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IN SILICO MONKEYPOX RESEARCH: PAVING THE WAY FOR NEXT-GENERATION TREATMENTS AND THERAPIES

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Abstract
The global re-emergence of the Monkeypox Virus (MPXV) has sparked renewed efforts to identify effective antiviral treatments. In this study, we conducted an in silico docking analysis using CB Dock2 to evaluate natural products and repurposed antiviral drugs. We focused on key viral targets for inhibition, including VP39 2'-O Methyltransferase, viral topoisomerase-DNA complexes, and poxin. Our findings highlighted several natural compounds, such as Physalin A, Sitoindoside IX, Withanolide, Shatavarin 1, Kutkoside, and Berberine HCl, as potential inhibitors. Additionally, Tecovirimat emerged as the most effective repurposed antiviral. Among the natural products, Withanolide, Sitoindoside IX, and Physalin A demonstrated promising inhibitory potential based on their Vina scores and binding affinities, offering hope for alternative therapeutic options. Tecovirimat remained the most potent inhibitor across all tested targets, underscoring its continued relevance in the fight against MPXV.

Keywords: Monkeypox Virus, VP39 2'-O Methyltransferase, Physalin A, Sitoindoside IX, Withanolide.

Introduction

Monkeypox virus (MPXV), a zoonotic Orthopoxvirus, has recently become a global health concern due to its spread beyond its traditional endemic regions in Central and West Africa. First identified in humans in 1970, MPXV has historically been limited to these regions, where it is transmitted primarily through animal reservoirs. However, recent outbreaks in Europe, North America, and other parts of the world have heightened global alarm. In response, the World Health Organization (WHO) declared monkeypox a Public Health Emergency of International Concern (PHEIC) in July 2022[1]. MPXV poses a serious health threat, particularly because it has a higher fatality rate than smallpox and can cause severe symptoms, including painful skin lesions, respiratory distress, and systemic

complications [2]. Currently, approved antiviral treatments for MPXV are limited, underscoring the urgent need for new therapeutic approaches. While vaccines, such as the smallpox vaccine, offer some protection against MPXV, they are not widely accessible, and the zoonotic nature of the virus complicates eradication efforts. MPXV can infect a wide range of animal species, challenging containment and increasing the potential for recurrent outbreaks [3]. Studies suggest that MPXV may adapt to different hosts over time, further complicating the development of effective control measures [4].

Public health agencies, including the Centers for Disease Control and Prevention (CDC) and WHO, have emphasized the need for intensified research efforts to discover new antiviral therapies, particularly for vulnerable populations such as immunocompromised individuals. The similarities between MPXV and the variola virus, which causes smallpox, make it a critical target for antiviral drug development [5]. Targeting viral proteins essential for replication, genome maintenance, and immune evasion could yield promising therapeutic strategies. Recent studies have identified three key proteins in MPXV that serve as potential drug targets: VP39 2'-O Methyltransferase, viral topoisomerase, and Poxin. Each of these proteins plays a distinct and essential role in the virus's life cycle. VP39 2'-O Methyltransferase is involved in viral RNA capping, a process crucial for stabilizing viral mRNA and ensuring efficient replication [6].

Viral topoisomerase facilitates DNA unwinding during replication, while Poxin aids in immune evasion by degrading host defense molecules. Inhibiting these proteins could disrupt the virus's ability to replicate and spread, offering a promising avenue for new antiviral drug development. By targeting these critical proteins, researchers hope to develop therapies that will not only manage current outbreaks but also prevent future ones [7].

1. VP39 2'-O Methyltransferase (PDBID-8B07)

Role in Mpox Pathogenesis

VP39 is a critical component in viral mRNA processing [8]. This enzyme carries out 2'-O methylation of the 5' cap of viral mRNA, a modification necessary for the proper recognition of viral mRNAs by the host cell's translation machinery. Without this modification, the host’s immune system can more easily recognize and degrade the viral RNA, significantly impairing the virus's ability to replicate [9]. In addition to enhancing translation efficiency, VP39-mediated methylation protects viral RNA from being detected by host antiviral defenses, such as the interferon response. This dual role in ensuring efficient translation and immune evasion makes VP39 an attractive drug target. Inhibiting VP39 can reduce viral protein synthesis and expose the virus to the host’s innate immune system [10].

