Introduction

Monkeypox Virus (MPXV), an enveloped double-stranded DNA virus, belongs to the Orthopoxvirus genus and Poxviridae family. MPXV is the causative agent of the emerging zoonotic disease Monkeypox (MPX) [1]. Since May 2022, cases of MPXV infection have been reported in non-endemic nations, such as the USA, the UK, Germany, and Australia (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385). These cases are alarming and indicate an emerging epidemic. MPXV has the ability to infect large mammalian species. This virus can be spread through saliva or respiratory substances, as well as skin contact with lesion exudate or crust material. MPXV can be shed from the feces of infected people [2, 3]. From the contaminated sources, Orthopoxvirus such as MPXV generally interacts with host glycosaminoglycans (GAGs) and enters into the host cell via endocytosis [4]. In the early phase of infection, the viral DNA gets transcribed in the host cytoplasm via viral RNA polymerase. In the Intermediate phase, the uncoated virus proceeds to replicate the double-stranded DNA. After the synthesis of structural proteins in the late phase, it constructs an intracellular mature virion (IMV). The IMV virion can be released by cell lysis or it can acquire a layer of the double membrane by trans-Golgi and come out as an external enveloped virion (EEV) (https://viralzone.expasy.org/149). The immune system responds when it

Keywords  Monkeypox · Zidovudine · Fludarabine · L2R · I4L · MD simulation
faces the viral DNA. This is critical for both defense and the establishment of adaptive unity. Usually cyclic GMP–AMP synthase (cGAS), IFN-γ-inducible protein 16 (IFI16), and DNA-dependent protein kinase (DNA-PK) sense viral DNA and initiate an innate response [5]. The clinical symptoms of MPX and Smallpox infection are not very distinguishable. Except for lymph node enlargement, which occurs early, often at the onset of fever, the majority of clinical characteristics of human MPX infection are similar to those of Smallpox [6].

Cidofovir, chemical name [(2S)-1-(4-amino-2-oxopyrimidin-1-yl)-3-hydroxypropan-2-yl] oxymethylphosphonic acid, is commonly used to treat MPX patients. Sometimes Brincidofovir (prodrug of Cidofovir) is also given. Cidofovir is an antiviral medicine that works against a variety of DNA viruses, including smallpox, MPX, cowpox, variola, and vaccinia. This medication can be given intravenously or topically [7]. Cidofovir is phosphorylated intracellularly after administrations and transforms into a cytosine analog. Because this cytosine analog competes with cytosine, viral DNA chain synthesis is stopped. Although the efficacy of the drug is satisfactory, this compound can lead to hepatotoxicity or nephrotoxicity in some patients [8]. Another antiviral drug called Tecovirimat is effective against MPX. This compound inhibits the functional role of the viral envelope protein. This inhibition leads to a reduced amount of extracellular virus; hence, the viral spreading decreases after treatment [9]. However, the drug is under investigation. It might take a long time to get established for the usage of this drug for MPX treatment.

MPX infections can be handled more efficiently using safer drugs. Drug repurposing and rational exploration of new drugs using bioinformatics tools are a fast and prospective approach to achieving this goal [10, 11]. In this study, we searched the proteome of MPXV to identify new drugs that are approved or very similar to the approved drugs. We conducted virtual screening and measured the Absorption, Distribution, Metabolism, and Toxicity (ADMET) values of the selected drugs. Using molecular docking and molecular dynamics (MD) simulations, we finally predicted some plausible anti-MPXV drugs.

**Method**

**Drug Compound Selection**

The total reference proteome of Monkeypox virus Zaire-96-I-16 (NCBI Accession ID: NC_003310.1) was collected from the National Center for Biotechnology Information (NCBI) Virus database. All proteins went under Basic Local Alignment Tool (BLAST)-based searching using DrugBank BlastP (https://go.drugbank.com/structures/search/bonds/sequence) for detecting druggable candidates. Drug targets and drugs with bit score higher than 100 and E value less than 0.001 were taken as a potential drugs and drug targets [12]. Only approved drugs (except Tecovirimat which is currently administered as an antiviral against MPX) were taken for further explorations.

**Similar Drug Search**

Drugs with similar structures were detected using Swiss-Similarity (http://www.swisssimilarity.ch/). Here, the SMILES of the selected approved drugs were given. Using the Bioactive Class of Compounds, Three-Dimensional (3D) parameters, and the ChEMBL database (https://www.ebi.ac.uk/chembl/), similar structures were detected. Compound library and screening method was Electroshape. Compounds with ≥ 0.9 score were considered very similar structures [13]. The SMILES were converted into pdb via Online SMILES Translator and Structure File Generator (https://cactus.nci.nih.gov/translate/).

