Recent Advances in the Research of HIV-1 RNase H Inhibitors

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Abstract: Reverse transcription is a crucial step in the life cycle of human immunodeficiency virus type 1 (HIV-1). In this process, multiple functional enzymes including RNA-dependent DNA polymerase, DNA-dependent DNA polymerase and RNase H are indispensable. The RNase H functions to degrade RNA of the RNA–DNA heteroduplex into small fragment. These properties of HIV-1 RNase H make it an attractive target for rational anti-HIV-1 drug design and development. In this review, we summarized the HIV-1 RNase H inhibitors that were recently reported in the literature, including their chemical structure, mechanism and structure-activity relationship. It seems likely that HIV-1 RNase H as a prominent non-traditional target may lead to the development of anti-HIV agents which could be used alone or in the combination with other HIV inhibitors in AIDS chemotherapy.

Key Words：HIV-1, AIDS, RNase H inhibitor, mechanism, SAR.

1. INTRODUCTION

Human immunodeficiency virus (HIV) is the pathogen of the acquired immunodeficiency syndrome (AIDS) that remains one of the world′s most serious health problems. In 2007, there were 33.2 millions people living with HIV, 2.5 millions of which were newly infected individuals, and 2.1 million patients died from AIDS globally [1]. Therefore, the prevention and therapy of AIDS are presently still a major medical challenge.

HIV consists of two types, HIV-1 and HIV-2, and the most frequent and virulent one is HIV-1. So far, the clinically approved HIV-1 inhibitors contain nucleoside/nucleotide reverse transcriptase inhibitors (N(t)RTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors (INIs), protease inhibitors (PIs) and entry inhibitors. Although the combination regimens of highly active anti-retroviral therapy (HAART) have dramatically decreased the morbidity and mortality from infection by HIV, the benefits of this approach are often compromised by the emergence of drug-resistant viral strains, severe side effects and poor patient compliance. Clearly, more endeavors should be made to develop new inhibitors with different targets and mechanisms.

Ribonuclease H (RNase H) plays an important role in the HIV-1 life cycle, and is a prominent target for the design and discovery of new anti-HIV agents that might provide new opportunities in the combination with other kinds of inhibitors in AIDS chemotherapy.

2. BIOLOGICAL FUNCTIONS OF THE HIV-1 RNASE H

RNase H appears to be ubiquitous in eukaryotes and bacteria. As a sequence-nonspecific endonuclease it belongs to nucleotidyl-transferase superfamily, in which, transposase, retroviral integrase, Holliday junction resolvase, and RNA-induced silencing complex (RISC) nuclease Argonaute are involved [2]. The mature HIV-1 reverse transcriptase (RT) is multi-functional enzyme, functioning as RNA-dependent DNA polymerase (RDDP) and DNA-dependent DNA polymerase (DDDP), and in strand displacement, strand transfer, and RNase H activities [3]. HIV-1 RT is a heterodimer composed of two subunits, p66 and p51, the latter being derived from p66 by proteolytic cleavage [4]. The two subunits are composed of four subdomains, namely, finger, palm, thumb and connection. The polymerase active site is located near the N-amino terminus of the p66, whereas the RNase H active site is near the carboxyl terminus starting from Tyr^{427} of p66 [5,6]. The structure of HIV-1 RT/DNA/primer complex is shown in Fig. (**1**).

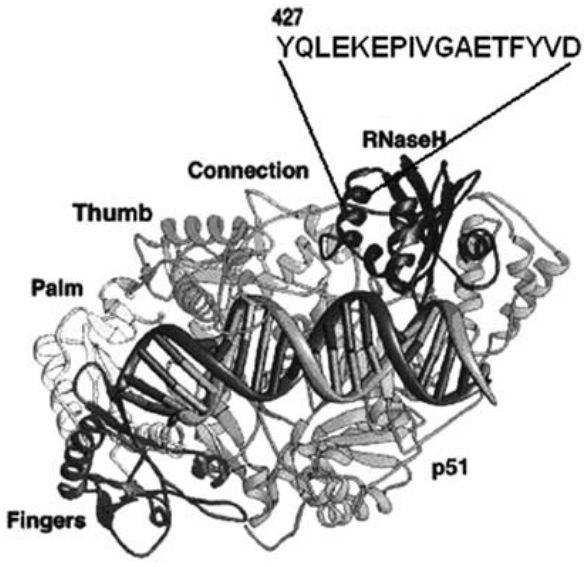

Fig. (1). The structure of HIV-1 RT/DNA/primer complex [5-6].

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2.1. The Function of HIV-1 RNase H

The RNase H of HIV-1 has two distinct functions: one is polymerization-dependent activity which is accompanied with RNA-dependent DNA synthesis and the nascent DNA 3′ primer terminus cleavages [7]; Another one is the polymerization-independent activity.

The conversion of the single-stranded HIV-1 RNA genome into the double-stranded DNA of the provirus requires the following activities of HIV-1 RNase H: (1) During minus-strand DNA synthesis, RNase H degrades the 5′ end sequence of the template RNA. (2) RNase H almost degrades the entire template RNA after the first strand transfers only leaving the polypurine tract (PPT) as primer for the plus-strand DNA synthesis. (3) RNase H then removes the PPT and tRNA primers respectively [8].

