

## HDAC-Targeted Library

Medicinal and Computational Chemistry Dept., ChemDiv, Inc., 6605 Nancy Ridge Drive, San Diego, CA 92121 USA, Service: +1 877 ChemDiv, Tel: +1 858-794-4860, Fax: +1 858-794-4931, Email:

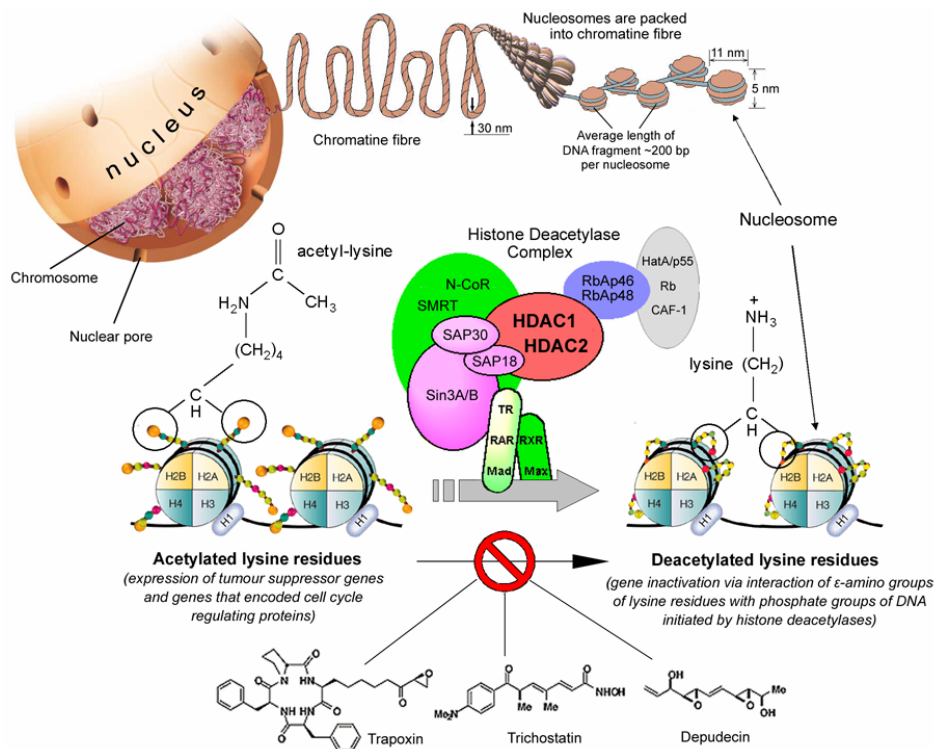
[ChemDiv@chemdiv.com](mailto:ChemDiv@chemdiv.com)

### **Histone acetylases and deacetylases as promising therapeutic targets**

Regulation of gene expression is mediated by several mechanisms such as DNA methylation, ATP-dependent chromatin remodeling, and post-translational modifications of histones. The latter mechanism includes dynamic acetylation and deacetylation of  $\epsilon$ -amino groups of lysine residues present in the tail of the core histones. Histones are the predominant protein components of chromatin, which stabilize the nucleosome core. They are subjected to a variety of specific post-translational modifications. Reversible acetylation and deacetylation of nucleosomal histones are critical in the modulation of chromatin structure, chromatin function and in the regulation of gene expression. Enzymes responsible for the reversible acetylation/deacetylation processes are histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively [<sup>i</sup>]. HATs act as transcriptional coactivators, and HDACs are a part of transcriptional corepressor complexes. Moreover, these enzymes also target non-histone protein substrates, including transcription factors, nuclear import factors, cytoskeleton, and chaperon proteins. They were first described almost 30 years ago [<sup>ii</sup>]. These enzymes have been identified in numerous biological systems, including multiple mammalian cell lines and tissues [<sup>iii</sup><sup>a</sup>], fungi [<sup>3b</sup>] plants [<sup>iv</sup>] and yeasts [<sup>v</sup>]. In particular, they have been received much attention as promising therapeutic targets for the treatment of many solid and hematological cancers in addition to diabetes, arthritis, polyglutamine and Huntington's diseases. It is now becoming clear that HDACs play critical role in controlling transcription, cell cycle, cell motility, DNA damage response and senescence by deacetylating histones and non-histone proteins.

It became evidently clear, that aberrant gene expression through epigenetic changes represents a major area for the study of cancer initiation and progression [<sup>vi</sup>]. Research data accumulated over two decades suggest that errors in the regulation of histone acetylation are associated with carcinogenesis, cancer progression as well as with other malignancies [<sup>vii</sup>]. Cancer is a complicated process involving genetic and epigenetic events, which result in neoplastic transformation. Numerous alterations of pathways involving HDACs were identified in tumor cells. In general, histone acetylation leads to chromatin remodeling and de-repression of molecular transcription mechanism. Mechanistically, interaction of the positively charged  $\epsilon$ -amino groups of lysine residues of histone *N*-terminal tails with

the negatively charged phosphate backbone of DNA results in chromatin condensation, leading to transcription silencing possibly by disallowing access to the transcription mechanism (Fig. (1)) [viii].



