Interactions Eucalyptus Oil with the Hcov-229E as New Potential Anti-Coronavirus: Molecular Docking Studies

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Abstract

Chemical composition in eucalyptus essential oil was evaluated for protein 6U7H HCoV-229E as anti-coronavirus. Six compounds and two anti-viral drugs approved by the FDA were tested in this study. The designed eucalyptus oil component chemistry significantly enhances HCoV-229E enzyme selectivity. The two ligands of 1.8-cyneol and favipiravir could dock into the active site of HCoV-229E successfully. The binding energies of -4.5 and -4.4 kcal/mol were obtained for two compounds respectively. Molecular docking study revealed the binding orientations of compounds in the active sites of HCoV-229E towards the design of potent inhibitors.

Keywords: *coronavirus, eucalyptus oil, HCoV-229E, molecular docking*

INTRODUCTION

Coronavirus (CoV) is a genus of the family Coronaviridae, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). 2019-nCoV is highly contagious and can cause mild to severe respiratory infections (Zhou et al., 2020). This transmission characteristic leads to the possibility of transmission from animals to humans. This virus first appeared in the city of Wuhan, China, and has gradually developed into a severe pandemic (Lupia et al., 2019). The deployment of 2019-nCoV has attracted great attention and caused concern around the world. To date, more than thirty-one thousand cases have been recorded and more than 1 million deaths as of October 12, 2020. SARS-CoV-2 continues to be a serious threat to human life as long as the outbreak continues and the lack of effective treatment.

In silico tools are nowadays well integrated into the drug development process and are considered a complementary approach to experimental methods. They have proved to successfully identify ligand-target interactions (Rao and Knaus, 2008) and to enrich active compounds in the libraries selected for biological testing (Tanrikulu et al., 2013). Although they were originally used for the prediction of novel disease-modulating compounds for a specific target, additional strategies arise (Jenkins et al., 2003). Besides classical lead-identification, in silico tools can also be deployed for the investigation of off-target interactions. These additional interaction predictions can comprise basically all targets for which sufficient data, structural or ligand-based, is available for model building. Via the parallel screening of multiple targets against one compound, so-called bioactivity profiles can be generated. They can help to predict adverse events as well as pharmacokinetic properties (Liu et al., 2010; AbdulHameed et al., 2012; Lounkine et al., 2012) and may aid to prioritize and identify the most promising drug candidates and to exclude compounds with a bad risk profile (Gleeson et al., 2011). In principle, a lot of different approaches can be used to address these issues. Among the most commonly used in silico tools are docking. The main prerequisite for docking is 3D structural information about the target derived from e.g. X-ray crystallography, NMR studies, or homology modeling. Basically, docking comprises two steps. First, the ligand is fitted into the binding site, and second the "quality" of the interaction pose is evaluated with scoring functions. The results can then be ranked according to their scores with compounds more likely to be active ranked at the top (Kitchen et al., 2004).

Since the emergence of SARS-CoV-2, various in silico tests have been carried out to see the molecular docking activity of various natural and synthetic compounds as drug candidates (Yu et al., 2020; Peele et al., 2020). The genome of the novel coronavirus (SARS-COV-2) encodes many proteins essential for its replication in the host genome namely, nucleocapsid protein, spike protein (S), an envelope protein (E), membrane protein (M), and the main coronavirus protease, which play an important role in gene expression, and cleave polyproteins into proteins associated with replication (Graham et al., 2008; Prasad & Prasad, 2020). Phytochemical-based compounds that occur naturally have been shown to exhibit several

anti-viral effects including other pharmacological properties (Perez, 2003), one of which is eucalyptus essential oil (Usachev et al., 2013). Several chemical compositions in eucalyptus essential oil that has been reported include 1.8-Cyneol, α-pinene, limonene, α-terpineol, myrcene, β-caryophyllene (Ogunwande et al, 2003), which have anti-viral effects (Li et al., 2016; Astani & Schnitzler, 2014). Therefore, in this study an in silico test was conducted to evaluate the effect of chemical composition in eucalyptus essential oil and anti-viral drugs approved by the FDA with the protein 6U7H HCoV-229E.

