Study the interaction pattern of molecular compound with DNMT1 and BDNF protein: An *in silico* predictive approach towards Type 2 Diabetic Retinopathy

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ABSTRACT

Type 2 Diabetic retinopathy (T2DR) is a microvascular complication affecting the eye of Type 2 Diabetes mellitus patients. It is one of the leading causes of visual impairment in industrialized countries in the working age group and one of the frequent causes of blindness in developing countries like India. According to World Health Organisation; diabetic retinopathy is 4.8% of the 37 million cases of blindness throughout the world. One of the main risk factors associated with development of T2DR is poorly controlled blood sugar as assessed by glycated haemoglobin levels (HbA1c)—the higher the HbA1c, the greater the risk of developing retinopathy. Legal blindness due to DR is estimated to be 25 times more common among the diabetic population than in those without diabetes. In India with the epidemic rise in type 2 diabetes mellitus as reported by the World Health Organization (WHO) diabetic retinopathy is fast becoming an important cause of visual disability. The study showed that genetic factors is one of the factor to promote the onset of retinopathy in diabetic patients. It has been shows that transient peaks of hyperglycemia is one of the risk factor for progression of Type 2 Diabetic retinopathy and that hyperglycemic peaks may cause persistent epigenetic changes despite subsequent normoglycemia . Epigenetic modifications, such as DNA methylation influence many cellular processes including regulation of gene transcription.DNAmethylation is exerted by DNMTs at the 5'position of cytosine residues in CpGdinucleotides (the p denotes the intervening phosphate group) by transferring methyl groups from S-adenosyl methionine (SAM), thus 5-methylcytosine is formed.
The main aim of this investigation was to analyse the role and mechanism of DNA methylation on BDNF and T2DR complications which inspired critical implication for the early prevention of T2DR.
An *In silico* approaches i.e. DNA methylation analysis through QUMA and docking (Schrodinger, Auto dock, Swiss dock, Cluspro server were used to analyse the interaction pattern of molecular compounds and DNMT1 and BDNF. Based on glide score result best molecular compound was selected to target DNMT1and which alternatively controls the methylation pattern on BDNF and may help in prevention of T2DR.
Keywords: Type 2 Diabetic retinopathy, Epigenetic modification, DNA methylation, 5-methylcytosine, Brain derived neurotrophic factor

INTRODUCTION

Type 2 Diabetic retinopathy is a potentially blinding complication of diabetes mellitus. Reasons for loss of vision are diabetic maculopathy and complications of proliferative diabetic retinopathy (PDR) such as vitreous hemorrhage, tractional retinal detachment, and neovascular glaucoma. By 2030 developing countries will face an increase by 69% and industrialized countries by 20% of the number of patients with diabetes compared to 2010(Shaw *et al*., 2010).Type 2 Diabetic retinopathy remains the leading cause of blindness in young adults affecting over 90% patients with 20 years of diabetes. The disease carries a heavy burden on our society as it is responsible for 4.8%of the 37million cases of eye disease related blindness worldwide. With the incidence of diabetes increasing at an alarming rate, the number of people with Type 2 Diabetic retinopathy is expected to grow from126.6 million in 2010 to 191.0million by 2030 (Zheng*et al*., 2012).T2DR is the most frightful of all the diabetes related ocular complications, as it could result in irreversible blindness if left untreated. This slow progressing diseases mainly characterized by damage of the microvasculature of the retina. Some of the early histopathological changes of this slow progressing disease include micro aneurysms, haemorrhages, cotton wool spots, intra retinal microvascular abnormalities, and venous bleeding. But, in more advanced stages, new fragile vessels are formed along the retina and on the posterior surface of the vitreous, and if not treated, they result in the detachment of the retina, leading to blindness (Frank *et al*., 2004).The possible mechanisms implicated in the development of diabetic retinopathy include oxidative stress, increased formation of advanced glycation end products, and activation of protein kinase-C, polyol production, and hexosamine pathways (Kitada*et al*., 2010; Santos *et al*., 2011). These pathways appear to be interlinked (Kowluru*et al*., 2001), which further complicates the strategies to prevent the development/progression of this devastating complication of diabetes.

Epigenetics is also known as "prefix genetics," "external genetics," or "post-genetics," indicating that in the absence of changes in the DNA sequence, the function of genes could undergo changes that are reversible and heritable (Moosavi*et al*., 2016).DNA

methylation is the earliest discovered mechanism in epigenetic modification. It refers to the addition of methyl groups of S-adenosylmethionine (SAM) to DNA molecules catalyzed by DNA methyl transferase (DNMT). In mammalian cells, CpG exists mainly in two forms: one evenly distributed throughout the DNA sequences (60–90% are methylated), and the other grouped in clusters known as the CpG islands (generally protected and remain unmethylated). In the eukaryotic cells, CpG islands are often found in the regulatory regions of coding genes and are involved in gene expression and chromatin structure modification (Portela*et al*., 2010). Hitherto, five enzymes have been identified in the DNMT family, namely DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L; however, among them, only DNMT1, DNMT3a, and DNMT3b are active for DNA methylation. DNA methylation deactivates the target genes or causes conformational changes in DNA, thereby affecting the protein-DNA interaction.In human DNA, 5-methylcytosine is found in approximately 1.5% of genomic DNA. The biological importance of 5-mC as a major epigenetic modification in phenotype and gene expression has been widely recognized.The quantification of 5-mC content or global methylation in diseased or environmentally impacted cells could provide useful information for detection and analysis of disease (https://www.whatisepigenetics.com/dna-methylation/).

