Bioorganic & Medicinal Chemistry Letters 18 (2008) 6093–6096

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of KD5170: A novel mercaptoketone-based histone deacetylase inhibitor

Joseph E. Payne [a], Céline Bonnefous [a], Christian A. Hassig [b], Kent T. Symons [b], Xin Guo [c], Phan-Manh Nguyen [b], Tami Annable [c], Paul L. Wash [a], Timothy Z. Hoffman [a], Tadimeti S. Rao [c], Andrew K. Shiau [b], James W. Malecha [a], Stewart A. Noble [a], Jeffrey H. Hager [c], Nicholas D. Smith [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

ARTICLE INFO

Article history:
Received 26 August 2008
Revised 6 October 2008
Accepted 7 October 2008
Available online 11 October 2008

Keywords:
Histone deacetylase inhibitor
HDAC
Mercaptoketone
Prodrug
HCT-116
KD5170
Sulfonamide

ABSTRACT

We report the identification of KD5170, a potent mercaptoketone-based Class I and II-histone deacetylase inhibitor that demonstrates broad spectrum cytotoxic activity against a range of human tumor-derived cell lines. KD5170 exhibits robust and sustained histone H3 hyperacetylation in HCT-116 xenograft tumors following single oral or iv dose and inhibition of tumor growth following chronic dosing.

The acetylation state of the terminal NH_2 of lysine residues in the histone chromatin core is a key regulator of gene transcription.[1] Histone acetyltransferases (HATs) and histone deacytalases (HDACs) maintain this balance and have been shown to act as transcriptional coactivators and transcriptional repressors, respectively.[2] Recently HDACs have emerged as a promising new target in cancer therapy as tumor cells up-regulate or recruit HDACs to regions in the genome that harbor tumor suppressor genes, resulting in their silencing.[3,4]

The majority of HDAC inhibitors (HDACi), including the recently approved SAHA (Zolinza™),[5] use a hydroxamic acid motif to chelate zinc in the active site of the enzyme (Fig. 1).[6] However, this motif typically confers undesired properties such as poor pharmacokinetics, off-target cross-reactivity and poor solubility.[7] Additional HDACi in clinical trials include the benzamide class[8] such as SNDX-275 and the macrocyclic peptide FK-228,[9,10] which undergoes intracellular disulfide reduction to liberate an alkylthiol, a functionality well established as a potent inhibitor of zinc-dependent enzymatic processes (Fig. 1).[7]

KD5150 (Fig. 1) is a novel HDACi that was identified from an ultra high-throughput screen aimed at identifying non-hydroxamate, non-benzamide small-molecule HDACis.[11] KD5150 is a thioacetate prodrug that undergoes facile hydrolysis in vivo to release a mercaptoketone that binds to the zinc in the active site of the HDAC enzyme.[12]

Although KD5150 was effective at reducing tumor burden in a mouse xenograft model following oral administration,[11] it suffered from poor aqueous solubility (0.2 mg/mL), precluding *intra-venous* (iv) administration and perhaps sub-optimal oral bioavailability. To improve the pharmaceutical potential of the series and allow for iv dosing an effort was undertaken to increase the aqueous solubility of KD5150. In addition, it was felt that improved physicochemical properties would potentially increase the biological activity of series, thereby increasing therapeutic potential. Herein, we describe our attempts to improve the physicochemical properties of KD5150 leading to the identification of the clinical candidate KD5170.

KD5150 contains a catechol group distal to zinc-binding mercaptoketone group and it was proposed that this site could allow for the incorporation groups to improve the water solubility via an ether linkage. Compounds of this type were prepared as shown in Scheme 1.

* Corresponding author.
E-mail address: Nicholas_Smith77@yahoo.com (N.D. Smith).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.029

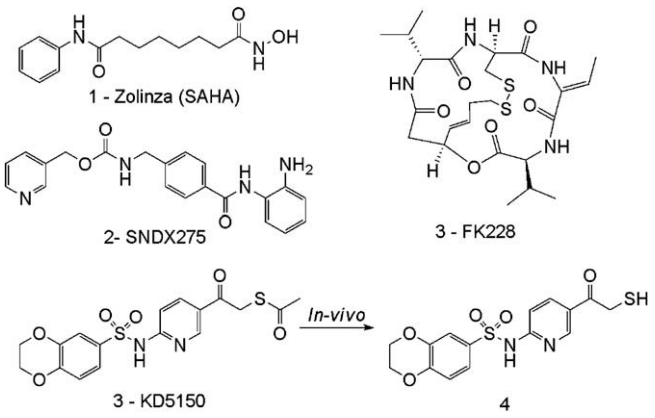


Figure 1. HDAC Inhibitors.

