African Journal of Biotechnology Vol. 9(25), pp. 3923-3931, 21 June, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1985
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Ligand based pharmacophore modelling of anticancer histone deacetylase inhibitors

Nighat Noureen[1], Saima Kalsoom[2] and Hamid Rashid[1]*

[1]Department of Bioinformatics, Mohammad Ali Jinnah University Islamabad, Pakistan.
[2]Department of Chemistry, Quaid-e-Azam University, Islamabad, Pakistan.

Accepted 23 March, 2010

Histone deacetylases have emerged as an important therapeutic target for the treatment of cancer. Genome-wide histone hypoacetylation causes many cancers. Recently, inhibitors of histone deacetylases (HDAC) have emerged as an important class of anticancer agents. Various side effects like myocardium damage and bone marrow depression even leading to cell death have been observed in the treatment of caner cells using HDAC inhibitors. The discovery and development of type-specific HDAC inhibitors is of both research and clinical interests. Ligand based pharmacophore modelling is playing a key role for the identification of ligand features for the particular targets. We present a model for designing the pharmacophore onto the set of 70 compounds of three different classes and two subclasses. The ligand based pharmacophore model has been identified in order to facilitate the discovery of type specific anticancer HDAC inhibitors. The result indicates that the *in silico* methods are useful in predicting the biological activity of the compound or compound library by screening it against a predicted pharmacophore. Ligand Scout 2.02 has been used to predict the pharmacophore features for anticancer HDAC inhibitors and the distances between pharmacophore features have been calculated through the software Jmol. The proposed model has been validated by docking the MS275 compound into the binding pocket of Human HDAC8. Our discovery will help in the identification of more specific anticancer human HDAC inhibitors.

Key words: Histone deacetylases, histone deacetylase inhibitors, cancer, anticancer, pharmacophore, therapeutic, target.

INTRODUCTION

More effective anticancer drug production with novel modes of action is today's need (Sikora et al., 1999; Gelmon et al., 1999). Oncologists are aware of the fact that new drug discovery must target the developing mechanism of tumour in order to improve the therapeutic efficiency (Qianbin and Wenfang, 2005). Many transcription regulating proteins are themselves deregulated in cancer at the level of expression or activity.

*Corresponding author. E-mail: drhamid@jinnah.edu.pk. Tel: +92-301-5453378.

Abbreviations: HDACs, Histone deacetylases; **HDACI's,** histone deacetylase inhibitors; **HBA,** hydrogen-bond acceptor; **HBD,** hydrogen-bond donor; **HDLP,** histone deacetylase like protein.

Histone deacetylases (HDACs) is one of such important family of proteins which are deregulated in cancer (Walkinshaw and Yang, 2008). Cancer can result from aberrations in chromatin modifying proteins such as HDACs and chromatin (Walkinshaw and Yang, 2008). Disruption of HDAC activity may play an important role in the uncontrolled cell growth of cancer (Chen et al., 2003). In the discovery of drugs, HDAC has become a novel target for the treatment of cancer and other diseases (Marks et al., 2001). Over the past few years, the number of HDAC enzyme subtypes has expanded considerably offering opportunities for the development of HDAC inhibitors with improved specificity (Juvale et al., 2006). With continued research and development, in the near future, histone deacetylase inhibitors (HDACI's) are likely to figure prominently in cancer treatment plans (Walkinshaw and Yang, 2008).

The first and only approved (in 2006) histone deacetylase

inhibitor, Zolinza by the U.S Food and Drug Administration is used for the treatment of Cutaneous T-cell Lymphoma (Walkinshaw and Yang, 2008). In general, histone deacetylase inhibitors are associated with certain toxicities (Minucci and Pelicci, 2006; Karagiannis and Osta, 2007). Intense research activities are ongoing in the pharmaceutical and academic laboratories toward improving the pharmacokinetic and therapeutic indices of current HDAC inhibitors (Chen et al., 2008a). The wide variety of structural HDAC inhibitors includes three molecular fragments, a linker domain which occupies the channel, a metal binding domain which interacts with the active site and surface recognition domain which interacts with the residues on the rim of the active site. In developing potent histone deacetylase inhibitors, this three-component concept has proved to be successful (Jung et al., 1999; Sternson et al., 2001).

The pharmacophore model may provide guidance for the rational design to discover novel histone deacetylase inhibitors by highlighting the important binding features of HDAC ligands (Chen et al., 2008b).

The knowledge of common properties of the binding group is essential for the determination of the type of inhibitor binding the target. Major goal of modern drug design is identification and development of new ligands with high affinity of binding toward a given protein receptor. A very useful model for achieving this goal is pharmacophore.

Many scientists have achieved utilization of a ligand-based approach like QSAR and 3D pharmacophores to provide an alternative and complementary tool for drug design of HDAC inhibitors (Katritzky et al., 2007; Ragno et al., 2006; Wagh et al., 2006; Chen et al., 2008b; Vadivelan et al., 2008). In order to gain further insight into the structural requirements of HDAC inhibitors, a novel three dimensional quantitative structure-activity relationship pharmacophore model is developed and further evaluated (Chen et al., 2008b). The pharmacophore model was derived from hydroxamic acid derivatives (Chen et al., 2008b). The pharmacophore presented consists of one hydrogen-bond acceptor (HBA), one hydrogen bond donor (HBD) and three hydrophobic features (Chen et al., 2008b). Compound MS-275 has been docked into the binding pocket of histone deacetylase like protein (HDLP) in order to check the proposed pharmacophore (Chen et al., 2008b).

A pharmacophore model of hydroxamic acid derivatives which was identified consisted of two HBAs and three hydrophobic features (Chen et al., 2009). Another pharmacophore model highlights important binding features of HDAC ligands and may provide guidance for the rational design to discover novel hydroxamate HDAC 1 selective inhibitors (Liqin et al., 2009). This pharmacophore hypothesis consists of one HBA, one aromatic ring and two hydrophobic groups (Liqin et al., 2009).

