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***In silico* study to find out the antagonist of *EHMT2* (G9A) to regulate DNA methylation in case of Type 2 Diabetic Retinopathy**

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Abstract: Type 2 Diabetic Retinopathy (T2DR) associated with major cause of loss of vision throughout world in diabetic individual is the advanced stage of diabetic retinopathy that anguishes retinal blood vessels. Brain derived neurotrophic factor (BDNF) play key role in defending retinas from unusual high concentration of glucose allied with diabetes in vitro by activating high affinity specific receptor tropomyosin-related kinase B (TrkB). Epigenetic modifications usually involve the interaction between genetic and environmental factors leading to development of disease. Histone methyltransferases regulate gene expression by altering chromatin structure through histone lysine methylation. It has been found that G9A act as the biomarker in various types of cancer like lung cancer, cervical cancer thereby down regulating tumour suppressor genes. Moreover, down regulation of BDNF gene has been found to be associated with the overexpression of genes like G9A (EHMT2), GLP in schizophrenia disease which leads to H3K9me2 (Histone 3 Lysine 9) dimethylation, the repressive mark associated with the suppression of gene expression. As per the available literature, it can be hypothesized that inhibition of H3K9 methylation repressive mark with the help of inhibitor might regulate BDNF expression thereby modulating signaling pathway of BDNF which in turn would prevent the damage of retinal cells in Type 2 Diabetic Retinopathy. An *in silico* approach before performing *in vitro* experiment is imperative and is helpful. The present study involved the bioinformatics analysis of compounds as an inhibitor of G9A (EHMT2) enzyme. Docking was performed on compounds obtained from NCBI PubChem database using UCSF Chimera, Swissdock, ADME (Absorption, Distribution, Metabolism and Excretion) analysis of compounds was done with the help of SwissADME.

Keywords: Type 2 Diabetic retinopathy, Brain Derived Neurotrophic Factor, HKMTi (Histone lysine methyltransferase inhibitor), Molecular docking, SwissADME.

I. INTRODUCTION

Type 2 Diabetic Retinopathy is the prominent and foremost cause of blindness worldwide associated with micro vascular hurdle, capillary non-perfusion, hemorrhages and neovascularization. These complications affects eyes leading to diabetic retinopathy. The progress of the disease leads to the formation of new veins in the retina and on the back surface of vitreous causing loss of vision (Curtis *et al.*, 2009). The patients suffering from Type 1 and Type 2 diabetes accompanied with micro vascular complexities develop diabetic nephropathy, neuropathy including retinopathy along with cardiovascular infections (Villeneuve *et al.*, 2010). Diabetic retinopathy is estimated to increase from 127 million to 191 million by 2030 (Zheng *et al.*, 2012). Hyperglycemia is one of the principal actor in the development and progress of diabetic retinopathy complications leading to abnormal metabolic activities-homeostatic abnormalities, oxidative stress, retinal blood's alteration, neoangiogenesis and activation of inflammatory pathways (Petrovic, 2013). Hyperglycemia mediated oxidative stress seems to be one of the linking component between neurodegeneration and early micro vascular complications (Stem *et al.*, 2013).

Retinal neurons and glial cells produces brain derived neurotrophic factor which is essential for the protection of neuronal cells (Ola *et al.*, 2013). Downstream signaling pathways of BDNF are carried out through two specific receptors -high affinity tropomyosin-related kinase B (TrkB) receptor and small affinity p75receptor. BDNF/TrkB signaling (Ras-Raf-MEK-ERK, also known as MAPK pathway) is very much crucial for neurons and retinal cells growth, differentiation and survival (Andero *et al.*, 2014). BDNF during initial development of T2DR repress apoptosis of retinal ganglion cells of rats (Ferryhough *et al.*, 2003). Decreased level of BDNF in the serum of diabetic patients and animals was found to be in association with resistance to insulin and less metabolism of glucose and lipids (Ola *et al.*, 2013).

Histone methylation is one of the stable epigenetic mark which is catalyzed by histone methyltransferase (HMTs) and histone demethyltransferases (HDMs) and is associated with the pathogenicity and complications of diabetes by altering chromatin structure (Villeneuve *et al.*, 2010). Histone methylation results in the suppression or expression of gene depending on the site it takes place. H3K9, H3K27 & H4K20 methylation are associated with the silenced chromatin states leading to gene suppression. The principle histone lysine methyltransferase involved in histone lysine methylation are G9A and GLP. These are the major HKMTs responsible for H3K9me1 and H3K9me2 found in mammals (Yokochi *et al.*, 2009). Number of genes in lymphocytes of diabetic condition were associated with increased concentration of H3K9me2 linked to activation of inflammatory & immune pathways associated with Type 1 diabetes complications (Miao *et al.*, 2008). Several studies revealed functional interactions between G9A and DNMT1. G9A and DNMT1 has been known to colocalized at the replication fork implicates H3K9 methylation during DNA replication (Rothbart *et al.*, 2012).

After going through the available literature, five compounds that has already been used in cases of diabetes were selected as inhibitor of G9A & DNMT1 which might up regulate expression of *BDNF* thereby preventing apoptosis of retinal cells in Type 2 Diabetic Retinopathy. Rutaecarpine has been shown with protective effect in high glucose condition in streptozotocin treated rats through IRS-1/ PI3K/AKT signaling pathway in liver and AMPK signaling pathway in skeletal muscles in type 2 diabetes (Nie *et al.*, 2016). It also reduces the injury of renal ischemia reperfusion in rats by reducing oxidative stress, apoptosis through repression of JNK/p38 MAPK pathway (Wang *et al.*, 2017).

Since H3K9 (histone lysine methylation) is the stable, repressive epigenetic mark through various studies including cancer, schizophrenia and cerebral ischemia, it can be hypothesized that inhibition of histone methyltransferase enzyme G9A could provide therapeutic effect by up regulating *BDNF* gene which might work as a potential treatment target in Type 2 Diabetic Retinopathy.

