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Thiol-based SAHA analogues as potent histone deacetylase inhibitors

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Abstract—In order to find novel nonhydroxamate histone deacetylase (HDAC) inhibitors, a series of thiol-based compounds modeled after suberoylanilide hydroxamic acid (SAHA) was synthesized, and their inhibitory effect on HDACs was evaluated. Compound **6**, in which the hydroxamic acid of SAHA was replaced by a thiol, was found to be as potent as SAHA, and optimization of this series led to the identification of HDAC inhibitors more potent than SAHA.

The reversible acetylation of the side chain of specific histone lysine residues by histone deacetylases (HDACs) and histone acetyl transferases (HATs) is an important regulator of gene expression.[1] Histone hyperacetylation by HDAC inhibition neutralizes the positive charge of the lysine side chain, and is thought to be associated with change of the chromatin structure and the consequential transcriptional activation of a number of genes.[2] One important outcome of histone hyperacetylation is induction of the cyclin-dependent kinase inhibitory protein $p21^{Waf1/Cip1}$, which causes cell cycle arrest.[3] Indeed, HDAC inhibitors such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) (Fig. 1) have been reported to inhibit cell growth,[4–6] induce terminal differentiation in tumor cells,[4,5] and prevent the formation of tumors in mice.[7] Therefore, HDACs have been viewed as attractive targets for anticancer drug development. A number of structurally diverse HDAC inhibitors have been reported[8] and most of them belong to hydroxamic acid derivatives, typified by TSA and SAHA, which chelate the zinc ion in the active site in a bidentate fashion through its CO and OH groups.[9] Although hydroxamic acids are frequently employed as zinc-binding groups (ZBGs), they often present metabolic and pharmacokinetic problems such as glucuronidation, sulfation, and enzymatic hydrolysis that result in a short in vivo half-life.[10,11] Because of such concerns with the metabolic stability associated with hydroxamic acids, it has become increasingly desirable to find replacement groups that possess strong inhibitory action against HDACs. Thus far, *o*-aminoanilides,[12–14] electrophilic ketones,[15–17] bromoacetamides,[18] semicarbazides[18] (Fig. 2), and *N*-formyl hydroxylamines[19] have been reported as small molecule nonhydroxamate HDAC inhibitors. However, they have reduced potency as compared to hydroxamate inhibitors, and unfortunately, electrophilic ketones have a metabolic disadvantage in that they are readily reduced to inactive alcohols.[15–17] We therefore initiated a search for

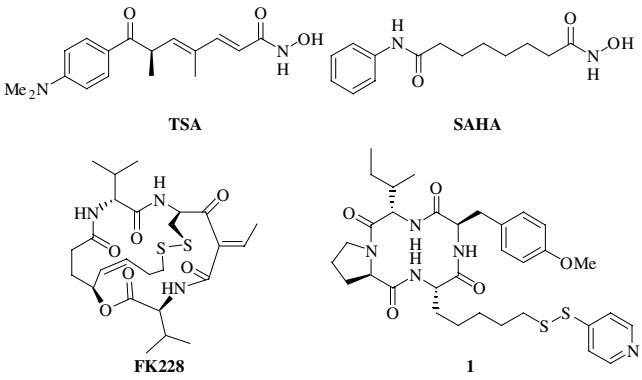

Figure 1. HDAC inhibitors.

Keywords: HDAC; SAHA; Enzyme inhibitor; Zinc protein.
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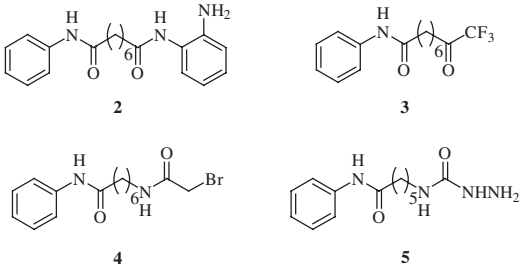


Figure 2. SAHA-based nonhydroxamate HDAC inhibitors.