In our studies, a 3D pharmacophore model is developed in order to assist the discovery of type specific and potent histone deacetylase inhibitors for the treatment of human cancer which has not been reported earlier. Various previously performed studies identified the pharmacophore of only hydroxamate derivatives of HDACIs. Current studies involved the pharmacophore identification of not only hydroxamates but also benzamide and bi-phenyl derivatives. So a unique pharmacophore based upon three major groups of HDACIs has been proposed in order to gain further insight into the structural requirements of HDACs. The proposed model is validated by docking the MS-275 compound into the binding pocket of Human HDAC8.

Our studies will help towards the identification of more potent human anticancer histone deacetylase inhibitors. With continued research and development, in the near future, HDACIs are likely to figure prominently in cancer treatment plans.

MATERIALS AND METHODS

The study was carried out using the software Ligand Scout (version 2.03[i2_001]). Ligand Scout is a software tool that allows the rapid and transparently deriving 3D chemical feature-based pharmacophores from structural data of macromolecule ligand complexes in a fully automated and convenient way (Steindl et al., 2006). Ligand Scout runs on all common operating systems and several successful application examples have been published (Schuster and Langer, 2005; Schuster et al., 2006).

The training set consisted of 70 compounds and was selected to generate the ligand based pharmacophore model. The compounds present in the set were different groups of hydroxamate, benzamide (Suzuki et al., 1999) and bi-phenyl derivatives (Dallavalle et al., 2009). The two groups chosen are basically the aliphatic and aromatic hydroxamates (Remiszewski et al., 2002; Woo et al., 2002; Lavoie et al., 2001; Massa et al., 2001).

Ligand based pharmacophore model generation was performed with Ligand Scout using default settings. The pharmacophore for each group of compounds and MS-275 has been generated and the distances among the pharmacophoric features of the ligands have been calculated using the software Jmol (jmol.org). Jmol is a Java viewer which is open source and is used for three dimensional structures of compounds, with their features, materials, biomolecules and crystals (Herráez, 2007).

The pharmacophore of the above mentioned groups and subgroups have been superimposed in order to get the common pharmacophore of anticancer HDAC inhibitors. The distances among the pharmacophoric features of the common and unique pharmacophore were then calculated.

Model validation has been performed through molecular docking studies which have been performed through Autodock 4.0 (Morris et al., 1998) and VMD (Humphrey et al., 1996). Compound MS-275 have been docked into the binding pocket of Human HDAC8 with PDB id 1T69 obtained from Protein Data Bank (rcsb.org). Autodock has been applied with great success in the prediction of bound conformations of enzyme inhibitor complexes, peptide antibody complexes and even protein-protein interactions (Morris et al., 1998).

RESULTS AND DISCUSSION

The pharmacophore generated by Ligand Scout for the

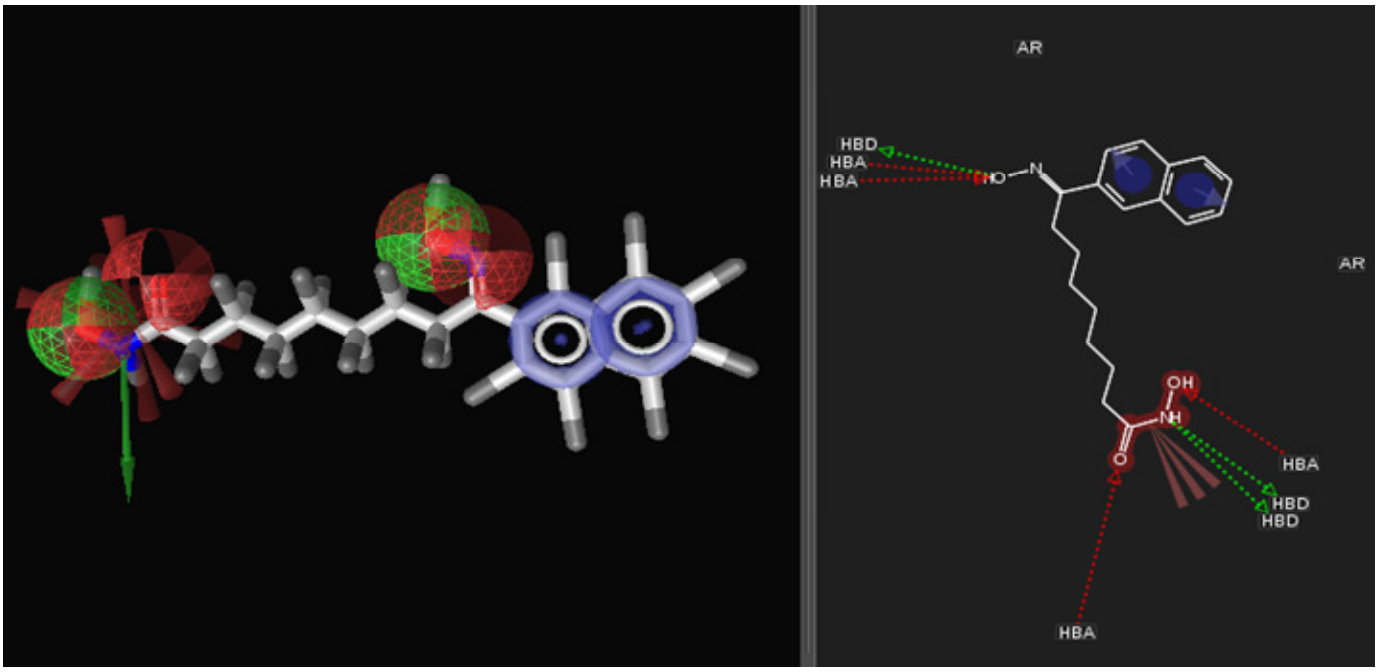

Figure1. A hydroxamate derivative containing the aliphatic chain linker pharmacophore.

