OPENING MINDS:
RESEARCH FOR SUSTAINABLE
DEVELOPMENT

Computational Studies on Inhibition of Histone Deacetylation by Hydroxamic Acid Derivatives: An *In-Silico* Approach

R. Dushanan[1], G.R. Ranawaka[2], S. Weerasinghe[3] and R. Senthilnithy[1*]

[1]*Department of Chemistry, The Open University of Sri Lanka, Nugegoda, Sri Lanka*
[2]*Department of Zoology, The Open University of Sri Lanka, Nugegoda, Sri Lanka*
[3]*Department of Chemistry, University of Colombo, Sri Lanka*

**Corresponding author: Email: rsent@ou.ac.lk*

1 INTRODUCTION

Both genetic and epigenetic modifications of DNA can lead to cancers. Acetyl and methyl histone modifications and DNA methylation are the most studied epigenetic modifications which are now recognized as a common hallmark of human tumours.

Histone is acetylated in its lysine residues within the N- terminal tail protruding from the histone core of the nucleosome. This removes positive charges, thereby reducing the affinity between histones and DNA. As a result, it becomes easier for RNA polymerase and transcription factors to access the promoter region. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.

There is no evidence that abnormal histone modification can cause cancer, but drugs that alter histone modifications help to turn on genes which help to control cell growth and division. Histone Deacetylase (HDAC) inhibitors cause an increase of the acetylated level of histones, which in turn promotes the re-expression of the silenced regulatory genes in cancer cells and reverses the malignant phenotype.

Interactions of drugs with HDAC, by covalent or non-covalent binding, may inhibit replication and interfere with transcription by recruiting essential transcription factors from their native binding sites, thus causing the death of cancerous cells. The main objective of cancer therapy is to focus on the interruption of the cellular reproductive cycle. Therefore, if Histone Deacetylation was prevented or controlled in its tracks, an effective anticancer therapy could be developed.

However, a major challenge is the lack of in-depth understanding of the biological function of the structurally diverse HDAC isoforms. Their participation and the mechanism in the process of tumour genesis are also not clearly understood. Computational studies based on Molecular Dynamics (MD) simulations can provide new information regarding the interaction of a drug with HDAC which will provide a better understanding of gene mutation.

This work presents an in-silico approach to study the inhibitory effect of a new hydroxamic acid derivative as a potential anti-cancer drug. Here we report MD simulation studies carried out on the Histone Deacetylase-Drug (HDAC-Drug)

complexes in order to study the stability of the complexes. The secondary structural changes in the HDAC were analysed from the trajectories.

2 METHODOLOGY

X-ray crystal structure of Histone Deacetylase (PDB ID 1ZZ1) was downloaded from the protein data bank. The structures of two drug compounds (derivatives of hydroxamic acids) shown in Figure 1 were generated in Gauss view and optimized with ab-initio calculation with CBS-QB3 basis set using Gaussian G09 software in Linux operating system and each structure was converted to .PDB format using Avagadro software and saved.

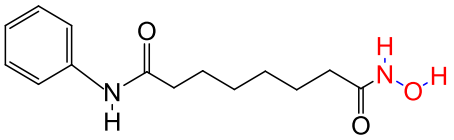
AutoDock Tools 1.5.6 docking software package, Vina with 9 modes and an exhaustiveness value of 8 were used to dock each optimized drug on to HDAC enzyme. Enzyme-drug complexes with the best binding score obtained from the molecular docking process were selected as the starting configurations for molecular dynamics studies. In the MD simulation, GROMOS53a6 all atom force field was employed for the enzyme while the force field parameters for drugs were obtained from PRODRG server. The enzyme-drug complex was placed at the centre of a 8.8 x 8.8 x 8.8 nm^3 cubical box. Then the complex was solvated by filling the simulation box with about 20,000 SPC/E water molecules and 11 Na+ ions were added to maintain electrical neutrality of the system.

Electrostatic interactions were modelled with particle mesh Ewald (PME) with short range cut-off of 1.2 nm. Temperature and pressure of the system were modulated at 300 K and 1 bar using Berendsen's weak coupling algorithm. All bonds were constrained at their equilibrium distances using LINCS algorithm.

