Repositioning of ligands that target spike glycoprotein as potential drugs against SARS-CoV-2

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Research Article

Keywords: SARS-CoV-2, COVID-19, docking studies, FDA-approved drugs

Posted Date: August 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-52025/v1>

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Abstract

The worldwide health emergency of the SARS-CoV-2 pandemic and the absence of a specific treatment for this new coronavirus have led to the use of computational strategies (drug repositioning) to search for treatments. The aim of this work is to identify FDA-approved drugs with the potential for binding to the spike structural glycoprotein at the hinge site, receptor binding motif (RBM), and fusion peptide (FP) using molecular docking simulations. It was identified drugs that binding to amino acids crucial for down to up conformational change, receptor recognition, and fusion of the viral membrane with the cell membrane. Results show some drugs that bind to hinge site amino acids (Varenicline, or even steroids as Betamethasone) while other drugs bind to crucial amino acids for RBM (Naldemedine, Atovaquone, Cefotetan) or FP (Edarbi, Maraviroc, Difluprednate); and highlights the Saquinavir that binds both the RBM and the FP. Therefore, these drugs could inhibit spike glycoprotein and prevent viral entry (possible anti-COVID-19 drugs). Several drugs are in clinical studies; focused on another pharmacological target (candesartan, Atovaquone, Losartan, Maviroc and Ritonavir) in this work we propose an additional target, the spike glycoprotein. These results can impact in the proposal of treatments that can inhibit the first steps virus replication cycle.

Introduction

The COVID-19 disease is produced by the virus called SARS-COV-2 and emerged in China at the end of 2019. Currently, it has spread worldwide, so the World Health Organization (WHO) has declared a pandemic for which there is no treatment or vaccine [24].

The process to generate and test a vaccine will take at least a year [8]. However, by computational tools such as repositioning, already known drugs (known information on pharmacokinetics, pharmacodynamics and toxicity) and used for other pathologies can be proposed.

Repositioning drugs shows several advantages, for example, the time and the production costs of the drugs are dismissed because the drugs are already available in the market [17]. In the case of aspirin, this drug was initially used as analgesic and antipyretic and derived from the repositioning strategies; currently its new indication is as a treatment for colorectal cancer. Another example is Hydroxychloroquine an antiparasitic and it is now used in the treatment of anti-arthritis systemic lupus erythematosus [33].

The SARS-COV-2 belongs to coronaviruses family, the genome is +ssRNA, non-segmented, with a size of 27 to 32 kilobases [18]. The genome encodes four major structural proteins including spike (S), nucleocapsid (N), membrane (M) and envelope (E) which required to make complete virus particle (see Fig.1A) [7, 18].

Spike protein comprised of S1 and S2 subunits. The S1 subunit contains a signal peptide (SS), followed by an N-terminal domain (NTD) and receptor-binding domain (RBD). The RBD contains a core structure and a receptor-binding motif (RBM). While the S2 subunit contains conserved fusion peptide (FP), heptad repeat 1 (HR1), a central helix (CH), a connector domain (CD), a heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT). At the boundary between S1 and S2 (S1/S2) there is a furin cleavage site, positions 681-684 (see Fig.1 B) [30, 32].

Conformationally, spike glycoprotein is organized on the viral surface in homotrimers [29]. When the RBM are hidden, the conformation is called down (receptor-inaccessible) (see Fig.1C). However, the homotrimer is asymmetric, because they constantly undergo structural rearrangement (up conformation) to fuse the viral membrane with the host cell membrane [13]. When two RBD domains are hidden (receptor-inaccessible) one RBD domain is exposed (receptor-accessible), named up conformation (see Fig.1D). This is due to the fact that the RBD of S1 undergoes hinge-like movements [32]. In SARS-CoV two hinge sites were characterized (hinge 1 site (354-361) and hinge 2 site (552-563), which are responsible for the switch in the up and down conformation [25]. The RBD in up position recognizes the receptor, through the receptor-binding motif (RBM), which binds to the outer surface of the claw-like structure of angiotensin-converting enzyme 2 (ACE2). The importance of RBM was demonstrated by comparing the sequences between SARS-CoV and SARS-CoV-2; and the (Leu455, Phe486, Gln493, Ser494 and Asn501) five amino acid mutation improves affinity for the receptor. Therefore, it is believed that SARS-CoV-2 is more infectious than SARS-CoV [30].

After recognition and binding of the RBM by the ACE2, the spike glycoprotein needs to be proteolytically activated at the S1/S2 boundary, such that S1 dissociates and S2 undergoes a dramatic structural change. These SARS-CoV-2 entry-activating proteases include cell surface type II transmembrane serine proteases (TMPRSS2) and lysosomal proteases cathepsins. The structural changes that S2 shows when dissociated is the exposure of the fusion peptide (FP) located at the amino acid position 816 to 855. Subsequently triggering the insertion of the FP into the host membrane and allowing the fusion of membranes (viral and cellular) to release the viral genome into the cytoplasm. [26].

As mentioned above, the spike glycoprotein is in first contact with the host cell and it is crucial for various processes such as attachment, receptor binding, membrane fusion via conformational changes, internalization of the virus, host tissue tropism [7, 30]. Therefore, it is one of the main targets for the development of vaccines and antivirals.

In this work, we repositioned drugs that have been previously approved by the FDA for other therapeutic use, which could be used as a treatment against COVID-19 disease, targeting the spike glycoprotein involved in three specific events (conformational change down to up, recognition of the ACE2 enzyme and membrane fusion).

Methodology

Molecular modeling of spike glycoprotein SARS-CoV-2

Two crystal structures which represent down and up conformational states of the spike glycoprotein were used. The ID PDB: 6VYB represents the up conformation whereas ID PDB: 6VXX constitutes the down conformation. Both crystals showed missing fragments and two disulfide bridges (480-488 and 840-851) which are important in the RBM and FP region respectively, thus, these were completed using the Program Modeller 9.23 (scripts: loop.py, segment.py and model-disulfide.py) [31]. After adding the missing residues and two disulfide bridges, the resultant tridimensional (3D) models were denominated as 6VXX-fill and 6VYB-fill down and up conformation correspondingly. In the model 6VYB-fill the chain B the site RBD is exposed. The crystals glycosylation's were preserved and added by the Chimera program [20] to the models 6VXX-fill and 6VYB-fill.

Selection of drugs to be repositioned

The 3D structures of the drugs were obtained from the database Zinc (<http://zinc.docking.org/>), accessed March 2020, download the subset fda, drugs which are approved by the FDA, per DrugBank selecting those that comply with the rules of Lipinski and have the properties ADMET (Absorption, Distribution, Metabolism, Elimination and Toxicity), binding to albumin, and solubility in water and in DMSO [14]. Also, the subset fda was submitted to Data Warrior program to predict drugs that were mutagenic, tumorigenic, reproductive or irritants effect. The drugs that presented those effects were eliminated and selecting for docking studies only those drugs that were safe were docked. In total were analyzed 1372 drugs; according to the criteria, they were finally chosen 1322 drugs were selected to be coupled to spike glycoprotein by docking studies.

Molecular docking

Molecular docking studies were carried out using the AutoDock Vina program [28]. The spike glycoprotein was rigid and the ligands flexible. The drugs with the highest affinity and those that reach key amino acids that influences the spike glycoprotein function were selected. The dockings simulation were focused to the hinge sites, the RBM and FP, for the 3 chains of the spike glycoprotein trimmer in the up and down conformation. In total, 1322 dockings were achieved for the A, B and C chain for the down and up conformation.

