Docking study of transmembrane serine protease type 2 inhibitors for the treatment of COVID-19

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1. Introduction

A novel coronavirus emerged in December 2019 and has spread to 210 countries around the world. Over 4,498,579 people have got infected till date; of whom, 304,631 have died [1]. Drugs that slow or kill the novel coronavirus could save the lives of severely ill patients and also might be applied prophylactically. So instead of moving up with compounds from scratch, public health departments are reviewing for repurpose drugs [2]. It includes already approved drugs for treatment of other diseases or previously established promising molecules that performed efficiently in animal studies of coronavirus infections, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS).

The coronavirus genome encodes four major structural proteins: spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and envelope (E) protein. Out of these structural proteins, S protein mediates the initial high-affinity binding to host cell-surface receptors and the subsequent fusion between the viral and host cell membranes to facilitate viral entry into host cell. It plays the key role in the early steps of viral infection. In addition, S protein consists of two main receptor-binding domains: the S1 domain responsible for binding of the S protein to a cellular receptor and the S2 domain allows the fusion of viral and cellular membranes [3]. In the case of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the binding of the S protein to the angiotensin-converting enzyme 2 receptor leads to proteolytic cleavage at the S1-S2 boundary by transmembrane serine protease type 2 (TMPRSS2), and this is a prerequisite for virus fusion and propagation [4]. So coronavirus infections can be controlled by targeting the host cell protease, TMPRSS2 [5].

Existing literature already provides evidence that one promising drug target for SARS-CoV-2 is the TMPRSS2 inhibitors [6]. Coronavirus needs TMPRSS2 to prime the viral S

protein for cell entry [7]. Camostat mesilate is an effective serine protease inhibitor. In mice, camostat mesilate dosed at concentrations comparable to the clinically feasible concentration in humans reduced mortality following SARS-CoV-2 infection from 100% to 30%−35% [8]. A double-blinded clinical trial shows primary efficacy outcome is the number of patients discharged by 14 days.

From the "Comparative Analysis of Antiviral Efficacy of FDA-Approved Drugs Against SARS-CoV-2" carried out in Vero cells isolated from the African green monkey's kidney, nafamostat showed an antiviral effect "hundreds of times" higher than that of remdesivir [9].

Nafamostat is a synthetic serine protease inhibitor approved in Japan for the treatment of acute pancreatitis and intravascular coagulation and has been used as an anticoagulant in extracorporeal circulation [10]. The "RACONA Study" clinical trials are underway to test the speculation that nafamostat can lower lung function decomposition and be under the necessity for intensive-care admission in coronavirus disease 2019 (COVID-19) patients [1]. This preclinical and clinical data inspired drug discovery interest in TMPRSS2 inhibitors.

Apart from the abovementioned drugs, peptidomimetic TMPRSS2 inhibitors such as Benzyl-D-ArgPro-4-Amidinopenicillamic (BAPA) derivatives showed antiviral effects on both influenza A and B viruses in human bronchial epithelial cells [2]. These kinds of peptidomimetic inhibitors present as an effective arsenal for the development of antiviral therapeutics against SARS-CoV-2.

By targeting the host cells rather than the viral proteins, it is possible to develop broad-spectrum antiviral drugs and also minimize drug resistance due to mutation [11]. From clinical data, it was found that coagulative abnormality is one of the complicating manifestations in patients with COVID-19 [12]; TMPRSS2 inhibitors may play dual roles not only as an antiviral agent to block viral entry but also as an anticoagulant [13].

The binding poses and affinity between a ligand and an enzyme are very important pieces of information for computer-aided drug design. In the initial stage of a drug discovery project, this information is often obtained by using molecular docking methods [14]. The suggested study utilizes molecular docking approach to identify TMPRSS2 inhibitors, which can act as promising antiviral medicines. TMPRSS2 inhibitors might act by two different mechanisms: reversible binding to the active site or interference with an allosteric domain. Understanding their inhibitory mechanism through molecular docking study helps us to identify the potential drug molecules through virtual screening.

2.   Materials and methods

2.1   Homology modeling and model validation

Amino acid sequence for the TMPRSS2 catalytic domain was obtained from UniProtKB [15]. It was searched for homology using blast protein (UCSF Chimera) against the structural database of Protein Data Bank (PDB) [16].

