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IN SILICO DOCKING STUDIES OF CYCLOOXYGENASE INHIBITORY ACTIVITY OF COMMERCIALLY AVAILABLE FLAVONOIDS

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Abstract

New drug discovery is considered broadly in terms of two kinds of investigational activities such as exploration and exploitation. 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. The current study is deals with the evaluation of the cyclooxygenase inhibitory activity of flavonoids using in silico docking studies. In this perspective, flavonoids like Silbinin, Galangin, Scopoletin, Hesperitin, Genistein, Daidzein, Esculatin, Taxifolin, Naringenin and Celecoxib were selected. Celecoxib, a known cyclooxygenase inhibitor was used as the standard. In silico docking studies were carried out using AutoDock 4.2, based on the Lamarckian genetic algorithm principle. Three important parameters like binding energy, inhibition constant and intermolecular energy were determined. The results showed that all the selected flavonoids showed binding energy ranging between -8.77 kcal/mol to -6.24 kcal/mol when compared with that of the standard (-8.30 kcal/mol). Intermolecular energy (-11.15 kcal/mol to -6.83 kcal/mol) and inhibition constant (374.69 nM to 26.83 μM) of the ligands also coincide with the binding energy. All the selected flavonoids contributed cyclooxygenase inhibitory activity because of its structural parameters. These molecular docking analyses could lead to the further development of potent cyclooxygenase inhibitors for the treatment of inflammation.

Key words

Binding energy, Flavonoids, Intermolecular energy, Cyclooxygenase.

Introduction

Drug design is an important tool in the field of medicinal chemistry where new compounds are synthesized by molecular or chemical manipulation of the lead moiety in order to produce highly active compounds with minimum steric effect [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].

Inflammation is a process involved in the pathogenesis of several disorders like arthritis and cardiovascular disease [8]. Cyclooxygenase (COX) is an endogenous enzyme which catalyses the conversion of arachidonic acid into Prostaglandins and thromboxanes [9, 10]. The enzyme exists in atleast two isoforms, COX-1 and COX-2. Although both the isoforms catalyze the same biochemical transformation, the two isoforms are subject to a different expression regulation [11]. COX-1 is a constitutive enzyme and is responsible for the supply of prostaglandins which maintain the integrity of the gastric mucosa and provide adequate vascular homeostasis whereas COX-2 is an inducible enzyme and is expressed only after an inflammatory stimulus [12, 13].

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine [14]. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves [15]. 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 [16]. Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease [15].

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

Materials and Methods

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

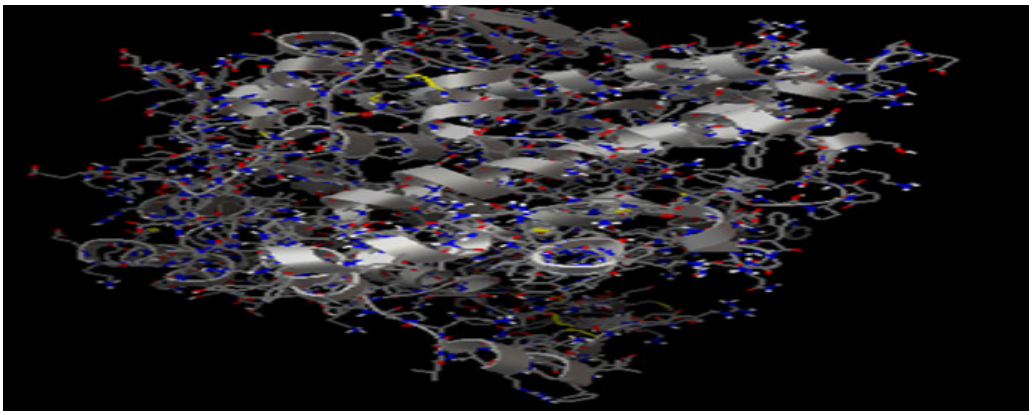

Fig. 1 Cyclooxygenase enzyme from RCSB (4COX)

The flavonoid ligands like Silbinin, Galangin, Scopoletin, Hesperitin, Genistein, Daidzein, Esculatin, Taxifolin, Naringenin and Celecoxib were built using Chemsketch and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies. The optimized ligand molecules were docked into refined cyclooxygenase model using "LigandFit" in the AutoDock 4.2 [18].

