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α-Keto Amides as Inhibitors of Histone Deacetylase

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Abstract—α-Keto ester and amides were found to be potent inhibitors of histone deacetylase. Nanomolar inhibitors against the isolated enzyme and sub-micromolar inhibitors of cellular proliferation were obtained. The α-keto amide **30** also exhibited significant anti-tumor effects in an in vivo tumor model.

Histone deacetylases (HDACs) catalyze the removal of acetyl groups from the ε-amino groups of lysine residues at the N-terminus of nucleosomal histones. The alteration of the acetylation status of histone proteins complexed with DNA in the nucleosome determines the accessibility of DNA within chromatin and consequently influences gene expression.[1] Recent studies indicate that inappropriate recruitment and expression of HDACs are implicated in several human cancers. The effects of HDAC inhibitors provide the basis for HDAC inhibition as a compelling anticancer target. Inhibitors of HDAC have been reported to induce differentiation of cancer cells and suppress cell proliferation.[2]

A variety of HDAC inhibitors have been reported in the literature (Fig. 1). Several are undergoing clinical evaluation.[2] The majority of these structures have a metal chelator attached to an aromatic group via a hydrophobic linker. The HDAC inhibitors trichostatin A (TSA) (lit. IC_{50} 3.4 nM)[3] and suberoyl anilide hydroxamic acid (SAHA) (lit. IC_{50} 10 nM)[4] both contain a hydroxamic acid moiety as the zinc-binding group. X-ray crystal structures of TSA and SAHA bound to an HDAC homologue show that the metal-binding group interacts with the active site zinc, the linker spans the tube-like portion of the binding pocket, and the aromatic 'cap group' makes contacts with the pocket entrance, analogous to the binding of the acetyl group, lysine side chain, and peptide backbone of the natural substrate.[6]

Screening of the Abbott compound library also revealed a hydroxamic acid-containing HDAC inhibitor (**1**, Fig. 1).[7] Due to the short half-life and low oral bioavailability of the hydroxamic acids, several substitutes for the hydroxamic acid were investigated. Electrophilic ketones, such as trifluoromethyl ketones and α-keto esters and amides, are known to be readily hydrated.[8] They have

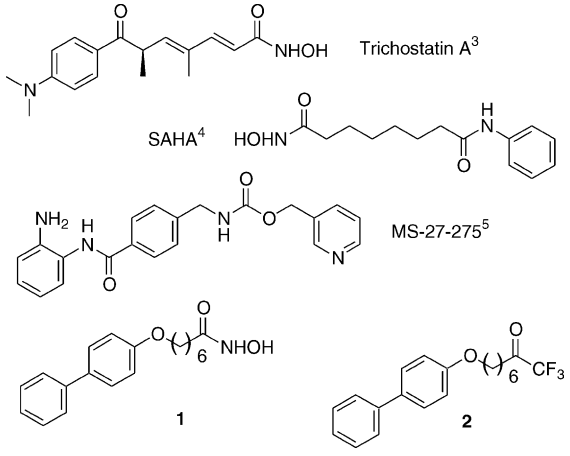

Figure 1. HDAC inhibitors.

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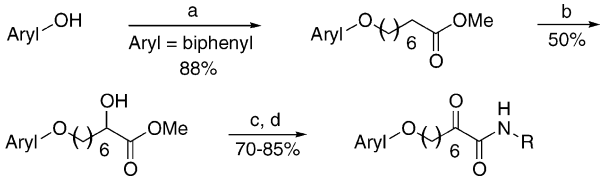
been reported to be inhibitors of lipases[9] and serine or cysteine proteases,[10] and trifluoromethyl ketones are reported to inhibit zinc-dependent enzymes.[11] We have previously reported that trifluoromethyl ketones such as **2** are inhibitors of HDAC.[12] However, the ketone was found to be readily metabolized to the alcohol in vivo. Other electrophilic ketones were then investigated. It was found that α-keto esters and amides are potent inhibitors of HDAC, and a series of these compounds were synthesized.

The ether-linked compounds (Scheme 1) were prepared by alkylation of biphenyl-4-ol with a bromo ester of the appropriate linker length. α-Hydroxylation of the ester was accomplished by deprotonation using LiHMDS and treatment with Davis' oxaziridine.[13] Oxidation of the alcohol with Dess–Martin periodinane gave the α-keto esters. To prepare the α-keto amides, the α-hydroxy esters were first treated with the appropriate amine to give the α-hydroxy amides, which were then oxidized to the ketones.

