

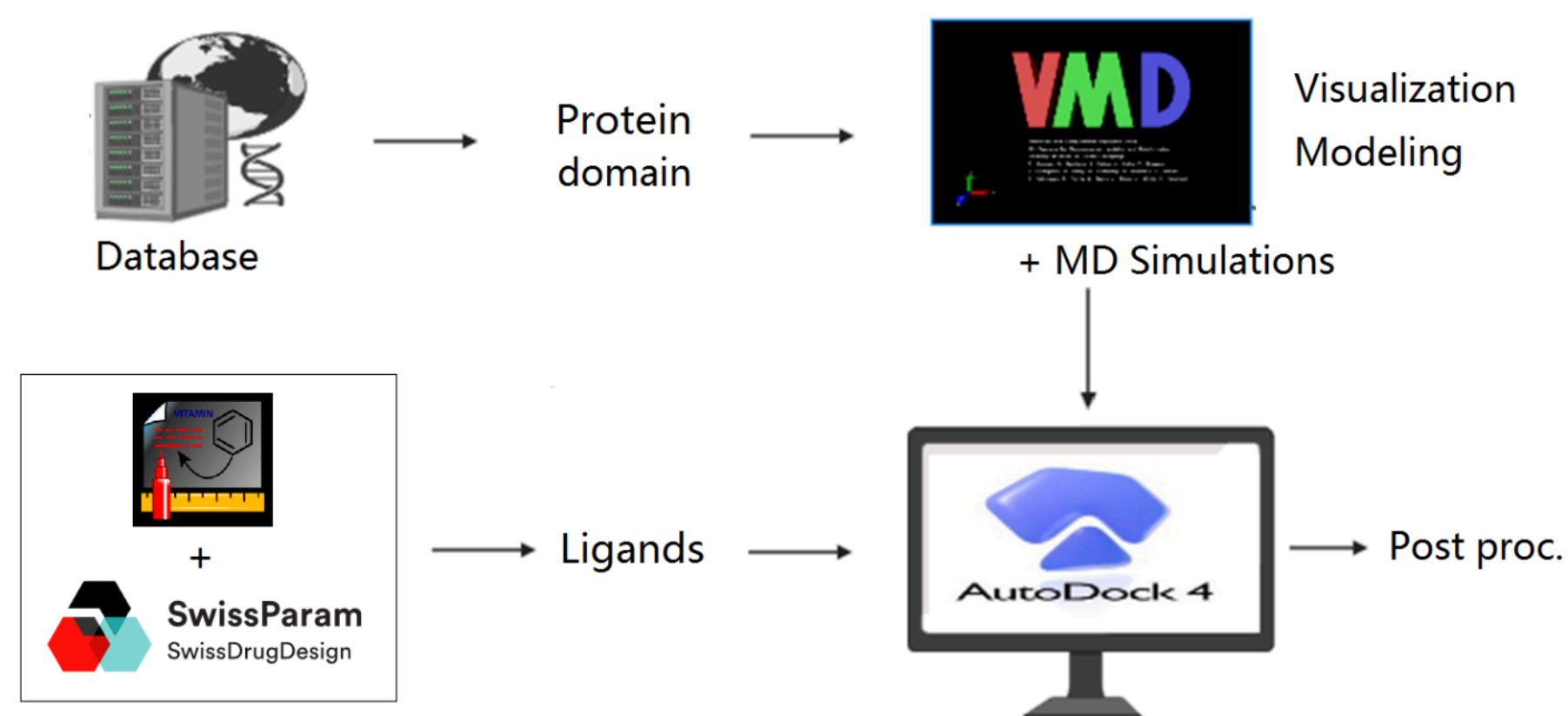
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Abstract

The study uses computational techniques, specifically molecular docking and molecular dynamics simulations, to identify potential inhibitors for SARS-CoV-2. The study focuses on the human ACE2 receptor, which binds to the SARS-CoV-2 spike protein. The aim is to identify the most effective binding scores for each drug, allowing a comparative analysis of their potencies against known antiviral agents. This approach aims to explore innovative drug candidates for natural COVID-19 treatments.

