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ORIGINAL RESEARCH

Exploring the Potential of HIV Integrase Inhibitors as Therapeutic Agents Against HSV and HCMV: A Molecular Docking Study

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Purpose: The worldwide threat of herpes virus infections and their resistance against the existing drugs creates an urgent need for the development of novel drug candidates. An RNase H like domain is present in the protein encoded by the highly conserved *ul15* gene (UL15) of Herpes Simplex Virus, *ul89* gene encoded protein (UL89) of Human Cytomegalo Virus and Human Immunodeficiency Virus (HIV) integrase proteins. This provided a way repurpose HIV integrase inhibitors as HSV UL15 and HCMV UL89 inhibitors.

Materials and Methods: The protein sequences were aligned using the Clustal Omega software to determine the conserved amino acid positions. Schrodinger software was used for docking studies, protein–ligand interaction and simulation studies. The selected drugs were screened to analyse their anti- HSV-1 and HSV-2 activities by Cytopathic effect (CPE) inhibition assay and RT-PCR using Vero cells as host. The experiments were further analyzed by the two-way ANOVA test using Bonferroni's post-HOC using GraphPad Prism software version 8.0.2. The RT-PCR experimental results were analyzed using the $2^{-\Delta\Delta Ct}$ Livak method to determine the fold reduction in viral gene expression.

Results: The in-silico studies showed the binding abilities of anti-HIV drugs to the active site of UL15 and UL89 by blocking their metal ions, similar to HIV integrase. Among the anti-HIV drugs tested, Elvitegravir and raltegravir were most effective in controlling the HSV-1 and HSV-2 infections at concentrations as low as 1.6 µg/mL when tested with $10TCID_{50}$ viral challenge dose. Elvitegravir was most effective in reducing the viral gene expression as tested by RT-PCR.

Conclusion: The tested FDA approved drug molecules showed the potential to be repurposed as an antiHSV and antiHCMV agent. The in silico and in vitro results warrant further investigation in preclinical and clinical studies to validate its therapeutic potential and safety for HSV-related infections.

Keywords: herpes simplex virus, drug repurposing, HIV integrase inhibitors, HCMV

Background

Herpes simplex virus (type 1 (HSV-1) and type 2 (HSV-2) and Human cytomegalovirus (HCMV) belong to *Herpesviridae* family of viruses. It is estimated that about 67% of the worldwide population is affected by HSV 1, 11.3% by HSV 2, and 83% seroprevalence is estimated for HCMV.[1–3] These viruses can cause diseases like genital sores, encephalitis, retinitis, hepatitis, nephritis, and colitis.[4,5] Immunocompromised patients are at a fatal risk of recurring HSV and HCMV infections and it can cause severe diseases, as pneumonia.[6–8]

DNA polymerase and thymidine kinase are the key proteins in HSV and HCMV against which many antiviral drugs act. Acyclovir and its derivatives and ganciclovir, the first line of drugs used against HSV and HCMV, act by inhibiting thymidine kinase and DNA polymerase, respectively. These drugs, however, are becoming ineffective as DNA polymerase and viral kinase are prone to mutations.[9,10] Therefore, there is an urgent need to expand the existing arsenal of antivirals against HSV-1, HSV-2, and HCMV by focussing on viral protein targets with low mutation rates. One such

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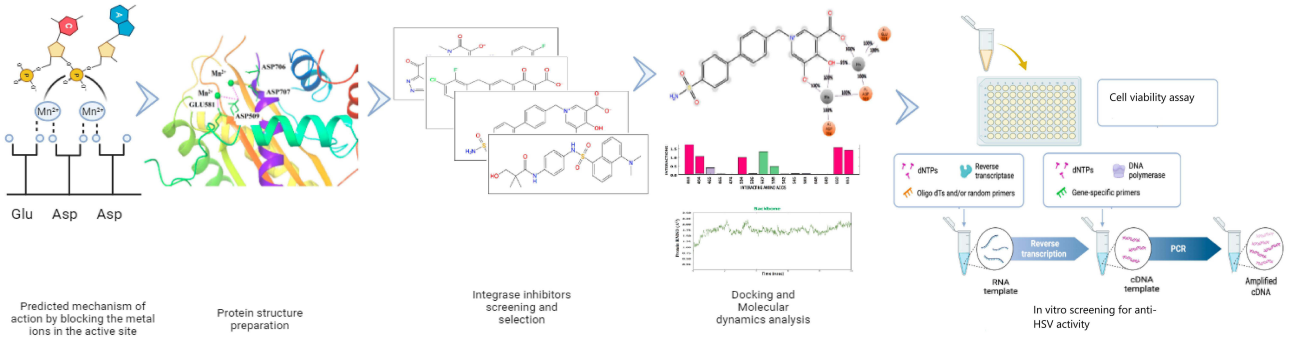
Graphical Abstract



target is the DNA encapsidation protein, encoded by the *ul15* gene in HSV-1, HSV-2, and its homologue UL89 gene in HCMV. These are the most conserved genes in the *Herpesviridae* family.[11,12] The *ul15* and *ul89* encoded proteins perform an important role during the packaging of viral DNA into mature capsids, by cutting the genomic material from viral concatemers.[13,14] These proteins have a conserved RNase H like domain, which makes use of the metal ion catalysis mechanism in the DNA cleavage and packaging activity as a part of the terminase complex.[15] Knockdown studies on *ul15* and UL89 genes have demonstrated that they are indispensable for viral propagation.[16,17]

