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RNA-dependent RNA polymerase (RdRp) inhibitors: The current landscape and repurposing for the COVID-19 pandemic

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1. Introduction

1.1. RNA virus infections

Among the major groups of infections, RNA virus infections contribute considerably to the worldwide death and morbidity index related to viral infection. Chronic illness related to persistent RNA virus infections represents a crucial public health worry [1]. While human immunodeficiency virus-1 as well as hepatitis C virus (HCV) are perhaps the most popular instances of persistent RNA viruses that cause chronic disease, proof recommends that numerous other RNA viruses, consisting of re-emerging viruses such as Ebola virus and Zika virus, develop persistent infections [2]. Furthermore, swine and avian influenza infections, together with Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV), stand for substantial pandemic risks to the general populace [3].

Considering the high frequency and vast circulation of RNA viruses, their huge genetic diversity as well as the frequent recombination of their genomes and raising activity at the human-animal interface, these viruses are identified as a recurring hazard to human health and wellness [4]. This truth once again became noticeably obvious in late 2019 and very early 2020, when severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was found to be the root cause of a large and quickly spreading outbreak of lower respiratory tract infection and disease, consisting of potentially fatal pneumonia, in Wuhan, China [5,6]. This novel coronavirus-induced febrile respiratory system disease was formally named coronavirus disease 2019 (COVID-19) by the WHO. Presently, the COVID-19 pandemic is still spreading worldwide. Along with vaccinations, people are also hoping for particular medicines [7,8]. An increasing number of medication prospects have come to the attention of clinical scientists, and many clinical trials are being performed throughout the world. However, there is...
1.2. RNA virus and SARS-CoV-2

An RNA virus is a virus that utilizes RNA as its genetic material. Based on their genome and mode of replication, three distinct groups of RNA viruses have been classified: double-stranded RNA viruses (dsRNA), single-stranded RNA (ssRNA) and retroviruses [13,14]. dsRNA viruses contain one to a dozen different RNA molecules, each of which encodes one or more viral proteins [15]. The genome of positive-sense ssRNA viruses is directly used as mRNA, and the host ribosomes translate this genome into a single protein that is modified by host and viral proteins to form the various proteins needed for replication [16–18]. One of these includes RdRp, which copies the viral RNA to develop a double-stranded replicative type [19,20]. Consequently, this dsRNA routes the development of brand-new viral RNA. The genome of negative-sense ssRNA viruses must be copied by an RNA replicase to form positive-sense RNA [21,22]. The positive-sense RNA molecule then acts as viral mRNA, and this mRNA is translated into proteins by host ribosomes. In addition, retroviruses have a ssRNA genome but are generally not considered RNA viruses because they utilize DNA intermediates for replication [23]. The nucleic acid of a pathogenic virus, such as coronaviruses, hepacivirus, influenza viruses and respiratory syncytial virus (RSV), is typically ssRNA (Table 1) [24,25]. These RNA viruses can create lower respiratory system tract infections that cause bronchiolitis as well as pneumonia [26]. Young children, the elderly, and patients with compromised heart, lung, or immune systems are at the highest risk for significant disease related to these RNA virus-related breathing infections [27,28].

SARS-CoV-2 is a positive-sense ssRNA virus that can infect humans [29]. The infection largely spreads from human to human through close contact and by breathing droplets generated from sneezes or coughs. The 30-kb genome of SARS-CoV-2 contains 14 open reading frames (ORFs) that can encode at least 27 proteins [30–32]. The 3' end of the genome encodes four structural proteins, namely, spike, envelope, membrane, and nucleocapsid proteins, and eight accessory proteins that disrupt the host's inherent immune responses. The ORF1ab region at the 5' end encodes a polyprotein, which is hydrolysed into 16 nonstructural proteins (nsp 1-16) to create a replicase/transcriptase complex (RTC). The main RTC is RdRp (nsp12) (Fig. 1). In RNA viruses, such as SARS-CoV-2, RdRp creates the machinery needed for RNA synthesis and the organized replication and transcription of genomic RNA.

1.3. RNA-dependent RNA polymerase

The genomic replication process of RNA infections is controlled by RdRp, which is inscribed by the virus itself [30,33]. After the virus attacks a host cell, the viral genomic RNA is directly utilized as a template, and the host cell protein synthesis system is utilized for the translation of RdRp. RdRp is consequently used to complete the transcriptional synthesis of negative-strand subgenomic RNA, the synthesis of different structural protein-related mRNAs, and the replication of viral genomic RNA. RdRp can properly and efficiently synthesize tens of thousands of nucleotides and thus facilitates all other biological activities that occur after the virus invades a host cell.

The structure of RdRp of positive-strand RNA viruses resembles that of a cupped right hand and includes fingers, palm and thumb subdomains that are largely associated with binding to the template, polymerization, nucleoside triphosphate (NTP) access and associated features [34–36]. In addition to these three central subdomains, an N-terminal subdomain that bridges the fingers and thumb subdomains is located in all RdRps [37], and this subdomain serves as the active site of RdRp. The completely encircled active site cavity is responsible for considerable communication between the finger and thumb subdomains. The active site of RdRp is extremely well preserved. The finger subdomain plays a considerable role in establishing the geometry of the active site [38] by holding the template RNA in place and facilitating polymerization. The thumb subdomain harbours residues that are involved in packing against the template RNA and stabilizing the initiating NTP on the template [39]. This subdomain also facilitates the translocation of the template following polymerization by accommodating large conformational rearrangements. The thumb subdomain exhibits the greatest diversity among the identified RdRp and differences in size and complexity based on the mode of replication initiation. The palm subdomain translocates to the junction of the finger and thumb subdomains and houses many

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Table 1

| Demonstrative virus          | Family                 | RNA                  |
|-----------------------------|------------------------|----------------------|
| Norovirus                   | Caliciviridae          | (+) ssRNA            |
| SARS-CoV                    | Coronaviridae          | (+) ssRNA            |
| MERS-CoV                    | Coronaviridae          | (+) ssRNA            |
| SARS-CoV-2                  | Coronaviridae          | (+) ssRNA            |
| Hepatitis C virus           | Flaviviridae           | (+) ssRNA            |
| Dengue virus                | Flaviviridae           | (+) ssRNA            |
| Zika virus                  | Flaviviridae           | (+) ssRNA            |
| Poliovirus                  | Picornaviridae         | (+) ssRNA            |
| Venezuelan equine encephalitis virus | Togaviridae | (+) ssRNA            |
| Influenza A virus           | Orthomyxoviridae       | (+) ssRNA            |
| Influenza B virus           | Orthomyxoviridae       | (+) ssRNA            |
| Influenza C virus           | Orthomyxoviridae       | (+) ssRNA            |
| Ebola virus                 | Filoviridae            | (+) ssRNA            |
| Marburg virus               | Filoviridae            | (+) ssRNA            |
| Vesicular stomatitis virus  | Caliciviridae          | (+) ssRNA            |
| Respiratory syncytial virus | Paramyxoviridae        | (+) ssRNA            |

SARS-CoV: severe acute respiratory syndrome coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; (+) ssRNA: positive-sense single-stranded RNA; (-) ssRNA: negative-sense single-stranded RNA.

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Fig. 1. Domain organization of SARS-CoV-2 nsp 12 (RdRp). The interdomain borders are labelled with residue numbers. RdRp: RNA-dependent RNA polymerase.
structurally conserved elements associated with catalysis [40]. The palm subdomain is involved in the selection of NTP over deoxyribonucleoside triphosphate (dTTP) and catalyses the phosphoryl transfer reaction by coordinating with two metal ions (Mg$^{2+}$ and/or Mn$^{2+}$). The best-known RdRps of positive-strand RNA viruses are the polioviral 3Dpol and hepatitis C virus nonstructural 5B (NS5B) proteins as well as the SARS-CoV-2 RdRp, which has recently attracted much attention.

