IJPSR (2019), Volume 10, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 December 2018; received in revised form, 29 April 2019; accepted, 11 May 2019; published 01 September 2019

IN-SILICO MOLECULAR DOCKING ANALYSIS OF ISOLATED HOMOISOFLAVANONES FROM BULBS OF *LEDABOURIA REVOLUTA* AS $GABA_A$ RECEPTOR INHIBITORS

B. Soni Veena and E. Sujatha *

Department of Botany, University College of Science, Osmania University, Hyderabad - 500007, Telangana, India.

Keywords:

Homoisoflavanones, $GABA_A$ receptors, *Ledabouria revoluta*, Molecular docking

Correspondence to Author:
Dr. E. Sujatha

Assistant Professor,
Department of Botany,
University College of Science,
Osmania University - 500007,
Telangana, India.

E-mail: sresearchou@gmail.com

ABSTRACT: γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate central nervous system (CNS); it activates the $GABA_A$ receptors that play a role in epilepsy. Targeting $GABA_A$ receptors through specific enhancement of neuronal inhibition involving GABA can be a better mechanism for identification of novel antiepileptic drugs. In this report, screening of novel isolated homoisoflavanones from bulbs of *Ledabouria revoluta* against human GABRB3 active site using molecular docking studies. The docking analysis reveals the identification of leads with favorable binding energy and hydrogen bond interactions confirmed the effective modulation of the receptor. Based on the dock score and number of hydrogen bond interactions, compound 3 observed to be the most potent.

INTRODUCTION: Epilepsy is an incessant and regularly convincing neurological issue symbolized by the discontinuous and indeterminate scenes of epileptic seizures that are expected to the extraordinary cerebral neuronal discharge that outcome in relatively prompt dissent of sensation and loss of consciousness [1, 2, 3]. This neurological issue has been a standout amongst the most explored restorative condition primarily because of its intricate horribleness relationship with generously high death rates [4, 5]. Epilepsy, being the best three acclaimed supporters of the worldwide weight of the neurological issue, stimulating effect around 65 million people worldwide other than widely shifting its pervasiveness and occurrence from 2.8 to 19.5 per 1000 of the all-inclusive community all through the world [6].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(9).4315-21

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(9).4315-21

The reason for most instances of epilepsy is obscure. A few cases happen as the aftereffect of cerebrum damage, brain tumors, stroke, and birth surrenders known as epileptogenesis [7, 8]. Realized hereditary changes are straightforwardly connected to a little extent of cases. The adequate pathogenesis of epileptic seizures is the awkwardness of excitatory and inhibitory synapses in the focal sensory system, which leads an unusual nerve cell action and neuronal release bringing about seizures. Consequently, reduction in duration and also the beginning of seizures by tweaking these synapses was the fundamental methodology for epilepsy treatment [9, 10].

The craving for pharmacological control of GABAergic neurotransmission has produced a plenty of xenobiotics which are valuable in prescription, including anticonvulsants, sedatives, anxiolytics, muscle relaxants and meds for treating pain [11]. γ-Aminobutyric acid (GABA), the major inhibitory synapse in vertebrate focal sensory system, applies its activity essentially by actuating the GABAA receptors (GABAARs).

GABA atoms or GABA-like compounds tie to the receptor and actuate it. Upon initiation, the GABA-A receptor specifically leads Cl− through its pore, resulting in hyperpolarization of the neuron. This causes an inhibitory impact on neurotransmission by lessening the shot of a fruitful activity potential [12, 13]. This aggregate neuronal restraint caused by GABA official to numerous neurons results in anticonvulsive properties [14, 15]. $GABA_A$ receptors are established by a pseudosymmetrical gathering of five indistinguishable or homologous subunits shaping a chloride-conducting particle pore [16, 17, 18]. Every subunit contains a 200 to 250-amino acids long extracellular N-terminal domain, a free heap of four membrane-spanning α-helices (TM1–TM4), a huge intracellular circle between the TM3 and TM4 area (somewhere in the range of 85 and 255 corrosive amino buildups) and a short C-terminal fragment. Buildups from the TM2 domain line the ion-conducting pore.

