

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

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## Research Article

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# Abstract

Our earlier experimental and computational report produced the evidence on anti-viral 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 SARS-CoV-2 virus, an *in silico* effort to evaluate the potentiality of the seselin has been made to test its efficacy against multiple targets of SARS-CoV-2 such as SARS-CoV-2S spike protein, 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 SARS-CoV-2S protein, COVID-19 main protease and free enzyme of the SARS-CoV-2 (2019-nCoV) main protease with a binding energy of -6.6 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 aminoacid residues involved in interactions are THR111 and GLN110 for spike protein, SER1003, ALA958 and THR961 for COVID-19 main protease, and for SARS-CoV-2 (2019-nCoV) main protease, it is THR111, GLN110 and THR292. The outcome of pharmacokinetic analysis suggests that the compound had favourable drugability properties by obeying Lipinski rule of five with efficient ADME properties and exhibiting high affinity towards the binding site that it was directed to. The results suggest that the seselin has inhibitory potential over multiple SARS-CoV-2 targets and holds a high potential to work effectively as a novel drug for COVID-19, if evaluated in experimental set ups in foreseeable future.

## Introduction

The outbreak of corona virus disease 2019 (COVID-19) disease recently has transitioned into pandemic state and consumed numerous human lives due to its contagious nature. This dreadful disease has infected more than 3.15 million people and the reported deaths have been over 0.22 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 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 opted by testing batches of patients across the world; nevertheless it is still in biased and anecdotal state [1].

The genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is composed of 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 replication of virus such as polymerase, helicase and endoribonuclease [2]. In a short period of time, documentation on molecular docking of naturally occurring plant compounds with SARS-CoV-2 proteins have been reported with plausible lower binding

energy. Among this, particular attention has been given to spike protein and proteases of SARS-CoV-2 [3]. 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 3C-like serine protease forms the replication enzymes of virus [4-7]. The resulted information of reported docking studies highly facilitates the categorization of drugs and takes a call on proceedings 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 the life saving drugs from them [8]. The definitive approach of ethnopharmacology is to classify the evidence of medicinal plants and isolate the drugs that take the edge off the human illness [9]. In the process of 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 with several bioactive compounds such as aegelin, lupeol, cuminaldehyde, eugenol, skimmianine, cineol, citral, marmesinin, marmelide,  $\beta$ -sitosterol, flavone, glycoside and marmeline is chosen for the study for the following reasons [10-13]. *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), meningoencephalitis (types 1-5), myocarditis (types 1-5), cardiomyopathy, pancreatitis etc., [14, 15]. Interestingly, a high throughput screening of approximately 6800 small molecules identified that proteases of picornavirus and coronavirus have a common inhibitors [16]. Importantly, in our earlier report, we have successfully purified the molecule seselin from the leaf extracts of *A. marmelos* [17]. It is noteworthy that it exhibited anti-viral 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 pay off endeavour ultimately [18]. In the midst of pandemic scenario, rapid and prompt report analysis of drug's eligibility by means of *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 anti-viral nature, this study is aimed at analyzing 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 [17] 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. The atomic co-ordinate and quality of structures were verified by CORINA-SMILES notations. To prepare the ligand for docking, Open Babel [19] in PyRx 0.8 [20] 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 SARS-CoV-2S protein (PDB-ID: 6VYB) [21], the crystal structure of COVID-19 main protease in complex with an inhibitor N3 (PDB ID: 6LU7) [22] and the free enzyme of the SARS-CoV-2 (2019-nCoV) main protease (PDB ID: 6Y2E) [23] were downloaded from the RCSB protein data bank (<https://www.rcsb.org/search>). The receptors preparation was performed by optimizing protein model geometry, removal of ligands and other heteroatoms using Biovia Discovery studio 20.1.0.

### **Prediction of potential ligand binding site**

Prior to docking analysis, the ligand binding sites on protein surface were identified using Metapocket 2.0 (<https://projects.biotec.tu-dresden.de/metapocket/index.php>). MetaPocket 2.0 uses consensus method in which the predicted binding sites from eight methods such as LIGSITE<sup>CS</sup>, PASS, QSiteFinder, SURFNET, Fpocket, GHECOM, Concavity and POCASA are combined together 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 for analysis of 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 [24], PyRx 0.8. Binding site amino acids were selected and the docking site on 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.

