INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 10, Issue 4, 2022

ISSN - 2321-4406

Research Article

IN SILICO ADME ANALYSIS AND MOLECULAR DOCKING APPLIED TO FLAVONOIDS TO FIND DRUG LEAD COMPOUNDS TARGETING DRD4

Mildred Garcia[1], Vaeeshnavi Buwa[2]*

[1]Department of Biology, Mindanao State University – Iligan Institute of Technology, Iligan, Philippines, [2]Department of Bioinformatics, BioNome, Bengaluru, Karnataka, India. Email: info@bionome.in

Received: 25 May 2022, Revised and Accepted: 25 June 2022

ABSTRACT

Objective: The aim of this present study is to find flavonoids that can be potential drug lead compounds targeting the human D(4) Dopamine receptor (DRD4). Thirty-nine flavonoids were collected from the literature survey, and 23 of them were predicted by SwissTargetPrediction to have bioactivity toward DRD4.

Methods: ADME properties were evaluated, and molecular docking was executed. Among the flavonoids studied, Isovitexin, Glabridin, and Glabrone have shown better binding energy than the native ligand, Nemonapride. However, ADME analysis has demonstrated that Isovitexin has low GI absorption and is in the grey zone of the BOILED-egg. Glabridin is a BBB permeant but is a P-gp substrate. Glabrone has high GI absorption, and a P-gp non-substrate but not a BBB permeant.

Results and Conclusion: The experimental investigations and clinical evaluations are recommended to examine the mechanisms of their actions and other pharmacological effects and to validate the results of this *in silico* study. The scaffolds of these compounds can also be optimized to improve the few lapses and have better attributes as CNS drug lead candidates.

Keywords: ADME, Molecular docking, Flavonoids, DRD4, Dopamine receptors, Glabrone.

 DOI: http://dx.doi.org/10.22159/ijms.2022v10i4.45114. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

INTRODUCTION

The Human Dopamine Receptor D4 (DRD4) is a member of the G-protein coupled receptor (GPCR) family and is known for its importance in neuronal signaling in the brain's mesolimbic system, an area vital for the regulation of emotion and complex behavior [1]. DRD4 receptor plays multiple essential roles in the central nervous system (CNS), such as the mediation of corticostriatal neurotransmission by controlling the activity of glutamate receptors, carrying out phospholipid methylation, and affecting the kinetics of ion channels [2,3] which are vital for the synaptic strength and the modulation of neuronal firing activity that is impaired in Attention Deficit Hyper Disorder. A network biology studies approach conducted by Verma *et al*. [4] have shown DRD4 as a target protein based on network parameters for attention-deficit/hyperactivity disorder (ADHD) using the STRING 10.0 Database.

Other studies have also shown the possible association of DRD4 in schizophrenia [5,6], novelty-seeking traits [7,8], addiction to psychostimulants [9], mood disorders [10], eating disorders [11,12], and obesity [13,14]. According to Yet [15], DRD4 has garnered attention as a pharmacological target for treating schizophrenia, Parkinson's disease, depression, and ADHD.

A class of phytochemicals known as flavonoids are secondary metabolites found in plants with a polyphenolic structure and are often found in fruits, vegetables, and certain beverages such as tea, coffee, and wine [16,17]. Like conventional antidepressant medicines, flavonoids may act pharmacologically on the CNS to modulate emotional and mood states linked to plastic and neurochemical changes [18-22]. Furthermore, flavonoids have been shown to have a variety of neuroprotective effects in the brain, including the ability to protect neurons against injuries inflicted by neurotoxins, the ability to reduce neuroinflammation, and the potential to enhance memory and cognitive performance [23].

According to multiple reports [24], molecular docking studies are essential for identifying potential flavonoid compounds for treating a variety of diseases prevalent in the human health system. Molecular docking predicts a molecule's binding affinity and optimum binding pose with the receptor's active site and has become a vital tool for drug discovery [25]. In addition, *in silico* approaches for investigating the absorption, distribution, metabolism, and excretion (ADME) features and compounds' pharmacokinetics are also vital components of the current industrial drug discovery paradigm [26]. The chemical properties of a potential drug candidate may be profiled using a combination of several different molecular descriptors [27].

In the present study, a literature search was performed to find relevant research publications highlighting the beneficial effects of flavonoids in neuropsychiatric and neurocognitive conditions. These flavonoids were screened and evaluated for ADME and drug-likeness properties. In addition, molecular docking with DRD4 as the target was also executed to find possible leads as a template to design new hypothetical molecules with improved binding affinities and better molecular residual interactions with it.

MATERIALS AND METHODS

Literature search and SwissTargetPrediction

A literature search was conducted using the keywords "Flavonoid," combined with "DRD4," "neuroprotectants," "cognitive health," "neuropsychiatric disorders," and "neurocognitive disorders." A list of flavonoids was obtained, and their canonical smiles were inputted in the SwissTargetPrediction to see if they target the DRD4. The search was specific for *Homo sapiens*.

Evaluation of pharmacokinetics and drug likeness of the flavonoids

Canonical smiles of the flavonoids that target the DRD4 protein were inputted in the SwissADME server [57] to check for physicochemical properties, lipophilicity, solubility, pharmacokinetics, drug-likeness, and medicinal chemistry.

Flavonoid structure retrieval
The structure of flavonoids with 0 and 1 Lipinski violation and bioavailability score of 0.55 was retrieved from the PubChem database [58]. SDF formats were downloaded and converted to Protein Data Bank (PDB) format using PyMOL 2.5 [59].

