Received: 24 July 2018 | Revised: 13 November 2018 | Accepted: 24 November 2018

DOI: 10.1111/cbdd.13455

WILEY

RESEARCH ARTICLE

Design, synthesis, and biologic evaluation of novel galloyl derivatives as HIV-1 RNase H inhibitors

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Funding information
Major Project of Science and Technology of Shandong Province, Grant/Award Number: No. 2015ZDJS04001; Ministry of Science, Innovation and Universities, Grant/Award Number: BIO2016-76716-R (AEI/FEDER, UE); National Natural Science Foundation of China, Grant/Award Number: 81273354; Young Scholars Program of Shandong University, Grant/Award Number: YSPSDU No. 2016WLJH32, to P. Z.; Key Project of NSFC for International Cooperation, Grant/Award Number: 81420108027; Division of Intramural Research, National Institute of Allergy and Infectious Diseases, Grant/Award Number: NIH grant AI033066;

Abstract

Human immunodeficiency virus (HIV) reverse transcriptase (RT)-associated ribonuclease H (RNase H) remains as the only enzyme encoded within the viral genome not targeted by current antiviral drugs. In this work, we report the design, synthesis, and biologic evaluation of a novel series of galloyl derivatives with HIV-1 RNase H inhibitory activity. Most of them showed IC_{50}s at sub- to low-micromolar concentrations in enzymatic assays. The most potent compound was **II-25** that showed an IC_{50} of 0.72 ± 0.07 μM in RNase H inhibition assays carried out with the HIV-1_{BH10} RT. **II-25** was 2.8 times more potent than β-thujaplicinol in these assays. Interestingly, **II-25** and other galloyl derivatives were also found to inhibit the HIV IN strand transfer activity in vitro. Structure–activity relationships (SAR) studies and molecular modeling analysis predict key interactions with RT residues His539 and Arg557, while providing helpful insight for further optimization of selected compounds.

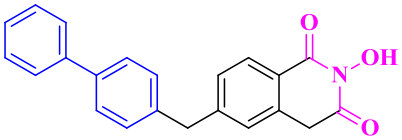
KEYWORDS
galloyl derivatives, HIV-1, RNase H inhibitors

Key Research and Development Project of Shandong Province, Grant/Award Number: No. 2017CXGC1401; Shandong Province Key R&D Plan, Grant/Award Number: No. 2017GSF218030

1 | INTRODUCTION

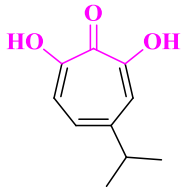
The global pandemic of human immunodeficiency virus (HIV) remains as a major threat to human health worldwide. Standard treatments against HIV infection, including highly active antiretroviral therapies (HAART), often include combinations of drugs targeting all three HIV type 1 (HIV-1)-encoded enzymes: protease, reverse transcriptase (RT), and integrase (IN). While HAART has proven to be effective in suppressing viral replication, its long-term efficacy could be limited by adverse effects due to drug toxicity and the emergence of drug resistance. Therefore, there is still a need for anti-HIV drug candidates, particularly for those acting on novel targets or showing mechanisms of action different from those used by currently approved drugs (Zhan, Pannecouque, De Clercq, & Liu, 2016).

HIV-1 RT is a well-established target that plays a key role in viral replication by converting the viral genomic



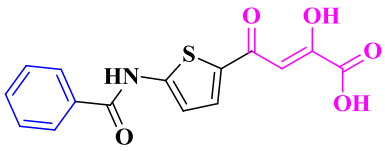
1. Hydroxylisoquinoline

$IC_{50}^{\ RNase\ H} = 0.8\ \mu M$
HIV-1: $EC_{50} = 3.0\ \mu M$



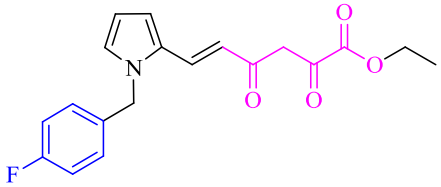
2. β-Thujaplicinol (BTP)

