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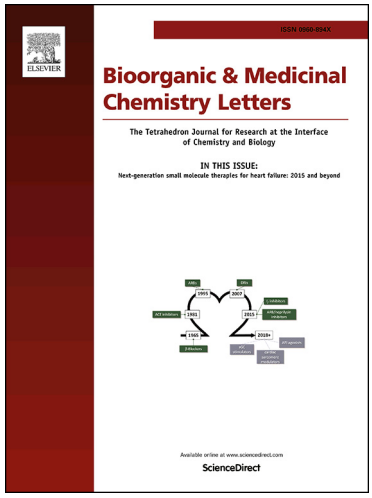

Discovery of novel *N*-hydroxy-2-arylisoindoline-4-carboxamides as potent and selective inhibitors of HDAC11

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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.

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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*-hydroxy-tetrahydroisoquinoline-7-carboxamide **1** was identified as a potent inhibitor of HDAC6 with modest activity against HDAC11 (Figure 1). The discovery of compound **1** provided an opportunity to identify selective HDAC11 inhibitors, and efforts to explore this hypothesis were initiated.

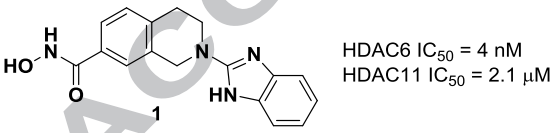
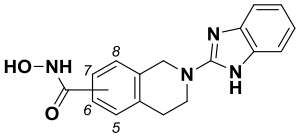


Figure 1. Structure and activity of Compound **1**

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 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 HDAC11 inhibition.

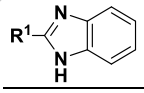
Table 1. SAR of the Hydroxamic Acid Regiochemistry

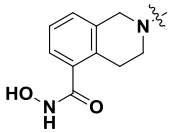
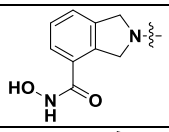
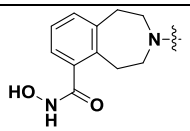


	Position of Hydroxamic Acid	HDAC 11 IC_{50} (μM)[a]	HDAC 6 IC_{50} (μM)[a]
1	7	2.1	0.004
2	5	1.2	>10
3	6	>10	0.002
4	8	4.6	0.082

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

Table 2. Optimization of Hydroxamic Acid core



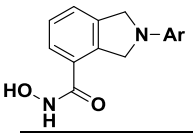
	R^1	HDAC11[a] IC_{50} (μM)	HDAC6[a] IC_{50} (μM)	m-CL_{int}[b] (μL/min/mg)	LipE[c]
2		1.2	>10	<7	4.5
5		0.17	>10	17	5.7
6		0.03	2.8	161	5.2

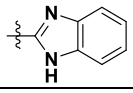
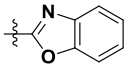
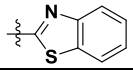
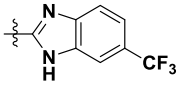
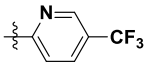
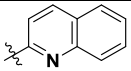
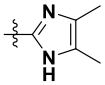
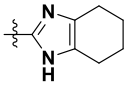
[a]Activity was measured using electrophoretic mobility shift assays with full length human recombinant HDAC proteins and fluorescently labeled peptide substrates. Reported as the mean of at least two separate assay runs. [b]In vitro intrinsic clearance after incubation with mouse liver microsomes. [c]Lipophilic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

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).

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 fluorescently 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 – $clogD_{7.4}$.

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 homology model supported a lipophilic binding hypothesis with the trifluoromethyl group predicted to efficiently fill a small pocket adjacent to the zinc binding site formed by hydrophobic residues and backbone carbonyls.

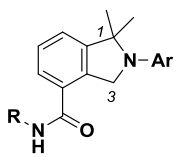
While altering the core and *N*-aryl substituent led to improvements in both potency and LipE, these changes also resulted in compounds with poor microsomal stability. It was hypothesized that the two benzylic methylene groups present in the isoindoline core were the most likely sites of metabolism and hypothesized that blocking these sites would afford compounds with improved microsomal stability.

Initial efforts focused on substitution of the benzyl group at the 1-position (Table 4). The less-hindered benzylic group was expected to be more prone to oxidation relative to the 3-position (which is presumably shielded by the neighboring hydroxamic acid). Thus, introduction of the gem-dimethyl group afforded compound **14** which retained the potency and LipE of the parent compound (**9**) with a significant increase in stability (CL_{int} = 12 μL/min/mg vs. 140 μL/min/mg after incubation with mouse liver microsomes). This trend was consistent across all analogs, including benzoxazole **15** and pyridines **17** and **18**. The solubility and permeability of the isoindolines was also examined in hopes of identifying a compound suitable for *in vivo* studies. Pyridines, such as **17** and **18** showed a nice balance of properties, as did the related pyrimidine (**19** and **20**) and pyrazine (**FT895**) analogs.

