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The design, synthesis and structure–activity relationships of novel isoindoline-based histone deacetylase inhibitors

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ABSTRACT

The design, synthesis and biological evaluation of a novel series of isoindoline-based hydroxamates is described. Several analogs were shown to inhibit HDAC1 with IC_{50} values in the low nanomolar range and inhibit cellular proliferation of HCT116 human colon cancer cells in the sub-micromolar range. The cellular potency of compound 17e was found to have greater in vitro anti-proliferative activity than several compounds in late stage clinical trials for the treatment of cancer. The in vitro safety profiles of selected compounds were assessed and shown to be suitable for further lead optimization.

Histone acetyltransferases (HATs) catalyze the acetylation of lysine residues on histone and non-histone proteins. The reverse reaction is catalyzed by histone deacetylases (HDACs) thus promoting a more closed chromatin structure where transcription is repressed.[1,2] HDAC inhibitors have found utility for the treatment of human cancers; Zolinza (vorinostat, SAHA, (**1**), Merck) was approved by the FDA for the treatment of advanced cutaneous T-cell lymphoma (CTCL). Several small molecule HDAC inhibitors such as belinostat (PXD-101, (**2**), Curagen),[3,4] entinostat (SNDX-275 (**3**), Syndax),[5,6] mocetinostat (MGCD-0103 (**4**), Methylgene)[7–10] (Fig. 1) and dacinostat (LAQ824 (**5**), Novartis AG)[11] (Fig. 2) have entered clinical trials for the treatment of a variety hematological and solid tumors.

Since the discovery of LAQ824 (**5**), further studies led us to identify both hydroxamate based (**6**)[12] and non-hydroxamate-based compounds ((**7**) and (**8**), Fig. 2)[13] which demonstrate dissimilar HDAC isoform selectivity profiles. In an effort to identify additional HDAC inhibitors, isoindoline based scaffolds have been explored. The synthesis and in vitro anti-proliferative activities of these analogs will be discussed.

Our goal was to generate highly potent compounds that have an acceptable solubility and safety profile for clinical development, and have sufficient oral exposure in preclinical animal toxicology models for rigorous evaluation. Building on earlier efforts that generated compound **6**, we sought compounds that minimized the number of rotatable bonds in order to increase oral exposure in rodent models.[14] To this end, a series of cyclic analogs via the linker nitrogen were explored.[15] One such cyclization of the linker nitrogen of **9** with the core phenyl ring results in the isoindoline scaffold (**10**) (Fig. 3). Syntheses of isoindolines of this nature that are amenable to rapid analog synthesis were lacking, therefore, new synthetic approaches to key intermediates were required.

An efficient lead optimization campaign required compound **13** as a late stage intermediate that could be rapidly diversified (Scheme 1). Conventional reaction conditions were explored to convert 5-bromophthalamide (**11**) to 5-bromo-2,3-dihydro-1H-isoindole (**12**), but only pretreatment with BF_3OEt_2 followed by borane-THF reduction generated the free amine while leaving the bromide intact. The resulting free amine was difficult to isolate and was therefore converted to the *tert*-butyl carbamate **12** prior to isolation and purification. A subsequent Heck reaction with methyl acrylate followed by removal of the Boc protecting group provided the desired intermediate **13**. Conversion of the secondary amine **13** to amides, sulfonamides or tertiary amines proceeded

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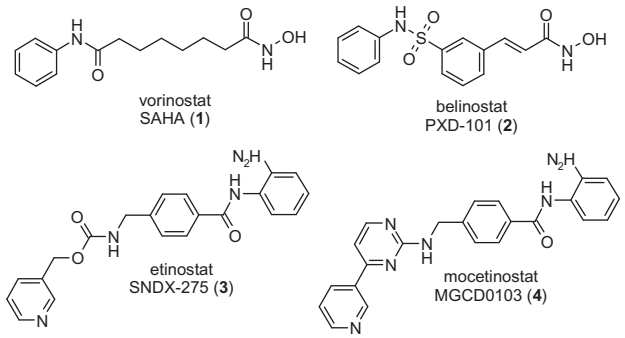


Fig. 1. Selection of HDAC inhibitors in clinical trials.

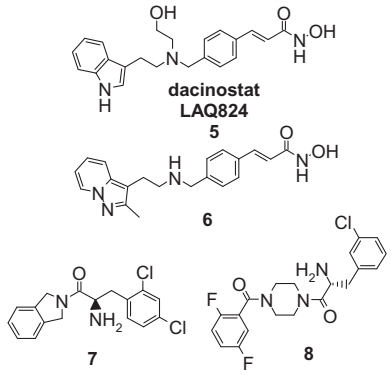


Fig. 2. Selection of HDAC inhibitors from previous internal efforts.