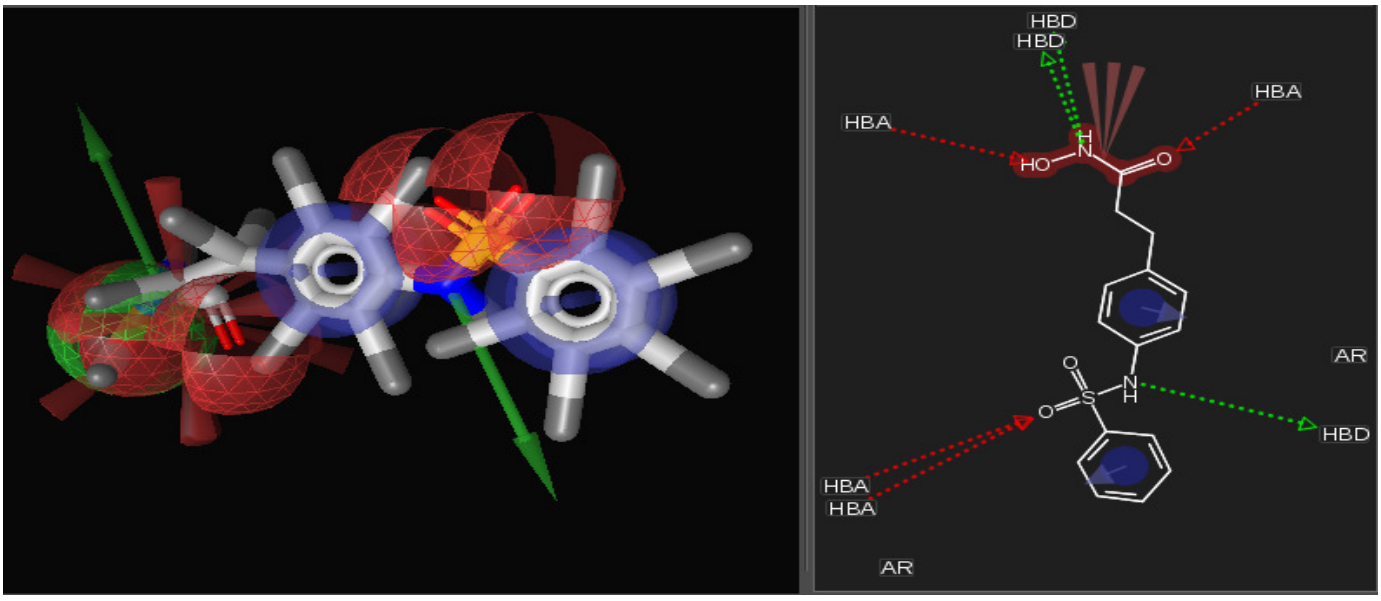

Figure 2. A hydroxamate derivative containing the aromatic chain linker pharmacophore.

training set showed three main features as hydrogen bond acceptors, hydrogen bond donors and aromatic rings. The pharmacophore generated for the chosen group of compounds showed consistency in the above features. The representative pharmacophores of each group and subgroups are shown in Figures 1, 2, 3 and 4. The pharmacophore of MS-275 are shown in Figure 5.

These figures show the 3D and 2D views of the pharmacophores. The features identified in green colors are the HBDs, red colored are HBAs and the aromatic rings are shown in blue color in both views. All the ligands showed consistency in these three features. Similarly, all 70 ligands showed similar features. On the whole, the pharmacophoric features for each are shown in Table 1.

The pharmacophores of all the compounds were then matched and a unique pharmacophore was identified after a detailed analysis. Similar features were identified after analysing the pharmacophores of all compounds

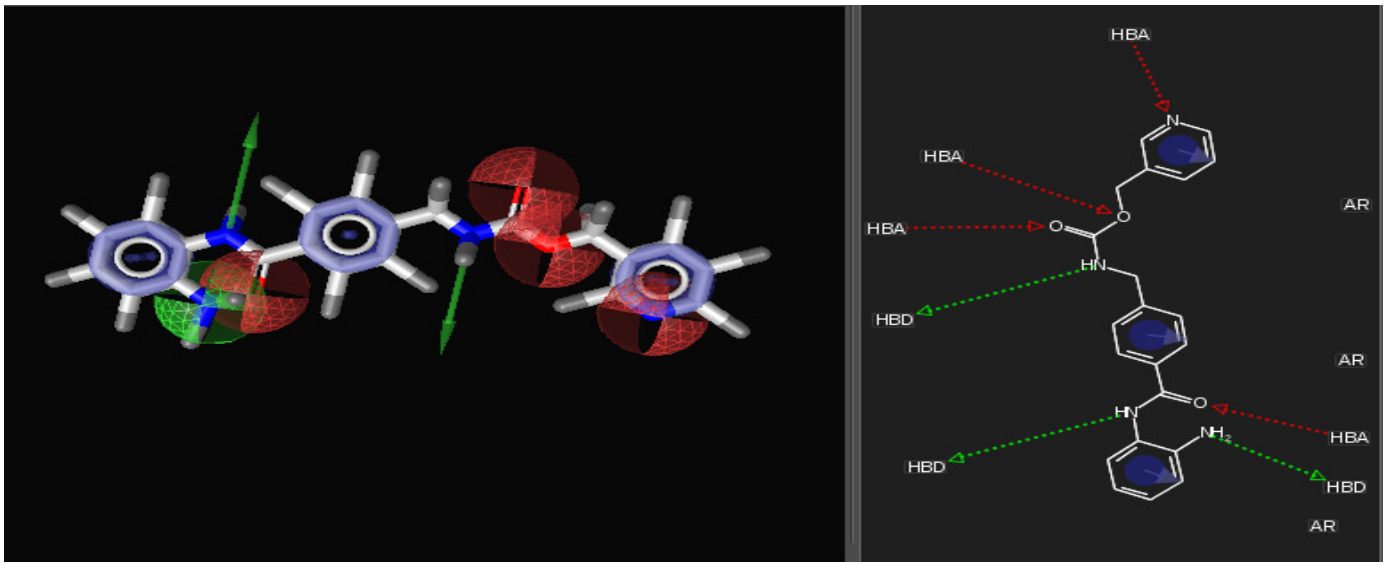

Figure 3. A benzamide derivative pharmacophore.

