Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Enzyme inhibition as a potential therapeutic strategy to treat COVID-19 infection

Lukas Paulsson-Habegger, Andrew K. Snabaitis, Stephen P. Wren *

School of Life Sciences, Pharmacy and Chemistry, Faculty of Science, Engineering and Computing, Kingston University, Penrhyn Road, Kingston-upon-Thames KT1 2EE, UK

ARTICLE INFO

Keywords:
Coronavirus
SARS-CoV-2
COVID-19
Spike protein
Enzymes
Inhibition
ACE2
RAAS
Furin
TMPRSS2

ABSTRACT

With the emergence of the third infectious and virulent coronavirus within the past two decades, it has become increasingly important to understand how the virus causes infection. This will inform therapeutic strategies that target vulnerabilities in the vital processes through which the virus enters cells. This review identifies enzymes responsible for SARS-CoV-2 viral entry into cells (ACE2, Furin, TMPRSS2) and discuss compounds proposed to inhibit viral entry with the end goal of treating COVID-19 infection. We argue that TMPRSS2 inhibitors show the most promise in potentially treating COVID-19, in addition to being a pre-existing medication with fewer predicted side-effects.

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a positive, single-stranded ribonucleic acid (+ssRNA) virus of the family Coronaviridae, more commonly known as 'coronavirus'.[1,2] An outbreak in Wuhan China reported in December 2019 spread quickly worldwide and the World Health Organisation (WHO) declared the associated coronavirus disease 2019 (COVID-19) a global pandemic on 11th March 2020.[1–3] As of 27th May 2021, 168,040,871 people are confirmed to have been infected with the virus worldwide, with 3,494,758 deaths,[4] affecting all but a handful of isolated countries with these numbers expected to increase for the foreseeable future. In the last two decades, two other members of the family Coronaviridae viruses have emerged that can cause severe, sometimes fatal, illness in humans. In 2002, SARS-CoV-1 caused an epidemic that lasted two years with 8,069 infections and 774 deaths before it died out.[5,6] Middle East Respiratory Syndrome Coronavirus (MERS-CoV) emerged in 2012, and has caused sporadic outbreaks which have caused 2,562 infections and 881 deaths.[7] Given the significance of SARS-CoV-2 as a human pathogen that causes significant mortality and morbidity, there is great interest in the discovery of drugs that prevent or treat COVID-19. In particular, targeting the viral entry mechanism to impede SARS-CoV-2 from entering cells may better enable the body to combat an infective process, as the severity of infection correlates with intracellular viral load.[8]

The structure of both SARS-CoV-2 and SARS-CoV-1, include a ‘spike protein’ that binds to angiotensin-converting enzyme 2 (ACE2).[9,10] Both viruses use ACE2 as their host receptor, in order to facilitate entry into cells. The SARS-CoV-2 spike has been shown to use both a very similar receptor binding domain (RBD) and receptor binding motif (RBM) as SARS-CoV-1, with the similarity of the whole protein being 76–78%, 73–76% for the RBD and 50–53% for the RBM, the ranges being due to differences between species.[3] This provides a good starting point to investigate the enzymes responsible for allowing viral entry, and the potential for inhibition to reduce the severity or prevent COVID-19 infection.

2. SARS-CoV-2

2.1. Structure of the virus

Here, we now consider the mechanism of viral entry to identify additional targets of relevance for drug development. SARS-CoV-1, MERS-CoV and SARS-CoV-2 are all + ssRNA viruses (meaning that the coding genetic material can be directly translated by ribosomes into:
proteins. There are 4 different proteins that make up the structure and function of the viral particle: spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The N protein surrounds the strand of RNA, whilst the S, E and M proteins together form the viral envelope that contains the genetic material. Of these structural aspects, the most relevant is the spike protein, as this is the ‘key’ to infecting a cell, interacting with the necessary receptors to gain entry. As such, it will subsequently be looked at in more detail.

The spike protein is a trimeric structure, with each protomer being a single, Y-shaped peptide chain that are joined together to give the whole structure. This structure can then be divided into two further functional components, or subunits: S1 and S2. S1 is responsible for binding to ACE2, whilst S2 interacts with the transmembrane serine protease type II (TMPRSS2) enzyme, which causes the membranes of the virus and cell to fuse. As the S2 subunit is responsible for binding to receptors, there is a great deal of variability between Coronavirus viruses, as there are numerous different receptors used by the different members of the family. Even between viruses that utilise the same receptor, i.e., SARS-CoV and SARS-CoV-2, which use ACE2 as a route of entry, there is slight variability. The S2 subunit, however, is more conserved across viral variants, suggestive of a critical role in the fusion of the viral and cellular membranes.

This structure, however, is not fixed, which has multiple implications as to how effective the virus is at binding to cells and also in evading the immune system. First, it is helpful to note that each protomer S1 subunit can be further divided into S\(^\text{A}\) and S\(^\text{B}\), with the former being the externally facing part of each S1 subunit, whilst the latter is the internally facing part. S\(^\text{A}\) is the critical RBD for interaction with ACE2. The S1 subunit can also exist as different conformations, which affect its ability to bind to ACE2. The S\(^\text{A}\) domain is typically in a ‘closed’ position, where each of the three domains are folded down, pointing inwards towards each other. In this conformation, the spike cannot bind to ACE2. But as the RBD is not being displayed to the immune system (antibodies, leukocytes, dendritic cells, etc.), it improves the ability of the virus to evade an immune response. When the S\(^\text{A}\) domain is in the open position it is able to bind to ACE2, it can begin the process of viral entry.

