Int. J. Drug Dev. & Res.
| January - March 2015 | Vol. 7 | Issue 1 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Protein-Protein Interfaces Mimics and Inhibitors Design for Cancers Caused by the disruption of HDAC-3

K. Rajaganapathy*

P. Balaji

V. K. Kalaichelvan

Department of Pharmacy, Annamalai University, Annamalai Nagar - 608002, Chidambaram, Tamil Nadu, India.

Corresponding Authors:
K. Rajaganapathy. M. Pharm.
Research Scholar
Department of Pharmacy,
Annamalai University,
Annamalai Nagar-608002,
Tamil Nadu, India.
Email:
vkrajaganapathy@gmail.com

Abstract:
Protein-Protein interactions are deregulated or disrupted; it's a new target for an anti-cancer agent development. In this work, the protein-protein interfaces mimics on a small molecule inhibitors of a molecular combinatorial ligand library (as a similar structure of protein-protein interfaces) was designed for disruption of NcoR-SIN3-HDAC3 complexes. And molecular docking study was performed with Schrodinger-Maestro-9.3.5-Version, the designed five Ligands was shown good binding interactions and their docking score was around -11.9, As a result of five ligand of a novel analogue is showing superior anti- cancerous histone deacetylase inhibitor caused by the disruption of HDAC-3.

Keywords: NcoR-SIN3-HDAC3, Histone deacetylase Inhibitors, Protein-Protein Interfaces.

Introduction

Histone Deacetylase enzymes play a significant role in inflicting cancer and dreadful human diseases. The basic repetition sub-units of all eukaryotic chromatin granule were nucleosome. It's created by DNA (146 base pairs) wrapped around octomer of histone proteins. The altered histone proteins, acetylation and deacetylation happen that optimized enzymes are referred to as HATs and HDAC1-3. Generally, hyperacetylation of e-amino teams of essential amino acid residues, that are within the tail of the core histones, facilitates gene expression, whereas histone deacetylation correlates with transcriptional repression. The equilibrium between the acetylation and deacetylation of histones plays a vital role in cell cycle management. Perturbations of balance between the activities of HATs and HDACs are joined to cancer and different diseases, like the hypoacetylated or hyper deacetylated chromatin granule by over expression of HDACs becomes inaccessible to transcription factors and affects the chromatin granule transforming method that is termed improper gene activation or silence transcription or repression. (Some mutations are going to be created for instance PML-RAR, RXR-RAR and PML united cells)1-3.

Human HDAC families consist of four categories, which incorporates Class-I, II, III and IV class I, II and IV HDACs are mediated by Zn-dependent mechanism and class III HDACs are mediated by an NAD-dependent mechanism(4).

The class I HDACs include HDAC one, 2, 3 and 8, expressing high sequence similarity to the yeast macromolecule Rpd3

(Reduced potassium dependency protein-3). It's

comparatively little size enzymes travel between 42–45 kDa, sole factor expressed within the nucleus. Class II HDACs share homologies with the yeast HDA1 macromolecule and consists of HDAC four, 5, 6, 7, 9, and 10. Class II HDACs are larger proteins travel between 120–130 kDa and its shuttling between the cytoplasm and also the nucleus class III HDACs are structurally unrelated to the human class I and class II HDACs, and share similarity with Sir 2 (Silent information regulator-2) macromolecule (Sir 1-7). And Class-IV comprises of HDAC114.

Histone deacetylase inhibitors (HDACIs) correspond to the hopeful new category of compounds for the treatment of cancers5-6. HDAC inhibitors target the gene expression while not modifying the deoxyribonucleic acid sequence. HDAC's bind deoxyribonucleic acid tightly to histones so preventing the transcription of various growth suppressor genes. HDAC inhibitors become potent inducers of growth arrest, programmed cell death of remodeling cells and differentiation by regulation the gene expression. A normal set of genes regulated by all HDAC inhibitors was found to be the foremost half concerned in cell-cycle, programmed cell death and deoxyribonucleic acid synthesis. The substrate access is blocked, inflicting AN accumulation of acetylated histones once HDAC inhibitors bind on to the HDAC active site. HDACIs comprising of four main structural categories together with hydroxamates, short-chain fatty acids, benzamides and cyclic tetrapeptides signify a broad family of chemical compounds. These compounds even have completely different affinities for numerous HDACs beside the structural dissimilarities. Hydroxamic acids are the biggest category of HDACIs with great therapeutic potential[5-6].