### **Pharmacokinetic analysis**

The drug likeness of the ligand was predicted by Lipinski filter at pH 7.0 which helps in distinguishing 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) [25]. The properties of the ligand with respect to absorption, distribution, metabolism and excretion

(ADME) were analyzed using SwissADME [26] 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 [27] was used for toxicity prediction. It can provide information on water solubility, intestinal absorption (human), skin permeability, 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 [28] was used. This online tool can predict the macromolecular targets (proteins from human, mouse and rat) of bioactive small molecules.

## Results

### Docking analysis

Docking of the molecule, seselin (Fig. 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 -6.6 kcal/mol for SARS-CoV-2S protein (PDB-ID: 6VYB), -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 (Fig. 2). The results demonstrated clearly that the molecule, seselin accurately interacted with all the three receptors. High binding affinity was predicted for all the receptors. Formation of hydrogen bonding was observed by the molecule seselin with THR111 and GLN110 residues of SARS-CoV-2S protein (Fig. 3). In the case of COVID-19 main protease it was found that seselin formed hydrogen bonding with the residue SER1003, a  $\pi$ -alkyl interaction with ALA958 and  $\pi$  -  $\sigma$  interaction with the residue THR961 (Fig. 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 (Fig. 5). Information about the atoms involved in bonding with ligands, bond lengths and docking energies of all the three receptors were given in the Table 1.

### Pharmacokinetic analysis

Analysis of pharmacokinetic properties showed that the molecule, seselin obeyed 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's mentioned in Lipinski's rule of five (Table 2). This suggested that the seselin had the great potential to work effectively as 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 aqueous medium. The physicochemical properties computed by swiss ADME were presented in Table 3. Toxicity of the molecule predicted using pkCSM online machine-learning platform suggested an optimal pharmacokinetic profile. It seemed to be characterized by a better water solubility and metabolic stability. In addition to this, there was no AMES toxicity and the maximum tolerated level in human was about 0.033 log/mg/kg/day. It was found that human ether-a-go-go-related protein (hERG1) and hERG2 were not inhibited by this compound. The molecule was not hepatotoxic and caused no skin sensitisation. Swiss Target Prediction was used to validate and estimate the most probable targets of the bioactive molecule, seselin (Fig. 6). Probability score computed for off-targets suggested that the molecule, seselin had high affinity towards the binding site that it was directed to. These details are useful to understand the molecular mechanism underlying the bioactivity, rationalize possible side-effects and to assess the possibility of repurposing therapeutically-relevant compound.

## Discussion

Pharmacophore modeling of bioactive compounds against the respiratory deteriorating virus has been initiated ever since the outbreak of SARS way back at 2002, providing new research ideas for drug discovery projects [29]. Also, extensive investigations on therapeutic targets to SARS-CoV-2 particularly towards spike protein and protease for discovery of potential drugs by screening the library of phytochemical compounds through computational methods have also been reporting linearly since the emergence of this virulent strain lately [3, 30, 31]. The molecular docking is a modeling approach which virtualizes the interaction between ligand and its receptor specifically in 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 a rate of energy. The thumb rule is that lower the binding energy, higher the affinity towards the target [32, 33]. Notably, the binding energy tested for seselin against multiple targets on SARS-CoV-2 in this study was found to be lower i.e. -6.6 kcal/mol, -6.9 kcal/mol and -6.7 kcal/mol for SARS-CoV-2S spike protein, 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 kcal/mol, -7.1 kcal/mol, -6.7 kcal/mol, -5.6 kcal/mol, -8.5 kcal/mol, -8.5 kcal/mol, -8.3 kcal/mol, -8.2 kcal/mol, -8.2 kcal/mol, -7.9 kcal/mol and -7.7 kcal/mol and -3.09 kcal/mol, respectively. Similarly, the binding energies of lopinavir, oseltamivir, ritonavir, talampicillin, lurasidone, apigenin, curcumin, glabridi, glycoumarin, glycyrrhizin, hederagenin, liquiritigenin, oleanolic acid, quercetin, rosmarinic acid, safficinolide, sageone, ursolic acid, glucobrassicin, saquinavir, amaranthin and andrographolide reported against the SARS-CoV-2 main protease (6LU7) are -4.1 kcal/mol, -4.65 kcal/mol, -5.11 kcal/mol, -11.17 kcal/mol, -11.17 kcal/mol, -7.8 kcal/mol, -7.0 kcal/mol, -8.0 kcal/mol, -7.5 kcal/mol,

