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Potent and selective HIV-1 ribonuclease H inhibitors based on a 1-hydroxy-1,8-naphthyridin-2(1*H*)-one scaffold

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ABSTRACT

Optimization studies using an HIV RNase H active site inhibitor containing a 1-hydroxy-1,8-naphthyridin-2(1*H*)-one core identified 4-position substituents that provided several potent and selective inhibitors. The best compound was potent and selective in biochemical assays (IC_{50} = 0.045 μM, HIV RT RNase H; 13 μM, HIV RT-polymerase; 24 μM, HIV integrase) and showed antiviral efficacy in a single-cycle viral replication assay in P4-2 cells (IC_{50} = 0.19 μM) with a modest window with respect to cytotoxicity (CC_{50} = 3.3 μM).

The causative agent of acquired immune deficiency syndrome (AIDS) is the human immunodeficiency virus type 1 (HIV-1). Resistance to marketed anti-HIV drugs continues to increase and thus there is a need to improve existing agents and to develop new antiviral agents that work by different mechanisms. Reverse transcriptase (RT) is one of three virally encoded enzymes and plays an essential role in viral replication by converting the single stranded RNA viral genome into double stranded proviral DNA. RT possesses two enzymatic activities, DNA polymerase and ribonuclease H (RNase H), the active sites for which are located in two distinct domains separated by ~60 Å. Both activities are required for successful reverse transcription and viral replication. Inhibitors of RT which act at the polymerase active site, for example, nucleoside and non-nucleoside inhibitors, have become essential components in the current armamentarium of antiretroviral therapy.[1] There has been considerable interest in developing a specific inhibitor of the RNase H activity of RT as a novel mechanism for the treatment of HIV infection.[2,3] The RNase H active site binds two magnesium ions which are believed to be involved in the catalytic mechanism of RNA phosphodiester bond hydrolysis.[4] Four structural classes of RNase H inhibitors containing heteroatoms suitably positioned to bind active site metals have been reported: diketoacids,[5] *N*-hydroxyimides,[6] hydroxytropolones,[7] and hydroxypyrimidinone acids.[8] We recently reported a new class of active site inhibitors based on a 1-hydroxy-1,8-naphthyridin-2(1*H*)-one (**1**, Fig. 1),[9] and herein we report structure–activity studies in this new class to obtain potent and selective RNase H inhibitors.

The compounds in Table 1 were prepared as shown in Scheme 1. Ester **2**[10] was saponified using NaOH. The resulting carboxylate salt underwent decarboxylation upon acidification to pH 2–3 using aqueous HCl, and the resulting phenol **3** was converted to triflate **4** using standard conditions. The triflate and benzyl groups in **4** were hydrogenolyzed using hydrogen gas and palladium on carbon at

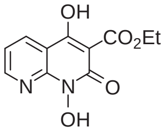

Figure 1. Lead compound **1**, RNase H IC_{50} = 0.11 μM.

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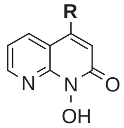
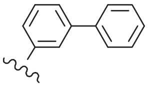
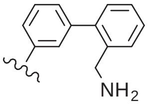
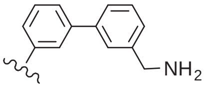
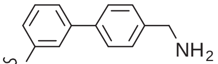
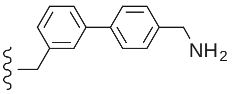
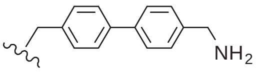
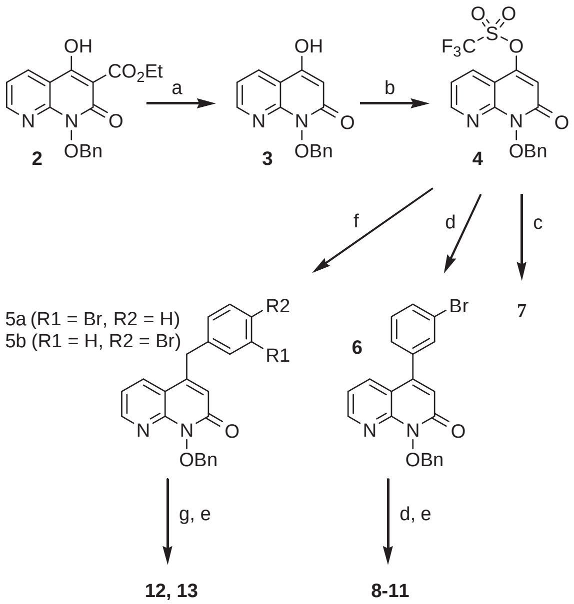
Table 1
Structure–activity relationships of 4-substituted 1-hydroxy-1,8-naphthyridin-2-ones[f]

