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Amino acid derivatives as histone deacetylase inhibitors

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Abstract—Ongoing clinical studies indicate that inhibitors of Class I and Class II histone deacetylase (HDAC) enzymes show great promise for the treatment of cancer. Zolinza® (SAHA, Zolinza®) was recently approved by the FDA for the treatment of the cutaneous manifestations of cutaneous T-cell lymphoma. As a part of an ongoing effort to identify novel small molecules to target these important enzymes, we have prepared several classes of amino acid-derived HDAC1 inhibitors. The design rationale and in vitro activity against the HDAC1 enzyme and HCT116 cell line are described in this letter.

Histone deacetylase (HDAC) enzymes are becoming an increasingly important therapeutic target for the treatment of cancer and other diseases. These enzymes catalyze the removal of acetyl groups from the lysine residues of proteins including histones. In some cancer cells, there is an overexpression of HDACs causing hypoacetylation of histones.[1] Hypoacetylation of histones is associated with a condensed chromatin structure and repression of gene transcription.[1] Inhibition of HDAC activity allows for the accumulation of acetyl groups on the histone lysine residues resulting in an open chromatin structure and transcriptional activation.[1] In vitro, the HDAC inhibitor Zolinza® causes the accumulation of acetylated histones and induces cell cycle arrest and/or apoptosis of some transformed cells.[2] In vivo xenograft studies have demonstrated many of these agents are effective in inhibiting tumor cell growth.[3] In the clinic, Zolinza® was effective in decreasing skin lesions resulting from cutaneous T-cell lymphoma and was recently approved for treatment of the disease.[4] In addition to Zolinza®, a wide range of structures inhibit the activity of Class I/II HDAC enzymes. These inhibitors can be characterized by a common pharmacophore comprised of metal binding, linker, and surface recognition domains (Fig. 1).[5]

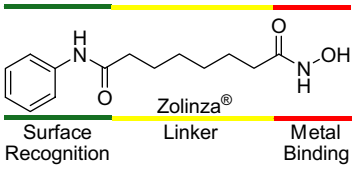

Figure 1. Pharmacophore for HDAC inhibitors.

Ongoing efforts to find HDAC1 inhibitors more potent than Zolinza® (**1**, $IC_{50} \sim 48$ nM) led to the development of aminosuberoyl hydroxamic acids such as **2**.[6] These inhibitors were prepared based on the notion that the amino acid moiety in the surface recognition domain more closely mimics the natural protein substrates for the HDAC1 enzyme. Concurrent efforts, based on the premise that the linker region could itself impart favorable interactions with the enzyme, led to the development of benzothiophene hydroxamic acids exemplified by **3**.[7] We began our work with the idea that favorable pharmacological properties conveyed by these two improvements could be combined. We found that this was the case when we prepared benzothiophene deriva-

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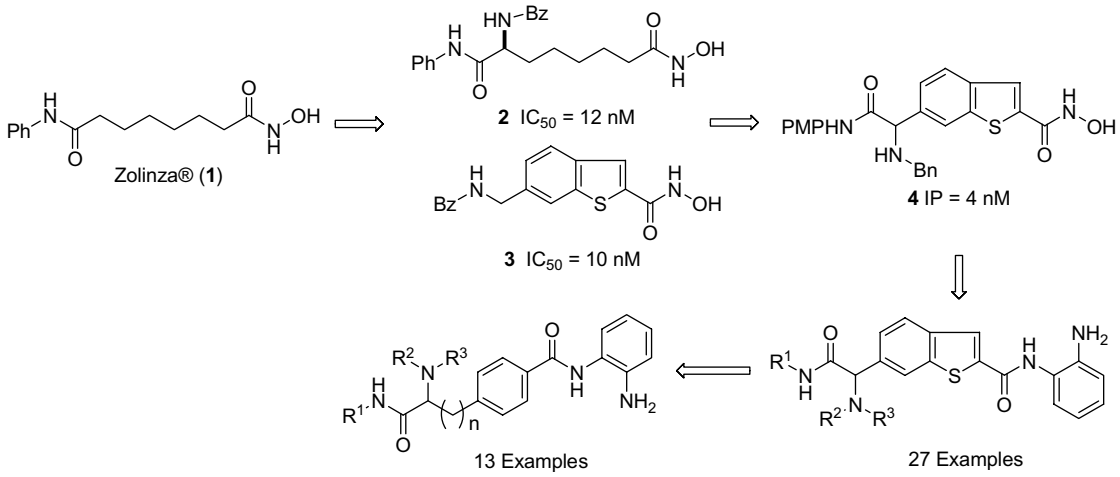


