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In silico allicin induced *S*-thioallylation of SARS-CoV-2 main protease

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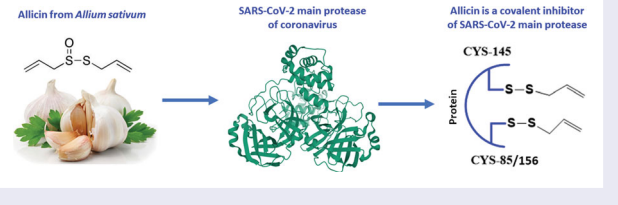
ABSTRACT

Coronavirus disease 2019 (COVID-19) is an ongoing pandemic caused due to new coronavirus infection with ^{3}716075 deaths across the world as reported by the World Health Organization (WHO). SARS-CoV-2 main protease (M^{pro}) plays a vital role in the replication of coronavirus and thus an attractive target for the screening of inhibitors for the therapy of COVID-19. The preclinical drugs ebselen and PX-12 are potent inhibitors of SARS-CoV-2 M^{pro} and covalently modifies the active site Cys-145 residue of M^{pro} through selenosulfide/disulfide. In the current report, using virtual screening methods, reactive sulfur species allicin is subjecting for covalent docking at the active site of SARS-CoV-2 M^{pro} using PX-12 as a benchmark reference compound. The results indicate that allicin induces dual S-thioallylation of Cys-145 and Cys-85/ Cys-156 residues of SARS-CoV-2 M^{pro}. Using density functional theory (DFT), Gibbs free energy change (DG) is calculated for the putative reactions between N-acetylcysteine amide thiol and allicin/allyl sulfenic acid. The overall reaction is exergonic and allyl disulfide of Cys-145 residue of M^{pro} is involved in a sulfur mediated hydrogen bond. The results indicate that allicin causes dual S-thioallylation of SARS-CoV-2 M^{pro} which may be of interest for treatment and attenuation of ongoing coronavirus infection.



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1. Introduction

Coronavirus (COVID-19) infection is a global emergency with 19,187,943 confirmed cases and 716,075 deaths across the world as on 8th August 2020 reported by World Health

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Organization (WHO)[1]. The disease caused due to SARS-CoV-2 virus which spreads rapidly through aerosols of cough and sneezes or secretions of bodily fluids of infected persons [2–4]. The uncontrolled spreading of the corona infection is a great threat for mankind which demands immediate development of therapies to combat the disease. It is a great challenge to treat the deadly infection due to a lack of specific drugs, viable vaccines to immunize against the virus, and unproven history of using ayurvedic medicine to control the disease [5,6]. In the independent reports, Zhang et al., 7 and Jin et al., 8 have reported the crystal structure of the SARS-CoV-2 main protease that plays a crucial role in replication and proliferation of the coronavirus [7,8]. SARS-CoV-2 main protease (M^{pro}) is involved in proteolytic processing of translated polyproteins of the coronavirus genome into the functional polypeptides which eventually assemble to form new coronavirus [7,8]. The active site of SARS-CoV-2 M^{pro} contains catalytic dyad motif involving His-41 and Cys-145 residues which facilitates the hydrolysis of peptide bonds at $>$ 11 sites of the viral genome polyprotein [7,8]. The recognition site for cleavage of the peptide bond by SARS-CoV-2 M^{pro} is the C-terminus of glutamine of sequence -Leu-Gln-Ser/Ala/Gly- in the polyprotein replicase lab of coronavirus [7]. Inhibition of the function of SARS-CoV-2 M^{pro} has a direct impact on the formation of functional polypeptides which are critical in the assembly of new viruses thereby attenuating the replication and proliferation of coronavirus. Interestingly, proteases with similar specificity for cleavage of peptide bond are not evident in humans [7] which is an added advantage in choosing the SARS-CoV-2 M^{pro} as a target for the screening of drugs to combat COVID-19.

Attempts have been made through a structure-based drug design approach to identify the inhibitors for SARS-CoV-2 M^{pro} to attenuate coronavirus infection [8]. The approved drugs and natural products have been virtually screened against SARS-CoV-2 M^{pro} to assess their ability to attenuate coronavirus replication and treatment of COVID-19 disease [9–11]. The natural product extract of garlic *Allium sativum* has a long-documented history in the human civilization as food spices, traditional medicine, antibacterial/antiviral and antioxidant agent and also for the treatment of common cold and infection [12]. Allicin is the heart of garlic extract which was isolated and characterized by Cavallito and Bailey in 1944 and accounts for the large section of pharmacological activity of garlic extract [13,14]. Allicin is a thiosulfinate containing organosulfur species produced by the *Allium sativum* as part of a defense mechanism to protect garlic plants against pathogens and predators [12,15]. Allicin is most abundant in garlic and formed through condensation of two molecules of allyl sulfenic acid in an enzymatic reaction during tissue damage of raw garlic or wetting of dried/pulverized garlic powder [16]. Allicin is an oxidizing agent and potentially reacts with cellular protein thiols and glutathione leading to the formation of *S*-allyl-mercapto-proteins/glutathione [17]. The multi-faceted role of allicin as antiviral agent, antimicrobial agent, modulator of the immune system, lowering risks of cardiovascular diseases may be useful in combating the on-going COVID-19 pandemic [12,15,18]. The oxidizing nature of allicin through *S*-thioallylation may be of special interest due to the presence of cysteine thiol at the active site of SARS-CoV-2 M^{pro} [7,8]. Jin et.al., 2020 have reported tethering of active site Cys-145 residue of SARS-CoV-2 M^{pro} with inhibitors ebselen and PX-12 through selenosulfide/disulfide. In the current report, virtual screening methods were used to assess the ability of allicin to covalently modify cysteine residues of SARS-CoV-2 M^{pro}. The report indicates the allicin as a covalent inhibitor of SARS-CoV-2 M^{pro} and may be useful in attenuating the coronavirus infection.

