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Class I histone deacetylase inhibition by aryl butenoic acid derivatives: *In silico* and *in vitro* studies

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ABSTRACT: Histone deacetylases (HDAC) are evolutionary conserved enzymes, which catalyze removal of acetyl groups from histone and non-histone proteins, therefore, control multiple biological processes. Inhibition of their activities have been investigated to modify gene expression and/or protein functions not only for treatment of certain diseases but also for understanding functions of deacetylase isoforms. We previously synthesized aryl butenoic acid derivatives and identified their pan-HDAC inhibition activities. In this study, we investigated selective inhibition activities of these derivatives (C1, C3, C4) on class I HDACs using *in silico* and *in vitro* approaches. Molecular docking studies of the three aryl butenoic acid derivatives were performed on the crystal structures of HDAC 1, 2, 3 and 8, which were obtained from RCSB protein databank, using Glide software. *In vitro* inhibition activities of the compounds at two different concentrations were tested using fluorometric assay. *In silico* results indicated that all the compounds showed higher affinity to HDAC 1 and 8 than other class I deacetylases. *In vitro* analysis showed that the compounds inhibit HDAC 8 more effectively than HDAC 1. It was shown that C1 had higher binding affinity and inhibition activity to both enzymes. We concluded that, C1 inhibited both HDAC 1 and 8, however, C3 and C4 showed slight selectivity for HDAC 8 over HDAC 1, which was in agreement with the docking studies. Further cell culture studies will be valuable to determine increased acetylation on target proteins in response to compound treatment.

KEYWORDS: Histone deacetylase inhibitors; aryl butenoic acid derivatives; molecular docking; *in vitro* activity screening.

1. INTRODUCTION

Genomic information is regulated by epigenome, which consists of covalent modifications of DNA and histone proteins. These modifications are controlled by various enzymes known as writers, readers and erasers, and interplay of these protein groups set specific marks on chromatin. While writer proteins introduce modifications, readers recognize them and remodel chromatin to regulate gene expression [1, 2] Some modifications are reversible and removed by eraser proteins. One of the reversible modifications is acetylation of histones which are small and positively charged proteins playing role in DNA packaging. Histones are acetylated at lysine amino acids, mostly in amino terminal tail of histones by a group of writer enzymes, histone acetyltransferases (HATs). Acetylation creates an open chromatin structure by altering packaging of DNA and histones, which allows gene expression. Acetyl groups of histones are removed by histone deacetylase enzymes (HDACs) and deacetylation represses transcription through condensation of chromatin, which prevents accessibility of transcription factors on genomic regions. To date, 18 HDAC subtypes have been identified in human and grouped into four classes according to their sequence and structural homologies. Class I (HDAC 1, 2, 3, 8), II (HDAC IIa; 4, 5, 7, 9, IIb; 6, 10) and IV (HDAC11) enzymes require zinc, although class III (SIRT 1-7) require NAD+ for their catalytic activities [3-5]. Classical HDACs (class I and II) remove acetyl groups from both histone and non-histone proteins (transcription factors, structural proteins, enzymes etc). HDACs have different catalytic activities such that class IIa HDACs have minimal deacetylase activities, which can function in the presence of class I HDACs. In parallel to their functions, subcellular localization of class I HDACs is primarily nucleus, class II enzymes shuttle between nucleus and cytoplasm [6, 7]. HDACs control multiple cellular processes such as proliferation, differentiation and apoptosis by either regulating

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gene expression directly or interacting with non-histone regulatory/structural proteins [8, 9]. These properties make HDACs suitable targets for modifying molecular and cellular mechanisms. Since it has been known that HDACs are druggable targets, several pan or selective inhibitors with different potencies have been developed and tested for the treatment of various neurodegerative diseases such as spinal muscular atrophy, amyotrophic lateral sclerosis, Huntington's and Alzheimer's diseases as well as different types of cancer [9-16]. Classical HDAC inhibitors are structurally divided as hydroxamic acids, short-chain fatty acids, benzamides and cyclic tetrapeptides. Several pre-clinical and clinical studies have been performed with these inhibitors to evaluate their therapeutic potentials, especially for cancers. Besides, three hydroxamic acids (Vorinostat, Belinostat, Panobinostat) and one cyclic tetrapeptide (Romidepsin) have been approved by FDA as anti-cancer drugs for lymphoma and myeloid myeloma (Figure 1).

