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REVIEW

Plant-Derived Natural Non-Nucleoside Analog Inhibitors (NNAIs) against *RNA-Dependent RNA Polymerase* Complex (nsp7/nsp8/nsp12) of SARS-CoV-2

Sreus A. G. Naidu[a], Ghulam Mustafa[b], Roger A. Clemens[c] and A. Satyanarayan Naidu[a]

[a]N-terminus Research Laboratory, Yorba Linda, CA, USA; [b]Department of Biochemistry, Government College University, Faisalabad, Pakistan; [c]Department of International Regulatory Science, University of Southern California School of Pharmacy, Los Angeles, CA, USA

ABSTRACT

The emergence of fast-spreading SARS-CoV-2 mutants has sparked a new phase of COVID-19 pandemic. There is a dire necessity for antivirals targeting highly conserved genomic domains on SARS-CoV-2 that are less prone to mutation. The *nsp12*, also known as the *RNA-dependent RNA-polymerase* (RdRp), the core component of 'SARS-CoV-2 replication-transcription complex', is a potential well-conserved druggable antiviral target. Several FDA-approved RdRp 'nucleotide analog inhibitors (NAIs)' such as remdesivir, have been repurposed to treat COVID-19 infections. The NAIs target RdRp protein translation and competitively block the nucleotide insertion into the RNA chain, resulting in the inhibition of viral replication. However, the replication proofreading function of *nsp14-ExoN* could provide resistance to SARS-CoV-2 against many NAIs. Conversely, the 'non-nucleoside analog inhibitors (NNAIs)' bind to allosteric sites on viral polymerase surface, change the redox state; thereby, exert antiviral activity by altering interactions between the enzyme substrate and active core catalytic site of the RdRp. NNAIs neither require metabolic activation (unlike NAIs) nor compete with intracellular pool of nucleotide triphosphates (NTPs) for anti-RdRp activity. The NNAIs from phytonutrient origin are potential antiviral candidates compared to their synthetic counterparts. Several *in-silico* studies reported the antiviral spectrum of natural phytonutrient-NNAIs such as *Suramin*, *Silibinin* (flavonolignan), *Theaflavin* (tea polyphenol), *Baicalein* (5,6,7-trihydroxyflavone), *Corilagin* (gallotannin), *Hesperidin* (citrus bioflavonoid), *Lycorine* (pyrrolidine alkaloid), with superior redox characteristics (free binding energy, hydrogen-bonds, etc.) than antiviral drugs (i.e. remdesivir, favipiravir). These phytonutrient-NNAIs also exert anti-inflammatory, antioxidant, immunomodulatory and cardioprotective functions, with multifunctional therapeutic benefits in the clinical management of COVID-19.

KEYWORDS

Antiviral; COVID-19 Non-nucleoside analog inhibitors (NNAIs); nsp14-ExoN; Phytonutrients; Redox; Remdesivir; RNA-dependent RNA polymerase (RdRp); SARS-CoV-2

CONTACT A. Satyanarayan Naidu asnaidu@nterminus.com N-terminus Research Laboratory, 23659 Via del Rio, Yorba Linda, CA 92887, USA.

Introduction

'Mutation is the driving force of evolution' – it is the variation upon which natural selection builds its biological diversity during evolution (1). RNA viruses have high mutation rates—up to a million-fold higher than their hosts—such extreme genetic rearrangement correlates with enhanced adaptability and virulence, a trait considered beneficial for the virus (2). Coronaviruses (CoVs) are potential cross-species pathogens with unique ability to mutate, adapt, transmit into new host species, and cause severe clinical outcomes as witnessed during the *Severe Acute Respiratory Syndrome* (SARS) in 2002, the *Middle East Respiratory Syndrome* (MERS) in 2012, and the ongoing *Coronavirus Disease 2019* (COVID-19), a challenge to global health (3, 4). The SARS-CoV and MERS-CoV were also appeared as epidemics in China, South Korea and United Arab Emirates which were emerged by CoVs specifically alpha- and beta-coronaviruses (5). The life cycle of CoVs consists of four steps. In the first step the spike (S) protein mediated cellular fusion occurs following its interaction with *dipeptidyl peptidase 4* (DPP4). In next stages the replicase enzyme is expressed which helps in the replication and then the transcription and finally the release of new virions (6). *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2), the etiological agent for COVID-19, is a single-stranded positive-sense RNA virus. Its life cycle is characterized by short replication times with high viral yields. In general, the CoV replication machinery is highly error-prone without correction systems; therefore, these viruses are prone to several genetic alterations during an infectious life cycle (7). Accordingly, CoVs evolve at a rapid pace, pose high risk of zoonotic transmission, and frequently develop resistance to therapeutics as well as evade vaccine-induced immunity (2, 8). This genomic advantage makes the SARS-CoV-2 pathogen a major challenge to develop antiviral strategies and control COVID-19. Due to this genomic advantage, SARS-CoV-2 is considered a challenge for developing any effective antiviral strategies.

Emergence of SAR-CoV-2 variants: The emergence of fast-spreading SARS-CoV-2 mutants has sparked a new phase of COVID-19 pandemic (9). Prevalence of SARS-CoV-2 variants have an evolutionary advantage over their ancestral (wild) type strains with could pose a major threat to global health (10). Clinical consequences may include: i) rapid viral transmission, ii) increased disease severity with high mortality rates, iii) escape detection by current diagnostic tests, iv) decreased susceptibility or resistance to antiviral drugs, and v) evasion of natural or vaccine-induced immunity. Among these risk factors, the ability of SARS-CoV-2 to evade vaccine-induced immunity—is an extreme concern; since a large population has been vaccinated and the herd immune pressure may drive genomic adaptation to evolve novel viral variants as 'escape' mutants. Such genetic drift in tandem with the evasion of immune recognition, certain sub-strains with different mutations could compromise effectiveness of vaccines against COVID-19 (11).

Based on epidemiological update by the WHO, five SARS-CoV-2 variants – *Alpha*-(B.1.1.7); *Beta*-(B.1.351); *Gamma*-(P.1) *Delta*-(B.1.617.2), and *Mu*-(B.1.621) have been identified since the beginning of the pandemic, all with mutations in the spike (S) protein (12, 13). The S-protein has been the most preferred target for COVID-19 vaccine development. However, emergence of variants with mutations in S-protein may disrupt several vaccine development protocols. Unfortunately, there is no effective 'multivalent vaccine' yet that could provide immune protection against multiple

SARS-CoV-2 variants. This global scenario highlights the dire necessity for identification and characterization of specific and potent antivirals that target highly conserved domains, which are less likely to mutate in the SARS-CoV-2 genome (14). Two specific enzymes of SARS-CoV-2 replication cycle: the *main protease* (M^{Pro}) during proteolytic activation, and *the RNA-dependent RNA polymerase (RdRp)* during transcription are considered as potential conserved druggable antiviral targets (15).

Conserved viral replicase enzymes as druggable targets: The RdRp activity in viral transcription and replication has been recognized as an attractive target to develop antiviral strategies against COVID-19 (16, 17). SARS-CoV-2 shares less genetic similarity with SARS-CoV (~79%) and MERS-CoV (~50%); however, the respective RdRps of these CoVs are highly conserved, suggesting that RdRp is a robust antiviral target for COVID-19 control (18, 19). Since RdRp is a viral enzyme without any host protein homologs, specific SARS-CoV-2 RdRp inhibitors with high potency and fewer side effects could be developed for COVID-19 management (20)

Several FDA-approved RdRp nucleotide analog inhibitors (NAIs) with established track record to treat RNA viral pathogens have been repurposed, which are currently undergoing stringent tests for safety/efficacy in the treatment of COVID-19 infections (21). Furthermore, high through-put screenings and *in-silico* studies are elucidating the anti-RdRp activity of several FDA-approved non-nucleotide inhibitor (NNAI) drugs (16). Unfortunately, the first open label, randomized, controlled trials with popular repurposed NAI drugs (i.e. remdesivir, favipiravir, lopinavir-ritonavir, ribavirin, sofosbuvir, etc.) showed poor efficacy against SARS-CoV-2 infections (22).