Drug Target Potential

Inhibitors of VP39 methyltransferase activity would prevent the virus from effectively replicating within host cells. By obstructing this enzyme, the virus’s ability to mask its RNA from the immune system is diminished, potentially leading to rapid viral clearance by the host [11].

2. Viral Topoisomerase-DNA Complex (PDBID-3IGC)

Role in Mpox Pathogenesis

The viral topoisomerase is a crucial enzyme that resolves topological issues arising during DNA replication and transcription. In the case of the Mpox virus, which possesses a large and complex DNA genome, topoisomerase ensures that the viral DNA can be unwound and rewound appropriately [12]. The Mpox topoisomerase interacts with the viral genome, introducing temporary breaks to relieve torsional stress during replication. If the enzyme's activity is disrupted, the replication fork may stall, resulting in incomplete viral DNA replication. Given the need for efficient replication of the viral genome, the viral topoisomerase is integral to the successful production of progeny viruses. This enzyme also plays a role in reducing supercoiling and ensuring that transcription proceeds smoothly, further emphasizing its importance in pathogenesis [13].

Drug Target Potential

Targeting the viral topoisomerase can disrupt MPXV's ability to replicate its genome, thereby reducing viral propagation. Drugs designed to stabilize the topoisomerase-DNA complex or inhibit its function could lead to irreversible DNA damage in the virus, halting its life cycle[13].

3. Poxin (PDBID-8C9K)

Role in Mpox Pathogenesis

Poxin proteins are key mediators in viral immune evasion. MPXV uses Poxins to degrade host immune signalling molecules like cyclic GMP-AMP (cGAMP), a critical molecule in the STING (Stimulator of Interferon Genes) pathway. This pathway is essential for initiating an antiviral state in host cells. By degrading cGAMP, Poxins prevent the activation of the STING pathway, allowing the virus to evade detection and avoid triggering the host's antiviral responses, such as the production of interferons and inflammatory cytokines [17]. This ability to suppress the innate immune response is vital for the virus to establish infection and spread within the host. Without functional Poxins, the virus would be subject to rapid immune clearance, making Poxin an essential player in MPXV pathogenesis.

Drug Target Potential

Inhibiting Poxin function could restore the host's ability to recognize and mount an immune response against the virus. Blocking Poxin would allow cGAMP to accumulate, leading to STING activation and the subsequent production of interferons that limit viral spread. Developing inhibitors for Poxins could enhance host immunity and reduce viral persistence [18]. The structural and functional roles of VP39 2'-O Methyltransferase, viral topoisomerase, and Poxin in MPXV pathogenesis highlight their importance as drug targets [19]. Inhibiting these proteins could interfere with viral replication, transcription, and immune evasion mechanisms, making them critical focal points for antiviral drug development. Strategies that target these proteins can potentially curtail Mpox virus infections, providing therapeutic options to manage outbreaks [20]. Tecovirimat (TPOXX) is currently the only FDA-approved antiviral for treating pox [21]. It targets the VP37 protein, which is essential for viral particle formation and spread in orthopoxviruses, including pox [22]. The drug is especially recommended for severe cases, immunocompromised patients, and others with higher risks of complications. Another drug, Brincidofovir (Tembexa), is an antiviral used experimentally against DNA viruses like smallpox and mpox [23]. However, its use is less frequent due to limited clinical data on pox-specific efficacy. Tecovirimat, an FDA-approved antiviral for smallpox, has shown promise against MPXV due to its inhibition of the VP39 2'-O Methyltransferase [24]. However, there is a growing interest in natural products as alternative or complementary antiviral therapies. Exploring phytochemicals, known for their wide range of bioactive properties, could provide new insights into drug discovery. In this study, we leverage the power of in silico docking using CB Dock2 to evaluate the binding affinities of natural products alongside Tecovirimat.