-7.2 kcal/mol, -7.6 kcal/mol, -7.7 kcal/mol, -7.8 kcal/mol, -7.3 kcal/mol, -7.1 kcal/mol, -6.8 kcal/mol, -7.1 kcal/mol, -7.6 kcal/mol, -8.1 kcal/mol, -9.2 kcal/mol, -12.67 kcal/mol and -3.09 kcal/mol, respectively. Likewise, the binding energies of apigenin, curcumin, glabridi, glycoumarin, glycyrrhizin, hederagenin, liquiritigenin, oleanolic acid and quercetin reported against the free enzyme of SARS-CoV-2 (2019-nCoV) main protease (6Y2E) are -7.0 kcal/mol, -6.4 kcal/mol, -7.1 kcal/mol, -7.1 kcal/mol, -8.4 kcal/mol, -7.7 kcal/mol, -6.9 kcal/mol, -8.0 kcal/mol and -7.4 kcal/mol, respectively [31, 34-38]. 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 important role in determining conformational stability and biological activity [39]. Hydrogen bonding can affect membrane transport and the distribution of the drug within the biological system [40]. In the strategy of bioisosterism, hydrogen bond capacity is an important factor for drug design and optimization [41]. On the other hand, amino acid residues are critical for the virus protein to interact with a host receptor and this can create a better application for site-directed mutagenesis to involve in testing the hypothesis for developing an effective antiviral therapies. A docking study on protein complex of SARS-CoV-S1 with ACE2 suggested that proposing a mutated residue would effectively block the receptor binding in turn preventing cellular entry of SARS-CoV [42]. This reported computational study was even strengthened by experimental proof earlier [43].

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 drug and the target. It appraises the tendency of a desired compound to fall under certain essential categories. It states that an orally active drug should comply to a minimum of four of the five laid down criteria that includes (a) molecular mass <500 Dalton (b) high lipophilicity (expressed as  $\text{LogP} < 5$ ) (c) less than 5 hydrogen bond donors (d) less than 10 hydrogen bond acceptors (e) molar refractivity between 40-130 [25]. It assures the recognition of absorption of drug, tissue distribution, fate of metabolism, its excretion and toxicity (ADMET) which can optimize the selection of suitable drug candidates for development [44]. Our results on this concern showed that the seselin has potentially cleared all the criteria put forth.

Drug safety assessment is very important 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 [45]. Cytochrome p450 (CYP) enzymes are important 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 may influence the pharmacokinetics of the drug altering their efficacy or toxicity. Another important target of many drugs is hERG (human ether-a-go-go-related protein responsible for  $\text{K}_v11.1$ , the alpha subunit of a potassium ion channel), the blockage of which can lead to sudden death [46].

An optimum pharmacokinetic profile is desired because it can prevent unforeseen toxic effects on human [47]. 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 [48]. One of the most decisive steps in rationalizing a biomolecule is predicting or mapping their 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 probable targets of the bioactive molecule can also help in understanding how a molecule can be chemically modified to improve its bioactivity towards a particular target protein. Probability score calculated can thus provide information on how specific the ligand is to the target that it is directed to [28]. Water solubility (LogS), Caco-2 permeability and topological polar surface area (TPSA) all fell within the reference range. Seselin as an anti-SARS-COV-2 molecule achieved with satisfactory binding affinity and ADMET. The outcome of pharmacokinetic analysis suggests that the compound had favourable drugability properties that further help to eliminate expensive reformulation later.

