Structural Analysis of Experimental Drugs Binding to the COVID-19 Target TMPRSS2

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Abstract

The emergence of SARS-CoV-2 has prompted a worldwide health emergency. There is an urgent need for therapeutics, both through the repurposing of approved drugs and the development of new treatments. In addition to the viral drug targets, a number of human drug targets have been suggested. In theory, targeting human proteins should provide an advantage over targeting viral proteins in terms of drug resistance, which is commonly a problem in treating RNA viruses. This paper focuses on the human protein TMPRSS2, which supports coronavirus life cycles by cleaving viral spike proteins. The three-dimensional structure of TMPRSS2 is not known and so we have generated models of the TMPRSS2 in the apo state as well as in complex with a peptide substrate and putative inhibitors to aid future work. Importantly, many related human proteases have 80% or higher identity with TMPRSS2 in the S1-S1' subsites, with plasminogen and urokinase-type plasminogen activator (uPA) having 95% identity. We highlight 376 approved, investigational or experimental drugs targeting S1A serine proteases that may inhibit TMPRSS2. Whilst the presence of a relatively uncommon lysine residue in the S2/S3 subsites means that many serine protease inhibitors may not inhibit TMPRSS2, this is likely to provide a handle for selective targeting. We discuss how experimental drugs targeting related serine proteases might be repurposed as TMPRSS2 inhibitors to treat coronaviruses.

Introduction

There is an urgent need to identify drugs and drug targets which are effective in treating COVID-19. Numerous drug targets have been suggested (Li and De Clercq 2020) and a plethora of drug repurposing efforts are underway. (J. Wang 2020; Zhou et al. 2020) Transmembrane Serine Protease 2 (TMPRSS2) is a membrane bound serine protease also known as Epitheliasin. TMPRSS2 belongs to the S1A class of serine proteases alongside proteins such as factor Xa and trypsin. Whilst there is evidence that TMPRSS2 autocleaves to generate a secreted protease,(Afar et al. 2001) its physiological function has not been clearly identified, However, it is known to play a crucial role in facilitating entry of coronavirus particles into cells by cleaving the spike protein.(Hoffmann, Kleine-Weber, et al. 2020; Bestle et al. 2020) Coronavirus spike proteins are thought to be cleaved at two sites, termed S1/S2 and S2'.(Kleine-Weber et al. 2018; Millet and Whittaker 2015; Belouzard, Chu, and Whittaker 2009) The proteases furin, trypsin, cathepsin, TMPRSS2, TMPRSS4, TMPRSS11, and human airway trypsin-like protease have all been implicated in these cleavages.(Kam et al. 2009; Belouzard, Chu, and Whittaker 2009; Ruochen Zang et al. 2020; Meyer et al. 2013; Bosch, Bartelink, and Rottier 2008) For SARS-CoV, the S2' cleavage site has a sequence motif (PTKR|S) that appears suitable for cleavage by trypsin-like proteases such as TMPRSS2 whereas the S1/S2 cleavage site has a sequence motif (SLLR|S) that appears suitable for cleavage by cathepsin or trypsin-like proteases. Whilst the SARS-CoV-2 S2' cleavage site has a similar sequence motif to SARS-CoV (PSKR|S) and would thus be suitable for cleavage by trypsin-like proteases, insertions of additional arginine residues at the the SARS-CoV-2 S1/S2 cleavage site (RRAR|S) clearly generate a furin cleavage site.(Coutard et al. 2020; Jaimes et al. 2020) Interestingly, this difference has been implicated in viral transmissibility of SARS-CoV-2.(Q. Wang et al. 2020)

There is good evidence that TMPRSS2 represents a good drug target for coronaviruses. TMPRSS2-expressing cells are more susceptible to SARS-CoV-2 infection and knockout mouse models show that lack of TMPRSS2 in the airways reduces the severity of lung pathology after SARS-CoV and MERS-CoV infection.(Iwata-Yoshikawa et al. 2019) For this reason, it has been suggested as a potential drug target for coronaviruses (Shen et al. 2017; Kawase et al. 2012) such as SARS-CoV-2. (Stopsack et al. 2020) TMPRSS2 is highly expressed in lung tissue (Lukassen et al. 2020) and it has been suggested that differential expression in males may lead to higher risk in male patients. (Asselta et al. 2020)(Qi et al., n.d.)(Stopsack et al. 2020) Peptidic inhibitors of TMPRSS2 have been described (Meyer et al. 2013) and the covalent TMPRSS2 inhibitor Camostat is being tested in a clinical trial against COVID-19. However, in this study we identify a number of experimental drugs with the potential to target TMPRSS2. MEROPS, the peptide database, lists 219 members of the S1A family in humans.(Rawlings et al. 2018) Many of the members in this family have been studied in detail, yielding numerous high resolution crystal structures, known inhibitors, and licensed drugs. (Maryanoff 2004) Importantly, the S1A family has a conserved fold with an arginine binding site that is targeted by the majority of small molecule inhibitors and drugs with its three catalytic residues (aspartate, histidine, and serine) in close proximity to the arginine binding site. In this study we generate homology models of human TMPRSS2 in the apo state as well as in complex with substrate peptide and a number of small molecule experimental drugs.

