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Utilizing Virtual Screening and Structure-Based Drug Design to Repurpose Approved Medications as Possible Inhibitors of the 3CL-Protease of the SARS-Cov-2

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Abstract

Since there is currently no approved vaccine or small molecule therapeutic on the market, the recent global outbreak of SARS-CoV-2, which has nearly 15 million infected people and more than 600,000 fatalities (accessed on 20th July 2020), poses a significant challenge to all nations and societies. Nearly 1500 investigations are actively enrolling patients by invitation for clinical research against COVID-19 because to the urgent need for a causative therapy.

The main protease of Coronaviruses, chymotrypsin-like protease (3CLpro), processes the large polyprotein 1ab releasing several further enzymes that are crucial for viral replication. Moreover, 3CLpro is unique for Coronaviruses and not found in higher organisms. This predestines 3CLpro as most attractive target for the development of anti-infective agents against SARS-CoV-2 and related Coronaviruses [1]. Consequently, several inhibitors of 3CLpro were developed mostly during the last 17 years right after the first wave of infection caused by the SARS-CoV-1. However, no experimental compound was developed further and reached the market. Most compounds were designed as covalent inactivators that react with the catalytic Cys145.

These guidelines, which are sometimes referred to as features, are made up of spheres where a heavy ligand atom with specific properties, such as hydrogen donor or acceptor properties, is meant to be positioned in order to make a specific interaction with a neighbouring amino acid. It is best to simulate the binding pocket using a sequence of overlapping excluded volume spheres in order to reduce the likelihood of receiving an excessive number of false positive hits for branched or bulky molecules that meet the requirements but would interfere with the receptor protein. These were produced utilising a radius of 2 from particular pocket amino acids [2]. Hits are not counted for molecules whose structures conflict with those of the excluded volume spheres. Using a pharmacophore model, one Very quick search for hits that satisfy all or some of the feature requirements across big 3D structure databases. The search process should very effectively enrich those chemical entities that provide high docking scores and have a higher likelihood of interacting with the relevant target protein if the pharmacophore model is adequately described. The MOE pharmacophore editor was used to launch the pharmacophore search [3]. All of the hits from the pharmacophore search were put through the previously mentioned follow-up virtual screening process using MOE to evaluate their binding affinity.

Introduction

This is reflected in the majority of the 3CLpro enzyme x-ray structures from different Coronaviruses. As opposed to covalent inhibitors, which are common, small reversible inhibitors are typically chosen since they have fewer toxicities and side effects. SARS-3CLpro CoV-2 recently showed two crystal structures, both of which were bound to covalent inhibitors). The crystal structures of the 3CLpro-complexes' PDB-IDs 6LU7 and 6Y2F show total overlap with an RMSD-value of 0.48 over more than 300 amino acids. The most significant crystal structure of 3CLpro of SARS-CoV-1 with a non-covalent inhibitor is found in PDB-ID: 3V3M.It's intriguing how many drugs that have been authorised for use in other ailments also demonstrated success against coronaviruses. The antiquated malaria drug hydroxychloroquine, which has shown effectiveness in clinical trials and appeared to be effective in inhibiting the multiplication of SARS-CoV-2 in vitro, is one of the active ingredients.Gao and colleagues 2020. These findings triggered a passionate debate about the hydroxylchloroquine's possible benefits for COVID-19 sufferers [4-15]. A significant retrospective study, however, discovered that COVID-19 patients who took chloroquine or hydroxychloroquine developed severe dysrhythmias and received no benefit.

Subjective Heading

In addition, a very recent FDA review of the safety concerns associated with the use of these chloroquine derivates highlights the connection between treatment and serious heart rhythm problems as well as other safety concerns, such as blood and lymph system disorders, kidney injuries, and liver failure (https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroqu Because of this, the official NIH treatment recommendation (accessed on July 20, 2020) advises against using chloroquine or hydroxychloroquine to treat COVID-19, with the exception of in clinical setting up tests. A corticosteroid called dexamethasone is used to treat a wide range of inflammatory illnesses, including allergy disorders, ulcerative colitis, arthritis, lupus, psoriasis, and respiratory problems. Dexamethasone is suggested for

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the treatment of COVID-19 patients in the NIH's actual COVID-19 treatment guideline due to lower mortality rates compared to patients who received conventional care.

In cell culture, other medications like mefloquine, selamectin, and cepharanthin also demonstrated positive benefits (Fan et al., 2020). The National Health Commission in China also advises using ribavirin and interferon together to treat COVID-19 because to its impact on MERS-CoV.Remdesivir, a broad-spectrum antiviral medication, is currently being studied in a clinical trial to treat COVID-19. Remdesivir was previously tested on humans who had the Ebola virus sickness. Initial findings in MERS-CoV-infected animal models were encouraging. Additionally, the clinically validated camostat mesilate has demonstrated that inhibition of the serine protease prevents SARS-CoV-2 from entering cells.

