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Discovery of potential xanthine oxidase inhibitors using *in silico* docking studies

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ABSTRACT

New drugs for the inhibition of the enzyme xanthine oxidase are in development and they have to be screened before being considered for preclinical and clinical evaluation. In order to understand the mechanism of ligand binding and to identify potent xanthine oxidase inhibitors, a study involving molecular docking and virtual screening has been performed. The objective of the current study is to evaluate the xanthine oxidase inhibitory activity of flavonoids using in silico docking studies. In this perspective, flavonoids like Butein, Fisetin, Diosmetin, Tricetin, Genistein, Tricin, Vitexycarpin, Herbacetin, Biochanin, Rhamnetin, Isorhamnetin, Robinetin, Peonidin and Okanin were selected. Allopurinol, a known xanthine oxidase inhibitor was used as the standard. In silico docking studies were carried out using AutoDock 4.2, based on the Lamarckian genetic algorithm principle. The three important parameters like binding energy, inhibition constant and intermolecular energy were determined. The results showed that all the selected flavonoids showed lesser binding energy ranging between -7.86 kcal/mol to -5.40 kcal/mol when compared with that of the standard (-4.47 kcal/mol). Intermolecular energy (-9.95 kcal/mol to -7.49 kcal/mol) and inhibition constant (1.72 µM to 110.19 µM) of the ligands also coincide with the binding energy. All the selected flavonoids consist of benzopyran ring in its basic nucleus, which would have contributed to its xanthine oxidase inhibitory activity. These molecular docking analyses could lead to the further development of potent xanthine oxidase inhibitors for the prevention and treatment of gout and related inflammatory conditions.

Key words: Docking, Flavonoids, Gout, Xanthine oxidase.

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]. New drug discovery is considered broadly in

terms of two kinds of investigational activities such as exploration and exploitation [2]. 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 [3].

Nowadays, the use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component of the drug discovery process [4, 5]. There is a wide range of software packages available for the conduct of molecular docking simulations like, AutoDock and DOCK, GOLD, FlexX and ICM [6, 7]. AutoDock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed [8, 9]. 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 [10]. 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.

Xanthine oxidase (XO) is a highly versatile enzyme that is widely distributed among different species from bacteria to man and within the various tissues of mammals. It is a member of group of enzymes known as molybdenum iron – sulphur flavin hydroxylases [11]. It catalyses the oxidation of hypoxanthine to xanthine and then to uric acid, the final reactions in the metabolism of purine bases [12]. The accumulation of uric acid in the body is responsible for the formation of several diseases and thus it plays a vital role in producing hyperuraecimia and gout [13]. Inherited xanthine oxidase reductase (XOR) deficiency leads to xanthineuria and multiple organ failure syndrome caused by the accumulation of xanthine in different tissues [14].

Gout is one of the most common metabolic disorders with a worldwide distribution and continues to be a major health problem. It is characterized by an excessive concentration of uric acid in the blood, causing the accumulation of monosodium urate crystals in the joints and kidneys leading to acute goutry arthritis, tophi of the joints and extremities and uric acid nephrolithiasis [15]. Elevated levels of uric acid not only leads to gout but also results in the development of hypertension, cardiovascular diseases, diabetes, obesity, cancer and hyperlipidemia [16].

Xanthine oxidase inhibitors (XOI) are much useful, since they possess lesser side effects compared to uricosuric and anti-inflammatory agents [17]. Allopurinol is the only clinically available XOI, which also suffers from many serious side effects such as hypersensitivity reactions, Steven's Johnson syndrome and renal toxicity. Thus, there is a necessary to develop compounds with XOI activity with lesser side effects when compared to allopurinol.

Flavonoids are widespread phytochemical constituents present in plants and they contribute to the flavour and colour of fruits and vegetables. They consist of 15 carbons of 2 phenolic rings connected by a 3-carbon unit, and grouped according to presence of various functional groups on the rings and the degree of ring saturation [18]. They are usually attached with sugar moiety to increase their water solubility. Most of the flavonoids are known to possess various pharmacological activities, such as antioxidant, antiviral, antibacterial and antimutagenic effects [19].

