 doi:10.20944/preprints202004.0015.v2

Computational Screening of molecules approved in Phase-I clinical trials to identify 3CL Protease inhibitors to treat COVID-19

Kamlesh Kumar Sahu[1,5*], Sergei Noskov[2], Jack Tuszynski[3,4], Michael Houghton[1,5], D. Lorne Tyrrell[1,5]

[1]Li Ka Shing Applied Virology Institute, Dept. of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada

[2]Centre for Molecular Simulation, Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada

[3]Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada

[4]Department of Physics and Experimental Oncology, University of Alberta, Edmonton, AB T6G 2J1, Canada

[5]Li Ka Shing Institute of Virology, Dept. of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada

Abstract – Ligand and structure based virtual screening approaches were applied to clinical stage drugs as well as those approved for human use in an attempt to repurpose drugs for potential use against COVID-19. This approach involved ligand-based shape similarity searches, structure-based docking and pharmacophore searches with the help of pharmacophore queries derived from available ligands and receptor structures. Several compounds appeared as hits in pharmacophore and shape similarity searches and those docking to the SARS-CoV-2 viral 3CL protease were then ranked on the basis of docking scores.

 doi:10.20944/preprints202004.0015.v2

Keywords – virtual screening, COVID-19, Protease 3CL pro,

*Corresponding Authors – ksahu@ualberta.ca

1. Introduction-

A novel coronavirus identified in late 2019 causes Severe Acute Respiratory Syndrome (SARS) [1]. Its main protease, also called Proteinase 3CL pro, contains 306 residues and is a key enzyme for its replication. The replication of this positive stranded RNA virus can be potentially controlled by targeting this protease.

Computational methods are less time-consuming and more cost-effective than physically testing large numbers of potential drugs in biochemical or cell-based assays, which has been the historical practice in the pharmaceutical industry. Emerging computational methods can effectively filter the number of compounds to be evaluated by biochemical and biological screening to a small subset of molecules that are more likely to yield active hits. In the case of COVID19 where it is crucial to find safe and therapeutic drugs very quickly, computational screening of drug libraries could identify drug candidates for immediate clinical testing. Indeed, virtual screening (VS) of chemically available ligand databases has become an important tool with which to explore chemical space [2, 3] [4, 5] and to accelerate the initial stages of drug discovery. The aim is to rapidly identify potential hit molecules which can then be evaluated experimentally and clinically.

VS has been used to identify inhibitors in cases where crystal structures were not available and homology models are used [6] and in cases where crystal structures were available and VS is used by docking drug fragment libraries [7]. Molecular docking powered VS is also used for prioritizing compounds if the 3D structure of the target's binding site is

 doi:10.20944/preprints202004.0015.v2

available. Similarity search powered VS can be used where no information of receptor is available. Molecular shape comparison and similarity searches have been used in drug lead identification from time to time and have been successful in finding inhibitors with minimum time and resources. In this study, we have applied ligand and structure based VS to screen chemical databases in an attempt to identify inhibitors of the SARS-CoV-2 3CL protease.

2. Methods

Different Virtual screening methods were used to select a consensus for potential inhibitors of the 3CL protease: An outline of our virtual screening protocol is shown in Figure 1. Virtual screening workflow involved first docking all 2827 phase-I approved small molecules on the rigid protease 3CL pro (i.e. flexible ligands but rigid receptor allowing fast docking and scoring of all molecules). This was followed by pharmacophore, shape matching and fingerprint search using queries made from existing known protease inhibitors. Flexible docking was used to rank order the compounds. We exploited properties of existing HIV protease inhibitors to identify potential viral protease inhibitors as this is one of the promising options [8].

2.1 Shape similarity search-

Commercial databases were screened by ROCS (Rapid Overlay of Chemical Structures) [9, 10], a Gaussian-shape volume overlap filter that can identify shapes that match the query molecule. Shape queries were made and databases were screened to find a match to the shape of existing HIV protease inhibitors (namely Lopinavir, Ritonavir and Darunavir). Conformations for this search were generated by OMEGA [11], from Open eye.

 doi:10.20944/preprints202004.0015.v2

2.2 Docking-

The structure of the viral protease was obtained from RCSB protein databank (pdb 6W63). Compounds from database were docked to this protease using MOE (Molecular Operating Environment) software [12]. 230560 conformations of 2827 molecules that passed phase-I clinical trials were docked with rigid receptor and high scoring compounds were selected for further screening. Compounds with high scores that satisfied at least one of the three pharmacophores were selected.

