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# Discovering small-molecule therapeutics against SARS-CoV-2

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**Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly become a global health pandemic. The lack of effective treatments, coupled with its etiology, has resulted in more than 400,000 deaths at the time of writing. The SARS-CoV-2 genome is highly homologous to that of SARS-CoV, the causative agent behind the 2003 SARS outbreak. Based on prior reports, clinicians have pursued the off-label use of several antiviral drugs, while the scientific community has responded by seeking agents against traditional targets, especially viral proteases. However, several avenues remain unexplored, including disrupting E and M protein oligomerization, outcompeting host glycan–virus interactions, interfering with the heparan sulfate proteoglycans–virus interaction, and others. In this review, we highlight some of these opportunities while summarizing the drugs currently in use against coronavirus 2019 (COVID-19).**

## Introduction

SARS-CoV-2 is an enveloped positive-sense RNA virus that belongs to the *Coronavirinae* [1]. SARS-CoV-2 has rapidly become a global pandemic, distinguishing it as the most infectious agent in a century [2]. Coronaviruses, which contribute to nearly one third of common cold infections in humans, have zoonotic origins in a range of mammals and birds [3]. In humans, respiratory infections, such as pneumonia and bronchiolitis, tend to turn fatal, especially in older, pediatric, immunocompromised, or co-morbid patients [4]. The current SARS-CoV-2 pandemic appears to be targeting not only similar populations, but also the young and the healthy. It might also have a gender bias, as was previously shown for SARS-CoV [5].

Four groups, the alpha, beta, gamma, and delta coronaviruses, are categorized based on phylogenetic clustering [1]. The current pandemic causing COVID-19 is of the beta type. The unique feature of all coronaviruses is the 'club-like' projection from the surface of the virion, a feature that mimics a crown, or *corona*, in Latin. The nonsegmented, positive-sense, single-stranded RNA genome of coronavirus is large (~32 kb) and encodes four structural and 16 nonstructural proteins (NSPs). The NSPs occupy two-thirds (20 kb) of the viral genome, with the remaining third comprising structural and accessory proteins [6]. The NSPs are widely implicated in the modulation of human innate immunity by suppressing interferon (IFN) synthesis and regulating its signaling. The four main structural proteins are Spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins, of which S, M, and E are membrane bound, whereas the N protein is located within the virions in complex with the genomic RNA.

## Coronavirus attachment and entry into host cells

### *Overview*

The primary pathways for host cell entry of enveloped viruses include either (i) a pH-independent, receptor-mediated pathway, where the viral envelope fuses with the host cell membrane to initiate viral uncoating; or (ii) a pH-dependent, endocytic

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https://doi.org/10.1016/j.drudis.2020.06.017

pathway, where the virus is transported to the endosome (low pH environment) through either clathrin- or caveolin-dependent processes [1,7]. The receptor-mediated pathway is a multistep process that is initiated by virus adherence to the host cell through the receptor-binding domain (RBD) of the S glycoprotein, which forms the prefusion trimeric S-receptor complex [8]. The S glycoprotein participates in two key events during the coronavirus life cycle. First, the S glycoprotein binds to cell surface heparan sulfate (HS) chains of the heparan sulfate proteoglycan (HSPG) receptor and other cellular receptor(s), which initiates internalization. Second, the S glycoprotein promotes fusion between viral and cellular membranes [9]. The accomplishment of these two events drives the release of the viral RNA genome into the host cell and subsequently triggers the viral replication cycle [1].

In addition to mediating viral entry, the S glycoprotein is the principal antigenic determinant and the key target of neutralizing antibodies [10]. Nearly 22 glycosyl chains are present on the S glycoprotein [11], which suggests that the nature of these sugar chains on Asn/Ser/Thr residues determines the specificity of different antibodies and holds the key to developing vaccines [12]. Furthermore, two unexpected *O*-glycosyl chains have been characterized on the S glycoprotein [13]. Given that glycosylation of influenza virus [14] has posed challenges for vaccine development, similar issues might arise for anti-SARS-CoV-2 vaccine strategies. Thus, the design and/or development of small molecules that limit viral infection is crucial. Interestingly, the membrane fusion domain is highly conserved in the S glycoprotein, which makes it an attractive target to prevent membrane fusion against SARS-CoV outbreaks [15]. Alternatively, panning the S glycoprotein with fusion domains using a small-molecule library is likely to be an attractive approach to develop novel inhibitors of viral entry.

### *Host cell surface receptors*

To date, multiple host cell receptors belonging to different families have been reported to facilitate the attachment and fusion of coronaviruses. For SARS-CoV, the major receptor is ACE2, a zinc metalloprotease and carboxypeptidase expressed as an ectoenzyme in a variety of organs and/or tissues, such as the kidneys, intestinal endothelium, lung, and heart. The ACE2 receptor binds to the RBD of SARS-CoV-2 through an extended set of interactions that span at least eight residues of helix α1 of ACE2 and seven residues of S1 [16,17]. Interestingly, superposition of the RBDs of SARS-CoV and SARS-CoV-2 in complex with ACE2 show striking similarity (RMSD = 0.68 Å), despite the large number of substitutions in the interfacial residues.

Other receptors with a role in coronavirus entry include the human aminopeptidase N, a cell-surface metalloprotease expressed on intestine, lung, and kidney epithelial cells. Aminopeptidase N is utilized by human coronavirus-229E [18]. Likewise, dipeptidyl peptidase 4 (DPP4), a serine exopeptidase expressed on the surface of most cell types, is utilized by MERS-CoV [19]. Finally, HCoV-OC43 and HCoV-HKU1 use glycan-based receptors carrying 9-*O*-acetylated sialic acid residues [20,21]. Coronaviruses of zoonotic origin (i.e., NL63, 229E, HKU1, OC43, and SARS-CoV) have successfully breached the species barrier to become true human pathogens [22]. One reason why SARS-CoV-2 might have transited from bats to humans is the commonality of ACE2 as a receptor [17]. Although not shown as yet for SARS-CoV-2, other coronaviruses exploit DC-SIGN, a receptor highly expressed by macrophages and dendritic cells [23].

