Chapter

Repurposed Therapeutic Strategies towards COVID-19 Potential Targets Based on Genomics and Protein Structure Remodeling

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Abstract

Target recognition is important for the identification of drugs with a high target specificity and/or for the development of existing drugs that could be replicated for the treatment of SARS-CoV-2 infections. Since SARS-CoV-2 is a pathogen recently discovered, no specific medicines have been identified or are available at present. The scientific community had proposed list of current drugs with therapeutic potential for COVID-19 on the basis of genomic sequence information coupled with protein structure modeling, posing an effective and productive therapeutic approach for repurposing existing drugs. The possible therapeutics for the treatment of COVID-19 involves a wide range of alternatives, encompassing nucleic acid-based treatments directed at the expression of genes of viruses, cytokine therapy, genetic engineered and vectored antibodies, and different formulations of vaccines. The future prospective in the treatment approaches the exploration of antiviral therapy, such as screening of prevailing molecules or libraries, testing of existing broad-spectrum antiviral medications, modern drug discovery focused on genomic knowledge and biochemical properties of various coronaviruses to create new targeted drugs.

Keywords: SARS-CoV-2, drug repurposing, molecular docking, mRNA vaccine, monoclonal Antibodies

1. Introduction

The challenge of the epidemic outbreak has reached alarming levels, shocking national healthcare systems to unpreparedness and causing international deployment. No drug therapy has been found to be effective in the treatment of the virus, despite COVID-19 being declared a global pandemic by the World Health Organization (WHO). In contrast, several randomized controlled trials conducted towards treatment have not yet provided practical guidance on therapeutic choices and pharmacologic therapy. Several successful research tests for therapy are currently underway. Other emerging, non-conventional drug discovery approaches include alternative ways to discover potent anti-SARS-CoV2 drugs that are quicker and less expensive. In addition, while drugs for COVID-19 are being repurposed and discovered, new drug delivery systems will play a major role in developing

effective delivery systems that have the potential to attack viruses, enhance physico-chemical characteristics, and avoid possible drug resistance that contributes to superior therapies. The best way to produce pharmaceutical drugs that cure SARS-CoV-2 is to find potential molecules from the medicines available for sale [1].

Coronavirus disease 19 (COVID-19) is a remarkably highly contagious and pathogenic infectious disease caused by severe acute respiratory syndrome- coronavirus-2 (SARS-CoV-2), which originated in Wuhan, China in December 2019 and spread worldwide. The infectibility of these viruses could only be in the wild before the outbreak of extreme acute respiratory syndrome (SARS) in 2002 and middle-eastern respiratory syndrome (MERS) in 2012 as spotted by the world. While the disease dissipated across the globe throughout the natural environment, the mode of transmission to humans from the wild was insignificant and still unknown. Analysis of the whole genome sequence of the bats, however, was shown to be 96% similar with a severe acute respiratory syndrome-like (SARS-like) bat virus and 79.5% comparable with SARS-CoV, indicating that it is the possible route of transmission to humans [2]. There are four genera of CoVs: Alphacoronavirus, Betacoronavirus (βCoV), Gammacoronavirus, and Deltacoronavirus. Two new βCoVs, extreme acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV), have appeared over the past 12 years, and these viruses can cause significant human illnesses. The absence of adequate drug treatment and related elevated morbidity and death rates of these two CoVs, as well as their ability to cause epidemics, illustrate the need for innovative drug development for the prevention of diseases of CoV [3].

2. Genomic characterization of SARS-CoV-2

The size of the genome of the coronavirus ranges from 26 to 32 kb and contains 6 to 11 open reading frames (ORFs) encoding polyproteins with 9680 amino acids [4]. About 80 percent of the SARS-CoV-2 genome has been studied to be similar to the previous human coronavirus (SARS-like bat CoV) while, notable differences in SARS-CoV and SARS-CoV-2 genome have been reported in various studies, such as the lack of 8a protein and perturbations in the number of amino acids in 8b and 3c protein in SARS-CoV-2 [5]. The SARS-CoV2 genome is a polycistronic single-stranded RNA (+ssRNA) with a 5′-cap structure and 3′-poly-A tail (~30 kb), utilized as a template for translating polyproteins (pp1a/pp1ab) into the replication/transcription machinery (RTM) of a double membrane vesicle (**Figure 1**) (6) [6]. RTM subsequently synthesizes a nested set of subgenomic RNAs (sgRNAs) in a discontinuous manner. These subgenomic messenger RNAs (mRNAs) have standard 5′-leader and 3′-terminal sequences between open reading frames (ORFs) on transcription regulatory sequences, where transcription termination and subsequent acquisition of a leader RNA occurs. Such minus-strand sgRNAs serve as models for subgenomic mRNA growth. At least six ORFs comprise the genome and subgenomes of a standard CoVs [7].

For the ORFs from the 5′ end, a region of about 20 kb corresponds to the two ORFs; ORF1a and ORF1b encoding 11 and 5 non-structural proteins: nsp1 to nsp11 and nsp12 to 16, respectively. The largest SARS CoV2 ORF1 gene (about two-thirds of the total length of the gene) contains −1 frameshift between ORF1a and ORF1b, resulting in the formation of two conserved polypeptide domains: pp1a and pp1ab. The ribosomal frameshift is involved in the translation of ORF1a directly from the RNA genome, near to the bottom of ORF1, which contains one ORF1ab polypeptide. There are ORFs encoding a few to more than ten structural/non-structural proteins downstream from the ORF1ab [8]. In ORF1ab as well as in other ORFs, CoVs

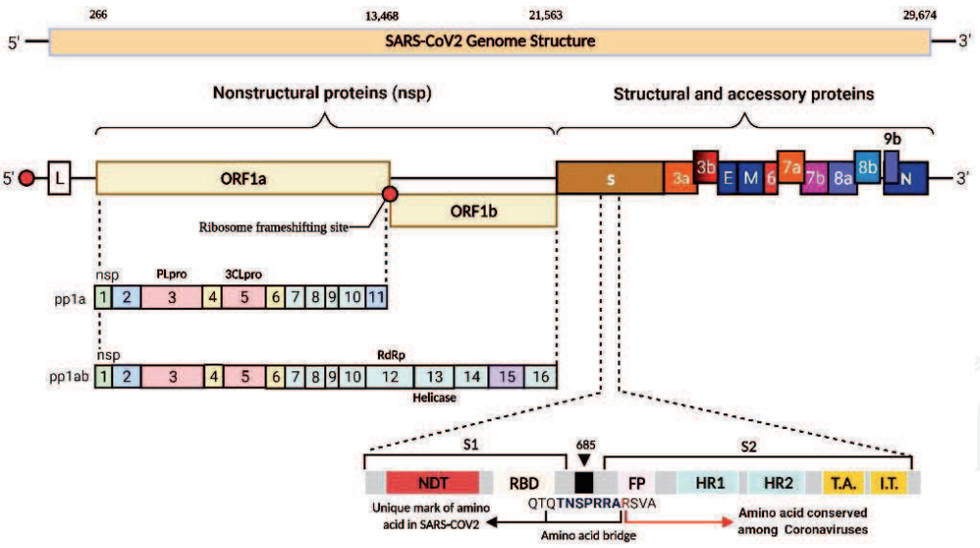

Figure 1.
Genomic organization of SARS-CoV-2. Schematic genomic structure of SARS-CoV-2 based on the SARS-CoV-2 Wuhan-Hu-1. The genome is categorized into two domains: Non-structural proteins and structural and accessory proteins. The S protein contains an S1 and S2 subunit, which are divided by the S cleavage site. Abbreviations: ORF, open reading frame; S, spike; E, envelope; M, membrane; N, nucleocapsid; NTD, N-terminal domain; RBD, receptor-binding domain; FP, fusion peptide; HR1 & 2, heptad repeat 1 and heptad repeat 2, containing the core binding motif in the external subdomain; TA, transmembrane anchor; IT, intracellular tail.

