

Original Research Article

DOI - 10.26479/2017.0304.01

## COMPUTATIONAL STUDIES ON THE IDENTIFICATION OF POTENTIAL LEADS FROM WINE POLYPHENOLS FOR METABOTROPIC GLUTAMATE RECEPTOR4 (MGLUR4)

S Devarajan<sup>1\*</sup>, A Subhash<sup>2</sup>

1.School of Biotechnology & Bioinformatics, D Y Patil University, Navi Mumbai 400614

2.School of Biotechnology & Bioinformatics, D Y Patil University, Navi Mumbai 400614

---

**ABSTRACT:** Parkinson's disease (PD) is caused by the alteration of dopamine neurons in the basal ganglia and results in motor symptoms such as tremor and bradykinesia. Activation of metabotropic glutamate receptor 4 (mGluR4) has been shown to modulate neurotransmission in the basal ganglia and results in antiparkinsonian effects in rodent PD models. There are numerous studies indicating that a moderate consumption of red wine provides the protection against neurodegenerative diseases due to the presence of phenolic compounds in wine. Studies have showed that polyphenols found in grape seed extract affects the metabotropic glutamate receptor agonist response in group I mGlu receptors. However, the molecular interaction and conformation between the wine polyphenol and mGluR4 is not available. In our presented study, we designed the 3D model for mGluR4, performed virtual screening and docking studies against wine polyphenols. Further we performed a molecular dynamics simulation to study the conformation. We have proposed Myricitin compound which showed the minimum energy score (-7.3 Kcal/Mol) considered as a potential positive allosteric modulator for mGluR4.

---

**KEYWORDS:** metabotropic glutamate receptor 4, wine polyphenols, flavonoids, 2D fingerprints, 3D pharmacophore, molecular docking, molecular dynamics simulation..

---

**\*Corresponding Author: Dr. Shine Devarajan Ph.D.**

School of Biotechnology & Bioinformatics, D Y Patil University, Navi Mumbai 400614

\* Email Address: sdshine@gmail.com

## 1. INTRODUCTION

Metabotropic glutamate (mGlu) receptors belong to the GPCR family and mediate slow, modulatory neurotransmission of glutamate, the primary excitatory neurotransmitter in the mammalian brain [1]. The identified eight mGlu receptors (mGlu1-mGlu8) have been categorized into three groups, I, II, and III, based on sequence homology, second messenger coupling and pharmacological characterization. The mGlu4 receptor together with mGlu6, mGlu7 and mGlu8 receptors belongs to group III mGlu receptors, is a predominantly presynaptic receptor, regulating release of glutamate (as an auto receptor) or GABA (as a heteroreceptor). As abnormalities in the balance of glutamate and GABA neurotransmission have been linked to the etiology of several neuropsychiatric and movement disorders, activation of the mGlu4 receptor may help to restore the balance between these two key neurotransmission systems and lead to therapeutic outcomes. This activation has been considered as a promising therapeutic approach for the treatment of Parkinson's disease, anxiety, schizophrenia, depression, chronic pain, epilepsy and addiction [2]. Three group III mGluR subtypes (mGluRs 4, 7, and 8) are expressed in the basal ganglia, a group of brain nuclei that are involved in the control of motor function and are critical to the motor deficits observed in Parkinson's disease (PD). It is interesting that activation of mGluR4 reduces transmission at a key basal ganglia synapse (striatopallidal synapse) that is believed to be overactive in patients with PD, and this effect is lost in mGluR4 knockout animals [3]. Due to the lack of selectivity of orthosteric ligands for the mGluRs, much effort has now been directed at identifying and examining the utility of compounds that act via allosteric sites on the receptor. This strategy has been successfully employed for other G-protein-coupled receptors, including mGluR 1, 2 and 5. These allosteric compounds can regulate receptor function in both positive and negative directions. Positive allosteric modulators (PAMs) have little or no effect on the receptor alone but can dramatically potentiate the effects of the endogenous ligand [4]. Wine and grape vine polyphenols are mainly flavonoids (flavanols, flavonols, and anthocyanins) and nonflavonoids (phenolic acids, hydrolysable tannins, and stilbenes) [5]. Extensive investigations have been undertaken to determine the neuroprotective effects of wine polyphenols [6, 7, 8] Several neuroprotective mechanisms of action have been proposed, suggesting that polyphenols exert their activities by reducing the production and the accumulation of Reactive oxygen species (ROS), whose accumulation is likely to play a crucial pathological role in brain aging, reducing oxidative stress and inflammation and modulating the activity of intracellular signal transduction molecules. There is evidence that polyphenols found in grape seed extract affects the metabotropic glutamate receptor agonist response in group I mGlu receptors [9]. In the present research, we aim to study the novel drug target for Parkinson's disease: (1) to predict the structural model of mGluR4, (2) to perform virtual screening and docking to identify agonist molecules from wine polyphenols, and (3) to investigate structural analysis of mGluR4-ligand complex using molecular dynamics simulation studies to study the conformation of the structure.

## **2. MATERIALS AND METHODS**

### **Ligand selection and Dataset**

Phenol-Explorer [10] is a comprehensive database on polyphenol which has the contents especially present in food. The ligands were retrieved from Phenol-Explorer for our analysis. 46 polyphenolic compounds under Flavonoids class of Red Wine were retrieved from the Phenol-Explorer database. The ligands were downloaded as .sdf file format from Phenol-Explorer. These files were converted into the SMILES strings using Open Babel software [12]. The chemical structure of the Flavonoids class of the Red Wine Polyphenol was collected from previous published literature [11]. These structures were used as an initial data set during computational docking procedure to study interactions with the binding site of the mGluR4.

