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REVIEW

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RNA-dependent RNA polymerase of SARS-CoV-2 as a therapeutic target

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Abstract

The global pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), named coronavirus disease 2019, has infected more than 8.9 million people worldwide. This calls for urgent effective therapeutic measures. RNA-dependent RNA polymerase (RdRp) activity in viral transcription and replication has been recognized as an attractive target to design novel antiviral strategies. Although SARS-CoV-2 shares less genetic similarity with SARS-CoV (~79%) and Middle East respiratory syndrome coronavirus (~50%), the respective RdRps of the three coronaviruses are highly conserved, suggesting that RdRp is a good broad-spectrum antiviral target for coronaviruses. In this review, we discuss the antiviral potential of RdRp inhibitors (mainly nucleoside analogs) with an aim to provide a comprehensive account of drug discovery on SARS-CoV-2.

KEYWORDS
coronavirus, COVID-19, drug target, RdRp, SARS-CoV-2

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a newly emerged zoonotic coronavirus that has thus far resulted in around 470 thousand deaths with the first infected case identified in December 2019. According to the World Health Organization, as of 10th May 2020, SARS-CoV-2 has infected around 8.9 million individuals in more than 200 countries and territories.[1] Although the world first witnessed an outbreak caused by the zoonotic coronavirus SARS-CoV in 2002, followed by another caused by Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, no drug or vaccine has been approved to treat these highly infectious coronaviruses.[2-5] This emphasizes an urgent need to find effective anti-SARS-CoV-2 drugs for the treatment of COVID-19 to alleviate the current global public health threat.

SARS-CoV-2 is a member of *Betacoronaviruses* of the *Coronaviridae* family which possesses a large genome made up of a single-stranded, positive-sense RNA of approximately 30 kb.[6,7] Following their entry into the host cell, the viral genome, which has at least 14 open reading frames (ORFs), is released into the cytoplasm for transcription and replication.[8,9] First, the ORFs 1a and 1b express two large replicase polyproteins (PP1a and PP1ab) which are further cleaved by papain-like cysteine protease and 3 chymotrypsin-like cysteine protease to produce the nonstructural proteins (nsps). And two important proteins, nsp12, also known as RNA-dependent RNA polymerase (RdRp), and nsp13 (helicase), are involved in directing viral genomes and proteins synthesis.[10,11] ORFs 2 to 14 encode four viral structural proteins: spike (S) protein, envelope (E) protein, membrane (M) protein and nucleocapsid (N) protein, and nine accessory factors which together function in the virion formation (Figure 1).

RdRp, the enzyme that is most conserved across several viral species, such as influenza virus, hepatitis C virus (HCV), Zika virus (ZIKV), and coronavirus (CoV), plays an essential role in the life cycle