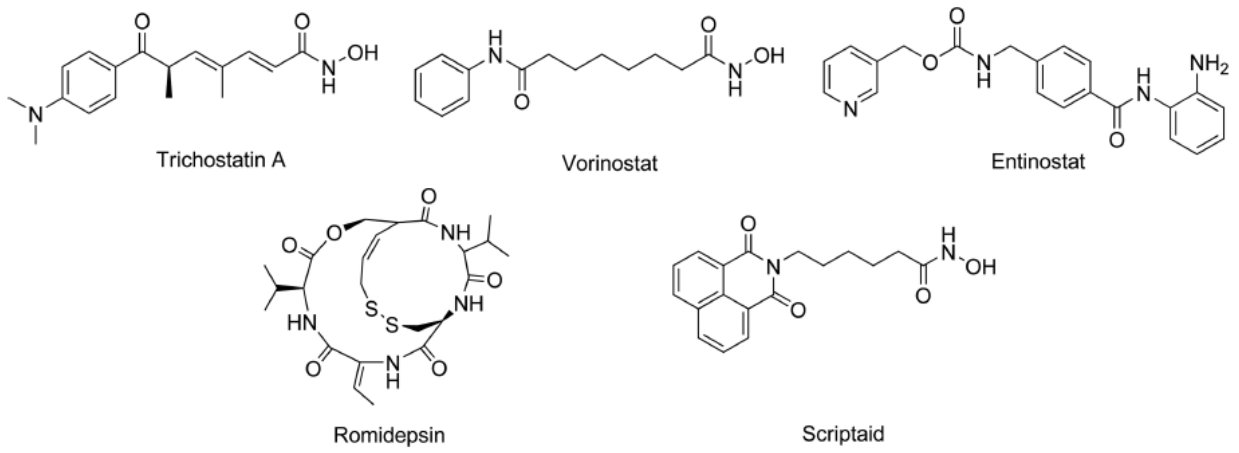


Figure 1: Current HDAC inhibitors

An important feature of HDAC is that it requires several components of other multi-protein complexes to perform its cellular activities. This includes transcriptional co-repressors such as mSin3A, N-CoR/ SMRT–SIN3–HDAC complex, PML and others in AML[5]. The SIN3–HDAC is a ubiquitous, abundant and large protein complex with highly conserved functional domains[7]. The complex contains HDAC-1, HDAC-3 and the scaffolding protein SIN3, and is involved to influence several key regulatory signals. The N-CoR/SMRTs are bridging proteins released by RAR

along with SIN3-HDAC complex. The N-CoR/SMRTs act as a linker for the interaction of RAR-RXR with SIN3-HDAC complex. The RAR-alpha is an element of nuclear hormone receptor family that functions as a transcription factor by binding to DNA and regulating transcription of its target genes. While the carboxyl terminal of the N-CoR/SMRT is responsible for non-redundant external interactions, the amino terminal mediates active transcriptional repression by mediating repression pathways in Acute Promyelocytic leukemia (APL) and Acute Myelogenous Leukemia (AML). Upon binding of ligand RA to its receptor, the N-CoR–SIN3–HDAC co-repressor complex is released and exchanged for the binding of a co-activator complex, such as TIF2–CBP HAT and gene transcription is activated and is masked in the absence of ligand[7].

This gift study concerned with planning of a novel histone deacetylase inhibitors by mimicking on a protein-protein interfaces. And inhibitors disturbed through protein-protein interactions of NcoR-SIN3-HDAC3 complexes by using the techniques of molecular docking tools Schrodinger (Maestro) – 9.3.5 version was used for finding out molecular docking and ligand–protein interactions. In these views, three combinatorial ligand libraries was designed and the studies were disbursed so as to spot a good, selective and superior anticancerous HDACi and its novel analogues for the treatment of human cancers caused by HDAC3 abnormality.

METHODOLOGY:

Protein-Protein Docking: To facilitate mimic the NcoR-SIN3A-HDAC-3 complex, the 3D crystal structures of proteins: NCoR (PDB ID: 1xc5), SIN3A (PDB ID: 1G1E) and HDAC3 (PDB ID: 4A69) were retrieved from the Protein Data Bank (PDB). First, the NcoR-SIN3 complex was mimicked by docking NcoR and SIN3 using Patch Dock. Similarly, the HDAC3 was docked with the NcoR-SIN3 complex to obtain a mimicked structure. The three protein complex of NCoR-SIN3A-HDAC-3 obtained by Patch Dock is shown in the Fig-2.