Drugs selection

Once finishing the docking simulations, Discovery Studio Visualizer (<https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/>) and Chimera [20] programs were used to visualize the docking results. Drugs with the highest affinity (more negative) and that interact with key amino acids for function at the interest sites (hinge, RBM and FP) were selected.

Results

Molecular modeling of spike glycoprotein

Fig. 2 shows the conformational structure of the models obtained by molecular modeling. The 6VXX-fill model is seen in the down conformation, and the up conformation is seen in the 6VYB- fill model.

Molecular docking and drug selection

Once the molecular docking was performed, the drugs with the highest affinity for the spike protein (mainly with amino acids belonging to the hinge site, the RBM and the FP) were selected. The drugs are listed in tables. The tables show the drug name, database Zinc identification number, use according to currently described pharmacological action, FDA approval number, inclusion in clinical studies related to COVID-19, affinity in kcal/mol (from highest to lowest affinity) and amino acid interactions from spike glycoprotein are indicated. The general scheme shows the possible sites of interaction (Fig.1).

Docking simulations on the hinge site

Docking simulations were performed on hinge sites (1 and 2), however only drug interactions were found at hinge site 1 (see table 1 and table 2). The affinity binding scores and molecular interactions of the drugs for down conformation are detailed in the Table1.

Fig. 3A shows Varenicline which binds to chain B and C in the hinge site 1, RBM and NTD with an affinity of -7.6 kcal/mol in down conformation, interacting by Van der Waals forces (Pro230C, Lys458B and Arg466B), π -interactions (Ile231C and Tyr200C), alkyl interactions (Trp353B and Arg355B), π -cation interactions (Asp467B and Arg355B) and finally forming hydrogen bonds (Gly232C, Glu465B, Asp198C and Gly199C). In addition Fig. 3B shows that Docosahexaenoic acid interacts by Van der Waals forces with the amino acids Pro230B, Val227B, His207B, Asp228B, Leu229B, Phe168B, Tyr170B, Ile128B, Ile203B, Phe194B, Ile119B, Phe192B, Trp104B and Asn121B, by an electrostatic interaction and a hydrogen bonds with Arg357B, and finally alkyl interactions with Leu179B and Val126B. Other interesting drug was sulbactam (Fig. 3C) which interacts with in the hinge 1 site, the RBM and the NTD by Van der Waals forces with the amino acid Asp198C, Pro426B, Leu518B, Glu516B, Phe515B, Tyr396B and Ser514B, and two hydrogen bonds with the amino acids Arg466B and Arg355B.

The table 2 shows compounds that interact mainly with amino acids belonging to the hinge site 1, the RBM site and the NTD of the up conformation. In Fig. 4A and table 2 is show that Accolate binds to chain A and hinge 1 site (-10.3 kcal/mol) in the up conformation. Accolate make Van der Waals interactions (Phe168B, Arg357A, Ile128B, Leu176B, Ile119B, Asn121B, Arg102B, Phe175B, Phe192B, Ser172B and Gln173B), hydrogen bonds (Pro174B, Asp228B and Val227B), π - π interactions (Tyr170B), π -sigma interactions (Leu226B) and alkyl interactions (Pro174B, Leu226B, Val126B, Met177B and Pro230B). Tigecycline (Fig. 4B) joins the A-chain at the hinge 1 site with an affinity of -9.7 kcal/mol in the up conformation. The drug interacts through Van der Waals forces with the amino acids Glu516A, Tyr200B, Arg357A, Pro426A and Gly232B, hydrogen bonds with Pro230B, Gly199B, Ile231B, Phe429A, Phe515A, Thr430A, Phe464A and Asp198B, and π - π interactions with Tyr396A and π -Cation with Arg355A.

Docking simulations on the RBM

Table 3 and 4 show the drugs selected for the RBM in the down and up conformation, respectively.

The drug the highest binding score for the RBM site in down conformation is Naldemedine. Which (Fig. 5A) interacts by Van der Waals forces with Gln493C, NAG1303A, Leu492C, Gly476C, Ala475C, Tyr473C, Asp467C, Asp420C, Glu465C, Tyr453C, Phe456C and Arg457C; makes hydrogen bonds with Arg454C, Leu455C and Tyr421C; alkyl interactions with Pro491C and Lys458C and π -cation interactions with Lys417C and Arg454C. The second drug that showed the highest affinity was Conivaptan (Fig. 5B) which interacts in the chain C at RBM region by Van der Waals forces (Pro491C, Gln493C, Tyr453C, Lys458C, Ile472C, Asn422C and Asp420C), hydrogen bonds (Phe456C, Arg457C and Tyr421C) and π interactions (Asp467C, Leu455C, Lys417C and Tyr421C).

Regarding the up conformation the Fig. 6A shows that Tedizolid Phosphate binds to chain B in the RBM displays an affinity of -9.7 kcal/mol. The drug interacts through Van der Waals forces (Asp405, Gly416B, Ile402B, Ile418B, Gln493B, Ser494B, Tyr495B, Leu452B, Tyr451B and Tyr453B), π -cation interactions (Arg408B and Lys417B) and also forms hydrogen bonds with (Gln409B, Gln414B, Lys417B and Arg403B). The drug Atovaquone (Fig. 6B) binds to the B chain in the RBM interacting through Van der Waals forces (Leu455B, Ser494B, Ala352B, Asp467B, Phe456B, Asn450B, Pro491B, Thr478B, Gly476B and Ser477B), π - π interactions (Tyr351B), hydrophobic interactions (Leu492B and Leu452B) and hydrogen bonds (Arg454B and Pro479). This drug showed the second highest binding affinity as can be seen in Table 4.

Regarding the drug Cefotetan, although it did not show one of the highest affinities, this drug is the one that interacts with the most crucial amino acids for the formation of the spike-ACE2 complex. The drug Cefotetan (Fig. 6C) is bound in the up conformation by Van der Waals forces (Ser349B, Tyr489B, Leu455B, Pro491B, Gly476B, Ser477B, Phe490B, Gln493B, Leu492B, Tyr351B, Tyr449B and Asp467B), forms hydrogen bonds (Tyr449B, Asn450B, Arg454B and Thr478B), alkyl interactions (Pro479B and Leu452B) and π -sulfur interactions (Phe456B and Tyr449B).

Docking simulations on the FP

The drugs which displayed the highest affinity for the fusion peptide in both the down and up conformation are summarized in tables 5 and 6 respectively. Results from the docking analyses are ordered from highest to lowest affinity. Some representative drug interactions are described below.

The drug Saquinavir (Table 5 and Fig. 7A) shows the highest affinity to the FP site (-11.1 kcal/mol), binding to the amino acids Cys851A and Leu849A in the down conformation. Saquinavir interacts through Van der Waals forces (Lys835A, Cys851A, Ala852A, Leu828A, Gly832A, Ile834A, Pro862A, Pro863A, Lys854A, Arg646C, Ile850A, Glu619C, Ser591C and NAG1309C), forms hydrogen bonds (Asp843A, Tyr837A, Asp614C, Asn616C, Val615C, Gln644C, Gly648C and Thr645C), π -Alkyl Interactions (Val860A and Tyr837A), π -Sigma Interactions (Leu849A) and π -Cation Interactions with (Tyr837A). While the drug Difluprednate showed less affinity to the same site (-8.9 Kcal/mol) (Table 5 and Fig. 7B) and in the down conformation, with Van der Waals interactions in amino acids Ala829A, Leu849A, Lys835A, Ala852A, Gly832A, Ile834A, Val860A, Asp614C, Ala668C, Thr866A, Ala647C and Cys840C, forms hydrogen bonds with amino acids Phe833A and Arg646C, halogen interactions with Ile850A and finally π -Alkyl interactions (Cys851A, Leu828A and Tyr837A).

