Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

VIRTUAL SCREENING AND MOLECULAR DOCKING STUDIES OF NOVEL INHIBITORS FOR HIV REVERSE TRANSCRIPTASE

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ABSTRACT

Human Immunodeficiency Virus is caused by retrovirus in human beings, where the overall immune system fails resulting in opportunistic infections, HIV-1 Reverse Transcriptase (RT) is an important enzyme supporting replication cycle of HIV. Reverse Transcriptase protein an essential focus in the current medications for HIV-1, because of flexible nature of the target proteins, there is a basic need to find novel, powerful medications against HIV. Henceforth, an endeavor has been made to screen ZINC compound Library, Using e-HiTS software in docking process with nevirapine bound HIV-RT, 884 ligands with alike properties were tested for their binding affinity towards targeted enzyme and at last 39 hits were reported as effective inhibitors of HIV-RT Virus.

KEYWORDS: **Retrovirus, Nevirapine, Virtual Screening, e-HITS and ZINC database.**

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INTRODUCTION

AIDS has become one of the most disturbing diseases humankind has ever faced and sixth-largest cause of death worldwide caused by Human Immunodeficiency Virus[1], Around 33.2 million people were suffering with HIV[2,3] according to WHO analysis, HIV is coming with type1 and type2 [4], where HIV-2 has been found to be less pathogenic than HIV-1.In HIV-2 transmission rate is much lower than HIV-1. To attack any type of virus human body's immune system releases antibodies through T-cells and CD4+ cells called as helper cells play a vital role in the body's immune response, orchestrating other cells in the immune system to carry out their specific protective functions.HIV-1 main targets are CD4+ T cells and macrophages, although these two cell types display different types of infection characteristics, like differences in viral uptake, the rate of HIV replication, capacity to form viral reservoirs[5]and cell fate. Infected macrophages with HIV-1 represent a stable viral reservoir with continuouslyproduced virus, thus contributing to the spread of HIV-1 to other cells and to the immune pathogenesis[6]. In its invasion of CD4+ cells, HIV recognizes and attaches itself to the outer surface of the cell, penetrating into cell releasing its 15 viral proteins and a ribonucleic acid and proceeds further to replicate itself in massive numbers, the single most devastating thing is integration of viral DNA into human DNA, As the infected T-cells die, the immune system of body becoming incapable to shield itself from infected virus, Search for new inhibitors was the need of hour to overcome above mentioned reasons, Enzymes like protease, reverse transcriptase and integrase encoded by the gag and gag pol genes[7] of HIV play an vital role in the virus replication cycle. The HIV genes are located in the central region of the pro-viral DNA and encode at least nine proteins and these proteins are divided into three classes. Class1: structural proteins, Pol, Gag, and Env, Class2: Regulatory proteins, Tat and Rev and Class3: Accessory proteins, Vpu, Vpr, Vif, and Nef [8]. Our paper deals with reverse transcriptase which catalyzes the formation of proviral DNA from viral RNA, the key stage in viral replication making reverse transcriptase a prime target for anti–HIV–therapy.Among the HIV proteins which have been genuinely characterized as major drug targets is the reverse transcriptase.

MATERIALS AND METHODS

Structure of HIV reverse transcriptase in complex with nevirapine was downloaded from Protein Data Bank having PDB ID as 1VRT, which is used as receptor structure for virtual screening purpose, A chemical library ZINC database[9] used for virtual screening containing over 4.6 million compounds in ready-to-dock three dimensional formats and around 727,842 Molecules each with 3D structure using catalogs of compounds from different vendors. The Molecules have been assigned biologically related prorogation states and were annotated with properties, such as LogP (solubility), Molecular Weight, number of rotatable bonds and library also contains complete details of each Molecule. In this library about 494,915 Molecules are Lipinski compliant out of which 134 are lead-like Molecules having Molecular weight between 150 and 350, calculated LogP less than four, number of Hydrogen-Bond Donors less than or equal to three, and number of Hydrogen-Bond Acceptors less than or equal to six, which are acceptable range. A total of 34,224 molecules with calculated Molecular weight less than 250,LogP values between − 2 and 3, less than six Hydrogen-Bond Acceptors, less than three Hydrogen-Bond Donors, less than three Rotatable Bonds .It should be possible to annotate Molecules using both numeric and alphanumeric data. It should be easy to add new Molecules or removed those that are no longer available and delete those having errors. When property based search applied on ZINC database retrieved 64,000 compounds, all these may not be functionally effective molecules against receptor, hence further screening was done to eliminate poor hits, Based on existing drug nevirapine, it is a non-nucleoside reverse transcriptase inhibitor which stops HIV from multiplying by preventing the reverse transcriptase enzyme from working. This enzyme changes HIV's genetic material (RNA) into the form of DNA.The ligand associated properties were identified using ADME-Tox, Lipinski[10] property data for Nevirapine shown in below in table 1.1.and compatible structure search was drawn by using software tools on ZINC database. Nevirapine Structure shown figure 1.1, there were no similar structures available in ZINC database. Hence, property based search was used for screening for similar compounds in ZINC database.

