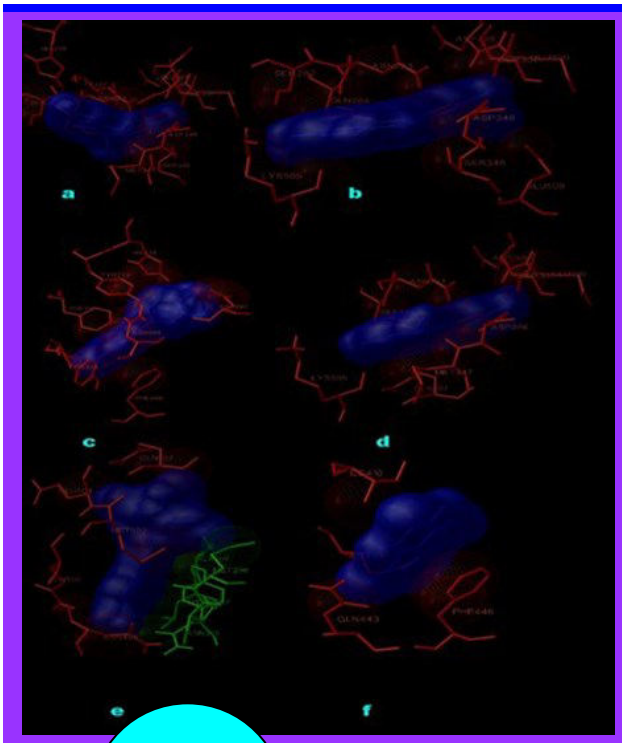

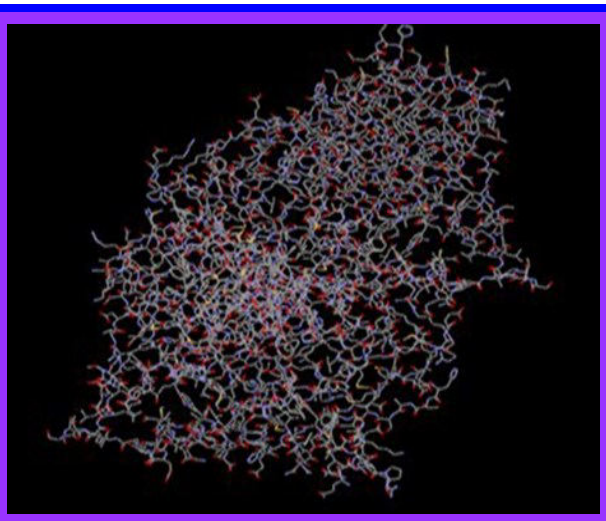

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Docking studies: *In silico* phosphodiesterase inhibitory activity of commercially available flavonoids

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Abstract

The objective of the current study is to evaluate the phosphodiesterase 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. The results showed that all the selected flavonoids showed binding energy ranging between -7.5 to -6.6 kcal/mol when compared with that of the standard (-4.77 kcal/mol). Inhibition constant (3.2 to 14.4 µM) and intermolecular energy (-9.3 to -8.7 kcal/mol) of the ligands also coincide with the binding energy. All the selected flavonoids contributed better phosphodiesterase inhibitory activity because of its structural parameters. Benzopyran ring in the flavonoids are majorly contributed its activity. These molecular docking analyses could lead to the further development of potent phosphodiesterase inhibitors for the treatment of inflammatory diseases.

Introduction

Docking is finding the binding ability of two interacting molecules with known structures. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Sandeep et al., 2011). Currently, the use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component in the drug discovery process (Koppen, 2009).

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; Prakhov et al., 2010). 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. Each docking is comprised of multiple independent executions of LGA and a poten-tial way to increase its performance is to parallelize the aspects for execution. Docking of small molecules in the receptor binding site and estimation of binding affinity of the complex is a vital part of structure based drug design (Cosconati et al., 2010; Seeliger and Groot, 2010).

A phosphodiesterase is an enzyme that breaks a phosphodiester bond. Generally, people speaking of phosphodiesterase are referring to cyclic nucleotide phosphodiesterases, which have huge clinical significance (Zhang et al., 2004). It regulates the amplitude and duration of responses triggered by the second messengers, cAMP and cGMP. In doing so, they regulate a wide range of biological responses triggered by light, hormones, neurotransmitters and odorants (Jeon et al., 2005).