The acknowledgment that the GABAA receptor framework is a composition gotten from 6α, 3β, 3γ, δ, θ, ε, π and 3ρ subunits 2, 3 and that distinctive recombinant subunits are especially critical in certain physiologic occasions intervened by GABA. This has created GABAA receptors with particular electrophysiological along with pharmacological properties including agonist affectability and its affectability for medications. Besides, these unmistakable receptor subtypes invigorated a scan for synthetic substances that have selectivity for $GABA_A$ receptors with a specific blend of subunits [19, 20]. Even though seizures are controlled with right now accessible AEDs yet over 30% of patients still have medicinally stubborn epilepsy. Moreover, around 30-40% of epileptic patients are as yet influenced by many side effects. These conditions have spurred the scientists to create novel ways to deal with treat epilepsy like antiepileptic constituents from natural prescriptions [21, 22].

Plants are a good source for the bioactive compounds and are used as traditional medicines. Phytochemical isolation of plants emphasizes in traditional medicines has yielded various bioactive compounds with different pharmacological activities. *Ledebouria* is a genus of weakly evergreen bulbs in the Hyacinthaceae family. The phytochemistry of several members of *Ledebouria* has been investigated previously [23, 24, 25]. Phytochemicals from a plant origin of *Ledebouria* genus are widely used in traditional medicine. Molecular docking is a method which predicts the orientation of one macromolecule of protein to the synthesized ligand when bound to each other, hence forming a stable complex at atomic level [26].

Drug discovery program is oriented in the direction of the search for lead structures, and thus virtual screening/molecular docking program constitutes a great tool to find hit which further undergoes limited optimization to identify the promising lead molecule. By taking this diversity of phytochemicals from a plant origin of *Ledebouria*, our present study initiates a docking methodology aiming at the discovery of these compounds as $GABA_A$ receptor agonists as the targets of epilepsy. We are interested in studying the possible interactions of isolated homoisoflavanones (3-Benzyl- 4-chromanones) from bulbs of *Ledabouria revoluta* derived compounds with the first reported three-dimensional structure of a GABAAR.

MATERIALS AND METHODS:

Protein Preparation: The crystal structure of a human gamma-aminobutyric acid receptor, the GABA(A)R-beta3 homopentamer (GABRB3) was retrieved from Protein Data Bank [27] (PDB) with PDB ID: 4COF. The X-Ray diffraction structure of this receptor had a resolution of 2.97 Å, R-value of 0.206, and R free value of 0.226. In the first step, the protein preparation protocol of Discovery Studio (DS) was used to prepare the protein structure retrieved from the PDB. The water molecules and the heteroatom were removed before the docking study. Hydrogen atoms were added to the protein structures corresponding to a pH value of 7.4. Then the protocol performs protein structure refinement that corrects their bond orders, missing loop regions, inserting missing atoms in incomplete residues, deleting alternate conformations, standardizing names of the atoms, and protonating titratable residues.

Finally, all-atom restrained energy minimization of the protein structure was carried out using CHARMm force field with steepest descent algorithm followed by conjugant gradient algorithm until the convergence gradient satisfied with a root mean square deviation (RMSD)

tolerance of 0.01 Å. After energy minimization, Using Define and Edit Binding Site tools in DS, the active site of the protein were selected based on the bound ligand benzamidine conformation, and an active site sphere was defined with a radius of 10 Å respectively.

Ligand Preparation and Optimization: The isolated homoisoflavanones (3R)-5,7-dihydroxy-3-(4′-methoxybenzyl) -4-chromanone (1), (3R)-5,7-dihydroxy-3-(4′hydroxybenzyl)-4-chromanone (2), (3R)-5 -hydroxy-7, 8-dimethoxy-3 -(4′-hydroxy-benzyl)-4-chromanone (3) and (3R)-5,7-dihydroxy-8-methoxy-3-(4′-hydroxybenzyl)-4-chromanone (4) **Fig. 1** from underground bulbs of *Ledabouria revoluta* were selected [28] for this study. The chemical structures of the compounds were sketched by using ChemBiooffice Ultra 14.0 software and saved in mol2 format. These ligands were then subjected to prepare ligands protocol of DS. Then, these compounds were converted from 2D to 3D structures by including stereochemical, ionization, tautomeric variations as well as energy minimization and optimized for their geometry, desalted and corrected for their chiralities and missing hydrogen atoms. The bonds order of these ligands were fixed, and the charged groups were neutralized. The ionization and tautomeric states were generated between a pH of 6.8 to 7.2.

