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REVIEW

DRUG DEVELOPMENT RESEARCH WILEY

Therapeutic dilemma in the repression of severe acute respiratory syndrome coronavirus-2 proteome

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Abstract

Currently, the pandemic coronavirus disease 2019 (COVID-19) has unprecedentedly captivated its human hosts by causing respiratory illnesses because of evolution of the genetic makeup of novel coronavirus (CoV) known as severe acute respiratory syndrome coronavirus-2 (SARS CoV-2). As much as the researchers are inundated for the quest of effective treatments from available drugs, the discovery and trials of new experimental drugs are also at a threshold for clinical trials. There has been much concern regarding the new and targeted drugs considering the comprehensive ambiguity regarding the mechanism and pathway of the drug action with respect to the new and unpredictable structural and nonstructural proteins (NSPs) of SARS CoV-2. This study was aimed to discuss functional pathways related to NSPs in CoVs with updated knowledge regarding SARS CoV-2, mechanisms of action of certain approved and investigational drugs for correct orientation regarding the treatment strategies, including nucleotide analog mechanism, receptor analog mechanism, and peptide–peptide interactions, along with the impact of COVID-19 on a global scale. Although there is a dire need for targeted drugs against SARS CoV-2, the practical achievement of its cure is possible by only using effective drugs with appropriate mechanisms to eliminate the disease.

KEYWORDS

antiviral drugs, COVID-19, cytokines, nonstructural proteins, pneumonia, SARS CoV-2, severe acute respiratory syndrome, viremia

1 | INTRODUCTION

Viruses have been known to mankind since the discovery of tobacco mosaic virus in the late 1890s (Lecoq, 2001) and the first viral epidemic of respiratory-tract infection was recorded in 412 BC. Influenza pandemic occurred in 1918 and swine flu in 2009, both caused by the H1N1 virus (Garten et al., 2009). In history, pandemics caused by viruses involved respiratory-tract infections and led to recorded thousands to millions of fatalities (Potter, 2001). Coronaviruses are known to cause zoonotic infections that are transmitted from animals to humans. The name "coronavirus" was given to these viruses because of crown-like spikes on their surface. Human Coronaviruses (HCoVs) belong to the family *Coronaviridae*, and the respiratory-tract-infection causative viruses were discovered in the 1960s when HCoV 229E and HCoV OC43 were identified. HCoV 229E and HCoV OC43 nurture in bats and rodents, respectively, and transmitted infections in humans (Cui, Li, & Shi, 2019). However, severe acute respiratory syndrome (SARS) caused by SARS CoV in 2002, respiratory infection by HCoV NL63 in 2004, Middle East respiratory syndrome (MERS) by MERS CoV in 2012 and COVID-19 by severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) in December 2019 are severe zoonotic infections of humans (Figure 1) (Cui et al., 2019; van der Hoek, Pyrc, & Berkhout, 2006). Furthermore, HCoV NL63, SARS CoV, MERS CoV, and SARS CoV-2 are also primarily nurtured in bats, but they may take other secondary hosts (Zhou et al., 2020).