Along with the antiviral nature of 'seselin' in our earlier report as well as other cited reports, it has also been proven to be inhibitor of indole acetic oxidase and peroxidase enzyme [49], ovidical [50], tumor suppressive and anti-HIV [51], cytotoxic [52], antinociceptive and vasodilatory [53], anti-fungal [54], anti-feedant and larvicidal [55,56] and DNA binding specific [57], that by showcasing itself in a broad manner of acquiring bioactive potentials. 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 in *in vitro* and *in vivo* studies to hold its candidacy in therapeutics and drug discovery for COVID-19.

## Declarations

### Compliance with Ethical Standards

The authors declare that they have no conflict of interest. There were no human participants involved

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**Consent to participate:** There were no humans participants on the study and thus, consent is not needed.

**Consent for publication:** Authors can provide the signed approval upon publisher's request

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**Authors' contributions:** All authors have contributed equally.

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## Tables

**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	SARS-CoV-2S protein	THR11	3.55Å	-6.6 kcal/mol
		GLN110	3.98Å	
	COVID-19 main protease	SER1003	3.66Å	-6.9 kcal/mol
		ALA958	5.12Å	
		THR961	4.50Å	
	Free enzyme of the SARS-CoV-2 (2019-nCoV) main protease	THR111	3.73Å	-6.7 kcal/mol
		GLN110	5.28Å	
		THR292	5.41Å	

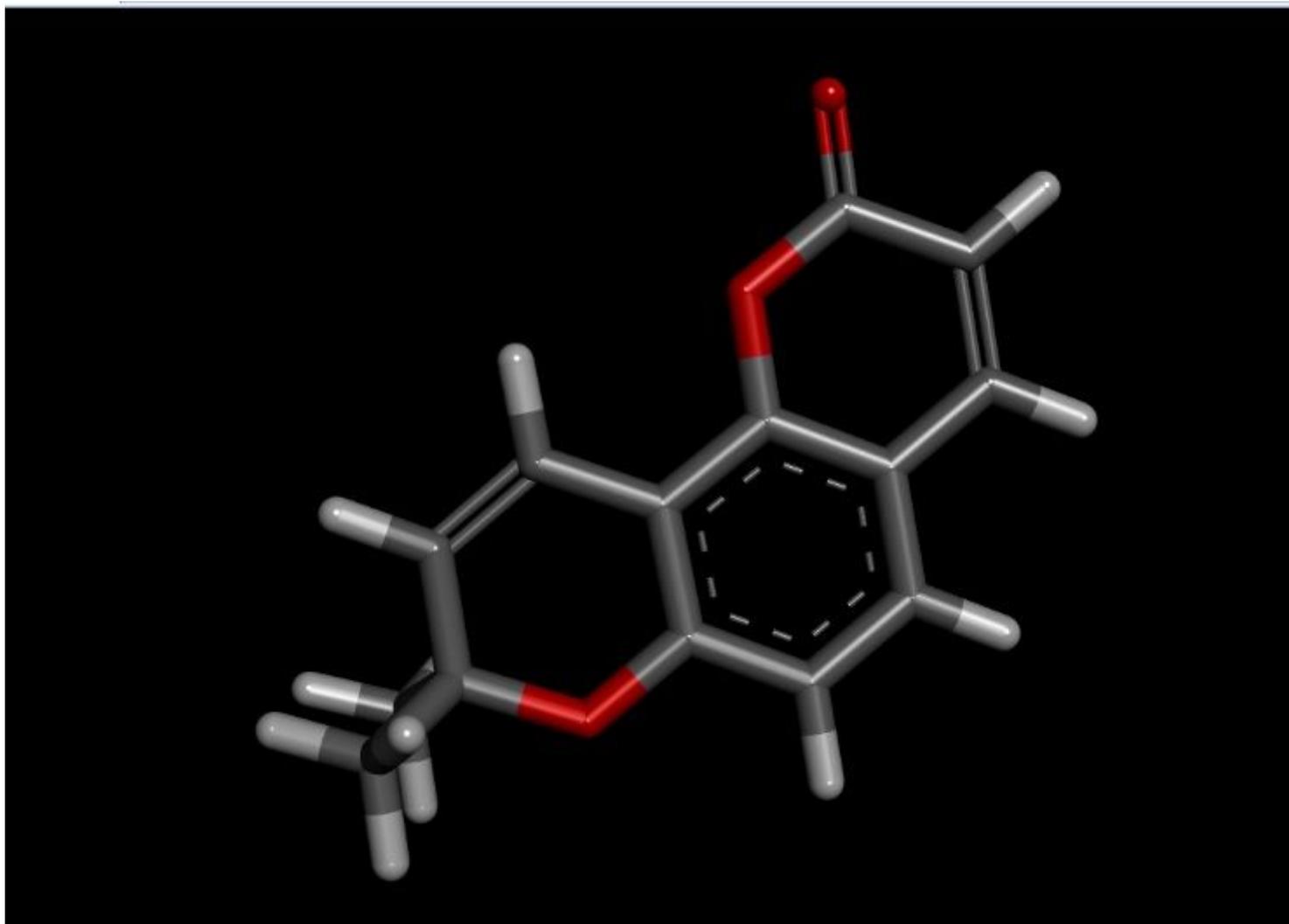
**Table 2** Various parameters of ligand seselin computed by Lipinski filter