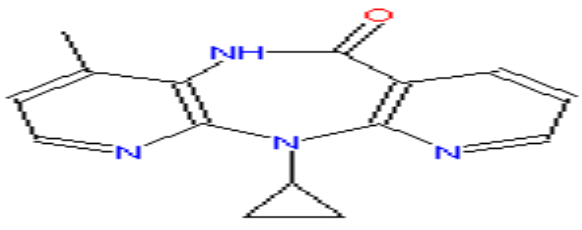

Figure 1.1
Nevirapine Structure

Table 1.1
Lipinski Property data of nevirapine

Molecule	Mol .Wt.	HBA	HBD	Log P	Rotatable Bonds
Nevirapine	266.2-600	5-10	1-5	2.6-3.6	1-10

Mol.Wt: Molecular Weight HBA: Hydrogen Bond Acceptors HBD: Hydrogen Bond Donors LogP: Solubility

After searching the database the above property based search retrieved 64,000 compounds and all 64,000 compounds may not be successful molecules against receptor, hence, further screening has been done to eliminate poor hits and after as shown in table 1.2 for changed data.

Table 1.2
Lipinski Property Data (Changed data for nevirapine)

Molecule	Mol .Wt.	HBA	HBD	Log P	Rotatable Bonds
Nevirapine	266.2-280	5-6	1-2	2.6-3.6	1-2

Mol.Wt: Molecular Weight HBA: Hydrogen Bond Acceptors HBD: Hydrogen Bond Donors LogP: Solubility

RESULTS AND DISCUSSION

We have used the software's like Molinspiration, Molegro Virtual Docker and eHiTS in our research, where molinspiration used for calculation of various molecular properties, for molecular modeling and helpful in data visualization. Protein - Ligand interactions, potential binding sites of the target protein can be known effectively through Molegro Virtual Docker tool and also we have used eHiTS a powerful tool for virtual high throughput screening. Before screening ZINC database, the eHiTS[11] docking protocol was validated. 1VRT protein bound ligand nevirapine was docked into the binding pocket and the Root Mean Square Deviation of the docked pose was 0.55A° with co-crystallized ligand, indicated that the parameters for docking simulation are good in reproducing the X-Ray crystal structure. A structure based search using structural features that are similar to nevirapine resulted in no hits. Hence, a Molecular constraint search was employed using physico-chemical properties of nevirapine which resulted in 884 ligands, all compounds are docked and the binding compatibility of each pose with the receptor was evaluated based on docked energies. The technique used in the study identified diverse geometrical ligands but specific in displaying binding compatibilities with the receptor active site region. From the screening analysis of 884 ligands, a total of 59 Molecules resulted in high dock scores (>6.581 to 8.179 kcal.Mol) than the original nevirapine molecule (6.5818 kcal/Mol). The ZINC id's along with binding energy scores for top three molecules are given in Table 1.3, which are better than niverapine which is used as a drug for HIV-RT.