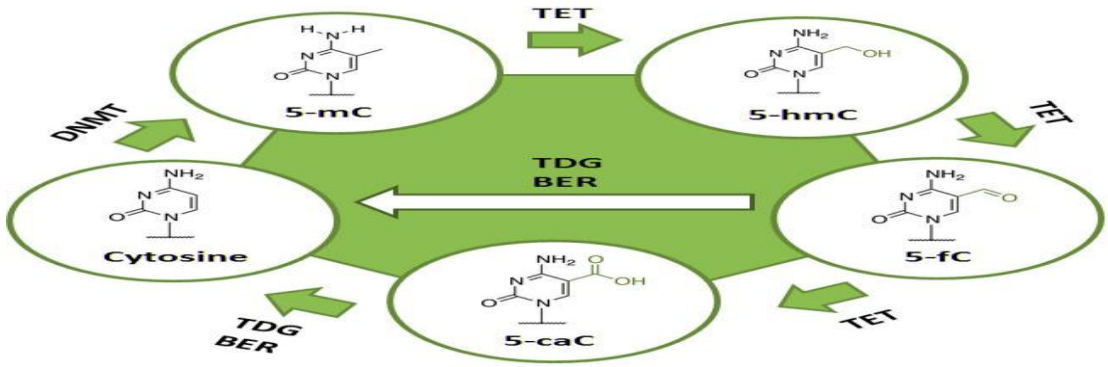


Figure.1: DNA Methylation Pathway

Retina is a neuronal tissue, it produces a substantial amount of neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4.BDNF a member of the neurotrophin family of growth factors and is important in the development, differentiation and maintenance of neurons.Hyperglycemia play a major role in the development of diabetic retinopathy and because of hyperglycemia biochemical alteration occurred such as in oxidative stress, increased formation of advanced glycation end products, and activation of protein kinase-C, polyol production, and hexosamine pathways. These pathways appear to be interlinked which further complicates the strategies to prevent the development/progression of this devastating complication of diabetes. The epigenetic factors i.e DNA methylation. The major disease caused by this factor is Type 2 diabetes and Type 2 diabetic retinopathy. Alteration in biochemical like oxidative stress, will alter the DNA methylation pathway of gene through which changes in protein expression is occurred which cause type 2 diabetic retinopathy. In Type 2 diabetic retinopathy hyper-methylation is caused due to increase in the level of DNMT1and DNMT1 enzymes is responsible for maintaining methylation status in a gene. In past study, a novel mechanism was provided for imbalance in retinal mitochondrial homeostasis in the development of T2DR, and showed the significant role of mtDNA methylation (Tewari*et al*., 2012). Diabetes facilitates the translocation of DNMT1 inside the retinal mitochondria, and DNMT1 in the mitochondria hypermethylatedmtDNA. Methylation is much higher at the D-loop region, the region with transcription and replication elements, than at other regions of mtDNA. Due to hypermethylation of the D-loop, the transcription of the mtDNA encoded genes that are important in the maintenance of ETC system is compromised, and the leakage of electrons in the ETC complexes is increased. These side reactions of the mitochondrial ETC directly generate superoxide radicals, further fuelling into the vicious cycle of superoxide radicals generated by the hyperglycaemic milieu. Diabetes also activates the enzymes responsible for maintaining DNA methylation status in the retina; the activities of DNMTs and TETs are increased, and DNA of many genes responsible in mitochondrial homeostasis is either hyper- or hypo- methylated (Tewari*et al*., 2012; Zhong*et al*.,2013; Mishra *et al.*, 2015; Kowluru*et al*., 2016). The CpG sites in the regulatory region of DNA polymerase gamma (POLG), a gene that encodes DNA polymerase gamma for mitochondrial biogenesis, are hypermethylated, resulting in impaired mitochondrial biogenesis, and decreasing mitochondrial copy numbers (Tewari*et al*., 2012). A dynamic DNA methylation process of MMP-9 is shown to maintain its transcriptional activation, though the transcription factor binding sites of the MMP-9 promoter are hypermethylated in diabetes, due to concomitant increased binding of TET at the same site, MMP-9 DNA remains hypermethylated resulting in its transcriptional activation (Kowluru*et al*., 2016), and this continues to fuel into the mitochondrial damage. In diabetes, due to activation of retinal DNMTs and their increased accumulation in the mitochondria, mtDNA is hypermethylated and 5mC levels are elevated. The D-loop region experiences more damage in diabetes, and also present higher cytosine methylation than other regions of the mtDNA. Regulation of DNMT1 by molecular or pharmacological means prevents mtDNA damage and ameliorates diabetes-induced transcriptional repression of mtDNA encoded genes important for functioning of the electron transport chain (Mishra *et al*., 2015). Thus, mitochondrial epigenetic appears to have a critical role in the development of Type 2 Diabetic Retinopathy.

According to past literature number of studies has been done on DNMT1 in cases of different diseases, such as in case of cancer changes occurred in DNMT1 (Paganon*et al*., 2011) and in case of diabetes, the activity of retinal DNMT is increased and the mtDNA replication enzyme, polymerase γ-1 (*POLG1),* is hypermethylated and its binding at the *D-loop* is impaired, resulting in decreased mtDNA biogenesis (Tewari*et al*., 2012). But no study has reported about DNMT1 and BDNF in contest of T2DR.

BDNF protein which is very important for maturation and proliferation of retinal cells. Therefore, it was in need to study DNMT1 and BDNF for the regulation of T2DR.

Therefore, there was a need to analysed DNA methylation analysis on BDNF gene and to design molecular compound which could controls the methylation pattern on BDNF gene and therefore the results obtained could be helpful in case of T2DR. In the present study the software's which were used for DNA Methylation analysis was QUMA and interaction pattern of molecules and DNMT1 (PDB file used 4WXX) was studied with the help of tools i.e. Swiss dock, Auto dock, Schrodinger. Thus, modulation of DNMT1 by pharmaceutical or molecular means could help maintain mitochondrial DNA integrity and serve as a potential strategy to regulate the development of Type 2 Diabetic Retinopathy (Mishrav*et al*., 2015).

MATERIALS AND METHODS

Computer with high speed internet access and online Bioinformatics tools were required.

METHODS:

1. **CpG Island Finder(DBCAT)**

Data Base of CpG islands and Analytical Tool (DBCAT) is developed in order to recognize comprehensive methylation profiles of DNA alteration in human diseases (http://dbcat.cgm.ntu.edu.tw/). The website (http://dbcat.cgm.ntu.edu.tw/) for CpG Island was browsed. The fasta format of BDNF gene was retrieved from NCBI. FASTA formatted sequence of BDNF gene was copied from NCBI page and pasted into the large text box. Clicked on submitted button and result was obtained.

2. **Promoter 2.0 Prediction Server**

Promoter2.0 predicts transcription start sites of vertebrate Pol II promoters in DNA sequences. It has been developed as an evolution of simulated transcription factors that interact with sequences in promoter regions. It builds on principles that are common to neural networks and genetic algorithms (http://www.cbs.dtu.dk/services/Promoter/).Theweb site (http://www.cbs.dtu.dk/services/Promoter/) was browsed for promoter prediction on BDNF gene.The fasta format of BDNF gene was retrieved from NCBI. FASTA formatted sequence of BDNF gene was copied from NCBI page and pasted into the large text box. Clicked on submit button and result was obtained.

3. **Meth primer**

Meth primer was a program for designing bisulfite-conversion-based Methylation PCR Primers. Currently, it can design primers for two types of bisulfite PCR: 1) Methylation-Specific PCR (MSP) and 2) Bisulfite-Sequencing PCR (BSP) or Bisulfite-Restriction PCR. Meth primer can also predict CpG islands in DNA sequences. The web site (http://www.urogene.org/methprimer/)was browsed for methylation analysis on BDNF gene.The fasta format of BDNF gene was retrieved from NCBI.FASTA formatted sequence of BDNF gene was copied from NCBI page and pasted into the large text box.Clicked on submit button and result was obtained.

4. **Quantification tool for Methylation Analysis**

QUMA includes most of the data-processing functions necessary for the analysis of bisulfite sequences. It also provides a platform for consistent quality control of the analysis. The QUMA web server is available at (http://quma.cdb.riken.jp/). The web site(http://quma.cdb.riken.jp/) was browsed for DNA methylation analysis on BDNF gene. The BDNF sequence which contain CpG site was selected and pasted in the box. The sequence was treated with bisulfite to convert unmethylated cytosine to thymine. The converted sequence was again pasted in the bisulfite sequence box. Then clicked on submitted button and result was obtained.

5. **Swiss Dock**

The web site (http://www.swissdock.ch/docking) was browsed for ligand protein interaction.PDB file of a protein was downloaded and uploaded on target selection box.clicked on search button and protein with their different chain was obtained. Based on Amino acid present on chain one chain was selected for docking.Ligand name was pasted on ligand selection box Clicked on search button and ligand with their different structure similarity was obtained. One ligand was selected from many structures for docking. Clicked on start docking button and result was obtained. Prediction file was downloaded and visualization was done through chimera software (http://www.swissdock.ch/docking).