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

**Table 3** Various physicochemical properties of ligand seselin computed by swiss ADME

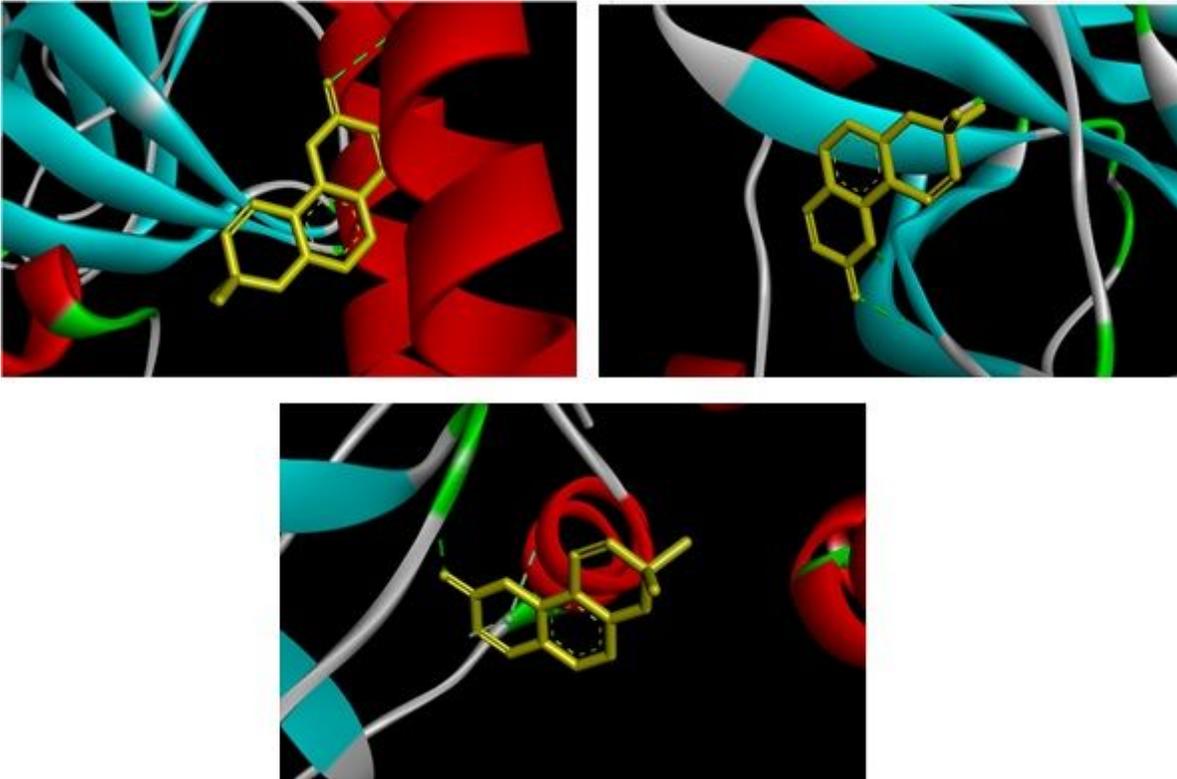
Formula	C <sub>14</sub> H <sub>12</sub> O <sub>3</sub>
Molecular weight	228.24 g/mol
Num. heavy atoms	17
Num. arom. heavy atoms	10
Fraction Csp3	0.21
Num. rotatable bonds	0
Num. H-bond acceptors	3
Num. H-bond donors	0
Molar Refractivity	66.61
TPSA	39.44 Å <sup>2</sup>

