RESEARCH ARTICLE

Identification of potential inhibitors of SARS-CoV-2 S protein-ACE2 interaction by *in silico* drug repurposing [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new coronavirus discovered that appeared in Wuhan, China, in December 2019, causes COVID-19 disease which have resulted in cases similar to SARS-atypical pneumonia. As of March 1, 2021, Mexico had reached 2.11 million cases of COVID-19 and 189 thousand deaths; around 116 million cases and 2.57 million deaths are reported worldwide with new cases and increasing mortality every day. To date, there is no specific commercial treatment to control the infection. Repurpose drugs targeting the angiotensin-converting enzyme 2 (ACE2) receptor represents an alternative strategy to block the binding of SARS-CoV-2 protein S and forestall virus adhesion, internalization and replication in the host cell.

Methods: Rigid molecular docking was performed using receptor binding domain of the S1 subunit of S protein (RBD_{S1})-ACE2 (PDB ID: 6VW1) interaction site and 1,283 drugs FDA approved and prescribed by the Mexican Public Health System. The results were analyzed by docking score, frequency of the drug in receptor site and the types of interactions at the binding site residues.

Results: About 40 drugs were identified as a potential inhibitor of RBD_{S1}-ACE2 interaction. Within the top-ranked drugs, we identified ipratropium, formoterol and fexofenadine, which stands out as they are used as therapies to treat chronic obstructive pulmonary disease, asthma and virtually any respiratory infection.

Conclusions: Our results will serve as the basis for *in vitro* and *in vivo*

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studies to evaluate the potential use of those drugs to generate affordable and convenient therapies to treat COVID-19.

Keywords

COVID-19, SARS-CoV-2, ACE2, Molecular Docking, Drug Repurposing

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Introduction

Emerging viruses can be defined as those whose incidence has increased in the last twenty years or whose presence has a high probability of increasing in the near future. Diseases caused by emerging viruses are one of the biggest public health threats globally. Some of the viruses that fall within this catalog are the avian influenza virus subtype H5N1, severe acute respiratory syndrome (SARS), Ebola, Zika, and MERS-CoV, to name a few¹. Coronaviruses (CoVs) are classified into four genera, α -CoV, β -CoV, γ -CoV, and δ -CoV2. α and β infect mammals, γ birds and δ birds and mammals, respectively². These viruses are of public health importance because they cause enteric, renal, and neurological respiratory diseases that range from asymptomatic to fatal^{3,4}.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appeared in Wuhan, China, in December 2019, causing cases of SARS-like atypical pneumonia^{5,6}, with a clinical picture of fever, general malaise, dry cough, shortness of breath and was called the coronavirus disease 2019 (COVID-19)⁷. It can be asymptomatic, develop mild-to-severe symptoms, or cause death in elderly individuals or in patients with chronic diseases, such as hypertension, diabetes, and obesity⁸. On January 31st 2020, the World Health Organization (WHO) declared COVID-19 a public health emergency of international concern and, on March 12th, it was declared a global pandemic⁹. In Mexico, local transmission (phase 2 of transmission) was declared on March 24th 2020, which resulted in the suspension of non-essential activities in the country, generating economic losses in addition to public health problems and deaths associated with the disease. As of March 1st 2021, Mexico had reached 2.1 million cases of COVID-19 and 189 thousand deaths; around 63.6 million cases and 1.47 million deaths have been reported worldwide.

To date, there is no specific commercial treatment to control the infection^{10–12}. Measures such as early detection, blocking the route of transmission through social isolation, isolation of suspected cases, disinfection of objects, as well as frequent hand washing with soap, in addition to the use of biosafety equipment such as surgical masks for health personnel, which has been effective in reducing the transmission of COVID-19.

Coronaviruses, such as SARS-CoV-2, are positive-stranded RNA viruses enveloped on a membrane. The coronaviral genome is composed of approximately 30,000 nucleotides containing the envelope (E), membrane (M), spike (S), nucleocapsid (N) and ORFs, that encode non-structural proteins, including enzymes that appear during their in-host reproductive cycle-genes¹³.

This virus measures 70 to 100 nm and belongs to the genus β -CoV¹⁴ and it has been proposed that any of the aforementioned proteins that make up CoVs may be targets for the development of vaccines or drugs⁴. Protein S plays an essential role on COVID-19 infection as it mediates the internalization on host cell and for the spread of the virus in the infected host^{15,16}. This starts when the receptor binding domain of the S1 subunit (RBD_{S1}) of S protein binds to the peptidase domain

of angiotensin-converting enzyme 2 receptor (ACE2)¹⁷ and it is known that disrupting the binding of S protein to ACE2 prevents the attaching and the later internalization of the virus to the host cell¹⁸.

This protein interaction has recently been crystallized and deposited in the Protein Data Bank database¹⁹, allowing us to use it as a model of study to test different strategies to counter SARS-CoV-2 infection, like blocking S glycoprotein-ACE2 interaction through the discovery of sites of potential pharmaceutical interest.

