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Design, synthesis, and biological evaluation of novel histone deacetylase 1 inhibitors through click chemistry

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ABSTRACT

We report the design, synthesis, and biological evaluation of a new series of HDAC1 inhibitors using click chemistry. Compound **17** bearing a phenyl ring at *meta*-position was identified to show much better selectivity for HDAC1 over HDAC7 than SAHA. The compond **17** also showed better in vitro anticancer activities against several cancer cell lines than that of SAHA. This work could serve as a foundation for further exploration of selective HDAC inhibitors using the compound **17** molecular scaffold.

Epigenetics is classically defined as reversible heritable changes in gene expression that occur without a change in DNA sequence. DNA methylation and histone acetylation are two well known epigenetic chromatin modifications, although ethyl, phosphoryl, ubiquitylation and other modifications of histones have been described.[1,2] The steady state of histone acetylation was balanced by histone deacetylases (HDACs) and histone acetyltransferases (HATs). HATs add acetyl groups to lysine residues of histone tails causing localized relaxation of chromatin and transcriptional activation of nearby genes, while HDACs remove the acetyl groups of acetylated histones leading to transcriptional repression.[3] HDACs play important roles in the upstream control of gene transcription, cell cycle progression, and apoptosis. It has been widely recognized that HDACs are promising targets for cancer therapy. Thus far, 18 Mammalian HDAC subtypes have been discovered and accordingly classified into four classes based upon their size, number of catalytic active sites, subcellular localization, and sequence homology to yeast models.[4,5] Classes I HDACs (1, 2, 3, and 8), class IIa HDACs (4, 5, 7, and 9), class IIb HDACs (6 and 10), and the class IV HDAC (11) are Zn^{2+}-dependent metallohydrolases, whereas class III HDACs (sirtuins 1–7) are NAD+-dependent Sir2-like deacetylases.[5] HDAC isoforms are highly expressed in various cancers, including gastric cancer, pancreatic cancer, colorectal cancer, prostate cancer and hepatocellular carcinoma.[6] HDACs are part of a transcriptional corepressor complex that plays a critical role in a variety of transcriptional regulatory pathways involving various tumor suppressor genes. HDAC inhibitors have been shown to induce cell growth arrest, differentiation and/or apoptosis in different cancer cell lines. HDAC inhibitors are emerging as a new class of chemotherapeutic agents.[7]

Since the suberoylanilide hydroxamic acid (SAHA, Vorinostat) has been licensed for the treatment of cutaneous T cell lymphoma treatment (CTCL) on 2006, more than 11 new HDACi are in various stages of clinical development for therapy of multiple cancer types.[8] Although HDAC inhibitors showed potent antitumor effects, they have exhibited side effects that might limit their clinical potential.[9] Therefore, looking for new HDAC inhibitors that are specific-inhibition to one kind HDAC subtype is extremely urgent and necessary.

Although a lot of HDAC inhibitors have been investigated, few class selective or isoform selective HDACi was found.[10] Not only the discovery of novel isozyme selective inhibitors are likely to provide more effective chemotherapy compared to pan-inhibitors, but also are useful as tools for probing the biology of the enzyme. Compound **1** is a HDAC inhibitor reported by Oyelere et al in 2008, which showed good binding affinity against HDACs.[11] Compound **1** are composed by three parts: zinc binding group (ZBG), linker and capping group (Fig. 1). Molecular modeling of the complex structure of HDAC1 with compound **1** indicated that the capping group region of compound **1** plays important role for HDAC isoform

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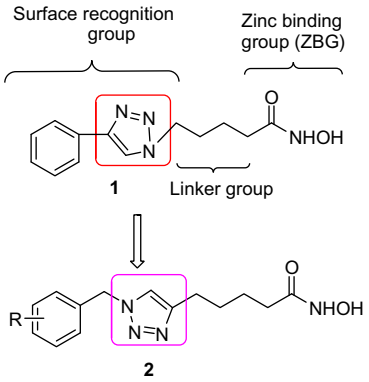


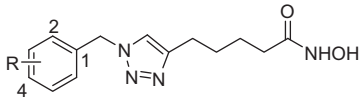
Figure 1. Design of new class I histone deacetylase inhibitors using a scaffold-hopping strategy.

selectivity. We envisioned that changing the position of substituents on the triazole ring would increase the selectivity for HDAC1. In this paper, we report a series of triazole-based compounds built by Click chemistry (Huisgen 1,3-dipolar cycloaddition).

