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Heterocyclic Ketones as Inhibitors of Histone Deacetylase

Anil Vasudevan,[a,*] Zhiqin Ji,[b] Robin R. Frey,[b] Carol K. Wada,[b] Douglas Steinman,[b] H. Robin Heyman,[b] Yan Guo,[b] Michael L. Curtin,[b] Jun Guo,[b] Junling Li,[b] Lori Pease,[b] Keith B. Glaser,[b] Patrick A. Marcotte,[b] Jennifer J. Bouska,[b] Steven K. Davidsen[b] and Michael R. Michaelides[b]

[a]*Medicinal Chemistry Technologies, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA*
[b]*Cancer Research, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA*

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Abstract—Several heterocyclic ketones were investigated as potential inhibitors of histone deacetylase. Nanomolar inhibitors such as **22** and **25** were obtained, the anti-proliferative activity of which were shown to be mediated by HDAC inhibition.

A balance between Histone Deacetylase (HDAC) and Histone Acetyl Transferase (HAT) determines the extent of acetylation of chromatin proteins. HDAC-mediated hypoacetylation increases positive charges at histone lysine residues and condenses the chromatin structure, generally leading to transcriptional repression. Recent studies indicate that HDACs are not only involved in the regulation of chromatin structure and gene expression, but also the regulation of cell cycle progression and tumorigenesis (Fig. 1).[1]

Several classes of compounds that inhibit the activity of partially purified HDACs, cause growth arrest of a wide range of transformed cells in culture and inhibit the growth of human xenografts in tumor growth studies have been identified.[2] Some of these inhibitors have been shown to act selectively, altering the transcription of less than 2% of expressed genes[3a] and several are undergoing clinical evaluation.[3b] Because HDAC is a zinc metalloenzyme, the majority of inhibitors have a metal chelating element attached to an aromatic group via a hydrophobic linker. The HDAC inhibitors trichostatin A (TSA) (lit. IC_{50} 3.4 nM)[4] and suberoyl anilide hydroxamic acid (SAHA) (lit. IC_{50} 10 nM)[5] both contain a hydroxamic acid moiety as the zinc-binding group.[6] The X-ray crystal structures of TSA and SAHA bound to an HDAC homologue have been reported and confirm that the metal-binding group interacts with the active site zinc, with the linker spanning the tube-like portion of the binding pocket and positioning the aromatic groups so as to make contact with the pocket entrance.[7]

Previous publications have delineated efforts aimed at optimizing hydroxamic acid containing compounds such as **1** identified via screening of the Abbott compound collection.[8] Recently, non-hydroxamate HDAC inhibitors containing electrophilic ketones such as trifluoromethyl ketones and α-keto amides, exemplified by **2** and **3** have been reported.[9] As a continuation of our quest for non-hydroxamate HDAC inhibitors, a range of electrophilic ketones were synthesized and evaluated in this study, some of which are shown in Table 1. The synthesis of these compounds was accomplished by one

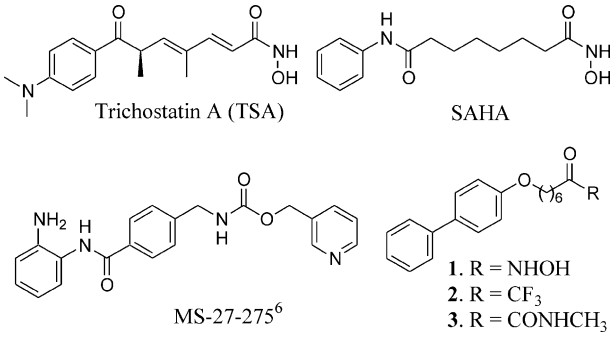

Figure 1. HDAC inhibitors.

