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HIV-1 REVERSE TRANSCRIPTASE INHIBIT THE NON-NUCLEOSIDE REVERSE TRANSCRIPTASE BY INSILICO METHOD

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ABSTRACT

The retrovirus HIV, and its subsequent progression to AIDS, is a rapidly growing worldwide epidemic. HIV-1 reverse transcriptase is one of the key players in the mechanism of infection by this retrovirus. Nearly half of the anti-AIDS drugs target the polymerase activity of RT. NNTRI NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS strongly inhibit wild-type HIV-1RT and drug-resistant variants, including Tyr181Cys and Lys103Asn RT. NNRTIs bind at the NNRTI-binding pocket (NNIBP), a hydrophobic pocket adjacent to the polymerase active site (~10 Å). The structure of those ligands is determined and these structures are minimized using the OPLS force field. Minimized structures are subjected to High throughput virtual screening against the protein taken from PDB (2BE2). Ligands with the best energy and score were selected for induced fit docking. Selected ligands are NNTRI NON-NUCLEOSIDE derivatives. The interactions between the ligands and protein were observed for different poses. The final docking results were also analyzed. The best ligands having the best docking score and interactions with the protein were studied and ADME properties were also analysed. All these computational docking studies are performed in molecular modelling software Schrödinger suites 2009.

1. INTRODUCTION:

Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS) is a disease of the human immune system caused by HIV. Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. HIV-1 reverse transcriptase is one of the key players in the mechanism of infection by this retrovirus. HIV-1 RT has been a key target for the development of antiviral therapies because it catalyses several steps in the replication of HIV virus, the causative agent of AIDS. Non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs) are a mainstay of combination therapies for the treatment of HIV infection.1 Non-nucleoside reverse-transcriptase inhibitors (NNRTIs) are antiretroviral drugs used in the treatment of human immunodeficiency virus (HIV). NNRTIs inhibit reverse transcriptase (RT), an enzyme that controls the replication of the genetic material of HIV. RT is one of the most popular targets in the field of antiretroviral drug development. The prevalence of NRTI resistance mutations among HIV-infected individuals with persistent viremia is estimated to be as high as 70% (-Pillay, D., H. Green, R. Matthias, D. Dunn, A. Phillips, C. Sabin, and B. Evans. 2005; .Richman, D. D., S. C. Morton, T. Wrin, N. Hellmann, S. Berry, M. F. Shapiro, and S. A. Bozzette. 2004; - Tamalet, C., J. Fantini, C. Tourres, and N. Yahi. 2003), and an increasing rate of transmission of drug-resistant HIV variants has been observed among newly infected patients (Geretti, A. M. 2007). In the present study intermolecular flexible docking had been carried out with the target protein (PDB ID: 2BE2) HIV-1 Reverse Transcriptase with NNRTI Non-Nucleoside inhibitor using the Induced Fit module available in the commercial package Schrodinger USA. Some of these toxicities are likely to be related to the effects that NRTIs have on mitochondria due to the ability of the active metabolites to interfere with the replication of mitochondrial DNA (mtDNA) (Kakuda, T. N. 2000; White, A. J. 2001). Therefore, a significant need remains for novel NRTIs with favorable resistance profiles, improved safety, long-term tolerability, and the potential for once-daily dosing to facilitate patient compliance. Totally seven compounds of nnrti non nucleoside derivatives(Anil R. Ekkati, Mariela Bollini, Robert A. Domao, Krasimir A. Spasov, Karen S.

Anderson and William L.Jorgensen-2012) including dimeric inhibitors in an NNRTI-linker-NNRTI motifs against target protein HIV-1 Reverse Transcriptase [PDB ID:2BE2]are docked at the defined active site. The best docked conformation of each compound is chosen based on the Glide Score, Glide Energy and binding of NNRTI at the NNRTI-binding pocket (NNIBP), a hydrophobic pocket adjacent to the polymerase active site (~10 Å).

