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Virtual screening, molecular docking and ADME/T properties analysis of neuroprotective property present in root extract of chinese skullcap against β-site amyloid precursor protein cleaving enzyme 1 (BACE1) in case of alzheimer's disease

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Abstract
Alzheimer's disease (AD) is one the most prevalent and common neurodegenerative disorder affecting a large population globally. The AD is characterized and diagnosed by the aggregation of Aβ plaques between neuronal synapses. Numerous causative factors are suggested by the researchers for the onset and progression of the AD. The anomaly in the glycosylation mechanism is investigated to play major role in formation of amyloid beta plaque (Aβ) from amyloid precursor protein (APP). The β-site amyloid precursor protein cleaving enzyme 1 (BACE1) which is also known as β-secretase is found to be prime initiator of Aβ as it cleaves APP's extracellular domain and further leads to formation of toxic plaques. BACE1 draw attention of the researcher around the world as the potent therapeutic target for the treatment of AD as its overexpression is directly associated with pathophysiology of the disorder. The biggest hurdle in therapeutic strategies in context to neurological disorder is the ability of the drug compound to cross blood brain barrier (BBB). The aim of our study is to virtually screen naturally occurring active compounds in the *Scutellaria baicalensis* plant's root extract as possible candidate for BACE1 inhibitor drugs. We retrieved the three-dimensional model of BACE1 protein (PDB ID: 2ZHV) from protein data bank. Using *in-silico* molecular docking programs and models, we performed virtual screening of the compounds found in the *Scutellaria* root extract and observed good docking score ranging from-5.9 to-7.7. The best docking score was found to be of isoscutellarin followed by norwogonin, apigenin, carthamidin, dihydrooxylin and genkwanin. Interestingly, five compounds were found to be blood brain permeable namely pellitorine, futoamide, dihydropiperlonguminin, chrysin and dihydrooroxylin. We conclude that selected compounds may be a potential candidate for novel therapeutic agent to treat and slow down the progression of AD.

Keywords: Alzheimer's, *in silico*, neuroprotective, cleaving enzyme, flavonoids

1. Introduction
We are living in the era where there is continuous strife for the betterment of life standard and quality and increasing its expectancy. In recent years, the biological sciences have taken a giant leap with the advent of the cutting-edge instrumentation facilities and computational tools. But it's obvious that this journey of human welfare particularly in the field of health and clinical sciences is never ending. With changing lifestyle and the environment, the challenges change too. Nowadays, AD is considered as one of the major public health concerns as it is the major cause of dementia in around 70% of the cases around the world [1]. Alzheimer's disease (AD) is the most common cause of dementia in the elderly, with a prevalence of 5% after 65 years of age. The disease was originally described by Alois Alzheimer and Gaetano Perusini in 1906, and it is clinically characterized by a progressive cognitive impairment, including impaired judgment, decision making and orientation, often accompanied, in later stages, by psycho-behavioural disturbances as well as language impairment [2]. Age is the most important risk factor, and as the life span of the population increases, there will be a steep increase in the number of AD cases in the coming years. Presently, only symptomatic treatment is available, but there is ongoing massive research effort aimed at finding disease-modifying drugs. The cognitive function of an AD patient deteriorates irreversibly over time and complete care is required for basic daily activities in the late stages of the disease [3]. The exact molecular mechanisms leading to the clinical symptoms and neuropathological changes associated with AD remain unclear. Of all the known causative factors, the most widely accepted is "amyloid hypothesis" which states that AD is caused due to aggregation of Aβ plaques between neuronal synapse [4].

Aβ act as neurotoxic agents and their interaction with several neuronal receptors triggers and initiates a cascade responsible for endoplasmic reticulum stress [5], mitochondrial dysfunction, oxidative stress [6], DNA damage, neuroinflammation and finally leads to neurodegeneration [7]. The Aβ plaques are formed by the cleavage of extracellular domain of APP by BACE1. BACE1 is a type I transmembrane aspartyl protease that cleaves APP at the β-site [8]. In healthy brain, BACE1 cleaves the transmembrane APP to release the β-stubs and represents the rate-limiting catalytic step for Aβ production. It is observed that, in case of AD patients, BACE1 concentration is found to be elevated [9]. As the proteolytic cleavage of APP by BACE1 is the initial step in the formation of Aβ plaques, various clinical studies are being conducted to design and formulate drug which can act as BACE1 inhibitors to stop or slow down the progression of AD. Since ages, plants extracts are used for treatment of medical anomalies and ailments in both traditional and modern medicines. Phytochemicals derived from medicinal plants are widely used for the purpose of therapeutic interventions. Studies have shown that root extract of *Scutellaria baicalensis* are rich in flavones, phenylethanoid glycosides and possess antibacterial, antiviral, antioxidant, hepatoprotective, anti-cancer and neuroprotective effects [10]. The active phytochemicals present in root extract of *Scutellaria baicalensis* are mentioned in Table 1 [11]. It has been proposed through the clinical studies that the root extract of *Scutellaria* can be used to alleviate the aggregation of Aβ plaques [12]. The molecular docking tools aid in virtually screening of these active compounds to sort out lead drug candidates among them and later they are tested *in-vitro* and *in-vivo* to establish them as potent therapeutic drug. We have performed in-silico study on active compounds present in root extract of *Scutellaria baicalensis* against BACE1.