2.2. Mechanism of viral entry

In order for SARS-CoV-2 to infect a cell, it first must bind to ACE2 and then merge its own membrane with the membrane of the cell so as to allow the contents of the virus to enter. ACE2 is a dimer of two complexes, each complex being made up of an ACE2 and a protein called B\(^\text{AT1}\). It is apt to note that each ACE2-B\(^\text{AT1}\) complex can bind to one spike protein as there are two ACE2 domains, meaning each complete enzyme can bind with two S proteins. Analysis of the crystal structure of the viral RBD and ACE2 complex suggests that once the virus has bound to ACE2, the virus is in an optimal position to fuse the viral envelope with the cell membrane. Further understanding of the mechanism of viral entry relies on existing research into SARS-CoV-1, which has been comparatively better studied. For membrane fusion to occur, a series of cleavages and conformational changes have to take place. This is believed to begin with a ‘pre-activation’ or ‘priming’ process of the spike protein by an enzyme called furin. Furin is a pro-protein convertase, which is a type of enzyme that converts inactive proteins to their biologically active counterpart and is able to cleave at a dibasic motif comprising any combination of Arg and Lys (K\(\text{K}\), R\(\text{K}\), R\(\text{K}\), R\(\text{R}\)), but is able to recognise this even with up to two to three other amino acids in between these residues. This motif is found in the SARS-CoV-2 spike protein at Arg\(_{682}\) to Arg\(_{686}\) (R\(_{682}\)-R\(_{687}\)-Arg\(_{684}\)-Phe\(_{686}\) \(\text{17,19,21,22}\)). This multibasic cleavage site is known as S\(_{1}/\text{S}_{2}\), indicating that this cleavage causes the separation of the two subunits. They do, however, remain non-covalently bound. Subsequently, another cleavage occurs, this time at a site within the S\(_{2}\) subunit denoted as S\(_{2}\). Unlike the S\(_{1}/\text{S}_{2}\), S\(_{2}\) is not necessarily denoted by a motif, instead relying on a single residue which can be either arginine or lysine as a so-called monobasic cleavage site. In SARS-CoV and SARS-CoV-2, this site consists of a dibasic Lys-Arg residue (Lys\(_{814}\)-Arg\(_{815}\) \(\text{17,19,23}\)). This cleavage, however, is not mediated by furin, but instead by TMPRSS2 (transmembrane protease, serine 2). This is an enzyme of which little is known regarding its exact biological function, despite being present in numerous tissues. It is implicated in prostate cancer and performs a similar role in multiple viruses including influenza, cleaving hemagglutinin to facilitate viral entry. Both of these two cleavages are vital for cell entry and thus provide another target for potential therapeutics through their inhibition.

At this point, the S\(_{1}\) subunit dissociates, and the fusion protein (FP), located at the top of the S\(_{2}\) subunit, becomes exposed to the membrane of the cell, into which it inserts itself. Through a cascade of conformational changes in the S\(_{2}\) subunit, the membranes come in to ever closer proximity and begin to merge, with the outer layers combining first (hemi-fusion) before both combine to result in a fusion pore, which gradually enlarges as the membranes continue to merge. The viral genetic material can now enter the cell.

2.3. Downregulation of ACE2 in COVID-19 infection

An important point to note around the discussion of the infection of the cell is the effect the infection itself has on ACE2. This is highlighted because, as reported by Kuba et al. (2005), ACE2 is in fact down-regulated due to coronavirus infection. As determined from these results, the quantity of ACE2 present in cells after infection is severely decreased. The authors suggest that the enzyme sheddase plays an important role in the loss of ACE2 expression. To examine this further, Glowacka et al. (2009) used the phorbol ester, phorbol myristate acetate (PMA), which induces shedding of ACE2, before comparing it to the effects of inactive viral-like particles (VLPS), SARS-CoV VLPs and NL63-CoV VLPs. The PMA (sheddng positive control) and the coronaviruses all caused ACE2 to be removed from the cell to become the free-floating soluble form, which was partitioned into the supernatant, supporting the shedding theory.

3. ACE2

3.1. Introduction to ACE2

ACE2 is a zinc metalloprotease that plays an important part in the renin-angiotensin-aldosterone system (RAAS), which is responsible for the management of blood pressure within the human body. It is a homologue of the related angiotensin-converting enzyme (ACE), which is also an important constituent of the RAAS system, with a 42% identical catalytic domain purposes, and 33% similarity between the two enzymes. Despite their relation, they have somewhat opposing with ACE responsible for increasing blood pressure, whilst ACE2 generally lowers it by countering the action of ACE.

3.2. Function of ACE2

ACE2’s related homologue, ACE, cleaves angiotensin-I (Ang-I) to form angiotensin-II (Ang-II), a powerful vasoconstrictor and mitogen that mediates high blood pressure. ACE2 acts as a counterbalance to this; it converts Ang-I to angiotensin-1–9 (Ang-(1–9)) by cleaving only the His amino acid on the C-terminus, thus preventing ACE from converting Ang-I to Ang-II. It also converts Ang-II to angiotensin-1–7 (Ang-(1–7)) by cleaving the Phe amino acid from the C-terminus, which prevents the potent vasoconstrictive effects of Ang-II from occurring. Ang-(1–7) is also a vasodilator, increasing the effectiveness of ACE2 in decreasing blood pressure.
3.3. Structure of ACE2 and its active site

ACE2 has a high similarity in its structure to the closely related ACE but has some important distinctions that cause the difference in the exhibited enzymatic activity. It is a transmembrane protein with a single extracellular catalytic domain (amino acids 147–555). The most critical residues have been determined to be Arg273, which binds to a known ACE2 inhibitor, MLN-4760. His505 and especially His504 have also been shown to be important in substrate binding; His505 assists in the hydrogen bonding of the nearby Tyr515, which itself hydrogen bonds to the substrate in order to stabilise the carbonyl tetrahedral intermediate that forms at the catalytic site. His505 is closer to the substrate and thus is able to directly hydrogen bond to the substrate, providing stability. ACE2 uses a motif known as HEXXH in which two histidine residues (His574 and His378) and one glutamate residue (Glu402) chelate the catalytic zinc ion.

