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In Silico Determination of Potential Vaccine and Drug Targets for SARS-CoV-2

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Abstract:
In December 2019, a deadly disease caused by SARS-CoV-2 appeared in, China. The disease, spread globally and was declared a global pandemic by the WHO. Covid-19 is responsible for more than 403 million confirmed cases and 5.78 million deaths. The worldwide scientific community released the complete genome sequences.
Objective
A new drug takes at least a year to develop. Thus, it is necessary to treat the disease with FDA-approved medications. This study aimed at creating a docking-based screening of a library made up of accepted compounds using QMDS of a library constructed from approved drugs and compounds from clinical trials, against SARS-CoV-2 papain-like Protease. The rational selection of these drugs was made by testing their ability to inhibit any COVID-19 proteins essential for the viral life-cycle.
Results
The homology model of the proteins was built based on the SARS-CoV structure and the drugs docked in S3/S4 pockets of the active site of the enzyme. Fifteen FDA-approved drugs, including Dalfampidrine, Chloroquine and Formoterol, bind the target enzyme with significant affinity and good geometry, implying that they could be used to treat the virus. We believe that these findings will aid in the development of rational anti-COVID-19 drugs.

Keywords: *Covid 19, SARS-CoV-2, docking scores, FDA, WHO, binding affinity*

1. Introduction

The occurrence of the 2019 novel coronavirus in China has caused major concern among the international community. The number of people infected with the virus has been steadily increasing with a broad topographical spread. The virus spread very rapidly from China to all countries, and on March 11th, 2020, it was declared a pandemic by the World Health Organization (WHO) (Cavasotto & Filippo, 2021). The virus was first isolated from infected patients in Wuhan, Hubei province on the 7th of January 2020(Khan et al., 2020). This coronavirus strain was identified as the severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) and also referred to as 2019-nCoV. The infection caused by this virus is known as coronavirus disease-2019 (COVID-19). Coronaviruses are members of the Coronaviridae family and are RNA viruses with positive-sense envelopes. These zoonotic pathogens infect birds, animals, and humans and may cause disease in Intestinal, liver, respiratory and nervous systems. These viruses mostly cause mild to serious respiratory tract infections. Among known coronaviruses, CoV-229E (alpha coronavirus), CoV-NL63 (alpha coronavirus), CoV-OC43 (beta coronavirus), CoV-HKU1 (beta coronavirus), severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and current SARS-CoV-2 can infect humans (Wu et al., 2020).The Family of coronaviruses includes four genera i.e. Alpha coronavirus, Beta coronavirus, Delta coronavirus, and Gamma coronavirus (Wu et al., 2020). SARS-CoV 2 is a beta-coronavirus, and so are the viruses that cause SARS-CoV and MERS-CoV. These two highly pathogenic Corona Viruses (SARS-CoV and MERS-CoV) triggered global epidemics in 2003 and 2012 respectively, with high mortality rates(Paules et al., 2020). Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) (Kuiken et al., 2003) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Zaki et al., 2012) are estimated to have infected over 10,000 people worldwide in the last two decades. These outbreaks were preceded by high mortality rates (9.6% for SARS-CoV and 34.4 % for MERS-CoV), highlighting the critical need for timely care early in the epidemic to prevent the spread of the disease (WHO 2003). The mortality rate of SARS-CoV 2 is currently estimated in the range of 0.5% – 6%, which is less deadly than SARS -10% and MERS -40%. However, SARS-CoV-2 is highly contagious with a reproductive number (R^0) in the range of 2.0 – 6.5 which is higher than both SARS and MERS. This explains its velocity in propagation. As of 5th May 2021, WHO reported 153,690,803 confirmed cases of infection globally with 3,218,861 deaths. Several strategies have been adopted to discover new COVID-19 therapeutics. The National Health Commission of China had advised the use of HIV-1 protease inhibitors, lopinavir, and ritonavir as ad hoc treatment for the infection whereas Wang tested seven approved drugs *in vitro* against the clinical isolate of the virus (Wang et al., 2020). However, none of this provided an effective treatment against the new disease. The discovery of the new vaccines has raised doubtful questions with their record 60-90% effectiveness. However, there have also been several challenges in administering

these vaccines, including a lack of reason to assume that the complications of vaccination for pregnant women will outweigh the benefits of vaccination, allergic reactions towards the vaccines and side effects to patients (CDC). Thus, the need to perform comparative genomic studies and explore the possibilities of re-purposing an FDA approved drug for treatment of COVID 19.

