In Silico Docking studies of Aldose Reductase Inhibitory activity of selected Flavonoids

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Abstract

New drugs for the inhibition of the enzyme aldose reductase are in development and they have to be screened before being considered for preclinical and clinical evaluation. The current study deals with the evaluation of the cyclooxygenase inhibitory activity of flavonoids using *in silico* docking studies. In this perspective, flavonoids like Farobin-A, Gericudranin-B, Glaziovianin-A, Rutin, and Xanthotoxin were selected. Epalrestat, a known aldose reductase inhibitor was used as the standard. Docking results showed that all the selected flavonoids showed binding energy ranging between -7.91 kcal/mol to -5.08 kcal/mol when compared with that of the standard (-5.59 kcal/mol). Intermolecular energy (-9.11 kcal/mol to -8.66 kcal/mol) and inhibition constant (1.58 μM to 187.37 μM) of the ligands also coincide with the binding energy. Xanthotoxin contributed better aldose reductase inhibitory activity because of its structural parameters. Further studies are required to develop potent aldose reductase inhibitors for the treatment of diabetes.

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INTRODUCTION

The number of compounds entering the drug discovery process at the initial stage is usually very

high. Among these only a very few candidate molecules makes it to the final step and emerges as the potential new therapy. The failure of a candidate molecule may occur due to different factors such as adverse effects, poor pharmacokinetics, lack of efficacy, commercial reasons etc. [1]. The AutoDock offers different types of search algorithms to search the conformational space. Among these, the Genetic Algorithm is the most modern and sophisticated. Genetic Algorithms are a family of powerful mathematical functions derived from the concepts of language of molecular genetics. Other types of search algorithms in AutoDock include Simulated Annealing and Local Search [2, 3].

Aldose reductase (ALR2; EC: 1.1.1.21) belongs to aldo-keto reductases super family. It is the first rate limiting enzyme in polyol pathway and reduces glucose to sorbitol by utilizing NADPH as a cofactor. sorbitol dehydrogenase is the enzyme responsible for the conversion of Sorbitol into fructose [4, 5]. The polyol pathway represents a minor route of glucose utilization, accounting for less than 3% of glucose consumption. However, in the presence of high glucose, the activity of this pathway is increased and could represent up to 30% of total glucose consumption [6]. Evidence for the involvement of ALR2 in diabetic neuropathy, retinopathy, nephropathy and cataract emerged from several independent studies [7]. Abnormal activation of the polyol pathway during diabetes leads to accumulation of osmotically active sorbitol leading to osmotic as well as oxidative stress, resulting in tissue injury [8, 9].

Flavonoids are natural substances with variable phenolic structures which are widely distributed in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Over 4000 structurally different flavonoids have been discovered from plant sources**.** These have raised considerable interest recently because of their beneficial effects on human health and are found to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities. Research done on flavonoids led to the discovery of the French paradox, ie, low incidence of coronary heart disease, despite having a diet relatively rich in Mediterranean populations in association with red wine consumption and a high saturated fat intake [10, 11].

The stereochemistry of binding of the flavonoids on aldose reductase has not yet been characterized. In the present study, the structural models of the ligands in the aldose reductase binding sites has been carried out, which may facilitate further development of more potent anti diabetic 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 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

Lamarckian genetic algorithm (LGA) for ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm was employed in present study. 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.