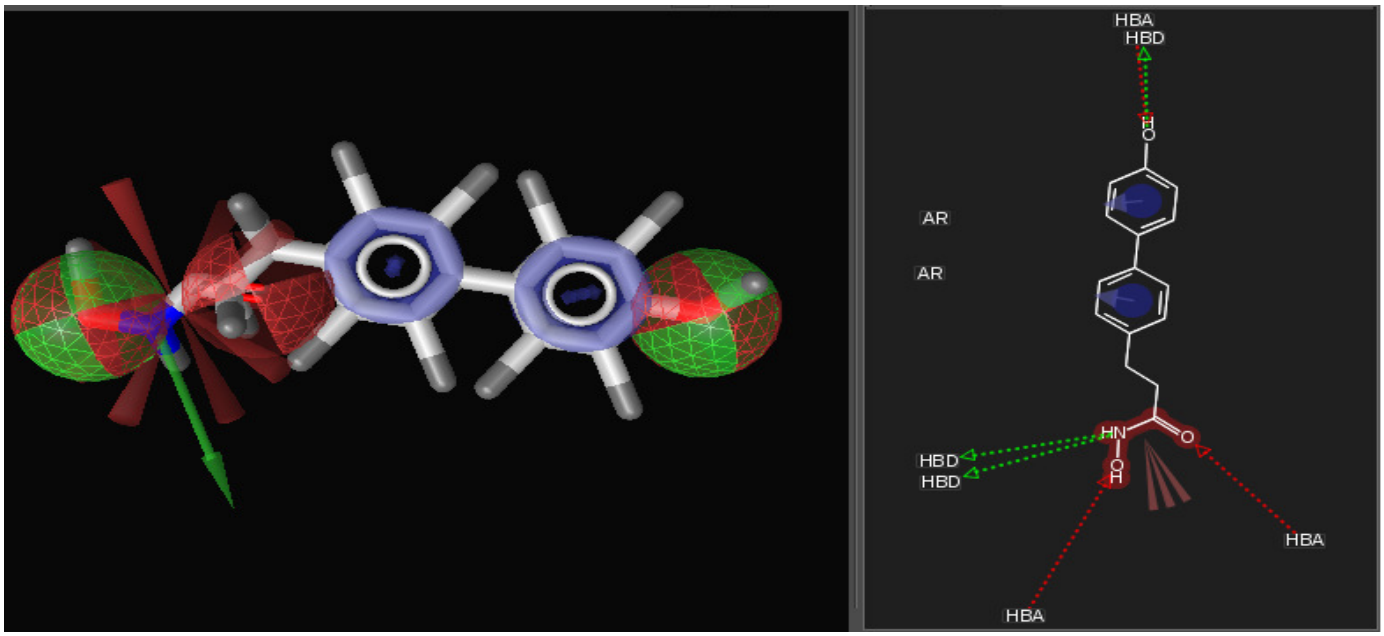

Figure 4. A bi-phenyl derivative pharmacophore.

generated by Ligand Scout. The similar features of all the compounds were then superimposed and merged into a single pharmacophore. Superimposed ligands (all) along with MS275 are shown in Figure 6.

The orange colored circles in Figure 6 show the superimposed features in the ligands. The uniquely identified pharmacophore are shown in Figure 7.

On the whole, the representative pharmacophoric features for each group and MS275 are shown in Table 2.

Our common featured pharmacophore predicted for three groups and two sub-groups of anticancer HDAC inhibitors and MS275 (Table 2 and Figure 7) is as; three HBAs (shown by red circles), three HBDs (two green circles and one green arrow) and two aromatic rings (shown by blue circles).

Chen et al. (2008) presented the pharmacophore of HDAC inhibitors as having one hydrogen bond acceptor, one hydrogen bond donor and three hydrophobic features. Like wise Chen et al. (2009) and Liqin et al. (2009) presented the pharmacophores of anticancer HDAC inhibitors using only hydroxamates derivatives.

