Indian Journal of Biochemistry & Biophysics
Vol. 62, May 2025, pp. 544-559
DOI: 10.56042/ijbb.v62i5.4034

In silico approach for uncovering inhibitors of SARS-CoV-2 by targeting TMPRSS2 *via* molecular networking-based strategies

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Received 15 July 2023; *revised 12 December 2024*

Understanding the pathogenesis of COVID-19 is vital for developing more effective therapeutic strategies. Among the key proteases involved in the disease progression are Transmembrane Serine Protease 2 (TMPRSS2) and Disintegrin and Metalloproteinase 17 (ADAM17), which play critical roles in viral entry and infection. TMPRSS2 facilitates the priming of the SARS-CoV-2 spike (S) protein, making it a promising target for therapeutic intervention. Alpha-1-antitrypsin (A1AT), a natural tissue protector with antiviral and anti-inflammatory properties, inhibits TMPRSS2, further underscoring its importance as a drug target. Given the urgency of addressing the COVID-19 pandemic, repurposing existing FDA-approved drugs offers a faster and more practical approach than developing new drugs from scratch. This study utilized molecular networking strategies via Cytoscape version 3.9.1 to screen FDA-approved drugs for potential interactions with TMPRSS2. A pharmacophore model was subsequently generated, followed by virtual screening and docking studies. From the molecular networking analysis, 22 compounds were selected based on their binding interactions with TMPRSS2. These compounds were evaluated using pharmacophore modeling and virtual screening, with further selection based on Lipinski's rule of five and low RMSD values (below 0.07 Å). Docking studies identified six top-performing molecules from the ZINC database, with ZINC00896543 and ZINC05316843 exhibiting the highest binding affinities (-22.0254 and -21.676 kcal/mol, respectively), surpassing the co-crystal ligand (-12.8236 kcal/mol). The findings highlight the potential of these repurposed compounds for integrated COVID-19 management. Further pharmacokinetic, pharmacodynamic, preclinical, and clinical studies are warranted to validate these candidates and pave the way for designing new agents with minimal side effects and enhanced efficacy.

Keywords: Computational study, COVID-19, Drug repurposing, Molecular docking, Molecular networking, Pharmacophore modeling, TMPRSS2

Coronaviruses (CoVs) are positive-strand RNA viruses belonging to the family Coronaviridae. These viruses possess glycoproteins in their viral envelopes[1]. CoVs are known to cause a wide range of respiratory and gastrointestinal diseases in vertebrates, including humans[2]. The ongoing COVID-19 pandemic highlights the urgent need to discover novel preventive and curative measures to combat this global health crisis. In response to this pressing issue, the World Health Organization (WHO) emphasizes the importance of exploring diverse techniques such as immune system modulation, vaccine development, drug repurposing, and other innovative approaches to combat this virus effectively.

Drug repositioning is the process of identifying new therapeutic applications for already approved medications, thereby addressing diseases that currently lack effective treatments[3,4]. This approach offers significant advantages in terms of cost and time compared to conventional de novo drug discovery processes. The rapid expansion of publicly accessible large-scale biomedical and electronic health-related data has accelerated the advancement of computational drug repositioning methods. Researchers and scientists from multidisciplinary fields have made various attempts to computationally evaluate the potential of repurposing existing

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pharmaceuticals for alternative pharmacological applications[5]. Given the current global outbreak of COVID-19, the strategy of drug repositioning has become even more critical and time-sensitive[6,7].

The entry of the SARS-CoV virus into cells can occur through either an endosomal or non-endosomal route. For the non-endosomal route to take place, the virus's spike (S) protein must bind to the host's angiotensin II converting enzyme receptor (ACE2). Subsequently, the S protein is cleaved into S1 and S2 subunits by the proteolytic activity of transmembrane serine protease 2 (TMPRSS2)[8]. Alternatively, the S protein can be processed through the less efficient and non-essential endosomal route, which relies on cathepsin L, a pH-dependent host-cell protease[9,10]. Alpha-1-antitrypsin (A1AT), an intrinsic tissue protector with antiviral and anti-inflammatory effects, is encoded by SERPINA1 and is associated with more than 100 known allelic variants that affect A1AT serum levels[11-13].

TMPRSS2 and disintegrin and metalloproteinase 17 (ADAM17) are two significant proteases involved in the pathogenesis of COVID-19. A1AT inhibits the activity of TMPRSS2, which is crucial for priming the SARS-CoV-2 S protein and facilitating viral infection. A1AT also prevents the activity of pro-inflammatory molecules such as neutrophil elastase, TNF-α, and IL-8. Notably, the discovery of an elastase cleavage site near the S1-S2 protein in the A2a SARS-CoV subtype (the D614G mutation) suggests the potential involvement of neutrophil elastase in this infection[14]. Furthermore, individuals with COVID-19 who exhibit decreased IL-6 and A1AT levels are associated with a poorer prognosis[15].

The endoplasmic reticulum is responsible for producing serine protease inhibitor A1AT, which is subsequently released by the Golgi apparatus. A1AT is expressed in various cells, including those found in the eye, lungs, digestive tract, neutrophils, and macrophages. Under normal circumstances, the liver synthesizes and secretes A1AT once a day, resulting in a normal plasma level ranging from 0.9 to 2.23 mg/mL. However, during an inflammatory acute-phase response, individuals with the "normal variant" can experience A1AT levels rising by more than four times[15].

The ACE2 receptor, a type I transmembrane protein, is primarily expressed in the testicles, cardiovascular system, colon, brain, mucous membrane, and lungs[16]. Smokers, as well as individuals with diabetes, chronic obstructive pulmonary disease (COPD), and various COVID-19 comorbidities, have been found to have higher levels of circulating ACE2 in their nasal and bronchial airways compared to nonsmokers[17]. These findings support the notion that ACE2 tissue levels play a role in the severity of COVID-19[18]. Furthermore, the activation of the ACE2 extracellular domain in the blood through ADAM17 may contribute to viral neutralization[19]. Figure 1 illustrates the critical role of

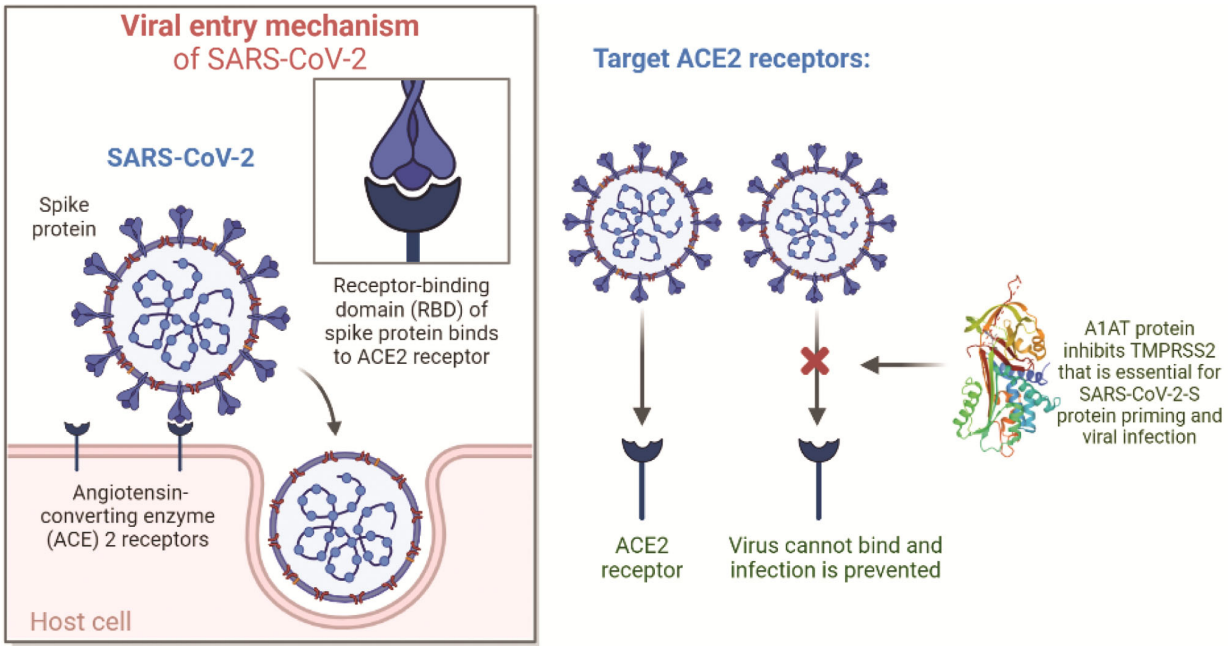


Fig. 1 — The function of A1AT in SARS-CoV-2 viral entrance into host cell machinery

