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α-Mercaptoketone based histone deacetylase inhibitors

Paul L. Wash [a], Timothy Z. Hoffman [a], Brandon M. Wiley [a], Céline Bonnefous [a], Nicholas D. Smith [a], Michael S. Sertic [a], Charles M. Lawrence [a], Kent T. Symons [b], Phan-Manh Nguyen [b], Kevin D. Lustig [b], Xin Guo [c], Tami Annable [c], Stewart A. Noble [a], Jeffrey H. Hager [c], Christian A. Hassig [b], James W. Malecha [a,*]

[a] Department of Chemistry, Kalypsys, Inc., 10420 Wateridge Circle, San Diego, CA 92121, USA
[b] Department of Biology, Kalypsys, Inc., 10420 Wateridge Circle, San Diego, CA 92121, USA
[c] Department of Pharmacology, Kalypsys, Inc., 10420 Wateridge Circle, San Diego, CA 92121, USA

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ABSTRACT

In an effort to discover novel non-hydroxamic acid histone deacetylase (HDAC) inhibitors, a novel α-mercaptoketone was identified in a high-throughput screen. Lead optimization of the screening hit, led to a number of potent HDAC inhibitors. In particular, α-mercaptoketone **19y** (KD5150) exhibited nanomolar in vitro activity and inhibition of tumor growth in vivo.

Over the past several years, a number of histone deacetylase (HDAC) inhibitors have entered clinical trials as potential cancer therapeutics (Fig. 1).[1] Recently, Vorinostat **1** was approved for treatment of cutaneous T-cell lymphoma.[2] A common structural feature of many of these compounds is the presence of a hydroxamic acid which serves to chelate zinc in the active site of the enzyme. Hydroxamates, however, have been shown to exhibit undesirable pharmaceutical properties including poor pharmacokinetics, low solubility, and the potential for chronic toxicities.[3,4] We sought to discover a series of HDAC inhibitors devoid of the issues often present in the hydroxamic acid class of compounds by introducing a novel zinc chelator.

A high-throughput screen of a 600,000 small molecule library was carried out in a fluorescence based biochemical assay that utilized the HDAC activity of a partially purified HeLa cell nuclear extract.[5] Disulfide **5** was identified in the screen. Analogous to the HDAC inhibitor FK228 **4** which undergoes a disulfide bond cleavage in cells to give the active reduced form[6], disulfide **5** is converted to the active α-mercaptoketone **6** under the reducing conditions of our assay (Fig. 2). The proposed binding mode is related to that of the hydroxamic acids with the α-mercaptoketone functioning as a monodentate or bidentate zinc chelating group.[7]

Replacement of the hydroxamate zinc chelating group with an α-mercaptoketone is not without precedent. While our

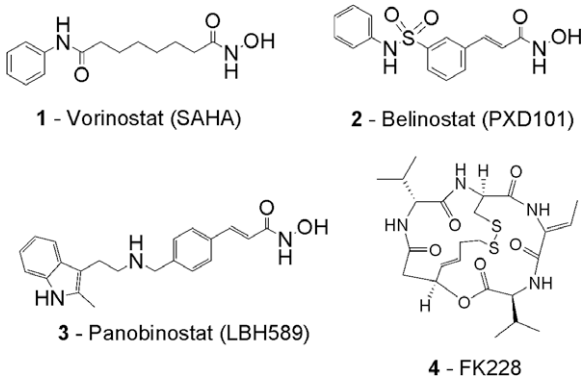

Figure 1. A sample of known HDAC inhibitors.

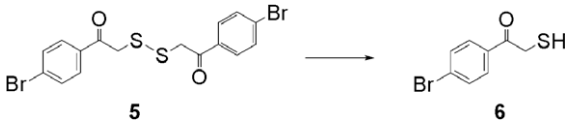

Figure 2. Conversion of screening hit to α-mercaptoketone.

* Corresponding author. Tel.: +1 858 754 3381; fax: +1 858 754 3301.
E-mail address: jmalecha@kalypsys.com (J.W. Malecha).

