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# Polymerases of Coronaviruses: Structure, Function, and Inhibitors

*Anand Gaurav and Mayasah Al-Nema*

Faculty of Pharmaceutical Sciences, UCSI University, Jalan Menara Gading,  
Taman Connaught, Cheras, Kuala Lumpur, Malaysia

## 10.1 INTRODUCTION

Viral diseases are widespread throughout the world and can range from minor infections to plagues that alter the course of history. The burden of diseases induced by viral infections is enormous, with most of the deadly infectious diseases being caused by viral infections. Among the viruses, coronaviruses (CoVs; subfamily *Coronavirinae*, family *Coronaviridae*, order *Nidovirales*) represent a major group of viruses known to be responsible for respiratory, enteric, hepatic, and neurological diseases in multiple species (Pene et al., 2003; Woo et al., 2009; Chan et al., 2012). The CoVs affecting human population are referred to as human coronaviruses (HCoVs). They lead to multiple respiratory diseases, such as common cold, pneumonia, and bronchitis (Lim et al., 2016). This century has seen rapid evolution of HCoVs, the contributory factors being urbanization and poultry farming. These factors allowed crossing of species barrier and genomic recombination of these viruses (Jones et al., 2013). Six HCoVs have been identified so far, namely severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1. The latter four viruses (HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) are mainly responsible for one-third of common cold infections in human, which in severe cases can lead to life-threatening pneumonia and bronchitis

(van der Hoek, 2007). The former two viruses (SARS-CoV and MERS-CoV) are responsible for life-threatening conditions, i.e., SARS and MERS (Graham et al., 2013; Kim et al., 2016; Hemida et al., 2017). Both SARS and MERS epidemics have surfaced for first time in the present century. The symptoms of both these conditions are similar, i.e., pneumonia marked by fever, headache, and subsequent onset of respiratory symptoms, such as cough, which may later develop into life-threatening respiratory failure and acute respiratory distress syndrome (de Wit et al., 2016). However, MERS has a much higher mortality (~35%) as compared to SARS (~10%) (Rabaan, 2017).

All CoVs are enveloped, nonsegmented positive-sense RNA viruses. They all contain very large genomes approximately of 30 kb. Other common features of CoVs include: (1) a highly conserved genomic organization, with a large replicase–transcriptase gene preceding structural and accessory genes; (2) expression of many nonstructural genes by ribosomal frameshifting; (3) several unique or unusual enzymatic activities encoded within the large replicase–transcriptase polyprotein; and (4) expression of downstream genes by synthesis of 3' nested subgenomic mRNAs (Taguchi, 2011; Fehr and Perlman, 2015).

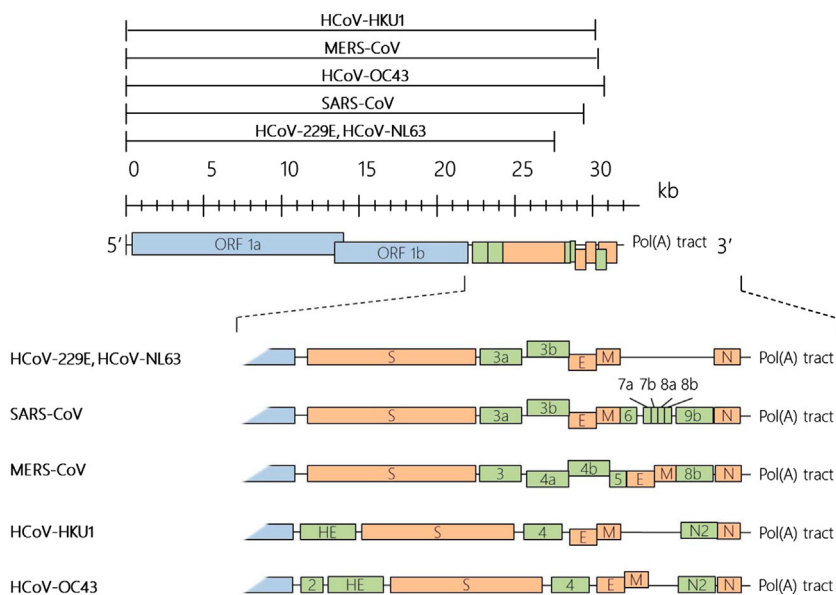
Just like other viral diseases, the development of therapies for CoV-borne diseases has been largely unsatisfactory despite the enormous investment into research in this area (Zumla et al., 2016). The major reason for this is the enormous variations in viruses and in their epidemiology and pathogenesis. Thus, unlike bacterial and fungal infectious diseases there is no single, magic-bullet approach to control viral infections. The most remarkable progress so far has involved vaccines and interferon therapy (Durai et al., 2015; Tang and Marasco, 2015; Enjuanes et al., 2016).

However, viral polymerases have also emerged as potential targets for design of drugs for viral diseases. Activity of viral polymerases is needed in several steps of the virus life cycle (Shi et al., 2013; Zhao et al., 2013; Deval et al., 2014; Velkov et al., 2014). Viral polymerases are known to play vital role in viral genome replication and transcription. They are thus critical for the survival and multiplication of viral particles. Two-thirds portion of the CoV genome from the 5'-end, expresses large replicase polyproteins 1a and 1ab (pp1a and pp1ab) which are cleaved by viral proteases to produce the RNA-dependent RNA polymerase (RdRP) and helicase (Hel). RdRP is thus the core enzyme of multiprotein replicase–transcriptase complex (RTC) required for transcription as well as replication of CoVs. One of the approaches for discovering anti-CoV drugs is to target the viral proteases thus inhibiting the production of RdRp and Hel, thereby blocking replication and transcription of CoV genome (Choi, 2012; Moradpour and Penin, 2013; Te Velthuis and Fodor, 2016). In this chapter, the structure and functions of

CoV RdRP will be discussed in detail along with a survey of design and development of RDRP inhibitors so far.

## 10.2 STRUCTURE OF HCoV RdRP

Although all the viral polymerases show some homology and share structural features and catalytic mechanism, they also have significant differences that reflect diverse virus replication strategies. Over the last few years lot of new knowledge has been acquired about the structure and function of viral polymerases including the CoV RdRP. Any discussion of the CoV RdRP structure must be preceded by the description of the structure of CoV genome. The typical CoV genome is a single-stranded, nonsegmented RNA genome, which is approximately 26–32 kb. It contains 5'-methylated caps and 3'-polyadenylated tails and is arranged in the order of 5'-end, replicase genes, genes encoding structural proteins (spike glycoprotein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N)), polyadenylated tail and then the 3'-end (Fig. 10.1) (Marra et al., 2003; Snijder et al., 2003; Lim et al., 2016; Shi et al., 2016).

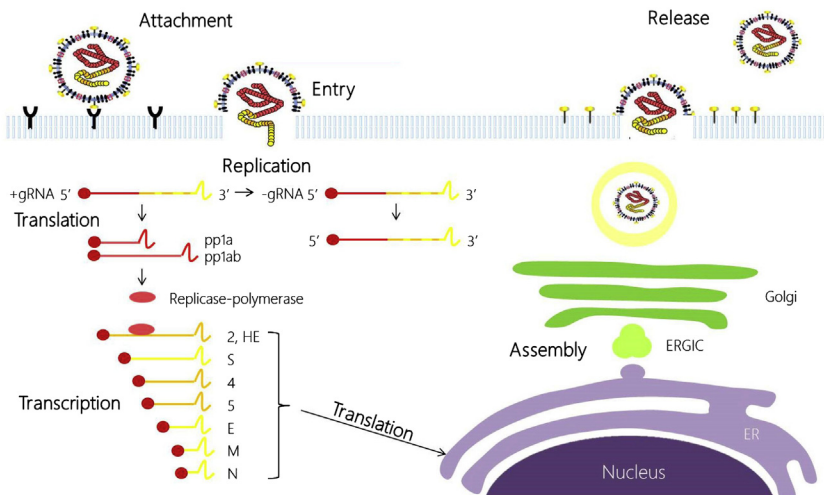


**FIGURE 10.1** Genome organization of human coronaviruses (HCoVs). Source: Reprinted from Lim, Y., Ng, Y., Tam, J., Liu, D., 2016. Human coronaviruses: a review of virus–host interactions. *Diseases* 4, 26. Open Access Publication.

