http://dx.doi.org/10.5012/bkcs.2012.33.6.2063

Structure-Activity Relationship of Novel Lactam Based Histone Deacetylase Inhibitors as Potential Anticancer Drugs

Jee Sun Yang,[†,*] Tae-Gyu Chun,[‡] Ky-youb Nam,[§] Hwan Mook Kim,[#,*] and Gyoonhee Han[†,¶,*]

[†]*Translational Research Center for Protein Function Control, Department of Biotechnology, Yonsei University, Seoul 120-740, Korea. [*]E-mail: connaitre@yonsei.ac.kr*
[‡]*Electronic Chemical Materials Research Institute, Cheil Industries Inc., Gyeonggi-do 437-711, Korea*
[§]*Research Institute of Bioinformatics & Molecular Design, Seoul 120-740, Korea*
[#]*College of Pharmacy, Gachon University of Medicine and Science, Incheon 406-799, Korea. [*]E-mail: hwanmook@gachon.ac.kr*
[¶]*Department of integrated OMICS for Biomedical Sciences (WCU Program), Yonsei University, Seoul 120-740, Korea*
[*]*E-mail: gyoonhee@yonsei.ac.kr*
Received September 26, 2011, Accepted March 12, 2012

Key Words : Histone deacetylase (HDAC), Quantitative structure-activity relationship (QSAR), Lactam based histone deacetylase inhibitor

Histone acetyltransferases (HAT) and histone deacetylases (HDAC) regulate the chromatin structure related to transcriptional activity; epigenetic control.[1] Repression or inhibition of HDAC causes histone hyperacetylation and unfolds DNA-histone complex so that transcription factors can bind to DNA.[2] Abnormal recruitment of HDAC is related to pathogenic progress, such as cancer development.[3] Over expression of HDAC1 had been found in gastric cancers, oesophageal squamous cell carcinoma, and hormone refractory prostate cancer.[4] Over expression over HDAC2 and HDAC6 were found in colon cancer and breast cancer, respectively.[5]

The efforts to discover HDAC inhibitors have been continued for 30 years since some studies have been designed to understand the mechanism of terminal differentiation of murine erythroleukemia cells.[6] This early observation gave the great inspiration to the discovery of novel pharmacological agents of chromatin remodeling. It has been reported that SAHA (**1a**) and PXD101 (**1b**) were potent class I and class II HDAC inhibitors,[7] while FK228 (**1c**)[8] and the 2-aminophenylamide derivatives, MS-275 (**1d**) and Cl-994 (**1f**), were somewhat selective to class I HDACs (Figure 1).[9] In October 2006, the FDA approved the first HDAC inhibitor, Vorinostat (SAHA, **1a**), to treat cutaneous T-cell lymphoma (CTCL). In the meantime, there had been aggressive efforts to discovery of the new HDAC inhibitors for every major tumor types; hematological and solid tumors. A number of chemical entities were currently undergoing preclinical and clinical trials. PXD101 (**1b**) is being evaluated in clinical trials and treat potentially to treat a wide range of solid and hematologic cancer either as a monotherapy or in combination with others, and both an oral and intravenous formulation of the drug are being evaluated in clinical trials. FK228 (**1c**), also known as depsipeptide, was isolated from Chromobacterium violaceum and showed potent *in vivo* antitumor activity against both human tumor xenografts and murine tumors. MS-275 (**1d**) induced TGF-β type II receptor

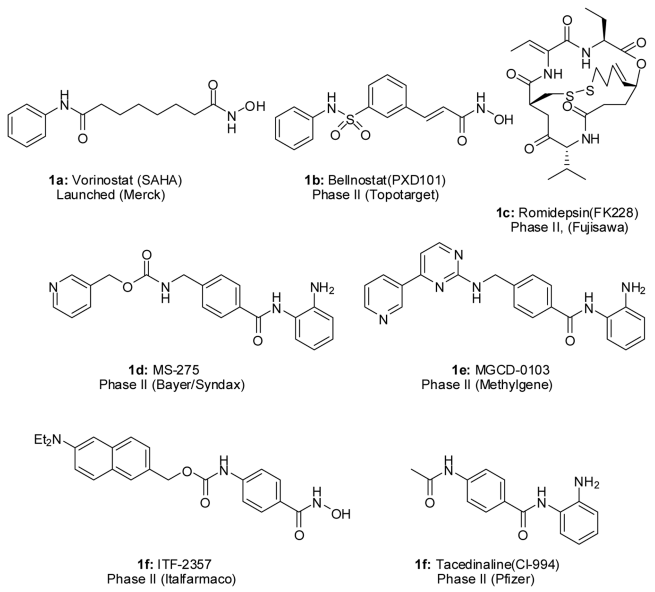

Figure 1. HDAC Inhibitors in the clinical trials.