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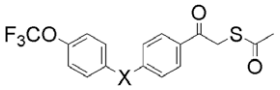
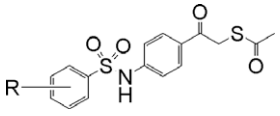
Table 1
Cell based HDAC inhibition and cytotoxicity of selected compounds.



Compound	X	Cell H3[a] EC_{50} (μM)	HCT-116 EC_{50} (μM)
7	$-CH_2NH-$	1.5	NT
8	–NHC(O)–	67.1	NT
9	–NHC(O)NH–	11.2	NT
10	$-NHSO_2-$	1.0	16.1
11a	$-SO_2NH-$	0.20	8.8
12	$-SO_2N(CH_3)-$	4.3	NT
1	SAHA	0.55	1.6

NT, not tested.
[a] HeLa cell-based histone H3 hyperacetylation assay.

Table 2
Cell based HDAC inhibition and cytotoxicity of selected compounds.



Compound	R	Cell H3[a] EC_{50} (μM)	HCT-116 EC_{50} (μM)
11b	H	0.33	15.4
11c	2-Cl	0.85	NT
l1d	3-Cl	0.15	NT
11e	4-Cl	0.28	30.8
11f	3,4-di-Cl	0.42	NT
11g	3-F	0.46	NT
11h	4-F	0.95	NT
11i	3-OCF_3	0.79	NT
11a	4-OCF_3	0.20	8.8
11j	4-CF_3	2.1	NT
11k	4-CH_3	0.10	6.5
11l	3-OCH_3	0.17	2.6
11m	4-OCH_3	0.05	0.5
11n	3,4-di-OCH_3	0.28	3.2

NT, not tested.
[a] HeLa cell-based histone H3 hyperacetylation assay.

work was in progress, an α-mercaptoketone SAHA analog was shown to be a potent HDAC inhibitor.[8] Mercaptoacetamide[9,10] and mercaptoethylamide[11] SAHA derivatives have also been shown to inhibit HDAC activity. However, our screening hit **5** represented a novel lead for the identification of new HDAC inhibitors; an α-mercaptoketone with a non-SAHA like core structure.

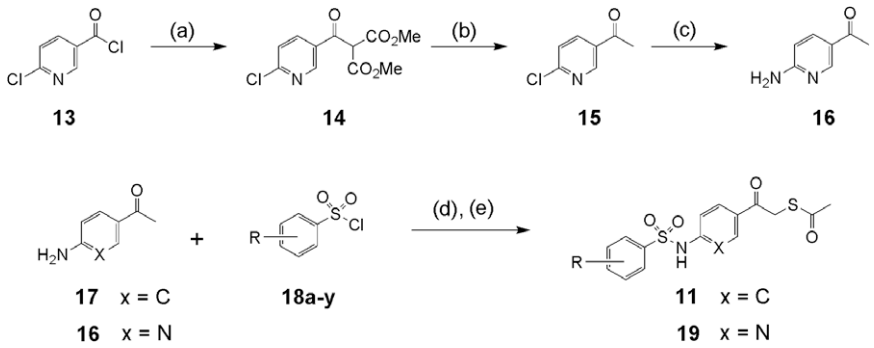
Most HDAC inhibitors consist of four structural units[1]; (a) a zinc chelating group attached to, (b) a hydrophobic spacer connected by, (c) a linker to, (d) a terminal aromatic tail. Our screening hit contained a novel α-mercaptoketone zinc chelating group with a phenyl spacer, but was devoid of the linker and terminal aromatic group which have been shown to improve potency in known HDAC inhibitors. Thus, the lead optimization program focused on the linker and terminal aromatic units.

The propensity of thiols to oxidatively dimerize in air or solution necessitated the use of a prodrug approach. While the disulfide linkage serves as the prodrug for the α-mercaptoketone in our screening hit, we chose to incorporate a thioacetate prodrug in our lead optimization program. The thioacetate prodrug hydrolyzes relatively slowly under neutral and acidic conditions, but undergoes facile enzymatic hydrolysis by esterases, such as those found in serum, to unmask the α-mercaptoketone.