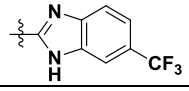
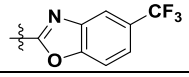
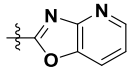
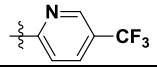
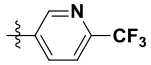
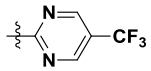
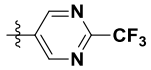
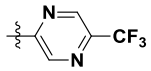
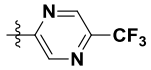
Having identified a series of potent compounds with suitable *in vitro* ADME properties, the cellular activity of these HDAC11 inhibitors was examined. Cellular activity was measured via a bioluminescence resonance energy transfer (BRET) target engagement assay using HDAC11 fused to Nanoluc luciferase and a proprietary compound labeled with a fluorescent tracer.[12] In general, compounds exhibited a five- to twenty-fold shift between the biochemical and cellular assays, with many having cellular IC_{50} values of less than 100 nM. Specifically, (trifluoromethyl)pyridine (**18**) and (trifluoromethyl)pyrazine (**FT895**) analogs, displayed IC_{50} values of less than 20 nM.

FT895 was envisioned as a potentially useful tool compound based on its overall potency and *in vitro* ADME profile. To enable a more thorough exploration of HDAC11 biology, a structurally matched companion inactive control analog was sought to use in tandem with **FT895**. Thus, compound **21** was synthesized and profiled for this purpose, and, as expected, replacing the hydroxamic acid necessary for zinc binding in the active site with a primary amide resulted in loss of all HDAC11 activity (Table 4). Furthermore, **FT895** was determined to be a highly-selective HDAC11 inhibitor showing greater than 1000-fold selectivity against the other 10 members of the HDAC family (Table 5), while **21** was found to be inactive against all HDACs.

Table 4. Optimization of *N*-Arylisoindolines



Ar	**R**	HDAC11[a] IC_{50} (μM)	HDAC11 BRET[b] IC_{50} (μM)	m-CL_{int}[c] (μL/min/mg)	Solubility[d] (μM)	PAMPA[e] (10^{-6} cm/s)	$clogD_{7.4}$[f]	LipE[g]

14		**OH**	0.002	NT	12	51	0.1	2.7	6.0
15		**OH**	0.001	NT	10	4	7	3.1	6.1
16		**OH**	0.002	0.020	7	78	0.7	1.6	7.2
17		**OH**	0.006	0.13	28	8	12	2.6	5.7
18		**OH**	0.003	0.012	12	51	8	2.5	6.1
19		**OH**	0.005	0.085	21	32	18	2.2	6.1
20		**OH**	0.027	0.27	7	82	NT	1.1	6.5
FT895		**OH**	0.003	0.015	22	31	13	2.3	6.3
21		**H**	>10	>25	7	0.3	17	2.7	2.3

[a]Activity was measured using electrophoretic mobility shift assays with full length human recombinant HDAC proteins and fluorescently labeled peptide substrates. Reported as the mean of at least two separate assay runs. NT = not tested. [b]BRET Cell assay. [c]In vitro intrinsic clearance after incubation with mouse liver microsomes. [d]Kinetic solubility at pH 7.4. [e]Permeability measured via parallel artificial membrane permeability assay. [f]Calculated logD value at pH 7.4. [g]Lipophilic efficiency = pIC_{50} HDAC11 – $clogD_{7.4}$.

Based on its overall *in vitro* profile, **FT895** was advanced to mouse PK studies to measure its suitability as an *in vivo* tool compound. The pharmacokinetic properties of **FT895** were assessed in male Balb/c nude mice following both intravenous (i.v.) and intraperitoneal (i.p.) dosing (Table 6).[13] The compound displayed a moderate clearance (42 mL/min/kg) and high volume of distribution, resulting in a half-life of 9.4 h after i.v. dosing. When dosed i.p., **FT895** had a similar $t_{1/2}$ (10.2 h) and improved exposure, resulting in a bioavailability of 81%. **FT895** also maintained free drug levels over the cellular IC_{50} for up to 4 h after a single 5 mg/kg i.p. dose, thus providing a potentially useful tool for further understanding the biology of HDAC11 *in vitro* and *in vivo*.

Table 5. HDAC Activity Profiles of **FT895** and **21**[a]

HDAC IC_{50} (μM)											
	1	2	3	4	5	6	7	8	9	10	11
FT895	>10	>10	>10	>10	>10	>10	>10	5.6	>10	>10	0.003
21	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10