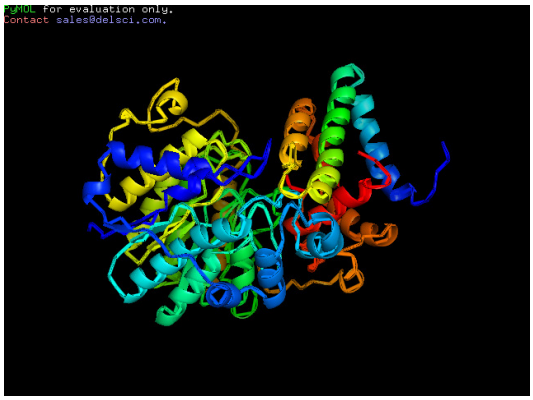


Fig 2: Protein complex of NcoR-SIN3A-HDAC3-The complex is 1156 amino-acids long and consists of 22 alpha-helix and 12 beta-pleated sheets.

Protein-Protein Interfaces Mimics and Small Molecule Designing: The obtained 3D complex of NCoR-SIN3A-HDAC3 was introduced to the online metta-PPISP server for the prediction of protein-protein interface region[8-10]. The interface region was interpreted and analyzed by SwissPDBViewer. The possible small molecular structures that mimic the interface region were identified drown from Marvin Sketch. MarvinSketch has advanced chemical editor for drawing chemical structures and it has a rich list of editing features. The Protein-protein interface mimics[8-10] was used for predicting ligands similar to the interface region. There were three positive interface regions predicted. The Interface Region-1 contained the amino-acids Asparagine, Glycine and Lysine at the residues 299, 300 and 301 while The Interface Region-2 contained the amino acids Methionine and Alanine at the residues 303 and 304 respectively and the

interface region-3 contained the amino acids Aspartic acid, Proline and Methionine respectively. And all the interface regions were present in the A-chain.

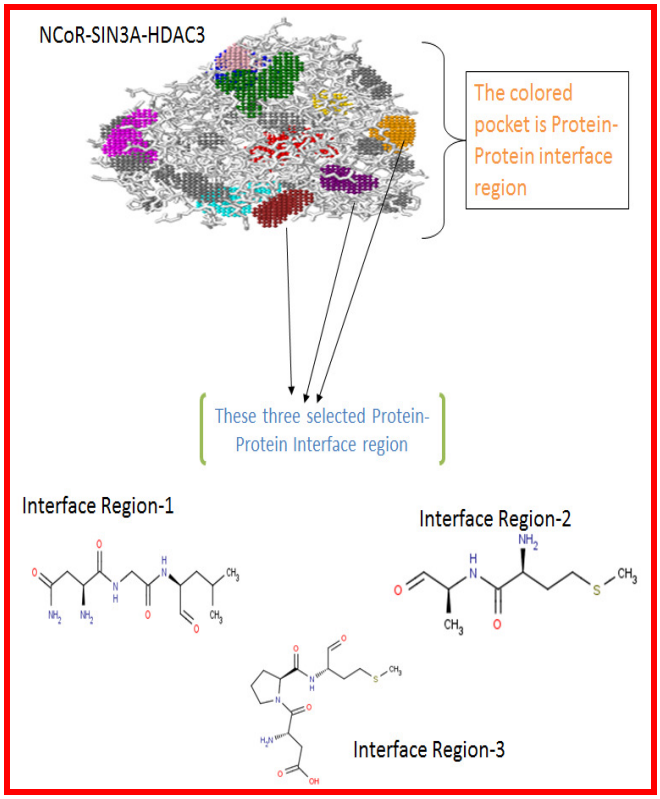


Fig-3: Protein – Protein Interface region Mimics On a small molecules

Results and Discussion

Combinatorial Ligand Library Designed From Interface Region of a Small Molecule:

The combinatorial ligand library-1, 2 and 3 were designed from Protein-Protein Interface region-1, 2 and 3 respectively and it was illustrated in Fig 4, 5 and 6. In this present study, the ligand library-1 was created by slight chemical modification of interface region-1 thus, first take away the corner region of -CH(CHO)-CH_2-$CH(CH_3)_2$, further amine and acyl halide coupling of the second position amine officious with the acyl halide like 7-(hydroxycarbamoyl) heptanoyl chloride and when amine-acid halide coupling finally can opt for alkylation of the eleventh position of amide and imidazole, they formed final confirmation.

