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Targeting with Allicin via Dual S-Thioallylation as a Potential Therapeutic Approach for COVID-19

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Abstract

According to the World Health Organization (WHO), coronavirus disease 2019 (COVID-19) is an ongoing epidemic caused by new coronavirus infections that has resulted in 3716075 deaths worldwide.The SARS-CoV-2 major protease (Mpro) is an important component of coronavirus replication and is hence a prime candidate for inhibitor discovery in COVID-19 treatment. The SARS-CoV-2 Mpro is effectively inhibited by the preclinical medicines ebselen and PX-12, which covalently modify the Mpro active site Cys-145 residue by selenosul-fide/disulfide. The reactive sulfur species allicin is subjected to covalent docking at the active site of SARS-CoV-2 M in the current work using PX-12 as a reference molecule and virtual screening methods. According to the findings, allicin causes the SARS-CoV-2 Mpro's Cys-145 and Cys-85/Cys-156 residues to undergo dual S-thioallylation. The hypothesized reactions between N-acetylcysteine amide thiol and allicin/allyl sulfenic acid are computed using density functional theory (DFT) to determine the Gibbs free energychange (DG). Overall, the reaction is exergonic, and the Mpro's Cys-145 residue's allyl disulfide is implicated in a hydrogen bond that is mediated by sulfur. According to the findings, allicin induces dual S-thioallylation of SARS-CoV-2 Mpro, which may be useful for treating and reducing persistent coronavirus infection.

Keywords: S-Thioallylation , COVID-19, Bioinformatic, Allicin, MDS.

استهداف Dual S-Thioallylation عبر الأليسين

كنهج علاجي محتمل لـ *COVID-19*

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الخلاصة

وفقا لمنظمة الصحة العالمية (WHO)، فإن مرض فيروس كورونا 2019 (COVID-19) هو وباء مستمر ناجم عن عدوى فيروسات تاجية جديدة أدت إلى وفاة 3716075 شخصًا في جميع أنحاء العالم. ويعد البروتياز الرئيسي لـ SARS-CoV-2 (Mpro) مكونًا مهمًا لتكاثر فيروس كورونا، وبالتالي فهو مرشح رئيسي لاكتشاف المثبط في علاج كوفيد-19. يتم تثبيط SARS-CoV-2 Mpro بشكل فعال بواسطة الأدوية قبل السريرية ebselen وPX-12، والتي تعدّل تساهميًا بقايا موقع Mpro النشط Cys-145 بواسطة سيلينوسول-فيد/ثاني كبريتيد. يتعرض نوع الأليسين الكبريتي التفاعلي إلى الالتحام التساهمي في الموقع النشط لـ SARS-CoV-2 M في العمل الحالي باستخدام PX-12 كجزيء مرجعي وطرق فحص افتراضية. وفقًا للنتائج، يتسبب الأليسين في خضوع بقايا Cys-145 وCys-85/Cys-156 الخاصة بـ SARS-CoV-2 Mpro للخضوع لثنائي S-thioallylation. يتم حساب التفاعلات المفترضة بين N-أسيتيل سيستئين أميد ثيول وحمض الأليسين/الأليل سلفينيك باستخدام نظرية الكثافة الوظيفية (DFT) لتحديد تغير الطاقة الحر لجيبس (DG). عمومًا، يكون التفاعل طاردًا للطاقة، ويتورط ثاني كبريتيد الأليل في بقايا Mpro Cys-145 في رابطة هيدروجينية يتوسطها الكبريت.

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وفقًا للنتائج، يحفز الأليسين تحلل S-thioallylation المزدوج لـ SARS-CoV-2 Mpro، والذي قد يكون مفيدًا في علاج وتقليل عدوى فيروسات التاجية المستمرة.

1. Introduction

Throughout human history, the edible plant known as garlic has attracted considerable interest as a potential treatment for sickness. It has been proven that formulations including crushed garlic are harmful to a variety of microorganisms, including bacteria, fungus, protozoa, and viruses. Garlic's health benefits include the ability to fight cancer and lower blood cholesterol levels. The containing sulfur chemical content in garlic cloves is exceptionally high (1-3%), according to chemical investigations [1].

