chemistry proceedings

MDPI

Proceeding Paper

Pharmacoinformatics Based Drug Design for the Blocking of Deadly Nucleoprotein of Lassa Pathogenic Virus †

Mohammed Emon [1], Noimul Hasan Siddique [2], Trisad Arman Ovi [3] and Md Sarafat Ali [1,*]

[1] Department of Biotechnology and Genetic Engineering, Faculty of Life Science, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj 8100, Bangladesh; 18bge034@bsmrstu.edu.bd
[2] Department of Microbiology, Faculty of Science, Noakhali Science and Technology University, Noakhali 38154, Bangladesh; noimul0514@student.nstu.edu.bd
[3] Department of Biotechnology, Faculty of Engineering, National Institute of Technology Warangal, Warangal 506004, India; ta872068@student.nitw.ac.in
* Correspondence: sarafatbiotech@bsmrstu.edu.bd
† Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: https://ecsoc-27.sciforum.net/.

Abstract: Lassa fever is an animal-borne, or zoonotic, acute viral hemorrhagic illness caused by Lassa virus, a member of the arenavirus family of viruses. There is currently no licensed vaccine for Lassa fever, but several potential vaccines are in development. However, there is still a need for a safe treatment with proven efficacy and a range of potential treatments. In both viral RNA synthesis and immunological suppression, the Lassa virus' nucleoprotein (NP) plays a crucial role. An arenaviral NP that demonstrates surprising capabilities and suggests particular mechanisms for immune evasion and cap binding. These discoveries have a lot of potential for therapeutic development. Based on cheminformatics approach, we focused on designing a computational drug for the targeting of viral nucleoprotein of human pathogenic Lassa fever virus. We accomplished several vital steps including target protein and ligand identification and refinement, active site analysis, ADMET analysis, molecular docking, protein-ligand interaction analysis, etc. We retrieved Lassa viral nucleoprotein receptor from widely used RSCB PDB website as well as control ligand molecules including Arbidol, Ribavirin, Favipiravir, UTP (Uridine 5′-Triphosphate) from significant chemical database PubChem. ADMET property analysis by SwissAdme and pkCSM server, molecular docking study done by PyRx virtual screening tools, and Discovery studio used for further protein-ligand interaction analysis. We found a very significant molecular binding affinity score that promises the designed drug is more stable and reliable. However, it will demand to be considered considerably in vivo and in vitro experimental drug design models.

Keywords: Lassa virus; viral nucleoprotein; molecular docking; In Silico drug design; Pharmacoinformatics

Citation: Emon, M.; Siddique, N.H.; Ovi, T.A.; Ali, M.S. Pharmacoinformatics Based Drug Design for the Blocking of Deadly Nucleoprotein of Lassa Pathogenic Virus. *Chem. Proc.* **2023**, *14*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2023

1. Introduction

Lassa fever virus illnesses is comprehended as viral hemorrhagic fever [1]. The virus that causes Lassa fever is only found in West Africa, and it was originally identified in 1969 [2,3]. LFV outbreak began to grow in size in Nigeria in 2015, reaching new maxima in 2016, 2017, and 2018 [4]. In Nigeria 2019, there were 554 cases with laboratory confirmation and 124 fatalities, for a case fatality rate (CFR) of 22% [4]. Between epidemiological weeks 1 to 15 of 2023 (week ending 16 April), there have been 4702 suspected cases, five probable cases, and 877 confirmed cases of Lassa fever in Nigeria [5]. There have been 152 deaths among confirmed cases, representing a 17% case fatality rate [5]. LASV is caused by rodent-to-human LASV transmissions from direct infected animals or animal excreta, but there has also infected human- to-human [6].

The incubation period for LF is approximately 7–21 days, individuals infected with LASV are asymptomatic 80%, whereas 20% of infected individuals experience severe and multi system disease [6,7]. However, there have also been human-to-human infections and transmitted from rodents to humans by direct contact with sick animals or animal excrement [6]. A few days later, symptoms including nausea, vomiting, diarrhea, coughing, head ache, muscle pain, chest pain, nausea, and sore throat may occur [8]. The initial phases of illness can be detected using significant RT-PCR method, ELISA are the most frequently used to diagnose Lassa fever [8]. There is currently no vaccine that protect against Lassa fever [8]. The emergence of potential new therapeutics for Lassa fever, such as ribavirin, favipiravir and monoclonal antibodies play promising role against LASV [4,9,10].

