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STRUCTURAL CHARACTERIZATION AND MOLECULAR DYNAMICS OF PROTEIN KINASE C EPSILON IMPLICATED IN BIPOLAR DISORDER.

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ABSTRACT

Bipolar disorder (BPD) is a condition in which people go back and forth between periods of a very good or irritable mood and depression. Recently accumulated evidence indicates that an alteration in PKC activity plays a significant role in pathophysiology of BPD. Protein kinase C (PKC) is a group of calcium and phospholipid-dependent enzymes, which plays a pivotal role in cell signaling systems. Inhibition of PKC plays an important role in neuroprotection against BPD. Hence to design and study the inhibitors for BPD we modeled 3D structure of PKC using Accelrys Discovery studio software package and five models were generated. The model with best PDF total energy (-9291.1621) and best Dope score (-47210.160156) was selected and analyzed for its validation using Ramachandran plot and for prediction of active site for ligand building. This model is submitted to PMDB and can be downloaded for docking studies using accession number PM0078502 to design potent inhibitors of PKC.

KEYWORDS: Bipolar disorder, Protein Kinase C, Homology modeling, Active site prediction.

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INTRODUCTION

Brain disorder is a psychological pattern, potentially reflected in behavior that is associated with distress. The disorder is defined by how a person feels, acts, or thinks. It is associated with particular functions of the brain or rest of the nervous system. Bipolar disorder (BPD) is one of the major depressive disorders affecting most of the population. According to the World Health Organization (WHO),[1] over a third of people in most countries report problems at some time in their life which meet criteria for diagnosis of one or more of the common types of mental disorder. At least 10 crore people suffer from mental illness in India[2]. The brain systems receiving the greatest attention in neurobiological studies of BPD have been the monoaminergic neurotransmitter systems, i.e., the serotonergic, noradrenergic, and dopaminergic neurotransmitter systems[3]. Despite evidence showing that many of these systems or circuits are likely to be involved, no obvious degeneration or complete dysfunction of any single neurotransmitter system has been identified. In this regard, the biological underpinnings of BPD appear to differ from classic neurodegenerative disorders such as Parkinson's and Alzheimer's disease. However, as we discuss here, many researchers believe that BPD arises from modulation of synaptic and neural plasticity in critical circuits mediating affective and cognitive function. The two main neuroanatomic circuits involved in mood regulation are the limbic-thalamic-cortical circuit and the limbic-striatal-pallidal-cortical circuit and dysfunction in any of these circuits may cause BPD. The effectiveness of the cell functioning under the modification and control of neurotransmitters underlies the patho-aetiology of mood disorders. Dopamine is particularly interesting because when it is decreased, in illnesses such as Parkinson's disease, is associated with low mood and loss of ability to enjoy things [4]. The dopamine system, therefore, has the capability for producing low and high emotions, the range of symptoms that one can see in the depression and in the manic phase of bipolar disorder. Various drugs like Olanzapine belongs to the class of newer atypical antipsychotic drugs, bearing thieno [2,3-b][1,5]benzodiazepine moiety. These atypical antipsychotic drugs shows affinity towards the blockade of serotonin 5HT2 and dopamine D2 receptors, and used to treat schizophrenia and bipolar disorders.[5]

The main brain areas involved in bipolar disorder include the frontal and temporal lobes of the forebrain, the prefrontal cortex, the basal ganglia and parts of the limbic system.[5] Investigation into the cAMP/protein kinase C (PKC) system found that the concentrations of the PKA regulatory subunits in the cytoplasm are significantly lower in cells of the frontal, temporal, occipital and parietal cortex, cerebellum and thalamus of bipolar disorder patients. The family of PKC isozymes is highly enriched in brain, and plays a major role in regulating both pre and postsynaptic aspects of neurotransmission.[6] PKC isozymes are major intracellular mediators of signals generated on external stimulation of cells via a variety of neurotransmitter receptor subtypes, which induce the hydrolysis of membrane phospholipids. PKC is known to be involved in signal transduction associated with the control of brain functions, such as ion channel regulation, receptor modulation, neurotransmitters release, synaptic potentiation/depression, and neuronal survival. PKC is now known to exist as a family of closely related subspecies, has a heterogeneous distribution in brain (with particularly high levels in presynaptic nerve terminals), and plays a major role in the regulation of neuronal excitability, neurotransmitter release and long-term alterations in gene expression and plasticity. Accumulating evidence from various laboratories has identified the family of protein kinase C (PKC) isozymes as a shared target in

