RESEARCH ARTICLE

Pharmacological Analysis of Vorinostat Analogues as Potential Anti-tumor Agents Targeting Human Histone Deacetylases: an Epigenetic Treatment Stratagem for Cancers

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Abstract

Alteration of the acetylation status of chromatin and other non-histone proteins by HDAC inhibitors has evolved as an excellent epigenetic strategy in treatment of cancers. The present study was sought to identify compounds with positive pharmacological profiles targeting HDAC1. Analogues of Vorinostat synthesized by Cai et al, 2015 formed the test compounds for the present pharmacological evaluation. Hydroxamte analogue 6H showed superior pharmacological profile in comparison to all the compounds in the analogue dataset owing to its better electrostatic interactions and hydrogen bonding patterns. In order to identify compounds with even better high affinity and pharmacological profile than 6H and Vorinostat, virtual screening was performed. A total of 83 compounds similar to Vorinostat and 154 compounds akin to analogue 6H were retrieved. SCHEMBL15675695 (PubCid: 15739209) and AKOS019005527 (PubCid: 80442147) similar to Vorinostat and 6H, were the best docked compounds among the virtually screened compounds. However, in spite of having good affinity, none of the virtually screened compounds had better affinity than that of 6H. In addition SCHEMBL15675695 was predicted to be a carcinogen while AKOS019005527 is Ames toxic. From, our extensive analysis involving binding affinity analysis, ADMET properties predictions and pharmacophoric mappings, we report Vorinostat hydroxamate analogue 6H to be a potential candidate for HDAC inhibition in treatment of cancers through an epigenetic strategy.

Keywords: Histone deacetylases - Vorinostat - virtual screening - molecular docking - pharmacological profiling

Asian Pac J Cancer Prev, **17 (3)**, 1571-1576

Introduction

Epigenetic regulation has gained global attention in cancer pathogenesis wherein the genetic mutations have fewer roles to play in tumour development. The epigenetic events involving acetylation and deacetylation of histones have surfaced as important cellular processes that regulate the fate of gene expressions observed in cancer progression (Kelly et al., 2010; Blair et al., 2012). The acetylation states of histones are determined by Histone deacetylases (HDACs) that removes acetyl moiety from histone proteins for chromatin remodeling and therefore regulates gene expression at the post transcriptional level (Johnstone et al., 2002; Ruijter et al., 2003; Clayton et al., 2006). Histone deacetylation carried out by HDAC's at Lys16 and trimethylation at Lys20 of histone H4 leads to compacting of the chromatin structure and tight folding of the nucleosome, thus preventing the binding of transcription factors to their respective DNA binding sites, leading to gene silencing of important tumor suppressor genes like p53 (Fraga et al., 2005) and since tumor suppressor genes ensures proper regulation of the cell, silencing of tumor suppressors leads to "loss of check" on cell cycle regulation thereby leading to abnormal cell proliferations (Sheh et al., 2002; Agrawal et al., 2007).

Alterations in HDAC activity have been observed in numerous cancers including haematological malignancies and solid tumors (Dokmanovic et al., 2007). Altered expression and aberrant recruitment of HDACs have been reported in various tumours. For example, overexpression of HDAC1, HDAC2, HDAC3, HDAC6 and SIRT7 (Glozak et al., 2007; Mariadason et al., 2008) have been identified in colon, breast, prostate, thyroid, cervical and gastric cancers and promyelocytic leukaemia and non-Hodgkin's lymphoma (Dokmanovic et al., 2005; Bolden et al., 2006; Fouladi et al., 2006; Lafon-Hughes et al., 2008). In fact, perturbations in histone acetylation with a number of well-characterized cellular oncogenes

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and tumor-suppressor genes became so pertinent that it prompted the search for pharmacologic agents capable of inhibiting HDAC on a hope that inhibitors might induce reactivation of tumor suppressor genes that have been silenced during the course of neoplastic transformation.

Alteration of the acetylation status of chromatin and other non-histone proteins by HDAC inhibitors has evolved as excellent epigenetic treatment strategies in treatment of cancers. The functional inhibition of HDAC's results in various molecular modifications including change in gene expression, induction of cell death, apoptosis, cell cycle arrest, and inhibition of angiogenesis and metastasis. Evidence suggests that HDAC inhibitors successfully induced polyploidy (Xu et al., 2005) and aberrant mitosis such as mitotic slippage (Stevens et al., 2008) and premature sister chromatid separation (Magnaghi et al., 2007) all of these events which lead to loss of cancer cell proliferation.