Materials and Methods

Development of a TMPRSS2 homology model

TMPRSS2 is in the S1A serine protease family. Sequences for all the human members of the family are available on Github:

https://github.com/djhuggins/TMPRSS2/tree/master/Sequences

We aligned the complete sequences with Clustal Omega. (Sievers et al. 2011) The overall sequence identity between TMPRSS2 and the other S1A family proteases is available on Github:

https://github.com/djhuggins/TMPRSS2/tree/master/Alignments

We selected a subset of sequences for alignment based on availability of structural data and similarity between sequences in the active site. We looked in particular at the identity in the active site of the enzymes to inform model and compound selection. We identified 20 residues close to the active site (S1-S1') as well as a larger set of 34 residues spanning the whole binding site (S4-S3-S2-S1-S1'-S2'-S3'-S4'). These residues are identified in Figure 1. The percentage identities between TMPRSS2 and this subset are given in Table 1:

Protein Name	Uniprot Name	TMPRSS2 Protease Domain % Identity	TMPRSS2 S4-S4' % Identity	TMPRSS2 S1-S1' % Identity
Plasminogen	PLMN_HUMAN	41.07	64.71	95.00
uPA	UROK_HUMAN	33.77	58.82	95.00
Trypsin-1	TRY1_HUMAN	38.60	58.82	90.00
Plasma kallikrein	KLKB1_HUMAN	41.99	61.76	85.00
Coagulation factor VII	FA7_HUMAN	38.16	61.76	85.00
Hepsin	HEPS_HUMAN	41.88	58.82	85.00
TMPRSS15	TMPRSS15_HUMAN	41.30	58.82	85.00
Coagulation factor XI	FA11_HUMAN	42.17	55.88	85.00
TMPRSS11E	TM11E_HUMAN	40.27	55.88	80.00
Tryptase gamma	TRYG1_HUMAN	39.82	52.94	80.00

Coagulation factor IX	FA9_HUMAN	39.19	52.94	80.00
Coagulation factor XII	FA12_HUMAN	36.89	50.00	80.00
Coagulation factor X	FA10_HUMAN	36.77	47.06	80.00
Chymotrypsin B	CTRB2_HUMAN	40.91	44.12	65.00

Table 1 - A subset of human S1A serine proteases and the percentage identity with TMPRSS2 of their protease domains, S1-S1' subsites, and S4-S3-S2-S1-S1'-S2'-S3'-S4' subsites.

