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Research paper

# 6-Arylthio-3-hydroxypyrimidine-2,4-diones potently inhibited HIV reverse transcriptase-associated RNase H with antiviral activity

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ABSTRACT

Human immunodeficiency virus (HIV) reverse transcriptase (RT) associated ribonuclease H (RNase H) remains the only virally encoded enzymatic function not targeted by current drugs. Although a few chemotypes have been reported to inhibit HIV RNase H in biochemical assays, their general lack of significant antiviral activity in cell culture necessitates continued efforts in identifying highly potent RNase H inhibitors to confer antiviral activity. We report herein the design, synthesis, biochemical and antiviral evaluations of a new 6-arylthio subtype of the 3-hydroxypyrimidine-2,4-dione (HPD) chemotype. In biochemical assays these new analogues inhibited RT RNase H in single-digit nanomolar range without inhibiting RT polymerase (pol) at concentrations up to 10 μM, amounting to exceptional biochemical inhibitory selectivity. Many analogues also inhibited integrase strand transfer (INST) activity in low to sub micromolar range. More importantly, most analogues inhibited HIV in low micromolar range without cytotoxicity. In the end, compound **13j** (RNase H $IC_{50} = 0.005$ μM; RT pol $IC_{50} = 10$ μM; INST $IC_{50} = 4.0$ μM; antiviral $EC_{50} = 7.7$ μM; $CC_{50} > 100$ μM) represents the best analogues within this series. These results characterize the new 6-arylthio-HPD subtype as a promising scaffold for HIV RNase H inhibitor discovery.



## 1. Introduction

HIV antiviral therapy relies primarily on inhibitors of three virally encoded enzymes: RT, integrase (IN), and protease (PR) [1]. Combinations of these inhibitors form the highly active antiretroviral therapy (HAART), which renders HIV infection clinically manageable [2]. However, current drugs do not cure HIV and HAART can be plagued by the selection of resistant viral strains after long term use. Therefore, antivirals with a novel molecular target and a distinct antiviral mechanism of action are constantly needed to provide new options for HAART in combating drug-resistant viruses. HIV RT has two distinct domains [3]: a pol domain which carries out both RNA-dependent and DNA-dependent viral DNA polymerization and is targeted by all currently known nucleoside RT inhibitors (NRTIs) [4] and non-nucleoside RT inhibitors (NNRTIs) [5]; and an RNase H domain [3,6] which is required to degrade the RNA strand from the RNA/DNA reverse transcription intermediate, and process both the tRNA primer for minus strand DNA synthesis and the polypurine tract (PPT) primer for plus strand DNA synthesis. Significantly, active site mutations associated with attenuated RNase H biochemical activity *in vitro* conferred reduced HIV replication in cell culture [7], suggesting that RNase H functions are essential for HIV genome replication and that small molecules with potent and selective

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RNase H inhibition should inhibit HIV replication. Unfortunately, despite decades of medicinal chemistry efforts, compounds conferring antiviral activity *via* targeting RNase H have yet to enter clinical development of any stage. As such, HIV RT-associated RNase H remains unvalidated as a drug target.

A few chemotypes (Fig. 1, A) have been reported to inhibit RNase H in biochemical assays [8], including 2-hydroxyisoquinolinedione (HID, **1**) [9], β-thujaplicinol (**2**) [10], dihydroxycoumarin (**3**) [11], diketoacid (DKA) **4** [12], pyrimidinol carboxylic acid **5** [13], hydroxynaphthyridine **6** [14] and pyridopyrimidone **7** (Fig. 1, A) [15]. These inhibitor types all feature a chelating triad (magenta) for binding two divalent metal ions. Structurally more elaborate chemotypes **4**–**7** also contain a hydrophobic aromatic moiety (cyan), which generally leads to more potent and selective RNase H inhibition in *in vitro* biochemical assays. HIV RNase H and IN share a similar active site fold as well as divalent metal dependence for catalytic activity [16]. Therefore, the chelating triad and the hydrophobic aromatic moiety embedded in inhibitor types **4**–**7** as well as typical INSTIs [17,18] may represent the minimal pharmacophore requirements for RNase H inhibitors. Unfortunately, the potent *in vitro* biochemical inhibition of RNase H observed with these inhibitor types typically does not confer significant antiviral activity in cell culture, possibly reflecting a steep biochemical barrier of small molecules competing against much larger RNA/DNA substrates [15]. Achieving RNase H inhibition in cell culture remains a challenge and likely requires tight RNase H binding and improved biochemical RNase H inhibition. We have long been interested in discovering antiviral compounds targeting HIV RNase H. Our efforts based on the aforementioned pharmacophore model have led to the discovery of four distinct chemotypes (**8**–**11**, Fig. 1, B), including the redesigned HID subtype **8** [19], the hydroxypyridonecarboxylic acid (HPCA) chemotype **9** [20], the redesigned HPD subtype **10** [21], and the *N*-hydroxy thienopyrimidine-2,4-dione chemotype **11** [22]. Among these inhibitor types HPD subtype **10** did not show appreciable HIV inhibition in cell culture despite potent and selective RNase H inhibition in *in vitro* biochemical assays [21]. Extended structure-activity relationship (SAR) analysis on **10** led to the design of subtypes **12** and **13** featuring a thio linkage at C-6 in lieu of the amino linkage (Fig. 1, C). Interestingly, **12** and **13** exhibited biochemical inhibitory profiles drastically different from that of **10**, as well as consistent antiviral activity in low micromolar range. We report herein the synthesis, biochemical and antiviral studies, and molecular modeling of **12** and **13**.

