Structural analysis of experimental drugs binding to the SARS-CoV-2 target TMPRSS2

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A B S T R A C T

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 also inhibit TMPRSS2. Whilst the presence of a relatively uncommon lysine residue in the S2/S3 subsites means that some serine protease inhibitors will not inhibit TMPRSS2, this residue is likely to provide a handle for selective targeting in a focused drug discovery project. We discuss how experimental drugs targeting related serine proteases might be repurposed as TMPRSS2 inhibitors to treat coronaviruses.

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1. 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 [1] and a plethora of drug repurposing efforts are underway [2,3]. 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 autoclaves to generate a secreted protease [4], 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 [5,6]. Coronavirus spike proteins are thought to be cleaved at two sites, termed S1/S2 and S2' [7—9]. The proteases furin, trypsin, cathepsin, TMPRSS2, TMPRSS4, TMPRSS11, and human airway trypsin-like protease have all been implicated in these cleavages [9—13]. 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 SARS-CoV-2 S1/S2 cleavage site (RRAR|S) clearly generate a furin cleavage site [14,15]. Interestingly, this difference has been implicated in viral transmissibility of SARS-CoV-2 [16]. In theory, preventing cleavage at either site should be deleterious to viral invasion.

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 [17]. For this reason, it has been suggested as a potential drug target for coronaviruses [18,19] such as SARS-CoV-2 [20]. TMPRSS2 is highly expressed in lung tissue [21] and it has been suggested that

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

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

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

TMPRSS2    VDSCQGDSGGPLVTSKNNIWWLIGDTSWGSGCAKAYRPGVYGNVMVFTDWIYRQMR
TMPRSS15   IDSCQGDSGGPLMCQENNRWFLAGVTSFGYKCALPNRPGVYARVSRFTEWIQSFLH
           :************: .:** *:* * **:*  **  *****..* **:** ::
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Fig. 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. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

differential expression in males may lead to higher risk in male patients [20,22,23] Peptidic inhibitors of TMPRSS2 have been described [12] 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 [24]. Many of the members in this family have been studied in detail, yielding numerous high resolution crystal structures, known inhibitors, and licensed drugs [25]. 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.

2. Materials and methods

2.1. 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 [26]. The overall sequence identity between TMPRSS2 and the other S1A family proteases is available on Github: https://github.com/djhuggins/TMPRSS2/tree/master/Alignments.
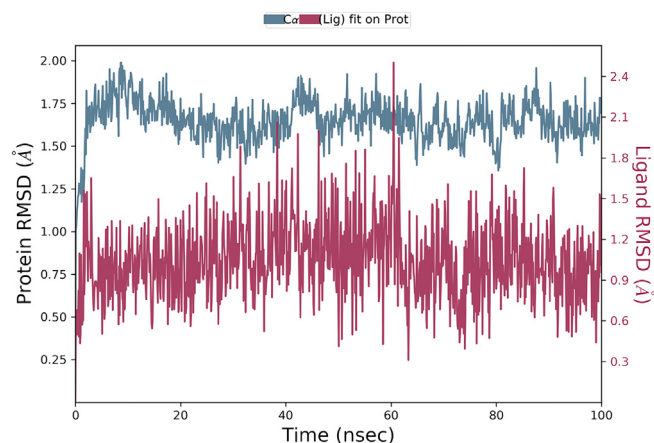

Fig. 2. The RMSD of the protein alpha carbons relative to the original structure (blue) and the RMSD of the ligand relative to the original structure (red) across the 100 ns simulation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

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

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.

