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Indole Amide Hydroxamic Acids as Potent Inhibitors of Histone Deacetylases

Yujia Dai,* Yan Guo, Jun Guo, Lori J. Pease, Junling Li, Patrick A. Marcotte, Keith B. Glaser, Paul Tapang, Daniel H. Albert, Paul L. Richardson, Steven K. Davidsen and Michael R. Michaelides

Cancer Research, Abbott Laboratories, Dept. R47J, Bldg. AP10, 100 Abbott Park Road, Abbott Park, IL 60064-6100, USA

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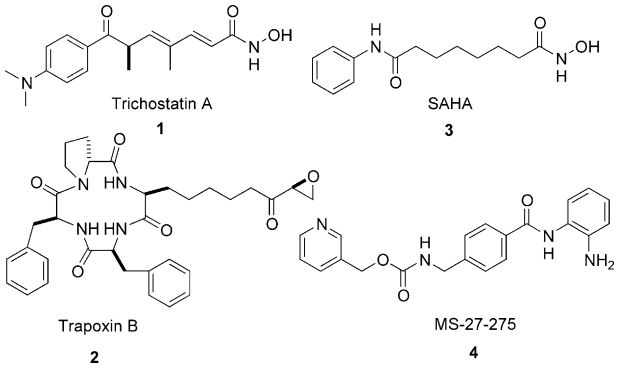
Abstract—A series of hydroxamic acid-based HDAC inhibitors with an indole amide residue at the terminus have been synthesized and evaluated. Compounds with a 2-indole amide moiety have been found as the most active inhibitors among the different regioisomers. Introduction of substituents on the indole ring further improved the potency and generated a series of very potent inhibitors with significant antiproliferative activity. A representative compound in the series, **7b**, has been found to be orally active in tumor growth inhibition model.

In eukaryotic cells, histone proteins are vital building blocks to the packaging of DNA into repeating nucleosomal units that are folded into high-order chromatin fibers.[1] It has been well documented that an elaborate collection of post-translational modifications including acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation take place on the 'tail' domains of histones. Among these modifications, histone acetylation of specific lysine residues in the N-terminal of the core histones plays a fundamental role in the regulation of gene expression by controlling the accessibility of DNA within chromatin to RNA-polymerase and co-factors.[2] The status of acetylation is regulated by the opposing activities of two families of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs).[3]

Inappropriate recruitment of HDACs can lead to the repression of a subset of genes resulting in excessive proliferation[4] and is implicated in a number of malignant diseases.[5] Consequently, the identification of potent HDAC inhibitors represents a compelling opportunity for the development of therapeutics for treatment of cancers.[6] A number of natural products including trichostatin A (**1**),[7] trapoxin B (**2**),[8] and synthetic small molecules such as suberoylanilide hydroxamic acid (SAHA, **3**)[9] and MS-27-275 (**4**)[10] have been identified as potent HDAC inhibitors and demonstrated anti-tumor activity.

Although there is limited structural information on human HDACs, a crystal structure of a bacterial HDAC homologue (HDLP) bound to TSA has shed light on the active site and the surrounding region which are conserved across the HDAC family.[11]



The active site consists of a tubular pocket leading to a zinc atom and two asparagine-histidine charge-relay

*Corresponding author. Fax: +1-847-935-5165; e-mail: yujia.dai@abbott.com

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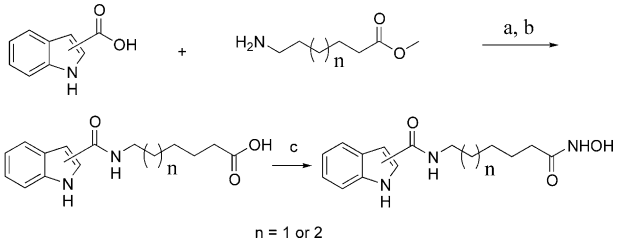
systems. Consequently, reversible HDAC inhibitors reported so far generally have a chelating group that forms a complex with the zinc atom and a hydrophobic spacer that fits into the tubular pocket.[12] Therefore, options for the structural modification of the chelating group and the hydrophobic spacer are limited. However, modification of the terminal residue ('cap') attached to the other end of the spacer, which presumably interacts with the entrance of the pocket, provides opportunities to discover potent and possibly even selective HDAC inhibitors.[13]

