

The Categories, Mechanisms and Features of Nonnucleoside Reverse Transcriptase Inhibitors of HIV-1

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Abstract. AIDS, or acquired immune deficiency syndrome is a dangerous disease of our age, and is mainly caused by HIV-1. In the last decades, researchers have paid attention to the inhibitors of reverse transcriptase (RT) of HIV-1 as a promising candidate for antiviral drugs. The reverse transcriptase (RT) is a crucial enzyme in the life cycle of HIV-1, responsible for the conversion of viral RNA to proviral DNA which will be later integrated with the genome of infected cells. RT is composed of two function domains: an RNA and DNA-dependent polymerase domain and an RNase H domain, which are respectively responsible for the synthesis and hydrolysis of proviral DNA strands. A number of drugs targeting one of the domains or both have been designed, tested or approved for clinical use, among which the nonnucleoside reverse transcriptase inhibitors (NNRTIs) have gained their status for various advantages. Herein, the molecular mechanism of four kinds of main RT inhibitors-polymerase inhibitors, RNase H active site inhibitors, RNase H allosteric inhibitors and dual inhibitors are introduced, as well as the advantages, drawbacks and challenges of these drugs. Their mechanisms and challenges are discussed to promote a comprehensive understanding of the development of NNRTIs.

Keywords: Enter key words or phrases in alphabetical order, separated by commas.

1. Introduction

AIDS, or acquired immune deficiency syndrome is an acute disease threatening an increasing number of populations. The main causative agent of AIDS is referred to as HIV-1, or human immunodeficiency virus type 1, which has infected approximate 38.4 million people at the end of 2021 [1] and only 28.7 million or 75% of the infected were receiving antiviral treatment. The data seems improved comparing with the infecting peak in 2004, though new challenges are emerging as well. The prevailing treatment to AIDS is highly active antiretroviral therapy or HAART, which combines a myriad of antiviral drugs for different replication periods of HIV-1. Though it greatly reduces the mortality and morbidity of AIDS, HAART requires a life-long intake of antiviral drugs and thus exposes several serious side effects of this therapy, including acute and chronic toxicity [2] and the occurrence of drug resistance virus variations [3,4]. Therefore, exploiting and utilizing novel mechanisms and expanding the drug design ideas are becoming increasingly necessary.

Since the discovery of the replication process of HIV-1, reverse transcriptase (RT) of the virus has been paid attention to as a promising target for inhibitors. HIV-1 is a typical reverse transcription virus, which indicates that its hereditary substance is RNA rather than DNA. Thus, to integrate its hereditary information into the genome of the infected cells, HIV-1 needs to manufacture a DNA strand corresponding to the viral RNA strand, and this is called reverse transcription. HIV-1 reverse transcriptase (RT) is a pivotal multifunctional protein involved in the process, which is responsible for the synthesis of (-)-DNA strand and the hydrolysis of viral RNA after the synthesis as shown in Fig 1.