2. Methods

2.1. General

Crystal structures of SARS-CoV-2 M^{pro} were retrieved from the protein databank (PDB). The PDB code of SARS-CoV-2 M^{pro} used in the studies: apo form is 6Y2E and inhibitor bound form are 6LU7, 5RFV, 5RFW. Three-dimensional structures of SARS-CoV-2 M^{pro} were processed using the Maestro Version 12.0.012 platform of Schrödinger software. The alignment of structures of M^{pro} and measurement of the distance between the atoms was achieved using option quick align and measurement of distance, respectively. The residue around 3 A° distance to the cysteine in the structure was identified using Maestro Version 12.0.012. The relative surface accessibility of the cysteine residues in the 3D-structure of M^{pro} was determined using the software Get-Access (http://curie.utmb.edu/getarea.html) [19]. The pka of cysteine thiols of M^{pro} were calculated using the protein preparation wizard of Glide, Maestro Version 12.0.012 Platform of Schrödinger software. The graph is plotted using Origin-Pro software.

2.2. Molecular docking of allicin and allyl sulfenic acid into the SARS-CoV-2 main protease

The virtual screening of allicin as an inhibitor of SARS-CoV-2 M^{pro} was performed using Glide, Maestro Version 12.0.012 Platform of Schrödinger software. Screening of allicin was conducted against the four PDB co-crystal structures of SARS-CoV-2 M^{pro}. The crystal structure of SARS-CoV-2 M^{pro} was processed using the protein preparation wizard. Hydrogen atoms were added, sample water orientations were achieved using PROPKA at pH 7, and waters with less than three hydrogen bonds to non-waters were removed from the M^{pro}. The restrained minimization of M^{pro} -ligand complex was achieved using OPLS3e force field. The ligand allicin was processed using LigPrep of Schrödinger software with OPLS3e force field. Docking simulations were achieved using the default option of the Glide docking process of Schrödinger software. Receptor grid was generated by choosing the centroid of the workspace ligand. Allicin docking was performed using standard precision (SP) mode with flexible ligand sampling by adding Epik state penalties for the docking score. Using the custom covalent reaction type provided by the Schrödinger, covalent docking was performed using pose prediction docking mode of Schrödinger software. The covalent docking affinity score was used to prioritize the binding site of the allyl sulfenic acid.

2.3. Computation of reaction between cysteine thiol and allicin

Gibbs free energy change (ΔG) for putative reactions between N-acetylcysteine amide and allicin were calculated by density functional theory (DFT) on the Maestro Materials Science 3.4.012 platform of Schrödinger software. Molecules were optimized using B3LYP-D3 on the Jaguar platform (version: 10.2, Schrödinger release 2019-2) with a 6-31G** basis set and polarization function on all atoms. Accuracy level was set to ultrafine and maximum iteration steps of 200 with the switch to analytical integrals near convergence. Gibbs free energy change (ΔG) for the reaction was calculated using the pre-optimized molecules on Jaguar Reaction (release 2019-2) platform. The accuracy level was set to ultrafine with default convergence criteria and the solvent model was none. Gibbs Free energies are stated in (kcal/mol) assuming standard condition ($T = 298.15$ K and $p = 1.0$ bar).

3. Results and discussion

3.1. Covalent docking of allicin into the active site of SARS-CoV-2 main protease

In independent studies, Zhang et.al., 2020 and Jin et.al., 2020 have reported crystal structures of SARS-CoV-2 M^{pro} (or) COVID-19 virus M^{pro}. Structure of SARS-CoV-2 M^{pro} is a homodimer with active site projecting outside the dimer interface (Figure-S1). Thus, the monomer structure is used for virtual screening of inhibitor against the SARS-CoV-2 M^{pro} [8,10,11]. Figure 1a shows the structure of SARS-CoV-2 M^{pro} with free cysteine thiols and active site dyad residues.