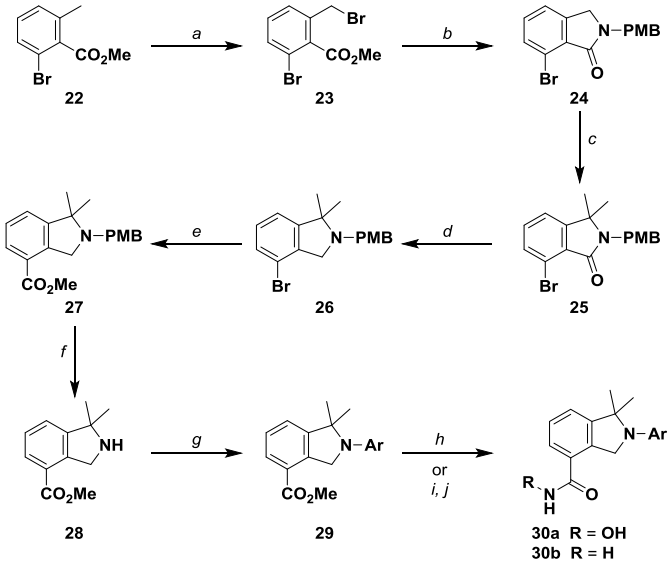
[a]Activity was measured using electrophoretic mobility shift assays with full length human recombinant HDAC proteins and fluorescently labeled peptide substrates. Reported as the mean of at least two separate assay runs.

Table 6. Pharmacokinetic Profile of **FT895**[a]

IV Dose (mg/kg)	$t_{1/2}$ (h)	C_0 (μM)	AUC_{last} (μM*h)	V_{ss} (L/kg)	CL (mL/min/kg)
1	9.4	3.5	1.0	18	42

IP Dose (mg/kg)	$t_{1/2}$ (h)	C_{max} (μM)	AUC_{last} (μM*h)	Bioavailability (%F)
5	10.2	4.2	3.8	81

[a]Dosed in male Balb/c nude mice (*n*=3). Dosing formulation: 5% DMA/1% Tween 80/94% sterile water



Scheme 1. Synthesis of 2-arylisoindoline-4-carboxamides. Reagents and conditions: (a) NBS, benzoyl peroxide, CCl_4, 80 °C, 16 h, 100%; (b) *p*-methoxybenzylamine, Et_3N, MeOH, 40 °C, 4 h, 37%; (c) NaH, THF, rt, 3 h then MeI, rt, 16 h, 61 %; (d) BH_3-THF, 80 °C, 16 h, 83%; (e) CO (60 atm), $PdCl_2$(dppf), Et_3N, MeOH, 130 °C, 24 h, 47%; (f) H_2, Pd/C, conc. HCl, MeOH, rt, 16 h, 79%; (g) ArCl, RuPhos, RuPhos 2G precatalyst, Cs_2CO_3, toluene, 100 °C, 16 h, 44-88%; (h) NH_2OH, NaOH, THF, MeOH, rt, 4 h, 12-48%; (i) LiOH, THF, H_2O, rt, 16 h, 78-94%; (j) NH_4Cl, HATU, (*i*-Pr_2)NEt, DMF, rt, 16 h, 20-34%. PMB = *p*-methoxybenzyl.

The *N*-hydroxy-2-arylisoindoline-4-carboxamides described in Tables 3 and 4 were synthesized according to the route illustrated in Scheme 1. Bromination of ethyl 2-bromo-6-methylbenzoate (**22**) with NBS afforded benzyl bromide **23 i**n quantitative yield. Amination and intramolecular cyclization provided isoindolinone **24** which was subsequently deprotonated with sodium hydride and alkylated with methyl iodide to afford **25**. Chemoselective reduction of the carbonyl group with borane provided isoindoline **26**, which underwent a palladium-catalyzed carbonylation in the presence of carbon monoxide and methanol to yield **27**. Removal of the *p*-methoxybenzyl protecting group afforded isoindoline **28**. Palladium-catalyzed Buchwald coupling[14] of **28** with the appropriate aryl chloride and the second generation RuPhos precatalyst yielded isoindoline carboxylate **29**. Treatment of **29** with hydroxyl amine generated the hydroxamic acid and provided the desired *N*-hydroxy-2-arylisoindoline-4-carboxamides (**30a**) in good yield. For the preparation of amides, such as **21**, ester **29** was saponified with lithium hydroxide, and the resulting acid was coupled with ammonium chloride in the presence of HATU to give amide **30b**.

In summary, a novel series of *N*-hydroxy-2-arylisoindoline-4-carboxamides have been identified as potent and highly selective inhibitors of HDAC11. These first-in-class inhibitors show promising cellular activity and pharmacokinetic properties that make them useful tools to better understand the biology of HDAC11 and its potential use as a therapeutic target for oncology and inflammation indications. Future work will include further optimization of **FT895** and its utility in *in vivo* models.

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Supplementary Material

Supplementary material associated with this article (including details of the in vitro HDAC 11 assay and experimental procedures for the synthesis of compounds **FT895** and **21**) can be found in the online version.

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Graphical Abstract

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

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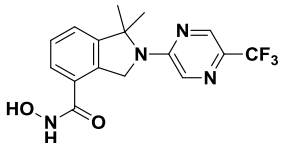

FT895
HDAC 11 IC_{50} = 3 nM
HDACs 1-10 IC_{50} >5 μM

Highlights

- Identified novel, potent, and selective first-in-class inhibitors of HDAC11
- Synthesis and structure activity relationships of compounds are reported
- **FT895** displays promising cellular activity and pharmacokinetic properties
- **FT895** is a valuable tool for further study of the biology of HDAC11