Figure 2. The evolution of amino acid-derived HDAC1 inhibitors.

tive **4**, which showed potent HDAC1 enzyme inhibition. Having established that we could use this concept to prepare highly potent compounds, we wished to examine other potential binding groups, particularly the 2-aminoanilide. The preparation and structure–activity relationship of benzothiophene derivatives with this binding group and subsequent development of phenylglycine- and phenylalanine-derived HDAC1 inhibitors are described herein (Fig. 2).

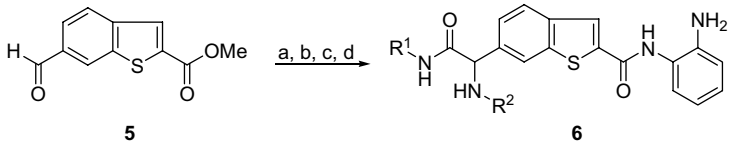
The desired benzothiophene amino acids were prepared using an Ugi reaction-based strategy.[8] In designing these inhibitors, experience suggested that a basic amine in the amino acid product would enhance cell permeability and growth inhibition. For this reason, trifluoroacetic acid (TFA) was used as the acid coupling partner in this multicomponent reaction in order to afford readily cleavable trifluoroacetamide products. With this strategy in mind, we prepared the amino acid derivatives as shown in Scheme 1. Aldehyde **5** underwent Ugi reaction with an isonitrile and a primary amine to give the amino acid trifluoroacetamide. The TFA group was removed with $NaBH_4$ in MeOH then the methyl ester was hydrolyzed with LiOH to give the acid. The acid, in turn, was coupled with 1,2-diaminobenzene to give anilide **6**.

These amino acid derivatives exhibited good inhibitory potency against the HDAC1 enzyme, with similar results observed for the *p*-methoxyphenyl (PMP) and benzyl amides.[9] For example, PMP amide **6c** and benzyl amide **6g** had an inflection point (IP) of 71 and 70 nM, respectively. Morpholinoethyl amide **6f** was slightly less potent. A focus on the benzyl amides identified compound **6m**, bearing a 4-pyridylethyl substituent on the amino acid nitrogen, as the most potent derivative with an IP of 39 nM (Table 1).

Additionally, we focused on piperazine-based amino acid amides because we expected them to have improved physical chemical properties, and because they could be synthesized more efficiently. These compounds could be prepared by first carrying out the Ugi reaction of aldehyde **5** in the presence of a piperazine and TFA to give the tertiary amino acid amide (Scheme 2). The anilide could then be installed by LiOH hydrolysis followed by coupling with 1,2-diaminobenzene to give the corresponding HDAC1 inhibitor **7**.

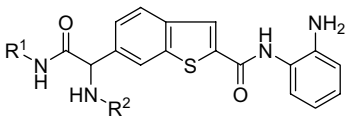
Using this chemistry, a number of piperazine substituents (R^2) were incorporated into the benzothiophene scaffold to prepare analogs **7a**–**g**, where R^1 was held constant as a benzyl group. The alkyl- and aryl-substituted compounds (**7a**–**e**) were more potent in the HDAC1 assay than those with a carbamate substituent (**7f** and **7g**). The more potent methyl (**7a**) and ethyl (**7b**) substituted compounds were screened against the human colon cancer cell line HCT116 and showed cell growth inhibition in the 400 nM range at 72 h. We further examined the effect of the amide substituent (R^1) on potency and found that aryl groups (**7h**–**m**) afforded more potent compounds than the benzyl group, with the 2-naphthyl-(**7i**) and 4-MePh-(**7k**) substituted compounds giving the best enzyme inhibition and cell-growth inhibition. Compound **7k** was resolved to give enantiomers **7l** and **7m** and isomer **7l** showed excellent HDAC1 inhibition and cell potency (Table 2).

Concurrent efforts indicated that compounds with a truncated linker could afford highly effective HDAC1 inhibitors. For example, malonyl substituted benzamide **8** showed excellent HDAC1 inhibition, with an IC_{50} of 41 nM, and good cell proliferation inhibition, with an HCT116 IC_{50} of 170 nM.[10] This prompted us to exam-



Scheme 1. Reagents and conditions: (a) R^1NC, R^2NH_2, TFA, MeOH; (b) $NaBH_4$, MeOH; (c) LiOH, H_2O, MeOH, THF; (d) 1,2-diaminobenzene, EDC, HOBT, DMF.