**Figure 1.** Role of HDAC inhibitors in regulation of tumor suppressor genes expression.

It has been reported that the inhibitors of HDACs induce hyperacetylation of histones that modulate chromatin structure and gene expression. These inhibitors also induce growth arrest, cell differentiation, and apoptosis of tumor cells. They also affect cell cycle progression, inducing cellular growth and arrest at both G and G/M phases. HDAC inhibitors enhance efficacy of anticancer agents that target DNA.

### Histone deacetylase inhibitors

Histone deacetylase inhibitors (HDIs) are of interest for cancer treatment as they reactivate the expression of epigenetically silenced genes, including those involved in differentiation, cell cycle regulation, apoptosis, angiogenesis, invasion, and metastasis. Early clinical trials with the first generation of HDIs have demonstrated promising therapeutic activity against both growth and survival of tumors *ex vivo* and *in vivo*. Several HDIs also display broad spectrum of antiprotozoal [ix], antidiabetic [x], antifibrogenic [xi] and immunosuppressant activity [xii]. Some of these inhibitors are used for the treatment of polyglutamine disorders [xiii], Huntington's disease [xiv] and rheumatoid arthritis [xv]. They also promote antigen-specific antibody production [xvi] and regulate cellular life span [xvii].

A number of structurally diverse classes of organic compounds have been identified as HDAC inhibitors. HDIs including short chain fatty and hydroxamic acids, such as suberoylanilide hydroxamic acid and pyroxamide, benzamides and cyclic tetrapeptides are novel classes of anti-neoplastic agents undergoing clinical evaluation. Natural products and dietary chemopreventive agents including butyrate derivative 4-PBA, diallyl disulfide, and sulforaphane have been shown to possess HDAC inhibitory activity [<sup>xviii</sup>]. These molecules demonstrate strong anti-neoplastic effects *in vitro* and *in vivo* by inducing growth arrest, differentiation and programmed cell death, inhibiting cell migration, invasion and metastasis as well as suppressing angiogenesis. They promote the accumulation of acetylated histone proteins by the inhibition of HDAC enzymes in both tumor and in normal cells. Notably, they arrest cell cycle in G1 and/or G2 mitotic phase and induce apoptosis selectively in transformed or cancer cells. So, it is generally believed that HDIs alter the acetylation of histone tails, modifying the expression of oncogenes and tumor suppressor genes, and rescuing normal cell growth and differentiation. In addition, these molecule display significant immunomodulatory and anti-inflammatory potential affect life span and stimulate antigen-specific antibody production.

### Concept and Applications

HDAC-targeted library design at CDL involves:

- *A combined profiling methodology that provides a consensus score and decision based on various advanced computational tools:*

1. Bioisosteric morphing and funneling procedures in designing novel potential HDAC inhibitors with high IP value. We apply CDL's proprietary Chemosoft<sup>TM</sup> software and commercially available solutions from Accelrys, MOE, Daylight and other platforms.
2. A molecular docking approach to focused library design.
3. Computational-based *in silico* ADME/Tox assessment for novel compounds includes prediction of human CYP P450-mediated metabolism and toxicity as well as many pharmacokinetic parameters, such as Brain-Blood Barrier (BBB) permeability, Human Intestinal Absorption (HIA), Plasma Protein binding (PPB), Plasma half-life time ( $T_{1/2}$ ), Volume of distribution in human plasma ( $V_d$ ), etc.

The fundamentals for these applications are described in a series of our recent articles on the design of exploratory small molecule chemistry for bioscreening [for related data visit ChemDiv. Inc. online source: [www.chemdiv.com](http://www.chemdiv.com)].

- *Synthesis, biological evaluation and SAR study for the selected structures:*

1. High-throughput synthesis with multiple parallel library validation. Synthetic protocols, building blocks and chemical strategies are available.
2. Library activity validation via bioscreening; SAR is implemented in the next library generation.