In the final stage of ligand preparation, compounds were minimized using CHARMm force field until a root mean square deviation of 0.01 was achieved. Steepest descent followed by conjugate gradient algorithm of DS was used for minimization method. A single low energy confirmation per ligand was generated, and the optimized ligands were used for docking analysis.

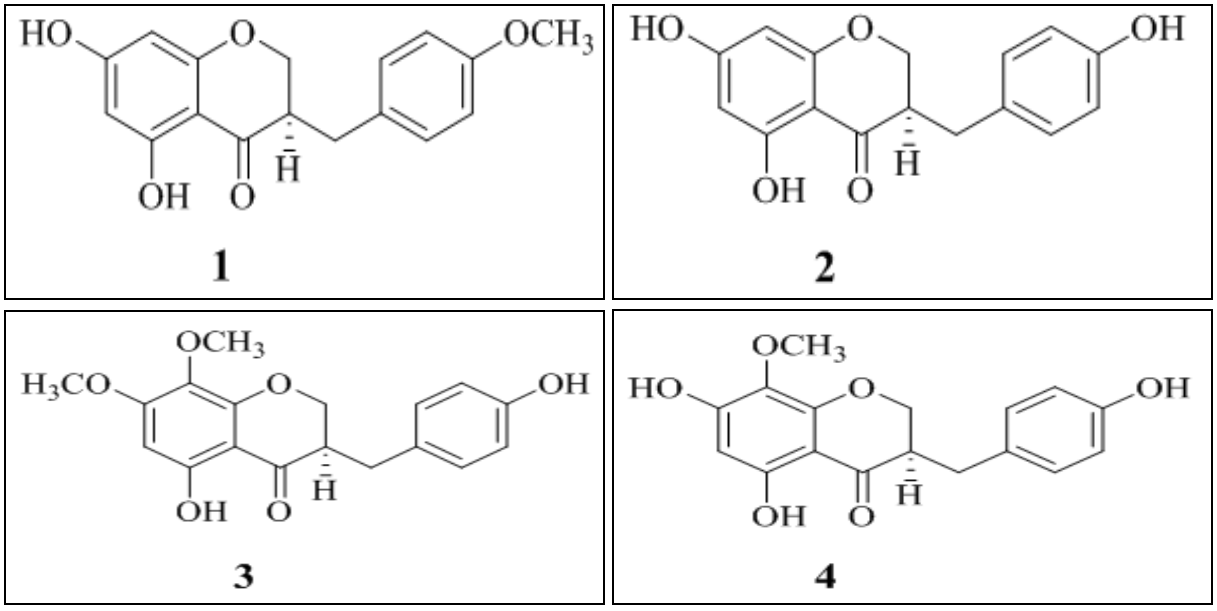


FIG. 1: STRUCTURES OF HOMOISOFLAVANONES ISOLATED FROM *LEDABOURIA REVOLUTA*

Docking Studies: The molecular docking studies were performed with the help of the DS LibDock program, which estimates the appropriate binding conformations of compounds in the defined active site of human GABRB3 (PDB: 4COF). LibDock uses protein site features, referred to as hot spots, consisting of two types states (polar and apolar). The LibDock algorithm is a flexible docking module of DS. The ligand poses are placed into the polar and apolar receptor interactions site. A polar hotspot is preferred by a polar ligand atom (*e.g.*, a hydrogen bond donor or acceptor), and an apolar hotspot is preferred by an apolar atom (*e.g.*, a carbon atom). The protocol allows the user to specify several modes for generating ligand conformations for docking. The scoring function of the LibDock calculates the binding affinity score or docking score (LibDock score) of the protein-ligand complex.

