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MODELLING THE THYROID CAUSING PROTEIN AND THEIR INHIBITOR STUDIES USING COMPUTATIONAL METHODS

***[1]Pallavi Kaulwar and [2]Prof. Archana Panche**

[1]Bsc Bioinformatics 3rd yr, MGM- Institute of Biosciences and Technology, Aurangabad.
[2]Professor, MGM- Institute of Biosciences and Technology, Aurangabad.

***Corresponding Author: Pallavi Kaulwar**

Bsc Bioinformatics 3rd yr, MGM- Institute of Biosciences and Technology, Aurangabad.

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ABSTRACT

Types of thyroid are. Causes of thyroid. Advantages by using some techniques or solutions for cure for thyroid. Disadvantages of thyroid. Drugs available for thyroid. Genes involved in thyroid. Receptors that play role during thyroid. Proteins that play role in thyroid disease are SIRT7- NAD dependent protein deacetylase that specifically mediates deacetylation of histone. GRB14- Adapter protein which modulates coupling of cell surface receptor kinases with specific signaling pathways. RPS6KB1- Regulates protein synthesis. ABCA1- cAMP dependent and sulfonylurea, sensitive anioin transporter. SLC5A5/NIS protein- Mediates iodine uptake in the thyroid gland. BCL6- Play role in immunoglobulin. MRAS- May serve as an important signal transducer for a novel upstream stimuli in controlling cell proliferation. P21 Capsid protein- Regulate immune response and prevents destruction of infected cells by cytotoxic. Nef protein- Bypasses host T-cell signaling by inducing, cell activation.

KEYWORDS: SIRT7- NAD, ABCA1- cAMP, SLC5A5/NIS.

1. INTRODUCTION

The thyroid gland is an endocrine gland in your neck. It makes two hormones that are secreted into the blood: thyroxine (T4) and triiodothyronine (T3). These hormones are necessary for all the cells in your body to work normally.

Thyroid disorders are very common and tend mainly to occur in women, although anybody - men, teenagers, children and babies, too - can be affected. About one in 20 people has some kind of thyroid disorder, which may be temporary or permanent.

The thyroid gland lies in the front of your neck in a position just below your Adam's apple. It is made up of two lobes - the right lobe and the left lobe, each about the size of a plum cut in half - and these two lobes are joined by a small bridge of thyroid tissue called the isthmus. The two lobes lie on either side of your wind-pipe.

1.1 Types of thyroid.

1) Thyroid Paragangliomas are rare neuroendocrine tumors
2) Hashimoto's Thyroiditis
3) Autoimmune thyroiditis
4) Differentiated thyroid cancer
5) Papillary thyroid cancer
6) Thyroidectomy
7) Thyroid eye disease (Grave's hypothyroidism)
8) Papillary-Type carcinoma of the Thyroglossal Duct Cyst: The case for conservative management
9) Subclinical hypothyroidism(SCH)
10) Congenital Hypothyroidism

1.2 Causes of Thyroid

Sex bias play a role for dissatisfied patients with hypothyroidism, age, weight, menopausal status, body mass index, High doses of EDCs(Endocrine disrupting chemicals), Tris(1,3-dichloro-2-propyl) phosphate given in high dosages, uptake of 18F-choline PET-CT.

1.3 Advantages by using some techniques or solutions for cure for Thyroid

- Function of carbon nano particles to improve lymph node dissection and identification of parathyroid glands during thyroid reoperation for carcinoma.
- Preoperative preparation of Hyperthyroidism for Thyroidectomy role of supersaturated Iodine and lithium carbonate.
- Thyroid hormones were significally decreased in undialyzed CKD patients as compared to healthy control. Because of free triiodothyronine (FT3) and free thyroxine (FT4) were found to be significantly reduced in undialyzed CKD patients.
- 3, 5-Diiodothyronine a novel thyroid hormone metabolite and potent modulation of energy metabolism.

- 99cm Tc WBS is a useful imaging modality in detecting remnant thyroid tissue, nodal and distant metastases before 1311 therapy.
- RAS protein mutation analysis holds great promise as a preoperative diagnostic tool for predicting FVPTC (Follicular variant of papillary thyroid carcinoma) in cytologically and sonographically intermediate nodules negative for BRAF mutations.