In 2019, Research and Development (R&D) spending in the pharmaceutical industry totaled 186 billion U.S. dollars globally and its projected to reach 233 billion U.S. dollars to 2026. Unfortunately, drug development takes large time and financial resources that not all countries possess, especially developing countries, like Mexico.

In this sense, drug repurposing or repositioning allow us to integrate all evidence, pharmacodynamics/kinetics, bioavailability, among other important parameters, from an existing and approved drug in order to manage emerging diseases, like COVID-19. All this translates into a considerable decrease in research time and investment of resources in R&D.

Different approaches have been taken in order to disrupt SARS-CoV-2 protein S-ACE2 interaction, as an example, many works have focus on finding potential binding sites on protein S structure, however, new variant strains have been detected worldwide, like B117 in UK, P1351 in South Africa, P1 and P2 in Brazil. All variant strains display the N501Y mutation, which is located on the RBD of the S protein, making the interaction more effective. In this sense, targeting RBD may be a transitory approach, therefore, an alternative strategy would be aiming at the ACE2 receptor. Some authors have pointed out some concerns about using drugs that targeting the renin-angiotensin signaling (RAS) pathway^{20–22}, but Jia and collaborators²³ highlight current efforts of exploiting ACE2 as therapeutic target, like the use of pseudo-ligands to dominate the binding site for SARS-CoV-2 as an example. Therefore, inhibition of the SARS-CoV-2 protein S-ACE2 interaction through aiming ACE2 receptor it is a plausible strategy. In this study, we screened a library consisting of 1300 FDA-approved drugs and acquired by Ministry of Health of Mexico in order to identify potential inhibitors of SARS-CoV-2-ACE2 interaction.

Methods

Molecule selection for docking

In order to run the docking simulation, we selected the X-ray crystal structure of SARS-CoV-2 RBD_{S1} in a complex with the ACE2 (PDB ID: 6VW1) which makes it have a more compact conformation in comparison with the SARS-CoV-2 RBD alone¹⁹. Chains B, E, F, and oligosaccharides and crystallographic water molecules were removed from the system; hydrogen atoms were added, and the receptor structure was protonated at pH 7.0 and 300 K using the Protonate 3D tool. Partial charges were assigned using the Amber10:EHT force-field. Later, the protein structure was subjected to energy

minimization using the same forcefield. Site Finder MOE was employed to analyze potential binding sites near to the receptor binding ridge on ACE2 and docking simulation were carried out. The docking parameters were set to take each ligand conformation as unique molecule, using the Alpha Triangle algorithm as placement method (at least 100 different orientations or poses on potential binding site) and further evaluation keeping the thirty best poses accordingly the London scoring function for binding affinity with a second refinement as a Rigid Receptor using Affinity dG algorithm keeping the ten best poses. The results were analyzed by docking score, frequency of the chemical compound as a stable conformation and the types of interactions at the binding site residues.

Database preparation

Data repositories such DrugBank, ZINC and PubChem were used to download the dataset from an updated list of reference medicine and The National Compendium of Health Supplies of Mexico (June 2020 update). The structures (sdf) dataset comprises 1,283 unique drugs, which the later were converted into Molecular Database format (mdb) using the Conformation Import MOE, feature available in MOE 2020.09 software (Molecular Operating Environment, Chemical Computing Group, Montreal, Canada) in MOE with an imposed limit of 3 kcal/mol strain energy and a maximum of 250 conformations per molecule and hydrogen atoms were added. Minimum energy configuration was calculated using the Amber10:EHT forcefield.

Results

Structural analysis of SARS-CoV-2 – ACE2 interaction

The structural analysis for the SARS-CoV-2 RBD_{S1} of the spike protein in a complex with the ACE2 (PDB ID: 6VW1; Figure 1A) revealing a potential site for ligand binding inside ACE2 structure (Table 1). The identified receptor site (Figure 1B) is proximal to the binding site of RBD_{S1} with a size of 86, therefore it can be used for simulating rigid molecular docking since receptor atoms are in an exposed region of the structure, which could be in favor of drug binding.

Virtual screening and molecular docking

An average of 78 conformations were generated for each ligand by Conformation Import MOE, generating 100,450 ligand conformations of the FDA approved and prescript drugs by the Mexican Public Health System.

The docking results were sorted and analyzed based on their S score, binding frequency which the drug binds to the receptor site and type of interactions, preferably, hydrogen bond, of the ligand with the selected site.

We selected 38 drugs (Table 2) that presents the best docking score between –10.04 and –4.04. Subsequently, we short-listed nine drugs based on their risk of teratogenicity, route of administration, interaction with other drugs, side effects and by their background as pharmacological therapy for the treatment of respiratory diseases.