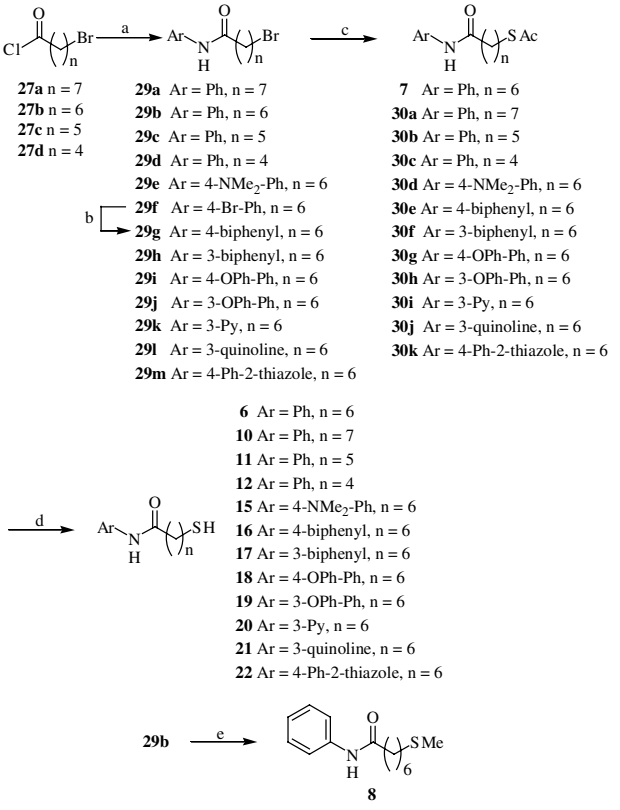
replacement groups for hydroxamic acid with the goal of drug discovery as well as finding new tools for biological research.

In the search for a suitable hydroxamic acid replacement, thiols seemed to be reasonable targets, because they have been reported to inhibit zinc-dependent enzymes such as angiotensin converting enzyme[20] and matrix metalloproteinase.[21] Recently, Furumai et al. demonstrated that the disulfide bond of FK228 (Fig. 1), a cyclic peptide HDAC inhibitor, is reduced in the cellular environment, releasing the free thiol analogue as the active species,[22] and Nishino et al. reported that cyclic tetrapeptides bearing disulfide group such as **1** (Fig. 1) inhibit HDACs under reductive conditions.[23] These reports prompted us to report on the synthesis and HDAC inhibition of thiol-based small molecule analogues.

The compounds prepared for this study are shown in Tables 1–3. Syntheses were accomplished as illustrated in Schemes 1–3. Compounds **6**–**8**, **10**–**12**, **15**–**21**, and **22** were synthesized from the corresponding acid chlorides **27a**–**d** by the route shown in Scheme 1. The amino group of aromatic amines **28** was acylated with an appropriate acid chloride **27** to give the amides **29a**–**f** and **29h**–**m**. Suzuki coupling[24] of bromobenzene **29f** with phenylboronic acid provided the biphenyl **29g**. Bromides **29** were treated with potassium thioacetate to give compounds **7** and **30a**–**k**, after which hydrolysis of the thioacetates under alkaline conditions gave the desired compounds **6**, **10**–**12**, **15**–**21**, and **22**. Sulfide **8** was obtained by the alkylation of sodium methanethiolate with bromide **29b**.

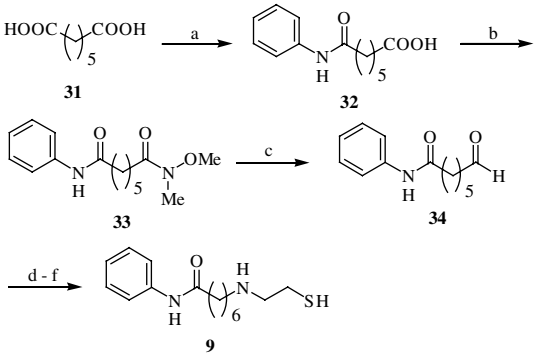
Synthesis of aminoethanethiol **9** was accomplished via aldehyde **34** (Scheme 2). The condensation of dicarboxylic acid **31** with an equivalent amount of aniline gave mono-anilide **32**. The carboxylic acid **32** was converted to Weinreb amide[25] **33** in the presence of EDCI and HOBt. Compound **33** was allowed to react with lithium aluminum hydride at 0 °C to give aldehyde **34** and subsequent reductive amination afforded aminoethanethiol **9**.

Compounds **13**, **14**, **23**–**25**, and **26** were prepared from alcohol **35** or **36** by the procedure outlined in Scheme 3. Treatment of bromide **35** with phenol in the presence of K_2CO_3 gave ether **38a**, and condensation of amine **36** with an appropriate aromatic carboxylic acid **37** afforded amides **38b**–**f**. Alcohols **38a**–**f** were converted to thiols **13**, **14**, **23**–**25**, and **26** in a three-step sequence: conversion of the alcohols to bromides, treatment of the bromides with potassium thioacetate, and hydrolysis of the resulting thioacetates.