6. **Schrodinger (Mastero):**

To perform the docking with Glide, you need to perform: Protein Preparation, Grid Generation, Ligand Prepapration, Ligand Docking (Screening). All files were provided so that each step could be performed independently and a project with all results for the different steps is provided: Strasbourg-Chemoinformatics-Docking-Training.prjzip (Pettersen*et al*., 2004)

7. **Auto Dock:**

The first step was to prepare the ligand and receptor coordinate files which included the information needed by AutoGrid and AutoDock. These coordinate files are created in an AutoDock-specific coordinate file format, termed PDBQT, which includes:i) Polar hydrogen atoms;ii) Partial charges;iii) Atom types;iv) Information on the articulation of flexible molecules.Thefirst two steps may be performed using the tools in the Edit menu of AutoDockTools, or withother molecular modelingprograms:i) Hydrogen atoms to the molecule was added.ii) Partial charges was added.Then, the molecule was read into theAutoDockTools using the Ligand (for the ligand) or Grid (forthe receptor) menus, and create the PDBQT file:iii) Deleted non-polar hydrogens and merge their charges with the carbon atoms.iv) Assigned atom types, defining hydrogen bond acceptors and donors and aromatic and aliphaticcarbonatoms.v) Chooseda root atom that will act as the root for the torsion tree description of flexibility.vi) Defined rotatable bonds and build the torsion tree.AutoDock requires pre-calculated *grid maps*, one for each atom type present in the ligand beingdocked. This helps to make the docking calculations fast.

8. Cluspro Server

Rigid body docking was performed by selecting Dot or ZDOCK from ClusProalgorithm(Ritchie *et al*., 2000; Comeau*et al*., 2004; Mandell*et al*., 2001; Chen *et al*., 2003).PDB file of receptor was pasted.PDB file of ligand was pasted.Clicked on submit buttom and result was obtained

RESULTS AND DISCUSSION

1. CpG Island finder tools: It is a tool used to predict the CpG Island in any gene.

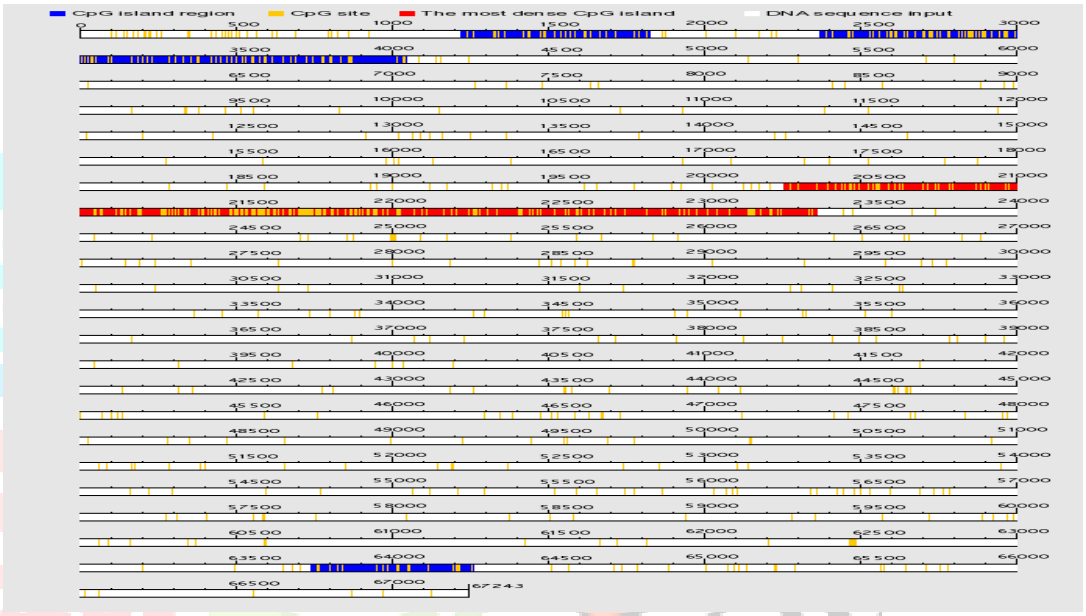


Figure 1: CpG Island present on BDNF gene

CpG Island and CpG plot are the first step for further analysis of DNA methylation. CpG Island typically occurs at or near the transcription start site of the gene. DNA methylation was a frequently used epigenetic signaling tool that can fix genes in the "off" position. As it was know that the CpG Island was the region where methylation takes place as the content of CG nucleotide was more as compare to other region, and methylation always occurs in the 5' position of cytosine. Hence, these two tools were used to know the CpG Island present on BDNF gene. In the figure.1, it was shows that total six CpG regions were present on BDNF gene, four CpG Island regions and two dense CpG Island was present in whole sequence of a gene. Normal CpG Island region shows in blue colour and dense region shows in red colour.

2. PREDICTED TRANSCRIPTION START SITES:gi_568815587_c27722058-27654893 Homo sapiens chromosome 11,GRCh38.p7 PrimaryAssemblyATTATTAAAGCGGTAGTCTGCCGGCGCTGATAAGCAACAAGTTCCCCAGCGGTCTTC CCGCCCTAGCCTGACAAGGCGAAGGTTTTCTTACCTGGCGACAGGGAAATCTCCCGA,53439 nucleotides

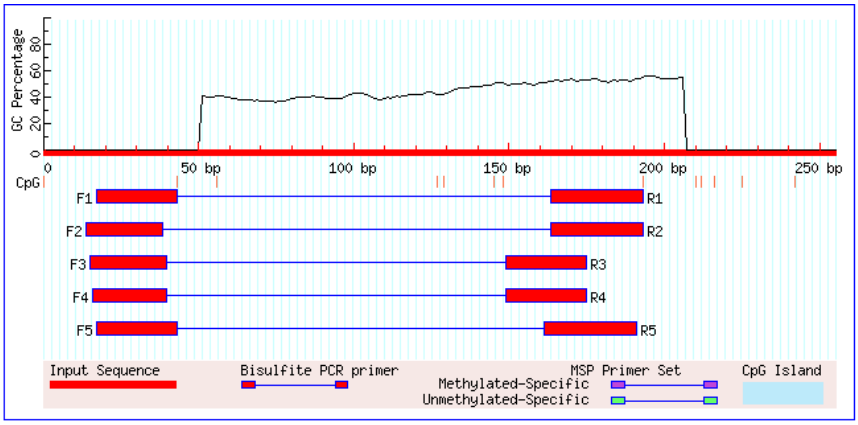
Position	Score	Likelihood
1500	0.642	Marginal prediction
2000	0.546	Marginal prediction
3800	0.723	Marginal prediction
5400	1.105	Highly likely prediction
6100	0.628	Marginal prediction

```
10300  1.128  Highly likely prediction
  11300  0.698  Marginal prediction
  12000  0.618  Marginal prediction
14000  1.096  Highly likely prediction
16800  1.213  Highly likely prediction
  20300  0.732  Marginal prediction
  21000  0.642  Marginal prediction
26300  1.301  Highly likely prediction
29900  1.092  Highly likely prediction
33100  1.166  Highly likely prediction
  33600  0.676  Marginal prediction
  34000  0.592  Marginal prediction
  35000  0.628  Marginal prediction
36000  1.152  Highly likely prediction
38600  1.126  Highly likely prediction
39300  1.233  Highly likely prediction
40800  1.098  Highly likely prediction
  41700  0.633  Marginal prediction
  42300  0.606  Marginal prediction
  42800  0.608  Marginal prediction
  43400  0.574  Marginal prediction
  44000  0.523  Marginal prediction
46000  1.107  Highly likely prediction
47400  1.127  Highly likely prediction
  48100  0.535  Marginal prediction
  50000  0.727  Marginal prediction
51400  1.092  Highly likely prediction
52200  1.037  Highly likely prediction
53900  1.073  Highly likely prediction
  54300  0.531  Marginal prediction
  56500  0.555  Marginal prediction
  57100  0.569  Marginal prediction
  58100  0.637  Marginal prediction
59000  1.081  Highly likely prediction
  59900  0.623  Marginal prediction
60600  1.138  Highly likely prediction
61500  1.093  Highly likely prediction
  63400  0.636  Marginal prediction
65300  1.213  Highly likely prediction
  65800  0.667  Marginal prediction
```