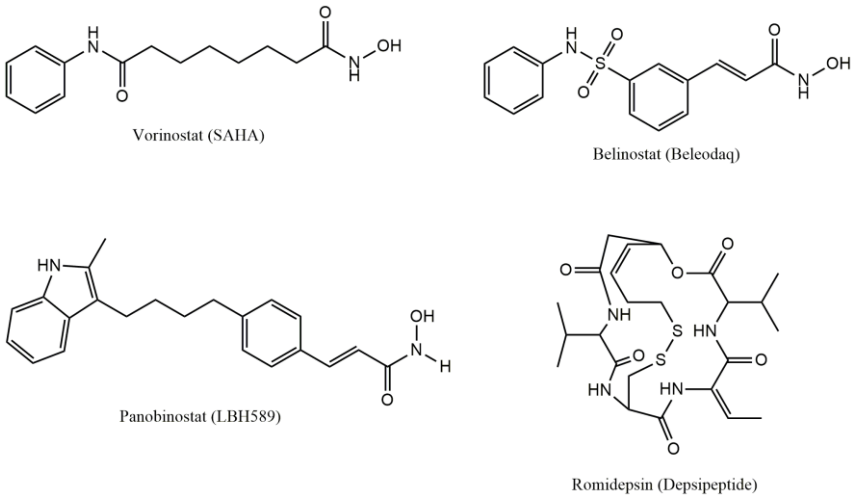

Figure 1. Chemical structures of FDA-approved HDAC inhibitors.

It has been known that therapeutic efficacies of HDAC inhibitors are limited due to resistance and side effects [17-20]. Therefore identification of selective HDAC inhibitors are important for both increasing efficacy and reducing side effects. Our group previously synthesized three aryl butenoic acid derivatives and identified their pan-HDAC inhibition activities [21, 22]. In this study, we focused on class I HDACs (1, 2, 3, 8) due to their catalytic potencies and analyzed selective inhibition activities of three aryl butenoic acid derivatives (C1, C3 and C4) by using *in silico* molecular modelling and *in vitro* activity screening approaches.

2. RESULTS

Class I HDACs are zinc-containing enzymes including HDAC 1, 2, 3, and 8 showing high sequence identity and catalytic site similarity. Among them, HDAC 8 functions alone, however others need to recruit into multi-subunit co-repressor complexes for activity [23]. Inhibitors of class I HDACs are known to bind to the catalytic site by interacting as the fourth ligand of Zn^{+2}, which form the other three coordination bonds with the side chains of two aspartic acid and one histidine residues [24]. Among our previously reported compounds, C1, C3, and C4, which showed promising pan-HDAC inhibition were docked to the crystal structures of class I HDACs to predict their affinity to these enzymes [21]. C1 was found to bind to HDAC 1 stronger than C3 and C4 (Table 1), by interacting with the Zn^{+2} through the carbonyl oxygen at the 2nd position of its butanoate moiety. C1 was stabilized in the catalytic site through π-π interactions with Phe150. C3 and C4 interacted with the Zn^{+2} through the oxygen of their p-methoxybenzene ring. C3 accepted an H bond via its morpholine oxygen from Asn95 side chain, and C4 from Gly149 backbone via the carbonyl oxygen at the 1st position of its butenedione moiety (Figure 2).

Binding orientations of the compounds were very similar in the catalytic site of HDAC 2 compared to HDAC 1, except C1, which interacted with Zn^{+2} through the 4-methoxybenzene oxygen like C3 and C4 (see Supplementary Material for details). This lowered its affinity to the receptor probably due to the steric hindrance caused by the methyl group linked to the oxygen compared to its carbonyl oxygen (Table 1). In the case of HDAC 3, however, C1's binding mode was very similar to that with HDAC 1, and so were the docking scores. In the shallower active cavity of HDAC 3, however, the morpholine moieties of C3 and C4 were partly solvent exposed, which lead to low binding affinities. C1 showed higher affinity to HDAC 8 than C3 and C4,

