



Original article

In silico QSAR analysis of quercetin reveals its potential as therapeutic drug for Alzheimer's disease

Md. Rezaul Islam^{a,e,*}, Aubhishek Zaman^b, Iffat Jahan^c, Rajib Chakravorty^d, Sajib Chakraborty^{e,**}

^a International Max Planck Research School for Neurosciences, University of Göttingen, 37077 Göttingen, Germany

^b University of Texas Southwestern Medical Center, Dallas, TX 75235, USA

^c Molecular Biology Lab, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh

^d Department of EEE, University of Melbourne, National ICT Australia, Victoria 3010, Australia

^e Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh

ARTICLE INFO

Article history:

Received 11 August 2013

Accepted 17 November 2013

Available online 15 December 2013

Keywords:

Alzheimer's disease

Molecular docking

In silico

Quercetin

Cholinesterase inhibitors

ABSTRACT

Acetylcholine-esterase (AChE) inhibitors are one of the most potent drug molecules against Alzheimer's disease (AD). But, patients treated with current AChE inhibitors often experience severe side effects. Quercetin is a plant flavonoid compound which can act as AChE inhibitor and it may be a better alternative to current AChE inhibitors in terms of effectiveness with no or fewer side effects.

Aims: The aim of the study was to compare quercetin with conventional AChE inhibitors to search for a better drug candidate.

Methods and materials: Physico-chemical properties of conventional drugs and quercetin were predicted using bioinformatics tools. Molecular docking of these compounds on the active site of AChE was performed using AutoDock and comparative analysis was performed. Later, modification on the basic structure of quercetin with different functional groups was done to perform QSAR analysis.

Result and discussion: Quercetin showed a similar drug likeness score to the conventional drugs. The binding strength for quercetin in the active site of the enzyme was -8.8 kcal/mol, which was considerably higher than binding scores for some of the drugs such as donepezil (binding score -7.9 kcal/mol). Fifteen hydrogen bonds were predicted between quercetin and the enzyme whereas conventional drugs had fewer or even no hydrogen bonds. It implies that quercetin can act as a better inhibitor than conventional drugs. To find out even better inhibitor, similar structures of quercetin were searched through SIMCOMP database and a methylation in the 4-OH position of the molecule showed better binding affinity than parent quercetin. Quantitative structure activity relationship study indicated that O-4 methylation was specifically responsible for better affinity.

Conclusion: This *in silico* study has conclusively predicted the superiority of the natural compound quercetin over the conventional drugs as AChE inhibitor and it sets the need for further *in-vitro* study of this compound in future.

Copyright © 2013, InPharm Association, Published by Reed Elsevier India Pvt. Ltd. All rights reserved.

1. Introduction

Alzheimer's disease (AD) impacts the part of brain that controls cognitive function affecting the memory, reasoning capabilities, learning abilities and language capabilities. Moreover, it can also bring non-cognitive disabilities into the patients like depression,

agitation, hallucinations and delusions. According to a recent estimate, more than 35 million people are affected by Alzheimer's disease worldwide¹ while another study estimated 4.5 million U.S. cases in the year 2000 alone and predicted that the number will rise 3-fold to 13.2 million by 2050. Despite the genetic predisposition to AD, several factors including diabetes, obesity etc. has been associated with Alzheimer's disease.^{2–5} Old age has been considered as one of the major risk factors for AD and the probability of being affected by it doubles every 5 years after mid-seventies and the rate is even higher for the people at an age of 85.^{6,7}

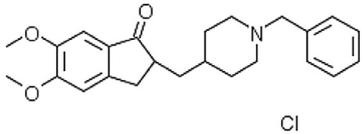
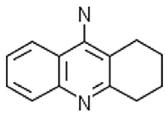
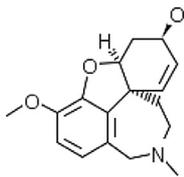
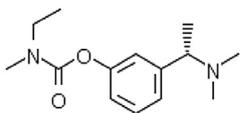
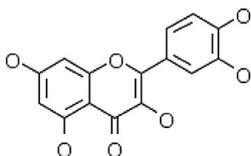
Molecular research suggests that the main cause of AD is the genesis and uncanny deposition of abnormal proteins (senile

* Corresponding author. International Max Planck Research School for Neurosciences, University of Göttingen, 37077 Göttingen, Germany.

** Corresponding author

E-mail addresses: rezaul.nayeem@gmail.com (M.R. Islam), sajib@univdhaka.edu (S. Chakraborty).

Table 1
List of conventional drugs and natural inhibitor along with their identity and 2D conformer.