	R	RT-RNH[a] IC_{50} (μM)	RT-Pol[b] IC_{50} (μM)	InST[c] IC_{50} (μM)	Viral replication[d] IC_{50} (μM)	Cytotoxicity[e] CC_{50} (μM)
7	H	1.4 ± 0.8	>50	15	4.5	20 ± 3.9
8		0.64	9.0	2.4	34	>50
9		0.67 ± 0.08	36	18	2.8 ± 1.9	9.1 ± 2.1
10		0.15 ± 0.03	5.1	9.1	0.85 ± 0.12	4.9
11		0.13 ± 0.07	16	7.8	0.62 ± 0.07	2.3 ± 0.72
12		0.10 ± 0.04	11	8.1	0.43 ± 0.17	4.6 ± 0.68
13		0.045 ± 0.02	13 ± 1.3	24	0.19 ± 0.12	3.3 ± 2.3

[a] RNase H assay using wild type HIV-1 reverse transcriptase.
[b] Polymerase assay using D443N HIV-1 reverse transcriptase.
[c] Strand-transfer assay using wild type HIV-1 integrase.
[d] HIV-1 HXB2 single-cycle viral replication assay in HeLa P4-2 cells.
[e] CC_{50} = concentration for 50% cytotoxic response using Alamar Blue indicator in HeLa P4-2 cells.
[f] Assay results with three or more independent determinations are represented as mean ±SEM.



Scheme 1. Synthesis of compounds in Table 1. Reagents and conditions: (a) aq NaOH, reflux, aq HCl; (b) $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2, 0 °C; (c) H_2, Pd–C, EtOAc; (d) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, microwave 120 °C; (e) 33% HBr in HOAc, water, 80 °C; (f) $ArCH_2ZnBr$, $Pd(PPh_3)_4$, Na_2CO_3, DME, microwave 110 °C; (g) $ArB(OH)_2$, Pd dppf, K_2CO_3, DMF/H_2O, microwave 120 °C.

ambient temperature and pressure to give **7**. Suzuki coupling of **4** with 3-bromophenylboronic acid gave naphthyridinone **6**. Limiting the stoichiometry of 3-bromophenylboronic acid to 1 equiv minimized formation of a double coupling product. Bromide **6** was used in Suzuki couplings with phenylboronic acid and the three regioisomers of *N*-Boc-aminomethyl-phenylboronic acid. Removal of the *O*-benzyl and *N*-Boc protecting groups was accomplished by heating in 33% HBr in acetic acid containing a small amount of water to provide **8–11**. Negishi coupling of **4** with the benzylzinc reagents obtained by treating 3- and 4-bromobenzyl bromide with diethylzinc gave **5a** and **5b**, respectively. Suzuki coupling of these bromides with 4-(*N*-Boc-aminomethyl)phenylboronic acid followed by deprotection with HBr gave **12** and **13**.