**Receptor Preparation**

Proteins that were potential targets for the approved compounds went under homology modeling. The primary sequences of the proteins were taken from the NCBI protein database. The primary sequences were given in AlphaFold2. This software was implemented via Colabfold [14, 15]. The models were refined by GalaxyRefine [16]. The receptors were visualized by University of California San Francisco (UCSF) ChimeraX [17]. The refined models were evaluated by SAVES v6.0 (https://saves.mbi.ucla.edu/) using ERRAT, Verify3D, and PROCHECK [18–20].

**Molecular Docking**

The selected receptors and ligands were imported in PyRx (https://pyrx.sourceforge.io/). PyRx is an open source tool that uses OpenBabel, AutoDock 4, and AutoDock Vina [21–24]. The ligands and receptors were converted into pdbqt format via OpenBabel. To draw the box for docking, the maximize option was selected. The maximize option covered the entire receptor. The molecular docking was performed to identify binding affinity of the selected molecules. To identify the inhibition constant (Ki) of the selected compounds, formula $Ki = \exp(\Delta G/RT)$ is implemented. Here, $\Delta G$ is the binding affinity or binding energy, $R$ is the universal gas constant ($1.985 \times 10^{-3} \text{ kcal/mol/K}$), and the value of temperature $T$ was 25 °C (298.15 K) (Ortiz et al. 25).
ADMET Screening

Approved drugs were taken as control. Analogs of these control drugs went under molecular docking. Analogs that showed more binding affinity scores than their corresponding control drugs were selected for Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analysis. To do so, the Canonical Simplified Molecular Input Line Entry System (SMILES) that were generated by SwissSimilarity were given in pkCSM (http://biosig.unimelb.edu.au/pkcsm/). Analogs with AMES test negative, hepatotoxicity negative, and highest intestinal absorption were visualized.

Ligand–Receptor Visualization

The pdbqt output of the selected ligands and receptors are imported in PyMol [26]. The ligand–receptor complexes were generated in PyMol. Finally, the molecular visualization was conducted via BIOVIA Discovery Studio Visualizer (https://discover.3ds.com/).

Molecular Dynamics Simulations

The ligand–receptor complexes were uploaded in Chemistry at Harvard Macromolecular Mechanics-Graphical User Interface (CHARMM-GUI)(https://www.charmm-gui.org/) input generator using the solution builder option [27]. Inputs were generated for GRÖningen MAchine for Chemical Simulations (GROMACS) 2021[28]. A transferable intermolecular potential with 3 points (TIP3) water box was created with 1-nm distance. CHARMM36m force field was implemented for energy minimization along with 100-ps NVT and NPT simulations at 303.15-K temperature [29]. Afterward, 100-ns production simulation was conducted. To analyze the secondary structure contents during simulation, gmx do_dssp of GROMACS was implemented.

Results

The workflow of the total study is presented in Fig. 1.

E8L and I4L Showed Three of Potential Approved Drugs Against Them

Monkeypox virus complete genome (NCBI Reference Sequence: NC_003310.1) had 181 proteins. All of these proteins went through Drug Bank BlastP. Among them, E8L or IMV surface membrane protein (32 kDa) that binds to cell surface chondroitin sulfate (glycosaminoglycan) and I4L or ribonucleotide reductase large subunit R1 showed highest (3) matches with approved drugs. E8L showed Brinzolamide, Dorzolamide, and Methazolamide. I4L matched with Gemcitabine, Hydroxyurea, and Fludarabine. Other proteins, such as L2R and Phospholipase-D-like protein, matched with Zidovudine and Tencovirimat, respectively (Table 1).

![Fig. 1 Schematic representation of the total study](image-url)
Antineoplastic Fludarabine and Antiretroviral Zidovudine Had Highest Amount of Analogos

SwissSimilarity detected compounds with different scores with the Electroshape method. The Electroshape method calculated fast molecular similarity calculations with chirality, shape and electrostatics in the ChEMBL database (https://www.ebi.ac.uk/chembl/) [30]. Here, most of the approved drugs did not have enormous analogs with 90% similarity. Only Fludarabine and Zidovudine presented 70 and 28 analogs, respectively (Table 2). Total 112 compounds were taken for molecular docking (Fig. 2).

