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Homology modelling and molecular docking study of TMPRSS2 with small-molecule protease inhibitors to control SARS-CoV-2

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ABSTRACT

Due to the urgent need of drugs to control the COVID-19 pandemic, repositioning of already marketed drugs could be a fast and convenient option to identify agents to aid in controlling and treating COVID-19. This work presented a computational work regarding homology modeling and molecular docking of repurposing drugs related to the SARS-CoV-2. We have created a homology model of the cell surface transmembrane protease serine 2 protein (TMPRSS2) in order to investigate and analyze the interactions of already known small-molecules. This study indicates the most active inhibitors, poceprevir, simeprevir and neoandrgrapholide, that can be used further to search for better TMPRSS2 inhibitors. Moreover, we analyzed the most important atomistic connections between these compounds and the modeled protein pockets. This study will focus on TMPRSS2-targeted drugs by comparing the binding mode of approved and experimentally used TMRSS2 inhibitors with other agents with TMPRSS2 inhibitory activity and could potentially inhibit SARS-CoV-2 and therefore could lead to the identification of new agents for further clinical evaluation of SARS-CoV-2 and potential treatment of COVID-19.

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INTRODUCTION

Coronaviruses are enveloped positive single-strand RNA viruses that encode four main structural proteins: M, Membrane; N, Nucleocapsid; E, Envelope and S, Spike [1]. SQ Recent studies found that entrance of SARS-CoV-2 to the host cell was facilitated by the interaction of its spike glycoprotein with cell surface transmembrane protease serine 2 (TMPRSS2) and angiotensin-converting enzyme-2 (ACE-2) [2, 3].

Several studies confirmed that the pathogenicity of coronavirus infections could be manipulated by targeting these host receptors [4–6]. Knockout of TMPRSS2 reduces coronavirus growth in the lungs, the proinflammatory response and the severity of lung pathology [6]. Another study confirmed that pharmacological inhibition of TMPRSS2 by a serine protease inhibitor such as Camostat also reduces SARS-CoV-2 entry into host cells [4]. In addition, several inhibitors of TMPRSS2 with nanomolar affinity were reported in another study [7], but

the safety of these molecules in humans has not been tested. Targeting TMPRSS2 could be a practical pathway to treat SARS-CoV-2 infections by using currently available molecules that are confirmed as inhibitors of TMPRSS2. An efficient way to predict the binding of drug molecules to protein targets is via computational modelling studies of protein-ligand interactions.

In this work, due to a lack of available three-dimensional (3D) models of TMPRSS2 in the literature, we used the homology modelling technique to generate a 3D model of TMPRSS2 and examine the activity of a small-molecule serine protease against TMPRSS2. Screening of known serine protease inhibitors has several advantages; for example, the safety profile of these approved inhibitors has been studied extensively, thus promoting their clinical use. In this study, different classes of promising proteases inhibitors, an antibiotic and a naturally occurring drug, were identified with predicted binding scores approximately equal to those of identified inhibitors of TMPRESS2. Additional experimental studies could be used to confirm the suitability of these molecules as an effective treatment for SARS-CoV-2.

MATERIALS AND METHODS

Evaluation of TMPRSS2 Homology

To construct the 3D structure of TMPRSS2 with 492 amino acids, the SWISS-MODEL template library (SMTL version 2020-09-16, PDB release 2020-09-11) was searched with BLAST [8] for evolutionarily related structures matching the target sequence (UniProtKB accession code (O15393-1). The target sequence was searched with BLAST against the primary amino acid sequence contained in the SMTL. A total of 809 templates were identified, and the target-template alignment features were used to predict the template's quality. The quality of the resulting model was estimated via GMQE (Global Model Quality Estimation) [9]. Higher numbers indicate higher reliability. The resulting models were ranked according to their GMQE values.

The homology models of TMPRSS2 with the highest GMQE values were selected for further evaluation, as shown in Figure 1A. Two extracellular domains were identified in the homology model of TMPRSS2: residues 144–231 for the cysteine-rich domain and residues 232–492 for the serine protease domain. SWISS-MODEL and cross-checked in MolProbity server were used to validate the model, and 94.22% of the residues were observed in the Ramachandran-favoured region, 34%–0.58% in the allowed region and 0.34% residues in the outlier region, suggesting that the model of TMPRSS2 was of good quality. Figure 1B shows the 3D structure of the model identified, and the alignment of the target protein and the template (PDB ID: 1z8g.1.A) is shown in Figure 1C.