2.2. The Mechanism of HIV-1 RNase H Action

There are two conceivable mechanism of RNase H activity of HIV-1 RT during the degradation of the template strand: RT aligns to the RNase H active site approximately 18nt from the 5′ end of the RNA making the primary cut. The first possible pathway is that the enzyme repositions to the 5′ end of the RNA to make a secondary cut approximately in the middle of the 18-nt segment. The two products which are 8–9nt long are small enough for rapid dissociation. The second possible pathway is that the enzyme rebinds or slides toward the 3′ end of the RNA, cutting a product which is 5nt long. Afterwards, another primary cut, secondary cut or 5-nucleotide cut is carried out tautologically [9-11]. The mechanism of HIV-1 RNase H is shown in Fig. (**2**).

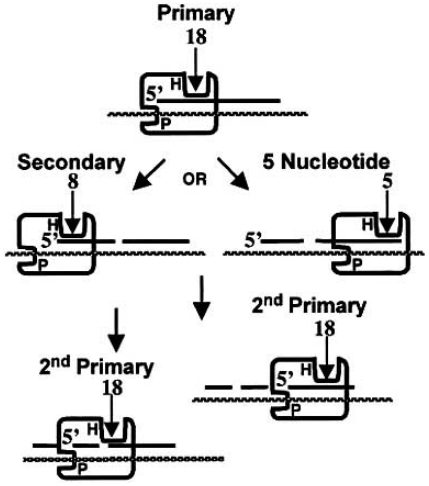


Fig. (2). A stepwise mechanism of HIV-1 RNase H [9].

Pattern lines represent DNA, whereas the solid lines represent RNA. The rectangle represents RT with the indents corresponding to the polymerase (P) and the RNase H (H) active sites.

Metal ions are prerequisite cofactors for the catalytic activities of HIV-1 RNase H domains; Klumpp [12] *et al.* found that the polymerization-independent activity of RNase H depended on the presence of metal ions. Mn^{2+} bind to the active sites of both polymerase and RNase H, while Mg^{2+} can only bind to the RNase H active site [13]. Crystallographic studies have confirmed that the Mn^{2+} binding pockets are formed by D443 and D549, and D443, E478, and D498, respectively. A cluster of four conserved carboxylates (D443, E478, D498, and D549) form a complex with the Mn^{2+} ions. If the divalent metal ion can not bind to the active site of RNase H, the RNase H will show low activity or even no activity [14]. The mechanism of requirement for metal-dependence and specificity has not been fully confirmed, but it provides an original way for drug design targeted at HIV-1 RNase H by using divalent metal ion chelators (Fig. (**3**)).

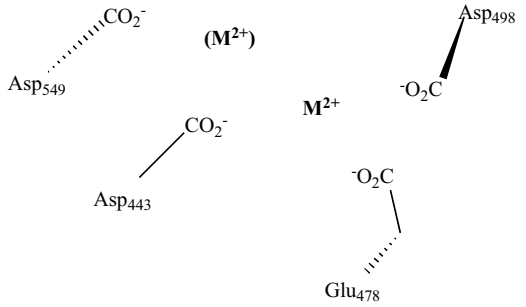
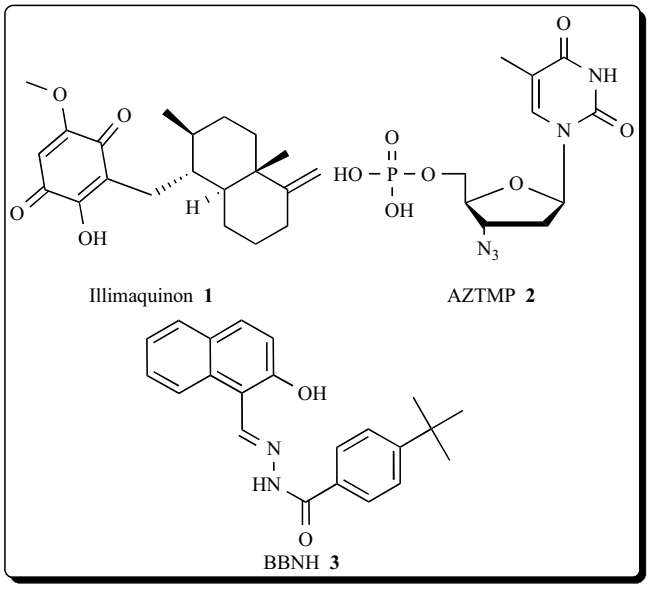


Fig. (3). The amino acid residue combined with metal ion at the active site of the HIV-1 RNase H [14].

3. HIV-1 RNASE H INHIBITORS

3.1. The Early Studies in HIV-1 RNase H Inhibitors

The search for HIV-1 RNase H inhibitors has been carried out since the 1990's, and earlier studies identified a few as leading compounds such as: dextran sulfate (DS) [15], Heparin, Illimaquinone (**1**) [16], AZTMP (**2**) [17] and phenylhydrazone derivatives represented by N-(4-tert-Butylbenzoyl)-2-hydroxy-1-naphthaldehyde hydrazone **(**BBNH, **3**) [18]. These compounds inhibited RNase H *in vitro* to some extent, but further studies indicated that these compounds were not selectively active against RNase H. These compounds, except for BBNH, did not exhibit inhibitory activity against RNase H in the HIV-1 replication process [19-21].



