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Computational screening of FDA-approved and natural compounds against Mpox Dual specificity protein phosphatase (H1)

Harshit Tiwari, Ashal Ilyas, Shashank Upadhyay, Pankaj Kumar Rai & Subhomoi Borkotoky*

Department of Biotechnology, Invertis University, Bareilly-243 123, Uttar Pradesh, India

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The resurgence of mpox as a global public health issue emphasizes the urgent need for innovative treatment approaches. Mpox dual specificity protein phosphatase (H1) is an interesting anti-poxvirus therapeutic target due to its role in controlling interferon signaling and viral replication. Inhibiting the operation of Mpox H1 would prevent the virus from multiplying and hence aid in disease management. Leveraging the repurposing potential of FDA-approved drugs and natural products, this study screened two compound libraries for their ability to bind Mpox H1. The docking scores were compared to a standard compound, NSC-62914, a compound known to inhibit the dual-specificity H1 phosphatase of *Variola major* virus. The top scoring complexes were further processed using MD simulations. Among the tested compounds, DB00358 from the FDA-approved library and TN1406 from the natural products library respectively were identified with the highest binding affinity and better stability. Given the effectiveness of NSC-62914 against a homologous dual-specificity H1 phosphatase in the *Variola major* virus, it is also proposed as a potential inhibitor for Mpox H1. These findings based on computational analysis could lead to the development of new potential antiviral treatments to combat Mpox, contributing to global efforts against this emerging infectious disease.

Keywords: Antiviral compounds, Molecular docking, Molecular dynamics simulation, Natural products, Phosphatase inhibitors, Viral replication inhibition

Monkeypox (Mpox) is a disease that was first identified in 1958 in monkeys. It is a viral disease caused by Mpox virus[1,2]. The virus belongs to the genus Orthopoxvirus and family poxviridae. This genus also includes Variola virus, which causes smallpox disease[3]. The mpox, or monkeypox, virus was classified into three clades: Clade I- found in the Congo Basin, with up to 10% human mortality with a primary transmission *via* rodents and limited human-to-human spread. Clade IIa is the one of West Africa, which holds low mortality and remains strongly zoonotic. Conversely, Clade IIb is currently spreading globally because of human-to-human infection[4].A global outbreak of mpox that mainly involved Clade IIb led to the declaration of a Public Health Emergency of International Concern by WHO in 2022. Mpox returned to Africa in 2024 and, in the DRC alone, over 21,000 cases have been reported as it involved a more virulent Clade Ib variant. The WHO declared it a PHEIC for the second time in August 2024. New mutations in Clade Ib appear to be more virulent, and sexual contact has been identified as the main mode of transmission. Cases have also been reported outside Africa, including Sweden, Thailand, and India[5,6]. In humans, Mpox disease shows a similar pattern of symptoms as that of smallpox[3]. The genome of the virus has similarities over 96.3% to that of the variola virus causing smallpox[7]. For Mpox, smallpox vaccines are proven to be 85% effective however there is a limitation to the availability of vaccines due to eradication of the smallpox in 1980[8]. Also, the drugs used against smallpox like vaccinia immune globulin are primary recommendations against monkeypox. These drugs have yet to be established as safe and effective and this has led to the emergence of disease-specific drugs that are effective and safe in terms of their mechanism of action and function[9,10]. The ~197.2 kb double-stranded DNA virus that causes mumps encodes 181 different proteins. The cytoplasm of the infected host cell is where the Mpox replicates. There are various ways that MPXV can spread, including human-to-human, animal-to-human, and animal-to-

