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Computational Drug Discovery of Potential Cyclooxygenase Inhibitors Using *in Silico* Studies

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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 part of structure-based drug design. The current study deals with the evaluation of the cyclooxygenase inhibitory activity of flavonoids using *in silico* docking studies. In this perspective, flavonoids like 4-methyl esculatin, vitexycarpin, wogonin, kaempferol, acacatechin, catechin, quercetin 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 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 -7.71 and -6.09 kcal/mol when compared with that of the standard (-5.95 kcal/mol). Intermolecular energy (-9.20 to -8.18 kcal/mol) and inhibition constant (2.23 to 34.37 μM) of the ligands also coincided with the binding energy. All the selected flavonoids contributed cyclooxygenase inhibitory activity because of their structural parameters. These molecular docking analyses could lead to the further development of potent cyclooxygenase inhibitors for the treatment of inflammation.

Keywords: binding energy, flavonoids, inhibition constant, intermolecular energy

INTRODUCTION

Flavonoids are the most abundant and most studied class of polyphenols. Flavonoids have been considered promising plant secondary metabolites with antioxidative and immunomodulatory properties. Foods and beverages rich in flavonoids have been associated, in several epidemiologic studies, with a decreased risk of age-related diseases (Ciz *et al.* 2012). 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 (Cushnie and Lamb 2005). Flavonoids are the most abundant and most studied class of polyphenols. In the last decades, flavonoids have been considered promising plant secondary metabolites with antioxidative and immunomodulatory properties.

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. Flavonoids can exert beneficial health effects through multiple mechanisms (Oteiza *et al.* 2005).

Inflammation is a process involved in the pathogenesis of several disorders like arthritis and cardiovascular disease (Adrouche-Amrani *et al.* 2012). Cyclooxygenase (COX)-2 is a key regulatory enzyme in the synthesis of prostanoids associated with trauma and inflammation. Cyclooxygenase (COX) is an endogenous enzyme which catalyses the conversion of arachidonic acid into Prostaglandins and thromboxanes (Papafili *et al.* 2002; Santilli *et al.* 2009). The enzyme exists in at least two isoforms, COX-1 and COX-2. Although both isoforms catalyze the same biochemical transformation, they are subject to a different expression regulation. Increased expression of the cyclooxygenase-2 enzyme (COX2) is one of the main characteristics of gastric cancer (GC), which is a leading cause of death in the world, particularly in Asia and South America (Nunez *et al.* 2011). 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 (Peretz *et al.* 2007; Wen *et al.* 2011).

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 (Cavasotto and Abagyan 2004). 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 (Schoichet 2004; Koppen 2009). There is a wide range of software packages available for the conduct of molecular docking simulations like, AutoDock, GOLD, FlexX (Collignon *et al.* 2011).

AutoDock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Schames *et al.* 2004). 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 (Cosconati *et al.* 2010). 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 (Seeliger and Groot 2010).

There is no conclusive report as to whether flavonoids have anti-inflammatory activity. The binding of the flavonoids with the cyclooxygenase shows that the ability of

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inhibiting the cyclooxygenase, thereby it shows the anti-inflammatory activity. The stereochemistry of the binding of flavonoids to cyclooxygenase has not yet been characterized. In the present study, the structural models of ligands in the cyclooxygenase binding sites were analyzed, which may facilitate further development of more potent anti-inflammatory agents.

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 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) and ChemSketch were downloaded from www.acdlabs.com. Online smiles translation was carried out using cactus.nci.nih.gov/translate/.

Docking methodology

We employed 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. The local search algorithm 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 the PDBQT file, was used for coordinate files which includes atomic partial charges. AutoDock Tools was used for creating PDBQT files from traditional PDB files (Khodade *et al.* 2007). The crystal structure of cyclooxygenase enzyme was downloaded from the Brookhaeven protein data bank (**Fig. 1**).

Celecoxib is a sulfa non-steroidal anti-inflammatory drug (NSAID) and selective COX-2 inhibitor used in the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms, and to reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis. In this study, Celecoxib was used as a standard.

Flavonoid ligands like 4-methyl esculatin, vitexycarpin, wogonin, kaempferol, acacatechin, catechin, quercetin and celecoxib were built using Chemsketch (**Fig. 2**). The chemical structure was drawn in the ChemSketch and SMILES notation for the drawn compound was generated. Then with the help of Online SMILES Translator the mol form of the ligands was then converted into pdb form. The pdb form of the ligands was used in the AutoDock 4.2 for the further study. 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. Thereby the ligands druglikeness scores should be monitored before enter into the docking studies.

The optimized ligand molecules were docked into refined enzyme model using "LigandFit" in the AutoDock 4.2 (Goodsell *et al.* 1996). Thereby, the optimization of the ligands was carried out using using "LigandFit" in the AutoDock 4.2 and then optimized ligands further used for the docking studies.