Table 1 Drug Bank BlastP results of approved drugs against the monkeypox virus proteome

| Protein (NCBI_accession)                  | $E$-value | Bit score | Drug name                      | Drug group       |
|------------------------------------------|-----------|-----------|--------------------------------|------------------|
| E8L (NP_536532.1)                        | 2.53E-42  | 145.591   | Brinzolamide, Dorzolamide, Methazolamide | Approved         |
| L2R (NP_536513.1)                        | 8.99E-87  | 253.447   | Zidovudine                      | Approved         |
| I4L (NP_536492.1)                        | 0         | 1161.75   | Gemcitabine, Hydroxyurea, Fludarabine | Approved         |
| Phospholipase-D-like protein (NP_536457) | 6.05E-28  | 112.464   | Tecovirimat                     | Approved & investigational |

Molecular Docking Followed by ADMET Screening Predicted Potential Fludarabine and Zidovudine Analogos

The approved drugs and the model validations were conducted. All of the proteins showed $>90\%$ of their amino acids in most favored regions. Analogs were docked against their corresponding receptors. To do so, 3D models of the viral proteins were generated and refined. All of them had an ERRAT score more than 90. Except for E8L, all of them passed the Verify3D test (Fig. 3).

Fifteen Zidovudine analogs scored better than Zidovudine. One analog showed an equal score. Twenty-Five Fludarabine analogs performed better than the parent drug. Eight compounds showed the same scores. Tecovirimat demonstrated one better compound with higher binding affinity. E8L had four ligands with more binding affinity than approved drugs.

Except CHEBI 43411, CHEBI 6822, and Methazolamide, all of the compounds demonstrated Ki value less than 100 $\mu$M.

The outperformed ligands and the approved drugs of Table 3 went under ADMET screening. The SMILES were given in pkCSM. pkCSM predicted the ADMET properties via graph-based signatures. Ligands with Hepatotoxic and mutagenic (AMES-positive) properties were discarded.

(2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butane triol or CHEBI: 173933 had highest intestinal absorption (92.641$\%$). 5’-Dehydroadenosine or CHEBI: 1958 showed 51.647$\%$ intestinal absorption. CHEBI: 173933 had highest Caco2 permeability 1.085. The unbound fraction (Fu) values were 0.755, 0.137, 0.86, and 0.81 for Zidovudine, CHEBI: 173933, Fludarabine, CHEBI: 1958, respectively. All of these molecules cannot cross the blood–brain barrier or Central Nervous System (CNS). Only CHEBI: 173933 might act as a CYP1A2 inhibitor. Total Clearance (log ml/min/kg) were 0.052, 0.663, 0.703 and 0.793 for Zidovudine, (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butane triol, Fludarabine, and 5’-Dehydroadenosine, respectively. Only Fludarabine was AMES-positive and other drugs were AMES negative. Both approved drugs Zidovudine and Fludarabine had been classified as hepatotoxic by pkCSM. However, 5’-Dehydroadenosine and (2S,4R)-4-(9H-Pyrido[3,4-b] indol-1-yl)-1,2,4-butanetriol had no hepatotoxic properties.

Ligand–receptor visualization demonstrated that (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol engaged more residues and generated less unflavored bonds with L2R (Thymidine Kinase) than Zidovudine. Especially, the Harmala alkaloid part of this compound generated Pi-sigma and Amide-Pi-stacked bond with the Thymidine kinase and allowed more solvent-accessible area (Sky blue shades in the benzene ring of Fig. 4b). This was not observed for Zidovudine (Fig. 4) (https://hmdb.ca/metabolites/HMDB0035191).

MD Simulations Based Insights Were Very Similar for All Druggable Candidates

Molecular dynamics simulation was carried out for the selected ligand–receptor complexes. The simulations generated Root Mean Square Deviation (RMSD), Root Mean Square Fluctuations (RMSF), Radius of Gyration (Rg), and Solvent-Accessible Surface Area (SASA) (Fig. 5).
RMSD of L2R (Thymidine Kinase) C-alpha backbone with Zidovudine (Green) and (2S,4R)-4-(9H-Pyrido[3,4-b] indol-1-yl)-1,2,4-butanetriol (CHEBI: 173933) (Golden) showed around similar value of RM hiSD from 0 to 50 ns. After 50 ns, around 0.1 ns or 1 Å (Fig. 6a). The RMSF values or protein mobility were very similar for both of the compounds (Fig. 6b). The values of protein compactness (Rg) increased by the time and with more SASA (Fig. 6c and d).

I4L (Ribonucleotide reductase large subunit R1) with Fludarabine (Green) and 5′-Dehydroadenosine (CHEBI: 1958) (Golden) showed nearly same RMSD value in his final steps of 100-ns simulation (Fig. 7a). The mobility of the protein (RMSF) values were not dissimilar except in some small regions (Fig. 7b). Protein compactness (Rg) and SASA decreased by the time (Fig. 7c and d).

The secondary structure contents were also similar between the approved drug–ligand complex and their corresponding proposed drug–ligand complex (Fig. 8). However, after ~20 ns a slight and stable increase in the 3-Helix was observed in L2R (Fig. 8a, b). After ~10-ns Coil structure was elevated in I4L (Fig. 8c, d).

