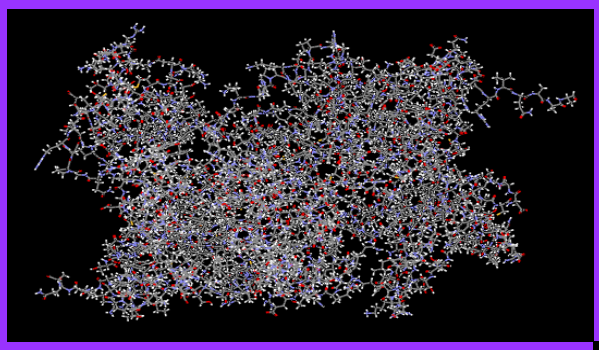

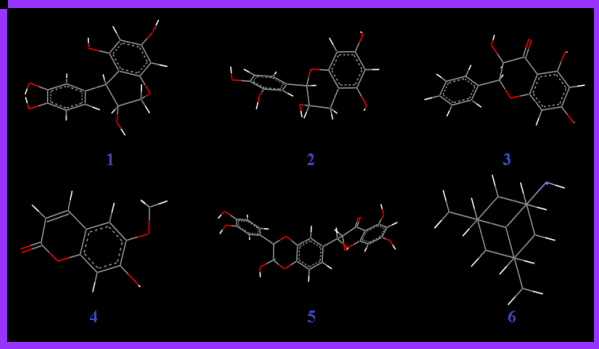

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Computational drug discovery of potential TAU protein kinase I inhibitors using *in silico* docking studies

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Abstract

The objective of the current study is to evaluate the tau protein kinase I inhibitory activity of flavonoids using *in silico* docking studies. *In silico* docking studies were carried out using AutoDock 4.2, based on the Lamarckian genetic algorithm principle. Memantine, a known neuroreceptor antagonist is currently used in the treatment of Alzheimer's disease. The results showed that all the selected flavonoids showed binding energy ranging between -7.1 to -4.9 kcal/mol when compared with that of the standard (-5.9 kcal/mol). Inhibition constant (6.6 to 280.1 µM) and intermolecular energy (-9.5 to -6.6 kcal/mol) of the ligands also coincide with the binding energy. These molecular docking analyses could lead to the further development of potent tau protein kinase I inhibitors for the treatment of Alzheimer's disease. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of Alzheimer's disease.

Introduction

Rational drug design is the inventive process of finding new medications based on the knowledge of the biological target. Drug research aims at the development of a novel therapeutic agent which is done by designing molecules that are complementary in shape and charge to the biomolecular target with which they interact and bind. Drug discovery and develop-ment is an intense, lengthy and an interdisciplinary endeavor. It is a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical *in vitro* and *in vivo* studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development (Ekins et al., 2005).

AutoDock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Collignon et al., 2011). Its default search function is based on Lamarckian Genetic Algorithm (LGA), a hybrid genetic algorithm with local optimization that uses a parameterized free-energy scoring function to estimate the binding energy (Madeswaran et al., 2012).

Tau proteins belong to the family of microtubule-associated proteins. Majorly expressed in neurons where it has an important role in the assembly of tubulin monomers into microtubules to make up the neuronal microtubules network (Billingsley and Kincaid, 1997). Tau is a phosphoprotein with 79 potential serine (Ser) and threonine (Thr) phosphorylation sites on the longest tau isoform. Phosphorylation has been reported on approximately 30 of these sites in normal tau proteins. Microtubules are involved in maintaining the cell shape and serve as tracks for axonal transport (Shin et al., 1991).

Tau proteins are the major constituents of intraneuronal and glial fibrillar lesions described in Alzheimer's disease and numerous neurodegenerative disorders referred to as 'tauopathies'. A direct correlation has been

recognized between the progressive involvement of the neocortical areas and the increasing severity of dementia, suggesting that pathological tau proteins are reliable marker of the neurodegenerative process (Galluzzo et al., 2006; Buee et al., 2000). Memantine, a known neuro-receptor antagonist is currently used in the treatment of Alzheimer's disease. Therefore, memantine is used as a standard for the current docking study.