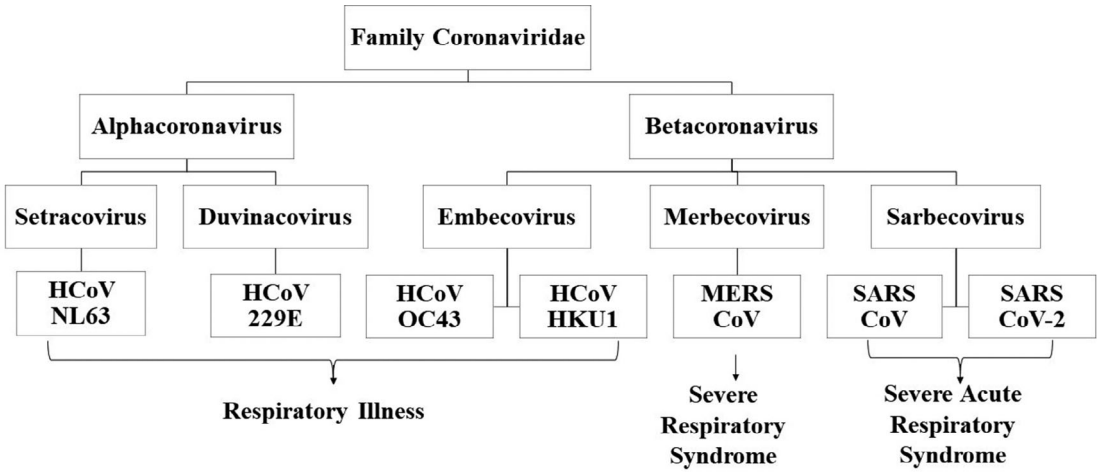

FIGURE 1 Phylogenetic family tree of coronaviruses infecting human hosts

The SARS CoV-2, which erupted originally from Wuhan city of China, has a reproductive number R_o ranging from 2.24 to 3.58 as compared to 2.7 to 3.7 of MERS, and 2 to 5 of SARS, whereas the transmission rate of COVID-19 is considered 2.2% and the official case fatality rate is 3.17% in China (Sun, Lu, Xu, Sun, & Pan, 2020; Zhao et al., 2020). The COVID-19 is characterized by SARS CoV-2 mediated sore throat, fever (38–39 °C), cough, body fatigue, and viremia that might lead to pneumonia and eventually ventilator-associated pneumonia in severe cases (Rothan & Byrareddy, 2020). The World Health Organization (WHO) has reported 6,535,354 confirmed cases of COVID-19 pandemic and 387,155 fatalities across the globe as of June 5, 2020, which is much higher than the combined fatalities (1632) for SARS and MERS, and the COVID-19 pandemic continues to rise (Mahase, 2020). Owing to the lack of prophylaxis or curative treatment strategies against COVID-19, the WHO has recommended social distancing, restrictions on direct contact, self-isolation, use of alcohol-based hand sanitizers with ethanol (80%) or isopropyl alcohol (75%), and antiseptic products such as soaps to avoid the SARS CoV-2 transmission within humans (World Health Organization, 2020). Many countries have officially imposed partial to complete lockdown according to the prevalence of COVID-19 in their respective areas, aiming to flatten the curve of COVID-19 spike (Lau et al., 2020).

The historic engulfment of such a large number of people in the current pandemic COVID-19 has brought the medical setups and developing countries in the world under crisis. Above that, the lack of prophylaxis and drug resistance associated with SARS CoV-2 has rendered all the available antiviral management to undergo retrials, creating an enigma across the globe.

2 | STRUCTURAL BACKGROUND ON SARS CoVs

The genome of CoVs ranges from 26 to 32 kbps in length with the open reading frames (ORFs) varying from 6 to 11; there are 14 ORFs in SARS CoV-2 (Song et al., 2019). There are two parts of the first ORF in SARS CoV-2, ORF1a and ORF1b, constituting about 67% of the viral RNA and are translated into polyproteins, pp1a and pp1ab. The pp1a and pp1ab polyproteins encode 10 (NSPs 1–10) and 15 (NSPs 1–10 and NSPs 12–16) NSPs, respectively. These exhibit 72–99.8% homology with SARS CoV (Wu et al., 2020; Wu et al., 2020). ORFs 3, 6, 7a, 7b, 8, and 9b code for the accessory proteins (Dong et al. (2020); Shereen, Khan, Kazmi, Bashir, & Siddique, 2020). ORFs 2, 4, 5, and 9a constitute about one-third of the CoVs RNA, which is translated into four structural proteins that ensemble as spike glycoprotein (S), envelope (E), membrane (M), and nucleocapsid (N), respectively (Dong et al. (2020); Guo et al., 2020). Other than these ORFs, the viroid RNA comprises untranslated regions. NSPs play a role in replication and viability of the viroid and upregulate the cytokine storm, whereas the structural proteins mediate viral pathogenesis in the host (Wu, Peng, et al., 2020).

