

Core Hopping of Bis-indole Derivatives to Identify an Effective Inhibitor for the Treatment of Bipolar Disorder

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ABSTRACT

Bipolar disorder is found to be one of the crucial mental disorders with severe side effects. The suicide rate among patients with bipolar disorder is likely greater than with patients of major depression and up to 17%-19% of patients die by suicide. Various proofs identify that family of protein kinase C (PKC) can act as target in the treatment of BPD. Different medications for treating bipolar disorder may cause different side effects. Due to its higher severity of side effects there is a need for the development of novel drugs. In the present study, core hopping technique is employed to obtain a new compound with higher activity. X-ray crystal structure of PKC- ϵ protein was downloaded from Protein Data Bank (PDB ID: 1GMI). The target protein was obtained pre-processed for docking analysis. By using core-hopping method 21 novel structures were developed from bis-indole derivative. All the obtained compounds were subjected to molecular docking and molecular dynamics simulations. From the docking analysis a novel protocoore 2-(3a,7a-dihydro-1H-indol-3-yl)-3-(1H-indol-3-yl)-1,2,3,3a,4,6a-hexahydropyrazolo[3,4-d] [1,2,3]triazol-6-amine showed significantly higher binding energy (-12.8 kcal/mol) than the normal bis-indole derivative (-4.5 kcal/mol). From the molecular dynamics study, the protein-ligand complex were found to be highly stable and the structure of protein remains intact and undergoes no deformation after binding with the ligand. Thus, the novel compound obtained from core hopping technique can be used for the development of effective drugs for the treatment of bipolar disorder with lesser side effects.

Key words: Bipolar disorder, Protein Kinase C, Core hopping, Molecular docking, Molecular dynamics.

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INTRODUCTION

Bipolar disorder is a genetic disorder which shows pathological disturbance in mood ranging from extreme elation or mania to severe depression with visual disturbances in thinking and behaviour. BPD is subclassified into Bipolar I disorder (Clear cut Mania) and Bipolar II disorder (Milder forms of Mania called hypomania). A diagnostic prevalence study revealed a life time prevalence of 1.0% and 1.1% for BPD-I and

BPD-II respectively with a common age of onset of bipolar disorder between 17-21 years. It is a highly disabling illness indicated by a study conducted by the World Health Organization that identified bipolar disorder as the 6th leading cause of disability world-wide in the 17-44 years age group.^[1] The suicide rate among patients with bipolar disorder is likely greater than with patients of major depression and up to 17%-19% of patients die by suicide. The lifetime risk of bipolar disorder includes monozygotic co-twin 40-70%, first degree relative 5-10% and unrelated person 0.5-1.5%. Certain genes plays the major role in bipolar disorder, but interaction of multiple genes (epistasis) or more complex genetic mechanisms (mutation) also play a vital role in BPD.^[2]

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Majority of intracellular mediators of signals are PKC isozymes which are generated on external stimulation of cells through a variety of neurotransmitter receptor subtypes, which include the hydrolysis of membrane phospholipids. PKC's function is involved in signal transduction associated with the control of brain functions such as ion channel regulation, receptor modulation, neurotransmitters release and neuronal survival. Various proofs identify that family of protein kinase C (PKC) can act as target in the treatment of BPD. PKC is a group of calcium and phospholipid dependent enzymes, which play a pivotal role in cell signalling systems and many evidences indicate that alterations in PKC activity plays significant role in the pathophysiology of Bipolar Disorder.^[3]

Different medications for treating bipolar disorder may cause different side effects. Some medications used for treating bipolar disorder largely are lithium and valproic acid^[4,5] although they have been linked to unique and serious symptoms. For example side effects of lithium include loss of coordination, excessive thirst, frequent urination, blackouts, seizures, slurred speech, fast, slow, irregular, or pounding heartbeat, hallucinations (seeing things or hearing voices that do not exist), changes in vision, itching, rash and swelling of the eyes, face, lips, tongue, throat, hands, feet, ankles, or lower legs. Valproic acid may affect young girls and women in unique ways. Sometimes, valproic acid may increase testosterone (a male hormone) levels in teenage girls and lead to a condition called polycystic ovarian syndrome (PCOS) and valproic acid/divalproex sodium result in changes in weight, nausea, stomach pain, vomiting, anorexia and loss of appetite.^[6,7] Thus, there is a need for the development of new drugs with higher activity and lesser side effects.