2.3 Pharmacophore modeling –

Pharmacophore models were developed using docked conformations of Ritonavir, Lopinavir and Darunavir. Figure 2 shows the site where all top conformations dock in a blind docking experiment and Figure 3 shows the pharmacophores. First pharmacophore query (Table-1) was based on top 5 docked conformations of Darunavir on 3CL protease and consisted of 8 features. A compound is chosen for docking if it satisfies 6 of these 8 features. The second pharmacophore (Table-2) was based on top 5 docked conformations of Ritonavir on 3CL protease and consisted of 6 features. A compound is chosen for docking if it satisfies 5 of these 6 features. The third pharmacophore (Table-3) was based on top 5 docked conformations of Lopinavir on 3CL protease and consisted of 5 features. A compound is chosen for docking if it satisfied 4 of these 5 features. The database compounds were screened by using these pharmacophore queries.

The conformations of the ligands used to develop pharmacophores were obtained from docking Lopinavir, Ritonavir and Darunavir to protease 3CL pro. A ligand library (drug repurposing library consisting of 2827 compounds obtained from selleckchem

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2020 doi:10.20944/preprints202004.0015.v2

https://www.selleckchem.com/screening/drug-repurposing-library.html) was prepared using MOE. The conformations of database compounds to be docked were generated using OMEGA [11], from OpenEye Scientific Software, Inc.

2.4 Fingerprinting –

The structures of existing protease inhibitors are available. Similarity searches were performed using different fingerprinting to obtain compounds from the drug repurposing library. This was done using MOE (Chemical Computing Group). The idea behind calculating MOE fingerprints is to identify substructures in the database, which are similar to active molecules.

3. Results and discussion

We tried to apply virtual screening protocols to identify inhibitors of protease 3CL based on molecular docking of 2827 small molecules that have passed into at least phase-I clinical trials. Pharmacophore and shape match searches of these molecules were based on the known existing protease inhibitors in general. The first docking run involved docking of all 2827 molecules to the rigid receptor (Protease 3CL pro) where side chains were not allowed to move. This was followed by pharmacophore search, shape match and fingerprint searches. For a compound to be considered as a potential hit, the following criteria were applied:

- It should either satisfy features of at least one of the three pharmacophore queries and should have a good docking score.

 doi:10.20944/preprints202004.0015.v2

- Or, it should have at least a 65% value of the Tanimoto similarity coefficient (fingerprinting method) with respect to the existing protease inhibitors and should have a good docking score.
- Or, it should have shape similarity to existing protease inhibitors and should have a good docking score.

The top scoring compounds from the shape similarity, fingerprint similarity, and pharmacophore searches that survived the above-mentioned criteria were then used for blind docking runs with a flexible receptor (where side chains were free to move) in order to rank them with the existing protease inhibitors. MOE [12, 13] was used to generate phamacophores. Docking helped us to identify complementary orientations of small molecules in the binding site of our target protease and to evaluate the generated docked poses with scoring functions for ligand binding strength predictions. Table 4 compares docking score of our top hits with docking scores of Lopinavir, Darunavir and Ritonavir.

In this way, 2 compounds were identified as binding to the 3CL protease with a high value of the binding free energy; Cobicistat and Amprenavir (Figure 4). Cobicistat is a cytochrome P450 3A (CYP3A) inhibitor that to the best of our knowledge, has not been previously identified as a protease inhibitor. Moreover, our study identified Amprenavir as a potential blocker of the SARS-CoV02 3CL protease. This compound is known to inhibit the HIV-1 protease and was approved for human use in 1992. A prodrug version, Fosamprenavir, is now used in the clinic. Given that at the time of writing, no drugs have yet to be proven effective in treating COVID-19 disease, our work suggests that Fosamprenavir be considered as a potential treatment for COVID-19. Initially, antiviral activity against SARS-CoV-2

 doi:10.20944/preprints202004.0015.v2

needs to be demonstrated in infected cell cultures (work in progress). Very recently, other computational groups have shown independently that Amprenavir and related HIV-1 protease inhibitors can bind the 3CL protease meaning that these molecules deserve immediate attention in the treatment of COVID-19.

This computational approach has been applied to rapidly identify drugs to treat COVID-19 and is based on limited information available so far. Authors wish to caution readers against the use of these drugs without prescription till these are experimentally tested and approved for use to treat COVID-19.

