Bioorganic & Medicinal Chemistry xxx (xxxx) xxx-xxx



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# Structure-activity relationship study of thiazolyl-hydroxamate derivatives as selective histone deacetylase 6 inhibitors

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ARTICLE INFO	A B S T R A C T
Keywords: Computer-aided drug design SAR of thiazolyl-hydroxamates Selective HDAC6 inhibitors	Several human diseases are associated with aberrant epigenetic pathways mediated by histone deacetylases (HDACs), especially HDAC6, a class IIb HDACs, which has emerged as an attractive target for neurodegenerative and autoimmune disease therapeutics. In a previous study, we developed the novel HDAC6-selective inhibitor <b>9a</b> (( <i>E</i> )- <i>N</i> -hydroxy-4-(2-styrylthiazol-4-yl)butanamide) and showed that it has anti-sepsis activity <i>in vivo</i> . In this study, we conducted structure-activity relationship (SAR) studies to optimize the activity and selectivity of HDAC6, synthesizing its derivatives with various aliphatic linker sizes and cap structures. We identified <b>6u</b> (( <i>E</i> )- <i>N</i> -hydroxy-3-(2-(4-fluorostyryl)thiazol-4-yl)propanamide), which has nanomolar inhibition activity and a 126-fold selectivity for HDAC6 over HDAC1. Through the docking analyses of <b>6u</b> against HDAC subtypes, we revealed the importance of the optimal aliphatic linker size, as well as the electronic substituent effect and rigidity
	of the aryl cap group. Thus, we suggest a new rationale for the design of HDAC6-selective inhibitors.

#### 1. Introduction

Post-translational modification (PTM) of histones dramatically effects gene expression. Since the emergence of epigenetics and PTMs, numerous genes and diseases have been found to be associated with histone PTMs. The control of the modification processes has been an attractive approach in drug development,<sup>1</sup> and, to date, histone acetylation and methylation modulators have mainly been developed as cancer therapeutics.<sup>2</sup> Histone acetylation is modulated by histone acetyltransferase (HAT) and deacetylase (HDAC). HDAC subtypes are grouped into 4 classes and 18 subtypes. Class I, II, and IV are zinc-dependent enzymes: class I (HDAC 1, 2, 3, and 8), class IIa (HDAC 4, 5, 7, and 9), class IIb (HDAC 6, and 10), and class IV (HDAC11). Class III members are NAD<sup>+</sup>-dependent enzymes known as sirtuins. The characteristics of each subtype and their selective inhibitors have been well investigated.<sup>3,4</sup> A majority of the FDA-approved anticancer HDAC inhibitors.

HDAC6 is a unique subtype of HDACs. It is a class IIb HDAC, with two catalytic domains mainly expressed in the cytoplasm.<sup>5</sup> Unlike other HDACs, HDAC6 is a non-specific histone deacetylase and modifies cytoplasmic proteins such as  $\alpha$ -tubulin,<sup>6</sup> Hsp90, and cortactin. The acetylation of  $\alpha$ -tubulin regulates the structure of the cytoskeleton.<sup>7</sup> The

genetic ablation of HDAC1-3 is lethal, but the knockout of HDCA6 does not affect mice. Therefore, HDAC6 inhibitor-induced side effects may be few, permitting their application in various diseases, including neurodegenerative and autoimmune diseases. In neurodegenerative diseases like Huntington's and Charcot-Marie-Tooth neuropathy, tubulin and microtubule acetylation increases intracellular axonal transport.<sup>8,9</sup> Increasing ubiquitination by Hsp90 acetylation is another suggested therapeutic strategy.<sup>10</sup> The mechanism of the anti-inflammatory effect of HDAC6 inhibitors is also intensely being studied. HDAC6 inhibitors block MAPK signaling by MKP-1 acetylation,<sup>11,12</sup> enhance the suppressive capacity of regulatory T cells,<sup>13,14</sup> and inhibit immune cells by altering microtubule dynamics and ubiquitination,<sup>15-17</sup> Our interest also lies in the development of selective HDAC6 inhibitors with less cytotoxicity, applicable to the disease models described above.

In our previous efforts, we identified the novel selective HDAC6 inhibitor **9a** (Fig. 1), with a thiazole linker and phenyl cap group, by computational database screening and molecular design.<sup>18</sup>

The IC<sub>50</sub> values of **9a** was found to be 199 nM for HDAC6 and 13.8  $\mu$ M for HDAC1.<sup>18</sup> Most zinc-dependent HDAC inhibitors consist of three pharmacophoric elements: the surface capping group, the aliphatic linker unit for channel binding, and the catalytic zinc-binding group. In this study, we optimized the scaffold of **9a** by altering not

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#### Known HDAC6 selective inhibitors





Tubastatin A

ACY-1215 (R=H), ACY-241 (R=Cl)



Fig. 2. HDAC6 inhibitor scaffold.

only the length of aliphatic linker, but also the structure and rigidity of the cap group (Fig. 2).

The designed derivatives were evaluated by preliminary docking analyses against HDAC6 (PDB: 5EDU)<sup>19</sup> and HDAC1 (PDB: 4BKX),<sup>20</sup> synthesized and *in vitro* HDAC1 and HDAC6 enzymatic assays were performed. As a result, we identified the more potent and selective HDAC6 inhibitor **6u**, with margin of safety and HDAC6 selectivity at the cellular level.

#### 2. Results and discussion

#### 2.1. Synthesis of derivatives of 9a

We designed 27 HDAC6 selective inhibitor candidates, using **9a** as a lead structure (Table 1), to evaluate the effects of the aliphatic chain length (m and n, Fig. 2), presence of a single or double bond in the m position, and modification of the cap group (R), on the selectivity and inhibition activity for HDAC6.

We previously confirmed that the *cis*-double bond in the *m* position, which is part of the cap group, is detrimental to HDAC6 activity (Data not shown). Our previous docking model of **9a** also revealed that if the linker *m* contains a *cis*-double bond, the drug will not fit in the active site pocket, due to huge steric crash.<sup>18</sup> Therefore, all compounds with a double bond in the *m* position are in the *trans* form.

As depicted in Scheme 1, the synthesis of desired thiazole hydroxamate 6 was initiated from readily available amides 1 and 4-ketopentanoic acid (3). Treatment of amide 1 with Laswsson's reagent yielded thioamide 2.<sup>18</sup> Bromination and esterification from 3 gave bromo ester 4. The thiazole esters 5a-5x were obtained by cyclization of 2 with 4 in MeOH solvent.<sup>21</sup> Finally, the thiazole esters 5a-5x were reacted with hydroxyl amine to give thiazole hydroxamates 6a-6x.<sup>22</sup>

#### 2.2. Structure-activity relationship of HDAC6 inhibitors

To measure inhibition activity, *in vitro* HDAC1 and HDAC6 enzymatic assays were performed, and their activities were compared using their IC<sub>50</sub> and selectivity indexes (IC<sub>50, HDAC1</sub>/IC<sub>50, HDAC6</sub>) (Table 1). In compounds with a phenyl cap group (**6a-6d**), C chain linker length changes (m = 1 or 2; n = 2 or 3) dramatically affected inhibition activity, but not HDAC6 selectivity. Therefore, the total length of the aliphatic chain (m + n) was optimized to 4, permitting the derivative (**6a**) with m = n = 2 to show the highest activity. After optimizing the aliphatic chain length to m = n = 2, we evaluated the derivatives with different bond states of m and aromatic cap groups.

The binding pocket of HDAC can be divided into three parts: zinccontaining catalytic site, hydrophobic channel, and solvent-accessible entrance. As illustrated in Supplementary Fig. S1, as opposed to different pocket size, the amino acid residues inside the binding pocket of all published human HDACs are highly conserved,<sup>23</sup> whereas those in the entrance region are diverse. Therefore, to improve HDAC6 Bioorganic & Medicinal Chemistry xxx (xxxx) xxx-xxx

Fig. 1. Known HDAC6 selective inhibitors and our HDAC6 inhibitor lead **9a**.

9a (Ref.21)

selectivity, we focused on modifying the cap group (m and R).

In this study, the *para*-fluorophenethyl cap group dramatically increased the inhibition potency of HDAC6 (**6e** and **6u**). In comparing the HDAC6 IC<sub>50</sub> of analogues with *para*-substituted phenethyl moieties (Table 1), we found that electronegative hydrogen bond acceptor substituents potentiate or sustain HDAC6 activity (**6f**, **6g** and **6l**), while donor groups decrease it (**6i** and **6o'**). The *meta*-fluorophenethyl-containing analogue (**6m**) showed somewhat decreased activity and selectivity for HDAC6. Analogues having di-/tri-substituted fluoro, hydroxyl, and methoxy phenyl groups did not show improved inhibition activity. Electronic features of substituents in the phenyl ring seem a more critical factor for activity than steric bulkiness. However, substitution of the phenethyl cap group had no significantly effect on HDAC6 selectivity. Derivatives with a heteroaryl ethyl cap group (**6q**, **6r**, and **6s**) also showed low HDAC6 selectivity.