The partially overlapping 5′-terminal open reading frames 1a and 1b (ORF1a and ORF1b) are within the two-thirds part of the CoV genome from the 5′-end and constitute the large replicase gene. After the entry of the virions into the host cell, the replicase gene is translated to produce pp1a and pp1ab (Fig. 10.2). The polyproteins are cleaved by papain-like cysteine protease (PLpro) and 3C-like serine protease (3CLpro or Mpro) to produce nonstructural proteins (nsps). The pp1a produces nsps 1–11, while pp1ab produces nsps 12–16. Many of these nsps assemble into the RTC to create a viable and suitable environment for RNA synthesis, leading to the replication and transcription of subgenomic RNA (Lim et al., 2016; Shi et al., 2016).

Apart from RdRP, the other nsps coded by the replicase gene also play a crucial role in CoV replication and transcription. The nsp12 encodes the RdRP domain, nsp13 encodes the helicase (Hel) domain, while the remaining nsps encode other enzyme domain and proteins such as proteinases, a putative RNA primase, a superfamily 1 helicase, an exo- and an endoribonuclease, single-stranded RNA (ssRNA)-binding proteins, and two methyltransferases (Adedeji et al., 2012; Subissi et al., 2014; Snijder et al., 2016).

The one-third part of the CoV genome from the 3′-end, and the subgenomic sequences encode the structural proteins, i.e., spike protein (S), envelop protein (E), membrane protein (M), and nucleocapsid



**FIGURE 10.2** Replication and transcription of coronavirus (CoV) genome, followed by translation to CoV structural proteins and assembly and release of new virus particles. Source: Reprinted from Lim, Y., Ng, Y., Tam, J., Liu, D., 2016. Human coronaviruses: a review of virus–host interactions. *Diseases* 4, 26. Open Access Publication.

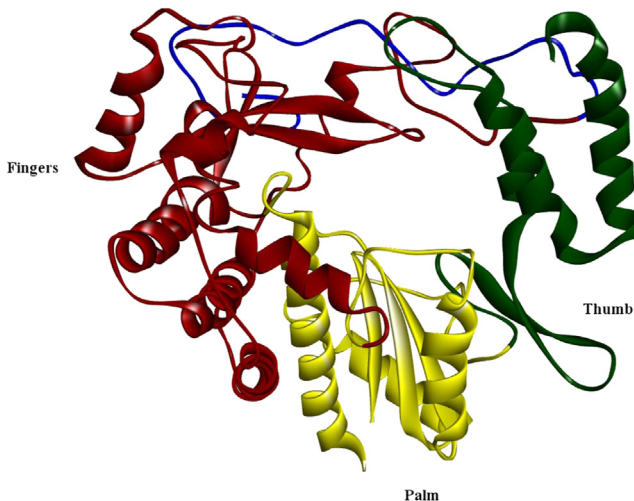
protein (N), which are essential for virus cell-receptor binding and virion assembly, and other nsps and accessory proteins that may have immunomodulatory effects (Ying et al., 2004). These regions are transcribed by the replicase–transcriptase complex followed by the translation of the mRNA to produce the respective proteins (Fig. 10.2) (Lim et al., 2016).

The RdRP is the central catalytic subunit of the RNA-synthesizing machinery of CoVs. Usually, the RdRP domain is readily identifiable by comparative sequence analysis, however biochemical characterization has been hindered by intrinsic protein properties and technical complications. It is now a well-known fact that replication and transcription of the ~30 kb CoV RNA genome is catalyzed by an RdRP domain in the C-terminal part of nsp12, one of 16 replicase subunits (Imbert et al., 2006; Sexton et al., 2016). However, thus far full-length, nsp12 has proven refractory to expression in bacterial systems, which hinder both the biochemical characterization of coronavirus RNA synthesis and RdRP-targeted antiviral drug design. Interestingly, for SARS-CoV a second protein, 22-kDa nsp8, with RdRP activity has been identified. This enzyme is capable of de novo initiation and has been proposed to operate as a primase. Interestingly, this protein has only been crystallized together with the 10-kDa nsp7, forming a hexadecameric, dsRNA-encircling ring structure (i.e., nsp(7 + 8), consisting of eight copies of both nsps) (te Velthuis et al., 2012).

More than two decades ago, the sequence analysis of the first CoV genomes was performed, and more recently comparative sequence analysis has allowed identification of the putative RdRP domain of the CoV RTC (Bournsnel et al., 1987; Gorbalenya et al., 1989; te Velthuis et al., 2010). The C-terminal, i.e., the two-thirds of the 930-odd-amino-acid subunit, known as nsp12 was successfully aligned with the conserved motifs of already known RdRPs (Gorbalenya et al., 1989; Xu et al., 2003). The unprecedented size of nsp12 among viral RdRP subunits, commonly ~500–600 amino acids, suggests that it may possess other functional domains in its as yet uncharacterized N-terminal domain (NTD). Biochemical information on the CoV RdRP has, however, remained scarce so far, particularly because full-length nsp12 was refractory to expression in bacterial systems. Preliminary evidence for the in vitro RdRP activity of nsp12 came from a study of the SARS-CoV enzyme by Cheng et al. (2005). The experiments involved the use of a glutathione S-transferase (GST)–nsp12 fusion protein. However, because of protein instability, the protein fragmented into three parts, hampering the studies. The studies also led to identification of primer-dependent activity on poly(A) template using filter-binding assays, however enzyme biochemistry was not further analyzed in detail (Cheng et al., 2005). Given its pivotal role in viral replication and the efficacy of polymerase

inhibitors used to combat other virus infections, the RdRP is widely regarded as an important and attractive target for the rational design of anti-CoV drugs (Xu et al., 2003; De Clercq, 2004). Therefore, several research groups have tried to solve the technical issues involved in the stable expression, purification and in vitro activity of the full-length CoV nsp12. Reasonable success has been achieved in development of methods to generate recombinant nsp12 protein containing its natural N-terminus. This was found to be a critical step on the road toward the purification of a stable recombinant CoV RdRP (te Velthuis et al., 2010).

However, homology model for SARS-CoV RdRP has been attempted. Based on the conserved sequence motifs and reliable sequence alignments, a three-dimensional homology model of the catalytic domain of SARS-CoV RdRP was developed by Xu et al. (2003) (Fig. 10.3). The homology model was based on the crystal structures of poliovirus 1 (PV1) strain Mahoney (PDB code: 1RDR), rabbit hemorrhagic disease virus (RHDV) (PDB code: 1KHV), hepatitis C virus (HCV) (PDB code: 1QUV), reovirus (RV) (PDB codes: 1N35 and 1N1H) and bacteriophage  $\phi$ 6 (Phi6) (PDB codes: 1HI0 and 1HI1), and HIV-1 RT (HIV1) (PDB code: 1RTD). The homology model of SARS-CoV RdRP showed the presence of the catalytic domain consisting of fingers, palm, and thumb



**FIGURE 10.3** Ribbon diagram of the homology model of severe acute respiratory syndrome coronavirus RNA-dependent RNA polymerase (SARS-CoV RdRP).  $\alpha$ -Helices are shown as spirals and  $\beta$ -strands as arrows. In catalytic domain, the N-terminal portion of the fingers subdomain is shown in blue, the base of the fingers in red, palm in yellow, and thumb in green. Source: Reprinted from Xu, X., Liu, Y., Weiss, S., Arnold, E., Sarafianos, S.G. Ding, J., 2003. Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design. *Nucleic Acids Res.* 31, 7117–7130. Open Access Publication.

subdomains that form an encircled nucleic acid-binding tunnel (Fig. 10.3). The structural analysis of the model provided critical information about the potential functional roles of the conserved motifs and specific residues in polymerization (Table 10.1).

