UNIVERSITY OF BIRMINGHAM
University of Birmingham Research at Birmingham

Targeting host cell proteases as a potential treatment strategy to limit the spread of SARS-CoV-2 in the respiratory tract

El-Shimy, Ismail A; Mohamed, Mahmoud M A; Hasan, Syed Shahzad; Hadi, Muhammad A

DOI:
10.1002/prp2.698

License:
Creative Commons: Attribution-NonCommercial (CC BY-NC)

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
El-Shimy, IA, Mohamed, MMA, Hasan, SS & Hadi, MA 2021, 'Targeting host cell proteases as a potential treatment strategy to limit the spread of SARS-CoV-2 in the respiratory tract', *Pharmacology Research and Perspectives*, vol. 9, no. 1, e00698. https://doi.org/10.1002/prp2.698

Link to publication on Research at Birmingham portal

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• User may use extracts from the document in line with the concept of ‘fair dealing’ under the Copyright, Designs and Patents Act 1988 (?)
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 28. Jun. 2022

Received: 19 July 2020 | Accepted: 13 November 2020

DOI: 10.1002/prp2.698

REVIEW

Targeting host cell proteases as a potential treatment strategy to limit the spread of SARS-CoV-2 in the respiratory tract

Ismail A. El-Shimy[1,2] | Mahmoud M. A. Mohamed[3] | Syed Shahzad Hasan[4] | Muhammad A. Hadi[5]

[1]Integrative Research Institute (IRI) for Life Sciences, Humboldt University Berlin, Berlin, Germany

[2]Institute of Pathology, Charité - Universitätsmedizin Berlin, Berlin, Germany

[3]Department of Radiology, Charité - Universitätsmedizin Berlin, Berlin, Germany

[4]School of Applied Sciences, University of Huddersfield, Huddersfield, UK

[5]School of Pharmacy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

Correspondence
Muhammad Abdul Hadi, School of Pharmacy, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
Email: m.a.hadi@bham.ac.uk

Abstract

As the death toll of Coronavirus disease 19 (COVID-19) continues to rise worldwide, it is imperative to explore novel molecular mechanisms for targeting SARS-CoV-2. Rather than looking for drugs that directly interact with key viral proteins inhibiting its replication, an alternative and possibly add-on approach is to dismantle the host cell machinery that enables the virus to infect the host cell and spread from one cell to another. Excellent examples of such machinery are host cell proteases whose role in viral pathogenesis has been demonstrated in numerous coronaviruses. In this review, we propose two therapeutic modalities to tackle SARS-CoV-2 infections; the first is to transcriptionally modulate the expression of cellular proteases and their endogenous inhibitors and the second is to directly inhibit their enzymatic activity. We present a nonexhaustive collection of clinically investigated drugs that act by one of these mechanisms and thus represent promising candidates for preclinical in vitro testing and hopefully clinical testing in COVID-19 patients.

KEYWORDS
adjunctive therapy, clinical trial, COVID-19, proteases, SARS-CoV-2

1 | INTRODUCTION

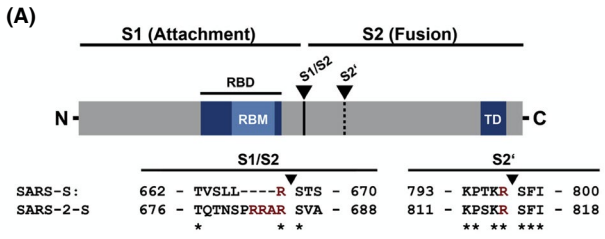
Coronavirus disease 19 (COVID-19) pandemic is one of the major challenges that is currently facing human societies throughout the globe. At the time of writing this review, the number of confirmed cases according to the World Health Organization (WHO) is approaching 55 million and the death toll is exceeding 1.3 million worldwide.[1] COVID-19 is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection in the respiratory tract. Many susceptible patients progress to a severe form of the disease that is characterized by an acute respiratory distress syndrome (ARDS),[2] which severely compromises their respiratory function and often causes organ failure and death. Understanding the mechanism by which the virus infects its host cells in the respiratory tract and spreads through the lung tissue causing its damage is of extreme importance to find an effective treatment. The key viral protein that enables members of the coronavirus family including SARS-CoV-2 to infect their host cells is the spike (S) protein. It is the largest structural protein existing in the virus particles with an overall length ranging between 1200 and 1400 amino acids and is organized into

Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, Coronavirus disease 19; SARS-CoV-2, syndrome coronavirus-2; WHO, World Health Organization.

Ismail Amr El-Shimy and Mahmoud M A Mohamed contributed equally.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2020 The Authors. *Pharmacology Research & Perspectives* published by John Wiley & Sons Ltd, British Pharmacological Society and American Society for Pharmacology and Experimental Therapeutics.

homotrimers that are integrated into the viral envelope giving rise to its characteristic corona-shaped structure.[3] It possesses a large ectodomain, a small transmembrane domain, and a short endodomain. Like all class I viral fusion proteins, the S protein ectodomain is divided into two functional domains: S1 domain which binds to host cell receptors and S2 domain which mediates the fusion between the viral envelope and the host cell membrane. In order for the fusion machinery of the S2 domain to be active, the S protein has to undergo proteolytic processing by a variety of host cell protease enzymes that are able to cleave its ectodomain at a number of specific sites (Figure 1B). Two of these cleavage sites have been well characterized in SARS-CoV-1, namely the S1/S2 site at the boundary between these two domains and the S2' site which exists within the S2 domain itself[4] (Figure 1A). Likewise, a multibasic cleavage site at the S1/S2 boundary has been characterized in the SARS-CoV-2 S protein.[5] It is worth to mention that these protease-catalyzed cleavage events can take place on the cell surface before viral entry into its host cell, inside endosomes during viral entry and in the cytosol during viral protein synthesis by the infected cell. This is why host cell proteases are believed to play a pivotal role in the pathogenesis of many human coronaviruses as well as other pneumotropic viruses such as influenza.



(B)

Protease	NC-IUBMB classification	Subcellular location	Preferential cleavage site P6-P5-P4-P3-P2-P1↓P1'-P2'-P3'-P4'
TMPRSS2	NA	Plasma membrane & extracellular space	NA
Cathepsin L	Cysteine endopeptidase	Plasma membrane & lysosome	X-X-X-hydrophobic-Phe-Arg ↓ X-X-X-X X-X-X-aromatic-Phe-Arg ↓ X-X-X-X X-X-X-hydrophobic-Arg-Arg ↓ X-X-X-X X-X-X-aromatic-Arg-Arg ↓ X-X-X-X
Cathepsin B	Cysteine endopeptidase	Plasma membrane, lysosome & extracellular space	X-X-X-X-Arg-Arg ↓ X-X-X-X
Trypsin	Serine endopeptidase	Extracellular space	X-X-X-X-X-Arg ↓ X-X-X-X X-X-X-X-X-Lys ↓ X-X-X-X
Neutrophil Elastase	Serine endopeptidase	Cytoplasmic vesicles & phagolysosomes in neutrophils	X-X-X-X-X-Val ↓ X-X-X-X X-X-X-X-X-Ala ↓ X-X-X-X
Furin	Serine endopeptidase	Golgi apparatus, plasma membrane, endosome & extracellular space	X-X-Arg-X-Lys-Arg ↓ X-X-X-X X-X-Arg-X-Arg-Arg ↓ X-X-X-X Arg-X-positive-X-X-Arg ↓ X-X-X-X Arg-X-X-X-positive-Arg ↓ X-X-X-X
Factor Xa	Serine endopeptidase	Extracellular space	X-X-Ile-Glu-Gly-Arg ↓ X-X-X-X X-X-Ile-Asp-Gly-Arg ↓ X-X-X-X

FIGURE 1 An illustration of the two cleavage sites of the Spike (S) proteins of SARS-CoV-1 and SARS-CoV-2 and the host cell proteases that can possibly cleave them. (A) Schematic representation of SARS-CoV-1 S protein with its functional domains (RBD, receptor binding domain; RBM, receptor binding motif; TD, transmembrane domain) and its two proteolytic cleavage sites (S1/S2, S2′). Amino acid sequences around the two protease recognition sites (red) are indicated for both S proteins of SARS-CoV-1 and SARS-CoV-2 (asterisks indicate conserved residues). Arrowheads indicate the cleavage site. (copied with permission from Figure 1A by Hoffmann and colleagues[6]). (B) A table listing host cell proteases that are reported to cleave S proteins of coronaviruses together with their subcellular locations, classification according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) and their common recognition sequences with the cleavage site indicated by a downward arrow. Amino acid residues on the N-terminal end of the cleavage site are designated P1, P2, P3,...etc, while those on the C-terminal end are designated P1', P2', P3',...etc "X" denotes any amino acid residue, "hydrophobic" denotes Ala, Val, Leu, Ile, Phe, Trp or Tyr, "aromatic" denotes Phe, Trp, His or Tyr and "positive" denotes Lys, Arg or His[7]