Cap group rigidity was used to evaluate HDAC6 selectivity. The  $IC_{50}$  and selectivity ratio of each pair of derivatives with an ethyl or ethenyl group in their *m* position were compared (Fig. 3). Although compounds with a phenethyl cap (**6a**, **6e**, **6f**, and **6g**) have higher HDAC inhibitory activity, all compounds with a phenylethene cap (**6t**, **6u**, **6v** and **6w**) show increased HDAC6 selectivity, revealing that cap group rigidification improves HDAC subtype selectivity.

The HDAC6 selectivity of derivatives with flexible phenethyl caps is unaffected by *para*-halogen substituents, which affect both the HDAC6 inhibition activity and selectivity of derivatives with rigid phenylethene caps; the activity and selectivity decrease in the order  $F > Br \ge Cl > H$ . The electronic and steric effects of the halogen substituent may play a more crucial role in the interaction of the rigid cap group with the binding site of HDAC6, compared with that of the flexible cap group.

We therefore performed extensive docking analysis of 6u to elucidate its HDAC6 selectivity, by examining possible binding poses of 6u in HDAC6.

#### 2.3. Docking simulation to elucidate the HDAC6 selectivity of 6u

The correlation between the cap group rigidity and HDAC6 selectivity is demonstrated by comparing the flexible docking results of **6u** with those of **6e**. The flexible docking of **6e** with a *p*-fluorophenethyl cap generated an ensemble of diverse conformations spread out at the active site pocket entrance (Fig. 4). In contrast, the poses of **6u** with a *p*-fluorophenylethene cap consisted of limited conformations aligned in the restricted area. Therefore, we examined in detail whether this cap group interacts with unconserved residues between HDAC1 and HDAC6.

The residues composing the rim of the catalytic channel, where most HDACs, including HDAC1 and HDAC6, possess two hydrophobic grooves, are also generally conserved with minor differences (Fig. 5A and B). A large groove is composed of His, Pro, Leu residues, and highly conserved in all HDACs. A small groove is located right next to the Leu residue of a large groove and is composed of a single Tyr (HDAC1 and 2) or Phe (HDAC3, 4, 6, 7, and 8) residue. In the X-ray crystal structures of complexes of HDAC inhibitors and class I HDACs, the cap moiety of inhibitors is mostly positioned at a large hydrophobic groove.<sup>19,25</sup> There are two distinctive residues present at the pocket entrance of HDAC6: Ser568 and Met682. Ser568 is located between two parallel

#### Bioorganic & Medicinal Chemistry xxx (xxxx) xxx-xxx

#### G. Nam, et al.

#### Table 1

Structures and the HDAC1 and HDAC6 inhibition activity of compounds.

Name	Structure	HDAC1 IC <sub>50</sub> (nM)	HDAC6 IC <sub>50</sub> (nM)	Selectivity index (HDAC1/HDAC6)
ба	N O	118.4 ± 14.20	30.89 ± 8.705	3.83
6b	н́м-он о N H H	19101 ± 7567	1455 ± 212.5	13.13
бс	S	15915 ± 7329	839.1 ± 391.9	18.97
6d	ули о	1158 ± 283.2	95.04 ± 60.98	12.18
6e	F N S	296.2 ± 42.30	21.23 ± 8.275	13.95
6f	CI N O	90.05 ± 43.92	$15.33 \pm 6.200$	5.87
бд	Br N O	79.81 ± 20.42	15.89 ± 6.330	5.02
6h	MeO S- S- MeO	83.71 ± 51.90	50.68 ± 15.93	1.65
6h′		470.1 ± 150.2	56.89 ± 17.22	6.86
6i	HN-OH H2N S HN-OH	1060 ± 179.3	85.35 ± 37.70	12.42
6j		119.7 ± 30.04	110.4 ± 35.36	1.08
6k	F <sub>3</sub> C N S HN-OH	164.6 ± 63.05	39.07 ± 9.235	4.21
61	O <sub>2</sub> N S HN-OH	98.02 ± 51.69	43.6 ± 12.14	2.25
om	r N S HN-OH	209.0 ± 85.75	37.95 ± 10.33	o.84

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#### Bioorganic & Medicinal Chemistry xxx (xxxx) xxx-xxx

#### Table 1 (continued)

G. Nam, et al.

Name

6n

60

60'

6р

6p'

6q

6r

6s

6t

6u

6v

6w

6x

TSA

SAHA

Structure	HDAC1 IC <sub>50</sub> (nM)	HDAC6 $IC_{50}$ (nM)	Selectivity index (HDAC1/HDAC6)
	278.3 ± 130.4	20.22 ± 5.075	13.76
MeO	103 ± 38.37	61.42 ± 17.69	1.68
S HN-OH HO	1132 ± 435.6	561.8 ± 210.9	2.01
OMe MeO	291.6 ± 118.1	97.07 ± 26.95	3.00
	15707 ± 13980	19531 ± 11130	0.80
HO' S HN-OH	558.1 ± 382.5	101 ± 42.13	6.53
S N S S S S S S S S S S S S S S S S S S	141.4 ± 152.4	33.77 ± 9.375	7.27
HN-OH	335.6 ± 91.35	54.2 ± 13.19	6.19
	25486 ± 19010	1386 ± 785.8	18.39
	5432 ± 695.0	42.98 ± 7.870	126.38
	19573 ± 7316	592.1 ± 192.4	33.06
Br N S HN-OH	16610 ± 8612	309.4 ± 145.6	3 38
	3.511 ± 1.456	8.922 ± 4.422	0.39
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1.86

 $131.5 \pm 21.40$ 

 $70.83 \pm 13.93$ 

Ĭ

н 0 ,C N\_OH H

0 K\_\_OH H

#### G. Nam, et al.

#### Table 1 (continued)

Name	Structure	HDAC1 IC <sub>50</sub> (nM)	HDAC6 IC <sub>50</sub> (nM)	Selectivity index (HDAC1/HDAC6)
Tubastatin A [24]	O N N O H	2977 ± 880.0	11.14 ± 2.614	267.24



Scheme 1. Reagents and conditions: (a) Lawesson's reagent, THF, rt, 2 h, 100%; (b) Br<sub>2</sub>, MeOH, 0 °C, 2 h, then 80 °C, 2 h, 55%; (c) MeOH, overnight reflux, 2 h, 40 ~ 60% (d) NH<sub>2</sub>OH  $\cdot$  HCl, 8 N NaOH, MeOH, 10 ~ 20%

side-chain phenyl rings of Phe620 and Phe680, conserved as Asp in other HDACs. Met682, a unique residue of HDAC6, is distinctively orientated in the next loop structure position, to provide additional surface in the small hydrophobic groove. These unique structural features of HDAC6 may enable ligand selectivity. The results of docking modeling suggest the active conformation of **6u** poses in the small hydrophobic groove at the pocket entrance.

In examining the molecular interactions of **6u** with the small groove residues of HDAC6 (Fig. 5C), we found that the *p*-fluorophenyl ring in the cap group of **6u** interacts with Phe679 forms ( $\pi$ - $\pi$  interaction) (angle = 31.1°, distance = 4.2 Å). With respect to electrostatic interactions, we found that an electropositive guanidinium side chain of Arg673 is located above Phe679, where the *para*-substituted halogen atoms can form favorable attractive interactions. The fluorine atom aligns with Arg673, and they work as counterparts. The corresponding residue in HDAC1 is Cys273, which is unfavorable to electrostatic interactions. The observed dramatic decrease in the potency of the *p*-chloro (**6v**) and *p*-bromo (**6w**) derivatives may be owed to the decreased electronegativity and effect of atom size.

To investigate the binding pose in the catalytic channel (Fig. 5D), the hydroxamate group forms a bidentate chelation with Zn ion, and the thiazole linker stacks in between Phe620 and Phe680. The orientation of thiazole was fixed to the sulfur atom of Ser568, which is a unique residue of HDAC6, and orients the cap group toward the small hydrophobic groove. In derivatives with longer linkers (n = 3C) such as **6c** and **6d**, the thiazole ring deviates from the space between two Phe residues, and the  $\pi$ - $\pi$  interaction cannot be formed (results not shown). The docking results therefore confirm that the optimal aliphatic chain length of m = n = 2 is important for the HDAC6 inhibitor to fit in the zinc-binding domain and cap group of the appropriate region. It especially confirms that m = trans-ethenyl is important for cap moiety localization to the small groove, which permits HDAC6 selectivity.

#### 2.4. Cellular assays of compound 6u

Western blot analysis was conducted to confirm HDAC6 inhibition of **6u** at cellular level, using cell extracts of HeLa and RAW 264.7 cells. respectively. We compared the effect of **6u** with that of the well-known HDAC6 inhibitor tubastatin A,<sup>24</sup> on tublin acetylation in HeLa cells (Fig. 6). Treatment with both compounds increased tubulin acetylation and equivalently increased histone acetylation, due to mild class I HDAC inhibition. These results suggest that the HDAC6 inhibitor ability of 6u is equivalent to that of tubastatin A in cells. Previous study results showed that the HDAC6 inhibitor 9a (see Fig. 1) suppresses the expression of LPS treatment-induced pro-inflammatory cytokines in RAW 264.7 cells, and has anti-sepsis effects in vivo.<sup>12,18</sup> To compare the cellular HDAC6 inhibition ability of 6u with that of the pan-HDAC inhibitor SAHA, we examined their effects on a-tubulin acetylation in RAW 264.7 cells.<sup>6</sup> As shown in Fig. 7, **6u** increases tubulin acetylation in a concentration-dependent manner. It also increased histone H3 acetylation, but at higher concentrations than SAHA.



