Supplementary Appendix. 1.

Molecular docking and dynamics simulations of NAC with M^{pro}

Methodology

Preprocessing and Optimization of Protein Structure

Molecular docking helps identify new therapeutic compounds for emerging infectious diseases by predicting receptor-ligand interactions (Munikumar et al. 2012, 2018, 2019; Pradhan et al. 2014; Manne et al. 2018; Ungarala et al. 2022; Goudar et al. 2023). For this study, the three-dimensional structure of 2019-nCoV Mpro bound to the N3 peptide (PDB ID: 6LU7) was obtained from the Protein Data Bank (Jin et al. 2020). AutoDock 4.2, an automated docking software, was used to dock selected inhibitors with the Mpro structure.

The receptor and ligand files were prepared in PDBQT format, which includes atomic charges, atom types, and rotatable bond information. The receptor molecule was preprocessed by adding polar hydrogens and Kollman charges, while Gasteiger charges were assigned to the ligands (Morris et al. 2009; Azam and Abbasi 2013; Sliwoski et al. 2014). A grid box with dimensions of 40 × 40 × 40 Å and 1 Å spacing defined the docking site. The receptor was kept rigid, while the ligand was flexible.

Docking simulations used the Lamarckian genetic algorithm to identify optimal conformations. Binding energy, expressed in kcal/mol, and receptor-ligand interactions were analyzed. The docked structures were visualized in 3D using Maestro Schrodinger 2023, and molecular dynamics simulations were performed to assess the stability of the docked complexes under biologically relevant conditions.

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations were performed using the Desmond 2022 module by Schrödinger LLC. The simple point charge (SPC) model was used for water molecules, and orthorhombic periodic boundary conditions were applied along the X, Y, and Z axes, with a 10 Å buffer. The Mpro-NAC docked complex was electrically neutralized by adding randomly placed counterions (Na+/Cl−) to balance the system's charge.

After constructing the solvated system, the protein-ligand complex underwent minimization and relaxation using Desmond's nine-stage protocol under the constant Number of atoms, Pressure, and Temperature (NPT) ensemble (Vilar et al., 2011). These stages included initial setup, restrained and unrestrained minimizations, simulations at 10 K with restraints, and gradual equilibration, culminating in a 100 ns production run using the OPLS 2005 force field. Trajectories were recorded every 4.8 ps (Leimkuhler & Sweet, 2004).

The stability of the MD simulation was analyzed using root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF). These parameters assessed the system's stability and protein dynamics over the 100 ns simulation, accounting for temperature, pressure, and volume conditions.

Results and Discussion

The analysis of the Mpro-NAC docked complex was performed using AutoDock 4.2. The selected NAC showed a docking score of -4.99 kcal/mol (Table 1; Supplementary Appendix 1), indicating strong binding affinity to clefts in domains I and II (Figure 4A, 4B). NAC formed five hydrogen bonds with key residues—Asn142, Ser144, Gly143, Cys145, and His163—with bond lengths between 2.63 and 2.79 Å, all within 4 Å of NAC. Additionally, residues including Asn28, Leu27, His41, Met49, Tyr118, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, His164, Glu166, Met165, His172, and Gln189 contributed van der Waals interactions within 4 Å (Figure 4C).

Post-docking molecular dynamics (MD) simulations assessed the conformational stability of the Mpro-NAC complex using Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and hydrogen bond (H-bond) patterns (Table 1; Supplementary Appendix 1). The RMSD analysis showed average deviations of 2.93 Å for Mpro and 3.60 Å for NAC. RMSF values for Mpro's α-carbons, sidechains, and heavy atoms averaged 2.93 Å, 3.97 Å, and 3.40 Å, respectively, indicating minor conformational changes and a stable interaction.

Superimposed configurations highlighted interactions between Mpro (cyan) and NAC (blue) post-docking, and during MD simulations (Mpro in red, NAC in green). RMSD values for Mpro and NAC during simulations were 2.96 Å and 3.86 Å, respectively (Figure 4A), confirming complex stability. During MD simulations, NAC formed six hydrogen bonds with residues His41, Asn142, Ser144, Gly143, and His163, with bond lengths ranging from 1.56 to 2.19 Å (Figure 4D). His41 and Asn142 also showed consistent water-mediated interactions with NAC throughout the simulation. Van der Waals interactions involving residues His41, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, His164, Met165, Glu166, and His172 contributed significantly to the stability of the complex (Figure 1D).

