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Discovery of novel *N*-hydroxy-2-arylisoindoline-4-carboxamides as potent and selective inhibitors of HDAC11

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ABSTRACT

N-Hydroxy-2-arylisoindoline-4-carboxamides are potent and selective inhibitors of HDAC11. The discovery, synthesis, and structure activity relationships of this novel series of inhibitors are reported. An advanced analog (**FT895**) displays promising cellular activity and pharmacokinetic properties that make it a useful tool to study the biology of HDAC11 and its potential use as a therapeutic target for oncology and inflammation indications.

Acetylation of lysine residues is an important post-translational modification that occurs on cellular proteins including, but not limited to, histones. Protein acetylation levels are controlled by histone deacetylases (HDACs) that catalyze the removal of acetyl groups and histone acetyltransferases (HATs) that are responsible for the addition of acetyl groups. The 11 HDAC family members require zinc as a cofactor for deacetylase activity and are grouped into four classes: class I (HDAC1, HDAC2, HDAC3, HDAC8), class IIa (HDAC4, HDAC5, HDAC7, HDAC9), class IIb (HDAC6, HDAC10), and class IV (HDAC11). HDACs regulate a range of cellular processes including gene expression, transcription factor activity, cell signaling pathways, and protein degradation.[1]

As a result, HDACs are associated with cancer and other diseases and have been an active area of drug development. Currently, four HDAC inhibitors have been approved for use in hematological cancers (vorinostat, romidepsin, belinostat, and panobinostat) and many more are currently in clinical trials.[2] Most of the reported HDAC inhibitors are pan-inhibitors, meaning that they inhibit most if not all HDAC isoforms, and their full utility has been limited by toxicity thought to be attributable to broad HDAC inhibition.[3] Therefore, isoform-selective inhibitors have the potential to maximize therapeutic benefit while minimizing side effects.

HDAC11 is the most recently identified HDAC[4] and has been reported to regulate various immune cells, such as antigen-presenting cells, neutrophils, myeloid-derived suppressor cells, T cells, and regulatory T cells,[5–9] and function in RNA splicing.[10] HDAC11-selective inhibitors could have utility for cancer or inflammatory and immunological diseases. In addition to deacetylase activity, HDAC11 has also been reported to have fatty acid deacylase activity.[11] Herein, we describe work to identify selective HDAC11 deacetylase inhibitors with cellular activity and pharmacokinetic properties in mice that can be used to further interrogate HDAC11 biology.

As part of our broad efforts to design and synthesize potent and selective inhibitors of the various HDAC family members, *N*-hydroxytetrahydroisoquinoline-7-carboxamide **1** was identified as a potent inhibitor of HDAC6 with modest activity against HDAC11 (Fig. 1). The discovery of compound **1** provided an opportunity to identify selective HDAC11 inhibitors, and efforts to explore this hypothesis were initiated.

Hydroxamic acids are well known as one of the common chemotypes found in HDAC inhibitors.[2] As hypothesized for compounds such as **1**, this group coordinates to the zinc atom located within the active site of all members of the HDAC family and is essential for HDAC activity.[3] Initial studies explored the impact of the hydroxamic acid position on both the HDAC6 and HDAC11 activities. As shown in Table 1, the regiochemistry of the hydroxamic acid substituent affected the potency of both HDAC11 and HDAC6. Substitution at the 6-position (compound **3**) afforded similar HDAC6 activity but resulted in loss of HDAC11 potency relative to the initial lead (**1**). Installing the hydroxamic acid group at the 8-position (**4**), resulted in a 20-fold loss in

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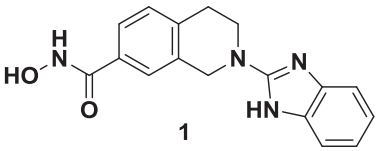
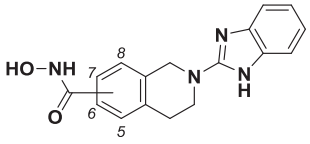


Fig. 1. Structure and activity of Compound **1**.

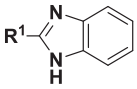
Table 1
SAR of the Hydroxamic Acid Regiochemistry.

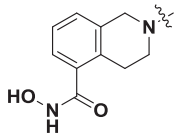
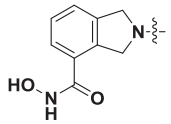
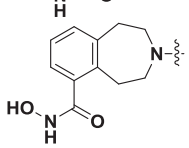


	Position of Hydroxamic Acid	HDAC 11 IC_{50} (μM)[a]	HDAC 6 IC_{50} (μM)[a]
1	7	2.1	0.004
2	5	1.2	> 10
3	6	> 10	0.002
4	8	4.6	0.082

[a] Activity was measured using electrophoretic mobility shift assays with full length human recombinant HDAC proteins and fluorescent-labeled peptide substrates. Reported as the mean of at least two separate assay runs.