Sequence alignment between model TMPRSS2 and homologous protein (PDB: 105E_H) was determined by multiple sequence alignments. Multiple sequence alignments were carried out using Clustal Omega [17].

Discrete optimized protein energy (DOPE) score was used for the ranking and scoring of the protein model generated by the MODELLER [18]; as per rule, out of the 50 models with the lowest DOPE score, the first three were selected and were evaluated using PROCHECK [19] and Verify3D [20]. The Chiron portal uses the discrete molecular dynamics for every-atom representation for each residue in the protein to perform rapid energy minimization of protein molecules [21]. Model evaluation was performed consistently before and after minimization using the PROCHECK program.

The TMPRSS2 homology model and template structure were compared using PyMOL molecular viewers [22] to check the root-mean-square deviation (RMSD) between the positioning of the carbon atoms. The RMSD value is used to illustrate the closeness of the carbon atom relatively. A low RSMD value is desired for the degree of relativity between the structures [23].

2.2   Molecular docking by AutoDock Vina

In this chapter, the AutoDock Vina docking software is used for the docking procedure. It is released under a free software license. The software has good accuracy in proficiency to find bioactive conformations. In AutoDock Vina, the parameter of binding energy has been used to determine which ligand has a stable complex interaction with protein; the more the negative value or lower binding affinity, the more stable the ligand-receptor complex is [24].

The ligands were prepared using ChemDraw. We have also performed our own energy minimizations of the ligands, with the methods implemented in Vina (–local_ only option). With these optimized structures, the goal of decreasing the number of unsuccessful dockings is achieved. Peptidomimetic inhibitors [25] (inhibitor "92" and inhibitor "50") and allosteric inhibitors (nafamostat and camostat mesilate) were docked against TMPRSS2 catalytic domain model. AutoDock Vina results were analyzed using the binding energy scoring function.

The grid points in X, Y, and Z axes were set at $22 \times 22 \times 22$. The grid center was placed in the active site pocket center at $(13.58 \times -3.24 \times 30.31)$. The entire binding site of the enzyme and provided enough space for the ligand translational and rotational walks were included in the grid boxes.

The Vina scoring function is fully empirical and calculates the affinity between ligand and protein, including Gaussian steric interaction terms and hydrophobic and hydrogen bond interaction terms [26]. For each calculation, nine poses ranked according to the scoring function of AutoDock Vina were obtained. AutoDock Tools, PyMOL, and LigPlot [27] were used for the postdocking analyses.

3. Results

3.1   Template identification and sequence alignment

For best results, the TMPRSS2 catalytic domain (255−493 residues) was considered for homology modeling. Blast proteins (UCSF Chimera) against the structural database of PDB results were taken into account for the selection of suitable template structures. The X-ray crystal structure of human serine protease hepsin chain H solved at 1.75 Å (PDB ID: 1O5E_H) resolution was selected as the template for the TMPRSS2 catalytic domain [28]. The template (PDB ID: 1O5E_H) was selected because it has serine protease activity and 42.19% sequence identity (Fig. 2.1).

3.2   Homology modeling

The homology model of TMPRSS2 was developed by applying Modeller v9.22. Predicted models, derived by Modeller9.22, were sorted according to the DOPE score and the top three models were verified using PROCHECK, Verify3D.

We confirmed the stereochemical quality and accuracy of the models utilizing PROCHECK. The results from PROCHECK are reported as Ramachandran plots (Fig. 2.2). To determine the quality of protein structure, Ramachandran plot is one of the most reliable methods. A structure of Ramachandran plot having 89.9% of its residues in the most favored regions A, B, and L has equivalent accuracy as 2Å-resolution crystal structure. Also, the Verify3D plot of the modeled protein (Fig. 2.2C) was obtained for