Table 1: Active phytochemicals present in root extract of *Scutellaria baicalensis*

S. No.	Ligand Name	Pubchem ID	Molecular Weight	Molecular formula
1.	Apigenin	5280443	270.24	C15H10O5
2.	Baicalein	5281605	270.24	C15H10O5
3.	Carthamidin	188308	288.25	C15H12O6
4.	Chrysin	5281607	254.24	C15H10O4
5.	Crotepoxide	161314	362.3	C18H18O8
6.	Dihydropiperlonguminin	12682184	275.34	C16H21NO3
7.	Dihydrooroxylin	177032	286.28	C16H14O5
8.	Futoamide	15596445	301.4	C18H23NO3
9.	Genkwanin	5281617	284.26	C16H12O5
10.	Hispidulin	5281628	300.26	C16H12O6
11.	Isoscutellarin	11996857	462.4	C21H18O12
12.	Norwogonin	5281674	270.24	C15H10O5
13.	Pellitorine	5318516	223.35	C14H25NO
14.	Rivularin	13889022	344.3	C18H16O7
15.	Negletein	471719	284.26	C16H12O5

***Source*:** Wang *et al*., 2018

2. Materials and Methods

In-silico based study was performed using Hewlett Packard laptop with hardware configuration 8 GB RAM, Intel i3 11th generation. The molecular docking tool used for the *in-silico* study was AutoDock Vina tool [13] in PyRx [19]. PyMOL 2.4.1 was used for the purpose of protein preparation and visualisation of the docked ligand with protein. Ligplot+ was used to analyse protein-ligand interaction. For the purpose of ADME/T studies, Swiss ADME webserver was used.

2.1 Retrieval and preparation of ligand

The active chemical compounds present in root extract of the *Scutellaria baicalensis* were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in Structural Data Format (SDF). A total of 15 active compounds were retrieved and their detail is mentioned in Table 1. Ligand preparation was done using PyRx software. The energy of the ligands was minimised using Open Babel tool within the PyRx software and force field used for energy minimisation was MMFF94. Finally, all the ligands were converted to AutoDock pdbqt format.

2.2 Preparation of the target protein

Three-dimensional structure in high resolution of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) was download from protein data bank (PDB) database (https://www.rcsb.org/) with PDB ID- 2ZHV. For the purpose of protein preparation, water molecules were removed to eliminate its hinderance in protein-ligand docking and polar hydrogen were added using PyMOL software. Finally, the PDB format of protein was converted to pdbqt format for executing AutoDock Vina.

2.3 Virtual screening of the ligands

AutoDock Vina tool within PyRx software was used for the purpose of virtual screening of the retrieved active compounds against the desired target protein. Blind docking was performed with grid box dimension (43.50 Å ×39.01 Å ×60.70 Å) and centre (-33.23, 13.08, 29.71). The exhaustiveness was set to 8 by default. After the completion of screening analysis, the candidates with good docking score were visualised using PyMOL. Further, ligands with lowest binding energy (kcal/mol) were selected for the purpose of other analysis.

2.4 Protein-ligand interactions

Ligplot+ software was used to make Ligplot for the prediction of hydrophobic and hydrogen bond interaction between the ligand and the target protein [15].

2.5 ADME/T analysis

The ADME/T analysis provides information regarding the absorption, distribution, metabolism, excretion and toxicity of the tested ligand or chemical compound. The analysis is done to knock out undesired compounds with insignificant drug likeness in order to make the study feasible and time-friendly. The canonical SMILES of the retrieved compounds or hits were used to analyse pharmacological and physiochemical

profile of the hits with the help of SwissADME webserver (http://www.swissadme.ch/) [16]. The parameters which were taken into consideration for pharmacological validation were molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donor, blood brain barrier permeability, topological polar surface area (TPSA) value and LogS value. BOILED-Egg tool of SWISSADME was used to analyse and predict GI absorption and brain penetration of the drug molecules [17].

3. Results

3.1 Virtual screening result analysis

The three-dimensional crystal structure of BACE1 (PDB ID: 2ZHV) was retrieved from protein data bank for docking analysis. The 23 active compounds of *Scutellaria baicalensis* root extract were retrieved from the PubChem database in Structural Data Format (SDF). The hit ligands or active compounds were screened against the desired target BACE1 using the AutoDock Vina tool of PyRx. We found that out of 23 docked compounds, 12 compounds namely apigenin, baicalein, carthamidine, chrysin, dihydropiperlonguminin, dihydrooroxylin, futoamide, genkwanin, hispidulin, isoscutellarin, norwogonin, pellitorine with docking score -7.5, -7.4, -7.5, -7.4, -6.9, -7.4, -6.6, -7.4, -7.3, -7.7, -7.6 and -5.9 kcal/mol respectively showed good docking result (Table 2). Interestingly, out of these twelve compounds, five candidates namely chrysin, dihydropiperlonguminin, dihydrooroxylin, futoamide and pellitorine showed blood brain permeability (Table 3).

Table 2: Phytochemicals showing good docking score against BACE1 target

S. No.	Ligand Name	PubChem ID	Molecular Weight	Molecular Formula	Docking Score
1.	Apigenin	5280443	270.24	C15H10O5	-7.5
2.	Baicalein	5281605	270.24	C15H10O5	-7.4
3.	Carthamidin	188308	288.25	C15H12O6	-7.5
4.	Chrysin	5281607	254.24	C15H10O4	-7.4
5.	Dihydropiperlonguminin	12682184	275.34	C16H21NO3	-6.9
6.	Dihydrooroxylin	177032	286.28	C16H14O5	-7.4
7.	Futoamide	15596445	301.4	C18H23NO3	-6.6
8.	Genkwanin	5281617	284.26	C16H12O5	-7.4
9.	Hispidulin	5281628	300.26	C16H12O6	-7.3
10.	Isoscutellarin	11996857	462.4	C21H18O12	-7.7
11.	Norwogonin	5281674	270.24	C15H10O5	-7.6
12.	Pellitorine	5318516	223.35	C14H25NO	-5.9

