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Prediction of anti-Alzheimer's activity of flavonoids targeting acetylcholinesterase in silico

Prediction of anti-Alzheimer's activity of flavonoids targeting acetylcholinesterase *in silico*

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ABSTRACT:

Introduction – Prenylated and pyrano-flavonoids of the genus *Artocarpus* J. R. Forster & G. Forster are well known for their acetylcholinesterase (AchE) inhibitory, anticholinergic, antiinflammatory, antimicrobial, antioxidant, antiproliferative and tyrosinase inhibitory activities. Some of these compounds have also been shown to be effective against Alzheimer's disease.

Objective – The aim of the *in silico* study was to establish protocols to predict the most effective flavonoid from prenylated and pyrano-flavonoid classes for AchE inhibition linking to the potential treatment of Alzheimer's disease.

Methodology – Three flavonoids isolated from *Artocarpus anisophyllus* Miq. were selected for the study. With these compounds, Lipinski filter, ADME/Tox screening, molecular docking and QSAR were performed *in silico*. *In vitro* activity was evaluated by bioactivity staining based on the Ellman's method.

Results – In the Lipinski filter and ADME/Tox screening, all test compounds produced positive results, but in the target fishing, only one flavonoid could successfully target AchE. Molecular docking was performed on this flavonoid, and this compound gained the score as -13.5762. From the QSAR analysis the IC_{50} was found to be 1659.59 nM. Again, 100 derivatives were generated from the parent compound and docking was performed. The derivative number 20 was the best scorer i.e., -31.6392 and IC_{50} was predicted as 6.025 nM.

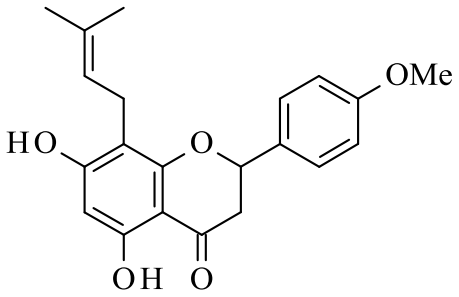
Conclusion – Results indicated that flavonoids could be efficient inhibitors of AchE and thus, could be useful in the management of Alzheimer's disease.

Keywords: *Artocarpus anisophyllus*; Alzheimer's disease; acetylcholinesterase; Lipinski filter**;** ADME/Tox screening; QSAR.

Introduction

Alzheimer's disease (AD) is known as the most common form of dementia and is a progressive neural disorder, characterized by memory loss and severe impairment of other intellectual capabilities (Thompson *et al.,* 2012). AD is connected with the reduced level of acetylcholine (Ach) and loss of cholinergic neurons in the brain (Lane *et al.,* 2006). Ach was the first discovered neurotransmitter that, transfers neural signal at all autonomic ganglia including neuromuscular junction and synapses in the central nervous system. In autonomic nervous system the neurotransmission of signal is governed by Ach between the preganglionic sympathetic and parasympathetic neurons. It is also responsible for stimulation of muscles, which include the muscles of gastro-intestinal muscles. The loss of function of Ach is implicated to the development of AD (Perry *et al.,* 1999). The AChE, an enzyme that breaks the neurotransmitter Ach into acetate and choline, hampers the normal neurotransmission. Cholinergic hypothesis of the disease states that the inhibition of AChE action may be one of the realistic approaches to the symptomatic management of AD (Weinstock, 1995). Acetylcholinesterase acts as one of the most significant targets against AD (Giacobini, 2004). Some of the known inhibitors of AchE are donepezil, galantamine tacrine, huperzine, and 7-methoxytacrine (Mirjana *et al.,* 2013).

5, 7-Dihydroxy-4'-methoxy-8-prenylflavanone (**1**), isobavachalcone (**2**) and 5-hydroxy-7, 8-(2, 2-dimethylchromano)-4'-methoxyflavanone (**3**) (Figure 1), isolated for the first time from the leaves of *Artocarpus anisophyllus* Miq. (Lathiff *et al.,* 2015), were selected for the present *in silico* bioactivity analysis. *Artocarpus anisophyllus* is a Malaysian plant, found in the lowland forests of Negeri Sembilan and Johor States of Malaysia, Sumatra and Philippines. This mid-canopy tree is locally known as "keledang babi" in Peninsular Malaysia or "mentawa" in Borneo. The *Artocarpus* species are rich in phenolic compounds especially prenylated and pyrano-flavonoids with various bioactivities including antimicrobial, anti-inflammatory, antioxidant, antiproliferative, anticholinergic, acetylcholinesterase (AchE) inhibitory and tyrosinase inhibitory activities (Arung *et al.,* 2006; Fang, *et al.,* 2008; Lin *et al.,* 2009; Ma *et al.,* 2010; Okoth *et al.,* 2013; Somashekhar *et al.,* 2013). Compound **1** is inactive towards DPPH free radicals, but possesses significant tyrosinase inhibitory activity (Lathiff *et al.,* 2015). In this present work, the bioactivity of the isolated compounds was studied *in silico* using several screening methods, which include drug likeness, ADME/Tox screening, molecular docking and QSAR. The goal of the study was to establish protocols to find out suitable target for the compounds in AD, and to check whether the isolated compounds could act as a better option for inhibition of AchE. After identifying the suitable target for the isolated compounds *in silico*, *in vitro* activity of these compounds was studied using bioactivity staining method (Ellman's method) for validating the *in silico* findings.