The combinatorial ligand library-2 was designed by the modification of interface region-2 thus, first the reaction carried out by formation of imine from aldehydes and amine of the starting material then the reaction mass was further reacted with AgNO3 and NH4OH they shaped COOH cluster within the position of eleven of the chemical masses then, acid amine coupling to form a final chemical mass.

The combinatorial library-3 was designed from the interface-3. First, the starting material was reacted with BOC-anhydride they formed Boc amine bond then reacted with EDC. Hcl, HoBt, DIPEA and DCM with the chemical reagent of benzimidazole, imidazole and morpholine they formed pre-final confirmation, then they reacted with Hcl they removed BOC anhydride to form a final chemical mass.

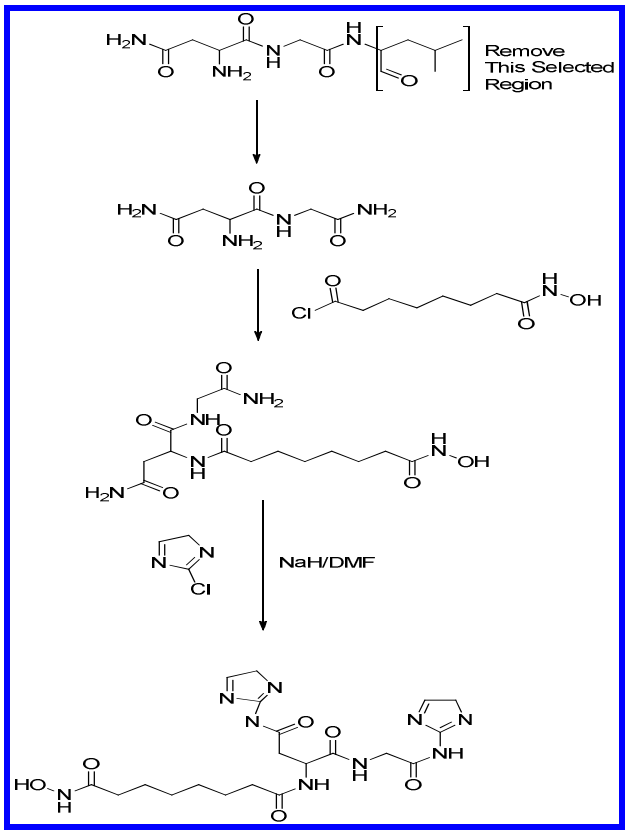


Fig-4: Combinatorial Ligand Library-1, from Interface Region-1

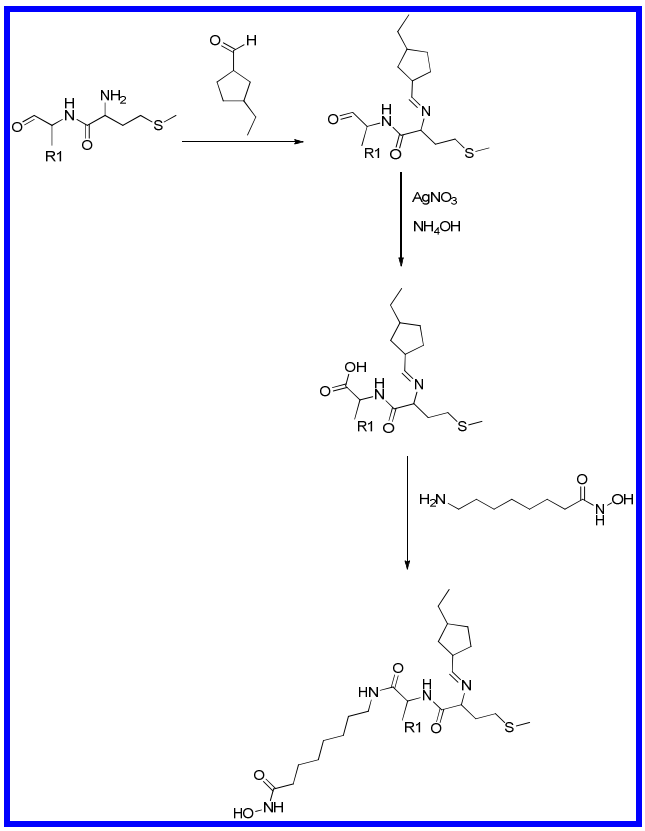


Fig-5: Combinatorial Ligand Library-2, from Interface Region-2

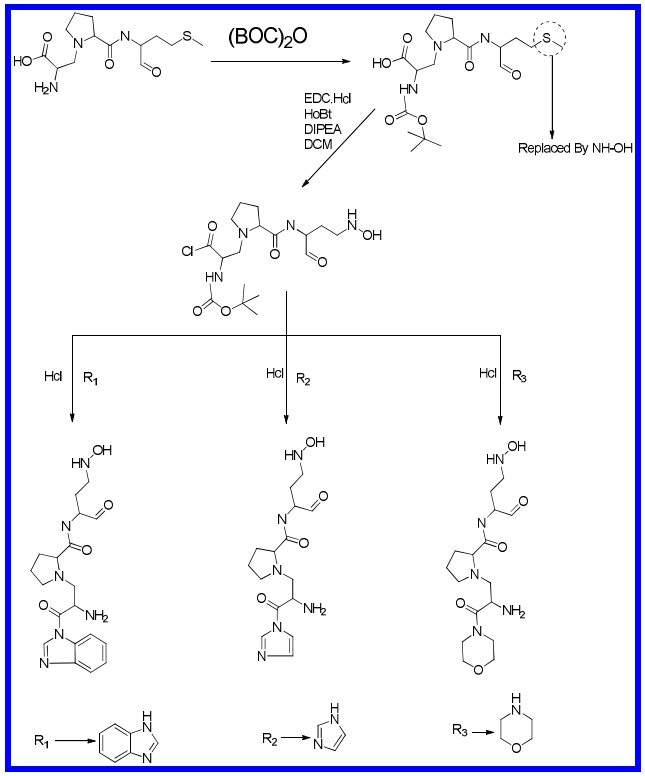


Fig-6: Combinatorial Ligand Library-3, from Interface Region-3