Comparative genomic studies of COVID-19, consisting of its genome sequence and epidemiology, have proven vital in providing useful insights into the virus (Lu et al., 2020) (Chan et al., 2020). Genomic sequence analyses have revealed that SARS CoV-2 shares more nucleotide homology with SARS-CoV than MERS-CoV (X. Xu et al., 2020) (Zheng, 2020).Complete genome sequencing of SARS-CoV 2 shows that the virus encodes for two large polyprotein that are further processed by virally encoded cysteine proteins, the Papain-Like Protease (PLpro) and the main protease(3-chymotrypsin like protease). Additionally, the RBD of SARS-CoV-2 binds to the human ACE2 receptor in a higher affinity than that of SARS-CoV. The presence of an insertion in the polybasic cleavage region in SARS-CoV-2 increases the infectivity of the virus (Ye et al., 2020) (Andersen et al., 2020) (Boni et al., 2020). It is also noted that the processing of the viral proteins is crucial for the infection and maturation of the virus in the host (Chen et al., 2020). Due to their various key roles in the viral life-cycle, these two proteases (PL Pro and M Pro) are important targets for antiviral drug development.COVID-19 Mpro has been used as a target to screen for potential inhibitors in clinically approved drugs (Z. Xu et al., 2020) (Lu et al., 2020) (Liu & Wang, 2020). This study has explored the protease PL pro as a target for development of an effective treatment against SARS-CoV-2 because of its essential roles in virus replication and immune evasion(Gao et al., 2021) . The safety profile of FDA-approved drugs is well documented, and the effectiveness of selected few can be quickly tested against a viral culture therefore, Drug repurposing could be an efficient approach to finding therapeutics against viral infections. This study involved the analysis 4,121 drugs approved by the FDA. The rational selection of these drugs was made by testing their ability to inhibit PL pro COVID-19 proteins essential for the viral life-cycle. We performed a virtual screening of these drugs against COVID-19 PLpro in order to identify potential inhibitors of its catalytic domain. This study displays a docking-based screening of a library made up of accepted compounds using quantum mechanical scoring of a library constructed from approved drugs and compounds from clinical trials, against SARS-CoV-2 target protein; the papain-like Protease.

2. Materials and Methods

2.1. Homology Modelling and Protein Sequences

The protein sequence of COVID -19 Papain Like proteinase used in this study was obtained from GenBank (Accession QHD: 43415). The catalytic domain of PLpro was delineated by comparing it with already known Corona Viruses sequences (SARS-CoV). The Homology model of the Papain-like Protease catalytic domain was generated using the SWISS-MODEL workspace (swissmodel.expasy.org/workspace) using the SARS-CoV PLpro crystal structure (PDB Id: 3E9S) as a template. The homology model was in closed flap conformation with GMQE and QMean scores of 0.95 and -0.22 respectively. Thus, the template ligand was used to define the binding site for docking.

2.2. The FDA Approved Drugs

A total of 4,121 approved drugs used in this study were obtained from four different libraries. This consists of FDA approved drugs from ChEMBL (version 26), DrugBank, DrugCentral databases and Selleck Chem. These drugs were then downloaded in sdf format from the ZINC15 library.
Redundant entries were eliminated.
Metals and molecules with more than 120 atoms were deleted.