FIGURE 2.1 Alignment of the amino acid sequences of transmembrane serine protease type 2 (TMPRSS2) catalytic domain and the crystal structure of 105E_H.
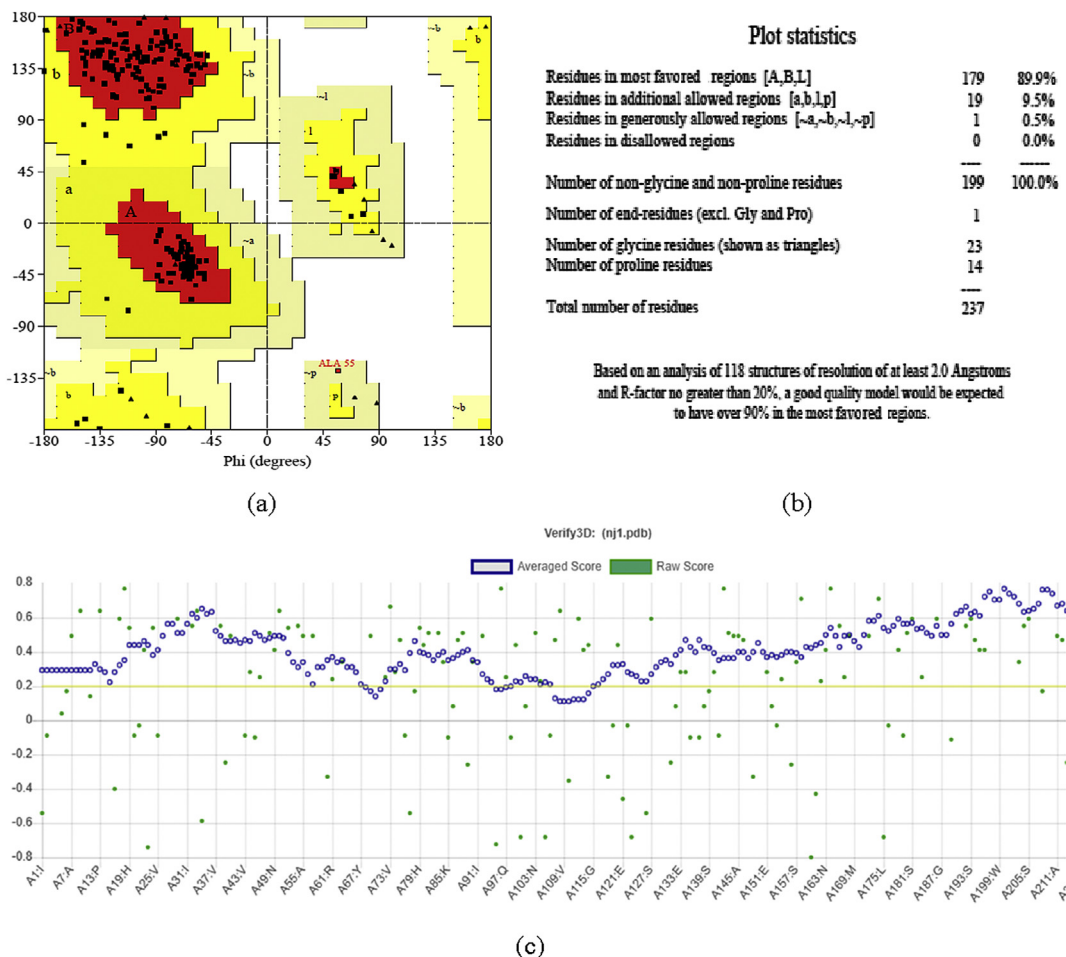
(a)

(b)

(c)

FIGURE 2.2 Structure validation using (A) the Ramachandran plot, (B) the Ramachandran plot statistics of the transmembrane serine protease type 2 (TMPRSS2) homology model, and (C) Verify3D.

structure validation, and it showed as PASS. The 3D environment profile shows that 80.0% of the residues have averaged 3D-1D score ≥ 0.2, which suggests the validity of the modeled protein. An RMSD value of 0.106 Å was obtained from the alignment computed using PyMOL molecular viewer, showing that the structures were closely related (Fig. 2.3). The TMPRSS2 homology model is represented by the cyan (gray in print version) helices, whereas the protein template (PDB ID: 1O5E_H) is represented by the purple (*black in print version*) helices. Meanwhile, it was observed from the molecular viewer that in TMPRSS2 homology, the model amino acid residues HIS41, ASP90, SER186, ASP180, and GLY209 also corresponded with the amino acid residues of the protein template at HIS41, ASP97, SER193, ASP187, and GLY220.