### **Structure Similarity search**

The structural similarity search was implemented using Open Babel [12]. The Tanimoto coefficient [13] is used to measure the 2D similarity. It compares the structural similarity between the reference molecule and the lead compounds using a concatenated fingerprint. The MACCS fingerprint as implemented in Open Babel. The MACCS fingerprint is a bit string registering the presence or absence of structural features (MACCS stands for Molecular ACCESS System). The MACCS fingerprints are widely used and have been found to be among the best 2D fingerprints, even surpassing 3D search methods [14,15]. The Tanimoto coefficient generates a number between 0 and 1 (1 corresponding to 'maximum similarity') [16]. Hesperetin (DB01094) was taken as reference for similarity search against 46 flavonoids because it is an approved drug in DrugBank database [17].

### **3D Pharmacophore analysis**

Training set of 10 polyphenols were selected from the results of a previous study. LigandScout [18] was used to build the pharmacophore model. In the present study, four features i.e., hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), ring aromatic (RA) and hydrophobic (HY) were selected to generate the pharmacophore hypotheses. It generates pharmacophore model based on chemical features of active compounds in training set. The generated pharmacophore model was compared and select based on the Pharmacophore fit score.

### **Target Structure prediction**

The SWISS-MODEL based homology modeling program [19] was used for the development of the mGluR4 receptor model. A sequence alignment was performed using the BLAST algorithm. mGluR7 receptor (PDB ID: 2E4Z) [20] was used as the template structure because it had the highest percentage of sequence identity in the sequence alignment (68%). Protein models were generated from the alignment in a stepwise manner. The backbone coordinates for the aligned positions were extracted from the template and the regions of insertions/deletions in the alignment were found by searching either a loop library or a conformational space search using constraint space programming. The templates were weighted by their sequence similarity to the target sequence and outlier atomic

S Devarajan & A Subhash RJLBPCS 2017 www.rjlbpcs.com Life Science Informatics Publications  
positions were excluded. Predicted model was refined by minimizing the energy through Swiss PDB viewer [21].

### **Active site prediction**

Potential active sites and respective amino acids were identified by Computed Atlas of Surface Topography of protein (CASTp) server (<http://sts-fw.bioengr.uic.edu/castp/>) [22] which is essential for Virtual screening and Docking. CASTP identified and measure pockets and pocket mouth openings, as well as cavities. The CASTP server predicts the amino acids crucial for binding interactions. Studies showed that amino acids Arg78, Thr182 and Ser159 are key residues of ligand binding domain [23]. The active pocket (active site) with more volume and area was taken for further analysis.

### **Virtual Screening**

iGEMDOCK [24] is an integrated virtual screening (VS) environment for screening purpose. It performed through post-screening analysis with pharmacological interactions. This provides interactive interfaces to prepare both the binding site of the target protein and the screening compound library. Each compound in the library is then docked into the binding site by using the in-house docking tool iGEMDOCK. Subsequently, it generates protein-compound interaction profiles of electrostatic (E), hydrogen-bonding (H), and Van der Waal's (V) interactions. Based on these profiles and compound structures, the program infers the pharmacological interactions and clusters the screening compounds for the post-screening analysis. It give models based on ranking and visualized the screening compounds by combining the pharmacological interactions and energy-based scoring function of iGEMDOCK. From the most favored compounds in the binding site, two structures were selected based on H-bond interaction and Van der Waals energy in the ligand binding domain.

### **Docking studies**

Metabotropic glutamate receptor 4 protein was used for the docking studies. AutoDock Vina [25] was used for all docking analysis in the present study. In general, the docking parameters for AutoDock and Vina were kept to their default values. Intermediary steps, such as .pdbqt files for protein and ligands preparation and grid box creation were performed using Graphical User Interface program known as AutoDock tools. It assigned polar hydrogens and added Kollman charges to the protein. AutoGrid was used for the preparation of the grid map using a grid box. AutoDock/Vina was employed for docking using protein and ligand structures along with grid box properties in the configuration file. AutoDock/Vina employs iterated local search global optimizer [26, 27]. The poses with lowest energy of binding or binding affinity were extracted and aligned with the receptor structure for further analysis.

### **Molecular dynamics stimulation**

MD simulation was conducted for modeled system (i.e. mGluR4) and ligand (i.e. Myricitin) in explicit solvent using the GROMACS 4.5.5 [28, 29] package with GROMOS96 43A1 force field [30].

S Devarajan & A Subhash RJLBPCS 2017 www.rjlbpcs.com Life Science Informatics Publications  
The Dundee PRODRG2.5 servers was used to generate the topology parameters of Myricitin [31]. The complex structure was solvated with water molecules in a dodecahedron box with edges that were 1.0 nm from the molecular boundary. To obtain a neutral system, six CL ions were added (charge +6.00) to the system by replacing solvent molecules. The solvated system was then subjected to further energy minimizations (maximum number of steps: 50000) to remove steric conflicts between the protein and water molecules, using the steepest descent integrator. Convergence was achieved when maximum force was smaller than 1000 kJ mol<sup>-1</sup> nm<sup>-1</sup>. The energy-minimized models were subjected to position-restrained MD under NVT and NPT conditions, keeping the number of particles (N), the volume (V), the system pressure (P) and the temperature (T) constant. We subsequently applied LINCS [32] constraints for all bonds, keeping the whole protein molecule fixed and allowing only the water molecule to move to equilibrate with respect to the protein structure. This was carried out for 50,000 steps for a total of 100 ps. Snapshots of the trajectory were taken every 1 ps. The final MD of 500,000 steps was carried out for 1,000 ps (1 ns) using the particle mesh Ewald (PME) electrostatics method under NVT and NPT conditions [33].