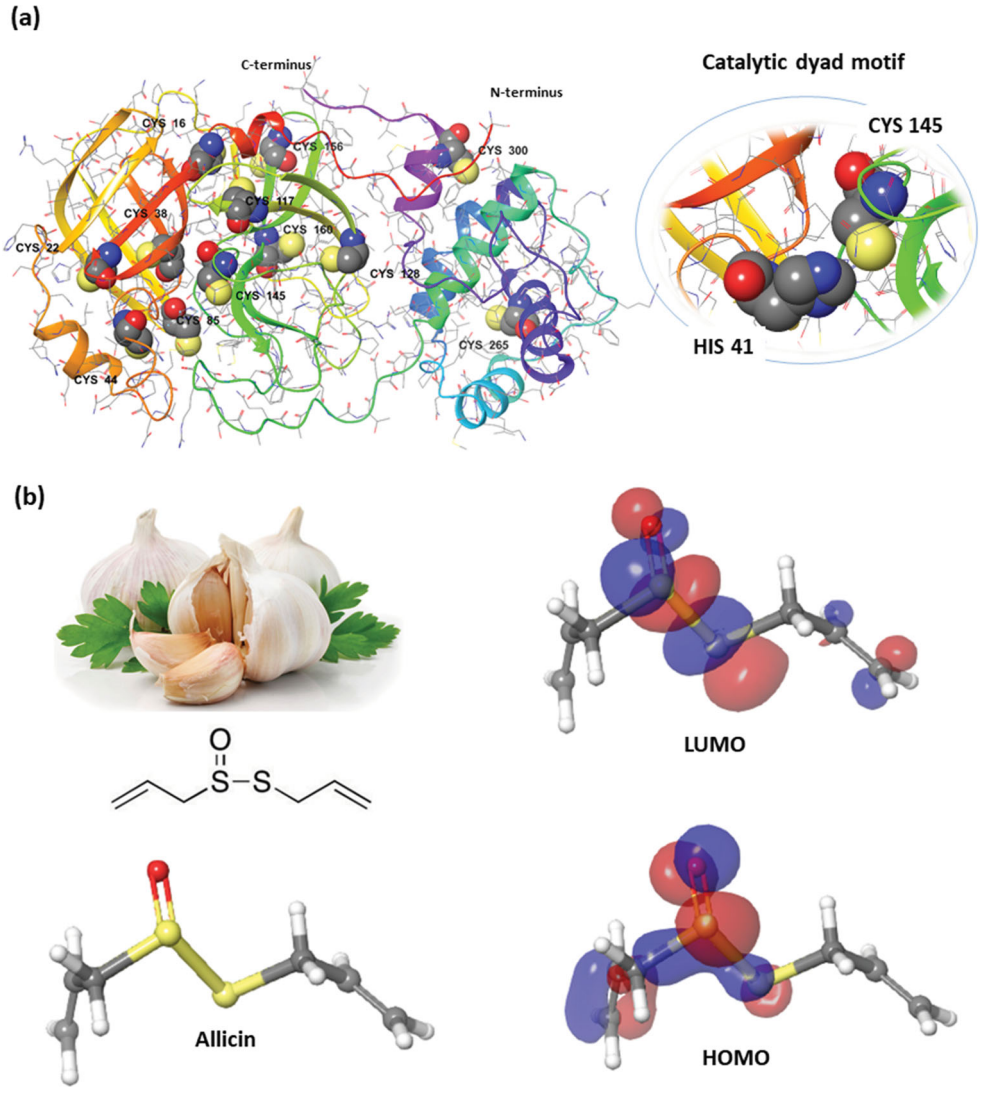

Figure 1. (a) Structure of SARS-CoV-2 M^{pro} (PDB ID: 6Y2E) [7] with the cysteine free thiols and the active site dyad motif. Cysteine thiols and catalytic residues are highlighted through the ball representation of Schrödinger software. N-and C-terminus of the protein is also indicated. (b) Allicin derived from garlic (*Allium sativum*) and optimized structure of allicin with HOMO and LUMO orbitals by DFT (Density Functional Theory). The 2D-structure of allicin is also indicated.

Interestingly, both the reports have documented the covalent binding of the inhibitors, both peptidomimetic and sulfur/selenium contain small molecules, at the catalytic Cys-145 residue of SARS-CoV-2 M^{pro}. Specific interest is ebselen and PX-12 which covalently bind to Cys-145 residue of SARS-CoV-2 M^{pro} through selenosulfide and disulfide bond, respectively. Virtual screening methods have revealed various natural products as inhibitors of SARS-CoV-2 M^{pro} including bioactive sulfur compounds derived from garlic essential oils [15]. Allicin is of special interest due to the antiviral activity and the reactive nature of the thiosulfinate group [12]. Figure 1b shows allicin natural products derived from garlic (*Allium sativum*) and its optimized structure with HOMO and LUMO orbitals by density functional theory (DFT). HOMO and LUMO orbitals indicate the nucleophilic attack at the thiosulfinate group of allicin. The current report has evaluated allicin as a covalent inhibitor of SARS-CoV-2 M^{pro} by virtual screening method on Glide, Maestro Version 12.0.012 Platform of Schrödinger software. Jin et.al., 2020 have demonstrated through tandem MS/MS analysis the PX-12 covalently modifies the Cys-145 residue of SARS-CoV-2 M^{pro} through a disulfide bond [8]. Hence, PX-12 is used as a benchmark reference in assessing the results of *in silico* docking of allicin to SARS-CoV-2 M^{pro}. Four representative co-crystals containing covalently bound ligands in the active site of SARS-CoV-2 M^{pro} were chosen for virtual screening of allicin: PDB ID 6LU7 and 6Y2F contains peptidomimetic and PDB ID 5RFV and 5RFW contains small molecule inhibitors. Figure-S2 shows the structure of ligands that are covalently bound to the Cys-145 residue in the co-crystals of SARS-CoV-2 M^{pro} retrieved from PDB. Conventional (or) non-covalent docking was performed to identify the binding of allicin to the active site of SARS-CoV-2 M^{pro}.