The present research concentrates on identification of new compounds for the treatment of BPD by core hopping method. Based on the core hopping of Bis-indole derivatives, the obtained structures were subjected to docking against PKC- ϵ protein and compared with the native structure. Further the compounds were screened for ADMET properties. The significant compound was identified and molecular dynamics analysis was carried out to determine the equilibrium and dynamic properties of the Protein-Ligand complex.

MATERIALS AND METHODS

Target Protein preparation

X-ray crystal structure of PKC- ϵ protein was downloaded from Protein Data Bank (PDB) with PDB ID: 1GMI.^[8] The target protein was obtained and pre-

processed using Protein Prep Wizard application of Schrödinger suite. The process of preparing receptors included assigning bond orders, adding hydrogen, treating metals, treating disulfides, deleting waters, alleviating potential steric clashes and adjusting formal charges. The refined protein structure is further optimised and energy minimised using incorporated OPLS (Optimized Potentials for Liquid Simulation) 2005 force field and refining the protein by limiting value of root mean square deviation (RMSD) to 0.50 Å as the constraint.

Core hopping Bis-indole derivatives

The general scaffold of Bis-indole derivative and the core of the molecule is shown in the Figure 1. Core Hopping module of Schrödinger suite^[9] was employed to obtain new molecule containing distinct core with improvised binding ability with the receptor. This technique aims to search for novel compound by substituting core with the protocores i.e., the scaffold against docked lead compound. Identified attachment points by defining attachment grow bonds from where the side chains are cleaved from the core. The Protocores were prepared by generating 3D structure, stereochemistry by using LigPrep application.

Molecular docking

The grid for PKC- ϵ was set up and generated from the Receptor Grid Generation panel of Glide module. The prepared ligands were then docked with the receptor by importing the generated grid file. Molecular docking of ligands obtained from core hopping with PKC- ϵ target was performed using Glide module of Schrödinger suite.^[10,11] The original compound is used as positive control. The conformations generated for the ligands were checked to determine the best ligand pose which is scored and ranked by scoring function termed as GlideScore.

Molecular dynamics simulation

The best interacting ligand obtained by Core Hopping screening method was subjected to molecular dynamics simulation run using MacroModel application of Schrödinger suite.^[12] Molecular dynamics simulation was performed for 100 ps. Total trajectory for 100 samples were generated. The charge and potential energy calculation was performed using OPLS_2005 force field. The trajectories were obtained for 500 iterations with 1.5 fs time step and 0.05 convergence thresholds. The calculation was performed in water at 300 K temperature.

RESULTS AND DISCUSSION

Core hopping and docking analysis

As from Figure 1 it can be observed that the core compounds has to be defined and bis-indole parent compound can be divided into three distinct parts among which yellow shaded region here referred as *core* was used to modify by screening against available in-built core library which was shown in the Figure 2 a, b and c. As interaction of one of the indole ring with PKC ϵ is the key interaction the centre region was subjected to modification by defining linker attachment at 3rd and 4th position of pyrrole ring. Twenty one compounds containing novel *Protocores* were generated for Bis-indole parent compound i.e., prepared template core by substituting the core from inbuilt core library. A library of 21 compounds were shown in Figure 3. In order to determine lead compound from these 21 compounds and to understand the binding mode, compounds were docked with the crystal structure of PKC ϵ . The cumulative binding score analysis showed that among 21 compounds, binding score obtained for compound, 2-(3a,7a-dihydro-1H-indol-3-yl)-3-(1H-indol-3-yl)-1,2,3,3a,4,6a-hexahydropyrazolo [3,4-d] [1,2,3] triazol-6-amine was observed to be highest, -12.8 kcal/mol (Table 1). The binding score for the native compound was found to be -4.5 kcal/mol (Table 2). The interaction profile of compound obtained after docking with protein is given in Figure 4.