Autodock 4.2 software was used to prepare the identified homology model of TMPRSS2 for molecular docking analysis. The protein was 3D protonated; the model was refined, and the energy was minimized using autodocking 4.2.

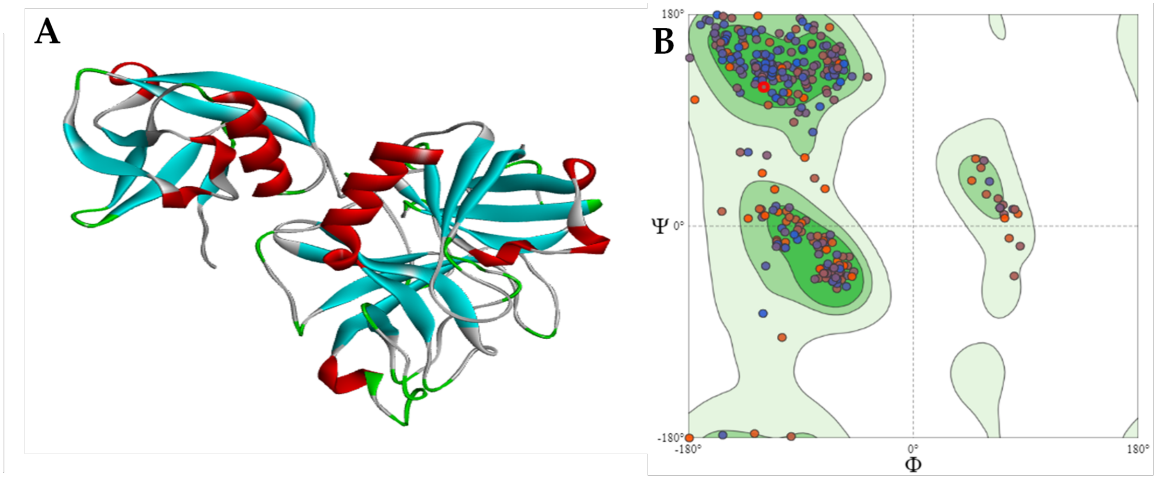
Active site residues of *TMPRSS2*

The catalytic triad of the active site of serine proteases is generally composed of SER, HIS and ASP [10]. After energy minimization, the CATSp online server was used to predict the binding pocket of the protein and to identify the active site residues in the binding pocket. The key residues in the predicted binding pocket of TMPRSS2 are ASP144, GLU145, ASN 146, ARG147, CYS148, VAL149, TYR161, SER163, ASP187, MET188, ASP220, ILE 221, TYR222, LYS223, PRO367, ASN368, PRO369, GLY370, MET371, MET372, LEU373, GLN374, GLN377, LEU404, TYR447, LYS449, TRP454, ILE456 and MET478 (Figure 2).

TMPRSS2 inhibitor key binding interactions in the docked complex

We aimed to dock and study the binding mode of approved TMPRSS2 inhibitors and experimentally used antiviral agents in COVID-19 (bromhexine hydrochloride, camostat mesylate and nafamostat) and compare the results with other agents with TMPRSS2 inhibitory activity, which could potentially inhibit SARS-CoV-2. This includes first: other approved serine protease inhibitors (gabexate, boceprevir and simeprevir), second: other protease inhibitors (calpain inhibitor II (ALLM), GC-376, MG-132 and Leuptine), third: protease inhibitors (lactacystin) and fourth: compounds with potential TMPRSS2 activity identified through virtual screening [11] (the antibacterial agent pivampicillin and the antiviral natural compound neoandrgrapholide).