Conventional drug/natural inhibitor name	PubChem compound CID	2D conformer
Donepezil	3152	
Tacrine	1935	
Galantamine	908828	
Rivastigmine	77991	
Quercetin	5280343	

plaques and neuro-fibrillary tangles) in aging brain resulting in oxidative and inflammatory damage and finally leading to energy failure and extensive neuronal loss.⁸ There is also a confirmed link between AD and cholinergic signaling of the brain in abundant available evidences.⁹ Degeneration of nuclei in the basal forebrain results in a loss of excitatory cholinergic transmission to the cerebral cortex and this is thought to contribute significantly to the cognitive deficits present in Alzheimer's disease.¹⁰

Depletion of the neurotransmitter acetylcholine in AD due to reduced number of acetylcholine receptor and increased activity of acetylcholine-esterase are considered as prominent features of AD. In Alzheimer's disease, cortical cholinergic innervations are reduced leading to shifting of tau protein to its hyper-phosphorylated form. Once the tau is hyper-phosphorylated, it reduces secretion of soluble APP (Amyloid Precursor Protein) and thus increases the production of β -amyloid protein. Increased accumulation of these proteins leads to form plaque that causes the toxic effect to the neuronal cell leading to its severe damage or death if this situation persists.^{11–13}

Different AChE inhibitors are highly prescribed as medicine because they serve to restore neurotransmission by blocking the action of acetylcholine-esterase. This action cleaves endogenously produced acetylcholine in synaptic clefts.¹⁴ These inhibitors subsequently play a role in the reduction of phosphorylation of tau protein causing a reduction of amyloid plaque deposition. Moreover, these drugs can show anti-oxidant properties which impede

the lesion formations of toxic substances and thus bring a pause in the promotion of Alzheimer's disease.¹⁵

Currently acetylcholine-esterase inhibitors such as tacrine, galantamine, rivastigmine and donepezil are widely used as conventional drugs for the therapeutic purpose of AD and have been found to improve cognitive function in AD patients.^{16–18} But these AChE inhibitors are known to have side effects such as hepatotoxicity and cardiovascular disease.¹⁹ Because of these side effects along with other shortcoming such as short life of the inhibitors and poor binding with the acetylcholine-esterase, the search for better acetylcholine-esterase inhibitors is still going on.

A recent study reports the isolation of a biologically active acetylcholine-esterase inhibitors from medicinal plants named quercetin from its natural source *Agrimonia pilosa*.²⁰ Quercetin, a major flavonoid under the class of flavonols, is found in many foods like cauliflower, nuts, tea, apples, berries, and beverages. This natural flavonoid compound has the anti-oxidant property similar to other chemically synthesized acetylcholine-esterase inhibitors and thereby it has the potential to be a good alternative candidate for Alzheimer's disease treatment through choline-esterase inhibition. In present study, we have performed an *in silico* comparison between the natural compound quercetin and the widely used conventional drugs (tacrine, galantamine, rivastigmine and donepezil) as an acetylcholine-esterase inhibitor. We have also performed an *in silico* quantitative structure activity relationship study of quercetin to investigate the functional groups that may help to design a better inhibitor of acetylcholine-esterase. To the best of our knowledge, this is the first report comparing a natural acetylcholine-esterase inhibitor with conventional inhibitors widely used.

2. Materials and methods

2.1. Retrieval and construction of dataset

2.1.1. Receptor protein and ligand

The three dimensional structure of Human Acetylcholine-esterase complex with FASCICULIN-II (PDB ID 1B41) was obtained from RCSB Protein Databank (<http://www.rcsb.org/pdb/home/home.do>). PDB structure of only Acetyl-cholinesterase was obtained using Pymol²¹ after removing the atomic coordinates of FASCICULIN-II. The structure was visualized in Raswin and UCSF-Chimera 1.6.1 UCSF.^{22,23} Quercetin was chosen as ligand and its structure was firstly retrieved as SDF format from PubChem compound (Table 1) followed by conversion into PDB format to perform molecular docking studies.

2.1.2. Conventional acetylcholine-esterase inhibitors

For detailed general information about the drugs, Drug Bank Database (www.drugbank.ca) was explored by drug names. For further analysis, 3D structure of the drugs was obtained from PubChem (<http://pubchem.ncbi.nlm.nih.gov>) database as SDF format which was later converted to pdb format by Open Babel 2.3.1.²⁴ The structures of the conventional drugs along with their PDB IDs are given in Table 1.

2.2. Molecular properties and drug likeness

Molecular properties and drug likeness of each selected compound was analyzed by Molinspiration WebME Editor 3.81 (<http://molinspiration.com:9080/webme/webme.html>) and Molsoft L.L.C.: Drug-Likeness and molecular property prediction (molsoft.com/mprop/) which predicts the molecular properties from the given valid structures and based on these molecular properties it gives an overall drug likeness score.