Table 2 ChEMBL IDs of the approved drug Analogs that were detected using SwissSimilarity

| Protein | Drugs (Commercial Name/ ChEMBL ID) |
|---------|-----------------------------------|
| E8L (IMV surface membrane protein) | Brinzolamide CHEBI: 3176  
CHEBI: 43411  
Dorzolamide CHEBI: 4702  
Methazolamide CHEBI: 6822 |
| L2R (Thymidine Kinase) | Zidovudine CHEBI: 10110  
CHEBI: 149753  
CHEBI: 490877  
CHEBI: 59847  
CHEBI: 117980  
CHEBI: 4728  
CHEBI: 59846  
CHEBI: 117936  
CHEBI: 118100  
CHEBI: 175144  
CHEBI: 117931  
CHEBI: 75473  
CHEBI: 169114  
CHEBI: 174448  
CHEBI: 68294  
CHEBI: 173933  
CHEBI: 66159  
CHEBI: 149757  
CHEBI: 118107  
CHEBI: 118121  
CHEBI: 3578  
CHEBI: 2453  
CHEBI: 63581  
CHEBI: 133856  
CHEBI: 69770  
CHEBI: 94793  
CHEBI: 145220  
CHEBI: 118075 |
| I4L (Ribonucleotide reductase large subunit R1) | Gemcitabine CHEBI: 175901  
Fludarabine CHEBI: 94701  
CHEBI: 94359  
CHEBI: 39740  
CHEBI: 9978  
CHEBI: 93913  
CHEBI: 45327  
CHEBI: 55419  
CHEBI: 140569  
CHEBI: 46315  
CHEBI: 95180  
CHEBI: 125640  
CHEBI: 125449  
CHEBI: 1014  
CHEBI: 16335  
CHEBI: 49751  
CHEBI: 90031  
CHEBI: 42452  
CHEBI: 2310  
CHEBI: 73141  
CHEBI: 19688  
CHEBI: 16750  
CHEBI: 174609  
CHEBI: 85997  
CHEBI: 681569  
CHEBI: 3927  
CHEBI: 93920  
CHEBI: 28498  
CHEBI: 93817  
CHEBI: 45448  
CHEBI: 83079  
CHEBI: 70972  
CHEBI: 83080  
CHEBI: 1958  
CHEBI: 95153  
CHEBI: 2312  
CHEBI: 31858  
CHEBI: 94463  
CHEBI: 134989  
CHEBI: 8612  
CHEBI: 1196  
CHEBI: 165834  
CHEBI: 94738  
CHEBI: 91901  
CHEBI: 95211  
CHEBI: 134606  
CHEBI: 40167  
CHEBI: 42575  
CHEBI: 147281  
CHEBI: 69426  
CHEBI: 83081  
CHEBI: 12060  
CHEBI: 141077  
CHEBI: 2038  
CHEBI: 111177  
CHEBI: 94323  
CHEBI: 3202  
CHEBI: 63580  
CHEBI: 62005  
CHEBI: 44081  
CHEBI: 63612  
CHEBI: 145115  
CHEBI: 90239  
CHEBI: 16299  
CHEBI: 76278  
CHEBI: 9976  
CHEBI: 168049  
CHEBI: 21891  
CHEBI: 131374  
CHEBI: 168240  
CHEBI: 8098  
CHEBI: 83194  
CHEBI: 80011  
CHEBI: 108514  
CHEBI: 111390 |
| Phospholipase-D-like protein | Tecovirimat CHEBI: 83194  
CHEBI: 80011  
CHEBI: 108514  
CHEBI: 111390 |
Discussion

The monkeypox disease is difficult to control, especially in developing nations. Poor management and improper treatment might result in long-term illness with severe negative effects (6). For diagnosis, the virus can be detected with Polymerase Chain Reaction (PCR) tests. But treatment includes mainly Cidofovir and Tecovirimat. These drugs can reduce the duration of viral shedding with several side effects (30) (8). However, Tecovirimat is still on trial and depending solely on these drugs may exacerbate the situation with costs and drug-resistant variants. As a result, new drugs are required to control the spread of MPXV in both endemic and non-endemic countries.

