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Design of inhibitors using a combinatorial library for HIV-Nef and human SH3 domain interaction

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Abstract:
The HIV-1 Nef protein has the ability to down regulate important molecules at the immune synapse. These include class I and class II (Human Leukocyte Antigen) HLA on the Antigen Presenting Cells (APC). The receptors in these molecules consist of SH-3 domain and their interaction with the HIV-1 Nef is critical. Therefore, it is important to inhibit this HIV-Nef and human SH3 domain interaction. Thus, we used a combinatorial library to screen for molecules to inhibit this interaction. The exercise identified a group of top ranking compounds for further consideration.

Keywords: Nef protein, combinatorial library, virtual screening, Hydrogen bond interaction.

Background:
AIDS stands for Acquired Immuno Deficiency Syndrome mainly caused by HIV virus **[1, 2].** According to WHO report in year 2010 around 34 million people were affected out of which 16.8 million were women and 3.4 million people were of less than 15 years age group **[3].** Two types of antivirals are available i.e., reverse transcriptase inhibitors it inhibit reverse transcriptase enzyme and nucleosides analogues for example Zidovudine, Lamivudine etc. Protease inhibitor act by inhibiting HIV protease and thus inhibiting virus assembly examples are Nelfinavir, Ritonavir **[4].** However combination of both the types proves to be effective in most cases better combination is Zidovudine (AZT) and Ritonavir **[5].** HIV-Nef plays an important role by down regulating the surface expression of CD4, MHC-I, MHC-II, and CD28 which are critically involved in immune synapse formation **[6].** It also increases the survival rate of infected cells by avoiding outside-in and inside-in death signal that may be p53 independent or dependent **[7].** Number of residues are involved in interaction with CD4 and WL (57-58) in flexible loop, and residues Glycine 95, Glycine 96, Leucine 97, Arginine 106, leucine 110 present in Nef core region **(Figure 1)** **[8].** The dileucine motif 165/166 and acidic motif EDE 174-176 are critical for down regulation of CD4 **[9].** Hence Nef protein has been selected and subjected for drug designing.

Methodology:
Multiple Sequnce alignment (MSA)
The amino acid sequences of various strains of HIV-Nef protein were selected from National Centre of Biotechnology Information (www.ncbi.nlm.nih.gov) database. Clustal W and the distance tree analysis have been performed to find mutations in Nef- SH3 domain interactive region by using MEGA (Molecular Evolutionary Genetics Analysis) software. Various residues of Nef and SH-3 domain participated in the interaction were diagrammatically presented **(Figure 2).**

Virtual Screening for ligand selection
PubChem Id- 308963 produced highest cell inhibition **[8].** Hence above said molecule has been used as a reference molecule to generate combinatorial library of molecules. For the high quality molecular diversity library generation, we have selected the molecules on the basis of their structural similarity and biochemical properties **[10].** The molecules were selected fom Pubchem database and Zinc database which are having

structural similarity upto 70% to the reference molecule. Further the molecules were filtered based on biochemical properties similarity to reference compound like log P, molecular weight, rotable bonds, net charges, number of hydrogen bonds donors and acceptors, polar desolvation energy. The molecules were futhur filtered by using the Lipinski's rule of five for differentiating between drug-like and non drug-like molecules **[11].** Molecules in SDF file format was converted into Mol2 format with the help of Open Babel software. The generated combinatorial library was used for virtual screening for using Molegro Virtual Docker **[12].** After screening top ten molecules were selected on the basis of their Mol Dock score and further analysis was done.