We started by using an online blind docking server to predict the binding sites of the approved inhibitors of TMPRSS2 (bromhexine hydrochloride, camostat mesylate and nafamostat) with TMPRSS2. Bromhexine hydrochloride showed a hydrogen bond interaction with the Asp482 residue of TMPRSS2 and formed two Van der Waals interactions with Val479 and Ile221 residues (Figure 3). The binding energy of TMPRSS2 with bromhexine hydrochloride was -5.00 kcal/mol. Camostat mesylate formed hydrogen bond interactions with



C

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Model_01 MALNSGSPPAIGPYYENHGYQPENPYPAQPTVVPTVYEVHPAQYYPSPVPQYAPRVLTQASNPVVCTQPKSPSGTVCTSKTKKALCITLT  90
1z8g.1.A ------------------------------------------------------------------------------------------

Model_01 LGTFLVGAALAAGLLWKFMGSKCSNSGIECDSSGTCINPSNWCDGVSHCPGGEDENRCVRLYGPNFILQVYSSQRKSWHPVCQDDWNENY 180
1z8g.1.A ----------------------------------------------SDQEPLYPVQVSSADARLMVFDKTEGTWRLLCSSRSNARV  40

Model_01 GRAACRDMGYKNNFYSSQGIVDDS---GSTSFMKLNTS-AGNVDIYKKLYHSDACSSKAVVSLRCIACGVNLNSSRQSRIVGGESALPGA 266
1z8g.1.A AGLSCEEMGFLRALTHSELDVRTAGAAGTSGFFCVDEGRLPHTQRLLEVISVCDCPRGRFLAAICQDCGRRKL--PVDRIVGGRDTSLGR 128

Model_01 WPWQVSLHVQNVHVCGGSIITPEWIVTAAHCVEKPLNNPWHWTAFAGILRQSFMFYGAGYQVEKVISHPNYD------SKTKNNDIALMK 350
1z8g.1.A WPWQVSLRYDGAHLCGGSLLSGDWVLTAAHCFPERNRVLSRWRVFAGAVAQA-SPHGLQLGVQAVVYHGGYLPFRDPNSEENSNDIALVH 217

Model_01 LQKPLTFNDLVKPVCLPNPGMMLQPEQLCWISGWGATEEKGKTSEVLNAAKVLLIETQRCNSRYVYDNLITPAMICAGFLQGNVDSCQGD 440
1z8g.1.A LSSPLPLTEYIQPVCLPAAGQALVDGKICTVTGWGNTQYYGQQAGVLQEARVPIISNDVCNGADFYGNQIKPKMFCAGYPEGGIDACQGD 307

Model_01 SGGPLVTSKN----NIWWLIGDTSWGSGCAKAYRPGVYGNVMVFTDWIYRQMRADG                                  492
 1z8g.1.ASGGPFVCEDSISRTPRWRLCGIVSWGTGCALAQKPGVYTKVSDFREWIFQAIKTH-                                  362
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Figure 1: A 3Dstructure of the (TMPRSS2) homology model

Pro335, Asn336, Asp482 and Trp483 and showed couples of Van der Waals interactions with Asn336, Val479 and Phe480 (Figure 3). Its binding energy with the enzyme was -6.20 kcal/mol. Nafamostat, the structurally related analogue of camostat mesylate, showed hydrogen bonds with Asp220 and Gly370 and Van der Walls interactions via Val479 and Thr481 residues (Figure 3). It showed similar binding energy to camostat mesylate of -6.50 kcal/mol.

These three inhibitors have been docked and studied previously by Kailas Sonawane et al., 2020 [12]. The results of their work showed similar binding interactions with the active site of TMPRSS2 and similar binding affinity (in the present work, we found the binding affinity to be -5.00, -6.20 and -6.50 kcal/mol for bromhexine hydrochloride, camostat mesylate, and nafamostat, respectively. Kailas Sonawane et al. found the binding affinity to be -5.96, -7.94 and -7.21 kcal/mol for bromhexine hydrochloride, camostat mesylate, and nafamostat, respectively). This consistency in the results gives confidence in the protocol used to generate homology models and docking results applied to the other inhibitors.