RNase H like domain belongs to the nucleotidyltransferase (NT) superfamily and it is also present in the Human Immunodeficiency Virus (HIV) integrase protein, which performs the DNA cleavage, aiding the viral DNA integration into the host genome.[18] The RNase H like domain of HSV and HCMV are similar to HIV integrase's RNase H like domain.[14,19] The DNA cleavage event is brought about by three important conserved amino acids namely, Asp509, Glu581, and Asp707 in UL15 of HSV 1 and 2, Asp463, Glu534, and Asp651 in UL89 of HCMV, and Asp64, Asp116 and Glu152 in HIV integrase protein.[20,21] These amino acids help in the coordination of 2 divalent metal ions that in turn coordinate the oxygen atom of the scissile phosphate group.[18] The HIV integrase protein is inhibited by small-molecule drugs like raltegravir by blocking the metal ion-binding capacity and similarly, the activity of the RNase H like domain can be blocked (Figure 1).[22]

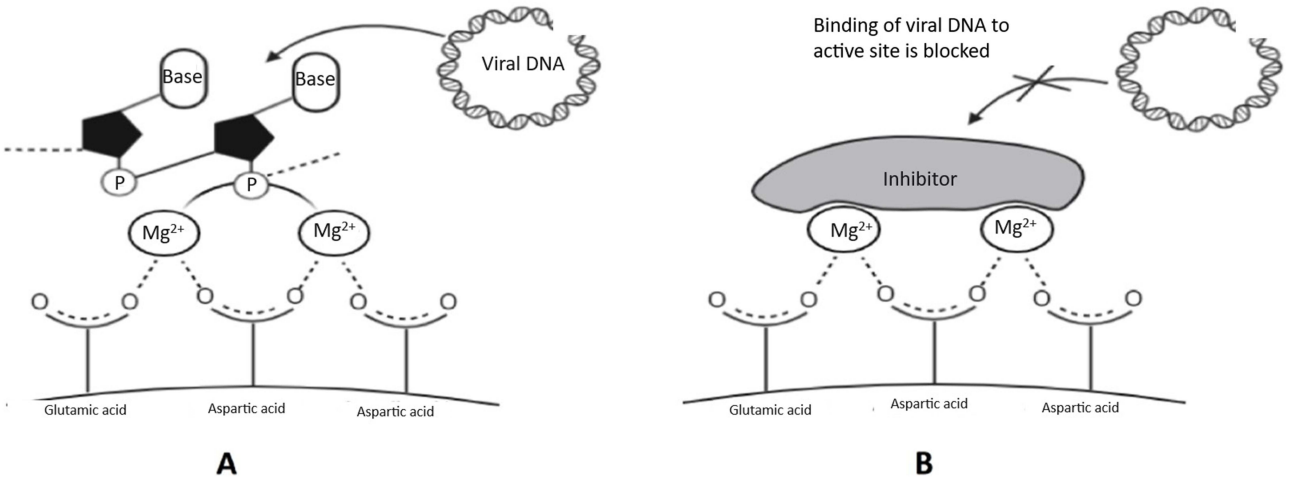


Figure 1 Proposed mechanism of inhibition of RNase H like domain in HSV and HCMV (**A**) The divalent cation coordinated by the three amino acids in the terminase active site is responsible for cleaving the phosphodiester bond in the DNA molecule (**B**) Blocking the metal ions by the inhibitor molecule, thus preventing nuclease activity of RNase H like domain.

Several small molecular drugs such as raltegravir, tomeglovir, bictegravir, and elvitegravir have been reported to effectively inhibit the HIV integrase and HIV reverse transcriptase protein by binding onto the RNase H like domain. HIV integrase inhibitor drugs are also found to inhibit HSV and HCMV replication in a few repurposing studies.[14,23–26] Raltegravir and elvitegravir have shown an inhibitory effect on both HSV and HCMV,[23,27] whereas tomeglovir[24,26] and letermovir, a known inhibitor or terminase complex in HCMV[25,26] have shown their activity against HCMV only. Bictegravir is a known inhibitor of HIV integrase protein that causes enzyme inhibition due to the metal coordination at the RNase H like domain which is similar to the inhibition mechanism of raltegravir, elvitegravir, tomeglovir, and letermovir.[28] However, the mechanism of action of these drugs against HSV and HCMV is not known. In this study, we predict that these drugs might interact with the RNase H like domain at the active site of UL15 and UL89, as seen in HIV integrase. A preliminary antiviral assay is conducted to determine the potential anti-HSV activity of these drug molecules.

Materials and Methods

Structure of UL15 and UL89

The crystal structure of HSV1 UL15 (PDB ID: 4IOX) lacked the metal ions, essential for the DNA cleavage activity. Since the inhibitors bind to the RNase H like domain through the metal ions, the presence of these metals is essential for the protein–ligand interactions. The metal ions were placed in UL15 by superposing it with UL89 (PDB ID: 3N4P) and copying the atomic coordinates using Maestro from the Schrodinger suite (SchrödingerLLC).

Sequence and Secondary Structure Comparison

The sequences of the integrase, UL89 and UL15 from HIV, HCMV, and HSV respectively were aligned using Clustal Omega.[29] In addition, secondary structures of these proteins were compared using DALI.[30] The DALI server is an online tool for comparing protein structures in 3D. The study involved a comparison between the selected 3D structures of the RNAse H like domains from the HSV UL15, HCMV UL89, and HIV integrase domain proteins which aids in analyzing the secondary structure and provides a possibility of HIV integrase inhibitors binding site in HSV and HCMV proteins.