1.4 Disadvantages of Thyroid

Microcalcifications and fast growth of the TN (Thyroid nodules) could therefore be used as predictive factors for the development of TC (Thyroid cells) in patients with Atypia of undetermined significance/ follicular lesion of undetermined significance (AUS/FLUS).

1.5 Proteins involved in Thyroid

- SIRT7 NAD dependent protein deacetylase that specifically mediates deacetylation of histone H3 at 'Lys-18' (H3K18AC).
- DBC1-Deleted in bladder cancer protein 1.
- RPS6KB1-Ribosomal protein S6 kinase beta-1(S6K1)/P70S6 kinase).
- ABCA1-ATP binding cassette transporter ABCA1.
- NIS protein Ensures iodine from the diet accumulates in the thyroid gland for production of thyroid hormones.

1.6 Drugs available for Thyroid

- Dexmedetomidine used in perioperative medicine.
- Dabrafenib (drug- drug interactions) given for amaplastic thyroid cancer (BRAFV600E) mutation, dosage-150mg twice daily.
- Edaravone a hydroxyl radical scavenging agent, dosage 10mg/kg, 20mg/kg, 30mg/kg, 40mg/kg
- Triciribine/STAT3 inhibitor WP 1066

1.7 Genes involved in Thyroid

- TFG/NTRK- [Hybrid/ mutated gene].
- SLC5A5 gene- provides instructions for making a protein called sodium(Na) iodide symported or NIS.

1.8 Receptors/ Antigens that play role during thyroid

CD45- [Lymphocyte common antigen- Receptor linked protein tyrosine phosphate].

2. REVIEW OF LITERATURE

2.1 List of proteins are

- **SIRT7** – NAD-dependent protein deacetylase that specifically mediates deacetylation of histone H3 at 'Lys-18' (H3K18Ac). In contrast to other histone deacetylases, displays selectivity for a single histone mark, H3K18Ac, directly linked to control of gene expression. H3K18Ac is mainly present around the transcription start site of genes and has been linked to activation of nuclear hormone receptors. SIRT7 thereby acts as a transcription repressor. Moreover, H3K18 hypoacetylation has been reported as a marker of malignancy in various cancers and seems to maintain the transformed phenotype of cancer cells. These data suggest that SIRT7 may play a key role in oncogenic transformation by suppresses expression of tumor suppressor genes by locus-specific deacetylation of H3K18Ac at promoter regions. Also required to restore the transcription of ribosomal RNA (rRNA) at the exit from mitosis: promotes the association of RNA polymerase I with the rDNA promoter region and coding region. Stimulates transcription activity of the RNA polymerase I complex. May also deacetylate p53/TP53 and promotes cell survival, however such data neeadditional confirmation. [Michishita-Kioi E, Xi Y, Tasselli L, Kioi M, Moqtaderi Z Tennen RI, Paredes S, Young NL, Chen K, Struhl K, Garcia BA, Gozani O, Li W, Chua KF, 2012 "SIRT7 links H3K18 deacetylation to maintenance of oncogenic transformation," Nature].

- **GRB14** - Adapter protein which modulates coupling of cell surface receptor kinases with specific signaling pathways. Binds to, and suppresses signals from, the activated insulin receptor (INSR). Potent inhibitor of insulin-stimulated MAPK3 phosphorylation. Plays a critical role regulating PDPK1 membrane translocation in response to insulin stimulation and serves as an adapter protein to recruit PDPK1 to activated insulin receptor, thus promoting PKB/AKT1 phosphorylation and transduction of the insulin signal. [King CC, Newton AC, 2004 "The adaptor protein Grb14 regulates the localization of 3-phosphoinositide-dependent kinase-1", J biol Chem].