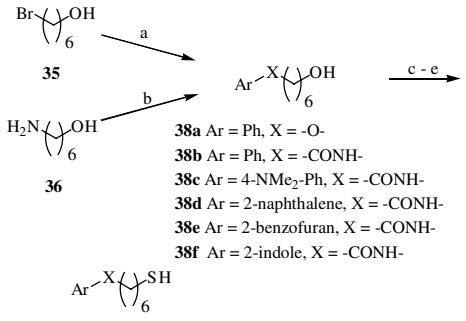


Scheme 1. Reagents and conditions: (a) $ArNH_2$ (**28**), Et_3N, CH_2Cl_2, rt, 25–99%; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, 1-methyl-2-pyrrolidinone, H_2O, 80 °C, 18%; (c) AcSK, EtOH, 60 °C, 84–99%; (d) 2 N aq NaOH, EtOH, THF, rt, 47–99%; (e) 15% aq NaSMe, EtOH, rt, 99%.

The compounds synthesized in this study were tested with an in vitro assay using a HeLa nuclear extract rich in HDAC activity.[26] The results are summarized in Tables 1–3 as IC_{50} values.



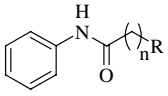
Scheme 2. Reagents and conditions: (a) Aniline, 180 °C, 43%; (b) *N*,*O*-dimethylhydroxylamine hydrochloride, Et_3N, EDCI, HOBt, DMF, rt, 94%; (c) $LiAlH_4$, THF, 0 °C, 72%; (d) 2-aminoethanol, $NaBH(OAc)_3$, THF, AcOH, rt; (e) $(Boc)_2O$, Et_3N, rt; (f) TFA, CH_2Cl_2, rt, 44% (three steps).

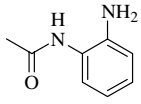


Scheme 3. Reagents and conditions: (a) Phenol, K_2CO_3, DMF, 80 °C, 96%; (b) ArCOOH (**37**), EDCI, HOBt, DMF, rt, 61–96%; (c) CBr_4, PPh_3, CH_2Cl_2, 0 °C, 25–99%; (d) AcSK, EtOH, 60 °C, 47–99%; (e) 2 N aq NaOH, EtOH, THF, rt, 28–74%.

As seen in Table 1, the IC_{50} values of SAHA, *o*-aminoanilide **2**, bromoacetamide **4**, and semicarbazide **5** were 0.28, 120, 14, and 150 μM, respectively (entries 1, 2, 4, and 5). Trifluoromethyl ketone **3** was reported previously to inhibit HDACs with an IC_{50} of 6.7 μM (entry 3).[15] In our study, changing the hydroxamic acid of SAHA to thiol yielded fruitful results. A pronounced inhibitory effect ($IC_{50} = 0.21$ μM) was observed with thiol **6**, which was about 30–700-fold more active than the previously reported nonhydroxamates, and as potent as SAHA (entry 6). To confirm that the thiol group plays an important role in anti-HDAC activity, thioacetate **7**, and sulfide **8** were tested. As expected, thiol transformation into thioacetate and sulfide led to an inhibitor that was about 30-fold less potent and a compound devoid of anti-HDAC activity, respectively (entries 7 and 8). These results suggest that although the inherent affinity of a monodentate thiol ZBG is less than that of bidentate ZBGs such as hydroxamate and hydrated electrophilic ketone, its ease of ionization makes thiol **6** as powerful as SAHA.[27] In order to find more potent ZBGs, we examined the activity of aminoethanethiol **9**, which was expected to coordinate zinc ion in a bidentate fashion. Although aminoethanethiols have been reported to inhibit zinc proteins,[28] this functional group led to a loss of HDAC inhibitory activity (entry 9).

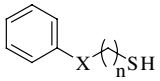
Table 1. HDAC inhibition data for SAHA and SAHA-based nonhydroxamates[a]



Entry	Compd	*n*	R	IC_{50} (μM)
1	SAHA[b]	6	–CONHOH	0.28
2	**2**[c]	6		120
3	**3**	6	$-COCF_3$	6.7[d]
4	**4**[e]	6	$-NHCOCH_2Br$	14
5	**5**[e]	5	$-NHCONHNH_2$	150
6	**6**	6	–SH	0.21
7	**7**	6	–SAc	7.1
8	**8**	6	–SMe	>100
9	**9**[f]	6	$-NHCH_2CH_2SH$	>100

[a] Values are means of at least three experiments.
[b] Prepared as described in Ref. 31.
[c] Prepared as described in Ref. 13.
[d] Data taken from the literature (Ref. 15).
[e] Prepared as described in Ref. 18.
[f] Trifluoroacetic acid salt.