expression selectively in human breast cancer cells. The additional novel therapeutic applications, such as the treatment of neurodegenerative diseases[10] and auto-immune diseases,[11] had been proposed for the use of histone deacetylase inhibitors.

In this paper, we report the 3-dimensional quantitative structure-activity relationship (QSAR) equation of a series of lactam based HDAC inhibitors for further evaluation of novel lactam based HDAC inhibitors based on the equation. In order to perform QSAR study, 3D structure of the HDAC inhibitors was prepared. All of 62 lactam based HDAC inhibitors[12-14] were built to the fragment units in Builder/Insight II[15] and hydrogen was added. Discover/Insight II was used for minimization of the conformation at the ground state. At the first time, only energy minimization of inhibitors was carried out by the consistent valence force field (CVFF)

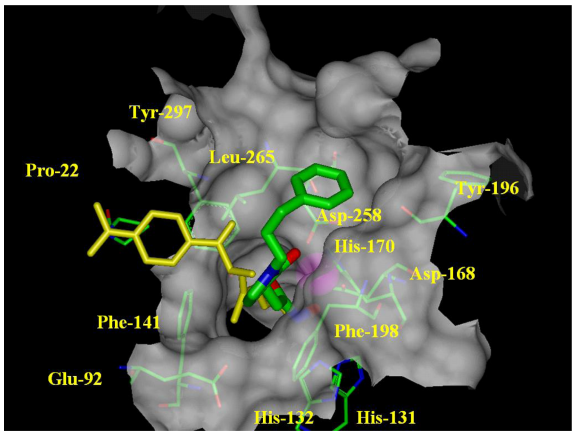

Figure 2. Docked orientations for **43** and TSA (yellow) bound to HDLP.

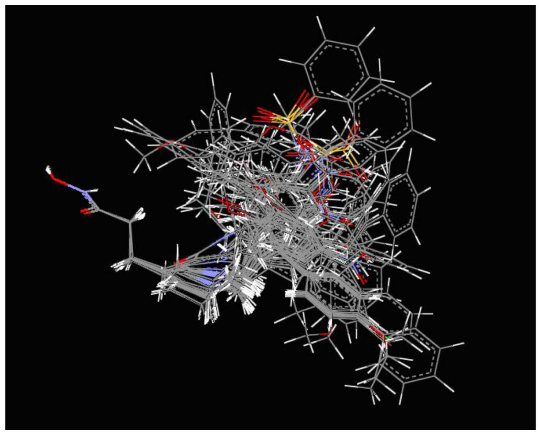

Figure 3. Aligned molecules of the training set and the test set.

of Discover program to reach the final derivative to 0.001 KJ/mol. The genetic function approximation (GFA) was performed with 62 compounds (population = 100, Generation = 5000, dependent variable: pIC_{50}, independent variables: 54 descriptors) and resulted in 100 QSAR equations. However, there were no meaningful equations, and showed only poor relationship between the structure and the activities ($r^2 < 0.2$).

By changing the idea, the crystal structure of histone deacetylase-like protein (HDLP) binding with Trichostatin A (TSA) was examined for the analysis of enzyme-ligand binding mode. The assumption was that the ligand with similar structure could display similar binding mode. Novel lactam based inhibitors had the similar binding moiety with TSA. The hydroxamic acid moiety showed same conformation and the enzyme was fixed except hydrogen. The conformation of HDAC inhibitors was modified to that of TSA, while hydroxamic acid moiety was remained as original conformation. The hydroxamic acid moiety of the lactam based HDAC inhibitor and all atoms of HDLP except hydrogen were fixed, while the rest of the inhibitor was free to relax during the minimization steps.