FIGURE 2.3 Alignment of the transmembrane serine protease type 2 (TMPRSS2) homology model shown as cyan (*gray in print version*) helices, and the 1O5E_H template structure shown as purple (*black in print version*) helices.

3.3   Active site identification of TMPRSS2

The TMPRSS2 homology model demonstrates that there is a degree of structural homology in the active site regions of serine protease, which contain the catalytic triad HIS41, ASP90, and SER186 (Fig. 2.4). Outside this catalytic domain, there are several allosteric binding sites, which possess a critical role in substrate binding and recognition [29]. These allosteric binding sites contain ASP180, and GLY209 forms the S1 pocket.

3.4   Molecular docking

Docking of peptidomimetic substrate analogues (inhibitor "92" and inhibitor "50") and allosteric inhibitors (nafamostat and camostat mesilate) in this study was carried out in the active site of the TMPRSS2 homology model (Figs. 2.5−2.9). Nine top poses for each ligand were returned in the simulation, out of which we selected one best pose for each ligand based on the binding energy calculation score. Lower scores indicate better docking, and scores below −7.5 are considered promising. Known active ligands (nafamostat and camostat mesilate) had low docking scores (Table 2.1).

4. Discussion

SARS-CoV-2 is a highly infective and often lethal virus. Marketed drugs that can reduce the infectivity of the virus are the fastest path to potential treatment. In this chapter, we
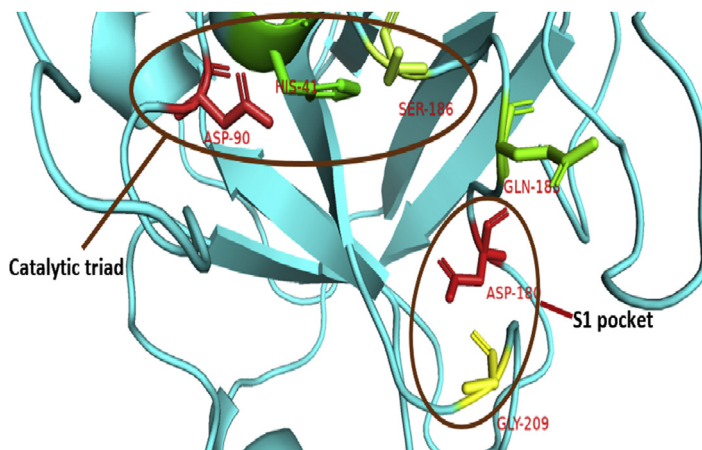
FIGURE 2.4 Active sites of transmembrane serine protease type 2 (TMPRSS2) homology model of HIS41, ASP90, and SER186 residues shown in a stick model represent the catalytic triad and ASP180 and GLY209 residues in the S1 specificity pocket. Performed with the PyMol molecular graphics system.
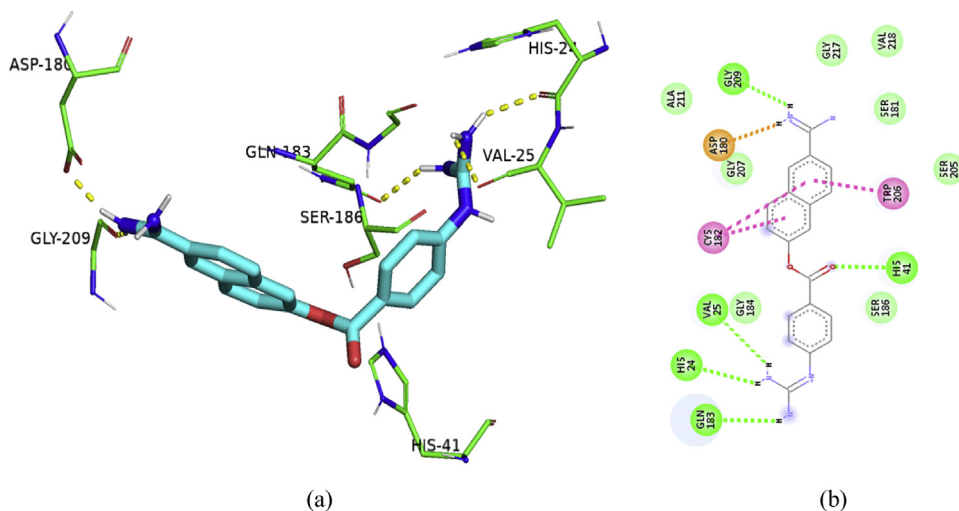