The stereochemistry of binding of the flavonoids on xanthine oxidase has not been characterized. In the present study, the structural models of the ligands in the xanthine oxidase binding sites has been carried out, which may facilitate further development of more potent anti gout 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/.

Coordinate File Preparation

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 [20]. Crystal structure of xanthine oxidase enzyme from bovine milk source was downloaded from the Brookhaeven protein data bank (Fig. 1).

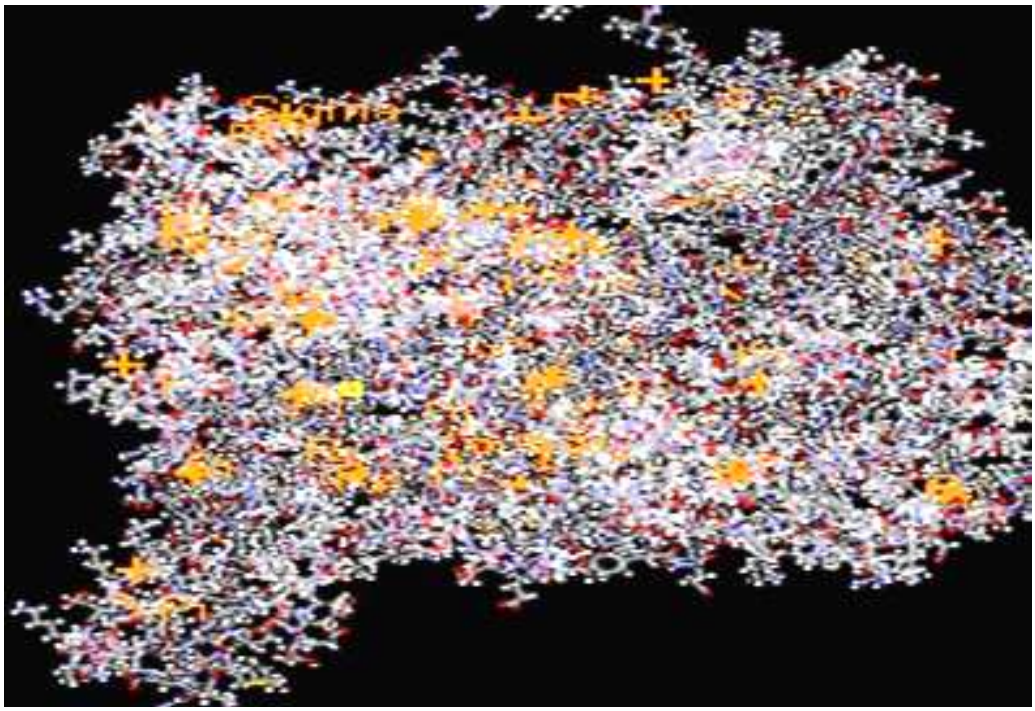

Fig. 1 Xanthine oxidase from bovine milk source (3BDJ)

The flavonoid ligands like Butein, Fisetin, Diosmetin, Tricetin, Genistein, Tricin, Vitexycarpin, Herbacetin, Biochanin, Rhamnetin, Isorhamnetin, Robinetin, Peonidin and Okanin and the standard allopurinol 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 xanthine oxidase model using “LigandFit” in the AutoDock 4.2 [21].