Given the high morbidity and mortality of COVID-19 pandemic and lack of effective antiviral drugs, the repurposing of traditional antiviral phyto-therapeutics and natural bioactives is a promising strategy (5). Large-scale phenotypic screening of natural compound libraries could isolate potential RdRp inhibitors through molecular docking and molecular dynamic simulations, *in-silico* ADMET (i.e. absorption, distribution, metabolism, excretion, and toxicity) and drug-likeness prediction analyses. Effective phytonutrient inhibitors could be identified and compared with antiviral prescription drugs (i.e. remdesivir), based on specific interactions of their ligand structures with core catalytic domains of SARS-CoV-2 RdRp. This review is aimed at collating data on potential role of such natural bioactive compounds derived from medicinal herbs and phytonutrient extracts in blocking the SARS-CoV-2 RdRp enzyme activity and inhibit the viral replication cycle. This information may help guide the discovery process to formulate antiviral interventions from potential natural plant-based bioactives for COVID-19 control.

Genomic organization of SARS-CoV-2 replication machinery

The 30-kb genome of SARS-CoV-2 consists of 14 *open reading frames* (ORFs) that encode at least 27 proteins (23, 24). The ORF1ab region at the 5′ end transcribes a polyprotein that cleaves into 16 *nonstructural proteins (nsp 1-16)* to create a *replicase/transcriptase complex* (RTC).

Viral replicase/transcriptase complex (RTC)

The *nsp12*, also known as the *RNA-dependent RNA-polymerase* (RdRp), is the core component of SARS-CoV-2 RTC that operates replication and transcription of the viral RNA (17, 25, 26). The *nsp12*/RdRp has limited or no catalytic activity; however, when it forms a complex with specific viral proteins, a significant polymerase function is acquired (27, 28). Accordingly, the *nsp12*/RdRp forms a complex with two cofactors *nsp7* and *nsp8* for structure-functional support (29); *nsp9,* the dimer forming RNA-binding protein; *nsp10*, the cofactor in viral replication; and *nsp14*, the *exoribonuclease (ExoN)* (23, 29–31). The *nsp14/ExoN* serves as a CoV-specific intrinsic mechanism (absent in other RNA viruses) that effectively removes incorporated nucleoside analogs and restores the viral polymerase function [Neogi *et al.* 2020] (32). This proofreading ability of *nsp14/ExoN* is a setback for NA inhibitors such as remdesivir to block the RNA replication in SARS-CoV-2. Although the interaction between all these CoV-*nsp*s is important for optimal replication of the viral RNA, the *nsp7-nsp8-nsp12* complex constitutes the minimal core for RTC function (33). After host cell invasion, the viral genomic RNA serves as a template, and reprograms the host metabolism (including the protein synthesis machinery) to facilitate the translation of RdRp enzyme. Subsequently, the RdRp polymerizes a high quantity of nucleotides to support an uninterrupted viral replication.

RNA-dependent RNA polymerase (RdRp)/nsp12

The *RNA-dependent RNA polymerase* (RdRp)/*nsp12* plays a central role in the replication and transcription cycles of SARS-CoV-2 *via* catalytic synthesis (polymerization) of the viral RNA (23). Due to its high evolutionary stability, RdRp has no counterpart in human cells; therefore, represents a unique antiviral target (34,35). The core structural features of RdRps are highly conserved across several viral species, despite the divergence in their sequences (36). The protein sequence homology between SARS-CoV-2 and SARS-CoV RdRp is about 96% and structural disparities exist only in the catalytically inactive domains (37).

Structure-function of SARS-CoV-2 RdRp complex

The RdRp complex of SARS-CoV-2 consists of a *nsp12* core catalytic unit, a *nsp7-nsp8 (nsp8-1)* heterodimer, and an additional *nsp8* subunit (*nsp8-2*) (23, 38). The RdRp structure has a 'polymerase' domain (residues Ser_{367} to Phe_{920}) that resembles a cupped 'right hand' and a unique *nidovirus RdRp-associated nucleotidyltransferase* (NiRAN) domain (residues Asp_{60} to Arg_{249}), where both interact through an 'interface' domain (residues Ala_{250} to Arg_{365}) (23, 39). This domain also consists of the 'fingers' subdomain (residues Leu_{366}-Ala_{581} and Lys_{621}-Gly_{679}), the 'palm' subdomain (residues Thr_{582}-Pro_{620} and residues Thr_{680}-Gln_{815}), and the 'thumb' subdomain (residues His_{816}-Glu_{920}) (40). The 'finger' subdomain stabilizes the template RNA and facilitates specific interactions with major residues in the active enzymatic site (41). The 'thumb' subdomain harbors residues that pack against the template RNA and stabilizes the *nucleoside triphosphates* (NTPs) on the template (42). This subdomain translocates the template RNA after polymerization and accommodates any conformational rearrangements. There is an

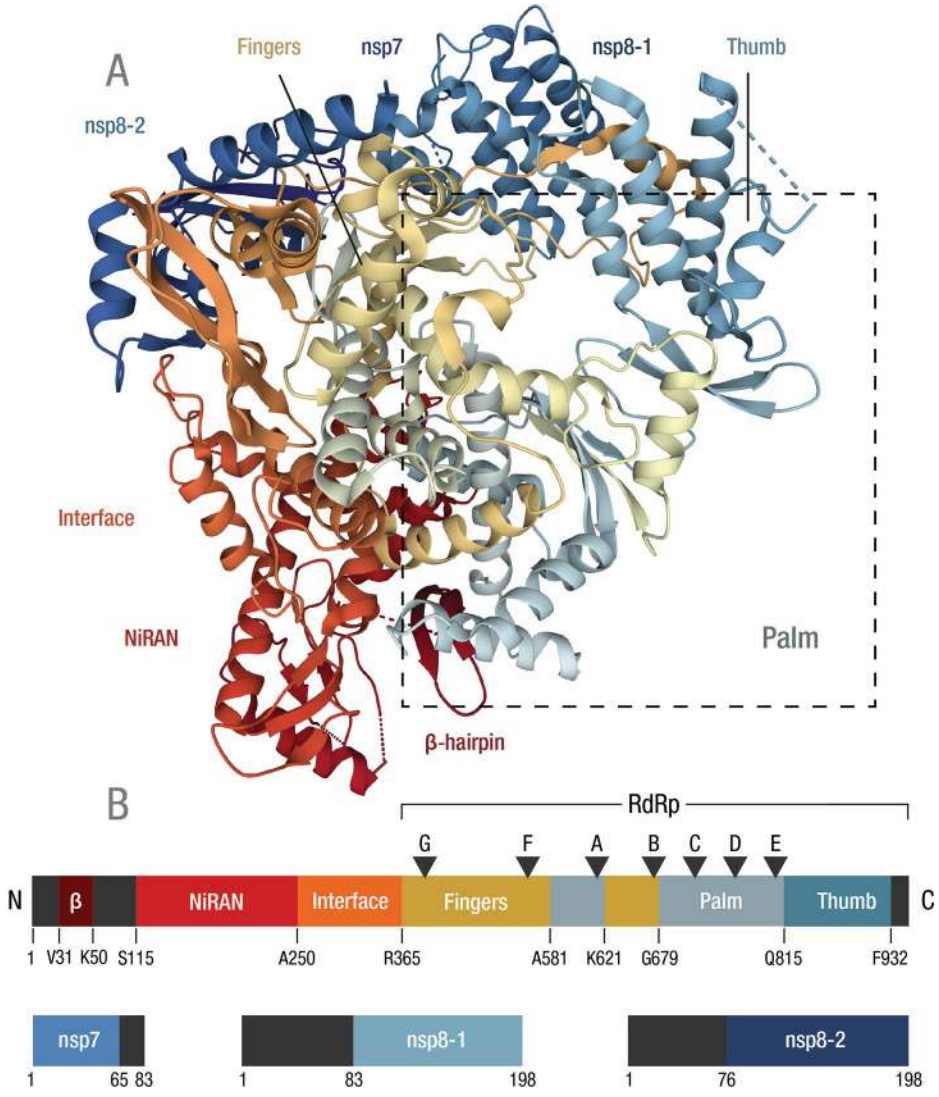

Figure 1. **The RdRp complex of SARS-CoV-2** consists of a *nsp12* core catalytic unit, a *nsp7-nsp8 (nsp8-1)* heterodimer, and an additional *nsp8* subunit (*nsp8-2*). The RdRp structure resembles a cupped 'right hand' and a unique NiRAN domain, both interacting *via* an 'interface' domain that consists of subdomains – the 'fingers', the 'palm', and the 'thumb'. An N-terminal β-hairpin is located between the 'palm' and the 'NiRAN' domain. The active enzymatic site of the RdRp is formed by seven conserved catalytic motifs, from A to G.