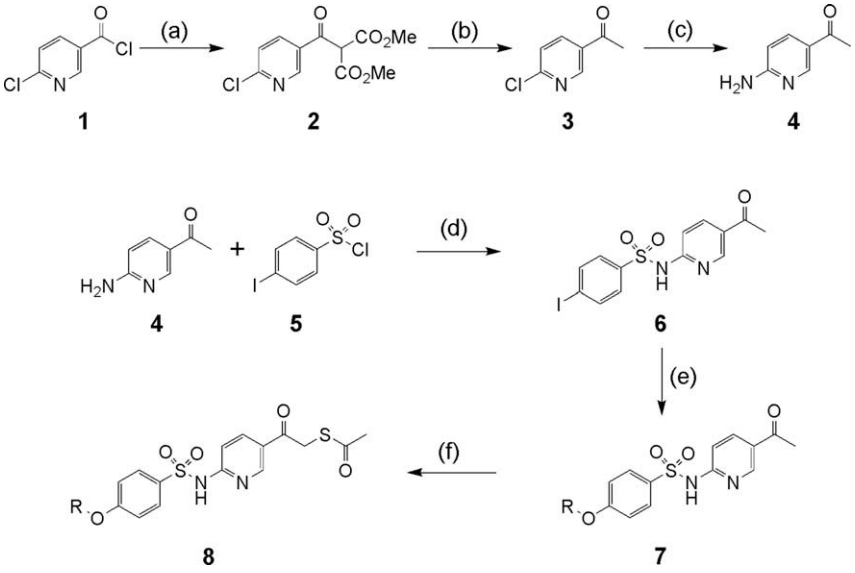
Aniline **4** was prepared from the commercially available acid chloride **1** in 3 steps. Thus addition of the anion of dimethylmalonate to acid chloride **1** gave keto-malonate **2** which was doubly decarboxylated to give ketone **3**. Finally, high pressure amination with ammonia gave the desired aniline **4**.

Aniline **4** was reacted with pipsyl iodide (**5**) in pyridine to give sulfonamide **6**. The iodide of **6** was readily derivatized with commercial alcohols using copper catalyzed chemistry[13] to give ethers **7** wherein groups aimed at improving aqueous solubility were appended at the *para*-position of the phenyl ring. Subsequently **7** was converted to the mercaptoketone prodrug **8** using pyrrolidone hydrotribromide in HBr/AcOH to affect ketone bromination (HBr/AcOH was required to minimize ring bromination). Displacement of the bromine with potassium thioacetate then gave the final thioesters **8**. Using this sequence, the structure–activity relationship (SAR) of this region of the molecule was rapidly investigated with a particular focus on the incorporation of groups that would improve aqueous solubility. Compounds prepared in this manor were tested for their pan-HDAC inhibitor activity by their ability to induce histone H3 hyperacetylation in a HeLA cell-based assay.[14] Additionally, the compounds were tested for cytotoxicity against the colorectal cancer line HCT-116 and the myeloma line U266.

KD5150 is a potent HDACi in the cell based assay with an EC_{50} = 0.038 μM and cytotoxicity of 1.2 μM against the HCT-116 and 2.0 μM against the U266 tumor lines (Table 1). Previous SAR had shown that a methoxy substituent at the *meta*-position (entry 2) lost activity relative to KD5150 (EC_{50} = 0.19 μM), whereas a *para*-methoxy substation (entry 3) maintained activity (EC_{50} = 0.029 μM). Hence the *para*-position was chosen as the primary focus for the attachment of functionality that would be expected to improve aqueous solubility. Thus, incorporation of a pyridine ring via a 1, 2, or 3 carbon chain (entries 4, 5, and 6) led to a loss in potency in the cell-based assay ($EC_{50} \sim 0.20$ μM) compared to the *para*-methoxy ether (entry 3). Similarly, use of a tethered alcohol (entry 7) or ether (entry 8) gave a loss in potency to ($EC_{50} \sim 0.10$ μM) and a significant decrease in cytotoxicity against the HCT-116 and U266 cell lines relative to KD-5150.

