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Design and campaign synthesis of piperidine- and thiazole-based histone deacetylase inhibitors

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Abstract—A lead benzamide, **3**, was identified as a potent and low molecular weight histone deacetylase (HDAC) inhibitor. Optimization led to **16d**, demonstrating an excellent balance of efficacy and non-efficacy properties, along with very desirable in vivo DMPK. The final compounds presented are >1000-fold more potent than the initial screen hit, an improvement in potency which was achieved with a concomitant significant improvement in all the main non-efficacy properties.

In the eukaryotic cell, DNA is routinely compacted to prevent transcription factor accessibility. When the cell is activated this compacted DNA is made available to DNA-binding proteins, thereby allowing the induction of gene transcription.[1] Nuclear DNA associates with histones to form chromatin and the N-terminal tails of the core histones contain lysine residues that are sites for acetylation.[2,3] This reversible process is important in transcriptional regulation and cell-cycle progression.[4] Histone deacetylases (HDACs) are zinc-containing enzymes which catalyze the removal of acetyl groups from the ε-amino termini of lysine residues clustered near the amino terminus of nucleosomal histones and inhibition of this process is intimately linked to the induction of gene transcription.[5] In addition, HDAC disregulation has been associated with several cancers and HDAC inhibitors such as the commercially launched Zolinza (**1**)[2] and MS-275 (**2**)[6] are undergoing study for the potential treatment of cutaneous T-cell lymphoma and various hematological malignancies (Fig. 1).[7–9] Recent data highlight the impressive response rates in the treatment of Hodgkin lymphoma.[10]

As part of an ongoing effort to identify novel HDAC inhibitors, compound **3**[11] was identified as an active,

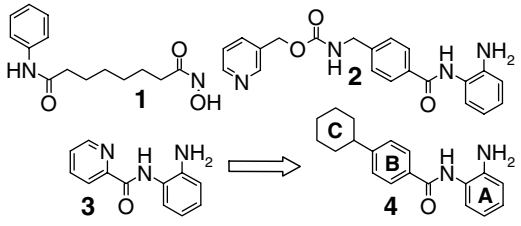

Figure 1. Zolinza (**1**), MS-275 (**2**) and pyridine lead **3**.

low molecular weight, but moderately soluble lead, capable of being elaborated into drug-like compounds. Although this compound demonstrated activity in a pooled HDAC enzyme assay,[12] cellular activity in an HCT116 proliferation assay[13] could not be reproducibly demonstrated. We hypothesized that the relatively modest enzyme potency of this compound leads to variable cellular activity, but this point was not conclusively proven. However, knowledge of postulated HDAC binding modes led us to suggest that elaboration of the 'B' ring of **3** with a 'C' ring, would provide a template for making compounds (**4**) of greater potency by being capable of making hydrophobic contacts in the cap group region of the HDAC protein (Fig. 2).

Compounds **7a**–**e** were therefore synthesized by coupling the amine **6** with substituted benzoic acid derivatives **5a**–**e** using *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)-*N*-methylmorpholinium chloride (DMTMM) in

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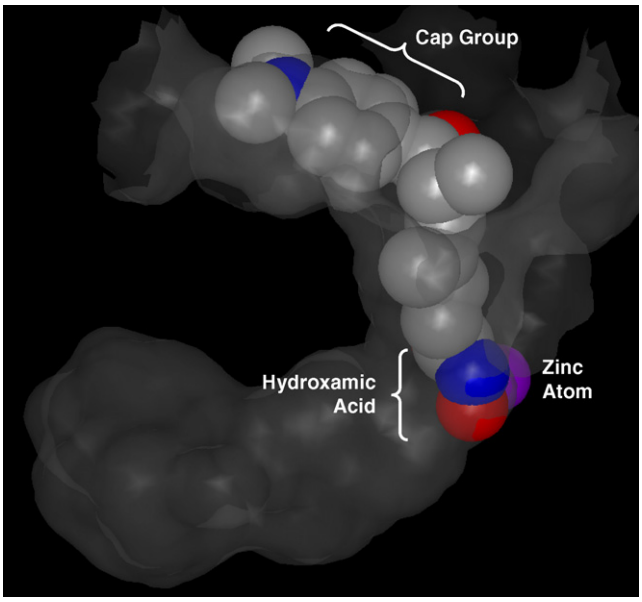


Figure 2. Crystal structure of Zolinza bound to HDAC-like protein.[14]

acetonitrile at room temperature, followed by overnight deprotection using HCl in dioxane. Compounds **8a–e** generally precipitated from the reaction mixture, and were isolated by filtration and washing with a non-polar solvent. Where free bases were required, they were usually further subjected to SCX cartridge ion-exchange chromatography to obtain the free base (Fig. 3).

