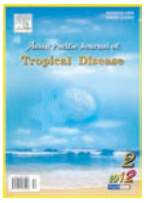
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd



Document heading doi:

Computational drug discovery of potential phosphodiesterase inhibitors using *in silico* studies

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ARTICLE INFO

Article history:
Received 9 August 2012
Received in revised from 11 September 2012
Accepted 9 December 2012
Available online 28 December 2012

Keywords:
Binding energy
Inhibition constant
Intermolecular energy

ABSTRACT

Objective: To evaluate the phosphodiesterase inhibitory activity of flavonoids using *in silico* docking studies. **Methods:** In this perspective, flavonoids like Aromadedrin, Biochanin, Eriodictyol, Isorhamnetin, and Okanin were selected. Caffeine, a known phosphodiesterase inhibitor was used as the standard. *In silico* docking study, was carried out to identify the inhibiting potential of the selected flavonoids against phosphodiesterase enzyme using AutoDock 4.2. The basic principle employed in the AutoDock 4.2 was Lamarckian genetic algorithm. **Results:** Docking results showed that all the selected flavonoids showed binding energy ranging between −7.57 kcal/mol to −5.79 kcal/mol when compared with that of the standard (−4.77 kcal/mol). Intermolecular energy (−9.06 kcal/mol to −8.17 kcal/mol) and inhibition constant (2.82 μmol to 57.41 μmol) of the ligands also coincide with the binding energy. **Conclusions:** Eriodictyol contributed better phosphodiesterase inhibitory activity because of its structural parameters. Further investigations on the above compounds and in vivo studies are necessary to develop potential chemical entities for the prevention and treatment of inflammatory disorders.

1. Introduction

Docking is finding the binding geometry 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[1]. Nowadays, 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[2, 3]. There is a wide range of software packages available for the conduct of molecular docking simulations like, AutoDock, GOLD, FlexX[4].

AutoDock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed[5]. 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 potential way to increase its performance is to parallelize the aspects for execution[6]. 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[7].

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, stems, flowers, tea, and wine[8]. 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[9]. 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[10].

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[11]. However, there are many other families of phosphodiesterases, including phospholipases C and D, autotaxin, sphingomyelin phosphodiesterase, DNases, RNases, andrestriction endonucleases (which all break

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the phosphodiester backbone of DNA or RNA), as well as numerous fewer -well-characterized small-molecule phosphodiesterases. They regulate 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[12].

Different PDEs of the same family are functionally related despite the fact that their amino acid sequences can show considerable divergence[13]. 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[14].

Phosphodiesterase enzymes are often targets for pharmacological inhibition due to their unique tissue distribution, structural properties, and functional properties[15].http://en.wikipedia.org/wiki/Phosphodiesterase - cite_note-7 Inhibitors of PDE can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE.

PDE inhibitors have been identified as new potential therapeutics in areas such as pulmonary arterial hypertension, coronary heart disease, dementia, depression, and schizophrenia[11]. 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[16].

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.

2. Materials and methods

2.1. 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 AutoDock4.2 was downloaded from 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/.

2.2. 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[17]. Crystal structure of cyclooxygenase enzyme was downloaded from the Brookhaeven protein data bank (Fig. 1).

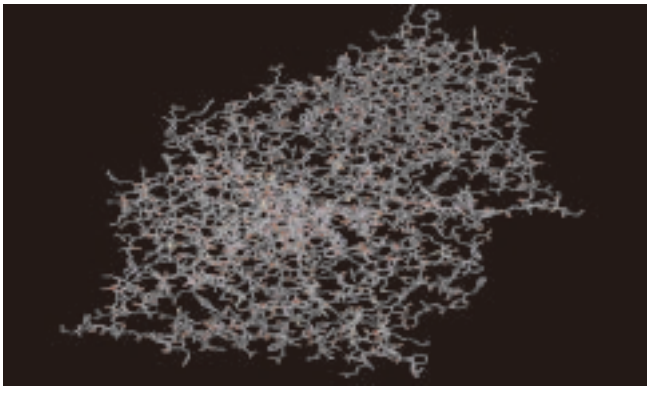

Figure 1. Phosphodiesterase enzyme from RCSB (3HMV).

The flavonoid ligands like aromadedrin, biochanin, eriodictyol, isorhamnetin, okanin and caffeine were built using Chemsketch and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies.