References

Azam SS, Abbasi SW (2013) Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines. Theor Biol Med Model 10:63. https://doi.org/10.1186/1742-4682-10-63

Goudar G, Manne M, Sathisha GJ, et al (2023) Phenolic, nutritional and molecular interaction study among different millet varieties. Food Chemistry Advances 2:100150. https://doi.org/10.1016/j.focha.2022.100150

Jin Z, Du X, Xu Y, et al (2020) Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature 582:289–293. https://doi.org/10.1038/s41586-020-2223-y

Manne M, Validandi V, Khandare AL (2018) Reduction of fluoride toxicity by tamarind components: An in silico study. Fluoride 51:122–136

Morris GM, Ruth H, Lindstrom W, et al (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 30:2785–2791. https://doi.org/10.1002/JCC.21256

Munikumar M, Krishna VS, Reddy VS, et al (2018) In silico design of small peptides antagonist against leptin receptor for the treatment of obesity and its associated immune-mediated diseases. J Mol Graph Model 82:. https://doi.org/10.1016/j.jmgm.2018.04.002

Munikumar M, Natarajan P, Amineni U, Radha Krishna KVV V (2019) Discovery of potential lumazine synthase antagonists for pathogens involved in bacterial meningitis: In silico study. Inform Med Unlocked 15:100187. https://doi.org/10.1016/j.imu.2019.100187

Munikumar M, Priyadarshini IV, Pradhan D, et al (2012) In Silico Identification of Common Putative Drug Targets among the Pathogens of Bacterial Meningitis. Biochemistry & Analytical Biochemistry 01:1–8. https://doi.org/10.4172/2161-1009.1000123

Pradhan D, Priyadarshini V, Munikumar M, et al (2014) Para-(benzoyl)-phenylalanine as a potential inhibitor against LpxC of Leptospira spp.: homology modeling, docking, and molecular dynamics study. J Biomol Struct Dyn 32:37–41. https://doi.org/10.1080/07391102.2012.758056

Sliwoski G, Kothiwale S, Meiler J, Lowe EW (2014) Computational methods in drug discovery. Pharmacol Rev 66:334–395

Ungarala R, Munikumar M, Sinha SN, et al (2022) Assessment of Antioxidant, Immunomodulatory Activity of Oxidised Epigallocatechin-3-Gallate (Green Tea Polyphenol) and Its Action on the Main Protease of SARS-CoV-2—An In Vitro and In Silico Approach. Antioxidants 11:294. https://doi.org/10.3390/antiox11020294

Supplementary appendix.1: Table 1. Molecular docking and dynamics simulations of NAC with Mpro of SARS-CoV-2

Before MD simulations				**After MD simulations (100ns)**				
Docking score (Kcal/mol)	H-Bond (Å)	Bond length (Å)	VdW interactions (4Å)	RMSD and RMSF (Å)		H-Bond (Å)	Bond length (Å)	VdW interactions (4Å)
				Mpro	NAC			
4.99	Asn142*:C=O← NH	2.63	Asn28, Leu27, His41*, Met49, Tyr118, Phe140* Leu141*, Asn142*, Gly143*, Ser144*, Cys145*, His163*, His164* Glu166*, Met165*, His172*, Gln189	Cα: 2.93 Side-Chain: 3.97 Heavy atoms: 3.40	3.6	His41: NH → HOH ← SH	2.14, 1.58	His41*, Phe140*, Leu141*, Asn142*, Gly143*, Ser144*, Cys145*, His163*, His164* Met165*, Glu166*, His172*
	Ser144*:CO←OH	2.16				Asn142*: C=O→ HOH← NH	1.56, 1.50	
	Gly143*:CO→ NH	2.03				Ser144*: HO → NH	2.00	
	Cys145: NH→ CO	2.79				Ser144*:CO ← OH	1.80	
	His163*: CO← NH	2.47				Gly143*→ CO	2.19	
						His163*: NH → C=O	1.78	

*Stable amino acids in before and after MD simulations