Brief Report

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Design and synthesis of novel histone deacetylase 6 inhibitors with benzyl-triazole as the core skeleton

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Summary **In the field of epigenetics, histone deacetylases (HDACs) are important members and well validated targets for anti-cancer drugs discovery. In this study, we designed and synthesized twenty-seven novel hydroxamic acid-based HDAC inhibitors (HDACis) with benzyl-triazole as the core skeleton. Most target compounds displayed excellent inhibition rates toward HDACs. Among them, compounds ZM-22 to ZM-27 with inhibition rates more than 90% toward HDACs exhibited potent inhibitory activity toward HDAC6, and ZM-23 possessed the best selectivity to HDAC6 over HDAC1. The high potency of compound ZM-23 toward HDAC6 was rationalized by molecular docking simulation. This series of compounds is worthy for further anti-cancer activity evaluation and structural optimization works.**

Keywords: Histone deacetylase, isoform, selective, inhibitor, anti-tumor

1. Introduction

Modulating the activities of enzymes in the field of epigenetics including "writers", "erasers" and "readers" has emerged as an attractive therapeutic strategy fighting against human diseases (*1*). During the translation process, the "loosen" and "condensed" forms of DNA can be regulated by the acetylation level of lysine residues (*2*). As one kind of the most important epigenetic erasers, histone deacetylases (HDACs) are responsible for the removal of the acetyl group located on the lysine residues of histones (*3*), the progress frees the genetic component and triggers the transcriptions (*4*). Besides histones, HDACs can also act on numerous non-histone substrates such as α-tubulin and p53 (*5-7*). The crucial roles played by HDACs make them promising targets for various human diseases treatment, especially cancers.

Till now, 18 mammalian HDACs with different structures, locations and functions are reported, which can be categorized into four classes: class I (HDAC1, 2, 3, and 8), class II (class IIa: HDAC4, 5, 7, and 9 and class IIb: HDAC6 and 10), and class IV (HDAC11) are all zinc ion (Zn^{2+}) dependent deacetylases that are mechanistically distinct from NAD^{+}-dependent class III HDACs (*8*). Following the function investigation of HDACs, numerous HDACs inhibitors (HDACis) are developed. Four HDACis, vorinostat (SAHA), romidepsin, belinostat, and panobinostat, have gained FDA approvals for the treatment of hematologic tumors (*9*). A survey of the results of clinical trials indicated that use of pan-HDACis or partially selective HDACis results in unwanted side effects such as fatigue, nausea/vomiting, diarrhea, cardiotoxicity and hematological toxicity, and thrombocytopenia (*10,11*), all of which severely limit their utilization in clinics.

With the purpose of eradicating the side effects, increasing number of researches are focusing on the development of isoform selective HDACis, especially HDAC6 selective inhibitors. In contrast to the lethal effect of HDAC1-3 genetic ablation, mice with HDAC6 knocked out are effectively normal (*12*). Several researches also demonstrated that HDAC6 selective

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inhibitors have fewer side effects than pan-HDACis (*13,14*). So, the development of selective HDAC6 inhibitors should be advantageous as a therapeutic approach, which has encouraged scientists to develop potent and selective HDAC6 inhibitors (*15-17*). ACY-1215, a first-in-class selective HDAC6 inhibitor, exhibits anti-tumor effects alone or in combination with other drugs in various cancers and is currently undergoing clinical trials for the treatment of breast cancer (*18*).

The canonical pharmacophore of HDACis consists of three parts: a surface recognition region (cap) that interacts with the entrance of active pocket of HDACs; Zn^{2+} binding group (ZBG) that chelates with Zn^{2+}; a linker that conjugates the cap and ZBG and interacts with the hydrophobic channel of the active site (Figure 1) (*19*). It is well studied that the delicate design of "cap" and "linker" parts can contribute to the selectivity towards specific HDAC isoforms (*2*). In this study, with the aim of searching for novel selective HDAC6 inhibitors, a series of HDACis with benzyl-triazole as the core skeleton was designed and synthesized. Hydroxamic acid was selected as the ZBG. The linker length was fixed to 7 atoms according to the known structure-activity relationship (SAR) that 7-8 atoms long linker usually displays best HDACs inhibitory activity.