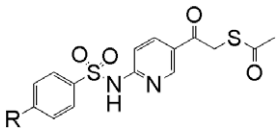
However, the use of tethered aliphatic amines gave more encouraging results. Thus although a 2-carbon linked dimethylamino group (entry 9) eroded potency (EC_{50} = 0.093 μM) compared to KD5150, extending the chain to 3- (entry 10) or 4-carbons (entry 11) gave compounds with equivalent cell based potencies ($EC_{50} \sim 0.040$ μM) to KD5150. Further, the 3-carbon tethered dimethylamino group (entry 10), had an improved cytotoxicity against HCT-116 (EC_{50} = 0.14 μM) and U266 (EC_{50} = 0.18 μM) cell lines compared to KD5150. The SAR of **10** was examined more closely; thus cyclization of the dimethylamino group of **10** by conversion to a pyrrolidinyl (entry 12), piperidinyl (entry 13) or morpholinyl (entry 14) group led to a slight loss in potency in the H3 cell-based assay (EC_{50} = 0.05–0.07 μM) and a corresponding reduction of cytotoxicity against HCT-116 and U266 lines. Restricting the confirmation of **10** as in piperidines **15** (EC_{50} = 0.11 μM) and **16** (EC_{50} = 0.044 μM) led to a general loss in potency, although conformation **16** was clearly preferred to **15**.

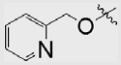
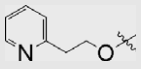
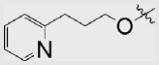
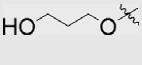
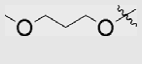
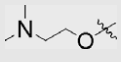
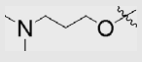
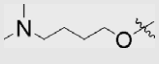
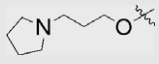
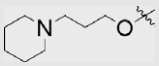
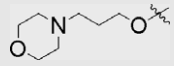
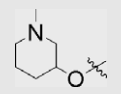
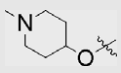
With robust potency in the histone H3 hyper-acetylation cell-based assay and sub-micromolar activity against the HCT-116 and U-266 tumor lines, the dimethylamino-tethered analog **10** (forthwith KD5170) was selected for further profiling. As shown



Scheme 1. Synthesis of α-thioketones incorporating a water solubilizing group. 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, 69%; (e) CuI (5 mol %), 1,10-phenanthralene (10 mol %), Cs_2CO_3 (2.5 equiv), ROH (20 equiv), 120 °C, 6 h, 40–80%; (f) i—Pyrrolidone hydrotribromide, HBr/AcOH, DMF, 50 °C, 2 h; ii—Potassium thioacetate, DMF, rt, 1 h, 45–70%.

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



Entry	R	Cell H3[a] EC_{50} (μM)	HCT-116 EC_{50} (μM)	U226 EC_{50} (μM)
1	KD5150	0.038	1.2	2.0
2	*m*-oMe	0.190	4.3	NT
3	*p*-oMe	0.029	0.54	NT
4		0.22	0.42	NT
5		0.19	0.75	9.6
6		0.22	NT	NT
7		0.11	8.6	NA
8		0.12	5.3	29
9		0.093	0.66	NT
10		0.045	0.14	0.18
11		0.029	0.28	0.12
12		0.061	0.82	0.4
13		0.053	1.0	0.8
14		0.067	0.33	19
15		0.110	3.7	1.2
16		0.044	0.18	0.30

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

in Table 2, the HBr salt of KD5170 is highly soluble in aqueous media (10 mg/mL) compared to KD5150 (<0.2 mg/mL). Additionally, KD5170 showed minimal inhibition of the five major cytochrome P450 enzymes (IC_{50} > 50 μM) and minimal inhibition of the hERG channel in cell-based patch clamp assays (EC_{50} > 30 μM).

To determine the cytotoxicity of KD5170 against cell lines derived from a variety of human tumor types, KD5170 was screened against the NCI panel of 60 human tumor-derived cell lines (NCI-60). As can be seen in Table 3 from a selection of the results,[14] KD5170 displayed micromolar to submicromolar activity against a broad spectrum of tumor cell lines.[15]

A key assay utilized in the program was the pharmacodynamic assessment of histone H3 hyperacetylation following single dose administration of HCT-116 tumor-bearing nude mice. Briefly, mice were dosed with compound po or iv, and tumors excised at 4 h and fold increase in H3 hyperacetylation over control determined by quantitative immunoblotting of tumor lysates.[14] As shown in Figure 2, KD5170 (42 mg/kg po) induced robust histone H3 acetylation in HCT-116 xenograft tumors. Importantly, the magnitude of this effect was greater that than observed with KD5150 (50 mg/kg po), (9-fold versus 5-fold over control). Furthermore, as the aqueous solubility of KD5170 allowed for iv administration, this

Table 2
Aqueous solubility, CYP P450 and hERG channel inhibition for KD5150 and KD5170

	Aq solubility (mg/mL)	EC_{50}	
		CYP inhibition[b] (μM)	hERG[d] (μM)
KD5170[a]	10	>50	>30
KD5150	0.2	>50[c]	>30

[a] HBr salt.
[b] Against 3A4, 2D6, 2C9, 2C19, and 1A2.
[c] 2C9 = 10 μM.
[d] Patch clamp.

