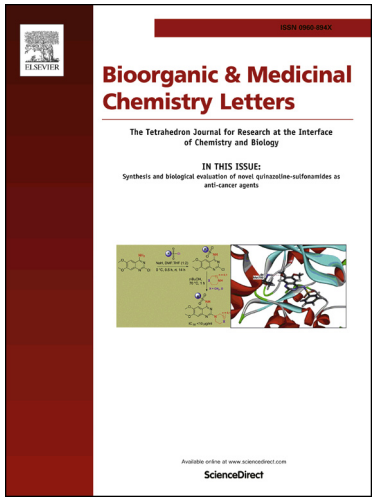


Acetanilide and bromoacetyl-lysine derivatives as activators for human histone deacetylase 8

Yusif M. Mukhtar, Yajun Huang, Jiajia Liu, Di Chen, Weiping Zheng

PII: S0960-894X(17)30414-6
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.04.037
Reference: BMCL 24890

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 30 December 2016
Revised Date: 8 April 2017
Accepted Date: 12 April 2017

Please cite this article as: Mukhtar, Y.M., Huang, Y., Liu, J., Chen, D., Zheng, W., Acetanilide and bromoacetyl-lysine derivatives as activators for human histone deacetylase 8, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.04.037

Acetanilide and bromoacetyl-lysine derivatives as activators for human histone deacetylase 8

Yusif M. Mukhtar[§], Yajun Huang[§], Jiajia Liu, Di Chen, Weiping Zheng*

School of Pharmacy, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, Jiangsu Province, P. R. China

*Correspondence: Tel, +86-15189129171; Fax, +86-511-88795939; E-mail, wzheng@ujs.edu.cn

[§]These authors contributed equally to this work.

Abstract

In the current study, seven compounds (i.e. **1**-**7**) were found to be novel activators for the N^{ε}-acetyl-lysine deacetylation reaction catalyzed by human histone deacetylase 8 (HDAC8). When assessed with the commercially available HDAC8 peptide substrate Fluor-de-Lys®-HDAC8 that harbors the unnatural 7-amino-4-methylcoumarin (AMC) residue immediately C-terminal to the N^{ε}-acetyl-lysine residue to be deacetylated, our compounds exhibited comparable activation potency to that of TM-2-51, the strongest HDAC8 activator reported in the current literature. However, when assessed with an AMC-less peptide substrate derived from the native HDAC8 non-histone substrate protein Zinc finger protein ZNF318, while our compounds were all found to be able to activate HDAC8 deacetylation reaction, TM-2-51 was found not to be able to. Our compounds also seemed to be largely selective for HDAC8 over other classical HDACs. Moreover, treatment with the strongest activator among our compounds (i.e. **7**) was found to decrease the K_M of the above AMC-less HDAC8 substrate, while nearly maintaining the k_{cat} of the HDAC8-catalyzed deacetylation on this substrate.

Key words: HDAC8; activator; classical HDAC; K_M depression.

HDAC8 belongs to the class I family of histone deacetylases (HDACs), one of the three classical families of HDACs (i.e. classes I, II, and IV) that are Zn^{2+}-containing metalloenzymes.[1] The class III HDACs constitute the sirtuin family of enzymes that use coenzyme β-nicotinamide adenine dinucleotide (β-NAD^+) as the co-substrate.[2,3] Since the discovery of HDAC8 in 2000,[4-6] more and more research has furthered our understanding of this class I HDAC.[1,5-11] Specifically, (i) while HDAC8 has a widespread expression in human tissues/organs, higher levels of expression were observed in brain, pancreas, kidney, and prostate *via* Northern blot analysis; moreover, HDAC8 expression is significantly enhanced in many cancer cells (e.g. SW480, A549, and G361 cells) as compared to normal cells. (ii) HDAC8 has been identified as a new marker for smooth muscle cell differentiation. (iii) while other class I HDACs (i.e. HDAC1/2/3) each requires its complex formation with other protein partners to be catalytically active, HDAC8 seems to be catalytically active by its own, as suggested by the *in vitro* experiment; and because of this property, HDAC8 has been extensively employed as a model HDAC in structure/function studies. It should be noted that HDAC8 was also found in complexes with other proteins (e.g. inv(16) fusion protein, cAMP response element-binding protein (CREB), human ever-shorter telomeres 1B (hEST1B), and α-actin) to participate in regulating distinct cellular functions, however, it is currently unknown whether the catalytic activity of HDAC8 was also regulated by such complex formation. (iv) while Zn^{2+} was originally discovered to be the catalytically essential cofactor for HDAC8, later studies suggested that Fe^{2+} may also be chosen by HDAC8 *in vivo* as the catalytic cofactor; (v) it is now known that HDAC8 takes both histone (*in vitro*) and