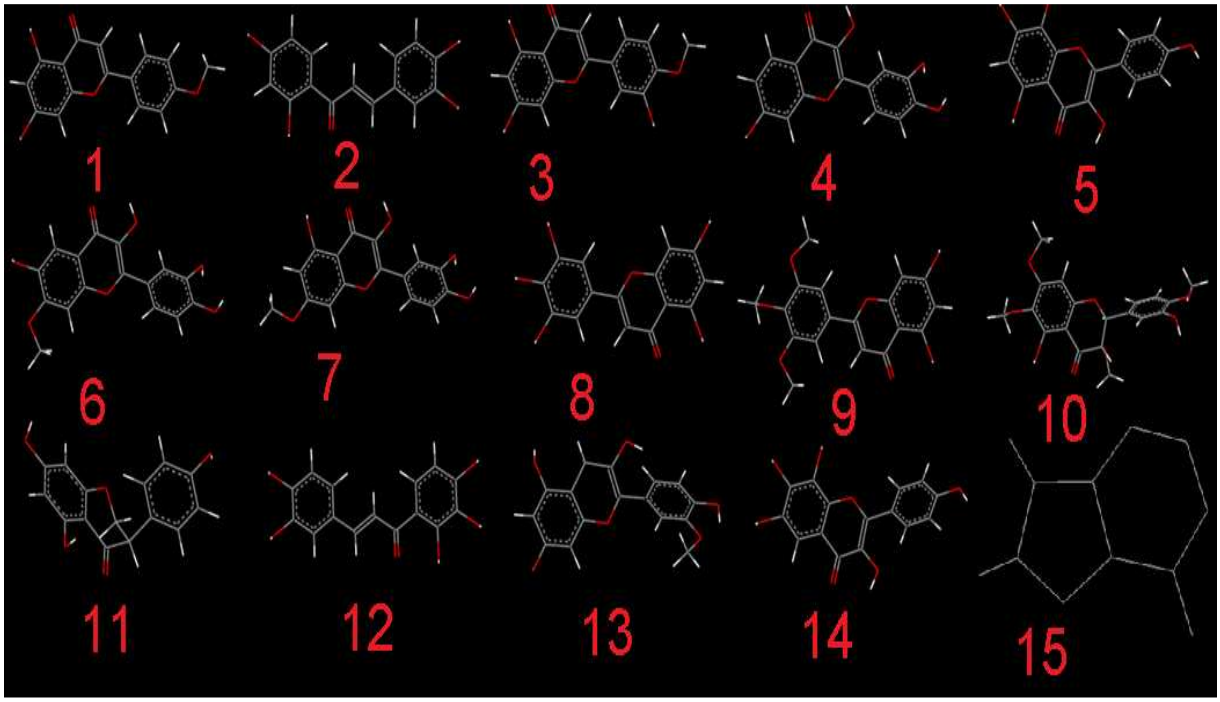

Fig. 2 The optimized ligand molecules (1 Butein, 2 Fisetin, 3 Isorhamnetin, 4 Rhamnetin, 5 Robinetin, 6 Herbacetin, 7 Diosmetin, 8 Biochanin, 9 Okanin, 10 Tricetin, 11 Peonidin, 12 Genistein, 13 Tricin, 14 Vitexycarpin and 15 Allopurinol)

AutoGrid calculation

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 [22]. The energy of interaction of each atom in the ligand was encountered.

AutoDock calculation

Docking can be carried out by various methods. But, the most efficient method is Lamarckian genetic algorithm. 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 [23].

Analysis using AutoDock Tools

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 [24].

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 [25]. In Fig. 3, docked pose of xanthine

oxidase enzyme with Butein and Fisetin ligands clearly demonstrated the binding positions of the ligand with the enzyme. Binding energy of the individual compound were calculated using the following formula,

Binding energy = A+B+C-D

where**,** A denotes final intermolecular energy + Wander valls 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).

