Advances in *in silico* drug discovery for rabies virus: Innovations in ligand identification and therapeutic mechanisms (Review)

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Abstract. Rabies remains a nearly universally fatal zoonotic disease once clinical symptoms manifest, despite the availability of effective vaccines. The lack of viable therapeutic interventions post-symptom onset has fueled the exploration of novel strategies, particularly computational approaches. The present review highlights recent advancements in *in silico* drug discovery targeting rabies virus, focusing on structure-based drug design, ligand screening, molecular dynamics and artificial intelligence-assisted repurposing. Specific viral proteins, including nucleoprotein (N), glycoprotein (G) and RNA-dependent RNA polymerase (L), have been computationally analyzed as promising therapeutic targets. Several FDA-approved drugs, such as emtricitabine and micafungin, along with phytochemicals such as (+)-catechin, have demonstrated strong binding affinities in docking simulations. Additionally, the development of multi-epitope peptide vaccines and RNA-based platforms, including mRNA vaccines and RNA interference, provides innovative preventive strategies. The integration of bioinformatics tools, such as AlphaFold, IEDB, CB-Dock2 and PLIP has streamlined target identification and validation. Despite notable computational progress, translational gaps remain due to limited experimental validation. The present review emphasizes the importance of combining *in silico* predictions with laboratory research and clinical data to accelerate the development of effective rabies therapies. With an increasing global health burden, particularly in resource-limited regions, computational drug discovery holds promise for bridging critical treatment gaps in rabies management.

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Contents

1. Introduction
2. Overview of structure-based drug discovery methods
3. Structural and functional insights into rabies virus proteins
4. AI and ML in rabies treatment
5. Drug repurposing
6. Multi-epitope peptide vaccines
7. Phytochemical-based compound candidates
8. RNA-based therapeutics
9. Regional variations and implications for treatment
10. Bioinformatics and integrative strategies to fight rabies virus
11. Conclusion and future perspectives

1. Introduction

Rabies is an acute, often fatal viral disease that primarily affects the central nervous system. It is typically transmitted through bites from infected domestic dogs and wild carnivorous animals. All warm-blooded animals, including humans, are susceptible to rabies infection. The rabies virus (RABV), a member of the *Lyssavirus* genus, is commonly found in the salivary glands of rabid animals and is excreted in their saliva. When an infected animal bites, the virus enters the blood through a fresh wound. Once introduced, RABV propagates along nerve tissue to the brain, establishing itself within the central nervous system. Subsequently, it spreads via nerves to the salivary glands, often resulting in symptoms such as excessive salivation. The disease initially manifests with excitation of the central nervous system, characterized by irritability and aggression. Infected animals may be highly contagious even before symptoms manifest (1). Despite effective vaccines for pre- and post-exposure prophylaxis (PEP), rabies remains a significant public health challenge, particularly in developing countries where access to PEP is limited. Once clinical symptoms appear, rabies is almost always fatal, underscoring the urgent need for effective therapeutic interventions. Rabies is prevalent in >150 countries, mainly in Asia and Africa, where exposure without timely PEP results in a mortality rate >99%. The RABV genome encodes five essential proteins: Nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L). These proteins play critical roles in the viral life cycle, facilitating attachment to

host cells, entry via endocytosis, uncoating and replication within the host cytoplasm.

Rabies poses a severe threat to human health and economic stability, particularly in developing countries. According to the World Health Organization (WHO) 2023 report, rabies causes ~59,000 human deaths annually, with 99% of cases resulting from dog bites (2). The highest mortality rates are reported in Asia and Africa, where access to PEP is often limited. India alone accounts for almost 36% of global rabies-related deaths, translating to an estimated 20,000 fatalities per year (3). By contrast, developed nations have largely controlled rabies through extensive vaccination programs and improved surveillance measures.

The economic impact of rabies is profound, resulting in over $8.6 billion in global annual losses. These losses stem primarily from the costs associated with PEP, the loss of livestock, and reduced productivity due to illness and death. The financial burden of rabies is particularly acute for low-income populations, where the average estimated cost of PEP can be prohibitive (4). This situation emphasizes the urgent need for novel therapeutic interventions, as there are currently no effective antiviral treatments available once clinical symptoms appear.

Recent epidemiological studies highlight the ongoing challenges in controlling rabies in specific regions. For instance, Malaysia has faced recurrent outbreaks of dog-mediated human rabies, with a significant association between low vaccination rates and increased incidence of cases (5). Another study conducted from 2015 to 2023 revealed that dogs accounted for 89.35% of confirmed rabies cases in animals, emphasizing the importance of vaccination campaigns (6). Therefore, rabies continues to pose a significant public health threat globally, particularly in developing regions where access to preventive measures is limited. Enhanced vaccination efforts and improved public health strategies are essential to combat this preventable disease effectively. The urgent need for therapeutic interventions for symptomatic patients further highlights the complexities involved in managing rabies as a public health concern.

Effective vaccines have been developed for PEP; timely administration can prevent rabies development following exposure. PEP includes wound cleansing, vaccine administration and equine or human rabies immune globulins (ERIG or HRIG). However, PEP is ineffective once neurological signs appear. Although there are rare reports of human rabies survivors, no established therapies exist. Advances in treatment often stem from key studies on rabies pathogenesis in animal models (7).