Protein-Ligand Docking: The complex of HDAC3 was taken as the target protein. A molecular docking study by Schrodinger (Maestro 9.3.5 Version) was performed for the calculation of binding energy at the active site for all the small molecules of the analogues from the combinatorial ligand library totally five ligands. The ligand-protein interaction images were viewed, which shows the type and distance of interacting atoms between ligand and protein. It also helps to identify the pocket information or functional sites of protein that are interacting with the ligand molecule.

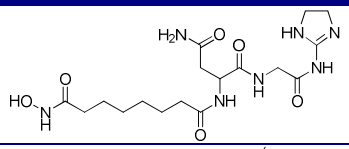
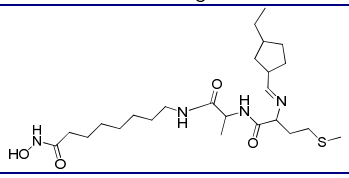
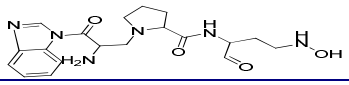
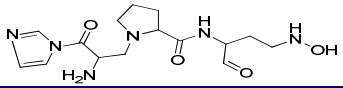
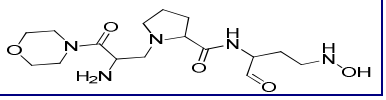
In these present investigations, docking result was shown on Table-I and the five designed ligands (Showing Table-II) exposed best and excellent protein – ligand interaction, it's showing more than seven hydrogen bond interactions, including a backbone and side chain, charged positive, one mild polar interactions and zing binding motif its shown on Fig-7, 8, 9, 10 and 11.

Table 1: Docking Result

S. No	Ligand (small molecule)	Docking Score $E_{bind\ (MM)}$
1.	Ligand - 2	-11.9
2.	Ligand – 3a	-11.6
3.	Ligand – 3b	-11.0
4.	Ligand – 3c	-10.9
5.	Ligand - 1	-9.9

*Arround or above -9 E_{bind} is a good result of docking score.
*Arround or above five H-bond interactions shown optimum docking result.

