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COMPUTATIONAL ANALYSIS OF MUTANTS OF CORE GP41 RESISTANCE TO FUSION INHIBITOR-ENFUVIRITIDE IN HUMAN IMMUNO DEFICIENCY VIRUS TYPE-1

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ABSTRACT: Modeling studies based on the generation of mutants which may lead to the cause of drug resistance in connection to structural analysis of inhibitor-enzyme complexes may provide a better understanding of drug-resistance mutations, influencing their effects at a structural level. One of the important factors in HIV resistance could be an altered drug binding in mutant forms. In line with this, the differences in the binding affinity between wild-type (WT) and mutants of HIV gp41 and fusion inhibitor (FI) such as enfuvirtide (EVT) were investigated, in the present study. The generation of four mutants (MTs) of HIV-1 gp41 such as G36V, V38M, N43S, and L44M with the help of software-MODELLER was carried out. Further, the mutants were docked with EVT using the software-Flexp Pep Dock. The WT protein showed higher affinity than the MTs, suggesting the favorable binding of EVT with the WT protein compared to MT types. This could be attributed to the presence of more number of H bond compared to other MTs. These models provide the first *in silico* evidence for the binding interaction of HIV-1 gp41 and it's MT with EVT, to our knowledge.

Key words: HIV, gp41, Fusion inhibitor, EVT, Modeling, Docking.

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INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV-1) is known to be a major killer disease. Since, no effective vaccine is available; the chemotherapy is the only way by which the rate of morbidity and mortality of HIV infected individuals has been reduced, significantly. More importantly, the introduction of antiviral therapy with a combination of three different drugs known as Highly Active Antiretroviral Therapy (HAART) the progression of viral progeny is suppressed to a greater extent. While mono-therapy commonly results in resistance due to the escape mechanism of HIV from a single drug, is not under present practice. The current treatment based on HAART has improved the life expectancy of HIV patient's considerably. Five classes of drugs inhibit HIV progression such as nucleoside analogue reverse transcriptase (RT) inhibitors (NRTIs), non-nucleoside RT inhibitors (NNRTIs), protease inhibitors (PI), integrase inhibitors (II) and fusion inhibitors (FI).These are directed against four virus-specific processes namely i) cell entry, ii) reverse transcription, iii) integration and iv) maturation (De Clercq, 2009; Mehellou and De Clercq, 2010).

Entry inhibitors are directed against the process of type–I HIV viral entry and can either inhibit attachment of the virus to the cellular receptors or the conformational changes necessary for subsequent membrane fusion. The envelope glycoprotein complex (Env) of the HIV-1 is responsible for entry into host cells. Env consists of three HIV surface glycoprotein (gp120) subunits that mediate receptor (CD4) and co-receptor (CCR5/X4) attachment and three gp41 subunits responsible for membrane fusion (Fung and Guo, 2004; Nagashima et al., 2001). Depending on the nature of inhibition, entry inhibitors are classified into 3 categories: (1) attachment inhibitors, which interfere with the attachment of the gp120 to CD4 receptors; (2) coreceptor inhibitors, which inhibit the interaction of the gp120-CD4 complex with the coreceptor (CCR5 or CXCR4); and (3) fusion inhibitors, which inhibit the fusion of viral and plasma membranes. Fusion inhibitors (FI) induce conformational changes within gp41 required for membrane fusion and inhibit attachment of gp120 to the receptor or co-receptor. Enfuvirtide (T-20, formerly DP-178, pentafuside), are peptides corresponding to the amino acid sequence of gp41 of the HXB2 isolate and is the only approved FI. It prevents membrane fusion by competitively binding to gp41 and blocking the formation of the post-fusion structure (Eggink et al., 2010; Reeves et al., 2002). Therefore, it is of interest for us to determine interactions of enfuvirtide EVT with gp41, in line with this in our study, the differences in the binding affinity between wild-type (WT) and four mutants (MTs) of HIV-1 core gp41 protein with EVT (FI) were investigated to understand the effect of blocking activity of EVT.

MATERIALS AND METHODS

Homology modeling of gp41 proteins

The gp41 proteins, WT and four MTs (G36V, V38M, N43S and L44M) were generated using MODELLER9v14 programme (Sali, 1997).

