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Repurposing of renin inhibitors as SARS-COV-2 main protease inhibitors: A computational study

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A B S T R A C T
The COVID-19 pandemic has urged for the repurposing of existing drugs for rapid management and treatment. Renin inhibitors down regulation of ACE2, which is an essential receptor for SARS-CoV-2 infection that is responsible for COVID-19, in addition to their ability to act as protease inhibitors were encouraging aspects for their investigation as possible inhibitors of main protease of SARS-CoV-2 via computational studies. A Pharmacophore model was generated using the newly released SARS-COV-2 main protease inhibitors. Virtual screening was performed on renin inhibitors, and Drug likeness filter identified remikiren and 0IU as hits. Molecular docking for both compounds showed that the orally active renin inhibitor remikiren (Ro 42-5892) of Hoffmann-La Roche exhibited good molecular interaction with Cys145 and His41 in the catalytic site of SARS-CoV-2 main protease. Molecular dynamics simulation suggested that the drug is stable in the active site of the enzyme.

1. Introduction

Since the Spanish flu pandemic in 1918, the modern world has never faced a challenge like the outbreak of severe acute respiratory syndrome related to coronavirus-2 (SARS-CoV-2) infection that causes coronavirus diseases-2019 (COVID-19) (Gorbalenya et al., 2020). The world health organization has announced that the viral infection related to the new strain of corona virus as pandemic in March, 2020 (Mahase, 2020). Many measures and precautions were adopted by healthcare officials worldwide in order to contain the infection (Jin et al., 2020a). The whole world has turned into a huge prison for human kind in “quarantine” (Parmet and Sinha, 2020). SARS-CoV-2 is the third respiratory syndrome to affect human after severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (Su et al., 2016). How the virus infects human cells has been published in many reports (Wrapp et al., 2020) with a key step involving the binding of the spike protein of the virus (S) to the trans-membranal angiotensin converting enzyme 2 (ACE2) (Yan et al., 2020). This has revealed the first biological target in fighting infection. The second target was human serine protease TMPRSS211 that has a crucial role in S protein priming (Matsuyama et al., 2020). Another target was the RNA dependent RNA polymerase responsible for replication of viral RNA (Elfiky, 2020). Finally there are two protease viral enzymes that are responsible for the release of essential proteins for viral structures (Stobart and Moore, 2014), main protease (MPro), also known as 3-chymotrypsin-like cysteine protease; 3CLPro & papain-like protease (PLPro), presenting an additional target (Báez-Santos et al., 2015; Zhang et al., 2020).

The ongoing research for developing a vaccine may be the ultimate solution to this pandemic. However, vaccine development has not succeeded with many RNA viruses including SARS and MERS, which are closely related to SARS-CoV-2. On the other hand, several reports originating from pharmaceutical industry expected that the vaccine will not be out till 2021 (Amanat and Krammer, 2020).

The design of new molecules using artificial intelligence and molecular software techniques has been launched by many companies (Emanuel and Wachter, 2019). Almost every day since the announcement of this pandemic, an article, a study or a report is discussing design suggestions (Yassine and Shah, 2020). The problem is that any new molecule cannot be approved for human use in controlling this infection until it passes all safety and efficacy requirements through clinical trials which may take a very long time (Hughes et al., 2011).

Drug repurposing of existing drugs with an established safety profile may comprise a solution in dealing with such a dilemma (Pushpakom et al., 2019). Drug repurposing is based on computational techniques...
including pharmacophore, molecular docking, homology modeling and molecular dynamics for the virtual screening to the aforementioned targets (Liu et al., 2013). The published protein structure of main protease (MP) with an inhibitor was a breakthrough for medicinal chemists to act swiftly to find an inhibitor from already known drugs (Jin et al., 2020b).