3.2. The Recent Research in HIV-1 RNase H Inhibitors

In recent years, with the development of molecular and structural biology, and medicinal chemistry, the study of HIV-1 RNase H inhibitors has been boosted, in particular with regard to the structural dynamics, and crystal structures of the complex of active site binding with RNase H inhibitors [22]. The newly reported inhibitors can be classified as follows: the first type of compounds such as the small molecule RNA fragment inhibitors competitively binds to the RNase H active site so as to interrupt the hydrolyzation of the DNA/RNA substrate by RNase H; the second type of compounds bind to a site near the RNase H active site so as to limit RNase H activity; the third type of compounds acted as metal-ion chelators.

3.2.1. Small Molecule RNA Fragment Inhibitors

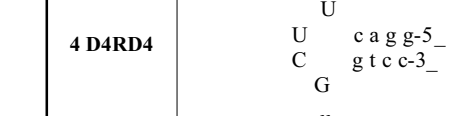
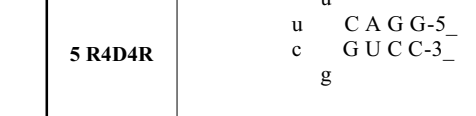
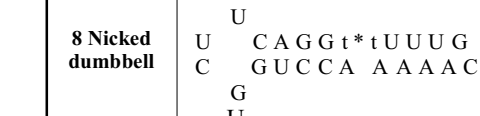
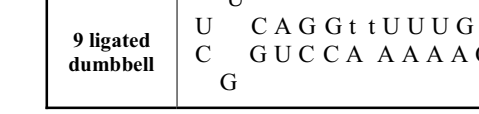
Recently, it has been reported that the oligonucleotide with 35 base pairs based on the G-quartet motif inhibit the RNase H and the polymerase at an IC_{50} of 500nM. Similarly to the other oligonucleotides, this kind of inhibitors has serious defaults, e.g. poor selectivity, and facile hydrolysis by the widespread nucleases [23,24]. Meanwhile, some DNA thioaptamers having certain proportion G-residues as consensus sequence and being modified by sulfur substitutions of the phosphoryl oxygens proved to inhibit RNase H activity and viral replication *in vitro* [25].

Hannoush and his colleagues [26] designed some small molecule oligonucleotides with structural characters of hairpins and dumbbells. These inhibitors were found to effectively inhibit the HIV-1 RNase H. Experiments showed that these oligonucleotides with high stability were not easily hydrolyzed by ubiquitous enzymes. In addition, with the structural feature of hairpins and dumbbells, these oligonucleotides did not inhibit the activity of the polymerase related to the HIV-1 RT, and, importantly, these oligonucleotides virtually did not inhibit the RNase H of mammals, which demonstrated that they had selectivity to the HIV-1 RNase H, and, therefore, should be further explored as potential anti-HIV drug candidates. The structures and the results of their inhibitory activities against HIV-1 RNase H are shown in Table **1**.

The basic structure of this aptamer was a ring of UUCG and a stem contained 4-6 base pairs. The structure activity relationship (SAR) showed that the factors influencing their activities included the helical configuration of short chain RNA, the number of the base pairs in the stem, RNA residues or DNA residues in the ring and stem, the sort of linkage between the RNA residues [26].

As shown in the Table **1**, if the RNA residues were replaced by the DNA residues, namely **D4RD4** and **R4D4R**, their activities disappeared thoroughly. The aptamer with α-helical configuration had higher activity. In comparison with **R4RR4**, ***R4*R*R4*** having the 2′ 5′-phosphodiester linkages of RNA residues in the stem did not show a change in activity. Binding the RNA residues with 2′ 5′-phosphodiester linkage in the loop generated **R4*R*R4** with three-fold lower activity. Addition of the base pairs in the stem generated the **R6RR6** with three-fold higher activity in comparison with **R4RR4**

Table 1. Potency of Hairpin and Dumbbell Inhibitors in Enzymatic Assays [26]

No.	Oligonucleotide	IC_{50}/μmol.L^{-1}
1 R4RR4		25.8
2 *R4RR4*		26.2
3 R4*R*R4		69.3
4 D4RD4		>>100
5 R4D4R		>>100
6 R6RR6		7.8
7 R6*R*R6		29.7
8 Nicked dumbbell		40.4
9 ligated dumbbell		3.3

Note: RNA residues are represented by capital letters, whereas small letters indicate DNA residue. Capital underlined letters represent 2′-5′-RNA residues. The asterisk represents a nicked stem (i.e. no linkage exists between the neighboring residues, resulting in open 5′-OH and 3′-monophosphate ends).

[26]. **Ligated dumbbell(9)** exhibited the best activity against HIV-1 RNase H with an IC_{50} value of 3.3μM.

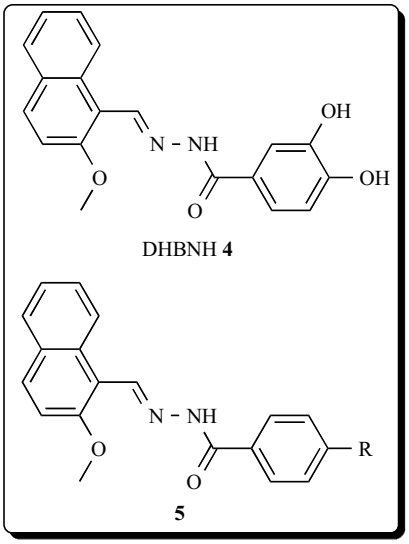
3.2.2. Dihydroxybenzoylnaphthyl Hydrazone (DHBNH)

Recently, dihydroxybenzoylnaphthyl hydrazone (DHBNH **4**) was found to be a new lead compound of HIV-1 RNase H inhibitors targeting a novel site [27] with an IC_{50} of 0.5μM [28]. DHBNH **4** was also found to have inhibitory activity

against drug-resistant HIV-1 RT variants Y181C RT and Y188L RT with an IC_{50} of 0.65μM and 1.2μM respectively, while the NNRTI Efavirenz showed no inhibitory activity under the same condition.

