Identification of a novel dual-target scaffold for 3CLpro and RdRp proteins of SARS-CoV-2 using 3D-similarity search, molecular docking, molecular dynamics and ADMET evaluation

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ABSTRACT
The new SARS-CoV-2 coronavirus is the causative agent of the COVID-19 pandemic outbreak that affected whole the world with more than 6 million confirmed cases and over 370,000 deaths. At present, there are no effective treatments or vaccine for this disease, which constitutes a serious global health crisis. As the pandemic still spreading around the globe, it is of interest to use computational methods to identify potential inhibitors for the virus. The crystallographic structures of 3CLpro (PDB: 6LU7) and RdRp (PDB: 6ML7) were used in virtual screening of 50000 chemical compounds obtained from the CAS Antiviral COVID19 database using 3D-similarity search and standard molecular docking followed by ranking and selection of compounds based on their binding affinity, computational techniques for the sake of details on the binding interactions, absorption, distribution, metabolism, excretion, and toxicity prediction; we report three 4-(morpholin-4-yl)-1,3,5-triazin-2-amine derivatives; two compounds (2001083-68-5 and 2001083-69-6) with optimal binding features to the active site of the main protease and one compound (833463-19-7) with optimal binding features to the active site of the polymerase for further consideration to fight COVID-19. The structural stability and dynamics of lead compounds at the active site of 3CLpro and RdRp were examined using molecular dynamics (MD) simulation. Essential dynamics demonstrated that the three complexes remain stable during simulation of 20 ns, which may be suitable candidates for further experimental analysis. As the identified leads share the same scaffold, they may serve as promising leads in the development of dual 3CLpro and RdRp inhibitors against SARS-CoV-2.

1. Introduction

The recent emerged coronavirus outbreak of severe asymptomatic pneumonia has geographically appeared as a human pathogen in the city of Wuhan, China. This virus was designated as 2019-nCoV at the very beginning of its emergency in China (Wang et al., 2020). Then, it is named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group (CSG) of International Committee on Taxonomy of Viruses (ICTV) based on the novelty and comparative genomic analysis. The SARS-CoV-2 belongs to the family Coronaviridae, which includes RNA viruses, and it is the third highest deadly human coronavirus reported in the 21st century followed by the SARS and MERS, expressing the highest fatality rate (Wu et al., 2020). The new virus is closely related (89.1% and 60% nucleotide sequence similarity) to SARS and MERS coronaviruses, respectively. On January 31, 2020, the (world Health Organization) WHO declared COVID-19 as a public health emergency of international concern. We have witnessed the rapid spread of COVID-19; which is the official name of diseases caused by SARS-CoV-2; that has already gripped all the continents of the globe with multiple epicenters, but the death curve is noticeably higher in America and Europe, with the worst hit reported in USA, Spain and Italy. By June, 01 2020, COVID-19 the pandemic has affected more than 6,230,000 confirmed cases, and lead to at least 374,000 deaths over 213 countries and territories.

Some studies investigated potential of combinations of human immunodeficiency virus protease inhibitor lopinavir/ritonavir as antiviral therapy to treat COVID-19. Along with the effort of the respected researchers, we put the credit to Liu et al. who have successfully established crystal structure of main protease (Mpro) or chymotrypsin-like protease (3CLpro) from COVID- 19, and deposited in the Protein Data Bank (PDB) for public access (https://www.rcsb.org/structure/6LU7). This enzyme plays an essential role in processing of translated polyproteins, such as protease inhibitors are
believed to be the right choice to halt the virus life cycle. Xu et al. reported that nelfinavir was identified as the best potential inhibitor against SARS-CoV-2 3CLpro, based on molecular docking studies among 4 drug compounds (nelfinavir, perampanel, praziquantel and pitavastatin). In addition, RNA-dependent RNA polymerase (RdRp), an essential enzyme in viruses replication, which make it, another key target to find therapeutic agents to COVID-19. Literature studies reported that antiviral drug Remdesivir as a potent inhibitor to virus replication.

While certain physical treatment has been shown to assist patients to fight this disease with their own immune systems, till no there is no proven remedies (antiviral drugs or vaccines) for COVID-19, which is aggravating the situation. Thus, identification or discovery of new effective antivirals is urgently needed to fight the worldwide corona crisis.

Computer-aided drug discovery/design methods (CADD) have played a major role in the development of therapeutically important small molecules for over three decade, screening chemical virtual libraries using computational methods as molecular docking can save money and reduce time (Aouidate et al., 2018a), consequently, speed up the identification of potential drug candidates (Aouidate et al., 2018b; Lipinski et al., 1997). Several research groups have come up with interesting strategies such as repurposing existing drugs or natural products to fight against COVID19(Das et al., 2020; Khan et al., 2020; Mittal et al., 2020), identification of peptide like small molecules using virtual screening of large chemical databases (Pant et al., 2020) or using fragment based approach to design new binders of main protease corona virus (Choudhury, 2020). As we are running of time and the virus is spreading quickly, we have screened the CAS COVID-19 Antiviral Compound Dataset, which includes ~50000 chemical compounds against 3CLpro and RNA-dependent RNA polymerase (RdRp) using computational methods and ligand and structure based screening and in this study, we report the identification of different compounds with CAS IDs (2001083-69-6, 2001083-68-5, 63248-75-9, 264621-13-8, 1025098-90-1, 1253912-09-2) as potent inhibitors of 3CLpro and (833463-10-8, 833463-11-9, 833465-33-1, 2001083-69-6, 833463-19-7, 833464-45-2) as potent inhibitors RNA-dependent RNA-polymerase (RdRp), most compounds are 4-(morpholin-4-yl)-1,3,5-triazin-2-amine derivatives, the analysis of SARS-CoV-2 main protease and RNA-dependent RNA polymerase binding sites reveals are combinations of hydrophobic, hydrophilic and charged residues holding with hydrogen bonds in excess, therefore, the 1,3,5-triazine that is aligned centrally in both proteins binding pockets could be a good choice to occupy the central part of the molecules to be substituted by different hydrophobic, hydrophilic and charged fragments.

