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Saporin, a Polynucleotide–Adenosine Nucleosidase, May Be an Efficacious Therapeutic Agent for SARS-CoV-2 Infection

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Abstract

Saporin, a type I ribosome-inactivating protein from soapwort plant, is a potent protein synthesis inhibitor. Catalytically, saporin is a characteristic *N*-glycosidase, and it depurinates a specific adenine residue from a universally conserved loop of the major ribosomal RNA (rRNA) of eukaryotic cells. It is well-known that saporin induces apoptosis through different pathways, including ribotoxic stress response, cell signal transduction, genomic DNA fragmentation and RNA abasic lyase (RAlyase) activity, and NAD^+ depletion by poly-(ADP)-ribose polymerase hyperactivation. Saporin's high enzymatic activity, high stability, and resistance to conjugation procedures make it a well-suited tool for immunotherapy approaches.

In the present study, we focus on saporin-based targeted toxins that may be efficacious therapeutic agents for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Our discussed points suggest that saporin may be a strategic molecule for therapeutic knockout treatments and a powerful candidate for novel drugs in the struggle against coronavirus 2019 (COVID-19).

Keywords

SARS-CoV-2, saporin, abasic site, rRNA, *N*-glycosidase

Introduction

Due to a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a major outbreak of extraordinary viral pneumonia [coronavirus 2019 (COVID-19)] started in Wuhan, China, in late December 2019. The World Health Organization (WHO) declared the epidemic of COVID-19 as a pandemic on March 11, 2020.[1] Coronaviruses are pathogenic in humans and other mammals (e.g., bats and pangolins). As the virus has spread around the world, countries have reached different stages of their outbreaks at different times. Across the globe, COVID-19 remains a global leading cause of death, with a still-rising incidence.

The main reason for this situation is that despite the well-known genetic and molecular features of SARS-CoV-2, there are still no therapeutic knockout treatments or vaccines. To date, even though various clinical trials are ongoing, no licensed and effective antiviral vaccine or drug is available against COVID-19. A recent paper reported that understanding the genetic and phenotypic basics of SARS-CoV-2 in pathogenesis is considerably important for drug discovery. The genomes of coronaviruses (CoVs) are single-stranded positive-sense RNA (+ssRNA) with a 5′-cap structure and 3′-poly-A tails. The SARS-CoV-2 genome of approximately 30 kilobase pairs (kb) includes a minimum of six open reading frames (ORFs). The first ORF (ORF1a/b) is approximately two-thirds of the whole genome length and encodes 16 nonstructural proteins. ORFs near the 3′-end of the genome encode four major structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (**Fig. 1**).[2]

Saporin is a toxic RNA *N*-glycosidase [Enzyme Commission no. (EC): 3.2.2.22] that depurinates eukaryotic ribosomal RNAs (rRNAs), thereby arresting protein

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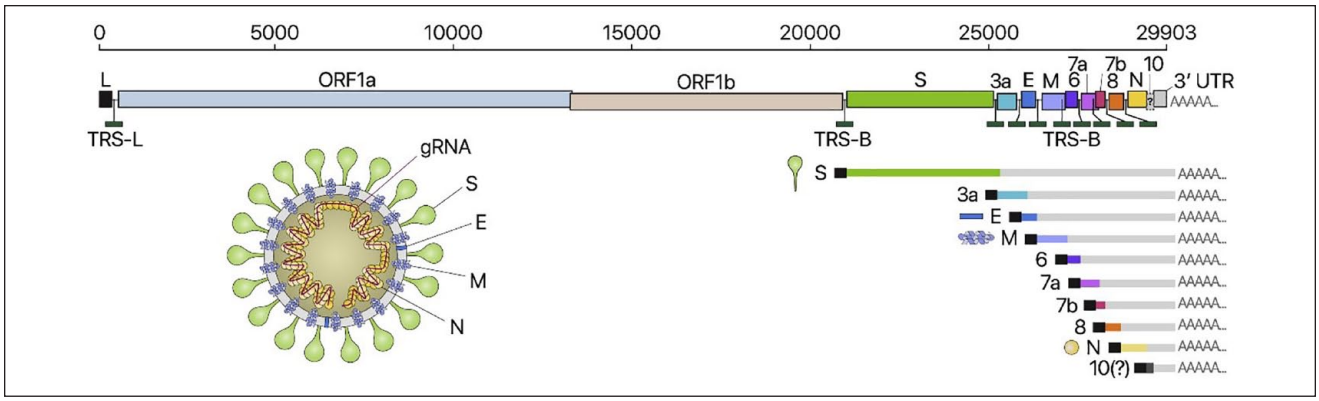