Zheng and colleagues have published an article (COVID-19 and the cardiovascular system) (Zheng et al., 2020) that highlighted the role of ACE2 in COVID-19 infection. They claimed that ACE inhibitors and Angiotensin Receptor (AT1) blockers (ARBs) will elevate the severity of infection in cardiovascular patients who are treated with such drugs, the over-expressed ACE2 in those patients may explain that finding (Xu et al., 2020). ACE2 acts on both Angiotensin I (deca-peptide) and Angiotensin II (octa peptide) to hydrolyze them into Angiotensin I (1–9) and Angiotensin II (1–7), respectively (Clarke and Turner, 2012). This action is considered a counter action to ACE in forming Angiotensin II, which is considered as one of the molecules that is responsible for elevated blood pressure in hypertensive patients (Crackower et al., 2002). Hence they claimed that blockers of the renin–angiotensin–aldosterone system (RAAS) may contribute to the high mortality rate of cardiovascular patients (Atlas, 2007).

This article received a correspondence by Mourad and Levy who stressed on the need to differentiate between the different blockers of the renin–angiotensin–aldosterone system on the expression of ACE2 (Mourad and Levy, 2020), where different level of inhibition of RAAS will affect the level of ACE2 differently. In a closer look, inhibition of renin will lead to the down regulation of ACE2 (Ferrario et al., 2005). In this context Mourad and Levy has suggested the well-known renin inhibitor aliskiren as a possible candidate to aid in the management of COVID-19 infection (Mourad and Levy, 2020).

In this study, we do agree with Mourad and Levy in their suggestion on the possible use of renin inhibitors. Remikiren, a second generation peptidomimetic renin inhibitor, failed the clinical trials because of its low bioavailability and weak blood pressure-lowering activity (Stanton, 2003) but passed several drug discovery process stages and thus is an excellent candidate for drug repurposing. The fact that renin is a pro dyad. In this system, cysteine thiol functions as the nucleophile in the catalytic active site of cysteine protease.

2. Results and discussion

2.1. Pharmacophore elucidation

Pharmacophore model generation was adopted using the ligands co-crystallized (OEW, N3, X77 & O6k) in PDB IDs (6W63, 6L7U, 6Y2F and 6Y7M). The essential binding interactions of the co-crystallized main protease ligands are illustrated in (Fig. 2) through MOE generation of Protein-Ligand Interaction Fingerprint (PLIF) of the four PDB files, the figure shows the main interacting amino acids (His41, Phe140, Asn142, Gly143, Cys145, His163, His164, Glu166, Pro168, Gln189 and Thr190). Upon pharmacophore automatic elucidation, 1036 pharmacophores were generated. For model selection; further narrowing down steps were adapted for choosing the best representative model. Firstly, we selected the models that covers all four used ligands which reduced the number to 910 models. Secondly, according to query size (QSsize), models with higher features number (five) were chosen to increase the model specificity which further reduced the number to 411. Finally, through comparing the overlap score of the selected models, only two models exceed the score of 2.00, those models were then used for virtual screening. The final selected model was the one with lower number of hits upon screening the database, see (Fig. 3).

The chosen model has five features; three hydrophobic features two of which are close by, separated by a distance of 2.72 Å, the third is separated by a distance of 9.59 Å and 10.44 Å. The other two features are H-bond acceptor which are separated by 4.83 Å and are away from the third hydrophobic feature by 2.91 Å and 6.10 Å (Fig. 4).

In order to find an approved drug that complies with this model for repurposing, a list of FDA-approved drugs (2684 drugs) was virtually screened using the above pharmacophore but unfortunately no hits were identified. Another database of 84 renin inhibitors, whose chemical structures and PDB IDs are listed in (supplementary data: Table S1), was compiled and was screened using the pharmacophore model. Ten ligands were identified as hits (R32, R31, 0GM, 0IU, 0QB, C60, REM, 3OX, L1A and 2Y2).

