Archives of Pharmaceutical Sciences Ain Shams University 2018; Vol. 2(1):22-30

OPEN ACCESS

Medicinal Chemistry

Review Article

HIV-1 RT-Associated RNase H review

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ABSTRACT

HIV-RT is an essential enzyme for HIV replication it comprises two associated functions: RNA- and DNA-dependent DNA polymerase (RDDP & DDDP) and ribonuclease H (RNase H). The RNase H is function to cleave RNA strand in RNA/DNA hybrid intermediate in the reverse transcription process. Currently, successful inhibitors target the polymerase function of HIV-RT. RNase H evolves to be a promising target for anti-HIV drug research. This review describes the compounds reported as HIV-1 RNase H inhibitors.

Keywords: *HIV-1; ribonuclease H; reverse transcriptase; tropolone; hydrazone*

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Citation | Madonna MAM, Nermin SA, Rabah ATS. 2018. HIV-1 RT-Associated RNase H review. Arch Pharm Sci ASU 2(1): 22-30
DOI: 10.21608/APS.2018.18731
Online ISSN: 2356-8380. **Print ISSN:** 2356-8399.
Received 08 February 2018. **Accepted** 12 April 2018.

Published by: Ain Shams University, Faculty of Pharmacy

1. INTRODUCTION

An estimate of 36.7 million HIV infected patients was reported in 2016 by the World Health Organization (WHO), this number includes 1.8 million infected children and the global HIV prevalence among adults was recorded 0.8% **[1]**. HIV is a retrovirus (single-stranded positive-sense RNA virus), it attacks and destroys the CD4 lymphocytic cells of the immune system which are responsible for the cellular adaptive immunity. Accordingly, loss of the CD4 cells weakens the body defense against infections and increases the risk of HIV-related cancers.

The highly active antiretroviral therapy (HAART), the current management for HIV infection, is classified into six drug classes based on how each drug interferes with the HIV life cycle. The WHO recommends the use of multiple classes as a combination therapy. These classes include the nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion and entry inhibitors, and integrase strand transfer inhibitors (INSTIs) **[2]**. The HIV-1 reverse transcriptase (RT) is an essential enzyme for HIV replication, it converts the single-stranded viral genomic RNA into a linear double-stranded DNA that can be subsequently translocated into the infected cell nucleus and integrated into the cell host DNA. This enzyme has two distinct activities, DNA polymerase activity, which can be RNA dependent (RDDP) or DNA dependent (DDDP) according to the template used and the RNase H **[3-5]**.

Several classes of RT inhibitors have been discovered and approved but so far, all of the RT inhibitors (RTIs) approved for the treatment of HIV-1 infection inhibit only the polymerase function of RT. However, the rapid emergence of drug-resistant strains of HIV limits the efficacy of the approved anti-AIDS treatments **[6]** and increase the need for identification of new RT inhibitors that may target another RT-associated function as RNase H and this might provide a new opportunity to treat patients resistant to currently available antiretroviral therapies.

RT-associated RNase H plays an essential role in the HIV-1 lifecycle and being the only HIV enzymatic function not targeted by current approved antiviral drugs makes it an attractive target to improve the anti-HIV treatments **[7]**.

2. REVERSE TRANSCRIPTION PROCESS AND RNase H ROLE

Reverse transcription of the viral genome is initiated by elongation of primer tRNALys3 annealed to a specific sequence in the viral RNA near the 5'end called primer binding site (PBS) **[8]**. The RT-associated DNA polymerase function starts the minus strand DNA synthesis and tRNA elongation continue till the ssRNA 5'end. During this process, the minus strand DNA with the template viral RNA generates an RNA: DNA heteroduplex which acts as a substrate for RNase H function to selectively degrades RNA strand of the RNA:DNA hybrid. This minus strand DNA molecule is then translocated to the 3'end of RNA genome through strand transfer for further elongation. During which the RNase H function continue to cleave the replicated viral RNA leaving two specific purine-rich sequences that are resistant to the RNase H cleavage known as polypurine tracts (PPTs). With this two PPTs annealed to the minus strand DNA, they serve as a primer for the plus strand DNA synthesis to initiate and continue elongation through U3, R and U5 viral sequence **[4, 8-10]**.