## We practice a multi-step approach for building HDAC-focused libraries:

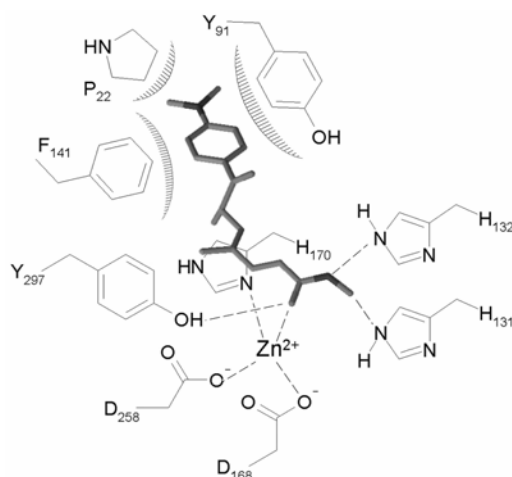
### *Virtual screening*

High-throughput screening of large diversity-based libraries still remains a common strategy within many pharmaceutical companies for the discovery of HDAC inhibitors. However, as noted by many researchers in the field, there is no evidence that high-throughput technologies, including parallel synthesis/combinatorial chemistry and HTS provided the expected impedance to the lead discovery process. Therefore, a number of approaches have been used for the design of more focused screening libraries. These range from pharmacophore and target structure-based design through combinatorial approaches to various QSAR methods. Thus, we have used some of the mentioned *in silico* strategies to design our HDAC-focused library. In particular, we have disclosed, how the knowledge obtained from receptor-ligand interaction models and structures of known ligands can be applied for the design of pharmaceutically relevant small-molecule HDIs.

### *Target-based Design*

3D Models of HDACs were recently developed based on crystallographic data. Major insight into the molecular mechanisms of HDAC activity and inhibition came from the crystal structure of HDLP, an archaeobacterium (*Aquifex aeolicus*) homolog of a eukaryotic deacetylase. In 1999, the crystal structure of HDLP, bound to an HDAC inhibitor, trichostatin A, was reported [<sup>xix</sup>]. The catalytic domain of HDLP is closely related to both classes of HDACs (I and II). Based on this information, it was presumed that the mechanism of deacetylation for these enzymes is similar. Recently, the structure of a catalytic core of class I and class II HDACs was revealed. It was noted that HDACs share an approximately 390-amino acid region of homology, referred to as the deacetylase core, arranged in a tubular deep pocket (the active site) with an adjacent internal cavity and a wider bottom. Residues that form the active site are conserved across all HDACs. The hydroxamic acids (*ex.*, TSA and SAHA) bind to the pocket.

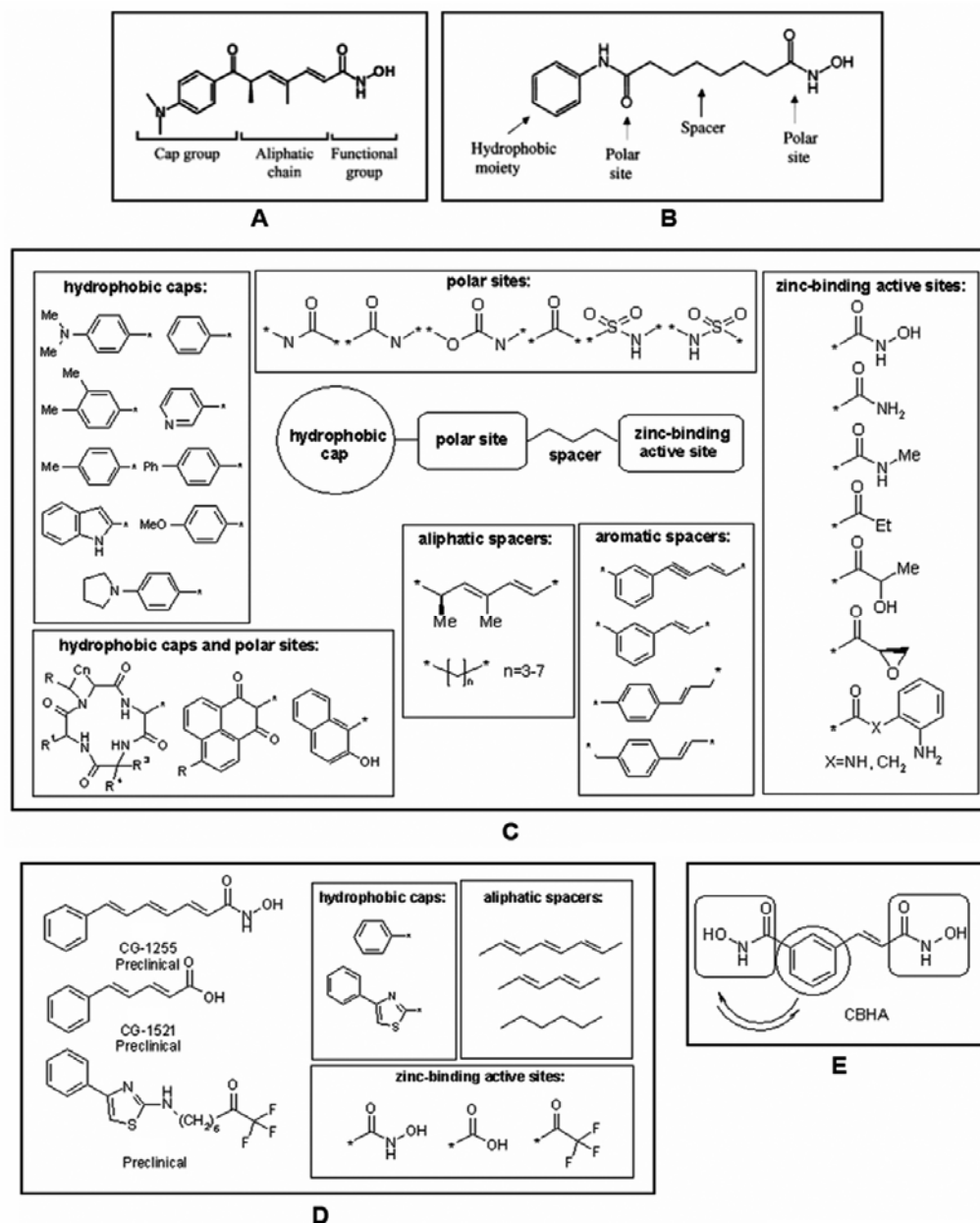
From the X-ray data on HDLP–TSA complex (Fig. (2)), the active catalytic site in this enzyme is believed to be formed by a tubular pocket, a zinc-binding site, and two asparagine–histidine charge-relay systems. The hydroxamic acid moieties of TSA bind to the Zn<sup>2+</sup> in the tubular pocket. The cavity leading to the active site is surrounded by hydrophobic residues, allowing for the proper alignment with the aliphatic chain of the acetyl-lysine residue.