2. Material and Methods

2.1. Receptor Protein Preparation

The crystal 3D structure of the target nucleoprotein (PDB ID: 3MX5) of infectious Lassa fever virus structure was obtained from the RSCB protein data bank (PDB) (https://www.rcsb.org/) [11–13]. The Biovia Discovery Studio visualization tool was per formed for the protein preparation [14].

2.2. Ligands Preparation

We select some FDA approved ligands such as Uridine 5′-triphosphate (PubChem CID: 6133), Arbidol (PubChem CID 131411), Ribavirin (PubChem CID: 37542), and Favipiravir (PubChem CID: 492405) were obtained from the PubChem data base (https://pubchem.ncbi.nlm.nih.gov/).

2.3. Active Site Determination

The widely significant CASTp v.3.0 server was used to predict the active sites of the Lassa virus refined nucleoprotein 3MX5 to the specific on chain C [15].

2.4. Molecular Docking Study

All docking studies were performed using the PyRx, which identifies good associations between the ligand and the protein [16]. A grid was constructed at the site of a co-crystallized ligand employing the PyRx tool [16]. This grid reflects features of focused protein and curvature utilized to produce more comprehensive ligand, poses assessment.

2.5. ADMET Analysis

We used computational way to anticipate ADMET properties of the ligands and their impurities to assist in quality monitoring of drugs. The SwissADME [17] and pkCSM [18] servers were used to predict the ADMET properties of the selected ligands.

3. Results and Discussion

3.1. Receptor Protein Retrieval and Preparation

Lassa Fever nucleoprotein 3MX5 [13] was chosen for this study and this 3D theoretical protein model was resolved using the X-ray diffraction method with a resolution factor of 1.90 Å [12]. Employing Discovery Studio 4.0 program [14], the ligands molecule, chain A, chain B, hetero atom, and water from PDB files were deleted, and only chain C of 3MX5 was utilized to screen and choose drug interaction for further examination [13].

3.2. Ligand Molecules Retrieval

Four FDA-approved antiviral drugs were selected after existing literature study review and databases were explored for potential small-molecule drug compounds against Lassa fever virus [4,9,12]. The ligand molecules including Uridine 5′-triphosphate

(DrugBank Accession No.: DB04005), Arbidol (DrugBank Accession No.: DB13609), Ribavirin (DrugBank Accession No.: DB00811), and Favipiravir (DrugBank Accession No.: DB12466) were retrieved as 2D and 3D conformer in SDF format from PubChem source.

3.3. Active Site Determination and Grid Box Set Up

CASTp webserver was used to generated active site of nucleoprotein structure 3MX5 refined protein of Lassa virus which is carrying specific target chain C [15]. In CASTp server we found most area (SA) $Å^2$ 1919.831 coverage in pocket ID 1 with most volume (SA) $Å^3$ 1818.156. According to AS sequence ID alignment and manipulation in PyRx docking environment we set specific grid box center x, y, z is 60.6760676459, 54.6751522917, 19.7695301284 and size x, y, z is 41.5732287602, 54.9212097324, and 40.1680733943 respectively.

3.4. ADMET Properties Prediction

We investigated the ADME characteristics by the SwissADME [17] of UTP, Arbidol, Ribavirin, and Favipiravir with their specific SMILE ID retrieved from PubChem. MW, heavy atoms, H-bond acceptors, rotatable bonds, H-bond donors, consensus Log Po/w, Log S (ESOL), GI absorption, BBB permeant, and Lipinski's rule etc. characteristics studied significantly. (Table 1) Accessibility and drug likelihood traits, also known as Pfizer's rule of five, are trustworthy criteria for assessing the possibility that a prescription will be prescribed, as indicated in Lipinski's rule of five criteria [19], that hydrogen bond donors must be 5>, hydrogen bond acceptors must be 10>, molar refractivity must be between 40 to 130, molecular mass must be less than 500 Da, and CLogP value must less than 5 [19].

Table 1. Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of four ligand compounds resulted by using swissADME and pkCSM tools.