Importantly, many of the proteases have 80% or higher identity with TMPRSS2 in the S1-S1' subsites, with plasminogen and urokinase-type plasminogen activator (uPA) having 95% identity. We selected the protein TMPRSS15 to generate a homology model (also known as enteropeptidase). TMPRSS15 provides a good template for building a homology model of TMPRSS2, with a deletion or two residues and an insertion of one residue. It also features a lysine residue in the S2/S3 subsites, which is unique to TMPRSS2 and TMPRSS15. The alignment of the TMPRSS2 and TMPRSS15 protease domains is shown in Figure 1.

```
TMPRSS2   IVGGESALPGAWPWQVSLHVQNVHVCGGSIITPEWIVTAAHCVEKPLNNPWHWTAFAGIL
TMPRSS15  IVGGSNAKEGAWPWVVGLYYGGRLLCGASLVSSDWLVSAAHCVYGRNLEPSKWTAILGLH
          ****..*  ***** *.*:  .  :**.*::: :*:*:*****     :* :***: *:

TMPRSS2   RQSF--MFYGAGYQVEKVISHPNYDSKTKNNDIALMKLQKPLTFNDLVKPVCLPNPGMML
TMPRSS15  MKSNLTSPQTVPRLIDEIVINPHYNRRRKDNDIAMMHLEFKVNYTDYIQPICLPEENQVF
           :*       .   ::::: :*:*: : *:****:*:*:  :.:.* ::*:***: . ::

TMPRSS2   QPEQLCWISGWGATEEKGKTSEVLNAAKVLLIETQRCNSRYVYDNLITPAMICAGFLQGN
TMPRSS15  PPGRNCSIAGWGTVVYQGTTANILQEADVPLLSNERCQQQMPEYN-ITENMICAGYEEGG
           * : * *:***:.  :*.*:::*: *.* *:..:**:.:    * **  *****: :*.

TMPRSS2   VDSCQGDSGGPLVTSKNNIWWLIGDTSWGSGCAKAYRPGVYGNVMVFTDWIYRQMR
TMPRSS15  IDSCQGDSGGPLMCQENNRWFLAGVTSFGYKCALPNRPGVYARVSRFTEWIQSFLH
          :***********: .:** *:* * **:*  **   *****..*  **:**   ::
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Figure 1 - Sequence alignment between the serine protease domains of TMPRSS2 and TMPRSS15. The loop regions that were remodelled are highlighted by green bars. TMPRSS2 residues in the S4-S3-S2-S1-S1'-S2'-S3'-S4' subsites are colored red. TMPRSS2 residues in the S1-S1' subsites are further highlighted in bold. The lysine residue between the S2/S3 subsites is highlighted with a blue arrow.

To build a homology model, the structure of TMPRSS15 was taken from PDB ID 4DGJ. (Simeonov et al. 2012) Selenomethionines were changed to methionines and missing side-chains were added using Schrödinger's Preparation Wizard, which was also used to evaluate the orientations of the asparagine, glutamine, and histidine residues, as well as the protonation state of all ionizable residues. All heteroatomic species such as buffer solvents and ions were removed. Water molecules in the arginine binding site were retained. The TMPRSS2 loop sequences EKPLNNPWH, QSFMSY, and VYDNLITPA (see Figure 1) were remodelled using Schrodinger's Prime and the whole protein was then energy minimized with the heavy atoms converged to an RMSD of 0.3 Angstroms. The homology model of TMPRS22 is available on Github overlaid with the benzamidine molecule from PDBID 2OQ5 (Kyrieleis et al. 2007):

https://github.com/djhuggins/TMPRSS2/blob/master/HomologyModels/TMPRSS2_HomologyModel_Enteropeptidase4DGJ_BenzamidineOverlay.pdb

We also generated a model of TMPRS22 bound to an eight residue sequence from the SARS-CoV-2 S2' cleavage site (PSKR|SFIE). This was based on the structure of bovine pancreatic trypsin inhibitor (BPTI) bound to anionic trypsin from PDBID 3FP6.(Zakharova, Horvath, and Goldenberg 2009) We used the backbone residues of BPTI and generated a homology model of the SARS-CoV-2 S2' sequence. The structure of the peptide was then energy minimized. Figure 2 shows the model, highlighting the eight subsites.

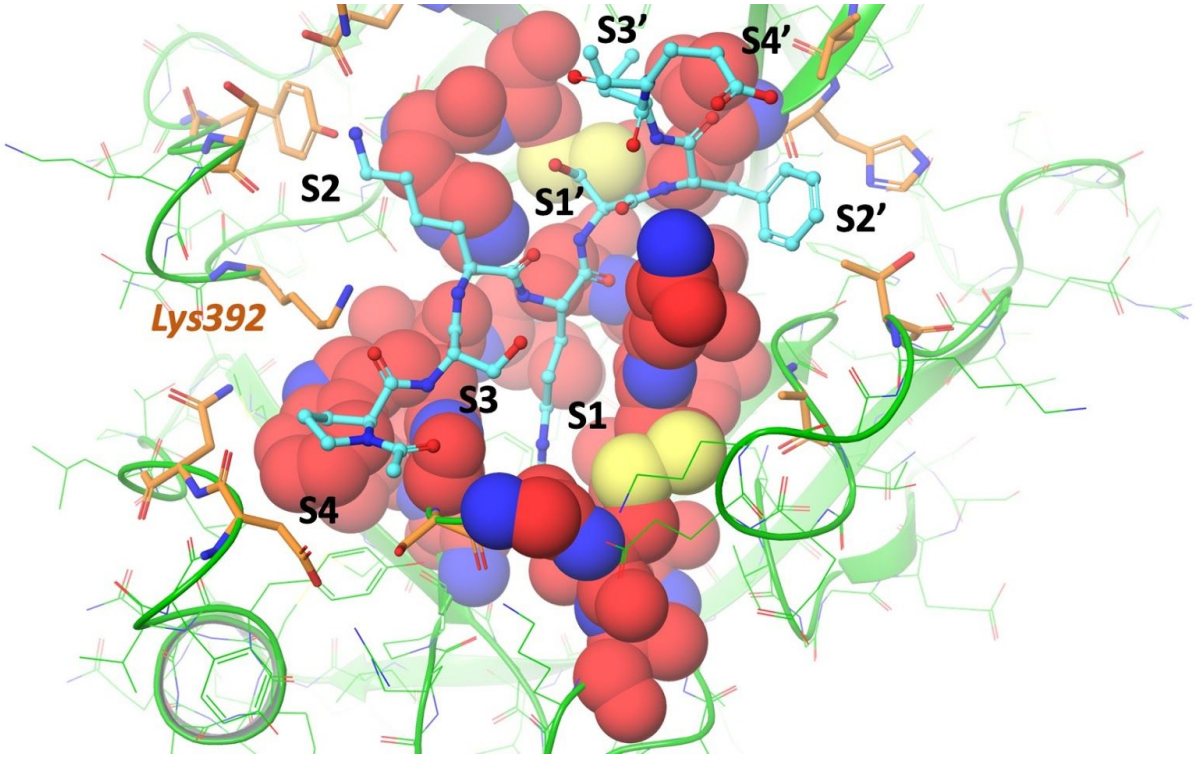

Figure 2 - Predicted structure of the SARS-CoV-2 S2' cleavage site sequence PSKRSFIE bound to the homology model of TMPRS22. The TMPRSS2 protein is displayed as green ribbons with

atoms in green wire. The peptide is displayed as cyan balls and sticks. Residues within 4 Ångström of the S1-S1' sites are displayed as red space filling. Residues within 4 Ångström of the S4-S3-S2-S1-S1'-S2'-S3'-S4' sites are displayed as orange sticks. The eight subsites and residue Lys392 are denoted.

The homology model of TMPRS22 overlaid with the SARS-CoV-2 S2' cleavage site PSKRSFIE 8-mer is available on Github:

https://github.com/djhuggins/TMPRSS2/blob/master/HomologyModels/TMPRSS2_HomologyModelFrom4DGJ_SubstrateOverlay.pdb

Identification of small molecule drugs targeting S1A serine proteases

