Review of Non-Nucleoside Reverse Transcriptase Inhibitors' Structure and Activity with HIV-1 Reverse Transcriptase

Anvi Surapaneni*[1], Atri Surapaneni[1], Ananya Vittaladevuni[2], Rohan Adwankar[3]

[1]The Quarry Lane School, 6363 Tassajara Rd, Pleasanton, CA 94588; [2]Irvington High School 41800 Blacow Rd, Fremont, CA 94538; [3]Dougherty Valley High School 10550 Albion Road, San Ramon CA 94582

HIV-1 Reverse transcriptase, Non-nucleoside reverse transcriptase inhibitors

ABSTRACT: The human immunodeficiency virus (HIV) weakens the body's immune system leading to the onset of acquired immunodeficiency syndrome (AIDS). Once the progression of HIV has led to AIDS, certain infections can be severely life-threatening when they normally are not. For years, researchers have been attempting to develop a complete cure for HIV, but current treatment includes various drugs to limit the spread of the disease. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are one class of antiretroviral drugs applied to treat HIV. HIV-1, being a retrovirus, requires an enzyme, reverse transcriptase (RT), to replicate and spread throughout its host. NNRTIs act as non-competitive inhibitors, binding allosterically to RT, and prevent the virus from replicating further due to the conformational change of the enzyme induced by the inhibitor. Due to the more rigid "butterfly-like" structures of first-generation NNRTIs, they are less active against frequent mutations developed in drug-resistant strains of HIV-1. Second-generation NNRTIs, consisting of larger aromatic systems, are more "flexible" with further contacts with main-chain residues or other residues in the NNRTI binding pocket, so they are more successful. NNRTIs have ranged from large systems to small chains, and the interactions and design intentions are important to understand for the creation of stronger inhibitors. The purpose of this article is to explore the structure and function of first, second, and new generations of NNRTIs against RT.

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Introduction:

The HIV virus affects people across the globe, including in 3rd world countries where it is difficult for people to access treatment options. Therefore, the pursuit of treatment options for this retrovirus proves significant. It is estimated that 37.9 million people have HIV as of 2019.[1]

Without treatment, the virus spreads and weakens the immune system of the host because it infects and damages CD4+ T cells, which are part of the immune system. CD4+ are cells that regulate and suppress the immune system response. HIV can replicate billions of times a day, which quickly impairs an individual's immune system and leads to acquired immune deficiency syndrome (AIDS). People infected with HIV/AIDS can have opportunistic illnesses (OIs) and cancers that would not grow in an uninfected person. These illnesses are known as AIDS-defining illnesses because they can only infect the host if the host's immune system is severely weakened.[2] Some AIDS-defining illnesses and cancers can even be fatal and are the main cause of death for HIV-infected patients.[3]

Treatment for HIV generally includes a regimen with multiple and different types of drugs to attack the virus at different stages in its life cycle, known as highly active antiretroviral therapy (HAART). Non-nucleoside reverse transcriptase inhibitors specifically block the function of the reverse transcriptase (RT) enzyme of HIV-1. This enzyme converts HIV's viral RNA into DNA, which then allows the virus to infect the host's DNA and then spread through the body. RT is a key enzyme for HIV replication, so it is a common target for anti-HIV drugs. NNRTIs inhibit RT by binding to an allosteric site on the enzyme, called the NNRTI "binding pocket" or (NNRTI-BP). This then causes a conformational change in the shape of the enzyme, which prevents it from carrying out its role in the transcription of viral RNA to DNA. NNRTIs have a wide range of structures with unique binding modes because the NNRTI-BP is in a closed conformation. Only once an NNRTI is bound to the RT enzyme does the binding "pocket" exist, so each ligand forms slightly different interactions with the amino acid residues.

Among the FDA-approved NNRTIs, there are several generations of NNRTIs from different drug classes. (All NNRTIs referenced as part of the first or second generation include only FDA-approved NNNRTIs). The first-generation NNRTIs are nevirapine, efavirenz, and delavirdine, which are characterized by their "butterfly-like" conformation when bound to RT. This first-generation of NNRTIs are effective against wild type (WT) HIV-1, but their potency dramatically decreases