often code different non-structural proteins, particularly near the 3′ end, although the specifics of the exact genes in the SARS-CoV-2 genome are still unclear primarily due to overlapping genes encoded in a different coding frame [9]. Two viral proteases, papain-like protease (PLpro) and 3C-like protease (3CLpro), are cleaved by the large replicase polyprotein 1a (pp1a) and pp1ab encoded by the 5 terminal open reading frame 1a/b (ORF1a/b) to produce non-structural proteins (NSPs) [10]. The NSPs contain two viral cysteine proteases (nsp3), chymotrypsin like, 3C like, or main protease (nsp5), RNA-dependent RNA polymerase (nsp12), helicase (nsp13) and others that may be involved in SARS-CoV-2 transcription and replication. [4]. In addition, four major structural proteins are coded by ORFs on a third of the genome near the 3′ terminus: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (**Figure 2**). Different CoVs encode unique structural and accessory proteins, such as HE protein, 3a/b protein, and 4a/b protein, in addition to these four major structural proteins. The sgRNAs of CoVs is translated back from both these structural and accessory proteins [11–13].

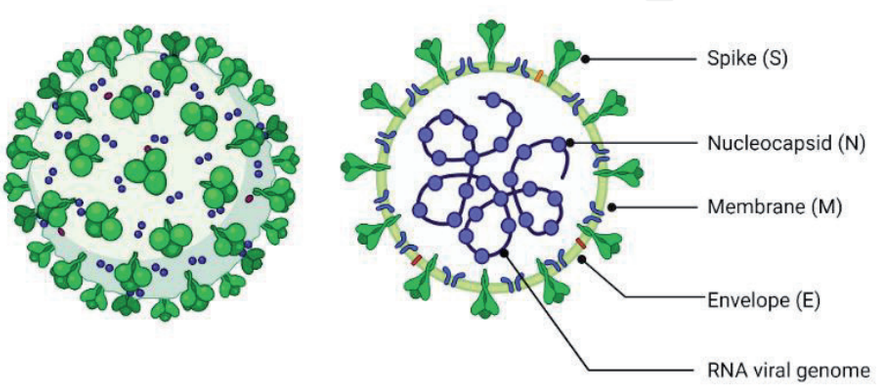

Figure 2.
The virion structure of SARS-CoV-2. The spike (S), envelope (E), membrane (M) proteins form the envelope of the CoV, and the nucleocapsid (N) proteins form the capsid to pack the genomic RNA.

3. Structural insights of potential SARS-CoV-2 proteins

The structural research was initiated by studying in silico modeling Wuhan-Hu1 market seafood pneumonia virus genome [14, 15]. Focused on reported studies on SARS-CoV-2, we highlighted the number of nsp structures of this virus available: the papain-like protease (PLpro, nsp3; PDB code: 7D47), the main protease (3CLpro, nsp5; PDB code: 6LZE), the RNA-dependent RNA polymerase (RdRp, nsp12) in complex with cofactors nsp7 and nsp8 (PDB code: 6M71), the helicase (nsp 13; PDB code: 6ZSL), the Spike glycoprotein (S) (PDB code: 6XR8) and the RNA binding domain of nucleocapsid (N) phosphoprotein (PDB code: 6VYO).

3.1 SARS CoV2 nsp3 PLpro

For SARS-CoV2, the papain-like proteinase (PLpro) domain is included in nsp3 (1945 amino acids; located in the polyprotein, from 818 to 2819 aa), a 217.28 kDa membrane-associated replicase product [16]. Nsp3 consists of two transmembrane regions and approximately 10–16 recognizable domains, nine of which are conserved, responsible for cleavages at the polyprotein replicate's N-terminus and assembly of viral-induced double-membrane cytoplasmic vesicles and viral replication, with nsp4 (**Figure 3**). It prevents the holding of NF-kappa-B signals. The inhibition of IRF3 host phosphorylation and dimerization and subsequent nuclear translocation was antagonized with type 1 interferon innate immune stimulation [17]. Additionally, PLpro has a deubiquitinating/deISGyling activity and processes cellular substrates from both 'Lys-48'-and' Lys-63′-linked polyubiquitin chains [18]. The role of Nsp3 is important to CoV replication and its domains include several predicted or demonstrated RNA replication accessories, such as ssRNA binding and unwinding domains, as well as those for which no separate function has yet been determined [19], cleaves ISG15 in vitro preferentially from substrates and utilizes host ADP-ribosylation to bind ADP-ribose [20].

3.2 SARS CoV2 nsp5 M^{pro}

The SARS-CoV-2 nsp5 contains 306 amino acids (located in the replicase polyprotein pp1ab, 3264 to 3569 aa), a 33.8-kDa main protease (M^{pro}), which is also referred to as 3C-like protease ($3CL^{pro}$) [21, 22]. The active site in SARS-CoV2 M^{pro} organized in between Domain I (8–99 aa) and Domain II (100–183 aa) was shown to be structurally similar to SARS-CoV M^{pro}. Both domains contribute one residue to the catalytic dyad (His41 and Cys145), linked to the helical domain IIII (200–306 aa)

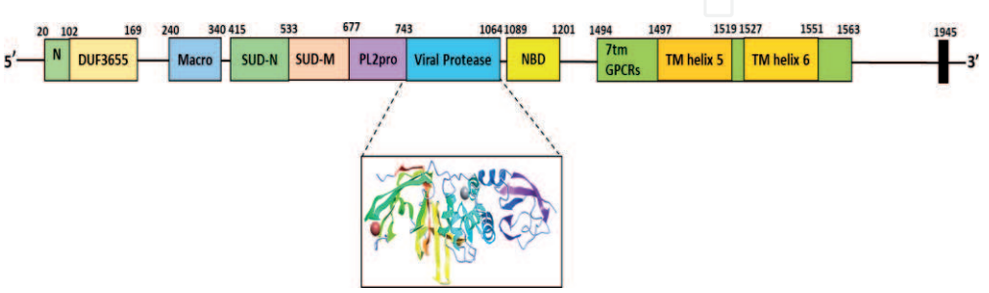

Figure 3.
Overview of the SARS-CoV2 nsp3 structural and genomic organization along with crystal structure of SARS-CoV-2 papain-like protease (PLpro). (PDB ID: 7D47). Abbreviations: DUF3655, protein of unknown function; macro, macro domain; SUD-N, SARS-unique domain binding G-quadruplexes; SUD-M, single-stranded poly(a) binding domain; PL2pro, coronavirus polyprotein cleavage domain; viral protease, papain like viral protease; NBD, nucleic-acid binding domain; 7tm GPCRs, seven-transmembrane G protein-coupled receptor superfamily; TM helix 5&6, transmembrane helix 5&6.

by a long loop region (184–199 aa) (**Figure 4**) [6]. Several co-crystalized SARS-M^{pro} structures bound with inhibitors, 11a and 11b (6LZE), I2 (2D2D), N1 (1WOF), N3 (2AMQ), and N9 (2AMD), revealed the position of S1, S2, and S4 subsites, especially in the active site close to His41 and Cys145, which is crucial for substrate recognition containing the core sequence [ILMVF]-Q-|-[SGACN] along with Tyr161 and His163 in the substrate-binding pocket [23]. Studies have estimated that the M^{pro} pp1ab protein has at least 11 conserved restriction sites, beginning with its autolytic cleavage site and even able to bind ADP-ribose-1′ [24, 25].