2.1 | Structural proteins: trigger of immune response

Structural proteins S, E, M, and N perform roles in host immune response such that the N-protein, one of the fundamental structural proteins, binds to the RNA genome to form a nucleocapsid that is imperative for virus replication. It inhibits the promotor of the nuclear factor kappa-B (NF-κB) and interferon-β (IFN-β), which eventually leads to disruption in the balance between biosynthesis of proinflammatory and anti-inflammatory cytokines. E is involved in host cell recognition as an integral component of the viroid envelope. M and E together manifest the transmembrane transportation, multiplication, assembly, and release of the viroids. Coronaviruses, especially SARS CoV and SARS CoV-2, attach to angiotensin converting enzyme receptor-2 (ACE-2) present on the lower respiratory-tract cells through S. Genetic mapping and phylogenetic analyses revealed the evolution of SARS CoV-2 because of mutations in its genome as

TABLE 1 Infectious viral proteins of human coronaviruses and their treatment drugs

Viral protein	Role in COVID-19 progression	Drug				Ref.
		Name	Class	Effective against	Mechanism of action	
NSP1	↓Host gene expression, ↓IFN-β	Tacrolimus[a]	Macrolide antibiotic	SARS CoV, HCoV NL63, HCoV 229E	Receptor analog mechanism	(Carbajo-Lozoya et al., 2012)
NSP2	↓Host signaling	Lopinavir/Ritonavir[a]	Protease inhibitor	SARS CoV, MERS CoV	Receptor analog mechanism	(Arabi et al., 2020; Chu et al., 2004)
NSP3	↑RTC ↑NSP1-4	Lopinavir[a]	Protease inhibitor	SARS CoV, MERS CoV	Receptor analog mechanism	(Sheahan et al., 2020)
NSP4	↑RTC	Cyclosporin A[a]	Immuno-suppressant	SARS CoV, HCoV 229E	↓Inflammation	(de Wilde et al., 2011)
NSP5	↑Replicases ↑NSP5-16	Lopinavir[a]	Protease inhibitor	SARS CoV, MERS CoV	Receptor analog mechanism	(Sheahan et al., 2020)
NSP6	↑RTC ↑Autophagy	Chloroquine[a]	Antiparasitic	SARS CoV, MERS CoV, SARS CoV-2	↓Lysosomal autophagy	(Yang & Shen, 2020)