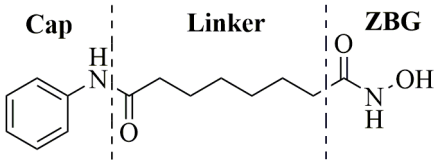


Figure 1. The pharmacophore of HDACis with SAHA as an example.

2. Materials and Methods

2.1. *Chemistry*

All of the chemical solvents and reagents, which were analytically pure without further purification, were purchased from Energy Chemical (Shanghai, China). Thin-layer chromatography was performed on 0.20 mm Silica Gel 60 F254 plates (Qingdao Haiyang Chemical, China). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (Bruker Company, Germany) or a Varian spectrometer (Varian, Palo Alto, CA, USA), using tetramethylsilane as an internal standard. Chemical shifts were given in parts per million. Mass spectra were recorded on a Q-TOF Premier mass spectrometer (Micromass, Manchester, U.K.).

2.2. *In vitro HDAC inhibition fluorescence assay*

In brief, 10 μL of enzyme solution (HeLa cell nuclear extract, HDAC1, or HDAC6) was mixed with different concentrations of tested compounds (50 μL). The mixture was incubated at 37°C for 5 min, followed by adding 40 μL fluorogenic substrate (Boc-Lys(acetyl)-AMC). After incubation at 37°C for 30 min, the mixture was quenched by addition of 100 μL of developer containing trypsin and trichostatin A (TSA). Over another incubation at 37°C for 20 min, fluorescence intensity was measured using a microplate reader at excitation and emission wavelengths of 390 and 460 nm, respectively. The inhibition ratios were calculated from the fluorescence intensity readings of tested wells relative to those of control wells, and the IC_{50} values were calculated using a regression analysis of the concentration/inhibition data.