In this work we identified a number of drug-like compounds, which led to the discovery of several chemical entities that provide new scaffolds that could serve as the core of novel 3CLpro and RdRp inhibitor families. All the four compounds were employed for 20ns MDS study. On the basis of various parameters like RMSD, RMSF, Rg and SASA, we report 2001083-68-5, 2001083-69-6 and 833463-19-7 (Figure 6) that they present the same scaffold as lead compounds, which could serve as dual inhibitors for 3CLpro and RdRp, respectively. Though, further in vitro and/or in vivo research is required to validate the CADD results.

2. Material and methods

2.1. Collection and curation of the chemical library

For VS, the chemical structures of 50000 compound include antiviral drugs and related compounds that are structurally similar to known antivirals (CAS COVID-19 Antiviral Compound Dataset) were downloaded as 3D sdf files from the Chemical Abstract Service database (https://www.cas.org/covid-19-antiviral-compounds-dataset) and prepared with an in-house KNIME workflow (using RdKit and 3D-e-Chem nodes) (Kooistra et al., 2018; Landrum, n.d.), first duplicated molecules, compounds with Pan Interference Assay Structures (PAINS) (Dahlin et al., 2015) have been deleted and salts have been stripped out, then best tautomer for every molecule has been generated and optimized, next a modified Lipinski’s rule(Lipinski, 2004; Lipinski et al., 1997) (with 300 ≤ Mw ≤ 700 g/mol, 5 ≤ Number of rotatable bonds ≤ 12) has been applied to filter out non drug like molecules, which allowed us to narrow down the database to 5378 drug like compound.

This filtered database has been submitted to a simple-pharmacophore based VS (3D-similarity search (Dobi et al., 2014)) workflow as a first step, and obtained molecules from this step have finally used in a VS using molecular docking.

2.2. 3D-similarity virtual screening

A multi-conformational version of the database was generated; 30 conformer per molecule were generated from the 5378 molecule left from the curation and preparation step; this resulted in a virtual library containing a total of 161256 conformers. An in-house KNIME workflow was employed as a first step of VS process, using 3D-generated pharmacophores from N3 and Remdesivir (Figure 1) as search terms to screen the database.

As there are just few inhibitors known for the two new studied proteins of SARS-CoV-2, here we suggest a simple approach to obtain focused databases for 3CLpro (N3 Cocystalized ligand) and RdRp SARS-CoV-2 targets based on single ligand pharmacophore, unlike other ligand-based VS methods, the main feature of our approach; is using similarity search in 3D (Dobi et al., 2014); is based on two fitting methods, which could enhance the accuracy, the first one based on overlapping the pharmacophores generated from the candidate structure (B from a database) and the query (reference molecule A), then rank them based on the Root Mean Squared Deviation (RMSD). The formula of RMSD is given by the Equation (1):

\[
RMSD = \sqrt{\frac{\sum_{i=1}^{n} (x_i - y_i)^2}{n}}
\]  

The second one is using T_{AB} score, which is a similarity between the reference structure, A and database aligned
molecules, B are represented by vector, $x$, of length $n$ with the $i$th property having the value $x_i$ (Sheridan, 2007). The formula of Tanimoto coefficient (Dobi et al., 2014) is given by the Equation (2):

$$T_{A,B} = \frac{\sum_{i=1}^{n} x_i A \cdot y_i B}{\sum_{i=1}^{n} x_i A^2 + \sum_{i=1}^{n} y_i B^2 - \sum_{i=1}^{n} x_i A \cdot y_i B}$$

(2)

Keeping only the first best 1000 mapped structures from every scoring method, concatenate the results, and finally select the common mapped structures (intersection) between the two databases, which has reduced the database to 205 and 186 molecules for 3CLpro and RdRp, respectively.