Molecular dynamics

Molecular dynamics parameter window was shown in Figure 5. A lead compound, 2-(3a,7a-dihydro-1H-indol-3-yl)-3-(1H-indol-3-yl)-1,2,3,3a,4,6a hexahydropyrazolo[3,4-d][1,2,3] triazol-6-amine obtained from core hopping method through various screening methods, was subjected for 100 ps molecular dynamics simulation run to predict protein-ligand complex stability (Figure 6). The complex was checked for its stability at pH ± 7 , 300K temperature and water solvent. The new trajectories for 100 samples were written for 100 ps MD simulation run. For convenience, only initial protein-ligand conformation at time, t=0 ps and final conformation at time, t=100 ps is depicted in Figure 7 and 8 by RMSD plot. The synthesizability compound 2-(3a,7a-dihydro-1H-indol-3-yl)-3-(1H-indol-3-yl)-1,2,3,3a,4, 6a-hexahydropyrazolo[3,4-d] [1,2,3] triazol-6-amine which acts against PKC E target was shown in the Figure 9.

DISCUSSION

Bipolar disorder is found to be one of the crucial mental disorders with severe side effects. Some of the serious

Table 1: Molecular docking score of obtained compounds.

S. No.	Title	Docking score
1	St 1 from N1NNc(c12)[nH][nH+]c2N	-12.8
2	St 1 from C[NH+](C1)CC12CN(C2)S	-11.32
3	St 1 from C[NH+](C1)CC12CN(C2)S	-10.56
4	St 1 from C[NH+](C1)CC12CN(C2)S	-10.32
5	St 1 from C[C@]1(O)C=NNC1	-10.21
6	St 4 from c1cc[n+](n12)C[C@@H](C2)O	-10.11
7	St 4 from c1cc[n+](n12)C[C@@H](C2)O	-10.01
8	St 6 from N#C[C@@]([O-])(C12)C[N@@H+](CC1)CC2	-9.57
9	St 1 from O=C1C=C(N)NCC1	-8.23
10	St 4 from c1cc[n+](n12)C[C@@H](C2)O	-6.42
11	St 6 from N#C[C@@]([O-])(C12)C[N@@H+](CC1)CC2	-5.43
12	St 2 from N1C[C@@H](C)[N@H+](C)c(c12)cc(F)c(F)c2	-4.58
13	St 6 from O[C@H]1[C@@H](O)COC[C@@H]1O	-4.45
14	St 1 from C[C@]1(O)C=NNC1	-4.44
15	St 6 from N#C[C@@]([O-])(C12)C[N@@H+](CC1)CC2	-4.31
16	St 6 from N#C[C@@]([O-])(C12)C[N@@H+](CC1)CC2	-4.2
17	St 6 from N#C[C@@]([O-])(C12)C[N@@H+](CC1)CC2	-4.18
18	St 1 from C[C@@H]1CCC(=C12)SCC(=O)N2	-4.13
19	St 2 from o1nc2[C@H]([NH3+])CN(C)c(c3)ccc1c23	-4.1
20	St 1 from C[NH+](C1)CC12CN(C2)S	-4.3
21	St 6 from O[C@H]1[C@@H](O)COC[C@@H]1O	-4.1

Table 2: Comparative score details of reference compounds docked with PKC ϵ protein.

S. No	Docking method	Compound	Aminoacid	GScore
1	Native Docking	(5S)-5-amino-1-(hydroxymethyl)-3,4-di(1H-indol-3-yl)-1,5-dihydro-2H-pyrrol-2-one	Tryptophan-116 Aspartic acid-118	-4.5
4	Core hopping	2-(3a,7a-dihydro-1H-indol-3-yl)-3-(1H-indol-3-yl)-1,2,3,3a,4,6a-hexahydropyrazolo[3,4-d][1,2,3] triazol-6-amine	Aspartic acid-922 Tryptophan-755 Glutamic acid-943	-12.8

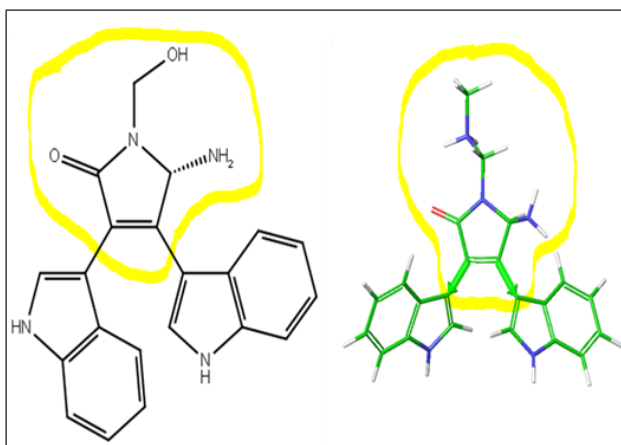


Figure 1: General scaffold of Bis-Indole derivative. The yellow highlighted area is determined to be the core of the molecule