Potency for inhibition of the RNase H activity of HIV-1 RT was determined using a high throughput adaptation of a previously described polymerase-independent assay.[5] In this assay, cleavage of the RNA strand in an RNA/DNA duplex substrate caused dissociation of the DNA strand biotinylated on the 5′ end. The released biotinylated DNA strand was annealed to a complimentary DNA strand tagged with fluorescein on the 5′ end. The resulting DNA duplex was then quantified using an ALPHA screen format. In this assay, the active site inhibitor 2-hydroxy-isoquinoline-1,3(2*H*,4*H*)-dione reported by Klumpp et al.[6] had an IC_{50} value of 0.86 μM. Potency for inhibition of the DNA polymerase activity of HIV-1 RT was determined using a previously described RNA-dependent DNA polymerase assay.[11] To determine inhibition of polymerase activity due to inhibitor binding outside of the RNase H active site,[12] assays were conducted with RT containing the D443N mutation, a mutation that perturbs binding of one of the two magnesium ions in the active site that are essential both for RNase H function and for inhibitor binding. This mutation abolished RNase H enzymatic activity and RNase H active site inhibitor binding while having no significant impact on the potency of several known non-nucleoside HIV-1 RT-polymerase inhibitors. Potency for inhibition of the HIV-1 integrase catalyzed strand-transfer reaction was determined using previously described methods.[13] The RNase H, polymerase, and integrase strand-transfer biochemical assays were conducted in the presence of 6 mM $MgCl_2$. Potency for inhibition of HIV-1 replication in HeLa P4-2 cells in a single-cycle infectivity assay was determined as previously described.[14] Infectious virus was produced by transfecting 293T cells with HIV-1 proviral DNA isolated from an HXB2 strain. Cytotoxicity assays were conducted in HeLa P4-2 cells using the redox sensitive indicator, Alamar Blue.[15]

Lead compound **1** had good potency in the RNase H cleavage assay ($IC_{50} = 0.11\ \mu M$) and provided an attractive starting point for optimization. Two magnesium-dependent viral enzymes, HIV-1 RT-polymerase and HIV-1 integrase, were used as selectivity counterscreens. In these assays, **1** showed no measurable inhibition of the polymerase activity of RT ($IC_{50} > 50\ \mu M$) and modest inhibition of integrase strand-transfer activity ($IC_{50} = 0.9\ \mu M$). Compound **1** inhibited viral replication in a single-cycle infectivity assay in HeLa P4-2 cells ($IC_{50} = 2.5\ \mu M$) and did not display cytotoxicity in these cells up to a maximum concentration of 50 μM as assessed using an Alamar Blue assay. Because of the highly polar nature of **1** ($\log P = -2.2$) and possible detrimental effect this might have on cell penetration, one optimization strategy involved pursuit of analogs with greater lipophilicity. Enzyme kinetics studies with **1** indicated that the inhibitor binds to an enzyme–substrate or enzyme–product complex.[9a] A crystal structure of **1** with full length RT[9b] showed the inhibitor binding to active site metals with an open space above the inhibitor. From this we hypothesized that substituents at positions 4 or 5 of the naphthyridinone core could potentially access other parts of the enzyme or nucleic acid substrate or product that would presumably be nearby. Toward this end, triflate **4** (Scheme 1) served as an ideal intermediate for preparing a variety of analogs containing lipophilic groups attached at the 4-position of the naphthyridinone core. Structure–activity results for several of these analogs are presented in Table 1.