Other approved serine protease inhibitors (gabexate, boceprevir and simeprevir) were also docked and studied in the present work and showed highly interesting results. Gabexate showed hydrogen bond interactions with Gly370, Met371, Thr481 and Asp482 and formed Van der Waals interactions with Va479, Phe480, Asp482 and Trp483. The binding energy of gabexate with TMPRSS2 was -5.70 kcal/mol. Boceprevir showed good and tight interaction with TMPRSS2 via numerous hydrogen bonds with Gly370, Met371, Val479, Thr481, Asp482 and Trp483 residues of the active site of TMPRSS2 and Van der Waals interactions with Ile221, Pro369, Al423, Val479, Thr481 and Trp483. It showed a high binding affinity with the enzyme of -8.40 kcal/mol. Simeprevir formed hydrogen bonds with Gly370, Asn476, Thr481 and Asp482 residues and Van der Waals interactions with Leu373, Val479, Thr481, Asp482 and Trp483.

Figure 2: Active site prediction using the CastP server

The binding affinity of simeprevir with TMPRSS2 was -8.90 kcal/mol (Figure 4).

The third class of inhibitors studied in this work were other protease inhibitors, including calpain inhibitor II (ALLM), GC-376, MG-132 and Leuptine. Calpain inhibitor II (ALLM) formed hydrogen bonds with Asp482, Thr481 and Val479 residues and Van der Waals interactions with Phe480 and Val479. The binding energy was -5.60 kcal/mol. GC-376 forms hydrogen bonds with Gly370, Thr481, Asp482 and Val479 residues and Van der Waals interaction with Met478. Its binding energy with the enzyme was -5.90 kcal/mol. MC-376 showed hydrogen bonds with Thr481, Asp482, Val479 and Gly370 and binding energy of 6.90 kcal/mol. MG-132 showed hydrogen bond interactions with Asp482, Val479, Thr481 and Gly370 and Van der Waals interactions with Met478, Ala423, Phe480 and Ile221.

The binding energy with TMPRSS2 was -5.60 kcal/mol. Leuptine showed hydrogen bond interactions with Asp482, Val479, Met371, Asn368 and Pro367 and formed hydrophobic interactions with Trp483. Its binding energy with TMPRSS2 was -5.50 kcal/mol.

The protease inhibitor lactacystin showed hydrogen bond interactions with Trp483, Asp482 and Val479 residues and low binding affinity for the enzyme of -4.8 kcal/mol.

The antibacterial agent pivampicillin and the antiviral natural compound neoandrgrapholide, identified by Wu et al. in 2020 using computational methods as potential inhibitors of TMPSS2 [11], were selected to be docked and studied in this work. They showed exciting results in terms of binding interactions and energy affinity. Pivampicillin formed hydrogen bond interactions with Asp482, Met478 and Trp483 of the active site of the TMPRSS2 and formed Van der Waals interactions with Met372, Val479 and Trp483 (Figure 5, Table 2).

Its binding energy was -5.20 kcal/mol. Neoandrgrapholide formed hydrogen bond interactions with Asp482 and Gly370 residues and Van der Waals interactions with Val479 and Met478. Its binding energy with the enzyme was -6.0 kcal/mol (Figure 5, Table 2).

Among these inhibitors, poceprevir, simeprevir and neoandrgrapholide show good binding affinity to TMPRSS2 of -8.40, -8.90 and -6.0 kcal/mol, respectively. The IC_{50} of poceprevir and simeprevir are 4.13 and 9.60 μM (Table 1), respectively.

In comparison to the experimentally used antiviral agents (bromhexine hydrochloride, camostat mesylate and nafamostat), they showed better binding affinity (-5.0, -6.20 and -6.50 kcal/mol, respectively) and comparable IC_{50} values (0.75, 0.042 and 3.16 μM, respectively) (Table 1).