Also, the possible binding energies, possible hydrogen bonding, and various interaction poses are calculated. The top-ranked docked complexes of each compound are selected based on LibDock Score. Binding poses with highest LibDock Score and lowest binding energy are preferred as the best

pose, and further binding interactions of the best pose for each compound are analyzed.

***In-silico* Pharmacokinetics Studies:** Different pharmacokinetics parameters, namely, absorption, distribution, metabolism, excretion, and toxicity, were calculated. ADMET profiling of compounds was performed by applying the ADMET descriptors protocol of DS. This study includes the quantitative measurement of drug-like properties include aqueous solubility, blood-brain barrier (BBB), plasma protein binding (PPB), CYP2D6 binding, intestinal absorption, and hepatotoxicity. Also, AlogP98 and PSA_2D were used in plotting the confidence ellipses. Analysis of the results is based on the standard parameters according to the software limitations.

RESULTS AND DISCUSSION:

Molecular Docking Studies: Molecular docking study was performed for the isolated homoisoflavanones 1, 2, 3 and 4 from *Ledabouria revoluta* using the human GABRB3 enzyme as a receptor with the aid of Libdock module of DS [29]. Binding affinity evaluation and inhibitory potential of these compounds were measured through LibDock docking score and H-bond interactions. Of all the conformations generated for each compound, the compound with the highest LibDock score is taken for interaction analysis of the hydrogen bonding. The hydrogen bond interaction is significant for the bioactivity of compounds. The stability of the best docked pose of these compounds was evaluated by determining the hydrogen bonding interactions of the protein with compounds which revealed the critical amino acids involved in hydrogen bond formation. The high LibDock score of the ligand pose with the least binding energy was taken into account for the prediction of the best ligand binding conformation. Apart from hydrogen bonding interactions, other non-bonded interactions like hydrophobic bonding were also observed. **Table 1** depicts the LibDock scores, interaction data and binding energies of homoisoflavanones. Structural model of *human* GABRB3 binding site and binding pattern of candidate compounds in the binding site have represented in **Fig. 2**. Docked compounds 1, 2, 3 and 4 into the binding site of human GABRB3 have shown with LibDock scores of 100.605, 102.811, 106.275 and 105.271 respectively.

After scrutinizing all the results of docking and interaction analysis, all the docked compounds were formed a hydrogen bond with the TYR143 amino acid residue of active site pocket of selected protein except the compound 2. But the compound 2 has formed a hydrogen bond with GLN224 amino acid residue of active site pocket with a bond distance of 2.390 Å. Receptor-ligand bonding interactions of compounds 1, 2, 3 and 4 with active site residues of human GABRB3 have shown in **Fig. 3**.

TABLE 1: CALCULATED DOCKING SCORES, BINDING ENERGIES AND INTERACTION ATOMS ALONG WITH THEIR BOND LENGTHS OF THE TARGETED COMPOUNDS INSIDE HUMAN GABRB3 (4COF) ACTIVE SITE

Compound	LibDock score	Interacting atoms	Bond Distance (A^0)	Donor	Acceptor	No. of H-bonds
1	100.605	B:TYR143: HH - 1: O10	2.059000	HH	O10	1
		1: H33 - B:LEU268: HD12	1.755000	H33	HD12	
		1: H32 - B:LEU268: HB1	1.701000	H32	HB1	
		1: H30 - B:GLN224: NE2	2.054000	H30	NE2	
		1: H30 - B:GLN224: HE21	1.472000	H30	HE21	
2	102.811	B:GLN224: HE21 - 2: O21	2.390000	HE21	O21	1
		2: H24 - B:TYR220: HE2	1.420000	H24	HE2	
		2: H29 - B:TYR220: CE2	1.973000	H29	CE2	
3	106.275	B:TYR143: HH - 3: O10	2.454000	HH	O10	2
		B:TYR143: HH - 3: O23	2.252000	HH	O23	
		3: H33 - B: ILE264:O	1.909000	H33	O	
		3: O19 - B: ILE264 HA	2.038000	O19	HA	
		3: H34 - B:LEU268: HB1	1.605000	H34	HB1	
4	105.271	B:TYR143: HH - 4: O22	2.429000	HH	O22	2
		B:GLN224: HE21 - 4: O18	2.473000	HE21	O18	
		4: H28 - B:GLN224: HB2	1.835000	H28	HB2	
		4: H30 - B:LEU268: HB1	1.599000	H30	HB1	
		4: H31 - B: ILE264:O	1.870000	H31	O	
		4: H34 - B:ILE264: HA	1.310000	H34	HA	