3.4. RAAS and its function and mechanism of action

Further discussion of the physiological role of the RAAS is necessary to illustrate the potential impact of ACE2 drug targets in modulating SARS-CoV-2 viral entry in to cells. The RAAS begins in the macula densa of the juxtaglomerular (JG) apparatus, found in the glomerulus of the kidneys, from which the aspartyl protease renin, is released, initiating the beginning of the RAAS hormone cascade. Renin cleaves angiotensinogen (AGT), a protein belonging to the serpin superfamily. The first 10 residues in the N-terminal region of AGT are cleaved off in this process between the leucine and valine residues, to form the decapeptide known as angiotensin-I (Ang-I). Ang-I, also known as proangiotensin, has little to no biological activity and acts solely as a precursor to angiotensin-II (Ang-II) in the beginning of a complex, interconnecting sequence of cleavages. From Ang-I, two enzymes can act on it to form two different products: ACE will convert Ang-I into Ang-II by cleaving off two residues, whilst ACE2 will convert it into Ang-(1–9) by cleaving just one residue, both from the C-terminus. Ang-(1–9) can then be converted into Ang-(1–7) by ACE by cleaving two C-terminal residues, but it can also be formed from Ang-II by ACE2, which cleaves one residue, again from the C-terminus. ACE can then cleave Ang-(1–7) to form angiotensin-(1–5) (Ang-(1–5)) by removing the two residues. Ang-II can also be converted by another enzyme, aminopeptidase A (AMPA) to angiotensin-(3–8) through cleavage of an N-terminal residue, before being converted to angiotensin-IV by aminopeptidase M (AMPMP) by cleavage of another N-terminal residue.

Ang-II is a potent vasoconstrictor that acts on the AT1R and AT2R G protein-coupled receptors. AT1R activation is responsible for the vasoconstrictive effects of Ang-II. It causes the constriction of blood vessels, anti-natriuresis, hypertrophy, cell proliferation, aldosterone secretion and oxidative stress. These actions allow RAAS to increase blood pressure within the cardiovascular system. Conversely, AT2R activation causes the dilation of blood vessels, natriuresis, and has anti-hypertrophic and anti-proliferative effects. There are a few potential reasons as to why Ang-II has a hypertensive effect despite acting on both, though the most likely explanation is the different amounts and locations of each receptor. AT1R, for example, is found in high concentrations within the kidneys and smooth muscles cells (i.e., blood vessels), whilst AT2R is found more concentrated in heart cells. In general, however, there are much fewer of the latter relative to the former, with AT1R being much more prevalent in foetuses, before diminishing rapidly after birth. This, along with differences in the structures of the receptors affecting how well each angiotensin peptide binds to them, likely accounts for the prohypertensive nature of Ang-II.

From Ang-II, two other angiotensin molecules can be produced: angiotensin-III and -IV (Ang-III and -IV). Ang-III is reported to have similar effects to Ang-II, in that it has the same aldosterone stimulating ability, but only 40% the vasopressor efficacy of Ang-II. Ang-III also targets the AT1 and AT2 receptors like Ang-II, but as shown by its different activity, performs differently. This could be explained by the ability of Ang-III to induce natriuresis mediated by AT2, whilst Ang-II does not cause this. Ang-IV is formed from Ang-III, and has a fairly distinct mode of action, acting on the receptor AT4, which is an insulin-regulated aminopeptidase receptor (giving its other acronym, IRAP), as opposed to the G protein-coupled receptors AT1 and AT2.

Activation of AT4R causes vasodilation through increased nitric oxide synthesis, particularly in the brain and kidneys where there are higher concentrations of this receptor. AT4R activation also moderates cell proliferation and cardiac contractility and modulates cellular glucose uptake (important for learning and memory processing).

The ACE2 axis is a more recent discovery to RAAS. There is the conversion of Ang-I into Ang-(1–9) and subsequently Ang-(1–7), as well as the direct conversion of Ang-II into Ang-(1–7). This has a twofold effect: firstly, it prevents the vasoconstrictive effect of Ang-II itself by removing it or preventing it from being synthesised in the first place. Secondly, the molecules formed as a result of the actions of ACE2, more specifically Ang-(1–7), in fact have vasodilative and cardioprotective effects through acting on the MAS1 receptor (MasR). Activation of MasR stimulates the synthesis of nitric oxide, cyclic guanosine monophosphate (cGMP) and endothelium-derived relaxation factor, among other agents that have a vasodilative effect. In addition, activation also has anti-hypertrophic and anti-proliferative effects, which in general are favourable for the body.

4. Potential medications against SARS-CoV-2

In appreciating their critical role in facilitating viral entry, the cleavage proteins ACE2, furin and TMPRSS2 will now be explored as potential targets for drug development.

4.1. Inhibitors of ACE2

Molecules that inhibit ACE2 may also block the interaction of SARS-CoV-2 with ACE and prevent viral entry. Here, we evaluate the drug-likeness of two compounds; MLN-4760 and the polypeptide DX600.

4.1.1. Analysis of Drug-likeness and experimental data of MLN-4760

To determine the drug-likeness of MLN-4760, structural analysis using SwissADME can be performed. As MLN-4760 is stereoeactive, each isomer should be looked at individually to consider any differences. Both isomers are predicted to have good to moderate solubility in water, each isomer should be looked at individually to consider any differences. No significant issues are found with the MLN-4760 score, a combination of these predictions, is also acceptable (at 55/55%). The bioavailability score, a combination of these predictions, is also acceptable (at 55/55%) for both compounds. No significant issues are found with the MLN-4760 structure in terms of potentially problematic fragments that are known or predicted to be toxic/reactive/instable/etc., through the Structural Alert feature of SwissADME. The results from this analysis suggest that MLN-4760 shows good potential as a lead compound for further pharmaceutical development. Further experimental testing would have to be performed to determine its pharmacokinetic and pharmacodynamic profile.

Another aspect that must be looked at is how well MLN-4760 binds to ACE2 and how selective it is, especially concerning the closely related ACE. Joshi et al. (2016) reported a great deal of data on this (note: the stereoisomers A and B of MLN-4760 as described within the paper refer to the R- and S-isomers respectively, as described within this article).

They looked at both the activity and selectivity of both stereoisomers of MLN-4760, as well as the racemic mixture for both ACE and ACE2.

It can be said that MLN-4790, regardless of stereoisomerism, is an inhibitor for recombinant-human ACE2 (rhACE2), as the hydrolysis of...
the substrate was completely prevented by this compound. However, the compound also shows some activity in inhibiting recombinant-human ACE (rhACE), which also indicates that it is not a selective inhibitor of just rhACE2. Table 1 displays the maximum inhibition (I_max) results of both rhACE and rhACE2 by different concentrations of the -S, -R and racemic mixture of MLN-4760, which also shows that the compound inhibits rhACE, in addition to rhACE2.