according to the docking studies (Table 1). The orientation of C1 regarding the zinc was same as that in HDAC 1 (Figure 2). We observed two H bonds accepted by the carbonyl oxygens of C1 from His142 and Tyr306 side chains. The co-crystallized ligand, N-hydroxy-4-(naphthalen-1-yl)benzamide, in HDAC 8's catalytic site also makes H bond with the former. Docking scores of C3 and C4 were better for HDAC 8, although their binding modes were very similar to those in HDAC 1 catalytic site. Both compounds made π-π contacts with Phe152 side chain, which also engages in hydrophobic contacts with the co-crystallized ligand.

Table 1. Docking scores (kcal/mol) of C1, C3, and C4 in class I HDACs' catalytic sites.

Compound	HDAC 1	HDAC 2	HDAC 3	HDAC 8
C1	-7.89	-5.80	-7.32	-7.59
C3	-5.35	-6.28	-3.93	-6.66
C4	-5.04	-6.19	-3.66	-6.14

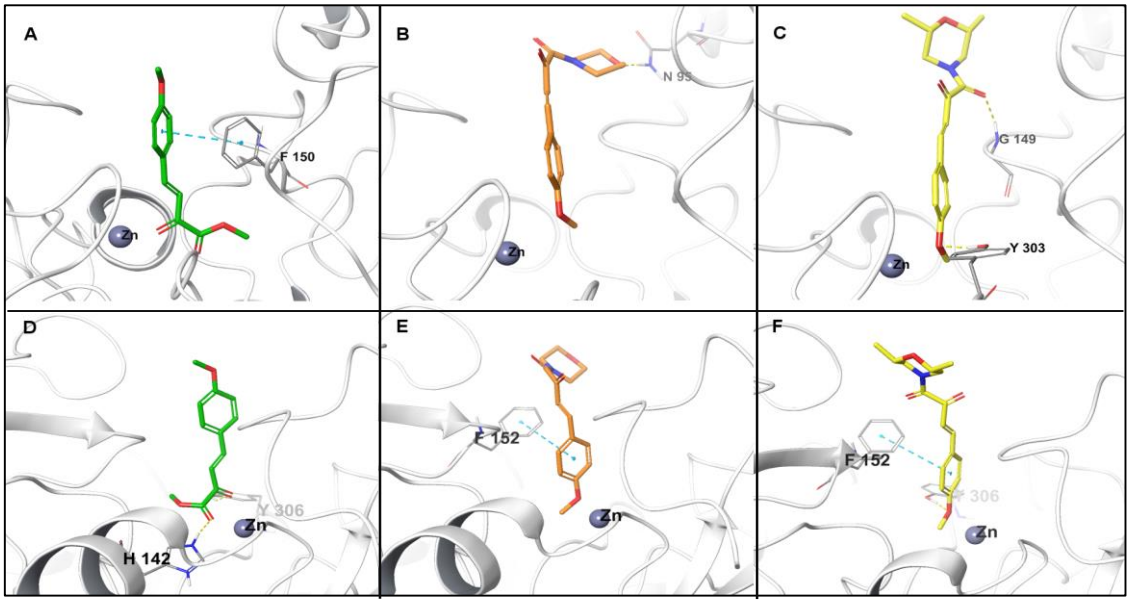

Figure 2. Docking poses of C1 (green), C3 (orange), and C4 (yellow) in the catalytic site of HDAC 1 (A, B, and C, respectively) and HDAC 8 (D, E, and F respectively).

In silico results showed that all the compounds bound to the zinc-containing catalytic site of HDAC 1 and HDAC 8, however C1 showed higher affinity to both enzymes. Their binding modes revealed favorable interactions with Zn^{+2} and a number of catalytically relevant residues. Therefore, HDAC 1 and HDAC 8 inhibition activities of the compounds were tested *in vitro* using recombinant enzymes in a fluorometric assay. We found that both HDAC 1 and HDAC 8 inhibition activities of the compounds were close to sodium phenylbutyrate (NaPBA), which is short chain fatty acid-based pan-HDAC inhibitor at 50 µM concentration. However, inhibition potencies of compounds were higher than NaPBA at 500 µM concentration. We found that compounds generally inhibit HDAC 8 more effectively than HDAC 1. C1 has better HDAC 1 inhibition activity than C3 and C4, however, HDAC 8 inhibition activities of compounds were similar (Table 2, Figure 3).