The RNase H hydrolyzes the PPT segments and the junctions of tRNA:DNA hybrid exposing the PBS of the plus strand DNA, this allows a second strand transfer of PBS sequence of plus strand DNA to anneal with the complementary PBS on the minus strand DNA and a bidirectional synthesis continues to complete the dsDNA. The rate of RNase H cleavage is approximately 7 folds slower than polymerization and this leads to believe that polymerase and RNase H activities are spatially but temporally coordinated during polymerase-dependent activity **[9-13]**.

3. RT STRUCTURE

RT is an asymmetric heterodimer enzyme encoded by the viral pol gene and derived from a gag-pol protein that is cleaved by the viral protease to yield the two subunits P66 and P51 **[14]**. Both subunits have a common N-terminus, P66 subunit contains the polymerase and RNase H active sites, where the polymerase domain comprises four subdomains: finger (residues1-85 and 118-155), palm (residues 86-117 and 156-237), thumb (residues 238-318) and connection (residues 319-426) while the RNase H domain comprises residues from (427-560) **Fig (1) [5,12,15,16].**

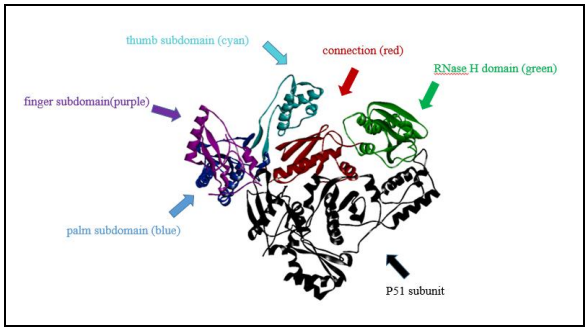

Fig. 1. HIV-1 RT structure showing the two subunits P66 (colored) and P51 (black). P66 subunit comprises the polymerase domain resembling a right hand in its structure with finger subdomain (purple), palm subdomain (blue), thumb subdomain (cyan) and connection (red) and the

RNase H domain (green). Figure generated using accelrys discovery studio client (PDB code: 6BSH) **[34]**.

4. HIV-1 RNase H INHIBITORS

Many HIV-1 RNase H inhibitors have been reported but none of them reached the clinical stages yet and due to its crucial roles in reverse transcription, RNase H remains an attractive target for anti-HIV drug research and development **[17-18]**.

4.1. Natural products and their derivatives

One of the first classes discovered to have good inhibition on RNase H function was the polyanions, in the late 80's, experiments were done on dextran, dextran sulfate, xylan polysulfate, DEAE-dextran, chondroitin sulfate, and heparin to test their inhibitory activity on HIV-1 RNase H and RT functions **[19]**.

Heparin inhibits RNase H 5.000-fold more efficiently than does RT. Xylan polysulfate and dextran sulfate inhibit RNase H about 250 to 1.000-fold more efficiently, with IC_{50} range between 0.1-8 nm. These compounds were isolated from algae and fungi and were suggested to have inhibitory activity on HIV-1 replication by two modes of action either interfering with virus uptake by the host cell or interfering with virus replication inside the host cell. To interfere with virus replication, it requires their uptake inside cells through macrophages which are considered as HIV carriers through the infected body.

Illimaquinone **(1) (Fig. 2)**, a 2^{ry} metabolites isolated from Red sea sponges *Smenospongia sp*, was tested, it didn't show inhibitory action on RDDP and DDDP at concentrations up to 10 µg/mL, but the RNase H activity was reduced by 62% at the same concentration showing IC_{50}= 15 µm. Two synthetic derivatives, 6'-acetate illimaquinone **(2)** and 6'-methyl illimaquinone **(3)** were synthesized for further study **(Fig. 2).**

Replacement of 6-OH group ortho to carbonyl on quinone ring by either acetyl or methoxy groups polished the anti-RNase activity and the IC_{50} exceeded 50 µg/mL **[20].**

Six derivatives of the sesquiterpenoid avarol, isolated from the marine sponge *Dysidea cinerea* keller from the Gulf of Eilat in the Red Sea were studied.

