Egyptian Journal of Medical Human Genetics

RESEARCH Open Access

Identification of the most potent acetylcholinesterase inhibitors from plants for possible treatment of Alzheimer's disease: a computational approach

Bishajit Sarkar[1*], Sayka Alam[1†], Tiluttoma Khan Rajib[1†], Syed Sajidul Islam[1†], Yusha Araf[2] and Md. Asad Ullah[1]

Abstract

Background: Being one of the rapidly growing dementia type diseases in the world, Alzheimer's disease (AD) has gained much attention from researchers in the recent decades. Many hypotheses have been developed that describe different reasons for the development of AD. Among them, the cholinergic hypothesis depicts that the degradation of an important neurotransmitter, acetylcholine by the enzyme acetylcholinesterase (AChE), is responsible for the development of AD. Although, many anti-AChE drugs are already available in the market, their performance sometimes yields unexpected results. For this reason, research works are going on to find out potential anti-AChE agents both from natural and synthetic sources. In this study, 50 potential anti-AChE phytochemicals were analyzed using numerous tools of bioinformatics and in silico biology to find out the best possible anti-AChE agents among the selected 50 ligands through molecular docking, determination of the druglikeness properties, conducting the ADMET test, PASS and P450 site of metabolism prediction, and DFT calculations.

Result: The predictions of this study suggested that among the selected 50 ligands, bellidifolin, naringenin, apigenin, and coptisine were the 4 best compounds with quite similar and sound performance in most of the experiments.

Conclusion: In this study, bellidifolin, naringenin, apigenin, and coptisine were found to be the most effective agents for treating the AD targeting AChE. However, more in vivo and in vitro analyses are required to finally confirm the outcomes of this research.

Keywords: Alzheimer's disease, Acetylcholinesterase, Molecular docking, Phytochemicals, Druglikeness properties, ADMET, PASS prediction, Naringenin

* Correspondence: sarkarbishajit@gmail.com
†The authors Sayka Alam, Tiluttoma Khan Rajib, and Syed Sajidul Islam have contributed equally to the work and jointly hold the second authorship.
[1]Department of Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Jahangirnagar University, Dhaka, Bangladesh
Full list of author information is available at the end of the article

Background

First described by Alois Alzheimer in 1907, Alzheimer's Disease (AD) has become one of the most prevalent dementia type diseases in the world which is increasing its numbers rapidly [1, 2]. Intellectual morbidity, psychomotor dysregulation, delusions, hallucinations etc., are some of the familiar symptoms of AD [3]. In the familial and congenital cases of AD, genetic factors play critical roles [4]. Different hypotheses have been developed by the scientists that shed light on several reasons for AD onset and development. One of these hypotheses is the amyloid cascade hypothesis, which describes that the deposition of β-amyloid plaques in the brain is mainly responsible for the development of AD which is the result of abnormal processing of the amyloid precursor protein (APP) by the β-secretase enzyme. These plaques interfere with the normal functions of the brain [5]. On the other hand, another hypothesis called the oxidative stress hypothesis describes that because of the deposition of increased amounts of ions like iron, aluminium, and mercury, free radicals and reactive oxygen species (ROS) are generated very rapidly in the brain which are responsible for increased lipid peroxidation and protein and DNA oxidation. The stresses produced by these oxidation events lead the way for the AD onset [6]. There is another hypothesis of AD development which is known as the cholinergic hypothesis. According to this hypothesis, the loss of functions of the cholinergic neurons and thus the cholinergic signaling and neurotransmission in the brain maybe responsible for AD [7]. Table 1 lists the current status of therapeutic agents that are intended to or being used to alleviate the complications related to AD. This experiment was conducted focusing on the cholinergic hypothesis of AD development.

The cholinergic hypothesis and the development of Alzheimer's disease

The cholinergic hypothesis concerns with one of the major neurotransmitters, acetylcholine (ACh) which is regulated by two enzymes, acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) [13]. ACh is involved in many important functions of the brain like learning and memory generation processes. It performs its functions through binding to two types of receptors, i.e., nicotinic (α7 and α4β2) and muscarinic receptors (M1 muscarinic receptor). ACh is synthesized by the enzyme choline acetyltransferase (ChAT) which catalyzes the transfer of an acetyl group from acetyl coenzyme A (Ac-CoA) to choline (Ch) in the pre-synaptic neuron and thus synthesizes the ACh. Thereafter, the ACh is secreted by the pre-synaptic neuron into the synapse where it mediates its effects by binding to either the nicotinic receptor or the muscarinic receptor. To maintain the optimal concentrations of ACh required for proper functioning of the brain, another enzyme called acetylcholinesterase (AChE) is synthesized by a serine hydrolase enzyme which hydrolyzes ACh to acetate and Ch. Then the Ch is again taken up by the pre-synaptic neuron for recycling and reusing. Thus, the balance of ACh is maintained in the normal brain. However, there is evidence that, in the brain of AD patients, the overexpression of AChE occurs. This phenomenon decreases the amount of ACh required for proper functioning of the brain which is why the neuron cells cannot operate properly and complications like brain damage as well as memory loss occur. These complications lead to the onset of AD development (Fig. 1) [14–18].

Donepezil and rivastigmine are two FDA-approved drugs that are currently used for mild to moderate AD treatment targeting the AChE enzyme. However, both of them have several side effects like nausea, diarrhea, anorexia, syncope, abdominal pain, and vomiting [19, 20]. Therefore, scientists are searching for more effective agents that can provide more efficacy than these drugs with much lesser side effects. Scientists have also focused on the natural resources for potential anti-AChE agents since the natural agents are generally much safer than synthetic chemicals. Galantamine is such a natural drug isolated from *Galanthus woronowii* which is also currently used for AD treatment alongside other approved chemical drugs. But since none of these drugs are found to be quite satisfactory to stop the progression or development of AD, research is going on to find out new compounds from natural sources with anti-AChE properties [21–23].

Molecular docking is a widely accepted and used technique in drug R&D which reduces both time and costs of lead discovery processes. This method is also known as computational drug design which has already been used for designing over 50 novel drugs, and many of them have also gained FDA approval for marketing. By simulating the interaction between ligand and receptor in the computer software, the docking system assigns scoring functions to the bound ligands which reflect their binding affinity. The lower docking score represents the greater binding affinity and vice-versa [24, 25]. The current study was designed to predict the best ligands among 50 selected phytochemicals with potential anti-AChE activities based on the molecular docking analysis (Table 2). Thereafter, the pharmacodynamics and physicochemical characteristics of the best selected ligands were predicted by determining their druglikeness properties, conducting the ADMET test, PASS, and P450 site of metabolism prediction and DFT calculations.

Methods

Total 50 phytochemicals were selected as ligands in this study by reviewing numerous literatures along with their

Table 1 Examples of AD treating agents, their mechanism of treatment, and their current status

Hypothesis	Treating agents	Mechanism of available treatments	Current status	Remarks	References
Amyloid cascade hypothesis	Plasma exchange with albumin 1 immunoglobulin	Exchange of plasma to remove the amyloid	Phase-III clinical trial	Ongoing research	[8, 9]
	ALZT-OP1a + ALZT-OP1b	Alleviation of amyloid plaque related neuroinflammation	Phase-III clinical trial	Ongoing research	
	Elenbecestat	Removal of amyloid by inhibiting Beta-secretase 1	Phase-III clinical trial	Ongoing research	
	Solanezumab	Monoclonal antibodies targeting amyloid plaque	Failed	Lack of efficacy	
	Verubecestat	Removal of amyloid by inhibiting Beta-secretase 1	Failed	Lack of efficacy	
	Lanabecestat	Removal of amyloid by inhibiting Beta-secretase 1	Failed	Lack of efficacy	
	CAD106	Vaccine-based therapy	Phase-II clinical trial	Ongoing research	
	CNP520	Inhibition of Beta-secretase 1	Phase-II clinical trial	Ongoing research	
	Crenezumab	Monoclonal antibody targeting amyloid plaque	Ongoing investigation	Ongoing research	
	Neflamapimod	Inhibition of inflammatory protein p38α MAPK	Ongoing investigation	Ongoing research	
	Aducanumab	Monoclonal antibody targeting amyloid plaque	Phase-III clinical trial	Ongoing research	
Oxidative stress hypothesis	Supplementation of Vitamin C and Vitamin E	Antioxidant to neutralize the ROS	Current investigational treatment	Ongoing study	[10, 11]
	Selenium	Antioxidant to neutralize the ROS	Ongoing investigation	Ongoing study	
	Curcumin	Antioxidant to neutralize the ROS	Current investigational treatment	Ongoing study	
	Ginko biloba	Antioxidant to neutralize the ROS	Current treatment	Most effective among the available treatments	
	Ebenone	Antioxidant to neutralize the ROS	Ongoing investigation	Ongoing study	
	Estrogen	Antioxidant to neutralize the ROS	Ongoing investigation	Ongoing study	
	Colostrinin	Antioxidant to neutralize the ROS	Ongoing investigation	Ongoing study	
Cholinergic hypothesis	Tacrine	Inhibition of acetylcholinesterase	Current treatment	Approved by FDA	[12]
	Donepezil	Inhibition of acetylcholinesterase	Current treatment	Approved by FDA	
	Galantamine	Inhibition of acetylcholinesterase	Current treatment	Approved by FDA	
	Rivastigmine	Inhibition of acetylcholinesterase	Current treatment	Approved by FDA	

ROS reactive oxygen species, *FDA* Food and Drug Administration

IC50 values. On sequential docking experiment, four best ligands were selected as the best inhibitors of AChE. Thereafter, their different drug-like parameters were analysed in different experiments. Donepezil and galantamine were used as the positive controls in the study.

Protein preparation and ramachandran plot generation

A three-dimensional structure of AChE (PDB ID: 1ACJ) was downloaded in PDB format from protein data bank (www.rcsb.org). The proteins were then prepared and refined using the Protein Preparation Wizard in Maestro Schrödinger Suite 2018-4 [60]. During protein preparation, the bond orders were assigned and hydrogen molecules were added to heavy atoms as well as all the waters were deleted and the side chains were adjusted using Prime [61]. After that, the structure was optimized and minimized using force field OPLS_2005, which was conducted setting the maximum heavy atom RMSD (root-mean-square-deviation) to 30 Å and any

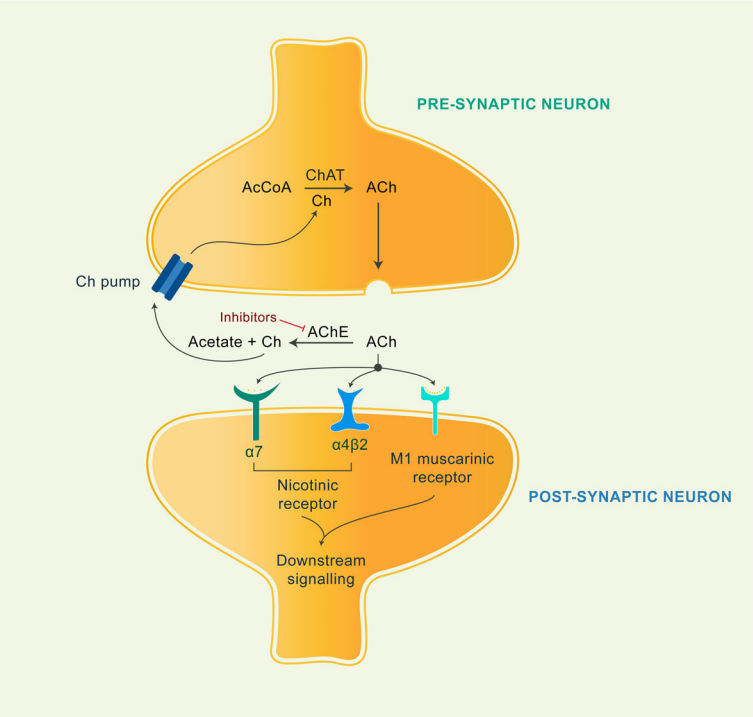


Fig. 1 Cholinergic hypothesis and role of AChE in AD development. ACh is synthesized by ChAT and released from the pre-synaptic neuron. The ACh mediates its effects on the post-synaptic neuron through nicotinic and/or muscarinic receptors. The ACh then performs the downstream signaling in the post-synaptic neuron. AChE enzyme breaks down the ACh and overexpression of AChE lowers the amount of ACh in the brain which leads to the AD onset. AChE inhibitors repress the AChE activity, thus aid in the AD treatment

remaining water less than 3 H- bonds to non-water was again deleted during the minimization step.