Molecular docking using PyRx
The native ligand (Nemonapride) and the cleaned DRD4 structure were uploaded to CB Dock [60] to obtain the binding site and calculates the center and size of the active site dimensions. PyRx virtual screening software was used for the initial docking of ligands. Active site dimensions were set closest to the values obtained from CB-Dock results which were x = −17.00, y = 17.00, and z = −18.00. The box was centered at x = −17.1622, y = 17.0545, and z = −18.0336. Dimensions of XYZ coordinates were x = 35.7485, y = 30.5631, and z = 32.6383. Before initiation of docking operation, energy minimization was done to the ligands. Exhaustiveness was set to 8.

Molecular docking using AutoDock 4.2.6
Before docking, the starting directory was set to the desired folder. The cleaned DRD4 protein was loaded into the AutoDock 4.2.6 workspace [61]. The polar hydrogen atoms and the Kollman charges were added to the protein. The protein was, then, saved in PDBQT format that was then used as the target. The ligand was imported into the workstation; the torsion tree was defined by choosing the root; and the number of rotatable bonds was identified and saved in PDBQT format. The ligand and protein were imported in PDBQT format into the workspace for further simulation process.

The ligands were docked one at a time to the protein. Grid spacing was set to 0.375 Å (default). Center grid box values obtained from CB-Dock results were utilized and set to x = −17.00, y = 17.00, and z = −18.00. The number of grid points along the x, y, and z dimensions was set as 40 × 40 × 40 to provide enough space for the rotational and translational movements of the ligands.

The AutoGrid was executed by providing the AutoGrid executable and GPF files as input and converted to the grid log file (GLG). The grid was then launched. After the successful execution of AutoGrid, the genetic algorithm was set to default and is as follows: (i) The number of GA runs: 10; (ii) population size: 300; (iii) the number of energy evaluations: 2.5 million (2.0 Å clustered tolerance); and (iv) the number of generations: 27000. The Lamarckian genetic algorithm was used, and the output was saved in docking parameter file (DPF) file format. The AutoDock was executed by providing the AutoDock executable and DPF files as input, converted to the docking log file (DLG), and docking was launched. The final DLG file, which contained the top ten free binding energy energies for every run and inhibitory constant, was generated. The lowest binding energy complex for each ligand was saved in PDB format for viewing of interacting residues.

Ligand interactions
The PDB format of the complex was uploaded to PLIP server [62] to view the Hydrogen Bonds and other interacting residues between the ligand and DRD4.

RESULTS

DRD4 structure retrieval and validation
The structure for human DRD4 in complex with Nemonapride (PDB ID 5WIU, at a resolution of 1.96 Å) was downloaded from PDB [28]. Water and heteroatoms were deleted, and the native ligand Nemonapride was separated using Discovery Studio 2021 Client [29]. The cleaned DRD4 was, then, validated using PROCHECK [30] and ERRAT [31] servers.

The cleaned DRD4 model (Fig. 1a) has shown 95.5% of the residues in the most favored regions of the Ramachandran plot (Fig. 1b), and the remaining 4.5% are in the additional allowed regions, which confirms that the model is of good quality. ERRAT is a so-called "overall quality factor" for non-bonded atomic interactions, with higher scores indicating higher quality. The generally accepted range is >50 for a high-quality model. For the cleaned DRD4 model, the overall quality factor predicted by the ERRAT server was 100 (Fig. 1c).

Literature search and SwissTargetPrediction
A total of 39 flavonoids were obtained from the literatures and undergone screening through the SwissTargetPrediction. The SwissTargetPrediction is based on the observation that similar bioactive molecules are more likely to share similar targets [32]. Therefore, the targets of a molecule can be predicted by identifying proteins with known ligands that are highly similar to the query molecule. Twenty-three of the flavonoids are predicted to target DRD4 (Table 1).

Evaluation of pharmacokinetics and drug likeness of the flavonoids
The passive human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeation predictions are both shown in the brain or intestinal estimated permeation method or BOILED-Egg model (Fig. 2). It is proposed as a predictive model that works by computing the lipophilicity and polarity of small molecules [33]. The points in the white area reflect substances that have a high likelihood of being passively absorbed by the gastrointestinal tract. The points in the

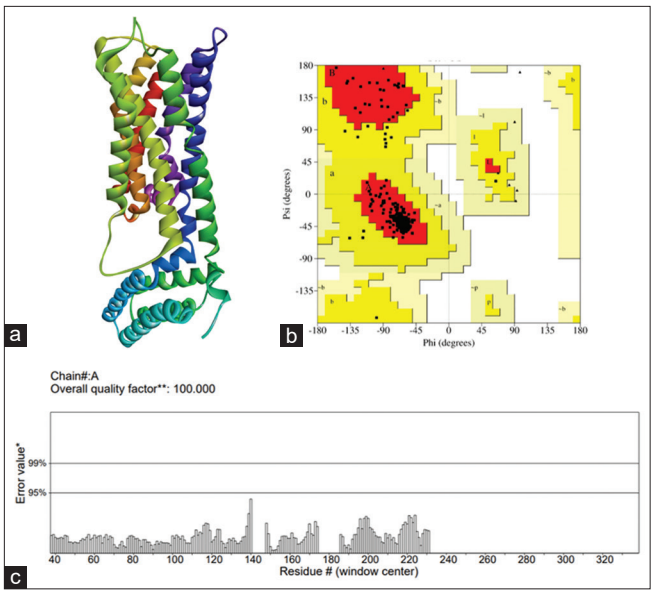

Fig. 1: (a) Cleaned D(4) Dopamine receptor using BIOVIA Discovery Studio. (b) Ramachandran plot obtained from PROCHECK. (c) ERRAT value