Figure 1. Coronaviruses include the largest genomes among all RNA viruses (26–32 kb).[2]

synthesis irreversibly during translation. It is one of the type I ribosome-inactivating proteins (RIPs), and it is synthesized from *Saponaria officinalis* L. (family Caryophyllaceae, common name: soapwort).[3] Several isoforms of saporin have been isolated from various parts of the plant, such as saporin-L1 (SAP) and saporin SO6 (Sap-SO6) purified from soapwort leaves and seeds, respectively. The crystal structure of Sap-SO6 [Protein Data Bank (PDB) code: 1QI7] has revealed that this enzyme contains two main domains: an N-terminal domain with a predominantly β-sheet, and a C-terminal domain with a prevalent α-helix structure (**Fig. 2**).[4,5]

RIPs are potent toxins and can cause irreversible cell damage leading to apoptosis and necrosis.[5] RIPs were first found in higher plants, and many RIPs have been identified from natural sources (e.g., higher plants, fungi, algae, and bacteria). Examples of plant RIPs include ricin, abrin, gelonin, momordin, and saporin.[6–8]

Saporin catalyzes the depurination of invariant adenosine residue (A_{4324}) from the $GA_{4234}GA$ tetraloop motif within the universally conserved α-sarcin–ricin recognition loop of the eukaryotic 28S rRNA molecule.[9] Saporin includes a single catalytic chain (domain A) and lacks a lectin-like binding domain; therefore, it is classified as a type I RIP.

Depurination of adenine residue causes a permanent inhibition of the 28S rRNA subunit, arresting the recognition and binding of elongation factor-1 (EF1) and the following creation of the elongation factor-2–guanosine triphosphate (EF2–GTP)–ribosome complex, thereby prohibiting translocation of the transfer RNA (tRNA) from the A site to the P site of the ribosomal complex, thus irreversibly blocking protein synthesis.[10]

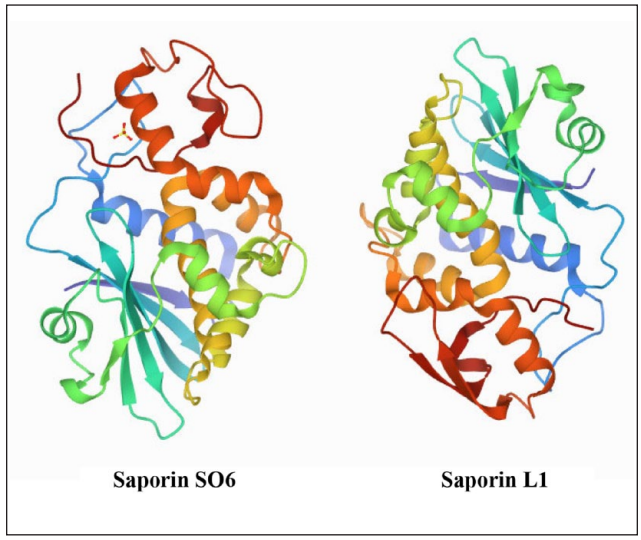

Figure 2. Protein structures of saporin isoforms from the Protein Data Bank (PDB): Saporin O6 (Sap-SO6; PDB code: 1QI7) and saporin-L1 (SAP; PDB code: 3HIS).

saporin removed more than one adenine residue from rRNA, unlike most RIPs.[11] In addition, saporin-R1 depurinated adenine not only from rRNA but also from messenger RNA (mRNA), tRNA, and poly(A) and other cellular DNAs, but not from adenosine triphosphate (ATP) or deoxyadenosine triphosphate (dATP).[12] Saporin removes multiple adenines from rRNAs (**Fig. 4**), whereas other RIPs exhibit exquisite specificity. This is undoubtedly one of the most important characteristics of saporin proteins. Therefore, it was suggested that saporin proteins would be more appropriately called polynucleotide–adenosine nucleosidases.[13]