Currently, numerous new agents that promise a new paradigm shift in cancer management are being investigated in phase I, II and III clinical trials (Piekarz et al., 2007). Several HDAC inhibitors were recently investigated in clinical trials as single agents or in combination therapy with other chemotherapeutic agents for haematological and/or solid tumors (Piekarz et al., 2007).

The first ever HDAC inhibitor approved by FDA was Vorinostat (SAHA, Zolinza TM; Merck) that enhanced tumor reversal in refractory peripheral T-cell lymphoma (Grant et al., 2007). Structure activity studies have revealed that Vorinostat (and other HDACs as well) harbors Zn2+ binding group (ZBG), chelating Zn2+ at the bottom of the active binding site of HDACs; a hydrophobic linker occupying the long and narrow tube-like channel; a surface recognition group (cap), essential for recognizing and interacting with amino acid residues of the HDACs active site; and a polar connection unit (CU) linking "cap group" with the hydrophobic linker. The keen perusal at common pharmacophores of Vorinostat led to the development of novel HDAC inhibitors by Cai et al (2015). They synthesized series of HDAC inhibitors with 1, 2, 4-oxadiazole–containing Vorinostat analogues. The analogue manifests efficient anti proliferative activities against cancer cells and demonstrated elevated potency in inhibiting HDAC enzymes.

Therefore, in the view of given observation, the present study focuses on computer based pharmacological profiling, evaluation and identification of high affinity Vorinostat analogues from the dataset of compound synthesized by Cai et al (2015). In addition, a possible attempt has also been pursued to identify still better compound other than analogues from the present dataset through virtual screening approaches.

Materials and Methods

Selection of compound dataset

1, 2, 4-oxadiazole–containing Vorinostat analogues designed by Cai et al., 2015 formed the dataset of compounds for the present study (Table 1).

Preparation of protein and compounds

The crystal structure of HDAC was retrieved from Protein Data Bank (PDB) with PDB ID: 4LXZ (Lauffer et al., 2013) (Figure 2). The structure was downloaded in pdb format and was further prepared for docking process. The protein was prepared using the PrepWiz module of Schrodinger suite, 2013 (Schrodinger. LLC, New York, NY). In the preparation procedure, the protein was first preprocessed by assigning the bond orders and hydrogens, creating zero order bonds to metals and adding disulphide bonds. The missing side chains and loops were filled using Prime Module of Schrodinger. Further all the water molecules were deleted beyond 5 Å from hetero groups. Once the protein structure was preprocessed, H bonds were assigned which was followed by energy minimization by OPLS 2005 force field (Jorgensen et al., 2005). The final structure obtained was saved in.pdb format for further studies. All the ligands were optimized through OPLS 2005 force field algorithm embedded in the LigPrep module of Schrodinger suite. The ionizations of the ligand were retained at the original state and were further desalted. The structures thus optimized were saved in sdf format for docking procedures.

Structure Similarity search

The compound with superior pharmacological profile

Table 1. Affinity (Rerank) scores and IC_{50} values (predicted by Cai et al., 2015) of Vorinostat analogues.

Compounds	Moldock Score	Rerank Score	Hdac Ic_{50} (Mm)
6a	-129.008	-105.72	0.38
6b	-134.486	-109.929	0.49
6c	-132.912	-106.06	2.18
6d	-135.575	-107.789	1.92
6e	-135.539	-106.922	1.15
6f	-130.43	-105.806	0.68
6g	-129.268	-100.058	0.19
6h	-133.353	-134.992	0.07
6i	-132.133	-109.166	0.35
6j	-133.519	-100.247	0.12
6k	-127.336	-103.077	0.08
9a	-150.699	-111.533	0.21
9b	-133.655	-98.1817	0.14
9c	-154.428	-100.507	0.91
9d	-146.144	-111.099	3.04
9e	-152.774	-119.649	0.88
9f	-161.269	-106.109	1.56
9g	-142.292	-105.977	4.13
9h	-155.474	-123.977	8.67
9i	-152.381	-121.652	0.82
9j	-148.093	-111.07	0.76
9k	-149.596	-117.621	1.05
10a	-130.845	-106.573	5.32
10b	-127.253	-102.103	>10
10c	-128.922	-107.405	9.63
10d	-139.271	-115.017	8.25
10e	-127.864	-103.141	>10
10f	-128.268	-104.738	>10
10g	-123.394	-101.018	7.65
10h	-117.207	-96.7902	>10
10i	-129.155	-107.737	3.98
10j	-130.813	-101.752	5.17

from the dataset was further used as query molecule in pursuit to identify still better drug like compound than any molecules mentioned in the dataset. Similarity search was supervised by Binary Finger Print Based Tanimoto similarity equation to retrieve compounds with similarity threshold of 95 % against NCBI's Pubchem compound database (Bandaru et al., 2014).