the brain for treatment of BPD. Protein kinase C (PKC) is a group of calcium and phospholipid-dependent enzymes, which plays a pivotal role in cell signaling systems. Recently many evidence indicates that alterations in PKC activity play a significant role in the pathophysiology of bipolar disorder.[7] Hence PKC remains as a potential target for drug delivery and in this study we attempt to construct a model structure for PKC by taking the crystal structure of 1GMI_A chain and 3PFQ_A as templates. 3D-profiles verification, Procheck analysis and aligning structures to calculate RMSD were performed as validation methods. The predicted 3D structure of PKC can be used to carry out the binding site and docking studies so as to alleviate the symptoms of the Bipolar disorder. The overall study design is depicted in the figure 1.

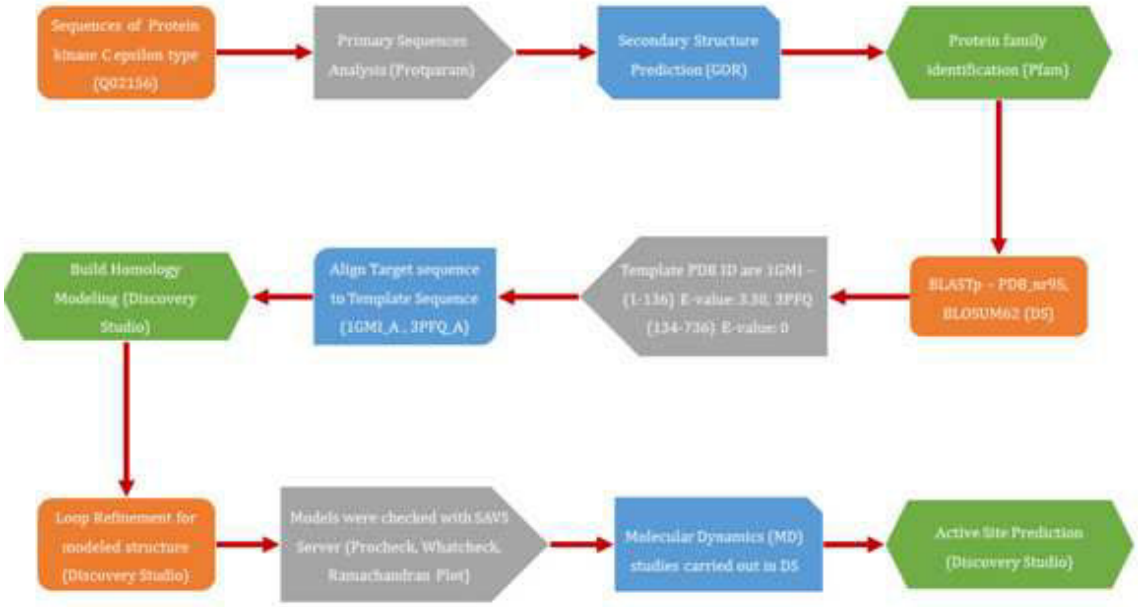

Figure 1
Schematic representation of the steps used for homology modeling, Molecular Dynamics analysis and active site prediction

MATERIALS AND METHODS

Sequence retrieval and analysis

Protein kinase C epsilon is implicated recently as target protein in Bipolar disorder. The amino acid sequence of PKC epsilon type (UniprotId: Q02156) was obtained from Uniprot_KB. The target protein is made up of 737 amino acid residues, which belongs to the family of STKc_nPKC_epsilon. The physicochemical properties of the protein were determined using CLC genomic workbench by using protein report protocol[8]. The characteristic values of the protein are as follows: Molecular weight of 83.673 kDa, isoelectric point of 7.01, aliphatic index is 78.684, 345 hydrophobic residues and with 172 Hydrophilic residues. Secondary structure prediction analysis was carried out by using GOR[9] and the structural details were found to contain Alpha helix formed by 228 aminoacids (aa), extended strand contributed by 135 aminoacids (aa) and random coils by 374 aminoacids. The BLAST program against Protein Data Bank (PDB) available at NCBI was used to identify a template structure for homology modeling of human PKC epsilon type. The crystal structure of human protein kinase C epsilon type obtained from PDB

database[10],1GMI_A showed 98% sequence identity and 3PFQ_A showed 45% sequence identity with target protein. The query coverage for 1GMI_A and 3PFQ_A extends over a range of 1-136 and 134-736 respectively. The first requirement in the construction of human PKC epsilon type structural model is the multiple sequence alignment between these templates and the target sequences. The sequence alignment is based on identifying structurally conserved regions (SCR) common to the template and target,[11] The multiple sequence alignment was performed in the Discovery Studio 3.5(DS 3.5) sequence analysis protocol called "Align multiple sequences". The query sequence and two template sequences were given as input in multiple sequence alignment protocol[12].