Mode of Action of Saporin

Endo et al. (1987) first discovered the enzymatic activity of RIPs, and all types of RIPs were called rRNA *N*-glycosidase (EC 3.2.2.22) (**Fig. 3**). Studies showed that isoforms of

Basics of Depurination Mechanism

Abasic sites are the lack of a purine or pyrimidine base in a DNA/RNA and arise from spontaneous damage or toxins (e.g., RIPs). The glycosidic bond is mostly unstable and

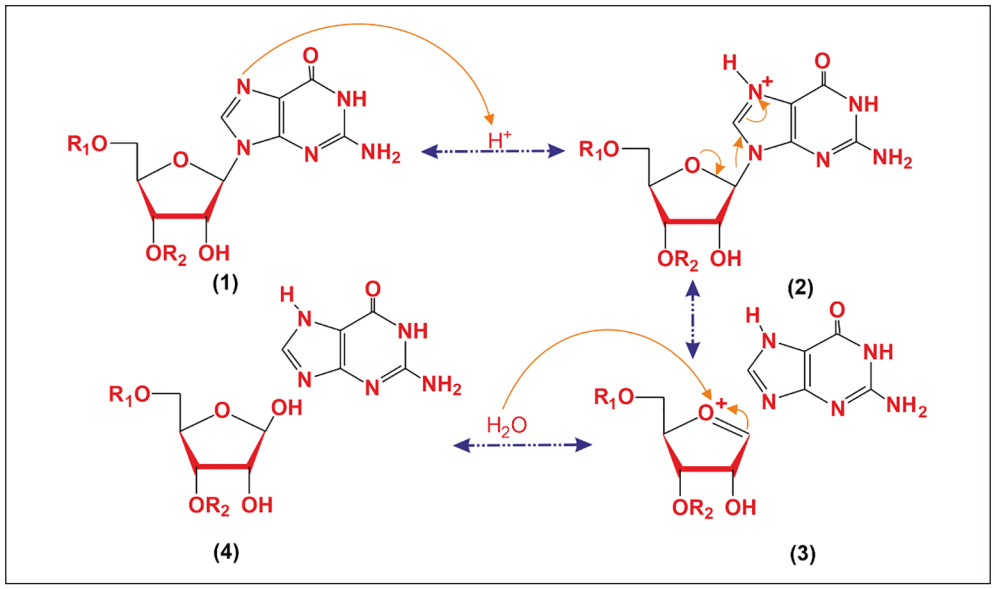

Figure 3. Schematic overview of construction of an abasic site in ribosomal RNA (rRNA).