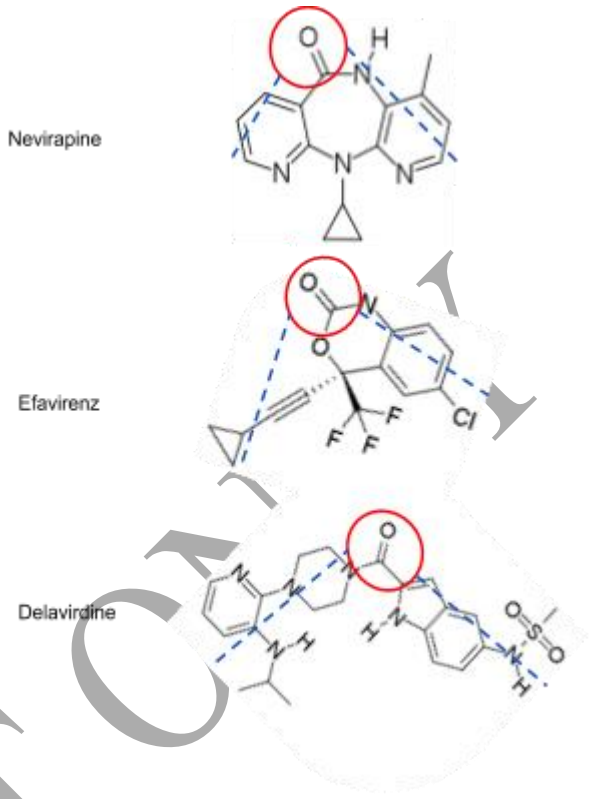
when used with mutant HIV-1 RTs. The rapid emergence of mutations in HIV-1 RT is a problem in NNRTI development because these mutations confer resistance to current drugs. Second generation NNRTIs are far more potent against resistant strains of HIV-1 RT because greater contacts are formed with residues that are less susceptible to becoming mutated side-chains. Further, the smaller size and torsional flexibility of second-generation NNRTIs contribute to their greater ability to adapt to mutations in the binding pocket. These include rilpivirine and delavirdine. Then, investigational drugs, part of the next generation of NNRTIs, have structural features that interact well with the NNRTI binding pocket, which could be used to develop better analogs. Still, the great number of resistant HIV-1 RTs requires stronger, more potent NNRTIs for treatment, and other anti-HIV compounds that target RT are also used in conjunction with NNRTIs for more effective treatment.

The Middle:

First Generation NNRTIs:

Each NNRTI creates interactions with different amino acids due to their differences in structure and molecular makeup. A key feature of first-generation NNRTIs is their "butterfly-like" conformation when bound to RT(figure 1); compounds possessing this structure have been shown to be particularly effective for inhibition of HIV-1 RT. This butterfly-like geometry when bound is supported by the structure of the NNRTI, which usually has a hydrophilic center and two hydrophobic moieties, which resemble the "body" and "wings", respectively. One of the wings is a heteroaromatic ring and the other is usually a phenyl or allyl substitute.[4] NNRTIs sterically inhibit the rigid motion between the thumb and palm subregions of the p66 subunit of the RT enzyme. HIV-1 easily mutates to evade first-generation NNRTIs, especially when taken individually. Cross-resistance, which occurs when a virus is immune to the effect of one molecule because of exposure to a similar molecule in the past, is also a common reason for treatment failure and is part of the reason why HIV can evade many first-generation NNRTIs through one mutation.[5, 6, 7]

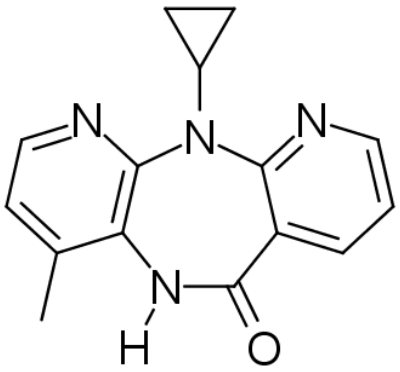
Figure 1. "Butterfly-like" geometry of first-generation NNRTIs



Nevirapine (NVP)

Nevirapine (figure 2) was the first NNRTI approved for HIV treatment. It has a very visible butterfly conformation, seen in its dipyridodiazepino structure. Its methyl group and cyclopropyl group aid in creating hydrophobic contacts.

Figure 2. Chemical structure and 3d modeled structure of nevirapine and binding interactions of nevirapine in NNRTI-BP (PDB: 4PUO)



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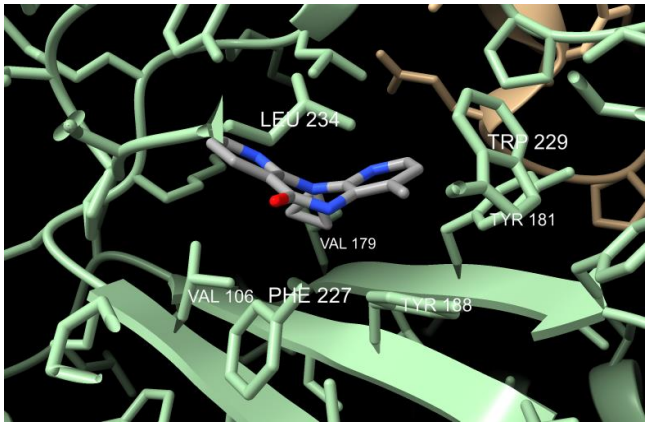
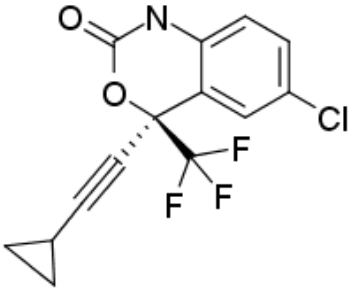