- **RPS6KB1** – Serine/threonine-protein kinase that acts downstream of mTOR signaling in response to growth factors and nutrients to promote cell proliferation, cell growth and cell cycle progression. Regulates protein synthesis through phosphorylation of EIF4B, RPS6 and EEF2K, and contributes to cell survival by repressing the pro-apoptotic function of BAD. Under conditions of nutrient depletion, the inactive form associates with the EIF3 translation initiation complex. Upon mitogenic stimulation, phosphorylation by the mammalian target of rapamycin complex 1 (mTORC1) leads to dissociation from the EIF3 complex and activation. The active form then phosphorylates and activates several substrates in the pre-initiation complex, including the EIF2B complex and the cap-binding complex component EIF4B. Also controls translation initiation by phosphorylating a negative regulator of EIF4A, PDCD4, targeting it for ubiquitination and subsequent proteolysis. Promotes initiation of the pioneer round of protein synthesis by phosphorylating POLDIP3/SKAR. In response to IGF1, activates translation elongation by phosphorylating EEF2 kinase (EEF2K), which leads to its inhibition and thus activation of EEF2. Also plays a role in feedback regulation of mTORC2 by

mTORC1 by phosphorylating RICTOR, resulting in the inhibition of mTORC2 and AKT1 signaling. Mediates cell survival by phosphorylating the pro-apoptotic protein BAD and suppressing its pro-apoptotic function. Phosphorylates mitochondrial URI1 leading to dissociation of a URI1-PPP1CC complex. The free mitochondrial PPP1CC can then dephosphorylate RPS6KB1 at Thr-412, which is proposed to be a negative feedback mechanism for the RPS6KB1 anti-apoptotic function. Mediates TNF-alpha-induced insulin resistance by phosphorylating IRS1 at multiple serine residues, resulting in accelerated degradation of IRS1. In cells lacking functional TSC1-2 complex, constitutively phosphorylates and inhibits GSK3B. May be involved in cytoskeletal rearrangement through binding to neurabin. Phosphorylates and activates the pyrimidine biosynthesis enzyme CAD, downstream of MTOR. [Fleckenstein DS, Dirks WG, Drexler HG, Quentmeier H, 2003, "Tumor necrosis factor receptor-associated factor (TRAF) 4 is a new binding partner for the p70S6 serine/threonine kinase" Leuk Res].

- **ABCA1**- cAMP-dependent and sulfonylurea-sensitive anion transporter. Involved in the efflux of intracellular cholesterol and phospholipids and their transfer to apoliproteins to form nascent high density lipoproteins/HDLs. [Krimbou L, Denis M, Haidar B, Carrier M, Marcil, Genest J Jr, 2004 "Molecular interactions between apoE and ABCA1: impact on apoE lipidation," J Lipid res].

- **SLC5A5/NIS protein** – Mediates iodide uptake in the thyroid gland. [Pohlenz J, Rosenthal IM, Weiss RE, Jhiang SM, Burant C, Refetoff S, 1998, "Congenital hypothyroidism due to mutations in the sodium/iodide symporter. Identification of a nonsense mutation producing a downstream cryptic 3' splice site," J Clin Invest].

- **BCL6** - Transcriptional repressor mainly required for germinal center (GC) formation and antibody affinity maturation which has different mechanisms of action specific to the lineage and biological functions. Forms complexes with different corepressors and histone deacetylases to repress the transcriptional expression of different subsets of target genes. Represses its target genes by binding directly to the DNA sequence 5'-TTCCTAGAA-3' (BCL6-binding site) or indirectly by repressing the transcriptional activity of transcription factors. In GC B-cells, represses genes that function in differentiation, inflammation, apoptosis and cell cycle control, also autoregulates its transcriptional expression and up-regulates, indirectly, the expression of some genes important for GC reactions, such as AICDA, through the repression of microRNAs expression, like miR155. An important function is to allow GC B-cells to proliferate very rapidly in response to T-cell dependent antigens and tolerate the physiological DNA breaks required for immunglobulin class switch recombination and somatic hypermutation without inducing a p53/TP53-dependent apoptotic response. In follicular helper $CD4^+$ T-cells (T(FH) cells), promotes the expression of T(FH)-related genes but inhibits the differentiation of T(H)1, T(H)2 and T(H)17 cells. Also required for the establishment and maintenance of immunological memory for both T- and B-cells. Suppresses macrophage proliferation through competition with STAT5 for STAT-binding motifs binding on certain target genes, such as CCL2 and CCND2. In response to genotoxic stress, controls cell cycle arrest in GC B-cells in both p^{53}/TP53-dependedent and -independent manners. Besides, also controls neurogenesis through the alteration of the composition of NOTCH-dependent transcriptional complexes at selective NOTCH targets, such as HES5, including the recruitment of the deacetylase SIRT1 and resulting in an epigenetic silencing leading to neuronal differentiation. [Shaffer AL, Yu X, He Y, Boldrick J, Chan EP, Staudt LM, 2000 "BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control," Immunity].

