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Original Article

DESIGNING OF ANTI-CANCEROUS HISTONE DEACETYLASE INHIBITORS THROUGH MIMICKING OF PROTEIN-PROTEIN INTERFACES

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ABSTRACT

Objective: The objective of the study was to come up with of the small molecular modulators that inhibit protein – protein interfaces or interaction site in HDAC complexes. The main focus is on the mimicking or forming of tiny molecule wherever by inhibiting the protein-protein interactions in specifically HDAC protein complexes.

Methods: By mimicking of the interface of the protein interaction site like SIN3A-SMRT complex as well as SIN3A-NcoR complexes.

Results: Totally 10 molecular structures were designed through molecular docking with HDAC2 PDB Id 3MAX and were downloaded from protein data bank.

Conclusion: The results clearly indicate that before synthesis and biochemical testing of new lead and its analogs; one can use molecular modeling based methods for qualitative assessment.

Keywords: HDAC, SIN3-NcoR, Docking and protein-protein interfaces.

INTRODUCTION

Nucleosomes are the elemental units of chromatin and area unit comprised of a histone protein octamer around that 146 base pairs of deoxyribonucleic acid are wound. The octamer contains two copies of the four core histones: H2A, H2B, H3 and H4. Linker histones clamp entry and exit sites of deoxyribonucleic acid round the core histone octamer and, with different nuclear proteins, contribute to the formation of a condensed higher order chromatin structure (1). Core and linker histones are the foremost regulators of chromatin condensation, so their modifications regulate the structural stability of the Chromatin fiber. The orderly packing of eukaryotic deoxyribonucleic acid depends on enzymatic modification of histones by the covalent addition of methyl, acetyl group or phosphate teams to extend or decrease transcription. These epigenetic changes (mitotically hereditary alterations in gene expression that don't seem to be caused by changes within the deoxyribonucleic acid sequence) observed because the "histone code" influence chromatin structure and so regulate the accessibility of transcription factors to deoxyribonucleic acid. Histone acetylation results in chromatin de-condensation and increased transcription of the gene. This is accomplished by 2 enzymes known as HAT and HDAC enzymes alter the four core Histone proteins via acetylation and deacetylation at specific lysine (K) residues in the N-terminal tail of the histones in the nucleus [2,3]. HAT and HDACs are present together at transcriptionally active genes. While the HDACs are involved in the transcriptional repression and removal of acetyl groups from histones to cause condensation of chromatin, the HATs are involved in acetylation and de-condensation of the chromatin resulting in increased gene transcription. Thus, HDACs possess negative regulatory mechanisms to that of the HATs. Protein-protein interactions are exclusively changed or deregulated in cancer cells.

There are four HDAC classes with several different HDACs in each class. Class I HDACs are homologous to yeast Rpd3 and include HDACs 1, 2, 3 and 8. Class I HDACs are expressed ubiquitously in human cell lines and tissues and are found primarily in the nucleus. Class II HDACs are homologous to yeast Hda1 and can be subdivided into two subclasses: ClassIIa (HDAC 4, 7, and9) and ClassIIb (HDAC 6 and 10). Class II HDACs exhibit tissue-specific expressions and can shuttle between the nucleus and cytoplasm. Class I and II HDACs share homology in their catalytic sites. Class III HDACs or sirtuins (SIRT1-7) include a group of proteins that are homologous with the yeast Sir2 family of proteins. Class IV HDACs contain HDAC11 and are homologous with both classes I and II. The role of HDACs is not restricted to their contribution to histone deacetylation; they also play a role in the de acetylation of non-histone proteins. HDACs are key elements in the regulation of gene expression, differentiation and development, and the maintenance of cellular homeostasis [4].

HDAC2 is gift within the nucleus of human cells. it's concerned in transcriptional repression regulated by the retinoblastoma protein Rb and is recruited to deoxyribonucleic acid through interaction with transcription factors and nuclear receptors. HDAC2 is additionally found in giant multi-protein complexes known as co-repressor complexes that are recruited to promoters of genes by specific transcription factors to silence gene activity. HDAC2 is one among the histone-modifying enzymes that regulates gene expression by transforming chromatin structure and is gift within the Sin3A and nucleosome remodeling and de acetylase (NuRD) co-repressor complexes (5, 6).