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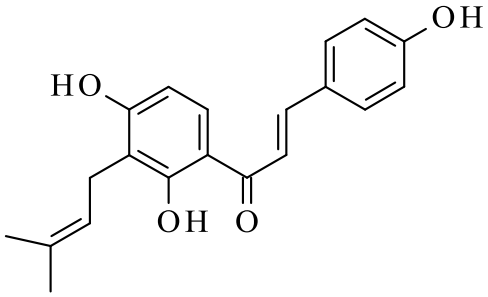

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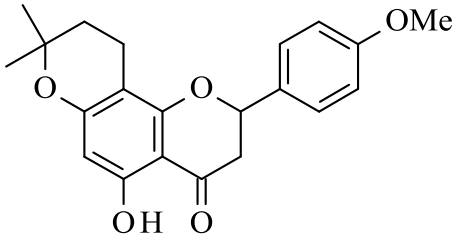

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Figure 1. Structures of flavonoids isolated from the leaves of *Artocarpus anisophyllus*

Experimental

Structural details of the selected compounds

The isolation and characterization of the flavonoids were achieved by vacuum liquid chromatography (VLC), gravity column chromatography (CC), 1D and 2D NMR, FTIR, UV, MS and by direct comparison with literature data (Lathiff *et al.,* 2015).

In silico approach for bioactivity analysis of the selected compounds

Ligand preparation

In the present study, ligands are the compounds isolated from the *A. anisophyllus* (Figure 1). Their structures were drawn with the ChemDraw Ultra 8.0 software and then the structures were converted to 3D structures of 'smiles' and 'sdf' formats with software *viz.* OpenBabel.

Toxicity and drug likeness

For ADME/Tox screening of the selected ligands, Mobyle@rpbs online portal (Lagorce *et al.,* 2008) was used. Molsoft L. L. C. online portal (www.molsoft.com) was used to screen the drug likeness of the compounds.

Target selection

In computer-aided drug designing, drug target search is an essential part of the work. A probable target for these selected compounds in relation to AD was anticipated with the help of PharmMapper (Figure 3). Of the flavonoids (Figure 1), only compound **1** showed any activity towards the expected target for AD, and the rest were discarded as they were inactive towards the target. For docking study the 3D structure of the target protein was obtained from Protein Data Bank (http://rcsb.org/pdb).

Molecular docking

Molecular docking was performed using FlexX of Biosolveit LeadIT with 5,7-Dihydroxy-4'-methoxy-8-prenylflavanone (**1**) and the target, and a separate docking was also performed with the target and known inhibitors to compare the efficacy of **1**. Docking results *i.e.,* docking energy, docked amino acid residues, hydrogen bond, and bond energy were recorded using LeadIT.

Quantitative structure activity relationship (QSAR) studies

The QSAR analysis was performed by taking 21 known inhibitors (Table 1). The QSAR descriptors *viz*. Molar Refractivity, Index of Refraction, Surface Tension, Density, Polarity and LogP were generated for each of the molecule using ACD ChemSketch software. The activities were measured by taking the inverse logarithm of IC_{50} values. The descriptors were tabulated in a MS Excel sheet against their bioactivities (log IC_{50}^{-1}). The descriptors and activities were loaded in Easy QSAR software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the activity of 5, 7-dihydroxy-4'-methoxy-8-prenylflavanone was predicted.

Table 1. Compounds and the known inhibitors (including marketed drugs) in SMILES

Compounds	Simplified Molecular Input Line Entry Specification (SMILES)
5, 7-Dihydroxy-4'-methoxy-8-prenylflavanone (**1**)	c1c(ccc(c1)OC)C1Oc2c(C(=O)C1)c(cc(c2CC=C(C)C)O)O
Donepezil	COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC
Galantamine	CN1CCC23C=CC(CC2OC4=C(C=CC(=C34)C1)OC)O
Rivastigmine	CCN(C)C(=O)OC1=CC=CC(=C1)C(C)N(C)C
Tacrine	C1CCC2=NC3=CC=CC=C3C(=C2C1)N