2.3. Virtual Screening

Prediction of drug-target interactions using AutoDock Vina. AutoDock Vina (Version 1.1.2), which is a molecular docking and virtual screening application, was used to predict binding affinities (Kcal/mol) between the Papain-Like proteinase of SARS-CoV-2 and 4,121 FDA approved Drugs. These drugs were converted to PDBQT format using Open Babel (Version 2.3.2) with the following option: --gen3d and –p 7. 4. The Papain Like protease is loaded to AutoDock Vina in PDB format and the FDA approved drugs in SDF format. Energy minimizations parameters were set at Force field UFF, optimization Algorithm Conjugate gradients. The total number of steps was set at 200, number of steps for update at 1 and stop if energy difference is less than 0.1. The Exhaustiveness parameter was set at 8. The lowest binding affinity with a Root Mean Square Division of Lower bound and upper bound at 0 and mode at 0 is selected and updated in the curated database.

2.4. Molecular Docking

Molecular Docking and Prediction of drug-target interactions using Ligand Efficiency, Lipophilic Ligand Efficiency, Torsion Quality, Inter-molecular and Intra-Molecular clashes by SeeSAR. Docking was performed using the SEESAR suite of programs from BiosolveIT (www.biosolveit.de/SeeSAR). The Papain Like Protease was loaded into the SeeSAR suite in the protein mode. The PLpro was then transferred to the Binding site Mode to define the Binding sites. Unoccupied pockets were detected. Residues were added to binding site surrounding selected molecule or pocket. Selected pockets are shown then selection of new binding site confirmed.

Selected drugs were loaded through to the Docking mode. Possess were generated and equilibrium properties were calculated as a standard for SeeSAR. The hydes (binding assessment) for Estimated affinity were then calculated.

3. Results

Both SARS-CoV and SARS-CoV-2 PL ^{pro} have similarly conserved catalytic residues His41 and Cys145 as well as Cys111, His272 and ASP286 along with oxyanion hole-stabilizing Trp106 respectively in the active region which is responsible for its enzymatic activity to process the newly synthesized polypeptide chain of the virus for functional and non-structural proteins (Báez-Santos et al., 2015): (Pillaiyar et al., 2016) .The COVID-19 Papain-like Protease is responsible for processing three cleavage sites of the viral polyprotein to release mature non-structural proteins 1, 2 and 3. Besides proteolytic processing, PLpro of corona viruses also has a deubiquitinating (DUB) and deISGylating activity which is hypothesized to modify the innate immune response to infection(Mielech et al., 2014). Inhibition of this protease will not only stop the production of functional polypeptides of SARS-CoV-2 but also help to reduce the viral replication and its load on the host. There have been several attempts to design inhibitors against SARS-CoV PLpro structure and they provided promising results thus the need to choose SARS-COV2 PLpro structure to virtually screen FDA approved drugs to find therapeutics.

Virtual screening of a chemical library of 4,121 FDA approved drugs (2,650 approved small molecules and 1,417 approved biologics) was docked onto the Papain-Like Protease structure of SARS-CoV2. Molecules were scored according to Binding affinity, Ligand Efficiency, Lipophilic Ligand Efficiency, Clashes (Inter-molecular and Intra-molecular slashes) and Quantum Mechanical Docking scores. QM scores capture the underlying physics of the molecular system accounting for all energy contributions including electronic polarization, covalent bond formation and charge transfer.

The Covid-19PL^{pro} structure was modelled based on the experimental crystal structure of SARS-CoV PL^{pro} PDB: 3E9S. Both proteases share 82% sequence identity and 100% within the binding site (Cavasotto & Filippo, 2021). The homology model is in close conformation with Global Model Quality Estimates (GMQE) and Qualitative Model Energy Analysis (QMEAN) scores. The homology model could accommodate the ligand from the template.