For further refinement of these hits, drug-likeness concept was applied. Walters and Murcko define ‘drug-like’ compounds as ‘molecules which contain functional groups and/or have physical properties consistent with the majority of known drugs’ (Walters and Murcko, 2002). Lipinski defines drug-like “as those compounds that have sufficiently acceptable ADMET properties and sufficiently acceptable toxicity properties to survive through the completion of human Phase I clinical trials” (Lipinski et al., 1997). Lipinski’s rule of five states that for a compound to be drug-like with acceptable physicochemical features, it should have a molecule weight <500, log P value < 5, not more than 5 HBD (H-bond donor) groups (OH and NH) and not more than 10 HBA (H-bond acceptor) groups (O and N) (Lipinski, 2004). Analysis of the ten selected hits was performed through measurement of molecular weight, count of HBD and HBA groups and consensus log P o/w (obtained from SwissADME web server), the results are summarized in (Table 1). The two hits that fulfill the rule are highlighted.

Consequently, only two ligands (0IU & Remikiren) were selected for further investigation. The chemical structures and the superimposition of 0IU & Remikiren on the pharmacophoric features is displayed in

Fig. 1. Catalytic active site of cysteine protease.
The zero hits count on FDA approved drugs and only 10 hits from 84 renin inhibitors can highlight the selectivity of the generated model.

2.2. Molecular docking

Before the docking of the renin inhibitors, a validation experiment was carried out to ensure that the docking protocol is acceptable. The validation results were found to be satisfactory, where the relative position of the co-crystallized ligand and the docked ligand were found to

### Table 1: Properties of the ten selected hits for drug-likeness filter.

| Ligand ID | Molecular weight (g/mol) | Consensus Log P_o/w | HBD count | HBA count | Drug-Likeness |
|-----------|--------------------------|---------------------|------------|-----------|---------------|
| R32       | 526.12                   | 0.02                | 8          | 9         | No; 2 violations: MW > 500, HBD > 5 |
| R31       | 551.73                   | 0.83                | 8          | 11        | No; 3 violations: MW > 500, HBA > 10, HBD > 5 |
| 0GM       | 840.08                   | 5.41                | 6          | 11        | No; 3 violations: MW > 500, HBA > 10, HBD > 5 |
| 0IU       | 643.86                   | 3.88                | 5          | 10        | Yes; 1 violation: MW > 500 |
| 0QB       | 682.93                   | 3.64                | 4          | 11        | No; 2 violations: MW > 500, HBA > 10 |
| C60       | 730.03                   | 4.50                | 5          | 11        | No; 2 violations: MW > 500, HBA > 10 |
| REM       | 630.85                   | 3.42                | 5          | 10        | Yes; 1 violation: MW > 500 |
| 3OX       | 692.88                   | 3.43                | 4          | 11        | No; 2 violations: MW > 500, HBA > 10 |
| L1A       | 706.93                   | 2.34                | 7          | 13        | No; 3 violations: MW > 500, HBA > 10, HBD > 5 |
| 2Y2       | 641.86                   | 2.03                | 6          | 11        | No; 3 violations: MW > 500, HBA > 10, HBD > 5 |

(Fig. 5). The zero hits count on FDA approved drugs and only 10 hits from 84 renin inhibitors can highlight the selectivity of the generated model.

2.2. Molecular docking

Before the docking of the renin inhibitors, a validation experiment was carried out to ensure that the docking protocol is acceptable. The validation results were found to be satisfactory, where the relative position of the co-crystallized ligand and the docked ligand were found to
be similar (Fig. 6). RMSD Between the atoms of the co-crystallized ligand and the docked ligand was calculated to be 0.8851 Å.

Docking of the two renin inhibitors identified by the pharmacophore model was carried into the active pocket of the SARS-CoV-2 main protease to examine the binding modes of both compounds within the active site. Of the two compounds, remikiren was found to bind through an extensive hydrogen bond network (Fig. 7).

