Potential phytochemicals as efficient protease inhibitors of 2019-nCoV

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Abstract

The novel coronavirus 2019 (nCov-2019/Covid-2019/2019-nCov) has become a pandemic in a very short span of time. It has caused significant loss to human lives, economy, daily life. The key development against the nCov-2019 remains apprehended when it comes to discovery of its vaccine or medicines for the treatment. Drugs used for the treatment of HIV (inhibitors of HIV protease) are being largely used for the treatment of nCov-2019. Therefore, we conducted a study by docking a set of natural compounds with reported protease activity against HIV or SARS coronavirus against the protease of nCov-2019. The Bavachinin ranked the top among natural compounds with binding energy of -7.74±0.152 Kcal/mol, RMSD 0.823±0.024 Å, predicted pKd 5.59 and predicted dG of -7.56 Kcal/mol. The finding infers that these three compounds could have the potential to inhibit the nCov-2019 protease. The finding was supported with reputed research publications.

Introduction

In past, the World has witnessed a number of viral pandemics across the continents and consequences have been very devastating and deadly (Cunha, 2004; Levy-Blitchtein and del Valle-Mendoza, 2016; Murray, 2015). The infection caused by any virus is highly contagious and poses a major risk to health as well as management as the genome of viruses keeps changing/mutating (Yasuhara-Bell et al., 2010). This phenomenon leads to emergence of new or resistant viral strains and therefore the search and demand of novel antiviral drug/agent is always on (Levy-Blitchtein and del Valle-Mendoza, 2016). Very handful of successful antiviral drugs has been developed till date which are very effective in case of viral diseases (Al-Hazmi, 2016; Blankson et al., 2002; Holmes, 2003). Anti-viral drugs which are already approved, most of them do possess adverse side effects. It is reported that long term treatments also causes viral resistance (Graham et al., 1990; Marzolini et al., 2001; Negro, 2010).

In the end of year 2019, many cases of pneumonia caused by corona virus were identified in Wuhan (Hubei Province of China). This became outbreak very shortly with 10,048 (by March 19th 2020) cases of death worldwide. China, Italy and Spain are severely with this outbreak. In India first case was reported on January 30th 2020 and by the end of 3rd week of March 2020 India has already entered in stage 2 of the outbreak with 326 confirmed cases, 4 deaths and...
more than 0.1 million people under observation. (www.worldometers.info and Ministry of Health and Family Welfare Government of India).

In addition to china, Italy and Spain the infection cases were also confirmed and reported globally which clearly signify that the it is an indeed a potential global health threat (Hui et al., 2020) and has been designated a global health emergency by the World Health Organization (WHO). The number of causalities and confirmed cases have been increasing and yet there are no effective drugs or vaccines are approved.

The causative agent behind this outbreak is identified as the “The 2019 novel coronavirus” i.e., 2019-nCoV or the Wuhan coronavirus which is a single-stranded RNA (positive-sense) coronavirus (Figure 1)(Lu et al., 2020). Comparative study shows that SARS-CoV and 2019-nCoV they both are from a common β-genus. Entry of coronavirus into host cells is mediated by a host receptor angiotensin-converting enzyme 2 (ACE2). Spike like protein anchored with viral envelop forms the receptor-binding domain (RBD) which is responsible for host receptor recognition and fusion of viral particle into host membrane (Li, 2016, 2015; Li et al., 2003). Host susceptibility is defined by the affinity of RBD and against ACE2 (Ghosh et al., 2009).

![Figure 1: Structural details of Covin-2019, source Seah et al., 2020](image-url)

Plants produce a variety of metabolites which are useful for therapeutic purposes. Some metabolites have the potential to stop viral proliferations by regulating its adsorption, binding to host cell receptors, inhibition of fusion of virus into the host cell membrane and by modulating intracellular signals (Ghosh et al., 2009; Khan et al., 2005).

In this research we have tried to identify the efficient inhibitor for the nCoV-2019 which is effective and possess very less or no side effects. Therefore, we have selected a number of
phytochemicals with reported anti-viral activity/protease inhibitor activity for the study along with few reported approved drugs which are being used to treat nCoV-2019 specially Ritonavir and Lopinavir in combination. We performed the molecular docking to identify the top ranked compound(s). The free energy was also predicted for the top ranked compound to assess their binding affinity towards the main protease of nCoV-2019.