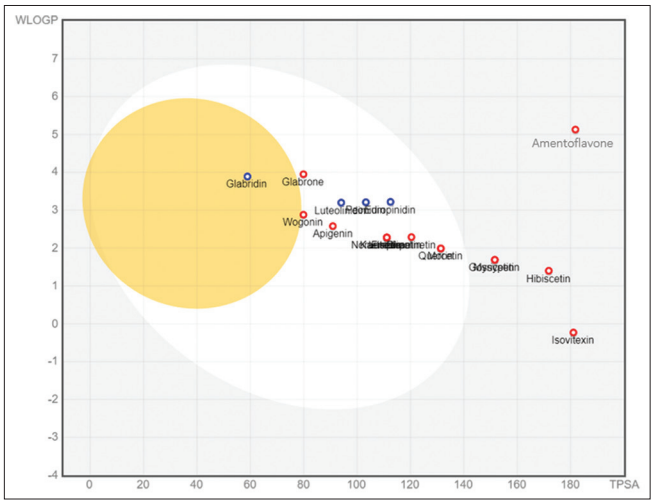

Fig. 2: BOILED-egg

Table 1: Flavonoids and results of the SwissTargetPrediction

Ligands	PubChem ID	Canonical Smiles	Swiss Target Prediction for DRD4
8-Prenylnaringenin	480764	CC(=CCC1=C2C(=C (C=C1O) O) C(=O) CC (O2) C3=CC=C (C=C3) O) C	No
Amentoflavone	5281600	C1=CC(=CC=C1C2=CC(=O) C3=C (O2) C(=C (C=C3O) O) C4=C (C=CC(=C4) C5=CC(=O) C6=C (C=C (C=C6O5) O) O) O) O	Yes
Apigenin	5280443	C1=CC(=CC=C1C2=CC(=O) C3=C (C=C (C=C3O2) O) O) O	Yes
Aspalathin	11282394	C1=CC(=C (C=C1CCC(=O) C2=C (C=C (C(=C2O) C3C (C (C (C (O3) CO) O) O) O) O) O) O) O	Yes
Astilbin	119258	CC1C (C (C (C (O1) OC2C (OC3=CC(=CC(=C3C2=O) O) O) C4=CC(=C (C=C4) O) O) O) O) O	No
Baicalein	5281605	C1=CC=C (C=C1) C2=CC(=O) C3=C (O2) C=C (C(=C3O) O) O	No
Butin	92775	C1C (OC2=C (C1=O) C=CC(=C2) O) C3=CC(=C (C=C3) O) O	No
Chrysin	5281607	C1=CC=C (C=C1) C2=CC(=O) C3=C (C=C (C=C3O2) O) O	No
Cosmosiin	5280704	C1=CC(=CC=C1C2=CC(=O) C3=C (C=C (C=C3O2) OC4C (C (C (C (O4) CO) O) O) O) O) O	No
Daidzein	5281708	C1=CC(=CC=C1C2=COC3=C (C2=O) C=CC(=C3) O) O	No
Diosmin	5281613	CC1C (C (C (C (O1) OCC2C (C (C (C (O2) OC3=CC(=C4C(=C3) OC(=CC4=O) C5=CC(=C (C=C5) OC) O) O) O) O) O) O) O	No
Echinatin	6442675	COC1=C (C=CC(=C1) O) C=CC(=O) C2=CC=C (C=C2) O	No
Epicatechin gallate	107905	C1C (C (OC2=CC(=CC(=C21) O) O) C3=CC(=C (C=C3) O) O) OC(=O) C4=CC(=C (C(=C4) O) O) O	No
Europinidin	14496547	COC1=CC(=CC(=C1O) O) C2=C (C=C3C(=CC(=CC3=[O+]2) O) OC) O	Yes
Fisetin	5281614	C1=CC(=C (C=C1C2=C (C(=O) C3=C (O2) C=C (C=C3) O) O) O) O	Yes
Formononetin	5280378	COC1=CC=C (C=C1) C2=COC3=C (C2=O) C=CC(=C3) O	No
Genistein	5280961	OC1=CC=C (C=C1) C1=COC2=CC (O)=CC (O)=C2C1=O	No
Glabridin	5318980	CC1(C=CC2=C (O1) C=CC3=C2OC[C@H](C3) C4=C (C=C (C=C4) O) O) C	Yes
Glabrone	5317652	CC1(C=CC2=C (O1) C=CC(=C2O) C3=COC4=C (C3=O) C=CC(=C4) O) C	Yes
Glabrol	480768	CC(=CCC1=C (C=CC(=C1)[C@@H] 2CC(=O) C3=C (O2) C(=C (C=C3) O) CC=C (C) C) O) C	No
Gossypetin	5280647	C1=CC(=C (C=C1C2=C (C(=O) C3=C (O2) C(=C (C=C3O) O) O) O) O) O	Yes
Hibiscetin	15559735	C1=C (C=C (C(=C1O) O) O) C2=C (C(=O) C3=C (O2) C(=C (C=C3O) O) O) O	Yes
Homoeriodictyol	73635	COC1=C (C=CC(=C1) C2CC(=O) C3=C (C=C (C=C3O2) O) O) O	No
Hyperoside	5281643	C1=CC(=C (C=C1C2=C (C(=O) C3=C (C=C (C=C3O2) O) O) OC4C (C (C (C (O4) CO) O) O) O) O) O	Yes
Isovitexin	162350	C1=CC(=CC=C1C2=CC(=O) C3=C (O2) C=C (C(=C3O) C4C (C (C (C (O4) CO) O) O) O) O) O	Yes
Kaempferitrin	5486199	CC1C (C (C (C (O1) OC2=CC(=C3C(=C2) OC(=C (C3=O) OC4C (C (C (C (O4) C) O) O) O) C5=CC=C (C=C5) O) O) O) O) O	Yes
Kaempferol	5280863	C1=CC(=CC=C1C2=C (C(=O) C3=C (C=C (C=C3O2) O) O) O) O	Yes
Luteolin	5280445	C1=CC(=C (C=C1C2=CC(=O) C3=C (C=C (C=C3O2) O) O) O) O	Yes
Luteolinidin	441701	C1=CC(=C (C=C1C2=[O+]C3=CC(=CC(=C3C=C2) O) O) O) O	Yes
Morin	5281670	C1=CC(=C (C=C1O) O) C2=C (C(=O) C3=C (C=C (C=C3O2) O) O) O	Yes
Myricetin	5281672	C1=C (C=C (C(=C1O) O) O) C2=C (C(=O) C3=C (C=C (C=C3O2) O) O) O	Yes
Naringin	442428	CC1C (C (C (C (O1) OC2C (C (C (OC2OC3=CC(=C4C(=O) CC (OC4=C3) C5=CC=C (C=C5) O) O) CO) O) O) O) O) O	No
Norartocarpetin	5481970	C1=CC(=C (C=C1O) O) C2=CC(=O) C3=C (C=C (C=C3O2) O) O	Yes
Peonidin	441773	COC1=C (C=CC(=C1) C2=[O+]C3=CC(=CC(=C3C=C2O) O) O) O	Yes
Quercetin	5280343	C1=CC(=C (C=C1C2=C (C(=O) C3=C (C=C (C=C3O2) O) O) O) O) O	Yes
Rhamnetin	5281691	COC1=CC(=C2C(=C1) OC(=C (C2=O) O) C3=CC(=C (C=C3) O) O) O	Yes
Rutin	5280805	CC1C (C (C (C (O1) OCC2C (C (C (C (O2) OC3=C (OC4=CC(=CC(=C4C3=O) O) O) C5=CC(=C (C=C5) O) O) O) O) O) O) O) O	Yes
Sylimarin	5213	COC1=C (C=CC(=C1) C2C (OC3=C (O2) C=C (C=C3) C4C (C(=O) C5=C (C=C (C=C5O4) O) O) O) CO) O	No
Wogonin	5281703	COC1=C (C=C (C2=C1OC(=CC2=O) C3=CC=CC=C3) O) O	Yes