Huperzine	CC=C1C2CC3=C(C1(CC(=C2)C)N)C=CC(=O)N3
7-Methoxytacrine	COC1=CC2=C(C=C1)N=C3CCCCC3=C2N
1,3-*Bis*[3-(2-imidazolin-2-yl)phenyl]urea;propionic acid	O=C(Nc1cccc(c1)C1=NCCN1)Nc1cccc(c1)C1=NCCN1
4-(2-(3-(4-Hydroxyphenyl)-4-methylcyclohexyl)propyl)phenol	CC(Cc1ccc(O)cc1)C1CCC(C)C(C1)c1ccc(O)cc1
4-Amino-*N*-(1-benzyl-piperidin-4-yl)-5-chloro-2-methoxy-benzamide	COc1cc(N)c(Cl)cc1C(=O)NC1CCN(Cc2ccccc2)CC1
3-(2-Amino-phenylsulfanyl)-1-(4-isobutyl-phenyl)-3-(3-nitro-phenyl)-propan-1-one	CC(C)Cc1ccc(cc1)C(=O)CC(Sc1ccccc1N)c1cccc(c1)[N+]([O-])=O
2,4'-(2,3-Dihydrobenzo[b][1,4]thiazepine-2,4-diyl)diphenol	Oc1ccc(cc1)C1CC(=Nc2ccccc2S1)c1ccccc1O
3-(2-Chloro-phenyl)-5-methyl-isoxazole-4-carboxylic acid [2-chloro-5-(1-hydroxyimino-ethyl)-phenyl]-amide	CC(N=O)c1ccc(Cl)c(NC(=O)c2c(C)onc2-c2ccccc2Cl)c1
1-Benzo[1,3]dioxol-5-ylmethyl-1-(4-fluoro-phenyl)-3-(3-trifluoromethyl-phenyl)-thiourea	Fc1ccc(cc1)N(Cc1ccc2OCOc2c1)C(=S)Nc1cccc(c1)C(F)(F)F
N-(4-(*N*-(4-(4-(dimethylamino)phenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)sulfamoyl)phenyl)acetamide	COc1ccc(cc1)-c1cc(nc(NS(=O)(=O)c2ccc(NC(C)=O)cc2)n1)-c1ccc(cc1)N(C)C
N''-{[2-propyl-4-(1H-pyrazol-1-yl)benzoyl]oxy}-4-(trifluoromethyl)benzenecarboximidamide	CCCc1cc(ccc1C(=O)ON=C(N)c1ccc(cc1)C(F)(F)F)-n1cccn1
N,N'-(1-(4-methoxyphenyl)-1H-pyrrole-3,4-diyl)bis(methan-1-yl-1-ylidene)bis(4-methoxyaniline)	COc1ccc(cc1)\N=C\c1cn(cc1\C=N\c1ccc(OC)cc1)-c1ccc(OC)cc1
4,4'-(Cyclohexane-1,1-diyl)bis(2,6-dimethylphenol)	Cc1cc(cc(C)c1O)C1(CCCCC1)c1cc(C)c(O)c(C)c1
2-((7-Amino-1,1,3,3,6-pentamethyl-2,3-dihydro-1H-inden-5-ylimino)methyl)phenol	Cc1c(N)c2c(cc1\N=C\c1ccccc1O)C(C)(C)CC2(C)C

9-(4-(Dimethylamino)phenyl)-2,6,7-trihydroxy-3H-xanthen-3-one	CN(C)c1ccc(cc1)-c1c2cc(O)c(O)cc2oc2cc(=O)c(O)cc12
5-(1-(Biphenyl-4-yl)-1H-tetrazol-5-yl)-4-methylpyrimidin-2-amine	Cc1nc(N)ncc1-c1nnnn1-c1ccc(cc1)-c1ccccc1
3,4,5-Trihydroxy-benzoic acid 2-(3,4-dihydroxy-phenyl)-5,7-dihydroxy-chroman-3-yl ester	Oc1cc(O)c2C[C@@H](OC(=O)c3cc(O)c(O)c(O)c3)[C@H](Oc2c1)c1ccc(O)c(O)c1

***In vitro* activity**

AchE inhibitory activity of three flavonoids isolated from *A. anisophyllus* was assessed using the AchE inhibition assay using TLC with bioactivity staining based on the Ellman's method developed by (Rhee *et al.*, 2003). Detection limit was established by applying nine spots with various concentrations (2000, 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/mL) onto the TLC plate. The concentration that produced a spot with the least observable whiteness after being sprayed with mixtures of acetylthiocholine iodide as substrate, Ellman's reagent and AChE enzyme solution was the detection limit. Detection limit is defined as the minimum concentration where the white spot is visible by eye (Rhee *et al.,* 2001).