*Corresponding author. Tel.: +1-847-938-6594; fax: +1-847-935-0310; e-mail: anil.vasudevan@abbott.com

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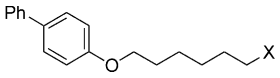
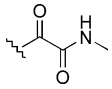
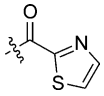
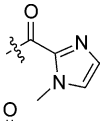
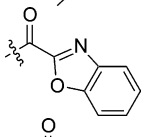
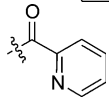
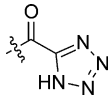
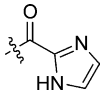
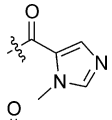
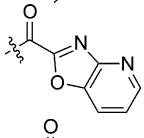
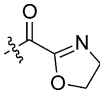
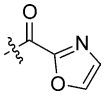
of several methods outlined below. Alkylation of 4-phenylphenol with bromomethyl heptanoate followed by saponification afforded the carboxylic acid, which was then converted to the *N*-methoxy-*N*-methyl amide as shown in Scheme 1. Treatment of this intermediate with the corresponding lithiated heterocycles afforded **4**–**7**.

Attempts at synthesizing the α-keto tetrazole **8** via alkylation of BOM-protected 2-lithio tetrazole with 7-chlorocarbonyl-heptanoic acid methyl ester were unsuccessful.

Table 1. HDAC inhibitory potency of α-keto heterocycles

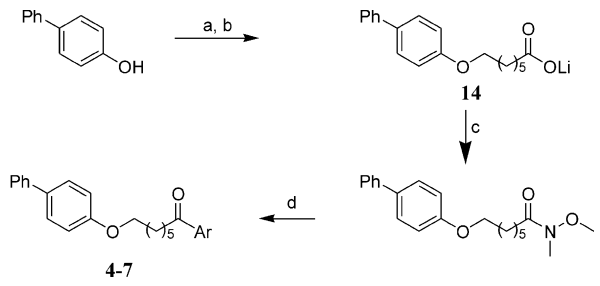


Compd	X	IC_{50} (μM)
2[9]		2.9
3[9]		0.11
4[a]		> 50
5[a]		> 50
6[a]		> 50
7[a]		> 50
8[b]		2.0
10[a]		> 50
11[a]		> 50
12[a]		> 50
13[b]		1.8
15[b]		1.1

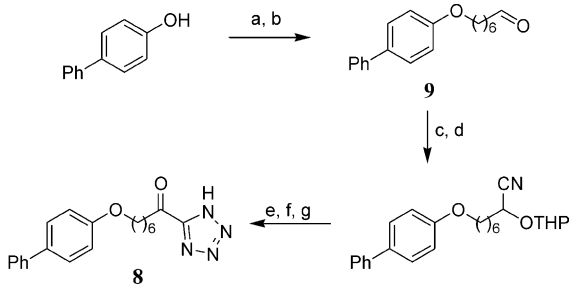
[a]Radioactivity-based HDAC assay.[13]
[b]Fluorescence-based HDAC assay.[14]

However, conversion of aldehyde **9** to the cyanohydrin followed by treatment with sodium azide, protecting group removal and Jones oxidation afforded the α-keto 1(*H*)-tetrazole (Scheme 2).

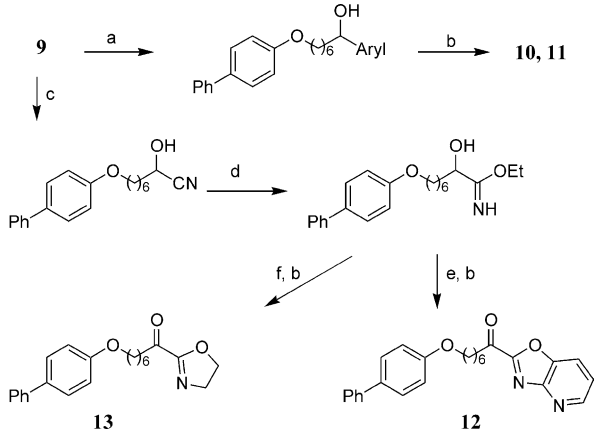
Treatment of aldehyde **9** (Scheme 3) with 2-lithio heterocycles followed by oxidation with manganese dioxide afforded compounds **10** and **11**. The cyanohydrin obtained from aldehyde **9** was treated with EtOH/HCl to afford the iminoether, which when heated with 2-amino-3-hydroxypyridine, followed by Dess–Martin



Scheme 1. (a) $Br(CH_2)_6COOCH_3$, Cs_2CO_3, DMF, 92%; (b) LiOH, H_2O, THF, 95%; (c) $HN(CH_3)OCH_3$, DMF, 68%; (d) *n*-BuLi, THF, ArH, 0 °C to rt.