Ligand preparation

Three-dimensional structures of the 50 selected ligand molecules as well as the control were downloaded from PubChem database (www.pubchem.ncbi.nlm.nih.gov). These structures were prepared for docking using the LigPrep module of Maestro Schrödinger Suite [62]. Minimized 3D structures of ligands were generated by Epik2.2 using OPLS_2005 force field and within pH 7.0 ± 2.0 [63].

Receptor grid generation

Grid confines the active site of the receptor protein to a shortened specific area for the ligand to dock specifically. In glide, a grid was generated where the default Van der Waals radius scaling factor 1.0 and charge cutoff 0.25 was used. The grid was then subjected to OPLS_2005 force field. A cubic box was generated around the active site (reference ligand active site). Then, the grid box volume was adjusted to 14 × 14 × 14 for docking test.

Glide standard precision (SP) and extra precision (XP) ligand docking, Prime MM-GBSA calculation and induced fit docking

SP and XP adaptable glide dockings were conducted using the Glide module in Maestro Schrödinger Suite [64]. The Van der Waals radius scaling factor and charge cutoff were kept at 0.80 and 0.15, respectively, for all the ligand molecules. Final score was assigned by the module by analyzing the pose of docked ligand within the active site of the receptor.

After SP and XP ligand docking, the docked complexes were subjected to molecular mechanics—generalized born and surface area (MM_GBSA) rescoring with the help of Prime module of Maestro Schrödinger suite for further evaluation. This technique utilizes the docked complex and uses an implicit solvent that assigns more accurate scoring function and improves the overall free-binding affinity score upon the reprocessing of the complex. It combines OPLS molecular mechanics energies (E_{MM}), surface generalized born solvation model for polar solvation (G_{SGB}), and a nonpolar salvation term (G_{NP}) for total free energy (ΔG_{bind}) calculation. The total free energy of binding was calculated by the following equation [65]:

Table 2 List of the 50 anti-AChE ligands with their experimental IC50 values used in the experiment

No	Name of the compound	PubChem CID	Source plant	IC50 value	References
01	Geraniol	637566	*Boesenbergia Pandurata*	200±0.21 μg/ml	[26, 27]
02	Bellidifolin	5281623	*Swertia chirata*	18.47 ± 0.47 μg/ml	[28]
03	Mangiferin	5281647	*S. chirata*	4.07 ± 0.06 μg/ml	[28]
04	Allocryptopine	98570	*Chelidonium majus*	250 ± 2.5 μM	[29]
05	Chelidonine	197810	*Chelidonium majus*	26.8 ± 1.2 μM	[30]
06	Isorhamnetin	5281654	*Calendula officinalis*	24.18 ± 0.74 μM	[31]
07	Quercetin	5280343	*Calendula officinalis*	14.37 ± 0.34 μM	[30]
08	Myricetin	5281672	*Scabiosa arenaria*	190 ± 0.41 μg/ml	[26, 27]
09	Ostruthin	5281420	*Peucedanum ostruthium*	–	[32]
10	Ostruthol	6441273	*Peucedanum ostruthium*	–	[32]
11	Corynoline	177014	*Corydalis incisa*	30.6 μM/mL	[33]
12	Imperatorin	10212	*Peucedanum ostruthium, Angelica dahurica*	63.7 μM	[32, 33]
13	Rupicoline	633439	*Tabernaemontana australis*	–	[34]
14	Stylopine	6770	*Corydalis crispa, Chelidonium majus*	114 ± 2.9 μM	[29, 35]
15	Scoulerine	22955	*Corydalis dubia*	245 μM	[35]
16	Ochrobirine	629543	*Corydalis crispa*	–	[35]
17	Estragole	8815	*O. basilicum, O. africanum, O.americanum, and O. minimum*	0.337 μM	[36]
18	Naringenin	932	*Citrus junos*	28.2 to 134.5 μM	[37, 38]
19	Carvacrol	10364	*Thymus vulgaris*	0.175 mM	[39–41]
20	Myrtenal	61130	*Taxus baccata*	0.17 ± 0.01 mM	[41]
21	Verbenone	29025	*Taxus baccata*	2.66 ± 1.04 mM	[41]
22	Cyclonataminol	53316925	*Buxus natalensis*	23 μM	[42, 43]
23	Buxaminol A	53324855	*Buxus natalensis*	29.8 ± 4.4 μM	[43]
24	Skimmianine	6760	*Zanthoxylum nitidum*	74.09 ± 0.33 mg/mL	[44, 45]
25	Harmaline	3564	*Peganum harmala*	8.4 μg/mL	[46]
26	Harmine	5280953	*Peganum harmala*	10.9 μg/mL	[46]
27	Isoimperatorin	68081	*Ruta graveolens, angelica dahurica*	74.6 μM	[33, 47]
28	Xanthotoxin	4114	*Ruta graveolens*	5.4×10^{-5} M	[47, 48]
29	Marmesin	334704	*Ruta graveolens, angelica dahurica*	13.3 mM	[46, 49]
30.	1,8-cineole	2758	*Inula graveolens*	0.015 mg/mL	[50]
31	Eugenol	3314	*Inula graveolens*	0.48 mg/mL	[50]
32	α-terpineol	17100	*Inula graveolens*	1.3 mg/mL	[50]
33	Apigenin	5280443	*Sideritis cesarea*	7.72 ± 0.15 μM	[51, 52]
34	Linearol	497896	*Sideritis congesta*	2.66 μg/mL	[52]
35	Sidol	194142985	*Sideritis congesta*	0.92 μg/mL	[52]
36	Sideridiol	12315541	*Sideritis congesta*	8.04 μg/mL	[52]
37	Sudachitin	12443122	*Micromeria cilicica*	65.2 ± 0.82 μg/mL	[52]
38	Ursolic acid	64945	*Nepeta sorgerae*	39.19 μg/mL	[52]
39	α-pinene	6654	*Salvia potentillifolia*	0.022 mg/mL	[50, 52]
40	Liriodenine	10144	*Beilschmiedia alloiophylla*	3.5 ± 1.0 μM	[53]
41	Secoboldine	10359075	*Beilschmiedia alloiophylla*	10.0 ± 0.6 μM	[54]
42	Lauro-tetanine	31415	*Beilschmiedia alloiophylla*	3.2 ± 0.3 μM	[55]

Table 2 List of the 50 anti-AChE ligands with their experimental IC50 values used in the experiment *(Continued)*

No	Name of the compound	PubChem CID	Source plant	IC50 value	References
43	Asimilobine	160875	*Beilschmiedia alloiophylla*	8.7 ± 1.5 μM	[53]
44	β-amyrone	12306160	*Beilschmiedia alloiophylla*	8.4 ± 2.0 μM	[53]
45	Berberine	2353	*Coptis chinensis, Berberis bealei and Phellodendron chinense*	1.48 ± 0.07 mg/ml	[54]
46	Coptisine	72322	*Coptis chinensis, Berberis bealei and Phellodendron chinense*	1.27 ± 0.06 mg/ml	[54]
47	Palmatine	19009	*Coptis chinensis, Berberis bealei and Phellodendron chinense*	5.21 ± 0.48 mg/ml	[54]
48	Luteolin	5280445	*Thymus vulgaris*	135 ± 0.18 μg/ml	[26, 55]
49	Rutin	5280805	*Micromeria cilicica, Fraxinus angustifolia*	149.0 ± 6.6 μM	[52, 56]
50	Kaempferol	5280863	*Cleistocalyx operculatus*	30.4 μM	[57]
Positive control 1	Donepezil	3152	NA	6.7 nM	[58]
Positive Control 2	Galantamine	9651	*Galanthus woronowii*	0.35 μmol/L	[59]

NA data not available

$$\Delta G_{bind} = G_{complex} - \left(G_{protein} - G_{ligand}\right), \text{where}, G = E_{MM} + G_{SGB} + G_{NP}.$$

The agents with best results in the SP and XP docking were selected for the MM_GBSA and IFD studies.

Thereafter, to carry out the IFD of the selected ligand molecules from SP and XP docking, again OPLS_2005 force field was applied after generating grid around the co-crystallized ligand of the receptor, and this time, the best five ligands were docked rigidly. Receptor and ligand Van Der Waals screening was set at 0.70 and 0.50, respectively, and residues within 2 Å were refined to generate 2 best possible posses with extra precision. Four best performing ligands were selected according to their MM_GBSA score, IFD score, and XP_{Gscore}. The 3D representations of the best pose interactions between the best four ligands and their respective receptors were obtained using Discovery Studio Visualizer [66]. At these stages, the docking parameters of the compounds under investigation were compared with the control

Ligand-based drug likeness property and ADMET prediction

The drug likeness properties of the 4 selected ligand molecules were analyzed using SWISSADME server (http://www.swissadme.ch/) [67]. After that, the ADME T for each of the ligand molecules was conducted using the online based server, ADMETlab (http://admet.scbdd.com/) to predict various pharmacokinetic and pharmacodynamic properties [68, 69]. The numeric and categorical values of the results generated by the ADME Tlab server were converted into qualitative values according to the documentation and explanation described in the ADMETlab server (http://admet.scbdd.com/home/interpretation/) for the convenience of interpretation.

PASS and SOM prediction

The PASS (Prediction of Activity Spectra for Substances) prediction of the best four selected ligands were carried out by the PASS-Way2Drug server (http://www.pharmaexpert.ru/passonline/), using the canonical SMILES from PubChem server (https://pubchem.ncbi.nlm.nih.gov/) [70]. While carrying out PASS prediction, the P_a (probability to be active) was kept greater than 70%, since the P_a > 70% threshold generates highly reliable prediction [71]. In the PASS prediction study, 15 possible biological activities were predicted. The P450 Site of Metabolism (SOM) of the four best selected ligand molecules was determined by online tool, RS-WebPredictor 1.0 (http://reccr.chem.rpi.edu/Software/RS-WebPredictor/) [72]. Moreover, the LD50 value and toxicity class of the compounds were predicted using the ProTox-II server (http://tox.charite.de/protox_II/) [73].