SARS-CoV RdRP contains an NTD (approximately residues 1–375) that is expected to form at least one protein domain. Some of the CoV-specific replicase transcription activities may be associated with region. This domain may be involved in interactions with the leader or

**TABLE 10.1** Conserved Motifs Identified To Be Present in SARS-CoV RdRP Homology Model and Their Potential Functions

Motifs	Amino acid sequence	Probable functions
A	612 PHLMGWDYPKCDRAM	Asp618: metal ion chelation  Asp623: recognition of rNTP sugar ring
B	678 GGTSSGDATTAYANSVFNICQAVTANVNALLST	Ser682 and Thr687: recognition of template–primer  Ser682, Thr687, and Asn691: help sugar selection of rNTP
C	753 FSMMILSDDAVVCYN	Asp760 and Asp761: metal ion chelation Ser759: binding of 3' $\epsilon$ -primer terminus or priming nucleotide
D	771 AAQGLVASIKNFKAVLYYQNNVFMSE	Stabilize the core structure; may also help position Asp618
E	810 HEFCSQHTMLV	Control the flexibility of the thumb Cys813 and Ser814: positioning of priming nucleotide
F	544 LKYAISAKNRARTVAGV	Lys545, Lys551, and Arg553: rNTP binding and positioning of template overhang
G	499 DKSAGFPFNKWGK	Positioning of template overhang

Source: Adapted from Xu, X., Liu, Y., Weiss, S., Arnold, E., Sarafianos, S.G., Ding, J., 2003. Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design. *Nucleic Acids Res.* 31, 7117–7130.



intrinsic sequences during transcription of the characteristic nested mRNAs of SARS-CoV and other CoVs and/or in protein–protein interactions involving the viral Hel or other viral and/or host proteins involved in virus replication (Tao et al., 2002).

The sequence and structure of the fingers subdomain are less conserved than those of the palm subdomain among different viral RdRPs. The fingers subdomain of SARS-CoV RdRP extends approximately from residues 376 to 584 and 626 to 679 and consists of two polypeptide segments, an N-terminal segment and a segment covering motifs A and B of the palm subdomain. The finger subdomain is predicted to have different secondary structural composition for the base and tips of the fingers. The base consists of  $\alpha$ -helices and the tip consists of  $\beta$ -strands and coils (Fig. 10.3). Two conserved sequence motifs (F and G) are also present in the SARS CoV model. These motifs play important functional roles in the mechanism of polymerization (Butcher et al., 2001; Tao et al., 2002).

The fingers subdomain of SARS-CoV RdRP comprises an N-terminal portion (residues 405–444) that forms a long loop originating from the fingertip to bridge the fingers and thumb subdomains (Fig. 10.3). Because of these interactions, an encircled nucleic acid-binding “tunnel” is formed that can accommodate binding and translocation of a nucleic acid without major conformational changes in the enzyme. The interaction of finger–thumb subdomains is supposed to play critical role in movement and modulation of initiation, elongation, and termination of RNA synthesis, ensuring high processivity (Butcher et al., 2001; Tao et al., 2002). The N-terminal region of the fingers subdomain is also suggested to be involved in recognition of nucleotide substrate, protein–protein interactions, and oligomerization of the polymerase (Hobson et al., 2001; Pathak et al., 2002; Bruenn, 2003).

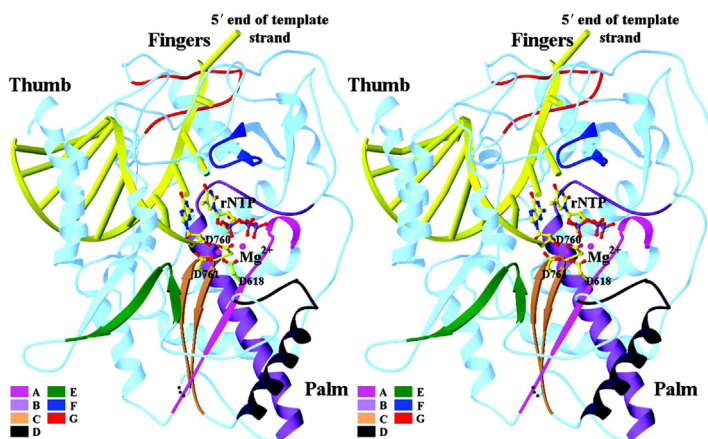
A motif F has been proposed to consist of two submotifs, F1 and F3 (Bruenn, 2003). In SARS-CoV RdRP, motif F1 contains several highly conserved basic residues, including Lys545, and motif F3 contains residues Lys551 and Arg553 (Table 10.1). In the structural model of SARS-CoV RdRP, residues of motif F are also predicted to form part of the ribonucleoside triphosphate (rNTP)-binding pocket and help position the template overhang (Table 10.1).

Another motif G is also found in SARS-CoV, which corresponds to Ser501, Gly503, Pro505, and Lys511 residues that are predicted to be involved in positioning of the 5' template strand in the structural model of SARS-CoV RdRp (Table 10.1). However, the palm subdomain of SARS-CoV RdRP (residues 585–625 and 680–807) is the most important structural element for the polymerase activity, as it constitutes the catalytic core of polymerase. It is comprised of five highly conserved sequence motifs (A–E) (Poch et al., 1989). The core structure of the

palm subdomain includes a central three-stranded  $\beta$ -sheet flanked by two  $\alpha$ -helices on one side and a  $\beta$ -sheet and an  $\alpha$ -helix on the other (Fig. 10.3). Residues forming the catalytic active site are found within motifs A and C.

Motif A of SARS-CoV RdRP contains two highly conserved aspartic acid residues, Asp618 and Asp623, separated by four residues (Table 10.1). Motif A consists of a  $\beta$ -strand and short  $\alpha$ -helix structure. The  $\beta$ -strand of motif A, along with the  $\beta$ -strands of motif C, forms the central  $\beta$ -sheet (Fig. 10.3). The first aspartate (Asp618) along with the two aspartates in motif C (Asp760 and Asp761) forms the catalytic core of SARS-CoV RdRP (Fig. 10.4). The corresponding three aspartates are probably involved in binding divalent metal ions required for catalysis (Beese and Steitz, 1991; Huang et al., 1998; Butcher et al., 2001; Bressanelli et al., 2002; Tao et al., 2002). Mutation of any of these aspartates in SARS-CoV RdRP is expected to annul the polymerase activity. Asp623 of motif A in RdRP is also expected to be involved in sugar selection (Table 10.1).

Motif B of SARS-CoV RdRP forms a “loop and  $\alpha$ -helix” structure and contains several highly conserved residues (Ser682, Gly683, Thr687, and Asn691) that appear to participate in recognition of the correct nucleic acid and selection of the correct substrate (Table 10.1).



