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Novel cyclohexanone compound as a potential ligand against SARS-CoV-2 main-protease

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ABSTRACT

No commercially available drug candidate has yet been devised which is unique to and not repurposed against SARS-CoV-2 and has high efficacy or safe toxicity profile or both. Taking curcumin as a reference compound, we identified a new commercially available cyclohexanone compound, ZINC07333416 with binding energy (~8.72 kcal/mol) better than that of popularly devised anti-Covid-19 drugs like viral protease inhibitor Lopinavir, nucleoside analogue Remdesivir and the repurposed drug hydroxychloroquine when targeted to the active-site of SARS-CoV-2 Main protease (Mpro) through docking studies. The ligand ZINC07333416 exhibits crucial interactions with major active site residues of SARS-CoV-2 Mpro viz. Cys145 and His41 involving in the protease activity; as well as GLU-166 and ASN-142 which plays the pivotal role in the protein-dimerization. The protein-ligand stable interaction was further confirmed with molecular dynamics simulation (MDS) studies. Based on virtual assessment, ZINC07333416 also have significant values in terms of medicinal chemistry, pharmacokinetics, synthetic accessibility and anti-viral activity that encourage its experimental applications against COVID-19.

The novel coronavirus SARS-CoV-2, etiological agent of COVID-19 have engendered a pandemic with higher morbidity and mortality rates. Millions of registered victims and several hundred thousand deaths have been reported worldwide due to COVID-19 [1]. Although many compounds have been advised and tested against COVID-19, most of them were either repurposed drugs or they lacked efficacy or lacked safe toxicity profile or all of them [2,3]. Drug repurposing against a pandemic may result in sudden crisis of an essential drug; it may also provide a long term efficacy or may result in unfavourable off-site interactions [3]. In our present study, we have focused on the identification of a novel compound targeted to SARS-CoV-2 main protease (Mpro) since, protease inhibitor drugs have shown improved outcome in COVID-19 victims [3]. We also objectified on comparing the same with popular drugs devised against COVID-19 [3–5] in terms of interaction, toxicity profiles and drug properties in-silico.

Curcumin was chosen in our study as a reference to screen analogous compounds since it has anti-viral activity and a safe toxicity profile [6,7]. The three-dimensional (3D) structure of SARS-CoV-2 Mpro (6LU7) was retrieved from RCSB-protein databank and all ligands (drugs/compounds) were obtained from PubChem database. The 3D formatting of ligands was performed using Openbabel tool [8]. 400 commercially available curcumin analogues were identified through SwissSimilarity server based on their stereochemistry, structural alignment and pharmacophore model [9]. Screened compounds (similarity scores with curcumin > 0.7) and known reference compounds were docked onto SARS-COV-2 Mpro using AutoDock version 4.2 [10]. Prior to the docking analysis, structure of the target protein was optimized by removing crystallographic water molecules and unwanted hetero-atoms or other bound ligands. Polar hydrogens were added and non-polar hydrogens were merged thereafter to the protein in ideal geometry. Requisite Kollman charges (4.0) were added to the protein to finally stabilize its structure. The torsions were fixed for the ligands. The initial parameters and van der Waals well depth of 0.100 kcal/mol were assigned for the protein. The important active site residue His41 [11] was centred to construct affinity grid-box of 60 Å³. Lamarckian Genetic Algorithm was employed to generate SARS-COV-2 Mpro-ligand complexes in 10 different poses. Based on best poses and lowest of binding energies we identified the phenolic compound ZINC07333416 having a 2, 6 di-methylene-cyclohexanone group Fig. 1 a. The binding energy of our lead compound ZINC07333416 (~8.72 kcal/mol) is
better than that of our reference compound Curcumin (−6.9 kcal/mol),
tested viral protease inhibitor Lopinavir [2] (−8.29 kcal/mol), newly
identified Lopinavir analogue ZINC541677852 [3] (−7.72 kcal/mol),
popular anti-viral nucleoside analogue Remdesivir [5] (−6.18 kcal/
 mol) and the repurposed drug hydroxychloroquine [4] (−6.36 kcal/
 mol) when targeted to the active-site of SARS-COV-2 Mpro (Table 1).