Figure 1. SARS-CoV-2 RBD_{S1} interaction with human ACE2 receptor. A) Crystallographic structure (PDB ID: 6VW1) of RBD_{S1} (red) and ACE2 receptor (blue); **B)** Molecular surface of the selected binding site in ACE2.

Table 1. General characteristics of RBD_{S1}-ACE2 receptor site.

Size.	PLB	Hyd.	Side	Residues
86	0.84	20	55	Gln81 Tyr83 Pro84 Leu85 Gln86 Leu95 Gln98 Ala99 Gln101 Gln102 Asn103 Ala193 Asn194 His195 Tyr196 Gly205 Asp206 Tyr207 Glu208 Asn210 Arg219 Lys562

Table 2. List of potential inhibitors of the RBD_{s1}-ACE2 interaction.

Drug name	S-Score	Interaction type (number)		
		Hydrogen bond	Ionic bond	Pi-bond
Uridine, trisodium salt	-9.53	10	3	0
Methotrexate sodium	-10.04	8	2	1
Raltritedex	-8.93	8	2	0
Folotylin	-8.19	8	2	0
CDP-choline(1-)	-8.10	8	0	0
Cefuroxime	-8.06	7	2	0
Fexofenadine	-7.89	7	0	0
Fludarabine phosphate	-7.99	6	0	1
Cefixime	-9.02	5	3	0
Aloin	-8.16	5	0	0
Domperidone	-6.63	5	1	0
Tamsulosin	-6.62	5	2	0
Cromoglycic acid	-8.63	4	0	1
Macitentan	-8.06	4	0	2
Tafluprost -Taflutan	-7.94	4	0	1
Thiopental(1-)	-5.42	4	0	0
Metoprolol	-5.10	4	0	0
Irinotecan	-8.72	3	0	1
Pitavastatin(1-)	-8.40	3	0	1
Amlodipine	-5.94	3	0	0
Verapamil	-5.85	3	1	0
Tolterodine	-4.77	3	0	0
Lopinavir	-8.62	2	0	0
Glimepiride	-8.47	2	1	0
Arformoterol	-6.85	2	0	0
Formoterol	-6.15	2	2	1
Ipratropium	-5.38	2	0	1
Pargaverine	-4.53	2	2	0
Pyrimilamine	-4.26	2	1	0
Biperiden	-4.23	2	0	1
Orlistat	-8.16	1	0	0
Glyburide	-8.10	1	0	0
Ribociclib	-7.96	1	0	1
Ibesartan	-7.17	1	0	3
Cholecalciferol	-5.81	1	0	0
Testosterone enanthate and estradiol valerate	-5.39	1	0	0
Disopyramide phosphate	-4.22	1	0	2
Primaquine	-4.04	1	1	2

Fexofenadine showed interactions of hydrogen bond with Lys 74, Ala 99, Ser 105, Ser 106, Trp 203 and Asp 509

(Figure 2A) with a docking score of -7.89 . Pitavastatin displays hydrogen bond interaction with Gln 102, Tyr 196, Asp

Figure 2. Two-dimensional representation of the interactions of the selected drugs with the ACE2 receptor binding site. The blue arrows indicate the structural hydrogen bridge bonds and the green arrows are the hydrogen bridge bonds with the side chain. **A)** Fexofenadine, **B)** pitavastatine, **C)** aformoterol, **D)** formoterol, **E)** ipratropium, **F)** pargeverine, **G)** cholecalciferol, **H)** lopinavir and **I)** cefixime.

206 and pi-H stacking with Leu 73 (Figure 2B) and a docking score of -8.40. Arformoterol showed hydrogen bond interactions with Tyr 202 and Asp 206 (Figure 2C) with a docking score of -6.85. Formoterol presented hydrogen bond interactions with Gln 98, Asn 194, ionic interaction with Glu 208 and pi-H interaction with Leu 85 (Figure 2D) and presents a docking score of -6.15. Ipratropium exhibited hydrogen bond interaction with Gln 98, Gln 208 and pi-H stacking with Asp 206 (Figure 2E) and a docking score of -5.38. Pargerverine shows hydrogen bond interactions with Gln 98, Gly 205, Glu 208 and ionic interaction with Glu 208 (Figure 2F) and presents a docking score of -4.53. Cholecalciferol presented hydrogen bond interaction with Gln 102 (Figure 2G) and had a docking score of -5.81. Lopinavir displays hydrogen bond interaction with Gln 102, Tyr 196 (Figure 2H) and a docking score of -8.62. Cefixime showed hydrogen bond interaction with Gln 98, Tyr 202, Glu 208, Arg 219, Lys 562 and ionic interaction with Arg 219, Lys 562 (Figure 2I) and a docking score of -9.02.

Some pharmacokinetics characteristics of the shortlisted potential inhibitors of the RBD_{S1}-ACE2 interaction are summarized in Table 3 and schematic representations of the interaction maps of these drugs with the selected binding site are shown in Figure 2.