Figure 2a shows the binding of allicin at the active site of the SARS-CoV-2 M^{pro}. Figure-S3 shows interacting residues at the binding region of allicin in the SARS-CoV-2 M^{pro}. Table-S1a provides a summary of the docking of allicin into the four PDB crystal structures of SARS-CoV-2 M^{pro}. The observed interaction network of allicin with residues in the binding region of M^{pro} (Figure-S3 and Table-S1a) are similar to the reported results of docking of allyl disulfide at the active site of M^{pro}[15]. The distance between sulfur of Cys-145 of M^{pro} and sulfur of allicin varies by 3.5-7.3 A°. Figure-S4a shows the binding of the reference compound PX-12 at the active site of the SARS-CoV-2 M^{pro}. Figure-S4b shows interacting residues at the binding region of PX-12 in the SARS-CoV-2 M^{pro}. Table-S1b provides a summary of the docking of PX-12 into the four PDB crystal structures of SARS-CoV-2 M^{pro}. The observed results are similar between allicin and the reference compound PX-12. The distance between sulfur of Cys-145 of M^{pro} and sulfur of PX-12 varies by 5.1-11.5 A°. It is evident from the comparison of non-covalent docking of allicin and reference compound PX-12, sulfur of allicin is closer to the active site sulfur of Cys-145 residue of M^{pro} than PX-12. The reference compound was experimentally shown by Jin et.al., 2020 to covalently modify the Cys-145 of M^{pro} through a disulfide bond. These observations indicate that like PX-12 modifying the active site Cys-145 residue of M^{pro} through disulfide, allicin may cause *S*-thioallylation of Cys-145 of SARS-CoV-2 M^{pro}. The current report has assessed the possibility of allicin induced *S*-thioallylation of Cys-145 of SARS-CoV-2 M^{pro} using *in silico* approach in the background of PX-12 as reference. Using the custom-made covalent reaction type provided by Schrödinger for reactions-1 and reaction-2 (Scheme-S1), covalent docking was performed between allicin/PX-12 and active site of SARS-CoV-2 M^{pro}. Figure 2b shows the formation of cysteine allyl disulfide at the Cys-145 residue of SARS-CoV-2 M^{pro} after covalent docking with allicin. Similar

results are also observed with PX-12 (Figure-S5). These observations support that allicin covalently modifies the Cys-145 residue of SARS-CoV-2 M^{pro} through the formation of a disulfide bond. The by-product of the reaction between Cys-145 thiol and the allicin is an allyl sulfenic acid which is a reactive sulfur species that can potentially react with thiols to form corresponding allyl disulfide. SARS-CoV-2 M^{pro} contains a total of twelve cysteine residues around the binding site of allicin; Cys-16, Cys-22, Cys-38, Cys-44, Cys-85, Cys-117, Cys-128, Cys-156, Cys-160, Cys-265, and Cys-300. Interestingly, all these cysteine residues are present in the reduced state of the thiol form. Figure 3 shows the relative accessible surface area of cysteine residues around the binding site of allicin of SARS-CoV-2 M^{pro}. Except for Cys-85, Cys-156, and Cys-300, the remaining cysteine residues are buried in the protein (RASA is < 5 %) and may not be accessible for the reaction with allyl sulfenic acid. The Cys-85 residue is accessible to external agents and independent of binding of the inhibitor to the M^{pro}. The Cys-156 residue is accessible to external agents and the extent of accessibility is affected due to the binding of the inhibitor to the M^{pro}. Figure-S6 shows the residues in the vicinity of Cys-156 in unbound and bound form of SARS-CoV-2 M^{pro}. Particularly, in the N3 bound form of SARS-CoV-2 M^{pro}, Lys-100 and Tyr-154 residues move over Cys-156 retarding its accessibility to the external agent. The Cys-300 residue is exposed in the apo form and buried in the inhibitor bound form of SARS-CoV-2 M^{pro}. The flanking residues to the C-terminus of Cys-300 are unstructured in nature and projects in opposite orientation between the apo and inhibitor bound form of SARS-CoV-2 M^{pro} (Figure-S7). Such moments are affecting the solvent accessibility of Cys-300 residue. Figure-S8 shows pKa of thiols of cysteine residues in the unbound and ligand-bound form of SARS-CoV-2 M^{pro}. The pKa of Cys-85, Cys-145, and Cys-156 are (11.6-11.8), 10.0, and (8.9-9.5), respectively. The pKa of thiol of Cys-145 and Cys-156 are comparable indicating similar reactivity towards *S*-thioallylation. In the case of Cys-156, relatively higher pH conditions may be required for cysteine thiol to exist in thiolate form. Based on the above information, allyl sulfenic acid was covalently docked at Cys-85 and Cys-156 residue of SARS-CoV-2 M^{pro}. Using a custom-made covalent reaction type provided by Schrödinger for the reactions-3 (Scheme-S1), covalent docking was performed between allyl sulfenic acid and solvent accessible Cys-85/Cys-156 thiol of SARS-CoV-2 M^{pro}. Figure 4b and c show the formation of cysteine allyl disulfide at Cys-85 and Cys-156 residue of SARS-CoV-2 M^{pro} after covalent docking with allyl sulfenic acid.