Build homology modelling and structural refinement

The protein homology model was constructed using "Build Homology Models" protocol[13] (Modeler) in DS 3.5. The output of "Align multiple sequences" was given as input to build homology modelling protocol. Ten models were generated, among which five were loop refined. Models were obtained by setting the parameter "True" for Refine loops in advanced settings of "Build Homology Models" protocol[14]. After the energy refinement the newly built model was refined with all its conserved regions, loop regions or structurally variable regions in modelled structure[15].The protein health report was found out for each modelled protein on the 3D window by selecting the "validate protein structure" sub-panel available in the main 'Tools' panel. The properties such as bond angle, bond length deviation, main chain residue conformation corrections and conformation corrections for each modelled protein structure were obtained by using the option "check structure" from the "Tools" panel.

Model validation and Molecular Dynamics

Model validation was carried out using SAVS server**.** To verify the protein model, the coordinates of the protein model were submitted to PROCHECK[16]. The stereo chemical quality of the protein structures was examined by Ramachandran plot using the PROCHECK program, the number of residues that are in the allowed or disallowed regions of Ramachandran plot[17]determines the quality of the model, which indicates the region of possible angel formation of (phi) Φ and (psi) Ψ angles and distribution of backbone conformational angles for each residue of the refined structure. Modelled structure was submitted to Protein Model Database, a repository for 3D protein models obtained by structure prediction methods. Potential energy of the modelled protein was analyzed before and after minimization by using calculate energy and minimization protocol respectively in DS 3.5. Parameters of minimization protocols were smart minimizer algorithm with 200 maximum steps, 0.1 RMS gradient value and 0.0 energy changes value and electrostatic based on spherical cut off. The Standard dynamics cascade protocol performs the following steps: minimization with steepest descent method, minimization with conjugate gradient, dynamics with heating, equilibration dynamics, and production dynamics. The minimization protocol minimizes the energy of a structure through geometry optimization. For the simulation cascade, following parameter were used: steepest descents minimization (500steps, RMS gradient 0.1) in first minimization step and in second steepest Descents minimization (500 steps, RMS gradient 0.0001), heating (2000 steps , initial temperature 50K, final temperature 300K), equilibration (120 ps, 1fs time step, coordinates saved every 1000 steps) and Production (120 ps, 1fs time step, 300 K, NVT ensemble, non-bond cutoff 14A, switching function applied between 10 and 12A, coordinates saved every 1000 steps)[18].

Active site prediction

Identification of active site regions were carried out using DS 3.5 based on receptor cavity method (by eraser algorithm)[19]. The physical properties of the active site region were viz.,

area volume: 743, XYZ coordinates -54.925 Å, 20.689Å and -16.024Å.

RESULTS AND DISCUSSION

According to Chen *et al*., PKC remains to be one of the most interesting target for mood disorders, especially Bipolar disorder and PKC can be used as a long term drug target for Bipolar disorder.[20] Indeed, accumulating evidence from Manji *et al*., has clearly demonstrated that targeting PKC signaling pathways play a significant impact in the treatment of Bipolar disorder. A major barrier for *In silico* targeting of PKC is the unavailability of 3D structure of PKC and hence the goal of our work is to create a best 3D protein structure for drug development. Similar work was done by Bagchi *et al*., who modeled RGS4 protein for Schizophrenia. [21] Choosing the correct target remains a challenging task since wrong target lead to various side effects like iatrogenesis. [23] The amino acid sequence of our desired protein was obtained from UniProt and the physicochemical properties of the protein were studied. The search for best template for modeling of human PKC epsilon type was carried out in the PDB using BLAST which searches for similar sequences protein structure and C2 domain from novel protein kinase C epsilon (PDB id. 1GMI) showed 98% identity and Protein kinase C β2 (PDB id. 3PFQ) showed 45% identity. They were found to have the best sequence similarity, identity and query coverage with human protein kinase C epsilon. The multiple sequence alignment of template 1GMI_A chain and 3PFQ_A chain sequences with human PKC epsilon type is as shown in (Figure 2).

