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DOCKING STUDIES OF FEBUXOSTAT BY USING MOLDOCK SOFTWARE

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ABSTRACT

Febuxostat is a selective, novel, non-purine analog xanthine oxidase (XO) inhibitor for the treatment of chronic hyperuricemia in patients with gout. This enzyme is involved in oxidation of Hypoxanthine and Xanthine to uric acid and the compounds that inhibits the action of xanthine oxidase enzyme helps in lowering the production of uric acid in the body. In this study, molecular docking of Febuxostat has been performed against XO (PDB ID: 3NVY) by using Moldock software and found that the amino acids Glu802, Ala1079 and 880 in the active site of XO are involved in the hydrogen bond formation with Febuxostat. The best conformation of this compound is reported here in which it is active as XO inhibitor and lower the production of Uric acid in body effectively and thus be used for the treatment of Gout.

Key words: Febuxostat, Molecular Docking, MolDock, Xanthine Oxidase

INTRODUCTION

Febuxostat is a selective, novel, non-purine analog XO inhibitor (Takano *et al.*, 2005) for the treatment of chronic hyperuricemia in patients with gout (Uloric, 2011). Gout is a disease which is characterized by elevated level or uric acid in the body. This condition is known as hyperuricemia and is caused by either overproduction or less elimination of uric acid. This uric acid can be accumulated in and around joints and tissues in the forms of crystals and cause pain, inflammation, along with that it may also cause kidney diseases and stones.

Febuxostat selectively inhibits XO independent of the redox state and does not affect other enzymatic pathways in purine/pyrimidine metabolism (Tom *et al.*, 2010). Febuxostat 10-120 mg/day rapidly and sustainably reduces serum uric acid by 25-70% in uric acid under-excretors and overproducers (Khosravan *et al.*,2006; Schumacher, 2005). It acts by binding into a channel in the molybdenum center of the enzyme, leading to a very stable and long-lived enzyme-inhibitor interaction with both the oxidized and reduced forms of the enzyme and, as a consequence, a strong inhibition of substrate binding (Okamoto *et al.*, 2003; Gaffo and Saag, 2009).

Xanthine oxidase (XO) enzyme have been isolated from a wide range of organisms, from bacteria to man, which accelerate the hydroxylation of a wide variety of purine, pyrimidine, pterin, and aldehyde substrates. All of these proteins have similar molecular weights and composition of redox centers (Nishino, 1994; Hille and Nishino, 1995). In humans, the enzyme catalyzes the last two steps of purine catabolism, the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. This reaction occurs at a molybdenum-pterin center and from there the electrons are transferred via two Fe_2S_2clusters to the isoalloxazine ring of FAD, which then passes them on to the second substrate NAD+ (Nishino, 1994; Hille and Nishino, 1995; Bray, 1975; Hille and Massey, 1985; Hille, 1996).

Molecular docking is a simulation process to predict the conformation of a receptor-ligand complex, where the receptor can be a protein and the ligand a small molecule. It can also be defined as a simulation process where a ligand position is estimated in a predicted or pre-defined site in the receptor molecule. All structural based virtual screening projects are based on the hypothesis that it is computationally determining the three-dimensional structure of binary complexes involving a protein and a ligand.

In this study, MolDock software has been used for docking studies. It is based on a new hybrid search algorithm, called Guided differential evolution. Differential evolution was introduced by Storn and Price (1995) and has been applied previously for docking purpose (Thomsen, 2003). The docking scoring function of MolDock is based on a piecewise linear potential (PLP) introduced by Gehlhaar *et al*., 1995; 1998) and further extended in GEMDOCK by Yang and Chen (2004). The scoring function was further improved to include new hydrogen bonding term and new charge schemes (Thomsen and Christensen, 2006). This MolDock software has shown a higher docking accuracy as compare to the other available docking softwares (Table 1) with respect to the identification of ligand-binding modes (Thomsen and Christensen, 2006).

MATERIALS AND METHODS

CHEMSKETCH software is used to draw the structure of Febuxostat. The three dimensional structure of Xanthine Oxidase was retrieved from the Protein Data Bank (http://www.rcsb/pdb; accession code: 3NVY). For docking purpose, MolDock software has been used in this study because of its higher docking accuracy (Table 1) over the other available docking softwares. This software automatically identifies the potential binding site by using the cavity detection algorithm which also includes the bound ligand.

In this study ten independent docking runs have been performed and each of these docking run come up with one pose (docked conformation). Thus 10 solutions (poses) were obtained from 10 independent docking runs (Table 2). Each pose was inspected individually and the interactions of the docked compound with the amino acids in the binding site are viewed. The pose selected as the best is the one with the highest MolDock score. Moldock software also calculates the re-ranked score which is refined score but we are only considering the Moldock score here.