Table-S2 provides a summary of covalent docking of allicin/PX-12/allyl sulfenic acid at cysteine thiols of four different co-crystal structures of SARS-CoV-2 M^{pro}. Covalent docking affinity score and retention of accessibility in inhibitor bound form supports the formation of cysteine allyl disulfide at Cys-85 over Cys-156 residue (Table-S2). However, as shown in Figure-S6, the pKa of Cys-145 is comparable with that of Cys-156 than Cys-85. The covalent docking studies indicate the dual *S*-thioallylation of Cys-145 and Cys-85/Cys-156 residue of SARS-CoV-2 M^{pro} by the allicin. These observations further supported by the recent report of Gruhlke et.al.,2019 on *S*-thioallylation of proteins while mapping the human allicin proteome [20].

3.2. Accessing the feasibility of the reaction between cysteine thiol and allicin

To support the observed covalent docking results between allicin and cysteine thiols of SARS-CoV-2 M^{pro}, the energetics of reactions between cysteine thiol with allicin was

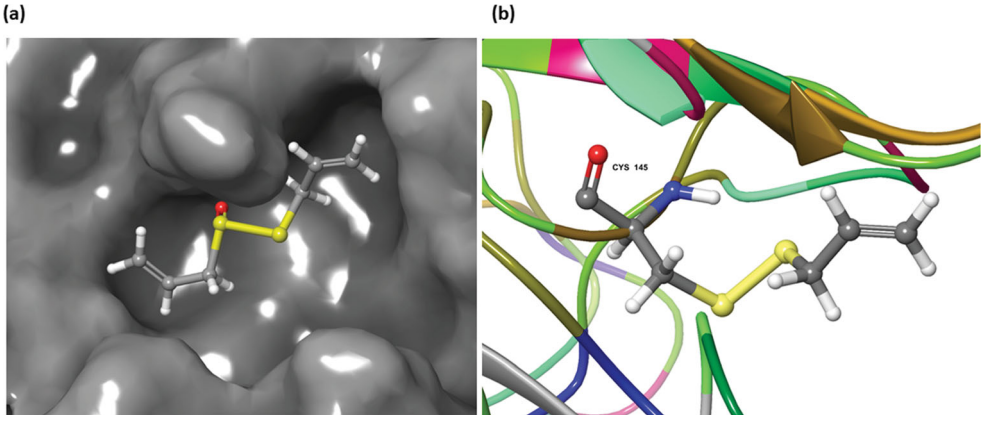

Figure 2. (a) Conventional/non-covalent docking and (b) Covalent docking of allicin at the active site of SARS-CoV-2 M^{pro}. The (S-S) distance in Figure-2b is 2.04 A°. As stated in Schrödinger's software, Non-covalent docking is 'docking ligands to a protein target using pre-generated receptor grids. Includes settings for ligand sampling, pose filtering and post-processing; constrains to the receptor, a ligand core, or on ligand torsions; and using similarity and dissimilarity screening'. Covalent docking is 'the reactive functional group on the ligand and reactive residues in the receptor are identified, and the bond is formed between the correct atoms on each'.