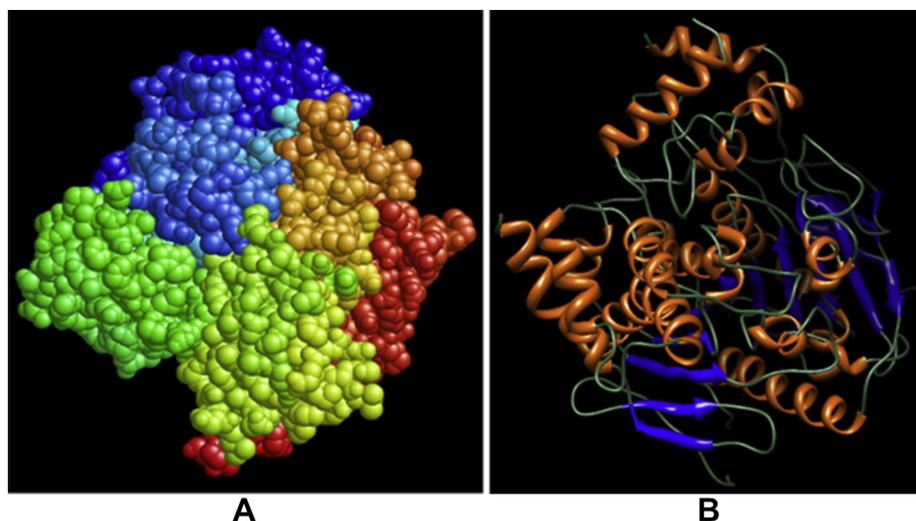


Fig. 1. Structural representation of acetylcholine-esterase. (A) Space-fill representation of 3D structure of acetylcholine-esterase. Different groups on the surface of the protein are highlighted by different color combinations and (B) Helix-loop-helix structural representation of 3-D model of acetylcholine-esterase. α -helices (blue) and β pleated sheets (light yellow) are connected by loops. These images were generated by using Pymol and UCSF-CHIMERA respectively.

2.3. Molecular docking and visualization

Docking studies with conventional inhibitory drugs and quercetin against Acetylcholine esterase were done by AutoDock. Protein and ligands were analyzed and modified for docking purpose using AutoDock Tools which is incorporated in the MGL tools (<http://mgltools.scripps.edu>). The grid box size was set at 44, 60, and 72 Å towards X, Y and Z axis respectively to include all the amino acid residues present in the rigid acetylcholinesterase protein for docking study. Values of X, Y, and Z center were set at 116.077, 103.442 and –143.125 Å respectively and the spacing was 1.000 Å during AutoDock analysis. AutoDock Vina reads the protein and ligand as PDBQT and performs the actual docking on the basis of configuration prepared by AutoDock Tools.²⁵ The output result which was also in PDBQT format was later converted to PDB by using Open Babel 2.3.1 tool. The output files for every conventional drug along with quercetin were visualized by Pymol Molecular Visualization Software. The best conformer for each inhibitor was selected on the basis of lower free energy and lower root mean square value.

2.4. Study of molecular interactions

Molecular interactions, such as like the number of hydrogen bonds, between the inhibitors and acetylcholinesterase were analyzed by Pymol Molecular Visualization system. During these analyses, the cutoff distance of hydrogen bond was set at 3.2.

Table 2
Molecular properties of conventional drugs and quercetin.

Property	Details	Value				
		Rivastigmine	Donepezil	Galantamine	Tacrine	Quercetin
log P	Octanol-water partition coefficient	2.276	4.1	1.987	3.05	1.683
TPSA	Polar surface area	32.781	38.777	41.934	38.915	131.351
N atoms	Number of non-hydrogen atoms	18.0	28.0	22.0	15.0	22.0
MW	Molecular weight	250.342	379.5	301.386	198.269	302.238
n ON	Number of hydrogen-bond acceptors (O and N atoms)	4	4	4	2	7
n OHNH	Number of hydrogen-bond donors (OH and NH groups)	0	0	1	2	5
n violations	Number of rule of 5 violations	0	0	0	0	0
n rotb	Number of rotatable bonds	5	6	1	0	1
Volume	Molecular volume	254.014	367.895	284.431	191.533	240.084

2.5. QSAR analysis

Similar compounds of Quercetin were searched in the SIMCOMP database.²⁶ Among the retrieved compounds, those having similarity score in a range 0.4–1.0 were chosen and their structures were downloaded in SDF format from the PubChem database using their corresponding IDs. Converting these SDF files to PDB, for every compound chosen from SIMCOMP database, a similar molecular docking study was performed as stated above. The result is summarized in [Supplementary Table 1](#). The single modified compound (see [Supplementary Table 1](#)) showing better affinity towards acetylcholine-esterase than quercetin was chosen for further analysis. To identify whether the better binding affinity was specific for modification to special group or it was just a result of any other group interacting in that position, a variety of quercetin structures with groups other than that particular group to the specific atom were prepared. Chlorination, bromination, sulfation and carboxylation were some of the modifications done to the structure. The structures were then made 3D with the help of molecular drawing tools ChemSketch²⁷ and docking studies were performed afterward.

3. Results and discussion

3.1. Comparative analysis of molecular properties reveals drug like property of quercetin

To compare the binding affinity of quercetin with conventional drugs to human acetylcholine esterase enzyme ([Fig. 1](#)), the atomic

Table 3
Drug likeness score for different inhibitors.

Name of inhibitors	Drug likeness score
Donepezil	0.91
Galantamine	0.46
Rivastigmine	0.97
Tacrine	0.97
Quercetin	1.00

Table 4
Binding affinity for different inhibitors calculated when they were docked with acetylcholine-esterase.