In order to identify potential drugs that might inhibit TMPRSS2, we exploited structural modelling data from related serine proteases. We used the MEROPS database to identify all proteins in the S1A family. (Rawlings et al. 2018) We then used Drugbank (Wishart et al. 2018) to identify 36 drug targets in the S1A family for which approved, investigational or experimental drugs are available. A list of the target names, UniProt identifiers, and Drugbank of these proteins is available on Github:

https://github.com/djhuggins/TMPRSS2/blob/master/DrugBankData/S1ASerineProtease_DrugBank_TargetIDs.csv

A list of all the 376 Drugbank drugs targeting S1A serine proteases is also available on Github:

https://github.com/djhuggins/TMPRSS2/blob/master/DrugBankData/S1ASerineProtease_DrugBank_DrugIDs.csv

Included with this is the list of DrugBank molecules for each target and a list of the Protein Databank PDB identifiers (Berman et al. 2000) of these drugs bound to the targets. Of these 36 targets, the 32 targets for which PDB structural data is available are reported in Table 2.

Protein Name	**UniProt ID**	**UniProt Gene Name**	**DrugBank Target ID**
Apolipoprotein(a)	P08519	APOA_HUMAN	1283
Cathepsin G	P08311	CATG_HUMAN	1010
Chymase	P23946	CMA1_HUMAN	1038
Coagulation factor IX	P00740	FA9_HUMAN	364
Coagulation factor VII	P08709	FA7_HUMAN	333

Coagulation factor X	P00742	FA10_HUMAN	216
Coagulation factor XI	P03951	FA11_HUMAN	1021
Coagulation factor XII	P00748	FA12_HUMAN	4672
Complement C1r subcomponent	P00736	C1R_HUMAN	2093
Complement C1s subcomponent	P09871	C1S_HUMAN	1529
Complement factor B	P00751	CFAB_HUMAN	1701
Complement factor D	P00746	CFAD_HUMAN	1840
Complement factor I	P05156	CFAI_HUMAN	8979
Haptoglobin	P00738	HPT_HUMAN	10260
Hepatocyte growth factor	P14210	HGF_HUMAN	1121
Kallikrein-6	Q92876	KLK6_HUMAN	1586
Kallikrein-7	P49862	KLK7_HUMAN	4150
Myeloblastin	P24158	PRTN3_HUMAN	954
Neutrophil elastase	P08246	ELNE_HUMAN	394
Plasma kallikrein	P03952	KLKB1_HUMAN	2440
Plasminogen	P00747	PLMN_HUMAN	211
Prostasin	Q16651	PRSS8_HUMAN	3746
Prostate-specific antigen	P07288	KLK3_HUMAN	8908
Prothrombin	P00734	THRB_HUMAN	48
Serine protease hepsin	P05981	HEPS_HUMAN	2128
Tissue-type plasminogen activator	P00750	TPA_HUMAN	1088
Trypsin-1	P07477	TRY1_HUMAN	1739
Trypsin-3	P35030	TRY3_HUMAN	1583
uPA	P00749	UROK_HUMAN	895
Vitamin K-dependent protein C	P04070	PROC_HUMAN	380
Vitamin K-dependent protein Z	P22891	PROZ_HUMAN	547

Table 2 - Human protein targets in the S1A serine protease family with structural data for approved, investigational or experimental drugs.

We identified 250 approved, investigational or experimental drugs targeting S1A proteases for which structural data is available. These drugs correspond to 479 PDB structures. We downloaded the structural data for these drugs in complex with their targets and aligned them in the same reference frame. The aligned structures are available on Github:

https://github.com/djhuggins/TMPRSS2/tree/master/AlignedPDBs

We overlaid the homology model of TMPRSS2 with the PDB structures containing the approved, investigational or experimental small-molecule drugs for S1A serine proteases. We selected a subset of these molecules based on fit within the homology model of TMPRSS2. We then used Embrace minimization with GBSA solvation to test whether all molecules fit with the homology model of TMPRSS2.(Guvench et al. 2002)

Results

Based on the homology model described above, the S2-S3 subsites of TMPRSS2 appear to be different than that of related proteases due to the presence of a charge residue Lys392 (see Figure 2). Whilst this suggests that many existing serine protease inhibitors which fill these subsites will not bind to TMPRSS2, it presents an opportunity to develop selective inhibitors in the future to exploit TMPRSS2 selectively in therapeutic settings. Figure 3 presents the predicted TMPRSS2 binding modes for a set of non-covalent S1A serine protease experimental drugs overlaid with the experimental crystal structures of the drugs bound to their known target. These drugs target the S1-S1' subsites where identity between the S1A serine proteases is very high (see Table 2).

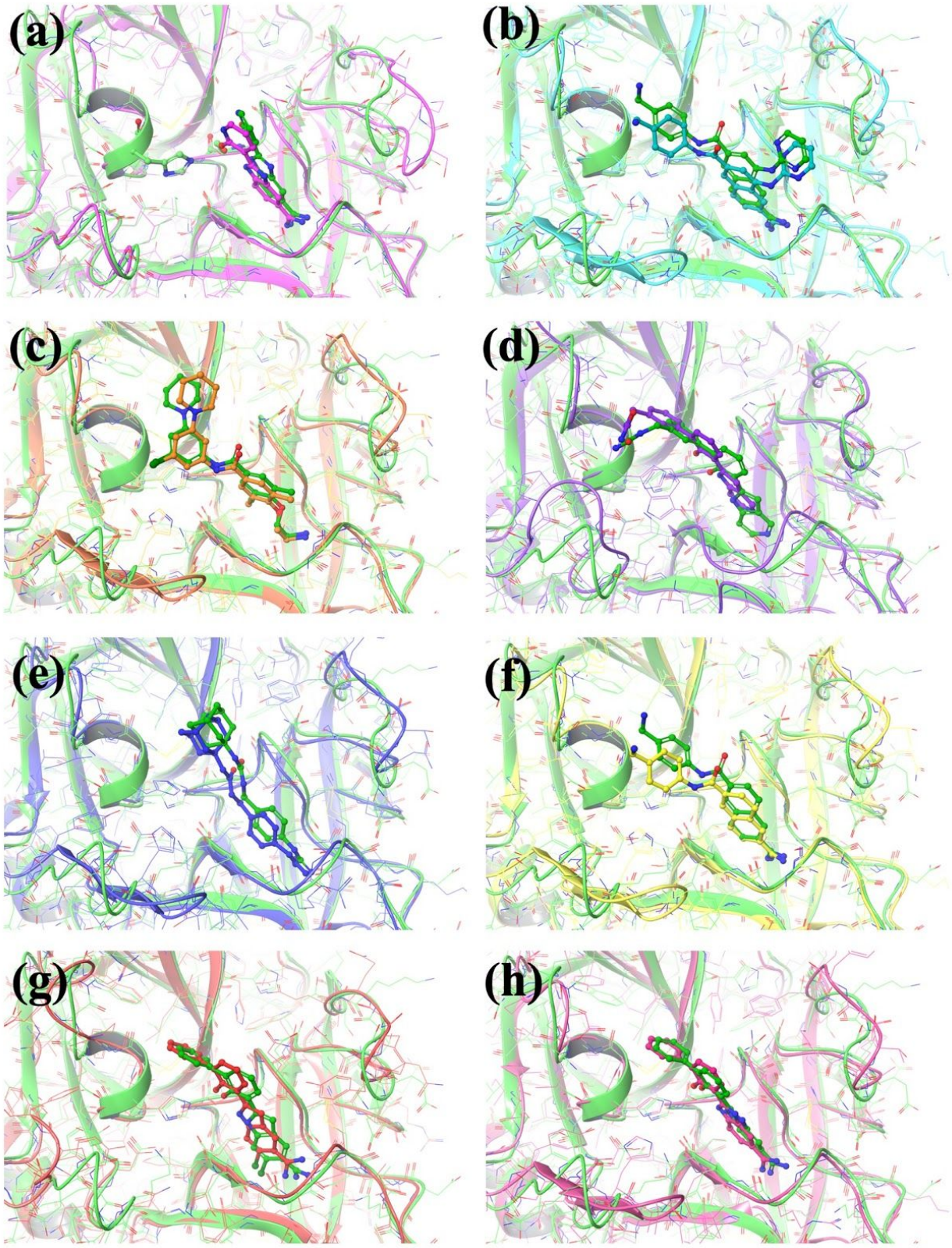
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