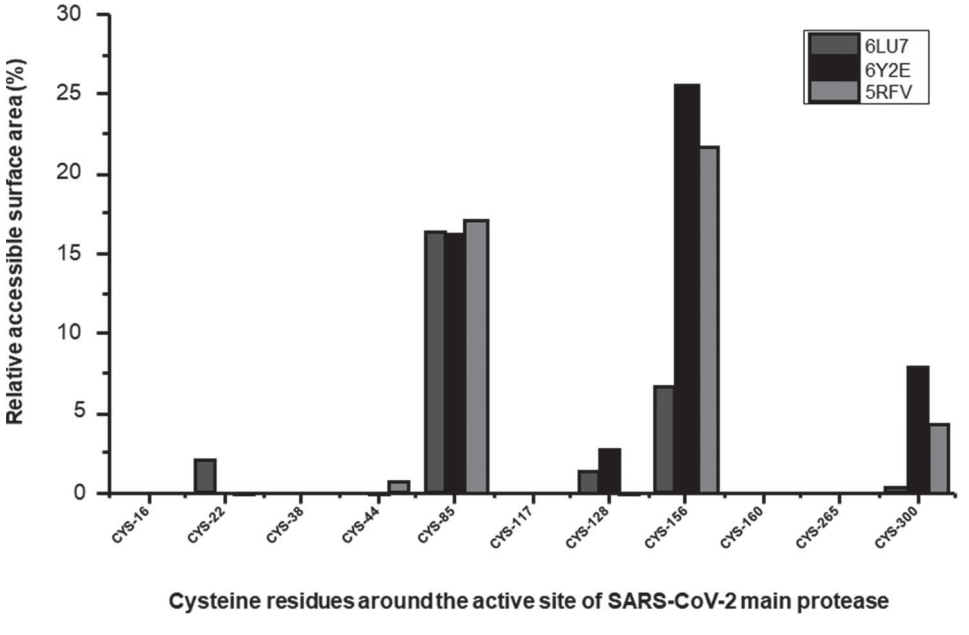

Figure 3. Relative accessible surface area of cysteine residues around the binding pocket of allicin in the SARS-CoV-2 main protease. Structures of SARS-CoV-2 M^{pro} (PDB ID) are indicated. PDB ID: 6Y2E is unbound form and that of PDB ID: 6LU7 and 5RFV are a ligand-bound form of SARS-CoV-2 M^{pro} [7,8].

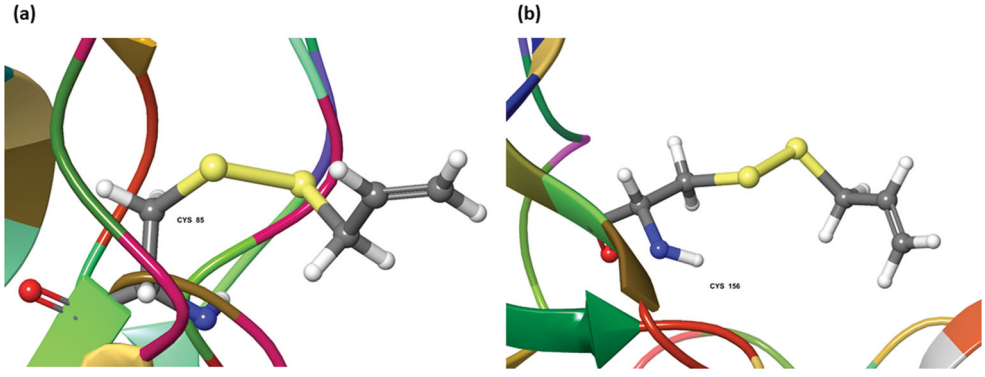

Figure 4. Covalent docking of allyl sulfenic acid at (a) Cys-85 reside and (b) Cys-156 residue of SARS-CoV-2 main protease. PDB ID: 6LU7 of SARS-CoV-2 M^{pro} is a template in the above structures and processed using Maestro Version 12.0.012 Platform of Schrödinger software. The (S-S) distance in Figure-4a and Figure-4b is 2.04 A°

calculated by density functional theory (DFT) using *N*-acetylcysteine amide as a model compound. Figure 5a shows the possible reactions of cysteine thiols with allicin and their reactive intermediates. Figure-S9 shows the optimized structures of reactants and products of putative reaction by DFT. The initial reaction of cysteine thiol with allicin results in the formation of products cysteine allyl disulfide and allyl thioaldehyde. The Gibbs free energy change for the initial reaction of cysteine thiol with allicin is + 4.79 kcal/mol indicating the endergonic and non-spontaneous nature of the reaction. These features suggest that additional energy is required to facilitate the occurrence of the reaction in the form of input energy or through the stabilization of the products. In the context of present studies, the later may be possible at the binding site of the allicin in SARS-CoV-2 M^{pro}. Figure 5b shows the sulfur mediated hydrogen bonding by the sulfur of allyl disulfide formed after covalent docking of allicin at the active site of SARS-CoV-2 M^{pro}. The sulfur mediated hydrogen bonds are well documented in peptides and proteins [21,22]. Sulfur centered H-bonds (SCHBs) are increasingly evident in proteins; N−H···S H-bonds in thioredoxins, O−H···S H-bonds are found in catalase and S−H···O H-bonds in globular proteins [19]. The role of sulfur mediated hydrogen bonding is becoming very significant in molecular assemblies, structural biology, and functional materials [19]. SCHBs are now considered as worthy participants in hydrogen bonding with distinct features compared to the conventional X−H···Y (X, Y = O, N) hydrogen bonds [23]. The sulfur of allyl disulfide is involved in bifurcated hydrogen bond; hydrogen bond distance between Gly-143 NH−S-Allyl is 2.43 A° with the angle of 147.8° and Cys-145 NH−S-Allyl is 2.33 A° with the angle of 153.4°. In addition to the hydrogen bond, hydrophobic and van der Waals interaction also contributes to stabilizing the newly formed product of cysteine allyl disulfide. It is worth noting that in the absence of such stabilization of product or input energy, the initial reaction may not be feasible between cysteine thiol and allicin. Rather, all the exposed cysteine residues in the 3D-structure of proteins may not react with allicin to yield the product of cysteine allyl disulfide. Newly formed product of allyl thioaldehyde may undergo tautomerization to allyl sulfenic acid which is an exergonic reaction with ΔG of −19.59 kcal/mol. Net reaction between cysteine thiol and allicin to yield cysteine allyl disulfide and allyl