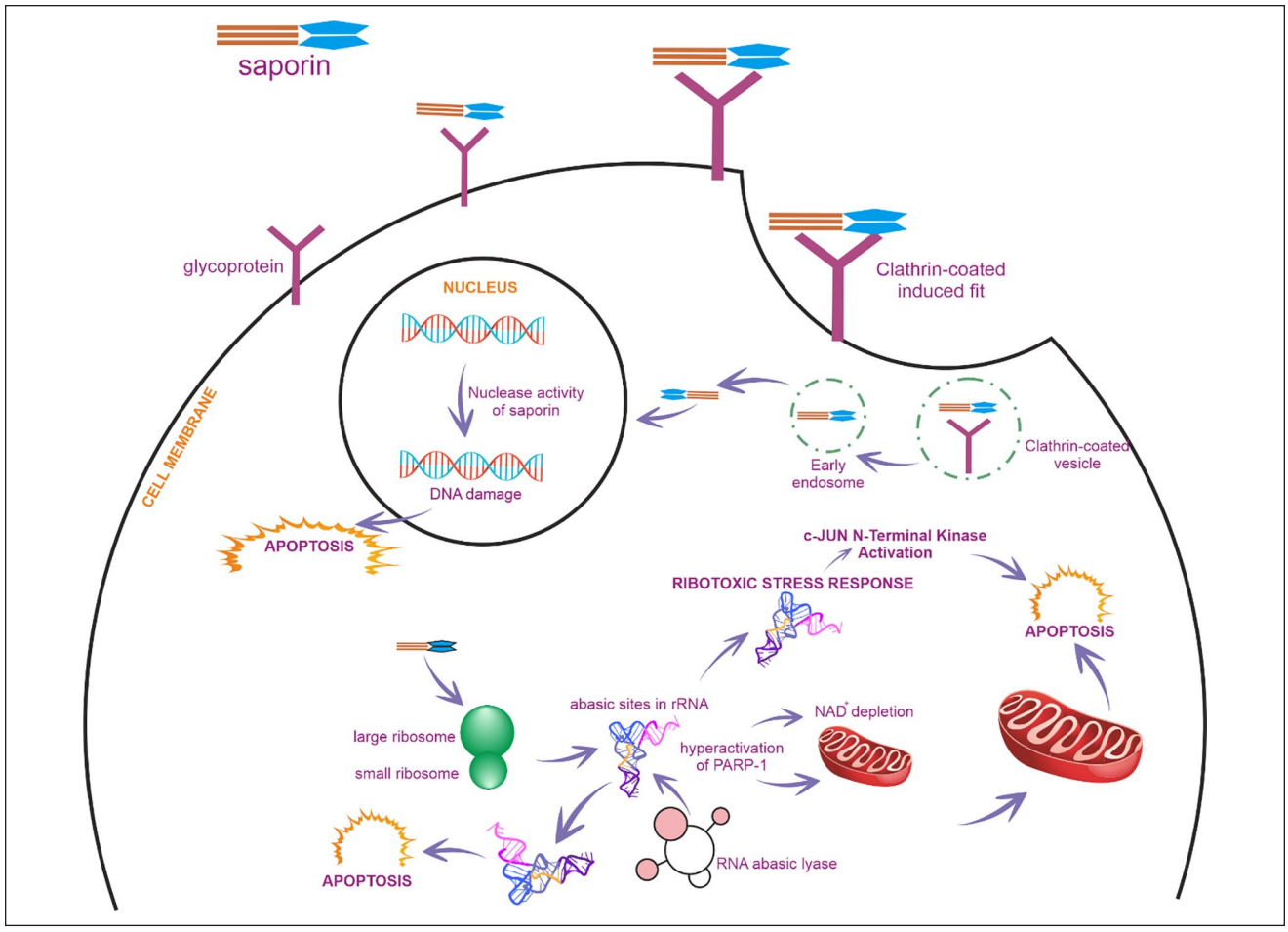

Figure 4. Schematic overview of different mechanisms of action of saporin isoforms.

susceptible to hydrolysis by diluted acids and enzymes (e.g., glucosidases). The *N*-glycosidic bond, particularly that created by nitrogenous bases, is one of the most reactive covalent bonds in DNA and RNA. Saporin-based depurination is a chemical reaction of releasing an adenine residue from nucleic acids and includes the hydrolysis of the *β*-*N*-glycosidic bond between the ribose sugar and nitrogenous base (**Fig. 3**). Hydrolysis reaction commences the protonation of the N3-position of the adenine base that forms a positive charge on the ring backbone and destabilizes the *N*-glycosidic bond. This leads to β-elimination of the nitrogenous base and creation of a ribofuranosyl oxocarbenium ion intermediate. Ultimately, it builds a ribofuranose hemiacetal via nucleophilic attack of H_2O at the position of C1′, and then the hydrolysis process is finalized.[14,15] Abasic sites as a consequence of base depurination on nucleic acids are vital if unrepaired. Abasic RNA structures were found to be more resistant to β-elimination than abasic DNAs,[16]suggesting that abasic sites may modify the 3D structure of an RNA molecule and all its interactions in a cell. Abasic RNA molecules can also be substrate for RNA abasic lyase (RAlyase), which is thought to be concerned with apoptosis.[17] Saporin induces apoptosis through different pathways, including ribotoxic stress response, cell signal transduction, RAlyase activity, and NAD^+ depletion by poly-[adenosine diphosphte (ADP)-ribose] polymerase hyperactivation.