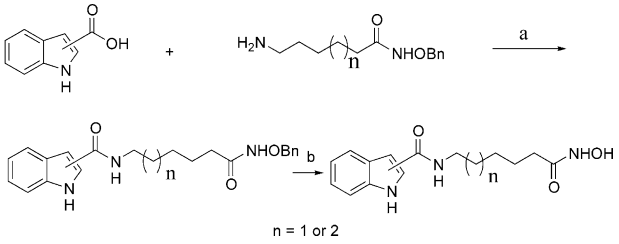
Recently, a series of SAHA analogues with a reversed amide linker derived from substituted benzoic acids were reported.[14] Most of these compounds are submicromolar HDAC inhibitors. During the course of developing potent and selective HDAC inhibitors, we envisioned that amide moieties derived from hetero aromatic acids might serve as useful terminal residues for HDAC inhibitors. In this paper, we report the discovery of a series of very potent hydroxamic acid-based HDAC inhibitors, which feature an indole amide moiety at the opposite end of the spacer from the hydroxamate group.

Scheme 1 shows a general synthetic route to the desired indole amide hydroxamates (Method A). The indole acids were coupled with methyl 6-aminocaproate or methyl 7-aminoheptoate under a standard HOBT-EDC mediated coupling condition. The methyl ester of the formed adduct was hydrolyzed to give the corresponding acids, which were then treated with *i*-PrOCOCl in the presence of Et_3N in DMF followed by the addition of 50% aqueous hydroxylamine solution to afford the desired hydroxamic acids in 32–65% yields.

The desired hydroxamic acids could also be synthesized using Method B as outlined in Scheme 2. Again, the



Scheme 1. Synthesis of indole amide hydroxamates. Method A: (a) HOBt, EDC, NMM, DMF, 62–99%; (b) NaOH, CH_3OH, H_2O, 82–100%; (c) *i*-PrOCOCl, Et_3N; then 50% aqueous NH_2OH, 32–65%.



Scheme 2. Synthesis of indole amide hydroxamates. Method B: (a) HOBt, EDC, NMM, DMF, 34–90%; (b) 10% Pd/C, THF, 62–95%.

HOBT-EDC mediated coupling condition was applied for the reaction of the indole acids with amines, in which an *O*-benzyl-protected hydroxamate functionality had already been incorporated. Hydrogenation removal of the benzyl group gave rise to the hydroxamic acids in good yields.

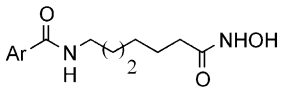
The HDAC inhibition activity of the compounds reported here was assayed using a peptide substrate and a mixture of HDACs, predominantly HDAC1 and HDAC2.[15]

Initially, the investigation started with screening amides derived from simple hetero aromatic acids (Table 1).

Amide hydroxamate **5**, which was prepared from picolinic acid and methyl 7-aminoheptoate, is a micromolar HDAC inhibitor and its activity is comparable to that of the simple phenyl analogue (**9**). Replacing the pyridine ring of **5** with a pyrrole residue (**6**) led to a slight improvement of the activity. A large increase in potency was achieved when an indole ring was incorporated. Compound **7b**, with an IC_{50} value of 14.6 nM, is more potent than **6**, **5** and **9** by factors of 36-, 69-, and 115-fold, respectively. The indole ring can be replaced by a benzofuran ring, although compound **8** is about 2-fold less active than **7b**. A hetero aromatic system appears significant for the compounds to retain high potency. This was indicated by the much weaker activity of indane carbocycle **11** compared to that of benzofuran analogue **10**.