The structure of the SARS-CoV-2 RdRp complex consists of a nsp 12 core catalytic unit, a nsp7-nsp8 (nsp8-1) heterodimer, and an additional nsp8 subunit (nsp8-2), and nsp12-nsp7-nsp8 is defined as the minimal core component for virus RNA replication [31]. The N-terminal portion of nsp12 contains a β-hairpin (V31–K50) and a nidovirus-specific extension domain (D60-R249). The β-hairpin is sandwiched by the palm subdomain in the RdRp core and nidovirus RdRp-associated nucleotidyltransferase (NiRAN), a configuration not observed in other coronavirus RdRp structures [41]. The C-terminal catalytic domain of nsp12 (A250-F369) connects to NiRAN through an interface subdomain. The C-terminal catalytic domain of nsp12 (S367–F920) adopts a canonical cupped right-handed configuration of all viral RdRp, composed of the finger, palm, and thumb subdomains. Catalytic metal ions are not observed in the absence of primer-template RNA and NTPs, although they are present in several structures of viral polymerases that synthesize RNA. The nsp7-nsp8 heterodimer binds above the thumb subdomain and stabilizes the thumb-finger interface. Nsp7 makes a major contribution to the binding of the heterodimer to nsp12, while nsp8 only contacts a few residues from nsp12. The other copy of nsp8 (nsp8-2) sits atop the finger subdomain and forms additional interactions with the interaction subdomain. In this structure, similar to other positive-strand RNA viruses RdRps, the template/primer entry channel, NTP entry channel, and nascent strand exit channel are all positively charged and converge in a central cavity, which is the active site of the SARS-CoV-2 RdRp that is formed by seven conserved catalytic motifs (A to G). In this central cavity, these RdRp motifs mediate template-guided RNA synthesis. Motif A (T611-M626) houses the catalytic motif DX2-4D, in which the first aspartic acid D618 is invariant in most viral polymerases. The flexible loop in Motif B (T680-T710) serves as a hinge to undergo conformational arrangement associated with template RNA and substrate binding. Motif C (F753–N767) contains the catalytic motif SDD, which is essential for binding the metal ion. Motif D contains residues L775-E796. Motif E contains residues H810-V820 and combines with the palm subdomain to support the primer chain. Motif F (K912-E921) interacts with the phosphates of incoming NTP. The NTP entry channel is formed by a set of hydrophilic residues, including K545, R553 and R555 in motif F [42]. Motif G (K500–S518) interacts with the template strand. The RNA template strand enters the active site composed of motifs A and C through the groove sandwiched by motifs F and G. The product-template hybrid exits this active site through the RNA exit channel on the front side of the RdRp (Fig. 2).

The polymerase of segmented negative-strand RNA viruses (sNSVs), including influenza virus, is composed of three polyptides: PB1, PB2 and PA/P3. PB1 contains the polymerase active site, whereas PB2 and PA/P3 have cap-binding and endonuclease domains, respectively, needed for transcription initiation by cap snatching [43–45]. In addition, the catalytic core of nonsegmented negative-strand RNA viruses (NNVs), including vesicular stomatitis virus (VSV), RSV and Ebola virus, is the L protein, which catalyses RNA polymerization during both replication and transcription, cap addition, and cap methylation of nascent viral mRNAs [36,46–49]. The RdRp domain is a functional domain of the L protein, and the L proteins of nonsegmented negative-sense single-stranded RNA viruses share six conserved regions and three functional domains (RdRp, capping, and cap methyltransferase) (Figs. 3 and 4).

RdRp is an important therapeutic target because it plays a pivotal role in replication of the RNA genome and because the host lacks a functional equivalent to this protein. In addition, due to the absence of a counterpart to RdRp in mammalian cells, its inhibition is not expected to cause target-related side effects, and thus, RdRp is considered an attractive target in drug discovery and development. The development of effective RdRp inhibitors to block viral replication has long been a research topic in many scientific institutions and pharmaceutical companies. There are two known classes of RdRp inhibitors: nucleoside analogue inhibitors (NIs) and nonnucleoside analogue inhibitors (NNIs). The classes show differences in structure and the location that bind to RdRp: enzyme active site (NIs) and allosteric sites (NNIs). This review summarizes the promising RdRp inhibitors that have been launched or are currently in clinical studies for the treatment of RNA virus infections, including COVID-19.

### 2. Nucleoside inhibitors

NIs terminate the RNA synthesis step, which is essential for RNA replication, through their incorporation by RdRp, which prevents incoming nucleotides from being added to the RNA chain [50]. It has been proposed that steric hindrance by nucleoside inhibitors, which contain a 3′-hydroxyl group, is responsible for the observed termination of chain elongation [51]. Due to this mechanism, NIs are sometimes called chain termination inhibitors [52]. NIs of RNA viruses, which have been developed as prodrugs, eventually become cleaved at their site of action in the liver by hepatic enzymes and undergo phosphorylation into a triphosphate form, which targets the polymerase at its highly conserved active site (Fig. 5). NIs of RdRp are classified into three major classes: pyrimidine nucleoside inhibitors, purine nucleoside inhibitors and miscellaneous nucleoside inhibitors (Table 2).

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*Fig. 2. Structure of RdRps of positive-strand RNA viruses. A: poliovirus 3Dpol (1RDR); B: HCV NS5B polymerase (1NB4); and C: SARS-CoV-2 RdRp (7BW4). Nsp7 and nsp8 act as cofactors to promote the activity of RdRp (grey). The palm, finger and thumb subdomains are shown in purple, green and yellow, respectively. RdRp: RNA-dependent RNA polymerase.*
2.1. Pyrimidine nucleoside inhibitors

2.1.1. Cytosine analogue inhibitors

Cytidine analogues are metabolized to both cytidine and uridine triphosphates through the action of cytidine deaminase. 1 (NHC, EIDD-1931, β-D-N4-hydroxycytidine) is an orally bioavailable ribonucleoside analogue with broad-spectrum antiviral activity against various unrelated RNA viruses, including SARS-CoV-2 (IC50 = 0.30 μM), MERS-CoV (EC50 = 0.56 μM), mouse hepatitis virus (MHV, EC50 = 0.17 μM) and venezuelan equine encephalitis virus (VEEV, EC50 = 1.00 μM), and increased potency against a coronavirus bearing resistance mutations to the nucleoside analogue inhibitor remdesivir [53–57]. 1 acts as a weak alternative substrate for cytidine triphosphate (CTP) to potentially modulate virus replication steps that are dependent on these structures, such as encapsidation, translation and replication [58]. 2 (EIDD-2801) is the 5′-isopropylester of 1 that exhibits broad activities against influenza viruses and multiple coronaviruses. In phase II clinical trials, Ridgeback Biotherapeutics is evaluating various candidates for the treatment of newly hospitalized adults with COVID-19 and symptomatic adult outpatients with COVID-19. Preclinical studies are also ongoing to determine
its potential as a treatment for influenza and MERS-CoV infection.

The study of pyrimidine nucleoside inhibitors as potential antiviral drugs revealed that the unique substituents at the C2’ or C4’ position of the nucleoside exhibit obvious antiviral activity. The incorporation of 2’-C-modified monophosphates onto the 3’ termini of growing virus RNA strands promotes the termination of elongation due to steric hindrance between the incoming natural nucleotide and the unnatural 2’-C-group of the inhibitor [59,60]. 3 (NM-107) is a 2’-C-methylcytidine that was initially identified as a competitive inhibitor of NS5B polymerase, and the EC50 of 3 in wild-type replicon cells is 1.85 μM [61]. Upon phosphorylation into its 5-triphosphate form, this metabolite inhibits viral RNA chain elongation and viral RdRp activity, and these effects block the viral production of HCV RNA and thus viral replication. In addition to HCV, this compound inhibits the replication of a variety of other viruses, such as dengue virus (DENV) and norovirus [62,63]. 4 (valopicitabine, NM-283), which is the 3’-O-valinyl ester of 3, was synthesized to obtain a compound with improved oral bioavailability compared with that of its parent compound 2’-C-methylcytidine. Physicochemical, pharmacokinetic, and toxicokinetic studies have shown that 4 is an acid-stable prodrug of 2’-C-methylcytidine with excellent pharmacokinetic and toxicokinetic profiles [64]. 4 is currently being evaluated in phase II clinical trials for the treatment of chronic hepatitis virus C (HCV) infection.