II. MATERIALS AND METHODS

A. Literature Mining

- 1) Overexpression of EHMT2 has been found in various types of cancer- bladder cancer, lung cancer, esophageal cancer, cervical cancer, suppressing tumor suppression gene through histone methylation. Hence through studies EHMT2 has been identified as potent therapeutic target in cancer. EHMT2 in association with DNMT1 contribute in DNA methylation and histone methylation (Ryuji *et al.*, 2012).
- 2) A study by Schweizer *et al.*, revealed that the inhibition of histone 3 lysine 9 (H3K9) methyltransferase enzymes – G9A (EHMT2) and SUV39H1 results in the up regulation of *BDNF* by regulating histone 3 lysine 9 methylation (repressive mark) around *BDNF* promoter with the help of inhibitor which in turn promotes the survival of neuronal cells in rats in cerebral ischemia (Schweizer *et al.*, 2015).
- 3) Inhibition of histone methyltransferases- G9A, GLP and Setdb1 with the help of inhibitor results in reduction of total and promoter specific H3K9me2 (histone 3 lysine 9 di-methylation) levels in *BDNF* gene by altering chromatin structure. This results in the increase of expression of *BDNF* in mice neuronal cell culture and in human lymphocyte cell culture which is essential for neuroprotection in schizophrenia. One of every five patient develop T2DM (Type 2 Diabetes Mellitus) and are more prone to the risk of T2DM with respect to healthy individual (Chase *et al.*, 2012).
- 4) Epigenetic modification contribute a lot in the development of disease by chromatin remodeling. G9A is responsible for H3K9 dimethylation leading to transcriptionally inactive epigenome. Inhibition of G9A results in the up regulation of hypoxia inducible factors- HIF1a and HIF2a by activating hypoxia signaling pathway thereby suppressing tumor in hypoxic condition in human cervical cancer (HeLa) and breast cancer (MCF-7) cell lines (Casciello *et al.*, 2017).

B. Downloading the structure of compound and 3D structure of protein of Homo sapien: The structure of compound for docking was taken from pubchem database using <https://pubchem.ncbi.nlm.nih.gov/> and 3D structure of protein was downloaded from Protein Data Bank which was browsed at site <https://www.rcsb.org>.

C. Ramachandran Plot Analysis: The analysis of Ramachandran plot was done by using Rampage. Rampage is a free online tool which can be browsed at site <http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>.

D. Preparation of Histone Lysine Methyltransferase Inhibitor ligand file: The ligand file of compounds- rutaecarpine, rhesinine, evodiamine and skimmianine that has been studied in case of diabetes was prepared in UCSF Chimera. The structure of compound was collected from pubchem database and then opened in UCSF Chimera. The structure of each compound was minimized and

hydrogen bond was added. Finally, the ligands files were saved in Protein Data Bank (PDB) and mole 2 file structure format for docking.

E. Molecular Docking and Visualization of docked Results: The docking of ligand and protein was done using “Swissdock” and the docked result was visualized using “UCSF Chimera” (Huang *et al.*, 2014).

F. ADME (Absorption, Distribution, Metabolism and Excretion) analysis of compounds: The analysis of ADME (Absorption, Distribution, Metabolism and Excretion) of compounds was done using “SwissADME” tool. SwissADME was browsed at <http://www.swissadme.ch>. Lipinski rule indicates safety of inhibitor for human use as well as their drug likeliness. According to Lipinski rule of five, molecular weight of inhibitor should be between 150g/mol and 500 g/mol, number of hydrogen bond donor should be less than or equal to five, number of hydrogen bond acceptor should be less than ten, molar refractivity should be between 40 to 130 and lipophilicity less than five are considered good (Lipinski *et al.*, 1997; Daina *et al.*, 2017).

III. RESULTS AND DISCUSSION

A. Ramachandran Plot Analysis of G9A, DNMT1

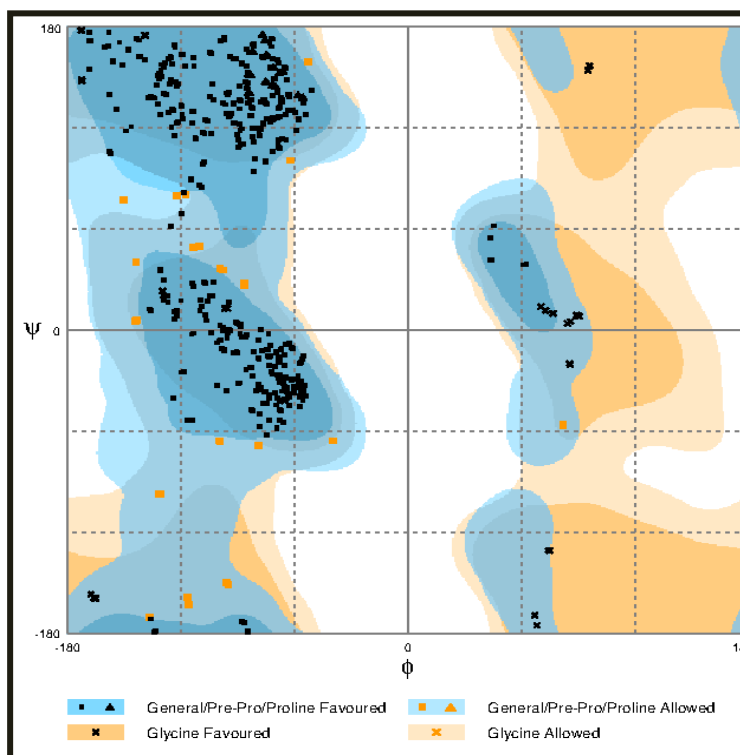
Analysis of ramachandran plot of EHMT2 (Euchromatic Histone Lysine Methyltransferase 2) also known as G9A, DNMT1 (DNA methyltransferase 1) having their PDB ID- 3RJW and 4WXX respectively has been shown in figure 1 and figure 2 including their values in Table 1. Ramachandran plot determines the quality of protein structure based on phi (ϕ), psi (ψ) and omega (ω) angles which helps in giving appropriate docking results. The favoured region (>90%) having dense number of residues gives the measurement of good quality of protein structure (Bilal *et al.*, 2009).

B. Ramachandran Plot of EHMT2 (3RJW) protein by Rampage

The present examination encapsulate that EHMT2 protein contain total 502 (94.7%) amino acid residues in favoured region along with 28 (5.3%) in allowed region and 0(0.0%) in outlier region.