Table 1.3
Top three ZINC hits

Sl.No	ZINC ID	e-HiTS Score(K.Cal/mol)
1	Nevirapine	-6.581
2	ZINC04923148	-8.179
3	ZINC05442451	-7.886
4	ZINC01558139	-6.954

From the above analysis as shown in table 1.3, it is obvious that there existed about four best ligands represents a high score than bound ligand in 1VRT protein. That means against IVRT Protein these four ZINC ligands acts as effective inhibitors for virus replication, ZINC id: ZINC04923148, which represents efficient orientation and the possible interactions are reported to be 19 and the Lipinski complaint data are: H-Bond Donors 1, H-Bond Acceptors 5, Molecular weight of 277.69, Log P 3.17 and number of Rotatable Bonds as 2, respectively as shown in figure 1.2 and it is also evident that the Molecule exhibited more number of interactions than the original ligand. The highest score obtained for ZINC04923148 and the eHiTS Score -8.179 kcal/Mol shown in figure1.3.

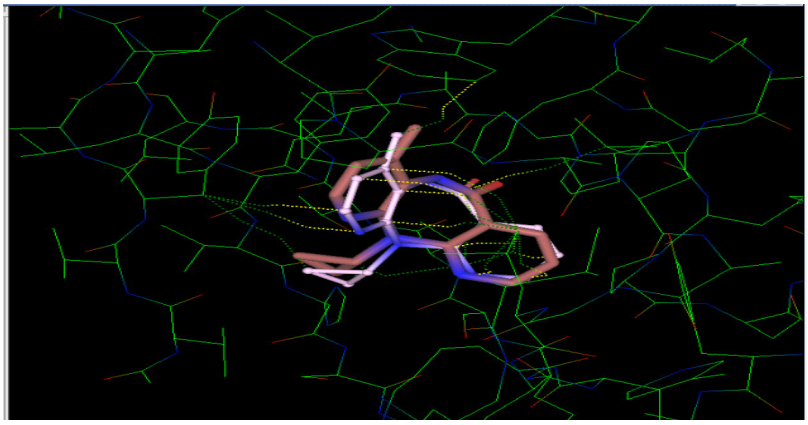

Figure 1.2
Image showing superimposition of Nevirapine with docked Ligand

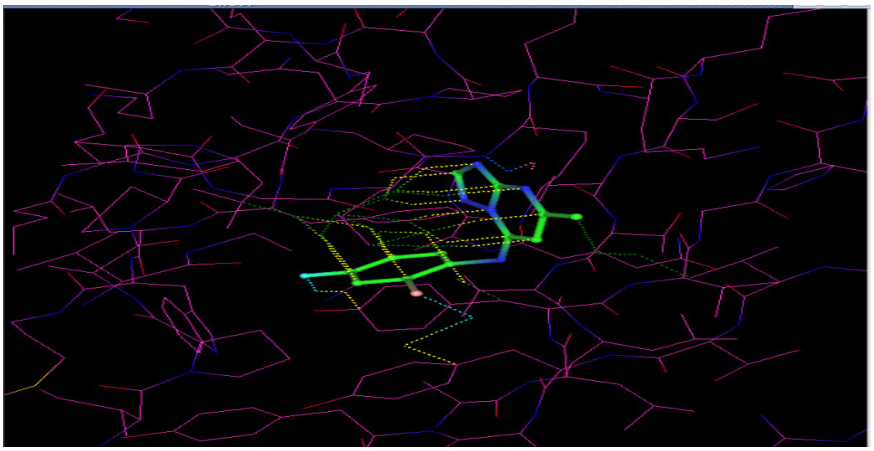

Figure 1.3
ZINC 04923148 showing Score of -8.179 KCal/Mol.

ZINC 04923148 represented better orientation as shown in Figure 1.3 with possible H-Bond interactions being 19 and the Lipinski data are: H-Bond Donors as 1, H-Bond Acceptors as 5, Molecular Weight as 277.69, number of Rotatable Bonds as 2 and LogP of 3.17 respectively. From the table 1.3 it is also evident that the molecule has more number of interactions than the original ligand. In order to study the feasible reason behind difference in number of interactions, the residue wise atomic interactions for each molecule was evaluated. From the interaction list, individual interactions between atomic coordinates of 1VRT active site residues and ZINC ligand displayed the high binding affinity for 18th interaction shows TYR-188 residue CZ atom interaction with ligand. This was mainly due to the Lone electron pair of a halogen atom of ligand and Pi electron of an aromatic ring of 1VRT. The best interaction from 19 interacting atoms between receptor and ligand of ZINC04923148 was:

Interaction 1
==========
Receptor SPT [16] Pi electron of an aromatic ring
Ligand SPT [21] Lone electron pair of a halogen atom (F,Cl,I)
Receptor angle 11.49
Dihedral angle 162.20
Ligand angle 11.85
Distance 3.9245
Score -2.5779
Receptor atom Index:206 Residue: CZ TYR-188 Type:C
Ligand atom Index:18 Type:F

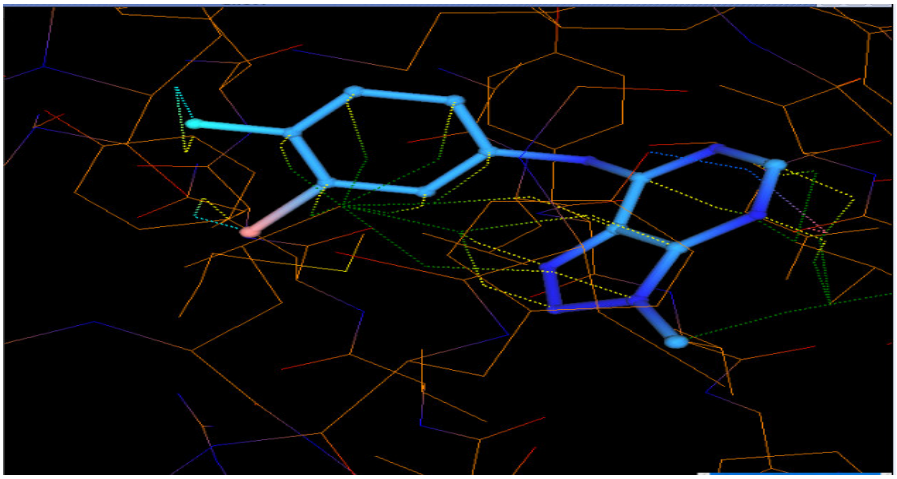

Figure 1.4
1VRT versus ZINC05442451 showing e-HiTS Score of -7.886 kcal/Mol.

ZINC 05442451 ligand (Figure 1.4, -7.886 kcal/Mol) with about 19 interactions and the Lipinski data are: H-Bond Donors as1, H-Bond Acceptor as 6 and Molecular weight as 278.678, logP of 2.63 and number of rotatable bonds as 2, respectively. Individual interactions between atomic coordinates of 1VRT active site residues and ZINC ligand displayed the high score for 15th interaction showing TYR-181 residue CG atom interaction with ligand. The best interaction from 19 interacting atoms between receptor and ligand of ZINC05442451 was:

Interaction 2
==========
Receptor SPT [16] Pi electron of an aromatic ring
Ligand SPT [21] Lone electron pair of a halogen atom (F,Cl,I)
Receptor angle 6.76
Dihedral angle 166.51
Ligand angle 25.22
Distance 3.2061
Score -1.7645
Receptor atom Index:154 Residue: CG TYR-181 Type:C
Ligand atom Index:15 Type:Cl