Results and discussion

Acetylcholinesterase inhibitory activity

AD is characterized by the loss of memory function that hampers the normal living and also the intellectual ability of the brain. During neural transmission the Ach breaks down into acetyl and choline by AchE on the post synaptic membrane. This degradation of Ach breaks the normal nervous transmission in the synaptic cleft and leading to the development of cholinergic AD symptoms. Restoring the damage caused by AchE can block the occurrence of AD and that can be gained through the inhibition of AchE function. Inhibitors of AchE or anti-cholinesterase prevent the breakdown of Ach and in turn maximize both level and duration of neurotransmitter action. In the present study, 5, 7-dihydroxy-4'-methoxy-8-prenylflavanone (**1**) was identified as an AchE inhibitor.

The *in silico* study was performed to check whether the selected compound could target the AchE more efficiently than the other inhibitors or drugs presently available in the market. There are a few drugs, *e.g.,* donepezil, rivastigmine, galantamine, tacrine, huperzine, and 7-methoxytacrine, available in the market, (Mirjana *et al.,* 2013). Donepezil, rivastigmine and galantamine are regarded as the phase-4 drugs (Mangialasche *et al*., 2010), and known as the most effective cholinergic drugs. Use of these medications was approved by regulatory organizations such as the U.S. Food and Drug Administration (FDA) and the European Medicines

Agency (EMA) to treat the mental manifestations of AD and to improve life quality of the patients (Birks, 2006; Hyde *et al.,* 2013). Though these drugs were approved by various regulatory agencies, those still display several side effects. The use of tacrine has been abandoned because of several side effects including hepatotoxicity (Watkins *et al.,* 1994; Birks *et al.,* 2009) and donepezil antagonistic effects include diarrhoea, anorexia, abdominal pain, gastrointestinal anomalies-nausea, as well as an increase in cardiac vagal tone causing bradycardia (Tayeb *et al.,* 2012). Adverse effects of rivastigmine are consistent with the cholinergic actions of the drug, and include nausea, vomiting, diarrhoea, anorexia, headache, syncope, abdominal pain and dizziness (Inglis, 2002; Birks *et al.,* 2009). Galantamine, huperzine and 7-methoxytacrine also show similar type of toxicities (Birks, 2006). However, compared to other AChE inhibitors, huperzine shows better permeation through blood-brain-barrier has higher oral bioavailability and longer AChE inhibition (Ebrahimi *et al.,* 2012). Considering the clinical effects of all the marketed drugs, there is no indication that any of these medicines is superior to other in efficacy (Tayeb *et al.,* 2012). The current study was about searching a potential inhibitor with higher efficiency than the marketed drugs for AD. In this connection, the *in silico* establishment of the isolated compound **1** was studied and it was validated by *in vitro* study against acetylcholinesterase.

In silico study

In silico study involved ADME/Tox screening, drug likeness prediction, target prediction, molecular docking and QSAR analysis. ADME/Tox screening is the initial step for selection of potential drug molecule, which helps in predicting the *in vivo* behaviour of such compound (Yu and Adedoyin, 2003). To be an efficient lead molecule, it should follow some basic criteria – viz, drug molecule must be bioavailable (absorption), distributed to the particular site of action, metabolically active (non-toxic) and should be eliminated from the body (Wan, 2013). ADME/Tox screening can be performed through online web server like FAF-Drugs3 maintains under the Mobyle@rpbs, OSIRIS Property Explorer, ADMET Predictor, ADMET Modeler etc. In the present context, compound **1** was found nontoxic i.e, could sustain the standard for absorption, digestion, metabolism and excretion in FAF-Drugs3 screening and also followed the drug likeness properties in the Molsoft (Figure 2) described by the Lipinski Rule of 5 (RO5). Molecules maintaining the Lipinski Rule of 5 are mainly orally administered drugs (Lipinski, 2004). The RO5 demonstrate mainly four physicochemical properties of a molecule to become a drug likely Molecular weight (MW) should be ≤ 500, logP ≤ 5, H-bond donors ≤ 5 and H-bond acceptor ≤ 10. Poor absorption, bioavailability and solubility is observed if a molecule shows MW more than 500 (Miteva *et al.*, 2006). However, in exceptional cases fulfilling the RO5 does not guarantee that a molecule is drug like. Moreover, it provides an idea about the solubility and oral bioavailability of a compound. Following the Lipinski Rule of 5, compound **1** showed molecular weight as 354.40 Dalton, logP value 4.64, H-bond donor 2, and H-bond acceptor 5.

The findings pointed that, compound **1** has drug like properties with drug likeness model score 1.28 (Lipinski *et al.,* 1997) and this can be an orally active drugs. Based on its physicochemical parameters shown in Rule of 5 it could be water soluble, permeable through intestine and orally bioavailable (Lipinski, 2004).

