Current Potential Therapeutic Approaches against SARS-CoV-2

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Contributor: Dharmendra Yadav

The ongoing SARS-CoV-2 pandemic is a serious threat to public health worldwide and, to date, no effective treatment is available. Thus, we herein review the pharmaceutical approaches to SARS-CoV-2 infection treatment. Numerous candidate medicines that can prevent SARS-CoV-2 infection and replication have been proposed. These medicines include inhibitors of serine protease TMPRSS2 and angiotensin converting enzyme 2 (ACE2). The S protein of SARS-CoV-2 binds to the receptor in host cells. ACE2 inhibitors block TMPRSS2 and S protein priming, thus preventing SARS-CoV-2 entry to host cells. Moreover, antiviral medicines (including the nucleotide analogue remdesivir, the HIV protease inhibitors lopinavir and ritonavir, and wide-spectrum antiviral antibiotics arbidol and favipiravir) have been shown to reduce the dissemination of SARS-CoV-2 as well as morbidity and mortality associated with COVID-19

Keywords: therapy ; SARS-CoV-2 ; combination therapy ; virus-based therapy ; host-based therapy ; SARS-CoV-2 cell entry inhibitors

1. Introduction

Coronaviruses contain positive-sense, single-stranded RNA, with a genome size ranging from 26–32 kb, and five structural proteins, and are classified into four categories: alpha, beta, gamma, and delta [1][2]. Human coronaviruses are alpha and beta coronaviruses which can cause respiratory and gastrointestinal tract infections [2]. The severe acute respiratory syndrome (SARS) outbreak between November 2002 and July 2003 (nine months) resulted in more than 8000 total cases and 774 deaths, with a fatality rate of 9.6% [3]. Middle East respiratory syndrome (MERS) was reported in 2012 resulting in more than 2400 cases and 858 deaths, with a fatality rate of 34.4%. Subsequently, in late December 2019, an unspecified case of pneumonia was reported in Wuhan, Hubei Province, the People's Republic of China [1][2][3]. COVID-19 is the official name given by the WHO to the disease caused by SARS-CoV-2 infection. It has since been observed that the virus could spread from human to human [4]. Its incubation period is 2 to 14 days with various clinical presentations: asymptomatic, mild to severe illness, and mortality [5]. Symptoms include fever, cough, difficulty breathing, malaise and fatigue, gastrointestinal symptoms (decreased appetite, vomiting, watery diarrhea, and dehydration), loss of taste and smell, sore throat, rhinorrhoea, severe pneumonia, and acute respiratory distress, which can lead to multiple organ failure and death. The SARS-CoV-2 virus is mainly spread via airborne/aerosol particles; the virus has been observed to remain viable and infective for over 3 h in the air [6][7]. SARS-CoV-2 infection is a highly communicable disease, and this pandemic has been designated a world public health emergency by the World Health Organization (WHO) [7]. However, SARS-CoV-2 has many potential natural, intermediate, and final hosts, as do other viruses; thus, major problems in the prevention and diagnosis of viral infection are raised [8]. In this paper we discuss the genetic structure of SARS-CoV-2 and its mechanism of pathogenesis. We include consideration of the phylogenetic analysis of the SARS-CoV-2 genome, multiple sequence alignment analysis, and therapeutic approaches to SAR-Co-V-2 infection.

2. SARS-CoV-2 Genetic Structure and Pathogenic Mechanism

The SARS-CoV-2 genome codes for more than 20 distinct proteins. At least four structural proteins are present in coronaviruses, namely spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (**Figure 1**). S proteins, which are involved in host attachment and virus-cell membrane fusion, determine the host range for viral infection (**Figure 2**) [9].

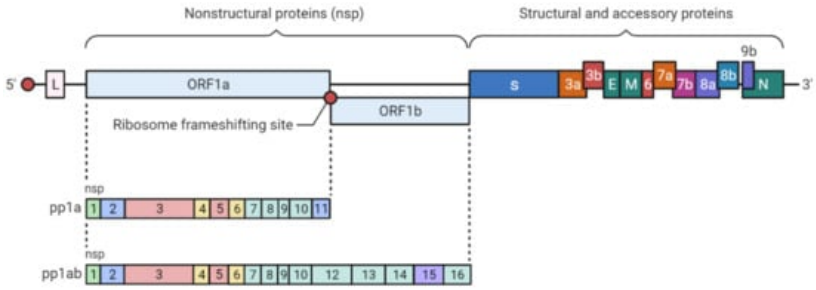

Figure 1. Genome structure of SARS-CoV-2. Figure was created by using BioRender (https://biorender.com, accessed on 15 September 2021).

