Letters in Drug Design & Discovery 22 (2025) 100030

Contents lists available at ScienceDirect

Letters in Drug Design & Discovery

journal homepage: www.keaipublishing.com/en/journals/lddd/

AI-driven in silico discovery, design, and evaluation of novel antiviral candidates targeting Marburg virus nucleoprotein for advanced therapeutic development

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ARTICLE INFO

Keywords:
Marburg virus disease
Nucleo-protein
Crizotinib
Drug design
In-silico tools
Artificial intelligence

ABSTRACT

Background: Marburg virus disease (MVD) is a highly virulent hemorrhagic fever caused by the Marburg virus (MARV), with case fatality rates reaching up to 90 %. Currently, no FDA-approved antiviral drugs exist for MVD, and available treatments are limited to supportive care. This highlights an urgent need for effective and targeted antiviral therapies.
Objective: The aim of this study was to design, screen, and evaluate novel antiviral candidates targeting the nucleoprotein of MARV using AI-assisted in silico drug design and molecular docking approaches.
Methods: The 3D structure of the MARV nucleoprotein (PDB ID: 5F5M) was retrieved and analyzed to predict active binding sites using computational tools. Crizotinib, an FDA-approved tyrosine kinase inhibitor, was selected as the lead compound based on virtual screening against the AXL gene—a key target associated with MARV infection. Using the WADDAICA AI platform, three novel Crizotinib-derived molecules were generated. Molecular docking was performed via CB-Dock2 to assess binding affinity with the nucleoprotein. Pharmacokinetic and toxicity profiles of the designed molecules were evaluated through ADMET and ProTox-II analyses. Molecular dynamics (MD) simulation was conducted using the iMODS server to validate the stability and interactions of the docked complexes.
Results: Among the three designed molecules, Molecule 3 demonstrated the highest binding affinity with the MARV nucleoprotein (− 8.3 kJ/mol). ADMET analysis indicated that Molecule 3 exhibited favorable absorption, distribution, and elimination properties, and was non-mutagenic, non-carcinogenic, and non-hepatotoxic. Toxicity analysis further supported its low-risk profile. Molecular dynamics simulations confirmed stable interactions with low deformability and consistent eigenvalues, validating its structural compatibility as a drug candidate.
Conclusion: This AI-driven in silico study successfully identified a promising Crizotinib-derived molecule (Molecule 3) with high binding affinity, favorable pharmacokinetic properties, and minimal toxicity. These findings support the potential of Molecule 3 as a lead antiviral candidate against MVD, warranting further in vitro and in vivo validation to assess its therapeutic efficacy.

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https://doi.org/10.1016/j.lddd.2025.100030
Received 7 March 2025; Received in revised form 7 April 2025; Accepted 22 April 2025
Available online 16 May 2025
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Introduction

Marburg virus disease (MVD) is a severe viral hemorrhagic fever caused by the highly pathogenic Marburg virus (MARV), which affects humans and primates with a high fatality rate.[1] MARV primarily spreads from fruit bats to humans and subsequently through direct contact with infected individuals or contaminated materials. MVD is a rare disease with high fatality rate ranging from ranging from 23 % to 90 %. The estimated incubation period of Marburg virus is 3–21 days.[2]

Despite being first identified in 1967, the Marburg virus remains a neglected pathogen with limited therapeutic advancements, largely due to its sporadic outbreaks and high biosafety requirements for laboratory research.[3] Recent re-emergence of MARV in various African regions, along with its high transmissibility and mortality rate, has highlighted the urgent need for novel antiviral strategies. Traditional drug development approaches are time-consuming and resource-intensive, which underscores the importance of integrating computational and AI-driven platforms to accelerate the discovery process.[4] Among the viral components, the nucleoprotein (NP) has emerged as a particularly promising drug target due to its essential role in viral genome encapsulation, replication, and interaction with other viral elements. Targeting NP could disrupt the life cycle of the virus at a fundamental level, making it a rational focus for antiviral development.[5]

MVD initially presents with flu-like symptoms such as high fever, chills, and severe headache. As the disease progresses, symptoms may include nausea, diarrhea, severe abdominal pain, dysphagia, leukopenia, neurological complications, and, in severe cases, bleeding from various body sites.[6] Advanced stages of the disease are characterized by dehydration and acute renal failure. Most patients develop rashes. Severe damage to the nervous system can lead to restlessness, confusion, and unconsciousness, which may progress to deep coma and ultimately result in death.[7]

The Marburg virus is enveloped and features glycoprotein spikes on its surface. The nucleocapsid, composed of RNA and approximately 29 nucleoprotein molecules, facilitates viral replication and transcription. The replication and transcription activity of MARV depends on the nucleoprotein (NP), which wraps the viral RNA genome.[8] Currently, vaccines or drugs for antiviral treatment are not approved for MVD. However, to improve survival, treatment with fluids for rehydration, supportive care and symptom-specific medications can be taken. Given the absence of approved vaccines or antiviral treatments for MVD, there is a pressing need to develop effective therapeutic options to mitigate the significant public health risk posed by this lethal virus.[9]