With indole amide **7b** showing outstanding HDAC activity, a series of hydroxamates with the spacer attached to the different positions of the indole ring were then examined (Table 2). It was found that all these compounds are very potent HDAC inhibitors, with activity being between 12.5 and 87.6 nM. They are all not only much more potent than **9**, but also more potent than SAHA (**3**), which exhibits an IC_{50} value of

Table 1. HDAC inhibition of amide hydroxamic acids



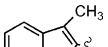
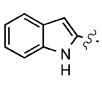
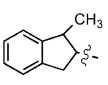
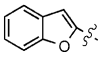
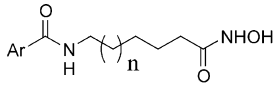
Compd	Ar	HDAC[15] IC_{50}, nM	Compd	Ar	HDAC[15] IC_{50}, nM
5		1680	**9**		1006
6		524	**10**		12.5
7b		14.6	**11**		732
8		29.6	—	—	—

Table 2. HDAC inhibition of indole amide hydroxamic acids



Compd	$n=1$ Ar	$n=1$ HDAC[15] IC_{50}, nM	Compd	$n=2$ Ar	$n=2$ HDAC[15] IC_{50}, nM
7a		12.5	**7b**		14.6
12a		39.8	**12b**		37.5
13a		87.6	**13b**		80.3
14a		17.0	**14b**		31.4
15a		36.4	**15b**		56.5
16a		37.6	**16b**		41.0

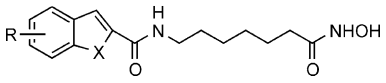
143 nM against HDACs in the same assay.[14] Compound **7a** and **7b**, in particular, are over 10-fold more potent than SAHA. Changing the spacer length from five to six methylene units made little difference in HDAC activity. The potency of compounds **7b**, **12b**, **13b** and **16b** are comparable to that of their five carbon atom chain analogues **7a**, **12a**, **13a** and **16a**, whereas **14b** and **15b** are slightly less active than **14a** and **15a**, respectively. Among the regioisomers derived from moving the amide linkage to the different positions of the indole ring, the two 2-indolyl analogues (**7a** and **7b**) are the two most potent compounds. Attaching the chains to other positions led to a 2- to 3-fold drop in activity except in the case of **14a**, which is almost equipotent to **7a**.

Based upon the finding that the 2-indole amide hydroxamates are the most potent compounds among the different position analogues, we directed our attention towards investigating the impact of substitution on the 2-indolyl residue. Thus, a series of analogues of **7b** were prepared and evaluated (Table 3). It was found that almost all the substituents investigated, except 5-BnO and 5-CF_3O (**24** and **25**), resulted in an improvement in the activity. Compounds **17**–**23** are all single-digit nanomolar HDAC inhibitors. A 4-fold increase of potency was seen when substituting with 4-CH_3O, 5-Br, or 4-CH_3O-6-CH_3O (**17**, **20**, and **23**). Consistent with the trend in the indole series, substitution on the benzofuran ring also boosted activity. All the three substituted compounds (**26**–**28**) are about 2-fold more active than the parent compound (**8**).

The compounds in Table 3 were also evaluated for their antiproliferative activity against human HT1080 fibrosarcoma cell line and human MDA435 breast carcinoma cell line.[16] All the compounds except **18** and **24** displayed submicromolar IC_{50} values with respect to growth of HT1080 and MDA435 cells. Significant antiproliferative effect was observed in both cell lines with **7b**, **17**, **21**, and **28**, which are all about 10-fold more potent than SAHA (HT1080: 2.4 μM; MDA435: 1.9 μM).[16]

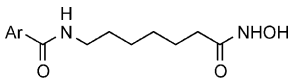
We also evaluated the influence of substitution at the indole nitrogen on the HDAC inhibition activity (Table 4). Introduction of a methyl group on the indole nitrogen of **7b** caused a 3-fold drop in activity (**29**). This trend was also seen in the 3-indoly analogues (**30** vs **12b**). However, the activity deterioration was reversed when a benzyl group was introduced to the nitrogen atom in **12b**. Compound **31** exibited an IC_{50} value of 12 nM. An even more robust boost was achieved when a phenyl group was attached to the indole nitrogen (**32**,

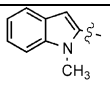
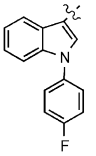
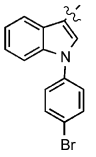
Table 3. HDAC inhibition and antiproliferation profiles of substituted indole and benzofuran amide hydroxamates



