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Review article

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

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ABSTRACT

The widespread nature of several viruses is greatly credited to their rapidly altering RNA genomes that enable the infection to persist despite challenges presented by host cells. Within the RNA genome of infections is RNA-dependent RNA polymerase (RdRp), which is an essential enzyme that helps in RNA synthesis by catalysing the RNA template-dependent development of phosphodiester bonds. Therefore, RdRp is an important therapeutic target in RNA virus-caused diseases, including SARS-CoV-2. In this review, we describe the promising RdRp inhibitors that have been launched or are currently in clinical studies for the treatment of RNA virus infections. Structurally, nucleoside inhibitors (NIs) bind to the RdRp protein at the enzyme active site, and nonnucleoside inhibitors (NNIs) bind to the RdRp protein at allosteric sites. By reviewing these inhibitors, more precise guidelines for the development of more promising anti-RNA virus drugs should be set, and due to the current health emergency, they will eventually be used for COVID-19 treatment.

Contents

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Abbreviation index

AEs	Adverse events
AUC_{0-t}	Area under the blood level-time curve
BID	Bis in die
C—H	Carbon-hydrogen bonds
C-D	Carbon-deuterium bonds
COVID-19	Coronavirus Disease 2019
CTP	Cytidine triphosphate
DENV	Dengue virus
dNTP	Deoxyribonucleoside triphosphate
DIEs	Deuterium isotope effects
DAAs	Direct-acting antiviral agents
dsRNA	Double-stranded RNA viruses
GT	Genotype
$t_{1/2}$	Half-life
HCV	Hepatitis virus C
C_{max}	Maximum plasma concentrations
MERS-CoV	Middle East respiratory syndrome coronavirus
MHV	Mouse hepatitis virus
NIAID	National Institute of Allergy and Infectious Diseases
(−) ssRNA	Negative-sense ssRNA
NNIs	Non-nucleoside inhibitors
NNVs	Nonsegmented negative-sense RNA genome
NS5B	Nonstructural 5B
Nsp	Non-structural proteins
NIs	Nucleoside inhibitors
NTP	Nucleoside triphosphate
NiRAN	Nucleotidyltransferase
ORFs	Open reading frames
F	Oral bioavailability
Peg-IFNα	Pegylated interferon-α
PSA	Polar surface area
(+) ssRNA	Positive-sense ssRNA viruses
QD	Quaque die
FDA	The United States Food and Drug Administration
RTC	Replicase/transcriptase complex
RSV	Respiratory syncytial virus
RBV	Ribavirin
RdRp	RNA-dependent RNA polymerase
sNSVs	Segmented negative strand RNA viruses
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SARS-CoV	Severe acute respiratory syndrome coronavirus
ssRNA	Single-stranded RNA
SOC	Standard of care
SAR	Structure-activity relationship
TID	Ter in die
TP	Triphosphate
VEEV	Venezuelan equine encephalitis virus
VSV	Vesicular stomatitis virus
ZIKV	Zika virus

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

Table 1
Highly pathogenic single-stranded RNA viruses.

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
Marburgvirus	*Filoviridae*	(−) ssRNA
Vesicular stomatitis virus	*Rhabdoviridae*	(−) 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.

currently only one RNA-dependent RNA polymerase (RdRp) small molecule inhibitor, remdesivir, that has been approved by the United States Food and Drug Administration (FDA) for the treatment of COVID-19 [9–12].

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 inscribes 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 design 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

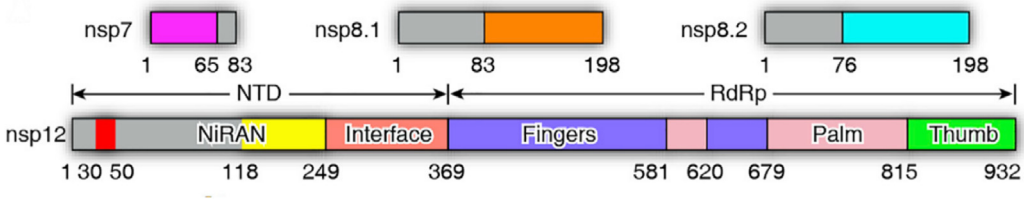


Fig. 1. Domain organization of SARS-CoV-2 nsp 12 (RdRp). The interdomain borders are labelled with residue numbers. RdRp: RNA-dependent RNA polymerase.

structurally conserved elements associated with catalysis [40]. The palm subdomain is involved in the selection of NTP over deoxyribonucleoside triphosphate (dNTP) 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 b-hairpin (V31–K50) and a nidovirus-specific extension domain (D60-R249). The b-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 RdRp, 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 phosphate group 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 polypeptides: 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).