$IC_{50}^{\ RNase\ H} = 0.21\ \mu M$



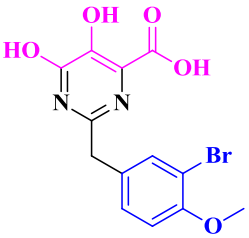
3. Diketoacid

$IC_{50}^{\ RNase\ H} = 3.2\ \mu M$



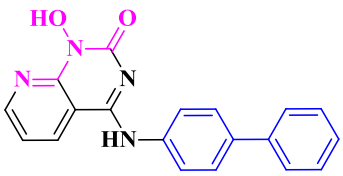
4. Diketoacid ester

$IC_{50}^{\ RNase\ H} = 13\ \mu M$
HIV-1: $EC_{50} = 14\ \mu M$



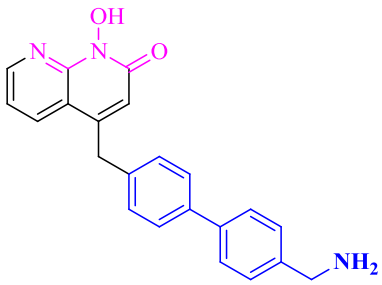
5. Pyrimidinol carboxylic acid

$IC_{50}^{\ RNase\ H} = 0.9\ \mu M$



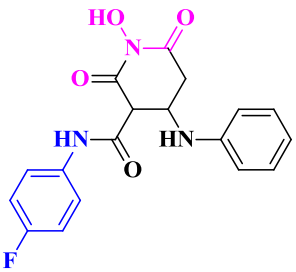
6. Naphthyridinone

$IC_{50}^{\ RNase\ H} = 0.032\ \mu M$
HIV-1: $EC_{50} = 0.10\ \mu M$



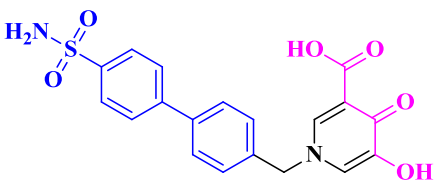
7. Pyridopyrimidinone

$IC_{50}^{\ RNase\ H} = 0.045\ \mu M$
HIV-1: $EC_{50} = 0.19\ \mu M$



8. 3-hydroxypyrimidine-2,4-dione (HPD)

$IC_{50}^{\ RNase\ H} = 0.004\ \mu M$
HIV-1: $EC_{50} = 6.1\ \mu M$



9. Hydroxypyridonecarboxylic acid

$IC_{50}^{\ RNase\ H} = 0.65\ \mu M$

FIGURE 1 Representative RNase H inhibitors [Colour figure can be viewed at wileyonlinelibrary.com]

single-stranded RNA into double-stranded DNA (Menéndez-Arias, 2013; Menéndez-Arias, Sebastian-Martin, & Alvarez, 2017). The viral RT possesses two distinct enzymatic functions: a DNA polymerase activity that synthesizes the proviral DNA and a ribonuclease H (RNase H) activity that selectively degrades the RNA strand in RNA/DNA replication intermediates (Tang et al., 2017). All currently approved antiretroviral drugs targeting the RT are DNA polymerase inhibitors. HIV RNase H inhibitors have not been approved for clinical use. Due to its important role in viral replication as well as its highly conserved catalytic site, the RNase H is a potentially important and underexploited target for designing novel anti-HIV drugs.

According to their mechanism of action, HIV-1 RNase H inhibitors can be classified into active-site or allosteric inhibitors (Cao, Song, De Clercq, Zhan, & Liu, 2014). Notably, the search for RNase H active-site inhibitors is becoming an important topic in the field of antiviral drug research. RNase H active-site inhibitors act by chelating the two divalent metal ions essential for the degradation of the RNA strand in RNA/DNA hybrids. Examples of compounds with such a mechanism of action are given in Figure 1 and include *N*-hydroxylisoquinolinediones (HID; Billamboz et al., 2008; Kankanala et al., 2017; Vernekar et al., 2015), β-thujaplicinol (Budihas et al., 2005), diketoacid derivatives (Tramontano et al., 2005), pyrimidinol carboxylic acids (Lansdon et al., 2011), naphthyridinones (Williams et al., 2010), pyridopyrimidinones (Beilhartz et al., 2014), hydroxypyrimidines (Tang et al., 2016), 3-hydroxypyrimidine-2,4-diones (HPD; Tang et al., 2017; Vernekar et al., 2017; Wang et al., 2018a,b; Wu et al., 2016), and hydroxypyridonecarboxylic acids (Kankanala et al., 2016). For reviews, see references (Yu, Liu, Zhan, & De Clercq, 2008; Wang, Gao, Menéndez-Arias, Liu, & Zhan, 2018c).