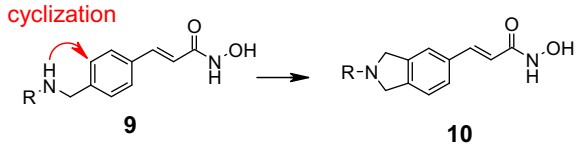
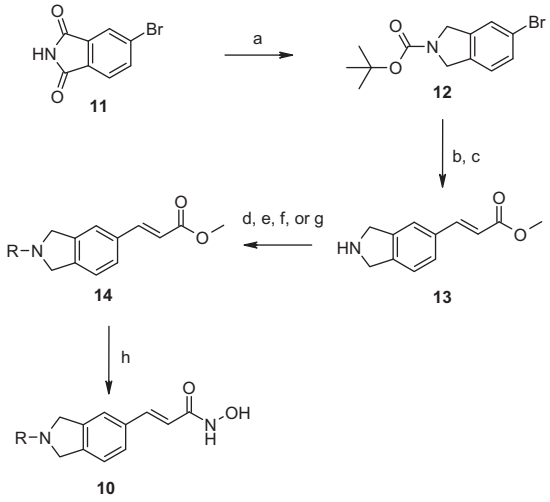


Fig. 3. Design of isoindoline based HDAC inhibitors.



Scheme 1. Reagents and conditions: (a) i. BF_3OEt_2, ii. BH_3 THF 40 °C, iii. Boc_2O, DMAP, THF (53% yield over two steps); (b) methyl acrylate, $Pd(OAc)_2$, $P(o\text{-}tol)_3$, Et_3N, DMF (42% yield); (c) i. TFA/CH_2Cl_2; (d) RCOCl, NMM, CH_2Cl_2 ii. HCl, dioxane (97% yield); (e) $ROCOCO_2R$, Et_3N, CH_2Cl_2; (f) RSO_2Cl, Et_3N, CH_2Cl_2; (f) RCH_2Br, Et_3N, CH_2Cl_2; (g) RCHO, $NaBH_3CN$, AcOH; (h) NH_2OH, NaOMe, MeOH.

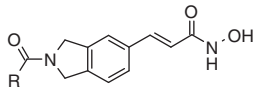
with relative ease. The desired hydroxamate analogs were obtained in good yields by treating the methyl esters with hydroxylamine in the presence of sodium methoxide.

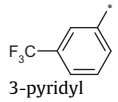
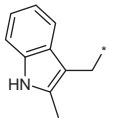
Purified HDAC-1 was used to assess the biochemical activity of each compound.[11] The anti-proliferative activities of promising compounds were determined in the HCT116 human colon cancer cell line. Simple alkyl carbamate and amide derivatives (compounds **15a–e**, Table 1) demonstrated sub-micromolar HDAC IC_{50} values, but these were generally poorly soluble with low activity in the cellular assay. Aromatic analogs did not improve the potency or solubility (compounds **15f** and **15g**). Incorporation of a methylene spacer (compound **15h**) improved the cellular activity to a level comparable to vorinostat (HCT116 IC_{50} = 0.81 μM). Replacement of the phenyl group with an indole (compound **15i**) resulted in a four-fold improvement in cellular potency and with an acceptable hERG safety profile (hERG radioligand binding IC_{50} >30 μM), however thermodynamic solubility was low (<20 μM at pH 6.8).

We next investigated sulfonamides. In general, alkyl and cycloalkyl sulfonamides (compounds **16a–16d**, Table 2) were weakly active. The phenyl sulfonamide derivative **16e** had moderate activity which was sensitive to steric and electronic effects on the phenyl ring. Substitution at the ortho position (**16l**) was tolerated, while improved potency was obtained with meta- and para-substituted analogs (e.g., compounds **16j** and **16k**). Modifications that reduced the aryl ring electron density (compounds **16g–16i**, 16m and **16n**) generally resulted in lower HDAC-1 potency, while analogs with greater electron density in the aromatic ring (compounds **16f**, **16o**, and **16p**) had improved affinity in the enzymatic assay. Several analogs were profiled to assess their solubility and in vitro safety profile. The analogs from the amide and sulfonamide series that were tested in the hERG patch clamp assay demonstrated IC_{50} values greater than 30 μM, but all suffered from a significantly lower than desired solubility (data not shown).

