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Discovery, synthesis and biochemical profiling of purine-2,6-dione derivatives as inhibitors of the human poly(A)-selective ribonuclease Caf1

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ABSTRACT

Eukaryotic mRNA contains a 3′ poly(A) tail, which plays important roles in the regulation of mRNA stability and translation. Well-characterized enzymes involved in the shortening of the poly(A) tail include the multi-subunit Ccr4-Not deadenylase, which contains the Caf1 (Pop2) and Ccr4 catalytic components, and poly(A)-specific ribonuclease (PARN). Two Mg^{2+} ions present in the active sites of these ribonucleases are required for RNA cleavage. Here, we report the discovery, synthesis and biochemical profiling of purine-2,6-dione derivatives as (sub)micromolar inhibitors of Caf1.

In eukaryotic cells, cytoplasmic mRNA is characterized by the presence of a 3′ poly(A) tail. The median length of the tail varies from 27 to 28 nucleotides in yeast to 60–100 nucleotides in mammalian cells.[1,2] The tail is important for the control of gene expression: enzymatic shortening of the poly(A) tail (deadenylation) can initiate mRNA degradation and repress translation.[3] An important enzyme involved in cytoplasmic deadenylation is the multi-component Ccr4-Not complex.[4,5] In addition to six non-catalytic subunits, the complex contains two subunits with ribonuclease activity: both Caf1 and Ccr4 display Mg^{2+}-dependent 3′–5′ *exo*-ribonuclease activity with a preference for poly(A). However, whereas the enzymatic activity of Caf1 is associated with an RNAse D/DEDD (Asp-Glu-Asp-Asp) domain,[6–8] the enzymatic activity of Ccr4 is provided by an EEP (endonuclease-exonuclease-phosphatase) domain.[9,10]

The analysis of genetically modified mice has identified the importance of Ccr4-Not subunits in the regulation of physiological functions such as bone formation and male fertility[11,12] as well as obesity and heart disease.[13,14] Moreover, mutations in *CNOT3* are frequently identified in acute lymphoblastic leukemia.[15] Currently, it is unclear whether the ribonuclease activities of the Ccr4-Not complex are involved in the regulation of these processes and to what extent the Caf1 and Ccr4 subunits have unique roles, or cooperate in deadenylation.[16–18] Because the structural complexity of the Ccr4-Not deadenylase is a significant barrier to distinguishing between catalytic and structural roles of the complex, additional tools to study Ccr4-Not function are required. In particular, cell-permeable small-molecule inhibitors that selectively inhibit the enzyme activities of Caf1 or Ccr4 are desirable as a complementary approach to RNAi and genetic techniques. Such molecules will be highly useful as pharmacological tools to study the involvement of the catalytic activity of Ccr4-Not in physiological processes, and contribute to the evaluation of this complex as a potential therapeutic target.

While nucleoside analogues have been reported as inhibitors of the poly(A)-specific ribonuclease PARN, whose DEDD-type nuclease domain is closely related to that of Caf1,[19,20] non-nucleoside inhibitors have only been reported for a limited number of Mg^{2+}-dependent ribonucleases. These include influenza RNA

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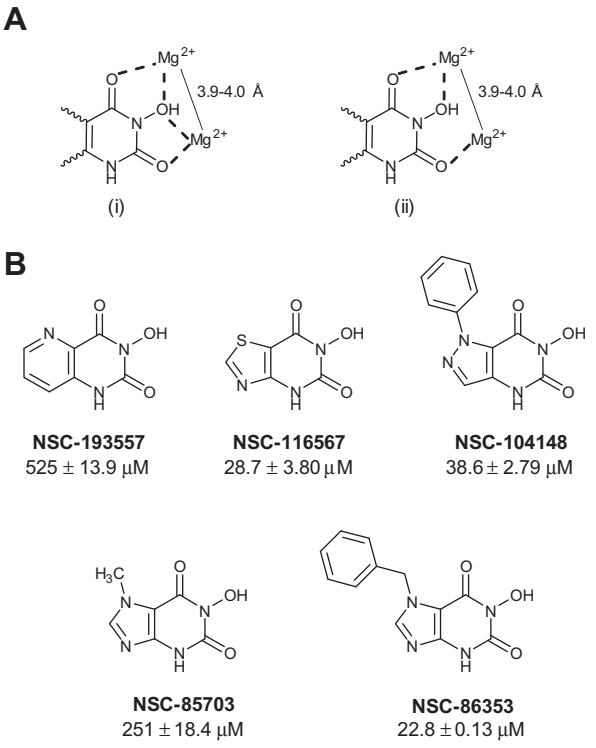


Figure 1. Discovery of 3-hydroxy-pyrimidine-2,4-dione compounds as inhibitors of the Caf1 ribonuclease. (A) Possible interaction modes of 3-hydroxy-pyrimidine-2,4-dione compounds with two divalent metal ions in the active site of Caf1. (B) Structure and activity of compounds selected from the Open Chemical Repository Collection (NCI, Bethesda). IC_{50} values refer to inhibition of Caf1. Also indicated is the standard error of the mean ($n = 3$).