Protein Preparation

Protein structures were prepared using Schrodinger (SchrödingerLLC). All the heteroatoms (except metal ions) were removed from the protein file. The protein structures were modified by assigning the correct bond orders, deleting the water molecules beyond 5 Å from the heterogeneous groups, and adding missing hydrogen atoms. The ionization and tautomeric states of amino acid residues were corrected by the addition of hydrogen atoms. Using the OPLS3e force field, the minimization of protein structures was carried out to the root mean square deviation (RMSD) value of 0.30 Å.

Identification of Binding Sites

The binding site was proposed to contain Asp509, Glu581, and Asp707 in UL15 of HSV, while Asp463, Glu534, and Asp651 in UL89 of HCMV. These amino acids are involved in the coordination of metal ions in the divalent ion catalysis.[18] The presence of a potential binding site containing the specified amino acids in UL15 and UL89 was confirmed using the SiteMap module (Version 2.2, Schrodinger LLC), which aids in the identification of probable binding sites and druggability of the protein targets.[31] This calculation allows the determination of the regions around the protein surface that might be used for the ligand binding. The hydrophobic and hydrophilic maps are also used to divide the protein into metal-binding regions, h-bond donor, and acceptor regions. The Site scores are generated based on site size, enclosure degree by the protein and solvent exposed area, tightness of interaction between site points and the ligand, the potential of donation or acceptance of hydrogen bonding of the ligand, and also the hydrophobic and hydrophilic characters of the site.

Ligand Preparation

Raltegravir (PubChem Id: 54671008), tomeglovir (PubChem Id: 475330), letermovir (PubChem Id: 45138674), elvitegravir (PubChem Id: 5277135) and bictegravir (PubChem ID: 90311989) were retrieved from the PubChem database. Ligand preparation was carried out using the LigPrep module (Schrodinger, LLC) by assigning the suitable protonation

states at physiological pH 7.2 ± 0.2. The chiralities of the original compounds were not changed. The low-energy conformers of the ligands were produced using ConfGen torsional sampling by using OPLS3e force field.

Docking

Raltegravir, elvitegravir, letermovir, tomeglovir, and bictegravir were docked with the RNase H like domains of UL15 and UL89. The extra precision (XP) mode of Glide (Schrodinger, LLC) was used for docking calculations with the default parameters as described earlier.[32] The initial minimization step size was done with 0.05 Å and the maximum step size was 1.0 Å. This was maintained in the case of conjugate gradient minimization and steepest descent minimizations. The value of 10^{-7} kcal/mol was used for energy change and 0.001 kcal/mol was used for gradient criteria in the convergence minimization criteria. All the conformations generated were considered for docking studies. The best conformation for each ligand was selected based on the Glide scoring function (G-score) and the protein–ligand interactions, which were further used in the molecular dynamics (MD) studies.

Molecular Dynamics (MD) Simulation

The interactions and stability of the ligands with the protein were studied through MD simulation. Protein-ligand complexes (UL15 and UL89 with raltegravir, tomeglovir, bictegravir, letermovir, and elvitegravir) were subjected to MD simulations using Desmond simulation package (Schrodinger LLC) and OPLS3 force field. The system was set up by placing the protein-ligand complex in an orthorhombic simulation box filled with a predefined SPC water model. The system was treated by adding the required number of Na^+ and Cl^- ions along with NaCl salt concentration of 0.15 M. The model system was then relaxed using NVT and NPT ensembles. Finally, MD simulation was carried out for 250 ns in an NPT ensemble at 300K temperature and 1 atm pressure.

Cell Culture and Virus Preparation

Vero cells were obtained from National centre for cell sciences Pune (NCCS) and were grown using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). DMEM was procured from GIBCO and FBS and was procured from Sigma Aldrich (St. Louis, MO). HSV 1 (Sc-16) and 2 (133,953–03) viruses were procured from Christian Medical College, Vellore, Tamil Nadu. The virus stocks were prepared by inoculating Vero cells maintained in DMEM containing 1% FBS with virus.

Virus Titration

The virus titration was conducted by inoculating 100 µL of Vero cells at 0.01×10^6 cells/well concentration using 96 well plates. Once a monolayer of cells at ~80% confluency was seen, it was washed with phosphate buffered saline (PBS) (pH 7.2) 3 times. This was followed by inoculation with viral samples. The 50% Tissue Culture Infectivity Dose ($TCID_{50}$) was calculated using the method given by Reed and Muench method.[33] The virus titration was performed by 10 fold serial diluting the virus stock solution (10^{-1} to 10^{-10}). 0.1 mL of each viral dilution was added to 8 wells per column of the plate thereby inoculating the monolayer cells. At every 24h post inoculation, the cytopathic effect (CPE) was microscopically observed and were given "+" per 25% based on the extent of CPE (+ 25%, ++ 50%, +++ 75% and ++++ 100% CPE).

Antiviral Drugs

Acyclovir, raltegravir, bictegravir, and elvitegravir were procured from Cayman Chemical Company (Ann Arbor, MI. USA). (with purity ≥ 98%). Stock solutions were prepared from accurately weighed powder which was dissolved in tissue culture grade DMSO (1 mg/mL) and stored at 4°C. Working standard solutions were prepared by appropriate dilutions of the stock solution in DMEM to obtain the required concentrations.