non-histone proteins (*in vitro* and *in vivo*) as its substrates; (vi) it is now also known that, besides catalyzing N^{ε}-acetyl-lysine deacetylation, HDAC8 is able to more proficiently (several-fold higher k_{cat}/K_M ratios) catalyze N^{ε}-fatty acyl-lysine defatty acylation *in vitro*. (vii) Despite the lack of complex formation with Notch1 protein, HDAC8 was recently shown to be able to indirectly increase the stability of Notch1 protein, so as to promote the Notch1 signaling in breast cancer cells. **Figure 1A** depicts the deacylation catalyzed by HDAC8.

The identification of *in vivo* non-histone HDAC8 substrates implied that HDAC8 regulates cellular processes beyond epigenetic mechanisms. And HDAC8 deacylation activity modulation may offer a viable avenue to developing therapeutics for human diseases such as cancer. In line with this, HDAC8 inhibitors have been pursued and quite a few fairly potent and selective HDAC8 inhibitors have been discovered.[10,12] These compounds would also be valuable in assessing further the biology and pharmacology of HDAC8. On the other hand, HDAC8 activators could constitute another class of compounds also able to potentially offer therapeutic benefits to human diseases and to be employed for a further deciphering of the biology and pharmacology of HDAC8.[10] Indeed, HDAC8 activation could be therapeutically beneficial to neuroblastoma and Cornelia de Lange syndrome spectrum disorders.[10,13] However, only one series of HDAC8 activators have been documented in current literature.[14,15] Specifically, a series of N,N'-disubstituted thioureas were recently found to be HDAC8 activators, with the strongest being TM-2-51 whose chemical structure is shown in **Figure 1B**. However, the HDAC8 activation effect of the N,N'-disubstituted thiourea compounds (including TM-2-51) was only examined with the HDAC8-catalyzed

deacetylation on Fluor-de-Lys®, a commercially available (from Enzo Life Sciences, Inc.) HDAC peptide substrate that harbors the unnatural 7-amino-4-methylcoumarin (AMC) residue immediately C-terminal to the N^{ε}-acetyl-lysine residue to be deacetylated, not on AMC-less substrates.[14]

Here we would like to report our discovery of novel activators for the deacetylase activity of human HDAC8, i.e. compounds **1**-**7** shown in **Figure 1B**. These compounds were found to be able to activate HDAC8 whether an AMC-containing (or AMC) peptide substrate or an AMC-less peptide substrate was used.