HSPGs represent an important, but underappreciated, group of host cell surface receptors. They contribute to the adherence and infectivity of multiple viruses [24–28]. Structurally, HSPGs are massive proteoglycans that carry highly sulfated, linear polysaccharide chains, which recruit extracellular signaling ligands, such as growth factors. Nearly all enveloped viruses use their surface glycoproteins to bind to the HS component of the HSPGs to enhance their probability of cell targeting, adherence, and fusion. For coronaviruses, the S glycoprotein has the ability to bind to HS. Enzymatic cleavage of HS or addition of exogenous heparin prevented S glycoprotein of SARS-CoV from binding to the host cell surface and resulted in reduced infectivity [29]. Likewise, a SARS-CoV strain isolated from a severely infected patient was inhibited by ~50% when treated with 100 mg/ml exogenous heparin [30]. Similarly, avian and murine coronavirus strains also use HSPGs to gain host cell entry [26,28]. Although HSPGs have not been specifically implicated in SARS-CoV-2 entry as yet, several studies using recombinant S1 protein of SARS-CoV-2 have shown tight binding to heparin and/or HS [31–33]. Additionally, this binding appears to induce a conformational change in the RBD, similar to that involved in viral entry (see below), which suggests a role in host cell entry [31].

### *Molecular players in host cell internalization*

Following initial virus adherence to primary host cell receptor (i.e., ACE2 in the case of SARS-CoV), a multistep process ensues, where proteolytic processing of the S glycoprotein and conformational changes are required for high efficiency fusion with the cell membrane [34]. One group of host proteases involved in this process are the type II transmembrane serine proteases TMPRSS2 and TMPRSS11D. There are also several other lung airway serine proteases that support the pH-independent mode of viral entry [35,36]. By contrast, the low pH environment pathway of viral entry involves activation of endosomal proteases, such as cathepsins, which are a family of cysteine proteases. Of interest are cathepsins B and L, which become active in the early and late endosome, respectively, in facilitating fusion with the endosomal membrane to support viral entry [37]. In addition, other host factors could also facilitate virus internalization [38].

A protein with a key role in viral life cycle is actin, which orchestrates rearrangements of the cytoskeleton during endosome formation. Interestingly, previous studies identified imatinib, an inhibitor of Abelson kinase (a tyrosine kinase), as an antiviral agent against SARS-CoV and MERS-CoV [39]. Inhibition of actin cytoskeleton rearrangement has been proposed as the mechanism of action for this agent [40]. However, the efficacy of imatinib in patients with SARS-CoV-2 is yet to be assessed.

## Discovering small molecules against SARS-CoV-2

### *Overview*

The number of expressed genes for SARS-CoV-2 (16 NSPs and four structural proteins), identical to that of SARS-CoV, offers multiple avenues for discovering antagonists (Fig. 1). More importantly, the two proteomes are ~95% homologous [41], which implies a high probability of cross-reactive agents. Only six regions of difference in the genome were identified between the two viruses, which