Additionally Saquinavir (table 6 and Fig. 8A) show the highest affinity in the up conformation. Saquinavir interacts by Van der Waals forces (Asn856C, Asn960C, Gln853C, Leu858C, Ala852C, Thr859C, Asp614B, Leu959C, Thr732C, Phe833C, Val952C, Asn955C, Ile834C, Gly832C, Ala831C, Arg646B, Leu849C, Gln836C, Ala570B and Tyr837C), forming hydrogen bonds with the following residues Arg847C, Asp848C and Thr732C; alkyl interactions (Lys854C, Val860C, Ala956C, Lys835C and Cys851C) and π -sulfur interactions (Cys851C and Cys840C). Other drug that reach the up conformation was Maraviroc (Fig. 8B) which interacts through Van der Waals forces with the amino acids Val952C, Asn955C, Leu959C, Thr732C, Leu858C, Ala570B, Asn856C, Val860C, Asp848C, Arg847C, Tyr837C and Gln836C through Van der Waals forces, forming hydrogen bonds with the amino acids Arg646B and Gln836C and Alkyl interactions with amino acids the Lys835C, Ala956C, Phe833C, Val963C, Ala852C, Cys851C, Leu849C and Cys840C.

Discussion

Spike glycoprotein is the structural protein of the SARS-CoV-2 virus that allows adhesion and binding to the receptor, making it crucial in the first step of infection [34]. Currently it has been considered as one of the main targets for vaccine design. However, this protein evades the immune response due to the dynamic structure, for example, when is found in the down conformation (receptor-inaccessible) where it hides RBD (hidden RBD) and prevents the binding of neutralizing antibodies. Additionally, when is found in the up conformation (accessible receptor) shows the RBD which has high affinity for the receptor (higher affinity than SARS-CoV). In addition to the above, the virus can enter through two routes, by receptor-mediated endocytosis and by direct fusion to the cell membrane because the fusion peptide can be exposed by cell surface proteases (depends on cell type). So can be developed vaccines with target the membrane fusion S2 subunit, however S2 subunit is less immunogenic than S1 subunit [6, 23].

Therefore, in this work, we have employed an in silico strategy called drug repositioning, in which we looked for drugs available and could target the spike glycoprotein (hinge sites, RBM and FP of interest). From this analysis, it was possible to identify 43 drugs, of which 8 are included in reported clinical trials (<https://clinicaltrials.gov/>).

Regarding the hinge region in the down conformation, 3 drugs were found with the possibility of binding and preventing the change to the up conformation and therefore avoiding exposure of the receptor binding site. The drug with highest affinity is Varenicline (-7.6 kcal/mol), however it is not found in any clinical study, unlike Docosahexaenoic acid (DHA) and Sulbactam, which are being studied as a complementary part of a main treatment. In the DHA clinical trial, the main treatment with Fenretinide (LAU-7b) was a synthetic retinoid derivative and has been used in the treatment of some types of cancer and cystic fibrosis. DHA is an omega-3 fatty acid and can be used as part of the diet. Another fatty acid that has been reported in 5 clinical trials is Icosapent Ethyl (Vascepa™) but not as part of a dietary food supplement. The effect is found on Inflammatory Biomarkers in Individuals with COVID-19. Arachidonic acid (AA) and other unsaturated fatty acids (especially eicosapentaenoic acid, EPA and DHA) are known to inactivate enveloped viruses and inhibit proliferation so they can serve as endogenous antivirals [5]. Furthermore, Sulbactam is an antibiotic inhibitor of the beta lactamase enzyme and was used in conjunction with intravenous ampicillin [9] to avoid secondary infection in patients with Cytokine Adsorption in Severe COVID-19 Pneumonia Requiring Extracorporeal Membrane Oxygenation (CYCOV).

As mentioned before, spike glycoprotein has been shown to be very dynamic due to the hinge sites, preventing switch up to down conformation can help to avoid hiding the receptor binding site and in therapy together (with neutralizing antibodies) can prevent the viral attachment. The best evaluated drugs to bind at the hinge site in the up position were 5 drugs (Accolate, Tigecycline, Betamethasone, Triamcinolone acetonide, Atazanavir), which two are in clinical trials (Betamethasone and Triamcinolone acetonide; both corticosteroids). Betamethasone is a steroid from the group of corticosteroids, it was used in pregnancies complicated by SARS-CoV-2 for its immunosuppressive and anti-inflammatory properties. However, a potential diabetogenic effect was found, in addition to that SARS-CoV-2 and other coronavirus may have effects on glycemia [10].

Therefore, its use in clinical trials is not recommended and has not been reported. In contrast, the use of Dexamethasone for the short-term in severe, intubated, COVID-19 patients has recently been recommended due to the anti-inflammatory effect that would limit the production of and damaging effect of the cytokines, but will also inhibit the protective function of T cells and block B cells from making antibodies [27]. The use of Betamethasone and Dexamethasone effect on hinge site blockage not been explored, the two corticosteroids have the same molecular weight and high structural similarity (the only difference is the orientation of the methyl group on carbon 16) so which could have similar effects. Use of inhaled corticosteroids had been associated with lower expression of ACE2 and TMPRSS2, but treatment with Triamcinolone Acetonide did not decrease expression of either gene [19]. On the other hand, the use of Atazanavir (protease inhibitor) has been reported in computer studies with inhibitory potency activity with K_d of 94.94 nM against the SARS-CoV-2 3C-like protease inhibitor [2].

On the other hand, we have found 10 better evaluated drugs from the docking studies at the RBM site in the down conformation, which although it would not prevent the change to the up conformation, would prevent receptor binding. Nine of the drugs show interaction with two amino acids (Leu455 and Gln493), of the 5 reported (Leu455, Phe486, Gln493, Ser494 and Asn501) as crucial for the RBM site in the formation of a stable complex between spike viral glycoprotein and human ACE2 [15, 30]. Only Saquinavir drug shows interaction with a crucial amino acid Leu455. Despite these interactions, the affinity for the glycoprotein is different, with the highest affinity being Naldemedine (-12.7 kcal/mol) and the lowest affinity Suvorexant, Riociguat, Diabeta and Candesartan drugs (-10.6 kcal/mol). Candesartan is included in three clinical studies due to the selective antagonist function of angiotensin II AT1 receptors; therefore, the present study reports an additional mechanism of this drug to avoid SARS-CoV-2 infection.

When the conformation of the spike glycoprotein in the S1 domain changes to the up position, it shows high affinity for the receptor, which can be blocked by drugs that bind to the RBM in up position. 6 drugs were found to show affinity for the RBM site in up position. However, the affinity values are lower compared to the affinity shown in down position (-9.7 kcal/mol in up vs -12.7 kcal/mol in down position). The drugs with the highest affinity were Tedizolid Phosphate and Atovaquone with (-9.7 kcal/mol). Atovaquone is in a clinical trial (in combination with Azithromycin) to treat COVID-19, due to its ability to treat or prevent

pneumonia caused by *Pneumocystis carinii* (clinical trials). On the other hand, the drug with the lowest affinity was Rosiglitazone (-8.3 kcal/mol) and was used to regulate glucose in type 2 diabetes, however its use is discontinued [21].

