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[Manos C. Vlasiou](#) *

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Article

A One Health Computational Framework for Identifying PA Endonuclease Inhibitors Against Contemporary H5N1 Avian Influenza

Manos C. Vlasίου

Department of Veterinary Medicine, University of Nicosia School of Veterinary Medicine, 2414, Nicosia, Cyprus; vlasίου.m@unic.ac.cy

Highlights

- A One Health computational pipeline was created to identify priority PA endonuclease inhibitors for current H5N1 clade 2.3.4.4b variants.
- Cross-host structural modelling showed that the architecture of the catalytic pocket is conserved between PA endonuclease variants associated with poultry and mammals.
- Docking and 170-ns molecular dynamics simulations revealed entecavir as a promising and stable PA endonuclease inhibitor candidate.
- Physicochemical suitability filtering identified antiviral scaffolds suitable for potential deployment strategies in poultry environments.

Abstract

Highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b continues to circulate extensively among wild birds, poultry, and mammals, presenting ongoing risks at the intersection of human, animal, and environmental health. Antiviral approaches tailored for poultry farming or farm settings are still largely under investigation. The influenza A polymerase acidic (PA) endonuclease, which plays a key role in cap-snatching during viral transcription, is a conserved antiviral target across different host species. This research introduces a computational workflow to detect PA endonuclease inhibitors suitable for veterinary and environmental use. Homology modelling of recent H5N1 clade 2.3.4.4b PA sequences (2021–2025) was performed based on the crystallographic structure 6FS8. A curated ligand library, including reference inhibitors, hydrophilic metal-binding scaffolds, and repurposed antivirals, was screened via cross-host molecular docking against poultry- and mammalian-specific PA models. Docking results highlighted baloxavir and entecavir as top candidates due to their strong and consistent binding profiles across multiple targets. Entecavir showed particularly promising binding affinity in the poultry PA model (−100.6), similar to the reference inhibitor baloxavir (−97.5 to −97.7). A 170 ns molecular dynamics simulation of the poultry PA–entecavir complex indicated stable structural behavior, with RMSD below 1.1 Å, and MM/PBSA calculations gave a binding free energy of $\Delta G = -85.1 \pm 0.8$ kJ/mol. Physicochemical analysis revealed entecavir's high polarity and predicted water solubility, suggesting its suitability for water-based or environmental delivery within poultry facilities. Overall, this study introduces a One Health–focused computational framework that integrates cross-host structural modeling, docking, molecular dynamics, and agrochemical suitability filtering to identify promising antiviral candidates for poultry outbreak control. The results point to entecavir as a promising candidate for further evaluation in veterinary antiviral studies.

Keywords: H5N1; Avian Influenza; antiviral approaches

1. Introduction

Highly pathogenic avian influenza (HPAI) H5N1 remains one of the most consequential viral threats at the animal–human interface [1–3]. Since 2021, the expansion of clade 2.3.4.4b across Europe, Asia, Africa, and the Americas has led to unprecedented levels of viral persistence in wild birds and repeated incursions into commercial poultry flocks [4,5]. The same period has seen an increase in spillover events into mammals, including farmed mink, domestic cats, marine mammals, and, most recently, dairy cattle, raising renewed concerns for zoonotic potential and pandemic preparedness [6–8]. Although vaccination strategies are expanding, effective antiviral countermeasures for poultry do not exist, and environmental contamination in barns and processing facilities remains a major driver of transmission during outbreaks [9–11].

The PA endonuclease, a component of the influenza polymerase complex, is essential for viral transcription by mediating cap-snatching [12–14]. Because this catalytic domain is structurally conserved across avian and mammalian isolates, it has emerged as a high-value antiviral target [15–17]. Baloxavir marboxil, a PA endonuclease inhibitor approved for human use, demonstrates the tractability of the target but also highlights limitations: reduced efficacy against specific variants, potential for resistance, and, importantly, unsuitability for use in poultry due to regulatory, residue, and pharmacokinetic constraints [18,19]. As a result, there is significant interest in identifying novel small molecules that inhibit the PA endonuclease but may exhibit (i) greater chemical simplicity, (ii) improved solubility for water-based delivery, (iii) reduced persistence in animal tissues, or (iv) compatibility with environmental or surface applications in poultry production systems [20–22]. Recent computational studies have evaluated potential PA endonuclease inhibitors using docking and molecular dynamics simulations [23–25]. However, existing work has three significant gaps:

1. Most studies focus on older H5N1 or H1N1 isolates, not the contemporary clade 2.3.4.4b lineage responsible for the current global spread [26,27].
2. To the best of our knowledge, no study incorporates cross-host structural comparisons (poultry vs mammalian variants) to ensure antiviral candidates are robust across One Health transmission interfaces.
3. Critically, no computational pipeline has evaluated antiviral candidates in the context of poultry/agrochemical feasibility, including solubility, environmental safety, residue risk, and suitability for in-barn, water, aerosol, or surface delivery systems.

These unaddressed gaps limit the translational relevance of existing computational research. In a real outbreak, an antiviral compound must be biophysically potent but also chemically appropriate for field deployment in poultry operations, whether as a therapeutic adjunct, an environmental antiviral in barns and equipment, or as a tool to reduce viral shedding and thereby lower occupational exposure risk for workers [28–31].

In this study, we introduce a novel, integrative computational workflow designed explicitly for a One Health antiviral strategy. We (i) model the PA endonuclease from contemporary poultry-associated and mammalian-associated H5N1 2.3.4.4b variants, (ii) screen a library of repurposed antivirals and hydrophilic, low-residue scaffolds using metal-aware docking, (iii) evaluate stability using molecular dynamics and MM/GBSA free-energy calculations, (iv) assess resistance robustness, and (v) apply a poultry environment suitability filter incorporating solubility, toxicity, and predicted environmental behaviour. Finally, we classify candidate inhibitors by translational use potential: poultry-directed antivirals, cross-host inhibitors, worker-protection candidates, and environmental antiviral agents (Figure 1).