Name of inhibitors	Affinity (Kcal/mol)
Donepezil	-7.9
Galantamine	-8.0
Rivastigmine	-8.6
Tacrine	-8.6
Quercetin	-8.8

co-ordinates of the 4 different commercial drugs along with the quercetin were retrieved from the PubChem database. The structural information of these compounds is summarized in Table 1. Molecular properties of quercetin in comparison to the conventional drugs, predicted by Molinspiration WebME Editor Tool (Table 2), show that the number of hydrogen bond donors and acceptors in quercetin outnumbers that of all the conventional drugs (tacrine, galantamine, donepezil and rivastigmine). Specifically quercetin has 7 hydrogen bond acceptors and 5 hydrogen bond donors (Table 2) in its structure according to this prediction. Besides the status of hydrogen bonds, it has a molecular weight of 302.238 and a log *P* value 1.683 (Table 2) which imply that similar to other conventional drugs, quercetin does not violate the rule of Lipinski's "rule of five"²⁸ which is an important criterion to predict a compound as a potential drug molecule to act in the biological system. Lipinski's "rule of five" molecules having molecular weight > 500, log *P* > 5, hydrogen bond acceptors > 10 and hydrogen bond donors > 5 show poor absorption or permeation rate. The volume and molecular weight of quercetin is moderate compared to the

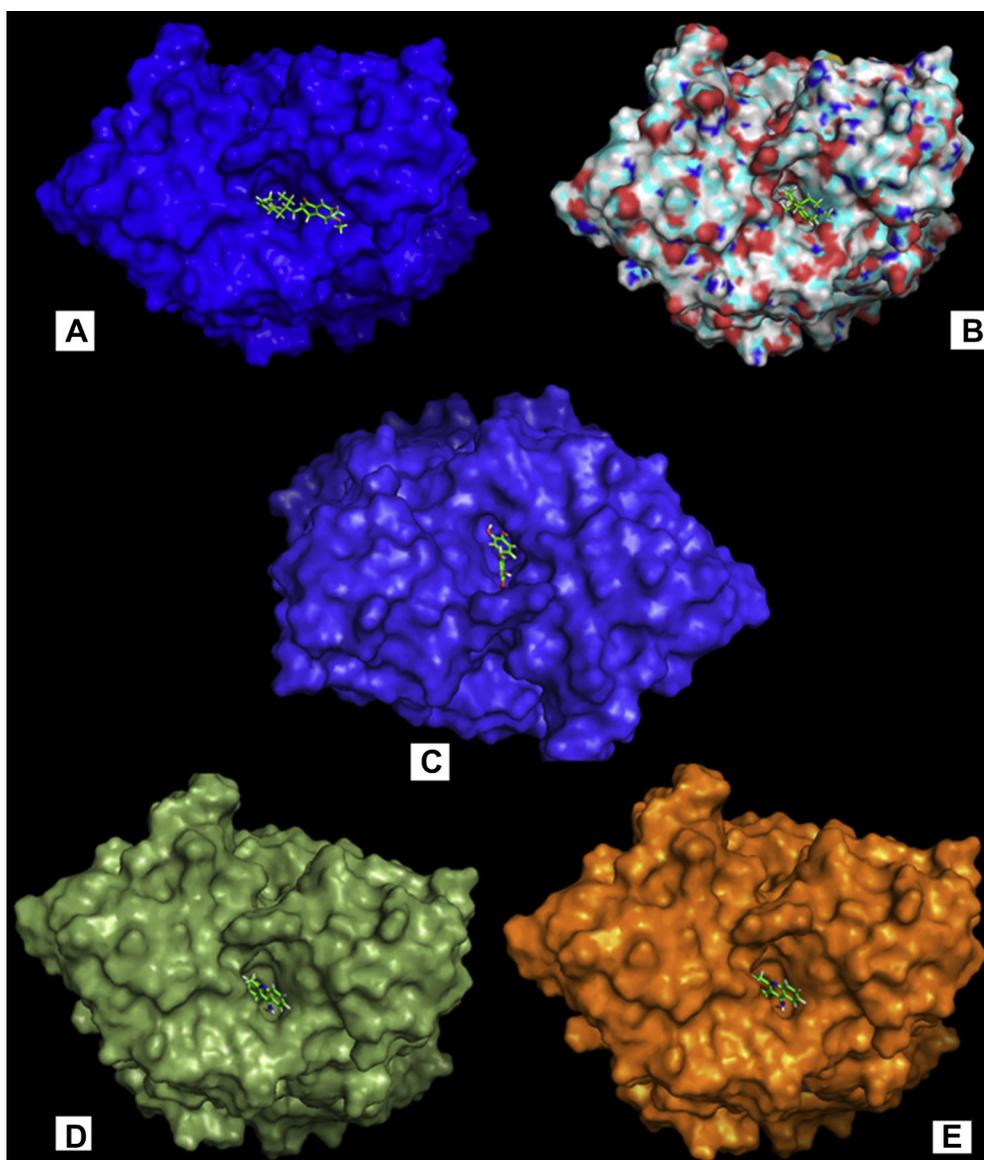


Fig. 2. Molecular docking of acetylcholinesterase inhibitors (A–E) represent the binding of donepezil, galantamine, quercetin, rivastigmine, and tacrine respectively in the same pocket on the surface of acetylcholinesterase protein. The surface of the protein is highlighted by different colors for each case.