Discussion

It has been established that S protein of SARS-CoV-2 virus plays a major role during viral infection. The S protein

mediates receptor recognition, cell attachment and fusion of viral membrane with host cell membrane²⁴⁻²⁹. The S protein binds to ACE2 receptor through the RBD_{S1}, mediating viral attachment to host cell³⁰. ACE2 expression is distributed mainly in lung, intestine, heart and kidney, also alveolar epithelial type II cells had higher expression levels²¹. The RBD region is a critical therapeutic target (vaccines and drugs) due to its indispensable function; however, it is suggested that mutations in this region may render pharmacological or immunological therapies ineffective^{31,32}.

In order to block this event, we propose an *in silico* approach to identify potential inhibitors of the SARS-CoV-2-ACE2 interaction aiming at the ACE2 receptor, blocking the virus accessibility to the membrane-bound ACE2. Here, we screened a drug library consisting of 1300 drugs, FDA approved and prescribed by the Mexican Public Health System, for potential SARS-CoV-2-ACE2 inhibitors, using a rigid receptor docking approach. Utilization of an FDA-approved drug library is an effective and ideal tool for drug repurposing in antiviral research^{33,34}, such as zika virus³⁵, human rhinovirus³⁶ and hepatitis B virus³⁷. We identify 38 potentially inhibitor drugs of SARS-CoV-2-ACE2 interaction and these are listed on Table 2. Several of those drugs were previously reported to be used for the treatment of respiratory diseases.

Within this list of potential inhibitors of the SARS-CoV-2-ACE2 interaction, is fexofenadine, a third-generation antihistamine

Table 3. Potential inhibitors of RBD_{S1}-ACE2 interaction selected according to desired characteristics.

Drug	Pharmacokinetics					Route of administration	Drug Type/ Teratogenic risk
	Bioavailability (%)	Protein binding (%)	Metabolism	Half-life (hours)	Excretion		
Fexofenadine	30-41	60-70	Hepatic	14.4	Urine and feces	Oral	Antihistaminic (third generation) / B
Cefixime	30-50	60	Hepatic	3 a 4	Urine and bile	Oral	Antibiotic (Cephalosporin; third generation) / B
Pitavastatine	60	96	Hepatic	11	Feces	Oral	Statin / X
Lopinavir	25	98-99	Hepatic	2-3	Urine and feces	Oral	Antiviral -Protease inhibitor / C
Arformoterol	21-37	61-64	Hepatic	26	Urine	Inhaled	Long lasting β agonist / C
Formoterol	61	50	Hepatic	17	Urine	Inhaled	Long lasting β agonist / C
Ipratropium	2	0-9	Gastrointestinal	1.6	Urine	Inhaled	Anticholinergic bronchodilators / B
Pargerverine	80	90	Hepatic	1.5-2	Urine	Oral, intravenous, intramuscular, rectal	Opium alkaloid antispasmodic / D
Cholecalciferol	NA	NA	Hepatic	NA	Urine	Oral, intramuscular	Vitamin

whose therapeutic indication is the treatment of symptoms of stationary allergies through the selective blockade of H1 receptors³⁸. *In silico* evidence^{39,40} suggest that it may interact with the SARS-CoV-2 main protease enzyme M^{Pro}, a key enzyme in viral replication⁴¹, acting as a potential inhibitor. Cefixime is a third-generation antibiotic derived from cephalosporin whose use is indicated for the treatment of infections in the upper and lower respiratory tract, otorhinolaryngological⁴² and urinary tract⁴³, inhibiting the synthesis of the bacterial wall by binding to specific binding proteins for penicillin and is currently used as a secondary therapy to prevent opportunistic infections during the development of COVID-19⁴⁴. Pitavastatin is a statin indicated for lowering blood cholesterol levels by inhibiting HMG-CoA reductase, preventing cholesterol synthesis⁴⁵, and it has also been observed that statin treatments can interfere with viral infectivity through inhibition of glycoprotein processing⁴⁶ also, they modulates the inflammatory process at cellular level⁴⁷, which is a remarkable characteristic of the SARS-CoV-2 infection. Additionally, *in silico* findings suggest that could be an efficient inhibitor of SARS-CoV-2 M^{Pro}⁴⁸ and SARS-CoV-2 RNA-dependent RNA polymerase (RdRp)⁴⁹ thru active site binding. Lopinavir is a protease inhibitor indicated as first barrier therapy, in conjunction with Ritonavir, to treat infection caused by the HIV virus by inhibiting the HIV-1 protease⁵⁰ in addition, studies in cell cultures have shown its effectiveness as an inhibitor of the replication of the MERS-CoV virus⁵¹ and SARS-CoV-1⁵², while in severe cases of SARS-CoV-2 infection, the results of clinical trials indicate that it is not useful⁵³. Formoterol and aformoterol, an enantiomer of formoterol, are long-lasting selective β agonists indicated for the treatment of chronic obstructive pulmonary disease (COPD) and bronchospasms⁵⁴, in the same way there is evidence of the use of these drugs as a partial inhibitor of viral replication in primary epithelial cells cultures⁵⁵ and *in silico* data suggest their binding to the papain-like protease PL^{Pro}, a coronavirus enzyme essential for viral spread⁵⁶. Ipratropium is a bronchodilator anticholinergic indicated for the treatment of asthma, shortness of breath, cough and tightness in the chest in patients with COPD^{57,58}. Inhalation therapy with ipratropium is currently in use to dilate bronchioles in COVID-19 patients to increase oxygen saturation levels (from <80% to 94%)⁵⁹. Pargerverine is an antispasmodic opioid alkaloid whose therapeutic indication is aimed at the treatment of painful spasms⁶⁰, also, acts as anticholinergic and has a moderate and non-selective blockade of muscarinic cholinergic fibers⁶¹. Since cholinergic activity contribute to airway narrowing, this might be a potential agent to open airway obstruction. Cholecalciferol, is a form of vitamin D (vitamin D3) that can be synthesized naturally in the skin and acts as a hormonal precursor, being converted into calcitriol, and therapeutically is used as a vitamin supplement to treat deficiencies of this vitamin⁶². In addition, it has been observed that vitamin D supplementation is favorable to reduce viral infections such as influenza^{63,64} or more aggressive cases such as HIV⁶⁵ and it has recently been suggested that it also presents favorable effects before and during the infection caused by SARS-CoV-2⁶⁶.