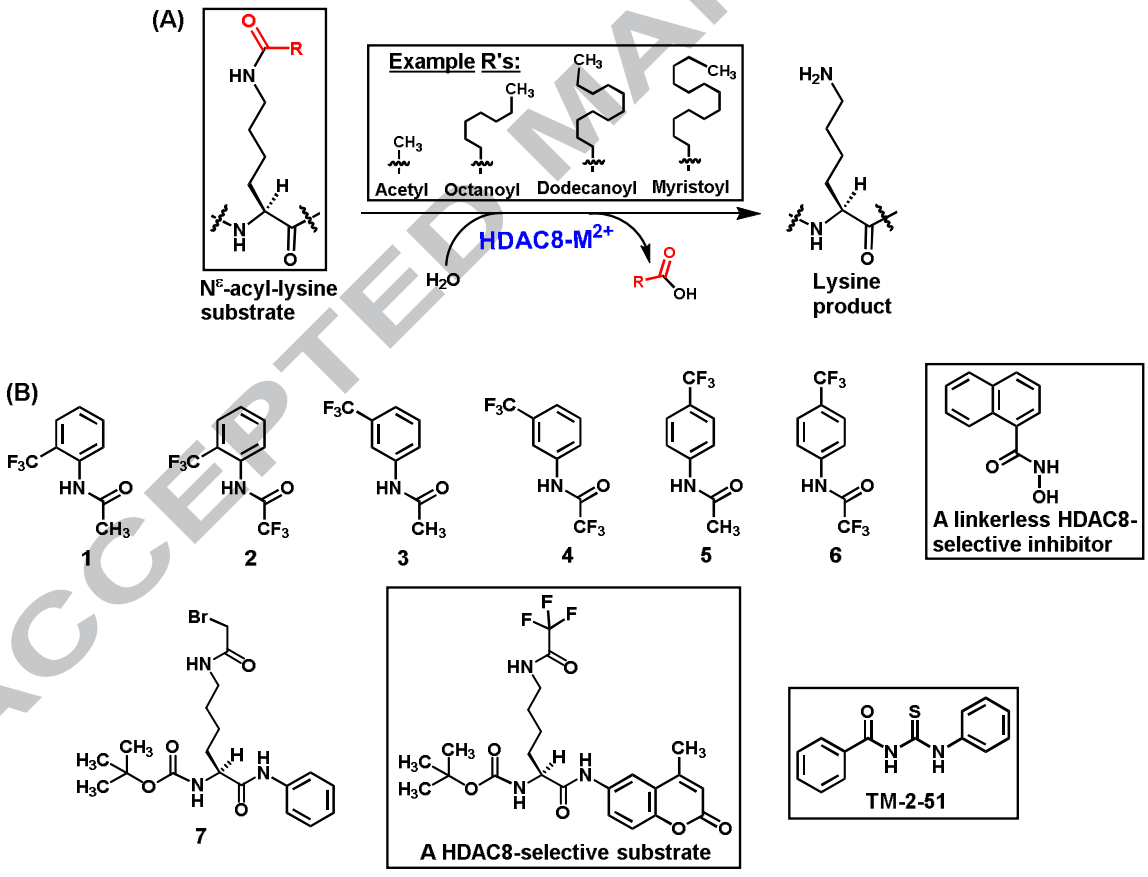
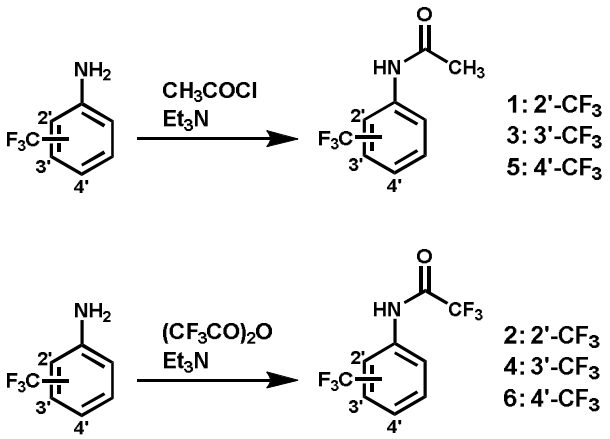


Figure 1. (A) The N^{ε}-acyl-lysine deacylation catalyzed by HDAC8. M^{2+} refers to a divalent cation (e.g. Zn^{2+}) that serves as the catalytically essential cofactor for the HDAC8 deacylation

reaction. **(B)** The chemical structures of compounds **1**-**7** prepared and assessed in the current study. Also shown are the chemical structures of TM-2-51 (the strongest HDAC8 activator from current literature), a linkerless HDAC8-selective inhibitor (structurally similar to compounds **1**-**6**), and a HDAC8-selective substrate (structurally similar to compound **7**). **Note:** these latter two compounds are the ones from which the designs of compounds **1**-**6** and **7** were respectively inspired.