DFT calculations

Minimized ligand structures obtained from LigPrep were used for DFT calculation using the Jaguar panel of Maestro Schrödinger Suite using Becke's three-parameter exchange potential and Lee-Yang-Parr correlation functional (B3LYP) theory with 6-31G* basis set [74–76]. Quantum chemical properties such as surface properties (MO, density, potential) and multipole moments were calculated along with HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy. Then, the global frontier orbital was analyzed and hardness (**η**) and softness (**S**) of selected molecules were calculated using the

following equation as per Parr and Pearson interpretation and Koopmans theorem [77, 78]. The DFT calculation was done for the 3 best ligand molecules.

$$\eta = (\mathrm{HOMO}\varepsilon - \mathrm{LUMO}\varepsilon)/2,$$
$$S = 1/\eta$$

Result

Molecular docking study

All the 50 selected ligands were docked successfully with their receptor protein, AchE. The ligand molecules that had the lowest binding energy were considered the best ligand molecules because lower binding energy or docking score represents higher binding affinity [79]. In the MM-GBSA study, the most negative ΔGBind score is also considered as the best ΔGBind score [80]. IFD study was conducted to determine the accurate binding mode and accuracy of active site geometry. The lowest values of IFD score and XP GScore represent the best results [81–84]. From the initial 50 ligands, the ligands that had both SP and XP scores of less than − 8.00 Kcal/mol were selected for MM_GBSA analysis and IFD study. Thirteen ligands were found to have both SP and XP scores less than − 8.00 Kcal/mol: bellidifolin, isoharmnetin, quercetin, myricetin, imperatorin, naringenin, harmaline, harmine, isoimperatorin, apigenin, coptisine, liriodenine, and scoulerine (Table 3). From these 13 ligands, 4 ligands were finally selected based on their lower scores in both MM_GBSA (ΔG_{bind} score) and IFD studies (XP G_{score} and IFD score), i.e., bellidifolin, naringenin, apigenin, and coptisine (Table 4). These four ligands were selected for further analysis to predict and determine their drug potentials. When compared to the two positive controls: donepezil and galantamine, it can be declared that these four compounds generated sound results in the molecular docking study.

Binding mode of best ligands with respective targets

The 3D representations as well as the interaction of different amino acids of the binding pocket of AChE with bellidifolin, naringenin, apigenin, and coptisine are illustrated in Fig. 2 and listed in Supplementary Table S1.

Bellidifolin generated an MM_GBSA score of − 55.23 Kcal/mol when docked with acetylcholinesterase. It also generated the IFD score of − 1155.67 Kcal/mol and XP G_{score} of − 10.58 Kcal/mol against acetylcholinesterase. It formed two hydrogen bonds with glutamic acid 199 and histidine 440 at 2.18 Å and 2.44 Å distance apart, respectively. It was also reported to form multiple pi-pi stacked hydrophobic interactions with phenylalanine 330 (×2) and tryptophan 84 (×6) amino acid residues within the binding pocket of AChE.

Naringenin was docked with acetylcholinesterase where it generated the MM_GBSA score of − 42.02 Kcal/mol, IFD score of − 1156.52 Kcal/mol, and XP G_{score} of − 12.24 Kcal/mol. It formed four hydrogen bonds with histidine 440 (×2), tyrosine 70, and proline 86 at 1.69 Å, 2.38 Å, 1.66 Å, and 2.99 Å distance apart, respectively. It was also found to form multiple pi-pi stacked hydrophobic interactions with phenylalanine 330 and tryptophan 84 (×2) amino acid residues within the binding pocket of AChE.

Apigenin was docked with acetylcholinesterase where it generated the MM_GBSA score of − 49.07 Kcal/mol, IFD score of − 1154.41 Kcal/mol, and XP G_{score} of − 11.78 Kcal/mol. It formed six hydrogen bonds with glutamic acid 199 (×2), glycine 117, glycine 123, serine 122, and tyrosine 130 at 2.55 Å, 2.26 Å, 2.77 Å, 2.90 Å, 2.73 Å, and 2.76 Å distance apart, respectively. It was also formed multiple pi-pi stacked hydrophobic interactions with tryptophan 84 (×5), histidine 440, and phenylalanine 330 (×2) amino acid residues within the binding pocket of AChE.

Coptisine was docked with acetylcholinesterase, and it was found to generate the MM_GBSA score of − 59.41 Kcal/mol, IFD score of − 1160.39 Kcal/mol, and XP G_{score} of − 16.24 Kcal/mol. It formed six hydrogen bonds with histidine 440 (×2), serine 122, and glycine 117 at 2.21 Å, 2.13 Å, 3.01 Å, 2.90 Å, and 2.53 Å distance apart, respectively. It was also predicted to form multiple hydrophobic interactions with tryptophan 84 (×7), tryptophan 432, and phenylalanine 330 (×3) amino acid residues within the binding pocket of AChE.

Druglikeness properties

The druglikeness property experiment was conducted for only the 4 best selected ligands: bellidifolin, naringenin, apigenin, and coptisine. All the 4 ligands were predicted to follow the Lipinski's rule of five. Moreover, bellidifolin was found to have the highest topological polar surface area (TPSA) value of 100.13 $Å^2$. Again, coptisine was predicted to have the highest molar refractivity of 87.95 as well as the highest concensus Log $P_{o/w}$ of 2.40 (Table 5). All the found compounds generated quite sound performance in the analysis when compared with the two positive controls, donepezil and galantamine.

ADMET prediction

The results of the ADMET test are summarized in Table 6 along with their comparison with donepezil and galantamine. All the four ligands performed quite similarly in the ADMET test. In the absorption section, all the ligands were predicted to be optimal Caco-2 permeable, whereas all of them were P-gp (P-glycoprotein) non-inhibitors and P-gp non-substrates. In the distribution

Table 3 The results of the SP and XP docking study of the selected ligands along with their respective glide energies

No	Name of the compound	Standard Precision (SP) Docking score/ Binding energy (Kcal/mol)	Extra Precision (XP) Docking score/ Binding energy (Kcal/mol)	Glide energy (Kcal/mol)
01	Coptisine	− 11.12	− 15.98	− 48.31
02	Myricetin	− 10.37	− 12.43	− 42.546
03	Quercetin	− 9.82	− 11.22	− 44.872
04	Isorhamnetin	− 9.66	− 10.31	− 47.76
05	Apigenin	− 9.51	− 9.96	− 44.67
06	Naringenin	− 9.36	− 10.22	− 53.39
07	Bellidifolin	− 8.96	− 9.79	− 49.191
08	Harmine	− 8.73	− 8.97	− 49.149
09	Imperatorin	− 8.49	− 8.81	− 42.072
10	Harmaline	− 8.34	− 9.07	− 38.62
11	Scoulerine	− 8.33	− 8.14	− 41.25
12	Marmesin	− 8.10	− 7.73	− 23.41
13	Isoimperatorin	− 8.06	− 7.46	− 33.972
14	Liriodenine	− 8.01	− 7.31	− 45.69
15	Carvacrol	− 7.99	− 7.19	− 27.98
16	Xanthotoxin	− 7.84	− 7.78	− 39.22
17	Corynoline	− 7.69	− 7.11	− 39.95
18	Ostruthin	− 7.59	− 6.90	− 48.51
19	β-amyrone	− 7.41	− 7.63	− 40.65
20	Lauro-tetanine	− 7.31	− 6.33	− 31.50
21	Ostruthol	− 7.28	− 9.52	− 42.707
22	Sidol	− 7.23	− 6.65	− 36.53
23	Berberine	− 7.13	− 6.63	− 47.86
24	Buxaminol A	− 6.84	− 7.44	− 24.70
25	Stylopine	− 6.74	− 6.12	− 32.544
26	Ochrobirine	− 6.74	− 7.87	− 28.776
27	Secoboldine	− 6.71	− 8.48	− 33.87
28	Skimmianine	− 6.62	− 6.13	− 40.65
29	Palmatine	− 6.45	− 6.90	− 30.91
30	α-pinene	− 6.33	− 8.09	− 30.32
31	Sudachitin	− 6.31	− 8.26	− 21.28
32	Eugenol	− 6.30	− 6.42	− 24.55
33	Kaempferol	− 6.27	− 9.25	− 31.34
34	Myrtenal	− 6.15	− 7.95	− 43.53
35	Cyclonataminol	− 6.11	− 5.41	− 33.46
36	Rupicoline	− 5.99	− 7.03	− 48.430
37	α-terpineol	− 5.84	− 6.32	− 36.76
38	Estragole	− 5.77	− 6.24	− 44.384
39	Luteolin	− 5.74	− 6.87	− 28.76
40	Geraniol	− 5.69	− 7.19	− 34.712
41	1,8-cineole	− 5.67	− 4.05	− 44.68
42	Asimilobine	− 5.66	− 7.05	− 22.69
43	Rutin	− 5.64	− 7.30	− 43.66

Table 3 The results of the SP and XP docking study of the selected ligands along with their respective glide energies *(Continued)*

No	Name of the compound	Standard Precision (SP) Docking score/ Binding energy (Kcal/mol)	Extra Precision (XP) Docking score/ Binding energy (Kcal/mol)	Glide energy (Kcal/mol)
44	Sideridiol	− 5.54	− 8.94	− 28.92
45	Ursolic acid	− 5.40	− 6.95	− 34.12
46	Linearol	− 5.24	− 6.56	− 36.59
47	Chelidonine	− 5.15	− 5.89	− 40.963
48	Verbenone	− 4.80	− 4.17	− 27.52
49	Mangiferin	− 4.65	− 4.22	− 29.058
50	Allocryptopine	− 4.39	− 2.66	− 32.638
Positive control 1	Donepezil	− 7.34	− 9.10	− 43.91
Positive Control 2	Galantamine	− 6.15	− 8.21	− 41.29

section, apigenin showed relatively poor performance with no capacity to be blood-brain barrier (BBB) permeable. In the metabolism section, all of the ligands were predicted to be CYP450 1A2 inhibitor and only apigenin was found to be CYP450 1A2 non-substrate. However, all of them were CYP450 2C9 and CYP450 2C19 non-inhibitors and bellidifolin and apigenin were substrates for CYP450 2C9. Moreover, only bellidifolin and coptisine were found to be substrates for CYP450 2C19 and CYP450 2D6. In the excretion section, coptisine was found to have the highest half-life of 1.8 h. In the toxicity section, all the four ligands were found to be drug induced liver toxicity (DILI) positive and only bellidifolin was Ames positive as well as hERG non-blocker. Moreover, both naringenin and coptisine were found to have human hepatotoxicity. The performance of the 4 finally selected ligands was not very different from that of the two positive controls.

PASS and P450 site of metabolism prediction

The predicted LD50 value for bellidifolin, naringenin, apigenin, and coptisine were 4000 mg/kg, 2000 mg/kg, 2500 mg/kg, and 1000 mg/kg, respectively. Moreover, both bellidifolin and apigenin were predicted to be in toxicity class 5, whereas naringenin and coptisine were found to be in toxicity class 4. The prediction of activity spectra for substances (PASS prediction) was conducted for the 4 ligands to identify 15 intended biological activities. The PASS prediction results of the 4 selected ligands along with the positive control donepezil are listed in Table 7. Coptisine did not show any of the activities; however, both bellidifolin and apigenin were

Table 4 The results of the MM_GBSA and IFD studies of the 13 ligands selected from the SP and XP docking studies along with the positive controls

No	Name	MM_GBSA ΔG_{bind} score (Kcal/mol)	XP G_{score} (Kcal/mol)	IFD score (Kcal/mol)
01	Coptisine	− 59.41	− 16.24	− 1160.39
02	Bellidifolin	− 55.23	− 10.58	− 1155.67
03	Apigenin	− 49.07	− 11.78	− 1154.41
04	Narigenin	− 42.02	− 12.24	− 1156.52
05	Harmine	− 41.20	− 10.24	− 1153.45
06	Imperatorin	− 38.81	− 8.70	− 1152.22
07	Myricetin	− 36.53	− 11.72	− 1149.29
08	Isoharmnetin	− 35.29	− 9.69	− 1153.95
09	Scoulerine	− 33.43	− 9.22	− 1140.92
10	Harmaline	− 32.24	− 6.78	− 1159.54
11	Isoimperatorin	− 32.96	− 7.54	− 1155.66
12	Quercetin	− 30.54	− 8.01	− 1151.03
13	Liriodenine	− 29.19	− 7.80	− 1152.97
Positive control 1	Donepezil	− 47.06	− 8.21	− 1148.97
Positive Control 2	Galantamine	− 40.92	− 7.40	− 1148.23