PDEs have different substrate specificities. Some are cAMP-selective hydrolases (PDE4, 7 and 8); others are

cGMP-selective (PDE5, 6, and 9). Others can hydrolyse both cAMP and cGMP (PDE1, 2, 3, 10, and 11). PDE3 is sometimes referred to as cGMP-inhibited phosphodiesterase. Although PDE2 can hydrolyze both cyclic nucleotides, binding of cGMP to the regulatory GAF-B domain will increase cAMP affinity and hydrolysis to the detriment of cGMP. This mechanism, as well as others, allows for cross-regulation of the cAMP and cGMP pathways (Wang, 2010).

Phosphodiesterase enzymes are often targets for pharmacological inhibition due to their unique tissue distribution, structural properties, and functional properties. Inhibitors of PDE can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE (Rotella, 2012).

PDE inhibitors have been identified as new potential therapeutics in areas such as pulmonary arterial hypertension, coronary heart disease, dementia, depression, and schizophrenia. Xanthines, caffeine, theobromine, and thyroid hormone are phosphodiesterase inhibitors which enhance lipolysis as inhibition of phosphodiesterase enzyme, thereby preserving cAMP, also activating kinase enzyme, which phosphorylates hormone-sensitive lipase and activates lipolysis (Saito et al., 2012).

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, stems, flowers, tea, and wine. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. Many of the flavonoids are responsible for the attractive colors of flowers, fruit, and leaves (Middleton, 1998). Research on flavonoids received an added impulse with the discovery of the French paradox, the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. The flavonoids in red wine are responsible, at least in part, for this effect (Groot and Rauen, 1998).

Flavonoids and their related compounds are low molecular weight substances, which are a group of natural products which exhibits various biological and pharmacological activities like antibacterial, antiviral, anti-oxidant, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral and antimutagenic effects and inhibition of several enzymes (Madeswaran et al., 2012; Formica and Regelson, 1995).

However, there is no conclusive report as to whether the phosphodiesterase activity of the flavonoids. The stereochemistry of binding of the flavonoids on phosphodiesterase has not yet been characterized. In the present study, the structural models of the ligands in the phosphodiesterase binding sites has been carried out, which may facilitate further development of more potent phosphodiesterase inhibitory agents.

Materials and Methods

Softwares required

Python 2.7- language was downloaded from www.python.com, Cygwin (a data storage) c:\program and Python 2.5 were simultaneously downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and AutoDock 4.2 was downloaded from www.scripps.edu, Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com, Molecular orbital package (MOPAC), ChemSketch was downloaded 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 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). Crystal structure of phosphodiesterase enzyme was downloaded from the Brookhaeven protein data bank (Figure 1). The flavonoid ligands like butein, diosmetin, fisetin, tricetin, tricin and caffeine were built using ChemSketch and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies (Bikadi and Hazai, 2009; Figure 2).

Lead optimization of the selected compounds was done by computation of drug likeness 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 number of torsions were tabulated in Table I.

The preparation of the target protein 3HMV (unbound target) with the AutoDock Tools software involved

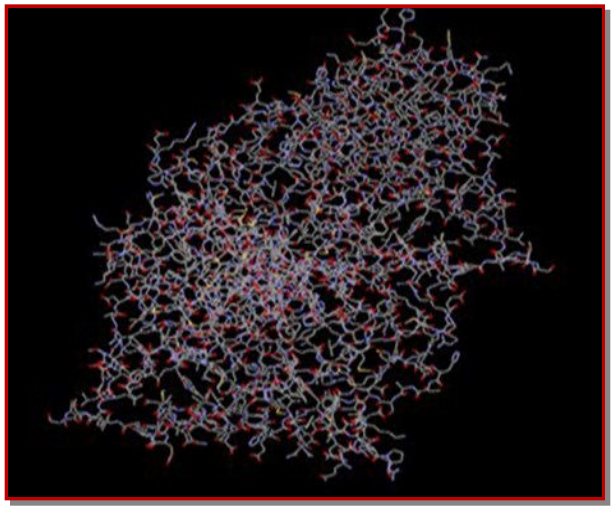

Figure 1: Phosphodiesterase enzyme from Brookhaeven protein data bank (3HMV)

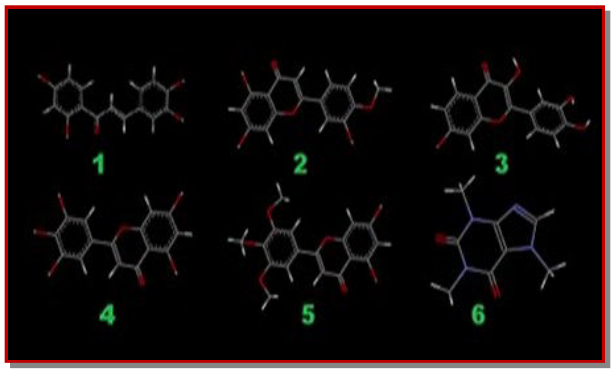

Figure 2: The optimized ligand molecules (1 Butein, 2 Diosmetin, 3 Fisetin, 4 Tricetin, 5 Tricin, and 6 Caffeine)

adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Gasteiger 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 (Umamaheswari et al., 2011). 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 27,000 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.

