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Research paper

6-Biphenylmethyl-3-hydroxypyrimidine-2,4-diones potently and selectively inhibited HIV reverse transcriptase-associated RNase H

Lei Wang [a], Jing Tang [a], Andrew D. Huber [b], Mary C. Casey [c], Karen A. Kirby [c, d], Daniel J. Wilson [a], Jayakanth Kankanala [a], Michael A. Parniak [e], Stefan G. Sarafianos [c, d, f], Zhengqiang Wang [a, *]

[a] Center for Drug Design, Academic Health Center, University of Minnesota, Minneapolis, MN 55455, USA
[b] Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Christopher S. Bond Life Sciences Center, Columbia, MO 65211, USA
[c] Department of Molecular Microbiology and Immunology, University of Missouri School of Medicine, Christopher S. Bond Life Sciences Center, Columbia, MO 65211, USA
[d] Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA
[e] Department of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15219, USA
[f] Department of Biochemistry, University of Missouri, Columbia, MO 65211, USA

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ABSTRACT

Human immunodeficiency virus (HIV) reverse transcriptase (RT)-associated ribonuclease H (RNase H) remains an unvalidated drug target. Reported HIV RNase H inhibitors generally lack significant antiviral activity. We report herein the design, synthesis, biochemical and antiviral evaluations of a new 6-biphenylmethyl subtype of the 3-hydroxypyrimidine-2,4-dione (HPD) chemotype. In biochemical assays, analogues of this new subtype potently inhibited RT RNase H in low nanomolar range without inhibiting RT polymerase (pol) or integrase strand transfer (INST) at the highest concentrations tested. In cell-based assays, a few analogues inhibited HIV in low micromolar range without cytotoxicity at concentrations up to 100 μM.

1. Introduction

Treatment of HIV infection relies on a large repertoire of FDA-approved single agents and fixed dose combinations consisting primarily of inhibitors of three virally encoded enzymes: RT, IN and protease (PR) [1]. However, as current drugs do not cure HIV, their long term use can be plagued by the selection of resistant viral strains. Combating drug-resistant HIV necessitates the discovery of antivirals with a novel molecular target and a distinct antiviral mechanism of action. HIV RT-associated RNase H represents such a novel target. RT comprises two distinct domains [2]: a pol domain responsible for both RNA-dependent and DNA-dependent viral DNA polymerization and targeted by all currently known nucleoside RT inhibitors (NRTIs) [3] and non-nucleoside RT inhibitors (NNRTIs) [4]; and an RNase H domain [2,5] required to degrade the RNA strand from the RNA/DNA heteroduplex reverse transcription intermediate. Significantly, attenuated RNase H enzymatic activity was found to correlate well with reduced HIV replication in cell culture [6], suggesting that RNase H enzymatic activity is required for HIV replication and that selectively inhibiting RNase H with small molecules should confer a similar antiviral phenotype. However, medicinal chemistry efforts targeting HIV RNase H have yet to deliver the first in-class antiviral candidate in the development pipeline at any stage. As such, HIV RT-associated RNase H remains unvalidated as a drug target.

Major HIV RNase H inhibitor types (Fig. 1, A) [7] reported by others include 2-hydroxyisoquinolinedione (HID, **1**) [8], β-thujaplicinol (**2**) [9], dihydroxycoumarin (**3**) [10], diketoacid (DKA) **4** [11], pyrimidinol carboxylic acid **5** [12], hydroxynaphthyridine **6** [13] and pyridopyrimidone **7** (Fig. 1, A) [14]. Key to these chemotypes is a chelating triad (magenta) capable of binding two divalent metal ions, a structural moiety also featured in inhibitors of HIV INST

* Corresponding author.
E-mail address: wangx472@umn.edu (Z. Wang).

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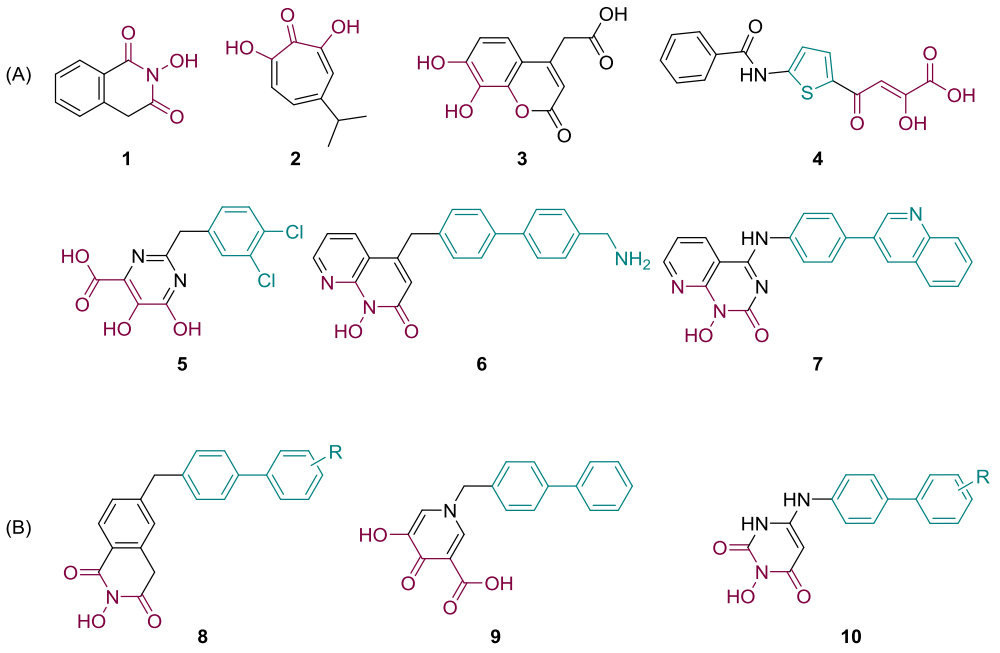