The action mechanism of action of DHBNH is probably based on preventing RNA strand from binding to the RNase H active site by locating at the novel site next to the polymerase and NNRTI binding pockets, so that the RNase H could not hydrolyze the RNA strand of the RNA–DNA duplex.

On the basis of the structure of DHBNH, some *para*–substituted DHBNH derivatives (**5**) on the benzoyl ring were designed and synthesized, which were confirmed to inhibit polymerase activities of HIV-1 RT by binding to the NNRTI-binding pocket without the anti-RNase H activity.



Studies on DHBNH (**4**) and *para*–substituted DHBNH derivatives (**5**) provided an important proof of the new strategy for design and development of "dual inhibitors" in anti-HIV drug research, that is drugs that could simultaneously inhibit the HIV-1 polymerase and HIV-1 RNase H activity.

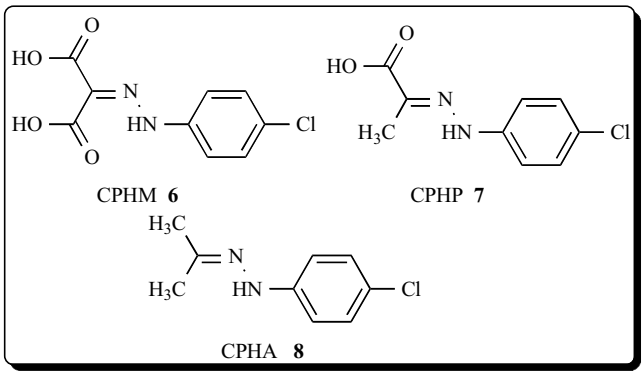
3.2.3. Inhibitors Based on Metal-Ion Co-Factor Mechanism

Divalent metal-ions are essential for RNase H activity. There are three possible models of actions by which metal ion chelators could act as HIV-1 RNase H inhibitors. First, HIV-1 RNase H inhibitors may inhibit enzyme activities by blocking access of the metal ion to the active site of RNase H. Second, they may block the recruitment of metal ions to the active site of RNase H. Third, the chelators may bind to the metal ions at the active site of enzyme, and remain bound to inhibit the enzymatic functions. HIV-1 RNase H inhibitors should at the same time be potent and selective, and if the first and the second models would operate specific RNase H inhibitors may not exist. Thus, the third model is the more likely. It is possible that the enzyme-inhibitor interaction is dependent on the multiple interactions of the chelator with RNase H. To improve the selectivity of RNase H inhibitors it may be desirable to design drugs that have no metals involved in the interaction with the enzyme.

So far, there are four series of chelators reported to act as potent HIV-1 RNase H inhibitors, i.e. CPHM and its derivatives (**6-8**), tropolone and its derivatives (**9-16**), N-hydroxyimides (**17-20**) and diketo acids (**22-24**).

(1). 4-chlorophenylhydrazone of Mesoxalic Acid (CPHM)

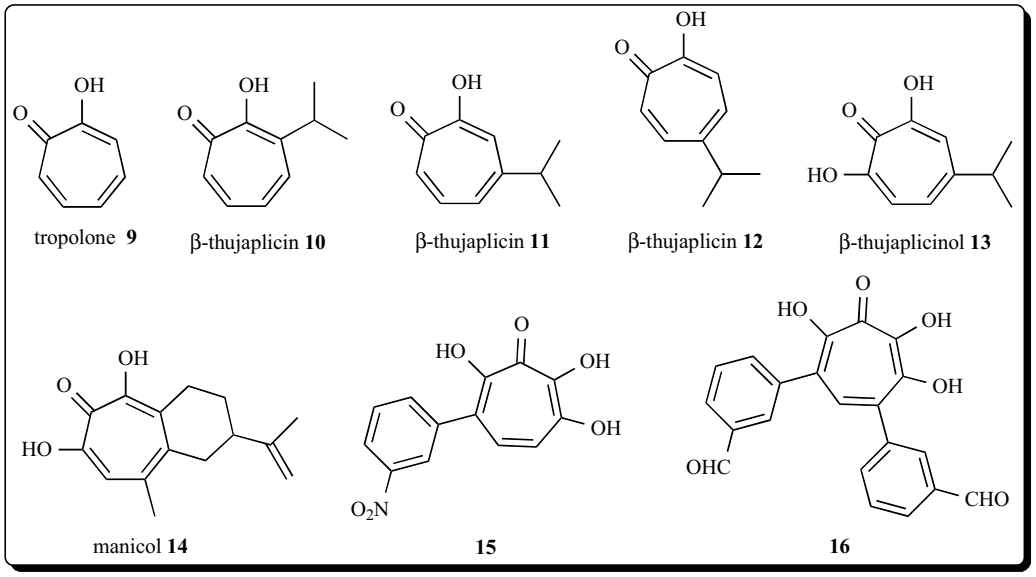
CPHM (**6**) belongs to dicarboxylic acid compound which showed high activity against polymerase-independent RNase H activity with an IC_{50} of 3 μM *in vitro* [29]. The specificity of CPHM binding target was proved by excluding inhibition against HIV-1 RT activity. Furthermore, the enzymatic binding assays indicated that CPHM (**6**) only had weak inhibitory activity against HIV-1 polymerase, but also had less or no inhibitory activity against DNA polymerase I, T7 DNA polymerase and murine leukemia virus (MLV) or avian myeloblastosis virus (AMV) reverse transcriptase. All experimental results demonstrated that CPHM (**6**) was targeted at RNase H activities by inhibiting the DNA strand transfer process [29]. Although the activity and selectivity of CPHM has been verified *in vitro*, CPHM inhibitory activity against HIV-1 RNase H *in vivo* and toxicity in cell culture or *in vivo* still need to be investigated.