Template selection

In the present study, the target protein fragment of HIV-1 gp41 sequence ID-Q53I19 obtained from the uniprot database, was submitted to protein- Basic Local Alignment Search Tool (BLASTp) (Altschul et al.,1997) and searched against protein data base (PDB).

Model Building

The crystal structure of gp41 protein from HIV-1 (PDB code-3CP1) (Bai et al., 2008) was considered as template. The WT protein was generated by substitution of D back to N at codon 43. In the template 3CP1, A chain was retained and the heteroatoms and water were removed. Command line options were provided for sequence alignment between template and WT. Following this, other commands were provided for model building using MODELLER9v14. To generate four different MT proteins of the gp41 protein residues at position 36, 38, 43 and 44 were substituted with indicated amino acids (G36V, V38M, N43S and L44M) in the WT sequence. Commands were given for sequence alignment between WT and MTs. Then, the MT models were generated as mentioned above for WT.

Model Evaluation and Energy minimization

Validation was done in order to eliminate the structural errors and to improve the quality and stability in the generated model. This was performed by Ramachandran plot and superimposition method on the models (Krissinel and Henrick, 2011; Lovell et al., 2003).

Ligand

The EVT was obtained from Chemspider database (ACD/Chemsketch, 2006) with ID number 16743716 having Molecular formula $C_{204}H_{301}N_{51}O_{64}$, was converted to Mol2 and then to PDB format for docking purpose and it was saved as a PDB file.

Visualization

The Discovery Studio (DS) and BIOVIA-2016 softwares were used for visualization purpose of modelled proteins and docking data (Discovery studio, 2007).

Docking

Docking was performed using the online free software FlexPepDock, a high-resolution peptide-protein docking (refinement) protocol for the modeling of peptide-protein complexes, implemented in the Rosetta framework (London et al., 2011). The Rosetta FlexPepDock protocol mainly consists of two alternating modules that optimize the peptide backbone and rigid body orientation, using the Monte-Carlo with minimization approach, respectively. The starting structure is refined in 200 independent FlexPepDock simulations. 100 of the simulations are carried out strictly in high-resolution mode, while 100 of the simulations include a low-resolution pre-optimization step, followed by the high-resolution refinement.

A total of 200 models are thus created and then ranked based on their Rosetta generic full-atom energy score. FlexPepDock was thoroughly benchmarked against a set of perturbed peptide-protein complexes and an effective range of sampling was defined.

The complex of proteins (WT and four MTs) and ligand (EVT) was provided as input files in PDB format to the FlexPepDock server-http://flexpepdock.furmanlab.cs.huji.ac.il/.The output flex report was further analyzed using DS and BIOVIA.

RESULTS

Validation of Models

In this study, gp41 core proteins were modeled with the help of MODELLER9v14 and the models were built based on the WT sequence of gp41 core protein. From the results of BLAST search against PDB, 3CP1 was identified as template protein (Figure 1). The models were validated by the following methods: (a) Ramachandran plot: Evaluation of the models (WT and three mutants) was performed using Ramachandran plot computed with RAMPAGE. Structural evaluation with RAMPAGE showed 83% of residues in the most favored region, suggesting a good quality (Figure 2) of the WT model. (b) Superimposition method: Superimposition of WT and template showed a deviation of 0.5 Å; this shows the change in single amino acid in the template sequence did not produce any significant change in the overall conformation of the model protein (Figure 3).

NCBI Blast:Protein Sequence (143 letters)

Chain A, Structure Of A Longer Thermalstable Core Domain Of Hiv-1 Gp41 Containing The Enfuvirtide Resistance Mutation N43d

Sequence ID: 3CP1_A Length: 86 Number of Matches: 1

Range 1: 1 to 86 GenPept Graphics Next Match Previous Match

Related Information

Structure - 3D structure displays

Score	Expect	Method	Identities	Positives	Gaps
122 bits(307)	3e-37	Compositional matrix adjust.	76/128(59%)	80/128(62%)	42/128(32%)

```
Query  7    TLTVQARQLLSGIVQQQNDLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGI  66
            TLTVQARQLLSGIVQQQNDLLRAIEAQQHLLQLTVWGIKQLQAR
Sbjct  1    TLTVQARQLLSGIVQQQNDLLRAIEAQQHLLQLTVWGIKQLQARS--------------  45

Query  67   WGCSGKLICTTAVPWNSSWSNKSQDQIWHNMTWMEWEREIENYTDLIYTLIEKSQNQQEK  126
             G  G                           WMEW+REI NYT LI++LIE+SQNQQEK
Sbjct  46   -GGRG-------------------------GWMEWDREINNYTSLIHSLIEESQNQQEK  78

Query  127  NEQELLEL  134
            NEQELLEL
Sbjct  79   NEQELLEL  86
```