Janssen screened a series of 4’-cytosine nucleoside analogues based on their pharmacodynamics and pharmacokinetic properties and found that 4’-chloromethyl-2’-deoxy-2’-fluorocytidine (5, ALS-8112) exhibited the most promising activity in the RSV replicon assay, with an EC50 of 0.15 μM [65]. 5 enters various types of epithelial cells in the respiratory tract and is subsequently phosphorylated to form an intracellular nucleoside triphosphate with a half-life of approximately 29 h [66]. The 5’-triphosphate of 5 is the active form of the drug and inhibits RSV polymerase with an IC50 of 0.02 μM, and no appreciable inhibition of human DNA and RNA polymerases was detected at a concentration of 100 μM [67]. 6 (lumicitabine, ALS-8176), the 3’-5’-di-O-isobutyryl prodrug of 5, is a first-in-class nucleoside RSV polymerase inhibitor that demonstrated excellent anti-RSV efficacy and safety in phase II clinical trials. Moreover, a number of 2’-fluoro-4’-substituted cytidine nucleosides exhibited potent inhibition of the RSV replicon with a wide selectivity window in these studies, and their 5’-triphosphates effectively inhibited RSV polymerase with high selectivity with respect to the host polymerases [65].
These findings indicate that access to the F atom might allow the synthesis of first-in-class antiviral agents against RSV infection.

7 (4'-Azido-2'-deoxy-2'-C-methylcytidine) is a potent nucleoside inhibitor of the NS5B polymerase that displays an EC_{50} value of 1.2 μM and shows moderate in vivo bioavailability in rats (F = 14%) [69]. 8 (TMC-649128) is the di-isobutyryl ester of 7, and its pharmacokinetic properties in rats can potentially be improved by introducing prodrug esters. Isobutyryl ester 8 exhibits greatly improved mean maximum plasma concentrations (C_{max} = 4.65 μM) and hence a larger area under the blood level-time curve (AUC_{0-1} = 12.7 μM/h) and greater oral bioavailability (F = 65%).

Fluorinated nucleosides are well known for their antiviral and anticancer properties. Pharmasset screened a series of 2'-cytosine nucleoside analogues with obvious anti-HCV activity, β-D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (9, PSI-6130), in which the C2' position of uridine is substituted by an F atom and methyl group, exerts a highly effective inhibitory effect on HCV replication [70,71]. Unfortunately, clinical phase I trials showed that 9 does not have good pharmacokinetic characteristics. The low oral bioavailability of 9 might be due to the presence of hydroxyl and amino groups, which are more polar, exhibit poor fat solubility, and cannot easily penetrate biofilms in its structure [72]. In addition, its amino group at position 4 is unstable under acidic conditions, and this amino group can be easily removed to generate carbonyl groups. To resolve the problem of bioavailability and metabolism, the 3',5'-disobutylate prodrug 10 (mericitabine, RG-7128) was designed to promote the absorption and metabolism of the compound in the intestine [73–76]. 10 is rapidly absorbed via the oral route and converted to 9, which is subsequently metabolized to its metabolite. The results also showed that 10 improves the pharmacokinetic parameters, and clinical trials have also shown that the drug exerts a certain effect. However, 10 is not the most ideal medicine due to its low general efficacy and short t_{1/2}.

2.1.2. Uracil analogue inhibitors

β-D-2'-deoxy-2'-fluoro-2'-C-methyluridine (11, PSI-6206) is a metabolite of 9 in vivo [77] that can significantly inhibit HCV replication. The patients with hepatitis C treated with 11 generally show good tolerance. However, the bioavailability of 11 is too low (25%) because 11 cannot be converted into 11-triphosphate (11-TP). However, observations of the metabolites of 9 revealed that monophosphate 9 can be further transformed into 11-TP, which has a long t_{1/2} and better activity than 9-TP [77,78]. This major discovery indicates that uridine monophosphate derivatives might be ideal direct-acting antiviral agents (DAAs). To ensure the safety and efficiency of the drug, the F atom and methyl group at the C2' position are retained. Because compounds containing phosphoric acid groups are negatively charged, the related compounds cannot be easily absorbed by the human body. The prodrug design was finally adopted, and the first prodrug 12 (PSI-7672) was designed [77]. Since then, a large number of derivatives have been synthesized, and some of the related experience has been previously summarized [79]. The isomeric form of the amino acid is significant because the d-alanine derivative is inactive, which means that the natural L-amino acid is required for activity. Observations of the amino acid side chain (R1) have revealed that a small alkyl group is a viable substitution, but significant decreases in potency are observed with substitutions larger than ethyl. Methyl results in the greatest potency and is therefore a viable substitution. If the amino acid is alanine and the phosphate ester is a phenyl substituent, the groups at the carboxylic acid ester (R2) that provide the desired submicromolar activity are small alkyl groups and branched alkyl groups. However, cytotoxicity was observed with n-butyl, 2-butyl and n-pentyl esters. Phenyl and halogenated alkyl groups do not provide sufficient improvements in potency. The evaluation of the phosphoramidate ester substituent (R2) revealed that a derivative with phenyl as a substituent exhibits good potency and is not cytotoxic (Table 3).

It was finally determined that 13 (PSI-7851) is an ideal DAA with a favourable pharmacokinetic profile for inhibiting HCV [77,78,80]. 13 contains a chiral phosphorous atom and is therefore a mixture of two diastereomers, S, diastereoisomer 14 (PSI-7976) and R, diastereoisomer 15 (sofosbuvir, PSI-7977) [81]. The activity of 15 is significantly better than that of 14, and this difference might be due to the different binding orientations of 14 and 15 to the enzyme, which are productive and nonproductive, respectively. 15 might preferentially bind in the nonproductive orientation and form a dead-end complex to exert a significant antiviral effect [82]. Compound 15 is an approved NS5B polymerase inhibitor (EC_{50} = 0.42 μM) and exerts pangenotypic antiviral effects against HCV genotypes (GTs) 1–6. The potent antiviral activities of 15 are higher than 90% [83]. In addition, 15 has the ability to suppress different families of viruses, including Zika virus (ZIKV), DENV and chikungunya virus [84–86]. Moreover, 15 exhibits a rapid response, and over 0.8 and 2 days, this compound presented the 2'-deoxy-2'-a-fluoro-β-C-methyluridine-5'-monophosphate analogue.

### Table 3
Structure–activity relationship of the 2'-deoxy-2'-a-fluoro-β-C-methyluridine-5'-monophosphate analogue.

| R1          | R2              | R3       | EC_{50} | Inhibition of cellular rRNA replication at 50 μM (%) |
|-------------|-----------------|----------|---------|---------------------------------------------------|
| Methyl      | Isopropyl       | Phenyl   | 0.52    | 25.9                                              |
| Methyl      | Methyl          | Phenyl   | 1.62    | 0.0                                               |
| Methyl      | c-Hexyl         | Phenyl   | 0.25    | 61.1                                              |
| Methyl      | Ethyl           | 4-F Phenyl | 0.76    | 55.3                                              |
| Methyl      | Isopropyl       | 4-F Phenyl | 0.77    | 0.0                                               |
| Methyl      | Isopropyl       | 4-Cl Phenyl | 0.42    | 0.0                                               |
| Methyl      | c-Hexyl         | 4-Cl Phenyl | 0.04    | 52.1                                              |
exerts estimated potencies of 99 and 99.9%, respectively [87]. The bioavailability of 15 is high, and maximum plasma concentrations (Cmax) were detected 0.5–2 h after oral administration [88]. 15 has been considered a potential effective drug for inhibiting SARS-CoV-2 infection since the emergence of the COVID-19 pandemic. The administration of 15 and daclatasvir in combination with standard of care (SOC) for the treatment of patients with COVID-19 resulted in better 14-day recovery rates and a shorter hospital stay. The patients in the therapy group experienced a shorter duration of hospital stay and a shorter median time to discharge than the control group (6 vs 8 days and 6 vs 11 days, respectively). Recently, Pinar Mesci et al. [89] reported that 15 can potentially be used to alleviate COVID-19-related neurological symptoms.