- **MRAS** - May serve as an important signal transducer for a novel upstream stimuli in controlling cell proliferation. Weakly activates the MAP kinase pathway. [Rodriguez-Viciana P, Oses-Prieto J, Burlingame A, Fried M, McCormick F, 2006 "A phosphatase holoenzyme comprised of Shoc2/Sur8 and the catalytic subunit of PP1 functions as an M-Ras effector to modulate Raf activity," Mol cell].

- **P21 Capsid protein** - May regulate immune response to the intracellular capsid in acting as a T-cell tolerogen, by having an immunoregulatory effect which prevents destruction of infected cells by cytotoxic T-cells.

- **Nef protein** - Bypasses host T-cell signaling by inducing a transcriptional program nearly identical to that of anti-CD3 cell activation. Interaction with TCR-zeta chain up-regulates the Fas ligand (FasL). Increasing surface FasL molecules and decreasing surface MHC-I molecules on infected $CD4^+$ cells send attacking cytotoxic CD8+ T-lymphocytes into apoptosis.

3. OBJECTIVE

The objective of our study is.

- To predict the structure and search potential inhibitors of these proteins.(Homology Modelling & Virtual Screening & Visualization).
- Molecular dynamics of the protein complexes with higher binding affinity.

4. METHODOLOGY

4.1 STEP 1- Literature read from peer reviewed research paper

Types of thyroid

1) Thyroid Paragangliomas are rare neuroendocrine tumors.
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Receptors/ Antigens that play role during thyroid

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Analysis of thyroid on the basis of statistical

- Kaplan-Meier analyses
- Eox proportional hazards regressions
- DNA methylation anlaysis
- Prognostic analysis

Methods used to study/analysis on the basis of thyroid

- PCR(Polymerase chain reaction)
- Sanger sequencing
- Chronic kidney disease(CKD)
- Molecular marker identification
- Next generation sequencing
- Microarrays
- Retrospective analysis was performed
- Univariate and multivariate logistics regression analysis

4.2 STEP 2- Screening of the proteins- We Checked the structures are present or not from the PDB database and we checked sequences are present or not from the Uniprotkb database.

There are 9 proteins found that play role in thyroid. They are as follows.

- SIRT7- NAD dependent protein acetylase sirutin-7, sequence also present, structure present in PDB database(5IQZ).
- RPS6KB1- Ribosomal protein S6 kinase beta-1 structure present in PDB, Sequence also present.
- ABCA1- ATP-binding cassette sub family A member 1, structure present.
- SLC5A5- Sodium iodide cotransporter, no structure present.
- BCL6- B-cell lymphoma 6 protein, structure present.

- Mras- RAS related protein, structure not present.
- GRAB14- Growth factor receptor bound protein 14, structure present
- PreC- Capsid protein, structure not present.
- Nef – protein nef, structure not present.

From this 9 proteins we focused on 4 proteins whose structure is not present in PDB database and they are 1) SLC5A5 2) MRas 3) p21(Capsid protein) 4) nef protein.

4.3 STEP 3- Homology Modelling- We checked the proteins with natural compounds

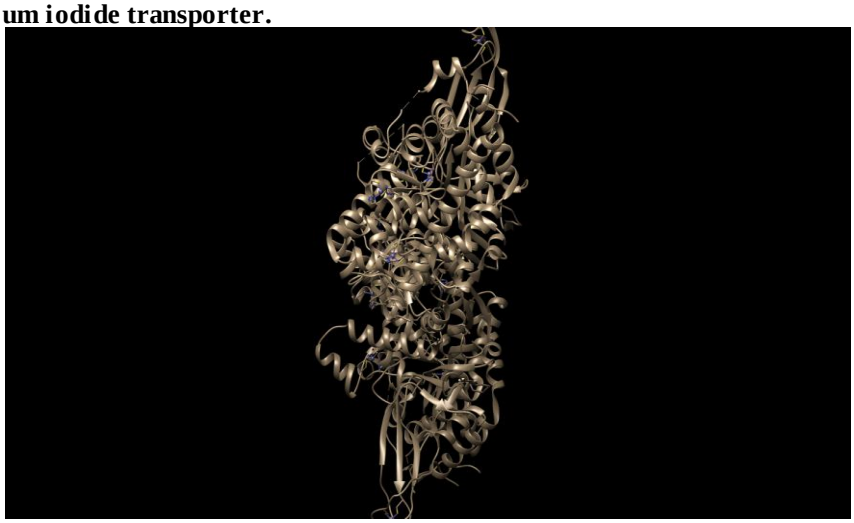
Homology modelling- It is a technique which allows to construct an unknown atomic-resolution model of the target protein.