Scheme 2. (a) $Br(CH_2)_7OH$, Cs_2CO_3, DMF, 92%; (b) Swern oxdn, 65%; (c) KCN, $NaHSO_3$, THF/H_2O, 90%; (d) THP, TsOH, 90%; (e) NaN_3, NH_4Cl, DMF, 120 °C, 5 h, 32%; (f) TsOH, CH_3OH, 83%; (g) Jones reagent, 60%.



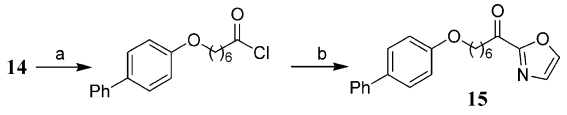
Scheme 3. (a) ArLi, THF, −78 °C to rt; (b) Dess–Martin periodinane, CH_2Cl_2; (c) KCN, $NaHSO_3$, H_2O/THF, 90%; (d) AcCl, EtOH, 100%; (e) 2-amino-3-hydroxypyridine, 2-ethoxyethanol, 130 °C, 40%; (f) 2-aminoethanol, CH_2Cl_2; TsOH, $CHCl_3$, 60 °C, 30%.

periodinane oxidation afforded the α-keto azabenzoxazole **12**.[11] Further, treatment of the iminoether with 2-aminoethanol followed by oxidation afforded the keto-oxazoline **13**.

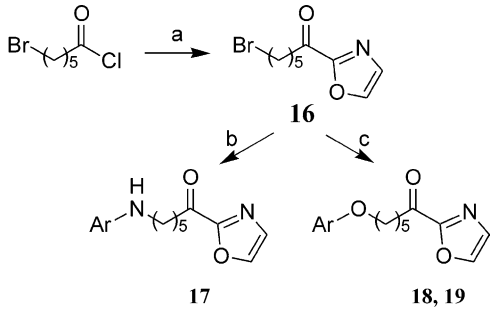
For the synthesis of the α-keto oxazoles, 2-lithio oxazole was treated first with $ZnCl_2$ at −78 °C and then with CuI at 0 °C, followed by the acid chloride obtained from carboxylate **14** (Scheme 4).

Table 1 lists several of the α-keto heterocycles investigated in this study along with the corresponding trifluoromethyl ketone and keto-amide. Initial results obtained with mono- and bicyclic heterocycles were not encouraging. However, the α-keto oxazoline **13** and the corresponding α-keto oxazole analogue **15** displayed single-digit micromolar potency. The sharp difference in potency between the α-keto oxazole and α-keto thiazole was somewhat surprising given their similar σ values,[12] though a similar trend has been observed in elastase inhibitors,[10b] suggesting that subtle differences within the catalytic site can lead to dramatic changes in potency. The α-keto tetrazole **8** was equipotent with the corresponding α-keto acid (data not shown). The lack of HDAC activity exhibited by the α-keto benzoxazole and α-keto azabenzoxazole analogues **6** and **12** would suggest a steric constraint within the active site of HDAC. As a result, it was decided to focus on the α-keto oxazole **15** and explore various changes in the aliphatic linker as well as the terminal hydrophobic moiety to improve the HDAC inhibitory potency of this class of compounds.

The synthesis of several nitrogen and ether linked α-keto oxazoles was achieved via the modified synthetic route shown in Scheme 5. Nucleophilic displacement of the bromine in intermediate **16** with anilines was performed in a commercial single-mode microwave unit[15] at 160° in DMF to afford compounds such as **17**. A series of hydrophobic aryl ether substituents were synthesized in a one-pot reaction of the phenol, polystyrene-supported



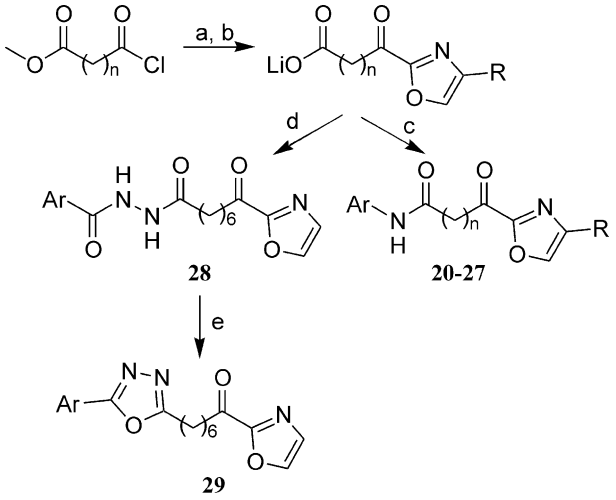
Scheme 4. (a) $SOCl_2$, DMF, reflux, 94%; (b) oxazole, BuLi, THF, −78 °C; $ZnCl_2$, −78 °C to rt, CuI, then acid chloride, 49%.