So our pharmacophore features based on the studies

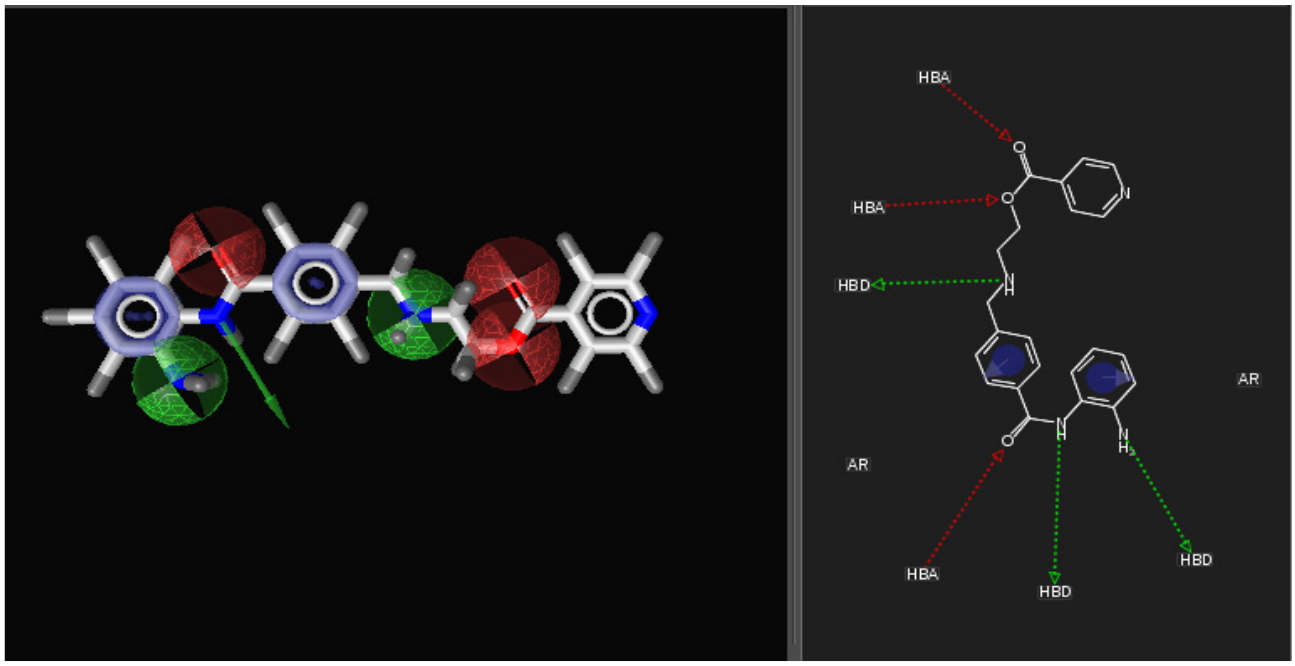

Figure 5. 3D and 2D pharmacophore of standard ligand MS275.

Table 1. Pharmacophoric features of each group of ligands.

Compounds	Hydrogen bond acceptors	Hydrogen bond donors	Hydrophobic aromatic rings
Aliphatic hydroxamtes	+	+	+
Aromatic hydroxamtes	+	+	+
Benzamides derivatives	+	+	+
Bi-phenyl derivatives	+	+	+

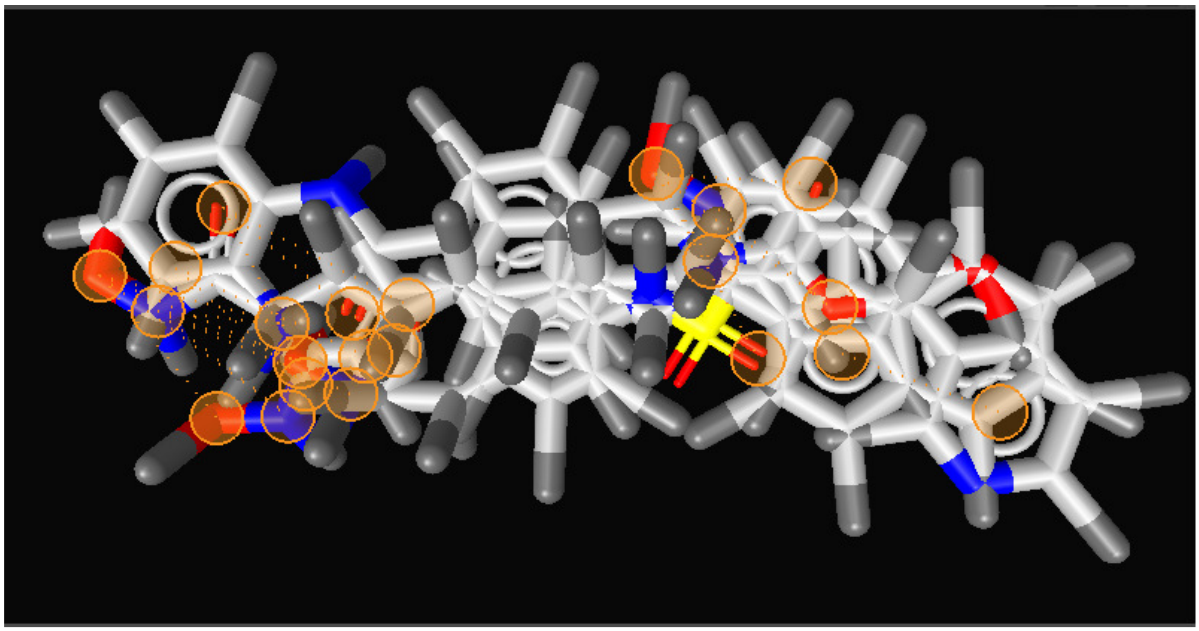

Figure 6. Aligned features of the all compounds.