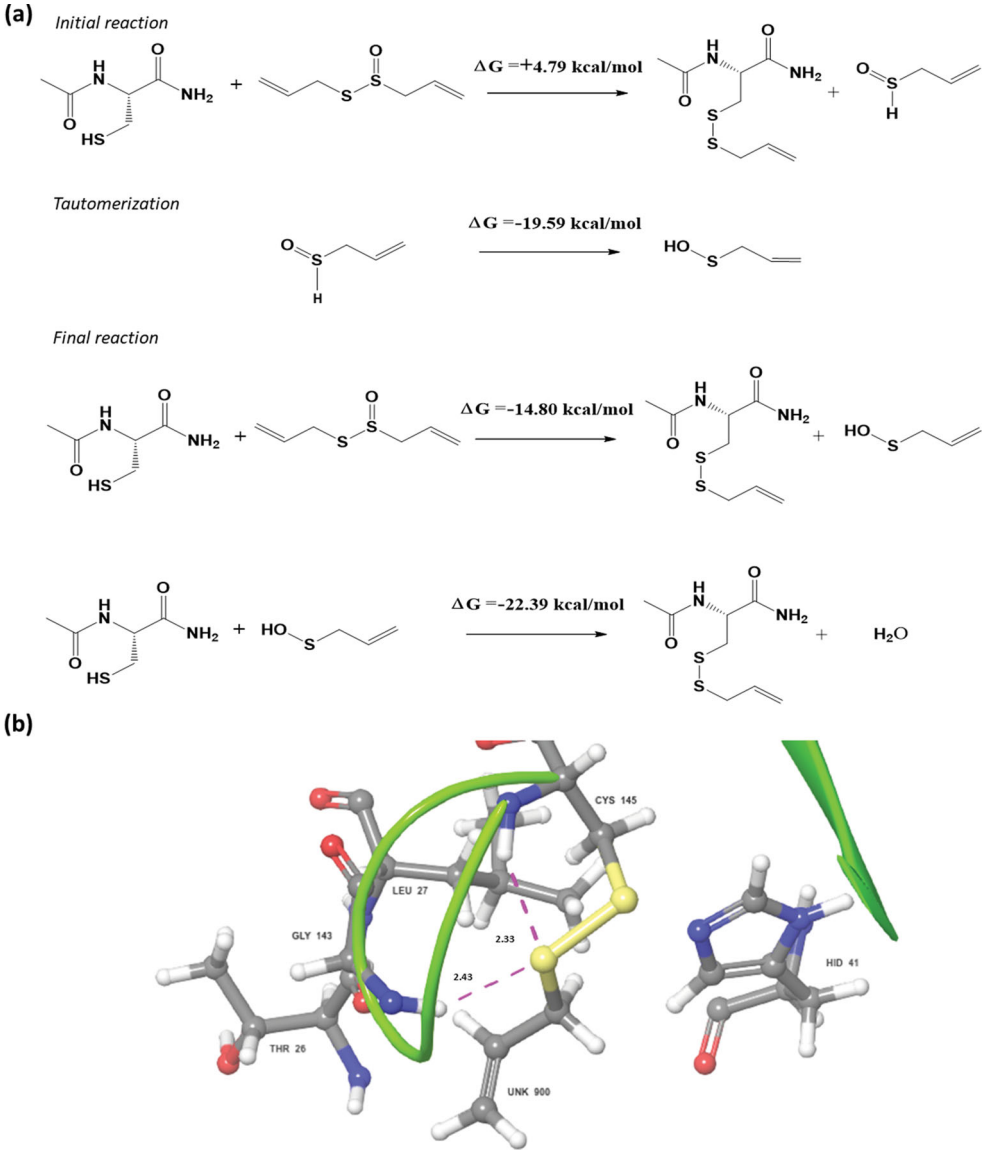

Figure 5. Calculation of ΔG value for corresponding putative reactions by density functional theory (DFT). (a) The reaction between *N*-acetylcysteine amide and allicin (or) allyl sulfenic acid. Tautomerization of allyl thioaldehyde to allyl sulfenic acid and ΔG value for corresponding reactions are indicated. (b) Sulfur mediated hydrogen bonding of the covalent product at the active site of SARS-CoV-2 M^{pro}.