### **3. RESULTS AND DISCUSSION**

#### **Structure validation of modeled structure and active site prediction of mGluR4**

In our study, we predicted the model of mGluR4 (Fig. 1) and validated using PROSA server. [34, 35]. We evaluated the structure using Ramachandran plot [36] and observed that the phi/psi angles of the majority of the residues (88.7%) are located in the most favored regions, followed by 9.2% residues, were present in the additional allowed regions, whereas 2.1% residues present in the disallowed regions. The present findings gave the impression that the proposed model is stereochemically stable. We further evaluated Z-score for the overall quality calibration of the model. All the Z-scores of the experimentally determined protein chains in current PDB were plotted (Fig. 2). In this plot, groups of structures from different sources (X-ray, NMR) are distinguished using different colors where dark blue represents NMR and light blue represents X-ray scores respectively. The Z-score of the predicted mGluR4 model is found to be within the range of scores, which are typically predicted for native proteins of similar size. The energy plot of the predicted model show local model quality as visualized by plotting energies as a function of amino acid sequence position (Fig. 3). The calculated energy for the predicted model of mGluR4 is negative, which indicates an error free model of the mGluR4 as positive values correspond to problematic or erroneous parts of the input structure.

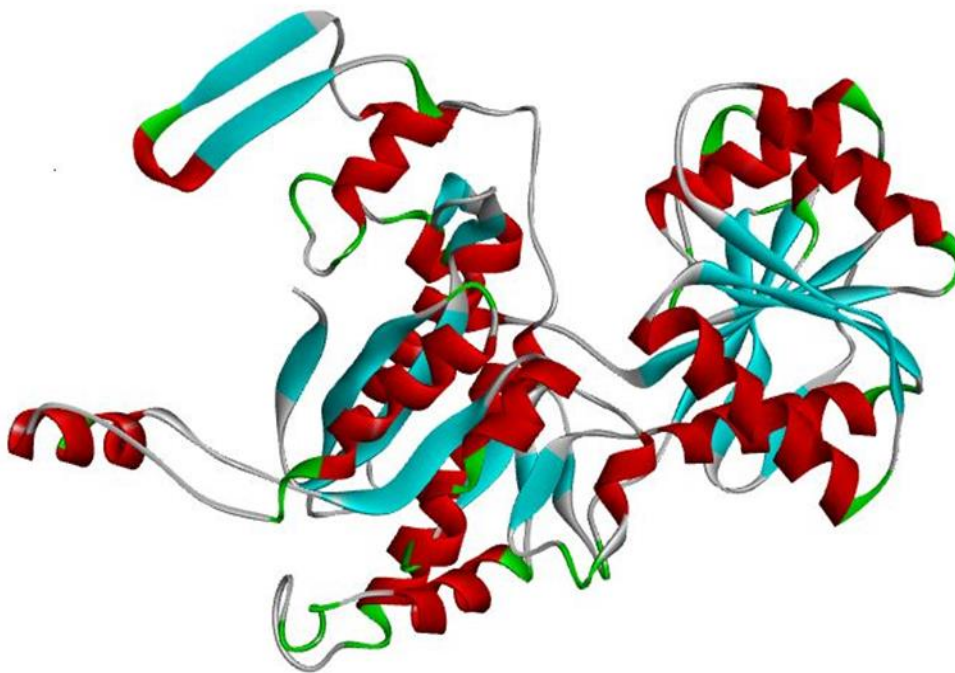


Fig.1. Predicted model of mGluR4 represented in cartoon representation

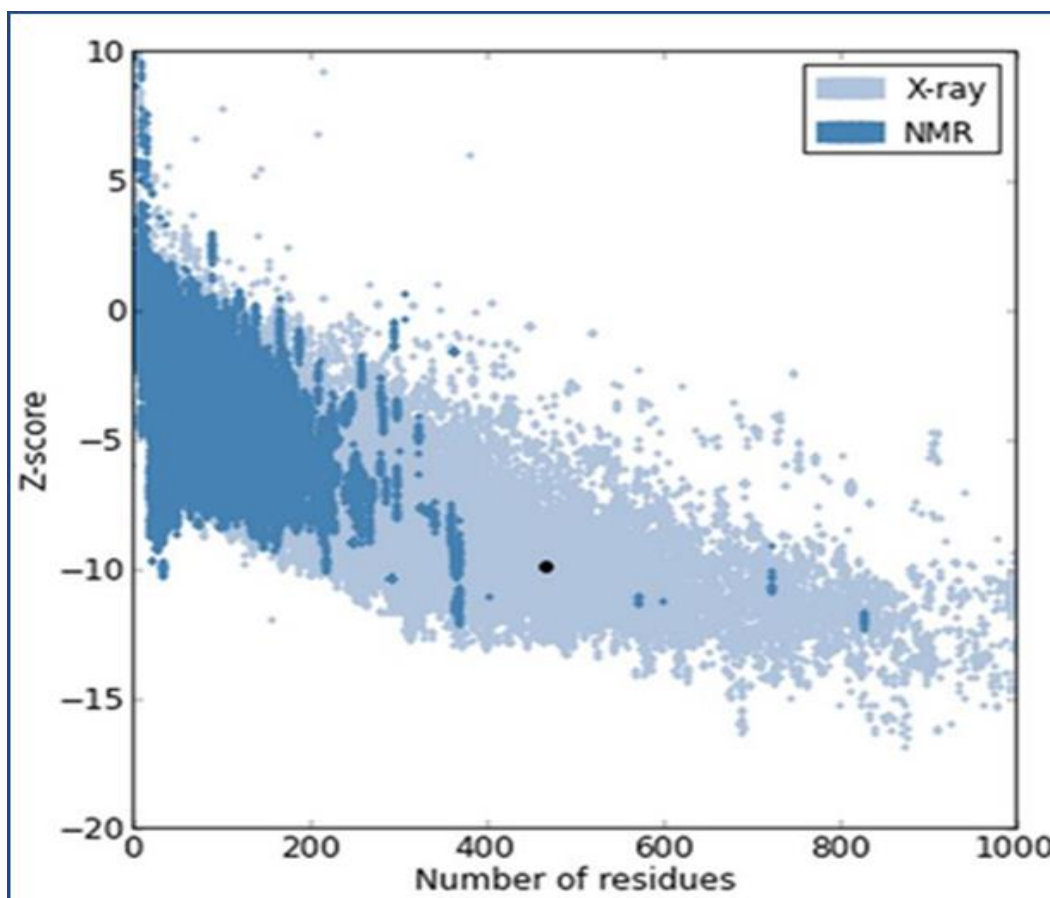


Fig.2. Z-score calculated for predicted model of mGluR4 by PROSA. The model can be visualized as a dark spot in the light blue shaded region.

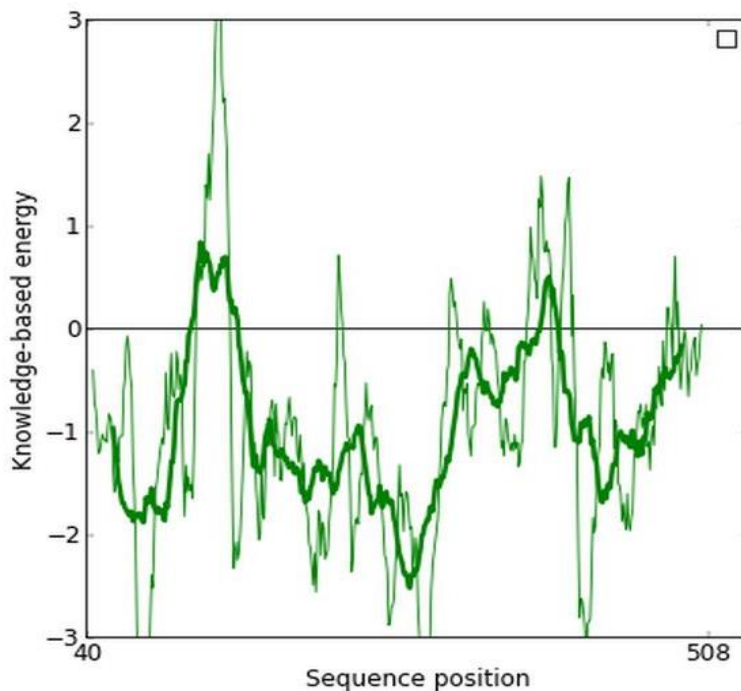


Fig.3. Energy calculated for predicted model of mGluR4 by PROSA.

CastP web server (<http://sts-fw.bi+oengr.uic.edu/castp/calculation.php>) is used to predict the active site of mGluR4. The potential binding pockets are identified and displayed (Fig. 4). The active site residues involved in ligand binding site are serine159, arginine78, and threonine182 [23].

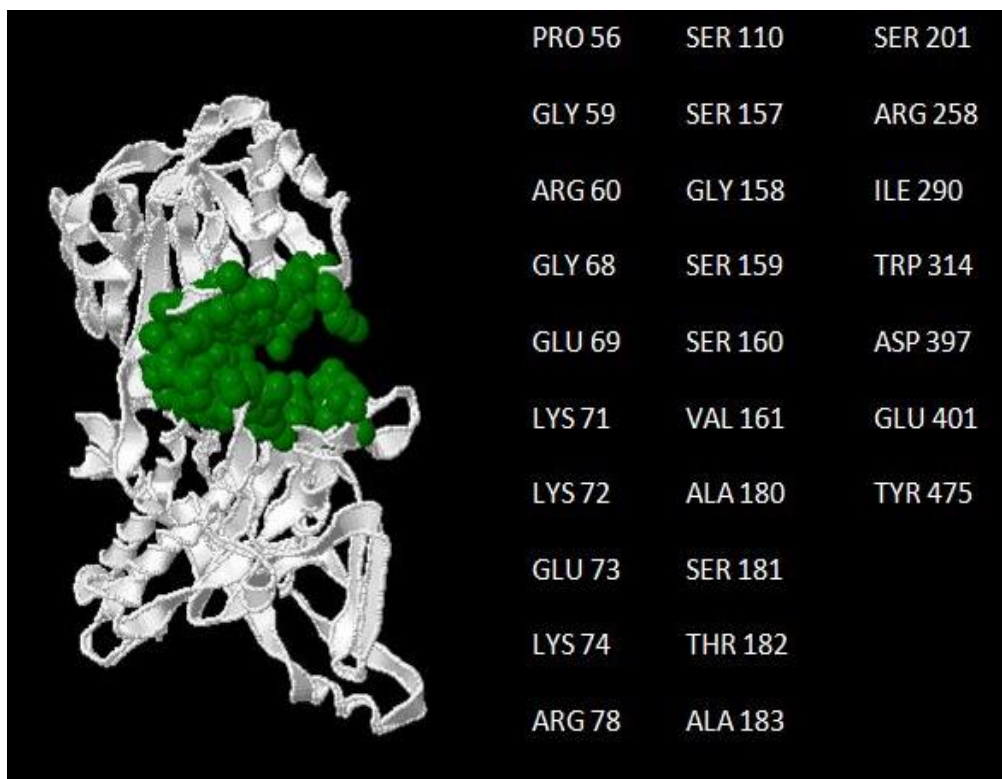


Fig.4. Active site amino acids of mGluR4 receptor identified by CastP

**Structure Similarity search and Pharmacophore analysis**

MACCS Fingerprints for 46 compounds was generated and the corresponding Tanimoto values were calculated using OpenBabel [12]. Top 10 molecules were selected whose Tanimoto coefficient was 0.8 and above for which Hesperetin compound kept as a reference (Table 1). Data sets of 10 molecules were selected to develop pharmacophore in Ligandscout. Four features hydrogen bond donor (HBD), hydrogen bond acceptors (HBA), ring aromatics (RA) and hydrophobic (HY) were selected. All 10 hypotheses were generated with the following features - HBA, HBD, RA and HY. The Pharmacophore feature patterns summarized in Fig. 5. Top 9 compounds were selected based fit score (Table 2).