Figure2: Promoter prediction of BDNF gene

Promoter was region of DNA that initiates the transcription of a particular gene. More than 0.6 score obtained were highly predicted promoter and chances of transcription initiation was high. Results obtained from Promoter 2.0 prediction figure.2 shows that some sequence contain less than 0.6 and some contain greater than 0.6 and the sequence which were contain greater than 0.6 where highly predicted promoter that initiates the transcription. In both the tools results obtained were in two different form , in first tool i.e. promoter prediction tool promoter region was present in a sequence in red colour and in case of promoter 2.0 prediction tool the result was obtained were in two different form i.e. highly likely prediction and marginal prediction and result obtained from this tool was based on calculation cut-off values, cut-off value i.e. 0.8 and in the sequence the cut-off value which contain 0.8 and greater than this value was contain promoter.

3. Meth Primer




```
Sequence Name:
Sequence Length: 255

CpG island prediction results
(Criteria used: Island size > 100, GC Percent > 50.0, Obs/Exp > 0.6)
No CpG islands were found in your sequence
Primer picking results for bisulfite sequencing (or restriction) PCR
```

	Primer	Start	Size	Tm	GC%	'C's	Sequence
1	Left primer	17	26	59.95	46.15	4	TTGGTTGGAATAAGTAATTTGGTGTA
	Right primer	193	30	55.85	50.00	9	CACAAAACATAAAAACTATTAATTTACTCT
	Product size: 177, Tm: 68.1, CpGs in product: 6						
2	Left primer	14	25	59.60	48.00	5	TTTTTGGTTGGAATAAGTAATTTGG
	Right primer	193	30	55.85	50.00	9	CACAAAACATAAAAACTATTAATTTACTCT
	Product size: 180, Tm: 68.2, CpGs in product: 6						
3	Left primer	15	25	59.18	48.00	5	TTTTGGTTGGAATAAGTAATTTGGT
	Right primer	175	26	50.27	42.31	4	TTAATTTACTCTACTACCTTAAACAC
	Product size: 161, Tm: 68.1, CpGs in product: 6						
4	Left primer	16	24	58.03	50.00	5	TTTGGTTGGAATAAGTAATTTGGT
	Right primer	175	26	50.27	42.31	4	TTAATTTACTCTACTACCTTAAACAC
	Product size: 160, Tm: 68.2, CpGs in product: 6						
5	Left primer	17	26	59.95	46.15	4	TTGGTTGGAATAAGTAATTTGGTGTA
	Right primer	191	30	53.97	53.33	10	CAAAACATAAAAACTATTAATTTACTCTAC
	Product size: 175, Tm: 68.2, CpGs in product: 6						

```
  1 TCATTGCCTGGGCTTCCTGGCTGGAACAAGTAACTTGGTGTAACGTTATCTGGGGGCGTT
    |:|||||:|||||:|||:|||:||||||:||||||:||||||||||++||||:|||||||++||
  1 TTATTGTTTGGGTTTTTTGGTTGGAATAAGTAATTTGGTGTAACGTTATTTGGGGGCGTT
                    >>>>>>>>>>>>>>>>>>>>>>>>>>>>

 61 CATCAATAAAAAATGCTGTTATTATCTTGATTGAATTCCTATTAGGCAAACTCTAGAGAG
    :||:||||||||||||:|||||||:|||||||||||:|||||||:||||:|||||||||
 61 TATTAATAAAAAAATGTTGTTATTATTTTGATTGAATTTTTATTAGGTAAATTTTAGAGAG

121 GTCAGTGCGCGAACTCTGTTTAAGCCGGCGTGTTTAAGGCAGCAGAGTAAACCAATAGCC
    ||:||||++++||:|:|||||||||:++|++|||||||||:||:|||||||||::||||::
121 GTTAGTGCGCGAATTTTGTTTAAGTCGGCGTGTTTAAGGTAGTAGAGTAAATTAATAGTT
                                                  <<<<<<<<<<<<<<<<<<

181 CCCATGCTCTGTGCGATTTCATTGTGTGCTCGCGTTCGCAAGCTCCGTAGTGCAGGAAGG
    :::|||:|:|||||++||||:|||||||||:|++++||++:|||:|:++||||||:|||||||