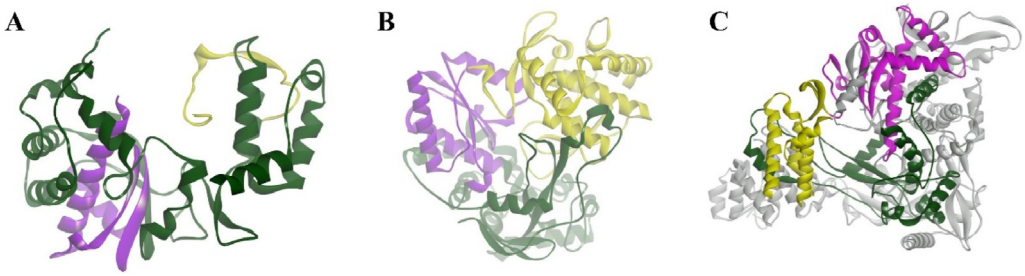


Fig. 2. Structure of RdRps of positive-strand RNA viruses. A: poliovirus 3Dpol (1RDR); B: HCV NS5B polymerase (1NB4); and C: nsp 12-nsp7-nsp8 complexes of SARS-CoV-2 (7BW4). Nsp7 and nsp8 act as cofactors to promote the activity of RdRp (grey). The palm, finger and thumb subdomains are shown in purple, green and yellow, respectively. RdRp: RNA-dependent RNA polymerase.

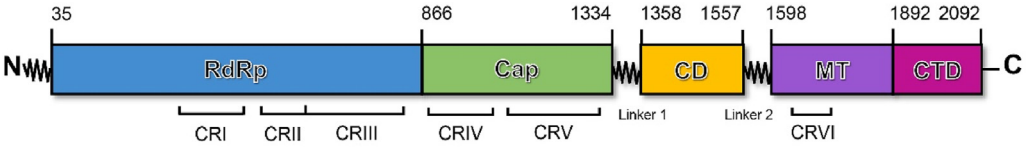


Fig. 3. Domain organization of the L protein of vesicular stomatitis virus. The conserved regions within L proteins of nonsegmented negative-strand RNA viruses are labelled CR I–VI.

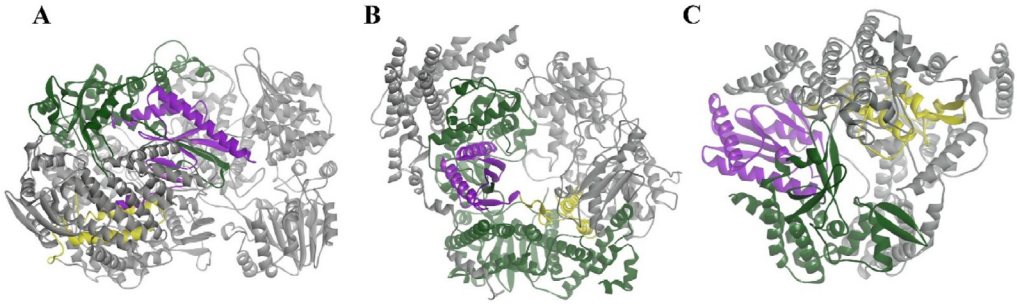


Fig. 4. Structure of the RdRp domain of nonsegmented negative-strand RNA virus polymerases. A: Vesicular stomatitis virus L protein (5A22); B: respiratory syncytial virus protein L protein (6UEN); and C: rotavirus VP1 protein (2R7Q). The palm, finger and thumb subdomains are shown in purple, green and yellow, respectively. RdRp: RNA-dependent RNA polymerase.