## Figures



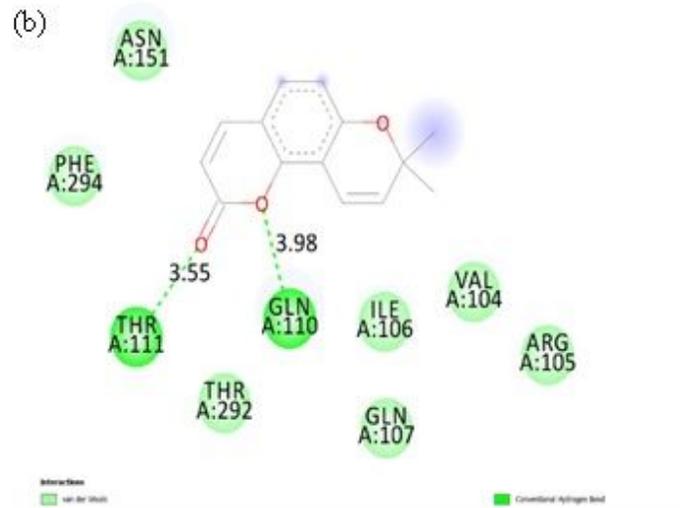
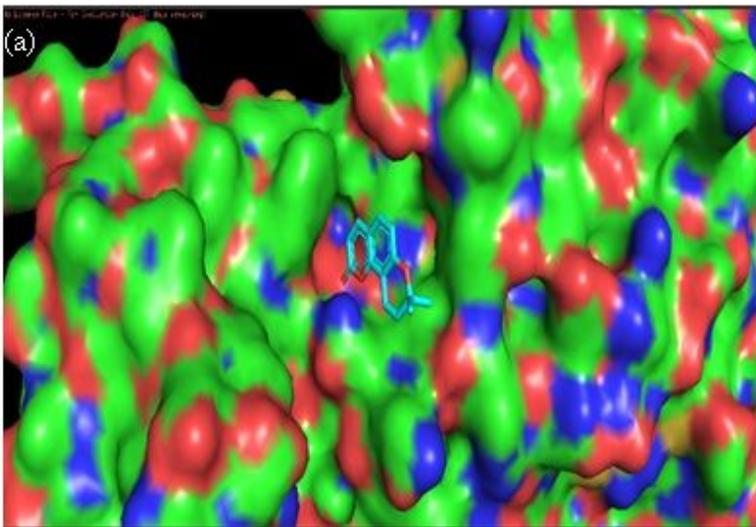
**Figure 1**

Three dimensional (3D) structure of seselin (Discovery Studio 20.0.1)