After a garlic clove is crushed, alliin quickly changes into the medically important allicin molecule. Alliin is lysed by the two-subunited pyridoxal 5-phosphate-dependent glycoprotein alliinase, also known as alliin-lyase (E.C.4.4.1.4) [2]. At least 10% of the protein in the form of alliinase (10 mg/g fresh mass) may be found in garlic cloves. The enzyme's gene has been cloned, and translational analysis has revealed that it has a molecular mass of 51.45 kDa and 448 amino acids. The resulting protein is 55 000 kDa and contains 5.5–6% carbohydrates. [3]. When a garlic clove is smashed, alliin quickly changes into the physiologically active allicin molecule. Alliin is lysed by the two-subunited pyridoxal 5-phosphate-dependent glycoprotein alliinase, also known as alliin-lyase (E.C.4.4.1.4) [4]. Garlic cloves contain a significant quantity of alliinase—at approximately 10% of the entire protein content (10 mg/g fresh weight). The gene encoding the enzyme has been cloned, and translation has shown that it contains 448 amino acids and has a molecular mass of 51.45 kDa. This yields a 55 000 kDa protein with a 5.5–6% carbohydrate content [5]. Additionally, the LC-HRMS records are supported by the heatmap data (Figure 1). One of the numerous types of bacteria that allicin and other antibacterial medicines successfully eradicate is antibiotic-resistant bacteria [6].

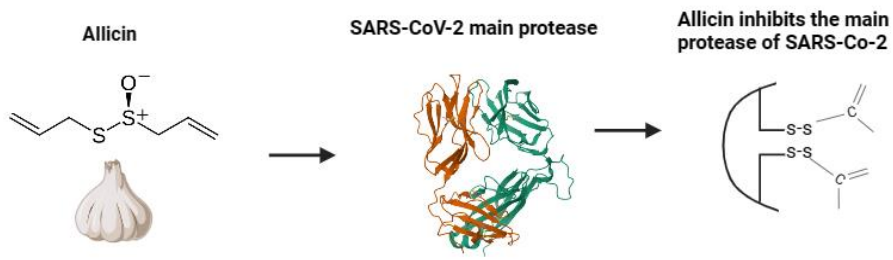

Figure -1 Shows the summary of the study that focuses on the Allicin from garlic to target the main protease of COVID-19.

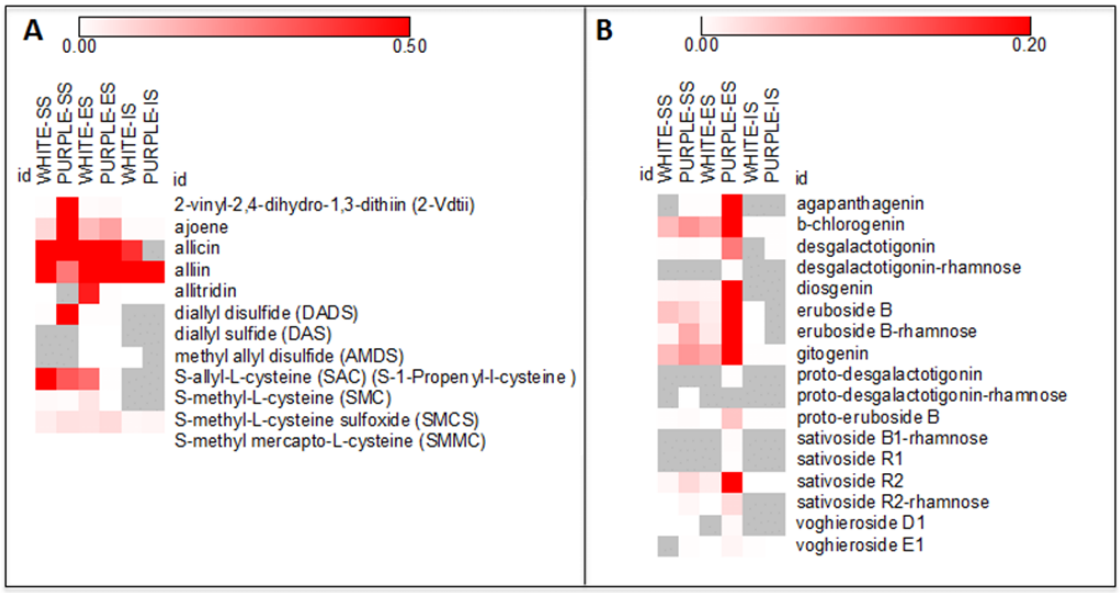

Figure -2 Sulfur-containing compounds are displayed in a heat map (A). Allicin and alliin were the sulphur compounds with the greatest signals in different varieties of garlic (Adopted from [7]).