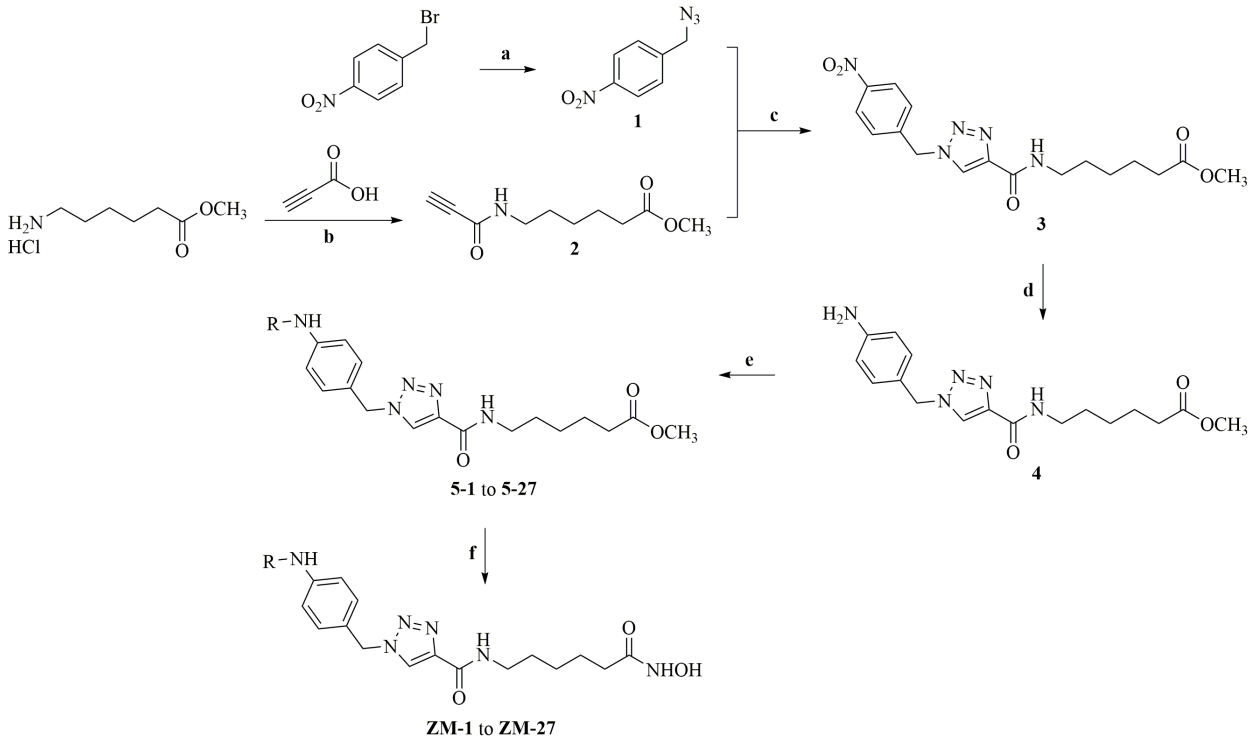
2.3. *Molecular docking*

Compounds were docked into the active site of HDAC6 (PDB entry: 5WGL) using Tripos SYBYL-X 2.1. Before docking process, the HDAC6 structure retrieved from PDB website was treated by deleting water molecules, adding FF99 charges. A 100-step minimization process was performed to further optimize the protein structure. The molecular structures were generated with Sybyl/Sketch module and optimized using Powell's method with the Tripos force field with convergence criterion set at 0.05 kcal/(Å mol) and assigned charges with the Gasteiger_Hückel method. Molecular docking was carried out *via* the Sybyl/SurflexDock module. Other docking parameters were kept to the default values.

3. Results and Discussion

3.1. *Chemical synthesis of target compounds*

The synthesis route is outlined in Scheme 1. 4-Nitrobenzyl azide (**1**) was synthesized from mixture of 4-nitrobenzyl bromide and sodium azide (NaN_3) using dimethyl sulfoxide (DMSO) as the solvent. In the other hand, the methyl 6-propiolamidohexanoate (**2**) was obtained by the condensation of methyl 6-aminocaproate hydrochloride and propiolic acid in the presence triethylamine (TEA) and dicyclohexylcarbodiimide (DCC). The Click reaction between **1** and **2** catalyzed by CuI could give the compound **3**. Reduction of the nitro group to amine group catalyzed by 5% Pd/C at the atmosphere of hydrogen could give the important intermediate **4**. In the next step, compound **4** reacted with various acyl chlorides or sulfonyl chlorides catalyzed by TEA to give compounds **5-1** to **5-27**. The methyl ester of compounds **5-1** to **5-27** was transferred to the hydroxamic acid by reacting with NH_2OH in anhydrous MeOH to get the final products, ZM-1 to ZM-27. Specific synthetic



Scheme 1. Reagents and conditions. a. NaN_3, DMSO, 30℃, 24 h; **b.** DCC, TEA, dry DCM, RT, overnight; **c.** CuI, anhydrous THF, nitrogen, RT, overnight; **d.** 5% Pd/C, hydrogen, CH_3OH, RT, 10 h; **e.** TEA, DCM, 0°C, 1 h, then RT, 8 h; **f.** NH_2OH, CH_3OH, RT, 2 h.

procedures and spectroscopy data of the all compounds see supplementary data (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=43*).

