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Structured based Drug Repurposing for Porcine Delta and Epidemic Diarrhea virus

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INTRODUCTION & AIM

Emerging and re-emerging viral infections in livestock remain a pressing concern for global food security, animal welfare and zoonotic preparedness. Porcine Delta Coronavirus (PDCoV) and Porcine Epidemic Diarrhoea virus (PEDV) are emerging porcine enteric coronaviruses, with high mortality (¬80%) and morbidity (100%), with no current licensed antiviral treatment. Vaccination, which poses the most effective available treatment, is challenged by the known rapid evolution and antigenic drift of coronaviruses. The economic and animal welfare impacts have escalated over the last decade due to the rapid spread of diseases, underscoring the need for innovative antiviral approaches that align with One Health principles. 3C-like main protease (3CLpro), exhibits high structural homology and stability across coronaviruses such as SARS-CoV-2 and FIP, PDCoV and PEDV. Inhibition of this protease has been shown to block virus replication across different species. Previous studies have explored coronavirus protease inhibition in human and companion-animal models, identifying promising scaffolds such as GC376, nirmatrelvir, and α-ketoamide derivatives. Saquinavir, a proven inhibitor of HIV, was effectively repurposed for human and animal coronaviruses, including SARS-COV-2 and FIP.

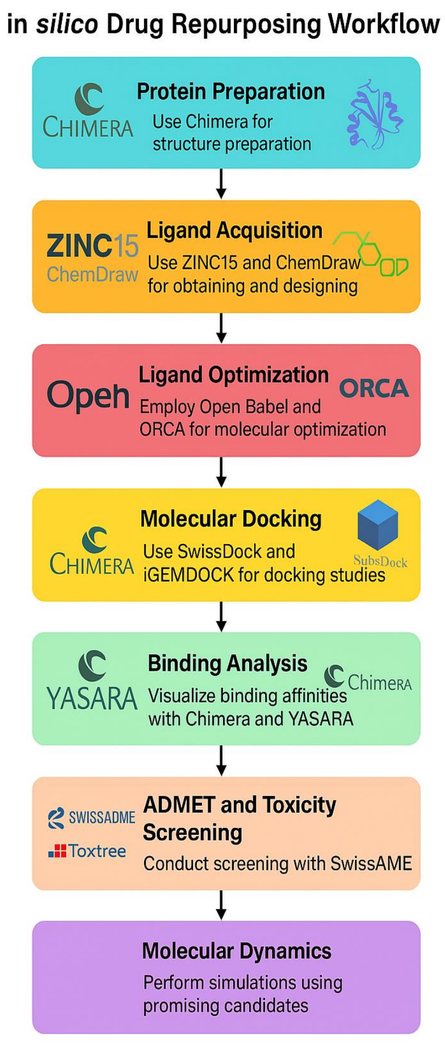
The One Health paradigm provides the conceptual framework for this study by linking animal disease control with broader public health resilience. Drug repurposing screening across a commercially available drug bank offers an efficient and low-cost alternative for treatment discovery. The similarity and conservation of the 3CLpro protease between the two coronaviruses enable shared molecular analysis to identify potential inhibitors that target both viruses. For this purpose, a validated computational approach to PEDV's and PEDCoV's 3CL-like protein, combining ligand-based similarity searches, molecular docking, molecular dynamics, and pharmacokinetic prediction, was applied based on our previous experience with SARS-CoV-2 and FIP in machine-learning-assisted docking and molecular docking. This integrated approach supports a reproducible *in silico* and *in vivo* pipeline for veterinary antiviral discovery.

The purpose of this study is to screen and identify potential inhibitors among commercially available drugs for 3CLpro of PEDCoV and PEDV, evaluate their binding affinity and assess their ADME and toxicity profiles within the One Health context. Among the screened compounds, saquinavir emerged as a promising lead and warrants further experimental validation.