*Correspondence:
E-mail: subhomoy.bk@gmail.com

animal. Viral entrance *via* the nasopharyngeal, oropharyngeal, subcutaneous, intradermal, and intramuscular pathways is facilitated by micropinocytosis, viral endocytosis, and cell membrane fusion. Mpox multiplication at the site of inoculation results in Mpox spreading to the blood, lymph nodes, tonsils, bone marrow, spleen, and other organs. This is what initiates inflammatory immune-mediated phagocytosis. Mpox mature virions and enveloped virions regulate the release of the Mpox genome and proteins into host cells[11]. In poxviruses, an important protein expresses known as H1 is a dual-specific phosphatase[12]. The phosphatase H1 enables the replication of the virus in cell culture[13]. The main function of phosphatase is the dephosphorylation of the signal transducer. It also blocks the interferon signal transduction pathway and activates transcription-1 (STAT1)[14,15]. The protein activates at an early stage of virus infection and inhibition of phosphatase may lead to diminished viral infection[13]. This protein is conserved in all poxviruses and forms multiple copies inside newly formed viral particles[13]. Owing to its predominant role in virus replication, infection, and interferon signal transduction, it is often read and researched as an important drug target against poxvirus.

The 171 amino acids Mpox H1 crystal structure was solved at 1.8 Åresolution[12]. A single asymmetric unit has one H1 molecule. Two such molecules of H1 form a dimer with domain swapping. The entire structure is composed of 6 α-helices and 4 β-strands. The H1 active site consists of a Cys-Arg-Asp catalytic triad. Cys and Arg are also conserved amino acids that are found in the phosphate binding loop between β4 and α4 (109-HCVAGVNRS-117)[12]. Among the other proteins, H1 is a crucial protein that could be used for developing effective drugs. By interfering with the protein dimerization, H1 could lose the ability to form dimer and hence could interfere the viral replication. In addition, the active site built around the phosphate-binding loop is also a potential target for drug development[12].

Drug discovery is a time-consuming process that requires *in vitro* analysis and animal testing before hitting the market for an effective, safe, and fast cure for the disease[16]. In patients with higher disease risk, anti-viral agents like cidofovir and tecovirimat are used to treat the infection of Mpox [17]. With the new advancements in research and development, the computational-based method of drug screenings is adopted to find new small molecules that can be used to treat the disease among the larger group of libraries of compounds. The approach has enabled the research to move in a promising direction by finding new molecules that can be used to treat disease more effectively and with safe results in humans. The FDA-approved drugs were previously screened based on the minimum binding energy with cysteine proteinase. The screening of the drugs was based on computational methods like molecular docking to find the drugs with the best binding affinity to the target[18]. In addition, natural compounds offer unique and diverse chemical structures that can be used as templates for developing new drugs. This diversity is challenging to achieve through synthetic methods alone. They may avoid the side effects of synthetic drugs because they must accumulate within living cells[19]. Various natural compounds have been screened against various drug targets of Mpox[20-22]. Many of the drug targets of the Mpox proteome have been targeted by various studies[20-22], however, Mpox H1 is less explored. In this study, we have screened multiple FDA-approved compounds and natural compounds against Mpox H1 using computational approaches. The compounds identified in this study binds effectively to the Mpox H1 active site. The binding modes of the reported compounds are also compared with a reference compound. Hence, it is proposed that these compounds can pave the way for anti-Mpox therapeutics development.

Materials & Methods

Data collection

The three-dimensional structure of Mpox H1 was obtained from theProtein Data Bank (PDB)[23], a comprehensive database of experimental structures with PDB ID: 8GZ4[12]. To prepare the receptor for docking analysis, UCSF Chimerav.1.17.3[24], a highly used tool for visualization and analysis of molecular structure, was used to remove the surplus water molecules and chains from the protein.

Molecular docking

Molecular docking is a widely used computational method to predict the binding mode of a ligand within the binding site of a protein target as well as virtual screening[18,21,25,26]. The DrugRep virtual screening server[27] was used in order to perform molecular docking. The DrugRep (https://cao.labshare.cn/drugrep/)is a freely available server for the virtual screening of