3.3 SARS CoV2 nsp12 RdRp

The SARS-CoV-2 nsp12 is a key component of the replication/transcription machinery sharing a strong structural homology with nsp12 of SARS-CoV, indicating that its function and mode of action may be well conserved [26]. SARS-CoV-2 nsp12, is a 106.7 kDa molecular weight protein with a chain length of 932 amino acids (located on the replicase polyprotein 1ab, 4393 to 5324 aa.), also referred to as RNA dependent RNA polymerase (RdRp) [27], has the capacity to synthesize the entire (−) RNA chains as a positive sense RNAs, as templates for additional genomic RNA (gNR) and subgenomic RNAs generation (sgRNAs) of the virus [21]. RNA-dependent RNA polymerases (RdRps) are a multi-domain protein that catalyzes polymerization of RNA-template (phosphodiesters formation between ribonucleotides), in the presence of divalent metal ions and thus play a major role in the viral replication and transcription of the SARS-CoV2 [28, 29]. The SARS-CoV-2 nsp12 (S1 to Q932) exists in complex with cofactors nsp7 (S1 to Q83) and nsp8 (A1 to Q198) heteromer with the second nsp8 attached to the distinct binding site. nsp12 contains the right-hand RdRp domain (S367 to F920) and the nidovirus-specific N-terminal β hairpin (D29 to K50) and extension domain (D60 to R249) which adopts the nidovirus RdRp-associated nucleotidyltransferase (NiRAN) architecture (**Figure 5**). The RdRp domain is divided into 3 sub-domains; the finger subdomain (L366–A581 and K621–G679), the palm subdomain (T582–P620 and T680–Q815), and the thumb subdomain (H816–E920) [30]. The nsp7-nsp8 heterodimer binding site is well conserved in the palm domain and overlaps with the functional domains of the preserved polymerase regions (fingers and thumb domains). In addition, seven preserved motifs (A-G) arranged in the polymerase active site domain, involved in template and nucleotide binding and catalysis, were also revealed by SARS-CoV-2 nsp12 structure [31]. Motif A contains the residue of classical divalent ion-binding glutamate (D) in position (618) & (623) of the conserved sequence

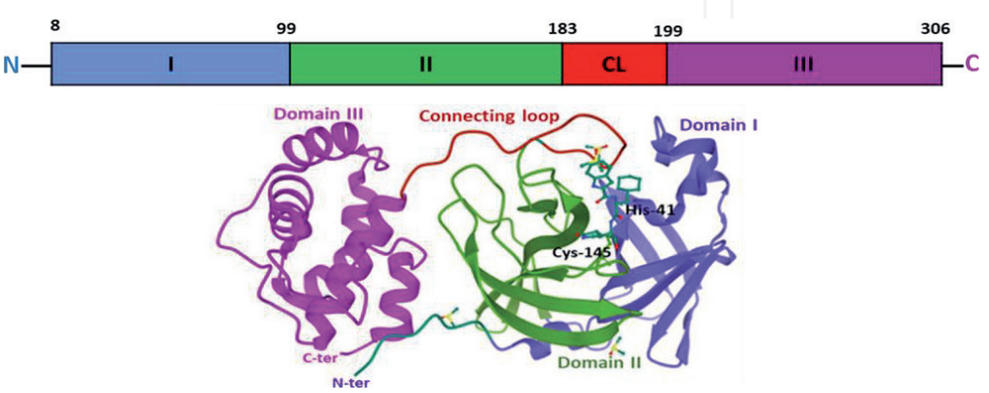

Figure 4.
Structural representation of SARS-CoV-2 M^{pro} monomer (PDB ID: 6LZE) (ribbon representation) composed of: N-terminal domain I (cornflower blue), domain II (green), and C-terminal domain III (pink). Substrate recognition site in (green and red) and catalytic dyad residues, His41 and Cys145 are highlighted and labeled.

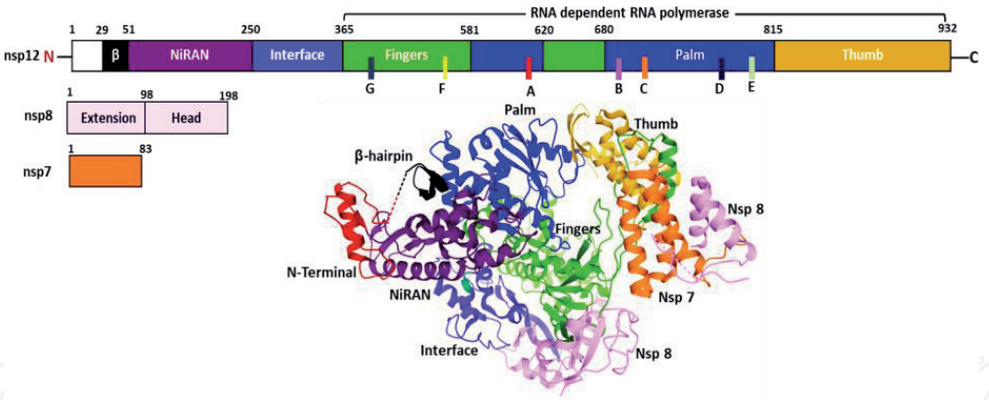

Figure 5.
Domain organization of COVID-19 virus nsp12 and structural ribbon representation of the SARS-Cov-2 RNA-dependent RNA polymerase in complex with cofactors nsp7-nsp8 (PDB ID: 6M71) displaying domains, conserved motifs (A-G), and protein regions in the structure. A broad N-terminal extension (red) consisting of the NiRAN domain (violet) and the interface domain (cornflower blue) adjacent to the polymerase domain comprises SARS-CoV nsp12-nsp7(orange)-nsp8 (light pink) complex. The interdomain borders are labeled with residue numbers.

611-TPHLMGW**D**YPKC**D**RAM-626. In motif C, (753- FSMMIL**SDD**AVVCFN-767), the catalytic residues of 759-SDD-761 (amino acids 759,760 and 761 are positioned between two β strands. Many other catalytic residues such as 317-**GDD**-319 and 327-**GDD**-329 are also conserved among other viruses [32].