Flavonoids are a large group of non-nutrient compounds naturally obtained from plants as part of their protective mechanisms against stresses of various origins. They emerged from being measured an agricultural oddity only after it was monitored that these compounds possess a potential defensive function against several human degenerative diseases (Budakoti et al., 2009). Flavonoids possess various biological activities like, anti-amoebic activity, anti-inflammatory, anti-coagulant, anti-cancer, anti-oxidants and anti-spasmodic (Aung et al., 2011; Beker et al., 2011; Chassany et al., 2007; Formica and Regelson, 1995).

Pharmacological treatments for neurodegenerative disorders are symptomatic and do not change the progression of the neurodegenerative disorders. These treatments are less satisfactory and may direct to serious side effects. Hence, the goal of current research is to develop potential compounds that could inhibit the tau protein and thereby it can be used for the treatment for neurodegenerative diseases.

Materials and Methods

Software required

Python 2.7-language was downloaded from www.python.com, Cygwin (a data storage) c:\program and Python 2.5 were simultaneously down-loaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and AutoDock 4.2 was down-loaded from www.scripps.edu, Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com, Molecular orbital package (MOPAC), ChemSketch was down-loaded from www.acdlabs.com. Online smiles translation was carried out using cactus.nci.nih.gov/translate/.

Docking methodology

We employed the Lamarckian genetic algorithm (LGA) for ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm. This algorithm first builds a population of individuals (genes), each being a different random conformation of the docked molecule. Each individual is then mutated to acquire a slightly different translation and rotation and the local search algorithm then performs energy minimizations on a user-specified proportion of the population of individuals. The individuals with the low

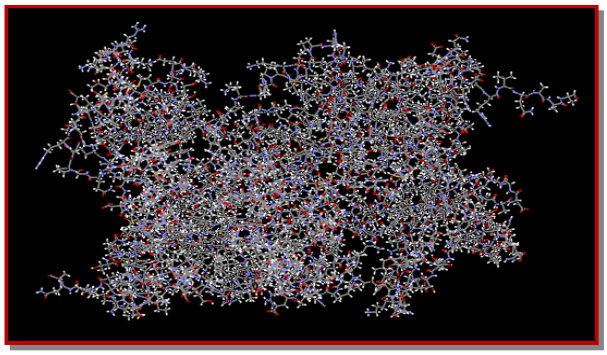

Figure 1: The refined structure of tau protein kinase I enzyme

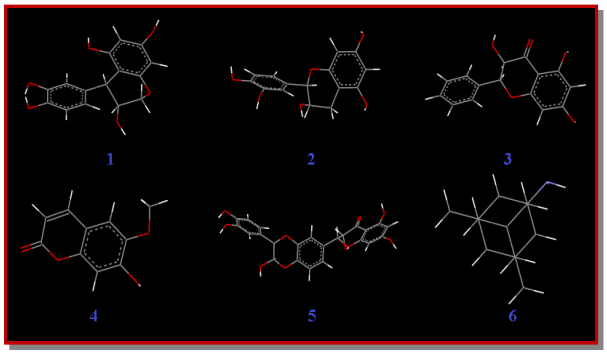

Figure 2: The optimized ligand molecules (1 acacatechin, 2 catechin, 3 galangin, 4 scopoletin, 5 silbinin, and 6 memantine)

resulting energy are transferred to the next generation and the process is then repeated. The algorithm is called Lamarckian because every new generation of individuals is allowed to inherit the local search adaptations of their parents.

An extended PDB format, termed as PDBQT file was used for coordinate files which includes atomic partial charges. AutoDock Tools was used for creating PDBQT files from traditional PDB files (Khairallah et al., 2008). Binary complex structure of tau protein kinase I enzyme (1J1C) was downloaded from the Brookhaeven protein data bank (Figure 1).

The flavonoid ligands like acacatechin, catechin, galangin, scopoletin, silbinin and memantine (Figure 2)were built using ChemSketch and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies (Bikadi and Hazai, 2009).