This study aims to design and evaluate potential antiviral candidates for MVD through an AI-assisted in-silico approach. The focus is on targeting MARV nucleoprotein, a critical component in viral replication and transcription that interacts with other viral proteins. Firstly, the 3D structure of the nucleoprotein was retrieved along with identification of its binding sites. Then, a selectivity analysis against the AXL gene was performed, selecting Crizotinib as the primary compound against nucleoprotein after screening for toxicity and allergenicity, to aid in the design of an efficient drug against MARV. Crizotinib is a kinase receptor inhibitor. It can block the viral replication by interfering with the nucleoprotein of MARV. In AI-assisted drug design against MARV, three de novo drug combinations targeting nuclear proteins were developed using crizotinib. Molecular docking and molecular dynamics simulations identified the most promising molecule based on binding affinity, stability, and chemical properties, including an evaluation of hepatotoxicity.[10,11]

This study highlights the novel possibilities to solve the negative problems of MARV by creating the combination of well-designed products with artificial intelligence. The designed molecule is an effective drug target against nucleoprotein of MARV thus can interfere with the replication and transcription of viral genome, suppressing its pathogenicity. The proposed drug candidate demonstrates potential to reduce MVD mortality and mitigate the public health impact of future MARV outbreaks. Further in vitro and in vivo studies are required to validate its clinical efficacy.

Material and methods

Target protein selection and structural data acquisition

The nucleoprotein was selected as the target for drug design against MVD because of its critical role in the replication and transcription of the viral genome. Targeting this protein offers a promising strategy to disrupt the propagation of the Marburg virus in host cells.[12] The 3D structure of the Marburg virus nucleoprotein (PDB ID: 5F5M) was downloaded from PDB (http://www.rcsb.org/pdb/, accessed on 10 December 2023), global database of structural data for biological macromolecules.[13]

Protein binding site prediction

Identifying protein-ligand binding sites is crucial for understanding protein function and guiding drug discovery. Using the COACH metaserver for protein-ligand binding sites prediction, ligand-protein binding site predictions using S-SITE and TM-SITE methods were generated. The PDB data for the nucleoprotein were uploaded to the COACH server.[14]

Ligand selection and virtual screening

The NIH and HiTS Small Molecule Suite (https://lsp.connect.hms.harvard.edu/smallmoleculessuite) is an open-source platform used for ligand screening and drug discovery. This tool was employed to identify potential molecules targeting the AXL gene, a receptor tyrosine kinase implicated in viral replication. Crizotinib, a known kinase receptor inhibitor, was selected as a promising candidate due to its compatibility with the target site and its favorable pharmacological properties. Its selection was based on its compatibility with the target site and its favorable pharmacological properties for translation into an antiviral drug.[15]

AI-driven de novo drug design

WADDAICA is an open-source AI platform that provides deep learning models for drug development. It is used by users to modify or create new drugs. Using Crizotinib as a guide, three drug molecules were generated by the AI server.[16]

Toxicity and pharmacokinetic assessment

Toxicity screening of Crizotinib and the AI-generated molecules was performed using ProTox 3.0 (http://tox.charite.de/protox_II accessed on December 20, 2023). This platform estimates the biological properties and potential toxicity of small molecules. The evaluation focused on LD50 values, hepatotoxicity, and other key safety parameters, validated using an external dataset.[17] Data for the three AI-generated molecules were input into Vnnadmet can be assessed by using https://vnnadmet.bhsai.org/vnnadmet/home.xhtml for distribution, metabolism, excretion and toxicity analysis. ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties are crucial in assessing the drug potential of a molecule.[18]

Molecular docking studies

Molecular docking was performed using CB-Dock (http://clab.labshare.cn/cb-dock), a docking tool that integrates AutoDock Vina. The binding pocket size and docking box dimensions were optimized based on ligand size. Input files for the nucleoprotein and ligands were provided in PDB format, and the docking results were analyzed to assess binding affinity and stability. Proteins and ligands are provided as input in PDB format for docking in CB-Dock. The docking results of our intelligence-generating molecules and nuclear proteins were obtained and analyzed.[19]

Molecular dynamics and stability assessment

IMOD (http://imods.chaconlab.org) is a platform for investigating protein aggregation and simulating macromolecular dynamics, helping to visualize 3D bioimaging data. The program represents complex dynamics in macromolecules and explores possible transformations, flexible network possibilities, and solutions with a variety of coarse atomic descriptions and model accuracy. Molecular Dynamics simulations of the docking area were performed and the results were analyzed.[20]

Results

Retrieval and visualization of Marburg virus nucleoprotein

The 3D structure of the Marburg virus nucleoprotein (PDB ID: 5F5M) was retrieved from the Protein Data Bank (PDB), a widely recognized repository for structural data of biological macromolecules. The structure provides atomic-level insights into the nucleoprotein's architecture, critical for understanding its function and potential drug-binding interaction sites. Visualization of the retrieved structure was performed using Discovery Studio, a robust software suite for molecular modeling and visualization. The structure was analyzed to identify key domains and structural features relevant to binding site prediction and drug design, as depicted in Fig. 1.