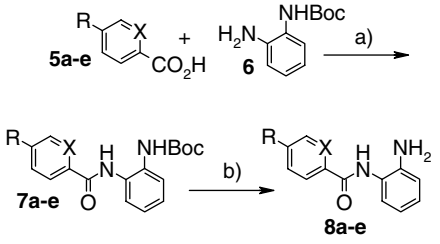


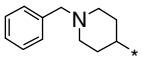
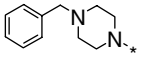
Figure 3. Synthesis of benzamide derivatives. (a) DMTMM, acetonitrile, rt, 50–82%; (b) 4 M HCl/dioxane, rt, 20 h, 42–82%. For the identity of R, see Table 1.

Compounds **8a–e** validated our hypothesis by showing a significant increase in potency compared to the starting hit **3**, albeit with a commensurate increase in log *D* (data not shown). We therefore sought to maintain or increase potency and to improve drug-like properties by appending a solubility-enhancing group to the 'C' ring (Table 1).

Our synthetic strategy relied upon the ability to access the key boronate ester **9**[16] in good yield and couple it in a Suzuki reaction with benzyl 4-{[(trifluoromethyl)sulfonyl]oxy}-3,6-dihydropyridine-1(2*H*)-carboxylate **10**[17] or commercially available 2-chloro-1,3-thiazole-5-carbaldehyde. Both reactions proved amenable to scale-up on multi-gramme scale, yielding 82% and 68% of the late-stage, divergent intermediates **11** and **12**, respectively. Compound **12** could be used in a series of reductive aminations in multiple parallel synthesis format with a large set of amines; as an alternative, reduction to **13**, mesylation and amine displacement were also highly effective. Compound **11** was simultaneously reduced and deprotected using H_2/10% Pd/C to yield the free amine **14** which could be alkylated or subjected to reductive amination, again in multiple parallel synthesis format. The final test compounds were liberated in 70–95% yields by Boc deprotection using TFA/DCM and were characterized by LC–MS and proton NMR. Approximately 300 compounds were made and tested using this synthetic strategy (Figs. 4 and 5).

The synthetic library of thiazole and piperidine phenyl benzamides were subjected to testing in the primary assay cascade. Gratifyingly, a large proportion of the compounds synthesized met the potency criteria and were therefore selected for progression into other discriminating in vitro cascade assays. Acquired long QT syndrome causes significant cardiac side effects and represents a major problem in clinical studies of drug candidates. One of the reasons for development of arrhythmias related to long QT is inhibition of the human ether-a-go-go-related-gene (hERG) potassium

Table 1. Enzyme and cell potency of benzamide HDAC inhibitors

Compound	R	X	Mean log *D*	HDAC enzyme pIC_{50}	HDAC1 enzyme pIC_{50}[15]	HCT116 proliferation pIC_{50}
3	H	N	1.0	4.29	5.61	—
8a		C	2.3	5.02	6.91	5.74
8b		C	1.2	4.78	NT	5.13
8c		C	<0.5	5.23	6.86	6.08
8d		C	2.51	4.57	7.35	6.36
8e		C	NT	4.44	NT	5.63