		Chlor-promazine[a]	Lysosomo-tropic		Inhibits CoVs entry in host cell	(Yang & Shen, 2020)
NSP7	↑Viroid RNA replication	Remdesivir[b]	Antiviral	Ebola, SARS CoV, MERS CoV, SARS CoV-2	Nucleotide analog mechanism	(Yang & Shen, 2020)
NSP8	↑Viroid RNA replication	Cyclosporin A[a]	Immuno-suppressant	SARS CoV, HCoV 229E	↓Inflammation	(de Wilde et al., 2011)
NSP9	↑Viroid RNA replication	Remdesivir[b]	Antiviral	Ebola, SARS CoV, MERS CoV, SARS CoV-2	Nucleotide analog mechanism	(Lau et al., 2020)
NSP10	↑Proofreading ↑Replicases	Remdesivir[b]	Antiviral	Ebola, SARS CoV, MERS CoV, SARS CoV-2	Nucleotide analog mechanism	(Shannon et al., 2020)
NSP12	↑Viroid RNA replication	Remdesivir[b]	Antiviral	Ebola, SARS CoV, MERS CoV, SARS CoV-2	Nucleotide analog mechanism	(Shannon et al., 2020)
NSP13	↓Host gene expression	Remdesivir[b]	Antiviral	SARS CoV-2	Nucleotide analog mechanism	(Shannon et al., 2020)
NSP14	↑Proofreading	Remdesivir[b]	Antiviral	SARS CoV-2	Nucleotide analog mechanism	(Shannon et al., 2020)
NSP15	↓Host RNA ↑Viroid RTC ↑IL-1β, IL-4, IL-10, MCP-1, IFN-γ, CD^{4+}, and CD^{6+} T-cells	Ciclesonide[a]	Steroid	SARS CoV-2	↓ Inflammation	(Matsuyama et al., 2020)
		Baracitinib[a]	DMARD	SARS CoV-2	↓ JAK-kinase, inflammation	(Richardson et al., 2020)
NSP16	↓Host immune balance	TP29[b]	Peptide	SARS CoV	↓Viral RNA methylation	(Yi Wang et al., 2015)
E	Formation and release of viroid	Chloroquine[a]	Antiparasitic	Malaria, SARS CoV-2	↓Lysosomal autophagy	(Yang & Shen, 2020)
		Hydroxy-chloroquine[a]	DMARD			(Schrezenmeier & Dörner, 2020)
N	↓NF-κB ↓IFN-β	Cyclosporin A[a]	Immuno-suppressant	SARS CoV, HCoV 229E	↓Inflammation	(de Wilde et al., 2011)
M	Formation and release of viroid	Chloroquine[a]	Antiparasitic	Malaria, SARS CoV-2	↓Lysosomal autophagy	(Yang & Shen, 2020)
		Hydroxy-chloroquine[a]	DMARD			(Schrezenmeier & Dörner, 2020)
S	Adherence of CoV on receptor	Arabidol[a] with Lopinavir/ Ritonavir[a]	Protease inhibitor	SARS CoV, MERS CoV	Receptor analog mechanism	(Deng et al., 2020)