METHOD

***In silico* methods:**

- Computational work was conducted in workstations equipped with Intel® Core™ i9-12900K processors (32 GB RAM) and dual NVIDIA® RTX 3060 GPUs.
- Operating system; Windows 11 Pro (64-bit) verified by recoding version numbers, database release dates, and stimulation parameters.
- PDCoV and PEDV main proteases crystal structures, 8E7C and 4XFQ respectively, were retrieved via RCSB Protein Data Bank (https://www.resb.org).
- Water molecules and co-crystallized ligands were removed before energy minimization
- SwissSimilarity 2023.1 (SIB Swiss Institute of Bioinformatics) was used for ligand-based screening
- Zinc Database (2023 updated version) and DrugBank v5.1.11 were used to identify approved or investigational compounds structural similar to native inhibitors
- SMILES were retrieved from PubChem (accessed March 2025) and converted to 3D PDBQT format using Open Babel v3.1.1
- FDA approved molecules were retained
- Docking studies were performed with AutoDock Vina 1.2.0 [3] integrated in PyRx 0.9.8
- For validation, the co-crystallized inhibitor GC376 from PDB 4XFQ was re-docked into its native pocket, yielding a re-docking RMSD = 1.84 Å, confirming protocol reliability
- The docking grid was centered at the catalytic dyad (Cys-His) and extended 25 × 25 × 25 Å. Exhaustiveness = 16, energy range = 4 kcal/mol, Exhaustiveness = 16, energy range = 4 kcal/mol.
- Binding energies below –7 kcal/mol were considered significant; the top 20 ligands were shortlisted for further evaluation based on (i) docking energy, (ii) hydrogen-bonding and hydrophobic contacts with conserved residues, and (iii) pharmacological precedent
- The top three candidates (saquinavir, eravacycline, irinotecan) with PEDV and PDCoV 3CLpro were subjected to 100-ns MD simulations using YASARA Structure 21.12.24, which integrates the AMBER14 force field for both protein and ligand.
- Solvation was achieved in a cubic water box with a 10 Å buffer, TIP3P water model, 0.15 M NaCl, pH 7.4
- Periodic boundary conditions and particle-mesh Ewald electrostatics were applied.
- Temperature was maintained at 298 K with a 2 fs timestep using Berendsen thermostat coupling.
- The root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen-bond count were monitored throughout [28-30].
- The pharmacokinetic and toxicity properties of the top-ranked ligands were assessed via SwissADME (2024 release) and Endocrine Disruptome v2.0.
- Parameters analyzed included molecular weight, topological polar surface area (TPSA), gastrointestinal (GI) absorption, blood-brain-barrier (BBB) permeability, cytochrome P450 inhibition profiles, and potential nuclear-receptor binding.



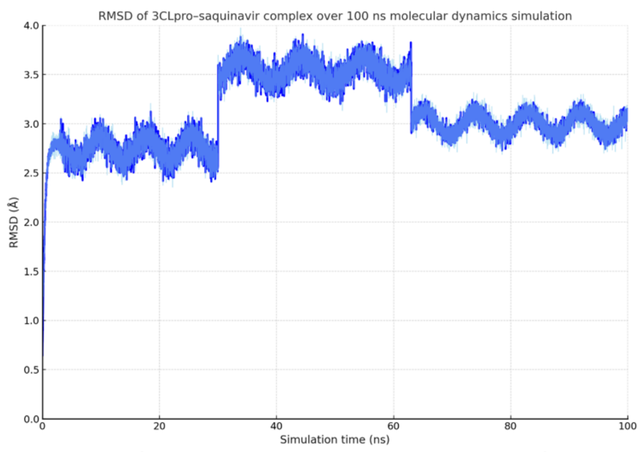
RESULTS & DISCUSSION

Molecular docking:

- From the 20 screened FDA-approved compounds, saquinavir, eravacycline and irinotecan were selected as the top three ligands interacting with 3CLpro.
- Docking scores for PEDV ranged from -9.8 and 10.7 kcal/mol and for PEDCoV ranged between -8.9 to 9.3 kcal/mol.

Target Protein	Inhibitor	Binding Affinity (Kcal/mol)	Amino Acid Residue
Porcine epidemic diarrhea 3C-like protease	Saquinavir	-126.2	GLN276 H/S, LEU283 H/M, ASP285 H/S, GLN276 V/S, GLY279 V/M, ARG136 V/S, TYR280 V/S, THR281 V/M, THR281 V/S, THR284 V/M, THR284 V/S.
Porcine Deltacoronavirus (HKU-15) Mpro	Saquinavir	-107.2	SER25 H/S, ALA26 H/M, LYS45 H/S, GLY142 H/M, SER25 V/M, ALA26 V/M, LEU27 V/M, HIS41 V/S, GLY44 V/S, LYS45 V/S, ASN141 V/M, ASN141 V/S, GLY142 V/M.

- Despite eravacycline's and irinotecan's higher negative docking scores, saquinavir was selected based on a multi-criterion approach:
  - Viral antiviral mechanisms.
  - Optimal hydrogen-bonding geometry within the catalytic dyad (Cys144-His41).
  - Hydrophobic contact with residues critical for substrate recognition.
  - Superior complex stability during MD stimulation.
- The 100 ns MD simulations of the PEDV and PDCoV 3CLpro confirmed stable binding.
- The RMSD plots showed rapid equilibration within the first five ns, maintaining mean values of 2.6–2.9 Å thereafter, indicative of equilibrium stability.