**FIGURE 10.4** Stereoview of the polymerase active site and the rNTP-binding site. The conserved sequence motifs (A–G) are highlighted. A docked rNTP substrate is shown as a ball-and-stick model. The catalytic active site is defined by the three conserved aspartates, Asp618, Asp760, and Asp761 (shown with side chains) that are coordinated with two divalent metal ions (shown as magenta spheres). *Source: Reprinted from Xu, X., Liu, Y., Weiss, S., Arnold, E., Sarafianos, S.G., Ding, J., 2003. Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design. Nucleic Acids Res. 31, 7117–7130. Open Access Publication.*

The N-terminal loop of motif B contains three conserved residues (Ser682, Gly683, and Thr687) that seem to interact with the nucleotide that forms base-pairing with the incoming rNTP (21, 25–27 (Ago et al., 1999; Butcher et al., 2001; Ng et al., 2002; Tao et al., 2002)). The  $\alpha$ -helical part of motif B, along with the  $\alpha$ -helix formed by motif D, is stacked beneath the central  $\beta$ -sheet. The conserved asparagine on motif B  $\alpha$ -helix is considered to contribute to the specificity of RdRP for rNTPs versus deoxyribose nucleoside triphosphates (dNTPs). This is proposed to be due to hydrogen-bonding with the second conserved aspartate of motif A which in turn hydrogen-bonds to the 2'-OH of rNTP SARS-CoV RdRP. As seen in Table 10.1, Asn691 of motif B appears to interact with Asp623 of motif A through a hydrogen bond. Thus, Asn691 is likely to play similar role as Asn623, i.e., help sugar selection of rNTP.

SARS-CoV RdRP contains the highly conserved motif C (Leu758–Ser759–Asp760–Asp761) at the active site. This motif forms a  $\beta$ -strand, turn, and  $\beta$ -strand hairpin structure, and the two conserved aspartates are located at the turn (Figs. 10.3 and 10.4). The first leucine is invariant in all CoV RdRPs and has no apparent functional role in the molecular model of SARS-CoV RdRP. In the molecular model of SARS-CoV RdRP, the possible role of Ser759 is to assist the positioning of the 3'-primer terminus and/or priming nucleotide (Table 10.1). Asp618 of motif A, along with the two aspartates of motif C (Asp760 and Asp761), forms the active site of SARS-CoV RdRP. The role of first aspartate (Asp760) is to coordinate with the metal ions during catalysis (Beese and Steitz, 1991; Huang et al., 1998; Butcher et al., 2001; Bressanelli et al., 2002; Tao et al., 2002).

Primary sequence of motif D is not well conserved, but always forms an  $\alpha$ -helix, turn, and short  $\beta$ -strand in all known RdRPs. The  $\alpha$ -helix of this motif flanks the central  $\beta$ -sheet containing the catalytic aspartates. The exact functional role(s) of motif D is not yet clear. It is likely that motif D is involved in stabilizing the core structure of the catalytic domain and in helping to position motif A in all viral RdRPs, including SARS-CoV RdRP (Table 10.1).

Primary sequence in motif E is also not well conserved (Fig. 10.3) (Poch et al., 1989), but motif E has a conserved  $\beta$ -strand, turn and  $\beta$ -strand structure. Motif E is located at the intersection of the palm and thumb subdomains and its proposed role is to control the flexibility of the thumb during DNA polymerization (Jacobo-Molina et al., 1993; Ding et al., 1998; Huang et al., 1998). In the molecular model of SARS-CoV RdRP, motif E comprises residues 810–820, and the residues at the turn (Cys813 and Ser814) assist in positioning of the primer strand at the polymerase active site, thereby contributing to the fidelity of polymerization process (Table 10.1).

The C-terminal region of SARS-CoV RdRP (residues 808–932) comprises only the thumb subdomain. The sequence of the thumb subdomain is less conserved in all polymerases. The conformation of the thumb subdomain of SARS-CoV RdRP leaves the nucleic acid cleft unobstructed allowing the entry of double-stranded RNA (dsRNA).

The structural information of SARS-CoV RdRP availed from the homology model described above has revealed critical information, which can be extended in general to all HCoV RdRPs. The inhibition of HCoV RdRPs is a potential pharmacological intervention for the therapy of diseases caused by HCoV infection. The structural information can be very useful for design and development of many small molecule inhibitors of HCoV RdRP. However, the success in this direction still demands more information about CoV RdRP.

### 10.3 FUNCTION OF HCoV RdRP

Virus life cycle involves transcription of their genomes into mRNA, which can be translated into viral proteins, and synthesis of identical copies of the genomes for encapsidation into newly assembled virus capsids. Most of the viruses spend their entire life cycle in host cell cytoplasm and thus do not have access to host polymerases and thus must encode their own polymerases for transcription and replication. Based on the type of genome, replication strategy, and need for transcription, viruses are classified into seven classes, while based on their function viral polymerases are classified into four classes, i.e., DNA-dependent DNA polymerase (DdDP), DNA-dependent RNA polymerase (DdRP), RdRP, and RNA-dependent DNA polymerase (RdDP) (reverse transcriptase). RdRP and reverse transcriptase are unique to virus, as host cell need not replicate RNA or perform reverse transcription, while other polymerases are present in host cell, too.

CoVs being RNA viruses use RdRP for replication as well as transcription of their genomes. The RdRPs perform the same basic function as all the other viral polymerases, i.e., to copy a nucleic acid template strand to yield a daughter strand. This activity involves transfer of a nucleotidyl moiety of incoming NTP (complementary to template strand) to the 3'-end of a growing daughter strand of DNA or RNA. The polymerase activity requires two divalent metal cations, i.e.,  $Mg^{2+}/Mn^{2+}$ . The polymerase active site consists of binding sites for the template strand, primer (*i*) and the incoming NTP (*i* + 1). The reaction steps include binding of template–primer and NTP, incorporation of nucleoside monophosphate into the growing daughter strand with the release of pyrophosphate, and translocation of the template strand and growing

daughter strand from  $i + 1$  site to  $i$  site. The metal ions bound to the aspartate residues play their roles by (1) facilitating the reaction between 3'-OH of terminal RNA or DNA primer and the alpha phosphate group of incoming nucleotide through binding to 3'-OH and increasing its nucleophilicity and (2) positioning the incoming NTP by binding to its phosphates and stabilizing the pyrophosphate leaving group.

Among the RdRPs of CoVs, one of the most well studied in terms of mechanism of action is that of SARS-CoV. There are two replicase subunits with RdRP activities that are believed to be involved in replication of SARS-CoV genome. The first one is 106-kDa nsp12 while the second one involves the 22-kDa nsp8. As discussed previously, RNA genome replication is a crucial step in SARS-CoV propagation and is mediated by the RNA replicase, which is composed of viral nsps and host proteins. SARS-CoV nsp12 is the first protein encoded by ORF1b and generated by cleavage of pp1ab by PLpro and 3CLpro. It is considered the catalytic core of the RNA replicase due to the presence of the SDD motif that is common to coronavirus RdRPs (Oh and Ahn, 2013). During the synthesis of viral genome and subgenomes, nsp12 initiates the RNA synthesis from the 3' ends of the viral genome and subgenomes in plus and minus senses (Selisko et al., 2006). The correct initiation of the synthesis of RNA is crucial for maintenance of the viral genome's integrity. Generally, RNA viruses utilize two mechanisms to initiate the RNA synthesis: de novo initiation and primer-dependent initiation (Kao et al., 2001). In de novo initiation the starting nucleotide provides the 3' hydroxyl group for the addition of the next nucleotide, whereas in primer-dependent initiation a separate oligonucleotide or protein primer is required to provide the hydroxyl nucleophile (Masters, 2006).

The possibility of belonging of the SARS-CoV RdRP to the primer-dependent polymerase class can be justified by the presence of a G motif (a signature that exists in all primer-dependent RdRPs, and is implicated in the recognition of primer-template RNA complex) in the central domain of the structure of SARS-CoV.