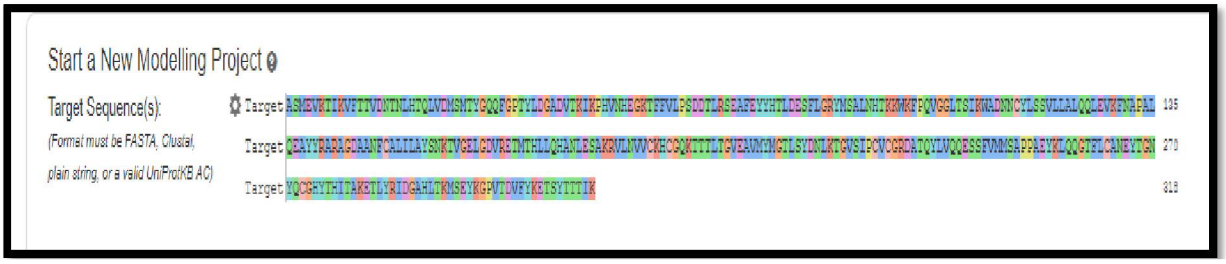


Figure 1: PDB 3E9S Sequence Used to Model for SARS-Cov-2 PL ^{Pro} Structure

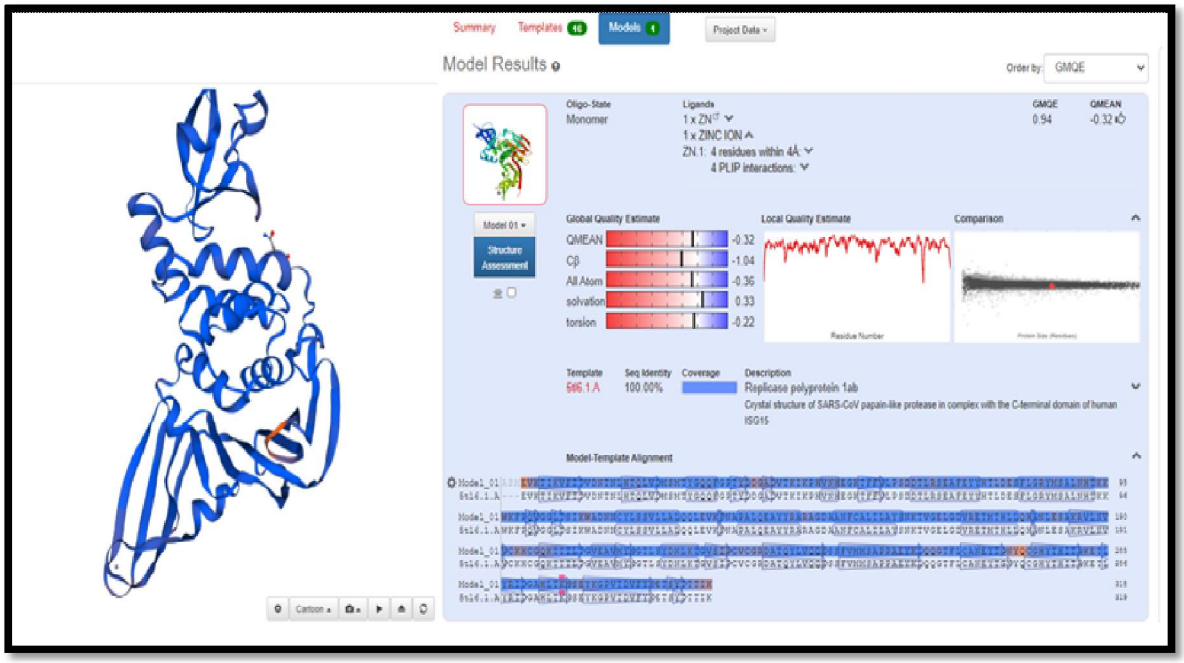


Figure 2: PL Pro Structure Modeled

The binding site contained more S3/S4 pockets, rather than the restrictive S1/S2 pockets close to the catalytic residues. The S3/S4 pockets contained residues Asp164, Val165, Arg166, Gly271, Tyr273, Thr301, Glu167, Met 208, Ala246, Pro247, Pro248, Asp302, Tyr 264, Gly266, Asn267, Tyr 268, Gln269 and Cys217.