2.3. Structure-based virtual screening

The smina software (Koes et al., 2013) was used to carry out molecular docking. The recently resolved 3D structures of SARS-CoV-2 main protease (PDB code: 6LU7 (Chang et al., 2020)) in complex with a the covalent peptide N3 and SARS-CoV-2 RNA dependent RNA polymerase (PDB code: 6LM7) (Gao, 2020) were downloaded from the Protein Data Bank (www.rcsb.org). To prepare the selected proteins for molecular docking, the co-crystallized water and small molecules were removed, protein structures were protonated using a grid box with a size of $(x=117.9, y=116.9, z=130.6)$ and a center of $(x=21.3, y=23.9, z=31.1)$ was set to cover the N3 binding site in the 6LU7. While a grid box with a size of $(x=115.7, y=18.2, z=21.5)$ and center of $(x=-10.782, y=15.787, z=6.277)$ was set to cover the N3 binding site in the 6LU7. Ligand Topology files were generated using PRODRG server (http://prodrg2.dlyndns.org/submit.html). 3CLpro–ligand and RdRp–ligand complexes were solvated and fully immersed in the center of a cubic box prior using TIP3P water model. Protonation and structure minimization were performed using Charm36 force field, where hydrogens were added for optimal hydrogen bond network by default.

The top hits with best pharmacokinetics properties were shortlisted and further analyzed by molecular dynamic simulations using GROMACS 2018 (Groningen Machine for Chemical Simulations) (Páll et al., 2015; Van Der Spoel et al., 2005). Protonation and structure minimization were performed using the charmm36 force field, where hydrogens were added for optimal hydrogen bond network by default. 3CLpro–ligand and RdRp–ligand complexes were solvated and fully immersed in the center of a cubic box prior using TIP3P water model. The number of water molecules adopted to solvate the complex were as follow: 3CLpro_2001083-69-6 (20,052 molecules); 3CLpro_2001083-68-5 (20,052 molecules); RdRp_833463-10-8 (57,268 molecules); RdRp_833463-19-7 (57,268 molecules). Four and eleven Na+ ions were added for the neutralization of the 3CLpro and RdRp systems, respectively, and subsequent energy minimization was performed. The energy

2.5. In silico pharmacokinetics (PK)/pharmacodynamics (PD) evaluation

The success of a drug journey through the body is measured regarding its pharmacokinetics parameters (Adsorption, distribution, metabolism, excretion and toxicity; ADMET). For this purpose, the best-identified hits were evaluated for their in silico pharmacokinetis parameters using pkCSM (Pires et al., 2015) to prevent the failure of those compounds during clinical studies and enhance their chances to reach the stage of being candidates drugs in future.

2.6. Molecular dynamics

The results from the structure based VS (for 3CLpro and RdRp) were separately processed using an in-house KNIME workflow (Berthold et al., 2007), firstly molecules were ranked according to the binding energy of their best scoring conformation then the 30 top ranked candidates were inspected visually for their interactions with the hot spot residues, finally selected molecules were submitted for further analysis (ADMET prediction and molecular dynamics). Visual inspection of docking poses and the analysis of protein ligand interactions were performed in Biovia Discovery Studio Visualizer version 2016 (Dassault systems Biovia corp) (Dassault Systèmes BIOVIA, 2016). Visualization images were rendered by PyMOL 2.3 (Schrodinger L.L.C)(DeLano, 2002).

Figure 1. a) 3D-pharmacophores generated from Remdesivir and N3 inhibitors for RNA and 3CLpro respectively. With Green: Aromatic and lipophilic features, Magenta: Hydrogen bond acceptor features, Cyan: Hydrogen bond donor features, Orchid: Hydrogen bond donor and acceptor features.
minimization of systems was performed using the steepest descent minimization of 5,000 steps (maximum number of minimization steps to perform) to remove the week Van der Waals contacts. Energy minimization was stopped when the maximum force was less than 1.0 kJ/mol. The system was further equilibrated for 50 ps at constant volume and a temperature of 300 K. The molecular dynamic simulations were run for 20,000 ps for each protein–ligand complex, where the coordinates were saved every two ps interval. The calculation of electrostatic interactions were treated by the Particle-Mesh Ewald (PME) (Darden et al., 1993) method. Van der Waals interactions were set at 1.2 nm. LINear Constraint Solver (LINCS) algorithm was applied to constraint the covalent bonds, including heavy atom-H bonds during the molecular dynamics (MD) simulations. The gmx rms, rmsf, gyrate, gmx hbond, gmx sasa were used for the calculation of RMSD, RMSF, Rg, Hydrogen bonds and SASA respectively.

Lastly, the trajectories were saved for further analysis using the Xmgrace (Turner, n.d.), pymol (DeLano, 2002) and UCSF Chimera (Pettersen et al., 2004).

3. Results and discussion

The aim of the current study is to identify novel potential lead compounds as inhibitors for 3CLpro and RdRp proteins. In silico VS of ~50 thousand molecules was performed by establishing a pipeline of PAINS and drug-like filters to assess drug likeness, then N3 and Remdesivir were used as simple-pharmacophores targets for 3D-similarity search to ensure the preparation of focused databases for both proteins, after that compounds that showed a strong binding affinity for 3CLpro and RdRp proteins using molecular docking were selected for further investigations. Further, the ADMET properties were predicted and analyzed to filter top hits and identify suitable lead candidates, the conformational stability of the best ligands in complex with proteins were studied using MD simulation. The current in silico study was undertaken to identify efficient antivirals compounds for COVID19.