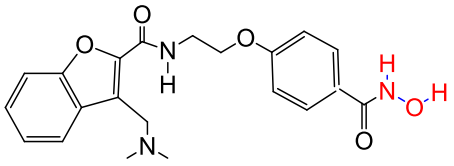
Similar simulation protocol was employed for the wild type enzyme (without drug) to compare the behaviour of wild type enzyme with that of complexes in aqueous medium. All the systems were subjected to 500 steps of energy minimization with steepest decent algorithm followed by 100 ps molecular dynamics simulation to equilibrate the system.

After the equilibration step, molecular dynamic simulations were performed for 100 ns for all studied enzyme-drug systems with a 0.002 ps time step using GROMACS molecular dynamic simulation package running in LINUX operating system. The trajectories of the outcomes were saved at every 2 ps intervals for further analysis. All the simulations were carried out using a high configuration Dell Power edge T130 Server.

All calculated properties of the inhibiter PCI-24781 were compared with those of Suberoylanilide hydroxamic acid (SAHA) since it is the well-known inhibiter drug in the clinical practice.



(a)



(b)

Figure 1: The two HDAC inhibiters used in this study; (a) Suberoylanilide hydroxamic acid (SAHA), (b) PCI-24781

ISSN 2012-9916 © The Open University of Sri Lanka

3 RESULTS AND DISCUSSION

The molecular dynamics trajectory files were analysed to study the structural changes of the enzyme with simulation time. The enzyme-drug interaction energies, root mean square deviation (RMSD), radius of gyration (Rg), solvent accessible surface area (SASA) and the root mean square fluctuation (RMSF) of the enzyme were analysed as a function of time.

Root mean square deviation and radius of gyration: The RMSD and Rg of the enzyme in each enzyme-drug complex were compared with the RMSD and Rg of the wild type enzyme and the comparison is shown in the Figure 2.

The RMSD of both the studied drugs slowly increased up to 40 ns of the trajectory and then attained the RMSD value of around 0.3 nm and it remained at this value for the rest of the simulation time.

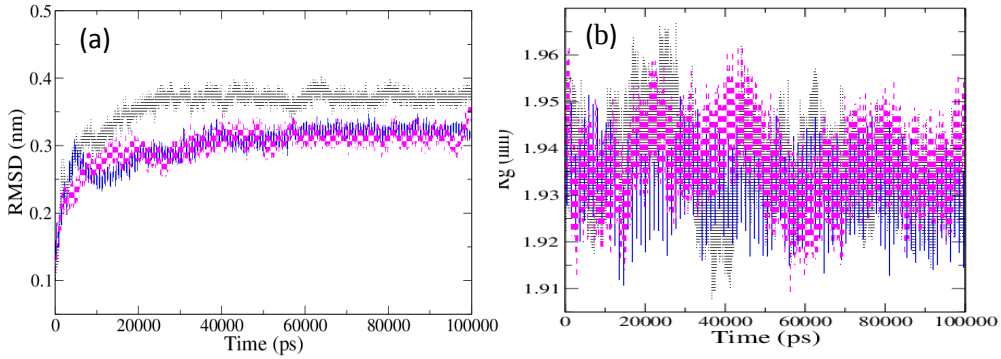


Figure 2: Comparison of (a) RMSD and (b) Radius of gyration (Rg) of the enzyme-drug complexes with wild type enzyme (Wild type enzyme, SAHA, PCI-24781)

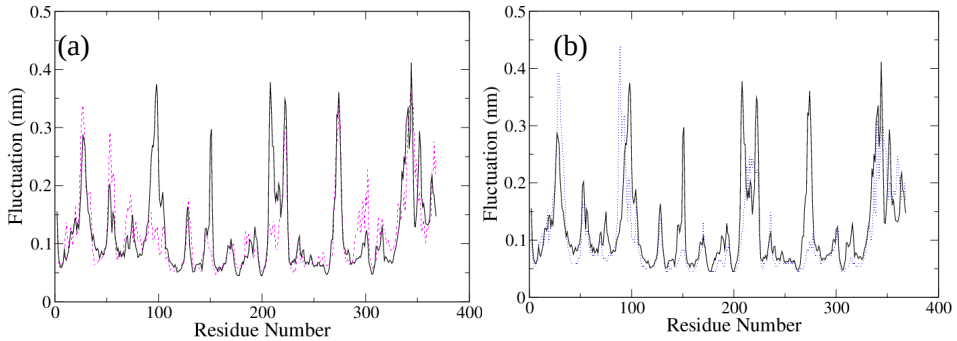


Figure 3: Comparison of RMSF of (a) SAHA – HDAC complex with wild type HDAC, (b) PCI-24781 – HDAC complex with wild type HDAC (Wild type enzyme, SAHA, PCI-24781