Figure 1: 3D structure of HIV-NEF Protein and SH3 domain complex

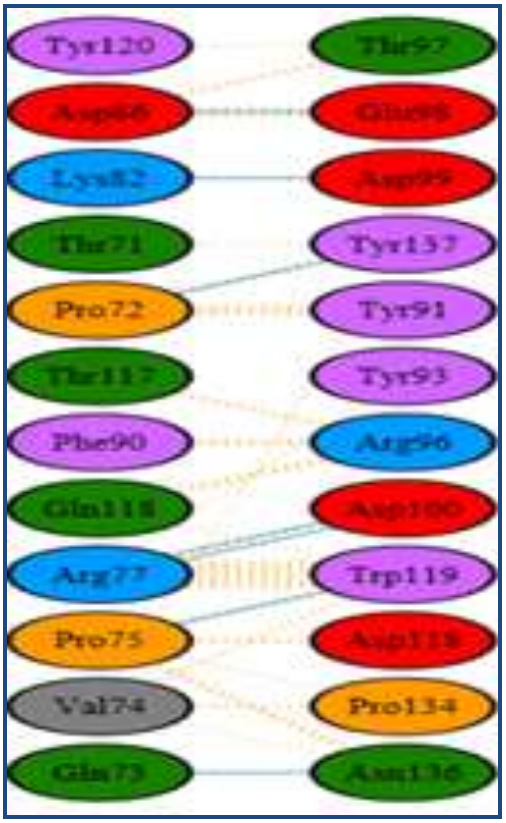

Figure 2: Nef and SH-3 domain Proteins binding regions with interacting residues.

Molecular Docking

Molecular docking has been performed between receptor protein HIV-Nef and reference molecule CID 308963, (5-[(4-tert-butylphenoxy) carbonylamino]-2-hydroxybenzoic acid). Top five screened ligand molecule have also been docked with HIV-Nef protein individually.

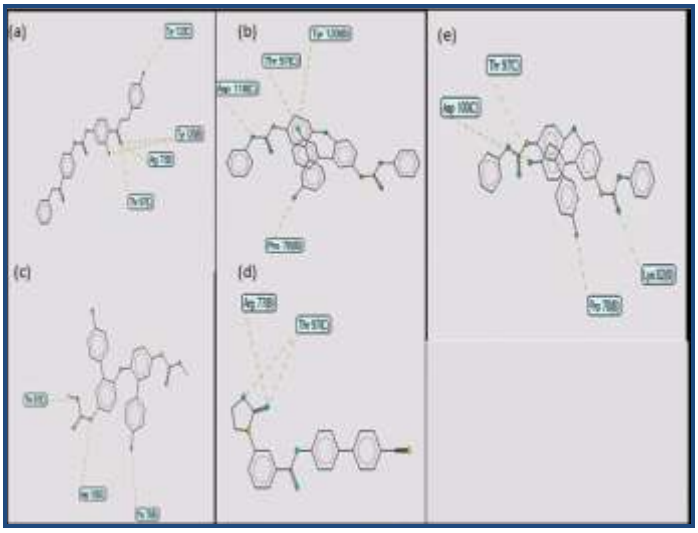

Figure 3: Structural Interaction of top five molecules with Nef protein active site; **a**) Molecule ZINC 059063351; **b**) Molecule ZINC 059062850; **c)** Molecule ZINC 059062913; **d**) Molecule ZINC 071918781; **e**) Molecule ZINC 059063033.