(a)	(b)

FIGURE 2.5 Molecular docking interactions between nafamostat and the binding sites in the transmembrane serine protease type 2 (TMPRSS2) homology model: (A) 3D model of the interactions and (B) 2D model of the interactions. Nafamostat blocks access to the catalytic triad (HIS 41, ASP 90, and SER 186) by forming hydrogen bonds with ASP 180, GLN 183, and GLY 209 in the S1 specificity pocket.

applied computational methods to identify inhibitors for TMPRSS2, a target found in already published works for its role in infection by coronaviruses. We generated a homology model and used a standard docking protocol to evaluate the binding of known peptidomimetic inhibitors and allosteric inhibitors of TMPRSS2. These include docking study of nafamostat and camostat, and both are considered for clinical trials in April 2020 by the University of Tokyo [29]. From our docking investigation, it was noticed that peptidomimetic inhibitors block the catalytic triad HIS 41 and ASP 90 by
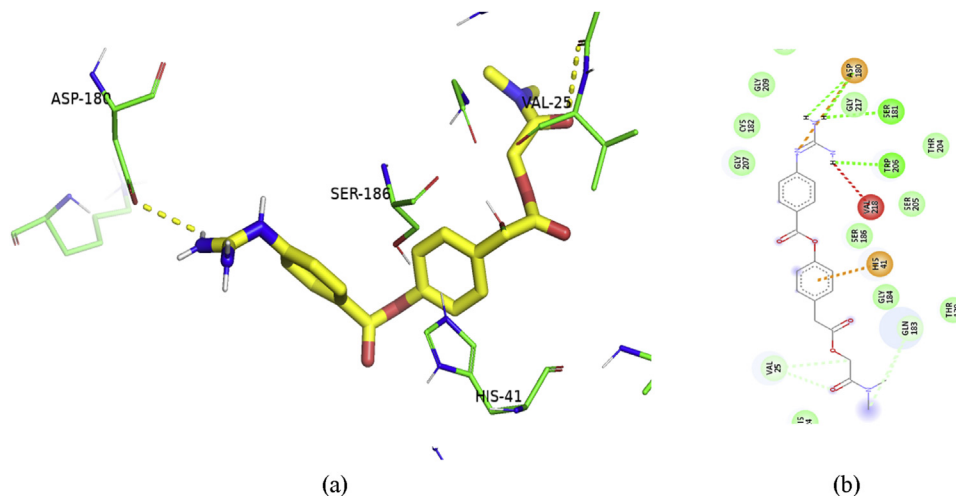
FIGURE 2.6 Molecular docking interactions between camostat and the binding sites in the transmembrane serine protease type 2 (TMPRSS2) homology model: (A) 3D model of the interactions and (B) 2D model of the interactions. Camostat blocks access to the catalytic triad (HIS 41, ASP 90, and SER 186) by forming a pi stacking interaction with His 41, hydrogen bonds with ASP 180 and GLN 183 in the S1 specificity pocket.
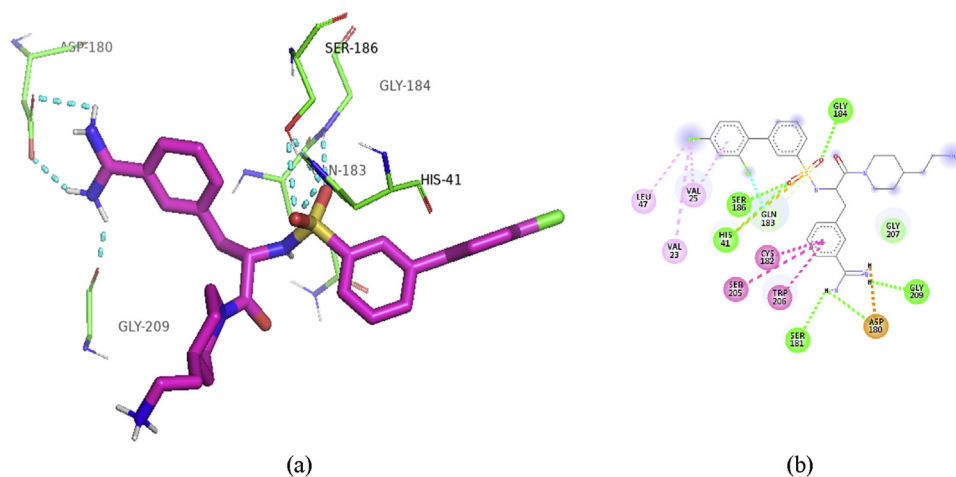