sulfenic acid has ΔG of − 14.80 kcal/mol indicating preference for the forward reaction. The allyl sulfenic acid is a reactive sulfur species and may participate in the reaction with nearby cysteine thiol to yield another molecule of cysteine allyl disulfide and water. This consecutive reaction is also exergonic with ΔG of −22.39 kcal/mol. The reaction of multiple thiol-containing M^{pro} with a single molecule of allicin may result in the formation of two cysteine allyl disulfide with the elimination of water. These calculations support the formation of Cys-145 allyl disulfide during the covalent docking of allicin at the active site

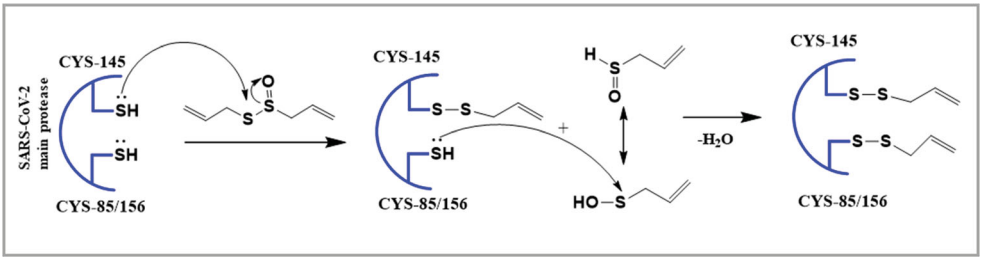

Figure 6. The schematic representation of dual *S*-thioallylation of SARS-CoV-2 main protease in virtual screening by the allicin.

of SARS-CoV-2 M^{pro}. The dual modification of cysteine allyl disulfide may be possible at Cys-145 and Cys-85/Cys-156 thiol of SARS-CoV-2 M^{pro} by the allicin. Figure 6 shows steps involved in the dual modification of cysteine residues of SARS-CoV-2 M^{pro} as cysteine allyl disulfide by the allicin.

The coronavirus genome encodes for 29 proteins (one may not get expressed): Four structural proteins (Spike, envelop, membrane, nucleocapsid) and 25 non-structural proteins [24,25]. The Spike protein/S-protein binds to cell surface angiotensin-converting enzyme 2 (ACE2) receptors of the host cells for entry into the host cells [26]. The non-structural proteins are mainly involved in the virus assembly, replication, and modulation of the host system and initially expressed as two long polyprotein pp1a and pp1ab. These polyproteins were further protested into 16 smaller proteins with the help of SARS-CoV-2 M^{pro} and papain-like protease. Interestingly, SARS-CoV-2 M^{pro} performs 11 of these cleavages, thus, it is an attractive target for the drug discovery against COVID-19 [7,8]. The other non-structural proteins involved in the life-cycle of coronavirus is RNA-dependent RNA polymerase (RdRp), helicase and NSP3 protein that associated with immunomodulation of host system to protect the virus. The structural spike glycoprotein and non-structural SARS-CoV-2 M^{pro}, papain-like protease, RdRp, helicase, and NSP3 are an important target for anti-COVID drugs [27]. The spike protein contains an exposed Cys-136 residue and twelve disulfides (PDB ID:6VXX) and further, Cys-136 residue is the distance from the binding surface with ACE2. Interestingly, the active site of SARS-CoV-2 M^{pro} and papain-like protease contains cysteine residue. The catalytic motif of M^{pro} consists of dyad residues (His-41 and Cys-145) [7,8] and papain-like protease consists of triad residue (Cys-111, His-272, and Asp-287) [28]. The covalent modification of active site cysteine residues in these proteases results in functional loss which eventually terminates the formation of non-structural proteins and replication of coronavirus. Using in silico approach, the present studies have evaluated the allicin as a covalent inhibitor of SARS-CoV-2 M^{pro}. The reactive nature of allicin and its reaction by-products with cysteine thiols results in the dual *S*-thioallylation of SARS-CoV-2 M^{pro}. Thus, allicin may attenuate the replication of coronavirus and may be useful in attenuating the COVID-19. Synergistic effects of allicin induced *S*-thioallylation of coronavirus proteins may play a role in attenuating the replication and propagation of coronavirus.

4. Conclusion

The active site of SARS-CoV-2 main protease that cleaves eleven sites of long polyprotein to release functional proteins required for assembly/replication of coronavirus contains cysteine free thiol. Allicin natural product derived from *Allium sativum* has proven medicinal properties including antiviral and antimicrobial activity and lowering risks of cardiovascular diseases contains reactive thiosulfinate which can cause protein S-thioallyllation. In the current report, using *in silico* non-covalent and covalent docking screening methods, allicin is evaluated as a covalent inhibitor of SARS-CoV-2 M^{pro}. Allicin causes dual *S*-thioallylation of Cys-145 and solvent-exposed Cys-85/Cys-156 residue of SARS-CoV-2 M^{pro} thereby acts as a potent inhibitor of SARS-CoV-2 M^{pro}. The multi-faceted roles of allicin as a booster of immune system [29] and covalent inhibitor of coronavirus protease may be useful in treating COVID-19 infection.

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Disclosure statement

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