A very important feature of HDAC is that it needs many elements of different multi-protein complexes to perform its cellular activities. This includes transcriptional co-repressors like mSin3A, N-CoR/ SMRT–SIN3–HDACadvanced, PML and others in AML (7, 8). The SIN3–HDAC could be a ubiquitous, abundant and enormous macromolecule advanced with extremely preserved useful domains.

The advanced contains HDAC-1, HDAC-2 and also the system macromolecule SIN3, and is concerned to influence many key restrictive signals. The N-CoR / area unit bridging proteins discharged by RAR together with SIN3-HDAC advanced. The N-CoR/SMRTs act as a linker for the interaction of RAR-RXR with SIN3-HDAC advanced is a. The RAR- a part of the nuclear hormone receptor family that functions as a transcription issue by binding to deoxyribonucleic acid and regulation transcription of its target genes. Where as the carboxyl terminal of the N-CoR /SMRT is to blame 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 advanced is discharged and changed for the binding of a co-activator advanced like TIF2–CBP HAT and factor transcription is activated and is covert within the absence of ligand [9].

Objective

The objective of the study has been coming up with of the small molecular modulators that inhibit protein – protein interfaces or interaction site in HDAC complexes. One protein combines with another protein and forms protein-protein interactions and produce protein complexes. Our aim is to produce (mimic) a small molecule about protein interfaces and reacting the molecule with macromolecule (protein) complexes wherever by inhibiting the protein-protein interactions. The main focus is on the mimicking or forming of tiny molecule wherever by inhibiting the protein-protein interactions in specifically HDAC protein complexes.

Experimentation

The discovery of small molecules that regulate protein–protein binding interactions in designing a new molecule and its practical importance. Accordingly, the prevailing approaches have been structure-based design and combinatorial methods (selection or screening of libraries). Often, design is aimed at mimicking peptide or protein structural elements in a smaller form. A successful design may then become the scaffold for a combinatorial library. Combinatorial method, on the other hand, allows quick evaluation of many possible ligands and frequently yields unexpected solutions. These may be structurally characterized and used as templates for further design. In addition, previous reports have appeared describing particular protein–protein interactions that can be inhibited by small molecules or by very short peptides.

Prediction of protein complexes

Patch Dock is an algorithm for molecular protein-protein docking. The input is two molecules of any type: proteins, DNA, peptides, drugs. The output is a list of potential complexes sorted by shape complementarily criteria. In order to mimic the SIN3A-SMRT complex as well as SIN3A-NcoR complexes, the 3D crystal structures of proteins: NCoR (PDB ID: 1xc5), SIN3A (PDB ID: 1G1E) and SMRT (PDB ID: 2ODD) were retrieved from the Protein Data Bank (PDB). First, the SIN3A-NcoR complex was mimicked by docking SIN3A and NcoR using PatchDock (10-12). Similarly, the SIN3A-SMRT complex was mimicked by docking with the SIN3A and SMRT to obtain a mimicked two protein complex structure SIN3A-NcoR and SIN3A-SMRT complexes).

Table 1: Proteins interacting with Sin3 have a wide range of functions. For those characterized, most commonly interact with an α-helix in PAH pocket.

Factor	Functions	SIN3 Interacting domain	SIN3 Interfaces
N-CoR	Co-repressor for nuclear hormone receptors	DALA alpha helix Motif	PAH1; PAH3-HID, SAP30
SMRT	Co-repressor for nuclear hormone receptors	Extensive (SIN3A only)	

The above table has shown the Sin3 complex interfaces such as PAH1, mSIN3A, mSIN3B and PAH2 which are scaffolding proteins and are interacting with seven factors. Among the seven factors which are mention in that table no-1, only two factors have been selected for protein-protein docking or prediction of protein complexes. And this protein-protein interactions to carry out with patchdock.

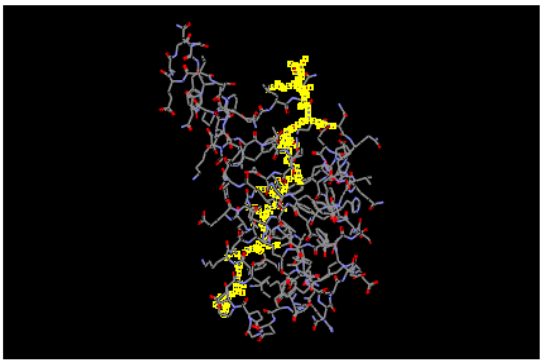

Fig. 1: Docked Structure of SIN3A PAH2 domain complexed with SMRT viewed by Argus lab