Alpha-1 Antitrypsin (A1AT) in modulating the entry of the SARS-CoV-2 virus into host cell machinery. Specifically, it demonstrates how A1AT inhibits the activity of the transmembrane protease serine 2 (TMPRSS2), an enzyme crucial for priming the viral spike (S) protein. TMPRSS2 facilitates the fusion of the viral envelope with the host cell membrane, enabling the virus to enter the host cell.

The Figure 1 outlines the following key steps:

- Binding of SARS-CoV-2 to ACE2 Receptor: The viral S protein attaches to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of host cells, initiating the infection process.
- TMPRSS2 Role in S Protein Activation: TMPRSS2 cleaves the S protein, activating it for membrane fusion and subsequent viral entry.
- Inhibitory Action of A1AT: A1AT suppresses TMPRSS2 activity, thereby preventing the cleavage and activation of the S protein, effectively blocking the virus's entry pathway.

This mechanism underscores A1AT's therapeutic potential as a candidate for mitigating SARS-CoV-2 infection by targeting the TMPRSS2-mediated entry process. However, the destruction of the cell membrane-active ACE2 by ADAM17, TMPRSS2, and virus uptake may result in increased production of Ang II and activation of AT1R. This disruption of the renin-angiotensin system homeostasis could have negative effects in COVID-19 patients. It is crucial to identify cellular inhibitors for TMPRSS2, ADAM17, and the inflammatory cytokines associated with COVID-19 due to their harmful effects. As mentioned previously, the host protein A1AT emerges as a key player in the infection of COVID-19 and SARS-CoV-2.

COVID-19 is associated with various comorbidities such as cardiovascular disease, lung conditions, hypertension, diabetes, liver and kidney damage, among others[20]. The prognosis of COVID-19 patients can vary widely and is influenced by multiple internal and external factors[21]. A1AT, as a regulator of the innate immune system, plays a role in preventing viral infections, maintaining lung homeostasis, and potentially impacting the renin-angiotensin system's stability[22]. Understanding the role of A1AT in SARS-CoV-2 infection and its interference with the progression of COVID-19 can provide valuable insights into the disease's pathophysiology and open avenues for novel therapeutic approaches.

TMPRSS2-mediated binding of the SARS-CoV-2 S protein is crucial for viral entry into host cells[8]. A1AT has shown efficient inhibition of TMPRSS2 in *in vitro* assays, making it a strong candidate for anti-COVID-19 therapy, as it can prevent viral entry[23]. Recent studies, such as Bai *et al*. (2023)[24], have confirmed the hypothesis that A1AT can inhibit SARS-CoV-2 entry.

Based on the information and discussions presented above, our research proposes a hypothesis aimed at identifying substances that can induce the production of alpha-1-antitrypsin (A1AT) and repurposing FDA-approved drugs for the treatment of COVID-19. To achieve this, we will employ molecular networking studies using Cytoscape version 3.9.1, a powerful computational tool. This approach will allow us to analyze and explore the connections between different molecules and identify drugs that interact with the transmembrane serine protease 2 (TMPRSS2), a key protein involved in the entry of the SARS-CoV-2 virus into host cells. Additionally, we will generate a pharmacophore model, a three-dimensional representation of the chemical features necessary for a molecule to bind to a specific target, using known ligands that have demonstrated inhibitory or interacting properties with TMPRSS2. By employing this model, we can screen and identify potential novel compounds from existing drug databases that possess similar chemical features and could be repurposed as therapeutics for COVID-19 treatment. To further assess the suitability of these identified compounds, we will conduct ADMET (absorption, distribution, metabolism, excretion, and toxicity) studies. These studies evaluate important pharmacokinetic properties of a compound, such as how it is absorbed, distributed within the body, metabolized, excreted, and its potential toxicity. This analysis will help us understand the compound's behavior in the body and predict its efficacy and safety as a potential therapeutic for inhibiting TMPRSS2 and combating SARS-CoV-2 infection. Furthermore, docking studies against the TMPRSS2 protein will be performed. Docking is a computational technique used to predict the binding mode and affinity between a small molecule (ligand) and a target protein. By virtually docking the potential candidate molecules against the structure of TMPRSS2, we can identify the best-fit compounds that exhibit strong binding interactions and have the potential to inhibit the activity of TMPRSS2 effectively. In summary, our research approach involves using molecular networking, pharmacophore modeling, ADMET studies, and docking simulations to

identify and evaluate repurposed drugs that can interact with TMPRSS2, a critical protein involved in the entry of the SARS-CoV-2 virus. This methodology aims to provide a clearer understanding of the potential therapeutic options for COVID-19 and pave the way for the discovery of novel compounds that could effectively combat the virus.

Materials and Methods

Devices used

We conducted *in silico* research utilizing various online and offline bioinformatics tools[25]. The online tools employed include the PubChem Database and Zinc Database, which were used to obtain ligands. The Protein Data Bank (PDB) was utilized to access the crystal structure of the protein of interest. For pharmacophore modeling, we utilized the PharmaGist Webserver and ZINCPharmer. PreADMET was employed for ADMET studies. In addition, Cytoscape 3.9.1 was utilized for biological networking and drug repurposing, while the Molecular Operating Environment (MOE) was employed for ligand and protein preparation as well as docking studies[26].

Molecular networking

For molecular networking, we utilized Cytoscape version 3.9.1, an open-source bioinformatics tool that enables the dynamic exploration and comprehension of complex biological networks. Our primary objective was to create a network of protein-drug interactions (PDI) to gain insights into potential interactions between our protein of interest, TMPRSS2, and various drugs. We utilized specific modules within Cytoscape, including CytoHubba and String Network, which provide diverse parameters for analysis. CytoHubba analyzes and characterizes nodes within a biological network using 11 different topological parameters such as degree, bottleneck, eccentricity, closeness, radiality, betweenness, stress, maximum clique centrality, maximum neighborhood component, edge percolated component, and density of the maximum neighborhood component based on shortest paths[27,28]. We focused particularly on degree, betweenness, and proximity criteria. The STITCH protein/compound query library contains information about chemical compounds and their interactions with various proteins[29]. By searching for a specific protein in the STITCH protein/compound query, the database generates a network that provides information on chemicals that interact with the protein of interest[30,31]. In our study, we screened over 1461 FDA-approved drugs using Cytoscape 3.9.1 to investigate potential interactions with our protein of interest, TMPRSS2. Figure 2 illustrates the workflow of our drug repurposing approach for SARS-CoV-2 infection. By utilizing these tools and methods, we aimed to