Although HIV-1 RNase H inhibitors include molecules showing a wide range of structurally diverse scaffolds, the RNase H active-site inhibitors have a common pharmacophore structure containing a divalent metal ion chelating group, a linker, and a hydrophobic moiety.

Nowadays, natural products have become an important source of novel lead compounds. Among them, galloyl derivatives show diverse physiologic activities, acting as antioxidants and agents protecting against cardiovascular disease, as well as antitumoral and antiviral drugs. β-Thujaplicinol is a natural monoterpenoid found in the wood of trees of the family *Cupressaceae* (Chedgy, Lim, & Breuil, 2009). This compound inhibits HIV-1 RNase H activity in vitro with an IC_{50} of around 0.2 μM, but is less potent against human and bacterial (*Escherichia coli*) RNases H with IC_{50}s of 5.7 and 50 μM, respectively (Budihas et al., 2005). Our approach involved the substitution of the original heptameric ring of β-thujaplicinol by an aromatic galloyl group. Previously, the chelating capacity of this galloyl ring had been shown in the crystal structure of compound **10** bound to H1N1 influenza virus polymerase acidic endonuclease (Figure 2; Carcelli et al., 2016). Two series of galloyl derivatives were designed by introducing diversely substituted non-aromatic aliphatic rings (piperidine and piperazine) as the peripheric substituent groups on the key pharmacophore element (galloyl group) and synthesized *via* simple and convenient procedures in order to explore the structure–activity relationships (SAR) and improve their inhibitory activity and physicochemical properties (Figure 3).

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

To obtain series I compounds **I-(1-24)**, a straightforward synthetic route was followed (depicted in Scheme 1). Briefly, the commercially available 3,4,5-tris(benzyloxy) benzoic acid (**I-a**) was converted to intermediate **I-b** through an acylation reaction with commercial 4-amino-1-Boc-piperidine (further details given in Supplementary Information). Then, the Boc group of **I-b** was removed in an ethyl ether solution of HCl at room temperature to obtain intermediate **I-c** in good yield. **I-d** was obtained by treating **I-c** with RSO_2Cl/RCOCl. The final step of the synthesis procedure involved debenzylation to generate the designed analogues **I-(1-24)**. The synthetic route for compounds of series II (**II-(1-27)**) was similar to that of **I-(1-24),** but we used 1-Boc-piperazine instead of 4-amino-1-Boc-piperidine (Scheme 2). The structure of all the synthesized compounds was confirmed after analysis of their spectral data (ESI-MS, ^{1}H NMR, and ^{13}C NMR). These data were consistent with the assumed structures.

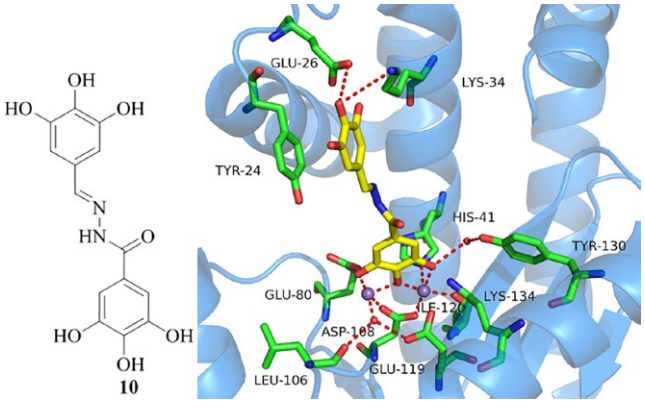


FIGURE 2 Formula of compound **10** and crystal structure of the compound bound to H1N1 influenza virus polymerase acidic endonuclease (PDB code: 5EGA; Carcelli et al., 2016) [Colour figure can be viewed at wileyonlinelibrary.com]