**Figure 2.** HDLP–TSA complex revealed by X-ray crystallography

### *Topological pharmacophores*

Co-crystallization of TSA and SAHA with HDLP allowed for the structural insight into the structural properties of other HDIs. Both TSA and SAHA contain a cap group (hydrophobic moiety and polar site), an aliphatic chain (spacer), and a terminal functional group (polar site), such as hydroxamic acid functional group (Fig. (3A,B)). The hydroxamic acid-based HDIs coordinate  $Zn^{2+}$  in a bidentate fashion. The aliphatic chain makes van der Waals interactions with the cavity residues. The cap group interacts with the moieties on the rim of the pocket. It is likely to mimic the amino acids adjacent to the acetylated lysine residue in the histone. The binding of TSA and SAHA causes conformational changes in a tyrosine residue on this rim allowing for the tighter packing of the cap group [xx]. In general, the topological pharmacophore for this class of compounds contains four basic components: hydrophobic cap (surface domain) that blocks the entrance to the active site, polar site, aliphatic or aromatic spacer of a specific length and metal-binding domain (Fig. (3C)).

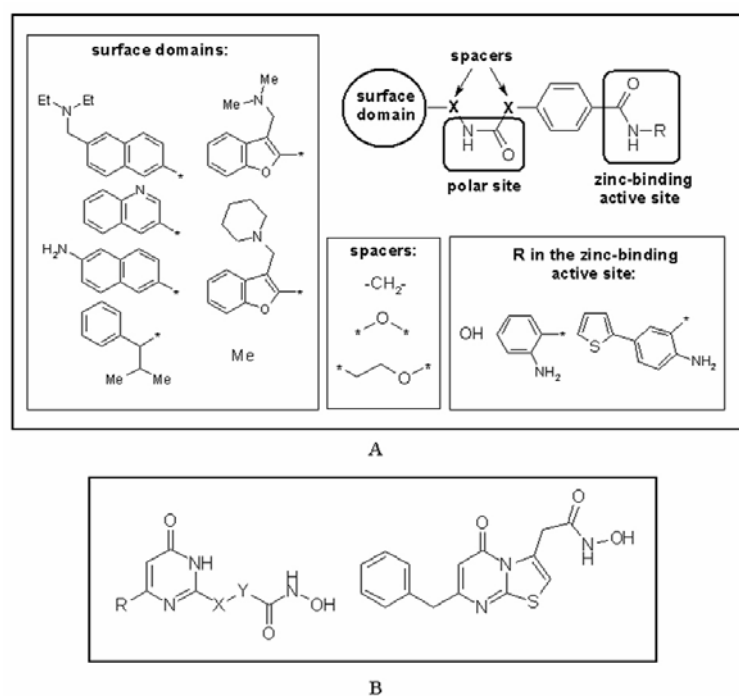


**Figure 3.** Topological pharmacophores within HDIs family

Arguably, three fragments play critical role in pharmacophore composition, namely hydrophobic cap, spacer and moiety interacting with the  $Zn^{2+}$  (Fig. (3D)). In some cases, the polar site and hydrophobic cap are located in the same fragment, for example in cyclic tetrapeptides (for example: Apicidine, depsipeptide, Trapoxin-A and Trapoxin-B as well as CHAPs) and in Scriptaid. The mirror composition of polar site and hydrophobic cap was featured in several HDIs, for example in CBHA (Fig. (3E)).