The unsubstituted core **7** had modest potency in the RNase H cleavage assay with >30-fold selectivity versus RT-Pol and ~10-fold selectivity versus integrase. Compound **7** also inhibited viral replication with an IC_{50} value of 4.5 μM, but at ~fourfold higher concentration, there was evidence of cytotoxicity (Alamar Blue $CC_{50} = 20\ \mu M$). Potency for inhibiting RNase H in the cleavage assay improved with the introduction of a *meta*-biphenyl group (**8**), however there was a proportionately greater increase in activity in the RT-polymerase and integrase assays. The limited solubility of **8** made it difficult to accurately assess potency in the viral replication assay, and therefore more water soluble analogs of **8** were sought. Introduction of an aminomethyl group on the distal phenyl ring provided analogs with improved physical properties and interesting structure–activity relationships. The *ortho*-aminomethyl biphenyl analog **9** was equipotent to **8** in the RNase H cleavage assay, however improved selectivity versus both RT-polymerase and integrase was obtained, as well as improved potency in the viral replication assay. Further improvements were realized with the *meta*- and *para*-aminomethyl analogs **10** and **11**, respectively. Homologation of **11** to the corresponding benzyl linked analog **12** produced little change in any of the assays. However, the *para*, *para* analog **13** showed improvements in all assays. Compound **13** had good potency in the RNase H cleavage assay ($IC_{50} = 45$ nM), selectivity versus both RT-polymerase and integrase (290- and 530-fold, respectively), and good potency in the single-cycle viral replication assay ($IC_{50} = 190$ nM) with an improved window versus cytotoxicity (17-fold). For the series of aminomethyl compounds **9**–**13**, the antiviral potency tracked well with the potency in the RNase H cleavage assay. The cytotoxic effects of **9**–**13** remained relatively constant with CC_{50} values in the range of 2–9 μM. For compounds **9**–**11**, the window between antiviral IC_{50} and cytotoxicity CC_{50} was small (3–5-fold). The more potent RNase H inhibitors **12** and **13** showed a larger window between antiviral efficacy and cellular toxicity (11- and 17-fold, respectively). Compound **13** is twofold more potent than lead compound **1** in the RNase H cleavage assay and 13-fold more potent than **1** in the viral replication assay. The greater than proportional increase in potency of **13** compared to **1** in the cellular assay suggests better cell penetration of the former which may be related to its greater lipophilicity ($\log P = 0.15$ and -2.2 for **13** and **1**, respectively). Compound **13** did not exhibit a significant shift in antiviral potency using virus pseudotyped with vesicular stomatitis virus glycoprotein (fold shift in IC_{50} value was 0.9), suggesting that the antiviral activity of **13** is not related to inhibition of viral entry into the cell. Compound **13** also did not show significant shifts in antiviral potency using viruses containing mutations in the integrase enzyme that confer resistance to several structural classes of integrase inhibitors (fold shift in IC_{50} values for the N155H, Q148R, and Y143R integrase mutants were 1.1, 1.9, and 0.84, respectively), suggesting that the antiviral potency of **13** is not related to inhibition of integrase. In a multi-cycle viral replication assay in MT-4 cells,[16] none of the compounds in Table 1 exhibited a significant window between antiviral efficacy and cellular toxicity, for example, **13** had an antiviral IC_{95} value of 0.92 μM and the first observation of cellular toxicity was at a concentration of 1.5 μM.

The aminomethylbiphenylmethyl group in **13** provided a 30-fold increase in potency in the RNase H cleavage assay compared to the unsubstituted analog **7**. The exact role of the aminomethylbiphenyl motif for improving potency in the RNase H cleavage assay is not known. Crystal soaking experiments using conditions that provided density for **1** in full length RT were unsuccessful using **13**. Molecular dynamics simulations of inhibitor–enzyme–substrate and inhibitor–enzyme–product complexes with **13** did not provide conclusive results; interactions of the biaryl moiety with nucleic acid substrate/product or with protein were possible.

In summary, 1-hydroxy-1,8-naphthyridin-2(1*H*)-one **1**, an HIV-1 RT RNase H active site inhibitor, was used as a starting point for optimization. A series of more lipophilic analogs containing a biaryl motif attached at the 4-position of the naphthyridinone core was prepared. An optimal analog in this series, **13**, was found to be a potent RNase H inhibitor in biochemical assays ($IC_{50} = 45$ nM) with good selectivity for inhibiting RNase H versus two other magnesium-dependent enzymes, HIV RT-polymerase and HIV integrase ($IC_{50} = 13\ \mu M$ and 24 μM, respectively). In a single-cycle viral replication assay in HeLa P4-2 cells, **13** inhibited viral growth at concentrations 17-fold lower than its cytotoxic concentration (antiviral $IC_{50} = 0.19\ \mu M$; $CC_{50} = 3.3\ \mu M$). Molecular dynamics simulations indicated that the biaryl group in **13** can interact with protein or nucleic acid substrate or product.