N-terminal β-hairpin (residues Asp_{29} to Lys_{50}) between the 'palm' subdomain and the 'NiRAN' domain that help stabilize the RdRp structure (Figure 1A) (23).

Core catalytic site of SARS-CoV-2 RdRp complex

The active enzymatic site of RdRp is formed by seven conserved catalytic motifs, from A to G. Five of these motifs (A-E) are in the 'palm' subdomain and the other

two (F and G) are located in the 'finger' subdomain (Figure 1B). Motif A (residues Thr_{611} to Met_{626}) houses the catalytic motif 'DX2-4D', in which the first aspartic acid (Asp_{618}) residue is common to most viral polymerases. The flexible loop in motif B (residues Gly_{678} to Thr_{710}) serves as a hinge that undergoes conformational modification with template RNA during the substrate binding (43). Motif C (residues Phe_{753} to Asn_{767}) with its catalytic motif SDD (residues Ser_{759} to Asp_{761}), is essential for binding to metal ions (44, 45). The aspartate residues Asp_{760} and Asp_{761} are involved in the coordination of two magnesium (Mg^{2+}) ions at the catalytic center (38). These conserved aspartic acids in the catalytic motif DX2-4D and SDD motifs regulate the polymerase activity. Motif F (residues Leu_{544} to Val_{557}) interacts with the phosphate group of incoming NTP, and its side chain (residues Lys_{545} and Arg_{555}) directs the NTPs to specific positions for catalysis. Motif G (residues Asp_{499} to Lys_{514}) interacts with the template strand and directs the RNA template to the active catalytic site. The active site has a highly conserved architecture of α-helices, antiparallel β-strands, RNA recognizing motifs (46) and the enzymatic catalysis requires both aspartates and divalent metal ions (47).

The RdRp mediates a template-directed RNA synthesis for SARS-CoV-2 replication, where entry of the RNA template, the NTP, and exit of the nascent RNA strand, all converge into a positively charged central cavity (23, 48). The NTP entry channel is separated by the hydrophilic motif F (residues Lys_{545}, Arg_{553}, and Arg_{555}) (23). The RNA template enters from a channel between motifs F and G into the active site, formed by motifs A and C, and held by motifs B and D (28).

In CoVs, RdRp catalyzes the synthesis of the RNA genome using the (+)RNA strand as a template to produce a complementary (−)RNA strand starting from 3′-poly-A tail (19). There are two plausible molecular mechanisms to initiate the genomic RNA synthesis by RdRp: i) the primer-independent *(de novo)* synthesizes the genomic RNA by forming a phosphodiester bond with 3′-hydroxyl group linked to 5′-phosphate group of the adjacent nucleotide (49); and ii) the primer-dependent synthesis generates a new RNA complementary to the template with base pairing under the guidance of either an oligonucleotide or a protein primer (50). Furthermore, four cellular ribonucleotide triphosphates (rNTPs), ATP, GTP, CTP, and UTP provide the template substrates, which are recognized by RdRp. Divalent metal ions magnesium (Mg^{2+}) and manganese (Mn^{2+}) act as essential cofactors in the polymerization reaction and coordinate the catalytic aspartates to promote reactivity with rNTPs (51).

Inhibitors of SARS-CoV-2 RdRp complex

Upon infection, SARS-CoV-2 releases its RNA into the host cell and reprograms host metabolic machinery to replicate its viral genome and produce viral progeny to infect new cells. In a host-targeted approach, the inhibition of specific enzymes or factors of the infected cell may block viral propagation. A broad-spectrum antiviral activity could be achieved with this approach, since the host-based cellular processes hijacked by most viral pathogens are somewhat similar (52). Virus-specific genomic/protein components are critical for viral life cycle. Also, the host cellular machinery that viral pathogen has reprogrammed during infection process is fundamental for its

propagation. Therefore, both the viral-targeted interventions as well as the host-targeted prevention strategies cumulatively define the therapeutic efficacy (53). A viral infection could be controlled with compounds that either directly or indirectly block the viral nucleic acid synthesis, or that drive the viral mutation rate over a threshold to prevent viral replication (often referred to as 'lethal mutagenesis') (3, 54).

Direct-acting antiviral agents (DAAs) represent a class of compounds that target viral proteins, i.e. nucleobases, nucleoside or nucleotide analogs that after activation of their corresponding non-natural *nucleoside 5′-triphosphate* (NTP) form *via* host cell pathways – are inserted 'erroneously' into the viral genome by the viral polymerase. However, the DAAs pose several inherent limitations, including their narrow-spectrum antiviral activity and vulnerability to drug resistance.

Host-targeted antiviral (HTA) compounds block cellular pathways that generate natural NTPs, thereby deprive viral polymerases of their natural substrates – a therapeutic strategy widely practiced in anticancer treatment. The HTA strategies exhibit a broad-antiviral spectrum independent of viral genetic control; therefore, possess a higher genetic barrier to drug resistance compared to the DAAs (55).

Molecular machinery involved in every stage of viral replication cycle has been targeted for drug development (56). In particular, the RdRp shares similar catalytic mechanisms and displays active site conservation among different positive-sense RNA viruses; therefore, it is a potential drug target (42). Evolutionary studies of whole-genome sequences of SARS-CoV-2 represent high degree identity (>90%) with other SARS viruses. Targeting the RdRp active sites, Asp_{760} and Asp_{761}, by antiviral drugs could be a potential therapeutic option for inhibition of CoV RdRp, and thereby the viral replication (57). Given the 98% amino acid similarity of the SARS-CoV and SARS-CoV-2 RdRps, repurposing of these enzyme inhibitors from the SARS outbreak may also effectively inhibit the SARS-CoV-2 polymerase (58).

Redox biochemistry of viral RdRp-ligand (inhibitor) interactions

Charge-induced conformational modifications: The polymerase activity of RdRp complex is regulated by switching between various structural conformations. The modular nature of RdRp complex to shift between various conformations and energy states is governed by redox transitions of the milieu and the interaction of core catalytic site of the enzyme protein with its ligand (substrate or inhibitor). Redox transitions are intrinsic to several biological systems that play a regulatory role in the transmission of cellular signals (59). Essentially, a functional RdRp complex requires both structural rigidity and flexibility to allow optimal interaction of active residues in its catalytic binding site with the ligands (substrate or inhibitor). The redox transitional states support the bioenergy demand for conformational association and dissociation of molecules, a prerequisite for functional outcomes of a protein–ligand interaction (59, 60).

Hydrogen (H)-bonds are responsible for secondary and tertiary structural protein motifs. In protein environments, redox (H^+ proton transfer) reactions occur along polar or charged residues and isolated water molecules. These compounds consist of H-bond networks that serve as redox sensors; therefore, an in-depth understanding of redox mechanism(s) is essential to elucidate H-bond energetics in protein-ligand interactions

(61). Since, protons (H^+) are redox sensors, the formation of H-bonds between a ligand and a protein motif explains the binding affinity of an inhibitor toward the RdRp protein target in molecular dynamic simulations; accordingly, more number of H-bonds reflect a stronger interaction (62). The active site of the SARS-CoV-2 RdRp is formed by conserved polymerase motifs (A-G), where the motifs A and C have the divalent-cation-binding amino acid Asp_{618}, and the catalytic residues Ser_{759}-Asp_{760}-Asp_{761}, respectively (23). The cellular redox state governs the van der Waals and π-Sulfur interactions with amino acid residues of the catalytic center and the NTP entry channel of the SARS-CoV-2 RdRp-RNA complex (59, 63).

Almost all viruses have polymerases that play a pivotal role both in viral replication and in the genetic evolution of viral RNAs. After binding to an RNA template and incorporating 5′-triphosphate ribonucleosides, viral polymerases synthesize an RNA copy according to the Watson-Crick base-pairing rules. The copying process sometimes may deviate from both the base-pairing rules specified by the template and the natural ribose selectivity and, thus, the process is error-prone due to the intrinsic (in)fidelity of viral polymerases (64). This genomic infidelity increases the possibilities for a polymerase enzyme to accept modified nucleotide analogs as substrates. Accordingly, nucleoside analogs that inhibit polymerases have emerged as important class of antiviral agents (65).

