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Virtual Screening Multitarget-Based Against 3CL^{pro} and TMPRSS2 Reveals New Promising Drugs Against SARS-CoV-2

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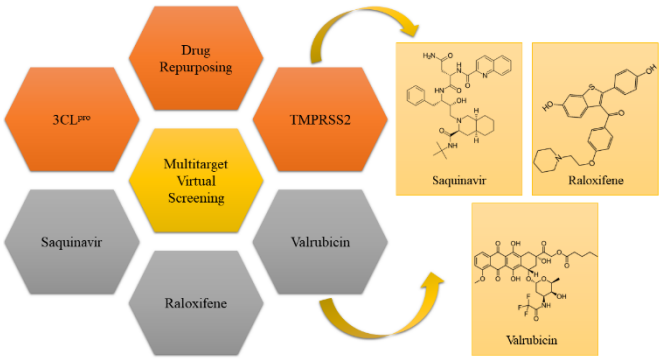
Graphical Abstract



Abstract. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) has spread worldwide and was declared a pandemic by the World Health Organization (WHO) on 11 March 2020. Responsible for various damages to public health, social life, and the economy of countries, this stimulates researchers and pharmaceutical companies to search for new therapies. The findings in recent years regarding the structure and biochemistry of SARS-CoV2 are remarkable. 3CL^{pro} is a potential target as an anti-SARS agent as it plays a vital role in the viral life cycle. In addition, recent studies point to transmembrane protease serine 2 (TMPRSS2) as one of the main targets responsible for a viral entry related to the cleavage of the S protein. In this way, using these drug targets in a multitarget approach can be promising in drug discovery. The design and identification of drugs that act on multiple targets can be the next stage in drug discovery campaigns. Finally, here using a virtual screening protocol docking-based in a multitarget approach, the drugs Raloxifene, Saquinavir, Tafluprost, Orlistat, and Valrubicin show a potential inhibition of 3CL^{pro} and TMPRSS2 and can be evaluated *in vitro* assay to prove your potential

1. Introduction

The new coronavirus (COVID-19) is a worldwide public health emergency announced by the World Health Organization (WHO), caused by the pathological agent Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This agent was previously identified in Wuhan (China) at the end of 2019, providing a global outbreak. The infection has since spread globally and disrupted everyday human life significantly [1–3].

SARS-CoV and SARS-CoV-2 share similar structural features. Coronaviruses are broad, single-stranded ribonucleic acid (RNA) viruses with a diameter of around 125 nm, which are enclosed by a capsid helical symmetry protein or envelope structured by nucleocapsid protein, and then surrounded by a bulbous bilayer lipid surface that contains various structural proteins (spike, envelope, and membrane) [4,5]. After SARS-CoV invasion, there is a hyperinflammatory response by the host's immune system, leading to acute respiratory distress and multiple organ failure. It is essential to block or reduce the inflammatory action caused by COVID-19 to improve the patient's health and reduce the casualty [6].

Due to its threatening potential, there is an urgent need to develop new therapies against this agent [7]. Computational approaches are practical tools to find new drug targets or repurpose existing drugs [8]. Also, various computational techniques and software programs are typically used in drug repurposing [9]. *In silico* methods (molecular docking, molecular dynamics, pharmacophore models, and others) could be used to identify and design drug candidates to study their interactions with their targets [4,10–12].

The critical viral proteins of SARS-CoV-2 are papain-like protease (PL^{pro}), 3-chymotrypsin-like protease (or $3CL^{pro}$), spike S proteins, and RNA-dependent RNA polymerase. The SARS-CoV $3CL^{pro}$ is a potential target as an anti-SARS agent as it plays a vital role in the viral life cycle. The $3CL^{pro}$ (or M^{pro}) gene is the main peptidase of SARS-CoV and is responsible for 11 cleavage sites in viral propeptide. As a result, it is an essential target for viral replication and structural assembly for the viral cycle [10,13]. Another critical drug target is the Transmembrane Serine Protease 2 (TMPRSS2), which facility viral entry with the function of cleaving the S protein and preventing the binding of the S protein to the ACE2 (angiotensin-converting enzyme 2) receptor [14–16].