181 TTTATGTTTTGTGCGATTTTATTGTGTGTTCGCGTTCGTAAGTTTCGTAGTGTAGGAAGG
    <<<<<<<<<<<<<<
```

```
241 TGCGGGAAGGTGTGT
    ||++|||||||||||
241 TGCGGGAAGGTGTGT


**********************************************************************
  Explanations                                                       *
----------------------------------------------------------------------*
  Upper row: Original sequence                                       *
  Lower row: Bisulfite modified sequence                             *
             (For display, assume all CpG sites are methylated)      *
  ++         CpG sites                                               *
  ::::       Non-CpG 'C' converted to 'T'                            *
  >>>>>>     Left  primer                                            *
  <<<<<<     Right primer                                            *
                                                                     *
**********************************************************************
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Figure 3: Meth primer result

Meth primer tool was used to study methylation. From the figure.3, it was shows that the methylated cytosine will not converted to thymine i.e. T and plus sign shows the CpG site. It was cleared that the C which was not changed in lower row of the sequence was methylated and from these technique it was analysed that total 12 C was not converted to T.

4. Quantification tool for Methylation analysis

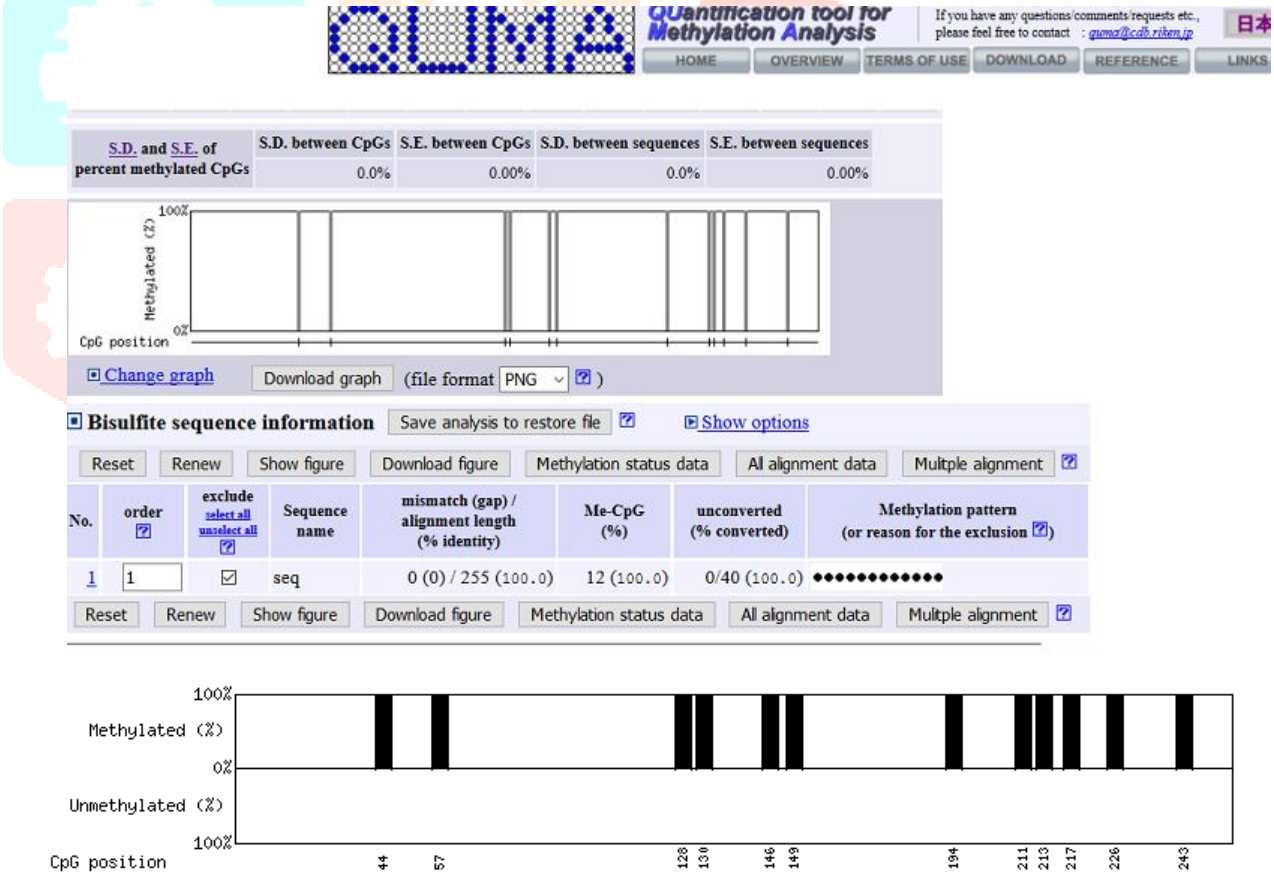

Figure 4: DNA methylation analysis

This result showed that total 12 CpG site or position were present in the sequence and black dot showed the methylation pattern. As it was known that DNMT1 enzyme playsan important role in DNA methylation. Therefore there was a need to study the interaction pattern of DNMT1 with some molecular compound (Decitabine, Zebularine, Hydralazine,Azacitidine, Procianamide).

5. Swiss Doc

1.DNMT1 AND DECITABINE INTERACTION

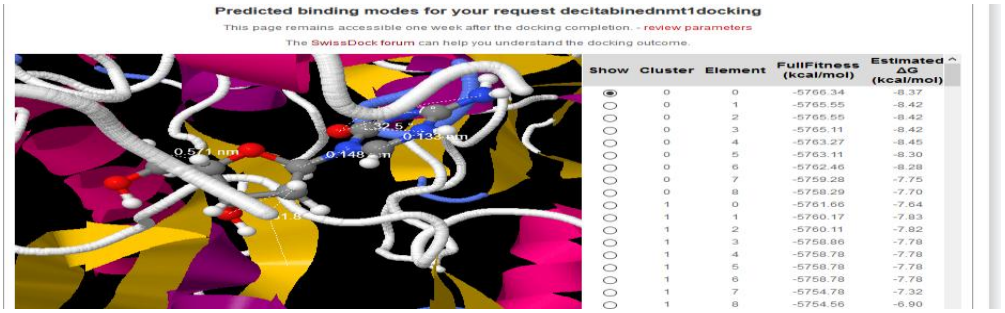

Figure.5: Decitabine interaction with DNMT1 .The structure shows the binding energy i.e. in the form of delta G and the value is -8.37.

2.DNMT1 AND ZEBULARINE INTERACTION

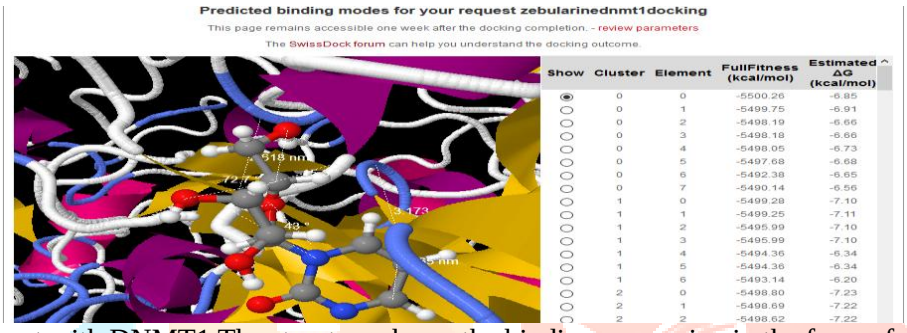

Figure.6: Zebularine interact with DNMT1.The structure shows the binding energy i.e. in the form of delta G and the value is -6.85.

3.DNMT1 AND HYDRALAZINE INTERACTION

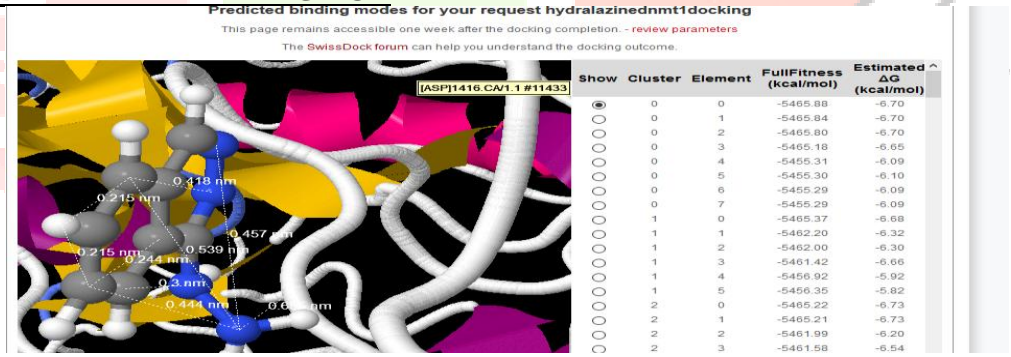

Figure.7: Hydralazine interact with DNMT1.The structure shows the binding energy i.e. in the form of delta G and the value is -6.70.

4.DNMT1 AND AZACITIDINE INTERACTION

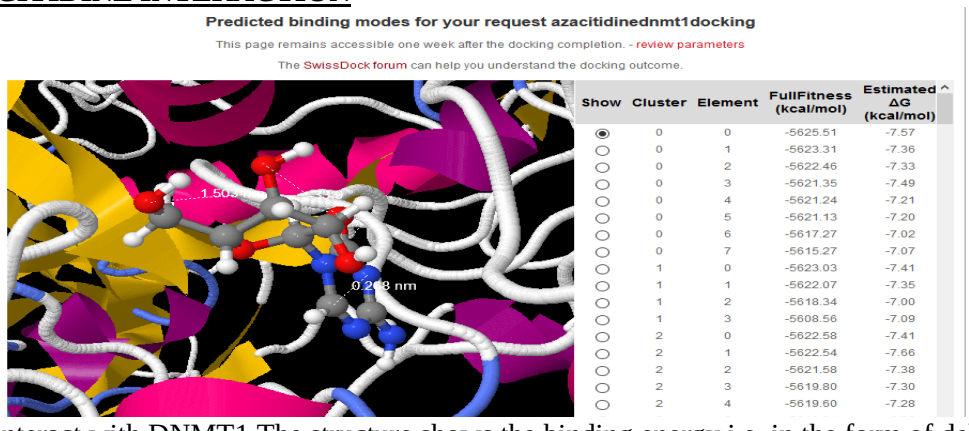

Figure.8: Azacitidine interact with DNMT1.The structure shows the binding energy i.e. in the form of delta G and the value is -7.57.

5.DNMT1 AND PROCAINAMIDE INTERACTION

Figure.9: Procainamide interact with DNMT1.The structure shows the binding energy i.e. in the form of delta G and the value is -7.02.