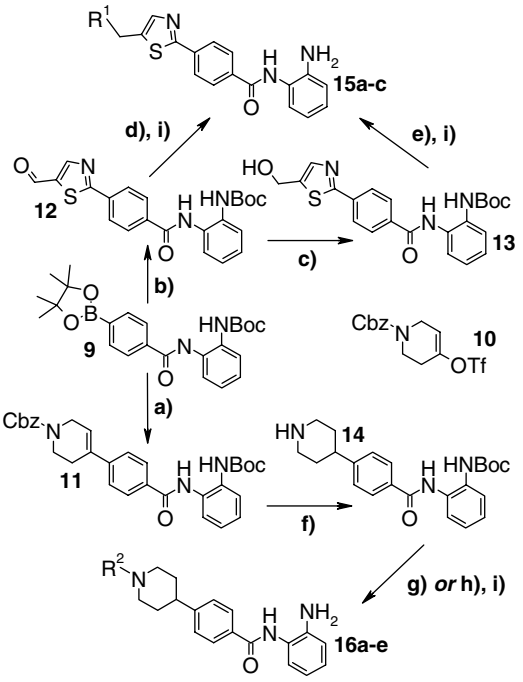


Figure 4. Synthesis of thiazole and piperidine-based benzamide derivatives. (a) $PdCl_2$(dppf), $NaHCO_3$, DME, H_2O, 60 °C, 5–9 h, 82%; (b) 2-chloro-1,3-thiazole-5-carbaldehyde, $PdCl_2$(dppf), $NaHCO_3$, DME, H_2O, 60 °C, 5–9 h, 68%; (c) $NaBH_4$, MeOH, water, 10 °C to rt, 18 h, 97%; (d) piperidine, $NaBH(OAc)_3$, DCM, rt, 3 h, H_2O 80%; (e) MsCl, DCM, Et_3N, 30 °C, cool to 10 °C, add ethylamine or propylamine in THF, rt, 22 h 49–60%; (f) 10% Pd/C, MeOH, H_2, 5 bar, 50 °C, 2 h 81%; (g) R^2CHO, $NaBH(OAc)_3$, DCM, rt, 3 h, H_2O 50–80%; (h) R^2 alkyl halide, DIPEA, IPA, reflux, 4 h 50–82%; (i) DCM, TFA, rt, 30 min, SCX2 cartridge elution–DCM, MeOH, 2 M NH_3/MeOH.

channel.[18] Therefore, early identification of hERG affinity is becoming increasingly important. We therefore chose to profile the compounds early in the cascade using a high-throughput patch-clamp hERG assay[19]; plotting the HCT116 proliferation pIC_{50} against hERG pIC_{50} allowed rapid identification of compounds worthy of progression to more detailed in vitro and in vivo studies. Compounds of high potency and low hERG liability are boxed in blue in Figure 5; the results of detailed testing for several of these compounds are described in Tables 2–4.

Compounds **15b**, **16b** and **16d** offer optimal in vitro efficacy and non-efficacy characteristics and were therefore selected for further in vivo study.

The thiazole **15a** demonstrates an encouraging increase in potency; however, its solubility is still modest (37 μM at pH 7.4). The secondary amines **15b** and **15c** were marginally less potent in the cellular proliferation assay, but showed solubility greater than 500 μM with minimal hERG inhibition (data not shown).

Compound **16a** emerged as one of the most potent piperidine-based inhibitors in the cellular assay (Table 3), however, unusually for this series, the compound shows significant inhibition of more than one cyto-

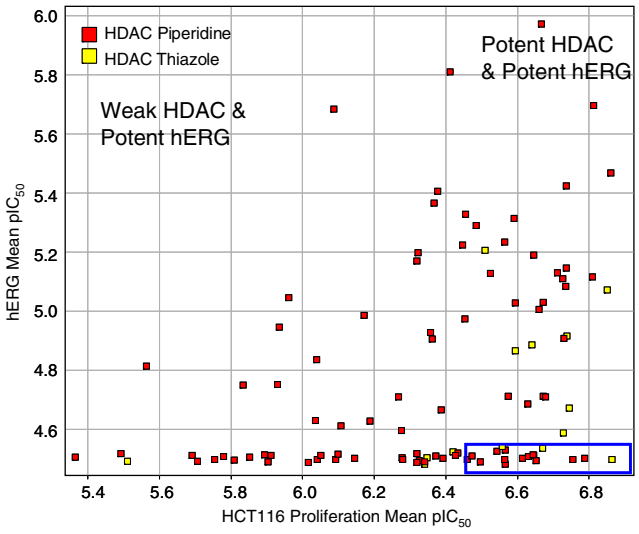


Figure 5. HCT116 cell potency versus hERG pIC_{50} for a series of thiazole and piperidine-based benzamide HDAC inhibitors.

chrome P450 isoform. A concern arising from P450 inhibition by an HDAC inhibitor is possible drug interactions that can be the result of abrogation of the P450 pathway(s) of metabolism causing toxicity due to elevated exposures of other drugs metabolized by these pathways. Compound **16a** shows pIC_{50} between 5 and 6 for 3A4, 2D6, 2C9 and 2C19, whereas **16b–e** demonstrate $pIC_{50} < 5$ against all isoforms tested. The most likely explanation of the increased P450 inhibition by **16a** is its higher flexibility, providing greater opportunity for it to adopt a conformation for cytochrome binding, coupled with its increased log *D*.