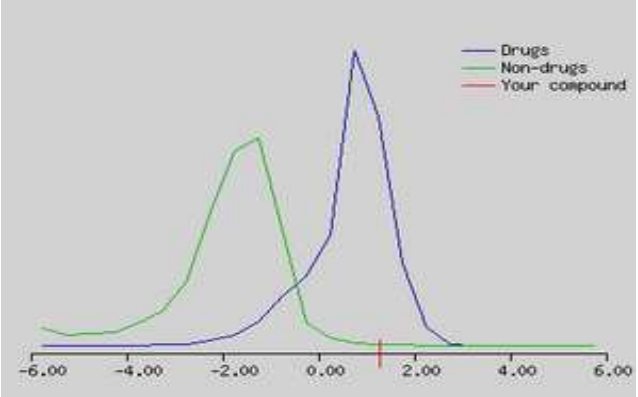
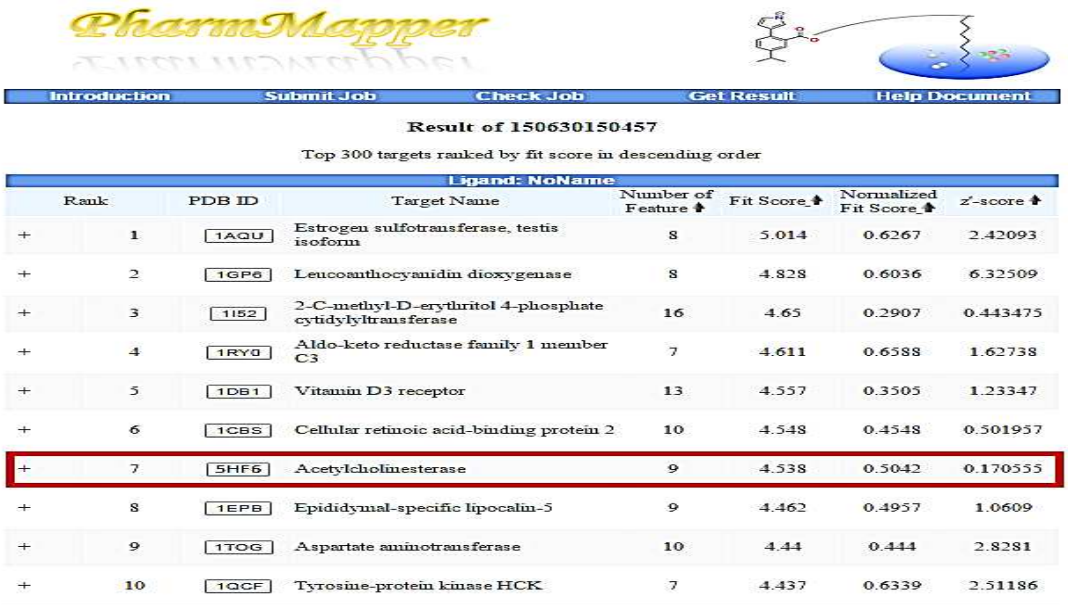


Figure 2. MolSoft drug likeliness properties of the isolated compound **1**

Target prediction in PharmMapper, ligand (compound **1**) generated about 300 probable targets and information about the binding score. PhamMapper is a web-based server and relies on high throughput screening process for target identification. Swiss Target Prediction, SEA, ChemProt and Target Hunter of Small Molecule etc. are other web-based server for target prediction. From the PharmMapper result, AchE was chosen as the target for the selected compound **1** by thoroughly studying all the consequences among the probable targets in the PharmMapper (Figure 3).



	Rank	PDB ID	Target Name	Number of Feature	Fit Score	Normalized Fit Score	z'-score
+	1	1AQU	Estrogen sulfotransferase, testis isoform	8	5.014	0.6267	2.42093
+	2	1GP6	Leucoanthocyanidin dioxygenase	8	4.828	0.6036	6.32509
+	3	1I52	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	16	4.65	0.2907	0.443475
+	4	1RY0	Aldo-keto reductase family 1 member C3	7	4.611	0.6588	1.62738
+	5	1DB1	Vitamin D3 receptor	13	4.557	0.3505	1.23347
+	6	1CBS	Cellular retinoic acid-binding protein 2	10	4.548	0.4548	0.501957
+	7	5HF6	Acetylcholinesterase	9	4.538	0.5042	0.170555
+	8	1EPB	Epididymal-specific lipocalin-5	9	4.462	0.4957	1.0609
+	9	1TOG	Aspartate aminotransferase	10	4.44	0.444	2.8281
+	10	1QCF	Tyrosine-protein kinase HCK	7	4.437	0.6339	2.51186

Figure 3. Target prediction in PharmMapper.