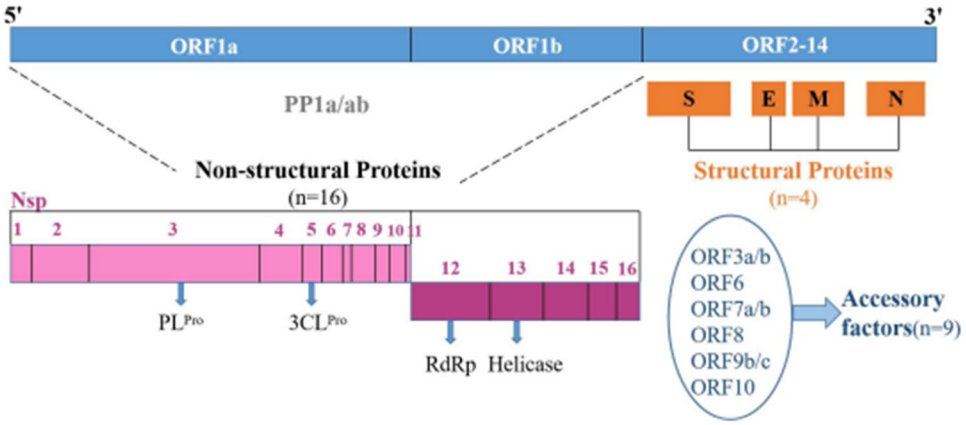


FIGURE 1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome organization. Expression of the 5′-open reading frame 1ab is initiates translation of two polyproteins (PP1a/ab) which are hydrolyzed into 16 nonstructural proteins (nsps). Open reading frame 1a (ORF1a) encodes nsp1-11 and ORF1b encodes nsp12-16. Four key proteolytic enzymes are labeled at their corresponding domain: papain-like proteinase (PL^{pro}), 3C-like proteinase ($3CL^{pro}$), RNA-dependent RNA polymerase (RdRp) and helicase. At the 3′-end of the viral genome structural proteins (orange) and accessory factors (blue) are encoded by ORFs 2 to 14

of RNA viruses.[12-16] Before producing progeny viral genome based on the (+)single-stranded RNA (ssRNA) template, CoV RdRp must first be synthesized; this signifies the indispensable role of RdRp in CoV replication. RdRp has a common function across different virus genera owing to its conserved protein structure, amino acid residues and motifs. For example, most (+)ssRNA viruses possess a specified structure of RdRp with three well-defined entrance and exit paths responsible for its function.[16] Additionally, motif G which functions as a part of the template entrance tunnel in ss (+) RNA viruses is also a component of the polymerase acidic subunit of influenza A/B virus.[16,17] Specifically, the protein sequence similarity between SARS-CoV-2 and SARS-CoV RdRp is up to 96% and the existing structural disparities are found in the catalytically inactive area.[18] Thus, broad-spectrum antiviral drugs acting on RdRp may block viral replication of different RNA viruses. Here we summarize the importance of RdRp structure-function relationship in CoV replication and discuss the potential of RdRp-targeting compounds and drug candidates against SARS-CoV-2.

2 | THE STRUCTURE-FUNCTION RELATIONSHIP OF RNA POLYMERASE IN CORONAVIRUSES

RdRp is predicted to be the central enzyme responsible for viral replication.[19] In CoVs, RdRp catalyzes the synthesis of the RNA genome by using the (+)RNA strand as a template to produce a complementary (−)RNA strand starting from 3′-poly-A tail. There are two plausible molecular mechanisms for the initiation of genomic RNA synthesis by RdRp: de novo (primer-independent) and primer-dependent RNA synthesis.[20-22] During de novo synthesis, genomic RNA is gradually synthesized through the formation of a phosphodiester bond composed of a 3′-hydroxyl group bound to the 5′-phosphate group of the next nucleotide. In the case of primer-dependent synthesis, new RNA complementary to the template is generated by base pairing under the guidance of either an oligonucleotide or a protein primer. In addition, four cellular ribonucleotide triphosphates (rNTPs), ATP, GTP, CTP, and UTP provide the template substrates recognized by RdRp. Acting as essential cofactors in the polymerization reaction, divalent metal ions magnesium (Mg) and manganese (Mn) coordinate the catalytic aspartates and promote the reactions with rNTPs.[23]

Previous data indicate that all RNA polymerases share a similar structure and mechanism of catalysis and also indicate the inextricable relationship between their structure and function.[16,24-27] Within the core structure of RdRp, there is a large and deep groove domain which resembles a cupped right-hand interconnected by "fingers," "palm," and "thumb" subdomains surrounding the active site cavity of RNA synthesis (Figure 2). Seven classic RdRp structural motifs with a relatively immobile arrangement (A to E pitched at conserved palm subdomain, F and G within the fingers) impact the catalytic process. Three pivotal channels defined as the entry channel for the template RNA and rNTPs and a central channel for the newly-synthesized double-stranded RNA (dsRNA) to exit together aid in the trafficking of the replication reactant between the catalytic centers and the exterior.[16,25] The entry tunnels lined with positively charged residues successfully allow template RNA and rNTPs into catalytic cavity and participate in the release of the pyrophosphate moiety after polymerization.[28,29] The intertwined fingers and flexible thumb aid in the formation of the template channel which extends across the surface of the fingers to drive incoming nucleotides towards the active site; they also regulate the recognition of the initiation site.[29-31] The conserved structural motif G arrays the entry of the template channel while the base is formed by motif B.[25,32] In *Reoviridae*, this channel binds the 3′ end of (−)RNA strand for transcription and (+) RNA strand during genome replication.[23] Overall, the template channel with variable conformations guarantees the authenticity and accuracy of template RNA during the replication progress and has