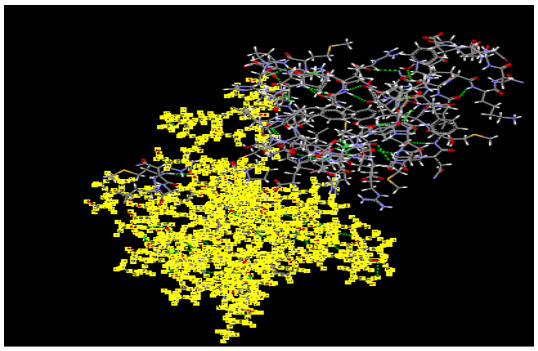

Fig. 2: Docked structure of mSin3A PAH1 domain complexed with N-CoR viewed by arguslab

Prediction of protein-protein interfaces: mimics and ligand design

Protein-Protein interfaces or interaction sites were predicted the small molecules were designed which has similar structure as protein-protein interfaces by using the online tool metappisp (A Meta server for protein-protein interaction site prediction). The obtained 3D complexes SIN3A- SMRT and SIN3A-NcoR was introduced to the online metta-PPISP server for the prediction of protein-protein interface region.

The interface region were interpreted and analyzed by Swiss PDB Viewer. The possible small molecule structures that mimic the interface region were identified and obtained structures were drawn by using Marvin Sketch. Marvin Sketch has advanced chemical editor for drawing chemical structures and it has a rich list of editing features.

Table 2: Selected protein-protein interface region

S. No	Interface ID	Interface Region
1.	Ligand -1	PHE, GLU.
2.	Ligand -2	TYR, GLN.
3.	Ligand -3	PRO, ASP, ALA.
4.	Ligand -4	TYR, TYR, LEU.
5.	Ligand -5	LYS, LYS.

Chemical substituent analog of the novel lead molecules

The five newly designed lead molecules of the drugs were created by replacing the hydrophilic region on the target molecule with other functional groups (considered at random). Five analogues of the small molecule of the newly designed lead molecules has been studied by performing various molecular properties calculation, ADME as well as protein-ligand interactions.

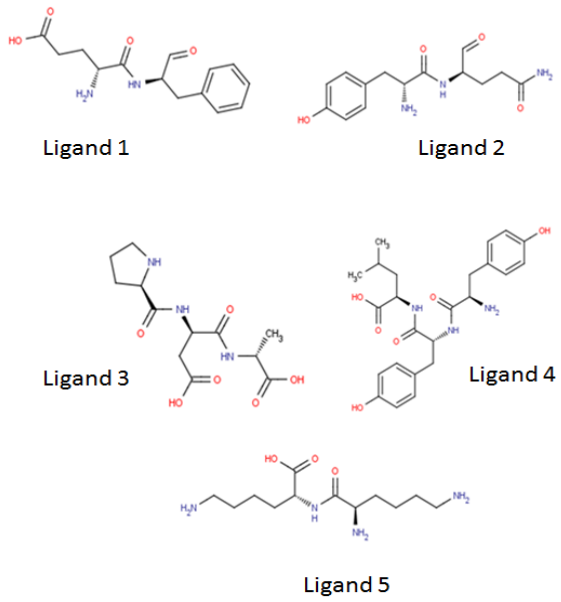

Fig. 3: Novel Lead Molecules of Mimicked Protein-protein Interfaces

Table 3: Chemical substituted analog

S. No.	Small molecule id	Possible position	Chemical subsitituted analog
1.	Ligand-1	CHO	NH_2OH (R_1)
		NH_2	CH_3COCH_2Br (R_2)
2.	Ligand-2	NH_2	CH_3-CO-CH_2-Br(R_1)
		NH_2	NH-OH(R_2)
3.	Ligand-3	NH	CH_3COCH_2Cl(R_1)
		COOH	OCH_3 (R2)
		NH	N-OH(R_3)
5.	Ligand-4	COOH	NH-OH-CO-CH_2-Cl (R1)
6.	Ligand-5	NH_2	(R_1)
		NH_2	NH-OH (R_2)

RESULT AND DISCUSSION

Molecular docking study

A molecular docking study by Schrodinger-Maestro 9.3.5 Version was performed to calculate of binding energy and H-bond interaction, H-bond donor & acceptors, hydrobhobicity and lipobobicity at the active site for all the newly designed small molecules and its chemical substituted analogues, totally 10 molecular structures were separately docked with HDAC2 PDB Id 3MAX were downloaded from protein data bank.