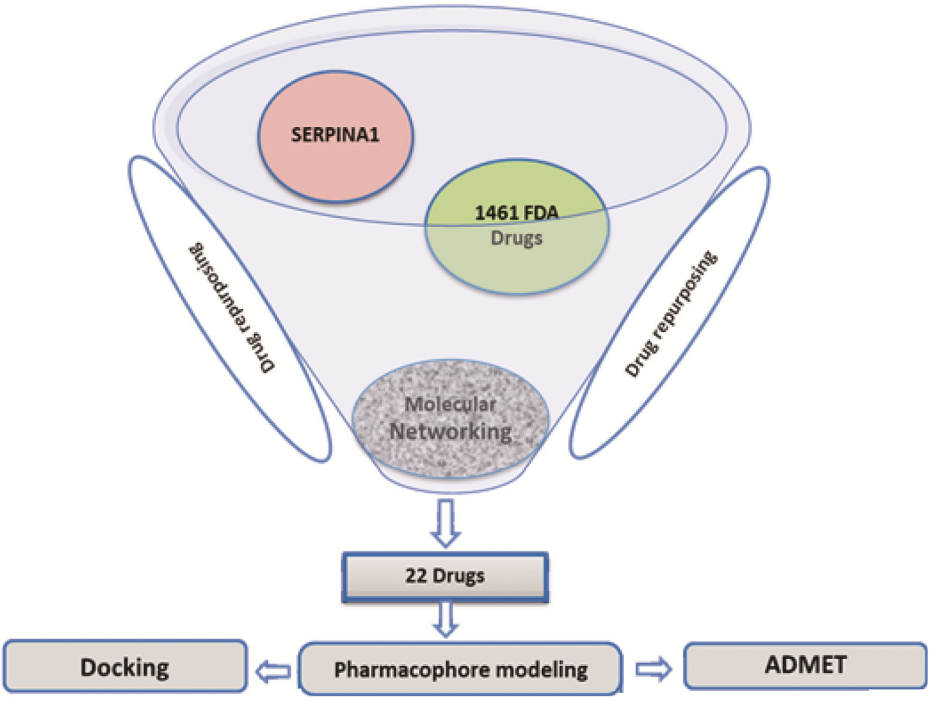


Fig. 2 — Workflow of drug repurposing approaches for SARS-CoV-2 infection

identify potential drug candidates from the FDA-approved drug pool that could interact with TMPRSS2 and be repurposed for the treatment of SARS-CoV-2 infection.

Pharmacophore modelling

Pharmacophore modeling is a relatively recent area of research that investigates the systematic impact of various characteristics present in drug-like molecules on their ability to bind to specific targets. In our study, we employed the web-based tool PharmaGist for ligand-based pharmacophore simulation. Rather than requiring the structure of the target receptor, this approach involves adding compounds that resemble drugs known to bind to the receptors[31]. For our research, we utilized PharmaGist and selected 22 drug compounds gathered from different literature surveys, including FDA-approved medications[32]. PharmaGist is an online server that generates 3D pharmacophores for molecules resembling drugs known to bind to specific target receptors. The server employs multiple flexible alignments of input ligands to identify candidate pharmacophores. During the alignment process, the flexibility of the ligands is taken into account explicitly and deterministically. In our study, we created a single model using nearly 22 drug compounds mainly obtained from literature surveys, including FDA-approved medications. As a validation step, we chose every fifth compound and created it as a reference pharmacophore. The reference pharmacophore was extracted from a 3D alignment of the bound ligands and matched the top-scoring pharmacophore candidate identified by PharmaGist.

Virtual screening

ZINCPharmer is a free online resource that functions as a pharmacophore web browser for chemical space that may be purchased. The ZINCPharmer is used to identify hydrophilic, hydrophobic, hydrogen bond donor, hydrogen bond acceptor, positive and negative ions, and aromatic pharmacophoric characteristics[33].

Molecular docking

Preparation of ligand files

The ligand files for eight proposed co-drugs were constructed using the builder module of MOE-DOCK, and their energies were minimized. Molecular geometries were drawn, and the 3D structures were optimized using the MMFF94 force field with a 0.0001 kcal/mol energy gradient convergence criterion. The MOE molecule builder module was employed for ligand construction, energy minimization, and correction of potential and partial energy. The ligand files were saved as molecular database (.mdb) files in a local directory for further processing.

Preparation of macromolecules

To evaluate the hit compounds, all compounds from the ZINCPharmer database were docked into the binding site of human TMPRSS2 in complex with Nafamostat (PDB ID: 7MEQ), which was retrieved from the RCSB Protein Data Bank. The PDB files were imported into the MOE suite, and the receptor preparation module was applied to prepare the proteins. Bound water molecules and hetero atoms were removed, polar and non-polar hydrogens were added, and 3D structures were corrected. The binding pocket was identified using MOE-Alpha Site Finder, and alpha spheres were generated to visualize the binding pocket. Dummy atoms were placed at the centers of these spheres, revealing deep small canyons lined with key residues, including hydrophobic and hydrophilic amino acids[34,35].

Docking methodology

For docking simulations, the placement was set as a triangular matcher, rescoring was set as London dG, the number of retainers was set as 10, and the refinement was set as a force field to generate 10 poses for each target ligand conformation. The docking run resulted in mdb.output files containing scoring and multiple conformations of each compound. The docked conformations were analyzed, and the best-scoring pose for each compound was selected for further ligand interaction studies. Ligand-receptor interactions were analyzed, and the selected best pose ligand molecule was subjected to surface analysis for interpretation[34].

In summary, pharmacophore modeling was performed using PharmaGist, virtual screening was conducted using ZINCPharmer, and molecular docking studies were carried out using MOE-DOCK. These methods allowed us to explore the interactions between ligands and the target protein TMPRSS2, providing valuable insights into potential drug candidates for the treatment of COVID-19.

Results and Discussion

Molecular networking

The molecular networking analysis using the "STITCH" module of the Cytoscape chemical query

database allowed us to explore the potential interactions between TMPRSS2 and a wide range of FDA-approved drugs. The resulting biological network provided a comprehensive visualization of the molecules that directly interact with TMPRSS2. In this network, each drug and protein molecule was represented as a node, while the interactions between them were depicted as edges (Fig. 3). The analysis yielded intriguing results, revealing 12 drugs that exhibited potential interactions with TMPRSS2 (Table 1). These drugs encompassed various therapeutic classes, including opioid analgesics (Codeine), antidotes (Cysteine), anticancer drugs (Doxorubicin, Cisplatin, Thalidomide, and Genistein), nonsteroidal anti-inflammatory drugs (NSAIDs) (Indomethacin), antihypertensives (Norepinephrine), amino acids (dl-O-Phosphoserine), anti-inflammatory drugs (Dexamethasone), antiviral protease inhibitors

