Inhibition of multiple SARS-CoV-2 proteins by an antiviral biomolecule, seselin from Aegle marmelos deciphered using molecular docking analysis

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ABSTRACT

Our earlier experimental and computational report produced evidence on the antiviral nature of the compound seselin purified from the leaf extracts of Aegle marmelos against Bombyx mori Nuclear Polyhedrosis Virus (BmNPV). In the pandemic situation of COVID-19 caused by the SARS-COV-2 virus, an in silico effort to evaluate the potentiality of the seselin was made to test its efficacy against multiple targets of SARS-COV-2 such as spike protein S2, COVID-19 main protease and free enzyme of the SARS-CoV-2 (2019-nCoV) main protease. The ligand seselin showed the best interaction with receptors, spike protein S2, COVID-19 main protease and free enzyme of the SARS-CoV-2 (2019-nCoV) main protease with a binding energy of 6.3 kcal/mol, 6.9 kcal/mol and 6.7 kcal/mol, respectively. Docking analysis with three different receptors identified that all the computationally predicted lowest energy complexes were stabilized by intermolecular hydrogen bonds and stacking interactions. The amino acid residues involved in interactions were ASP1184, GLU1182, ARG1185 and SER943 for spike protein, SER1003, ALA958 and THR961 for COVID-19 main protease, and for SARS-CoV-2 (2019-nCoV) main protease, it was THR111, GLN110 and THR292. The MD simulation and MM/PBSA analysis showed that the compound seselin could effectively bind with the target receptors. The outcome of pharmacokinetic analysis suggested that the compound had favourable drugability properties. The results suggested that the seselin had inhibitory potential over multiple SARS-CoV-2 targets and hold a high potential to work effectively as a novel drug for COVID-19 if evaluated in experimental setups in the foreseeable future.

Abbreviations: ALA: Alanine; ARG: Arginine; ASN: Asparagine; ASP: Aspartic acid; COVID-19: Coronavirus disease of 2019; CYS: Cysteine; GLN: Glutamine; GLU: Glutamic acid; GLY: Glycine; kcal/mol: Kilocalorie per mole; MD: Molecular Dynamics; MM/PBSA: Molecular Mechanics Poisson-Boltzmann Surface Area; PDB: Protein Data Bank; PDBQT: Protein Data Bank, Partial Charge (Q) & Atom Type (T); PHE: Phenylalanine; PRO: Proline; RCSB: The Research Collaboratory for Structural Bioinformatics; RMSD: Root-mean-square deviation; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SER: Serine; THR: Threonine; TPSA: Topological polar surface area; TRP: Tryptophan; VAL: Valine

Introduction

The outbreak of coronavirus disease 2019 (COVID-19) recently has transitioned into a pandemic state and consumed numerous human lives due to its contagious nature. This dreadful disease has infected more than 182 million people, and the reported deaths have been over 4 million, till date [World Health Organization (WHO), Coronavirus, COVID-19 update - https://covid19.who.int/]. The antiviral drugs treated for many diseases have been tested onto COVID-19 as well, reckoning by its potentiality. But these current medications, which include administration of repositioned Food and Drug Administration (FDA) approved drugs, namely lopinavir/ritonavir (experimental, retrovirus protease inhibitor), remdesivir (experimental, viral polymerase inhibitor) and favipiravir [experimental, viral ribonucleic acid (RNA) polymerase inhibitor] etc., are based on in vitro studies reported for COVID-19 and recommended by testing batches of patients across the world. Nevertheless, it is still in a biased and anecdotal state (Scavone et al., 2020).

The genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) comprises single-stranded (+) RNA that encodes for structural and non-structural proteins. Spike glycoprotein, envelope protein, membrane protein, and nucleocapsid protein comes under structural proteins. The non-structural proteins are the enzymes involved in the replication of virus. Those are polymerase, helicase and endoribonuclease (Jogalekar et al., 2020). In a short period, documentations on molecular docking of naturally occurring plant compounds with SARS-CoV-2 proteins have been...
reported with plausible lower binding energy. Among these, particular attention has been given to spike protein and proteases of SARS-CoV-2 (Hall & Ji, 2020). This is because, upon the molecular studies on virus transmission, it is evident that i) the spike protein binds to the host cellular receptor angiotensin converting enzyme-2 (ACE2) and responsible for the fusion between the viral envelope and the cellular membrane ii) the proteolytic cleavage of polyprotein by a papain-like cysteine protease and 3-C-like serine protease forms the replication enzymes of virus (Hofmann & Pöhlmann, 2004; Wan et al., 2020; Wrapp et al., 2020; Zhang et al., 2020). The resulted information of reported docking studies highly facilitates the categorization of drugs and takes a call on proceeding with potential candidates for in vitro and follow up studies.

Plants especially possessing medicinal values, have been a preferable source for human healthcare purposes, particularly by discovering lifesaving drugs (Jimenez-Garcia et al., 2013). Therefore, the ideal approach of ethnopharmacology is to classify the evidence of medicinal plants and isolate the drugs that take the edge off human illness (Farnsworth, 1994). In screening for bioactive compounds, the assortment of the plant species to be studied is a decisive factor for the eventual success of the investigation. In that way, Aegle marmelos, commonly called as bael (Family: Rutaceae), reported to be rich in several bioactive compounds such as aegelin, lupeol, cuminaldehyde, eugenol, skimmianine, cineol, citral, marmesinin, marmelide, β-sitosterol, flavone, glycoside and marmeline, was chosen to the study for the following reasons (Arul et al., 1999; Farooq, 2005; Govindachari & Premila, 1983; Manandhar et al., 1978). A. marmelos exhibited antiviral activity against Human coxsackieviruses B1-B6, an enterovirus (that also includes polioviruses and echoviruses) belongs to the picornaviridae which causes clinical manifestations such as respiratory illness (types 2-5), meningoencephalaphitis (types 1-5), myocarditis (types 1-5), myocardio-ophathy, pancreatitis etc., (Badam et al., 2002; Melnick, 1984). Interestingly, a high throughput screening of approximately 6800 small molecules identified that proteases of picomavirus and coronavirus have a common inhibitor (Kuo et al., 2009).