Fig. 3. Compounds with alkane-alkene linker pairs in their m position, and their IC<sub>50</sub> values.

#### Bioorganic & Medicinal Chemistry xxx (xxxx) xxx-xxx



Fig. 4. Binding poses of 6e and 6u in the CDII of HDAC6 (PDB code 5EDU) generated using Schrödinger XP-Glide flexible docking. Rigidification of the cap group localized the cap moiety to a smaller hydrophobic groove. The Connolly surface of binding site was generated and rendered in opaque solid gray.

#### 2.5. Microsomal stability assay

To evaluate the *in vivo* stability of **6g** and **6u**, we performed microsomal stability assays in both mice and human microsomes. The residual concentration of **6g** in mice and human microsomes significantly decreased after 30 min, regardless of the presence of NADPH. In contrast, **6u** concentrations in human microsomes decreased negligibly, and it showed low intrinsic clearance (Table 2). Overall, **6u** has acceptable submicromolar cytotoxicity (result not shown) and human hepatic tolerance *in vitro*.

#### 3. Conclusion

Based on the structure of our lead HDAC6 inhibitor 9a, we designed 27 compounds for further optimization of its activity and selectivity. We aimed at optimizing the linker length, and the substituents and bond state of the cap group. HDAC1 and HDAC6 enzymatic assays were performed to evaluate the potency of their HDAC6 selectivity. The linker length optimized to m = n = 2 and cap moiety rigidification were critical for improving HDAC6 selectivity. In this study, only the *para*-fluoro phenyl cap in conjugation with the *trans*-ethenyl group (6u) was found to sustain nanomolar HDAC6 potency and increase HDAC6 selectivity over 126-fold. To establish the molecular basis for the HDAC6 selectivity of 6u, we performed intensive molecular docking analyses, using the structures of all available HDAC subtypes. Docking results suggested that cap rigidification permitted HDAC6 selectivity by localizing the cap group to the small hydrophobic groove, and interacting with unconserved residues in HDAC6. Although our scaffold is a classical linear hydroxamate pan-HDAC inhibitor like SAHA, with the manipulation of the aliphatic linker length and the substituent and simple rigidification of the aryl cap group, we constructed the novel HDAC6-selective scaffold 6u. Because compound 6u showed submicromolar cytotoxicity in HeLA cells (Data now shown) and negligibly metabolization in human microsomes, we are currently conducting in vivo therapeutic application of 6u in a mouse model of neurodegenerative disease, the results of which will be published in due course.

#### 4. Material and methods

#### 4.1. Chemistry

#### 4.1.1. General methods

Commercially available reagents were used without additional

purification, unless otherwise stated. All reactions were performed under an inert atmosphere (nitrogen). All melting points were obtained on an Electrothermal IA9000 series digital melting point apparatus and were uncorrected. Mass spectra (MS) were obtained with ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry, using electrospray ionization (ESI). The instrument consists of an Acquity UPLC system (Waters Co., Milford, MA, USA) coupled to a Waters Acquity Xevo G2 Q-TOF system (Waters Corp., Manchester, UK). Nuclear magnetic resonance spectra (<sup>1</sup>H and <sup>13</sup>C NMR) were obtained for  $CDCl_3$ ,  $CD_3OD$ , and  $DMSO-d_6$  on a Bruker Unity 300 MHz and Varian Unit 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to, respectively, residual CDCl<sub>3</sub>  $\delta_{\rm H}$  (7.26 ppm), CDCl<sub>3</sub>  $\delta_{\rm C}$  (77.0 ppm), CD<sub>3</sub>OD  $\delta_{\rm H}$  (3.31 ppm), CD<sub>3</sub>OD  $\delta_C$  (49.00 ppm), DMSO- $d_6 \delta_H$  (2.50 ppm), and DMSO- $d_6 \delta_C$ (39.5 ppm) as internal standards. Resonance patterns are reported with the notations s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are reported in hertz (Hz). For thin layer chromatography, Kieselgel 60 F254 (Merck) was coated on the plates, and E. Merck Kieselgel 60 (230-400 mesh) was used for flash column chromatography.

4.1.1.1. *Methyl* 5-*bromo-4-oxopentanoate*. To a solution of levulinic acid (10 g, 86.12 mmol) in methanol (80 mL), bromine (4.41 mL, 86.12 mmol) was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, and then for 2 h at 80 °C. The methanol was evaporated, and the solution was neutralized to pH 7 using saturated sodium bicarbonate. The aqueous layer was extracted using methylene chloride, and the organic layer was washed with water and brine, dried over magnesium sulfate and concentrated. The residue was purified using column chromatography (Hex:EtOAc = 5:1) to afford a pale-yellow liquid compound (8.8 g, 48% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.96 (s, 2H), 3.69 (s, 3H), 2.98–2.95 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  200.51, 172.78, 51.79, 34.39, 34.12, 27.99.

4.1.1.2. Methyl 6-bromo-5-oxohexanoate. To a solution of 4acetylbutyric acid (5 g, 38.42 mmol) in methanol (48 mL), bromine (1.98 mL, 38.42 mmol) was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, and then for 2 h at 80 °C. The solution was evaporated and neutralized to pH 7 using saturated sodium bicarbonate. The aqueous layer was extracted with methylene chloride, and the organic layer was washed with water and brine, dried over magnesium sulfate, and concentrated. The residue was purified



**Fig. 5.** Superposition of the cocrystalized TSA (yellow carbon) in HDAC6 (PDB id 5EDU) with the QPLD docking model **6u** (green carbon). (A) Overlay of the X-ray structures of HDAC subtypes. The two unique residues, Ser568 and Met 682 in HDAC6 CD2 are indicated. Ser568 locates between two Phe residues. In other HDACs, the Ser568 residue is conserved as Asp, and Met682 as Gly. (B) Top view showing two hydrophobic grooves with lipophilic potential at the binding pocket entrance. (C) Electrostatic potential surfaces around the pocket entrance and composing residues. The distance and angle plane between the cap group of **6u** and Phe679 are shown (figures are drawn in Schrödinger release 2018-2). (D) H-bond and  $\pi$ - $\pi$  interaction are represented as yellow and blue dashed lines, respectively. Zn ion, gray sphere.

using column chromatography (Hex:EtOAc = 5:1) to afford a compound as pale-yellow liquid (4.63 g, 54% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.87 (s, 2H), 3.67 (s, 3H), 2.77–2.66 (m, 2H), 2.40–2.35 (t, *J* = 15 Hz, 2H), 2.00–1.90 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  215.45, 201.31, 51.72, 38.66, 34.12, 32.79, 18.92.

4.1.2. General procedure for the preparation of thiazole  $esters^{21}$ 

To a solution of the appropriate thioamide (1 equiv.) in methanol

(0.16 mmol mL<sup>-1</sup>), bromo ester (1–1.5 equiv.) was added and stirred at 70 °C under reflux overnight. After the reaction was completed, methanol was removed under reduced pressure. The residue was extracted with ethyl acetate and washed with water and brine. The organic layer was dried with MgSO<sub>4</sub> and concentrated. The crude product was purified by flash chromatography on a silica gel (hexane–ethyl acetate) to obtain a thiazole compound.



Fig. 6. Western blotting results of the HDAC6 inhibition effect of 6u and tubastatin A in HeLa cells.

Bioorganic & Medicinal Chemistry xxx (xxxx) xxx-xxx



Fig. 7. Western blotting results of the HDAC6 inhibition effect of 6u and SAHA in RAW 264.7 cells.

4.1.2.1. Methyl 3-(2-phenethylthiazol-4-yl)propanoate (**5a**). Colorless oil in 52% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.30–7.16 (m, 5H), 6.82 (s, 1H), 3.80 (s, 3H), 3.30–2.25 (t, J = 15 Hz, 2H), 3.11–3.06 (m, 4H), 2.76–2.71 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 196.54, 196.37, 176.48, 160.52, 158.94, 152.36, 152.30, 140.78, 70.52, 51.79, 34.12, 32.63, 27.19.

4.1.2.2. Methyl-3-(2-benzylthiazol-4-yl)propanoate (**5b**). Colorless oil in 50% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.36–7.25 (m, 5H), 6.89 (s, 1H), 4.24 (s, 3H), 3.63 (s, 3H), 3.22–3.17 (t, J = 15 Hz, 2H), 3.00–2.95 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 200.51, 190.47, 170.97, 159.43, 156.32, 153.27, 152.64, 150.22, 140.53, 140.53, 51.79, 34.12, 27.19.

4.1.2.3. Methyl 4-(2-benzylthiazol-4-yl)butanoate (5c). Colorless oil in 51% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.34–7.23 (m, 5H), 6.83 (s, 1H), 4.36 (s, 2H), 3.69 (s, 3H), 3.24–3.19 (t, *J* = 15 Hz, 2H), 3.05–3.00 (t, *J* = 15 Hz, 2H), 2.86–2.76 (m,2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 200.51, 190.47, 170.97, 159.43, 156.32, 153.27, 152.64, 150.22, 140.53, 51.79, 34.47, 34.12, 27.19.