Drug repurposing is a fast and viable solution to this problem. Bioinformatics-based screening can be used to repurpose existing medications at a preliminary level. In this study, we wanted to propose several approved drugs and other compounds that are similar to them with better mutagenicity and toxicity profiles. To do so, we explored the proteome of MPXV. Then selected E8L/IMV surface membrane protein and I4L/ribonucleotide reductase large subunit R1 showed as druggable candidates. These proteins have strong sequence similarity and domains that can be targeted with Brinzolamide, Dorzolamide, Methazolamid, Gemcitabine, Hydroxyurea, and Fludarabine. For example, according to pfam (https://pfam.xfam.org/), E8L has a Eukaryotic-type carbonic anhydrase domain. Brinzolamide, Dorzolamide, or Methazolamide are carbonic anhydrase inhibitors that are used to treat glaucoma [30–32]. These carbonic anhydrase inhibitors also demonstrated antiviral properties against influenza virus and Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [33–35]. Therefore, Brinzolamide, Dorzolamide, or Methazolamide could potentially interfere
in cell surface binding of IMV by inhibiting E8L Eukaryotic-type carbonic anhydrase domain. These drugs can be used as lead compounds to develop less toxic and mutagenic anti-MPXV agents. Moreover, I4L has Ribonucleotide reductase family (barrel domain), Ribonucleotide reductase (all-alpha domain) and ATP cone domain. Gemcitabine, Hydroxyurea, and Fludarabine-type nucleoside analogs could react with I4L and stop viral replication. Ribonucleotide Reductase is essential for generating DNA precursors and pathogenesis in poxviruses [36]. Gemcitabine, Hydroxyurea, and Fludarabine inhibit Ribonucleotide reductase in mammalian cells or interfere in DNA replication process. Gemcitabine and hydroxyurea have broad-spectrum antiviral or anti-Ribonucleotide reductase activity [37, 38]. Fludarabine can reduce the secretion of hepatitis B virus progeny DNA in HepG2-NTCPsec + cells [39]. Reasonably, these approved nucleoside analogs should reduce MPXV pathogenesis by decreasing MPXV DNA synthesis that are mediated by I4L. L2R protein has Thymidine kinase domain and Zidovudine, a dideoxynucleoside, can interact with this viral protein (https://go.drugbank.com/). Zidovudine has broad-spectrum thymidine kinase interference capability. This drug can inhibit thymidine incorporation in different mammalian, bacterial, and viral genomes [40–42]. Hence, if Zidovudine gets phosphorylated by L2R, it potentiates chain reaction termination during MPXV DNA replication. 

To develop less hepatotoxic and non-mutagenic drugs, we searched for similar compounds using the 3D electroshape algorithm of SwissSimilarity. After collecting the compounds, molecular docking was conducted with their corresponding receptors. The molecular docking simulations revealed compounds that can interact with the receptors with satisfactory binding affinity scores (binding affinity score more than or equal to the approved drugs) (Table 3). Ki values more than 100 μM can be considered as potent inhibitors [43]. Except CHEBI 43411, CHEBI 6822 and Methazolamide, all of Table 3 compounds were potent inhibitors. The most potent inhibitor was Tecovirimat analog 2-((4-Methoxy-3-methyl-2-pyridylmethyl)sulfo)-5-trifluoromethyl-1H-benzimidazole (CHEBI:80011) with Ki value 0.412833. This benzimidazole can be further modified to develop better anti-MPXV drugs.

To execute the docking, the receptors were modeled using DeepMind’s AlphaFold 2 (14). AlphaFold 2 is an artificial intelligence (AI)-based groundbreaking software that can model any protein from amino acid sequences with a very high level of accuracy. The models were refined for molecular docking. Compounds with equal or higher binding affinity scores were selected for pkCSM-based ADMET analysis. pkCSM demonstrated a harmala alkaloid (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butane triol (CHEBI: 173933) and 5′-Dehydrodadenosine (CHEBI: 1958) showed decent intestinal absorption. After absorption, except this harmala alkaloid, all of these drugs will disseminate in unbound form (more than 70% of the absorbed drugs). During this circulation, they cannot cross...
Table 3 Name of the approved drugs and their ChEMBL IDs. ChEMBL IDs of the analogs that exhibited higher binding scores with their corresponding receptors than the approved drugs

| Ligand    | Binding Affinity (kcal/mol) with L2R | Inhibition constant, µM ($T = 298.15$ K) |
|-----------|--------------------------------------|------------------------------------------|
| CHEBI: 68294 | −7.6                                 | 2.64835                                  |
| CHEBI: 145220 | −7.2                                 | 5.20598                                  |
| CHEBI: 175144 | −6.7                                 | 12.1175                                  |
| CHEBI: 133856 | −6.6                                 | 14.3481                                  |
| CHEBI: 173933 | −6.6                                 | 14.3481                                  |
| CHEBI: 66159 | −6.5                                 | 16.9893                                  |
| CHEBI: 149753 | −6.5                                 | 16.9893                                  |
| CHEBI: 3578  | −6.2                                 | 28.2047                                  |
| CHEBI: 75473 | −6.2                                 | 28.2047                                  |
| CHEBI: 174448 | −6.2                                 | 28.2047                                  |
| CHEBI: 490877 | −6.2                                 | 28.2047                                  |
| CHEBI: 149757 | −6.1                                 | 33.3967                                  |
| CHEBI: 10110 | −6                                   | 39.5445                                  |
| CHEBI: 94793 | −6                                   | 39.5445                                  |
| CHEBI: 117936 | −6                                   | 39.5445                                  |
| CHEBI: 4728  | −5.9                                 | 46.8239                                  |
| Zidovudine   | −5.9                                 | 46.8239                                  |