Davis and his colleagues [30] found that CPHM inhibited HIV-1 RNase H by directly chelating Mg^{2+}. The dicarboxylic acid moiety of this kind of compounds was crucial for preserving their activities. 4-chlorophenyhydrazone of pyruvic acid (CPHP, **7**) and 4-chlorophenylhydrazone of acetone (CPHA, **8**) showed poor inhibitory activity against HIV-1 RNase H because of the shortage of one or two carboxylic acid moieties.

(2). Tropolone and its Derivatives

Tropolone (**9**) and its derivatives (**10-14**), a series of natural products, were obtained from the heartwood of several cupressaceous plants which have many biological effects, for example, antitumor, antifungal, insecticide, antimicrobial effects. Recent investigations showed that these compounds effectively inhibited HIV-1 RNase H activity *in vitro* [31]. The most effective derivative was β-thujaplicinol (**13**) (2,7-dihydroxy-4-1 (methylethyl)-2,4,6-cycloheptatrien-1-one) with the inhibitory activity against both HIV-1 RNase H and *E.coli* RNase H at a concentration of 0.2 μM and 50 μM respectively. In addition, β-thujaplicinol (**13**) was found to inhibit HIV-1 integrase markedly in biochemical assays and had appreciable cytoprotective activity against HIV-1-induced cytopathogenicity in a cell-based assay [32]. Another derivative manicol (**14**) had inhibitory activity gainst



HIV-1 RNase H and *E.coli* RNase H with an IC_{50} of 1.5μM and 40μM respectively. Both β-thujaplicinol (**13**) and manicol (**14**) inhibited DNA-dependent DNA polymerase of HIV-1 RT at a concentration of more than 50μM, suggesting that they are specific inhibitors of the HIV-1 RNase H.

Structure-activity relationship analysis confirmed that the 7-hydroxy group was necessary for ensuring the inhibitory activity of these kinds of compounds. Tropolone (**9**) and the derivatives of α, β and γ-thujaplicins (**10-12**) had no or very weak activity against HIV-1 RNase H, in comparison with the 7-hydroxytropolone derivatives β-thujaplicinol (**13**) and manicol (**14**).

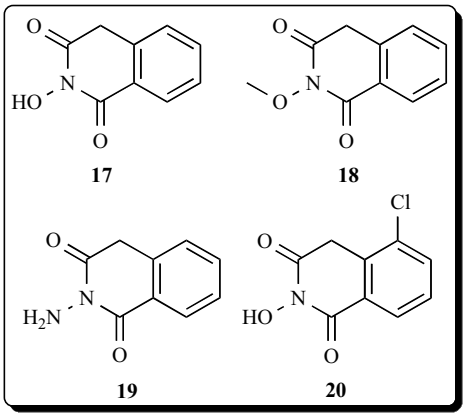
When β-thujaplicinol (**13**) was used in combination with calanolide A, a non-nucleoside inhibitor of HIV-1 RT, synergism was found in the anti-HIV-1 activity experiments, which reflects its possible mechanism of the dual-action by targeting both HIV-1 RT and RNase H.

Some new hydroxytropolone derivatives were designed, synthesized and found having enhanced inhibitory potency against HIV-1 RNase H, compared with that of β-thujaplicinol (**13**) [31]. For example, monosubstituted 3,7-dihydroxytropolone (**15**) and unsubstituted 3,7-dihydroxy- tropolone (**16**) inhibited HIV-1 RNase H with IC_{50} values of 1.3μM and 4.7μM respectively [33]. However, these two compounds showed high cellular toxicity, which limits their further investigation. Thus, further molecular modification will be an inevitable approach for improving their potency and decreasing their toxicity.

(3). N-Hydroxyimides Inhibitors

N-hydroxyimides were previously designed as influenza endonuclease inhibitors by binding to the divalent metal ions at the active site of the enzyme, which were found to inhibit HIV-1 replication by targeting at both the independent domain of RNase H and full-length HIV-RT protein. Compound **17** was the best of all compounds with high selectivity and activity in the assay using CGK1 as substrate, exhibiting an IC_{50} of 0.6μM against HIV-1 RNase H. In another assay, using poly(dC:rG) as a template, compound **17** showed significant inhibitory activity against HIV-1 RNase H with an IC_{50} value of 1 μM, without the inhibitory effect on the *E.coli* RNase H [34]. Similarly, compound **17** inhibited the isolated HIV RNase H domain with an IC_{50} of 0.43μM in the assay using substrate CGK1. Under the same conditions, compound **20** also showed significant inhibition of the isolated HIV-1 RNase H domain (IC_{50} = 0.38 μM) [34].