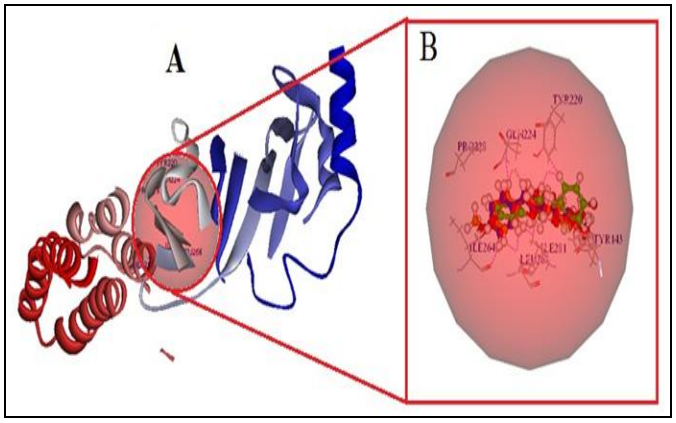
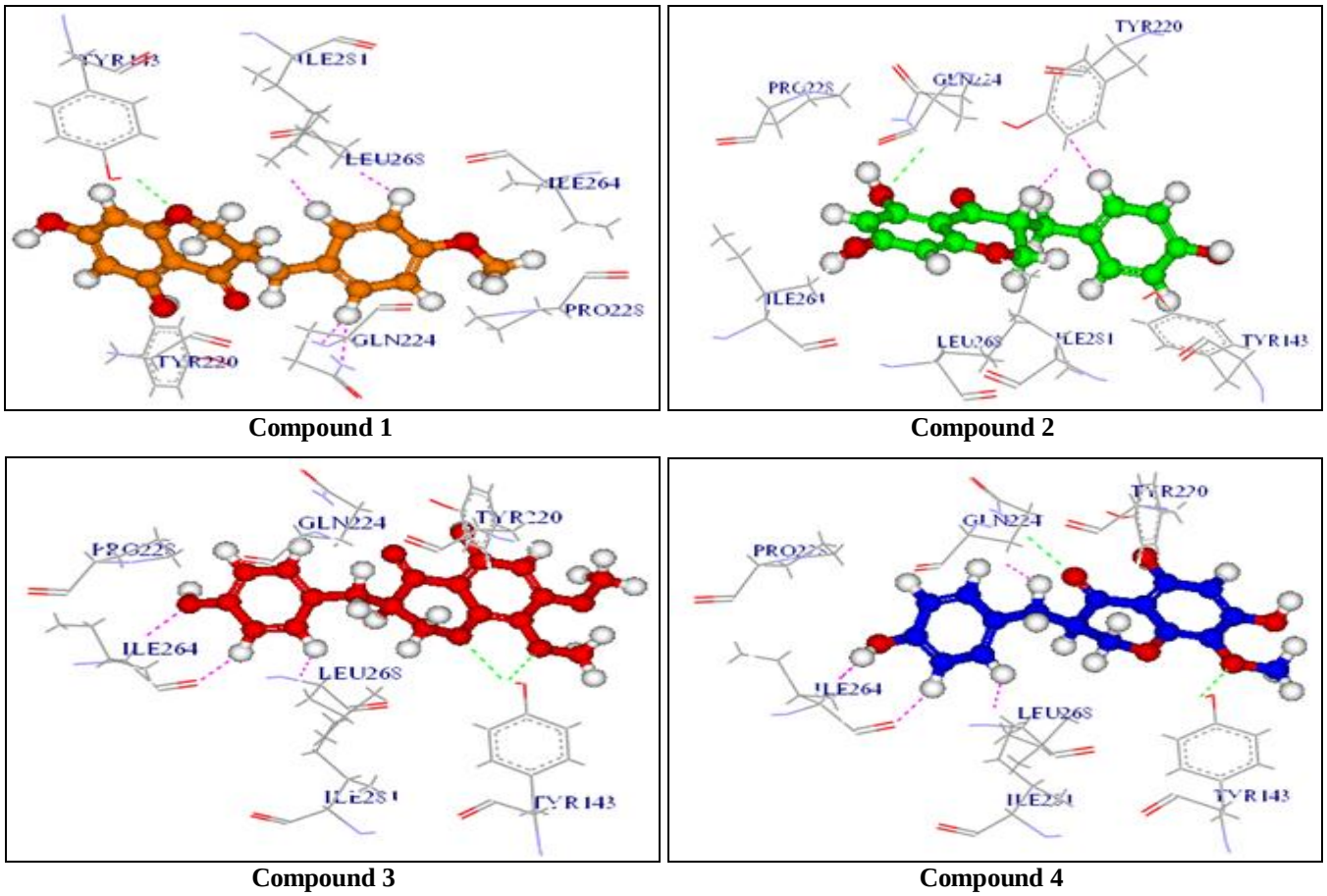