Lead optimization of the selected compounds was done by computation of druglikeness properties. The druglikeness scores of the compounds were evaluated with the help of Lipinski's rule. The various parameters of the ligands like molecular formula, molecular weight, aromatic carbons, rotatable bonds and no. of torsions were tabulated in Table I.

The preparation of the target protein 1J1C with the AutoDock Tools software involved adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Gas-

Table I

Ligand parameters

	Molecular formula	Molecular weight	Aromatic carbons	Rotatable bonds	No. of torsions
Acacatechin	$C_{15}H_{14}O_6$	290.277	12	6	6
Catechin	$C_{15}H_{14}O_6$	290.277	12	6	6
Galangin	$C_{15}H_{12}O_5$	272.261	12	4	4
Scopoletin	$C_{10}H_8O_4$	192.174	9	2	2
Silbinin	$C_{23}H_{18}O_{10}$	454.397	18	8	8
Memantine	$C_{12}H_{21}N$	179.300	19	1	1

teiger charges are calculated for each atom of the macromolecule in AutoDock 4.2 instead of Kollman charges which were used in the previous versions of this program. Three-dimensional affinity grids of size 277 × 277 × 277 Å with 0.6 Å spacing were centered on the geometric center of the target protein and were calculated for each of the following atom types: HD, C, A, N, OA, and SA, representing all possible atom types in a protein. Additionally, an electrostatic map and a desolvation map were also calculated (Konc et al., 2011).

Rapid energy evaluation was achieved by precalculating atomic affinity potentials for each atom in the ligand molecule. In the AutoGrid procedure, the target enzyme was embedded on a three dimensional grid point. The energy of interaction of each atom in the ligand was encountered.

We have selected important docking parameters for the LGA as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on an individual in the population was set to 0.06 (Madeswaran et al., 2013).

AutoDock was run several times to get various docked conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates. AutoDock Tools provide various methods to analyze the results of docking simulations such as, conformational similarity, visualizing the binding site and its energy and other parameters like intermolecular energy and inhibition constant. For each ligand, 10 best poses were generated and scored using AutoDock 4.2 scoring functions (Umamaheswari et al., 2011).

Results and Discussion

In silico docking study, was carried out to identify the inhibiting potential of selected flavonoids against tau protein kinase I enzyme. In this study 5 different flavornoids were selected for the *in silico* docking studies. The docking studies were performed by the use of AutoDock 4.2. In the docking studies, if a compound shows lesser binding energy compared to the standard it proves that the compound has higher activity (Chang et al., 2010).

Hyperphosphorylation of the tau protein result in the self-assembly of tangles of paired helical filaments and straight filaments, which are involved in the pathogenesis of Alzheimer's disease and other tauopathies (Alonso et al., 2001). Recent research suggests that tau protein may be released extracellularly by an exosome based mechanism in Alzheimer's disease. Some aspects of how the disease functions also suggest that it has some similarities to prion proteins (Hall and Patuto, 2012).

Flavonoids are the excellent anti-oxidants when compared to other compounds. Extracts from onion and different flavonoids activate the cellular anti-oxidant system. Analysis of the receptor/ligand complex models generated after successful docking of the flavornoids was based on the various parameters such as hydrogen bond interactions, binding energy, inhibition constant and orientation of the docked compound within the active site (Madeswaran et al., 2013).

Binding energy of the individual compounds were calculated using the following formula:

Binding energy = A + B + C - D

Where, A denotes final intermolecular energy + van der Walls energy (vdW) + hydrogen bonds + desolvation energy + electrostatic energy (kcal/mol), B denotes final total internal energy (kcal/mol), C denotes torsional free energy (kcal/mol), D denotes unbound system's energy (kcal/mol)

Flavonoids showed binding energy ranging between -7.1 to -4.9 kcal/mol (Table II). Silbinin showed better binding energy -7.1 kcal/mol than the standard memantine (-5.9 kcal/mol). All the selected flavonoids had showed binding energy compared to that of standard. This proves that flavonoids consist of potential tau protein kinase I inhibitory binding sites similar to that of the standard.