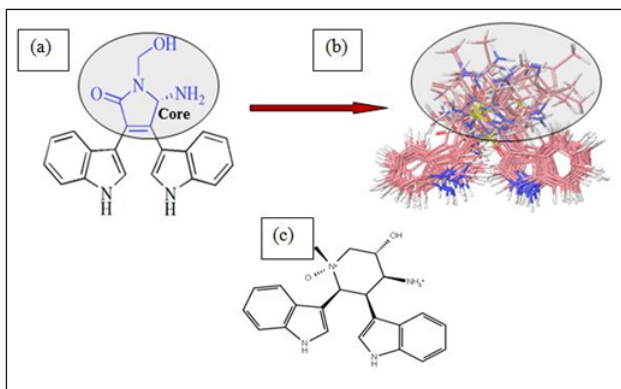


Figure 2: (a) Structure of template core, (b) aligned compounds obtained after core hopping and (c) structure of lead compound obtained after core hopping. Core molecule from (a) was replaced with (c) molecule where (b) shows the combination of conformation generated from differential rmsd frequencies

effects are Increased physical and mental activity and energy; hyperactivity, Significant changes in appetite and sleep patterns, Trouble breathing, Social withdrawal, Loss of energy, persistent lethargy; aches and pains, Weight gain; blood pressure and heart problems; diabetes etc.,^[13] In particular, mania is associated with overactive protein kinase C (PKC) intracellular signalling and recent genome-wide association studies of bipolar disorder have implicated an enzyme that reduces the activation of PKC. Importantly, the current mainstays in the treatment of mania, lithium (a monovalent cation) and valproate (a small fatty acid) indirectly inhibit PKC. Hence PKC is considered to be a potential target in treatment of bipolar disorder.^[14]

There are several drugs in treatment of bipolar disorder and mostly commonly used is bis-indole derivatives. There are several side effects associated with these

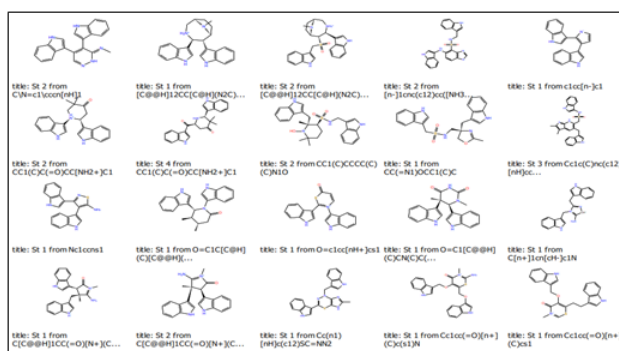


Figure 3: Structure of compounds obtained from core hopping method. The compounds are obtained by the synthesizability score

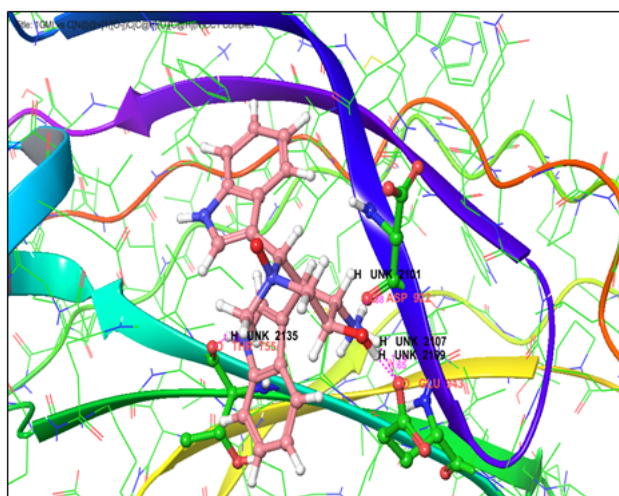


Figure 4: Interaction profile of highly interacting compound with PKC ε protein obtained through molecular docking with core hopping method. Interaction of compound with PKC ε protein along with the residue to which it interacted and the distance was shown

drugs and there is a need for the development of new drugs with lesser side effects and higher activity. “Core Hopping” docking method was employed to obtain new core containing compounds, which were ranked by molecular docking to yield lead compounds. In the present study, Twenty one compounds containing novel *Protocores* were generated for Bis-indole parent compound i.e., prepared template core by substituting the core from inbuilt core library. A library of 21 compounds were obtained based on side chain RMSD score, overlapped score and synthesizability score and alignment values. All the 21 compounds along with the native compound were used for docking against PKC enzyme.