Figure 3 - Putative model of TMPRSS2 bound to the experimental drugs from (a) Trypsin in PDBID 1GHV,(B. A. Katz, Elrod, et al. 2001) (b) uPA in PDBID 1SQA,(Wendt, Geyer, et al. 2004) (c) uPA in PDBID 2VIV,(Frederickson et al. 2008) (d) factor VII in PDBID 2FLR,(Riggs et al. 2006) (e) uPA in PDBID 1EJN,(Sperl et al. 2000) (f) uPA in PDBID 1OWH,(Wendt, Rockway, et al. 2004) (g) hepsin in PDBID 1O5E,(Bradley A. Katz et al. 2004) and (h) uPA in PDBID 1GJC.(B. A. Katz, Sprengeler, et al. 2001) The TMPRSS2 protein is displayed as green ribbons with atoms in green wire and the predicted binding modes of the experimental drugs in TMPRSS2 are shown in green balls and sticks. The experimental protein structures are displayed as different colored colored ribbons with wire atoms and the binding modes of the experimental drugs are shown as balls and sticks.

The predicted complexes and a file with their Embrace MMGBSA scores are available on Github:

https://github.com/djhuggins/TMPRSS2/tree/master/PredictedBindingModes

The S1-S1' subsites of TMPRSS2 has a high similarity to the known targets of all these drugs (see Table 1). Table 3 reports further details for the drugs in Figure 3.

Drugbank ID	Crystallized Protein	PDB ID	Notes
DB04442	Trypsin	1GHZ	
DB03082	uPA	1SQA	Abbott compound
DB08697	uPA	2VIV	Astex compound
DB07247	Factor VII	2FLR	
DB03782	uPA	1EJN	
DB02398	uPA	1OWH	
DB03865	Hepsin	1O5E	Trypsin/thrombin/uPA/hepsin inhibitor
DB01725	uPA	1GJC	Trypsin/thrombin/uPA inhibitor

Table 3 - A set of experimental drugs targeting S1A serine proteases that may inhibit TMPRSS2 and are modelled in Figure 2.

All the small molecule drugs with an experimental crystal structure are reported on Github:

https://github.com/djhuggins/TMPRSS2/blob/master/DrugBankData/S1ASerineProtease_DrugBank_DrugIDs_WithPDBData.csv

We predict that a reasonable number of these may also inhibit TMPRSS2 and could prove effective as treatments for COVID-19. If hitting numerous host proteases is important then simple molecules such as DB04442 may prove more effective.

Discussion

A number of drug targets have been suggested for coronaviruses(Zumla et al. 2016) such as SARS-CoV-2. A recent study highlights that three covalent inhibitors of the drug target TMPRSS2 blocked SARS-CoV-2 infection of human lung cells, with nafamostat (Fujii and Hitomi 1981) showing better than camostat or gabexate.(Hoffmann, Schroeder, et al. 2020) Non-viral drug targets such as TMPRSS2 have the advantage that the virus cannot develop resistance mutations that reduce the affinity of the drug for the target. Mutations that allow the virus to utilize alternative host proteases are possible, but the corresponding change in pathogenesis leads to a higher likelihood of being deleterious to viral fitness. TMPRSS2 has the additional advantage that the drug discovery community has significant experience in developing drugs targeting serine proteases. For instance, diverse, high affinity inhibitors have been synthesized for widely studied S1A serine protease targets such as Thrombin and Factor Xa .(Vukovic and Huggins 2018)

A number of host proteases have been implicated in cleavage of the coronavirus including furin, trypsin, cathepsins, TMPRSS2, TMPRSS4, and human airway trypsin-like protease.(Ou et al. 2020) Given the furin cleavage in SARS-CoV-2 it seems likely that furin-targeted drugs would prove useful in treating the virus.(Coutard et al. 2020) Whilst there are no drugs that specifically target furin, it is possible that Camostat targets furin in addition to S1A proteases given a mechanism of action where it covalently binds to the arginine binding site. However, at this stage there is significantly more evidence that targeting TMPRSS2 will effectively treat SARS-CoV-2. The known TMPRSS2 inhibitor Camostat is being assessed in a clinical trial against COVID-19 and other inhibitors, such as Namafostat, look to be effective in cell-based studies. However, it seems highly likely that these simple covalent binders may inhibit other S1A serine proteases and this may lead to unwanted side effects for these drugs. TMPRSS2 knockout mice develop normally with no observable phenotype suggesting that it may be safely targeted.(Kim et al. 2006) To maximize safety, it would be very useful to identify exactly which proteases are key to cleaving the SARS-CoV-2 spike protein. Recent work suggests that TMPRSS2 and TMPRSS4 may both be important.(R. Zang et al. 2020)