Likewise, it is important to take into account that ACE2 plays an important biological role since regulates cardiovascular

functions and innate immune system and, therefore cautions must be taken. Another point to take into account is the delivery method of the drug, since the primary target must be smooth muscle, like the one surrounding the bronchioles, and lung epithelial cells in the airway and airspace compartments, hence, inhalable delivery would be the acceptable choice to deliver the drug in a selectively and localized manner.

Given these characteristics, the results obtained through our *in silico* approach, we consider that the aforementioned drugs are outlined as possible inhibitors of the RBD_{S1}-ACE2 interaction. These drugs are well tolerated, commonly used and affordable, hence, most of the drugs on this list can be tested *in vitro*, and even *in vivo* and, consequently, in clinical trials for the development of adjuvant therapies to treat COVID-19.

Conclusion

In the absence of approved therapies for treatment or prevention, drug repurposing has provided fast and valuable insight into the treatment of COVID-19. Targeting ACE2 receptor as a COVID-19 therapy it is a conceivable approach since it is essential for the viral internalization. However, this approach requires an integrative evaluation of the pros and cons by a clinical context since ACE2 is a multifunctional protein. Jia and collaborators²³ present an extensive review for this underexplored approach to treat COVID-19, pointing that it is imperative to determine, by clinicians, the stage of the disease and comorbidities that could prove consequential for an ACE2-targeting regimen. Several drugs are currently investigated by clinical trials or are already in use to treat COVID-19 patients, like lopinavir or ipratropium. In this *in silico* study using structure-bases virtual screening, we identified potential inhibitors of SARS-CoV-2-ACE2 by their interaction with ACE2 receptor. We identify the uridine trisodium salt, methotrexate sodium, raltritedex, folotyn, CDP-choline(1-), cefuroxime, fexofenadine, fludarabine phosphate, cefixime, aloin, domperidone, tamsulosin, cromoglycic acid, macitentan, tafluprost -taflutan, thiopental(1-), metoprolol, irinotecan, pitavastatin(1-), amlodipine, verapamil, tolterodine, lopinavir, glimepiride, arformoterol, formoterol, ipratropium, pargerverine, pyrilamine, biperiden, orlistat, glyburide, ribociclib, ibesartan, cholecalciferol, gravignost, disopyramide phosphate and primaquine. Based on desired characteristics like pharmacokinetics, route of administration or by their background as pharmacological therapy, we propose a shortlist of drugs suitable for testing their potential RBD_{S1}-ACE2 inhibitory activity: fexofenadine, cefixime, pitavastatine, lopinavir, arformoterol, formoterol, ipratropium, pargerverine and cholecalciferol. Our identification of potential inhibitors of the SARS-CoV-2-ACE2 interaction among commonly use drugs highlights their potential use for treating COVID-19. Further *in vitro*, *in vivo* or clinical trial are needed to validate their potential use as inhibitors of SARS-CoV-2-ACE2 interaction.

Data availability

Source data

Protein Data Bank: Crystal structure of SARS-CoV-2 receptor binding domain (RBD_{S1}) of the spike protein in a complex with the ACE2 receptor. <https://identifiers.org/pdb:6vww1>

Ministry of Health of Mexico: Drug list used by the Ministry of Health of Mexico (June 2020 version). <http://www.csg.gob.mx/Compendio/CNIS/cnis.html>.