The study of SAR showed that the N-hydroxyl group acting as the bridge/ligand was essential for keeping inhibitory activity. With the substitution of the hydroxyl group by the methoxy or amino group, the inhibitory effects of compounds **18** and **19** disappeared completely. Substitutions on the different positions of the phenyl moiety, e.g. compound **20** with the 4-chloro substitution, might improve the activity and the selectivity [34].



(4). Diketo Acid Inhibitors

The discovery of the diketo acid inhibitors is based on the hypothesis of metal cation/carboxylate coordination, and this principle has already been successfully used in the research of HIV-1 integrase inhibitors. Because of the similarities in structures of HIV-1 intergrase and RNase H, which belong to the superfamily of the nucleotidyl-transferase, diketo acids might be active against HIV-1 RNase H [35].

Compound **21** was the best model of the interaction between the divalence metal ion and the integrase diketo acid inhibitor [36] (Fig. (**4**)).

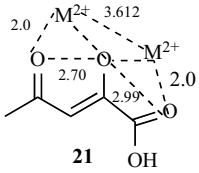


Fig. (4). Interaction between the metal ion and the integrase diketo acid inhibitor [36].

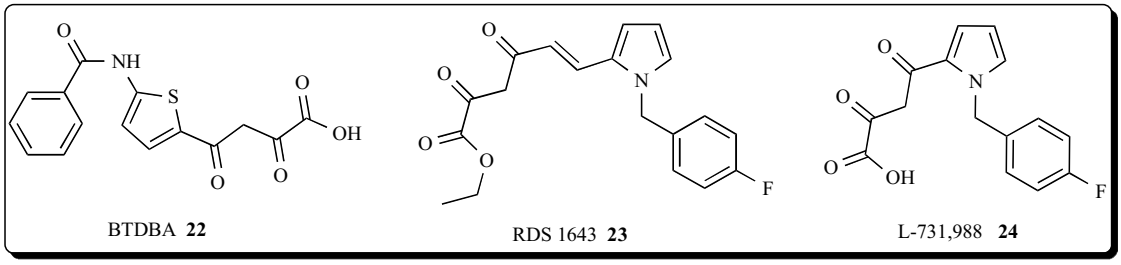
Diketo acid derivative, 4-[5-(benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid (BTDBA, **22**), was the first reported HIV-1 RNase H inhibitor, which inhibited the HIV-1 RNase H as part of HIV-RT with an IC_{50} of 3.2μM without influencing the activity of HIV-1-dependent RNA and DNA polymerase, and inhibited the isolated HIV-RNase H domain with a similar IC_{50} value of 4.7 μM. Also, BTDBA **22** was found to inhibit the HIV-1 integrase at a samilar concentration.

Tramontano *et al.* [37] demonstrated that a new diketo acid derivative, RDS 1643 (**23**), could selectively inhibit the activity of HIV-1 RNase H without influencing the activity of avian myeloblastosis virus (AMV) RT-RNase H, *E.coli* RNase H and had little influence on the HIV-1 integrase. The results showed that RDS 1643 (**23**) inhibited HIV-1 RNase H with an IC_{50} of 13μM. The inhibition to the HIV-1 integrase needed the concentration of 92-98μM.L^{-1}, while the HIV-1 integrase inhibitor, L-731 988 (**24**) inhibited the activity of integrase with a IC_{50} of 0.2μM.

RDS 1643 (**23**) selectively inhibited the activity of polymerase-independent HIV-1 RNase H *in vitro*; it also inhibited the virus replication including the drug-resistant HIV-1 RT variants in the cell culture assay. Taken together, the obtained results from all experiments indicated that RDS 1643 (**23**) was the first promising compound with good selectivity and high potency in enzyme and cell culture assay until now.

3.2.4. Other Inhibitors with Unknown Binding Sites

Min *et al.* [38] found that RNase H activity was peaceably inhibited by some naphthoquinones such as 1,4-naphthoquinone (**25**), vitamin K_3 (**26**), juglone (**27**) and plumbagin (**29**), while other compounds such as naphthazarin (**28**) and shikonins (**30–32, 33–34**) inhibited RNase H activity weakly. The most active compound **25** showed inhibitory activity against HIV-1 RNase H with an IC_{50} of 9.5μM without marked inhibitory activity on RDDP and DDDP. However, RDDP and DDDP activities can be inhibited effectively by compound **27** and **29** with an IC_{50} of 8μM and 10μM, and 5μM and 7μM, respectively.



The SAR study of the naphthoquinones confirmed that compounds without hydroxyl substituents (**25** and **26**) had notable inhibitory activity against HIV-1 RNase H, while one- hydroxylated naphthoquinones (**27** and **29**) showed poor inhibitory activity and two-hydroxylated naphthoqui-

