Microbes and Infectious Diseases 2021; 2(3): 415-422

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Review article

Potential chemical compounds can inhibit SARS CoV-2 replication and open the way for the production of anti-SARS CoV-2 therapy

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ARTICLE INFO

Article history:
Received 27 May 2021
Received in revised form 28 June 2021
Accepted 29 June 2021

Keywords:
COVID-19
PPMO
BAPA
Therapy
Untranslated region
TMPRSS2
Subgenomic RNA

ABSTRACT

The genome of severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) has an untranslated region (UTR) that enhances the replication and transcription of RNA, and subgenomic RNA (sgRNA) which encodes the 4 structural proteins that are essential for viral replication. The host cell of SARS CoV-2 expresses an important enzyme called TMPRSS2 protease that allows the virus to invade the cell. Peptide conjugated phosphorodiamidate morpholino oligomers (PPMOs) and Benzylesulfonyl-d-arginine- proline-4-amidinobenzylamide (BAPA) chemicals inhibit the expression of the TMPRSS2 in the influenza host cell. Peptide conjugated phosphorodiamidate morpholino oligomers also inhibited the UTR of the influenza genome, sgRNA of the porcine reproductive and respiratory syndrome virus (PRRSV), and other locations in other respiratory viruses. The combination of these chemicals can inhibit the UTR and sgRNA of SARS CoV-2 and prevent the expression of the TMPRSS2 in the SARS CoV-2 host cell to stop SARS CoV-2 replication, which may help the production of a drug against the SARS CoV-2 virus.

Introduction

Several infected people with unknown pneumonia cases that have appeared at the beginning of 2019 in some hospitals in Wuhan [1], it was found that they were infected by a new coronavirus, called by the International Committee on taxonomy of viruses a severe acute respiratory syndrome coronavirus-2 (SARS CoV-2). This virus causes a respiratory disease called by World Health Organization COVID- 19 [2]. Now, the virus has become a pandemic and spread all over the world [1]. It is not the first coronavirus, but SARS CoV and MERS CoV have appeared before it [3], and coronaviruses are seven species,where SARS CoV-2 is the 7th and latest one and has 70% genetic sequence similarity to SARS CoV, and its symptoms include fever, fatigue, dry cough, dyspnea, and hard breathing [1].

The genome of SARS CoV-2 is 30 Kb in length and has a 5' cap structure and 3' poly (A) tail [2] with UTR that regulates RNA replication and transcription and has 14 open reading frames (ORFs) [4]. One is called ORF1ab that is the first of these ORFs consists of the majority of the SARS CoV-2 genome and encodes two polyproteins (PP1a & PP1ab) that are lysed by Chymotrypsin- like cysteine protease (3CL proteases) [2] EC [3.4.22.69] and papain-like protease (PL proteases) [2] EC [3.4.22.2] at 11 distinct sites to form the 16 nonstructural

DOI: 10.21608/MID.2021.77910.1161
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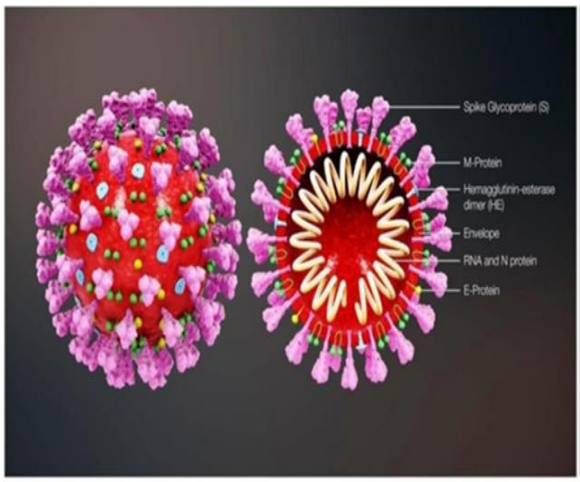
proteins (NSPs) [5] that are essential for the formation of sgRNAs, which expresses the 4 main structural proteins that are spike protein (S protein), envelop protein (E protein), nucleocapsid protein (N protein), and membrane protein (M protein). So, the subgenomic RNA is necessary for the SARS CoV-2 replication process [2].