METHODOLOGY

Preparation of target protein X-ray structure

The crystal structure of coronavirus HCoV-229E (PDB code: 6U7H, http://www.rcsb.org/) was selected as the protein target model in this study. A small regularization was performed, water positions and symmetry were corrected, and hydrogens were added (Sohilait 2018). The repaired pdb file was evaluated and passed to AutodockTools for pdbqt file preparation. Thus, water molecules and non-standard residues were removed, only polar hydrogens were maintained, and Gasteiger charges were computed for protein atoms by ADT.

Ligands preparation

All of the structures were constructed using ChemSketch-12.01 for Windows and these geometries were optimized using the DFT-B3LYP/6-31G(d). The geometry of built compound was optimized, partial charges were also calculated, and saved as mol2 files that was passed to ADT for pdbqt file preparation (Sohilait, 2017).

Protein–ligand docking using AutoDock-Vina

The scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the protein spike 6U7H HCoV-229E. Autodock4 (ver. 4.2.6) (Morris et al., 2009) was employed for docking simulations. Lamarckian genetic algorithm with local search (GALS) was used as search engine, with a total of 100 runs. The region of interest, used by Autodock4 for docking runs and by Autogrid4 for affinity grid maps preparation, was defined in such a way to comprise the whole catalytic binding site using a grid of 25 x 25 x 25 points with a gridspace of 0.375 Å, centers of grid box: x = 155.07; y = 186.45; z = 177.03. The more energetically favourable cluster poses were evaluated by using Phyton Molecule Viewer (PMV ver.1.5.6) and PyMol ver.1.7.4 (DeLano Scientific LLC)

RESULT and DISCUSSION

The process, which brings and binds the two molecular structures together, is called docking. Docking has been acknowledged with significant attention among all the virtual screening methods. Docking has been a capable choice for the modeling of the 3-dimensional structure of the receptor-ligand complex and evaluating the stability of the complex that determines the specific biological recognition. The docking problem can be subdivided into two steps (Koehler and Villar, 2000; Verdonk et al., 2004; Kellenberger et al., 2004; Rester, 2006). Exploring the conformational space of ligands that bind to target molecules and scoring this set, i.e. ranking it in accordance with the estimated binding affinity. That is a conformation of the ligand is typically generated, and with the help of scoring function compared to the earlier conformations. The current conformation is then accepted or rejected on the basis of the score for that respective conformation. Then again a new conformation is generated, and the search process iterates to an endpoint. Thus, searching and scoring can be tightly coupled in docking (Shoichet et al., 2002). Hence it becomes important to possess better scoring functions so that the highest rank-ordered conformation would have a higher experimental binding affinity with the receptor. The terms “library” and “drug-likeness”, for the compounds are also associated, so as to carry out a comparison with a subset of chemical compounds by eliminating inferior active and toxic compounds based on a set of undesirable characters (Yang and Shen, 2005). Molecular docking is a computational procedure that attempts to predict the noncovalent binding of macromolecules or, more frequently, of a macromolecule (receptor) and a small molecule (ligand) efficiently, starting with their unbound structures, structures obtained from MD simulations, or homology modeling, etc. The goal is to predict the bound conformations and the binding affinity. The prediction of

binding of small molecules to proteins is of particular practical importance because it is used to screen virtual libraries of drug-like molecules in order to obtain leads for further drug development.

To predict the human coronavirus HCoV-229E data on a structural basis, automated docking studies were carried out using the AutoDock software program. The scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities, and the orientation of these compounds at the active site of the HCoV-229E enzyme. Virtual screening was carried out through docking the designed compounds into the HCoV-229E binding site to predict if these compounds have an analogous binding mode to the coronavirus inhibitors. The scoring functions of the compounds were calculated from minimized ligand-protein complexes. In order to compare the binding affinity of the eucalyptus oil components, chloroquine, and favipiravir were docked into the empty binding site of HCoV-229E enzyme and the results are listed in Table 1.