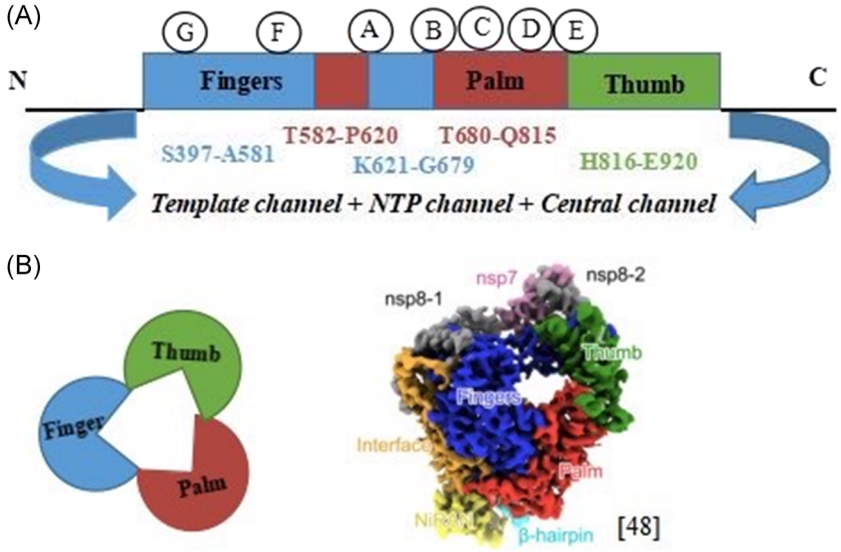


FIGURE 2 The organization of RdRp core domain. A, The annotation of SARS-CoV-2 RdRp. The "Fingers," "Palm," and "Thumb" subdomains corporately offer template, NTP and double-strand RNA (dsRNA) channels by interaction. Motifs A through E are mainly distributed in palm domain while motifs G and F are parts of the finger domain. B, A cartoon structure (left) and ribbon diagram (right, quoted from Gao et al) of RdRp. In both, the three subdomains are represented by the colors blue (finger), green (thumb) and red (palm). RdRp, RNA-dependent RNA polymerase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

been proposed to be a significant target for the development of antivirals.[25,28,33-35] Substrate rNTPs selectively enter through the NTP channel via specific interactions between the ribose 2′- and 3′-hydroxyl groups of the incoming nucleotides and conserved residues of motifs A and B.[36] The conformation of the replication complex varies between different states of nucleotide binding.[25,37] Another central channel is formed by the palm and the thumb subdomains where export of the newly generated dsRNA intermediate product takes place.

Being the most functionally diverse domain, the size of the RdRp thumb domain differs between two replication modes. RdRps with a primer-depended initiation mechanism, as in the case of *Picornaviridae* and *Caliciviridae*, have small thumb subdomains whereas that of *Flaviviridae* and bacteriophage polymerase are significantly larger, and this has been shown to correlate with de novo polymerization initiation.[22] The thumb subdomain, as a bridge connecting the fingers and the palm subdomains, consists of the folded C-terminal region of the polypeptide chain.[16,25,36] It serves as a specific functional element (called "priming loops") to correctly position the rNTPs for catalysis, acting as a stabilizing platform between two ribonucleotides during de novo initiation. Large conformational rearrangements often occurring in the thumb domain results in the movement of primer RNA strand and nucleotide; it also functions in translocating template and exiting nascent RNA.[11,16,25] Dengue virus-3 (DENV-3) and West Nile virus RdRps possess primer-independent closed conformation sharing two cavities located in the thumb subdomain which facilitates the initiation of viral template.[35]