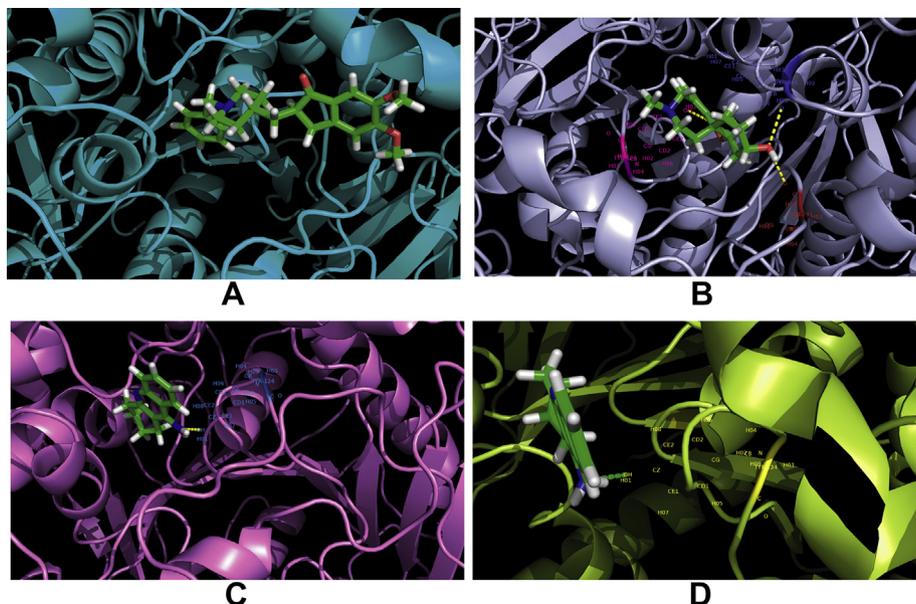


Fig. 3. Docked chemical compounds in the acetylcholinesterase pocket. (A) Acetylcholinesterase-donepezil bound form. No hydrogen bond formed in the hydrogen bond parameter used. (B) Galantamine-acetylcholinesterase complex. Yellow dashes indicate hydrogen bonds formed between the enzyme and the drug molecule. Amino acid residues at Tyr 124, Tyr 341 and Ser 293 of acetylcholinesterase participate in these intermolecular interactions and they are labeled along with their atoms. (C) Rivastigmine-acetylcholinesterase complex. Yellow dash indicates the hydrogen bond between the drug molecule and Tyr 124 residue of acetylcholinesterase. All the atoms of Tyr 124 are labeled in the diagram. (D) Tacrine-acetylcholinesterase complex. Green dash indicates a single hydrogen bond between the drug compound and enzyme. The distance of the hydrogen bond measured is 3.0.

conventional drugs used (Table 2). But interestingly it has a polar surface area of 131.351 which is higher than any other conventional drugs used. A detail of other properties like octanol–water partition coefficient, number of non-hydrogen atoms, and the number of rotatable bonds is summarized in Table 2.

3.2. High drug likeness score of quercetin

Overall drug likeness score for drug molecules constitute a distribution that is skewed to right and peaks in the range of 0.8–1.2 on a drug likeness score.^{29–33} In this study, drug likeness score

for all of the conventional drugs was predicted by Molsoft L.L.C.: Drug-Likeness and molecular property prediction tool. The result is summarized in Table 3. Interestingly, the natural compound quercetin has the highest drug likeness score (1.0) while the FDA approved drug tacrine has a value of 0.97 according to the prediction (Table 3).

3.3. Molecular docking shows higher binding affinity of quercetin

Natural compound quercetin along with other conventional inhibitors of acetylcholinesterase was allowed to bind at the