yellow area are for compounds with a high probability to permeate through the BBB to access the CNS. Molecules that are not projected to be well absorbed or BBB permeant are in the grey zone or beyond the reference range. The points are colored in blue if predicted as actively effluxed by P-gp (PGP^+) and red if predicted as a non-substrate of P-gp (PGP^-). Aspalathin, Hyperoside, Kaempferitin, and Rutin were out of range in the BOILED-Egg model. Table 2 shows the pharmacokinetic properties of the flavonoids. Glabridin was only the BBB permeant among the flavonoids. Most flavonoids have HIA. Europinidin, Glabridin, Kaempferitin, Luteolinidin, Peonidin, and Rutin were P-gp substrates, the rest which are in red dots are non P-gp substrates.

The Lipinski's Rule of Five distinguishes between the drug like and non-drug like molecules. It predicts high probability of failure of molecules due to non-drug-likeness for the molecules not complying with 2 or more of the following rules: (1) molecular weight <500; (2) logP <5; (3) H-bond donors <5; and (4) H-bond acceptors <10 [34].

It was reported that the bioavailability score should be 0.55 for a neutral organic compound that satisfies Lipinski's rule to act as a good oral drug [35]. Amentoflavone and Aspalathin obtained two Lipinski violations. Kaempferitin and Rutin obtained three, and their bioavailability score is 0.17. The rest of the flavonoids which have 0-1 violations and a bioavailability score of 0.55 and were considered for molecular docking. The results of Lipinski filter analysis is documented in Table 3.

Molecular docking

Table 4 shows the PyRx results. The more negative the numerical values for the binding affinity, the better is the predicted binding between a ligand and the macromolecule [36]. Glabrone and Isovitexin showed

better binding affinity and Glabridin has the same value compared to the native ligand, Nemonapride.

Table 5 shows the Autodock results. The binding energy obtained from Autodock ranges from −9.37 kcal/mol to −7.17 kcal/mol. Isovitexin, Glabridin, and Glabrone still showed as the top three which has the best binding energy.

Ligand interactions

Ligand interactions are shown in Figs. 3-5 and list of amino acids that are interacting with the ligand are shown in Tables 6-8. Isovitexin has shown the greatest number of hydrogen bonds compared to Glabridin and Glabrone. Glabridin has shown the greatest number of hydrophobic interactions among Isovitexin and Glabrone.

Table 2: Pharmacokinetics properties of the flavonoids

Ligands	Blood Brain Barrier	GI Absorption	Permeability Glycoprotein Substrate
Amentoflavone	No	Low	No
Apigenin	No	High	No
Aspalathin	No	Low	No
Europinidin	No	High	Yes
Fisetin	No	High	No
Glabridin	Yes	High	Yes
Glabrone	No	High	No
Gossypetin	No	Low	No
Hibiscetin	No	Low	No
Hyperoside	No	Low	No
Isovitexin	No	Low	No
Kaempferitrin	No	Low	Yes
Kaempferol	No	High	No
Luteolin	No	High	No
Luteolinidin	No	High	Yes
Morin	No	High	No
Myricetin	No	Low	No
Norartocarpetin	No	High	No
Peonidin	No	High	Yes
Quercetin	No	High	No
Rhamnetin	No	High	No
Rutin	No	Low	Yes
Wogonin	No	High	No

DISCUSSION

Dopamine receptors are involved in a variety of biological processes, which are primarily the CNS [37-40], including cognition, memory, learning, and motor control, as well as neuroendocrine signaling modulation [41], and are thus associated with a variety of psychiatric and neurological disorders. DRD4 is a target for the most common neuroleptic medications [42]. Neuroleptics, also known as antipsychotic medications, are used to treat and manage symptoms of many psychiatric disorders. It is a target for drugs that treat schizophrenia and Parkinson's disease. In addition, DRD4 is mainly considered to affect treatment response by stimulants in ADHD [43].