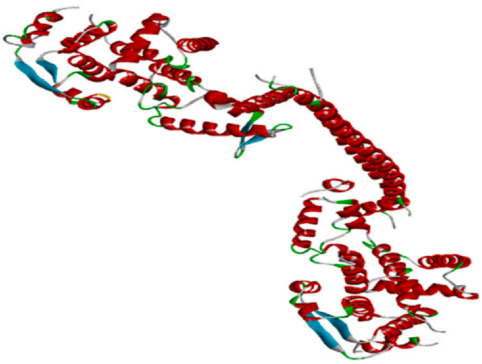

Fig. 1. 3D structure of the Marburg virus nucleoprotein (PDB ID: 5F5M) visualized using Discovery Studio, highlighting its detailed molecular architecture essential for binding site prediction and drug design.

Prediction of ligand binding sites in nucleoprotein structure

Binding site prediction for the Marburg virus nucleoprotein identified several potential ligand-binding regions based on C-scores, cluster sizes, and consensus binding residues. The top-ranking site (C-score: 0.18) is associated with nucleic acid binding, as identified in PDB structure 2yhmG, and includes key residues such as 141, 145, 153, 231, and 291. This site, the largest cluster with 12 binding residues, is significant for interactions. The second-ranking site (C-score: 0.06) is associated with peptide binding, as seen in structure 4ypiB, with residues 222, 230, 256, and 344 involved. Other notable sites include interactions with small molecules such as calcium (CA), magnesium (MG), mannose (MAN), and S-adenosylmethionine (SAM) in lower-ranking predictions. The Table 1 below represents the Predicted Binding Sites of Marburg Virus Nucleoprotein. Each site provides valuable insights into functional hotspots that can be targeted for therapeutic interventions. Screening of compounds targeting the AXL gene for Marburg virus disease (MVD) using the Small Molecule Suite is depicted in the Fig. 2.

Identification of AXL gene as a potential therapeutic target

The AXL gene was identified as a crucial target for Marburg virus disease (MVD) due to its role in various viral processes Compounds were screened to identify potential inhibitors of this gene using the Small Molecule Suite, a platform for virtual screening against specific targets. Among the compounds tested, Crizotinib (PubChem ID: 11626560) stood out as a promising selective inhibitor of the AXL gene. Crizotinib, a tyrosine kinase inhibitor, has been shown to target cancer-related genes, and its potential as an AXL inhibitor suggests it may also play a role in inhibiting MVD progression. This result highlights Crizotinib as a candidate for further investigation as a therapeutic agent against Marburg virus infection.

Crizotinib characterization as a candidate antiviral compound

Crizotinib is an FDA-approved tyrosine kinase inhibitor, originally developed for the treatment of ALK-positive tumors. The 3D structure of Crizotinib (Fig. 3) was retrieved and analyzed for its relevance to Marburg virus disease (MVD). Its physio-chemical properties, detailed in Table 2, were assessed using data from PubChem (PubChem ID: 11626560), providing insight into its potential as a selective AXL inhibitor for MVD treatment.

AI-based design of novel crizotinib-derived therapeutic molecules

Leveraging the AI capabilities of the WADDAICA platform, Crizotinib was used as a foundational molecule to design novel therapeutic candidates for MVD. Through the competition model, crizotinib underwent strategic modifications to create three distinct chemical entities, each optimized for targeting the AXL gene. The resulting AI-designed molecules, depicted in Fig. 4, illustrate the structural alterations. These modifications enhance binding affinity, specificity, and therapeutic potential against MVD.

ADMET profiling of crizotinib and AI-designed molecules

VNN-ADMET was used to conduct ADMET (Absorption, Distribution, Metabolism, Elimination, and Toxicity) analysis of drug targets. As shown in Table 3, Crizotinib was found to be hepatotoxic, and the AMES carcinogenicity test returned a positive result, indicating potential risks.

In contrast, Table 4 demonstrates that AI-generated Molecule 1 is neither hepatotoxic nor carcinogenic, with a negative AMES result, suggesting it is a safer candidate. Molecules 2 and 3, while predicted to be hepatotoxic, yielded positive AMES results. Despite this, AI-generated molecules are overall non-toxic and show promise as potential drug candidates.

Table 1
Predicted binding sites of Marburg virus nucleoprotein.