Table 2: IUPAC Name of the ligands

Ligand No	IUPAC Name	Structure
Ligand-1	N-[2-carbamoyl-1-({[(4,5-dihydro-1H-imidazol-2-yl)carbamoyl]methyl}carbamoyl)ethyl]-N'-hydroxyoctanediamide	
Ligand-2	8-(2-{2-[(E)-[(3-ethylcyclopentyl)methylidene]amino]-4-(methylsulfanyl)butanamido}propanamido)-N-hydroxyoctanamide	
Ligand-3a	1-[2-amino-3-(1H-1,3-benzodiazol-1-yl)-3-oxopropyl]-N-[4-(hydroxyamino)-1-oxobutan-2-yl]pyrrolidine-2-carboxamide	
Ligand-3b	1-[2-amino-3-(1H-imidazol-1-yl)-3-oxopropyl]-N-[4-(hydroxyamino)-1-oxobutan-2-yl]pyrrolidine-2-carboxamide	
Ligand-3c	1-[2-amino-3-(morpholin-4-yl)-3-oxopropyl]-N-[4-(hydroxyamino)-1-oxobutan-2-yl]pyrrolidine-2-carboxamide	

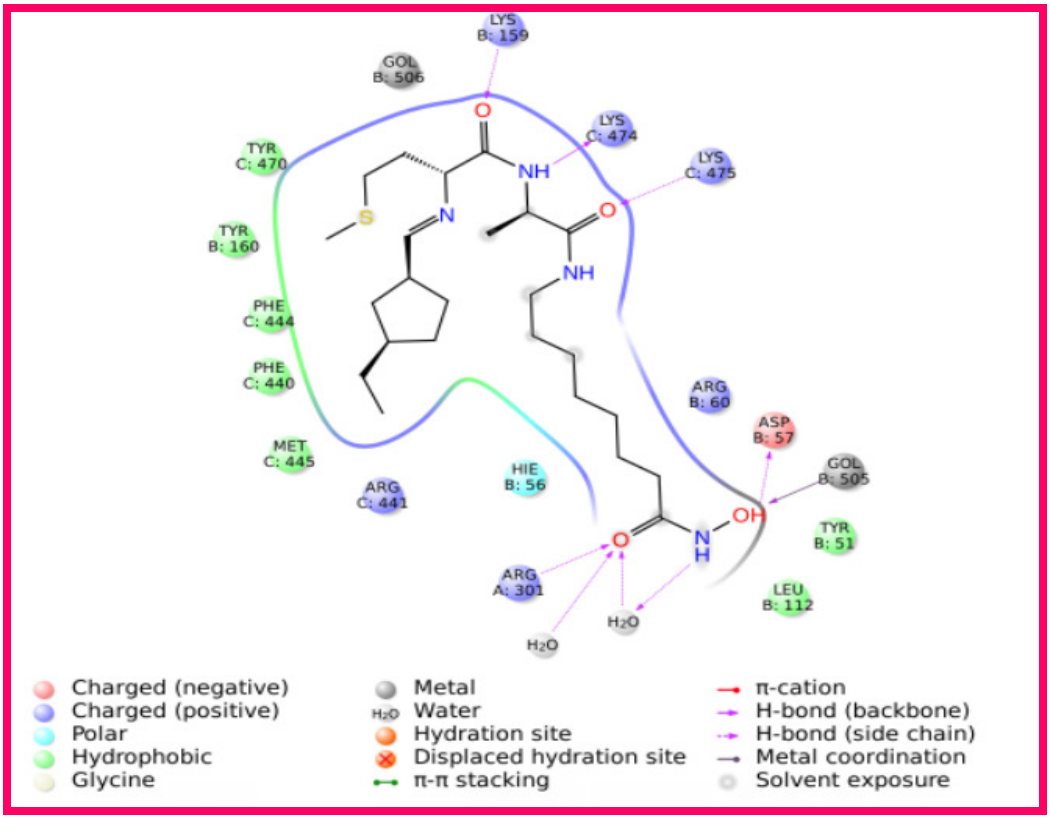


Fig-7: Interaction of HDAC3 with Ligand 2

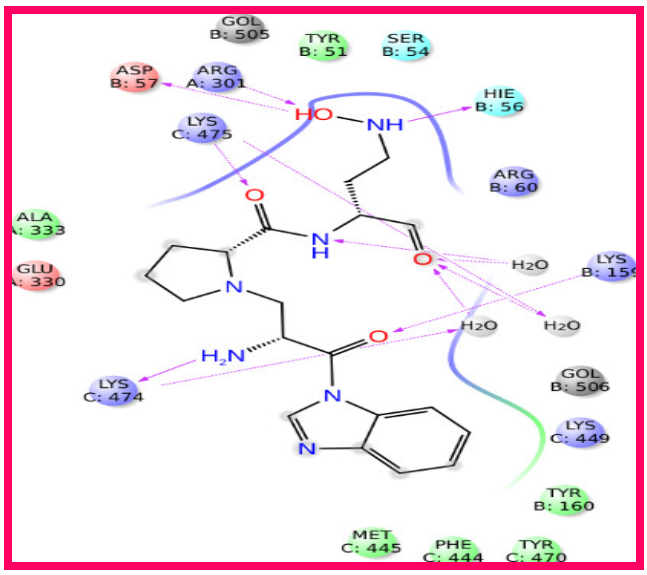


Fig-8: Interaction of HDAC3 with Ligand 3a

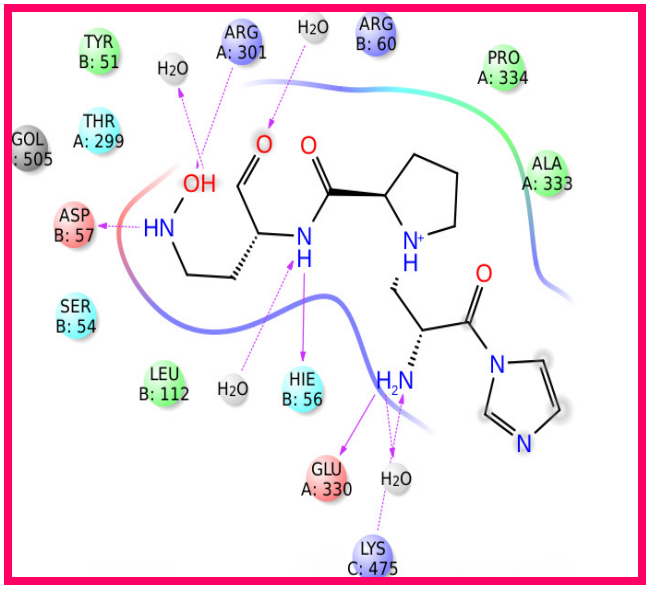


Fig-9: Interaction of HDAC3 with Ligand 3b

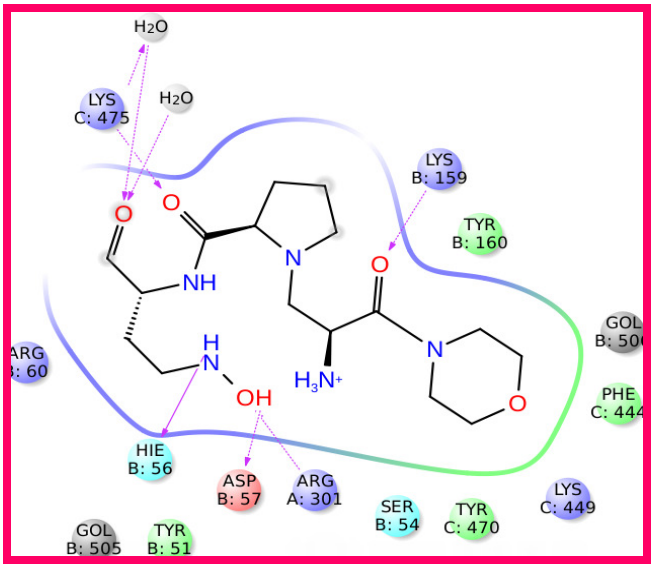


Fig-10: Interaction of HDAC3 with Ligand 3c

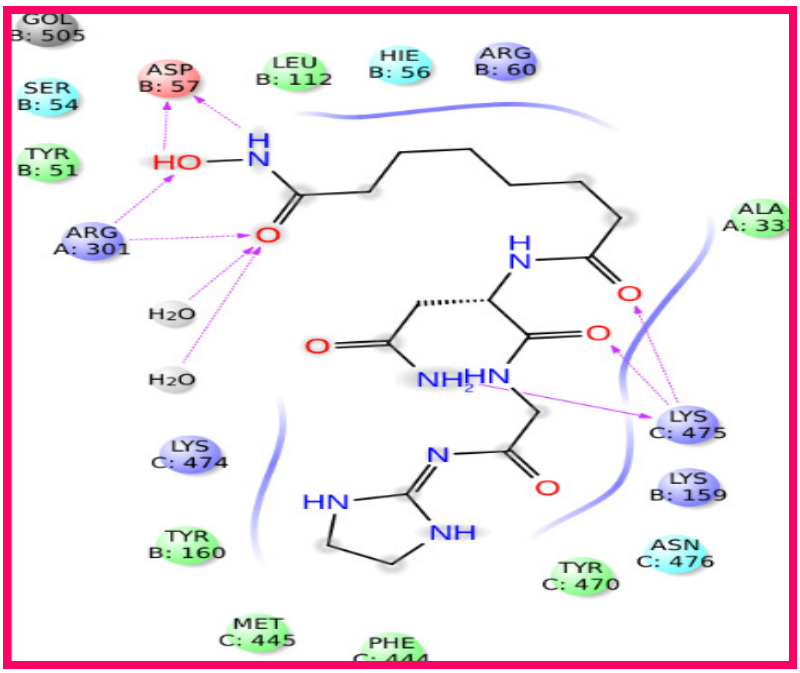


Fig-11: Interaction of HDAC3 with Ligand1

Conclusion