2 | HOST CELL PROTEASES AS POTENTIAL DRUG TARGETS

Here, the focus is on protease enzymes that have been implicated in the proteolytic activation of SARS-CoV-1 and 2 S proteins since both strains were shown to be very similar in terms of genomic sequence homology, infection mechanism and ensuing pathology.[8] These enzymes include trypsin, elastase, thermolysin, cathepsin B, cathepsin L, transmembrane serine proteases (TMPRSS), plasmin, and factor Xa. All of these proteases were reported to cleave the S protein in vitro at the S1/S2 or S2' sites or both.[9] Of particular importance is TMPRSS2 which was shown to associate with ACE2, a host cell receptor for SARS-CoV-1, and form complexes that improve viral entry at the cell surface.[10] Recently, SARS-CoV-2 was also shown to utilize the same ACE2

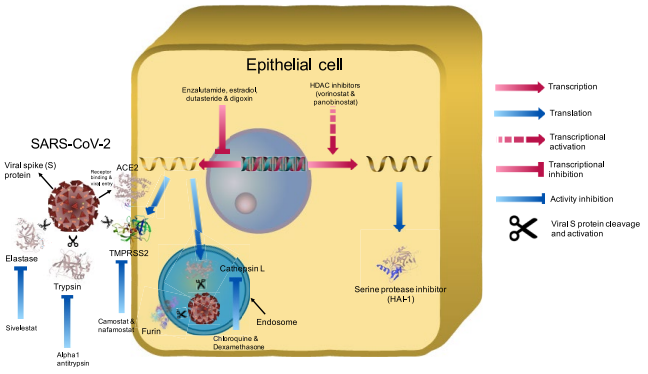


FIGURE 2 A diagram illustrating the different drug candidates, their targets and mechanisms of action. Host cell proteases depicted here are reported to proteolytically cleave the S protein of coronaviruses which is an essential step to initiate the fusion process between viral and epithelial cell membranes. Three categories of drugs are described; drugs that downregulate the expression of protease enzymes, drugs that directly inhibit their enzymatic activity, and drugs that upregulate the expression of endogenous protease inhibitors. All elements used in this illustration come from the Reactome icon library (https://reactome.org/icon-lib). Protein structures of host cell proteases and protease inhibitors were obtained from UniProt knowledgebase (https://www.uniprot.org/) and Protein Data Bank (https://www.rcsb.org/). Abbreviations: TMPRSS, transmembrane serine protease; HDAC, histone deacetylase; HAI-1, hepatocyte growth factor activator inhibitor type 1

receptor for entry and TMPRSS2 protease activity to prime its S protein[6] (Figure 2). In addition, the host cell protease furin was shown to cleave the SARS-CoV-2 S protein at the S1/S2 site which is an essential event for spike-driven viral entry into lung cells.[5] Moreover, the endosomal cysteine proteases cathepsin B and L were found to participate in processing SARS-CoV-1 and 2 S proteins enabling them to be primed even in the absence of TMPRSS2 activity.[6,11] Beside proteases, host cells are equipped with a collection of natural protease inhibitors that control the activity of many of the above-mentioned proteolytic enzymes such as alpha1 antitrypsin (AAT) which inhibits trypsin and elastase and hepatocyte growth factor activator inhibitor type 1 (HAI-1) and type 2 (HAI-2) which regulate activities of transmembrane serine proteases including TMPRSS2.[12] Interestingly, HAI-2 was reported to inhibit influenza virus H1N1 infection in cell culture, and its administration showed protective effects in a mouse model of influenza.[13] This has been attributed to its effective inhibition of the proteolytic cleavage of influenza virus hemagglutinin (HA) which is another class I viral fusion protein that shares many common features with the coronavirus S protein. Similar to the S protein, HA is synthesized as an intact precursor that gets cleaved by host cell proteases giving rise to two functional subunits HA1 and HA2. HA1 resembles the S1 domain of S protein where it binds to sialic acid receptors on the host cell surface, while HA2—just as the S2 domain—contains a fusion peptide that gets exposed upon cleavage initiating the fusion process between viral and host cell membranes.[9] Beside HAI-1 and HAI-2, the serine protease inhibitor, plasminogen activator inhibitor 1 (PAI-1) was likewise shown to inhibit HA cleavage and prevent H1N1 influenza virus replication both ex vivo and in vivo.[14]

In light of what is known so far about proteases and their inhibitors, two treatment approaches are proposed to control SARS-CoV-2 infections. The first approach is to use transcriptional suppressors that downregulate the expression of protease enzymes on the gene level or alternatively transcriptional activators that enhance the expression of the naturally occurring protease inhibitors. The second approach is to target host cell proteases directly through the use of small-molecule compounds or proteins that are known to inhibit their enzymatic activity. In this review, we discuss these two approaches and present a number of drug candidates that act by one or more of these mechanisms, and as such have a great potential for clinical use to limit viral infectivity and spread.

One obvious limitation of these targeting approaches is the fact that host cells are equipped with a wide range of proteolytic enzymes—as discussed above—that were found to cleave coronavirus S protein. Targeting one specific enzyme with an inhibiting molecule may not be sufficient to stop viral spread due to the abundance of other proteases. Despite this drawback, it is obvious at least from a number of in vitro and in vivo studies that cleavage of S protein by specific proteases appears to be more important for viral pathogenicity than by others and that inhibiting one of these enzymes can significantly block viral entry into its host cells. One particular example is TMPRSS2 where its inhibition in human lung cell lines clearly blocked their infection with SARS-CoV-2 in vitro.[6] The same concept applies to other kinds of viruses that possess class I viral fusion proteins as influenza A where inhibition of trypsin-like serine proteases using the naturally occurring protease inhibitors HAI-2 and PAI-1 displayed antiviral activity both in vitro and in vivo.[13,14] Furthermore, combination antiprotease therapy targeting more than one protease enzyme simultaneously is another possibility to enhance the antiviral activity of such drugs. Some drug combinations with acceptable safety profiles are discussed below to serve this purpose.

3 | DRUGS AFFECTING TRANSCRIPTIONAL REGULATION OF HOST CELL PROTEASES OR PROTEASE INHIBITORS

3.1 | Drugs targeting androgen and estrogen receptors

TMPRSS2 gene expression is known to be activated by the androgen receptor.[15-17] For this reason, drugs that block this receptor are believed to interfere with TMPRSS2 expression resulting in its downregulation. In line with this, enzalutamide (an androgen receptor antagonist), estradiol, and the phytoestrogen genistein were recently reported to downregulate the expression of TMPRSS2 using RNA sequencing data derived from human prostate, breast, and endometrial cancer cell lines.[18] On the other hand, testosterone and metribolone (a synthetic androgen) were shown to significantly increase its expression in human prostate cancer cells. Consistent with these findings, treatment of prostate cancer cell lines with enzalutamide again resulted in reduced TMPRSS2 mRNA expression measured by real-time quantitative polymerase chain reaction (qPCR).[19] Moreover, fulvestrant (an estrogen receptor antagonist) was found to upregulate TMPRSS2 expression in a breast cancer cell line using single-cell RNA sequencing data.[18] Notably, the authors demonstrated that genes that were highly correlated with TMPRSS2 expression in RNA sequencing data obtained from human lung tissues were significantly enriched for androgen and estrogen response hallmark genes. This provides sound evidence that androgen and estrogen receptors are important transcriptional regulators of TMPRSS2 gene in human lung cells. It is worth mentioning here that estrogen receptor signaling played a protective role in female mice infected with SARS-CoV-1 where ovariectomy or treating the mice with an estrogen receptor antagonist led to increased mortality.[20] Moreover, there is a growing body of evidence suggesting that male sex is a predisposing factor to COVID-19. One possible mechanism that could contribute to this predisposition is the increased expression of transmembrane serine proteases, particularly TMPRSS2, in response to male sex hormones.[21,22]

Regarding their clinical utility, both enzalutamide and estradiol have a well-established clinical profile making them suitable for trial in COVID-19 patients. Enzalutamide is clinically approved for treatment of patients with castration-resistant prostate cancer and

metastatic castration-sensitive prostate cancer.[23] The most common adverse effects associated with its use are peripheral edema, hyperglycemia, hyponatremia, hypermagnesemia, upper respiratory tract infection, asthenia, back pain, disturbed bowel movement, and arthralgia.[23] On the other hand, oral administration of estrogen and its synthetic derivatives has long been used for contraception and treatment of menopause-related conditions such as vaginal atrophy and osteoporosis.[24] Adverse effects of systemic estradiol include vaginal hemorrhage, edema, headache, gastrointestinal discomfort, mastalgia, deep vein thrombosis, and nasopharyngitis.[25,26] Given such toxicity profiles, we propose that enzalutamide can be used at an oral daily dose of 160 mg in male patients especially those with a compelling indication as prostate cancer. Likewise, estradiol combined with a progestin can be administered at an oral daily dose of 1-2 mg to female patients.