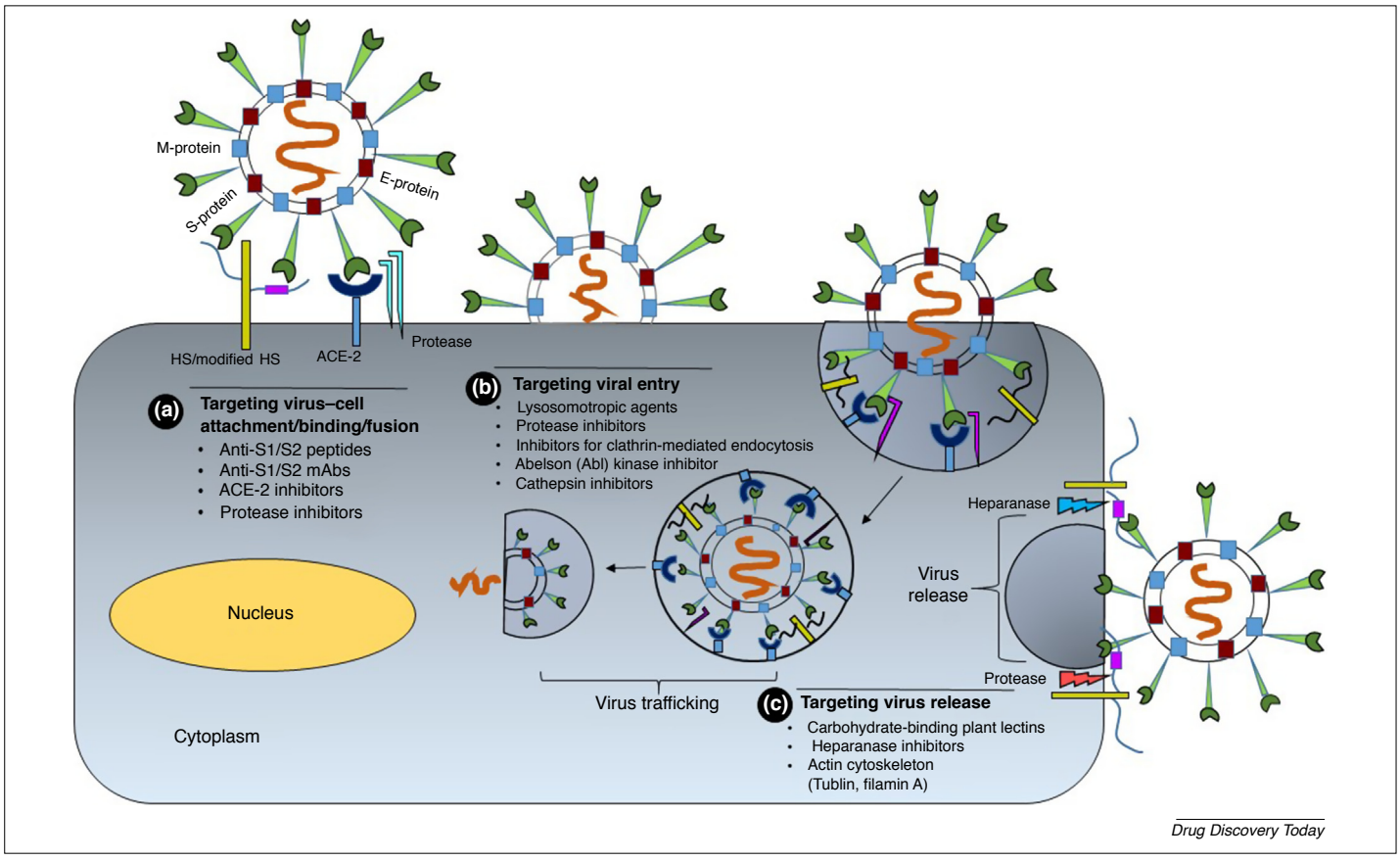


**FIGURE 1**

Developing candidates that interfere with the early stages of virus-receptor attachment and internalization. **(a)** The early severe acute respiratory syndrome coronavirus (SARS-CoV-2) spike protein–host cell surface heparan sulfate proteoglycan (HSPG) interaction could be targeted by peptide antagonists that mimic S1/S2 subunits, or ACE2 sequence, or are heparin sulfate (HS) mimetics, or anti-Spike glycoprotein (S) antibodies. Likewise, protease inhibitors could prevent fusion with the angiotensin-converting enzyme 2 (ACE2) receptor. **(b)** The next step of SARS-CoV-2 invasion involving S binding to its fusion receptor (ACE2) offers the possibilities of using soluble ACE2 peptide or anti-ACE2 antibodies. Likewise, inhibitors of endocytosis or cathepsin L could reduce the efficiency of virus–cell fusion. The release of virus from the host cell could also be targeted through inhibitors of proteases or heparanase, which contribute to the process. For example, HS mimetics combined with a cocktail of protease inhibitors might block virus egress. Not shown is the role of other enzymes involved in viral replication, including helicase and RNA-dependent RNA polymerase, which offer a major route to small-molecule discovery. Abbreviation: mAb, monoclonal antibody.

could provide additional targets for anti-SARS-CoV-2 agents. Two recently published reviews provide an excellent summary of agents discovered against coronaviruses other than SARS-CoV-2 [42,43]. Here, we discuss recent developments in the direction of anti-SARS-CoV-2 agents and highlight opportunities waiting to be explored.

*A priori*, one of the simplest early approaches would be to target the S glycoprotein–host cell surface HSPG interaction (Fig. 1). Modifiers of S1 and/or S2 subunits could include hydrophobic small molecules, heparin/HS-based oligosaccharides, or HS mimetics. The next step involving ACE2 receptor interactions offers the possibilities of using soluble ACE2 and/or S1 subunit-based peptides or peptidomimetics. The following step involves proteolytic cleavage of the S glycoprotein, which could be prevented by inhibitors of serine or cysteine proteases. Likewise, inhibitors of proteases involved in endocytosis (e.g., cathepsin L) would reduce viral infectivity. Subsequent processes involve several enzymes and nonenzymatic proteins involved in viral replication, such as RNA-dependent RNA polymerase (RdRp) and the E and M proteins, which offer diverse and virus-specific routes to small-molecule discovery. Finally, virus release from the host cell surface involves heparanase and other proteases, which could offer a novel route to effective anti-SARS-CoV-2 agents (Fig. 1). Here, we discuss various aspects of these putative molecular approaches.

### *Viral enzymes*

An often-used approach to discover drugs quickly is to identify inhibitors of key enzymes involved viral entry and propagation. For SARS-CoV-2, this group would include members of the NSP family, especially the main or chymotrypsin-like protease (Mpro and CLpro) and the papain-like protease (PLpro). Inhibiting Mpro is likely to be rewarding because it would stop the processing of a large polyprotein, replicase 1ab, which is essential for replication. Additionally, its substrate specificity is unlike that of any human protease. Relying on its 96% sequence identity with Mpro from SARS-CoV, the Hilgenfield group transformed a flexible amide bond of a parent peptide into a structurally restrained, pyridone-containing peptidomimetic **13b** (Fig. 2), which inhibited SARS-CoV-2 with 670 nM potency and exhibited substantial lung

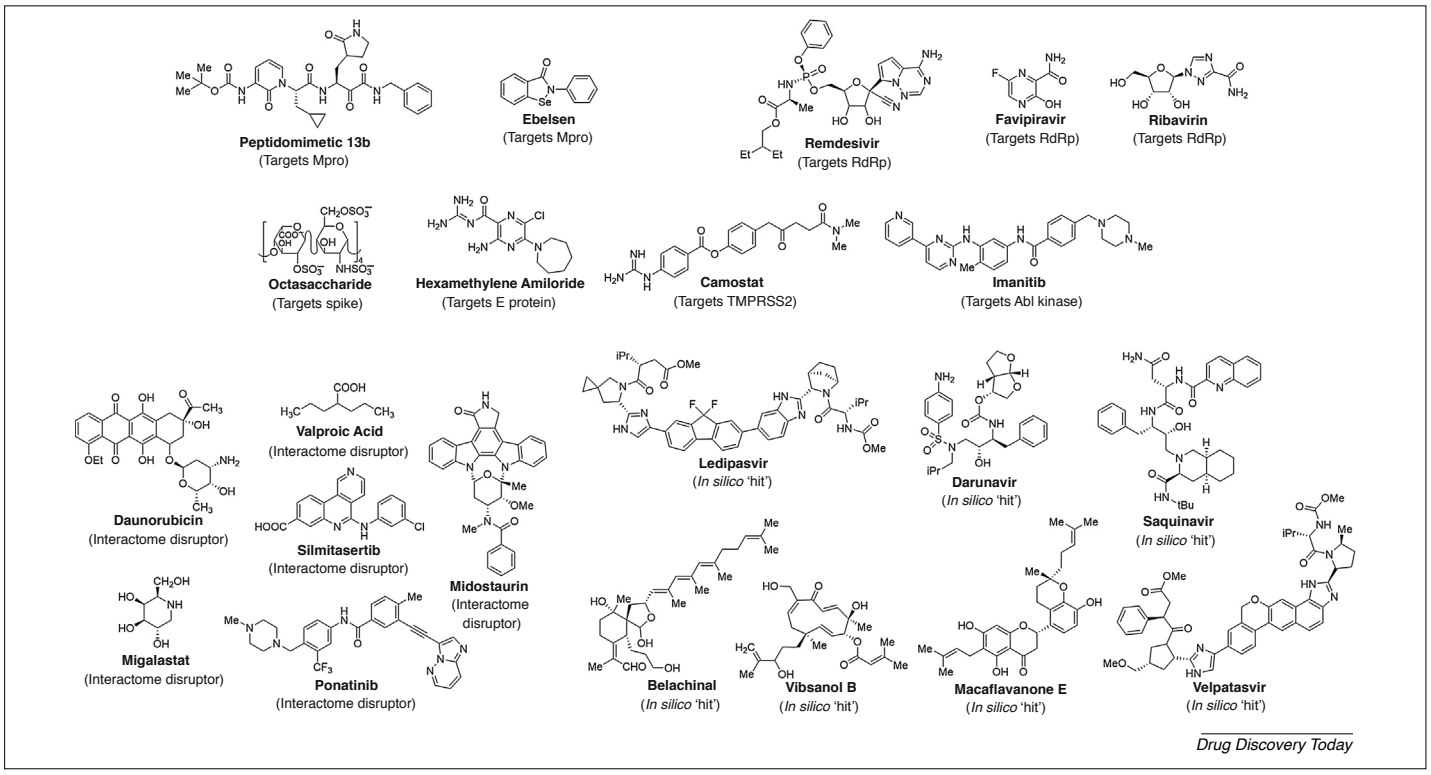


**FIGURE 2**

Structures of in-clinical use and putative anti-severe acute respiratory syndrome coronavirus (SARS-CoV-2) molecules. The putative agents were identified through computational screens, high-throughput interactome analysis, rational design of protease inhibitor analogs, or other approaches. Also shown are agents that were discovered earlier as inhibitors of SARS-CoV, such as hexamethylene amiloride, which are likely to inhibit SARS-CoV-2 owing to their similarity. See main text for details. Abbreviations: Abl, Abelson; E, envelope protein; RdRp, RNA-dependent RNA polymerase; S, Spike protein.