2. Materials and method

SARS-CoV-2 Mpro crystal structures were obtained from the protein databank (PDB).SARS-CoV-2 Mproused in the studies' PDB code is: The inhibitorbound forms are 6LU7, 5RFV, and 5RFW, and the apo form is 6Y2E. The Maestro Version 12.0.012 platform of Schrödinger software was used to process the three-dimensional structures of the SARS-CoV-2 Mpro. Using the rapid align option and the distance measurement, respectively, the alignment of the Mpro structures and the measurement of the distance between the atoms were accomplished. Maestro Version 12.0.012 was used to locate the residue in the structure that was about 3 A° away from the cysteine. Utilizing the software Get-Access (http://curie.utmb.edu/getarea.html), it was possible to assess the relative surface accessibility of the cysteine residues in the 3D-structure of Mpro[8]. The protein preparation wizard of Glide, Maestro Version 12.0.012 Platform of Schrödinger software was used to determine the pka of cysteine thiols in Mpro.

Allicin and allyl sulfenic acid molecular docking into the SARS-CoV-2 mainprotease Using the Glide, Maestro Version 12.0.012 Platform of Schrödinger software, allicin's potential as a SARS-CoV-2 Mpro inhibitor was virtually screened. The four PDB co-crystal structures of SARS-CoV-2 Mpro were tested against allicin. The protein preparation wizard was used to process the SARS-CoV-2 Mpro crystal structure.Waters having less than three hydrogen bonds to non-waters were eliminated from the Mpro after hydrogen atoms were introduced, sample water orientations were produced using PROPKA at pH 7, and hydrogen atoms were added. Using the OPLS3e force field, the Mpro-ligand complex minimization was constrained. LigPrep from the Schrödinger software was used to prepare the ligand allicin with an OPLS3e force field. The Glide docking procedure of the Schrödinger software was used to simulate docking operations using its default setting. The centroid of the workspace ligand was selected

to create the receptor grid. Standard precision (SP) mode with flexible ligand sampling was used to dock allicin, and Epik state penalties were added to the docking score. Covalent docking was carried out utilizing the pose prediction docking mode of the Schrödinger software using the custom covalent reaction type made available by Schrödinger. The binding location of the allylsulfenic acid was given priority based on the covalent docking affinity score.

Computation of reaction between cysteine thiol and allicin on the Maestro MaterialsScience 3.4.012 platform of Schrödinger software, the Gibbs free energy change (G) for speculative reactions between N-acetylcysteineamide and allicin was estimated using density functional theory (DFT). With a 6-31G** basisset and a polarization function on each atom, molecules were optimized using B3LYP-D3 on the Jaguar platform (version: 10.2, Schrödinger release 2019-2). Near convergence, the use of analytical integrals was switched to, with an accuracy level set to ultrafine and a maximum iteration step size of 200. The reaction's Gibbsfree energy change (G) was computed using the platform's pre-optimized molecules and Jaguar Reaction (version 2019-2). The default convergence criteria were used, the accuracy level was set to ultrafine, and there was no solvent model. The usual conditions (T=298.15 K and p=1.0 bar) are used to calculate the Gibbs Free energies, which are expressed in (kcal/mol).

3. Results and discussion

Allicin's covalent docking onto the SARS-CoV-2 major protease's active site Zhang et al., 2020 and Jin et al., 2020 both published crystal structures of the COVID-19 or SARS-CoV-2 virus Mpro. The SARS-CoV-2 Mproisa homodimer's structure shows that the active site extends beyond the dimer interface . In order to virtually screen inhibitors against the SARS-CoV-2 Mpro, the monomer structure is used [9][11]. The structure of SARS-CoV-2 Mpro is depicted in Figure 1a along with free cysteine thiols and active site dyad residues.Image 1.(a) The structure of SARS-CoV-2 Mpro (PDB ID: 6Y2E) [12] with the active-site dyad motif and cysteine-free thiols. The ball representation of Schrödinger program highlights cysteine thiols and catalytic residues. The protein's N- and C-termini are also shown. (b) Allicin is obtained from garlic (Allium sativum), and the DFT (Density Functional Theory) optimized structure of allicin with HOMO and LUMO orbitals.