1.1. CLASSIFICATION OF HIV:

HIV is a member of the genus Lentivirus, part of the family of Retroviridae. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period. There are two species of HIV known to exist: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed LAV. It is more virulent, more infective, and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa. Both types are transmitted by sexual contact, through blood, and from mother to child, and they appear to cause clinically indistinguishable AIDS. However, it seems that HIV-2 is less easily transmitted, and the period between initial infection and illness is longer in the case of HIV-2. The strains of HIV-1 can be classified into four groups: the "major" group M, the "outlier" group O and two new groups, N and P. These four groups may represent four separate introductions of simian immunodeficiency virus into humans.

1.2. STRUCTURE OF HIV:

Outside of a human cell, HIV exists as roughly spherical particles (sometimes called virions). The surface of each particle is studded with lots of little spikes. An HIV particle is around 100-150 billionths of a metre in diameter. That's about the same as:

- 0.1 microns
- 4 millionths of an inch
- One twentieth of the length of an E. coli bacterium
- One seventieth of the diameter of a human CD4+ white blood cell.

Unlike most bacteria, HIV particles are much too small to be seen through an ordinary microscope. However, they can be seen clearly with an electron microscope.HIV particles surround themselves with a coat of fatty material known as the viral envelope. Projecting from this are around 72 little spikes, which are formed from the proteins gp120 and gp41. Just below the viral envelope is a layer called the matrix, which is made from the protein p17. The proteins gp120 and gp41 together make up the spikes that project from HIV particles, while p17 forms the matrix and p24 forms the core. The viral core is usually bullet-shaped and is made from the protein p24. Inside the core are three enzymes required for HIV replication called reverse transcriptase, integrase and protease. Also held within the core is HIV's genetic material, which consists of two identical strands of RNA. The morphologically mature virions, the group-specific antigens (gag) form two distinct constituents, the membrane-associated matrix protein (MA) layer and the viral core, which are made up of capsid protein (CA). The core capsid in morphologically mature virions is organized as either an isometric, or as a cone-shaped body, and protects two identical molecules of genomic viral RNA. The RNA together with a nucleic-acid associated basic protein (NC), makes up the ribonucleoprotein portion (RNP) [De Clerck, E-2005].

1.4 THERAPY OF HIV

Several distinct classes of drugs are now used in combination to treat HIV infection, commonly referred to as HAART, "Highly Active Antiretroviral Therapy:

(i) Nucleoside-Analog Reverse Transcriptase Inhibitors (NRTI). These drugs inhibit viral RNA-dependent DNA polymerase (reverse transcriptase) and are incorporated into viral DNA.

- Zidovudine (AZT = ZDV, Retrovir) first approved in 1987
- Didanosine (ddI, Videx)
- Zalcitabine (ddC, Hivid)
- Stavudine (d4T, Zerit)
- Lamivudine (3TC, Epivir)

(ii) Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). In contrast to NRTIs, NNRTIs are not incorporated into viral DNA; they inhibit HIV replication directly by binding non-competitively to reverse transcriptase.

- Nevirapine (Viramune)
- Delavirdine (Rescriptor)

(iii) Protease Inhibitors. These drugs are specific for the HIV-1 protease and competitively inhibit the enzyme, preventing the maturation of virions capable of infecting other cells, e.g: Saquinavir (Invirase) first approved in 1995, Ritonavir (Norvir), Indinavir (Crixivan), Nelfinavir (Viracept).

(vi) Low-dose oral alpha-interferon, used quite widely for the treatment of patients with HIV infection, does not appear to have significant effect on the signs and symptoms of the disease.

2. MATERIALS AND METHODS

2.1 Mechanics of Docking

To perform docking, the first requirement is the structure of the protein. Usually the structure has been determined in the lab using a biophysical technique such as x-ray crystallography, or less often, NMR spectroscopy. This protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program depends on two components namely the search algorithm and the scoring function.