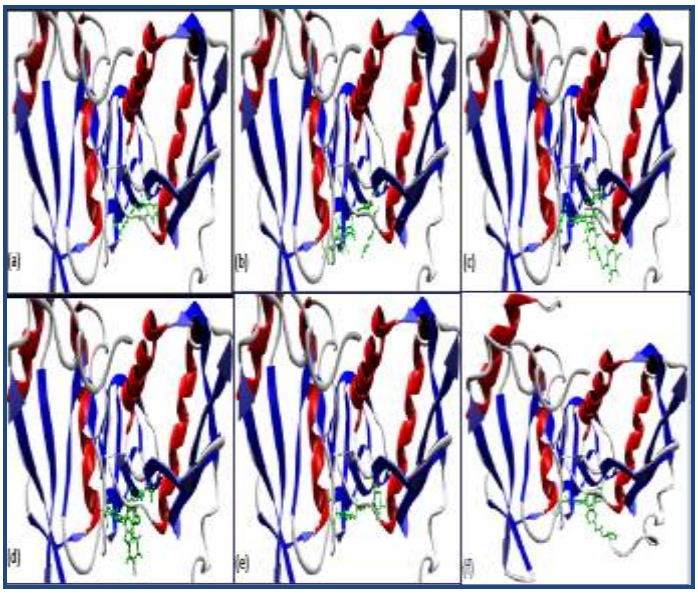

Figure 4: Reference molecule **a)** and top five molecules; **b-f)** docking results with Nef Protein by using AUTODOCK. **a)** CID 308963; **b)** ZINC059063351; **c)** ZINC059062850; **d)** ZINC059062913; **e)** ZINC071918781; **f)** ZINC059063033.

Result:

MSA Analysis

Nef protein sequences of various HIV 1 strains were subjected to multipl alignment by using MEGA software. Multiple sequence alignment did not reveal much changes in the active site region between 70 – 120 amino acids. Most of the amino acids in this region mainly remained conserved. This implies that there was no significant change present in active site

region. Distance tree results also implies that large number of sequences arising from same parent node, hence the above said active site has been used for virtual screening against generated combinatorial library.

Virtual Screening Analysis

The reference molecule PubChem Id 308963: (5-[(4-tert-butylphenoxy) carbonylamino]-2-hydroxybenzoic acid) molecular weight is 329.24 Da, three hydrogen bond donors and five hydrogen bond acceptors, log P of 5.3 and five rotatable bonds. It has shown MolDock score of -40.36 and having hydrogen bond energy of -1.24 KJ/mol. After virtual screening against HIV-Nef active site, top ten molecules from 1780 molecules were selected based on MolDock score **Table 1 (see supplementary material).** ZINC059063351 molecule has proved as best ligand because of its least score (-157.04KJ/mol). It showed four hydrogen bonds interaction and three strong electrostatic interaction within the active site **(Figure 3).** This protein-ligand complex showed having hydrogen bond energy of -4.17 and Ligand efficiency 1 of -4.24 with molecular weight of 495.5. Out of these top ten molecules top five molecules have been chosen for, further detail docking study with HIV- Nef protein on Autodock software.

Molecular Docking Analysis

The molecular docking has been performed between HIV-Nef protein and reference molecule as well as top five selected molecules obtained after virtual screening (**Figure 4**). Analysis results have been compared and found ZINC 059063351 molecule and HIV-Nef is showing best interaction with least binding energy (-7.52 KJ/Mol), which is better than reference molecule CID 308963 (-4.57 KJ/Mol) **Table 2 (see supplementary material).**

Discussion:

HIV-Nef protein helps in the down regulation of T – helper cells by mainly acting on CD4, MHC I, MHC II receptors and thus decreases the immunity of the host **[3, 6].** Only a single reference molecule is available till date that inhibits the interaction of Nef with human receptor **[8].** The main objective of this study was to find the molecules that are similar to known inhibitor in structure and passing through the Lipinski rule of five, similarly above said filters were used by Das et al., & Lipinski *et al*, **[7, 11].** A combinatorial library comprising of 1780 molecules was generated. Molecules were docked against target by using Molegro Virtual Docker and sorted according to their Mol Dock Score in decending order, similar work has been performed by Langer *et al.,* **[12, 13].** On the basis of this score the top ten molecules were selected and further studied. These molecules showed binding energy in range of-142.11 to-157.04 KJ/Mol. The average of total binding energy of top ten molecules around -149.57 KJ/Mol. The hydrogen binding energy is in range of –1.31 to -6.90 and the average found to be -4.5. The ZINC 059063351 molecule showed lowest binding energy of -7.52 KJ/mol which is better than reference molecule, hence it can be suggested as a best inhibitor for Nef protein. It may be tested further experimentally.