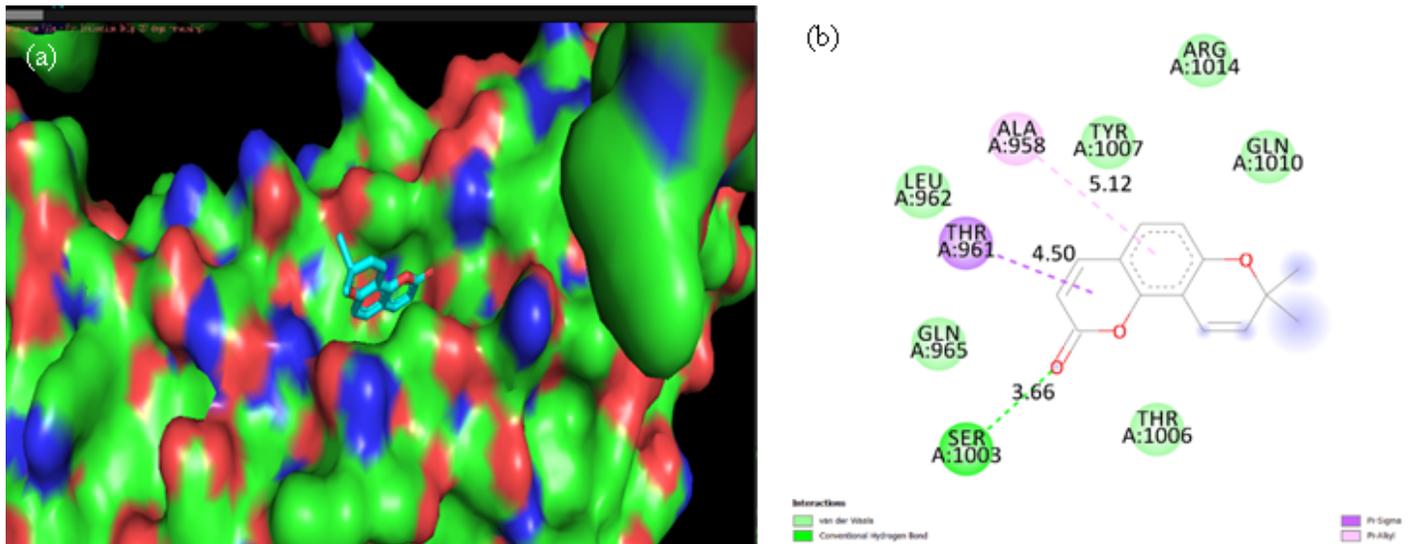
**Figure 2**

3D view of (a) SARS-CoV-2S, (b) COVID-19 main protease and (c) Free enzyme of the SARS-CoV-2 (2019-nCoV) main protease with ligand (seselin) docked in the binding site visualised using Discovery Studio 20.0.1. (Green lines indicating hydrogen bonds)