3.1. Virtual screening

The prepared chemical database was submitted to a simple 3D-similarity search filter workflow, which was designed by identifying the main features of the N3 and Remdesivir ligands that are involved in interactions with 3CLpro and RdRp (Figure 1), in order to generate focused databases for both proteins. As there is no ligand in complex with the RdRp protein of SARS-CoV-2, which is known to be a target for antiviral drugs. Recommendations of Goa et al.; the authors resolved the structure and in many other studies (Cao et al., 2020; Gao, 2020; Shannon et al., 2020); prompted us to consider Remdesivir as reference molecule for our ligand-based screening company against this target. For 3LCpro the N3 has been considered as reference molecule for our ligand-based VS. The pharmacophore features generated from Remdesivir and N3 are shown in (Figure 1).

Results from the first ligand based VS generated total of 255 and 186 compounds that showed positive result from the two ranking methods RMSD and TA,B, so they have been selected as focused databases for 3LCpro and RdRp, respectively.

Smina was used to dock the focused databases from CAS-COVID19-antivirals database. This resulted in a final ranked list, the predicted binding modes of the top 30 molecules were inspected manually for final selection. 6 compounds for 3LCpro (Figure 2) and 6 compounds for RdRp (Figure 4) of this list were chosen for acquisition and ADMET prediction. Table 1 lists their details.
3.1.1. 3CLpro-ligand complexes

For 3CLpro we observed that most of the hit compounds show common interactions and are involved in H-bond interaction with residues Phe140, Glu143, Asn142, Ser144, Cys145, His163, Glu166, His172, Gln189, Thr190 and hydrophobic interaction with His41, Met49, Met165 which is found consistent with recent studies (Macchiagodena et al., 2020; Ul Qamar et al., 2020).

Among the top 6 hit molecules from CAS COVID19 Dataset, compounds 2001083-69-6 and 2001083-68-5 show the highest affinity \( -9.064 \text{ Kcal/mol} \) and \( -8.879 \text{ Kcal/mol} \) respectively and also good ADMET analysis (Table 2). Binding interactions of top hit molecules, compounds 2001083-69-6 and 2001083-68-5 with 3CLpro binding pocket are shown in Figure 3(a, b).

Compound 2001083-69-6 forms three strong hydrogen bonds, the first one between the \(-\text{NH} \) of the Gly143 residue and the Fluorine of the difluoromethyl group (\( \text{NH} - \text{F} \) at distance of 2.73 Å), the second one between Oxygen of the carboxyl group substituent on the piperidinyl moiety and the \(-\text{SH} \) of the Cys145 dyad residue (\( \text{O} - \text{SH} \) at distance of 3.65 Å), the third one between the Oxygen of OH in the carboxyl group substituent on the piperidinyl moiety and the hydrogen of the OH group of the Ser144 residue, which can be seen in green dotted lines as shown in Figures 2(a) and 3(a).

Also, 1,3,5-triazine moiety and morpholine ring are aligned centrally to the binding pocket are involved in hydrophobic interaction with dyad residue His41 and Met49 residues.

Compound 2001083-68-5 is stabilized by four strong hydrogen bonds, the two first one are between OH of the carboxyl substituent of the piperidinyl moiety and Oxygen of the carbonyl of the Phe140 and the Glu166 residues (\( \text{OH} - \text{O} \) at distance of 1.89 and 2.48 Å) and the third and fourth ones are between Oxygen of the carbonyl group in the piperidinyl moiety and the \(-\text{NH} \) of the His163 and His172 residues (\( \text{O} - \text{NH} \) at distance of 2.42 and 3.08 Å), which can be seen in green dotted lines. The molecule is also stabilized by different pi-pi alkyl interactions dyad residues His41 and Cys145 as shown in Figures 2(b) and 3(b).
Figure 4. 3D-Binding modes of the six hits compounds in the substrate-binding site of RdRp. (A) 833463-10-86 (Δ Δ 9.529 Kcal/mol), (B) 833463-11-9 (Δ Δ 9.205 Kcal/mol), (C) 833463-33-1 (Δ Δ 9.162 Kcal/mol), (D) 178477-46-8 (Δ Δ 8.895 Kcal/mol), (E) 2001083-69-6 (Δ Δ 8.850 Kcal/mol) and (F) 833463-19-7 (Δ Δ 8.816 Kcal/mol). The motifs (A-G) are colored in (A) green, (B) light pink, (C) white green, (D) Magenta, (E) Red, (F) Yellow and (G) Cyan respectively.

Table 1. List of Smina binding affinities for potential COVID19 3CLpro and RdRp.