4.1.2.4. Methyl 4-(2-phenethylthiazol-4-yl)butanoate (**5d**). Colorless oil in 51% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) &: 7.34–7.20 (m, 5H), 6.79 (s, 1H), 3.72 (s, 3H), 3.43–3.38 (t, J = 15 Hz, 2H), 3.22–3.17 (t, J = 15 Hz, 2H), 3.04–2.99 (t, J = 15 Hz, 2H), 2.54–2.49 (t, J = 15 Hz, 2H), 2.2–2.10 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) &: 196.54, 196.37, 176.48, 160.52, 158.94, 152.36, 152.30, 140.78, 70.52, 51.79, 36.47, 34.12, 32.63, 27.19.

4.1.2.5. Methyl 3-(2-(4-fluorophenethyl)thiazol-4-yl)propanoate (5e). Yellow oil in 46% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.29–7.16 (m, 4H), 6.82 (s, 1H), 3.67 (s, 3H), 3.30–2.25 (t, J = 15 Hz, 2H), 3.11–3.06 (m, 4H), 2.76–2.71 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 196.52, 176.48, 160.52, 158.94, 152.36, 152.30, 140.78, 70.52, 51.79, 34.12, 32.63, 27.19.

4.1.2.6. Methyl 3-(2-(4-chlorophenethyl)thiazol-4-yl)propanoate (5f). Colorless oil in 65% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.26–7.24 (d, J = 8 Hz, 2H), 7.13–7.11 (d, J = 8 Hz, 2H), 6.78 (s, 1H), 3.68 (s, 3H), 3.26–3.22 (t, J = 15 Hz, 2H), 3.08–3.04 (m, 4H), 2.76–2.72 (t, J = 15 Hz, 2H).

4.1.2.7. Methyl 3-(2-(4-bromophenethyl)thiazol-4-yl)propanoate (5g). Colorless oil in 96% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.41–7.39 (d, J = 8 Hz, 2H), 7.08–7.06 (d, J = 8 Hz, 2H), 6.78 (s, 1H), 3.68 (s, 3H), 3.26–3.22 (t, J = 15 Hz, 2H), 3.09–3.03 (m, 4H), 2.75–2.71 (t, J = 15 Hz, 2H).

4.1.2.8. Methyl 3-(2-(4-methoxyphenethyl)thiazol-4-yl)propanoate (5h). Colorless oil in 95% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.13–7.11 (d, J = 8 Hz, 2H), 6.84–6.82 (d, J = 8 Hz, 2H), 6.78 (s, 1H), 3.79 (s, 3H), 3.69 (s, 3H), 3.25–3.21 (t, J = 15 Hz, 2H), 3.09–3.00 (m, 4H), 2.76–2.72 (t, J = 15 Hz, 2H).

4.1.2.9. Methyl 3-(2-(4-((tert-butoxycarbonyl)amino)phenethyl)thiazol-4yl)propanoate (5i). Colorless oil in 41% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.31–7.29 (d, J = 8 Hz, 2H), 7.11–7.08 (d, J = 8 Hz, 2H), 7.01 (s, 1H), 3.68 (s, 3H), 3.28–3.24 (t, J = 15 Hz, 2H), 3.05–3.00 (m, 4H), 2.75–2.71 (t, J = 15 Hz, 2H), 1.53 (s, 9H).

4.1.2.10. Methyl 3-(2-(4-cyanophenethyl)thiazol-4-yl)propanoate (5j). Colorless oil in 44% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.58–7.56 (d, J = 8 Hz, 2H), 7.30–7.28 (d, J = 8 Hz, 2H), 6.79 (s, 1H), 3.68 (s, 3H), 3.30–3.26 (t, J = 15 Hz, 2H), 3.18–3.14 (t, J = 15 Hz, 2H), 3.08–3.04 (t, J = 15 Hz, 2H), 2.75–2.71 (t, J = 15 Hz, 2H).

4.1.2.11. Methyl 3-(2-(4-(trifluoromethyl)phenethyl)thiazol-4-yl)propanoate (**5k**). Colorless oil in 74% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) & 7.55–7.53 (d, J = 8 Hz, 2H), 7.32–7.30 (d, J = 8 Hz, 2H), 6.80 (s, 1H), 3.68 (s, 3H), 3.30–3.26 (t, J = 15 Hz, 2H), 3.18–3.14 (t, J = 15 Hz, 2H), 3.09–3.05 (t, J = 15 Hz, 2H), 2.75–2.71 (t, J = 15 Hz, 2H).

4.1.2.12. Methyl 3-(2-(4-nitrophenethyl)thiazol-4-yl)propanoate (51). Colorless oil in 91% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.15–8.13 (d, J = 8 Hz, 2H), 7.35–7.33 (d, J = 8 Hz, 2H), 6.80 (s, 1H), 3.68 (s, 3H), 3.33–3.29 (t, J = 15 Hz, 2H), 3.23–3.20 (t, J = 15 Hz, 2H), 3.08–3.05 (t, J = 15 Hz, 2H), 2.75–2.71 (t, J = 15 Hz, 2H).

4.1.2.13. Methyl 3-(2-(3-fluorophenethyl)thiazol-4-yl)propanoate (5m). Colorless oil in 65% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.27–7.21 (m, 1H), 6.98–6.96 (d, J = 12 Hz, 1H), 6.92–6.88 (m,

Table 2

merosoniai stability assay results. The memore clearance and residual concentrations of verapanni, ou, and og after so min of meabatio	Microsomal stability	assay rest	sults. The intrinsic	clearance and re	sidual concentration	s of verapamil	, <b>6u</b> , and <b>6</b> g	after 30 min	of incubation
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Mouse								Human						
Compound	NADPH(-	-)	NADPH(+)			CL <sub>h,int</sub>	NADPH(-	NADPH(-) NADPH(+)				CL <sub>h,int</sub>		
	0 min	30 min	0 min	5 min	15 min	30 min	(µL/ IIII/ IIIg)	0 min	30 min	0 min	5 min	15 min	30 min	(µL/ IIIII/ IIIg)
Veraparmil 6u 6g	100.0% 100.0% 100.0%	99.3% 95.8% 79.5%	92.6% 106.9% 96.9%	52.1% 99.8% 83.0%	18.1% 68.6% 61.2%	N.D. 25.2% 33.8%	216.8 98.5 70.1	100.0% 100.0% 100.0%	96.2% 112.4% 66.4%	90.6% 106.3% 105.0%	61.6% 105.1% 81.8%	40.6% 103.4% 70.9%	13.0% 94.6% 38.3%	125.9 7.7 63.8

2H), 6.79 (s, 1H), 3.68 (s, 3H), 3.28–3.24 (t, J = 15 Hz, 2H), 3.11–3.05 (m, 4H), 2.76–2.72 (t, J = 15 Hz, 2H).

4.1.2.14. Methyl 3-(2-(3,4-difluorophenethyl)thiazol-4-yl)propanoate (5n). Colorless oil in 67% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.09–6.96 (m, 2H), 6.90–6.87 (m, 1H), 6.79 (s, 1H), 3.68 (s, 3H), 3.26–3.22 (t, J = 15 Hz, 2H), 3.09–3.03 (m, 4H), 2.75–2.71 (t, J = 15 Hz, 2H).

4.1.2.15. Methyl 3-(2-(3,4-dimethoxyphenethyl)thiazol-4-yl)propanoate (50). Colorless oil in 94% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 6.81–6.72 (m, 3H), 6.79 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.68 (s, 3H), 3.27–3.23 (t, J = 15 Hz, 2H), 3.10–3.01 (m, 4H), 2.76–2.72 (t, J = 15 Hz, 2H).

4.1.2.16. Methyl 3-(2-(3,4,5-trimethoxyphenethyl)thiazol-4-yl)propanoate (**5p**). Colorless oil in 83% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) &: 6.80 (s, 1H), 6.42 (s, 2H), 3.83 (s, 6H), 3.82 (s, 3H), 3.68 (s, 3H), 3.28–3.24 (t, J = 15 Hz, 2H), 3.10–3.01 (m, 4H), 2.76–2.72 (t, J = 15 Hz, 2H).

4.1.2.17. Methyl 3-(2-(2-(furan-2-yl)ethyl)thiazol-4-yl)propanoate (5q). Colorless oil in 42% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.33–7.32 (dd, J = 2, 0.8 Hz, 1H), 6.79 (s, 1H), 6.28–6.27 (dd, J = 3.2, 2 Hz, 1H), 6.02–6.01 (dd, J = 3.2, 2 Hz, 1H), 3.68 (s, 3H), 3.33–3.29 (t, J = 15 Hz, 2H), 3.13–3.05 (m, 4H), 2.76–2.72 (t, J = 15 Hz, 2H).