The third series that was investigated was the amine based isoindolines (Table 3). Attempts to synthesize a more diverse set of isoindoline analogs via reductive amination and alkylation were unsuccessful while identical approaches worked with scaffolds

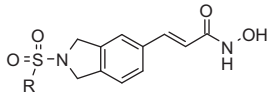
Table 1
Amide and carbamate series



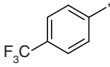
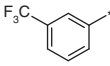
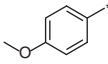
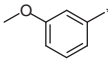
Compound	R	HDAC-1[a] IC_{50} (μM)	HCT116[b] IC_{50} (μM)
15a	$(CH_3)_3CO$	0.52	nd
15b	$(CH_3)_3C$	0.42	nd
15c	Cyclopropyl	0.42	nd
15d	Cyclopentyl	0.35	nd
15e	Cyclohexyl	0.39	nd
15f		0.20	5.82
15g	3-pyridyl	0.38	nd
15h	benzyl	0.13	0.83
15i		0.020	0.19

nd – not determined
[a] Inhibition of recombinant HDAC1.
[b] Anti-proliferative activity in human colon cancer HCT116 cell line.

Table 2
Sulfonamide series



16

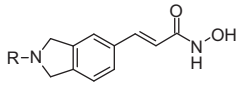
Compound	R	HDAC-1[a] IC_{50} (μM)	HCT116[b] IC_{50} (μM)
16a	Methyl	0.63	nd
16b	Butyl	0.15	3.21
16c	Cyclopropyl	0.79	nd
16d	Benzyl	0.12	1.12
16e	Phenyl	0.19	3.42
16f	2-Thiophenyl	0.089	0.80
16g	4-Pyridyl	0.48	
16h	3-Pyridyl	0.15	4.26
16i		0.14	8.84
16j		0.047	1.58
16k		0.062	1.42
16l		0.13	2.23
16m		0.16	2.43
16n		0.16	2.05
16o		0.035	0.93
16p		0.092	1.13

nd – not determined
[a] Inhibition of recombinant HDAC1.
[b] Anti-proliferative activity in human colon cancer HCT116 cell line.

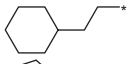
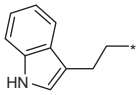
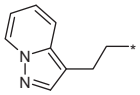
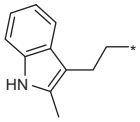
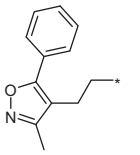
containing larger ring sizes than the isoindoline.[16] Synthetic issues in this series included highly variable yields and multiple by-products that hampered purification. Even though a complete SAR trend in the amine series remains frustratingly out of reach, several key analogs provide valuable information. The benzyl analog **17a** was moderately potent against HDAC-1 enzyme and had improved solubility (106 μM at pH 6.8). Potency was further improved with the cyclohexylethyl moiety (**17b**) however, hERG inhibition appeared. Potency was further enhanced with the incorporation of the indole derivative **17c** with a concomitant decrease in hERG activity. Incorporation of a methyl group at the 2-position of the indole (**17e**) resulted in a five-fold increase in cellular potency, a similar increase in the hERG patch clamp activity was also observed. Compound **17f** was found to be less active than other analogs in this series.

To determine if a full lead optimization campaign with this series was warranted, in vitro profiling in comparison to several clinical compounds was performed (Tables 4–6). Cancer patients often take multiple medications and therefore drug–drug interactions are a major concern,[17] especially with the potential to use an HDAC inhibitor as part of a combination therapy regime.[18] CYP3A4 is generally regarded as the most relevant CYP450 implicated in drug–drug interactions and thus was the focus of our profiling.[19] All compounds tested had CYP3A4 IC_{50} values greater than 10 μM and with some having IC_{50} values greater than 50 μM. The potency of CYP3A4 inhibition of all compounds tested in this series appears significantly less than benzamide class of HDAC inhibitors.

Table 3
Amine series



17

Compound	R	HDAC-1[a] IC_{50} (μM)	HCT116[b] IC_{50} (μM)	hERG patch clamp[c] IC_{50} (μM)
17a		0.20	1.04	27.5
17b		0.10	0.90	22
17c		0.016	0.22	>30
17d		0.078	0.53	5.2
17e		0.026	0.042	4.8
17f		0.17	2.2	>30

[a] Inhibition of recombinant HDAC1.
[b] Anti-proliferative activity in human colon cancer HCT116 cell line.
[c] Activity in automated patch clamp assay for hERG activity.

