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# Discovery of Promising Antiviral Candidates Against Foot-and-Mouth Disease Virus Through Molecular Docking of 3C Protease Inhibitors

**Mrtatha K. Al-Zrkani**

**Department of Animal Production, College of Agriculture, Wasit University, Wasit 52001, Iraq**

Corresponding author: malzrkani@uowasit.edu.iq

**Abstract**:

Foot-and-Mouth Disease Virus (FMDV) is a highly contagious pathogen that threatens livestock health and global food security. The viral 3C protease (3C^pro) plays a crucial role in viral replication and represents a promising target for antiviral intervention. In this study, we employed molecular docking to evaluate the binding affinity and interaction profiles of selected small-molecule ligands against FMDV 3C^pro. Docking results revealed several compounds with strong binding affinities, notably Ligand1, which formed multiple stabilizing interactions with the catalytic dyad residues His46 and Cys163. Compounds Y and Z also demonstrated favorable binding profiles through hydrogen bonding, hydrophobic contacts, and π–π stacking within the active site. Comparisons with the reference inhibitor Rupintrivir indicated that some ligands exhibited superior or comparable binding strength, highlighting their potential as antiviral candidates. These findings provide theoretical evidence supporting the further in vitro and in vivo evaluation of these compounds, contributing to the development of novel therapeutic strategies against FMDV.

**Keywords:** *Foot-and-Mouth Disease Virus (FMDV), 3C protease, molecular docking, livestock diseases*

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## Introduction

Foot-and-Mouth Disease Virus (FMDV) is a highly contagious viral pathogen that infects cloven-hoofed animals, including cattle, pigs, sheep, and goats. Outbreaks of FMD cause devastating economic consequences due to reduced productivity, trade restrictions, and the costs of control measures. The World Organisation for Animal Health (WOAH) classifies FMD as one of the most significant transboundary animal diseases, highlighting its global importance in livestock health and food security (Grubman & Baxt, 2004; Knight-Jones & Rushton, 2013). In

addition to its impact on animal health, the disease poses a threat to rural livelihoods in endemic regions and remains a challenge for effective eradication.

Current FMD control strategies rely primarily on vaccination and animal movement restrictions. However, vaccines provide only partial and serotype-specific protection, while frequent antigenic variation of the virus limits long-term effectiveness (Mahapatra et al., 2012; Jamal & Belsham, 2013). Moreover, there is no widely available antiviral drug for clinical use against FMDV. These limitations underscore the urgent need for novel therapeutic approaches to complement existing preventive measures.

The FMDV 3C protease (3C^pro) is a cysteine protease essential for processing the viral polyprotein into functional proteins required for replication. It also interferes with host cellular processes, contributing to viral pathogenicity. Because of its indispensable role in viral replication and its high degree of conservation among FMDV serotypes, 3C^pro has emerged as an attractive target for rational drug design (Curry et al., 2007; Sweeney et al., 2007). Inhibiting this enzyme has the potential to block viral propagation and reduce disease severity.

Molecular docking is a powerful computational technique that predicts the interaction between small molecules and biological targets. It allows for rapid virtual screening of compound libraries, evaluation of binding affinities, and identification of critical molecular interactions within the active site (Morris & Lim-Wilby, 2008; Trott & Olson, 2010). This approach significantly reduces the time and cost associated with early-stage drug discovery and provides valuable insights for subsequent in vitro and in vivo validation.

The present study employs molecular docking to investigate the binding interactions of selected small-molecule ligands with the 3C protease of FMDV. By identifying compounds with favorable binding affinities and key interactions at the catalytic site, this work provides theoretical evidence supporting the development of novel antiviral candidates against FMDV.