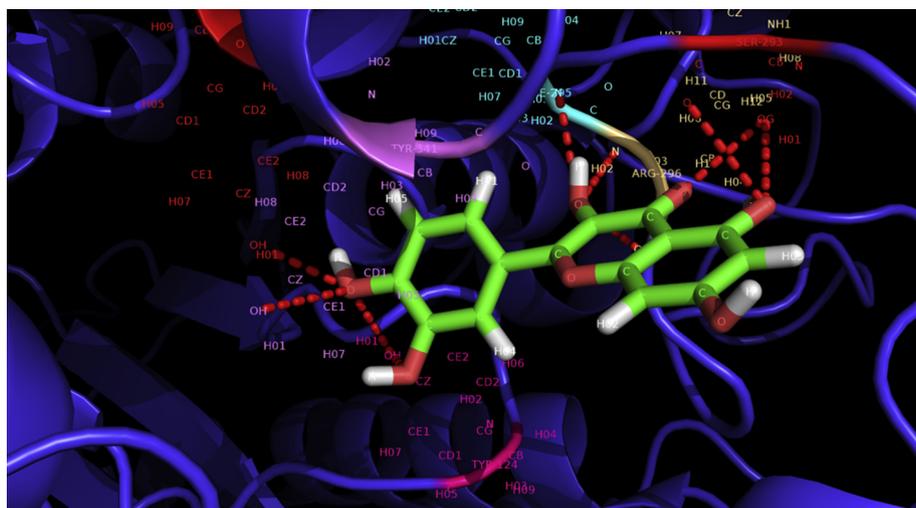


Fig. 4. Deep view at the Quercetin-acetylcholinesterase complex. Different atoms of the quercetin structure participate in a number of hydrogen bonds (highlighted in red dashes) formed with acetylcholinesterase which have been labeled. Tyr 124, Ser 293, Phe 295, Arg 296 and Tyr 341 are the participated amino acid residues of the enzyme to form ten hydrogen bonds which outnumber the interactions for other (e.g. tacrine, donepezil, galantamine, and rivastigmine) drug–enzyme complex. This figure clearly implies the strong binding of Quercetin in the active pocket of acetylcholinesterase. This image has been developed using Pymol Molecular Visualization system.

Table 5
Detail of molecular interactions of different inhibitors with acetylcholine-esterase.

Ligand–receptor complex	No. of hydrogen bonds	Hydrogen bond donor	Hydrogen bond acceptor	Length of hydrogen bond (Å)
Quercetin–acetylcholine-esterase	10	Quercetin: 1: O	Acetyl-cholinesterase: A:Tyr 124: OH	2.8
		Quercetin: 1: O	Acetyl-cholinesterase: A:Tyr 341: OH	3.2
		Quercetin: 1: O	Acetyl-cholinesterase: A:Tyr 337: HO1	2.7
		Quercetin: 1: H	Acetyl-cholinesterase: A: Phe 295: N	3.0
		Quercetin: 1: O	Acetyl-cholinesterase: A: Arg 296: N	2.8
		Quercetin: 1: O	Acetyl-cholinesterase: A: Ser 293: OG	2.7
		Acetyl-cholinesterase: A: Tyr 124: OH	Quercetin: 1: O	2.9
		Acetyl-cholinesterase: A: Ser 293: OG	Quercetin: 1: O	2.9
		Acetyl-cholinesterase: A: Arg 296: O	Quercetin: 1: O	2.7
		Quercetin: 1: H	Acetyl-cholinesterase: A: Ser 293: OG	2.9
Rivastigmine–acetylcholine-esterase	1	Rivastigmine: 1: O	Acetyl-cholinesterase: A:Tyr 124: OH	3.0
Tacrine–acetylcholine-esterase	1	Tacrine: 1: O	Acetyl-cholinesterase: A:Tyr 124: OH	3.0
Galantamine–acetylcholine-esterase	3	Acetyl-cholinesterase: A:Tyr 124: OH	Galantamine: 1: O	3.0
		Galantamine: 1: OH	Acetyl-cholinesterase: A:Tyr 341: O	2.8
		Galantamine: 1: OH	Acetyl-cholinesterase: A: Ser 293: O	3.0
Donepezil–acetylcholine-esterase	0			

active site using same parameter as mentioned in method section 2.3. The binding score of these compounds is summarized in Table 4. Quercetin has higher binding score than the conventional drugs when allowed to bind at the same pocket of the acetylcholine-esterase as conventional drugs. The higher binding energy (–8.8 kcal/mol) of quercetin at the active site of acetylcholine-esterase potentiates its ability to act as an inhibitor against this protein. Tacrine and galantamine show a closer score (–8.6 kcal/mol each) to quercetin in case of binding at the active site of acetylcholine-esterase, while the conventional drugs donepezil and rivastigmine show lower binding energy of –7.6 kcal/mol and –8.0 kcal/mol respectively. The binding of the conventional drugs and the natural compound quercetin on the surface of the acetylcholine-esterase is illustrated in Fig. 2. The surface of the protein is colored differently for each of the inhibitor to distinguish one from another. This figure shows that all of the docked inhibitors (including quercetin) bind at the same active site pocket of acetylcholine-esterase.

3.4. Molecular interactions between acetylcholine-esterase and docked ligands

While binding at the active site, a number of hydrogen bonds between different atoms of acetylcholine-esterase and the inhibitors participate to interact with each other to make the binding

stronger. But an exception is found for donepezil that has no hydrogen bond to interact with the acetylcholine-esterase. Moreover, tacrine and rivastigmine have only one hydrogen bond and they both interact with Tyr 124 amino acid residue of acetylcholine-esterase. Galantamine forms a moderate number of hydrogen bonds with acetylcholine-esterase that involves Tyr 124, Ser 293, Tyr 341 residues of this protein (Fig. 3). While the conventional drugs form fewer to moderate number of hydrogen bonds with active site residues of acetylcholine-esterase, the natural compound quercetin forms a higher number of hydrogen bonds.