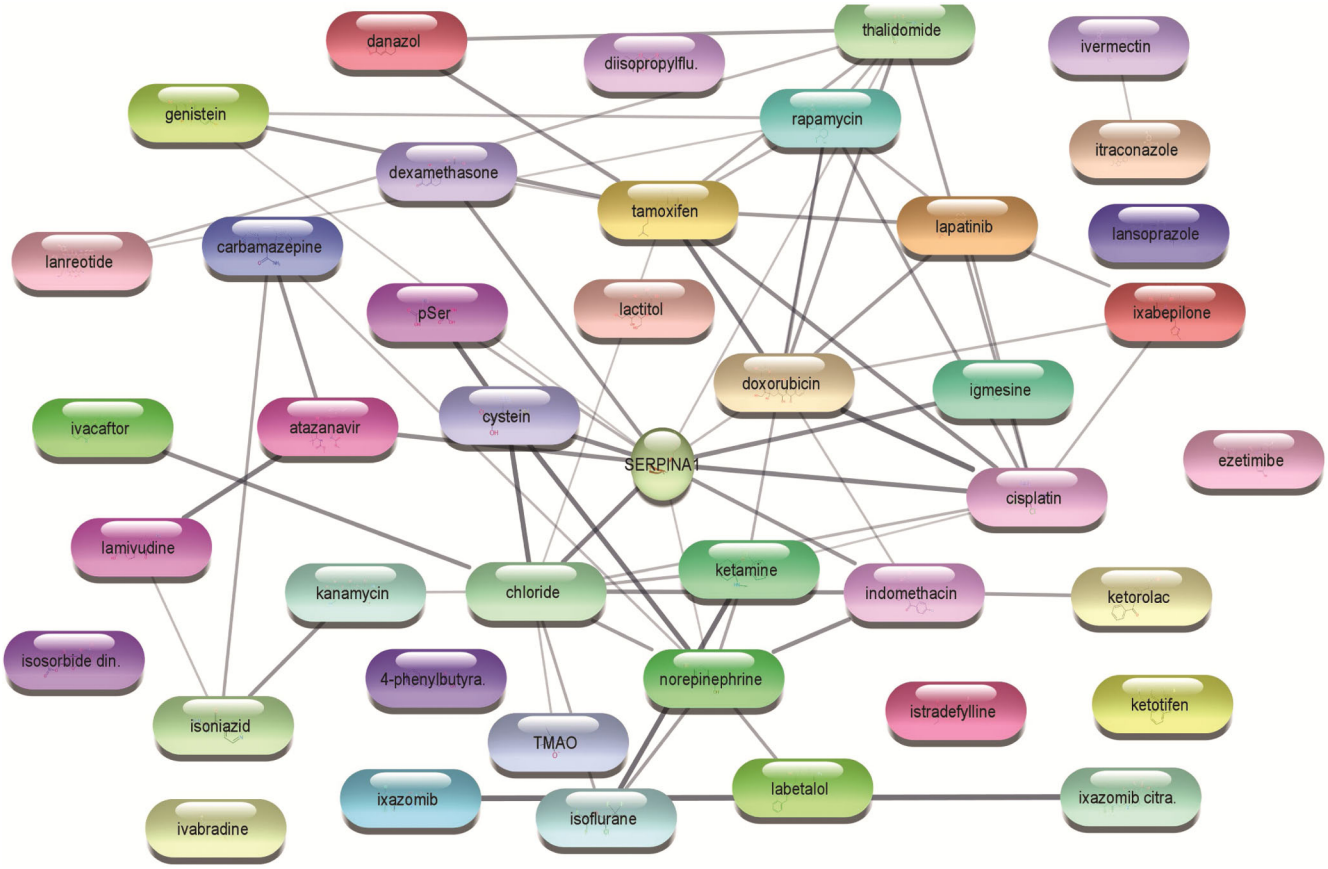


Fig. 3 — Protein druginteractions view via molecular networking interface

Table 1 — Molecular networking analysis yielded 12 drugs that exhibited potential interactions with TMPRSS2

S. No	Compound Name	Class of drug	Degree	Closeness	Betweenness	MCC	Bottleneck	Eccentricity
1	Codeine	Opioid Analgesic	5	8.5	3.33333	14	1	0.5
2	Cysteine	Antidote	4	8	2	8	1	0.5
3	Doxorubicin	Anticancer	4	8	1.66667	8	1	0.5
4	Indomethacin	NSAID	4	8	1.66667	8	1	0.5
5	Cisplatin	Anticancer	4	8	1.66667	8	1	0.5
6	Norepinephrine	Antihypertensive	4	8	0.66667	12	1	0.5
7	Thalidomide	Anticancer	3	7.5	0	6	1	0.5
8	dl-O-Phosphoserine	Amino acid	2	7	0	2	1	0.5
9	Dexamethasone	Anti-inflammatory drug	1	6.5	0	1	1	0.5
10	Genistein	Anticancer	1	6.5	0	1	1	0.5
11	Atazanavir	Antiviral Protease inhibitor	1	6.5	0	1	1	0.5
12	Igmesine	Neuroprotective agent	1	6.5	0	1	1	0.5

(Atazanavir), and neuroprotective agents (Igmesine). The identification of these potential drug candidates highlights the possibility of repurposing existing medications to target TMPRSS2 and potentially interfere with its activity. Repurposing FDA-approved drugs offers several advantages, including a shorter timeline for clinical translation, as these drugs have already undergone safety and efficacy assessments. However, further experimental studies are necessary to confirm the interactions and evaluate the effectiveness of these compounds in inhibiting TMPRSS2 and combatting SARS-CoV-2 infection. The utilization of CytoHubba within the molecular networking analysis provided additional insights into the connectivity of the network. Parameters such as degree, closeness, and betweenness allowed us to assess the significance of each node within the network. Degree represents the number of edges connected to a node, providing an indication of its interaction potential. Betweenness measures the frequency with which a node acts as a bridge along the shortest path between other nodes, while closeness determines the average distance between a node of interest and all other connected nodes[36]. The findings from the molecular networking analysis offer valuable information for further investigations and the development of potential therapeutic strategies. The identified drugs may possess unique properties that enable them to interact with TMPRSS2, potentially inhibiting its function and mitigating the infectivity of SARS-CoV-2. However, it is important to note that these results are preliminary, and additional studies, including *in vitro* and *in vivo* experiments, are necessary to confirm the effectiveness and safety of these drug candidates.

In conclusion, the molecular networking analysis provided insights into the potential interactions between FDA-approved drugs and TMPRSS2. The identification of 12 drugs with potential interactions offers exciting possibilities for repurposing existing medications as potential treatments for COVID-19. These findings pave the way for further investigations and highlight the importance of repurposing strategies in the development of novel therapeutic approaches against SARS-CoV-2.

Pharmacophore modelling

To investigate the potential binding characteristics of the selected drug molecules, we performed pharmacophore modelling using PharmaGist. A total of 22 drug molecules were included in the analysis, consisting of 12 drugs selected from the molecular networking results and an additional 10 drugs from diverse literature reports (Fig. 4). The literature-selected drugs were known to interact with TMPRSS2, the protein of interest in our study. The pharmacophore features generated from the selected 22 drug molecules included one aromatic feature (AR) and two acceptor features (ACC). These features provide insights into the essential molecular properties required for potential interaction with TMPRSS2 (Table 2). Table 2 presents the primary PharmaGist report page, providing valuable information about the selected drug molecules and their physicochemical attributes. The table includes details such as the number of atoms, spatial features, aromatic properties, hydrophobicity, donors, acceptors, negatives, and positives. The "Jmol" file with the highest number of molecules aligned and the highest J score was retrieved for further analysis. Figure 5 depicts the generated pharmacophore model for the selected drug molecules, visualized using PyMOL, providing a detailed representation of the critical molecular features essential for binding to TMPRSS2. The pharmacophore model acts as a template for identifying and optimizing compounds with desired binding characteristics. Key features illustrated in the (Fig. 5) include:

- Aromatic Center:

The model highlights one aromatic center, depicted as a flat, planar region, which represents an aromatic ring in the molecule. This aromatic feature facilitates π-π interactions or hydrophobic stacking with specific residues in the TMPRSS2 active site, contributing to stable binding.

- Hydrogen Bond Acceptors:

Two hydrogen bond acceptor regions are marked in the pharmacophore model. These regions signify atoms or groups within the molecule capable of accepting hydrogen bonds from donor groups in the TMPRSS2 enzyme. These interactions are critical for anchoring the molecule to the binding pocket and enhancing specificity.

- Spatial Arrangement:

The relative spatial orientation of these features is carefully aligned to mimic the binding requirements of TMPRSS2. The distances and angles between the aromatic center and hydrogen bond acceptors are optimized to maximize interaction strength and ensure a snug fit within the enzyme's active site.

