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Screening of camphene as a potential inhibitor targeting SARS-CoV-2 various structural and functional mutants: Through reverse docking approach

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Abstract

Background: SARS-CoV was first identified in 2003 but SARS-CoV-2, which gained its recognition again in 2019 as COVID-19, has been a crucial threat worldwide and has caused more death rates than the SARS-CoV but till now no confined treatments are available. The present study aimed to investigate the efficacy of camphene against various structural and functional mutants of SARS-CoV-2 using reverse docking protocol.

Methods: To investigate the efficacy of camphene as a potential antiviral drug against COVID-19, against of all possible target proteins in SARS-CoV-2, which could lead to a new platform for drug discovery. Reverse pharmacology (Reverse docking) approach was performed, which involved docking of camphene and 20 structural and non-structural proteins (NSPs) of SARS-CoV-2 performed using maestro 12.8 of Schrödinger.

Results: The results were evaluated since the minimum binding energy obtained after docking and camphene was effective against most of the proteins responsible for SARS-CoV-2, but camphene showed greater efficacy against the main protease (protease 9), which is main functional protein of SARS-CoV-2. Hence, the study proves that camphene can be a good drug candidate for different mutants of SARS-CoV-2.

Conclusion: Protease 9, which is the main functional protein of SARS-CoV-2, expressed the best binding affinity with camphene having the minimum binding energy (-5.616). Hence, it is concluded that camphene could be the drug contender against protease 9 as it is a more potent target in SARS-CoV-2. This could be a major finding, as camphene is related to camphor, which is already very beneficial against many respiratory problems.

Keywords: SARS-CoV-2, Functional mutants, Reverse pharmacology, Binding affinity, Drug discovery
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Introduction

The whole world is battling hard with Coronavirus disease since 2019, and it has caused a global health concern across the world, leaving many people's lives at risk. Coronavirus was first identified in 2003 as SARS-CoV (1) and another coronavirus in 2012, designated as the Middle East respiratory syndrome coronavirus (MERS-CoV), was recognized in Saudi Arabia in 2012 (2). SARS as well as MERS were the most spread strains of coronaviruses (2) but again in December 2019, there was an eruption of new coronavirus (SARS-CoV-2), in Wuhan, China, which caused serious pulmonary disease (3-6) and since then, it is spreading widely all around the world.

The disease caused by SARS-CoV-2 is called as coronavirus disease 2019. This is a large, enveloped, positive single-stranded RNA virus with the virion size of 50-200 nm in diameter, which infects humans together with a broad range of animals (7). According to their structural appearance, as virions are spherical, with a central nucleus and solar corona-like projection on the surface, the name is suggested as coronavirus (8).

Coronavirus belongs to the Coronaviridae family and is fractioned into the four subfamilies including alpha, beta,

gamma, and delta coronavirus (7). SARS-CoV-2 belongs to the B-lineage of beta-coronaviruses and is firmly related to the SARS-CoV virus (7). The RNA genome of Coronavirus has 29 811 nucleotides, and it encodes 29 proteins as per the phylogenetic analysis, it is originated from bat (5). The virus has four structural proteins, known as S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. An envelope-anchored SARS-CoV-2 spike (S) glycoprotein facilitates coronavirus entry into host cells by binding to the cell membrane receptor, angiotensin-converting enzyme 2 (ACE), to enter human cells (9-11). The binding affinity between the receptor-binding domain of the virus and host ACE2 helps in predicting the infectivity of hosts to the SARS-CoV infection (12-14). Surface plasmon resonance (SPR) study proved that SARS-CoV-2 spike (S) protein binds to ACE2 with 10- to 20-fold higher affinity than the other SARS-CoV S proteins (15), which is a possible reason for SARS-CoV-2 high infectivity. Thus, viral entry into the host cell is a critical step in viral infection and could be experimented with for therapeutics developments. After the entry and binding of ACE2 and S proteins, the virus requires the host cells' protein synthesis, which in turn requires viral proteases. The virus uses its RdRp to synthesize the viral-RNA. Numerous structural and assembly proteins are synthesized during this infection process and are further responsible for the release of progeny viral particles by exocytosis (16). There are 16 other non-structural proteins (NSPs) that can be considered primarily from the perspective of drug design, including RNA-dependent RNA polymerase (RdRp), Coronavirus main protease (3CLpro), and papain-like protease (PLpro). These proteins are important because upon entering host cells, the viral genome is unleashed as a single-stranded positive RNA. β-CoVs produce pp1a and pp1ab by translation of the genomic RNA. They are proteolytically splitted into structural and NSPs by main protease (Mopar), also known as 3-chymotrypsin-like protease (3CLpro), and by papain-like protease (PLpro) (17). Thus, the main protease plays an important role in the processing of polyproteins, which are the viral RNA transcription products. Thus, the inhibition of the main protease can be the main target for drug discovery (18,19). Therefore, the main structural proteins and NSP can play a crucial role in the perspective of drug design.