Table 2. *In vitro* HDAC 1 and HDAC 8 inhibition activities of C1, C3 and C4.

Compound	HDAC 1 activity (%)		HDAC 8 activity (%)	
	50 µM	500 µM	50 µM	500 µM
NaPBA	9	28	34	72
C1	18	89	31	91
C3	9	49	37	95
C4	9	43	41	89

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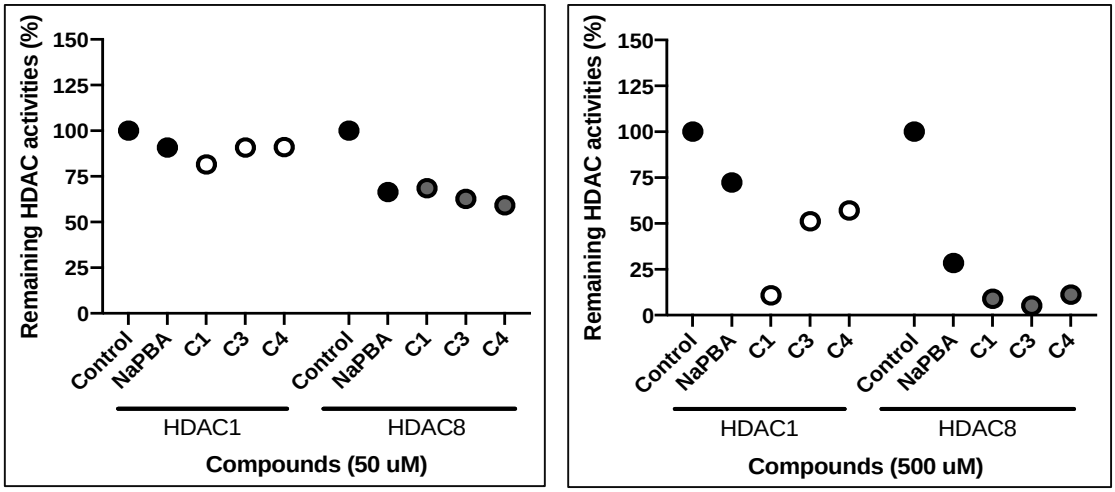

Figure 3. *In vitro* inhibition activities of C1, C3 and C4 at 50 and 500 µM concentrations. Remaining HDAC 1 and HDAC 8 activities in inhibitor-treated wells were calculated according to no inhibitor "controls".

3. DISCUSSION

HDACs are important players of epigenetic regulation via histone modifications. Preventing activities of HDACs by natural or synthetic inhibitors have been investigated to alter gene expression for the treatment of several diseases such as cancer and neurodegenerative diseases. Besides, deacetylation of non-histone proteins makes HDACs as popular therapeutic targets to modify different biological processes. Therefore, several molecules have been identified or developed as selective/non-selective inhibitors of HDACs [25-29]. Depending on chemical structures, these inhibitors interact with HDACs differently, which affects their potencies, toxicities and eventually efficacies. Previously, we identified three aryl butenoic acid derivatives, C1, C3, and C4, which showed more than 50% inhibition against pan-HDAC panel at 500 µM concentration and their binding properties to human HDAC8 were evaluated [21]. In this study, due to the availability of isoform-specific contemporary crystal structures and *in vitro* screening platforms, we could identified class I HDAC isoform-specific inhibition profiles of the compounds in the light of the molecular docking studies, which was performed using various HDAC isoforms. We focused on class I HDACs due to their potent deacetylase activities. Our molecular docking studies showed that HDAC 1 and HDAC 8 binding affinities of the compounds were higher than the other class I HDACs. In the case of HDAC 2, binding orientation of C1 changed and its affinity reduced. Relatively smaller cavity of HDAC 3 was unable to accommodate C3 and C4 effectively, which was apparent with their docking scores. Therefore, HDAC 1 and HDAC 8 inhibition activities of the compounds were tested using an *in vitro* fluorometric assay. Similar to *in silico* results, we found that the compounds generally inhibited HDAC 8 more effectively than HDAC 1. At low concentration (50 µM), the compounds weakly inhibited HDAC enzymes, however, inhibition potencies increased at high concentration (500 µM), especially for HDAC 8. Among the three compounds, C1 non-selectively inhibited both HDAC 1 and HDAC 8, however, C3 and C4 showed slight selectivity for HDAC 8 over HDAC 1. This selectivity was also apparent in their docking scores with these enzymes. Concerning the HDAC8 binding profiles, the results of the current study clearly shows better correlation with the *in vitro* results than those of Ayhan Eşiyok et. al [21], probably owing to Glide, the preferred current crystal structure and HDAC8-specific *in vitro* screening assay that was used.