Table 4
In vitro drug–drug interaction profile of select compounds

Compound	HCT116[a] IC_{50} (μM)	CYP3A4 IC_{50} (μM)	CYP3A4/HCT116 ratio
SAHA (**1**)	0.81	>10	>12
Belinostat (**2**)	0.16	>10	>62
SNDX-275 (**3**)	0.67	0.73	1.1
MGCD0103 (**4**)	0.31	0.57	1.8
LAQ824 (**5**)	0.019	14	736
15h	0.83	>50	>60
15i	0.19	nd	
16f	0.80	>50	>63
16o	0.93	>50	>54
17b	0.90	>50	>56
17c	0.22	>10	>45
17d	0.53	>50	>94
17e	0.042	>10	>238

nd – not determined
[a] Anti-proliferative activity in human colon cancer HCT116 cell line.

Additional profiling included the hERG automated patch clamp (QPatch) assay (Table 5). To allow for rapid decision making on cardiac safety, we utilized the in vitro cardiac safety index (iCSI).[12] The iCSI is defined as a ratio of the hERG and the cellular anti-proliferative IC_{50} values. All amides and sulfonamides tested were inactive and relatively few amines (compounds **17b**, 17d and **17e**) demonstrated activity in the patch clamp assay. While the late stage clinical HDAC inhibitors disclosed below are inactive in the patch clamp assay, their moderate cellular activity makes it difficult to precisely determine iCSI values. Compound **17e** is the most

Table 5
In vitro cardiac safety index of select compounds

Compound	HDAC1 IC_{50} (μM)	HCT116[a] IC_{50} (μM)	hERG patch clamp[b] IC_{50} (μM)	iCSI
SAHA (**1**)	0.077	0.81	>30	>37
Belinostat (**2**)	0.015	0.16	>30	>187
SNDX-275 (**3**)	0.485	0.67	>30	>44
MGCD0103 (**4**)	0.152	0.31	>30	>97
LAQ824 (**5**)	0.015	0.019	10.3	542
15h	0.13	0.83	>30	>36
15i	0.020	0.19	>30	>158
17b		0.90	22.2	25
17c		0.22	>30	>136
17d		0.53	3.9	7.4
17e		0.042	4.8	114
17f		2.1	>30	>15

[a] Anti-proliferative activity in human colon cancer HCT116 cell line.
[b] Activity in automated patch clamp assay for hERG activity.

Table 6
Solubility and in vitro microsomal stability profile

Compound	Rat t1/2 (min)	Human t1/2 (min)	Thermodynamic solubility at pH 6.8 (μM)
SAHA (**1**)	114	405	19
Belinostat (**2**)	18	405	734
SNDX-275 (**3**)	405	405	401
MGCD0103 (**4**)	31.3	37.8	33
LAQ824 (**5**)	2.3	12	97
15a	14	131	99
15i	15	173	20
16f	nd	nd	34
16j	4.1	11	20
16k	7	15	20
16o	8.5	19	<5
17a	29	50	517
17c	5	nd	490
17d	9	112	765
17e	2	30	263
17f	6	17	490

potent HDAC inhibitor identified in this series and has an iCSI of 114. The hERG SAR in this series appears to track with HDAC activity and additional efforts to improve this safety margin would be warranted during a lead optimization campaign.

$$\text{iCSI} = \frac{\text{hERG } IC_{50}}{\text{cellular } IC_{50}} = \frac{\text{hERG } IC_{50}}{\text{HCT 116 } IC_{50}}$$

While the hydroxamate class of clinical HDAC inhibitors appears to be more potent and less susceptible to CYP450 inhibition than the benzamide class, hydroxamates generally have poor PK properties in rodent species.[20] This creates an additional challenge in the development of anti-cancer agents, which generally rely on rodent based xenograft models. The microsomal stability and solubility of select compounds were evaluated and are reported below (Table 6). All the analogs in the isoindoline series have poor microsomal stability in rat liver microsomes, whereas several analogs show enhanced stability in human liver microsomes. Compounds **17d** and **17e** have a reasonable combination of potency, solubility and human microsomal to justify further investigation.

In summary, a novel class of hydroxamate based HDAC inhibitors with the general structure **10** has been identified. Synthetic routes that enable late stage diversification, including a previously unreported method of efficient phthalamide carbonyl reduction in the presence of an aryl bromide functionality have been developed. A systematic approach to improve solubility, potency and metabolic stability resulted in several compounds with potency superior to several HDAC inhibitors in late stage clinical trials. Compound **17e** was identified as a candidate for lead optimization with promising drug-like properties. This compound possesses low nanomolar in vitro anti-proliferative activity, good solubility and an acceptable CYP3A4 profile. The low micromolar activity in the hERG patch clamp assay would have to be addressed by either improving HDAC potency, reducing hERG affinity or a combination thereof. Efforts toward this end will be reported in due course.

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