The thiazole **15b** shows good in vivo properties, clearance being higher than desirable, but quite reasonable in light of its very high plasma free levels. Of the three compounds, however, it demonstrates the lowest exposure after oral dosing to the nude mouse.

Compound **16d** demonstrates superior exposure in the nude mouse and was therefore progressed to a nude mouse xenograft study, in which a statistically significant difference in tumor weight can be demonstrated, by comparison to sham-dosed control animals (Fig. 6). An excellent dose–response profile was observed, with significant growth inhibition observed at 12.5 mg/kg orally.

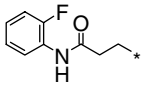
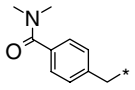
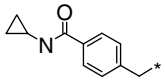
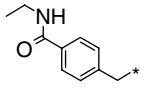
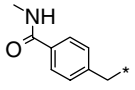
In summary, **16d**[20] therefore emerged as the compound demonstrating the most favorable balance of efficacy and non-efficacy properties, along with very desirable in vivo DMPK properties across the species tested. The final leading series of compounds are >1000-fold more potent than the initial screen hit, an improvement in potency which was achieved with a concomitant significant improvement in all the main non-efficacy properties. Finally, **16d** can be assembled in a highly convergent manner from readily available precursors, enabling further detailed studies, which will be reported in due course.

Table 2. Enzyme and cell potency of thiazole benzamide HDAC inhibitors

Compound	R^1=	MW	HDAC1 enzyme pIC_{50}	HCT116 proliferation pIC_{50}	ACD log *D*
15a		393	7.66	6.84	2.94
15b	CH_3CH_2NH–	352	7.42	6.69	1.79
15c	$CH_3(CH_2)_2NH$–	366	7.56	6.73	2.4

Use R1 definition in conjunction with Figure 4.

Table 3. Enzyme and cell potency of piperidine benzamide HDAC inhibitors

Compound	R^2=	Method	HDAC1 enzyme pIC_{50}	HCT116 proliferation pIC_{50}	Mean log *D*
16a		h	7.17	6.52	2.76
16b		g	7.07	6.32	1.53
16c		h	7.24	6.38	2.31
16d		h	7.46	6.44	2.28
16e		h	7.51	6.36	1.99

Use R2 definition in conjunction with Figure 4.

Table 4. DMPK properties of the HDAC inhibitors **15b** and **16d** in Han Wistar rat, nude mouse and beagle dog

Compound/species	Dose IV [μmol/kg]	CL [ml/min/kg]	$t_{1/2}$ IV [h]	Vss [l/kg]	$AUC_{(0-t)}$ IV norm [h kg/L]	Dose oral [μmol/kg]	$t_{1/2}$ oral [h]	$AUC_{(0-t)}$ oral norm [h kg/L]	Plasma % free
15b/rat (HW)	5.0	38	5.2	7.3	0.44	70.9	2.1	0.31	54
15b/mouse (nude)	—	—	—	—	—	70.9	1.1	0.06	—
16b/rat (HW)	5.0	49	1.6	3.1	0.34	10.0	2.1	0.07	28
16b/mouse (nude)	—	—	—	—	—	54.8	3.8	0.23	40
16d/rat (HW)	2.5	16	1.7	3.5	1.08	54.8	3.7	0.74	21
16d/mouse (nude)	5.0	36	4.8	5.0	0.46	54.8	3.2	0.59	—
16d/dog (beagle)	0.8	7	10.2	4.2	4.9	3.0	7.2	8.4	33

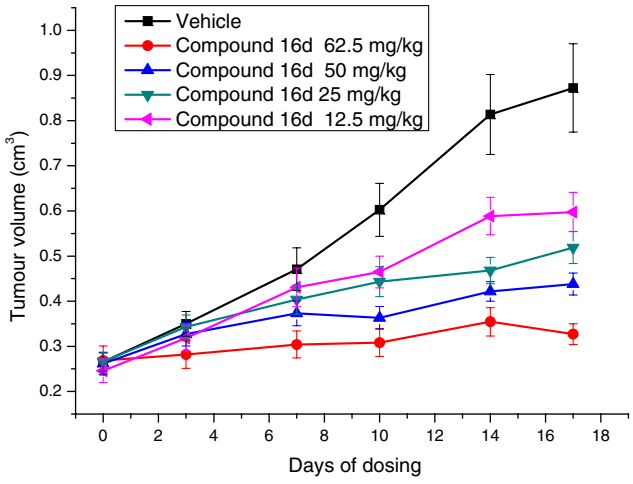


Figure 6. Nude mouse A549a xenograft efficacy for **16d** when administered orally once daily (5 d/week) for 18 days.