Increased expression of HDAC 1 and HDAC 8 was reported in some cancer types such as myeloma and neuroblastoma [30, 31]. Additionally, several reports indicated both neurotoxic or neuroprotective properties of HDAC 1 [8]. This is also the case for other HDACs, except HDAC 8, since there is lack of information about neurodegeneration. The possible reason is that HDACs form multiprotein complexes and depending on interaction partners, HDACs can deacetylate various proteins and involve in different biological processes. Therefore, same HDAC can have opposing functions depending on the cell type, subcellular localization and its targets. Although HDACs are suitable as drug targets, this makes inhibitor design complicated and indicates that elucidating functions of HDAC subtypes are essential to evaluate the effects of inhibitors. Selective inhibitors can also be used as tools to understand functions of HDAC subtypes, as well [8].

4. CONCLUSION

We concluded that aryl butenoic acid derivatives have different inhibition potencies and selectivities over class I HDACs. According to our *in silico* and *in vitro* results, C1 inhibits both HDAC 1 and HDAC 8 enzymes however, C3 and C4 are slightly selective for HDAC 8 over HDAC 1. Molecular modelling and *in vitro* screening are powerful approaches for inhibitor design, however cell culture studies are required to determine both inhibition activities on native HDAC conformations and increased acetylation of target proteins.

5. MATERIALS AND METHODS

5.1. Chemistry

Compounds 1a, C1, C3 and C4 were synthesized as shown in Figure 4. The construction of aryl butenoic acid ester involved the usual condensation reactions of corresponding p-anisaldehyde with pyruvic acid under basic conditions followed by esterification with HCl. The ester moiety was functionalized with morpholines in the presence of triethylaluminum. Details about synthesis of the compounds were both given in patent description and Esiyok et al. [21, 22]. NMR spectra of the compounds are given in Supplementary Material (Figure S1-4).

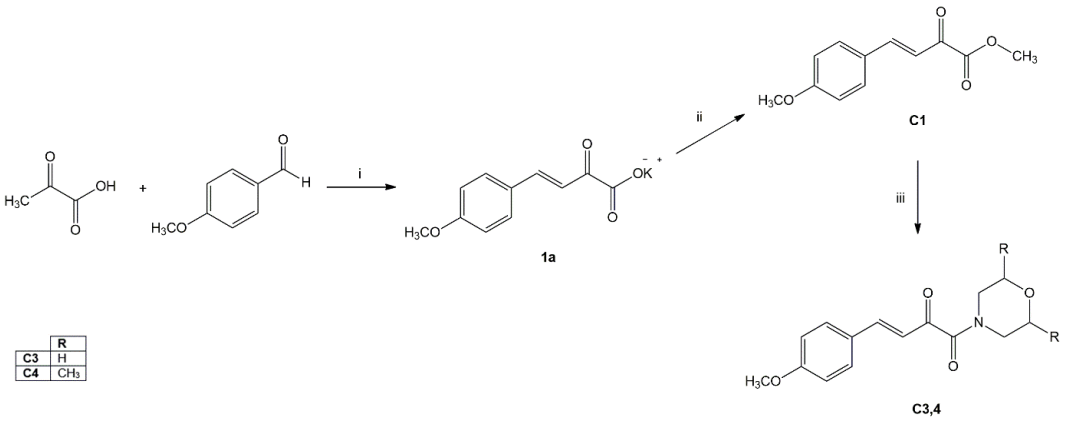

Figure 4. Synthesis diagram of 1a, C1, C3 and C4. Reagents and conditions: *(i) KOH, MeOH, 0 °C; (ii) CH_3COCl, MeOH, 65 °C; (iii) morpholine / 2,6-dimethyl morpholine, $Al(C_2H_5)_3$, toluene, heptane, 80 °C.*