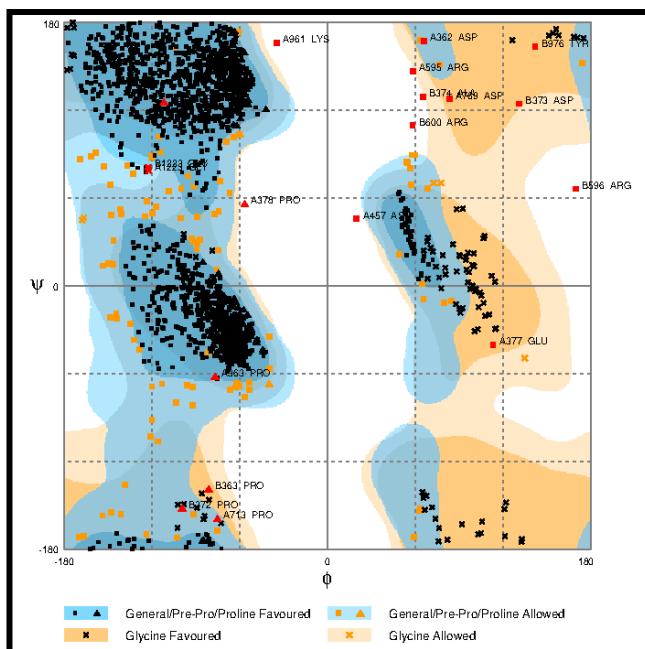
C. Ramachandran Plot of DNMT1 (4WXX) protein by Rampage

DNMT1 protein contain 2183(94.5%) total number of amino acid residues in favoured region, 107 (4.6%) in allowed region and 19 (0.8%) in outlier region.



Number of residues in favoured region (~98.0% expected) 502 (94.7 %)
 Number of residues in allowed region (~2.0% expected) 28 (5.3%)
 Number of residues in allowed region 0 (0.0%)

Fig 1: Rampage of EHMT2 (3RJW) Protein



Number of residues in favoured region (~98.0% expected) 2183 (94.5 %)
 Number of residues in allowed region (~2.0% expected) 107 (4.6%)
 Number of residues in allowed region 19(0.8%)

Fig 2: Rampage of DNMT1 protein

Table 1: Ramachandran plot analysis of protein by Rampage

Protein	Favoured Region	Allowed Region	Outlier Region
Ehmt2	502 (94.7 %)	28 (5.3%)	0 (0.0%)
Dnmt1	2183 (94.5 %)	107 (4.6%)	19(0.8%)

Table 1 encapsulates Ramachandran plot result of proteins using Rampage. EHMT2 (G9A) protein contain total 502 (94.7%) of amino acid residues in favoured region, 28 (5.3%) of amino acid residues in allowed region and 0 (0.0%) in outlier region. DNMT1 protein contain total 2183 (94.5%) amino acid residues in favoured region along with 107 (4.6%) amino acid residues in allowed region and 19 (0.8%) in outlier region. Favoured region having dense number of residues i.e, (>90%) is the indication of good quality of a protein.

D. Molecular Docking

Docking of protein- ligand was done using SwissDock and visualized through UCSF Chimera. The interaction of protein-ligand having binding energy greater than -5 was considered good for further analysis. Visualization of protein-ligand complex was done using UCSF Chimera. Ligand minimization and addition of hydrogen bond was done in Chimera. After the preparation of ligand, appropriate chain of protein was selected by opening the protein structure in Chimera followed by structure minimization using minimization menu. Finally, docking was performed using docking option. Results obtained were saved in PDB form (Grosdidier *et al.*, 2011).

Docking of EHMT2 (G9A) protein was done with ligand inhibitors using computer with good internet connection. As per the docking results, best selected ligands are Rutaecarpine, Rhetsinine, Evodiamine and Skimmianine. The binding energy (ΔG) of the best ligand inhibitor is shown in Table 3. After this, docking of DNMT1 protein with best selected ligand inhibitor was done using

SwissDock having PDB ID 4WXX. Docking of selected ligands with both EHMT2 and DNMT1 protein shows that the inhibitors act as the dual inhibitor of both EHMT2 and DNMT1. Among all, rutaecarpine and rhetsinine was found having binding energy greater than -5 as best with binding energy -7.60kcal/mol and -7.45 kcal/mol respectively with respect to receptor protein EHMT2 (G9A) having PDB ID 3RJW.

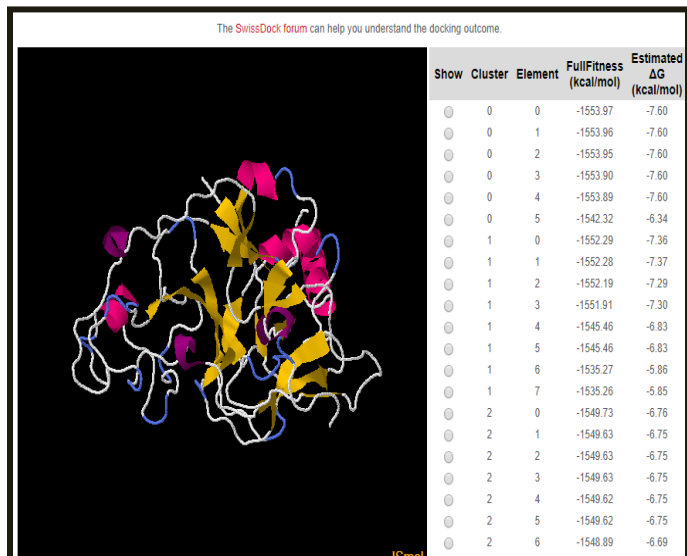


Fig 3: SwissDock interaction result of EHMT2 (3RJW) with Rutaecarpine

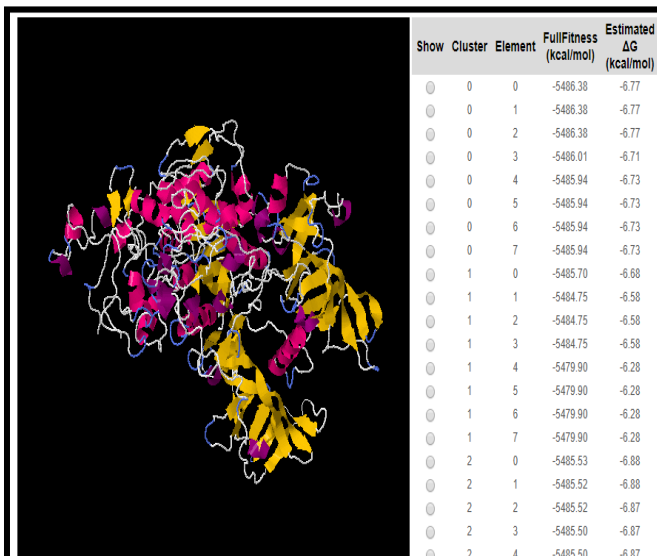


Fig 4: SwissDock interaction result of DNMT1 (4WXX) with Rutaecarpine

The figure 3 shows the interaction result of EHMT2 (3RJW) with Rutaecarpine and the obtained delta G value was -7.60 kcal/mol which was greater negative value than -5 and the fullfitness score was -1553.97 kcal/mol. Figure 4 shows the result of DNMT1 (4WXX) with Rutaecarpine and the delta G value was -6.77 kcal/mol which was greater negative value than -5 and the fullfitness score was -5486.38 kcal/mol.