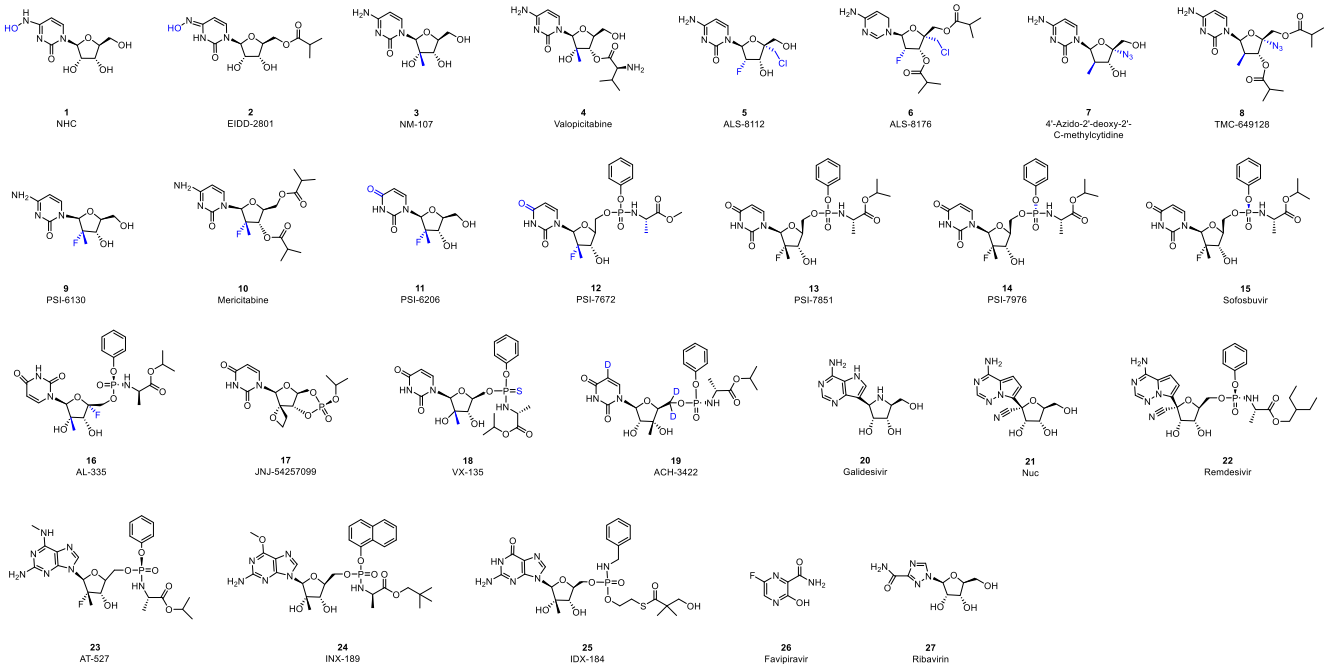


Fig. 5. Structures of nucleoside analogue inhibitors.

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-N^4-hydroxycytidine) is an orally bioavailable ribonucleoside analogue with broad-spectrum antiviral activity against various unrelated RNA viruses, including SARS-CoV-2 (IC_{50} = 0.30 μM), MERS-CoV (EC_{50} = 0.56 μM), mouse hepatitis virus (MHV, EC_{50} = 0.17 μM) and venezuelan equine encephalitis virus (VEEV, EC_{50} = 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

Table 2
RNA-dependent RNA polymerase nucleoside inhibitors.