Compd	R	X	HDAC[15] IC_{50}, nM	HT1080[16] proliferation IC_{50}, μM	MDA435[16] proliferation IC_{50}, μM
7b	H	NH	14.6	0.14	0.15
17	4-CH_3O	NH	3.1	0.12	0.13
18	5-CH_3O	NH	6.5	0.72	1.2
19	6-CH_3O	NH	9.9	0.34	0.23
20	5-Br	NH	3.5	0.48	0.47
21	5-CH_3	NH	6.3	0.20	0.17
22	5-F	NH	5.4	0.48	0.25
23	4-CH_3O, 6-CH_3O	NH	3.1	0.26	0.60
24	5-BnO	NH	13.6	1.5	0.80
25	5-CF_3O	NH	16	0.89	0.30
8	H	O	29.6	0.76	0.49
26	5-CH_3O	O	15	0.38	0.30
27	7-CH_3O	O	17	0.71	0.32
28	5-Cl	O	15	0.18	0.19

Table 4. HDAC inhibiton and antiproliferative profiles of amide hydroxamates



Compd	Ar	HDAC[15] IC_{50}, nM	HT1080[16] proliferation IC_{50}, μM	MDA435[16] proliferation IC_{50}, μM
29		47	0.84	0.61
30		56	0.78	0.39
31		12	1.9	0.85
32		3.5	2.5	0.76
33		11.8	0.99	0.38
34		18.9	0.49	0.34

3.5 nM). Nonetheless, incorporation of a *p*-halogenated phenyl group brought about a decrease in potency, with a larger bromo group resulting in a more dramatic drop than a smaller fluorine atom. The antiproliferative activity of these *N*-substituted indole hydroxamates against HT1080 and MDA435 cell lines were also determined (Table 4). *N*-(*p*-bromophenyl) indole hydroxamate **34** is the most potent one among the investigated *N*-substituted analogues against growth of both HT1080 and MDA435 cells.

Compound **7b** has been tested for its antitumor activity in an HT1080 mouse xenograft model. **7b** displayed 36 and 39% tumor growth inhibition, respectively, when orally dosed once every other day at 30 and 100 mg/kg. The results are comparable to those shown by SAHA (25% at 30 mg/kg and 44% at 100 mg/kg) when dosed once a day in the same model.

In summary, a series of hydroxamic acid-based HDAC inhibitors with an indole amide moiety at the terminus have been developed. These inhibitors have displayed very potent HDAC inhibition activity and significant antiproliferative effect against two human cancer cell lines. Compound **7b**, as a representative in the series, inhibited tumor growth when dosed orally in a cancer animal model. These results further confirm the hypothesis that structural modification of the terminal residues of HDAC inhibitors can lead to potent HDAC inhibitors that may have potential as antitumor agents. Currently, isolation of individual HDAC enzymes is under way with the objective of assessing their HDAC selectivity profiles.

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15. Procedure for HDAC inhibition assay: activity is monitored by the deacetylation of the peptide substrate (QSY-7)-RGGRGLGK(Ac)-GGARRHRK(TAMRA)NH_2 by a mixture of HDACs, predominantly HDAC1 and HDAC2, which is prepared from nuclear extraction of K562 erythroleukemia cells followed by partial purification. After incubation with the HDAC enzymes for 30 min, the extent of deacetylation product is determined by addition of endoproteinase Lys-C with trichostatin A (TSA). The TSA stops further deacetylation and the endoproteinase Lys-C cleaves only the deacetylated (product) peptide. The fluorescence of the wells is read 2 h after addition of the second enzyme at which time the fluorescence of each well is increasing linearly. Compounds are

screened over a 1000-fold concentration range in half-log dilutions. The fluorescence of the wells is measured with a microtiter plate fluorescence reader (ex WL, 544 nm; em WL, 590 nm), and the% inhibition is calculated from the fluorescence readings of inhibited wells relative to those of control wells. The concentration of compound which results in 50% inhibition is determined by plotting the log[Inh] versus the logit function of the % inhibition. IC_{50} values are determined using a regression analysis of the concentration/inhibition data. The IC_{50} values for all compounds are from a single determination.

16. The given IC_{50} of cell growth inhibition was determined using the procedure described by note 19 in ref 13a.