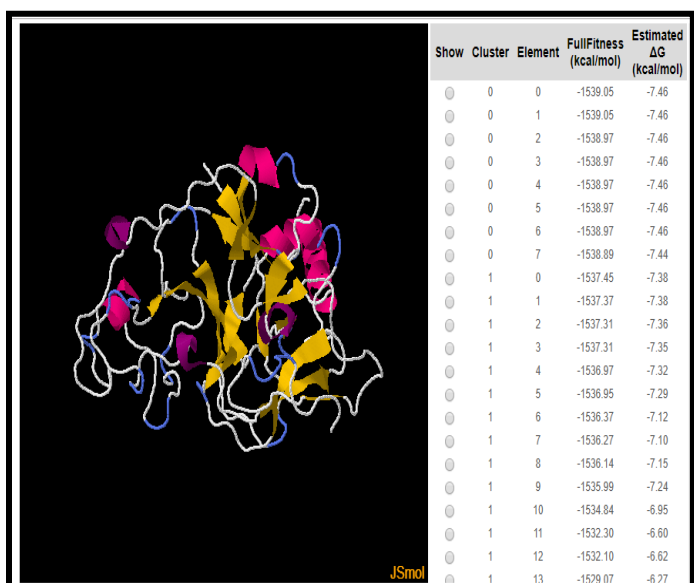


Fig 5: SwissDock interaction result of EHMT2 (3RJW) with Rhetsinine

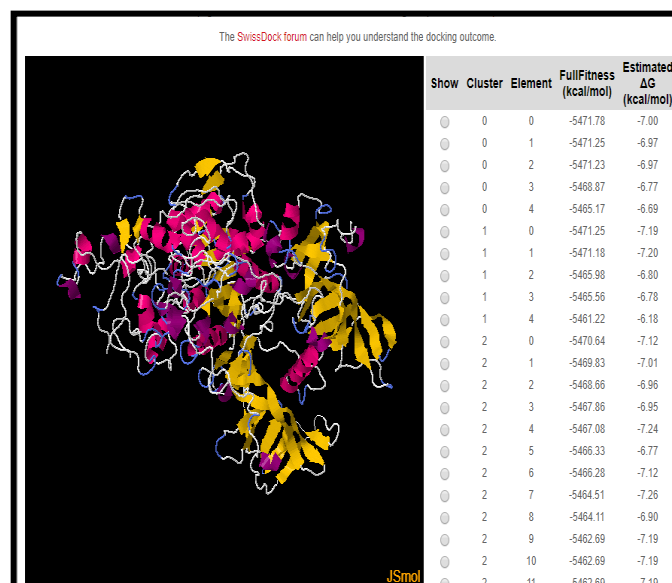


Fig 6: SwissDock interaction result of DNMT1 (4WXX) with Rhetsinine

Result obtained from figure 5 shows that after interaction of EHMT2 (3RJW) with Rhetsinine, delta G obtained was -7.46 kcal/mol which was greater negative value than -5 and the fullfitness score obtained was -1539.05 kcal/mol. Figure 6 shows the interaction result of DNMT1 (4WXX) with Rhetsinine, obtained delta G was -7 kcal/mol which was greater negative value than -5 and the fullfitness score was -5471.78 kcal/mol.

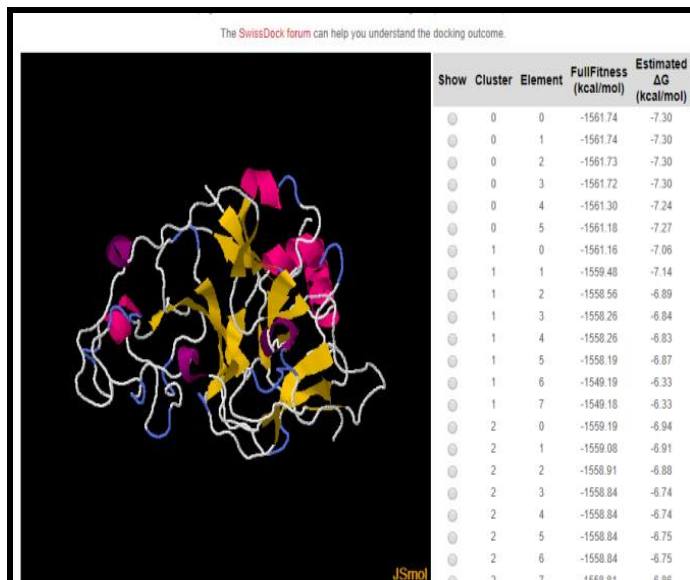


Fig 7: SwissDock interaction result of EHMT2 (3RJW) with Evodiamine

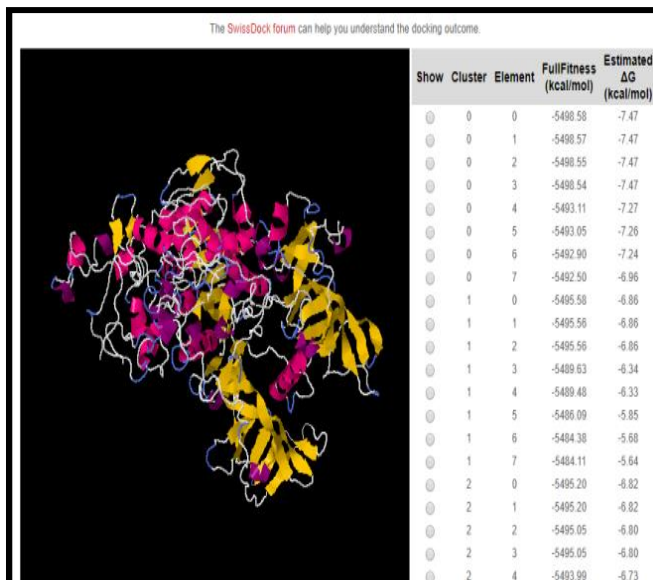


Fig 8: SwissDock interaction result of DNMT1 (4WXX) with Evodiamine

Figure 7 shows the result after interaction of EHMT2 (3RJW) with Evodiamine having delta G -7.30 kcal/mol which was greater negative value than -5 and the fullfitness score was -1561.74 kcal/mol. Figure 8 shows the interaction result of DNMT1 (4WXX) with Evodiamine obtained delta G was -7.47 kcal/mol which was greater negative value than -5 and the fullfitness score was -5498.58 kcal/mol.