Number	Compound	CAS registry number	Classification	Condition and highest clinical phase
1	NHC	3258-02-4	Pyrimidine nucleoside	Biological test
2	EIDD-2801	2349386-89-4	Pyrimidine nucleoside	Phase II - COVID-19- 2020
3	NM-107	20724-73-6	Pyrimidine nucleoside	Phase II - Hepatitis C - 2003
4	Valopicitabine	640281-90-9	Pyrimidine nucleoside	Phase II - Hepatitis C - 2004
5	ALS-8112	798009-58-2	Pyrimidine nucleoside	Phase I - Infection, RSV -2014
6	Lumicitabine	1445385-02-3	Pyrimidine nucleoside	Discontinued - Infection, RSV - 2019 Phase II - Infection, RSV -2014
7	4′-Azido-2′-deoxy-2′-C-methylcytidine	1019639-20-3	Pyrimidine nucleoside	Biological test
8	TMC-649128	1019639-33-8	Pyrimidine nucleoside	Discontinued - Hepatitis C - 2011 Phase I - Hepatitis C - 2011
9	PSI-6130	817204-33-4	Pyrimidine nucleoside	Phase I - Hepatitis C - 2006
10	Mericitabine	940908-79-2	Pyrimidine nucleoside	Phase II - Hepatitis C - 2011
11	PSI-6206	1064684-44-1	Pyrimidine nucleoside	Phase I - Hepatitis C - 2009
12	PSI-7672	1015255-46-5	Pyrimidine nucleoside	Biological test
13	PSI-7851	1064684-44-1	Pyrimidine nucleoside	Biological test
14	PSI-7976	1190308-01-0	Pyrimidine nucleoside	Biological test
15	Sofosbuvir	1190307-88-0	Pyrimidine nucleoside	Phase II - Porphyria cutanea tarda - 2017 Launched - Hepatitis C - 2014 Phase III - Hepatitis B - 2015
16	AL-335	1613589-09-5	Pyrimidine nucleoside	Phase II - Hepatitis C - 2015
17	JNJ-54257099	1255860-33-3	Pyrimidine nucleoside	Discontinued - Hepatitis C - 2015 Phase I - Hepatitis C - 2015
18	VX-135	798007-79-1	Pyrimidine nucleoside	Phase II - Hepatitis C - 2013
19	ACH-3422	798779-31-4	Pyrimidine nucleoside	Phase I - Hepatitis C - 2014
20	Galidesivir	249503-25-1	Purine nucleoside	Phase I - COVID-19 -2020 Phase I - Viral haemorrhagic fever −2019
21	Nuc	1191237-69-0	Purine nucleoside	Preclinical
22	Remdesivir	1809249-37-3	Purine nucleoside	Launched - COVID-19 -2020 Phase II/III - Ebola virus disease - 2018
23	AT-527	2241337-84-6	Purine nucleoside	Phase II - COVID-19 - 2020 Phase II - Hepatitis C −2019
24	INX-189	1234490-83-5	Purine nucleoside	Discontinued - Hepatitis C −2012 Phase II - Hepatitis C −2011
25	IDX-184	1036915-08-8	Purine nucleoside	Discontinued- Hepatitis C −2013 Phase II - Hepatitis C −2009
26	Favipiravir	259793-96-9	Miscellaneous nucleoside	Phase III - COVID-19 - 2020 Launched - Influenza A - 2017 Launched - Influenza B - 2017 Phase II - Ebola virus disease - 2014
27	Ribavirin	36791-04-5	Miscellaneous nucleoside	Phase I - COVID-19 - 2020 Phase I/II - Prostate cancer-2016 Phase II - Hepatitis E - 2012 Launched - Hepatitis C - 2001 Launched - Infection, RSV -1986

RSV: respiratory syncytial virus.

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 EC_{50} 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 EC_{50} 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 ($t_{1/2}$) of approximately 29 h [66]. The 5′-triphosphate of **5** is the active form of the drug and inhibits RSV polymerase with an IC_{50} 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 a phase II clinical trial for the treatment of RSV [68]. 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-t} = 12.7 μM/h) and greater oral bioavailability (F = 65%).