Scheme 5. (a) Oxazole, BuLi, THF, −78 °C; $ZnCl_2$, −78 °C to rt, CuI, then acid chloride, 59%; (b) Ar-NH_2, DMF, 160 °C, 30 min; (c) PTBD, Ar–OH, acetonitrile, 175 °C, 15 min.

1,5,7-triazabicyclo[4.4.0]dec-5-ene (PTBD)[16] and **16** at 175 °C in acetonitrile using a single-mode microwave unit to afford compounds such as **18** and **19**. [CAUTION: Heating solvents in a closed-vessel in a microwave to temperatures above their boiling point should only be performed in instruments equipped with appropriate safety features.] Compounds containing an amide, hydrazide or diacylhydrazine linker (**20**–**28**) were synthesized using a suitably protected acid chloride as shown in Scheme 6. A set of 1,3,4-oxadiazole linked compounds were also synthesized via a dehydrative cyclization using the Burgess reagent in a single-mode microwave.

The *meta* phenyl substitution pattern in **18** affords a slight improvement in potency compared to the *para* phenyl substitution in case of the ether-linked compounds, and is equipotent with the 2-naphthyl substituent (**15**) in case of the ether and amine-linked compounds, **17** and **19**. The amide linked compounds tended to afford significantly better inhibitory potency against HDAC with **22** being 10-fold more potent that the corresponding ether linked compound **18**. In general, compounds with a six-atom tether between the ketone and amide carbonyl were about 5-fold more potent than compounds with a five-atom tether, consistent with SAR trends observed with trifluoromethyl



Scheme 6. (a) Oxazole, BuLi, THF, −78 °C; $ZnCl_2$, −78 °C to rt, CuI, then acid chloride, 59%; (b) LiOH, THF/H_2O, 95%; (c) $ArNH_2$, EDC, HOBt, 87%; (d) $ArCONHNH_2$, EDC, HOBt, 62%; (e) $(C_2H_5)_3NSO_2NHCO_2CH_3$, THF, 150 °C, 15 min, microwave, 38%.

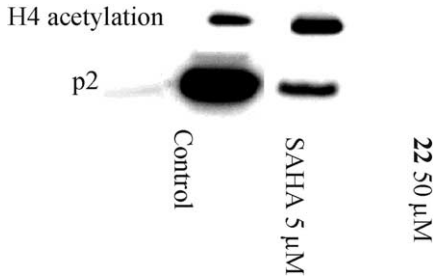


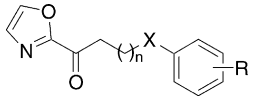
Figure 2. Western blot analysis of the effect of **22** and SAHA on H4 acetylation and p21 induction in MDA435 cells.

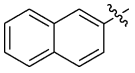
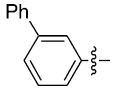
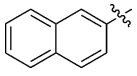
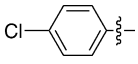
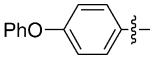
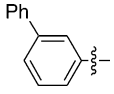
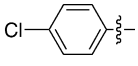
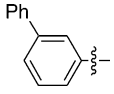
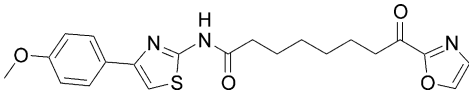
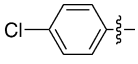
ketones and α-keto amides.[9] Several potent HDAC inhibitors were obtained via incorporation of thiazole containing substituents into the side chain as shown in **25**. The hydrazide-linked compound (**27**) was devoid of HDAC activity whereas the 1,3,4-oxadiazole (**29**) linker afforded a more potent analogue than the corresponding diacylhydrazine linked analogue (**28**). The sulfur and sulfone linked compounds (**30** and **31**, respectively) were equipotent with the nitrogen linked compounds, as were analogues in which the heteroatom linker was replaced with an ethylene moiety (**32**). Reverse amides **33** and **34** with indole substituents in the side chain were also found to be potent inhibitors of HDAC. Some of the more potent compounds from this study were found to have anti-proliferative activity in MDA435 cells in the micromolar range, with **25** being the most potent compound (IC_{50} 2.3 μM). Figure 2 shows a Western blot analysis of histone hyperacetylation and p21 induction in MDA435 cells produced by **22** at 50 μM, consistent with the notion that the anti-proliferative activity is mediated by HDAC inhibition (Table 2).[17]