Significantly, in our earlier report, we have successfully purified the molecule seselin from the leaf extracts of A. marmelos (Somu et al., 2019). It is noteworthy that it exhibited antiviral activity against Bombyx mori nuclear polyhedrosis virus (BmNPV). The process of drug development is a high risk associated platform with numerous evaluations but a high payoff endeavor ultimately (Chadwick & Marsh, 2008). Amid pandemic scenario, rapid and prompt report analysis of drug’s eligibility using in silico approach would serve as an initiating factor for a study to pursue on its basis. In this context, on presuming the potentiality of seselin against the SARS-CoV-2 based on its antiviral nature, this study aims to analyse the in silico structural inhibition of SARS-CoV-2 proteins by seselin.

Materials and methods

Preparation of ligand

The structure of the ligand seselin, an anti-BmNPV compound obtained from the purified crystal in our earlier report (Somu et al., 2019) was drawn using MarvinSketch program (MarvinSketch, ChemAxon). PRODRG server was used to describe the ligand molecules, and a variety of topologies for use with GROMACS, WHAT IF, Autodock, HEX etc., were generated. CORINA-SMILES notations verified the atomic coordinate and quality of structures. To prepare the ligand for docking, Open Babel (O’Boyle et al., 2011) in PyRx 0.8 (Dallakyan, 2008) was used. The Graphical User Interface was used to change energy minimization parameters, and the energy of the ligand was minimized. Finally, the ligand was converted into Autodock ligand (PDBQT) format for docking.

Preparation of receptor

The structures of Spike protein S2 (PDB-ID: 6LXT) (Zhu & Sun, 2020), the crystal structure of COVID-19 main protease in complex with an inhibitor N3 (PDB ID: 6LU7) (Liu et al., 2020) and the free enzyme of the SARS-CoV-2 (2019-nCoV) main protease (PDB ID: 6Y2E) (Zhang et al., 2020) were downloaded from the RCSB protein data bank (https://www.rcsb.org/search). The receptors preparation was performed by optimizing protein model geometry, removing ligands and other heteroatoms using Biovia Discovery studio 20.1.0.

Prediction of potential ligand binding site

Before docking analysis, the ligand-binding sites on protein surface were identified using Metapocket 2.0 (https://projects.biotectu-dresden.de/metapocket/index.php). MetaPocket 2.0 uses a consensus method in which the predicted binding sites from eight methods such as LIGSITEc, PASS, QSiteFinder, SURFNET, Fpocket, GHECOM, Concavity and POCASA are combined to improve the success rate of prediction. A z-score is calculated separately for each pocket site in different predictors. Top 10 potential ligand-binding sites were retrieved to analyze active site binding residues and comparison of docking results.

Virtual screening and visualization

PyRx 0.8 was used for virtual screening that uses Vina and AutoDock 4.2 as docking softwares with Lamarckian Genetic Algorithm (LGA) as the scoring function. Docking analysis of seselin with three different receptors was carried out using AutoDock vina (Trott & Olson, 2010), PyRx 0.8. Binding site amino acids were selected, and the docking site on the protein target was defined by generating a grid box that allows selecting the search space. All bonds of ligands were set to be rotatable. Charges were added for both ligand and receptors. The calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The best conformation for each receptor was chosen with the lowest docked energy once after the docking search was completed. The interactions of protein-ligand conformations, including hydrogen bonds and the bond length, were analyzed using Discovery Studio 20.1.0 and PyMOL Molecular Graphics system, Schrodinger, LLC. The 2D structure of the protein-ligand interaction was visualized using Biovia Discovery studio 20.1.0.
systems. On the other hand, \(2Na^+\) were added to neutralize the charge of the 6LU7 and 6Y2E systems. The counter ions of \(4Na^+\) were supplied as counter ions to the 6LXT system. All the three systems were subjected to energy minimization using the steepest descent algorithm for 10000 nsteps. The system was exposed to periodic boundary conditions, and the canonical ensemble (NVT) and isothermal-isobaric ensemble (NPT) were employed for 50000 nsteps at normal temperature (300K) and 1 bar of pressure. Berendsen temperature coupling and standard pressure coupling were used for keeping the system in a stable environment (300K, 1 bar). To deal with long-range Coulomb interactions of the system, the particle mesh Ewald method was used. The LINCS algorithm was used to constrain all the bonds. For each system, MD simulation was performed for 130 nanosecond (ns), and trajectories were retrieved for further analysis. For instance, the different structural parameters such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuations (RMSF), Inter-molecular Hydrogen Bonding and Free Energy Landscape (FEL) were computed as a function of time to explore the dynamic behavior of the protein-ligand binding pattern.

In essence, the binding energy calculations of the complexes were calculated using molecular mechanics Poisson-Boltzmann surface area (MM/PBSA) method. A protocol developed by the Rashmi Kumari and GROMACS utilities were used to calculate the different energy terms of the system (http://rashmikumari.github.io/g_mmpbsa/) (Kumari & Kumar, 2014). The equilibrated trajectory information, the last 5 ns of each MD trajectories, were used as an input for MM/PBSA analysis. The binding free energies of each system were calculated as the amount of free energy difference between the unbound component and the complex structures. The MM/PBSA analysis provides an individual contribution to the binding energy and a per-residue contribution that provides the key amino acid residues, which might be helpful in better understanding the binding pattern of the compound.