Through a study, Ahn and coworkers proposed that SARS-CoV nsp12 can use both primer-dependent and primer-independent RNA synthesis activities (Ahn et al., 2012). In their study, they characterized the biochemical properties of a full-length SARS-CoV nsp12 that can initiate de novo synthesis using viral RNA templates corresponding to the 3' ends of both the plus and minus strands of the SARS-CoV genome. In addition, they mapped the minimal cis-acting regions required for initiation of the synthesis of plus and minus RNA strands. The results of their study concluded that: (1) nsp12 needs a small region of less than 40 nucleotides at the termini of the viral genome and its complementary minus-strand RNA for initiation of RNA synthesis;

(2) poly(A) tail at the 3' end of viral genome plays a regulatory role in initiation of RNA synthesis during minus-strand RNA synthesis, and (3) the purine nucleotides are preferentially used by nsp12 for de novo initiation of RNA synthesis (Ahn et al., 2012).

Structural studies of PV RdRPs revealed that a native N-terminus is considered essential for the proper folding of their fingers subdomain and locating of their active site. Any additional residues, even a single methionine residue, if added, can negatively affect the activity of the enzyme (Gohara et al., 1999; Thompson and Peersen, 2004). These findings proposed that any addition to N-terminal might hinder the proper folding of SARS-CoV RdRP and thus cause subsequent degradation (te Velthuis et al., 2010).

te Velthuis et al. tried to qualitatively assess the polymerase activity of the nsp12. They developed a reliable protocol for successful expression, purification, and in vitro activity of stable SARA-CoV nsp12 (te Velthuis et al., 2010). The first part of their study involved description of a combined strategy that involved bacterial expression of nsp12 fusion protein and its in vivo cleavage by ubiquitin carboxyl-terminal hydrolase 1 (Ubp1), which recognizes the cleavage site in nsp12 that is located between ubiquitin (Ub) moiety and N-terminus. Proteolytic cleavage of nsp12 fusion protein generates purified and stable SARS-CoV nsp12, containing its natural N-terminus. In the second part of the study, Aartjan et al. assessed the polymerase activity of the nsp12 by monitoring the incorporation of nucleotides on a synthetic RNA template. The extension of the primer was observed by incorporation of [ $\alpha$ - $^{32}$ P] ATP and the extension of the  $^{32}$ P-labeled primer. The sequence alignments of SARS-CoV nsp12 with a known structure of an RdRP revealed that the nsp12 residues 618, 760 and 761 are the canonical aspartic acids responsible for the coordination of the divalent cation in the active site of RdRP. In other viral RdRPs, the substitution of equivalent residues with asparagine or alanine resulted in changing or abolishing the activity of polymerase. In order to obtain an active site mutant that could be used as negative control, Aartjan et al. engineered an nsp12 mutant D618A. The mutant protein showed only  $8 \pm 3\%$  residual primer extension activity. Therefore, it confirms the nsp12-specific nature of the RdRP activity and supports the previous hypothesis that this residue is an essential player in the active site of the enzyme (te Velthuis et al., 2010).

nsp8 is the second polymerase involved in the replication of SARS-CoV genome. It is a small protein that is unique for CoVs and was reported to be only capable of synthesizing RNA de novo with a low fidelity on ssRNA templates. nsp8 forms a hexadameric ring structure with the 10-kDa nsp7 that resides immediately upstream in the replicase polyprotein precursors. nsp8 would serve as RNA primase by

synthesizing short oligonucleotide which would act as primers for subsequent extension by nsp12 (Imbert et al., 2006). The formation of nsp7–nsp8 complex boosts the RdRP activity and increases the RNA binding with nsp8 (Marcotte et al., 2007; te Velthuis et al., 2012).

te Velthuis et al. demonstrated that the nsp7–nsp8 hexadecamer is the most probable conformation of the second SARS-CoV polymerase (te Velthuis et al., 2012). They tried to find whether the dsRNA-binding channel of this complex plays any role in the RdRP activity of nsp8 and whether this activity is influenced by nsp7 (Imbert et al., 2006). Therefore, they generated and purified recombinant forms of SARS-CoV nsp8 and nsp7–nsp8 complex that have natural N-terminal residues. Their finding proved that exposure of the natural N-terminus is crucial for the enzymatic activity of the complex on partially dsRNA templates, demonstrating that nsp7–nsp8 complex is multimeric RNA polymerase that is capable of primer extension as well. The activity is 20-fold weaker than that of the primer-dependent nsp12 RdRp at equal monomer concentration. Moreover, they performed a site-directed mutagenesis of nsp8 to identify residues that may contribute to the catalytic center of the nsp7–nsp8 complex polymerase. Their efforts resulted in two observations: (1) mutation of the conserved N-terminal D/ExD/E motif, including SARS-CoV residues D50 and D52, abolished the polymerase activity, while mutation of the C-terminal motif, comprising of D161 and D163 in SARS-CoV, did not affect RdRP activity; (2) the primer extension activity of nsp8 is severely affected by the presence of N-terminal extensions, i.e., ubiquitin and His6, due to changing its oligomeric state. The strong activity of nsp7–nsp8 complex indicates that the activity of nsp8 is unlikely to be directly controlled by an N-terminal cleavage event, as was observed for the PV polymerase (Marcotte et al., 2007). These observations propose that a more diverse array of nsp8-containing RdRPs may be involved in CoV transcription and replication (te Velthuis et al., 2012).

The mechanism of transcription and replication of SARS-CoV can be understood from the nsp7–nsp8 complex. Zhai et al. presented the crystal structure of the hexadecameric nsp7–nsp8 super-complex of SARS-CoV at 2.4 Å resolutions (Zhai et al., 2005). The structure of this super-complex displayed the atomic interactions between CoV nsps and the architecture of the CoV replication and transcription machinery. Their experiments propose that the super-complex could encircle RNA and function as a general processivity factor for RdRP (nsp12).

The super-complex is a hollow cylinder-like structure with a central channel. It is assembled from eight copies of nsp8 and bound tightly together by eight copies of nsp7. The role of nsp7–nsp8 super-complex is to bind the nucleic acids. The phosphate backbone of nucleic acids passes through the channel without electrostatic repulsion, because the inner channel of the complex has positive potential while the outer surface is covered by negative potential.

CoV replication takes place in the cytoplasm of the infected cells where no DNA is involved. Therefore, the presence of dsRNA intermediate is important for the genomic replication of all CoVs during the RNA synthesis (Zhai et al., 2005; Enjuanes et al., 2006; Van Hemert et al., 2008). The function of the hollow cylindrical structure of the hexadecamer is to encircle and stabilize the dsRNA by holding the nascent and template strands together for transcription and replication. The high conservation of nsp7 and nsp8 in CoVs indicates that they are general components for all CoVs. Thus, the structural and functional analysis of the SARA-CoV nsp7–nsp8 complex might enable the scientists to decode the mechanism of SARS-CoV genome replication and even use them to develop new strategies for the prevention or treatment of SARS in animals and humans (Zhai et al., 2005; te Velthuis et al., 2012).

## 10.4 CLINICAL THERAPIES FOR HCoV INFECTIONS

As discussed previously, the major life-threatening HCoV infections are SARS and MERS, thus the discussion in this section will include the current therapies of SARS and MERS with primary focus on the RdRP inhibitors. At present, no specific antiviral therapy or vaccine has been proven to be effective for treating or preventing of HCoV infections (Geller et al., 2012). Numerous tested compounds have exhibited effectiveness in inhibiting the entry or replication of SARS-CoV in cell culture and animal models but failed in randomized placebo controlled trials. During the epidemic of SARS-CoV, various approaches were used but none were found to be successful. Since then, research has been conducted to discover effective therapeutic agents for SARS-CoV infection based on understanding the virology of CoVs. The current clinical management strategy for SARS is the supportive care, which involves organ support and prevention of complications including organ failure, acute respiratory distress syndrome, and secondary nosocomial infections (Cheng et al., 2007; Chan et al., 2015; Zumla et al., 2016).