The active site of HDAC is consisted of a curved and opened tubular pocket. The presence of a zinc ion at HDAC active site was important in the mode of action of HDAC inhibitors. The binding mode of lactam based HDAC inhibitor **43** and TSA were shown in Figure 2. The lactam ring of **43** and TSA passes through a narrow channel in the binding pocket which was formed by two hydrophobic aromatic side chains of Phe141 and Phe198. The hydroxamic acid moiety of **43** and TSA chelated to the catalytic Zn^{2+} ion bound in the active site. The docking data suggested that the chain length between the zinc binder group and the lactam ring is quite important to form the flexible conformation in the binding pocket. Especially, the carbonyl group in the lactam ring forms the weak hydrogen bond with the residue of His170 and the aromatic hydrophobic cap group gives the extra stabilization with hydrophobic pocket on the enzyme surface.

By using the optimal structures of all 62 HDAC inhibitors, the final 3D QSAR equation was generated again through GFA method. The conformational structures of all 62 HDAC inhibitors were obtained by generating binding mode in HDLP (PDB code: 1C3R[16]) as mentioned above (Figure 3). 3D structures of the lactam based inhibitors were used for 3D QSAR study by using QSAR+/Cerius 2 (Accerlys Inc., San Diego CA, 2005). The following equation was obtained after performing GFA method twice.

$$pIC_{50} = 11.4961 + 0.452472 \times \text{"}S\_dsCH\text{"} + 0.032028 \times \text{"}Jurs\text{-}PPSA\text{-}3\text{"} - 2.27903 \times \text{"}JX\text{"} - 0.037669 \times \text{"}Shadow\text{-}YZ\text{"} - 0.324032 \times \text{"}Sr\text{"} - 0.87024 \times \text{"}Atype\_C\_6\text{"}$$

S-dsCH: Electrotopological state index;
Jurs PPSA-3: Jurs Partial Positive Surface Area 3;
A-type C-6: count of atom types;
Sr: superdelocalizability;
Jx: Balaban Indices (covalent radii);
Shadow YZ: YZ plane area of molecular shadow

All predicted activities of a series of lactam based HDAC inhibitors were obtained by using above equation. The equation from 45 compounds of the training set (Supplementary Table 1) with 6 descriptors shows good correlation coefficient with 77% of the variance. To confirm the accuracy of this equation, 15 compounds which are regularly distributed with biological activities were used as test set (Supplementary Table 2). The correlation coefficient (r^2) between observed and predicted activity of test set was found to be 0.76 (Figure 4). It shows that HDAC enzyme inhibitory activities of the newly designed lactam base HDAC inhibitors would be well predicted.

Six descriptors, *S-dsCH, Jurs PPSA-3, A-type C-6, Sr, Jx,* and *Shadow YZ*, were used as independent variables for the given QSAR equation. The *Jx* and *Shadow YZ* is related with the correlation of molecular size and activity. The equation suggests that the increase of radii and YZ shadow area of inhibitors induce the decrease of activity, and it could be explained by the narrow channel of HDLP which could cause steric repulsion with bulky compounds. However, the increase of surface area of aromatic cap group and the positive part induced substituent displayed the increase of activity in the equation. The hydrophobic interaction between

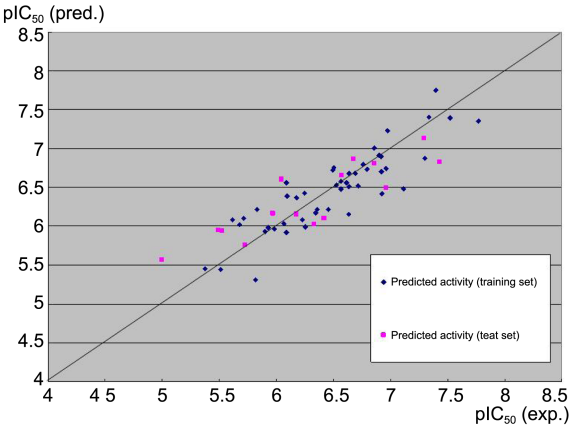

Figure 4. 62 compounds of QSAR result.

the surface region of HDLP and aromatic cap group offers a big stabilized energy. Because *Sr* stands for the index of reactivity in aromatic hydrocarbon, the more stable aromatic group would promise the better inhibitory activity. The range variance of *S-dsCH* and *A-type C_6* was not wide, so these factors were considered to be not much effective to activity than other descriptors.

