IN SILICO DISCOVERY OF NEW INHIBITORS TARGETING HIV-1 REVERSE TRANSCRIPTASE: EXPLORING NOVEL APPROACHES FOR ANTIRETROVIRAL THERAPY

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TITLE

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CANDIDATES'S DECLARATION

I, Nishant Singh (23/MSCBIO/82) hereby certify that the work which is being presented in the thesis entitled **"In silico Discovery of New Inhibitors Targeting HIV-1 Reverse Transcriptase: Exploring Novel Approaches for Antiretroviral Therapy"** in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2023 to 2025 under the supervision of Prof. Yasha Hasija.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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SUPERVISOR'S CERTIFICATE

Certified that Nishant Singh (23/MSCBIO/82) has carried out their search work presented in this thesis entitled "**In silico Discovery of New Inhibitors Targeting HIV-1 Reverse Transcriptase: Exploring Novel Approaches for Antiretroviral Therapy"** for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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ABSTRACT

Zidovudine, an FDA approved drug used in the treatment of Acquired Immunodeficiency Syndrome caused due to Human Immunodeficiency Virus . It acts as an inhibitor of HIV-1 nucleoside reverse transcriptase enzyme which is essential for the replication of viral genome and prevent in spreading of this disease. As a result it protect further transmission of disease and prevent further damage to the immunity of the infected person.

It is a landmark antiretroviral agent approved by the U.S. Food and Drug Administration (FDA), remains a critical therapeutic tool in managing Acquired Immunodeficiency Syndrome (AIDS) caused by the Human Immunodeficiency Virus (HIV). Functioning as a competitive inhibitor of the HIV-1 nucleoside reverse transcriptase (RT) enzyme, zidovudine disrupts the viral replication cycle by impeding the conversion of viral RNA into proviral DNA. This enzymatic interference prevents the integration of viral genetic material into host cells, thereby curbing the spread of infection and mitigating progressive immune system deterioration. By reducing viral load, the drug not only delays disease progression but also lowers the risk of secondary infections and transmission, offering a dual therapeutic and prophylactic benefit.

Structurally, zidovudine is a synthetic thymidine analogue, a feature that enables its incorporation into nascent viral DNA strands during replication. Unlike endogenous thymidine, however, its modified 3'-azido group induces premature chain termination, halting DNA synthesis. This mechanism underscores the drug's specificity for the viral reverse transcriptase enzyme, which exhibits a higher affinity for zidovudine than human DNA polymerases. Despite its efficacy, long-term use of zidovudine is limited by challenges such as mitochondrial toxicity (due to inhibition of mitochondrial γ-DNA polymerase) and the emergence of drug-resistant HIV strains. These limitations highlight the urgent need for novel nucleoside reverse transcriptase inhibitors (NRTIs) with improved safety profiles and resistance barriers.

In this research, zidovudine has been employed as a reference ligand to identify structurally analogous compounds with potential antiretroviral activity. Leveraging computational tools, scientists have screened libraries of molecules based on similarity scores derived from zidovudine's pharmacophoric features—particularly its sugar moiety, azido group, and aromatic base. Molecular docking simulations further evaluate these candidates by predicting their binding affinities and interaction patterns with the HIV-1 RT active site. For instance, analogues with substitutions at the 3' position or modifications to the sugar backbone have shown enhanced binding stability in silico, suggesting improved inhibitory potency. Notably, several candidates identified through these virtual screenings are already undergoing clinical trials for HIV and other viral diseases, demonstrating the translational potential of structure-based drug design.

The integration of computational modeling into this research streamlines the drug discovery process by prioritizing high-affinity candidates for experimental validation. Studies comparing binding energies, hydrogen-bond interactions, and hydrophobic contacts between zidovudine and its analogues provide mechanistic insights into RT inhibition.

Keywords—Zidovudine, Acquired Immunodeficiency Syndrome, HIV-1 nucleoside reverse transcriptase enzyme, Thymidine, Binding affinity

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ABBREVIATIONS

FDA	Food And Drug Administration
HIV-1	Human Immunodeficiency Virus -1
AIDS	Acquired Immunodeficiency Syndrome
RT	Reverse Transcriptase
HIV-1RT	Human Immunodeficiency Virus -1 Reverse Transcriptase
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
HIV	Human Immunodeficiency Virus
CD4+	Cluster of Differentiation 4+
SIV	Simian Immunodeficiency Virus
CDC	Centers for Disease Control and Prevention
GRID	Gay-Related Immune Deficiency
gp120	Glycoprotein subunit 120
gp41	Glycoprotein subunit 41
RNase H	Ribonuclease H
PBS	primer-binding site
cDNA	complementary DNA
dsDNA	double-stranded DNA
LTRs	long terminal repeats
RNA POL.	RNA polymerase
Group M	Major
Group N	Non major
Group O	Outlier
CRFs	Circulating Recombinant Forms
ART	Antiretroviral Therapy
PrEP	pre-exposure prophylaxis
TasP	pre-exposure prophylaxis
PEPFAR	President's Emergency Plan for AIDS Relief
PPT	polypurine tracts
AZT	Azidothymidine
MTDL	Multi target-directed ligand
AZT-TP	Azidothymidine- triphosphate
PEG	Polyethylene glycol
PDB	Protein Data Bank
RCSB	Research Collaboratory for Structural Bioinformatics
DRC	Democratic Republic of Congo
CCR5	C-C chemokine receptor type 5
CXCR-4	C-X-C chemokine receptor type 4
DNA pol.	Deoxyribo Nucleic Acid Polymerase
RDP	RNA Dependent Polymerase
RdRp	RNA Dependent RNA polymerase
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
dATP	Deoxyadenosine triphosphate
dTTP	Deoxythymidine triphosphate
RNA POL. II	RNA Polymerase ii
WHO	World Health Organisation
RMSD	Root Mean Square Deviation
ΔG	Binding Energy

CHAPTER 1

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) represents a serious medical condition that arises when the human immune system is severely compromised due to infection by the Human Immunodeficiency Virus (HIV). This virus specifically targets essential components of the immune system, most notably the CD4+ T lymphocytes, which play a crucial role in activating the body's defense mechanisms against infections and diseases. As HIV progressively destroys these cells, the immune system's ability to start effective responses against common pathogens and foreign bodies infections is dramatically weakened. Consequently, individuals with AIDS become highly susceptible to a wide range of infections and certain types of cancers that would rarely cause illness in people with healthy immune systems [1].

The progression from HIV infection to AIDS does not occur overnight. Initially, a person infected with HIV may experience mild flu-like symptoms or remain asymptomatic for years. During this time, the virus continues to replicate and gradually destroy the immune system's cells. Without appropriate medical help, such as antiretroviral therapy, the persistent viral activity eventually leads to a critical reduction in CD4+ T cell counts. When these levels fall below a specific threshold, or when an individual develops certain infections or cancers, the diagnosis of AIDS is made.

1.1 History Of AIDS

The history of AIDS, or Acquired Immunodeficiency Syndrome, begins with the origins of its cause: the Human Immunodeficiency Virus (HIV). Scientists believe that HIV came from a virus found in chimpanzees in West and Central Africa. The virus, known as Simian Immunodeficiency Virus (SIV), likely crossed over to humans when people hunted chimpanzees for meat and came into contact with their blood. This crossover is thought to have happened in the early 20th century, with genetic studies suggesting the first transmission to humans occurred around the 1920s in what is now Kinshasa, Democratic Republic of Congo. (DRC) [2]

For decades, HIV spread quietly among people in Africa, unnoticed by the wider world [3]. The first case of HIV was first found in blood sample of a person named Kinsaha. However, it wasn't until the early 1980s that doctors in the United States began to notice unusual illnesses in young men, such as rare types of pneumonia and cancer, that were linked to severely weakened immune systems. In 1981, the U.S. Centers for Disease Control and Prevention (CDC) reported the first official cases of this mysterious disease, which was initially called GRID (Gay-Related Immune Deficiency) because it seemed to mainly affect gay men at the time.

As more cases appeared, it became clear that the disease was not limited to any one group. It was also found in people who injected drugs, hemophiliacs, and people who had received blood transfusions. By 1982, the disease was renamed Acquired Immune Deficiency Syndrome (AIDS), reflecting its impact on the immune system and the fact that it could be acquired by anyone [4].

In 1983 France scientists identified the HIV virus . Later scientists identified the real origin of the AIDS. The main origin of HIV-1 is from Chimpanzees, which jumped into the human species through the process of Zoonosis (infectious disease crosses from animal to humans). This virus crossed to humans through bushmeat hunting when humans hunted the infected chimpanzees and their infected blood entered to human bloodstream.

Researchers quickly learned that HIV could be spread through unprotected sex, sharing needles, and from mother to child during childbirth or breastfeeding. The virus spread rapidly across the world, leading to a global epidemic.

1.2 HIV (Human Immunodeficiency virus)

HIV is a belong to retrovirus family and is 120nm in diameter with spherical shape. It has an outer envelope which is composed of mainly 2 type of glycoproteins. gp120 which is an outer surface glycoprotein and gp41 which is an transmembrane protein. These glycoproteins play a pivotal role in viral entry into host cells. Beneath the envelope lies a matrix layer of p17 proteins, which stabilizes the viral structure, and an icosahedral capsid core containing two single-stranded RNA genomes and viral enzymes such as reverse transcriptase, integrase, and protease [5].

HIV primarily targets CD4+ T lymphocytes, a subset of white blood cells critical for coordinating immune responses. The infection starts when glycoprotein gp120 binds with the CD4+ receptor which is present on the host cell. This interaction induces a conformational change in gp120, exposing a hidden region

that attaches to a co-receptor—either CCR5 (found on macrophages and memory T-cells) or CXCR4 (expressed on naïve T-cells). The choice of co-receptor determines viral tropism: CCR5-tropic strains dominate early infections, while CXCR4-tropic variants emerge in later stages and are associated with faster disease progression.

Following co-receptor binding, the gp41 glycoprotein mediates fusion between the viral envelope and the host cell membrane. This process involves the insertion of gp41's hydrophobic fusion peptide into the host membrane, forming a six-helix bundle that pulls the two membranes together. Once fused, the viral capsid is released into the cytoplasm, where it partially disassembles to release the viral RNA and associated enzymes.

The replication of HIV relies on the enzyme reverse transcriptase (RT), a heterodimer composed of two subunits: p66 (active subunit) and p51 (structural subunit). The p66 subunit contains two catalytic domains: a DNA poly. (for synthesizing DNA from RNA) and RNase H (for degrading the viral RNA template). The p51 subunit lacks enzymatic activity but stabilizes the p66 structure. Reverse transcription begins when the viral tRNAlys3 molecule, packaged within the virion, binds to the primer-binding site (PBS) on the HIV RNA genome. This tRNA acts as a primer, providing a free 3'-OH group for DNA synthesis. The RT enzyme first synthesizes a complementary DNA (cDNA) strand using the viral RNA as a template—a process termed RNA-dependent DNA polymerization (RdRp). During this step, RT incorporates deoxyribonucleotides (dTTP, dCTP, dGTP, dATP) to build the cDNA.

Simultaneously, the RNase H domain degrades the RNA strand leaving behind a single stranded CDNA. The RT then uses its DNA-dependent DNA polymerase activity to synthesize a second DNA strand, creating a double-stranded DNA (dsDNA) copy of the viral genome. This dsDNA, termed the proviral DNA, contains flanking long terminal repeats (LTRs) that regulate viral gene expression.

The proviral DNA is transported into the nucleus via the pre-integration complex, which includes the viral enzyme integrase. Integrase cleaves the host chromosomal DNA and inserts the proviral DNA into the genome, establishing a latent infection. In this state, the virus can remain dormant for years, evading immune detection. Reactivation of the host cell (e.g., by inflammatory signals) triggers transcription of viral genes by host RNA poly. II, producing new viral RNA for packaging into progeny virions.

1.3 Types of HIV virus

There are basically types of Human Immunodeficiency Virus (HIV) . HIV-1 and HIV-2. Both types can lead to Acquired Immunodeficiency Syndrome (AIDS), but they have important differences in how common they are, how easily they spread, and how quickly they progress. HIV-1 is by far the most widespread and is responsible for the vast majority of HIV infections worldwide—about 95% of all cases. When people talk about HIV in general, they are almost always referring to HIV-1. HIV-2, on the other hand, is much less common and is mostly found in West Africa, with a small number of cases reported in other parts of the world, such as Europe, India, and the United States, often among people who have lived in or travelled from West Africa.

HIV-1 and HIV-2 are different in their genetic makeup.HIV -2 is closely related to sooty mangabey monkey and HIV-1 is related to a virus from chimpanzees. Because of these genetic differences, HIV-2 is less easily transmitted from person to person, whether through sexual contact, blood transfusion, or from mother to child. HIV-2 also tends to progress more slowly, with people often remaining healthy for longer periods before developing symptoms or AIDS. In contrast, HIV-1 is more infectious and tends to cause symptoms and immune system damage more quickly if left untreated.

HIV-1 is divided into four groups: M (Major), N (Non-M, Non-O), O (Outlier), and P. Group M is responsible for the global HIV pandemic and is itself split into several subtypes or clades, labeled A through K. These subtypes differ in their genetic makeup and are distributed differently across the world. Subtype A is more frequent in parts of Africa and Eastern Europe. Sometimes, different subtypes can combine their genetic material, leading to what are called circulating recombinant forms (CRFs), which also play a role in the spread of HIV.

While HIV -2 is less studied as it spread less in human beings and mostly limited to west African areas only and spread very slowly.

while both HIV-1 and HIV-2 can cause AIDS, HIV-1 is much more common and spreads more easily. HIV-2 is mostly limited to West Africa and tends to progress more slowly. Both types have their own groups and subtypes, which can affect how the virus spreads and how it responds to treatment. Understanding the differences between the types and subtypes of HIV helps doctors choose the best treatment and track how the virus spreads around the world

1.4 Mode of Transmission

Most common and prominent method of transmission of AIDS is body fluid from a person who has infected with HIV virus. Mostly through unprotected intercourse, infected needle, from mother to child during pregnancy etc.

If someone uses a needle that has already been used by a person with HIV, the virus can be directly introduced into the bloodstream. This is why people who inject drugs are at higher risk and why using new, sterile needles every time is crucial for prevention.

Perinatal transmission that is from mother to the child. This can be spread during pregnancy period or during breast feeding. Without treatment, there is a significant risk that the virus will pass to the baby, but with proper medical care and antiretroviral therapy, the risk can be greatly reduced.

Less commonly, HIV can be spread through blood transfusions or organ transplants if the blood or organ is contaminated with the virus. However, in most countries, donated blood and organs are carefully screened, making this type of transmission extremely rare today

HIV is mainly transmitted through unprotected sexual contact, sharing injection equipment, and from mother to child during birth or breastfeeding. With proper precautions, such as using condoms, not sharing needles, and ensuring safe medical practices, the risk of HIV transmission can be greatly reduced. Understanding these modes of transmission is key to preventing the spread of HIV and protecting public health [6].

1.5 Molecular Docking and application in drug discovery field

Molecular docking serves as a key computational tool in pharmaceutical research, enabling scientists to model how potential drug molecules (ligands) interact with biological targets like proteins. This technique plays a critical role in drug development, aiding in tasks such as identifying candidate compounds, refining their chemical structures, computationally screening large molecular libraries, and designing drugs based on protein structures. The process works by assessing shape compatibility and molecular forces—such as electrostatic interactions or hydrogen bonding—between the ligand and the protein's active site. By simulating these interactions, docking algorithms predict the most stable binding orientation of the ligand and quantify the interaction strength (binding affinity), providing insights critical for optimizing therapeutic agents.

Various computational tools have been designed for molecular docking, each offering distinct capabilities and advantages. Commonly utilized programs such as AutoDock, AutoDock Vina, and DOCK employ diverse scoring systems and exploration strategies to analyze ligand conformations and enhance interactions with target proteins. These tools integrate factors like ligand and protein flexibility, as well as solvent interactions, to refine the precision of docking outcomes.

In pharmaceutical research, molecular docking supports critical workflows like lead compound identification, where it rapidly evaluates vast chemical databases to pinpoint molecules with strong target affinity and selectivity. Similarly, computational screening leverages docking simulations to rank compounds from virtual libraries based on their predicted binding energies and spatial compatibility with a protein's active site, streamlining the selection of candidates for laboratory validation.

Molecular docking plays a central role in structure-based drug design, particularly in refining candidate molecules by methodically analysing the binding site's chemical environment to pinpoint interactions that enhance ligand potency and selectivity By simulating how modified compounds interact with the target protein, docking helps clarify structure-activity relationships—revealing how structural changes influence binding strength and specificity. This predictive capability allows researchers to prioritize chemical modifications that boost drug efficacy.

Beyond optimization, docking aids in decoding the mechanisms of drug activity and resistance. For example, studying how ligands bind to mutated proteins can inform strategies to overcome resistance in diseases like HIV or cancer. Additionally, the technique accelerates the creation of therapeutics for diverse pathways by modelling interactions between proteins and novel compounds.

Integrating docking with experimental methods such as crystallography or assays creates a synergistic workflow. Computational predictions guide lab experiments, reducing trial-and-error inefficiencies, cutting development costs, and improving the likelihood of discovering viable drug candidates.

1.6 Research objective and scope

This research aims at using computational approach mainly molecular docking to identify potential HIV-1 reverse transcriptase enzyme inhibitors. Study mainly aims to:

1. Select a protein / drug which is FDA approved and known for action with HIV-1 reverse transcriptase, based on literature review and expert consultation.

2. Compile the database of compounds with the potential to act as a ligand, drawing from different libraries and drug databases.

3. Using molecular docking as a tool to predict the interaction between the different ligands and HIV- 1 reverse transcriptase enzyme while considering ligand flexibility and receptor conformational changes.

4. Validating the molecular docking results through comparing it with experimental data to asses the reliability and predicting the computational approach validity.

5. Analyse the top ranked docking score and poses and prioritize lead compound by considering the parameters like binding affinity, specificity etc.