3.2 | 5-alpha reductase inhibitors

5-alpha reductase inhibitors are another class of drugs that influence intracellular androgen signaling and are clinically used to treat conditions with excessive androgen production such as benign prostatic hyperplasia and male pattern hair loss.[27] By inhibiting 5-alpha reductase enzyme, these drugs prevent the conversion of testosterone to the more active dihydrotestosterone and hence attenuate androgen-mediated cellular responses.[27] This in turn can lead to a reduction in the expression of androgen-regulated genes including TMPRSS2. Indeed, treatment with the clinically approved inhibitor dutasteride was found to reduce TMPRSS2 expression in microdissected prostate epithelial tumor tissues as measured by both gene expression microarrays and real-time PCR.[28] This drug also significantly decreased TMPRSS2 staining in the neoplastic prostate epithelium. With regard to its safety, dutasteride is generally well tolerated and causes no serious adverse effects. Its use is associated with decreased libido, ejaculation disorder, and erectile dysfunction, however, these effects are usually mild and decrease over time.[29] In terms of applicability to COVID-19 patients, we propose this drug can be used at an oral daily dose of 0.5 mg. However, it is important to mention that it is contraindicated in pregnant women or women who may become pregnant due to potential risk to a male fetus.[30]

3.3 | Histone deacetylase (HDAC) inhibitors

HDAC inhibitors are compounds that inhibit histone deacetylase enzyme which catalyzes the removal of acetyl groups from both histone and nonhistone proteins including transcription factors. Inhibition of these deacetylation reactions alters the compactness of the chromatin structure and the transcriptional regulation of many genes that control the cell cycle, proliferation, and apoptosis.[31] For this reason, HDAC inhibitors have been used as anticancer drugs. Among the genes whose expression is altered by HDAC inhibition are those encoding the serine protease inhibitors HAI-1 and HAI-2. By querying the connectivity map (CMAP) drug perturbation signatures,[32] we found that the classical HDAC inhibitors apicidin and trichostatin A increased the expression of SPINT1 and SPINT2 genes which encode HAI-1 and HAI-2, respectively, in human lung cancer cell lines as measured by L1000 gene expression assay. Similarly, a number of second-generation HDAC inhibitors (vorinostat, panobinostat, and mocetinostat) were found to increase the expression of one of these two genes or both in the same cell lines.

In the matter of clinical relevance, vorinostat and panobinostat are both clinically approved for treatment of T-cell lymphoma and multiple myeloma, respectively. In terms of safety, the most common adverse effects associated with vorinostat are fatigue, nausea, diarrhea, thrombocytopenia, anorexia, and dysgeusia. Pulmonary embolism, anemia, and squamous cell carcinoma of the skin are more severe toxicities that were reported in less than 5% of patients.[33,34] Similarly, panobinostat use is linked to asthenia, fatigue, and diarrhea, however, it is also associated with pneumonia, peripheral neuropathy and the more serious myelosuppression which manifests as thrombocytopenia, neutropenia, and lymphopenia.[35] Given the blood toxicity profiles of these drugs, we give them less priority for clinical investigation in COVID-19 despite their seemingly desirable effects on host cell proteases as we believe their risk-benefit ratio does not favor their use.

3.4 | Cardiac glycosides

Long known for their positive inotropic effect, cardiac glycosides have been used for managing patients with cardiac muscle failure for a very long time. However, because of their narrow therapeutic index and relatively high risk of toxicity associated with their prolonged use, their clinical indications are now restricted.[36] Interestingly, these compounds may still provide some benefits for patients with coronavirus infections. By examining a large collection of RNA sequencing data, Wang and colleagues showed that digitalis-like compounds including digoxin and proscillaridin A markedly reduced expression of the endosomal cathepsins B and L in human thyroid cancer cells.[37] This effect can limit S protein processing and priming in the endosomal compartments of infected host cells.

Digoxin use has long been associated with gastrointestinal adverse effects, visual disturbances, and cardiovascular toxicity including cardiac arrhythmia, tachycardia, and heart block.[38] Despite its potential for cardiovascular toxicity, we suggest that digoxin can be tested in COVID-19 patients at an oral daily dose of less than 0.125 mg such that its serum concentration is kept below 1 ng/mL. At such low concentration, digoxin is not only associated with minimal adverse effects, but also exerts beneficial hemodynamic, neurohormonal, and clinical effects in patients with congestive heart failure and other cardiovascular diseases.[39] Accordingly, we encourage testing digoxin in COVID-19 patients with preexisting cardiovascular conditions such as heart failure.

4 | DRUGS THAT DIRECTLY INHIBIT HOST CELL PROTEASE ACTIVITY

4.1 | Human neutrophil elastase (HNE) inhibitors

Elastase and other serine proteases released from neutrophils at inflammation sites are believed to play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), pneumonia, and acute lung injury which may result from viral infections.[40,41] These diseases are usually accompanied by a local imbalance between proteases (serine proteases, cathepsins, and metalloproteases) and antiproteases (alpha1 antitrypsin, a2-macroglobulin, cystatins, and tissue inhibitors of metalloproteinases). This increased activity of proteolytic enzymes particularly elastase leads to the breakdown of elastin and other components of the lung connective tissue and induce the release of pro-inflammatory cytokines augmenting the destructive inflammatory response.[42] In addition, elastase was shown to activate the SARS-CoV-1 S protein and shift the viral entry to a low pH-independent route.[43,44] Therefore, inhibition of elastase would serve two purposes; the first is to prevent S protein cleavage and activation and the second is to ameliorate elastase-mediated lung damage. A vast range of HNE inhibitors have already been developed to treat various lung inflammatory conditions including COPD, CF, bronchiectasis, acute lung injury and ARDS[41] and are now being tested in clinical trials. We believe these candidates would be extremely beneficial in the management of patients with severe SARS-CoV-2 infection.

To date, there are no known severe toxicities that could limit the use of the HNE inhibitor sivelestat in COVID-19 patients. Adverse effects with the highest reported incidence in clinical trials are hepatobiliary disorders including elevated blood bilirubin and liver enzymes.[45] Such safety profile makes this drug of particular interest for clinical testing in COVID-19 patients upon hospital admission. Intravenous administration of sivelestat to hospitalized patients at a dose of 0.2 mg/kg per hour is proposed as a preventative treatment option to attenuate ARDS associated with the viral infection and improve the mortality rate in patients with advanced disease.[46,47]

4.2 | Alpha1 antitrypsin (AAT)

This alpha globulin glycoprotein is one of the most widely known members of the SERPIN superfamily of serine protease inhibitors. It is mainly synthesized in the liver by hepatocytes and released into the bloodstream. It is also synthesized by the pancreas, lung alveolar cells, vascular endothelium, and intestinal epithelium.[48] Genetic deficiency of AAT leads to a wide range of pathologies affecting mainly the lungs, liver, and blood vessels.[48] AAT possesses anti-inflammatory, immunomodulatory, and anti-infection activity. It promotes tissue repair and protects tissues from damage induced by proteolytic enzymes released from inflammatory cells.[48] Equally important, AAT inhibits the activity of trypsin and elastase which are known to cleave and activate the S protein of SARS-CoV-1[9] and possibly also SARS-CoV-2. Similar to HNE inhibitors, these properties make AAT a promising candidate for managing COVID-19 patients.[49]

Like sivelestat, the toxicity profile of AAT therapy should not limit its use in COVID-19 patients. The most common adverse reactions are typical of intravenous infusion of proteins and include fever, chills, urticaria, nausea, vomiting, and fatigue.[50] Dyspnea, anaphylactic reactions, and exacerbation of heart failure were also reported in patients receiving AAT therapy, however, incidence of these events was rather low.[50] Accordingly, intravenous infusion of AAT at a dose of 60 mg/kg weekly is proposed as another preventative measure that can protect against ARDS and reduce mortality in hospitalized COVID-19 patients.