FIG. 2: (A) STRUCTURAL MODEL OF *HUMAN* GABRB3 (PDB: 4COF) BINDING SITE (SPHERE); (B) BINDING SITE AND BINDING PATTERN OF CANDIDATE COMPOUND

The best docking score of for compound 3 was achieved by forming two hydrogen bonds with the TYR143 amino acid residue of the active site of the human GABRB3 receptor protein. From **Fig. 3** it is observed that, compound 3 has formed two hydrogen bonds from the hydrogen atom of TYR143 with the 10th oxygen atom of the compound 3 (B:TYR143:HH-3:O10) with a hydrogen bond distance of 2.454 Å and 23rd oxygen atom of the compound 3 (B:TYR143:HH-3:O23) with a hydrogen bond distance of 2.252 Å. Some of the non-bonded interactions are also found between compound 3 and ILE264 and LEU268 amino acid residues.



Compound 1 **Compound 2**

Compound 3 **Compound 4**

FIG. 3: RECEPTOR-LIGAND HYDROGEN BONDS (GREEN COLOUR) AND BUMPS (PINK COLOUR) OF COMPOUNDS WITH ACTIVE SITE RESIDUES OF HUMAN GABRB3 (PDB: 4COF)

***In-silico* Pharmacokinetics Studies:** ADMET studies of homoisoflavanones 1, 2, 3, and 4 predicted using ADMET descriptor module of DS to provide insight into the pharmacokinetic property of the compounds. All the parameters calculated are tabulated in **Table 2**. According to the DS parameters, standard analysis for the compounds, the value like level 0 indicates good absorption for human intestinal absorption, level 3 for good solubility, level 1 for toxicity, level 0 for non-inhibitory and level 1 for an inhibitory property with CYP450 2D6. All the compounds are showing value 3 for lower BBB penetration except compound 1. The compound 1 showing value 2 for medium BBB penetration. ADMET descriptors, the 2D polar surface area in A2 per compound, are

plotted against their consonant estimated atom-type partition coefficient (ALogP98). The space covered by the ellipse is a prophecy of excellent absorption without any violation of ADMET properties. Ellipses indicate the absorption model at 95% and 99% confidence limit to the BBB and intestinal absorption models. The plot of polar surface area and ALogP for CA compounds are represented in **Fig. 4**.