We used the tool for homology modelling is UCSF Chimera-1.13.1.

UCSF chimera- It is a program for interactive visualization and analysis of molecular structures and related data. We performed comparative modelling of the 4 listed proteins. Steps are – 1) Background and caveats
2) Blast search for templates
3) Verifying the alignment
4) Running Modeller

Results obtained from chimera are

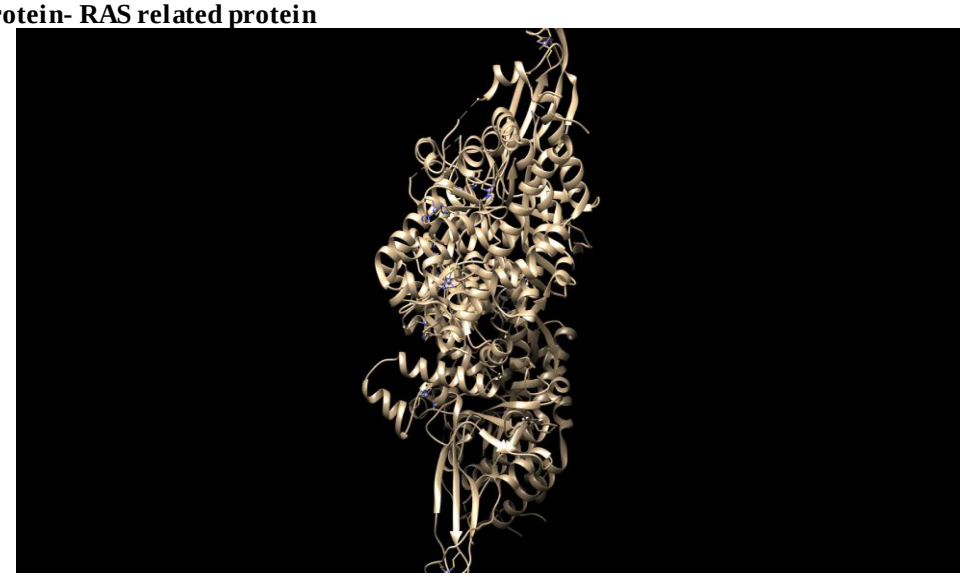
1) SLC5A5- Sodium iodide transporter.



4.4 a): Sodium Iodide Transporter Protein.

From the Fig1, the information we got is Uniprot ID- Q92911,Target- 5NVA, Template-p42866 (Oprm_mouse), E-value 9.327, Score- 119, Resolution- 1.95, Chain names- Sodium/glucose co transporter, Identity- 100%, Secondary Structure- Helix, Matrix- Blosum62, P-value null, R-value 0.201, B-value 29.97.