While vaccines have proven effective in preventing rabies transmission, there is a pressing need for therapeutic strategies to combat the disease in symptomatic patients. This need is particularly critical in resource-limited settings where vaccination coverage remains insufficient. The WHO has initiated efforts to improve access to rabies vaccines through partnerships with organizations such as Gavi, focusing on strengthening surveillance and reporting systems while encouraging multisectoral collaborations (8).

Conventional treatment methods include pharmacological therapies, surgical intervention and physical therapy. These methods have been the cornerstone of medical practice for decades, as they are often backed by extensive clinical research, providing established protocols for healthcare providers. In addition, this approach allows for direct patient assessment, enabling healthcare providers to tailor treatments based on individual patient needs (9). In a number of cases, conventional treatments can yield immediate results, such as pain relief or symptom management. On the contrary, these approaches can be resource-intensive, requiring significant time and personnel for administration and monitoring, and can sometimes be costly in resource-limited settings (10). Therefore, treatment strategies have incorporated *in silico* approaches, as they provide a better understanding of the treatment design, disease modelling and drug discovery, which include molecular docking, virtual screening and machine learning (ML) algorithms that analyze large datasets to predict treatment outcomes.

In silico methods can process vast amounts of data quickly, significantly reducing the time required for drug discovery and development compared to traditional laboratory methods. These approaches can easily scale to accommodate larger datasets without a corresponding increase in resource requirements. This scalability is particularly beneficial in addressing complex diseases with multifactorial causes in a cost-effective manner, while minimizing laboratory work and animal testing. However, computational predictions need to be validated through experimental studies, as there is a risk of false positives or negatives. Their effectiveness relies heavily on the availability and quality of data. They may not always accurately reflect the complexities of human biology or disease progression (11).

In recent years, computational methods have emerged as powerful tools in drug discovery and vaccine design for viral infections such as rabies. *In silico* approaches, such as molecular docking, homology modelling, virtual screening, molecular dynamics simulations and quantitative structure-activity relationship (QSAR) models, offer cost-effective and time-efficient means of identifying potential antiviral compounds and understanding their mechanisms of action. These methods can predict interactions between viral proteins and potential inhibitors, facilitating targeted therapy development (12). However, systematic clinical research into combination therapies faces challenges due to the sporadic occurrence of rabies cases. There is a pressing need for medical approaches that accelerate effective therapy development through veterinary care and investigational treatments of naturally infected dogs when appropriate. Understanding the pathogenesis of RABV in both humans and dogs has advanced significantly, providing critical insight into severe neurological dysfunction associated with the virus. Of note, four disease processes need to be managed: Viral propagation, neuronal degeneration, inflammation and systemic compromise (13).

The present review aimed to provide a comprehensive overview of recent advancements in *in silico* research focused on RABV and focusses on novel ligand identification, structural elucidation of key viral proteins, drug repurposing strategies as potential treatments for rabies, and explores the design of multi-epitope peptide vaccines. By emphasizing these developments, we aim to illustrate the potential of computational approaches in combating rabies and inspire further research in this vital area. While the biological mechanisms of rabies are well-documented, addressing its therapeutic challenges

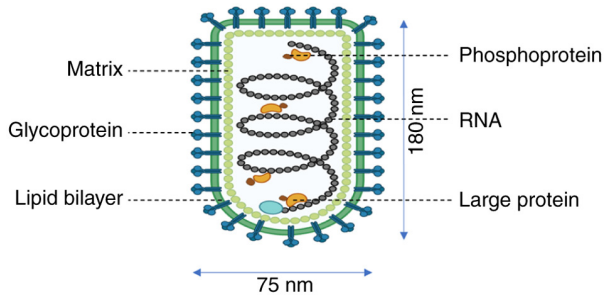

Figure 1. Structure of the *Lyssavirus*.

necessitates innovative approaches. Computational methods, with their cost-effectiveness and predictive capabilities, have emerged as a powerful solution in the quest for novel treatments.

2. Overview of structure-based drug discovery methods

Given the lack of effective treatments post-infection, structure-based drug discovery offers a promising avenue by exploiting detailed molecular insights into the key proteins of the virus. *In silico* methods, which encompass computer-based techniques, have become essential in drug discovery. These methods utilize computational tools and algorithms to predict, analyze and optimize interactions between ligands (potential drug molecules) and biological targets, such as proteins or nucleic acids. By simulating molecular interactions and screening extensive libraries of compounds, *in silico* approaches significantly expedite the identification of promising drug candidates, while reducing the costs and time associated with traditional experimental methods. Structure-based drug discovery has emerged as a promising and efficient strategy for identifying novel and potent drug candidates in a more efficient and cost-effective manner than conventional methods (14). This approach relies on the three-dimensional structures of biological protein targets to elucidate the molecular basis of diseases. To virtually identify drug candidates, several preparatory steps for proteins and compounds are essential for obtaining accurate results. Key steps include homology modelling, virtual screening, QSAR models and pharmacophore modelling, molecular docking and molecular dynamics simulations (12,15). Molecular docking predicts the preferred orientation of one molecule when bound to another to form a stable complex. By contrast, molecular dynamics simulations study the physical movements of atoms and molecules, providing a dynamic view of molecular interactions. These techniques are cost-effective and precise enough to predict mechanical characteristics, optimize structures, and simulate the natural motion of biological macromolecules (16). Structure-based virtual screening involves several computational phases, including target and database preparation, docking, post-docking analysis, and prioritization of compounds for testing. Homology modelling predicts protein structures from amino acid sequences by aligning them with known templates, constructing models and refining them through various steps (14). QSAR models establish associations between the properties of chemical substances and their biological activities to predict the activities of new chemical entities. Moreover, pharmacophore modelling identifies essential structural features necessary for a molecule to interact with a specific biological target, aiding in virtual screening and lead compound optimization (15). An overview of the *in silico* methods used in the drug discovery process, with their advantages and disadvantages is provided in Table I.