2.2 The Search Algorithm

The search space consists of all possible orientations and conformations of the protein paired with the ligand. With the present computing resources, it is impossible to exhaustively explore the search space. This would involve enumerating all possible distortions of each molecule since molecules are dynamic and exist in an ensemble of conformational states and all possible rotational and translational orientations of the ligand relative to the protein at a given level of granularity. Most docking programs in use account for a flexible ligand, and several are attempting to model a flexible protein receptor. Each snapshot of the pair is referred to as a pose.

2.3 The Scoring Function

The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favorable binding interaction. Most scoring functions are physics-based molecular mechanics force fields that estimate the energy of the pose where a low or negative energy indicates a stable system and thus a likely binding interaction. An alternative approach is to derive a statistical potential for interactions from a large database of protein-ligand complexes, such as the Protein Data Bank, and evaluate the fit of the pose according to this inferred potential.

2.4 PACKAGES:

There are many software packages available for docking. Some of them are free and some are commercial. The below table shows a list of some docking software packages available with the developer's names,

2.5. STEPS INVOLVED FOR DOCKING:

2.5.1. PROTEIN AND LIGAND PREPARATION:

The target protein procured from PDB bank. After selected, the protein put into the Maestro which is docking software, Then, Pre-process, Optimization and minimization process are involved that protein. After finished those processes, the result was saved as .impref.mae. The synthetic ligands are put into the software Page. Then the select the project table for more ligands and then select the generate ligands. After finished those processes, the result was saved as .out.mae.

2.5.2. GLIDE DOCKING:

In the receptor grid generation, select the ligand and grid is formed. Then the result was saved as .glide grid.maegz.
In the ligand docking, both the ligand preparation result file and grid generation file are selected and the program is started. After finished those processes, the result was saved as .pv.maegz.

2.5.3. INDUCED FIT DOCKING:

Based on glide docking results, the best ligand take for induce fit docking. After choose the best ligands, the minimization is started. After that, both ligand file and ligand minimization file is select and program is started. After finished those processes, the result was saved as. Finally, the docking score and glide energy analyzed for identify the best ligands.

2.6. MARVINSKETCH

MarvinSketch allows users to quickly draw molecules through basic functions on the GUI and advanced functionalities such as sprout drawing, customizable shortcuts, abbreviated groups, default and user defined templates and context sensitive popup menus. MarvinSketch has a rich support for atom and bond properties. Users can assign stereochemistry, charge, valence, radicals and isotopes to each atom. Single, double, triple bonds and aromatic forms are supported. Moreover using wedge bonds user can assign stereochemistry to atoms. Additional data fields can also be attached to atoms, via "S-group" logic so that any user defined information can be stored directly with the structural information. You can draw single step reactions in MarvinSketch by placing a reaction arrow in any position, pointing in any direction in relation to reaction products. The structures 'in front' of the arrow will be recognized as reactants, structures 'above' the arrow as agents, and structures behind as products. Atoms can be automatically or manually mapped using the arrow function.