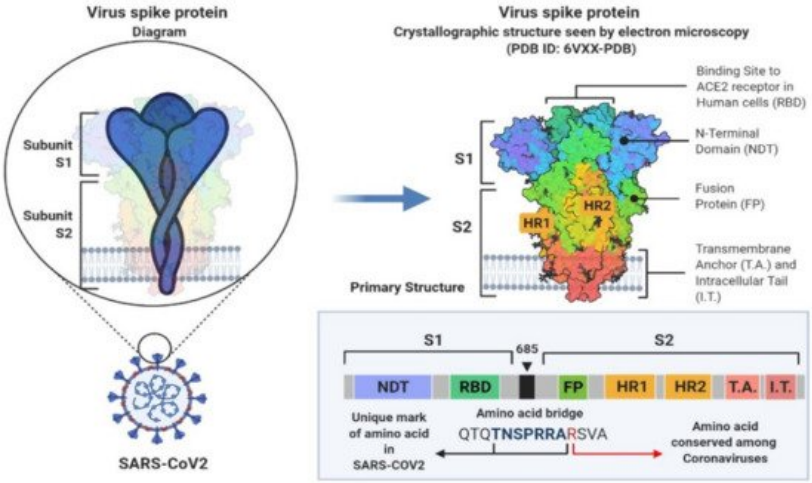

Figure 2. Crystallographic structure SARS-CoV-2. Figure was created using by BioRender (https://biorender.com, accessed on 15 September 2021).

The SARS-CoV-2 main protease (Mpro) is recognised as one of the most essential viral proteins. SARS-CoV-2 Mpro is more than 96% similar to SARS-CoV Mpro. During viral translation, SARS-CoV-2 Mpro cleaves 11 polyproteins to polypeptides that are required for transcription and replication [10]. Some of the candidate drugs that can prevent SARS-CoV-2 viral replication target Mpro, such as remdesivir, griffithsin, nafamostat, disulfiram, lopinavir/ritonavir, nelfinavir, danoprevir and favipiravir [11].

3. Phylogenetic Analysis of SARS-CoV-2 Genome

A sequence alignment and phylogenetic analysis of SARS-CoV-2 genome is shown in **Figure 3**. The phylogenetic tree is primarily divided into three clades [12]. Clade I consist of SARS-CoV and Bat-SL-CoV genomes which share a sequence identity ranging from 88% to 99%. Clade II consist of 13 complete genomes of coronavirus and MERS-CoV genomes which share a sequence identity from 78% to 89%. Clade III consist of 23 SARS-CoV-2 and Bat-SL-CoV complete genomes which share a sequence identity ranging from 89% to 100%; the SARS-CoV-2 genomes isolated from human samples show a sequence identity ranging from 98% to 100% [13]. A particularly interesting observation from the analysis was that there is no major divergence in the SARS-CoV-2 genome sequence of different SARS-CoV-2 virus genomes isolated from different countries, as shown in **Figure 3**. The sequence alignment of the SARS-CoV-1 (Bat, PDB ID: 3TNT) and the SARS-CoV-2 (human, PDB ID: 7MBI) main proteases reveals that the amino acid sequence is conserved with a sequence identity of 96%; differences between these genomes are shown in **Figure 4** at specific positions [13][14].