Four main structural features in benzamides (for example, MS-275, Tacedinaline and ITF-2357) and CRA-024781 as well as BB2 can be identified. These are surface domain, polar site, aliphatic spacer and  $Zn^{2+}$ -binding group (Fig. (4A)). Topological organization of these inhibitors is different from

hydroxamic acid-based compounds. Some modifications of this pharmacophore can be found in MGCD-0103 where the polar site is replaced by pyrimidine fragment.



**Figure 4.** Topological pharmacophore benzamide-based HDIs

This pharmacophore model yielded several potent HDIs. For example, a series of hydroxamates bearing uracil moiety for a linker have been recently introduced [169] (Fig. (4B)).

### 3D-QSAR approaches

Docking simulation and three-dimensional quantitative structure-activity relationships (3D-QSARs) analyses were recently conducted on four series of HDAC inhibitors [xxi]. These studies were performed with the GRID/GOLPE combination using structure-based alignment.

Indole amide HDIs, LBH-589, and NVP-LAQ824 were developed using similar approach [xxii]. Studies included comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). Selected ligands were docked into the active site of human HDAC1. Based on the docking results, novel binding mode of indole amide analogues in the human HDAC1 catalytic core was presented. As a result, authors identified two molecules active against HDAC1 [xxiii]. Using the same approach, QSAR models were derived from a training set of 40 molecules [xxiv]. An external test set consisting of 17 molecules was used to validate CoMFA and CoMSIA models. All molecules were superimposed on the template structure by atom-based, multifit and the SYBYL QSAR rigid body field fit alignments. The statistical quality of the QSAR models was assessed using the parameters  $r^2$  (conv),  $r^2$  (cv) and  $r^2$  (pred). In addition to steric and electronic fields, ClogP was also taken as descriptor to

account for lipophilicity. The resulting models exhibited good conventional and cross-validated values up to 0.910 and 0.502 for CoMFA and 0.987 and 0.534 for CoMSIA.

In order to facilitate design of isoform-specific HDIs, structural differences between class I HDACs were studied using molecular docking. Three-dimensional models of four class I histone deacetylases, namely HDAC1, HDAC2, HDAC3, and HDAC8 were assembled [xxv]. A series of HDIs were docked to the homology models to understand the similarities and differences between the binding modes. Molecular dynamic simulations of these HDAC-inhibitor complexes indicated that the interaction between the protein surface and inhibitor is the key factor for proper fit.

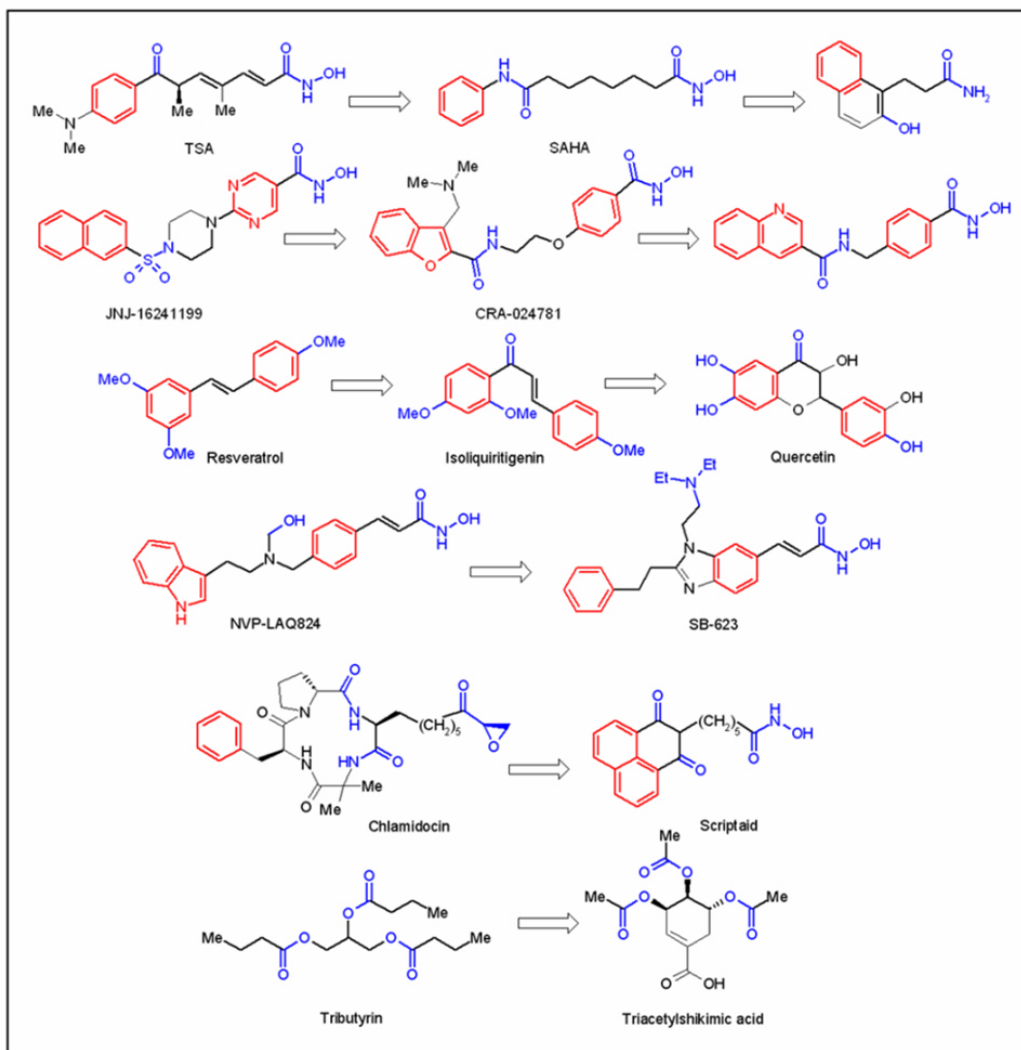
Homology modeling, force field design, and free energy simulation studies were recently described to optimize the activities of HDIs [xxvi]. In the optimization effort, authors used a computational protocol employing sequentially homology modeling, docking, molecular dynamics simulation, and free energy perturbation calculations for known HDIs. This effort yielded newly developed force field parameters for the coordination environment of Zn<sup>2+</sup>. It was suggested that free energy of inhibitor in aqueous solution is an important factor in determining its binding energy.