Molecular docking with FlexX was performed between ligand and the target along with some known inhibitors including a few drug molecules available in the market. In this approach, the ligand is allowed to interact with the specific target and it is the basic step of Ligand Based Drug Design (LBDD) method (Acharya *et al.,* 2011). Docking involves an algorithm of molecular interactions (intermolecular interaction) between the ligand and the specific target. Ligand molecule interacts with the target at specific binding site by searching favourable conformation of the protein. The ligand target binding is determined by mode of bonding between the amino acid residues of the target protein and the ligand molecule. Molecular docking furnishes quantitative prediction of binding energetics and also provides the ranking (score) of docked compounds on the basis of binding affinity of ligand-target complex (Huang and Zou, 2010). The binding energetics mainly based on the hydrogen – bonding pattern of amino acid residues and ligand molecule. Autodock, DOCK, GOLD, FlexAID, HYBRID and idock etc. are some other softwares for performing molecular docking. The docking study showed that the ligand-target binding complex for **1** (Figure 4) was more significant than that with the phase-4 drugs except donepezil and also few known inhibitors shown higher docking score than the molecule **1**. The score shown by compound **1** and donepezil was -13.5762 and -15.4974 respectively; other inhibitors' scores were within the ranges from 1.0000 to -23.4139 (Table 2).

Table 2. Different parameters in the comparison of drug efficacy of 5,7-dihydroxy-4'-methoxy-8-prenylflavanone in LeadIT

Molecule Name	**Score**	**Bonding Pattern**	**Bond Energy**	**Bond Length**
5,7-Dihydroxy-4'-methoxy-8-prenylflavanone(**1**)	-13.5762	H48-O GLY-523-A	-4.7	1.71A
		O14-H GLN-527-A	-4.5	1.64A
		O26-H ALA-526-A	-3.9	1.87A
Donepezil	-15.4974	O2-HD22 ASN 533-A	-4.7	1.98A
		H58-OE2 GLU-313-A	-6.0	1.95A
Galantamine	-11.1766	H43-O ASN-533-A	-4.7	1.89A
		O11-HE ARG-517-A	-3.4	1.87A
Rivastigmine	-10.1986	H41-O GLY-523-A	-4.7	1.78A
		O6-H ALA-528-A	-4.7	1.64A
Tacrine	-14.3769	H28-OD1 ASN-533-A	-4.4	1.90A
		H29-OE1 GLN-413-A	-4.1	2.01A
Huperzine	-0.0000	Not Docked	-	-
7-Methoxytacrine	-18.3407	H33-OE1 GLN-508A	-4.6	1.66A
		N9-HE ARG-534-A	-4.7	1.78A
1,3-Bis[3-(2-imidazolin-2-yl)phenyl]urea; propionic acid	1.000	H47-O GLY-523-A	-4.7	1.64A
		O1-H ALA-528-A	-4.7	1.84A

4-(2-(3-(4-Hydroxyphenyl)-4 methylcyclohexyl)propyl)phenol	-9.9479	H33-O ALA-412-A O8-HH TYR-503-A	-4.7 -4.7	1.66A 1.74A
4-Amino-N-(1-benzyl-piperidin-4-yl)-5-chloro-2-methoxy-benzamide	-17.1618	O12-H ALA-528-A	-4.7	2.17A
3-(2-Amino-phenylsulfanyl)-1-(4-isobutyl-phenyl)-3-(3-nitro-phenyl)-propan-1-one	-18.3317	O12-H ALA-528-A O31-HH TYR-510-A	-4.7 -3.7	1.76A 1.62A
2,4'-(2,3-Dihydrobenzo[b][1,4]thiazepine-2,4-diyl)diphenol	-23.4139	H26-OH TYR-503-A O1-H LEU-524-A	-4.4 -4.7	1.69A 1.62A
3-(2-Chloro-phenyl)-5-methyl-isoxazole-4-carboxylic acid [2-chloro-5-(1-hydroxyimino-ethyl)-phenyl]-amide	-16.9822	O4-H ALA-528-A H34-O GLY-523-A	-4.7 -4.7	1.76A 2.04A
1-Benzo[1,3]dioxol-5-ylmethyl-1-(4-fluoro-phenyl)-3-(3-trifluoromethyl-phenyl)-thiourea	-17.0306	O16-HE GLN-413-A O14-HE ARG-417-A	-4.3 -3.7	1.85A 2.09A
N-(4-(N-(4-(4-(dimethylamino)phenyl)-6-(4-methoxyphenyl)pyrimidin-2-yl)sulfamoyl)phenyl)acetamide	-18.6011	O2-HD21 ASN-317-A H48-O ALA-505-A	-4.6 -3.8	2.20A 1.81A
N''-{[2-propyl-4-(1H-pyrazol-1-yl)benzoyl]oxy}-4-(trifluoromethyl)benzenecarboximidamide	-12.7710	N13-H GLN-527-A O11-H ALA-528-A	-4.5 -4.2	1.98A 1.90A
N,N'-(1-(4-methoxyphenyl)-1H-pyrrole-3,4-diyl)bis(methan-1-yl-1-ylidene)bis(4-methoxyaniline)	-18.5407	N17-H ARG-525-A	-4.3	1.78A
4,4'-(Cyclohexane-1,1-diyl)bis(2,6-dimethylphenol)	0.2879	O21-HD21 ASN-317-A	-4.4	1.90A
2-((7-Amino-1,1,3,3,6-pentamethyl-2,3-dihydro-1H-inden-5-ylimino)methyl)phenol	-9.0876	H36-O GLN-413-A N9-HE ARG-417-A	-3.6 -3.7	2.27A 1.61A
9-(4-(Dimethylamino)phenyl)-2,6,7-trihydroxy-3H-xanthen-3-one	-10.4276	H43-O GLY-523-A O19-H GLN-527-A	-4.7 -4.5	1.63A 2.07A
5-(1-(Biphenyl-4-yl)-1H-tetrazol-5-yl)-4-methylpyrimidin-2-amine	-21.0719	H29-OH TYR-503-A N10-HD22 ASN-533-A	-4.7 -4.5	1.73A 2.11A
3,4,5-Trihydroxy-benzoic acid 2-(3,4-dihydroxy-phenyl)-5,7-dihydroxy-chroman-3-yl ester	-17.9143	O31-H LEU-524-A H48-OH TYR-503-A H49-O LEU-524-A	-4.7 -4.6 -3.2	1.89A 1.81A 1.52A