Swiss dock is one of the docking tools which was used for interaction of ligand with molecular compound and chimera is a software used to validate the results obtained through swiss dock .There are total five different molecular compound was used and they are zebularine ,hydralazine ,decitabine,5-azacitidineand procinamide and these molecular compound was interacted with protein DNMT1 (4WXX).
Interactions of these molecular compounds with protein were correct.

6.SCHRODINGER (MASTERO)DOCKING RESULTS

1.DNMT1 AND DECITABINE INTERACTION

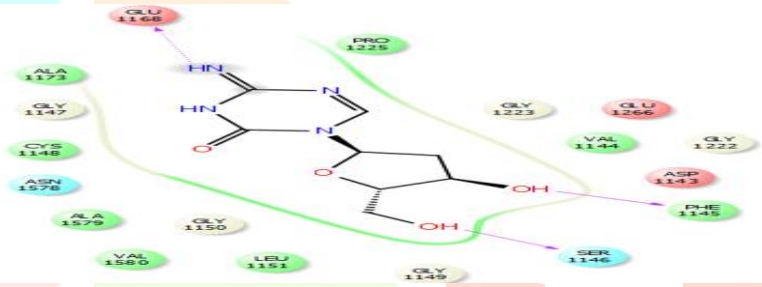

Figure.10: Hydrophobic interaction of Decitabine with DNMT1.The docking score of these protein and ligand interaction is -6.468 and the interaction showed are (GLU-1168)NH,(SER-146)OH,(PHE-1145)OH.

2.DNMT1 AND ZEBULARINE INTERACTION

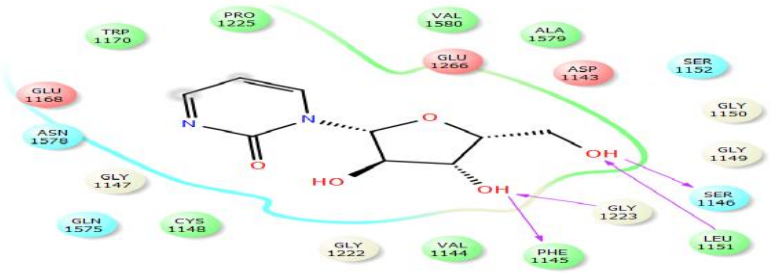

Figure.11: Hydrophobic interaction of Zebularinewith DNMT1.The docking score of these protein and ligand interaction is -6.682 and the interaction showed are (PHE-1145)OH,(SER-1146)OH,(GLY-1223)OH,(LEU-1151)OH.

3.DNMT1 AND HYDRALAZINE INTERACTION

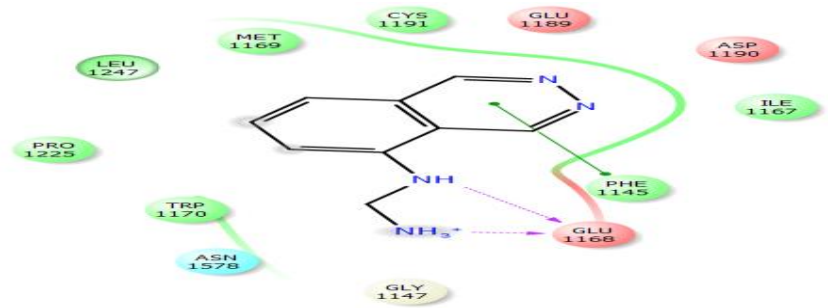

Figure.12: Hydrophobic interaction of Hydralazine with DNMT1.The docking score of these protein and ligand interaction is -4.801 and the interaction showed are (GLU-1168)NH,(GLU-1168)NH3.

4.DNMT1 AND AZACITIDINE INTERACTION

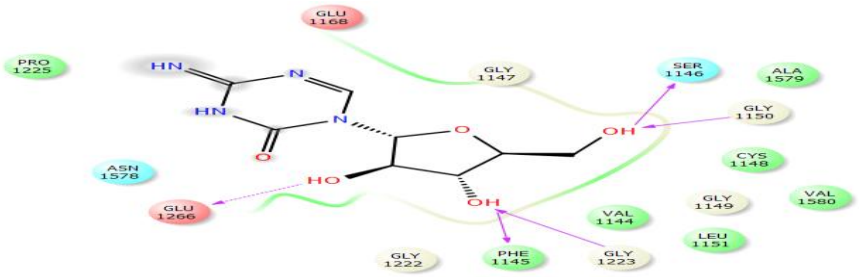

Figure.13: Hydrophobic interaction of Azacitidinewith Dnmt1.The docking score of these protein and ligand interaction is -5.816 and the interaction showed are (PHE-1145)OH,(GLY-1223)OH,(SER-1146)OH,(GLY-1150).

5.DNMT1 AND PROCIANAMIDE INTERACTION

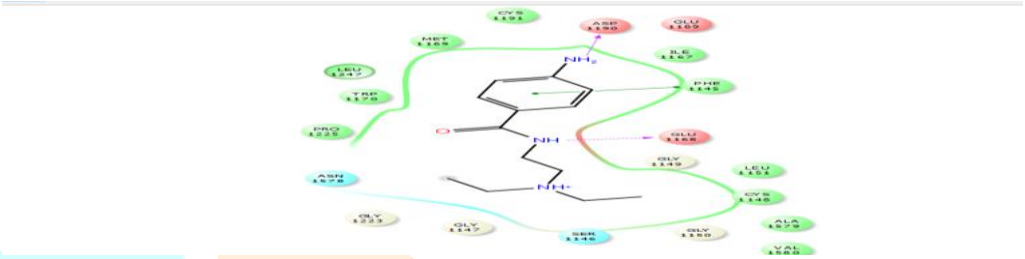

Figure.14: Hydrophobic interaction of Procianamidewith DNMT1.The docking score of these protein and ligand interaction is -3.973 and the interaction showed are (GlU-1168)NH,(ASP-1190)NH2.

Protein-ligand interaction was also analysed by another tool which was commonly used for docking i.e. Schrodinger Glide docking. Glide docking provided more accurate value than Swiss dock because it follows 3 step to select the best grid for docking and the 3 steps are HTVS to SP to XP.

7.Auto Dock results

1.DNMT1 AND DECITABINE INTERACTION

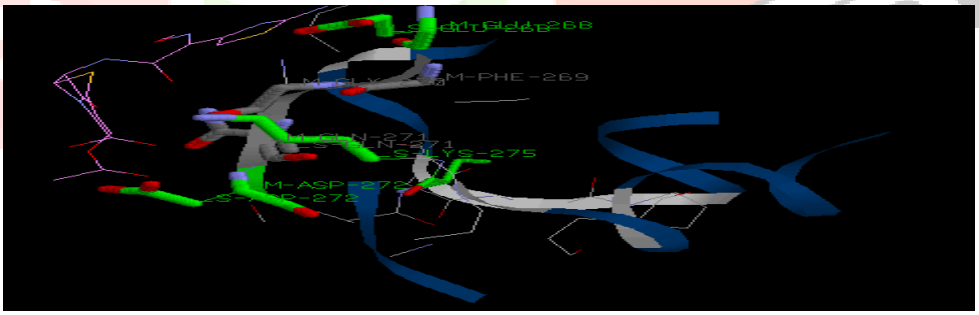

Figure.15: Decitabine interaction with DNMT1.The ligand structure which shows the binding energy -5.28 with their interaction was viewed by PyMol viewer and the interaction shows are (GLU-268),(PHE-269),(LYS-275),(ASP-272),(GLN-271).

2.DNMT1 AND ZEBULARINE INTERACTION

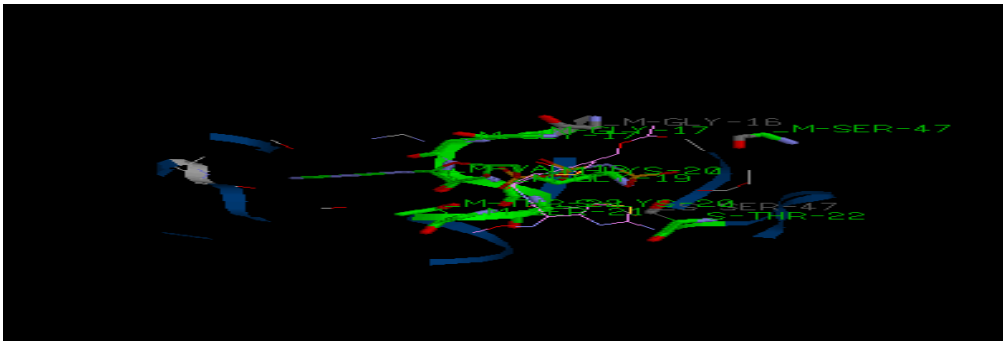

Figure.16: Figure: Zebularine interaction with DNMT1.The ligand structure which shows the binding energy -6.37 with their interaction was viewed by PyMol viewer and the interaction shows are (GLY-16),(THR-22),(LYS-20),(SER-47).

3.DNMT1 AND HYDRALAZINE INTERACTION

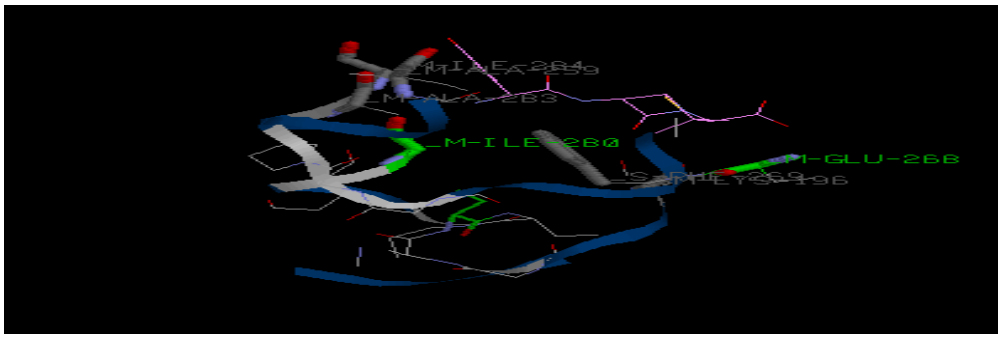

Figure.17: Hydralazine interaction with DNMT1.The ligand structure which shows the binding energy -4.46 with their interaction was viewed by PyMol viewer and the interaction shows are (GLU-268),(LYS-169),(ILE-280),(ALA-283).