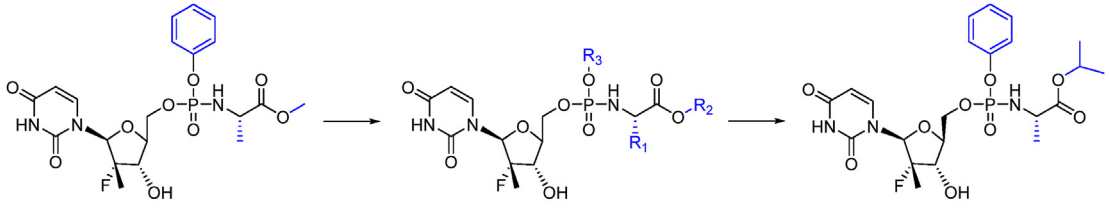
Fluorinated nucleosides are well known for their antiviral and anticancer properties. Pharmasset screened a series of 2′-cytosine nucleoside analogues with obvious anti-HCV activity. β-D-2′-Deoxy-2′-fluoro-2′-*C*-methylcytidine (**9**, PSI-6130), in which the C2′ position of uridine is substituted by 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′-diisobutyrate 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 (R_1) 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 (R_2) 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 (R_3) 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_p diastereoisomer **14** (PSI-7976) and R_p 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_{90} = 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

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



12
PSI-7672

2'-deoxy-2'-a-fluoro-β -C-methyluridine-5'-monophosphate analog

13
PSI-7851

R_1	R_2	R_3	EC_{90}	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 (C_{max}) 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 IC_{50} values as low as 27 nM. In an HCV subgenomic replicon assay, the phosphoramidate prodrug of **16** demonstrated very potent activity with EC_{50} values as low as 20 nM [91]. The administration of **16** in combination with simeprevir 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** (JNJ-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 (EC_{50} 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 diarrhoea (two subjects each), and no severe adverse events were recorded. Compound **18** demonstrated potent antiviral activity with a 4.5 $\log_{10}$ 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 $\log_{10}$, 4.2 $\log_{10}$, and 4.6 $\log_{10}$ 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, BCX4430) 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-5734) is the *Sp* isomer of the 2-ethylbutyl L-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 (EC_{50} = 0.77 μM) and exhibits low cytotoxicity (CC_{50} > 100 μM). In addition, the EC_{90} 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 to or 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 EC_{95} 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 $\log_{10}$ 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 (EC_{50} = 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_{50} = 0.31 mM, Ki = 52.3 nM) but does not inhibit human polymerases a, b or g (IC_{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-naïve HCV GT 1-infected subjects, respectively. **25** in combination with pegylated interferon-α (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** also exhibits 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_{50} = 61.88 μM, CC_{50} > 400 μM, 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, treatment with **26** significantly shortened the times to fever and cough relief in the patients with hypertension/diabetes [119]. Phase III clinical trials of **26** for the treatment of hospitalized patients with SARS-CoV-2 infection are outgoing. Future large-scale clinical trials will help verify the effectiveness and safety of **26** as a drug for the treatment of COVID-19.

27 (ribavirin, ICN-1229) can directly induce antiviral activity against a number of RNA viruses by increasing the mutation frequency in the genomes of several RNA viruses [120]. This compound is primarily indicated for the treatment of hepatitis C and viral haemorrhagic fevers. **27**-TP also exhibits an inhibitory action on viral mRNA guanylyltransferase and mRNA 2′-O-methyltransferase of DENV [121] and was used as a therapeutic drug in the SARS outbreak in 2003 [122]. Tong et al. compared **27** and supportive therapies for patients with severe COVID-19 and found that ribavirin therapy is not associated with an improved negative conversion time in the SARS-CoV-2 test or with an improved mortality rate in patients with severe COVID-19 [123]. In addition, a clinical trial studied the efficacy and safety of the combination of interferon beta-1b, lopinavir-ritonavir, and **27** for the treatment of patients with COVID-19. The results showed that early triple antiviral therapy was safe and superior to lopinavir-ritonavir alone in alleviating symptoms and shortening the durations of viral shedding and hospital stay in patients with mild symptoms, and the side effects were mild and controllable [124]. However, the results from the trial need to be further verified by an expanded double-blind 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. Non-nucleoside inhibitors

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 PB2 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 thumbs I and II and palms I, II and III) (Table 4).