Materials and Methods
1. Target preparation and Selection of phytochemicals with reported Anti-viral activity
The crystal structure of 2019-nCoV (PDB ID 6LU7) was retrieved from the structural database of protein (Protein Data Bank), the structure was co-crystalized with a protease inhibitor and other heteroatoms. Structure was imported in an open source molecular editor (Discovery studio visualizer 4.0). Co-crystal ligands and heteroatoms were removed and structure was stored as .pdb format. The structure was optimized with the help of Chimera UCSF by running 100 steps of Steepest descent followed by 100 steps of Conjugate gradient of energy minimization algorithm. The optimized model was verified on PROCHECK server for protein structure verification (http://servicesn.mbi.ucla.edu/PROCHECK/). The optimized model was observed visually by aligning with its crystal structure and later this optimized structure was used as receptor for further docking experiments. The phytochemicals with reported antiviral activity were retrieved from PubChem PubChem in .sdf format. These ligand structures were imported in DS visualizer and saved in .pdb format (table 1).

Molecular Docking Studies
The receptor protein to be used for docking was prepared in AutoDock MGL tool. The residues around (1Å) the protease inhibitor attached with the co-crystal of nCov-2019 was used to prepare the receptor grid. Using MGL tool, Receptor and ligands were stored in .pdbqt format for later use. Vina was run using command line in command prompt. The default grid point spacing of 0.375Å and exhaustiveness of 8 was used in configuration. The output files was obtained in .pdbqt format and they analysed Pymol and using Discovery studio visualizer. The co-crystal ligand was used for validating and optimization of the ligand binding (Figure 2).

In-silico Validation of ligand binding:

The lead compound obtained after docking was validated using a tool, KDEEP on the webserver www.playmolecule.org, which is based on 3D-convolutional neural networks (CNN). It is an open source tool. It compares the approach with other machine-learning and scoring methods using several diverse data sets. It is helpful in predicting the binding affinity...
(pKd), Free energy for ligand binding (ΔG, in Kcal/mol). After the docking, the best docked pose of the natural compound/ligands was retrieved using PyMol and stored in .sdf formats. The tool requires both receptor and docked pose of ligand to estimate the pKd and ΔG. Both receptor and docked pose of the ligand were uploaded on Kdeep and result was obtained within few minutes in .csv format.

Figure 2: Optimization of Autodock vina program by docking the co-crystal ligand in same pocket. (Docking score of -6.7 ±0.115 Kcal/mol)

**Results and Discussion**

15 natural compounds with reported anti-viral activity was docked with main protease of nCov-2019. Three drugs which have been reported to treat the nCov-2019 patients were used as positive controls. Dock score (overall binding energy) revealed the potentials of these naturals compounds. The top ranked natural compound based on dock score is Bavachinin which has binding energy of -7.74+/−0.152 and RMSD 0.823+/−0.023. The second ranked compound was Kaempferol with dock score of -7.68 ± 0.021 Kcal/mol and RMSD 1.88±0.108 followed by Luteolin dock score -7.58 ± 0.112 Kcal/mol and RMSD 1.303±0.083.

Only positive control drug Nelfinavir has best docking score ( -7.9 ± 0.057, RMSD 1.97±0.72) than Bavachinin. Rest two positive controls Lopinavir and Ritonavir are ranked below the Bavachinin and Kaempferol with dock score (-7.52 ± 0.022 Kcal/mol , RMSD 1.76 ± 0.012 and -7.42 ± 0.68 Kcal/mol, RMSD 1.97±0.072 respectively. Apart from these two natural compounds Hinokinin, Luteolin and Sinigrin exhibited better dock score (-7.51 ± 0.021, -7.58 ± 0.112 and -7.49 ± 0.152 Kcal/mol. The Bavachinin had docked with a RMSD of 0.823±0.024 Å. Table 1 includes the list of compound with IC50. Table 2 includes the details of dock score,
RMSD and interacting residues. Since, residues around the co-crystal ligand was considered as the binding site of the receptor therefore, the key residues which we had looked forward to have interaction with natural compounds were compared with interaction co-crystal ligands and approved drugs with the receptor.

| Compound                  | IC50* value (µM) | Activity against                     | Reference                  |
|---------------------------|------------------|--------------------------------------|----------------------------|
| Bavachinin                | 38.4             | SARS-CoV                             | Kim et al., 2014           |
| Neobavaisoflavone         | 18.3             |                                      |                            |
| Isobavachalcone           | 7.3              |                                      |                            |
| 4′-O-methylbavachalcone   | 10.1             |                                      |                            |
| Psoraladin                | 4.2              |                                      |                            |
| Corylifol A               | 32.3             |                                      |                            |
| Lycorine                  | 15.7 ± 1.2 nM    | SARS-CoV                             | Shi-you, et al 2005        |
| Kappa-Carrageenan         |                  |                                      |                            |
| Lamda-Carrageenan         |                  |                                      |                            |
| Beta-Carrageenan          |                  |                                      |                            |
| Nelfinavir                | 30nM             | HIV Protease & SARS-CoV              | Zhijian Xu et al., 2020    |
| Esculetin ethyl ester     |                  |                                      | Mayer et al., 2010         |
| Hinokinin                 | 10.1             | HIV Protease & SARS-CoV              | Cui et al., 2020           |
| Luteolin                  | 116 µM           | HIV Protease & SARS-CoV              | Schwarz et al., 2014       |
| Kaempferol                |                  | HIV Protease & SARS-CoV              | Yi, L et al., 2004         |
| Aloe-emodin               | 366 µM           | SARS coronavirus                     | Lin et al., 2005           |
| Hesperetin                | 8.3 µM           | SARS coronavirus                     |                            |
| Sinigrin                  | 217              |                                      |                            |
| 18β-glycyrrhetinic acid   | 46 µM            |                                      |                            |