Considering another point in the viral replication cycle, we looked for drugs capable of binding to FP and taking into account that it is a conserved sequence with SARS-CoV that shows two FP sites. FP1 (amino acid 798 to 818) and FP2 (amino acid 816 to 835) which have characteristics of an active fusion region working, furthermore, they postulate that the regions function cooperatively as an extended FP (FP1-2) [12, 16]. For the case of SARS-CoV-2 spike glycoprotein, Tang and collaborators recently determined that FP is found in the region located in amino acids 816 to 855, which corresponds to the region 798-835 for SARS-CoV spike glycoprotein. The amino acids Leu803, Leu804, Phe823, Cys822, Cys833, Asp830 and Leu831 in SARS-CoV (corresponding to the amino acids Leu821, Leu822, Phe823, Cys840, Asp848, Leu849, and Cys851 in SARS-CoV-2) have been shown by biophysical, site-directed mutagenicity functional assays to be essential for membrane fusion function [12](Lai et al, 2017). In addition, these amino acids are conserved in SARS-CoV, MERS-CoV and SARS-CoV-2 [26].

Of worthy interest is that nine drugs were found to be bound in the down conformation, within the FP and interact mainly with two highly conserved amino acids (Cys851A and Leu849A) which are crucial for the process of membrane fusion in the family of the coronavirus [26]. However, no drug has been reported in clinical studies. The drug with the highest affinity was Saquinavir (-11.1 kcal/mol) and the lowest affinity was Difluprednate (-8.9 kcal/mol). However Difluprednate interacts with an additional amino acid to modulate the membrane fusion process (Cys840A) in total three critical amino acids (Cys840A, Cys851A and Leu849A). In order to be able to use these drugs in future clinical studies (in addition to the interactions found), the commercial presentation must be considered. Such is the case of Terconazole which can be administered as a cream and suppository vaginal presentation (<https://www.accessdata.fda.gov>), so it could not be used for treatment of COVID-19 in that presentation.

On the other hand, when the protein is in the up position, the best evaluated drugs to bind FP are 10, of which 5 (Maraviroc, Ritonavir, Bosentan, Fosinopril sodium, Ceftazidime) are found in clinical trials. The Maraviroc, a C-C Chemokine Receptor 5 (CCR5) antagonist, is well-tolerated without significant side effects in its current use in patients with HIV; in patients with COVID-19 has been used as a drug against the main protease (Mpro) of SARS-CoV-2 (determined by computer methods) [22]. The drug found in the largest number of clinical trials is Ritonavir, most of it is used in combination with Lopinavir both are protease inhibitors to treat HIV and had been used against other coronaviruses (SARS-CoV and MERS-CoV) [4]. The use in SARS-CoV-2 also seeks to inhibit cellular proteases to prevent completion of the viral replication cycle; however some clinical trials have not been concluded and others have concluded that there is no difference in using ritonavir in hospitalized adult patients with severe COVID-19 and standard medical care treatment [3]. Another class of drugs that have been used for the treatment of COVID-19 are ACE inhibitors such as Fosinopril sodium, which is being studied in a clinical trial (<https://clinicaltrials.gov/>), which seeks to block the virus receptor. Therefore, these 3 drugs, in addition to the effect described in the clinical trial, could have an effect in preventing the fusion of the viral membrane with the cell membrane.

Furthermore, the drug Bosentan is a dual endothelin receptor antagonist, used in the treatment of pulmonary hypertension (PHT) (<https://clinicaltrials.gov/>), and Ceftazidime is an antibiotic used to treat lower respiratory tract infections, both drugs and others (ie Budesonide, Cefdinir, Cefepime, Clindamycin, Clobazam, Dexamethasone, Dexmedetomidine, Fosfomycin, Dextroamphetamine, etc.) are part of a clinical study called Pharmacokinetics, Pharmacodynamics, and Safety Profile of Understudied Drugs Administered to Children Per Standard of Care (POPS or POP02) in the treatment of COVID-19.

Interesting, Saquinavir has been found to be the most affinity for the FP for both conformations (up and down), and showing an affinity of -11 kcal/mol and in addition it binds to four crucial amino acids in the membrane fusion process (Cys851C, Cys840C, Leu849C and Asp848C). Furthermore it can bind to the RBM in down position. Therefore, this drug is a potential candidate to inhibit the FP and RBM sites of the spike glycoprotein. It has also been reported by computational methods effect on 3C-like protease (3CL pro) also called the main protease (Mpro) [1, 11], so it could be considered a multi-target drug, which confers a greater probability of success.

Considering the data reported, the use of multi-target drugs can be proposed, and by interfering at different points in the replication cycle, it could have a better effect. It is even possible to propose combined therapies that prevent both viral entry (spike target) and replication cycle events inside the host cell to be used as a preventive and therapeutic treatment.

Conclusion

By using in silico tools, a subgroup of drugs previously approved by the FDA for use in various human diseases has been analyzed. From this group of drugs, we were able to identify potential drugs that could inhibit the function of spike glycoprotein interfering, the entry of SARS-CoV-2 on host cell inhibiting the viral infection. Among these potential drugs some are reported in clinical trials as therapy in conjunction with other drugs (DHA, Sulbactam and Atovaquone); others are part of the main therapy due to its effect on the ACE2 receptor (Fosinopril sodium and Candesartan), anti-inflammatory effect (Betamethasone, Triamcinolone acetonide); effect on proteases (Atazanavir and Ritonavir). Additionally, Maraviroc is used as a CCR5 antagonist and determined by computer methods as a possible protease inhibitor. On the other hand, even though Saquinavir has not been reported in clinical trials it has been suggested from theoretical and in silico studies, a 3C-like protease effect was determined. Therefore, in this work, we propose other therapeutic targets that could bind the spike protein and which may have a summative effect with the other reported targets/uses.

Declarations

Acknowledgements

We gratefully acknowledge to CONACYT (Grants: CB-254600 and SEP-CONACYT-ANUIES- ECOS Francia: 296636), to Instituto Politécnico Nacional (Grant: Proyectos Insignia IPN-2015), and to COFAA-SIP/IPN (SIP 20200568).

Author contributions

All authors contributed to the development, analysis, and drafting of this article.

Compliance with Ethical Standards

This study was funded by Grants: CB-254600 and SEP-CONACYT-ANUIES-ECOS Francia: 296636), to Instituto Politécnico Nacional (Grant: Proyectos Insignia IPN-2015), and to COFAA- SIP/IPN (SIP 20200568).

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

<http://zinc.docking.org/>, accessed march, 2020

<https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/>

<https://www.accessdata.fda.gov>, accessed june, 2020.

<https://clinicaltrials.gov/>, accessed june, 2020.

