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Identification of novel HDAC inhibitors through cell based screening and their evaluation as potential anticancer agents

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ABSTRACT

A series of benzimidazole based HDAC inhibitors have been rationally designed, synthesized and screened. The SAR of this new chemotype is described. The lead compound, **11e**, showed strong activity in several cellular assays and demonstrated in vivo efficacy in mouse xenograft pancreatic cancer models.

In eukaryotic cells, DNA is wrapped by histones to form a complex called chromatin. Histone deacetylases (HDACs) regulate histone deacetylation, and play important roles in chromatin folding. By altering the structure of the chromatin, HDACs affect the interaction of transcription factors with the DNA.[1,2] Thus, inhibiting HDACs may halt cell cycle progression, inducing differentiation and proliferation arrest. Several proof-of-concept HDAC inhibitors have advanced to clinical stage.[3,4] One of these agents, suberoylanilide hydroxamic acid (SAHA, Fig. 1), has been approved to treat cutaneous T-cell lymphoma since 2006. Despite the recent progress, more structurally diversified and potent HDAC inhibitors are still highly desirable for treating cancer. Herein, we would like to report a new family of suberic acid derived HDAC inhibitors with strong anti-tumor activity.

SAHA inhibits HDAC enzyme activity and has moderate antiproliferation activities in cellular assays. Metabolic studies have unveiled that the benzamide bond of SAHA may be cleaved in vivo, generating hydroxyaniline and other toxic secondary metabolites.[5] We envisioned that eliminating the benzamide bond may block this metabolic pathway, and minimize the potential toxicity and side effects. The hypothesis inspired us to design and prepare benzimidazole derived inhibitor **11e** and its analogs.

The synthesis of the benzimidazole analogs **11** started with making aniline diamines through the synthetic routes outlined in Scheme 1. For N-substituted diamine **3**, amine addition to 1-fluoro-2-nitrobenzene **1** afforded aniline **2** and the reduction of nitro group generated diamine **3**. For 4-substituted diamine **6**, Suzuki coupling between boronic acids and 5-bromo-2-nitroaniline **4** followed by the hydrogenation yielded**6**.

The assembly of the hydroxymic acid **11** started with amide formation (Scheme 2).[6] Treatment of **3**, **6** or commercially available aniline diamine with methyl 8-chloro-8-oxooctanoate **7** generated a mixture of isomeric amides **8** and **9**, which subsequently underwent cyclization mediated by acetic acid in toluene at 100 °C to provide benzimidazole **10**. Conversion of the methyl ester to hydroxymic acid **11** was accomplished by treating **10** with hydroxylamine in the presence of NaOH solution in THF and methanol.

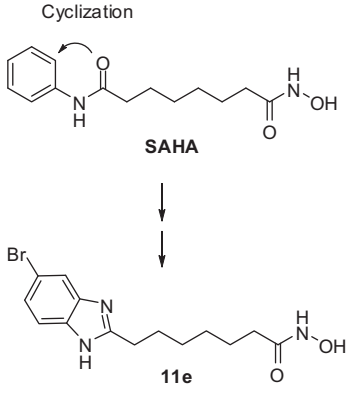

Figure 1. Design of **11e**.