drugs especially drug repurposing. The binding pocket, which has been determined using an integrated CurPocket tool, was located in the server's receptor-based screening module[27]. CurPocket[28] is a protein-ligand binding site prediction method that calculate curvature factors to find cavities on the protein surface. The server uses AutoDock Vina[29] to perform the docking calculations. The virtual screening was performed using two in-built chemical libraries of DrugRep: the DrugBank database and the Traditional Chinese Medicine (TCM) library. There are 2470 FDA-approved compounds in the DrugBank library which has been approved safe and effective against different diseases. On the other hand, 2390 natural compounds are found in the TCM library representing a wide range of traditional Chinese medicinal compounds. UCSF Chimaera was used to dock a reference compound, NSC-62914 (PubChem ID: 66662), in addition to the library compounds. Using both *in vitro* and *in silico* techniques, NSC-62914 was previously discovered to be a prospective inhibitor of the dual-specificity H1 phosphatase of the *Variola major* virus[30]. To assess the binding effectiveness of the compounds from the virtual screening study, NSC-62914was used as a standard. The docked complexes were visualized and analyzed using the UCSF Chimera tool.

Molecular dynamics (MD) simulations

The top scoring complexes were subjected to MD simulation using GROMACS (GROningen MAchine for Chemical Simulations) v. 2021[31] with CHARMM36 force field[32]. The ligand parameters were generated using CHARMM General Force Field (CGenFF) program v. 4.6[33,34] (https://app.cgenff.com/homepage). The TIP3P water model was used to solvate the systems were in 1.2 nm cubic box and were neutralized with 150 mM NaCl. The systems were minimized using steepest-descent method comprising of 5000 steps. The Particle Mesh Ewald (PME) method[35] was used to account for electrostatic forces. The LINCS algorithm[36] was used to constrain the hydrogen bonds. Each system was subjected to equilibration through 1 ns NVT/NPT ensembles at 300K, maintaining a constant pressure of 1.0 bar. Temperature coupling was achieved using the V-rescale[37] thermostat, while pressure was controlled using the Berendsen pressure coupling[38]. Final production runs of each system were carried out for 100 ns each. The post-MD analyses including calculation of root mean square deviation (RMSD), radius of gyration (Rg), and hydrogen bonds (H-bonds) were carried out using the in-built GROMACS utilities.

Results

Interactions of the ligands with Mpox H1

The binding site predicted by the DrugRep server was centred around the Mpox H1 active site (Asp 79, Cys 110, Val 111, Asp 112, Gly 113, Val 114, Asn 115, Arg 116). The dimensions (Å) of the grid box for the targeted site were taken as X: 12 Å, Y: 11 Å, Z: 10 Å, and coordinates (center) were set as X: -9 Å, Y: -33 Å, Z: -24 Å (Fig. 1A). The reference compound NSC-62914 interacted with the Mpox H1 with a binding affinity of -6.0 kcal/mol (Table 1). Each screening with FDA-approved and natural compound libraries using DrugRep returned the top 100 molecules based on estimated binding affinities (kcal/mol). The top 10 compounds obtained from each library are shown in (Table 2). The top compounds with binding affinity > -6.0 kcal/mol were selected (Table 1). One compound from the DrugBank library Mefloquine (DB00358) interacted with the highest binding affinity of -6.5 kcal/mol, followed by one compound from the TCM library Aromadendrin (TN1406) interacted with a score of -6.2 kcal/mol. NSC-62914 formed two hydrogen bonds with residues Asn 115 and Arg 116 and 04 hydrophobic interactions with the residues Asp 79, Val 111, Asn 155, and Phe 158 (Figs 1B & 2A). DB00358 formed two hydrogen bonds with the residues Asp 80 and Asn 155 and 05 hydrophobic interactions with the residues Asp 79, Ala 112, Asn 115, Arg 116, and Phe 158 (Figs 1C & 2B). TN1406 formed 04 hydrogen bonds with residues Asp 80, Arg 116, and Asn 155 and 07 hydrophobic interactions with the residues Asp 79, Cys 110, Ala 112, Val 114, Asn 115, Ala 151, and Glu 154 (Figs 1D & 2C). The chemical structures of these three compounds are shown in (Fig. 3).