Nucleotide analog inhibitors (NAIs)

Nucleotide analog inhibitors (NAIs) exhibit spectral antiviral activity and target the active site of viral RdRps. Many NAIs are active against various CoV RdRp genotypes, an indication that the catalytic active sites bound by this type of inhibitors are highly conserved (21). The NAIs target RdRp protein translation and competitively block the nucleotide insertion into the RNA chain, resulting in the inhibition of viral RNA replication (16, 66). Classical antiviral NAIs are obligate RNA chain terminators, and the lack of a reactive 3′-hydroxyl (3′-OH) group makes these compounds potent RdRp inhibitors (67). NAIs of RNA viruses, developed mostly as prodrugs, require a phosphorylation step, convert into a triphosphate (TP) form to target the highly conserved active site of RdRp (16). NAIs against RdRp are classified into three major categories: i) pyrimidine nucleoside inhibitors, ii) purine nucleoside inhibitors, and iii) miscellaneous nucleoside inhibitors (Figure 2).

Pyrimidine nucleoside (CTP/UTP/TTP) inhibitors: Cytidine (C)-NAIs are metabolized to both CTP and UTP through cytidine deaminase activity. The UMP derivatives are also potent direct-acting antiviral agents (DAAs). The thymine (TTP)-NAIs display pangenotypic activity with a high *in vitro* barrier to resistance (16, 68).

Purine nucleoside (ATP/GTP) inhibitors: Adenine (A)-NAIs interfere with viral RdRp activity; however, their monophosphorylation kinetics are slow. The use of a parent nucleoside modified with monophosphate could elevate the intracellular NTP levels. Remdesivir is an A-NAI that effectively bypasses this rate-limiting step of monomer phosphorylation (69, 70). Remdesivir occupies the central position of the catalytic active site and forms a covalent bond with the primer RNA strand, and blocks replication by non-obligate RNA chain termination (23, 71). Remdesivir in triphosphate

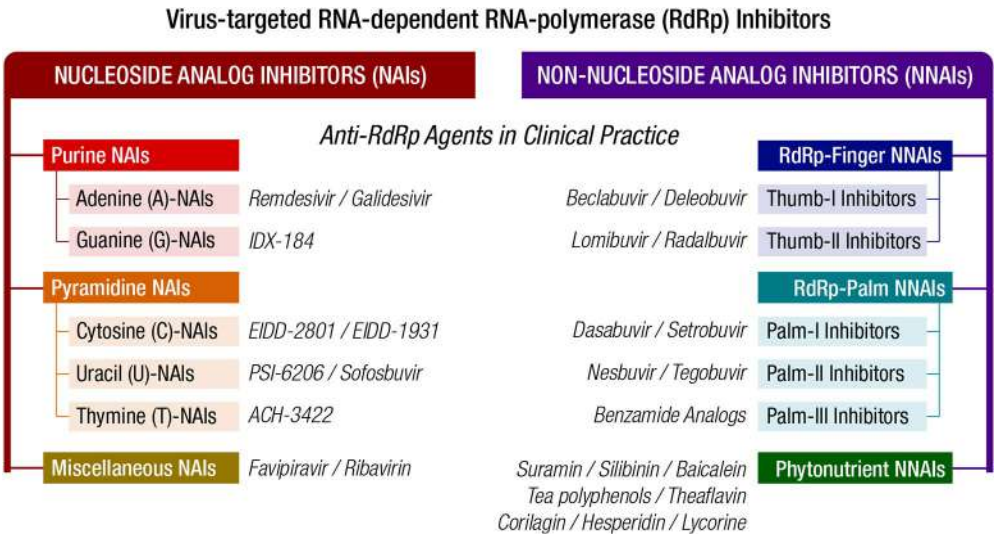

Figure 2. Virus targeted RNA-dependent RNA-polymerase (RdRp) inhibitors.

(TP) form incorporates into RNA, replaces the ATP binding with counterpart template uridine (U) and blocks the replication of SARS-, MERS-, and SARS-CoV-2 pathogens (34). Guanine (G)-NAIs are preferentially cleaved by hepatic enzymes to form TP, which selectively inhibit NS5B polymerase but does not inhibit human polymerases *a, b* or *g* (16).

Limitations in the development of NAI-based interventions: Since the genomic size of CoVs are unusually large, their replication is more complex compared to other RNA viruses. Replication of large RNA requires an evolved RTC machinery, which has been achieved through the structural combination of *nsp7+nsp8+nsp12 (RdRp complex)* [Gorbalenya 2006] (28, 72). The large-sized RNA replication also involves *nsp14*, a *3′ to 5′ exoribonuclease (nsp14-ExoN)* to remove any mis-incorporated nucleotides (i.e. NAIs) to minimize high error rates typical of viral RNA polymerases to prevent negative fitness effects [Ferron *et al.* 2018, Ogando *et al.* 2019]. This replication proofreading function of *nsp14-ExoN* has significantly hampered the development of NAI-based drug development against COVID-19. The *nsp14-ExoN* excises the incorporated NAIs and provides resistance to SARS-CoV-2 against many anti-RdRp inhibitors (32). The *nsp14-ExoN* confers up to 20-fold increase in replication fidelity compared to other RNA viruses and responsible for developing resistance to many NAIs against CoV pathogens (73). For example, the anti-RdRp efficacy of the NAI drug remdesivir could be compromised by the ExoN that abrogates the NAI-induced lethal mutagenesis and prevents the incorporation of any mismatched nucleotides into the viral genome (29, 30). A combination therapy with an NAI drug (i.e., favipiravir, remdesivir, ribavirin, or galidesivir) with a phytonutrient inhibitor (i.e. conivaptan or hesperidin) of ExoN domain of SARS-CoV-2-*nsp14* could be effective in increasing the efficacy of the RdRp inhibitors (74).

Another limitation in the NAI development is the presence of intracellular natural nucleotide triphosphates (NTPs) at high levels. Therefore, the triphosphorylated (TP)

form of NAI must compete with the highly concentrated cellular pool of NTP for antiviral activity. Accordingly, the effective dosage of NAI needs to be adjusted high, which may increase the risk of drug toxicity (16, 17).

Non-nucleoside analog inhibitors (NNAIs)

Non-nucleoside analog inhibitors (NNAIs) bind to allosteric sites on the surface of polymerase enzyme, change its spatial conformation; thereby, exert antiviral activity by altering interactions between the enzyme substrate and the active core catalytic site of the RdRp (75). Such inhibitors neither require metabolic activation (unlike triphosphorylation of NAIs) nor compete with intracellular pool of NTP for anti-RdRp activity. The structures of NNAIs, especially of the phytonutrient category are diverse, which makes these natural bioactive compounds as potential antiviral candidates compared to their synthetic counterparts (i.e. becklabuvir, lomibuvir, nesbuvir). However, the structural variability and non-conservation of adjacent allosteric sites may also allow CoV pathogens to develop resistance against allosteric site inhibitors (16, 17, 76). NNAIs against RdRp are classified into three major categories: i) RdRp-Finger NNAIs, ii) RdRp-Palm NNAIs, and iii) Phytonutrient NNAIs (Figure 2).

Thumb Inhibitors: Benzimidazole and indole compounds are prominent *Thumb I inhibitors* that bind *via* hydrophobic interactions and salt bridge/H-bonds between the ester group or carbonyl group of the compound and the guanidine group of the amino acid residue (77). *Thumb II inhibitors* are dihydropyrones, thiophene carboxylic acids and pyranoindole compounds with lipophilic groups that occupy the shallow grooves formed by the amino acid residues Leu_{419}, Tyr_{477} and Trp_{528} in the thumb II site. The acidic groups of these compounds generate H-bonds with the backbone amide bonds with amino acid residues Ser_{476} and Tyr_{477} (78).

Palm Inhibitors: N-aryl uracil (U) analogs, benzothiadiazines and acyl pyrrolidines are *Palm I inhibitors* that bind 'palm I' site located between the active site of the RdRp enzyme and the 'palm II' site which contains a deep hydrophobic pocket (79). *Palm II inhibitors* such as benzofurans bind to the 'palm II' site mainly composed of a large hydrophobic pocket in the palm area. The palm II site inhibitors differ from other NNAIs in that they exhibit potent activity against genotypes 1 to four and NS5B polymerase (80).