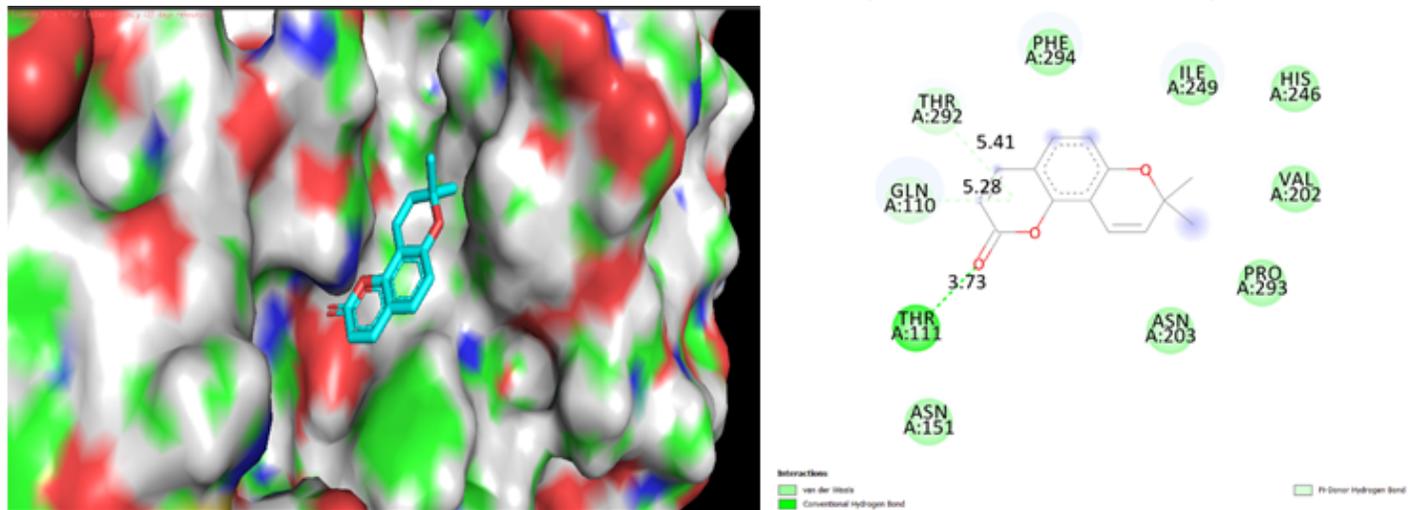
**Figure 3**

(a) Visualization of molecular surface of the SARS-CoV-2S with ligand docked in the binding site using Pymol (b) 2D structure - The ligand forming hydrogen bonding with the residues THR11 and GLN110 (Discovery Studio 20.0.1.)



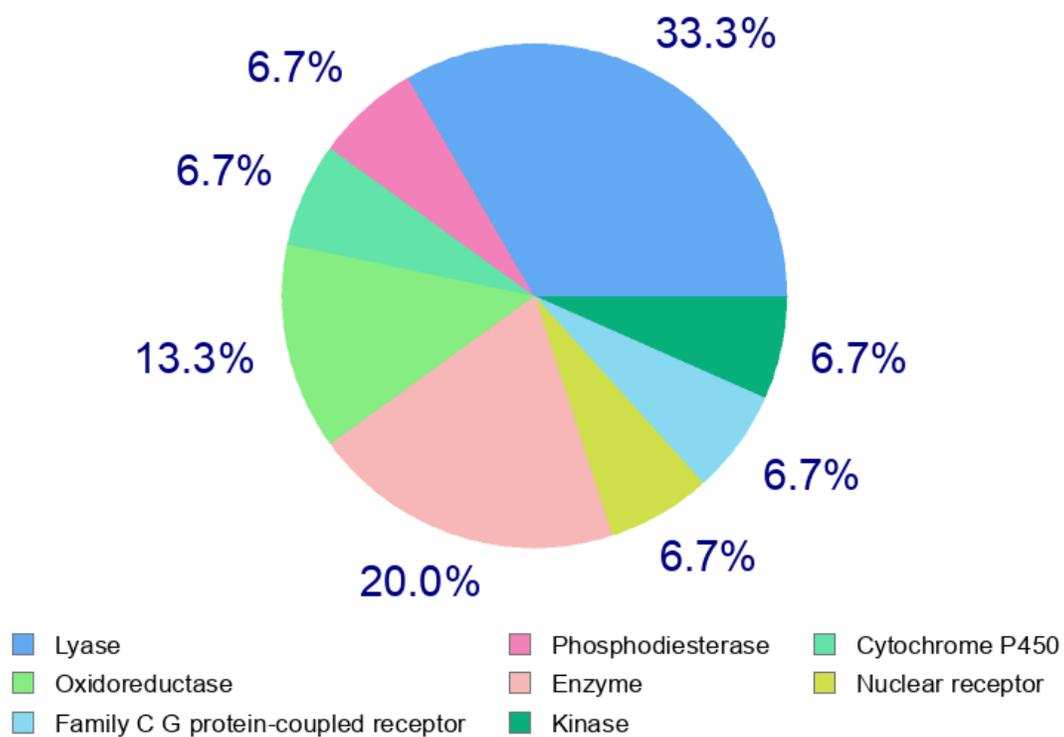
**Figure 4**

(a) Visualization of molecular surface of COVID-19 main protease with ligand docked in the binding site using Pymol (b) 2D structure - The ligand forming hydrogen bonding with the residue SER1003. ALA958 showing Pi-alkyl and THR961 showing Pi-sigma interactions (Discovery Studio 20.0.1.)



**Figure 5**

(a) Visualization of molecular surface of the free enzyme of SARS-CoV-2 (2019-nCoV) main protease with ligand docked in the binding site using Pymol (b) 2D structure - The ligand forming hydrogen bonding with the residue THR111. GLN110 showing Van der Waals interactions and THR292 showing Pi-donor hydrogen interactions



**Figure 6**

Probable targets of the compound predicted by Swiss Target Prediction server