RMSF analysis showed that loop residues 240–260 displayed elevated flexibility (>2.5 Å), whereas the catalytic domain remained rigid (<1.5 Å), indicating preservation of the protease's structural core during ligand binding. Hydrogen-bond analysis further demonstrated the presence of 3–5 persistent interactions between saquinavir and key catalytic-pocket residues throughout the MD trajectory, supporting sustained binding stability.

The radius of gyration decreased from 5.7 Å to 5.2 Å by 20 ns, reflecting compaction of the enzyme–ligand complex, while SASA values stabilised near 155 nm², consistent with a folded, solvent-shielded conformation. Together, these parameters confirm that saquinavir maintains stable interactions without inducing significant structural distortion of 3CLpro.

ADMET predictions indicated low gastrointestinal absorption and no blood–brain barrier penetration, although the compound exhibited acceptable lipophilicity (logP ≈ 2.9) and no PAINS or Brenk structural alerts. Its classification as a P-glycoprotein substrate and CYP3A4 inhibitor is consistent with its well-characterised pharmacokinetic profile in humans and represents a potential limitation for veterinary use.

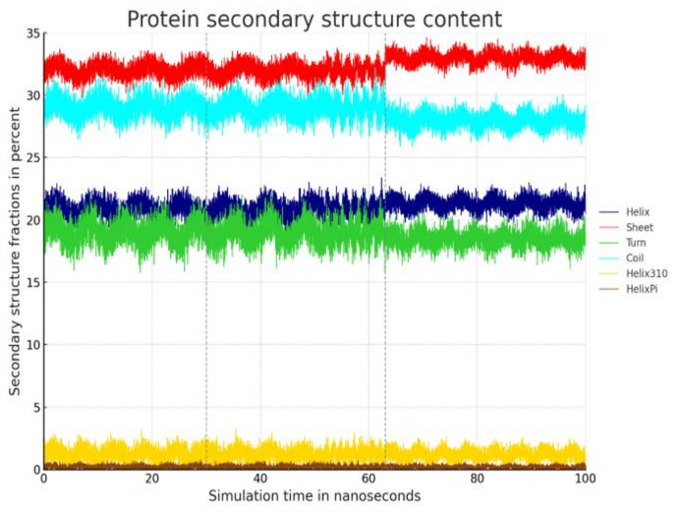
Nevertheless, its polar surface area and molecular weight are within 20% of those of clinically validated coronavirus protease inhibitors (e.g., GC376), suggesting it is feasible to optimise for veterinary applications.

Notably, the 3CLpro active site is highly conserved across coronaviruses and lacks close homologues in mammalian hosts, minimising the risk of off-target protease inhibition. No shared catalytic dyad motifs were detected between 3CLpro and porcine cathepsins or caspases.

However, amino-acid substitutions within the S1/S2 sub-sites may influence affinity, and expanded in silico screening of viral variants would enhance predictive robustness. Overall, this study highlights the value of an integrated computational–toxicological workflow, combining docking, molecular dynamics, and ADMET profiling, to accelerate the discovery of veterinary antivirals.

The observed complex stability and favourable energetics position saquinavir as a promising cross-species antiviral prototype, bridging human and veterinary drug development.

These findings further support the One Health paradigm by demonstrating how computational drug repurposing can rapidly generate mechanistic insights for animal diseases with zoonotic or pandemic potential and can be adapted to other veterinary coronaviruses or RNA viruses with conserved protease architectures.



CONCLUSION

- The present study establishes a validated computational-toxicology workflow for identifying antiviral candidates against PEDV and PDCoV, leveraging in silico drug repurposing.
- Focusing on the conserved 3C-like proteases (3CLpro) of both viruses, molecular docking, 100-ns MD simulations, and ADMET profiling identified saquinavir as a promising lead candidate.
- Despite moderate violations of traditional drug-likeness rules, saquinavir displayed stable enzyme–ligand interactions, persistent hydrogen bonding, and a pharmacological mechanism already proven in viral protease inhibition.
- Its clinical safety record and structural similarity to veterinary antiviral scaffolds make it an attractive candidate for repurposing.
- The study also proposes practical mitigation strategies for pharmacokinetic limitations in swine, including nanoformulation, depot injection, and prodrug design, which can enhance bioavailability and therapeutic duration.

FUTURE WORK / REFERENCES

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