Computational applications have been applied to track potential drugs against SARS-CoV-2 (16). Bioinformatics technology such as molecular docking and dynamic simulation has initiated a new direction of research in advanced drug discovery as it predicts the bioactivity of the compound/drug against various infectious diseases as well as many other common diseases by showing the interaction between receptor proteins and ligands (20). Many plant-derived drugs have been registered for the treatment of various infectious diseases caused by bacteria, viruses, and fungi (21,22). Camphene is a plant-derived cyclic monoterpene presents in many conifers as well as in much cannabis chemovars. In 1981, Schäfer and Schäfer suggested that camphene has reduced experimentally induced bronchospasm in animals, and also, it was applied in the human chronic obstructive pulmonary disease, which was quite fruitful against this pulmonary disease (23). So, with this perspective, in the present study, camphene was docked as an antiviral against COVID-19 structural and NSPs through reverse docking, which is the novelty of the present research and was found as a promising approach against the COVID-19.

Materials and Methods

Virtual screening

Virtual screening is the most important in-silico technology in the pharmaceutical industry. Through extensive literature survey and protein data bank search, 20 target proteins were screened based on their structural and functional integrity in SARS-CoV-2 virus.

Drug-likeness properties by molinspiration

The details of commercially available molecular already published antiviral agent camphene are also prepared along with their chemical formula, molecular weight, and PubChem ID. The smart screening was done using Molinspiration tool that followed Lipinski's rule of five, and then, camphene was ready for molecular docking studies (23,24).

Reverse docking studies

Reverse docking is a computational methodology and an important in-silico technique in which we can predict the most preferred orientation of one molecule binding to a second molecule to form a stable complex model for a potential target. As in this case, camphene binding with the best possible target proteins.

A reverse docking study was performed between the camphene and 20 structural and NSPs of SARS-CoV-2 using maestro 12.8 Schrödinger. A study was conducted to predict the best binding score, affinity, and confirmation. Maestro 12.8 is an automated suite of protein-ligand docking tools used to predict the protein interactions with small molecules such as drug molecules and substrates. It analyses, the interaction of ligand molecules at the specified target site of the protein. Schrödinger uses OPLS4 forcefield covers 8% of chemical space, which is the best in class and linear regression to predict the free binding energies (24-26).

Schrödinger ligand preparation product, Ligprep, was used to prepare high-quality, all-atom 3D structures. The ligand preparation included 2D-3D conversions, generating variations, correction, verification, and optimization (27).

3D structure of SARS-CoV-2 proteins was downloaded

in PDF format from the PDB. Maestro protein preparation wizard was used for the preparation of protein structure by adding hydrogen bonds, filling missing loop, and side-chain residue, etc. Protein preparation also refines the structure by optimizing and minimizing the energy of the structure. Receptor grid was generated using receptor grid generation in the Glide application (Glide, version 12.8, Schrödinger, LLC, and New York-2) of Maestro (Schrödinger, LLC, New York, NY, version 2014-2). The receptor grid for protein was generated by stipulating the binding (active) site residues, which were recognized by the Site Map tool. Once the receptor grid is generated, the ligands are docked to the protein using Glide version 12.8 (Grid-based Ligand Docking with Energetics) docking protocol. The ligands were docked using "Extra precision mode" (XP). The docked conformers were evaluated using the Glide (G) Score. The G Score is calculated as follows:

G Score = a*vdW + b*Coul + Lipo + Hbond + Metal + Bury P + RotB + Site

Wherein *vdW* denotes van der Waals energy, *Coul* denotes Coulomb energy, *Lipo* denotes lipophilic contact, *HBond* indicates hydrogen-bonding, *Metal* indicates metal-binding, *BuryP* indicates penalty for buried polar groups, *RotB* indicates forfeit for freezing rotatable bonds, *Site* denotes polar interactions in the active site, and a = 0.065 and b = 0.130 are coefficients of vdW and Coul, respectively (28).