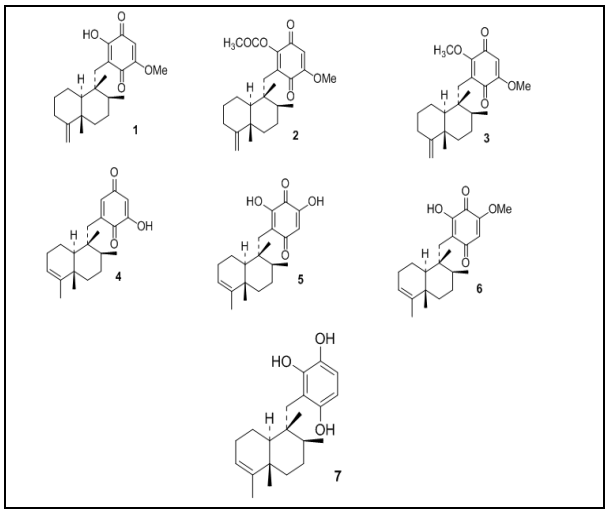

Fig. 2. Chemical structures of HIV-1 RNase H inhibitors of the natural source and their derivatives

Avarone A **(4)** and Avarone B **(5) (Fig. 2.)**, showed about 70% inhibition on RDDP function at IC_{50}= 6.8 µg/mL and 5 µg/mL, respectively and showed moderate activity on RNase H function with inhibitory percentages of 54% for Avarone A and 41% for Avarone B.

Avarol C and Avarone D showed almost no inhibitory action on RDDP function and poor activity on RNase H function with inhibitory percentages of 14% and 4%, respectively.

Avarone E **(6)** and Avarol F **(7) (Fig. 2)** were the best ones with inhibitory percentages of 91% and 72%, respectively and IC_{50} of 1 µg/mL and 7 µg/mL, respectively on RPPD function. Additionally, they showed inhibitory percentages of 94% and 100%, respectively on RNase H

function. They proved to be the most potent out of the 6 tested compounds so they were further tested for activity on the 3 catalytic functions of RT (RDDP, DDDP, and RNaseH) the result showed in case of Avarone E= 1 µg/mL, 6 µg/mL, 14 µg/mL and in case of Avarol F= 7 µg/mL, 4.5 µg/mL, 14.5 µg/mL for RDDP, DDDP, and RNase H activities, respectively **[21]**.

4.2. Hydroxylated Tropolones

β-thujaplicinol **(8) (Fig. 3)**, derived from the heartwood of cupressaceous trees, inhibited HIV-1 RNase H activity at IC_{50}= 0.21 µM without affecting the DNA polymerase function of the enzyme. To eliminate the possibility that the planar tropolone derivatives were intercalated into DNA/RNA hybrid and make the substrate unavailable for enzyme binding, surface plasmon resonance study was made and showed that the inhibition was not due to the intercalation of analog into the nucleic acid substrate **[22]**.

Yonetani-Theorell plot of RNase H inhibition using a non-nucleoside coumarin derivative, calanolide A, in the presence of β-thujaplicinol showed that the inhibitors bind to two independent sites.

β-thujaplicin **(9) (Fig. 3)**, a β-thujaplicinol derivative which lacks the hydroxyl function at position 7 of the heptatriene ring was inactive which suggests the importance of the 2, 7 dihydroxy function of these tropolone analogs possibly to act by two-metal catalyzed catalytic mechanism **[22]**.

In 2009, more studies were made to demonstrate the binding and inhibitory properties of β-thujaplicinol **(8)**. Results showed that β-thujaplicinol is a time-dependent RNase H inhibitor with noncompetitive kinetics that doesn't affect the polymerase activity of HIV-RT. Results also suggested that β-thujaplicinol need the presence of a bound RNA:DNA substrate to act properly on RT as it showed weak affinity to the free enzyme.

SPR studies showed that in absence of divalent metal ions, no inhibitor binding was detected **[23]**.

Another tropolones analogs (3, 7dihydroxytropolones) were reported to have good inhibitory action on RNase H activity. The 6-monosubstituted 3,7-dihydroxytropolone **(10)** and the 4, 6-disubstituted 3,7-dihydroxytropolone **(11) (Fig. 3)** inhibited HIV-1 RT RNase H activity showing IC_{50}= 1.3 and 4.7 µM, respectively **[24]**.