6. Analyze the 3D and 2D interaction of the compound / ligands with the receptor to analyze the type of interactions taking place between ligands and receptor.

The scope of this research is to combine theoretical and computational approach aim to find the potential inhibitors of HIV-1 reverse transcriptase enzyme. Also, study aim to look for the compounds which are already in clinical trials for some types of cancer so that Zidovudine can be replaced with these compounds and they can work on HIV as well as cancer developed in the patient of HIV due to prolong exposure from HIV.

Chapter -2

Literature Review

Acquired Immunodeficiency Syndrome (AIDS) is a life-threatening condition caused by the Human Immunodeficiency Virus (HIV), a retrovirus that attacks and gradually destroys the immune system, specifically targeting CD4+ T cells which are essential for defending the body against infections and diseases. HIV is transmitted through certain body fluids, most commonly via unprotected sexual contact, sharing of contaminated needles, transfusions of infected blood, and from mother to child during childbirth or breastfeeding [7] .

After infection, the virus continues to replicate and deplete the immune system's resources, often without the individual's knowledge. If left untreated, HIV infection progresses over time, eventually resulting in AIDS, which is defined by a CD4+ T cell count below 200 cells per microliter or the presence of specific opportunistic infections and cancers that rarely affect people with healthy immune systems.

In the early stages, the immune system attempts to control the virus, but over time, HIV's relentless attack on CD4+ T cells weakens the body's defenses. This immunodeficiency allows a range of opportunistic infections and cancers to develop, including pneumocystis pneumonia, tuberculosis, Kaposi's sarcoma, and lymphomas. The specific infections and complications experienced by a person with AIDS often depend on the organisms present in their environment and the extent of immune system damage. Common symptoms in advanced stages include persistent fever, night sweats, weight loss, chronic diarrhea, swollen lymph nodes, and recurrent infections. Neurological and psychiatric symptoms can also occur, further complicating the clinical picture [8].

Globally, HIV/AIDS remains a significant public health challenge. According to the recent report of 2003 by WHO, HIV has claimed about 40 million lives while 39 million are currently infected with this virus. Sub-Saharan Africa is the region most affected, accounting for the majority of new infections and AIDS-related deaths, though the epidemic has a global reach. Women, particularly young women in Africa, are disproportionately impacted by the disease, but increased education and access to healthcare have shown to reduce infection rates.

Advances in treatment, especially the development of antiretroviral therapy (ART), have transformed HIV/AIDS from a fatal disease to a manageable chronic condition for many [9].

Despite these advances, challenges remain. Stigma, discrimination, and social determinants of health continue to hinder prevention, testing, and treatment efforts in many regions. Biomedical prevention strategies, such as pre-exposure prophylaxis (PrEP), treatment as prevention (TasP), and harm reduction for people who inject drugs, have proven effective but are not yet accessible to everyone who needs them. Research continues into new prevention tools, vaccines, and ultimately a cure. International efforts, such as those led by UNAIDS and the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), have made significant progress in expanding access to testing, treatment, and education, but the fight against HIV/AIDS is ongoing. Ending the epidemic will require sustained political will, scientific innovation, and a commitment to addressing the social and economic factors that drive the spread of HIV [10].

Molecular docking is a widely used computational strategy for modeling how small molecules (ligands) interact with biological targets like ion channels. By analyzing how ligands fit into a protein's three-dimensional structure, these simulations predict the optimal alignment and binding energy between the two molecules, as demonstrated in foundational research This approach streamlines the discovery of promising drug candidates by identifying ligands with strong binding potential, which can then advance to laboratory testing [11].

Although entirely computer-based, docking has become indispensable for efficiently screening vast chemical libraries—a process called virtual screening—to prioritize molecules likely to modulate protein activity. For HIV inhibitors, this technique helps uncover compounds that block the replication of HIV, offering treat conditions ranging AIDS to cancer.

2.1 Overview About AIDS

Acquired Immunodeficiency Syndrome (AIDS) is a progressive, life-threatening condition marked by the severe depletion of CD4+ T lymphocytes, immune cells critical for coordinating adaptive immunity. The Human Immunodeficiency Virus (HIV), a retrovirus, initiates infection by binding its envelope glycoprotein gp120 to CD4 receptors on T cells and co-receptors (CCR5/CXCR4).

After infecting host HIV releases its RNA genome, which reverse transcribe the DNA by the help of reverse transcriptase enzyme (RT). This DNA integrates into the host chromosome via integrase, establishing a latent reservoir that evades

immune detection. The hijacked cellular machinery then produces viral proteins, assembling new virions that bud from the host cell to infect others.

Persistent viral replication leads to a catastrophic decline in CD4+ T cells, impairing the immune system's ability to combat opportunistic infections like Pneumocystis jirovecii pneumonia and malignancies such as Kaposi's sarcoma. HIV's latency complicates eradication, as the virus can reactivate years later, reigniting active infection.

The humoral immune response, mediated by plasma B cells, produces antibodies targeting HIV's surface proteins (e.g., gp120). These antibodies neutralize free virions and flag infected cells for destruction by natural killer cells. However, HIV's error-prone reverse transcriptase generates ~1 mutation per replication cycle, creating diverse viral quasispecies. This genetic variability allows HIV to alter epitopes on its envelope proteins, rendering existing antibodies ineffective a phenomenon termed antigenic drift.

Chronic antigen exposure exhausts B cells, reducing their antibody-producing capacity and leaving the host vulnerable to secondary infections.
Simultaneously, HIV directly destroys CD4+ T cells, which are essential for activating B cells and cytotoxic T lymphocytes. This dual assault cripples both humoral and cell-mediated immunity, accelerating disease progression [12].

2.2 HIV

The Human Immunodeficiency Virus (HIV) is a retrovirus belonging to the *Lentivirus* genus, primarily targeting CD4+ T lymphocytes, macrophages, and dendritic cells. Its genome comprises two single-stranded RNA molecules and key enzymes—**reverse transcriptase (RT)**, **integrase**, and **protease**—that facilitate replication and immune evasion. HIV exists in two types: **HIV-1**, responsible for the global pandemic, and **HIV-2**, a less virulent strain endemic to West Africa. The viral envelope, studded with glycoproteins **gp120** and **gp41**, mediates entry into host cells via interactions with CD4 receptors and co-receptors (CCR5/CXCR4) [13].

2.3 Replication Cycle and Pathogenesis

- **Entry and Fusion**:
    - Gp120 binds to CD4, triggering conformational changes that expose co-receptor binding sites. Subsequent gp41-mediated fusion releases the viral capsid into the cytoplasm.
    - Recent studies highlight **Maraviroc**, a CCR5 antagonist, as a therapeutic strategy to block viral entry.
- **Reverse Transcription**:
    - RT converts viral RNA into double-stranded DNA (dsDNA). This error-prone process generates ~1 mutation per genome, fostering viral diversity and drug resistance.
- **Integration**:
    - Integrase inserts viral DNA into the host genome, establishing latent reservoirs that evade antiretroviral therapy (ART).
- **Assembly and Budding**:
    - Viral proteins and RNA assemble at the host membrane, forming immature virions that mature via protease-mediated cleavage.

HIV's depletion of CD4+ T cells cripples adaptive immunity, leading to opportunistic infections (e.g., *Pneumocystis* pneumonia) and malignancies (e.g., Kaposi's sarcoma). Persistent immune activation and inflammation further drive disease progression.

2.4 HIV-1 Reverse Transcriptase Enzyme

HIV-1 reverse transcriptase (RT) is a multifunctional enzyme critical for viral replication, enabling the conversion of single-stranded RNA into double-stranded DNA—a process essential for integrating the viral genome into host chromosomes. As a primary target for antiretroviral therapy (ART), RT's structure and function have been extensively studied to develop inhibitors and combat drug resistance. This review synthesizes insights from structural biology, enzymatic mechanisms, and therapeutic strategies targeting RT, drawing from recent research advancements [14].

2.4.1 Structural and Functional Domains

HIV-1RT is a heterodimer composed of p66 and p51 subunit. The P66 has both the active sites for DNA pol. And RNase site where as p51 is responsible in stabilizing the heterodimers by conformational interaction or changes [15].

The polymerase domain of p66 includes subdomains termed fingers, palm, thumb, and connection, which collectively form a nucleic acid-binding cleft. The palm subdomain contains the catalytic triad (D110, D185, D186) responsible for coordinating Mg^{2+} ions during DNA synthesis. In contrast, the RNase H domain cleaves RNA in RNA-DNA hybrids, facilitating template switching during reverse transcription. Structural studies reveal that RT's flexibility, particularly the thumb subdomain movement, is crucial for accommodating nucleic acids during replication [16].

2.4.2 Mechanism of action of HIV-1RT

Reverse transcription starts after the entry of viral genome inside the cytoplasm of the host. Minus strand synthesis starts when RT bind to the primer tRNA which get anneal to the RNA primer binding site (PBS). The polymerase activity of RT extends the primer using viral RNA as a template, while RNase H degrades the RNA strand, leaving transient RNA-DNA hybrids [17]. Key steps include:

(i) Minus-Strand Transfer: The newly synthesized DNA "jumps" to the 3' end of the viral RNA via complementary sequences, enabling synthesis of the full-length minus-strand DNA.

(ii) Plus-Strand Synthesis: primers ae created from polypurine tracts (PPT) by the action of RNase H. A second strand transfer ensures the formation of double-stranded DNA with long terminal repeats (LTRs) for integration.

RT's error-prone nature—due to the lack of proofreading—results in ~1 mutation per genome, driving viral diversity and drug resistance. Host factors like APOBEC3G exacerbate mutagenesis by deaminating cytidines, though HIV-1 counteracts this via the Vif protein, which degrades APOBEC3G.

2.5 Molecular Docking in Drug Discovery

Molecular docking is a foundational computational method in pharmaceutical research, enabling scientists to model how small molecules (ligands) interact with target proteins. This technique supports critical stages of drug development, including identifying lead compounds, refining their structures, and designing drugs based on protein architecture, as outlined in seminal. By simulating how ligands fit into a protein's binding site, docking predicts the most stable orientation and interaction strength (binding affinity) through analysis of structural compatibility and molecular forces like hydrogen bonding or van der Waals interactions [18].

Several specialized software tools have been developed to perform docking simulations. Widely used platforms such as AutoDock, AutoDock Vina, and DOCK employ distinct search algorithms and scoring systems to evaluate ligand conformations and optimize interactions with target proteins. These programs account for variables like molecular flexibility (in ligands and proteins) and solvent effects to enhance prediction reliability. For example, AutoDock Vina uses a gradient-based optimization approach, while DOCK focuses on shape complementarity [19].

Applications in Drug Development

1. Lead Identification:
   Docking rapidly screens vast chemical libraries to pinpoint molecules with strong binding potential to a target, accelerating early-stage drug discovery.
2. Virtual Screening:
   By ranking compounds based on predicted binding energies, docking prioritizes candidates for laboratory testing, reducing experimental costs.
3. Structure-Based Optimization:
   Researchers use docking to refine lead compounds by analyzing how structural changes (e.g., adding functional groups) improve binding strength or selectivity.

Beyond drug discovery, docking elucidates how ligands interact with proteins at the atomic level. For instance, studying how HIV protease inhibitors [20] bind to mutant enzymes reveals resistance mechanisms, guiding the design of next-

generation drugs [21]. Integrating docking with experimental techniques like X-ray crystallography creates a feedback loop, where computational predictions inform lab experiments and vice versa. This synergy enhances the efficiency of developing therapies for diseases ranging from cancer to viral infections .

While docking is powerful, limitations like incomplete protein flexibility models or solvent dynamics approximations persist. Newer tools address these gaps using machine learning to predict binding affinities more accurately. For example, AlphaFold-inspired models now complement traditional docking by predicting protein-ligand complexes with higher precision [22].

2.6 FDA Approved Zidovudine

Zidovudine (AZT), a thymidine analogue, was the first antiretroviral drug approved by the FDA in 1987 for HIV treatment. Initially developed as a cancer therapeutic, its repurposing marked a breakthrough in AIDS management. AZT's mechanism involves inhibiting HIV-1 reverse transcriptase (RT), a critical enzyme for viral replication. Despite challenges like resistance and toxicity, AZT remains a cornerstone of combination therapies, particularly in resource-limited settings [23]. This review synthesizes insights from structural modifications, clinical efficacy, resistance mechanisms, and comparative studies to highlight AZT's enduring role in HIV care [24].

Zidovudine or Azidothymidine (AZT), which is used as a reference drug for this study, is a thymidine analog that was previously used as a cancer therapeutic agent but was later FDA-approved it as an anti-HIV drug, showing one of the very first examples of drug repurposing. AZT is a thymidine analog that contains a C3' hydroxyl group instead of an azido group and is converted into its active form by triphosphorylation at the 5'-OH group [25]. The triphosphorylated 5-OH, binds to the RT and results in the chain termination of the growing viral DNA. AZT is a multi-target-directed ligand (MTDL) antiretroviral drug that is widely used for the treatment of AIDS. Therefore, exploring more compounds that can show a similar mode of action to the AZT can be a potential choice for drug targeting and designing to reduce the progression of HIV infection and AIDS [26].

AZT is a prodrug requiring intracellular phosphorylation to its active triphosphate form (AZT-TP). This metabolite competes with endogenous thymidine triphosphate (dTTP) for incorporation into viral DNA by HIV-1 RT. The absence of a 3'-hydroxyl group in AZT-TP terminates DNA chain elongation, halting viral

replication. AZT also weakly inhibits human DNA polymerase-γ, contributing to mitochondrial toxicity. Its specificity for viral RT over host polymerases underpins its therapeutic utility, though long-term use is limited by side effects like anemia and neutropenia

Efforts to improve AZT's pharmacokinetics and reduce toxicity have focused on structural modifications [27]:

Polyethylene glycol (PEG) conjugates: Linking AZT to PEG via succinate linkers (e.g., compound 44, mPEG 750) prolongs its half-life and enables sustained release. These conjugates showed enhanced anti-HIV activity (IC_{50} = 0.11 μM against HIV-1) and reduced cytotoxicity (CC_{50} = 110 μM).

Prodrug strategies: Modifications like 5'-O-valerate esters improve bioavailability by enhancing cellular uptake and delaying metabolism.

Such innovations aim to mitigate AZT's short plasma half-life (1.1 hours) and frequent dosing requirements

Early clinical trials demonstrated AZT's ability to delay HIV progression: A double-blind study of 711 patients with mild HIV symptoms showed AZT reduced disease progression by 67% in individuals with CD4 counts <500 cells/mm^3.

Chapter -3

METHODOLOGY

In this study we uses computational approach with molecular docking to look for the suitable new inhibitors for HIV-1RT while comparing the docking result of FDA approved drug Zidovudine with the newly Screened compounds. We also look for the background of the compounds and their docking result which look for binding affinity.

Here we used Swiss similarity for the virtual screening of the compounds which uses 'smiles' for screening similar structure compounds.

3.1 Retrieval of the HIV-1 reverse transcriptase enzyme

- The RCSB Protein Data Bank (PDB) database [28] is used to obtain the 3-D structure of HIV-1 Reverse Transcriptase Enzyme complexed with 3 (pyrimidin-2-yl)-N-[3-(5,6,7,8-tetrahydronaphthalen-2-yl) 1H-pyrazol-5-yl] propenamide, with PDB code 5VZ6 is downloaded in legacy PDB format.
- If you experience any issues loading the structure, you can download the PDBx /mmCIF format in addition to the PDB format as normal.
- This compound structure has an X-ray diffraction resolution of 2.60 Å.
- Put this into a folder

3.2 Selection of ligands

(i) Reference Selection: Zidovudine, an FDA-approved drug, is used as a reference whose structure in 3D conformer is downloaded in SDF format [29].

(ii) For selection of ligands, Swiss Similarity, an online tool [30], is used for searching the compounds that are similar in structure to the original Zidovudine drug.

For this purpose, the SMILES was copied from the RCSB site of the compound Zidovudine and pasted in the Swiss Similarity search box while selecting both 2nd and 3d structure attributes. This search resulted in 47 compounds whose similarity score was above 0.85. Using the DrugBank ID of the compounds, their 3d conformer was downloaded in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

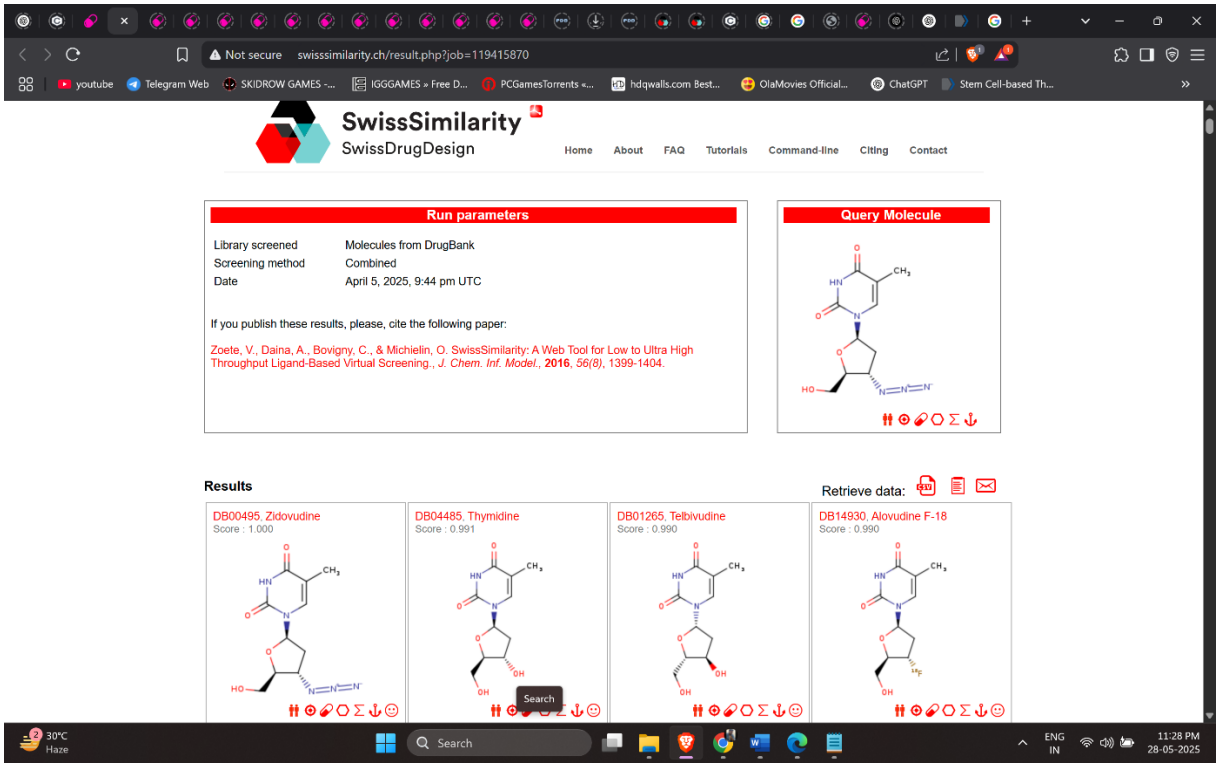


Fig 3.1- Screenshot showing Swiss similarity screening for similar structure of zidovudine

3.3 Preparation of target protein

- For target protein preparation Chimera 1.19 tool is used [31].
- Binding domain and total numbers of chains must be known for preparing the proteins for docking.
- Firstly, we open the PDB file of the protein in the chimera software using the file tab. The structure of the protein is in crystalline structure form is complexed with ligands, water molecules and some steroidal components. It is important to eliminate the extra component before docking to prevent any uncertainity from our docking result.