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_{50} = 1.6 μM) against HCV GT 1 NS5B polymerase, and its EC_{50} value is higher than 10 μM 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_{50} value of 0.022 μM and an EC_{50} value of 0.62 μM [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_{50} = 0.3 μM) with an EC_{50} value of 1.7 μM [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_{50} value of **32** in inhibiting HCV GT 1b NS5B polymerase is 0.016 μM, and the EC_{50} value is 4.2 μM [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_{50} value of **33** in inhibiting HCV GT 1b NS5B polymerase is 0.045 μM, and the EC_{50} value is 0.084 μM [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

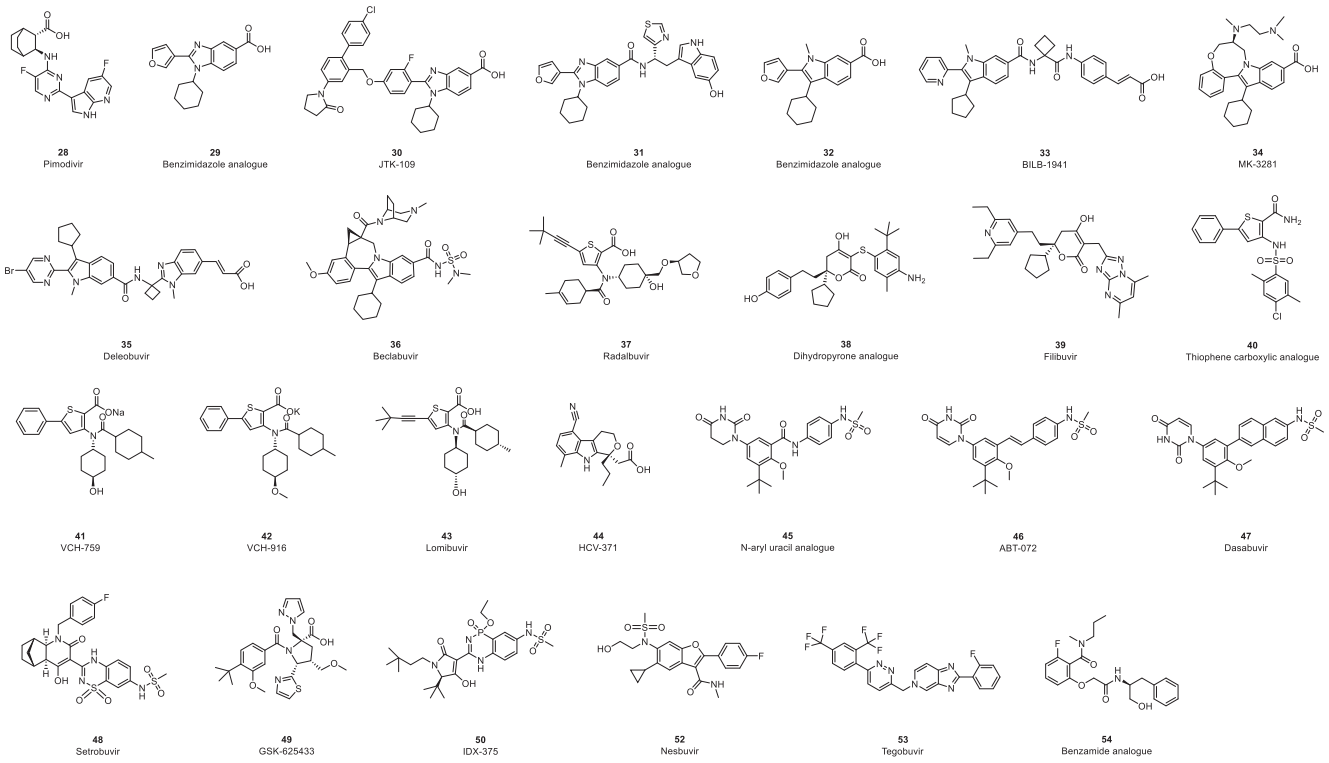


Fig. 6. Structures of nonnucleoside analogue inhibitors.

