Theoretical Investigation of Inhibitory Action of Flavanoids against Nipha Virus through Molecular Docking

Discipline: Chemistry

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INTRODUCTION

Nipah virus is a bat-borne, zoonotic virus that causes Nipah virus infection in humans and other animals, a disease with a very high mortality rate (40-75%). Numerous disease outbreaks caused by Nipah virus have occurred in South east Africa and South east Asia. Nipah virus belongs to the genus Henipavirus along with the Hendra virus, which has also caused disease outbreaks[1]. Nipah virus (NiV) is an RNA virus belonging to family Paramyxoviridae. It belongs to the genus Henipavirus which also contains Hendra virus (HeV) and the recently described Cedar virus. Bats are the natural reservoir of Henipaviruses. It first emerged in Malaysia in 1998 and has since caused several outbreaks in South and Southeast Asia. NiV is highly pathogenic to a broad range of mammals and is considered to have pandemic potential due to its zoonotic as well as person to person transmission[2]. A recent outbreak in a new geographical area in Kerala, India is just the latest such event[4]. Research into this disease has been hampered by the relatively small number of cases as well as difficulties in diagnosis[3]. Research into epidemiology, modes of transmission and potential prevention and control strategies is needed urgently.

Nipah virus belongs to the family Paramyxoviridae, genus Henipavirus. It is an enveloped, negative-sense, single-stranded RNA virus. The NiV genome is approximately 18.2 kb in length and encodes six structural proteins: nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G), and large polymerase protein (L). The virus's envelope is derived from the host cell membrane and contains the F and G proteins, which are essential for viral entry into host cells. The N protein encapsulates the RNA genome, forming a helical ribonucleo capsid complex that associates with the P and L proteins to form the viral replication complex. The M protein plays a crucial role in virus assembly and budding. The F and G proteins facilitate

the initial attachment and fusion of the virus with the host cell membrane, initiating infection[4,5].

There had been various efforts to manage NiV infection by searching for small molecule therapeutics against various structural proteins[6]. The virus attachment glycoprotein (NiV-G) present on the virus surface is essential for the recognition of host cell-surface receptors ephrin-B2 (EFNB2) and ephrin-B3 (EFNB3) mediating cellular attachment, followed by some conformational changes that result in triggering of NiV fusion glycoprotein (NiV-F) that leads to membrane fusion and virus entry into host[7] .Hence molecular inhibition of NiV-G is one of the prime strategies for treating NiV infection. Also,as an effective strategy to interrupt virus adhesion with host, the inferences regarding inhibition of interactions between NiV-G and host receptors have been well established , which provide the structural basis for screening antiviral therapeutic inhibitors against NiV-G.

In the current scenario, there is a need to find lead compounds that result in anti-NiV drug development. The entire research conducted in this project makes use of a wide variety of computational methods such as molecular docking , protein-ligand molecular dynamics simulations, post-MD protein-ligand interaction studies[8,9], MM-PBSA binding free energy calculations[10], etc. for finding novel flavonoid and flavonoid derivatives inhibitors acting against specific targets of NiV. Due to the disease's complexity, interdisciplinary approaches are generally necessary to address the many components of drug discovery, including resistance, permeability, toxicity, drug efflux, compatibility with other viral therapies, etc. In these situations, computational techniques are highly feasible and adaptable for accelerating the drug development process and effectively tackling challenges at the atomic and molecular levels[12,15].

Here we focused on investigating the efficacy of bioactive compounds like flavonoids in inhibiting the NiV-G glycoprotein. Flavonoids are powerful antioxidants and they are found to be effective against diseases such as cancer, obesity, hypertension, and other diseases[16]. The literature studies also underline the effectiveness of flavonoids as potential antiviral compounds. In this work, fifty-three hundred (5300) flavonoid phytochemicals and the control drug, were tested for Nipah G protein inhibition potential

through an in silico molecular docking study. Further, drug-like properties of the selected phytochemicals were also evaluated. We combined statistical and pharmacophore approaches to identify functional descriptors responsible for protein–ligand interaction. Finally, the stability of the ligand–protein complex was studied using the molecular dynamic simulation technique[22].