| Ligand    | Binding Affinity (kcal/mol) with I4L | Inhibition constant, µM ($T = 298.15$ K) |
|-----------|--------------------------------------|------------------------------------------|
| CHEBI: 95211 | −7.7                                 | 2.23663                                  |
| CHEBI: 3202  | −7.3                                 | 4.39664                                  |
| CHEBI: 168240 | −7.3                                | 4.39664                                  |
| CHEBI: 3927  | −7.2                                 | 5.20598                                  |
| CHEBI: 95153 | −7.2                                 | 5.20598                                  |
| CHEBI: 9976  | −7.1                                 | 6.16431                                  |
| CHEBI: 55419 | −7                                   | 7.29905                                  |
| CHEBI: 93920 | −7                                   | 7.29905                                  |
| CHEBI: 45448 | −6.9                                 | 8.64268                                  |
| CHEBI: 90031 | −6.9                                 | 8.64268                                  |
| CHEBI: 94359 | −6.9                                 | 8.64268                                  |
| CHEBI: 1196  | −6.8                                 | 10.2336                                  |
| CHEBI: 16299 | −6.8                                 | 10.2336                                  |
| CHEBI: 73141 | −6.8                                 | 10.2336                                  |
| CHEBI: 85997 | −6.8                                 | 10.2336                                  |
| CHEBI: 147281 | −6.8                               | 10.2336                                  |
| CHEBI: 2310  | −6.7                                 | 12.1175                                  |
| CHEBI: 63580 | −6.7                                 | 12.1175                                  |
| CHEBI: 94463 | −6.6                                 | 14.3481                                  |
| CHEBI: 1014  | −6.5                                 | 16.9893                                  |
| CHEBI: 31858 | −6.5                                 | 16.9893                                  |
| CHEBI: 62005 | −6.5                                 | 16.9893                                  |
| CHEBI: 83079 | −6.5                                 | 16.9893                                  |
| CHEBI: 90239 | −6.5                                 | 16.9893                                  |
| CHEBI: 168049 | −6.5                               | 16.9893                                  |
| CHEBI: 1958  | −6.4                                 | 20.1168                                  |
| CHEBI: 8098  | −6.4                                 | 20.1168                                  |
| CHEBI: 19688 | −6.4                                 | 20.1168                                  |
| CHEBI: 42575 | −6.4                                 | 20.1168                                  |
the blood–brain barrier or Central Nervous System (CNS). Plausibly, these drugs cannot interfere with the nervous system. None of these drugs can interact with cytochrome P450 (CYP) enzymes except (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol (CHEBI: 173933). For this compound, drug–drug interactions and personalized medication might be more important. All of the drugs showed higher total clearance than 0.6 except Zidovudine

Table 3 (continued)

| Ligand | Binding Affinity (kcal/mol) with I4L | Inhibition constant, µM (T=298.15 K) |
|--------|--------------------------------------|--------------------------------------|
| CHEBI: 69426 | −6.4 | 20.1168 |
| CHEBI: 83081 | −6.4 | 20.1168 |
| CHEBI: 94738 | −6.4 | 20.1168 |
| CHEBI: 165834 | −6.4 | 20.1168 |
| Fludarabine | −6.4 | 20.1168 |

| Ligand | Binding Affinity (kcal/mol) with Phospholipase-D-like protein | Inhibition constant, µM (T=298.15 K) |
|--------|---------------------------------------------------------------|--------------------------------------|
| CHEBI: 80011 | −8.7 | 0.412833 |
| Tecovirimat | −8.6 | 0.488828 |

| Ligand | Binding Affinity (kcal/mol) with E8L | Inhibition constant, µM (T=298.15 K) |
|--------|--------------------------------------|--------------------------------------|
| CHEBI__4702 | −6.4 | 20.1168 |
| Dorzolamide | −6.3 | 23.8199 |
| CHEBI__3176 | −6 | 39.5445 |
| Brinzolamide | −5.6 | 77.7344 |
| CHEBI__43411 | −5.4 | 108.988 |
| CHEBI__6822 | −5.2 | 152.806 |
| Methazolamide | −4.9 | 253.68 |

**Fig. 4** L2R (Thymidine Kinase) interactions with a Zidovudine (binding affinity − 5.9 kcal/mol) and b (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol (CHEBI: 173933) (binding affinity − 6.6 kcal/mol)
Hence, to maintain a good bioavailability, these drugs should be administered in a higher amount than Zidovudine. Only Fludarabine tested positive for AMES, whereas all other medicines tested negative. pkCSM classified both the approved medications Zidovudine and Fludarabine as hepatotoxic. 5′-Dehydroadenosine and (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol (CHEBI: 173933) had no hepatotoxic properties.