In addition, Table 2 shows the results of the inhibitory concentrations (IC_{50}) of each enzyme and compound. The results suggest a 600–10,000-fold selectivity towards ACE2. The results show that the racemate and the R-isomer are roughly equivalent in activity and selective in regard to rhACE2, whilst the S-isomer is about 20% less efficacious and also less selective. However, these data arise through the use of recombinant-human versions of both ACE and ACE2, which are produced artificially using bacteria or yeast. The authors also describe results using human bone marrow cells, specifically mononuclear (MNCs) and CD34 + cells. Interestingly, there seems to be a reversal in the efficacy and selectivity of the stereoisomers.

As can be seen from Table 3, there has been a stark reversal in the efficacies and selectivity of the stereoisomers, with the S-isomer now being much more efficacious and more selective, having a 20-fold selectivity for ACE2 over ACE compared to only a 3-fold selectivity for the R-isomer and racemate. The S-isomer was also tested in CD34 + cells, which also displays this reversal with an I_max of 19 ± 2% in ACE and 38 ± 4% in ACE2, with a 63-fold selectivity of ACE2 over ACE. This suggests that under physiological conditions, the isomers behave differently, which would need further investigation to elucidate further details.

The results, along with the analysis using SwissADME, with good pharmacodynamics and few predicted issues related to toxicity or negative interactions, suggest that there is promising potential for further development of this compound. Improvements to increase its potency and selectivity towards ACE2, as well as assessing the need for the molecule to be able to cross the BBB would be ideal places to start.

### 4.1.2. Analysis of drug-likeness and experimental data of DX600

DX600 was discovered as part of a search through peptide libraries, and as such, has very different characteristics compared to MLN-4760. It has been shown to strongly inhibit ACE2, in addition to good selectivity for it versus ACE.[58]

To date, DX600 has not been profiled for its potential therapeutic usage. DX600 is a relatively large polypeptide which causes a number of concerns, such as a high price or difficulty of the manufacture of the molecule, as well as limited routes for administration (e.g., subcutaneous or intravenous routes). This is because peptides are susceptible to hydrolysis in the stomach, as well as having limited absorption due to their bulkiness. Whilst peptides have valuable potential due to their excellent selectivity and binding affinity towards the target enzyme, strategies such as enhancing the stability of the peptide, preventing hydrolysis, and improving their absorption are necessary to enable their use.

### 4.1.3. Pre-existing drugs with inhibitory activity towards ACE2

An alternative strategy to find potential therapeutics is through substrate-based searches and computational design. Early research shortly after the discovery of ACE2, and the revelation that it was responsible for allowing SARS-CoV to enter cells, as well as more recent exploration due to the coronavirus pandemic has provided details into small molecule compounds and peptide-based ones that have an inhibitory effect against the enzyme.[59,60] Huentelman et al. (2004) searched pre-existing databases (NCI/DTP) during the SARS epidemic using structure-based virtual screening (SBVS) and found a lead compound, N-(2-aminoethyl)-1-aziridineethanamine, which displayed micromolar levels of activity (57 ± 7 μM).[61] Terali et al. also used SBVS to find eight compounds that were determined to have activity towards ACE2 in silico.[62] None of these drugs have yet been used in clinical trials to treat COVID-19.

#### 4.2. Potential inhibitors of furin

Research by Becker et al. discussed compounds that mimic the arginine that furin uses as its substrate.[63,64] Also important to note is that there are already furin inhibitors that are in use for research, one of the most reported being decanoyl-Arg-Val-Lys-Arg-CMK (dec-RVKR-CMK).[64] However, many are unsuitable for further use in drug design due to the vulnerability of the P1 ketone. As such, Becker et al. looked at a variety of groups to replace the P1 Arg of dec-RVKR-CMK and assessed their activity, using the general formula R-Arg-Val-P2-P1 (with P2 almost always being Arg) being used.

Importantly, all of these compounds were measured to be reversible competitive inhibitors, which is typically a desirable trait when permanent inhibition is unwanted. Worthy to note is the K_i value of each inhibitor: a lower value means a more potent inhibition of furin. This highlights compounds 15–18, all of which exhibit good potency values, especially 15 and 17. Becker et al. then moved on to determine the selectivity of these molecules towards other proprotein convertases (PCs), as well as serine proteases. Some activity was exhibited towards some of the PCs tested, but not all, whilst practically no activity was shown to occur towards the serine proteases. The study moved on to test molecule 15, the most potent found, against an avian influenza virus (H7 subtype). However, despite the excellent in vitro activity of the compound, the in vivo activity was found to be diminished.[63,65]

Becker et al. (2012) in a later study went on to improve upon their previous work.[59] They took the most potent inhibitor, denoted previously as compound 15, now compound 1, and instead looked at the P5 residue, having established the effectiveness of 4-amidinobenzylamide at P1. The aims were to improve the lipophilicity of the compound in order to allow it to better permeate the membrane of cells by incorporating fatty acid residues into the structure. They also tested the effects of substituting the P5 position with hydrophobic cyclic groups, as well as a broader variety of groups, though these proved less successful.[64]

---

1 National Cancer Institute/Development Therapeutics Program.

---

| Enzyme  | Compound | MLN-4760-S | MLN-4760-R | MLN-4760-S/R |
|---------|----------|------------|------------|-------------|
| rhACE   | 5.0 ± 0.1| 4.4 ± 0.3  | 4.4 ± 0.2  |
| rhACE2  | 8.0 ± 0.1| 8.9 ± 0.1  | 8.5 ± 0.1  |

| Enzyme  | Compound | MLN-4760-S | MLN-4760-R | MLN-4760-S/R |
|---------|----------|------------|------------|-------------|
| ACE     | 34 ± 1%  | 29 ± 3%    | 22 ± 2%    |
| ACE2    | 63 ± 2%  | 35 ± 1%    | 34 ± 2%    |

---

Table 2 The results from Fig. 5 displaying the pIC₅₀ of each enzyme for the different isomers and the mixture.[56]

Table 3 The results from Fig. 18 displaying the I_max as percentages of each enzyme for the isomer and the mixture.[56]
Becker et al. then evaluated substituents that primarily consisted of amines and their guanylated analogues (20–28). These compounds immediately showed much-improved inhibition constant values, with 20–26 being in the picomolar range of K_i values. Selectivity showed a similar pattern, with high selectivity for some of the PCs, less so for others, and poor inhibition of other enzymes such as serine proteases. After performing similar testing of 22 and 24, compound 24 especially was found to be very effective against the H7N1 virus, which, due to both requiring furin for cleavage, would indicate that this compound would likely function against SARS-CoV-2. It requires further in vivo trials and preclinical evaluation to determine whether it has any therapeutic potential.