Table 3
Selected cytotoxic activities of KD5170 against cell lines from the NCI-60 panel

Line	Origin	EC_{50} (μM)	Line	Origin	EC_{50} (μM)
HT29	Colon	1.0	PC3	Prostate	0.74
MB435	Breast	0.18	768-0	Kidney	0.59
H460	Lung	0.23	HL60	Leukemia	0.39
SKOV3	Ovary	0.76	MJ	CTCL	0.17

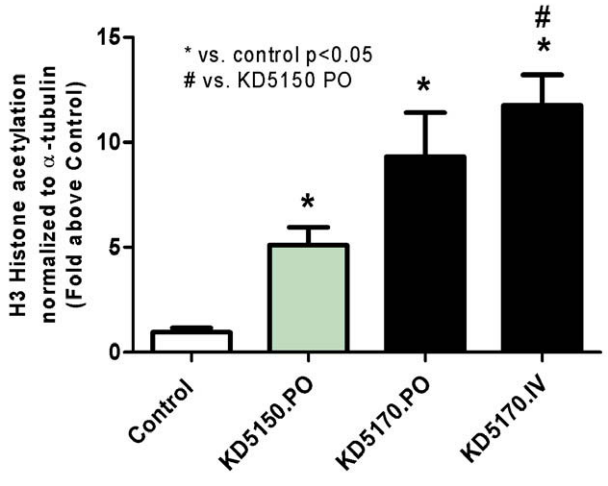


Figure 2. Histone H3 hyperacetylation pharmacodynamic biomarker: comparison of KD5150 (po) and KD5170 (po and iv).

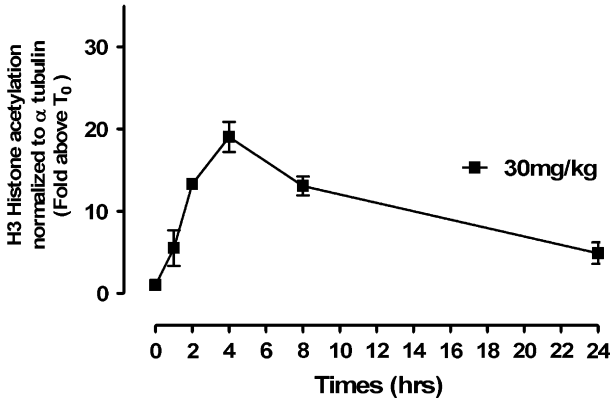


Figure 3. KD5170 induces a robust and sustained pharmacodynamic response in xenograft tumor tissues.

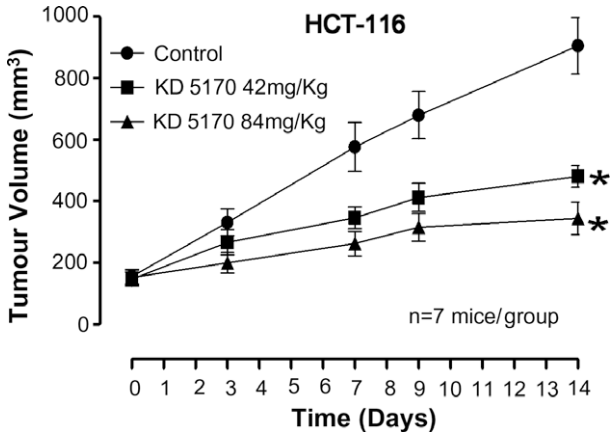


Figure 4. KD-5170 demonstrates tumor growth regression in HCT-116 tumor bearing mice (po dosing).

dosing route was examined and gave a similar robust pharmacodynamic response (12-fold over control).

The time course of histone H3 hyperacetylation in HCT-116 tumors was also examined as shown in Figure 3. Following a 30 mg/kg oral dose of KD5170, tumor H3 hyperacetylation rapidly increased to reach a maximum at 4 h. Significant hyperacetylation was also observed at 8 hours with levels decreasing by 24 h (although still above T_0 levels).