![Fig. 5](image1)

**Fig. 5** I4L (Ribonucleotide reductase large subunit R1) interactions with a Fludarabine (binding affinity – 6.4 kcal/mol) and b 5′-Dehydroadenosine (CHEBI: 1958) (binding affinity – 6.4 kcal/mol)

![Fig. 6](image2)

**Fig. 6** Molecular dynamics simulation of L2R (Thymidine Kinase) with Zidovudine (Green) and (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol (CHEBI: 173933) (Golden) where a 100-ns Root Mean Square Deviation (RMSD), b Root Mean Square Fluctuations (RMSF) of all residues, c Radius of Gyration (Rg) for 100,000 ps, and d Solvent-Accessible Surface Area (SASA) profile have been demonstrated. Here, RMSD represents the protein stability with the drugs inside the dynamic system. The RMSF graph depicts the mobility of the receptors with the drugs. Rg graph showing alteration of protein compactness during the simulation and SASA representing the change of solvent accessible surface in dynamic condition (Table 4). Hence, to maintain a good bioavailability, these drugs should be administered in a higher amount than Zidovudine. Only Fludarabine tested positive for AMES, whereas all other medicines tested negative. pkCSM
Therefore, the similar compounds should be safe to use. Ligand–receptor Visualization revealed these analogs made less unfavorable bonds with the receptors and more conventional H bonds than their approved counterparts (Fig. 4 and 5). These bonds presented static interactions. To analyze them in dynamic conditions, MD simulation was conducted. MD simulation results suggested that these drugs can modulate the function of the viral proteins by manipulating the c-alpha backbones. These manipulations can change the mobility, compactness, and solvent accessible surface of the proteins. The secondary structure contents further support structural changes of the protein. GROMACS gmx do_dssp results of approved and proposed drugs were not significantly different (Fig. 8). L2R 3-Helix and I4L Coil structures demonstrated slight elevation during the simulation. Therefore, according to these results, inside the host cell, the I4L (Ribonucleotide reductase large subunit R1) and L2R (Thymidine Kinase) will not be able to process the DNA replication properly. Consequently, in the intermediate phase of the viral life cycle (~100-min’ post-infection) the MPXV protein will not be able to generate new IMVs. A Smaller amount of viral double-stranded DNA will not trigger cGAS: IFI16 and DNA-PK in an abrupt way (5). Previous studies reported that (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol (CHEBI: 173933)-type harmala alkaloids containing Peganum harmala L. seeds have antiviral activities in in vivo and in vitro models [44]. The approved candidate of this compound, Zidovudine or Azidothymidine, is a well-studied viral polymerase inhibitor (https://go.drugbank.com/drugs/DB00495). The other approved drug Fludarabine, fluorinated purine analog, inhibited Zika virus, Enterovirus A71, and Severe fever with thrombocytopenia syndrome virus (IC50 values below 1 μM) different cell lines such as Vero, U251 MG, BHK21, and HMC3 cells [45]. Similar candidate, 5’-Dehydroadenosine, is a non-fluorinated purine analog and has potential properties to act as an antiviral agent (7). In future studies, researchers and clinicians can use this drug discovery pipeline/methodology, approved and proposed drugs to treat MPXV infected in vitro and in vivo models.
Conclusion

The MPVX infection can be targeted with Brinzolamide, Dorzolamide, Methazolamide, Zidovudine, Gemcitabine, Hydroxyurea, Fludarabine, and Tecovirimat. However, Zidovudine and Fludarabine offer better druggable properties than others. Especially, our results suggest that Zidovudine, (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol harmala alkaloid, Fludarabine, and 5′-Dehydroadenosine purine analog are potent antiviral agent and they can impede MPXV DNA synthesis by inhibiting or reacting with I4L (Ribonucleotide reductase large subunit R1) and L2R (Thymidine Kinase). This inhibition/reaction should prevent IMV and, eventually, EMV formation by depleting or masking A, T, G, and C. This in silico analysis encourages these drugs to be explored in in vivo and in vitro models for clinical applications.