Physicochemical Properties	**UTP**	**Arbidol**	**Ribavirin**	**Favipiravir**
Molecular weight (g/mol)	484.14	477.41	244.20	157.10
Heavy atoms	29	29	17	11
Ratable bonds	8	8	3	1
H-bonds acceptors	15	4	7	4
H-bonds donors	7	1	4	2
Lipophilicity:				
Consensus Log $P_{o/w}$	−3.75	4.26	−2.18	−0.27
Water Solubility:				
Log S (ESOL)	1.20	−5.45	−0.21	−0.80
Class	Highly Soluble	Moderately Soluble	Very soluble	Very soluble
Pharmacokinetics:				
GI absorption	Low	High	Low	High
BBB permeant	No	No	No	No
Druglikeness:				
Lipinsky	No; 2 violations: NorO > 10, NHorOH > 5	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Medicinal Chemistry:				
Leadlikeness	No; 2 violations: MW > 350, Rotors > 7	No; 3 violations: MW > 350, Rotors > 7, XLOGP3 > 3.5	No; 1 violation: MW < 250	No; 1 violation: MW < 250
Synthetic accessibility	5.02	3.57	3.89	2.08
Toxicological Properties:				
AMES toxicity	No	No	No	Yes

Oral Rat Acute Toxicity (LD50) (mol/kg)	2.373	2.958	1.988	1.941
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	5.151	0.737	3.096	2.023
Hepatotoxicity	No	Yes	No	No
Skin Sensitization	No	No	No	No

The pkCSM server was used to evaluate the toxicity of four ligand molecules, toxicity is a crucial part of computational drug discovery. AMES toxicity, skin sensitization, hepatotoxicity, oral rat chronic toxicity (LOAEL), and oral rat acute toxicity (LD50) result were investigated (Table 1).

3.5. Molecular Docking Study and Protein-Ligand Interaction Analysis

In molecular docking study through PyRx virtual screening tools predicted several binding affinity scores with 8 different models but we collected data for only RMSD 0 resulted model, which is the most reliable and acceptable. UTP, Arbidol, Ribavirin and Favipiravir compounds with Lassa viral refined nucleoprotein 3MX5 showed their binding affinity score −8.8 (Kcal/mol), −7.1 (Kcal/mol), −6.8 (Kcal/mol), and −5.8 (Kcal/mol) respectively as shown in Table 2.

Table 2. The receptor ligand docking scores with different binding interactions.

Protein-Ligand Complex	**Docking Score (Kcal/mol)**	**H-Bond**	**Non-Bonding Interaction**
3MX5-UTP	−8.8	ASN173, ALA, ARG118, VAL166, ALA552, ASP557	**van der Waals:** LEU172, GLU170, SER121, TRP164, ARG162, ASP165, ALA122, LEU550, GLN379, ARG556, PRO555, ILE114, TRP331 **Unfavorable Positive-Positive: (**ARG551) **Pi-Cation: (**ARG329)
3MX5-Ribavirin	−6.8	ASN173, ASP557	**van der Waals:** GLU117, LEU172, ARG329, GLU170, ARH556, ILE114, PRO555, ARG118, ALA169, SER121
3MX5-Favipiravir	−5.8	SER121, ARG329,	**van der Waals:** ALA169, ARG118, GLU117, ASN173, PHE173, TRP164 **Halogen (Fluorine): (**LEU172) **Pi-Sigma: (**LEU172) **Pi-Alkyl: (**LEU120)

Further protein-ligand-interaction was carried out by Discovery Studio, the 3D and 2D diagram is shown in Figure 1 [14].