RNA-viruses, such as the CoVs, have short generation time (81); therefore, require high amounts of NTPs from host metabolic reserves to sustain their high replication rate. Any deprivation or imbalance in the cellular NTP pool could severely compromise the viral genome synthesis and inhibit viral replication. However, a balanced inhibition of the cellular pathway is critical to maintain viability of the host cells while blocking the viral replication (55, 82).

The ongoing COVID-19 pandemic has globally initiated an extensive high through-put screening for potent plant-based natural NNAIs against SARS-CoV-2 RdRp enzyme. Potent and promising bioactive phytonutrients are being identified by *in silico* molecular docking studies and evaluated for viral protein-ligand ('inhibitor') binding affinities to SARS-CoV-2 gene-encoded molecular targets. Finally, taking advantage of nontoxic

properties or absence of side effects in human applications, phytonutrients are directly compared for functional efficacy with prominent antiviral drugs (i.e. 'remdesivir', the US-FDA approved drug for treatment of severe COVID-19 cases), and such promising natural bioactive compounds derived from plant-sources are currently subjected for human clinical testing worldwide.

Phytonutrient NNAIs against the SARS-CoV-2 RdRp complex

Phytonutrients and bioactives from medicinal herbs that are extensively characterized, could provide a new direction in the development of novel anti-COVID-19 prophylactics and therapeutics (83, 84). Notably, several efficient drugs designed in the past are based on the structure of natural compounds with desired biological activities. Almost half the drugs approved between 1981 and 2014 by the US-FDA, were derived from or mimicked a natural compound (85). Based on high diversity, complex molecular structure(s), broad-spectrum activity including inhibition of viral transcription/translation, as well as considering their overall safety and non-cytotoxicity, phytonutrients could be potential candidates for anti-COVID-19 interventions (86, 87). During this COVID-19 pandemic, several clinical practices have integrated complementary or traditional medicine, as adjuvant therapeutic protocol with the Western medicine (88, 89). Herein a few promising phytonutrient RdRp inhibitors are described, that deserve further attention and evaluation as possible NNAI intervention(s) for COVID-19 management (Figure 3).

Suramin

Suramin, a phytonutrient naturally found in tea from eastern white pine tree needles (*Pinus strobus*) has been widely used to treat African sleeping sickness and river blindness for over a century (90). Suramin is a polyanionic compound that binds to positively charged patches in DNA or RNA binding proteins (91). Also, suramin is a potent antiviral agent with wide range of effects, including inhibition of viral attachment, viral entry, and viral release from host cells *via* interactions with viral capsid proteins (92).

Suramin inhibits SARS-CoV-2 infection in cell cultures by blocking cellular entry of the virus (93). In a recent study, a dosage of 8–32 μM suramin nearly abolished the elongation of the RNA primer strand compared to 100–1,000 μM dosage of remdesivir, for a comparable degree of inhibition. Also, 100 μM of suramin totally blocked formation of RdRp–RNA complex compared to a 5 mM dosage of remdesivir; thus, the RdRp inhibition potency of suramin is at least 20-fold more than remdesivir (38)

Suramin is a direct and potent NNAI of the SARS-CoV-2 RdRp enzyme. The cryo-structural analysis of the viral RdRp-suramin complex revealed two binding sites. One site that directly blocks binding of the RNA template strand and the other site clashes with the RNA primer strand adjacent to the RdRp catalytic site, both result in RdRp inhibition (94). Structural comparison of the RdRp–suramin complex with the RdRp-remdesivir complex revealed that the mode of RdRp inhibition of the phytonutrient-NNAI is different from the pharmaceutical-NAI. If the base position of

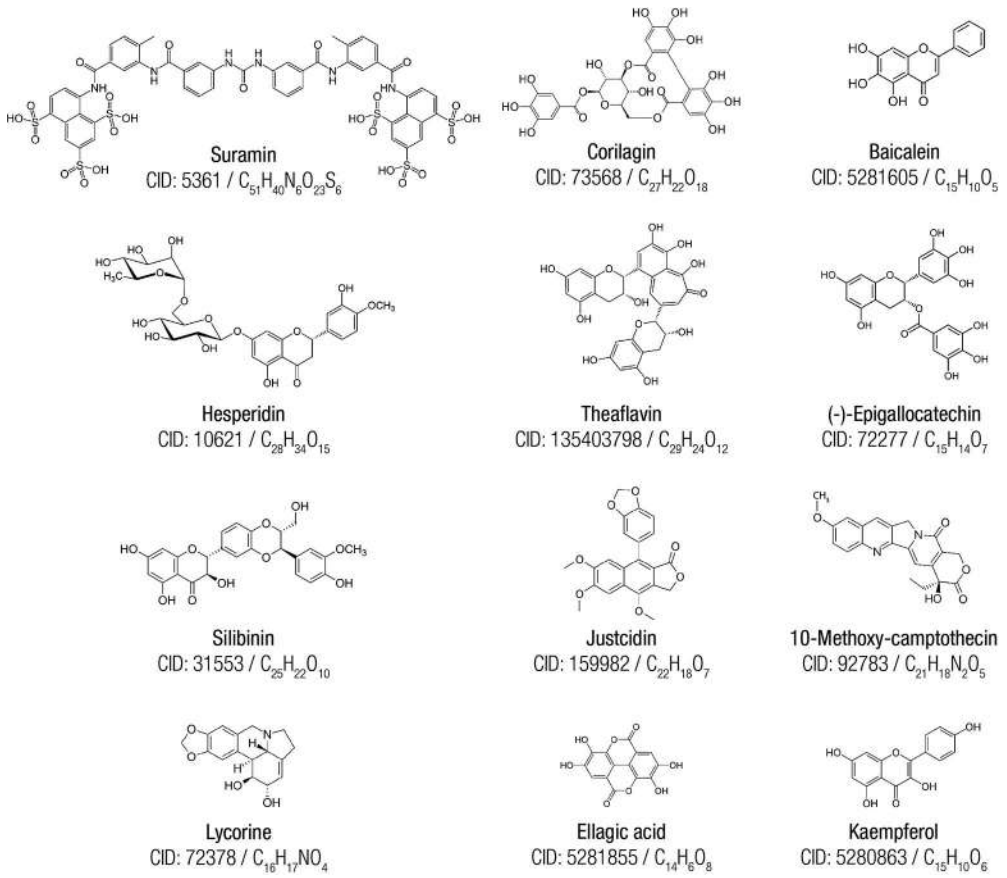

Figure 3. Structures of phytonutrient non-nucleoside analog inhibitors (NNAIs) against SARS-CoV-2 RdRp enzyme. The most important structural attributes of potential anti-SARS-CoV RdRp NNAIs include hydrogen (H)-bonding capacity for the 2′ and 3′ groups of the sugar ring and C3′ endo sugar puckering, and the absence of a hydrophobic binding pocket for NNAIs (76).

remdesivir is defined as +1 position, then the first suramin molecule occupied the space of −1 to −3 positions of the RNA template strand. The second suramin molecule at the active site occupies the space of the primer strand ranging from −4 to +1 positions. Binding of two suramin molecules block the RNA template–primer duplex interaction with the active site as well as the entry of nucleotide triphosphate into the catalytic site, resulting in direct inhibition of the RdRp catalytic activity. In a recent UK study, suramin (50 μM) demonstrated total inhibition of RNA duplex formation. Suramin and its derivatives also blocked the activity of both SARS-CoV-2 RdRp and *nsp13/helicase* in cell-based assays (38, 72).

Silibinin

Silibinin, the flavonolignan component of the silymarin extract obtained from the 'milk thistle' herb (*Silybum marianum*) (95), directly inhibits the SARS-CoV-2 RdRp enzyme, as well as reduces the *signal transducer and activator of transcription* (STAT3)-induced

lung and systemic inflammation in the infected host (96). The dual ability of silibinin to target both the viral replication machinery and the host cytokine storm provides a strong rationale for the clinical testing of this phytonutrient for COVID-19 management.