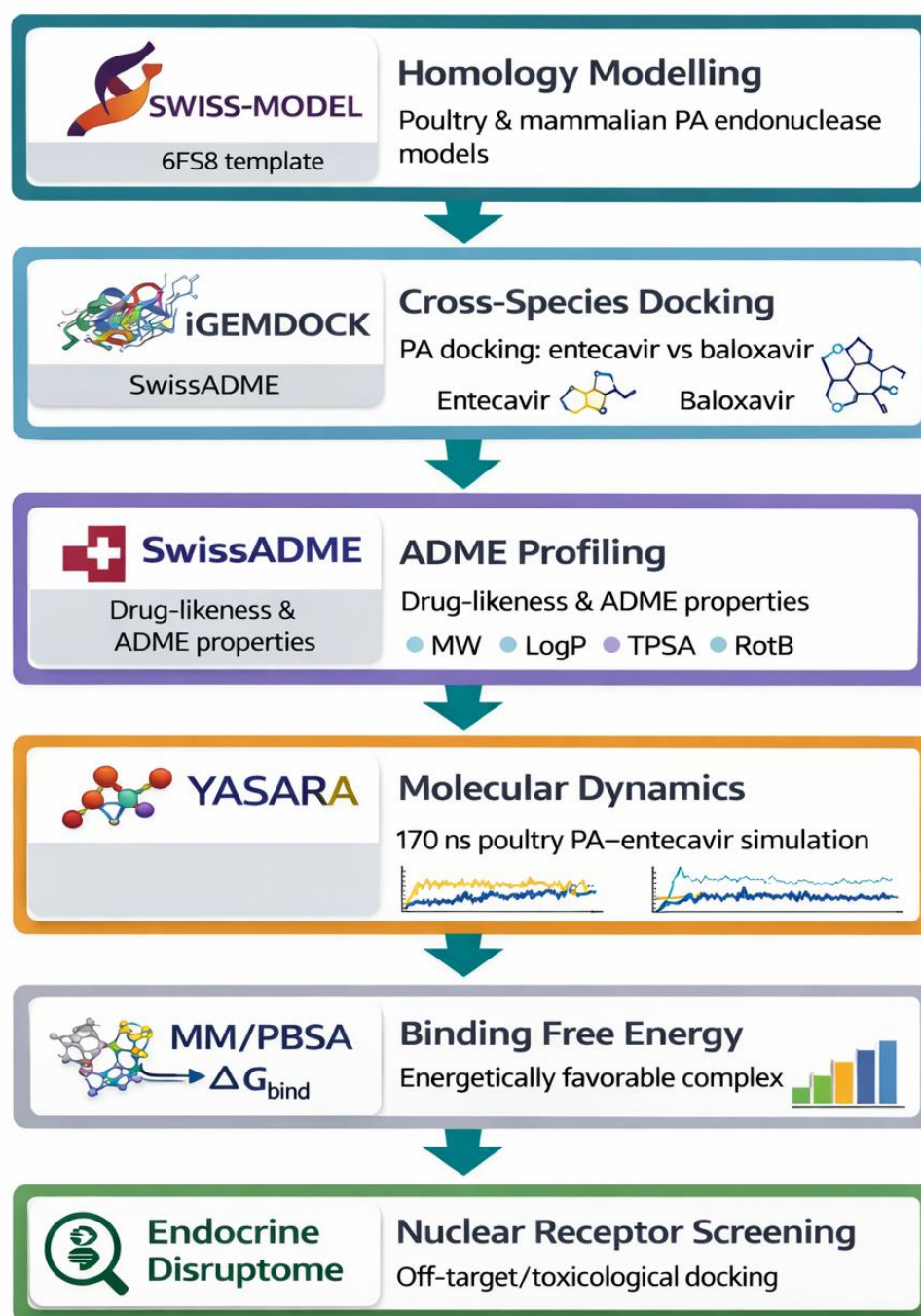


Figure 1. Overview of the computational pipeline used to evaluate entecavir versus baloxavir against influenza A PA endonuclease. The workflow integrates template-based homology modelling (SWISS-MODEL; template 6FS8), cross-species docking (iGEMDOCK), ADME profiling (SwissADME), molecular dynamics simulation (YASARA; 170 ns for poultry PA-entecavir), MM/PBSA binding free energy estimation, and nuclear receptor screening (Endocrine Disruptome).

Together, these components form a computational and translational pipeline aimed at identifying antiviral compounds aligned with the practical realities of controlling H5N1 across poultry, environment, and human exposure pathways. This approach supports both veterinary public health and pandemic preparedness by targeting a conserved viral function while grounding compound selection in real-world field constraints.

2. Methods

2.1. PA Sequence Retrieval and Alignment

PA sequences from H5N1 clade 2.3.4.4b (2021–2025) were retrieved from GenBank/GISAID. Sequences representing wild birds, poultry, and mammalian spillover hosts were aligned (MAFFT v7). Variants with complete coding regions and no ambiguous nucleotides were selected. Representative poultry and mammalian PA variants were chosen for homology modelling.

2.2. Structural Template Identification

High-resolution PA endonuclease structures were screened in the Protein Data Bank. PDB 6FS8 (1.9 Å, inhibitor-bound, with intact Mn²⁺ ions) was selected as the primary template due to structural completeness and well-resolved catalytic geometry. Additional structures (4E5G, 5E6X, 3EBE, 3HW6) were used as secondary references to cross-validate the alignment and active-site geometry.

2.3. Homology Modelling

SWISSMODEL and MODELLER were used to generate models of poultry- and mammalian-associated PA endonuclease (residues 1–193). Models were ranked using GMQE, QMEAN, MolProbity, and catalytic-site inspection. Only models retaining correct metal coordination and without steric clashes were accepted for docking [32–34].

2.4. Active-Site Definition and Protein Preparation

The catalytic site was defined using conserved PA residues (H41, E80, D108, E119) and supporting residues (K34, T20, W88, R124, T123, K134). A 10–12 Å radius around metal ions defined the docking search space. All structures were prepared using Auto Dock Tools. Protonation states were assigned at pH 7.4. Metal ions were retained. Crystallographic waters were removed unless contributing bridging interactions. Minimization was performed to remove clashes.