Figure 1: BLASTp result showing 59% identity between template (3CP1) and the target WT-gp41 sequence (Q53I19)

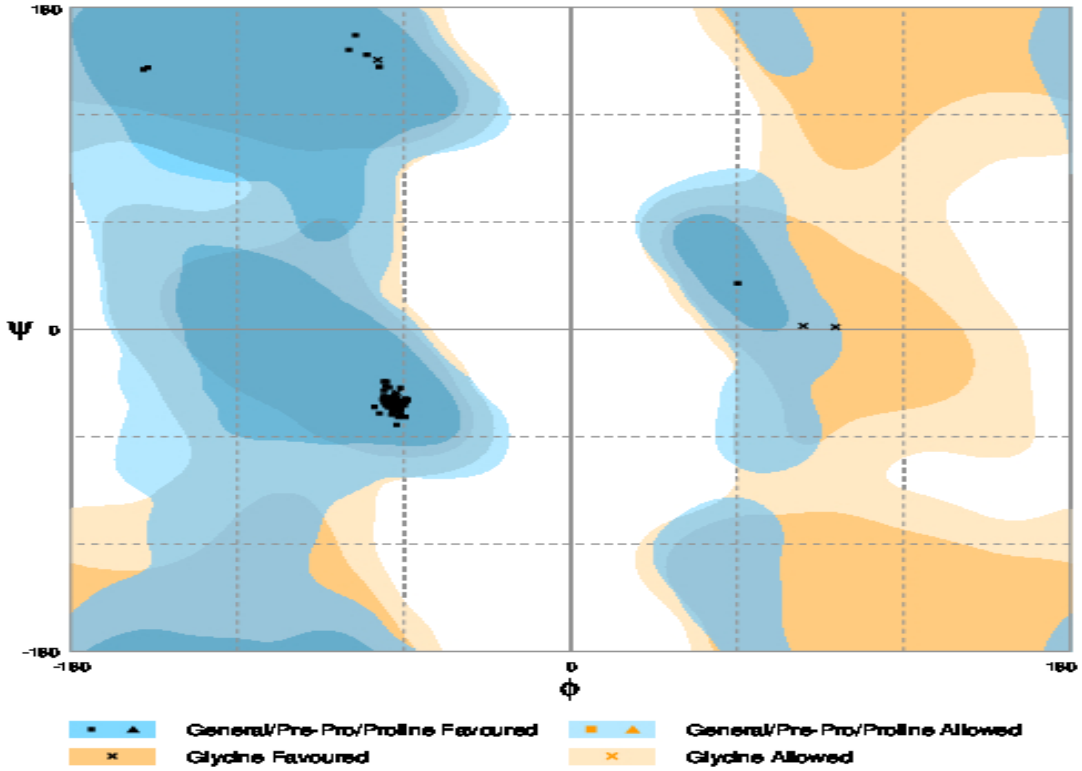

Figure 2: Ramachandran Plot

```
Evaluation of residues
Number of residues in favoured region     (~98.0% expected)  :    83 (100.0%)
Number of residues in allowed region      (~2.0% expected)   :     0 (  0.0%)
Number of residues in outlier region                         :     0 (  0.0%)
```