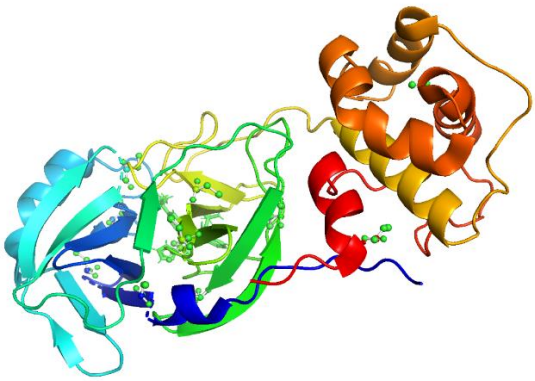

Figure -3 demonstrates the SARS-CoV-2 Mpro structure (PDB ID: 6Y2E), which includes cysteine free thiols and the active site dyad motif. The cysteine thiols and catalytic residues are highlighted in the Schrödinger program's ball representation. The N- and C-termini of the protein are also displayed.

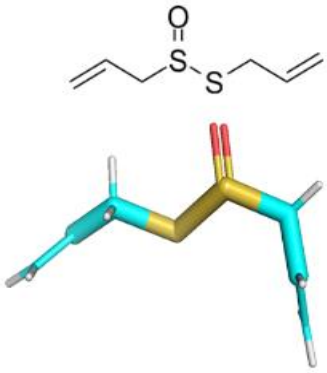

Figure -4 showcases the Allicin is derived from the garlic plant (Allium sativum). Allicin has the thiosulfinate group of functions R-S-(O)-S-R. Garlic doesn't have the substance until there is tissue damage.

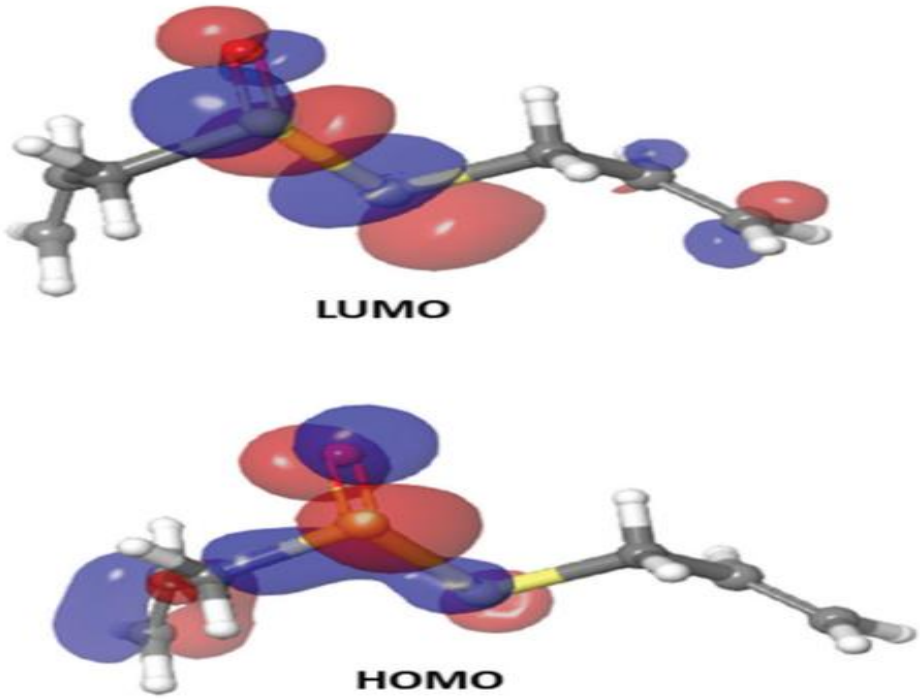

Figure -5 explains how HOMO and LUMO orbitals were exploited by density functional theory to optimize the molecular structure of allicin. Another interesting aspect of allicin is its 2D structure.