Rank	C-score	Cluster Size	PDB Hit	Ligand Name	Download Complex	Consensus Binding Residues
1	0.18	12	2yhmG	Nuc_Acid	Rep, Multi	141, 142, 144, 145, 153, 156, 157, 213, 216, 225, 226, 227, 231, 291, 293, 313, 315, 317, 318
2	0.06	4	4ypiB	PEPTIDEd	Rep, Multi	222, 223, 230, 234, 235, 237, 238, 256, 259, 260, 263, 266, 267, 269, 270, 273, 274, 276, 344
3	0.03	2	4b5wA	CA	N/A	147, 153
4	0.02	3	3hiyB	MG	N/A	68, 70
5	0.02	3	N/A	N/A	N/A	148, 391, 394
6	0.02	1	2vm4A	MAN	N/A	477, 482
7	0.01	2	2wi8A	Nuc_Acid	Rep, Multi	157, 161, 213, 216
8	0.01	1	N/A	N/A	N/A	407, 409, 410, 412, 414
9	0.02	1	N/A	CA	N/A	510, 514, 515, 517, 518, 521
10	0.02	1	3nmuA	SAM	N/A	274, 275

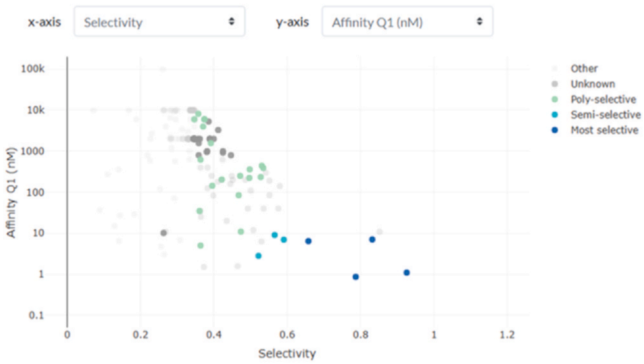

Fig. 2. Screening of compounds targeting the AXL gene for Marburg virus disease (MVD) using the Small Molecule Suite. Crizotinib (PubChem ID: 11626560) was identified as a selective inhibitor of the AXL gene, showing promise for further investigation as a potential therapeutic agent for MVD.

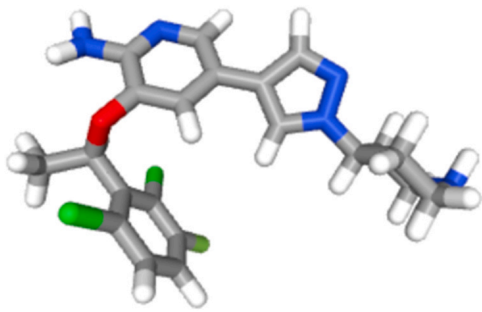

Fig. 3. 3D structure of crizotinib, an FDA-approved drug. Crizotinib, a tyrosine kinase inhibitor, has been identified as a selective inhibitor of the AXL gene in the context of Marburg virus disease (MVD). This figure highlights the molecular structure of Crizotinib and its relevance in the study of MVD treatment.

Table 2
Physio-chemical properties of crizotinib.

Property Name	Property Value
Molecular weight	450.3 g/mol
XLogp3-AA	3.7
Hydrogen bond donor count	2
Hydrogen bond acceptor count	6
Rotatable bond count	5
Exact mass	449.1185439 g/mol
Monoisotopic mass	449.1185439 g/mol
Topological polar surface area	78 Å
Heavy atom count	30
Formal charge	0
Complexity	558
Isotope atom count	0
Defined atom stereocenter count	1
Undefined atom stereocenter count	0
Defined bond stereocenter count	0
Undefined bond stereocenter count	0
Covalently-bonded unit count	1
Compound is canonicalized	Yes

Toxicological evaluation of crizotinib and derivative molecules

The toxicity of crizotinib was evaluated using ProTox and the results showed possible immunosuppression and carcinogenicity. Meanwhile, hepatotoxicity, cytotoxicity, and mutagenicity scores are low. Inactivity against toxic receptor signaling pathways and stress response pathways is shown in the table below. Table 5 below represents the Predicted Toxicity Endpoints and Classification for Various Targets.

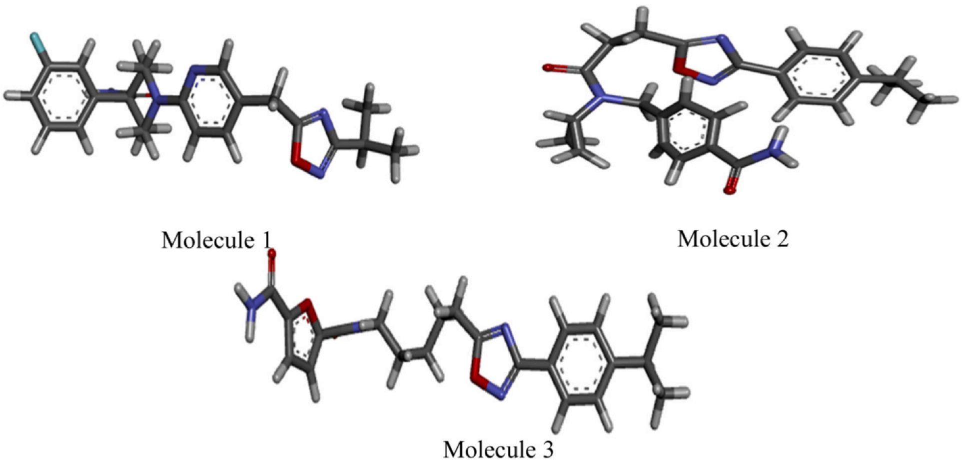

Fig. 4. Structural representation of AI-designed molecules derived from crizotinib, optimized for enhanced binding affinity to the AXL gene. These structures were generated and refined using the WADDAICA platform, highlighting molecular modifications aimed at improving target specificity and binding efficiency.