**Table 1. Compounds and Tanimoto Coefficients**

No.	Compounds	Tanimoto coefficient with hesperetin
1	Isorhamnetin	0.9375
2	Naringenin	0.84375
3	Kaempferol	0.84375
4	Dihydromyricetin 3-O-rhamnoside	0.828571
5	Dihydroquercetin 3-O-rhamnoside	0.828571
6	catechin 5-O-gallate	0.828571
7	Quercetin 3-O-rhamnoside	0.828571
8	Myricetin	0.818182
9	Quercetin	0.818182
10	Isorhamnetin	0.8

**Table 2. Compounds and Pharmacophore fit score**

No.	Compounds	Pharmacophore Fit score
1	Kaempferol	110.97
2	Quercetin	110.93
3	Isorhamnetin	110.42
4	Isorhamnetin 3-O-glucoside	110.41
5	Dihydromyricetin 3-O-rhamnoside	110.32
6	Dihydroquercetin 3-O-rhamnoside	104.01
7	Quercetin 3-O-rhamnoside	103.1
8	Naringenin	102.84
9	Myricetin	102.76
10	Hesperetin	101.74



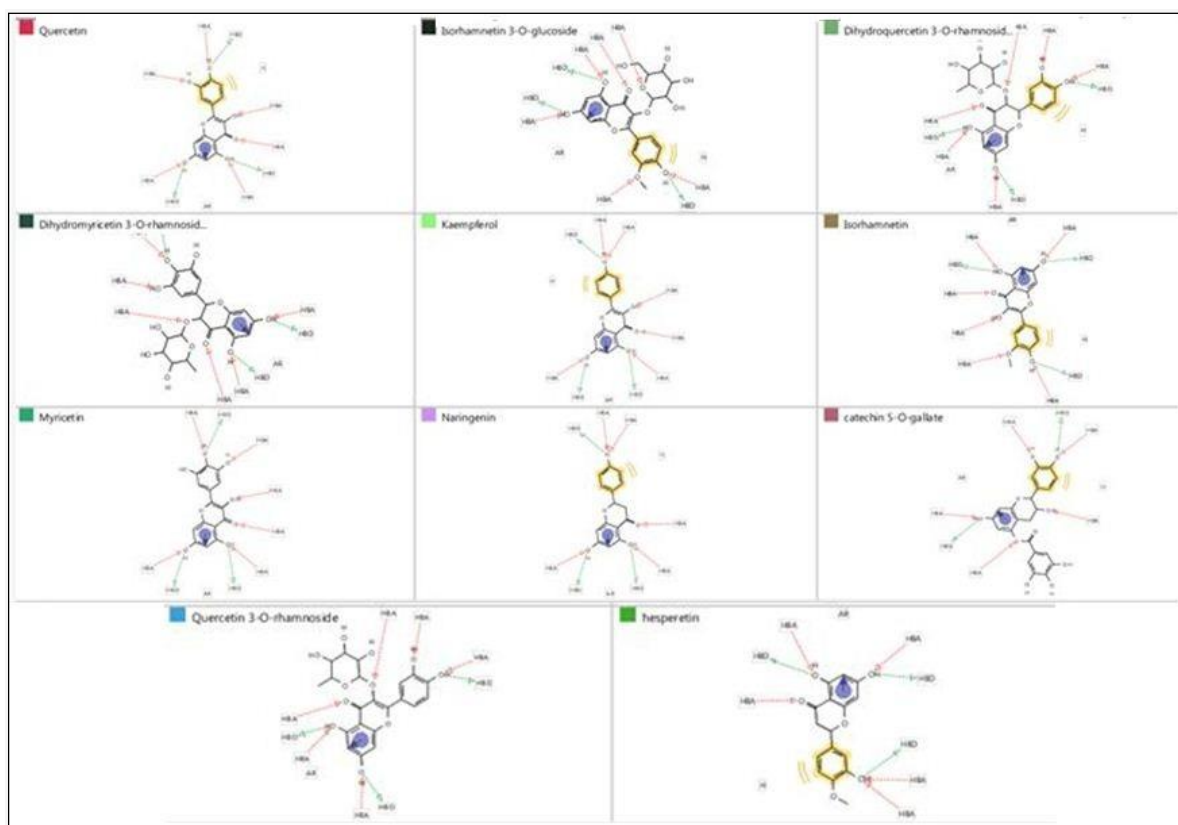


Fig.5. Pharmacophore features of compounds generated by LigandScout.

### Virtual screening and Docking Studies

The virtual screening technique was used for the screening purpose and we select Myricetin and Hesperetin which can possess good interaction profile with the target protein mGluR4. The ligands were interacting with the key amino acids Arg78, Thr182 and Ser159 which are residues of ligand binding domain (Fig. 6). Myricetin and Hesperetin were used for further docking analysis. Using AD4 and Vina, Hesperetin and Myricetin were docked against mGluR4. Autodock Vina treats docking as a stochastic global optimization of the scoring function and precalculating grid maps and interaction between all atom type pair at every distance. From the results of each program, the compounds were ranked based on their predicted binding energies (Table 3). Results suggest that Myricetin had a binding energy of -7.3 kcal/mol, which is comparatively lesser than Hesperetin compound. Docked pose was visualized using Chimera [37] and shown in (Fig. 7).