16 (AL-335) is a type of uridine analogue with 4′-fluoro-2′-C-substituted sugar moieties [90]. 16-TP exhibits potent inhibition of NS5B polymerase with IC50 values as low as 27 nM. In an HCV subgenomic replicon assay, the phosphoramidate prodrug of 16 demonstrated very potent activity with EC50 values as low as 20 nM [91]. The administration of 16 in combination with simprevir and odalasvir has been evaluated in human phase II clinical trials and has shown promising efficacy and safety results. 16 is well tolerated when administered as single and multiple doses and exhibits an acceptable pharmacokinetic profile [92]. 17 (NJ-54257099) is a cyclic phosphate ester derivative belonging to the class of 2′-deoxy-2′-spirooxetane uridine nucleotide prodrugs [93]. This compound profoundly dose-dependently decreases HCV RNA levels in mouse models of HCV GT 1a and 3a infections. 17 was terminated following completion of phase I clinical studies conducted by Janssen Pharmaceutical. This is because the clinical antiviral activity of 17 in patients infected with HCV GT 1 was insufficient to justify further clinical studies. Compound 18 (VX-135) exhibited pronounced antiviral activity against GTs 1–6 (EC50 values between 12 and 390 nM) in vitro [94] and has been evaluated in phase II clinical studies for the treatment of hepatitis C. The phase I study evaluated the pharmacokinetics, safety and antiviral activity of 18 in 48 healthy controls and 30 patients with HCV GT 1 infection. The most common adverse events were headache and diarrhea (two subjects each), and no severe adverse events were recorded. Compound 18 demonstrated potent antiviral activity with a 4.5 log10 decrease in HCV RNA over 7 days at a dose of 200 mg quaque die (QD) in patients infected with chronic hepatitis C [95].

2.1.3. Thymine analogue inhibitors

19 (ACH-3422) is an NS5B polymerase inhibitor that displays pangenotypic activity and a high in vitro barrier to resistance. 19 is designed to introduce three deuteriums on the side chains of pyrimidine and ribose groups. The incorporation of deuterium into pharmacologically active agents according to the principle of deuterium isotope effects (DIEs) offers potential benefits, such as improved exposure profiles and decreased production of toxic metabolites that could yield improvements in efficacy, tolerability, or safety [96]. Therefore, 19 is well tolerated and induces no serious adverse events in healthy volunteers and hepatitis C patients [97]. Among active patients, increasing doses of 19 resulted in increased viral decline. In the proof of concept group administered 700 mg of the antiviral, mean decreases in the maximum viral load of 3.4 log10, 4.2 log10, and 4.6 log10 were obtained after 7, 10, and 14 days of treatment, respectively. Three of six patients (50%) achieved viral clearance after the administration of 700 mg for 14 days.

2.2. Purine nucleoside inhibitors

2.2.1. Adenine analogue inhibitors

20 (galidesivir, BXC4430) is an adenosine nucleoside analogue developed by BioCryst Pharmaceuticals. This compound was originally intended as a treatment for HCV but was subsequently developed as a potential treatment for deadly filovirus infections [98,99]. Studies have shown that 20 protects against both Ebola and Marburg viruses in both rodents and monkeys [99]. This compound also shows broad-spectrum antiviral effectiveness against a range of other RNA virus families, including SARS-CoV and MERS-CoV [100]. 20 can bind SARS-CoV-2 RdRp, with a binding energy of ~7.0 kcal/mol [101]. In April 2020, BioCryst opened enrolment into a randomized, double-blind, placebo-controlled clinical trial aiming to assess the safety, clinical impact and antiviral effects of 20 in patients with COVID-19.

21 (Nuc, GS-441524) is a 1′-CN-modified adenosine C-nucleoside analogue that exhibits antiviral activity against a variety of RNA viruses [102]. Structurally, the 1′-CN group provides potency and selectivity for viral RdRp. A study conducted in 2019 revealed that 21 can potentially be used for the treatment of feline infectious peritonitis caused by a coronavirus [103]. 21 is synthesized into 21-TP through intracellular metabolism, and 21-TP can interfere with the activity of viral RdRp. However, the kinetics of the monophosphorylation of 21 are slow, and the use of a parent nucleoside modified with monophosphate might greatly increase the intracellular NTP concentration [102]. Compound 22 (remdesivir, GS-7714) is the 5p isomeric 1′-C-alanine phosphoramidate prodrug and effectively bypasses the rate-limiting step of 21 monomer phosphorylation [104]. Compound 22 is activated more rapidly than 21 in human cells infected with SARS-CoV and MERS-CoV, and multiple uses of this compound have been explored with the aim of helping to address urgent and unmet medical needs around the world, including Ebola disease, SARS, MERS and most recently COVID-19 [105]. In Vero E6 cells, 22 effectively blocks SARS-CoV-2 infection at low concentrations (EC50 = 0.77 μM) and exhibits low cytotoxicity (CC50 > 100 μM). In addition, the EC50 value of 22 against SARS-CoV-2 in Vero E6 cells is 1.76 μM [10]. A study using the rhesus macaque model of SARS-CoV-2 infection revealed that therapeutic treatment with 22 initiated early during infection results in a clear clinical benefit in SARS-CoV-2-infected rhesus macaques [106,107]. NIAID reported that remdesivir was superior to placebo in shortening the time to recovery in adults who were hospitalized with COVID-19 and had evidence of lower respiratory tract infection in phase III clinical trials [108]. Recently, the FDA approved 22 for the treatment of patients with COVID-19 requiring hospitalization. To date, 22 is the first and only FDA-approved treatment for COVID-19 in the United States. In addition, some researchers have argued for the direct administration of 21 as a COVID-19 treatment because 21 exhibits either similar or to more potency than 22 against SARS-CoV-2 in cell culture [109].

23 (AT-527) is a novel modified guanosine nucleotide prodrug inhibitor of the NS5B polymerase that belongs to the same category as 22. Compound 23 exhibits higher in vitro antiviral activity than compound 15. The free base of 23 had an EC50 value of 25 nM and thus exhibited 10-fold higher potency than 15 in Huh-7 cells bearing the HCV GT 1b replicon [110]. The antiviral activity and safety of 23 has been demonstrated in phase II clinical studies of hepatitis C patients. The mean maximum reductions observed in noncirrhotic subjects with HCV GT 1b, noncirrhotic subjects with HCV GT 3, and subjects with compensated cirrhosis after 7 days of treatment were 4.4, 4.5, and 4.6 log10 IU/mL, respectively [111]. A phase II clinical trial was established to evaluate the safety and efficacy of 23 for the treatment of adult patients hospitalized with moderate COVID-19 disease. 24 (INX-189), the phosphoramidate nucleoside analogue prodrug of 2′-C-methylguanosine, is a potent HCV replication inhibitor (EC50 = 35 nM) that is currently being investigated in a phase II clinical trial by Bristol-Myers Squibb for the oral treatment of hepatitis C virus infections [112].
2.2.2. Guanine analogue inhibitors

25 (IDX-184), a highly potent inhibitor of HCV replication in vitro, was designed to achieve enhanced targeting to the liver through monophosphorylation and reduce the exposure of other tissues to the drug. Compound 25 is preferentially cleaved by hepatic enzymes to form TP, and 25-TP potently inhibits NS5B polymerase (IC\textsubscript{50} = 0.31 mM, Ki = 52.3 nM) but does not inhibit human polymerases a, b, or g (IC\textsubscript{50} > 50 mM) [113]. The administration of 25 at single and multiple doses of up to 100 mg/day for three days revealed that the compound is safe and well tolerated in both healthy volunteers and treatment-naive HCV GT-1 infected subjects, respectively. 25 in combination with pegylated interferon-\alpha (Peg-IFN) and ribavirin (RBV) was generally safe and well tolerated in HCV GT-1-infected subjects, and its lowest dose of 50 mg QD resulted in marked viral load reductions compared with those obtained with Peg-IFN/RBV alone [114].