This pharmacophore model provides a framework for screening and designing molecules with the desired interaction profile, aiding in the identification of inhibitors with high binding affinity and specificity for TMPRSS2. It serves as a crucial tool in the rational drug design process, streamlining the discovery of potential therapeutic agents. The pharmacophore model provides insights into the potential binding sites and molecular interactions between the drugs and the protein. The visualization in PyMOL allows for a clear representation of these features, aiding in the understanding of the molecular properties that are important for potential drug-protein interactions. The analysis of the pharmacophore

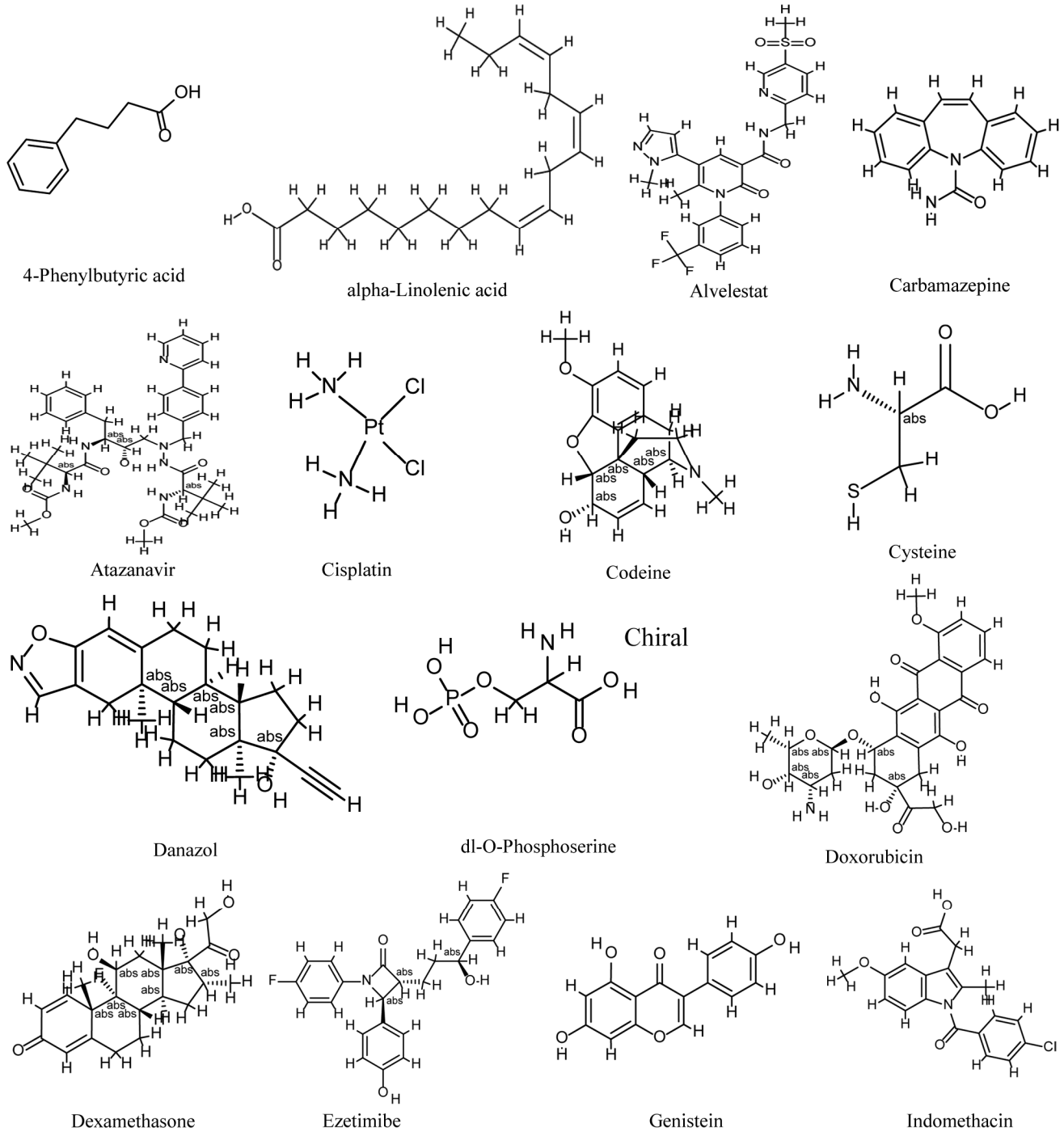


Fig. 4 — Selected drug molecules for pharmacophore modelling (*Contd.*)

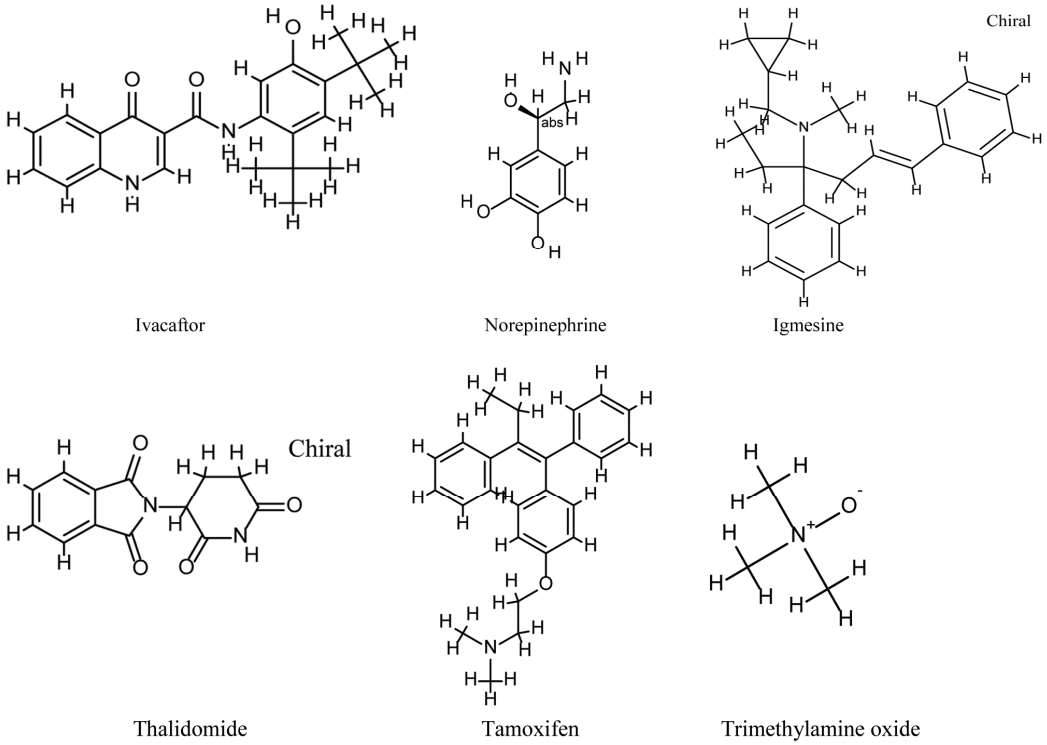


Fig. 4 — Selected drug molecules for pharmacophore modelling

Table 2 — The primary PharmaGist report page (Visualization of the detected features)