From the analysis the native compound showed binding score of -4.5 kcal/mol, whereas a novel compound from core hopping method showed -12.8 kcal/mol. It was

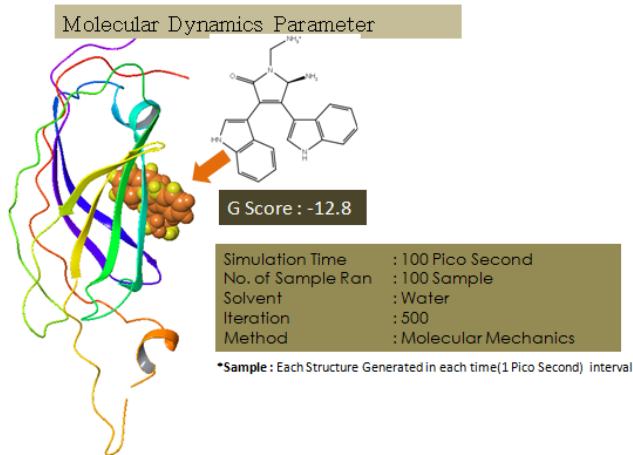


Figure 5: Molecular Dynamics parameter. Figure showing the molecular dynamics parameter with simulation time and number of sample run

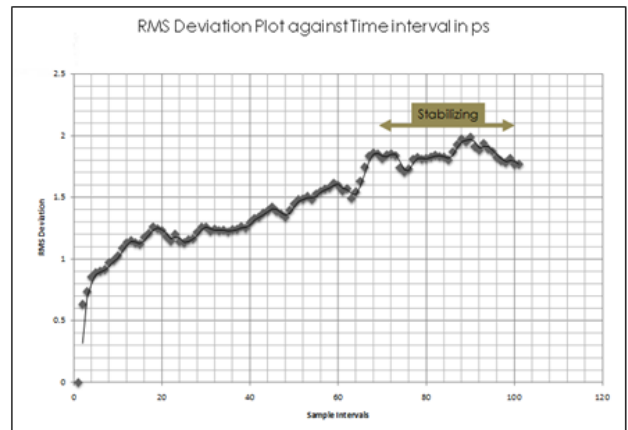


Figure 8: RMSD Deviation plot against Time interval in ps. RMSD Deviation plot against Time interval in ps which shows a stabilized state.

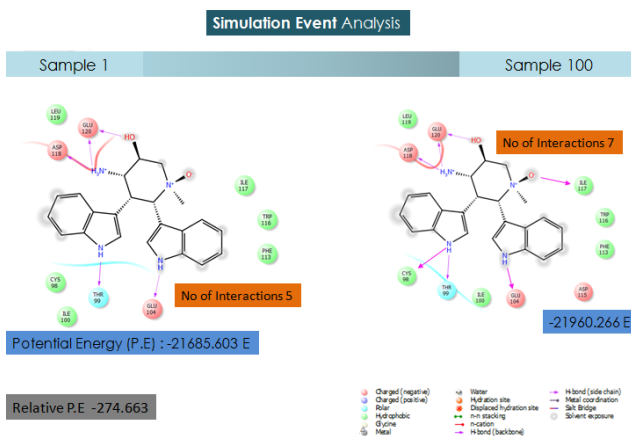


Figure 6: Simulation Event Analysis. Figure showing Simulation Event Analysis between 1st to 100th sample

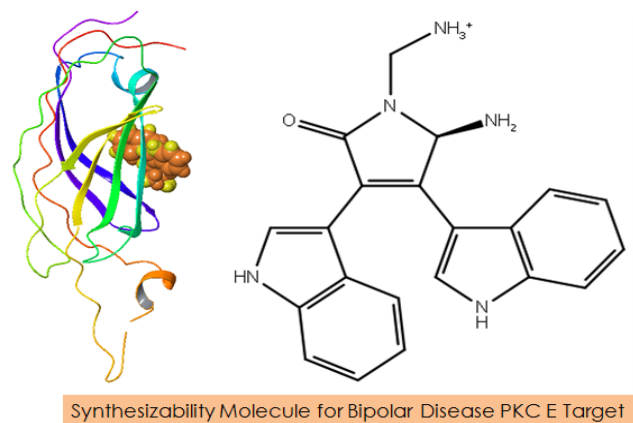


Figure 9: Synthesizability compound against PKC E target. Figure showing synthesizability core molecule for Bipolar Disorder.