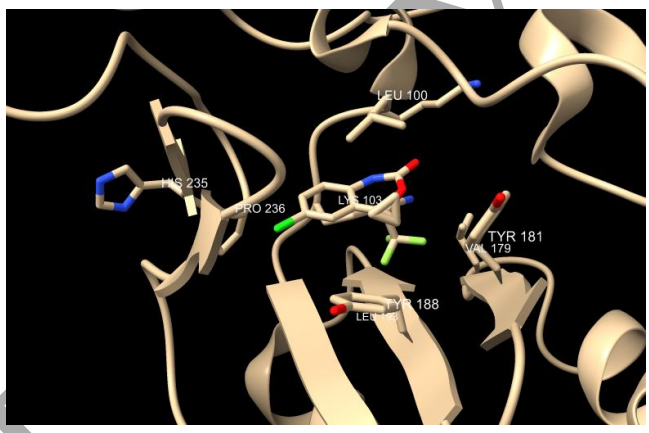
The heteroaromatic ring has a functional group that can be both an electron donor and acceptor, which interacts with the main chain K101 and K103. The binding of nevirapine disrupts the reverse transcriptase's DNA polymerization activity by forming a pocket in the enzyme. Pocket formation prevents the ability of reverse transcriptase to add nucleotides and translocate.[8, 9]

Efavirenz (EFV)

Efavirenz is one of the first-generation NNRTIs that serve as the first line of the regiment for HIV-1. Its structure (figure 3) still has a butterfly conformation and its overall structure is a benzoxazine.

Figure 3. Chemical structure and 3d modeled structure of efavirenz and binding interactions of efavirenz in NNRTI-BP (PDB: 1FK9)





In comparison to NRTI therapy, efavirenz has proven to be more efficient. Despite higher efficiency, a single mutation on the reverse transcriptase of the virus is sufficient to confer resistance towards the drug, including the K103N mutation. Aside from mutations inducing this resistance and affecting the efficiency of efavirenz, the drug has also shown side effects affecting the central nervous system.[7, 10]

Delavirdine (DLV)

Delavirdine (figure 4) also resembles a butterfly structure consisting of two main structures, an indole and pyridine nucleus. These two heterocyclic components have been identified as having important anti-HIV activity, and have led to the development of more novel inhibitors.[11] Because its efficacy is relatively weak compared to other inhibitors, it is mostly used in conjunction with other inhibitors in treatment.[11]

Figure 4. Chemical structure and 3d modeled structure of delavirdine and binding interactions of delavirdine in NNRTI-BP (PDB: 1KLM)

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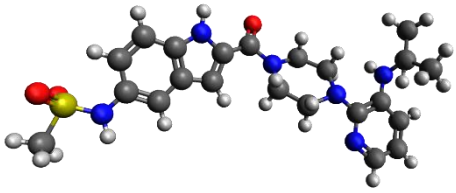
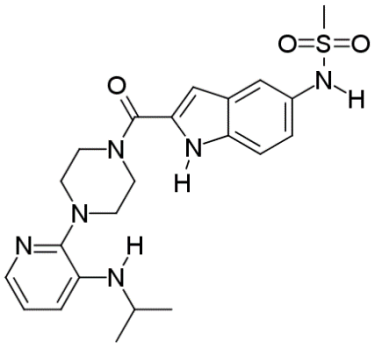
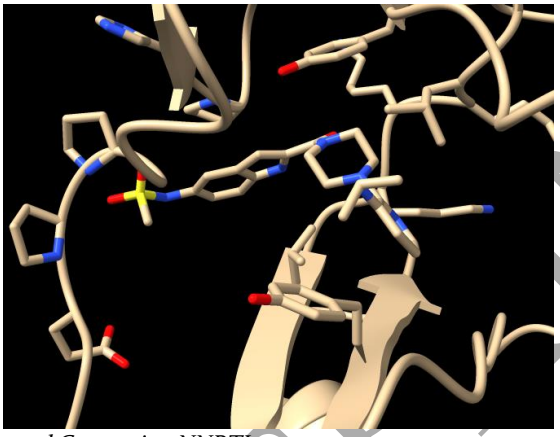






Second Generation NNRTIs:

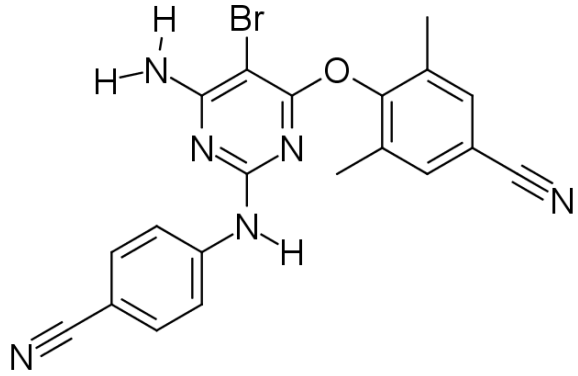
In comparison to first-generation NNRTIs, second-generation NNRTIs have achieved high levels of inhibition even when tested against mutated HIV-1 strains.[12] The structures of second-generation NNRTIs are part of the class of diaryl pyrimidines (DAPY) compounds. Specifically, second-generation compounds have unique binding modes as opposed to the butterfly-like conformation of first-generation NNRTIs.[13] It is important to note that second-generation NNRTIs are still allosteric inhibitors and bind to the same binding pocket as first-generation NNRTIs, although they have different modes of binding.