The triazole analogues **2**–**29** in table 1 were synthesized by using the procedure described in Figure 2. The *N*-hydroxyhept-6-ynamide was easily synthesized from the commercially available 6-heptynoic acid.[12] Then all compounds were prepared by a convenient one-pot procedure from a variety of readily available substituted benzyl bromide, sodium azide and *N*-hydroxyhept-6-ynamide in 27% ~ 68% yields.

The HDAC inhibition activity of the compounds reported herein were examined based on fluorescent-based HDAC biochemical activity assay using recombinant human HDACs. It is noteworthy that this set of structures show promising selectivity for HDAC1, as they are no activity in the inhibition of HDAC7. As shown in table 1, compound 2 bound to the HDAC1 with an IC_{50} of 1.68 μM, which is three times more potent than compound **1**. The position of substitution might have great impact on the effectiveness of the set of compounds against HDAC1. It is observed that *ortho*-position substitution in the phenyl ring did not improve the inhibition activity (**3**–**5**). For instance, when a bromide group was introduced at *ortho*-position of compound **2**, the inhibitory activity against HDAC1 was decreased two times with an IC_{50} = 3.58 μM in comparison with original compound **2**. Interestingly, different functional groups at the *para*-position of phenyl ring show different inhibition activities (**6**–**15**). When a variety of functional groups such as *tert*-butyl, methyl, trifluoromethyl, fluoro, CN, phenyloxy or phenyl groups were introduced at para-position of compound **2**, respectively, the inhibition activities of HDAC1 were significantly increased (**9**–**15**). Among them, the compound **13** with phenoxy at *para*-position displayed IC_{50} value of 0.122 μM against HDAC1, which was about 13.7 times more potent than the original compound **2**. However, it was disappointing that chloro, bromo and methoxy substitutions did not improve the potency (**6**–**8**). To our delight, the functional groups at the *meta*-position of phenyl ring could significantly increase inhibition activities, which suggested that *meta*-position might be a feasible position for further optimization (**16** and **17**). For example, when a phenyl group was introduced at *meta*-position of compound **2**, the inhibitory activity against HDAC1 was obviously improved and the resulting compound **17** displayed IC_{50} value of 0.058 μM, which was about 29 times more potent than the compound **2**. Molecular modeling indicates that the phenyl ring adopted a coplanar geometry in order to fit within hydrophobic pocket (Fig. 3). However, these improved interactions can be overweighed by increasing exposed hydrophobic surface area under some circumstances. For

Table 1
IC_{50} Values for HDAC1 and HDAC7 Inhibition (μM)



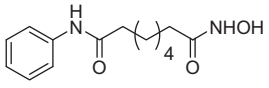
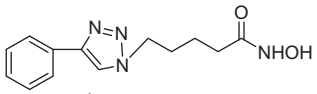
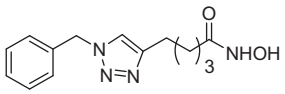
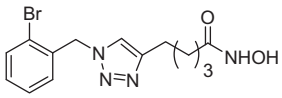
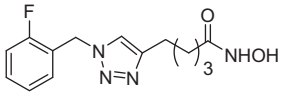
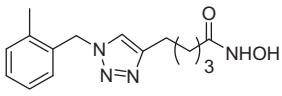
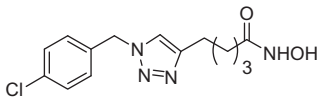
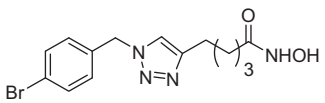
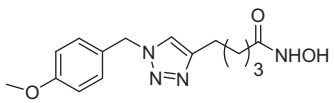
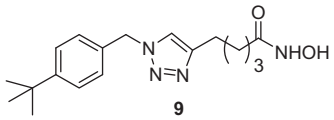
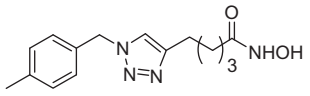
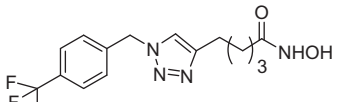
Sructure	IC_{50}(μM)	
	HDAC1	HDAC7
 **SAHA**	0.131	38.9
 **1**	5.13	18.7
 **2**	1.68	NA
 **3**	3.58	NA
 **4**	1.36	NA
 **5**	1.05	NA
 **6**	2.21	NA
 **7**	1.60	NA
 **8**	1.85	NA
 **9**	0.601	NA
 **10**	0.440	NA
 **11**	0.440	NA

Table 1 (*continued*)