Introduction

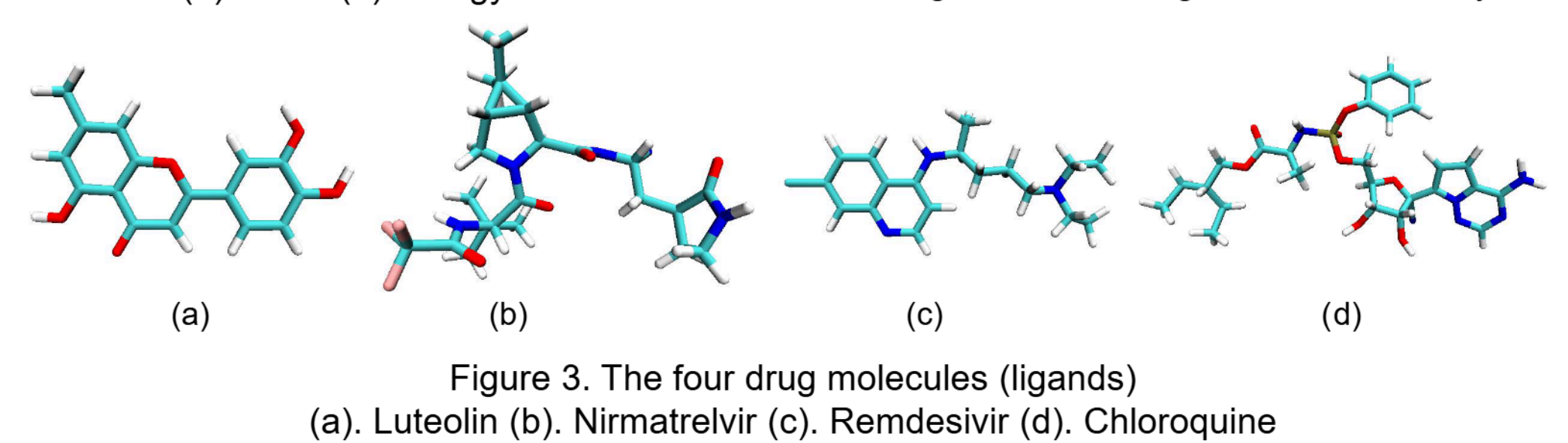
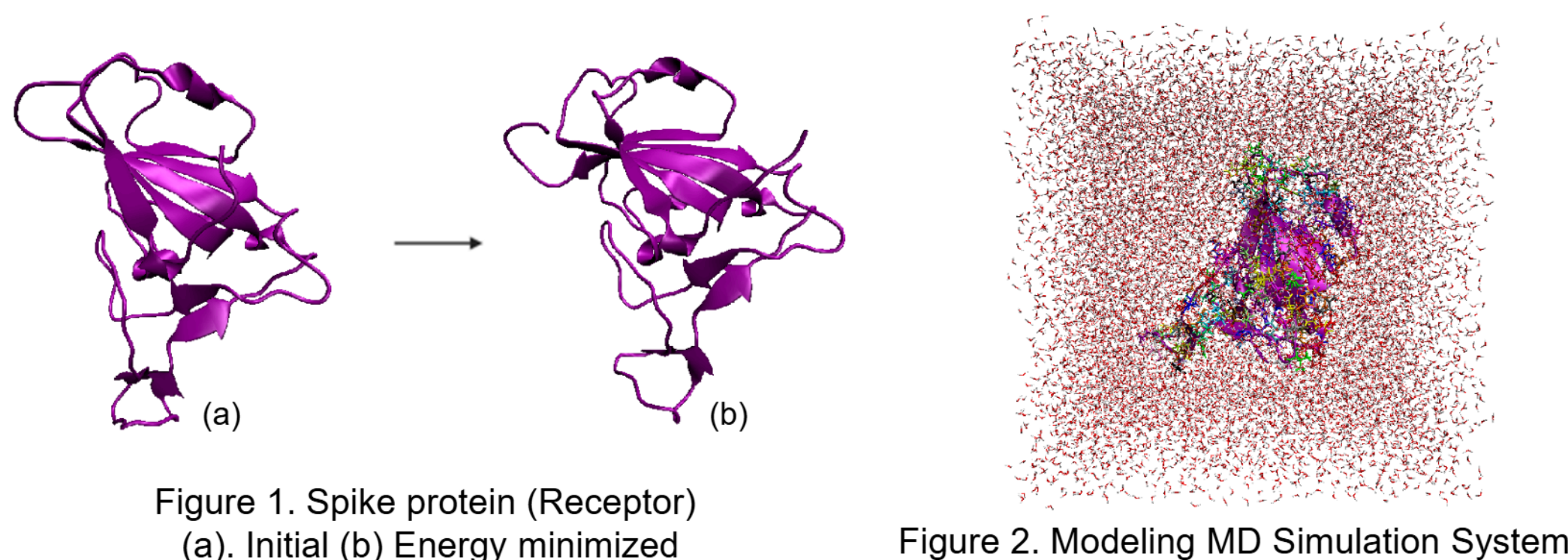
AD4 and AutoDock Tools are automated docking software packages developed by the Scripps Research Institute and the University of California, San Diego. They are robust, cost-free, and can be completed within a GUI environment. Molecular docking is a computer-aided drug design technique that helps predict 3D structures of macromolecules. SARS-CoV-2 can infect the human respiratory epithelial cells through interaction with the human ACE2 receptor. The spike protein is a large type I transmembrane protein containing two subunits, S1 and S2. S1 mainly contains a receptor binding domain (RBD), which is responsible for recognizing the cell surface receptor. S2 contains basic elements needed for the membrane fusion. The S protein plays key parts in the induction of neutralizing-antibody and T-cell responses, as well as protective immunity.