4.3 | Inhibitors of the clotting factor Xa

The clotting protein factor Xa is a serine protease that is derived from the hydrolysis of its precursor factor X which takes place via two principal pathways.[51] The first is the extrinsic pathway which occurs at the surface of a damaged endothelium and macrophages and involves activation of factor X by factor VII/VIIa in association with a membrane-bound cofactor, tissue factor (TF). The second is the intrinsic pathway in which factor X is activated on the platelet surface by a membrane-bound tenase complex comprising factor IXa, its cofactor factor VIIIa, and calcium ions. Factor Xa then activates prothrombin to thrombin eventually leading to blood clotting. In the context of SARS-CoV-1 infections, factor Xa was found to cleave the viral S protein at the S1/S2 boundary and promote entry into host cells.[52] Hence, drugs that act as inhibitors of factor Xa are considered protective against S protein priming and viral entry. Three factor Xa inhibitors (rivaroxaban, apixaban, and edoxaban) are now approved as novel oral anticoagulants and can be used as a replacement for the vitamin K antagonist warfarin.[53]

Given that COVID-19-associated coagulopathy (CAC) has been reported in several studies,[54,55] the use of factor Xa inhibitors may offer additional benefit by preventing development of venous thromboembolism (more common in COVID-19 patients) and arterial thromboembolism (less common) especially among seriously or critically ill patients with COVID-19.[56] A recently published retrospective analysis involving 4389 hospitalized COVID-19 patients reported a lower mortality and intubation in the anticoagulation group compared to the control group.[57] A number of clinical trials are underway to investigate the effectiveness of heparin and low molecular weight heparin both in prophylactic and therapeutic doses to prevent thromboembolism in COVID-19 patients.[58-64]

Although the pathogenesis of hypercoagulability in COVID-19 patients is not fully understood, it has been proposed that all three components of Virchow's triad including endothelial injury, stasis, and hypercoagulable state are included.[54,56] Elevated levels of a number of prothrombin factors including von Willebrand factor (vWF), factor VIII, D-dimer, fibrinogen, neutrophil extracellular traps, prothrombotic microparticles, and anionic phospholipids are believed to be responsible for CAC . We believe that Xa inhibitors

TABLE 1 A list of clinically approved and/or investigational drug candidates that target host cell proteases via transcriptional regulation or direct inhibition of protease activity together with their clinical indications, available routes of administration, and ongoing clinical trials in COVID-19 patients

Drug candidates	Targeted host cell factor	Reported mechanism	Clinical indications	Routes of administration	Clinical trials in COVID-19 patients**
Androgen receptor antagonists (enzalutamide)	TMPRSS2	Downregulation of protease expression[18,19]	*Treatment of metastatic castration-resistant prostate cancer	Oral	NCT04475601 NCT04456049
Estrogen receptor agonists (estradiol)	TMPRSS2	Downregulation of protease expression[18]	*Treatment of moderate to severe vasomotor symptoms and vulvar and vaginal atrophy due to menopause *Prevention of postmenopausal osteoporosis *Treatment of estrogen deficiency due to hypogonadism, castration or primary ovarian failure *Oral contraception for preventing pregnancy (in combination with synthetic progestins)	Oral, topical, transdermal and vaginal	NCT04359329 NCT04539626
5-alpha reductase inhibitors (dutasteride)	TMPRSS2	Downregulation of protease expression[28]	*Treatment of symptomatic benign prostatic hyperplasia	Oral	NCT04446429
Camostat and nafamostat mesilate	TMPRSS2	Inhibition of protease activity[10,72]	†Treatment of chronic pancreatitis in Japan[94-96] †Treatment of COVID-19 (indication under investigation)	Oral	Camostat mesilate (149 trials eg, NCT04583592, NCT04353284 & NCT04455815) Nafamostat mesilate (6 trials eg, NCT04418128, NCT04623021 & NCT04352400)
HDAC inhibitors (trichostatin A, vorinostat and panobinostat)	HAI-1 and HAI-2	Upregulation of protease inhibitor expression (through query of CMAP drug perturbation signatures[32])	*Vorinostat is approved for treatment of cutaneous manifestations in patients with progressive cutaneous T-cell lymphoma *Panobinostat is approved for treatment of multiple myeloma	Oral	None
Cardiac glycosides (Digoxin)	Cathepsin B and L	Downregulation of protease expression[18]	*Treatment of mild to moderate heart failure in adults *Control of ventricular rate in patients with chronic atrial fibrillation *Increasing myocardial contraction in children with heart failure	Oral, intramuscular and intravenous	None
Glycopeptide antibiotics (Teicoplanin)	Cathepsin L	Inhibition of protease activity[79,80]	*Treatment and/or prophylaxis of bacterial infections caused by susceptible microorganisms	Oral, intramuscular and intravenous	None

(Continues)

Table 1 (Continued)

Drug candidates	Targeted host cell factor	Reported mechanism	Clinical indications	Routes of administration	Clinical trials in COVID-19 patients**
Dexamethasone	Cathepsin B and L	Inhibition of protease activity[83,84]	*As otic suspension for treating inflammation associated with bacterial infections in acute otitis media and acute otitis externa (in combination with ciprofloxacin) *As intramuscular and intravenous injections for treating a variety of endocrine, rheumatic, dermatologic, allergic, ophthalmic, gastrointestinal, respiratory, hematologic, neoplastic, edematous, and other conditions. *As oral tablets for treatment of multiple myeloma *As intravitreal implant for treatment of some forms of macular edema and non-infectious posterior uveitis *As various ophthalmic formulations for treating inflammatory conditions of the eye †Management of COVID-19 patients with severe respiratory symptoms (indication under investigation[97])	Numerous routes of administration are available	2114 trials eg, NCT04347980, NCT04513184 & NCT04509973
Chloroquine and hydroxychloroquine	Lysosomal cathepsins	Inhibition of protease activity by increasing endosomal pH[85,86]	*Treatment of infections by *Plasmodium malariae*, *P vivax*, *P ovale*, and susceptible strains of *P falciparum* *Treatment of extraintestinal amebiasis *Treatment of rheumatic diseases †Treatment and prophylaxis of Zika virus infection[98,99] †Treatment of COVID-19 (Hydroxychloroquine's effect on mortality of hospitalized COVID-19 patients was not significant [87,88])	Oral	Chloroquine (27 trials eg, NCT04331600, NCT04428268 & NCT04420247) Hydroxychloroquine (151 trials eg, NCT04345692, NCT04351620 & NCT04466540)
HNE inhibitors (sivelestat)	Elastase	Inhibition of protease activity[41]	†Treatment of acute lung injury and ARDS in adults[100]	Intravenous	None
Alpha1 antitrypsin	Trypsin and elastase	Inhibition of protease activity[48]	*Treatment of alpha 1 antitrypsin deficiency †Treatment of atopic dermatitis[101] and COPD[102]	Intravenous, topical, and inhalation	NCT04547140 NCT04495101 NCT04385836
Factor Xa inhibitors (rivaroxaban, apixaban, and edoxaban)	Factor Xa	Inhibition of protease activity[53]	*Prevention of venous thromboembolism after total knee or hip replacement surgeries *Prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation *Treatment of deep vein thrombosis and pulmonary embolism *Prophylaxis against major cardiovascular events in coronary artery disease or peripheral artery disease patients (in combination with aspirin)	Oral	Rivaroxaban (7 trials eg, NCT04504032, NCT04508023, NCT04416048 & NCT04324463) Apixaban (NCT04498273 & NCT04512079) Edoxaban (NCT04542408 & NCT04516941)

Note: Indications denoted by * are FDA approved and those denoted by † are currently under investigation. Information concerning clinical indications and available routes of administration was obtained from the DrugBank online database (https://www.drugbank.com/). **All clinical trial information for the drug candidates was obtained from ClinicalTrials.gov, number of clinical trials and their identifiers are specified whenever applicable (https://clinicaltrials.gov/, accessed 17 November 2020).