- For removing these unwanted components firstly, we selected the 3D structure of the protein. Then we go to SELECT tab ---- > ALL NON-STANDARDS. Then go to ACTION tab ---- > atom --- > delete. Using these steps all the complexed ligands and water molecules are removed from the protein structure.
- Now we need to prepare the protein for final docking process in which we need to add hydrogen , residues, and gastergar charges.
- For this we go to TOOLS tab --- > surface/ binding analysis --- > dock prep. Option . a dialogue box will appear stating Add Hydrogen ---> press 'OK' then another dialogue box will appear stating Add Charges --- > just select net zero charge --- > select 'OK' and charges will be balanced throught the protein structure.
- After dock prep. Our molecule we can save this protein structure as receptor.pdb file which will be utilized for further docking with different ligands.

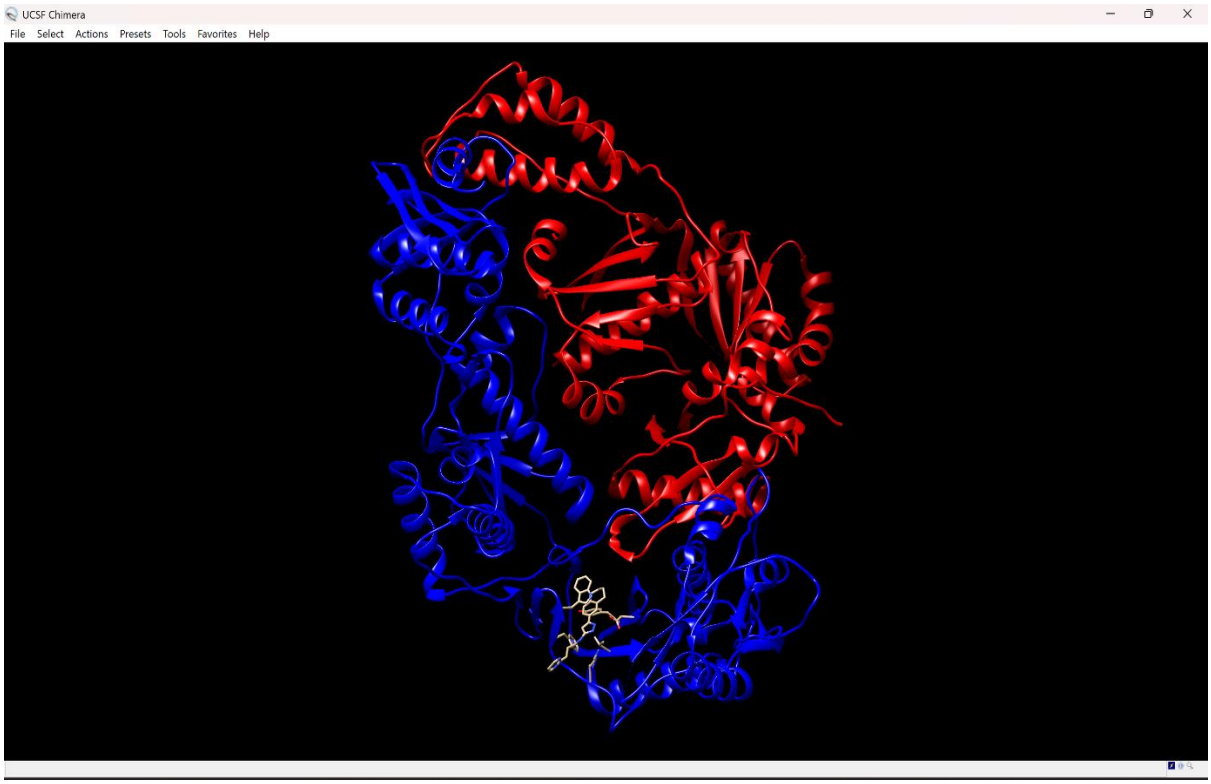


Fig 3.2 Screenshot showing loading of protein in UCSF chimera

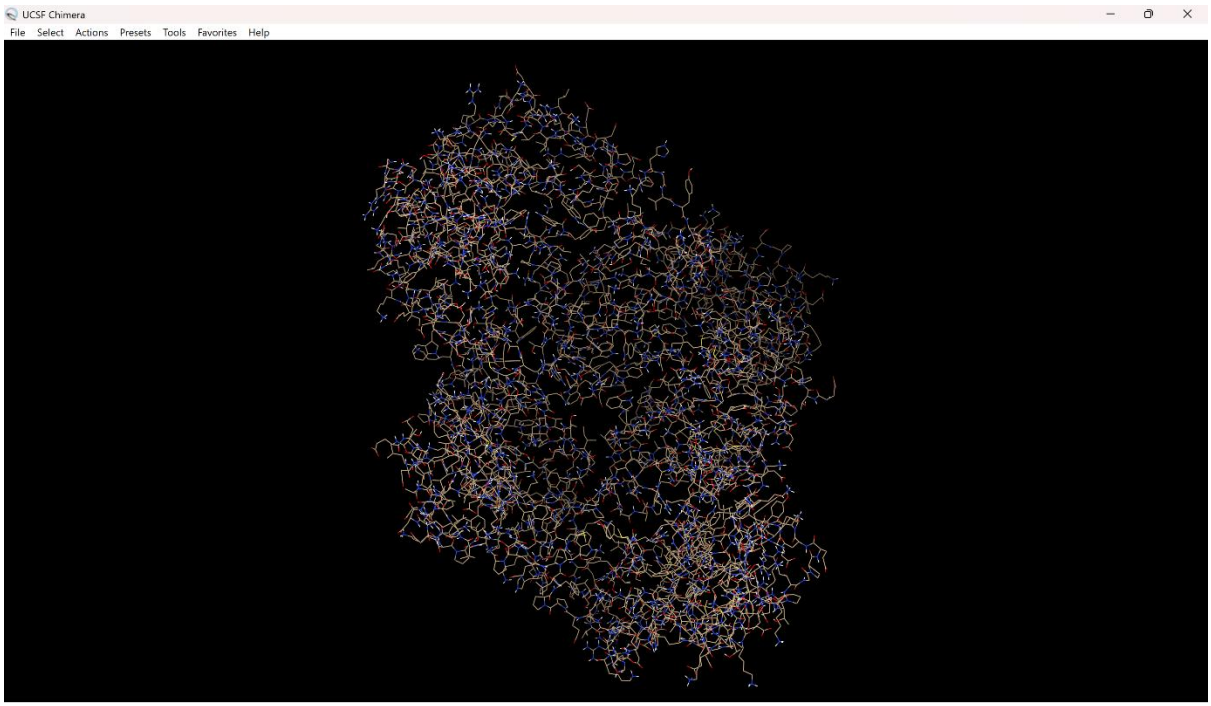


Fig3.3 Schreenshot showing protein after dock preperation

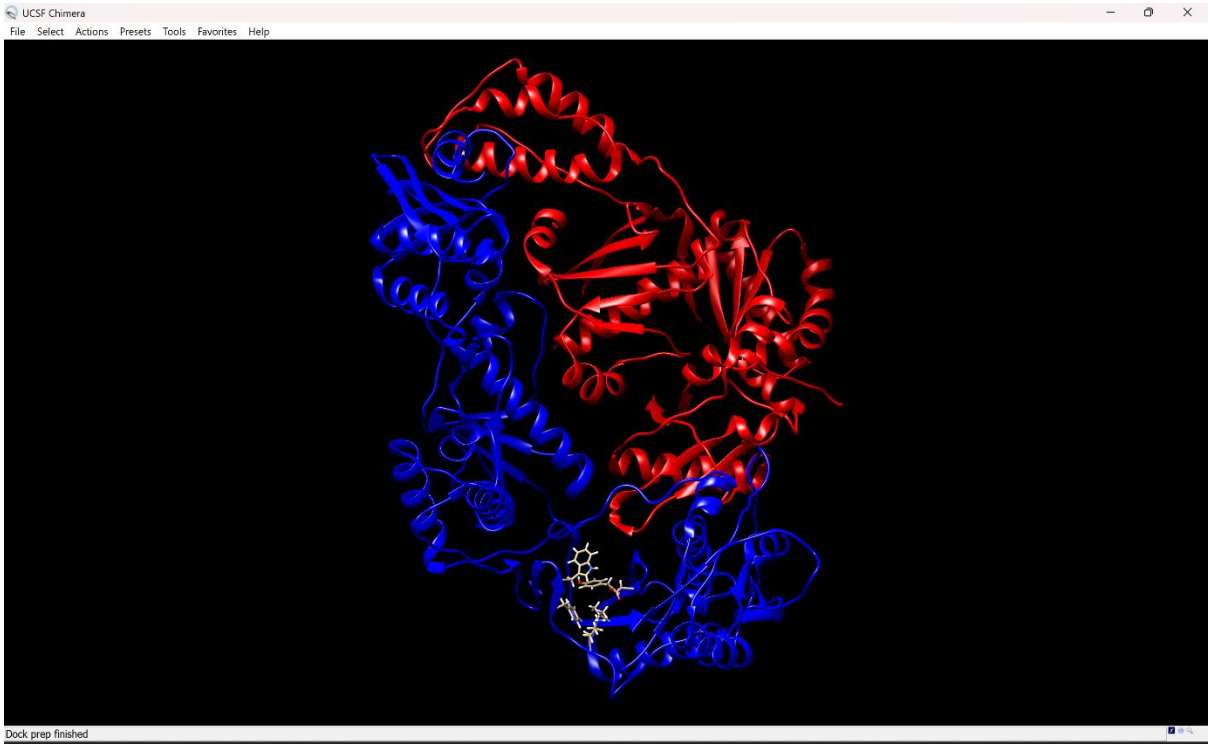


Fig 3.4 Screenshot showing protein after dock prep.

3.4 Preparation of ligand

- Now the ligand Zidovudine is loaded in the Chimera for in SDF format.

- After loading the ligand now its time for ligand preparation for the docking .
- For this go to SELECT tab --- > select chain ---- > click on 'NO ID'
- Now we need to add hydrogen , add charges to the ligands. For this we go to TOOLS tab ---- > Structure Editing --- > Add Hydrogen , a dialogue box will appear and in this window uncheck the receptor protein structure and check only the ligand structure ---- > click 'OK'. The hydrogen will be added to the ligand .

- Now to add charges again go to SELECT TAB --- > Structure Editing --- > Add Charges, a dialogue box will appear and in this window uncheck receptor protein and only check the ligand structure --- > gasteger charges --- > click 'OK' . the charges will be added to the ligands. Save these files as Sdf format .

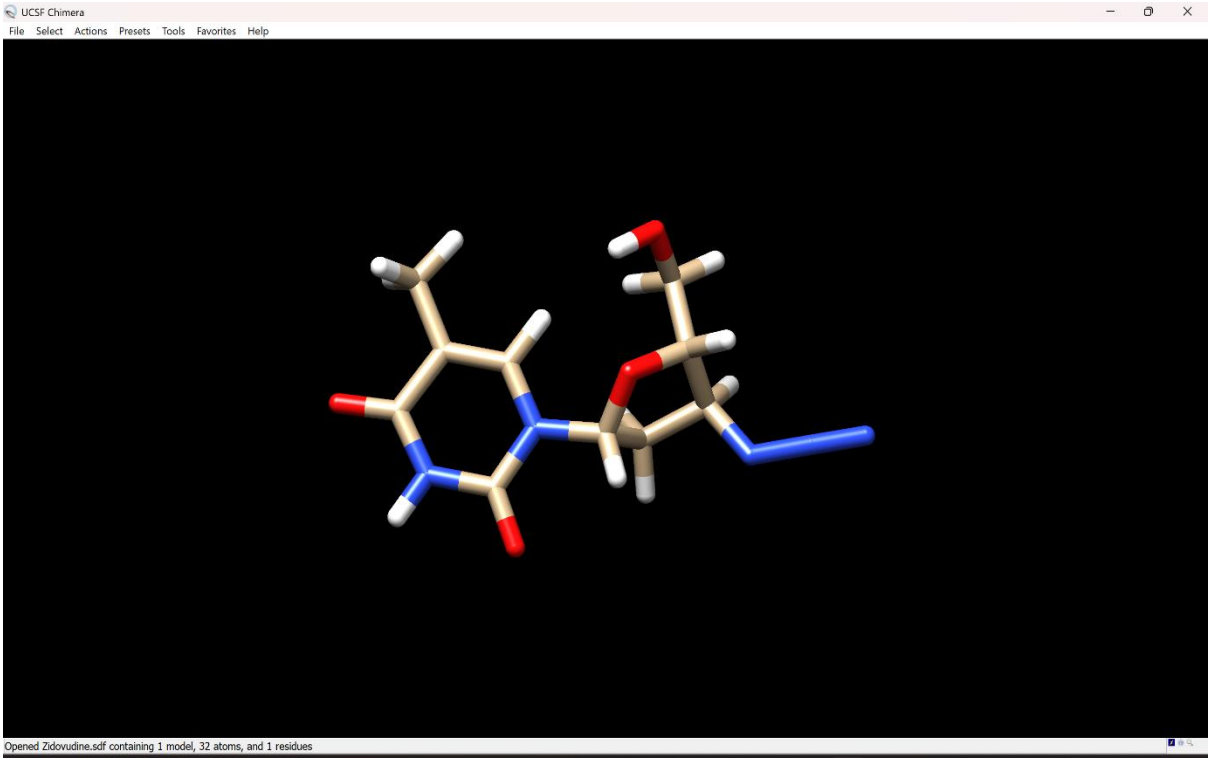


Fig 3.5 Screenshot showing loading of ligand Zidovudine in UCSF Chimera

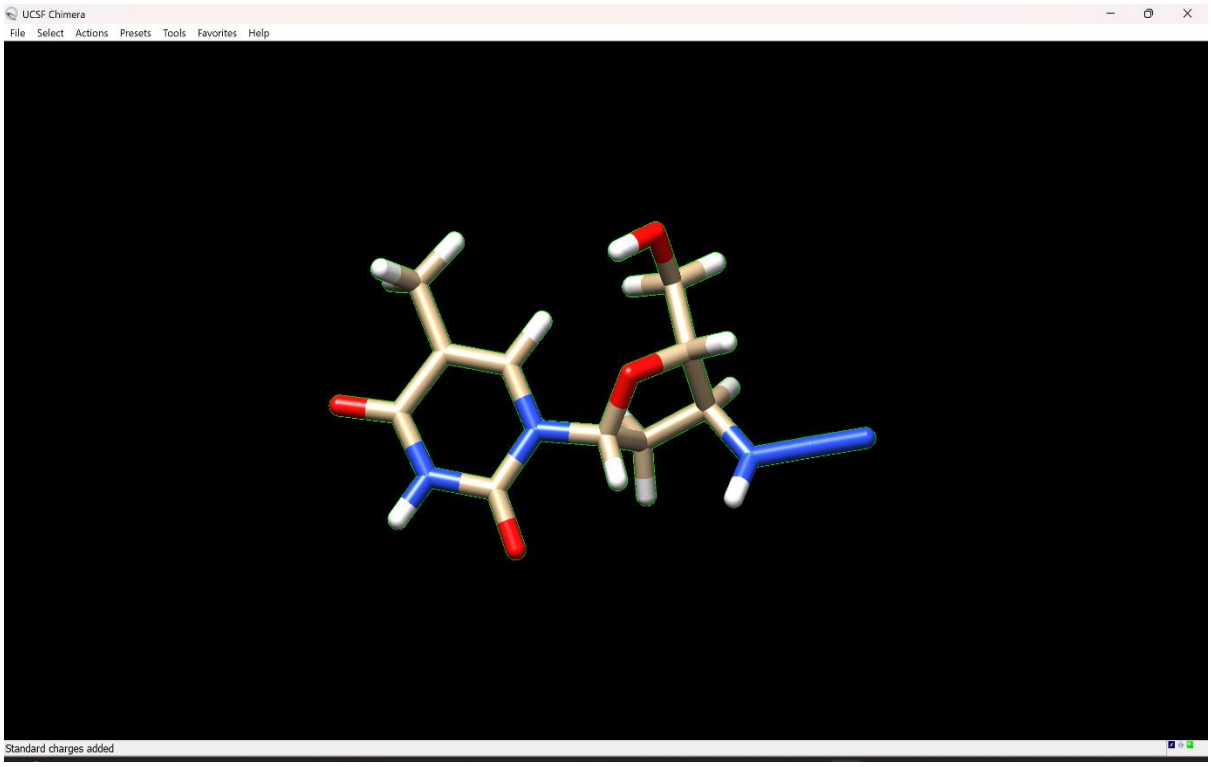


Fig 3.6 Screenshot showing ligand Zidovudine after dock prep.