**Pharmacokinetic analysis**

The drug-likeness of the ligand was predicted by Lipinski filter at pH 7.0, which helps distinguish drug-like molecules from non-drug like molecules. According to this, a drug should comply with a minimum of four of the five laid down criteria, which include molecular mass (< 500 Dalton), lipophilicity (< 5), hydrogen bond donors (< 5), hydrogen bond acceptors (< 10) and molar refractivity (between 40 and 130) (Lipinski, 2004).

The ligand properties concerning absorption, distribution, metabolism and excretion (ADME) were analyzed using SwissADME (Daina et al., 2017). In which the user can either draw their ligand or include SMILES. This website allows computing the ADME parameters, pharmacokinetic properties, drug-likeness that can support drug discovery. pkCSM online machine-learning platform (Pires et al., 2015) was used for toxicity prediction. It can provide information on water solubility, intestinal absorption (human), skin permeability, the volume of distribution, total clearance, maximum tolerated dose, skin sensitization, chronic toxicity, hepatotoxicity etc. To estimate the most probable targets of the compound SwissTargetPrediction server (Gfeller et al., 2014) was used. This online tool can predict the macromolecular targets (proteins from humans, mouse and rats) of small bioactive molecules.

**Results**

**Docking analysis**

Docking of the molecule, seselin (Figure 1) identified and purified from the leaves of A. marmelos with three different receptors using docking procedure showed that all the computationally predicted lowest energy complexes were stabilized by intermolecular hydrogen bonds and stacking interactions. The ligand seselin showed the best interaction with target receptors based on the RMSD values. In addition to RMSD clustering, AutoDockVina was also used to calculate the binding free energies of these interactive molecules to find the best binding mode. The calculated final docked energies were \(-6.3\) kcal/mol for Spike protein S2 (PDB-ID: 6LXT), \(-6.9\) kcal/mol for COVID-19 main protease (PDB ID: 6LU7) and for the free enzyme of SARS-CoV-2 (2019-nCoV) main protease (PDB ID: 6Y2E), it was \(-6.7\) kcal/mol. Docking results identified that the molecule seselin could enter the substrate-binding region of the active site (Figure 2). The results demonstrated clearly that the molecule seselin accurately interacted with all three receptors. High binding affinity was predicted for all the receptors. The molecule seselin observed the formation of hydrogen bonding with ARG1185 for spike protein S2 (Figure 3).

In COVID-19 main protease, seselin formed hydrogen bonding with the residue SER1003, a \(\pi\)-alkyl interaction with...
ALA958 and π - σ interaction with the residue THR961 (Figure 4). The ligand showing one hydrogen bond formation with the residue THR111, a Van der Waals interaction with GLN110 and a Pi-donor hydrogen interaction with THR292 was observed with free enzyme of the SARS-CoV-2 (2019-nCoV) main protease (Figure 5). Information about the atoms involved in bonding with ligands, bond lengths and docking energies of all the three receptors were given in Table 1.

**Molecular dynamics simulations**

MD simulation studies were performed to identify the structural and dynamic behaviour of the complex system. The RMSD (Root-mean-square deviation) were calculated for the backbone atoms for all the complexes. The results of RMSD were plotted in Figure 6. It was evident from the figure that all the complexes were showing up drift during the initial stage of MD simulation. It was important to note that all the systems were started to maintain a plateau after 100 ns of simulation. The RMSD value of the 6LU7 system was ended with ~0.45 nm, the 6Y2E system ended with ~0.40 nm, and the 6LXT system were ended with ~2 nm.

Further, the fluctuation of Cα residues of the target structure was monitored using RMSF (Root-mean-square fluctuation) analysis, and the results were represented in Figure 7. Notably, all the complexes were showing RMS fluctuation between ~ 0.05 and ~ 0.15 nm. Further, the intermolecular hydrogen bonding was assessed during the dynamic simulation using GROMACS’s g_hbond tool. The results were plotted in Figure 8. Importantly, all the complexes had a maximum of 3 hydrogen bond interactions with the respective target receptor.

The binding energy values obtained from the MM/PBSA analysis were represented in Table 2 and Figure 9. It is evident from the table that the van der Waals interaction energy majorly contributed binding free energies of all three systems. The compound seselin exhibited a total binding free energy of $-35.994 \pm 54.785$, $-50.697 \pm 59.195$ and $-26.121 \pm 39.985$ kJ/mol for 6LU7, 6Y2E and 6LXT, respectively. Moreover, the energy contributed by each amino acid residue with the respective target receptors were plotted in Figure 10. It was observed from the figure that CYS-22, PHE-66, ASN-65 and HIS-64 were the major contributing residues of the 6LU7 system. ASN-1178, ASN-953 and VAL-1176 major contributing residues of the system 6LXT.

The amino acid residues such as PRO-96, TRP-31,ALA-70, VAL-18, CYS-16, ASN-95, VAL-73, GLY-15 and GLY-71 were the key contributors to the binding of seselin to the 6Y2E receptor.

**Pharmacokinetic analysis**

Analysis of pharmacokinetic properties showed that the molecule seselin obeyed the Lipinski rule of five and had
efficient ADME properties. The biological molecule used in this present study was found to pass all the five criteria mentioned in Lipinski’s rule of five (Table 3). This suggested that the seselin had the great potential to work effectively as a novel drug. This information about the ADME properties of the seselin would be highly advantageous during the early process of drug discovery indeed intended to be the first step. Physicochemical descriptors such as ADME parameters,
pharmacokinetic properties, drug-like nature and medicinal chemistry friendliness that support drug discovery for the molecule used in the study were computed. Properties including intestinal absorption and blood-brain barrier penetration were also identified. It was predicted that the molecule could be transported across the intestinal epithelium, could cross the blood-brain barrier and soluble in an aqueous medium. The physicochemical properties computed by swiss ADME were presented in Table 4. Toxicity of the molecule predicted using the pkCSM online machine-learning platform suggested an optimal pharmacokinetic profile. It seemed to be characterized by better water solubility and metabolic stability. In addition to this, there was no AMES toxicity, and the maximum tolerated level in a human was about 0.033 log/mg/kg/day. It was found that human ether-a-go-go-related protein (hERGI) and hERGII were not inhibited by this compound. The molecule was not hepatotoxic and caused no skin sensitization. Swiss Target Prediction was used to validate and estimate the most probable targets of the bioactive molecule, seselin (Figure 11). The probability score computed for off-targets suggested that the molecule seselin had a high affinity towards the binding site that it was directed to. These details help understand the molecular mechanism underlying bioactivity, rationalizing possible side-effects, and assessing the possibility of repurposing therapeutically relevant compounds.