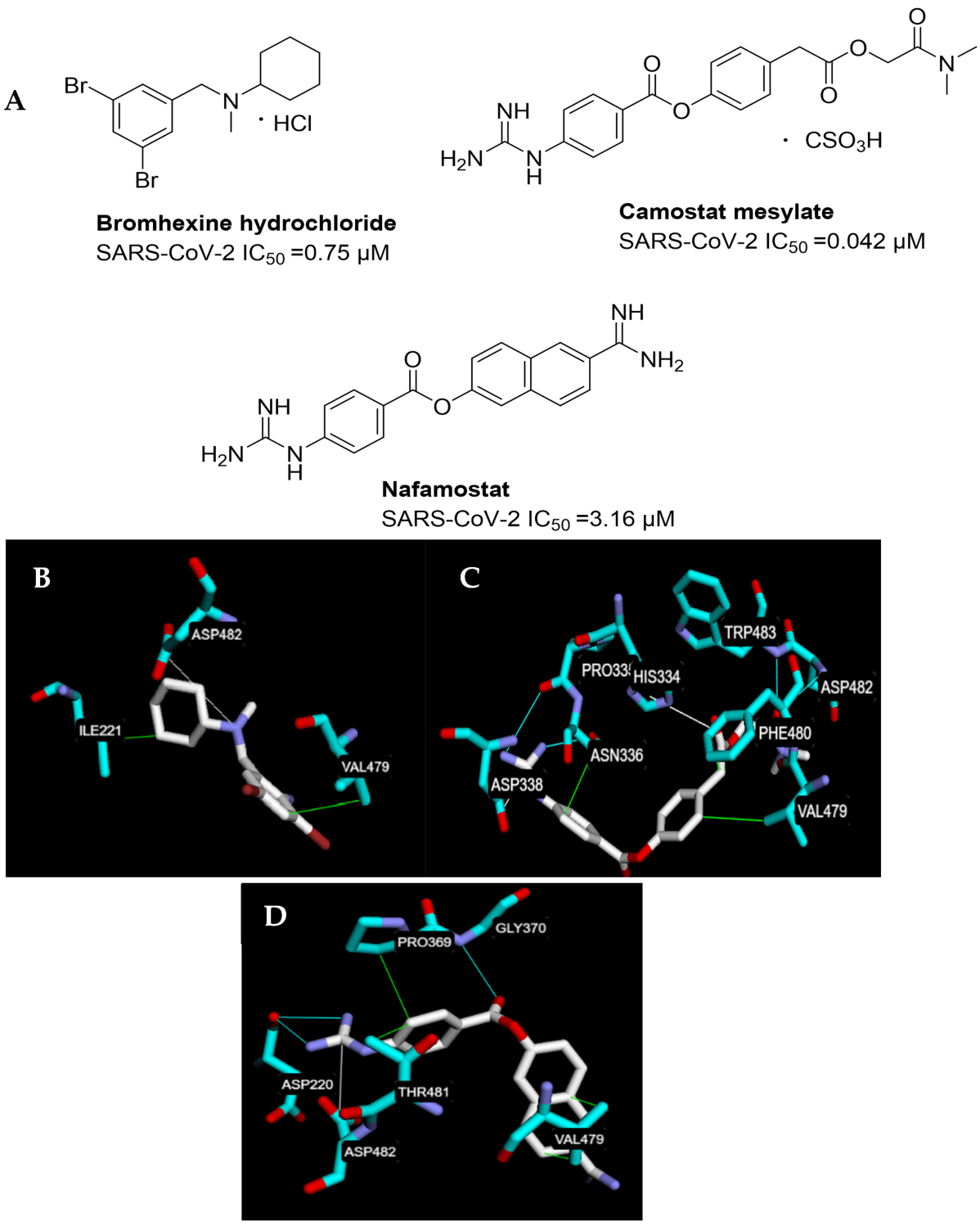

Figure 3: Structures of bromhexine hydrochloride, camostat mesylate and nafamostat SARS-CoV-2 inhibitory data expressed IC_{50}values