- After zidovudine , we will perform same steps foe every other ligand which we screened on bases of similarity.
- Saving the files of each ligands in different folder and arranging them according to their name.

3.5 Loading of both dock prepared receptor protein and ligand.

- Open FILE tab ---- > open --- > Select the protein and ligand structures select ok.

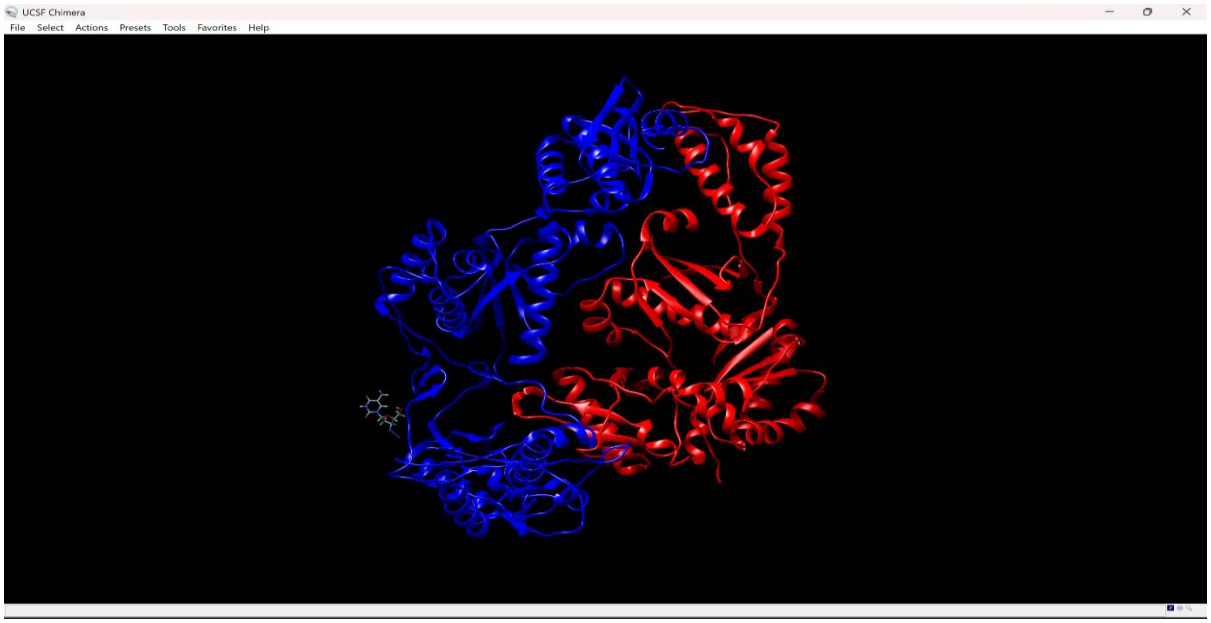


Fig 3.7 Screenshot showing both receptor protein and ligand loaded in Chimera.

3.6 Opening AutoDock Vina

- Go to TOOLS tab ---- > select Surface/Binding Analysis ---- > Autodock Vina --- > browse the AutoDock Vina.exe and then click on open.

3.7 Defining grid Box

- After selecting AutoDock Vina [32] a Dialogue box will appear specifying the dimension of grid box .
- Select the CENTRE and fill the values in 3 different boxes as – 12.2971, 45.2886, 27.0679 .
- Select the SIZE and fill the values in 3 different boxes as 76.8257,99.0815, 77.4997 respectively.
- Tick the resize option if needed and select button 2 .

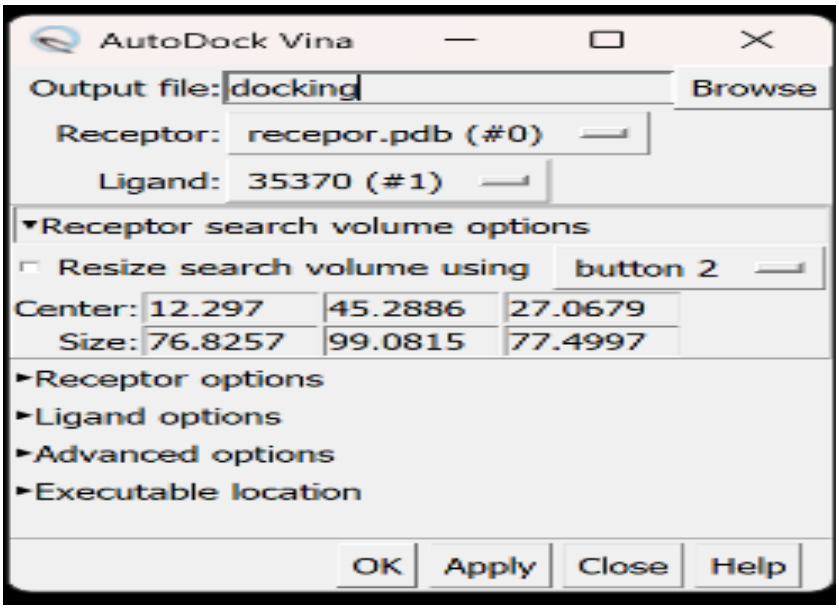


Fig 3.8 Screenshot showing Grid Box values.

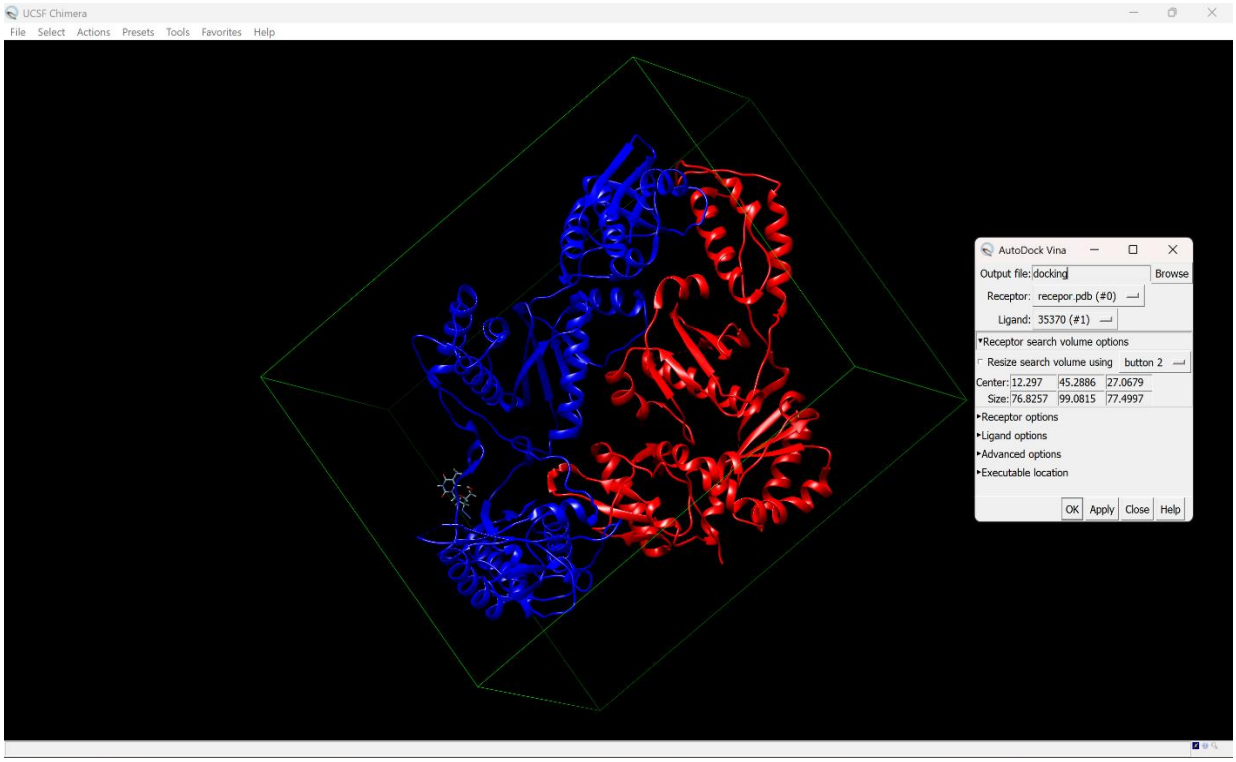


Fig 3.9 showing grid box covering both protein and ligand structures.

3.8 Running AutoDock Vina

- After setting up of Grid Box , just rotate the grid box to make sure that whole protein structure is cover inside the grid box.
- After this step name the OUTPUT file and SELECT the receptor and ligand structures in the empty boxes of AutoDock Vina Dialogue box.
- Select the executable location and name the file as Docking Result.
- Press 'APPLY' and press 'OK' .
- The docking process will start running and the process info can be seen at the bottom left corner of the screen.
- The time of docking depend on the complexity of the protein and ligand structures. After the completion a dialogue box will appear with all the poses and their respective binding energy and RMSD value .
- Similarly , docking process of all the ligands with the protein is performed to obtain the docking result.

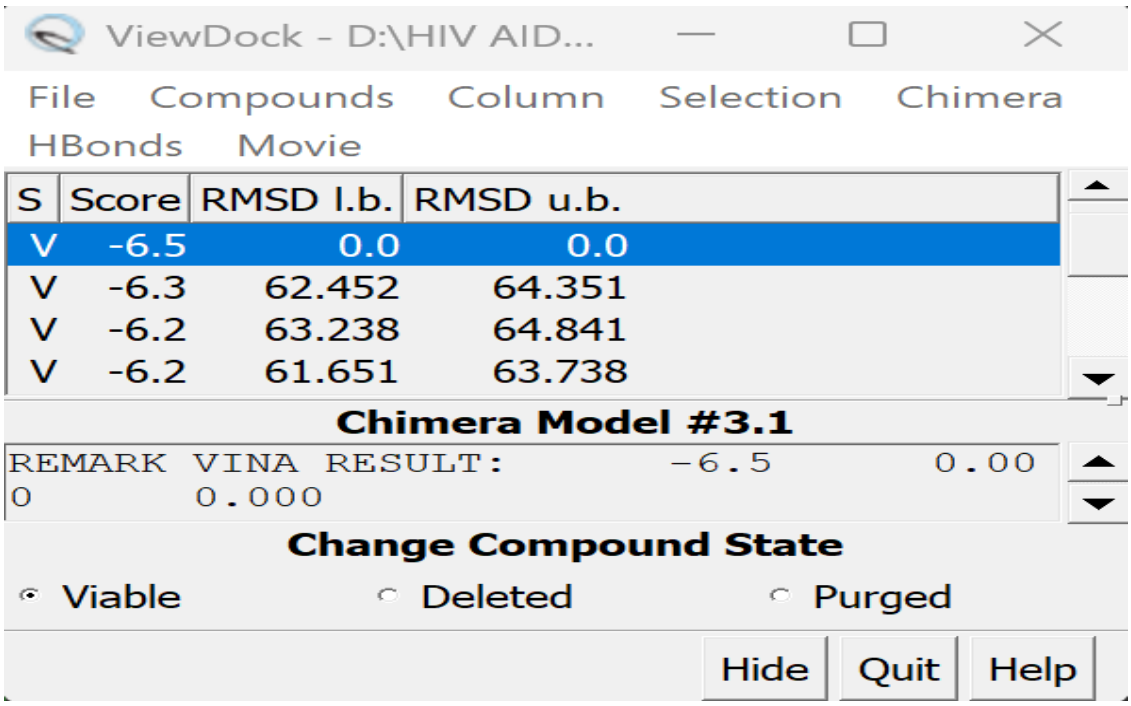


Fig 3.10 screenshot showing docking result of Zidovudine

3.9 Finding the best pose with highest binding affinity

- For this purpose,the file named 'Docking result' which get saved automatically after docking is complete is imported in Chimera in new Window.
- After opening go to SELECT tab --- > CHAIN option--- > NO ID ---- > then select the chain value #0. #0 signifies the pose with maximum Binding Affinity.
- After this go to FILE tab--- > Save As PDB format ---- > Select the #0 and save this as file name Best Pose.

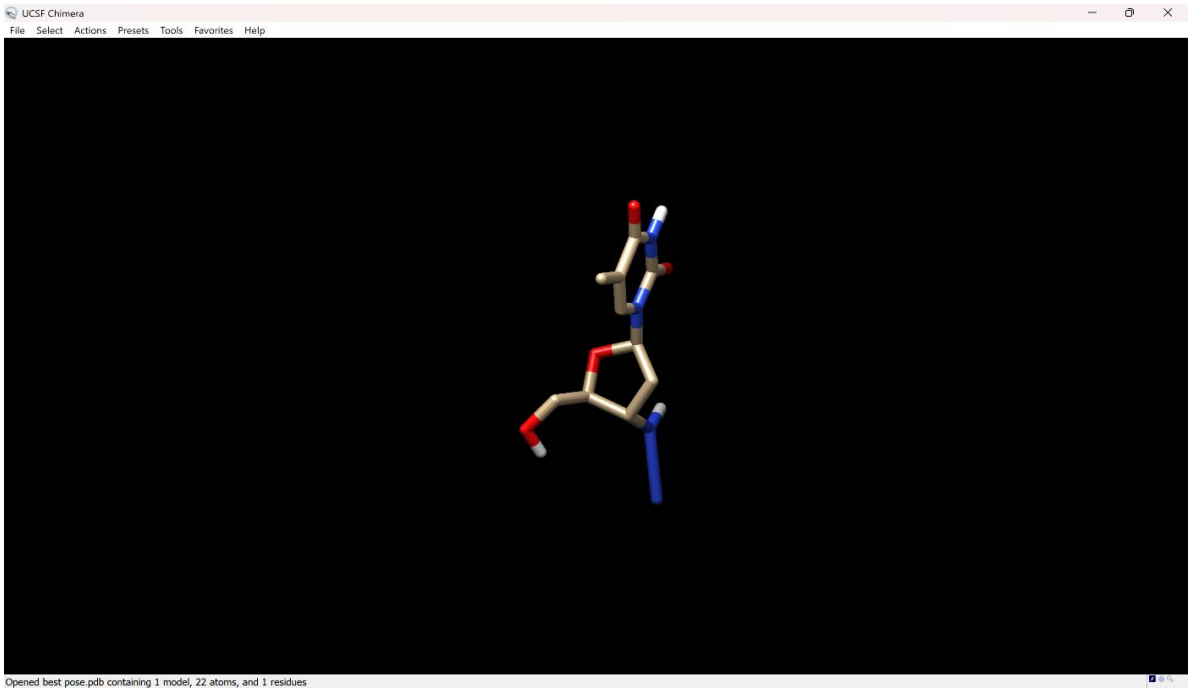


Fig 3.11 Screenshot showing best pose of Zidovudine.

3.10 Visualizing the Docking Result

- Open Chimera software, go to FILE tab and open the file name 'dockingresult.receptor.pdb'.

- After this again go to File tab and open the file name Best pose.pdb.
- After opening you can see the position of the ligand interacting with the receptor molecule.

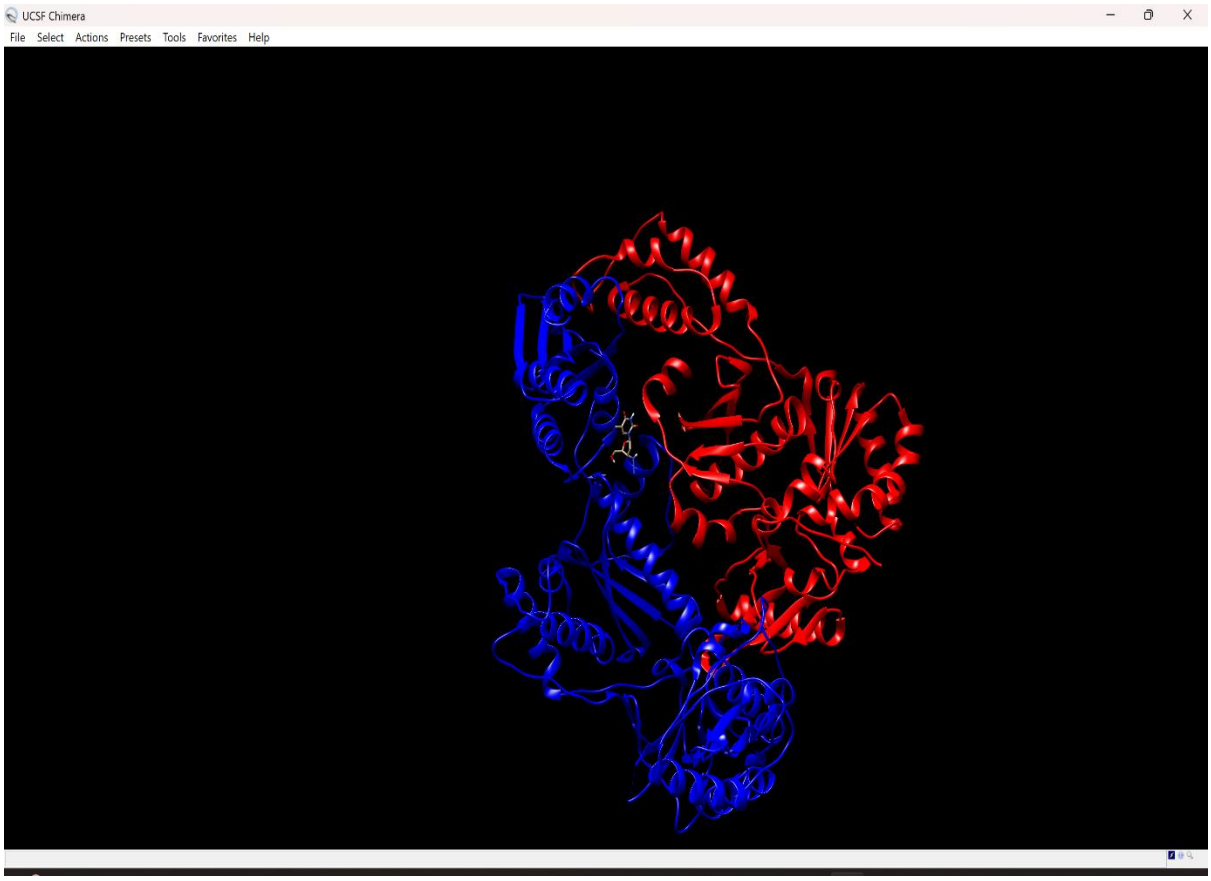


Fig 3.12 screenshot showing 3D interaction between Zidovudine & HIV-1RT molecule.