In this present work, designed five small molecular Novel analogues through the molecular combinatorial ligand library as a similar structure of protein-protein interface region of NcoR-SIN3-HDAC3 protein complexes. And these five Novel analogues were docked with HDAC-3 protein and its PDB Id was 4A69. A molecular docking was done at Schrodinger-Maestro-9.3.5-Version. In this view, identified various intermolecular interactions such as Hydrogen bonding including side chain and backbone, charged positive, polar and zing binding interactions. As a result of this ligands shown good binding interactions. And these five ligands are a novel analogue for anti-cancerous histone deacetylase inhibitor, caused by the disruption of HDAC-3.

Acknowledgement:

I express my deep sense of gratitude to Dr.S. Kabilan, Professor, Department of chemistry, Annamalai University, Annamalai Nagar-608002 to given as by using the facility of Drug Discovery Lab, Schrodinger.

References

1) Jessica E. Bolden, Melissa J. Peart, Ricky W. Johnstone. Anticancer activities of histone deacetylase inhibitors. Natural Reviews, 2006; 5:269-284.
2) Bradley R.C. Emerging roles for chromatin remodeling in cancer biology. *Trends in cell biology,* 2001; 11: 515-521.
3) Zdenko Herceg, Thomas Vaissière. Epigenetic mechanisms and cancer An interface between the environment and the genome. Epigenetics, 2011; 6(7): 804-819.