Docking analysis of AI-generated ligands with nucleoprotein

Crizotinib and AI-developed nuclear proteins were docked using the blind docking option of CB-Dock2. AI-designed molecules 1, 2, and 3 were docked one-to-one with nuclear proteins, and their docking complexes were analyzed separately. The results showed that the binding affinity of the ligands to the nucleoprotein docking complex ranged from 7 to 8 kcal/mol, indicating strong and effective binding. AI-generated molecules 1, 2, and 3 formed docked complexes with nucleoprotein, with binding affinities of $-$ 8.3 kJ/mol, $-$ 8.4 kJ/mol, and $-$ 8.4 kJ/mol, respectively. The docking complexes and 2D bonding interactions are shown in Fig. 5.

Selection of optimal ligand based on multi-parameter evaluation

Ligand selection was performed based on ADMET analysis results, toxicity screening, and bonding interactions between the ligand and nucleoprotein in the docking complex. All three ligand molecules were screened and analyzed according to these parameters. AI-generated molecule 3 from Crizotinib was selected as the best candidate for drug development due to its strong binding interactions with the Marburg virus nucleoprotein. It was also predicted to be non-mutagenic according to the AMES test. Furthermore, molecule 3 is not a HERG blocker, is non-hepatotoxic, and is not carcinogenic.

Molecular dynamics simulation of the nucleoprotein–ligand complex

iMODS was used to evaluate several parameters characterizing the docking complex between the AI-generated molecule 3 (based on crizotinib) and the nuclear protein. Fig. 6A illustrates the deformability of the docked complex, while Fig. 6B presents the B-factor, reflecting the mobility of each residue. The eigenvalues, which indicate the stiffness of the complex and its resistance to deformation, are shown in Fig. 6C. Fig. 6D displays the number of variants in the junctional complex, where individual variants are highlighted in purple and distinct variants in green. The modes of motion across the structure are presented in Fig. 6E, with interacting regions marked in red and anti-interacting regions in blue. Finally, Fig. 6F shows the elastic network, where fixed regions are indicated in gray. Notably, enhanced interactions between individual residues are associated with low RMSD values. The calculated charge of the docked complex was approximately 5.775623×10^{-7}.

Discussion

Marburg virus causes hemorrhagic fever, which is highly contagious and fatal in humans and nonhuman primates. MARV is the virus that causes Marburg disease (MVD) in humans, with a fatality rate ranging from 23 % to 90 %, depending on the dose. Given these high fatality rates and the lack of targeted therapeutics, identifying antiviral agents that target specific viral proteins is crucial.[21] According to an in-silico study, the receptor binding sites of MARV nucleoprotein are promising candidates for drug design aimed at controlling the spread of MARV by suppressing its replication in the host. The replication and transcription of the MARV genome depend on the presence of the nucleoprotein (NP).[22]

Compared to previous studies, this in-silico analysis stands out by targeting the nucleoprotein (NP) of Marburg virus (MARV) as the primary inhibitory site. Previous studies have predominantly focused on targeting other viral proteins, such as the glycoprotein (GP) or polymerase (L) proteins, which play crucial roles in MARV's entry into host cells and genome replication.[23] For instance, glycoprotein inhibitors have been shown to disrupt MARV entry, and polymerase inhibitors aim to interfere with viral replication. However, while effective, these approaches have certain limitations. In contrast, targeting NP, a unique viral protein, minimizes overlap with host cellular machinery, potentially reducing adverse effects.[24]

Table 3
Crizotinib's liver toxicity, metabolism, and interactions with cytochrome P450 enzymes, membrane transporters, and other pharmacological parameters.

Query	Liver Toxicity (DILI)	Cyto-toxicity	Cyp 1A2	Cyp 3A4	Cyp 2D6	Cyp 2C9	Cyp 2C19	BBB	P-gp Inhibitor	P-gp Substrate	Herg Blocker
Mol 1	Yes	No	No	No	No	No	No	Yes	Yes	Yes	Yes
Mol 2	Yes	Yes	No	No	No	No	No	Yes	No	Yes	Yes
Mol 3	No	Yes	No	No	No	No	No	Yes	No	Yes	Yes

Table 4
Pharmacological properties of the three designed molecules, including liver toxicity, metabolism, cytochrome P450 inhibition, membrane transporter interactions, and other key parameters such as HERG blocking, MMP activity, AMES test, and predicted oral bioavailability (MRTD).