Potential natural antiviral drugs:

Several natural products have demonstrated promising antiviral properties in various studies, showing their potential as therapeutic agents against viral infections [25]. Physalin A, a bioactive compound isolated from Physalis species, has shown significant antiviral activity, particularly against the hepatitis C virus (HCV) and Epstein-Barr virus (EBV), by inhibiting viral replication and modulating immune responses [26]. Sitoindoside IX, a bioactive steroidal lactone found in *Withania somnifera* (Ashwagandha), has also been investigated for its antiviral potential, showing inhibitory effects against herpes simplex virus (HSV) through immunomodulatory mechanisms [27]. Withanolide, another compound derived from *Withania somnifera*, has been explored for its antiviral activity against multiple viruses, including the influenza virus and HCV [28]. This compound primarily works by disrupting viral entry and replication pathways, making it a candidate for further investigation in antiviral drug development. Shatavarin 1, a saponin glycoside found in *Asparagus racemosus*, has exhibited antiviral properties against respiratory viruses by strengthening the host's immune response

and preventing viral multiplication [29]. Kutkoside, an iridoid glycoside isolated from *Picrorhiza kurroa*, has shown potent antiviral activity, particularly against the hepatitis B virus (HBV) [30]. Studies suggest that it exerts its effect by inhibiting viral DNA replication. Lastly, Berberine HCl, an alkaloid present in Berberis species, has been extensively researched for its antiviral properties [31], with demonstrated efficacy against human immunodeficiency virus (HIV), influenza, and herpes viruses. Berberine inhibits viral replication and modulates inflammatory pathways, making it a versatile antiviral agent with broad-spectrum activity.

Global declaration:

Eurosurveillance recently released articles addressing the ongoing outbreak. In a rapid communication dated 14 March 2024, findings from genomic sequencing of viral genomes were shared to shed light on the strains responsible for the outbreak [32]. The global response to the MPXV outbreak, as emphasized by organizations like the WHO and CDC, calls for a comprehensive strategy that includes not only vaccination but also the development of new antiviral therapies [33]. This study contributes to that narrative by identifying viable candidates for drug development and reinforces the importance of exploring diverse sources for antiviral compounds.

Methodology

Protein and Ligand Preparation

Protein structures of VP39 2'-O Methyltransferase (PDB ID-8B07), viral topoisomerase-DNA complex (PDB ID-3IGC), and Poxin (PDB ID-8C9K) as shown **in Figure 3 A, B, C** were retrieved from the Protein Data Bank. The natural ligands, including Physalin A, Withanolide, Shatavarin 1, and other natural products, were obtained from the PubChem database and prepared in their 3D conformations as shown in **Figure 2**. These ligands were energy minimized using the MMFF94 force field, ensuring that the ligands were in their most stable and optimal form for docking.

Docking Protocol using CB Dock2

CB Dock2 is a popular tool for performing docking studies and utilizes the AutoDock Vina algorithm. CB Dock2 operates by automatically identifying binding cavities in the protein structure through a grid-based cavity prediction algorithm. Once these cavities are identified, the ligands are prepared by minimizing their energy to avoid any high-energy conformations that could interfere with the docking process [34]. The software uses a scoring function that evaluates binding affinities based on intermolecular forces, including hydrogen bonds, van der Waals interactions, and hydrophobic contacts. The Vina algorithm iteratively improves the ligand's binding pose by adjusting its position and orientation within the binding site, with the final score representing the free binding energy in kcal/mol.

CB Dock2 Algorithm and Workflow

CB Dock2 employs a unique combination of cavity detection and docking algorithms. First, the cavity detection algorithm analyses the protein's 3D structure, identifying the most likely binding sites based on geometric features, such as crevices and pockets on the protein surface. The ligand is then docked into these predicted cavities, and the Vina algorithm refines the ligand's orientation based on the calculated interaction energy [35]. The Vina algorithm uses an optimization technique that minimizes the predicted binding energy by evaluating multiple possible orientations of the ligand within the binding site. The final docking pose represents the orientation with the lowest energy, which is assumed to correspond to the most favorable binding interaction. The algorithm evaluates multiple factors, including hydrogen bonding, hydrophobic effects, and electrostatic interactions, to produce a binding score in kcal/mol [34] [35]. CB-Dock2 improves molecular docking by combining cavity detection and ligand docking into a streamlined process. The methodology involves multiple steps, beginning with cavity detection using a fast Fourier transform (FFT) algorithm to analyse the protein surface [34, 35]. This method identifies potential binding pockets based on the surface's geometric concavities. Cavities are ranked by volume and shape, determining the most likely binding site for

ligands. Once the cavity is identified, Auto Dock Vina performs docking simulations. Vina uses a stochastic global search algorithm to find optimal ligand poses by minimizing the free energy of binding. The algorithm combines various interaction forces, including van der Waals, hydrophobic interactions, hydrogen bonding, and torsional penalties to calculate the binding energy.