Table 3: Phytochemical having the property of being blood brain permeant

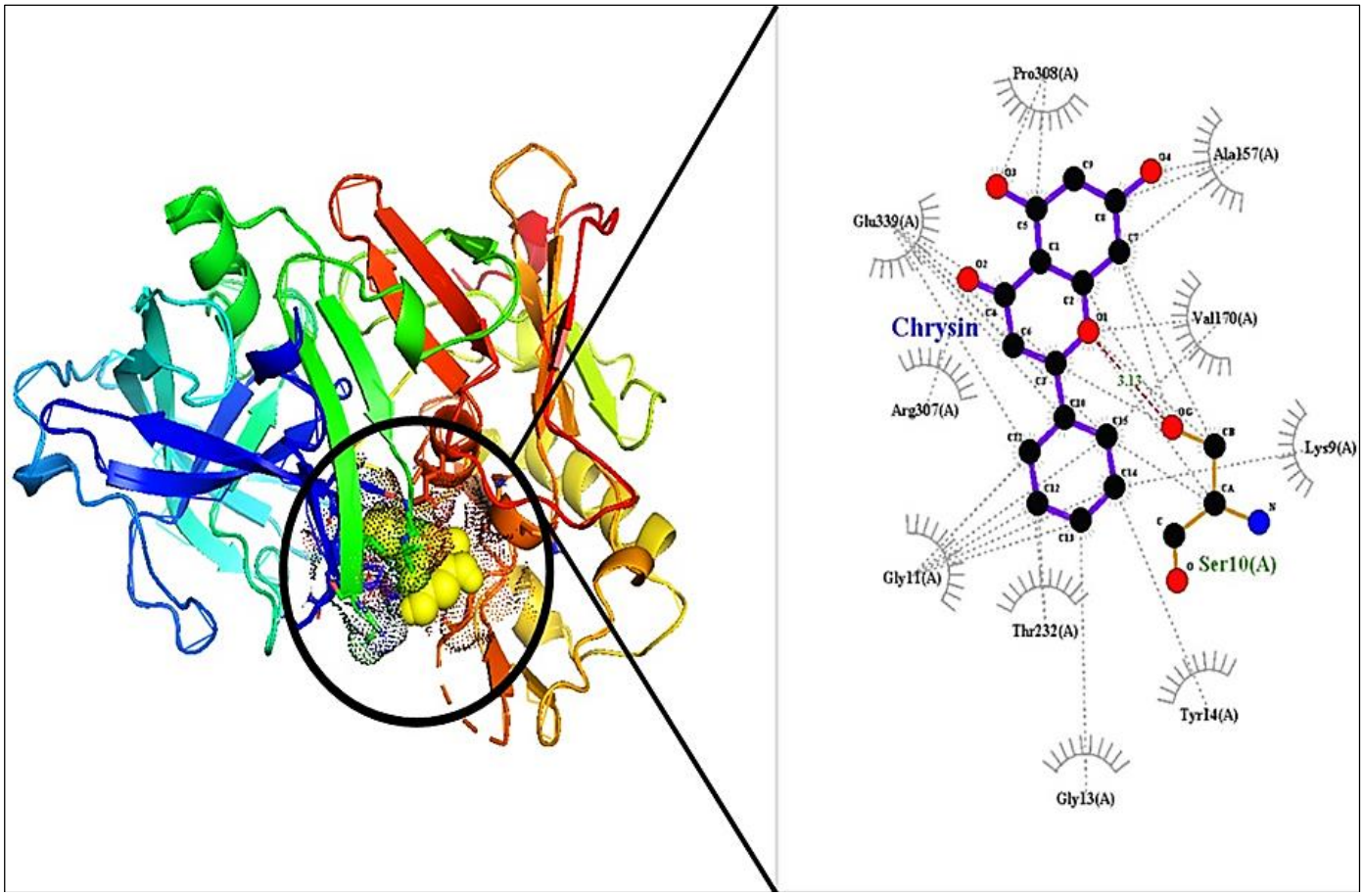
S. No.	Ligand Name	PubChem ID	Molecular Weight	Molecular Formula	Docking Score
1	Chrysin	5281607	254.24	C15H10O4	-7.4
2	Dihydropiperlonguminin	12682184	275.34	C16H21NO3	-6.9
3	Dihydrooroxylin	177032	286.28	C16H14O5	-7.4
4	Futoamide	15596445	301.4	C18H23NO3	-6.6
5	Pellitorine	5318516	223.35	C14H25NO	-5.9

3.2 Protein-ligand interaction analysis

Ligplot+ software for protein-ligand has been used for analysis and highlighted the hydrogen bonds and hydrophobic interaction between the amino acids of the protein and the ligand. The analysis aid in predicting the binding affinity between the ligand and the target protein. Different types of interactions exhibited by the selected compounds is given in Table 4. The schematic 2D representation of interaction between the blood brain permeant ligands and the target protein is given in Figure 1.