Severe acute respiratory syndrome coronavirus 2 UTR and sgRNA are potential targets of drugs to stop the virus replication, so PPMO and BAPA chemicals that inhibited the UTR of the influenza genome [6] and sgRNA of the PRRSV [7,8] may be potential also in case of SARS CoV- and stop its replication process. They also inhibited the expression of TMPRSS2 that enhances the invasion process in the influenza host cell, so they may inhibit the expression of this enzyme in the SARS CoV-2 genome to harden its invasion process. This may open the way for the production of a potential drug against SARS CoV-2.

SARS CoV-2 structure

Severe acute respiratory syndrome coronavirus 2 consists of genetic material called positive-sense, single-stranded genomic RNA, and structural proteins such as spike protein, membrane protein, envelop protein, nucleocapsid protein, hemagglutinin- esterase dimmer, and other important proteins that are RNA-dependent RNA polymerase (RdRp), chymotrypsin-like cysteine protease (3CL proteases), papain-like protease (PL proteases), and nonstructural proteins.

Figure 1 The schematic diagram shows the virus genetic material and the structural proteins, which are the spike protein, membrane protein, envelope protein, nucleocapsid protein, and hemagglutinin-esterase dimer [9].



Positive-sense, single-stranded genomic RNA

The SARS-CoV-2 genome is one of the longest RNA genomes because it has about 30 Kb. The genomic RNA (gRNA) consists of 14 ORFs and plays an important role in the translation of the viral polyproteins, where it serves as mRNA and has a 5'-cap and a 3'-poly (A) tail that represents a highly structured UTR that regulates RNA proliferation. The 3'-UTR has a stem-loop and pseudoknot that is hypothesized to participate in transcriptional regulation. The ORF1ab that is the first of the 14 ORFs forms most coronavirus genome length (two thirds) and encodes two polyproteins (PP1a & PP1ab) that are proceeded by SARS VoV-2 main proteases to produce the structural proteins that participate in the formation of new viruses [2,5].

RNA dependent RNA polymerase (RdRp)

The RdRp enzyme of SARS CoV-2 is similar to that of SARS-CoV and MERS-CoV and participates in the virus replication from the RNA template [10], and the replication and transmission take place by a multimeric RdRp non-structural protein complex [11].

Chymotrypsin- like cysteine protease (3CL protease)

The 3CL protease plays an important role in the SARS CoV-2 life cycle, maturation, and replication, so it is a significant target of drugs, where it participates in the composition of the structural proteins, which are used in the production of new viruses. It consists of three domains; domain I, II, and III, and its substrate-binding region locates at the cleft of domain I and II. It is composed of the conserved His 41 and Cys 145 catalytic dyad. The SARS main protease contains other buried subunits such as S1 andS2 [3,11]. The S1 subunit consists of amino acid residues named His163, Cys145, Glu166, His172,Gly143, and Phe140, and the S2 subunit consists of amino acid residues named Cys145, His41, and Thr25. The SARS also contains other 3 shallow subunits such as, S3, S4, S5 that are consisted of amino acid residues named Met49, His41, Met165, Glu166, and Gln189. Due to the function of the 3CL protease and PL protease in the life cycle of the virus, maturation, and replication, they became an important target for the development of an effective anti-SARS CoV-2 therapy [3].

Papain-like protease (PL protease)

The PLpro plays a significant role in viral replication because it cleaves the virus polyproteins into 16 nonstructural proteins, that participate in the formation of the sgRNA, which encodes the SARS CoV-2 structural proteins allowing the formation of viral- like particles. It also helps the virus to overcome the immune response to be able to cause

the COVID-19 disease, so it is an important target of drugs [12].

Spike protein (S protein)

The S protein of SARS CoV-2 enhances the viral invasion into the host cell [13] and has amino acids range from 1104 to 1273, amino (N)- terminal S1 subunit, and carboxyl (C)-terminal S2 subunit, and each of them has a specific function [14]. The S1 subunit enables the virus to attach to the virus receptor which is the Angiotensin-converting enzyme-2 (ACE2) [13], and the S2 subunit enables the virus to invade the host cell [15]. The S protein two subunits are cleaved from each other by the proteases to perform their functions [13].

Membrane protein (M protein)

This protein plays an important role in the shape of the virus and its assembly. It is considered the dominant protein in the virus and contains 80 amino acids [16]. It cannot alone do its function accurately, so it interacts with all other large structural coronavirus proteins [17] and can be neutralized by the antibodies, so it is a significant target of vaccines [16].