4.DNMT1 AND AZACITIDINE INTERACTION

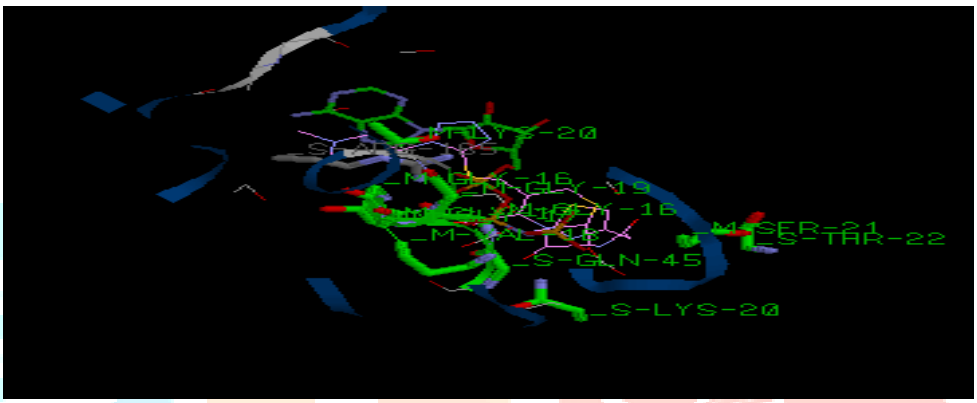

Figure.18: Azacitidine interaction with DNMT1.The ligand structure which shows the binding energy -3.44 with their interaction was viewed by PyMol viewer and the interaction shows are (GLY-16),(THR-22),(LYS-20),(SER-21)(GLN-45).

5.DNMT1 AND PROCAINAMIDE INTERACTION

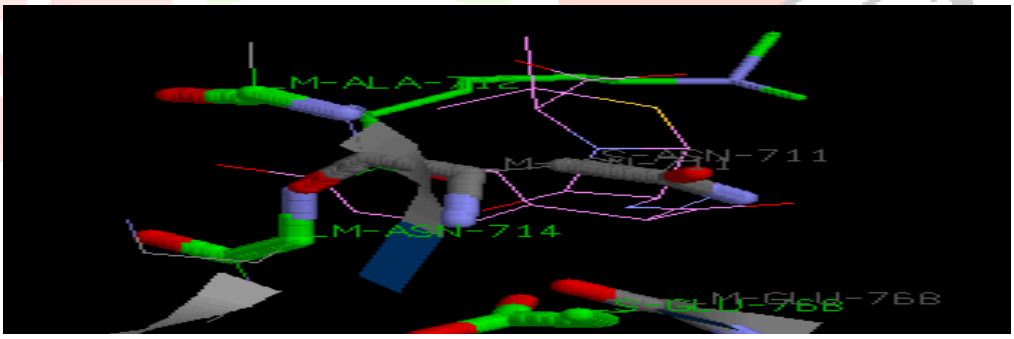

Figure.19: Procianamide interaction with DNMT1.The ligand structure which shows the binding energy -3.68 with their interaction was viewed by PyMol viewer and the interaction shows are (GLU-768),(ASN-714),(ALA-712).

Auto dock is one of the best docking tools. It is designed to predict how small molecules, such as molecular compound or substrates or drug candidates, bind to a receptor of known 3D structure. Auto dock also uses PyMolviwer software to analyses the hydrophobic interaction of ligand protein and binding of protein to ligand with binding energy.

8.Cluspro server:

1.INTERACTION OF DOCKED DNMT1 AND BDNF

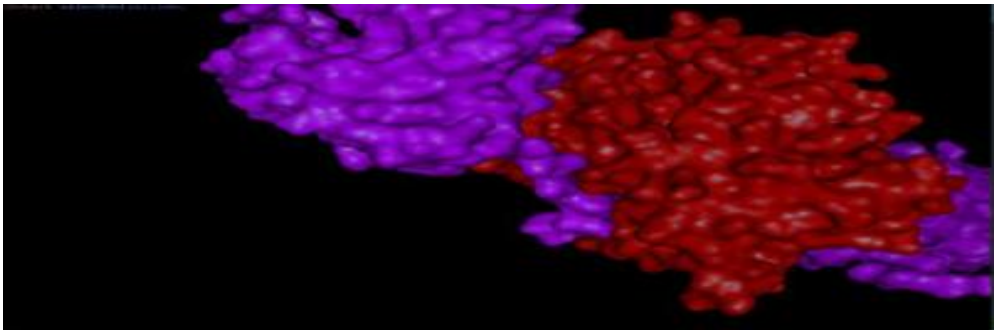

Figure.20: Modified DNMT1 docked with BDNF. The two proteins had the interaction with the residues of GLU 1168, PHE1145, LEU1151, GLY1223, SER 1146, and SER 1146, PHE 246, THR 239, GLY 268, ARG 288. Magenta colour = DNMT1; Red = BDNF.

The ligand molecules are showing the interaction with the active site residues and it has the binding region as in the methylasetransferase binding domain.

Protein - protein complexes are the most commonly attempted targets of such modelling. So this protein - protein docking or interaction has the effect to change one protein's function or structure or some of the properties. Based on this technique, the docked DNMT1 protein gets again docked with the BDNF protein which may change the hypermethylation process of BDNF Protein (Marcotte*et al*., 1999). So this protein-protein interaction work was carried out by ClusPro Server.

CONCLUSION

The methylation pattern on BDNF gene predicted the DNMT1 role, as DNMT1 play a important role in DNA methylation and in case of Diabetes and diabetic retinopathy DNMT1 level increases. Therefore the interaction pattern of the proteins i.e. BDNF protein and DNMT1 protein was studied with the help of different tools, tools which were used for docking are swissdock, autodock, clusproserver. On the basis of result obtained from these tools best ligand molecule was selected i.e. Decitabine and Zebularine, which may control the level of DNMT1 through which BDNF level may get controlled and it may help in controlling T2DR.

REFERENCES

Akio K, Tibor A, Rauch b, Ivan T, Hsun T (2009). "Insulin Gene Expression Is Regulated by DNA Methylation" *PLoS ONE.*, **9**: 6953. Goffin J, Eisenhauer E (2002). DNA methyltransferase inhibitors—state of the art. Ann. Oncol. 13: 1699–1716.

Chen, R., &Weng, Z. (2003). A novel shape complementarity scoring function for protein-protein docking. *Proteins: Structure, Function, and Bioinformatics*, *51*(3), 397-408.