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13. HCT116 cells are adherent epithelial human colon carcinoma cell lines. Inhibition of proliferation in whole cells was assayed using the Promega cell titer 96 aqueous proliferation assay (Promega #G5421). The HCT116 cells were seeded in 96-well plates at 1×10^3 cells/well, and allowed to adhere overnight. They were treated with inhibitors for 72 h. The 20 μL of the tetrazolium dye MTS was added to each well and the plates were reincubated for 3 h. Absorbance was then measured on a 96-well plate reader at 490 nm. The IC_{50} values for HDAC inhibitors were determined by performing dose–response curves with individual compounds and determining the concentration of inhibitor producing 50% decrease in maximal signal (diluent control). For other examples of the use of HCT116 cells in the evaluation of HDAC inhibitors, see also: (a) Nagaoka, Y.; Maeda, T.; Kawai, Y.; Nakashima, D.; Oikawa, T.; Shimoke, K.; Ikeuchi, T.; Kuwajima, H.; Uesato, S. *Eur. J. Med. Chem.* **2006**, *41*, 697; (b) Remiszewski, S. W.; Sambucetti, L. C.; Bair, K. W.; Bontempo, J.; Cesarz, D.; Chandramouli, N.; Chen, R.; Cheung, M.; Cornell-Kennon, S.; Dean, K.; France, G. D.; Green, M. A.; Howell, K. L.; Kashi, R.; Kwon, P.; Lassota, P.; Martin, M. S.; Mou, Y.; Perez, L. B.; Sharma, S.; Smith, T.; Sorensen, E.; Taplin, F.; Trogani, N.; Versace, R.; Walker, H.; Weltchek-Engler, S.; Wood, A.; Wu, A.; Atadja, P. *J. Med. Chem.* **2003**, *46*, 4609; (c) Kim, D.-K.; Lee, J. Y.; Kim, J.-S.; Ryu, J.-H.; Choi, J.-Y.; Lee, J. W.; Im, G.-J.; Kim, T.-K.; Seo, J. W.; Park, H.-J.; Yoo, J.; Park, J.-H.; Kim, T.-Y.; Bang, Y.-J. *J. Med. Chem.* **2003**, *46*, 5745; (d) Moradei, O. M.; Mallais, T. C.; Frechette, S.; Paquin, I.; Tessier, P. E.; Leit, S. M.; Fournel, M.; Bonfils, C.; Trachy-Bourget, M.-C.; Liu, J.; Yan, T. P.; Lu, A.-H.; Rahil, J.; Wang, J.; Lefebvre, S.; Li, Z.; Vaisburg, A. F.; Besterman, J. M. *J. Med. Chem.* **2007**, *50*, 5543.
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20. (DMSO-d_6) 1.13 (t, 3H, CH_3CH_2), 1.73 (m, 4H, piperidine 3-CH_2), 2.10 (t, 2H, piperidine 2-*axial* CH), 2.60 (m, 1H, piperidine 4-CH), 2.94 (d, 2H, piperidine 2-equiv CH), 3.28 (m, 2H, CH_3CH_2) 3.55 (s, 2H, phenyl-CH_2-N), 4.85 (s, 2H, NH_2), 6.59 (m, 1H, CONHCHC*H*), 6.77 (d, 1H, NH_2CH), 6.96 (m, 1H, NH_2CHCH), 7.17 (d, 1H, CONHC*H*), 7.39 (m, 4H, piperidine-phenyl *o*-CH and ethylbenzamide *m*-CH), 7.80 (d, 2H, ethylbenzamide *o*-CH), 7.90 (d, 2H, piperidine-phenyl *m*-CH), 8.38 (t, 1H, CH_3CH_2NH), 9.55 (s, 1H, CON*H*); mass spectrum: $M+H^+$ 457.