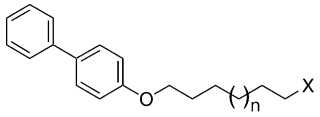
To vary the aryl group in the amide-linked series the α-keto amide group was installed first (Scheme 2). The α-keto ester was prepared via a Horner-Wadsworth-Emmons olefination[14] followed by treatment with CsF. The ester was then converted to the amide by treatment with methylamine without concomitant conversion of the ketone to the imine. The arylamide was then installed using resin-bound coupling reagents, which allowed the preparation of several arylamides in parallel.

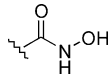
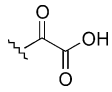
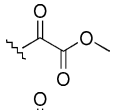
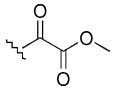
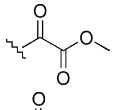
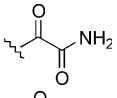
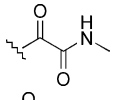
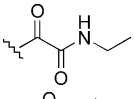
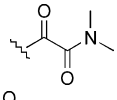
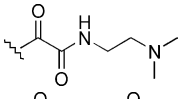
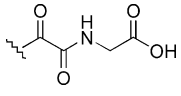
Compounds **15**, **16**, and **18** were prepared as in Scheme 2 starting with the alkylation of the appropriate aryl thiol and aryl oxime with *O*-silyl protected 6-bromo-1-hexanol. Compound **20** was prepared as in Scheme 2 using *N*-BOC protected 6-amino-1-hexanol.



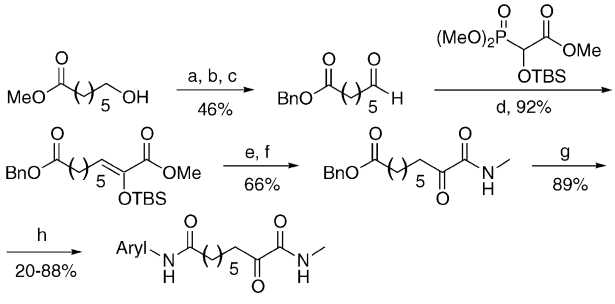
Scheme 1. (a) Cs_2CO_3, methyl 7-bromoheptanoate, DMF; (b) LiHMDS, Davis' oxaziridine, THF −78 °C; (c) RNH_2, methanol; (d) Dess–Martin periodinane, CH_2Cl_2.

Table 1. Electrophilic ketones

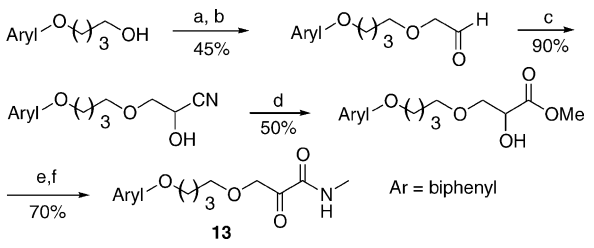


Compd	*n*	X	IC_{50} (μM)[b]
1[a]	1		0.020
2[a]	1		2.9
3[b]	1		3.7
4[b]	1		0.062
5[b]	0		0.082
6[b]	2		0.109
7[a]	1		> 50
8[b]	1		0.11
9[a]	1		4.9
10[a]	1		> 5
11[a]	1		> 50
12[a]	1		> 50

[a]Mixture of HDAC1 and HDAC2 from nuclear extraction of K562 erythroleukemia cells.[15]
[b]Fluorescence-based HDAC assay.[16]

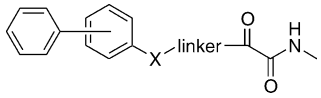


Scheme 2. (a) LiOH, H_2O, THF; (b) BnBr, $NaHCO_3$, DMF; (c) Swern; (d) LiCl, DBU, THF; (e) CsF, HOAc, CH_3CN; (f) $MeNH_{2(l)}$, THF, Et_3N; (g) $ArylNH_2$, carbodiimide-PS resin, HOAt, DMF.



Scheme 3. (a) NaH, bromoacetaldehyde dimethyl acetal, DMF; (b) H_2SO_4, acetone, H_2O; (c) KCN, $NaHSO_3$, H_2O, THF; (d) $HBr_{(g)}$, MeOH; (e) RNH_2, methanol; (f) Dess–Martin periodinane, CH_2Cl_2.