Molecule	Liver Toxicity (DILI)	Cyto-toxicity	HLM	CYP Inhibitors	BBB	P-gp Inhibitor	P-gp Substrate	HERG Blocker	MMP	AMES	MRTD
Mol 1	No	No	Yes	1A2: No, 34A: No, 2D6: No, 2C9: No, 2C19: No	No	No	Yes	Yes	No	No	193
Mol 2	Yes	No	Yes	1A2: No, 34A: No, 2D6: No, 2C9: No, 2C19: No	No	No	Yes	Yes	No	No	217
Mol 3	Yes	No	Yes	1A2: No, 34A: No, 2D6: No, 2C9: No, 2C19: No	No	No	Yes	No	No	Yes	

Our findings build on those of Suraya et al.,[25] who emphasized the pivotal role of NP in MARV replication, and further demonstrate the potential of specific inhibitors designed via computational approaches. Inhibiting NP could offer a unique advantage, as it directly impacts the stability and replication of the viral genome, potentially leading to a more specific and potent antiviral effect. Unlike glycoprotein or polymerase inhibitors, targeting the nucleoprotein may present fewer side effects, as it is a unique viral component without direct analogs in host cells, reducing the likelihood of host cell toxicity.[26,27]

Furthermore, our selection and optimization of Molecule 3, based on ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) and molecular dynamics (MD) simulations, aligns with recent computational drug discovery approaches. Studies by Zhang et al. (2024) demonstrated the importance of ADMET screening in identifying safe and bioavailable drug candidates.[28,29] This highlights that Molecule 3, with favorable ADMET properties such as non-hepatotoxicity and high bioavailability, adheres to best practices in computational drug design.[30,31]

Our approach, combining computational docking and ADMET analysis with a focus on the nucleoprotein, presents a novel direction in MARV therapy.[32] While further experimental validation is needed, this strategy holds promise for developing a more specific antiviral

Table 5
Predicted toxicity endpoints and classification for various targets. The table presents the classification, prediction, and associated probability for organ toxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and signaling pathways based on the Tox21 database analysis.

Toxicity Model Report			
Classification	Target	Prediction	Probability
Organ toxicity	Hepatotoxicity	Inactive	0.53
Toxicity end points	Carcinogenicity	Active	0.55
Toxicity end points	Immunotoxicity	Active	0.65
Toxicity end points	Mutagenicity	Inactive	0.56
Toxicity end points	Cytotoxicity	Inactive	0.64
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon receptor	Inactive	0.80
Tox21-Nuclear receptor signaling pathways	Androgen receptor	Inactive	0.96
Tox21-Nuclear receptor signaling pathways	Androgen receptor ligand binding domain	Inactive	0.95
Tox21-Nuclear receptor signaling pathways	Aromatase	Inactive	0.84
Tox21-Nuclear receptor signaling pathways	Estrogen receptor Alpha	Inactive	0.85
Tox21-Nuclear receptor signaling pathways	Estrogen receptor ligand binding domain	Inactive	0.94
Tox21-Nuclear receptor signaling pathways	Peroxisome proliferator activated receptor gamma	Inactive	0.94
Tox21-Stress response pathways	Nuclear factor	Inactive	0.92
Tox21-Stress response pathways	Heat shock factor response element	Inactive	0.92
Tox21-Stress response pathways	Mitochondrial membrane potential	Inactive	0.80
Tox21-Stress response pathways	Phosphoprotein	Inactive	0.91
Tox21-Stress response pathways	ATPase family AAA-domain containing protein 5	Inactive	0.93