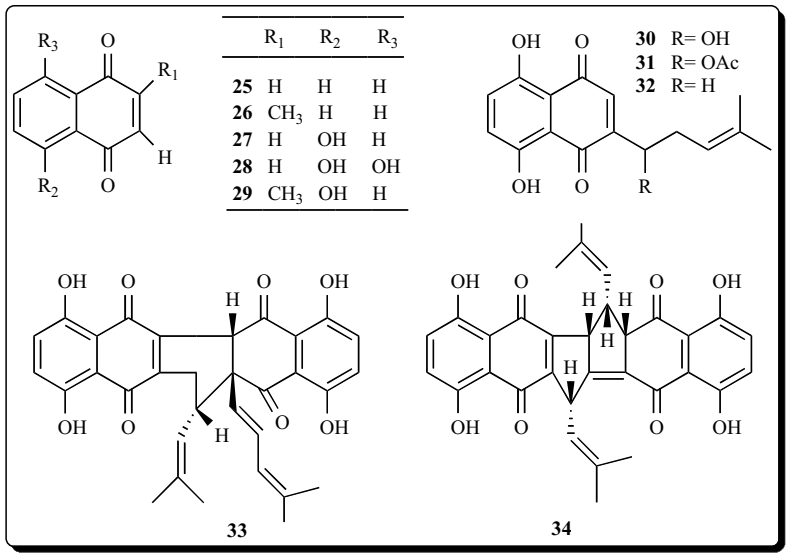


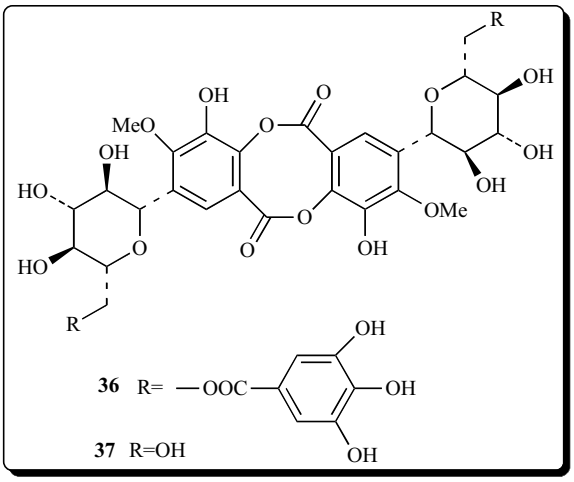
Table 2. Inhibitory Effects of Quinones on HIV-1 RNase H Activity

Compounds	IC_{50}/μmol.L^{-1}
1,4-Naphthoquinone (**25**)	9.5
Vitamin K3 (**26**)	75
Juglone (**27**)	95
Naphthazarin (**28**)	>100
Plumbagin (**29**)	80
Shikonin (**30**)	>100
Acetylshikonin (**31**)	>100
Deoxyshikonin (**32**)	>100
Shikometabolin C (**33**)	>100
Shikometabolin D (**34**)	>100

nones(**28, 32-34**) had no inhibitory activity at all. RDDP and DDDP activities could be inhibited by 5-hydroxylated or 5,8-hydroxylated naphthoquinones. Some trimeric naphthoquinone derivatives were reported to inhibit HIV-1 intergrase *in vitro* by binding with metal cations [39], indirectly reflecting that hydroxylated naphthoquinone derivatives inhibit HIV-1 RNase H by the metal-ion co-factor pathway.

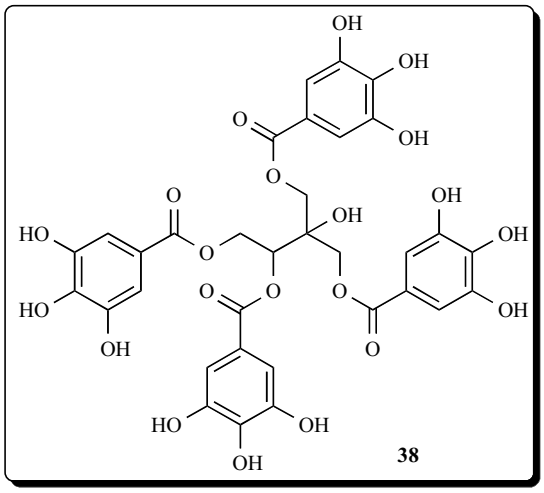
A series of naphtalenesulfonic acid derivatives has been obtained by structure-based design and a combinatorial medicinal chemistry approach [40]. The most potent of these compounds was compound **35**, which, in enzyme assays, inhibited the HIV-1 RNase H and RDDP activities at 25-100 nM and 90 nM, respectively; it had a Kd value for DNA-RT binding of 40 nM, and in cell-based assays it showed an EC_{50} value of 2.5 μM and a CC_{50} value of 112 μM. Further enzymeatic studies showed that (**35**) did not affect T7, T4 and Klenow DNA polymerases while it inhibited the MLV RDDP activity [40]. Probably, this compound prevented retroviral RT interaction with the substrate heteroduplex, even though the two orders of magnitude between the inhibitory potencies obtained in enzyme and cell-based studies did not allow to exclude that its *in vivo* mode of action could involve viral components other than RT.

Ardimerin digallate (**36**), a new natural compound which is extracted from natural plant of *Ardisia japonica,* was certified to inhibit HIV-1 RNase H with an IC_{50} of 1.5μM *in vitro* without inhibiting both human and *E. coli* RNase H at a concentration of 200μM.L^{-1} [41]. the galloyl group is an important pharmacophore for anti-HIV activity [42,43], thus the galloyl moiety in Ardimerin digallate molecule is indispensable for reserving the inhibitory activity. Ardimerin (**37**) had no inhibitory activity against HIV-1 RNase H because of the absence of galloyl unit.