References -

1. Anand, K., et al., *Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs.* Science, 2003. **300**(5626): p. 1763-7.
2. Lauro, G., et al., *A Combinatorial Virtual Screening Approach Driving the Synthesis of 2,4-Thiazolidinedione-Based Molecules as New Dual mPGES-1/5-LO Inhibitors.* ChemMedChem, 2020. **15**(6): p. 481-489.
3. Kouman, K.C., et al., *Structure-Based Design and in Silico Screening of Virtual Combinatorial Library of Benzamides Inhibiting 2-trans Enoyl-Acyl Carrier Protein Reductase of Mycobacterium tuberculosis with Favorable Predicted Pharmacokinetic Profiles.* Int J Mol Sci, 2019. **20**(19).
4. Crochet, R.B., et al., *Investigating combinatorial approaches in virtual screening on human inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3): a case study for small molecule kinases.* Anal Biochem. **418**(1): p. 143-8.
5. Tanrikulu, Y., B. Kruger, and E. Proschak, *The holistic integration of virtual screening in drug discovery.* Drug Discov Today.
6. Ahmed, A., et al., *Identification of novel serotonin 2C receptor ligands by sequential virtual screening.* Bioorg Med Chem, 2009. **17**(13): p. 4559-68.
7. Mochalkin, I., et al., *Discovery of antibacterial biotin carboxylase inhibitors by virtual screening and fragment-based approaches.* ACS Chem Biol, 2009. **4**(6): p. 473-83.
8. Li, G. and E. De Clercq, *Therapeutic options for the 2019 novel coronavirus (2019-nCoV).* Nat Rev Drug Discov, 2020. **19**(3): p. 149-150.
9. Rush, T.S., 3rd, et al., *A shape-based 3-D scaffold hopping method and its application to a bacterial protein-protein interaction.* J Med Chem, 2005. **48**(5): p. 1489-95.
10. Hawkins, P.C., A.G. Skillman, and A. Nicholls, *Comparison of shape-matching and docking as virtual screening tools.* J Med Chem, 2007. **50**(1): p. 74-82.

11. *OMEGA 3.1.2.2: OpenEye Scientific Software, Santa Fe, NM, USA. http://www.eyesopen.com, 2019.*
12. *Molecular Operating Environment (MOE), 2019.01; Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2019.*
13. Kaserer, T., et al., *Pharmacophore Models and Pharmacophore-Based Virtual Screening: Concepts and Applications Exemplified on Hydroxysteroid Dehydrogenases.* Molecules, 2015. **20**(12): p. 22799-832.

Tables -

Table 1 -Description of features in Pharmacophore based on top 5 docked Darunavir poses

Feature ID	Feature	Description
F1	Aro|Hyd	Annotates aromatic rings and hydrophobe
F2	Hyd	Annotates Hydrophobe
F3	Aro|Hyd	Annotates aromatic rings and hydrophobe
F4	Acc2	Annotates projected locations of potential H-bond donors
F5	Acc2	Annotates projected locations of potential H-bond donors
F6	Acc2	Annotates projected locations of potential H-bond donors
F7	Acc2	Annotates projected locations of potential H-bond donors
F8	Acc2	Annotates projected locations of potential H-bond donors

Table 2 -Description of features in Pharmacophore based on top 5 docked Ritonavir poses

F1	PiN	the π-system plane normal
F2	PiN	the π-system plane normal
F3	Aro|Hyd	Annotates aromatic rings and hydrophobe
F4	Aro|Hyd	Annotates aromatic rings and hydrophobe
F5	Aro|Hyd	Annotates aromatic rings and hydrophobe
F6	Hyd	Annotates Hydrophobe

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2020 doi:10.20944/preprints202004.0015.v2

Table 3 -Description of features in Pharmacophore based on top 5 docked Lopinavir poses

F1	PiN	the π-system plane normal
F2	Aro|Hyd	Annotates aromatic rings and hydrophobe
F3	Aro|Hyd	Annotates aromatic rings and hydrophobe
F4	Aro|Hyd	Annotates aromatic rings and hydrophobe
F5	Don	Annotates an H-bond donor heavy atom