Table 4: Interaction of phytochemical compound with active site of the target protein

S. No.	Ligand Name	PubChem ID	Hydrophobic Interaction	Hydrogen Bond Interaction	Docking Score
1.	Chrysin	5281607	Lys9, Gly11, Gly13, Tyr14, Ala157, Val170, Thr232, Arg307, Pro308, Glu 339	Ser10	-7.4
2.	Dihydropiperlonguminin	12682184	Ser10, Gly11, Tyr14, Gly156, Val170, Trp277, Gln303, Leu306, Arg307, Pro308, Ala335, Glu339, Val361	Gly13, Thr232	-6.9
3.	Dihydrooroxylin	177032	Ala157, Val170, Trp277, Gln303, Arg307, Pro308, Glu339, Val361	Ser10	-7.4
4.	Futoamide	15596445	Ser10, Gly11, Tyr14, Val170, Gln303, Arg307, Pro308, Tyr320, Ala335, Glu339, Val361	Thr232	-6.6
5.	Pellitorine	5318516	Gly13, Tyr14, Gly156, Thr232, Trp277, Gln303, Arg307, Pro308, Ala335, Val361	Ser10, Gly11, Glu339	-5.9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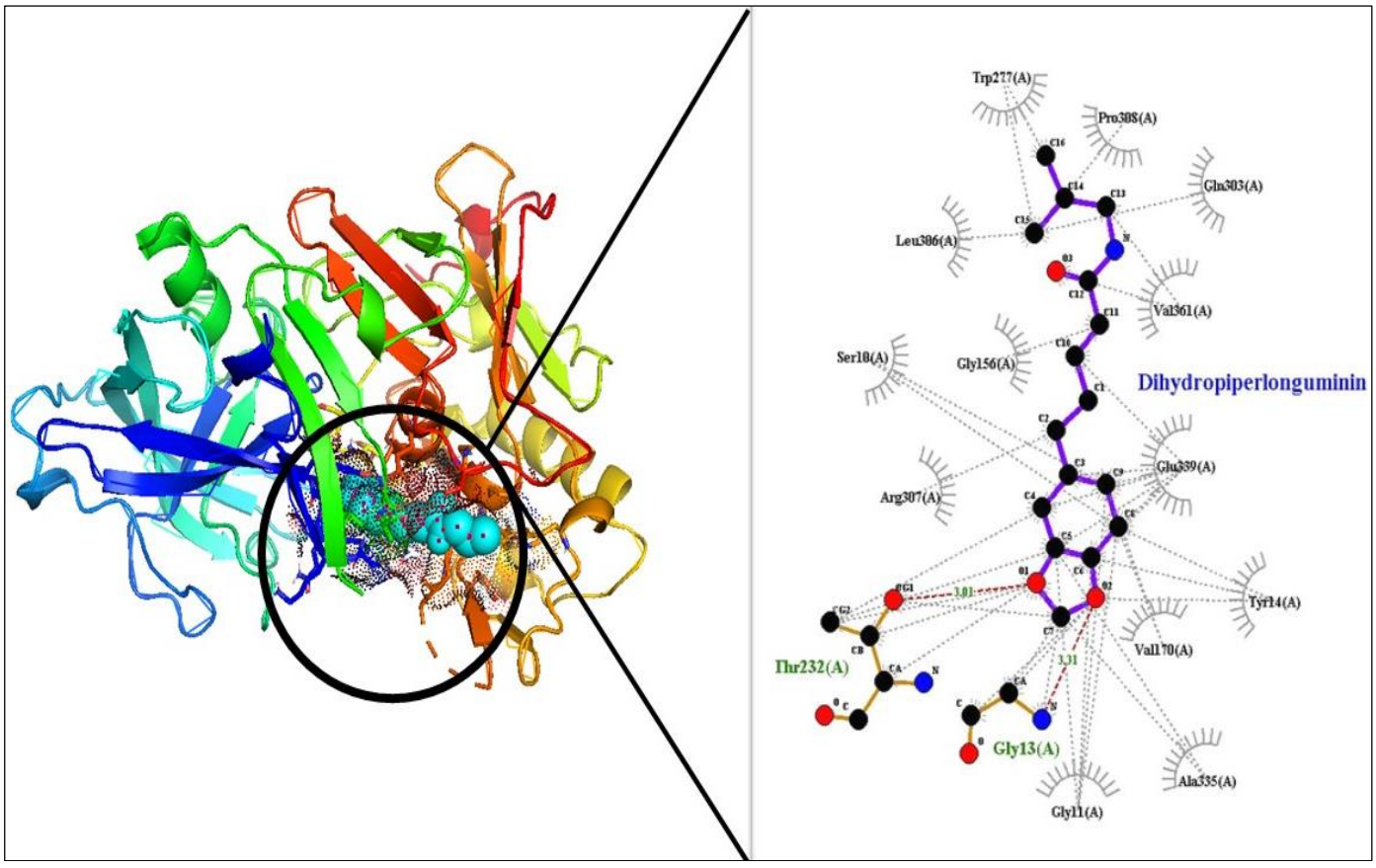
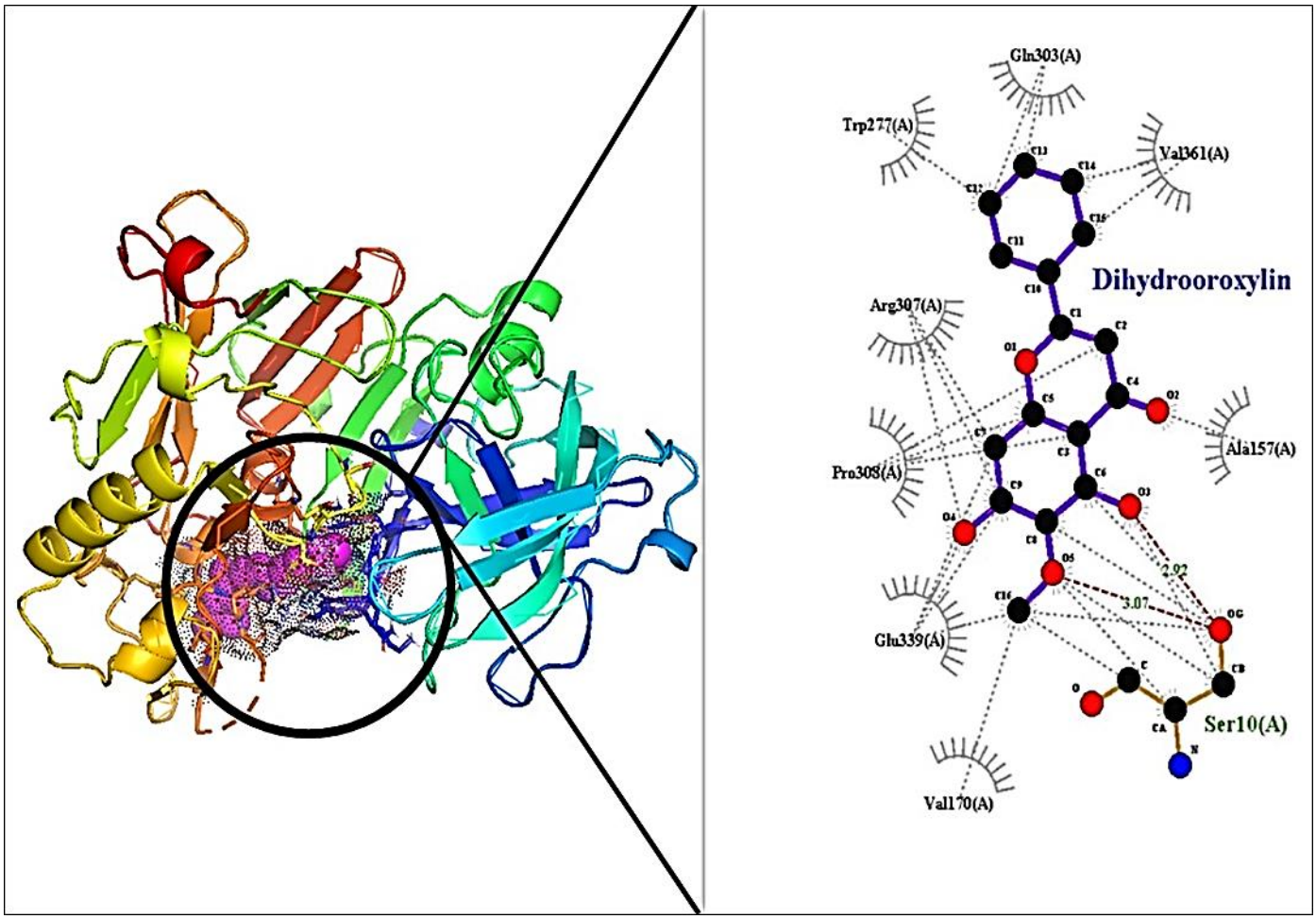
Ala157(A)
Glu339(A)
Chrysin
Val170(A)
Arg307(A)
Lys9(A)
Ser10(A)
Gly11(A)
Thr232(A)
Tyr14(A)
Gly13(A)