### 10.4.1 Approaches to Identify the Suitable Treatment for SARS

HCoV-229E and HCoV-OC43 were the only HCoVs known before the SARS epidemic. These two viruses cause self-limiting upper respiratory tract infections. In 2003 when SARS-CoV suddenly emerged, the researchers especially those involved in antiviral research were not so well prepared. The improvement in SARS treatment depends on identifying effective antivirals for viral load suppression. Thus, three general



approaches were used to identify potential antiviral treatment for HCoV infections (Barnard and Kumaki, 2011; Kilianski and Baker, 2014). The first and fastest approach to drug discovery was to test drugs with broad-spectrum antiviral activity that were used to treat CoV associated human infections. Numerous agents were identified by this approach including interferon alpha, beta, and gamma, ribavirin, and cyclophilin inhibitors (de Wilde et al., 2013; Falzarano et al., 2013; Frausto et al., 2013; Chan et al., 2015; Zumla et al., 2016). The second drug discovery approach to identify antivirals for SARS involved screening of chemical libraries that comprised numerous existing compounds or databases, containing information on transcriptional signatures in different cell lines. Various classes of drugs were identified by this approach including those that affect neurotransmitter regulation, kinase signaling, protein processing, estrogen receptor, lipid metabolism, and DNA synthesis or repair (Chan et al., 2013; de Wilde et al., 2014; Elshabrawy et al., 2014). The third approach to identify treatment for SARS included de novo development of specific antiviral agents based on viral genome and structural biology of individual CoVs. The development of such candidate drugs was more time-consuming than that in the first two approaches. Many viral inhibitors were identified by this method including mAbs that target the host receptor, siRNA inhibitors that target specific viral enzymes involved in the viral replication cycle, inhibitors of virus endocytosis by the host cell, and inhibitors of host cellular proteases (Du et al., 2014; Jiang et al., 2014; Tang et al., 2014; Ying et al., 2014; Chan et al., 2015).

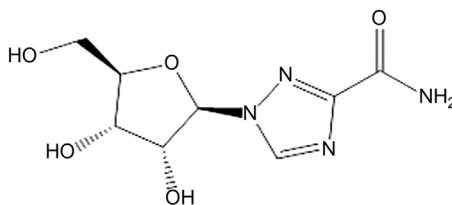
#### 10.4.2 RNA-Dependent RNA Polymerase Inhibitors

Despite high species diversity of CoVs, they all have common genomic elements that are important for the development of new therapeutic agents. The large replicase polyprotein 1a and 1b are cleaved by two viral proteases, 3C-like protease and papain-like protease, to produce nsps, i.e., RdRP and Hel. RdRP is an essential part of the CoV replication–transcription complex and is involved in the production of genomic RNA. RdRP as well as Hel are the key targets for the development of potent anti-CoV drugs. The strong binding of chemicals to the target proteins in the virus disrupt the viral cell cycle and reduce the viral infection (van Boheemen et al., 2012; Chan et al., 2015).

#### 10.4.3 Marketed RdRP Inhibitors

The most well-known broad-spectrum antiviral agent with proven virus inhibition activity is ribavirin (**1**), a purine nucleoside analog.

Although its mechanism of action is still a debatable issue, it blocks the replication of various RNA and DNA viruses by inhibiting inosine monophosphate (IMP) dehydrogenase which is a key enzyme involved in guanosine triphosphate (GTP) biosynthesis. Ribavirin also inhibits the viral RdRP activity by blocking mRNA capping and induction of mutations in RNA-dependent viral replication. However, ribavirin did not show any significant activity against SARS-CoV replication in cell culture—its required inhibitory concentration is much higher (500–5000  $\mu\text{g}/\text{mL}$ ) than that needed to inhibit other viruses (50–100  $\mu\text{g}/\text{mL}$ ). Thus, it does not appear to represent a viable treatment option. However, high doses of ribavirin have been used in treating SARS patients, but its clinical application has been limited due to severe side effects associated with the high doses (Booth et al., 2003; Lee et al., 2003; De Clercq, 2006; Cheng et al., 2013; Peters et al., 2015).

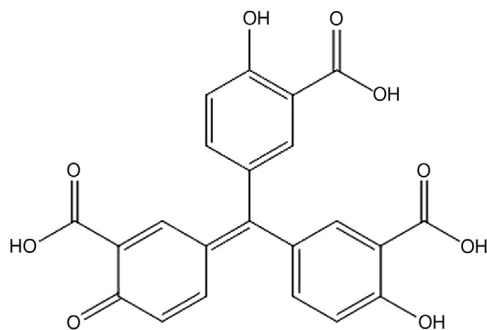


1, Ribavirin

#### 10.4.4 Preclinical RdRP Inhibitors

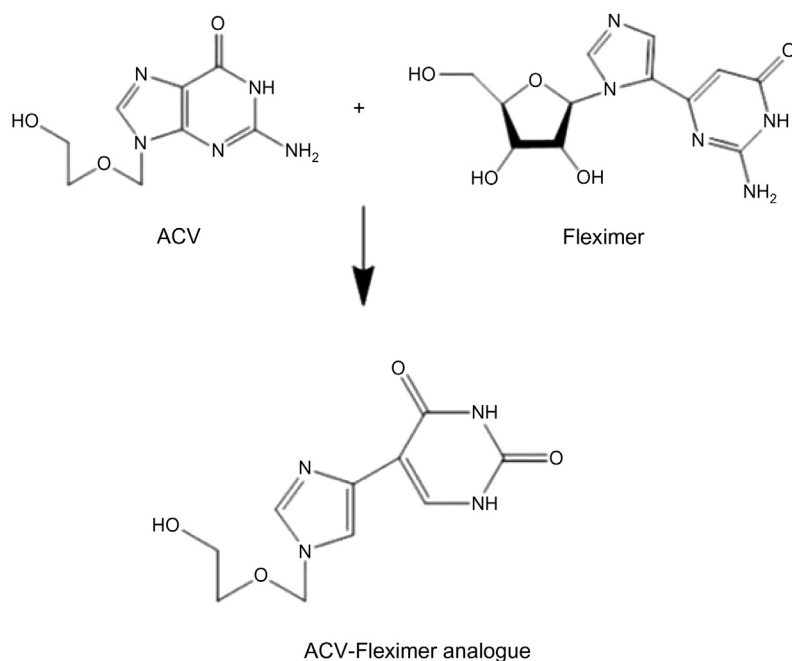
Notwithstanding to 3C-like protease and S proteins, only limited studies on small molecule inhibitors for RdRP, which is critical for the viral RNA replication inside the host cells, are available. Yap et al. have experimentally shown that aurintricarboxylic acid (ATA) (2) could selectively inhibit SARS-CoV replication inside host cells, leading to viral mRNA transcripts production 1000-fold less than that in untreated control (Yap et al., 2005). This is possibly related to the inhibition of SARS-CoV RdRP (Anand et al., 2003; Bermejo Martin et al., 2003; Koren et al., 2003; He et al., 2004; Yap et al., 2005). In this study, Yap et al. incorporated multiple bioinformatics tools to investigate the potential binding modes/sites of ATA with RdRP of SARS-CoV and other pathogenic positive-strand RNA viruses as well as with several other proteins. The positive controls used in this study constituted of three proteins for which crystal structures are available:  $\text{Ca}^{2+}$ -activated neutral protease (*m*-calpain), protein tyrosine phosphatase (PTP) of *Yersinia pestis*, and HIV integrase. Eight regions with homologous 3D-conformation were

obtained for 10 proteins of interest.  $R_{\text{binding}}$  (754–766) is one of these regions located in the palm subdomain of SARS-CoV RdRP, which was predicted by molecular docking studies to be important binding motif recognized by ATA (Cushman and Sherman, 1992; Posner et al., 1995; Liang et al., 2003; Sun et al., 2003; Yap et al., 2005). The results of this experiment showed that ATA could bind to RdRP as well as other proteins in SARS-CoV where it can inhibit their activity by incorporating its binding motifs to the catalytic domains of the specific proteins. This inhibitory binding mechanism, especially for RdRPs, could explain why mRNA transcripts were reduced by 1000-folds when SARS-infected cell culture was treated with ATA compared to untreated control. Thus, ATA could be developed as potent antiviral drug targeting the replication machinery in possibly over a large group of positive-strand RNA viruses (Yap et al., 2005).