The design of compounds **1**-**6** was initially inspired by the previously reported linkerless HDAC8 inhibitor shown in **Figure 1B**.[16] Specifically, it was our initial intent to potentially develop a mechanism-based HDAC8 inhibitor by converting the hydroxamic acid functionality of the depicted linkerless inhibitor to an acetanilide and in the meantime installing a trifluoromethyl group *ortho* to the acetanilide functionality in the resulting analog **1**. However, when compound **1** was prepared (according to **Scheme 1**) and subjected to an *in vitro* HDAC8 assay with Fluor-de-Lys®-HDAC8, a commercially available (from Enzo Life Sciences, Inc.) AMC HDAC8 peptide substrate, we found that this compound did not inhibit rather reproducibly promoted the deacetylation reaction catalyzed by human HDAC8 with ~1.2-fold of activation at 50 μM (**Table 1**). Of note, even though Fluor-de-Lys®-HDAC8 and the afore-mentioned Fluor-de-Lys® both harbor an AMC residue immediately C-terminal to the N^{ϵ}-acetyl-lysine residue to be deacetylated, the former is known to be a superior HDAC8 substrate to the latter, according to the work by Enzo Life Sciences, Inc. It should also be noted that, when assessed with compound **1** side-by-side, TM-2-51 was found to exhibit ~2.2-fold of HDAC8 activation (**Table 1**). Prompted by these findings, we subsequently prepared five analogs of compound **1** also according to **Scheme 1**, i.e. compounds **2**-**6** depicted in **Figure 1B**, to see if the observed HDAC8 activation by compound **1** was sort of a



Scheme 1. The synthesis of compounds **1**-**6**.

Table 1. The effect of TM-2-51 and compounds **1**-**7** on the deacetylation reaction catalyzed by HDAC8.[a,b,c]

Compound	AMC substrate[d]	AMC-less substrate[e]
TM-2-51	2.2 ± 0.7[f]	0.5 ± 0.01
1	1.2 ± 0.1	1.5 ± 0.3
2	1.4 ± 0.5	1.2 ± 0.1
3	1.7 ± 0.4	1.3 ± 0.4
4	1.7 ± 0.7	2.7 ± 1.1
5	1.7 ± 0.1	1.4 ± 0.1
6	1.1 ± 0.5	1.4 ± 0.4
7	2.8 ± 1.5	5.6 ± 0.4

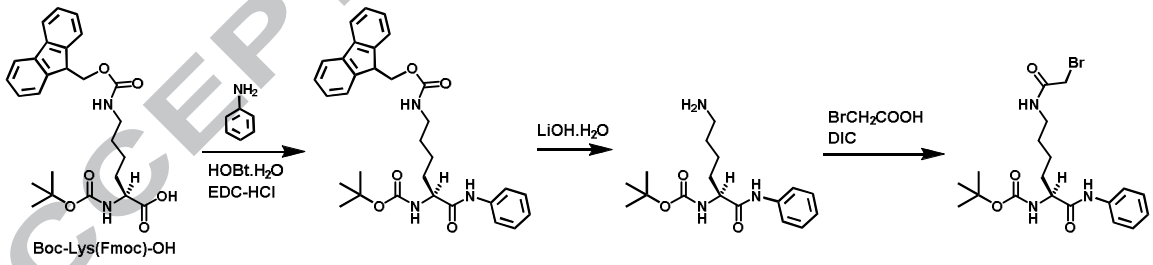
[a] See "Supplementary Material" for the HDAC8 deacetylation assay details.
[b] The concentration of TM-2-51 and compounds **1**-**7** used was all 50 μM. Since a decent fold of HDAC8 activation was reproducibly obtainable at 50 μM of TM-2-51 with the AMC substrate, this concentration was used in the current study for all the assays with TM-2-51 and with compounds **1**-**7** as well, so that a direct comparison among the assay data from the TM-2-51 treatment and those from the compounds **1**-**7** treatment can be made.
[c] Each number is the ratio of the HDAC8 deacetylation reaction velocity with compound treatment over that without compound treatment.
[d] The AMC substrate used was Fluor-de-Lys®-HDAC8 from Enzo Life Sciences, Inc.
[e] The AMC-less substrate used was CH_3CONH-FG-(N^{ε}-acetyl-lysine)-FSW-$CONH_2$.[8]
[f] The fold of activation determined in the current study.