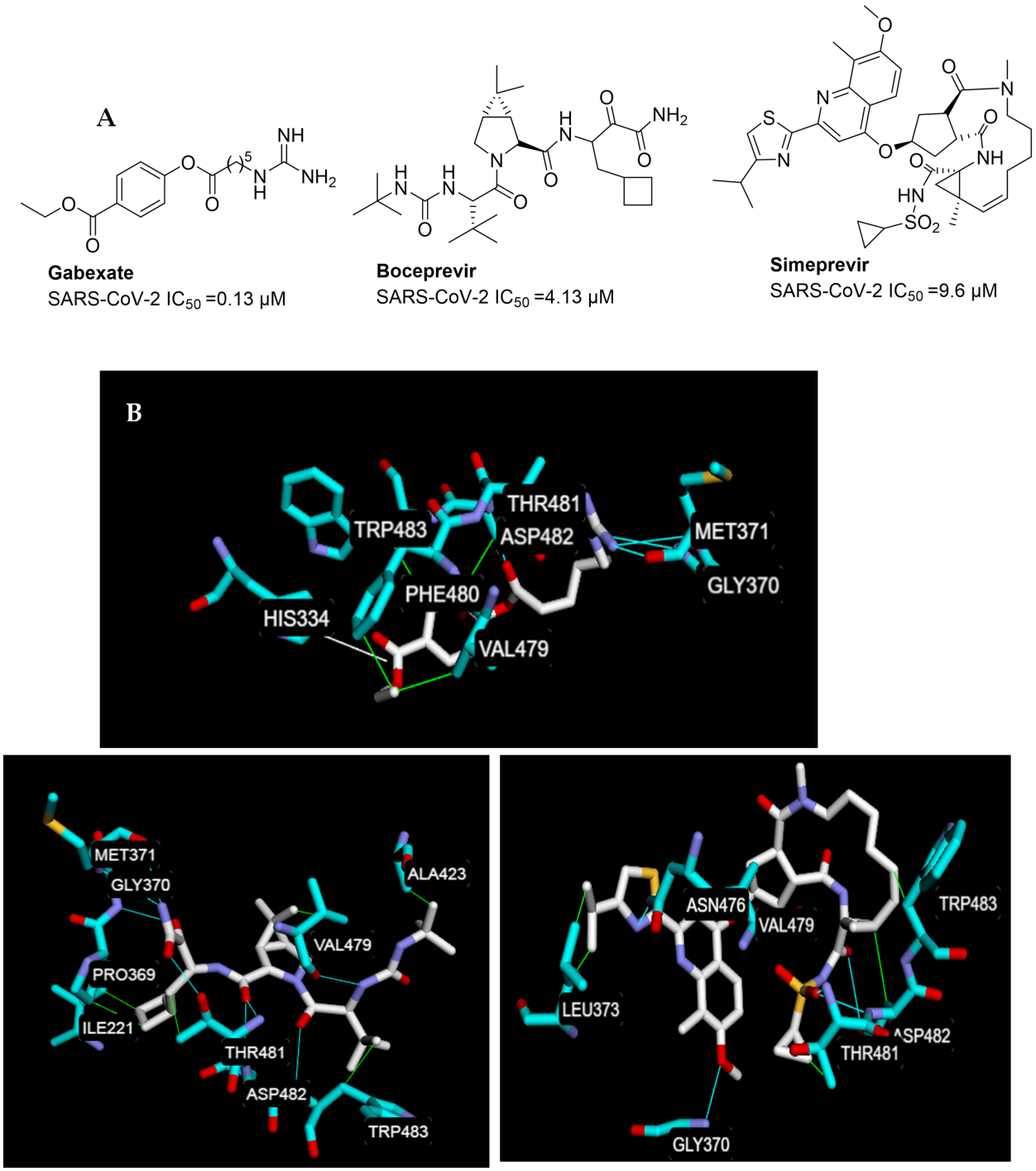

Figure 4: Structures of gabexate, boceprevir and simeprevir; SARS-CoV-2 inhibitory data expressed as IC_{50} values

Therefore, boceprevir, simeprevir and neoandrgrapholide could be suitable inhibitors of TMPRSS2 and reasonable candidates for further biological evaluation.

RESULTS AND DISCUSSION

For the analysis, we selected the amino acid sequence of human transmembrane protease serine 2 (Uniprot accession no: O15393) iso form-2 (492 amino acids). A homology model of TMPRESS2 was uploaded to the online server (SWISS-MODEL) after retrieval in FASTA format. The Continuous Automated Model Evaluation (CAMEO) platform was employed to subject the model to continuous evaluation [17]. The 3D-modelled structure was validated against the results obtained using *MolProbity* version 4.4 [18] . After verification, a homology model of TMPRSS2 of high quality was chosen for molecular docking studies.