Docking simulations of silibinin with SARS-CoV-2 RdRp complex yielded eleven possible clusters, of which three have occupied the RNA template tunnel of the viral polymerase enzyme. Based on the free binding energies (ΔG up to −9.4 kcal/mol), silibinin clusters with highest affinity were in the low (~100 nmol/L) nanomolar range, like that of remdesivir. Silibinin interacted with well-characterized catalytic aspartate residues (i.e. Asp_{618}, Asp_{623}, Asp_{760}, Asp_{761}) as well as with other key residues (Arg_{555}, Val_{557}, Thr_{680}, Ser_{682}, Asn_{691}) involved in the RdRp interaction with the entry path of RNA template/NTPs (96)

The macrophage- and neutrophil-dependent activation of STAT3 plays a critical role in acute lung inflammation and lung tissue damage (97). Accordingly, increased alveolar epithelial death and phosphorylated STAT3 are common phenotypic traits in COVID-19 with *acute respiratory distress syndrome* (ARDS) (98). Therefore, STAT3-mediated cellular modulation of pulmonary inflammation could relieve the severity of ARDS symptoms in COVID-19 patients (36). As a potent inhibitor of STAT3 and a master regulator of inflammatory cytokine signaling and immune response (97), Vendura et al. (100), silibinin can be expected to limit the cytokine storm and T-cell lymphopenia in severe COVID-19 patients. Pretreatment with silibinin inhibits LPS-induced recruitment of airway inflammatory cells as well as the production of specific pro-inflammatory cytokines (i.e. IL-1β, TNFα), which may provide protection against lung injury (101). Silibinin may protect damaged lung tissue by regulating the inflammatory cell cascade (i.e. macrophages, T-cells, and astrocytes) during tissue repair (102). Thus, silibinin may also act as an immune therapeutic to alleviate cytokine storm and T-cell lymphopenia in severe COVID-19.

In earlier clinical trials with HIV-infected patients, co-administration of silibinin with multiple antiretrovirals (i.e. darunavir-ritonavir) showed safety and well tolerance profile without any need for dose adjustment with anti-viral therapy (103). Currently, a cohort study at the Catalan Institute of Oncology in Catalonia, Spain will evaluate the oral bioavailability, single and multidose pharmacokinetics, and safety of a 'milk thistle' (Eurosil85) formulation for clinical management of COVID-19. This randomized, open-label, phase II multicenter clinical trial (SIL-COVID19), will also test the therapeutic efficacy of silibinin to prevent ARDS in moderate-to-severe COVID-19-positive onco-hematological patients (94).

Tea polyphenols

Tea is a rich source of bioactives and known to exert broad spectrum activities against human viral pathogens, including HIV, herpes simplex virus, influenza, hepatitis B, and hepatitis C (104). Potent antiviral activities of bioactive tea molecules against several protein drug targets of SARS-CoV-2 were reported (105–107). Three bioactive molecules isolated from tea demonstrated specific interaction with the active site of the SARS-CoV-2 RdRp-RNA complex and the NTP entry channel of the viral enzyme,

both values were higher compared to the FDA-approved antiviral drugs remdesivir and favipiravir (63). The catalytic center of SARS-CoV-2 RdRp is a highly conserved domain that contains residues Ser_{759}, Asp_{760}, and Asp_{761} (23, 38). Furthermore, the residues Lys_{545}, Arg_{553}, and Arg_{555} in the RdRp-RNA complex facilitate formation of the NTP entry channel. The first tea bioactive, *Epicatechin-3,5-di-O-gallate* forms three H-bonds and a π-anion interaction with the Uracil at position 20 of the primer RNA strand and stabilizes inside the active pocket of RdRp-RNA complex by eight H-bonds with π-anion and π-Lone Pair interactions. Several residues of RdRp along with the adenine (A) and uracil (U) of the template RNA strand at positions 11 and 10 show van der Waals interactions. The second molecule from tea, *Epigallocatechin-3,5-di -O-gallate*, forms two H-bonds with uracil (U) at position 20 of primer RNA and binding to the active pocket of RdRp-RNA complex involves nine H-bonds with π-alkyl and van der Waals interactions. The third tea polyphenol, *Epigallocatechin-3,4-di -O-gallate* binds to the primer strand uracil (U) at position 10 *via* two H-bonds, while the adenine (A) at position 20 of the same RNA strand forms one H-bond with three π-anion, two π-alkyl, and one π Lone Pair interaction(s) at the RdRp active site. In comparison, both remdesivir and favipiravir form significantly weaker van der Waals and π-Sulfur interactions with amino acid residues of the catalytic center and the NTP entry channel of the SARS-CoV-2 RdRp-RNA complex (63).

Theaflavin

Theaflavin and *Theaflavin gallate*, the black tea polyphenols, show broad-spectrum antiviral activity (109). *Theaflavin 3,3′-digallate* (TF3), *Theaflavin 3-gallate* (TF2a) and *Procyanidin B2* inhibit specific targets of SARS-CoV-2 and considered as promising intervention candidates for COVID-19 management (108). TF3 (ΔG = −14.92 kcal/mol), Procyanidin B2 (ΔG = −11.68 kcal/mol) and TF2a (ΔG = −10.90 kcal/mol) exhibit high binding affinities toward RdRp of SARS-CoV-2 with low docking scores (110). Theaflavin and its derivatives from traditional Chinese medicine were docked into the catalytic pocket near the active site of RdRp in SARS-CoV-2 (ΔG = −9.11 kcal/mol), SARS-CoV ((ΔG = −8.03 kcal/mol), and MERS-CoV ((ΔG = −8.26 kcal/mol). Theaflavin forms H-bonds and π-cation interaction with Arg_{553} in the catalytic pocket of SARS-CoV-2 RdRp complex. Theaflavin also inhibits the RdRp activity *via* blocking the active site in the enzymatic scaffold (111).

Baicalein

Baicalein (5,6,7-trihydroxyflavone) could block the viral replication in cell culture systems by inhibiting the RdRp activity of SARS-CoV-2 (113, 114). This natural bioactive flavone is found in the root of *Scutellaria baicalensis*, an 'East Asian skullcap' plant, widely used in traditional Chinese medicine for the treatment of hyperlipidemia, hypertension, atherosclerosis, and common cold (115, 116). Baicalein, along with its analog baicalin, could inhibit certain types of lipoxygenases (LOs) [i.e. human platelet 12-LO (12-hLO) and human reticulocyte 15-LO (15-hLO)], and act as anti-inflammatory

agents (117). Both compounds are also potent inhibitors of several RNA viral pathogens such as Dengue and Zika viruses (118, 119).

In the antiviral assay, baicalein and baicalin at 20 μM dose showed 99.8% and 98% inhibition of SARS-CoV-2, respectively. In dose-dependent inhibition assay, baicalein (EC_{50} 4.5 μM) demonstrated more potent antiviral activity than baicalin (EC_{50} 9.0 μM) against SARS-CoV-2 replication in Vero cells. At 30 μM concentration, only baicalein (14.6%), but not baicalin (−3.3%), could inhibit the SARS-CoV-2 pseudovirus entry (113). In an experimental murine model (LPS-induced acute lung injury), oral administration of baicalein (200 mg/kg) in crystal β form showed improvement in the respiratory function, inhibition of inflammatory cell infiltration in the lung, and decreased serum levels of IL-1β and TNF-α (112).

Baicalein (ΔG = −8.7 kcal/mol) and baicalin (ΔG = −7.8 kcal/mol) interact with SARS-CoV-2 RdRp with stronger binding energy than remdesivir (ΔG = −6.5 kcal/mol). Baicalein seems to bind His_{133} residue in the *nucleotidyl-transferase* domain and Asn_{705} residue in the palm domain of SARS-CoV-2 RdRp enzyme, which differs from the specific binding sites for remdesivir (113, 119). Since, the anti-RdRp mode of action of remdesivir (the NAI agent), seems to be different from baicalein (the NNAI agent), a combinational treatment with these two classes of antiviral agents could be a potential option for COVID-19 management.

Corilagin

Corilagin (RAI-S-37), is a gallotannin from plants such as *Caesalpinia coriaria*, *Alchornea glandulosa* and found in the leaves of *Punica granatum* (pomegranate) (120). This phytonutrient is known for potent anti-tumor, anti-inflammatory and hepatoprotective activities (121). As an NNAI of SARS-CoV-2 RdRp, corilagin binds directly to RdRp and effectively inhibits viral polymerase activity in both cell-free and cell-based assays, fully resists the proofreading activity and potently inhibits SARS-CoV-2 infection with a low 50% effective concentration (EC_{50}) value of 0.13 μmol/L (122). Based on computation modeling, corilagin binds to the palm domain of RdRp and prevents conformational changes required for nucleotide incorporation by RdRp.