TABLE 2: PREDICTED ADMET VALUES OF CA DERIVED COMPOUNDS

Compound	ADMET_BBB_ Level	ADMET_Absorption _Level	ADMET_Solubility _Level	ADMET_Hepato toxicity	ADMET_CYP2 D6
1	2	0	3	1	0
2	3	0	3	1	1
3	3	0	3	1	0
4	3	0	3	1	0

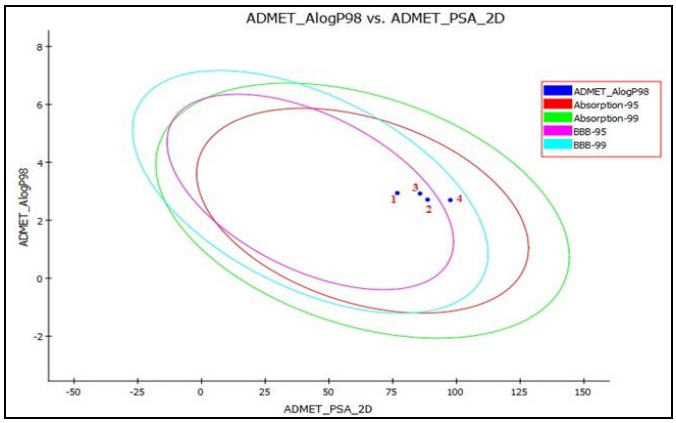


FIG. 4: PLOT OF PSA *vs.* ALogP FOR CANDIDATE COMPOUNDS

CONCLUSION: In conclusion, the modulation of GABAA receptors by motivating GABA mediated neuronal inhibition, promises to be a constructive therapeutic approach in the treatment of epilepsy. The present study initiates an attempt to find the potential compound of homoisoflavanones 1, 2, 3 and 4 from the bulbs of the *Ledebouria revoluta*.

The molecular docking study on human GABRB3 (4COF) confirmed the active modulation by compounds 1, 2, 3, and 4. The compound 3 shown good docking interactions with the docking score of 106.275 can acts as specific leads for receptor modulation, and the results suggested that they would be potential anti-epileptic agents. Our ADMET studies allow us to evaluate these homoisoflavanones and to assess the parameter that will be essential for further lead optimization studies.

ACKNOWLEDGEMENT: We are grateful to the Head, Department of Botany, Osmania University for providing necessary laboratory facilities. We would like to thank Dr. PVA Lakshmi and Dr. BS Kumar for fruitful discussions in data analysis.

CONFLICT OF INTEREST: The authors are no conflict of interest.

REFERENCES:

1. Altshuler LL, Devinsky O, Post RM and Theodore W: Depression, anxiety and temporal lobe epilepsy. Laterality of focus and symptoms. Arch Neurol 1990; 4: 284-88.
2. Kabir M, Iliyasu Z, Abubakar IS, Kabir ZS and FarinYaro AU: Knowledge, attitude and belief about epilepsy among adults in a Northern Nigeria Urban community Ann Africa Med 2012; 4: 107-12.
3. Zayed MF, Ihmaid SK, Ahmed HEA, El-Adl K, Asiri AM and Omar AM: Synthesis, modeling, and anticonvulsant studies of new quinazolines showing three highly active compounds with low toxicity and high affinity to the GABA-A receptor. Molecules 2017; 22: 188.
4. Yuen AC, Thompson PJ, Flugel D, Bell GS and Sander JW: Mortality and morbidity rates are increased in people with epilepsy: Is stress part of the equation? Epilepsy Behav 2007; 10: 1-7.
5. Mohanraj R, Norrie J, Stephen LJ, Kelly K, Hitiris N and Brodie MJ: Mortality in adults with newly diagnosed and chronic epilepsy: a retrospective comparative study. Lancet Neurol 2006; 5: 481-87.
6. Devinsky O, Vezzani A, Brien TJ, Jette N, Scheffer IE, de Curtis M and Perucca P: Epilepsy. Nat Rev Dis Prim 2018; 4: 18024.
7. Fisher R, van Emde Boas W, Blume W, Elger C, Genton P, Lee P and Engel J: Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 2005; 46: 470-72.
8. De Boer HM, Mula M and Sander JW: The global burden and stigma of epilepsy. Epilepsy Behav 2008; 12: 540-546.
9. Scharfman HE: The neurobiology of epilepsy. Curr Neurol Neurosci Rep 2007; 7: 348-54.
10. Goldenberg MM: Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. Pharmacy and Therapeutics 2010; 35: 392-15.
11. Borghese CM, Herman M, Snell LD, Lawrence KJ, Lee H-Y, Backos DS, Vanderlinden LA, Harris RA, Roberto M, Hoffman PL and Tabakoff B: Novel molecule exhibiting selective affinity for $GABA_A$ receptor subtypes. Sci Rep 2017; 7: 6230.
12. Czuczwar SJ and Patsalos PN: The new generation of GABA enhancers. Potential in the treatment of epilepsy. CNS Drugs 2001; 15: 339-50.
13. Steiger JL and Russek SJ: $GABA_A$ receptors: building the bridge between subunit mRNAs, their promoters, and