For each Ligand ten poses were generated and docked into the binding site. All these poses were sorted according to their binding affinities. Candidates were selected in terms of their Binding affinities, Ligand Efficiency, Torsion quality, absence of Intra/Inter-molecular clashes (Quantum mechanical Docking scores).

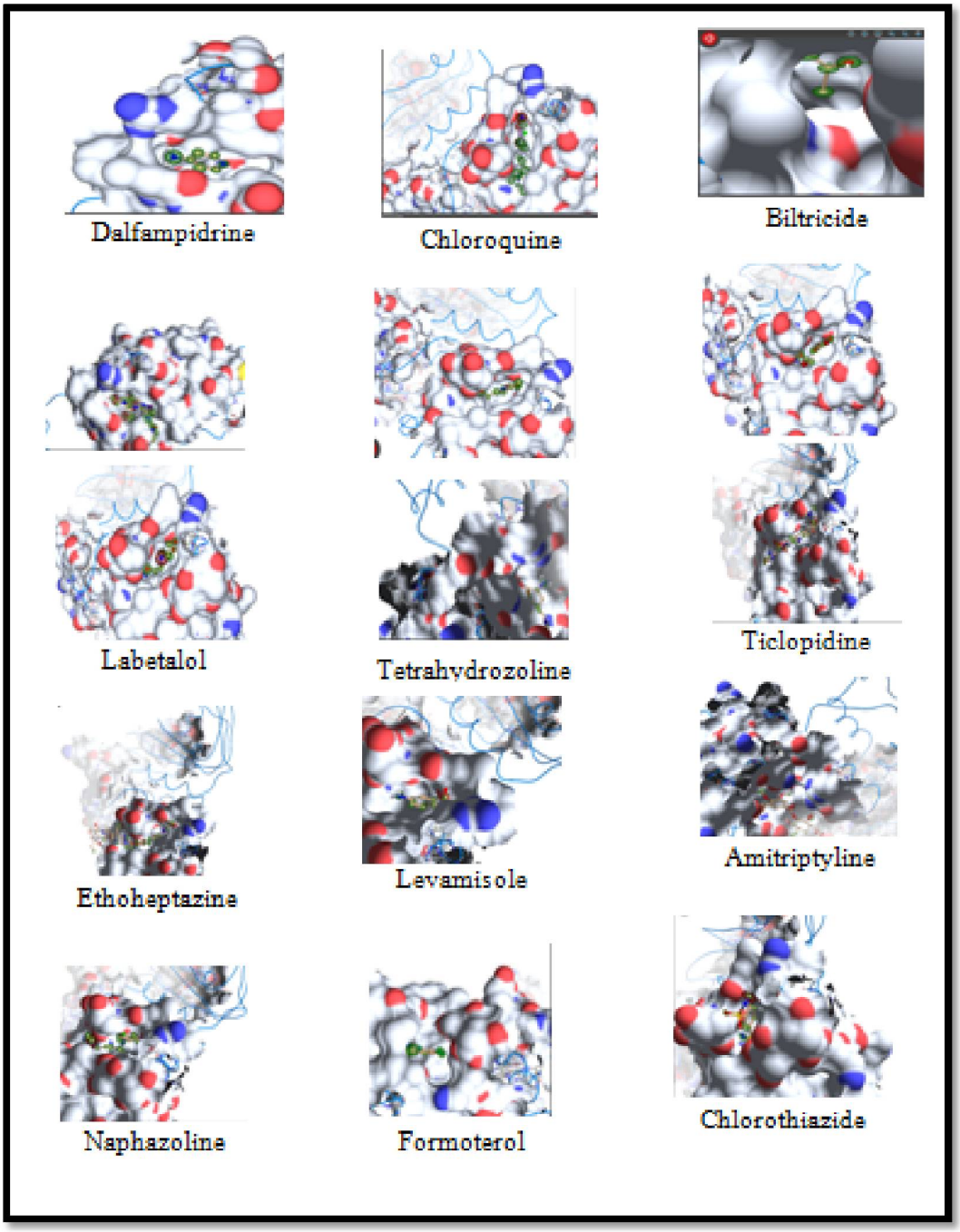
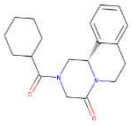
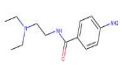
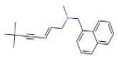
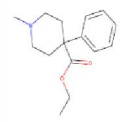
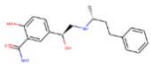
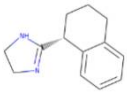
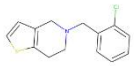
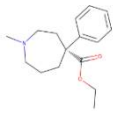
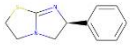
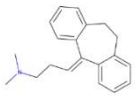
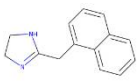
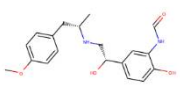
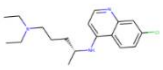