4.1.2.18. Methyl 3-(2-(2-(thiophen-2-yl)ethyl)thiazol-4-yl)propanoate (5r). Colorless oil in 75% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.15–7.13 (dd, J = 5.2, 1.2 Hz, 1H), 6.93–6.91 (dd, J = 5.2, 3.6 Hz, 1H), 6.83–6.82 (dd, J = 3.6, 1.2 Hz, 1H), 6.82 (s, 1H), 3.68 (s, 3H), 3.36–3.33 (m, 4H), 3.11–3.07 (t, J = 15 Hz, 2H), 2.78–2.74 (t, J = 15 Hz, 2H).

4.1.2.19. Methyl 3-(2-(2-(1H-indol-3-yl)ethyl)thiazol-4-yl)propanoate (5s). Red oil in 46% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.17 (s, 1H), 7.62–7.59 (d, J = 9 Hz, 1H), 7.37–7.34 (d, J = 9 Hz, 1H), 7.22–7.08 (m, 2H), 7.00 (s, 1H), 6.81 (s, 1H), 3.68 (s, 3H), 3.39–3.34 (t, J = 15 Hz, 2H), 3.24–3.19 (t, J = 15 Hz, 2H), 2.71–2.66 (t, J = 15 Hz, 2H), 2.24–2.19 (t, J = 15 Hz, 2H), 2.71–2.66 (t, J = 15 Hz, 2H), 2.24–2.19 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 210.78, 190.72, 162.48, 155.28, 154.32, 152.47, 150.35, 150.20, 138.79, 135.44, 129.87, 125.44, 50.62, 46.42, 35.29, 31.52, 26.49.

4.1.2.20. (*E*)-*Methyl* 3-(2-strylthiazol-4-yl)propanoate (5t). Colorless oil in 60% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) & 7.56–7.53 (d, J = 9 Hz, 2H), 7.43–7.24 (m, 5H), 6.90 (s, 1H), 3.65 (s, 3H), 3.14–3.09 (t, J = 15 Hz, 2H), 2.81–2.76 (t, J = 9 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) & 172.64, 168.93, 158.27, 137.22, 136.11, 130.29, 128.35, 121.95, 114.92, 51.27, 33.25, 31.52, 26.49.

4.1.2.21. (E)-Methyl 3-(2-(4-fluorostyryl)thiazol-4-yl)propanoate (**5u**). Yellow oil in 44% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.54–7.51 (d, *J* = 9 Hz, 2H), 7.32–7.18 (m, 4H), 6.90 (s, 1H), 3.65 (s, 3H), 2.81–2.76 (t, *J* = 15 Hz, 2H), 2.63–2.58 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 172.64, 158.27, 137.22, 136.11, 130.29, 128.35, 121.95, 114.92, 51.27, 33.25, 31.52, 26.49.

4.1.2.22. Methyl 3-(2-(4-chlorostyryl)thiazol-4-yl)propanoate (5v). Colorless oil in 46% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) & 7.34–7.29 (d, J = 8 Hz, 2H), 7.13–7.11 (d, J = 8 Hz, 2H), 6.92 (s, 1H), 3.68 (s, 3H), 3.26–3.22 (t, J = 15 Hz, 2H), 3.08–3.04 (m, 4H), 2.76–2.72 (t, J = 15 Hz, 2H).

4.1.2.23. (E)-Methyl 3-(2-(4-bromostyryl)thiazol-4-yl)propanoate (5w). Colorless oil in 32% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.54–7.47 (m, 2H), 7.43–7.18 (m, 5H), 6.90 (s, 1H), 3.69 (s, 3H), 3.11 (t, J = 7.4 Hz, 2H), 2.78 (t, J = 7.5 Hz, 2H).

4.1.2.24. (*E*)-Methyl 3-(2-(2-(1*H*-indol-3-yl)vinyl)thiazol-4-yl)propanoate (**5x**). Red oil in 44% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) & 8.31 (bs, 1H), 7.67–7.65 (d, J = 6 Hz, 2H), 7.62–7.59 (d, J = 9 Hz, 1H), 7.36–7.33 (d, J = 9 Hz, 1H), 7.21–7.08 (m, 2H), 6.89 (s, 1H), 6.79 (s, 1H), 3.68 (s, 3H), 2.82–2.77 (t, J = 15 Hz, 2H), 2.39–2.34 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) & 200.98, 195.46, 175.81, 160.42, 154.52, 150.99, 149.34, 142.65, 140.23, 138.48, 136.57, 120.48, 114.73, 113.28, 50.64, 35.29, 31.52.

#### 4.1.3. General procedure for the preparation of hydroxamate<sup>22</sup>

To a solution of the appropriate thiazole ester (1 equiv.) in methanol (0.1 mmol mL<sup>-1</sup>), hydroxylamine hydrochloride (2 equiv.) and 8 *N*-sodium hydroxide (0.5 mmol mL<sup>-1</sup>) were added at 0 °C. The reaction mixture was stirred for 1.5 h at room temperature. After the reaction was completed, methanol was removed under reduced pressure. The residue was extracted with ethyl acetate and washed with water and brine. The organic layer was dried with MgSO<sub>4</sub> and concentrated. The crude product was recrystallized with ethyl acetate or methylene chloride.

4.1.3.1. *N*-Hydroxy-3-(2-phenethylthiazol-4-yl)propanamide (**6a**). White solid in 19% yield. mp 124.4–125.3 °C. HRMS: calcd for  $C_{14}H_{16}N_2O_2S$  [M – H]<sup>+</sup> 275.0862, found 275.0854. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ) &: 7.32–7.18 (m, *J* = 15 , 9 Hz, 5H), 6.78 (s, 1H), 3.30–2.25 (t, *J* = 15 Hz, 2H), 3.11–3.06 (m, 4H), 2.76–2.71 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ ) &: 196.54, 196.37, 176.48, 160.52, 158.94, 152.36, 152.30, 140.78, 70.52, 34.12, 32.63, 27.19.

4.1.3.2. *N*-Hydroxy-3-(2-benzylthiazol-4-yl)propanamide (**6b**). White solid in 17% yield. mp 135.1–135.3 °C. HRMS: calcd for  $C_{13}H_{14}N_2O_2S$  [M – H]<sup>+</sup> 261.0705, found 261.0698. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 7.32–7.21 (m, *J* = 15, 6 Hz, 5H), 6.76 (s, 1H), 4.24 (s, 2H), 2.83–2.78 (t, *J* = 15 Hz, 2H); 2.39–2.34 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 200.51, 190.47, 170.97, 159.43, 156.32, 153.27, 152.64, 150.22, 140.53, 140.53, 34.12, 31.14.

4.1.3.3. *N*-Hydroxy-4-(2-benzylthiazol-4-yl)butanamide (6c). White solid in 19% yield. mp 137.3–137.6 °C. HRMS: calcd for  $C_{14}H_{16}N_2O_2S$  [M – H]<sup>+</sup> 275.0834, found 275.0647. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 7.31–7.20 (m, *J* = 15, 6 Hz, 5H), 6.75 (s, 1H), 4.25 (s, 2H), 2.82–2.77 (t, *J* = 15 Hz, 2H), 2.39–2.34 (t, *J* = 15 Hz, 2H), 2.10–2.00 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 200.51, 190.47, 170.97, 159.43, 156.32, 153.27, 152.64, 150.22, 140.53, 140.53, 34.47, 34.12, 27.19.

4.1.3.4. *N*-Hydroxy-4-(2-phenethylthiazol-4-yl)butanamide (6d). White solid in 22% yield. mp 154.7–155.3 °C. HRMS: calcd for  $C_{15}H_{18}N_2O_2S$  [M – H]<sup>+</sup> 289.1146, found 289.1137. <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) &: 7.31–7.20 (m, *J* = 15, 9 Hz, 5H), 6.75 (s, 1H), 3.31–2.26 (t, *J* = 15 Hz, 2H), 3.12–3.07 (t, *J* = 15 Hz, 9 Hz, 5H), 2.82–2.77 (t, *J* = 15 Hz, 2H), 2.39–2.34 (t, *J* = 15 Hz, 2H), 2.09–1.99 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) &: 196.54, 196.37, 176.48, 160.52, 158.94, 152.36, 152.30, 140.78, 70.52, 51.79, 36.47, 34.12, 32.63, 27.19.

4.1.3.5. *N*-Hydroxy-3-(2-(4-fluorophenethyl)thiazol-4-yl)propanamide (**6e**). Light yellow solid in 32% yield. mp 118.4–118.9 °C. HRMS: calcd for  $C_{14}H_{16}FN_2O_2S$  [M+H]<sup>+</sup> 295.0918, found 295.0917. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ) & 7.32–7.19 (m, 4H), 6.78 (s, 1H), 3.30–3.25 (t, *J* = 15 Hz, 2H), 3.11–3.06 (m, 4H), 2.76–2.71 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ ) & 196.54, 176.48, 160.52, 158.94, 152.36, 152.30, 140.78, 70.52, 34.12, 32.63, 27.19.