2.5. Ligand Library Preparation

Three ligand sets were examined: 1. Reference PA inhibitors. 2. Hydrophilic, poultry/environment-compatible chemotypes. 3. FDA-approved antivirals or chelators. Ligands were protonated at pH 7.4, minimized via MMFF94, and converted into 3D conformers (Table 1 a, b, c).

Table 1. (a) Ligand library used for initial screening against H5N1 PA endonuclease Group A – Reference PA endonuclease inhibitors (protocol validation, n = 10); (b) Ligand library used for initial screening against H5N1 PA endonuclease Group B– Hydrophilic, metal-binding “poultry-friendly” candidates (n = 20); (c) Ligand library used for initial screening against H5N1 PA endonuclease Group C – Environmental / food-system comparators (One Health context, n = 10).

(a)			
ID	Compound name	Chemical class / role	Rationale
A1	Baloxavir (baloxavir acid)	Approved PA endonuclease inhibitor	Gold-standard positive control
A2	Baloxavir marboxil	Prodrug of baloxavir	Comparator; shows prodrug vs active
A3	L-742,001	Experimental PA inhibitor	Widely cited research inhibitor
A4	2,4-Dioxo-4-phenylbutanoic acid	2,4-Dioxobutanoic acid	Classic PA metal-chelating scaffold
A5	4-(4-Chlorophenyl)-2,4-dioxobutanoic acid	2,4-Dioxobutanoic acid	Aryl-substituted PA inhibitor

A6	4-(4-Fluorophenyl)-2,4-dioxobutanoic acid	2,4-Dioxobutanoic acid	Aryl-substituted PA inhibitor
A7	4-(4-Bromophenyl)-2,4-dioxobutanoic acid	2,4-Dioxobutanoic acid	Aryl-substituted PA inhibitor
A8	3-Hydroxyquinolin-2(1H)-one	Hydroxyquinolinone	PA inhibitor pharmacophore
A9	3-Hydroxypyridin-2(1H)-one	Hydroxypyridinone	PA inhibitor pharmacophore
A10	Flutimide	Historic PA-inhibitor scaffold	Literature comparator
(b)			
ID	Compound name	Chemical class	Rationale
B1	Gallic acid	Polyphenolic acid	Strong metal chelation, food-adjacent
B2	Caffeic acid	Phenolic acid	Metal binding, antioxidant
B3	Ferulic acid	Phenolic acid	Hydrophilic, feed-relevant
B4	p-Coumaric acid	Phenolic acid	Small, polar aromatic acid
B5	Protocatechuic acid	Dihydroxybenzoic acid	Catechol-type chelator
B6	Gentisic acid	Dihydroxybenzoic acid	Metal binding, polar
B7	Chlorogenic acid	Polyphenol	Potent chelator, larger scaffold
B8	Catechol	Simple diol	Minimal chelation motif
B9	Pyrogallol	Trihydroxybenzene	Strong chelation motif
B10	Salicylic acid	Hydroxybenzoic acid	Classic chelating pharmacophore
B11	Acetohydroxamic acid	Hydroxamate	Strong metalloenzyme binder
B12	Benzohydroxamic acid	Hydroxamate	Drug-like chelator
B13	Deferiprone	Hydroxypyridinone	Potent metal chelator
B14	Maltol	Hydroxypyryone	Moderate chelator
B15	Kojic acid	Hydroxypyryone	Metal-binding scaffold
B16	Pyridine-2,4-dicarboxylic acid	Heteroaromatic diacid	PA-relevant chelation geometry
B17	Pyridine-2,6-dicarboxylic acid	Heteroaromatic diacid	Dipicolinic acid, a strong chelator
B18	Pyridine-3,5-dicarboxylic acid	Heteroaromatic diacid	Symmetric chelation
B19	Phthalic acid	Aromatic diacid	Compact diacid scaffold
B20	Isophthalic acid	Aromatic diacid	Positional isomer comparator
(c)			
ID	Compound name	Chemical class	Rationale
C1	Citric acid	Tricarboxylic acid	GRAS chelator
C2	Lactic acid	Organic acid	Food-system relevance
C3	Malic acid	Dicarboxylic acid	GRAS, polar
C4	Tartaric acid	Dicarboxylic acid	GRAS, chelating
C5	Succinic acid	Dicarboxylic acid	Simple aliphatic diacid
C6	Fumaric acid	Dicarboxylic acid	Unsaturated diacid
C7	Gluconic acid	Polyhydroxy acid	Food and sanitation use
C8	Ascorbic acid	Vitamin C	Redox-active, chelating
C9	EDTA	Polyaminocarboxylate	Strong metal chelator (reference)
C10	Phytic acid	Polyphosphate	Strong chelator, feed relevance

2.6. Docking

Docking was performed using iGEMDOCK. Each ligand was docked into poultry, mammalian, and crystal PA structures in triplicate. Poses were selected based on predicted affinity, correct metal-chelating geometry, catalytic-residue interactions, and cross-host pose conservation (RMSD <2 Å). The docking protocol was validated by redocking baloxavir into the crystallographic PA endonuclease structure (PDB 6FS8). The predicted pose reproduced the crystallographic orientation with RMSD <2 Å, confirming the reliability of the docking settings.

2.7. ADMET and Poultry/Environmental Suitability

SwissADME, pkCSM, and ProTox-II were used for ADMET prediction. Chemical suitability criteria included: high solubility, low lipophilicity, low predicted avian/human toxicity, low environmental persistence and minimal bioaccumulation risk. Compounds were classified into Tier 1 (poultry/environment-compatible), Tier 2 (uncertain), or Tier 3 (excluded).