The active site region of RdRps is located entirely in the palm domain which contains a highly conserved architecture of α-helices, antiparallel β-strands, RNA recognizing motif, and catalytic aspartates.[32,33,38] This dynamic subdomain of picornavirus and calicivirus RdRps selectively unites with rNTPs to catalyze phosphoryl transfer reaction with the help of metal ions.[25,39] Motif E, at the junction between the palm and thumb subdomains, forms a tight loop that projects into the active site cavity, which in turn conduces to the proper positioning of the 3′-hydroxyl of the RNA primer strand for the catalysis of rNTPs.[16,40] The finger subdomain, located at the RdRp N-terminal portion, contains two conserved motifs: motif G which comprises a loop that is part of the template entrance channel, and motif F which houses a conserved arginine residue. The finger domain plays a key role in stabilizing template RNA in place and facilitates interaction with major residues thus favoring the recognition of active site.[25] Furthermore, in vitro and in vivo assays of nucleotide discrimination suggest that the mutations in palm domain have strong correlation with elongation rates and fidelity; the fingertips primarily affect elongation to alter virus replication rates.[39,40] In addition, the role of RdRps to aid escape of the virus from the host defense mechanisms has important implications during viral evolution.[16,40-42]

Although RdRps are considered good antiviral targets, progress has been hampered due to difficulties in protein expression, purification, and catalytic activity in vitro.[43,44] With regard to SARS-CoV studies, this obstacle has been mitigated by utilizing accessory factors nsp7 and nsp8 complexed with nsp12 which increase the RdRp template binding activity and processivity.[45] Nsp8 can de novo synthesize up to 6 nucleotides in a sequence-specific fashion and can subsequently be used as a primer for RdRp RNA synthesis (a non-classical biosynthesis route).[46] The nsp7-nsp8 complex has been shown to cooperate in activating and enhancing processivity of the primer-dependent activity of RdRp in vitro.[47] Gao et al[48] obtained

the cryogenic electron microscopy structure of the full-length SARS-CoV-2 nsp12 complexed with one nsp7 and two nsp8 at a resolution of 2.9 Å. They identified a unique β-hairpin structure positioned at the N-terminal domain, uncovered the interface between nsp7-nsp8 and nsp12, and they also reported the key residues for viral replication and transcription (Figure 2B). In accordance with the data from this report, in vitro purified RdRp complex was constructed with residues 397 to 920; its closed conformation was further stabilized by the nsp7-nsp8 heterodimer which is packed against the thumb-index finger interface.[49] Furthermore, they revealed the structural mechanism of action of remdesivir (RDV) and other nucleotide analogs that target SARS-CoV-2 RdRp, which is critical for the discovery of potential COVID-19 curative drugs.

3 | EVALUATION AND DEVELOPMENT OF POTENTIAL SRAS-CoV-2 RdRp INHIBITORS

Different approaches are being used to identify, evaluate, and develop potential inhibitors targeting the SARS-CoV-2 RdRp as therapeutics against COVID-19. Here we review the current status of six RdRp-targeting drugs against SARS-CoV-2 (Table 1).

3.1 | Remdesivir

A small molecule GS-5734, referred to as RDV, is a monophosphoramidate prodrug of an adenosine analog currently under investigation against SARS-CoV-2. Although not approved by the FDA, RDV has exhibited highly efficacious broad-spectrum activity against diverse viruses in both cultured cells and animal models. The half-maximal effective concentration (EC_{50}) values of RDV has been reported for HCoV-229E (0.024 ± 0.018 μM in human hepatoma [Huh7] cells), HCoV-OC43 (0.15 ± 0.015 μM in Huh7 cells), MERS-CoV (0.074 μM in primary human airway epithelial [HAE] cells, 0.09 μM in Calu-3 cells), SARS-CoV (0.069 μM in HAE cells), porcine deltacoronavirus (0.02 μM in Huh7 cells), and murine hepatitis virus (MHV; 0.03 μM in delayed brain tumor cells).[54-57] Based on the favorable in vitro antiviral activity of RDV, it was further tested in animal models of different viral infections. In a mouse model of SARS-CoV infection and a rhesus macaque model of MERS disease, administration of RDV effectively reduced the pulmonary viral loads and improved pathological symptoms.[54,58] Additionally, RDV has also been shown to act against ebola virus (EBOV) in nonhuman primate models.[59-61] In addition, it appears that there is a high genetic barrier for the development of GS-5734 resistant mutations due to the conserved functional residues (F476L and V553L in fingers domain of nsp12) in MHV or SARS-CoV.[55]