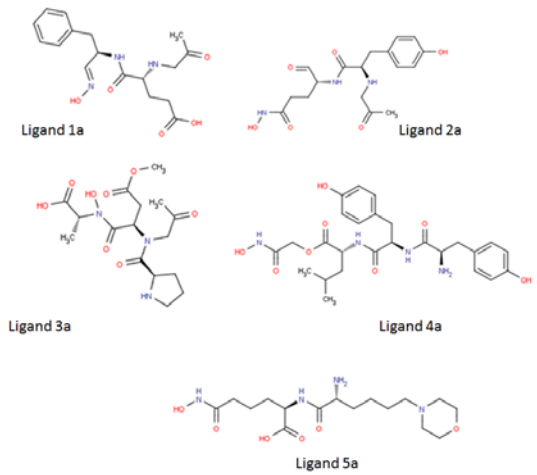

Fig. 4: Chemical substituted Analogues Of the Novel Lead Molecules

Table 3: Docking Result

S. No.	Ligand(small molecule)	Docking Score E_{bind} (MM)
1.	Ligand - 2	-10.9
2.	Ligand - 5	-10.6
3.	Ligand - 4	-10.0
4.	Ligand – 4 a	-9.8
5.	Ligand - 5a	-9.6
6.	Ligand -1	-9.5
7.	Ligand - 3	-8.3