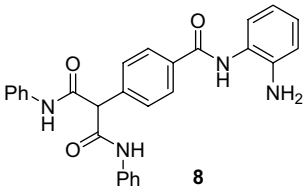
Table 1. Benzothiophene amino acid derivatives-secondary amines



Compound	R^1	R^2	HDAC1 IP[a] (nM)
6a	PMP	PMB	90
6b	PMP	*c*-C_6H_{11}	74
6c	PMP	CH_2CH_2Ph	71
6d	PMP	Bn	79
6e	PMP	CH_2CH_2-4-pyridyl	32
6f	CH_2CH_2-1-morpholino	CH_2CH_2Ph	230
6g	Bn	CH_2CH_2Ph	70
6h	Bn	CH_2CH_2OMe	170
6i	Bn	CH_2CH_2OH	150
6j	Bn	$CH_2CH_2NMe_2$	150
6k	Bn	CH_2CH_2-2-imidazolyl	520
6l	Bn	CH_2-3-pyridyl	180
6m	Bn	CH_2CH_2-4-pyridyl	39
6n	Bn	CH_2CH_2-2-pyridyl	110

[a] IP values are from a single determination or the average of two determinations.

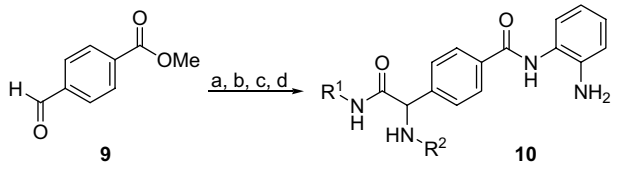
ine phenylglycine analogs of **8** as potential HDAC1 inhibitors.



8

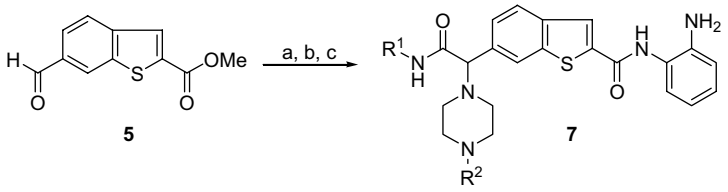
HDAC1 IC_{50} = 41 nM
HCT116-72hr IC_{50} = 170 nM

The desired phenylglycine anilides could be prepared using a similar Ugi strategy (Scheme 3). In this series, we first pursued compounds where the amine of the amino



9 **10**

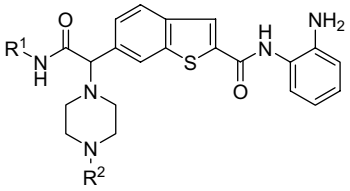
Scheme 3. Reagents and conditions: (a) LiOH, H_2O, MeOH, THF; (b) *N*-Boc-1,2-diaminobenzene, EDC, HOBT, DMF; (c) R^1NC, 2,4-$MeOPhCH_2NH_2$, acid, MeOH; (d) TFA, CH_2Cl_2.



5 **7**

Scheme 2. Reagents and conditions: (a) R^1NC, amine, TFA, trifluoroethanol; (b) LiOH, H_2O, MeOH, THF; (c) 1,2-diaminobenzene, EDC, HOBT, DMF.

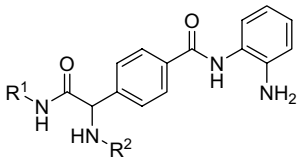
Table 2. Benzothiophene amino acid derivatives: piperazines



Compound	R^1	R^2	HDAC1 IP (nM)	HCT116—72 h IP[a] (nM)
7a	Bn	Me	110	430
7b	Bn	Et	80	490
7c	Bn	$CH_2CHCHPh$	230	ND[b]
7d	Bn	Ph	150	ND
7e	Bn	CH_2CH_2Ph	180	ND
7f	Bn	Boc	260	ND
7g	Bn	Cbz	470	ND
7h	4-ClPh	Me	62	340
7i	2-Naphthyl	Me	25	580
7j	4-MeOPh	Me	43	410
7k	4-MePh (±)	Me	25	240
7l	4-MePh	Me[c]	21	130
7m	4-MePh	Me[d]	18	560

[a] IP values are from a single or the average of two determinations.
[b] Not determined.
[c] Enantiomer 1.
[d] Enantiomer 2.