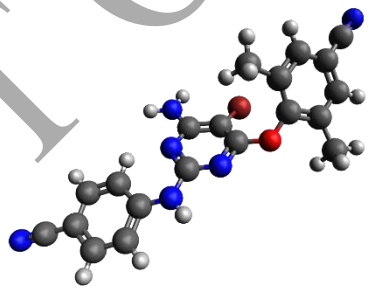
Etravirine (ETV)

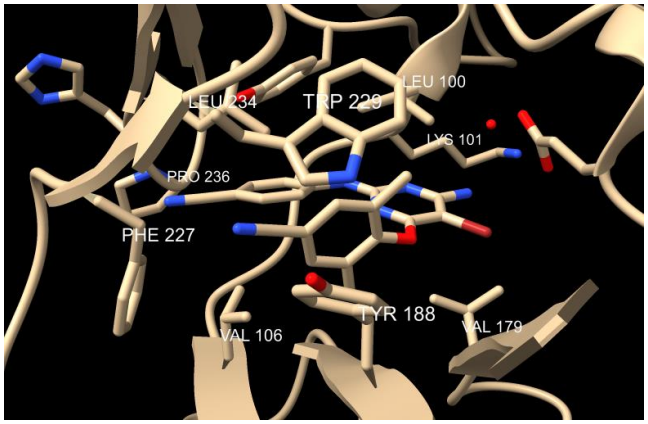
Etravirine (figure 5) provided very strong inhibitory results compared to first-generation NNRTIs. The molecule displayed promising activity not only on HIV-1 RT but also on HIV-2 RT, activity which was not present in the first-generation substrate. It is a DAPY and aromatic ether containing three heterocyclic rings, a central diaminopyrimidine with a substituted bromine, and two aromatic rings with a cyano group.[14]

Etravirine's flexibility allows it to sustain activity even against the mutations that have prevented first-generation NNRTIs from functioning, specifically, L100I and K103N.

Figure 5. Chemical structure and 3d modeled structure of etravirine and binding interactions of etravirine in NNRTI-BP (PDB: 3MEC)







Rilpivirine (RPV)

Rilpivirine (figure 6), initially known as TMC-278, is among the second generation of NNRTIs that have demonstrated greater genetic barriers to resistance conferred by mutations.[15] Key components of its DAPY structures are its aromatic rings, multiple methyl side chains, aniline ring, and cyano groups. Rilpivirine's aniline ring and cyanovinyl group form a flexible dihedral angle, allowing for torsional flexibility in the binding pocket. A more flexible ligand suggests not

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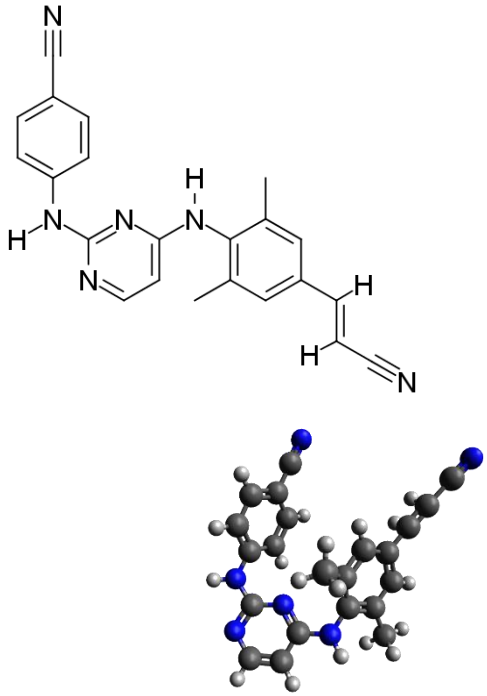
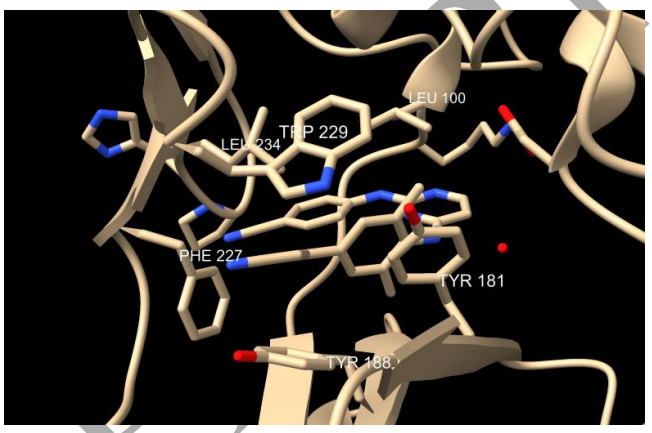
only greater potency, but a greater genetic barrier of resistance from reverse transcriptase, which has been supported by increased activity shown by rilpivirine with mutations L100I, K103N, Y181C, and Y181L.[16]

Figure 6: Chemical structure and 3d modeled structure of rilpivirine and binding interactions of rilpivirine in NNRTI-BP (PDB: 2ZD1)





When analyzing the crystal structure of rilpivirine bound in mutant RT, the cyanovinyl group is observed to have strong interactions with Y183 following the very common Y181C mutation. The various crystal structures of rilpivirine with different mutations on RT show different conformations of the ligand in the binding pocket, but still with strong inhibitory effects. This highlights the importance of torsional flexibility to maintain resistance to mutations.