4.1.3.6. *N*-Hydroxy-3-(2-(4-chlorophenethyl)thiazol-4-yl)propanamide (**6***f*). White solid in 38% yield. mp 160.6–162.5 °C. HRMS: calcd for  $C_{14}H_{14}ClN_2O_2S$  [M-H]<sup>+</sup> 309.0475, found 309.0465. <sup>1</sup>H NMR

(400 MHz, DMSO- $d_6$ )  $\delta$ : 7.28–7.26 (d, J = 8 Hz, 2H), 7.19–7.17 (d, J = 8 Hz, 2H), 7.02 (s, 1H), 3.29–3.26 (t, J = 15 Hz, 2H), 3.09–3.05 (t, J = 15 Hz, 2H), 3.06–3.02 (t, J = 15 Hz, 2H), 2.50–2.46 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 170.41, 170.25, 154.53, 138.93, 131.76, 129.80, 128.13, 113.41, 34.92, 34.06, 31.87, 26.52.

#### 4.1.3.7. N-Hydroxy-3-(2-(4-bromophenethyl)thiazol-4-yl)propanamide

(**6***g*). White solid in 55% yield. mp 159.3–161.3 °C. HRMS: calcd for  $C_{14}H_{14}BrN_2O_2S$  [M – H]<sup>+</sup> 352.9960, found 352.9959. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 7.47–7.45 (d, *J* = 8 Hz, 2H), 7.23–7.21 (d, *J* = 8 Hz, 2H), 7.08 (s, 1H), 3.24–3.20 (t, *J* = 15 Hz, 2H), 3.02–2.98 (t, *J* = 15 Hz, 2H), 2.90–2.86 (t, *J* = 15 Hz, 2H), 2.35–2.31 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 168.70, 155.41, 140.33, 131.59, 131.25, 119.65, 113.60, 34.75, 34.34, 32.21, 27.28.

4.1.3.8. *N*-Hydroxy-3-(2-(4-methoxyphenethyl)thiazol-4-yl)propanamide (**6**h). White solid in 64% yield. mp 140.4–142.5 °C. HRMS: calcd for  $C_{15}H_{17}N_2O_3S$  [M – H]<sup>+</sup> 305.0965, found 305.0960. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 7.16–7.14 (d, J = 8 Hz, 2H), 7.05 (s, 1H), 6.88–6.86 (d, J = 8 Hz, 2H), 3.80 (s, 3H), 3.30–3.27 (t, J = 15 Hz, 2H), 3.10, 3.03 (m, 4H), 2.53–2.50 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 174.95, 174.18, 162.26, 158.31, 136.01, 133.02, 117.43, 117.24, 58.17, 38.83, 38.55, 35.81, 30.45.

#### 4.1.3.9. N-Hydroxy-3-(2-(4-aminophenethyl)thiazol-4-yl)propanamide

(*6i*). White solid in 28% yield. mp 154.2–155.1 °C. HRMS: calcd for  $C_{14}H_{16}N_3O_2S$  [M – H]<sup>+</sup> 290.0971, found 290.0963. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) & 6.88 (s, 1H), 6.84–6.82 (d, *J* = 8 Hz, 2H), 6.56–6.54 (d, *J* = 8 Hz, 2H), 3.11–3.07 (t, *J* = 15 Hz, 2H), 2.93–2.89 (t, *J* = 15 Hz, 2H), 2.84–2.80 (t, *J* = 15 Hz, 2H), 2.37–2.33 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-*d*<sub>6</sub>) & 171.27, 170.25, 154.29, 145.40, 129.82, 128.74, 115.55, 113.26, 35.09, 34.78, 31.89, 26.50.

#### 4.1.3.10. N-Hydroxy-3-(2-(4-cyanophenethyl)thiazol-4-yl)propanamide

(6j). White solid in 29% yield. mp 149.5–152.4 °C. HRMS: calcd for  $C_{15}H_{14}N_3O_2S$  [M – H]<sup>+</sup> 300.0805, found 300.0807. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) & 7.76–7.74 (d, J = 8 Hz, 2H), 7.48–7.46 (d, J = 8 Hz, 2H), 7.09 (s, 1H), 3.29–3.25 (t, J = 15 Hz, 2H), 3.14–3.10 (t, J = 15 Hz, 2H), 2.90–2.86 (t, J = 15 Hz, 2H), 2.35–2.31 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ) & 170.23, 169.86, 154.66, 146.24, 131.98, 129.36, 118.41, 113.50, 109.79, 35.50, 33.50, 31.84, 26.50.

#### 4.1.3.11. N-Hydroxy-3-(2-(4-(trifluoromethyl)phenethyl)thiazol-4-yl)

propanamide (**6k**). White solid in 63% yield. mp 140.4–142.5 °C. HRMS: calcd for  $C_{15}H_{14}F_{3}N_2O_2S$  [M–H]<sup>+</sup> 343.0735, found 343.0728. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) & 7.59–7.57 (d, J = 8 Hz, 2H), 7.42–7.40 (d, J = 8 Hz, 2H), 7.03 (s, 1H), 3.35–3.31 (t, J = 15 Hz, 2H), 3.20–3.16 (t, J = 15 Hz, 2H), 3.07–3.03 (t, J = 15 Hz, 2H), 2.50–2.46 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ) & 170.23, 170.13, 154.61, 144.84, 144.83, 128.86, 124.96, 113.44, 35.30, 33.78, 31.85, 26.53.

#### 4.1.3.12. N-Hydroxy-3-(2-(4-nitrophenethyl)thiazol-4-yl)propanamide

(6l). White solid in 44% yield. mp 163.2–164.1 °C. HRMS: calcd for  $C_{14}H_{14}N_3O_4S$  [M – H]<sup>+</sup> 320.0715, found 320.0705. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) & 8.16–8.14 (d, J = 8 Hz, 2H), 7.56–7.54 (d, J = 8 Hz, 2H), 7.10 (s, 1H), 3.32–3.28 (t, J = 15 Hz, 2H), 3.20–3.16 (t, J = 15 Hz, 2H), 2.90–2.86 (t, J = 15 Hz, 2H), 2.35–2.31 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ) & 168.62, 155.49, 149.31, 146.53, 130.32, 123.86, 113.73, 35.06, 33.86, 32.20, 27.28.

#### 4.1.3.13. N-Hydroxy-3-(2-(3-fluorophenethyl)thiazol-4-yl)propanamide

(6m). White solid in 62% yield. mp 144.9–145.7 °C. HRMS: calcd for  $C_{14}H_{14}FN_2O_2S$  [M – H]<sup>+</sup> 293.0764, found 293.0760. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 7.31–7.26 (m, 1H), 7.03 (s, 1H), 7.01–6.91 (m, 3H), 3.31–3.27 (t, *J* = 15 Hz, 2H), 3.12–3.08 (t, *J* = 15 Hz, 2H),

3.06–3.02 (t, J = 15 Hz, 2H), 2.50–2.46 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ) & 170.39, 170.26, 164.15, 161.73, 154.54, 143.03, 142.95, 129.82, 129.74, 124.07, 124.04, 114.93, 114.72, 113.39, 112.77, 112.55, 35.28, 33.96, 31.88, 26.54.

#### 4.1.3.14. N-Hydroxy-3-(2-(3,4-difluorophenethyl)thiazol-4-yl)

propanamide (6n). White solid in 36% yield. mp 143.9–145.6 °C. HRMS: calcd for  $C_{14}H_{13}F_2N_2O_2S$  [M-H]<sup>+</sup> 311.0670, found 311.0666. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 7.18–7.11 (m, 2H), 7.03 (s, 1H), 7.03–6.98 (m, 1H), 3.30–3.26 (t, *J* = 15 Hz, 2H), 3.09–3.05 (t, *J* = 15 Hz, 2H), 3.06–3.02 (t, *J* = 15 Hz, 2H), 2.50–2.46 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 170.24, 170.14, 154.60, 124.64, 124.61, 124.58, 124.55, 117.04, 116.87, 116.81, 116.63, 113.42, 34.66, 33.95, 31.86, 26.53.

#### 4.1.3.15. N-Hydroxy-3-(2-(3,4-dimethoxyphenethyl)thiazol-4-yl)

propanamide (60). White solid in 66% yield. mp 135.6–136.8 °C. HRMS: calcd for  $C_{16}H_{19}N_2O_4S$   $[M-H]^+$  335.1066, found 335.1066. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) &: 7.02 (s, 1H), 6.87–6.85 (d, J = 8 Hz, 1H), 6.80–6.79 (d, J = 4 Hz, 1H), 6.75–6.73 (dd, J = 8, 1.6 Hz, 1H), 3.80 (s, 3H), 3.80 (s, 3H), 3.28–3.25 (t, J = 15 Hz, 2H), 3.06–3.00 (m, 4H), 2.50–2.46 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ) &: 170.97, 170.24, 154.41, 148.99, 147.66, 133.07, 120.48, 113.31, 112.16, 111.74, 55.12, 55.03, 35.32, 34.52, 31.87, 26.52.

#### 4.1.3.16. N-Hydroxy-3-(2-(3,4,5-trimethoxyphenethyl)thiazol-4-yl)

propanamide (**6p**). White solid in 44% yield. mp 150.3–150.6 °C. HRMS: calcd for  $C_{17}H_{21}N_2O_5S$  [M–H]<sup>+</sup> 365.1173, found 365.1171. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) & 7.10 (s, 1H), 6.51 (s, 2H), 3.74 (s, 6H), 3.61(s, 3H), 3.25–3.21 (t, *J* = 15 Hz, 2H), 2.97–2.93 (t, *J* = 15 Hz, 2H), 2.91–2.88 (t, *J* = 15 Hz, 2H), 2.36–2.32 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-*d*<sub>6</sub>) & 169.46, 168.73, 155.40, 153.17, 136.59, 136.24, 113.52, 106.14, 60.42, 56.24, 35.99, 34.77, 32.22, 27.31.