In an attempt to identify pre-existing drugs able to interact with furin, Wu et al. screened multiple databases and found several compounds with good affinity for furin. Many of these drugs are available already as medications, including aminopterin, silybin, diminozene and methotrexate, among others. The most important part of this is that these drugs are already approved, on the market and have a well-studied side effect profile in humans, meaning their theoretical application in the treatment of COVID-19 is possible should there be clinical trial evidence of a satisfactory risk/benefit profile.

4.3. Potential inhibitors of TMPRSS2

TMPRSS2 has also been the target of research, particularly for influenza and coronaviruses such as SARS-CoV AND MERS-CoV. The field of TMPRSS2-specific inhibitors is quite nascent, with the earliest article describing the first synthetic compounds being published by Meyer et al. in 2013. Here, they discuss previously found inhibitors, including ovomucoid trypsin inhibitor and 4-(2-aminoethyl)-benzenesulfonylfuoride, but note that they have limited potential as lead compounds. Another issue was the lack of understanding surrounding the substrate specificity of the enzyme. Therefore, the authors screened a number of substrates of serine proteases against TMPRSS2, and determined the importance of a glycine-like residue in the P2 position and a hydrophobic P3 position. From the screened substrates, methylsulfonyl/methoxy carbonyl groups were found to be well tolerated at P4.

Once the basic characteristics of the enzyme’s substrate specificity were established, Meyer et al. began the process of developing the inhibitor. Previous publications have made light on the ability of 4-amidinobenzylamide in the P1 position as being important to inhibiting other serine proteases, and as such was selected as the base for testing. For P3, α-Arg and α-Asp(OtBu) were found to have the highest K_i values. For P2, proline was initially chosen as the main option, as it is the preferred P2 residue for a number of different serine proteases. This, however, could therefore negatively affect the selectivity of the final compound. Testing other residues indicated that either alanine or arginine were suitable replacements for this position. For P4, benzylsulfonyl was used due to it also being preferred by many other serine proteases. Though removing the group in most cases was detrimental to the inhibitory activity, some compounds still showed acceptable K_i values. Further experimentation whereby the side chain α-butyI ester group of α-glutamic acid or α-aspartic acid (at P3) was replaced with various different groups did not yield any improvements.

Throughout, the P1 position was occupied by 4-amidinobenzylamide, but other groups, specifically 3-amidinophenylalanine, have also been reported as having potential to improve the characteristics of the compound due to their activity on other enzymes. Using previously described inhibitors of the enzymes matriptase and thrombin, they showed that the matriptase inhibitor displayed even lower K_i values (8 nM) than found in the previous series of compounds (~19 nM). Meyer et. al. discussed, however, that the highly hydrophilic character of the compound could limit its bioavailability, and subsequently made modifications to the P1 group (corresponding to the C-terminal region). A number of these analogues had as good or only slightly worsened K_i values (generally < 20 nM). After this, the N-terminal was also amended, using two different piperide residues at the C-terminus. The resulting analogues generally showed excellent activity (<10 nM), with an N-terminal 1,3-dichlorobenzyl group (compound 92) giving a K_i value of 0.9 nM, and 1,3-dimethoxy benzyl (compound 94) giving 1.0 nM. Final alterations to the N-terminal region gave compounds 111–114 which also exhibited highly promising K_i values (3–5 nM).

Testing of four of the most potent inhibitors (92, 93, 113, 114) for their effect on cell viability resulted in two (93 and 113) causing a decrease of ~ 20% in viability, whilst the other two had little to no effect. Meyer et. al. decided to further investigate compound 92, and thus studied the effect it had on the propagation of influenza viruses (H1N1 and H3N2). The results showed that there was a dose-dependent suppression of the virus titres compared to the control, with a 10 μM concentration causing a 10- and 100-fold decrease in virus titres at 24 h for H1N1 and H3N2 respectively, and a 50 μM concentration causing a 100–1000-fold decrease at 24 h. This demonstrated both the fact that the influenza viruses utilise TMPRSS2 to enter cells and thus replicate, as well as showing the efficacy of inhibiting the enzyme as a method of preventing viral entry into cells. Whilst the authors focused on the influenza virus, the inhibitor should also be able to prevent entry into cells by TMPRSS2-dependent coronaviruses, including SARS-CoV-2.

As with ACE2 and furin, it is always worthwhile looking at pre-existing drugs to determine whether any exist that are able to inhibit the enzyme in question. As TMPRSS2 is a serine protease, it is most pertinent to look at serine protease inhibitors, a field of medications that is significantly more developed than furin inhibitors and encompasses many different drug types, including antivirals, anti-inflammatory, anticanicance, to name just a few. This is highly beneficial, as it gives a much larger basis to screen for a compound that will inhibit TMPRSS2 specifically. It will also likely enable the ability to select one that has a known dosage/side effect profile, as well as affording the opportunity to be able to co-administer multiple medications in order to increase their combined effect. Numerous sources have initially indicated that camostat mesylate, a drug used in the treatment of chronic pancreatitis, is an inhibitor of TMPRSS2 and is able to partially block SARS-CoV (by 65%), another coronavirus (NL63) and tested influenza viruses (H1N1, H3N2) from entering cells. The compound is rapidly hydrolysed at the side chain ester when absorbed, with a half-life of < 1 min, to form GBP. Whilst GBP is not as effective as camostat at TMPRSS2 inhibition, it is still potent enough to give a therapeutic effect. As such, further research into improving camostat to make it more resistant to hydrolysis could prove beneficial to the pharmacokinetics of the compound. Such is the evidence that camostat has good potential in treating COVID-19 that numerous clinical trials are currently underway, though results are not yet available.