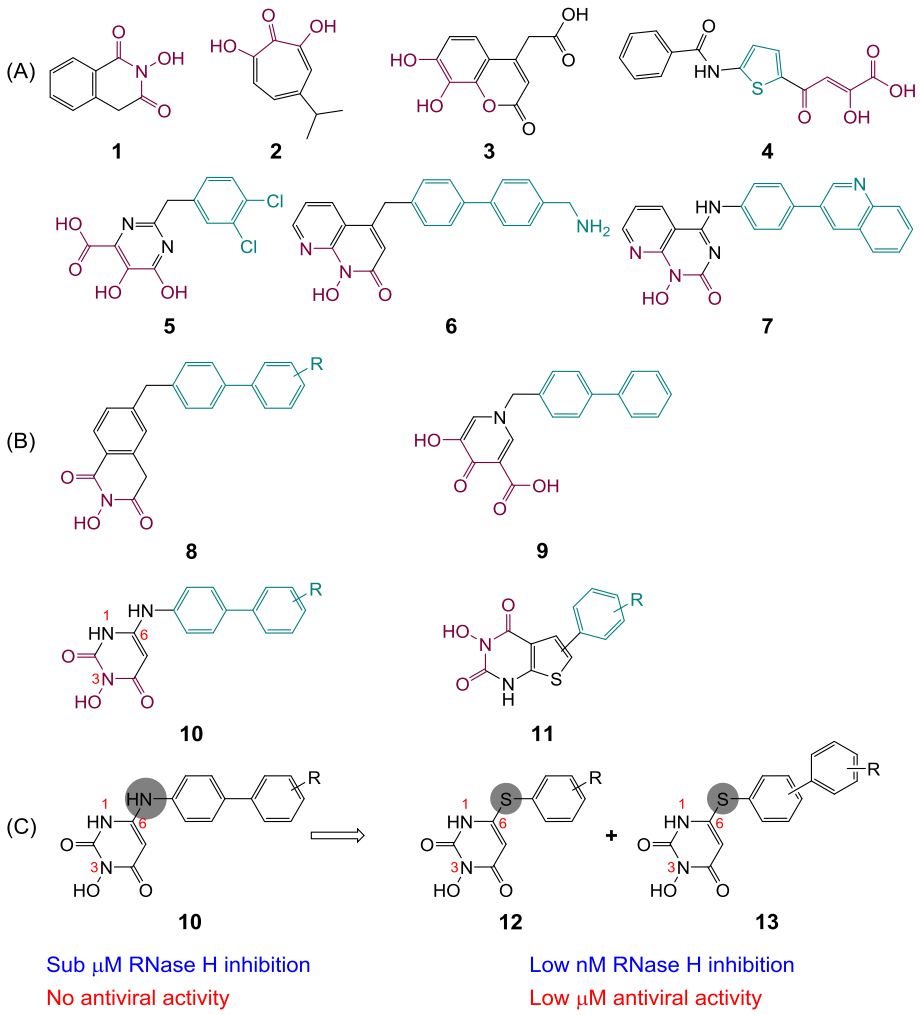


**Fig. 1.** Design of active site RNase H inhibitors. (A) Major chemotypes reported as HIV RNase H active site inhibitors. All chemotypes contain a chelating triad (magenta); scaffolds **4**–**7** also feature an aryl or biaryl moiety (cyan) connected through a methylene or amino linker; (B) our previously reported RNase H inhibitor chemotypes **8**–**11**; (C) the design of 6-phenylthio-HPD (**12**) and 6-biphenylthio-HPD (**13**) subtypes based on 6-biphenylamino-HPD (**10**). Subtypes **12** and **13** showed drastically improved biochemical potency *in vitro* and significant antiviral activity.