The primary model of human protein kinase c epsilon type was created using Modeler in DS 3.5. The initial model was energy refined during generation by selecting the option "True" in 'refine loops' parameter. The generated model was again subjected to loop refinement in order to refine all the main chain conformations of those residues which occur in the structurally variable regions or loops in modelled structure. The residues with incorrect main chain and side chain conformations were revealed by protein health check report. The model that resulted after loop refinement was validated as having low energy conformation of all other models generated (Figure 3). The modeled proteins before and after loop refinement were validated primarily by the lowest PDF (Probability Density Function energy) and lowest DOPE (Discrete Optimized Protein Energy) score. The loop model scores of the modelled structures were as shown in the (Table 1). The best model named as Untitled1.M0001L001 had a lowest PDF total energy of -9291.162 and lowest DOPE score of -47210 (Figure 4). After initial validation, the modeled protein was verified using 3D profiles verify[22] algorithm available in DS 3.5. The verified score was observed as 165.21 that lie between expected high score of 226.61 and expected low score of 101.974. The graphical representation of 3D profile score is depicted in (Figure 5).Model validation was carried out using SAVS server**.** The percentage of residues in the most favorable regions of Ramachandran plot was 79.5% and of those lying in disallowed region was 1.1% (Figures 6). The volume of protein was assessed using PROVE (PROtein Volume Evaluation), the characteristic protein volume were Z-Score mean of 1.767, Z-Score of RMS-41.012 and remaining values are as shown in the Figure 7. Modelled structure was deposited with the Protein Model Database[23] (PM0078502), which is a repository for 3D protein models obtained by structure prediction methods.

Molecular Dynamic simulation and Active site prediction

Potential energy of the modeled protein[24] (PM0078502) was analyzed before and after minimization by using calculate energy and minimization protocol respectively in DS 3.5 and it was found to be 101265.27565 Kcal/mol and -30972.10032 Kcal/mol respectively. The standard dynamics cascade protocol was performed using DS 3.5 and the results are as shown in Table 2. 56 amino acids were found

in ligand binding pocket, 28 hydrophobic residues were found in the 5Å region of the best ligand binding pocket, which are VAL496, LEU497, PHE502, VAL505, MET506, LEU507, ALA518, VAL519, VAL521, LEU522, LEU565, PHE566, PHE567, VAL568, MET569, VAL572, LEU577, LEU616, LEU618, ILE621, LEU622, LEU623, LEU631, ALA632, PHE634, MET636, CYS637 and CYS651 respectively which are involved in the hydrophobic interaction with ligand compounds (Figure 8).

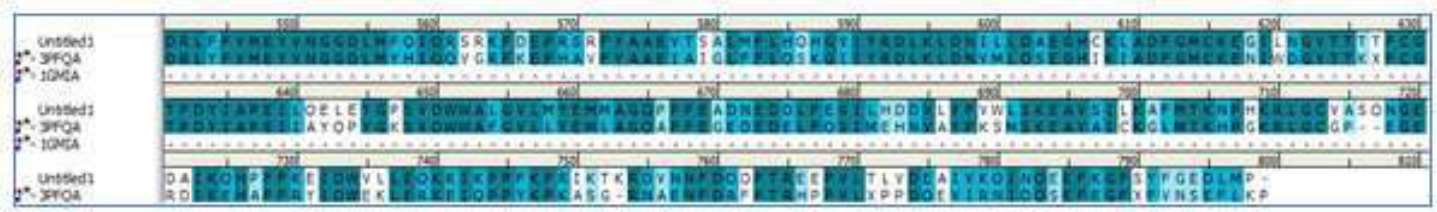

Figure 2

Shows Multiple sequence alignment of human protein kinase c epsilon type with template sequences 1GMI and 3PFQ. Deep green color shows conserved residue in all three sequences.