3. Structural and functional insights into rabies virus proteins

RABV, a member of the *Rhabdoviridae* family, encodes five structural proteins: Nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the large RNA-dependent RNA polymerase (L or RdRp) (Fig. 1). Each protein plays a critical role in viral replication, immune evasion and pathogenesis. Understanding their structural and functional properties provides essential insight into antiviral drug targeting. The N protein encapsulates the viral RNA genome, forming the ribonucleoprotein complex crucial for viral transcription and replication, shielding the viral genome from host immune responses (17). The G protein mediates viral entry by interacting with host cell receptors, such as the nicotinic acetylcholine receptor and p75 neurotrophin receptor. It is also the primary target for neutralizing antibodies, rendering it a key focus for vaccine and antiviral drug development (18). The L protein, in association with the P protein, carries out viral genome replication and transcription, rendering it a promising target for broad-spectrum antivirals (19).

Computational approaches in targeting RABV proteins. Modern computational techniques have significantly advanced rabies antiviral research. Structure-based drug design, homology modelling via SWISS-MODEL (https://swissmodel.expasy.org/), molecular docking using AutoDock Vina (https://vina.scripps.edu/) and GOLD (https://www.ccdc.cam.ac.uk/discover/blog/gold-the-all-in-one-molecular-docking-package/), and molecular dynamics simulations have provided valuable insight into protein-ligand interactions. Additionally, artificial intelligence (AI) and ML are enhancing ligand identification and optimization, expediting therapeutic discoveries. However, *in vitro* experiments to validate the *in silico* results are mandatory for robust drug discovery.

Key viral targets for drug discovery. Viral proteins serve as crucial targets in antiviral drug discovery due to their vital roles in the viral life cycle, including replication, transcription and host cell entry. Targeting these proteins can disrupt viral propagation and provide effective therapeutic strategies (19).

The nucleoprotein is critical for encapsulating viral RNA and forming a ribonucleoprotein complex essential for replication and transcription. Structural analysis (PDB ID: 2GTT) has revealed its strong RNA-binding affinity, rendering it a viable target for small-molecule inhibitors. Molecular docking studies identified ZINC01530604 and ZINC01530605 as promising candidates targeting phosphorylation sites essential for nucleoprotein function. Additionally, QSAR modelling analyzed >450 physicochemical parameters, leading to the identification of peptide inhibitors, such as P16b6, that can effectively bind to the phosphoprotein and suppress viral

Table I. *In silico* methods, applications and tools.

Method	Description	Application in rabies drug discovery	Commonly used tools	Advantages	Limitations	(Refs.)
Molecular docking	Predicts the preferred binding orientation of a ligand (drug candidate) with a target protein.	Identifies potential inhibitors for rabies nucleoprotein (N), glycoprotein (G), and RNA-dependent RNA polymerase (RdRp).	AutoDock, AutoDock Vina, SwissDock, Glide (Schrödinger), GOLD	Cost-effective, high-throughput screening.	May not account for protein flexibility and solvent effects.	(23,58)
Molecular dynamics (MD) simulations	Simulates the physical movements of atoms and molecules over time.	Evaluates the stability and dynamic interactions of drug candidates with rabies virus proteins.	GROMACS, AMBER, CHARMM, NAMD	Provides insight into binding stability and conformational changes.	Computationally intensive, requires high-performance computing.	(12)
Virtual screening	Screens large compound libraries for potential drug candidates based on binding affinity.	Identifies small molecules with potential antiviral activity against rabies virus proteins.	PyRx, Schrodinger's Glide, MOE, OpenEye FRED, LigandScout	Rapidly analyzes thousands of compounds.	Requires accurate target structures for reliable predictions.	(12,15)
Homology modeling	Predicts the 3D structure of proteins when an experimentally determined structure is unavailable.	Used to model the structure of rabies virus RdRp, nucleoprotein, and glycoprotein for drug discovery.	SWISS-MODEL, MODELLER, Phyre2, I-TASSER	Provides structural insights when crystal structures are unavailable.	Accuracy depends on template selection and alignment quality.	(26)
Quantitative structure-activity relationship (QSAR) models	Establishes mathematical relationships between chemical properties and biological activity.	Predicts the effectiveness of new rabies antiviral compounds before experimental testing.	KNIME, DeepChem, PaDEL-Descriptor, ChemOffice, QSARINS	Enables optimization of drug candidates.	Requires extensive datasets for training reliable models.	(12,15)
Pharmacophore modeling	Identifies essential molecular features required for ligand-receptor interactions.	Helps in the design of novel inhibitors targeting rabies virus proteins.	LigandScout, PharmaGist, Discovery Studio, MOE	Highlights key functional groups for drug interaction.	May miss non-obvious interactions not included in the model.	(12,58)

replication (20). While molecular docking is a powerful tool for narrowing down candidates, further validation steps are essential for confirming antiviral activity.