Figure 3

Ligands that had a positive binding affinity in AutoDock Vina were discarded from the study as the lower the binding affinity/ binding energy the more stable ligand-receptor interaction we get. 126 drugs had a –kcal/mol binding affinity towards the PL ^{pro} structure and thus considered as possible therapeutics against COVID-19. 44 had relaxed torsions, 28 tolerable torsions and 48 had strained torsions. 66 had no Intra/Inter-molecular clashes, 30 had possible clashes and 29 had severe clashes, 60 had Quality Lipophilic Ligand Efficiency and Ligand Efficiency while 34 had Good Quality LLE and LE. Among the selected set, there were several anesthetics, antineoplastic agents, appetite depressants, skin ointments, diagnostic imaging agents and other unsuitable drugs, and hence were removed from consideration. Finally, fifteen drugs were left with an estimated binding affinity within -4Kcal/mol to -8Kcal/mol range, having no torsional strain, intra- and inter-molecular clashes, Quality Lipohilic Ligand Efficiency and Ligand efficiency. These promising inhibitors of COVID-19 PLpro are listed.

Number	ZINC ID	Structure And Molecular Formula	Drug Name/Commercial Name	Pharmacological Action	Route of Administration	Binding Energy (Kcal/Mol)	SEESAR
1.	ZINC403566	C19H24N2O2 	Biltricide	An anthelmintic used in most schistosome and many cestode infestations.	ORAL	-7.6	Relaxed torsions, No Intra/Inter-molecular clashes, Good LLE and LE
2.	ZINC1530756	C13H21N3O 	Procainamide	A medication used to treat life threatening ventricular arrhythmias.	Oral/Intra-Muscular/ Intravenous	-5.0	Tolerable torsions, Intra-molecular clashes, Good Quality LLE and LE
3.	ZINC1520981	C21H25N 	Terbinafine	An ally amine antifungal used to treat dermatophyte infections of toenails and fingernails as well as other fungal skin infections	Oral/Topical	-6.5	No Torsional Strain, Inter-molecular clashes, Quality LLE and LE
4.	ZINC1681	C15H21NO2 	Pethidine	A narcotic analgesic that can be used for the relief of most types of moderate to severe pain, including postoperative pain and the pain of labor.	Oral/Sub-cutaneous/Intra-muscular	-4.8	Relaxed Torsions, No Inter/Intra-molecular clashes, Quality LLE and LE
5.	ZINC416	C19H24N2O3 	Labetalol	An alpha- and beta-adrenergic antagonist used to treat hypertension, angina, and sympathetic over activity syndrome	Oral/Intravenous	-5.5	No Torsional Strain, Intra-molecular clashes, Quality LLE and LE
6.	ZINC113426	C13H16N2 	Tetrahydrozoline	An alpha-adrenergic agonist used in the temporary symptomatic relief of discomfort and redness of the eyes due to minor irritations, as well as alleviate nasal congestion.	Opthalmic/Oral, Respiratory (Inhalation)	-6.9	No Intra/Inter-molecular clashes, Good LLE and LE, Relaxed Torsions