Table 2. Varying the hydrophobic linker and linking group



Compd	Regiochem	X	Linker	IC_{50} (nM)
6[a]	*para*	O	$-(CH_2)_6-$	114
13[b]	*para*	O	$-(CH_2)_4OCH_2-$	>10,000
14[b]	*para*	O	$-(CH_2)_4C\equiv C-$	>1000
15[b]	*para*	S	$-(CH_2)_6-$	526
16[b]	*para*	SO_2	$-(CH_2)_6-$	143
17[b]	*para*	SO_2NH	$-(CH_2)_6-$	241
18[b]	*para*	CH=NO	$-(CH_2)_5-$	853
19[b]	*para*	NHC(O)	$-(CH_2)_6-$	201
20[b]	*meta*	C(O)NH	$-(CH_2)_6-$	21
21[b]	*meta*	NHC(O)	$-(CH_2)_6-$	3.7

[a]Mixture of HDAC1 and HDAC2 from nuclear extraction of K562 erythroleukemia cells.[15]
[b]Fluorescence-based HDAC assay.[16]

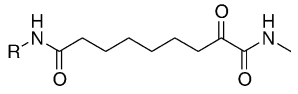
Compounds **13** and **14** were synthesized via the cyanohydrin as shown for **13** in Scheme 3. The cyanohydrin was converted to the α-hydroxy ester then carried on to the α-keto amide as described in Scheme 1. All compounds were characterized by ^1H NMR, MS, and combustion analysis.

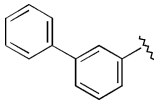
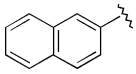
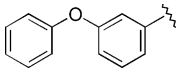
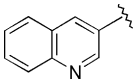
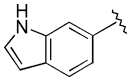
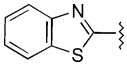
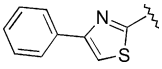
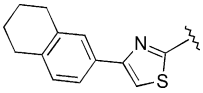
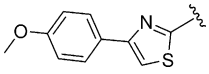
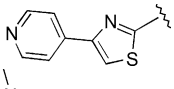
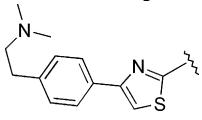
Table 1 lists several of the electrophilic ketones which were investigated. The α-keto ester **4** and the α-keto amide **8** were found to be potent inhibitors of HDAC. Interestingly, only the methyl ester and methyl amide derivatives were active. The corresponding acid, primary amide, ethyl amide, and dimethyl amide were all inactive. X-ray crystal structures of an HDAC homologue show a water pocket near the active site[6] which could perhaps be accessed by extending off the methyl amide. However, attaching groups such as a dimethylamine (**11**) or carboxylic acid (**12**) led to a loss of HDAC activity. The structure activity relationship of the α-keto esters and amides correlates with the hydroxamates with regard to linker length with six carbons being optimal, indicating a similar binding mode.

As with the trifluoromethyl ketones, the α-keto amides are readily metabolized to the alcohol in vivo. The α-keto esters are also subject to hydrolysis. In an effort to find more a metabolically stable α-keto amide and increase potency, changes in the hydrophobic linker and the group (X) attaching the aryl moiety were investigated (Table 2). A heteroatom (**13**) was added to the linker. It was hoped that the electron-withdrawing oxygen group would increase the hydration state of the ketone and therefore influence its metabolism. However, it was found to exist primarily as the enol form which was inactive. Insertion of an alkyne linker (**14**) also led to a loss of activity. *para*-Sufide, sulfone, sulfonamide, oxime, and amide (**15**–**19**) were slightly less potent, while the *meta*-amide linked compounds **20** and **21** increased potency dramatically.

We then investigated the effect of different aryl groups in place of the biphenyl of **21**. The cellular activities of these compounds are shown as IC_{50} values for inhibition of

Table 3. Varying the aromatic group of amide-linked compounds



Compd	R	IC_{50} (nM)[a]	PLF-HT1080 (μM)	PLF-MDA435 (μM)
21		3.7	6.9	14
22		338	nd[b]	nd
23		9.4	17	22
24		1041	35	39
25		9.5	2.9	4.0
26		18	3.4	4.5
27		133	13	14
28		11	1.2	3.1
29		1.4	>50	>50
30		9.1	2.7	5.8
31[c]		4.3	0.67	2.0
32		3.1	0.12	0.16

[a]Fluorescence-based HDAC assay.[16]
[b]nd, not determined.
[c]Trifluoroacetic acid salt.

Figure 2. Western blot analysis the effect of **30** and SAHA on H4 acetyliation and p21 induction in MDA435 cells.