3.2. *Activity of target compounds against HDACs extracted from Hela cell nucleus*

We first screened the inhibitory activities of all 27 final products against Hela cell nucleus extracts whose main component is class I HDACs. Single concentration (1 μM) was used and the inhibition rate (%) was calculated. The results are presented in Table 1. All 27 compounds we obtained presented moderate to excellent inhibitory activities against HDACs demonstrating that both the benzyl-triazole fragment as the "cap" part and the linker length are appropriate for HDAC inhibition. Among all 27 compounds, six of them (ZM-22 to ZM-27) displayed robust inhibitory activity against HDACs with the inhibition rates higher than 90% at 1 μM. Notably, the compound ZM-26 is slightly more potent than the marketed drug SAHA. Surprisingly, these 6 compounds have a uniform sulfamide moiety in their structures, which is speculated to form extra interactions with HDAC compared with other amide-based compounds. This might be the reason why these 6 compounds possess higher inhibitory activities against HDACs than others.

3.3. *HDAC isoform specificity of compounds ZM-22 to ZM-27*

With these 6 potent compounds in hand, their isoform selectivity was further investigated against HDAC1 (class I) and HDAC6 (class IIb). The pan-HDACi SAHA and HDAC6 selective inhibitor ACY1215 were utilized as the positive controls. The result is showed in Table 2. Consistent with published data, SAHA as a pan-HDACi is almost equipotent toward HDAC1 and 6 without conspicuous selectivity. ACY1215 as a well-studied HDAC6 selective inhibitor displayed high potency to HDAC6 with the IC_{50} value of 8.0 nM. Encouragingly, all of our 6 compounds are strong HDAC6 inhibitors with the IC_{50} values ranging from 8.4 to 50 nM. Compounds ZM-22 to ZM-25 display selectivity towards HDAC6 over HDAC1 with different degrees. The selectivity factor for HDAC6 over HDAC1 (SF6/1) of ZM-23 is 9.37, slightly better than that of ACY1215 (SF6/1 = 9.13).

3.5. *Molecular simulation result of compound ZM-23 towards HDAC6*

To rationalize our biological experiment findings, molecular docking simulation was performed. Considering that compound ZM-23 displayed potent HDAC6 inhibitory activity as well as the best selectivity toward HDAC6 over HDAC1, we investigated the proposed binding mode of ZM-23 with HDAC6. The crystal structure of *Danio rerio* HDAC6 catalytic domain 2 in complex with ACY1215 (PDB code 5WGL) was used as the template. The result is showed

Table 1. Structures all 27 target compounds and their inhibition rates against HDACs at 1 μM

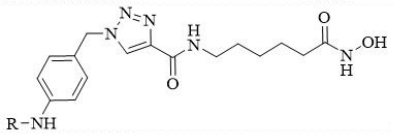
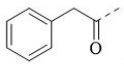
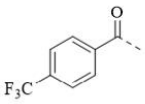
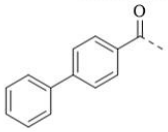
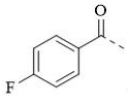
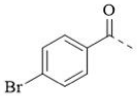
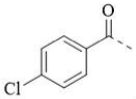
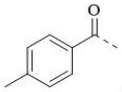
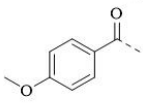
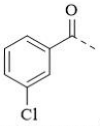
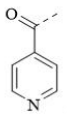
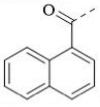
Compd	 R =	Inhibition rate at 1 μM
ZM-1		84.9%
ZM-2		88.5%
ZM-3		71.4%
ZM-4		47.9%
ZM-5		53.9%
ZM-6		87.5%
ZM-7		64.5%
ZM-8		74.3%
ZM-9		67.7%
ZM-10		69.1%
ZM-11		71.4%
ZM-12		76.6%
ZM-13		86.0%
ZM-14		72.8%

Table 1. Structures all 27 target compounds and their inhibition rates against HDACs at 1 μM (continued)