CNS drug discovery studies must determine if a compound will penetrate the BBB and be distributed throughout, because efficacy is primarily dependent on sufficient exposure within the CNS. Based on comparing the physicochemical properties of marketed CNS and CNS-inactive drugs, van de Waterbeemd *et al*. [44] concluded that a compound should have a molecular weight below 450 g/mol to enhance CNS penetration.

Out of the three flavonoids which showed the best binding toward DRD4, only Glabridin was a BBB permeant and had a molecular weight of 324.37 g/mol. However, it was also suggested that drug molecules intended for the treatment of CNS disorders must also be capable of bypassing the P-gp efflux pump at the intestinal and BBB levels to achieve efficacy [45]. P-gp is a member ABC superfamily membrane transporter found in both the intestinal epithelium and the BBB, where it plays a critical role in the bioavailability of orally taken medicines used to treat diseases in the brain [46].

From the BOILED-egg, it can be seen that Glabridin is a blue dot which means that as a substrate of the P-gp, it might be ejected from the brain. Yu *et al*. [47] have investigated the role of P-gp in Glabridin penetration across the BBB through *in vitro* and *in vivo* models. Glabridin was found to have a limited brain penetration in rats but increased when coadministered with P-gp inhibitors. Despite this, additional mechanisms of bypassing the P-gp transporters [48] can be used to

Table 3: Lipinski's rule of five and bioavailability score of the flavonoids

Ligands	Lipinski's Rule of 5					Bioavailability Score
	Molecular weight <500	Consensus LogP <5	Hydrogen Bond Donor <5	Hydrogen Bond Acceptor <10	Violation/s	
Amentoflavone	538.46 g/mol	3.62	6	10	2	0.17
Apigenin	270.24 g/mol	2.11	3	5	0	0.55
Aspalathin	452.41 g/mol	−0.78	9	11	2	0.17
Europinidin	331.30 g/mol	1.05	4	7	0	0.55
Fisetin	286.24 g/mol	1.55	4	6	0	0.55
Glabridin	324.37 g/mol	3.45	2	4	0	0.55
Glabrone	336.34 g/mol	3.13	2	5	0	0.55
Gossypetin	318.24 g/mol	0.96	6	8	1	0.55
Hibiscetin	334.23 g/mol	0.63	7	9	1	0.55
Hyperoside	464.38 g/mol	−0.38	8	12	2	0.17
Isovitexin	432.38 g/mol	0.05	7	10	1	0.55
Kaempferitrin	578.52 g/mol	−0.42	8	14	3	0.17
Kaempferol	286.24 g/mol	1.58	4	6	0	0.55
Luteolin	286.24 g/mol	1.73	4	6	0	0.55
Luteolinidin	271.24 g/mol	0.85	4	5	0	0.55
Morin	302.24 g/mol	1.2	5	7	0	0.55
Myricetin	318.24 g/mol	0.79	6	8	1	0.55
Norartocarpetin	286.24 g/mol	1.74	4	6	0	0.55
Peonidin	301.27 g/mol	0.97	4	6	0	0.55
Quercetin	302.24 g/mol	1.23	5	7	0	0.55
Rhamnetin	316.26 g/mol	1.63	4	7	0	0.55
Rutin	610.52 g/mol	−1.51	10	16	3	0.17
Wogonin	284.26 g/mol	2.54	2	5	0	0.55

Table 4: PyRx results

Ligand	Binding Affinity
Apigenin	−8.9
Europinidin	−8.9
Fisetin	−9.1
Glabridin	−10.1
Glabrone	−10.6
Gossypetin	−9
Hibiscetin	−8.9
Isovitexin	−10.2
Kaempferol	−8.9
Luteolin	−9
Luteolinidin	−9
Morin	9.1
Myricetin	−9.2
Norartocarpetin	−8.9
Peonidin	8.7
Quercetin	−9.3
Rhamnetin	8.5
Wogonin	8.9
Nemonapride (Native Ligand)	−10.1

Table 5: Autodock results

Ligands	Binding Energy
Apigenin	−7.68 kcal/mol
Europinidin	−8.03 kcal/mol
Fisetin	−6.81 kcal/mol
Glabridin	−8.56 kcal/mol
Glabrone	−8.48 kcal/mol
Gossypetin	−7.97 kcal/mol
Hibiscetin	−8.25 kcal/mol
Isovitexin	−9.37 kcal/mol
Kaempferol	−7.63 kcal/mol
Luteolin	−7.81 kcal/mol
Luteolinidin	−7.43 kcal/mol
Morin	−7.54 kcal/mol
Myricetin	−7.46 kcal/mol
Norartocarpetin	−7.40 kcal/mol
Peonidin	−7.28 kcal/mol
Quercetin	−7.17 kcal/mol
Rhamnetin	−7.47 kcal/mol
Wogonin	−7.88 kcal/mol
Nemonapride (Native Ligand)	−8.24 kcal/mol

Table 6: List of amino acids and residue number that are interacting with Isovitexin

	Index	Residue	Amino Acid
Hydrophobic Interactions	1	116A	VAL
	2	187A	LEU
	3	187A	LEU
	4	193A	VAL
	5	410A	PHE
	6	414A	HIS
Hydrogen Bonds	1	94A	SER
	2	95A	GLU
	3	95A	GLU
	4	185A	CYS
	5	185A	CYS
	6	187A	LEU
	7	197A	SER
	8	414A	HIS
	9	434A	THR
	10	434A	THR
	11	438A	TYR
	12	438A	TYR

optimize Glabridin's potential as a CNS drug candidate. Cui *et al.* [49] demonstrated in their mice experiment that Glabridin appears to be a