non-specific effect. As shown in **Table 1**, all these five analogs were also found to be able to activate HDAC8 deacetylation activity *in vitro* on Fluor-de-Lys®-HDAC8. More importantly, differential folds of activation were observed with different compounds, with **3**, **4**, and **5** exhibiting the highest degrees of HDAC8 activation. This further finding tends to rule out the possibility that the observed HDAC8 activation by compounds **1**-**6** was sort of a non-specific effect.

Given that the above-described HDAC8 activation by compounds **1**-**6** was observed when the AMC peptide substrate Fluor-de-Lys®-HDAC8 was used, we subsequently would like to see if these compounds would also be able to activate the HDAC8 dacetylation reaction on an AMC-less peptide substrate. For this, we picked CH_3CONH-FG-(N^{ε}-acetyl-lysine)-FSW-$CONH_2$ since this peptide derived from the zinc finger protein ZNF318, a native HDAC8 non-histone substrate, represents the up-to-date most proficient peptide substrate for the HDAC8 deacetylation reaction with a measured k_{cat}/K_M of 4,826 $M^{-1}S^{-1}$.[8] When compounds **1**-**6** were assayed side-by-side with TM-2-51 on an *in vitro* HDAC8 assay with this substrate, TM-2-51 was found to be unable to activate the HDAC8 deacetylation, however, our compounds **1**-**6** were all able to activate with various folds of activation, with compound **4** being the strongest HDAC8 activator (**Table 1**).

While the mechanism of HDAC8 activation by compounds **1**-**6** are to be investigated, in the current study, we also found that compound **7** depicted in **Figure 1B** was also able to activate HDAC8 deacetylation activity on both the AMC Fluor-de-Lys®-HDAC8 and the AMC-less CH_3CONH-FG-(N^{ε}-acetyl-lysine)-FSW-$CONH_2$ (**Table 1**). In fact, compound **7**

turned out to be a stronger HDAC8 activator than any of compounds **1**-**6** on either substrate. Of note, the design of compound **7** was initially inspired by the previously reported HDAC8-selective substrate shown in **Figure 1B**.[17] Specifically, it was also our initial intent to potentially develop a mechanism-based HDAC8 inhibitor by converting the side chain trifluoroacetyl at the central position of the depicted substrate molecule to bromoacetyl, hoping that the electrophilic bromoacetyl will be captured by an amino acid side chain nucleophile after compound **7** binding to HDAC8 and/or being debromoacetylated by HDAC8, thus covalently labeling HDAC8. However, when compound **7** was prepared (according to **Scheme 2** *via* a three-step synthesis) and subjected to an *in vitro* HDAC8 assay with the AMC substrate Fluor-de-Lys®-HDAC8, we found that this compound did not inhibit rather reproducibly promoted the deacetylation reaction catalyzed by HDAC8. Compound **7** was subsequently found to also activate the HDAC8 deacetylation on the AMC-less substrate CH_3CONH-FG-(N^{ε}-acetyl-lysine)-FSW-$CONH_2$.



Scheme 2. The synthesis of compound **7**.

Next, we would like to see if compounds **1**-**7** were also able to activate the classical human HDACs other than HDAC8; for this, we employed the HeLa nuclear extract as a

source of these HDACs. Of note, HeLa nuclear extract is a rich source of human nuclear HDACs and is thus expected to have a rich presence of HDAC1 and HDAC2 that are exclusively nuclear.[7] When compounds **1**-**7** were assayed side-by-side with TM-2-51 with the afore-mentioned AMC Fluor-de-Lys®-HDAC8, a known substrate also for the HDACs present in HeLa nuclear extract (according to the work by Enzo Life Sciences, Inc.), except for a slight HDAC activation by compound **7**, no HDAC activation was observed for TM-2-51 and compounds **1**-**6** (**Table 2**). Of note, our observed lack of HDAC activation by TM-2-51 is consistent with the literature finding.[14]