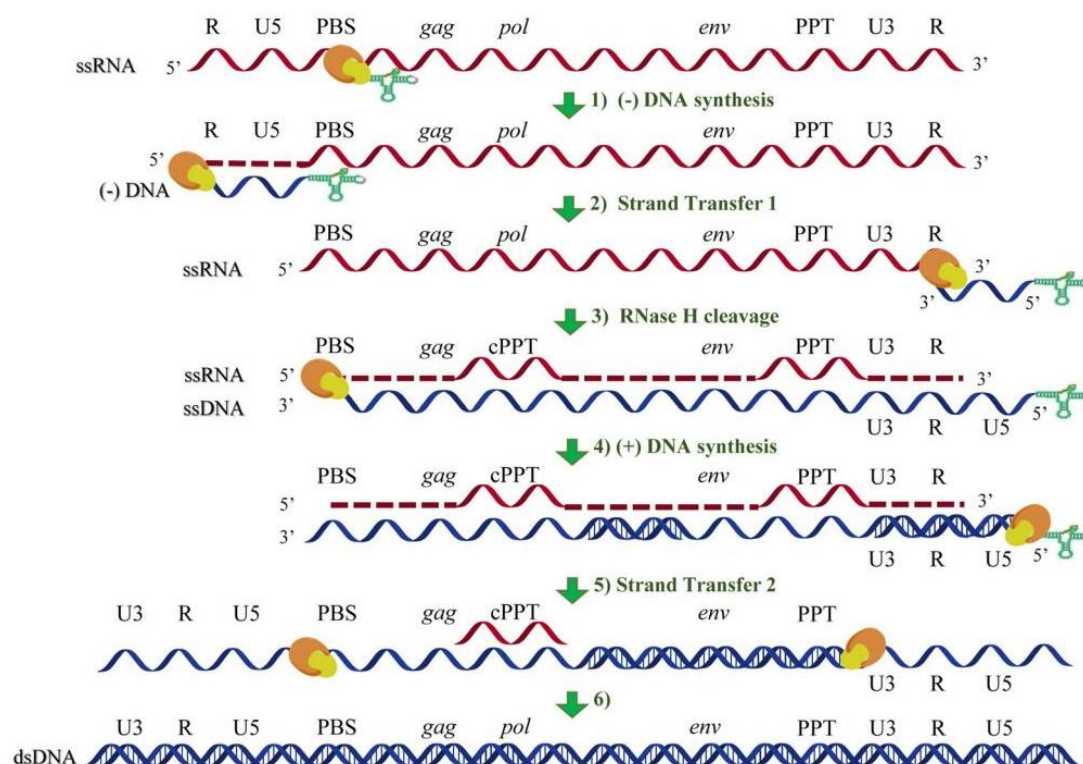


Figure 1. The reverse transcription process of HIV-1 mediated by RT [5].

It is revealed that two functional domains enable RT to complete its tasks: an RNA- and DNA-dependent DNA polymerase domain, and an endonuclease or RNase H domain. The former one catalyzes the synthesis of (-)-DNA strand from corresponding viral RNA and the latter one is responsible for the hydrolysis of viral RNA. Traditional RT inhibitors imitates the structure of deoxyribonucleotide to combine with the polymerase domain, competing with normal dNTPs to block the synthesis of DNA strand. This classical version of inhibitors is called nucleoside reverse transcriptase inhibitors, or NRTIs. However, such idea encounters obstacles because of drug resistance caused by mutations of virus and severe liver, kidney and bone marrow toxicity [6,7]. Newly-developed inhibitors, on the contrary, avoiding the structure of dNTPs thus partially solve the problem. They are given the name “nonnucleoside reverse transcriptase inhibitors”, or NRRTIs, and are gaining status in the field of HIV-1 inhibitors. Inhibiting mechanism of NRRTIS are more variable and flexible, targeting a myriad of active locus on RT. The most prevailing NRRTIs target an allosteric site adjacent to the polymerase active center to change the structure of the polymerase to defect the enzyme thus prevent the synthesis of DNA strand. Comparing with the classical nucleoside inhibitors, this allosteric-site targeting inhibitor is specific rather than competitive, which leads to improved selectivity, and it can function extracellularly [8]. Another idea of drug design is to set the RNase H domain as target: this kind of inhibitors interfere with the hydrolysis of RNA strand to block the replication of virus. This is because viral RNA strand will combine tightly with newly synthesized (-)-DNA strand through hydrogen bond and RT is required to cleave and decompose the RNA strand to release the (-)-DNA strand as the substrate for the synthesis of (+)-DNA [9]. If RNase H domain is blocked, the RNA strand will not be decomposed, thus restricts the (-)-DNA strand to involve in the generation of (+)-DNA strand. Some other inhibitors target more than one locus and even involve in multiple processes in virus replication-not only reverse transcription, but also integration of viral DNA-and they are referred to dual inhibitors, indicating their multi-target property [10].

Having a grasp of the characteristics, mechanisms and advantages or challenges of these inhibitors is beneficial to understanding the HIV-1 inhibitor researches from a birds' eye perspective. Herein, the mechanism of nonnucleoside polymerase inhibitors, RNase H inhibitors and dual inhibitors will be introduced in detail as well as some candidate substance discovered in research. We hope this

review provide you some comprehensive understanding of the development, challenges and possible future directions of HIV-1 inhibitors.

2. Polymerase Inhibitors

2.1. Mechanism, characteristic and challenges

As a widely-used clinic medicine, nonnucleoside polymerase inhibitors interfere with the spatial structure of the catalyzing site of the RT to defect the polymerase domain. To catalyze the synthesis of (-)-DNA strand, 3 aspartic acid residues-named D110, D185 and D186 –which are attached to a β -sheet structure are required to be in correct location to combine with substrates [11,12]. However, the presence of polymerase inhibitors can interpret this by combining with an allosteric site approximate 10Å from the catalyzing site. Candidate substances for polymerase inhibitors are tend to be strong hydrogen bond acceptors or donors to bind with some amino residues at this site. In most cases, they also possess a hydrophobic structure to fit in the hydrophobic environment at the binding site. It is revealed that most polymerase inhibitors can induce the formation of a pocket structure-or NRRTIs binding pocket-which means that the protein skeletons of polymerase domain will move and set room for the inhibitor. This leads to a 2Å displacement of the β -sheet structure and the three aspartic acid residues, hence defects the polymerase domain.

One apparent advantage of nonnucleoside polymerase inhibitors is that they combine specifically with polymerase domain of RT, which requires less amount of drug intake and ensures higher efficiency. Moreover, this inhibitor targets RT directly rather than involving in the synthesis process, which means it can effect on extracellular RT and reduce the concentration of virus outside the cells. However, the challenge to the application of this inhibitor is that drug resistance occurs frequently. The binding process between inhibitors and the allosteric site is highly dependent on kind of the amino residues at the site, which are very sensitive to mutations. Discovered drug resistance mutations such as K103N/S, V106A/M, Y181C/I/V, Y188L/C/H, and G190A/ S/E can decrease the activity of inhibitors drastically to 10% even 1%. [12-14] Hence, discovering new substance effective to the mutated types of viruses is a demanding task at present.