PubChem: Ligands. <https://pubchem.ncbi.nlm.nih.gov>.

Drugbank: Ligands. <https://go.drugbank.com>.

ZINC database: Ligands. <http://zinc.docking.org/>.

Extended data

Figshare: Identification of potential inhibitors of SARS-CoV-2 S protein-ACE2 interaction by in silico drug repurposing, <https://doi.org/10.6084/m9.figshare.14466333>⁶⁷

This project contains the underlying data file:

- Table_E1_DrugsAccessionNumber.xlsx (Accession numbers of drugs used for docking simulations)

Data is available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0)

Author contributions

FET-F and DC-A performed most of the bioinformatics work, shared first authorship, equal contribution to this work; FET-F wrote the initial manuscript draft; JAC-M and RP-A generated the ligand data base and assisted bioinformatic analysis; GA-G revising critically the initial manuscript draft and provision facility resources; P.G.-G, DC-A and GAS-M contributed intellectually to the project conceptualization and participate in the initial manuscript draft; DC-A and G.A.S.-M designed and supervised the project. G.A.S.-M conceptualize, acquire the financial support for the project leading to this publication, supervised the bioinformatic work and wrote the final version of the manuscript; all authors read and approved the final manuscript version.

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Krishnaprasad Baby

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I am pleased to write the review for this manuscript. In this manuscript, authors have used computational tools to predict the activity of a suitable drug to treat/ prevent COVID-19. The specific target can prevent the entry of SARS-CoV-2 in human body. Thus keeping the receptor for virus entry ACE2, the available drugs are screened for their binding efficacy, which indirectly correlates the biological activity. Though the computational predictions are widely accepted, this manuscript has few limitations in its current form. Hence, I would suggest minor changes or amendments might be necessary before acceptance. Followings are a few of them:

Abstract:

- As the pandemic is dynamically changing the numbers, giving a country specific might not hold good. I suggest to add the data related to the world-wide data can be put. An important observation is the number of drugs from US-FDA can be written the same number as it was mentioned later in the manuscript (1,283 may not be correct).

Introduction:

- I feel many places few more additional support from the literature is required. Which can be strengthened by citing references.

Methods:

- Molecule selection for docking appears incorrect. It is protein selection for ligand docking. Moreover, authors have optimised the protein structure, which should have been more appropriate. The resolution of 6VW1 is missing. The rationale for taking 6VW1 appears missing in methodology. Database preparation appears incorrect. It can be dataset/ or ligand preparation. When DrugBank/ZINC15 was accessed is missing (month of accession). Which version of ZINC library was used is missing. Most importantly, the reason why ligands conformation was

generated is missing.

Results:

- Table 3, the details regarding generation of data appears missing. I feel the detailed legends can be given.
- The current gold standard for molecular activity prediction is MD simulation, this can be tried by authors.

Discussion:

- In the methodology the number of drugs selected were 1283, but here it changed to 1300. This looks inconsistent.
- In the methodology, authors have mentioned that they have generated some conformers. Which conformation of drugs were active?
- Overall, I feel the discussion part appears very weak and lacks substantial evidence for how these drugs could be used in COVID-19.

Conclusions:

- The conclusion can be made simple by reducing the number of sentences. Most of the journals will not accept citations (references) in conclusions. Hence, the authors can give a thought to modify the conclusion by keeping their views only on objectives/ aims.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacy and Pharmacology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 29 Oct 2021

Guillermo A Silva-Martinez

We thank the reviewer for the detailed revision of our paper and for suggesting improvements.

Abstract:

- *As the pandemic is dynamically changing the numbers, giving a country specific might not hold good. I suggest to add the data related to the world-wide data can be put.*
- *An important observation is the number of drugs from US-FDA can be written the same number as it was mentioned later in the manuscript (1,283 may not be correct).*

Response: We thank the reviewer for this suggestion to improve the impact of our abstract. US-FDA approved drug number was corrected.

Introduction:

- *I feel many places few more additional support from the literature is required. Which can be strengthened by citing references.*

Response: We agree with the reviewer's observation, however, we believe that the introduction provides the necessary information to be in context to the overall objective of our work.

Methods:

- *Molecule selection for docking appears incorrect. It is protein selection for ligand docking. Moreover, authors have optimized the protein structure, which should have been more appropriate. The resolution of 6VW1 is missing. The rationale for taking 6VW1 appears missing in methodology.*
Database preparation appears incorrect. It can be dataset/ or ligand preparation. When DrugBank/ZINC15 was accessed is missing (month of accession). Which version of ZINC library was used is missing. Most importantly, the reason why ligands conformation was generated is missing.

Response: We thank the reviewer for the comments and for suggesting improvements. Changes on the subsections mentioned above were made. Resolution of 6VW1, the rationale of using that structure and accession date to databases were added to the manuscript. In the "Protein selection for ligand docking", it is now clearly specified that rigid molecular docking is carrying out. In this type of docking, protein orientation is fixed and only ligands are allowed to vary their orientation, therefore each conformer allow to simulate this variation. We assumed that no further explanation was needed.