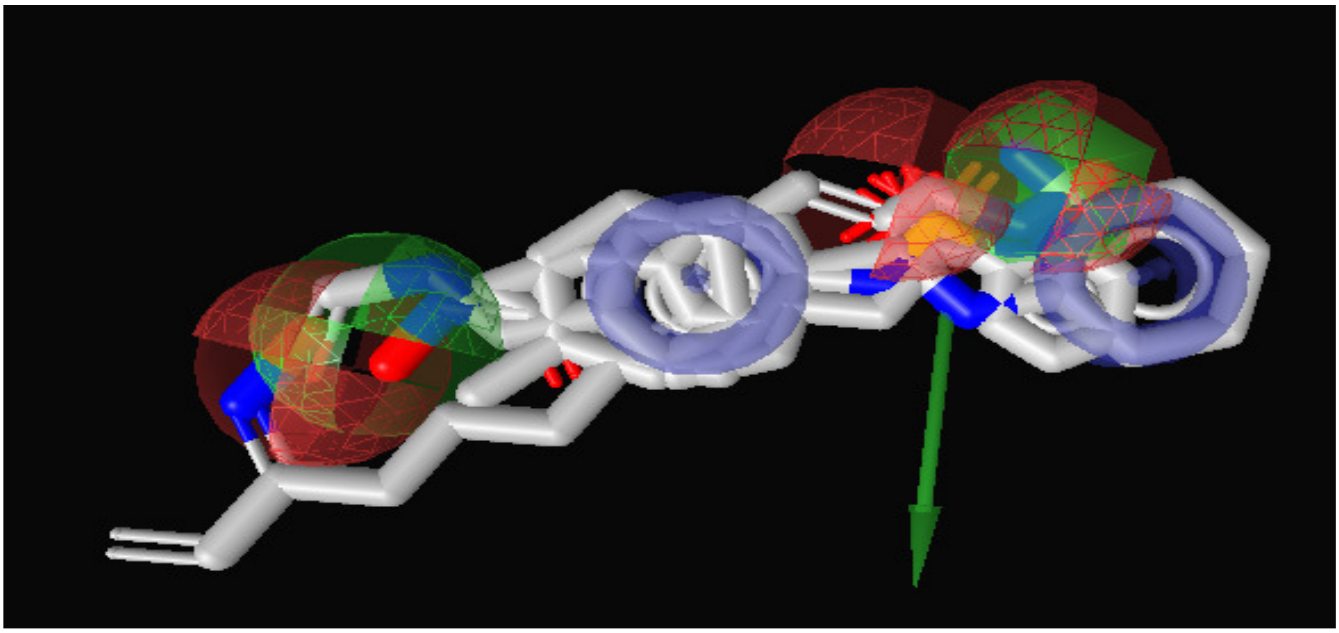

Figure 7. Proposed 3D pharmacophore of anticancer HDAC inhibitors.

Table 2. Total pharmacophoric features of each group of ligands and MS-275.

Compounds	Hydrogen bond acceptors	Hydrogen bond donors	Hydrophobic aromatic rings
Aliphatic Hydroxamtes	Four	Three	Two
Aromatic Hydroxamtes	Four	Three	Two
Benzamides Derivatives	Four	Three	Three
Bi-phenyl Derivatives	Three	Three	Two
MS-275	Three	Three	Two

Table 3. Pharmacophoric triangle distances of each group of ligands.

Ligands	HBA-HBD (nm)	HBD-Ar (nm)	HBA-Ar (nm)
Aliphatic hydroxamtes	0.0816 – 0.6543	0.3655 – 0.9214	0.8011 – 0.99
Aromatic hydroxamtes	0.1393 – 0.5194	0.1936 – 0.3462	0.1549 – 0.4664
Benzamides derivatives	0.1427 – 0. 6221	0.2971- 0.8912	0.1427 – 0.3776
Bi-phenyl derivatives	0.1824 – 0.6664	0.1429 – 0.696	0.251 – 0.7748
MS275	0.1427 – 0. 6664	0.1429- 0.8912	0.1427 – 0.4664

of three major groups have improved the features more. In this way, the pharmacophore has not only been restricted to hydroxamate derivatives but other groups have also been included.

The distance triangle measured between the common pharmacophore features of each group of compounds and MS275 using Jmol is shown in Table 3. The distance ranges from minimum to maximum and have been measured between the HBA and HBD, HBA and aromatic ring and HBD and aromatic ring. The distances among common pharmacophoric features between the predicted pharmacophore are shown in Figure 8. The distances between aromatic and HBD range from 0.1429 to 0.9214 nm, between aromatic to HBA range from 0.1427 to 0.99 nm and between HBA to HBD range from 0.0816 to 0.6664 nm.

The comparison of the pharmacophoric features at each step along with the standard MS275 shows the validation of predicted pharmacophore model. As the standard MS275 showed similar pharmacophoric features

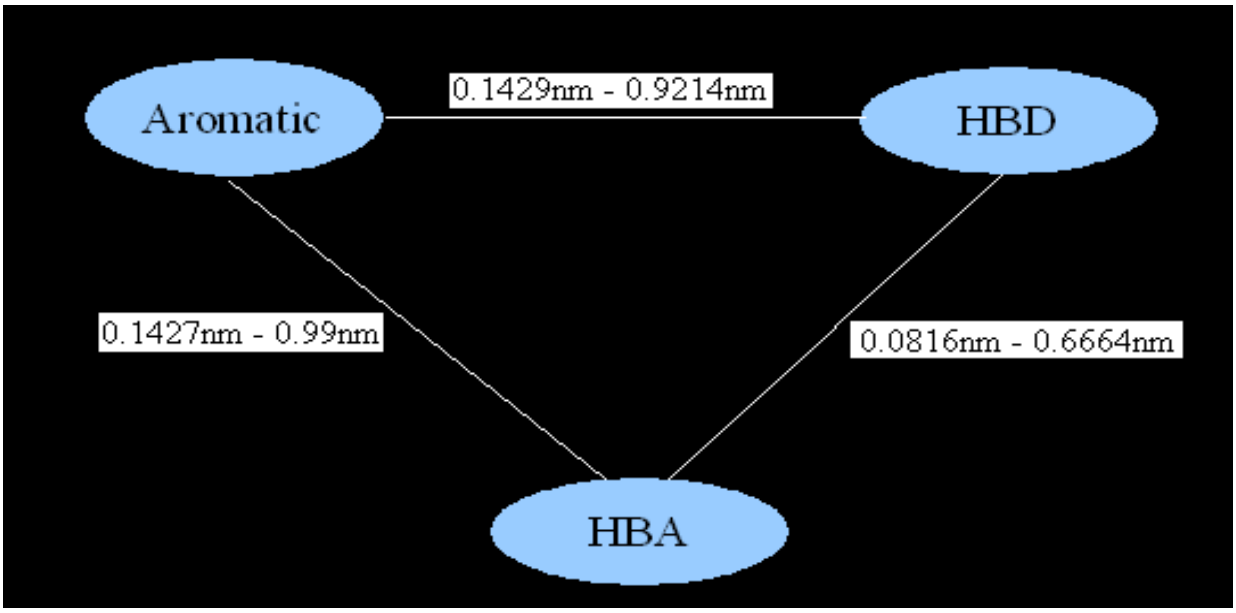

Figure 8. Distance ranges among pharmacophoric features in predicted pharmacophore.