Fig. 1. Major active site RNase H inhibitors. (A) RNase H inhibitor types reported by others. All chemotypes contain a chelating triad (magenta); scaffolds **4**–**7** also feature an aryl or biaryl moiety (cyan) connected through a methylene or amino linker; (B) our previously reported RNase H inhibitor chemotypes **8**–**10**. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

which shares a similar active site fold and a divalent metal dependence for catalytic activity [15]. Importantly, inhibitor types **4**–**7**, which contain an additional hydrophobic aromatic moiety (cyan), exhibited more potent and selective RNase H inhibition, indicating that the chelating triad and the hydrophobic aromatic moiety may represent the minimal pharmacophore requirements for RNase H inhibitors. A similar pharmacophore model was observed with canonical INSTIs [16,17]. Nevertheless, reported RNase H inhibitors generally lack antiviral activity in cell culture, presumably due to the biochemical barrier of small molecules competing against much larger RNA/DNA substrates [14,18]. In addition, many of these RNase H inhibitors also lack a selective inhibitory profile biochemically over the inhibition of RT pol and INST. Therefore, there is a need to continue to identify novel chemotypes with potent and selective biochemical RNase H inhibition that could confer antiviral activity. Toward this end, we have discovered three distinct chemotypes (**8**–**10**, Fig. 1, B) based on the afore-mentioned pharmacophore model, including the redesigned HID subtype **8** [19], the hydroxypyridonecarboxylic acid (HPCA) chemotype **9** [20], and the redesigned HPD subtype **10** [21]. We describe herein the evolution of another HPD subtype **17** from the original HPD subtypes **11** and **12** (Fig. 2) which were synthesized as INST and RT pol dual inhibitors [22,23], and later found to inhibit HIV RNase H in our primary screening assay. Brief structure-activity-relationship (SAR) analysis on **11** and **12** concerning the N-3 substitution (subtype **13**) and the C5 and C6 sites (subtype **14**) led to subtype **15** with a biaryl moiety at C-6 (Fig. 2, A). Further structural modifications on **15** aimed to minimize the peripheral binding interactions important for RT pol binding (Fig. 2, B) [24,25]. As such the sequential removal of the C5 alkyl group (subtype **16**) and the N-1 substituent resulted in the design of subtype **17** which demonstrated highly potent and selective RNase H inhibition, with some analogues also inhibiting HIV in cell culture without cytotoxicity. Interestingly, subtype **17** is a drastically improved congener of HPD subtype **10** which did not demonstrate significant HIV inhibition in cell culture [21]. We report herein the synthesis, biochemical and antiviral studies, and molecular modeling of **17**.

2. Results and discussion

2.1. Chemistry

All compounds of the original HPD subtypes **11**–**12** were synthesized according reported procedures [22,23]. Subtype **13** was synthesized from a previously synthesized intermediate **18** [23] according to Scheme 1. Direct methylation of **18** afforded compound **13a**, whereas **13b** was prepared via a two-step synthetic sequence: an N-3 hydroxylation and the subsequent methylation of intermediate **19**.

Analogues of subtype **14** were synthesized based on procedures described in Scheme 2. The synthesis involved key β-ketoesters (**20** and **23**) which were synthesized as reported [23]. Cyclization of β-ketoesters with thiourea followed by the conversion of thiocarbonyl into carbonyl produced pyrimidine-2,4-diones **21** and **24**. The N-1 ether linkage was introduced via a highly regioselective reaction between a bissilylated pyrimidine-2,4-dione and a chloromethyl alkyl ether, an activated alkylating agent, to yield advanced intermediates **22** and **25**. Finally, N-3 hydroxylation with *m*CPBA under basic condition furnished desired compounds **14a** and **14b**.

The synthesis of subtype **15** critically featured a brominated pyrimidine-2,4-dione intermediate (**30**) which was prepared from β-ketoester **27** based on a similar procedure used for the synthesis of subtype **14** (Scheme 3). Suzuki coupling [26] of **30** with an aryl boronic acid yielded intermediate **31** which was subjected to N-3 hydroxylation to complete the synthesis of **15**. It is noteworthy that β-ketoesters involved in the synthesis of HPD core are typically prepared from a Blaise reaction [27] between an appropriate cyano compound and a bromoester. However, in this synthesis β-ketoester **27** was prepared using a different reaction [28] from much cheaper carboxylic acid **26** and diethyl methylmalonate.

Subtype **17**, the major subtype for this study, was synthesized as described in Scheme 4. The synthetic approach utilized was adapted from a known synthesis of pyrimidine-2,4-dione analogues [29]. The synthesis began with a Blaise reaction to prepare the key β-ketoesters **33** which were subsequently converted to