In vitro Cytotoxicity Study

The cytotoxicity of the drug molecules was checked at concentrations of 100, 25, and 6.25 µg/mL. Readings were taken in triplicates and the data is expressed as mean ± standard deviation. The cytotoxicity of the drugs was tested on uninfected Vero cells for 3 days. The cell viability was determined by the addition of 100 µL of

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) to the cells after removing the media. After 4 hours of incubation, the formazan produced by the viable cells were estimated after dissolving them in 50 µL DMSO. The absorbance was measured at 570 nm in a microplate reader (ELx800, BioTek Instruments, Inc., Winooski, VT, USA).[34] The experiments were repeated at least thrice to obtain statistically significant results.

$$\%viable\ cells = \left(\frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ cell\ control\ sample}\right) \times 100$$

Cytopathic Effect (CPE) Inhibition Assay

Antiviral screening of selected antiviral drugs against HSV-1 and HSV-2 was carried out using 10 $TCID_{50}$ virus challenge dose. The tested concentrations were 25, 5, 1.6, 0.4, and 0.1 µg/mL. The screening was carried out using the post-infection model. The cells were pre-infected with the virus 2 hours prior to the drug treatment at different concentrations. The percent antiviral activity of the test drugs was determined by calculating the proportion of viable cells by MTT assay.[35] Experiments were repeated at least thrice to obtain statistically significant results. All readings were taken in triplicates, and statistical differences were evaluated one-way ANOVA test using Bonferroni's post-HOC analysis.

$$\%\ Antiviral\ activity = \left(\frac{Absorbance\ of\ test\ sample - Absorbance\ of\ virus\ control}{Absorbance\ of\ cell\ control - Absorbance\ of\ virus\ control}\right) \times 100$$

Analysis of ul15 Expression Levels by Real Time Polymerase Chain Reaction (RT-PCR)

Vero cells were cultured in 6 well microtitre plates and the monolayer culture was infected with 10$TCID_{50}$ dose of the HSV-1 for 2 hours. The cells were treated with 6 µg/mL of acyclovir and raltegravir while elvitegravir and bictegravir were added at 5 µg/mL. The treatment was maintained for 72 hours. The TRIzol reagent (Invitrogen) was used for extracting total RNA and iScript cDNA Synthesis Kit (Bio-Rad) was used for cDNA synthesis. Applied Biosystems® QuantStudio® 5 Real-Time PCR System (Thermo Fisher, Waltham, MA, USA) was used for conducting qRT-PCR with TB green® Premix Ex Taq™ II (TII RnaseH Plus) (TaKaRa, Kusatsu-shi, Japan). Primers targeting *ul15* gene expression were: Forward primer: 5'TTGTCGACGAGGCCAACTTT3'; Reverse primer: 5′AAAGCTCGTACTGGCCTTCC3′. HUEL gene was used as the housekeeping gene and primers targeting gene expression were as follows: Forward primer: 5′TCAGACGACGAAGTCCCCATGAAG3′; Reverse primer: 5′TCCTTACGCAATTTTTTCTCTCTGGC3′.[36] The Ct values generated as duplicates were averaged, normalized against housekeeping genes and then compared against biological controls (untreated) using the ΔΔCt method of comparison. Fold change in the target gene mRNA expression was expressed as.–ΔΔCT[37]

Results

Protein Structures

The crystal structure of UL89 had two Mn^{2+} ions at the binding site, which were modeled into the UL15. The modeled UL15 structure exhibited metal coordination with the three desired amino acids Asp509, Glu581, and Asp707 which are known to bind the metal ion in UL15 active site (Figure 2). Two metal ions are required in the RNase H like domain for the binding of inhibitor molecules. This ensured that the metal ions were placed at the correct position in the binding site.

Secondary Structure Analysis

The structure alignment of UL15, UL89, and HIV integrase proteins exhibited better conservation of secondary structures compared to their sequences (Figure 3). The amino acids Asp64, Asp116, and Glu152 in HIV integrase; Asp509, Glu581, and Asp707in HSV UL15; and Asp463, Glu534, and Asp651 in HCMV UL89 were conserved in the sequence and structure. These amino acids are involved in the metal coordination at the binding site. However, the secondary structures of UL15 and UL89 did not show a good alignment with the HIV integrase as it is not a part of Herpesvirus family. The conservation of secondary structures and the metal coordinating amino acid triad explains the similar function of UL15, UL89, and HIV integrase protein's RNase H like domains.