Molecular docking of compounds

Molecular docking program- Molegro Virtual Docker (MVD) which incorporates highly efficient PLP (Piece wise Linear potential) and MolDock scoring function provided a flexible docking platform (Yang et al., 2004, Thomsen et al., 2006). All the ligands were docked at the active site of HDAC. Docking parameters were set to 0.20Å as grid resolution, maximum iteration of 1500 and maximum population size of 50. Energy minimization and hydrogen bonds were optimized after the docking. Simplex evolution was set at maximum steps of 300 with neighborhood distance factor of 1. Binding affinity and interactions of ligands with protein were evaluated on the basis of the internal ES (Internal electrostatic Interaction), internal hydrogen bond interactions and sp2-sp2 torsions. Post dock energy of the ligand-receptor complex was minimized using Nelder Mead Simplex Minimization (using non-grid force field and H bond directionality) (Nelder et al., 1965). On the basis of rerank score, best interacting compound was selected from each dataset.

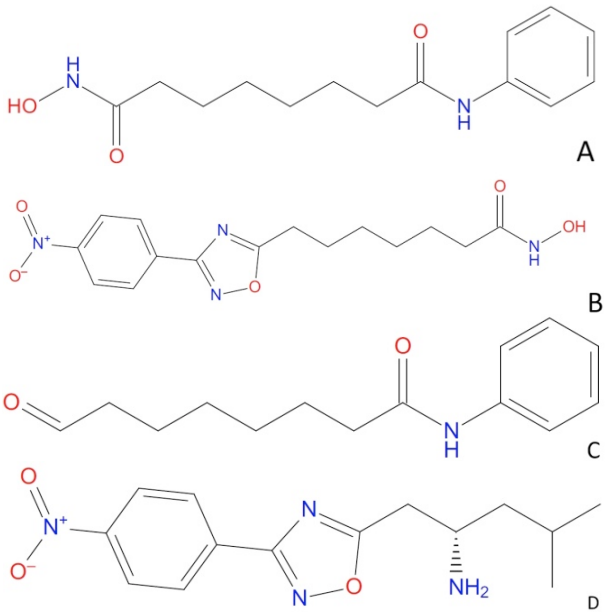

Figure 1. Structures of compounds (A) Vorinostat (B) 6H (C) Vorinostat Similar SCHEMBL15675695 (PubCid: 15739209) and (D) 6H Similar AKOS019005527 (PubCid: 80442147)

Bioactivity and ADMET profiling of compounds.

All the compounds were screened for its drug ability by Lipinski filters. Biological activity of the ligands was predicted using Molinspiration webserver (© Molinspiration Cheminformatics 2014). The complete ADMET properties were calculated using admetSAR (Cheng et al., 2012; Bandaru et al 2013).

Pharmacophoric Mapping

Pharmacophoric mapping involving ligand interaction patterns, hydrogen bond interaction, and hydrophobic interactions were evaluated using Accelrys Discovery Studio 3.5 DS Visualizer (Kelotra et al., 2014).

Results and Discussion

Table 1 shows the affinity (rerank) scores of various Vorinostat analogues of dataset1 along with the HDAC activity (IC_{50}) as assessed by the Cai et al. (2015). Evident from the docking (rerank) scores, hydroxamate compound

Table 2. Affinity Score Comparison of Vorinostat and Hydroxamate Analogue 6H with their Best Docked Similars

Compound	Rerank Score	No. of similar compounds retrieved	Similar compound with highest binding affinity	Rerank Score	Ratios of Rerank scores
Vorinostat	-106.281	154	SCHEMBL15675695 (Vorinostat Similar)	-115.62	6H: Vorinostat = 1.270
6H	-134.992	83	AKOS019005527 (6H Similar)	-111.396	6H:AKOS019005527 =1.211 6H: SCHEMBL15675695 =1.167