2, Aurintricarboxylic acid (ATA)

Another approach for inhibition of HCoV RdRP was identified based on the development of a series of doubly flexible nucleoside analogs. These analogs are based on flexible acyclic sugar scaffold of acyclovir (ACV) and flex-base motif found in fleximers that inhibit RdRP. These doubly flexible nucleoside analogs were evaluated for their antiviral activity and viewed as a novel class of drug candidates with potential of inhibiting several CoVs. Fleximers are unique nucleoside analogs that possess purine base scaffold where pyrimidine and imidazole rings are attached by a single carbon–carbon bond instead of being fused like in typical purines. The designed analogs retain all the essential purine hydrogen-bonding pattern, as well as they allow the nucleobase to explore alternative binding modes. These analogs have several advantages including binding affinity to atypical enzymes, ability to overcome resistance caused by point mutations in biologically important binding sites, and increased binding affinity compared to other inhibitors. Peters et al. described the design, synthesis, and preliminary screening of this



**FIGURE 10.5** Synthesis of target fleximer analogs based on flexible acyclic sugar scaffold of acyclovir and flex-base motif of fleximers. Source: Reprinted with permission from Peters, H.L., Jochmans, D., de Wilde, A.H., Posthuma, C.C., Snijder, E.J., Neyts, J., et al., 2015. Design, synthesis and evaluation of a series of acyclic fleximer nucleoside analogues with anti-coronavirus activity. *Bioorg. Med. Chem. Lett.* 25, 2923–2926. Copyright 2015 Elsevier.

new class of nucleoside analogs. Flex-base modifications of fleximers were combined with acyclic sugar scaffold of ACV, a nucleoside polymerase inhibitor (Fig. 10.5) (Peters et al., 2015).

ACV is approved for the treatment of varicella zoster virus (VZV) and herpes simplex virus (HSV) infections. It also has activity against human immunodeficiency virus (HIV-1) and suppresses the replication of HIV-1 as well as of HSV-2 in the submicromolar range of concentration in lymphoid and cervicovaginal human tissues. Peters et al. designed ACV analogs in the hope that they would possess this biological activity (Peters et al., 2015). The results of their study was that these acyclic fleximer nucleoside analogs could block the replication of MERS-CoV and HCoV-NL63 in cell culture at micromolar concentration but had no effect on SARS-CoV (McGuirt and Furman, 1982; Furman et al., 1984; Vanpouille et al., 2010; Peters et al., 2015).

In addition to nucleoside analog inhibitors of RdRP, regulation of gene expression by small interference RNA (siRNA) has been used to inhibit HCoVs in vitro. The ability of siRNA to silence the expression of

specific genes provides a novel approach to treat various human diseases. To explore the ability of siRNA in interrupting the replication of SARS-CoV, specific siRNAs were synthesized and introduced into mammalian cells. These siRNAs were targeting the viral spike, RdRP, envelop, protein, and nucleocapsid. The results showed that all anti-SARS siRNA inhibited viral gene expression. This finding suggested a novel approach for the inhibition of production of viral proteins leading to treatment of SARS (Meng et al., 2006; Bitko and Barik, 2007).

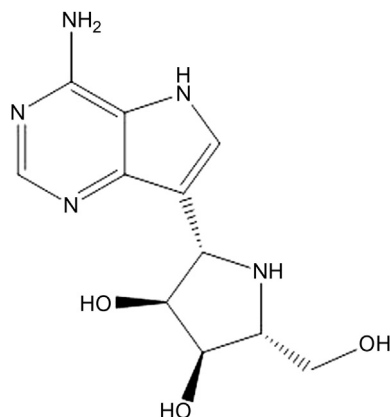
There are two approaches to produce siRNAs in mammalian cells, the first is under the control of specific promoters to form double-stranded siRNA, and the second is through a hairpin structure where siRNAs are expressed as hairpin structures and subsequently processed to siRNA intracellularly. Meng et al. employed both the approaches to produce several siRNA molecules to test against SARS-CoV RdRP and envelope E protein (Meng et al., 2006). In this study, a cell-based assay was developed for screening siRNA to inhibit the expression of two genes that encode envelope E protein and RdRP of SARS-CoV. Envelope E protein plays important role in envelope formation and virus assembly, while RdRP is essential for viral RNA replication.

Both envelope E and RdRP genes were synthesized based on published sequences and cloned into mammalian expression vectors. Two siRNA sites for envelope E gene and four siRNA sites for RdRP gene were designed and tested. In addition, the siRNA or short hairpin RNA (shRNA) expression cassettes were cotransfected into NIH 3T3 cells with the SARS viral envelope E or RdRP expression vectors. Reverse transcription was used to examine the expression levels of envelope E genes and RdRP, followed by quantitative real-time polymerase chain reaction (PCR). Approximately 90% of envelope E gene expression was reduced by both siRNA expression cassettes for envelope E, while two of the siRNA expression cassettes for RdRP effectively blocked the expression of these genes (Kamath and Ahringer, 2003; Boutros et al., 2004; Paddison et al., 2004; Meng et al., 2006).

Both siRNA and shRNA for one of the siRNA sites of the RdRP gene were also examined to find that both of them inhibited exogenous RdRP expression in a dose-dependent manner. These siRNA molecules may be useful for further studies to test the function of these genes in SARS virus replication and assembly. Furthermore, these agents could potentially be developed into therapeutic options for SARS treatment (Meng et al., 2006).

Recently, a novel synthetic adenosine analog, BCX4430 (Immucillin-A) (3), that could inhibit RdRP by acting as a nonobligate RNA chain terminator, was developed by BioCryst Inc. It possesses broad-spectrum activity in multiple viruses and a preliminary preclinical safety profile. Originally, BCX4430 was intended for the treatment of hepatitis C, but was subsequently developed as a potential therapy for deadly filovirus

infections. In addition, it inhibits a wide range of RNA viruses including filoviruses, such as Marburg and Ebola, as well as CoVs, such as MERS-CoV and SARS-CoV. The development of this inhibitor has been fast-tracked to increase the number of therapies for recent Ebola virus epidemic in West Africa. Further studies on BCX4430 are required to develop it for the treatment of HCoV infections.



3, BCX4430 (Immucillin-A)

### 10.4.5 Design of SARS-CoV RdRP Inhibitors

Two classes of antiviral agents that target SARS-CoV polymerase have been identified: nucleoside analog and nonnucleoside analog inhibitors (Xu et al., 2003).