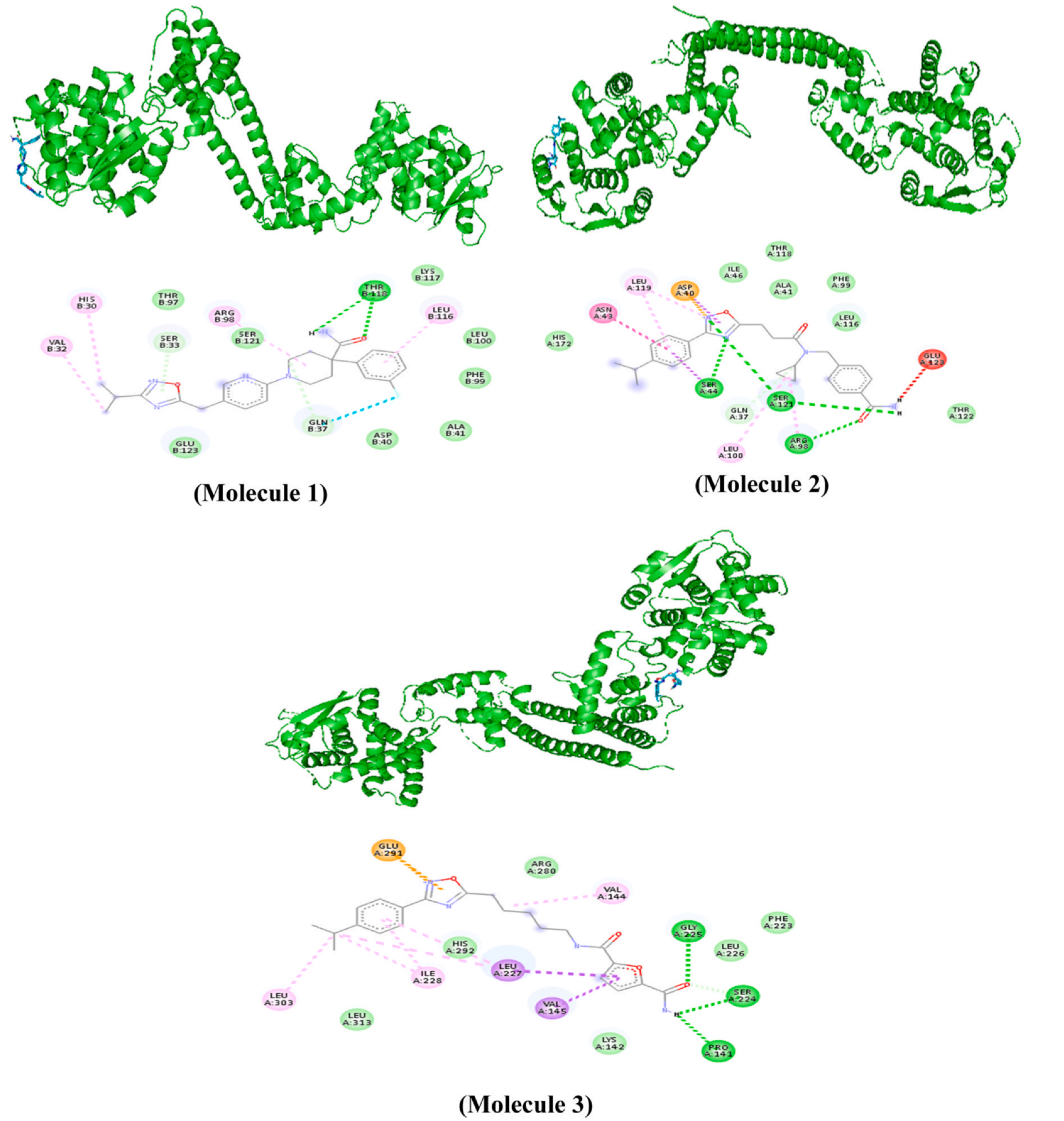

Fig. 5. Docked complexes and 2D bonding interactions between the nucleoprotein and AI-generated molecules. The 3D structure illustrates the overall binding conformation of each molecule within the active site of the nucleoprotein, while the 2D interaction diagrams highlight key hydrogen bonds, hydrophobic interactions, van der Waals forces, and other non-covalent interactions stabilizing the complex. These interactions provide insights into the binding affinity and specificity of the AI-designed ligands toward the target protein.

agent against MARV with potentially reduced toxicity, contributing to the expanding arsenal of antiviral strategies targeting the unique proteins of the virus rather than traditional entry or replication machinery.[33] This study provides a strong foundation for developing MARV-specific therapeutics by targeting the nucleoprotein, a strategy that may also be extendable to other filoviruses.[34,35]

Despite the promising outcomes, this study has several limitations. The conclusions are based solely on computational predictions, and no in vitro or in vivo validations have been conducted yet. The modeled binding affinities and ADMET predictions require experimental testing to confirm pharmacological efficacy and safety. Additionally, while the nucleoprotein is a compelling target, potential resistance mutations and host protein interactions were not assessed. Future studies should include high-throughput screening, protein crystallography, and cell-based assays to validate and refine the proposed antiviral candidates. These insights open avenues for the broader application of AI-assisted in-silico design in targeting structurally conserved proteins across other filoviruses, potentially accelerating therapeutic development pipelines.