Table 7: List of amino acids and residue number that are interacting with Glabridin

	Index	Residue	Amino Acid
Hydrophobic Interactions	1	90A	LEU
	2	91A	PHE
	3	91A	PHE
	4	101A	TRP
	5	111A	LEU
	6	111A	LEU
	7	187A	LEU
	8	187A	LEU
	9	193A	VAL
Hydrogen Bonds	1	115A	ASP
	2	187A	LEU
	3	187A	LEU

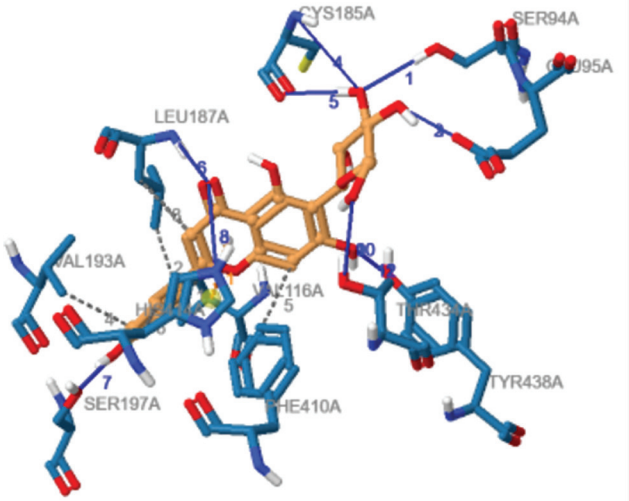

Fig. 3: Isovitexin interactions with amino acids

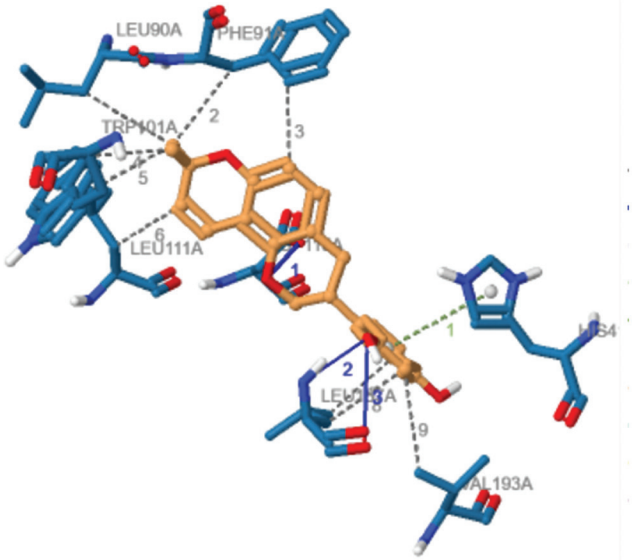

Fig. 4: Glabridin interactions with amino acids

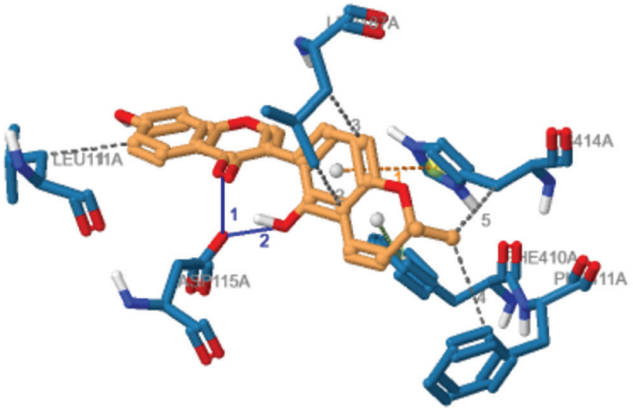

Fig. 5: Glabrone interactions with amino acids

Table 8: List of amino acids and residue number that are interacting with Glabrone

	Index	Residue	Amino Acid
Hydrophobic Interactions	1	111A	LEU
	2	187A	LEU
	3	187A	LEU
	4	411A	PHE
	5	414A	HIS
Hydrogen Bonds	1	115A	ASP
	2	115A	ASP

promising candidate for memory enhancement, and it will be beneficial to investigate its potential for use in the treatment of Alzheimer's disease.

Both Glabridin and Glabrone are essential bioactive components isolated from licorice (*Glycyrrhiza*) root extract, demonstrating anti-inflammatory, and neuroprotective effects and anti-depressant effects in many experimental studies [50]. On the other hand, Isovitexin is one of the main bioactive compounds of *Passiflora* species [51]. *Passiflora* species has, traditionally, been used to treat anxiety, insomnia, and nervousness [52-54]. Despite having a great binding energy score toward DRD4, Glabrone was not a BBB permeant. Isovitexin has low GI absorption and is also not a BBB permeant.

Insights about ADME properties of the flavonoids mentioned in this study can aid in the early stage of drug discovery and can help save time and resources. Drug developers may still make chemical modifications to drug candidates during the discovery and lead optimization stages in order to optimize the ADME properties of the compounds [55]. Furthermore, when using *in silico* methods for prediction, it is important to note that algorithms and tools applied are only models thus being only as good as the data and idea they are based on [56]. This implies that a continuous experimental validation and improvements are still necessary.