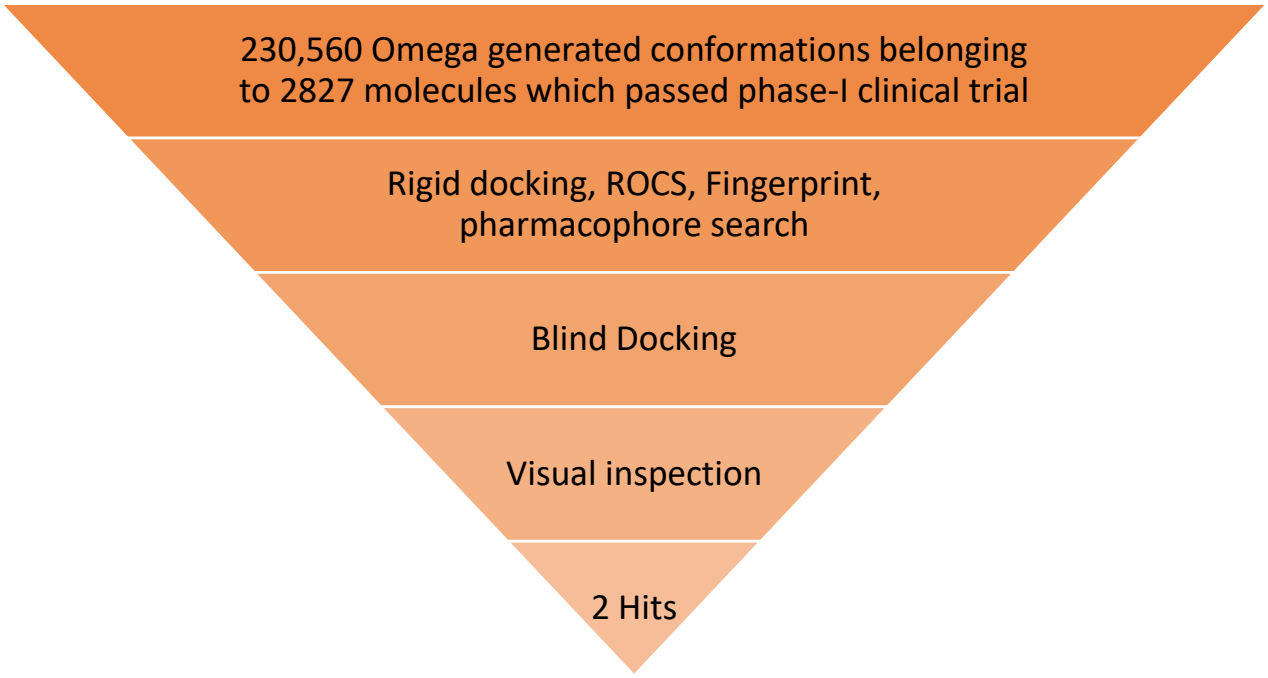
Table 4 - Comparison of Scores after blind flexible docking on Protease 3CL pro

Molecule name	Scores in Kcal/mol
Cobicistat	-16.4773
Amprenavir	-14.1705
Lopinavir	-13.9381
Darunavir	-13.7188
Ritonavir	-14.3478