Table II

Binding energies of the compounds based on their rank

Compounds	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Acacatechin	-6.6	-6.6	-6.3	-5.9	-6.2	-6.2	-6.1	-5.9	-5.7	-5.6
Catechin	-7.6	-7.6	-7.5	-7.3	-6.9	-7.2	-7.2	-6.8	-6.4	-6.3
Galangin	-7.7	-7.2	-6.9	-7.1	-6.8	-6.7	-6.6	-6.4	-6.3	-6.2
Scopoletin	-6.0	-6.0	-5.9	-5.9	-5.9	-5.8	-5.8	-5.4	-5.3	-5.2
Silbinin	-9.5	-8.6	-8.3	-7.6	-7.5	-7.2	-7.2	-7.1	-6.9	-6.6
Memantine	-6.2	-6.0	-6.0	-6.0	-5.9	-5.9	-5.9	-5.9	-5.9	-5.8

Table III

Inhibition constant of the compounds based on their rank

Compounds	Inhibition constant of the compounds based on their rank (μM, mM[a])									
	1	2	3	4	5	6	7	8	9	10
Acacatechin	280.1	302.5	533.7	1.0[a]	566.2	628.8	739.0	919.0	1.4[a]	1.6[a]
Catechin	51.1	52.4	67.2	97.2	190.3	100.6	116.0	229.1	419.3	488.0
Galangin	17.4	43.2	67.4	43.6	84.8	87.1	111.0	147.3	169.9	217.3
Scopoletin	104.8	105.1	124.3	129.4	132.0	163.2	164.1	283.0	338.3	394.0
Silbinin	6.6	29.4	48.3	156.3	181.1	296.7	312.2	329.2	473.3	797.7
Memantine	48.2	64.2	66.4	69.0	85.4	76.0	79.2	79.5	84.5	96.5

Table IV

Intermolecular energies of the compounds based on their rank

Compounds	Binding energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Acacatechin	-4.9	-4.8	-4.5	-4.1	-4.4	-4.4	-4.3	-4.1	-3.9	-3.8
Catechin	-5.9	-5.8	-5.7	-5.5	-5.1	-5.5	-5.4	-5.0	-4.6	-4.5
Galangin	-6.5	-6.0	-5.7	-6.0	-5.6	-5.5	-5.4	-5.2	-5.1	-5.0
Scopoletin	-5.4	-5.4	-5.3	-5.3	-5.3	-5.2	-5.2	-4.8	-4.7	-4.6
Silbinin	-7.1	-6.2	-5.9	-5.2	-5.1	-4.8	-4.8	-4.8	-4.5	-4.2
Memantine	-5.9	-5.7	-5.7	-5.7	-5.6	-5.6	-5.6	-5.6	-5.6	-5.5

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. Inhibition constant is directly proportional to binding energy. Flavonoids showed inhibition constant ranging from 6.6 to 280.1 μM (Table III). Silbinin showed excellent inhibition constant 6.6 μM than the standard memantine (48.2 μM). All the selected compounds had lesser inhibition constant when compared to the standard. Thus, the potential tau protein kinase I inhibitory activity of the flavonoids were compared with the memantine.

Intermolecular energy is also directly proportional to binding energy. Flavonoids showed intermolecular energy ranging between -9. to -6.6 kcal/mol which was lesser when compared to the standard (-6.2 kcal/mol; (Table IV). We found a decrease in intermolecular energy of all the selected compounds with a simultaneous decrease in the binding energy. This result further proved the tau protein kinase I inhibitory activity of all the selected flavonoids.

Based on the docking studies, thetau protein kinase I inhibitory activity of the selected compounds was found to be decreased in the order of silbinin, galangin, memantine, galangin, scopoletin and acacatechin. On the basis of the above study, silbinin and galangin possess potential tau protein kinase I inhibitory binding sites similar to that of the standard. This may be attributed due to the differences in the position of the functional groups in the compounds.