References and notes

1. Molina, J.-M. *Drugs* **2008**, *68*, 567.
2. Schultz, S. J.; Champoux, J. J. *Virus Res.* **2008**, *134*, 86.
3. Klumpp, K.; Mirzadegan, T. *Curr. Pharm. Des.* **2006**, *12*, 1909.
4. Nowotny, M.; Yang, W. *EMBO J.* **2006**, *25*, 1924.
5. Shaw-Reid, C. A.; Munshi, V.; Graham, P.; Wolfe, A.; Witmer, M.; Danzeisen, R.; Olsen, D. B.; Carroll, S. S.; Embrey, M.; Wai, J. S.; Miller, M. D.; Cole, J. L.; Hazuda, D. J. *J. Biol. Chem.* **2003**, *278*, 2777.
6. Klumpp, K.; Hang, J. Q.; Rajendran, S.; Yang, Y.; Derosier, A.; In, P. W. K.; Overton, H.; Parkes, K. E. B.; Cammack, N.; Martin, J. A. *Nucleic Acids Res.* **2003**, *31*, 6852.
7. Himmel, D. M.; Maegley, K. A.; Pauly, T. A.; Bauman, J. D.; Das, K.; Dharia, C.; Clark, A. D., Jr.; Kevin Ryan, K.; Hickey, M. J.; Love, R. A.; Hughes, S. H.; Bergqvist, S.; Arnold, E. *Structure* **2009**, *17*, 1625.
8. Kirschberg, T. A.; Balakrishnan, M.; Squires, N. H.; Barnes, T.; Brendza, K. M.; Chen, X.; Eisenberg, E. J.; Jin, W.; Kutty, N.; Leavitt, S.; Liclican, A.; Liu, Q.; Liu, X.; Mak, J.; Perry, J. K.; Wang, M.; Watkins, W. J.; Lansdon, E. B. *J. Med. Chem.* **2009**, *52*, 5781.
9. (a) Williams, P. D.; Staas, D. D.; Venkatraman, S.; Booth, T. M.; Loughran, H. M.; Ruzek, R. D.; Wai, J. S.; Vacca, J. P.; Prasad, S.; Munshi, S. K.; Bahnck, C.;

Dornadula, G.; Hrin, R. C.; Himmelberger, A. L.; Miller, M. D.; Hazuda, D. J.; Grobler, J. A. *16th Conference on Retroviruses and Opportunistic Infections*, poster 559, Feb 8–11, 2009.; (b) Su, H.-P.; Yan, Y.; Prasad, S.; Smith, R. F.; Daniels, C. L.; Abeywickrema, ; Reid, J. C.; Loughran, H. M.; Kornienko, M.; Sharma, S.; Grobler, J. A.; Xu, B.; Sardana, V.; Allison, T. J.; Williams, P. D.; Darke, P. L.; Hazuda, D. J.; Munshi, S. *J. Virol.* **2010**, *84*, 7625.

10. Pratt, J. K.; Donner, P.; McDaniel, K. F.; Maring, C. J.; Kati, W. M.; Mo, H.; Middleton, T.; Liu, Y.; Ng, T.; Xie, Q.; Zhang, R.; Montgomery, D.; Molla, A.; Kempf, D. J.; Kohlbrenner, W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1577.
11. Shaw-Reid, C. A.; Feuston, B.; Munshi, V.; Getty, K.; Krueger, J.; Hazuda, D. J.; Parniak, M. A.; Miller, M. D.; Lewis, D. *Biochemistry* **2005**, *44*, 1595.
12. During the course of this work it was found that RNase H inhibitors exhibited partial inhibition of DNA polymerase activity with RNA templates and not with DNA templates. The inhibition observed exclusively on RNA templates is believed to be an indirect effect due to formation of stable complexes in which inhibitor is bound to the RNase H active site. Grobler, J. A., unpublished results.
13. Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S.; Vacca, J.; Hazuda, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661.
14. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646.
15. Hamid, R.; Rotshteyn, Y.; Rabadi, L.; Parikh, R.; Bullock, P. *Toxicol. In Vitro* **2004**, *18*, 703.
16. Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096.