**Table 1.** Information about the atoms involved in bonding with ligands, bond lengths and docking energies of all the three receptors.

| Ligand                | Receptors               | Interacting residues | Bond length (Å) | Binding affinity (kcal/mol) |
|-----------------------|-------------------------|----------------------|-----------------|-----------------------------|
| Seselin               | Spike protein S2        | ASP1184, GLU1182, ARG1185, SER943 | 3.72 Å, 3.69 Å, 2.91 Å, 3.61 Å | −6.3 kcal/mol, −6.9 kcal/mol |
|                       | COVID-19 main protease  | SER1003, ALA958, THR961 | 3.66 Å, 5.12 Å, 4.50 Å | −6.9 kcal/mol |
|                       | Free enzyme of the SARS-CoV-2 main protease | THR111, GLN110, THR292 | 3.73 Å, 5.28 Å, 5.41 Å | −6.7 kcal/mol |

**Figure 6.** RMSD analysis of the compounds with CoV-2 receptors (a) 6LXT complex (b) 6LU7 complex (c) 6Y2E complex.
Discussion

Pharmacophore modelling of bioactive compounds against the respiration deteriorating virus has been initiated ever since the outbreak of SARS way back in 2002, providing new research ideas for drug discovery projects (Chou et al., 2006). Also, extensive investigations on therapeutic targets to SARS-CoV-2, particularly towards spike protein and protease, for the discovery of potential drugs by screening the library of phytochemical compounds through computational methods have been reporting linearly since the emergence of this virulent strain lately (Hall & Ji, 2020; Ul Qamar et al., 2020; Wu et al., 2020). Molecular docking is a modeling approach that virtualizes the interaction between ligand and its receptor, specifically at the atomic level. This tactic magnifies the characteristic properties of a molecule in terms of binding the target; thereby, one can understand the biochemical process involved. It basically predicts the conformation, position and orientation of a ligand in a binding site with an energy rate. The thumb rule is that the lower the binding energy, the higher the affinity towards the target (McConkey et al., 2002; Meng et al., 2011). Notably, the binding energy tested for seselin against multiple targets on SARS-CoV-2 in this study was found to be lower, i.e., $-6.3 \text{ kcal/mol}$, $-6.9 \text{ kcal/mol}$ and $-6.7 \text{ kcal/mol}$ for SARS-CoV-2S spike protein (6LXT), SARS-CoV-2 main protease (6LU7) and free enzyme of SARS-CoV-2 (2019-nCoV) main protease (6Y2E), respectively, which implies the higher degree of affinity. The binding energies of kamferol, curcumin, pterostilbene, hydroxychloroquine, fisetin, quercetin, isorhamnetin, genistein, luteolin, resveratrol and apigenin reported against the SARS-CoV-2S spike protein are $-7.4 \text{ kcal/mol}$, $-7.1 \text{ kcal/mol}$, $-6.7 \text{ kcal/mol}$, $-5.6 \text{ kcal/mol}$, $-8.5 \text{ kcal/mol}$, $-8.5 \text{ kcal/mol}$, $-8.3 \text{ kcal/mol}$, $-8.2 \text{ kcal/mol}$, $-8.2 \text{ kcal/mol}$, $-7.9 \text{ kcal/mol}$ and $-7.7 \text{ kcal/mol}$ and $-3.09 \text{ kcal/mol}$, respectively. Similarly, the binding energies of lopinavir, oseltamivir, ritonavir, talampicillin, lurasidone, apigenin, curcumin, glabridi, glycoumarin, glycyrrhizin, hederagenin, liquiritigenin, oleanolac acid, quercetin, rosmarinic acid, saffconilide, safficinolide, saquinavir, amaranthin and andrographolide reported against the SARS-CoV-2 main protease (6LU7) are $-4.1 \text{ kcal/mol}$, $-4.65 \text{ kcal/mol}$, $-5.11 \text{ kcal/mol}$, $-11.17 \text{ kcal/mol}$, $-11.17 \text{ kcal/mol}$, $-7.8 \text{ kcal/mol}$, $-7.0 \text{ kcal/mol}$, $-8.0 \text{ kcal/mol}$, $-7.5 \text{ kcal/mol}$, $-7.2 \text{ kcal/mol}$, $-7.6 \text{ kcal/mol}$, $-7.7 \text{ kcal/mol}$, $-7.8 \text{ kcal/mol}$, $-7.3 \text{ kcal/mol}$, $-7.1 \text{ kcal/mol}$, $-6.8 \text{ kcal/mol}$, $-7.1 \text{ kcal/mol}$, $-7.6 \text{ kcal/mol}$, $-8.1 \text{ kcal/mol}$, $-9.2 \text{ kcal/mol}$, $-12.67 \text{ kcal/mol}$ and $-3.09 \text{ kcal/mol}$, respectively. Likewise, the binding energies of apigenin, curcumin, glabridi, glycoumarin, glycyrrhizin, hederagenin, liquiritigenin, oleanolac acid and quercetin reported against the free enzyme of SARS-CoV-2 (2019-nCoV) main protease (6Y2E) are $-7.0 \text{ kcal/mol}$, $-6.4 \text{ kcal/mol}$, $-7.1 \text{ kcal/mol}$, $-7.1 \text{ kcal/mol}$, $-8.4 \text{ kcal/mol}$, $-7.7 \text{ kcal/mol}$, $-6.9 \text{ kcal/mol}$, $-8.0 \text{ kcal/mol}$ and $-7.4 \text{ kcal/mol}$, respectively (Elmezayen et al., 2020; Enmozhi et al., 2020; Muralidharan et al., 2020; Rane et al., 2020; Sampangi-Ramaiah et al., 2020; Ul Qamar et al., 2020). Intermolecular forces, including hydrogen bonding, determine the binding of a ligand to its receptor. Among various interactions, hydrogen bonding is considered to be crucial for interaction specificity. By participating in receptor-drug complexation, hydrogen bonds play an essential role in determining conformational stability and