**Table 3. Selected compound and Binding affinity with mGluR4**

No.	Compounds	Binding Affinity(kcal/mol)
1.	Myricetin	-7.3
2.	Hesperetin	-7.0



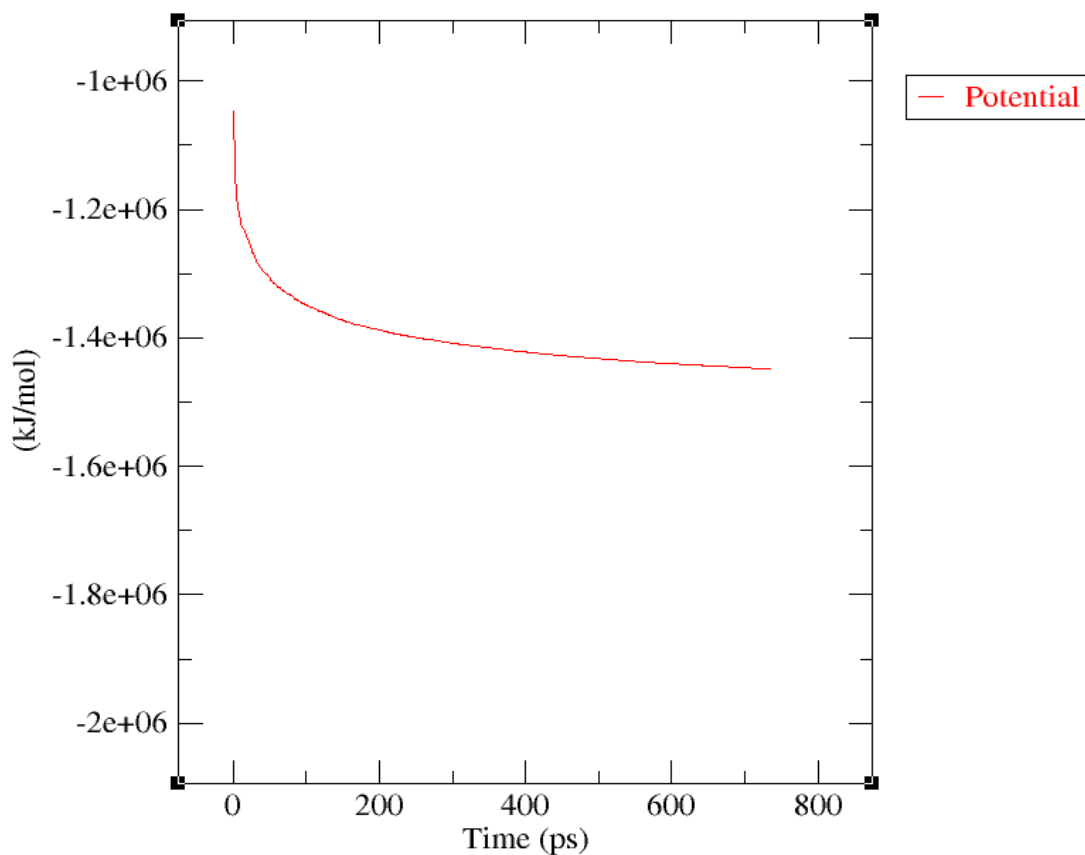


Fig.8. Energy of the mGluR4-Myricitin complex system during simulation for 1 ns

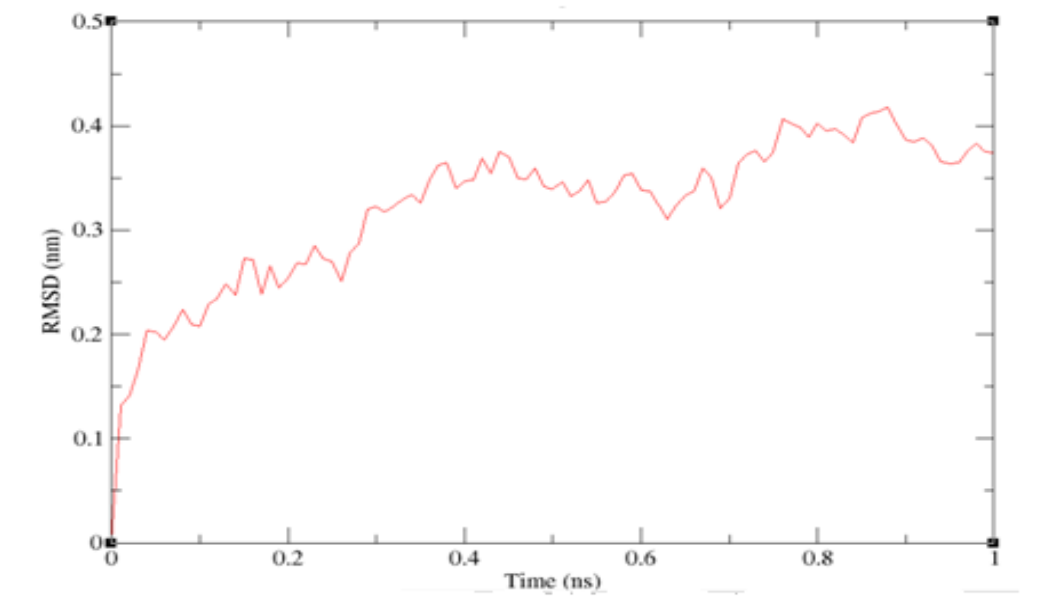


Fig.9. RMSD of mGluR4-Myricitin complex structure at 1 ns

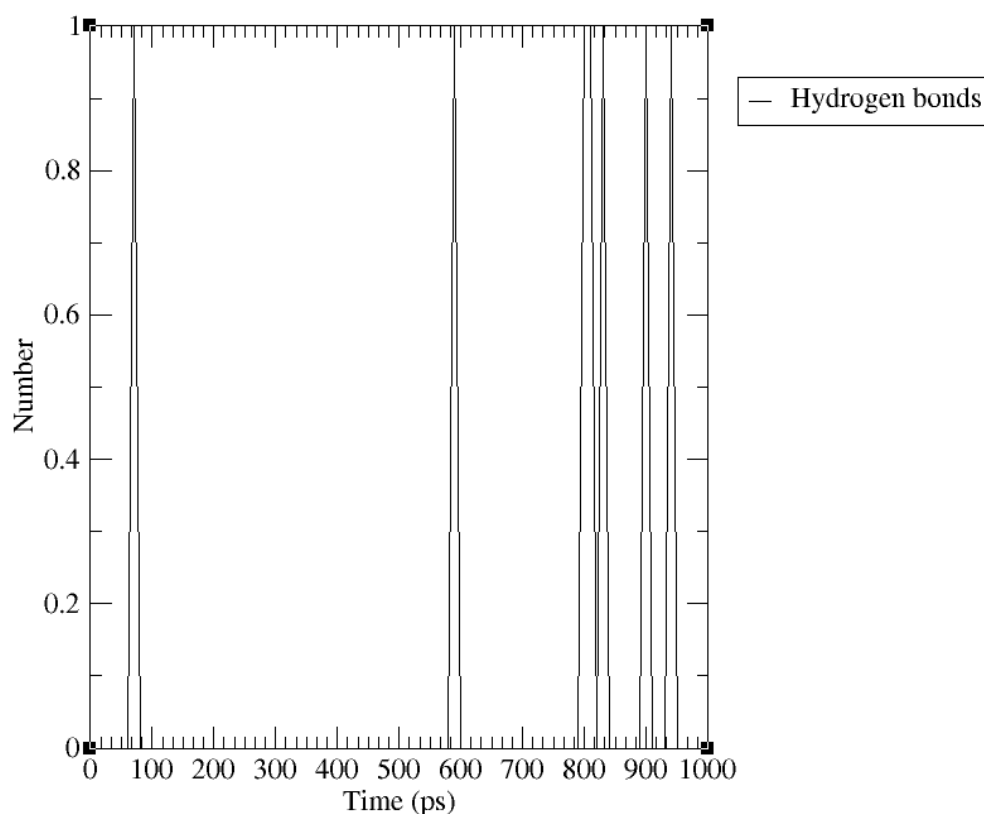


Fig.10. Hydrogen bonds shown in mGluR4-Myricitin complex structure after 1 ns MD simulation.

The peaks indicate the highest number of H-bonds at respective time intervals.

In our study, we have selected mGluR4 as the most promising drug target for the drug agonist designing. The PDB structure of mGluR4 was not available; therefore, it was predicted and validated with the help of comparative modeling. 2D fingerprint and 3D pharmacophore based screening was performed on Flavonoids class from Red Wine Polyphenol. The virtual screening for mGluR4 was carried out against the Flavonoids class, which revealed the top two candidates and indicated strong binding affinity for mGluR4. We further demonstrated that Myricitin, having the minimum energy score ( $-7.3$  Kcal/Mol), possesses the highest binding affinity towards the mGluR4 and can be a potential agonist on the basis of the interactions with residues of the active site of mGluR4. MD simulation showed that mGluR4-Myricitin complex has a good energy and comparatively stable structure. Notably, from the results we can conclude that myricitin is one of the promising agonist compounds for mGluR4 receptor and thus can be used for further in vitro studies. Thus, the present study shed light on the computational aspects of some favorable flavonoid compounds for the treatment of Parkinson's disease.

#### CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

#### ACKNOWLEDGEMENT

The authors are highly grateful to School of Biotechnology and Bioinformatics, D. Y. Patil University, Navi Mumbai for providing the resources and support for the current work.

## REFERENCES

1. Niswender CM, Johnson KA, Weaver CD, Jones CK, Xiang Z, Luo Q Rodriguez AL, Marlo JE, de Paulis T, Thompson AD, Days EL, Nalywajko T, Austin CA, Williams MB, Ayala JE, Williams R, Lindsley CW, Conn PJ. Discovery, Characterization, and Antiparkinsonian Effect of Novel Positive Allosteric Modulators of Metabotropic Glutamate Receptor 4. *Mol Pharmacol.* 2008; 74:1345–1358.
