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Active Site Prediction and Targeting Bipolar Disorder through Molecular Docking Techniques on Protein Kinase Epsilon

Bioinformatics

KEYWORDS : Bipolar disorder, Protein Kinase C, Protein Modeling Database, Inhibitor, Molecular Docking.

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ABSTRACT

Bipolar disorder (BPD) is a complex genetic disorder in which the core feature is pathological disturbance in mood ranging from extreme elation, or mania, to severe depression usually accompanied by disturbances in thinking and behavior. Recently 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, enriched in brain, where it plays a major role in regulating both pre-and postsynaptic aspects of neurotransmission. Inhibition of PKC plays an important role in neuroprotection against BPD. The modeled protein structure (PM0078502) was retrieved from PMD database and subjected to various bioinfromatics tools. The active site was predicted and to molecular docking studies was performed with ChEMBL database PKC inhibitors. Totally 80 compounds have been screened and after toxicity prediction ten compounds have been selected as candidate drugs for Bipolar Disorder. The best inhibitor which shows good interaction with target was [(3-{3-[4-(1-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1H-indol-1-yl}propyl)sulfanyl] methanimidamide.

1.Introduction

Bipolar is one of the most severely debilitating of all medical illness and affects the lives and functionating of millions worldwide. Patients with BPD generally experience high rates of relapse, chronicity, lingering residual symptoms, cognitive and functional impairment, psychological disability and diminished well being[1]. According to the world Health organization WHO[2],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. Atleast 10 crore people suffer from mental illness in India[3]. 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[4]. 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]. 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 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 retrieved the modeled structure for PKC form PMD Database and carried docking studies with PKC inhibitors.

2. MATERIALS AND METHODS

Target retrieval and Active site prediction

Modeled protein structure was retrieved from PMD Database (PM0078502) for molecular docking studies. The active sites of the protein were predicted using DS 3.5, which is based on the receptor cavity method ("Eraser" algorithm) [8]. This study reveals the key residues in the target protein which are responsible for ligand binding, which are present in the active site or elsewhere.

Ligand identification and minimization

The Lead compounds was obtained from chEMBL database. Around 80 compounds were obtained and finally ten compounds were identified. Ligand optimization was carried out using CHARMm and MMF force field by small molecules minimization protocol in DS.3.5[9].Various ligand conformations were generated based on Bond energy, CHARM energy, dihedral energy, electrostatic energy, initial potential energy and initial RMS gradient values.

Evaluation of drug likeliness and ADME-Toxicity investigation

The drug likeliness was evaluated using the Lipinski rule of 5 via Lipinski drug filter protocol using DS.3.5[10]. Lipophilicity plays a important role in the drug discovery and design. Lipophilicity plays a role in the determination of physicochemical property which has a major role in finding out the ADMET[11] (absorption, distribution, metabolism, excretion, and toxicity) and the over suitableness of drug candidates.

Molecular docking

The Molecular docking studies were carried to Identify the binding affinities and interaction between the inhibitors and the target using BioSolveIT FlexX [12]. The active site of the modeled protein was loaded in the BioSolveIT FlexX. The active site amino acids were defined in the target molecule during the target preparation step of FlexX. A sphere of 10Å radius was defined as an active site. The screened compounds were loaded in FlexX as docking library. The Protein Ligand clash was set to 2.9 Å and Intra Ligand clash was set to 0.6 in the docking. Maximum number of fragmentation and iterations were set to 200. The docked ligand-target complexes were analyzed carefully to identify the interactions and binding affinities. The docking score was recorded and docking poses were saved for reference.

3.Results and Discussion

Protein simulation and Active site prediction

Modeled protein potential energy was analyzed before and after minimization by using

Calculate energy protocol in DS 3.5 and it was found to be 0.80714Kcal/mol and -37892.85214 Kcal/mol respectively [Table 1]. Based on the receptor cavity method we identified 16 active sites in the modeled structure. Based on the size of the volume, we selected the first active site for further study.

Table: 1 Energy values of modeled protein before and after Minimization

Modeled Protein	Forcefield	Potential Energy (kcal/mol)	Vander waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	RMS Gradient (kcal/(mol x A)
Before Minimization	CHARMm	0.80715E+12	0.80715E+12	-0.23632E+05	0.18436E+12
After Minimization	CHARMm	-37892.85214	-2600.94541	-48183.20038	2.11025

Ligand retrieval, Minimization and ADME toxicity

The Lead compounds were retrieved from public databases such as chemicalize.org and ADMET(Absorption, Distribution, Excretion and Metabolism) property was analyzed. These results show that the Lead compounds (1-10) possess good pharmacokinetic properties and it satisfies all the parameters to be taken over as a good drug. The energy minimizations of the filtered ligands were performed using DS 3.5.

Docking studies

Molecular docking studies were performed using Flex X. The results of interaction between modeled protein with ten lead compounds and its docking results are shown in Table2 and the best docked complex is displayed in Figure1. All the amino acid and Ligand atom residues which involved in molecular interactions are displayed. The results show that a good interaction occurs between the protein and the Ligand. [(3-{3-[4-(1-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1H-indol-1-yl}propyl)sulfanyl]methanimidamide showed more binding capacity than the other compounds.