Table 2. The effect of TM-2-51 and compounds **1**-**7** on the deacetylation reaction catalyzed by the HDACs in HeLa nuclear extract.[a,b,c]

Compound	AMC substrate[d]	AMC-less substrate[e]
TM-2-51	0.69 ± 0.03	0.58 ± 0.08
1	0.98 ± 0.16	1.45 ± 0.08
2	0.87 ± 0.01	1.22 ± 0.24
3	0.89 ± 0.04	0.81 ± 0.27
4	0.96 ± 0.13	0.54 ± 0.09
5	0.93 ± 0.1	0.83 ± 0.37
6	0.93 ± 0.03	0.64 ± 0.42
7	1.09 ± 0.07	1.22 ± 0.1

[a] See "Supplementary Material" for the HDAC deacetylation assay details.
[b] The concentration of TM-2-51 and compounds **1**-**7** used was all 50 μM. See the footnote b of **Table 1** for reasons behind the use of this concentration.
[c] Each number is the ratio of the HDAC deacetylation reaction velocity with compound treatment over that without compound treatment.
[d] The AMC substrate used was Fluor-de-Lys®-HDAC8 from Enzo Life Sciences, Inc.
[e] The AMC-less substrate used was CH_3CONH-RH-(N^{ε}-acetyl-lysine)-(N^{ε}-acetyl-lysine)-$CONH_2$.[18]

Compounds **1**-**7** and TM-2-51 were also assayed with HeLa nuclear extract on CH_3CONH-RH-(N^{ε}-acetyl-lysine)-(N^{ε}-acetyl-lysine)-$CONH_2$, an AMC-less substrate for the HDACs present in HeLa nuclear extract previously discovered in our laboratory.[18] With this substrate, no HDAC activation was observed for TM-2-51 and compounds **3**-**6**, however, compounds **1**, **2**, and **7** did exhibit certain degrees of HDAC activation (**Table 2**). When compared with the above-described capability of compounds **1**-**7** to activate HDAC8 on the AMC-less substrate, the here observed differential HDAC activating efficacies of compounds **3**-**6** and **1/2/7** presumably results from their differential modes of binding to HDAC, with the binding of **1/2/7** able to promote the HDAC deacetylation catalysis (analogous to their promoting the HDAC8 deacetylation catalysis), whereas the binding of **3**-**6** is unable to (in contrast to their promoting the HDAC8 deacetylation catalysis).

It should be noted that, while the HDAC activation by compounds **1** and **2** on the AMC-less substrate (**Table 2**) was comparable in strength to their respective HDAC8 activation on both AMC and AMC-less substrates (**Table 1**), the HDAC activation by compound **7** on both AMC and AMC-less substrates (**Table 2**) was much weaker in strength than its HDAC8 activation on both AMC and AMC-less substrates (**Table 1**). Therefore, we still consider compound **7**, the strongest HDAC8 activator among compounds **1**-**7** and TM-2-51, as a selective activator for HDAC8 *versus* other classical HDACs. It should also be noted that, the second strongest HDAC8 activator among compounds **1**-**7**, i.e. compound **4**, seemed to have an absolute activation selectivity for HDAC8 *versus* other classical HDACs.

Next, we also performed a kinetic characterization of the HDAC8 activation by the strongest activator **7**. As shown in **Table 3**, treatment with compound **7** at 50 μM was found to decrease the K_M of AMC and AMC-less HDAC8 substrates respectively by ~3.5- and ~3.6-fold. However, while there was only a slight k_{cat} decrease (< 40%) for the HDAC8 deacetylation on the AMC-less substrate, there was a ~13-fold k_{cat} decrease for the HDAC8 deacetylation on the AMC substrate. Therefore, the catalytic efficiency (k_{cat}/K_M) was enhanced by ~2.6-fold when the AMC-less substrate was used, and was decreased by ~3.7-fold when the AMC substrate was used. One putative explanation for these findings is that, while the binding of compound **7** to HDAC8 can enhance the binding affinity of both AMC and AMC-less substrates, however, when the AMC substrate was used, the catalytically essential/important functionality/functionalities might have been shifted to new position(s) sub-optimal to catalysis, whereas when the AMC-less substrate was used, there was no such a deleterious impact on catalysis following the binding of **7** to HDAC8. As for the observed HDAC8 activation by compound **7** on the AMC substrate, we are currently unable to offer an explanation.