In this study we have highlighted a large number of S1A serine protease inhibitors with experimental drug status that may inhibit TMPRSS2. Assays have previously been reported that would allow these molecules to be tested against TMPRSS2(Meyer et al. 2013) and one or more of these may have the appropriate PK properties to attain sufficiently high concentrations in the lung and effectively treat COVID-19.

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Conflict of Interest Statement

The authors declare no conflicts of interest

References

Afar, D. E., I. Vivanco, R. S. Hubert, J. Kuo, E. Chen, D. C. Saffran, A. B. Raitano, and A. Jakobovits. 2001. "Catalytic Cleavage of the Androgen-Regulated TMPRSS2 Protease Results in Its Secretion by Prostate and Prostate Cancer Epithelia." *Cancer Research* 61 (4): 1686–92.

Asselta, Rosanna, Elvezia Maria Paraboschi, Alberto Mantovani, and Stefano Duga. 2020. "ACE2 and TMPRSS2 Variants and Expression as Candidates to Sex and Country Differences in COVID-19 Severity in Italy." https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3559608.

Belouzard, Sandrine, Victor C. Chu, and Gary R. Whittaker. 2009. "Activation of the SARS Coronavirus Spike Protein via Sequential Proteolytic Cleavage at Two Distinct Sites." *Proceedings of the National Academy of Sciences of the United States of America* 106 (14): 5871–76.

Berman, Helen M., John Westbrook, Zukang Feng, Gary Gilliland, T. N. Bhat, Helge Weissig, Ilya N. Shindyalov, and Philip E. Bourne. 2000. "The Protein Data Bank." *Nucleic Acids Research* 28 (1): 235–42.

Bestle, D., M. R. Heindl, H. Limburg, O. Pilgram, and H. Moulton. 2020. "TMPRSS2 and Furin Are Both Essential for Proteolytic Activation and Spread of SARS-CoV-2 in Human Airway Epithelial Cells and Provide Promising Drug Targets." *bioRxiv*. https://www.biorxiv.org/content/10.1101/2020.04.15.042085v1.abstract.

Bosch, Berend Jan, Willem Bartelink, and Peter J. M. Rottier. 2008. "Cathepsin L Functionally Cleaves the Severe Acute Respiratory Syndrome Coronavirus Class I Fusion Protein Upstream of rather than Adjacent to the Fusion Peptide." *Journal of Virology* 82 (17): 8887–90.

Coutard, B., C. Valle, X. de Lamballerie, B. Canard, N. G. Seidah, and E. Decroly. 2020. "The Spike Glycoprotein of the New Coronavirus 2019-nCoV Contains a Furin-like Cleavage Site Absent in CoV of the Same Clade." *Antiviral Research* 176 (April): 104742.

Frederickson, Martyn, Owen Callaghan, Gianni Chessari, Miles Congreve, Suzanna R. Cowan, Julia E. Matthews, Rachel McMenamin, Donna-Michelle Smith, Mladen Vinković, and Nicola G. Wallis. 2008. "Fragment-Based Discovery of Mexiletine Derivatives as Orally Bioavailable Inhibitors of Urokinase-Type Plasminogen Activator." *Journal of Medicinal Chemistry* 51 (2): 183–86.

Fujii, S., and Y. Hitomi. 1981. "New Synthetic Inhibitors of C1r, C1 Esterase, Thrombin, Plasmin, Kallikrein and Trypsin." *Biochimica et Biophysica Acta* 661 (2): 342–45.

Guvench, Olgun, Jörg Weiser, Peter Shenkin, István Kolossváry, and W. Clark Still. 2002. "Application of the Frozen Atom Approximation to the GB/SA Continuum Model for Solvation Free Energy." *Journal of Computational Chemistry* 23 (2): 214–21.

Hoffmann, Markus, Hannah Kleine-Weber, Simon Schroeder, Nadine Krüger, Tanja Herrler, Sandra Erichsen, Tobias S. Schiergens, et al. 2020. "SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor." *Cell* 181 (2): 271–80.e8.

Hoffmann, Markus, Simon Schroeder, Hannah Kleine-Weber, Marcel A. Müller, Christian Drosten, and Stefan Pöhlmann. 2020. "Nafamostat Mesylate Blocks Activation of SARS-CoV-2: New Treatment Option for COVID-19." *Antimicrobial Agents and*

Chemotherapy, April. https://doi.org/10.1128/AAC.00754-20.
Iwata-Yoshikawa, Naoko, Tadashi Okamura, Yukiko Shimizu, Hideki Hasegawa, Makoto Takeda, and Noriyo Nagata. 2019. "TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection." *Journal of Virology* 93 (6). https://doi.org/10.1128/JVI.01815-18.
Jaimes, Javier A., Nicole M. André, Joshua S. Chappie, Jean K. Millet, and Gary R. Whittaker. 2020. "Phylogenetic Analysis and Structural Modeling of SARS-CoV-2 Spike Protein Reveals an Evolutionary Distinct and Proteolytically Sensitive Activation Loop." *Journal of Molecular Biology*. https://doi.org/10.1016/j.jmb.2020.04.009.