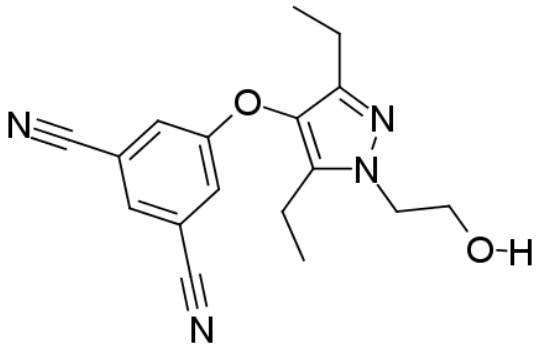
Further Structures:

Although the first two generations of NNRTIs have been relatively successful in inhibiting HIV and decreasing the viral load when multiple drugs are used in treatment, there are several new schemes and structures for further generations of NNRTIs.

Lersivirine

Levirisine (figure 7) has a much more unconventional structure compared to NNRTIs in the first two generations. The structure is centered around a substituted pyrazole ring joined to a six-membered aromatic ring by a central ether. The presence of only one aromatic ring may limit the strength of possible pi stacking interactions between the ligand and the reverse transcriptase enzyme. However, the design of this comparatively less bulky ligand would allow for greater flexibility in the binding pocket. Greater flexibility is important in the inhibition of mutated HIV-1 reverse transcriptase, which is a significant problem. Additionally, ethyl carbon chains attached to the pyrazole allow for hydrophobic interactions in the binding pocket, creating a more favorable binding affinity. Cyano groups on aromatic rings are present in other third-generation NNRTIs as well as both second-generation NNRTIs, etravirine and rilpivirine. The structure is designed to minimize interactions with the portions of the binding pocket that are prone to mutations while maximizing contacts with the amino acids that are not prone to mutations. This design mindset contrasts with other NNRTIs that have a much greater molecular weight and aim for stronger interactions by creating a larger contact area. Further, lersivirine showed inhibitory activity when tested against 14 out of 15 strains of HIV-1 RT that were mutated at a single amino acid in the binding pocket. When lersivirine was tested with NRTI drugs, synergistic interactions were observed. Overall, the molecule shows promise for inhibiting RT and this new pharmacophore of smaller ligands provides new insights for designing NNRTIs.[17]

Figure 7. Chemical structure and 3d modeled structure of lersivirine and binding interactions of lersivirine in NNRTI-BP (PDB: 2WOM)



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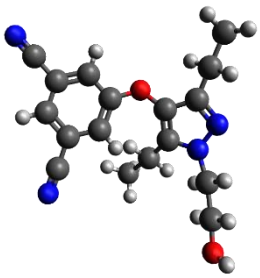
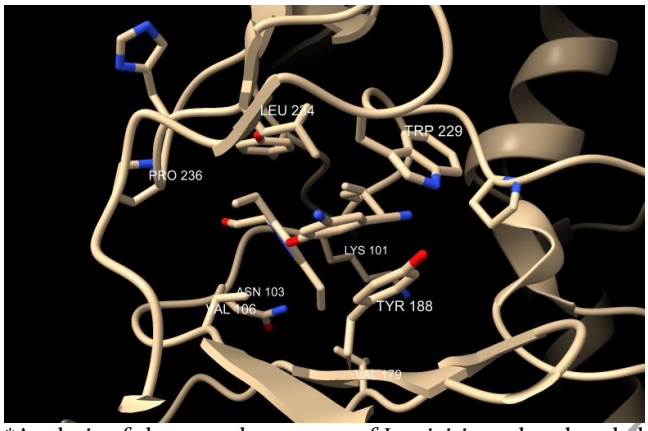




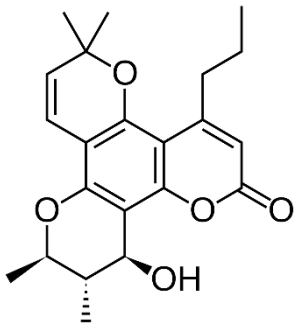
*Analysis of the crystal structure of Lersivirine when bonded to HIV-1 RT shows interactions with L100, V106, Y181, Y188, F227, W229, Y318, L234, and P236. Specifically, with Y181, Lersivirine creates a significant conformational change in the amino acid, similar to the change created by efavirenz. Two hydrogen bonds are formed between the ligand and L103 and P236.