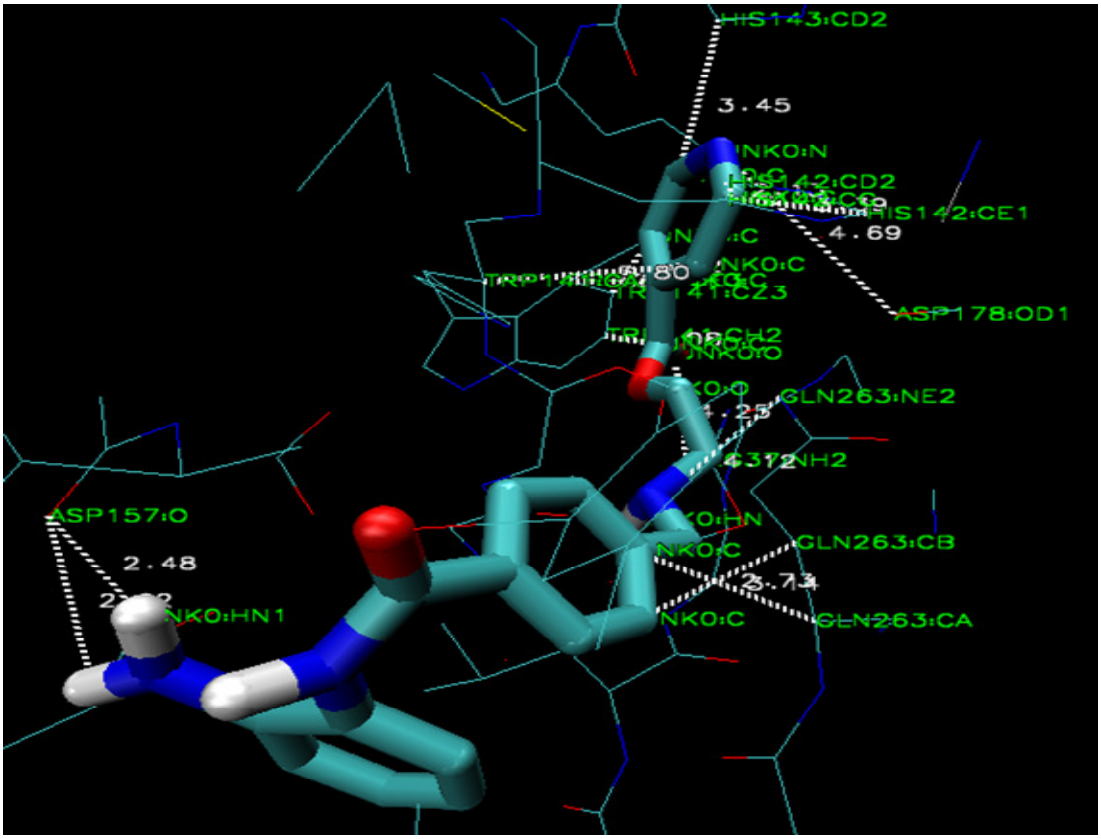

Figure 9. Actively docked conformation of MS275 into HDAC8 cavity.

like the test data, it has been docked into the binding cavity of the Human HDAC8. The docked ligand into the cavity and the binding interactions are shown in Figure 9. The important binding interactions which include hydrogen, hydrophobic and ionic interactions are shown in Figure 9. The figure shows the interactions at particular distances.

The binding interactions of MS275 include 6 hydrogen bonds, 9 hydrophobic interactions and 2 ionic bonds. The hydrogen bonds include H of the ligand with the O's of ASP157 at distances of 2.62Å and 2.48Å, with the O's of ASP101 at 3.04Å and 3.10Å, with the O of PHE152 at 3.45Å and with N of GLN263 at 4.13Å. The ionic interactions include N of ARG37 with the O of Ligand at 4.25Å and O of ASP178 with the N of ligand at 4.69Å. The hydrophobic interactions include the C's of ligand with the C's of TRP141 at 2.12Å, at 3.80Å, at 2.31Å and 3.08Å, of HIS142 at 2.13Å and 3.39Å, of GLN263 at 3.14Å and 2.73Å and last of the HIS143 at 3.45Å.

The actively docked conformation of the MS275 into the cavity and the strong binding interactions of the ligand with HDAC8 showed the validation of the proposed model. The strong interactions of the ligand with the human target protein show the validation of proposed pharmacophore model like Chen et al. (2008) did with the HDLP.

Our predicted pharmacophore for the three major groups and two sub groups of anticancer HDAC inhibitors will help in the identification of type specific drugs. This model has broadened the vision for the generation of more specific drugs for human cancers and it opens the way to produce and identify more effective drugs

ACKNOWLEDGEMENTS

Special thanks to Zohaib Hassan Waheed, student of MS Computer Science, for his great help and support. We are also grateful to Faculty of Engineering and applied Sciences, Mohammad Ali Jinnah University, Islamabad for support in the research work.

REFERENCES

Chen JS, Faller DV, Spanjaard RA (2003). Short-chain fatty acid inhibitors of histone deacetylases: promising anticancer therapeutics? Curr. Cancer Drug Targets, 3: 219-236.

Chen PC, Patil V, Guerrant W, Green P, Oyelere AK (2008a). Synthesis and structure–activity relationship of histone deacetylase (HDAC) inhibitors with triazole-linked cap group. Bioorg. Med. Chem. 16: 4839-4853.

Chen Y, Jiang YJ, Zhou JW, Yu QS, You QD (2008b). Identification of ligand features essential for HDACs inhibitors by pharmacophore modeling. J. MGM, 26: 1160-1168.

Chen Y, Li H, Tang W, Zhu C, Jiang Y, Zou J, Yu Q, You Q (2009). 3D-QSAR studies of HDACs inhibitors using pharmacophore-based alignment. Eur. J. Med. Chem. Pp. 1-9.