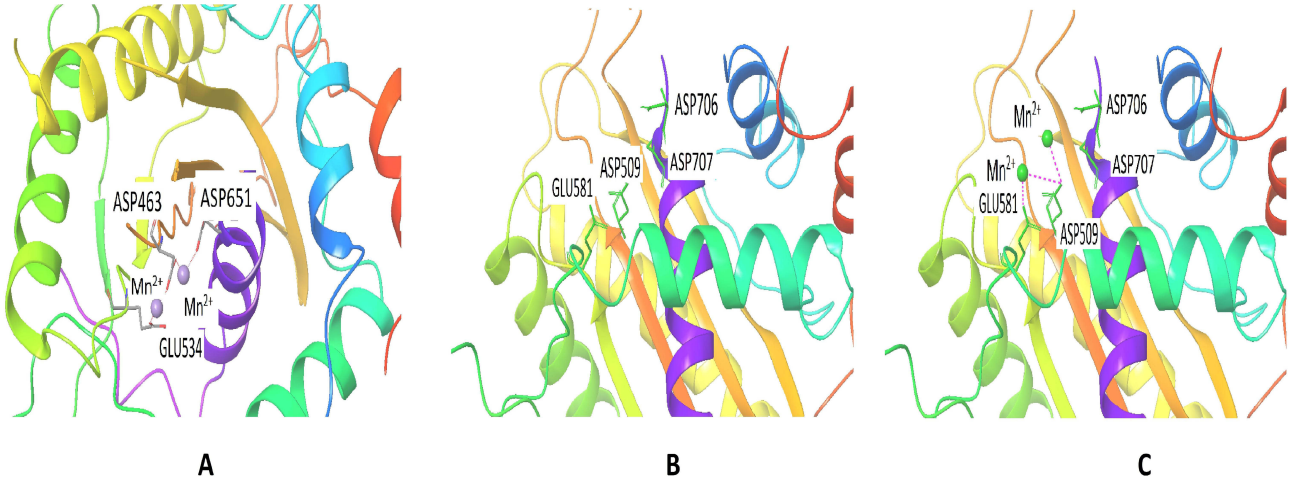


Figure 2 Modeling of selected viral proteins and metal ion interactions (**A**) Structure of HCMV UL89 with Mn^{2+} metal ions in the active site (PDB ID-3N4Q) along with the interacting amino acids; (**B**) Structure of HSV UL15 obtained from PDB database (PDBID- 4IOX) without the metal ions; (**C**) The structure of HSV UL15 with Mn^{2+} ions in the active site. The atomic coordinates from HCMV UL89 were used to model the metal ions into the HSV UL15 active site.

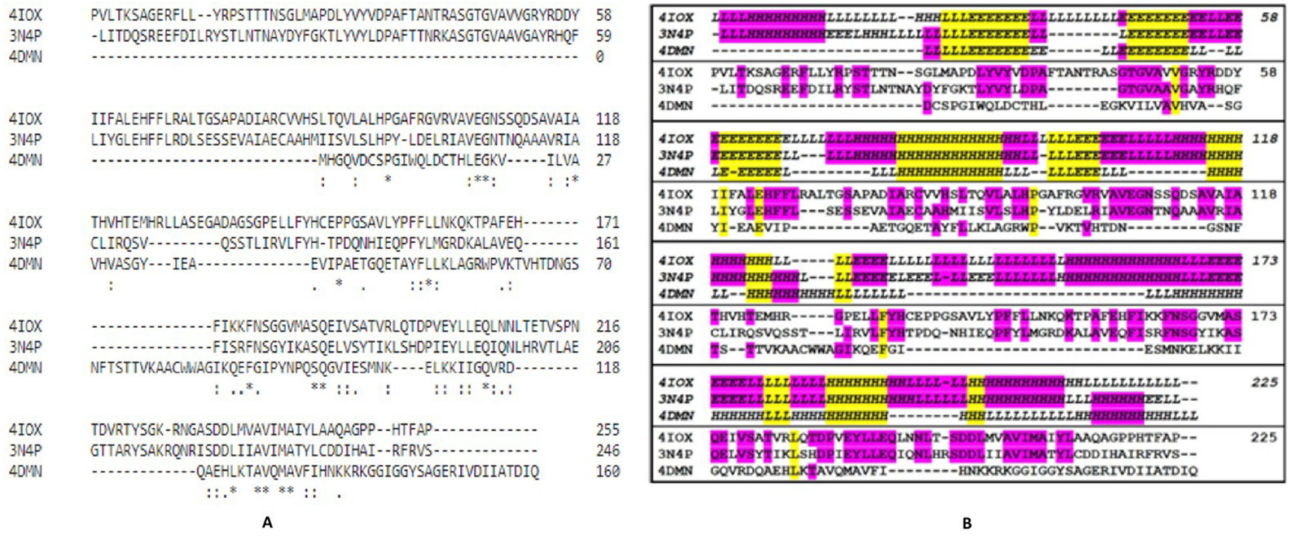


Figure 3 Alignment of HSV-UL15, HCMV-UL89, and HIV integrase (**A**) Sequence alignment of UL15 (PDBID:4IOX), UL89 (PDBID:3N4P), and HIV integrase (PDBID:4DMN) using Clustal Omega software. The figure showed the conservation of the amino acids involved in metal ion coordination at the RNase H like domain active site (Asp64, Asp116, and Glu152 in HIV integrase; Asp509, Glu581, and Asp707 in HSV UL15; and Asp463, Glu534, and Asp651 in HCMV UL89) (**B**) Secondary structure alignment of UL15 (PDBID:4IOX), UL89 (PDBID:3N4P) and HIV integrase (PDBID:4DMN) catalytic domain. The highly conserved secondary structure of the metal ion chelating triad points towards a similarity of function in UL15, UL89 and HIV integrase protein RNase H like domains.

Docking Studies

Raltegravir and elvitegravir are the reported inhibitors of HSV-1 and −2 and HCMV; letermovir and tomeglovir were reported to inhibit HCMV only and the activity of bictegravir on HSV is not explored. Since these drugs target the RNase H like domain of HIV integrase, HIV reverse transcriptase, and terminase complex of HCMV, we hypothesized that their inhibition of HSV and HCMV might be through the binding of RNase H like domain of UL15 and UL89, respectively (Figure S1). To confirm this, we have performed docking of these compounds with the RNase H like domain of UL15 and UL89. Docking results demonstrated that the binding mechanism of these compounds is similar to that of the proposed structure in HIV integrase.[21] The metal ion coordination in UL15 involves the amino acids Asp509, Glu581, and Asp706 (Figure S2) whereas in UL89 the amino acids involved are Asp463, Glu534, and Asp651. In the figure (Figures S2 and S3), the β-hydroxy-ketone structural motif of raltegravir, bictegravir, and elvitegravir are shown to