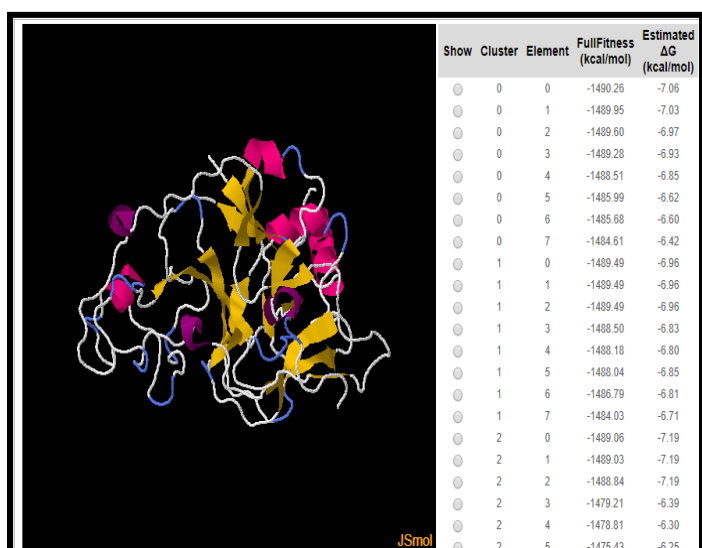


Fig 9: SwissDock interaction result of EHMT2 (3RJW) with Skimmianine

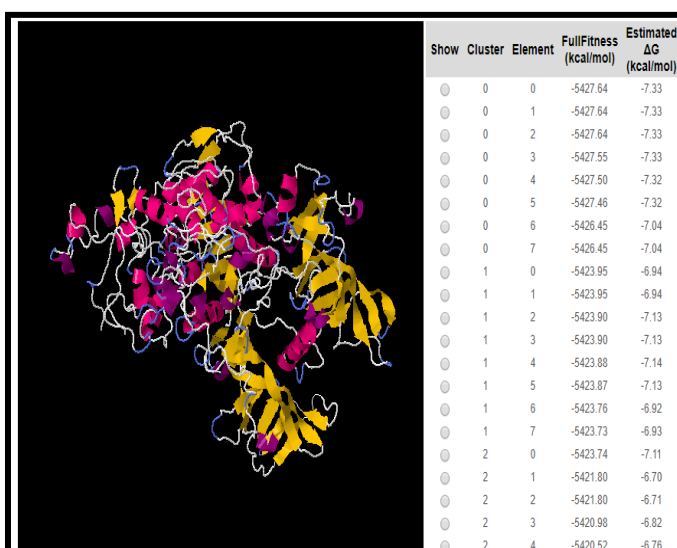


Fig 10: SwissDock interaction result of DNMT1 (4WXX) with Skimmianine

The figure 9 shows the interaction result of EHMT2 (3RJW) with Skimmianine and the delta G value obtained was -7.06 kcal/mol which was greater negative value than -5 and the fullfitness score was -1490.26 kcal/mol. Figure 10 shows the result of DNMT1 (4WXX) with Skimmianine and the delta G value was -7.33 kcal/mol which was greater negative value than -5 and fullfitness score was -5427.64 kcal/mol.

E. Visualization through UCSF Chimera tool

UCSF Chimera tool is useful for interactive visualization and analysis of molecular structures including density maps, sequence alignments, docking results, trajectories and conformational ensembles. High quality images can be generated (<https://www.cgl.ucsf.edu/chimera>).

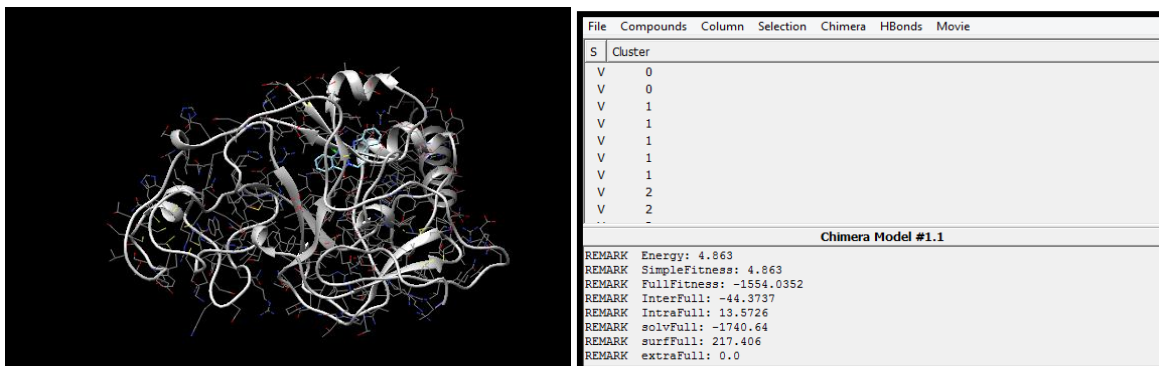


Fig 11: Visualization of of EHMT2 (3RJW) with Rutaecarpine through UCSF Chimera

The figure 11 shows the interaction result of EHMT2 (3RJW) with Rutaecarpine and the obtained delta G value was -7.60 kcal/mol which was greater negative value than -5 interaction and the fullfitness score was -1553.97 kcal/mol.