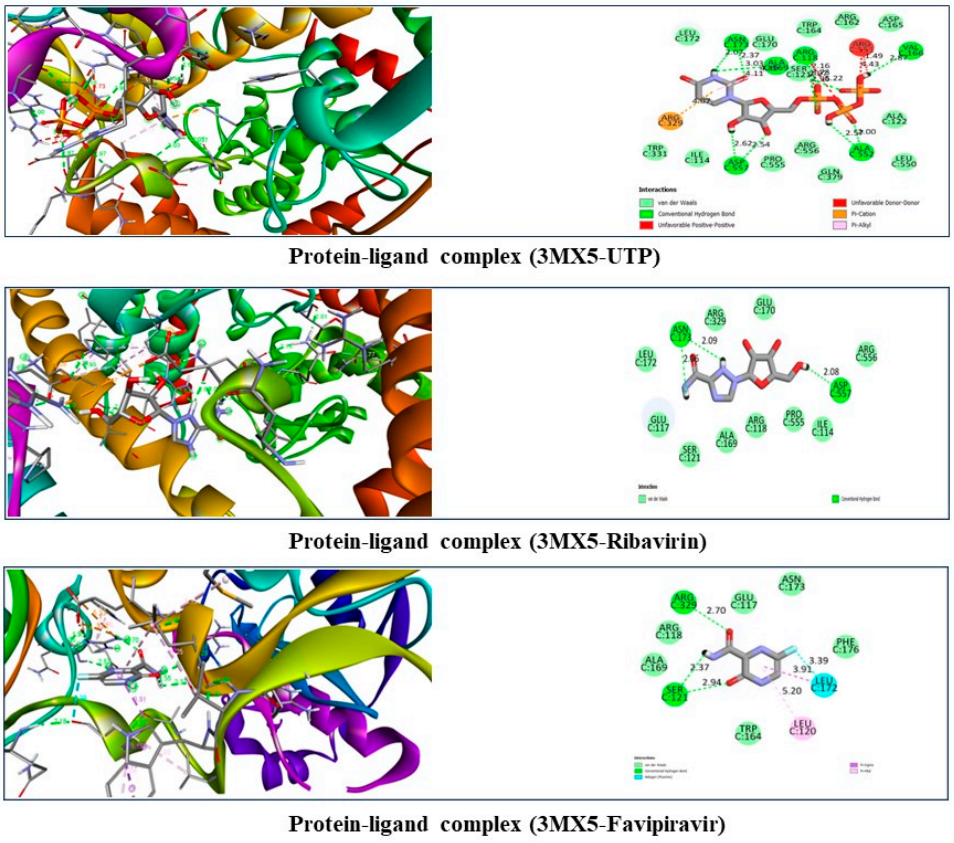

Figure 1. Protein-Ligand Interactions with nucleoprotein refined 3MX5 with UTP, Ribavirin and Favipiravir analysis by Discovery studio; (**a**) 3MX5-UTP (**b**) 3MX5-Ribavirin (**c**) 3MX5-Favipiravir.

4. Conclusions

Lassa fever is an often pernicious viral hemorrhagic fever, and occur endemic situation in populations in several countries, it is caused by Lassa virus. According to genetic dating, zoonotic transmissions of LASV to humans have been going on for generations. A significant high case fatality rate is linked to this human pathogenic infectious disease. Thus, it may pose a threat to life. The immunology, epidemiology, ecology, and pathophysiology of Lassa fever are still poorly understood, despite progress in these fields. The present drug design investigation used a computational chemistry strategy targeting potential inhibitors against the nucleoprotein protein (3MX5) for advancements in deadly Lassa fever virus treatment. UTP, Arbidol, Ribavirin, and Favipiravir were selected as promising ligand candidates for receptor-based molecular docking against 3MX5 with the PyRx. The SwissADME and the pkCSM anticipated the ADMET properties of the selected ligands. Analyzing the different binding interactions, UTP showed a high binding affinity with nucleoprotein 3MX5 compared to other molecules. However, further extended in vivo and in vitro investigations are required to evaluate the current CADD study.

Supplementary Materials: Upon request to corresponding author.