Materials and methods

In this study, we sought to find the flavonoid derivative inhibitors against NiV viruses that attach to the crystal structure of NiV-G glycoprotein with PDB ID: 2VSM.The protein crystal structure is a complex of NiV-G glycoprotein with human EFNB2, the crystal structure as shown in Fig:1

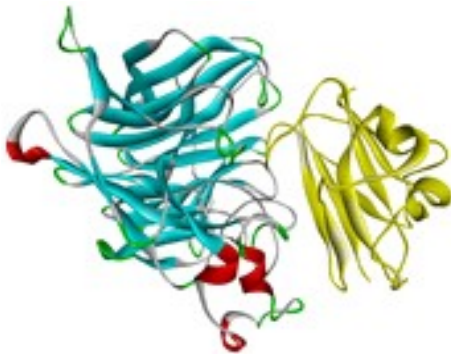


Fig 1. Crystal structure of 2VSM

Protein - Ligand Preparation and Molecular Docking

The initial preparation of the protein PDB structure was carried out by removing water, native inhibitors, complexed protein EFN B-2 and followed by the addition of missing hydrogens and charges. In the case of 2VSM crystal structure, the NAG (2-acetamido-2-deoxy-beta- D-glucopyranose) inhibitor is removed, which is present in binding site. On the addition of missing hydrogens, centroid of the site is located near the catalytic residues GLN559,GLU579 , TYR581 and ILE 588 in the crystal the structure was chosen as the docking site of the ligand. The 3D representations of proteins with binding sites of PDB ID : 2VSM is shown in figure 2 and its residue sequence is shown in the figure 3.

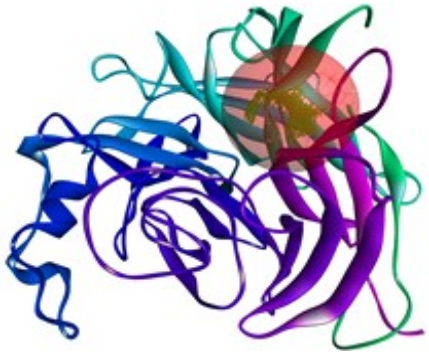


Fig 2. Crystal structure of 2VSM with binding site

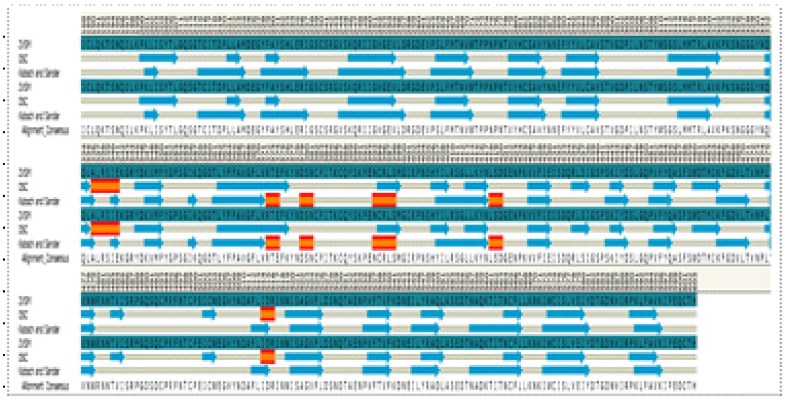


Fig 3. D Seaquence of 2VSM

The Cartesian coordinates of the centroid corresponding to the crystal structure of 2VSM was x = 16.09, y = 70.13 and z = -24.49. Grid box centred on these binding site coordinates is utilised for docking calculations. The grid box size for docking were fixed as 40 Å in x, y and z dimensions respectively and the number of grid points per map was 6400. The grid point spacing and exhaustiveness used in the current calculation is 1 Å and 8 respectively. After the each protein-ligand docking calculation, ten conformations (docking poses) and its corresponding docking score was obtained as output[30]. Out of the ten conformations, the best poses with higher binding energy (docking score) were selected for the further analysis.