can act by a dual mechanism; by inhibiting viral entry and preventing CAC.[65]

4.4 | Other important candidates with serine/cysteine protease inhibitory activity

An additional set of small molecule and protein inhibitors of serine proteases, especially TMPRSS2, have been thoroughly reviewed.[66] Of particular importance are the two TMPRSS2 inhibitors camostat and nafamostat mesilate. Camostat has shown remarkable in vitro antiviral activity against H1N1 influenza, SARS-CoV-1 and the Middle East respiratory syndrome-related coronavirus (MERS-CoV) infections in human tracheal epithelial cells, human lung cancer cells and monkey kidney epithelial cells.[10,67,68] Moreover, camostat exhibited in vivo antiviral activity against H1N1 influenza and SARS-CoV-1 in mouse models.[69-71] It was also recently shown that camostat can block SARS-CoV-2 entry into human lung cancer cells and therefore constitutes a potential therapeutic option.[6] Interestingly, nafamostat was found to be more effective than camostat in blocking MERS-CoV entry and replication in host cells.[72] Another specific TMPRSS2 inhibitor with great potential is the mucolytic agent bromhexine hydrochloride[73] whose high safety profile makes it an excellent candidate for clinical testing against SARS-CoV-2. In addition, other serine protease inhibitors showed remarkable antiviral effects against H1N1 influenza infections both in vitro and in vivo by virtue of their ability to block HA cleavage and activation, and thus are good candidates to be tested against SARS-CoV-2 infections. These include ovomucoid, aprotinin and 4-(2-aminomethyl) benzenesulfonyl fluoride hydrochloride.[71,74-76] It is worth mentioning here that a number of FDA-approved drugs are reported to inhibit the activity of cathepsins specifically cathepsin L and these have been recently reviewed and proposed as potential candidates for the treatment of COVID-19.[77] Examples include the antileprotic drug clofazimine,[78] the antibiotic teicoplanin,[79,80] the anti-HIV saquinavir,[81] the antituberculosis rifampicin,[78] the antioxidant astaxanthin,[82] the anti-inflammatory dexamethasone,[83,84] and the antimalarial chloroquine.[85,86] Although the latter chloroquine and its derivative hydroxychloroquine are currently used in treatment protocols of COVID-19, it was recently reported that hydroxychloroquine did not reduce mortality in hospitalized COVID-19 patients compared to standard of care.[87,88]

Among all aforementioned candidates, camostat and nafamostat are currently receiving the most attention in terms of clinical testing in COVID-19. Camostat administration was associated with mild adverse effects when used in 95 patients suffering from dyspepsia associated with nonalcoholic pancreatic disease at a dose of 200 mg three times daily for 2 weeks. Only two of the 95 patients developed very mild side effects.[89] Similarly, nafamostat did not show any serious adverse effects when administered to patients with severe acute pancreatitis at a dose of 240 mg/day intravenously or via arterial infusion.[90] Yet, the safety and efficacy profile of these two drugs in COVID-19 patients is still being assessed in 11 trials with larger sample sizes (Table 1). In addition to TMPRSS2 inhibitors, the endosomal cathepsin L inhibitor teicoplanin is considered a potential antibiotic for treating secondary bacterial infections associated with SARS-CoV-2. The adverse effects most frequently associated with teicoplanin treatment are local and hypersensitivity reactions, such as itching and drug fever; anaphylactic reactions including the "red man syndrome" are uncommon. Teicoplanin is also less likely than vancomycin to cause nephrotoxicity, especially when administered in combination with an aminoglycoside.[91-93]

5 | COMBINATION PROTEASE-TARGETING THERAPY

As discussed earlier, combinatorial protease-targeting therapy is propounded to tackle the problem of redundancy of host cell proteases that can process and activate coronavirus S protein. Targeting more than one protease enzyme using a drug combination is likely to be more effective in combating viral spread than inhibiting just a single enzyme. We propose here a number of protease-targeting drug combinations that we believe would provide potential therapeutic benefit for hospitalized COVID-19 patients. Inhibitors of the membrane-bound TMPRSS2 are of particular importance because of the key role this enzyme plays in early S protein processing at the cell surface.[6] Combining such inhibitors with drugs targeting the extracellular proteases as trypsin and elastase or those targeting the endosomal proteases as cathepsin L is a considerable option as such combinations would provide an adequate coverage of host cell proteases across the different compartments (Figure 2). A TMPRSS2 inhibitor as camostat or nafamostat and an elastase inhibitor as sivelestat are recommended to be administered to all hospitalized patients starting on day 1 of admission. This may help limit viral spread in the respiratory tract in the early days of admission and at the same time protect against ARDS and viral-induced inflammatory lung damage.[45,46] Another alternative combination with the same rationale would be oral dutasteride (which acts via TMPRSS2 downregulation) instead of camostat together with IV sivelestat. When it comes to targeting androgen receptor signaling and TMPRSS2 expression, the 5-alpha reductase inhibitor dutasteride is preferred over the androgen receptor antagonist enzalutamide because its adverse effects are generally less severe. IV AAT is also suggested for administration with TMPRSS2 inhibitors as an alternative to sivelestat since both of them are expected to exert similar beneficial effects in terms of mitigating elastase-induced lung damage and inflammation.[46,49] Dexamethasone (which inhibits cathepsins B and L[83,84]) can be added to these combinations once the patient develops a severe respiratory deficit and requires oxygen support since this drug has already been shown to reduce mortality in this particular clinical scenario.[97] Another potential candidate to add to such combinations is the oral secretolytic agent bromhexine hydrochloride which showed inhibitory activity against TMPRSS2 and

is generally safe to use.[73] Moreover, we recommend the use of the glycopeptide antibiotic teicoplanin in hospitalized patients who develop pneumonia as a result of secondary bacterial infections as this antibiotic was shown to inhibit cathepsin L[79,80] and thus may help to control the viral infection as well.

The question concerning the optimal timing for administration of antiprotease combinations is definitely pivotal. We propose that oral TMPRSS2-targeting drugs such as camostat, dutasteride, and bromhexine should be initiated as early as possible once a positive test of the virus is established as currently investigated in several clinical trials of camostat in COVID-19.[103-106] We hypothesize that the clinical outcome would be significantly improved if the drugs are administered early. The elastase inhibitors AAT and sivelestat should be administered only to hospitalized patients who suffer from a progressive disease and pronounced dyspnea. We encourage that these agents are to be administered—as stated above—together with camostat or dutasteride once patients are admitted to the hospital and continued throughout the hospitalization period to provide adequate prophylaxis against COVID-19 respiratory complications particularly ARDS.[46,47,49] We also emphasize here that such antiprotease treatment should always be tested as an add-on therapy to available antiviral drugs and not as replacement since it is vital to target the virus both directly and indirectly to achieve the best clinical outcome possible.

It is very important to point out that several antiviral drugs that are currently being tested against SARS-CoV-2 have not shown significant clinical benefit in COVID-19 patients. There are at least four published randomized controlled trials evaluating the effectiveness of the RNA polymerase inhibitor remdesivir in treating COVID-19 patients.[107-110] These trials reported mixed findings with no clear mortality benefits. Wang et al in their randomized controlled trial found that remdesivir did not significantly improve the time to clinical improvement although this time interval was numerically shorter in patients treated with remdesivir within 10 days of symptom onset compared to those treated with placebo.[107] Beigel et al in their randomized controlled trial reported an overall shorter recovery time among patients in the remdesivir group compared to those in the placebo group regardless of disease severity.[108] Goldman et al in their randomized phase III trial comparing a 5-day course with a 10-day standard course of remdesivir reported no significant differences in both groups after adjustment of imbalances in baseline clinical status.[109] Spinner et al also examined the effectiveness of a 5-day and a 10-day remdesivir course, compared with standard care at day 11 after treatment initiation in a randomized phase III trial involving hospitalized patients with moderate COVID-19.[110] The authors found that patients who received remdesivir for 5 days had significantly higher odds of better clinical status distribution compared to those who received standard care. Although the FDA has approved remdesivir for COVID-19 treatment, the current evidence is insufficient to support its use as a first-line therapy against COVID-19. Regarding the safety and efficacy of the viral protease inhibitors lopinavir-ritonavir in COVID-19 patients, there are at least six published randomized controlled trials.[111-116] Most of the trials are of small sample size and show either no benefit or only a mild reduction in time to symptoms resolution with low certainty.[117] A recent large-sized trial showed that lopinavir-ritonavir combination has no significant effect on the 28-day mortality, duration of hospital stay, risk of progression to invasive mechanical ventilation or death.[115] Consequently, the evidence available so far does not support the use of lopinavir-ritonavir combination for treating hospitalized COVID-19 patients. Taken together, the results of all these clinical trials of antiviral drugs clearly emphasize the need for alternative approaches to treat SARS-CoV-2 infections. One such approach discussed extensively in this review is targeting host cell protease enzymes, which we believe is worth exploring.