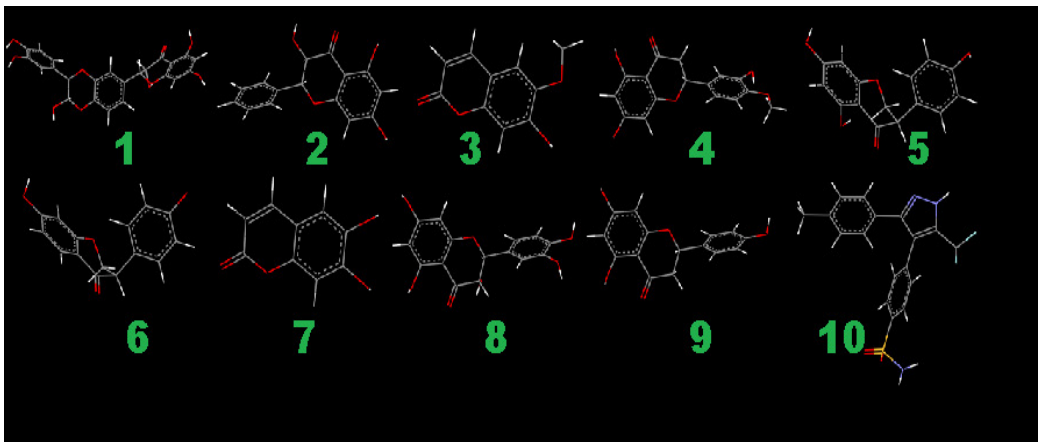

Fig. 2 The optimized ligand molecules (**1** Silbinin, **2** Galangin, **3** Scopoletin, **4** Hesperitin, **5** Genistein, **6** Daidzein, **7** Esculatin, **8** Taxifolin, **9** Naringenin and **10** Celecoxib)

The preparation of the target protein 3D3L (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 [19].

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 [20]. 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. Unbound target 3D3L and unbound ligands were both treated as rigid.

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 [21]. 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 [22].

Results and Discussion

Docking analysis: The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses [23]. In Fig. 3, docked pose of cyclooxygenase enzyme with the ligands Silbinin and Celecoxib clearly demonstrated the binding positions of the ligand with the enzyme. Binding energy of the individual compounds were calculated using the following formula,

Binding energy = A+B+C-D where**,** A denotes final intermolecular energy + Wandervalls 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).

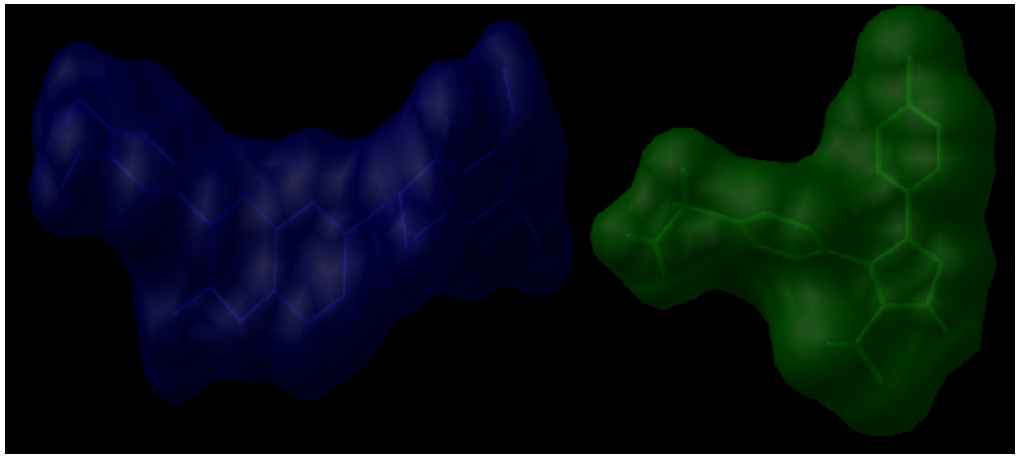

Fig. 3 Docked pose of cyclooxygenase enzyme (3D3L) with Silbinin and Celecoxib

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 [24]. 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 1, flavonoids showed binding energy ranging between -8.77 kcal/mol to -6.24 kcal/mol. All the selected flavonoids had showed binding energy compared to that of standard Celecoxib (-8.30 kcal/mol). This proves that flavonoids consist of potential cyclooxygenase inhibitory binding sites similar to that of the standard.

Table 1. 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**
Silbinin	-8.77	-8.33	-7.86	-7.64	-7.61	-7.35	-7.30	-7.30	-7.14	-7.04
Galangin	-8.10	-8.08	-7.03	-7.25	-7.24	-7.08	-7.10	-7.03	-6.70	-6.51
Scopoletin	-6.24	-6.10	-6.08	-6.08	-6.08	-6.08	-6.07	-6.04	-6.02	-6.01
Hesperitin	-7.73	-7.40	-7.34	-7.25	-7.10	-6.91	-6.28	-7.10	-6.72	-5.88
Genistein	-7.39	-7.34	-7.24	-6.28	-6.21	-6.20	-5.48	-5.66	-5.65	-5.31
Daidzein	-7.65	-7.37	-7.26	-7.62	-7.58	-6.66	-5.87	-5.86	-5.71	-5.69
Esculatin	-6.47	-6.42	-6.34	-6.27	-5.94	-5.93	-5.86	-5.74	-5.56	-5.55
Taxifolin	-6.44	-5.95	-6.41	-6.37	-6.25	-6.10	-5.98	-5.49	-5.44	-4.83
Naringenin	-7.68	-7.67	-7.56	-7.45	-7.32	-7.47	-7.31	-6.82	-7.39	-7.02
Celecoxib	-8.30	-5.90	-5.35	-5.39	-5.32	-5.22	-5.24	-5.14	-5.12	-5.03