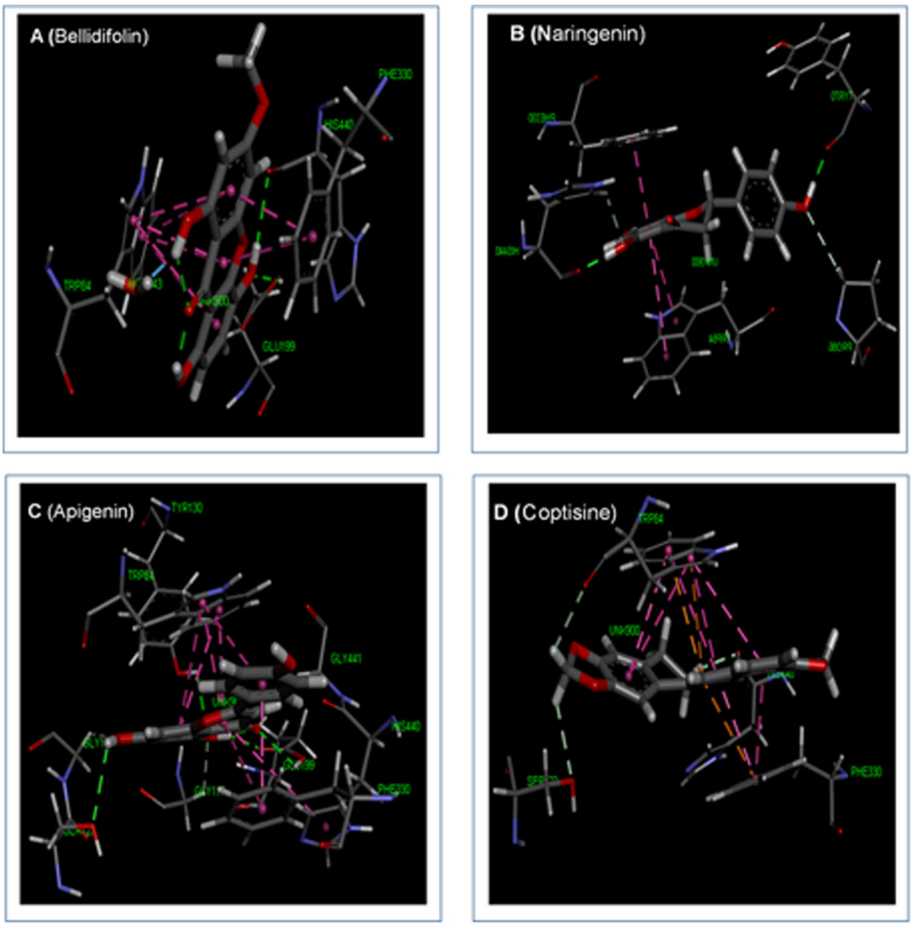


Fig. 2 Figure showing the various types of bonds and amino acids that take part in the interaction between the 4 best selected ligands (**a** bellidifolin, **b** naringenin, **c** apigenin, **d** coptisine) and their receptor, AChE. Interacting amino acid residues of target molecule are labeled in the diagram and dotted lines depict the interaction between the ligand and receptor. Green dotted lines—conventional bond; deep pink—Pi-Pi stacked bond; and orange—charge-charge interaction

Table 5 The druglikeness properties of the best 4 ligands and the positive controls

Drug Likeness Properties	Rules	Bellidifolin	Naringenin	Apigenin	Coptisine	Donepezil (positive control 1)	Galantamine (positive control 2)
Lipinski's rule of five	–	Yes	Yes	Yes	Yes	Yes	Yes
Molecular weight	< 500	274.23 g/mol	272.25 g/mol	270.24 g/mol	320.32 g/mol	379.49 g/mol	287.35 g/mol
Concensus log $P_{o/w}$	≤ 5	1.77	1.84	2.11	2.40	4.00	1.91
Num. H-bond acceptors	< 10	6	5	5	4	4	4
Num. H-bond donors	< 5	3	3	3	0	0	1
Molar refractivity	40–130	72.55	71.57	73.99	87.95	115.31	84.05
No of rotatable bonds	≤ 10	1	1	1	0	6	1
TPSA ($Å^2$)	–	100.13 $Å^2$	71.57	90.90 $Å^2$	40.80 $Å^2$	38.77	41.93

Table 6 The ADME/T test results of the best 4 ligand molecules along with the two positive controls

Class	Properties	Bellidifolin	Naringenin	Apigenin	Coptisine	Donepezil (positive control 1)	Galantamine (positive control 2)
Absorption	Caco-2 permeability	Optimal	Optimal	Optimal	Optimal	Optimal	Optimal
	Pgp-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor	Non-inhibitor
	Pgp-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate	Substrate
	Human Intestinal Absorption (HIA)	HIA positive	HIA positive	HIA positive	HIA negative	HIA positive	HIA positive
Distribution	Plasma Protein Binding	Moderate	Moderate	Optimal	Low	Optimal	Low
	BBB (Blood–Brain Barrier)	BBB positive	BBB positive	BBB negative	BBB positive	BBB positive	BBB positive
Metabolism	CYP450 1A2 inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor
	CYP450 1A2 substrate	Substrate	Substrate	Non-substrate	Substrate	Non-substrate	Substrate
	CYP450 3A4 inhibitor	Non-inhibitor	Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
	CYP450 3A4 substrate	Non-substrate	Non-substrate	Non-substrate	Substrate	Substrate	Substrate
	CYP450 2C9 inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
	CYP450 2C9 substrate	Substrate	Non-substrate	Substrate	Non-substrate	Non-substrate	Non-substrate
	CYP450 2C19 inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
	CYP450 2C19 substrate	Substrate	Non-substrate	Non-substrate	Substrate	Substrate	Non-substrate
	CYP450 2D6 inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
	CYP450 2D6 substrate	Substrate	Non-substrate	Non-substrate	Substrate	Substrate	Substrate
Excretion	$T_{1/2}$ (h)	1.0	0.9	1.3	1.8	1.6	1.7
Toxicity	hERG (hERG Blockers)	Non-blocker	Blocker	Blocker	Blocker	hERG positive	Non-blocker
	H-HT (Human Hepatotoxicity)	HHT positive	HHT negative	HHT positive	HHT negative	HHT positive	HHT positive
	Ames (Ames Mutagenicity)	Ames positive	Ames negative	Ames negative	Ames negative	Ames negative	Ames negative
	DILI (Drug Induced Liver Injury)	DILI positive	DILI positive	DILI positive	DILI positive	DILI negative	DILI negative

predicted to have 14 activities each. Naringenin showed all the 15 intended biological activities. On the other hand, the possible sites of metabolism (SOMs) by CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1, and 3A4 of bellidifolin, naringenin, apigenin, and coptisine were also determined, and the possible SOMs by these isoforms are indicated by circles on the chemical structure of the molecule [72] (Table 8). In both PASS and P450 SOM prediction, all the 4 compounds showed quite satisfactory performance which is comparable to the positive controls, donepezil and galantamine.

Analysis of frontier's orbitals

In the analysis of Frontier's orbitals, the DFT calculations were performed. The results of the DFT calculations are listed in Table 9. Coptisine showed the lowest gap energy of 0.047 eV as well as the highest dipole moment of 6.459 debye. On the other hand, apigenin generated the highest gap energy of 0.130 eV and the second highest dipole moment of 2.163 debye. The order of dipole moments of these 4 compounds were, bellidifolin < apigenin < naringenin < coptisine. Comparing with the positive controls, it can be declared that all the four compounds showed sound results in the DFT calculations (Fig. 3).

Table 7 The PASS prediction results showing the biological activities of the best 4 ligands and the positive controls

Sl no	Biological activities	Bellidifolin		Naringenin		Apigenin		Coptisine		Donepezil (positive control 1)		Galantamine (positive control 2)	
		Predicted LD50: 4000 mg/kg		Predicted LD50: 2000 mg/kg		Predicted LD50: 2500 mg/kg		Predicted LD50: 1000 mg/kg		Predicted LD50: 1500 mg/kg		Predicted LD50: 2000 mg/kg	
		Toxicity class: 5		Toxicity class: 4		Toxicity class: 5		Toxicity class: 4		Toxicity class: 4		Toxicity class: 4	
		Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
01	Antioxidant	–	–	0.794	0.003	0,732	0,004	–	–	0.893	0.003	0.781	0.010
02	Membrane integrity agonist	0.956	0.003	0.964	0.003	0.967	0.002	–	–	0.768	0.017	0.813	0.005
03	Antineoplastic	0.742	0.019	0.751	0.018	0.774	0.015	–	–	0.823	0.005	0.956	0.002
04	Antimutagenic	0.891	0.002	0.857	0.003	0.921	0.002	–	–	0.799	0.009	0.942	0.002
05	Mucomembranous protector	0.710	0.052	0.844	0.010	0.797	0.019	–	–	0.773	0.011	0.764	0.014
06	Membrane integrity agonist	0.754	0.010	0.924	0.003	0.967	0.002	–	–	0.834	0.004	0.720	0.015
07	TP53 expression enhancer	0.853	0.007	0.822	0.009	0.873	0.006	–	–	0.920	0.002	0.798	0.008
08	Lipid peroxidase inhibitor	0.755	0.004	0.815	0.003	-	-	–	–	0.875	0.009	0.909	0.005
09	HIF1A expression inhibitor	0.937	0.004	0.911	0.005	0.911	0.005	–	–	0.751	0.019	0.866	0.009
10	APOA1 expression enhancer	0.702	0.005	0.836	0.003	0.826	0.003	–	–	0.918	0.002	0.978	0.001
11	Free radical scavenger	0.719	0.004	0.769	0.003	0.719	0.004	–	–	0.911	0.005	0.782	0.010
12	Peroxidase inhibitor	0.908	0.003	0.818	0.004	0.924	0.002	–	–	0.912	0.008	0.930	0.003
13	Vasoprotector	0.758	0.007	0.707	0.010	0.891	0.003	–	–	0.711	0.022	0.748	0.013
14	Histidine kinase inhibitor	0.889	0.002	0.892	0.002	0.918	0.002	–	–	0.931	0.003	0.814	0.011
15	Apoptosis agonist	0.833	0.006	0.709	0.014	0.847	0.005		–	0.891	0.006	0.951	0.001

Discussion

Molecular docking is an effective strategy in computer-aided drug designing which works on some specific algorithms and assigns affinity scores depending on the poses of the ligands inside the binding pocket of a target. In molecular docking, the lowest docking score corresponds to the highest affinity which reflects that the complex remains more time in contact with good stability [85, 86].

In this study, 50 compounds that were proven to have anti-AChE properties were selected by literature reviewing. Initially, from these 50 compounds, 13 compounds were selected based on their SP and XP docking scores (compounds having both SP and XP docking scores over − 8.0 Kcal/mol). When compared with the positive controls, donepezil and galantamine, all the compounds were found to have relatively good results in molecular docking analysis. The 13 ligands selected from the initial docking analysis had predicted results better than donepezil. Later, from these 13 compounds, according to the MM_GBSA and IFD scores, 4 compounds, i.e., bellidifolin, naringenin, apigenin, and coptisine, were selected as final ligands for further analysis. These final compounds were also predicted to have better results than donepezil and galantamine in the MM_GBSA and IFD studies.

AChE possesses several notable amino acids in its active-site gorge, i.e., serine 200, glutamic acid 327, histidine 440, tryptophan 279, tyrosine 121, phenylalanine 330, and tryptophan 84. All the 4 finally selected ligands were found to form potential hydrogen and hydrophobic interactions with histidine 440, phenylalanine 330, and tryptophan 84 within the active site of AChE. Since hydrogen and hydrophobic interactions are important for strengthening the receptor-ligand interactions, it can be declared that these ligands might be able to effectively inhibit the AChE enzyme at its active site [87, 88].