Estimation of bioactivity score and drug likeliness properties

Molinspiration Cheminformatics is a molecular descriptor that evaluates drug likeliness properties using the Molinspiration server (http://www.molinspiration.com) following Lipinski's Rules of five, which states that molecules must have molecular weight ≤ 500, log P ≤ 5, number of hydrogen bond donors ≤ 5, and number of hydrogen bond acceptors ≤ 10 (29).

Results

Virtual screening

Virtual library of 20 proteins prepared through extensive literature survey compounds, which are kept as a Supplementary Table.

Drug likeness properties

Screening drug-likeness properties of camphene by molinspiration shown in Table 1 indicates that the calculated property of compound through molinspiration server and the compound was categorized based on the Lipinski's rule of five. Camphene obeys the Lipinski's rule of five and has drug-likeness property. These parameters play a vital role in the determination of the bioactivity of chemical compounds. Lipinski's rule of five describes the general rule of an orally active drug must have.

Table 1. Drug-like properties of camphene

Properties	Value
miLogP	3.33
TPSA	0
natoms	10
MW	136.24
nON	0
nOHNH	0
Violations	0
nrotb	0
Volume	152.37

logP: Partition coefficient/hydrophobicity measurement; nOHNH: The number of hydrogen bond donors; nON: Hydrogen bond acceptor; n violations: Number of Lipinski's rule-of-five violations; nrotb: Number of rotatable bonds; Volume: molecular volume; TPSA: Topological polar surface area.

Molecular docking studies

Maestro 12.8 was used for the docking studies. The docked complex having the lowest binding energy was selected as the most possible antiviral target protein. In the present study, *in silico* reverse docking studies were carried out to identify the effectiveness of antiviral agent camphene to various SARS-CoV-2 protein molecules that can fit into the most favorable binding mode, against camphene ligand. Protein structures were obtained in PDF file format from the protein data bank. The structures were subjected to protein preparation to add missing hydrogen, optimize the protonation states of histidine residues and orientation of hydroxyl groups. The structure was also subjected to energy minimization using the OPLS_2005 force field to constrain the heavy atoms.

The active site of proteins was obtained using the SiteMap tool, which provides a fast and effective means of identifying potential binding pockets of proteins. SiteMap identifies the character of binding sites using a novel search and assesses each site by calculating various properties like size, volume, amino acid exposure, enclosure, contact, hydrophobicity, hydrophilicity, and donor/acceptor ratio. As a result, five binding sites with site scores of more than 0.7014 were identified. Site 1, with site score 0.896, volume 246.617, exposure 0.722, enclosure 0.612, contact 0.743, phobic 0.374, philic 0.756, balance 0.495, and don/acc 0.942 was utilized for additional docking analysis. The anticipated amino acids in active site region are Chain A: 8, 106, 108, 109, 110, 111, 127, 151, 200, 202, 203, 240, 246, 246, 249, 250, 252, 253, 292, 293, 294, 297, 298A grid, which was generated all over the active site for effective binding. The glide docking tool of Schrödinger was used to dock camphene to proteins. The ligand docking calculations were done on the extra precision (XP) mode of Glide. The docking score for the

main protease 9 (PDB ID: 6WTJ) was -5.616 as given in Table 2 and Figure 1. In Figure 2, we have provided the molecular docking interaction f camphene with the other important proteins of SARS-CoV-2 such as NSP3, RdRP, and receptor binding domain (spike protein, NSP9, RNA replicase) is shown, which is in correlation with Table 2.

Table 2. Docking results of top 10 proteins

PDB ID	Name of protein	Docking score
6WTJ	Main protease of SARS-CoV-2	-5.616
6WOJ	Non-structural protein NSP3	-5.414
7B3B	RNA-dependent RNA polymerase	-4.978
7ORB	L452R mutant receptor binding domain of SARS-CoV-2 Spike	-4.534
6WC1	Nsp9 RNA-replicase	-4.225
7BV2	nsp12-nsp7-nsp8 complex	-4.103
7NXC	SARS-CoV-2 P.1 variant Spike	-4.069
7MSX	SARS-CoV-2 Nsp2	-3.98
7K3N	Crystal Structure of NSP1 from SARS-CoV-2	-3.341
6WXD	SARS-CoV-2 Nsp9 RNA-replicase	-3.307