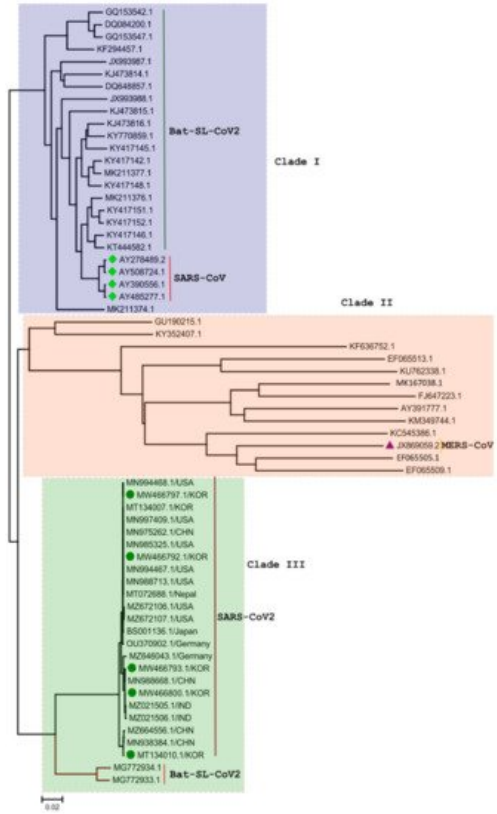

Figure 3. The phylogenetic tree was generated using the latest complete genome sequences of different neighbors, MERS-CoV, SARS-CoV, and Bat-SL-CoV. The tree is divided into three major clades according to the grouping of clusters: Clade I: Bat-SL-CoV-2 and SARS-CoV viruses showing a close evolutionary relationship with each other. Clade II: Human and bat coronaviruses, including MERS-CoV. Clade III: All of the SARS-CoV-2 genomes isolated from humans—it was observed that these genomes show a close evolutionary relationship with Bat-SL-CoV-2.

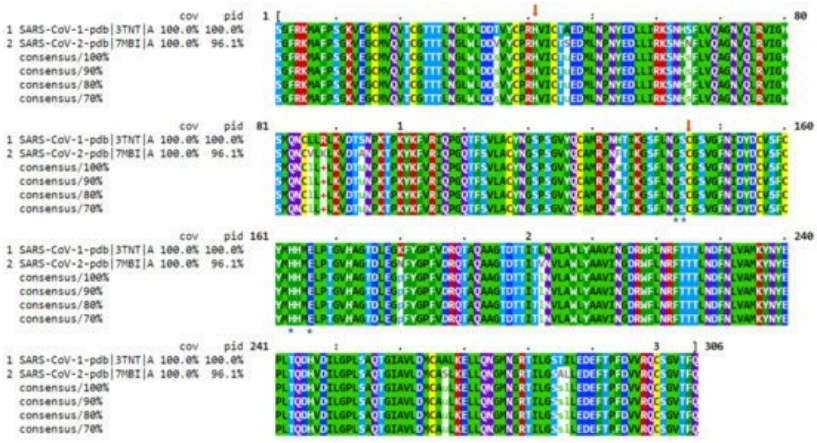

Figure 4. Multiple sequence alignment analysis of the amino acid sequence of SARS-CoV-1 and SARS-CoV-2 Mpro. Amino acids marked underneath with an arrow represent catalytic residues; residues marked underneath with * represent substrate-binding residues of various subsites.

4. Therapeutic Approaches to SAR-COV-2 Infection

To identify therapeutic agents that are effective against SARS-CoV-2 infection, extensive research on the structure and pathogenesis of COVID-19 is in progress [15]. Therapeutic approaches to COVID-19 can be categorized into virus-based therapy and host-based therapy, as shown in **Figure 5**.

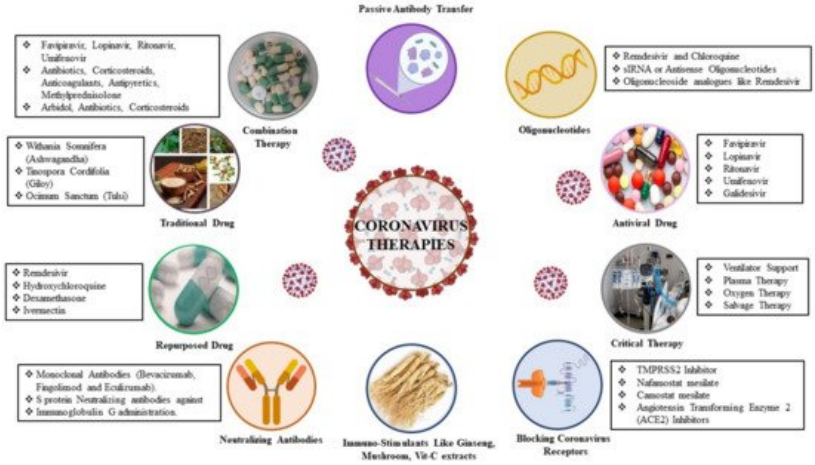

Figure 5. Therapeutic approaches to SARS-CoV-2 infection.

