including remdesivir, which has been shown to be effective in clinical trials [113, 114]. In silico studies have identified several potential targets within the non-structural proteins (NSPs) that could be exploited for the development of antiviral drugs against SARS-CoV-2. Several studies have identified potential monoclonal antibodies, inhibitors of S protein, Nsp12 an RNA polymerase that are essential for viral replication [115, 116]. Overall, in silico studies have been helpful in the hunt for possible SARS-CoV-2 inhibitors and targets, offering insightful information about the biology of the virus and facilitating the speeding up of the drug discovery process.

Conclusion and Future Prospects

The current review emphasizes on SARS-CoV-2, potential druggable targets, inhibitors, and deep insights into in silico studies that are being used to drug design and repurposing of existing FDA-approved drugs. The comprehensive collection of viral enzymes and their counter receptors that are involved in SARS-CoV-2 entry in human cells, replication of genomic RNA, and controlling the host cell physiology provide the future direction to medicinal chemists toward drug design. The detailed insights about FDA-approved and other potential inhibitors that have been identified through various studies and are under the clinical trial stage have also been complied and are of interest for the researchers and budding scientific brains. The application of computational approaches such as docking simulations, molecular dynamics, QSAR modeling, and homology modeling that have been used to find plausible inhibitors against SARS-CoV-2 will also provide the future directions for research to investigate the large mountains of untouched molecules. Overall, this review fulfills its objective providing vital resources of promising drug targets, potential inhibitors, and advanced computational approaches to strengthen the researchers working on drug discovery for COVID-19 and other lethal diseases. The application of in silico investigation prior to the laboratory experiments will reduce the time, cost, and excessive use of living organisms to identify the potential drug candidates and their effectiveness with lower toxicity.

Although, in silico studies offer significant advantages for drug discovery, they also have certain limitations. These limitations include limited accuracy, which depends on the quality of the data and models used, and can be impacted by various factors such as protein flexibility and solvation effects. Additionally, the findings of in silico studies need to be validated experimentally to confirm their accuracy and to ensure the efficacy of potential drug candidates. Inability to account for unknown factors is another limitation, as computational studies rely on existing data and knowledge, and may not be able to account for unknown factors that may impact the effectiveness of potential drug candidates. Finally, there are ethical considerations associated with in silico studies, as they can identify potential drug candidates with ethical implications, such as drugs derived from endangered species or that are not economically viable for widespread use.

Amidst the continuing evolution of the SARS-CoV-2, the development of effective treatments and the discovery of possible drug targets and inhibitors is of paramount significance. In silico research can play a significant role in the process of finding possible targets and inhibitors. Several future directions have been identified to advance the effectiveness of computational studies in drug discovery for SARS-CoV-2 including the integration of multiple computational techniques, the utilisation of machine learning and artificial intelligence, exploring the repurposing of existing drugs and integration of experimental validation. These directions highlight the potential for advanced computational techniques to identify novel therapeutic targets and candidates for COVID-19 treatment.

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Declarations

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