1. Al-Khafaji K, Al-Duhaidahawi D, Taskin Tok T (2020) Using integrated computational approaches to identify safe and rapid treatment for SARS- CoV-2. *J Biomol Struct Dyn*:1-9
2. Beck BR, Shin B, Choi Y, Park S, Kang K (2020) Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. *Comput Struct Biotechnol J* 18:784-790
3. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C (2020) A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 382:1787-1799

4. Chan JF, Yao Y, Yeung ML, Deng W, Bao L, Jia L, Li F, Xiao C, Gao H, Yu P, Cai JP, Chu H, Zhou J, Chen H, Qin C, Yuen KY (2015) Treatment With Lopinavir/Ritonavir or Interferon-beta1b Improves Outcome of MERS-CoV Infection in a Nonhuman Primate Model of Common Marmoset. *J Infect Dis* 212:1904-1913
5. Das UN (2020) Can Bioactive Lipids Inactivate Coronavirus (COVID- 19)? *Arch Med Res* 51:282-286
6. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S (2009) The spike protein of SARS-CoV—a target for vaccine and therapeutic development. *Nat Rev Microbiol* 7:226-236
7. Fehr AR, Perlman S (2015) Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol* 1282:1-23
8. Graham BS (2013) Advances in antiviral vaccine development. *Immunol Rev* 255:230-242
9. Hornuss D, Laubner K, Monasterio C, Thimme R, Wagner D (2020) [COVID-19 associated pneumonia despite repeatedly negative PCR- analysis from oropharyngeal swabs]. *Dtsch Med Wochenschr* 145:844-849
10. Kakoulidis I, Ilias I, Koukkou E (2020) SARS-CoV-2 infection and glucose homeostasis in pregnancy. What about antenatal corticosteroids? *Diabetes Metab Syndr* 14:519-520
11. Khan SA, Zia K, Ashraf S, Uddin R, Ul-Haq Z (2020) Identification of chymotrypsin-like protease inhibitors of SARS-CoV-2 via integrated computational approach. *J Biomol Struct Dyn*:1-10
12. Lai AL, Millet JK, Daniel S, Freed JH, Whittaker GR (2017) The SARS- CoV Fusion Peptide Forms an Extended Bipartite Fusion Platform that Perturbs Membrane Order in a Calcium-Dependent Manner. *J Mol Biol* 429:3875-3892
13. Li F (2016) Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu Rev Virol* 3:237-261
14. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46:3-26
15. Luan J, Lu Y, Jin X, Zhang L (2020) Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochem Biophys Res Commun* 526:165-169
16. Millet JK, Whittaker GR (2018) Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells. *Virology* 517:3-8
17. Novac N (2013) Challenges and opportunities of drug repositioning. *Trends Pharmacol Sci* 34:267-272
18. Palacios Cruz M, Santos E, Velazquez Cervantes MA, Leon Juarez M (2020) COVID-19, a worldwide public health emergency. *Rev Clin Esp*
19. Peters MC, Sajuthi S, Deford P, Christenson S, Rios CL, Montgomery MT, Woodruff PG, Mauger DT, Erzurum SC, Johansson MW, Denlinger LC, Jarjour NN, Castro M, Hastie AT, Moore W, Ortega VE, Bleecker ER, Wenzel SE, Israel E, Levy BD, Seibold MA, Fahy JV, National Heart L, Blood Institute Severe Asthma Research Program I (2020) COVID-19 Related Genes in Sputum Cells in Asthma: Relationship to Demographic Features and Corticosteroids. *Am J Respir Crit Care Med*
20. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 25:1605-1612
21. Ryan GJ, Foster KT, Jobe LJ (2011) Review of the therapeutic uses of liraglutide. *Clin Ther* 33:793-811
22. Shamsi A, Mohammad T, Anwar S, AlAjmi MF, Hussain A, Rehman MT, Islam A, Hassan MI (2020) Glecaprevir and Maraviroc are high-affinity inhibitors of SARS-CoV-2 main protease: possible implication in COVID- 19 therapy. *Biosci Rep* 40
23. Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F (2020) Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* 117:11727- 11734
24. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, Iosifidis C, Agha R (2020) World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). *Int J Surg* 76:71-76
25. Song W, Gui M, Wang X, Xiang Y (2018) Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. *PLoS Pathog* 14:e1007236
26. Tang T, Bidon M, Jaimes JA, Whittaker GR, Daniel S (2020) Coronavirus membrane fusion mechanism offers a potential target for antiviral development. *Antiviral Res* 178:104792
27. Theoharides TC, Conti P (2020) Dexamethasone for COVID-19? Not so fast. *J Biol Regul Homeost Agents* 34

28. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 31:455-461
29. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 181:281-292 e286
30. Wan Y, Shang J, Graham R, Baric RS, Li F (2020) Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade- Long Structural Studies of SARS Coronavirus. *J Virol* 94
31. Webb B, Sali A (2016) Comparative Protein Structure Modeling Using MODELLER. *Curr Protoc Bioinformatics* 54:5.6.1-5.6.37
32. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367:1260-1263
33. Xue H, Li J, Xie H, Wang Y (2018) Review of Drug Repositioning Approaches and Resources. *Int J Biol Sci* 14:1232-1244
34. Yoshimoto FK (2020) The Proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2 or n-COV19), the Cause of COVID-19. *Protein J* 39:198-216

Tables

Table1 Selected drugs for the hinge site in the down conformation

Drug	ID_ZINC ^a	Pharmacological action	FDA-Approved Drugs ^b	Clinical studies related to COVID-19 ^c	Affinity (kcal/mol)	Amino acid interactions with spike glycoprotein	
Varenicline	ZINC1481833	agonist for $\alpha 4\beta 2$ receptors	NDA #021928	NF	-7.6	Lys458B, Arg355B,	
						Pro230C, Arg466B,	
						Ile231C, Tyr200C,	
						Trp353B, Asp467B	
						Gly232C, Glu465B,	
						Asp198C, Gly199C	
Docosahexaenoic acid (DHA)	ZINC4474564	Omega-3 essential fatty acid	NF	NCT04417257	-7.2	Arg357A,	Pro230B,
						Val227B,	His207B,
						Asp228B,	Leu229B,
						Phe168B,	Tyr170B,
						Ile128B,	Ile203B,
						Phe194B,	Ile119B,
						Phe192,	Trp104B,
						Asn121B,	Leu179B,
						Val126B	
						Arg355B,	Asp198C,
Sulbactam	ZINC897244	β -lactamase inhibitors	ANDA #065074	NCT04324528	-7	Pro426B,	Leu518B,
						Glu516B,	Phe515B,
						Tyr396B,	Ser514B,
						Arg466B	

a ID_ZINC (<http://zinc.docking.org/>), accessed march, 2020.

b information retrieved from Drugs@FDA (<https://www.accessdata.fda.gov/>), accessed june, 2020. Latest New Drug Application (NDA) and Abbreviated New Drug Application (ANDA)

c information retrieved from <https://clinicaltrials.gov/>, accessed june, 2020

Table 2 Selected drugs for the hinge site in the up conformation

Drug	ID_ZINC ^a	Pharmacological action	FDA-Approved Drugs ^b	Clinical studies related to COVID-19 ^c	Affinity (kcal/mol)	Amino acid interactions with spike glycoprotein ^d
Accolate	ZINC896717	Leukotriene	NDA 020547	NF	-10.3	Arg357A, Phe168B,
		Receptor				Ile128B, Leu176B,
		Antagonists				Ile119B, Asn121B,
						Arg102B, Phe175B,
						Phe192B, Ser172B,
						Gln173B, Pro174B,
						Asp228B, Val227B,
						Tyr170B, Leu226B,
						Pro174B, Val126B,
						Met177B, Pro230B
Tigecycline*	ZINC14879972	Blocking entry	ANDA #091620	NF	-9.7	Arg357A, Arg355A,
		of amino-acyl				Glu516A, Tyr200B, ,
		transfer RNA				Pro426A, Gly232B,
						Pro230B, Gly199B,
						Ile231B, Phe429A,
						Phe515A, Thr430A,
						Phe464A, Asp198B,
						Tyr396A
Betamethasone	ZINC3876136	Secretory	NDA 020934	NF	-9.1	Arg355A, Ser514A,
		leukocyte				Tyr200B, Gly199B,
		protease				Asp198B, Asp428A,
		inhibitor				Glu516A, Phe529A,
						Leu517B, Phe515A,
						Thr430A, Tyr396A,
						Pro426A, Phe464A
Triamcinolone acetoneide	ZINC3882036	Activates the	NDA #012104	NCT04395482	-8.9	Arg355A, Gly199B,
		glucocorticoid				Asp198B, Phe464A,
		receptor				Pro426A, Phe429A,
						Ser514A, Glu516A,
						Asp428, Thr430A,
						Phe515A, Tyr396A
Atazanavir	ZINC3941496	Protease	ANDA #091673	NF	-8.9	Phe562A, Pro225B,
		inhibitors				Phe562A, Pro521A,