The preparation of the target protein 4COX (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 (Madeswaran *et al.* 2012). 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

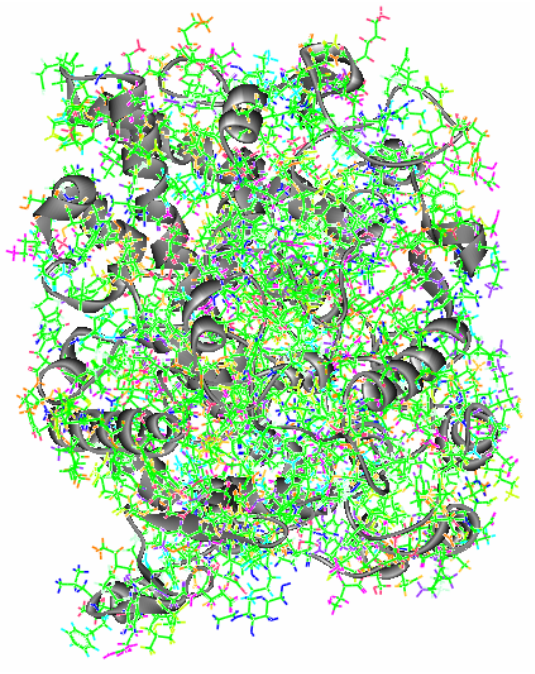

Fig. 1 Refined cyclooxygenase enzyme (4COX).

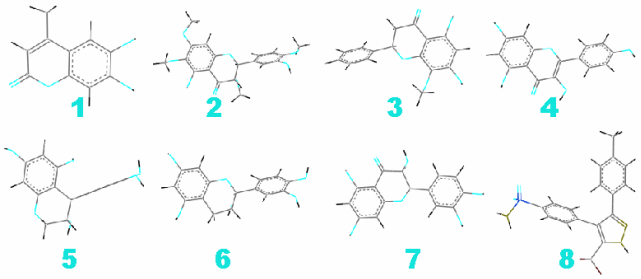

Fig. 2 Optimized ligand molecules. 1 4-methyl esculatin, **2** vitexycarpin, **3** wogonin, **4** kaempferol, **5** acacatechin, **6** catechin, **7** quercetin and **8** celecoxib.

calculated (Konc *et al.* 2011).

Rapid energy evaluation (kcal/mol) 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.

Important preset docking parameters in AutoDock 4.2 software for the LGA as follows: population size of 150 individuals (each flavonoid ligand), 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 ten 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 (Chang *et al.* 2010). 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 (Park *et al.* 2006).

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 Waal's energy (vdW) + hydrogen bonds + desolvation energy + electro-

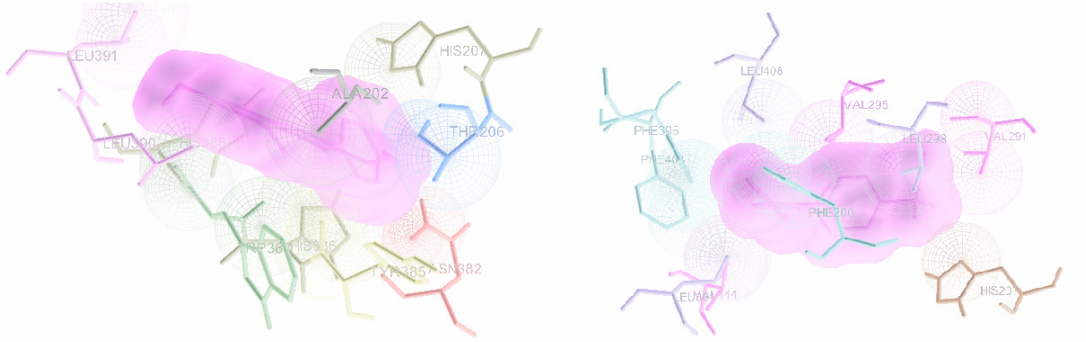

Fig. 3 Docked pose of cyclooxygenase enzyme with the ligands kaempferol and celecoxib.

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**
4-Methyl Esculatin	-6.59	-6.58	-6.56	-6.55	-6.53	-6.37	-6.31	-6.09	-6.08	-5.77
Vitexycarpin	-6.09	-6.08	-5.21	-4.84	-4.83	-4.79	-4.67	-4.83	-4.83	-4.39
Wogonin	-7.56	-7.55	-7.52	-7.41	-7.39	-7.12	-6.71	-6.38	-6.36	-5.62
Kaempferol	-7.71	-7.69	-7.65	-7.65	-6.96	-6.84	-6.65	-6.59	-6.64	-6.58
Acacatechin	-6.95	-6.85	-6.68	-6.52	-5.86	-5.86	-5.65	-4.58	-4.47	-4.37
Catechin	-7.13	-6.62	-6.52	-6.10	-5.84	-6.71	-6.33	-5.91	-5.88	-4.56
Quercetin	-7.22	-7.01	-6.84	-6.81	-6.46	-6.21	-6.20	-6.12	-5.77	-5.15
Celecoxib	-5.95	-5.90	-5.35	-5.39	-5.32	-5.22	-5.24	-5.14	-5.12	-5.03

Table 2 Inhibition Constant of the compounds by the docking studies.