| CAS ID      | Hydrogen bonds                          | Other interactions               | Binding affinity |
|-------------|-----------------------------------------|----------------------------------|------------------|
| 3CLpro inhibitors |                                          |                                  |                  |
| 2001083-69-6 | Glu143, Cys145, Ser144                  | His41, Leu27, Arg188, Met165, Thr26, Met49 | −9.064           |
| 2001083-68-5 | Glu166, Phe140, His163, His172          | His164, Arg188, Cys145, His41, Leu27, Thr26, Met49 | −8.879           |
| 63248-75-9  | Ser144, Cys145, Asn142, Thr190, Arg188  | His41, Leu27, Met165, Cys145     | −9.162           |
| 264621-13-8 | Thr26, Cys145, His163, Glu166           | His172, Met49, Met165            | −8.564           |
| 1025098-90-1 | Glu143, Cys145, Ser144, Glu166         | Met165, His41, Leu27, Met49      | −8.558           |
| 1253912-09-2 | Glu166, Glu143, Glu189                 | Cys145, Leu27, Asn142, His41, Met165, Pro168 | −8.400           |
| RdRp inhibitors |                                          |                                  |                  |
| 833463-10-8  | Tyr455, Arg553, Tyr619                  | Arg624, Asp623, Lys621, Asp670, Asp618 | −9.529           |
| 833463-11-9  | Arg553, Tyr619                          | Asp618, Tyr455, Arg624, Asp623, Lys621, Asp760 | −9.205           |
| 833465-33-1  | Lys621                                 | Asp618, Pro620, Tyr455, Arg553, Lys798 | −9.162           |
| 2001083-69-6 | Arg553, Arg624, Thr556, Asn691, Thr687, Thr680 | Lys621, Arg555                  | −8.850           |
| 833463-19-7  | Arg553, Tyr619, Ser682                 | Arg624, Lys621, Asp623, Asp618, Asp760 | −8.816           |
| 833464-45-2  | Tyr619                                 | Asp618, Asp760, Arg624, Lys621, Asp623, Arg553 | −8.751           |

Table 2. In silico ADMET prediction of identified 3CLpro and RdRp SARS-Cov-2 inhibitors.

| CAS ID      | Blood-Brain Barrier (LogBB) | Intestinal absorption | Caco-2 permeability | CYP2D6 Substrat | CYP3A4 Substrat | CYP2D6 Inhibitor | CYP3A4 Inhibitor | Clearance | AMES Tox | hERG inhibition |
|-------------|-----------------------------|-----------------------|---------------------|-----------------|-----------------|------------------|------------------|-----------|----------|-----------------|
| 3CLpro inhibitors |                              |                       |                     |                 |                 |                  |                  |           |          |                 |
| 2001083-69-6 | −2.06                        | 71.31                 | 0.99                | No              | No              | No               | No               | 0.032     | No       | No              |
| 2001083-68-5 | −2.04                        | 70.55                 | 1.012               | No              | No              | No               | No               | 0.068     | No       | No              |
| 63248-75-9  | −0.37                        | 86.04                 | 0.699               | No              | Yes             | No               | Yes              | 0.817     | No       | No              |
| 264621-13-8 | −1.19                        | 88.25                 | 0.972               | No              | Yes             | No               | Yes              | 0.284     | No       | No              |
| 1025098-90-1 | −0.35                        | 94.93                 | 1.285               | No              | Yes             | No               | Yes              | 0.385     | No       | No              |
| 1253912-09-2 | −0.88                        | 77.30                 | 0.898               | No              | Yes             | No               | Yes              | 0.696     | Yes      | No              |
| RdRp inhibitors |                              |                       |                     |                 |                 |                  |                  |           |          |                 |
| 833463-10-8  | −1.824                       | 96.449                | 0.84                | No              | Yes             | No               | No               | −0.473    | No       | No              |
| 833463-11-9  | −1.367                       | 99.29                 | 1.23                | No              | Yes             | No               | No               | −0.126    | No       | No              |
| 833465-33-1  | −0.947                       | 100                   | 1.33                | No              | Yes             | No               | Yes              | 0.26      | No       | No              |
| 2001083-69-6 | −2.06                        | 71.31                 | 0.99                | No              | No              | No               | No               | 0.032     | No       | No              |
| 833463-19-7  | −1.543                       | 100                   | 1.123               | No              | Yes             | No               | No               | 0.026     | No       | No              |
| 833464-45-2  | −1.570                       | 98.730                | 1.22                | Yes             | No              | No               | No               | 0.333     | Yes      | No              |
3.1.2. RdRp-ligand complexes

For RdRp we observed that most of the hit compounds are involved in interactions with residues functionally important aspartate residue of motif A (Asp623) and motif C (Asp760), together with conserved Arginine residues of motif F (Arg553), which were found highly interacted with the compounds as shown in Table 1. Among these, motif A and C are most strikingly conserved aspartate residues that bind divalent metal ions necessary for catalytic activity.

Based on different binding energies (Table 1) and ADMET properties (Table 2) of different hits, two tops hits 833463-10-8 and 833463-19-7, which show good ADMET properties in comparison to others were chosen for further investigations.

In the docking analysis, three hydrogen bonds between compound 833463-10-8 and RdRp SARS-CoV-2 binding pocket were formed. The first hydrogen bond 2.84 Å (Tyr455-N−F) was mediated by the Trifluoromethyl group, the second one 2.97 Å (Arg553-NH3+/C0N) was formed by the N of the 1,3,5-triazine moiety and the Asp553 of the motif F, and the third 2.36 Å (Tyr 619-NH–F) was formed between the Trifluoromethyl of the pyridine moiety and the NH of the Tyrosine backbone, which can be seen in green dotted lines in Figures 4(a) and 5(a).

The molecule is also stabilized by three carbon hydrogen bonds with Asp618 (motif A), Lys621 (motif A) and Asp760 (motif C), and two Pi-Anion interactions with Asp623 (motif A) and Asp760 (motif C) and two Pi-Cation interactions with Arg553 (motif C) and Lys621 (motif A).