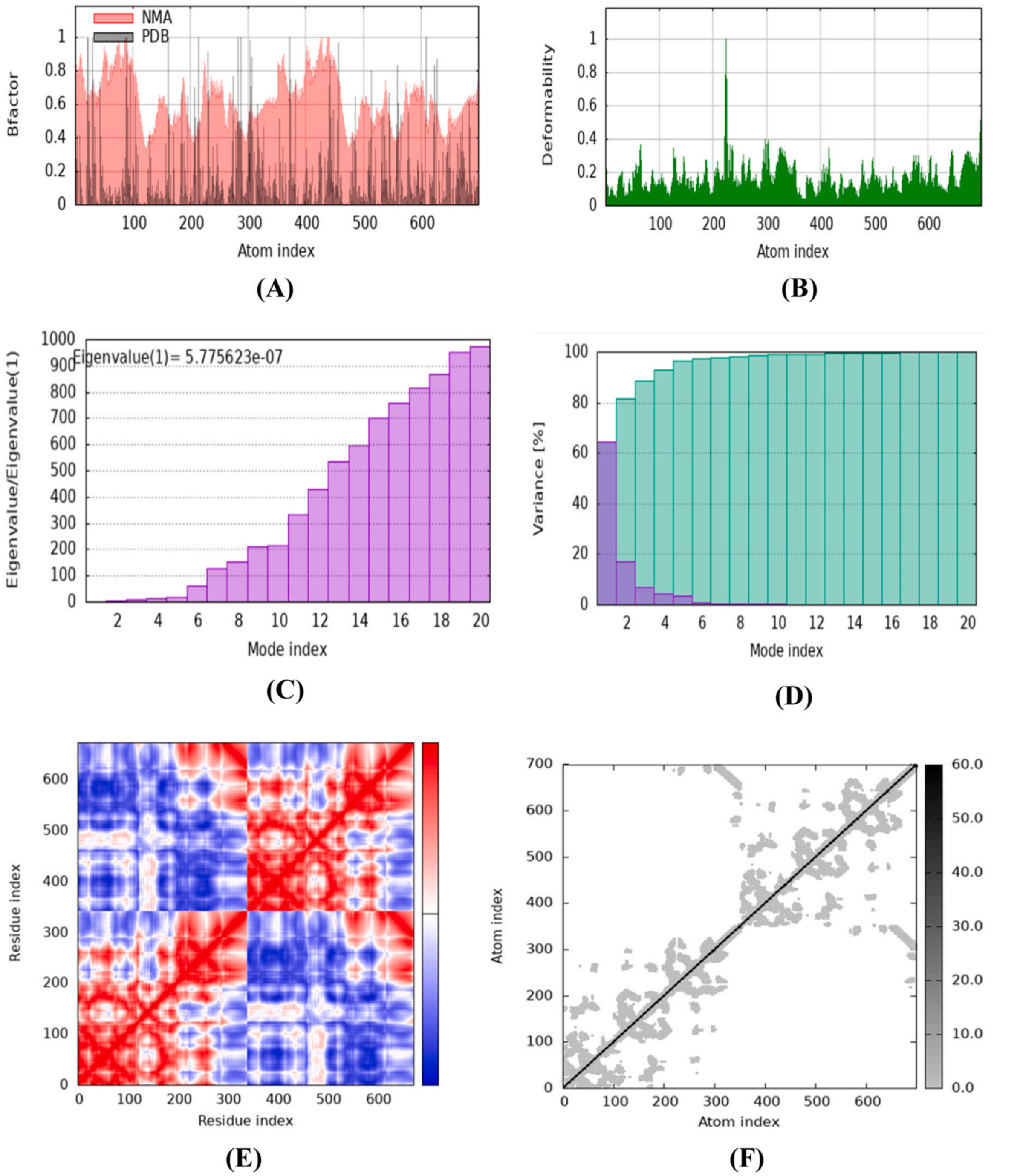

Fig. 6. iMODS analysis of the docking complex between AI-generated molecule 3 and the nucleoprotein. (A) Deformability of the complex; (B) B-factor plot indicating residue flexibility; (C) Eigenvalues representing structural stiffness; (D) Variants of the junctional complex, with individual variants shown in purple and different variants in green; (E) Co-variance map highlighting interacting regions in red and anti-interacting regions in blue; (F) Elastic network model showing fixed regions in grey.

Conclusion

This in-silico study employed advanced computational and bioinformatics tools to design a potential antiviral drug targeting the nucleoprotein of the Marburg virus. Three crizotinib-derived molecules were designed using an AI-driven approach to bind to the nucleoprotein of the Marburg virus, inhibiting its replication in host cells. The results showed that the AI-designed molecules not only demonstrated high binding affinity but also formed protective interactions with the nucleoprotein. These findings were confirmed by

ADMET analysis, which showed that the AI-designed molecules have a non-toxic profile and high bioavailability. This research holds significant public health implications, offering a potential therapeutic avenue to mitigate the mortality and risk associated with Marburg virus disease. However, further in vivo and in vitro studies are needed to validate these findings and assess the efficacy of these molecules against emerging global health threats.

CRediT authorship contribution statement

Krishnaraju Venkatesan: was involved in conceptualization, data curation, and formal analysis, and wrote original manuscript. **Premalatha Paulsamy**, **Saud Alqahtani**, **Yahya I.Asiri**, **Taha Alqahtani**, **Ali Alqahtani**, and **Khalid A Asseri** did formal analysis, data curation, and editing. **Durgaramani Sivadasan**, **Rehab Ahmed**, **Hassabelrasoul Elfadil**, **Absar A. Qureshi**, **Malarkodi Velraj** and **Kalpana Krishnaraju** did editing, review, and checked for grammar. All authors have read and agreed to the published version of the manuscript.

Ethics approval

No animal/humans has been used in this study, therefore, the ethics approval is not required.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Funding

This work is totally self-supported and no funding has been received from any source.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgements

The authors extend their sincere acknowledgment to the Deanship of Scientific Research at King Khalid University for funding this study through the Large Research Group Project under grant number "RGP 2/365/45".

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