Table 2. Effect of linker variation on HDAC inhibitory activity of thiols[a]



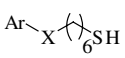
Entry	Compd	X	*n*	IC_{50} (μM)
1	**6**	–NHCO–	6	0.21
2	**10**	–NHCO–	7	1.5
3	**11**	–NHCO–	5	0.37
4	**12**	–NHCO–	4	6.2
5	**13**	–O–	6	11
6	**14**	–CONH–	6	0.36

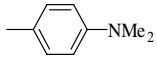
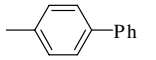
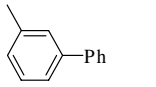
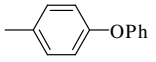
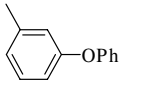
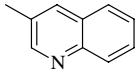
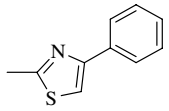
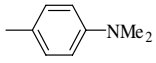
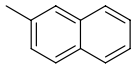
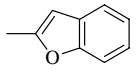
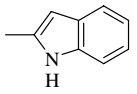
[a] Values are means of at least three experiments.

We next examined the effect of linker parts of thiol **6**. The results are shown in Table 2. HDAC inhibition was distinctly dependent on chain length, with $n = 7$ (**10**) and $n = 4$ (**12**) resulting in less potent inhibitors. However, compound **11**, in which $n = 5$, proved to be equally effective to **6**, in which $n = 6$ (entries 1–4). The similar structure–activity relationship (SAR) between thiols and hydroxamates, with $n = 6$ optimal,[29,30] indicates that thiols inhibit HDACs in a binding mode similar to that of hydroxamates. As for the group attaching the phenyl moiety, ether **13** displayed a moderate activity, whereas the activity of the reversed amide **14** was maintained (entries 5 and 6).

Having investigated the requirements for the ZBGs and linker parts, we next turned our attention to aromatic groups (Table 3). In the amide-linked series (entries 1–9), 4-substituted phenyl compounds tended to reduce the potency. Specifically, compounds **15** (Ar = 4-NMe_2-Ph), **16** (Ar = 4-biphenyl), and **18** (Ar = 4-PhO-Ph) showed about a 3–10-fold decrease in potency when compared to the parent thiol **6** (entries 2, 3, and 5). In contrast, when a phenyl group was introduced at the 3-position of the phenyl group of **6**, the IC_{50} of compound **17** was improved and reached 0.075 μM (entry 4). In addition, 3-phenoxy compound **19** was equipotent with compound **6** (entry 6). Next, we investigated the effect of the replacement of the phenyl group of compound **6** with heteroaryl rings (entries 7–9). While pyridine **20** and phenylthiazole **22** retained the potency of compound **6**, quinoline **21** was about 3-fold more active than compound **6** ($IC_{50} = 0.072$ μM). The reversed amide-linked series (entries 11–14) exhibited potencies similar to or

Table 3. Effect of aromatic group variation on HDAC inhibitory activity of thiols[a]



Entry	Compd	Ar	X	IC_{50} (μM)
1	**6**	–Ph	–NHCO–	0.21
2	**15**		–NHCO–	1.2
3	**16**		–NHCO–	1.1
4	**17**		–NHCO–	0.075
5	**18**		–NHCO–	0.62
6	**19**		–NHCO–	0.21
7	**20**		–NHCO–	0.11
8	**21**		–NHCO–	0.072
9	**22**		–NHCO–	0.17
10	**14**	–Ph	–CONH–	0.36
11	**23**		–CONH–	0.61
12	**24**		–CONH–	0.085
13	**25**		–CONH–	0.079
14	**26**		–CONH–	0.1

[a] Values are means of at least three experiments.

greater than the parent thiol **14**, except for **23** (Ar = 4-NMe_2-Ph), which resulted in a slightly less potent inhibitor. In particular, the reversed amides **24** with a naphthalene substituent and **25** with a benzofuran substituent showed stronger inhibition of HDACs with IC_{50}s of 0.085 and 0.079 μM, respectively. As a result, IC_{50}s in the double-digit nanomolar range were observed with 3-biphenyl **17**, quinoline **21**, naphthalene **24**, and benzofuran **25**, which were approximately 3–4-fold more potent than SAHA.

In summary, in order to find novel nonhydroxamate HDAC inhibitors, we designed and prepared a series of thiol-based SAHA analogues, and evaluated their inhibitory effect on HDACs. Compound **6**, in which the hydroxamic acid of SAHA was replaced by a thiol, was found to be as potent as SAHA. We have shown that the potency is related to chain length, with $n = 6$ optimal, and the amide and reversed amide were preferred as the group attaching the phenyl moiety. The conversion of the phenyl group of compound **6** to other aromatic groups led to the identification of inhibitors more potent than SAHA. The SAR results within the thiol series indicate that thiols inhibit HDACs in a manner similar to that of hydroxamates. As far as we could determine, this is the first report of nonmacrocyclic thiol inhibitors of HDACs. These small molecule thiols may be useful as tools for biological research and as orally bioavailable anticancer drugs. Currently, further detailed SAR studies and the next stage of evaluations are under way.

Acknowledgements

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