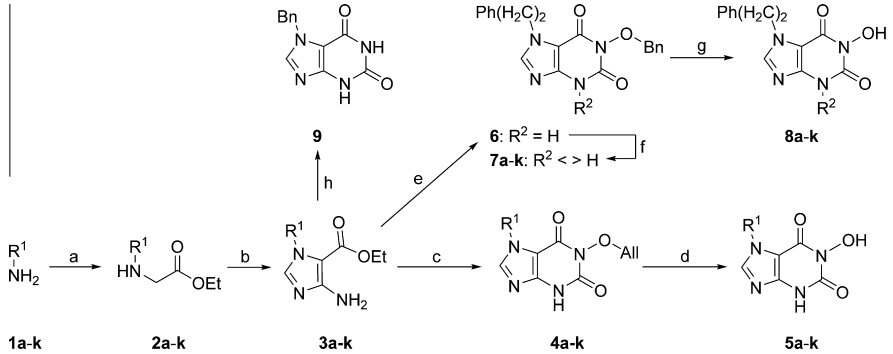
Table 1
Activity of 7-substituted 1-hydroxy-3,7-dihydro-1*H*-purine-2,6-diones **5**

Cmpd	R^1 in	IC_{50} (μM)[a] (Caf1)	IC_{50} (μM)[a] (PARN)
5a		10.4 ± 0.4	84.1 ± 6.7
5b		28.2 ± 7.6	n.d.
5c		10.6 ± 2.7	119 ± 25
5d		6.6 ± 0.7	125 ± 32
5e		23.3 ± 2.2	245 ± 20
5f		13.3 ± 2.3	n.d.
5g		20.6 ± 5.8	n.d.
5h		3.6 ± 1.1	n.d.
5i		10.6 ± 4.3	n.d.
5j		1.5 ± 0.3	n.d.
5k		4.0 ± 1.1	n.d.

[a] IC_{50} values were determined using a fluorescence-based biochemical assay as described.[22] Also indicated are the standard errors of the means ($n = 3$). N.d., not determined.

endonuclease (required for viral transcription) and human immunodeficiency virus (HIV) RNAse H (an RNA endonuclease involved in the degradation of RNA strands of RNA:DNA hybrids).[21,22] In addition, we recently reported the discovery of non-nucleoside inhibitors of Caf1, which were identified by screening a compound library.[22] Here, we describe an alternative approach, based on the discovery of *N*-hydroxyimide compounds as inhibitors of HIV RNAse H and human flap endonuclease FEN1, a structure-specific DNA endonuclease.[23,24] It was suggested that these compounds inhibited enzyme activity by coordination of the two divalent metal ions required for catalysis (Fig. 1A). Interestingly, *N*-hydroxyimides were also identified as inhibitors of the influenza RNA endonuclease PA.[21] Based on the distance between the Mg^{2+} ions in the active site of the Caf1 ribonuclease (3.9–4.0 Å; Fig. 2a and b),[25] we hypothesized that compounds containing this moiety may adopt a similar binding mode in the active site of Caf1, thereby blocking substrate binding.

We therefore searched the Open Chemical Repository Collection, a diverse set of more than 200,000 compounds (Developmental Therapeutics Program, National Cancer Institute, Bethesda), for the presence of *N*-hydroxyimides. To limit the number of compounds, we focused on 3-hydroxypyrimidine-2,4-diones and obtained seven compounds for further analysis. Following LC–MS and solubility analysis, five compounds were evaluated



Scheme 1. Synthesis of 7-substituted 1-hydroxy-3,7-dihydro-1*H*-purine-2,6-diones. Reagents and conditions: (a) ethyl 2-bromoacetate, $CHCl_3$, rt, 2 h (37–79%); (b) (ethoxymethylene)cyanamide, THF, Δ, 16 h, then $KOBu^t$, EtOH, Δ, 2 h (46–80% over 2 steps); (c) CDI, PhMe, Δ, 2 h, then *O*-allylhydroxylamine, aq NaOH, EtOH, Δ, 2 h (21–61% over 2 steps); (d) $Pd(OAc)_2$, PPh_3, HCOOH, EtOH–H_2O (8:2), 80 °C, 2 h (23–55%); (e) CDI, PhMe, Δ, 2 h, then *O*-benzylhydroxylamine, aq NaOH, EtOH, Δ, 2 h (74% over 2 steps); (f) R^2X (X = Br or I), K_2CO_3, DMF, 80 °C, 2–12 h, (67–98%); (g) H_2, 10% (w/w) Pd(C), CH_2Cl_2–MeOH (1:9) (33–53%); (h) urea, 2-methoxyethanol, 190 °C, 24 h, then aq NaOH, Δ, 3 h (33%). For definitions of R^1 and R^2 refer to Tables 1–3.