Several non-hydroxamic acid analogues of SAHA were designed on the basis of catalytic mechanism of HDACs [xxvii]. Kinetic enzyme assays and molecular modeling suggested that the mercaptoacetamide moiety of these compounds interacts with Zn<sup>2+</sup> in the active site of HDACs and removes a water molecule from the reactive site of the deacetylation.

### *Bioisosteric morphing*

Bioisosteric transformation is one of the tools that allow to balance different lead-like parameters including specificity, physicochemical and PKPD properties in the SAR studies. In addition, bioisosteric transformation approach provides insight into patentability of lead candidates. Numerous HDIs were designed using this technique [xxviii]. Typical examples of bioisosteric modifications for this class of molecules are shown in Fig. (5). The color marked structural motives are well recognized: hydrophobic caps (red), polar and zinc-binding active sites (blue) and aliphatic and aromatic spacers (black, bold type).





**Figure 5.** Bioisosteric transformations within HDIs family

### 2D-QSAR Modeling

Comprehensive 2D-QSAR studies of HDIs were recently reported [xxix]. For example, Xie *et al.* [29c] identified, collected, and verified structural and biological activity data for 124 compounds from various literature sources. The authors further performed an extensive QSAR study on this comprehensive data set by using various 2D-QSAR and classification methods. Highly predictive QSAR model with  $r^2$  of 0.76 and leave-one-out cross-validated  $r^2$  of 0.73 was obtained. The overall rate of cross-validated correct prediction of the classification model was around 92%. High-throughput biological screening can be followed by a computer-based QSAR study leading to the development of novel HDAC inhibitors [xxx].

We have effectively used all the strategies have been described to design our internal HDAC-targeted library with the prime focus on the structure-based design.

### Synthesis and biological evaluation

- (4) Novel HDAC-targeted libraries are synthesized according to the above criteria.
- (5) The subsets of HDAC library are validated by bioscreening in collaboration with academic institutions.

Our strategy has proven to be efficient for generation of protein class-targeted libraries. The higher hit rate over diverse libraries, along with identification of novel active chemotypes with optimized diversity and ADME properties, has been shown in multiple studies. Using the computational approaches listed above we have compiled HDAC-focused library consisted of more than 2000 small molecule compounds Representative set of HDAC-biased compounds is shown below.

Picture required

Examples of compounds from the HDAC-targeted library

## Conclusion

It should be especially noted that relatively modest progress in understanding pharmacology and clinical role of HDACs has been made since their discovery. From this point of view, specific natural and synthetic HDIs are useful tools for dissecting HDAC role in both normal and aberrant biological processes. Further optimization of these molecules into clinical candidates may yield drugs with enhanced efficacy against cancers, neurodegenerative and inflammatory diseases. As outlined in this paper, successful discovery of novel HDI leads relies on a combination of techniques from a wide range of disciplines, including molecular docking, pharmacophore-based design, data mining methods and traditional medicinal chemistry. The integration of high-throughput screening strategies with advanced virtual screening technologies holds great promise for more efficient discovery of HDI leads. Thus, here we provide efficient tools for *in silico* design of novel small molecule HDIs. Based on the accumulated knowledgebase as well as unique structure- and target-based models we have been designed more than 2000 small molecule compounds targeted specifically against HDAC family enzymes. As a result, the library is renewed each year, proprietary compounds comprising 50-75% of the entire set. Clients are invited to participate in the template selection process prior to launch of our synthetic effort.