2.8. Molecular Dynamics and Binding Energy

MD simulations were performed only for the top poultry-model complex: poultry PA–entecavir. The complex was simulated under NPT conditions (170 ns, YASARA AMBER 96 force field). Trajectories were analysed for RMSD, RMSF, total potential energy, and secondary structure. The binding free energy was calculated using gmx_MMPBSA. MD simulations were performed using YASARA Dynamics with the AMBER96 force field. YASARA trajectories were exported in compatible format and processed using gmx_MMPBSA. The system was solvated in a cubic water box using the TIP3P water model with periodic boundary conditions. Na⁺/Cl⁻ ions were added to neutralize the system. Temperature was maintained at 298 K using a Berendsen thermostat and pressure at 1 atm using a barostat. A timestep of 2 fs was used, and trajectories were recorded every 10 ps (Table 2).

Table 2. Molecular dynamics summary for poultry PA–entecavir.

Metric	Observation
Simulation length	170 ns
RMSD (solute vs start)	Stabilizes during trajectory; plateau below ~1.1 Å
Total potential energy	Fluctuates around stable mean; no systematic drift
RMSF	Expected local flexibility; no global destabilization indicated
Secondary structure	Helix/sheet content remains broadly stable

3. Results

3.1. Homology Modelling and Structural Readiness of PA Endonuclease Targets

Homology models of the poultry-associated and mammalian-associated PA endonuclease domains were successfully generated using the crystal structure PDB 6FS8 as the primary template. The modelling focused on residues 1–193, corresponding to the catalytically active endonuclease domain. Structural alignment of the two models with the template confirmed preservation of the characteristic α/β fold of the PA endonuclease catalytic domain and conservation of the metal-binding catalytic residues H41, E80, D108, and E119 (Figure 2).

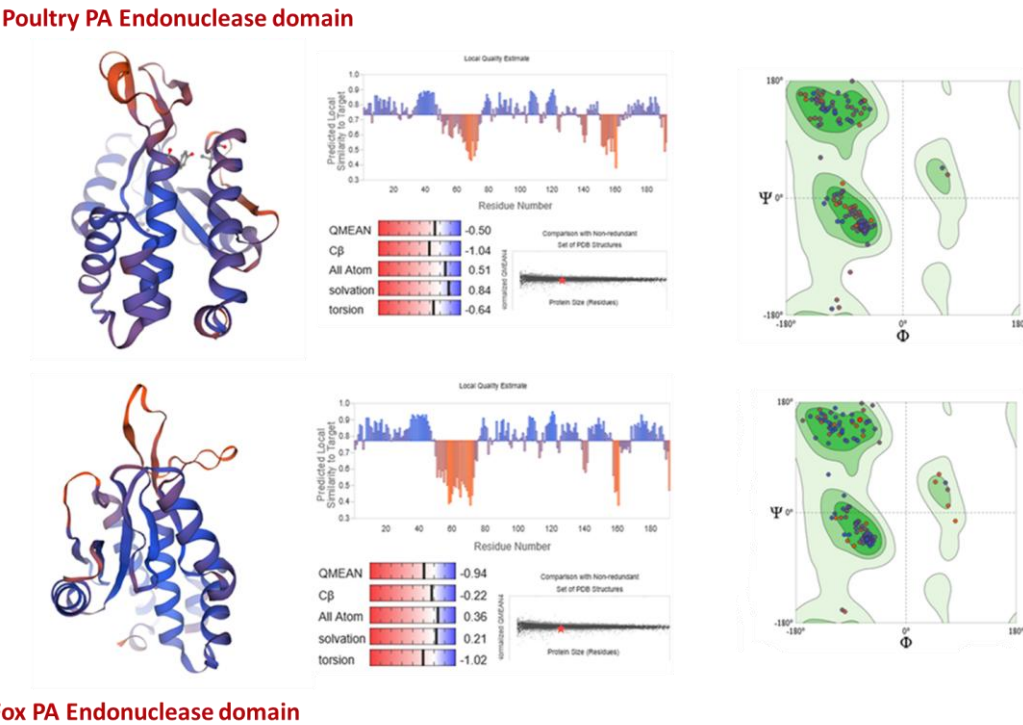


Figure 2. Structural alignment of SWISS-MODEL homology models of poultry- and mammalian-associated PA endonuclease domains (residues 1–193) against the crystallographic template (6FS8). The conserved catalytic pocket region is preserved in both models, supporting their suitability for structure-based docking.

The resulting models displayed high structural similarity to the template and retained the catalytic cavity geometry required for metal-dependent endonuclease activity. Visual inspection confirmed correct positioning of the catalytic pocket and absence of steric clashes that could interfere with ligand docking. These structural features indicate that the homology models were suitable for structure-based virtual screening and for cross-host comparison of ligand-binding behaviour.

3.2. Cross-Species Docking Performance and Interaction Residues

Docking energies indicated robust engagement of both ligands with PA endonuclease across crystal and model targets. Baloxavir produced strong docking energies on 6FS8 (–101.6 to –101.7 across repeated runs), and similarly favorable scores on poultry and mammalian models (–97.5 to –97.7). Entecavir showed the strongest docking in the poultry model (–100.6), while remaining favourable in the mammalian model (–95.0) and in 6FS8 (–83). Residue-level interaction analysis revealed that baloxavir consistently engages catalytic pocket residues in both model and crystal targets. For the mammalian model, recurrent interactions were observed with Glu80, Arg84, Tyr24, Phe105, and Leu106. For the poultry model, baloxavir binding also involved residues in the catalytic cavity including His41, Glu80, Asp108, Tyr24, and Arg84.

Entecavir docking produced target-specific residue signatures. In 6FS8, interactions included Arg85, Leu107, Glu120, Lys135, Tyr106. In the mammalian model, entecavir interacted frequently with His41, Glu80, Arg82, Leu106, Pro107, and Asp108. In the poultry model, the top entecavir pose engaged residues including His52, Ser60, Lys113, Ala159, Asp160, Thr162, Leu163, and Asp164, supporting stable positioning within the modelled pocket region (Table 3). Docking and interaction analysis support a consistent catalytic-pocket binding hypothesis and cross-species feasibility, with baloxavir serving as a strong positive control and entecavir showing particularly favorable docking in the poultry model.