	Ile128B, Ile203B,
	Ile119B, Asn121B,
	Arg102B, Trp104B,
	Phe175B, Phe192B,
	Pro174B, Gln173B,
	Ser172B, Tyr170B,
	Lys41B, Val227B,
	Asp228B Leu226B,
	Val126B, Met177B

*only powder for intravenous route administration

a ID_ZINC (<http://zinc.docking.org/>), accessed march 2020.

b information retrieved from Drugs@FDA (<https://www.accessdata.fda.gov>), accessed june, 2020. Latest New Drug Application (NDA) and Abbreviated New Drug Application (ANDA)

c information retrieved from <https://clinicaltrials.gov/>, accessed june, 2020

Table 3 Selected drugs for the RBM in the down conformation

Drug	ID_ZINC ^a	Pharmacological action	FDA-Approved Drugs ^b	Clinical studies related to COVID-19 ^c	Affinity (kcal/mol)	Interactions with spike glycoprotein ^d
Naldemedine	ZINC100378061	Opioid receptor antagonist	NDA 208854	NF	-12.7	Gln493C, Leu455C,Leu492C, Gly476C, Ala475C, Tyr473C, Asp467C, Asp420C, Glu465C, Tyr453C, Phe456C, Arg457C, Arg454C, Tyr421C, Pro491C,

						Lys458C, Lys417C, NAG1303A
Conivaptan	ZINC12503187	Vasopressin receptor antagonist	NDA #022016	NF	-12.1	Leu455C, Gln493C, Asp420C, Pro491C, Arg454C, Asp467C, Lys417C, Tyr421C, Tyr453C, Phe456C, Phe457C, Asn422C, Ile472C, Lys458C
Tipranavir	ZINC100022637	Protease inhibitor	NDA #021814	NF	-10.9	Leu455C, Gln493C, Tyr453C, Asp420C, Try421C, Ser477C, Asn422C, Gly476C; Ala475C, Pro491C, Lys417C, Tyr473C, Arg454C, Phe456C, Arg457C, Asp467C
Vorapaxar	ZINC3925861	Thrombin Receptor Antagonist	NDA 204886	NF	-10.9	Leu455C, Gln493C, Glu406C, Tyr453C, Tyr495C, Arg403C, Ala372A, Phe456C, Lys458C, Ile468C, Gly476C, Ser477C, Thr478C, Glu465C, Pro491C, Tyr421C, Lys417C, Arg454C, Asp467C, Arg457C
Pancuronium	ZINC4097383	Neuromuscular blocking	ANDA #072320	NF	-10.8	Leu455C, Gln493C, Asp420C, Phe456C, Ser477C, Glu465C, Lys458C, Arg457C, Asn370C, Arg454C, Tyr421C, Tyr473C
Saquinavir	ZINC29416466	Protease inhibitor	NDA #021785	NF	-10.7	Asp467C, Pro491C, Phe456C, Asn422C, Phe490C, Gln474C, Tyr473C, Lys458C, Glu465C, Try421C, Ile468C, Lys417C, Leu455C , Ala475C, Gly476C
Suvorexant	ZINC49036447	Orexin receptor antagonist	NDA 204569	NF	-10.6	Leu455C, Gln493C, Asn370C, Arg457C, Phe456C, Asp467C, Pro491C, Ile418C, Tyr421C, Tyr453C, Arg454C, Lys417C
Riociguat	ZINC3819392	Guanylate cyclase stimulator	NDA 204819	NF	-10.6	Leu455C, Gln493C, Glu465C, Ile468C, Asp420C, Tyr473C, Tyr453C, Tyr421C, Arg457C, Asp467C, Arg454C, Phe456C, Lys417C
Diabeta	ZINC537805	Insulin stimulator	NDA 017532	NF	-10.6	Leu455C, Gln493C, Tyr421C, Pro491C, Arg457C, Asp467C, Tyr473C, Glu465C,

						Arg454C, Phe456C, Tyr453C, Ile418C, Ile468C, Lys417C
Candesartan	ZINC4074875	ACEII antagonist	NDA	NCT04351724	-10.6	Leu455C, Gln493C,
			#020838	NCT04330300		Phe456C, Arg457C,
				NCT04394117		Glu465C, Lys458C,
						Asp467C, Ala475C,
						Ile468C, Phe490C,
						Gly476C, Tyr453C,
						Pro491C, Arg454C

a ID_ZINC (<http://zinc.docking.org/>), accessed March 2020.

b information retrieved from Drugs@FDA (<https://www.accessdata.fda.gov/>), accessed june, 2020. Latest New Drug Application (NDA) and Abbreviated New Drug Application (ANDA)

c information retrieved from <https://clinicaltrials.gov/>, accessed june, 2020

d bold letters indicate amino acids crucial for the formation of the spike glycoprotein-ACE2 complex

Table 4 Selected drugs for the RBM in the up conformation

Drug	ID_ZINC ^a	Pharmacological action	FDA-Approved Drugs ^b	Clinical studies related to COVID-19 ^c	Affinity (kcal/mol)	Amino acid interactions with spike glycoprotein ^d
	ZINC43100953	Inhibition of bacterial protein synthesis	NDA #205435	NF	-9.7	Gln493B, Ser494B, Asp405B, Gly416B, Ile402B, Ile418B, Tyr495B, Leu452B, Tyr451B, Tyr453B
Tedizolid Phosphate						,Arg408B, Lys417B, Gln409B, Gln414B, Arg403B
Atovaquone	ZINC116473771	Inhibitor of ubiquinol	ANDA #202960	NCT04339426	-9.7	Leu455B, Ser494B, Ala352B, Asp467B, Phe456B, Asn450B, Pro491B, Thr478B, Gly476B, Ser477B, Tyr351B, Leu492B, Leu452B, Tyr449B, Arg454B, Pro479B
Trimipramine	ZINC3831586	Selective serotonin reuptake	ANDA #077361	NF	-9	Gln493B, Leu492B, Tyr449B,

		inhibitors				Val483B, Pro479B, Gly482B, Asn481B, Phe490B, Tyr489B, Phe490B, Cys488B
Cefotetan	ZINC3830441	Inhibitors of	NDA	NF	-8.8	Leu455B,
		bacterial wall	#050588			Gln493B,
		synthesis				Ser494B,
						Tyr489B,
						Pro491B,
						Gly476B,
						Ser477B,
						Phe490B,
						Leu492B,
						Tyr351B,
						Asp467B,
						Tyr449B,
						Asn450B,
						Arg454B,
						Thr478B,
						Pro479B,
						Leu452B,
						Phe456B,
						Tyr449B
Losartan	ZINC3873160	ACE antagonist	NDA	14 studies	-8.5	Arg355B,
			#020386	found		Glu516B,
						Asp428B,
						Asp427B,
						Phe429B,
						Tyr396B, Lys462B,
						Leu425B,
						Phe464B,
						Leu461B,
						Tyr423B,
						Asp398B,
						Pro426B,
						Pro463B,
						Phe429B