TABLE 1 (Continued)

Viral protein	Role in COVID-19 progression	Drug				Ref.
		Name	Class	Effective against	Mechanism of action	
		Losartan[b], Olmesartan[b]	ACE-2 inhibitors	Hypertension	Receptor analog mechanism	(Gurwitz, 2020)
		Convalescent plasma[a]	Neutralizing antibodies	SARS CoV, MERS CoV, influenza, EBOLA	↑Immune response	(Chen, Xiong, Bao, & Shi, 2020)

Abbreviations: E, envelope; M, membrane; N, nucleocapsid; NSP, nonstructural protein; S, spike.
[a]FDA-approved drug.
[b]Experimental drug.

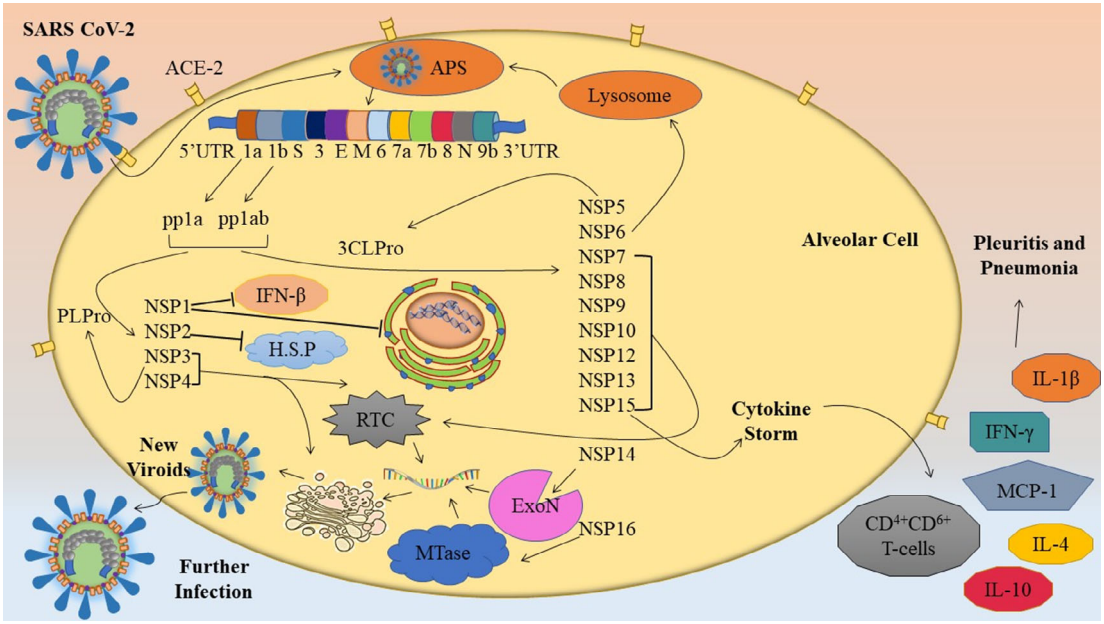

FIGURE 2 Infection and cytokine storm pathway of COVID-19 in host alveolar cell. 3CLPro, 3-Cysteine-like protease; ACE-2, angiotensin converting enzyme-2; APS, autophagosome; ExoN, exonuclease; H.S.P, host signaling protein; MTase, methyl transferase; NSP, nonstructural protein; PLPro, papain-like protease; RTC, restriction-transcription complex

compared to previously known coronaviruses, resulting in severe pathogenicity. A decreasing trend in pathogenicity might be associated with structural proteins such as S, E, M, and N. Protein S with 27 nucleotide substitutions accounts for the highest pathogenicity by inducing cellular infection. ACE-2 inhibition and convalescent plasma therapy involve S as a target in the treatment of COVID-19 (Table 1). Protein E in the viroid envelope mediates pathogenicity through intracellular trafficking and viral assembly leading to corporal spread of infection (Westerbeck & Machamer, 2015), whereas the glycoprotein M contributes to pathogenesis by packaging newly formed viroid in the host (Mousavizadeh & Ghasemi, 2020). The substitution of five nucleotides in N of SARS CoV-2 showed 90.5% amino acid sequence homology with SARS CoV and contributed to the pathogenesis of SARS CoV-2 by encapsulating and protecting the viroid RNA from hydrolytic enzymes of the host cells (Guo et al., 2020; Li, Geng, Peng, Meng, & Lu, 2020; Wu, Peng, et al., 2020).

2.2 | NSPs: Control of the cellular machinery and responses

After being released in the host cell cytoplasm, the NSP1 to NSP5 from ORF1a are cleaved by the viral proteases including papain-like proteases (PLPro), whereas NSP5 to NSP16 from ORF1ab by 3-Cystein proteases (3CLPro) (Kim et al., 2020). NSP1, a small protein with 180 amino acid residues, acts as a suppressor of host gene expression by degrading the host mRNAs and inhibiting the translational machinery of the cell. Previously, it was reported that NSP1 is involved in the suppression of IFN-β expression, which is essential for balanced biosynthesis of proinflammatory and anti-inflammatory cytokines in the body. The genetic studies of NSP1 revealed 81% structural conservation between SARS CoV and SARS CoV-2 owing to mutation of seven residues in SARS CoV-2 and exhibits 36–38% structural conservation in comparison with moderate to severely