6 | CONCLUSION AND OUTLOOK

In summary, we emphasize the importance of host cell proteases as potential drug targets for the treatment of SARS-CoV-2 infection and highlight therapeutic mechanisms that have not been sufficiently exploited to impair the virus capacity to spread through its host cells. We present a collection of clinically approved drugs as well as drugs under investigation that either suppress the transcription of proteases or increase the expression of their natural protease inhibitors. We also include drugs that are reported to possess protease inhibitory activity. These are believed to be potential candidates for drug repurposing and immediate preclinical testing in cell line models to verify their efficacy. As many of these candidates have been in clinical use for several years and their safety profiles are well established, we encourage their rapid clinical testing in patients with COVID-19 in combination with antiviral drugs. This would constitute a two-hit approach where not only viral proteins/enzymes necessary for viral replication are targeted but also the host cell machinery that the virus takes advantage of to facilitate its propagation in its infected host.

7 | Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[118] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[119]

DISCLOSURE

None.

AUTHOR CONTRIBUTIONS

IAS and MMAM conceptualized the idea in consultation with MAH. IAS and MMAM reviewed literature and wrote the initial draft. MAH and SSH critically reviewed the initial draft and expanded certain sections of the review. All authors contributed to revising the

manuscript in view of reviewers' comments. All authors have read and approved the final version for publication.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Ismail A. El-Shimy https://orcid.org/0000-0003-0587-5140
Syed Shahzad Hasan https://orcid.org/0000-0002-4058-2215
Muhammad A. Hadi https://orcid.org/0000-0003-0108-7833

REFERENCES

1. Coronavirus disease (COVID-19) outbreak. World Health Organization (WHO) https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
2. Hariri L, Hardin CC. Covid-19, angiogenesis, and ARDS endotypes. *N Engl J Med*. 2020;383:182-183.
3. Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses*. 2012;4:1011-1033.
4. Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc Natl Acad Sci USA*. 2009;106:5871-5876.
5. Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol Cell*. 2020;78:779-784.e5.
6. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181:271-280.e8.
7. Barrett A, Rawlings N, Woessner J. *Handbook of Proteolytic Enzymes*, 3rd edn. Elsevier Ltd; 2013.
8. Xu J, Zhao S, Teng T, et al. Systematic comparison of two animal-to-human transmitted human coronaviruses: SARS-CoV-2 and SARS-CoV. *Viruses*. 2020;12:244.
9. Millet JK, Whittaker GR. Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. *Virus Res*. 2015;202:120-134.
10. Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S, Gallagher T. A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. *J Virol*. 2011;85:873-882.
11. Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proc Natl Acad Sci USA*. 2005;102:11876-11881.
12. Kataoka H, Kawaguchi M, Fukushima T, Shimomura T. Hepatocyte growth factor activator inhibitors (HAI-1 and HAI-2): emerging key players in epithelial integrity and cancer: HAI-1 and HAI-2 in epithelial integrity. *Pathol. Int*. 2018;68:145-158.
13. Hamilton BS, Chung C, Cyphers SY, Rinaldi VD, Marcano VC, Whittaker GR. Inhibition of influenza virus infection and hemagglutinin cleavage by the protease inhibitor HAI-2. *Biochem Biophys Res Commun*. 2014;450:1070-1075.
14. Dittmann M, Hoffmann H-H, Scull MA, et al. A serpin shapes the extracellular environment to prevent influenza A virus maturation. *Cell*. 2015;160:631-643.
15. Menon T, Yates JA, Bochar DA. Regulation of androgen-responsive transcription by the chromatin remodeling factor CHD8. *Mol Endocrinol*. 2010;24:1165-1174.
16. Hägglöf C, Hammarsten P, Strömvall K, et al. TMPRSS2-ERG expression predicts prostate cancer survival and associates with stromal biomarkers. *PLoS One*. 2014;9:e86824.
17. Maitland NJ, Frame FM, Polson ES, Lewis JL, Collins AT. Prostate cancer stem cells: do they have a basal or luminal phenotype? *Horm Cancer*. 2011;2:47-61.
18. Wang X, et al. TMPRSS2 transcriptional inhibition as a therapeutic strategy for COVID-19. *Preprints*. 2020.
19. Kregel S, Chen JL, Tom W, et al. Acquired resistance to the second-generation androgen receptor antagonist enzalutamide in castration-resistant prostate cancer. *Oncotarget*. 2016;7:26259-26274.
20. Channappanavar R, Fett C, Mack M, et al. Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus infection. *J. Immunol*. 2017;198:4046-4053.
21. Palaiodimos L, Kokkinidis DG, Li W, et al. Severe obesity, increasing age and male sex are independently associated with worse in-hospital outcomes, and higher in-hospital mortality, in a cohort of patients with COVID-19 in the Bronx, New York. *Metabolism*. 2020;108:154262.
22. Cai H. Sex difference and smoking predisposition in patients with COVID-19. *The Lancet Respir Med*. 2020;8:e20.
23. Astellas Pharma US, Inc., Xtandi (enzalutamide) [package insert]. U.S. Food and Drug Administration (FDA) website https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/203415s015lbl.pdf. 2019.
24. Stefanick ML. Estrogens and progestins: background and history, trends in use, and guidelines and regimens approved by the US Food and Drug Administration. *Am J Med*. 2005;118(Suppl 12B):64-73.
25. Wyeth Laboratories Inc. Alesse (levonorgestrel and ethinyl estradiol) [package insert]. U.S. Food and Drug Administration (FDA) website https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/020683s011lbl.pdf. 2017.
26. Novo Nordisk Inc. Activella (estradiol/norethindrone acetate) [package insert]. U.S. Food and Drug Administration (FDA) website https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/020907s019lbl.pdf. 2017.
27. Aggarwal S, Thareja S, Verma A, Bhardwaj TR, Kumar M. An overview on 5α-reductase inhibitors. *Steroids*. 2010;75:109-153.
28. Mostaghel EA, Geng L, Holcomb I, et al. Variability in the androgen response of prostate epithelium to 5-reductase inhibition: implications for prostate cancer chemoprevention. *Cancer Res*. 2010;70:1286-1295.
29. Hirshburg JM, Kelsey PA, Therrien CA, Gavino AC, Reichenberg JS. Adverse effects and safety of 5-alpha reductase inhibitors (Finasteride, Dutasteride): a systematic review. *J Clin Aesthet Dermatol*. 2016;9:56-62.
30. GlaxoSmithKline. Avodart (dutasteride) [package insert]. U.S. Food and Drug Administration (FDA) website https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/021319s032lbl.pdf. 2020.
31. Khan O, La Thangue NB. HDAC inhibitors in cancer biology: emerging mechanisms and clinical applications. *Immunol Cell Biol*. 2012;90:85-94.
32. Subramanian A, Narayan R, Corsello SM, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. *Cell*. 2017;171:1437-1452.e17.
33. Kavanaugh SM, White LA, Kolesar JM. Vorinostat: a novel therapy for the treatment of cutaneous T-cell lymphoma. *Am J Health Syst Pharm*. 2010;67:793-797.
34. MERCK & CO., INC. Zolinza (vorinostat) [package insert]. U.S. Food and Drug Administration (FDA) website https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/021991s009lbl.pdf. 2018.
35. Greig SL. Panobinostat: a review in relapsed or refractory multiple myeloma. *Target Oncol*. 2016;11:107-114.
36. Prassas I, Diamandis EP. Novel therapeutic applications of cardiac glycosides. *Nat Rev Drug Discov*. 2008;7:926-935.