Fig. 8 Secondary structure contents of L2R (Thymidine Kinase) with a (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol (CHEBI: 173933) and b Zidovudine. I4L (Ribonucleotide reductase large subunit R1) secondary structure contents with c 5′-Dehydroadenosine (CHEBI: 1958) and d Fludarabine
## Table 4  Absorption, Distribution, Metabolism, Excretion, and Toxicity properties of approved drugs and selected drugs

| ADMET Properties                        | Zidovudine | (2S,4R)-4-(9H-Pyrido[3,4-b]indol-1-yl)-1,2,4-butanetriol [CHEBI: 173933] | Fludarabine | 5′-Dehydroadenosine [CHEBI: 1958] |
|------------------------------------------|------------|-----------------------------------------------------------------------------|------------|----------------------------------|
| SMILES                                   | CC1 = CN(C(= O)N[+][N−]) C1 = NC2C(C(O2)O)N[+][N−]                           | C1 = NC2C(N = NC1 = C2C = C1C = C1) | NC1 = C2N = CN(C@H30[C@H]C = O)[C@H](O)[C@H]30C2 = NC = N1 |
| MOL_WEIGHT                               | 267.245    | 272.304                                                                      | 285.235    | 265.229                          |
| LOGP                                     | −0.19628   | 1.4928                                                                        | −1.8409    | −1.7734                          |
| #ROTATABLE_BONDS                         | 3          | 4                                                                             | 2          | 2                                |
| #ACCEPTORS                               | 6          | 4                                                                             | 9          | 9                                |
| #DONORS                                  | 2          | 4                                                                             | 4          | 3                                |
| SURFACE_AREA                             | 106.629    | 115.066                                                                       | 111.012    | 106.213                          |
| Water solubility (log mol/L)             | −3.517     | −2.984                                                                        | −2.101     | −1.516                           |
| Caco2 permeability (log Papp in 10−6 cm/s) | −0.154     | 1.085                                                                         | −0.123     | 0.201                            |
| Intestinal absorption (human) (% Absorbed) | 70.225    | 92.641                                                                        | 58.223     | 51.647                           |
| Skin Permeability (log Kp)               | −3.142     | −2.742                                                                        | −2.735     | −2.735                           |
| P-glycoprotein substrate                 | No         | No                                                                            | No         | Yes                              |
| P-glycoprotein I inhibitor                | No         | No                                                                            | No         | No                               |
| P-glycoprotein II inhibitor               | No         | No                                                                            | No         | No                               |
| VDss (human) (log L/kg)                  | 0.036      | −0.013                                                                        | 0.524      | −0.108                           |
| Fraction unbound (human) (Fu)             | 0.755      | 0.137                                                                         | 0.86       | 0.81                             |
| BBB permeability                          | −1.166     | −1.001                                                                        | −1.33      | −1.217                           |
| CNS permeability                          | −3.236     | −3.02                                                                         | −3.727     | −3.714                           |
| CYP2D6 substrate                         | No         | No                                                                            | No         | No                               |
| CYP3A4 substrate                         | No         | No                                                                            | No         | No                               |
| CYP1A2 inhibitor                         | No         | Yes                                                                           | No         | No                               |
| CYP2C19 inhibitor                        | No         | No                                                                            | No         | No                               |
| CYP2C9 inhibitor                         | No         | No                                                                            | No         | No                               |
| CYP2D6 inhibitor                         | No         | No                                                                            | No         | No                               |
| CYP3A4 inhibitor                         | No         | No                                                                            | No         | No                               |
| Total Clearance (log ml/min/kg)           | 0.052      | 0.663                                                                         | 0.703      | 0.793                            |
| Renal OCT2 substrate                     | No         | No                                                                            | No         | No                               |
| AMES toxicity                            | No         | No                                                                            | Yes        | No                               |
| Max. tolerated dose (human)              | 0.656      | 0.748                                                                         | 0.823      | 0.702                            |
| hERG I inhibitor                         | No         | No                                                                            | No         | No                               |
| hERG II inhibitor                        | No         | Yes                                                                           | No         | No                               |
| Oral Rat Acute Toxicity (LD50) (mol/kg)   | 2.298      | 2.15                                                                           | 1.973      | 1.763                            |
| Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day) | 2.014 | 1.222                                                                         | 3.056      | 2.727                            |
| Hepatotoxicity                           | Yes        | No                                                                            | Yes        | No                               |
| Skin Sensitization                       | No         | No                                                                            | No         | No                               |

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**Note:** The SMILES strings and molecular properties are represented as they are given in the table.
Table 4 (continued)

| ADMET Properties | Zidovudine | Fludarabine | 5′-Dehydroadenosine [CHEBI: 1958] |
|-------------------|------------|-------------|----------------------------------|
| T. Pyriformis toxicity (log ug/L) | 0.118 | 0.333 | 0.285 | 0.285 |
| Minnow toxicity (log mM) | 3.145 | 0.131 | 4.504 | 3.083 |

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