It's important to point out that both investigations have shown that the inhibitors, including peptidomimetic chemicals and small compounds including sulfur or selenium, covalently attach to the catalytic Cys-145 region of the SARS-CoV-2 Mpro. Ebselen and PX-12 are two particularly intriguing substances that engage covalently to the Cys-145 residues of the SARS-CoV-2 Mpro through selenosulfide and disulfide bonds, respectively. Virtual screening approaches have identified several natural compounds as SARS-CoV-2 Mpro inhibitors, including the vital bioactive sulfur molecules derived from garlic oils [13]. Allicin's highly reactive and antiviral properties make it particularly noteworthy [14]. Figure 3 depicts the ideal structure of allicin, which is a chemical that occurs naturally that is derived from garlic

(Allium sativum). This structure includes HOMO and LUMO orbitals. The nucleophilic assault on the thiosulfinate group of allicin is seen in the HOMO and LUMO orbitals. The Glide, Maestro Version 12.0.012 Platforms of Schrödinger software was used in the current investigation to test allicin as a covalent inhibitor of SARS-CoV-2 Mpro. Jin et al., 2020 showed using tandem MS/MS analysis that PX-12 covalently changes the Cys-145 location of SARS-CoV-2 Mpro through a bond formed of disulfide [11]. Disulfide.To evaluate the outcomes of allicin's in silico docking to SARS-CoV-2 Mpro, PX-12 is employed as a standard. For a simulated screening of allicin, four typical co-crystals with bound by covalent ligands at the SARS-CoV-2 Mpro site of action were chosen. PDB ID 6LU7 and 6Y2F include peptidomimetic, whereas PDB ID 5RFV and 5RFW contain small molecule inhibitors. In the co-crystals of the SARS-CoV-2 Mpro that were acquired from the PDB, Figure 2 shows the structure of the ligands that are covalently bonded to the Cys-145 residue. Using traditional (or non-covalent) docking, the affinity of allicin for the SARS-CoV-2 Mpro active site was assessed.The affinity of allicin to the SARS-CoV-2 Mpro active site. The study also reveals interaction residues in the allicin binding area of the SARS-CoV-2 Mpro. Allicin was docked with the four SARS-CoV-2 Mpro crystal structures that were discovered in the PDB. It was discovered that allicin and the residues in the binding area of Mpro interact in a network resembling docking data for allyl disulfide at the location of Mpro's activity that had previously been made public [13].