As depicted in Figures 4(f) and 5(b), compound 833463-19-7 is stabilized by three hydrogen bonds between the NH of the backbone Tyr619 (motif A) and Trifluoromethyl group A (Tyr619-NH−F) at distance 2.34 Å and one hydrogen bond with Ser682. In addition 1,3,5-triazine moiety, which is aligned centrally to the binding pocket it its involved in H-bond and Pi-Cation interaction with Arg553 of motif F, one Pi-Cation interaction with Lys621 (motif A), also the molecule is stabilized by a Pi-Cation Asp623(motif A), one hydrogen carbon interaction with Asp760 (motif C).

3.2. ADMET properties

In drug discovery, the prediction of ADMET properties is an important study to escape the failure of drugs in the clinical phases (Kennedy, 1997). Pharmacokinetic and bioavailability predictions are an essential tool in drug discovery process and should be considered to develop a new drug. The absorption, distribution, metabolism, excretion and toxicity predictions results are shown in Table 2, for the pkCSM predictive model, a high caco-2 permeability would translate in predicted values > 0.90, Intestinal absorbance value below 30% indicates poor absorbance. Low value of total clearance...
(logCLtot) means high drug half lifetime. For a given compound a logBB < –1 considered poorly distributed to the brain. Positive result in AMES test suggests that compound could be mutagenic.

For 3CLpro inhibitors, compounds 2001083-69-6, 2001083-68-5 and 264621-13-8 show excellent absorption and distribution properties, which could be considered as permeable compounds with poor distribution into the brain, also they present good clearance property and show no hERG inhibition nor AMES toxicity. The subtypes of cytochrome P450 CYP2D6 and CYP3A4 indicate that compounds 2001083-69-6 and 2001083-68-5 could not be substrates or inhibitors for the two mains subtypes, therefore probably could not be metabolized, thus a low chance of drug-drug interactions. That we suggest them as promising inhibitors for 3CLpro main protease of SARS-CoV-2 and have been selected for MD to investigate their stability in RdRp binding site.

Based on the analysis of different ADMET properties of proposed RdRp inhibitors (Table 2), we have found that all six compounds present satisfactory ADMET properties, however, compounds 833463-10-8 and 833463-19-7 present a combination of high intestinal absorption, high Caco-2 permeability and low blood-brain barrier values, which indicate that they show a little chance to cross the blood-brain barrier. As known, cytochrome P450 (CYP) enzymes are heme-proteins that play a key role in the metabolism of drugs, consequently, exploring those parameters may prevent their failure in clinical studies and have a deep insight on how they react in the body, which allows medicinal chemist to introduce new functional groups on the molecule to prevent the metabolic pathways susceptible to give toxic or very polar compounds that can eliminated very easily from the body. Accordingly, that can help to synthesize metabolically stable drugs, as well as to avoid drug-drug interactions. As shown in Table 2 most compounds are substrate to CYP3AD4 but not inhibitor to CYP2D6 or CYP3A4. Finally, as compounds 833463-10-8 and 833463-19-7 present good absorption, distribution and metabolism properties, also show no AMES mutagenicity or hERG inhibition properties. Thus, they have been chosen as models for MD simulation to investigate their stability in RdRp binding site.

3.3. Conformational dynamics and stability of protein-ligand complex

3.3.1. Root mean squared deviation

The applied molecular docking based virtual screening and the extensive bioavailability analysis lead to the identification of four compounds as models for 3CLpro and RdRp inhibition of coronavirus for COVID19 as shown in Figure 6, 2001083-69-6 and 2001083-68-5 for 3CLpro and 833463-10-8 and 833463-19-7 for RpRd having the most favorable binding interactions, and best pharmacokinetics profiles. Four colors (Black, Red, Blue and Magenta) have been used to make explanation smoother and easier.
However, the stability of protein-ligand complexes depends on the solvent conditions around the protein and the molecular interactions. It has been observed in several cases, that compounds presenting better docking scores and molecular interactions may fail to bind with protein in experimental results (Azam & Abbasi, 2013). Due to the limitation in computing power, the structural behavior, dynamics and flexibility of 3CLpro-ligand and RdRp-ligand complexes were assessed for 20 ns of MD simulation. The root mean square deviation values of both proteins and ligands were calculated against the initial structure in the protein–ligand complexes and plotted using the Xmgrace software to compare the protein backbone stability.

The backbone of the 3CLpro-2001083-68-5 (Red) complex showed significant fluctuation compared to the 3CLpro-2001083-69-6 (Black) complex as shown in Figure 7(a). The substantial RMSD value can be explained by the mobility of the loops. At the same time, the visual analysis of the trajectory confirms the stability of the secondary structure of the protein in both complexes. The RMSD average value for 3CLpro-2001083-69-6 (Black) (1.6 Å) was lower than that of 3CLpro-2001083-68-5 (Reds) (2.12 Å) and it is interesting to see that the RMSD curve for 3CLpro-2001083-69-6 (Black) is remarkably more stable than this of 3CLpro-2001083-68-5 (Red).

For RdRp-ligand complexes RdRp-833463-10-8 (blue) and RdRp-833463-19-7 (Magenta) shown in Figure 7(b). Overall, the backbone of RdRp-833463-10-8 (blue) shows higher RMSD value compared to RdRp-833463-19-7 (Magenta) and it is interesting to see that the RMSD curves for both protein-ligand complexes after 18 ns present almost the same conformation changes pattern within the acceptable range.