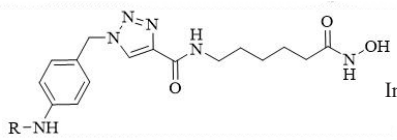
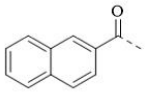
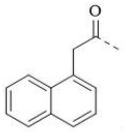
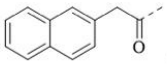
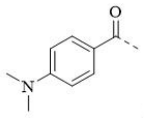
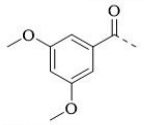
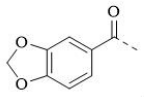
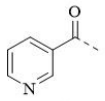
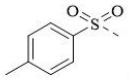
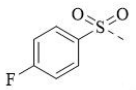
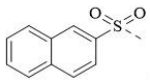
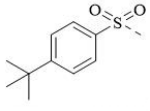
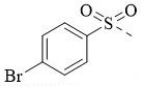
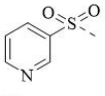
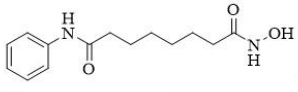
Compd	 R =	Inhibition rate at 1 μM
ZM-15		45.0%
ZM-16		88.0%
ZM-17		63.0%
ZM-18		80.0%
ZM-19		86.4%
ZM-20		71.0%
ZM-21		86.3%
ZM-22		93.1%
ZM-23		91.0%
ZM-24		92.3%
ZM-25		93.8%
ZM-26		97.1%
ZM-27		90.1%
SAHA		96.1%

Table 2. The inhibitory activities of selected compounds against HDAC1 and HDAC6

Compd	IC_{50}[a], nM		SF6/1[b]
	HDAC1	HDAC6	
ZM-22	104.3	14.6	7.14
ZM-23	206.2	22.0	9.37
ZM-24	55.4	11.8	4.69
ZM-25	66.1	14.9	4.44
ZM-26	5.6	8.4	0.67
ZM-27	144.0	50.0	2.88
SAHA	43.2	20.7	2.09
ACY1215	73.0	8.0	9.13

[a]The IC_{50} values are the means of three experiments. [b]SF6/1: selectivity factor for HDAC6 over HDAC1 (SF6/1 = IC_{50}(HDAC1)/ IC_{50}(HDAC6)).

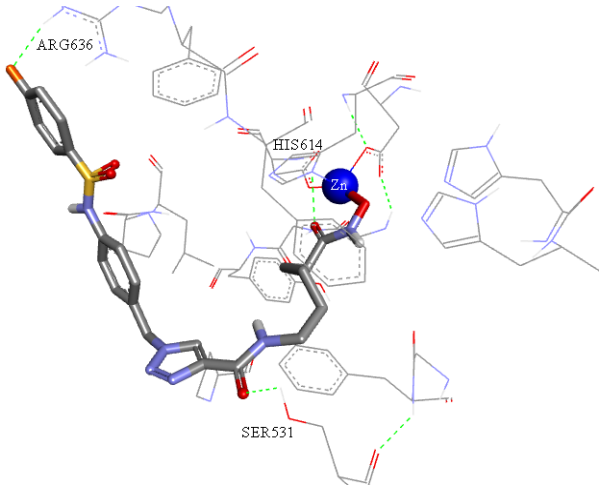


Figure 2. Proposed binding model of compound ZM-23 with HDAC6 (by modification of PDB code 5WGL using Tripos SYBYL-X 2.1). The Zn^{2+} is presented as a blue sphere. Hydrogen bonds are shown as green dashed lines. The figure was generated by Discovery Studio Visualizer.

in Figure 2. From the docking result, we can see that the hydroxamic acid moiety of ZM-23 can smoothly chelate with the Zn^{2+} in a monodentate manner. The oxygen atom of the carboxyl group in the hydroxamic acid formed a hydrogen bond with HIS614 residue. The fluorine atom on the terminal benzene ring formed a hydrogen bonds with ARG636 residue, and the oxygen atom of the amide group adjacent to the triazole formed another hydrogen bond with SER531 residue. All of these interactions can explain why ZM-23 possessed strong inhibitory activity toward HDAC6.