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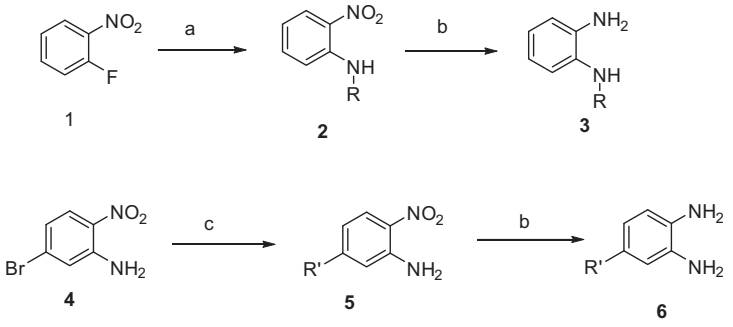
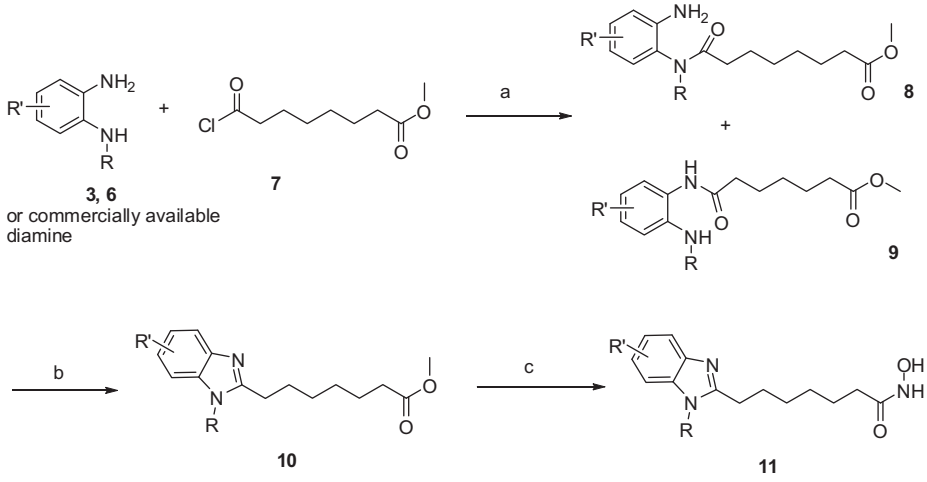


Scheme 1. Reagents and conditions: (a) RNH_2, EtOH; (b) Pd/C, ammonium formate, reflux; (c) $Pd(PPh_3)_4$, K_2CO_3, $R'B(OH)_2$.



Scheme 2. Reagents and conditions: (a) DCM, DIEA; (b) AcOH, toluene, heat; (c) NH_2OH (50% in water), NaOH (1 M), THF/MeOH.

All of the compounds were first screened in the human ovarian cancer SKOV3cell assay (Table 1). Compound **11a** inhibited the cell proliferation with an IC_{50} of 2.7 μM, equally potent as SAHA (IC_{50} = 2.3 μM) in the same assay. Shortening the floppy carbon chain appeared detrimental and compound **11b** with a five carbon linker was about four fold less potent than **11a**. Substitutions on the benzimidazole ring were explored next. Halogen substitution at the 6 position of the benzimidazole ring had beneficial effect and improved the cell activity with 6-F (**11c**, IC_{50} = 1.9 μM) and more remarkably, with 6-Cl (**11d**, IC_{50} = 0.32 μM) and 6-Br(**11e**, IC_{50} = 0.27 μM). Further enhancement of the cell activity was observed when a benzene ring (**11f**, IC_{50} = 0.1 μM) was installed at 6-position. Attempts to improve the activity by manipulating the terminal ring were not successful, and compounds **11g–l** were much less active than **11f**. Shifting the substitutions from the 6- to the 5-position appeared unfavorable, and compound **11m** was about 30-fold less active than **11d**. Electronic donating groups such as 6-methoxy **11n** and 6-dimethylamino **11o** did not enhance the activity and the imidazole[4,5-c]pyridine system **11p** killed the activity completely. Additional groups on the N-1 exemplified by **11q**,**11r**, **11s** and **11t** proved mostly disadvantageous. While compound **11q** had similar activity as **11a**, the other compounds, **11r–t,** with bigger groups were all less active.

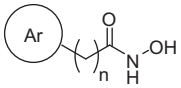
Compound **11e** (a.k.a **T009**) and **11f** emerged as the leads after the cell based screening. Because compound **11e** had better solubility than **11f**, it was chosen for further testing.