Although remdesivir has been shown to be more effective than other NAIs, it is sensitive to the proofreading activity of SARS-CoV-2 *nsp14/ExoN* domain (32). Expression of *nsp10-nsp14* leads to a 2.1-fold increase in EC_{50} value of remdesivir in cell-based RdRp activity assays. In contrast, the EC_{50} values of corilagin (RAI-S-37) sustains the same efficacy in the absence or presence of the *nsp10-nsp14*, which indicates that this phytonutrient-NNAI is unaffected by the proofreading activity of SARS-CoV-2 (115). Since, the anti-RdRp mode of action for corilagin and remdesivir are different, a combinational intervention with these two antivirals has been indicated for COVID-19 management.

Hesperidin

Hesperidin, a bioflavonoid mainly found in citrus fruits, shows high binding affinity and stability when complexed with the active sites in RdRp and M^{pro} enzymes of

SARS-CoV-2 (123). Hesperidin contains both the flavanone *hesperitin* (aglycone) and the disaccharide *rutinoside* (rhamnose linked to glucose), with widely known antiviral, and anti-inflammatory effects. The multifunctional activities of hesperidin are attributed to its antioxidant properties and inhibition of mitogen-activated protein kinase (MAPK)-dependent signaling pathways (124).

In molecular docking and molecular dynamic simulation studies the binding affinities and ligand stability of hesperidin with SARS-CoV-2 enzymes: M^{pro} (ΔG = −15.18 kcal/mol) and RdRp (ΔG = −9.46 kcal/mol) are superior to the docking scores for antiviral drugs: remdesivir (-8.2 kcal/mol for M^{pro} and −7.5 kcal/mol for RdRp); lopinavir (-7.9 kcal/mol for M^{pro} and −6.9 kcal/mol for RdRp) and ritonavir (-8.2 kcal/mol for M^{pro} and −7.2 kcal/mol for RdRp). Also, hesperidin strongly interacts with the amino acid residues Ser_{759}-Asp_{760}-Asp_{761} in the catalytic site as well as the divalent-cation-binding residue Asp_{618} in the SARS-CoV-2 RdRp enzyme (123). Based on the safety (low cytotoxicity), the predicted ADMET profile, and the ability to form high affinity/stable complexes with SARS-CoV-2 replicase enzymes, hesperidin could be a promising multitarget antiviral agent for COVID-19 management.

Lycorine

Lycorine, a bioactive pyrrolidine alkaloid isolated from the bulbs of *Lycoris radiata*, exhibits several pharmacological and broad-spectrum antiviral effects (125). Lycorine effectively inhibits several CoV pathogens (126, 127), including SARS-CoV (IC_{50} = 1.02 μM), MERS-CoV (IC_{50} = 2.12 μM) and SARS-CoV-2 (IC_{50} = 0.88 μM), more effective than the antiviral activity of remdesivir (IC_{50} = 6.5 μM) (128). The binding affinity of lycorine (ΔG = −6.2 kcal/mol) with SARS-CoV-2 RdRp protein is stronger than that of remdesivir (ΔG = −4.7 kcal/mol). Remdesivir is known to inhibit SARS-CoV-2 RdRp activity *via* non-obligate RNA chain termination by targeting the core catalytic active site on the RdRp enzyme (38). Lycorine shows similar binding position that overlaps with the nucleoside rings of remdesivir in the same pocket region of the catalytic active site on the viral polymerase. Lycorine forms H-bonds with Asp_{623}, Asn_{691}, and Ser_{759} residues on RdRp protein, similar to remdesivir. Lycorine is a potent NNAI against RdRp activity of several CoV pathogens and more effectively on SARS-CoV-2; therefore, may be a promising candidate for COVID-19 management.

Medicinal herbs and other natural products

Several phytonutrients from North-South African medicinal plants have demonstrated higher docking scores with RdRp than remdesivir, the reference antiviral drug. Based on molecular dynamic simulation data and free energy calculations, the docking scores for *3-O-α-l-arabinopyranosyl-echinocystic acid* (ΔG= −9.9 kcal/mol), *3′-epiafroside* (ΔG= −9.3 kcal/mol), and *Genkwanin 8-C-β-glucopyranoside* (ΔG= −9.1 kcal/mol), are reportedly several-fold higher than remdesivir (ΔG= −7.1 kcal/mol) (129). Also, *Argemone mexicana* L., known as 'Ghamoya' is used in herbal medicine as an anti-inflammatory, immune-modulator, anti-spasmodic and anti-HIV agent (130). Molecular docking data showed that *Protopine* (ΔG = −6.07 kcal/mol), *Allocryptopine* (ΔG = −5.75 kcal/mol)

and *(±) 6-Acetonyldihydrochelerythrine* (ΔG = −5.66 kcal/mol) from this plant are potential RdRp inhibitors of SARS-CoV-2 (131).

Several secondary metabolites from Indonesian herbal plants have shown potent antiviral activity (132). An *in silico* molecular docking study has identified several phytonutrient RdRp inhibitors such as *Justicidin* D (ΔG= −8.7 kcal/mol), *10-Methoxycamptothecin* (ΔG= −8.5 kcal/mol), *Inoxanthone* (ΔG= −8.3 kcal/mol), *and 3-O-Caffeoylquinic acid* (ΔG= −8.2 kcal/mol). Binding affinities of these plant-derived compounds were higher compared to the reference antiviral drugs remdesivir (ΔG= −8.2 kcal/mol), hydroxychloroquine (ΔG= −6.7 kcal/mol), and chloroquine (ΔG= −5.8 kcal/mol) (133).

Propolis from (honeybee products) contains bioactives including phenolic acids, flavonoids, and terpenes with broad spectrum antiviral effects. The *ellagic acid* interaction with RdRp utilizes five H-bonds with Gly_{808}, Pro_{809}, His_{816}, Thr_{817}, and Tyr_{831} while amino acid residues Asp_{761} and Glu_{811} are involved in H-bonding with *hesperitin*, and *kaempferol*. The aromatic ring of *ellagic acid*, *hesperetin*, and *kaempferol* is involved in π-ion hydrophobic interaction with Lys_{798} residue of SARS-CoV-2 RdRp. In molecular docking studies, phenolics such as *ellagic acid (ΔG= −6.4 kcal/mol), hesperetin (ΔG= −6.3 kcal/mol), and kaempferol (ΔG= −6.2 kcal/mol),* showed high-affinity interactions with the RdRp enzyme and considered effective COVID-19 inhibitors (134).

Apart from the NNAI spectrum of the specific phytonutrients against the SARS-CoV-2 RdRp, the potential health benefits of these natural compounds on regulation of various physiological functions has also been elucidated (Table 1). This bio-functional aspect of phytonutrient NNAIs has significant clinical relevance, since the pathobiology of SARS-CoV-2 involves host metabolic reprogramming that deregulates cellular redox homeostasis, affects mitochondrial function and its related bioenergetic pathways (146–148). Accordingly, these phytoceutical antiviral compounds could provide synergistic or additive benefits with pharmaceutical drugs in immune modulation, anti-inflammation, and relieve oxidative stress in COVID-19 patients (149).

Conclusions

Antiviral drugs with proven efficacy are not yet available to prevent transmission or facilitate treatment of COVID-19. The 'repurposing' of approved antiviral drugs with adjuvant combination(s) of well characterized phytonutrients could be one of the rapid and safe strategies to combat the COVID-19 pandemic. Computational drug repurposing is a promising alternative that enables prioritization of existing compounds through rapid high through-put screening analyses (150). Virtual *in silico* processing protocols could meet the current challenge of antiviral drug discovery considering comparative testing of both pharmaceutical and phytoceutical bioactive molecules. Large virtual compound libraries could be filtered by different computational screening methods such as molecular docking, ligand-based similarity searches or pharmacophore-based screening, which reduces the number of bioactive molecules to a smaller set of potential candidates for clinical evaluation (151). Some predicted drugs and potential phytoceutical compounds that target viral proteins such as RdRp and pathological host pathways are currently undergoing human clinical trials (152).