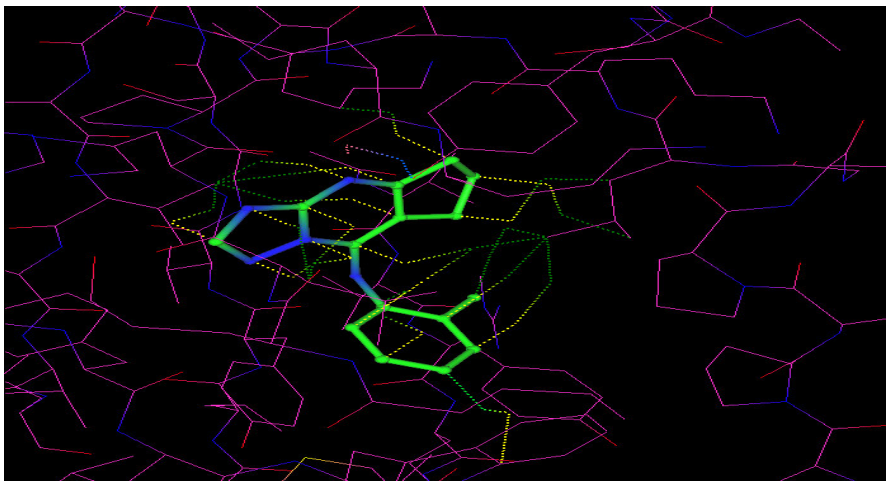

Figure 1.5
ZINC01558139 ligand showing e-HiTS Score of -6.954 kcal/mol

ZINC01558139
The fourth molecule score was -6.954 kcal/mol of ZINC01558139 ligand with about 20 interactions and the Lipinski data are: H-bond donors as 2, H-bond acceptor as 5 and Mol. Weight as 266.628, logP of 3.13, and number of rotatable bond as 1 respectively as shown in figure 5.12. From the interaction list shown it is evident that individual interactions between atomic coordinates of 1VRT active site residues and ZINC ligand displayed the high score for 1st interaction showing LEU-100 residue CD1 atom interaction with ligand. This was mainly due to the H on aliphatic (chain) hydrophobic carbon of ligand and H on aliphatic (chain) hydrophobic carbon Leu-100 of 1VRT. The best interaction from 20 interacting atoms between receptor and ligand of ZINC01558139 was:

Interaction 3
==========
Receptor SPT [12] H on aliphatic (chain) hydrophobic carbon
Ligand SPT [12] H on aliphatic (chain) hydrophobic carbon
Receptor angle 49.91
Dihedral angle 139.57
Ligand angle 40.57
Distance 4.3422
Score -0.6621
Receptor atom Index:46 Residue: CD1 LEU-100 Type: C
Ligand atom Index:0 Type: C

The number of interacting residues for Nevirapine, ZINC04923148, ZINC05442451 and ZINC01558139 molecules are given below.

Table 1.4
Number of interactions made by four molecules

S. No	ZINC ID	SCORE (Kcal/mol)	No. of INTERACTIONS	INTERACTING RESIDUES
1	NEVARIPINE	-6.5818	16	CB TYR-181 CD1 LEU-100 CG LEU-234 CG2 VAL-106 CZ3 TRP-229 CB TYR-181
2	ZINC04923148	-8.179	19	CG1 VAL-179 CD2 LEU-100 CD1 LEU-100 CB TYR-188 CG LEU-234 CG TYR-181 CZ TYR-188
3	ZINC05442451	-7.886	19	CA PRO-236 CD1 LEU-100 CG1 VAL-106 CG2 VAL-106 CG TYR-181 CE2 TYR-181 OH TYR-318
4	ZINC01558139	- 6.954	20	CD1 LEU-100 CE2 TRP-229 CG LEU-234 CG2 VAL-106 CB LEU-234 CG2 VAL-106 CG LYS-103 CD2 LEU-100 CB LEU-100 OH TYR-318

The number of interactions formed by nevirapine and ZINC ligand are 16 and 19 respectively, and when compared with the residue interactions between nevirapine and best ZINC ligand ZINC04923148, which is more effective than nevirapine, and the major active site residues that participated in interactions are: Leu100, Val106, Tyr181, Trp229 and Leu234, Lys-103, Tyr-318. Based on the scores as mentioned in table1.4, Molecules displayed not only the same type of amino acid interactions like standard one but also forming an extra hydrogen bonds with other amino residues of the binding pocket of the receptor. These interactions suggested that the specified molecule may also perform similar activity like the standard one. From the above observations, it was evident that all the molecules have shown better scores than the standard. And by the binding interactions, the molecule ZINC01558139 suggested the lead molecule.

CONCLUSION

In our research findings, we found effective molecules which can inhibit deadly virus by docking ligand and protein 1VRT taken from protein data bank, resulted in about 59 such molecules. 1VRT bound co-crystallized ligand displayed a score of -6.5818 kcal/Mol. Screening procedures carried out using selected criteria resulted in top three best molecules, represented by ZINC04923148, ZINC05442451 and ZINC01558139 with scores of -8.179, -7.886 and -6.954 kcal/Mol respectively.

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