Dallavalle S, Cincinelli R, Nannei R, Merlini L, Morini G, Penco S, Pisano C, Vesci L, Barbarino M, Zuco V, Cesare MD, Zunino F (2009). Design, synthesis, and evaluation of biphenyl-4-yl-acrylohydroxamic acid derivatives as histone deacetylase (HDAC) inhibitors. Eur. J. Med. Chem pp. 1-13

Gelmon KA, Eisenhauer EA, Harris AL, Ratain MJ, Workman P (1999). Anticancer agents targeting signalling molecules and cancer cell environment: challenges for drug development? J. Natl. Cancer Inst. 91: 1281-1287.

How to use Jmol to study and present molecular structures, ISBN 978-1-84799-259-8 Lulu Enterprises: Morrisville, NC, USA © Angel Herráez, 2007.

Humphrey W, Dalke A, Schulten K (1996).VMD: Visual molecular dynamics. J. Mol. Graphics, 14: 33-38.

Jmol: an open-source Java viewer for chemical structures in 3D. http://www.jmol.org/

Jung M, Brosch G, Kolle D, Scherf H, Gerhauser C, Loidl P (1999). Amide analogues of trichostatin A as inhibitors of histone deacetylase and inducers of terminal cell differentiation. J. Med. Chem. 42: 4669-4679.

Juvale DC, Kulkarni VV, Deokar HS, Wagh NK, Padhye SB, Kulkarni VM (2006).3D-QSAR of histone deacetylase inhibitors: hydroxamate analogues. Org. Biomol. Chem. 4: 2858-2868.

Karagiannis TC, El-Osta A (2007). Will broad-spectrum histone deacetylase inhibitors be superseded by more specific compounds?. Leukemia. 21: 61-65.

Katritzky AR, Slavov SH, Dobchev DA, Karelson M (2007). Comparison between 2D and 3D-QSAR. QSAR Comb. Science, 26: p. 333.

Lavoie R, Bouchain G, Frechette S, Woo SH, Khalil EA, Leit S, Fournel M, Yan PT, Trachy-Bourget MC, Beaulieu C, Li Z, Besterman J, Delorme D (2001). Design and synthesis of a novel class of histone deacetylase inhibitors. Bioorg. Med. Chem. Lett. 11(2001): 2847-2850.

Liqin Y, Fei L, Yadong C, Qidong Y (2009). Pharmacophore identification of hydroxamate HDAC 1 inhibitors. Chin. J. Chem. 27: 557-564.

Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK (2001). Histone deacetylases and cancer: causes and therapies. Nat. Rev. Cancer, 1: 194-202

Massa S, Mai A, Sbardella G, Esposito M, Ragno R, Loidl P, Brosch G (2001). 3-(4-Aroyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamides, a New Class of Synthetic Histone Deacetylase Inhibitors. J. Med. Chem. 44: 2069-2072.

Minucci S, Pelicci PG (2006). Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat. Rev. Cancer, 6: 38-51.

Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998). Automated docking using a Lamarkian Genetic algorithm and an empirical binding free energy function. J. Comp. Chem. 19(14): 1639-1662.

Qianbin L, Wenfang X (2005). Novel anticancer targets and drug discovery in post genomic age. Curr. Med.Chem- Anti-Cancer Agents, 5: 53-63.

Ragno R, Simeoni S, Valente S, Massa S, Mai A (2006). 3-D QSAR studies on histone deacetylase inhibitors. A GOLPE/GRID approach on different series of compounds. J. Chem. Info. Model, 46: p. 1420.

Remiszewski SW, Sambucetti LC, Atadja P, Bair KW, Cornell WD, Green MA, Howell KL, Jung M, Kwon P, Trogani N, Walker H (2002). Inhibitors of human histone deacetylase: synthesis and enzyme and cellular activity of straight chain hydroxamates. J. Med. Chem. 45: 753-757.

Schuster D, Langer T (2005). The identification of ligand features essential for PXR activation by pharmacophore modeling. J. Chem. Info. Model, 45: p. 431.

Schuster D, Laggner C, Palusczak A, Hartmann RW, Langer T (2006). Pharmacophore modeling and in silico screening for new P450 19 (aromatase) inhibitors. J. Chem. Info. Model, 46: p. 1301.

Sikora K, Advani S, Koroltchouk V, Magrath I, Levy L, Pinedol H (1999). Essential drugs for cancer therapy: A world health organization consultation. Ann. Oncol. 10: 385-390.

Steindl TM, Schuster D, Wolber G, Laggner C, Langer T (2006). High-throughput structure-based pharmacophore modelling as a basis for successful parallel virtual screening. J. Comp. Aided Mol. Design, 20: 703-715.

Sternson SM, Wong JC, Grozinger CM, Schreiber SL (2001). Synthesis of 7200 small molecules based on a substructural analysis of the histone deacetylase inhibitors trichostatin and trapoxin. Org. Lett. 3: 4239-4242.

Suzuki T, Ando T, Tsuchiya K, Fukazawa N (1999). Synthesis and Histone Deacetylase Inhibitory Activity of New Benzamide Derivatives. J. Med. Chem. 42: 3001-3003.

Vadivelan S, Sinha BN, Rambabu G, Boppana K, Jagarlapudi SA(2008). Pharmacophore modeling and virtual screening studies to design some potential histone deacetylase inhibitors as new leads. J.

Mol. Grap. Modeling, 26: p. 935.
Wagh NK, Deokar HS, Juvale DC, Kadam SS, Kulkarni VM (2006). 3D-QSAR of histone deacetylase inhibitors as anticancer agents by genetic function approximation. Indian J. Biochem. Biophys. 43: p. 360.
Walkinshaw DR, Yang XJ (2008).Histone deacetylase inhibitors as novel anticancer therapeutics. Curr. Oncol. 15(5).
Woo SW, Frechette S, Khalil EA, Bouchain G, Vaisburg A, Bernstein N, Moradei O, Leit S, Allan M, Fournel M, Trachy-Bourget MC, Li Z, Besterman JM, Delorme D (2002). Structurally simple trichostatin A-like straight chain hydroxamates as potent histone deacetylase inhibitors. J. Med. Chem. 45: 2877-2885.