Molecular docking analysis showed a value of −7.6 kcal/mol binding energy between RDV and SARS-CoV-2 RdRp.[50] In the generated low-energy binding conformation, RDV was fitted in the bottom of the RNA template channel and formed interactions with key amino acids located in the binding pocket.[8] Gordon et al showed that the triphosphate of RDV was incorporated into RNA replacing ATP bind with counterpart template uridine (U), and delayed termination at a specific position i + 3 to hamper the replication of SARS-, MERS-, and SARS-CoV-2 directly.[62] In Vero E6 cells, RDV potently blocked virus infection at low-micromolar concentration and showed high selectivity index (SI = CC_{50}/EC_{50}, EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87).[51] The data obtained from quantitative reverse transcription-polymerase chain reaction and Western blot analyses at a stage post virus entry indicated that SARS-CoV-2 virus yield was reduced greatly in the RDV treatment group, which is consistent with its putative antiviral mechanism. This potential antiviral drug also has been shown to reduce lung inflammation and virus titer in SARS-CoV-2 infected rhesus monkeys.[63] The first clinical case of SARS-CoV-2 in the United States also demonstrated promising results upon treatment using RDV.[64] Since then, seven clinical trials have been initiated worldwide to determine the safety and efficacy of RDV for the treatment of COVID-19. The beneficial role of RDV to treat patients with severe COVID-19 pneumonia symptoms was expounded by Grein et al[65]; they reported improvements in clinical symptoms to be observed in 68% of the cases. However, there are limitations to the compassionate use of drugs: small size of the treatment group, the relatively short follow-up time, the lack of a double-blinded randomized study and the absence of a control group. Several clinical trials have been halted in China due to insufficient patients. Therefore, updated clinical data is needed to demonstrate the full efficacy of this drug against SARS-CoV-2.

3.2 | Favipiravir

Favipiravir (FPV; T-705), an influenza-directed agent approved in Japan, is a guanine analog. It selectively inhibits viral RdRp thus disrupting the replication cycle of RNA viruses which implicates its broad antiviral activity. As an active generator of this prodrug, T-705-4-ribofuranosyl-5′-triphosphate was recognized as a purine nucleotide by RNA polymerase with no obvious effect on DNA virus or mammalian cells.[66,67] Previous studies showed in vitro and in vivo antiviral activities of FPV against influenza A, B, and C viruses, Ebola virus, Lassa, and other viruses. It also showed a synergistic effect with oseltamivir, an influenza virus NA inhibitor, in mice infected with H3N2, H1N1, and H5N1.[68-71]

The docking analysis discovered that the triphosphate of this clinically-approved antiviral drug forms five hydrogen bond and seven hydrophobic interactions with the crucial amino acids of SARS-CoV-2 RdRp, for example, Arg^{553} acting on rNTP binding, Asp^{760} and Asp^{761} positioned in proximity with the catalytic center of functional motif C.[52] Although with a low in vitro selectivity against SARS-CoV-2 (EC_{50} = 61.88 μM, CC_{50} > 400 μM, SI > 6.46), FPV showed protective effect against a wide range of RNA viral infections in animal models, suggesting that further in vivo studies of this drug against SARS-CoV-2 may be useful.[51] The data from phase I, II, and III clinical trials demonstrated that FPV exhibited good overall efficacy and safety.[72] In an open-label, randomized, multicenter clinical study