Fig. 1 c. shows the ligand ZINC07333416 exhibiting Pi-alkyl in
teractions with both of the crucial active site residues Cys145 and His41
that plays the major role in the protease activity [11]. Conventional
hydrogen bond was found with active site residues ASP-187 and TYR-54.
The ligand also exhibits van der Waals interaction with GLU-166 and
ASN-142 which plays the pivotal role in dimerization [11] of the pro-
tein. Hence, ZINC07333416 can efficiently bind to the active site of the
SARS-COV-2 Mpro by specifically interacting with residues responsible
for structural and functional integrity of the protease.

AVCpred server was employed to predict the general antiviral po-
tential of our lead as well as reference compounds (see Table 2). The
prediction uses integrated Quantitative-Structure-Activity-Relationship
(QSAR) and best-performing molecular descriptor based screening-
algorithm against 30 known viral pathogens including SARS

Table 1

| Protein Ligands | Binding energy (kcal/mol) |
|----------------|--------------------------|
| SARS CoV2 (2019-nCoV) Mpro Lead compound | ZINC07333416 | −8.72 |
| (Commercially available Curcumin analogue) | Hydroxychloroquine | −6.36 |
| Newly addressed compounds | ZINC541677852 | −7.72 |
| Curcumin (reference compound) | Remdesivir (nucleoside analogue) | −6.18 |
| Popular antiviral drugs devised | Lopinavir (viral protease inhibitor) | −8.29 |
with our docking results. The root mean square fluctuation (RMSF) was used to evaluate the amount of positional fluctuation of each residue of the protein-ligand backbone during MDS. It was observed that our RMSF values lie between 0.05 and 0.4 nm with an approximate average of 0.2 nm and minimum fluctuations of the crucial active-site residues Fig. 2 (F). The DSSP (Define Secondary Structure of Proteins) model further ensured the stability of the protein structure during simulation by ascertaining the changes in secondary structures. The study revealed that stable secondary structural conformation of our target protein with bound ligand was maintained throughout the simulation with respect to all structural patterns (helices, loops, bends etc.) Fig. 3 [17–19].

Therefore, we identified a new and commercially available compound having favourable drug-likeness, lead-likeness and synthetic accessibility. The identified compound showed stable molecular interactions when targeted to the active-site of SARS CoV-2 Mpro. It has no experimental or clinical report so far and hence can be uniquely addressed to COVID-19 to overcome the drawbacks with repurposed drugs. Furthermore, the anti-viral activity, medicinal chemistry and toxicity profiles of our lead compound are encouraging as compared to known/tested compounds and hence can be optimistically used for experimental trials against COVID-19.

Table 2

| Compounds             | Molecular weight | mlogP | TPSA   | Drug likeliness violations | Lead likeliness violations | GI absorption | BBB permeation | Synthetic accessibility Score [Scale: 1 (very easy) to 10 (difficult)] | LD50 (mg/kg) | Anti-viral activity (%) |
|-----------------------|------------------|-------|--------|------------------------------|----------------------------|----------------|----------------|-----------------------------------------------------------------|--------------|------------------------|
| ZINC07333416          | 320.38           | 3.11  | 46.53  | No                           | 1                          | High           | Yes            | 2.83                                                            | 2300         | 41.95                  |
| Hydroxychloroquine    | 335.87           | 2.35  | 48.39  | No                           | 2                          | High           | Yes            | 2.82                                                            | 1240         | 37.74                  |
| ZINC541677852         | 394.39           | 2.92  | 76.02  | No                           | 1                          | High           | Yes            | 3.60                                                            | 1000         | 72.54                  |
| Curcumin              | 368.38           | 1.47  | 93.07  | No                           | 2                          | High           | No             | 2.97                                                            | 2000         | 20.18                  |
| Remdesivir            | 602.59           | 2.82  | 203.57 | Yes                          | 2                          | Low            | No             | 6.33                                                            | 1000         | Proven                 |
| Lopinavir             | 628.80           | 2.93  | 120.00 | Yes                          | 3                          | High           | No             | 5.67                                                            | 5000         | Proven                 |

Fig. 2. (A) RMSD trajectory of SARS CoV-2 Mpro. (B) RMSD trajectory of ligand ZINC07333416. (C) Rg pattern of the protein-ligand complex during MDS. (D) SASA to evaluate stability of hydrophobic core of the complex backbone. (E) H-bond observed during MDS. (F) RMSF pattern of target protein during simulation.
Structural analysis by DSSP algorithm showing stable secondary structural conformation during 50 ns timescale.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2020.104546.

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