Schematic of the simulation and docking procedures

Methodology

The protein molecular modeling of the spike protein fragment for SARS CoV-2 was done using Gene Bank accession numbers QHR63250.2, LOCUS QHR63250, and Accession MN996527.1. The 3D structure of the S protein fragment was modeled using SWISSMODEL21 server, with template "8dnn.1.A" selected for sequence identity and coverage. The drug molecule (ligands) were modeled using the Chemscketch tool, and the structures were saved in .mol2 files and loaded to SWISSPARAM for PDB and PSF files. The molecular docking process involved preparing the ligand and receptor, obtaining 3D coordinates, and creating graphic representations.



Results

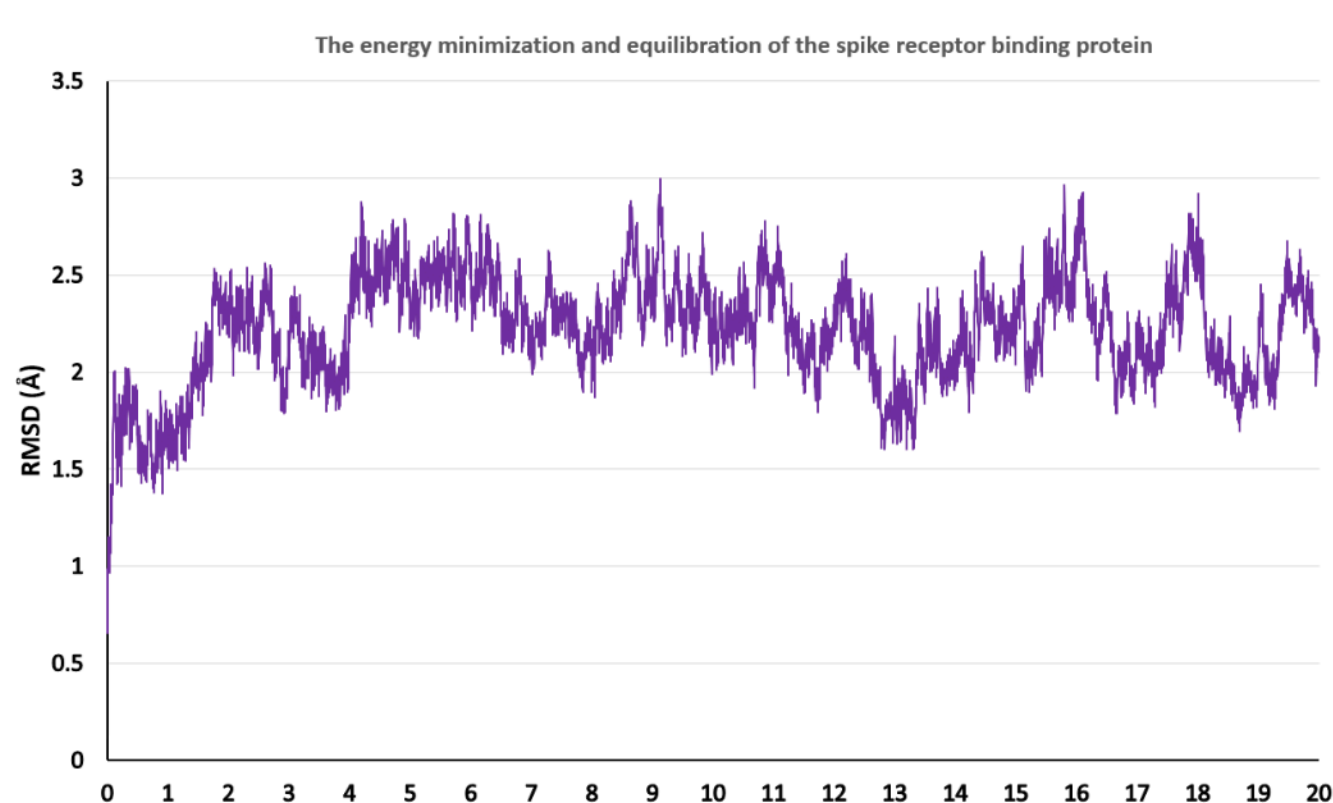


Figure 4. RMSD plot

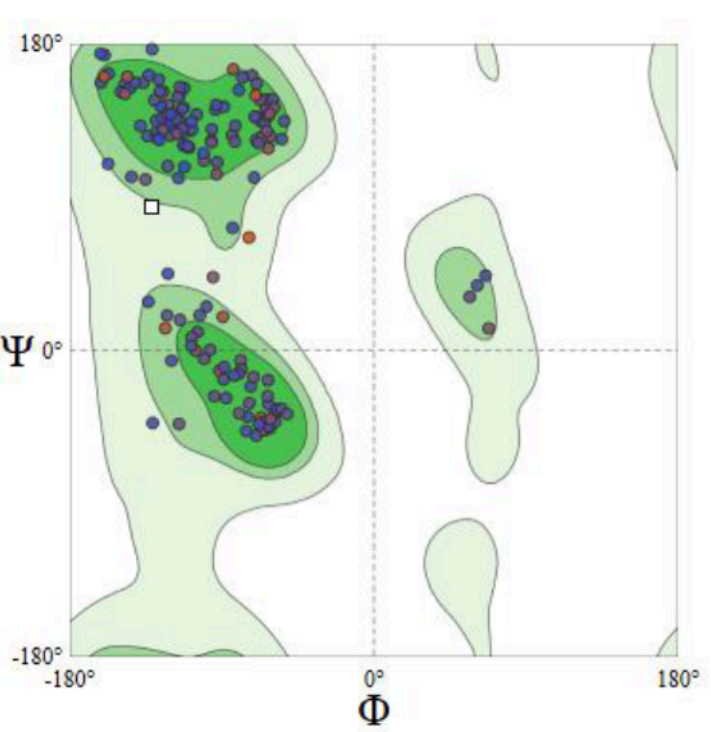


Figure 5. Ramachandran plot

The graph in Figure 4 shows the energy minimization and equilibration of the spike receptor binding protein. The RMSD increases from 0.5 to 2.5 with constant fluctuations, eventually reaching an equilibrium state of 2.5 over 20ns. This analysis confirms the simulation's equilibration and a consistent state, with a gradual increase in RMSD and stabilization indicating protein equilibration and conformational equilibrium. CHARMM force field was used for NAMD simulation.