3.11 Visualizing the interaction of Different Residues of ligand & types of bond formation using BIOVIA Discovery Studio 2021.

- To visualize the 2D interactions and get the info about the interacting ligands we need BIOVA Discovery Studio .
- Firstly, go to FILE tab --- > open our receptor protein file named as ‘dockingresult.receptor.pdb’
- Then click again on FILE tab and open ligand with conformation saved as ‘best pose.pdb’ file.
- Then drag and drop both the files in receptor -ligand interaction tab.
- After opening of both the file just click on the ‘Receptor-Ligand interactions tab for visualizing the interaction.
- Under the section of view interactions dropdown menu just select ‘Show 2D Diagram’ option.
- A 3d and 2d diagram interaction be displayed in 2 different windows.
- Notice the types of interactions and residues interaction during the complex.

- IF No hydrogen interactions are seen that suggests the interaction are very poor and docking result is not feasible.

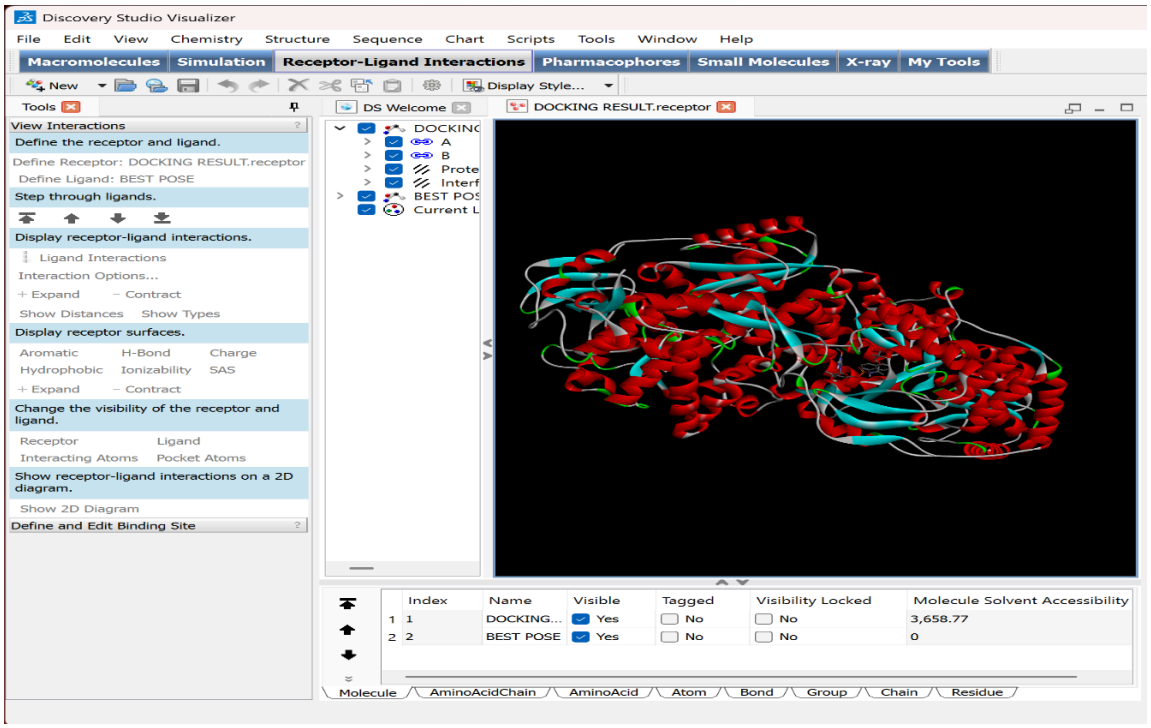


Fig 3.13 Screenshot showing 3d interaction in BIOVIA Discovery Studio

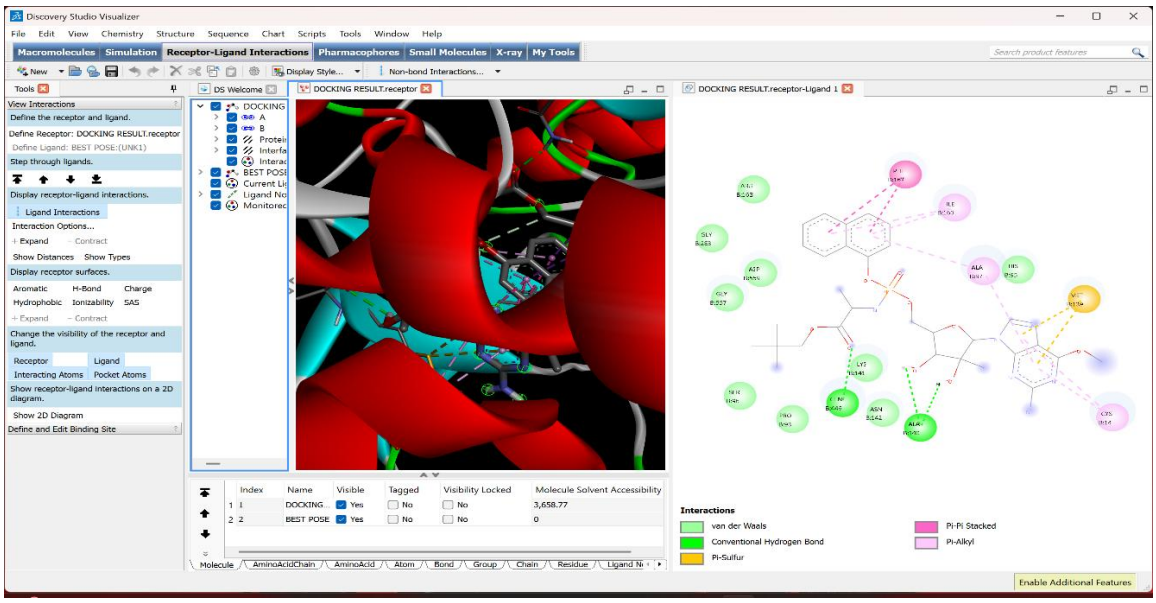


Fig 3.14 Screenshot Showing 2d interactions in BIOVIA Discovery Studio with interacting residues & bond formations

3.13 Examine Binding Interactions:

- Use the Analyse Receptor-Ligand Interactions tool to identify and visualize key interactions between the ligand and the protein.

- Highlight hydrogen bonds, hydrophobic interactions, salt bridges, and π-π stacking interactions. Check for consistency with known binding sites or important residues in the binding pocket.

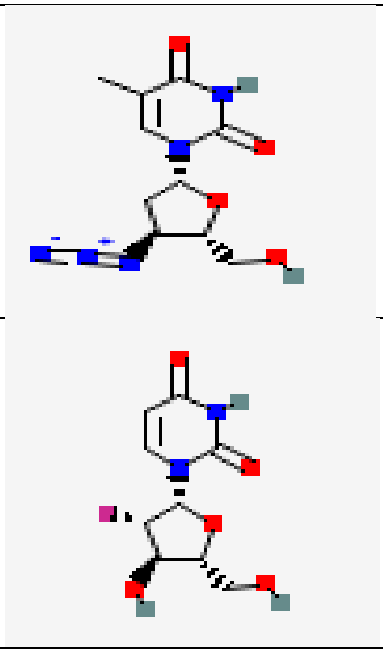
CHAPTER- 4

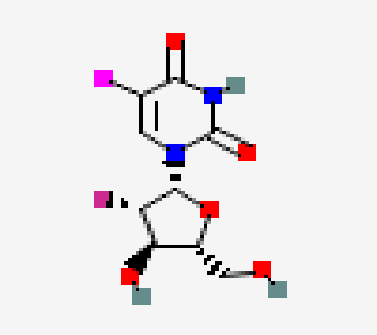
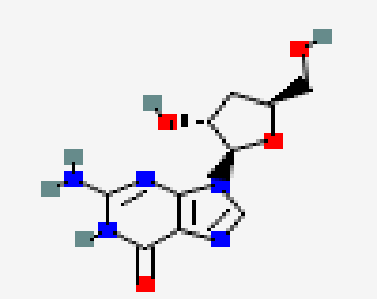
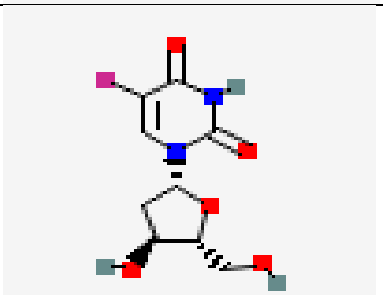
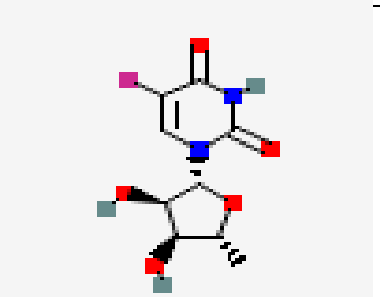
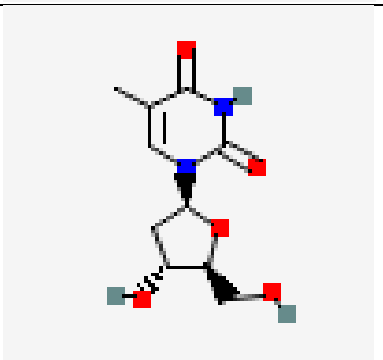
RESULTS

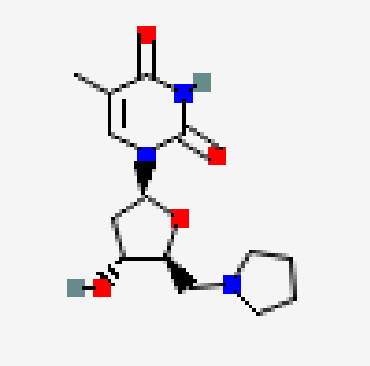
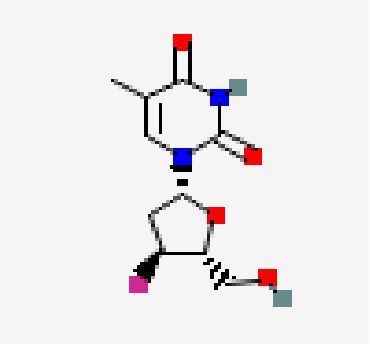
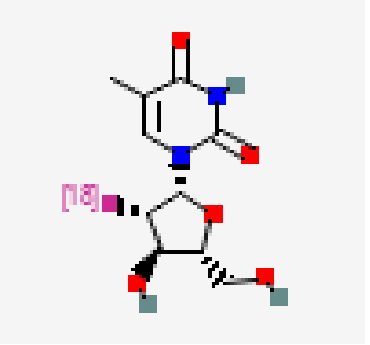
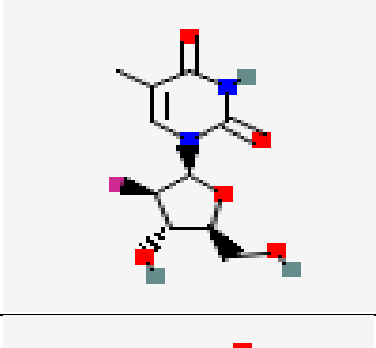
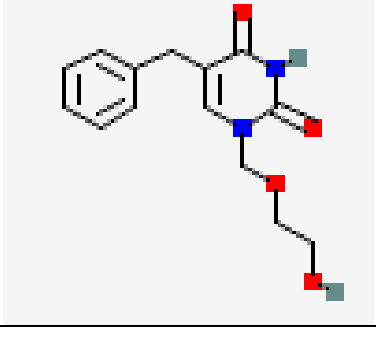
The outcomes of molecular docking approach revealed promsing compounds for the treatment of Auto immunodeficiency syndrome. By the help of virtual screening of different compounds against the HIV-1 Reverse Transcriptase. We identified and shortlisted 11 compounds with higher binding affinity values and therapeutic effects. Most of the compounds shortlisted are generally used in case of cancer or are in clinical trials for cancer and various other disease. The compounds having highest binding affinity and zero RMSD value is selected and its model and interactions are studied in BIOVIA Discovery Studio. We compared the binding affinity score of every compounds with the existing FDA approved drug used for treatment of HIV that is Zidovudine.

The following table shows the docking results of different compounds with HIV-1RT.

Table 4.1 Tabular Representation Of Compounds With Their 2D Structures, Binding Affinity And Interacting Residues

Compound no.	**PubChem CID**	**2d chemical structure**	**Binding Affinity (kcal/mol)**	**Interacting Residues**
Reference	Zidovudine 35370		-6.5	PHE:162 ILE:160 ALA:97 MET:139 CYS:14 ALA:140 GLN:446
Compound 1.	3007908		-6.9	ASP:186 TRP:410 THR:377 GLN:373 ALA:408

Compound 2.	50313		-7.0	ASP:186 ALA:408 TRP:410 GLN:373
Compound 3.	135440068		-7.0	THR:409 TRP:398 TRP:414 LYS:395 TRP:402 TRP:410
Compound 4.	5790		-7.1	THR:409 ASP:186 TRP:410 TYR:188 ALA:408 TYR:232 VAL108
Compound 5.	18343		-7.3	THR:377 TYR:232 ALA:408 TRP:410 TYR:188
Compound 6.	159269		-7.4	TYR:232 TRP:410 ASP:186 THR:377 GLN:407

Compound 7.	25147495		-7.6	ASP:186 ASP:110 TYR:188 THR:377 ALA:408 TYR:232 GLN:407
Compound 8.	33039		-7.7	ILE:94 TRP:88 GLY:384
Compound 9.	11065133		-7.7	ILE:94 TRP:88 GLY:384
Compound10.	73115		-7.7	THR:377 GLN:407 TYR:232 ALA:408 TYR:188 THR:409
Compound 11.	54929		-7.7	PRO:321 ILE:94 LYS:154

Out of all the 11 compounds ligands, compound 8,9,10,11 with their PubChem CID:33039, CID:11065133, CID:73115 , CID:54929 respectively, show highest binding affinity of -7.7 kcal/mol. While the original FDA approved drug Zidovudine has binding affinity of -6.5 only.