Table 1: List of natural compound with reported Antiviral activity with inhibitory concentration (IC50) and reference

**Positive Control Group**

In case of co-crystal ligand, the key interacting residues were Gly143, Cy145, His 164, Glu166, Gln 189 and Thr 190. Other positive controls were also having interactions with many of these residues either as H-bonds or Pi-interactions. For example, the Lopinavir shows interactions with receptor by one H-bond from Met163 and two H-bonds from Gln189, however, Glu166 is engaged in Pi-anion interaction with a benzene ring of the Lopinavir. Residues Leu17,
Cys145 and His41 were interestingly engaging a common benzene group by Pi-interaction as explained in figure 3.1a, 3.2b and 3.3c

Figure 3: Positive control group compounds (approved drugs) 3.1 Lopinavir, 3.2 Rotinavir and 3.3 Nelfinavir interaction of binding site residues of main protease of nCoV-2019.

Another positive control drug Ritonavir, interacts with Ser46 and Gln189 by H-bonds and residues Met49, Cys145, and Met165 interacts thorough the pi anion interaction (Figure 3.2). The third positive control drug Nelfinavir makes contact with thee residues through H-bonding namely, Gly143, Cys145 and Gln189. Residue Met49 is involved in Pi-Anion interaction with a benzene and His41 engage a benzene group of Pi-Pi stacking (Figure 3.3).

**Natural Compounds**

Interaction of natural compounds with the binding site residues are also interesting to analyse. Bavachinin shows interaction with Glu166 by a H-bond and two Pi-anion interaction with Cys145 and Met165. However, the residues Met165, Pro168 and Glu189 have shown interaction by Van der Waals force (figure 4.1). Bavachinin is top ranked compound among all the natural compounds and positive control drugs except Nelfinavir.
Figure 4: 2D (a), 3D(b) and surface depiction (c) of interaction natural compounds namely 4.1 Bavachinin, 4.2 Kaempferol, 4.3 Luteolin and 4.4 Hinokinin

Kaempferol is second ranked compound and has interaction with Leu141, Cys145, Gln189 by H-bonds and Cys145 also involved in a Pi-anion interaction. A very uncommon H-bond was also noticed which is imparted by Thr26. Key Van der Waals interaction in this case was observed with Asn142, Gly143 and Glu166 which are there in solvent accessible area too (figure 4.2). The third ranked compound was Luteolin. It has interaction with His163 by H-bond and Cys145 by two Pi-anion bonds with dihydroxychromen-4-one group of the compound. Key Van der Waals forces was observed by Met165, Glu166, Gln189 and they were in solvent accessible area (Figure 4.3). The fourth ranked compound was important and it might have ranked fourth but its interaction is significant. Three H-bonds by Gly143, Cys145,
and Ser45 while key Van der Waals interaction was imparted by Thr25, Asn142, Glu166 (Figure 4.4).

The dock score, RMSD and interacting residues for each positive control group and test compounds are described in table 2.

| Receptors→Ligand | 2019 nCov main protease Crystal structure (PDB ID 6LU7), Published on February 05, 2020 | Dock Score (Kcal/mol) | RMSD (Å) | pKd | dG (Kcal/mol) |
|------------------|------------------------------------------------------------------------------------------|-----------------------|---------|-----|---------------|
| Control          | Nelfinavir                                                                                | -7.92 ± 0.057         | 0.054 ± 0.041 | 5.61062 | -7.51         |
|                  | Lopinavir                                                                                 | -7.52 ± 0.022         | 1.76 ± 0.012  | 5.25348 | -7.18         |
|                  | Ritonavir                                                                                 | -7.42 ± 0.68          | 1.97±0.072    | 5.31465 | -7.06         |
|                  | Co-crystal inhibitor                                                                      | -6.72 ± 0.027         | 1.93±0.043    | 6.38342 | -7.67         |
| Natural compound | Bavachinin                                                                                | -7.74 ± 0.152         | 0.823±0.024   | 5.59971 | -7.56         |
| with reported    | Kaempferol                                                                                | -7.68 ± 0.021         | 1.88±0.108    | 5.351614 | -7.22         |
| antiviral        | Hinokinin                                                                                 | -7.51 ± 0.021         | 1.744±0.097   | 5.066619 | -6.84         |
| activity         | Luteolin                                                                                  | -7.58 ± 0.112         | 1.303±0.083   | 5.0643334 | -6.84         |

**Table 2:** List of natural compounds and approved drugs (as positive control) with dock score, RMSD of docked compounds, pKd and dG.