FIGURE 2.7 Molecular docking interactions between inhibitor "50" and the binding sites in the transmembrane serine protease type 2 (TMPRSS2) homology model: (A) 3D model of the interactions and (B) 2D model of the interactions. Inhibitor "50" blocks the catalytic triad HIS 41 and ASP 90 by forming strong hydrogen bonds. Additional hydrogen bonding interaction is also found with ASP 180 and GLN 209 in the S1 specificity pocket.

forming strong hydrogen bonds. Additional hydrogen bonding interaction was also found with ASP 180 and GLN 209 in the S1 specificity pocket. Allosteric inhibitors block access to the catalytic triad by forming a pi stacking interaction with His 41 and hydrogen bonds with ASP 180 and GLN 183 in the S1 specificity pocket. Our docking
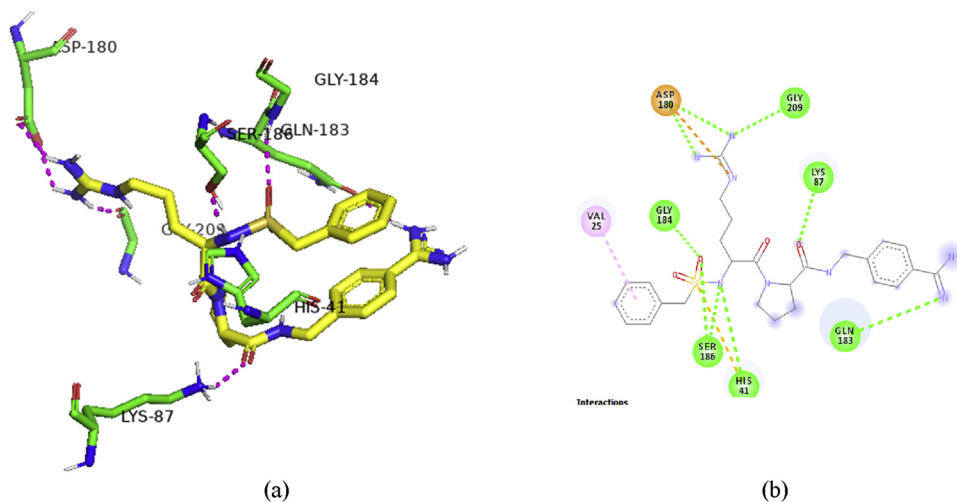
FIGURE 2.8 Molecular docking interactions between inhibitor "92" and the binding sites in the transmembrane serine protease type 2 (TMPRSS2) homology model: (A) 3D model of the interactions and (B) 2D model of the interactions. Inhibitor "50" blocks the catalytic triad HIS 41 and ASP 90 by forming strong hydrogen bonds. Additional hydrogen bonding interaction is also found with ASP 180 and GLN 209 in the S1 specificity pocket.
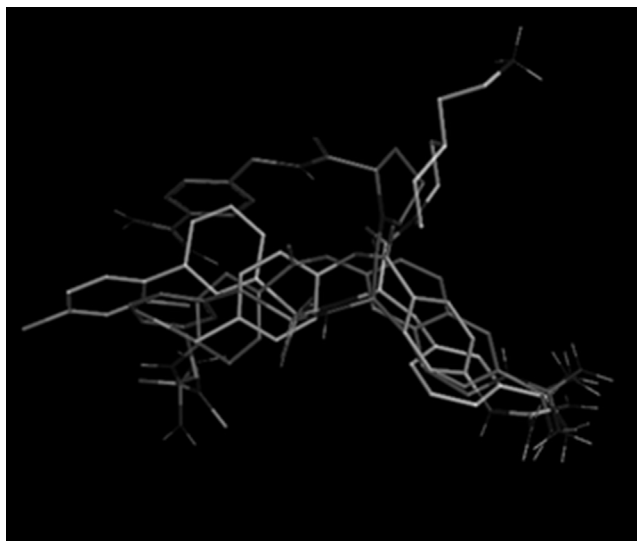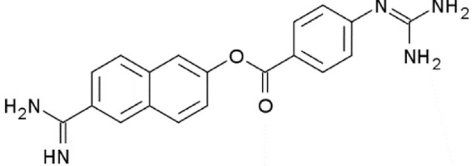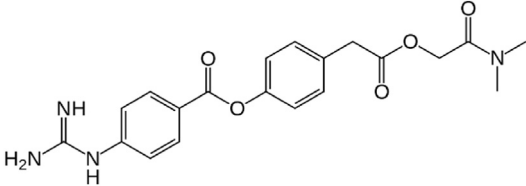