4.1.3.17. *N*-Hydroxy-3-(2-(2-(furan-2-yl)ethyl)thiazol-4-yl)propanamide (**6***q*). White solid in 33% yield. mp 127.0–128.1 °C. HRMS: calcd for  $C_{12}H_{13}N_2O_3S [M-H]^+$  265.0656, found 265.0647. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) &: 7.53–7.52 (dd, *J* = 2, 0.8 Hz, 1H), 7.11 (s, 1H), 6.35–6.34 (dd, *J* = 3.2, 2 Hz 1H), 6.14–6.13 (dd, *J* = 3.2, 0.8 Hz, 1H), 3.26–3.23 (t, *J* = 15 Hz, 2H), 3.06–3.03 (t, *J* = 15 Hz, 2H), 2.90–2.86 (t, *J* = 15 Hz, 2H), 2.35–2.31 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-*d*<sub>6</sub>) &: 168.70, 155.42, 154.19, 142.02, 113.73, 110.88, 106.39, 32.18, 31.59, 27.91, 27.18.

#### 4.1.3.18. N-Hydroxy-3-(2-(2-(thiophen-2-yl)ethyl)thiazol-4-yl)

propanamide (**6***r*). White solid in 56% yield. mp 122.9–123.2 °C. HRMS: calcd for  $C_{12}H_{13}N_2O_2S_2$  [M – H]<sup>+</sup> 281.0417, found 281.0418. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) &: 7.32–7.31 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.11 (s, 1H), 6.94–6.92 (dd, *J* = 4.8, 3.2 Hz 1H), 6.90–6.89 (dd, *J* = 3.2, 1.2 Hz, 1H), 3.27–3.25 (m, 4H), 2.90–2.86 (t, *J* = 15 Hz, 2H), 2.35–2.31 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-*d*<sub>6</sub>) &: 168.69, 168.65, 155.44, 143.18, 127.36, 125.64, 124.45, 113.74, 35.04, 32.22, 29.63, 27.31.

#### 4.1.3.19. N-Hydroxy-3-(2-(2-(1H-indol-3-yl)ethyl)thiazol-4-yl)

propanamide (6s). Light red solid in 21% yield. mp 129.3–131.2 °C. HRMS: calcd for  $C_{16}H_{16}N_3O_2S$  [M – H]<sup>+</sup> 312.0814, found 312.0807. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ) & 8.17 (s, 1H), 7.62–7.59 (d, J = 9 Hz, 1H), 7.37–7.34 (d, J = 9 Hz, 1H), 7.22–7.08 (m, 2H), 6.98 (s, 1H), 6.73 (s, 1H), 3.41–3.36 (t, J = 15 Hz, 2H), 3.27–3.22 (t, J = 15 Hz, 2H) 2.82–2.77 (t, J = 15 Hz, 2H), 2.39–2.34 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ ) & 210.78, 190.72, 162.48, 155.28, 154.32, 152.47, 150.35, 150.20, 138.79, 135.44, 129.87, 125.44, 50.64, 46.42, 35.29, 31.52, 26.49.

4.1.3.20. N-Hydroxy-(E)-3-(2-styrylthiazol-4-yl)propanamide

(*6t*). White solid in 20% yield. mp 166.9–167.8 °C. HRMS: calcd for  $C_{14}H_{15}N_2O_2S$  [M+H]<sup>+</sup> 275.0661, found 275.0643. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) &: 7.54–7.51 (d, *J* = 9 Hz, 2H), 7.41–7.22 (m, 5H), 6.88 (s, 1H), 3.14–3.09 (t, *J* = 15 Hz, 2H), 2.81–2.76 (t, *J* = 9 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) &: 172.64, 168.93, 158.27, 137.22, 136.11, 130.29, 128.35, 121.95, 114.92, 33.25, 31.52, 26.49.

#### 4.1.3.21. N-Hydroxy-(E)-3-(2-(4-fluorostyryl)thiazol-4-yl)propanamide

(*6u*). Light yellow solid in 28% yield. mp 154.7–155.3 °C. HRMS: calcd for  $C_{14}H_{14}FN_2O_2S$  [M – H]<sup>+</sup> 291.0615, found 291.0604. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 7.54–7.51 (d, *J* = 9 Hz, 2H), 7.32–7.18 (m, 4H), 6.90 (s, 1H), 2.75–2.70 (t, *J* = 15 Hz, 2H), 2.52–2.47 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 172.64, 158.27, 137.22, 136.11, 130.29, 128.35, 121.95, 114.92, 33.25, 31.52, 26.49.

#### 4.1.3.22. N-Hydroxy-(E)-3-(2-(4-chlorostyryl)thiazol-4-yl)propanamide

(*6ν*). Yellow solid in 27% yield. mp 176.2–176.8 °C. HRMS: calcd for  $C_{14}H_{12}Cln_2O_2S$  [M–H]<sup>+</sup> 307.0311, found 307.0308. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 10.05–10.35 (m, 1H), 8.85–8.61 (m, 1H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.45 (d, *J* = 2.8 Hz, 2H), 7.28 (s, 3H), 3.00–2.88 (m, 2H), 2.40–2.31(m, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 174.08, 165.69, 156.97, 135.11, 133.61, 132.38, 129.35, 129.30, 122.64, 114.75, 33.48, 26.86.

#### 4.1.3.23. N-Hydroxy-(E)-3-(2-(4-bromostyryl)thiazol-4-yl)propanamide

(*6w*). Yellow solid in 21% yield. mp 172.4–172.6 °C. HRMS: calcd for  $C_{14}H_{12}BrN_2O_2S$  [M-H]<sup>+</sup> 352.9791, found 352.9769. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 10.07–10.36 (m, 1H), 8.87–8.67 (m, 1H), 7.73–7.22 (m, 7H), 2.94 (t, *J* = 7.6 Hz, 2H), 2.37 (t, *J* = 7.7 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 168.00, 165.53, 157.13, 135.44, 132.40, 132.25, 129.62, 122.66, 114.84, 32.18, 27.34

#### 4.1.3.24. N-Hydroxy-(E)-3-(2-(2-(1H-indol-3-yl)vinyl)thiazol-4-yl)

propanamide (**6**x). Light red solid in 20% yield. mp 169.2–171.4 °C. HRMS: calcd for  $C_{16}H_{14}N_3O_2S$  [M – H] <sup>+</sup> 312.0815, found 312.0807. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ) & 8.31 (bs, 1H), 7.67–7.65 (d, J = 6 Hz, 2H), 7.62–7.59 (d, J = 9 Hz, 1H), 7.36–7.33 (d, J = 9 Hz, 1H), 7.21–7.08 (m, 2H), 6.89 (s, 1H), 6.79 (s, 1H), 2.82–2.77 (t, J = 15 Hz, 2H), 2.46–2.41 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (100 MHz, DMSO- $d_6$ ) & 200.98, 195.46, 175.81, 160.42, 154.52, 150.99, 149.34, 142.65, 140.23, 138.48, 136.57, 120.48, 114.73, 113.28, 35.29, 31.52.

#### 4.1.4. General procedure for **6h'**, **60'**, and **6p'** synthesis<sup>26</sup>

To a solution of the appropriate methoxyphenethyl thiazole (**6h**, **6o**, and **6p**) (1 equiv.) in methylene chloride ( $0.1 \text{ mmol mL}^{-1}$ ), boron tribromide (1.5 equiv) was added dropwise at 0 °C, and the reaction mixture was stirred for 10–30 min at room temperature. After the reaction was completed, the reaction mixture was cooled to 0 °C and quenched with methanol. Methanol was then removed under reduced pressure, and the residue was recrystallized with acetonitrile or methylene chloride.

## 4.1.4.1. N-Hydroxy-3-(2-(4-hydroxyphenethyl)thiazol-4-yl)propanamide (6h'). White solid in 32% yield. mp 156.3–157.1 °C. HRMS: calcd for

(67). White solid in 32% yield, mp 156.3–157.1 C. HRMS: calcd for  $C_{14}H_{15}N_2O_3S$  [M-H]<sup>+</sup> 291.0872, found 291.0837. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) & 7.30 (s, 1H), 7.04–7.02 (d, J = 8 Hz, 2H), 6.69–6.67 (d, J = 8 Hz, 2H), 3.31–3.27 (t, J = 15 Hz, 2H), 2.95–2.90 (m, 4H), 2.39–2.35 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ ) & 171.97, 168.35, 156.24, 152.11, 130.24, 129.82, 115.65, 115.61, 34.29, 33.91, 31.70, 25.85.