**Discussion**

The novel coronavirus nCoV-2019 or Covid-2019 was identified in Wuhan, China by end of the January 2020. Since then it has spread globally and have affected the human life largely. A number of drugs has been tested and being tested for the treatment of nCoV-2019. Various reported have concluded the use of drugs such as Nelfinavir, Lopinavir, Ritonavir, Hydroxychloroquine, azithromycin etc. (Colson et al., 2020; Li and De Clercq, 2020; Lin et al., 2020; Lu, 2020) against nCoV-2019. Interestingly, many of them are antiviral and used for the treatment of HIV (Kaldor et al., 1997; Markowitz et al., 1995; Walmsley et al., 2002). A case studies (yet to be published) from SMS hospital, Jaipur, India,) where an Italian citizen with nCoV-2019 infection was successfully treated with the combination of Lopinavir and Ritonavir.

In this study, we had planned to screened few natural compounds which have reported antiviral activity. The 3Dstructure of main protease of nCoV-2019 was newly released on Protein Data Bank (PDB ID 6LU7) which was co-crystalized with a protease inhibitor (PRD_002214). For
the optimization of docking program, we had used this co-crystal inhibitor for docking and comparison with its native pose in the crystal structure. We obtained a mixed result which was possibly because of higher flexibility of the ligand’s functional groups and presence of cavity the solvent accessible area.

Figure 5: superimposition of native and docked pose of co-crystal ligand.

The superimposition of Cavity 1 (C1) has shown a significant bind spot superimposition between native and test pose of co-crystal ligand functional group, while cavity 2 (C2) which has shown a stronger and very bad RMSD superimposition of functional group. Here we should take a note that very high flexibility of the ligand backbone might have generated the problem in obtaining the precise co-crystal pose. This certain situation leads to a logical concussion that fragment library can be used to screen better inhibitory drugs instead of conventional drug screening procedure. Fragment based drug screening and active library generation would help to form a portent inhibitor.

The top ranked natural compounds Bavachinin, Kaempferol and Luteolin are very potential and could be a competitive natural substitute for protease inhibition of nCov-2019. Bavachinin exhibited best pKd (5.59) and free energy of the ligand binding (-7.56 Kcal/mol) among all the natural compounds. Kim et al., 2014 have reported the activity of Bavachinin (a flavanone) against the papain-like protease of the SARS-Corona Virus. Kim et al., 2014 had isolated this compound from the seeds of Psoralea corylifolia and tested against the SARS Cov and concluded the IC50 of the compound was 38.4 µM. The common name of this plant in Sanskrit is Avalguja.

The second ranked compound Kaempferol which is a unique compound with multipurpose usage. This compound has been studied by (Al-Nour et al., 2019) against the replicase polyprotein 1 ab of the coronavirus. Another study by Schwarz have reported that derivatives
of Kaempferol can be used as an 3a channel protein inhibitor against the antiviral drugs against the coronavirus. Yang et al., 2014 have done in-vitro testing of Kaempferol along with other six compounds against the influenza B and interestingly this compound has shown the best activity among all. A research paper published by Chia, 2007 et al., who have identified the Kaempferol from the Toona sinensis which is later tested by Chen et al., 2008 with Toona sinensis extract suggests that it has potential to inhibit the replication of SARS coronavirus.

The third ranked compound is also valuable in terms of its activity. The luteolin which has been tested along with fifteen other compounds by Zhang & Chen, 2008 suggested that luteolin could be a potential anticomplementary agent against the SARS, which acts on the classical pathway and the alternative pathway (AP) of the complement system. A report by Ryu infers that luteolin potentially inhibits the SARS-CoV 3CL protease (IC50 = 20.2 μM).

Above studies suggests that these compounds potential with high therapeutic value against the nCov-2019 and they strongly support our findings. These compounds do have lower docking score and better binding affinity with the main protease than the lopinavir and ritonavir drugs. Therefore, to further explore the possibilities of potential of these compounds, their chemical/synthetic analogue or derivatives can be explored to obtain the potential antiviral compounds against the nCov-2019.

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