Different from other HDACs, HDAC6 is located mainly in cytoplasm and many non-histone proteins are its substrate (*20*). With regards to the structure, HDAC6 possess a wider entrance of the active pocket than other HDAC isoforms (*21*). This wide entrance can accommodate large size cap groups which make it possible to design specific HDAC6 inhibitors. With this in mind, we designed and synthesized a series of 27 novel compounds with large size cap groups by using benzyl-triazole as their core structures. Actually, many published potent HDACis contain the triazole fragment as the cap or linker part proving that it is favorable for binding with HDACs (*22*). In addition, triazole is a stable group that is able to resist metabolism and degradation under acidic/basic and oxidative/reductive conditions *in vivo*, and it can be used as the bioisostere of ester and amide groups (*23*). So, in this work, we also introduce a triazole moiety as the cap part. Hydroxamic acid was selected as the ZBG due to its high affinity to Zn^{2+}. Out of all 27 compounds we obtained, compounds ZM-22 to ZM-27 containing a sulfamide moiety in their cap parts exhibited excellent inhibition rate to HDACs derived from the HeLa cell extracts. In the isoform selectivity assay, compound ZM-26 displayed the most potent HDAC6 inhibitory activity and compound ZM-23 possessed the best selectivity toward HDAC6 with the selective index slightly higher than ACY1215. In the docking study, the proposed binding mode of ZM-23 showed that the hydroxamic acid coordinated well with the Zn^{2+}. The sulfamide may provide a suitable angle for the cap part to interact with the rim of the HDAC6. The anticancer activities of novel HDAC6 inhibitors warrant further investigation.

4. Conclusion

In this work, a series of 27 novel HDACis possessing benzyl-triazole as a central core were rationally designed based on the pharmacophore constituents of known HDACis. All of the target compounds displayed moderate to excellent inhibitory activities to HDACs. Out of them, compounds ZM-22 to ZM-27 with inhibition rates more than 90% toward HDACs exhibited potent inhibitory activity toward HDAC6, and ZM-23 possessed the best selectivity to HDAC6 over HDAC1. The proposed binding mode between ZM-23 and HDAC6 was also analyzed by docking simulation. All these results demonstrated that this series of compounds is worthy for further biological evaluation and structure optimization works.