4.1. Virus-Based Therapy

Viral nucleic acids consist of nucleosides and nucleotides. Drugs capable of attacking nucleotides, nucleosides, or viral nucleic acids can affect the activity of a broad range of coronaviruses and other viruses, as shown in **Table 1** [16]. Possible targets for antiviral therapy include major enzymes and proteins involved in SARS-CoV-2 viral replication. The PLpro enzymes and papain-like protease of SARS-CoV and MERS-CoV have been shown to exert proteolytic, deubiquitylating, and deISGylating activities. Studies have shown that lopinavir-ritonavir is the most potent protease inhibitor as shown in **Table 1** [17]. The main SARS-CoV-2 immunogen antigen is the Spike glycoprotein with membrane anchor, which plays an important role in the interaction between host cells and viruses. Studies have shown that certain monoclonal antibodies can target the receptor binding domain (RBD) subunit epitopes and inhibit viral cell receptor binding, whereas other monoclonal antibodies bind to the S2 subunit and disrupt viral cell fusion [18]. A study using the CR3022 neutralising antibody of SARS-CoV shown in **Table 1** [19]. Earlier trials also showed that adoptive transfer of plasma containing anti-MERS-COV-S antibodies had the ability to prevent infection and accelerate viral clearance.

Table 1. Virus-based therapy: Drugs capable of attacking nucleotides, nucleosides, or viral nucleic acids of a broad range of coronaviruses and other viruses.

Antiviral Agent	Drug Target	Mechanism of Action	Infectious Disease	References
Remdesivir	**RdRp**	**Terminates the non-obligate chain**	**SARS-CoV-2, MERS-CoV, SARS-CoV**	[20]
Favipiravir	**RdRp**	**Inhibits RdRp**	**SARS-CoV-2, Influenza**	[21]
siRNA	**RdRp**	**Short chains of dsRNA that interfere**	**SARS-CoV, MERS-CoVWu**	[22]
Galidesivir	**RdRp**	**Inhibits viral RNA polymerase function by**	**Galidesivir SARS-CoV-2,**	[23]
Ribavirin	**RdRp**	**Inhibits viral RNA synthesis and mRNA capping**	**SARS-CoV-2, MERS-CoV, SARS-CoV,**	[24]
LJ001 and JL103	**Lipid membrane**	**Membrane-binding photosensitizers that induce**	**Enveloped viruses (IAV, filoviruses, poxviruses, arenaviruses, bunyaviruses, paramyxoviruses, flaviviruses and HIV-1)**	[25]
CR3022	**Spike glycoprotein**	**Immunogenic antigen against Spike protein**	**SARS-CoV-2, SARS-CoV**	[26]
Griffithsin	**Spike glycoprotein**	**Griffithsin binds to the SARSCoV-2 spike**	**SARS-CoV-2**	[27]
Peptide (P9)	**Spike glycoprotein**	**Inhibits spike protein-mediated cell-cell entry or**	**Broad-spectrum (SARS-CoV, MERS-CoV, influenza)**	[28]
Nafamostat	**Spike glycoprotein**	**Inhibits spike-mediated membrane fusion A**	**SARS-CoV-2, MERS-CoV**	[29]
Ritonavir	**3CLpro**	**Inhibits 3CLpro**	**SARS-CoV-2, MERS-CoV**	[30]

Antiviral Agent	Drug Target	Mechanism of Action	Infectious Disease	References
Lopinavir	3CLpro	Inhibits 3CLpro	SARS-CoV-2, MERS-CoV, SARS-CoV, HCoV-229E, HIV, HPV	[31]
Darunavir and cobicistat	3CLpro	Inhibits 3CLpro	SARS-CoV-2	[32]

4.2. Host-Based Therapy

Viral entry of SARS-CoV-2 depends on the priming of its spike protein and on transmembrane protease 2 (TMPRSS2). Further studies have shown that camostat mesylate, a serine protease inhibitor, can block TMPRSS2 activity and is thus considered as a therapeutic candidate as shown in **Table 2** [33]. Other research indicates a pH- and receptor-dependent endocytosis when coronavirus is introduced into the host cell. AP-2-associated protein kinase 1 (AAK1), a host kinase, controls clathrin-mediated endocytosis [34]. Since the virus structure is now established, various inhibitors have been tested in cell-based systems for their ability to prevent viral entry and replication within the host body, as shown in **Table 2** [35]. These include spike (S) protein inhibitors, S-cleavage inhibitors, helicase and protease inhibitors, fusion core blockers, HCB monoclonal antibodies, RBD–ACE2 blockers, antiviral peptides, siRNAs, and antifreeze eutralizati antibodies [35][36]. The following section concentrates on the possible therapeutic treatment options based on our limited knowledge of SARS-CoV-2.