Table 2
Optimization of Hydroxamic Acid core.

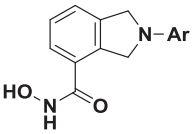


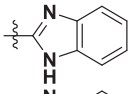
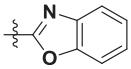
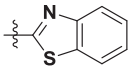
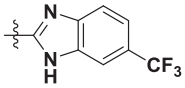
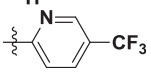
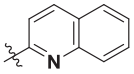
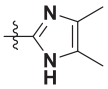
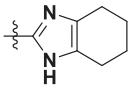
	R^1	HDAC11[a] IC_{50} (μM)	HDAC6[a] IC_{50} (μM)	m-CL_{int}[b] (μL/min/mg)	LipE[c]
2		1.2	> 10	< 7	4.5
5		0.17	> 10	17	5.7
6		0.03	2.8	161	5.2

[a] Activity was measured using electrophoretic mobility shift assays with full length human recombinant HDAC proteins and fluorescent-labeled peptide substrates. Reported as the mean of at least two separate assay runs.
[b] *In vitro* intrinsic clearance after incubation with mouse liver microsomes.
[c] Lipophilic efficiency = pIC_{50} HDAC11 – $clogD_{7.4}$.

potency vs. HDAC6 but only a 2-fold loss in activity vs. HDAC11, meaning it could potentially be tolerated. The largest impact was observed when the hydroxamic acid was substituted at the 5-position. While compound **2** exhibited only modest HDAC11 activity (IC_{50} = 1.2 μM), no significant HDAC6 potency was observed (IC_{50} > 10 μM). Having identified a preferred regiochemistry for the hydroxamic acid, optimization of the tetrahydroisoquinoline ring and the *N*-aryl substituent was initiated to identify the optimal shape for

Table 3
Optimization of *N*-Aryl substituent.



	Ar	HDAC11[a] IC_{50} (μM)	m-CL_{int}[b] (μL/min/mg)	LipE[c]
5		0.17	17	5.7
7		0.007	313	6.6
8		0.035	323	5.5
9		0.002	140	6.3
10		0.007	81	6.2
11		0.026	92	5.5
12		2.3	88	6.5
13		0.30	317	5.8

[a] Activity was measured using electrophoretic mobility shift assays with full length human recombinant HDAC proteins and fluorescent-labeled peptide substrates. Reported as the mean of at least two separate assay runs.
[b] *In vitro* intrinsic clearance after incubation with mouse liver microsomes.
[c] Lipophilic efficiency = pIC_{50} HDAC11 – $clogD_{7.4}$.

HDAC11 inhibition.

Table 2 highlights efforts to optimize the hydroxamic acid core. Working from the tetrahydroisoquinoline, changes to the ring size of the saturated ring were examined. Thus, isoindoline and 2,3,4,5-tetrahydrobenzodiazepine cores were explored. Both isoindolines (**5**) and benzodiazepine (**6**) cores were tolerated and exhibited > 10-fold increases in potency against HDAC11 (IC_{50} = 170 nM and 3 nM, respectively) while maintaining selectivity over HDAC6 (IC_{50} > 10 μM and 2.8 μM, respectively). While tetrahydrobenzodiazepine **6** showed better potency vs. HDAC11, optimization efforts were focused on the isoindoline **5** due to its combination of reasonable potency and significant microsomal stability relative to **6** (CL_{int} = 17 μL/min/mg vs. 161 μL/min/mg after incubation with mouse liver microsomes).

The optimization of the heterocyclic ring at the 2-position of the isoindoline ring is summarized in Table 3. A variety of replacements for the benzimidazole ring were explored, with benzoxazole (**7**), benzothiazole (**8**), pyridine (**10**), and quinoline (**11**) analogs all showing improved potency vs. HDAC11, albeit with a significant loss of microsomal stability. Saturating one of the aromatic rings (**13**) or eliminating one of the rings (to afford imidazole **12**) resulted in loss of potency and microsomal stability. Of note, introduction of the lipophilic trifluoromethyl group (compound **9**) resulted in a significant increase in both potency (2 nM vs. 170 nM) and LipE (6.3 vs. 5.7) but again with loss of microsomal stability relative to **5**. In the absence of a co-crystal structure of HDAC11, a homology model based on internal co-crystal structures of HDAC8 was generated. Modeling of compound **9** into the