S. No	Molecule	Atoms	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
1	Danazol	52	26	25	1	21	1	3	0	0
2	Tamoxifen	57	14	14	3	9	0	2	0	0
3	Cisplatin	9	4	2	0	0	2	2	0	0
4	Codeine	43	14	13	1	8	1	4	0	0
5	Dexamethasone	57	19	16	0	11	3	5	0	0
6	Indomethacin	40	11	11	3	2	0	4	1	1
7	Norepinephrine	23	9	5	1	0	4	4	0	0
8	Ezetimibe	51	9	7	3	1	2	3	0	0
9	Alvelestat	60	15	15	4	3	1	6	0	1
10	Igmesine	53	12	12	2	9	0	1	0	0
11	Alpha linolenic acid	49	19	19	0	16	0	2	1	0
12	Genistein	30	11	8	3	0	3	5	0	0
13	Ivacaftor	56	23	22	3	14	2	4	0	0
14	Atazanavir	103	32	31	3	15	5	9	0	0
15	4-phenyl butyric acid	23	6	6	1	2	0	2	1	0
16	Trimethylamine oxide	14	4	4	0	3	0	0	0	1
17	Thalidomide	29	7	7	2	0	1	4	0	0
18	dl-O-Phosphoserine	16	7	6	0	0	1	4	2	0
19	Carbamazepine	30	7	7	2	3	1	1	0	0
20	Doxorubicin	68	24	18	3	3	6	12	0	0
21	Rapamycin	144	48	29	0	32	3	13	0	0
22	Cysteine	13	7	5	0	0	2	4	1	0

features allows us to identify key molecular characteristics that are likely important for the interaction between the drug molecules and TMPRSS2. These features provide insights into the potential binding sites and molecular interactions that may occur between the drugs and the protein. The results of the pharmacophore modelling offer a foundation for further investigations, such as virtual

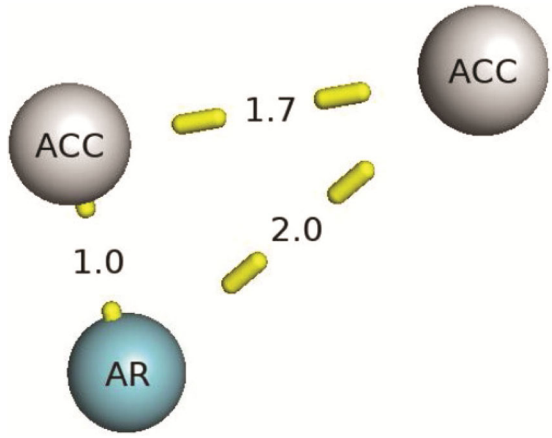


Fig. 5 — Generated pharmacophore (one aromatic center and two hydrogen bond acceptor) visualized using PyMol

screening and molecular docking studies. These techniques will help us identify potential drug candidates that possess similar pharmacophoric properties and have the potential to interact with TMPRSS2. In conclusion, the pharmacophore modelling analysis using PharmaGist revealed essential features required for potential interactions between the selected drug molecules and TMPRSS2. The identification of these features provides valuable insights into the molecular characteristics necessary for the development of drug candidates targeting TMPRSS2. Further investigations, including virtual screening and molecular docking, will allow us to identify potential drug candidates for repurposing in the treatment of COVID-19. These results pave the way for future studies that can explore the efficacy and safety of these repurposed drugs, potentially contributing to the development of novel therapeutic strategies against SARS-CoV-2.

Virtual screening

To identify potential drug candidates that align with the generated pharmacophore, we performed virtual screening using ZINCPharmer. The ZINC database was chosen for our research, and the pharmacophore data generated by PharmaGist was utilized for the virtual screening process. Using ZINCPharmer, we conducted virtual screening based on the generated pharmacophore model. From the initial screening, ZINCPharmer generated 672 hits, representing compounds that potentially match the pharmacophore features. To narrow down the selection, we focused on specific criteria for further analysis. We applied two criteria for selecting the compounds from the generated hits. First, we considered the RMSD (Root Mean Square Deviation) value, setting a threshold of below 0.07 Å. This criterion ensured that the selected compounds closely matched the pharmacophore features. Additionally, we adhered to Lipinski's rule, which states that drug-like molecules should have a molecular weight ≤ 500 g/mol. By applying this criterion, we further refined the selection to prioritize compounds that are more likely to possess favorable drug-like properties. Based on these criteria, we identified 22 drug molecules as potential hits for further analysis and characterization. These selected compounds represent promising candidates for interaction with TMPRSS2 and potential inhibition of its activity. To gain further insights into the interaction between the selected drug molecules and TMPRSS2, we performed docking studies. The crystal structure of human TMPRSS2 in complex with Nafamostat (PDB ID: 7MEQ) was used as the target for the docking simulations. By subjecting the 22 drug molecules to docking studies, we aimed to assess their binding affinity and potential interactions with the active site of TMPRSS2. The docking simulations provide valuable information on the spatial orientation and energetics of the drug-protein interactions.

In conclusion, the virtual screening process using ZINCPharmer based on the generated pharmacophore model resulted in the selection of 22 potential drug candidates. These compounds exhibit favorable matching to the pharmacophore features and adhere to Lipinski's rule, indicating their drug-like properties. These findings open up possibilities for further investigations and the development of potential therapeutics for COVID-19.

Molecular docking

The molecular docking study aimed to assess the interaction between the selected drug compounds and the target protein TMPRSS2. The binding affinity, which indicates the strength of the interaction between the ligand and the protein, was used as a measure to evaluate the potential efficacy of the compounds.

The results of the docking study revealed promising binding affinities for the selected compounds against TMPRSS2 (PDB ID: 7MEQ). The top six compounds exhibited the highest binding affinities, with scores of -22.0254, -21.676, -21.1944, -20.8837, -18.9221, and -18.6266 for ZINC00896543, ZINC05316843, ZINC00537805, ZINC03794794, ZINC11592625, and ZINC00601298, respectively.

Table 3 presents the binding affinity scores for the selected compounds from the ZINC database. These compounds have diverse clinical uses, ranging from antiarrhythmic agents to antineoplastic agents and antibiotics. The high negative binding affinity scores indicate strong interactions between the compounds and TMPRSS2, suggesting their potential as therapeutic candidates for COVID-19 treatment. Among the compounds, Flecainide (ZINC00896543), Cinitapride (ZINC05316843), and Diabeta (ZINC00537805) demonstrated the highest binding affinities. Flecainide is an antiarrhythmic agent, Cinitapride is a gastroprokinetic agent and antiulcer agent, and Diabeta is used for the treatment of non-insulin dependent diabetes mellitus. The high binding affinities of these compounds indicate successful coupling with the active site of TMPRSS2, suggesting their potential to directly affect the activity of TMPRSS2. This finding supports the repurposing of these drugs for the treatment of COVID-19, as they can potentially interfere with the infectivity of SARS-CoV-2 by targeting TMPRSS2. Additionally, the virtual screening using the generated pharmacophore led to the identification of novel molecules that have not been previously reported for their interaction with TMPRSS2. This suggests their potential as repurposed drugs, offering a new avenue for targeting TMPRSS2 in the context of COVID-19. The strong binding affinities observed in the molecular docking results further support the potential of these repurposed drugs. In summary, the combination of virtual screening and molecular docking has provided valuable information on potential drug candidates for targeting TMPRSS2. These findings warrant further investigation and highlight the importance of repurposing existing drugs and exploring new therapeutic approaches for combating COVID-19.