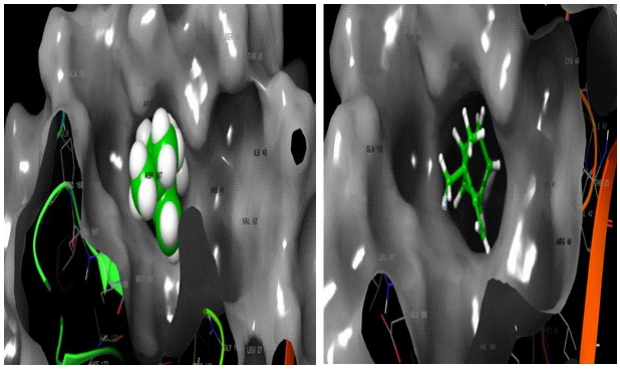

Figure 1. Docking score for the main protease 9 and camphene is shown in this figure (2 hydrogen bonds are shown with Glu126, Glu292)

Discussion

Reverse docking or reverse pharmacology has been proven as a very promising tool for drug designing as it includes the interaction of single drug against various target proteins by identifying the potential active binding sites (30). In this study, the reverse docking approach was used against various structural and NSPs involved in SARS-CoV-2 by interacting with camphene, which is widely known for antibacterial properties as well as in respiratory-related problems (23). 3D structure of SARS-CoV-2 proteins was downloaded from PDB in PDF format and the active site of protein was recognized using SiteMap tool. LigPrep was used to get 3D structure of camphene, and ligand preparation was used for 2D-3D conversions and validation of 3D structures (27). After docking camphene with various structural and functional mutants of COVID-19 using glide docking tool and after getting the docking score using XP (extra precision) mode of glide, the binding score was obtained between various SARS-CoV-2 proteins and camphene, as shown in Table 2. Protease 9, which is regarded as the main functional protein in the SARS-CoV-2 disease mechanism, expressed the minimum binding energy with camphene as shown in Figure 1 (which represents docking score of main protease 9 and camphene) and Figure 2 (which represents molecular docking interaction of main protease 9 with camphene) is shown in Figure 2. The binding energy of camphene with protease 9 is minimum as compared with the other proteins of SARS-CoV-2 as given in Table 2, and Figure 2, Figure 1 shows the docking score of protease and camphene which is -5.616, this

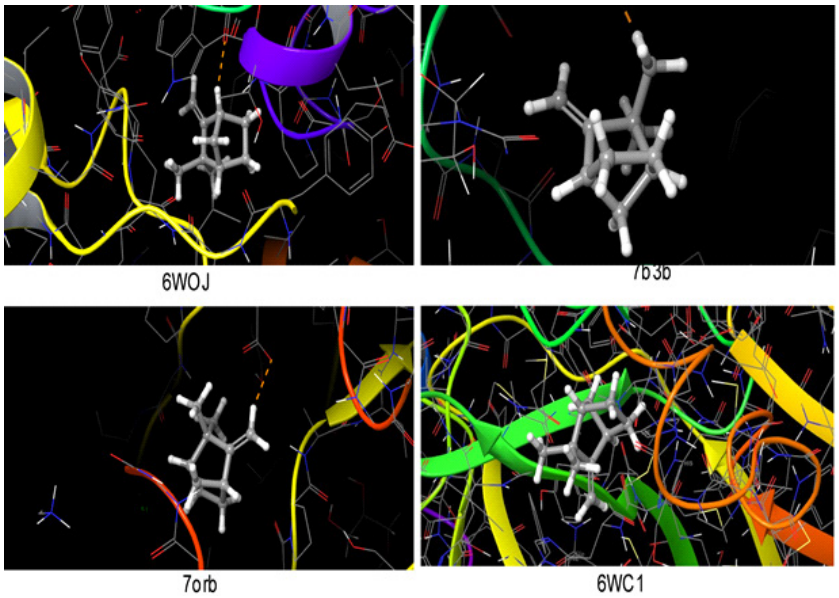

Figure 2. Molecular docking interactions between camphene and the binding sites of some important SARS Cov-2 Protein such as NSP3, RdRP, receptor binding domain (spike protein, NSP9, RNA replicase)

compound set-ups two hydrogen bonds with Glu126, Glu292, and *pi-pi* stacking with Arg130. Figure 3 shows the molecular docking interaction of camphene with the binding sites of main protease 9 (16). Hence, this result showed that camphene could be a better drug target against this main protease 9 and can facilitate the suppression of this fatal disease.