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 (Madeswaran et al., 2011). 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, ten best poses were generated and scored using AutoDock 4.2 scoring functions (Madeswaran et al., 2012).

Results and Discussion

In silico docking study, was carried out to identify the inhibiting potential of selected flavonoids against phosphodiesterase enzyme. In this study 5 different flavonoids 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

Table I

Ligand parameters

	Molecular Formula	Molecular Weight	Aromatic Carbons	Rotatable Bonds	No. of Torsions
Butein	$C_{15}H_{12}O_5$	272.3	12	7	7
Diosmetin	$C_{16}H_{12}O_6$	300.1	15	5	5
Fisetin	$C_{15}H_{10}O_6$	286.05	15	5	5
Tricetin	$C_{15}H_{10}O_7$	302.04	15	6	6
Tricin	$C_{18}H_{16}O_7$	344.05	15	6	6
Caffeine	$C_8H_{10}N_4O_2$	194.2	5	0	0

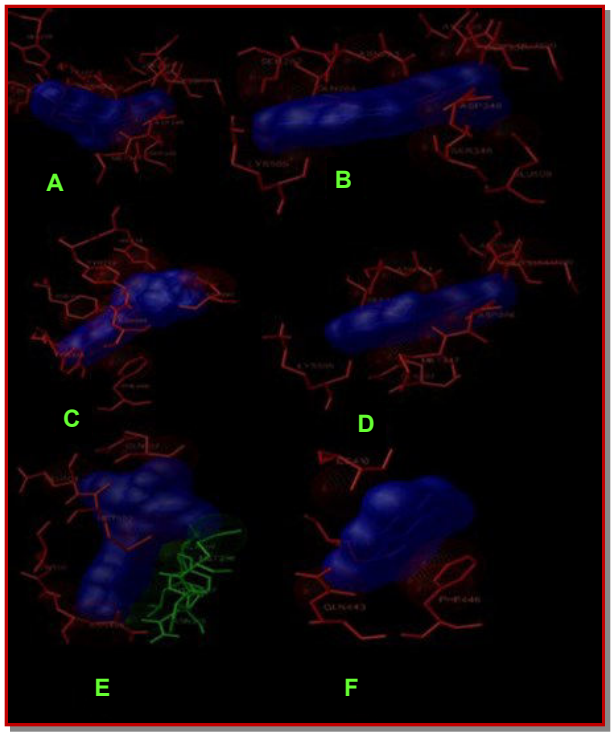

Figure 3: Binding orientations of phosphodiesterase enzyme (3HMV) with the ligands (a Butein, b Diosmetin, c Fisetin, d Tricetin, e Tricin, and f Caffeine)

shows lesser binding energy compared to the standard it proves that the compound has higher activity (Chang et al., 2010).

Analysis of the receptor/ligand complex models generated after successful docking of the flavonoids was based on the parameters such as hydrogen bond interactions, π – π interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active site (Madeswaran et al., 2012). As a general rule, in most of the potent anti inflammatory compounds, both hydrogen bond and π – π hydrophobic interactions between the compound and the active sites of the receptor have been found to be responsible for mediating the biological activity.

The binding mode of the flavonoids with in the active site of phosphodiesterase has been analyzed. The aminoacid residues responsible for the binding interactions of the Butein (Figure 3A) with the enzyme, HIS 278, SER 282, ILE 287, ASN 283, GLN 284, ASP 299, GLU 304, GLU 300, SER 301, ASP 346, SER 348, MET 347. The potential binding sites of the diosmetin (Figure 3B) was found that, SER 282, GLN 284, LYS 505, ASN 283, ASP 299, SER 301, GLU 300, ASP 346, SER 348, GLU 509. For the fisetin (Figure 3C), HIS 234, TYR 233, PHE 414, TYR 403, PHE 446, ASN 395, ASP 392.

The potential binding sites of the tricetin (Figure 3D) was found that, ASP 299, GLU 300, SER 301, GLY 505, GLN 507, MET 347, GLN 284, ASN 283, ASP 346. For the Tricin (Figure 3E), GLN 507, GLN 504, MET 503, GLN 500, ASN 496, ALA 309, MET 296, ASN 305, TYR 297.