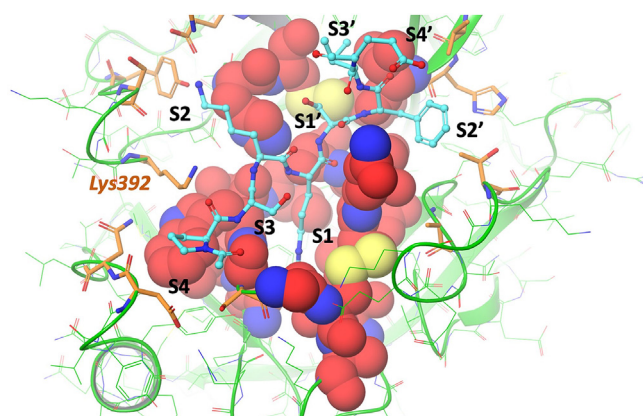| Protein Name	Uniprot Name	TMPRSS2 Protease Domain % Identity	TMPRSS2 S4—S4′ % Identity	TMPRSS2 S1—S1′ % Identity
Plasminogen	PLMN_HUMAN	41.07	64.71	95.00
uPA	UROK_HUMAN	33.77	58.82	95.00
Trypsin-1	TRY1_HUMAN	38.60	58.82	90.00
Plasma kallikrein	KLKB1_HUMAN	41.99	61.76	85.00
Coagulation factor VII	FA7_HUMAN	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

Fig. 3. 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. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

(S4−S3−S2−S1−S1′-S2′-S3′-S4′). These residues are identified in Fig. 1. The percentage identities between TMPRSS2 and this subset are given in Table 1:

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 of 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 Fig. 1.

To build a homology model, the structure of TMPRSS15 was taken from PDB ID 4DGJ [27]. 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 Fig. 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 Å. The homology model of TMPRS22 is available on Github overlaid with the benzamidine molecule from PDBID 2OQ5 [28]: https://github.com/djhuggins/TMPRSS2/blob/master/HomologyModels/TMPRSS2_HomologyModel_Enteropeptidase4DGJ_BenzamidineOverlay.pdb.

To assess the quality of the homology model, we performed 100 ns of molecular dynamics using the Desmond package. The system was set up as an orthorhombic box with a minimum distance of 10 Å from the protein to the box edge. Water was modelled with the SPC water model and the OPLS3e forcefield was used for the protein and ligand. 100 ns of simulation was then performed in the NPT ensemble at 300 K and 1.01325 bar. Fig. 2 shows the RMSD of the protein and the ligand during the simulation. Both stabilize within the first 10 ns? The protein remains stable, with an alpha carbon RMSD around 1.7 Å relative to the original structure and always below 2.0 Å. The ligand also remains stable, with an RMSD around

Table 2

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

| 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	10,260
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
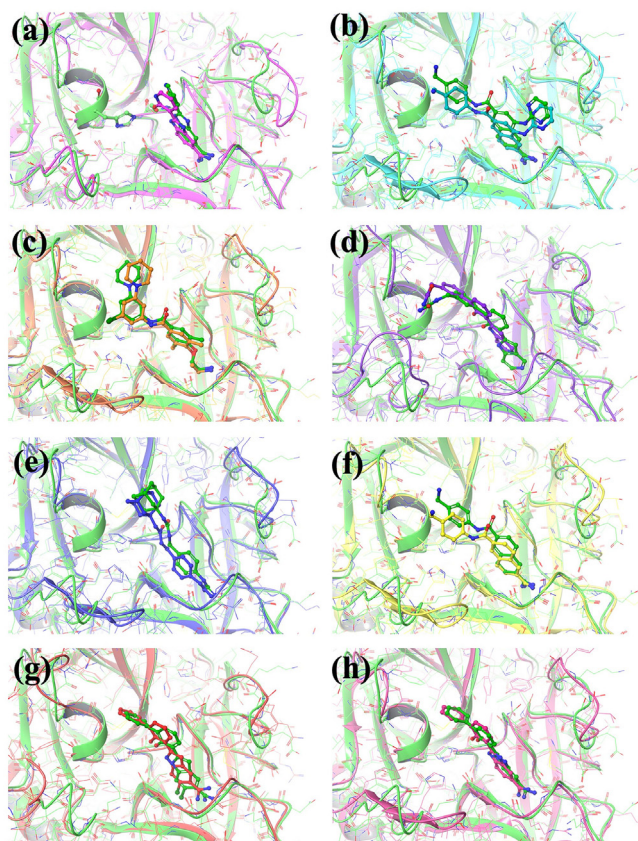
Fig. 4. Putative model of TMPRSS2 bound to the experimental drugs from (a) Trypsin in PDBID 1GHV [33], (b) uPA in PDBID 1SQA [34], (c) uPA in PDBID 2VIV [35], (d) factor VII in PDBID 2FLR [36], (e) uPA in PDBID 1EJN [37], (f) uPA in PDBID 1OWH [38], (g) hepsin in PDBID 1O5E [39], and (h) uPA in PDBID 1GJC [40]. 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 ribbons with wire atoms and the binding modes of the experimental drugs are shown as balls and sticks. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