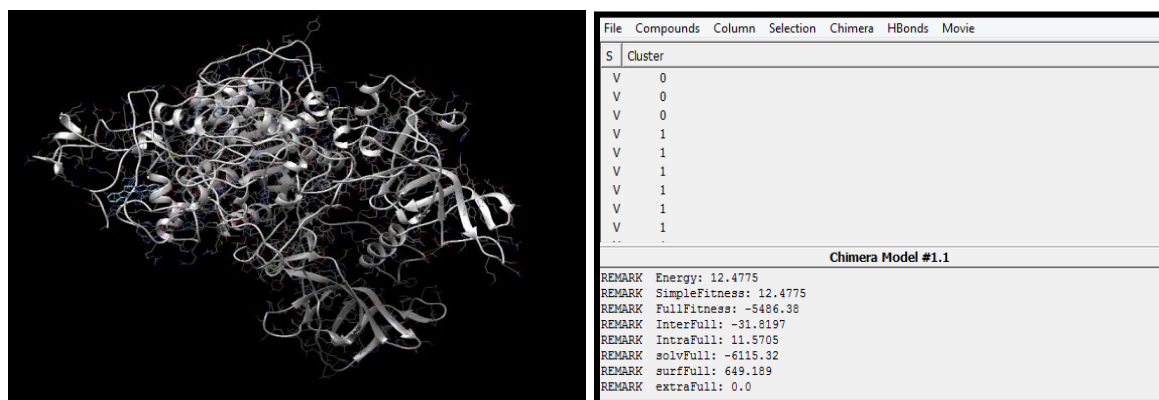


Fig 12: Visualization of interaction of DNMT1 (4WXX) with Rutaecarpine through UCSF Chimera

Figure 12 shows the result of interaction of DNMT1 (4WXX) with Rutaecarpine and the delta G value was -6.77 kcal/mol which was greater negative value than -5 and the fullfitness score was -5486.38 kcal/mol.

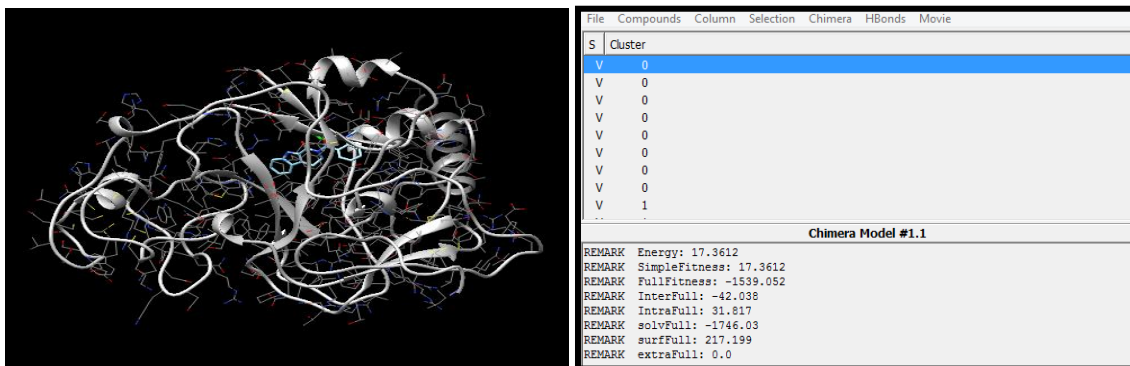


Fig 13: Visualization of interaction of EHMT2 (3RJW) with Rhetsinine through UCSF Chimera

Result obtained from figure 13 shows that after interaction of EHMT2 (3RJW) with Rhetsinine, delta G obtained was -7.46 kcal/mol which was greater negative value than -5 and the fullfitness score obtained was -1539.05 kcal/mol.

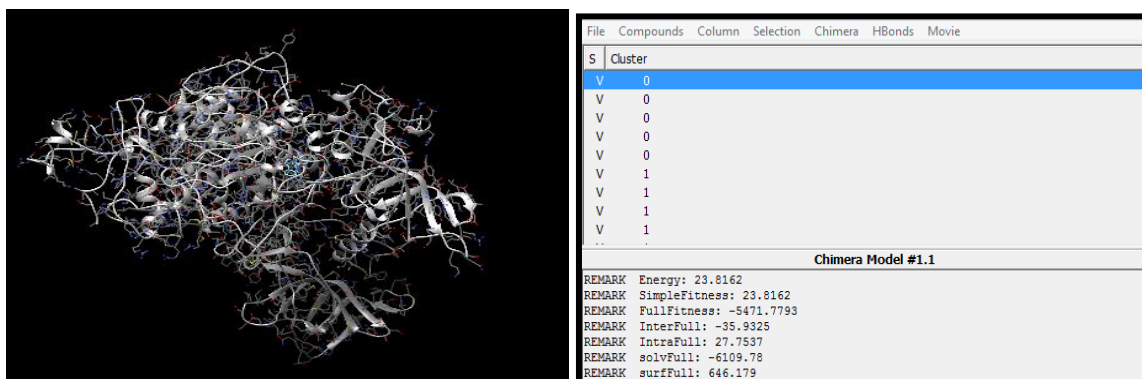


Fig 14 : Visualization of interaction of DNMT1 (4WXX) with Rhetsinine through UCSF Chimera

Figure 14 shows the interaction result of DNMT1 (4WXX) with Rhetsinine, obtained delta G was -7 kcal/mol which was greater negative value than -5 and the fullfitness score was -5471.78 kcal/mol.

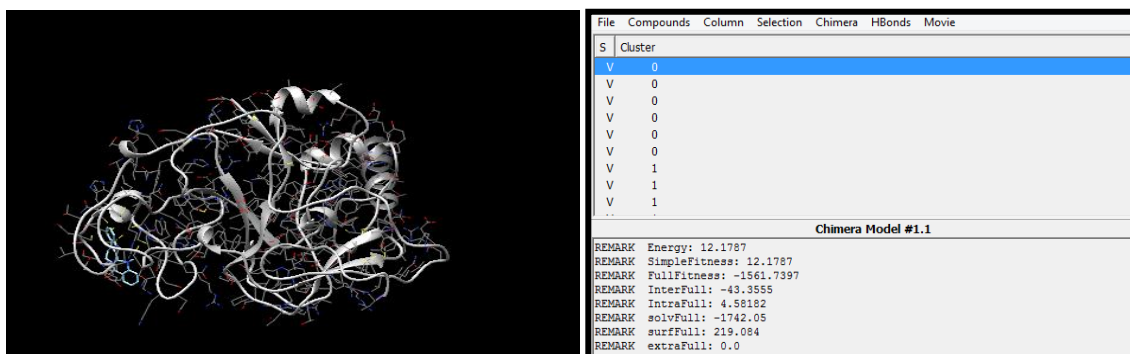


Fig 15 : Visualization of interaction of EHMT2 (3RJW) with Evodiamine through UCSF Chimera

Figure 15 shows the result after interaction of EHMT2 (3RJW) with Evodiamine having delta G -7.30 kcal/mol which was greater negative value than -5 and the fullfitness score was -1561.74 kcal/mol.