penetration in mice, with no significant adverse effects [44,45]. Peptidomimetic **13b** carries an alpha-ketoamide group that irreversibly modifies the active site cysteine of Mpro.

The Mpro has also been the subject of a recent high-throughput screening (HTS) approach [46]. Based on the structure of an earlier Mpro inhibitor, Jin *et al.* performed *in silico* drug discovery followed by an high-throughput sequencing (HTS) campaign of 10,000 compounds to identify six diverse molecules (ebselen, disulfiram, tideglusib, carmofur, shikonin, and PX-12) as covalent inhibitors of SARS-CoV-2. Of these, ebselen (Fig. 2) displayed good antiviral potency (4.67 μM). Unfortunately, these agents are likely to be promiscuous. Despite this, Mpro has been the subject of several efforts to identify active site inhibitors through computational and synthetic screening [47–49].

The PLpro of SARS-CoV is also a replicase-processing enzyme, in which Cys, His, and Asp form the catalytic triad. PLpro has been targeted by both covalent and noncovalent agents [50,51]. The most potent agent identified to date displayed an impressive potency of 150 nM against SARS-CoV, with a good therapeutic index, but with liver microsomal stability of only ~1 h [52]. Interestingly, despite the high homology (~95%) of PLpro from the two SARS coronaviruses [41], no inhibitors of the novel coronavirus have been reported as yet.

An enzyme that could be targeted for drug discovery is RdRp (nsp12), which is the target of several agents, including ribavirin, favipiravir, and remdesivir (Fig. 2) [53,54]. All three agents mimic the nucleoside substrate recognized by viral RNA polymerase, leading to inhibition. RdRp inhibition is also a superior approach because, once these substrate mimetics are incorporated, the virus cannot induce 'repair', thus permanently blocking replication. All three agents display fairly broad-spectrum antiviral activity because the viral RdRp is substantially conserved across multiple viruses. However, subtle amino acid differences can have profound consequences for the affinity of a particular drug. This is why these drugs exhibit varied *in vitro* inhibition potencies against different coronaviruses. In fact, early research against a clinical isolate of the SARS-CoV-2 [53] showed that, of the three, only remdesivir displayed good $IC_{50}$ (0.77 μM). Extensive clinical trials on all three agents are currently in progress. Nearly 80 Chinese patients displayed promising results with favipiravir, which contributed to its recommendation as a treatment option in China; however, the US Food and Drug Administration (FDA) has guided against its use because of its adverse consequences noted in treatment of patients with influenza A, such as anemia, which raises concerns regarding its use against COVID-19, especially at higher doses.

The first report regarding the use of remdesivir for treating COVID-19 was in a single US patient [55], which was followed by a subsequent report on 53 patients, of whom 36 exhibited clinical improvement [56]. Later, a randomized study on 237 patients reported faster time to clinical improvement with remdesivir than with a placebo, although statistical significance was not obtained [57]. The most extensive randomized, controlled trial thus far, involving 1063 patients, was performed by the US National Institutes of Health, which demonstrated a 31% faster time

to recovery [58]. This led the FDA to approve the use of remdesivir in emergency settings, especially for patients with advanced-stage disease in hospitals.

Remdesivir is a good drug but not likely to be the final drug of choice. Major improvements are needed in terms of its efficacy and toxicity. Furthermore, it is a parenteral drug, whereas an oral agent would be more suitable. Hopefully, the recent availability of the cryo-electron microscopy structure of RdRp, the target of remdesivir, should enable the discovery of advanced agents [59].

Another key enzyme is helicase (nsp13), which functions during the postfusion stage. RdRp and helicase belong to the replication-transcription complex that cooperates with other viral factors to rapidly transcribe and propagate in the host cell [60]. Although known to be crucial for replication, the design and/or discovery of inhibitors against helicase and the components of replication-transcription complex, except for RdRp, have been slow over the past decade.

### *Structural proteins*

#### ***S glycoprotein***

The S glycoprotein of coronaviruses is a distinguishing feature that is the basis not only for its name, but also for its myriad roles in facilitating host cell attachment and entry [61]. The S glycoprotein is a viral membrane-bound protein comprising three S1 subunits arranged in the form of a crown on top of three S2 stalks, each of which connect to a transmembrane segment followed by an intracellular tail. The S1 subunit carries the RBD that is recognized by one or more host cell receptors. The major receptor for SARS-CoV and SARS-CoV-2 is human ACE2 [16,17,62]. The trimeric S glycoprotein is cleaved into S1 and S2 subunits during the process of entry. Interestingly, the S glycoprotein of SARS-CoV-2 differs from that of other SARS-related coronaviruses in that it contains a furin-cleavable site at the S1/S2 boundary [63]. In fact, cleavage following, but not before, host cell receptor engagement enhances viral entry because of the importance of appropriate conformational changes for virus–host cell fusion [64]. Several human airway proteases, such as trypsin, plasmin, TMPRSS2, TMPRSS4, TMPRSS11a, and HAT, or endosomal cathepsin L, induce further cleavage of the S glycoprotein to enhance efficient virus attachment and fusion [65–67]. A recent report showed that SARS-CoV-2 utilizes a fusion and receptor-dependent syncytium formation for host cell entry, which might contribute to rapid virus spread [67].