In summary, docking simulation and 3D quantitative structure-activity relationship analysis were conducted on a series of novel lactam based HDAC inhibitors. Optimal conformation of the inhibitors was obtained from docking model in the active site of HDLP by using Insight II. The descriptors were examined for QSAR study by using Cerius 2. Finally, 6 descriptors were chosen as the independent variables for the generation of QSAR equation (training set: $r^2 = 0.77$, test set: $r^2 = 0.76$). The QSAR equation suggests that HDAC inhibitors which is entirely small but has big surface area of stabilized aromatic cap group would show better HDAC inhibitory activities. And it could be also explained by the molecular docking study of lactam core HDAC inhibitors in the active site of HDLP. On the basis of the descriptors thus obtained from the QSAR equation, novel lactam based HDAC inhibitors can be designed and developed that are predicted to attain improved HDAC inhibitory activity.

Experimental Section

Data Sets. A series of lactam core based HDAC inhibitors with a hydroxamic acid were used for generating QSAR equation in this study (Figure 5).[12-14] The HDAC inhibitor activity of the compounds reported was assayed using a mixture of HDACs, predominantly HDAC1 and HDAC2. These inhibitor activities were converted into the corresponding pIC_{50} (-log IC_{50}) for 3D QSAR analysis. The total set of HDAC inhibitors (62 compounds) was divided into training set (47 compounds) and test (15 compounds) set, which were selected randomly diverse molecules possessing activities of wide range.

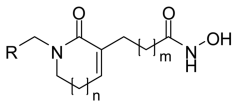

Figure 5. General structure of lactam based hydroxamic acid HDAC Inhibitors.

Molecular Docking Study. The binding mode of lactam based HDAC inhibitors with HDLP was studied using Discover/Insight II. Discover was used for energy minimization of HDAC inhibitors and the complex structure of the HDAC homologues and HDAC inhibitors. All calculations were performed on SGI Octane workstation with the IRIX 6.5 operation system. The crystal structure of HDLP (PDB code: 1C3R) in complex with TSA was recovered from Brookhaven protein data base (PDB, http://www.rcsb.org/pdb). HDLP from the hyperthermophilic bacterium *Aquifex aeolicus* shares 35.2% identity with human HDAC1 over 375 residues. Therefore HDLP was used for the homology model of HDAC1. The ligand binding mode and the binding affinity could be strongly pH-dependent. The protein structures were generated by using default values to determine the protonation states of the titratable groups. Amines were protonated and carboxylate groups were negatively charged, while zinc ion was 2+ charged and hydroxyl groups were neutral. For docking study, the bonding order of HDLP was fixed and hydrogen of HDLP was added at pH 7.4. The active site of HDLP was defined based on TSA and specified to all amino acids within 10 Å radius to all of the inhibitor atoms. The orientation of the lactam based HDAC inhibitors in the active site of HDLP was observed through the minimization of active site residue with the default set of Discover parameters. The structures of HDAC inhibitors were built by Builder/Insight II, and then the energy minimization of the complex structure of the HDLP and the inhibitors were performed by conjugate gradient method using the CVFF. The computational complex model was solvated using a solvent sphere of water extending 23.0 Å around the zinc ion, and residues only within 5.0 Å of lactam based inhibitors were allowed to move during the geometry optimizations using 500 steps of steepest decent and 3000 steps of conjugated gradient. The hydroxamic acid coordinates of HDAC inhibitors were restrained using 10.0 kcal/mol harmonic forces, the zinc ion VDW (Van der Waals) radius was taken from the work of Stote and Karplus.[17] The flexible docking module of Discover program was used for docking study of HDAC inhibitors and HDLP.

QSAR Study. 2D and 3D QSAR study was performed by using QSAR+/Cerius 2. More than 300 theoretical descriptors, representing diverse molecular physical properties, were used for calculating the equation. Calculation using many descriptors usually gives poor results and takes long time. Thus, the number of descriptors was required to be reduced by using correlation matrix.[18] Therefore, the descriptors with correlation score over 0.85 were eliminated, and through this procedure the number of descriptors was reduced to 54.