Table 1. Binding affinity and RMSD

Name	Binding Affinity	RMSD/UB	RMSD/LB
Native Ligand	-4.0	1.327	1.227
α-Pinene	-4.3	1.266	1.181
α -Terpineol	-3.8	0.637	0.637
β-Caryophyllene	-4.3	1.433	1.176
Limonene	-4.4	1.967	0.411
Myrcene	-4.3	1.609	0.645
1.8-Cyneol	-4.5	1.976	1.224
Chloroquine	-4.1	1.874	1.329
Favipiravir	-4.4	1.626	1.626

We also carried out flexible docking studies using the AutoDock program to predict binding modes, and we derived the correlations between activities and binding energies. Furthermore, analysis was performed to identify key residues for hydrogen bonds formed and nonbonded interaction energy between ligand and enzyme. AutoDock allows the flexible docking of ligands into its site of action. It has the ability to use all the rotatable bonds of the ligands to give a number of conformations from which the best mode could be achieved. All compounds were embedded in the hydrophobic pocket formed by the amino acids. The results of docking studies with respect to HCoV-229E are summarised in Table 2.

Table 2. Interaction with in amino acid

Bonding Distance (Å)	Amino Acid Residue				
	Asn440	Phe438	Gln430	Nag2981	Pro431
Native Ligand	3.6	2.7	2.3	4.7	3.1
α-Pinene	3.7	4.2	4.6	4.7	3.5
α -Terpineol	4.8	3.1	5.0	3.7	2.7
β -Caryophyllene	3.5	3.6	3.8	3.6	4.7
Limonene	3.7	3.5	3.2	3.6	3.5
Myrcene	3.3	4.0	3.5	3.4	3.6
1.8-Cyneol	3.7	3.6	3.6	3.6	3.4
Chloroquine	3.9	3.7	3.8	3.9	3.2
Favipiravir	2.9	4.2	3.1	2.0	3.0

Native ligand, α-Pinene, α -Terpineol, β -Caryophyllene, Limonene, Myrcene, 1.8-Cyneol, Chloroquine, and Favipiravir showed five interaction via hydrogen bonds similarly. Native ligand of HCoV-229E showed five hydrogen bond interactions with amino acids Asn440, Phe438, Gln430, Nag2981, and Pro431 with hydrogen bond length 3.6 Å, 2.7 Å, 2.3 Å, 4.7 Å, & 3.1 Å, respectively. The ligand favipiravir has bonding distance smaller than native ligand, with the results of favipiravir, it can be docking well on the protein side. Some residues are always in contact with the ligand, although the specific interactions might vary. The typical binding pocket and interactions observed are captured in Fig. 1a for the potent favipiravir in their best docked poses. 1.8-Cyneol showed hydrogen bond interactions with amino acids Asn440, Phe438, Gln430, Nag2981, and Pro431 with hydrogen bond length 3.7 Å, 3.6 Å, 3.6 Å, 3.6

Å, & 3.4 Å, respectively (Fig. 1b). Favipiravir and 1.8-Cyneol has a good binding affinity and is supported by very small amino acid bonds so it can be concluded that they can be new potential inhibitors.