2.2. Introduction to some inhibitors

2.2.1. Nevirapine

Nevirapine, or NVP is the first approved NRRTIs for clinical use. The binding structure of this substance is inferred to be its double-pyridine ring and a three-member alkyl ring[15]. The double-pyridine rings extend the delocalized π -system and cause a π - π stacking interaction between NVP and the binding pocket, while the lipophilic alkyl substitution “tail” can bind with other hydrophobic amino residues [15].

2.2.2. Delavirdine

As the second approved NRRTIs in 1997, Delavirdine, or DLV possess more interaction sites with the allosteric site comparing to NVP [16]. It is a relatively longer molecule consisting of several heterocycles to form various interactions with the binding pocket. The oxygen atom at the middle of the molecule and the nitrogen atom on the indole ring both form hydrogen bonds with surrounding amino residues, and the outreached aromatic rings are responsible for stacking interactions [17].

2.2.3. Efavirenz

Efavirenz, or EFV, is deemed as the second generation of NRRTIs because of its effects on drug-resistant viruses [18]. It should be partially attributed to the novel design of this molecule: more innovative structures are introduced into EFV, such as the alkynyl chain with a three-member ring, and the trifluoromethyl, to create a significant polarity difference in one molecule. Nowadays, EFV is one of the most widely used NRRTIs due to its broad-spectrum effect to various mutant viruses and its reduced possibility of drug resistance [18].

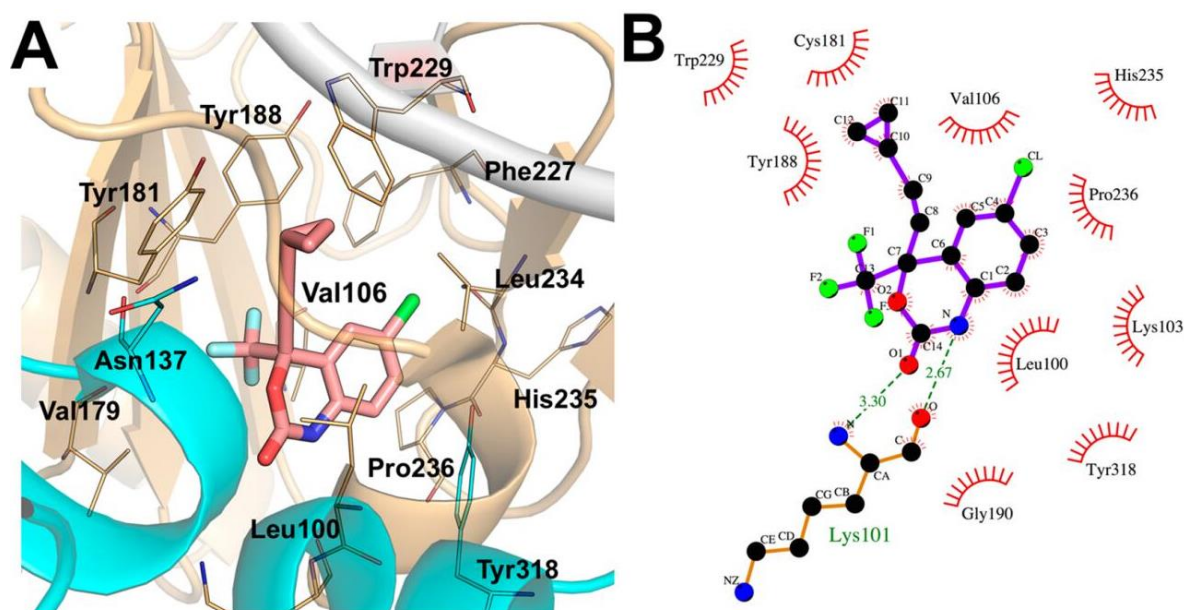


Figure 2. (A) The binding mode of EFV and (B) the ligand interactions of EFV [15].

2.3. Indole Aromatic Sulfone Inhibitors

Among all the drug resistant viruses, the Y181C variation is the most threatening and tricky mutation, whose 181 tyrosine at allosteric site mutates to cysteine [19]. Such change greatly blocks the interaction between many NNRTIs with the RT polymerase domain. To handle the problem, a creative solution was proposed, which is, to form a covalent bond between the mutated amino acid residue and the inhibitor molecule. Cysteine possesses an electron-rich pendant sulfhydryl suitable for nucleophilic agent to attack some electron-deficient small molecules [20,21]. Indole aromatic sulfone inhibitors, or IAS inhibitors, which contains an electron-deficient ring, are one of the attempts to overcome the obstacle of Y181C [22].