## References

- <sup>i</sup> Balakin Konstantin V; Ivanenkov Yan A; Kiselyov Alex S; Tkachenko Sergey E. Histone deacetylase inhibitors in cancer therapy: latest developments, trends and medicinal chemistry perspective. *Anti-cancer agents in medicinal chemistry* 2007;7(5):576-92.
- <sup>ii</sup> Inove, A.; Fujimoto, D. *Biochem. Biophys. Res. Commun.*, **1969**, 36, 146.
- <sup>iii</sup> (a) Taunton, J.; Hassig, C.A.; Schreiber, S.L. *Science*, **1996**, 272, 408; (b) Waterborg, J.M.; Matthews, H.R. *Anal. Biochem.*, **1982**, 122, 313.
- <sup>iv</sup> Sendra, R.; Rodrigo, L.; Salvador, L.; Franco, L. *Plant Mol. Biol.*, **1988**, 11, 857.
- <sup>v</sup> (a) Carmen, A.A.; Rundlett, S.E.; Grunstein, M. *J. Biol. Chem.*, **1996**, 271, 15837; (b) Yang, W-M.; Inouye, C.; Zeng, Y.; Bearss, D.; Seto, E. *Proc. Natl. Acad. Sci.*, **1996**, 93, 12845; (c) GenBank accession number U80780; (d) GenBank accession number AF026949; (e) GenBank accession number AF071559; (f) Johnson, C.A.; Barlow, A.L.; Turner, B.M. *Gene*, **1998**, 221, 127.
- <sup>vi</sup> (a) Zhang, W.; Bieker, J.J. *Proc. Natl. Acad. Sci.*, **1998**, 95, 9855; (b) Jenuwein, T.; Allis, C.D. *Science*, **2001**, 293, 1074; (c) Spotswood, H.T.; Turner, B.M. *J. Clin. Invest.*, **2002**, 110, 577; (d) Kristeleit, R.; Stimson, L.; Workman, P.; Aherne, W. *Expert Opin. Emerg. Drugs*, **2004**, 9, 135; (e) Bannister, A.J.; Schneider, R.; Kouzarides, T. *Cell*, **2002**, 109, 801; (f) Carmena, M.; Earnshaw, W.C. *Nat. Rev. Mol. Cell Biol.*, **2003**, 4, 842; (g) Goto, H.; Yasui, Y.; Nigg, E.A.; Inagaki, M. *Genes Cells*, **2002**, 7, 11; (i) Timmermann, S.; Lehrmann, H.; Polesskaya, A.; Harel-Bellan, A. *Cell Mol. Life Sci.*, **2001**, 58, 728.
- <sup>vii</sup> Di Gennaro, E.; Bruzzese, F.; Caraglia, M.; Abruzzese, A.; Budillon, A. *Amino Acids*, **2004**, 26, 435.
- <sup>viii</sup> (a) Allfrey, V.G., Faulkner, R.; Mirsky, A.E. *Proc. Natl. Acad. Sci.*, **1964**, 51, 768 ; (b) Grunstein, M. *Nature*, **1997**, 389, 349. [36] Marmorstein, R. *Nat. Rev. Mol. Cell Biol.*, **2001**, 2, 422.
- <sup>ix</sup> (a) Ouaisi, M.; Ouaisi, A. *J. Biomed. Biotechnol.*, **2006**, 2, 13474; (b) Kathy, T.A.; Anna, W.; Mike, J.K.; David, P.F.; Allan, S. *Int. J. for Parasitol.*, **2000**, 30, 761; (c) Colletti, S.L.; Myers, R.W.; Darkin-Rattray, S.J.; Gurnett, A.M.; Dulski, P.M.; Galuska, S.; Allocco, J.J.; Ayer, M.B.; Li, C.; Lim, J.; Crumely, T.M.; Cannova, C.; Schmatz, D.M.; Wyvratt, M.J.; Fisher, M.H.; Meinke, P.T. *Bioorganic & Medicinal Chemistry Letters*, **2001**, 11, 107.
- <sup>x</sup> (a) Takigawa-Imamura, H.; Sekine, T.; Murata, M.; Tskazawa, K.; Nakagawa, J. *Biosci. Biotechnol. Biochem.*, **2003**, 67, 1499; (b) Mosely, A.L.; Ozcan, S. *J. Biol. Chem.*, **2003**, 278, 19660.
- <sup>xi</sup> Rombouts, K.; Gebretsadik, K.; Chavez, M.; Geerts, A. *J. Hepatology*, **2002**, 36, 9.
- <sup>xii</sup> Skov, S.; Rieneck, K.; Skak, K.; Tomra, S.; Michelsen, B.K.; Odum, N. *Blood*, **2002**, 15, 101.
- <sup>xiii</sup> Taylor, P.J.; Kenneth, H.F. *Trends in Molecular Medicine*, **2002**, 8, 195.