Figure 7. RMSF analysis of the compounds with CoV-2 receptors (a) 6LXT complex (b) 6LU7 complex (c) 6Y2E complex.
biological activity (Hadži et al., 1990). Furthermore, hydrogen bonding can affect membrane transport and the distribution of the drug within the biological system (Kubinyi, 2001). In the strategy of bioisosterism, hydrogen bond capacity is an essential factor for drug design and optimization (Yunta, 2017). On the other hand, amino acid residues are critical for the virus protein to interact with a host receptor. This can create a better application for site-directed mutagenesis to test the hypothesis for developing an effective antiviral therapy. A docking study on the protein complex of SARS-CoV-S1 with ACE2 suggested that proposing a mutated residue would effectively block the receptor binding, preventing cellular entry of SARS-CoV (Zhang et al., 2005). This reported computational study was even strengthened by experimental proof earlier (Huentelman et al., 2004).

MD simulations are beneficial for studying the conformational space of proteins and visualizing dynamic structural changes (Liu et al., 2018). The lower fluctuations during the RMSD and RMSF analysis indicated the higher stability of the seselin in the binding pocket of target structures. It is worth noting that throughout the binding process, all three systems yielded minimal fluctuations (± ~0.05 nm) except the 6LXT system during the RMSD calculation. Also, the compound seselin displayed minimal RMS fluctuations at the binding site of respective target receptors. For instance, ASP1184, GLU1182, ARG1185 and SER943 showed RMSF

Figure 8. Inter-molecular hydrogen bond analysis of the compounds with CoV-2 receptors (a) 6LX7 complex (b) 6LU7 complex (c) 6Y2E complex.

Table 2. Interaction energy analysis of the complexes during MM/PBSA analysis.

| Energy Terms               | Compounds |
|----------------------------|-----------|
| Van der Waal energy (kJ/mol) | 6LU7      | 6Y2E     | 6LXT     |
| Electrostatic energy (kJ/mol)   | -31.207 +/- 68.219 | -73.140 +/- 56.998 | -28.324 +/- 45.266 |
| Polar solvation energy (kJ/mol) | -2.068 +/- 5.152  | -7.718 +/- 9.460  | -1.054 +/- 3.712  |
| SASA energy (kJ/mol)            | -0.000 +/- 0.000  | -6.109 +/- 4.847  | -2.491 +/- 4.063  |
| Binding energy (kJ/mol)          | -35.994 +/- 54.785 | -50.697 +/- 59.195 | -26.121 +/- 39.985 |
fluctuation of \( \sim 0.075 \) nm in the 6LXT system. In such a way that SER1003, ALA958 and THR961 in the 6LU7 system were displayed \( \sim 0.075 \) nm RMSF fluctuation and THR111, GLN110 and THR292 were present in the 6Y2E system exposed fluctuation of \( \sim 0.05 \) nm. The higher binding affinity of the system was also indicated by more H-bonds between the protein and the ligand molecule (Surti et al., 2020). Interestingly, all three systems produce three stable inter-molecular hydrogen bond interactions throughout the simulation period. We believe that this increased number of hydrogen bonds with a consistent interaction mode may result in tightly bound complex systems.

Moreover, the various energy terms calculated during the MM/PBSA analysis produce additional support to understand the system’s binding affinity. Each system has a large number of amino acid residues that contribute to the binding free energy. Notably, high contributing residues are more than the weakly contributing residues in all the systems. In particular, the 6Y2E system has 9 highly contributing residues which are better than the other two systems investigated in this study. Overall, these MD simulations and MM/PBSA results produce more incredible support for the effective binding of seselin with the SARS-CoV-2 target receptors.

The discovery and development of drugs are exorbitant and time-investing. Accurate predictions of the in vivo pharmacokinetics of the drug under study earlier in the process are paramount since this can prevent clinical phase drug development failures. The Lipinski rule assesses five factors that determine the possible and potential interactions between a drug and the target. It appraises the tendency of the desired compound to fall under specific essential categories. It states that an orally active drug should comply with a minimum of four of the five laid down criteria that include (a) molecular mass < 500 Dalton (b) high lipophilicity (expressed as LogP < 5) (c) less than 5 hydrogen bond donors (d) less than 10 hydrogen bond acceptors (e) molar refractivity between 40-130 (Lipinski, 2004). It assures the recognition of drug absorption, tissue distribution, the fate of metabolism, its excretion and toxicity (ADMET), which can optimize the selection of suitable drug candidates for development (Boobis et al., 2002). Our results on this concern showed that the seselin has potentially cleared all the criteria put forth.

Drug safety assessment is critical which should be evaluated in preclinical and clinical trial phases. Prediction of toxicity of the compound rationalizes possible side-effects and assesses the possibility of repurposing therapeutically relevant compounds (Yang et al., 2018). Cytochrome p450 (CYP) enzymes are essential oxidases that help in the metabolism of drugs. Induction or inhibition of the enzyme, CYP3A4 mainly found in the liver and intestine, oxidizes small foreign organic molecules such as toxins or drugs that may influence...
the drug’s pharmacokinetics altering their efficacy or toxicity. Another important target of many drugs is hERG (human ether-a-go-go-related protein responsible for K\textsubscript{s}11.1, the alpha subunit of a potassium ion channel), the blockage of which can lead to sudden death (Feng et al., 2018).