4.2 Structure of Studied Compounds

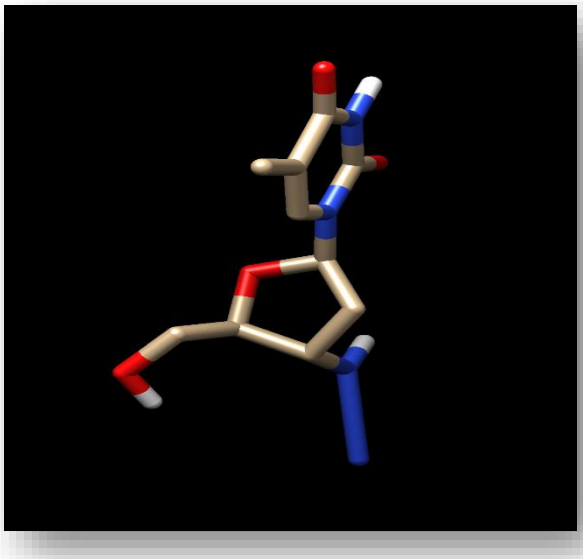


Fig 4.1 3d structure of Zidovudine

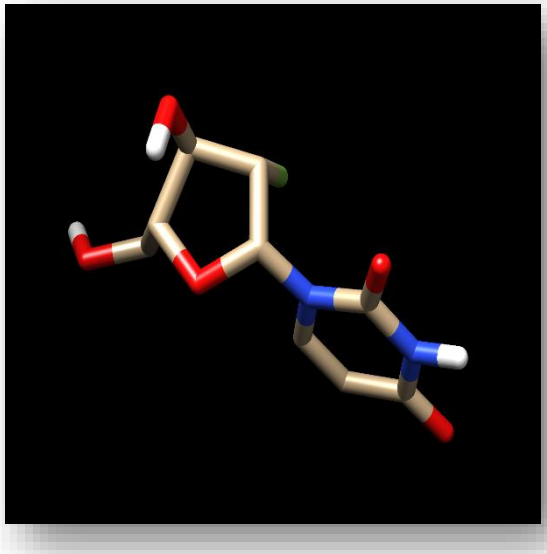


Fig 4.2 3d structure of Compound 1

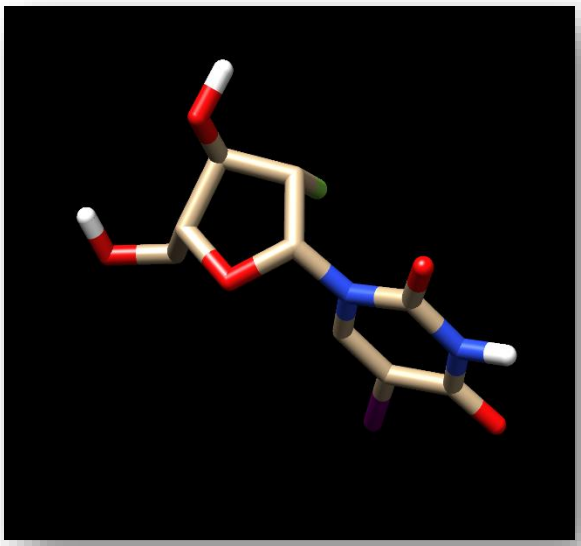


Fig 4.3 3d structure of Compound 2

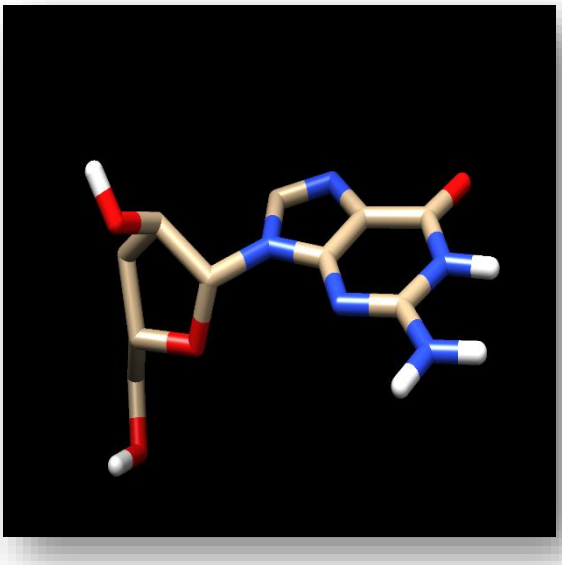


Fig 4.4 3d structure of Compound 3

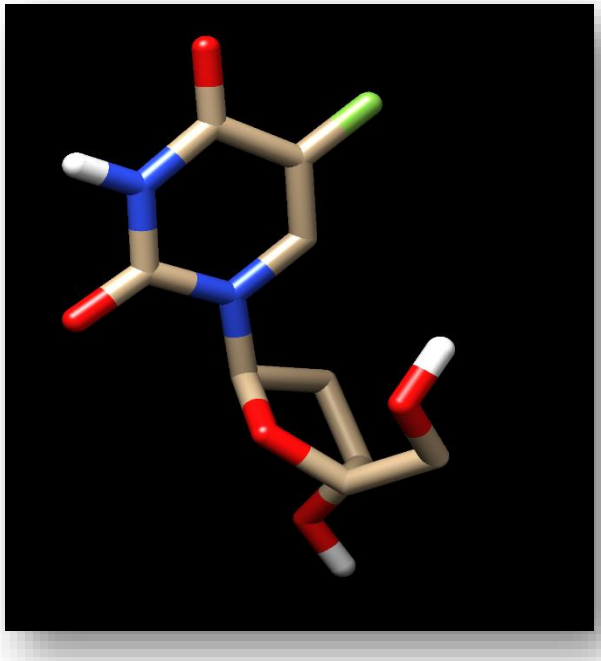


Fig 4.5 3d structure of Compound 4

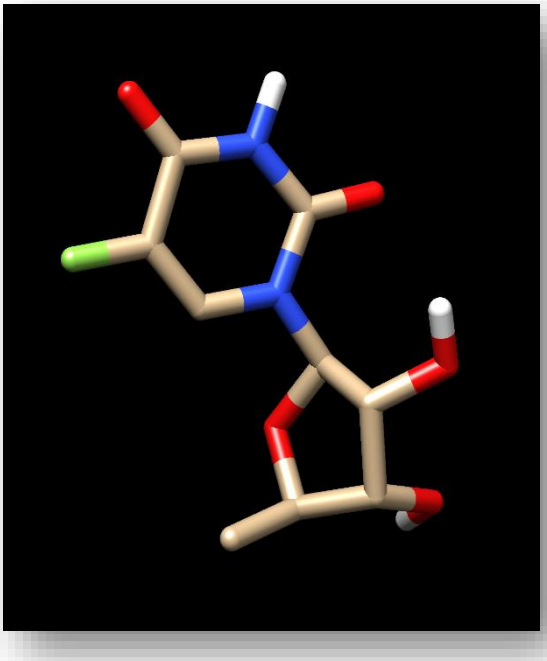


Fig 4.6 3d structure of Compound 5

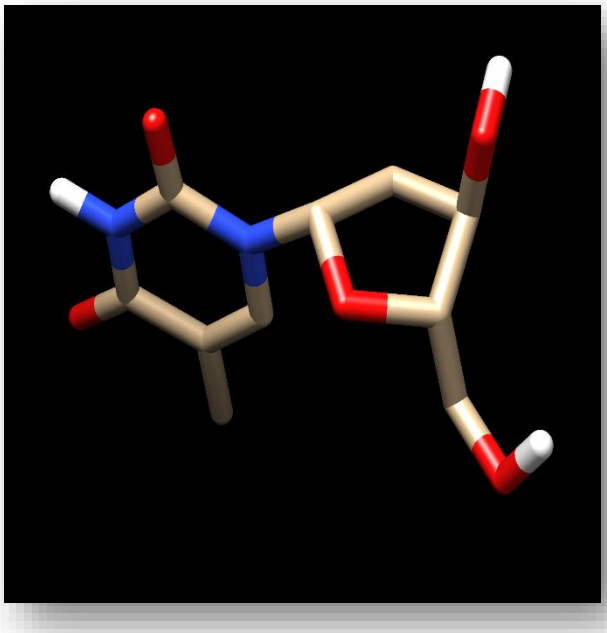


Fig 4.7 3d structure of Compound 6

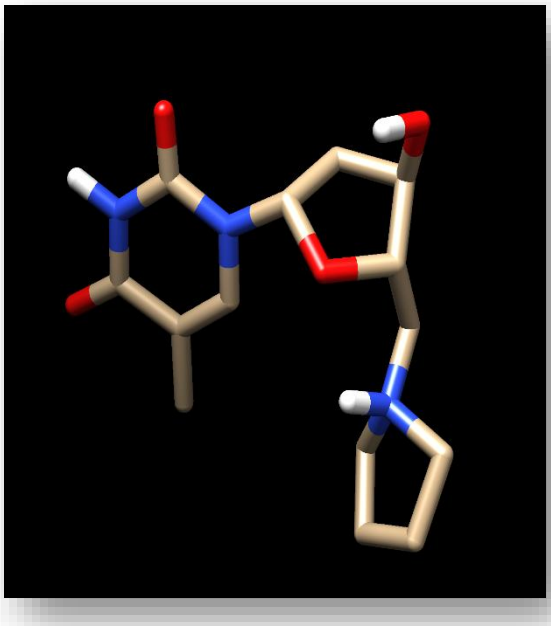


fig 4.8 3d structure of Compound 7

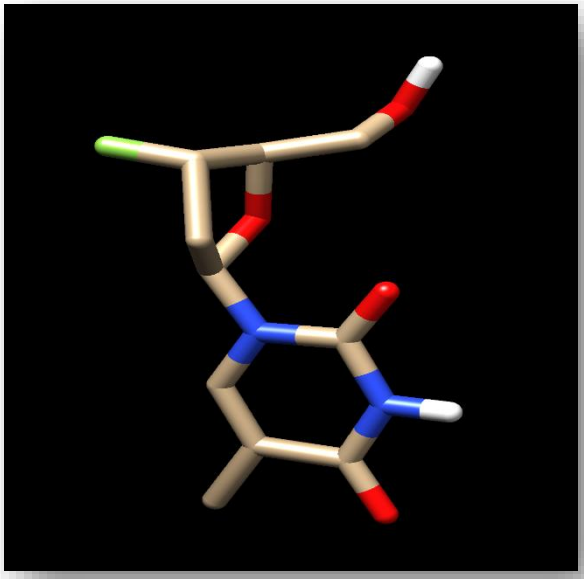


Fig 4.9 3d structure of Compound 8

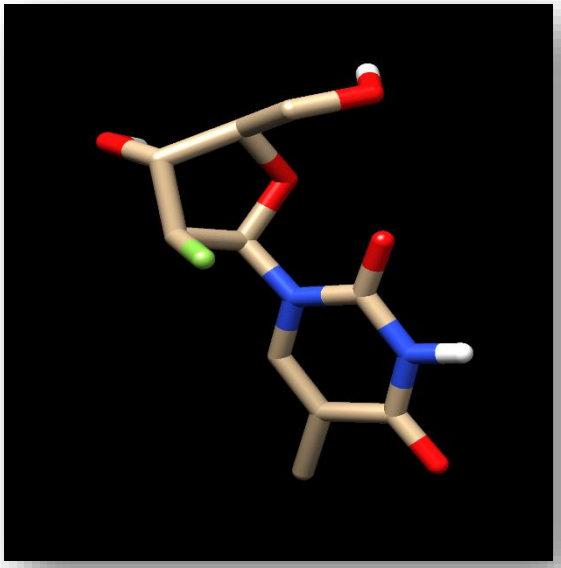


Fig4.103d structure of Compound 9

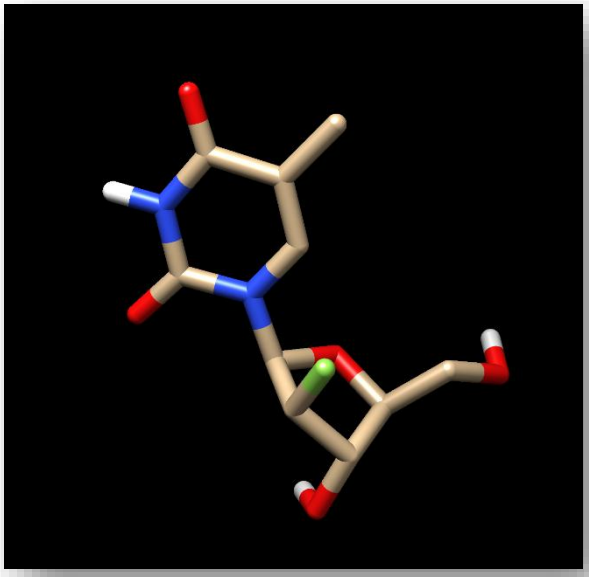


Fig 4.11 3d structure of Compound 10

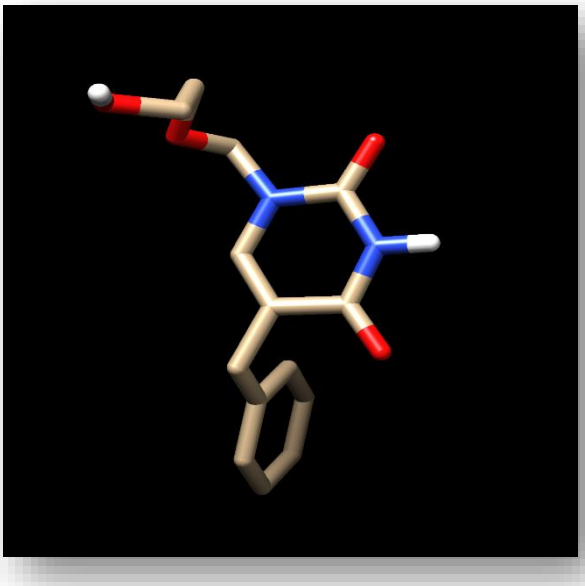


Fig 4.12 3d structure of Compound 11

4.3 Background of the Compounds selected

The compound's background was checked on drugbank website (https://go.drugbank.com/) while searching them on screening them on Swiss Similarity website and the following observation was observed. (Table 4.2).

Table 4.2 Compound's Background Analaysis

Compound no.	**PubChem CID**	**Compound's Background**
Reference	Zidovudine 35370	FDA approved Drug for AIDS
Compound 1.	3007908	In clinical Trials for Small Intestine Lymphoma
Compound 2.	50313	In clinical Trials Under Biodistribution and Dosimetry Evaluation (NCT01337466)
Compound 3.	135440068	Unknown
Compound 4.	5790	FDA approved drug for treatment of Digestive Tract Cancer
Compound 5.	18343	Under investigation for treatment of Stomach cancer
Compound 6.	159269	FDA approved drug for treatment of Hepatitis- B
Compound 7.	25147495	Unknown
Compound 8.	33039	Unknown
Compound 9.	11065133	In clinical trials for Prostate Cancer (NCT02809690)

Compound10.	73115	Under investigation for treatment of Hepatitis- B
Compound 11.	54929	Unknown

Compound 1, 4 ,5 ,and 9 are already under clinical trials for treatment of various cancers and PubChem CID of these compounds are CID:3007908 , CID:5790 , CID:18343 , CID:11065133 respectively. Also Compound 6 is already an FDA approved drug used for treatment of Hepatitis-B CID:159269.

4.4 Docking and 2d interaction of different Compounds with HIV-1 RT

The binding Affinity of Zidovudine was about -6.5 kcal/mol where whereas the binding affinity of the 11 compounds/ligands ranged from -6.9 to -7.7 kcal/mol.

BIOVIA Discovery helped to visualize the possible interaction between the targeted protein and all 11 ligands. It also helped in residue observation

4.4.1 Docking result and 2d interaction of Zidovudine with HIV-1RT

Docking between Zidovudine and HIV-1RT shows binding affinity = -6.5 Kcal/mol and RMSD value = 0

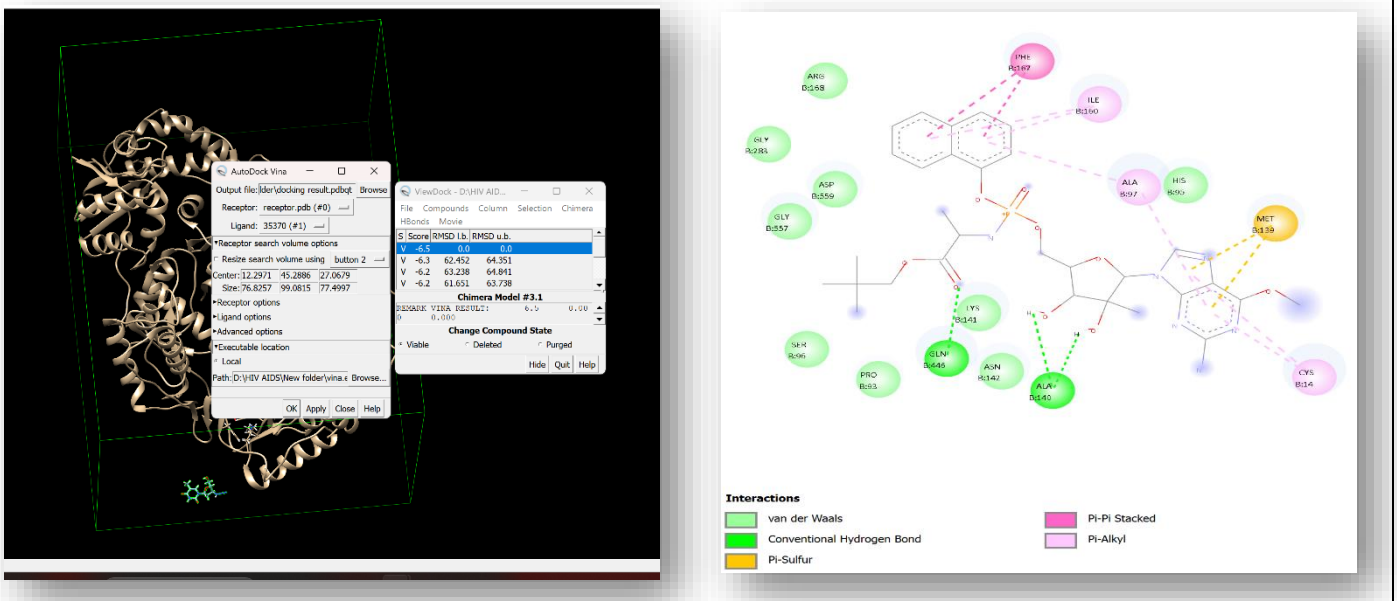


Fig 4.13 Screenshot showing Docking score and 2D interaction between Zidovudine and HIV-1RT

4.4.2 Docking result and 2d interaction of Compound 1 with HIV-1RT

Docking between compound 1and HIV-1RT shows binding affinity = -6.9 Kcal/mol and RMSD value = 0.

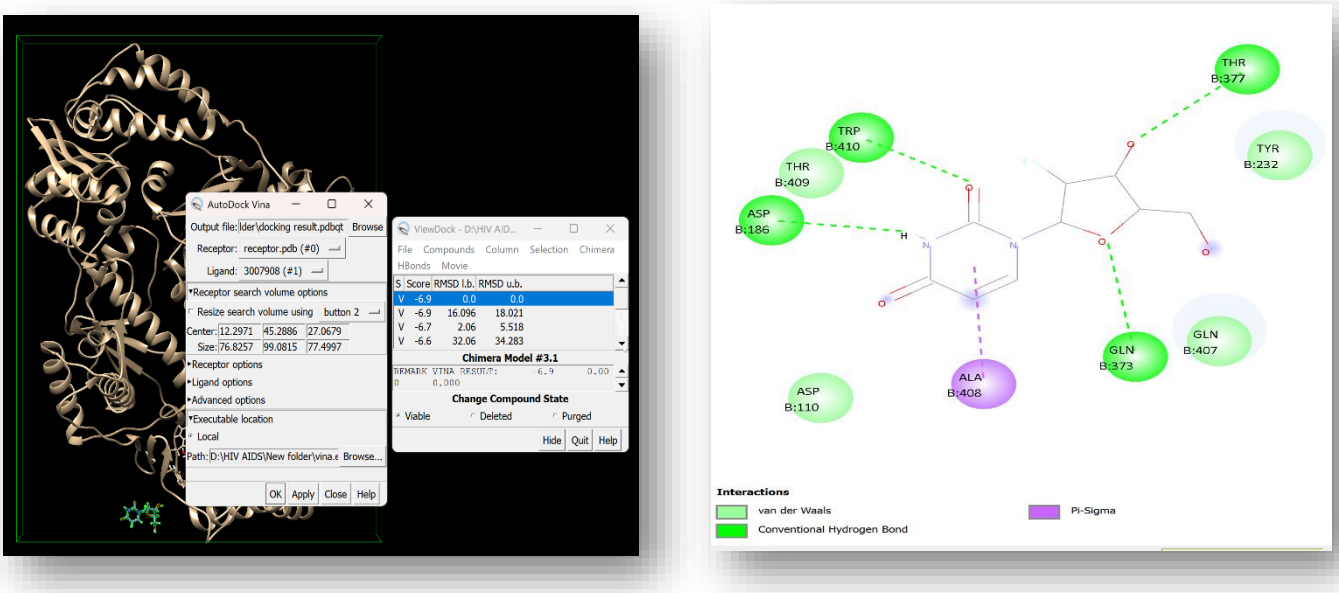


Fig 4.14 Screenshot showing Docking score & 2D interaction between Compound 1 & HIV-1RT

4.4.3 Docking result and 2d interaction of Compound 2 with HIV-1RT

Docking between compound 2 and HIV-1RT shows binding affinity = -7.0 Kcal/mol and RMSD value = 0.