3. RNase H Inhibitors

3.1. Active Site Inhibitors

The key to the hydrolysis function of RNase H lies in the unique structure of the catalyzing site of RNase H domain. RNase H is a metalloenzyme, with a highly conserved structure of active site where three amino acid residues-named D443, E478, D498, D549-coordinating with two metal ions, usually magnesium ions [23]. The metal ions are suitable electron acceptors which can bind the phosphate group in nucleoside and a water molecule, by which the catalyzing process could be done [24]. Thus an apparent drug design idea is to find out a biocompatible small molecule which can coordinate with the magnesium ions to block the binding of RNA and water molecule. Inhibitors in this mechanism-targeting the active site directly-are always modified ligands to form a chelation structure with the magnesium ions.

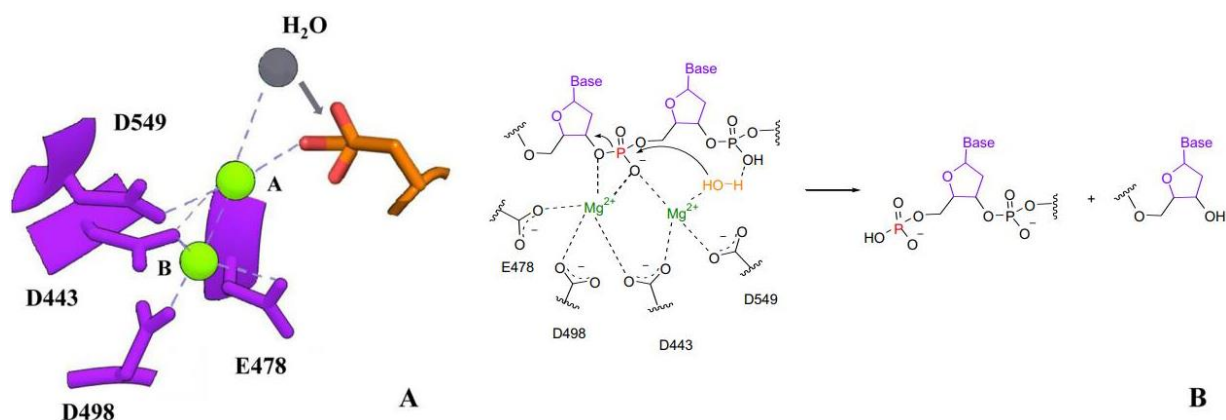


Figure 3. (A)The structure of the active site of the RNase H domain and (B) the detailed mechanism of hydrolysis catalyzing [5]

3.1.1. Diketo Acids(DKAs)

The diketo acids are the first known effective RNase H inhibitors, which was firstly reported effective to influenza virus integrase [26]. The discovery of diketo acids not only open a gate for RNase H inhibitors but also cast a light on the research of dual inhibitors. The reason why the diketo acids can work on both viruses is that the structure of influenza virus integrase shares remarkable similarity with the RNase H domain at the active site. They both present a metal ion-amino residues structure. Moreover, the structure of HIV-1 integrase is also similar to that of the RNase H domain, which means diketo acids may target two sites-the RNase H and the integrase-at the same time [26-28]. It is also found that the inhibitors' selectivity towards RNase H and HIV-1 integrase can be coordinated via changes on the charge of the molecule. By adding substitutions of different electron effects, an RNase H-preferred or integrase-preferred or dual-target inhibitor can be created [29].

3.1.2. Synthetic α -Hydroxytropolones

Though DKAs are proved effective, some serious problems still exist, including its unsatisfactory metabolism properties and low cell membrane penetration, which greatly restrict its application in clinical scenarios. Another problem is that, though the selectivity of DKAs can be coordinated, it is difficult to make it precisely target the RNase H site. Hydroxylated tropolones, which are the compounds separated from some natural vegetation, such as *Thuja Plicata* and *Dulacia Guianensis*, are described as the first RNase H selective inhibitors. However, these natural products seemed ineffective in blocking the replication of HIV-1 and showed significant cell toxicity partially due to their binding with cellular metalloenzymes. Fortunately, a series of compounds imitating the structures of hydroxy tropolones have been synthesized and proved effective with lower toxicity. [30].

3.2. Allosteric inhibitors

It seems that active site inhibitors are promising in novel antiviral drugs, but the fact is that a simple but serious question block their way to clinical use: they are not so effective. The reasons are various: the active site of RNase H domain is a depthless, open shape structure with few contacts site for inhibitors. Moreover, some HIV-1s are found to get rid of the dependence on a precise RNase H hydrolysis, which means that an interfered hydrolysis process causes limited influence on the replication. Therefore, inhibitors targeting the overall structure RNase H rather than the active site are designed and synthesized, which are given the name allosteric inhibitors.

Allosteric inhibitors possess various mechanisms which could not be divided into simple categories. Generally speaking, most allosteric inhibitors share a similar mechanism with polymerase inhibitors: binding with some certain amino acid residues and inducing the formation of a binding pocket to cause displacement of some key parts of RNase H [31]. However, most of the mechanisms are simulated rather than proved by experiment because the binding process is not easy to record, especially when the binding sites are various. For some candidate compounds, we can only infer that they are allosteric

inhibitors from the fact that they do not coordinate with metal ions [32]. Methods in computational chemistry are applied in selecting candidates because in this case a fixed binding site does not exist and potential binding sites are found via calculating the molecule docking energy and simulating the interaction patterns between RNase H and inhibitors [33].