Rosiglitazone	ZINC968330	Agonist of	Discontin	NF	-8.3	Gln493B,
		peroxisome	ed			Ser494B,
		proliferator-				Asp405B, Ile402B,
		activated				Tyr451B,
		receptor-				Tyr453B, Ile418B,
						Gly416B,
						Thr415B,
						Gln414B,
						Arg408B,
						Gln409B,
						Glu406B,
						Tyr495B,
						Arg403B, Lys417B

a ID_ZINC (<http://zinc.docking.org/>), accessed march 2020.

b information retrieved from Drugs@FDA (<https://www.accessdata.fda.gov/>), accessed june, 2020. Latest New Drug Application (NDA) and Abbreviated New Drug Application (ANDA)

c information retrieved from <https://clinicaltrials.gov/>, accession june, 2020

d bold letters indicate amino acids crucial for the formation of the spike glycoprotein-ACE2 complex

Table 5 Selected drugs for the fusion peptide in the down conformation

Drug	ID_ZINC ^a	Pharmacological action	FDA-Approved Drugs ^b	Clinical studies related to COVID-19 ^c	Affinity (kcal/ mol)	Interactions with spike glycoprotein ^d
Saquinavir	ZINC2941 6466	Protease inhibitor	NDA #021785	NF	-11.1	Cys851A, Leu849A,
						Lys835A, Ala852A,
						Leu828A, Gly832A, Ile834A,
						Pro862A, Pro863A,
						Lys854A, Arg646C, Ile850A,
						Glu619C, Ser591C,
						Asp843A, Tyr837A,
						Asp614C, Asn616C,
						Val615C, Gln644C, Gly648C,
						Thr645C, Val860A, Tyr837A,
Edarbi	ZINC1421 0642	ACE2 Blocker	NDA #200796	NF	-10.5	NAG1309C
						Cys851A, Leu849A,
						Leu861A, Asp614C,
						Val860A, Lys835A, Ile834A,
						Thr866A, Ala831A,
						Ala829A, Arg646C,
						Asp867A, Thr866A,
						Gly832A, Ile850A, Pro863A,
						Pro862A, Ala668C,
						Ala852A, Leu828A, Tyr837A
Loratadine	ZINC5379 31	H1-receptors inhibitor	NDA #021375	NF	-10.3	Cys851A, Leu849A,
						Asp614C, Gly832A,
						Pro862A, Pro863A,
						Thr866A, Ile850A, Arg646C,
						Asp867A,

	Ile834A,
	Phe833A, Lys835A,
	Leu828A, Val860A,
	Tyr843A, Ala668A

Perampanel	ZINC3069 1797	Non-competitive AMPA receptor antagonists	NDA #202834	NF	-10	Cys851A, Ile850A, Phe833A, Lys854A, Val860A,	Leu849A, Leu828A, Pro863A, Gly832A, Ala668A, Arg646C, Tyr837A, Lys835A
Emend	ZINC2742 8713	Substance P/neurokinin 1 receptor antagonist	NDA #021549	NF	-10	Cys851A, Ile834A, Ala831A, Thr866A, Asp614A, Cys851A, Tyr837A,	Leu849A, Ala668A, Leu861A, Gly832A, Ala829A, Asp843A, Leu849A, Val860A, Ala852A, Tyr837A, Ile850A, Lys835A, Asp830A, Arg646C
Nebivolol	ZINC4213 946	β_1 -adrenergic receptor antagonists	NDA #021742	NF	-9.8	Cys840B, Ala845B, Gly1059B, Val729B, Pro1057B, Arg815B, Leu828B,	Asp848B, Gly842B, Phe782B, Thr778B, Ser730B, Phe823B, Leu841B, Asp867B
Terconazole *	ZINC3873 936	Inhibiting <i>de novo</i> sterol biosynthesis	ANDA #075953	NF	-9.7	Cys851A, Asp614C, Pro863A, Ser813A, Ala831A, Glu868A, Asp867A, Tyr837A, Lys854A, Lys835C, Ile834A,	Leu849A, Pro862A, Thr866A, Gly836A, Ala852A, Gly832A, Arg646C, Leu849A, Ile850A, Val860A, Ala668C,
Rolapitant	ZINC3816 514	Substance P/neurokinin 1 receptor antagonist	NDA #206500	NF	-9.7	Cys851A, Ala647C, Leu861C, Ala668C, Pro863A, Ile834A, Gly832A, Tyr837A, Val869A, Arg646C, Pro862A, Val860A, Arg646C,	Leu849A, Leu828A, Asp614C, Arg646C, Pro862A, Ile850A, Ala852A,
Difluprednate	ZINC4212 945	Phospholipase A2 inhibitory	NDA #022212	NF	-8.9	Cys840A, Leu849A, Gly832A, Ile834A, Val860A, Asp614C, Ala668C, Thr866A, Ala647C, Phe833A, Arg646C, Ile850A, Leu828A, Tyr837A,	Cys851A, Ala829A, Lys835A, Ala852A,

*Only cream and suppository vaginal presentation

a ID_ZINC (<http://zinc.docking.org/>), accessed march 2020.

b information retrieved from Drugs@FDA (<https://www.accessdata.fda.gov>), accessed june, 2020. Latest New Drug Application (NDA) and Abbreviated New Drug Application (ANDA).

ci nformation retrieved from <https://clinicaltrials.gov/>, accessed june, 2020

d bold letters indicate amino acids crucial for the FP function of the spike glycoptotein

Table 6 Selected drugs for the fusion peptide in the up conformation

Drug	ID_ZINC ^a	Pharmacol ogical action	FDA- Approved Drugs ^b	Clinical studies related to COVID-19 ^c	Affinit y (kcal/ mol)	Amino acid interactions with spike glycoprotein ^d
Saquinavir	ZINC29416	Protease	NDA	NF	-11	Cys851C, Cys840C, Leu849C,
	466	inhibitor	#021785			Asp848C, Asn856C, Asn960C,
						Gln853C, Leu858C, Ala852C,
						Thr859C, Asp614B, Thr732C,
						Phe833C, Val952C, Asn955C,
						Ile834C, Gly832C, Ala831C,
						Gln836C, Tyr837C, Arg847C,
						Lys854C, Val860C, Ala956C,
						Lys835C, Arg646B, Leu959C,
						Ala570B
Maraviroc	ZINC10000	Receptor	NDA	NCT04435	-10.7	Cys851C, Cys840C, Leu849C,
	3902	CCR5	#022128	522		Asp848C, Val952C, Asn955C,
		Antagonist				Leu959C, Thr732C, Leu858C,
						Ala570B, Asn856C, Val860C,
						Arg847C, Tyr837C, Gln836C,
						Arg646B, Lys835C, Ala956C,
						Phe833C, Val963C, Ala852C
Azilsartan	ZINC14210	ACE2	NDA 2007	NF	-10.6	Cys851C, Cys840C, Leu849C,
	642	antagonist	96			Asp848C, Tyr837C, Gln836C,
						Arg646B, Pro862C, Thr732C,
						Val952C, Asn955C, Phe833C,
						Arg847C, Thr859C, Lys854C,
						Leu849C, Val860C, Ala852C,
						Lys835C, Ala956C, Leu959C,
						Ile834C
Tipranavir	ZINC10001	Protease	NDA	NF	-10.5	Cys851C, Cys840C, Leu849C,
	6058	inhibitor	#021814			Asp848C, Thr734C, Ile834C,
						Lys835C, Thr732C, Phe855C,