The scoring function is expressed as:

$$\Delta G_{\text{binding}} = \Delta G_{\text{vdW}} + \Delta G_{\text{hydrophobic}} + \Delta G_{\text{H-bond}} + \Delta G_{\text{torsion}}$$

Where:

- ΔG_{vdW} accounts for van der Waals interactions,
- $\Delta G_{\text{hydrophobic}}$ accounts for hydrophobic interactions,
- $\Delta G_{\text{H-bond}}$ represents hydrogen bonding energy, and
- $\Delta G_{\text{torsion}}$ reflects the penalty for ligand torsional flexibility.

CB-Dock2 also integrates genetic algorithms (GA) to handle ligand flexibility [34] [35]. The ligand is sampled in various conformations, and the GA explores the conformational space through iterative mutation, selection, and crossover, optimizing the best ligand poses. The process ranks docking poses based on their predicted binding energies, with the most energetically favourable conformations presented as the final results. By leveraging cavity detection and the flexibility of docking algorithms, CB-Dock2 enhances both accuracy and efficiency in predicting how ligands bind to protein targets, making it a useful tool in computational drug design [34] [35].

Results

The docking results revealed that several natural products exhibited promising binding affinities with the selected MPXV targets. Physalin A emerged as a potent inhibitor with a Vina score of -10.3 kcal/mol against VP39 2'-O Methyltransferase, indicating strong hydrogen bonding and hydrophobic interactions. Similarly, Withanolide showed a favourable binding score (-9.1 kcal/mol), making it a potential candidate for MPXV inhibition. These findings are significant as they suggest that natural products could play a vital role in developing alternative antiviral treatments for MPXV. The docking analysis for MPXV proteins with PDB IDs 8C9K and 8B07 revealed promising results for several natural compounds. Withanolide demonstrated the highest binding affinity, achieving a Vina score of -10.7 kcal/mol, indicating it as a strong potential inhibitor. Sitoindoside IX and Physalin A both followed closely with Vina scores of -10.3 kcal/mol, suggesting they also have significant potential as MPXV inhibitors. Tecovirimat, a known antiviral, displayed a Vina score of -9.6 kcal/mol, which was also matched by Kutkoside, showing that these natural products may perform comparably to existing antiviral treatments. Other compounds, including Shatavarin 1 and Berberine HCl, showed moderate binding affinities with Vina scores of -8.6 kcal/mol and -8.3 kcal/mol, respectively. These findings indicate that natural compounds, particularly Withanolide, Sitoindoside IX, and Physalin A, hold potential for the development of alternative antiviral treatments targeting MPXV. Tecovirimat, the chemical antiviral, displayed the s binding affinity across all targets, with a Vina score of -10.3 kcal/mol for VP39 2'-O Methyltransferase and -9.9 kcal/mol for the viral topoisomerase-DNA complex. Its ability to inhibit multiple critical viral enzymes makes it a highly effective antiviral agent. However, the growing concerns around antiviral resistance necessitate the search for complementary therapies, particularly from natural sources. This study provides preliminary evidence supporting the potential of natural compounds like Physalin A, Sitoindoside IX and Withanolide in inhibiting MPXV. These natural inhibitors demonstrated comparable binding affinities to Tecovirimat, particularly against viral topoisomerase-DNA complex (PDBID-3IGC), and Poxin

(PDBID-8C9K), VP39 2'-O Methyltransferase see **Figure 1& Table 1**. Further in vitro and in vivo studies are required to validate these findings, but the in-silico results are encouraging. The involvement of global health organizations such as the WHO and CDC in addressing the current MPXV outbreak highlights the importance of continued research into antiviral agents. The WHO has emphasized the need for a multi-faceted approach that includes not only vaccination but also the development of new antiviral treatments. By exploring natural products and their efficacy, this study contributes to the growing body of research aimed at finding novel solutions for MPXV.