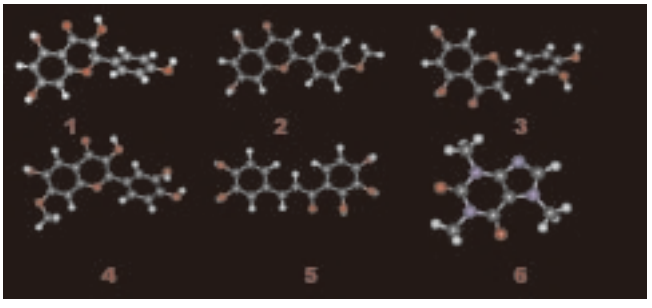

Figure 2. The optimized ligand molecules (1 Aromadedrin, 2 Biochanin, 3 Eriodictyol, 4 Isorhamnetin, 5 Okanin and 6 Caffeine).

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 1. The preparation of the target protein 3HMV (unbound target) 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. 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[18].

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[19]. 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.

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[20]. 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[21].

3. Results

3.1. Docking analysis

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 AutoDock4.2. In the docking studies, if a compound shows lesser binding energy compared to the standard it proves that the compound has higher activity[22].

Docking orientations of the Flavonoids

The binding mode of the flavonoids with in the active site of phosphodiesterase has been analyzed. The binding interactions of the Aromadedrin (figure 3a) with the enzyme, GLN 284, ASN 286, SER 301, ASP 345, MET 347, SER 348, LYS 505, GLN 507 and GLU 509.

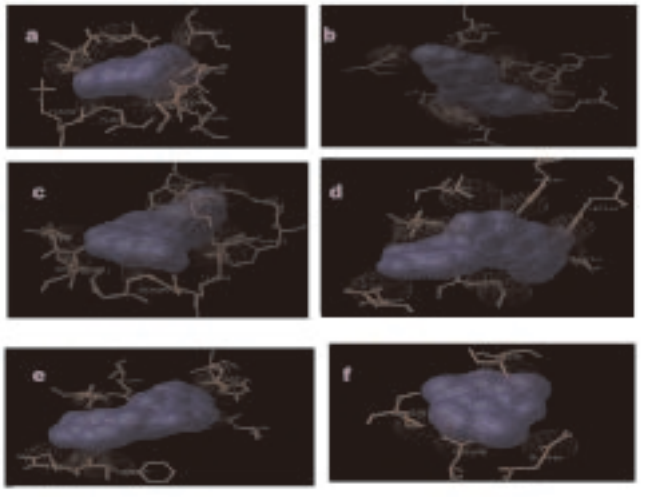

Figure 3 Binding orientations of phosphodiesterase enzyme (3HMV) with the ligands (a Aromadedrin, b Biochanin, c Eriodictyol, d Isorhamnetin, e Okanin and f Caffeine)

The potential binding sites of the Biochanin (figure 3b) was found that, HIS 234, ASP 302, PHE 314, ILE 410, MET 411, PHE 446 and MET 481. For the Eriodictyol (figure 3c), HIS 234, SER 282, ASP 346, MET 347, SER 348, ILE 410, GLU 413, PHE 414, LYS 505 and GLN 507.

The potential binding sites of the Isorhamnetin (figure 3d) was found that, ASN 203, HIS 234, HIS 278, ASP 345, MET 347, ASP 392, LEU 503 and GLN 507. For the Okanin (figure 3e), HIS 274, ASP 275, ASN 283, GLU 304, ASP 346, ASP 392, LYS 505 and GLN 507.

The binding site of the Caffeine (figure 3f) was found that,

Table 1

Ligand Parameters.

Ligands	Molecular Formula	Molecular Weight	Aromatic Carbons	Rotatable Bonds	No. of Torsions
Aromadedrin	$C_{15}H_{12}O_6$	288.261	12	5	5
Biochanin	$C_{16}H_{12}O_5$	284.272	15	4	4
Eriodictyol	$C_{15}H_{12}O_6$	288.261	12	5	5
Isorhamnetin	$C_{16}H_{12}O_7$	316.272	15	6	6
Okanin	$C_{15}H_{12}O_6$	288.261	12	8	8
Caffeine	$C_8H_{10}N_4O_2$	194.196	5	0	0

ILE 410, MET 431, GLN 443, PHE 446. This 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 Waals 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).

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[23]. 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.