3.4 SARS CoV2 nsp13 helicase

SARS-CoV-2 nsp13 is a 66.85 KDa protein, with a chain length of 601 aa. (located replicase polyprotein pp1ab, from 5325 to 5925 aa), also referred to as the Helicase [33]. SARS-CoV Nsp13 is a key enzyme in the disassembly of double stranded oligonucleotides into single strand using hydrolyzed energy of NTPs [34], having a N-terminal zinc-binding domain (ZBD) containing 3 zinc-finger motifs consisting of 2 tandem C-terminal RecA-like helicase domains (RecA1 and RecA2) and bridging stalk and 1B domains (**Figure 6**). The RecA-like domains catalyze the unwinding of the double stranded RNA and the NTP hydrolysis translocation of the complex. The stalk domain acts as a connection between the domain RecA-like/1B and the ZBD, which acts as an interface with other replicative machinery components [35, 36]. Nsp13 is strongly conserved among nidoviruses (percentage

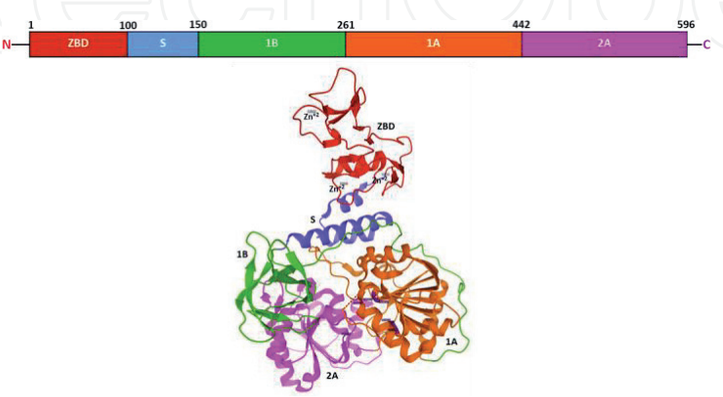

Figure 6.
Domain representation of SARS-CoV2 nsp13 and the structural ribbon representation of the SARS-CoV-2 helicase (PDB ID: 6ZSL). The interdomain borders are labeled with residue numbers. The colors of the protein domains are indicated in panel a (ZBD-red, stalk-cyan blue, 1B-green, 1A-orange and 2A-pink). Three zinc atoms are shown as black spheres. The NTPase active site (violet) consists of six residues-Lys288, Ser289, Asp374, Glu375, Gln404 and Arg567.

similarity of 99.8% with SARS-CoV, the only 1 amino acid substitution I570 V) consists of five domains that fold in a triangular pyramid shape [37]. These domains hold incredibly high conservation of protein sequences along with its essential role in viral replication, thus making it a vital target for a wide variety of therapeutics to target this viral family [38]. Helicase exhibits various enzymatic roles, including not only hydrolysis of NTPs required by the capping process, but also removal of 50–30 directionally RNA duplexes and 50-triphosphatase RNA activity. In addition, the association of Helicase nsp13 with RdRp Nsp12 promotes RNA unwinding activity and is a crucial viral replication enzyme, in all coronaviruses [39].

3.5 Spike glycoprotein (S)

The newly discovered SARS-CoV2 S-glycoprotein is a glycosylated trimer, each protomer with a chain length of 1260 amino acids (residues 14–1273), with a molecular weight of 141.1 kDa, consisting of two subunits, the surface subunit S1 and the transmembrane unit S2 [40, 41]. The surface subunit S1 is composed of 672 amino acids (residues 14–685) and differentiated into four divisions: N-terminal domain (NTD), a C-terminal domain (CTD, also known as the receptor-binding domain, RBD), and two subdomains (SD1 and SD2). The transmembrane S2 subunit is composed of 588 amino acids (residues 686–1273) and contains an N-terminal hydrophobic fusion peptide (FP), two heptad repeats (HR1 and HR2), a transmembrane anchor (TA), and an intracellular tail (IT), arranged as FP-HR1-HR2-TA-IT (**Figure 7**). A polybasic amino acid bridge (−QTQT-**NSPRRAR**-SVA-), essential for viral targeting studies, links S1 and S2 together (**Figure 1**). The SARS-CoV-2 S glycoprotein shares similar structural, topological and mechanistic features with other class I fusion proteins, including HIV envelope (Env) glycoprotein and influenza virus haemagglutinin (HA), as a standard class I viral fusion protein [42]. However, using crystallography, the actual structure of this protein can be studied. The Protein Data Bank (PDB) model of the glycoprotein shows various regions that are vital for the infection process compose the subunits [43]. Spike glycoprotein of

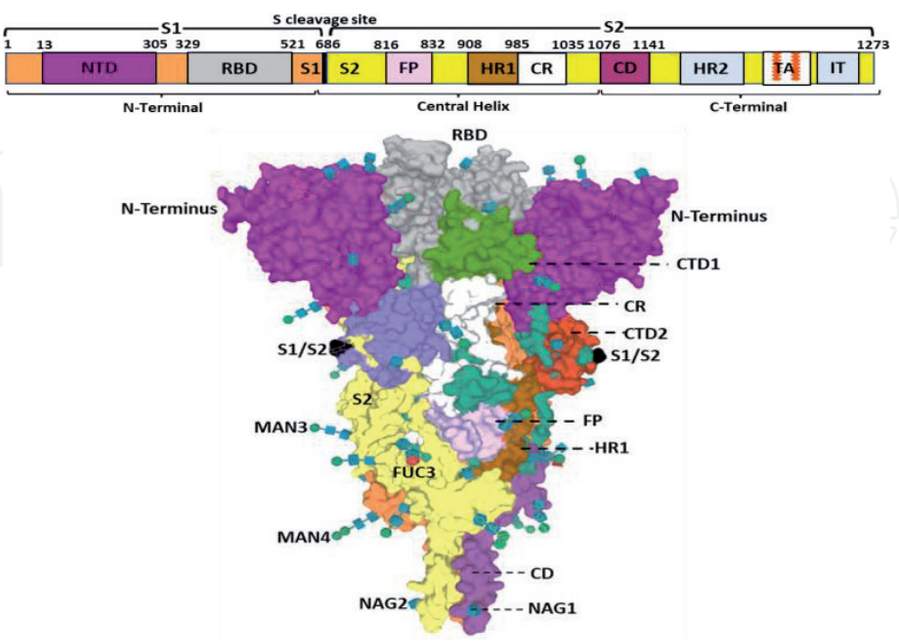

Figure 7.
Crystal structure of SARS-CoV 2 spike protein (S) PDB ID: 6XR8. Different domains of the spike protein that includes; signal sequence (SS), the N-terminal domain (NTD), receptor-binding domain (RBD), subdomain 1 and 2 (SD1&2), protease cleavage sites (S1/S2/S2′), fusion peptide (FP), heptad repeat 1 and 2 (HR1&2), central helix (CH), connector domain (CD), transmembrane anchor (TA), and intracellular tail (IT).

the Wuhan coronavirus is specified to be modified by homologous recombination which is supposed to be a combination of the SARS-CoV bat and the not identified Beta-CoV bat. The fluorescent study shows SARS-CoV-2 still uses the same ACE2 (angiotensin-converting enzyme 2) receptor and the entrance pathway previously used by SARS-CoV [44]. Both receptor binding expressed on the cell membranes of receptive cells and membrane fusion are responsible for the S protein. However, in the assembly and budding of viral particles, the proteins M and E are involved [9].

