



An Insilico approach in Mitigation of the Diabetic complications caused by BDNF V66M variant

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Abstract:

Brain Derived Neurotropic Factor (BDNF) is a protein well recognized for its involvement in the growth and development of Neuronal system and synaptic plasticity. It plays a key role in Energy metabolism and Food intake. Studies were carried on the involvement of BDNF in the treatment of Diabetes and Obesity. The present study is on the specific variant V66M of BDNF protein and its influence on the pathways which are connected to the insulin signaling. This mutation leads to the decrease in the secretion of the mature BDNF. This will affect the activation of the TrkB receptor which is involved in the PI3k pathway in turn affecting the Insulin signaling. The complete analysis of the V66M variant was done. Through Computer aided drug designing approach, the chemical compound 1-(((2-((5-cyanopyridin-2-yl)amino)ethyl)amino)acetyl)-2-cyano-(s)-pyrrolidine was found to be the potent drug candidate which can mitigate the diabetic effect caused due to the mutation.

Keywords: Brain Derived Neurotropic Factor, insulin signaling, Computer aided drug designing

Introduction:

Brain Derived Neurotropic Factor (BDNF) is a protein of Neurotrophin family, a group of structurally related growth factors. The involvement of BDNF in insulin signaling can be connected with its regulation on TrkB receptor activation. Binding of BDNF with the TrkB Receptor plays a prominent role in intracellular signaling for neuronal survival, morphogenesis and plasticity; TrkB activation in turn elicits various Intracellular signaling pathways like mitogen activated protein kinase/ extracellular signal protein kinase (MAPK/ERK), Phospholipase C α (PLC α) and phosphoinositide 3- Kinase (PI3K) pathway^[1]. The PI3K has a major role in the insulin function via; activation of the Akt/ Pk β and the PkC ζ cascades^[2]. Therefore BDNF plays a prominent role in Glucose metabolism. The importance of this protein is further emphasized by suggesting its role in diabetes where patients with

diabetes have found decreased concentration of BDNF. Both central and peripherally administered BDNF lowers blood glucose and increases energy expenditure in animal models of Type 2 Diabetes^[3].

The variant at 66th position of BDNF has substitution mutation which changes the amino acid Valine to Methionine. This polymorphism affects the intracellular packaging of proBDNF and secretion of mature BDNF^[4]. This leads to significant decrease in the BDNF Secretion. The mutation creates the disturbance in the interaction of ProBDNF with the sorting proteins thus leading to misorting of BDNF into the constitutive secretory pathway instead of regulated secretory pathway^[5]. This mechanism will result in the decrease in concentration of Mature BDNF which affects the TrkB receptor activated pathways thus affecting the insulin signaling leading to Type 2 Diabetes. The current work aims at study the molecular details of the mutation and finding a potent drug candidate for mitigating the effect of this mutation in diabetes using an Insilico approach.

Materials and Methods:

The BDNF sequence was retrieved from Uniprot^[6], the exclusive database of proteins. The sequence was analyzed for finding the specific features in the point of the mutational site. The influence of this mutation at molecular level was analyzed for finding the stability and disorderness of the protein due to mutation using the tools I Mutant^[7] and RoNN^[8] The protein analysis of BDNF was done using various tools for the prediction of ligand binding site, functional region of a protein, motif regions, signal peptides, protein patterns, stability changes upon mutation, domain analysis and the determination of primary, secondary, tertiary structure.

The structure of the protein was further analyzed using What If Server^[9] for its molecular structure property where the change in Interatomic distances, Bond angles, Residue accessibility, Torsion angles; Hydrogen bond networking was checked after the mutation. The

mutated structure was designed using the tool I Tasser^[10] by Ab Initio Approach. The suitable drug candidates were screened based on their efficiency towards the non toxic property, drug likeliness and ambiguity studies. Docking studies were performed using the software Argus lab^[11] with the selected drugs at the targeted site 66M. The potent drug was selected based on best binding affinity.