Table 2. Host-based therapy: Drug target and mechanism of action against infectious diseases.

Antiviral Agent	Drug Target	Mechanism of Action	Infectious Disease	References
Baricitinib	Clathrin-mediated endocytosis	Baricitinib	Clathrin-mediated endocytosis	[34]
Chloroquine	Endosomal acidification	A lysosomatropic base that appears to disrupt intracellular trafficking and viral fusion events	SARS-CoV-2, SARS-CoV, MERS-CoV	[33]
Convalescent plasma	-	Inhibits virus entry to the target cells	SARS-CoV, SARS-CoV-2, Influenza	[35][36]
Camostat Mesylate	Surface protease	Potent serine protease inhibitor	SARS-CoV, MERS-CoV, HcoV-229E	[33]
Corticosteroids	Pulsed methylprednisolone	Patients with severe MERS who were treated with systemic corticosteroid with or without antivirals and interferons had no favorable response	SARS-CoV, MERS-CoVL	[35]
Nitazoxanide	Interferon response	Induces the host innate immune response	Coronaviruses, SARS-CoV-2	[19]
Recombinant interferons	Interferon response	Exogenous interferons	SARS-CoV-2, SARS-CoV, MERS-CoV	[37]

4.2.1. Neutralizing Antibodies

In general, coronavirus infection begins with the entry of the viral S protein, which binds to the cell surface. This S protein fuses with the cell membrane and facilitates the syncytial development and transmission of viral nucleocapsids into the cell for further replication [35]. Studies have shown that neutralization of the S protein RBD of SARS-CoV [36] and MERS-CoV [38][39][40] by antibodies can be effective against these diseases. Neutralisation of antigens can be highly useful in COVID-19 treatment, given that the S protein RBD sequence of SARS-CoV-2 is similar to those of SARS-CoV and MERS-CoV [37]. Critical COVID-19 patients are currently treated with immunoglobulin G [35][36]. FcR plays a role in inflammation in the lung; therefore, inflammation in COVID-19 can be reduced by blocking FcR activation. Thus, intravenous administration of immunoglobulins can be effective in the treatment of pulmonary inflammation, as shown in **Table 3** and **Figure 6** [41].

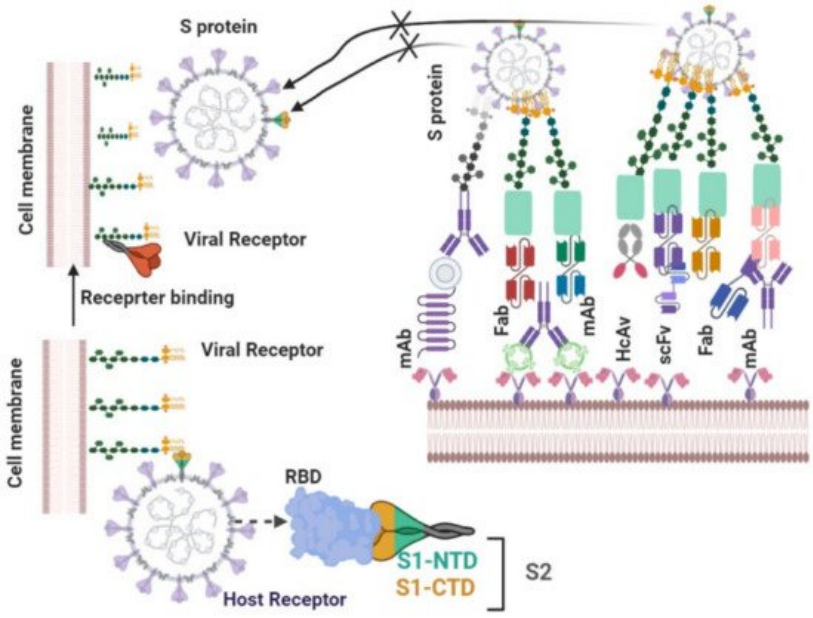

Figure 6. Schematic of binding mechanism of SARS-CoV-2 spike protein to the receptor.

Table 3. Neutralizing antibodies against SARS-CoV-2.