Molecular dynamic simulation studies

The stabilities of the docked complexes were evaluated using MD simulation analysis. The RMSD pattern (Fig. 4A) of the protein backbone of the apo-protein was compared with the docked complexes and it was observed that the backbone was stable without any strong deviations. The backbone RMSD of the docked complexes also found to attain a lower RMSD than the apo-protein. Further the radius of gyration profiles for all the complexes were calculated

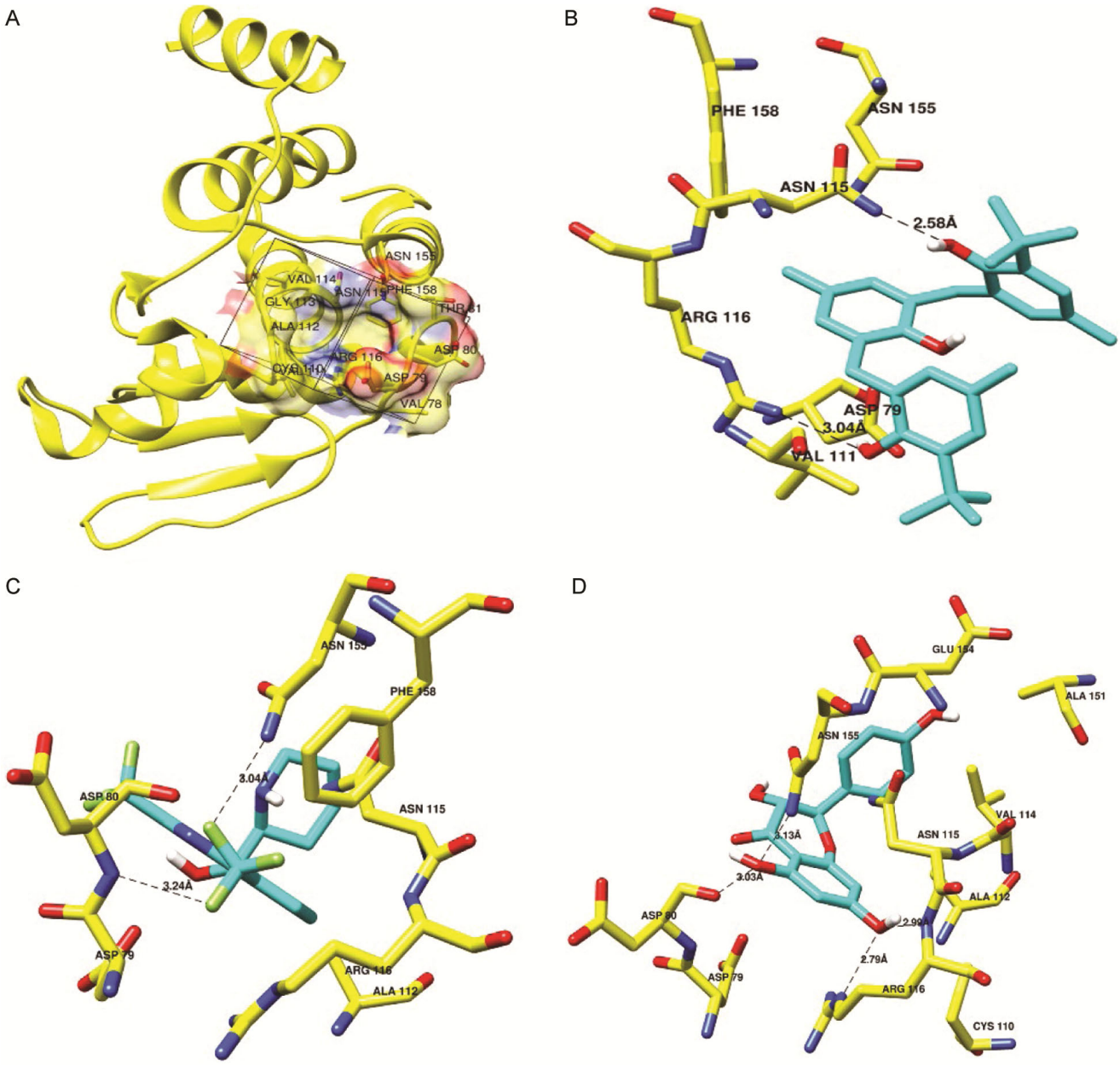

Fig. 1 — Molecular docking results: (A) binding site of Mpox H1 predicted by DrugRep, shown as a surface representation. Interactions of (B) NSC-62914;(C) DB00358; and (D) TN1406 with Mpox H1. The protein residues are shown in yellow and the ligands are shown in cyan. The hydrogen bonds are shown as dotted lines with their distances in Angstrom (Å)