3.3.2. Root mean squared fluctuation

The root-mean-square fluctuation (RMSF) values of backbones Cα atoms were calculated by averaging over all the conformations sampled during 20 ns simulation. RMSF values provide insight into structural fluctuations as well as flexibility of different regions of protein, the RMSF fluctuated less, which meant the residues are stable, otherwise the residues are unstable.

The RMSFs of 3CLpro-2001083-69-6 (Black) and 3CLpro-2001083-68-5 (Red) are calculated and shown in Figure 8(a),
most of the proteins’ residues in both protein-ligand complexes present RMSF values below 2 Å and the general shape of the fluctuation’s curve for both complexes are the same. However, the N-terminal and C-terminal show large conformational changes as seen in Figure 8(a). We found that there are no significant changes between both ligands in the 3CLpro binding pocket.

The RMSFs of RdRp-833463-10-8 (Blue) and RdRp-833463-19-7 (Magenta) are calculated and shown in Figure 8(b), the general shape of the fluctuation’s curve for both complexes are almost the same. However, the N-terminal and C-terminal show large conformational changes as seen in Figure 8(b). We found that there is no significant change between both ligands in the RdRp binding pocket.

3.3.3. Compactness

The radius of gyration (Rg) factor is used to describe the compactness of the protein-ligand complexes during MD. It is simply a measurement of the distance between the center of mass of the protein atoms with its terminal in a given time frame. Generally, the compact protein or globular protein shows less variation in the gyration value while the expanded form of the structure showed higher Rg value. In this study, variations occurring in Rg values were plotted against time for all the complexes as shown in Figure 9(a, b) for 3CLpro and RdRp, respectively.

For 3CLpro-ligand complexes shown in Figure 9(a), the Rg curves for 3CLpro-2001083-69-6 (Black) and 3CLpro-2001083-68-5 (Red) the same type of Rg pattern with a steady behavior during the MD simulation, which indicates that both ligands binding leads the compactness in the 3CLpro protein.

For Rg curves of RdRp-ligand complexes shown in Figure 9(b), after detailed observation and analysis of the Rg plot, we found that after 4 ns, the fluctuation curves of RdRp-833463-10-8 (Blue) was higher than that of RdRp-833463-19-7 (Magenta), which indicates the stability of RdRp-833463-10-8 (Blue) complex in comparison to RdRp-833463-19-7 (Magenta).

3.3.4. Hydrogen bond analysis

The binding stabilities of the studied top hits compounds in both proteins 3CLpro and RdRp of coronavirus for COVID19 were monitored during the trajectory period of the MD simulations. The stabilities of the protein–ligand complexes were evaluated by calculating the Hydrogen bond profiles using the g_hbond tool of Gromacs and the number of hydrogen bonds vs time are plotted in Figure 10.

For 3CLpro-ligand complexes, the analysis revealed that the average numbers of H_bonds observed in 2001083-69-6 (Black) and 2001083-68-5 (Red) in 3CLpro are 1.818 with a maximum of 5 H_bonds and 1.910 with a maximum of 4 H_bonds per timeframe during the MD simulation period (Figure 10(a, b)), respectively. From the above analysis, we conclude that both complexes present good stability.

For RdRp-ligand complexes, the analysis revealed that the average numbers of H_bonds observed in 833463-10-8 (Blue) and 833463-19-7 (Magenta) in RdRp are 0.645 with a maximum of 4 H_bonds and 0.241 with a maximum of 3 H_bonds per timeframe during the MD simulation period (Figure 10(c, d)), respectively. From the above analysis, we conclude that 833463-10-8 (Blue) forms more hydrogen bonds with RdRp in comparison to 833463-19-7 (Magenta), but other properties and MD trajectories suggest that 833463-10-8 (Blue) leaves the binding pocket while to 833463-19-7 (Magenta) remains inside the binding pocket of RdRp and the hydrogens bonds are mainly produced right there.

3.3.5. RMSD, radius gyration and SASA of ligands

As the RMSF and Radius gyrate of the proteins present similar patterns, In addition to H_bond analysis, we have tried to examine three properties to illustrate the stabilities of the selected ligands in the binding pocket of 3CLpro and RdRp proteins during the simulation of 20ns as shown in Figure 11(a–f) respectively: (1) Ligand Root mean square deviation of a ligand with respect to the reference conformation (the first frame at time = 0 is used as the reference) (RMSD); (2) Radius of Gyration (Rg) - It is used to measure the ‘extendedness’ of a ligand, and is equivalent to its principal moment of inertia; (3) Solvent Accessible Surface Area (SASA) - the surface area of a molecule accessible by a H2O molecule; which can help to understand more the behavior of ligands inside binding pockets.

Properties of ligands in 3CLpro are is shown in Figure 11(a, c, e):

RMSD values of both ligands 2001083-69-6 (black) and 2001083-68-5 (Red) in the initial stage show fluctuation from 0 ns to 5 ns but later maintains constant during the simulation. The overall RMSD of these ligands were below 2 Å. However, the RMSD value and fluctuation of 2001083-68-5 were higher than 2001083-69-6. This analysis indicates that compound 2001083-69-6 (Black) in 3CLpro maintained constant RMSD during the simulation after 5 ns compared to 2001083-68-5(Red).