2.3. Miscellaneous nucleoside inhibitors

26 (favipiravir, T-705) is a broad-spectrum anti-RNA virus drug that was approved in 2014 for the oral treatment of influenza A and for the treatment of influenza B infection. Studies have shown that 26 demonstrates good antiviral effects against a variety of RNA viruses, such as Ebola virus and rabies virus, in addition to influenza viruses [115–117]. Wang et al. [10] showed that 26 can effectively reduce SARS-CoV-2 infection in vitro (EC\textsubscript{50} = 61.88 \mu M, CC\textsubscript{50} > 400 \mu M, SI > 6.46). Ongoing clinical trials have shown that 26 can accelerate the recovery of COVID-19 patients, as demonstrated by a median cure time of 2.5–9 days, whereas that obtained with the control group is 11 days (8–13 days). Compared with the control group, the patients with nonsevere new coronavirus belonging to the 26 group exhibited a shorter virus clearance time, and chest CT also showed significant improvement. In addition, the patients in the 26 treatment group experienced fewer adverse reactions and better tolerance [118]. The results of the clinical trial conducted in Wuhan suggest that among patients with common COVID-19, the 7-day clinical recovery rate of the patients in the 26 treatment group was 71.43%, which was significantly higher than the rate of 55.68% obtained with the control group; in addition, the rate of 55.68% obtained with Peg-IFN alone [114]. The structures of NNIs are diverse. Most NNIs change the spatial conformation of RdRp by binding to allosteric sites on the surface of the enzyme and thereby inhibit its activity and the replication of RNA viruses (Fig. 6). 28 (pimodivir, JNJ-63623872) is an NNI of the PBZ domain of the RdRp of influenza A virus [126]. Phase I and II clinical trials have shown that 28 has the potential to not only reduce the viral load but also have a clinical impact on patients [127,128]. However, due to the unsatisfactory results of phase III clinical trials, the clinical study of 28 has been terminated. In addition, clinical studies of allosteric site inhibitors have mainly focused on anti-HCV infection. Five different allosteric binding sites for NNIs in HCV NS5B polymerase have been discovered [129,130]. Two of these sites are located in the thumb subdomain of the polymerase, and the other three are located in the palm subdomain. The NNIs of HCV can be divided into five different classes according to the location of their respective binding sites (referred to as thumb I and II and palms I, II and III) (Table 4).

3. Non-nucleoside inhibitors

The initially discovered benzimidazole 29 has weak inhibitory activity (IC\textsubscript{50} = 1.6 \mu M) against HCV GT 1 NS5B polymerase, and its EC\textsubscript{50} value is higher than 10 \mu M in a cell-based model for subgenomic replicon [131]. The structure has been optimized to improve the activity of benzimidazole compounds. JTK-109 (30) is a superior compound that was obtained by modifying position 2 of benzimidazole. 30 inhibits HCV GT 1NS5B polymerase with an IC\textsubscript{50} value of 0.022 \mu M and an EC\textsubscript{50} value of 0.62 \mu M [132]. In addition, the carboxyl group at position 5 of benzimidazole can be modified to enhance the antiviral activity of the compound. A longer side chain was linked by an amide bond to obtain 31, which exerts an inhibitory effect on NS5B polymerase GT 1 polymerase (IC\textsubscript{50} = 0.3 \mu M) with an EC\textsubscript{50} value of 1.7 \mu M [133]. To further improve the activity of the compounds on enzymes and cells, the structure of these compounds was further modified by replacing the benzimidazole ring with the indole ring, which yielded 32. The IC\textsubscript{50} value of 32 in inhibiting HCV GT 1b NS5B polymerase is 0.016 \mu M, and the EC\textsubscript{50} Value is 4.2 \mu M [134]. Compared with 29, the activity of 32 at the enzyme level was increased 100-fold, and the activity in the cell model was also improved. The structure of 32 was modified using the same strategy as that used to obtain 29, which yielded 33 (BILB-1941). The IC\textsubscript{50} value of 33 in inhibiting HCV GT 1b NS5B polymerase is 0.045 \mu M, and the EC\textsubscript{50} value is 0.084 \mu M [131]. Compound 33 was administered via a single oral dose in a clinical phase I trial, which revealed that this compound has antiviral activity against HCV GT 1. Adverse events (AEs) were mainly related to the gastrointestinal tract (most frequent diarrhoea), and the frequency increased with increasing dose [135]. However, at high doses (450 mg), all five actively treated patients were unable to tolerate the compound due to gastrointestinal reactions, and clinical studies have been discontinued [136].

Whether the compound has benzimidazole or indole as its core, the dihedral angle between the core and the 2-position aryl group trial. It is worth noting that the safety of ribavirin has been controversial. The adverse reactions that have been reported include teratogenicity and haemolytic anaemia [125].

3.1. Thumb I inhibitors

Thumb I inhibitors are mainly benzimidazole and indole compounds, and the compounds under clinical studies all show excellent anti-HCV activity. These types of compounds bind to thumb I mainly through hydrophobic interactions and salt bridge/hydrogen bonds between the ester group or carbonyl group of the compound and the guanidine group of the amino acid residue R503.

The initially discovered benzimidazole 29 has weak inhibitory activity (IC\textsubscript{50} = 1.6 \mu M) against HCV GT 1 NS5B polymerase, and its EC\textsubscript{50} value is higher than 10 \mu M in a cell-based model for subgenomic replicon [131]. The structure has been optimized to improve the activity of benzimidazole compounds. JTK-109 (30) is a superior compound that was obtained by modifying position 2 of benzimidazole. 30 inhibits HCV GT 1NS5B polymerase with an IC\textsubscript{50} value of 0.022 \mu M and an EC\textsubscript{50} value of 0.62 \mu M [132]. In addition, the carboxyl group at position 5 of benzimidazole can be modified to enhance the antiviral activity of the compound. A longer side chain was linked by an amide bond to obtain 31, which exerts an inhibitory effect on NS5B polymerase GT 1 polymerase (IC\textsubscript{50} = 0.3 \mu M) with an EC\textsubscript{50} value of 1.7 \mu M [133]. To further improve the activity of the compounds on enzymes and cells, the structure of these compounds was further modified by replacing the benzimidazole ring with the indole ring, which yielded 32. The IC\textsubscript{50} value of 32 in inhibiting HCV GT 1b NS5B polymerase is 0.016 \mu M, and the EC\textsubscript{50} Value is 4.2 \mu M [134]. Compared with 29, the activity of 32 at the enzyme level was increased 100-fold, and the activity in the cell model was also improved. The structure of 32 was modified using the same strategy as that used to obtain 29, which yielded 33 (BILB-1941). The IC\textsubscript{50} value of 33 in inhibiting HCV GT 1b NS5B polymerase is 0.045 \mu M, and the EC\textsubscript{50} value is 0.084 \mu M [131]. Compound 33 was administered via a single oral dose in a clinical phase I trial, which revealed that this compound has antiviral activity against HCV GT 1. Adverse events (AEs) were mainly related to the gastrointestinal tract (most frequent diarrhoea), and the frequency increased with increasing dose [135]. However, at high doses (450 mg), all five actively treated patients were unable to tolerate the compound due to gastrointestinal reactions, and clinical studies have been discontinued [136].