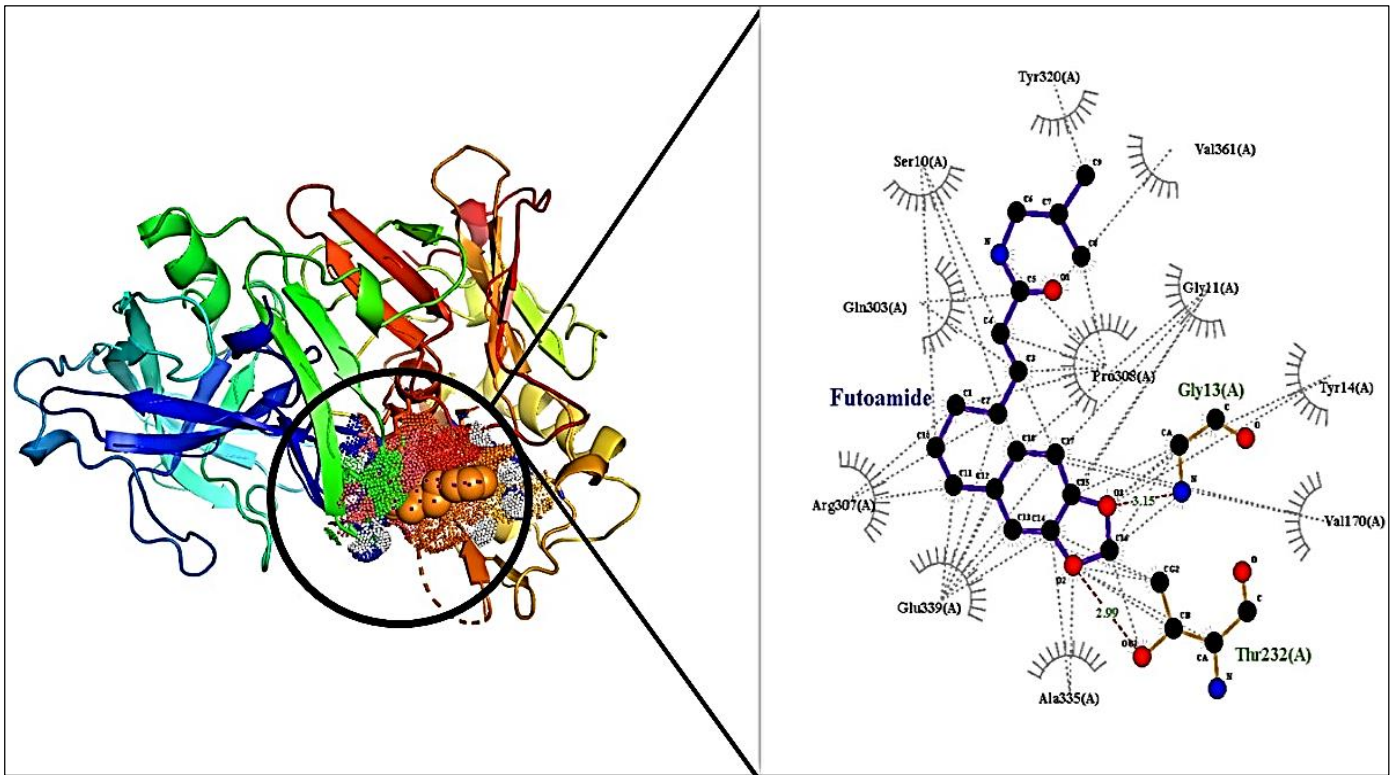
(A)