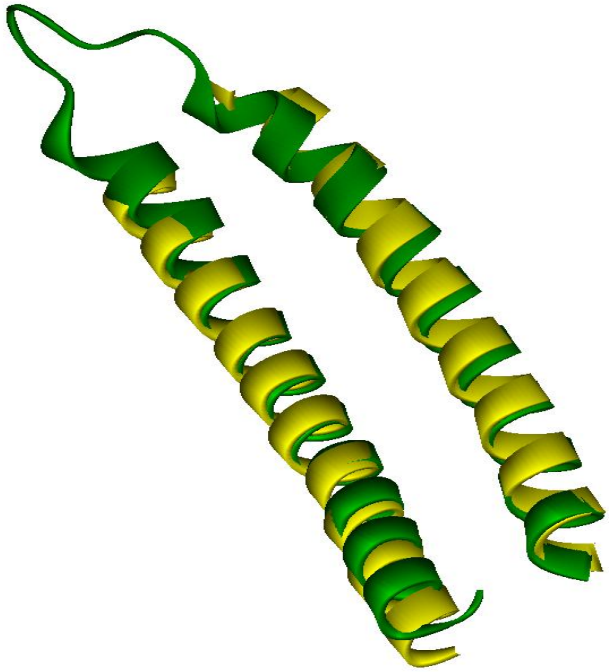

Figure 3: Superimposition of template-3CP1 (yellow) and WT (green) showing an RMSD of 0.5 Å

Docking of gp41 and EVT

The gp41 proteins (WT and 4 MTs) were docked with EVT using the software FlexPepDock. Of the ten poses produced, the best ligand pose was selected based on top Flex score. The WT protein showed the high score compared to other MTs. Among the four mutants, the high score of -19 kcal/mol was obtained for the putative mutants N43S, followed by V38M, L44M and G36V (Table 1). Further, the root mean square for protein back bone (rmsBB) was also provided along with the top scores. This indicates that the rmsBB was higher for WT followed by N43S, then V38M and less deviation was observed for MTs- L44M and G36V. The gp41 and EVT complex was visualized using DS between the peptide (EVT) and the targets (WT and MTs) (Figure 4 and 5).

Hydrogen bond interactions

The docked complexes were dissected using the software BIOVIA in order to get insights into the interaction between the proteins and the ligand. Of all other types of interactions such as hydrophobic, electrostatic, vander Waals, the presence of Hydrogen (H) bonds is of prime importance, due to their significant contribution towards stability and structural integrity of protein-ligand complex. Interestingly, a classical H bond and a pi-H bond were formed between Thr24 of WT protein and Glu80 and His77 of EVT, respectively. Followed by an H bond in salt bridge was formed between Arg37 and Glu80 of EVT (Figure 6A and Table 2). In case of MT-V38M, a classical H bond between Gln65 and Gln88 of EVT was formed (Figure 6B and Table 2). In case of another MT-N43S, two carbon H bonds were formed between Gln22 and Ala97; Ser98 and Gln18, respectively (Figure 6C and Table 2). Surprisingly, no H bonds were observed in MTs like G36V and L44M (Table 2).

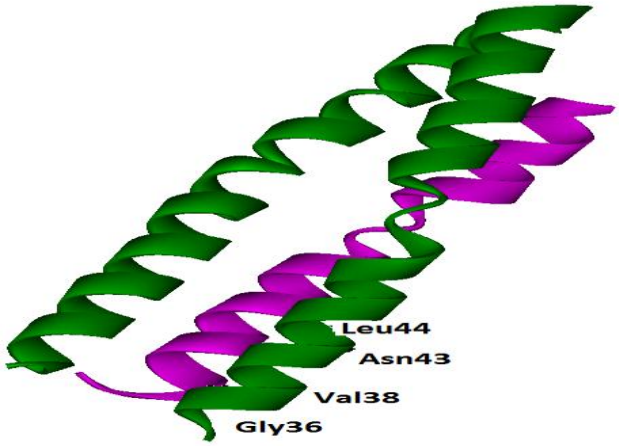

Figure 4: 3-D model structure of gp41 –WT (green) in complex with EVT (pink)