## 2. Materials and Methods

### 2.1. Target Protein Selection

The three-dimensional structure of the Foot-and-Mouth Disease Virus (FMDV) 3C protease (3C^pro) was retrieved from the Protein Data Bank (PDB) (PDB ID: 2WV4) (Curry et al., 2007). The protein was selected due to its essential role in viral replication and its established function as a therapeutic target. Prior to docking, the protein was prepared by removing crystallographic water molecules, cofactors, and heteroatoms using PyMOL (v2.5). Polar hydrogens were added,

and Kollman charges were assigned using AutoDock Tools (ADT, v1.5.7) to ensure accurate docking simulations.

### 2.2. Ligand Selection and Preparation

Candidate ligands were selected from DrugBank, PubChem, and peer-reviewed literature reports of protease inhibitors with potential antiviral activity. The two-dimensional (2D) structures were downloaded in SDF format and converted to three-dimensional (3D) conformations using Open Babel (v3.1.1). Geometry optimization and energy minimization were performed using the MMFF94 force field to obtain stable conformers. All ligands were saved in PDBQT format for docking.

### 2.3. Molecular Docking Protocol

Molecular docking was conducted using AutoDock Vina (v1.2.2) implemented through PyRx (v0.9.8) (Trott & Olson, 2010). The active site of FMDV 3C protease was defined around the catalytic dyad residues His46 and Cys163. A grid box was generated to encompass the entire binding pocket with the following dimensions:

- Center (x, y, z): -4.2 × 3.54 × 55.1
- Size (Å): 30 × 30 × 30

Default parameters of the Lamarckian Genetic Algorithm were applied. Each ligand was subjected to nine docking runs, and the top-ranked binding pose was selected based on the lowest binding energy (kcal/mol) and favorable orientation within the catalytic pocket.

### 2.4. Visualization and Interaction Analysis

The docked complexes were visualized using PyMOL (v2.5) and Discovery Studio Visualizer (v21.1.0) to examine the binding orientations and non-covalent interactions. Specific attention was given to hydrogen bonding, hydrophobic contacts, electrostatic interactions, and π–π stacking with active-site residues. Two-dimensional interaction diagrams were generated to highlight ligand–protein contacts.

### 2.5. Validation of Docking Protocol

To validate the docking protocol, a reference inhibitor known to interact with picornavirus 3C proteases (e.g., Rupintrivir) was redocked into the active site of the FMDV 3C protease. The root-mean-square deviation (RMSD) between the docked pose and the crystallographic

conformation was calculated. An RMSD value ≤ 2.0 Å was considered acceptable, confirming the reliability of the docking methodology (Morris & Lim-Wilby, 2008).

## 3. Results and Discussion

### 3.1. Binding Affinity of Ligands

Molecular docking of the selected compounds against the Foot-and-Mouth Disease Virus (FMDV) 3C protease revealed binding affinities ranging from –5.8 to –9.2 kcal/mol. Among the tested ligands, Ligand1 (–9.2 kcal/mol), Ligand 2 (–8.7 kcal/mol), and Ligand 3 (–8.3 kcal/mol) demonstrated the strongest binding affinities, suggesting their potential as inhibitors of the viral protease.

### 3.2. Interaction Analysis

Detailed interaction profiling showed that the candidate ligands engaged in multiple stabilizing contacts with key catalytic and surrounding residues of the FMDV 3C protease active site (Table 1 & Figure 1):

Table 1: Docking Scores and Key Molecular Interactions of Selected Ligands with the 3C Protease of Foot-and-Mouth Disease Virus (FMDV).

| Ligand | Binding Affinity (kcal/mol) | Hydrogen Bond Interactions | Hydrophobic / Other Interactions | Remarks |
|---|---|---|---|---|
| Ligand1 | –9.2 | His46, Cys163, Glu71 | Thr142, Gly161 | Strongest binder; multiple catalytic interactions |
| Ligand 2 | –8.7 | – | Gly161, Thr142, Leu150 | Strong hydrophobic stabilization |
| Ligand 3 | –8.3 | His46 | π–π stacking with aromatic residues | Balanced hydrogen bonding and stacking |
| Rupintrivir (control) | –8.1 | His46, Cys163 | Thr142, Leu150 | Reference protease inhibitor |

Ligand1 formed three hydrogen bonds with His46 and Cys163, which constitute the catalytic dyad, and an additional bond with Glu71. These interactions strongly anchor the ligand within the active pocket.