Trp277(A)
Pro308(A)
Gln303(A)
Leu306(A)
Val361(A)
Ser10(A)
Gly156(A)
Dihydropiperlonguminin
Glu339(A)
Arg307(A)
Tyr14(A)
Val170(A)
Thr232(A)
Gly13(A)
Ala335(A)
Gly11(A)

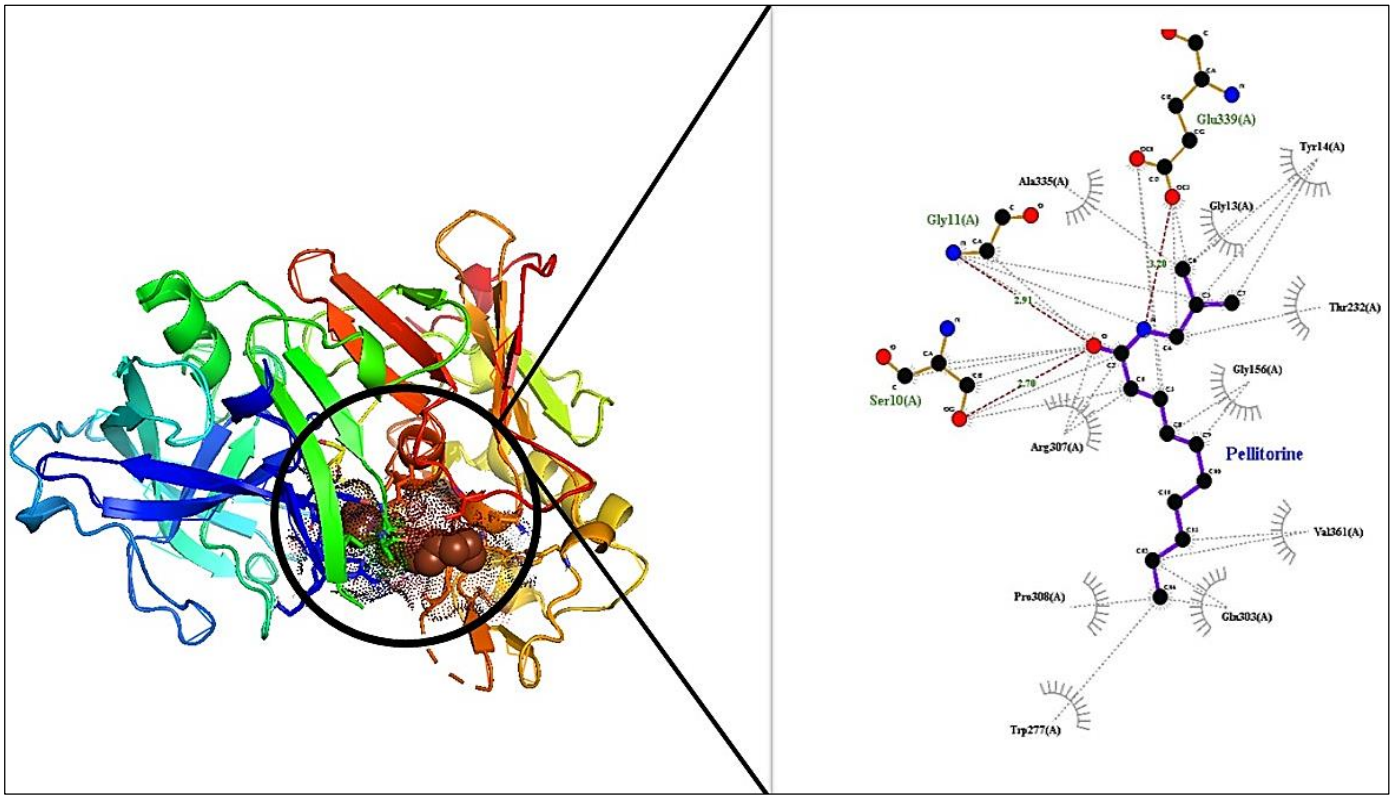
(B)


Gln303(A)
Trp277(A)
Val361(A)
Dihydrooroxylin
Arg307(A)
Ala157(A)
Pro308(A)
Glu339(A)
Ser10(A)
Val170(A)
2.92
3.07

(C)


Tyr320(A)
Ser10(A)
Val361(A)
Gln303(A)
Gly11(A)
Pro308(A)
Futoamide
Gly13(A)
Tyr14(A)
Arg307(A)
Val170(A)
Glu339(A)
Thr232(A)
Ala335(A)
3.15
2.99

(D)



(E)