- <sup>xiv</sup> Hockely, E.; Woodman, B.; Ghazi-Noori, S.; Mahal, A.; Marsh, J.L.; Lewis, C.M.; Bates, G.P.; Marks, P.A.; Thompson, L.M.; Steffa, J.S.; Lowden, P.A.; Sathasivam, K.; Rosa, E.; Zhou, X.; Smith, D.L. *Proc. Natl. Acad. Sci.*, **2003**, *18*, 2041.
- <sup>xv</sup> Chung, Y.-L.; Lee, M.-Y.; Wang, A.-J.; Lin-Fen. *Mol. Therapy*, **2003**, *8*, 707.
- <sup>xvi</sup> Kohge, T.; Gohda, E.; Okamura, T.; Yamamoto, I. *Biochemical Pharmacology*, **1998**, *56*, 1359.
- <sup>xvii</sup> Chang, K. T.; Min, K.-T. *Ageing Research Reviews*, **2002**, *1*, 313.
- <sup>xviii</sup> Myzak, M.C.; Dashwood, R.H. *Curr. Drug Targets*, **2006**, *7*, 443-52.
- <sup>xix</sup> Finnin, M.S.; Donigian, J.R.; Cohen, A.; Richon, V.M.; Rifkind, R.A.; Marks, P.A.; Breslow, R.; Pavletich, N.P. *Nature*, **1999**, *401*, 188.
- <sup>xx</sup> Miller, T.A.; Witter, D.J.; Belvedere, S. *J. Med. Chem.*, **2003**, *46*, 5097
- <sup>xxi</sup> Ragno, R.; Simeoni, S.; Valente, S.; Massa, S.; Mai, A. *J. Chem. Inf. Model.*, **2006**, *46*, 1420.
- <sup>xxii</sup> Guo, Y.; Xiao, J.; Guo, Z.; Chu, F.; Cheng, Y.; Wu, S. *Bioorg. Med. Chem.*, **2005**, *13*, 5424.
- <sup>xxiii</sup> (a) Ragno, R.; Mai, A.; Massa, S.; Cerbara, I.; Valente, S.; Bottoni, P.; Scatena, R.; Jesacher, F.; Loidl, P.; Brosch, G. *J. Med. Chem.*, **2004**, *47*, 1351; (b) Lu, Q.; Wang, D.S.; Chen, C.S.; Hu, Y.D.; Chen, C.S. *J. Med. Chem.*, **2005**, *48*, 5530.
- <sup>xxiv</sup> Juvale, D.C.; Kulkarni, V.V.; Deokar, H.S.; Wagh, N.K.; Padhye, S.B.; Kulkarni, V.M. *Org. Biomol. Chem.*, **2006**, *4*, 2858.
- <sup>xxv</sup> Wang, D.F.; Helquist, P.; Wiech, N.L.; Wiest, O. *J. Med. Chem.*, **2005**, *48*, 6936.
- <sup>xxvi</sup> Park, H.; Lee, S. *J. Comput. Aided. Mol. Des.*, **2004**, *18*, 375.
- <sup>xxvii</sup> Suzuki, T.; Matsuura, A.; Kouketsu, A.; Nakagawa, H.; Miyata, N. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 331.
- <sup>xxviii</sup> (a) Suzuki, T.; Nagano, Y.; Kouketsu, A.; Matsuura, A.; Maruyama, S.; Kurotaki, M.; Nakagawa, H.; Miyata, N. *J. Med. Chem.*, **2005**, *48*, 1019; (b) Shinji, C.; Maeda, S.; Imai, K.; Yoshida, M.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 4784; (c) Hanessian, S.; Vinci, V.; Auzzas, L.; Marzi, M.; Giannini, G. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 4784; (d) Suzuki, T.; Miyata, N. *Mini Rev. Med. Chem.*, **2006**, *6*, 515.
- <sup>xxix</sup> (a) Bouchain, G.; Delorme, D. *Curr. Med. Chem.*, **2003**, *10*, 2359; (b) Wang, D.F.; Wiest, O.; Helquist, P.; Lan-Hargest, H.Y.; Wiech, N.L. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 707; (c) Xie, A.; Liao, C.; Li, Z.; Ning, Z.; Hu, W.; Lu, X.; Shi, L.; Zhou, J. *Curr. Med. Chem. Anticancer Agents*, **2004**, *4*, 273.
- <sup>xxx</sup> Bouchain, G.; Leit, S.; Frechette, S.; Khalil, E.A.; Lavoie, R.; Moradei, O.; Woo, S.H.; Fournel, M.; Yan, P.T.; Kalita, A.; Trachy-Bourget, M.C.; Beaulieu, C.; Li, Z.; Robert, M.F.; MacLeod, A.R.; Besterman, J.M.; Delorme, D. *J. Med. Chem.*, **2003**, *46*, 820.