Another potentially viable medication already available that could treat COVID-19 infections is bromhexine, a drug mainly used as a mucolytic for productive coughs. It has been demonstrated to have good activity towards TMPRSS2, and as such it provides another prospective compound that would be effective for this purpose. A closely related analogue of bromhexine, ambroxol, also appears to have potential therapeutic benefit in treating COVID-19, but functions through a different mechanism. The structure/activity relationship of these compounds could be analysed to potentially establish more effective inhibitors in the future.

5. Discussion

A focus on pre-existing compound libraries and drug development targets has the potential to save time and money and may be the fastest route towards minimising COVID-19 associated morbidity and mortality. This paper has identified three therapeutic targets with better potential; ACE2, Furin and TMPRSS2.

ACE2 (Section 4.1) inhibition would prevent the virus from being
able to bind and subsequently enter cells, preventing infection. There exists a selection of potential and proven inhibitors, though currently no compound has been tested in vivo for antagonistic activity towards the enzyme. Another important question is the physiological consequences of ACE2 inhibition. ACE2 may have benefits in preventing excess hypertension, in addition to having anti-hypertrophic, anti-proliferative and antithrombotic properties. It should also be taken into consideration that ACE2 will be downregulated in an active COVID-19 infection, as detailed in Section 2.3, which would complicate the effects of inhibiting the enzyme. Such a substantial disruption of the RAAS would therefore likely be undesirable in a patient already at an elevated risk of severe hypertension and thromboembolic complications.

Another option is the inhibition of furin (Section 4.2), the enzyme responsible for ‘priming’ the SARS-CoV-2 spike protein prior to membrane fusion. Inhibition would prevent this, making it substantially more difficult or perhaps impossible for the virus to infect the cell. Like ACE2, there are a number of purposely designed molecules and potential medications that can be repurposed in order to fulfill the role desired. However, the physiological consequences of furin inhibition are poorly understood. Furin is a proprotein convertase that is responsible for activating through cleavage a wide variety of proteins. Whilst performing modelling will help provide a clearer picture, it is only with clinical trials that the activity of a furin inhibitor could be established.

TMPRSS2 (Section 4.3) inhibition could provide a better potential to the previous two options. Research has implicated TMPRSS2 in prostate cancer (Lucas et al., 2014), with significant upregulation found in tumours contributing to metastasis. Bromhexine, a widely available medication that inhibits TMPRSS2 was found to have little to no cytotoxicity. This provides a very promising lead in a potential method for treating COVID-19.

From what has been discussed, we argue the best option to explore further would be TMPRSS2 inhibitors. Inhibition of the TMPRSS2 enzyme, based on the research that has thus far been performed, appears to have the least theoretical drawbacks when compared to inhibiting ACE2 or furin, whilst still offering the potential ability to prevent SARS-CoV-2 from entering cells and thus treating a COVID-19 infection. Another benefit is that inhibitors of this enzyme have already been established, with the most promising being already approved medications, namely bromhexine and camostat. Clinical trials are being performed to monitor the efficacy of camostat in preventing or treating infections.

Also of interest, though not discussed within this paper, is the area of peptidomimetics, specifically ones that mimic ACE2. These would have the same advantages of an ACE2 inhibitor, by causing SARS-CoV-2 viruses to bind to it as opposed to the actual enzyme, whilst also benefitting from not interfering with ACE2 and the RAAS. [54-60]

6. Conclusion

There are a number of potential therapeutic targets for the treatment of COVID-19 infection through the inhibition of the enzymes related to the infection process. This review has described the important aspects of each of the potential targets and their physiological relevance and has discussed the advantages and disadvantages of potential candidates for further development. Critically, some of these candidates have been identified through computational approaches and from pre-existing drug libraries, in an effort to reduce valuable time for further preclinical assessment. Pre-existing medications able to inhibit TMPRSS2 appear to be the best candidates for profiling to determine their efficacy in treating COVID-19 infected individuals, whilst also providing lead compounds to further develop as TMPRSS2 inhibitors.

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Uncited references
33, 42.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We’d like to thank Dr Philip Gaughwin for assistance and suggestions with editing.