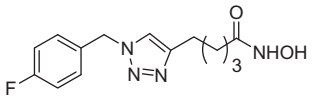
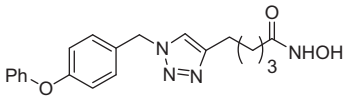
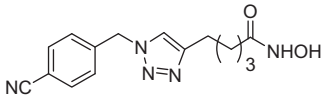
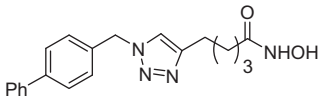
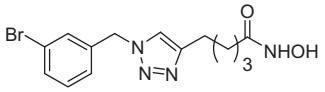
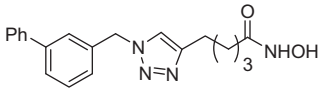
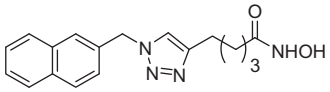
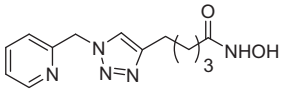
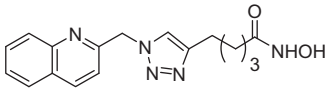
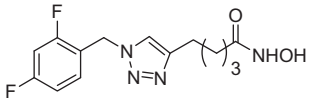
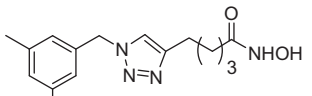
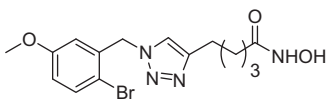
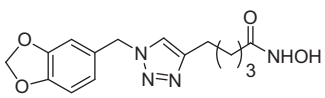
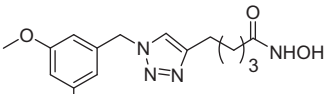
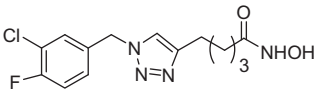
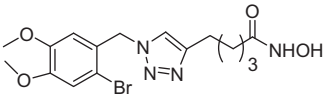
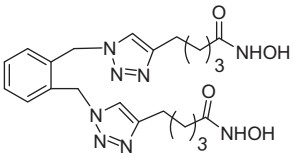
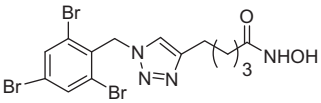
Sructure	IC_{50}(μM)	
	HDAC1	HDAC7
 **12**	0.645	NA
 **13**	0.122	NA
 **14**	0.701	NA
 **15**	0.202	NA
 **16**	0.396	NA
 **17**	0.058	NA
 **18**	0.107	NA
 **19**	2.57	NA
 **20**	0.246	NA
 **21**	1.22	NA
 **22**	0.269	NA
 **23**	0.159	NA
 **24**	1.90	NA

Table 1 (*continued*)

Sructure	IC_{50}(μM)	
	HDAC1	HDAC7
 **25**	0.252	NA
 **26**	0.649	NA
 **27**	0.862	NA
 **28**	0.807	NA
 **29**	NA	NA

[a]SAHA was used as a positive control. Values are means of three experiments, and standard error of the IC_{50} was generally less than 10%.
[b]NA, no activity observed at 10 μM concentration tested.

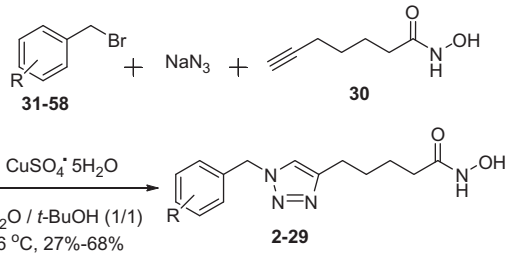


Figure 2. General procedure for the synthesis of compound **2**–**29**.

instance, the binding affinity of the resulting compound **18** (IC_{50} = 0.246 μM) was decreased when diphenyl was substituted with L-2-naphthylalanine (2-Nal). Incorporation of nitrogen into the phenyl ring did not improve the potency of the compound **2**. When the phenyl group was replaced with quinoline ring, the resulting compound **22** showed IC_{50} value of 0.246 μM, which was about 6.8 times more potent than the compound **2**. The impact of the *di*-substituted or *tri*-substituted compounds was also investigated, and the results indicated that the methyl group (**22**) or methoxy group (**23**, **25**) caused dramatic increase of the potency against HDAC1 (**21**–**29**). To our surprise, the 2,4,6-tribromo substituted compound **29** completely lost its inhibitory activities against HDAC1. Molecular modeling indicates that the *ortho*-position substituted bromo atom could not fit within hydrophobic pocket due to its large size.