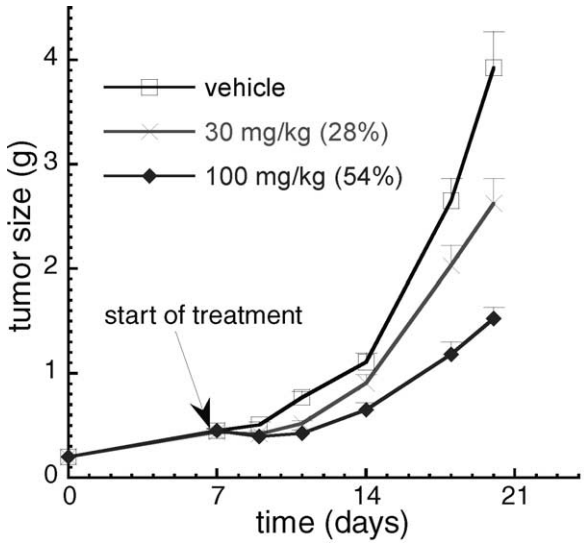


Figure 3. Effects of **30** on tumor growth in an HT1080 mouse tumor model ($n = 10$ per dose group). Percent inhibition of growth to 2 g relative to control is in parentheses of legend.

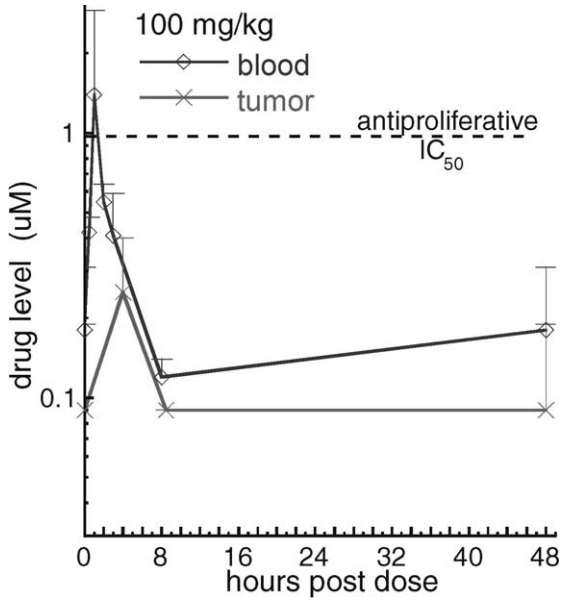


Figure 4. Drug levels of **30** post dose at 100 mg/kg.

proliferation of HT1080 and MDA435 cells.[17] Most aryl groups with the exception of phenoxyphenyl were tolerated. Several compounds with a 4-phenylthiazole aryl group (**30**, **31**, and **32**) maintained potency in the isolated enzyme assay while increasing cellular potency as compared to **21** (Table 3).

The anti-proliferative activity of these compounds was verified to be the result of inhibition of HDAC via Western blot analysis (Fig. 2).[18] Treatment of MDA435 cells with **30** at 50 μM produced hyperacetylation of histone H4 similar to the effect of SAHA. Treatment with **30** also resulted in the induction of $p21^{Waf1/Cip1}$ expression.

α-Keto amide **30** was also evaluated in an HT1080 mouse tumor model (Fig. 3).[19] The compound was administered intraperitoneal (ip), at 30 and 100 mg/kg, every other day (q2d). Significant inhibition of tumor growth to 2 g relative to control was achieved. However, some evidence of toxicity (rough coats, weight loss, lethality) was observed in the 100 mg dose group.

The α-keto amide **30** was observed to have a short half-life in vivo, in cell culture, and in whole blood due to its rapid reduction to the inactive α-hydroxy amide. Overall exposure was low with only transient concentrations of inhibitor above the cellular proliferation IC_{50} value (Fig. 4). Despite the poor pharmacokinetics of the α-keto amides, they exhibit significant anti-tumor effects in xenograft models, suggesting that transient exposure to HDAC inhibitors may be sufficient for anti-tumor effects.

Alpha-keto esters and amides have been shown to be potent inhibitors of HDAC. By altering the linker and aryl group, nanomolar HDAC inhibitors, some with single digit to sub-micromolar IC_{50} values against cellular proliferation, were obtained. Significant anti-tumor effects were observed in an in vivo tumor model with α-keto amide **30** despite the short half-life of this compound. In conclusion, it has been shown that alternatives to the hydroxamate zinc-binding group can lead to potent, single-digit nanomolar, inhibitors of HDAC.

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