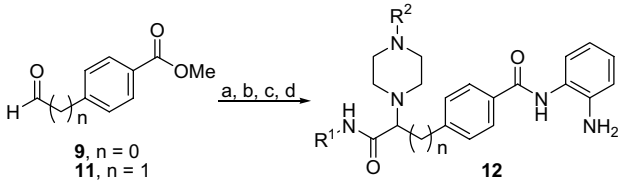
Table 3. Phenylglycine derivatives



Compound	R^1	R^2	HDAC1 IP (nM)	HCT116—72 h IP[a] (nM)
10a	4-MePh	$COCH_2Ph$	690	8100
10b	4-MeOPh	$COCH_2Ph$	870	6400
10c	2-Naphthyl	$COCH_2Ph$	170	2700
10d	4-MePh	$COCH_2CH_2Ph$	340	3000
10e	4-MeOPh	$COCH_2CH_2Ph$	260	2800
10f	2-Naphthyl	$COCH_2CH_2Ph$	250	2700

[a] IP values are from a single determination or the average of two determinations.

acid was acylated—these compounds differ from the malonates only by transposition of the carbonyl and the NH moiety of one amide unit. These inhibitors were prepared by first hydrolyzing ester **9** and then coupling to *N*-Boc-1,2-diaminobenzene. This amide could then undergo Ugi reaction with the appropriate acid and 2,4-dimethoxybenzylamine to give the protected HDAC1 inhibitor. The 2,4-dimethoxybenzyl and Boc protecting groups could then be removed by treating with TFA in CH_2Cl_2 to give amide **10**. These amino acid derivatives showed good to moderate inhibition of the HDAC1 enzyme (Table 3). However, they were relatively ineffective at inhibiting cell proliferation in the HCT116 assay.



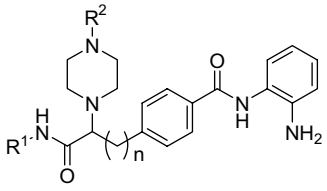
Scheme 4. Reagents and conditions: (a) R^1NC, amine, TFA, trifluoroethanol; (b) LiOH, H_2O, MeOH, THF; (c) 1,2-diaminobenzene, EDC, HOBT, DMF.

The cell-based activity imparted by the piperazine ring in the benzothiophene series suggested that we should examine its effect in the phenylglycine series. Such compounds were prepared as illustrated in Scheme 4 in a manner similar to the benzothiophenes.

We examined these piperazine-based compounds in the HDAC1 and HCT116 assays (Table 4). Although these inhibitors did not appear to be as potent as the amide-substituted compounds in the HDAC1 enzymatic assay (**12c** vs. **10c**), they showed much better cell potency. At this point, it seemed that the potency decrease observed in these compounds could result from a truncated linker relative to the benzothiophenes. With that in mind, the one-carbon homologs, phenylalanines, were prepared and a dramatic increase in potency was observed. For example, compound **12g** is approximately 10 times more potent in the HDAC1 assay than compound **12c** and is about five times more potent in the cell proliferation assay.

In conclusion, potent HDAC1 inhibitors were developed based on the premise that amino acid derivatives with optimal linker domains mimic the natural substrates of the HDAC1enzyme. We found that the benzothiophene and phenylalanine series, in particularly, afforded highly potent inhibitors, while the phenylgly-

Table 4. Phenylglycine and phenylalanine piperazine derivatives



Compound	R^1	R^2	*n*	HDAC1 IP (nM)	HCT116—72 h IP[a] (nM)
12a	4-ClPh	Me	0	6200	ND
12b	Bn	Me	0	860	ND
12c	2-Naphthyl	Me	0	360	640
12d	2-Naphthyl	Ph	0	620	ND
12e	2-Naphthyl	CH_2CH_2Ph	0	680	ND
12f	4-ClPh	Me	1	310	370
12g	2-Naphthyl	Me	1	37	220

[a] IP values are from a single determination or the average of two determinations.

cine series was less potent. These results suggest that an optimal linker length is necessary to maximize inhibitor potency. We also found that piperazine-based amino acids imparted the best inhibition of cell proliferation across the different scaffolds. These principles are being used to develop additional HDAC1 inhibitors. Those analogs will be reported in due course.

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