Whether the compound has benzimidazole or indole as its core, the dihedral angle between the core and the 2-position aryl group
Table 4
RNA-dependent RNA polymerase nonnucleoside inhibitors.

| Number | Compound | CAS registry number | Allosteric sites | Condition and highest clinical phase |
|--------|----------|---------------------|------------------|--------------------------------------|
| 28     | Pimodivir | 1629869-44-8        | PB2              | Discontinued - Influenza A - 2020    |
| 29     | Benzimidazole analogue | /                  | Thumb I          | Biological test                       |
| 30     | JTK-109   | 480462-62-2         | Thumb I          | Discontinued - Hepatitis C - 2003     |
| 31     | Benzimidazole analogue | /                  | Thumb I          | Biological test                       |
| 32     | Benzimidazole analogue | /                  | Thumb I          | Biological test                       |
| 33     | BILB-1941 | 494856-61-0         | Thumb I          | Discontinued - Hepatitis C - 2014     |
| 34     | MK-3281   | 886043-45-4         | Thumb I          | Discontinued - Hepatitis C - 2007     |
| 35     | Deleobuvir| 1221574-24-8        | Thumb I          | Discontinued - Hepatitis C - 2013     |
| 36     | Beclabuvir| 958002-33-0         | Thumb I          | Launched - Hepatitis C - 2017         |
| 37     | Radalbuvir| 1314795-11-3        | Thumb II         | Phase II - Hepatitis C - 2011         |
| 38     | Dihydropyrrone analogue | /                  | Thumb II         | Biological test                       |
| 39     | Filibuvir | 877130-28-4         | Thumb II         | Discontinued - Hepatitis C - 2013     |
| 40     | Thiophene carboxylic analogue | /                  | Thumb II         | Biological test                       |
| 41     | VCH-759   | 713139-25-4         | Thumb II         | Phase II - Hepatitis C - 2006         |
| 42     | VCH-916   | 1200133-34-1        | Thumb II         | Phase I - Hepatitis C - 2008          |
| 43     | Lomibuvir | 1026785-55-6        | Thumb II         | Licenced - Infections - 2016          |
|        | HCV-371   | 675184-27-7         | Thumb II         | Discontinued - Hepatitis C - 2010     |
|        | N-aryl uracil analogue | /                  | Palm I           | Biological test                       |
| 46     | ABT-072   | 1132936-00-5        | Palm I           | Phase II - Hepatitis C - 2009         |
| 47     | Dasabuvir | 1132935-63-7        | Palm I           | Launched - Hepatitis C - 2015         |
| 48     | Setrobuvir| 1071517-39-9        | Palm I           | Phase II - Hepatitis C - 2009         |
| 49     | GSK-625433| 885264-71-1         | Palm I           | Discontinued - Hepatitis C - 2009     |
| 50     | IDX-375   | 1256735-81-5        | Palm I           | Phase I - Hepatitis C - 2007          |
| 51     | CC-31244  | Undisclosed structure| Palm I           | Phase II - Hepatitis C - 2009         |
| 52     | Nesbuvir  | 1132935-63-7        | Palm II          | Phase II - Hepatitis C - 2019         |
| 53     | Tegobuvir | 1000787-75-6        | Palm II          | Phase II - Hepatitis C - 2008         |
| 54     | Benzamide analogue | /                  | Palm III         | Biological test                       |
exerts a greater impact on the activity of the compound during the process of structural modification [137]. Structure-activity relationship (SAR) studies involving an indolo-benzoxazocine scaffold led to the identification of 34 (MK-3281), an inhibitor that exhibits good potency in the HCV subgenomic replication assay and attractive molecular properties suitable for a clinical candidate [138]. Compound 34 inhibited HCV NS5B polymerase with an IC_{50} value of 0.006 μM, and an EC_{50} value of 0.038 μM was obtained in the replicator model (GT 1b). The compound caused a consistent decrease in viremia in vivo, as demonstrated with the chimeric mouse and chimpanzee model of HCV infection [139]. In a 7-day clinical trial of 34 monotherapies, patients with HCV GT 1b infection showed the greatest decrease in viral load and no viral load rebound, whereas patients with HCV GT 1a infection exhibited a small decrease in viral load, and their viral load appeared to rebound. One patient developed severe myoclonus side effects, and clinical trials of the compound were terminated. The EC_{50} values of 35 (deleobuvir, BI 207127) in the cell-based HCV GT 1b and GT 1a subgenomic replicons are 23 nM and 11 nM, respectively [140]. In a clinical trial of 35 combined with SOC for the treatment of HCV infection, the patients showed a significant reduction in viral load [141]. However, the use of 35 for the treatment of hepatitis C has been discontinued by Boehringer Ingelheim due to weak market competition. 36 (beclabuvir, BMS-791325) is a thumb I-NS5B polymerase ligand [142]. In cell culture, 36 inhibits the replication of HCV subgenomic replicons representing HCV GTs 1a and 1b at EC_{50} values of 3 nM and 6 nM, respectively, and similar values (3–18 nM) were obtained for GTs 3a, 4a, and 5a [143]. The oral bioavailability of 36 is 66%, and its volume of distribution is 2.7 L/kg; following intravenous administration, a plasma clearance of 3.5 mL/min/kg and an estimated plasma t_{1/2} of 8.3 h were obtained in a 24-h rat pharmacokinetic study [144]. 36 represents a valid drug that exhibits a good tolerability and safety profile, and together with other antivirals, this compound exhibits optimal efficacy against HCV in compensated phases of the diseases, as demonstrated in clinical studies [145,146]. At present, 36 in combination with asunaprevir and daclatasvir has been launched in Japan for the treatment of hepatitis C.

3.2. Thumb II inhibitors

The thumb II site is a spatially distinct allosteric site on the polymerase situated at the base of the thumb subdomain at a distance of 30 Å from the enzyme active site. Inhibitors acting on the thumb II site are mainly dihydropyrones, thiophene carboxylic acids and pyranoindole compounds. The lipophilic substituents of these compounds occupy the shallow grooves formed by the amino acid residues Leu 419, Tyr477 and Trp 528 in the thumb II site. The acidic groups of the compounds generate hydrogen bonds with the backbone amide bonds of the amino acid residues Ser 476 and Tyr477 [147,148].

37 (radalbuvir, GS-9669) is an inhibitor of NS5B polymerase that is currently in phase II clinical trials for the oral treatment of patients with HCV infection. In replicon cell lines, compound 37 exerts a high antiviral effect against HCV GT 1a (EC_{50} = 11 nM), HCV GT 1b (EC_{50} = 2.8 nM) and HCV GT 5a (EC_{50} = 8 nM) [149]. Due to its synergistic or additive effects with other antivirals and the lack of cross-resistance, this compound might be an important component of interferon-free combinations for the treatment of HCV infection [150].

38 is a seed compound identified by high-throughput screening. The compound has an IC_{50} value of 0.93 μM for HCV GT 1b NS5B polymerase inhibition, and an EC_{50} value of 48 μM was obtained in the replicon model [148]. To optimize the structure of the compound, one strategy is to introduce an aromatic group that can interact with the residue and undergo π–π stacking interactions, and another strategy is to replace the sulfur atom connecting the dihydropyrene and the aromatic group on the right with a carbon atom. These strategies aim to improve the membrane permeability and pharmacokinetic properties of the compound and yield highly active compound 39 (filibuvir, PF-868554) [151,152]. Compound 39 exhibits an IC_{50} value of 0.01 μM for HCV GT 1b NS5B polymerase inhibition and an EC_{50} value of 0.59 μM in the replicon model. In the phase I trial of patients with HCV GT 1 infection, the single administration of 39 resulted in decreases in HCV RNA. In a phase II clinical trial, the combination of 39 and Peg-IFN/RBV increased the rapid viral response rate and was well tolerated. However, compared with the development of Gilead Sciences' hepatitis C drug, Pfizer has been far behind, and continued development of 39 cannot take the lead in the market; thus, Pfizer terminated its clinical research of 39.

40 was the first discovered anti-HCV inhibitor of thiophene-2-carboxylic acid acting on the thumb II site, and its IC_{50} value for HCV GT 1b NS5B polymerase inhibition is 14 μM [153]. 41 (VCH-759), 42 (VCH-916) and 43 (lomibuvir, VCH-222) were obtained through the structural modification of 40. These three compounds have all entered clinical trials. Compound 41 inhibits GT1a NS5B polymerase and GT1b NS5B polymerase with IC_{50} values of 0.41 and 0.38 μM, respectively [154]. In a 1a clinical trial of 41, the average viral load of HCV type 1 patients who were treated with 800 mg TID was evaluated 10 days later, and the largest decrease in viral load was observed 2.5 log_{10} IU/mL [155]. Although more patients had diarrhea, no serious adverse reactions were observed. The EC_{50} value for 42 for HCV GT 1 in the replicon model was found to be equal to 0.1 μM [156]. In a clinical phase I trial, the compound rapidly reduced the viral load of HCV GT 1 patients after 3 days of treatment, and the maximum reduction in viral load obtained with these treatments was 1.5 log_{10} IU/mL [157]. The compound was well tolerated in healthy volunteers administered a single oral dose of 1500 mg and in patients with HCV orally administered 750 mg BID, and the maximum average viral load observed 3 days after the treatment was decreased by 3.7 log_{10} IU/mL. The phase II clinical trial of 43 in combination with telaprevir for the treatment of patients with HCV GT 1 was terminated [158,159].