Table 1 — Virtual screening results of FDA-approved and natural compounds against Mpox H1

Sl No	Database ID	Name	Binding affinity (kcal/mol)	H-bonds	Hydrophobic interactions
1	DB00358	Mefloquine	-6.5	Asp 80, Asn 155.	Asp 79, Ala 112, Asn 115, Arg 116, and Phe 158
2	TN1406	Aromadendrin	-6.2	Asp 80, Arg 116, Asn 155	Asp 79, Cys 110, Ala 112, Val 114, Asn 115, Ala 151, and Glu 154
3	NSC-62914	Antioxidant 80	-6.0	Asn 115, Arg 116	Asp 79, Val 111, Asn 155, and Phe 158

Table 2 — The top 10 compounds obtained from docking-based screening from each library: DrugBank and TCM. The DrugBank IDs are prefixed with DB and TCM IDs are prefixed with T

SL no	Database ID	Name	Binding affinity (kcal/mol)
1	DB00358	Mefloquine	-6.5
2	DB00434	Cyproheptadine	-6.0
3	DB00231	Temazepam	-5.9
4	DB00564	Carbamazepine	-5.8
5	DB00295	Morphine	-5.8
6	DB00327	Hydromorphone	-5.8
7	DB00842	Oxazepam	-5.8
8	DB00854	Levorphanol	-5.7
9	DB00776	Oxcarbazepine	-5.7
10	DB00719	Azatadine	-5.7
11	TN1406	Antioxidant 80	-6.2
12	T2O2770	Agarotetrol	-6.0
13	TN3861	Dihydropinosylvin	-5.8
14	T2771	Orcinol glucoside	-5.7
15	TN1369	Alloimperatorin	-5.6
16	T5S2347	Deoxyshikonin	-5.6
17	T2838	Naringenin	-5.6
18	T2949	Alizarin	-5.5
19	T3840	Dihydroresveratrol	-5.5
20	T0925	Allantoin	-5.5

for 100 ns trajectory (Fig. 4B). As compared to apo all the three complexes have shown a similar range of Rg values suggesting overall compactness or structural stability of the protein in the presence of the ligands. To stability between ligand and receptor was assessed through the hydrogen bonds (H-bonds) formed in the three complexes during the 100 ns simulation period (Fig. 4C). Among the complexes, the highest H-bonds are formed by TN1406 with maximum04 H-bonds over the 100 ns trajectory. The compound DB00358 formed maximum 03 H-bonds followed by the reference compound NSC-62914 with maximum 02 H-bonds over the 100 ns trajectory.

Discussion

Computational approaches can support the drug development process by rapid screening of large compound libraries and help in understanding the interaction details of ligands with drug targets[21]. Computational screenings have been used to find inhibitors against various other Mpox drug targets[21,26].