5.2. Molecular modeling

The compounds were modelled and minimized using MacroModel (2018-4, Schrödinger, LLC, New York, NY, 2018) and OPLS 2005 force field parameters [23]. The crystal structure of HDAC 1 (PDB ID: 5ICN), HDAC 2 (PDB ID: 4LXZ), HDAC 3 (PDB ID: 4A69) and HDAC 8 (PDB ID: 5FCW) was downloaded from RCSB Protein Data Bank (www.rcsb.org) and prepared for docking with the Protein Preparation Wizard (2018-4, Schrödinger, LLC, NY, 2018) of Maestro (2018-4, Schrödinger, LLC, NY, 2018) [23, 33-37]. In this procedure unwanted residues were removed and the protons were handled with Epik (2018-4, Schrödinger, LLC, New York, NY, 2018), water orientations were sampled and H bonds were set with Propka. Receptor grids was generated for the catalytic site of each enzyme structure taking the centroid coordinates of each co-crystallized ligand. Glide (2018-4, Schrödinger, LLC, New York, NY, 2018) was used to dock the ligands to these grids at standard precision with 50 runs for each ligand [38-40]. Docking scores are expressed as XP GScore in kcal/mol. The co-crystal ligands were re-docked to HDAC 1, 2, 3 and 8; the obtained binding poses were close their original conformations (2.43, 1.00, 0.26, and 0.35 Å, respectively).

5.3. *In vitro* HDAC inhibition activity assay

HDAC 1 and 8 inhibition activities of the compounds were tested using *in vitro* HDAC fluorometric drug discovery assay kits (Enzo). Briefly, recombinant HDAC 1 or HDAC 8 enzymes were mixed with compounds at 50 and 500 µM final concentrations. To initiate reactions, Fluor de Lys substrate was added and microplates were placed into 37°C incubator for 1h. Reactions were stopped by addition of developer II, containing 1 µM final concentration of trichostatin A (TSA), a potent hydroxamate-based pan-HDAC inhibitor.

Microplates were then kept in dark at room temperature for additional 45 min before measuring fluorophore at 360-460 nm wavelengths with a microplate reader (Molecular Devices Spectramax M2). TSA (5 μM) was also used as a positive control. Inhibition activity of sodium phenylbutyrate (NaPBA), a short-chain fatty acid-based pan-HDAC inhibitor was tested at the same concentrations together with the compounds due to structural similarities. Measurements were performed in triplicate and the percentage of remaining HDAC activities in inhibitor-containing wells were normalized to no inhibitor controls. Graphpad prism 8 software was used for data analysis.

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Conflict of interest statement: The authors declared no conflict of interest.

Appendix A. Supplementary Material

Supplementary material related to this article can be accessed at http://doi.org/10.35333/jrp.2019.42.

REFERENCES

[1] Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000; 403(6765): 41-45. [CrossRef]

[2] Nicholson TB, Veland N, Chen T. Writers, Readers, and Erasers of Epigenetic Marks. In: Gray SG. Epigenetic cancer therapy, first ed., Academic Press, Elsevier, 2015, pp. 31-66

[3] Seto E, Yoshida M. Erasers of histone acetylation: the histone deacetylase enzymes. Cold Spring Harb Perspect Biol. 2014; 6(4): a018713: 1-26. [CrossRef]

[4] Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res. 2011; 21(3): 381-395. [CrossRef]

[5] De Ruijter AJ, Van Gennip AH, Caron HN, Kemp S, Van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003; 370: 737-749. [CrossRef]

[6] Fischle W, Dequiedt F, Hendzel MJ, Guenther MG, Lazar MA, Voelter W, Verdin E. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell. 2002; 9(1): 45-57. [CrossRef]