Expect for the binding-pocket mechanism, there do exist some allosteric inhibitors function in other mechanisms. One of the examples is p66/51 interface inhibitor. In fact, the transcriptase of HIV-1 consists two subunits: p66 and p51. P66 subunit is the functional unit which contains the polymerase and RNase H domains, while p51 does not possess any catalytic site. However, p51 is responsible for the folding of p66 and it keeps the correct spatial configuration of p66. It is not thoroughly revealed that how p66 and p51 interact with each other, but a kind of Benzothienooxazinone derivative has been reported to have the ability to interfere with the process [34].

4. Dual Inhibitors

With the development of NRRTIs, the concept “dual inhibitors” is proposed as an inhibitor targeting two sites or two proteins at the same time. It is proved that multi-target drugs tend to be more effective than those only target one: the replication of virus is an interconnected process, which means that if you only block one step, there may exist some compromising mechanism to control the influence [35]. However, if you block several steps in virus replication at the same time, the protective mechanism of virus may be overloaded, which significantly reduces the rate of replication. Dual inhibitors, in this case, often target these two sites: the polymerase site and the RNase H site, or the RNase H site and the integrase.

4.1. IN/RNase H Dual Inhibitors

As mentioned in 3.1.1, the foundation of the IN/RNase H inhibitors is that RNase H domain and integrase share similarity in structure of active site, especially the folding motif of carboxyl residues and the metal ions. Expect for mentioned DKAs inhibitors, there are other candidates as well.

4.1.1. Galloyl Derivatives

This is a series of compounds consisting of an N-acylhydrazone scaffold with a galloyl group. The three oxhydroyls on the benzene ring serve as strong electron donors to coordinate with the metal ions. And there is an interesting fact: this kind of compounds have two coordination mode (shown in Fig 4), and they coordinate with RNase H in mode A while with integrase in mode B [36,37].

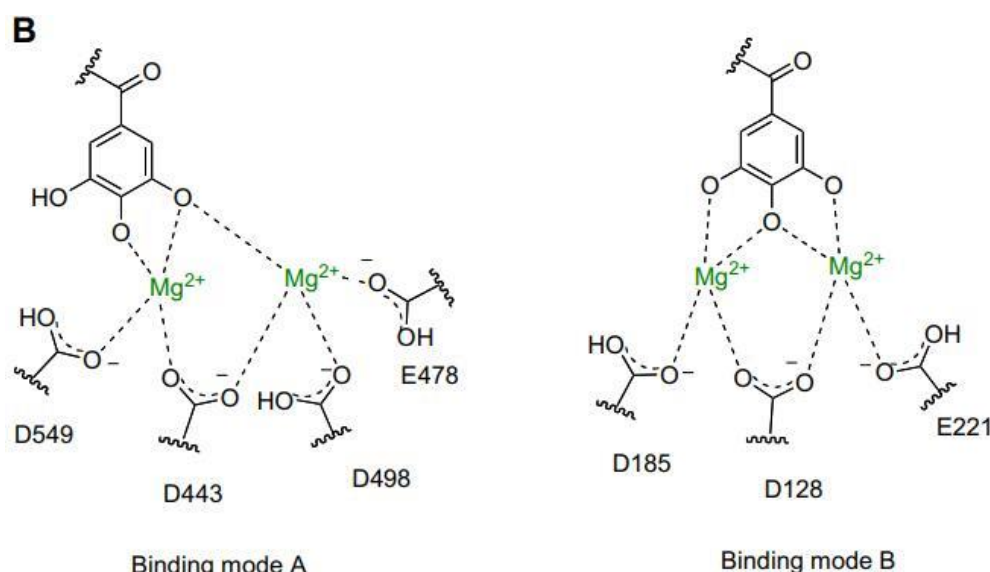


Figure 4. The chelating modes of galloyl derivatives [5]

4.2. RT-associated DNA Polymerase/RNase H Dual Inhibitors

The mechanism of polymerase/RNase H inhibitors are opaquer. The reason for this is that most polymerase/RNase H inhibitors are allosteric inhibitors for both sites, which means they combine with a myriad of docking sites varying from molecule to molecule. Some promising candidates for this kind of inhibitors are nicotinic acid derivatives [38], isatin derivatives [39] and heteroaryl carbothioamide derivatives [40].