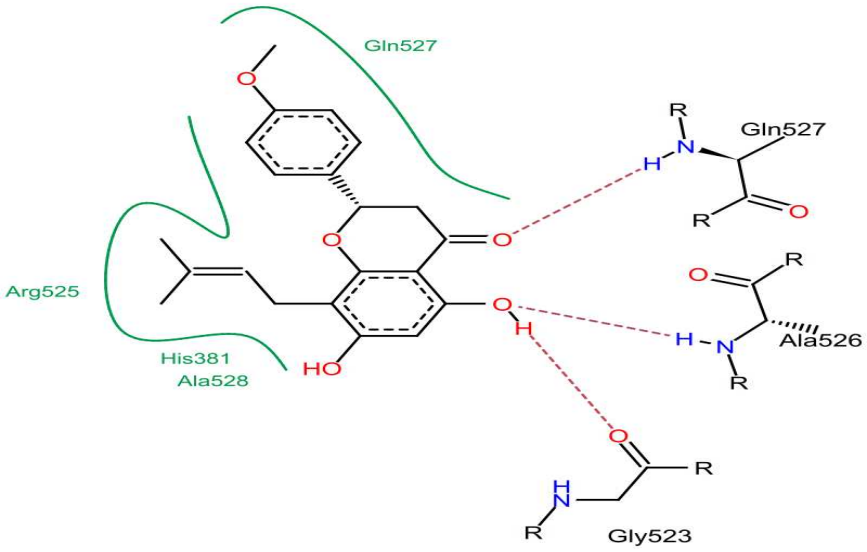


Figure 4. Flex X binding pattern of compound **1** with the target

QSAR models describe the relationship between a chemical structure and biological activities in a set of chemicals and based on this, it also predicts the activity of new chemicals. QSAR software predicts the biological activity based on statistical calculation such as regression analysis. Prediction of biological activities is achieved quantitatively as a concentration of a chemical substance required to express a biological response. Easy QSAR, cQSAR, QSARPro. and McQSAR etc. are some of the softwares for prediction of structure activity relationship. In the QSAR assay by Easy QSAR software, significant correlation with R square value of 73.55% was found. The Rsq value should be definitely high for a good QSAR equation, higher Rsq means higher fitting of the equation to the given data, hence better predictions it would provide for new test data. The Adjusted Rsq was 61.34%; therefore, the difference between Rsq and adjusted Rsq was less. High difference in Rsq and Adjusted Rsq indicates weaker overall prediction. The F statistics of the test was 6.02 and the critical F was 2.63. The F statistics of the test should be greater than Critical F, otherwise the generated equation is inefficient. The multiple regression plots (linear) for 21 AChE inhibitors are shown in (Figure 5).