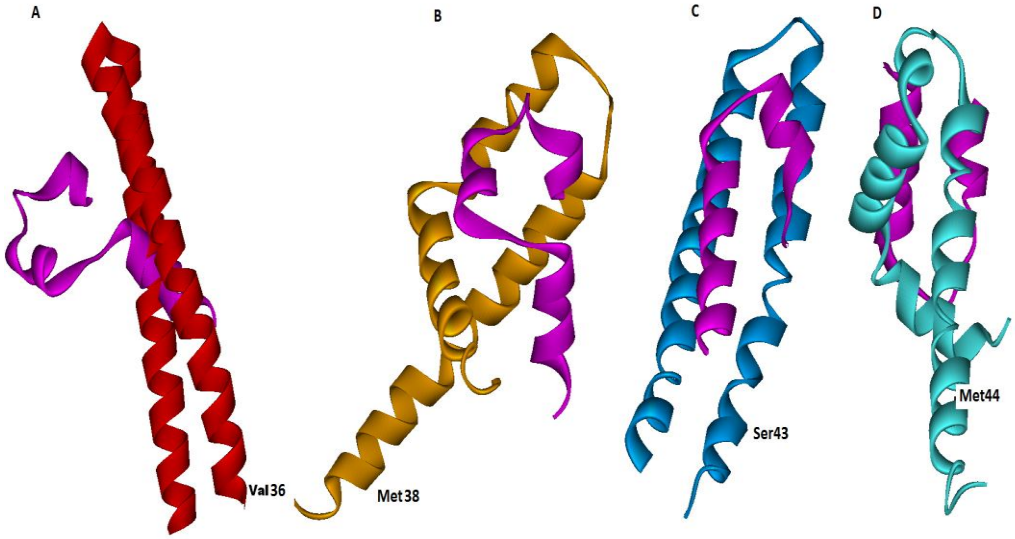

Figure 5: 3-D model structure of MTs of gp41 docked with EVT. A-G36V; B-V38M; C-N43S; D-L44M

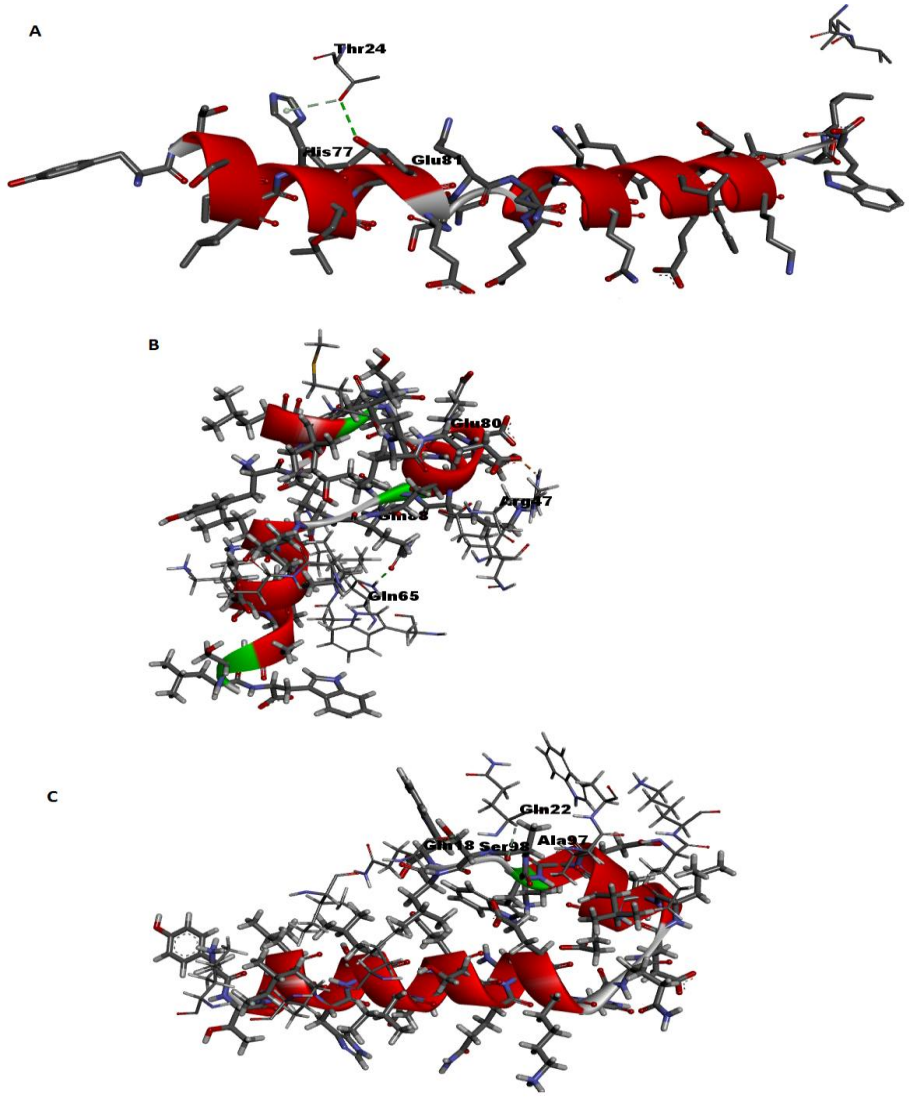

Figure 6: hydrogen bond interactions between the protein (WT and MTs) and the ligand(EVT). A-WT; B-V38M; C-N43S