HDAC biochemical assays demonstrated that compound **11e** was a pan-HDAC inhibitor with single digit nano-molar IC_{50} against HDAC3, 5, 6, 9 and 10 (Table 2). In contrast to SAHA, **11e** potently inhibited HDAC4, 7 and 9.[7] Additional cell based screening revealed that compound **11e** was a potent agent with broad anti-proliferative activities and outperformed SAHA in most of the cell lines we examined (Table 3).[8,9] Notably, compound **11e** potently inhibited the pancreatic cancer MiaPaca line with an IC_{50} = 89 nM, and had an IC_{50} of 173 nM in the resistant lung cancer H69-Gli1 line, a 25-fold improvement over standard cisplatin/etopside (1:2) treatment (IC_{50} = 4.5 μM).

Finally, compound **11e** was tested in the human pancreatic cancer MiaPaca xenograft mousemodel.[10,11] Daily oral administration of **11e** at 100 mg/kg for 22 days resulted in 74% tumor growth inhibition when compared to the vehicle control as measured on Day 22 (Fig. 2). It also showed comparable efficacy with Gemcitabine (80 mg/kg) on Day 25 when the study ended. No gross toxicity of **11e** was observed following daily oral dosing.

In conclusion, we have identified a series of benzimidazole based HDAC inhibitors through cell screening and most of these inhibitors exhibit potent cell activity. The lead compound **11e** demonstrates broad anti-proliferative activity in a variety of cell lines and is active in MiaPaca xenograft mouse model. The potential of this novel class of HDAC inhibitors for treating cancer and other HDAC related disorders in clinical is being explored.

Table 1



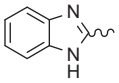
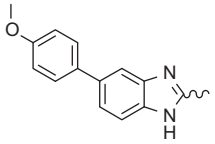
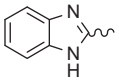
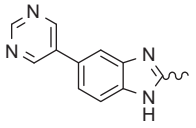
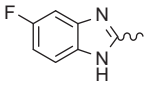
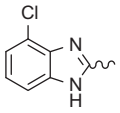
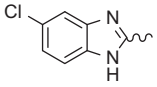
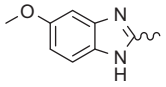
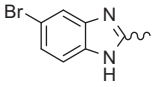
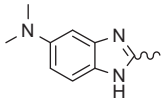
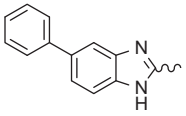
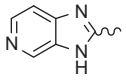
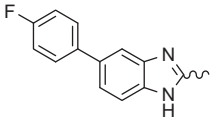
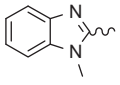
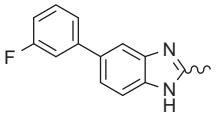
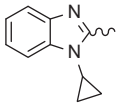
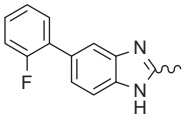
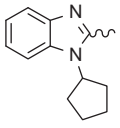
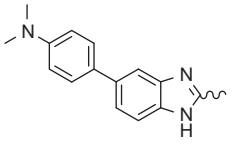
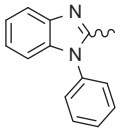
Compounds	Ar	*n*	SKOV3 IC_{50} (μM)	Compounds	Ar	*n*	SKOV3 IC_{50} (μM)
11a		6	2.7	**11k**		6	3.1
11b		5	11	**11l**		6	2.6
11c		6	1.9	**11m**		6	10
11d		6	0.32	**11n**		6	11
11e		6	0.27	**11o**		6	1.2
11f		6	0.1	**11p**		6	>20
11g		6	1.2	**11q**		6	3.0
11h		6	1.5	**11r**		6	9.5
11i		6	7.4	**11s**		6	4.8
11j		6	6.6	**11t**		6	>20
SAHA	—	—	2.3	—	—	—	—