5. Conclusion and Outlook

In this review, a simplified contour of the development in HIV-1 nonnucleoside reverse transcriptase inhibitors is present. The mechanisms of main types of nonnucleoside inhibitors are introduced on a molecular basis, including the polymerase inhibitors, RNase H active site inhibitors, RNase H allosteric inhibitor and dual inhibitors. The review shows and discusses the advantages, challenges and drawbacks of some of the inhibitors. For example, traditional allosteric polymerase inhibitors are proved most effective though troubled with the occurrence of drug resistance due to mutations of amino acid residues. RNase H active site inhibitors seem promising but encounters serious problem to bind with the target site. RNase H allosteric inhibitors share the same problem with dual inhibitors that they are not designed but accidentally found. The overall principle behind the occurrence of a potential docking site is still in dark at present, which means the discovery of a new RNase H allosteric inhibitor or a dual inhibitor involves possibilities. However, these two kinds of inhibitors are promising because they avoid many drawbacks of their ancestors: they are not vulnerable to drug resistance and can eradicate viruses more efficiently. Another significant obstacle, though not fully discussed in the review, is that most of the candidates are cellular toxic, which requires efforts to reduce their side effects. Therefore, several possible directions in future are proposed: 1) the development of a high-efficiency computational method to find potential docking sites for RNase H and polymerase domains; 2) the proposal and application of novel strategies against drug-resistant mutations, for example, the covalent inhibitors; and 3) the researches on the structure-function mechanism of cellular toxicity of small molecules.

References

- [1] WHO. HIV/AIDS data and statistics. Available at: <https://www.who.int/hiv/data/en/> (Accessed Date: October 4, 2022).
- [2] Deeks, S.G.; Lewin, S.R.; Havlir, D.V. The end of AIDS: HIV infection as a chronic disease. *Lancet*, 2013, 382 (9903), 1525 - 1533.
- [3] Kim, M.; Siliciano, R.F. Reservoir expansion by T-cell proliferation may be another barrier to curing HIV infection. *Proc. Natl. Acad. Sci. USA*, 2016, 11.
- [4] Basti, B. D.; Mahesh, V.; Bant, D.D.; Bathija, G.V. Factors affecting antiretroviral treatment adherence among people living with human immunodeficiency virus/acquired immunodeficiency syndrome: a prospective study. *J. Family Med. Prim. Care*, 2017, 6 (3), 482 - 486.
- [5] Valentina, N. M.; Antonella, M., et al. Small-molecule Inhibitors of HIV-1 Reverse Transcriptase-Associated Ribonuclease H Function: Challenges and Recent Developments [J] *Curr. Med. Chem.*, 2021, 28, 6146 - 6178.
- [6] Deval, J.; Courcambeck, J.; Selmi, B. et al. Structural determinants and molecular mechanisms for the resistance of HIV-1 RT to nucleoside analogues [J]. *Curr Drug Metab*, 2004, 5 (4): 305 — 316.
- [7] Ho, Y.C.; Shan, L.; Hosmane, N.N.; Wang, J.; Laskey, S.B.; Rosenbloom, D.I.; Lai, J.; Blankson, J.N.; Siliciano, J.D.; Siliciano, R.F. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell*, 2013, 155 (3), 540 - 551.
- [8] Gao Ping. Design, Synthesis and Biological Evaluation of Novel HIV-1 Integrase / Ribonuclease H Dual Inhibitors and NNRTTs [D]. Shandong, Shandong University, 2020.