The RABV virus glycoprotein (RVG) facilitates host cell entry and neurotropism, making it a main antiviral target. Docking studies have suggested polyethylene glycol 4000 as an inhibitor, effectively blocking receptor binding sites to prevent viral spread (21). Structural studies have also highlighted conserved tyrosine and threonine residues contributing to glycoprotein stability. Furthermore, computational techniques have facilitated the identification of neutralizing monoclonal antibodies, such as 523-11, which bind to RVG and prevent membrane fusion (22).

RABV RdRp is essential for viral RNA synthesis. Homology models based on vesicular stomatitis virus (PDB ID: 5A22) have provided structural insight into its catalytic

mechanism. Molecular docking analyses identified compounds binding to key catalytic residues (Met585, Glu620, Lys621, Trp622 and Glu696), inhibiting polymerase activity. A virtual screening of 2,045 compounds from the National Cancer Institute (NCI) identified several promising inhibitors with strong binding affinity to RdRp active sites, supporting further experimental validation (23).

Thus, these viral proteins, nucleoprotein, glycoprotein and RdRp, are promising targets for antiviral drug discovery due to their critical functions in the viral lifecycle and their amenability to targeted inhibition. Progress with small-molecule inhibitors, peptides, and neutralizing antibodies against these targets may lead to the development of effective antiviral therapies. Hence, focusing drug discovery efforts on critical viral proteins with validated roles in replication and infection, supported by structural and computational studies, are fundamental for developing potent antiviral agents that can effectively disrupt viral propagation and combat viral diseases.

Across the literature, there is a general consensus that structure-based computational methods accelerate the identification of antiviral candidates for RABV, particularly for targets, such as nucleoprotein, glycoprotein and RdRp. However, discrepancies remain as regards the translation of *in silico* findings to *in vitro* and *in vivo* efficacy. This emphasizes the necessity for integrated pipelines that combine computational screening with experimental validation, a direction increasingly advocated in recent reviews and original research articles.

4. AI and ML in rabies treatment

The advent of ML and AI has markedly facilitated the understanding of complex biological systems and accelerated the drug discovery process. One area where ML/AI has played a vital role is protein structure prediction, which is essential to understanding the biological mechanisms involved in the disease. DeepMind's AlphaFold is the game changer in this scenario, surpassing all other existing tools and recognized as the recipient of the 2024 Nobel Prize in Chemistry (24). Secondly, the role of AI in drug discovery has been monumental. In the field of RABV drug discovery, these technologies facilitate the analysis of genetic, epidemiological and pharmacological datasets to predict treatment efficiency, optimize antiviral drugs and identify novel therapeutic targets by significantly reducing the cost and time involved in developing new rabies treatments. AI-based computational models are speeding up the discovery of host factors and RABV proteins that can serve as therapeutic targets by analyzing viral genomes and host-pathogen interactions to detect the critical molecular pathways essential for RABV replication (25). In addition, predicting the conserved viral epitopes could enable the development of vaccines or anti-viral drugs (26). Likewise, AI also supports the identification of host proteins involved in immune evasion or viral invasion that could open new avenues in therapeutics (27).

Another area where AI is playing a transformative role is in the development of antiviral drugs for rabies. ML approaches enable rapid screening of millions of compounds to identify leads as potential inhibitors of viral replication by analyzing extensive molecular datasets and predicting drug-receptor interactions, followed by lead optimization to enhance their efficacy (28). Drug repurposing is another interesting area of research where AI can expedite the identification of FDA-approved drugs with activity against the RABV (29). AI also predicts drug-virus interactions, identifying promising antiviral candidates inhibiting replication. Additionally, the amalgamation of AI and CRISPR genome-editing technology is advancing research into RABV evolution, diagnosis, and treatment (30). Together, these efforts are paving the way for safer, more effective therapeutic options for rabies prevention and treatment.

5. Drug repurposing

Research into the molecular basis of rabies, which affects the central nervous system, has demonstrated that the virus targets neurons, leading to severe neurological symptoms (31). Currently, the only available treatment option is post-exposure prophylaxis, which comes with important drawbacks, including high costs and complicated administration. These limitations necessitate alternative strategies where drug repurposing could benefit rabies treatment. By re-examining existing drugs for novel therapeutic applications, the drug development process can be accelerated with reduced cost and potentially reveal new treatment pathways. Repurposed medications may provide a more rapid route to clinical application, addressing the urgent need for more accessible and effective therapies for rabies (10).

In a previous study, notable candidates, such as cidofovir, emtricitabine, famciclovir, acyclovir, and stavudine were shown to exhibit potential efficacy against the RABV glycoprotein (32). Advanced docking analyses were conducted using the CB-Dock2 program to assess these candidates. This sophisticated approach searched the database of the server for template ligands with strong topological similarity (FP2 $\geq$0.4) through an integrated homologous template-based blind docking mechanism (32). Following this, cavity detection was performed using an in-house technique known as FitDock to aid molecular docking with specific templates. The docking analyses identified five potential binding cavities for Emtricitabine within the target protein, with volumes ranging from 7209 Å^3 to 1703 Å with significant binding affinities, varying from -5.0 to -6.1; lower scores suggest stronger binding affinity. The root means square deviation values were observed around 10 Å, and the hydrogen bond count fluctuated around 25, indicating stable interactions with limited conformational deviation and persistent hydrogen bonding (32).