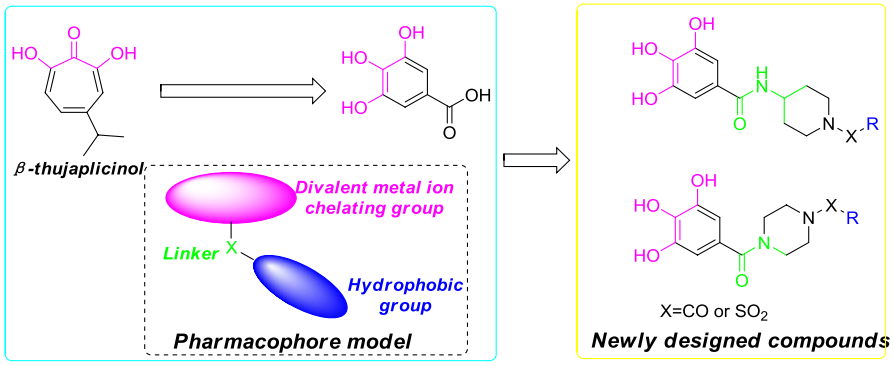
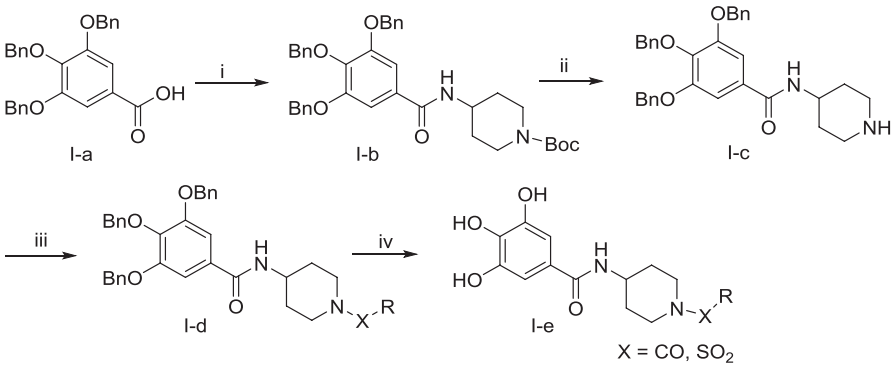


FIGURE 3 Design of galloyl derivatives as HIV-1 RNase H inhibitors [Colour figure can be viewed at wileyonlinelibrary.com]