References

1 Coronavirus Disease Study Group of the International Committee on Taxonomy of Viruses. The Species Severe Acute Respiratory Syndrome-Related Coronavirus: Classifying 2019-nCoV and Naming it SARS-CoV-2. Nat. Microbiol. 2020;5(4):536-44.
2 Who.int. 2020. Naming the Coronavirus Disease (COVID-19) and the Virus that Causes it. [online] Available at: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019) and-the-virus-that-causes-it>.
3 Wan Y, Shang J, Graham R, Baric RS, Li F, Gallagher T. Receptor Recognition by the Novel Coronavirus from Wuhan: An Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. J Virol. 2020;94(7). https://doi.org/10.1128/JVI.00127-20.
4 Covid19.who.int. 2020. WHO Coronavirus Disease (COVID-19) Dashboard. [online] Available at: <https://covid19.who.int/> . [Date Accessed: 27/05/2021].
5 Drosten C, Günter S, Preiser W, et al. Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. New Eng J Med. 2003;348(20):1967-1976.
6 Who.int. 2020. WHO | Summary of Probable SARS Cases with Onset of Illness From 1 November 2002 To 31 July 2003. [online] Available at: <https://www.who.int/csr/sars/country/table2004_04_21/en/>.
7 World Health Organization. 2020. Middle East Respiratory Syndrome Coronavirus (MERS-CoV). [online] Available at: <https://www.who.int/emergencies/mers-cov/en/>.
8 Liu Y, Liao W, Wan L, Xiang T, Zhang W. Correlation Between Relative Nasopharyngeal Virus RNA Load and Lymphocyte Count Disease Severity in Patients with COVID-19. Viral Immunol. 2020.
9 Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-Converting Enzyme 2 (ACE2) as a SARS-CoV-2 Receptor: Molecular Mechanisms and Potential Therapeutic Target. Intens Care Med. 2020;46(4):586-590.
10 Peder sen S, Ho Y. SARS-CoV-2: A Storm is Raging. J Clin Invest. 2020;130(5):2202–2205.
11 Bosch BJ, van der Zee R, de Haan CAM, Rottier PJM. The Coronavirus Spike Protein Is a Class I Virus Fusion Protein: Structural and Functional Characterization of the Fusion Core Complex. J Virol. 2003;77(16):8801–8811.
12 Tortorici M, Veesler D. Structural Insights into Coronavirus Entry. Adv Virus Res. 2019;105:93–116.
13 Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenticity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020;181(2):281–292.e6.
14 Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Ann Rev Virol. 2016;3(1):237–261.
15 Shang J, Wan Y, Luo C, et al. Cell Entry Mechanisms of SARS-CoV-2. Proc Natl Acad Sci. 2020;117(21):11727–11734.
16 Yan K, Zhang Y, Li Y, Xia Lu, Guo Y, Zhou Q. Structural Basis for the Recognition of SARS-CoV-2 by Full-Length Human ACE2. Science. 2020;367(6485):1444–1448.
17 Beste D, Heindl MR, Limburg H, et al. TMPRSS2 and Furin are Both Essential for Proteolytic Activation of SARS-CoV-2 in Human Airway Cells. Life Sci Alliance. 2020;3(9)e202000786. https://doi.org/10.26508/lsa.202000786.
18 Head-Sargent T, Gallagher T, Ready, Set, Fuse! The Coronavirus Spike Protein and Acquisition of Fusion Competence. Viruses. 2012;4(4):557-580.
19 Hoffmann M, Kleine-Weber H, Pohlmann 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(4):779–784.e5.
20 Boiticher-Friebertshauer E, Garten W, Klenk HD, eds. Activation of Viruses by Host Proteases. Cham: Springer International Publishing; 2018.
21 Wu C, Zheng M, Yang Y, Gu X, Yang K, Li M, et al. Furin: A Potential Therapeutic Target for COVID-19. & Science. 2020;23(10).
22 Benton DJ, Wrobel AG, Xu P, et al. Receptor Binding and Priming of the Spike Protein of SARS-CoV-2 for Membrane Fusion. Nature. 2020;580(7807):327–330.
23 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(2):271–280.e8.
24 TMPRSS2 Transmembrane Serine Protease 2 [Homo sapiens (human)] - Gene - NCBI [Internet]. National Center for Biotechnology Information. 2021 [cited 18 February 2021]. Available from: <https://www.ncbi.nlm.nih.gov/gene?db=gene&Cmd=ShowDetail&View=term&ToSearch=7113>.
25 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.

L. Paulsson-Habegger et al.
Bioorganic & Medicinal Chemistry 48 (2021) 116389
26 Paolo-Giobacchino A, Chen H, Peituch MC, Rossi C, Antonarakis SE. Cloning of the TMPRSS2 Gene, Which Encodes a Novel Serine Protease with Transmembrane, LDLR, and SR-BI Domain and Maps to 21q22.3. Genomics. 1997;43(3):309–320.

27 Böttcher-Friebertshäuser E, Freuer C, Siefaff F, et al. Cleavage of Influenza Virus Hemagglutinin by Airway Proteases TMPRSS2 and HAT Differs in Subcellular Localization and Susceptibility to Protease Inhibitors. J Virology. 2010;84(11): 5605–5614.

28 Lucas JM, Heinlein C, Kim T, et al. The Androgen-Regulated Protease TMPRSS2 Activates a Prosecretory Cascade Involving Components of the Tumor Microenvironment and Promotes Prostate Cancer Metastasis. Cancer Discov. 2014;4(13):1310–1325.

29 Belouzard S, Millet J, Licitra B, Whittaker G. Mechanisms of Coronavirus Cell Entry Mediated by the Viral Spike Protein. Viruses. 2012;4(6):1011–1033.

30 Vidal Y, Zhang J, Xu X, et al. Distinct-Combinatorial States of SARS-CoV-2 Spike Protein. Science. 2020;369(6511):1586–1592.

31 Walls A, Tortorici M, Snijder J, et al. Tectonic Conformational Changes of a Coronavirus Spike Glycoprotein Promote Membrane Fusion. Proc Natl Acad Sci. 2017;114(42):11157–11162.

32 Song W, Gui M, Wang X, Xiang Ye, Heise MT. Cyto-EM Structure of the SARS Coronavirus Spike Glycoprotein in Complex with its Host Cell Receptor ACE2. PLOS Path. 2018;14(8):e1007236.

33 White JM, Whitaker GR. Fusion of Enveloped Viruses in Endosomes. Traffic. 2017;18(5):693–714.

34 Kuba K, Imai Y, Rao S, et al. A Crucial Role of Angiotensin-Converting Enzyme 2 (ACE2) in SARS Coronavirus-Induced Lung Injury. Nat Med. 2005;11(8):875–879.

35 Glowacka I, Bertram S, Herzog P, et al. Differential Downregulation of ACE2 by the Spike Proteins of Severe Acute Respiratory Syndrome Coronavirus and Human Coronavirus NL63. J Virol. 2010;84(2):1198–1205.

36 Donoghue M, Huich F, Baroues E, et al. A Novel Angiotensin-Converting Enzyme-Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1-9. Circ Res. 2000;87(5):1–9.

37 Patel V, Zhong J, Grant M, Oudit G. Role of the ACE2/Angiotensin 1-7 Axis of the Renin-Angiotensin System in Heart Failure. Circ Res. 2016;118(23):1313–1326.

38 Eriksson U, Danbolt CA, Jönsson J, et al. ACE2-Targeting New Angiotensin Converting Enzyme-Inhibitors. Curr Biol. 2002;12(21):745–752.

39 Acton S, Robison K. 2001. Angiotensin Converting Enzyme Homolog and Therapeutic and Diagnostic Uses Therefor. US 6,194,556 B1.