Results:

- *Table 3, the details regarding generation of data appears missing. I feel the detailed legends can be given.*

Response: We shortlisted nine drugs based on their risk of teratogenicity, route of administration, interaction with other drugs, side effects and by their background as pharmacological therapy for the treatment of respiratory

diseases. It is described in the Results section, subsection Virtual Screening and Molecular docking, paragraph 3.

- *The current gold standard for molecular activity prediction is MD simulation, this can be tried by authors.*

Response: We agree with the reviewer comment. Unfortunately, we do not have the computational infrastructure or access to supercomputer cluster to run MD. We are trying to run MD on our workstations, but those simulations will take time. However, we feel that our work is a valid approximation for COVID19 drug repurposing.

Discussion:

- *In the methodology the number of drugs selected where 1283, but here it changed to 1300. this looks in consistency.*

Response: We thank reviewer comment. See response above.

- *In the methodology, authors have mentioned that they have generated some conformers. Which conformation of drugs were active?*

Response: In order to generate active conformers for docking simulation, we impose a 3 kcal/mol limit on strain energy, which means that conformation elucidates under these criteria can occur under physiological conditions and maintain their activity. Methodology section was restructured to be more clear.

- *Overall, I feel the discussion part appears very weak and lacks substantial evidence for how these drugs could be used in COVID-19.*

Response: We thank the reviewer for this comment. We feel that most of the relevant evidence -to date of manuscript submission- regarding COVID19 and the potential inhibitors, were discussed.

Conclusions:

- *The conclusion can be made simple by reducing the number of sentences. Most of the journals will not accept citations (references) in conclusions. Hence, the authors can give a thought to modify the conclusion by keeping their views only on objectives/ aims.*

Response: We thank the reviewer for this observation. This section was restructured.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 June 2021

<https://doi.org/10.5256/f1000research.55408.r85900>

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Fernando Yepes-Calderon

GYM Group SA, Cali, Colombia

Authors use molecular docking tools to test existing medicine in their efficacy to impede the S protein-ACE2 binding and thus, disabling the entry point of Cov2 to human cells.

The authors use a solid justification to support this project. Since medicine creation is an expensive and long-lasting task, verifying if some active molecules in already FDA-approved medicine might block the binding between the S-protein and the ACE2 is an excellent alternate path.

The document is generally well organized and pleasant to read. I suggest adding some corrections to the abstract as follows.

Background

A new coronavirus outbreak, firstly reported in Wuhan, China, in December 2019, causes COVID-19, which symptoms are similar to SARS-atypical pneumonia. Worldwide, around 116 million cases and 2.57 million deaths are reported, with new cases increasing mortality every day. To date, there is no specific commercial treatment to control the infection.

Repurpose drugs targeting the angiotensin-converting enzyme 2 (ACE2) receptor represent an alternative strategy to block the binding of CoV2's protein S and forestall virus adhesion, internalization, and replication in the host cell.

Methods

We performed rigid molecular docking using the receptor-binding domain at the S1 subunit of the S protein (RBDS1)-ACE2 (PDB ID:6VW1) interaction site and 1,283 FDA-approved drugs. The docking score, frequency of the drug in the receptor site, and interactions at the binding site residues were used as analyzing criteria.

Results

This research yielded 40 drugs labeled as potential inhibitors of RBD S1-ACE2 interaction. Among the inhibitors, compounds such as ipratropium, formoterol, and fexofenadine can be found. Specialists employ these drugs to treat chronic obstructive pulmonary diseases, asthma, and almost any respiratory infection.

Conclusions: Our results will serve as the basis for *in vitro* and *in vivo* studies to evaluate the potential use of those drugs to generate affordable and convenient therapies to treat COVID-19.

The proposed modifications focus on keeping the communications global. The CoV2 numbers in Mexico are absent in this suggested abstract since the authors provide worldwide numbers. I also shortened some sentences and fixed some wrongly used singulars.

1. Correct the hyphenation in the whole document. If the authors are using a latex template, use the command `\hyphenation{co-rrect, se-pa-ra-tion, sche-me}`.

Events: Janu-ary and associ-ated (intro, paragraph 2), lig-and (methods, paragraph 2).

2. Introduction, page 3, paragraph 2. Current events suggest that Covid19 is not only lethal in the elder or patients with chronic diseases. I might suggest deleting this statement.
3. Introduction, page 3, paragraph 2. something is missing in this paragraph. Also, the statement may be misleading; the actual spread and high contamination levels are proof of non-efficiency. If isolation and the other measurements were effective, what would be the sense for the proposal?
4. In subsection "Database preparation," you have information already provided in the first subsection of the methods.
5. Discussion, page 7, paragraph 1. In "ACE2 expression it is" the authors might have two nouns in the same sentence.