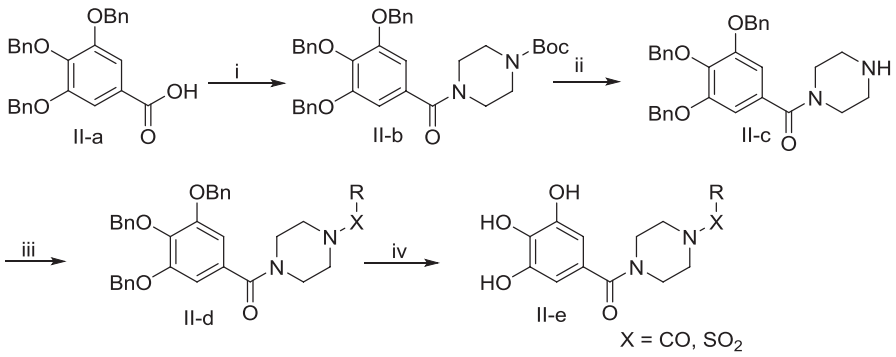
2.2 | HIV-1 RNase H inhibition

All compounds were first tested in vitro for their inhibitory activity against HIV-1 RT-associated RNase H. The substrate used was a template-primer containing a 31-nucleotide RNA labeled at its 5′ end with ^{32}P, bound to a 21-nucleotide DNA (Álvarez, Matamoros, & Menéndez-Arias, 2009). Assays were carried out with recombinant RT of HIV-1 group M subtype B BH10 strain (HIV-1_{BH10}), and β-thujaplicinol was used as control (Gao et al., 2017; Sun et al., 2018). The obtained IC_{50} values are given in Table S1. Six out of the 24 compounds of series I showed IC_{50}s in the range of 10.6–27.6 μM, while other molecules had poor inhibitory activity with IC_{50} values above 50 μM. Results indicate that the piperidine linker had a little impact on the observed inhibition.

In contrast to Series I compounds, all the compounds included in Series II (and containing the piperazine linker) were found to be potent inhibitors of HIV-1 RNase H. Their IC_{50} values were in the range of 0.72–2.67 μM, except **II-24** whose IC_{50} was 12.2 μM. With the exception of **II-21** and **II-24**, all of them were found to be more potent than the control compound β-thujaplicinol. Compound **II-25** was the most efficient RNase H inhibitor with an IC_{50} value of 0.72 ± 0.07 μM,



SCHEME 1 Synthetic route of compounds **I-(1-24)**. Reagents and conditions: (i) 4-Amino-1-Boc-piperidine, EDCl, HOBt, Et_3N, DMF, rt, 12 hr; (ii) HCl/EtOAc, rt, 2 hr; NaOH/H_2O; (iii) RSO_2Cl/RCOCl, Et_3N, DCM, rt, 12 hr; (iv) H_2, Pd/C, MeOH/DCM, rt, 24 hr



SCHEME 2 Synthetic route of compounds **II-(1-27)**. Reagents and conditions: (i) 1-Boc-piperazine, EDCl, HOBt, Et_3N, DMF, rt, 12 hr; (ii) HCl/EtOAc, rt, 2 hr; NaOH/H_2O; (iii) RSO_2Cl/RCOCl, Et_3N, DCM, rt, 12 hr; (iv) H_2, Pd/C, MeOH/DCM, rt, 24 hr

2.8 times better than β-thujaplicinol. In general, all inhibitors of this series had similar inhibitory activities. For compounds sharing the same terminal substituent, the sulfonamide linker conferred slightly higher inhibitory activity than the amide linker. Large substituents in the phenyl moiety decreased the interaction between the RNase H and the inhibitor, while the introduction of dimethyl, isopropyl, and especially *tert*-butyl groups caused a dramatic decline in inhibitory activity. The introduction of hydrogen bonds between donor and/or receptor contributes to the activity, as well as the position of substituents. The compounds with *meta*- and *ortho*- substituents were more potent than those having *para*-substituents. The results of the biologic evaluation and the SAR analysis described above will be beneficial in future to design new potent HIV-1 RNase H inhibitors.

2.3 | HIV-1 RNA-dependent DNA polymerase (RDDP) inhibition

HIV-1 RT's RDDP inhibition was determined by using a commercial ELISA-based assay developed by Roche (Suzuki, Craddock, Okamoto, Kano, & Steigbigel, 1993) or a DNA polymerization assay in which a 5′-labeled DNA primer hybridized to an RNA template was elongated in the presence of dNTPs (see Supporting information (Appendix S1) for details). The inhibitory activity of Series I compounds was initially determined with the ELISA-based assay (Table S1). The results of this analysis revealed that only four out of the 24 tested compounds showed some inhibitory activity in these assays ($IC_{50} < 100$ μM). **I-5**, the most potent RNase H inhibitor of the series (RNase H $IC_{50} = 10.6 \pm 1.4$ μM), showed excellent selectivity over the HIV-1 RT DNA polymerase ($IC_{50} \geq 293.3$ μM). In contrast, the most potent DNA polymerase inhibitor (**I-16**) was found to be a dual inhibitor, also active against the viral RNase H. For this compound, the DNA polymerase IC_{50} was 39.9 ± 2.3 μM, while the IC_{50} value obtained in RNase H inhibition assays was 13.1 ± 3.2 μM.