CONCLUSION

In this present study, *in silico* approach such as ADME analysis and molecular docking was employed to find flavonoids that may have potential as drug lead molecules to target the human DRD4. The molecular docking results showed a good docking score ranging from −9.37 kcal/mol to −7.17 kcal/mol. Among the flavonoids studied, Isovitexin, Glabridin, and Glabrone have shown better binding energy compared to the native ligand which is Nemonapride. However, ADME analysis has demonstrated that Isovitexin has low GI absorption and is in the grey zone of the BOILED-egg. Glabridin is a BBB permeant but is a P-gp substrate. Glabrone has high GI absorption, a non P-gp substrate but not a BBB permeant. However, further experimental investigations and clinical evaluations are recommended to examine the mechanisms of their actions and other pharmacological effects and to validate the results of this *in silico* study. The scaffolds of these compounds can also be optimized to improve the few lapses and have better attributes as CNS drug leads.

ACKNOWLEDGMENT

The author extends her appreciation to BioNome, India and to guide Ms. Vaeeshnavi Buwa for the guidance in using the different tools to carry out the study.

CONFLICTS OF INTEREST

The author declares that there is no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Woods AS. The dopamine D4 receptor, the ultimate disordered protein. J Recept Signal Trans 2010;30:331-6.
2. Kuznetsova AY, Deth RC. A model for modulation of neuronal synchronization by D4 dopamine receptor-mediated phospholipid methylation. J Computat Neurosci 2008;24:314-29.
3. Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M. How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis. Neurotoxicology 2008;29:190-201.
4. Verma A, Chauhan SS, Pankaj V, Srivastva N, Srivastava P. Network biology approaches to identify the drug lead molecule for neurodevelopmental disorders in human. Open Bioinform J 2020;13:15-24.
5. Seeman P, Guan HC, Van Tol HH. Dopamine D4 receptors elevated in schizophrenia. Nature 1993;365:441-5.
6. Tarazi FI, Zhang K, Baldessarini RJ. Dopamine D4 receptors: Beyond schizophrenia. J Recept Signal Transduct 2004;24:131-47.
7. Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. Nat Genet 1996;12:81-4.
8. Ebstein RP, Novick O, Umansky R, Priel B, Osher Y, Blaine D, *et al*. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of novelty seeking. Nat Genet 1996;12:78-80.
9. Di Ciano P, Grandy DK, Le Foll B. Dopamine D4 receptors in psychostimulant addiction. Adv Pharmacol 2014;69:301-21.
10. Manki H, Kanba S, Muramatsu T, Higuchi S, Suzuki E, Matsushita S, *et al*. Dopamine D2, D3 and D4 receptor and transporter gene polymorphisms and mood disorders. J Affect Disord 1996;40:7-13.
11. Bachner-Melman R, Lerer E, Zohar AH, Kremer I, Elizur Y, Nemanov L, *et al*. Anorexia nervosa, perfectionism, and dopamine D4 receptor (DRD4). Am J Med Genet Part B Neuropsychiatr Genet 2007;144:748-56.
12. Levitan RD, Masellis M, Basile VS, Lam RW, Kaplan AS, Davis C, *et al*. The dopamine-4 receptor gene associated with binge eating and weight gain in women with seasonal affective disorder: An evolutionary perspective. Biol Psychiatry 2004;56:665-9.
13. Levitan RD, Masellis M, Lam RW, Muglia P, Basile VS, Jain U, *et al*. Childhood inattention and dysphoria and adult obesity associated with the dopamine D4 receptor gene in overeating women with seasonal affective disorder. Neuropsychopharmacology 2004;29:179-86.
14. Poston WS, Ericsson M, Linder J, Haddock CK, Hanis CL, Nilsson T, *et al*. D4 dopamine receptor gene exon III polymorphism and obesity risk. Eating Weight Disord Stud Anorexia Bulimia Obes 1998;3:71-7.
15. Yet L. In: Katritzky AR, Ramsden CA, Scriven EF, Taylor RJ, editors. Comprehensive Heterocyclic Chemistry III. Netherlands: Elsevier; 2008.
16. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Sci World J 2013;2013:162750.
17. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. Am J Clin Nutr 2004;79:727-47.
18. Donato F, de Gomes MG, Goes AT, Borges Filho C, Del Fabbro L, Antunes MS, *et al*. Hesperidin exerts antidepressant-like effects in acute and chronic treatments in mice: Possible role of l-arginine-NO-cGMP pathway and BDNF levels. Brain Res Bull 2014;104:19-26.
19. Jesse CR, Donato F, Giacomeli R, Del Fabbro L, da Silva Antunes M, De Gomes MG, *et al*. Chronic unpredictable mild stress decreases BDNF and NGF levels and Na+, K+-ATPase activity in the