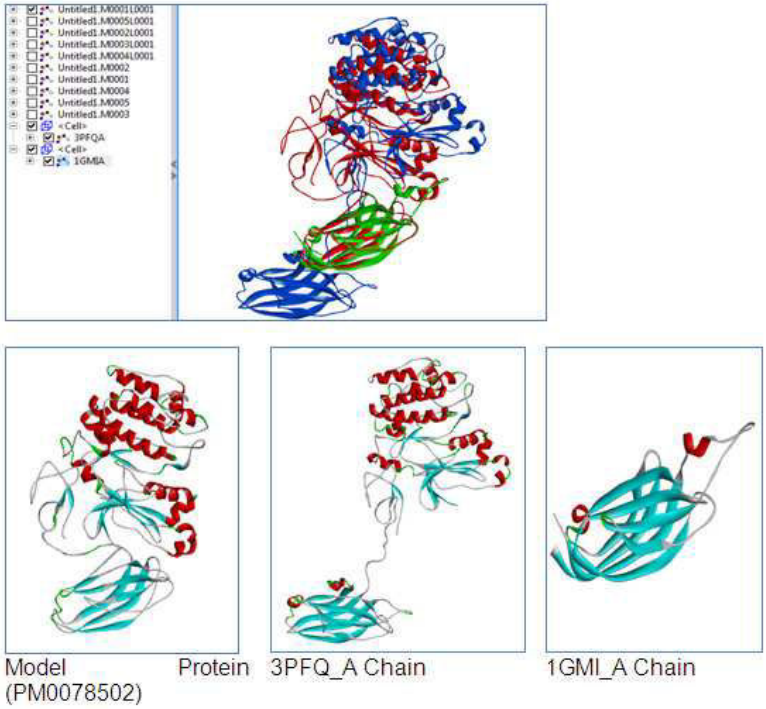

Figure 3

Superimposition of PM0078502 model (Red color) over template ((3PFQ, chain A) (Blue), (1GMI, Chain A) (green)).

Figure 4
Modeled PM0078502 after loop refinement
[Secondary rendering done in Discovery studio3.5]