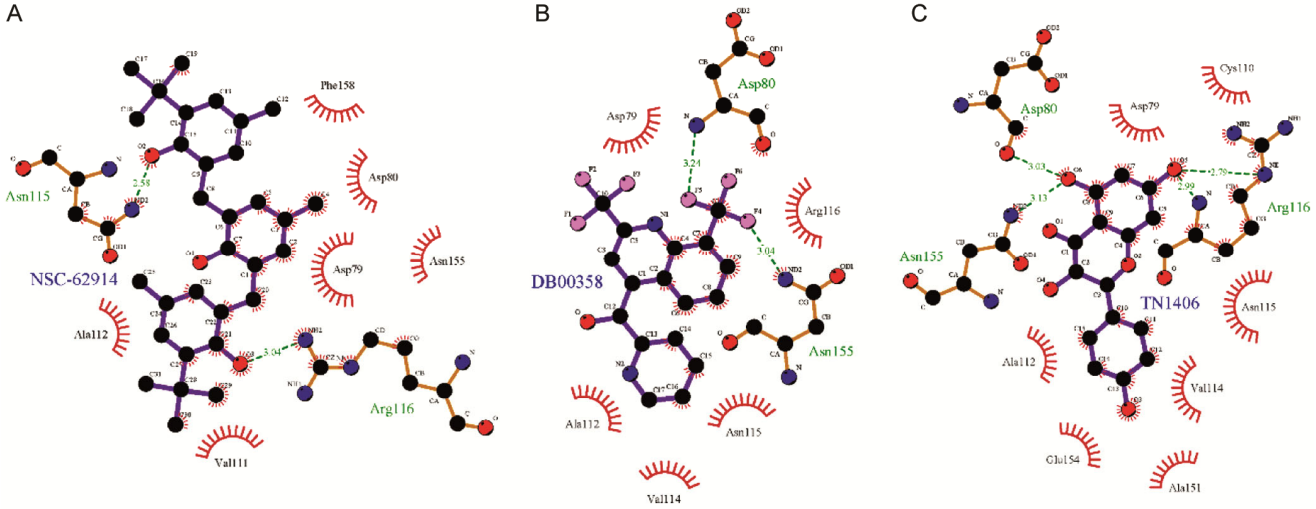

Fig. 2 — The two-dimensional (2D) interaction diagrams of the top ligands: (A) NSC-62914; (B) DB00358; and (C) TN1406. The hydrogen bonds are represented as green dotted lines, while hydrophobic interactions are shown as red arcs

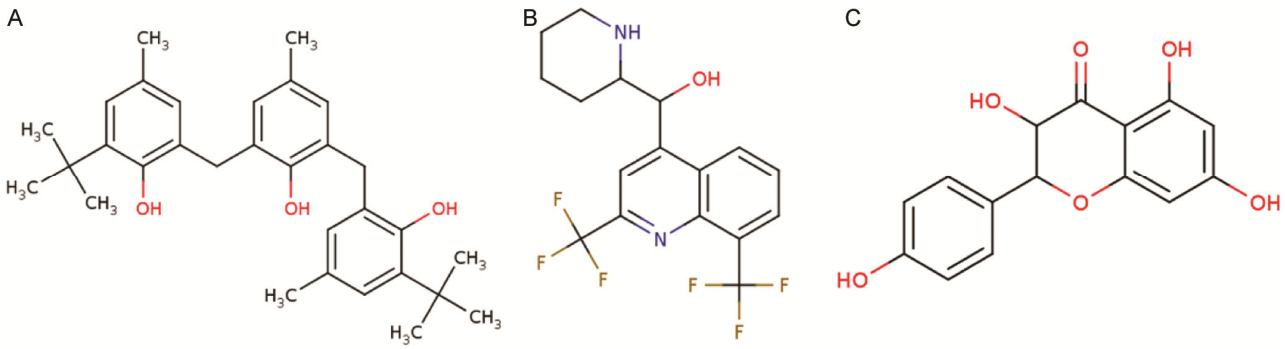

Fig. 3 — The 2D molecular structures of the three reported compounds: (A) NSC-62914; (B) DB00358; and (C) TN1406