Table 3. iGEMDOCK docking energies (total score) across three PA targets.

Target	Ligand	Docking energy (reported)	Notes
6FS8 (crystal)	Baloxavir	−101.7, −101.7, −101.6	replicate runs consistent
6FS8 (crystal)	Entecavir	−83.0, −83.0	replicate runs consistent
Poultry PA model	Baloxavir	−97.5, −97.7	replicate runs
Poultry PA model	Entecavir	−100.6, −100.6	replicate runs
Mammalian (fox) PA model	Baloxavir	−97.6 to −97.7	replicate runs
Mammalian (fox) PA model	Entecavir	−95.0 to −95.1	replicate runs

3.3. Interaction Profiles Within the Catalytic Pocket

Residue-level interaction analysis revealed that both ligands bind within the catalytic cavity of the PA endonuclease, engaging residues known to contribute to substrate recognition and metal-dependent catalysis. Baloxavir displayed highly consistent interaction profiles across the crystal and homology targets. In the mammalian PA model, the ligand formed recurrent interactions with Glu80, Arg84, Tyr24, Phe105, and Leu106, residues located within the catalytic pocket and surrounding hydrophobic cavity. In the poultry PA model, additional interactions were observed with His41 and Asp108, two residues directly involved in the metal-coordinating catalytic centre. These interactions support the established mechanism of PA inhibition, in which the ligand chelates catalytic metal ions and stabilizes its binding through hydrogen bonding and hydrophobic contacts.

Entecavir demonstrated a somewhat different interaction pattern. Within the crystal structure, interactions were observed with Arg85, Leu107, Glu120, Lys135, and Tyr106, suggesting accommodation within the catalytic pocket but in a different orientation than in baloxavir. In the mammalian model, entecavir established contacts with His41, Glu80, Arg82, Leu106, Pro107, and Asp108, placing the ligand in proximity to the catalytic metal-binding residues. Interestingly, in the poultry PA model the ligand engaged residues including His52, Ser60, Lys113, Ala159, Asp160, Thr162, Leu163, and Asp164, suggesting stabilization through interactions extending beyond the immediate catalytic residues into the surrounding pocket region (Figure 3). This expanded interaction network may explain the improved docking score observed for entecavir in the poultry model.

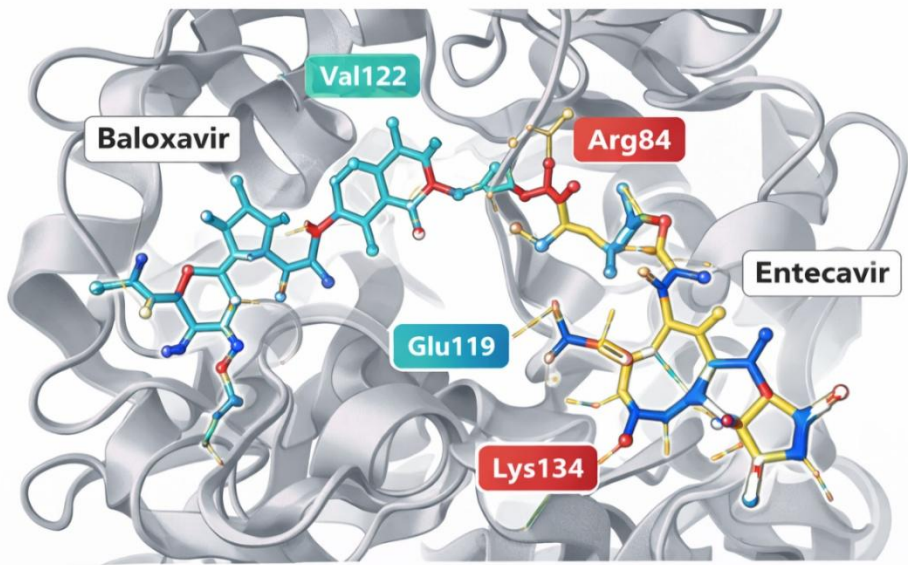


Figure 3. Representative iGEMDOCK docking pose of baloxavir and entecavir within the PA endonuclease active site. Key interacting residues identified by iGEMDOCK interaction analysis are highlighted. The pose illustrates binding within the catalytic cavity.

3.4. Molecular Dynamics Stability of Poultry PA–Entecavir Complex

To evaluate the stability of ligand binding under dynamic conditions, the poultry PA–entecavir complex was subjected to a 170-ns molecular dynamics simulation under explicit solvent conditions (Figure 4). Several trajectory-derived metrics were analysed to assess structural stability.