To summarize, in the current study, compounds **1**-**7** were found to be novel activators of the human HDAC8-catalyzed deacetylation reaction. When assessed with the AMC HDAC8 substrate, these compounds exhibited comparable activation potency to that of TM-2-51, the strongest HDAC8 activator reported in the current literature. However, when assessed with an AMC-less HDAC8 substrate, while these compounds were all found to be able to activate HDAC8 deacetylation reaction, TM-2-51 was found not to be able to. Our compounds also

Table 3. The steady-state kinetic parameter determination for the HDAC8 deacetylation reaction in the presence or absence of compound **7**.[a]

Treatment	AMC substrate[b]			AMC-less substrate[c]		
	k_{cat} (min^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1}S^{-1}$)	k_{cat} (min^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1}S^{-1}$)
No 7	106 ± 1.6	2990 ± 72	591	12.4 ± 2.2	74.5 ± 21.1	2774[d]
With 7[e]	8.1 ± 1.1	852.8 ± 27.4	158	9 ± 4.9	20.9 ± 5.5	7177

[a] See "Supplementary Material" for the HDAC8 deacetylation assay details.
[b] The AMC substrate used was Fluor-de-Lys®-HDAC8 from Enzo Life Sciences, Inc.
[c] The AMC-less substrate used was CH_3CONH-FG-(N^{ε}-acetyl-lysine)-FSW-$CONH_2$.[8]
[d] Close to the literature value of 4826 $M^{-1}S^{-1}$.[8]
[e] The concentration of compound **7** used was 50 μM.

seemed to be largely selective for HDAC8 over other classical HDACs. Moreover, treatment with the strongest HDAC8 activator **7** was found to decrease the K_M of both AMC and AMC-less HDAC8 substrates, while nearly maintaining the k_{cat} of the HDAC8 deacetylation on the AMC-less substrate. Future work includes: (i) a chemical mechanistic study on the HDAC8 activation by these compounds, especially the strongest activator **7** and the second strongest activator **4**, on both AMC and AMC-less HDAC8 substrates. A structural study on the complex of HDAC8 and these novel activators would be one rewarding approach toward an understanding of the chemical mechanism of these activating compounds.

(ii) a study on the possible activation effect of our compounds toward the defatty acylation activity of HDAC8. The findings from these future studies will be helpful in developing superior HDAC8 activators that can be subjected to further cell model- and animal model-based studies.

Acknowledgements

We are grateful for the financial support to this work from the following: the Jiangsu provincial specially appointed professorship, the Jiangsu provincial "innovation and venture talents" award plan.

References

1. Seto, E.; Yoshida, M. *Cold Spring Harb. Perspect. Biol*. **2014**, *6*, a018713.

2. Bheda, P.; Jing, H.; Wolberger, C.; Lin, H. *Annu. Rev. Biochem.* **2016**, *85*, 405.

3. Chen, B.; Zang, W.; Wang, J.; Huang, Y.; He, Y.; Yan, L.; Liu, J.; Zheng, W. *Chem. Soc. Rev*. **2015**, *44*, 5246.

4. Buggy, J. J.; Sideris, M. L.; Mak, P.; Lorimer, D. D.; McIntosh, B.; Clark, J. M. *Biochem. J*. **2000**, *350 Pt. 1*, 199.

5. Van den Wyngaert, I.; de Vries, W.; Kremer, A.; Neefs, J.; Verhasselt, P.; Luyten, W. H.; Kass, S. U. *FEBS Lett*. **2000**, *478*, 77.