The S glycoprotein is the primary target for the discovery of antiviral agents, especially antibodies [15,68,69]. At least three clinical trials (NCT04334980, NCT04283461, and NCT04324606) are in progress to develop vaccines that limit viral spread. Unfortunately, no small-molecule modulator of S glycoprotein function has been identified as yet. A promising idea is to antagonize RBD–ACE2 recognition through the use of soluble peptides. One of the earliest approaches in this direction was the design of a $^{438}$Tyr-Lys-Tyr-Arg-Tyr-Leu$^{443}$ sequence, derived from the RBD of SARS-CoV S glycoprotein, which bound to hACE2 receptor with ~46 μM affinity [70]. An improved approach was devised recently through the use of the 23-mer ACE2 α1 helix, which forms nearly seven selective interactions when binding to SARS-CoV-2 RBD. The affinity of this peptide was 47 nM [71]. The use of these peptides could limit binding and attachment, thereby limiting SARS-CoV-2 infection.

Another approach is to target the interactions of the S2 subunit during the fusion process, which is mediated by heptad repeat 1 (HR1) and HR2 domains. Crystal structure studies indicated that these interactions were enhanced in SARS-CoV-2 compared with SARS-CoV, which led to the design of a cholesterol-modified 36-mer peptide as a potent inhibitor of virus–cell fusion [72]. Although the *in vivo* stability of these all-natural sequences is not known, their high affinity makes for an attractive approach to design more stable analogs and/or peptidomimetics as competitive inhibitors.

A novel approach that might rapidly identify promising peptidic agents against SARS-CoV-2 is the filamentous bacteriophage surface display technology (Fig. 3). Earlier work on herpes simplex virus (HSV) identified multiple candidate peptides that competed with 3-*O*-sulfated HS and inhibited infection *in vivo* [73,74]. Likewise, library panning against fusion domains of SARS-CoV-2 viral glycoproteins S1 and S2 could help identify better, smaller peptidic agents.

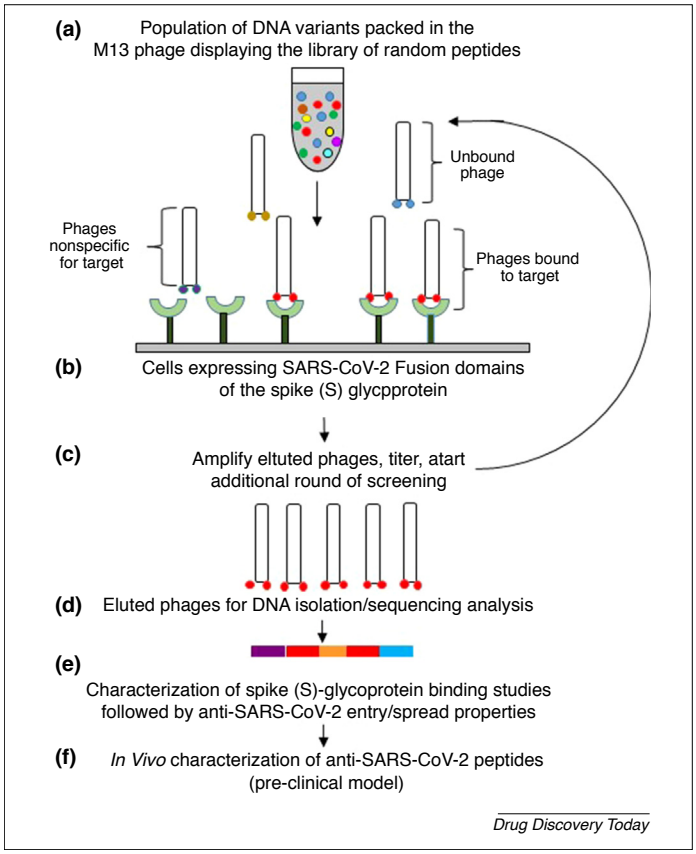


**FIGURE 3**

Diagram showing how a panning experiment **(a–d)** to identify anti-severe acute respiratory syndrome coronavirus (SARS-CoV-2) peptide could be identified using phage display screening of random peptide libraries, such as against fusion domains of the Spike (S) glycoprotein. The technology, implemented earlier for herpes simplex virus (HSV)-binding peptides, is an *in vitro* selection technique in which a peptide is genetically fused to a coat protein of a nonlytic bacteriophage (M13). This results in the display of the fused protein on the exterior of the phage virion, whereas the DNA encoding the fusion resides within the virion. The physical linkage between the displayed peptide and the DNA encoding it allows screening of more than 1 billion variant peptides against the SARS-CoV-2 S protein. The phages binding to the angiotensin-converting enzyme 2 (ACE2) receptor will have to be sequenced to generate peptides **(e,f)** for the development and characterization pf anti-S peptides to prevent SARS-CoV-2 infection.