40 Guy RJ, Jackson RM, Jenas HA, Hooper NM, Turner AJ. Identification of Critical Active-Site Residues in Angiotensin-Converting-Enzyme-2 (ACE2) by Site-Directed Mutagenesis. FEBS J. 2005;272(14):3521–3520.

41 Menach E, Hashida Y, Yasukawa K, Inouye K. Effects of Conversion of the Zinc-Containing Protease Metalloendopeptidase 17 into Dipeptidylpeptidase III, HEXXH, on the Activity and Stability of Thermolysin. Biochim Biophys Acta. 2013;1831(19):1901–1906.

42 FEBS Lett. 2014;580(18):4329–4332.

43 Lorenzo JN, Weihprecht H, Schnermann J, Skott O, Briggs JP. Renin Release from the Juxtaglomerular Apparatus. Am J Physiol-Renal Physiol. 2005;289(1):F49–F57.

44 Lorenz JN, Weihprecht H, Schnermann J, Skott O, Briggs JP. Renin Release from the Juxtaglomerular Apparatus. Am J Physiol-Renal Physiol. 2005;289(1):F49–F57.

45 Tucker T, Lumma W, Mulichak A, et al. Design of Highly Potent Noncovalent Inhibitors of Coronavirus Spike Glycoprotein Promote Membrane Fusion. J Am Chem Soc. 2008;130(21):7459–7469.

46 Becker G, Lu Y, Hardes K, et al. Highly Potent Inhibitors of Proprotein Convertase Furin as Potential Drugs for Treatment of Infectious Diseases. J Biol Chem. 2012;287(26):21992–22003.

47 Sato M, Shirato K, van der Hoek L, Taguchi F, Matsuyama S. Simultaneous Treatment of Human Bronchial Epithelial Cells with Serum and Corona-Peptide Inhibitors Prevents Severe Acute Respiratory Syndrome Coronavirus Entry. J Virol. 2012;86(12):6537–6545.

48 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. Palm Pharm Ther. 2015;33:66–74.

49 Zhou Y, Vedantham P, Lu K, et al. Protease Inhibitors Targeting Coronavirus and Influenza Virus. Annu. Rev. Pharmacol. Toxicol. 2016;56:76–84.

50 Meyer D, Siefaff F, Hammami M, Böttcher-Friebertshäuser E, Garten W, Steinmetzer T. Identification of the First Synthetic Inhibitors of the Type II Transmembrane Serine Protease TMPRSS2 Suitable for Inhibition of Influenza Virus Infection. Biochem J. 2013;452(2):313–343.

51 Boitßer E, Freuer C, Steinmetzer T, Klenk H, 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 Inactivation. Vaccine. 2009; 27(46):6324–6330.

52 Siefaff F, Böttcher-Friebertshäuser E, Meyer D, et al. Development of Substrate Analogue Inhibitors for the Human Airway Trypsin-like Protease HAT. Bioorg Med Chem. 2011;21(16):4860–4864.

53 Biela A, Siefaff F, Terwesten F, Heine A, Steinmetzer T, Klebe G. Ligand Binding Stepwise Disrupts Water Network in Thrombin: Enthalpic and Entropic Changes Reveal Classical Hydrophobic Effect. J Med Chem. 2012;55(15):6904–6910.

54 Jende M, Steinmetzer T, Biela A. Identification of New Substrate Analogue and Noncovalent Molecular-Weight Inhibitors of Matriptase. J Med Chem. 2010;53(15):5523–5535.

55 Tucker T, Lumma W, Mulichak A, et al. Design of Highly Potent Noncovalent Thrombin Inhibitors That Utilize a Novel Lipophilic Binding Pocket in the Thrombin Active Site. J Med Chem. 1997;40(16):3095–3102.

56 Stürzebecher J, Prasa D, Wilkstrom P, Viehwe U. Structure-Activity Relationships of Inhibitors Derived from 3-Aminophenylalanine. J Enzyme Inhib Med Chem. 1995;10(1):97–99.

57 Middel I, Hood A, Proctor P, Chasseaud L, Iorns S, Cheng K, et al. Metabolic Fate of 14C-Camostat Mesylate in Man, Rat and Dog After Intravenous Administration. Xenobiota. 1994;24(1):79–92.

58 Breining P, Frolund A, Hejen J, et al. Camostat Mesylate Against SARS-CoV-2 and COVID-19—Rationale, Dosing and Safety. Basic Clin Pharmacol. 2020;128(2):204–212.

59 Hoffmann M, Hoffmann-Winkler H, Smith J, et al. Camostat Mesylate Inhibits SARS-CoV-2 Activation by TMPRSS2-related Proteases and its Metabolite GBEA Exerts Antiviral Activity. Ebiomedicine. 2021;55:103255. https://doi.org/10.1016/j.ebiom.2021.103255.

60 Search of Camostat | Covid-19 | Results - ClinicalTrials.gov | National Institute of Health. 2021 [cited 18 February 2021]. Available from: https://www.clinicaltrials.gov/ct2/results?term=Å–cklondÅ–irnandÅ–camostatentry&–state=–city=–dist=–.

61 Depenhaus M, de Villiers D, Lemperle G, Meyer M, Di Somma S. Potential New Treatment Strategies for COVID-19: Is There a Role for Bromhexine as Add-on Therapy? Intern Emerg Med. 2020;15(5):801–812.

62 Alotajj M. Azithromycin and Ambroxol as Potential Pharmacotherapy for SARS-CoV-2. Int J Antimicrob Agents. 2020;56(6), 106192.
Larue R, Xing E, Kenney A, et al. Rationally Designed ACE2-Derived Peptides Inhibit SARS-CoV-2. Bioconjug Chem. 2021;32(1):215–223.

Alagumuthu M, Rajput S, Baig M. Structure-Based Design of Novel Peptidomimetics Targeting the SARS-CoV-2 Spike Protein. Cell Mol Bioeng. 2020;14(2):177–185.

VanPatten S, He M, Alhiti A, Cheng K, Ghanem M, Al-Abed Y. Evidence supporting the use of peptides and peptidomimetics as potential SARS-CoV-2 (COVID-19) therapeutics. Future Med. Chem. 2020;12(18):1647–1656.