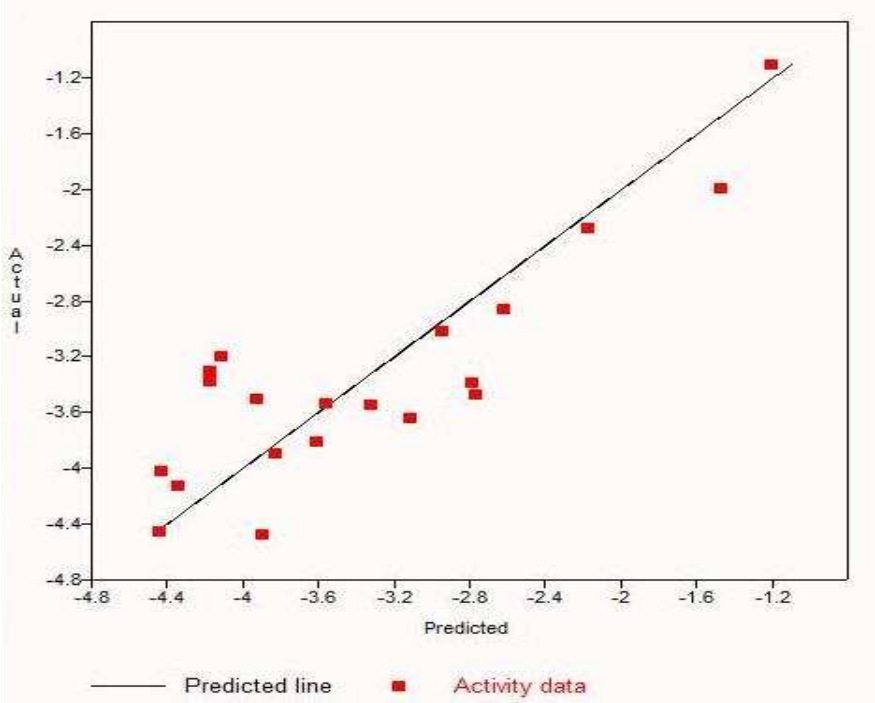


Figure 5. The Multiple regression plots (Linear) for the inhibitor

The equation generated out of QSAR analysis is as follows:
Activity = 1.0518E+001 + 8.8234E-001*(Molar Refractivity) + -7.4631E+000*(Index of Refraction) + 8.8064E-003*(Surface Tension) + -1.5087E+000*(Density) + -2.9761E-001*(logP) + -2.2007E+000*(Polarity)

From the above QSAR equation the IC_{50} value of 5, 7-dihydroxy-4'-methoxy-8-prenylflavanone (**1**) was predicted as 1659.59 nM. Validating the *in silico* result, an *in vitro* investigation on AchE inhibitory activity showed compound **1** as one of the AchE inhibitors with the detection limit of 125 µg/mL for inhibiting AChE enzyme and that of the positive control, galantamine hydrobromide was 7.81 µg/mL. Compound **1** was clearly an inhibitor of AChE, but it was less efficient than the standard. As a result, a family of 100 derivatives was prepared from the parent compound **1**, and again molecular docking was performed to get the higher efficiency compound. Several derived compounds showed much higher efficiency towards the target than that of donepezil, compound **1** and known inhibitor. In docking analysis, the compound **20** exhibited (score -31.6392) the highest efficiency towards the target binding site to form a stable configuration of ligand-target complex (Figure 6) to inhibit the activity of AchE. The IC_{50} value of this derivative was predicted as 6.025 nM from the QSAR study and it was much less than that of the parent compound. So, compound **20** might act as the efficient inhibitor of AchE (Mirjana *et al.,* 2013). AchE pathway is one of the pathways leading to Alzheimer's disease (Perry *et al.,* 1999). If compound **20** blocks the AchE activity during neurotransmission, the AchE pathway leading to Alzheimer's disease can potentially be

blocked. Further synthesis and *in vitro* and *in vivo* analyses are required to establish the AchE inhibition activity of compound **20**.

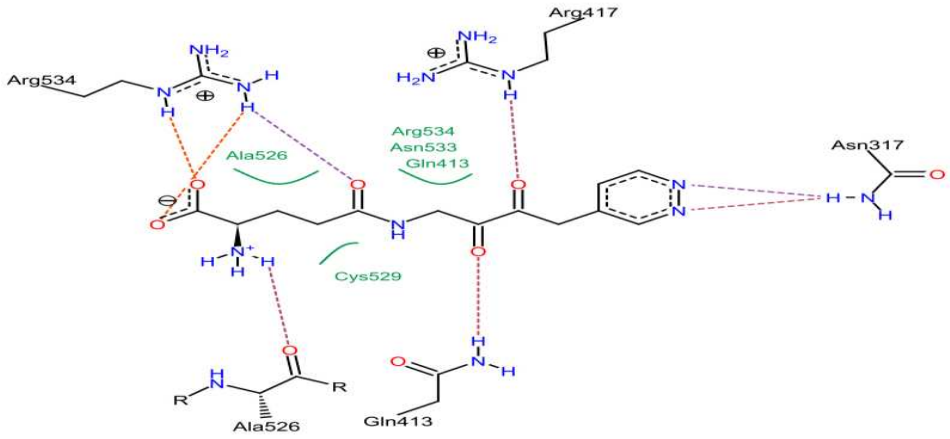


Figure 6. Flex X binding pattern of derived compound **20** with the target

Acknowledgment

The authors gratefully acknowledge Department of Biotechnology (DBT), Govt. of India sponsored Bioinformatics Infrastructure Facility (BIF) in Assam University Silchar-788011 for providing free access of DelCON's e-Journal library and the Ministry of Higher Education (MOHE) Malaysia for financial support under the Research University Grant (RUG) with vote number Q.J130000.2526.12H47.

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