Discussion

The findings from this study underscore the urgent need for effective therapeutic strategies against monkeypox virus (MPXV), particularly in light of its rising incidence and potential for severe health consequences. The comparative analysis of the binding affinities of natural compounds with key viral proteins—VP39 2'-O Methyltransferase, viral topoisomerase, and Poxin—reveals promising candidates for antiviral development. The selection of VP39, topoisomerase, and Poxin as drug targets is particularly relevant given their distinct roles in MPXV pathogenesis. VP39's function in mRNA cap methylation not only facilitates efficient translation of viral proteins but also aids in immune evasion, making it a critical target. Inhibitors that can disrupt this protein may enhance the host immune response, leading to improved viral clearance. The binding affinity of Physalin A (-10.3 kcal/mol) highlights its potential as a lead compound, suggesting that further exploration into its mechanism could yield effective antiviral therapies. Similarly, targeting the viral topoisomerase is crucial due to its involvement in DNA replication and transcription. The ability of natural products, particularly Withanolide and Sitoindoside IX, to achieve high binding affinities (-10.7 and -10.3 kcal/mol respectively) suggests they could serve as templates for developing new inhibitors that disrupt MPXV replication. These findings are promising, especially given the historical challenges of creating effective antivirals for DNA viruses. Poxin's role in immune suppression presents another therapeutic opportunity. By degrading cGAMP, Poxin prevents the activation of the STING pathway, which is vital for initiating antiviral immune responses. Inhibitors of Poxin could potentially restore the host's immune vigilance, making it a strategic target for drug development. Exploring natural products as antiviral agents represents an innovative approach to addressing the limitations of current therapies like Tecovirimat and Brincidofovir. The binding affinities exhibited by the natural compounds in this study indicate that they may not only complement existing treatments but could also help mitigate the risk of antiviral resistance—a significant concern as the MPXV outbreak continues. The results showing comparable binding affinities between natural compounds and established antivirals signal the potential for these natural products to be integrated into therapeutic regimens. For instance, Withanolide's potent binding suggests it could enhance therapeutic efficacy, particularly for high-risk populations, including immunocompromised individuals who are disproportionately affected by MPXV. The preliminary in silico findings necessitate further validation through in vitro and in vivo studies to establish the clinical relevance of these natural compounds. Investigating their pharmacokinetics, safety profiles, and mechanisms of action will be crucial steps in their development as therapeutic agents. Additionally, the synergistic potential of combining these natural products with existing antiviral drugs should be explored to enhance treatment efficacy and broaden the therapeutic arsenal against MPXV. The results of this study indicate that targeting VP39 2'-O Methyltransferase, viral topoisomerase, and Poxin with natural products holds promise for the development of effective antiviral therapies against MPXV. Given the ongoing threat posed by this emerging infectious disease, continued research and innovation in this area are imperative for public health.

Conclusion

This study highlights the promising potential of targeting key viral proteins—VP39 2'-O Methyltransferase, viral topoisomerase, and Poxin—as effective strategies for developing novel antiviral therapies against the Monkeypox Virus (MPXV). The compelling binding affinities observed with natural compounds such as Physalin A, Withanolide, and Sitoindoside IX offer hope for these

agents as viable therapeutic candidates. These compounds could play a pivotal role in either enhancing or complementing existing treatments, such as Tecovirimat, which has shown efficacy in MPXV management. The ability of these natural products to disrupt essential viral functions represents not only a significant scientific breakthrough but also introduces an innovative approach to addressing the virus. This becomes especially relevant as we face the rising challenge of resistance to conventional antivirals. The significance of these findings lies in their potential to expand the current arsenal of antiviral therapies. In a world where viral outbreaks continue to pose serious public health threats, relying solely on a limited number of treatments is insufficient. Natural products, with their diverse chemical structures and biological properties, present an untapped reservoir of antiviral potential. By targeting multiple viral proteins, these compounds may offer a multi-faceted approach to combating MPXV, thereby reducing the likelihood of resistance and ensuring more comprehensive viral inhibition. Furthermore, the urgency of the global health crisis posed by the ongoing MPXV outbreaks cannot be overstated. With cases increasing worldwide, the need for new, effective therapies is critical. While Tecovirimat remains a strong contender, this study suggests that natural compounds could either work synergistically with existing treatments or serve as alternatives in cases where traditional antivirals fail. Therefore, further research into these compounds, including in vitro and in vivo studies, is not only recommended but necessary. Such studies will help clarify their mechanisms of action, optimize their efficacy, and ensure their safety in clinical settings.