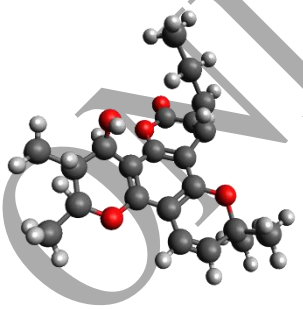
Calanolide A

Calanolide A (figure 8), being a natural product that was extracted from plants in a Malaysian rainforest, is arguably the most unique NNRTI. It is an organic tetracyclic heterocycle compound. The structure of these NNRTIs is what determines their activity and an important understanding with the Calanolide family is that they interact with reverse transcriptase in a mechanically different way. They are a mixed type inhibitor and do show activity when coupled with HIV-1 reverse transcriptase.

The diversity of NNRTIs and specifically the calanolide family of inhibitors compared to either of the past generations displays the variety of molecules in the NNRTI family. Although calanolide A is comparatively less potent than the other generations of NNRTIs, it showed very promising activity when inhibiting reverse transcriptase with a mutation at Y181. There is also evidence that the Calanolide class binds not only to the NNRTI-BP but also to another binding pocket, which could lead to greater inhibition when coupled with other NNRTIs. [18]

Figure 8. Structure of calanolide A visualized in Avogadro





Polypharmacy:

The high mutation rate of HIV RT necessitates the development of anti-HIV compounds. The future of HIV treatment firmly lies in the expansion of knowledge on novel NNRTIs. Yet another important area of emphasis is "synergistic inhibition". This is attained through a combination of NNRTIs and nucleoside reverse transcriptase inhibitors (NRTIs). NRTIs bind competitively to RT, directly at the active site, also inhibiting the enzyme. Although polypharmacy in HIV is often used, it is arguable that the usage is not ideal and rather problematic. 55% of people 50 years and older took 5 or more daily medications for HIV.[14] This excessive polypharmacy is not ideal as it can result in nonadherence and adverse drug events.[19, 20] There is also a one-pill ART option known as ADONE (adherence to one pill) that combines multiple antiretroviral drugs into one pill that increases adherence and quality of life on average. It also improves CD4 cell count as decreasing viral RNA production. Especially with chronic, asymptomatic diseases such as HIV, adherence is poor, which increases the risk of cardiovascular disease and the resistance of HIV to the medication.[21] The method of accomplishing this is to have drugs that are effective at targeting specific parts of the enzyme as the process of interference is observed when drugs attempt to attack the same binding spot. NRTIs function by mimicking DNA nucleotides, and therefore bind to the binary RT:DNA complex. Hence, an NNRTI that can precisely interact with the RT:DNA:dNTP complex is likely to be most ideal for synergistic inhibition.[22] Pyrrolobenzothiazepinones and Pyrrolobenzoxazepinones are examples of drugs that have been shown to be promising in this regard.[23] Hence, further research should be conducted regarding them.

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General History of HIV/AIDS & HIV Lifecycle:

Retroviruses are a class of viruses that use the host cell's machinery to replicate their proteins and RNA and proliferate. They use the reverse transcriptase enzyme to convert their RNA into DNA and insert the viral DNA into the host cell's genome, where it will be replicated and more copies of the virus can be made.

Reverse transcriptase is used to reverse transcribe the virus's RNA genome into DNA, which allows viruses to attack cells and inject this cDNA, the complementary DNA strand, into the host cell's genome, using the cell's mechanism to replicate itself. HIV infects CD4+ cells, or T-cells, in the immune system. The virus attacks the T-cell and then uses the cell's machinery to manufacture and release more viruses into the body through the bloodstream.[2]

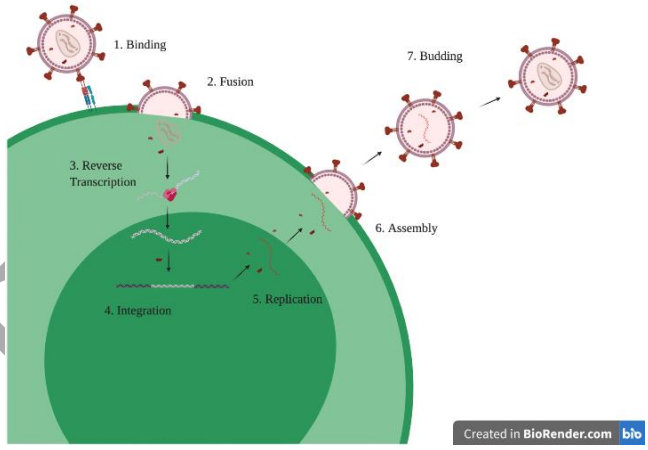
The seven steps of the life cycle of HIV are

1) binding, 2) fusion, 3) reverse transcription, 4) integration, 5) replication, 6) assembly 7) budding (figure 9).