Envelope protein (E protein)

The SARS CoV-2 virus contains about 20 copies of the SARS CoV-2 E protein that play an important role in the pathogenicity and invasion process, and each protein consists of amino acids range from 76–109, and it ranges in size from 8.4– 12 kDa. It consists of a small hydrophilic amino terminus (consists of 7–12 amino acids), large hydrophobic transmembrane domain TMD (consists of 25 amino acids), and long hydrophilic carboxyl terminus (consists of most proteins) [17]. The virus that lacks the E protein cannot invade the host cells or has very low pathogenicity. So, the work on the inhibition of this protein is very important [18].

Nucleocapsid protein (N protein)

The N protein of SARS CoV-2 plays an important role in the RNA synthesis, transcription, and translation, and contains three domains that form it such as, the N-terminal domain (~130 residues), central domain (~120 residues), and the C-terminal domain [11]. It regulates important interactions in the cell such as the reorganization of actin, progression of the host cell cycle, and apoptosis [19]. It is a good target of vaccines because of its immunogenicity and expression increase during the infection [11].

Hemagglutinin-esterase

Different coronaviruses have a receptor destroying enzyme (RDE) that contains acetylesterase and receptor-binding function, so it is called hemagglutinin- esterase (HE) [21], and this activity is believed to improve the invasion and spread of SARS CoV- 2 through the mucosa [22].

Nonstructural proteins (accessory proteins)

Severe acute respiratory syndrome coronavirus 2 encodes 1-16 nonstructural proteins (NSP) and the NSP16 forms a heterodimer with its cofactor nsp10 and modifies the virus genetic material to make it similar to human RNA and protects it from recognition by the immune response and MDA5 helicase enzyme [23]. The NSPs genes lie between the structural protein genes, and their number and sequence differ among different coronaviruses. These nonstructural proteins enhance the virus replication and pathogenicity because they play an important role in the formation of the structural proteins, but the specific function is unclear [24].

PPMO and BAPA

Figure 2. The chemical structure of the phosphorodiamidate morpholino oligomer (PMO), AVI-4658 [25].

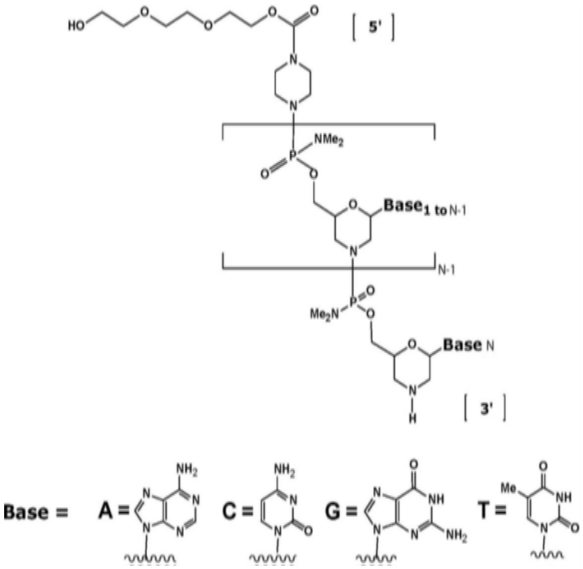
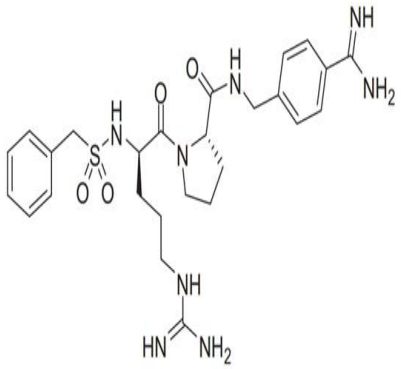


Figure 3. The chemical structure of BAPA (benzylsulfonyl-d-arginine-proline-4-amidinobenzylamide) [7].



Inhibition of untranslated region, subgenomic RNA, and TMPRSS2

PPMO

Phosphorodiamidate morpholino oligomer consists of DNA nucleobases A, G, T, and C, morpholine rings, and phosphorodiamidate intersubunit linkages. It blocks cRNA and interferes with the 5' UTR and mRNA translation start codon, AUG. It conjugates with an arginine-rich cell- penetrating peptide (CPP) to form PPMO that has antiviral activity against some types of positive and negative RNA viruses [6].