interact with the metal ions. A similar mechanism of RNase H like domain inhibition was reported for HIV integrase.[21,28] The 1-hydroxymethyl-2-methylpropyl group of elvitegravir is also involved in the metal ion coordination for HSV UL15 and this motif interacts with the Asp651 amino acid of UL89 in HCMV (Table 1). The interactions that persisted more than 90% of the simulation time were with the amino acid triad through the metal ion coordination (Supplementary material, Figure S2 and S3). This was observed in the case of all the compounds with UL15 and UL89 binding sites (Supplementary material, Figure S2 and S3). The graph in figures (Supplementary material, Figure S4 and S5) shows the RMSD changes of protein and ligand. The protein did not undergo any detectable changes throughout the simulation period as variations of RMSD were in the range of 1–3 Å. Further, the contribution of the amino acid triad was comparatively high in the interactions with the ligands (Supplementary material, Figure S6 and S7).

Binding Site Analysis

The amino acids, other than the catalytic triad, which might have the potential to interact with the ligands were explored through binding site investigation using SiteMap. A few amino acids that can probably act as hydrogen bond acceptors and donors were identified (Table 2). In addition, the amino acids having hydrophobic features were recognised. These amino acids might contribute to ligand interactions at the binding sites. Sitemap shows the amino acids involved in interaction and the space available for ligand docking. The resistance to small molecules drugs occurs due to mutant viral strains having substitutions in the amino acids generally targeted by drugs at the binding site. Therefore, other amino acids mentioned here can be focussed on during the drug designing studies. Inhibitors can be designed for this site which can be exploited further for drug designing.

Table 1 Docking Output for the HSV UL15 and HCMV UL89 with Selected Drug Anti-HIV Drugs

Virus	Drug	Docking Score (kcal/mol)
HSV	Raltegravir	−4.117
	Elvitegravir	−8.770
	Letermovir	−5.882
	Tomeglovir	−5.504
	Bictegravir	−5.776
HCMV	Raltegravir	−5.858
	Elvitegravir	−6.516
	Letermovir	−5.485
	Tomeglovir	−4.676
	Bictegravir	−6.663

Table 2 Potential Binding Sites Using SiteMap for HSV UL15 and HCMV UL89 Proteins

Virus Type	H Bond Acceptor Features and Complementary Amino Acids	H Bond Donor Features and Complementary Amino Acids	Hydrophobicity Features and Complementary Amino Acids
HSV	GLN680, ASP706, ASP707, ASP509, GLY522, ALA518, TYR507, TYR676	ALA518, THR521, ASP706, ASP707, TYR507, TYR676	ALA711, GLY522, HIS540
HCMV	LYS583, TYR461, ASN626, ASP651, HIS628, ASN536	MET579, ASN536, GLU534, ASP650, ASP651, ASP463, LYS583, ARG629, HIS628, GLN623	LYS583, MET658, HIS628

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In vitro Cytotoxicity Study

This study of drug cytotoxicity helped in determining the drug concentrations which could be safely administered to the Vero cells without killing them but still producing the antiHSV effect. Elvitegravir has a CC50 at concentrations above 3 µg/mL[38] while raltegravir is known to have mild cytotoxicity at 25 µg/mL.[39] Since data is not available on the Vero cells specific toxicity of Bictegravir, the assay was carried out at 6.25 µg/mL and below to maintain uniformity.[28,40] In the current study, the concentrations of 6.25 µg/mL and above for elvitegravir and bictegravir proved to be extremely cytotoxic to the Vero cells while raltegravir was mildly cytotoxic to the Vero cells at 25 µg/mL (Figure 4). Therefore, on the basis of these results, further antiviral assays were performed at concentrations <6 µg/mL for all the drug molecules.

Cytopathic Effect Inhibition Assay

The drug molecules showed potential antiHSV1 and 2 activity when given a 10TCID$_{50}$ virus challenge dose (Figure 5). In a study testing the antiHSV effect of raltegravir using CV1 cells as host cells, the inhibitory concentrations were ~30 µg/mL and ~38 µg/mL for HSV-1 and HSV-2, respectively.[41] Since the higher concentrations were found to be cytotoxic to Vero cells concentrations 25 µg/mL and below were tested. Raltegravir effectively inhibited HSV1 and 2 infections and even concentrations as low as 1.6 µg/mL could give >50% antiviral activity. Raltegravir showed highest CPE inhibition activity at 25 µg/mL while bictegravir and elvitegravir showed higher activity at 5 µg/mL.

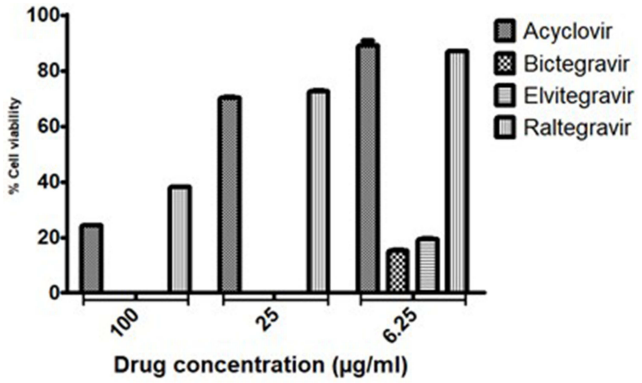

Figure 4 Drug cytotoxicity was determined using MTT assay. Raltegravir and Acyclovir were mildly cytotoxic compared to other drugs at concentrations of 100 and 25 µg/mL. However, elvitegravir and bictegravir were found to be highly cytotoxic for concentrations above 6.25 µg/mL. The results are represented as a mean of 3 independent experiments.