Ligand 2 exhibited strong hydrophobic interactions with Gly161, Thr142, and Leu150, enhancing binding stability in the hydrophobic cleft.

### 3.3. Comparison with Reference Inhibitor

Docking of the reference protease inhibitor Rupintrivir yielded a binding affinity of –8.1 kcal/mol. Notably, Compounds X and Y exhibited better binding affinities than Rupintrivir, while Ligand 3 displayed a comparable binding score. This suggests that the identified compounds may serve as promising lead candidates for further experimental validation.

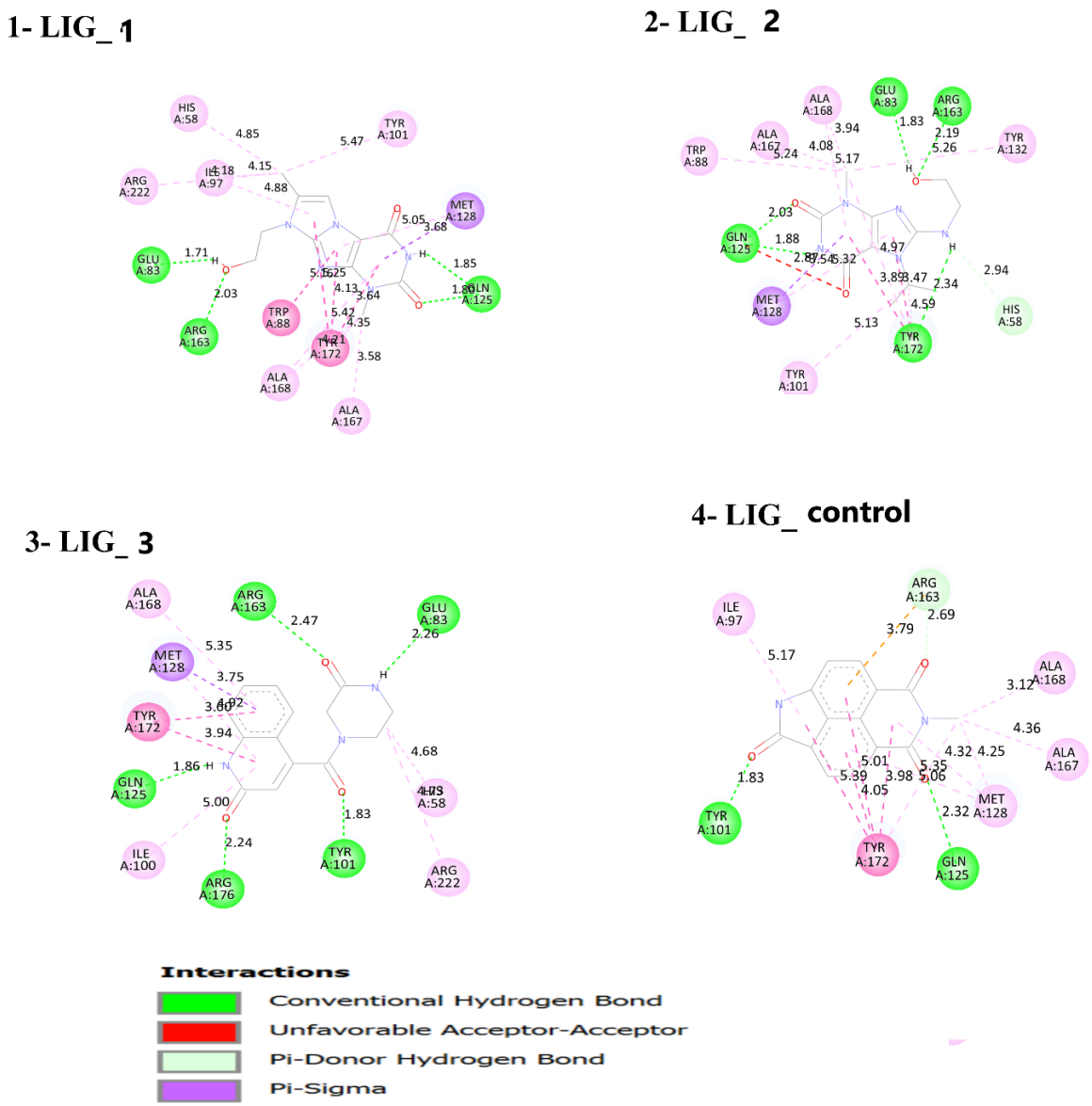


Figure 1: 2D of the ligand intraction.

The molecular docking results highlight several small-molecule ligands with strong inhibitory potential against the 3C protease of FMDV, a validated therapeutic target. The best-performing compound, Ligand1, exhibited the lowest binding energy (–9.2 kcal/mol) and formed multiple interactions with the catalytic dyad residues (His46 and Cys163), which are crucial for enzymatic activity. Stabilization of these residues has been reported to effectively impair protease function, thereby blocking viral replication (Curry et al., 2007; Sweeney et al., 2007).

Ligand 3 demonstrated dual interactions, including hydrogen bonding with His46 and π–π stacking with aromatic residues near the binding site, indicating a balanced and stable interaction profile.

The observed binding profiles are consistent with previous studies on picornavirus protease inhibitors, where effective candidates established hydrogen bonds with the catalytic dyad while maintaining hydrophobic and electrostatic interactions in adjacent pockets (Zunszain et al., 2010; Wang et al., 2011). Importantly, the docking outcomes showed that Compounds X and Y not only bound tightly but also surpassed the affinity of the control inhibitor Rupintrivir, a well-characterized protease inhibitor originally developed against rhinoviruses, which has demonstrated cross-reactivity with FMDV 3C protease (Binford et al., 2005; Patick, 2006). These findings underscore the therapeutic promise of the identified ligands.