In addition to computational methods for drug repurposing, the validation of these compounds is crucial for establishing their efficacy against rabies. Repositioning already approved medications can significantly lower drug development costs and timelines, while reducing the likelihood of unexpected side-effects. Computational repositioning allows for the rapid screening of candidates *in silico*, which renders it an attractive option for prioritizing potential treatments. In a previous study, the ChEMBL library was filtered from 2,354,965 compounds down to 6,554 that had passed through any phase of clinical trials to avoid cytotoxic inhibitors (33). The cryo-electron microscopy (cryo-EM) structure of the respiratory syncytial virus (RSV) strain A2 polymerase

(PDB: 6UEN) was prepared for AutoDock Vina docking studies. Compounds with flexible ligand structures or poor binding affinities were eliminated, narrowing the selection to 4,919 candidates. Protein-Ligand Interaction Profiler was used for interaction analyses with micafungin, trombopag, trovafloxacin, azlocillin Na, trospium, amiodarone HCl, perflubron, mephenesin and methadone. Among these candidates, micafungin demonstrated exceptional promise with a binding affinity of -14.32 kcal/mol and an inhibition constant of 0.0317 nM, outperforming existing inhibitors, such as AZ-27 (0.47997 nM), ALS-8112 (2.56 nM) and ribavirin (6.98 nM) (33). *In vitro* assays further confirmed the efficacy of micafungin by reducing RSV polymerase activity to only 29.6% of the control levels; by contrast, verubecestat (97.4%), ribavirin (98.4%) and ALS-8112 (89.5%) retained markedly higher levels of activity compared with the control. These findings collectively demonstrate that computational approaches can effectively identify promising repurposed compounds for rabies treatment while validating their efficacy through rigorous screening processes (33).

Phthalazine derivatives have gained attention in recent times due to their pharmacological properties (34). Their unique structural characteristics make them promising candidates in combating viral infections. High-throughput screening methods have played a key role in discovering compounds that inhibit RABV replication, while maintaining low cytotoxicity. Comprehending the mechanisms of action of these compounds is vital for augmenting their therapeutic potential. Phthalazine and its derivatives, nitrogen-containing heterocyclic compounds, have gathered attention in medicinal chemistry due to its versatile pharmacological properties. Of note, one phthalazine derivative compound, 7671954, was found to be particularly effective against RABV infections across different *Lyssavirus* species with low cytotoxicity (IC50 values <30 μM) (34). Experiments established that these compounds inhibited viral replication by targeting the replication complex (34). Furthermore, these derivatives interact with host receptor, such as the p75 neurotrophin receptor and nicotinic acetylcholine receptor, both of which play critical roles in viral entrance, potentially increasing immune responses while limiting viral interactions with host cells (18).

In summary, drug repurposing provides a cost-effective and accelerated path for rabies treatment, identifying promising candidates such as emtricitabine, famciclovir, acyclovir, stavudine and cidofovir. Computational screening and high-throughput assays have enabled the rapid identification of drugs with potent antiviral potential against rabies. These approaches address the urgent need for affordable and accessible therapies beyond current post-exposure prophylaxis, which remains costly and limited in availability. The continued research and validation of these repurposed drugs could transform rabies management and save countless lives globally.

6. Multi-epitope peptide vaccines

Generally, drug discovery focuses on treatment post-infection, whereas vaccines provide proactive protection. Computational methods have facilitated vaccine design through precise epitope mapping. Since viral entry is the key to infection, the RABV glycoprotein (RABV-G) is mainly targeted for vaccine development. Several immunoinformatic tools are utilized to identify the antigenic sites and further design multiepitope peptide vaccines (35).

The Immune Epitope Database (IEDB) is employed to predict epitopes based on the Bepipred Linear Epitope Prediction 2.0 method. Initial sequence analysis that includes antigenicity, allergenicity and virulence assessments is carried out using AllerTOP v2.0, DeepTMHMM, VirulentPred and VaxiJen. This is followed by BLASTP analysis to ascertain no cross reactivity with human proteins. Physico-chemical properties and structure prediction studies are performed using Protparam, PSIPRED and GalaxyRefine followed by the final prediction of immune response prediction using C-ImmSim server. Studies were carried out using this approach for RABV proteins (36,37).

In a previous study, AR16 and hPAB, two conserved RABV peptides, were combined with Gp96 adjuvant to create an oil-in-water emulsion. By day 14, the vaccine candidates produced virus-neutralizing antibodies and were safe. By day 28, they peaked at 5-6 IU/ml in mice and 7-9 IU/ml in beagles. The promise of multi-epitope peptide vaccines for rabies prevention was demonstrated by the 70-80% protective efficacy against a virulent rabies strain in mice (36). In another study, using computational immunoinformatic methods, B-cell epitopes from the RABV glycoprotein and nucleoprotein were found (37). The eukaryotic vector pcDNA3.1(-) was used to express the highly antigenic areas after they were put together into a multi-epitope construct. Oral delivery was facilitated by adopting food-grade *Escherichia coli* as a carrier. Experiments conducted both *in vitro* and *in vivo* demonstrated that the vaccination could produce a robust immune response in mice, markedly increasing survival following viral challenge without causing any negative side-effects. This method demonstrates a viable, scalable approach to rabies prevention, especially in populations of wild animals (37).