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. As shown in table 2, flavonoids showed inhibition constant ranging from 374.69 nM to 26.83 µM. All the selected compounds had lesser inhibition constant when compared to the standard (826.13 nM). Inhibition constant is directly proportional to binding energy. Thus, the cyclooxygenase inhibitory activity of the flavonoids were compared with the Celecoxib. As shown in table 3, flavonoids showed intermolecular energy ranging between -11.15 kcal/mol to -6.83 kcal/mol which was lesser when compared to the standard (-9.79 kcal/mol). Intermolecular energy is also directly proportional to binding energy. 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 cyclooxygenase inhibitory activity of all the selected flavonoids.

Table 2. Inhibition Constant of the compounds based on their rank

COMPOUNDS	Inhibition Constant of the compounds based on their rank (µM, mM*, nM**)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Silbinin	374.69**	779.47**	1.73	2.49	2.65	4.13	4.44	4.49	5.85	6.97
Galangin	1.16	1.19	7.08	4.89	4.92	6.51	6.25	7.05	12.33	17.03
Scopoletin	26.83	34.05	34.89	34.89	34.91	35.07	35.44	37.57	38.85	39.61
Hesperitin	2.14	3.77	4.13	4.82	6.21	8.54	24.87	6.26	11.78	48.94
Genistein	3.85	4.19	4.96	25.14	28.19	28.58	96.14	71.27	72.65	128.26
Daidzein	2.47	3.95	4.78	2.60	2.78	13.09	50.11	50.92	65.31	67.19
Esculatin	18.05	19.55	22.52	25.39	44.50	44.63	51.08	62.46	83.95	85.15
Taxifolin	18.93	43.15	20.08	21.36	26.10	34.02	41.44	93.86	102.48	286.26
Naringenin	2.36	2.40	2.87	3.44	4.29	3.33	4.38	10.00	3.80	7.12
Celecoxib	826.13**	47.38	119.26	111.53	126.99	148.51	144.53	170.01	177.14	205.18

Table 3. Intermolecular energies of the compounds based on their rank

COMPOUNDS	Inter molecular energies of the compounds based on their rank									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Silbinin	-11.15	-10.72	-10.25	-10.03	-10.00	-9.73	-9.69	-9.68	-9.52	-9.42
Galangin	-9.29	-9.28	-8.22	-8.44	-8.43	-8.27	-8.29	-8.22	-7.89	-7.70
Scopoletin	-6.83	-6.69	-6.68	-6.68	-6.68	-6.67	-6.67	-6.63	-6.61	-6.60
Hesperitin	-9.23	-8.89	-8.84	-8.74	-8.59	-8.41	-7.77	-8.59	-8.22	-7.37
Genistein	-8.58	-8.53	-8.43	-7.47	-7.40	-7.39	-6.67	-6.85	-6.84	-6.50
Daidzein	-8.54	-8.27	-8.15	-8.51	-8.47	-7.56	-6.76	-6.75	-6.60	-6.59
Esculatin	-7.07	-7.02	-6.94	-6.87	-6.53	-6.53	-6.45	-6.33	-6.16	-6.15
Taxifolin	-8.23	-7.74	-8.20	-8.16	-8.04	-7.89	-7.77	-7.28	-7.23	-6.62
Naringenin	-8.87	-8.86	-8.75	-8.65	-8.52	-8.67	-8.50	-8.01	-8.59	-8.22
Celecoxib	-9.79	-7.39	-6.84	-6.88	-6.81	-6.71	-6.73	-6.63	-6.61	-6.52

Based on the docking studies, the cyclooxygenase inhibitory activity of the selected compounds was found to be decreased in the order of Silbinin, Celecoxib, Galangin, Hesperitin, Naringenin, Daidzein, Genistein, Esculatin, Taxifolin and Scopoletin. On the basis of the above study, Silbinin, Galangin, Hesperitin, Naringenin and Daidzein possess potential and significant cyclooxygenase 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

In conclusion, the results of the present study clearly demonstrated the *in silico* molecular docking studies of Celecoxib and selected flavonoids with cyclooxygenase enzyme exhibited binding interactions and warrants further studies needed for the development of potent cyclooxygenase inhibitors for the treatment of inflammation. These results clearly indicate that the flavonoids especially, Silbinin, Galangin, Hesperitin, Naringenin and Daidzein have similar binding sites and interactions with cyclooxygenase compared to the standard. This *in silico* studies is actually an added advantage to screen the cyclooxygenase 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.

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