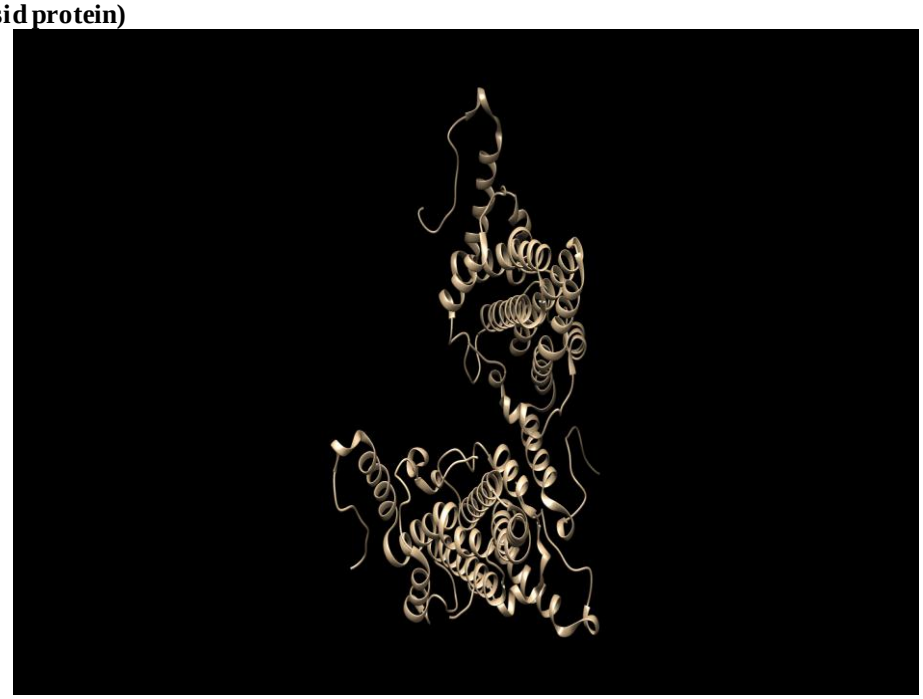
2) MRas Protein- RAS related protein



4.3 b): RAS related Protein

From the Fig2, the information we got is Uniprot ID- Q6R6M4, Target- 5GVI_A, Template- p42866 (Oprm_mouse), E-value 3.438, Score- 157, Resolution- 2.21, Identity - 33%, Secondary structure- Helix, sheet, Matrix- Blosum62, B-value null, R-value 0.20, P-value null.

3) P21- (Capsid protein)



4.3 c): Capsid Protein

From the Fig3, the information we got is Uniprot ID- Q15194, Target- 3j2V_C, Template- p42866 (oprm_mouse), E-value 7.287, Score- 376, Resolution- 3.5, Identity- 99.46%, Matrix- Blosum62, RMSD- 17.1, B-value null, P-value null, R-value null.

4) Nef protein

4.3 d): Nef Protein

From fig4, the information we got is Uniprot ID- E0A9W3, Target- 4EN2_A, Template- p42866 (oprm_mouse), E-value 4.186, Score- 354, Resolution- 2.58, Identity- 84.88%, Secondary structure- helix, sheet, Matrix- Blosum62, RMSD- 14.5, B-value 77.79, R-value 0.21, P-value null.

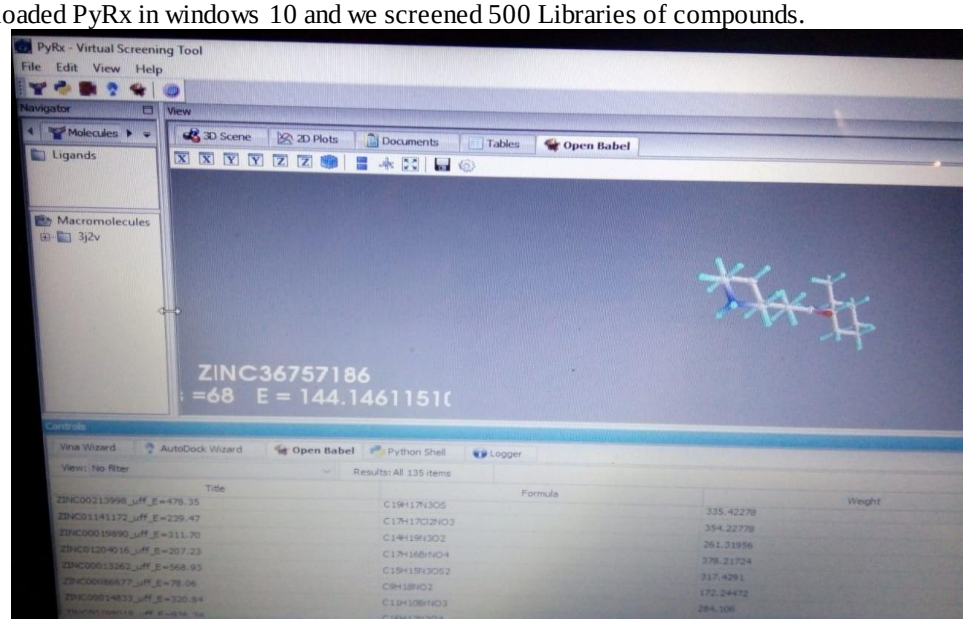
STEP 4- Virtual Screening & Molecular Docking

For virtual screening & Molecular Docking we used softwares that are.