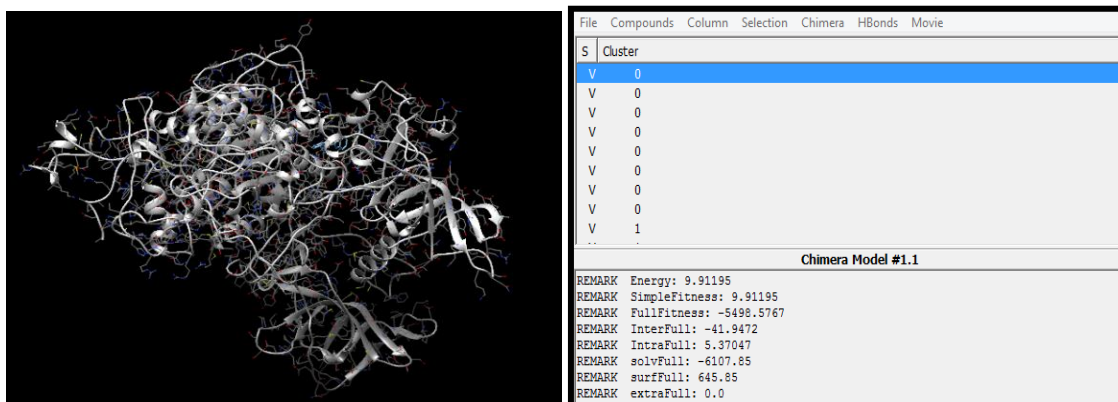


Fig 16 : Visualization of interaction of DNMT1 (4WXX) with Evodiamine through UCSF Chimera

Figure 16 shows the interaction result of DNMT1 (4WXX) with Evodiamine, obtained delta G was -7.47 kcal/mol which was greater negative value than -5 and the fullfitness score was -5498.58 kcal/mol.

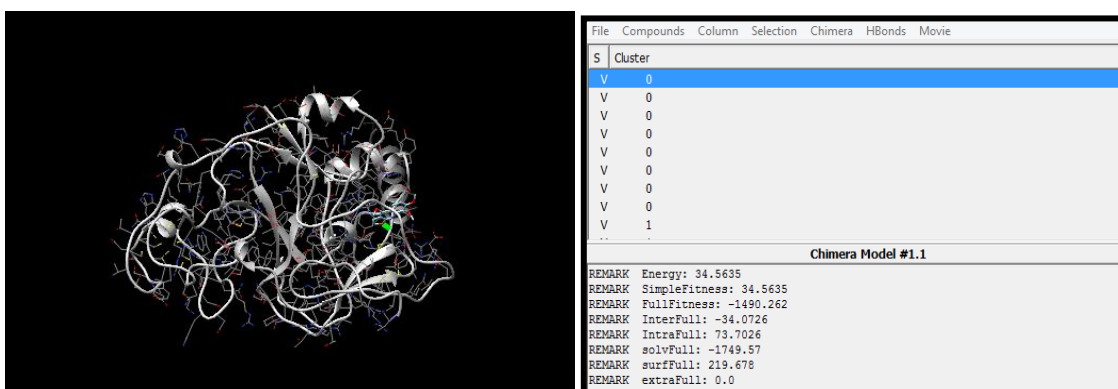


Fig 17 : Visualization of interaction of EHMT2 (3RJW) with Skimmianine through UCSF Chimera

The figure 17 shows the interaction result of EHMT2 (3RJW) with Skimmianine and the delta G value obtained was -7.06 kcal/mol which was greater negative value than -5 and the fullfitness score was -1490.26 kcal/mol.

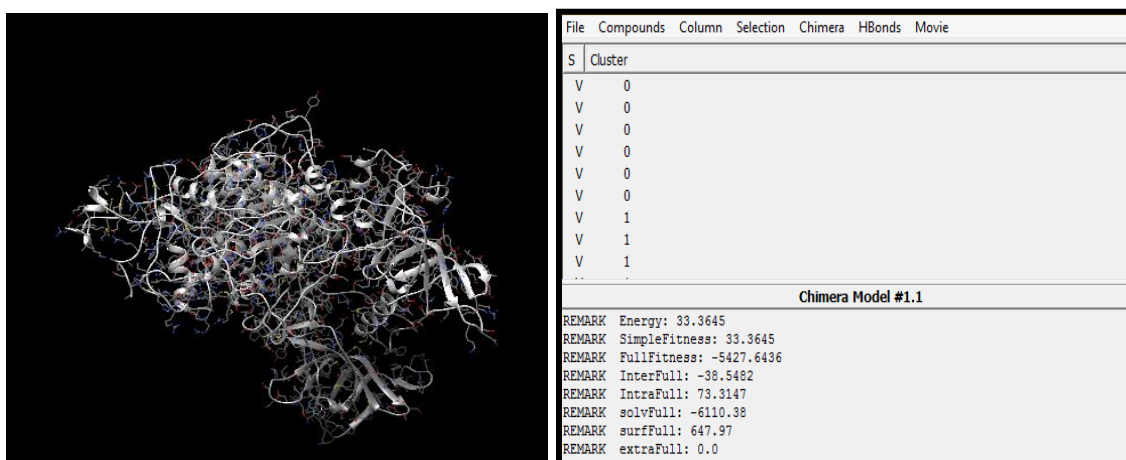


Fig 18 : Visualization of interaction of DNMT1 (4WXX) with Skimmianine through UCSF Chimera

Figure 18 shows the result of interaction DNMT1 (4WXX) with Skimmianine and the delta G value was -7.33kcal/mol which was greater negative value than -5 and fullfitness score was -5427.64 kcal/mol.

Table 2 : Interaction of EHMT2 and DNMT1 with molecular compound

EHMT2 PDB ID	Binding Energy (deltaG) (kcal/mol)[EHMT2]	Molecular Compound	DNMT1 PDB ID	Binding Energy (deltaG) (kcal/mol)[DNMT1]
3RJW	-7.60	Rutacarpine	4WXX	-6.77
3RJW	-7.46	Rhetsinine	4WXX	-7
3RJW	-7.30	Evodiamine	4WXX	-7.47
3RJW	-7.06	Skimmianine	4WXX	-7.33

The delta G values obtained shows the binding energy of molecular compounds. The best compound which interacts with protein EHMT2 was Rutacarpine and Rhetsinine having binding energy -7.60kcal /mol and -7.46 kcal/mol respectively. Moreover, the interaction of compounds were also good with protein DNMT1 which was greater negative value than -5.