7. Phytochemical-based compound candidates

In recent times, natural forms of therapy have gained importance based on popular beliefs, skills, and indigenous practices owing to their no-side-effect advantage. The role of traditional medicine has taken a center stage, primarily including plants as the source of innumerable compounds with enormous therapeutic benefits (38). Although this approach is used for rabies management, there are limited data available to support the efficacy of these methods. Phytochemicals provide an alternative approach for antiviral treatment as they are known to inhibit viral replication (39).

Compounds such as coumarins, lignans, alkaloids, flavonoids and terpenoids exhibit antioxidant activity and may suppress viral genome activity. A previous study identified anti-rabies phytochemicals from *Salix subserrata* and onion, screened for toxicity using SwissADME and Protox-II servers. Among the candidates, (S+)-catechin and kaempferol exhibited promising binding interactions with RABV glycoprotein based on docking results. (+)-Catechin displayed an improved binding affinity, full fitness and estimated ΔG values than kaempferol with the formation of conventional hydrogen bonds with the target protein, rendering it a potential natural

Table II. Identified ligands, drug repurposing and vaccine design.

Compound/drug	Type	Target protein	Binding affinity (Kcal/mol)	Predicted effect/ mechanism of action	*In silico* method used	(Refs.)
ZINC01530604	Ligand	Nucleoprotein (N)	-7.5	Inhibits viral replication	Molecular docking	(21)
ZINC01530605	Ligand	Nucleoprotein (N)	-7.2	Inhibits viral replication	Molecular docking	(21)
(+)-Catechin	Phytochemical	Nucleoprotein (N)	-8.1	Natural inhibitor	Molecular docking	(40)
Kaempferol	Phytochemical	Nucleoprotein (N)	-7.9	Natural inhibitor	Molecular docking	(40)
Emtricitabine	Drug repurposing	Polymerase (L)	-7.8	Potential inhibitor	Molecular docking	(32)
Famciclovir	Drug repurposing	Polymerase (L)	-7.5	Potential inhibitor	Molecular docking	(32)
Multi-epitope peptide 1	Vaccine design	Various	Varies	Induces immune response	IEDB, Bepipred	(36,37)
Multi- epitope peptide 2	Vaccine design	Various	Varies	Induces immune response	IEDB, Bepipred	(36,37)

inhibitor against rabies; this demonstrates the role of natural product-based drugs (40).

Likewise, dodecandrin from *Phytolacca dodecandra* L'Hérit., a ribosomal inactivating protein, is expected to have an antiviral effect by depurinating the α-sarcin/ricin loop of the ribosomal RNA, thereby halting protein synthesis and inhibiting viral replication (41). *Datura metel* Linn. contains anticholinergic agents such as atropine, which can affect nervous system function by acting as competitive antagonists of muscarinic acetylcholine receptors. This blockade can regulate nervous system activity, perhaps relieving neurological signs of rabies (42). *Salix subserrata* and *Silene macroselen* were also previously investigated for the ability of their extracts to extend the survival rates of mice infected with rabies, although no specific bioactive compounds were reported (40). Although these plants have exhibited some potential in laboratory settings, they have not been proven effective for human rabies treatment, setting the stage for additional pharmacological and clinical studies (9). Additionally, salviifoside A from *Alangium salviifolium*, was previously demonstrated to exhibit potential in selectively inhibiting RVG interactions with promising binding scores derived from docking analyses against RVG protein structures obtained from UniProt (Accession no. A3RM22) (43). That study utilized homology modelling with SWISS-MODEL to create potential structures for docking analysis.

In summary, phytochemical-derived compounds present an encouraging and diverse pool of natural antiviral substances with possible activity against RABV targets; however, their therapeutic promise has yet to be definitively confirmed by thorough pharmacological and clinical trials to convert these initial results into efficient human therapies. An overview of the type of ligands, their targets and the mechanism of action is presented in Table II.

8. RNA-based therapeutics

RNA-based therapeutics have emerged as a promising strategy for the prevention and treatment of rabies, leveraging advanced vaccine platforms such as mRNA and self-replicating RNA (srRNA) technologies.

RNA interference (RNAi). Initial studies investigated RNAi as a post-transcriptional gene silencing mechanism against rabies. A previous study demonstrated that there was a 5-fold decrease in the RABV titer in BHK-21 cells by using short-interfering RNAs (siRNAs) against the mRNA of the N protein (44). Research has highlighted that microRNAs can significantly reduce genomic RNA and mRNA of the proteins in replication and pathogenesis, indicating the potential of RNAi as an antiviral agent (44).