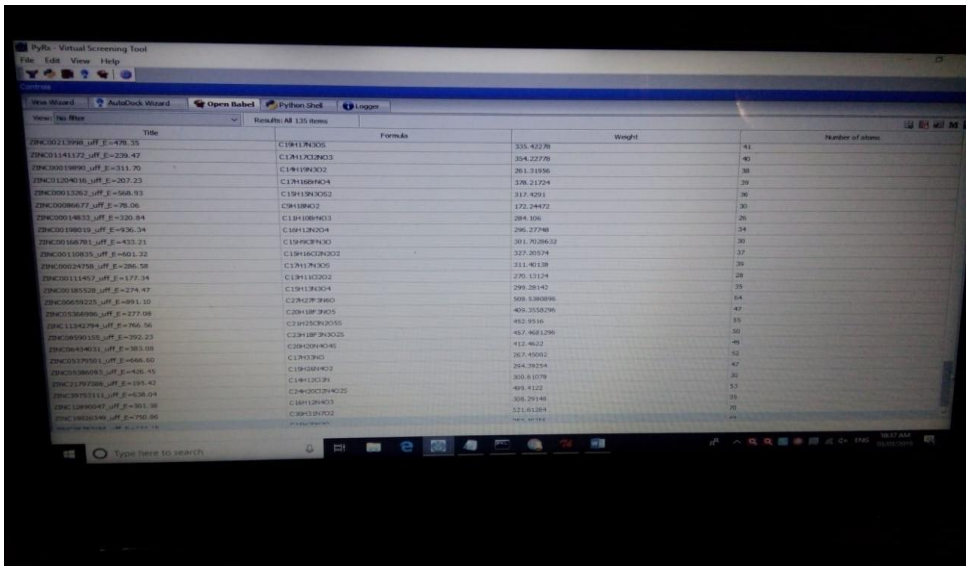
1) PyRx

PyRx is a virtual screening software for computational drug discovery that can be used to screen Libraries of compounds(EX- Pubchem database) against potential drug targets like G-Protein, nuclear receptors.

We had downloaded PyRx in windows 10 and we screened 500 Libraries of compounds.



4.3 e): PyRx Virtual Screening.



4.3 f): List of natural compounds.

2) AutoDock Vina

AutoDock Vina is an open- source program for doing molecular docking.

Why we are using this software?

It significantly improves the average accuracy of the binding mode predictions compared to AutoDock 4 & it is faster.

We had installed in Linux operating system- autodock_vina_1_1_2__linux_x86.tgz

3) MGL Tools

MGL Tools is a software developed at the Molecular graphics Laboratory (MGL) used for visualization & analysis of molecular structures.

We had downloaded in Linux operating system- ./mgltools_Linux-x86_64_1.5.6_install

This Tools include

i) ADT- AutoDock Tools

ii) PMV- Python molecular Viewer

iii) Vision- Visual Programming environment

5. RESULT AND DISCUSSIONS

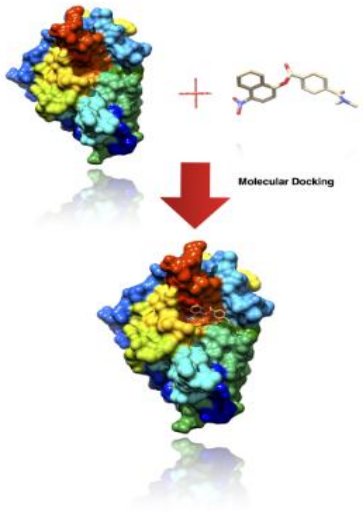


Fig 5 a): Molecular Docking.

We selected 5gvi (Ras Protein) and we selected 1 random ligand(Pubchem database) and we docked(Protein-ligand docking). After we docked we got the global energy value is -6.1.