Series II compounds were also found to be very weak inhibitors of the RT's RDDP activity in primer elongation assays. All of them showed IC_{50} values above 50 μM (Table S2), and their highest inhibitory activity at this concentration was 20.5% and 22.2%, observed with compounds **II-22** and **II-24**, respectively. It should be noted that at 50 μM, Series I compounds **I-16** and **I-23** were found to be less potent than **II-22** and **II-24**, showing inhibitory activities of 11.3% and 7.2%, respectively. In contrast, an IC_{50} of 0.42 ± 0.12 μM was determined for the approved non-nucleoside RT inhibitor efavirenz in assays carried out in the same conditions.

Taken together, our results show that galloyl derivatives described in this paper are inactive or weak inhibitors of the DNA polymerase activity of HIV-1 RT and might be considered as selective RNase H inhibitors.

2.4 | HIV IN strand transfer inhibition

Based on the similarity of the HIV RNase H and IN active sites, and the expected chelating activity of galloyl derivatives, we determined the inhibitory activity of selected Series II compounds (i.e., **II-21**, **II-24,** and **II-25**) in IN strand transfer assays. As shown in Figure 4, the three compounds were able to inhibit the viral IN but were less potent than raltegravir (an approved IN inhibitor). Interestingly, **II-25** was found to be the most potent inhibitor, followed by **II-21** and **II-24**. This order was consistent with the IC_{50} values obtained in RNase H inhibition assays. Although IC_{50}s were not accurately determined for all compounds, our data suggest that the galloyl derivatives described show similar potency against HIV RNase H and IN strand transfer activities. Interestingly, the HIV RNase H and IN catalytic sites have similar geometries with magnesium ions located at structurally equivalent positions in both enzymes. In addition, some of the key structural features displayed by the galloyl derivatives described in this work are shared by approved IN inhibitors such as raltegravir, elvitegravir, or dolutegravir that also contain adjacent hydroxyl groups in planar aromatic rings (Yu et al., 2008; Wang et al., 2018c; Sun et al., 2018).

2.5 | Anti-HIV activity evaluation

All Series I compounds were evaluated in cell culture for their antiviral activity against wild-type HIV-1 and HIV-2 strains (IIIB and ROD, respectively). Nevirapine, zidovudine, lamivudine, and didanosine were used as positive controls. EC_{50} values (anti-HIV activity) and CC_{50} values (cytotoxicity) for Series I compounds are given in Table S1. None of the compounds was active in phenotypic assays (EC_{50} values above 19.9 μM for all tested molecules). However, their cytotoxicity was low with CC_{50} values in the range of 19.9–178.4 μM.

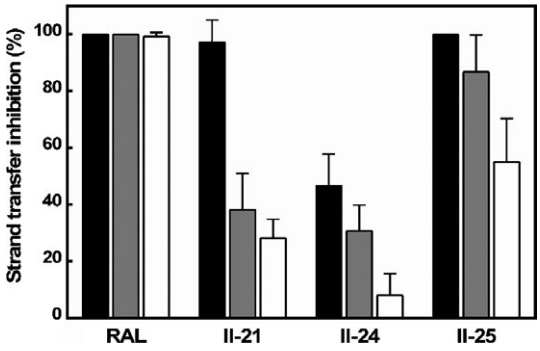


FIGURE 4 HIV IN strand transfer inhibition assays. Histograms represent percent inhibition of the strand transfer reaction in assays carried out with heteropolymeric hybrids (Chow, 1997) in the presence of compounds **II-21**, **II-24,** and **II-25** and raltegravir, at 50, 10, and 1 μM concentrations (black, gray, and white bars, respectively). RAL, raltegravir