RESULTS AND DISCUSSION

Figure 1 represents the structure of Febuxostat. The crystal structure of Xanthine oxidase enzyme (PDB ID: 3NVY) is used here for docking studies (Fig. 2). In this crystal structure the enzyme is bound to a ligand, quercetin, which forms hydrogen bonds with the amino acid residues Glu 802, Ala 1079, Arg 880 and Thr1010 within the active site of this enzyme (Fig. 3). Figure 4 shows the docking result of febuxostat against XO (3NVY) and represents the conformation of febuxostat within the active site of xanthine oxidase. In this conformation it forms hydrogen bond with the active site residues Glu 802, Ala 1079, Arg 880 (Fig. 4).

Table 1. Comparison of docking accuracy and average RMSD values of MolDock with other available docking programs (Yang *et al.*, 2004).

Method	Docking Accuracy	Average RMSD (Å)
MolDock	87.01%	1.38
Glide	81.82%	1.38
Surflex	75.32%	1.86
FlexX	57.89%	3.15

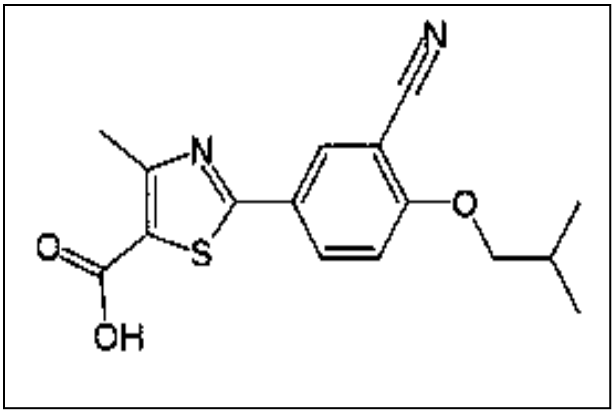

Fig. 1. Structure of Febuxostat

.Table 2. MolDock score for 10 poses obtained upon docking of Febuxostat

Name	Ligand	MolDock Score (Kcal/mol)	Rerank Score (Kcal/mol)	H-Bond Interaction
1	Pose 1	-121.325	-92.1752	-4.72481
2	Pose 2	-121.295	-96.8276	-4.01992
3	Pose 3	-116.819	-96.1296	-2.481
4	Pose 4	-114.223	-77.9041	-4.13136
5	Pose 5	-105.498	-87.8468	-1.97683
6	Pose 6	-102.84	23.1499	-6.08607
7	Pose 7	-121.146	10.8996	-1.83293
8	Pose 8	-92.7268	7.62667	-1.54852
9	Pose 9	-93.1528	-39.9885	-4.03662
10	Pose 10	-80.212	-20.623	-2.954

The comparison of the Figure 3 and 4, that presents the binding mode of the quercetin (bound ligand) and the docking results of febuxostat, respectively, shows that febuxostat forms the hydrogen bonds with most of the active site residues as observed in case of bound quercetin within the crystal structure. The amino acid residues within the active site that are involved in hydrogen bond formation in both the two cases are Glu 802, Ala 1079 and Arg 880

(Fig. 3 and 4). Figure 5 presents a comparison of binding mode of quercetin and febuxostat with in the active site of XO and it shows that the febuxostat was docked in the same position where quercetin was bound in the crystal structure. These docking results indicate the conformation of febuxostat in which it could be more potent and help in future studies to synthesize and formulate febuxostat that can be used for the treatment of hyperuricemia related gout.