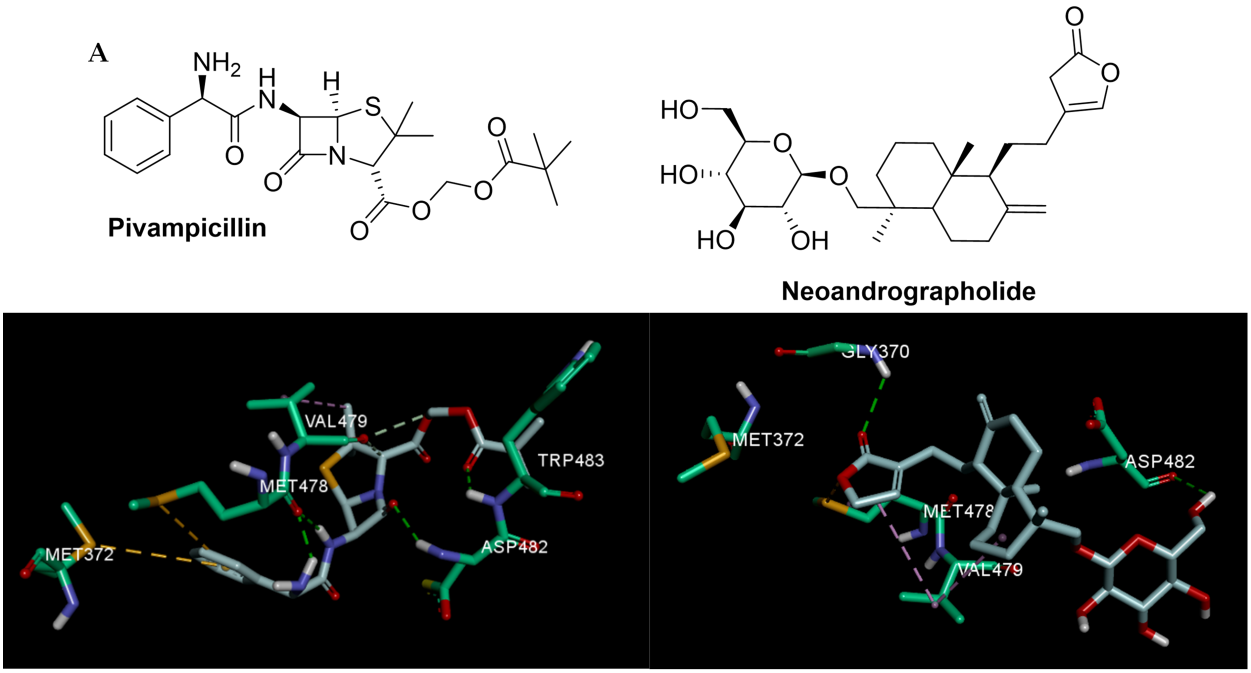

Figure 5: Structures of pivampicillin and neoandrgrapholide

Table 1: TMPRSS2 inhibitor data

Name	Class	Docking Score		
		SARS-CoV-2 IC_{50} (μM)	cal/mol	RMSD
Bromhexine	Amucolytic drug	0.75 [13]	-5.0	1.5
Camostat mesylate	Miscellaneous serine protease inhibitors	0.042 [3]	-6.20	3.2
Nafamostat	Miscellaneous serine protease inhibitors	3.16 [14]	-6.50	1.4
Gabexate	Miscellaneous serine protease inhibitors	0.13 [15]	-5.70	5.4
Boceprevir	HCV protease (serine protease) inhibitors	4.13 [16]	-8.40	
Simeprevir	HCV protease (serine protease) inhibitors	9.60 [17]	-8.90	
Calpain inhibitor II (ALLM)	calpain proteases inhibitors	0.97 [16]	-5.60	1.18
GC_376	calpain proteases inhibitors	0.03 [16]	-5.90	3.16
MG-132	Cathepsin and calpain protease (cysteine protease) inhibitors	3.90 [16]	-5.60	2.0
Leuptine	Miscellaneous cysteine protease inhibitors	NA	-5.50	1.88
lactacystin	proteasome inhibitor	NA	-4.8	3.16
Neoandrographolide	Antiviral natural compound	NA	-6.0	2.5
Pivampicillin	Ani-bacterial agent	NA	-5.2	1.74

Table 2: H-bonding of TMPRSS2 with selected inhibitors

Inhibitor	Hydrogen bond interactions	Distance (Å)
Boceprevir		
	Gly370	1.93
	Met371	3.05
	Met371	2.84
	Val479	2.29
	Thr481	2.82
	Thr481	1.87
	Asp482	2.22
	Trp483	3.18
Simeprevir		
	Gly370	2.33
	Asn476	3.40
	Thr481	2.61
	Asp482	2.77
	Asp482	2.00
Pivampicillin		
	Met478	2.20
	Met478	2.91
	Trp483	2.51
	Asp482	1.95
Neoandrographolide		
	Gly370	1.97
	Asp482	2.06

The quality of the refined models was validated by generating Ramachandran plots on PROCHECK [19].