The binding site of the caffeine (Figure 3F) was found that, ILE 410, MET 431, GLN 443, PHE 446. These results proves that the effective binding orientations were present in the selected flavonoids when compared with the standard caffeine.

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 -7.5 to -6.6 kcal/mol. Tricetin showed better binding energy -7.5 kcal/mol than the standard Caffeine (-4.8 kcal/mol; Table II). All the selected flavonoids had showed binding energy compared to that of standard. This proves that flavonoids consist of potential phosphodiesterase inhibitory binding sites similar to that of the standard.

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 3.2 to 14.4 µM (Table III). Tricetin showed excellent inhibition constant 3.17 µM than the standard caffeine (318.4 µM). All the selected compounds had lesser inhibition constant when compared to the standard. Thus, the potential phosphodiesterase inhibitory activity of the flavonoids were compared with the caffeine.

Intermolecular energy is also directly proportional to binding energy. Flavonoids showed intermolecular energy ranging between -9.29 to -8.70 kcal/ mol which was lesser when compared to the standard (-4.77 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 phosphodiesterase inhibitory activity of all the selected flavonoids.

Based on the docking studies, the phosphodiesterase inhibitory activity of the selected compounds was found to be decreased in the order of tricetin, tricin, diosmetin, fisetin, butein and caffeine. On the basis of the above study, tricetin, tricin, diosmetin, fisetin, and

Table II

Binding energies of the compounds based on their rank

Com-pounds	Binding energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Butein	-6.6	-6.4	-6.0	-5.9	-5.9	-5.9	-5.7	-5.2	-5.4	-4.6
Diosmetin	-6.8	-6.8	-5.7	-6.7	-6.5	-6.2	-6.5	-6.1	-6.1	-5.9
Fisetin	-6.7	-6.4	-6.3	-6.3	-5.9	-5.7	-5.8	-5.7	-5.6	-5.1
Tricetin	-7.5	-7.3	-7.2	-6.5	-5.7	-5.5	-5.0	-5.7	-5.4	-5.3
Tricin	-6.8	-5.9	-6.8	-5.8	-6.2	-5.8	-5.5	-5.2	-4.4	-4.2
Caffeine	-4.8	-4.8	-4.8	-4.8	-4.7	-4.7	-4.7	-4.1	-4.0	-3.7

Table III

Inhibition Constant of the compounds based on their rank

Com-pounds	Inhibition Constant of the compounds based on their rank (μM, mM[a])									
	1	2	3	4	5	6	7	8	9	10
Butein	14.4	21.7	38.0	45.2	45.7	50.6	61.7	148.7	105.1	421.5
Diosmetin	10.7	11.0	63.4	12.0	15.8	27.5	18.0	30.8	34.7	44.5
Fisetin	11.4	21.1	23.3	25.7	43.8	66.9	57.5	66.8	82.5	174.7
Tricetin	3.2	4.4	5.7	17.3	68.2	91.7	213.2	70.2	101.5	119.0
Tricin	10.1	48.5	10.8	53.2	29.6	51.8	84.9	154.7	581.7	812.8
Caffeine	318.4	318.7	321.9	323.9	377.7	377.8	384.2	905.8	1.2[a]	1.98[a]

Table IV

Intermolecular energies of the compounds based on their rank

Com-pounds	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	2	3	4	5	6	7	8	9	10
Butein	-8.7	-8.4	-8.1	-8.0	-8.0	-7.9	-7.8	-7.3	-7.5	-6.7
Diosmetin	-8.3	-8.3	-7.2	-8.2	-8.0	-7.7	-8.0	-7.6	-7.6	-7.4
Fisetin	-8.2	-7.9	-7.8	-7.7	-7.4	-7.2	-7.3	-7.2	-7.1	-6.6
Tricetin	-9.3	-9.1	-8.9	-8.3	-7.5	-7.3	-6.8	-7.5	-7.2	-7.1
Tricin	-8.6	-7.7	-8.6	-7.6	-8.0	-7.6	-7.3	-7.0	-6.2	-6.0
Caffeine	-4.8	-4.8	-4.8	-4.8	-4.7	-4.7	-4.7	-4.1	-4.0	-3.7

butein possess potential phosphodiesterase 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

Results clearly indicate that from the selected flavonoids, tricetin have better binding sites and interactions with phosphodiesterase enzyme and further investigations are necessary to develop potential chemical entity for the prevention and treatment of inflammatory disorders.

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