However, as we know during the COVID-19 pandemic, it was very hard to manage this deadly virus due to its highly infectious and contagious nature and hospitals were the main centers for the spread of the disease (31). Therefore, new treatments are needed to reduce the risk of progression of this virus, and thus, computational studies can be very efficient in finding and evaluating drug against this infectious virus, so as a bioinformatician we can highly contribute to this COVID-19 pandemic war, which is still going on, and we can evaluate the efficient drugs without being exposed to the virus through computation studies. There are various tools and software available for bioinformaticians among which reverse docking is one of the most efficient approaches and tools to identify unknown target/secondary target (30), which was applied in the present study. There is still a requirement to discover more antiviral drugs against COVID-19, therefore, using this approach, the efficacy of camphene as an antiviral agent against this virus was evaluated. In silico approaches to the drug discovery can pave the way for further in vitro studies.

One of the limitations of the present study is the lack of comparison of the efficacy of other antiviral drug which are evaluated till now against COVID-19, and there is need of in vitro studies for further evaluation of the efficacy of camphene against this deadly virus, which needs further approval by research committee and needs safety procedures and highly equipped lab (biosafety level 3).

The present study provides an overview that camphene could be an effective drug against structural and functional protein of SARS-CoV-2, as camphene exhibits binding energy against protease 9, so this can be further tested with dynamic simulation study and in vitro study. This study provided one more drug in terms of drug discovery against COVID-19.

Conclusion

In the molecular docking study, the binding sites of the functional and structural protein of different mutants of SARS-CoV-2 were compared and the affinity with ligand camphene was estimated. According to *in silico* analysis, a bicyclic scaffold provides efficient binding to the hydrophobic part of the binding site of the surface proteins under consideration, and protonated nitrogen provides electrostatic interactions. The main protease to be a potent drug target in SARS-CoV-2, camphene, is the most prominent drug candidate, a new inhibitor targeting the membrane fusion stage, and possessing a broad spectrum of antiviral activity against RNA viruses SARS-CoV-2. This could be a major finding, as camphene is related to camphor, which is already very beneficial against many respiratory problems. However, further trials are needed.

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Authors' contribution

Conceptualization: Prachi Srivastava, Ruby Singh.
Data curation: Mahendra Kumar Savita.
Formal analysis: Ruby Singh, Mahendra Kumar Savita, Prachi Srivastava.
Investigation: Prachi Srivastava.
Methodology: Prachi Srivastava, Ruby Singh, Mahendra Kumar Savita.
Project administration: Prachi Srivastava.
Resources: Mahendra Kumar Savita, Prachi Srivastava,

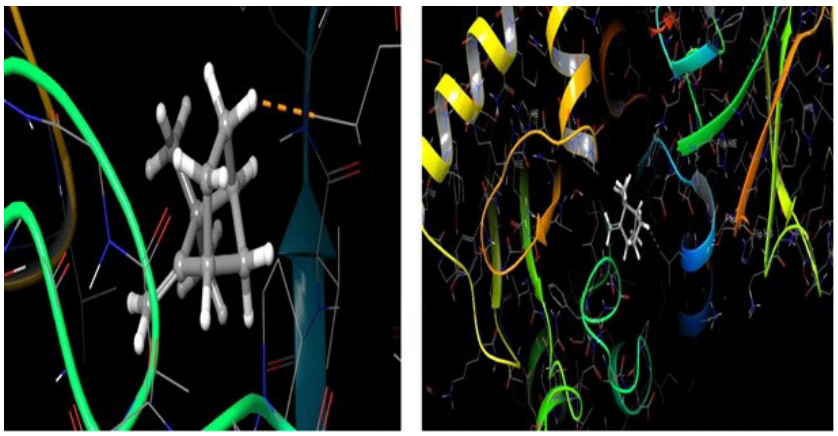

Figure 3. Molecular docking interactions between camphene and the binding sites of the main protease

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Software: Prachi Srivastava, Mahendra Kumar Savita.
Supervision: Prachi Srivastava.
Validation: Prachi Srivastva.
Visualization: Mahendra Kumar Savita.
Writing–original draft: Ruby Singh.
Writing–review & editing: Prachi Srivastva, Mahendra Kumar Savita, Ruby Singh, Neha Bora.

Competing interests

The authors declare that there is no conflict of interests, financial or otherwise.

Ethical issues

All work is insilco. No one was harmed.

Spplementary file

Supplementary table contain virtual library of 20 proteins prepared through extensive literature survey compounds.

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