Drug discovery strategies have been based on "one drug, one target" [17]. Nowadays, with the perception that an approved drug can present other medicinal actions, the premise "one drug, one target" is replaced by "one drug and multiple targets" [17,18]. The development of a multitarget drug will produce a small molecule drug with a lower affinity for the target than a single-target inhibitor [19]. However, this is not a disadvantage since its effect is related to interaction with all targets that will produce an adequate biological response [17,19]. The inhibition of multiple targets ($3CL^{pro}$ and TMPRSS2) can help design new anti-CoV drugs [20]. In this way, TMPRSS2 inhibition could prevent the viral entry event while $3CL^{pro}$ inhibition the viral multiplication [21]. Finally, this information makes it feasible to develop a drug that inhibits both targets. Here, we propose the drugs Raloxifene, Saquinavir, Tafluprost, Orlistat, and Valrubicin, promising multitarget inhibitors against $3CL^{pro}$ and TMPRSS2, that can be evaluated *in vitro* methods to probe their potential against SARS-CoV-2.

2. Materials and Methods

2.1 Target selection and preparation

3D-structure of $3CL^{pro}$ and TMPRSS2 cocrystallized with its ligands was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank database (RCSB PDB, https://www.rcsb.org/) [22] under the code 5RHD and 7MEQ, respectively. All hydrogens were added for these structures, while cocrystallized ligands, ions, and water molecules were removed. Subsequently, these were submitted to the re-docking procedure. This step was performed using the *GOLD*® v. 5.8.1 software [23]. For the re-docking, all four algorithms (Chemical Piecewise Linear Potential (ChemPLP), GoldScore, ChemScore, and Astex Statistical Potential - ASP) were applied to obtain FitScores and binding modes. The best binding pose was chosen for each ligand, and their Root-Mean-Square Deviation (RMSD) values were calculated using the *PyMol*® v. 0.99 software [24].

2.2 Selection and preparation of ligand dataset

From the ZINC database (https://zinc.docking.org/), 1,615 FDA-approved drugs were selected and submitted to the conformational analysis using the *MarvinSketch*® software [25]. Thus, ten conformations were generated for each ligand, choosing the conformation with the lowest energy value. The molecules were minimized using the *ArgusLab*® software [26] by applying the semi-empirical AM1 (Austin Model 1).

2.3 Molecular docking

Molecular docking studies were performed using the *GOLD*® v. 5.8.1 software by employing the ChemPLP for 5RHD and GoldScore for 7MEQ as scoring function, which was chosen from our initial redocking procedures. A 6 Å region around the cocrystallized ligand was selected using the maximum efficiency of the genetic algorithm (GA). Finally, ten binding poses were generated for each ligand, and the highest FitScore value was further analyzed. Furthermore, the complexes were analyzed for their interactions with the key residues using the *Discovery Studio*® software [27].

3. Results and Discussion

3.1 Validation of Screening Protocol

For both $3CL^{pro}$ and TMPRSS2 crystal structures, the re-docking protocol followed by the RMSD calculation was performed. Thus, according to Table **1**, for $3CL^{pro}$ (PDB id 5RHD), the best score function was ChemPLP (RMSD = 0.164), and for TMPRSS2 (PDB id 7MEQ) was GoldScore (RMSD = 0.625). In addition, the FitScore of cocrystallized ligands was used as a starting point for the virtual screening. Thus, the selected compounds showed values above 67 for the visual analysis of interactions with key residues, as demonstrated in the next topic.

Table 1. RMSD calculations and FitScore of cocrystallized ligands

PDB	ChemPLP		GoldScore		ChemScore		ASP	
	RMSD	FitScore	RMSD	FitScore	RMSD	FitScore	RMSD	FitScore
5RHD	0.164	50.30	0.197	44.27	0.212	44.31	0.224	44.33
7MEQ	0.789	32.00	0.625	40.85	0.811	17.48	0.834	33.42

3.2 Virtual Screening

After the validation protocol, the compounds were submitted to the virtual screening against the selected targets. The best compounds against TMPRSS2 present a FitScore between 67 – 77 and interactions with critical residues (His^{296} and Ser^{441}). In addition, the compounds against $3CL^{pro}$ showed similar FitScore and essential interactions with Cys^{145} and His^{41}. Thus, five drugs (Table **2**) were found promising against both targets, on the hypothesis that they are efficient inhibitors that can block the activity of TMPRSS2, increasing the resistance to the entry of the SARS-CoV-2 virus in the target human cells and against $3CL^{pro}$ preventing viral growth and maturation [20,21]. Our approach suggests these drugs as multitarget inhibitors, acting against viral entry (TMPRSS2) and replication ($3CL^{pro}$).