6. Hu, E.; Chen, Z.; Fredrickson, T.; Zhu, Y.; Kirkpatrick, R.; Zhang, G. F.; Johanson, K.; Sung, C. M.; Liu, R.; Winkler, J. *J. Biol. Chem*. **2000**, *275*, 15254.

7. Wolfson, N. A.; Pitcairn, C. A.; Fierke, C. A. *Biopolymers* **2013**, *99*, 112.

8. Alam, N.; Zimmerman, L.; Wolfson, N. A.; Joseph, C. G.; Fierke, C. A.; Schueler-Furman, O. *Structure* **2016**, *24*, 458.

9. Aramsangtienchai, P.; Spiegelman, N. A.; He, B.; Miller, S. P.; Dai, L.; Zhao, Y.; Lin, H. *ACS Chem. Biol*. **2016**, *11*, 2685.

10. Chakrabarti, A.; Melesina, J.; Kolbinger, F. R.; Oehme, I.; Senger, J.; Witt, O.; Sippl, W.; Jung, M. *Future Med. Chem.* **2016**, *8*, 1609.

11. Chao, M. W.; Chu, P. C.; Chuang, H. C.; Shen, F. H.; Chou, C. C.; Hsu, E. C.; Himmel, L. E.; Huang, H. L.; Tu, H. J.; Kulp, S. K.; Teng, C. M.; Chen, C. S. *Oncotarget* **2016**, *7*, 1796.

12. Li, Y.; Seto, E. *Cold Spring Harb. Perspect. Med*. **2016**, *6*, a026831.

13. Deardorff, M. A.; Porter, N. J.; Christianson, D. W. *Protein Sci*. **2016**, *25*, 1965.

14. Singh, R. K.; Mandal, T.; Balsubramanian, N.; Viaene, T.; Leedahl, T.; Sule, N.; Cook, G.; Srivastava, D. K. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 5920.

15. Singh, R. K.; Cho, K.; Padi, S. K.; Yu, J.; Haldar, M.; Mandal, T.; Yan, C.; Cook, G.; Guo, B.; Mallik, S.; Srivastava, D. K. *J. Biol. Chem*. **2015**, *290*, 6607.

16. Krennhrubec, K.; Marshall, B. L.; Hedglin, M.; Verdin, E.; Ulrich, S. M. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 2874.

17. Riester, D.; Wegener, D.; Hildmann, C.; Schwienhorst, A. *Biochem Biophys Res Commun*. **2004**, *324*, 1116.

18. Fatkins, D. G.; Zheng, W. *Anal. Biochem*. **2008**, *372*, 82.

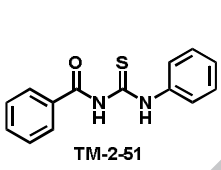
Graphical Abstract

Acetanilide and bromoacetyl-lysine derivatives as activators for human histone deacetylase 8

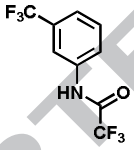
Yusif M. Mukhtar[§], Yajun Huang[§], Jiajia Liu, Di Chen, Weiping Zheng*

Seven compounds were found to be novel activators for the N^{ε}-acetyl-lysine deacetylation reaction catalyzed by human histone deacetylase 8, with **4** and **7** being the two strongest. Besides being HDAC8-selective activators *versus* other classical HDACs, **4** and **7** were also found to be strong HDAC8 activators on a 7-amino-4-methylcoumarin (AMC)-less substrate, which is in contrast to TM-2-51, the strongest HDAC8 activator reported in the current literature.

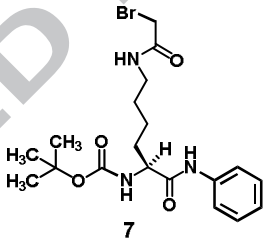
Compound	Fold of Activation		
	AMC substrate		AMC-les
	HDAC8	HDACs	HDAC8
TM-2-51	2.2 ± 0.7	0.69 ± 0.03	0.5 ± 0.01
4	1.7 ± 0.7	0.96 ± 0.13	2.7 ± 1.1
7	2.8 ± 1.5	1.09 ± 0.07	5.6 ± 0.4



TM-2-51



4



7