[7] Morris MJ, Monteggia LM. Unique functional roles for class I and class II histone deacetylases in central nervous system development and function. Int J Dev Neurosci. 2013; 31(6): 370-381. [CrossRef]

[8] Thomas EA, D'Mello SR. Complex neuroprotective and neurotoxic effects of histone deacetylases. J Neurochem. 2018; 145(2): 96-110. [CrossRef]

[9] Didonna A, Opal P. The promise and perils of HDAC inhibitors in neurodegeneration. Ann Clin Transl Neurol. 2015; 2(1): 79-101. [CrossRef]

[10] Jia H, Morris CD, Williams RM, Loring JF, Thomas EA. HDAC inhibition impairs beneficial transgenerational effects in Huntington's disease mice via altered DNA and histone methylation. Proc Natl Acad Sci U S A. 2015; 6; 112(1): E56-64. [CrossRef]

[11] Yang SS, Zhang R, Wang G, Zhang YF. The development prospection of HDAC inhibitors as a potential therapeutic direction in Alzheimer's disease. Transl Neurodegener. 2017; 6 (19): 1-6. [CrossRef]

[12] Lai JI, Leman LJ, Ku S, Vickers CJ, Olsen CA, Montero A, Ghadiri MR, Gottesfeld JM. Cyclic tetrapeptide HDAC inhibitors as potential therapeutics for spinal muscular atrophy: screening with IPSC-derived neuronal cells. Bioorg Med Chem Lett. 2017; 27(15): 3289-3293. [CrossRef]

[13] Lazo-Gómez R, Ramírez-Jarquín UN, Tovar-Y-Romo LB, Tapia R. Histone deacetylases and their role in motor neuron degeneration. Front Cell Neurosci. 2013; 7: 243, 1-7. [CrossRef]

[14] Jones PA, Issa JP, Baylin S. Targeting the cancer epigenome for therapy. Nat Rev Genet. 2016; 17(10): 630-641. [CrossRef]

[15] Bora-Tatar G, Erdem-Yurter H. Investigations of curcumin and resveratrol on neurite outgrowth: perpectives on spinal muscular atrophy. Biomed Res Int. 2014; 709108: 1-8. [CrossRef]

[16] Lunke S, El-Osta A. Applicability of histone deacetylase inhibition for the treatment of spinal muscular atrophy. Neurotherapeutics. 2013; 10(4): 677-687. [CrossRef]

[17] Abend A, Kehat I. Histone deacetylases as therapeutic targets: from cancer to cardiac disease. Pharmacol Ther. 2015; 147: 55-62. [CrossRef]

[18] Manal M, Chandrasekar MJ, Gomathi Priya J, Nanjan MJ. Inhibitors of histone deacetylase as antitumor agents: A critical review. Bioorg Chem. 2016; 67: 18-42. [CrossRef]

[19] Suraweera A, O'Byrne KJ, Richard DJ. Combination therapy with histone deacetylase inhibitors (HDACi) for treatment of cancer: Achieving the full potential of HDACi. Front Oncol. 2018; 8(92): 1-15. [CrossRef]

[20] Mehnert JM, Kelly WK. Histone deacetylase inhibitors: Biology and mechanism of action. Cancer J. 2007; 13(1): 23-29. [CrossRef]

[21] Esiyok PA, Seven O, Eymur G, Bora-Tatar G, Dayangaç-Erden D, Yelekci K, Yurter H, Demir AS. Aryl butenoic acid derivatives as a new class of histone deacetylase inhibitors: synthesis, in vitro evaluation, and molecular docking studies. Turk J Chem. 2014; 38(2): 338-344. [CrossRef]

[22] Türk Patent ve Marka Kurumu, Patent başvuru numarası: 2011/03844, Buluş başlığı; Aril bütanoik asit türevleri, bunların HDAC inhibitör aktiviteleri ve gen ekspresyonunu arttırıcı özellikleri. http://online.turkpatent.gov.tr/EPATENT/servlet/PreSearchRequestManager

[23] Watson PJ, Millard CJ, Riley AM, Robertson NS, Wright LC, Godage HY, Cowley SM, Jamieson AG, Potter BV, Schwabe JW. Insights into the activation mechanism of class I HDAC complexes by inositol phosphates. Nat Commun. 2016; 25(7): 11262. [CrossRef]