3.6 Nucleocapsid (N)

SARS-CoV-2's N (Nucleocapsid) protein is a putative RNA-binding protein responsible for gathering the viral RNA genome for encapsulation in the viral membrane into compact ribonucleoprotein (RNP) complexes [45]. N is the structural unit present in the nucleocapsid of the SARS-CoV-2 genome, 46 kDa protein and comprises of 419 amino acids (aa) containing three distinctly diverse domains: The N-terminal domain (NTD)/RNA-binding domain (46–174 aa), the serine/arginine-rich (SR-rich; 184–197 aa) linker region (LKR; 175–254 aa) and the C-terminal domain (CTD; 255–364 aa) (**Figure 8**) [46]. Both domains (NTD & CTD) are flanked by an inherently disordered region IDR, which is the central linker region (LKR) with a Ser/Arg (SR)-rich area containing alleged phosphorylation sites [47, 48]. An asymmetric unit crystal configuration of the N-terminal RNA nuclear protein binding field showed the sandwiched fold shared by the CoV N-NTD with the same right hand (loop) - (β-sheet core) - (loop), with the order β1-η1-β2- β3- β4- β5- β6- β7. The core β-sheet consists of five β-strands with just 3_{10} helix ahead of strand β2 and a β-strand between the strands β2 and β 5. The β-hairpin is functionally important in mutational analysis of amino acid residues at the RNA binding N-terminal domain of SARS-CoV2 [49]. The structures of the N-CTD RNA binding domain highlight the reserved architecture of 3_{10} helix acidic coil containing a β-sheet core of 5 antiparallel β-sheets, and an expanded β3–4 hairpin with the order η1-α1-α2-η2-α3-α4-β1-β2-α5-η3. Generally, the RNA binding domain of the N protein is essentially simple and sometimes defined as a right hand-like shape with a protruding fundamental finger, basic palm and acidic wrist [50].

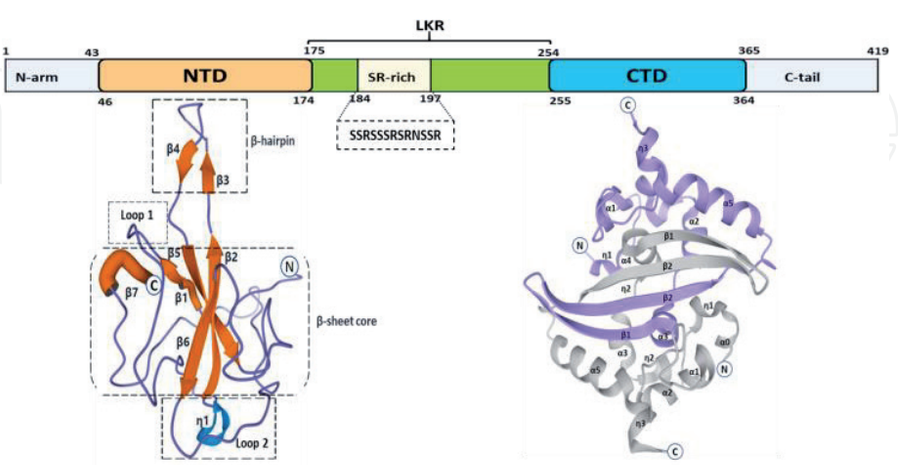

Figure 8.
Schematic representation of SARS-CoV-2 Nucleocapsid (N) protein domains. Three intrinsically disordered regions, i.e., N-arm, linker region (LKR) and C-tail, and the N-terminal domain (NTD) and C-terminal domain (CTD) are illustrated. The charged Ser/Arg (SR)-rich motif (colored light yellow) is shown. Cartoon view of the N protein NTD domain (PDB ID: 6M3M), a monomer, colored by the number variants at different positions, and CTD (PDB ID: 7C22), a heterodimer with one monomer colored gray and the other colored blue indicating various regions within the structure as observed in SARS-CoV-2 genomes.

4. Repurposing therapeutics towards SARS-CoV-2 potential targets

Therapeutics facilities aid physicians and scientist to help patients with the better cure for the disease and develop a vaccine. The production of novel therapeutics and vaccination is now at the earliest possible time to curb the epidemic. In addition, continuous attempts are being made at regional, national, and person levels to recognize the host, genomics, epidemiological interactions, and propagation modes of SARS-CoV-2 in order to control the epidemic. Scientists worldwide are actively working to establish an optimal antiviral agent and vaccine against SARS-CoV-2 [51]. One way of preventing this issue is to test two or three drugs with minimal prolixity that operate on various cellular signals with viral replication. A further approach that helps researchers to curb the variety of individual antimicrobials for emerging and re-emerging infectious diseases is the high-performance screening of host-virus interaction level composite libraries for synergistic combinations [52, 53]. Nevertheless, the screening of experimental small molecule drugs in combinations and other potent anti-SARS-CoV-22 agents with new and improved key characteristics can be effective which can be helpful in COVID-19 therapy. Potential medicines include previously used or tested medicines for diagnosing diseases and medicines recently discovered or developed. In most cases, the benefits of drug repurposing are safety evaluation, preclinical monitoring, and in some cases the development processes would not take much time and thus reduce the time needed for drug development. The chances of failure with repurposed drugs would be smaller as the early steps of medication efficacy and safety testing have already been completed and the treatment has already been shown to be sufficiently effective in a preclinical and human model [54]. Antiviral therapies are being investigated towards treatment of COVID-19 because the SARS-CoV-2 replication of leads to wide variation of the clinical manifestations. As significant functional proteins of SARS-CoV-2, Nsps and other structural and accessory proteins are involved in transcription of RNA, synthesis of protein, post-translational modification, viral duplication and contagion of the host. Among them, PLpro (nsp3), 3CLpro (nsp5), RdRp (nsp12), Helicase (nsp13), Spike glycoprotein (S) and Nucleocapsid (N) are the most important targets for the development of small-molecule inhibitors due to the enzyme active site and clear biological functions [55].

4.1 Protease inhibitors

Protease inhibitors are drugs that inhibit the activity of protease enzyme responsible for viral development, infection, and replication by cleaving it into smaller fragments. They bind to the active site of the enzyme, mediate and block the maturation of freshly formed virions [51]. Two major targets: PLpro and 3-CLpro in the replicase 1a and 1ab domain are essential component for virus reproduction and regulation of host cell response thus being the important target for the SARS-CoV-2 inhibitors (**Figure 9**). Ribavirin (SCH-18908) [NCT00578825], an antiviral drug acts on the replicase protein, preventing binding of the nucleotides, resulting in reduced viral replication or the formation of defective virions. Lopinavir [NCT04321174] and Ritonavir [NCT04330690], are protease inhibitors used to treat HIV infections by inhibiting the HIV protease enzyme to form an inhibitor-enzyme complex and proteolytic cleavage of the viral polyprotein precursors [3]. Darunavir [NCT01448707], another (HIV-1) nonpeptidic protease inhibitor with the inhibition activity of the dimerization and catalytic activity of HIV-1 protease. Saquinavir [DB01232] inhibiting HIV1/2 protease-mediated lysis of HIV gag and pol polyproteins was found cytotoxically active at conc. Above 50 μM [56]. Rupintrivir (DB05102) a broad-spectrum