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 Phase III - Influenza A - 2018
29	Benzimidazole analogue	/	Thumb I	Biological test
30	JTK-109	480462-62-2	Thumb I	Discontinued - Hepatitis C - 2003 Phase I - Hepatitis C — 2002
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 Phase I/II - Hepatitis C - 2014
34	MK-3281	886043-45-4	Thumb I	Discontinued - Hepatitis C - 2007 Phase I - Hepatitis C - 2007
35	Deleobuvir	1221574-24-8	Thumb I	Discontinued - Hepatitis C - 2014 Phase III - 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	Dihydropyrone analogue	/	Thumb II	Biological test
39	Filibuvir	877130-28-4	Thumb II	Discontinued - Hepatitis C - 2013 Phase II - Hepatitis C — 2009
40	Thiophene carboxylic analogue	/	Thumb II	Biological test
41	VCH-759	713139-25-4	Thumb II	Phase I/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 Discontinued- Hepatitis C —2010 Phase II - Hepatitis C - 2010
44	HCV-371	675184-27-7	Thumb II	Discontinued - Hepatitis C - 2003 Phase I - Hepatitis C - 2002
45	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 Phase I - Hepatitis C - 2007
50	IDX-375	1256735-81-5	Palm I	Phase II - Hepatitis C - 2009
51	CC-31244	Undisclosed structure	Palm I	Phase II - Hepatitis C - 2019
52	Nesbuvir	1132935-63-7	Palm II	Phase II - Hepatitis C - 2008
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 chimaeric 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 dihydropyrone 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 phase I 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 volume reached 2.5 $\log_{10}$ IU/mL [155]. Although more patients had diarrhoea, no serious adverse reactions were observed. The EC_{50} value of **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 EC_{50} 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 IC_{50} 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 EC_{50} 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 log_{10} 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 IC_{50} values of 1.1 (1a) and 0.028 (1b) μM and EC_{50} values of 0.29 (1a) and 0.003 (1b) μM in the replicon model [174]. **50** (IDX-375) demonstrates low nanomolar potency in vitro (EC_{50} 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 log_{10} 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 IC_{50} 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-IFNα 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 a phase II clinical trial for the treatment of HCV infection [182]. Its EC_{50} 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-oxy-6-fluoro-N-((S)-1-hydroxy-3-phenylpropan-2-yl)-benzamide-based scaffold. Among them, the first palm III inhibitor identified was **54**, and its IC_{50} 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

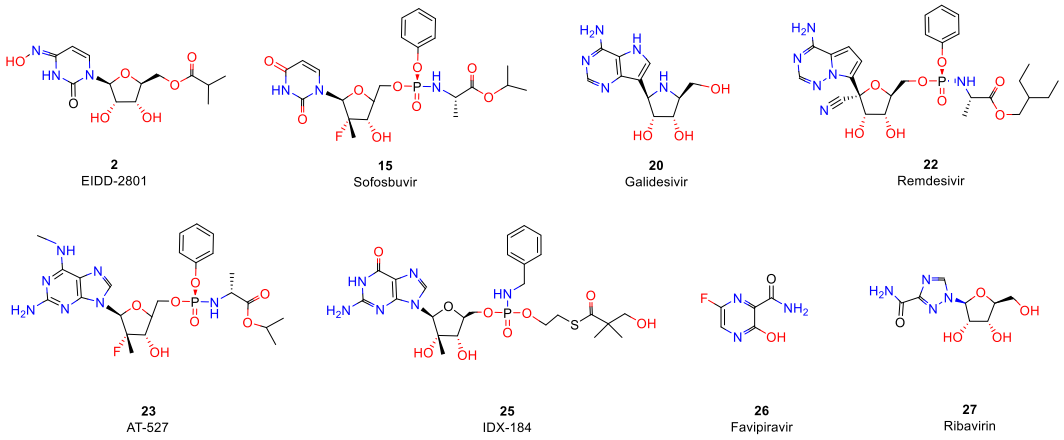


Fig. 7. Structure of the RdRp inhibitor repurposing for the COVID-19 pandemic.