Compound 44 (HCV-371) is a type of pyranoindole HCV thumb II inhibitor that has a structure that differs from that of the two abovementioned types of compounds [160–162]. For 90% HCV GTs 1a and 1b, the IC_{50} value was 0.3–1.4 μM, the IC_{50} value for HCV GT3a inhibition was 1.8 μM, and the EC_{50} values for HCV GTs 1a and 1b in the replicon model were 6.1 and 4.8 μM, respectively. The compound was well tolerated in clinical phase I trials, but due to a lack of significant antiviral activity, clinical trials of the compound have been terminated.

3.3. Palm I inhibitors

The palm I binding site is located between the active site and the palm II site and contains a deep hydrophobic pocket. The inhibitors that bind to this site mainly include N-aryl uracil analogues, benzothiadiazines and acyl pyrrolidines.

A series of N-aryl uracil analogues was reported as a novel structural class of NS5B polymerase NNIs [163]. Compound 45 is a potent inhibitor of GTs 1a (EC_{50} = 51 nM) and 1b (EC_{50} = 19 nM) NS5B polymerase [164]. Replicon activity was maintained when the assay was conducted in the presence of 40% human plasma [EC_{50} = 61 nM (1a), EC_{50} = 22 nM (1b)]. However, 45 exhibited poor pharmacokinetic properties in rats, with high plasma clearance and poor oral bioavailability (F = 1.4%). The physical properties of this compound that can be associated with poor oral exposure include low aqueous solubility and poor membrane permeability. The
solubility problem is likely related to the high melting point of the compound. Based on its excellent antiviral activity profile, 46 (ABT-072) was developed. The replacement of the amide linkage in 45 with a trans-olefin yielded a compound with improved permeability and solubility and markedly better pharmacokinetic properties in preclinical species. The replacement of the dihydrouracil in 45 with an N-linked uracil provided better potency in the HCV GT 1 replicon assay. Compound 46 is a potent inhibitor of HCV GT 1 replicons, with EC50 values of 1 nM and 0.3 nM against HCV GT 1a and GT 1b, respectively. The results from phase I clinical studies supported the once-daily oral dosing of HCV-infected patients with 46 [163,165]. A phase II clinical study that combined 46 with the HCV protease inhibitor ABT-450 revealed a sustained virologic response at 24 weeks after dosing (SVR24) in 10 of 11 patients who received treatment [166]. 47 (dasabuvir, ABT-333) is also an N-linked uracil derivative that was identified via throughput screening of the aryl dihydrouracil fragment [167]. This compound does not exhibit stereoisomerism, is thermodynamically stable and shows aqueous solubility, dissolution, and Caco-2 permeability. The IC50 for clinical isolates ranges between 2.2 and 10.7 nM for HCV GTs 1a and 1b [168]. In 2016, 47 in combination with ombitasvir/paritaprevir/ritonavir was launched for the treatment of chronic hepatitis C infection. However, 47 has limitations in terms of its limited genotypic coverage, and its administration to patients with advanced cirrhosis is difficult. This compound also represents a large pill burden when added to combination therapy [169].

Compounds with benzothiadiazine as the basic skeleton show strong activity at the enzyme level and in the cell model, and their physical and chemical properties are not ideal due to their special compound structure (intramolecular hydrogen bonds bring the aromatic ring close to the same plane), which results in poor pharmacokinetic properties in the body [170,171]. Such structures are optimized by introducing carbon-containing branches, reducing the number of aromatic rings, and reducing the polar surface area (PSA) of the molecule. 48 (setrobuvir, ANA598) is a benzothiadiazine analogue with a reduced aromatic ring that is currently in phase II clinical trials. In the replicon model, the EC50 values of 48 in inhibiting HCV GTs 1a and 1b NS5B polymerases were found to be equal to 0.05 and 0.003 μM, respectively [172]. In a phase I clinical trial, BID treatment with 48 at doses of 200 mg, 400 mg, and 800 mg for 4 days decreased the viral load by 2.4, 2.3 and 2.9 log10 IU/mL, respectively. In an earlier phase II study, 48 was administered in combination with Peg-IFN/RBV to naïve HCV GT 1-infected patients for 12 weeks, and the combination exhibited potent antiviral activity and good safety and tolerability [173]. 49 (GSK-625433) is a representative acyl pyrrolidine HCV polymerase inhibitor with IC50 values of 1.1 (1a) and 0.028 (1b) μM and EC50 values of 0.29 (1a) and 0.003 (1b) μM in the replicon model [174]. 50 (IDX-375) demonstrates low nanomolar potency in vitro (EC50 of 2.3 nM) in the HCV GT 1b replicon with a selectivity index of 43,000 [175]. 50 is well absorbed and well tolerated by all healthy male volunteers included in the phase I study. A single-day 200-mg BID dose resulted in exposure-related HCV activity with maximal 0.5 to 1.1 log10 reductions in plasma HCV RNA levels [176]. 51 (CC-31244, undisclosed structure) is a pangenotypic inhibitor of NS5B polymerase (GTs 1–5) that was designed for the treatment of hepatitis C infection. Compound 51 shows no significant cytotoxicity, CYP450 inhibition, or off-target or drug-drug interactions [177]. In the phase I study, a rapid and marked decline in HCV RNA levels, slow viral rebound after treatment, and no viral breakthrough during treatment were observed in the patients, which indicates that this compound is highly favourable compared with the currently approved NNIs [178].

3.4. Palm II inhibitors

The palm II site is mainly composed of a large hydrophobic pocket in the palm area, and the RdRp inhibitors that bind to this site include benzofurans. These palm II site inhibitors are different from other nonnucleoside inhibitors in that they exhibit potent activity against HCV GTs 1 to 4 and NS5B polymerase. 52 (nesbuvir, HCV-796) is the first palm II–NNI inhibitor to enter phase II clinical trials. In hepatoma cells containing an HCV GT 1b replicon, the IC50 value of 52 was found to be 9 nM [179]. In a phase I clinical trial, the greatest decreases in the average viral load, which reached 1.4 log IU/mL, were observed with the 1000 mg BID and 1500 mg BID treatments [180]. The initial results of phase II clinical trials showed that the combined treatment of 52 and PEG–IFNs can increase the therapeutic effect and reduce the occurrence of mutant strains. However, elevated liver enzyme levels were observed in some patients administered the combination treatment for at least 8 weeks [181]. The efficacy and safety of 52 for the treatment of hepatitis C via intravenous injection are currently being evaluated. 53 (tegobuvir, GS-9190) is currently in phase II clinical trials for the treatment of HCV infection [182]. Its EC50 values were lower than 16 nM against HCV GT 1 and higher than 100 nM for other GTs [183]. In the clinical studies of 53 for the treatment of HCV GT 1-infected patients (doses of 40 mg and 120 mg BID), the viral load decreased by 1.4 and 1.7 log IU/mL after 8 days, respectively.

3.5. Palm III inhibitors

Cliff C. Cheng et al. [184] described a novel series of NS5B polymerase inhibitors based on the 2-oxo-6-fluoro-N-[(5)-1-hydroxy-3-phenylpropan-2-yl]-benzamide-based scaffold. Among them, the first palm III inhibitor identified was 54, and its IC50 value related to the inhibition of HCV GT 1B NS5B polymerase was 0.8 μM. The X-ray crystal structure of a complex with 54 has provided structural insights into the mechanism of inhibition and aided the rationalization of the structure–activity relationships. Compound 54 binds within the active site cavity of NS5B polymerase near the top of the palm subdomain, and the binding site of this compound is a new binding site, which has been denoted the palm III site.