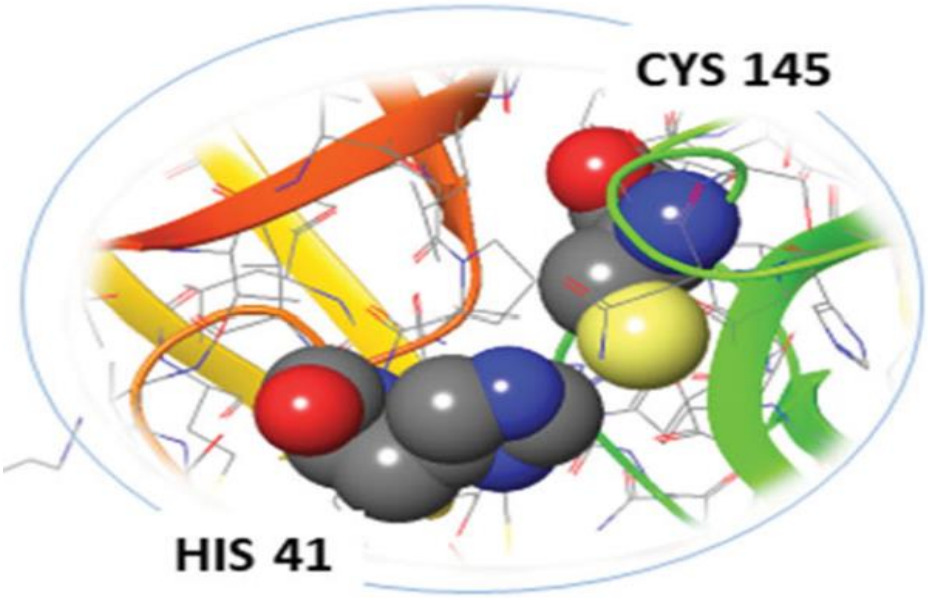

Figure -6 Shows the effects of allicin on the SARS-CoV-2 Mpro's active location

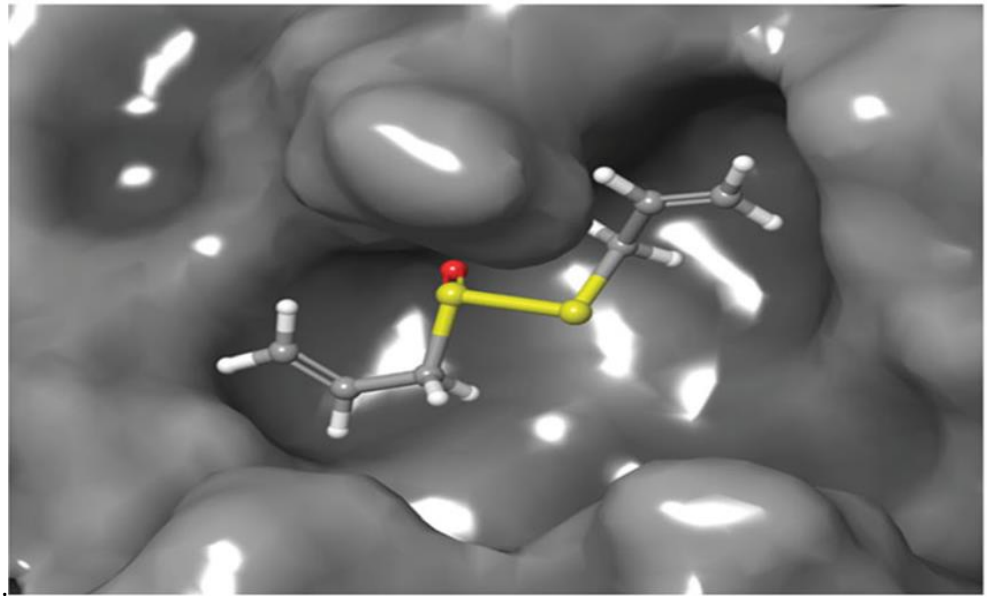

Figure -7 Demonstrates the covalent docking of allicin at the SARS-CoV-2 Mpro active site and its conventional/non-covalent docking

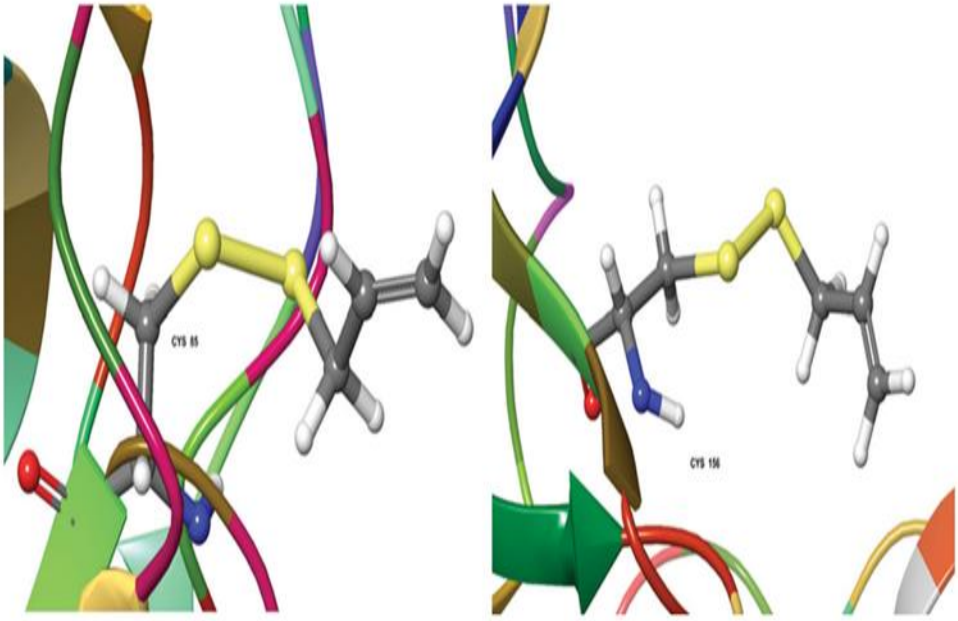

Figure -8 Demonstrates that the Cys-85 and Cys-156 sites of the SARS-CoV-2 main protease interacted into allyl sulfenic acid covalently. The Schrödinger Maestro Version 12.0.012 Platform was used to process the SARS-CoV-2 Mpro PDB ID: 6LU7 template. 2.04 A° separates (S-S).