37. Wang X, Dhindsa R, Povysil G, et al. TMPRSS2 transcriptional inhibition as a therapeutic strategy for COVID-19. *Preprints*. 2020; 2020030360. https://doi.org/10.20944/preprints202003.0360.v2.
38. Ehle M, Patel C, Giugliano RP. Digoxin: clinical highlights: a review of digoxin and its use in contemporary medicine. *Crit Pathw Cardiol*. 2011;10:93-98.
39. Gheorghiade M, Adams KF Jr, Colucci WS. Digoxin in the management of cardiovascular disorders. *Circulation*. 2004;109:2959-2964.
40. Groutas WC, Dou D, Alliston KR. Neutrophil elastase inhibitors. *Expert Opin Ther Pat*. 2011;21:339-354.
41. Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A. The role of neutrophil elastase inhibitors in lung diseases. *Chest*. 2017;152:249-262.
42. Kawabata K, Hagio T, Matsuoka S. The role of neutrophil elastase in acute lung injury. *Eur J Pharmacol*. 2002;451:1-10.
43. Belouzard S, Madu I, Whittaker GR. Elastase-mediated activation of the severe acute respiratory syndrome coronavirus spike protein at discrete sites within the S2 domain. *J Biol Chem*. 2010;285:22758-22763.
44. Matsuyama S, Ujike M, Morikawa S, Tashiro M, Taguchi F. Protease-mediated enhancement of severe acute respiratory syndrome coronavirus infection. *Proc Natl Acad Sci USA*. 2005;102:12543-12547.
45. Aikawa N, Kawasaki Y. Clinical utility of the neutrophil elastase inhibitor sivelestat for the treatment of acute respiratory distress syndrome. *Ther Clin Risk Manag*. 2014;10:621-629.
46. Sahebnasagh A, Saghafi F, Safdari M, et al. Neutrophil elastase inhibitor (sivelestat) may be a promising therapeutic option for management of acute lung injury/acute respiratory distress syndrome or disseminated intravascular coagulation in COVID-19. *Journal of Clinical Pharmacy and Therapeutics*. 2020;45:1515–1519.
47. Mohamed MMA, El-Shimy IA, Hadi MA. Neutrophil Elastase Inhibitors: a potential prophylactic treatment option for SARS-CoV-2-induced respiratory complications? *Crit Care*. 2020;24:311.
48. Kim M, Cai Q, Oh Y. Therapeutic potential of alpha-1 antitrypsin in human disease. *Ann Pediatr Endocrinol Metab*. 2018;23:131-135.
49. Oguntuyo KY, Stevens CS, Siddiquey MN, et al. In plain sight: the role of alpha-1-antitrypsin in COVID-19 pathogenesis and therapeutics. *bioRxiv*. 2020. https://doi.org/10.1101/2020.08.14.248880
50. Petrache I, Hajjar J, Campos M. Safety and efficacy of alpha-1-antitrypsin augmentation therapy in the treatment of patients with alpha-1-antitrypsin deficiency. *Biologics*. 2009;3:193-204.
51. Palta S, Saroa R, Palta A. Overview of the coagulation system. *Indian J. Anaesth*. 2014;58:515-523.
52. Du L, Kao RY, Zhou Y, et al. Cleavage of spike protein of SARS coronavirus by protease factor Xa is associated with viral infectivity. *Biochem Biophys Res Commun*. 2007;359:174-179.
53. Eriksson BI, Quinlan DJ, Eikelboom JW. Novel oral factor Xa and thrombin inhibitors in the management of thromboembolism. *Annu Rev Med*. 2011;62:41-57.
54. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395:1417-1418.
55. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. *N Engl J Med*. 2020;383:120-128.
56. Singhania N, Bansal S, Nimmatoori DP, Ejaz AA, McCullough PA, Singhania G. Current overview on hypercoagulability in COVID-19. *Am J Cardiovasc Drugs*. 2020;20(5):393-403. https://doi.org/10.1007/s40256-020-00431-z
57. Nadkarni GN, Lala A, Bagiella E, et al. Anticoagulation, mortality, bleeding and pathology among patients hospitalized with COVID-19: a single health system study. *J Am Coll Cardiol*. 2020;76:1815–1826. https://doi.org/10.1016/j.jacc.2020.08.041.
58. Eduardo M Rego, MD, PhD, D'Or Institute for Research and Education. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US).Identifier NCT04485429, Efficacy Assessment of Methylprednisolone and Heparin in Patients With COVID-19 Pneumonia. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04485429?term=heparin&cond=Covid19&draw=2&rank=2. 2020.
59. Quovadis Associazione & University of Padova. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Identifier NCT04393805, Heparins for Thromboprophylaxis in COVID-19 Patients: HETHICO Study in Veneto (HETHICO). ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04393805?term=heparin&cond=Covid19&draw=2&rank=4. 2020.
60. Alex Spyropoulos, Northwell Health. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Identifier NCT04401293, Full Dose Heparin Vs. Prophylactic Or Intermediate Dose Heparin in High Risk COVID-19 Patients. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04401293?term=heparin&cond=Covid19&draw=2&rank=6. 2020.
61. Matthew Neal MD, University of Pittsburgh. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US).Identifier NCT04505774, Anti-thrombotics for Adults Hospitalized With COVID-19. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04505774?term=heparin&cond=Covid19&draw=2&rank=7. 2020.
62. University of Manitoba and University Health Network. Toronto. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Identifier NCT04372589, Antithrombotic Therapy to Ameliorate Complications of COVID-19 (ATTACC). ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04372589?term=heparin&cond=Covid19&draw=2&rank=8. 2020.
63. University of Sao Paulo General Hospital. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Identifier NCT04487990, CoV-Hep Study: Regional Anticoagulation Modalities in Continuous Venous Venous Hemodialysis in Patients With COVID-19. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04487990?term=heparin&cond=Covid19&draw=2&rank=9. 2020.
64. de Paris AP-H.ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Identifier NCT04344756, Trial Evaluating Efficacy and Safety of Anticoagulation in Patients With COVID-19 Infection, Nested in the Corimmuno-19 Cohort (CORIMMUNO-COAG). ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04344756?term=heparin&cond=Covid19&draw=2&rank=10. 2020.
65. Ranucci M, Ballotta A, Di Dedda U, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *J Thromb Haemost*. 2020;18:1747-1751.
66. Shen LW, Mao HJ, Wu YL, Tanaka Y, Zhang W. TMPRSS2: a potential target for treatment of influenza virus and coronavirus infections. *Biochimie*. 2017;142:1-10.
67. Yamaya M, Shimotai Y, Hatachi Y, et al. The serine protease inhibitor camostat inhibits influenza virus replication and cytokine production in primary cultures of human tracheal epithelial cells. *Pulm Pharmacol Ther*. 2015;33:66-74.
68. Shirato K, Kawase M, Matsuyama S. Middle east respiratory syndrome coronavirus infection mediated by the transmembrane serine protease TMPRSS2. *J Virol*. 2013;87:12552-12561.
69. Zhou Y, Vedantham P, Lu K, et al. Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral Res*. 2015;116:76-84.
70. Lee MG, Kim KH, Park KY, Kim JS. Evaluation of anti-influenza effects of camostat in mice infected with non-adapted human influenza viruses. *Arch Virol*. 1996;141:1979-1989.
71. Bahgat MM, Błazejewska P, Schughart K. Inhibition of lung serine proteases in mice: a potentially new approach to control influenza infection. *Virol J*. 2011;8:27.
72. Yamamoto M, Matsuyama S, Li X, et al. Identification of nafamostat as a potent inhibitor of middle east respiratory syndrome