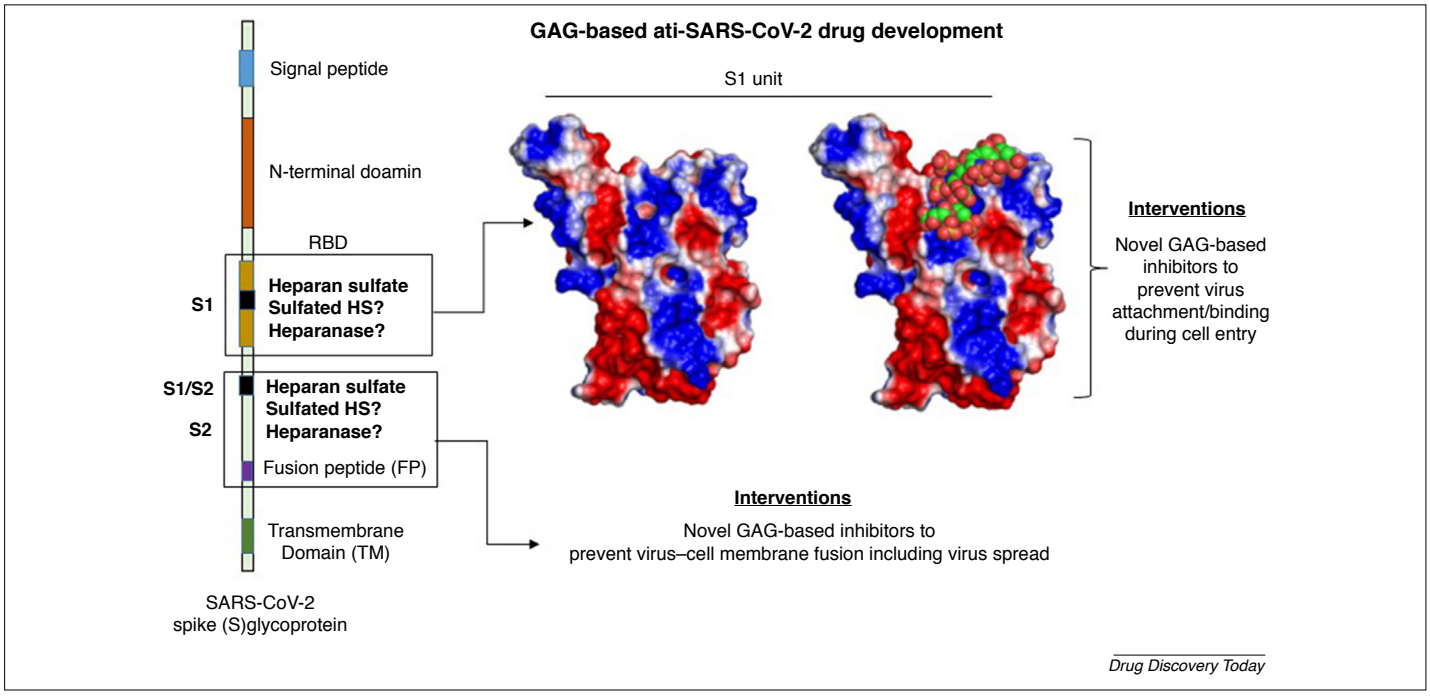


**FIGURE 4**

Glycosaminoglycan (GAG)-based interventions for targeting severe acute respiratory syndrome coronavirus (SARS-CoV-2). The spike (S) glycoprotein of SARS-CoV-2 contains three heparin sulfate (HS)-binding sites, including the receptor-binding domain (RBD) and S2. Whereas HS engagement of the RBD would directly compete with ACE2 and prevent viral adherence to host cells, HS engagement of the site in S2 would prevent virus–host cell fusion. Also shown is the computerized docking of a HS hexasaccharide binding to the S1 subunit. The model predicts strong interactions between the two. The protein surface is color coded using the electrostatic potential surface (positive in blue and negative in red) calculated through the APBS tool in PyMol. The heparin hexasaccharide sequence (spheres), shown in green, is colored by atom type.

Alternatively, an ACE2 receptor-based panning could isolate anti-fusogenic peptides that bind the S glycoprotein with high affinity.

A more recent approach to inhibit coronavirus infection is via competitive inhibition with heparin or HS. Typically, enveloped viruses as distinct as HSV, HIV, cytomegalovirus (CMV), and SARS utilize HSPGs on the host cell surface to facilitate cellular penetration [24–28,75,76]. Although much remains to be understood regarding the molecular underpinnings of these processes, the host cell HS–viral glycoprotein interactions might be selective, as exemplified in the case of HSV, in which a sulfated octasaccharide sequence was found to be important for binding to viral glycoprotein D [77]. Recently, the RBD of SARS-CoV-2 was found to interact with pharmaceutical heparin using circular dichroism [31–33]. Whereas the Skidmore lab [31] utilized circular dichroism to show heparin–S glycoprotein interaction, the Linhardt lab [32] showed that heparin is selectively recognized by the S glycoprotein among all the different glycosaminoglycans tested. Furthermore, the Boons lab [33] identified a common octasaccharide sequence (Fig. 2) as the most potent (38 nM) in inhibiting the S–heparin interaction. Interestingly, three possible sites of HS binding on the S glycoprotein, including the RBD, have been predicted [32]. A quick analysis of the electrostatic surface of S1 followed by molecular docking of a small library of HS hexasaccharides based on well-established literature protocols [78] shows high complementarity between the two binding partners (Fig. 4). This supports the expectation that heparin-like molecules, such as glycosaminoglycan mimetics, which have been found to potently inhibit HSV and HCMV [79,80], could also be good inhibitors of SARS-CoV-2.

An important area that deserves attention is the glycosylation of proteins involved in virus recognition and entry. All proteins (ACE2, S, etc.) carry multiple glycan chains, especially on Asn residues. In fact, numerous Asn residues on the S glycoprotein are glycosylated with high mannose and complex sugar types, which are predicted to have important roles in bypassing the host defense system [81]. It is possible that soluble glycans or glycan mimetics serve as effective competitors depressing viral attachment. Another possibility is to alter the expression of glycans through modulation of endoplasmic reticulum glucosidases, as exemplified by iminosugar-induced changes in structure of *N*-glycans of ACE2, which impaired the recognition of SARS-CoV Spike protein [82].

### *The E and M proteins*

The E and M proteins have key roles in virus integrity as well as morphogenesis, ion channel formation, the stabilization of other proteins, and the activation of host inflammatory mediators [83,84]. The E protein is a relatively short protein (76 residues) that forms a symmetric pentamer that yields an ion channel. In the absence of E protein, membrane permeability decreases significantly, which results in inefficient virus maturation. Consistent with this finding, the drug hexamethylene amiloride (Fig. 2) was shown to reduce ion conductance *in vitro* for several synthetic SARS coronaviruses [85]. The other structural protein, M glycoprotein, is the most abundant protein in coronaviruses. Its transmembrane domain oligomerizes and forms a lattice, which imparts strength for the assembly of the viral membrane and, in turn, the virus. Unfortunately, no small-molecule inhibitors targeting the M protein are known as yet.

A powerful approach that could lead to the discovery of a pan-CoV agent would be to destabilize the E or M proteins. These proteins tend to be highly conserved across many different species and/or strains because of their role in the propagation and completion of the virus life cycle. Thus, small molecules that disrupt the stability of these structural proteins would immediately result in the loss of virus integrity and viral infectivity. Another advantage with these targets is the high number of protein–protein interfaces that could be targeted. The feasibility of such an approach was demonstrated through the computational discovery of a small molecule (MW <500) that disrupts matrix protein 1 of influenza A [86]. To date, no agent has been designed based on this approach for either SARS-CoV-2 or SARS-CoV.

### *Host proteases*

Given that host proteases are required for the rapid internalization of the SARS virus, an effective strategy would be to inhibit these proteases to prevent amplification of infection. In the case of coronavirus entry, the interaction between ACE2 and the S glycoprotein requires priming by serine protease TMPRSS2, which generates S1 and S2 subunits required for successful viral entry. Given that lung epithelial cells express high levels of TMPRSS2 [87], SARS-CoV-2 is likely to use TMPRSS2 for entry. Therefore, the transmembrane protease TMPRSS2 is of major interest as a modulator of viral entry. In fact, the feasibility of this approach was indicated in a 2012 report that demonstrated the use of camostat, a TMPRSS2 inhibitor, and EST, a cathepsin L inhibitor, in efficiently blocking viral entry into human cells [88]. Likewise, studies on SARS-CoV-2 have also shown reasonable support for this approach [89]. This has led to camostat (Fig. 2) being studied in clinical trials (NCT04321096).