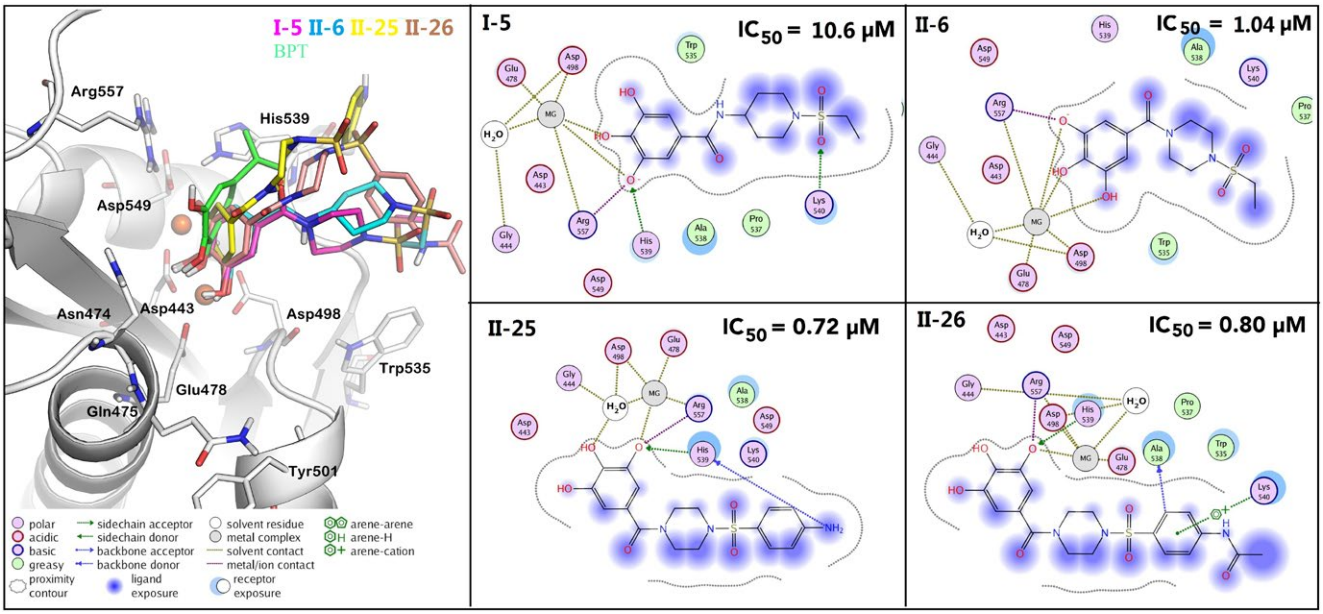


FIGURE 5 Predicted binding mode of compound **I-5**, **II-6**, **II-25,** and **II-26** with the active site of RNase H (PDB code: 3QIO). β-Thujaplicinol (BTP) binding is shown as reference on the left panel [Colour figure can be viewed at wileyonlinelibrary.com]

The Series II compounds showed similar properties, with EC_{50} values above 40 μM and no cytotoxicity when tested at 20 μg/ml concentration.

Analysis of the cytotoxicity of Series I compounds showed that the lowest toxicities were observed for those containing phenylsulfonyl side chains (**I-17** to **I-21** and **I-23** and **I-24**) or methoxy-substituted benzene rings (**I-14**, **I-15,** and **I-16**). The introduction of a halogen, like fluorine, reduced the toxicity in the phenylsulfonyl series.

Interestingly, cell permeability assays revealed that **II-25** had poor membrane permeability. Thus, in experiments carried out with Caco-2 cells, the apparent permeability (P_{app}) of **II-25** was below 1.4×10^{-7} cm/s, while reference compounds for low and high permeability, such as nadolol and metoprolol, showed P_{app} values of 1.1×10^{-7} cm/s and 1.63×10^{-5} cm/s, respectively. However, despite the high recovery of nadolol and metoprolol in these experiments (above 93%), for compound **II-25** we obtained recoveries below 32.4%, suggesting that cellular retention, metabolism, poor absorption, and other factors could also contribute to its poor efficacy in antiviral assays.

2.6 | Molecular modeling studies

In order to gain further insight into the preferred binding mode of the tested compounds and rationalize SAR results, molecular models of **I-5**, **II-6**, **II-25,** and **II-26** bound to HIV-1 RNase H were obtained. Docking was carried out with the molecular mechanics-generalized Born surface area (MM/GBSA) method, using the Glide/Schrödinger software (Poongavanam, Steinmann, & Kongsted, 2014; Poongavanam et al., 2018). The crystal structure of HIV-1 RNase H bound to an N-hydroxy-quinazolinedione inhibitor (PDB code: 3QIO) was used as reference.