Results and discussion

Molecular docking studies

The results of molecular docking of 5 selected flavonoid derivatives (shown in fig 5) to the binding sites of crystal structures of 2VSM is discussed in the following section. The docking scores (binding free energies) of the best docking poses is summarized in Table 1. The 3D representations of best docking poses of these 5 flavonoids in the crystal structures of NiV -2VSM were shown in figure respectively. The images of the docking poses interpret that like the native ligands all the selected flavonoids fit well into the active site of 2VSM crystal structures. As per the docking scoring function of AutoDock[18] the protein-ligand nonbonding interactions (van der Waals / hydrophobic & hydrogen bonding) plays a key role in the effective binding (docking) and contribute significantly to the docking score of a particular pose. The table 2 with 2D images of protein-ligand nonbonding interactions in these best docking poses of 2VSM are also presented here. The 3D docking poses with the interactions of 5 selected 5 ligands inside NiV-2VSM is shown in figure 5 respectively. The freeware Schrodinger Maestro visualizer was used to extract 2D/3D plots from these docking poses. The hydrogen bonding interactions, hydrophobic interactions and all other types of nonbonding interactions between protein and ligands were highlighted in these plot.

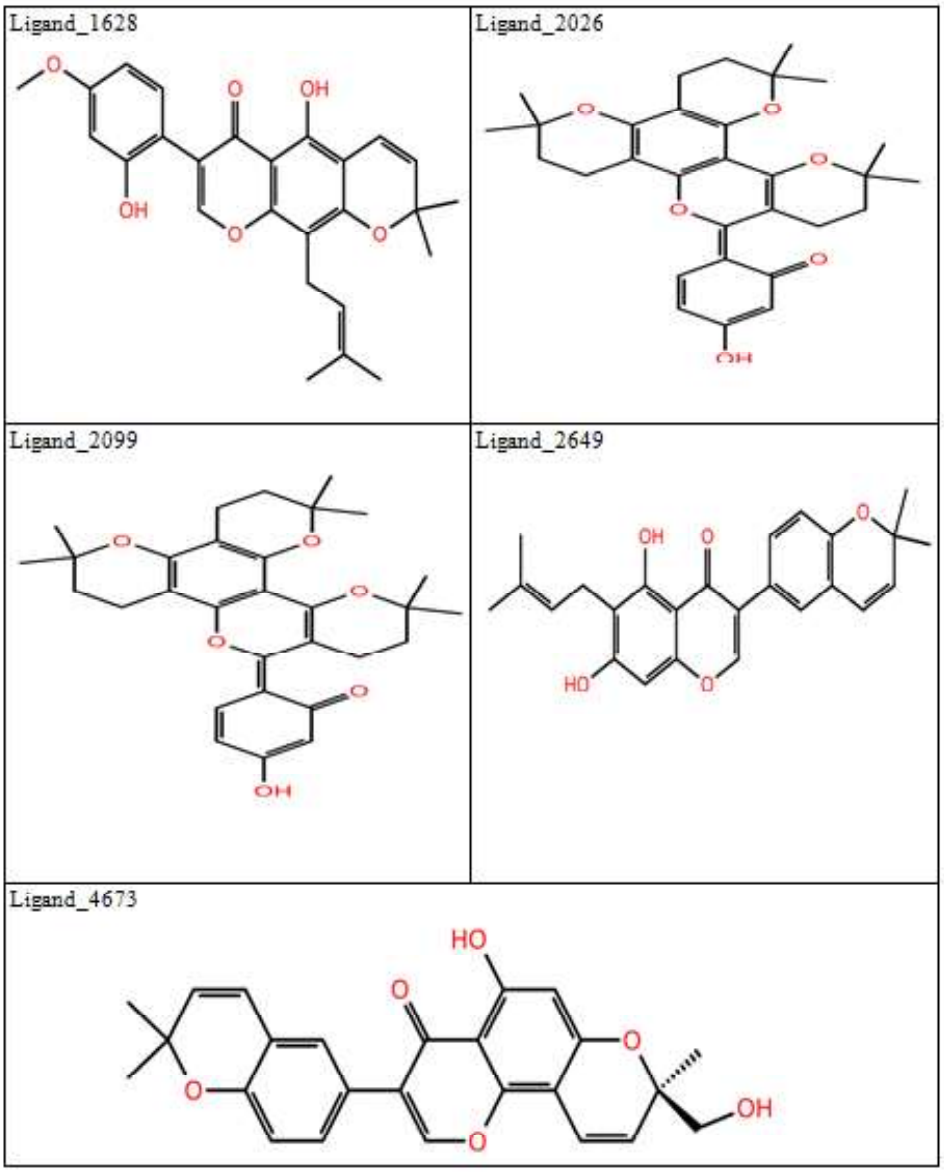


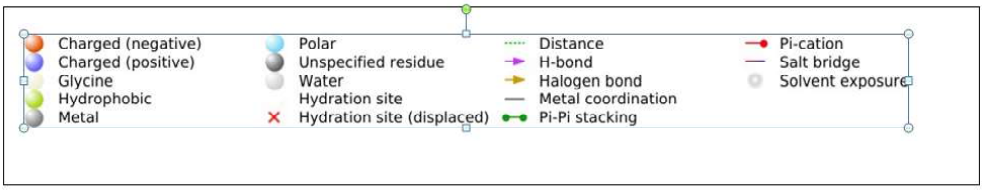
Fig 4. Structure of selected ligands

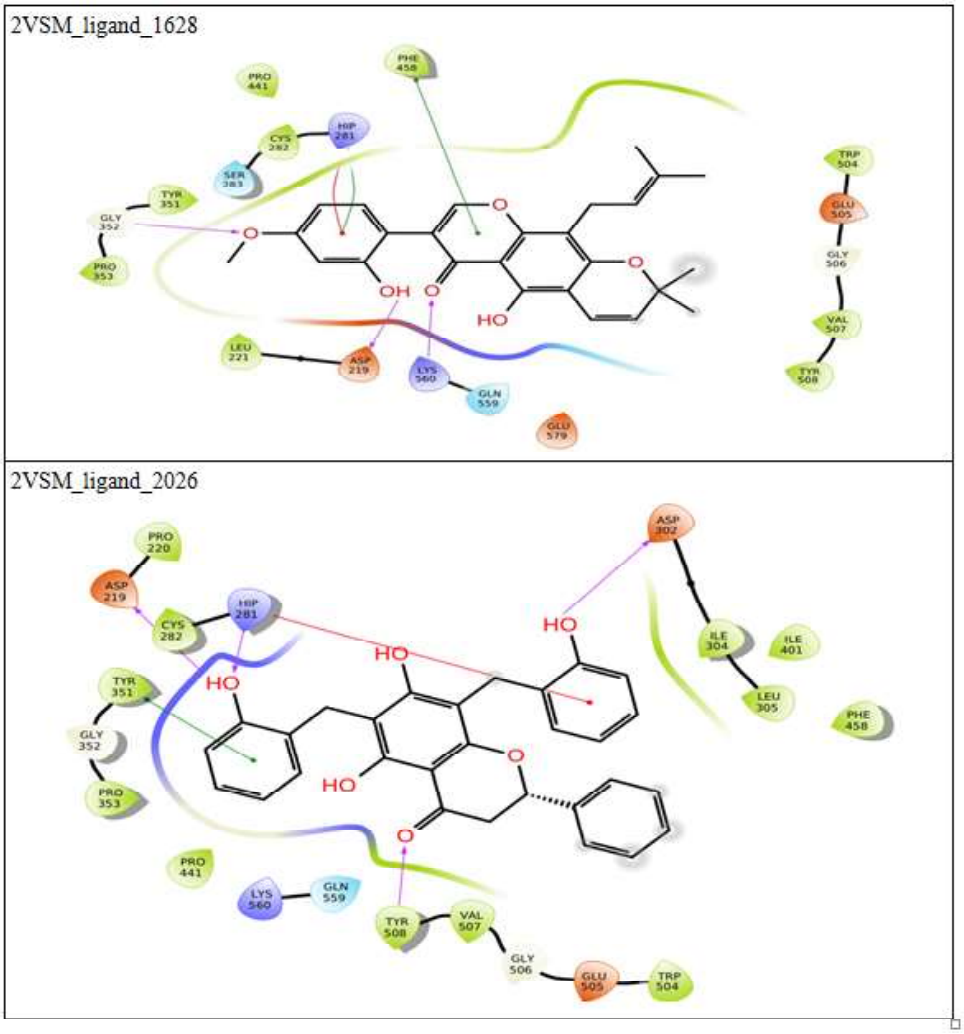
Fig 5. 3D Docked poses of selected ligands at the active site of 2VSM