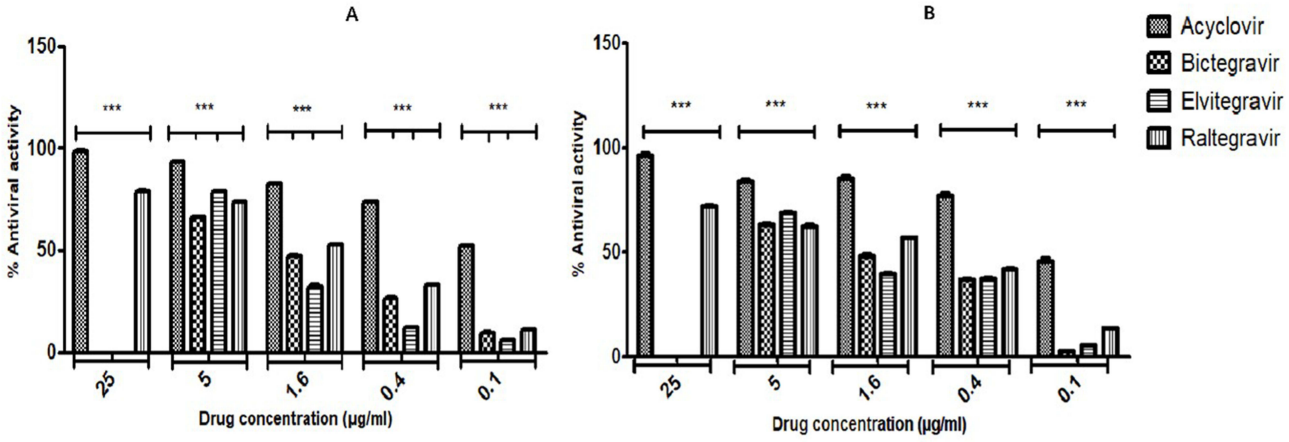

Figure 5 The anti-HSV activity of the selected anti-HIV drug molecules against (**A**) HSV1 and (**B**) HSV2 by CPE inhibition assay with 10 TCID$_{50}$ virus challenge dose. The percent antiviral activity shown is representative of the results from 3 independent experiments and expressed as mean ± Standard Error of the Mean. The two-way ANOVA test using Bonferroni's post-HOC keeping Acyclovir as the control, revealed that there was a significant relation between the groups (*** represents P>0.0001). Data is plotted using GraphPad Prism software version 8.0.2.

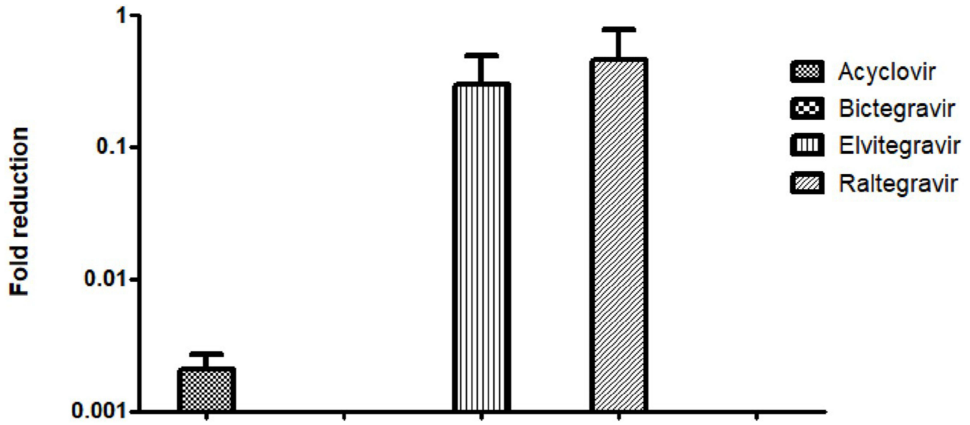

Figure 6 Reduction in viral gene expression levels of HSV-UL15 gene in the Vero cells treated with selected anti-HIV drugs, expressed as $2^{-\Delta\Delta Ct}$ value (Fold change). Among the tested drugs, apart from Acyclovir (6 µg/mL), which is used as standard, Raltegravir (6 µg/mL) and Elvitegravir (5 µg/mL) showed better anti-HSV activity. Bictegravir (5 µg/mL) did not show any reduction in viral gene expression.

Analysis of ul15 Expression Levels by RT-PCR

The RT-PCR study shed light on the capacity of the drug molecules to inhibit the *ul15* gene expression (Figure 6). The inhibition of gene expression is reflected by the increase in cycle number, as the gene copies reduce the cycle number increases. The Livak method was followed to calculate the fold change in gene expression. The *ul15* gene expression was reduced to 0.2% (Fold change of 0.002±0.001) by acyclovir which is the standard drug used for HSV infections. The highest reduction in *ul15* gene expression among the HIV inhibitors was elvitegravir which reduced the gene expression to 30% (Fold change of 0.299±0.959) followed by raltegravir which reduced the gene expression to 46% (Fold change of 0.455±0.322).

Discussion

In this work, a preliminary study was conducted to determine the capacity of a few anti-HIV integrase inhibitors to inhibit HSV. The study involved the use of in silico and in vitro methods to analyze the repurposing potential of anti-HIV drugs as anti-HSV drugs. The method employed in the current study was based on the concept of the structure-activity relationship, wherein the similarity in the protein structure of HIV integrase and the terminase complex of HSV was targeted.[14,19] Both of these viral proteins have RNase H like domain which forms the basis of this study.