The prediction of druglikeness properties facilitates the drug development processes. The molecular weight and TPSA are two important characteristics of a drug that affect its permeability through the biological barriers. Higher molecular weight and TPSA represent lower permeability of the drug molecule and vice-versa. Again, the lipophilicity (expressed as LogP) influences the absorption of the drug molecule in the body and the higher LogP of a drug represents its lower absorption in the body. The capability of a drug molecule to cross the cell membrane is also influenced by the number of hydrogen bond acceptors and donors beyond the acceptable range. Moreover, the number of rotatable bonds (acceptable range is less than 10) also influences the

Table 8 The P450 site of metabolism prediction of the best four ligand molecules and the positive controls

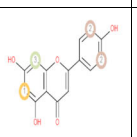
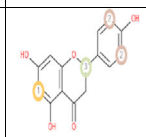
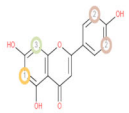
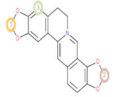
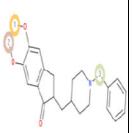
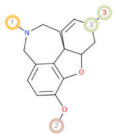
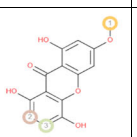
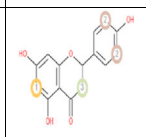
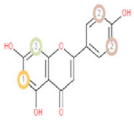
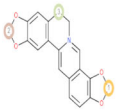
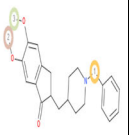
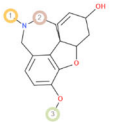
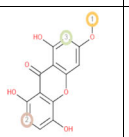
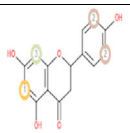
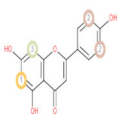
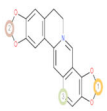
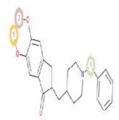
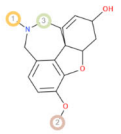
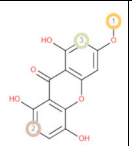
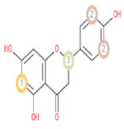
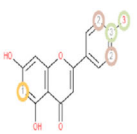
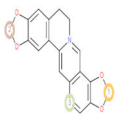
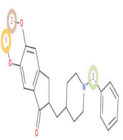
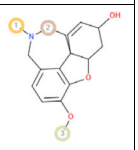
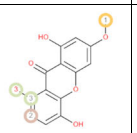
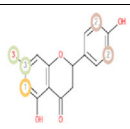
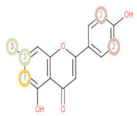
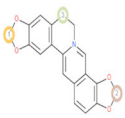
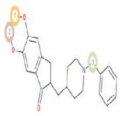
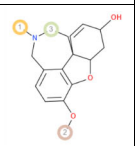
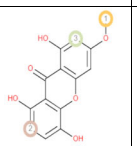
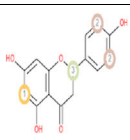
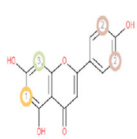
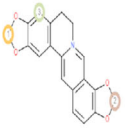
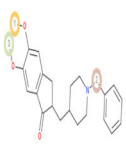
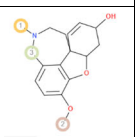
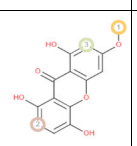
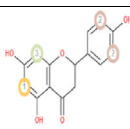
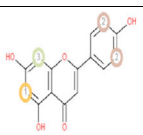
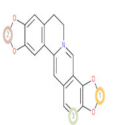
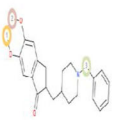
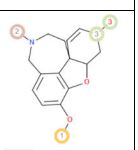
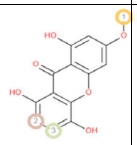
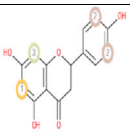
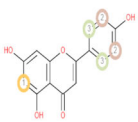
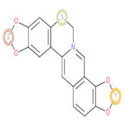
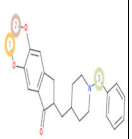
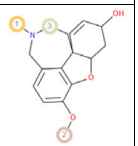
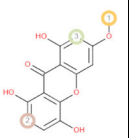
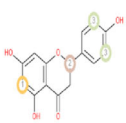
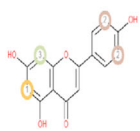
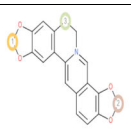
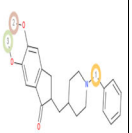
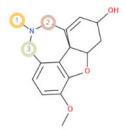
Names of P450 iso-enzymes	Bellidifolin	Naringenin	Apigenin	Coptisine	Donepezil (positive control 1)	Galantamine (positive control 2)
1A2						
2A6						
2B6						
2C8						
2C9						
2C19						
2D6						
2E1						
3A4						

Table 9 The results of the DFT calculations of the selected best 4 ligands and the positive controls

Compound name	HOMO energy (eV)	LUMO energy (eV)	Gap (eV)	Hardness (η) (eV)	Softness (S) (eV)	Dipole moment (Debye)
Bellidifolin	− 0.015	0.101	0.116	0.058	17.241	1.630
Naringenin	− 0.005	0.094	0.099	0.049	20.408	2.278
Apigenin	− 0.048	0.082	0.130	0.065	15.385	2.163
Coptisine	− 0.134	− 0.087	0.047	0.023	21.276	6.459
Donepezil (positive control 1)	− 0.028	0.076	0.104	0.032	18.560	2.398
Galantamine (positive control 2)	− 0.022	0.081	0.103	0.047	16.141	3.041

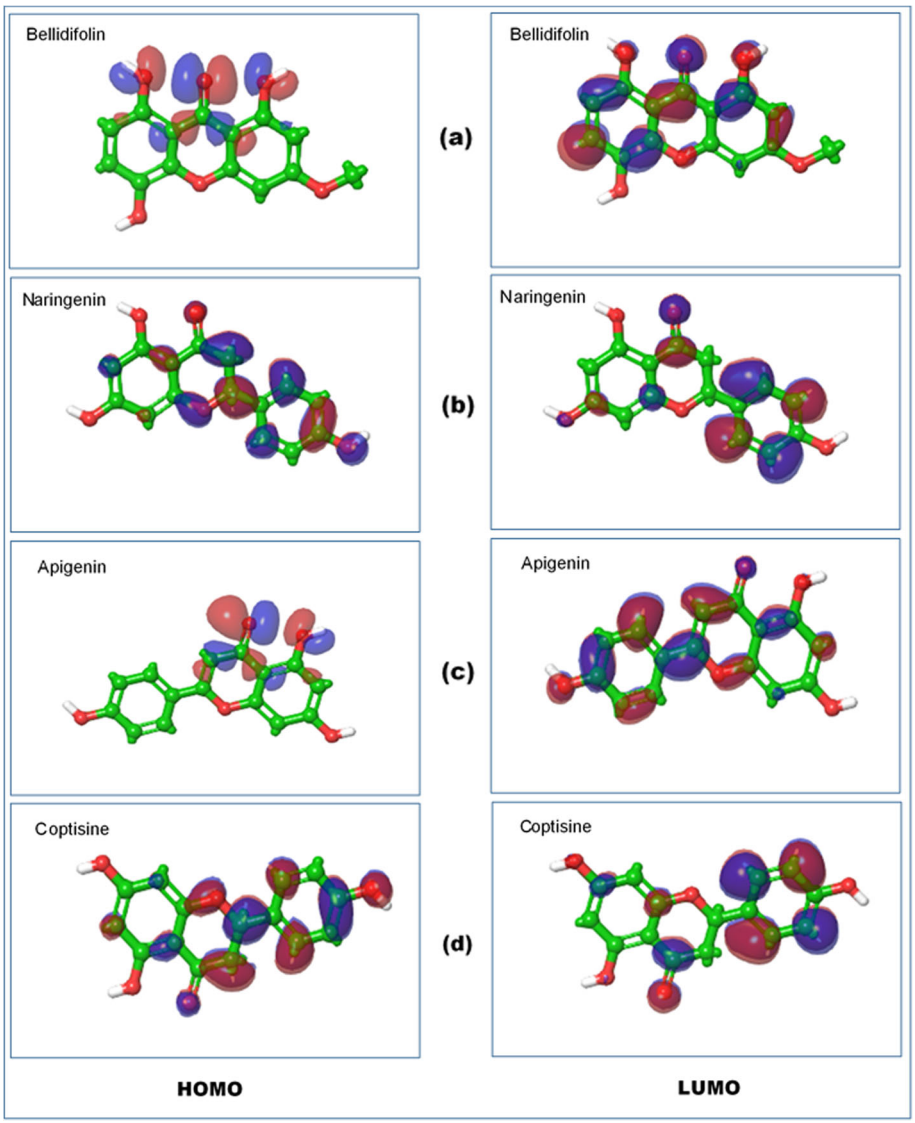


Fig. 3 The results of HOMO and LUMO studies; **a** bellidifolin, **b** naringenin, **c** apigenin, and **d** coptisine

druglikeness properties of a drug. Furthermore, the Lipinski's rule of five demonstrates that a successful drug should have properties within the acceptable range of the five Lipinski's rules, i.e., molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, number of hydrogen bond donors ≤ 5, molar refractivity from 40 to 130, and lipophilicity (expressed as LogP) ≤ 5 [89–92]. All the 4 selected ligands were found to follow the five Lipinski's rules of druglikeness properties. All of them showed quite good results in the druglikeness property experiment and for this reason, and all of them were considered as potential drug candidates.

ADMET prediction of a candidate drug aids in determining the pharmacological and pharmacodynamic properties of the drug molecule within a biological system. For this reason, the ADMET properties are important determinants for the success of a drug development process. BBB permeability is the most important characteristic of the drugs which primarily target the brain cells. Again, the transportation of drugs is influenced by the P-glycoprotein (P-gp) in the cell membrane; therefore, inhibition of this protein severely affects the drug transport. Moreover, if a drug is found to be permeable in the Caco-2 cell line, then that particular drug is considered to be absorbed well in the intestine. Orally absorbed drugs travel through the blood circulation and deposit in the liver where they are metabolized by group of enzymes of the Cytochrome P450 family and later excreted as bile or urine. Therefore, inhibition of any of these enzymes affects the metabolism and biodegradation of the drugs [93, 94]. Furthermore, if a drug is found to be a substrate for one or more CYP450 enzyme or enzymes, then that drug is considered to be metabolized well by the respective CYP450 enzyme or enzymes [95]. A drug's pharmacodynamic properties depend on the degree of its binding with the plasma protein. A drug can pass through the cell layers if it binds to the plasma proteins less efficiently and vice versa [96]. Human intestinal absorption (HIA) is another crucial determinant for the orally administered drugs [97–99]. The half-life of a drug describes that the greater the half-life, the longer it would stay in the body and the greater its potentiality [100–102].

Furthermore, HERG is a K+ channel which is found in the heart muscle. Blocking the hERG channel or signaling may cause cardiac arrhythmia leading to death [103, 104]. On the other hand, human hepatotoxicity (H-HT) refers to any type of acute liver injury which may lead to organ failure and death [105, 106]. The Ames test is a mutagenicity assay that is used to detect the potential mutagenic chemicals [107]. Drug induced liver injury (DILI) reflects the injuries in the liver that are caused by administration of drugs [108]. Bellidifolin, naringenin, and coptisine showed quite similar and sound performance in the AMDET prediction. However, apigenin was found to be BBB impermeable and also a good binder to the plasma membrane, which could affect its biodistribution throughout the body as a potential inhibitor of AChE. As a consequence, the performance of apigenin in ADMET test was declared to be non-satisfactory.