As shown in table 2, flavonoids showed binding energy ranging between −7.57 kcal/mol to −5.79 kcal/mol. Eriodictyol showed better binding energy −7.57 kcal/mol than the standard Caffeine (−4.77 kcal/mol). 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 (Ki) and intermolecular energy were also determined. Inhibition constant is directly proportional to binding energy. As shown in table 3, flavonoids showed inhibition constant ranging from 2.82 μmol to 57.41 μmol. Eriodictyol showed excellent inhibition constant 2.82 μmol than the standard Caffeine (318.37 μmol). 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. As shown in table 4, flavonoids showed intermolecular energy ranging between −9.06 kcal/mol to −8.17 kcal/mol which was lesser when compared to the standard (−4.77 kcal/mol). 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.

Table 2

Binding 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
Aromadedrin	−7.00	−6.96	−6.93	−6.46	−6.36	−5.86	−6.12	−5.23	−5.05	−4.86
Biochanin	−6.98	−6.94	−6.94	−6.76	−6.68	−6.63	−6.11	−6.09	−6.05	−5.98
Eriodictyol	−7.57	−7.55	−7.28	−7.18	−6.38	−6.36	−5.80	−5.70	−5.63	−5.68
Isorhamnetin	−6.71	−6.53	−6.56	−6.32	−6.23	−6.15	−5.94	−6.01	−5.33	−4.96
Okanin	−5.79	−5.37	−5.25	−4.94	−5.23	−5.08	−4.68	−4.52	−4.17	−3.66
Caffeine	−4.77	−4.77	−4.76	−4.76	−4.67	−4.67	−4.66	−4.15	−4.00	−3.69

Table 3

Inhibition Constant of the compounds based on their rank.

COMPOUNDS	Inhibition Constant of the compounds based on their rank (μmol, mmol*)									
	1	2	3	4	5	6	7	8	9	10
Aromadedrin	7.43	7.95	8.29	18.33	21.63	50.63	32.65	147.88	199.69	275.46
Biochanin	7.60	8.15	8.21	11.06	12.71	13.70	33.38	34.16	36.92	41.68
Eriodictyol	2.82	2.90	4.59	5.44	21.21	21.72	56.22	66.85	75.14	68.36
Isorhamnetin	12.03	16.31	15.47	23.21	26.91	31.15	43.91	39.04	124.01	230.58
Okanin	57.41	115.54	142.16	239.98	146.67	190.30	371.92	483.67	882.20	2.09*
Caffeine	318.37	318.69	321.95	323.88	377.73	377.79	384.18	905.85	1.17*	1.98*

Table 4

Intermolecular 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
Aromadedrin	−8.49	−8.45	−8.42	−7.95	−7.86	−7.35	−7.61	−6.72	−6.54	−6.35
Biochanin	−8.18	−8.14	−8.13	−7.95	−7.87	−7.83	−7.30	−7.29	−7.24	−7.17
Eriodictyol	−9.06	−9.05	−8.77	−8.67	−7.87	−7.85	−7.29	−7.19	−7.12	−7.17
Isorhamnetin	−8.50	−8.32	−8.35	−8.11	−8.02	−7.94	−7.73	−7.80	−7.12	−6.75
Okanin	−8.17	−7.76	−7.63	−7.32	−7.62	−7.46	−7.07	−6.91	−6.55	−6.04
Caffeine	−4.77	−4.77	−4.76	−4.76	−4.67	−4.67	−4.66	−4.15	−4.00	−3.69

Based on the docking studies, the phosphodiesterase inhibitory activity of the selected compounds was found to be decreased in the order of Eriodictyol, Aromadedrin, Biochanin, Isorhamnetin, Okanin and Caffeine. On the basis of the above study, Eriodictyol, Aromadedrin, Biochanin, Isorhamnetin and Okanin 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.

4. Discussion

In conclusion, the results of the present study clearly demonstrated the *in silico* molecular docking studies of Caffeine and selected flavonoids with phosphodiesterase enzyme exhibited binding interactions and warrants further studies needed for the development of potent phosphodiesterase inhibitors for the treatment of inflammation. These results clearly indicate that the flavonoids especially, Eriodictyol, Aromadedrin, Biochanin, Isorhamnetin and Okanin have similar binding sites and interactions with phosphodiesterase compared to the standard. This *in silico* studies is actually an added advantage to screen the phosphodiesterase inhibition. Further investigations on the above compounds and in vivo studies are necessary to develop potential chemical entities for the prevention and treatment of inflammatory disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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