- [9] Betancor, G.; Álvarez, M.; Marcelli, B.; Andrés, C.; Martínez, M. A.; Menéndez-Arias, L. Effects of HIV-1 reverse transcriptase connection subdomain mutations on poly purine tract removal and initiation of (+)-strand DNA synthesis. *Nucleic Acids Res.*, 2015, 43 (4), 2259 - 2270.
- [10] Das K, Arnold E. HIV1 reverse transcriptase and antiviral drug resistance. Part 2 [J]. *Curr Opin Virol*, 2013, 3 (2): 119 — 128.
- [11] Kohlstaedt LA, Wang J, Friedman JM, et al. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor [J]. *Science*, 1992, 256: 1783–1790. Luis-Cremer, N. The emerging profile of cross-resistance among the nonnucleoside HIV-1 reverse transcriptase inhibitors. *Viruses* 2014, 6 (8), 2960 – 2973.
- [12] Wensing, A. M.; Calvez, V.; Gunthard, H. F.; Johnson, V. A.; Paredes, R.; Pillay, D.; Shafer, R. W.; Richman, D. D. 2014 Update of the drug resistance mutations in HIV-1. *Top. Antivir. Med.* 2014, 22, 642 – 650.
- [13] Melikyan, G. L.; Rhee, S. Y.; Varghese, V.; Porter, D.; White, K.; Taylor, J.; Towner, W.; Troia, P.; Burack, J.; Dejesus, E.; Robbins, G. K.; Razzeca, K.; Kagan, R.; Liu, T. F.; Fessel, W. J.; Israelski, D.; Shafer, R. W. Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. *J. Antimicrob. Chemother.* 2014, 69 (1), 12 – 20.
- [14] Namasivayam V Vanangamudi M, et al. The Journey of HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) from Lab to Clinic[J]. *J. Med. Chem.*, 2019, 62, 4851 – 4883.
- [15] Scott, L. J.; Perry, C. M. Delavirdine. *Drugs* 2000, 60 (6), 1411 – 1444
- [16] Esnouf, R. M.; Ren, J.; Hopkins, A. L.; Ross, C. K.; Jones, E. Y.; Stammers, D. K.; Stuart, D. I. Unique features in the structure of the complex between HIV-1 reverse transcriptase and the bis-(heteroaryl) piperazine (BHAP) U-90152 explain resistance mutations for this nonnucleoside inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* 1997, 94 (8), 3984 – 3989.
- [17] Young, S. D.; Britcher, S. F.; Tran, L. O.; Payne, L. S.; Lumma, W. C.; Lyle, T. A.; Huff, J. R.; Anderson, P. S.; Olsen, D. B.; Carroll, S. S. L-743, 726 (DMP-266): a novel, highly potent nonnucleoside inhibitor of the human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* 1995, 39 (12), 2602 – 2605.
- [18] Rhee S Y, Blanco J L, Jordan M R, et al. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual—patient-and sequence-level meta-analysis [J]. *PLoS Med*, 2015, 12 (4): e1001810.
- [19] De Cesco, S., Kurian, J., Dufresne, C., et al. Covalent inhibitors design and discovery [J]. *Eur J Med Chem*, 2017, 138: 96 — 114.
- [20] Billamboz, M., Bailly, F., Barreca, M.L., et al. Design, synthesis, and biological evaluation of a series of 2-hydroxyisoquinoline-1, 3-(2H, 4H)-diones as dual inhibitors of human immunodeficiency virus type 1 integrase and the reverse transcriptase RNase H domain [J]. *J Med Chem*, 2008, 51 (24): 7717 - 7730.
- [21] Chan A.H, Lee W. G, Spasov K. A et al. Covalent inhibitors for eradication of drug-resistant HIV-1 reverse transcriptase: From design to protein crystallography[J]. *Proc Natl Acad Sci. USA* 2017, 114 (36): 9725 - 9730.
- [22] Beilhartz, G.L.; Götte, M. HIV-1 ribonuclease H: structure, catalytic mechanism and inhibitors. *Viruses*, 2010, 2 (4), 900 - 926.
- [23] Tramontano, E.; Corona, A.; Menéndez-Arias, L. Ribonuclease H, an unexploited target for antiviral intervention against HIV and hepatitis B virus. *Antiviral Res.*, 2019, 171, 104613.
- [24] Wai, J.S.; Egbertson, M.S.; Payne, L.S.; Fisher, T.E.; Embrey, M.W.; Tran, L.O.; Melamed, J.Y.; Langford, H.M.; Guare, J. P. Jr.; Zhuang, L.; Grey, V.E.; Vacca, J.P.; Holloway, M.K.; Naylor-Olsen, A.M.; Hazuda, D.J.; Felock, P.J.; Wolfe, A.L.; Stillmock, K.A.; Schleif, W.A.; Gabryelski, L.J.; Young, S.D. 4-Aryl-2, 4-dioxobutanoic acid inhibitors of HIV-1 integrase and viral replication in cells. *J. Med. Chem.*, 2000, 43 (26), 4923 - 4926.
- [25] Shaw-Reid, C.A.; Munshi, V.; Graham, P.; Wolfe, A.; Witmer, M.; Danzeisen, R.; Olsen, D.B.; Carroll, S.S.; Embrey, M.; Wai, J.S.; Miller, M.D.; Cole, J.L.; Hazuda, D.J. Inhibition of HIV-1 ribonuclease H by a novel diketo acid, 4-[5-(benzoylamino) thien-2-yl]-2,4-dioxobutanoic acid. *J. Biol. Chem.*, 2003, 278 (5), 2777 - 2780.