Fig 1: The ligands having property of blood brain permeability are shown in the figure. The grey dotted line depicts hydrophobic interaction of ligand with respective amino acid of the target protein while the red dotted line shows the hydrogen bonding of the phytochemical ligand with the amino acid of the target protein, BACE1. (A) Chrysin interaction with target protein BACE1 (B) Dihydropyridine interaction with target protein BACE1 (C) Dihydrooroxylin interaction with target protein BACE1 (D) Futoamide interaction with target protein BACE1 (E) Pellitorine interaction with target protein BACE1

3.3 ADME/T analysis of selected top compounds

Pharmacological and pharmacokinetic profiling is considered as an important step in drug development as it helps to predict the efficacy of drug such as its absorbability, bioavailability, its ability to reach site of action, metabolism and finally its excretion that too without posing significant side effect. Several factors are taken into consideration to determine a compound's drug likeness. Computational programs are used widely in the field of pharmaceuticals to test the ADME/T of a compound which aid in selection of top candidates. The striking aspect of the selected drug compound is their BBB permeability, molecular weight, topological polar surface area (TPSA), LogS value satisfy the Lipinski rule of five [18] (Table 5). Furthermore, polar surface area, high oral bioavailability, H-bond donors and acceptors are key characteristics for therapeutic agent development. All of these models unscrew the qualitative prediction and rating of absorption, the impacts of formulation on drug permeability, determining the mechanism(s) of permeability, and the possibility for transporter-mediated drug-drug interactions. Top five ligands which were chosen on the basis of good docking score and BBB permeability has drug likeness properties by Lipinski's rule of five. The BOILED-Egg used for prediction of gastrointestinal absorption and blood brain barrier permeability is given in figure 2.

Table 5: MW: Molecular Weight; LogS: Predicted aqueous solubility; Log0/w: Predicted Lipophilicity; Accept H: Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution; Donor H: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution; TPSA: Topological polar surface area, molecular descriptor for drug transport properties such as GI absorption and blood-brain barrier (BBB) penetration

Ligand Name	MW	Pubchem ID	Log S	Logo/w	Accept H	Donor H	TPSA (Å)	BBB Per meant
Pellitorine	223.35	5318516	-3.40	4.39	1	1	29.10	YES
Chrysin	254.24	5281607	-4.19	3.52	4	2	70.67	YES
Dihydropiperlonguminin	275.34	12682184	-3.52	3.51	3	1	47.56	YES
Dihydrooroxylin	286.28	177032	-3.70	2.85	5	2	75.99	YES
Futoamide	301.4	15596445	-4.48	4.91	3	1	47.56	YES

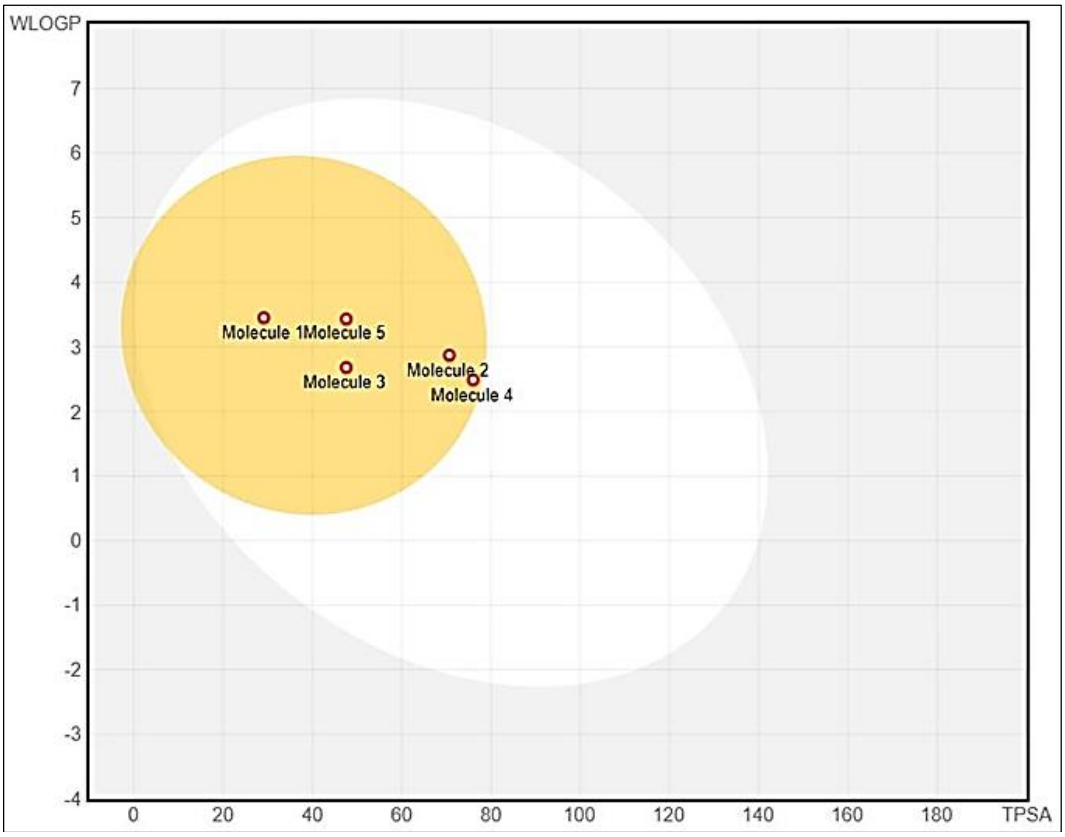


Fig 2: BOILED-Egg to assess gastric absorption and blood-brain barrier permeability Yolk: Blood brain permeability; White: GI absorbability; Molecule 1: Pellitorine; Molecule 2: Chrysin; Molecule 3: Dihydropiperlonguminin; Molecule 4: Dihydrooroxylin; Molecule 5: Futoamide