Prediction of Activity Spectra for Substances or PASS prediction is a process of determining the biological activities of drug candidates or drug-like molecules. The PASS prediction estimates biological activities by predicting two potential probabilities: Pa, which represents the probability of a compound to be active and Pi, which represents the probability of a compound to be inactive. By predicting these two probabilities, the PASS prediction determines potential biological activities of a drug-like molecule. The Pa value of greater than 0.7 represents the greater probability of exhibiting the activity of a substance in an experiment. The Pa > 70% threshold corresponds to a highly reliable prediction, while Pa > 0.5 gives quite reliable prediction and the Pa > 0.3 threshold describes that a compound is less likely to have the desired activity [70, 71, 109]. Most of the intended activities (13 activities) were common between bellidifolin, naringenin, and apigenin, for example, antineoplastic, antimutagenic, membrane integrity agonist, HIF1A expression inhibitor, and apoptosis agonist (Table 8).

The toxicity classes of the 4 compounds were determined by the ProTox-II server (http://tox.charite.de/protox_II/index.php?site=compound_input), which predicts the toxicity of a compound and then classifies the compound into a toxicity class that ranges from 1 to 6. To classify the compounds into toxicity classes, the server uses the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). According this system, since both bellidifolin and apigenin had toxicity class 4, they would be harmful if swallowed. Again with the toxicity class of 5, both naringenin and coptisine might be harmful if swallowed [73, 110]. Both the positive controls, donepezil and galantamine were found to have the toxicity class of 4.

The cytochrome P450 or CYP450 represents a family of 57 isoforms of the heme-containing P450 enzymes. This enzyme family is one of the most important enzyme family for proper metabolism within the human body, which is responsible for catalyzing the phase-I metabolism of about 90% of all the marketed drugs. They also convert the lipophilic drugs or compounds to more polar, hydrophilic substances. Among these 57 isoforms of the CYP450 enzymes, 9 isoforms are most important for biological activities, i.e., CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1, and 3A4 [111, 112]. When the P450 SOMs of the 4 best selected ligands: bellidifolin, naringenin, apigenin, and coptisine were predicted, all of them showed potential SOMs where these 9 isoforms of

Table 10 Overall comparison of the main features of bellidifolin, naringenin, apigenin, and coptisine as potential drugs to treat AD according to the study along with the 2 positive controls from comparison. All the compounds are ranked (excellent, good, and poor) based on their performance in every aspect

Features	Bellidifolin	Naringenin	Apigenin	Coptisine	Donepezil (positive control 1)	Galantamine (positive control 2)	Remarks (Best performers)
Molecular docking study	Good	Good	Good	Excellent	Good	Poor	Coptisine
Druglikeness properties	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	All
Absorption	Excellent	Excellent	Excellent	Poor	Good	Poor	Bellidifolin, naringenin, apigenin
Distribution	Excellent	Excellent	Poor	Poor	Excellent	Good	Bellidifolin, naringenin
Metabolism	Excellent	Poor	Good	Good	Good	Excellent	Bellidifolin
Excretion	Good	Good	Good	Excellent	Excellent	Excellent	Coptisine
Toxicity	Good	Excellent	Good	Excellent	Excellent	Excellent	Naringenin & apigenin
PASS Prediction	Good	Good	Good	Good	Good	Good	All
P450 SOM	Good	Excellent	Excellent	Poor	Good	Good	Naringenin, apigenin
DFT calculations	Good	Good	Poor	Excellent	Good	Good	Coptisine

P450 enzymes can act on. For this reason, all these ligands or compounds might be metabolized well by the body.

Frontier's orbitals study or DFT calculation is an important method to predict the pharmacological properties of small molecules. HOMO and LUMO help to study the chemical reactivity and kinetic stability of the query molecules. The term "HOMO" represents the regions on a small molecule which might donate electrons during the formation of a complex and the term "LUMO" corresponds to the regions on a molecule that might receive electrons from the electron donor. The difference in HOMO and LUMO energy is regarded as gap energy that corresponds to the electronic excitation energy. The molecule that has greater orbital gap energy tends to be energetically more unfavorable to undergo a chemical reaction and vice versa [76, 113–117]. All of the 4 ligands were predicted to have significant gap energy representing their possibility to undergo a chemical reaction. However, among the four best selected ligands, coptisine, with its lowest gap energy, was predicted to have higher probability to undergo a chemical reaction [25, 117] (Table 9 and Fig. 3).

Considering all the aspects of the study and comparing the four best selected compounds, it can be concluded that bellidifolin has the highest potentiality to be used as anti-AD drug. From Table 10, it is clear that bellidifolin generated the most sound and satisfactory performance among the four compounds, with no poor performance in any of the analysis. Even compared to the positive controls, donepezil and galantamine, bellidifolin generated very good results in almost all the analyses. Therefore, bellidifolin can be considered as the most suitable compound as a drug for AD treatment among the compounds considered in this study. However, further investigation could also be conducted on other compounds as they had also shown convincing results in the docking analysis (Table 3). And the authors recommend further in vivo and in vitro experiments to strengthen the findings of this study.

Conclusion

As Alzheimer's disease (AD) is one of the most rapidly growing dementia type diseases in the world, more and more research studies are being conducted to find out the best possible treatment for this disease. Although several treatments are already available, none of these treatments possesses significant satisfactory results. Plants have been known to contain many beneficial agents that can be used to treat a variety of diseases. In this experiment, 50 anti-AChE phytochemicals were selected to analyze their activities against the acetylcholinesterase (AChE) enzyme, which is a key enzyme responsible for the development of AD, by exploiting numerous approaches used in computer-aided drug design. Upon continuous computational experimentation, bellidifolin, naringenin, apigenin, and coptisine were predicted to be the best inhibitors of AChE. Then, their drug potentiality was checked in different post-screening studies where they were also predicted to show quite similar and sound performance, although in some aspects, their performances were not up to the mark. However, more wet-lab based studies must be performed on these 4 agents as well as the other remaining agents to finally confirm their potentiality, safety, and efficacy.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-020-00127-8.

Additional file 1 : **Supplementary Table S1**. List of different amino acids within the binding pocket of AChE that interact with the four best selected ligands, along with the bond distances and types of the interactions.

Abbreviations

AD: Alzheimer's disease; APP: Amyloid precursor protein; AChE: Acetylcholinesterase; ChAT: Choline acetyltransferase; Ac-CoA: Acetyl coenzyme A; Ch: Choline; PASS: Prediction of Activity Spectra for Substances; SOM: Site of metabolism; MM-GBSA: Molecular mechanics – generalized born and surface area; HOMO: Highest occupied molecular orbital; LUMO: Lowest unoccupied molecular orbital; TPSA: Topological polar surface area; BBB: Blood-brain barrier; HERG: Human ether-a-go-go-related gene

Acknowledgements

The authors are thankful to the Swift Integrity Computational Lab, Dhaka, Bangladesh, a virtual platform of young researchers, for providing the support.

Authors' contributions

BS supervised and designed the study; SA conducted the molecular docking study; TKR performed the druglikeness properties and ADMET test; SSI performed the PASS prediction and DFT calculations; YA wrote the manuscript; MAU edited the manuscript. The authors have read and approved the manuscript.

Funding

No funding was received for conducting the study.

Availability of data and materials

The tools used in this study are available on the Internet.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest among themselves.

Author details

[1]Department of Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Jahangirnagar University, Dhaka, Bangladesh. [2]Department of Genetic Engineering and Biotechnology, School of Life Sciences, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

Received: 10 September 2020 Accepted: 21 December 2020
Published online: 15 February 2021

References

1. Suh WH, Suslick KS, Suh YH (2005) Therapeutic agents for Alzheimer's disease. Curr Med Chem -Central Nervous Syst Agents 5:259–269. https://doi.org/10.2174/156801505774913035
2. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A (2005) Global prevalence of dementia: a Delphi consensus study. Lancet 366:2112–2117. https://doi.org/10.1016/s0140-6736(05)67889-0
3. Merriam AE, Aronson MK, Gaston P, Wey SL, Katz I (1988) The psychiatric symptoms of Alzheimer's disease. J Am Geriatr Soc 36:7–22. https://doi.org/10.1111/j.1532-5415.1988.tb03427.x
4. Nordberg A, Svensson AL (1998) Cholinesterase inhibitors in the treatment of Alzheimer's disease. Drug Saf 19:465–480. https://doi.org/10.2165/00002018-199819060-00004
5. Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256:184–186. https://doi.org/10.1126/science.1566067
6. Markesbery WR (1997) Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 23:134–147. https://doi.org/10.1016/s0891-5849(96)00629-6
7. Francis PT, Palmer AM, Snape M, Wilcock GK (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry 66:137–147. https://doi.org/10.1136/jnnp.66.2.137
8. Huang LK, Chao SP, Hu CJ (2020 Dec) Clinical trials of new drugs for Alzheimer disease. J Biomed Sci 27(1):1–3. https://doi.org/10.1186/s12929-019-0609-7
9. https://alzheimersnewstoday.com/experimental-treatments-for-alzheimers-disease-treatments-that-target-beta-amyloid-protein/?cn-reloaded=1. Accessed Jan 2020.
10. Padurariu M, Ciobica A, Lefter R, Lacramioara Serban I, Stefanescu C, Chirita R (2013) The oxidative stress hypothesis in Alzheimer's disease. Psychiatr Danub 25(4):0–409
11. Persson T, Popescu BO, Cedazo-Minguez A (2014) Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? Oxid Med Cell Longev 2014. https://doi.org/10.1155/2014/427318
12. Teixeira JP, de Castro AA, Soares FV, da Cunha EF, Ramalho TC (2019) Future therapeutic perspectives into the Alzheimer's disease targeting the oxidative stress hypothesis. Molecules. 24(23):4410. https://doi.org/10.3390/molecules24234410
13. Terry AV, Buccafusco JJ (2003) The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 306:821–827. https://doi.org/10.1124/jpet.102.041616
14. Talesa VN (2001) Acetylcholinesterase in Alzheimer's disease. Mech Ageing Dev 122:1961–1969. https://doi.org/10.1016/s0047-6374(01)00309-8
15. Viegas J, Bolzani VD, Barreiro EJ, Manssour Fraga CA (2005) New anti-Alzheimer drugs from biodiversity: the role of the natural acetyl cholinesterase inhibitors. Mini Rev Med Chem 5:915–926. https://doi.org/10.2174/138955705774329546
16. Bohnen NI, Kaufer DI, Hendrickson R, Ivanco LS, Lopresti B, Davis JG, Constantine G, Mathis CA, Moore RY, DeKosky ST (2005) Cognitive correlates of alterations in acetylcholinesterase in Alzheimer's disease. Neurosci Lett 380:127–132. https://doi.org/10.1016/j.neulet.2005.01.031
17. Hasselmo ME, Bower JM (1993) Acetylcholine and memory. Trends Neurosci 16:218–222. https://doi.org/10.1016/0166-2236(93)90159-j.
18. McGleenon BM, Dynan KB, Passmore AP (1999) Acetylcholinesterase inhibitors in Alzheimer's disease. Br J Clin Pharmacol 48:471. https://doi.org/10.1046/j.1365-2125.1999.00026.x
19. Tayeb HO, Yang HD, Price BH, Tarazi FI (2012) Pharmacotherapies for Alzheimer's disease: beyond cholinesterase inhibitors. Pharmacol Ther 134(1):8–25. https://doi.org/10.1016/j.pharmthera.2011.12.002
20. Farlow M, Veloso F, Moline M, Yardley J, Brand-Schieber E, Bibbiani F, Zou H, Hsu T, Satlin A (2011) Safety and tolerability of donepezil 23 mg in moderate to severe Alzheimer's disease. BMC Neurol 11(1):57. https://doi.org/10.1186/1471-2377-11-57
21. Winblad B, Grossberg G, Frölich L, Farlow M, Zechner S, Nagel J, Lane R (2007) IDEAL: a 6-month, double-blind, placebo-controlled study of the first skin patch for Alzheimer disease. Neurology 69(4 suppl 1):S14–S22. https://doi.org/10.1212/01.wnl.0000281847.17519.e0
22. Bartolucci C, Perola E, Pilger C, Fels G, Lamba D (2001) Three-dimensional structure of a complex of galanthamine (Nivalin®) with acetylcholinesterase from Torpedo californica: implications for the design of new anti-Alzheimer drugs. Proteins: Structure Function Bioinform 42(2):182–191. https://doi.org/10.1002/1097-0134(20010201)42:2<182::aid-prot50>3.0.co;2-1
23. Pilger C, Bartolucci C, Lamba D, Tropsha A, Fels G (2001) Accurate prediction of the bound conformation of galanthamine in the active site of Torpedo californica acetylcholinesterase using molecular docking. J Mol Graph Model 19(3-4):288–296. https://doi.org/10.1016/s1093-3263(00)00056-5
24. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7(2):146–157. https://doi.org/10.2174/157340911795677602
25. Sarkar B, Ullah MA, Islam SS, Rahman MH, Araf Y (2020) Analysis of plant-derived phytochemicals as anti-cancer agents targeting cyclin dependent kinase-2, human topoisomerase IIa and vascular endothelial growth factor receptor-2. J Recept Signal Transduct:1–7. https://doi.org/10.1080/10799893.2020.1805628
26. Dhananjayan K, Sumathy A, Palanisamy S (2013) Molecular docking studies and in-vitro acetylcholinesterase inhibition by terpenoids and flavonoids. Asian J Res Chem 6(11):1011–1017