TABLE 1 Potential inhibitors target SARS-CoV-2 RdRp

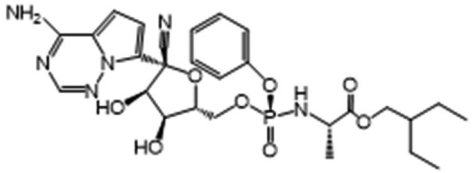
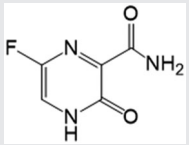
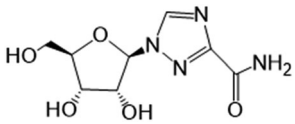
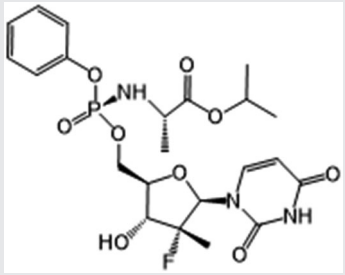
Compound	Structure	Status in progress of SARS-CoV-2			Pharmacological function
		In silicon	In vitro	Clinical trial	
Remdesivir (GS-5734)		Three hydrogen bonds and three hydrophobic interactions with RdRp,[8] the binding energy of −7.6 kcal/mol[50]	In Vero E6 cells, EC_{50} = 0.77 μM; CC_{50} > 100 μM; SI > 129.87[51]	NCT04302766;NCT04323761; NCT04257656;NCT04292730; NCT04280705; NCT04292899;manufacturer's compassionate use program	Treatment of ebola and coronavirus
Favipiravir (T-705)		Five hydrogen bond and seven hydrophobic interactions with RdRp[52]	In Vero E6 cells, EC_{50} = 61.88 μM, CC_{50} > 400 μM, SI > 6.46[51]	ChiCTR2000030254; ChiCTR2000029600; ChiCTR2000029544; NCT04336904	Anti-influenza
Ribavirin		Thirteen H-bonds with W508, Y510, K512, C513, D514, N582, D651 (3), A653 (3), and W691 of RdRp, the binding energy of −7.8 kcal/mol[50]	In Vero E6 cells, EC_{50} = 109.50 μM, CC_{50} > 400 μM, SI > 3.65[51]	ChiCTR2000029387; NCT04276688	With a broad antiviral spectrum, including HCV, RSV, et al
Sofosbuvir (GS-7977)		Seven H-bonds (W508 (3), K512 (2), A653, and W691) and 2 hydrophobic contacts (Y510 and D651) with Rdrp, the binding energy of −7.5 kcal/mol[50]	-	-	Anti-HCV

TABLE 1 (Continued)

Compound	Structure	Status in progress of SARS-CoV-2			Pharmacological function
		In silicon	In vitro	Clinical trial	
Galidesivir (BCX4430)		Six hydrogen bond and four hydrophobic interactions with RdRp,[52] the binding energy of −7.0 kcal/mol[50]	-	-	Anti-HCV
EIDD-2801 (β-D-N^4-hydroxycytidine-5′-isopropyl ester)		-	EC_{50} = 0.3 μM in vero cells, 0.08 μM in Calu-3 cells[53]	-	Anti-influenza virus and coronavirus

Abbreviations: HCV, hepatitis C virus; RdRp, RNA-dependent RNA polymerase; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

(ChiCTR2000030254), FPV showed superior efficacy to treat moderate COVID-19 pneumonia as compared with Arbidol (umifenovir, a membrane fusion inhibitor).[73] In a small-scale non-randomized controlled study at The Third People's Hospital of Shenzhen (ChiCTR2000029600), 35 patients treated with FPV (1600 mg orally twice daily on day 1, then 600 mg orally twice daily on days 2-14) in combination of interferon-α exerted a higher viral clearance and ameliorative chest computed tomography imaging compared with the control group receiving lopinavir/ritonavir (n = 45).[74] Additionally, an adaptive and double-blinded phase III clinical study of FPV combined with baloxavir marboxil (an accredited anti-influenza virus drug in Japan and USA) (ChiCTR2000029544) is ongoing with participants aged from 18 to 75. In Italy, a placebo-controlled trial to evaluate efficacy and safety of FPV in patients with moderate COVID-19 started on 25th March and it is anticipated to complete on 20th July 2020 (NCT04336904).[75,76]