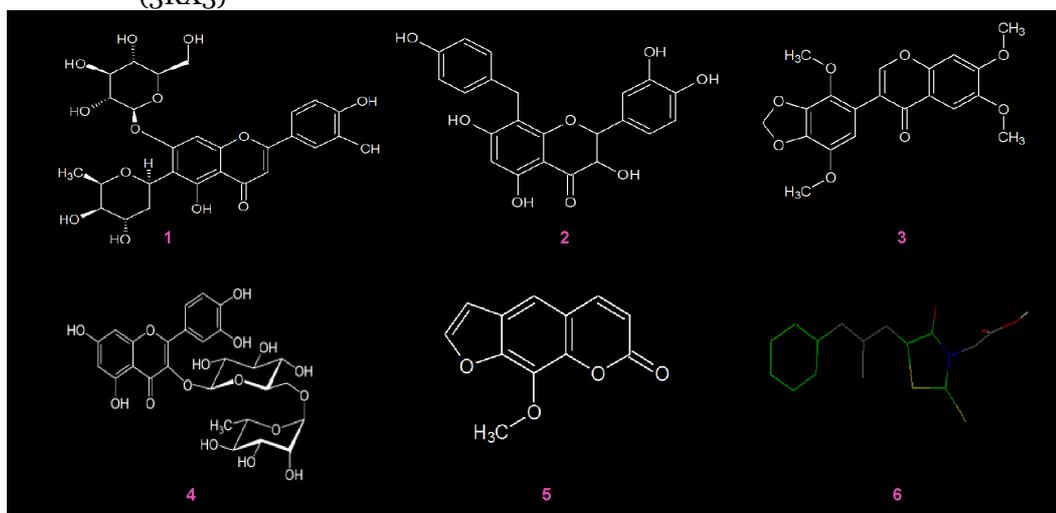**Fig. 1** Refined aldose reductase enzyme from RCSB (3RX3)

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 [11]. Crystal structure of aldose reductase enzyme was downloaded from the Brookhaeven protein data bank and refined with the help of Accelrys studio viewer (Fig. 1).

Fig. 2 The optimized ligand molecules (**1** Farobin-A, **2** Gericudranin-B, **3** Glaziovianin-A, **4** Rutin, **5** Xanthotoxin, and **6** Epalrestat)

In fig.2 the flavonoid ligands like Farobin-A, Gericudranin-B, Glaziovianin-A, Rutin, Xanthotoxin and Epalrestat 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 aldose reductase model using "LigandFit" in the AutoDock 4.2 [12].

The preparation of the target protein 3RX3 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 [13].

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 [14]. 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.

Auto Dock 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, ten best poses were generated and scored using AutoDock 4.2 scoring functions [15].

RESULTS AND DISCUSSION

Docking analysis

In silico docking study, was carried out to identify the inhibiting potential of selected flavonoids against aldose reductase enzyme. 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 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.

The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses [16]. In Fig. 3, docked pose of aldose reductase enzyme with the ligands Xanthotoxin and Epalrestat 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 + 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).

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 [17, 18]. 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.
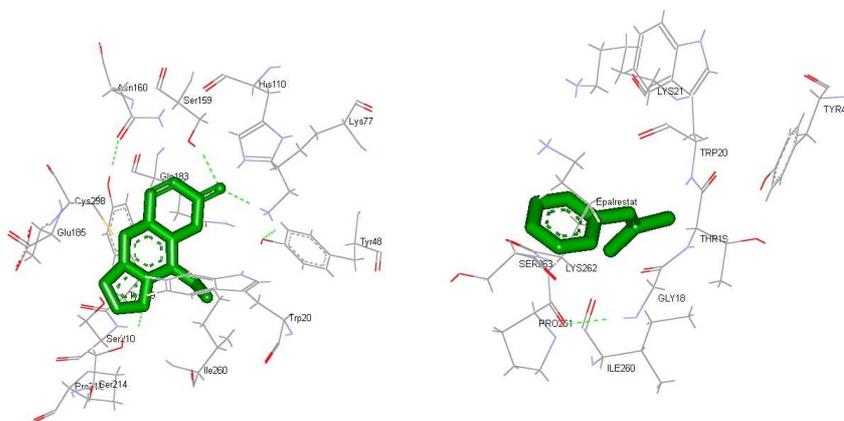

Fig. 3 Docked pose of aldose reductase enzyme (3RX3) with Xanthotoxin and Epalrestat