Table 1. Conserved motifs and residues in the SARS-CoV-2 RdRp enzyme and their potential interactions with the phytonutrient NNAI ligands. Additional health attributes of specific dietary bioactives is also depicted.

Phyto-NNAI	RdRp Binding Residues* (ΔG = kcal/mol)	Additional Health Attributes of Plant Bioactives
Suramin	$N_{497}/K_{500}/R_{569}/Q_{573}$ & $K_{551}/R_{553}/R_{555}/R_{836}$ (EC_{50} = 0.26 to 0.43 μM)	Prevents IL-1 mediated host response, PGE-2 synthesis, thymocyte proliferation, and IL-6 production (38, 135*, 72*).
Silibinin	$D_{618}/D_{623}/D_{760}/D_{761}$ & $R_{555}/V_{557}/T_{680}/S_{682}/N_{691}$ (-9.4 kcal/mol)	Inhibits secretion of pro-inflammatory cytokines, such as TNF-α, IL-6, activates NF-κB and upregulates intracellular cAMP level (96, 136)*
Theaflavin	R_{553} (-9.1 kcal/mol)	Suppresses LPS-induced ICAM-1 and VCAM-1 expressions *via* blockage of NF-κB and JNK activation in intestinal epithelia (110, 137*).
Epicatechin-3,5-di-O-gallate	$K_{545}/D_{623}/D_{425}/N_{691}$/ $S_{759}/S_{682}/S_{814}$ (-14.7 kcal/mol)	Potent anti-inflammatory, inhibits TNFα-induced activation of NF-κB and secretion of pro-inflammatory mediator IL-8, (63)*.
Epigallocatechin-3,5-di-O-gallate	$S_{682}/D_{623}/R_{553}/R_{55}$/ $K_{545}/I_{548}/D_{760}/S_{759}$ (-9.2 kcal/mol)	Inhibits leukocyte migration into endothelial cell monolayers and ameliorates chronic fatigue syndrome, (63)*
Epigallocatechin-3,4-di-O-gallate	$R_{836}/R_{555}/S_{759}/N_{691}$/ $D_{623}/S_{682}/D_{452}/R_{553}/K_{545}$ (-14.9 kcal/mol)	Potent antioxidant that protects erythrocyte calcium-ATPase and sodium/potassium-ATPases against oxidative stress (63, 138)*.
Baicalein	H_{133}/N_{705} (-8.7 kcal/mol)	Prevents apoptosis and neuroprotective *via* inhibiting oxidative stress, protein conjugation, and inflammation (119, 139*, 113*).
Corilagin	$G_{616}/D_{761}/K_{798}/W_{617}/W_{800}$/ $D_{618}/S_{814}/E_{811}/S_{549}/C_{799}/A_{550}$ (-8.9 kcal/mol)	Down-regulates pro-inflammatory mediators TNF-α, IL-1β, IL-6, NO (iNOS) and COX-2 by blocking NF-κB nuclear translocation (140)*.
Hesperidin	$S_{759}/D_{760}/D_{761}$ & D_{618} (-9.5 kcal/mol)	Reduces oxidative stress *via NADP-oxidase* inhibition in the vasculature, ameliorates endothelial dysfunction/hypertension (123, 141*).
Lycorine	$D_{623}/N_{691}/S_{759}$ (-6.2 kcal/mol)	Alle*via*tes LPS-induced lung injury of inflammation and oxidative stress by blocking the HMGB1/TLRs/NF-κB pathway (38, 138*).
Justicidin A	$W_{104}/I_{119}/V_{126}/I_{128}/W_{170}/F_{192}$/ $F_{194}/I_{203}/L_{226}/V_{227}$ (-8.7 kcal/mol)	Protects neuronal cell death by blocking hyperphosphorylation of tau and induces autophagy by regulating GSK-3β/AMPK activity (133, 143)*.
10-Methoxy-camptothecin	$V_{126}/W_{170}/V_{227}$ (-8.5 kcal/mol)	Regulates solute carrier transporters (SLCs), responsible for cellular influx of endogenous substrates and several clinically important drugs (133, 144)*.
Ellagic acid	$G_{808}/P_{809}/H_{816}/T_{817}/Y_{831}$ (-6.4 kcal/mol)	Reduces plasma alkaline phosphatase activity, calcium content, and hypertrophy in vascular tissues during hypertension, (134*).
Kaempferol	D_{761}/E_{811} (-6.2 kcal/mol)	Scavenges ROS and relieves oxidative stress related medical conditions that involve disturbed metabolism of redox metals such as copper (134, 145*).

Natural compounds, especially plant-derived bioactives, have emerged as adjuvant interventional options to overcome the limitations of existing antiviral drugs against COVID-19 (149, 153). Several ongoing *in silico* studies have demonstrated the antiviral

potential of natural compounds and these phytonutrients also have multifunctional effects such as anti-inflammatory, antiviral, antioxidant, cardioprotective, and exhibit potent therapeutic benefits in the treatment of COVID-19 associated clinical manifestations.

Chemical modification of natural bioactive compounds may be required to increase the potency of their antiviral activity to levels suitable for therapeutic application. For the drug discovery process, understanding the effects of redox sensor mechanisms, especially proton transfer systems (i.e. H-bonds) that modulate the structural conformity of phytonutrients could boost the development of effective antiviral RdRp interventions (145).

Disclosure statement

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.

About the authors

Dr. Sreus A.G. Naidu, has earned Doctorate in Pharmacy and MS in Regulatory Science from the University of Southern California. Sreus has over 15 years of experience working at N-terminus Research Laboratory based in California, which specializes in the isolation, purification, and activation of bioactive molecules. He is co-inventor on multiple patents with applications in human nutrition and animal healthcare.

Dr. Ghulam Mustafa, has earned Doctor of Philosophy in Biochemistry from University of Agriculture, Faisalabad, Pakistan with research work from Scripps Institution of Oceanography, University of California, San Diego, USA. Mustafa has a strong biochemistry background with extensive research and technology development experience in biochemistry, biotechnology, microbiology and bioinformatics. He has been working on drug discovery using both traditional and in silico approaches for more than 17 years. Dr. Mustafa has contributed more than 60 peer-reviewed research publications to the journals of international repute. Currently, he is working as an Assistant Professor of Biochemistry in Government College University Faisalabad, Pakistan.

Professor Roger A. Clemens, is Associate Director of the Regulatory Science program and Adjunct Professor of Pharmacology and Pharmaceutical Sciences within the USC School of Pharmacy. Dr. Clemens was the Director of Analytical Research at USC for 5 years, and the Scientific Advisor for Nestlé USA for more than 21 years. He has published more than 50 original manuscripts in nutrition and food science, participated in more than 200 invited domestic and international lectures, and served as an expert panel member for the food industry, scientific organizations, trade associations and regulatory agencies in the United States and Canada.

Professor A Satyanarayan Naidu, is the Director of N-terminus Research Laboratory in California, USA. After receiving PhD in Medical Microbiology (1985) from the Osmania University in India, Dr. Naidu served the Directorate of Public Health Services (DPHS), the Government of A.P., India and the World Health Organization (WHO) Surveillance program. He performed post-doctoral research at the Medical University of Pécs, Hungary and the Biomedical Center-Uppsala, Sweden. Dr. Naidu joined the faculty at the Lund University; Sweden (1988-1992), the University of North Carolina at Chapel Hill, USA (1993-1997). He was appointed as the Director at the Center for Antimicrobial Research, California State University-Pomona, USA (1998-2000). Dr. Naidu's discoveries on Staphylococcal toxic shock syndrome (TSS) and E. coli hemolytic uremic syndrome (HUS) have garnered international recognition. He was principal investigator for several NIH grants, published more than 100 peer-reviewed research publications, written over 30 book chapters, and authored 4 reference volumes in the field of medical sciences. He holds 24 core patents, and his technology transfers in biomedical technology reach worldwide.

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ORCID

Sreus A. G. Naidu http://orcid.org/0000-0003-3517-8135
Ghulam Mustafa http://orcid.org/0000-0001-6510-6496
Roger A. Clemens http://orcid.org/0000-0002-5898-9793
A. Satyanarayan Naidu http://orcid.org/0000-0002-6008-0482

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