2. RESULTS :

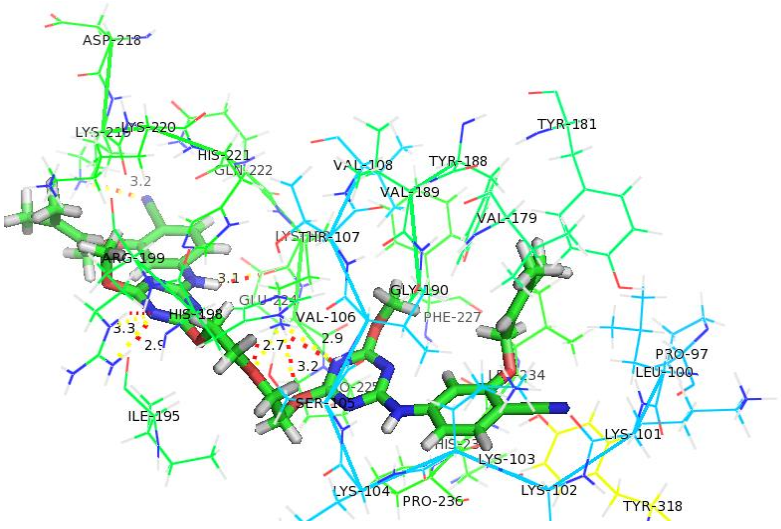
Comparative docking studies between NNRTI NON NUCLEOSIDE DERIVATIVES including dimeric inhibitors in an NNRTI-linker-NNRTI motifs such as COMPOUND 6dd, 6aa and 6cc with target protein HIV-1 REVERSE TRANSCRIPTASE reveals positive information. Docking score and the Glide energy between COMPOUNDS 6dd , 6aa ,6cc And the target protein PDB ID(2BE2) is -89.151,-11.052 ; -79.674,-10.163 & -78.641,-10.001 and these compound have high binding affinity for the target, binds at the NNRTI-binding pocket (NNIBP),hydrophobic pocket adjacent to the polymerase active site NNIBP consists of residues L100, K101, K103, V106, T107, V108, V179, Y181, Y188, V189, G190, F227, W229, L234, and Y318 of p66 and E138 of p51 . Hence the present study suggests that the compound 6dd & 6aa with further in vitro and in vivo testing can be emerged as promising Anti-viral drug candidates against AIDS.

Docking Program	Developed by
ClusPro	Camacho group at Boston University
SmoothDock	Carlos J. Camacho and P. Christoph Champ at University of Pittsburgh
AutoDock	Olson group at The Scripps Research Institute
Dock	Kuntz and Shoichet groups at the University of California, San Francisco
EhiTS	SimBioSys in Toronto, Canada
Glide	Schrödinger in the US

COMPOUND 6DD:

Poses	Hbond	Distance	Docking Score	Docking Energy kcal/mol
1	(LYS219)N-H...N (LYS223)N-H...O N-H...O(LYS223) (ARG199)N-H...N	3.168 2.705 3.094 2.85	-11.052	-89.151
2	(LYS223)N-H...O (LYS219)N-H...N (LYS223)N-H...O (ARG199)N-H...N	3.151 2.953 2.943 2.895	-10.973	-85.842
3	N-H...O(LYS223) (LYS223)N-H...O (LYS223)N-H...O (ARG199)N-H...O	3.194 2.889 3.185 2.866	-10.066	-83.644

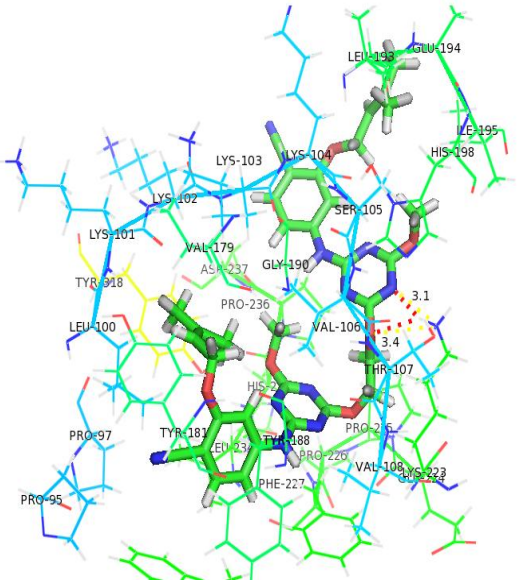
PYMOL RESULT FOR COMPOUND 6DD:



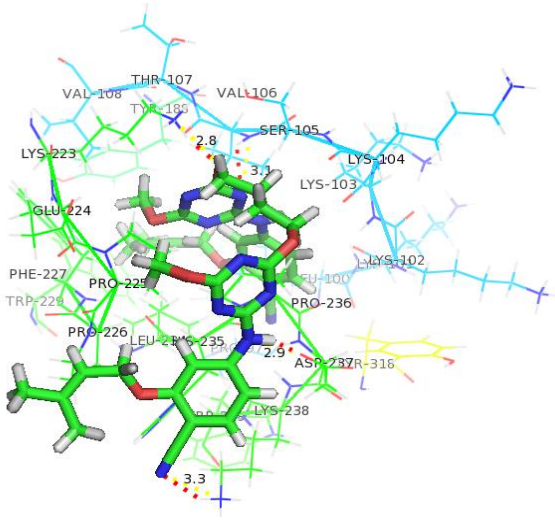
COMPOUND 6AA:

Poses	Hbond	Distance	Docking Score	Docking Energy kcal/mol
1	No interaction		-10.163	-79.674
2	(LYS223)N-H...O (LYS223)N-H...O (ARG199)N-H...N	3.199 3.146 3.060	-9.442	-75.189
3	No interaction		-9.455	-74.646

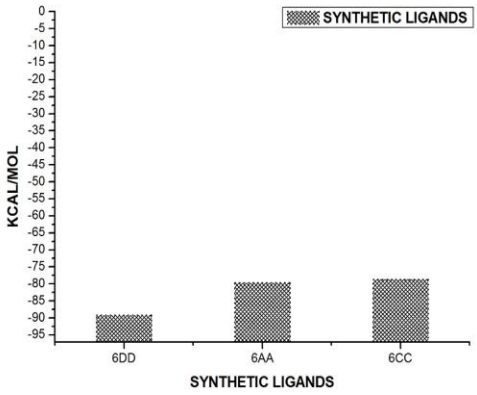
PYMOL RESULT FOR COMPOUND 6AA:



PYMOL RESULT FOR COMPOUND 6CC:



INDUCE FIT DOCKING RESULTS IN GRAPH:



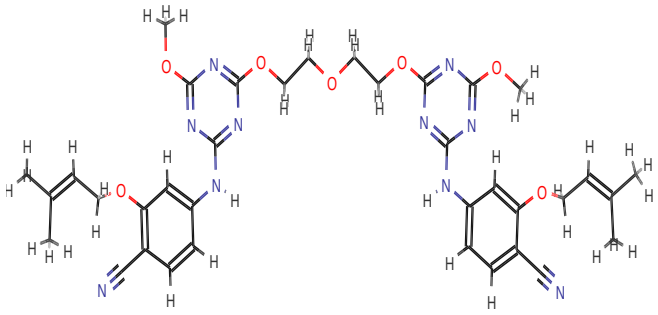
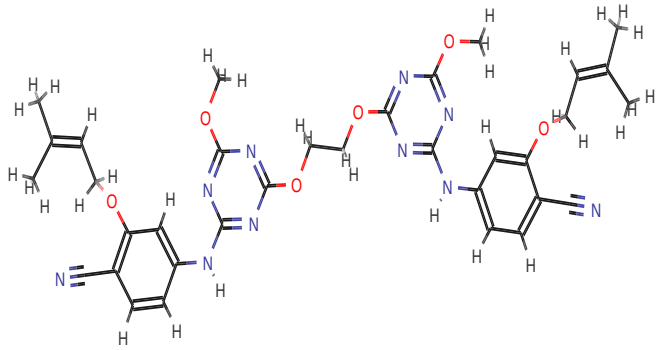
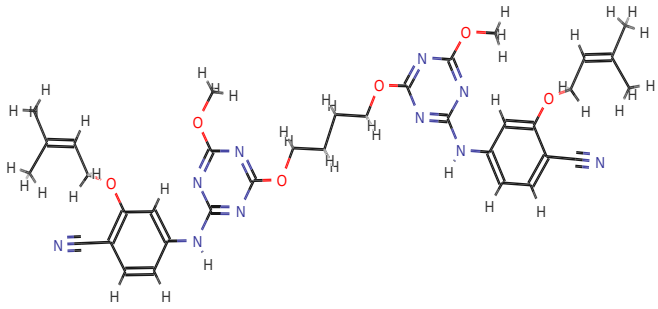
COMPOUND 6CC:

Poses	Hbond	Distance	Docking Score	Docking Energy kcal/mol
1.	(LYS223)NH...O (LYS238)N-H...N N-H...O(ASP237)	2.836 3.315 2.859	-10.001	-78.641
2	(TYR181)O-H...N (ARG199)N-H...O	2.810 2.790	-10.630	-75.687
3	N-H...O(LYS103) (ARG199)N-H...O	3.250 3.136	-9.180	-74.164

4.2 DISCUSION:

Induced Fit Docking between the target protein HIV-1 Reverse transcriptase and NNRTI NON- NUCLEOSIDE derivatives were carried out using Glide, and the images were obtained using PYMOL. The pymol images shows hydrophobic pocket residues such as L100, K101, K103, V106, T107, V108, V179, Y181, Y188, V189, G190, F227, W229, L234, and Y318 of p66 and E138 of p51 which are the active site of HIV-1 Reverse Transcriptase.

LIGANDS USED FOR INDUCED FIT DOCKING:

S.NO	Compound	Name	Chemical structure
1	6dd	4-({4-[2-(2-{[4-({4-cyano-3-[(3-methylbut-2-en-1-yl)oxy]phenyl}amino)-6-methoxy-1,3,5-triazin-2-yl]oxy}ethoxy)ethoxy]-6-methoxy-1,3,5-triazin-2-yl}amino)-2-[(3-methylbut-2-en-1-yl)oxy]benzonitrile.	
2	6aa	4-{[4-(2-{[4-({4-cyano-3-[(3-methylbut-2-en-1-yl)oxy]phenyl}amino)-6-methoxy-1,3,5-triazin-2-yl]oxy}ethoxy)-6-methoxy-1,3,5-triazin-2-yl]aminomethylbut-2-en-1-yl)oxy]benzonitrile.	
3	6cc	4-{[4-(4-{[4-({4-cyano-3-[(3-methylbut-2-en-1-yl)oxy]phenyl)amino)-6-methoxy-1,3,5-triazin-2-yl]oxy}butoxy)-6-methoxy-1,3,5-triazin-2-yl]amino]-2-[(3-methylbut-2-en-1-yl)oxy]benzonitrile.	

REFERENCE:

Anil R. Ekkati, Mariela Bollini, Robert A. Domao, Krasimir A. Spasov, Karen S. Anderson and William L.Jorgensen. Discovery of dimeric inhibitors by extension into the entrance channel of HIV-1 reverse transcriptase. Bioorg Med Chem Lett. 2012. 22(4): 1565-1568. Doi:10.1016/j.bmcl.2011.12.132.

De Clerck, E. New approaches toward Anti-HIV chemotherapy . J. Med. Chem. 48, (2005). 1297–1313.

Flexner C. Nature Rev Drug Disc. 2007; 6:959.

Geretti, A. M. 2007. Epidemiology of antiretroviral drug resistance in drugnaive persons. Curr. Opin. Infect. Dis. 20:22–32.

Kakuda, T. N. 2000. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochrondrial toxicity. Clin. Ther. 22:685– 708.

Pillay, D., H. Green, R. Matthias, D. Dunn, A. Phillips, C. Sabin, and B. Evans. 2005. Estimating HIV-1 drug resistance in antiretroviral-treated individuals in the United Kingdom. J. Infect. Dis. 192:967–973.

Richman, D. D., S. C. Morton, T. Wrin, N. Hellmann, S. Berry, M. F. Shapiro, and S. A. Bozzette. 2004. The prevalence of antiretroviral drug resistance in the United States. AIDS 18:1393–1401.

Tamalet, C., J. Fantini, C. Tourres, and N. Yahi. 2003. Resistance of HIV-1 to multiple antiretroviral drugs in France: a 6-year survey (1997-2002) based on an analysis of over 7000 genotypes. AIDS 17:2383–2388.

White, A. J. 2001. Mitochondrial toxicity and HIV therapy. Sex. Transm. Infect. 77:158–173.