The first series of compounds was prepared with a trifluoromethoxyphenyl tail and amine, amide and sulfonamide linkers (Table 1). Compounds were tested for their pan-HDAC inhibitor activity by their ability to induce histone H3 hyperacetylation in a HeLa cell line. Cytotoxicity for the more potent inhibitors was evaluated against the human colorectal cancer line HCT-116.[5] The sulfonamide analogs showed the best HDAC inhibition, but were not very potent in the cytotoxicity assay. The orientation of the sulfonamide linkage was very important for activity with sulfonamide **11a** five times more potent than the related compound **10**.

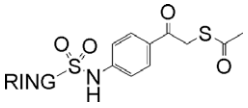
The preferred sulfonamide linker was incorporated into the next series of molecules targeted at modifications of the tail region. The general synthetic procedure used to prepare these analogs is illustrated in Scheme 1. Aniline **16** is prepared in three steps from 6-chloronicotinoyl chloride **13** (dimethylmalonate addition, decarboxylation, aminolysis). Aniline **16** or 4-aminoacetophenone **17** is coupled with sulfonyl chloride **18a-y** in pyridine followed by installation of the thioacetate prodrug (bromination, thioacetate addition) to give the corresponding phenyl **11** or pyridyl **19** α-mercaptoketones (masked as thioacetate prodrug).

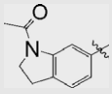
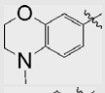
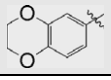
Using this chemistry, a variety of substituted phenyl compounds was synthesized to investigate structural modifications of the terminal aromatic group (Table 2). Ortho substitution decreased HDAC inhibitory activity whereas meta or para substitution was preferred depending on the substituent. Halo derivatives favored meta substitution, while other analogs favored para substitution. The 4-trifluoromethyl analog **11j** was a poor HDAC inhibitor, but the 4-methyl **11k** and 4-methoxy **11m** analogs were more potent than the unsubstituted compound **11b**. In addition, **11m** displayed significant activity in the HCT-116 assay. Encouraged by these results, a series of compounds with a bicyclic terminal aromatic group was examined (Table 3). In general, these compounds exhibited even more potent HDAC inhibition than the simple phenyl substituted derivatives. Indole derivatives **11p** and



Scheme 1. General synthesis of α-mercaptoketones. Reagents and conditions: (a) dimethylmalonate, $MgCl_2$, Et_3N, toluene, 5 h, 92%; (b) H_2O, DMSO, 130 °C, 2 h, 32%; (c) NH_3, 120 °C, 10 h, 89%; (d) pyridine, 60 °C, 1 h, 55–90%; (e) i—pyrrolidone hydrotribromide, HBr/AcOH, DMF, 50 °C, 2 h; ii—potassium thioacetate, DMF, rt, 1 h, 45–70%.

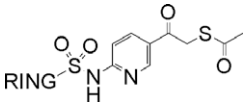
Table 3
Cell based HDAC inhibition and cytotoxicity of selected compounds.

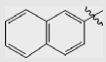
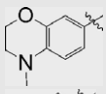
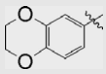


Compound	RING	Cell H3[a] EC_{50} (μM)	HCT-116 EC_{50}(μM)
11b		0.33	15.4
11o		0.029	2.6
11p		0.059	1.5
11q		0.16	1.1
11r		0.044	0.6
11s		0.047	3.3
11t		0.091	4.6
11u		0.079	1.8
11v		0.10	2.6
11w		0.082	3.2
11x		0.088	1.4
11y		0.026	0.4

[a] HeLa cell-based histone H3 hyperacetylation assay.

Table 4
Cell based HDAC inhibition and cytotoxicity of selected compounds.



Compound	RING	Cell H3[a] EC_{50} (μM)	HCT-116 EC_{50} (μM)
19b		0.15	5.3
19o		0.042	4.9
19u		0.24	4.9
19v		0.15	9.6
19w		0.095	6.4
19x		0.36	5.5
19y		0.038	1.2

[a] HeLa cell-based histone H3 hyperacetylation assay.