Finally, having demonstrated a sustained pharmacodynamic response, KD5170 was examined for its anti-tumor potential in HCT-116 tumor bearing nude mice. As shown in Figure 4, when dosed orally QOD in a 14-day xenograft, KD5170 demonstrated significant tumor growth inhibition at 42 and 84 mg/kg with T/C values[16] of 44% and 25%, respectively.

In summary, we have identified KD5170, a novel mercaptoketone-based broad spectrum Class I and II-HDAC inhibitor with potent activity in cell based assays that monitor histone acetylation, and broad spectrum activity against a variety of human tumor cell lines. KD5170 exhibits high aqueous solubility and when dosed orally or intravenously, gave a robust and sustained pharmacodynamic response as monitored by histone H3 hyperacetylation in xenograft tumors. This robust pharmacodynamnic response translated into tumor growth inhibition with chronic oral administration of nude mice bearing HCT-116 xenograft tumors. *These data and others support the continued pre-clinical and clinical development of KD5170 as an oncology therapeutic with potential across a wide range of cancer indications.*

References and notes

1. Wang, G. G.; Allis, C. D.; Chi, P. *Trends Mol. Med.* **2007**, *13*, 363.
2. Xu, W. S.; Parmigiani, R. B.; Marks, P. A. *Oncogene* **2007**, *26*(37), 5541.
3. Gallinari, P.; Di, M. S.; Jones, P.; Pallaoro, M.; Steinkuhler, C. *Cell Res.* **2007**, *17*, 195.
4. Glozak, M. A.; Seto, E. *Oncogene* **2007**, *26*, 5420.
5. (a) Marks, P. A. *Oncogene* **2007**, *26*, 1351; (b) Marks, P. A.; Breslow, R. *Nat. Biotechnol.* **2007**, *25*, 84.
6. Paris, M.; Porcelloni, M.; Binaschi, M.; Fattori, D. *J. Med. Chem.* **2008**, *51*(6), 1505.
7. Suzuki, T.; Miyata, N. *Curr. Med. Chem.* **2005**, *12*, 2867.
8. (a) Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Discov.* **2006**, *5*, 769; (b) Zhou, N.; Moradei, O.; Raeppel, S.; Leit, S.; Frechette, S.; Gaudetee, F.; Paquin, I.; Bernstein, N.; Bouchain, G.; Vaisburg, A.; Jin, Z.; Gillespie, J.; Wang, J.; Fournel, M.; Yan, P. T.; Trachy-Bourget, M. C.; Kalita, A.; Lu, A.; Rahil, J.; MacLeod, R.; Li, Z.; Besterman, J. M.; Delormw, D. *J. Med. Chem.* **2008**, *51*, 4072.
9. Konstantinopoulos, P. A.; Vanoros, G. P.; Papavassiliou, A. G. *Cancer Chemother. Pharmacol.* **2006**, *58*, 711.
10. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K. H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Res.* **2002**, *62*, 4916.
11. Wash, P. L.; Hoffman, T. Z.; Wiley, B.M.; Bonnefous, C.; Smith, N. D.; Sertic, M. S.; Lawrence, C. M.; Hassig, C. A.; Symons, K. T.; Guo, X.; Nguyen, P.; Annable, T.; Noble, S. A.; Hager, J. H.; Malecha, J. W. Bioorg. Med. Chem Lett., 2008 (preceding paper).
12. The binding mode of the mercaptoketone maybe mono- or bi-dentate: Vanommeslaeghe, K.; Loverix, S.; Geerlings, P.; Tourwe, D. *Bioorg. Med. Chem. Lett.* **2005**, *13*, 6070.
13. Wolter, M.; Nordmann, G.; Job, G. E.; Buchwald, S. L. *Org. Lett.* **2002**, *4*, 973.
14. Hassig, C. A.; Symons, K. T.; Guo, X.; Nguyen, P.; Annable, T.; Wash, P. L.; Payne, J. E.; Jenkins, D. A.; Bonnefous, C.; Trotter, C.; Wang, Y.; Anzola, J. V.; Milkova, E. L.; Hoffman, T. Z.; Dozier, S. J.; Wiley, B. M.; Saven, A.; Malecha, J. W.; Davis, R. L.; Shiau, A. K.; Noble, S. A.; Rao, T. S.; Smith, N. D.; Hager, J. H. *Mol. Cancer Ther.* **2008**, *7*, 1054.
15. The cytotoxic activities (EC_{50}) ranged from 0.1 to 7.7 μM with a mean and median of 1.6 and 0.9 μM, respectively.
16. *T*/*C*: (Treated final volume − Treated initial volume)/(Control final volume − Control initial volume) × 100. A *T*/*C* value of 0 equals tumor stasis.