The results are as below:

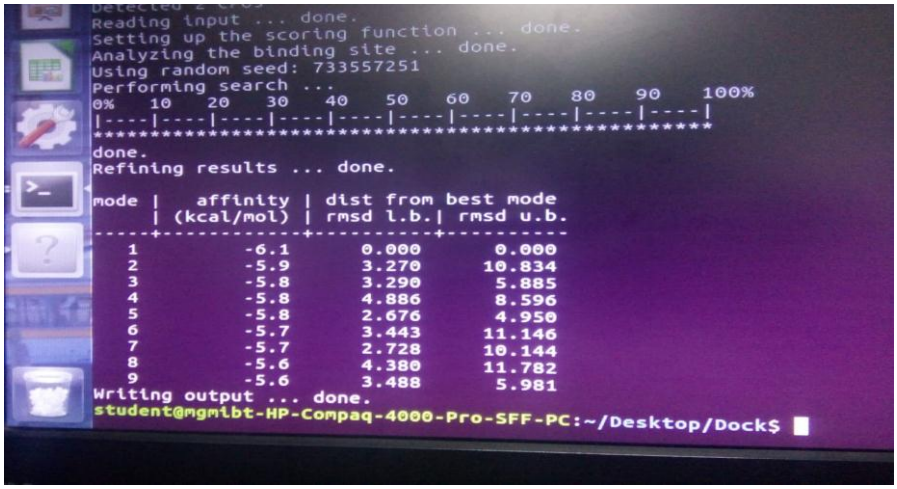


Fig 5 a) Docking Results.

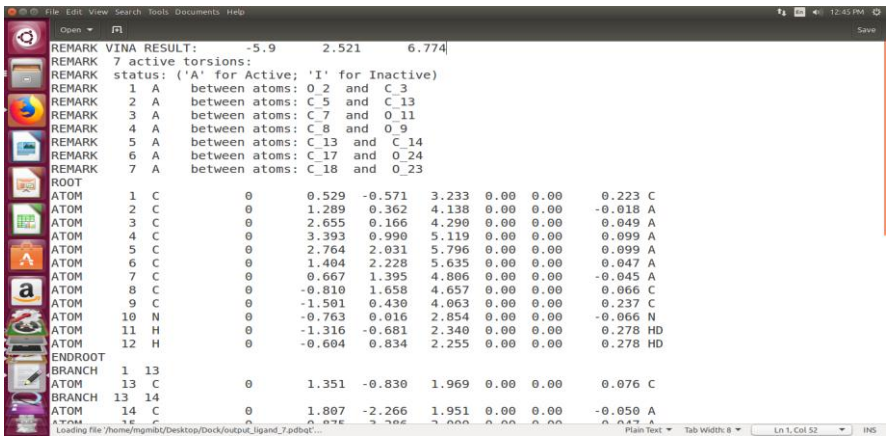


Fig 5 b) Vina Result.

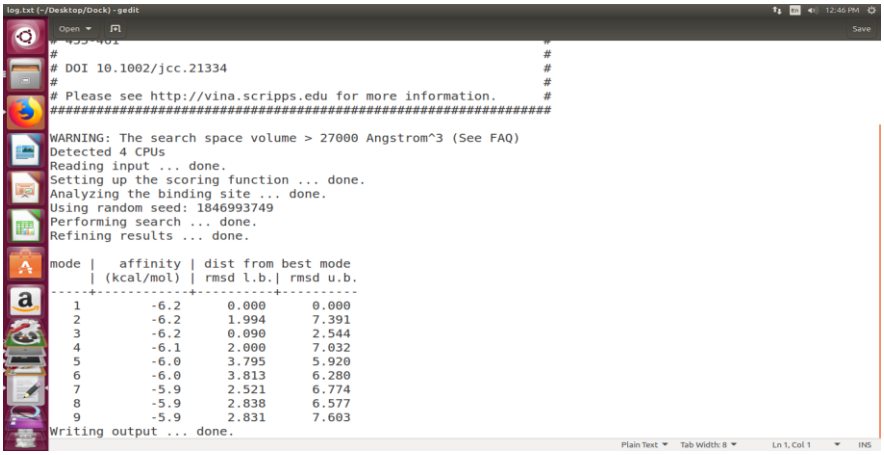


Fig 5 c) Log file

6. CONCLUSION AND FUTURE SCOPE

- Molecular docking give the promising contributions to identification and optimization of ligand in the modern drug discovery.
- The combination of chemical information of natural compounds (Pubchem database) with docking-based virtual screening by using PyRx software will be playing an important role in drug discovery in the coming era og genomics and will emerge new potential targets from the functional genomic studies.

FUTURE SCOPE

These inhibitors can be used as drug molecule for disease treatment using mentioned proteins/receptors as drug target.

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