Kam, Yiu-Wing, Yuushi Okumura, Hiroshi Kido, Lisa F. P. Ng, Roberto Bruzzone, and Ralf Altmeyer. 2009. "Cleavage of the SARS Coronavirus Spike Glycoprotein by Airway Proteases Enhances Virus Entry into Human Bronchial Epithelial Cells in Vitro." *PloS One* 4 (11): e7870.
Katz, B. A., K. Elrod, C. Luong, M. J. Rice, R. L. Mackman, P. A. Sprengeler, J. Spencer, et al. 2001. "A Novel Serine Protease Inhibition Motif Involving a Multi-Centered Short Hydrogen Bonding Network at the Active Site." *Journal of Molecular Biology* 307 (5): 1451–86.
Katz, B. A., P. A. Sprengeler, C. Luong, E. Verner, K. Elrod, M. Kirtley, J. Janc, et al. 2001. "Engineering Inhibitors Highly Selective for the S1 Sites of Ser190 Trypsin-like Serine Protease Drug Targets." *Chemistry & Biology* 8 (11): 1107–21.
Katz, Bradley A., Christine Luong, Joseph D. Ho, John R. Somoza, Erik Gjerstad, Jie Tang, Steven R. Williams, et al. 2004. "Dissecting and Designing Inhibitor Selectivity Determinants at the S1 Site Using an Artificial Ala190 Protease (Ala190 uPA)." *Journal of Molecular Biology* 344 (2): 527–47.
Kawase, Miyuki, Kazuya Shirato, Lia van der Hoek, Fumihiro Taguchi, and Shutoku Matsuyama. 2012. "Simultaneous Treatment of Human Bronchial Epithelial Cells with Serine and Cysteine Protease Inhibitors Prevents Severe Acute Respiratory Syndrome Coronavirus Entry." *Journal of Virology* 86 (12): 6537–45.
Kim, Tom S., Cynthia Heinlein, Robert C. Hackman, and Peter S. Nelson. 2006. "Phenotypic Analysis of Mice Lacking the Tmprss2-Encoded Protease." *Molecular and Cellular Biology* 26 (3): 965–75.
Kleine-Weber, Hannah, Mahmoud Tarek Elzayat, Markus Hoffmann, and Stefan Pöhlmann. 2018. "Functional Analysis of Potential Cleavage Sites in the MERS-Coronavirus Spike Protein." *Scientific Reports* 8 (1): 16597.
Kyrieleis, Otto J. P., Robert Huber, Edgar Ong, Ryan Oehler, Mike Hunter, Edwin L. Madison, and Uwe Jacob. 2007. "Crystal Structure of the Catalytic Domain of DESC1, a New Member of the Type II Transmembrane Serine Proteinase Family." *The FEBS Journal* 274 (8): 2148–60.
Li, Guangdi, and Erik De Clercq. 2020. "Therapeutic Options for the 2019 Novel Coronavirus (2019-nCoV)." *Nature Reviews. Drug Discovery* 19 (3): 149–50.
Lukassen, Soeren, Robert Lorenz Chua, Timo Trefzer, Nicolas C. Kahn, Marc A. Schneider, Thomas Muley, Hauke Winter, et al. 2020. "SARS-CoV-2 Receptor ACE2 and TMPRSS2 Are Primarily Expressed in Bronchial Transient Secretory Cells." *The EMBO Journal*. https://www.embopress.org/doi/abs/10.15252/embj.20105114.
Maryanoff, Bruce E. 2004. "Inhibitors of Serine Proteases as Potential Therapeutic Agents: The

Road from Thrombin to Tryptase to Cathepsin G." *Journal of Medicinal Chemistry* 47 (4): 769–87.

Meyer, Daniela, Frank Sielaff, Maya Hammami, Eva Böttcher-Friebertshäuser, Wolfgang Garten, and Torsten Steinmetzer. 2013. "Identification of the First Synthetic Inhibitors of the Type II Transmembrane Serine Protease TMPRSS2 Suitable for Inhibition of Influenza Virus Activation." *Biochemical Journal* 452 (2): 331–43.

Millet, Jean Kaoru, and Gary R. Whittaker. 2015. "Host Cell Proteases: Critical Determinants of Coronavirus Tropism and Pathogenesis." *Virus Research* 202 (April): 120–34.

Ou, Xiuyuan, Yan Liu, Xiaobo Lei, Pei Li, Dan Mi, Lili Ren, Li Guo, et al. 2020. "Characterization of Spike Glycoprotein of SARS-CoV-2 on Virus Entry and Its Immune Cross-Reactivity with SARS-CoV." *Nature Communications*. https://doi.org/10.1038/s41467-020-15562-9.

Qi, Jing, Yang Zhou, Jiao Hua, Liying Zhang, Jialin Bian, Beibei Liu, Zicen Zhao, and Shuilin Jin. n.d. "The scRNA-Seq Expression Profiling of the Receptor ACE2 and the Cellular Protease TMPRSS2 Reveals Human Organs Susceptible to COVID-19 Infection." https://doi.org/10.1101/2020.04.16.045690.

Rawlings, Neil D., Alan J. Barrett, Paul D. Thomas, Xiaosong Huang, Alex Bateman, and Robert D. Finn. 2018. "The MEROPS Database of Proteolytic Enzymes, Their Substrates and Inhibitors in 2017 and a Comparison with Peptidases in the PANTHER Database." *Nucleic Acids Research* 46 (D1): D624–32.