Conclusion:

Nef is one of the accessory proteins of HIV genome therfore it plays an important role in down regulation of host immunity. In current work, HIV-Nef protein has been subjected for virtual screening by the ligands collected through combinatorial library approach. Finally it was found that ZINC059063351 is a potential drug molecule with better binding energy and hydrogen bonding. The above said molecule may tested experimentally for future use.

References:

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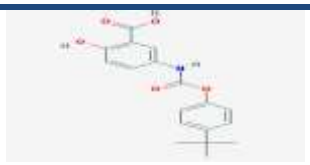
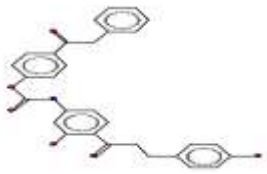
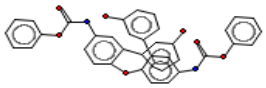
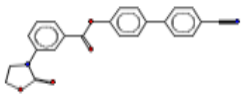
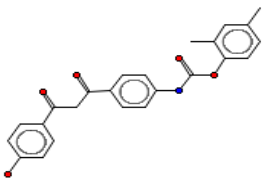
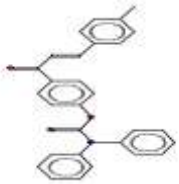
Supplementary material:

Table 1: Top ten molecules represented after virtual screening by Molegro Virtual Docker on Nef protein active site.

S.No	Name of molecule	3D Structure	E_{Inter}(kJ/Mol)	E_{Intra}(kJ/Mol)	Mol Dock Score
(I)	CID 308963		-29.12	-11.01	-40.73
1	ZINC59063351		-165.74	8.69	-157.04
2	ZINC59062850		-176.42	20.76	-155.66
3	ZINC59062913		-162.10	8.4	-153.61
4	ZINC71918781		-162.16	12.34	-149.82
5	ZINC59063033		-173.89	24.52	-149.37
6	ZINC59063231		-169.43	22.49	-147.23
7	ZINC34954557		-167.23	21.77	-145.36

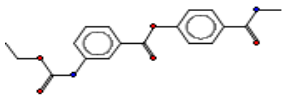
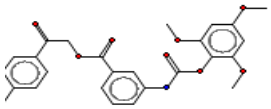
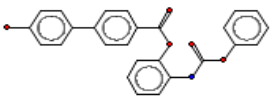
8	ZINC26994568		-159.70	14.80	-145.23
9	ZINC59062828		-153.20	9.90	-143.30
10	ZINC59063117		-147.18	5.77	-142.11

Table 2: AUTODOCK results obtained from docking of Nef protein with top five ligands along with reference molecule.

S. No.	Ligand	Electrostatic Energy(KJ/mol)	Ligand efficiency	Binding energy(KJ/mol)
1	CID 308963(Reference molecule) (5-[(4-tert-butylphenoxy)carbonylamino]-2-hydroxybenzoic acid)	-1.34	-2.24	-4.57
2	ZINC 059063351 (1-[4-(2-Oxo-2-phenylacetyl)phenyl]-2-phenylethane-1,2-dione)	-1.06	-4.24	-7.52
3	ZINC059062850(N-[3-(3-hydroxy phenyl)-4-[2-(3(hydroxyphenyl)-4-(phenoycarbonyl amino) phenoxy] phenyl] carbamate)	-1.2	-3.31	-5.53
4	ZINC059062913(N-[3-(4-hydroxy phenyl)-4-[2-(4-hydroy phenyl)-4-(methoxy carbonyl amino) phenoxy] phenyl] carbamate)	-1.15	-4.15	-6.79
5	ZINC071918781([4-(4-cyanophenyl)phenyl])	-2.36	-5.16	-4.48
6	ZINC059063033(N-[3-(4-hydroxyphenyl)-4-[2-(4- hydroxyl phenyl)-4 (phenoxycaronylamino)phenoxy]phenyl]carbamate)	-2.54	-3.17	-5.12