Discussion

The MOE 2019 programme (Chemical Computing Group, Montreal, Canada) and Autodock Vina (The Scripps Research Institute, La Jolla, USA) were used for two virtual screens.The latter was integrated into the PyRx environment's free version, which is accessible at https://sourceforge.net/projects/pyrx/. The entire AMBER14:EHT force field was utilised for the MOE-based virtual screen. The RCSB Protein Data Bank provided the SARS-CoV-2 3CLpro crystal structure (PDB-ID: 6LU7). The covalently attached ligand to the catalytic Cys145 was eliminated, and the Cys145 was then made available as a free thiol. After that, the free target protein underwent MOE's QuickPrep method, which included repairs for any missing atoms, misaligned geometries, or other 3D protonation, removing water molecules more than 4.5 from any receptor or ligand atom, and crystallographic aberrations. The binding pocket was established using the expelled covalent ligand. A MOE-specific database was imported and the SD-file containing the 3D structures of approved medications was chosen as the ligand in the docking setup for virtual screening. The stiff receptor and the triangular matcher were chosen as additional factors. Ten docking poses per ligand molecule were evaluated using the London dG score. The screening results were exported as SD files, and Osiris Datawarrior was used to integrate several findings for a single chemical structure to create a result file with distinct chemical structures. maintaining various docking scores for each molecule. The crystal structure of 3CLpro was likewise liberated from the covalent ligand for the virtual screen with Autodock Vina, and it was afterwards put through the Dockprep technique used by the UCSF Chimera programme (University of California, USA 2004). The procedure calls for the removal of solvent, the use of a rotamer library to replace missing side chains, protonation, and the insertion of charges.

PyRx was used to load an SD-file containing the 3D structures of the ligand library and convert it to the necessary pdbqt-format. The implemented Vina wizard was then used to pick the protein target and the compound library as numerous ligands in the following phase. An area cuboid was employed to specify the active site pocket before beginning the virtual screening process. The exhaustiveness, which measures docking precision, was set at 8 in the last stage. The Vina wizard then began the virtual screening process. Again, the data were acquired in pdbqt format, which needed to be changed for additional analysis into SD format.

Using MOE 2019 software, structural data visualisation and molecular docking were carried out (Chemical Computing Group, Montreal, Canada). RCSB Protein Data Bank provided the 3CLpro of SARS-CoV-2 crystal structure (PDB-ID: 6LU7). The structure file was imported into the computer and then underwent the previously described 3D protonation and structure preparation. The installed Amber14:EHT force field was used to determine the partial charges of all protein and ligand atoms. The ligand was placed in the binding site using the triangle matcher during molecular docking, and the London dG scoring function was used to rate the results. The best 50 poses were passed to the refinement and energy minimization in the pocket using the induced fit method and then rescored with the GBVI/WSA dG scoring function. Best poses were further refined by energy minimization of all amino acids in a radius of 10 Å around the ligand.

In order to facilitate the investigation of protein-ligand interactions, the complex structures of 3CLpro with the highest scoring ligands were superposed and subjected to the Quickprep process implemented in MOE using the AMBER14:EHT force field once more. Several docked protein-ligand complexes with ideal binding postures were used as the foundation for the generation and analysis of protein ligand interaction fingerprinting (PLIF) utilising the corresponding MOE tool. A panel displaying the precise connections of each ligand to a variety of protein-ligand complexes that the PLIFs have selected as the most significant relationships amino acids that are close to the binding pocket. Most crucially, a pharmacophore model encapsulating fundamental requirements that a possible ligand of the corresponding binding pocket must satisfy can be created using the PLIFs and frequency of protein-ligand contacts. These guidelines, which are sometimes referred to as features, are made up of spheres where a heavy ligand atom with specific properties, such as hydrogen donor or acceptor properties, is meant to be positioned in order to make a specific interaction with a neighbouring amino acid. It is best to simulate the binding pocket using a sequence of overlapping excluded volume spheres in order to reduce the likelihood of receiving an excessive number of false positive hits for branched or bulky molecules that meet the requirements but would interfere with the receptor protein. These were produced utilising a radius of 2 from particular pocket amino acids. Hits are not counted for molecules whose structures conflict with those of the excluded volume spheres. Large 3D structure databases can be quickly searched for matches that satisfy all or some of the feature requirements using a pharmacophore model. The search process should very effectively enrich those chemical entities that provide high docking scores and have a higher likelihood of interacting with the relevant target protein if the pharmacophore model is adequately described. The MOE pharmacophore editor was used to launch the pharmacophore search. All of the hits from the pharmacophore search were put through the previously mentioned follow-up virtual screening process using MOE to evaluate their binding affinity.