Table 1: Docking score between models of gp41 and EVT

FI models	Total score (kcal/mol)	rmsBB (Å)
WT	79.123	9.954
MT-G36V	-49.422	2.592
MT-V38M	-29.194	4.825
MT-N43S	-19.072	5.099
MT-L44M	-48.211	3.456

Table 2: H bond interactions between proteins and EVT

FI models	H bond donor	H bond acceptor	Bond distance (Å)
WT	A:THR24:OG1	B:GLU81:OE2	3.1
	A:THR24:OG1	B:HIS77	2.9
	A:ARG47:HH12	B:GLU80:OE2	2.1
MT-V38M	A:GLN65:HE21	B:GLN88:OE1	1.8
MT-N43S	A:GLN22:HA	B:ALA97:O	2.8
	B:SER98:HB1	A:GLN18:O	2.6

DISCUSSION

The first fusion inhibitors developed were peptide mimics of the heptade repeat (HR2) sequence of gp41 that act by competitively binding to HR1 in HIV-1. Enfuvirtide (T-20, formerly DP-178, pentafuside), SJ-2176 and C34 are peptides corresponding to the gp41 amino acid sequence of the HXB2 isolate. Therefore, EVT is the first and approved member in amongst the class of drugs known as fusion inhibitors (Kilby et al., 1998; Jiang et al 1993; Lu et al., 1995). It interferes with the process of host cells fusion with HIV-1through blocking the entry of virus into cells by binding to the HR1 domain of gp41 preventing formation of the 6-helix bundle (Gallo et al., 2001). Owing to which, EVT remains active against HIV-1 clinical isolates that are resistant to NRTIs, NNRTIs, and PIs. T20 (enfuvirtide, Fuzeon, DP-178) is a 36–amino acid peptide corresponding to residues 127–162 of the extracellular portion (ectodomain) of gp41 (or residues 643–678 in the gp160 precursor) of the HIV envelope glycoprotein (Reynes et al., 2007). Clinically, EVT based treatment regimens gave a better and more durable antiviral response than regimens without EVT (Rimsky et al., 2008). New generations of T20-like peptides have been developed with improved potency and stability. Besides T20 and derivatives, other fusion inhibitors have been developed that target different domains of gp41.

In this study, MTs were selected on the basis of clinical data obtained from literature (Zollner et al., 2001; Roman et al., 2003; Wei et al., 2002). Resistance to EVT is often associated with mutations at the tripeptide motif involving residues 36, 37, and 38 located in the HR1 domain of gp41. Secondary resistance to EVT generally involves mutations at residues between 36 and 45 in the HR1 domain of gp41, and may occur rapidly in patients who receive subtherapeutic doses of EVT or EVT monotherapy (Wei et al., 2002).

In the present study, the binding affinity of gp41 with EVT was found higher in case of WT compared to MTs. This could be attributed to the presence of more number of favorable interactions (hydrogen, hydrophobic and electrostatic) in WT, more particularly H bonds (3). Interestingly, the variant-N43S of the putative mutant N43D showed high score next to WT and showed 2 H bonds. While the other MTs -V38M, L44M and G36V displayed lower score (Table 1), owing to the absence of favorable bonding, this has been evident in MTs- L44M and G36V with no H bonding compared to other MTs- N43S and V38M**.** Further, in these MTs hydrophobic interactions were found. However, the limitation of this study is non-performance of molecular dynamics (MD) simulations. As MD is a reliable method to determine the protein binding activity during dynamic state of the protein in the current scenario. Therefore, in this study, an initial step was taken to see the effect of binding affinity of WT and MT proteins of gp41 with EVT, which showed more affinity was towards the WT compared to the resistant MTs. Since the mutant's displayed less affinity, the drug (EVT) could not bind efficiently with them to mediate its inhibitory activity, thereby leads to EVT resistance. Overall, the interactions between the membrane protein and the peptide proposed in this study may be useful for better understanding of fundamental molecular mechanism of mutation-acquired EVT resistance and suggest that further studies based on EVT are needed to expand our knowledge regarding EVT resistance mechanisms.

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CONFLICT OF INTEREST

None to be declared.

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