The active site of SARS-CoV-2 main protease has a Cys-His catalytic dyad formed by Cys-145 and His-41, similar to that reported in other SARS main protease enzymes (Anand, 2002; Yang et al., 2003). Known protease inhibitors are known to form covalent bonds with the Cys145 residue (Zhang et al., 2020; Tang et al., 2020). Visual examination of the chosen binding pose of remikiren within the binding pocket showed that the amide carbonyl is oriented towards the Cys145 residue, suggesting the possibility of covalent bond formation. Additionally, it has been reported that the catalysis reaction is stabilized through the formation of hydrogen bonds with His41, Gly143, Ser144 and/or Cys145 (Zhang et al., 2020). Remikiren was found to form hydrogen bonds with both His41 and Cys145 through hydrogen bonds, suggesting the stabilization of the complex. The active site of the SARS-CoV-2 main protease also has a S1 binding pocket consisting of Phe140, Leu141, His163, Glu166 and His172 (Jin et al., 2020b), the imidazole moiety of the remikiren was found to be oriented towards the S1 pocket and was found to form hydrogen bonds with both His163 and Glu166. Interactions of known inhibitors with the S1 pocket have been previously reported and are thought to be advantageous for inhibitor activity (Zhang et al., 2020; Tang et al., 2020). Furthermore, remikiren was found to form an additional hydrogen bond between the sulphonyl oxygen and Met49. Interactions between remikiren and the active site of SARS-CoV-2 main protease are displayed in (Table 2).

2.3. Molecular dynamics

A molecular dynamics simulation was carried out to confirm and further elaborate on the activity of remikiren in the active pocket of the SARS-CoV-2 main protease. Fig. 8 shows the root-mean-square deviation (RMSD) fluctuations of the protein (black) and the ligand (red) over 10 ns. The RMSD of protein and the ligand level off at around 0.3 nm (3 Å) and 0.15 nm (1.5 Å) respectively, which are quite small. This suggests that the binding of the ligand in the active site is stable. Additionally, visual inspection of the trajectory of the binding pose confirmed the stability of the ligand in the active pocket (Fig. 8).

3. Conclusion

There’s no doubt that COVID-19 panic pandemic through the past months forced all researchers to try to find a solution. Our search was focused on the fact that renin inhibitors downregulate ACE2 receptor which is a first target for SARS-COV-2 infection, as well as their inhibitory activity on proteases. Based on this knowledge, we utilized the cheminformatic computational techniques such as pharmacophore elucidation & search, virtual screening, molecular docking and molecular dynamics to identify a potential hit from known renin inhibitors to be repurposed against one of the most important SARS-COV-2 enzymes, the main protease. Remikiren was identified to fulfill the pharmacophore query and showed promising results in both molecular docking and molecular dynamics studies and is recommended for further clinical studies.
4. Experimental

4.1. Pharmacophore elucidation

The X-ray crystallographic structures of the co-crystallized SARS-
COV-2 main protease inhibitors with the following PDB IDs (6W63,
6LU7, 6Y2F and 6Y7M) were downloaded from protein data bank (htt
ps://www.rcsb.org/). Pharmacophore elucidation, validation, selec-
tion followed by virtual screening were performed by MOE (2019.0102)
software. The co-crystallized main protease inhibitors were first pre-
pared by conformational search and charges adjustment, they were
rigidly aligned, and pharmacophore models were automatically gener-
ated, the large number of pharmacophores were filtered based on cover,
QSize and the overlap score to choose a suitable one. The selected model
was then used for virtual screening on a list of 2684 FDA-approved drugs
(downloaded from Drugbank) and a database of 84 co-crystallized renin
inhibitors (downloaded from protein data bank). Lipinski’s drug-like
filter was applied to minimize the number of hits by calculating the
compounds’ properties from SwissADME free web server (http://www.
swissadme.ch/).