Then, a comparison and activity Cliff Analysis was used to visualise the chemical landscape, find clusters and singletons with high docking scores, and group related molecules together on a 2D surface.There are four significant clusters that stand out, including sizable flavonoid, tetracycline, aminoglycoside, and anthracycline clusters (Fig. 2B). Quercetin, oxytetracycline, kanamycin, and doxorubicin serve as illustrative medications for these clusters, respectively. There are other singletons or groups of two with high scores, such as raloxifen. It is in great agreement with the very recent publication by Jo et al., who provide experimental proof that flavonoids are in fact inhibitors, that numerous flavonoids are among the hits with the best docking scores. that 3CLpro.It should be mentioned that 6.5% of the FDA-approved medications and 4.5% of the flavonoids quercetin, rutin, homoorientin, all of which are flavonoids, eltrombopag, and doxorubicin, struck the virtual screen PAINS patterns. In order to solve the issue of frequent hitters in experimental high throughput screening campaigns—often

false positive hits—Baell and Holloway created the idea of PAINS. However, only six patented assays assessing protein-protein interaction (PPI) inhibition using the AlphaScreen detection technique were used to test the crucial substructural components of electronic PAINS filters. warn against using PAINS filters in a careless manner to identify and classify substances with liabilities and suggest that only orthogonal experiments should be used to reach such findings.All compounds were taken further to detailed docking analysis to elucidate the potential molecular interactions with 3Clpro, because a wide variety of orthogonal assays despite the fact that some of the approved drug molecules contain crucial substructures like labile ester (salvianolic acid B) or possibly redox active groups like electron rich scaffolds, tests have been conducted to demonstrate biological activity and safety of the molecules prior to drug approval (polyphenols). The virtual screen's best hits were subjected to a more thorough docking technique that sampled 50 docking postures utilising a secondary GBVI/WSA dG score that was introduced in MOE. Table 1 summarises the findings of the compounds that showed the greatest promise. Curiously, most high Antioxidants called flavonoids are also used medicinally as anti-cancer or antibiotics. The two medicines with the greatest docking scores, kanamycin and salvianolic acid B, display comparable binding postures by occupying the S1- and S2-subpockets Both ligands form H-bonds with the catalytic Cys145 despite the fact that kanamycin interacts with the thiolate moiety and salvianolic acid B with the backbone nitrogen of Cys145. The most effective protein-ligand interactions with 3CLpro for typical ligands are shown in Fig. S2, and they are further discussed in the section.

The development of a pharmacophore model was made possible by the thorough analysis of the key chemical interactions involving ligands and amino acids that border the active site pocket. A simplified version of this model describes the chemical properties in terms of molecular interactions, such as H-bond donator or acceptor, and their 3D layout within the receptor protein's active site pocket. By overlapping excluded volume spheres with the protein pocket shape, the pharmacophore model is finished. If any of a ligand's atom centres cross across an excluded volume, the ligand will not match. A pharmacophore model for 3CLpro was created using the results of the PLIF-analysis mentioned above. A heavy atom's position that can act as an H-bond donor, acceptor, or metal ligator is one of the model's five essential characteristics, or features.The pharmacophore model outperforms conventional virtual screens for screening larger compound libraries for chemical entities that satisfy the requirements of the chemically and spatially defined model characteristics, which rely on quick rigid model docking techniques.

Conclusion

This study was prompted by the present SARS-CoV-2 epidemic to quickly find currently approved medications that could be modified to target 3CLpro, a cysteine protease crucial for Coronavirus replication. Several scaffolds with a high potential to inhibit 3CLpro were discovered by a virtual screening method, including flavonoids, which were recently characterised as experimentally proven inhibitors of this enzyme, antibiotics, and anticancer drugs. most optimistic Oxytetracycline, naringin, kanamycin, cefpiramide, salvianolic acid-B, teniposide, etoposide, and doxorubicin were among the drugs that were impacted. A further pharmacophore search that screened 7.2 million compounds from the ZINC15 collection greatly expanded the tested chemical space, to well-known chemical groups like flavonoids. Furthermore, the detailed examination of the critical interactions between ligands and the amino acids in the active site pocket opened up new avenues for creating and enhancing 3CLpr inhibitors.

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Conflict of Interest

The authors declare that they are no conflict of interest.

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