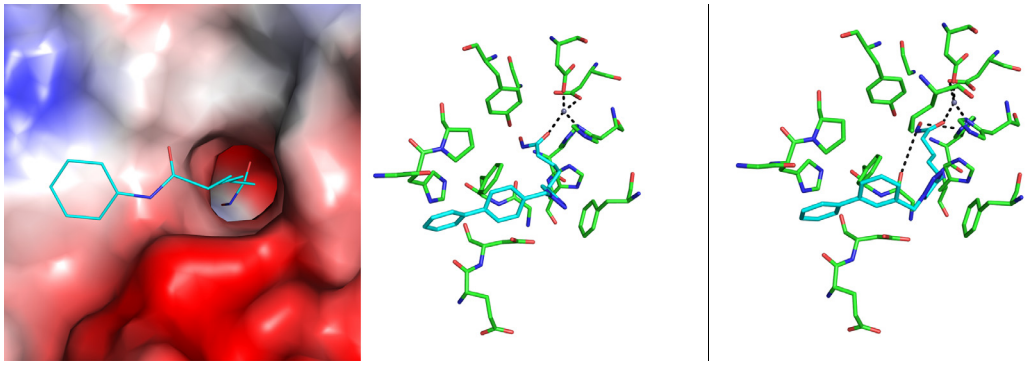


Figure 3. Plausible binding mode of SAHA, **15** and **17** to HDAC1.

To further investigate the antiproliferative activities of these derivatives, some of these compounds were selected to test their effects on the human tumor cell lines, such as Hela (human cervical cancer cell line), BGC823 (human stomach cancer cell line), A549 (human lung cancer cell line), HT1080 (human sarcoma cell line), A431 (human epithelial carcinoma cell line), and DU-145 (human prostate cancer cell line), with SAHA as the positive control. The results are summarized in table 2. Compounds **7**, **9**, **13**, **15**, **17**–**20**, **23** and **25** showed very good GI_{50} values in the micromolar range against DU145 cell lines. It is remarkable that compounds **15** and **17**, especially in view of their higher HDAC1 activities, have much better anti-cancer activities than that of SAHA against several cancer cell lines. In contrast, compound **3**, the poor inhibitor of HDAC1, shows much weaker activity against those cell lines. Thus, taken together, the results of the enzyme and cell-based assays verify the high correlation between the in vitro HDAC1 inhibitory activity and cellular cytotoxicity of HDAC inhibitors.

In summary, we have developed one-pot click chemistry approach to design a series of HDAC inhibitors, which show features of high potency and selectivity of HDAC1, as well as ability to inhibit several cancer cell growth. We identified a representative lead from this series, **17**, which is several folds more potent than SAHA against HDAC1. The compond **17** showed promising *in vitro* anticancer activities against several cancer cell lines. Biological results demonstrate those compounds which demonstrated a high degree of HDAC1 inhibition (**15**, **17** and SAHA) also exhibited high levels of potency in the cell-based assay. In contrast, the weaker inhibitors of HDAC1 also generally exhibited reduced potency against cancer cells. Inhibitory studies on metastatic tumors in animal models are currently in progress in our laboratory.

Table 2
Antiproliferative Activity of Selected Compounds

Compd	GI_{50} (μM)						
	Hela	BGC823	A549	HT1080	A431	HUVEC	Du145
3	NA	NA	NA	NA	NA	ND	NA
7	NA	NA	NA	NA	15.4	ND	7.91
9	NA	NA	NA	7.37	NA	NA	7.20
13	NA	7.77	NA	7.69	9.03	ND	4.49
15	3.72	1.80	2.15	2.69	2.99	5.48	1.21
16	NA	NA	NA	6.65	NA	NA	NA
17	3.85	3.05	1.47	3.14	4.65	1.13	1.19
18	NA	6.64	NA	4.61	5.68	NA	7.23
20	NA	NA	NA	10.2	NA	NA	4.06
22	NA	NA	NA	8.65	NA	NA	NA
23	NA	NA	NA	9.94	NA	NA	5.62
25	NA	NA	NA	8.04	6.80	NA	7.89
26	NA	NA	NA	10.2	NS	NA	NA
27	NA	NA	NA	NA	17.3	NA	NA
SAHA	3.36	3.09	1.78	4.87	4.48	8.13	6.86
1	NA	NA	7.69	NA	NA	NA	NA

[a]SAHA was used as a positive control.
[b]Inhibition of cell growth by the listed compounds was determined by using MTT assay.
[c]NA, no activity observed at 10 μM concentration tested.
[d]Values are means of three experiments, and standard error of the GI_{50} was generally less than 20%.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.102.

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2. Verdin, E.; Dequiedt, F.; Kasler, H. *Trends Genet.* **2003**, *19*, 286.
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