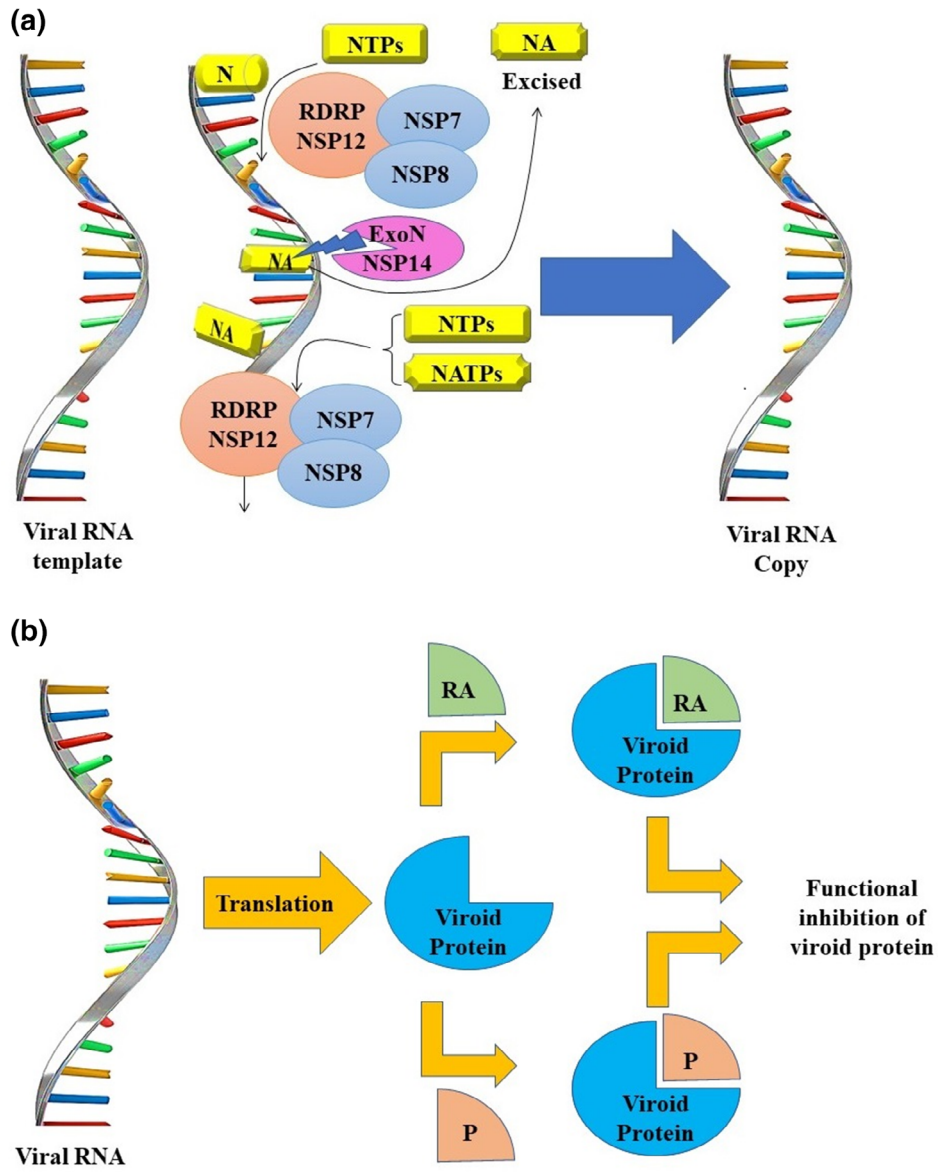

FIGURE 3 Schematic diagram of mechanisms of inhibition. (a) RNA-dependent RNA polymerase (RDRP-NSP12) along with NSP7 and NSP8 cofactors replicate viral RNA using incoming nucleoside triphosphates (NTPs) and nucleoside analog triphosphates (NATPs), while the proofreader NSP14 exonuclease detects and excises nucleotide analogs and the RDRP replaces them with corresponding nucleosides (N). (b) Translation of viral RNA into protein inhibited be receptor analog (RA) drugs or peptide inhibitor (p)

pathogenic CoVs (Wang et al., 2010; Wu, Peng, et al., 2020). NSP2 has been reported to suppress intracellular signaling by binding with ubiquitously expressed prohibitin proteins, PHB1 and PHB2, which modulate transcriptional activity and mitochondrial functioning. This suppression of PHBs aids in the pathogenesis of the virus in the host, causing fever and viremia. In SARS CoV-2, 60 of 304 amino acid residues of NSP2-protease have mutated, causing greater pathogenicity and virulence of SARS CoV-2 in the host (Angeletti et al., 2020; Cornillez-Ty, Liao, Yates, Kuhn, & Buchmeier, 2009).