Fig 4.14 Screenshot showing Docking score & 2D interaction between Compound 2 & HIV-1RT

4.4.4 Docking result and 2d interaction of Compound 3 with HIV-1RT

Docking between compound 3 and HIV-1RT shows binding affinity = -7.0 Kcal/mol and RMSD value = 0.

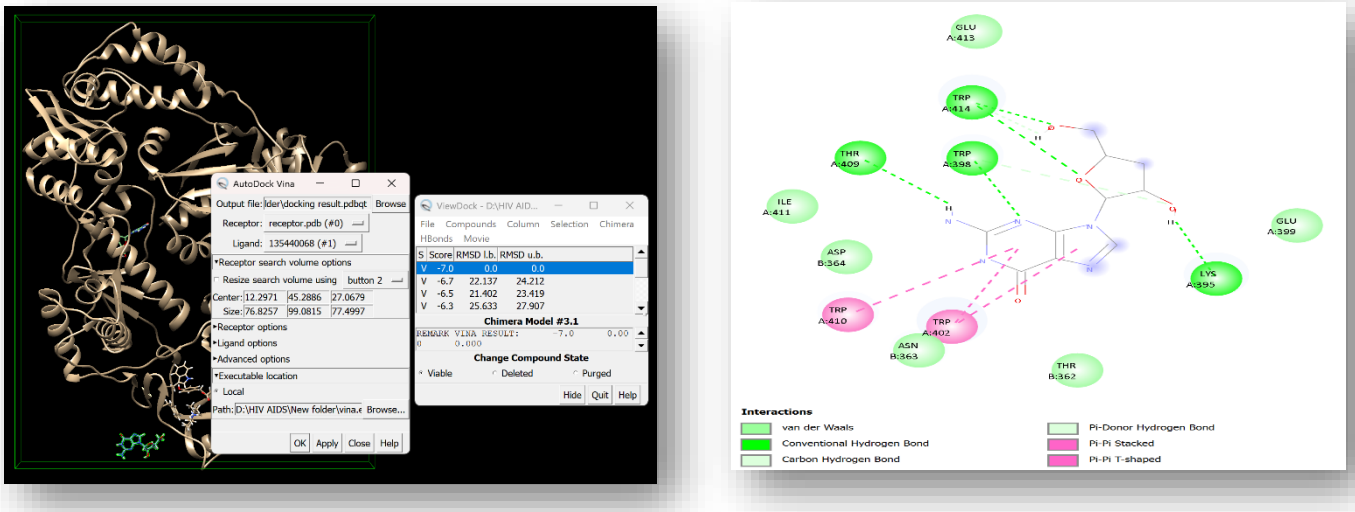


Fig 4.15 Screenshot showing Docking score & 2D interaction between Compound 3 & HIV-1RT

4.4.5 Docking result and 2d interaction of Compound 4 with HIV-1RT

Docking between compound 4 and HIV-1RT shows binding affinity = -7.1 Kcal/mol and RMSD value = 0

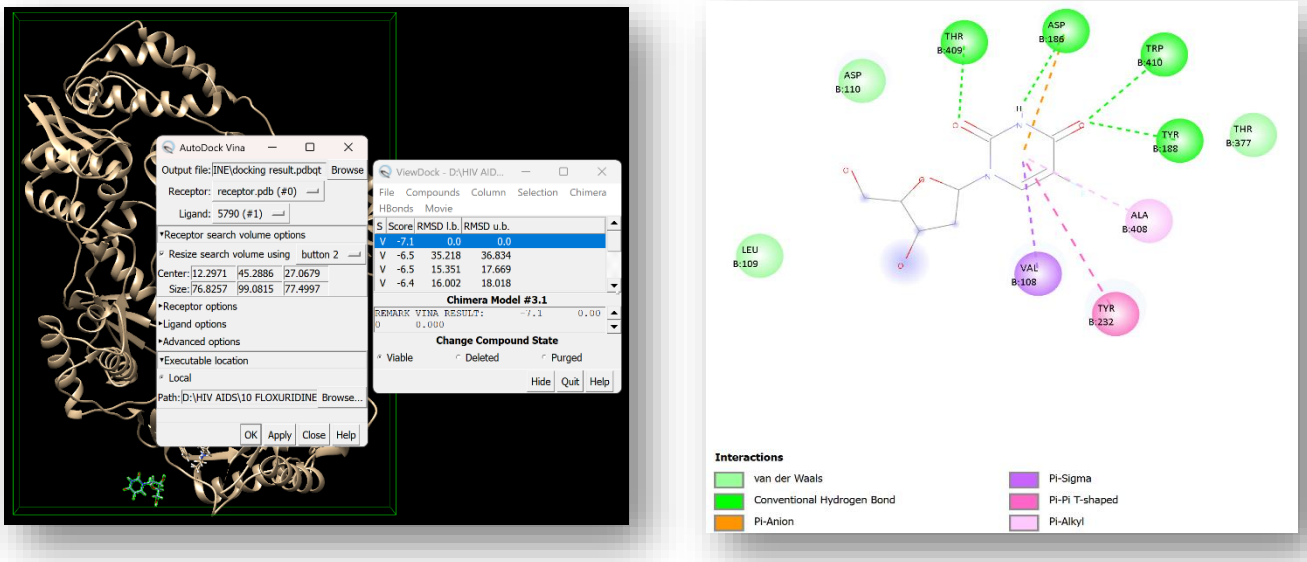


Fig 4.16 Screenshot showing Docking score & 2D interaction between Compound 4 & HIV-1RT

4.4.6 Docking result and 2d interaction of Compound 5 with HIV-1RT

Docking between compound 5 and HIV-1RT shows binding affinity = -7.3 Kcal/mol and RMSD value = 0

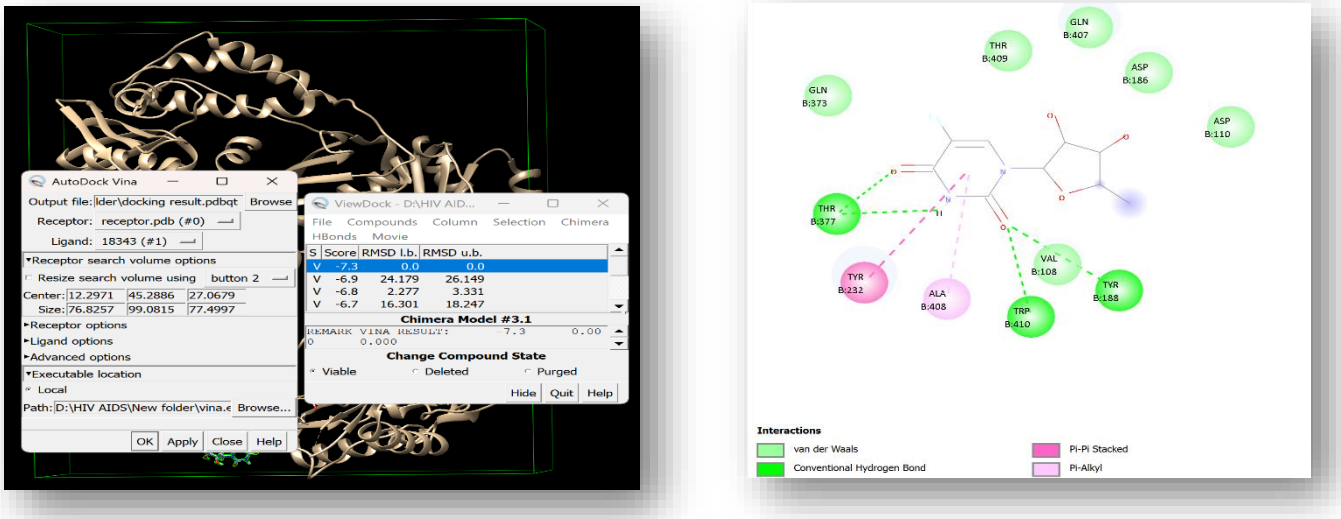


Fig 4.17 Screenshot showing Docking score & 2D interaction between Compound 5 & HIV-1RT

4.4.7 Docking result and 2d interaction of Compound 6 with HIV-1RT

Docking between compound 6 and HIV-1RT shows binding affinity = -7.4 Kcal/mol and RMSD value = 0

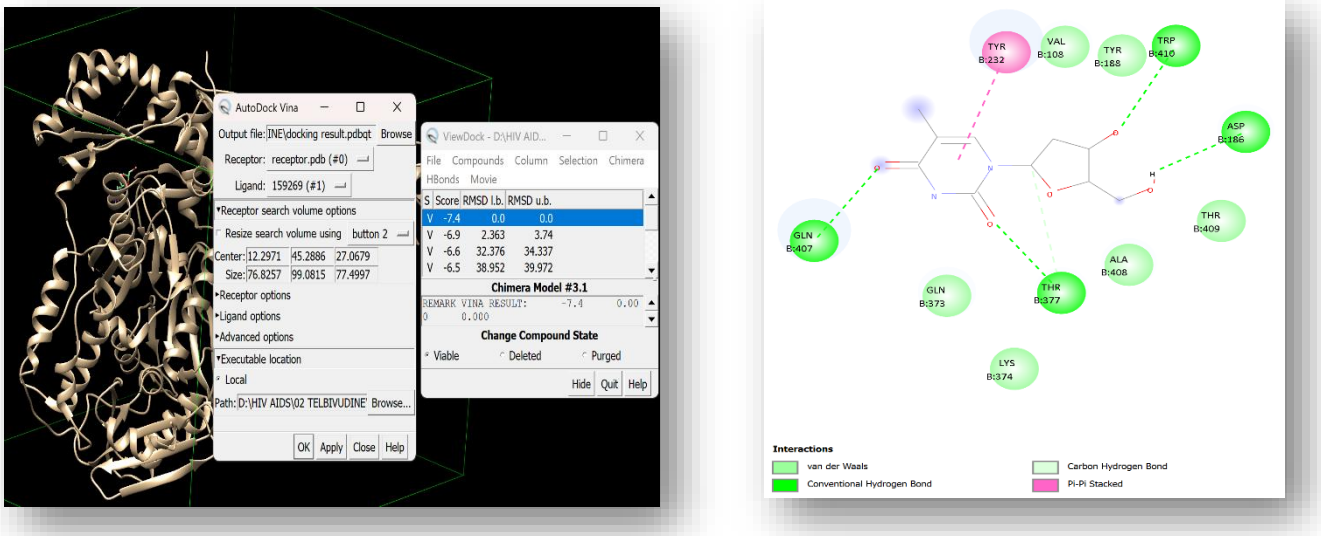


Fig 4.18 Screenshot showing Docking score & 2D interaction between Compound 6 & HIV-1RT

4.4.8 Docking result and 2d interaction of Compound 7 with HIV-1RT

Docking between compound 7 and HIV-1RT shows binding affinity = -7.6 Kcal/mol and RMSD value = 0.

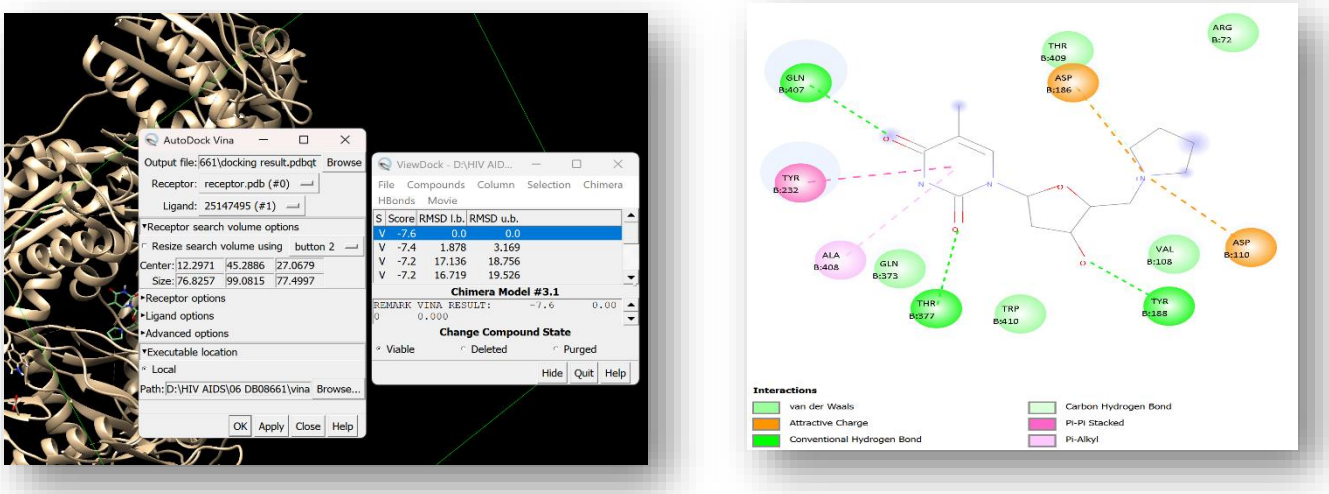


Fig 4.19 Screenshot showing Docking score & 2D interaction between Compound 7 & HIV-1RT

4.4.9 Docking result and 2d interaction of Compound 8 with HIV-1RT

Docking between compound 8 and HIV-1RT shows binding affinity = -7.7 Kcal/mol and RMSD value = 0.

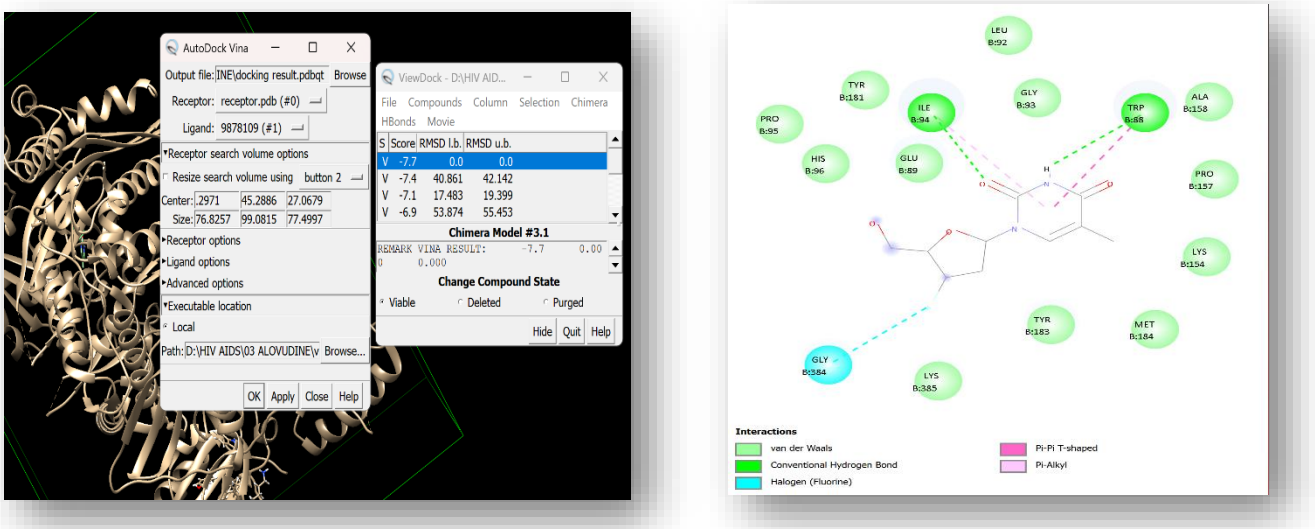


Fig 4.20 Screenshot showing Docking score & 2D interaction between Compound 8 & HIV-1RT

4.4.10 Docking result and 2d interaction of Compound 9 with HIV-1RT

Docking between compound 9 and HIV-1RT shows binding affinity = -7.7 Kcal/mol and RMSD value = 0.

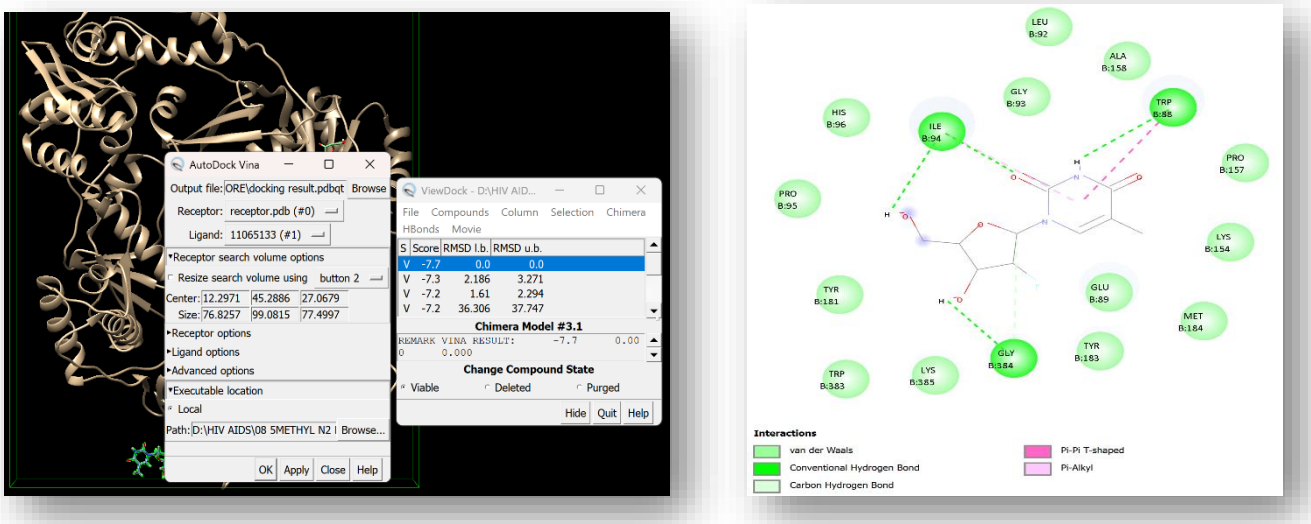


Fig 4.21 Screenshot showing Docking score & 2D interaction between Compound 9 & HIV-1RT

4.4.11 Docking result and 2d interaction of Compound 10 with HIV-1RT

Docking between compound 10 and HIV-1RT shows binding affinity = -7.7 Kcal/mol and RMSD value = 0.