4) Xiang-Jiao, Y.; Serge, G. Class II Histone Deacetylases: from sequence to function, regulation and clinical implication. Molecular and cellular biology, 2005; 25:2873-2884.
5) Saverio Minucci, Mario Varasi, Florian Thaler, Agnese Abate, Giacomo Carenzi, Ciro Mercurio. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for

cancer," Nature Reviews – Cancer, 2006; 6: 38-51.

6) Dong, H. K.; Minjung, K.; Ho Jeong, K. Histone Deacetylases in carcinogenesis and its inhibitors as anti cancer agents. Journal of biochemistry and molecular biology, 2003; 36(1):110 – 119.

7) Donald, E. A. Histone Deacetylases: transcriptional repression with SINers andNuRDs. Trends in Cell Biology, 1999; 9: 193-198.

8) Andrea, G. C. Protein-protein interfaces: mimics and inhibitors. Current opinion in chemical biology, 2001 ; 5:654 -659.

9) Reena Zutshi, Michelle Brickner, Jean Chmielewski; Inhibiting the assembly of protein-protein interfaces, Current Opinion in Chemical Biology, 1998 ;2:62-65.

10) Nurcan, T.; Gozde, K.; Ozlem, K.; Attila, G.; Ruth, N. A survey of available tools and web server for analysis of protein-protein interactions and interfaces. Briefings in Bioinformatics, 2009; 10: 1-16.

Article History: ----------

Date of Submission: 22-02-2015

Date of Acceptance: 02-03-2015

Conflict of Interest: NIL

Source of Support: NONE