Table 3. Binding energy profile of parent compounds and its respective similar against HDAC

	6H	6H similar (AKOS019005527)	Vorinostat	Vorinostat Similar (SCHEMBL15675695)
Energy overview: Descriptors	Rerank Score	Rerank Score	Rerank Score	Rerank Score
Total Energy	-134.992	-111.396	-106.281	-115.620
External Ligand interactions	-148.547	-124.440	-113.249	-132.630
Protein - Ligand interactions	-148.547	-124.440	-113.249	-132.630
Steric (by PLP)	2.424	-97.303	-87.705	-118.040
Steric (by LJ12-6)	7.505	-21.196	-19.498	-10.648
Hydrogen bonds	-6.588	0	-4.406	-3.942
Internal Ligand interactions	-13.554	-13.044	-6.969	-17.010
Torsional strain	0.226	7.460	3.118	6.412
Steric (by PLP)	0.692	1.307	0.419	2.300

6H (Figure 1b) demonstrated highest binding affinity among all the analogues in the given dataset. From keen perusal of the structural details of 6H reveals that the highest binding affinity (Rerank Score: 134.992)can be attributed for the presence of nitro group at C4 of the phenyl group, in addition the presence of such structural moieties may perhaps also explain the appreciable IC 50 values (0.07). Compound 6H can therefore be potentially bioactive against HDAC's both in terms of affinity and enzyme activity. Our observations with having binding affinity predictions and HDAC activity estimations correlate for some compounds including 6H. However for other compounds the correlation is not observed. The discrepancies observed are an important subject for further investigation. However, taking into consideration all the compounds, unarguably 6H demonstrated highest binding affinity (Figure 2) and in addition showed optimal in vitro activity.

In further approach, in pursuit to identify even better molecule endowed with superior pharmacological profile than compound 6H and Vorinostat, virtual screening was performed against Pubchem database. A total of 83

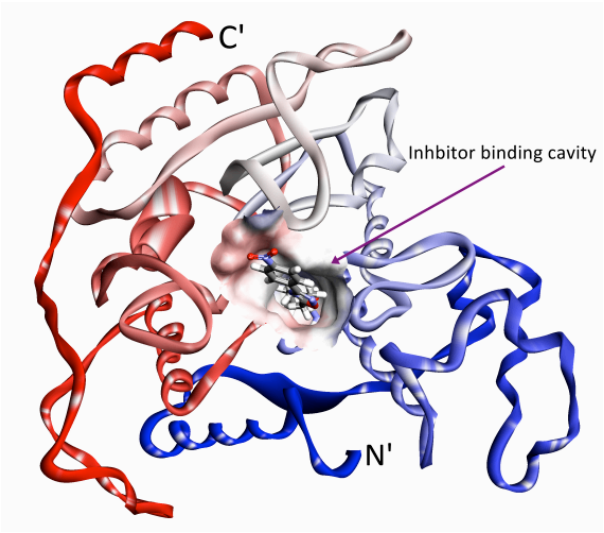

Figure 2. Compound 6H a Hydroxamate Analogue of Vorinostat in the Binding Pocket of HDAC1 (PDB ID: 1LXZ). Red to blue spectrum of the helix represent N to C terminal of the protein structure