Ribotoxic Stress Response and Cell Signal Transduction

RIPs are a family of highly potent toxins that inhibit protein synthesis by blocking ribosomes. In addition, recent studies suggest that RIPs are also capable of stimulating cell death by apoptosis. Apoptosis is an innate biochemical mechanism by which organisms exterminate undesired cells to maintain healthy cells.[18] One of the earliest reports that RIPs are capable of stimulating cell death came from Griffiths et al. (1987).[19] They discovered that intramuscular injection of abrin and ricin into rats resulted in the formation of apoptotic bodies and cell deaths.

Researchers discovered that the depurination of rRNA by RIP toxins triggered a ribotoxic stress response that activates c-Jun N-terminal kinase (JNK) (apoptosis) and can lead to cell death.[20] Not all forms of RIPs induce a kinase cascade; however, α-sarcin and ricin do, whereas emetine derivatives do not.

Mitogen-activated protein kinases (MAPKs) are a special group of protein kinases that regulate many cellular events, including transcription, translation, proliferation, differentiation, and apoptosis.[21] In humans, members of the MAPK superfamily can be divided into six groups: the extracellular signal-regulated protein kinases (ERK1, 2), JNKs (JNK1, 2, 3), *p*38s (α, β, γ, and δ), ERK5s, ERK3s, and ERK7s. Each member of the MAPKs can be stimulated by different signal transduction cascades concerning environmental stimulants such as ultraviolet radiation, inflammatory signals, and reactive oxygen species.

A recent study demonstrated that destruction to the 3′ end of large rRNA mediates a stress signal that is transduced to a JNK through keeping the ribosome in the *pre*-translocational state.[22] For example, trichosanthin was reported to inhibit HIV replication in T-lymphoblastoid cells and also decrease HIV *p*24 amounts in HIV-infected macrophages.[23] A recent paper discovered that CEP-11004, an effective inhibitor of JNKs, could restrain the antiviral activity of trichosanthin in C8166 cells. The inhibitor alone had no effect on HIV replication, however, and trichosanthin alone significantly inhibited the replication. These results revealed that the anti-HIV action of trichosanthin might be related to MAPK signal transduction downstream from the point of CEP inhibition.[24] In contrast, the antiviral activities of RIPs without specific depurination in rRNA also have been reported. Specifically, trichosanthin was shown to bind to chemokine receptors CCR5, CXCR4, CCR1, CCR2B, CCR3, and CCR4 on the surface of HEK293 cells. Thus, these data showed that the RIP enzyme synergizes the chemokines to trigger chemotaxis and G protein action without specific depurination.[25]

Saporin has been shown to stimulate cell death via apoptosis in a variety of different cell lines, including human peripheral blood B lymphocytes and neutrophils, the Daudi B-cell line, and hematopoietic cell lines HL60 and TF1.[12] Similarly, saporin has been linked to monoclonal antibodies against the CD30 antigen of human lymphocytes to form targeted toxins, all of which induced apoptosis in the $CD30^+$ L540 cell lines.[26] As highlighted in Ref. [27], the cytotoxicity of saporin-6 is administered by its RNA *N*-glycosidase and apoptotic properties and the residues tyrosine-16 and tryptophan-208, respectively.

Apoptosis Induction via NAD^+ Depletion by PARP [Poly-(ADP-ribose) Polymerase] Hyperactivation

Various studies on RIP-induced apoptosis include alternative pathways of apoptosis. Studies showed that hyperactivation of PARP1 enzymes can result in depletion of NAD^+ levels and then can induce mitochondrial damage and apoptosis.[28]

A recent study found that saporin-L2 and saporin-S6 have transforming activity that involves auto-ADP-ribosylation of the PARP enzyme. Moreover, they also revealed that saporins directly depurinate automodified PARP, removing adenine residue from the ADP-ribosyl group.[29,30] These results showed that saporin-L2 and saporin-S6 induced apoptosis via NAD^+ depletion by PARP hyperactivation, an alternative pathway of apoptosis.

Apoptosis Induction via Nuclease Activity. Another pathway of apoptosis stimulated by saporin is nuclease enzyme activity that leads to DNA damage and apoptosis. Saporin-6 has been shown to have deoxyribonuclease (DNase) activity by which it causes DNA damage and, indirectly, apoptosis.[31] In this study, saporin-6 possesses two catalytic activities: RNA *N*-glycosidase and genomic DNA fragmentation activity. These results suggest that saporin is also active on DNA and induces apoptosis.