The predicted binding mode of the selected four compounds to the HIV-1 RNase H was found to be similar to that previously reported for β-thujaplicinol (Figure 5). According to the obtained models, the phenolic hydroxyl group and one oxygen anion of compound **I-5** could chelate two metal cofactors (Mg^{2+}), and the oxygen anion could also interact with conserved Arg557 and His539 residues in the enzyme. Besides, one oxygen atom in the sulfonyl moiety could participate in a hydrogen bond with Lys540. Unlike in the case of **I-5**, both the oxygen anion and the phenolic hydroxyl group of **II-6** contribute to the chelation with Mg^{2+}. In the case of **II-25**, the oxygen anion could interact with Mg^{2+}, Arg557, and His539, while the phenolic hydroxyl group also shows potential interactions with Mg^{2+} although in this case *via* a water bridge. In addition, the terminal NH_2 could form a hydrogen bond with His539. Although there is only one oxygen anion in the **II-26** structure that interacts with Mg^{2+}, multiple additional interactions with Arg557 and His539 of such an oxygen and of the substituted benzene rings with Ala538 and Lys540 are predicted to contribute to increase the inhibitory activity of the compound. In agreement with our modeling studies, previous analyses predicted the involvement of ionic/π interactions between Arg557 and Mg^{2+}-binding RNase H inhibitors (Poongavanam et al., 2018; Corona et al., 2014).

3 | CONCLUSIONS

Successful combination antiretroviral therapy requires continued efforts to develop novel inhibitors targeting

different steps of HIV-1 replication cycle. Based on the summarized pharmacophore models of HIV-1 RNase H inhibitors (Figure 1), two series of novel galloyl derivatives were designed, synthesized, and evaluated for their biologic activities against HIV-1, and its enzymes RT DNA polymerase and RNase H. Most derivatives proved to be highly effective in inhibiting HIV-1 RNase H activity at sub- to low-micromolar concentrations and demonstrated high selectivity over HIV-1 RT DNA polymerase. Among them, **II-25** was the most promising compound and exhibited potent inhibitory activity against HIV-1 RNase H, with an IC_{50} value of 0.72 ± 0.07 μM, 2.8 times more potent than β-thujaplicinol in enzymatic assays. Interestingly, **II-25** and other tested galloyl derivatives were also found to inhibit the HIV IN strand transfer activity, a property shared by related compounds such as gallic acid flavon-3-yl esters (Desideri et al., 1998). Unfortunately, the designed compounds fail to exhibit significant inhibitory activity in infected cells, probably due in part to their poor permeability through the cell membrane. Still, tested compounds show low cytotoxicity and SAR studies and molecular modeling analysis are expected to be helpful in providing guidance for further optimization.

ACKNOWLEDGMENTS

Financial support from the National Natural Science Foundation of China (NSFC No. 81273354), the Key Project of NSFC for International Cooperation (No. 81420108027), the Key Research and Development Project of Shandong Province (No. 2017CXGC1401), the Young Scholars Program of Shandong University (YSPSDU No. 2016WLJH32, to P. Z.), the Major Project of Science and Technology of Shandong Province (No. 2015ZDJS04001), Shandong Province Key R&D Plan (No. 2017GSF218030), and NIH grant AI033066 from the National Institute of Allergy and Infectious Disease (awarded to K.H. L.) are gratefully acknowledged. Work in Madrid was supported by grant BIO2016-76716-R (AEI/FEDER, UE) (Spanish Ministry of Science, Innovation and Universities) and an institutional grant of Fundación Ramón Areces.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Gao P, Wang X, Sun L, et al. Design, synthesis, and biologic evaluation of novel galloyl derivatives as HIV-1 RNase H inhibitors. *Chem Biol Drug Des*. 2019;93:582–589. https://doi.org/10.1111/cbdd.13455