 doi:10.20944/preprints202004.0015.v2

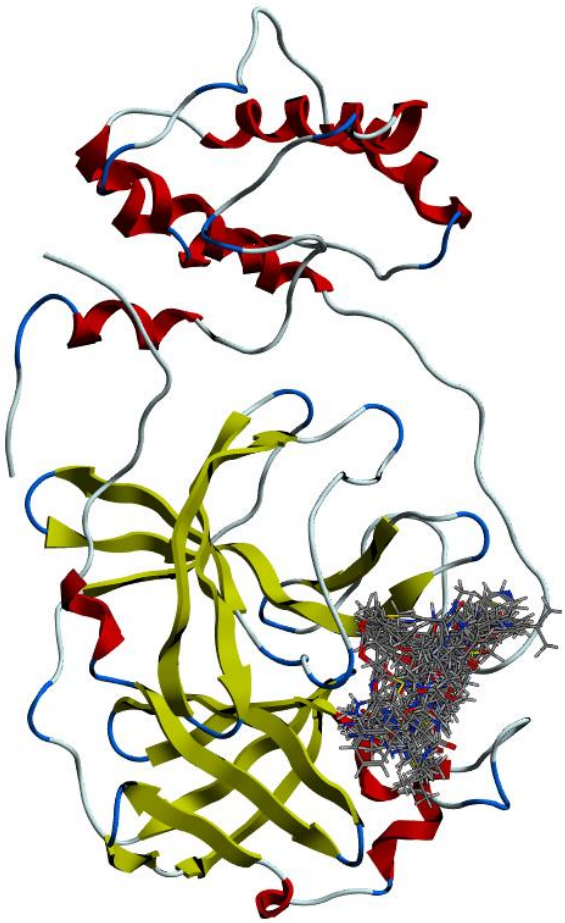
Figures -

Figure-1 Virtual screening protocol



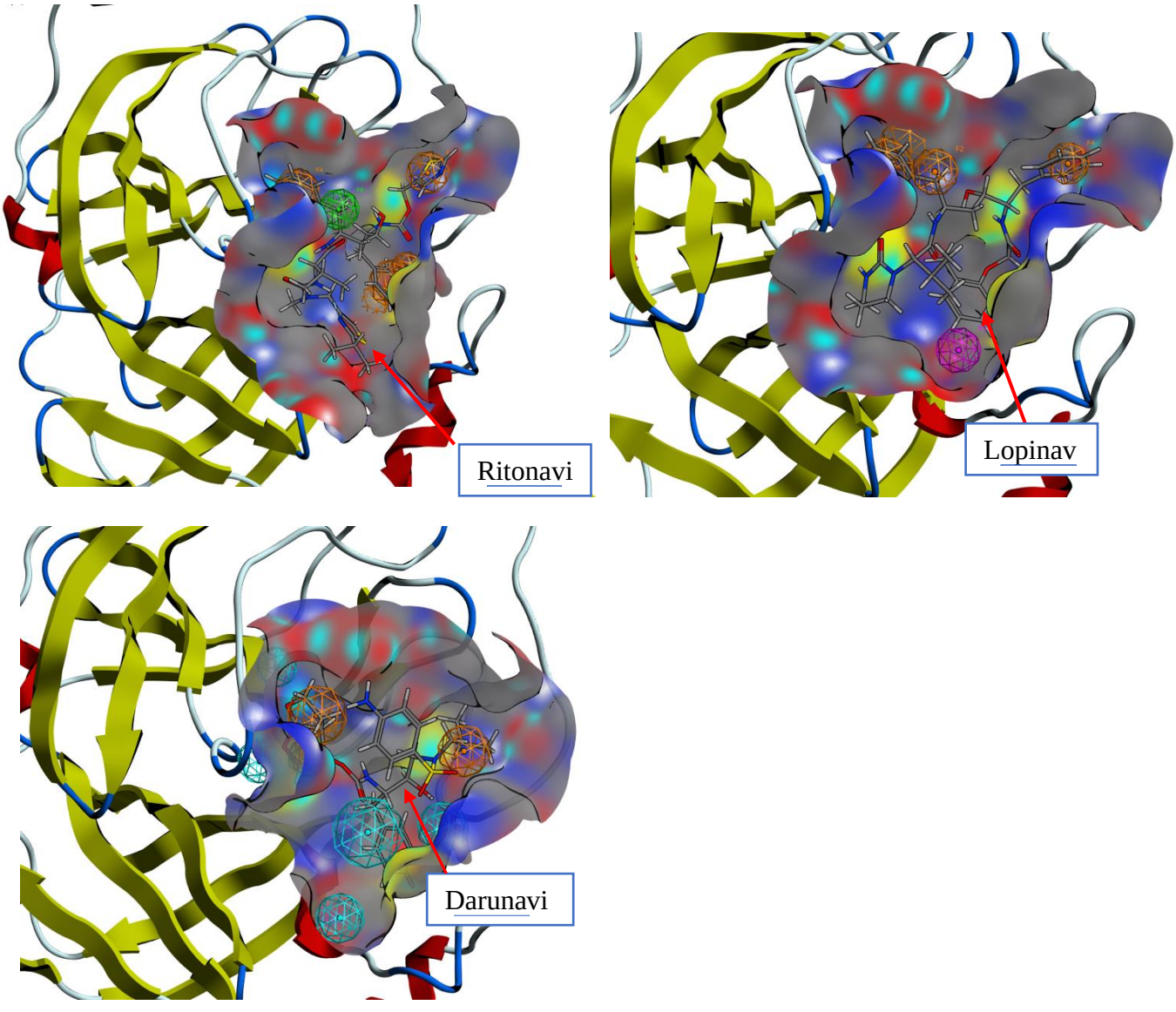
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2020 doi:10.20944/preprints202004.0015.v2

Figure-2 Site in Protease 3CL pro where top poses of existing inhibitors dock.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2020 doi:10.20944/preprints202004.0015.v2

Figure-3 Pharmacophores derived from top 5 conformations of Ritonavir, Lopinavir and Darunavir (Only one conformation is shown in Figure for better visibility of features)



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2020 doi:10.20944/preprints202004.0015.v2

Figure-4 Amprenavir in binding site of protease 3CL pro