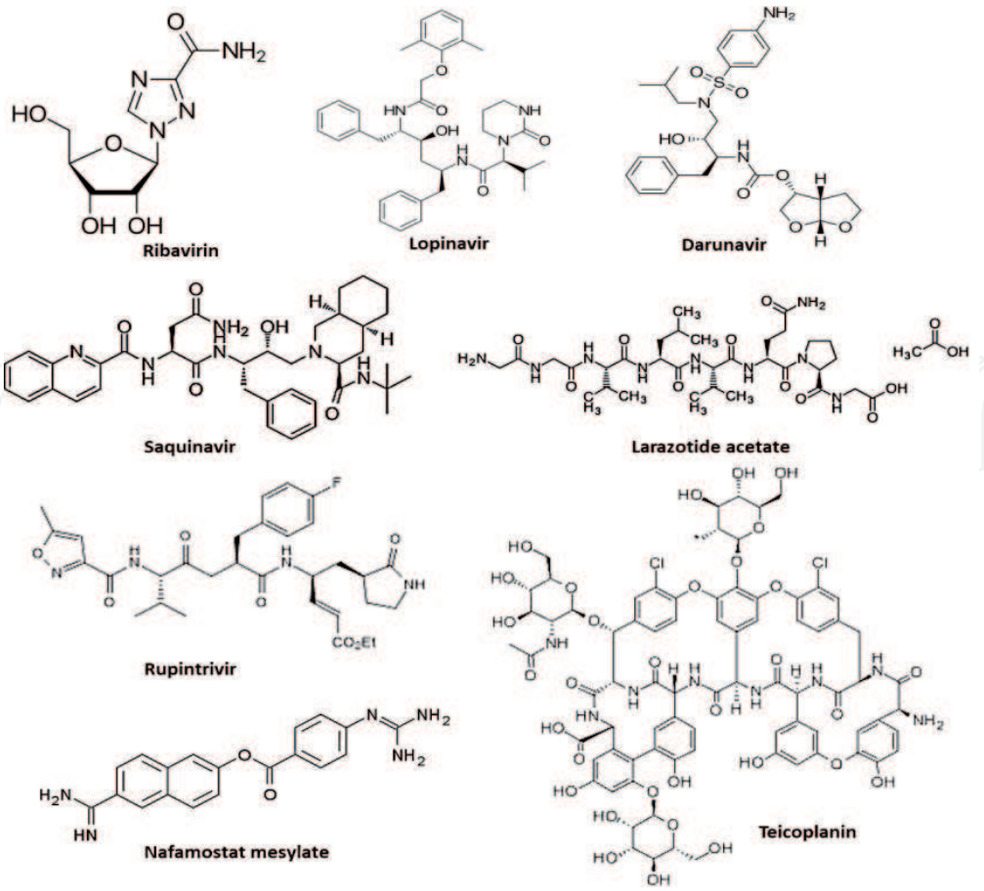

Figure 9.
Repurposed protease inhibitors for COVID-19 treatment.

antiviral agent is a potent 3C-Like protease inhibitor against norovirus, picornavirus, and coronavirus proteases in development against human rhinoviral (HRV) infections that has been recently co-crystallized with its target [57]. Larazotide acetate (AT1001) (NCT03569007) a paracellular permeability peptide inhibitor has been studied for the treatment of autoimmune diseases such as type I diabetes mellitus, gastrointestinal diseases and disorders. Studies have revealed that AT1001 potentially binds to the M^{pro} catalytic domain showing up intermolecular interactions [58]. Teicoplanin (DB06149), a glycopeptide antibiotic widely used to treat bacterial infections, has been reported to be active against SARS-CoV-2 in *in-vitro* studies inhibiting peptidoglycan polymerization, resulting in inhibition of bacterial cell wall synthesis and cell death [59, 60]. Nafamostat mesylate [NCT04418128], a clinical proven and synthetic serine protease inhibitor has been identified to inhibit the activity SARS-CoV-2 by targeting TMPRSS2-dependent host cell entry [61, 62]. However, *in-vitro* studies of active phytochemical compounds from the natural sources have revealed the SARS-CoV-2 proteolytic and inhibitory activity; Phaitanthrin D (*Isatis indigotica Fort*), Baicalin (*Scutellaria baicalensis*), Piceatannol (*Vitis vinifera*), Platycodin D (*Platycodon grandiflorus*), Betulonal (*Cassine xylocarpa*), 2b-Hydroxy-3,4-secofriedelolactone-27-oic acid (*Viola diffusa*), Cleistocaltone A (*Cleistocalyx operculatus*), Phyllaemblinol (*Phyllanthus emblica*) etc. [55].

4.2 Replicase inhibitors

RdRp is a critical non-structural protein component of the SARS-CoV-2 genome that uses a metal-ion-dependent mechanism to catalyze viral RNA synthesis. However, due to its comprehensive knowledge about the different domains and its functions, strong preservation among evolutionary RNA viruses and the lack

of homologous sequences in mammalian cells; the ease progress and consequent access to biochemical assays to quickly detect large collections of compounds [63]. Remdesivir (GS-5734) [NCT04252664], a nucleoside analogue acts on the replicase polyprotein 1ab of SARS-CoV-2 genome preventing RNA polymerase function resulting in ending the transcription of RNA and reduces the production of viral RNA. Penciclovir [NCT00820534], another nucleoside analogue, synthetic acyclic guanine derivative with antiviral activity targets the RdRp inhibiting the DNA synthesis of virus-infected cells and terminating viral replication (**Figure 10**). β-D-N4-hydroxycytidine, an orally bioavailable, broad-spectrum antiviral ribonucleoside analogous to multiple RNA viruses like influenza, CoV, equine encephalitis and Ebola viruses have been found in *in-vitro* studies of antiviral effect on SARS-CoV-2 primarily through mutagenesis of viral RNA [64, 65]. Cefuroxime, an anti-bacterial drug is prescribed in patients with COVID-19, as a potential inhibitor with the ability of binding tightly to the active site of the enzyme, with a highest ICM score of −41.30, and mfscore of −63.04, which when compared to Remdesivir had a score of −27.4 and a mfscore of −113 [66] The natural products and derivatives with anti-virus, anti-inflammation and anti-tumor effects exhibited high binding affinity to RdRp, such as 14-deoxy-11,12-didehydroandrographolide (*Andrographis paniculate*), Gnidicin (*Gnidia lamprantha*), 2b,30b-dihydroxy-3,4-seco-friedelolactone-27-lactone (*V. diffusa*), Theaflavin 3,30-di-O-gallate (*Camellia sinensis*), Betulonal (*C. xylocarpa*), 1,7-dihydroxy-3- methoxyxanthone (*Swerti apseudochinensis*) [67].