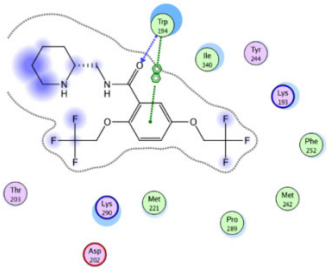
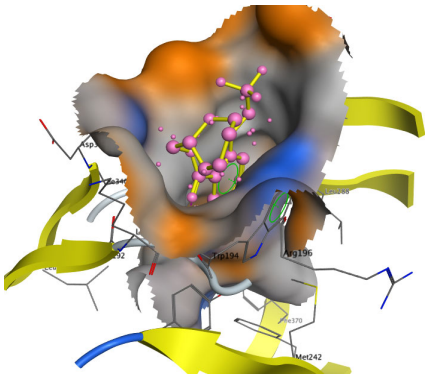
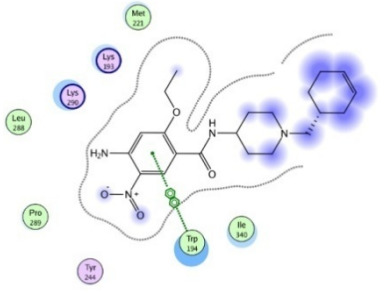
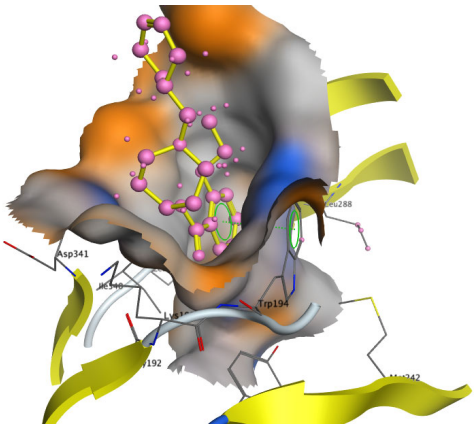
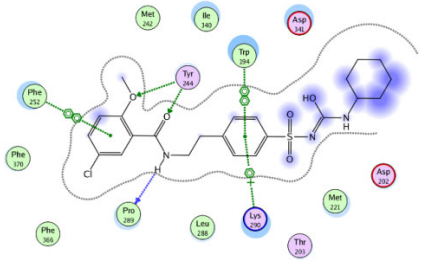
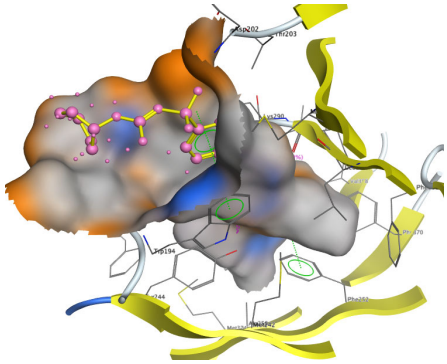
The active site of A1AT proteins contains specific amino acid residues that play a crucial role in ligand binding and interaction. These residues include backbone acceptor and arene-arene interactions at the carboxamide and aryl ring, as well as surrounding amino acid residues such as Trp 194, Ile 340, Tyr 244, Lys 193, Phe 252, Met 242, Thr 203, and Lys 290. The majority of these residues are hydrophobic, making them important contributors to the receptor-ligand interaction. In our study, all the selected molecules (ligands) exhibited favorable interactions with the active site of A1AT proteins. These ligands were well surrounded by both polar and greasy

Table 3 — Binding affinity score for the selected compounds from the ZINC database

ZINC ID	Drug Name	Clinical Use	Binding Affinity
ZINC00896543	Flecainide	Antiarrhythmic agent	-22.0254
ZINC05316843	Cinitapride	Gastroprokinetic agent and antiulcer agent	-21.676
ZINC00537805	Diabeta	Treatment of non-insulin dependent diabetes mellitus	-21.1944
ZINC03794794	Mitoxantrone	Antineoplastic agent	-20.8837
ZINC11592625	Oxytetracycline	Tetracycline antibiotic	-18.9221
ZINC00601298	Sultopride	Atypical antipsychotic agent	-18.6266
ZINC11592619	Tetracycline	Antibiotics	-18.3874
ZINC00388678	Floxuridine	Antineoplastic antimetabolite	-16.9175
ZINC11592624	Oxytetracycline	Tetracycline antibiotic	-15.8303
ZINC00057407	Methoxamine	Alpha adrenergic agonist used to treat hypotension	-15.7661
ZINC08586063	Tetracycline	Antibiotics	-15.7236
ZINC00057408	Methoxamine	alpha adrenergic agonist used to treat hypotension	-15.5654
ZINC03830728	Doxifluridine	Treatment of Stomach Cancer	-15.5303
ZINC00967922	Sultopride	Atypical antipsychotics	-14.9397
ZINC03830727	Doxifluridine	Treatment of Stomach Cancer	-13.1374
ZINC00025672	Telbivudine	Treatment of chronic hepatitis B	-12.7179
ZINC00057409	Methoxamine	Alpha adrenergic agonist used to treat hypotension	-11.4309
ZINC03830842	Flecainide	Antiarrhythmic agent	-10.318
ZINC01846088	Amisulpride	Dopamine D2 receptor antagonist	-7.612
ZINC03784384	Cinitapride	Gastroprokinetic agent and antiulcer	-6.2365
ZINC08586065	Tetracycline	Antibiotics	-6.0122
ZINC04574746	Oxytetracycline	Antibiotics	-5.9887
Co crystal	-	-	-12.8236

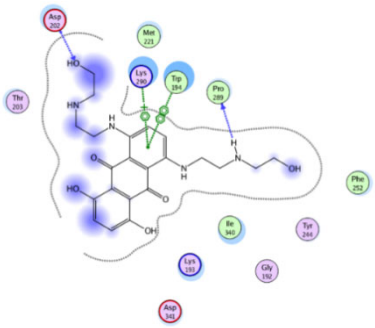
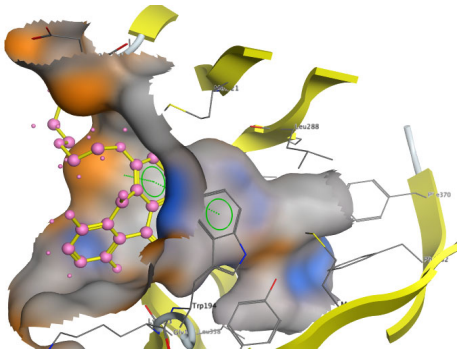
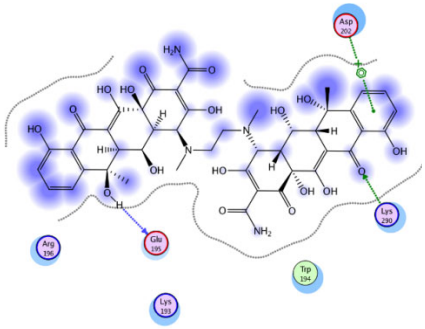
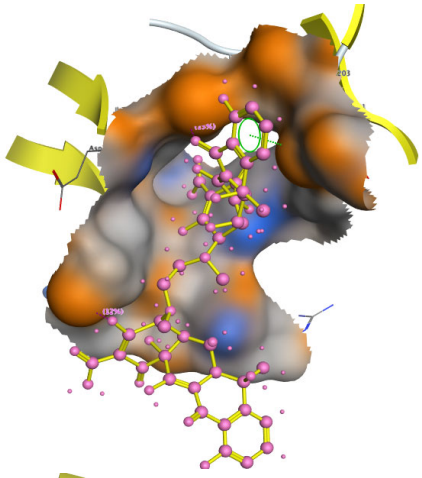
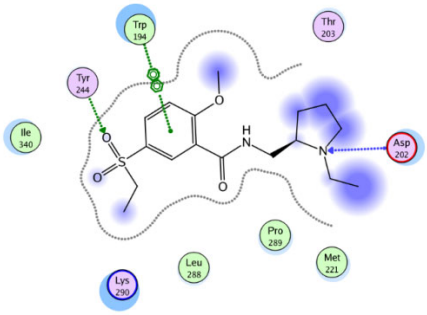
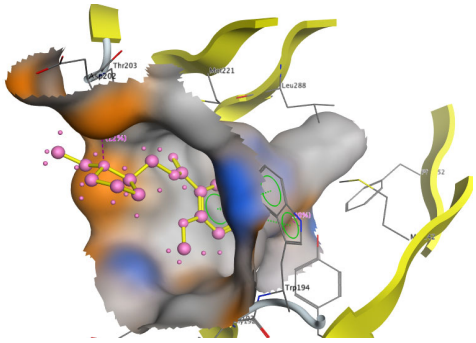
centers, indicating their potential for effective receptor-ligand interactions. The exposed nature of these ligands further enhances their accessibility to the active site and facilitates the formation of stable complexes. Table 4 provides an overview of the ligands and their respective characteristics in terms of receptor contact and exposure to polar and greasy centers. This information confirms the suitability of these ligands for interacting with the hydrophobic residues present in the active site of A1AT proteins. The favorable interactions observed between the selected ligands and the active site residues suggest their potential as therapeutic candidates for A1AT-related conditions or as regulators of the renin-angiotensin system. These findings provide valuable insights into the molecular mechanisms underlying the interaction between ligands and A1AT proteins, offering new possibilities for drug development and therapeutic interventions. In summary, our results highlight the importance of the active site residues in A1AT proteins for ligand binding and interaction.