Table 2

HDAC isoform	1	2	3	4	5	6	7	8	9	10	11
11e (IC_{50} nM)	20.2	34.1	6.4	279	2.5	1.9	313	282	1.1	3.0	33.4
SAHA (IC_{50} nM)	4	11	3	$>10^4$	8750	2	$>10^4$	1020	$>10^4$	N/A	N/A

Table 3

Cell lines	Skov-3	A2780	A2780C/P	MiaPaca	H295R	Mdamb231	VCAP
Primary site	Ovarian	Ovarian	Ovarian	Pancreas	Adrenal	Breast	Prostate
11e (IC_{50} μM)	0.26	1.9	2.0	0.089	0.18	0.82	2.4
SAHA(IC_{50} μM)	2.3	1.9	2.5	2.3		1.3	
Cell lines	H69	H69 Gil1	H23	MV522	MIHA	Hep3B	SNU880
Primary site	Lung	Lung	Lung	Lung	Liver	Liver	Liver
11e (IC_{50} μM)	0.15	0.17	0.56	2.2	0.23	0.61	0.98
SAHA(IC_{50} μM)	0.6	—	—	—	—	2.1[9]	—

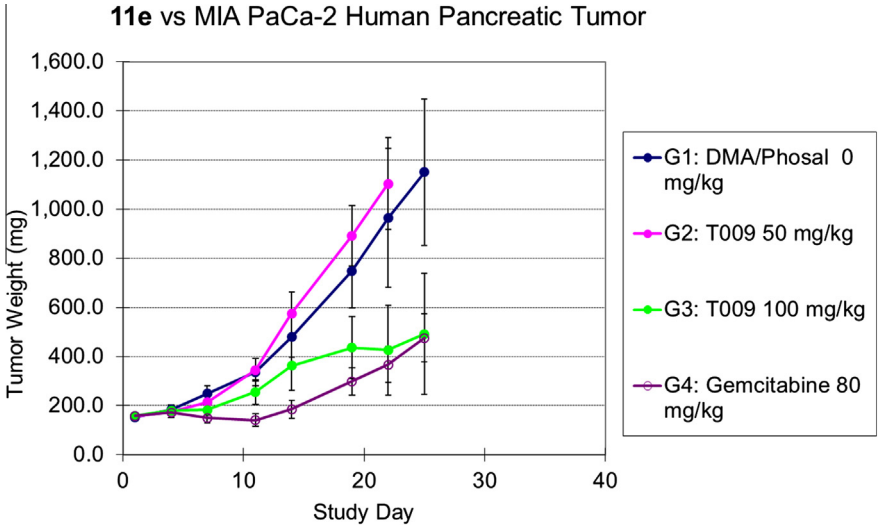


Figure 2. **11e** in MiaPaCa-2 tumor models.

Acknowledgments

The authors would like to thank Drs. Glen Weiss and Holly Hongwei Yin for testing some of the compounds.