4. Discussion

Alzheimer's disease is among the most common neurodegenerative disorders, impacting a vast population worldwide. The glycosylation process abnormality is thought to play a significant role in the production of amyloid beta plaque. The beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), also known as beta-secretase, has been identified as the primary initiator of amyloid beta plaque because it cleaves APP's extracellular domain, resulting in the production of plaques. The root extract of *Scutellaria baicalensis* (Chinese skullcap) are rich in flavonoids and possess neuroprotective property. In this study, the BACE1 is taken into consideration as the target protein and the active compound present in the root extract of Chinese skullcap are taken as the ligand. Out of 15 phytochemicals screened against the target protein BACE1, 12 of the compounds shown good docking score while 5 out of them possess blood brain barrier (BBB) permeability property as shown by BOILED-Egg. The top five compounds which have best docking score with BBB permeability were chosen. The chosen compounds exhibit strong hydrophobic interaction and some hydrogen bonding with drug likeness property as they follow Lipinski's rule of five. Thus, these five best docked compounds can be used for drug formulation against BACE1 to tackle Alzheimer's disease. The striking aspect of the study is that apart from showing drug-likeness and good docking score, the compounds have BBB property which is considered as important feature of the drugs used as therapeutic intervention in neurodegenerative disorder. The data from this *in silico* study can be used for *in vivo* and *in vitro* study for further validation. Further experimental research using the *in silico* may aid in formulation of novel therapeutic drug.

5. Disclosure statement

The authors declare no conflict of interest.

6. References

1. Alzheimer's Association. Alzheimer's disease facts and figures includes a special report on the financial and personal benefits of early diagnosis. Alzheimers Assoc. 2018;14:367-429. DOI: 10.1016/j.jalz.2016.03.001.
2. Coimbra J, Marques D, Baptista SJ, Pereira C, Moreira PI, Dinis T, *et al*. Highlights in BACE1 Inhibitors for Alzheimer's Disease Treatment. Frontiers in chemistry. 2018;6:178. https://doi.org/10.3389/fchem.2018.00178.
3. Alam M, Abbas K. An Insight into Neurodegenerative Disorders, their therapeutic approaches and drugs available for tackling with Neurodegeneration: A Review. IAR Journal of Medical Case Reports, 2021, 2(3).
4. Selkoe Dennis J, Hardy John. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Molecular Medicine. 2016;8(6):595-608. DOI: 10.15252/emmm.201606210.
5. Plácido AI, Oliveira CR, Moreira PI, Pereira CM. Enhanced amyloidogenic processing of amyloid precursor protein and cell death under prolonged endoplasmic reticulum stress in brain endothelial cells. Mol. Neurobiol. 2015;51:571-590. 10.1007/s12035-014-8819-1.
6. Carvalho C, Correia SC, Cardoso S, Plácido AI, Candeias E, Duarte AI, *et al*. The role of mitochondrial disturbances in Alzheimer, Parkinson and Huntington diseases. Expert Rev. Neurother. 2015;15:867-884. 10.1586/14737175.2015.1058160.
7. Correia SC, Resende R, Moreira PI, Pereira CM. Alzheimer's disease-related misfolded proteins and dysfunctional organelles on autophagy menu. DNA Cell Biol. 2015;34:261-273. 10.1089/dna.2014.2757.
8. Hampel H, Lista S, Vanmechelen E, *et al.* β-Secretase1 biological markers for Alzheimer's disease: state-of-art

of validation and qualification. Alz Res Therapy. 2020;12:130. https://doi.org/10.1186/s13195-020-00686-3.
9. Menting KW, Claassen JA. Beta-secretase inhibitor; a promising novel therapeutic drug in Alzheimer's disease. Front. Aging Neuro Sci. 2014;6:165. 10.3389/fnagi.2014.00165.
10. Zhao Q, Chen XY, Martin C. *Scutellaria baicalensis*, the golden herb from the garden of Chinese medicinal plants. Science bulletin. 2016;61(18):1391-1398. https://doi.org/10.1007/s11434-016-1136-5.
11. Wang ZL, Wang S, Kuang Y, Hu ZM, Qiao X, Ye M. A comprehensive review on phytochemistry, pharmacology and flavonoid biosynthesis of Scutellaria baicalensis. Pharm Biol. 2018 Dec;56(1):465-484. DOI: 10.1080/13880209.2018.1492620. PMID: 31070530; PMCID: PMC6292351.
12. Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim S. Neuroprotective and Cognitive Enhancement Potentials of Baicalin: A Review. Brain sciences. 2018;8(6):104. https://doi.org/10.3390/brainsci8060104.
13. Trott O, Olson AJ. Vina AD. Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010 Jan;31(2):455-61. 10.1002/jcc.21334.
14. Sruthi N, Nithyasree R, Sathya A. Drug reposition of Amoxycillin by molecular docking. Int. J Adv. Chem. Res. 2020;2(2):01-11. DOI: 10.33545/26646781.2020.v2.i2a.21
15. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem. Inf. Model, 51(10):2778-2786.
16. 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;7:42-717. https://doi.org/10.1038/srep42717.
17. Daina A, Zoete V. Chem. Med Chem. 2016;11:11-17.
18. Lipinski CA, Lombardo F, Dominy BW, *et al*. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivr. Rev. 1997;23(1-3):3-25.
19. Dallakyan S, Olson Arthur J. Small molecule library screening by docking with PyRx. Methods Mol. Biol. 2015;1263:243-50. 10.1111/j.1747-0285.2007.00471.x.