According to Figure 3, the RMSF of the amino acids of the two studied complexes did not show a drastic change to the RMSF of the amino acids of the wild type enzyme. However, some significant local conformational changes were observed. With both studied HDAC-drug complexes fluctuation of 28(ALA), 151(GLY) and 208(PHE) amino acids were stabilized with low RMSD.

This may be due to the coordination of water molecules with these amino acids in aqueous medium that led to the stabilization of the fluctuations of the said residues (RMSD of individual residues are not shown here). Similar reduction of fluctuations of the residues 53(SER), 73(LEU), 129(GLU), 274(PRO), 294(ASP), 297(ALA) 300(CYS) and 302(GLY) with the reference drug (SAHA) and of the residues 274(PRO), 208(PHE), 222(ASN) with the new drug PCI-24781 were observed and the RMSD of individual residues did not reveal significant structural rearrangements.

In both complexes, fluctuation of 98(ASP) residue was suppressed and the RMSD of individual residue indicates some structural rearrangements of the residue. While in the enzyme with the new drug, PCI-24781, fluctuations of the residues; 89(ASN), 93(GLY) were enhanced and the RMSD of the individual residues indicate some structural changes of 89(ASN) at about 17th ns and of 93(GLY) at 86th ns.

4 CONCLUSIONS

This *in-silico* study demonstrates that the new drug, PCI-24781 and the reference drug, SAHA exhibit a similar effect on the enzyme and therefore, it could be possible to use the new drug, PCI-24781 as an alternative to the reference drug, SAHA for the inhibition of histone deacetylation.

Acknowledgment

Faculty Research Grant (2016) of Faculty of Natural Sciences/OUSL is gratefully acknowledged for funds provided for this study. Department of Mathematics and computer science is acknowledged for providing a high-end computer.

REFERENCES

Armstrong L., (2014), *Epigenetics*, Garland Science, Taylor 7, Francis Group, LLC.

Maleszewsha M. and Kaminska B., (2013), Is Glioblastoma an Epigenetic Malignancy? *Cancers*, **5(3)**, 1120-1139.

Neto B.A.D and Lapis A.M.A., (2009), Recent Developments in the Chemistry of Deoxyribonucleic Acid (DNA) Intercalators: Principles, Design, Synthesis, Applications and Trends; *Molecules*, **14**, 1725 – 1746.

Nielsen T.K., Hildmann C., Dickmanns A., Schwienhorst A., Ficner R. (2005), Crystal structure of a bacterial class 2 histone deacetylase homologue, *J.Mol.Biol.* **354**: 107-120.

Schüttelkopf A.W. and Van Aalten D.M., (2004), "PRODRG: a tool for high-throughput crystallography of protein-ligand complexes", *Acta Crystallogr* **D60**, 1355-1363.

Berendsen H.J. C., Grigera J.R. and Straatsma T.P., (1987), "The missing term in effective pair potentials", *J. Phys. Chem.*, **91 (24)**, 6269-6271.

Essmann U., Perera L., Berkowitz M.L., Darden T., Lee H. and Pedersen L.G., (1995), "A smooth particle mesh Ewald method", *J. Chem. Phys.*, **103 (19)**, 8577-8593.

Berendsen H.J.C., Postma J.P.M., Van Gunsteren W.F., DiNola A. and Haak J.R., (1984), "Molecular dynamics with coupling to an external bath", *J. Phys. Chem.*, **81**, 3684-3690.

Hess B., (2007), "P-LINCS: A parallel linear constraint solver for molecular simulation", *J. Chem. Theory Comput.*, **4 (1)**, 116-122.

Pronk S., Pall S., Schulz R., Larsson P., Bjelkmar P., Apostolov R., Shirts M., Smith J., Kasson P., Vander Spoel D. and others, (2013), "Gromacs 4.5: a high-throughput and highly parallel open source molecular simulation toolkit', *Bioinformatics*, **29 (7)**, 845 – 854.

ISSN 2012-9916 © The Open University of Sri Lanka