3.3 | Ribavirin

Ribonucleoside analog ribavirin (1-β-*D*-ribofuranosyl-1,2,4-triazole-3-carboxamide) with a broad antiviral spectrum was first synthesized in the 1970s.[77] Despite the antiviral mechanism of ribavirin being controversial, one recognized mechanism of action is that its monophosphate metabolite leads to interruption of viral RNA replication by inhibiting the host inosine monophosphate dehydrogenase enzyme or enhance immune response.[78] It has been tested against respiratory RNA viruses including respiratory syncytial virus, influenza virus, several CoVs, HCV, and herpesviruses in animal and human cell lines.[78-82] Ribavirin is usually recommended in combination with interferon or other antivirals to treat viral infections. Acting as a marketed guanosine agent, ribavirin can be toxic to fetuses and is thus forbidden for pregnant women; it also causes anemia and lowers blood levels of calcium and magnesium, making it inappropriate for old patients.

The optimized active forms of ribavirin was estimated to bind SARS-CoV-2 RdRp with a binding energy of −7.8 kcal/mol which is comparable to native nucleotides and established 13 H-bonds interactions, suggesting the potential efficacy against SARS-CoV-2.[50] At a concentration of 109.50 μM, Ribavirin inhibited 50% CPE in Vero E6 caused by SARS-CoV-2.[51] Although ribavirin was included in the COVID-19 therapy Guidelines (Version 6), monotherapy is stagnated in preclinical studies due to the natural resistance to CoV genome and apparent side effects at higher doses. Therefore, clinical studies of ribavirin use in combination with interferon-α or other antivirals against SARS-CoV-2 infection is ongoing (ChiCTR2000029387, NCT04276688).[76]

3.4 | Sofosbuvir

Sofosbuvir (GS-7977; formerly PSI-7977), developed by Gilead in 2013, is an FDA-approved HCV inhibitor which was the first drug to

treat safely and effectively without use of interferon.[83] The coupling of sofosbuvir with velpatasvir (approved as EPCLUSA) is commonly applied in diverse HCV genotypes (GT 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6a).[84] This drug has also been shown to be effective against Yellow Fever, hepatitis A virus and ZIKV infection.[85-87] The robust in vitro activity (14-110 nM) of this drug with no obvious toxicity supports further in vivo exploration

Since the structure and replication mechanism of HCV RdRp is similar to that of SARS-CoV-2, it was suggested that it likely inhibited SARS-CoV-2.[88] In silico, sofosbuvir can tightly bind to SARS-CoV-2 RdRp with a −7.5 kcal/mol binding energy, forming seven H-bonds (W508 (3), K512 (2), A653, and W691) and two hydrophobic contacts (Y510 and D651).[50,89] As shown in RNA polymerase extension assays, the triphosphate form of sofosbuvir, as well as that of tenofovir (a nucleotide reverse transcriptase inhibitor used to treat HIV and HBV), alovudine and AZT (two anti-HIV agents), could be recognized mistakenly by SARS-CoV-2 RdRp and be incorporated into the newly-synthesized RNA chain to prohibit the progress of primer extension.[90-92] Based on the clinical data in treating HCV, Sayad et al suggested to include sofosbuvir in COVID-19 related registered clinical trial, and it has been proposed as a treatment option by EASL-ESCMID position paper.[93,94]

3.5 | Galidesivir

In preclinical studies, phosphorylated galidesivir (BCX4430) has been shown to act as a non-obligate RNA chain terminator which can inhibit viral RNA polymerases of a wide array of RNA viruses including flaviviruses (ZIKV and DENV), filoviruses (EBOV and Marburg virus) and CoVs, such as SARS-CoV and MERS-CoV.[95-97] Based on high-content image assays in HeLa cells, BCX4430 displayed inhibitory activity with an EC_{50} value of 57.7 μM and CC_{50} > 296 μM against SARS-CoV. BCX4430 possesses a rapid pharmacokinetics with a <5 minutes half-life ($t_{1/2}$) which, in the case of its metabolin BCX4430-triphosphate, is extended to 6.2 hours in vivo.