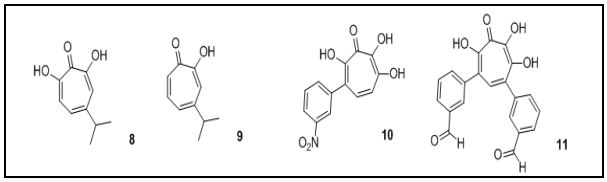

Fig. 3. Chemical structures of hydroxylated topolones active site HIV-1 RNase H inhibitors

4.3. Hydroxyquinoline (HID)

2-Hydroxyquinoline-1,3(2H,4H)-dione **(12)** (HID) **(Fig. 4)** previously reported **[25]** was designed as a targeted active site binding inhibitor with selectivity for two-metal-ion enzymes as influenza endonuclease. It was considered the prototype compound of a series of *N*-hydroxyimides for which three oxygen atoms present optimal distances for interaction with two divalent metal ions bound at a distance of 4-5 Å in the RT RNase H and IN active sites.

A series of 7-substituted 2-hydroxyquinoline-1,3(2H,4H)-diones was designed and biologically evaluated of dual IN/RNase H inhibition **[26]**. The prototype compound **(12)** showed HIV RNase H activity with IC_{50}= 5.9 µM which differs from which was reported by Klumpp (~1 µM) **[27]**, this may be attributed to the different

conditions of enzyme assay and the results showed that introduction of an arylcarbonylamino group or of a hydroxylated phenyl ring at position 7 retains the inhibitory activity of lead compound with IC_{50} range between 5.7-0.8 μM.

The rest of the substitutions enhanced the RNase H activity giving no clear SAR to be discussed, but they showed potential IN inhibition, unfortunately, all compounds showed high cytotoxicity in cell culture assays.

Also, the magnesium chelating properties of the compounds were suggesting that the anti-IN and anti-RNase H activities of 2-hydroxyquinoline-1,3(2H,4H)-diones can be related to their metal binding capacities. This proves that HID pharmacophore can be used as a chelating core for designing active site-directed RNase H inhibitors **[28]**.

Another series of C-6 benzyl and C-6 biarylmethyl HID analogs were synthesized and biologically evaluated **[28]**. All the analogs showed potent RNase H inhibition with an IC_{50} range between 0.4-2.9 μM but the C-6 biarylmethyl analogs were more potent than the C-6 benzyl ones suggesting that the additional aromatic ring can be advantageous for the inhibitory activity.

The most potent analogs were **(13)** and **(14)** (RNase H IC_{50}= 0.5 and 0.4 μM, respectively) **(Fig. 4)** featuring strong electron-withdrawing groups: cyano **(13)** and sulfonamide **(14)** at the terminal aromatic ring which suggests an inhibitor binding mode where the terminal aromatic group makes critical contacts with the RNA/DNA substrate. All the analogs showed good RT polymerase inhibition with IC_{50} values ranges between 6.5-0.5 μm. Analogs were further tested against NNRTI-resistant HIV RT mutants and results showed that the IC_{50} values were in range with those of wild-type which indicate that the compounds do not occupy the NNRTI pocket.

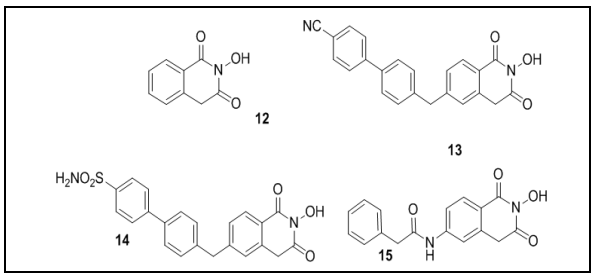

Fig. 4. Chemical structures of hydroxyquinoline active site HIV-1 RNase H inhibitors

Molecular modeling confirmed the interaction between the HID core and the 2 metal cofactors. Although the mechanism of RT polymerase inhibition of these compounds is not clear their potent dual inhibition against RT RNase H and polymerase make them very promising candidates for future development of HID-based anti-HIV drugs **[29]**.

Another study in which C-5, C-6 or C-7 substituted HID subtypes were synthesized and evaluated for both RNase H and RT polymerase activity showed that all HID subtypes tested inhibited RNase H in low micromolar range (IC_{50}= 0.20–2.0 μM) **[30]**.