Conclusion

These results clearly indicate that from the selected flavonoids, silbinin and galangin have better binding sites and interactions with tau protein kinase I enzyme and further investigations are necessary to develop potential chemical entity for the prevention and treatment of Alzheimer's disease.

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Conflict of Interest

Authors declare no conflict of interest

References

Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci USA. 2001; 98: 6923-28.

Aung HT, Furukawa T, Nikai T, Niwa M, Takaya Y. Contribution of cinnamic acid analogues in rosmarinic acid to inhibition of snake venom induced hemorrhage. Bioorg Med Chem. 2011; 19: 2392-96.

Beker BY, Bakir T, Sonmezoglu I, Imer F, Apak R. Anti-oxidant protective effect of flavonoids on linoleic acid peroxidation induced by copper (II)/ascorbic acid system. Chem Phys Lipids. 2011; 164: 732-39.

Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform. 2009; 1: 15.

Billingsley ML, Kincaid RL. Regulated phosphorylation and dephosphorylation of tau protein: Effects on microtubule interaction, intracellular trafficking and neurodegeneration. Biochem J. 1997; 323: 577-91.

Budakoti A, Bhat AR, Azam A. Synthesis of new 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1,3-thiazolino[5,4-b]quinoxaline derivatives and evaluation of their antiamoebic activity. Eur J Med Chem. 2009; 44: 1317-25.

Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Rev. 2000; 33: 95-130.

Chang MW, Ayeni C, Breuer S, Torbett BE. Virtual screening for HIV protease inhibitors: A comparison of AutoDock 4 and vina. PLoS One. 2010; 5: 119-55.

Chassany O, Bonaz B, Bruley DES Varannes S, Bueno L, Cargill G, Coffin B, Ducrotté P, Grangé V. Acute exacerbation of pain in irritable bowel syndrome: Efficacy of phloroglucinol and trimethyl phloroglucinol. A randomi-zed, double-blind, placebo-controlled study. Alimen Pharmacol Ther. 2007; 25: 1115-23.

Collignon B, Schulz R, Smith JC, Baudry J. Task-parallel message passing interface implementation of AutoDock 4 for docking of very large databases of compounds using high-performance super-computers. J Comput Chem. 2011; 32: 1202-09.

Ekins S, Nikolsky Y, Nikolskaya T. Techniques: Applications of systems biology to absorption, distribution, metabolism, excretion and toxicity. Trends Pharmacol Sci. 2005; 26: 202-09.

Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol. 1995; 33: 1061-80.

Galluzzo P, Marino M. Nutritional flavonoids impact on nuclear and extranuclear estrogen receptor activities. Genes Nutr. 2006; 1: 161-76.

Hall GF, Patuto BA. Is tau ready for admission to the prion club? Prion 2012; 6: 223-33.

Khairallah M, Khairallah RJ, Young ME, Allen BG, Gillis MA, Danialou G, Deschepper CF, Petrof BJ, Des Rosiers C. Sildenafil and cardio-myocyte-specific cGMP signaling prevent cardiomyopathic changes associated with dystrophin deficiency. Proc Nat Acad Sci. 2008; 105: 7028-33.

Konc J, Konc JT, Penca M, Janezic D. Binding-sites prediction assisting protein-protein docking. Acta Chim Slov. 2011; 58: 396-401.

Madeswaran A, Umamaheswari M, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. *In silico* docking studies of lipoxygenase inhibitory activity of commercially available flavonoids. Orient Pharm Exp Med. 2012; 12: 157-61.

Madeswaran A, Umamaheswari M, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. Docking studies of aldose reductase inhibitory activity of commercially available flavonoids. Int J Biomed Pharmaceut Sci. 2013; 7: 7-11.

Shin RW, Iwaki T, Kitamoto T, Tateishi J. Hydrated autoclave pretreatment enhances tau immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues. Lab Invest. 1991; 64: 693-702.

Umamaheswari M, Madeswaran A, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. Study of potential xanthine oxidase inhibitors: *In silico* and *in vitro* biological activity biological activity biological activity biological activity. Bangladesh J Pharmacol 2011; 6: 117-23.

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