The Ramachandran plot is a tool used to evaluate protein conformations, assess steric strain, and validate model accuracy. It categorizes residues into favored, allowed, and outlier regions, with a high percentage in the favored region indicating a stable structure, as Figure 5.

1. Interaction between Luteolin and Spike protein

Table 1. Clustering Histogram Table: Luteolin

Clus	Lowest	Run	Mean	Num	Histogram
-ter	Binding		Binding	in	
Rank	Energy		Energy	Clus	5 10
1	-4.84	5	-4.84	1	#
2	-4.50	9	-4.00	2	##
3	-4.41	3	-4.41	1	#
4	-4.30	2	-4.30	1	#
5	-4.18	6	-4.18	1	#
6	-4.17	10	-4.17	1	#
7	-4.11	1	-4.11	1	#
8	-3.86	4	-3.86	1	#
9	-3.57	8	-3.57	1	#

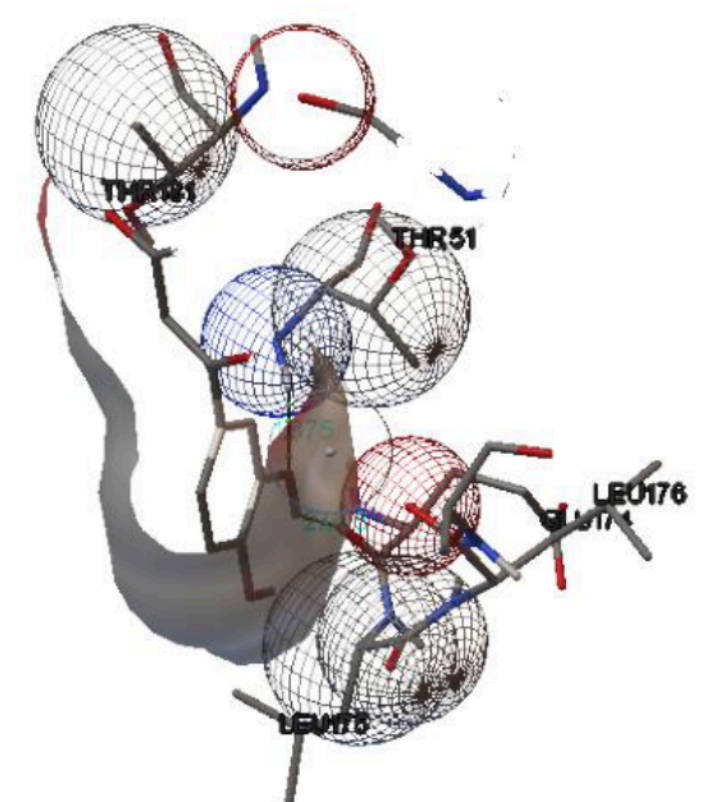


Figure 6. Luteolin-Spike protein Binding, Active sites: THR51,LEU176,LEU175

Figure 6 shows Luteolin's strongest binding energy with the spike protein at -4.84 kcal/mol, indicating a strong interaction between the receptor and ligand. The study confirms 10 luteolin-spike protein interactions in Table 1, with the strongest interaction and lowest binding energy indicating higher ligand efficacy.

2. Interaction between Nirmatrelvir and Spike protein

Table 2. Clustering Histogram Table: Nirmatrelvir

Clus	Lowest	Run	Mean	Num	Histogram
-ter	Binding		Binding	in	
Rank	Energy		Energy	Clus	5 10
1	-4.04	9	-4.04	1	#
2	-3.99	6	-3.79	2	##
3	-3.78	7	-3.78	1	#
4	-3.70	8	-3.70	1	#
5	-3.09	5	-3.09	1	#
6	-2.94	3	-2.94	1	#
7	-2.83	4	-2.83	1	#
8	-2.16	2	-2.16	1	#
9	-1.95	10	-1.95	1	#