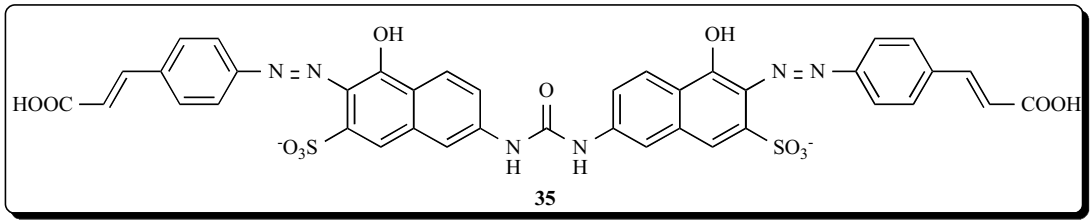


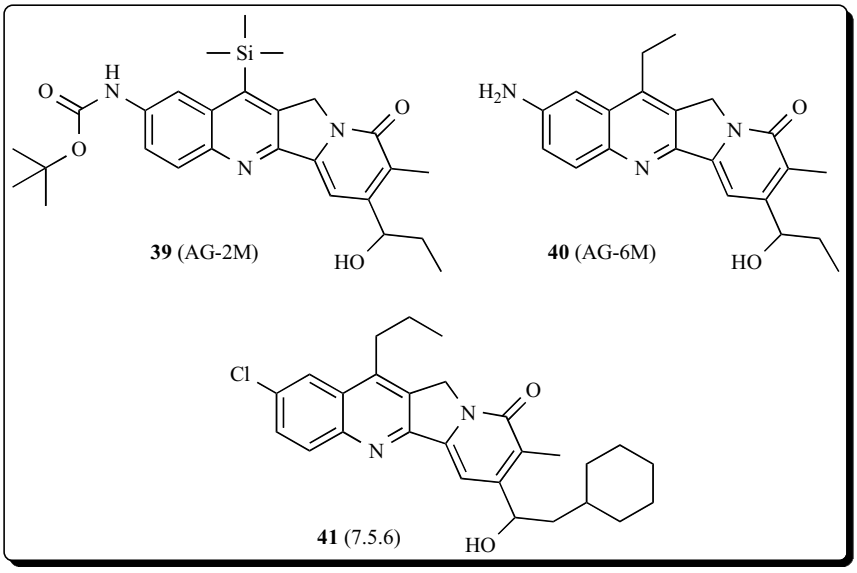
In addition, Ardimerin digallate could also effectively inhibit HIV-2 RNase H activity without inhibition of the DNA polymerase of reverse transcriptase at the same concentrations. Therefore, ardimerin digallate could be regarded as a novel anti-HIV drug candidate targeting both HIV-1 and HIV-2 RNase H.

A new natural compound, 1,3,4,5-tetragalloylapiitol (**38**), extracted from the plant *Hylodendron Gabunensis,*was found



to have significant inhibitiory activity against HIV-1 RNase H and HIV-2 RNase H with an IC_{50} of 0.24 and 0.13 μM, respectively. It had a poor selectivity with inhibitory activity





against human RNase H (IC_{50}=1.5μM) and the *E. coli* enzyme in a fluorescence resonance energy transfer (FRET) based assay [44].

Finally it is worth reporting that some mappicine analogues (**39**-**41**) inhibit HIV-1 RNase H activity, in enzyme assays, with IC_{50} values of 2-10 μM. They also inhibit replication, in cell based assays, of wild type and NNRTI resistant mutant HIV- 1 (RT mutations were K103N/Y181C and V106A/Y181C) [45].

4. PROSPECT

Since the 1990's, HIV-1 RNase H had been regarded as an important enzyme playing a crucial role in HIV-1 replication. However, there are still no RNase H inhibitors entering clinical trials. At the same time, the emergence of multi-drug resistance, cross resistance, and toxicity, including reverse transcriptase inhibitors, protease inhibitors and cell entry inhibitor, stimulate medicinal chemists for discovery and development of new HIV-1 inhibitors, especially those exhibiting new mechanisms of action. HIV-1 RNase H is an example of a viable target for development of novel HIV inhibitors. Recent studies have shown that the combinatory use of HIV-1 RNase H inhibitors with other type of anti-AIDS agents, for example, the HIV-1 RNase H inhibitor of diketo acid with efavirenz, one of the HIV-1 NNRTIs, have synergism on inhibition of the HIV-1 replication [46]. However, it should be taken into account that HIV-1 RNase H inhibitors might result in the enhancement of HIV-1 NRTIs resistance [47].

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ABBREVIATIONS

HAART	=	Highly active antiretroviral therapy
HIV(-1)	=	Human immunodeficiency virus (type-1)
AIDS	=	Acquired immune deficiency syndrome
NNRTIs	=	Non-nucleoside reverse transcriptase inhibitors
NRTIs/NtRTIs	=	Nucleoside (nucleotide) reverse transcriptase inhibitors
RT	=	Reverse transcriptase
INIs	=	Integrase inhibitors
PIs	=	Protease inhibitors
RNase H	=	Ribonuclease H
RISC	=	RNA-induced silencing complex
RDDP	=	RNA-dependent DNA polymerase
DDDP	=	DNA-dependent DNA polymerase
DS	=	Dextran sulfate
BBNH	=	N-(4-tert-butylbenzoyl)-2-hydroxy-1-naphthaldehyde hydrazone
DHBNH	=	Dihydroxybenzoylnaphthyl hydrazone
SAR	=	Structure activity relationship
CPHM	=	4-Chlorophenylhydrazone of mesoxalic acid
CPHP	=	4-Chlorophenyhydrazone of pyruvic acid
CPHA	=	4-Chlorophenylhydrazone of acetone
MLV	=	Murine leukemia virus
AMV	=	Avian myeloblastosis virus

DKA = Diketo acid

BTDBA = 4- [5-(Benzoylamino)thien-2-yl]-2,4-dioxobutanoic acid

FRET = Fluorescence resonance energy transfer

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