Another group of host proteases aiding viral fusion are Abelson (Abl) kinases, which are cytosolic, nonreceptor tyrosine kinases [90]. Previous work identified imatinib (Fig. 2), an anticancer agent, as an inhibitor of Ab1 kinase [91]. Imatinib reduces SARS-CoV virus fusion and syncytia formation, probably by interfering with the dynamics of actin movement during virus–cell fusion [92]. Unfortunately, this area of inhibitor design has not attracted much attention, probably because of the high possibility of adverse consequences that arise from knocking out host proteases.

### *Repurposing drugs, clinical candidates, or natural products*

Each protein, nucleic, and glycan component of the SARS-CoV-2 could be targeted for discovery of small molecules that modulate their interaction with human biomolecules. This 'human–virus biomolecular interactome' is likely to be massive and challenging to decipher. However, curating the interactome might yield the discovery of novel agents that alter key pathways for infection. A recent study of the likely human–virus biomolecular interactome for SARS-CoV-2 offers a peek into navigating this process and deriving new uses for approved drugs, agents in clinical trials, and preclinical agents.

A multilaboratory team assembled by Nevan Krogan of University of California–San Francisco identified using affinity purification–mass spectrometry 332 human protein binding partners to 27 of the 28 SARS-CoV-2 proteins (Fig. 5) [93]. The interactions spanned a range of functions, such as gene replication and

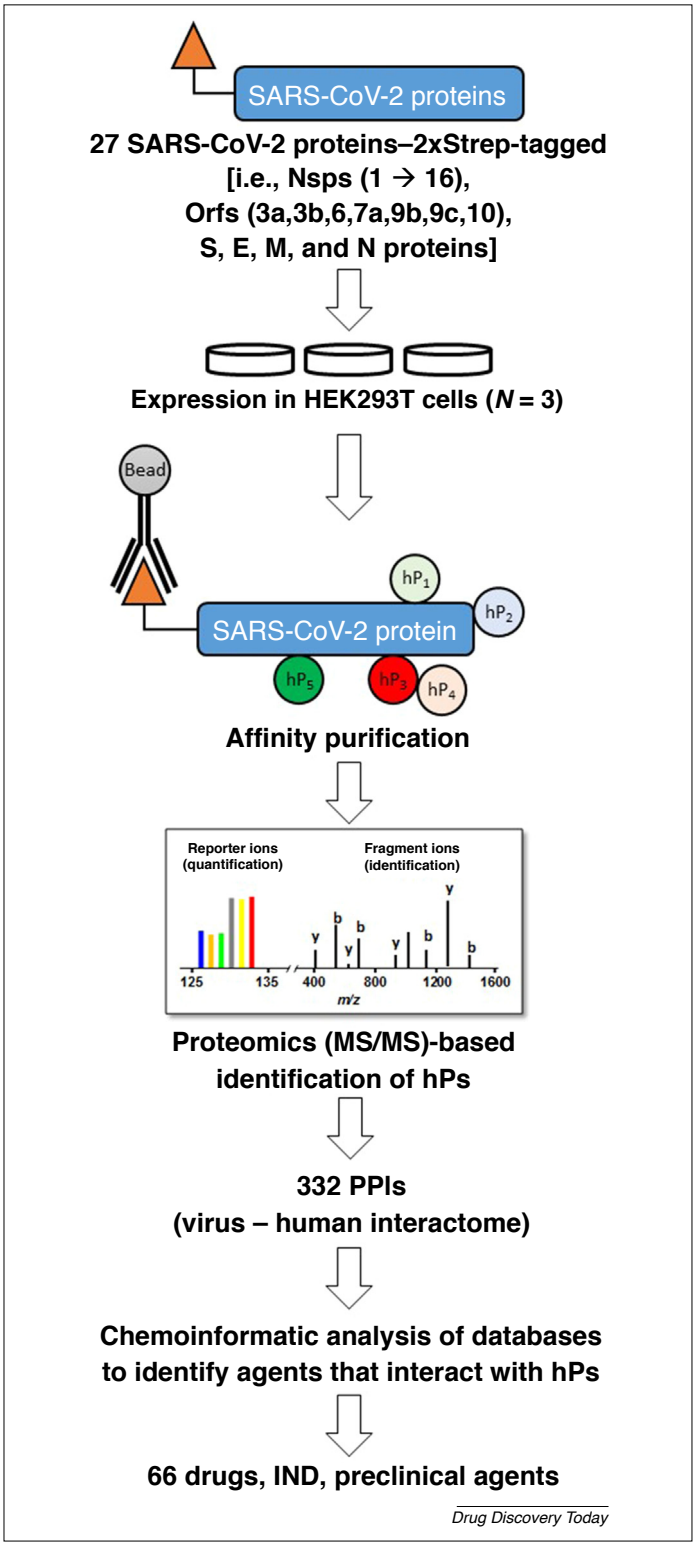


**FIGURE 5**

An affinity purification–mass spectrometry (MS) approach used to identify potential inhibitors of severe acute respiratory syndrome coronavirus (SARS-CoV-2) [93]. By affinity tagging (Strep) every gene of SARS-CoV-2, expressing the constructs in human HEK293T cells, affinity purifying on immobilized antibody beads, and identifying all the human proteins (hPs) that are co-isolated, a multidisciplinary team deduced 332 protein–protein interactions worth targeting. Using chemoinformatics, the team then identified 66 known drugs, or investigational new drug (IND) agents or preclinical promising molecules as plausible inhibitors of SARS-CoV-2. The structures of some of the agents are shown in Figure 2.

expression, trafficking between endoplasmic reticulum and Golgi, palmitoylation, interferon signaling, stress response modulation, and ubiquitinylation. The SARS-CoV-2 proteins that invoke these human pathways include ten NSPs (i.e., NSP1, 4, 5, 6, 7, 8, 9, 10, 13, and NSP15), two structural proteins (S and E), and six open-reading frames (i.e., Orf3a, 6, 8, 9b, 9c, and Orf10). Interestingly, Nsp6 of SARS-CoV-2 was found to interact with Sigma receptor, which is known to be targeted by chloroquine, the drug suggested during the early phase of pandemic for treating patients with COVID-19. Likewise, NSP8 binds to human mitochondrial ribosomes, which are known to be off-target partners of azithromycin [93], another drug used extensively to date.