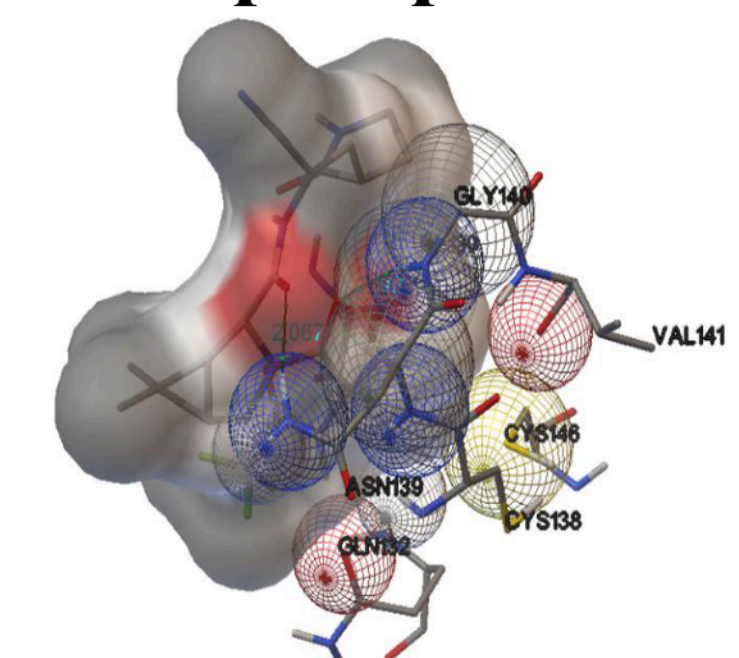


Figure 7. Nirmatrelvir-Spike Protein Binding, Active sites: ASN139, GLN132, CYS138, CYS146, VAL141, GLY140

In the 9th run, Nirmatrelvir's greatest binding energy with the spike protein was -4.04 kcal/mol, as shown in Figure 7, suggesting a robust receptor-ligand interaction. 10 nirmatrelvir-spike protein interactions are confirmed in Table 2; the highest ligand effectiveness is indicated by the strongest interaction and lowest binding energy.

3. Interaction between Remdesivir and Spike protein

Table 3. Clustering Histogram Table: Remdesivir

Clus	Lowest	Run	Mean	Num	Histogram
-ter	Binding		Binding	in	
Rank	Energy		Energy	Clus	5 10
1	-1.02	1	-1.02	1	#
2	-0.58	2	-0.58	1	#
3	+0.30	10	+0.30	1	#
4	+0.31	5	+0.31	1	#
5	+0.33	9	+0.33	1	#
6	+0.65	7	+0.65	1	#
7	+0.67	8	+0.67	1	#
8	+0.90	4	+0.90	1	#
9	+1.03	6	+1.03	1	#
10	+1.62	3	+1.62	1	#

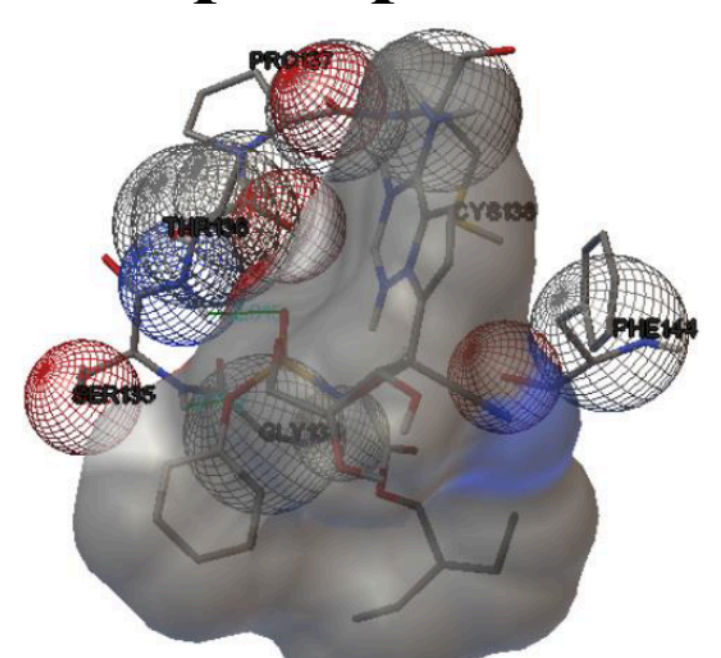


Figure 8. Remdesivir-Spike Protein Binding, Active sites: SER135, PHE144, CYS138, PRO138