Table 1: Docking results of the natural products and Tecovirimat against the monkeypox virus targets based on the data provided:

Product	VP39 2'-O Methyltransferase (PDBID-8B07)- Vina scores c	Viral Topoisomerase-DNA Complex (PDBID-3IGC) Vina score c	Poxin (PDBID-8C9K) Vina score c
Withanolide	-10.7	-10.7	11.3
Sitoindoside IX	-10.3	-10.3	9.6
Shatavarin 1	-8.6	9.3	8.4
Physalin A	-10.3	-10.6	9.6
Kutkoside	-9.6	8.2	7.8
Berberine HCl	-8.3	8.4	7.3
Chlorogenic Acid	7.3	7.8	7.9
Tecovirimat	-10.3	-9.9	9.1

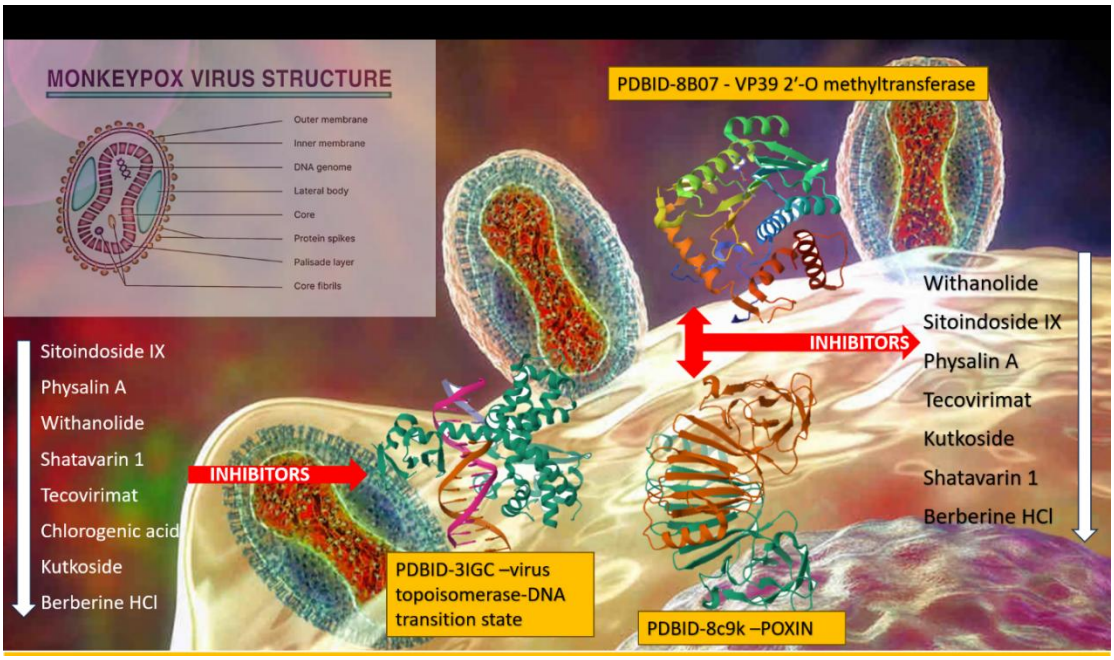

Figure1: In Silico High-Throughput Screening of Poxin, VP39 2'-O Methyltransferase, and Viral Topoisomerase-DNA Complexes as Drug Targets for Monkeypox Virus and their potential