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References

1. Luan Y, Li J, Bernatchez JA, Li R. Kinase and Histone Deacetylase Hybrid Inhibitors for Cancer Therapy. J Med

Chem. 2019; 62:3171-3183.
2. Lee HY, Fan SJ, Huang FI, Chao HY, Hsu KC, Lin TE, Yeh TK, Lai MJ, Li YH, Huang HL, Yang CR, Liou JP. 5-Aroylindoles Act as Selective Histone Deacetylase 6 Inhibitors Ameliorating Alzheimer's Disease Phenotypes. J Med Chem. 2018; 61:7087-7102.
3. Boyault C, Sadoul K, Pabion M, Khochbin S. HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. Oncogene. 2007; 26:5468-5476.
4. Lee HY, Nepali K, Huang FI, Chang CY, Lai MJ, Li YH, Huang HL, Yang CR, Liou JP. (N-Hydroxycarbonylbenylamino)quinolines as selective histone deacetylase 6 inhibitors suppress growth of multiple myeloma *in vitro* and *in vivo*. J Med Chem. 2018; 61:905-917.
5. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP. HDAC6 is a microtubule-associated deacetylase. Nature. 2002; 417:455-458.
6. Luo J, Su F, Chen D, Shiloh A, Gu W. Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature. 2000; 408:377-381.
7. Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, Guarente L, Weinberg RA. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell. 2001; 107:149-159.
8. Bian J, Luan Y, Wang C, Zhang L. Discovery of N-hydroxy-4-(^{1}H-indol-3-yl)butanamide as a histone deacetylase inhibitor. Drug Discov Ther. 2016; 10:163-166.
9. Li X, Xu W. HDAC1/3 dual selective inhibitors - new therapeutic agents for the potential treatment of cancer. Drug Discov Ther. 2014; 8:225-228.
10. Fraczek J, Vanhaecke T, Rogiers V. Toxicological and metabolic considerations for histone deacetylase inhibitors. Expert Opin Drug Metab Toxicol. 2013; 9:441-457.
11. Mackay HJ, Hirte H, Colgan T, Covens A, MacAlpine K, Grenci P, Wang L, Mason J, Pham PA, Tsao MS, Pan J, Zwiebel J, Oza AM. Phase II trial of the histone deacetylase inhibitor belinostat in women with platinum resistant epithelial ovarian cancer and micropapillary (LMP) ovarian tumours. Eur J Cancer. 2010; 46:1573-1579.
12. Govindarajan N, Rao P, Burkhardt S, Sananbenesi F, Schluter OM, Bradke F, Lu J, Fischer A. Reducing HDAC6 ameliorates cognitive deficits in a mouse model for Alzheimer's disease. EMBO Mol Med. 2013; 5:52-63.
13. Kalin JH, Bergman JA. Development and therapeutic implications of selective histone deacetylase 6 inhibitors. J Med Chem. 2013; 56:6297-6313.
14. Seidel C, Schnekenburger M, Dicato M, Diederich M. Histone deacetylase 6 in health and disease. Epigenomics. 2015; 7:103-118.
15. Miao H GJ, Mou ZS, Wang BL, Zhang L, Su L, Han YT, Luan YP. Design, synthesis and biological evaluation of 4-piperidin-4-yltriazole derivatives as novel histone deacetylase inhibitors. BioSci Trends. 2019; 13:7.
16. Zhao C GJ, Zhang L, Su L, Luan Y. Novel HDAC6 selective inhibitors with 4-aminopiperidine-1-carboxamide as the core structure enhanced growth inhibitory activity of bortezomib in MCF-7 cells. BioSci Trends. 2019; 13:7.
17. Hsieh YL, Tu HJ, Pan SL, Liou JP, Yang CR. Anti-metastatic activity of MPT0G211, a novel HDAC6 inhibitor, in human breast cancer cells *in vitro* and *in vivo*. Biochim Biophys Acta Mol Cell Res. 2019; 1866:992-1003.
18. Cao J, Lv W, Wang L, Xu J, Yuan P, Huang S, He Z, Hu J. Ricolinostat (ACY-1215) suppresses proliferation and promotes apoptosis in esophageal squamous cell carcinoma *via* miR-30d/PI3K/AKT/mTOR and ERK pathways. Cell Death Dis. 2018; 9:817.
19. Kalin JH, Butler KV, Kozikowski AP. Creating zinc monkey wrenches in the treatment of epigenetic disorders. Curr Opin Chem Biol. 2009; 13:263-271.
20. Senger J, Melesina J, Marek M, Romier C, Oehme I, Witt O, Sippl W, Jung M. Synthesis and Biological Investigation of Oxazole Hydroxamates as Highly Selective Histone Deacetylase 6 (HDAC6) Inhibitors. J Med Chem. 2016; 59:1545-1555.
21. Butler KV, Kalin J, Brochier C, Vistoli G, Langley B, Kozikowski AP. Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin A. J Am Chem Soc. 2010; 132:10842-10846.
22. Oyelere AK, Chen PC, Guerrant W, Mwakwari SC, Hood R, Zhang Y, Fan Y. Non-peptide macrocyclic histone deacetylase inhibitors. J Med Chem. 2009; 52:456-468.
23. Ding C, Chen S, Zhang C, Hu G, Zhang W, Li L, Chen YZ, Tan C, Jiang Y. Synthesis and investigation of novel 6-(1,2,3-triazol-4-yl)-4-aminoquinazolin derivatives possessing hydroxamic acid moiety for cancer therapy. Bioorg Med Chem. 2017; 25:27-37.

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