Different types of PPMOs can stop SARS CoV-2 replication, as they had a great influence on other viruses such as influenza and PRRSV, where they inhibited the UTR of the influenza genome, prevented the expression of the TMPRSS2 in the influenza host cell and inhibited the sgRNA of the PRRSV. They have targeted thecomplementary RNA to hinder the storage of the information in a single strand, acted against the 5' UTR and/or the start codon of translation (AUG) in mRNA to stop the translation process, and interfered with spliceosome protein that mediates mRNA maturation reactions. Different PPMOs target different locations in TMPRSS2 mRNA as followed:

- T-AUG with the sequence CAAAGCCATCTTGCTGTTATCAAC has interfered with the 42-65 nt (initiator AUG).
- Scramble with the sequence TGCTCTGTCTACAGTAGTGTCA has interfered with the nonsense sequence control.
- T-ex5 with the sequence CAGAGTTGGAGCACTTGCTGCCCA has interfered with the 382-405 nt (5_ end of exon 5, adjacent to splice site), reducing virus titers by 2 to 3 log10 units in calu-3 airway cell.
- T-ex4 with the sequence TGATGCACAGTGCTTTCTTAGTCT has interfered with the 295-318 nt (5_ end of exon 4, adjacent to splice site)[6].
- PPMO 5UP2 has interfered with the 5' terminal region of the PRRSV.
- PPMO 5HP has interfered with the 5' UTR of the genomic RNA.
- PPMO 6P1 has interfered with the ORF6.
- PPMO 7P1 has interfered with the ORF7.
- Dozen PPMO has interfered with variable sites in the PRRSV genome and its sgRNA[8].

Peptide conjugated phosphorodiamidate morpholino oligomers targeted the sequence that regulates the transcription of SARS CoV-2 genomic RNA, reducing viral titers by 4-6 log10 at 48-72 hours post-infection in cell culture in a nontoxic dose [26].

- 50END-1 PPMO with the sequence CCTGGGAAGGTATAAACCTTTAAT has targeted 1–24 nt.
- 50END-2 with the sequence TGTTACCTGGGAAGGTATAAACCTT has targeted 5–29 nt.
- TRS-1 with the sequence TTTTAAAGTTCGTTTAGAGAACAG has targeted 59–82 nt.
- TRS-2 with the sequence AAGTTCGTTTAGAGAACAGATCTAC has targeted 53–77 nt.
- AUGb with the sequence AGGCTCTCCATCTTACCTTTCGGT has targeted 251–275 nt [26].

A study was published in 2007 shows other different PPMOs able to target different locations in other coronaviruses as followed:

- 5TERM with the sequence CGGACGCCAATCACTCTTATA has targeted genomic 5' terminus (2–22).
- (+)19-40 with the sequence GAGTTGAGAGGGTACGTACGGA has targeted genomic leader stem-loop 1 (19–40).
- TRS1 with the sequence GTTTAGATTAGATTTAAACTAC has targeted genomic and subgenomic TRS region (51–72).
- TRS2 with the sequence CGTTTATAAAGTTTATATTAGAT has targeted genomic TRS region (60–82).
- 5UTR with the sequence TGACAAGACCAGGCCCGCGG has targeted genomic region between the leader and pp1ab AUG (104–123).

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- AUG with the sequence TCTTTGCCATTATGCAACCTA has targeted genomic pp1ab AUG region (200–220).
- 1ABFS with the sequence GACGGGCATTTACACTTGTAC has targeted genomic pp1ab ribosomal frameshift signal (13612–13632).
- MBTRS with the sequence GTACTACTCATAATGTTTAGAT has targeted subgenomic RNA 6 TRS region (28958–28979).
- (-)3TERM with the sequence TATAAGAGTGATTGGCGTCCG has targeted antigenomic 3' terminus (2–22).
- (-)19-40 with the sequence TCCGTACGTACCCTCTCAACTC has targeted antigenomic leader stem-loop 1 (18–39).
- RND with the sequence AGTCTCGACTTGCTACCTCA has targeted randomized control sequence.
- SARS-TRS1 with the sequence GTTCGTTTAGAGAACAGATC has targeted SARS-CoV genomic leader TRS region (53-72).
- SARS-5TERM with the sequence GGTAGGTAAAAACCTAATAT has targeted SARS-CoV genomic 5' terminus (1–20) [27].