27. Kitphati W, Wattanakamolkul K, Lomarat P, Phanthong P, Anantachoke N, Nukoolkarn V, Thirapanmethee K, Bunyapraphatsara N (2012) Anticholinesterase of essential oils and their constituents from Thai medicinal plants on purified and cellular enzymes
28. Nag G, Das S, Das S, Mandal S, De B (2015) Antioxidant, anti-acetylcholinesterase and anti-glycosidase properties of three species of Swertia, their xanthones and amarogentin: a comparative study. Pharm J 7(2). https://doi.org/10.5530/pj.2015.2.6
29. Cahlíková L, Opletal L, Kurfürst M, Macáková K, Kulhánková A, Hošt'álková A (2010) Acetylcholinesterase and butyrylcholinesterase inhibitory compounds from Chelidonium majus (Papaveraceae). Nat Prod Commun 5(11): 1934578X1000501110. https://doi.org/10.1177/1934578x1000501110
30. Olennikov DN, Kashchenko NI, Chirikova NK, Akobirshoeva A, Zilfikarov IN, Vennos C (2017) Isorhamnetin and quercetin derivatives as anti-acetylcholinesterase principles of marigold (Calendula officinalis) flowers and preparations. Int J Mol Sci 18(8):1685. https://doi.org/10.3390/ijms18081685
31. Hlila MB, Mosbah H, Mssada K, Jannet HB, Aouni M, Selmi B (2015) Acetylcholinesterase inhibitory and antioxidant properties of roots extracts from the Tunisian Scabiosa arenaria Forssk. Ind Crop Prod 67:62–69. https://doi.org/10.1016/j.indcrop.2015.01.009
32. Urbain A, Marston A, Hostettmann K (2005) Coumarins from Peucedanum ostruthium as inhibitors of acetylcholinesterase. Pharm Biol 43(8):647–650. https://doi.org/10.1080/13880200500382720
33. Kim DK (2002) Inhibitory effect of corynoline isolated from the aerial parts of corydalis incisa on the acetylcholinesterase. Arch Pharm Res 25(6):817. https://doi.org/10.1007/bf02976997
34. Andrade MT, Lima JA, Pinto AC, Rezende CM, Carvalho MP, Epifanio RA (2005) Indole alkaloids from Tabernaemontana australis (Müell. Arg) Miers that inhibit acetylcholinesterase enzyme. Bioorg Med Chem 13(12):4092–4095. https://doi.org/10.1016/j.bmc.2005.03.045
35. Wangchuk P, Keller PA, Pyne SG, Sastraruji T, Taweechotipatr M, Rattanajak R, Tonsomboon A, Kamchonwongpaisan S (2012) Phytochemical and biological activity. https://doi.org/10.1177/1934578x1200700507
36. Farag MA, Ezzat SM, Salama MM, Tadros MG, Serya RA (2016) Anti-acetylcholinesterase activity of essential oils and their major constituents from four Ocimum species. Z Naturforsch C 71(11-12):393–402. https://doi.org/10.1515/znc-2016-0030
37. Heo HJ, Kim MJ, Lee JM, Choi SJ, Cho HY, Hong B, Kim HK, Kim E, Shin DH (2004) Naringenin from Citrus junos has an inhibitory effect on acetylcholinesterase and a mitigating effect on amnesia. Dement Geriatr Cogn Disord 17(3):151–157. https://doi.org/10.1159/000076349
38. Tundis R, Bonesi M, Menichini F, Loizzo MR, Conforti F, Statti G, Pirisi FM, Menichini F (2012) Antioxidant and anti-cholinesterase activity of Globularia meridionalis extracts and isolated constituents. Nat Prod Commun 7(8): 1934578X1200700814. https://doi.org/10.1177/1934578x1200700814
39. Askin H, Yildiz M, Ayar A (2017) Effects of thymol and carvacrol on acetylcholinesterase from drosophila melanogaster. Acta Physica Polonica A 132(3):720–722. https://doi.org/10.12693/aphyspola.132.720
40. Jukic M, Politeo O, Maksimovic M, Milos M, Milos M (2007) In vitro acetylcholinesterase inhibitory properties of thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone. Phytother Res 21(3): 259–261. https://doi.org/10.1002/ptr.2063
41. Kaufmann D, Dogra AK, Wink M (2011) Myrtenal inhibits acetylcholinesterase, a known Alzheimer target. J Pharm Pharmacol 63(10): 1368–1371. https://doi.org/10.1111/j.2042-7158.2011.01344.x
42. Ndhlala AR, Aremu AO, Moyo M, Amoo SO, Van Staden J (2012) Acetylcholineterase inhibitors from plant sources: friends or foes. Cholinesterase: Production, Uses Health Effects:67–98. https://doi.org/10.1016/b978-0-12-405927-6.00016-3
43. Matochko WL, James A, Lam CW, Kozera DJ, Ata A, Gengan RM (2010) Triterpenoidal alkaloids from Buxus natalensis and their acetylcholinesterase inhibitory activity. J Nat Prod 73(11):1858–1862. https://doi.org/10.1021/np100494u
44. Yang ZD, Zhang DB, Ren J, Yang MJ (2012) Skimmianine, a furoquinoline alkaloid from Zanthoxylum nitidum as a potential acetylcholinesterase inhibitor. Med Chem Res 21(6):722–725. https://doi.org/10.1007/s00044-011-9581-9
45. Cabral RS, Sartori MC, Cordeiro I, Queiroga CL, Eberlin MN, Lago JH, Moreno PR, Young M (2012) Anticholinesterase activity evaluation of alkaloids and coumarin from stems of Conchocarpus fontanesianus. Rev Bras Farm 22(2): 374–380. https://doi.org/10.1590/s0102-695x2011005000219
46. Adhami HR, Farsam H, Krenn L (2011) Screening of medicinal plants from Iranian traditional medicine for acetylcholinesterase inhibition. Phytother Res 25(8):1148–1152. https://doi.org/10.1002/ptr.3409
47. Wszelaki N, Kuciun A, Kiss A (2010) Screening of traditional European herbal medicines for acetylcholinesterase and butyrylcholinesterase inhibitory activity. Acta Pharm 60(1):119–128. https://doi.org/10.2478/v10007-010-0006-y
48. Howes MJ, Houghton PJ (2009) Acetylcholinesterase inhibitors of natural origin. Int J Biomed Pharm Sci 3(SI1):67–86
49. Phoopichayanun C (2007) Acetylcholinesterase inhibitors from roots of Feroniella lucida (Doctoral dissertation)
50. Dohi S, Terasaki M, Makino M (2009) Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils. J Agric Food Chem 57(10):4313–4318. https://doi.org/10.1021/jf804013j
51. Balkis A, Tran K, Lee YZ, Ng K (2015) Screening flavonoids for inhibition of acetylcholinesterase identified baicalein as the most potent inhibitor. Int J Biol 7(9):26. https://doi.org/10.5539/jas.v7n9p26
52. Antikolinesteraz AHTP, Bitkileri BKOLF (2014) Lamiaceae family plants as a potential anticholinesterase source in the treatment of Alzheimer's disease. Bezmialem Sci 1:1–25
53. Mollataghi A, Coudiere E, Hadi AHA, Mukhtar MR, Awang K, Litaudon M, Ata A (2012) Anti-acetylcholinesterase, anti-α-glucosidase, anti-leishmanial and anti-fungal activities of chemical constituents of Beilschmiedia species. Fitoterapia 83(2):298–302. https://doi.org/10.1016/j.fitote.2011.11.009
54. Kaufmann D, Kaur Dogra A, Tahrani A, Herrmann F, Wink M (2016) Extracts from traditional Chinese medicinal plants inhibit acetylcholinesterase, a known Alzheimer's disease target. Molecules 21(9):1161. https://doi.org/10.3390/molecules21091161
55. Vladimir-Knežević S, Blažeković B, Kindl M, Vladić J, Lower-Nedza AD, Brantner AH (2014) Acetylcholinesterase inhibitory, antioxidant and phytochemical properties of selected medicinal plants of the Lamiaceae family. Molecules 19(1):767–782. https://doi.org/10.3390/molecules19010767
56. Falé PL, Ferreira C, Rodrigues AM, Cleto P, Madeira PA, Florêncio MH, Frazão FN, Serralheiro ML (2013) Antioxidant and anti-acetylcholinesterase activity of commercially available medicinal infusions after in vitro gastrointestinal digestion. J Med Plant Res 7(20):1370–1378. https://doi.org/10.5897/jmpr13.4438
57. Min BS, Cuong TD, Lee JS, Shin BS, Woo MH, Hung TM (2010) Cholinesterase inhibitors from Cleistocalyx operculatus buds. Arch Pharm Res 33(10):1665–1670. https://doi.org/10.1007/s12272-010-1016-5
58. Ogura H, Kosasa T, Kuriya Y, Yamanishi Y (2000) Comparison of inhibitory activities of donepezil and other cholinesterase inhibitors on acetylcholinesterase and butyrylcholinesterase in vitro. Methods Find Exp Clin Pharmacol 22(8):609–614. https://doi.org/10.1358/mf.2000.22.8.701373
59. Cummings JL, Geldmacher D, Farlow M, Sabbagh M, Christensen D, Betz P, Donepezil 23 mg Expert Working Group (2013) High-dose donepezil (23 mg/day) for the treatment of moderate and severe Alzheimer's disease: drug profile and clinical guidelines. CNS Neurosci Ther 19:294–301. https://doi.org/10.1111/cns.12076
60. Schrödinger Release 2018-4: protein preparation wizard; Epik, Schrödinger, LLC, New York, NY, 2016; Impact, Schrödinger, LLC, New York, NY, 2016; Prime, Schrödinger, LLC, New York, NY, 2018.
61. Schrödinger Release 2018-4: Prime, Schrödinger, LLC, New York, 2018.
62. Schrödinger Release 2018-4: LigPrep, Schrödinger, LLC, New York, 2018.
63. Schrödinger Release 2018-4: Epik, Schrödinger, LLC, New York, 2018.
64. Schrödinger Release 2018-4: Glide, Schrödinger, LLC, New York, 2018.
65. Dash R, Hosen SZ, Karim MR, Kabir MS, Hossain MM, Junaid M, Islam A, Paul A, Khan MA (2015) In silico analysis of indole-3-carbinol and its metabolite DIM as EGFR tyrosine kinase inhibitors in platinum resistant ovarian cancer vis a vis ADME/T property analysis. J App Pharm Sci 5(11):073–078. https://doi.org/10.7324/japs.2015.501112
66. Visualizer DS (2017) Release 4.1. Accelrys Inc., San Diego
67. Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7:42717. https://doi.org/10.1038/srep42717
68. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, Lee P, W.; Tang, Y. (2012) admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. https://doi.org/10.1021/ci300367a
69. Dong J, Wang NN, Yao ZJ, Zhang L, Cheng Y, Ouyang D, Lu AP, Cao DS (2018) ADMETlab: a platform for systematic ADMET evaluation based on a