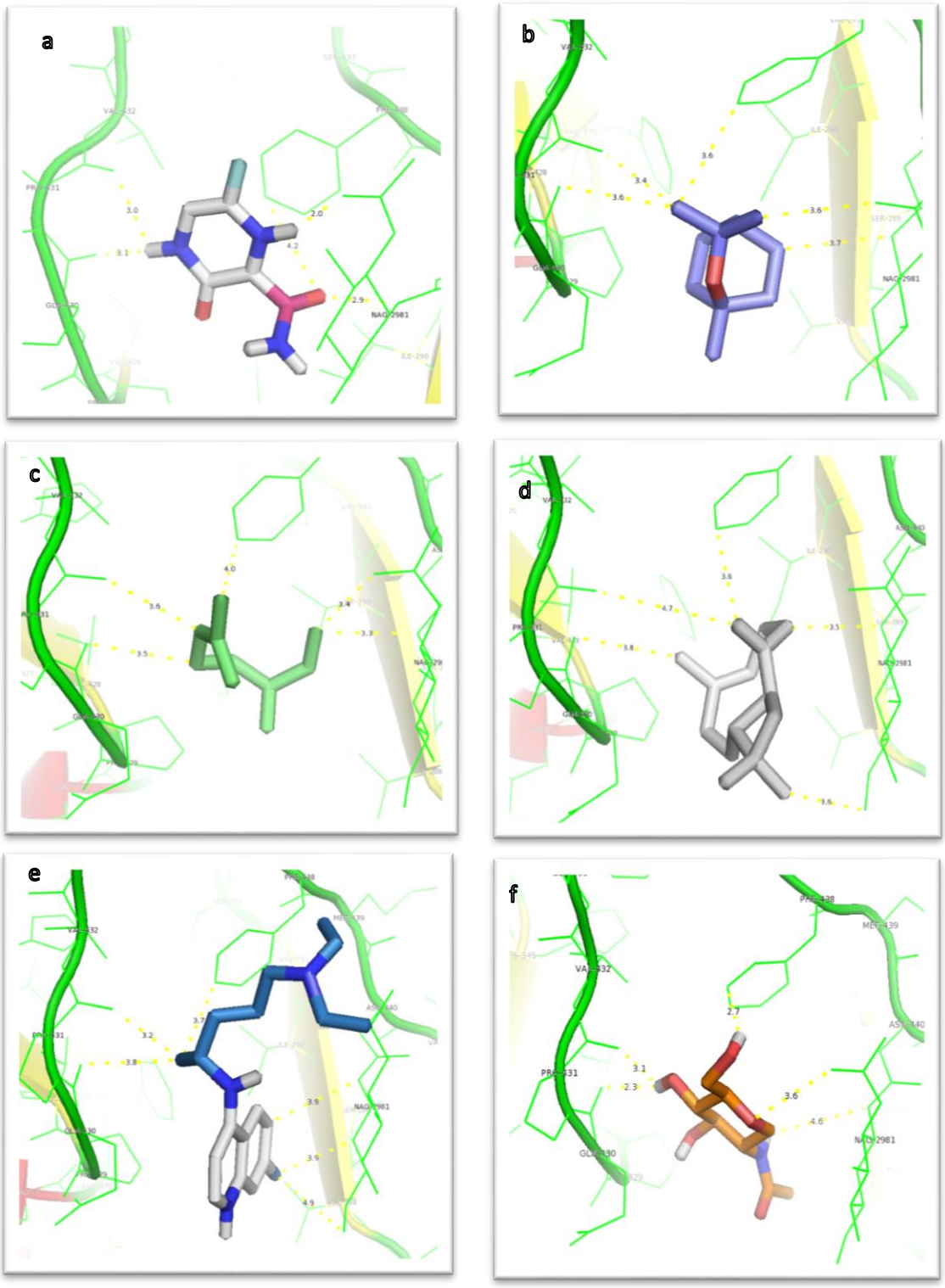

Figure 1. Docked pose of ligand (a) Favipiravir, (b) 1.8-cyneol, (c) Myrcene, (d) β-Caryophyllene, (e) Chloroquine, (f) Native Ligand with binding site of HCoV-229E

Computational methods can be applied in drug development for the identification of novel lead candidates, but also for the prediction of pharmacokinetic properties and potential adverse effects, thereby aiding to prioritize and identify the most promising compounds. Moreover, molecular docking revealed that optimum hydrogen bonds were determinant factors in the interactions of in silico between ligands and HCoV-229E.

CONCLUSION

The analysis results showed that the chemical components of eucalyptus oil significantly increased the selectivity of the enzyme HCoV-229E. All analyzed ligands showed interaction via hydrogen bonds similarly. Of all the ligands tested, 1,8-cyneol and favipiravir were successful in binding to the active HCoV-229E site so it can be concluded that they can be new potential inhibitors. However, evaluation studies in vitro and in vivo studies are required to test the ability of these compounds as anti-coronavirus.

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