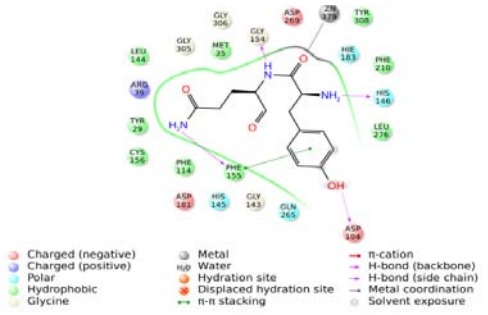

Fig. A

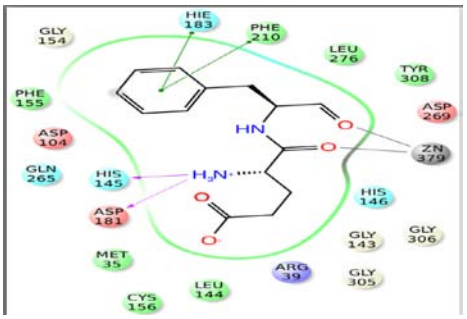

Fig. B

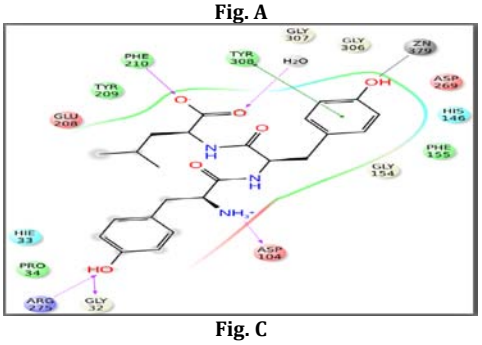

Fig. C

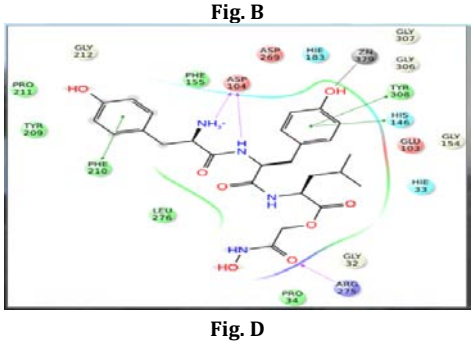

Fig. D

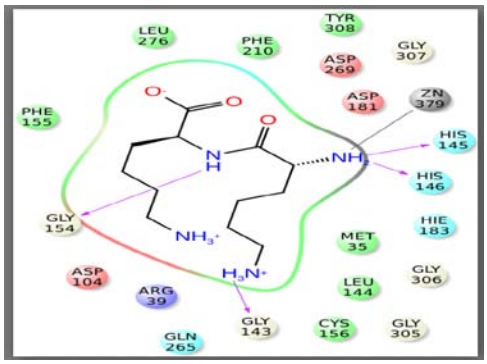

Fig. E

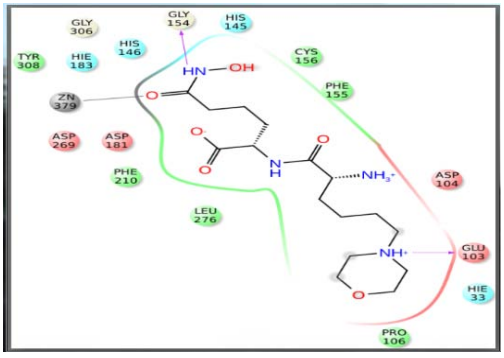

Fig. F

Fig. 5: Fig-a, b, c, d, e, f Showing Most active Protein –Ligand interactions of Ligand No-1,2,4,4a,5,5a.

ADME analysis

Present study identify through docking and binding interactions of various novel lead molecules (As similar to Protein-Protein Interfaces) and its analogues for anti-cancerous histone deacetylase inhibitors. After analyzing of the molecular docking and ADME (By using qikprop command in Maestro) the ligand no 1, 2, 4, 4a, 5, 5a showing most active binding energy of the targeted protein and its obeying good Lipinski rule of five and ADME test and results are shown. and its showing more than seven hydrogen bond interactions and its including π - π stacking, hydrogen bond (side chain and backbone) and metal ion (zing binding motif) interactions.

Table 9: ADME analysis

S. No	Ligand	Mol. wt	VD (L)	FA(%)	Coca2-permeability(cm/s)	Log p	Rotable bond
1.	Ligand-1	278.307	955.142	128.831	7E-05	1.2	11
2.	Ligand-2	349.386	1158.789	220.261	2E-06	-1	14
3.	Ligand-3	293.322	974.936	142.448	9E-08	-2.7	12
4.	Ligand-4	365.385	1179.198	231.229	2E-05	0.8	18
5.	Ligand-5	301.299	953.796	288.915	3E-05	2.6	18
6.	Ligand-1a	387.389	1192.889	424.07	4E-06	-1.3	18
7.	Ligand-2a	457.525	1460.759	240.084	2E-05	0.5	10
8.	Ligand-3a	530.577	1631.824	274.511	9E-08	-2.6	14
9.	Ligand-4a	274.362	988.378	284.704	6E-08	-3.3	18
10	Ligand-5a	374.364	1245.106	404.902	4E-07	-1.7	20

Predicted properties

- Fraction of oral dose absorbed (FA)
- Caco-2 permeability
- Volume of distribution (VD)
- Octanol/water distribution coefficient (LogP)

Table 10: The result of analysis ADME continue

Small molecule ID	S. A. A (A^2)	S. A. G (A^2)	Vol (A^3)	Hyd. ener Kcal/mol	Ref (A^3)	Polari (A^3)	Drs	Ars
Ligand-1	526.2	632.1	1198.34	-5.67	92.43	52.23	3	5
Ligand-2	546.41	620.03	1090.59	-5.53	88.65	33.75	4	8
Ligand-3	485.3	578.8	976.6	-16.41	82.76	33.31	5	8
Ligand-4	575.8	698.7	1305.7	-17.48	136.1	51.1	8	10
Ligand-5	630.9	652.8	1057.4	-18.7	82.2	32.8	6	9
Ligand-1a	654.8	673.8	1176.4	-5.57	108.5	41.7	3	6
Ligand-2a	602.8	671.9	1126.3	-22.45	93.7	37.6	5	9
Ligand-3a	552.5	623.8	1066.4	-34.6	88.6	36.3	7	11
Ligand-4a	692.5	771.7	1377.3	-21.15	123.5	49.2	4	10
Ligand-5a	618.08	624.2	1028.6	-11.49	83.5	31.03	5	9

Predicted properties

- S. S. A: - Surface area approximate
- S. S. G: - Surface area grid
- Vol: - Volume
- Hyd. ener: - Hydration energy
- Ref: - Refractivity
- Polari: - Polarizibility
- Drs: - Hydrogen bond donars
- Ars: - Hydrogen bond acceptors

CONCLUSION

These results clearly indicate that before synthesis and biochemical testing of new lead and its analogs; one can use molecular modeling based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis. The ligand No 1,2,4,4a,5,5a is a new lead and analogue for the anti-cancerous histone deacetylase inhibitor for disruption of HDAC2.

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CONFLICT OF INTEREST

None to declare

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