NSP3 ADP ribose phosphatase is the largest NSP that splits from the ORF1a-ORF1ab as PLPro and mediates the restriction-transcription complex (RTC) for synthesizing the viral genome in the host cell. The transmembrane domains of NSP3 act as a part of the scaffold, along with NSP4 and NSP6 for the assemblage of RTC (Imbert et al., 2008; Wu, Peng, et al., 2020). NSP4 is also a conserved protein of CoVs and contributes to the RTC of viral enzymes. Both NSP3 and NSP4 combine and catalyze the formation of double membrane vesicles (DMVs) in infected host cells to organize the newly produced virions prior to being released to the exterior (Alsaadi & Jones, 2019). NSP5 is a highly conserved protein in the CoVs series; it acts as 3CLPro, and cleaves the replicases downstream at 11 sites through NSP5 to NSP16; hence, it is also termed the main protease (MPro) (Wang, Zhang, Liu, Peng, & Mackey, 2020). NSP6 is a vital protein for the assemblage of DMVs as it aids NSP3 and NSP4 lead RTC and reinforces the autophagy genes causing the lysosomal-autophagosome fusion, resulting in viroid RNA release in the host cytoplasm (Yang & Shen, 2020). A cylindrical super-complex composed of two asymmetrical units, each containing 4 units of NSP7 and NSP8, both of which show structural conservation in SARS CoV-2 and SARS CoV with respect to the other CoVs. NSP8 is a noncanonical RNA-dependent RNA polymerase (RDRP) that provides RNA primers for viral RNA synthesis. The conservation of structure gives rise to entrance and exit paths for the nucleotide triphosphates (NTPs) and nascent RNA strands, respectively, in SARS CoV-2, similar to those reported for

SARS CoV (Gao et al., 2020). NSP9 is a replicase protein that catalyzes replication of viral RNA by the formation of dimers and preferential binding to single-stranded viroid RNA. Dimerization of NSP9 is essential for RNA binding and replication of viroid RNA (Figure 2) (Qiu & Xu, 2020; Wathelet, Orr, Frieman, & Baric, 2007).

NSP10 contains two zinc finger binding domains that are conserved in CoVs. It acts as a cofactor in viral replication through positive reinforcement of viroid RNA replicases and exonuclease NSP14, which provides the resistance of viroids against nucleotide analog drugs (Senanayake, 2020). NSP11 is a relatively small protein and currently has no known functions. NSP12 is an RNA-dependent RNA polymerase (RDRP), which is the chief component of the viroid RTC and catalyzes the synthesis of viral RNA. NSP12 works in compliance with the cofactors NSP7 and NSP8 to carry out the polymerization and building of the RNA strand (Gao et al., 2020). NSP13 contains an N-terminal zinc binding domain, whereas at the C-terminus it constitutes a helicase protein that performs its role in unwinding of the RNA or DNA; the NSP12-NSP13 interaction further promotes the unwinding activity (Shu et al., 2020). NSP14 is a guanine-N7-methyltransferase with 3′-5′ N terminus exonuclease activity that excises the nucleotide analogs from the viroid RNA transcripts and performs its role in proofreading of the synthesized viral RNA, leading to protein synthesis according to the RNA transcript. Furthermore, NSP14 eradicates the nucleoside analogs and interacts with the trimeric RNA polymerase complex (NSP12, NSP7, and NSP8) to perform its proofreading activity. The quality of the CoVs makes them resistant to nucleotide analog mechanism (NAM) treatment, causing uncontrollable growth and spread of the SARS CoV-2 in recent COVID-19 pandemic (Figure 3a) (Cao, Deng, & Dai, 2020; Ferron et al., 2018).

NSP15 is a hexameric, manganese-dependent, and uridine-specific endoribonuclease. It catalyzes the RNA replication process in RTC. It degenerates the single- or double-stranded RNA of the host to catalyze viral RNA polymerization (Kim et al., 2020; Matsuyama et al., 2020). The polyuridine sequences cleaved by NSP15 trigger the cytokine storm, with release of interleukin 1β, 4, and 10 (IL-1β, IL-4, and IL-10), monocyte chemoattractant protein-1 (MCP-1), interferon γ (IFN-γ), interferon γ-induced protein 10 (IP10), and $CD^{4+}$and CD^{6+} T-cells (Fu, Cheng, & Wu, 2020; Shang, Yang, Rao, & Rao, 2020). NSP16 is a 2′ O-methyltransferase that methylates the RNA cap 2′-O position of the ribose, bringing about the cap-1 form of the RNA. This proofreading strategy acquired by the CoVs helps them in overcoming the innate immune response and IFN-mediated antiviral responses (Decroly et al., 2011; Liao, Way, & Madahar, 2020).