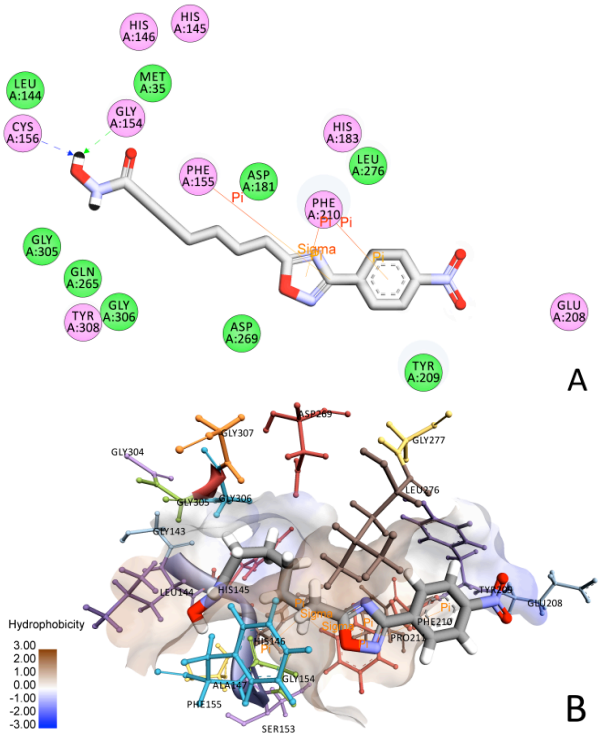

Figure 3. (A) Interactions of compound 6H in the active site of HDAC. Residues circled in green participate in van der Waals interaction with the ligand while residues in pink forms electrostatic interactions. Hydrogen bonds are shown as green and blue arrows between ligand and residues Cys 156 and Gly 154 (B) The active site of receptor is shown with hydrophobic intensities. The hydrophobic intensities of the binding site ranges from -3.00 (least hydrophobic area - blue shade) to 3.00 (highly hydrophobic area -brown shade)

Table 4. ADMET Profiles of Parent Compound and its Respective Similar

Model	6H	6H Similar (AKOS019005527)	Vorinostat	Vorinostat Similar (SCHEMBL15675695)
Absorption				
Blood-Brain Barrier	BBB+	BBB-	BBB+	BBB+
Human Intestinal Absorption	HIA+	HIA+	HIA+	HIA+
P-glycoprotein Substrate	Substrate	Substrate	Substrate	Substrate
P-glycoprotein Inhibitor	Inhibitor	Non-inhibitor	Inhibitor	Inhibitor
Distribution & Metabolism				
CYP450 2C9 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 Substrate	Substrate	Substrate	Substrate	Substrate
CYP450 1A2 Inhibitor	Non-inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4 Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
Excretion & Toxicity				
Human Ether-a-go-go-Related Gene Inhibition	Inhibitor	Inhibitor	Inhibitor	Inhibitor
AMES Toxicity	Non-AMES toxic	AMES toxic*	Non Ames toxic	Non AMES toxic
Carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Carcinogens*
Honey Bee Toxicity	Low HBT	Low HBT	Low HBT	Low HBT
Acute Oral Toxicity	III	III	III	III

Table 5. Bioactivity Prediction of Parent and Similar Compounds Against Various Drug Targets

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
6h	0.14	-0.14	-0.33	-0.12	-0.14	1.38*
Vorinostat	-0.08	-0.32	-0.21	-0.32	-0.09	0.16
6H similar (AKOS019005527)	0.17	0.07	-0.25	-0.20	-0.03	-0.02
Vorinostat Similar (SCHEMBL15675695)	-0.16	-0.56	-0.39	-0.47	-0.14	-0.09

*Compound 6h showing activity highest enzyme inhibition and least activity against other drug targets testifying its target specificity against enzymes (in the present case HDAC)

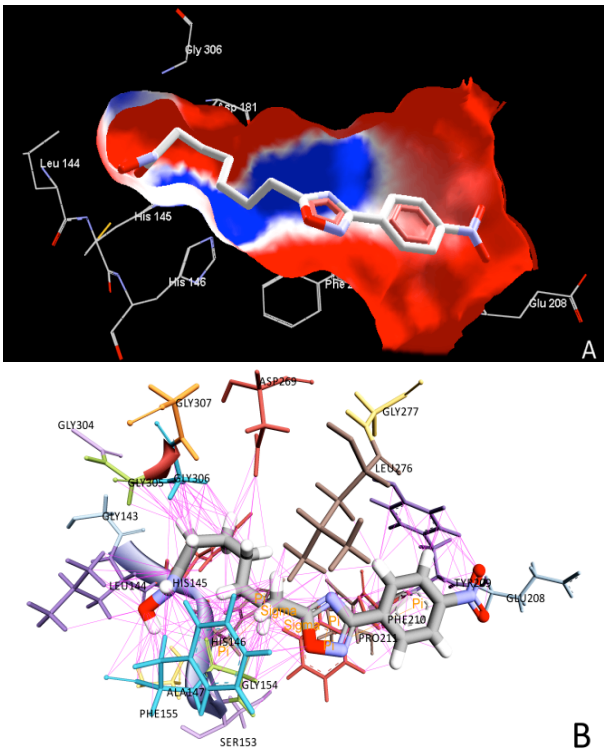

Figure 4. (A) Electrostatic interactions of 6H in the active site of HDAC1. The red surfaces of the protein in electrically negative while blue is positive and white represent electrically neutral surface of the binding surface (B) Binding pattern of 6H with HDAC. The pink lines represent various interactions like electrostatic, van der Waals, stearic, hydrogen bonding and hydrophobic interactions that enable energetically favourable binding of the ligand in the cavity