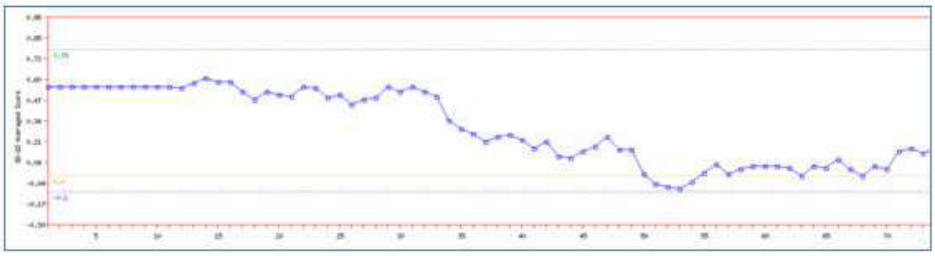

Figure 5
3D profiles verify graph

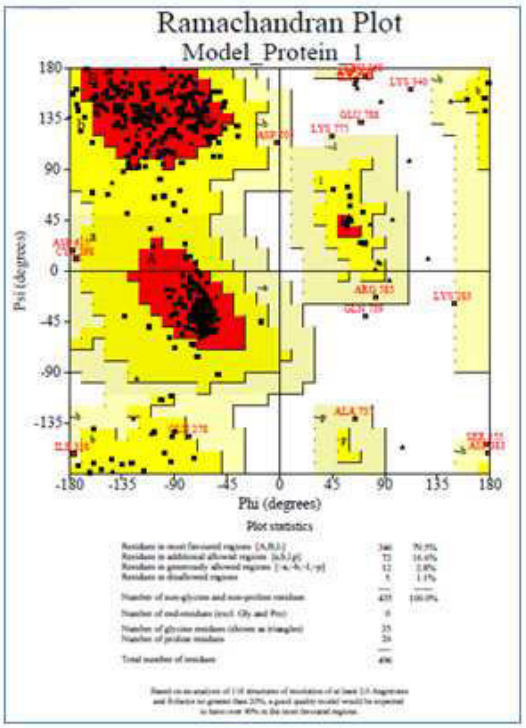

Figure 6
Ramachandran's Map of PM0078502 by PROCHECK program

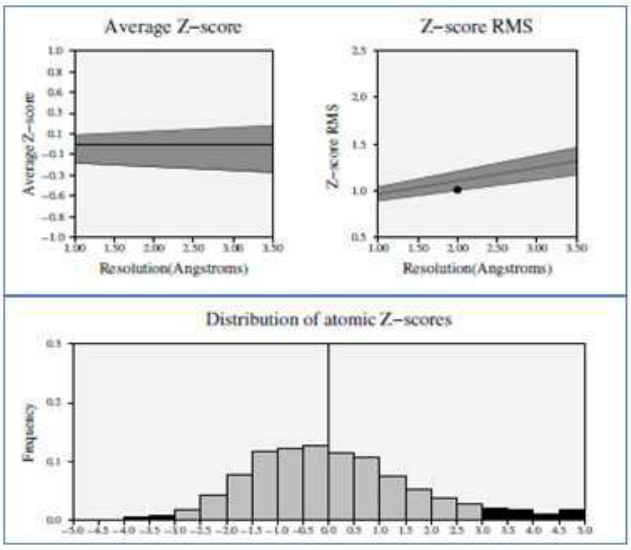

Figure 7
Main chain parameters for the model generated by PROVE

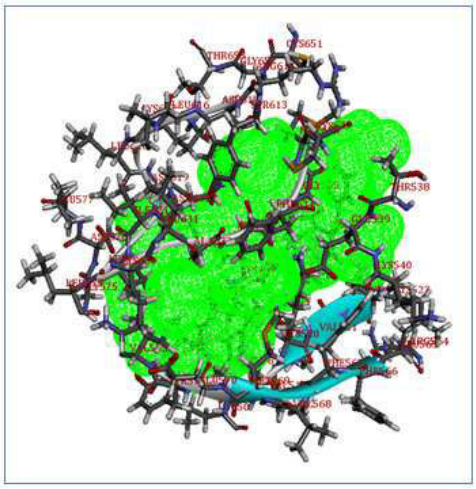

Figure 8
Active site of modeled protein (PM0078502) by Discovery studio 3.5
Note: green color indicates binding area of the active site of modeled protein (PM0078502)

Table 1
Loop model scores of 5 models

Loop Model Scores			
Name	**PDF Total Energy**	**PDF Physical Energy**	**DOPE Score**
Untitled1.M0001L0001	-9291.1621	-9475.14462	-47210.160156
Untitled1.M0005L0001	-9227.2471	-9445.09118	-47011.691406
Untitled1.M0002L0001	-9218.2305	-9520.2505064	-46957.167969
Untitled1.M0003L0001	-9202.7949	-9391.175635	-46731.859375
Untitled1.M0004L0001	-7211.4805	-8335.43058	-45952.937500

Table 2
Result of Standard Dynamics values.

Sl.No	Name	Total energy	Potential energy	Temperature	Van der waals energy	Electro statistic energy
1	Conformation	-19,649.90	-26,774.80	304.804	-2,814.81	-34,214.40
2	Conformation	-19,654.60	-26,897.40	309.849	-2,856.24	-34,310
3	Conformation	-19,659.80	-26,900.10	309.743	-2,832.06	-34,402.20
4	Conformation	-19,665	-26,924.50	310.566	-2,802.64	-34,400.10
5	Conformation	-19,670	-27,057	316.018	-2,826.19	-34,602.40
6	Conformation	-19,677.20	-27,073.30	316.409	-2,828.66	-34,517.60
7	Conformation	-19,682.70	-27,051.90	315.253	-2,870.48	-34,475
8	Conformation	-19,688.90	-27,015.80	313.45	-2,831.80	-34,533
9	Conformation	-19,700.90	-27,081.40	315.741	-2,791.57	-34,802.30
10	Conformation	-19,703.80	-26,967.60	310.746	-2,809.34	-34,730

CONCLUSION

Bipolar disorder is one of the most increasing mood disorders in modern world and it affects most of the people in developing countries. It is evident that PKC plays an important role in signaling systems and alteration in its system plays a major role in pathophysiology of BPD. Hence PKC remains to be promising target to combat BPD. Targeting PKC using drug requires a valid 3D protein model to analyse the binding pattern and interaction with the ligands *in silico.* So we modeled a 3D protein structure which can be a potent target for drug delivery. 1GMI and 3PFQ were chosen as templates for protein modeling and 5 models were generated using Discovery studio and the model with lowest PDF total energy (-9291.1621) and DOPE score -47210.160156 was chosen as the best model. Further model validation studies showed that the modeled protein is a reliable target. *In silico* drug targeting using this model will help in quick screening and identification of a potent drug for Bipolar disorder.

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