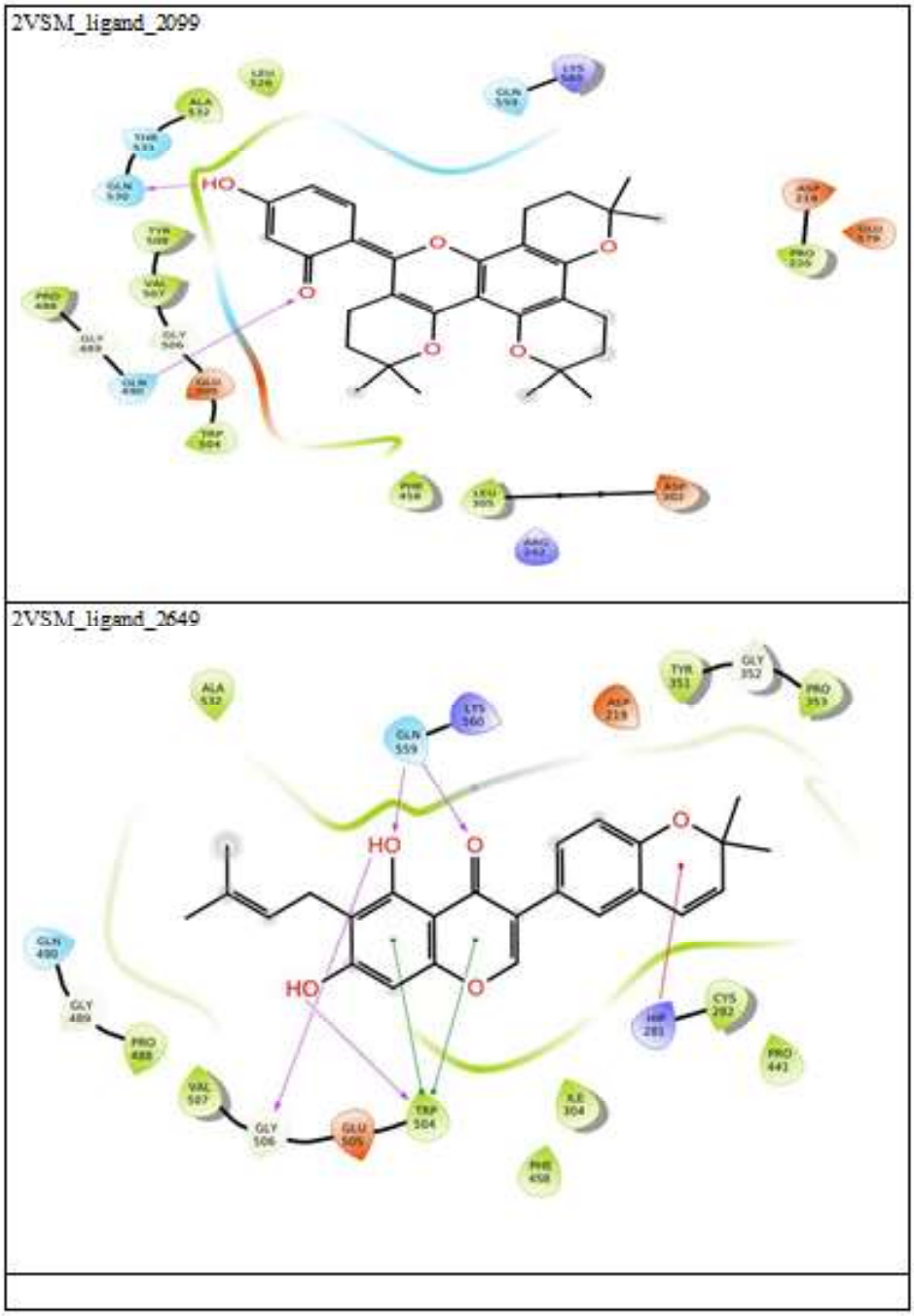
Ligands	*Docking Score (kcal/mol)*
Ligand_2026	-9.93
Ligand_2649	-9.66
Ligand_1628	-9.48
Ligand_4673	-9.31
Ligand_2099	-8.03

Table 1Docking scores of selected 8 flavonoids against 2VSM target

The higher negative binding energy values (docking scores) in all calculations is an indication of effective binding of these flavonoids to the active site of 2VSM crystal structures of NiV. The order of strength of binding of flavonoids to the 2VSM crystal structure is observed as *Ligand_2026* (*- 9.93*) > *Ligand_2649* (*-9.66*) > *Ligand_1628* (*-9.48*) >*Ligand_4673* (*-9.31*) > *Ligand_2099* (*-8.03*) On the analysis of the best docking poses (Table 1) all selected flavonoids has binding interactions with the amino acid residues GLN559,GLU579 along with other residues.