Number	ZINC ID	Structure And Molecular Formula	Drug Name/Commercial Name	Pharmacological Action	Route of Administration	Binding Energy (Kcal/Mol)	SEESAR
7.	ZINC19594599	C14H14ClNS 	Ticlopidine	A platelet aggregation inhibitor used in the prevention of conditions associated with thrombi, such as stroke and transient ischemic attacks	Oral	-7.5	No Torsional Strain, Intra-molecular clashes, Very Good Quality LLE and LE
8.	ZINC268	C16H23NO2 	Ethoheptazine	Is a phenazepine based opioid analgesic	Oral	-6.7	Relaxed Torsions, No clashes, Quality LLE and LE
9.	ZINC119839	C11H12N2S 	Levamisole	Anti-helminthic used for parasitic, viral, and bacterial infections.	Oral	-6.9	No Torsional Strain, Inter-molecular clashes, Quality LLE and LE
10.	ZINC968257	C20H23N 	Amitriptyline	An antidepressant indicated in the treatment of depressive illness, either endogenous or psychotic	Oral	-6.6	No Torsional Strain, Intra-molecular clashes, Quality LLE and LE
11.	ZINC119717	C14H14N2 	Naphazoline	Is a sympathomimetic vasoconstrictor used for the symptomatic relief of redness and itching of the eye, and nasal congestion	Opthalmic	-7.4	No Torsional Strain, Intra-molecular clashes
12.	ZINC856	C19H24N2O4 	Formoterol	Is an inhaled long-acting beta2-adrenergic receptor agonist used as a bronchodilator in the management of asthma and COPD	Respiratory (Inhalation)	-6.7	No Torsional Strain, Intra-molecular clashes, Quality LLE and LE
13.	ZINC19144226	C18H26ClN3 	Chloroquine	Is an antimalarial drug It is also used for second line treatment for rheumatoid arthritis	Intra-muscular/Sub Cutaneous	-5.4	No Torsional Strain, Intra-molecular clashes, Quality LLE and LE

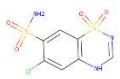
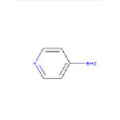
Number	ZINC ID	Structure And Molecular Formula	Drug Name/Commercial Name	Pharmacological Action	Route of Administration	Binding Energy (Kcal/Mol)	SEESAR
14.	ZINC3872055	C7H6ClN3O4S2 	Chlorothiazide	used to treat hypertension and edema in congestive heart failure	Oral/Intravenous	-6.1	No Torsional Strain, No Intra/Inter-molecular clashes, High Quality LLE and LE
15.	ZINC599985	C5H6N2 	Dalfampidrine	Potassium channel blocker used on patients with multiple sclerosis	Oral	-4.6	No Torsional Strain, No Intra/Inter-molecular clashes, Quality LLE and LE

Table 1

5. Discussion

The COVID-19 pandemic caused by SARS-CoV-2 has turned out to be a deadly and widespread virus after the 1918 influenza virus pandemic (JOHNSON & MUELLER, 2002):(Taubenberger & Morens, 2006). In the last two decades, outbreaks of human infecting coronaviruses such as SARS-CoV, MERS-CoV, and now this novel SARS-CoV-2 have become increasingly lethal, highlighting the lack of effective treatments. Further SARS-CoV-2 variant strains could pose a serious threat of recurrence in the near future, and one more failure in early medical planning will place humanity far behind in terms of global health and economic development. The current outbreak, as well as figures show an increase in COVID-19-related deaths around the world. This has already placed the global health system in a state of emergency, necessitating an urgent and successful therapeutic response to end the outbreak now and forever. Interestingly, the genetic identity of SARS-CoV-2 with other coronavirus strains, especially with humans infecting SARS-CoV, the similarity in host cell interaction, infection, and replication machinery, and the presence of conserved region in these major potential therapeutic targets such as Spike (S), glycoprotein receptor-binding domain (RBD), non-structural proteins (NSPs) cysteine proteases 3CLpro (NSP5) and PL ^{pro} (NSP3), RNA dependent RNA polymerase (RdRp) and others have supported at the great extent to use the range of therapeutic drugs being employed previously for other strains as well as to study for new or repurposed drugs and Vaccines (Chen et al., 2020): (Khailany et al., 2020).