Author Contributions: Conceptualization, M.E.; methodology, M.E.; software, M.E., N.H.S. and T.A.O.; validation, M.E.; formal analysis, M.E. and N.H.S.; investigation, M.E. and M.S.A.; resources, M.E.; data curation, M.E., N.H.S. and T.A.O.; writing—original draft preparation, M.E.; writing—review and editing, M.E.; visualization, M.E.; supervision, M.S.A.; project administration, M.E. and M.S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yun, N.E.; Walker, D.H. Pathogenesis of Lassa Fever. *Viruses* **2012**, *4*, 2031–2048. https://doi.org/10.3390/v4102031.
2. Cai, Y.; Ye, C.; Cheng, B.; Nogales, A.; Iwasaki, M.; Yu, S.; Cooper, K.; Liu, D.X.; Hart, R.; Adams, R.; et al. A Lassa Fever Live-Attenuated Vaccine Based on Codon Deoptimization of the Viral Glycoprotein Gene. *MBio* **2020**, *11*, 10–1128. https://doi.org/10.1128/mbio.00039-20.
3. Olayemi, A.; Fichet-Calvet, E. Systematics, Ecology, and Host Switching: Attributes Affecting Emergence of the Lassa Virus in Rodents across Western Africa. *Viruses* **2020**, *12*, 312. https://doi.org/10.3390/v12030312.
4. Salam, A.P.; Cheng, V.; Edwards, T.; Olliaro, P.; Sterne, J.; Horby, P. Time to reconsider the role of ribavirin in Lassa fever. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009522. https://doi.org/10.1371/journal.pntd.0009522.
5. Global Strategy on Human Resources for Health: Workforce 2030. World Health Organization. Available online: https://apps.who.int/iris/bitstream/handle/10665/250368/9789241511131-eng.pdf (accessed on 31 March 2023).
6. Murphy, H.L.; Ly, H. Pathogenicity and virulence mechanisms of Lassa virus and its animal modeling, diagnostic, prophylactic, and therapeutic developments. *Virulence* **2021**, *12*, 2989–3014. https://doi.org/10.1080/21505594.2021.2000290.
7. 'Efficacy Trials of Lassa Therapeutics: Endpoints, Trial Design, Site Selection' WHO Workshop Final Report. 2018. Available online: https://www.who.int/docs/default-source/blue-print/lassa-therapeutics-workshop-april-25-2018.pdf?sfvrsn=fb777089_2 (accessed on 15 October 2023).
8. User, C. Lassa Fever|CDC Fact Sheet. Available online: https://www.cdc.gov/vhf/lassa/pdf/factsheet.pdf (accessed on 15 October 2023).
9. Rosenke, K.; Feldmann, H.; Westover, J.B.; Hanley, P.W.; Martellaro, C.; Feldmann, F.; Saturday, G.; Lovaglio, J.; Scott, D.P.; Furuta, Y.; et al. Use of Favipiravir to Treat Lassa Virus Infection in Macaques. *Emerg. Infect. Dis.* **2018**, *24*, 1696–1699. https://doi.org/10.3201/eid2409.180233.
10. Mire, C.E.; Cross, R.W.; Geisbert, J.B.; Borisevich, V.; Agans, K.N.; Deer, D.J.; Heinrich, M.L.; Rowland, M.M.; Goba, A.; Momoh, M.; et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat. Med.* **2017**, *23*, 1146–1149. https://doi.org/10.1038/nm.4396.
11. Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235–242. https://doi.org/10.1093/nar/28.1.235.
12. RCSB PDB-3MX5: Lassa Fever Virus Nucleoprotein Complexed with UTP. Available online: https://www.rcsb.org/structure/3MX5 (accessed on 15 October 2023).
13. Qi, X.; Lan, S.; Wang, W.; Schelde, L.M.; Dong, H.; Wallat, G.D.; Ly, H.; Liang, Y.; Dong, C. Cap binding and immune evasion revealed by Lassa nucleoprotein structure. *Nature* **2010**, *468*, 779–783. https://doi.org/10.1038/nature09605.
14. BIOVIA Discovery Studio Visualizer-Dassault Systèmes: San Diego, CA, USA, 2021. Available online: https://discover.3ds.com/discovery-studio-visualizer-download (accessed on 21 August 2023).
15. Tian, W.; Chen, C.; Lei, X.; Zhao, J.; Liang, J. CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Res.* **2018**, *46*, W363–W367. https://doi.org/10.1093/nar/gky473.
16. Dallakyan, S.; Olson, A.J. Small-molecule library screening by docking with PyRx. *Methods Mol. Biol.* **2015**, *1263*, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19.
17. Daina, A.; Michielin, O.; Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* **2017**, *7*, 42717. https://doi.org/10.1038/srep42717.
18. Pires, D.E.V.; Blundell, T.L.; Ascher, D.B. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J. Med. Chem.* **2015**, *58*, 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104.
19. Lipinski, C.A. Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discov. Today Technol.* **2004**, *1*, 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007.