comprehensively collected ADMET database. J Chem 10(1):29. DOI. https://doi.org/10.1186/s13321-018-0283-x
70. Filimonov DA, Lagunin AA, Gloriozova TA, Rudik AV, Druzhilovskii DS, Pogodin PV, Poroikov VV (2014) Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chem Heterocycl Compd 50(3):444–457. https://doi.org/10.1007/s10593-014-1496-1
71. Geronikaki A, Poroikov V, Hadjipavlou-Litina D, Filimonov D, Lagunin A, Mgonzo R (1999) Computer aided predicting the biological activity spectra and experimental testing of new thiazole derivatives. Quant Struct-act Rel 18(1):16–25. https://doi.org/10.1002/(sici)1521-3838(199901)18:1<16::aid-qsar16>3.0.co;2-o
72. Zaretzki J, Bergeron C, Huang TW, Rydberg P, Swamidass SJ, Breneman CM (2012) RS-WebPredictor: a server for predicting CYP-mediated sites of metabolism on drug-like molecules. Bioinformatics 29(4):497–498. https://doi.org/10.1093/bioinformatics/bts705
73. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R (2014) ProTox: a web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Res 42(W1):W53–W58. https://doi.org/10.1093/nar/gku401
74. Schrödinger Release 2018-4: Jaguar, Schrödinger, LLC, New York, 2018.
75. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B 37(2):785. https://doi.org/10.1103/physrevb.37.785
76. Becke AD (1988) Density-functional exchange-energy approximation with correct asymptotic behavior. Phys Rev A 38(6):3098. https://doi.org/10.1103/physreva.38.3098
77. Pearson RG (1986) Absolute electronegativity and hardness correlated with molecular orbital theory. P Natl Acad Sci 83(22):8440–8441. https://doi.org/10.1073/pnas.83.22.8440
78. Parr RG, Yang W (1989) Density-functional theory of atoms and molecules, vol. 16 of International series of monographs on chemistry. Oxford University Press, New York
79. Yuriev E, Ramsland PA (2013) Latest developments in molecular docking: 2010–2011 in review. J Mol Recognit 26(5):215–239. https://doi.org/10.1002/jmr.2266
80. Zhang X, Perez-Sanchez H, C-Lightstone F (2017) A comprehensive docking and MM/GBSA rescoring study of ligand recognition upon binding antithrombin. Curr Top Med Chem 4v(14):1631–1639. https://doi.org/10.2174/1568026616666161117112604
81. Sherman W, Day T, Jacobson MP, Friesner RA, Farid R (2006) Novel procedure for modeling ligand/receptor induced fit effects. J Med Chem 49(2):534–553. DOI. https://doi.org/10.1021/jm050540c
82. Aamir M, Singh VK, Dubey MK, Meena M, Kashyap SP, Katari SK, Upadhyay RS, Singh S (2018) In silico prediction, characterization, molecular docking and dynamic studies on fungal SDRs as novel targets for searching potential fungicides against fusarium wilt in tomato. Front Pharmacol 9:1038. https://doi.org/10.3389/fphar.2018.01038
83. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes. J Med Chem 49(21):6177–6196. https://doi.org/10.1021/jm051256o
84. Priyadarshini V, Pradhan D, Munikumar M, Swargam S, Umamaheswari A, Rajasekhar D (2014) Genome-based approaches to develop epitope-driven subunit vaccines against pathogens of infective endocarditis. J Biomol Struct Dyn 32(6):876–889. https://doi.org/10.1080/07391102.2013.795871
85. Gohlke H, Hendlich M, Klebe G (2000) Knowledge-based scoring function to predict protein-ligand interactions. J Mol Biol 295(2):337–356. https://doi.org/10.1006/jmbi.1999.3371
86. Shoichet BK, McGovern SL, Wei B, Irwin JJ (2002) Lead discovery using molecular docking. Curr Opin Chem Biol 6(4):439–446. https://doi.org/10.1016/s1367-5931(02)00339-3
87. Dvir H, Silman I, Harel M, Rosenberry TL, Sussman JL (2010) Acetylcholinesterase: from 3D structure to function. Chem Biol Interact 187(1-3):10–22. DOI. https://doi.org/10.1016/j.cbi.2010.01.042
88. Klebe G (2015) Protein-ligand interactions as the basis for drug action. In: Multifaceted Roles of Crystallography in Modern Drug Discovery. Springer, Dordrecht, pp 83–92. https://doi.org/10.1007/978-94-017-9719-1_7
89. Lipinski CA (2004) Lead-and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol 1:337–341. https://doi.org/10.1016/j.ddtec.2004.11.007
90. Pollastri MP (2010) Overview on the rule of five. Curr Protoc Pharmacol 49:9–12. https://doi.org/10.1002/0471141755.ph0912s49
91. Ullah A, Prottoy NI, Araf Y, Hossain S, Sarkar B, Saha A (2019) Molecular docking and pharmacological property analysis of phytochemicals from Clitoria ternatea as potent inhibitors of cell cycle checkpoint proteins in the cyclin/CDK pathway in cancer cells. Comput Mol Biosci 9(03):81. DOI. https://doi.org/10.4236/cmb.2019.93007
92. Sarkar B, Islam SS, Ullah MA, Hossain S, Prottoy MN, Araf Y, Taniya MA (2019) Computational assessment and pharmacological property breakdown of eight patented and candidate drugs against four intended targets in Alzheimer's disease. Adv Biosci Biotechnol 10(11):405. https://doi.org/10.4236/abb.2019.1011030
93. Li AP (2001) Screening for human ADME/Tox drug properties in drug discovery. Drug Discov Today 6(7):357–366. https://doi.org/10.1016/s1359-6446(01)01712-3
94. Guengerich FP (1999) Cytochrome P-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol 39(1):1–17. https://doi.org/10.1146/annurev.pharmtox.39.1.1
95. Glue P, Clement RP (1999) Cytochrome P450 enzymes and drug metabolism—basic concepts and methods of assessment. Cell Mol Neurobiol 19(3):309–323. https://doi.org/10.1023/a:1006993631057
96. Dixit B (2017) A review on the effects of CMPF binding with human serum albumin. Bioinform Rev 3(9):9–18
97. Radchenko EV, Dyabina AS, Palyulin VA, Zefirov NS (2016) Prediction of human intestinal absorption of drug compounds. Russ Chem Bull 65(2):576–580. https://doi.org/10.1007/s11172-016-1340-0
98. Wessel MD, Jurs PC, Tolan JW, Muskal SM (1998) Prediction of human intestinal absorption of drug compounds from molecular structure. J Chem Inf Comput Sci 38(4):726–735. https://doi.org/10.1021/ci980029a
99. Basant N, Gupta S, Singh KP (2016) Predicting human intestinal absorption of diverse chemicals using ensemble learning based QSAR modeling approaches. Comput Biol Chem 61:178–196. https://doi.org/10.1016/j.compbiolchem.2016.01.005
100. Swierczewska M, Lee KC, Lee S (2015) What is the future of PEGylated therapies? https://doi.org/10.1517/14728214.2015.1113254
101. Smalling RW (1996) Molecular biology of plasminogen activators: what are the clinical implications of drug design? Am J Cardiol 78(12):2–7. https://doi.org/10.1016/s0002-9149(96)00736-9
102. Sahin S, Benet LZ (2008) The operational multiple dosing half-life: a key to defining drug accumulation in patients and to designing extended release dosage forms. Pharm Res 25(12):2869–2877. https://doi.org/10.1007/s11095-008-9787-9
103. Sanguinetti MC, Jiang C, Curran ME, Keating MT (1995) A mechanistic link between an inherited and an acquird cardiac arrthytmia: HERG encodes the IKr potassium channel. Cell 81(2):299–307. https://doi.org/10.1016/0092-8674(95)90340-2
104. Aronov AM (2005) Predictive in silico modeling for hERG channel blockers. Drug Discov Today 10(2):149–155. https://doi.org/10.1016/s1359-6446(04)03278-7
105. Cheng A, Dixon SL (2003) In silico models for the prediction of dose-dependent human hepatotoxicity. J Comput Aid Mol Des 17(12):811–823. https://doi.org/10.1023/b:jcam.0000021834.50768.c6
106. Xu JJ, Diaz D, O'Brien PJ (2004) Applications of cytotoxicity assays and pre-lethal mechanistic assays for assessment of human hepatotoxicity potential. Chem Biol Interact 150(1):115–128. https://doi.org/10.1016/j.cbi.2004.09.011
107. Mortelmans K, Zeiger E (2000) The Ames Salmonella/microsome mutagenicity assay. Mutat Res-Fund Mol M 455(1-2):29–60. https://doi.org/10.1016/s0027-5107(00)00064-6
108. Holt, M P, ; Ju, C. Mechanisms of drug-induced liver injury. AAPS J. 2006, 8(1), E48-E54. DOI: https://doi.org/10.1208/aapsj080106
109. Lagunin A, Stepanchikova A, Filimonov D, Poroikov V (2000) PASS: prediction of activity spectra for biologically active substances. Bioinformatics 16(8):747–748. https://doi.org/10.1093/bioinformatics/16.8.747
110. United Nations. Economic Commission for Europe. Secretariat, 2005. Globally harmonized system of classification and labelling of chemicals (GHS). United Nations Publications. https://unece.org/ghs-rev1-2005. Accessed 3 Dec 2020.
111. Tyzack JD, Mussa HY, Williamson MJ, Kirchmair J, Glen RC (2014) Cytochrome P450 site of metabolism prediction from 2D topological fingerprints using GPU accelerated probabilistic classifiers. J scinemrofnimehC 6(1):29. https://doi.org/10.1186/1758-2946-6-29
112. Danielson PB (2002) The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 3(6):561–597. https://doi.org/10.2174/1389200023337054

113. Matysiak J (2007) Evaluation of electronic, lipophilic and membrane affinity effects on antiproliferative activity of 5-substituted-2-(2, 4-dihydroxyphenyl)-1, 3, 4-thiadiazoles against various human cancer cells. Eur J Med Chem 42(7):940–947. https://doi.org/10.1016/j.ejmech.2006.12.033
114. Zhan CG, Nichols JA, Dixon DA (2003) Ionization potential, electron affinity, electronegativity, hardness, and electron excitation energy: molecular properties from density functional theory orbital energies. J Phys Chem A 107(20):4184–4195. DOI. https://doi.org/10.1021/jp0225774
115. Hoque MM, Halim MA, Sarwar MG, Khan MW (2015) Palladium-catalyzed cyclization of 2-alkynyl-N-ethanoyl anilines to indoles: synthesis, structural, spectroscopic, and mechanistic study. J Phys Org Chem 28(12):732–742. https://doi.org/10.1002/poc.3477
116. Ayers PW, Parr RG, Pearson RG (2006) Elucidating the hard/soft acid/base principle: a perspective based on half-reactions. J Chem Phys 124(19): 194107. https://doi.org/10.1063/1.2196882
117. Ullah MA, Johora FT, Sarkar B, Araf Y, Rahman MH (2020) Curcumin analogs as the inhibitors of TLR4 pathway in inflammation and their drug like potentialities: a computer-based study. J Recept Signal Transduct:1–5. https://doi.org/10.1080/10799893.2020.1742741

Publisher's Note