2VSM_ligand_1628
PHE 458
PRO 441
HIP 281
CYS 282
SER 283
TYR 351
GLY 352
PRO 353
TRP 504
GLU 505
GLY 506
VAL 507
TYR 508
OH
HO
LEU 221
ASP 219
LYS 560
GLN 559
GLU 579
2VSM_ligand_2026
PRO 220
ASP 219
HIP 281
CYS 282
ASP 302
ILE 304
ILE 401
LEU 305
PHE 458
TYR 351
GLY 352
PRO 353
PRO 441
LYS 560
GLN 559
TYR 508
VAL 507
GLY 506
GLU 505
TRP 504


2VSM_ligand_2099
2VSM_ligand_2649

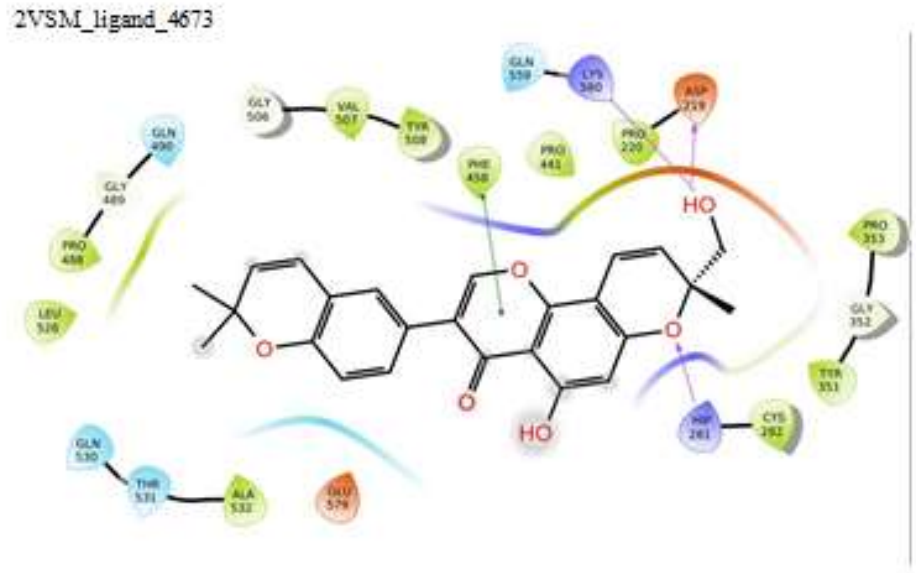


Table 2 : Two dimensional representation of interactions of five selected ligands with 2VSM

We further analysed the nonbonding interactions of each flavonoid with binding site residues of the studied targets. In the analysis of 2VSM-flavonoid docking poses, *Ligand-2026* showed prominent hydrophobic interactions (through van der Waals forces) with amino acid residues including GLN559 (the catalytic residue) including other residues .From the docking results it is confirmed that all selected 5 flavonoids possess bigger negative docking scores (>-8.0 kcal/mol) which is an indication of strong binding affinity of these flavonoids to active site of glycoprotein of NiV. The binding pockets of the flavonoids discussed here have active amino acid residues that reflected the binding sites of the previously reported NAG-2VSM complex. Therefore, these docking poses may be the best option for starting conformations in further molecular dynamics simulation studies.

Conclusion

There are no reported NiV-G inhibitors till date that has been taken into clinical development. From the virtual screening of the few naturally occurring flavonoid compounds using molecular docking calculations we selected 5 flavonoids which has

strong binding affinity and inhibitory activity against NiV-G of NiV. The bigger negative docking scores (>-8.0 kcal/mol) of selected flavonoids at active site NiV-G is an indication of its strong binding affinity and inhibitory activity by reducing its proteolytic activity. We found that the 5 selected flavonoids fit well into the active site domains of NiV-G through strong hydrophobic and hydrogen bonding interactions especially with GLN559 and GLU579 amino acid residues. Since these flavonoids are widely available or synthetically viable , nontoxic and dietary natural compounds with previous history of clinical use, these compounds later subjected to detailed investigations. With this intention, from the docking results, we generated the best docking poses of NiV-G-Flavonoid Complex structures for further MD simulations and post MD investigations. The results obtained from these computational calculations can be extended to experimental studies and the selected flavonoids may choose as lead compounds for the drug discovery of NiV diseases.

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