The team then identified 62 agents, either approved drugs, clinical, or preclinical molecules that interact with the human proteins as putative disruptors of the human–SARS-CoV-2 interactome. A number of these agents are anticancer drugs (e.g., ponatinib, silmitasertib, midostaurin and daunorubicin) (Fig. 2), whereas others are very simple agents, such as valproic acid and miglastat [93]. Despite these successes, the S1–ACE2 interaction was not picked up in this study. Likewise, the SARS-CoV-2 proteins involved in host cell glycan recognition (e.g., sialic acids and/or HS) would not be picked up because of its focus on protein–protein interactions. Finally, the computational screening used in the approach was directed toward repurposing drugs against human proteins, and not toward against SARS-CoV-2 proteins. Thus, the knowledge garnered in developing the interactome appears to have been only partially utilized thus far.

Another approach to repurpose approved drugs for SARS-CoV-2 was reported by Zhou *et al*. [94]. In this approach, a 'systems pharmacology-based network medicine platform' was developed for quantifying the projected interactions between SARS-CoV-2 proteins and drugs through calculation of proximity of known drug targets to the coronavirus–human interactome. The logic of this approach was to first identify human proteins that are known to interact with coronavirus proteins and then to sort the proximity of these proteins to known targets of clinically used drugs, which could then be expected to antagonize the interactions of SARS-CoV-2 with host proteins. Based on this network analysis, the authors identified several drugs and drug combinations as potential inhibitors of SARS-CoV-2, including melatonin, mercaptopurine, sirolimus, toremifene, emodin, and others [94].

A good strategy to rapidly discover antivirals against SARS-CoV-2 in a nontargeted manner would be through the phenotypic screening of clinically approved drugs. Examples of this strategy have been in the use of chloroquine/hydroxychloroquine, lopinavir/ritonavir, and remdesivir/favipiravir in patients with COVID-19. In fact, several clinical trials have begun with other broad-spectrum agents, including darunavir [54,95]. Since the COVID-19 outbreak, several groups have used computational tools to repurpose approved drugs against SARS-CoV-2 proteins, including Mpro [47,96,97]. Interestingly, these studies identified several different drugs, including remdesivir, saquinavir, darunavir, ledipasvir, and velpatasvir (Fig. 2).

Finally, a group with considerable promise in terms of preventing or treating viral infection is through the use of natural products. A large number of libraries containing natural products are and could be tested for their ability to limit SARS-CoV-2 infection. Following the SARS-CoV outbreak of 2000/2003, several groups pursued natural products as inhibitors of Mpro and realized modest levels of activities [98,99]. Recently, computational modeling using a library of natural products led to the identification of belachinal, macaflavanone E, and vibsanol B (Fig. 2) as possible modulators of SARS-CoV-2 protein E [100]. Yet, the use of these natural compound libraries in target-agnostic phenotypic HTS studies against SARS-CoV-2 has not been implemented so far.

## Concluding remarks

The global footprint and high fatality rate of SARS-CoV-2 is a clear and urgent call for the development of novel antiviral interventions. An ideal candidate will be an agent that blocks the early events of viral attachment and cell entry, thereby preventing viral spread. This would be advantageous for preventing human-to-human transmission, especially from asymptomatic individuals. In this regard, the most direct approach will arguably be to target inhibitors of key proteases, such as Mpro, PLpro, TMPRSSs, cathepsin L, RdRp, and helicase, involved in viral infection and spread. Targeting the attachment and fusion processes, involving structural glycoproteins, including S and M, would also stop the virus in its tracks. Unfortunately, research on small-molecule modulators of coronaviruses, especially SARS-CoV, had not progressed enough since the 2003 outbreak to yield a bounty of candidates to screen against SARS-CoV-2.

Yet, several clinically approved drugs have been rapidly evaluated in patients with COVID-19 based on reports from SARS-CoV studies. The pandemic nature of SARS-CoV-2 led to rapid off-label use of chloroquine/hydrochloroquine, lopinavir/ritonavir, ribavirin/favipiravir, and others. However, caution should be exercised regarding the interpretation of the results using these agents. Most off-label studies have been with a limited number of patients without the benefit of rigorous control cohorts. Additionally, the known adverse effects of these drugs introduce additional challenges in infected patients.

The scientific community has quickly responded to the COVID-19 crisis by developing a range of possible therapeutics. These agents include the pyridone-containing mimetic against Mpro, the 23-mer α1 peptide against the RBD, and the cholesterol-containing 36-mer sequence from the S2 subunit. This survey also reveals some major avenues of antiviral drug discovery not being pursued with vigor, including targeting the HSPG–virus interaction, disrupting the oligomerization of E and M proteins, outcompeting host glycan–virus interactions, and the comprehensive screening of all nonstructural and structural proteins of SARS-CoV-2. A glaring omission appears to be high-throughput phenotypic cellular entry-based screening against SARS-CoV-2, which is likely to yield better results because of its target-agnostic nature. Likewise, artificial intelligence (AI)-based drug discovery [101] has not yet been implemented for identifying potent binders of all 20 SARS-CoV-2 proteins.

There is also a possibility that several other promising targets are being sidestepped completely. One example is human heparanase, which is known in some enveloped viruses, such as HSV [102], to contribute to the removal of HS chains from cell surfaces, thereby facilitating virus release from host cells. It is likely that heparanase has a similar role for coronaviruses. Thus, heparanase inhibitors, such as PI-88 and SST0001, which are currently in clinical trials as

anticancer agents (NCT00268593 and NCT01764880), might also be effective against SARS-CoV-2.

Overall, the unprecedented COVD-19 pandemic raises the expectations that the scientific community should be ever-ready to rapidly discover and develop efficacious and quality agents to avoid the risk of a pandemic. Hopefully, this experience will ensure that emphasis on understanding the fundamentals of biology and chemistry of these infectious agents remains high for years to come.

## Author contributions

V.T. prepared and finalized the manuscript; J.C.B. searched literature and contributed to writing, N.V.S. performed molecular modeling and prepared figures; M.S.M. reviewed and revised the manuscript; and U.R.D. wrote sections and finalized the manuscript.

## Conflict of interest

None declared.

## Acknowledgments

We thank Rama Gunta and Adam Hawkridge for preparing the structure figure and generic MS image used in Fig. 5, respectively. We also thank the computational resources made available to us through a grant from the National Center for Research Resources (S10 RR027411) to V.C.U. This work was supported, in part, by grants from the NIH including HL107152, HL090586 and CA241951 (U.R.D.).



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