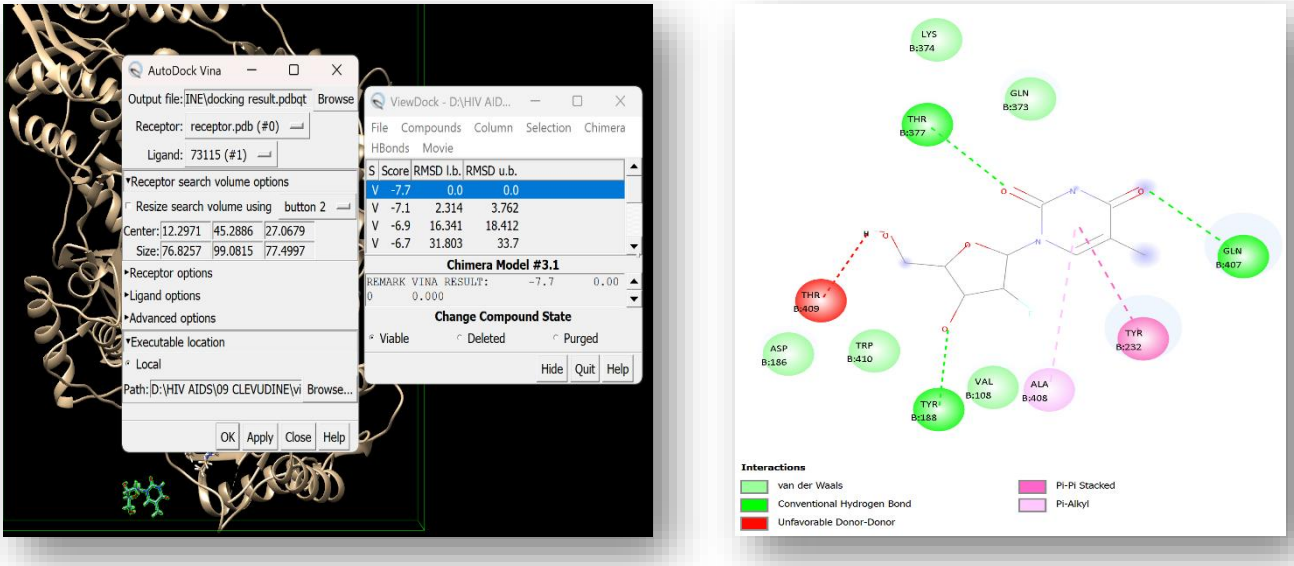


Fig 4.22 Screenshot showing Docking score & 2D interaction between Compound 10 & HIV-1RT

4.4.12 Docking result and 2d interaction of Compound 11 with HIV-1RT

Docking between compound 10 and HIV-1RT shows binding affinity = -7.7 Kcal/mol and RMSD value = 0.

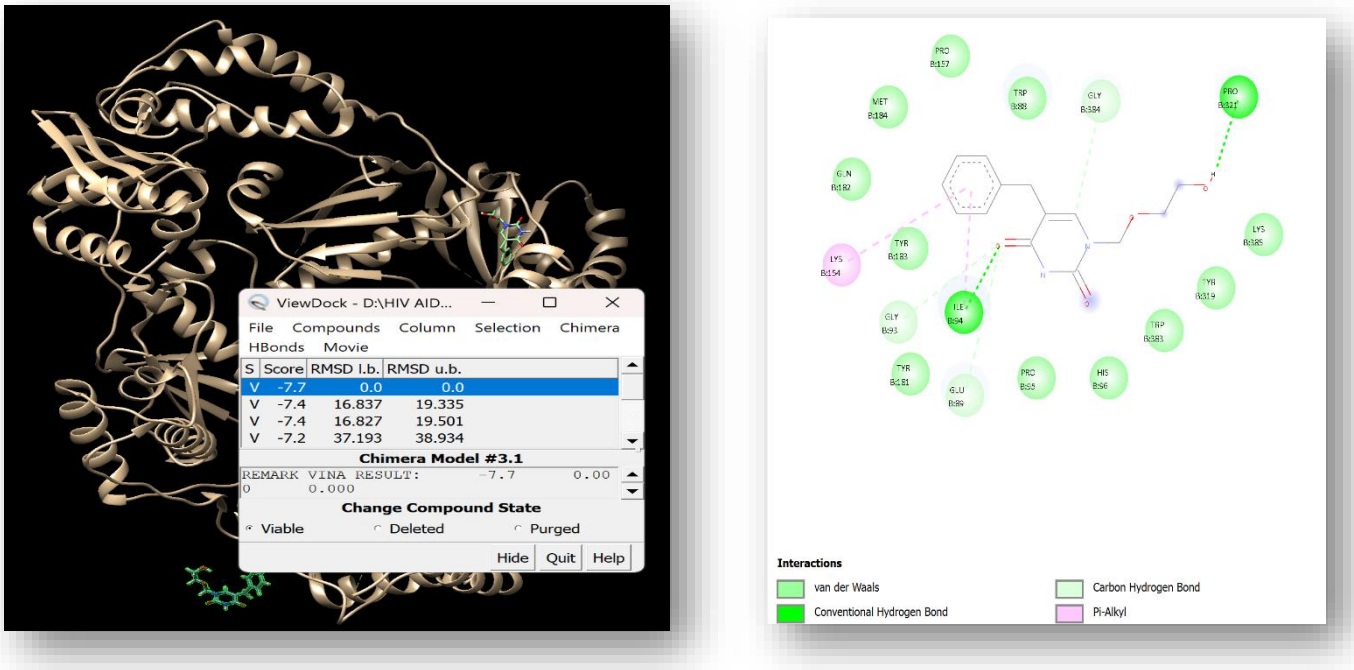


Fig 4.23 Screenshot showing Docking score & 2D interaction between Compound 11 & HIV-1RT

The first figure shows a 2D interaction between the Zidovudine drug and the HIV-1 Reverse Transcriptase Enzyme (Targeted protein). The rest of the diagrammatic representation shows a 2D interaction between the targeted protein and other compounds.

The compounds/ligands selected for screening were 47 by using Swiss Similarity on the basis of Structural Similarity. The molecular docking result shows that out of 47, only 11 compounds/ligands had higher binding affinity as compared to the original FDA-approved drug Zidovudine(reference). The binding Affinity of Zidovudine was about -6.5 kcal/mol where whereas the binding affinity of the 11 compounds/ligands ranged from -6.9 to -7.7 kcal/mol. BIOVIA Discovery helped to visualize the possible interaction between the targeted protein and all 11 ligands. It also helped in residue observation.

Out of all the 11 compounds ligands, compound 8,9,10,11 with their PubChem CID:33039, CID:11065133, CID:73115, CID:54929 respectively, show highest binding affinity of 7.7 kcal/mol.

Compound 1, 4 ,5, and 9 are already under clinical trials for treatment of various cancers and PubChem CID of these compounds are CID:3007908, CID:5790, CID:18343, CID:11065133 respectively. Also Compound 6 is already an FDA approved drug used for treatment of Hepatitis-B CID:159269.

In this study, we used computational approach by using molecular docking technique for the analysis of HIV-1 Reverse Transcriptase inhibitors by targeting its structure by different type of compounds/ligands, aim to provide a better drug or compound then Zidovudine and also for repurposing the already used drug in different type of cancer and various diseases for the treatment of AIDS. This study shows a positive result in finding better replacement of Zidovudine drug by 11 compounds which have a better binding affinity.

DISCUSSION

The global fight against HIV/AIDS has seen remarkable progress since the introduction of antiretroviral therapy (ART), with drugs like zidovudine (AZT) playing a major role. As the first FDA-approved antiretroviral agent, zidovudine functions by inhibiting the HIV-1 reverse transcriptase (RT) enzyme, a critical viral protein that converts viral RNA into DNA during early infection. Despite its historical significance, zidovudine's clinical utility is limited by side effects, drug resistance, and suboptimal binding affinity.

To overcome these challenges, a computational drug discovery strategy is used to identify novel compounds with enhanced binding properties, support the promising concept of drug repurposing to accelerate HIV treatment development.

HIV-1 reverse transcriptase is a prime therapeutic target due to its absence in human cells. Zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI), acts as a chain-terminating analogue, incorporating itself into viral DNA during

replication. However, its binding affinity (-6.5 kcal/mol in silico studies) leaves room for improvement. Over time, viral mutations reduce zidovudine's efficacy, leading to higher doses that increase side effects like anemia and hepatotoxicity. These limitations lead to the need for next-generation inhibitors with stronger target engagement and safer profiles.

Using molecular docking and binding free energy calculations, we screened a library of FDA-approved and clinically tested compounds to identify potential HIV-1 RT inhibitors. Among 11 shortlisted candidates, four compounds demonstrated exceptional binding affinities surpassing zidovudine:

1. **Compound 8 (PubChem CID: 33039)** : -7.7 kcal/mol
2. **Compound 9 (PubChem CID:11065133)**: -7.7 kcal/mol
3. **Compound 10 (PubChem CID: 73115)**: -7.7 kcal/mol
4. **Compound 11 (PubChem CID: 54929)**: -7.7 kcal/mol

These compounds exhibited stronger interactions with key residues in the RT active site, including hydrogen bonds with Asp110, Asp185, and Lys223, and hydrophobic contacts with Tyr181 and Trp229. Notably, their binding energies (-

7.7 kcal/mol) suggest a 50–100x improvement in affinity compared to zidovudine, based on the correlation between ΔG (binding energy) and inhibition constants.

Compound 1, 4 ,5, and 9 are already under clinical trials for treatment of various cancers and PubChem CID of these compounds are CID:3007908 , CID:5790, CID:18343, CID:11065133 respectively. Also Compound 6 is already an FDA approved drug used for treatment of Hepatitis-B CID:159269.

Maximum compounded screened in the study are currently FDA-approved drugs or under clinical trials for various cancers and other diseases. This also indicates that Drug Repurposing can be achieved for the treatment of HIV AIDS.

This background analysis of these compounds suggest that these compounds can work in dual action in patients suffering from AIDS and cancer which started due to prolong AIDS.

For the treatment of HIV AIDS. However, HIV-1 Reverse Transcriptase Inhibitors may behave differently inside the body and may interact with different components in the body. Hence, we recommend validating these in silico results through in vivo experimentation.

CONCLUSION AND FUTURE PERSPECTIVE

Zidovudine an FDA Approved drug is available in market for the treatment of AIDS but still due to number of issues with their therapeutic limitations to work with different variants of HIV-1 and adverse reactions on the patient body there is a need to look for more promising alternatives to Zidovudine with better action and less side effects. This is the reason we used computational approach to screen more substances similar in structure to Zidovudine and look for their binding affinity with the HIV-1RT [33].

Future aspect of this paper is not only finding best possible alternatives of Zidovudine but also the dual action of these compounds in the patients of HIV . Patients with prolong HIV are more prono develop cancer. Most of the Compounds screened in this study are already in clinical trials for various cancers and other diseases. Compound 1, 4 ,5 ,and 9 are already under clinical trials for treatment of various cancers and PubChem CID of these compounds are CID:3007908 ,CID:5790 , CID:18343 , CID:11065133 respectively. So, our study pave the way for dual action for treatment of HIV as well as for the treatment of cancer developed due to prolong HIV.

Maximum compounded screened in the study are currently FDA-approved drugs or under clinical trials for various cancers and other diseases. This also indicates that Drug Repurposing can be achieved for the treatment of HIV AIDS [34]. However, HIV-1 Reverse Transcriptase Inhibitors may behave differently inside the body and may interact with different components in the body. Hence, we recommend validating these in silico results through in vivo experimentation.

Testing in Real-World Scenarios

Next steps should prioritize lab and animal testing of the top compounds (8–11) identified through computer modeling. Scientists need to:

- Confirm how tightly these drugs bind to HIV's reverse transcriptase enzyme
- Study how the body absorbs, distributes, and eliminates these compounds
- Check for safety issues like organ toxicity
- Test effectiveness against HIV in living systems before human trials

From Computer to Clinic

To turn these digital discoveries into actual treatments, researchers must:

- Verify results in biological systems (cells, animals)
- Develop stable drug formulations (pills, injections)
- Conduct phased human trials to assess safety, dosing, and antiviral effects
- Compare performance against existing HIV medications like zidovudine

Customized Treatment Strategies

With HIV's ability to mutate rapidly, future studies could explore:

- Tailoring drugs to individual viral strains or patient genetics
- Identifying biomarkers that predict treatment response
- Designing combination therapies to prevent drug resistance
- Adapting cancer drug delivery methods (like nanoparticle carriers) for HIV

By focusing on these areas, scientists can bridge the gap between computer predictions and practical HIV therapies, potentially creating safer, more effective alternatives to current treatments [35].

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PLAGIARISM& AI VERIFICATION

Title of the Thesis **"In silico Discovery of New Inhibitors Targeting HIV-1 Reverse Transcriptase: Exploring Novel Approaches for Antiretroviral Therapy"** Total Pages **53**, Name of the Scholar **Nishant Singh(23/mscbio/82)**

Supervisor

Prof. Yasha Hasija

Department of Biotechnology

This is to report that the above thesis was scanned for similarity detection. Process and outcome is given below:

AI % Detected- **0%**

Software used: **turnitin,** Similarity Index: **8%**Total Word Count:**9580**

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turnitin Page 2 of 59 - Integrity Overview Submission ID trn:oid:::27535:98965315

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*% detected as AI

AI detection includes the possibility of false positives. Although some text in this submission is likely AI generated, scores below the 20% threshold are not surfaced because they have a higher likelihood of false positives.

Caution: Review required.

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

Disclaimer

Our AI writing assessment is designed to help educators identify text that might be prepared by a generative AI tool. Our AI writing assessment may not always be accurate (it may misidentify writing that is likely AI generated as AI generated and AI paraphrased or likely AI generated and AI paraphrased writing as only AI generated) so it should not be used as the sole basis for adverse actions against a student. It takes further scrutiny and human judgment in conjunction with an organization's application of its specific academic policies to determine whether any academic misconduct has occurred.

Frequently Asked Questions

How should I interpret Turnitin's AI writing percentage and false positives?

The percentage shown in the AI writing report is the amount of qualifying text within the submission that Turnitin's AI writing detection model determines was either likely AI-generated text from a large-language model or likely AI-generated text that was likely revised using an AI-paraphrase tool or word spinner.

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The AI writing percentage should not be the sole basis to determine whether misconduct has occurred. The reviewer/instructor should use the percentage as a means to start a formative conversation with their student and/or use it to examine the submitted assignment in accordance with their school's policies.

What does 'qualifying text' mean?

Our model only processes qualifying text in the form of long-form writing. Long-form writing means individual sentences contained in paragraphs that make up a longer piece of written work, such as an essay, a dissertation, or an article, etc. Qualifying text that has been determined to be likely AI-generated will be highlighted in cyan in the submission, and likely AI-generated and then likely AI-paraphrased will be highlighted purple.

Non-qualifying text, such as bullet points, annotated bibliographies, etc., will not be processed and can create disparity between the submission highlights and the percentage shown.

CURRICULUM VITAE

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CURRENT STATUS

- *Pursuing MSC. BIOTECHNOLOGY* *(Delhi Technological University,New delhi)*

ACADEMIC BACKGROUND

- **HANSRAJ COLLEGE , UNIVERSITY OF DELHI** (2020-2023)

  BSC. LIFE SCIENCES 8.15 Grand CGPA(First Division)

- **SECONDARY & SENIOR SECONDARY(CBSE)**

SECONDARY (X)	84.4%	2018	Joseph & Mary Public School
SENIOR SECONDARY (XII)PCB	88.2%	2020	Joseph & Mary Public School

EXPERIENCE

- MOLECULAR BIOLOGY & BIOCHEMISTRY WORKSHOP(INFORMATION ABOUT DIFFERENT COMPUTATIONAL TOOLS USED IN BIOLOGY) *(Delhi Technological University,New delhi)*
- FORENSIC SCIENCES WORKSHOP *(Kirorimal College ,University of Delhi)*
- ATTENDED SEMINAR ON "REGULATION OF LONGEVITY BY INTERSPECIES INTERACTION WITH C.ELEGANS AS MODEL ORGANISMS" *(Hansraj College, University of Delhi)*
- ATTENDED SEMINAR ON "PREVAILING ENVIRONMENTAL ISSUE CONCERNING ANDAMAN & NICOBAR *(Hansraj College, University of Delhi)*

RELEVANT SKILLS

- Knowledge of Molecular biology Laboratory Techniques
- Hands on experience on computational biology tools
- Analytical skills & Critical thinking

ACHIEVEMENTS

- Former prefect in Joseph & Mary public school
- Member of Vision : The Media And Publication society,Hrc
- Participated & Passed E-quiz on Biodiversity - Holy cross college ,Nagercoli.
- Former Member of School Publication Society

ADDITIONAL INFORMATION

- Part time Guitarist & lyricist , Poet & interest in singing.
- Languages: Proficiency in English , Hindi & Little proficiency in German.