4.2. Molecular docking

A docking experiment was carried out to examine the binding of the
renin inhibitors identified by the pharmacophore screening into the
active site of the SARS-CoV-2 main protease using Autodock Vina (Oleg
and Arthur J, 2010). The crystal structure of the SARS-CoV-2 main
protease (PDB code: 6W63) (Mesecar) was downloaded from the protein
databank (https://www.rcsb.org/). The downloaded structure of the
protein was prepared using Autodock tools 4 (J. Westbrook, 2002), by
the deletion of water molecules and assigning of partial charges using
Gasteiger charges. Before the docking procedure was performed, vali-
dation was carried by the redocking of the co-crystallized ligand. The
database of renin inhibitors was also prepared used Autodock tools 4 (J.
Westbrook, 2002), through the addition of Gasteiger charges. The active
pocket was defined using a grid box of dimensions 60 X 60 X 60 points
centered on the native ligand with a spacing of 0.375 Å. The Lamarckian
genetic algorithm was used to carry out a 100 docking runs for each
compound using the default Autodock parameters. The results were
analyzed and visualized using UCSF Chimera (Morris et al., 2009).

4.3. Molecular dynamics

A molecular dynamics simulation was performed for 10ns to further
analyze the binding of remikiren into the active site of the SARS-CoV-2
main protease using GROMACS (GROningen Machine for Chemical
Simulations) v. 5.1.2 (Pettersen et al., 2004). The topology and
coor-dinate file of the protein was generated using GROMACS, applying
the AMBER99SB force field (Berendsen et al., 1995). On the other hand,
the parameterization of remikiren was carried out using ACPYPE
(AnteChamber PYthon Parser Interface) module of AmberTools (Maier

Table 2
The docking score and interaction formed between remikiren and SARS-CoV-2
main protease.

| Energy Score (Kcal/mol) | Amino Acid | Interacting Group | Length (Å) |
|-------------------------|------------|-------------------|------------|
| –8.00                   | His41 (H-bond) | Amide Carbonyl | 4.29       |
|                         | Met49 (H-bond) | Hydroxyl Group   | 3.28       |
|                         | Cys145 (H-bond) | Amide Carbonyl | 3.20       |
|                         | His163 (H-bond) | Imidazole Nitrogen | 3.77    |
|                         | Glu166 (H-bond) | Imidazole Nitrogen | 4.20    |

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et al., 2015) and the automated topology builder (http://bio2byte.be/ acype/) (Case et al., 2020). 23046 TIP3P water molecules were added to the system, in addition to 68 Na+ and Cl− ions, in order to achieve a final concentration of 0.15 mM. Prior to the molecular dynamics simulations, the system was minimized using the steepest descent method, applying a force tolerance of 100 kJ/mol and for a maximum of 5000 steps. This was then followed by two restrained molecular dynamics simulations were carried out, each for 100 ps, serving to equilibrate the system. This was then followed by a 10 ns molecular dynamics simulation, using a leap frog algorithm. Temperature and pressure were kept constant at 300 K and 1 bar respectively using Berendsen temperature coupling and Berendsen pressure coupling set 0.1. Long-range electrostatic interactions were calculated with the Particle Mesh-Ewald (PME) method with a 1 nm short-range cut-off and short-range non-bonded interactions were computed only within a cut-off of 1.2 nm. Finally, the trajectories produced from the dynamics simulation were analyzed used XMGraice (Souza da Silva and Vranken, 2012) and visually inspected using VMD (Visual Molecular Dynamics) (Humphrey et al., 1996).

Availability of data and material
The downloaded PDB files from https://www.rcsb.org/
The ligands used in renin inhibitor database were downloaded from https://www.rcsb.org/
The FDA-approved drugs list was downloaded from https://www.drugbank.ca/releases/latest.

Authors’ contribution
All authors contributed equally in the concept and work design. Material preparation, collection of data, pharmacore modeling and virtual screening were performed by Y. M. Nissam and M. K. El-Ashrey. R. H. Refaey performed the molecular docking and molecular dynamics studies.

Declaration of competing interest
The authors declare no competing interests.

Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.virol.2020.12.008.

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