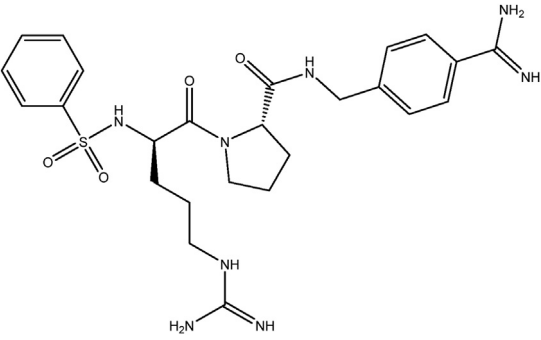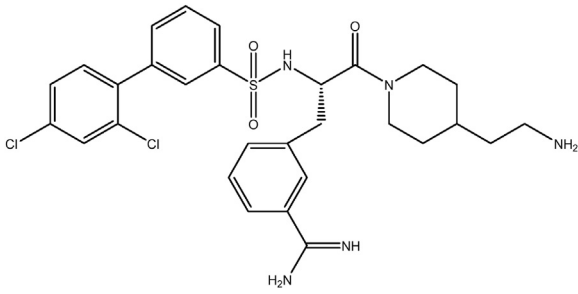**FIGURE 2.9** Superimposition of the relevant docked structures of nafamostat, camostat, inhibitor "50", and inhibitor "92".

study is only a small first step in determining the binding mechanism of these drugs. Finally, the outcomes of this study might help researchers and strengthen their efforts on discovering novel uses for existing medications and to develop antiviral therapeutics against SARS-CoV-2.

Table 2.1 Selected compounds with the highest minimum docking score, minimum docking score, and average docking score.

Name	Structure	Minimum score	Average score
Nafamostat		−7.9	−7.5
Camostat mesilate		−7.6	−7.3
Inhibitor "92"		−8.7	−8.0
Inhibitor "50"		−8.5	−7.8

5. Conclusions

Evaluation of new antiviral strategies might get benefited through progressively sophisticated in vivo SARS-CoV-2 models. Especially, the therapy delivery is promised by the infection models and the prototype antiviral agents that will both reveal broad-spectrum antiviral agents and guard against the potentially serious coronavirus; for example, TMPRSS2 inhibitors may be suitable therapeutic agents for a variety of respiratory viruses that rely on proteolysis for cell entry. Coronavirus research will endure to reveal features of virus operating mechanisms and clinically relevant antiviral strategies for the foreseeable future.

The existing data on TMPRSS2 inhibitor treatments can be summarized as a promising hypothesis. They require further and eventually rigorous testing for efficacy and safety by randomized controlled trials in humans. One unique benefit of blocking TMPRSS2 airway proteases is that, besides coronavirus, they could target several other respiratory viruses, which include influenza A and B viruses. In addition, TMPRSS2 inhibitors may act as an anticoagulant providing additional benefits to intensive-care COVID-19 patients. In this report, we used computational methods to identify drug presupposing candidates for TMPRSS2 inhibitors. This study provides an insight into the design and prediction of the possible interaction modes and binding affinities of peptidomimetic inhibitors (inhibitor "92" and inhibitor "50") and allosteric inhibitors (nafamostat and camostat mesilate) compounds with the TMPRSS2 homology model. Thus our results may provide a path to design new lead molecules to control the novel coronavirus 2 entry into human cells.

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