3 | CONTRAST OF DRUG ACTION MECHANISMS

The urgent requirement of potent drugs against COVID-19 is unmet because of limitations in drug design, targeted drug delivery, and controlled in vitro, in vivo, and clinical studies (Ahmadpoor & Rostaing, 2020; Dhama et al., 2020). Researchers from the globe are putting efforts in the quest of an effective treatment for COVID-19. Previously, both clinical and preclinical studies reported various mechanisms of action for antiviral activity. Receptor analog mechanism (RAM) causes inhibition of the target proteins by steric inhibition of binding or catalytic sites of the protein by receptor analog drugs, causing functional inhibition and poisoning of the target protein. RAM is shown by tacrolimus against NSP1 and lopinavir/ritonavir against NSP2, 3, and 5. However, cyclosporin A is found to be effective against NSP4, 8, and N-protein through an immuno-suppressant mechanism (Arabi et al., 2020; Carbajo-Lozoya et al., 2012; Chu et al., 2004; Matsuyama et al., 2020; Sheahan et al., 2020; Yang & Shen, 2020). Chloroquine and hydroxychloroquine work efficiently by increasing the pH of the lysosome and inhibiting proteins E and N. Moreover, these drugs along with losartan and olmesartan are also under investigation for the inhibition of ACE-2 (Gurwitz, 2020; Liu et al., 2020). Certain peptides have been shown to inhibit other proteins through specific interactions between the amino acids, which inhibit their binding and catalytic sites, and ultimately suppress their function. TP29 is a peptide experimental drug that inhibits NSP10 and 16 by making peptide–peptide interactions (PPIs), leading to cap-0 RNA strands instead of 2′ O-methylation (cap-1). This might result in increased immunity by IFN-1 stimulation (Figure 3b) (Yi Wang et al., 2015). Remdesivir, which is metabolized to C-adenosine triphosphate analog, inhibits NSP7, 9, 10, 12, 13, 14, and 15 of HCoVs by substitution of nucleotides during RNA polymerization through NAM. Experimental drugs with NAM mode of action are on the verge of clinical trials for their promising investigational studies (Table 1) (Lau et al., 2020; Shannon et al., 2020). However, the plausibility of RAM over NAM for inhibition of viral enzymes has an edge because of the NSP14 exonuclease that has the ability to proofread their synthesized RNA and polypeptide machinery (Figure 3a,b) (Cao et al., 2020; Dhama et al., 2020). Convalescent plasma, which provides neutralizing antibodies to target protein S, has been under clinical investigation and is sought as a last resort for curing COVID-19 patients (Chen et al., 2020). PPI is also a promising tool for further investigation as NSP16 is another important drug target that protects the synthesized viral RNA from cleavage or recognition by the host immune response by methylation to the cap-1 form. The resistance of CoVs, including SARS CoV-2, because of the genetically evolving NSPs, proofreading capabilities of NSP14, severity of NSP15-dependent cytokine storm, and NSP16-mediated viral RNA-capping make them highly commendable drug targets.

4 | CONCLUSION

The lack of vaccine, oriented medicine and socio-economic burdens are escalating around the world, especially in underdeveloped countries. The resurgence of the COVID-19 might be increased if the official lockdowns are lifted in underdeveloped countries, ultimately increasing the risks of pandemic. Presently, providing financial support to underdeveloped countries and utilizing existing medicine and genetics of SARS CoV-2 can be helpful in treatment. NSPs and structural proteins are equally important in antiviral therapy, as NSP14

exonuclease and NSP16 2′ O-methyl transferase are seemingly promising targets for anti-COVID-19 treatment. However, exceptional diligence is required for the selection of a suitable drug manifested by its mechanism of action against SARS CoV-2, along with the implementation of smart WHO recommended policies to effectively eradicate the current COVID-19 pandemic.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Aatika Sadia: Investigation; writing-original draft preparation. **Muhammad Asim Raza Basra:** Conceptualization; supervision; writing-review and editing.

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