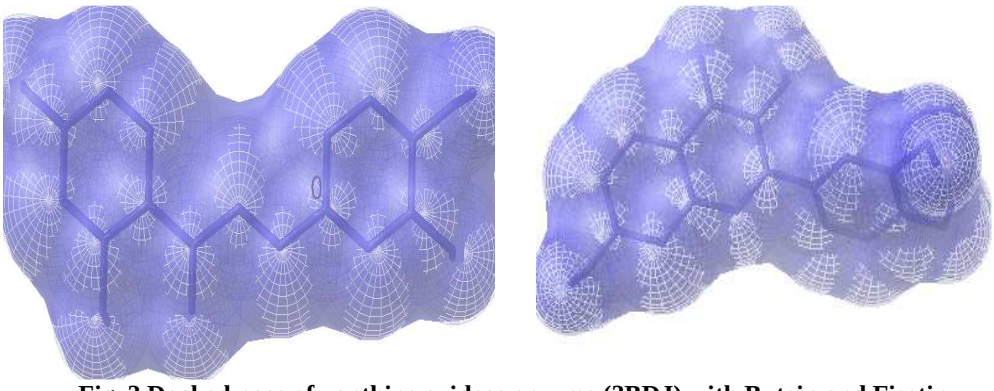

Fig. 3 Docked pose of xanthine oxidase enzyme (3BDJ) with Butein and Fisetin

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**
Biochanin	-7.03	-6.99	-6.61	-6.56	-6.44	-5.92	-5.63	-5.63	-5.60	-5.41
Butein	-7.86	-6.22	-6.03	-6.01	-5.07	-5.03	-4.75	-4.39	-4.37	-4.20
Diosmetin	-7.13	-6.34	-6.04	-5.89	-5.87	-5.84	-5.55	-5.42	-5.25	-5.25
Fisetin	-7.77	-7.01	-6.78	-6.03	-5.89	-5.84	-5.58	-5.34	-5.30	-4.80
Herbacetin	-7.42	-7.41	-7.07	-6.18	-5.79	-6.17	-5.25	-4.76	-4.61	-4.54
Isorhamnetin	-7.71	-5.99	-5.73	-5.59	-5.27	-5.17	-5.02	-4.96	-4.65	-4.49
Rhamnetin	-7.60	-7.37	-7.09	-7.05	-6.58	-5.95	-5.79	-5.57	-4.74	-4.45
Tricetin	-6.88	-6.36	-6.87	-6.24	-6.17	-6.06	-6.02	-5.09	-4.49	-4.06
Tricin	-5.91	-5.80	-5.55	-5.48	-5.41	-5.34	-5.30	-4.87	-4.83	-4.65
Vitexycarpin	-5.40	-5.28	-5.07	-4.63	-4.56	-4.03	-4.56	-4.29	-4.20	-4.10
Genistein	-5.99	-5.96	-5.59	-5.53	-5.52	-5.26	-5.44	-5.40	-5.22	-5.10
Okanin	-7.01	-6.42	-6.18	-5.24	-5.11	-4.93	-4.74	-4.25	-4.19	-4.14
Peonidin	-6.72	-6.63	-6.02	-6.56	-6.55	-6.31	-5.66	-5.61	-5.47	-5.19
Robinetin	-7.58	-7.51	-5.46	-5.40	-5.06	-5.04	-4.69	-4.51	-4.31	-4.19
Allopurinol	-4.47	-4.47	-4.46	-4.46	-4.45	-4.20	-4.09	-4.09	-3.99	-3.87

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 [26]. As a general rule, in most of the potent antigout 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 -7.86 kcal/mol to -5.40 kcal/mol. All the selected flavonoids had lesser binding energy when compared to the standard allopurinol (-4.47 kcal/mol). This proves that flavonoids consist of potential xanthine oxidase inhibitory binding sites when compared to 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 1.72 µM to 110.19 µM. All the selected compounds had lesser inhibition constant when compared to the standard (529.73 µM). Inhibition constant is directly proportional to binding energy. We found a decrease in inhibition constant of all the selected flavonoids with a simultaneous decrease in the binding energy. Thus, the xanthine oxidase inhibitory activity of the flavonoids were found to be higher compared to allopurinol.

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**
Biochanin	7.05	7.49	14.17	15.54	19.15	45.81	74.50	75.05	79.04	108.10
Butein	1.72	27.48	38.26	39.58	191.88	205.89	330.95	601.16	631.49	834.62
Diosmetin	5.93	22.55	37.65	48.50	49.62	52.03	85.69	107.26	142.28	142.52
Fisetin	2.02	7.30	9.13	38.09	48.04	52.23	81.47	121.69	131.34	303.40
Herbacetin	3.64	3.70	6.59	29.73	56.89	30.05	140.85	322.77	414.52	470.92
Isorhamnetin	2.24	40.48	62.95	80.19	136.13	163.20	207.76	230.78	389.23	514.95
Rhamnetin	2.69	3.99	6.35	6.83	15.08	43.75	56.58	83.17	335.96	546.04
Tricetin	9.02	21.83	9.13	26.75	29.83	35.90	38.93	184.65	509.05	1.06*
Tricin	46.92	55.83	84.94	95.69	108.54	121.04	129.72	267.21	289.36	392.29
Vitexycarpin	110.19	134.54	191.47	405.43	455.31	1.12*	455.87	721.88	834.91	989.47
Genistein	41.00	42.44	79.54	88.42	89.72	138.52	102.58	109.23	149.68	182.44
Okanin	7.24	19.63	29.75	145.29	178.69	243.74	337.43	770.62	853.01	915.64
Peonidin	11.85	13.85	38.74	15.49	15.76	23.74	71.42	77.58	97.43	156.49
Robinetin	2.78	3.13	98.70	110.64	195.26	201.97	366.76	491.60	698.10	848.43
Allopurinol	529.73	534.14	541.00	541.30	545.56	830.85	1.01*	1.01*	1.18*	1.45*