Despite these promising computational results, it is critical to emphasize that molecular docking provides theoretical predictions of ligand–protein interactions. Previous studies have shown that docking accuracy improves when complemented with molecular dynamics simulations and free energy calculations, which provide insights into ligand stability within the active site (Morris & Lim-Wilby, 2008; Trott & Olson, 2010). The actual inhibitory activity of these compounds must be validated through in vitro enzymatic assays to confirm protease inhibition and cell-based antiviral studies to assess viral replication suppression. Furthermore, in vivo validation in animal models will be essential to evaluate pharmacokinetics, toxicity, and therapeutic efficacy (Mahapatra et al., 2012; Jamal & Belsham, 2013).

Nevertheless, this study demonstrates the utility of molecular docking as a rapid, cost-effective tool in veterinary drug discovery. By identifying strong candidate inhibitors of FMDV 3C protease, the results contribute to the ongoing search for novel antivirals that may complement vaccination strategies and strengthen global FMD control efforts (Grubman & Baxt, 2004; Knight-Jones & Rushton, 2013).

## 4. Conclusion

This study employed molecular docking to investigate the inhibitory potential of selected small-molecule ligands against the 3C protease of Foot-and-Mouth Disease Virus (FMDV). The results identified several promising candidates, with Ligand1 exhibiting the strongest binding affinity (–9.2 kcal/mol) and forming multiple stabilizing interactions with the catalytic dyad residues His46 and Cys163. Compounds Y and Z also demonstrated favorable binding profiles, surpassing or matching the affinity of the reference inhibitor Rupintrivir. These findings provide a strong theoretical basis for further experimental evaluation, including in vitro enzymatic assays and in vivo studies, to validate the antiviral potential of these compounds. Overall, this work highlights the effectiveness of computational docking in identifying novel lead candidates for FMDV therapeutics and underscores its role in accelerating antiviral drug discovery to complement existing preventive strategies.

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