The first genetic function approximation (GFA) was performed (population = 100, Generation = 5000, dependent variable: pIC_{50}, 54 independent variables) and gave 100

QSAR equations. To increase the accuracy of the equation, 8 descriptors (*Jx, Jurs-PPSA-3, Atype_C_6, Shadow_YZ, Dipole-mag, S_dsCH, Sr, Shadow-Ylength*) were selected which displayed $r^2 > 0.5$ and lack-of-fit (LOF) < 0.2 in the equation. The final equation was obtained by performing GFA once again under following condition; population = 50, Generation = 500000, dependent variable: pIC50, independent variables: 8 descriptors.

Acknowledgments. This research was supported by National Research Foundation (2009-0092966), Korean Research WCU grant (R31-2008-000-10086-0) and the Brain Korea 21 project, the Republic of Korea.

References

1. Cosio, B. G.; Mann, B.; Ito, K.; Jazrawi, E.; Barnes, P. J.; Chung, K. F.; Adcock, I. M. *Am. J. Respir. Crit. Care Med*. **2004**, *170*(2), 141.
2. Kouzarides, T. *Curr. Opin. Genet. Dev*. **1999**, *9*(1), 40.
3. Mai, A.; Massa, S.; Pezzi, R.; Rotili, D.; Loidl, P.; Brosch, G. *J. Med. Chem*. **2003**, *46*(23), 4826.
4. Halkidou, K.; Gaughan, L.; Cook, S.; Leung, H. Y.; Neal, D. E.; Robson, C. N. *Prostate*. **2004**, *59*(2), 177.
5. Osada, H.; Tatematsu, Y.; Saito, H.; Yatabe, Y.; Mitsudomi, T.; Takahashi, T. *Int. J. Cancer* **2004**, *112*(1), 26.
6. Marks, P. A. *Oncogene* **2007**, *26*(9), 1351.
7. Paris, M.; Porcelloni, M.; Binaschi, M.; Fattori, D. *J. Med. Chem*. **2008**, *51*(6), 1505.
8. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K. H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Res*. **2002**, *62*(17), 4916.
9. Beckers, T.; Burkhardt, C.; Wieland, H.; Gimmnich, P.; Ciossek, T.; Maier, T.; Sanders, K. *Int. J. Cancer* **2007**, *121*(5), 1138.
10. The Huntington Study Group. *Neurology* **2001**, *57*(3), 397.
11. Leoni, F.; Fossati, G.; Lewis, E. C.; Lee, J. K.; Porro, G.; Pagani, P.; Modena, D.; Moras, M. L.; Pozzi, P.; Reznikov, L. L.; Siegmund, B.; Fantuzzi, G.; Dinarello, C. A.; Mascagni, P. *Mol. Med*. **2005**, *11*(1-12), 1.
12. Kim, H. M.; Hong, S. H.; Kim, M. S.; Lee, C. W.; Kang, J. S.; Lee, K.; Park, S. K.; Han, J. W.; Lee, H. Y.; Choi, Y.; Kwon, H. J.; Han, G. *Bioorg. Med. Chem. Lett*. **2007**, *17*(22), 6234.
13. Kim, H. M.; Lee, K.; Park, B. W.; Ryu, D. K.; Kim, K.; Lee, C. W.; Park, S. K.; Han, J. W.; Lee, H. Y.; Han, G. *Bioorg. Med. Chem. Lett*. **2006**, *16*(15), 4068.
14. Kim, H. M.; Ryu, D. K.; Choi, Y.; Park, B. W.; Lee, K.; Han, S. B.; Lee, C. W.; Kang, M. R.; Kang, J. S.; Boovanahalli, S. K.; Park, S. K.; Han, J. W.; Chun, T. G.; Lee, H. Y.; Nam, K. Y.; Choi, E. H.; Han, G. *J. Med. Chem*. **2007**, *50*(11), 2737.
15. Hagler, A. T.; Lifson, S.; Dauber, P. *J. Am. Chem. Soc*. **1979**, *101*(18), 5122.
16. 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*(6749), 188.
17. Stote, R. H.; Karplus, M. *Proteins* **1995**, *23*(1), 12.
18. Liu, X.; Tu, M.; Kelly, R. S.; Chen, C.; Smith, B. J. *Drug Metabolism and Disposition* **2004**, *32*(1), 132.