Apoptosis Induction via RNA Abasic Lyase (RAlyase) Activity. Abasic RNA molecules can also be substrate for RNA abasic lyase (RAlyase), which is thought to be concerned with apoptosis. RAlyase catalyzes a β-elimination reaction, producing a 5′-phosphate end of the 3′ fragment (α-fragment) of the 28S rRNA and α-hydroxy-α, an β-unsaturated aldehyde end of the 5′ fragment. It is likely that RAlyase constitutes a part of a molecular system that leads the apoptosis.[32] Saporin catalyzes the depurination of adenine residue (A_{4324}) from the $GA_{4234}GA$ tetraloop motif within the universally conserved α-sarcin–ricin recognition loop of the eukaryotic 28S rRNA molecule, indicating that saporin may indirectly induce apoptosis by RNA abasic lyase enzyme activity.[9]

Discussion

COVID-19 remains a global leading cause of death, with a still-rising incidence. This is basically caused by the fact that despite the well-known structural properties of SARS-CoV-2, there are still no therapeutic knockout treatments or vaccines. Scientists are racing, however, to discover the best drugs or vaccines to treat COVID-19 disease.

Antiviral activities of RIPs against many animal, human, and plant viruses are well-documented phenomena. Specifically, several RIPs were tested clinically for therapeutic use; for example, trichosanthin from the Chinese medicinal herb *Trichosanthes kirilowii* has been applied in clinical treatment of AIDS.[25]

Saporin, a type I RIP from the soapwort plant, is a potent protein synthesis inhibitor. Catalytically, saporin is a specific *N*-glycosidase and depurinates a specific adenine residue from a universally conserved loop of the major rRNA of eukaryotic cells.[3]

Ribotoxic stress response upregulates the transcription and expression of many proteins through its activation of MAPK in cells, suggesting that saporin may be a regulatory agent in the expression of target viral proteins. Saporin may also inhibit specifically the development of viruses in one or more points in their lifecycle, including infection, integration, replication, and translation. One of the most important features that makes saporin a powerful therapeutic agent against SARS-CoV-2 infection is the unusual stability of the protein and its ability to keep its enzymatic activity. The roles of putative active site residues Tyr^{72}, Tyr^{120}, Glu^{176}, Arg^{179}, and Trp^{208}, and two invariant residues Tyr^{16} and Arg^{24}, are proposed to be important for the structural stability of saporin.[31]

Scientists revealed that saporin has high coil content (>50%), and therefore it is extremely resistant to high temperature, to denaturation by urea or guanidine, to attack by proteolytic enzymes,[33] and to chemical modifications such as derivatization and conjugation procedures.[34]

Due to saporin lacking the cell-binding lectin domain, saporin is much less toxic than most type II RIPs. Throughout the past decade, our research group has been studying quillaic acid or gypsogenin-based *Gypsophila* saponins showing their toxicity-enhancing effects on Sap-SO6 without causing toxicity by themselves (up to 100,000-fold).[35–37]A recent paper reported that soapwort saponins trigger clathrin-mediated endocytosis of both saporin and saporin-associated immunotoxins.[38] Its extraordinary stability and its ability to get into cells by clathrin-mediated endocytosis of saporin or saporin-associated immunotoxins are extremely important for drug delivery.

Saporin's high enzymatic activity, high stability, and resistance to conjugation procedures make it a well-suited tool for immunotherapy approaches. Previous clinical study on saporin-based immunotoxins has shown that several critical issues must be taken into deeper consideration to fully exploit their therapeutic potential.[5]

Saporin-based immunotoxins might be used in the treatment of infectious diseases within the scope of targeted therapy. Here, we focus on how saporin-based targeted toxins may be efficacious therapeutic agents for SARS-CoV-2 infection. Our discussed points suggest that saporin might be a strategic molecule for therapeutic knockout treatments and a powerful candidate of novel drugs for the struggle against SARS-CoV-2 infection.

Declaration of Conflicting Interests

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