11r were potent HDAC inhibitors and cytotoxic against the HCT-116 cell line. Benzimidazole **11u** and benzoxazine **11v** also induced histone H3 hyperacetylation, but were less potent in the cytotoxicity assay. Napthyl **11o** and 1,4-dioxine **11y** were the most potent HDAC inhibitors. However, **11y** demonstrated the best combination of HDAC inhibition and in vitro cytotoxicity.

Although the bicyclic compounds were potent HDAC compounds and displayed potent cell killing activity, the initial in vivo results with these compounds were less encouraging. In an effort to improve the oral activity, compounds with increased aqueous solubility were investigated.[12] Thus, a series of compounds was prepared which incorporated the optimized sulfonamide linker and bicyclic tails, but replaced the phenyl spacer with a pyridyl group (Table 4). The in vitro data for the pyridyl series tracked with the results from the phenyl series with the 1,4-dioxine **19y** and napthyl **19o** as the most potent compounds.

Given the in vitro activity of the phenyl and pyridyl dioxins **11y** and **19y**, the compounds were tested for their anti-tumor activity in vivo. To this end, HCT-116 tumor xenograft bearing nude mice were dosed orally with pyridyl analog **19y** and phenyl analog **11y** at 50 mg/kg, once-a-day for 14 days (Fig. 3). Compound **19y** exhibited superior anti-tumor efficacy with a T/C value[13] of 40% compared to 87% for **11y.** At the end of the study, tumors were excised at 2 and 4 h after the last dose and histone H3 hyperacetylation was monitored by Western blotting and LiCor imaging technology. As depicted in Figure 4, the degree and duration of

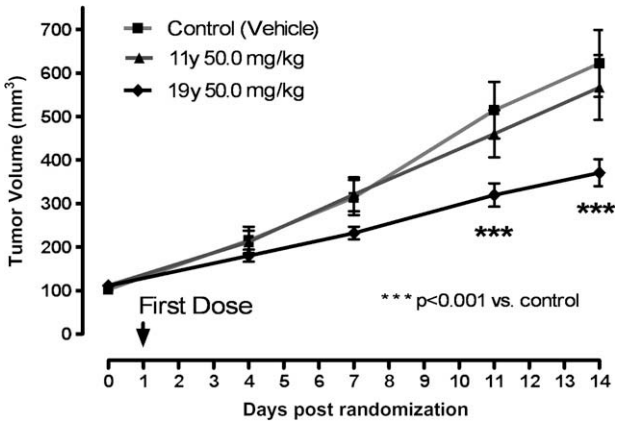


Figure 3. Human colon cancer (HCT-116) xenograft.

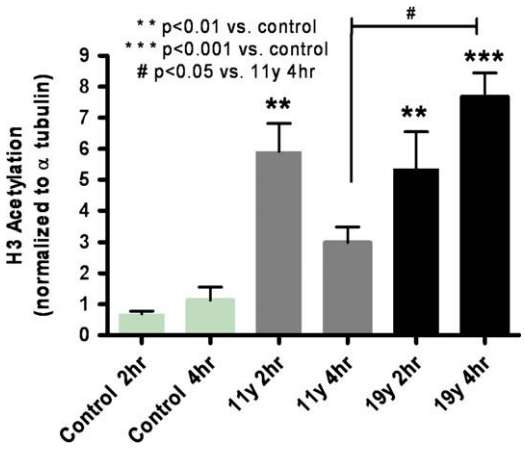


Figure 4. Time course of histone H3 hyperacetylation of end-stage HCT-116 tumors.

induction of hyperacetylation paralleled the anti-tumor efficacy. Robust histone H3 acetylation was observed with **19y** at both 2 and 4 h post-single dose. In contrast, **11y** treatment led to transient H3 hyperacetylation as evidenced by significant biomarker induction only at 2 h. Thus, the incorporation of the pyridyl spacer in **19y** effects improved target modulation and, as a result, improved anti-tumor activity.

A novel series of potent HDAC inhibitors incorporating an α-mercaptoketone has been identified. Based on superior in vivo activity, **19y** was advanced for further study and designated KD5150. These results support the continued investigation of the KD5150 series of α-mercaptoketone HDAC inhibitors as potential cancer therapeutics.[5,12,14]

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