When the same parameter used for other inhibitors, quercetin forms ten hydrogen bonds with active site residues of the protein (Fig. 4). The hydrogen bonds formed by quercetin involve the same amino acid residues Tyr 124, Ser 293 and Tyr 341 as for conventional drugs (galantamine, tacrine, rivastigmine). Moreover, different atoms of other amino acid residues such as Phe 295, Arg 296, Tyr 337 and Tyr 341 participate to form the hydrogen bonds. A detail about the hydrogen bond acceptors and donors and length of these hydrogen bonds for all the inhibitors is summarized in Table 5. Interactive views (Figs. 3 and 4) at the active site of the acetylcholine esterase for different inhibitors show the hydrogen bonds (dashed line) binding to different atoms of the amino acid residues in the active site (highlighted with different colors) of the protein. The parameter used (see the methods and material) for hydrogen bond visualization was same for all the inhibitors.

3.5. Quantitative structural activity relationships

The structures retrieved on the basis of similarity score via SIMCOMP search tool were all docked to the target molecule. The grid/search space for these molecules to perform molecular docking was kept the same as for the inhibitors mentioned earlier (see methods and materials). The name of the retrieved structures, their similarity score and binding score at the active site of acetylcholine-esterase is summarized in Supplementary Table 1. Among all of the compounds selected for docking at the active site of acetylcholine-

Table 6
Modifying groups for tacrine and quercetin and their normalized log values.

Modifying group	Log of tacrine values	Log of quercetin value
–Br	0.0086002	–0.0315171
–Cl	0.0043214	–0.0506100
–CH ₃	0.0170333	–0.0177288
–NO ₂	–0.0087739	–0.0757207
–SO ₃ H	0.0000001	–0.0555173

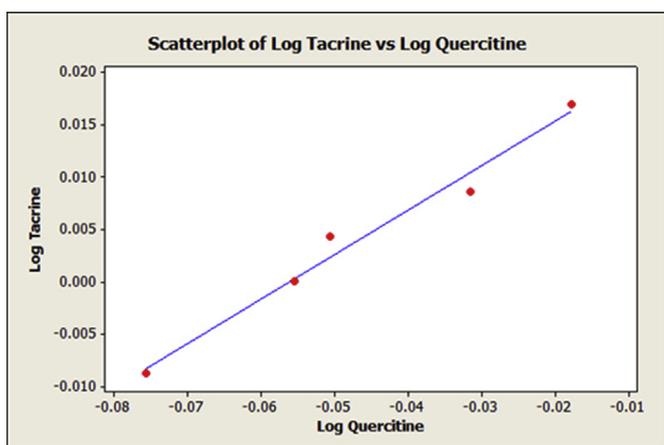


Fig. 5. Scatter plot of Log of different group attached Quercetin versus corresponding Log of the identical group attached Tacrine. The scatter plot shows a liner pattern.

esterase, azaleatin (better known as 4-OH methylated form of quercetin) shows a higher binding energy (−9.0 kcal/mol) than quercetin. To identify whether the better binding affinity was specific for methylation or it was just a result of any other group interacting in that position, a variety of quercetin structure with groups other than methyl attached to the O-4 atom were prepared. Chlorination, bromination, sulfation and carboxylation were some of the modifications done to the structure. The structures were then made 3D with the help of molecular drawing tools ChemSketch.

To carry out a Quantitative Structural Activity Relationships (QSAR) on the basis of binding score we had to set a reference structure for comparing binding scores. Among conventional drugs, tacrine was chosen as the reference molecule. Different groups were added to tacrine structure just as in for quercetin. For both conventional drug and quercetin the binding value for different groups were normalized by dividing the values with that of the binding value of the molecule without any groups added.

A scatter plot of the logarithm of normalized binding values of quercetin and tacrine was generated (Fig. 5). The expected result for the scatter plot was a liner graph with methylation showing optimum scores. The results found in the scatter plot meet the expectation to a good extent.

The scatter plot reveals the correlation between the values from two candidates was positive. The modifications caused similar changes to the binding values to both the parent candidates (Fig. 5). From the scores in Table 6 it is evident that the distant most point from the origin belongs to methylated structure. This predicts that the methylated form of quercetin can improve this molecule as drug molecule.

4. Conclusion

In overall analysis, docking techniques have predicted quercetin to act as a better natural drug molecule than current commercial ones. Quercetin fulfills all the natural prerequisites of a drug molecule- good binding affinity with the receptor, good absorptivity and low toxicity. The study employs docking technique to reaffirm the ability of the molecule as a drug and implies possible chemical modification in quercetin structure- a methylation at 4-OH position – to further improve the binding affinity. Although due to more hydrogen bond with the target molecule our structure was more stable, more hydrogen atoms add to the polarity (lower log *P*, more Hydrogen bond atom and greater polar surface area) of the structure and hence it is very critical to check the excretion and intrusion property inside the cell. This can only be answered by *in-vitro* ADMETox experimentation. Our *in silico* study has conclusively predicted the superiority of the natural compound quercetin over the conventional drugs as AchE inhibitor and it sets the need for further *in-vitro* study of this compound in future.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jyp.2013.11.005>.

Conflicts of interest

All authors have none to declare.