F. ADME (Absorption, Distribution, Metabolism and Excretion) analysis of molecules by SwissADME Tool

The ADME (absorption, distribution, metabolism and excretion) properties of molecules were calculated using online tool SwissADME. Canonical smiles of ligands were taken from Pubchem database and pasted in SwissADME analysis box and the result was obtained after clicking on run, shown in Tables. Ligands following Lipinski's Rule of Five (RO5) which includes molecular weight lower than 500 g/mol, TPSA (Topological Polar Surface Area) which indicates polarity having value between 20 and 140Å² is considered good (Lipinski *et al.*, 1997).

Table 3: Physiochemical properties of ligands

Ligands	Formula	Mol.Wt (g/mol)	Heavy atoms	H-bond acceptors	H-bond donors	MR	TPSA (Å ²)
Rutacarpine	C18H13N3O	287.32	22	2	1	87.41	50.68
Rhetsinine	C19H17N3O2	319.36	24	2	2	97.18	65.20
Evodiamine	C19H17N3O	303.36	23	1	1	97.67	39.34
Skimmianine	C14H13NO4	259.26	19	5	0	70.99	53.72

Table 3 shows the analysis of physiochemical properties of molecules having molecular weight 287.32g/mol, 319.36g/mol, 303.36g/mol and 259.26g/mol respectively which were less than 500g/mol, acceptable. Hydrogen bond acceptors and hydrogen bond donors of molecules were 2, 2, 1, 5 and 1, 2, 1 and 0 respectively. The value of Molecular refractivity (MR) obtained were 87.41, 97.18, 97.67 and 70.99 which was between 40 and 130, were acceptable. TPSA (Topological polar surface area) of molecules obtained were 50.68Å², 65.20Å², 39.34Å² and 53.72Å² respectively which was less than 140Å² and was acceptable.

Table 4: Lipophilicity of ligands

Ligands	Log P _{o/w} (iLOGP)	Log P _{o/w} XLOGP3	Log P _{o/w} (WLOGP)	Log P _{o/w} (MLOGP)	LogP _{o/w} (SILICOS-IT)	LogP _{o/w} (Consensus Log P _{o/w})
Rutacarpine	2.51	3.03	3.10	2.75	3.71	3.02
Rhetsinine	1.77	3.85	2.48	2.66	3.20	2.79
Evodiamine	2.25	3.10	2.23	3.16	2.79	2.71
Skimmianine	2.78	2.84	3.01	1.09	2.90	2.52

Table 4 shows the lipophilicity of ligands after SwissADME analysis, LogP_{o/w} (Consensus Log P_{o/w}) obtained for Rutacarpine, Rhetsinine, Evodiamine and Skimmianine were 3.02, 2.79, 2.71 and 2.52 respectively which was less than five and was acceptable.

Table 5: Drug likeliness of ligands

Ligands	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Rutacarpine	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes; 0 Violation	Yes;0 Violation	0.55
Rhetsinine	Yes	Yes	Yes	Yes	Yes	0.55
Evodiamine	Yes	Yes	Yes	Yes	Yes	0.55
Skimmianine	Yes	Yes	Yes	Yes	Yes	0.55

Table 5 shows the drug likeness of molecules- Rutaecarpine, Rhetsinine, Evodiamine and Skimmianine which were found to obey all the computational filters of SwissADME- Lipinski, Ghose, Veber, Egan and Muegge with 0 violation. The bioavailability score of all the molecules was 0.55 which was acceptable.

SwissADME is a online tool used to calculate the ADMET properties of molecule. ADMET analysis involves the calculation of absorption, distribution, metabolism, toxicity and excretion of molecule (Daina *et al.*, 2017). The compounds following Rule of Five (RO5) - calculated value of logP (clogP) i.e lipophilicity less than or equal to five, molecular weight less than or equal to 500g/mol, hydrogen bond donor less than or equal to five and hydrogen bond less than equal to ten including polar surface area (PSA) less than 140 (Å²) is considered good to be used as a drug (Lipinski *et al.*, 1997). Also, SwissADME contain computational filters- Ghose, Egan, Veber and Muegee which gives the quantitative estimation of molecules with respect to physiochemical properties and also discriminate between drug likeness and non drug likeness of molecule. The molecules obeying these filters is considered good to behave as a drug (Ndombera *et al.*, 2019). The present study includes the SwissADME analysis of four compounds- Rutaecarpine, Rhetsinine, Evodiamine and Skimmianine. The results concludes that all four compounds were having values in acceptable range along with bioavailability score 0.55 to be used as a drug.

IV. CONCLUSIONS

Type 2 Diabetic Retinopathy, one of the major causes of blindness worldwide results in retinal detachment leading to loss of vision. BDNF, which belongs to the neurotrophin family of growth factors is crucial for the growth, differentiation and survival of retina. Retinal neurons and glial cells produces BDNF which helps in the protection of neuronal cells. BDNF dysregulation was found to be associated with neurodegeneration resulting in angiogenesis in PDR (Proliferative diabetic retinopathy). However, the exact mechanism of Type 2 Diabetic Retinopathy pathogenesis still remains elusive. As per the available literature, it has been revealed that H3K9 methylation is a repressive mark associated with the suppression of gene expression. Previous studies revealed number of genes in lymphocytes in diabetic condition were having increased H3K9 dimethylation levels linked to immune and inflammatory pathways associated with type 1 diabetes. (Miao *et al.*, 2008). The effect of G9A inhibitor reduces mRNA expression of G9A leading to significant increase in the BDNF expression by reducing H3K9me2 in schizophrenia. The present study focused to find the inhibitor of EHMT2 in Type 2 Diabetic Retinopathy which includes *in-silico* analysis to study the interaction pattern of molecular compounds with EHMT2. The docking was done using SwissDock and was visualized through UCSF Chimera. The ADMET (Absorption, Distribution, Metabolism, Excretion & Toxicity) properties of compounds were analyzed by SwissADME. The result showed interaction of EHMT2 with Rutaecarpine and Rhetsinine after docking having delta G (binding energy) -7.60 kcal/mol and -7.46 kcal/mol respectively which was greater negative value than -5 as well as follows Lipinski rule of Five was best among all to be used as drug. Moreover, docking result of Dnmt1 with Rutaecarpine and Rhetsinine were also good-6.77 kcal/mol and -7 kcal/mol, greater negative value than -5.

The study concludes that these compounds may act as dual inhibitor of EHMT2 and DNMT1 thereby regulating expression of BDNF by promoting cell differentiation and survival and would act as an therapeutic approach in Type 2 Diabetic Retinopathy.

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