Riggs, Jennifer R., Huiyong Hu, Aleksandr Kolesnikov, Ellen M. Leahy, Kieron E. Wesson, William D. Shrader, Dange Vijaykumar, et al. 2006. "Novel 5-Azaindole Factor VIIa Inhibitors." *Bioorganic & Medicinal Chemistry Letters* 16 (12): 3197–3200.

Shen, Li Wen, Hui Juan Mao, Yan Ling Wu, Yoshimasa Tanaka, and Wen Zhang. 2017. "TMPRSS2: A Potential Target for Treatment of Influenza Virus and Coronavirus Infections." *Biochimie* 142 (November): 1–10.

Sievers, Fabian, Andreas Wilm, David Dineen, Toby J. Gibson, Kevin Karplus, Weizhong Li, Rodrigo Lopez, et al. 2011. "Fast, Scalable Generation of High-Quality Protein Multiple Sequence Alignments Using Clustal Omega." *Molecular Systems Biology* 7 (1). https://www.embopress.org/doi/abs/10.1038/msb.2011.75.

Simeonov, Peter, Michael Zahn, Norbert Sträter, and Thole Zuchner. 2012. "Crystal Structure of a Supercharged Variant of the Human Enteropeptidase Light Chain." *Proteins* 80 (7): 1907–10.

Sperl, S., U. Jacob, N. Arroyo de Prada, J. Sturzebecher, O. G. Wilhelm, W. Bode, V. Magdolen, R. Huber, and L. Moroder. 2000. "(4-Aminomethyl)phenylguanidine Derivatives as Nonpeptidic Highly Selective Inhibitors of Human Urokinase." *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.97.10.5113.

Stopsack, Konrad H., Lorelei A. Mucci, Emmanuel S. Antonarakis, Peter S. Nelson, and Philip W. Kantoff. 2020. "TMPRSS2 and COVID-19: Serendipity or Opportunity for Intervention?" *Cancer Discovery*, April. https://doi.org/10.1158/2159-8290.CD-20-0451.

Vukovic, S., and D. J. Huggins. 2018. "Quantitative Metrics for Drug–target Ligandability." *Drug Discovery Today*. https://www.sciencedirect.com/science/article/pii/S1359644617305895.

Wang, Junmei. 2020. "Fast Identification of Possible Drug Treatment of Coronavirus Disease-19 (COVID-19) through Computational Drug Repurposing Study." *Journal of Chemical*

Information and Modeling, May. https://doi.org/10.1021/acs.jcim.0c00179.
Wang, Qiong, Ye Qiu, Jin-Yan Li, Zhi-Jian Zhou, Ce-Heng Liao, and Xing-Yi Ge. 2020. "A Unique Protease Cleavage Site Predicted in the Spike Protein of the Novel Pneumonia Coronavirus (2019-nCoV) Potentially Related to Viral Transmissibility." *Virologica Sinica*, March. https://doi.org/10.1007/s12250-020-00212-7.
Wendt, Michael D., Andrew Geyer, William J. McClellan, Todd W. Rockway, Moshe Weitzberg, Xumiao Zhao, Robert Mantei, et al. 2004. "Interaction with the S1β-Pocket of Urokinase: 8-Heterocycle Substituted and 6,8-Disubstituted 2-Naphthamidine Urokinase Inhibitors." *Bioorganic & Medicinal Chemistry Letters*. https://doi.org/10.1016/j.bmcl.2004.04.030.
Wendt, Michael D., Todd W. Rockway, Andrew Geyer, William McClellan, Moshe Weitzberg, Xumiao Zhao, Robert Mantei, et al. 2004. "Identification of Novel Binding Interactions in the Development of Potent, Selective 2-Naphthamidine Inhibitors of Urokinase. Synthesis, Structural Analysis, and SAR of N-Phenyl Amide 6-Substitution." *Journal of Medicinal Chemistry* 47 (2): 303–24.
Wishart, David S., Yannick D. Feunang, An C. Guo, Elvis J. Lo, Ana Marcu, Jason R. Grant, Tanvir Sajed, et al. 2018. "DrugBank 5.0: A Major Update to the DrugBank Database for 2018." *Nucleic Acids Research* 46 (D1): D1074–82.
Zakharova, Elena, Martin P. Horvath, and David P. Goldenberg. 2009. "Structure of a Serine Protease Poised to Resynthesize a Peptide Bond." *Proceedings of the National Academy of Sciences of the United States of America* 106 (27): 11034–39.
Zang, R., M. F. G. Castro, B. T. McCune, and Q. Zeng. 2020. "TMPRSS2 and TMPRSS4 Mediate SARS-CoV-2 Infection of Human Small Intestinal Enterocytes." *bioRxiv*. https://www.biorxiv.org/content/10.1101/2020.04.21.054015v1.abstract.
Zang, Ruochen, Maria Florencia Gomez Castro, Broc T. McCune, Qiru Zeng, Paul W. Rothlauf, Naomi M. Sonnek, Zhuoming Liu, et al. 2020. "TMPRSS2 and TMPRSS4 Promote SARS-CoV-2 Infection of Human Small Intestinal Enterocytes." *Science Immunology*. https://doi.org/10.1126/sciimmunol.abc3582.
Zhou, Yadi, Yuan Hou, Jiayu Shen, Yin Huang, William Martin, and Feixiong Cheng. 2020. "Network-Based Drug Repurposing for Novel Coronavirus 2019-nCoV/SARS-CoV-2." *Cell Discovery*. https://doi.org/10.1038/s41421-020-0153-3.
Zumla, Alimuddin, Jasper F. W. Chan, Esam I. Azhar, David S. C. Hui, and Kwok-Yung Yuen. 2016. "Coronaviruses — Drug Discovery and Therapeutic Options." *Nature Reviews Drug Discovery*. https://doi.org/10.1038/nrd.2015.37.