Compounds	Inhibition Constant of the compounds based on their rank (µM)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4-Methyl Esculatin	14.89	15.07	15.64	15.93	16.40	21.57	23.66	34.61	34.86	59.30
Vitexycarpin	34.37	35.15	152.26	283.28	289.67	305.80	379.19	286.91	288.88	603.44
Wogonin	2.89	2.91	3.10	3.72	3.82	6.04	12.04	21.00	21.90	76.30
Kaempferol	2.23	2.30	2.45	2.47	7.87	9.77	13.29	14.82	13.52	15.13
Acacatechin	8.05	9.58	12.79	16.74	50.47	50.94	72.62	439.84	525.48	627.50
Catechin	5.97	14.02	16.65	34.05	52.46	12.02	22.87	46.42	48.78	453.93
Quercetin	5.13	7.26	9.75	10.24	18.54	28.23	28.71	32.60	59.18	167.14
Celecoxib	45.38	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 by the docking studies.

Compounds	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4-Methyl Esculatin	-7.18	-7.17	-7.15	-7.14	-7.12	-6.96	-6.91	-6.68	-6.68	-6.36
Vitexycarpin	-8.18	-8.16	-7.30	-6.93	-6.91	-6.88	-6.76	-6.92	-6.92	-6.48
Wogonin	-8.75	-8.75	-8.71	-8.60	-8.59	-8.31	-7.90	-7.57	-7.55	-6.81
Kaempferol	-9.20	-9.18	-9.14	-9.14	-8.45	-8.33	-8.14	-8.08	-8.13	-8.07
Acacatechin	-8.74	-8.64	-8.47	-8.31	-7.65	-7.65	-7.44	-6.37	-6.26	-6.16
Catechin	-8.92	-8.41	-8.31	-7.88	-7.63	-8.50	-8.12	-7.70	-7.67	-6.35
Quercetin	-9.01	-8.80	-8.63	-8.60	-8.25	-8.00	-7.99	-7.91	-7.56	-6.94
Celecoxib	-7.21	-7.39	-6.84	-6.88	-6.81	-6.71	-6.73	-6.63	-6.61	-6.52

static 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) (Madeswaran *et al.* 2012).

RESULTS AND DISCUSSION

Docking analysis

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 (Umamaheswari *et al.* 2012).

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

The best scoring ligands were identified by best docked scores obtained in comparitive docking studies of ligands with cyclooxygenase enzyme. Identified ligands can be explored further to generate more effective and potential drug molecules through ligand based drug designing approaches. These docking studies also provide indepth understanding of the interaction at their binding sites of ligand groups and receptor sites.

The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses (Madeswaran *et al.* 2012). In **Fig. 3**, docked pose of cyclooxygenase enzyme with the ligands kaemperol and celecoxib clearly demonstrated the binding positions of the ligand with the enzyme.

The potential binding sites of the kaemperol was found that, ALA 202, THR 206, HIS 207, VAL 291, VAL 295, LEU 298, ASN 382, TYR 385, TRP 387, HIS 388. The binding sites of the celecoxib was found to be PHE 200, HIS 207, VAL 291, VAL 295, LEU 298, LEU 390, PHE 395, PHE 404, LEU 408, VAL 444. This proves that the effective binding sites are present in the selected flavonoid kaemperol when compared with the standard celecoxib. It proves that the ability of inhibiting the cyclooxygenase enzyme by the selected flavonoid.

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.* 2011). 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 -7.71 and -6.09 kcal/mol. All the selected flavonoids had showed binding energy compared to that of standard Celecoxib (-5.95 kcal/mol). This proves that flavonoids consist of potential cyclooxygenase 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. As shown in **Table 2**, flavonoids showed inhibition constant ranging from 2.23 to 34.37 μM. All the selected compounds had lesser inhibition constant when compared to the standard (45.38 μM). 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 -9.20 and -8.18 kcal/mol which was lesser when compared to the standard (-7.21 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.

Based on the docking studies, the cyclooxygenase inhibitory activity of the selected compounds was found to be decreased in the order of kaempferol, wogonin, quercetin, catechin, acacatechin, 4 methyl esculatin, vitexycarpin and celecoxib. On the basis of the above study, kaempferol, wogonin, quercetin and catechin possess potential 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, kaempferol, wogonin, quercetin and catechin 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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