- [26] Sluis-Cremer, N.; Arion, D.; Parniak, M.A. Destabilization of the HIV-1 reverse transcriptase dimer upon interaction with N-acyl hydrazone inhibitors. *Mol. Pharmacol.*, 2002, 62 (2), 398 - 405.
- [27] Tramontano, E.; Esposito, F.; Badas, R.; Di Santo, R.; Costi, R.; La Colla, P. 6-[1-(4-fluorophenyl) methyl-1H-pyrrol-2-yl]-2, 4-dioxo-5-hexenoic acid ethyl ester a novel diketo acid derivative which selectively inhibits the HIV-1 viral replication in cell culture and the ribonuclease H activity in vitro. *Antiviral Res.*, 2005, 65 (2), 117 - 124.
- [28] Kim, M.; Siliciano, R.F. Reservoir expansion by T-cell proliferation may be another barrier to curing HIV infection. *Proc. Natl. Acad. Sci. USA*, 2016, 113 (7), 1692 – 1694.
- [29] Budihas, S.R.; Gorshkova, I.; Gaidamakov, S.; Wamiru, A.; Bona, M.K.; Parniak, M. A.; Crouch, R.J.; McMahon, J.B.; Beutler, J. A.; Le Grice, S.F. Selective inhibition of HIV-1 reverse transcriptase-associated ribonuclease H activity by hydroxylated tropolones. *Nucleic Acids Res.*, 2005, 33 (4), 1249 - 1256.
- [30] Christen, M. T.; Menon, L.; Myshakina, N.S.; Ahn, J.; Parniak, M. A.; Ishima, R. Structural basis of the allosteric inhibitor interaction on the HIV-1 reverse transcriptase RNase H domain. *Chem. Biol. Drug Des.*, 2012, 80 (5), 706 - 716.
- [31] Desantis, J.; Massari, S.; Corona, A.; Astolfi, A.; Sabatini, S.; Manfroni, G.; Palazzotti, D.; Cecchetti, V.; Pannecouque, C.; Tramontano, E.; Tabarrini, O. 1, 2, 4-triazolo[1,5-a] pyrimidines as a novel class of inhibitors of the HIV-1 reverse transcriptase-associated ribonuclease H activity. *Molecules*, 2020, 25 (5), E1183.
- [32] Himmel, D.M.; Sarafianos, S.G.; Dharmasena, S.; Hossain, M.M.; McCoy-Simandle, K.; Ilina, T.; Clark, A. D. Jr.; Knight, J.L.; Julias, J.G.; Clark, P.K.; Krogh-Jespersen, K.; Levy, R. M.; Hughes, S.H.; Parniak, M.A.; Arnold, E. HIV-1 reverse transcriptase structure with RNase H inhibitor dihydroxy benzoyl naphthyl hydrazone bound at a novel site. *ACS Chem. Biol.*, 2006, 1 (11), 702 - 712.
- [33] Massari, S.; Corona, A.; Distinto, S.; Desantis, J.; Caredda, A.; Sabatini, S.; Manfroni, G.; Felicetti, T.; Cecchetti, V.; Pannecouque, C.; Maccioni, E.; Tramontano, E.; Tabarrini, O. From cycloheptathiophene-3-carboxamide to oxazinone- based derivatives as allosteric HIV-1 ribonuclease H inhibitors. *J. Enzyme Inhib. Med. Chem.*, 2019, 34 (1), 55 – 74.
- [34] Gill, M. S. A.; Hassan, S.S.; Ahemad, N. Evolution of HIV-1 reverse transcriptase and integrase dual inhibitors: recent advances and developments. *Eur. J. Med. Chem.*, 2019, 179, 423 - 448.
- [35] Carcelli, M.; Rogolino, D.; Gatti, A.; Pala, N.; Corona, A.; Caredda, A.; Tramontano, E.; Pannecouque, C.; Naesens, L.; Esposito, F. Chelation motifs affecting metal-dependent viral enzymes: N'-acylhydrazone ligands as dual target inhibitors of HIV-1 integrase and reverse transcriptase ribonuclease H domain. *Front. Microbiol.*, 2017, 8, 44.
- [36] Gao, P.; Wang, X.; Sun, L.; Cheng, X.; Poongavanam, V.; Kongsted, J.; Álvarez, M.; Luczkowiak, J.; Pannecouque, C.; De Clercq, E.; Lee, K.H.; Chen, C.H.; Liu, H.; Menéndez-Arias, L.; Liu, X.; Zhan, P. Design, synthesis, and biologic evaluation of novel galloyl derivatives as HIV-1 RNase H inhibitors. *Chem. Biol. Drug Des.*, 2019, 93(4), 582-589.
- [37] Narang, R.; Narasimhan, B.; Sharma, S.; Sriram, D.; Yogeeswari, P.; De Clercq, E.; Pannecouque, C.; Balzarini, J. Synthesis, antimycobacterial, antiviral, antimicrobial activities, and QSAR studies of nicotinic acid benzylidene hydrazide derivatives. *Med. Chem. Res.*, 2012, 21(8), 1557-1576.
- [38] Meleddu, R.; Distinto, S.; Corona, A.; Bianco, G.; Cannas, V.; Esposito, F.; Artese, A.; Alcaro, S.; Matyus, P.; Bogdan, D.; Cottiglia, F.; Tramontano, E.; Maccioni, E. (3Z)-3-(2-[4-(aryl)-1, 3-thiazol-2-yl] hydrazin-1-ylidene)-2, 3-dihydro-1H-indol-2-one derivatives as dual inhibitors of HIV-1 reverse transcriptase. *Eur. J. Med. Chem.*, 2015, 93, 452 - 460.
- [39] Corona, A.; Onnis, V.; Deplano, A.; Bianco, G.; Demurtas, M.; Distinto, S.; Cheng, Y.C.; Alcaro, S.; Esposito, F.; Tramontano, E. Design, synthesis and antiviral evaluation of novel heteroaryl carbothioamide derivatives as dual inhibitors of HIV-1 reverse transcriptase-associated RNase H and RDDP functions. *Pathog. Dis.*, 2017, 75 (6), ftx078.