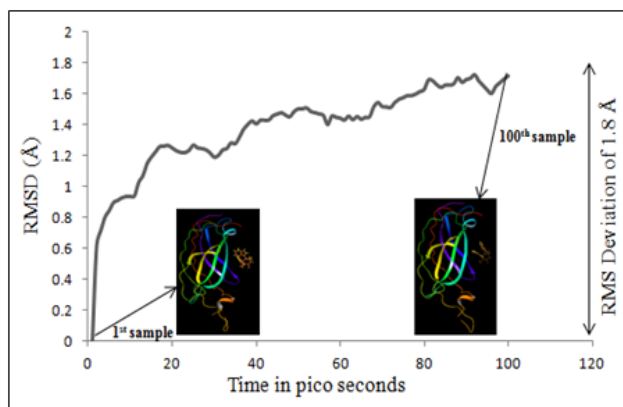


Figure 7(a): RMSD plot for 100 ps MD simulation run showing protein-ligand complex configuration for initial and final. RMSD plot showing deviation based on change in time for 100 ps molecular dynamic simulation

observed that apart from hydrogen bond interaction of indole ring with THR residue of protein, the ammonium functional group showed two sites of strong hydrogen bond interaction with ASP and GLU residues and hydroxyl group showed hydrogen bond interaction with GLU residue. The substitution of five member pyrrole ring with the six member cyclohexane moiety broke the conjugation between the two indole rings and pyrrole ring and between indole rings and carbonyl group of pyrrole ring. Due to substitution of cyclohexane ring the ammonium group and hydroxyl group were easily able to give their proton to participate in hydrogen bond donation. The enhanced interaction was attributed to the hydroxyl and ammonium functional groups of core.^[15] The hydrogen bond donating ability of these functional groups improved the binding ability of the ligand with protein which is depicted in Figure 4.

Further the selected compound in complex with target protein was subjected to molecular dynamics simulation. The dynamics study of protein-ligand complex showed that the complex was stable in given temperature and pressure condition. The stability was investigated by observing the root mean squared deviation (RMSD) of the complex.^[16] The RMSD showed that around 56 ps, the complex possess small fluctuation from its initial conformation with RMSD of 1 to 1.4 Å. After 57 ps, the complex begun gaining stability and was observed to be stable thereafter. The complex was found to be fluctuating around its mean conformation with RMSD of 1.8 Å. which indicates that the complex is highly stable no deformation after binding with the ligand was observed.

CONCLUSION

X-ray crystal structure of PKC- ϵ protein was downloaded from Protein Data Bank. The target protein was pre-processed by assigning bond orders, adding hydrogen, treating metals, treating disulfides, deleting waters, alleviating potential steric clashes and adjusting formal charges. By using core-hopping method 21 novel compounds were developed from bis-indole derivative. All the obtained compounds were subjected to molecular docking and molecular dynamics simulations. From the docking analysis a novel compound 2-(3a,7a-dihydro-1H-indol-3-yl)-3-(1H-indol-3-yl)-1,2,3,3a,4,6a-hexahydropyrazolo[3,4-d] [1,2,3]triazol-6-amine showed significantly higher binding energy (-12.8 kcal/mol) than the normal bis-indole derivative (-4.5 kcal/mol). From the molecular dynamics study, the protein-ligand complex was found to be highly stable and the structure of protein remains intact and undergoes no deformation after binding with the ligand. Thus, the novel compound obtained from core hopping technique can be used for the development of effective drugs for the treatment of bipolar disorder with lesser side effects.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

ABBREVIATIONS

BPD: Bipolar Disorder; **PKC:** Protein kinase C; **OPLS:** Optimized potential for liquid simulations; **RMSD:** Root mean square deviation; **ASP:** Aspartic acid; **GLU:** Glutamic acid.

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