coronavirus S protein-mediated membrane fusion using the split-protein-based cell-cell fusion assay. *Antimicrob Agents Chemother*. 2016;60:6532-6539.
73. Lucas JM, Heinlein C, Kim T, et al. The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. *Cancer Discov*. 2014;4:1310-1325.
74. Böttcher E, Freuer C, Steinmetzer T, Klenk H-D, Garten W. MDCK cells that express proteases TMPRSS2 and HAT provide a cell system to propagate influenza viruses in the absence of trypsin and to study cleavage of HA and its inhibition. *Vaccine*. 2009;27:6324-6329.
75. Ovcharenko AV, Zhirnov OP. Aprotinin aerosol treatment of influenza and paramyxovirus bronchopneumonia of mice. *Antiviral Res*. 1994;23:107-118.
76. Zhirnov OP, Klenk HD, Wright PF. Aprotinin and similar protease inhibitors as drugs against influenza. *Antiviral Res*. 2011;92:27-36.
77. Liu T, Luo S, Libby P, Shi G-P. Cathepsin L-selective inhibitors: a potentially promising treatment for COVID-19 patients. *Pharmacol Ther*. 2020;213:107587.
78. Kamboj RC, Raghav N, Mittal A, et al. Effects of some antitubercu-lous and anti-leprotic drugs on cathepsins B, H and L. *Indian J Clin Biochem*. 2003;18:39-47.
79. Zhou N, Pan T, Zhang J, et al. Glycopeptide antibiotics potently inhibit cathepsin L in the late endosome/lysosome and block the entry of ebola virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). *J Biol Chem*. 2016;291:9218-9232.
80. Zhang J, Ma X, Yu F, et al. Teicoplanin potently blocks the cell entry of 2019-nCoV. *bioRxiv*. 2020. https://doi.org/10.1101/2020.02.05.935387.
81. Cai J, Zhong H, Wu J, et al. Cathepsin L promotes vascular intimal hyperplasia after arterial injury. *Mol Med*. 2017;23:92-100.
82. Shibaguchi T, Yamaguchi Y, Miyaji N, et al. Astaxanthin intake attenuates muscle atrophy caused by immobilization in rats. *Physiol Rep*. 2016;4:e12885.
83. Nguyen-Ba G, Robert S, Dhalluin S, Tapiero H, Hornebeck W. Modulatory effect of dexamethasone on ornithine decarboxylase activity and gene expression: a possible post-transcriptional regulation by a neutral metalloprotease. *Cell Biochem. Funct*. 1994;12:121-128.
84. Crossland H, Constantin-Teodosiu D, Greenhaff PL, Gardiner SM. Low-dose dexamethasone prevents endotoxaemia-induced muscle protein loss and impairment of carbohydrate oxidation in rat skeletal muscle. *J Physiol*. 2010;588:1333-1347.
85. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*. 2020;30:269-271.
86. Porotto M, Orefice G, Yokoyama CC, et al. Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy. *J Virol*. 2009;83:5148-5155.
87. Horby P, Mafham M, Linsell L, et al. Effect of hydroxychloroquine in hospitalized patients with COVID-19: preliminary results from a multi-centre, randomized, controlled trial. *medRxiv*. 2020. https://doi.org/10.1101/2020.07.15.20151852
88. 'Solidarity' clinical trial for COVID-19 treatments. World Health Organization (WHO) https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments
89. Sai JK, Suyama M, Kubokawa Y, Matsumura Y, Inami K, Watanabe S. Efficacy of camostat mesilate against dyspepsia associated with non-alcoholic mild pancreatic disease. *J Gastroenterol*. 2010;45:335-341.
90. Hirota M, Shimosegawa T, Kitamura K, et al. Continuous regional arterial infusion versus intravenous administration of the protease inhibitor nafamostat mesilate for predicted severe acute pancreatitis: a multicenter, randomized, open-label, phase 2 trial. *J Gastroenterol*. 2020;55:342-352.
91. de Lalla F, Tramarin A. A risk-benefit assessment of teicoplanin in the treatment of infections. *Drug Saf*. 1995;13:317-328.
92. Wood MJ. The comparative efficacy and safety of teicoplanin and vancomycin. *J Antimicrob Chemother*. 1996;37:209-222.
93. Svetitsky S, Leibovici L, Paul M. Comparative efficacy and safety of vancomycin versus teicoplanin: systematic review and meta-analysis. *Antimicrob Agents Chemother*. 2009;53:4069-4079.
94. Su SB, Motoo Y, Iovanna JL, Xie MJ, Sawabu N. Effect of camostat mesilate on the expression of pancreatitis-associated protein (PAP), p8, and cytokines in rat spontaneous chronic pancreatitis. *Pancreas*. 2001;23:134-140.
95. Gibo J, Ito T, Kawabe K, et al. Camostat mesilate attenuates pancreatic fibrosis via inhibition of monocytes and pancreatic stellate cells activity. *Lab Invest*. 2005;85:75-89.
96. Sai JK, Suyama M, Kubokawa Y, Watanabe S. Efficacy of camostat mesilate against chronic upper abdominal pain associated with mild chronic pancreatitis. *Gastrointest Endosc*. 2007;65:AB299.
97. The RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with Covid-19 — preliminary report. *N Engl J Med*. 2020. https://doi.org/10.1056/NEJMoa2021436
98. Shiryaev SA, Mesci P, Pinto A, et al. Repurposing of the anti-malaria drug chloroquine for Zika Virus treatment and prophylaxis. *Sci Rep*. 2017;7:1885.
99. Li C, Zhu X, Ji X, et al. Chloroquine, a FDA-approved drug, prevents zika virus infection and its associated congenital microcephaly in mice. *EBioMedicine*. 2017;24:189-194.
100. Iwata K, et al. Effect of neutrophil elastase inhibitor (Sivelestat Sodium) in the treatment of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS): a systematic review and meta-analysis. *Intern Med*. 2010;49:2423-2432.
101. Brown WM. rAAt (dermatological) Arriva/ProMetic. *Curr Opin Mol Ther*. 2006;8:69-75.
102. Brown WM. rAAt (inhaled) arriva/hyland immuno. *Curr Opin Mol Ther*. 2006;8:76-82.
103. Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US).Identifier NCT00287391, Camostat and Artemisia Annua vs Placebo in COVID-19 Outpatients. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04530617?cond=NCT04530617&draw=2&rank=1. 2020.
104. Geoffrey Chupp, Yale University. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US).Identifier NCT04353284, Camostat Mesylate in COVID-19 Outpatients. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04353284?cond=NCT04353284&draw=2&rank=1. 2020.
105. Stanford University. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Identifier NCT04524663, Oral Camostat Compared With Standard Supportive Care in Mild COVID-19 Patients (COPS-2002). ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04524663?cond=NCT04524663&draw=2&rank=1. 2020.
106. Cancer Research UK. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US).Identifier NCT04455815, A Trial Looking at the Use of Camostat to Reduce Progression of Symptoms of Coronavirus (COVID-19) in People Who Have Tested Positive But Are Able to Stay at Home. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT04455815?cond=NCT04455815&draw=2&rank=1. 2020.
107. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 2020;395:1569-1578.
108. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19—final report. *N Engl J Med*. 2020;383:1813–1826. https://doi.org/10.1056/NEJMoa2007764.

109. Goldman JD, Lye DC, Hui DS, et al. Remdesivir for 5 or 10 Days in Patients with Severe Covid-19. *N Engl J Med*. 2020;383:1827–1837. https://doi.org/10.1056/NEJMoa2015301.
110. Spinner CD, Gottlieb RL, Criner GJ, et al. Effect of remdesivir vs standard care on clinical status at 11 days in patients with moderate COVID-19: a randomized clinical trial. *JAMA*. 2020;324:1048-1057.
111. Cao B, Wang Y, Wen D, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe covid-19. *N Engl J Med*. 2020;382:1787-1799.
112. Li Y, Xie Z, Lin W, et al. Efficacy and safety of lopinavir/ritonavir or arbidol in adult patients with mild/moderate COVID-19: an exploratory randomized controlled trial. *Med*. 2020. https://doi.org/10.1016/j.medj.2020.04.001
113. Huang YQ, Tang SQ, Xu XL, et al. No statistically apparent difference in antiviral effectiveness observed among ribavirin plus interferon-alpha, lopinavir/ritonavir plus interferon-alpha, and ribavirin plus lopinavir/ritonavir plus interferon-alpha in patients with mild to moderate coronavirus disease 2019: results of a randomized, Open-Labeled Prospective Study. *Front Pharmacol*. 2020;11:1071.
114. Hung IF, Lung KC, Tso EY, et al. Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet*. 2020;395:1695-1704.
115. Horby PW, Mafham M, Bell JL, et al. Lopinavir–ritonavir in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. 2020;396:1345-1352.
116. Huang M, Tang T, Pang P, et al. Treating COVID-19 with chloroquine. *J Mol Cell Biol*. 2020;12:322-325.
117. Siemieniuk RA, Bartoszko JJ, Ge L, et al. Drug treatments for covid-19: living systematic review and network meta-analysis. *BMJ*. 2020;370:m2980.
118. Harding SD, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res*. 2018;46:D1091-D1106.
119. Alexander SP, Fabbro D, Kelly E, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: enzymes. *Br J Pharmacol*. 2019;176:S297-S396.

How to cite this article: El-Shimy IA, Mohamed MM, Hasan SS, Hadi MA. Targeting host cell proteases as a potential treatment strategy to limit the spread of SARS-CoV-2 in the respiratory tract. *Pharmacol Res Perspect*. 2021;9:e00698. https://doi.org/10.1002/prp2.698