S.N.	Antibody Name	Antibody Type	Origin	PDB ID	Epitopes	Neutralizing Mechanism	Cross Neutralizing Activity	Protective Efficacy	Ref
1	CV30	Human IgG	Infected COVID-19 patients	6XE1	D420-Y421, Y453, L455-N460, Y473-S477, F486-N487, Y489, Q493, T500, G502, Y505	Block hACE2-RBD interaction	no	IC50 value of 0.03 μg/mL	[35]
2	REGN10933 Recombinant	full-human antibodies	Humanized mice and COVID-19-convalescent patients	6XDG	R403, K417, Y421, Y453, L455-F456, A475-G476, E484-Y489, Q493	Block hACE2-RBD interaction, ADCC & ADCP	no	IC50 value of 37.4 pM	

S.N.	Antibody Name	Antibody Type	Origin	PDB ID	Epitopes	Neutralizing Mechanism	Cross Neutralizing Activity	Protective Efficacy	Ref
3	B38	Human IgG	COVID-19-convalescent patient	7BZ5	R403, D405-E406, Q409, D420-Y421, Y452, L454-N460, Y473-S477, F486-N487, Y489-F490, Q493-G496, Q498, T500-V503, Y505	Block hACE2-RBD interaction	no	A single dose of B38 (25 mg/kg)	[35]
4	CC12.1	Human IgG	COVID-19-convalescent patient	6XC3	R403, D405-E406, R408-Q409, D420-Y421, Y453, L455-N460, Y473-S477, F486-N487, Y489, Q493-G496, Q498, T500-V503, Y505	Block hACE2-RBD interaction	no	IC50 value of 0.019 μg/mL	[36]
5	CB6	Human IgG	COVID-19-convalescent patient	7C01	R403, D405-E406, R408-Q409, D420-Y421, L455-N460, Y473-S477, F486-N487, Y489, Q493, Y495, N501-G502, G504-Y505	Block hACE2-RBD interaction	no	A single dose of CB6-LALA (50 mg/kg)	[37]

S.N.	Antibody Name	Antibody Type	Origin	PDB ID	Epitopes	Neutralizing Mechanism	Cross Neutralizing Activity	Protective Efficacy	Ref
6	C105	Human IgG	COVID-19-convalescent patient	6XCN, 6XCM	R403, D405, R408, D420-Y421, Y453, L455-N460, Y473, A475-G476, F486-N487, G502, Y505	Block hACE2-RBD interaction	no	IC50 value of 26.1 ng/mL	[41]
7	CC12.3	Human IgG	COVID-19-convalescent patient	6XC7	R403, D405, D420-Y421, Y453, L455-N460, Y473-S477, F486-N487, Y489, Q493, G496, N501, Y505	Block hACE2-RBD interaction	no	IC50 value of 0.018 μg/mL	[42]
8	CR3022	Human IgG	SARS-convalescent patient	6YOR, 6 W41	Y369-N370, F374-K386, L390, F392, D428, T430, F515-L517	Trapping RBD in the less stable up conformation while leading to destabilization of S	SARS-CoV, SARS-CoV-2	ND50 value of 0.114 μg/mL	[19]
9	EY6A	Human IgG	Late-stage COVID-19 patient	6ZDH, 6ZER, 6ZCZ	Y369, F374-S375, F377-K386, N388, L390, P412-G413, D427-F429, L517	destabilization of S	SARS-CoV, SARS-CoV-2	ND50 value of ~10.8 μg/mL	[26]
10	VHH-72	Llama single domain antibody	llama immunized with prefusionstabilized betacoronavirus spikes	6WAQ	Y356-T359, F361-C366, A371-T372, G391-D392, R395, N424, I489, Y494	Trapping RBD in the less stable up conformation while leading to destabilization of S, Block hACE2_RBD interaction	SARS-CoV, SARS-Co-V-2	IC50 values of 0.14 μg/mL and 0.2 mg/mL.	[19]

S.N.	Antibody Name	Antibody Type	Origin	PDB ID	Epitopes	Neutralizing Mechanism	Cross Neutralizing Activity	Protective Efficacy	Ref
11	BD23	Human IgG	COVID-19-convalescent patient	7BYR	G446, Y449, L452, T470, E484-F486, Y489-F490, L492-S494, G496, Q498, T500-N501, Y505	Block hACE-RBD2 interaction	no	IC50 value of 8.5 µg/mL	[26]
12	Fab 2–4	Human IgG	Infected COVID-19 patients	6XEY	Y449, Y453, L455-F456, E484-F486, Y489-F490, L492-S494, G496	Locking RBD in the down conformation while occluding access to ACE2	no	Neutralizing SARS-CoV-2 live virus with IC50 value of 0.057 µg/mL	[41]

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