SARS-CoV-2 replication enzyme, helicase has the properties of unwinding and splitting DNA and RNA into two single-stranded nucleic acids and these

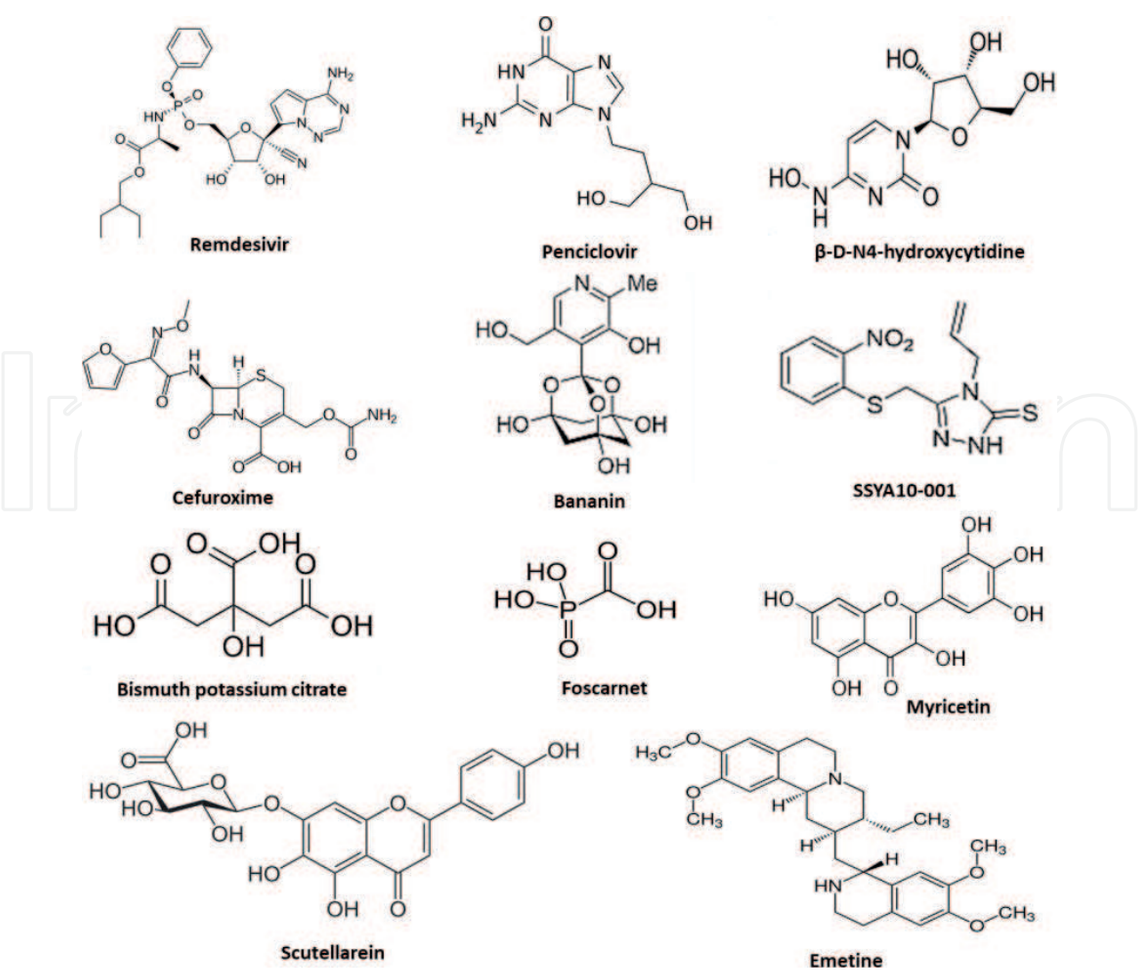

Figure 10.
Repurposed replicase inhibitors for COVID-19 treatment.

characteristic of the enzyme marks it as a potential target to be studied [68]. Bananins have been shown to inhibit SARS-CoV ATPase activity leading to inhibition of viral replication *in-vitro* with IC50 values far less than 10 mM [69] Bismuth salt, such as Bismuth potassium citrate (BPC) has been observed to dose-dependently inhibit both the NTPase and RNA helicase activities of SARS-CoV-2 nsp13. Further studies indicates that SARS-CoV-2 nsp13 may be a valuable target for antivirals, which could be beneficial in attempting to regulatory mechanism of this life-threatening virus [70]. Foscarnet, a class of antiviral drug approved for the treatment of HIV/AIDS-related cytomegalovirus (CMV) infections and herpes, functions as a pyrophosphate molecule by binding to viral DNA polymerase and preventing the elongation of the DNA chain [71]. *In vitro* studies have revealed that SSYA10–001, a small inhibitor molecule of SARS-CoV helicase, has an antiviral effect on several coronaviruses by likely targeting a conserved binding pocket in nsp13 domain and can lead the production of successful wide spectrum anti-coronavirus drugs including SARS-CoV-2 [72]. Scutellarein isolated from *Scutellaria baicalensis* has been historically used in the treatment of inflammation and respiratory diseases, and myricetin present in fruits such as cranberry and vegetables such as *Calamus scipionum* and garlic. Were found to inhibit SARS-CoV helicase (nsp13) via inhibition of ATPase activity being a potential phytochemical inhibitor [73, 74]. Emetine could be re-purposed to treat COVID-19 based on the *in-vitro* antiviral activity against SARS-CoV-2 and its ability to shield chicken embryos from IBV [75].

4.3 Structural protein inhibitors

For the entry of coronavirus into a host cell, transmembrane Spike (S) glycoprotein is essential. Fusion of the membrane and activation of the virus entry require cleavage at the S1–S2 junction and because of their vital role in the interaction between the virus and the cell receptor, spike protein can be an important potential target for antiviral agents [76]. The receptors that mediate the fusion of the S protein of SARS-CoV2 into the host cell are angiotensin converting enzyme-2 (ACE-2) and type 2 transmembrane protease serine (TMPRSS2). Cleavage via TMPRSS2 the fusion of the S protein to the ACE-2 receptor is achieved and thus are the potential drug targets in the SARS-CoV-2 therapeutics [51]. Umifenovir (Arbidol) [NCT04350684], an antiviral drug has been found to block and effectively prevent the trimerization and cell adherence and entry of SARS-CoV-2 spike glycoprotein interacting with the key residues in the target domain indicating as the potential target [77]. Griffithsin, a lectin protein has demonstrated antiviral properties and can potently inhibit viral entry and prevent binding to the S glycoprotein both in vitro and in vivo SARS-CoV infection with limited cytotoxic effects. Previous studies have shown griffithsin to inhibit MERS-CoV infectivity in *in vitro* assays without any noticeable cytotoxicity [78]. Camostat mesylate [NCT04608266] can feasible therapeutic choice for COVID-19 as it decreases the unregulated release of cytokine observed in extreme COVID-19, regardless of its antiviral function, because TMPRSS2 expression is necessary for vigorous secretion of cytokine when mice are exposed to polyIC [79]. Bromhexine hydrochloride [NCT04355026] (BRH) inhibits the viral entry of transmembrane protease serine 2 (TMPRSS2) and is potentially known to be protective against SARS-CoV-2 [80]. Eriodictyol, a *Herba santa* flavanone, is a popular herbal medicine used to cure asthma and colds. In silico studies predicts, eriodictyol binds to almost all targeted proteins with good energy and has shown its relevance for COVID 19 therapy [81].