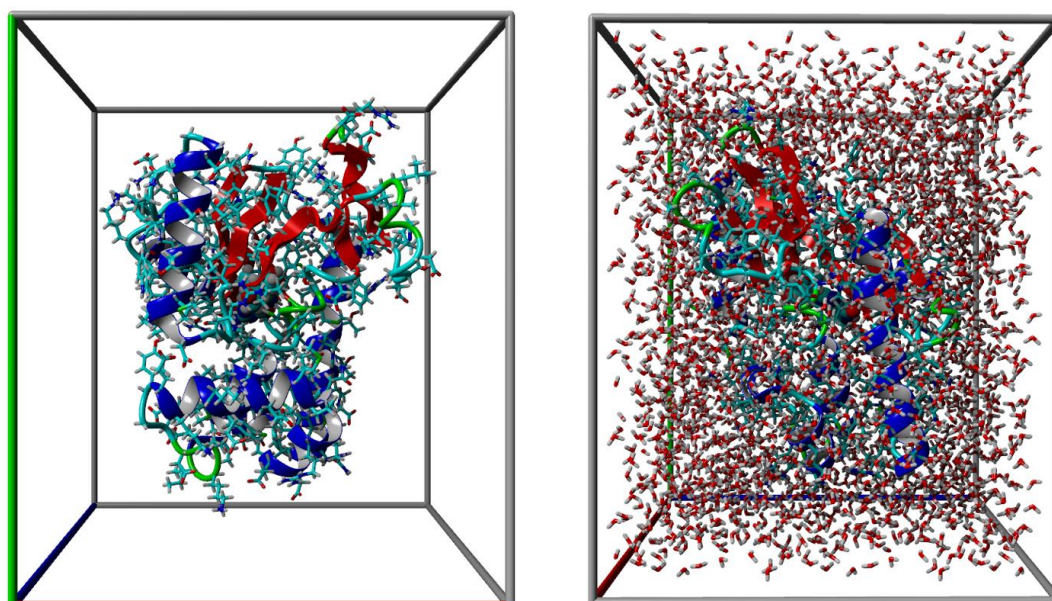


Figure 4. A ray-traced picture of the simulated system (snapshot). The simulation cell boundary is set to periodic. Atoms that stick out of the simulation cell will be wrapped to the opposite side of the cell during the simulation.

The root-mean-square deviation (RMSD) of the solute relative to the starting structure indicated rapid equilibration early in the trajectory, followed by stable fluctuations below approximately 1.1 Å throughout the remainder of the simulation (Figure 5). This low RMSD range suggests that the protein–ligand complex maintained a stable conformational state over the simulation timescale.

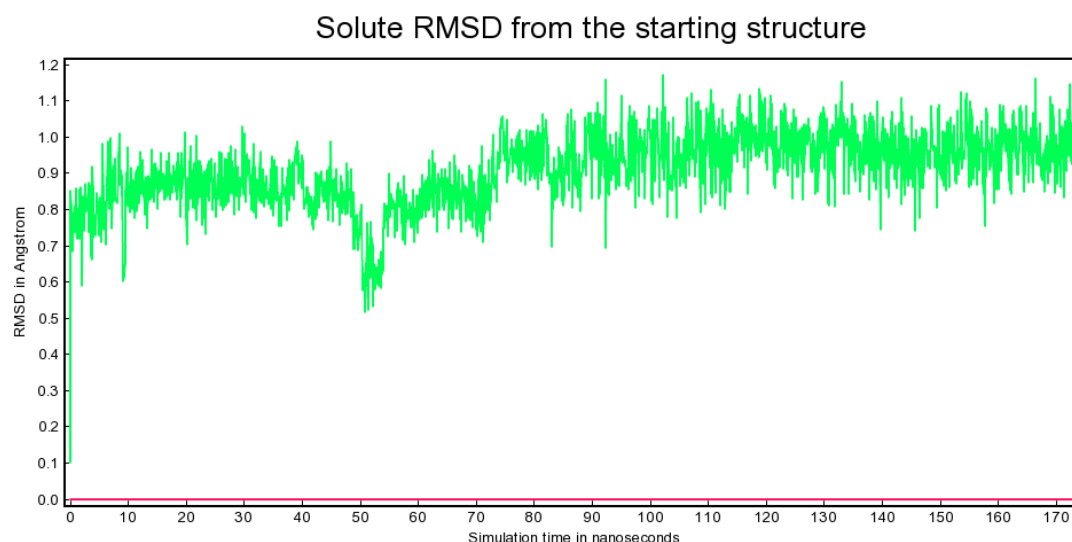


Figure 5. Solute RMSD from the starting structure for the poultry PA–entecavir complex over 170 ns YASARA MD simulation. RMSD stabilization indicates convergence and structural stability of the complex under explicit solvent conditions.

The total potential energy profile fluctuated around a stable mean value without systematic drift (Figure 6), indicating that the system remained thermodynamically stable under the selected simulation conditions.