Regarding the methodology, the authors used a standard: importing the macromolecule, edit or delete water molecules, add hydrogens, add charges, and the ligand. Did the authors have any particular issue during the process? In case affirmative, add it to assert reproducibility.

The list of potential inhibitors is highly appreciated. The authors presented the interactions of the selected drugs nicely.

I suggest the authors reinforcing their discussion with arguments derived from the mutation capacity of the retrovirus that might render current vaccination not entirely compelling. Therefore, the presented alternatives could set as plausible options for treatment.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical devices development, Medical Imaging, Methods development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 29 Oct 2021

Guillermo A Silva-Martinez

We thank you for the detailed revision of our paper and for suggesting improvements. Also, we want to apologize for the long delay on our response, we were waiting for a second reviewer report to edit our manuscript.

Authors use molecular docking tools to test existing medicine in their efficacy to impede the S protein-ACE2 binding and thus, disabling the entry point of Cov2 to human cells. The authors use a solid justification to support this project. Since medicine creation is an expensive and long-lasting task, verifying if some active molecules in already FDA-approved medicine might block the binding between the S-protein and the ACE2 is an excellent alternate path.

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Response: We thank the reviewer for the detailed revision of our paper and for suggesting improvements. We are pleased that you found the structure of the writing to be pleasant.

Background

A new coronavirus outbreak, firstly reported in Wuhan, China, in December 2019, causes COVID-19, which symptoms are similar to SARS-atypical pneumonia. Worldwide, around 116 million cases and 2.57 million deaths are reported, with new cases increasing mortality every day. To date, there is no specific commercial treatment to control the infection.

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We performed rigid molecular docking using the receptor-binding domain at the S1 subunit of the S protein (RBDS1)-ACE2 (PDB ID:6VW1) interaction site and 1,283 FDA-approved drugs. The docking score, frequency of the drug in the receptor site, and interactions at the binding site residues were used as analyzing criteria.

Results

This research yielded 40 drugs labeled as potential inhibitors of RBD S1-ACE2 interaction. Among the inhibitors, compounds such as ipratropium, formoterol, and fexofenadine can be found. Specialists employ these drugs to treat chronic obstructive pulmonary diseases, asthma, and almost any respiratory infection.

Conclusions: Our results will serve as the basis for in vitro and in vivo studies to evaluate the potential use of those drugs to generate affordable and convenient therapies to treat COVID-19.

- **Response: Taking into account your contribution, in the manuscript the abstract has been improved and better organized.**

The proposed modifications focus on keeping the communications global. The CoV2 numbers in Mexico are absent in this suggested abstract since the authors provide worldwide numbers. I also shortened some sentences and fixed some wrongly used singulars.

1. *Correct the hyphenation in the whole document. If the authors are using a latex template, use the command \hyphenation{co-rrect, se-pa-ra-tion, sche-me}. Events: Janu-ary and associ-ated (intro, paragraph 2), lig-and (methods, paragraph 2).*

Response: We thank the reviewer for this observation. The hyphenation has been corrected in the whole manuscript.

2. *Introduction, page 3, paragraph 2. Current events suggest that Covid19 is not only lethal in the elder or patients with chronic diseases. I might suggest deleting this statement.*

Response: We agree with the reviewer's observation, however it's important to remark that adult individuals (over 40 years) and patients with comorbidities are prone to death by COVID 19 disease. This paragraph was restructured.

3. *Introduction, page 3, paragraph 2. something is missing in this paragraph. Also, the statement may be misleading; the actual spread and high contamination levels are proof of non-efficiency. If isolation and the other measurements were effective, what would be the sense for the proposal?*

Response: We thank the reviewer for this comment. This paragraph was rewritten.

4. *In subsection "Database preparation," you have information already provided in the first subsection of the methods.*

Response: We thank to reviewer for this comment. Changes were made in this section.

5. *Discussion, page 7, paragraph 1. In "ACE2 expression it is" the authors might have two nouns in the same sentence.*

Response: We thank the reviewer for this comment. Changes were made in this section.

Regarding the methodology, the authors used a standard: importing the macromolecule, edit or delete water molecules, add hydrogens, add charges, and the ligand. Did the authors have any particular issue during the process? In case affirmative, add it to assert reproducibility.

Response: We do not have any issue during the structure preparation; therefore, reproducibility can be achieved without any problem.

The list of potential inhibitors is highly appreciated. The authors presented the interactions of the selected drugs nicely.

Response: We appreciate that the reviewer finds our data well-presented.

I suggest the authors reinforcing their discussion with arguments derived from the mutation capacity of the retrovirus that might render current vaccination not entirely compelling. Therefore, the presented alternatives could set as plausible options for treatment.

Response: We thank the reviewer for suggesting improvements to our discussion section. Reinforced of the discussion section were made in the manuscript taking reviewers suggestion.

Competing Interests: No competing interests were disclosed.

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