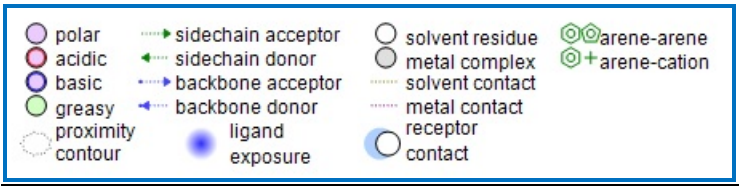
Table 4 — 2D and 3D interactions of the top six HITs from ZINC database compounds

Ligands	Binding affinity (kcal/mol)	2D interactions	3D interactions
ZINC00896543	-22.0254		
ZINC05316843	-21.676		
ZINC00537805	-21.1944		

(*Contd.*)

Table 4 — 2D and 3D interactions of the top six HITs from ZINC database compounds (*Contd*.)

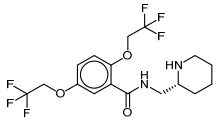
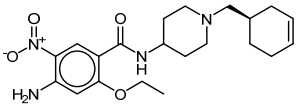
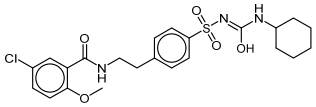
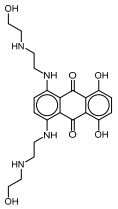
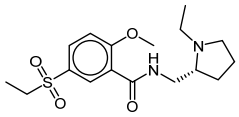
Ligands	Binding affinity (kcal/mol)	2D interactions	3D interactions
ZINC03794794	-20.8837		
ZINC11592625	-18.9221		
ZINC00601298	-18.6266		



Discussion

Our overall study focused on investigating the potential of repurposing FDA-approved drugs for targeting key proteins involved in COVID-19, namely ACE2 and TMPRSS2. By analyzing the literature and conducting molecular networking, pharmacophore modeling, virtual screening, and molecular docking studies, we aimed to identify drug candidates that could potentially interact with these proteins and inhibit their activity[37]. The molecular networking analysis revealed a network of drugs that could potentially interact with TMPRSS2, a protein known to facilitate viral entry into host cells. This provided valuable insights into the potential repurposing of

Table 5 — The top hit list of selected molecules from the ZINC database

S. No	ZINC ID	Drug	Structure	Class of Drug
1	ZINC00896543	Flecainide		Anti-arrhythmic drug
2	ZINC05316843	Cinitapride		Gastroprokinetic agent and antiemetic agent
3	ZINC00537805	Diabeta		Antidiabetic medication
4	ZINC03794794	Mitoxantrone		Antineoplastic agent
5	ZINC00601298	Sultopride		Antipsychotic agent

existing drugs for targeting TMPRSS2 and interfering with SARS-CoV-2 infectivity. Through pharmacophore modeling, we generated a pharmacophore representing the essential features required for ligand binding to TMPRSS2, including an aromatic center and two hydrogen bond acceptors. Virtual screening using the generated pharmacophore allowed us to screen a large database of compounds and identify potential hits that exhibited similar pharmacophoric features. The selected compounds were further subjected to molecular docking studies to assess their binding affinities with TMPRSS2. The docking results revealed several compounds with high binding affinities, indicating strong interactions with the active site of TMPRSS2. Among the top-ranked compounds, Flecainide, Cinitapride, and Diabeta exhibited the highest binding affinities. These compounds have diverse clinical uses, ranging from antiarrhythmic agents to gastroprokinetic agents and antidiabetic medications. The significant binding affinities observed for these compounds suggest their potential as repurposed drugs for targeting TMPRSS2 in the context of COVID-19. Furthermore, our study highlighted the importance of the active site residues in A1AT proteins for ligand binding and interaction. The selected ligands exhibited favorable interactions with the active site, indicating their potential as therapeutic candidates for A1AT-related conditions and as regulators of the renin-angiotensin system. These findings open up new possibilities for drug development and therapeutic interventions in the context of A1AT-related disorders. Overall, our study demonstrated the utility of *in silico* approaches for identifying and repurposing existing drugs for targeting key proteins involved in COVID-19. The combination of molecular networking, pharmacophore modeling, virtual screening, and molecular docking provided valuable insights into potential drug candidates that can directly interact with ACE2 and TMPRSS2. However, it is important to emphasize that further experimental validation, including *in vitro* and *in vivo* studies, is necessary to confirm the efficacy and safety of these repurposed drugs.

Conclusion

The study highlights the potential of repurposed compounds as promising candidates for addressing COVID-19 through innovative therapeutic approaches. Through this molecular networking model, we have come up with 12 drugs that interact well with the TMPRSS2 protein. Initially, we used 22 molecules (10 molecules were retrieved from a literature survey and 12 molecules were from FDA-approved drugs) for generating a pharmacophore. The pharmacophores generated are one aromatic center and two hydrogen

bond acceptors. By using these pharmacophore features, we have gone for virtual screening by using the ZINC database. ZINC's database threw 672 hits, from which, based on RMSD values, the top 22 molecules were further selected for docking.

Among the 22 compounds chosen from the ZINC database, the top six molecules were found to have a higher binding affinity for TMPRSS2when compared to the co-crystal ligand. The top six molecules giving the best score can be seen in the Table 5. Further, we have found that those drugs, which were not traditionally used for COVID-19 treatment, have been interacting with the TMPRSS2 protein via drug repurposing and molecular networking. Moreover, the top six binding affinities show that these selected ligands from the ZINC database are well bound in the protein pockets of proteins. The study identifies repurposed compounds as potential contributors to developing comprehensive therapeutic strategies for COVID-19. Further, the pharmacokinetics, pharmacodynamics, preclinical, and clinical studies on these drugs may permit to design the new agents without undesirable side effects and interactions. Moreover, a research on increasing ability on A1AT level by these drugs may help us to understand the complete mechanism of action. The repurposing of FDA-approved drugs offers a time-efficient and cost-effective approach to finding potential treatments for COVID-19. Leveraging existing drugs that have already undergone extensive testing and regulatory approval can expedite the development process and provide viable therapeutic options in the ongoing battle against the pandemic. Overall, our study contributes to the growing body of research aimed at identifying effective therapeutic strategies for COVID-19. The repurposed drugs identified in this study hold promise for further investigation and clinical evaluation. With continued research and collaborative efforts, these drug candidates have the potential to make a significant impact in mitigating the impact of COVID-19 and improving patient outcomes.

Acknowledgement

The authors are thankful to the Nandha College of Pharmacy, Erode, Tamil Nadu, India, the Government Medical College and Hospital, Pudukkottai, Tamil Nadu, India, and the JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Tamil Nadu, India, for providing the necessary facilities to carry out this research work.

Conflict of interest

All authors declare no conflict of interest.

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