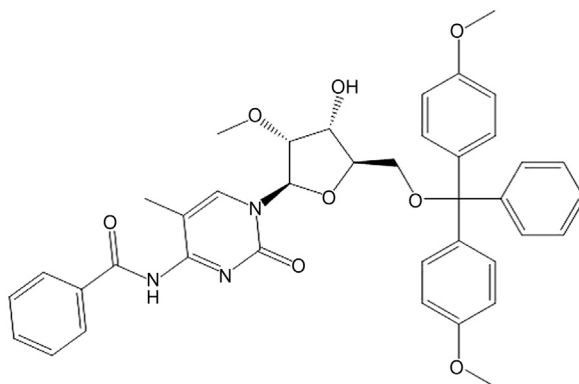
#### 10.4.5.1 Nucleoside Analog Inhibitors

Nucleoside analog inhibitors are dNTPs or rNTPs that lack 3'-OH group. These inhibitors compete with nucleotide substrate to bind to the active site of polymerase. Once they are incorporated into the elongation chain of nucleic acid, chain termination results. Nucleoside inhibitors are widely used as a treatment for HBV, HCV, HIV-1, and herpesvirus infections (De Francesco et al., 2003; Shim et al., 2003; Xu et al., 2003).

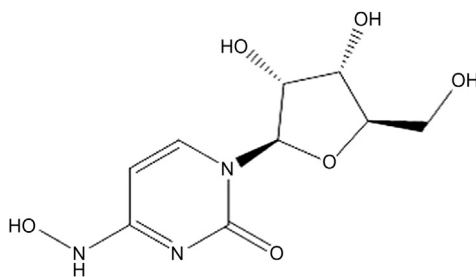
Xu et al. reported a type of nucleoside analogs that should inhibit SARS-CoV RdRP (Xu et al., 2003). They proposed that nucleoside analogs that could inhibit RdRP of SARS-CoV should contain substituents at 2' and 3' positions which may form hydrogen bonds with neighboring Asp623 and Asn691 residues in the active site of the polymerase. In addition, the analysis of molecular model of the structure of SARS-CoV RdRP suggested that potential nucleoside inhibitors should contain C3'

endo sugar puckering conformation to maintain its ability to involve its 3'-substituent in hydrogen-bond formation and avoid steric conflicts with 2'-substituent. Thus, the nucleoside analogs with 2'-C-methyladenosine and 2'-O-methylcytidine could be found to be effective SARS-CoV RdRP inhibitors (Carroll et al., 2003; Xu et al., 2003).

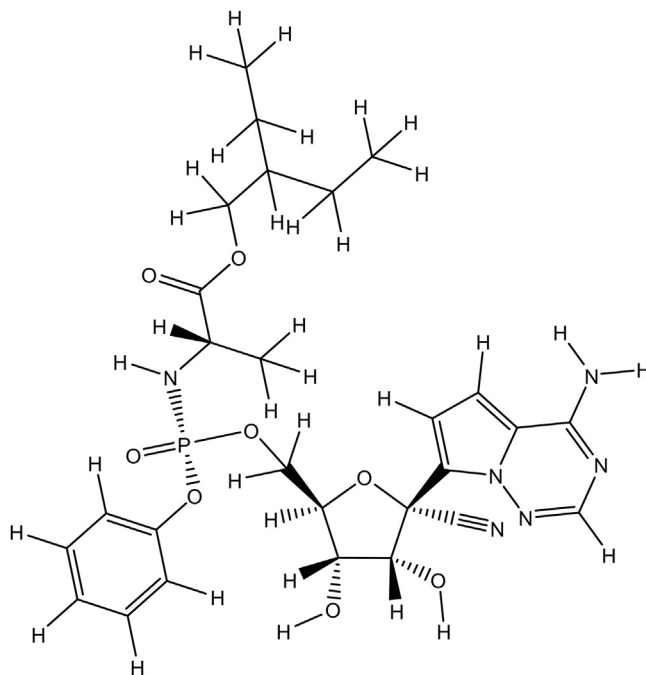
At present, only few nucleoside analogs are recognized as inhibitors of RdRP in SARS-CoV, e.g., a 2'-O-methylcytidine derivative, N<sub>4</sub>-Benzoyl-5'-O-(dimethoxytrityl)-5-methyl-2'-O-methylcytidine (Bz-DMT-dC) (4), has been found to have inhibitory activity toward SARS-CoV replication. In addition,  $\beta$ -D-N<sub>4</sub>-hydroxycytidine (5) is another nucleoside analog which has been accredited with both anti-HCV and anti-SARS-CoV effects. It partially fulfills the features of SARS RdRP inhibitors that have been described. It showed activity against SARA-CoV at EC<sub>50</sub> of 10  $\mu$ M (selectivity index  $\geq$  10). However, the mechanism of antiviral effect of  $\beta$ -D-N<sub>4</sub>-hydroxycytidine is still not clear, but it was postulated that it might inhibit the viral replication by affecting the thermodynamics of secondary structure of RdRP, but this speculation was not ascertained (Barnard et al., 2004; De Clercq, 2006).



4, Bz-DMT-dC

5,  $\beta$ -D-N<sub>4</sub>-hydroxycytidine

Recent Ebola virus outbreak in West Africa necessitated vigorous efforts to identify new antivirals targeting filoviruses. This led to the development of a new series of C-linked nucleoside analogs with anti-Ebola properties (Warren et al., 2016). One such analog remdesivir (GS-5734) (6) with good anti-Ebola property was reported to inhibit SARS-CoV and MERS-CoV replication in multiple in vitro systems, including primary human airway epithelial cell cultures with submicromolar EC<sub>50</sub> values (Sheahan et al., 2017). Experimental evaluation of GS-5734 in a mouse model of SARS-CoV infection showed that its prophylactic and early therapeutic administration reduced lung viral load and improved respiratory function along with other clinical signs. Although the mechanism of action of GS-5734 has not been unraveled completely, it is generally assumed that the molecule targets the RdRP function of the viral polymerase (Agostini et al., 2018).



6, Remdesivir (GS-5734)

#### 10.4.5.2 Nonnucleoside Analog Inhibitors

Nonnucleoside inhibitors of polymerases are effective therapeutics with large specificity against HIV-1, and currently they are under development as anti-HCV drugs. These inhibitors are hydrophobic in nature and act in a noncompetitive manner with respect to dNTP or rNTP



substrates, which is consistent with inhibitor binding at site different from nucleotide substrate. In HIV-1 RT, the nonnucleoside analog inhibitors bind to hydrophobic pocket near the polymerase active site, located at the palm–thumb subdomain interface, and cause conformational changes in the residues at the polymerase active site, displacement of the “primer grip,” and restriction on the movement of thumb. In HCV, they bind to hydrophobic pocket on the surface of thumb subdomain, producing allosteric effect that interferes with the conformational change in the thumb (Kohlstaedt et al., 1992; Das et al., 1996; Dhanak et al., 2002; Chan et al., 2003; Love et al., 2003; Xu et al., 2003).

Unlike in HIV-1 RT, the SARS-CoV RdRP does not contain hydrophobic pocket near its active site. Further, its thumb subdomain is smaller than that of HCV RdRP and the essential part of inhibitor binding pocket of HCV does not exist in it. Therefore, it seems that the nonnucleoside inhibitors for HIV-1 or HCV may not work for SARS-CoV polymerase. However, different allosteric site exists in SARS-CoV polymerase which can be targeted for the development of SARS-CoV polymerase inhibitors (Xu et al., 2003).

## 10.5 CONCLUSIONS

CoV RdRPs presents an exciting opportunity for the scientific community to develop effective therapeutic interventions for the treatment of deadly diseases, such as SARS and MERS caused by CoVs. Two nsp with RdRP activity have been identified for SARS CoV, which are nsp12 and nsp8 having de novo and primer-dependent polymerase activity, respectively. Studies have led to reveal many of the biochemical and biophysical properties of the polymerase domain facilitating the development of novel therapeutic strategies. Numerous approaches to develop RdRP inhibitors ranging from small molecule inhibitors to fleximers of ACV and siRNAs have been explored in the recent past. Fundamental research into CoV RdRP is thus proving to be invaluable, and will likely continue to be, in combating current and emerging CoV infections.

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