References

1. Querfurth HW, LaFerla FM. Mechanisms of disease. *N Engl J Med*. 2010;362:329–344.

2. Martins IJ, Hone E, Foster JK, et al. *Apolipoprotein E*, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease. *Mol Psychiatry*. 2006;11:721–736.
3. Priyadarshini M, Kamal MA, Greig NH, et al. Alzheimer's disease and type 2 diabetes: exploring the association to obesity and tyrosine Hydroxylase (Formerly Current Drug Targets *CNS Neurol Disord Drug Targets*. 2012;11:482–489.
4. Fewlass DC, Noboa K, Pi-Sunyer FX, Johnston JM, Yan SD, Tezapsidis N. Obesity-related leptin regulates Alzheimer's A β . *FASEB J*. 2004;18:1870–1878.
5. Profenno LA, Porsteinsson AP, Faraone SV. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. *Biol Psychiatry*. 2010;67:505–512.
6. Wilson RS, Arnold SE, Schneider JA, Kelly JF, Tang Y, Bennett DA. Chronic psychological distress and risk of Alzheimer's disease in old age. *Neuroepidemiology*. 2006;27:143–153.
7. 2012 Alzheimer's disease facts and figures. The Journal of The Alzheimer's Association *Alzheimer's Dement*. 2012;8:131–168.
8. Gómez-Isla T, Hollister R, West H, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol*. 1997;41:17–24.
9. Buckingham SD, Jones AK, Brown LA, Sattelle DB. Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection. *Pharmacol Rev*. 2009;61:39–61.
10. Babic T. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J Neurol Neurosurg Psychiatr*. 1999;67:558.
11. Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. *Behav Brain Res*. 2011;221:555.
12. Butterfield DA, Drake J, Pocernich C, Castegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid β -peptide. *Trends Mol Med*. 2001;7:548–554.
13. Meyer-Luehmann M, Spires-Jones TL, Prada C, et al. Rapid appearance and local toxicity of amyloid- β plaques in a mouse model of Alzheimer's disease. *Nature*. 2008;451:720–724.
14. Evans JG, Wilcock G, Birks J. Evidence-based pharmacotherapy of Alzheimer's disease. *Int J Neuropsychopharmacol*. 2004;7:351–369.
15. Gilgun-Sherki Y, Melamed E, Offen D. Antioxidant treatment in Alzheimer's disease. *J Mol Neurosci*. 2003;21:1–11.
16. Aguglia E, Onor ML, Saina M, Maso E. An open-label, comparative study of rivastigmine, donepezil and galantamine in a real-world setting. *Curr Med Res Opin*. 2004;20:1747–1752.
17. Ritchie CW, Ames D, Clayton T, Lai R. Metaanalysis of randomized trials of the efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer disease. *Am J Geriatr Psychiatry*. 2004;12:358.
18. Jeremy PR, Rigby John, Adam CH, Rowland Rosalind. Cardiovascular monitoring with acetylcholinesterase inhibitors: a clinical protocol. *Adv Psychiatr Treat*. 2007;13:178–184.
19. Kröger E, Berkers M, Carmichael PH, Souverein P, van Marum R, Egberts T. Use of rivastigmine or galantamine and risk of adverse cardiac events: a database study from the Netherlands. *Am J Geriatric Pharmacother*. 2012;10:373–380.
20. Jung M, Park M. Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. *Molecules*. 2007;12:2130–2139.
21. DeLano WL. *The PyMOL Molecular Graphics System*. 2002. <http://www.pymol.org/>: DeLano Scientific.
22. Sayle RA, Milner-White EJ. RASMOL: biomolecular graphics for all. *Trends Biochem Sci*. 1995;20:374.
23. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem*. 2004;25:1605–1612.
24. O'Boyle NM, Banck M, James CA, et al. Open Babel: an open chemical toolbox. *J Cheminformatics*. 2011;3:1–14.
25. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31:455–461.
26. Hattori M, Tanaka N, Kanehisa M, Goto S. SIMCOMP/SUBCOMP: chemical structure search servers for network analyses. *Nucleic Acids Res*. 2010;38(suppl 2):W652–W656.
27. Fan Y, Cheng M, Cheng Y. The reformation in our organic chemistry classes caused by the excellent software ACD/ChemSketch [J] (Natural Science Edition) *J Guangzhou Univ*. 2002;6.
28. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol Toxicol Methods*. 2000;44:235–249.
29. Egan WJ, Walters WP, Murcko MA. Guiding molecules towards drug-likeness. *Curr Opin Drug Discov Devel*. 2002;5:540–549.
30. Brüstle M, Beck B, Schindler T, King W, Mitchell T, Clark T. Descriptors, physical properties, and drug-likeness. *J Med Chem*. 2002;45:3345–3355.
31. Walters WP, Murcko MA. Prediction of 'drug-likeness'. *Adv Drug Deliv Rev*. 2002;54:255–271.
32. Clark DE, Pickett SD. Computational methods for the prediction of 'drug-likeness'. *Drug Discov Today*. 2000;5:49–58.
33. Müller KR, Rätsch G, Sonnenburg S, Mika S, Grimm M, Heinrich N. Classifying 'drug-likeness' with kernel-based learning methods. *J Chem Inf Model*. 2005;45:249–253.