Comeau, S. R., Gatchell, D. W., Vajda, S., & Camacho, C. J. (2004). ClusPro: an automated docking and discrimination method for the prediction of protein complexes. *Bioinformatics*, *20*(1), 45-50.

Deaton AM, Bird A(2011). CpG islands and the regulation of transcription. Genes Dev, 2510: 1010–1022

Dhe-Paganon, S., Syeda, F., & Park, L. (2011). DNA methyl transferase 1: regulatory mechanisms and implications in health and disease. *International journal of biochemistry and molecular biology*, *2*(1), 58.

Kitada, M., Zhang, Z., Mima, A., & King, G. L. (2010). Molecular mechanisms of diabetic vascular complications. Journal of diabetes investigation, 1(3), 77-89.

Kowluru, R. A. (2001). Diabetes-induced elevations in retinal oxidative stress, protein kinase C and nitric oxide are interrelated. Actadiabetologica, 38(4), 179-185.

Kowluru, R. A., & Mishra, M. (2015). Contribution of epigenetics in diabetic retinopathy. *Science China. Life Sciences*, *58*(6), 556.

Madsen–Bouterse, S. A., Mohammad, G., Kanwar, M., &Kowluru, R. A. (2010). Role of mitochondrial DNA damage in the development of diabetic retinopathy, and the metabolic memory phenomenon associated with its progression. *Antioxidants & redox signaling*, *13*(6), 797-805.

Marcotte E, Pellegrini M, RiceD, YeatesT, Eisenberg D (1999). Detecting protein function and protein-protein interactions from genome sequences. Science 285: 751-753

Mishra, M., &Kowluru, R. A. (2015). Epigenetic Modification of Mitochondrial DNA in the Development of Diabetic RetinopathyMethylation of mtDNA in Diabetic Retinopathy. *Investigative ophthalmology & visual science*, *56*(9), 5133-5142.

Moosavi, A., &Ardekani, A. M. (2016). Role of epigenetics in biology and human diseases. Iranian biomedical journal, 20(5), 246.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., &Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), 1605-1612.

Portela, A., &Esteller, M. (2010). Epigenetic modifications and human disease. Nature biotechnology, 28(10), 1057-1068.

Ritchie, D. W., & Kemp, G. J. (2000). Protein docking using spherical polar Fourier correlations. *Proteins: Structure, Function, and Bioinformatics*, *39*(2), 178-194.

Robertson, K. D., &Wolffe, A. P. (2000). DNA methylation in health and disease. *Nature Reviews Genetics*, *1*(1), 11-19.

Santos, J. M., &Kowluru, R. A. (2011). Role of mitochondria biogenesis in the metabolic memory associated with the continued progression of diabetic retinopathy and its regulation by lipoic acid. *Investigative ophthalmology & visual science*, *52*(12), 8791-8798.

Shaw, J. E., Sicree, R. A., &Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice*, *87*(1), 4-14.

Tewari, S., Zhong, Q., Santos, J. M., &Kowluru, R. A. (2012). Mitochondria DNA Replication and DNA Methylation in the Metabolic Memory Associated with Continued Progression of Diabetic RetinopathyMetabolic Memory and mtDNA. *Investigative ophthalmology & visual science*, *53*(8), 4881-4888.

Zheng, Y., He, M., &Congdon, N. (2012). The worldwide epidemic of diabetic retinopathy. Indian journal of ophthalmology, 60(5), 428