TABLE:2

Ligand-Protein interaction with docking scores

S. NO	IUPAC/SMILES	LEAD-IT (DOCKING)					
		LEAD-IT SCORE	HBOND	AMINO ACID	AMINO ACID ATOM	LIGAND ATOM	H-BOND LENGTH (Å)
1	6-[2-(piperazin-1-yl)pyridin-4-yl]-2-N-(pyrazin-2-yl)pyridine-2,4-diamine	-31.7363	4	TYR57	_HH	N15	2.32313
				GLU570	_O	H46	1.79642
				ASP619	_OD1	H47	1.98392
				ASP619	_OD2	H47	2.20471
2	N-cyclohexyl-4-[1-(piperazin-1-yl)-2,6-naphthyridin-3-yl]pyridin-2-amine	-30.1111	8	LYS499	_HN	O15	2.20723
				ASP619	_OD1	H31	1.54509
				ASP619	_OD2	H31	2.32313
				GLY503	_O	H34	1.79642
				LYS499	_O	H35	1.98392
				GLY503	_O	H57	2.25626
				ASP633	_OD1	H57	2.20471
				ASP633	_OD2	H57	2.09863
3	N-[(1R)-1-cyanoethyl]-3-[5-(4-{[(oxan-4-yl)amino]methyl} phenyl) -1,2-oxazol-3-yl] benzamide	-28.367	5	LYS499	_HN	N10	2.09919
				LYS499	_HZ3	N10	2.31811
				LEU497	_O	H42	1.98535
				ASP633	_OD1	H59	2.11812
				ASP633	_OD2	H59	1.93452
4	N-(1-benzylpiperidin-4-yl)-3-(6-methyl-1H-indol-2-yl)-1H-indazol-5-amine	-27.8141	4	GLY503	_O	H37	2.22424
				SER501	_OG	H38	1.80291
				ASP615	_OD1	H63	2.08465
				ASP615	_OD2	H63	1.93242
5	3-(6-methyl-1H-indol-2-yl)-N-(pyrrolidin-3-yl)-1H-indazol-5-amine	-26.5303	6	LYS499	_HN	N8	1.60749
				LYS499	_HZ3	N8	2.47721
				ASP619	_OD1	H35	2.07474
				ASP619	_O	H44	2.0666
				ASP619	_OD1	H47	1.83776
				ASP619	_O	H47	2.41915
6	Cn1cc(cn1)c2cccc(c2)c3cnc(N)c(n3)C(=O)N[C@@H]4C5CC6CC4C[C@]	-25.078	4	LYS499	_HN	O25	2.28314
				LYS499	_HZ3	O25	2.01027
				GLY503	_HN	O27	2.00625
				GLY503	_O	H56	1.9662
7	[(3-{3-[4-(1-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1H-indol-1-yl}propyl) sulfanyl]methanimidamide/ BISINDOLYLMALEIMIDE IX	-24.916	8	LYS499	_HN	O17	1.61379
				LYS499	_HZ3	O17	2.48194
				SER501	_HN	O	2.37283
				GLY503	_HN	O	2.19868
				LYS520	_HZ3	S32	1.96299
				THR536	_OG1	H55	2.21092
				THR536	_O	H55	2.27507
				GLU539	_OE2	H56	1.78341
8	N-cyclohexyl-4-[1-(piperazin-1-yl)-2,6-naphthyridin-3-yl]pyridin-2-amine	-23.9583	5	LYS499	_HN	N14	2.21512
				LYS499	_HZ3	N14	1.68341
				LYS520	_NZ	H31	2.27966
				ASP633	_OD1	H31	1.63002
				ASP619	_OD1	H58	1.89486
9	1,5-dimethyl-N-[3-(6-methyl-1H-indol-2-yl)-1H-indazol-5-yl]-1H-imidazole-4-sulfonamide	-22.7324	4	LYS499	_HN	O23	2.42084
				SER501	_HN	N	2.12842
				GLY503	_HN	O	2.30199
				ASP633	_OD2	H35	2.13654
10	1-(5-{1-[(2,5-dichlorophenyl) methyl]-1H,2H,3H,4H-pyrido[2,3-b] pyrazin-7-yl}pyridin-2-yl)-4-methylpiperazine	-20.2386	3	LYS499	_HN	N15	2.10495
				LYS499	_HZ3	N15	1.68895
				ASP576	_OD1	H59	1.76113

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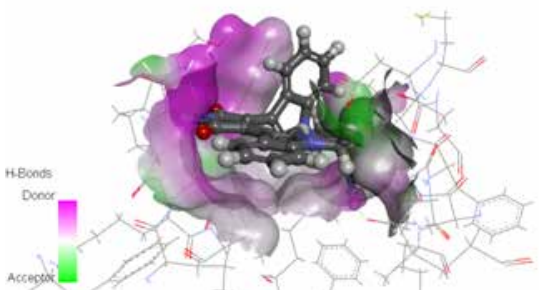
FIGURE:1
BEST DOCKED COMPLEX

PROETIN LIGAND INTERACTIONS		
LYS499	_HN	O17
LYS499	_HZ3	O17
SER501	_HN	O
GLY503	_HN	O
LYS520	_HZ3	S32
THR536	_OG1	H55
THR536	_O	H55



4. 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 a putative drug requires a valid 3D protein model to analyze the binding patterns and interactions with the ligands Insilico. So the modeled structure was downloaded from PMD database and Active site, Ligand preparation, ADMET properties, Docking studies were done by DS 3 server and the best Ligand was identified, which will have good pharmacological value.

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