Our findings are similar to the work of Baby *et al.* [28], in which approximately 2800 FDA-approved drugs were used in a virtual docking, finding Valrubicin as a potential dual-target inhibitor. In addition, Silvas *et al.* [29] found Orlistat as an inhibitor of autophagy and replication inhibitors of SARS-CoV-2 and MERS-CoV in Vero E6 and Calu-3 cells at a post-entry step. These data highlight our findings, in which the virtual screening protocol identified drugs with promising activity against SARS-CoV-2. On the other hand, our virtual screening identified Tafluprost, a prostaglandin analog used in glaucoma treatment. Several studies have proposed the potential of these analogs against COVID-19 [30].

Another critical drug finding in our protocol was Raloxifene, a selective estrogen receptor modulator (SERM) approved to treat and prevent osteoporosis in postmenopausal women. This drug showed evidence of effect in the primary virologic endpoint in treating early mild to moderate COVID-19 patients, shortening the viral shedding time [32,33]. Finally, the HIV protease inhibitor Saquinavir is documented as a multitarget drug repurposed by computational methods for COVID-19 therapy. Several studies have determined from *in silico* models that this compound can effectively inhibit SARS-CoV-2 [34,35]. *In silico* studies demonstrated that Saquinavir inhibited $3CL^{pro}$ activity in SARS-CoV-2 [35]. Another study showed that Saquinavir inhibits SARS-CoV-2 *in vitro* [36] and has been used to treat patients with COVID-19 [37]. In addition, our findings indicate that Saquinavir can inhibit the TMPRSS2 in a multitarget approach. These data highlight the effectiveness of our protocol in identifying promising drugs and proposing a multitarget inhibition.

Table 2. The fit score docking and main interactions of top 5 compounds from virtual screening protocol.

ZINC code	Drug	Clinical Indication	Main Interactions					
			3CL^{pro}			TMPRSS2		
			FitScore	Cys^{145}	His^{41}	FitScore	Ser^{441}	His^{296}
ZINC538275	Raloxifene	Osteoporosis	76.79	π-Alkyl	π-π T shaped	68,26	C-H-bond	π-π
ZINC3914596	Saquinavir	Antiviral	77.80	π-Sulfur / π-Alkyl	π-Alkyl	71,33	C-H-bond	C-H-bond
ZINC13912394	Tafluprost	Glaucoma	69.56	π-Sulfur	π-Alkyl	67,75	C-H-bond	π-Alkyl
ZINC8214635	Orlistat	Obesity	75.88	2 π-Alkyl	π-Alkyl	75,06	π-Alkyl	C-H-bond
ZINC11616852	Valrubicin	Anticancer	67.89	2 π-Sulfur / C-H-bond	VDW	73,73	C-H-bond	C-H-bond

Conclusions

The discovery of new drugs against SARS-CoV-2 is a tremendous challenge for medicinal chemistries. This is because the virus has still mutated, and we may go through another pandemic episode of this virus. Although the development of vaccines has controlled this viral threat, it is still necessary to discover an innovative drug that can be used in the symptomatic treatment of the disease. In this way, our findings indicate the drugs Valrubicin, Orlistat, Raloxifene, Saquinavir, and Tafluprost with the best molecular docking results and interactions with the central residues for the inhibition of TMPRSS2 and $3CL^{pro}$ proteins, proposing multitarget inhibitors. Thus, it is believed that these drugs may be collaborators in the treatment of COVID-19. However, it is worth mentioning that *in vitro* and *in vivo* assays are necessary for further proof of the efficiency of these drugs.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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