hippocampus and prefrontal cortex of mice: Antidepressant effect of chrysin. Neuroscience 2015;289:367-380.
20. Zhen L, Zhu J, Zhao X, Huang W, An Y, Li S, *et al*. The antidepressant-like effect of fisetin involves the serotonergic and noradrenergic system. Behav Brain Res 2012;228:359-66.
21. Zhang JC, Wu J, Fujita Y, Yao W, Ren Q, Yang C, *et al*. Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in mice after inflammation. Int J Neuropsychopharmacol 2015;18:pyu077.
22. Hritcu L, Ionita R, Postu PA, Gupta GK, Turkez H, Lima TC, *et al*. Antidepressant flavonoids and their relationship with oxidative stress. Oxid Med Cell Longev 2017;2017:5762172.
23. Vauzour D, Vafeiadou K, Rodriguez-Mateos A, Rendeiro C, Spencer JP. The neuroprotective potential of flavonoids: A multiplicity of effects. Genes Nutr 2008;3:115-26.
24. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci 2016;5:e47.
25. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Design 2011;7:146-57.
26. Lombardo F, Desai PV, Arimoto R, Desino KE, Fischer H, Keefer CE, *et al*. *In silico* absorption, distribution, metabolism, excretion, and pharmacokinetics (ADME-PK): Utility and best practices. An industry perspective from the international consortium for innovation through quality in pharmaceutical development: miniperspective. J Med Chem 2017;60:9097-113.
27. Kerns EH, Di L. Pharmaceutical profiling in drug discovery. Drug Discov Today 2003;8:316-23.
28. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al*. The protein data bank. Nucleic Acids Res 2000;28:235-42.
29. BIOVIA, Dassault Systèmes, Discovery Studio (Client Version), San Diego: Dassault Systèmes; 2021.
30. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Crystallogr 1993;26:283-91.
31. Colovos C, Yeates TO. Verification of protein structures: Patterns of nonbonded atomic interactions. Protein Sci 1993;2;1511-9.
32. Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: A web server for target prediction of bioactive small molecules. Nucleic Acids Res 2014;42:W32-8.
33. Daina A, Zoete V. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem 2016;11:1117.
34. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the rule of 5 and drugability. Adv Drug Deliv Rev 2016;101:89-98.
35. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2012;64:4-17.
36. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In: Chemical Biology. New York: Humana Press; 2015. p. 243-50.
37. Arai M. Increased plasma arginine vasopressin levels in dopamine agonist-treated Parkinson's disease patients. Neuroendocrinol Lett 2011;32:39.
38. Huang Y, Qiu AW, Peng YP, Liu Y, Huang HW, Qiu YH. Roles of dopamine receptor subtypes in mediating modulation of T lymphocyte function. Neuroendocrinol Lett 2010;31:782.
39. Matalka KZ, Attallah LJ, Qinna NA, Alhussainy T. Dopamine selectively modulates lipopolysaccharide-induced TNF-alpha, IFN-gamma and IL-10 within mice tissues. Neuroendocrinol Lett 2011;32:176-86.
40. Markianos M, Panas M, Kalfakis N, Hatzimanolis J, Vassilopoulos D. Neuroendocrine evidence of normal hypothalamus-pituitary dopaminergic function in Huntington's disease. Neuroendocrinol Lett 2010;31:359-62.
41. Esch T, Stefano GB. The neurobiology of stress management. Neuroendocrinol Lett 2010;31:19-39.
42. Wong AH, Van Tol HH. The dopamine D4 receptors and mechanisms of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1091-9.
43. Ptáček R, Kuželová H, Stefano GB. Dopamine D4 receptor gene DRD4 and its association with psychiatric disorders. Med Sci Monit 2011;17:RA215.
44. van de Waterbeemd H, Camenisch G, Folkers G, Chretien JR, Raevsky OA. Estimation of blood-brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. J Drug Target 1998;6:151-65.
45. Feng S. Neurorestoratologic strategies and mechanisms in the nervous system. Biomed Res Int 2015;2015:163170.
46. Demeule M, Régina A, Jodoin J, Laplante A, Dagenais C, Berthelet F, *et al*. Drug transport to the brain: Key roles for the efflux pump P-glycoprotein in the blood-brain barrier. Vasc Pharmacol 2002;38:339-48.
47. Yu XY, Lin SG, Zhou ZW, Chen X, Liang J, Yu XQ, *et al*. Role of P-glycoprotein in limiting the brain penetration of glabridin, an active isoflavan from the root of Glycyrrhiza glabra. Pharm Res 2007;24:1668-90.
48. Amin ML. P-glycoprotein inhibition for optimal drug delivery. Drug Target Insights 2013;7:DTI-S12519.
49. Cui YM, Ao MZ, Li W, Yu LJ. Effect of glabridin from *Glycyrrhiza glabra* on learning and memory in mice. Planta Med 2008;74:377-80.
50. Hosseinzadeh H, Nassiri-Asl M. Pharmacological effects of *Glycyrrhiza* spp. and its bioactive constituents: Update and review. Phytother Res 2015;29:1868-86.
51. Quercia V, Turchetto L, Pierini N, Cuozzo V, Percaccio G. Identification and determination of vitexin and isovitexin in *Passiflora incarnata* extracts. J Chromatogr A 1978;161:396-402.
52. Brasseur T, Angenot L. The pharmacognosy of the passion flower. J Pharm Belgiq 1984;39:15-22.
53. Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. Pharmacol Biochem Behav 1994;47:1-4.
54. Soulimani R, Younos C, Jarmouni S, Bousta D, Misslin R, Mortier F. Behavioural effects of *Passiflora incarnata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. J Ethnopharmacol 1997;57:11-20.
55. Loftsson T. Physicochemical properties and pharmacokinetics. In: Essential Pharmacokinetics-a Primer for Pharmaceutical Scientists. Netherlands: Elsevier; 2015. p. 85-104.
56. Krüger A, Gonçalves Maltarollo V, Wrenger C, Kronenberger T. ADME profiling in drug discovery and a new path paved on silica. In: Drug Discovery and Development: New Advances. India: IntechOpen; 2019. p. 1-32.
57. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:1-3.
58. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, *et al*. PubChem 2019 update: Improved access to chemical data. Nucleic Acids Res 2019;47:D1102-9.
59. Yuan S, Chan HS, Hu Z. Using PyMOL as a platform for computational drug design. Wiley Interdiscip Rev Comput Mol Sci 2017;7:e1298.
60. Liu Y, Grimm M, Dai WT, Hou MC, Xiao ZX, Cao Y. CB-Dock: A web server for cavity detection-guided protein–ligand blind docking. Acta Pharmacol Sin 2020;41:138-44.
61. Huey R, Morris GM, Forli S. Using AutoDock 4 and AutoDock vina with AutoDockTools: A tutorial. Scripps Res Inst Mol Graph Lab 2012;10550:92037.
62. Adasme MF, Linnemann KL, Bolz SN, Kaiser F, Salentin S, Haupt VJ, *et al*. PLIP 2021: Expanding the scope of the protein–ligand interaction profiler to DNA and RNA. Nucleic Acids Res 2021;49:W530-4.