2. Celanire S and Campo B. Recent advances in the drug discovery of metabotropic glutamate receptor 4 (mGluR4) activators for the treatment of CNS and non-CNS disorders. *Expert Opin Drug Discov.* 2012; 7:261-80. doi: 10.1517/17460441.2012.660914.
3. Valenti O, Mannaioni G, Seabrook GR, Conn PJ, and Marino MJ. Group III metabotropic glutamate-receptor-mediated modulation of excitatory transmission in rodent substantia nigra pars compacta dopamine neurons. *J Pharmacol Exp Ther.* 2005; 313:1296–1304.
4. Corey RH, Craig WL, Colleen MN. mGluR4-positive allosteric modulation as potential treatment for Parkinson's disease. *Future Med Chem.* 2009; 1: 501–513. doi:10.4155/fmc.09.38.
5. Waterhouse AL. Wine phenolics. *Annals of the New York Academy of Sciences.* 2002; 957:21–36.
6. Sun Y, Simonyi A, Sun G.Y. The French paradox and beyond: neuroprotective effects of polyphenols. *Free Radical Biology and Medicine.* 2002; 32:314–318.
7. Assuncao M, Santos-Marques MJ, De Freitas V, Carvalho F, Andrade JP, Lukoyanov NV, Paula-Barbosa MM. Red wine antioxidants protect hippocampal neurons against ethanol-induced damage: A biochemical, morphological and behavioral study. *Neuroscience.* 2007; 146:1581-1592.
8. Bastianetto S. Red wine consumption and brain aging. *Nutrition.* 2002; 18: 432–433.
9. Ahn SH, Kim HJ, Jeong I, Hong YJ, Kim MJ, Rhie DJ, Jo YH, Hahn SJ, Yoon SH. Grape seed proanthocyanidin extract inhibits glutamate-induced cell death through inhibition of calcium signals and nitric oxide formation in cultured rat hippocampal neurons. *BMC Neurosci.* 2011; 12-78.
10. Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalbert A. Phenol-Explorer: An online comprehensive database on polyphenol contents in foods. *Database.* 2010; bap024. doi: 10.1093/database/bap024.
11. Abdelkader B, Stéphanie S. Nassima C, Jean-Michel M, Mohamed C, Jean-Pierre M, Tristan R. Wine Polyphenols: Potential Agents in Neuroprotection Oxidative Medicine and Cellular Longevity. 2012; 2012.
12. O'Boyle NM, Banck M, James C, Morley C, Vandermeersch T, Hutchison GR. Open Babel: an open chemical toolbox. *J. Cheminform.* 2011; 3:33-47.