An optimum pharmacokinetic profile is desired because it can prevent unforeseen toxic effects on humans (Bugrim et al., 2004). In-depth knowledge of the toxicological profile of the drug molecule is crucial as the toxicity is the reason for the failure of the drug in many clinical trials (Cronin, 2001). One of the most decisive steps in rationalizing a bio-molecule is predicting or mapping its targets. This can, in turn, provide molecular insights into the mode of action of the drug candidate, possible side effects or cross-reactivity. For many proteins, including kinases and phosphatases, hundreds of ligand molecules are identified. Understanding the bioactive molecule’s potential targets can also help understand how a molecule can be chemically modified to improve its bioactivity towards a particular target protein. The probability score calculated can thus provide information on how specific the ligand is to the target that it is directed to (Gfeller et al., 2014). Water solubility (LogS), Caco-2 permeability and topological polar surface area (TPSA) all fell within

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**Table 3.** Various parameters of ligand seselin computed by Lipinski filter.

| Compound | Molecular weight | Lipophilicity | Hydrogen bond donors | Hydrogen bond acceptors | Molar refractivity |
|----------|------------------|---------------|----------------------|------------------------|------------------|
| Seselin  | 228.000000       | 2.803000      | 0                    | 3                      | 66.61            |

**Table 4.** Various physicochemical properties of ligand seselin computed by Swiss ADME.

- **Formula:** C\textsubscript{14}H\textsubscript{12}O\textsubscript{3}
- **Molecular weight:** 228.24 g/mol
- **Num. heavy atoms:** 17
- **Num. arom. heavy atoms:** 10
- **Fraction Csp3:** 0.21
- **Num. rotatable bonds:** 0
- **Num. H-bond acceptors:** 3
- **Num. H-bond donors:** 0
- **Molar Refractivity:** 66.61
- **TPSA:** 39.44 Å\textsuperscript{2}

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**Figure 10.** Contribution energy of amino acids of (a) 6XLT (b) 6LU7 (C) 6Y2E during the binding process.

**Figure 11.** Probable targets of the compound predicted by Swiss Target Prediction server.
the reference range. Seselin as an anti-SARS-COV-2 molecule achieved with good binding affinity and ADMET. The outcome of pharmacokinetic analysis suggests that the compound had favourable drugability properties that further help eliminate expensive reformulation later.

Along with the antiviral nature of ‘seselin’ in our earlier report, it has also been proven to be an inhibitor of indole acetic oxide and peroxidase enzyme (Goren & Tomer, 1971), oxicidal (Tanaka et al., 1985), tumour-suppressive and anti-HIV (Huang et al., 1994), cytotoxic (Gunatilaka et al., 1994), antinoicceptive and vasodilatory (Lima et al., 2006), anti-fungal (Ortega et al., 2007), antifeedant and larvicidal (Mukandiwa et al., 2013, 2015) and DNA binding specific (Parveen et al., 2016), that by showcasing itself in a broad manner of acquiring bioactive potentials. Thus, the findings of this study suggest that the in silico multiple target inhibition of seselin on SARS-COV-2 virus proteins predicted with higher binding affinity, non-toxicity, solubility and stability, is proficient and competent for pursuing the experiments to prove at in vitro and in vivo studies to hold its candidacy in therapeutics and drug discovery for COVID-19.