The main mechanism of action for the majority of antiviral drugs is to target a vital process in the virus life cycle. This requires the determination of a viable target that will be indispensable for the virus to complete its life cycle and produce virus progeny particles. The advantage of using the terminase protein subunits as a target against HSV is the importance of this protein in the packaging of virus genomic material in the viral capsid, making it vital to the virus life cycle.[13,14] The target used in the current study is the small subunit of terminase complex which is highly invariable and does not have off-target effects in the human host,[42] making it less prone to developing viral drug resistance.[9,10]

The anti-HIV drugs are HIV integrase inhibitors and some of them have shown activity against members of *Herpesviridae* family.[23,24,26,27,43] Therefore, the current study uses the subunit of terminase complex as a target for the repurposing of anti-HIV drug molecules. The application of drug repurposing by targeting the RNase H like domain has been tested earlier by an earlier study and here the advantage of suppressing acyclovir resistant HSV 1 and 2 strains was uncovered.[44]

The current study was divided into the in-silico screening and in vitro validation stages. The structure selected for the study was the small subunit of terminase protein encoded by the *ul15* gene from HSV1 (PDB ID: 4IOX). The protein sequence of HSV1 and HSV2 share ~94% homology and the amino acids required for the catalytic activity of DNA cleavage are conserved in HSV1 and 2 (Supplementary material and Figure S8). Therefore, the mechanism of inhibition can be assumed to be similar for both viruses. The in-silico analysis was able to establish the capacity of bictegravir, elvitegravir, letermovir, raltegravir and tomeglovir as suitable candidates for the anti-HSV activity via the docking and molecular dynamics simulation. These drugs are known and FDA approved inhibitors of HIV integrase protein. These drug molecules were analysed through the in-silico stages to determine their viability as potential anti-HSV and anti-

HCMV agents. Since the anti-HSV activity of bictegravir is not yet established, it was selected for the in vitro analysis stages. Raltegravir is a known inhibitor of *ul15* gene-encoded protein and therefore it was used for this study. Elvitegravir is known to act upon the UL89 gene-encoded protein in HCMV and therefore its effect on the *ul15*-encoded protein was tested in the *in-vitro* stages of the current study. This was further verified using in-vitro assays like cytopathic effect inhibition assay and RT-PCR by testing the drugs at different non-cytotoxic concentrations. The cytopathic effect inhibition assay demonstrated the antiviral activity at ~30-40% for bictegravir and elvitegravir to the HSV 1 and 2 infection at concentration of 1.6 µg/mL, whereas for raltegravir the antiviral activity improved as the concentration was increased against both HSV1 and 2. Higher concentrations proved to be cytotoxic for bictegravir and elvitegravir and therefore could not demonstrate the antiviral efficacy at higher concentrations. The RT-PCR experimental results were analyzed using the $2^{-\Delta\Delta Ct}$ Livak method on HSV1 infected Vero cells. Due to the high degree of conservation between HSV1 and 2, the experiment was carried out in HSV1 for the preliminary screening in this study. The ΔΔCt value was generated by comparing treated and untreated groups and further the ΔΔCt value was converted to fold change ($2^{-\Delta\Delta Ct}$). The experiment demonstrated acyclovir's high efficiency in reducing the viral gene expression. Elvitegravir and raltegravir also showed appreciable reduction in viral gene expression. However, the bictegravir did not have an appreciable effect on viral gene expression and therefore, the activity of *ul15* gene may not be highly affected by treatment of HSV1 infected cells with bictegravir as compared to elvitegravir and raltegravir. The higher activity of elvitegravir may be contributed to its specific ability to inhibit *ul15* gene expression, while raltegravir has higher inhibition capacity for HSV DNA polymerase. Bictegravir is an efficient inhibitor of HIV integrase protein however the differences in the overall protein structure of HSV UL15 and HIV integrase may be the reason the lower activity of bictegravir against HSV. However, since the activity of these known inhibitors of RNase H like domain has been established by this study, it provides a new direction towards the repurposing of drug molecules that may inhibit the terminase RNase H like domain in the *Herpesviridae* family of viruses.

Conclusion

The repurposing of anti-HIV drugs for HSV and HCMV could be an alternative option for designing new drug molecules. RNase H like domain belongs to the nucleotidyltransferase (NT) superfamily, and it is an unexplored target in HSV. This domain is present in several organisms like *Escherichia coli* RNase H 1 and 2, human RNase H 1 and 2, the RuvC Holliday junction resolvase, the Argonaute RNase, the Hepatitis B virus (HBV) RNase H, the HIV RNase H, and the HIV integrase.[45] The RNase H like domain as an effective antiviral target is established by various studies.[46,47] In the present study, elvitegravir, raltegravir, letermovir, bictegravir and tomeglovir have shown encouraging results in terms of binding energy and stability against the RNase H-like domain in UL15 and UL89 in HSV and HCMV, respectively. Raltegravir is a known inhibitor RNase H like domain even for SARS Covid 2.[48] The results of this study suggest that these drugs may be used for the treatment of HSV and HCMV after further in vitro and in vivo evaluations. The drug formulations may be optimized to design an effective treatment strategy. Combination studies may be designed to determine the effect of drug combinations on the HSV strains. With further exploratory studies on other viruses with NT domain, these anti-HIV drugs may be repurposed efficiently for treating other viral diseases.

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Disclosure

The authors declare no conflicts of interest in this work.

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