Animal studies have supported these findings. Gupta *et al* (14) documented extensive protection in mice intracerebrally infected with an adenovirus vector expressing siRNA coding-sequences against the N and L proteins, and subsequent challenge with fixed RABV. In addition, another study found decreased N protein synthesis, fewer clinical signs and reduced morbidity in mice intracerebrally inoculated with plasmids encoding for siRNAs against the N protein prior to challenge with the CVS fixed strain of RABV (45). Aptamers and siRNAs were tested by coupling them to each other and administering them with Lipofectamine. One of the siRNAs (N53) produced an 80.13% decrease in viral RNA, while aptamer UPRET 2.03 led to a 61.3% decrease when applied alone at 2 h post-infection (p.i). Chimera UPRET 2.03-N8 (aptamer-siRNA) at 24 h p.i. resulted in 36.5% inhibition of viral replication (46).

mRNA vaccines. mRNA technology has emerged as a rapid and scalable platform for the development of a rabies

Table III. Rabies virus strains and therapeutics

Strain	Geographical location	Host	Genetic variations	Impact on pathogenicity	Target for therapeutics	Therapeutic approach (Refs.)
RABV1	Africa	Domestic animals	Single nucleotide polymorphisms	Increased virulence in certain strains	Nucleoprotein (N)	Drug/ligand discovery (64-66)
RABV2	Asia	Bats	Insertions and deletions	Variable pathogenicity	Glycoprotein (G)	Vaccine design (64-66)
RABV3	Americas	Wild animals	Amino acid substitutions	Reduced efficacy of some vaccines	Polymerase (L)	Drug repurposing (64-66)

vaccine. In a previous study, an optimized mRNA vaccine construct (LVRNA001) encoding the RABV glycoprotein (RABV-G) induced neutralizing antibody titers and a robust Th1 cell-mediated immune response in mice (47). Protection against lethal challenge with RABV was demonstrated in mice and dogs. The post-exposure immunization of dogs with LVRNA001 yielded a 100% survival rate, compared to 33.33% for the inactivated vaccine (47).

The thermostability of mRNA rabies vaccines was also explored in another study. It was established that extended storage at temperatures between -80 and +70˚C, or exposure to alternating temperatures, did not impair the protective potential of the vaccine in mice, suggesting that a rigorous cold chain may not be necessary (48). Another study focused on nucleoside-modified mRNA-lipid nanoparticle (LNP) vaccines. A single immunization of RABV-G mRNA-LNP in mice induced stronger humoral and T-cell immune responses than three doses of an inactivated vaccine. This single-dose mRNA vaccine provided complete protection against rabies, with the immune response persisting for at least 25 weeks, and possibly longer with a two-dose regimen (49).

CureVac AG engineered a RABV (RABV) mRNA vaccine, CV7201, using the cationic protein protamine to encapsulate mRNA that encodes the RABV glycoprotein. CV7201 was thermostable and effectively elicited a WHO-defined antibody response in 70.3% of subjects through three intradermal immunizations (Clinical trial no. NCT02241135). Optimizing the LNP formulation from CV7201, CureVac AG engineered CV7202, which employs the identical mRNA antigen as CV7201. The optimized LNP contains an ionizable amino lipid, a PEG-lipid-modified one, phospholipid and cholesterol (50). CV7202 exhibited acceptable tolerance in a clinical trial (Clinical trial no. NCT03713086) (50). Stokes *et al* (51) used a cationic nanoemulsion to encapsulate saRNA coding for alphavirus RNA-dependent RNA polymerase and the rabies glycoprotein G.

RNA-based adjuvants. The application of RNA adjuvants to boost the immunogenicity of rabies vaccines has also been investigated (52). A first-in-concept human study evaluated the safety, tolerability and immunogenicity of the TLR 7/8/RIG-I agonist RNA adjuvant CV8102, used alone or blended with a commercial rabies vaccine (Rabipur®). The results revealed that CV8102 was safe and well-tolerated, with doses of 25 and 50 μg markedly increasing the immunogenicity of reduced doses of the rabies vaccine (52). Consequently, RNA-based therapeutics, including RNAi, mRNA vaccines and RNA-based adjuvants, exhibit tremendous potential in developing rabies prevention and treatment methods, with further research aimed at maximizing their efficacy, safety and accessibility for global use.

9. Regional variations and implications for treatment

Understanding the genetic diversity of the RABV (Table III) is crucial for designing effective vaccines. A previous study analyzing 50 dog-brain samples from suspected rabies cases in India provided insight into regional viral variations (53). PCR screening targeting the nucleoprotein (N) and glycoprotein (G) genes, followed by sequencing, revealed that six isolates from Mumbai belonged to a single Arctic lineage which traced back to an Indian lineage I from 2006. Bayesian coalescent analysis estimated the time to the most recent common ancestor (TMRCA) for these sequences to be 1993, indicating long-standing geographic clustering. These phylogenetic data underscore the importance of tracking viral evolution for epidemiological studies and vaccine development (53). Sequence analysis further revealed amino acid polymorphisms in G-protein epitopes, influencing antigenicity and pathogenicity. These findings highlight the necessity for region-specific vaccine strategies to account for genetic variations in circulating RABV strains (53).