The first step, binding, occurs when HIV binds to the CXCR4 receptor of a CD4 cell. After HIV binds to the receptor, glycoprotein 41 (GP41), unfolds from the virus and inserts its hydrophobic terminus into the cell membrane. GP41 then folds back on itself to bring the virus closer to its host cell and facilitates fusion between the cell and the virus. The full molecular mechanism of fusion of the virus and host cell is not completely understood as of now. However, scientists have found that fusion is triggered by activation of the CD4 receptor.[24] After the virus enters the cell, it initiates reverse transcription to convert its single-stranded RNA into double-stranded DNA. In reverse transcription, DNA is produced from an RNA template. Reverse transcriptase, one of the enzymes that facilitate reverse transcription makes an abnormal amount of mistakes during transcription; 1 in every 2000 base pairs is miscopied, or approximately 0.05%. The high rate of mutation is an advantage to HIV since it can easily and quickly mutate to NRTIs and NNRTIs if given independently.[24]

Antiretroviral therapy (ART) includes administering one drug to treat HIV; these are HIV medications. Highly active antiretroviral therapy (HAART), which includes administering 3 or more antiretroviral drugs, is more effective since it is more difficult for HIV to evade many antiretroviral medicines at once.[25, 26] One of the ssRNA strands carried by HIV is converted to a double helix containing one strand of RNA and one strand of DNA by passing through polymerase. Then, the double helix passes through ribonuclease H, which breaks down the RNA strand. The cDNA then passes through polymerase again to form dsDNA. Integrase then cleaves a portion of each 3' end of the DNA double helix to form "sticky ends". The "sticky ends" then attach to the cell's genome to be transcribed along with the rest of the genome when the cell is active. Cell activation triggers the conversion of viral DNA into mRNA, which then migrates into the cytoplasm and is transcribed along with the host cell's DNA, making viral proteins. Some of the large proteins are cleaved to make smaller, core proteins. After the viral proteins are assembled, HIV RNA and proteins assemble into immature viruses at the surface of the cell. 2 viral RNA strands, replication enzymes, and core proteins combine to form a capsid. The immature HIV capsid, which cannot infect a cell, leaves the host cell and simultaneously acquires a new viral envelope, which consists of viral and host cell proteins. The capsid matures outside the host cell and infects other cells. The budding of the viruses induces pyroptosis, which is a type of apoptosis that triggers an inflammatory response. [27, 28, 29]

Figure 9: HIV life cycle (Created with BioRender.com)



NNRTI Binding Pocket:

The heterodimeric HIV-1 reverse transcriptase (RT) enzyme consists of a p66 (66 kDa) subunit and a p51 (51 kDa) subunit. RT (figure 10) exhibits DNA polymerase activity, which produces dsDNA from the viral RNA and endonucleolytic ribonuclease H (RNaseH) activity, which degrades the viral genomic RNA strand after it is copied. The p66 chain contains a DNA polymerase domain and an RNase H domain, with both active sites while the p51 polypeptide has just a non-functional polymerase domain.

The polymerase domain in the p66 chain consists of multiple subdomains in the shape of a right hand, with fingers (residues 1–85 and 118–155), palm (residues 86–117 and 156–237), and thumb (residues 238–318) subdomains. Within the palm subdomain of the p66 subunit is the polymerase active site, with an aspartic acid catalytic triad (D110, D185, and D186).[12] The subdomains of the p66 subunit are flexible, allowing RT to take on different conformational states to bind nucleic acid template strands. The p51 chain has a different arrangement of these same subdomains, resulting in a rigid structure that provides structural support for p66.[4]

All NNRTIs interact with RT by binding to an allosteric site on the p66 subunit of RT to non-competitively inhibit reverse transcription of the HIV-1 RNA.[29] This site, the non-nucleoside reverse transcriptase inhibitor binding "pocket" or the NNRTI-BP (figure 11), is located on the palm subdomain of p66, approximately 10 Å away from the DNA polymerase

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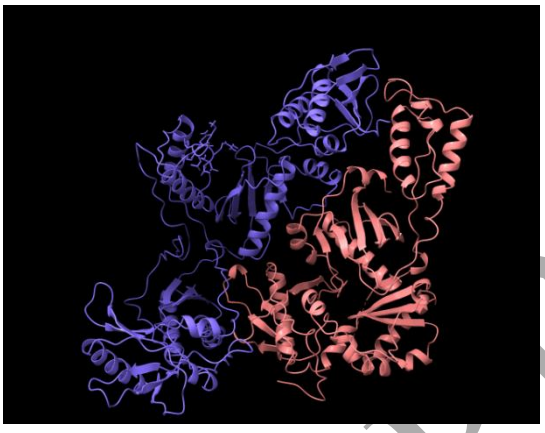
catalytic site. The NNRTI-BP's residues create hydrogen bonding interactions with NNRTIs at key hydrophobic residues (K101, K103, S105, D192, and E224 of the p66 subunit and E138 of the p51 subunit) and has the aromatic residues (Y181, Y188, F227, W229, and Y232).[12] Without NNRTI binding, the NNRTI-BP residues have a different conformation. In an unliganded RT, the side chains Y181 and Y188 point into the hydrophobic pocket, and there exists a surface depression at the putative entrance to the NNRTI-BP. The NNRTI-BP is flexible, adopting different conformations for different NNRTIs that bind to RT.