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References
Arul, V., Kumaraguru, S., & Dhananjayan, R. (1999). Effects of ageline and lupeol, the two cardioactive principles isolated from the leaves of Aegle marmelos Corr. Journal of Pharmacy and Pharmacology, 51, 252–252.
Badam, L., Bedekar, S. S., Sonawane, K. B., & Joshi, S. P. (2002). In vitro antiviral activity of bael (Aegle marmelos Corr) upon human coxsackieviruses B1-B6. The Journal of Communicable Diseases, 34(2), 88–99.
Boobis, A., Gundert-Remy, U., Kremer, P., Macheras, P., & Pelkonen, O. (2002). In silico prediction of ADME and pharmacokinetics: Report of an expert meeting organised by COST B15. European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences, 17(4–5), 183–193. https://doi.org/10.1016/S0928-0987(02)00185-9
Bugrim, A., Nikolaikaya, T., & Nikolsky, Y. (2004). Early prediction of drug metabolism and toxicity: Systems biology approach and modeling. Drug Discovery Today, 9(3), 127–135. https://doi.org/10.1016/S1359-6446(03)02971-4
Chadwick, D. J., & Marsh, J. (Eds.). (2008). Ethnobotany and the search for new drugs. Ciba Foundation Symposium (Vol. 185). John Wiley & Sons.
Chou, K. C., Wei, D. Q., Du, Q. S., Siras, S., & Zhong, W. Z. (2006). Progress in computational approach to drug development against SARS. Current Medicinal Chemistry, 13(27), 3263–3270. https://doi.org/10.2174/09298670778773077
Cronin, M. T. (2001). Prediction of drug toxicity. Farmaco (Societa Chimica Italiana: 1989, 56(1–2), 149–151. https://doi.org/10.1016/S0014-2707(01)01018-7
Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7, 42717 https://doi.org/10.1038/srep42717
Dallalayn, S. (2008). PyRx-python prescription v. 0.8. The Scripps Research Institute, 2010.
Elmezayen, A. D., Al-Obaidi, A., Şahin, A. T., & Yeleki, K. (2020). Drug repurposing for coronavirus (COVID-19): In silico screening of known drugs against coronavirus 3CL hydrolyase and protease enzymes. Journal of Biomolecular Structure and Dynamics, 39(8), 2980–2992. https://doi.org/10.1080/07391102.2020.1758791
Enmozi, S. K., Raja, K., Sebastine, I., & Joseph, J. (2020). Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: An in silico approach. Journal of Biomolecular Structure and Dynamics, 39(9), 3092–3098. https://doi.org/10.1080/07391102.2020.1760136
Farquhar, N. R. (1994). Ethnopharmacology and drug development. In Ciba foundation symposium 185-Ethnobotany and the search for new drugs: Ethnobotany and the Search for New Drugs: Ciba Foundation Symposium. John Wiley & Sons.
Faroog, S. (2005). 5SS medicinal plants. Field and laboratory manual (identification with its phytochemical and in vitro studies data. International Book Distributors.
Feng, P., Zhao, L., Guo, F., Zhang, B., Fang, L., Zhan, G., Xu, X., Fang, Q., Liang, Z., & Li, B. (2018). The enhancement of cardiotoxicity that results from inhibition of CYP 3A4 activity and hERG channel by berberine in combination with statins. Chemico-Biological Interactions, 293, 115–123. https://doi.org/10.1016/j.cbi.2018.07.022
Gefiler, D., Grosdidier, A., Wirth, M., Daina, A., Michielin, O., & Zoete, V. (2014). SwissTargetPrediction: A web server for target prediction of bioactive small molecules. Nucleic Acids Research, 42(1), 32–38.
Goren, R., & Tomer, E. (1971). Effects of seselin and coumarin on growth, indoleacetic acid Oxidase, and Peroxidase, with Special Reference to cucumber (Cucumis sativa L) Radicles. Plant Physiology, 47(2), 312–316. https://doi.org/10.1104/pp.47.2.312
Govindachari, T. R., & Premila, M. S. (1983). Some alkaloids from Aegle marmelos. Phytochemistry, 22(3), 755–757. https://doi.org/10.1016/S0031-1829(01)85977-0
Gunatilaka, A. A., Kingston, D. G., Wijeratne, E. M., Bandara, B. M., Hofmann, G. A., & Johnson, R. K. (1994). Biological activity of some coumarins from Sri Lankan Rutaceae. Journal of Natural Products, 57(4), 518–520. https://doi.org/10.1021/np50106a013
Hadzi, D., Kidric, J., Koller, J., & Mavri, J. (1990). The role of hydrogen bonding in drug-receptor interactions. Journal of Molecular Structure, 237, 139–150. https://doi.org/10.1016/0022-2860(89)80136-6
Hall, D. C., Jr, & Ji, H. F. (2020). A search for medications to treat COVID-19 via in silico molecular docking models of the SARS-COV-2 spike glycoprotein and 3CL protease. Travel Medicine and Infectious Disease, 35, 101646. https://doi.org/10.1016/j.tmaid.2020.101646
Hofmann, H., & Höflimann, S. (2004). Cellular entry of the SARS corona-virus. Trends in Microbiology, 12(1), 466–472. https://doi.org/10.1016/S0962-8924(04)00080-8
Huang, L., Kashiwada, Y., Cosentino, L. M., Fan, S., & Lee, K. H. (1994). 3’4’-Di-(c-o-()-camphanyloyl(-)()-ciskhellactone and related compounds: A new class of potent anti-HIV agents. Bioorganic and Medicinal Chemistry Letters, 4(4), 593–598. https://doi.org/10.1016/S0960-8944(01)80161-X
Huentelman, M. J., Zubcevic, J., Hernandez Prada, J. A., Xiao, X., Dimitrov, D. S., Razaida, M. K., & Ostrov, D. A. (2004). Structure-based discovery of a novel angiotensin-converting enzyme 2 inhibitor. Hypertension, 44(6), 903–906. https://doi.org/10.1161/01.HYP.0000146120.29648.36
Jimenez-Garcia, S. N., Vazquez-Cruz, M. A., Guevara-Gonzalez, R. G., Torres-Pacheco, I., Cruz-Hernandez, A., & Feregrino-Perez, A. A. (2013). Current approaches for enhanced expression of secondary metabolites as bioactive compounds in plants for agronomic and human health purposes—a review. Polish Journal of Food and Nutrition Sciences, 63(2), 67–78. https://doi.org/10.2478/v10220-012-0072-6
Kumari, R., & Kumar, R. (2014). g_mmpbsa—A GROMACS tool for high-throughput MM-PBSA calculations. Journal of Chemical Information and Modeling, 54(7), 1951–1962. https://doi.org/10.1021/ci500202m
Kuo, C. J., Liu, H. G., Lo, Y. K., Seong, C. M., Lee, K. I., Jung, Y. S., & Liang, P. H. (2009). Individual and common inhibitors of coronavirus and picornavirus main proteases. FEBS Letters, 583(3), 549–555. https://doi.org/10.1016/j.febslet.2008.12.059
Lima, V., Silva, C. B., Mafezoli, J., Bezerra, M. M., Moraes, M. O., Mourao, G. S., Silva, J. N., & Oliveira, M. C. (2006). Antinociceptive activity of the pyrazocumarin seselin in mice. Fitoterapia, 77(8–9), 574–578. https://doi.org/10.1016/j.fitote.2006.09.005

Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. Drug Discovery Today, Technologies, 1(4), 337–341. https://doi.org/10.1016/j.ddte.2004.11.007
Liu, X., Zhang, B., J., Yang, H., & Rao, Z. (2020). The crystal structure of COVID-19 main protease in complex with an inhibitor N3. RCSB PDB.
Manandhar, M. D., Shoeb, A., Kapil, R. S., & Popli, S. P. (1978). New alkaloids from Aegle marmelos. Phytochemistry, 17(10), 1814–1815. https://doi.org/10.1016/S0031-9422(00)88714-2
McConkey, B. J., Sobolev, V., & Edelman, M. (2002). The performance of current methods in ligand-protein docking. Current Science, 83(7), 845–855.
Melnick, J. L. (1984). Entoriversus. In A. S. Evans (Ed.), Viral infections of humans (pp. 187–251). Springer.
Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. Current Computer-Aided Drug Design, 7(2), 146–157. https://doi.org/10.2174/157340911795677602
Mukandiwa, L., Ahmed, A., Elloff, J. N., & Naidoo, V. (2013). Isolation of seselin from Clausena anisata (Rutaceae) leaves and its effects on the feeding and development of Lucilia cuprina larvae may explain its use in ethnoveterinary medicine. Journal of Ethnopharmacology, 150(3), 886–891. https://doi.org/10.1016/j.jep.2013.09.037
Mukandiwa, L., Elloff, J. N., & Naidoo, V. (2015). Larvicidal activity of leaf extracts and seselin from Clausena anisata (Rutaceae) against Aedes aegypti. South African Journal of Botany, 100, 169–173. https://doi.org/10.1016/j.sajb.2015.05.016
Muralidharan, N., Sakhivel, R., Velmurugan, D., & Gromiha, M. M. (2020). Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 main protease. Journal of Biomolecular Structure and Dynamics, 1–11. https://doi.org/10.26434/chemxriv.12094203v1
Sampangi-Ramaiah, M. H., Vishwakarma, R., & Shaanker, R. U. (2020). Molecular docking analysis of selected natural products from plants for inhibition of SARS-CoV-2 main protease. Current Science, 118(7), 1087–1092.
Scavone, C., Brusco, S., Bertini, M., Sportiello, L., Rafaniello, C., Zoccoli, A., Berrino, L., Racagni, G., Rossi, F., & Capuano, A. (2020). Current pharmacological treatments for COVID-19: What’s next? British Journal of Pharmacology, 177(21), 4813–4824. https://doi.org/10.1111/bph.15072
Singh, G., Jayadev Magani, S. K., Sharma, R., Bhat, B., Shrivastava, A., Chinthakindi, M., & Singh, A. (2019). Structural, functional and molecular dynamics analysis of cathepsin B gene SNPs associated with tropical calcific pancreatitis, a rare disease of tropics. PeerJ, 7, e7425. https://doi.org/10.7717/peerj.7425
Somo, C., Karuppiah, H., & Sundaram, J. (2019). Antiviral activity of seselin from Aegle marmelos against nuclear polyhedrosis virus infection in the larvae of silkworm, Bombyx mori. Journal of Ethnopharmacology, 245, 112155. https://doi.org/10.1016/j.jep.2019.112155
Surti, M., Patel, M., Adnan, M., Moin, A., Ashraf, S. A., Siddiqui, A. J., Snoussi, M., Deshpande, S., & Reddy, M. N. (2020). Ilimaquinone (marine sponge metabolite) as a novel inhibitor of SARS-CoV-2 key target proteins in comparison with suggested COVID-19 drugs: Designing, docking and molecular dynamics simulation study. RSC Advances, 10(62), 37707–37720. https://doi.org/10.1039/D0RA06379G
Tanaka, H., Ahn, J. W.,Katayama, M., Wada, K., Marumo, S., & Osaka, Y. (1985). Isolation of two ovicidal substances against two-spotted spider mite. Agricultural and Biological Chemistry, 49(7), 2189–2190. https://doi.org/10.1271/bbb1961.49.2189
Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455–461. https://doi.org/10.1002/jcc.21334
Ul Qamar, M. T., Alqahanti, S. M., Alamri, M. A., & Chen, L. L. (2020). Structural basis of SARS-CoV-2 3C_lpro and anti-COVID-19 drug discovery from medicinal plants. Journal of Pharmaceutical Analysis, 10(4), 313–319. https://doi.org/10.1016/j.jpha.2020.03.009
Wan, Y., Zhang, J., Graham, R., Baric, R. S., & Li, F. (2020). Receptor recognition by the novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS coronavirus. Journal of Virology, 94(7), e00127–20. https://doi.org/10.1128/JVI.00127-20
Wanap, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., Graham, B. S., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science (New York, N.Y.), 367(6483), 1260–1263. https://doi.org/10.1126/science.abb2507
Wu, C., Liu, Y., Yang, Z., Zhang, P., Zhong, W., Wang, Y., Wang, Q., Xu, Y., Li, M., Li, X., Zheng, M., & Zheng, M. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharmacologica Sinica B, 10(5), 766–788. https://doi.org/10.1016/j.apsb.2020.02.008
Yang, H., Sun, L., Li, W., Liu, G., & Tang, Y. (2018). In silico prediction of chemical toxicity for drug design using machine learning methods and structural alerts. Frontiers in Chemistry, 6, 30.
Yunta, M. J. (2017). It is important to compute intramolecular hydrogen bonding in drug design. Am J Model Optim, 5, 24–57.
Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauhering, L., Becker, S., Rox, K., & Hilgenfeld, R. (2020). Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved γ-ketoamide inhibitors. Science (New York, N.Y.), 368(6549), 409–412. https://doi.org/10.1126/science.abb3405
Zhang, L., Sun, X., & Hilgenfeld, R. (2020). Crystal structure of the free enzyme of the SARS-CoV-2 (2019-nCoV) main protease. RCSB PDB.
Zhang, Y., Zheng, N., Hao, P., Cao, Y., & Zhong, Y. (2005). A molecular docking model of SARS-CoV S1 protein in complex with its receptor, human ACE2. Computational Biology and Chemistry, 29(3), 254–257. https://doi.org/10.1016/j.compbiolchem.2005.04.008
Zhu, Y., & Sun, F. (2020). Structure of post fusion core of 2019-nCoV S2 subunit. RCSB PDB.