Peptide conjugated phosphorodiamidate morpholino oligomers target different locations in other positive and negative-sense RNA viruses.

Peptide conjugated phosphorodiamidate morpholino oligomers targets in positive-sense RNA viruses as followed:

- IRES in 5' terminal end of the genome in Coxsackievirus B2 and B3.
- IRES in 5' terminal end of the genome in Poliovirus type 1.
- IRES in 5' terminal end of the genome and RNA-dependent RNA polymerase in Enterovirus 71.
- The IRES 5' portion and Translation initiation codon region in Foot-and-mouth disease virus.
- The genomic RNA 5' terminus in Mouse hepatitis virus.• The 5' UTR of EAV genome in Equine virus (EAV).
- The 5' and 3' terminal of the genome in the West Nile virus.
- The 5' and 3' terminal of the genome and 3' stem-loop in Dengue virus.
- The 5' and 3' UTR of the genome in Japanese encephalitis virus.
- The AUG region of ORF1 in Chikungunya virus.
- The 5' terminal end and AUG translation start site regions in the Sindbis virus.
- The 5' terminal end and AUG translation start site regions in Venezuelan equine encephalitis virus.
- The AUG region of ORF1 near the 5' end of the genome in Noroviruses.
- The 5' UTR and translation initiation start region of ORF1, 3' UTR, and 5' terminus of antisense HEV RNA in hepatitis E virus [28].

Peptide conjugated phosphorodiamidate morpholino oligomers targets in negative-sense RNA virus as followed:

- The translation initiation codon region of VP24 and VP35 in the Ebola virus.
- The translation initiation codon region of VP24 and VP35 in Marburg virus.
- The 5' terminus and the translation start-site region of RSV L mRNA in Respiratory Syncytial Virus (RSV).
- The translation initiation codon region of the nucleocapsid protein mRNA in the Measles virus.
- The translation initiation codon regions of polymerase subunit PB1 mRNA, NP mRNA, and 3' end of NP viral genome
- RNA in influenza A virus.
- The 5' terminal of both genomic segments across different arenaviruses in Junín virus, Tacaribe virus, Pichinde virus, and lymphocytic choriomeningitis virus [28].

BAPA

BAPA has prevented the expression of TMPRSS2 in human airway epithelial cells and reduced virus titers of different influenza A and B viruses more than 1000 folds by 24-48 hours post-infection in a nontoxic concentration. "Calu-3 cells were infected by seasonal isolates A/Memphis/14/96(H1N1) or B/Massachusetts/11 at low Mol of 0,0001 and incubated in the presence of 20 or 50 μM of BAPA for 72 h)." 20 μM BAPA reduced virus titers in case of A/Memphis/14/96(H1N1) to 10-30 folds and in case of B/Massachusetts/11 to 100-400 folds. 50 μM BAPA reduced virus titers to 1000-10,000 folds in both viruses post-infection.

A study by llyushina and co-workers indicated that antivirals and protease inhibitors together can prevent drug resistance and drug doses, that may decrease the toxicity and dangerous side effects of the drug, so a combination of PPMO and BAPA is

very important and can prevent SARS CoV-2 replication in high efficacy [7].

Conclusion

There are no specific drugs against SARS CoV-2 until now and the world has become in a bad need to develop one against the virus. The inhibition of SARS CoV-2 UTR, subgenomic RNA, and TMPRSS2 of the virus-host cell can prevent SARS CoV-2 replication and overcome the pandemic, so the use of PPMO and BAPA chemicals in a combination can open the way for the development of a drug against the virus. Hence, researchers have to try to make a drug using these compounds and detect its effect against the virus in lung cell culture media.

Acknowledgments

I want to thank the Microbiology Department, Faculty of Science Tanta University, and Biochemistry Department professor (Prof. Dr. Tarek Mostafa), Faculty of Science, Tanta University. Also, I need to thank my college Abdallah Elwakeel, Chemistry Department, Faculty of Science, Tanta University for helping me doing this review.

Conflict of interest: None.

Financial disclosure: None to declare.

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