The 10 Remdesivir-spike protein interactions are displayed in Table 3. The ligand and the spike protein had the greatest binding contact, the lowest binding energy, and the highest ligand effectiveness with the spike protein during the 1st run of the confirmation. With a binding energy of -1.02 kcal/mol of the remdesivir, in the 1st run, which depicts the interaction between the receptor and the ligand, Figure 8 displays the binding energy displayed when it binds with the spike protein (receptor).

4. Interaction between Chloroquine and Spike protein

Table 4. Clustering Histogram Table: Chloroquine

Clus	Lowest	Run	Mean	Num	Histogram
-ter	Binding		Binding	in	
Rank	Energy		Energy	Clus	5 10
1	-3.49	9	-3.49	1	#
2	-3.18	5	-3.18	1	#
3	-3.12	6	-3.12	1	#
4	-2.71	1	-2.71	1	#
5	-2.69	10	-2.60	2	##
6	-2.69	2	-2.69	1	#
7	-2.39	4	-2.39	1	#
8	-2.23	3	-2.23	1	#
9	-1.98	7	-1.98	1	#

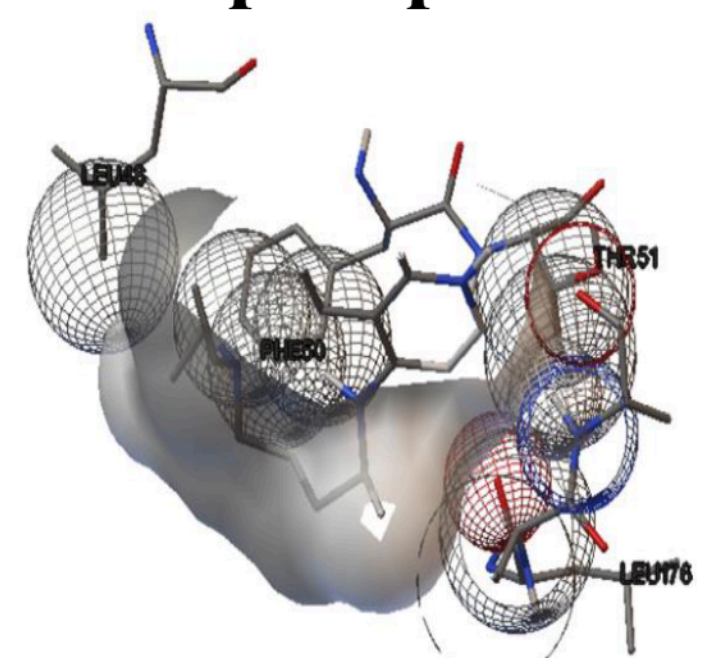


Figure 9. Luteolin-Spike Protein Binding, Active sites: LEU176, THR51, PHE50, LEU148

Figure 9 shows the binding energy exhibited by Chloroquine when binding with the spike protein(receptor) with a binding energy of -3.49 kcal/mol at the 9th run which portray a strong interaction between the receptor and the ligand. 10 nirmatrelvir-spike protein interactions are confirmed in Table 4.

Conclusion

Four drugs, Luteolin, Nirmatrelvir, Remdesivir and Chloroquine, have varying binding affinity and interaction profiles with the spike protein receptor, indicating their potential as therapeutic candidates against COVID-19. Luteolin exhibited the strongest binding affinity, while chloroquine and remdesivir showed weak binding energy scores. Key interactions, hydrogen bonds, hydrophobic contacts, and electrostatic interactions were identified. Further experimental validation, including in vitro and in vivo studies, is needed to confirm efficacy, safety, and pharmacokinetic properties.

Reference

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