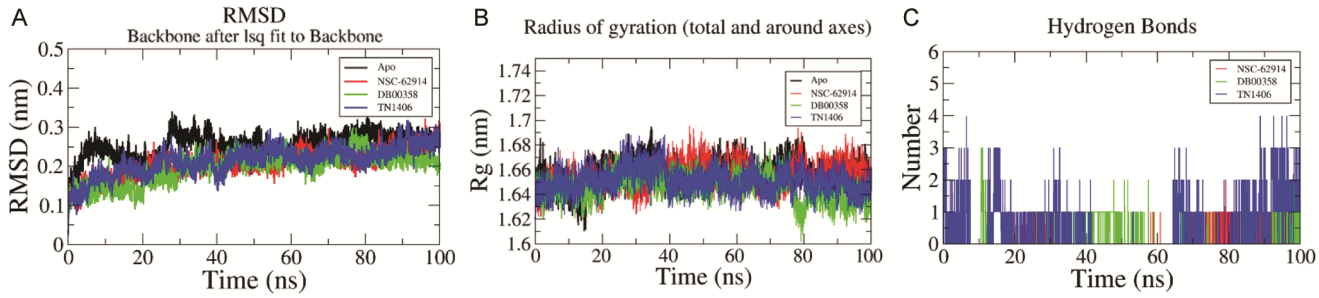

Fig. 4 — The MD simulation results of the docking complexes: (A) RMSD plot of the protein-ligand complexes over time; (B) Rg plot indicating protein compactness; and (C) Number of H-bonds between the ligands and Mpox H1 during the simulation

In this study, we have identified one FDA-approved compound DB00358 (Mefloquine), and one natural compound Aromadendrin (TN1406) that can bind strongly with the active site of Mpox H1. Both of them also have a higher score than the reference compound NSC-62914 (Antioxidant 80). Mefloquine,a4-quinolinemethanol that shares similar structural areas with chloroquine/hydroxychloroquine, is an antimalarial drug that has been also tested as an antiviral against SARS CoV-2 *in vitro*[39]. Aromadendrin is a flavonoid compound, known for its anti-inflammatory activities and also has potential antiviral activity against HIV[40]. All three compounds interact with some of the critical residues of phosphatase such as Asp 79 and Arg 116. As a general acid, Asp79 promotes the formation of the enzyme-phosphate intermediate as well as the process of its hydrolysis, whereas Arg116 ensures effective substrate binding and orientation by capturing the phosphate ion[12]. Aromadendrin additionally interacts with Cys110, which attacks the phosphorous atom during the de-phosphorylation reaction, resulting in a transient enzyme-phosphate intermediate[12]. These interactions support the possibility of DB00358 and TN1406 being inhibitors of Mpox dual specificity protein phosphatase. Moreover, the binding affinities of these two compounds are also higher than the reference compound NSC-62914, which has proven inhibitory activity against the *Variola major* virus dual-specificity H1 phosphatase with an IC_{50} value of 48 μM[30]. Furthermore, the MD simulation analysis also showed that the two reported compounds formed stable complexes with Mpox H1 and formed stronger interactions compared to NSC-62914 in terms of H-bonds. The sequence identity between *Variola major* virus dual-specificity H1 phosphatase and Mpox dual specificity protein phosphatase is more than 90%. Hence, NSC-62914 can also be effective against MpoxH1.

Limitations

Molecular docking and MD simulations, though important in drug discovery, with MD simulations providing valuable insights into the dynamic behaviour of proteins in simulated biological environments. However, despite accounting for conformational flexibility, these methods still have limitations as it does not completely capture the real-world solvent effects intricacies and the precise energy of molecular interactions. It may be useful to compare the results with a reference molecule like NSC-62914; however, they might not necessarily reflect clinical relevance or *in vivo* conditions. Subsequent validations *via* other approaches including the bioassays and experimental animal models should also be undertaken to support the findings of this study.

Conclusion

In conclusion, the computational analyses demonstrated that the compounds DB00358 and TN1406 bind more effectively to the Mpox H1, in comparison with the reference compound NSC-62914.These compounds appeared to bind to substantial functional groups of the protein, indicating the possibility of worthy inhibitors. Considering *Variola major* virus dual-specificity H1 phosphatase has over 90% sequence identity with the Mpox H1, then there is also a possibility of the efficacy of NSC-62914 against the latter. These results highlight the future application for drug repurposing, particularly focusing on validated candidates to drive experimental work to transform into antivirals against Mpox.

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Conflicts of interest

All authors declare no conflict of interest.

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