The potential binding sites of the Xanthotoxin was found that, TRP 20, TYR 48, LYS 77, HIS 110, SER 159, ASN 160, GLN 183, GLU 185, TYR 209, SER 210, PRO 211, SER 214, ILE 260 and CYS 298. The binding sites of the Epalrestat was found to be GLY 18, THR 19, TRP 20, LYS 21, SER 210, PRO 211, LEU 212, SER 214, ILE 260, PRO 261, LYS 262 and SER 263. This proves that the effective binding sites are present in the selected flavonoid Xanthotoxin when compared with the standard Epalrestat. It proves that the ability of inhibiting the aldose reductase enzyme by the selected compounds. As shown in table 1, flavonoids showed binding energy ranging between -7.25 kcal/mol to -3.84 kcal/mol. From the selected flavonoids Xanthotoxin had showed better binding energy (-7.25 kcal/mol) when compared to the standard Epalrestat (-5.59 kcal/mol). This proves that Xanthotoxin consist of potential aldose reductase inhibitory binding sites when compared to 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
Farobin-A	-4.26	-4.14	-3.77	-3.51	-2.92	-2.71	-2.67	-2.65	-2.52	-1.96
Gericudranin-B	-5.95	-5.65	-5.32	-5.00	-4.51	-4.77	-4.58	-4.29	-4.27	-3.81
Glaziovianin-A	-6.71	-5.26	-5.11	-5.02	-5.01	-4.90	-4.90	-5.08	-5.08	-4.84
Rutin	-3.84	-3.25	-2.49	-3.20	-3.01	-2.75	-2.67	-1.78	-1.67	-0.82
Xanthotoxin	-7.25	-7.24	-7.24	-7.24	-7.24	-7.24	-7.24	-7.23	-7.23	-7.16
Epalrestat	-5.59	-5.58	-5.58	-5.58	-5.58	-5.57	-5.57	-5.57	-5.56	-5.56

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. Inhibition constant is directly proportional to binding energy. Theoritical IC_{50} was calculated with the help of AutoDock 4.2. As shown in table 2, flavonoids showed inhibition constant ranging from 4.86 µM to 1.52 mM. Xanthotoxin had better inhibition constant (1.58 µM) when compared to the standard (80.08 µM). This implies that the Xanthotoxin were found to be higher activity against aldose reductase enzyme when compared to Epalrestat. We found a decrease in inhibition constant of all the selected flavonoids with a simultaneous decrease in the binding energy.

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

COMPOUNDS	Inhibition Constant of the compounds based on their rank (µM, * mM)									
	1	2	3	4	5	6	7	8	9	10
Farobin-A	752.00	922.34	1.71*	2.66*	7.21*	10.25*	11.05*	11.46*	14.31*	36.37*
Gericudranin-B	43.33	72.13	125.23	217.27	492.80	316.33	436.54	715.84	746.34	1.60*
Glaziovianin-A	11.99	139.92	178.35	207.50	211.13	255.00	257.26	187.91	188.22	284.25
Rutin	1.52*	4.12*	14.99*	4.48*	6.24*	9.70*	11.12*	49.84*	59.77*	250.05*
Xanthotoxin	4.86	4.91	4.91	4.92	4.95	4.95	4.97	4.98	5.00	5.67
Epalrestat	80.08	80.78	81.08	81.28	81.49	82.91	82.93	83.00	83.50	83.75

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. As shown in table 3, flavonoids showed intermolecular energy ranging between -7.55 kcal/mol to -8.62 kcal/mol. Xanthotoxin had better intermolecular energy (-7.55 kcal/mol) when

compared to the standard (-5.59 kcal/mol). This result further proved that Xanthotoxin consist of

better aldose reductase inhibitory activity compared to the standard.

Table 3: 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**
Farobin-A	-8.44	-8.32	-7.95	-7.69	-7.10	-6.89	-6.85	-6.82	-6.69	-6.14
Gericudranin-B	-8.34	-8.04	-7.71	-7.38	-6.90	-7.16	-6.97	-6.68	-6.65	-6.20
Glaziovianin-A	-8.21	-6.75	-6.61	-6.52	-6.51	-6.39	-6.39	-6.51	-6.57	-6.33
Rutin	-8.62	-8.03	-7.26	-7.98	-7.78	-7.52	-7.44	-6.55	-6.44	-5.59
Xanthotoxin	-7.55	-7.54	-7.54	-7.54	-7.54	-7.54	-7.53	-7.53	-7.53	-7.46
Epalrestat	-5.59	-5.88	-5.88	-5.88	-5.88	-5.87	-5.87	-5.87	-5.86	-5.86

Based on the docking studies, the aldose reductase inhibitory activity of the selected compounds was found to be decreased in the order of Xanthotoxin, Glaziovianin-A, Gericudranin-B, Epalrestat, Farobin-A, and Rutin. All the flavonoids possess binding sites with aldose reductase enzyme. But, the Xanthotoxin showed better binding interactions and docking parameters (binding energy, inhibition constant, intermolecular energy) than the other selected flavonoids and the standard.