Inhibitors

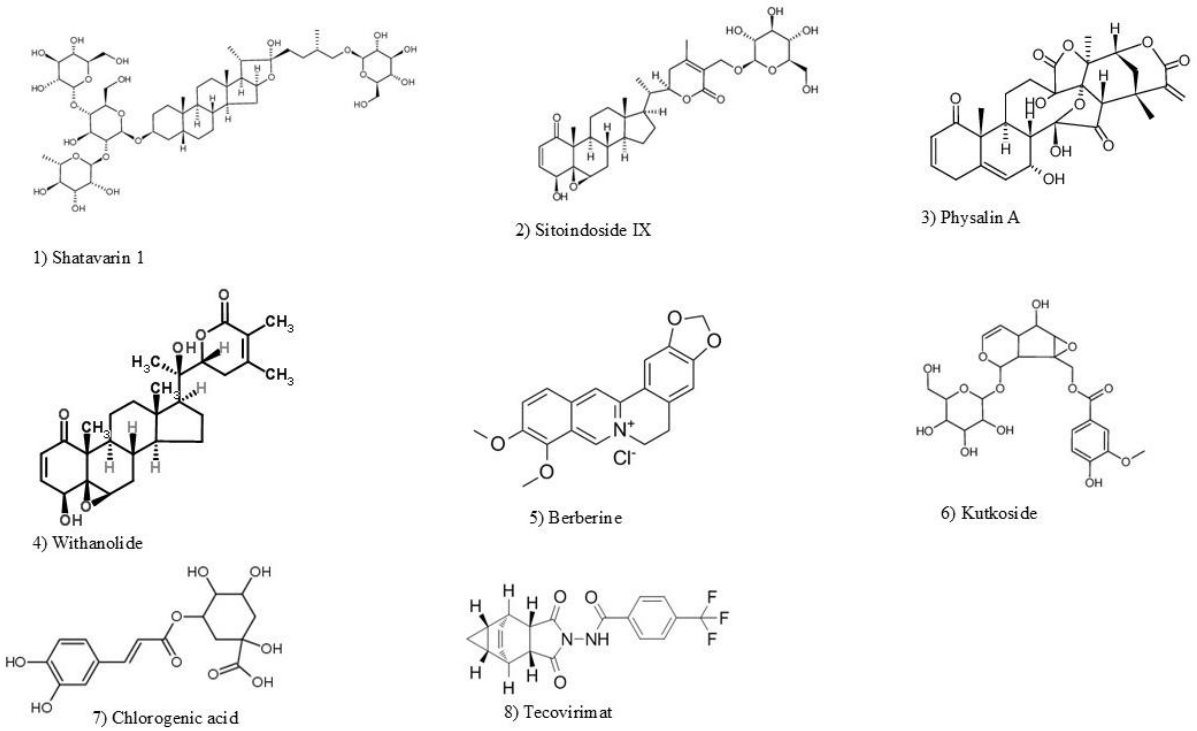

Figure 2: 1. Shatavarin 1, 2. Sitoindoside IX, 3. Physalin A, 4. Withanolide, 5. Berberine 6. Kutkoside, 7. Chlorogenic acid, 8. Tecovirimat

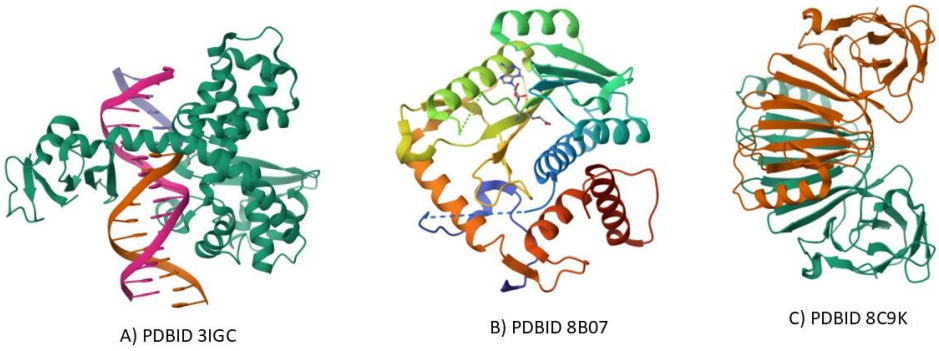

Figure 3: A : Viral Topoisomerase, **B:** VP39 2'-O Methyltransferase. **C:** Poxin

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