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

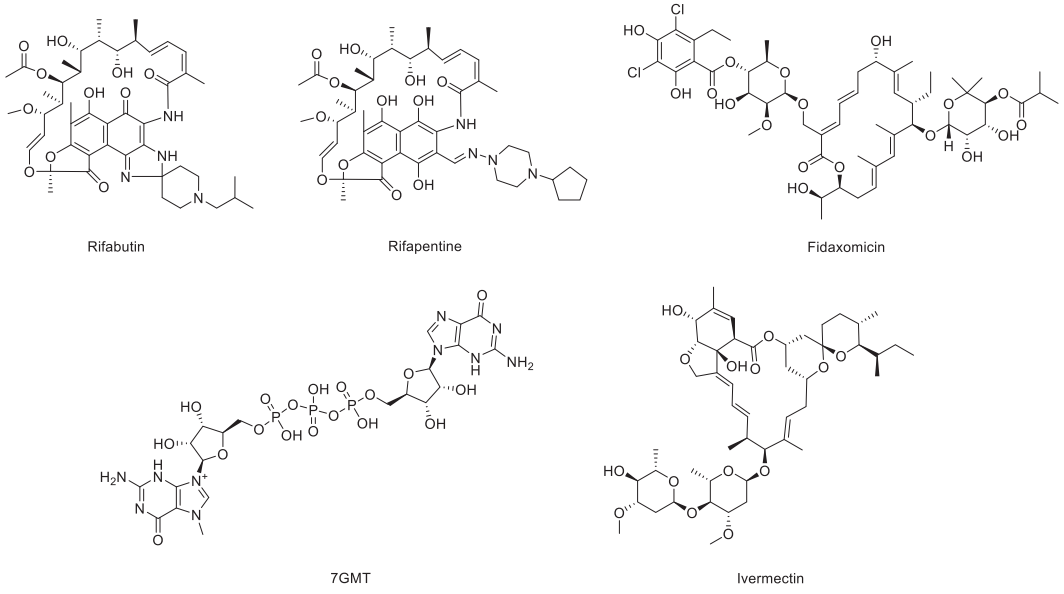


Fig. 8. Structure of the antibacterial drugs that have potential inhibitory interactions with RdRp of SARS-CoV-2.

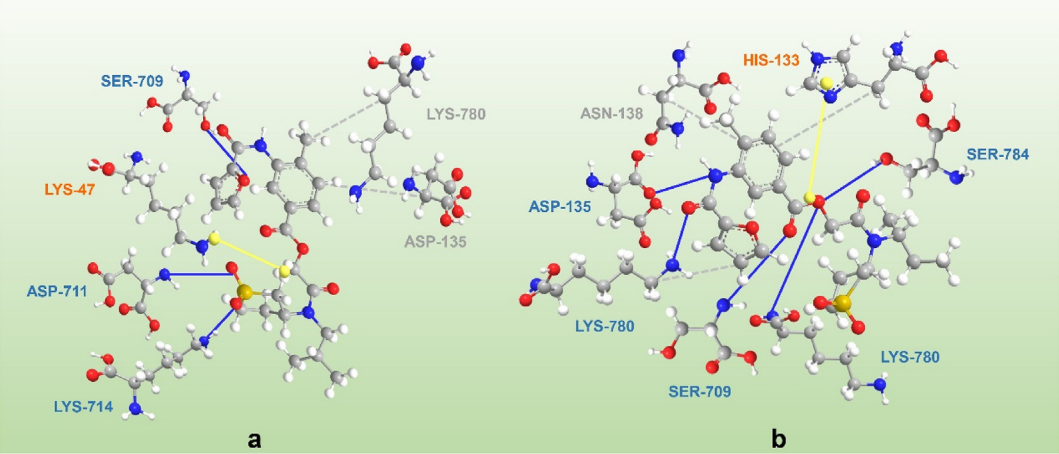


Fig. 9. The interaction of SRAS-CoV-2 RdRp with (a) ZINC09128258 and (b) ZINC09883305. Solid blue lines represent H-bonds, while hydrophobic interactions are grey dashed lines. In addition, π-cation stacking is represented by yellow spheres connected by dashed lines.

virtual screening and molecular docking, including candidate drugs that have been applied in other diseases (Fig. 8). M.S.A. Parvez et al. [187] revealed that antibacterial drugs, including rifabutin, rifapentine, 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 in vitro/vivo 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 $t_{1/2}$ 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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