Prediction of the Active Site of TMPRSS2

To predict the binding pocket of TMPRESS, we used the online server Computed Atlas of Surface Topography of proteins CASTp [20]. Then, we selected a potential binding pocket based on amino acid residue similarity with the binding pockets of other serine proteases.

Ligand Preparation

We used PubChem Database to retrieve the 3D coordinates of bromhexine hydrochloride (CID 5702220), camostat mesylate (CID 5284360), nafamostat (CID 4413), gabexate(CID 6604561), boceprevir (CID 10324367), simeprevir (CID 46866715), calpain inhibitor II ALLM (CID 4331), GC-376 (CID 131704475), MG-132 (CID 462382), Leuptine (CID 72429), lactacystin (CID 6610292), pivampicillin (CID 33477) and neoandrgrapholide (CID 9848024). The molecules were obtained in sdf format and then changed into PDB format with the help of Discovery Studio [21]. Autodock 4.2 software was used to convert all ligands into the PDBQT type and to prepare molecules for docking [22].

Docking of inhibitors with a homology model of TMPRSS2

Autodock vina (Version 1.1.2.) and PyRx option run at an 'exhaustiveness' of 8 were used to perform the molecular docking of inhibitors into the binding pocket of TMPRSS2. The Vina search space was defined by centring the grid box at X = 26.6017, Y = 1.5115, Z = 15.6421, with a grid dimension of 25. 0000 Å × 25.0000Å × 25. 0000 Å, thus enclosing the active site residues. Following a sequence of ligand-receptor docking runs by Vina, the results were evaluated, and the binding affinities of the ligands were calculated. Following the selection of the best pose from each cluster, the ligands were ranked on the basis of their binding affinities [23]. By employing a defaulting RMSD acceptance scale of 2.0 Å, the best docked complex was clustered [24]. The Achilles blind docking server was used to re-validate protein-ligand interactions and binding scores.

The Achilles blind docking server was also used to perform blind docking of the ligands to the TMPRSS2 model, which resulted in a comprehensive series of docking poses and calculations of the best binding affinities. Then, clusters of results were generated using a pose clustering algorithm,

and a representative pose in each cluster with the best binding affinity was selected. Protein-ligand interactions were analysed via the Protein-Ligand Interaction Profiler (PLIP) algorithm tool of BINDSURF. Then, representative clusters were manually inspected, and the best post at the predicted pocket was selected for each compound [25]. The Auto Dock Vina-generated results for the protein-ligand interactions were visualized with Discovery Studio.

CONCLUSIONS

Due to the urgent need of drugs to control the COVID-19 pandemic, repositioning of already marketed drugs could be a fast and convenient option to identify agents to aid in controlling and treating COVID-19. Hence, in the present study, we used the homology modelling technique to generate a 3D model of TMPRSS2 and computationally screen of a number of approved small molecule serine protease inhibitors for activity against TMPRSS2. Among these inhibitors, poceprevir, simeprevir and neoandrgrapholide showed good binding affinity to TMPRSS2 of -8.40, -8.90 and -6.0 kcal/mol, respectively. The IC_{50} of poceprevir and simeprevir were 4.13 and 9.60 μM, respectively. Therefore, boceprevir, simeprevir and neoandrgrapholide are good inhibitors of TMPRSS2 and reasonable candidates for further biological evaluations.

Author Contributions

Conceptualization; Asma Alsarrah and Salha Tawati, methodology; softwares.; validation; formal analysis, Salha Tawati, resources; data curation; visualization; writing—original draft preparation; Aisha Alsfouk and Salha Tawati.

Conflicts of Interest

The Authors declare that they have no conflict of interest.

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