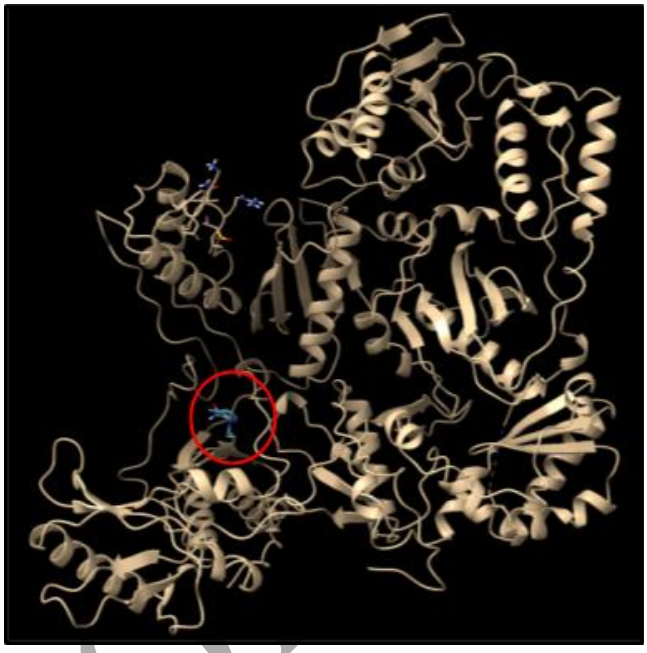
When an NNRTI binds to RT, this causes rotation of Y181 and Y188 away from the hydrophobic core to accommodate the inhibitor. The binding of an NNRTI to RT creates short-range and long-range distortions of the RT structure, which results in RT inhibition.[5]

Figure 10. HIV-1 reverse transcriptase (PDB: 1REV) visualized in ChimeraX with the p66 subunit in purple and p51 subunit in pink.



(Ren, et al. "The Structure of HIV-1 Reverse Transcriptase Complexed with 9-Chloro-TIBO: Lessons for Inhibitor Design." Structure 3, no. 9 (1995).)

Figure 11. Nevirapine, in blue, docked in NNRTI-BP, circled in yellow, on HIV-1 RT (PDB: 1REV) using Autodock Vina and visualized in ChimeraX.



Mechanisms of Inhibition:

There are multiple hypotheses for RT inhibition by NNRTIs. Because NNRTI binding results in multiple structural and conformational changes, it is unclear which of these conformational changes is operative in RT inhibition. Three of the possible mechanisms of inhibition of RT are:

i) change in direction of the p66 thumb or loss in mobility

ii) distortion of the catalytic triad at the polymerase active site

iii) displacement of the primer grip.

NNRTI binding distorts the primer grip, which prevents DNA from binding to the polymerase active site.[12] The binding of an NNRTI may affect the direction or degree of mobility that the p66 thumb subdomain possesses. A molecular dynamics study supports the theory that the "arthritic model", which suggests that NNRTI binding restricts the mobility of the thumb subdomain, is accurate.[30] However, computational studies suggest that NNRTI binding may not restrict mobility, instead change the direction that the thumb subunit can move. The binding of an NNRTI can also inhibit RT by preventing the conformational changes in the YMDD loop, which is a part of the catalytic triad, which is a subunit of the protease protein responsible for lysing large proteins into core proteins, which encase the virus. The YMDD loop contains 2 of the 3 aspartate residues in the triad, countering the formation of new viruses. NNRTI binding possibly induces restriction of p66 thumb mobility by changing the residues which make up the primer grip, preventing proper positioning of the primer 3' end in the polymerase active site.[4] The "primer grip" has been proposed to be part of positioning the 3' primer terminus at the polymerase binding site.[5]

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Conclusion

HIV is a retrovirus, a class of viruses that use the reverse transcriptase enzyme to synthesize double-stranded DNA from single-stranded RNA. They then integrate the viral DNA into a host cell's genome to produce more viruses. Antiretroviral drugs prevent the proliferation of HIV by inhibiting the entry and/or reverse transcription of HIV so that the virus cannot replicate. One class of antiretroviral drugs are NNRTIs, also known as non-nucleoside reverse transcriptase inhibitors. NNRTIs non-competitively inhibit the RT enzyme by binding to the NNRTI-BP and changing the short-range and long-range conformation of RT and distorting the palm and thumb subregions of RT. First-generation NNRTIs, which are less effective than second-generation NNRTIs, inhibit the motion of the palm and thumb subregions of the p66 unit. HIV mutates to the rigid structure of first-generation NNRTIs, so they are no longer effective. Second-generation NNRTIs have a similar mechanism of action, but are more flexible and therefore are more effective. HAART and ADONE, which use 3-4 ARVs, are used to minimize the chance that HIV will mutate by targeting many sites of entry or replication, therefore reducing the viral load. NNRTIs, especially the second generation, inhibit RT more effectively and for longer because of their flexibility.

AUTHOR INFORMATION

Corresponding Author

*To whom correspondence should be addressed. E-mail address: surapaneni.anvi@gmail.com.

Author Contributions

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ABBREVIATIONS

HIV, Human immunodeficiency virus; AIDS, Acquired Immunodeficiency syndrome; RT, reverse transcriptase; NNRTI, Non-nucleoside reverse transcriptase inhibitor; NNRTI-BP, Non-nucleoside reverse transcriptase inhibitor binding pocket.

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