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6. *Synthesis of compound* ***11e***: To a solution of methyl 8-chloro-8-oxooctanoate (229 mg, 1.11 mmol) in DMF at 0 °C was added a solution of 4-bromo-2-nitroaniline (200 mg, 0.922 mmol) and triethylamine (0.37 mL, 2.77 mmol) in DMF drop-wise. The reaction was stirred overnight at room temperature, diluted with ethyl acetate and washed with 1 N HCl, saturated sodium bicarbonate solution and brine. The separated organic layer was concentrated and purified by biotage column chromatography to give methyl 8-(4-bromo-2-nitrophenylamino)-8-oxooctanoate (290 mg) To a solution of methyl 8-(4-bromo-2-nitrophenylamino)-8-oxooctanoate (241 mg, 0.623 mmol) in EtOH was added SnCl2·H2O (490 mg, 2.37 mmol). The reaction washeated to reflux overnight and quenched by water. The mixture was filtered and the filtrate was extracted with ethyl acetate. The combined organic layer was dried, concentrated and purified by biotage column chromatography to afford methyl 8-(2-amino-4-bromophenylamino)-8-oxooctanoate (149 mg) To a solution of methyl 8-(2-amino-4-bromophenylamino)-8-oxooctanoate (140 mg, 0.395 mmol) in toluene was added a drop of acetic acid. The reaction was heated to 110 °C overnight and concentrated. The residue was purified by biotage column chromatography to give methyl 7-(5-bromo-1H-benzimidazol-2-yl)heptanoate (113 mg) To a solution of methyl 7-(5-bromo-1*H*-benzimidazol-2-yl)heptanoate (100 mg, 0.295 mmol) in THF at 0 °C was added 50% hydroxylamine solution (0.17 mL, 2.70 mmol). To this was addedKOH (0.74 mL, 1 M in MeOH, 0.74 mmol) dropwise. The reaction was stirred at 0 °C for 2 h, briefly concentrated and neutralized by adding 1 N HCl solution until pH was 7. The mixture was extracted with ethyl acetate, dried and concentrated. The residue was purified by column to give 7-(6-bromo-1*H*-benzimidazol-2-yl)-*N*-hydroxy heptanamide (**11e**, 69 mg). (MS M+ 1, found 341).
7. The IC_{50}s of SAHA have beenreported: Wagner, F. F.; Olson, D. E.; Gale, J. P.; Kaya, T.; Weïwer, M.; Aidoud, N.; Thomas, M.; Davoine, E. L.; Lemercier, B. C.; Zhang, Y.-L.; Holson, E. B. *J.Med. Chem.* **2013**, *56*, 1772.
8. All tumor cell lines were obtained from American Type Culture Collection (ATCC), with the exception of MV522 which was received as a gift from UCSD. The cultures were maintained in RPMI 1640, DMEM, or DMEM/F12, supplemented with 10% or 20% fetal bovine serum or ITS pre-mix with Nu-Serum, and housed in a 5% CO_2 atmosphere at 37 °C. 96-well plates used for IC_{50} determination were seeded at 5000 cells per well. Each drug was tested at a high concentration of 100 μM at a 1:10 dilution. Each drug was further refined (if applicable) at a high concentration of 10 μM at a 1:2 dilution.
9. Novotny-Diermayr, V.; Sangthongpitag, K.; Hu, C. Y.; Wu, X.; Sausgruber, N.; Yeo, P.; Greicius, G.; Pettersson, S.; Liang, A. L.; Loh, Y. K.; Bonday, Z.; Goh, K. C.; Hentze, H.; Hart, S.; Wang, H.; Ethirajulu, K.; Wood, J. M. *Mol.Cancer Ther.* **2010**, *9*(3), 642.
10. In vivo efficacy of the compound was tested through a human pancreas tumor xenograft model. Female Ncr nude mice were inoculated with 1X107 MIA PaCa-2 human pancreas cells suspended in a mixture of 50% Matrigel and 50% tissue culture media in a total volume of 100:l. When the tumors reached a mean weight of 157 mg, the mice were separated into four groups of 7–8 mice per group and treated. One group was treated with vehicle only. A second group was treated with Compound **11e** at 50 mg/kg. A third group was treated with Compound **11e** at 100 mg/kg and a fourth group was treated with Gemcitabine at 80 mg/kg. Vehicle and Compound **11e** were administered by mouth while Gemcitabine was administered via an intraperitoneal injection. Body weights and tumor size data were collected twice weekly. Tumor width and length were measured inmillimeters and converted to tumor weight (in milligrams) using the formula (width2 × Length/2) = tumor weight (mg).
11. An in vivo study of SAHA in MiaPaca xenograft models (25 mpg, ip, qd) can be found in: Fujisawa, T.; Joshi, B. H.; Puri, R. K. *J. Transl. Med.* **2011**, *9*, 37.