compounds structurally similar to compound 6H were retrieved while 154 structurally similar compounds were retrieved against its parent compound Vorinostat. All the similar compounds those akin to 6H and Vorinostat retrieved hitherto were docked against HDAC1. Compound AKOS019005527 (PubCid: 80442147) akin to 6H Similar (Figure 1d) showed superior binding affinity out of all the similar 83 compounds retrieved against its parent compound 26 d, while, compound SCHEMBL15675695 (PubCid: 15739209akin to Vorinostat (Figure 1c) demonstrated superior affinity .

It can be well noticed in Table 2 that none of the virtually screened compounds (neither the 6H similar nor Vorinostat similar) had higher binding affinity than 6H. The binding affinity of compound 6H is 1.3 times better than Vorinostat while it has 1.2 times better affinity than its similar compound AKOS019005527. In addition, 6H shows 1.16 folds higher binding affinity than Vorinostat similar SCHEMBL15675695. From the extensive analysis we presume that 6H may form a potential compound against HDAC at least in terms of binding affinity as deduced from our study.

Further we probed to reveal the rationale behind superior pharmacological profile of 6H. In terms of binding affinity, the appreciable binding of 6H can be attributed to its excellent interaction profile especially in terms of electrostatic and H-bonding interactions (Table 3). Apparent from the docking profile of compounds 6H, energy values of descriptors of external ligand interactions contributes 10.91 folds higher stability than internal ligand interactions. Further external ligand interactions were stabilized mostly by stearic energy guided by linear potentials while in internal ligand interactions, the strain due to torsion contributes for the stability of the ligand receptor interactions. As can be observed in other compounds like 6H similar AKOS019005527, Vorinostat and its similar SCHEMBL15675695, external ligand interactions as well as internal ligand interactions demonstrates less value than that of 6H.

The ADMET profiles (Table 4) of 6H and Vorinostat and their best docked respective similar revealed that compound 6H was a better compound and most likely druglike compared to rest of the three compounds. The predicted ADMET profile of compound 6H and Vorinostat was quite appreciable and proved to be non-toxic and a non-carcinogen. It can observed that 6H similar compound AKOS019005527 although was best docked similar among 83 molecules, however was predicted to be Ames Toxic, while Vorinostat similar SCHEMBL15675695 was predicted to be a carcinogen. Further when we predicted the bioactivity of all of these four compounds against different drug targets (Table 5). Compound 6H demonstrated highest enzyme inhibitor activity, testifying it to be specific and good potentiator of HDAC. Our bioactivity predictions are in coherence with in vitro testing performed by Cai et al., 2015, who also showed the most favorable IC_{50} value of 6H against HDACs.

Owing to optimal affinity, high enzyme inhibition activity and non-toxicity, 6H was further analyzed for pharmacophoric mappings. Comprehensively shown in Figure 3a, the compound 6h demonstrates van der Waals interactions with Leu 144, Met 35, Asp 181, Gly 305, Gln 265, Gly 306, Asp 269, Tyr 209, Leu 276 and electrostatic interactions with Cys 156, Gly 154, Phe 155, Phe 210, His 183, Tyr 308, His 145 and His 146. The compound is

a hydrogen bond acceptor from Gly 154 and Cys 156. In addition π-π interactions were observed with Phe 210 and Phe 155. The hydrophobic interaction of 6H in the active site is comprehensively shown in figure 3b in addition, electrostatic interaction and ligand binding pattern of 6H in the inhibitory site of HDAC1 is shown in figure 4a and 4b respectively.

Considering, optimal activity as experimentally predicted HDAC activity (predicted by Cai et al., 2015) and our analysis including better binding affinity, ADMET properties interaction profiles and pharmacophoric features, we anticipate compound 6H may form a potential candidate for HDAC inhibition in clinical treatment of cancers.

In conclusion, from, our extensive analysis involving binding affinity analysis, ADMET properties predictions and pharmacophoric mappings, we anticipate hydroxamate compound - 6H synthesized by Cai et al (2015) to be a potential candidate for HDAC inhibition which in addition can overcome narrow therapeutic window of present HDAC inhibitors in clinical treatment of cancers.

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