	Asn856C, Gln853C, Arg646C,
	Gln836C, Tyr837C, Ala956C,
	Leu858C, Thr859C, Val963C,
	Asn960C, Val860C, Asp614C,

						Lys854C, Ala852C, Leu959C
Ritonavir	ZINC39444	Protease	NDA	79 studies	-10.3	Cys851C, Cys840C, Leu849C,
	22	inhibitor	#022417	found		Asp848C, Asn955C, Ile834C,
						Arg646C, Thr827C, Arg847C,
						Gly838C, Thr859C, Leu858C,
						Asp568B, Ile569B, Asn960C,
						Thr732C, Leu959C, Gln836C,
						Asp848C, Thr572B, Asn856C,
						Val952C, Ala956C, Ala852C,
						Ala570B, Val963C, Lys854C,
						Val860C, Lys835C, Tyr837C,
						Phe833C
Isavuconaz	ZINC29571	Inhibit	NDA	NF	-9.7	Cys851C, Cys840C, Leu849C,
onium	072	fungal	#207500			Asp848C, Ala570B, Val963C,
		cytochrom				Leu959C, Val860C, Gly832C,
		e P450				Ala831C, Ile569B, Ser45C,
						Ser46C, Arg847C, Tyr837C,
						Gln836C, Lys835C, Asp57B,
						Asp586B, Gln853C, Ala852C,
						Thr572C, Asn856C, Lys557B,
						Asp568B, Asp568B, Phe833C
						,Ile834C
Bosentan	ZINC15388	endothelin	ANDA	NCT04278	-9.1	Cys851C, Cys840C, Leu849C,
	57	receptor	#205699	404		Asp848C, Thr827C, Ile834C,
		antagonist				Gly832C, Arg847C, Arg646B,
						Val860C, Phe833C, Leu858C,
						Ile569B, Ala852C, Asp830C,
						Ala831C, Leu959C, Phe833C,
						Ala852C, Tyr837C, Gln836C
Fosinopril	ZINC39203	ACE	NDA	NCT04330	-9	Cys851C, Cys840C, Leu849C,
sodium	55	inhibitor	#019915	300		Asp848C, Asn960C, Asn955C,
			Discontin			Thr732C, Ile834C, Tyr837C,
			ued			Lys835C, Asn960C, Val963C,
						Ala852C, Gln853C, Thr859C,

						Val860C, Gln836C, Lys854C,
						Arg646B, Ala956C, Leu959C,
						Leu858C, Phe833C, Arg847C
Ceftazidime	ZINC38719	β -	ANDA	NCT04278	-8.7	Cys851C, Cys840C, Leu849C,
	60	lactamase	#062655	404		Asp848C, Gly832C, Thr827C,
		inhibitors				Ile834C, Gln836C, Lys835C,
						Asn960C, Pro862C, Lys854C,
						Thr859C, Val860C, Arg847C,
						Arg646B, Tyr837C, Phe833C,
						Ala852C, Leu858C, Leu959C,
						Val963C
Cefditoren	ZINC42152	against	NDA	NF	-8.4	Cys851C, Cys840C, Leu849C,
pivoxil	34	MSSA	021222			Asp848C, Leu828C, Asp830C,
						Thr827C, Ala829C, Ile834C,
						Asp848C, Lys835C, Phe833C,
						Gly838C, Val860C, Tyr837C,
						Arg847C, Arg646B, Gln836C,
						Gly832C, Ala853C

a ID_ZINC (<http://zinc.docking.org/>), accessed march 2020.

b information retrieved from Drugs@FDA (<https://www.accessdata.fda.gov/>), accessed june, 2020. Latest New Drug Application (NDA) and Abbreviated New Drug Application

(ANDA).

c information retrieved from <https://clinicaltrials.gov/>, accessed june, 2020

d bold letters indicate amino acids crucial for the FP function of the spike glycoprotein

Figures

Figure 1

Structural spike glycoprotein A) Structural proteins, spike (S), nucleocapsid (N), membrane (M), envelope (E). B) Spike protein sequence; S1 subunit contains: Signal sequence (SS), N-terminal domain (NTD), receptor binding domain (RBD), receptor-binding motif (RBM); S2 subunit contains: S1/S2 cleavage site, fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM). C) Down conformation, homotrimer hidden the RBD (receptor-inaccessible). D) Up conformation, homotrimer is asymmetric, two RBD domains are hidden (receptor-inaccessible) one RBD domain is exposed (receptor-accessible). The sites of interest for this work are underlined and in bold. (figure created with BioRender.com).

Figure 2

Spike glycoprotein trimer top (left) and side (right) views A) the trimer is observed in the down conformation (6VXX-fill). B) The trimer is shown in the up conformation (6VYB-fill). The region of the ectodomain was modeled, the regions are observed: RBD (pink), NTD (blue), HR1 (yellow), FP (red), hinge 1(cyan) and hinge 2 (lime). Figure is shown in black and white colors for a better view of the chains. Chain A is colored in black, chain B is colored in dark grey and chain C is colored in light grey.

Figure 3

Molecular interactions of the drugs in the hinge 1 site with spike glycoprotein in the down conformation A) Interactions of the Varenicline-spike glycoprotein complex are observed. B) Binding mode of the Docosahexaenoic acid-spike glycoprotein complex. C) Molecular interactions with the drug Sulbactam and spike glycoprotein. It is observed that the best compounds directed to the hinge site bind preferentially to hinge site 1, because a cavity is formed composed of hinge site 1, RBM, and NTD, which the drugs studied bind with greater affinity to this cavity.

Figure 4

Molecular interactions of the drugs with the hinge 1 site spike glycoprotein in the up conformation A) Interactions of the Alcolate-spike glycoprotein complex are observed. B) Molecular interactions with the drug Tigecycline and in the spike glycoprotein.

Figure 5

Molecular interactions of the drugs with RBM in the spike glycoprotein in down conformation A) Interactions of the Naldemedine with spike glycoprotein complex are observed. B) Molecular interactions with Conivaptan and spike glycoprotein chain C.

Figure 6

Molecular interactions of the drugs with RBM in spike glycoprotein in the up conformation A) Interactions of the Tedizolid Phosphate and spike glycoprotein complex are observed. B) Interactions of Atovaquone-spike glycoprotein complex are shown. C) It is observed how Cefotetan interacts with spike glycoprotein.

A)

B)

Interactions

— Van der Waals	— Carbon Hydrogen Bond	— Alkyl	— Pi-Cation
— Conventional Hydrogen Bond	— Halogen (Fluorine)	— Pi-Alkyl	— Pi-Sigma

Figure 7

Molecular interactions of drugs with spike glycoprotein in FP (down conformation) A) Interactions of Saquinavir-spike glycoprotein complex are observed. B) Molecular interactions with the drug Difluprednate and the chain A of spike glycoprotein.

Figure 8

Molecular interactions of the drugs with spike glycoprotein in the FP (up conformation) A) Interactions of the Saquinavir-spike glycoprotein complex are observed. B) Molecular interactions with the drug Maraviroc with of spike glycoprotein.