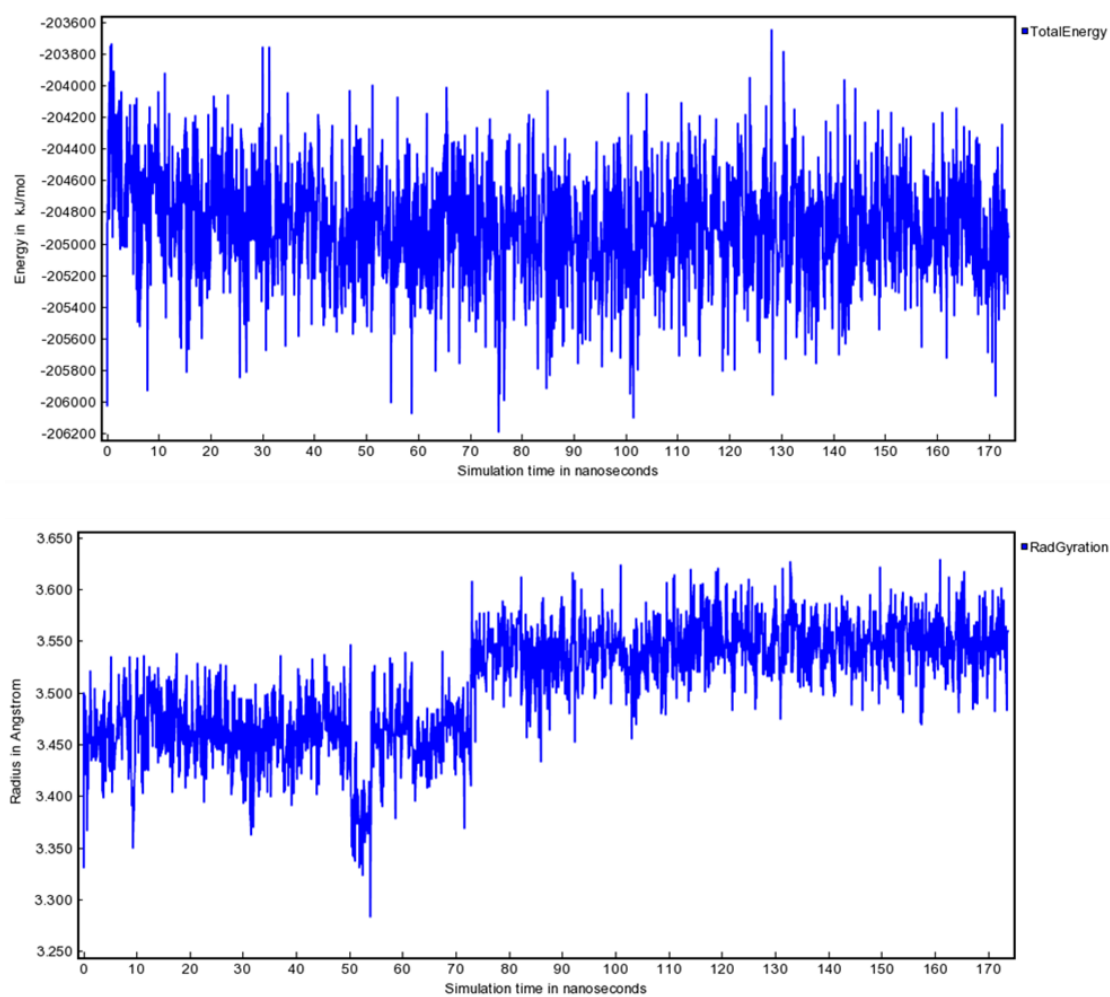


Figure 6. Total potential energy profile of the poultry PA–entecavir simulation system over 170 ns. Fluctuations occur around a stable mean without systematic drift, supporting stable simulation behavior. RMSD stabilization indicates convergence and structural stability of the complex under explicit solvent conditions.

Analysis of the secondary structure content during the simulation revealed no major changes in helix or β -sheet fractions, confirming that ligand binding did not induce destabilisation of the PA endonuclease fold (SFigure 1).

Residue-level RMSF analysis showed that most residues exhibited low fluctuation values, consistent with a structurally stable protein. Higher fluctuations were restricted primarily to solvent-exposed loop regions, a behaviour typical for flexible surface segments of globular proteins (SFigure 2).

Binding free energy estimation using the MM/PBSA approach yielded a mean $\Delta G_{\text{binding}}$ of -85.146 ± 0.836 kJ/mol, indicating energetically favourable complex formation and further supporting the stability of the entecavir-bound state throughout the simulation trajectory. The magnitude of the calculated binding free energy is consistent with stable protein–ligand complex formation and supports the docking results, indicating favourable interaction of entecavir with the avian PA catalytic site.

3.5. ADME Profiling and Selection Rationale

Physicochemical profiling performed using SwissADME highlighted notable differences between the two candidate compounds (Table 4).

Table 4. SwissADME summary.

Parameter	Entecavir	Baloxavir (acid)
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Molecular weight (g/mol)	277.28	483.49
TPSA (Å²)	130.05	100.31
Consensus LogP	-0.54	2.90
HBA / HBD	5 / 4	6 / 1
GI absorption	High	High
BBB permeant	No	No
P-gp substrate	No	No
PAINS	0 alerts	0 alerts
CYP inhibition (selected)	none predicted	CYP2C19/CYP2C9/CYP2D6 predicted
Solubility class	soluble/very soluble	moderately soluble

Entecavir exhibited a lower molecular weight (277.28 g/mol) compared with baloxavir (483.49 g/mol), along with a substantially higher polar surface area (TPSA 130.05 Å²) and markedly lower lipophilicity (consensus LogP -0.54). These characteristics correspond to a high predicted aqueous solubility, which may be advantageous for applications involving water-based delivery systems such as drinking water treatments or environmental formulations.

Baloxavir displayed moderate lipophilicity (LogP ≈ 2.9) and lower polarity (TPSA 100.31 Å²), characteristics consistent with its design as a systemically administered antiviral drug. Both molecules showed no PAINS alerts, suggesting a low likelihood of assay interference.

Predicted pharmacokinetic properties indicated high gastrointestinal absorption for both compounds. However, baloxavir demonstrated predicted inhibition of several CYP isoforms (CYP2C19, CYP2C9, and CYP2D6). In contrast, entecavir showed no predicted CYP inhibition, suggesting a potentially more favourable metabolic interaction profile for drug repurposing.

3.6. Concise Endocrine Nuclear Receptor Screening

To explore potential off-target interactions relevant to toxicological safety, both compounds were screened against human nuclear receptors using the Endocrine Disruptome platform (Table 5).

Table 5. Endocrine Disruptome nuclear receptor docking-based screening (Entecavir vs Baloxavir).

Receptor	Entecavir	Baloxavir
AR	-7.7	4.4
AR antagonist	-8.3	0.2
ERα	-8.0	-5.6
ERα antagonist	-8.0	-8.1
ERβ	-7.7	0.7
ERβ antagonist	-7.6	-6.5
GR	-7.5	-4.5
GR antagonist	-7.0	-7.8
MR	-8.3	1.0
LXRα	-8.0	-4.7
LXRβ	-7.9	-3.9
PPARα	-7.8	-4.2
PPARβ	-8.0	-4.9
PPARγ	-6.7	-6.5
PR	-3.0	-3.0
RXRα	-7.6	-7.1
TRα	-7.6	4.1
TRβ	-8.0	3.2

Entecavir demonstrated moderate predicted docking affinities across several receptors, including the androgen receptor (AR), estrogen receptor α (ERα), mineralocorticoid receptor (MR),

and thyroid receptor β (TR β), with docking scores typically ranging from -7 to -8 kcal/mol equivalents.

Baloxavir generally showed weaker interactions across most receptors, although moderate docking scores were observed in antagonist-mode predictions for ER α , GR, and RXR α .

It should be noted that these predictions represent docking-based affinity estimates rather than functional endocrine activity. Still, they provide a preliminary toxicological context for evaluating candidate compounds within a broader One Health drug repurposing pipeline.

4. Discussion

This study presents a computational–translational pipeline to identify influenza A PA endonuclease inhibitors that are both structurally effective and chemically appropriate for use across the One Health interface of H5N1 avian influenza. While previous *in silico* studies mainly aimed at finding high-affinity binders to influenza polymerase, few have addressed the practical needs for veterinary or environmental use. By integrating cross-host structural modelling, molecular docking, molecular dynamics, and physicochemical filters, this work expands the scope of computational antiviral discovery to include candidates relevant for poultry production and outbreak management [36].