Results and Discussion:

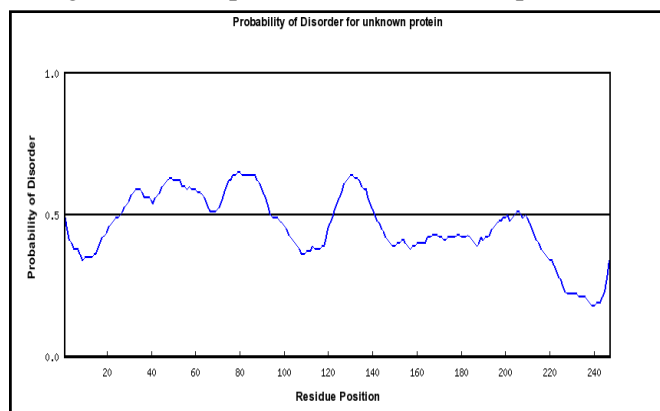
Mutation Analysis Using I Mutant

Table 1: I Mutant Results showing the changes in the stability after mutation

Position	W T	NE W	Stability	RI	p H	T
66	V	M	Decrease	5	7.0	25

Disorder Prediction Using RONN

Fig 1: Disorder prediction of the BDNF protein



Disordered regions:
27 - 93, 123 - 140, 205 - 206
Disorder Probability of Mutational site V – 0.52

Analysis of Structural changes in a Mutated BDNF Protein

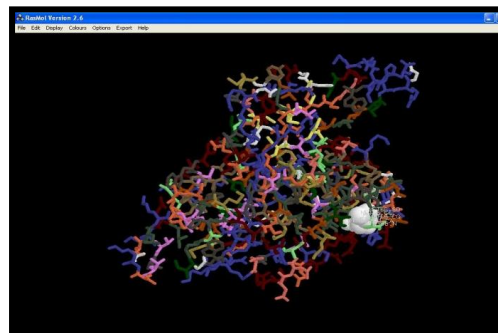
Table 2: Changes in the Molecular Structure of BDNF Protein after Mutation.

Sl.No	What if server	Normal BDNF protein	Mutated BDNF protein
1	Torsion angles	66 VAL (66) A Phi 177.6 Psi 174.5 Omega 176.4 Chi1-5	66 MET (66) A -65.2 phi -42.6 psi -176.0 omega -69.0 chi1 -178.5 chi 68.0 chi5
2	Bond angles	66 VAL (66) A 108.2663 N-CA-C 117.1704 CA-C-N 119.4481 CA-C-O 123.3771 O-C-N	66MET(66) A 113.6915N-CA-C 120.4090 CA-C-N 119.0724 CA-C-O 120.4706 O-C-N
3	Short atomic Distances	Inter CG1 <-->67 ILE A 0.24 2.76 INTRA	O <-->70 LEU A 0.14 2.56 INTRA
4	Optimal Hydrogen Bonding Network	N -> 63 PHE A	N -> 62 THR A

The comparative study of Normal and Mutated Structures of BDNF protein was done which shows the deviations from the Native protein in terms of Optimal hydrogen bonding network, Short inter atomic distances, Bond angles, and Torsion angles. These deviations might affect the structural and functional properties of the protein.

Mutated Structure of BDNF

Fig 2: Mutated structure designing in I Tasser and visualization in Rasmol highlighting the mutated site V66M



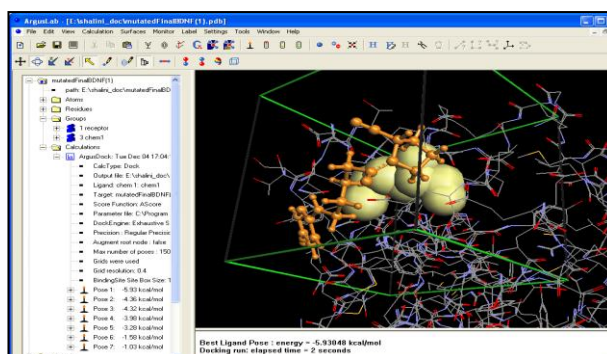
Drug Library

Table 3: Drugs selected after screening analysis:

S.NO	Ligand Name	Chemical ID	Toxicity Analysis	Docking Results
1.	1-(((2-((5-cyanopyridin-2-yl)amino)ethyl)amino)acetyl)-2-cyano-(S)-pyrrolidine	CID 9796290	MW : 298.2 Drs : 2 Ars : 7 FB : 9 RB : 14 #R : 2 RL : 6 C : 15 nC : 7 C/nC : 0.466667 #Chrg : 0 Chrg : 0 LogP : -0.100000 PSA : 104.840000	-5.93048kcal/mol
2.	N-alanylproline-O-(4-nitrobenzoyl)hydroxylamine	CID 137324	MW : 350.2 Drs : 2 Ars : 10 FB : 7 RB : 17 #R : 2 RL : 6 C : 15 nC : 10 C/nC : 0.666667 #Chrg : 2 Chrg : 0 LogP : 0.900000 PSA : 141.710000	-5.22663kcal/mol
3.	Saxagliptin	CID 11243969	MW : 315.2 Drs : 2 Ars : 5 FB : 4 RB : 21 #R : 5 RL : 6 C : 18 nC : 5 C/nC : 0.277778 #Chrg : 0 Chrg : 0 LogP : 0.730000 PSA : 90.350000	No Acceptable ligand poses
4.	1-(((3-hydroxy-1-adamantyl)amino)acetyl)-2-cyanopyrrolidine	CID24848920	MW : 303.2 Drs : 2 Ars : 5 FB : 5 RB : 19 #R : 4 RL : 6 C : 17 nC : 5 C/nC : 0.294118 #Chrg : 0 Chrg : 0 LogP : 0.790000 PSA : 76.360000	No Acceptable ligand poses
5.	Vildagliptin	CID 6918537	MW : 303.2 Drs : 2 Ars : 5 FB : 5 RB : 19 #R : 4 RL : 6 C : 17 nC : 5 C/nC : 0.294118 #Chrg : 0 Chrg : 0 LogP : 0.790000 PSA : 76.360000	No Acceptable ligand poses
6.	Diprotin A	CID 94701	MW : 341.2 Drs : 3 Ars : 7 FB : 9 RB : 9 #R : 1 RL : 5 C : 17 nC : 7 C/nC : 0.411765 #Chrg : 0 Chrg : 0 LogP : 1.010000 PSA : 112.730000	No Acceptable ligand poses
7.	Topiramate	CID 5284627	MW : 339.2 Drs : 1 Ars : 9 FB : 3 RB : 16 #R : 3 RL : 6 C : 12 nC : 10 C/nC : 0.833333 #Chrg : 0 Chrg : 0 LogP : -0.830000 PSA : 123.920000	No Acceptable ligand poses
8.	Chlorpropamide	CID 2727	MW : 276.6 Drs : 2 Ars : 5 FB : 4 RB : 11 #R : 1 RL : 6 C : 10 nC : 7 C/nC : 0.700000 #Chrg : 0 Chrg : 0 LogP : 1.800000 PSA : 83.650000	-5.40617kcal/mol

Docking Results:

Fig 3: Docking results of 1-(((2-((5-cyanopyridin-2-yl)amino)ethyl)amino)acetyl)-2-cyano-(S)-pyrrolidine with the targeted site 66M of BDNF protein showing the best binding energy of -5.93048kcal/mol



The Valine at 66th Position lies in the propeptide region of BDNF . The V66M polymorphism thus impairs activity-dependent secretion of BDNF by interfering with intracellular sorting of the protein⁽¹²⁾. The significance of the mutational site was analyzed. Changes in the stability of the structure and disorderness were checked and were found that the mutation creates instability in the structure. The disorder probability of the structure was found to be 0.52 which indicates the disorderness in the residue. Through the molecular dynamics study , the changes in the torsion angles, bond angles, interatomic distances and hydrogen bond networking was observed indicating the influence of this changes on the structure and functional properties of the protein. The mutation may affect the functional and binding property of BDNF with the sorting proteins which effect the intracellular trafficking of ProBDNF. Thus effecting the secretion of Mature BDNF. The chemicals which can be used as diabetic drugs were screened for drug property so as to block the misorting of the protein into the constitutive pathway rather than the regular secretory pathway thus mitigating the effect caused due to the mutation. The Drug screening was performed for toxicity studies, drug likeliness and ambiguity studies. 67 chemicals were screened for drug likeliness from different chemical databases and after performing the screening analysis finally 8 chemical compounds were found to be the efficient drug candidates. The chemical 1-(((2-((5-cyanopyridin-2-yl)amino)ethyl)amino)acetyl)-2-cyano-(S)-pyrrolidine was found to be the potent drug based on its best binding affinity.

Conclusion:

The work lays emphasis on the significance of the mutation V66M on molecular level. Drug Screening was performed to find the chemical compounds bearing the best drug property. By performing Docking studies , the chemical 1-(((2-((5-cyanopyridin-2-yl)amino)ethyl)amino)acetyl)-2-cyano-(S)-pyrrolidine was found to be the potent drug based on its best binding affinity which can be used in the treatment of Type 2 Diabetes.

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