SARS-CoV-2 nucleocapsid (N) protein is a multifunctional protein that plays a key role in the assembly of the virus and its transcription of the RNA. During

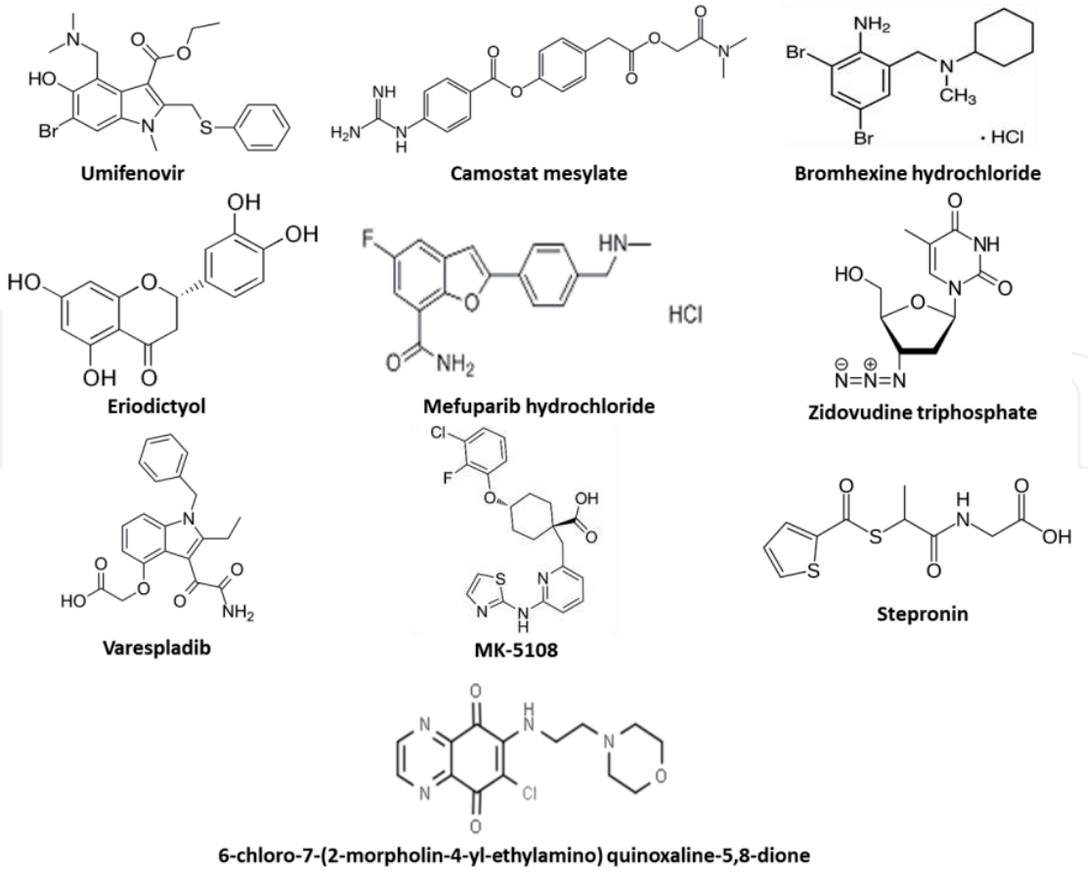

Figure 11.
Repurposed structural and accessory protein inhibitors for COVID-19 treatment.

the packing of the RNA genome, the N protein is critical in the creation of helical ribonucleoproteins, controlling viral RNA synthesis during replication and transcription. Infected host cells and their cellular functions are also regulated by the N protein [82]. Previous studies identified a special ribonucleotide-binding pocket composed of strongly conserved residues in the NTD by solving the complex structure of CoV NP-NTD bound with ribonucleotide monophosphate. Compounds that bind to this RNA-binding pocket may inhibit normal NP function and may be used to regulate CoV diseases. 6-chloro-7-(2-morpholin-4-yl-ethylamino) quinoxaline-5,8-dione (H3) (**Figure 11**) revealed inhibitory activity on the RNA-binding of NP [83]. Mefuparib hydrochloride, CVL218, a Poly [ADP-ribose] polymerase 1 (PARP1) inhibitor have shown to exhibit effective inhibitory activity against SARS-CoV-2 replication without apparent cytopathic effect in *in-vitro* and *in-silico* studies [84]. Zidovudine triphosphate, an anti-HIV drug, acts as a potential inhibitor of the N-terminal domain of SARS-CoV2 N-protein based on docking and simulation research, and can be considered to repurpose for therapeutic options to fight COVID-19 [85]. Out of 13 molecules analyzed for the molecular simulation study on SARS-CoV-2 nucleocapsid protein, 3 (Varespladib, MK-5108, Stepronin) have been found to be show inhibitory activity on SARS-CoV-2 N protein with high docking score [86].

5. Vaccines approved for emergency use authorization (EUA)

Various vaccines across the globe are in the progress of development against COVID-19, while the efficacy, productive and stability of most of them is still unclear. The latest report from the World Health Organization states about 69

S.No.	Candidate	Developer	Type	Approval Status (EUA)
1	BNT162b2	Pfizer–BioNTech	mRNA	FDA Approved
2	mRNA-1273	ModernaTX, Inc.	mRNA	FDA Approved
3	Sputnik-V	Gamaleya	Ad26, Ad5	EMA Approved
4	ChAdOx1 nCoV19	Oxford-AstraZeneca	Non-Replicating Viral Vector	WHO Approved
5	Covaxin (BBV152 A, B, C)	Serum Institute, Bharat Biotech	Inactivated	ICMR Approved (India)
6	CoronaVac	Sinovac	Inactivated	Approval limited (China, Indonesia)
7	Convidicea (Ad5-nCoV)	CanSino Biologics	Viral vector	Approval limited (China)
8	EpiVacCorona	Vector Institute	Protein vaccine	Approved for Early use (Russia)
9	Sinopharm	Sinopharm	Inactivated	Approval limited
10	BBIBP-CorV	Beijing Institute of Biological Products-Sinopharm	Inactivated	Approval limited

Table 1.
Vaccines against COVID-19 approved for emergency use authorization.

vaccine candidates in the progress of clinical trial and about 181 candidates in pre-clinical development [87]. About 7% of vaccinations in the state of preclinical trials performed are based on practice. Hereby, we list the current status of several vaccines across the globe approved by Food and Drug Administration (FDA), European Medicine Agency (EMA), World Health Organization (WHO), Indian Council of Medical Research (ICMR) and other countries medical advisory boards under progress of development for the emergency use authorization (EUA) in order to curb the pandemic (**Table 1**).

6. Conclusion

The principle of drug repurposing is very beneficial and may have proven success, although this may differ with the severity as well as the subjects in whom therapy is performed. In the case of vaccinations, several vaccines have shown promising effects throughout the globe and the proceeding steps will soon provide hope for the early commercial launch of COVID-19 vaccines. Since the structural organization and genomic constitution of the SARS-CoV-2 is similar to that of SARS-CoV, antiviral drugs and other therapeutic measures employed to control the disease at the time can be utilized in order to control this epidemic condition. However, the rate and amount of research and clinical COVID-19/SARS-CoV-2 studies to improve potential treatments and therapies for this disorder will surely help us in order to curb the disease soon. Consequently, the design and production of SARS-CoV-2 vaccines is similarly critical in comparison to the development of new medicines and clinical trials of old drugs. Experience from SARS-CoV and MERS-CoV suggests that there is a major focus on creating animal models that can summarize different forms of human disease and assess the safety and efficacy of vaccines.

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