13. Delaney JS. Assessing the ability of chemical similarity measures to discriminate between active and inactive compounds. *Mol. Divers.* 1996; 1:217-222.
14. Brown RD, Martin YC. The information content of 2D and 3D structural descriptors relevant to ligand-receptor binding. *J. Chem. Inf. Comput. Sci.* 1997; 37: 1-9.
15. Bender A, Mussa HY, Glen RC, Reiling, S. Molecular similarity searching using atomic environments, information-based feature selection and a naïve Bayesian classifier. *J. Chem. Inf. Comput. Sci.* 2004; 44: 170-178.
16. Martin YC, Kofron JL, Traphagen LM. Do structurally similar molecules have similar biological activity? *J. Med. Chem.* 2002; 45:4350-4358.
17. Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M. DrugBank: a knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Res.* 2008; 36:D901-6.
18. Gerhard W, Thierry L. LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters. *J. Chem. Inf. Model.* 2005; 45:160–169. DOI: 10.1021/ci049885e
19. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. *Bioinformatics.* 2006; 22: 195-201. doi: 10.1093/bioinformatics/bti770
20. Muto T, Tsuchiya D, Morikawa K, Jingami H. Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. *Proc Natl Acad Sci U S A.* 2007; 104:3759-64.
21. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis.* 1997; 18: 2714-2723
22. Binkowski TA, Naghibzadeh S, Liang J CASTp: Computed Atlas of Surface Topography of proteins. *Nucleic Acids Res.* 2003; 31:3352-55.
23. David RH, Xi-Ping H, Roman P, Vanya P, Geoffrey H, Christian T, Henning T. Probing the Ligand-binding Domain of the mGluR4 Subtype of Metabotropic Glutamate Receptor, *The Journal of Biological Chemistry.* 1999; 274:33478-33495.
24. Yang JM, Chen CC. GEMDOCK: a generic evolutionary method for molecular docking. *Proteins: Structure, Function, and Bioinformatics.* 2004; 55:288–304. doi: 10.1002/prot.20035
25. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *Journal of Computational Chemistry.* 2010; 31 455-461.
26. Baxter J. Local optima avoidance in depot location. *J Oper Res Soc.* 1981; 32:815-819.

27. Blum C, Blesa MJ, Roli A, Sampels M. Hybrid Metaheuristics: An Emerging Approach to Optimization. Studies in Computational Intelligence. Berlin Heidelberg: Springer-Verlag. 2008; 114.
28. Berendsen HJC, van der Spoel D, van Drunen R. GROMACS: A message-passing parallel molecular dynamics implementation. Computer Physics Communication. 1995; 91, 43–56. DOI: 10.1016/0010-4655(95)00042
29. Hess CK, Van Der Spoel D, Lindahl E. GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. Journal of Chemical Theory and Computation. 2008; 4: 435–447.
30. van Gunsteren WF, Billeter SR, Eising AA, Hünenberger PH, Krüger P, Mark AE, Scott WRP, Tironi IG. Biomolecular Simulation: The GROMOS96 Manual and User Guide. Vdf Hochschulverlag AG an der ETH Zürich, Zürich, Switzerland. 1996; 1-1042.
31. Schüttelkopf AW, van Aalten DMF. PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. Acta Crystallographica D. 2004; 60: 1355–1363. DOI: 10.1107/s0907444904011679.
32. Hess HB, Berendsen HJC. Fraaije JGEM. LINCS: a linear constraint solver for molecular simulations. Journal of Computational Chemistry. 1997; 18: 1463–1472.
33. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A smooth particle mesh Ewald method. The Journal of Chemical Physics. 1995; 103: 8577–8593.
34. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Research. 2007; 35, W407-W410.
35. Sippl M.J. Recognition of Errors in Three-Dimensional Structures of Proteins. Proteins. 1993; 17:355-62.
36. Lovell SC, Davis IW, Arendall WB 3rd, de Bakker PI, Word JM, Prisant MG, Richardson JS, Richardson DC. Structure validation by C alpha geometry: phi, psi and Cbeta deviation. Proteins Struct Funct Genet. 2003; 50:437–450. doi: 10.1002/prot.10286.
37. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin, TE. UCSF Chimera—a visualization system for exploratory research and analysis. J. Comput Chem. 2004; 25:251605–1612.