4. Conclusion

The SARS-CoV-2 main protease, which cleaves a long polyprotein into eleven functional proteins required for coronavirus assembling and replication, has cysteine free thiol in its active site. Allicin, a naturally occurring substance produced from the Allium sativum plant, has been found to offer health advantages, including antiviral and antibacterial activity as well as a decreased risk of cardiovascular diseases. Reactive thiosulfinate, whose can cause protein S-thioallylation, is also present. In the current paper, covalent and non-covalent docking screening techniques are used to evaluate allicin as a covalent inhibitor of SARS-CoV-2 Mpro. Allicin, an effective inhibitor of SARS-CoV-2 Mpro, causes a double sthioallylation of Cys-145 with the residue of Cys-85/Cys-156 that is exposed to solvent in SARS-CoV-2 Mpro.

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References:

[1] S. Ankri and D. Mirelman, “Antimicrobial properties of allicin from garlic,” *Microbes Infect.*, vol. 1, no. 2, pp. 125–129, Feb. 1999, doi: 10.1016/S1286-4579(99)80003-3.

[2] L. D. Lawson and C. D. Gardner, “Composition, Stability, and Bioavailability of Garlic Products Used in a Clinical Trial,” *J. Agric. Food Chem.*, vol. 53, no. 16, pp. 6254–6261, Aug. 2005, doi: 10.1021/jf050536+.

[3] M. S. Rahman, Q. H. Al-Shamsi, G. B. Bengtsson, S. S. Sablani, and A. Al-Alawi, “Drying kinetics and allicin potential in garlic slices during different methods of drying,” *Dry. Technol.*, vol. 27, no. 3, pp. 467–477, 2009.

[4] E. Touloupakis and D. F. Ghanotakis, “Nutraceutical use of garlic sulfur-containing compounds,” in *Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors*, Springer, 2010, pp. 110–121.

[5] H. A. R. Suleria *et al.*, “Garlic (Allium sativum): diet based therapy of 21st century–a review,” *Asian Pacific J. Trop. Dis.*, vol. 5, no. 4, pp. 271–278, 2015.

[6] X. Dong *et al.*, “Optimizing the synergistic effect of CuWO 4/CuS hybrid composites for photocatalytic inactivation of pathogenic bacteria,” *Environ. Sci. Nano*, vol. 9, no. 11, pp. 4283–4294, 2022.

[7] G. Diretto, A. Rubio-Moraga, J. Argandoña, P. Castillo, L. Gómez-Gómez, and O. Ahrazem, “Tissue-Specific Accumulation of Sulfur Compounds and Saponins in Different Parts of Garlic Cloves from Purple and White Ecotypes,” *Molecules*, vol. 22, no. 8. 2017. doi: 10.3390/molecules22081359.

[8] R. Fraczkiewicz and W. Braun, “Exact and efficient analytical calculation of the accessible surface areas and their gradients for macromolecules,” *J. Comput. Chem.*, vol. 19, no. 3, pp. 319–333, 1998.

[9] M. T. ul Qamar, S. M. Alqahtani, M. A. Alamri, and L.-L. Chen, “Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants,” *J. Pharm. Anal.*, vol. 10, no. 4, pp. 313–319, 2020.

[10] A. Ton, F. Gentile, M. Hsing, F. Ban, and A. Cherkasov, "Rapid identification of potential inhibitors of SARS-CoV-2 main protease by deep docking of 1.3 billion compounds," *Mol. Inform.*, vol. 39, no. 8, p. 2000028, 2020.

[11] Z. Jin *et al.*, "Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors," *Nature*, vol. 582, no. 7811, pp. 289–293, 2020.

[12] L. Zhang *et al.*, "Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors," *Science (80-.).*, vol. 368, no. 6489, pp. 409–412, 2020.

[13] B. T. P. Thuy *et al.*, "Investigation into SARS-CoV-2 resistance of compounds in garlic essential oil," *ACS omega*, vol. 5, no. 14, pp. 8312–8320, 2020.

[14] J. Borlinghaus, F. Albrecht, M. C. H. Gruhlke, I. D. Nwachukwu, and A. J. Slusarenko, "Allicin: chemistry and biological properties," *Molecules*, vol. 19, no. 8, pp. 12591–12618, 2014.