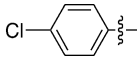
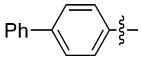
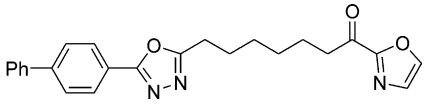
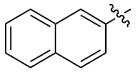
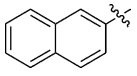
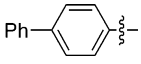
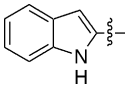
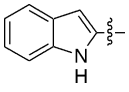
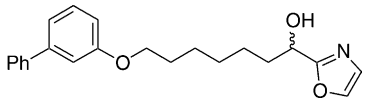
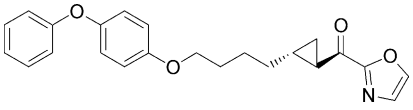
The anti-proliferative activity of this class of compounds was presumably compromised by their rapid reduction to the inactive alcohol, as shown in Figure 3. Further, in whole blood, representative α-keto oxazoles **15** and **18** were rapidly reduced to the corresponding alcohol with half-lives of 0.18 and 0.21 h, respectively.

Attempts to modulate this rapid reduction via incorporation of a cyclopropyl substituent alpha to the ketone (**35**) or substituents on the oxazole moiety (data not shown) were unsuccessful as these compounds were inactive against HDAC.

Table 2. Effect of linker variation on HDAC inhibitory activity of α-keto oxazoles



Compd	*n*	X	R	IC_{50} (μM)[b]
17[b]	5	–NH–		0.43
18[b]	5	–O–		0.62
19[b]	5	–O–		0.52
20[b]	5	–CONH–		0.32
21[b]	5	–CONH–		0.58
22[b]	5	–CONH–		0.06
23[b]	4	–CONH–		1.50
24[b]	4	–CONH–		0.31
25[b]				0.03
26[b]	4	–$CONHCH_2$–		6.40

Compd	*n*	X	R	IC_{50} (μM)[b]
27[a]	4	–CONHNH–		> 10
28[a]	5	–CONHNHCO–		1.83
29[a]				0.25
30[b]	5	–S–		0.54
31[b]	5	–SO_2–		0.61
32[b]	4	–CH=CH–		0.99
33[b]	5	–NHCO–		0.09
34[b]	4	–NHCO–		0.34
36[a]				> 50
35[a]				> 50

[a]Radioactivity-based HDAC assay.[13]
[b]Fluorescence-based HDAC assay.[14]

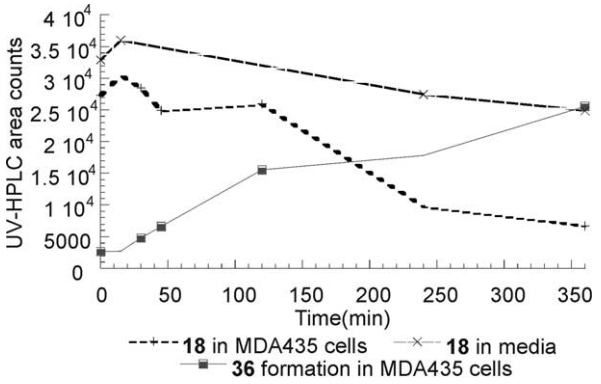


Figure 3. Metabolic fate of **18** in the presence of MDA435 cells with concomitant formation of **36**.

In summary, a series of novel, potent α-keto oxazoles were identified as HDAC inhibitors in this study. Efforts to mitigate the metabolic liability of these electrophilic carbonyl containing compounds will be the focus of future communications.

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