[24] Lombardi PM, Cole KE, Dowling DP, Christianson DW. Structure, mechanism, and inhibition of histone deacetylases and related metalloenzymes. Curr Opin Struct Biol. 2011; 21(6): 735-743. [CrossRef]

[25] Uba AI, Yelekçi K. Identification of potential isoform-selective histone deacetylase inhibitors for cancer therapy: a combined approach of structure-based virtual screening, ADMET prediction and molecular dynamics simulation assay. J Biomol Struct Dyn. 2018; 36(12): 3231-3245. [CrossRef]

[26] Hsu KC, Liu CY, Lin TE, Hsieh JH, Sung TY, Tseng HJ, Yang JM, Huang WJ. Novel class IIa-selective histone deacetylase ınhibitors discovered using an in silico virtual screening approach. Sci Rep. 2017; 7: 3228. [CrossRef]

[27] Ingham OJ, Paranal RM, Smith WB, Escobar RA, Yueh H, Synder T, Porco JA Jr, Bradner JE, Beeler AB. Development of a potent and selective HDAC8 inhibitor. ACS Med Chem Lett. 2016; 7(10): 929-932. [CrossRef]

[28] Noor Z, Afzal N, Rashid S. Exploration of novel inhibitors for class I histone deacetylase isoforms by QSAR Modeling and Molecular Dynamics Simulation Assay. PLoS One. 2015; 10(11): e0143155. [CrossRef]

[29] Bora-Tatar G, Dayangaç-Erden D, Demir AS, Dalkara S, Yelekçi K, Erdem-Yurter H. Molecular modifications on carboxylic acid derivatives as potent histone deacetylase inhibitors: activity and docking studies. Bioorg Med Chem. 2009; 17(14): 5219-5228. [CrossRef]

[30] Mithraprabhu S, Kalff A, Chow A, Khong T, Spencer A. Dysregulated Class I histone deacetylases are indicators of poor prognosis in multiple myeloma. Epigenetics. 2014; 9(11): 1511-1520. [CrossRef]

[31] Yoon S, Eom GH. HDAC and HDAC Inhibitor: From Cancer to Cardiovascular Diseases. Chonnam Med J. 2016; 52(1): 1–11. [CrossRef]

[32] Banks JL, Beard HS, Cao Y, Cho AE, Damm W, Farid R, Felts AK, Halgren TA, Mainz DT, Maple JR, Murphy R, Philipp DM, Repasky MP, Zhang LY, Berne BJ, Friesner RA, Gallicchio E, Levy RM. Integrated modeling program, applied chemical theory (IMPACT). J Comput Chem. 2005; 26(16): 1752-1780. [CrossRef]

[33] Lauffer BE, Mintzer R, Fong R, Mukund S, Tam C, Zilberleyb I, Flicke B, Ritscher A, Fedorowicz G, Vallero R, Ortwine DF, Gunzner J, Modrusan Z, Neumann L, Koth CM, Lupardus PJ, Kaminker JS, Heise CE, Steiner P. Histone deacetylase (HDAC) inhibitor kinetic rate constants correlate with cellular histone acetylation but not transcription and cell viability. J Biol Chem. 2013; 288(37): 26926-26943. [CrossRef]

[34] Watson PJ, Fairall L, Santos GM, Schwabe JW. Structure of HDAC3 bound to co-repressor and inositol tetraphosphate. Nature. 2012; 481(7381): 335-340. [CrossRef]

[35] Tabackman AA, Frankson R, Marsan ES, Perry K, Cole KE. Structure of 'linkerless' hydroxamic acid inhibitor-HDAC8 complex confirms the formation of an isoform-specific subpocket. J Struct Biol. 2016; 195(3): 373-378. [CrossRef]

[36] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res. 2000; 28(1): 235-242. [CrossRef]

[37] Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aided Mol Des. 2013; 27(3): 221-234. [CrossRef]

[38] Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem. 2004; 47(7): 1739-1749. [CrossRef]

[39] Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem. 2004; 47(7): 1750-1759. [CrossRef]

[40] Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem. 2006; 49(21): 6177-6196. [CrossRef]