Also, Rg-time variation shown in Figure 11(c) for both ligands 2001083-69-6 (Black) and 2001083-68-5 (Red) in the receptor binding pocket of 3CLpro protein were observed almost constants, the 2001083-69-6 (Black) compound Rg average is 4.2 Å, which is lower than this of 2001083-68-5 (Red) (Rg average 4.45 Å), However, the Rg-time diagrams suggested steady conformation changes for both ligands.

After detailed observation, for SASA plots shown ifFigure 11(e) for both ligands 2001083-69-6 (Black) and 2001083-68-5 (Red), we found that there is no significant change between them in the 3CLpro binding pocket, because they are both present the same type pattern.

Properties of ligands in RdRp are is shown in Figure 11(b, d, f):

RMSD values of both ligands 833463-10-8 (Blue) and 833463-19-7(Magenta) are shown in Figure 11(b). The fluctuations curves of 833463-10-8 (Blue) are higher than that of 833463-19-7 (Magenta) and it is of interest to see that the
Figure 9. Molecular dynamics simulation. Radius gyrate of the backbone over the 20 ns MDS at 300 K of the complexes systems, the color codes are for (a) 3CLpro-2001083-69-6 (Black) and 3CLpro-2001083-68-5 (Red), (b) RdRp-833463-10-8 (Blue) and RdRp-833463-19-7 (Magenta).

Figure 10. Number of hydrogen bonds of the complex of 3CLpro of coronavirus for COVID-19 with (a) 2001083-69-6 (Black) and (b) 2001083-68-5 (Red) and RdRp of coronavirus for COVID-19 with (c) 833463-10-8 and 833463-19-7 (Magenta) as a function of time are retained or broken during simulation time scale 20 ns.
RMSD curve for 683463-19-7 (Magenta) is remarkably stable than that of 833463-10-8 (Blue).

For Rg variation for both ligands 833463-10-8 (blue) and 833463-19-7 (Magenta) in the binding pocket of RdRp protein are shown in Figure 11(d). Rg variation of 833463-10-8 (blue) shows higher fluctuations during the 20 ns simulation when compared to 833463-19-7 (Magenta). The Rg-time plots suggested a steadier conformation of 833463-19-7 (Magenta) than 833463-10-8 (blue).

Also, SASA plot of both ligands 833463-10-8 (blue) and 833463-19-7 (Magenta) shown in Figure 11(d), indicates that variation of 833463-10-8 (blue) shows higher fluctuations during the 20 ns simulation when compared to 833463-19-7 (Magenta). For the ligand 833463-10-8 (blue), the average
value of SASA is 7.724 nm$^2$ during throughout the 20 ns simulation, while the ligand 833463-19-7 (Magenta) shows SASA of 7.603 nm$^2$ during the simulation. In overall 833463-19-7 (Magenta) presents less value of SASA compared to 833463-10-8 (blue) because of the consistency of the 833463-19-7 (Magenta) in the binding pocket of the protein.

MD simulation results indicate that, both compounds 2001083-68-5 and 2001083-69-6 form stable complexes with 3CLpro, with higher stability of 3CLpro-2001083-69-6 complex. Also 833463-19-7 forms a stable complex with RdRp, the three compounds remain close to their initial docking positions even in unrestrained simulations, hence suggesting formation of stable complexes. All of these indicating that the complexes of 3CLpro-2001083-68-5, 3CLpro-2001083-69-6 and RdRp-833463-10-8 are very likely to have an inhibition activity of SARS-CoV-2, especially they present similar scaffold (4-(morpholin-4-yl)-1,3,5-triazin-2-amine) as depicted in in bold (Figure 6), which could serve as dual inhibition of both proteins 3CLpro and RdRp of coronavirus COVID19.

4. Conclusions

In view of the fact that 3CLpro and RdRp inhibitors play a significant role for the treatment of COVID19, which is an international crisis, we have conducted a computer aided drug design study to rapidly and efficiently screen optimal ligands from the 50000 antiviral compounds in (CAS COVID-19 Antiviral Compound Chemical Database). Based on binding affinity and interactions analysis, six compounds were shortlisted for each protein most of them with a 4-(morpholin-4-yl)-1,3,5-triazin-2-amine scaffold. Further, based on ADME/T analysis, among the shortlisted, four molecules with best ADMET properties and least toxic behavior (2001083-69-6 and 2001083-68-5 in 3CLpro) and (833463-10-8 and 833463-19-7 in RdRp) were selected as models for MD simulation to illustrate the ligand-proteins stability. MD simulation and analysis of four lead compounds, two in complex with 3CLpro revealed that both of the them formed stable complexes in the ligand-binding pocket of 3CLpro, while for the two compounds in complex with RdRp, only one ligand formed a stable during 20-ns simulation.

It is expected that the new dual 3CLpro and RdRp inhibitors for coronavirus SARS-CoV-2 (COVID19 disease) may become potential drug candidates. Or at least, they may stimulate a new way for developing novel inhibitors against COVID19. However, further in vitro studies are required to validate the findings.

Disclosure statement

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