The PA endonuclease plays a critical role in the influenza replication cycle by mediating the cap-snatching mechanism required for viral transcription. Because this catalytic domain is structurally conserved across influenza A viruses, it has emerged as an attractive antiviral target. However, most previous computational studies evaluating PA inhibitors have focused on human influenza strains or historical H5N1 isolates. In contrast, the present study examines contemporary H5N1 clade 2.3.4.4b variants, which currently dominate global outbreaks in wild birds and poultry. Structural comparison of poultry-associated and mammalian-associated PA models confirmed that the catalytic architecture of the enzyme remains largely conserved while exhibiting subtle differences in pocket geometry and local flexibility. These structural variations can influence ligand binding behaviour and highlight the importance of evaluating antiviral candidates across host-associated variants when considering interventions at the animal–human interface [37–39].

Docking analysis demonstrated that both baloxavir and entecavir interact favourably with the catalytic cavity of the PA endonuclease. Baloxavir served as a strong positive control, consistently engaging catalytic residues and producing stable docking scores across the crystal structure and both homology models. Entecavir, a nucleoside analogue originally developed as an antiviral agent against the hepatitis B virus, displayed particularly favourable binding within the poultry-associated PA model. The predicted binding energies and interaction patterns suggest that entecavir may be capable of stabilising within the catalytic pocket through a combination of hydrogen bonding and electrostatic interactions involving residues that contribute to the metal-coordinating catalytic centre. These findings support the potential of nucleoside-based scaffolds as alternative PA endonuclease inhibitors beyond the hydroxypyridinone-based pharmacophores represented by baloxavir.

Molecular dynamics simulations provided further insight into the stability of the predicted protein–ligand complex. The poultry PA–entecavir system demonstrated rapid equilibration and stable structural behaviour throughout the simulation trajectory. Low RMSD fluctuations and stable potential energy profiles indicate that the complex remained conformationally stable under explicit solvent conditions. In addition, secondary structure analysis confirmed preservation of the overall protein fold, while residue-level fluctuation patterns suggested that flexibility remained largely confined to solvent-exposed loop regions. Together, these observations support the structural compatibility of entecavir with the PA catalytic pocket and reinforce the docking predictions indicating favourable ligand binding.

A notable feature of this study is the incorporation of physicochemical and ADMET criteria relevant to poultry production environments. Traditional computational drug discovery workflows typically prioritise compounds solely on the basis of binding affinity or pharmacokinetic properties optimised for human systemic administration. In contrast, antiviral compounds intended for use in

animal production systems may require different chemical characteristics, including higher aqueous solubility, reduced lipophilicity, limited tissue persistence, and minimal environmental accumulation. The physicochemical profile of entecavir, characterised by relatively high polarity and predicted aqueous solubility, suggests potential compatibility with water-based delivery systems or environmental applications within poultry facilities. Such properties may be advantageous for strategies aimed at reducing viral load in barns, drinking water systems, or contaminated surfaces during outbreaks.

The translational implications of this approach are particularly relevant in the context of H5N1 control strategies. Poultry outbreaks are currently managed primarily through biosecurity measures, culling, and vaccination campaigns. However, environmental contamination and rapid viral transmission within densely populated production systems remain significant challenges. Antiviral compounds that could reduce viral replication or environmental persistence may provide complementary tools for outbreak mitigation. The identification of candidate inhibitors that are compatible with poultry production conditions, therefore, represents an important step toward bridging the gap between computational discovery and practical disease control measures.

Beyond poultry health, the importance of discovering antivirals within the One Health framework is increasingly clear as H5N1 expands its host range. Recent spillovers into mammals, such as farmed mink and dairy cattle, highlight the need to find antiviral candidates effective across various species. The cross-host modeling approach used in this study helps identify compounds that can interact stably with both avian and mammalian PA variants. These host-agnostic inhibitors could protect poultry and reduce zoonotic transmission risks to farm workers and others. Overall, the study demonstrates how computational methods in antiviral discovery can address veterinary and environmental health challenges beyond traditional drug development. By combining structural modeling, docking, molecular dynamics, and physicochemical filtering tailored for poultry, the workflow prioritizes candidates that are mechanistically sound and practical. The identification of entecavir as a promising candidate showcases the potential for drug repurposing within this strategy. Future efforts should validate these candidates through biochemical PA endonuclease inhibition tests, antiviral studies in bird cell cultures, and environmental stability assessments relevant to poultry farming. Expanding this computational approach to other influenza polymerase targets and larger compound libraries could accelerate antiviral discovery, supporting integrated One Health strategies to combat highly pathogenic avian influenza.

5. Conclusions

This study introduces a detailed computational approach to identify influenza A PA endonuclease inhibitors effective against current H5N1 clade 2.3.4.4b variants, taking into account physicochemical factors relevant to poultry production. By combining cross-host structural modelling, molecular docking, molecular dynamics simulations, and physicochemical filtering, we found compounds that can stably interact with the catalytic pocket of the PA endonuclease in both avian and mammalian variants. Entecavir, among the screened compounds, displayed promising docking results, remained stable in molecular dynamics tests, and possessed physicochemical properties suitable for applications requiring high water solubility and low lipophilicity. These characteristics indicate that repurposed antiviral scaffolds may serve as promising starting points for developing inhibitors tailored for veterinary and environmental use.

Notably, the computational workflow presented here extends beyond conventional structure-based drug discovery by incorporating factors pertinent to poultry production settings and cross-host viral transmission. This approach provides a translational pathway for prioritising antiviral candidates that could facilitate integrated control strategies, addressing veterinary, environmental, and occupational concerns related to highly pathogenic avian influenza. Future work involving biochemical PA inhibition assays, avian cell culture models, and evaluations of compound behavior in poultry-associated environmental matrices will be essential for advancing these candidates toward effective antiviral treatments.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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