cognate transcription factors. Pharmacol Ther 2004; 101: 259-81.
14. Pollard JR and French J: Antiepileptic drugs in development. Lancet Neurol 2006; 5: 1064-67.
15. Macdonald RL and Kang JQ: Molecular pathology of genetic epilepsies associated with GABA (A) receptor subunit mutations. Epilepsy Curr 2009; 9: 18-23.
16. Tretter V, Ehya N, Fuchs K and Sieghart W: Stoichiometry and assembly of a recombinant GABAA receptor subtype. J Neurosci 1997; 17: 2728-37.
17. Baumann SW, Baur R and Sigel E: Forced subunit assembly in alpha1 beta2 gamma2 GABAA receptors. Insight into the absolute arrangement. J Biol Chem 2002; 277: 46020-25.
18. Sigel E and Steinmann ME: Structure, function and modulation of GABA(A) receptors. J Biol Chem 2012; 287: 40224-31.
19. Olsen RW and Sieghart W: International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric acid A Receptors: classification on the basis of subunit composition, pharmacology, and function. Update Pharmacol Rev 2008; 60: 243-60.
20. Olsen RW and Sieghart W: $GABA_A$ receptors: subtypes provide a diversity of function and pharmacology. Neuropharmacology. 2009; 56: 141-48.
21. Nsour WM, Lau CB and Wong IC: Review on phytotherapy in epilepsy. Seizure 2000; 9: 96-107.
22. Sorensen AT and Kokaia M: Novel approaches to epilepsy treatment. Epilepsia 2013; 54: 1-10.
23. Pohl T, Crouch NR and Mulholland DA: Southern African Hyacinthaceae: chemistry, bioactivity and ethnobotany. Curr Org Chem 2000; 4: 1287-24.
24. Pohl T, Koorbanally C, Crouch NR and Mulholland DA: Secondary metabolites of *Scilla plumbea*, *Ledebouria cooperi* and *Ledebouria ovatifolia* (Hyacinthaceae). Biochem Syst Ecol 2001; 29: 857-60.
25. Mutanyatta J, Matapa BG, Shushu DD and Abegaz BM: Homoisoflavonoids and xanthones from the tubers of wild and in vitro regenerated *Ledebouria graminifolia* and cytotoxic activities of some of the homoisoflavanoids. Phytochemistry 2003; 62: 797-04.
26. Meng XY, Zhang HX, Mezei M and Cui M: Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 2011; 7: 146-57.
27. Miller PS and Aricescu AR: Crystal structure of a human GABAA receptor. Nature 2014; 512: 270-75.
28. Moodley N, Crouch NR, Mulholland DA, Slade D and Ferreira D: 3-Benzyl-4-chromanones (homoisoflavanones) from bulbs of the ethnomedicinal geophyte *Ledebouria revoluta* (Hyacinthaceae). South African Journal of Botany 2006; 72: 517-20.
29. Rao SN, Head MS, Kulkarni A and Lalonde JM: Validation studies of the site-directed docking program LibDock. J Chem Inf Model 2007; 47: 2159-71.

How to cite this article:
Veena BS and Sujatha E: *In-silico* molecular docking analysis of isolated homoisoflavanones from bulbs of *Ledabouria revoluta* as $GABA_A$ receptor inhibitors. Int J Pharm Sci & Res 2019; 10(9): 4315-21. doi: 10.13040/IJPSR.0975-8232.10(9).4315-21.