#### 4.1.4.2. N-Hydroxy-3-(2-(3,4-dihydroxyphenethyl)thiazol-4-yl)

propanamide (**60'**). White solid in 28% yield. mp 162.3–162.6 °C. HRMS: calcd for  $C_{14}H_{14}N_2O_4S$   $[M-H]^+$  307.0757, found 307.0753. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) & 7.32 (s, 1H), 6.65–6.62 (m, 2H), 6.49–6.47 (d, J = 8 Hz, 1H), 3.29–3.25 (t, J = 15 Hz, 2H), 2.96–2.93 (t,

J = 15 Hz, 2H), 2.88–2.84 (t, J = 15 Hz, 2H), 2.40–2.36 (t, J = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 172.08, 168.32, 151.97, 145.58, 144.18, 130.91, 119.54, 116.29, 115.99, 115.56, 34.69, 33.86, 31.69, 25.82.

#### 4.1.4.3. N-Hydroxy-3-(2-(3,4,5-trihydroxyphenethyl)thiazol-4-yl)

propanamide (**6**p'). White solid in 33% yield. mp 165.2–166.3 °C. HRMS: calcd for  $C_{14}H_{15}N_2O_5S$  [M–H]<sup>+</sup> 323.0813, found 323.0854. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) &: 7.36 (s, 1H), 6.14 (s, 2H), 3.27–3.23 (t, *J* = 15 Hz, 2H), 2.96–2.93 (t, *J* = 15 Hz, 2H), 2.80–2.76 (t, *J* = 15 Hz, 2H), 2.66–2.63 (t, *J* = 15 Hz, 2H); <sup>13</sup>C NMR (400 MHz, DMSO-d<sub>6</sub>) &: 173.84, 172.33, 151.50, 146.50, 131.86, 130.15, 115.76, 107.59, 34.89, 33.70, 33.04, 25.

#### 4.2. Biology and computational studies

#### 4.2.1. Compounds and reagents

SAHA (SML0061), tubastatin A (SML0044), and anti- $\beta$ -actin antibody (a mouse monoclonal antibody) (A1978) were purchased from Sigma-Aldrich. Antibodies for acetyl K40  $\alpha$ -tubulin (ab179484),  $\alpha$ -tubulin (ab52866), and histone H3 (ab1791) were purchased from Abcam. Rabbit polyclonal anti-acetyl-histone H3 antibody (06-599) was purchased from Millipore. Secondary anti-rabbit and anti-mouse antibodies labeled with horseradish peroxide (sc-2030 and sc-2005) were purchased from Santa Cruz Biotechnology. DMEM high glucose media (SH30243.01), FBS (SH30084.03), and penicillin-streptomycin solution (SV30010) were purchased from Hyclone. Other chemicals and reagents were obtained from Sigma-Aldrich, unless otherwise mentioned.

#### 4.2.2. Ligand preparation for docking

Docking study were carried out by  $\text{Glide}^{27}$  in Schrödinger maestro  $11^{28}$  (operated under CentOS release 7 and windows 10) and Sybyl X 2.1.1<sup>29</sup> (operated under windows 7) software. In Schrödinger, sketched ligands were prepared using the LigPrep module. Ligands were charged and minimized using the OPLS3 force field. We set out to generate possible ionized states at pH 5–9, check metal binding state, and include original state options, to generate neutral and deprotonated states. All generated states were used for docking. In Sybyl, ligands were sketched in neutral states and energy minimized using the conjugated gradient method in the Tripos force field with the Gasteiger-Hückel charge method, until a convergence value of 0.001 kcal Å<sup>-1</sup> mol<sup>-1</sup>.

#### 4.2.3. Preparation of protein structures

The crystal structures of human HDAC1 (PDB: 4BKX) and HDAC6 (PDB: 5EDU) were downloaded from RCSB Protein Data Bank (https:// www.rcsb.org/). Downloaded structures were refined as follows: in Schrödinger, 'Protein Preparation' module was used. Missing side chains and loops were filled in using Prime, and metal ions included in protein were set to create zero-order bonds. For final refinements, Hbond assessment and restrained minimization were conducted in default option. In Sybyl, human HDAC6 (PDB ID: 5EDU) was prepared using 'Protein Preparation' module. All water molecules and unnecessary molecules were removed. Ligands were extracted and charged with Gasteiger-Hückel charge, and Kollman-all charge was used for protein.

#### 4.2.4. Glide docking

Grids were generated using receptor grid generation module from prepared protein structures. Center of grids were calculated from cocrystalized ligands or nearby residues. Grid box sizes were automatically defined or defined as 15 Å if the size was too small. Generated grids were used for both XP glide<sup>30</sup> and QPLD docking<sup>31</sup>. XP docking was proceeded in default setting and gerated 10 poses per ligand for comparison. For QPLD, precision setting of initial docking, and XP was used for redocking.

#### G. Nam, et al.

#### 4.2.5. Surflex-dock docking

The docking was conducted using prepared HDAC6 crystal structure, using Docking suite module. Protomol was generated with cocrystalized TSA structure as the template, with the threshold to 0.50 and bloat to 5. The Cscore calculation option was checked, and maximum output poses was set to 50. Only poses with Cscore > 3 were considered as docking hits.

#### 4.2.6. HDAC enzymatic assay

The inhibition activity of all the synthesized **9a** derivatives and the reference compounds against HDAC1 and HDAC6 were evaluated using BPS bioscience assay kits (Fluorogenic HDAC1 assay kit [50061] and Fluorogenic HDAC6 [50076]). Enzymatic assays were conducted in accordance with the manufacturer's protocols. After a reaction, half of the reactant volume (50 µl) was loaded into white 96-well plates (Costar 4693). Fluorescence was detected using a Glomax-Multi plus detection system from Promega, at an excitation wavelength range of 350-380 nm, and emission range of 440-470 nm. We designed the final concentration of DMSO to be 0.1 vol% in reaction volume to minimize the effects of DMSO.<sup>24</sup> For the reactions, all mixtures were incubated in a 37 °C water bath for 30 min, and at room temperature for 15 min after adding 50 µl of HDAC developer. All assays were repeated at least three times. Relative enzyme activity was calculated using Microsoft Excel. Dose-response curves and  $IC_{50}$  values were generated using the GraphPad Prism v5 software.

#### 4.2.7. Cell culture

Raw 264.7 and HeLa cells were purchased from the Korea Cell Line Bank and cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution. All cell lines were grown at 37 °C in a humidified incubator with an atmosphere of 95% air and 5%  $CO_2$ .

#### 4.2.8. Immunoblotting

Raw 264.7 and HeLa cells were seeded in 6-well plates (30006, SPL life science) at a density of  $2.5 \times 10^5$ /mL and incubated for 24 h. After washing with PBS, the drug-diluted media were replaced, and they were again incubated for 24 h. 6u, SAHA, and tubastatin A at concentrations of 0.3-3 µM and 0.1-10 µM were used to treat Raw 264.7 and HeLa cells, respectively. The final DMSO concentration was 0.1 vol % of reaction volume, in all control and drug-treated wells. Proteins in the Raw 264.7 cells were harvested using Ripa buffer (R0278, Sigma-Aldrich) and the EpiQuik<sup>TM</sup> nuclear extraction kit I (OP-0002, Epigentek), and the Nuclear extract kit (Active motif 40010) was used to extract proteins in the HeLa cells, following the manufacturer's protocols. These proteins were quantified using the Bradford assay (Biorad 500-0006). Protein samples were electrophoresed on 10 and 15% SDS-polyacrylamide gels, transferred onto PVDF membranes, and blocked with 8% skim milk in TBST (10 mM Tris-HCl, pH 7.5, 150 nM NaCl and 0.1% Tween 20) overnight. They were then probed with various concentrations of primary antibodies diluted in TBST for 2 h at room temperature, per the manufacturer's instructions. Various concentrations of secondary antibodies labeled with horseradish peroxidase and diluted with TBST (dilution ration, 1:4000) for 2 h at room temperature, were used. The immune-reactive bands were obtained using the enhanced chemiluminescence (ECL) (ECL-PS250, Dongin LS) system detection kit with a ChemiDOC imaging system (Dacinchchemi™). Acetyl α-tubulin, α-tubulin, and β-actin were detected in single membranes by repeating antibody detach using EzReprobe (WSE-7240, ATTO), and blocking. Acetyl histone H3 and histone H3 were also detected in single membranes using the same protocol.

#### 4.2.9. Microsomal stability assay

The microsomal assay was conducted as previously reported.<sup>32</sup> Various concentrations of the reaction solutions were prepared. Each reaction volume contained  $1 \mu M$  of the test (**6u** and **6g**) or reference

compound. (  $\pm$ )-Verapamil hydrochloride, 1 nM NADPH, 0.5 mg/ml total protein of microsomes (Human liver microsomes ultra pooled donor: Corning Gentest<sup>™</sup>, 452117; mouse liver microsome and CD-1 mice: Corning Gentest<sup>™</sup> 452701), and 0.1 M phosphate buffer (pH 7.4). The incubation plates were then incubated at 37 °C for 0, 5, 15, and 30 min respectively. Additionally, assays without NADPH were used to evaluate metabolic stability in the absence of NADPH at 37 °C for 0 and 30 min. The reaction was terminated by adding acetonitrile. The reacted solution was transferred onto a filter plate and analyzed using UPLC-MS. The assay was performed in duplicate.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2019.06.036.

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