0.9 Å relative to the original structure and always below 2.5 Å.

We also generated a model of TMPRS22 bound to an eight-residue sequence from the SARS-CoV-2 S2′ cleavage site (PSKR|SFIE). The structure of the peptide was based on the structure of bovine pancreatic trypsin inhibitor (BPTI) bound to anionic trypsin from PDBID 3FP6 [29]. 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. Fig. 3 shows the model, highlighting the eight subsites.

2.2. 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 [24]. We then used Drugbank [30] 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 ID 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 [31] 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.

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 [32]. The use of minimization ensures that the resulting complexes to do contain any clashes or bad contacts.

3. 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 Fig. 3). 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. Fig. 4 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).
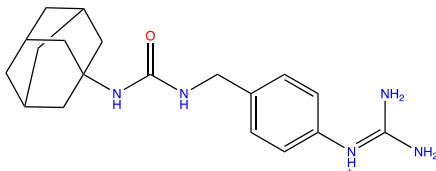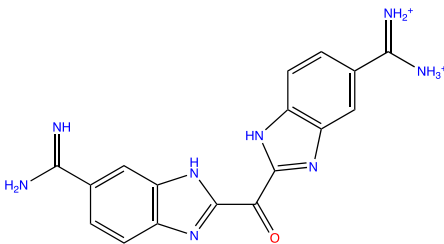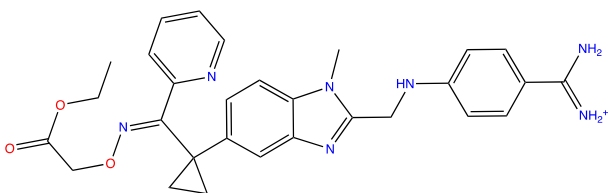
The predicted complexes and a file with their Embrace MMGBSA scores are available on Github: https://github.com/djhuggins/

Table 3
A set of experimental drugs targeting S1A serine proteases that may inhibit TMPRSS2 and are modelled in Fig. 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 4
Three of the TMPRSS2 Embrace top scoring serine protease inhibitors.

Drugbank ID	Structure	Protein Target
DB03782		Urokinase-type plasminogen activator
DB03213		Trypsin-1
DB04107		Trypsin-1

TMPRSS2/tree/master/Embrace.

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 Fig. 4.

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 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.

4. Discussion

A number of drug targets have been suggested for coronaviruses [41] 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 [42] showing better than camostat or gabexate [43]. 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. As a drug target TMPRSS2 has the additional advantage that the drug discovery community has significant experience in developing drugs targeting serine proteases. For instance, many diverse and high affinity inhibitors have been synthesized for widely studied S1A serine protease targets such as Thrombin and Factor Xa [44].

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 [45]. Given the furin cleavage in SARS-CoV-2 it seems likely that furin-targeted drugs would prove useful in treating the virus [14]. 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 [46]. 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 [47]. This work identifies a number of experimental drugs for serine proteases that may effectively inhibit TMPRSS2 based on homology and Embrace binding free energy calculations. Three are shown in Table 4.

All three molecules in Table 4 contain a positively charged warhead similarly to nafamostat, camostat, and gabexate (guanidine in the case of to DB03782, nafamostat, camostat, and gabexate versus amidine in the case of DB03213 and DB04107). The vast majority of the 376 serine protease inhibitors contain such a warhead.

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(12) [48] 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.

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.

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