Galidesivir inhibits SARS-CoV-2 by tightly binding to its RdRp (binding energy of −7.0 kcal/mol). It has been shown to establish connections with 10 different amino acid residues (Thr^{455}, Arg^{553}, Lys^{621}, Arg^{624}, Asp^{452}, Ala^{554}, Asp^{623}, Asn^{691}, Ser^{759}, Asp^{760}) with a 62.09 piecewise linear potential score closed to positive control ATP.[50,52] However, the effect of galidesivir against SARS-CoV-2 has not yet been reported at the cellular or animal level.

3.6 | EIDD-2801

EIDD-2801 is an orally bioavailable prodrug of the ribonucleoside analog β-D-N^4-hydroxycytidine (NHC; EIDD-1931) with high inhibitory potency against influenza virus, EBOV, and multiple CoVs as observed in cell culture and animal studies.[98-100] NHC was shown to have prophylactic effect in the treatment of MHV and MERS-CoV. Recently, it was shown that NHC was effective against SARS-CoV-2 in Vero cells (EC_{50} of 0.3 μM) and in Calu-3 cells (EC_{50} of 0.08 μM).[53] Sheahan et al also provided in vivo efficacy data of EIDD-2801 against SARS-CoV or MERS-CoV infected mice, which signed with improved pulmonary function and reduced viral load. Ridgeback Biotherapeutics issued that the phase 2 trials testing EIDD-2801 as potential treatment for COVID-19 have been launched following two randomized double-blind placebo-controlled phase 1 studies which showed safety and promising exposures in human.[101]

4 | SUMMARY AND FUTURE DIRECTIONS

Different nucleoside/nucleotide polymerase inhibitors have been reported or suggested to be effective against SARS-CoV-2 infection. Here we discussed two anti-HCV drugs (sofosbuvir and galidesivir), an acknowledged broad-spectrum antiviral inhibitor (ribavirin), an anti-influenza agent (favipiravir), and two EBOV and CoV targeting compounds (RDV and EIDD-2801). In a study just published, the detailed action mechanism of RDV-triphosphate metabolite was reported that a steric clash at the i + 3 position resulted in the premature termination of RdRp chain synthesis while mapping a key residue S861 during the translocated polymerase.[102] Until today, the results of three large clinical trials of RDV were announced, which are preliminary data from Gilead's open-label critical care group (available online), the critical care group from the randomized double-blind controlled trial in China (Lancet online), and the NIAID randomized double-blind controlled trial (available online).[103-105] However, these results are not encouraging in improving patients' condition as expected and appeared obvious adverse event, which indicates that RDV is not a cure for SARS-CoV-2. What controversial is that RDV showed a therapeutic effect in a 1063 clinical trial in America and Gilead's SIMPLE trial which the mortality and recovery time of patients are decreased. The other trials in China have been suspended due to low enrollment rate. Meanwhile, multiple clinical trials have been initiated in patients with COVID-19 in China, the US, Japan, and other countries to evaluate FPV alone or in conjunction with other agents. Recently, EIDD-2801 has received permission from the FDA to begin patient trials. Sofosbuvir, demonstrated from the functional framework and docking affinity, is also hopefully entering clinical testing. Cocktail therapy with RdRp in combination with other compounds or herbal medicines are also considered as a positive measure. An in vitro study indicated that emetine (an alkaloid derived from natural plant approved in China for use as an old anti-malarial drug) inhibited SARS-CoV-2 virus replication with an EC_{50} at around 0.5 μM; a synergistic effect between RDV (6.25 μM) and emetine hydrochloride (0.195 μM) could potentially achieve a 64.9% inhibition in SARS-CoV-2 viral yield.[106] In conclusion, although these drugs have potential as therapeutic options against SARS-CoV-2, discovering and developing new drugs targeting the SARS-CoV-2 RdRp is urgently needed to fight against COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOUR CONTRIBUTIONS

YW, QC, and LR conceived this review. YW wrote the initial draft of the review, VA, QC, RD, and LR revised and edited the manuscript.

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