HID analogues with an amide linkage at C-6 inhibited RNase H at submicromolar concentrations (IC_{50}= 0.20–0.30 μM), reflecting a 5–10 fold of improvement in potency over the ones without linkage, the most potent one was compound **(15)** (IC_{50}= 0.2 μM) **(Fig. 4)**, all analogs also showed RT polymerase inhibition in low micromolar range showing relatively no selectivity for RNase H **(15)** was considered the best not because it has the best selectivity for RNase H (7 folds) but because the other analogs showing better selectivity were biochemically less potent against RNase H inhibition. Molecular modeling studies suggested 2 possible binding

modes for RNase H **(15)**, the most probable one and showing the higher docking scores are metal coordination mode due to chelating properties of HID core **[30]**.

4.4. N-Acyl hydrazone [31]

One of the early classes to be reported as the N-acyl hydrazone, a new hydrazone derivative, *N*-(4-*tert*-Butylbenzyl)- 2-hydroxy-1 naphthaldehyde hydrazine (BBNH) **(16) (Fig. 5)**. It was reported to inhibit both the polymerase and the RNase H activities of HIV-1 RT *in vitro*. IC_{50} values for inhibition of RDDP were 0.8-3.4 µM, The DDDP IC_{50}~12 µM, while RNase H IC_{50} was 3.5 µM. It also inhibited HIV-1 replication in cell-based assays with EC_{50}= 1.5 µM and showed good activity against several mutant RT that have high-level resistance to other NNRTI **[31]**.

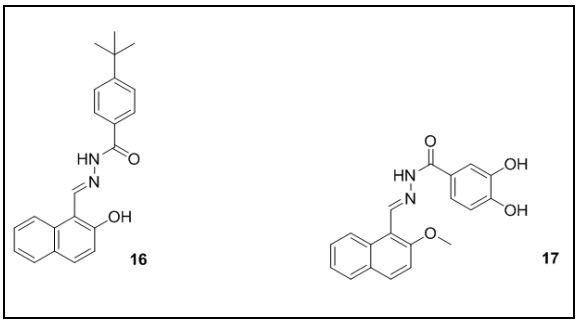

Fig. 5. Chemical structures of N-acyl hydrazone allosteric HIV-1 RNase H inhibitors

BBNH was hypothesized to bind to two different sites on HIV-1 RT. The first in the polymerase domain possibly nearby the NNRTI-binding site and the second one possibly located in the RNase H domain, the authors suggested that the time-dependent BBNH inhibition of RT RNase H may result from a slow conformational change in the RNase H domain of RT due to binding of BBNH to the NNRTI pocket **[32]**.

In 2006, a novel N-acyl hydrazone analog (*E*)-3,4-dihydroxy-*N*=-((2-methoxynaphthalen-1-yl)methylene) benzohydrazide (DHBNH) **(17) (Fig. 5)** was synthesized, it inhibited RNase H activity with IC_{50}= 0.5 µM in a non-competitive mechanism, it was unable to inhibit the RDDP activity of HIV-1 RT. But it showed ~40 fold decrease in potency (18.5 µM) at inhibiting a catalytically active isolated HIV-1 RT-RNase H domain which suggested that the binding pocket of DHBNH with RT may be outside the RNase H domain of the enzyme.

Crystal structure of RT/DHBNH complex showed that the inhibitor doesn't bind near RNase H active site, but it binds to a novel site >50 A° away, it is located in the palm of P66 subunit between the NNRTI-binding pocket and the polymerase active site. DHNBH is oriented with its benzoyl ring partially entering the NNRTI pocket and naphthyl ring near polymerase active site, SAR analysis predict that DHBNH derivatives with bulky substitutions at the *para* position of the benzoyl ring would extend to a part of the NNRTI-binding pocket and this may enhance the inhibition of the RT polymerase activity while retaining the ability to inhibit RNase H.

It was suggested that mode of action of DHNBH was through interference with correct positioning of DNA–RNA substrate, with the possibility that the inhibitor perturbs the trajectory of the template-primer so that RNase H cannot cleave the RNA strand of an RNA–DNA duplex **[33]**.

5. CONCLUSION

Over the last decade there has been a huge interest in the discovery and development of anti-HIV-1 RNase H compounds due to the emerging multi-drug resistance, a large number of scaffolds has been reported with different mechanisms of action, this provides promising chances for additional studies to be carried out to develop an

HIV-1 RT-associated RNase H inhibitor to reach clinical trials.

Conflict of Interest

The author(s) confirms that this article content has no conflicts of interest.

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