On the basis of the above study, Xanthotoxin, possess potential aldose reductase inhibitory binding sites and docking parameters compared to that of the standard. This may be attributed due to the differences in the position of the functional groups in that compound. Further development of Xanthotoxin and their derivatives could be a potential drug candidate for the aldose reductase inhibition in future.

CONCLUSION

These results clearly indicate that from the selected flavonoids, Xanthotoxin have better binding sites and interactions with aldose reductase enzyme and further investigations are necessary to develop potential chemical entity for the prevention and treatment of diabetes.

REFERENCES

1) Dias R, Azevedo WF. Molecular docking algorithms. Current Drug Targets. 2008; 9: 1040-1047.

2) Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Computational Chem 2009; 30: 2785-2791.

3) Hetenyi C, Van der Spoel D. Efficient docking of peptides to proteins without prior knowledge of the binding site. Protein Science. 2002; 11: 1729-1737.

4) Ravindranath TM, Mong PY, Ananthakrishnan R, Li Q, Quadri N, Schmidt MA, Ramasamy R, Wang Q. Novel Role for Aldose Reductase in Mediating Acute Inflammatory Responses in the Lung, J Immunol. 2009; 183: 8128-8137.

5) Hwang YC, Shaw S, Kaneko M. Aldose reductase pathway mediates JAK-STAT signaling: a novel axis in myocardial ischemic injury. J Fed Am Soc Exp Biol 2005; 19: 795-797.

6) Yadav UCS, Naura AS, Aguirre LA, Ramana KV, Boldogh I, Sur S, Boulares HA, Srivastava SK. Aldose reductase inhibition suppresses the expression of Th2 cytokines and airway inflammation in ovalbumin-induced asthma in mice. J Immunol 2009; 183: 4723-4732.

7) Guzman A, Guerrero OR. Inhibition of aldose reductase by herbs extracts and natural substances and their role in prevention of cataracts. Revista Cubana de Plantas Medicinales 2005; 10: 3-4.

8) Dong Y, Yang J, Ren X, Zhang H, He J. New Aldose Reductase Inhibitors N99-596 A and B

from *Streptomyces*. J Antibiotics 2005; 58: 737-739.

9) Saraswat M, Muthenna P, Suryanarayana P, Petrash JM, Reddy G. Dietary sources of aldose reductase inhibitors: prospects for alleviating diabetic complications, Asia Pacific J Clin Nut 2008; 17: 558-565.

10) Patel JM. A review of potential health benefits of flavonoids. Lethbridge Undergraduate Research J 2008; 3: 1-5.

11) Madeswaran A, Umamaheswari M, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. In Silico docking studies of lipoxygenase inhibitory activity of commercially available flavonoids J. Comput. Method. Mol. Des 2011; 1 (4): 65-72.

12) Goodsell DS, Morris GM, Olson AJ. Automated docking of flexible ligands: Applications of Autodock. J Mol Recog 1996; 9: 1-5.

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

14) 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. Bangladesh J Pharmacol 2011; 6: 117-123.

15) Madeswaran A, Umamaheswari M, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. Discovery of potential aldose reductase inhibitors using in silico docking studies. Orient Pharm Exp Med 2012; 12: 157-161.

16) Sivakumar R, Lokesh N, Rajashekhar A, Ramu N, Saikishore P, Venkatanarayanan R. Docking studies on PPARγ of novel α-phenoxy phenyl propionic acid derivatives as antidiabetic agent. Der Pharmacia Sinica. 2011; 2: 327-329.

17) Azam F, Prasad MVV, Thangavel N. Molecular docking studies of 1- (substituted phenyl) -3- (naphtha [1,2 –d] thiazol -2-yl) urea/thiourea derivatives with human adenosine A2A receptor. Bioinformation 2011; 6: 330-334.

18) Umamaheswari M, Madeswaran A, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. Docking studies: Search for possible phytoconstituents for the treatment of gout. Int J Biol Pharm Res 2012; 3, 6-11.