In our study, chloroquine, an anti-malarial drug, was identified as a potential inhibitor of viral PL pro. The antiviral activity of chloroquine has previously been reported. (Savarino et al., 2006): Yan et al., 2013). After being shown to block COVID-19 infection at a low micromolar concentration (EC50: 1.13 M) in cell culture experiments, China launched a few clinical trials of the drug in patients (Wang et al., 2020). Chloroquine has been shown to be effective against viral infection both at the entry and post-entry levels. The effect of chloroquine during the post-entry stages is most likely manifested by its inhibition of the critical viral protein, PLpro. Another intriguing molecule discovered during our research is formoterol, which relaxes airway muscles to improve breathing and is used to treat chronic obstructive pulmonary disease (COPD) as a bronchodilator and asthma. Our research predicted that Dalfampidrine, which is used to improve walking in MS patients, is one of the top drugs that could bind to the Papain-like proteinase receptor of SARS-CoV-2. However, there is no supporting evidence, and more research is required. If these drugs also inhibited viral PLpro activity, they would have a synergistic effect in treating patients. Antabitant, Pilaralisib, and Tiracizine were identified as suitable compounds targeting the SARS-CoV-2 PL pro structure in the study of (Cavasotto & Filippo, 2021).

6. Conclusion

There has not been any clinically approved medications for SARS-CoV since its outbreak in 2003 (Rajpoot et al., 2021), this possess a significant concern in the medical and scientific fields. Nevertheless, repurposing existing licensed drugs as an effective measure against SARS-CoV-2 is one of the best choices for rapid analysis and approval. Several anti-HIV medications with a wide range of activity, such as lopinavir and ritonavir, which are known to suppress HIV protease, are being tried against SARS-CoV-2 proteases. These drugs are being used as part of a combination drug to combat COVID-19 disease (Arabi et al., 2018): (Chu et al., 2004): (Lim et al., 2020): (Şimşek Yavuz & Ünal, 2020). However, the hypothesis that the combinations of these drugs block SARS-CoV-2 proteases and other targets, has shown a weak impact in terms of binding specificity to these targets as well as toxic side effects, necessitating the development of target-specific drugs with enhanced pharmacokinetic efficacy against SARS-CoV-2. Several structural studies have shown that the above-mentioned

broad-spectrum drugs (listed) individually acting on of SARS-CoV-2 PL ^{pro} structure are stronger and enhanced drug candidates than the anti-HIV broad-spectrum drugs. (Chen et al., 2020). Our structural findings centered on the repurposing of FDA-approved drugs that can function as SARS-COV-2 PL pro protease inhibitors. The 176 drugs obtained from the ZINC15 library were virtually screened by unbiased, blind docking studies against the crystal structure of PLpro. The drugs binding specifically with all the catalytic residues Trp106, Cys111, His272, and Asp286 were only considered as positive. Hence the 15 drugs selected had a positive binding to the catalytic residues.

An important parameter in the final phase of selecting the drugs that were already on the market was dependent on their oral or injectable route of administration. The majority of the medications tested in this study have a pharmacological effect for symptoms of cardiovascular and respiratory diseases, as well as decongestant, anti-inflammatory, antipyretic, and analgesic properties. Since COVID-19 patients show symptoms, such as running nose, fever, sore throat and those with pre-medical conditions have shown an increase in disease severity, re-purposing of drugs as an inhibitor of PL ^{pro} protease may also help as a risk reducer in the case of patients with a particular pre-medical condition.

7. Limitations

The study was carried out through the Retrospective In silico study design and therefore trials of the results have not been tested on animal models.

8. Ethics Approval

This research was approved by the Masinde Muliro University of Science and Technology ethical and approval committee on 18th November 2020.

9. Declaration of Interests

The authors declare that there are no competing interests in the publication of this paper.

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