As shown in table 3, flavonoids showed intermolecular energy ranging between -9.95 kcal/mol to -7.49 kcal/mol which was lesser when compared to the standard (-4.47 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 xanthine oxidase inhibitory activity of all the selected flavonoids.

Based on the docking studies, the xanthine oxidase inhibitory activity of the selected compounds was found to be decreased in the order of Butein, Fisetin, Isorhamnetin, Rhamnetin, Robinetin, Herbacetin, Diosmetin, Biochanin, Okanin, Tricetin, Peonidin, Genistein, Tricin, Vitexycarpin and Allopurinol. On the basis of the above study, Butein, Fisetin, Isorhamnetin, Rhamnetin, Robinetin and Herbacetin possess potential xanthine oxidase inhibitory binding sites when

compared to that of the standard. This may be attributed due to the differences in the position of the functional groups in the compounds. Flavonoids consist of benzopyran ring in its basic nucleus which could be responsible for the xanthine oxidase inhibitory activity [19].

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**
Biochanin	-8.22	-8.19	-7.81	-7.75	-7.63	-7.11	-6.82	-6.82	-6.79	-6.60
Butein	-9.95	-8.31	-8.11	-8.09	-7.16	-7.12	-6.84	-6.48	-6.45	-6.29
Diosmetin	-8.62	-7.83	-7.53	-7.38	-7.36	-7.34	-7.04	-6.91	-6.74	-6.74
Fisetin	-9.26	-8.50	-8.66	-7.52	-7.38	-7.33	-7.07	-6.83	-6.79	-6.29
Herbacetin	-9.21	-9.20	-8.86	-7.97	-7.58	-7.96	-7.04	-6.55	-6.40	-6.33
Isorhamnetin	-9.50	-7.78	-7.52	-7.38	-7.06	-6.96	-6.81	-6.75	-6.44	-6.28
Rhamnetin	-9.39	-9.15	-8.88	-8.84	-8.37	-7.74	-7.58	-7.36	-6.53	-6.24
Tricetin	-8.67	-8.15	-8.66	-8.03	-7.96	-7.85	-7.81	-6.88	-6.28	-5.85
Tricin	-7.70	-7.59	-7.34	-7.27	-7.20	-7.13	-7.09	-6.66	-6.62	-6.44
Vitexycarpin	-7.49	-7.37	-7.16	-6.72	-6.65	-6.11	-6.65	-6.37	-6.29	-6.19
Genistein	-7.18	-7.16	-6.79	-6.72	-6.71	-6.46	-6.64	-6.60	-6.41	-6.29
Okanin	-9.40	-8.81	-8.56	-7.62	-7.50	-7.32	-7.12	-6.63	-6.57	-6.53
Peonidin	-8.51	-8.42	-7.81	-8.35	-8.34	-8.10	-7.45	-7.40	-7.26	-6.98
Robinetin	-9.37	-9.30	-7.25	-7.19	-6.85	-6.83	-6.48	-6.30	-6.10	-5.98
Allopurinol	-4.47	-4.47	-4.46	-4.46	-4.45	-4.20	-4.09	-4.09	-3.99	-3.87

CONCLUSION

Molecular docking studies of allopurinol with xanthine oxidase enzyme exhibited binding interactions and warrants further studies needed for the development of potent xanthine oxidase inhibitors for the treatment of gout. These results clearly indicate that flavonoids especially, Butein, Fisetin, Isorhamnetin, Rhamnetin, Robinetin and Herbacetin have excellent binding interactions with xanthine oxidase. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of gout and related inflammatory disorders.

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