10. Bioinformatics and integrative strategies to fight rabies virus

Computational studies play a critical role in decoding the intricacies of RABV pathogenesis. Research into intrinsically disordered protein regions of the RABV proteome has yielded key insights (51). High intrinsic disorder levels in the phosphoprotein (P-protein) and nucleoprotein (N-protein) are linked to Negri body formation and host immune suppression. Proteomics analysis of highly purified RABV (RABV) has revealed 47 host proteins that become entrapped inside viral particles upon assembly. Of these, 11 are highly disordered. A comprehensive study on five of these highly disordered mouse proteins, neuromodulin, Chmp4b, DnaJB6, Vps37B and Wasl, utilized bioinformatics tools such as FuzDrop, D2P2, UniProt, RIDAO, STRING, AlphaFold and ELM to investigate their intrinsic disorder propensity (54). These disordered host proteins appear to play a major role in RABV pathogenicity, immune evasion, and possibly resistance

to antiviral drugs. That study highlights the complex interplay between virus and host, in which intrinsic disorder plays a key role in viral pathogenic processes. This suggests that these intrinsically disordered proteins and their corresponding host interactions might be good candidates for therapeutic targets (54).

In addition, dysregulation in the matrix (M) protein could play a role in viral budding and transmission, affecting the capacity of the virus to spread. Phylogenetic analysis, comparing RABV isolates from different host species and geographic regions, has revealed clear patterns of clustering consistent with host-specific adaptation of the virus (55). These patterns highlight the ecological niche of Lyssavirus, which is strongly shaped by their mammalian hosts, with implications for transmission dynamics and vaccine development.

Overcoming limitations of in silico approaches. While *in silico* approaches have revolutionized vaccine and drug discovery, it is important to recognize their limitations. One of the main challenges is the predictive reliability and accuracy of computational models, which are highly dependent on structural information. Flaws in this information, due to low-resolution inputs or errors in homology modelling, can undermine the efficacy of the models. Moreover, molecular docking and dynamics simulation tend to over-simplify biological interactions with the result of predictions being often at variance from actual practice. Incorporating high-resolution experimental information, e.g., cryo-electron microscopy, can effectively improve the fidelity of such models.

A key aspect of enhancing *in silico* approaches is strengthening the connection between computational methods and *in vitro* and *in vivo* confirmations. The inability of most predicted candidates to succeed experimentally demonstrates the need to improve the practical applicability of predictions. Additionally, present computational models lack consideration of host-pathogen interactions, immune systems, or PK/PD profiles, which are critical aspects. Using systems biology methods and AI-based simulations can make predictions more biologically relevant. Computational complexity and accessibility are also major challenges. Sophisticated approaches, including molecular dynamics simulations and quantum chemistry calculations, are computationally costly and hence scale poorly. Optimizing parallel processing algorithms and utilizing cloud-based platforms can help overcome these limitations. Furthermore, most sophisticated tools need specialized skills and hence access is restricted in low-resource environments where rabies is endemic. Providing improved user interfaces and training programs can facilitate access.

The use of combined *in silico* and *in vitro* approaches is crucial in the design of novel rabies therapeutics. Computational methods, such as molecular dynamics simulation and QSAR models, enable the prediction of the behavior of putative drug candidates before they are synthesized (56-60). These models can screen large compound libraries, thus accelerating the identification of lead candidates while reducing drug development time and costs. In addition, *in silico* approaches can model RABV interactions with host cells, providing insight into the life cycle of the virus and identifying novel therapeutic targets. The synergy of computational prediction and experimental confirmation is central to expediting the development of useful rabies vaccines and treatments.

Translation to practical application. Even with significant success in the use of computational resources towards therapeutic interventions for rabies, the translation of these *in silico* discoveries into viable treatments is a challenge. To address this issue, tackling the underreporting and misdiagnosis of cases, particularly in rural locations in endemic nations, calls for the creation of affordable, simple, and usable point-of-care diagnostic devices. Strengthening surveillance systems with a view to collecting accurate data in a timely manner is indispensable for successful control of rabies (61). Likewise, a One Health approach can be adopted which was proven successful in several countries such as Bhutan, Bangladesh and Sri Lanka (62). Further improvements in the prediction accuracy of epidemiological models, by incorporating real-time data and environmental factors, can result in more efficient resource deployment and enhanced predictive capabilities. Public education programs for raising awareness and adherence to rabies prevention strategies, like dog vaccination and prompt post-exposure prophylaxis, are also essential (63).

Finally, bioinformatics and computational approaches are crucial for progressing the knowledge on the pathogenesis of RABV and establishing efficient strategies for prevention and treatment. Overcoming the disconnect between computational prediction and experimental investigations is necessary for bridging *in silico* discovery and practical implementation and, eventually mitigating the global disease burden of rabies.

11. Conclusion and future perspectives

Addressing rabies at the therapeutic level remains a major scientific and public health challenge, particularly in regions with limited access to post-exposure interventions. While vaccines have substantially reduced transmission, effective treatments for symptomatic cases are still lacking. The present review highlighted how computational tools are transforming the drug discovery landscape for rabies by enabling the exploration of novel targets and mechanisms. Notably, *in silico* platforms have facilitated the rational design of peptide vaccines, the identification of antiviral leads, and re-evaluation of existing drug libraries with improved precision and efficiency. However, the success of these approaches centers on their integration with experimental validation pipelines. Future efforts are required to prioritize translational studies, interdisciplinary collaborations and capacity building in endemic regions to harness the full potential of bioinformatics-driven research. Ultimately, a data-driven, systems-level approach may provide the most promising route toward effective and equitable rabies control.

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Authors' contributions

RR drafted the manuscript. SS contributed to the conceptualization of the study, assisted in drafting the manuscript and supervised the study. Both authors were involved in the literature search. Both authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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