4. Discussion and perspectives

Currently, only 22 has been approved in the United States as the first COVID-19 treatment drug. The specific drug for the treatment of COVID-19 remains scarce, and the rapid identification of an effective strategy for the treatment of COVID-19 is currently a major challenge facing researchers. In terms of development time, research progress, safety and effectiveness, small-molecule drugs are the best choice compared with other therapies, such as monoclonal antibodies, oligonucleotide-based therapies, plasma therapies, and peptide therapies. However, the medicinal chemistry of SARS-CoV-2 infection remains in its infancy, and target-specific lead molecules remain to be identified. The existing antiviral drugs have established safety characteristics and effectiveness against related coronaviruses. The reuse of existing small molecule antiviral drugs is an important and promising strategy for addressing the COVID-19 epidemic. If the existing drugs can be repurposed for the treatment of SARS-CoV-2 infection, preclinical research (such as animal experiments and pharmacological research) and early clinical research can be bypassed, and the drugs can directly enter phase II or III clinical trials. RdRp is one of the most important viral proteins of RNA viruses for RNA synthesis and has been proposed as a valuable target for the development of antiviral therapeutics. Considering the similarity of
the key drug-binding pockets between SARS-CoV-2, SARS-CoV, and MERS-CoV RdRps, repurposing known RdRp inhibitors for SARS-CoV-2 remains a promising strategy [185]. W. Yin et al. reported the complex structure of 22 inhibiting SARS-CoV-2 RdRp, which provided insights into the mechanism of viral RNA replication and a rational template for drug design to combat viral infection [186]. In this study, the complex structure revealed that the partial double-stranded RNA template was inserted into the central channel of RdRp, where 22 was covalently incorporated into the primer strand at the first replicated base pair and terminated chain elongation. At present, the candidate drugs that have shown obvious anti-SARS-CoV-2 at the cellular level or in clinical trials are 2, 15, 20, 22, 23, 25, 26 and 27 (Fig. 7). Like 22, these nucleotide analogues can converge into a central cavity of viral RdRp and inhibit viral RdRp through nonobligate RNA chain termination, a mechanism that requires conversion of the parent compound to the triphosphate active form. 2, 20, 22, 26 and 27 retain the entire ribose group, so they may be able to form a stable hydrogen bond network similar to natural substrates [31]. In addition, the unmodified side chain hydroxyl groups on the 20 and 27 nucleosides can also form hydrogen bonds. In particular, 2 has been shown to be 3 to 10 times as potent as 22 in blocking SARS-CoV-2 replication [54]. The N4 hydroxyl group off the cytidine ring forms an extra hydrogen bond with the side chain of K545, and the cytidine base also forms an extra hydrogen bond with the guanine base from the template strand. These two extra hydrogen bonds may explain the apparent higher potency of 2 in inhibiting SARS-CoV-2 replication [186]. However, 15 only formed 7H-bonds and two hydrophobic contacts with the SARS-CoV-2 RdRp residues. This is because fluorine substitution occurs on the ribose group of 15, so they cannot form a hydrogen bond network, but this is necessary to keep the incoming natural nucleotides stable. The same phenomenon occurs when compound 23 is combined with SARS-CoV-2 RdRp. The guanosine triphosphate derivative 25 formed 10H-bonds with the SARS-CoV-2 RdRp residues and two metal interactions with the active site residue of RdRp [101]. Moreover, among all intracellular NTPs, cytidine triphosphate is found at a lower intracellular concentration than other NTPs. Therefore, pyrimidine nucleoside inhibitors such as compound 2 are more likely to be developed into antiviral drugs.

In addition, a variety of antiviral drugs were suggested as lead candidates against COVID-19 through homologue model-based
virtual screening and molecular docking, including candidate drugs that have been applied in other diseases [187], revealed that antibacterial drugs, including rifabutin, rifampicin, fidaxomicin, 7-methyl-guanosine-5'-triphosphate-5'-guanosine and ivermectin, have a potential inhibitory interaction with RdRp of SARS-CoV-2 and could be effective drugs for COVID-19. The drug surface hotspot study revealed that the molecular binding sites of all the compounds had a similar pattern. The vast number of noncovalent interactions between these screened compounds and RdRp suggests that the protein-inhibitor complexes are very stable. Considering that patients with COVID-19 may have combined bacterial or fungal infections, the proportion of antibiotics used in clinical treatment is relatively high [188]. If these antibacterial drugs that have inhibitory effects on RdRp can show a significant decrease in viral load in vitro studies, they would be good therapies and play a dual antiviral/antibacterial role.

For the more effective screening of candidate drugs, we compared the advantages and disadvantages of NIs and NNIs. NIs targeting the active site of virus RdRps and NNIs targeting allosteric sites have different biochemical properties. NIs mostly exhibit spectral antiviral activity. Many anti-HCV NIs are active against multiple HCV GTS, which indicates that the catalytic active sites bound by such inhibitors are relatively highly conserved. However, the problem faced in the development of NIs is the high concentration of intracellular natural NTP. For triphosphorylated NIs to compete with high concentrations of cellular NTP to exert their antiviral activity, the dose of the drug needs to be increased, which increases the risk of drug toxicity. In addition, NNIs acting on allosteric sites exert antiviral activity by affecting the binding of the catalytically active site of RdRp to the substrate. Such inhibitors do not need to undergo metabolic activation and do not need to compete with intracellular NTP. Combined with the structural diversity of such inhibitors, these compounds appear to be better antiviral drugs than nucleoside analogues. However, the structural variability and nonconservation of adjacent allosteric sites cause the RNA virus to rapidly develop resistance to allosteric site inhibitors.

As part of ongoing global efforts to prevent the spread of SARS-CoV-2 and treat the resulting infection, the use of approved drugs for nonapproved uses can alleviate urgent needs. However, to prevent viruses with similar genomic and pathological characteristics from returning a few years later, more specific, safe and effective drugs need to be developed. 2, the isopropyl ester prodrug of N4-hydroxycytidine, has been approved for use in clinical trials for the treatment of patients with COVID-19 and has shown positive effects. Since deuterium atoms are twice as heavy as hydrogen atoms, the vibrational zero-point energy of carbon-deuterium bonds (C-D) is lower than that of carbon-hydrogen bonds (C–H), and C-D is more stable than C–H [189]. Wen et al. replaced the hydrogen in the active molecular group with isotope deuterium to close the metabolic site and prolong the t1/2 of the drug, which further improves the metabolism of remdesivir in vivo and expands the scope of the treatment window and thereby reduces the therapeutic dose [190]. Additionally, the pharmacophore model is a ligand-based drug design tool that starts from the structure of known active compounds to find common pharmacodynamic feature information, thereby guiding the rational design or virtual screening of new compounds. M.S.A. Parvez et al. [187] designed a pharmacophore using 22, which was used further for screening the ZINC database. Molecular docking analysis revealed that two compounds (ZINC09128258 and ZINC09883305) with pharmacophore features that interact effectively with RdRp of SARS-CoV-2, indicating their potential as effective inhibitors of the enzyme (Fig. 9). In addition, in recent years, the research and development of RdRp inhibitors for RNA viruses has continued to be hot. Wang et al. reported the synthesis and biological evaluation of a series of 2,3’- and 2’,4’-substituted guanosine nucleotide analogues as HCV NS5B polymerase inhibitors [191]. 6’-Fluorinated aristeromycins were designed as dual-target antiviral compounds for the development of broad-spectrum antiviral agents that target RNA viruses [192]. These new compounds may also be a hope against the COVID-19 epidemic.

Undeniably, the development of efficient anti-SARS-CoV-2 drugs over a short time is associated with considerable obstacles and unidentified threats. Nonetheless, efforts to establish antiviral medicines to fight the novel coronavirus are urgently needed. Scientists from clinical research organizations and pharmaceutical companies along with front-line doctors should enhance their cooperation to jointly advertise pharmaceutical, scientific and preclinical tests of appropriate anti-coronavirus medications.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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