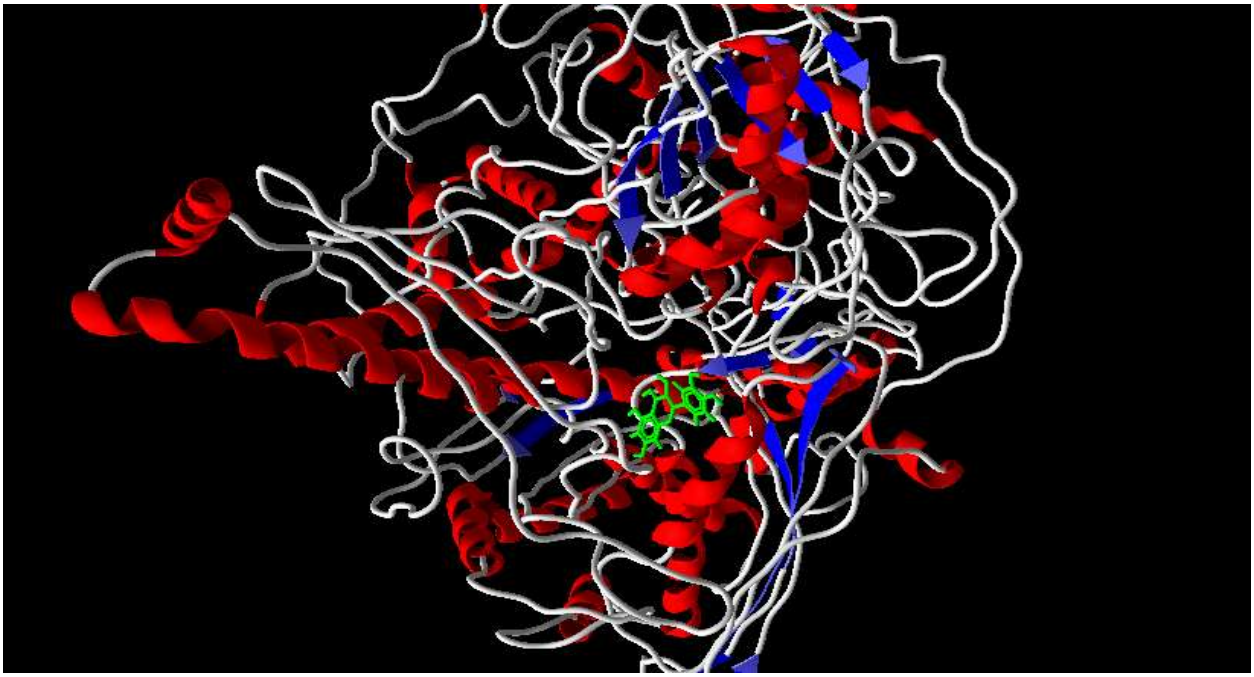

Fig. 2: Secondary structure of Xanthine Oxidase (PDB ID: 3NVY) with Quercetin (green colour) as bound ligand.

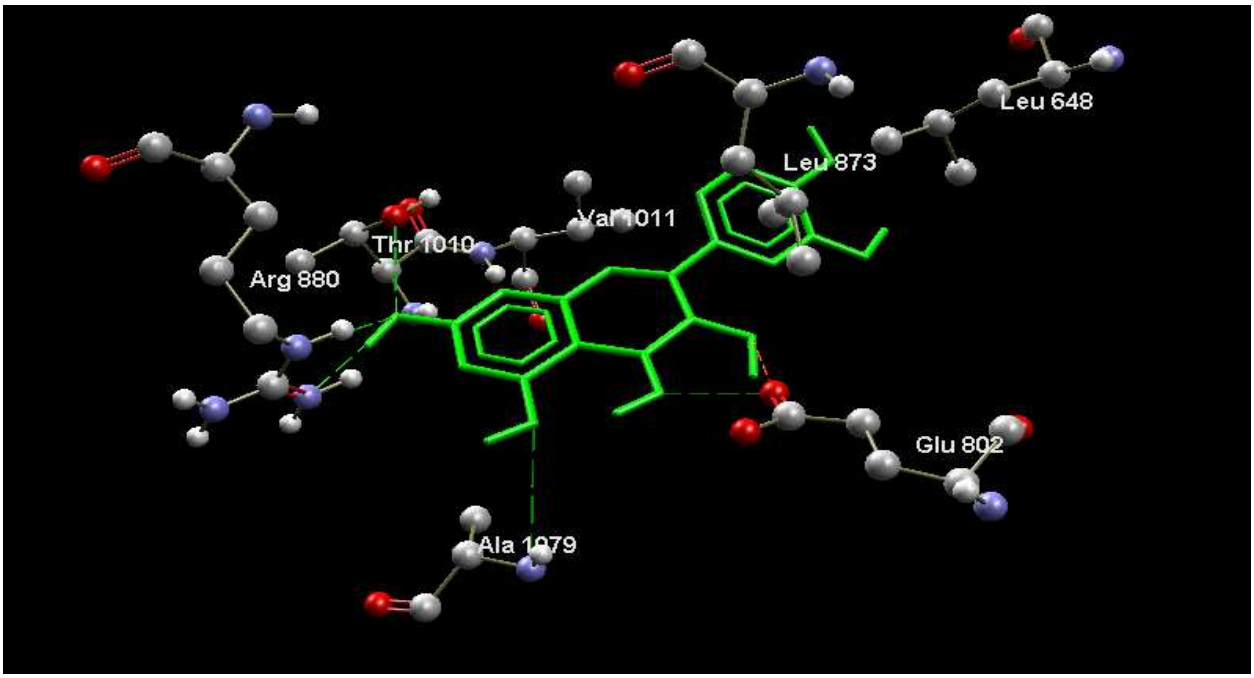

Fig. 3: Binding of quercetin with in the active site of Xanthine oxidase (PDB ID: 3NVY).
(Amino acids in the active site are presented in ball and stick by element colour and bound ligand (quercetin) is presented in green colour. Green lines shows hydrogen bonds)

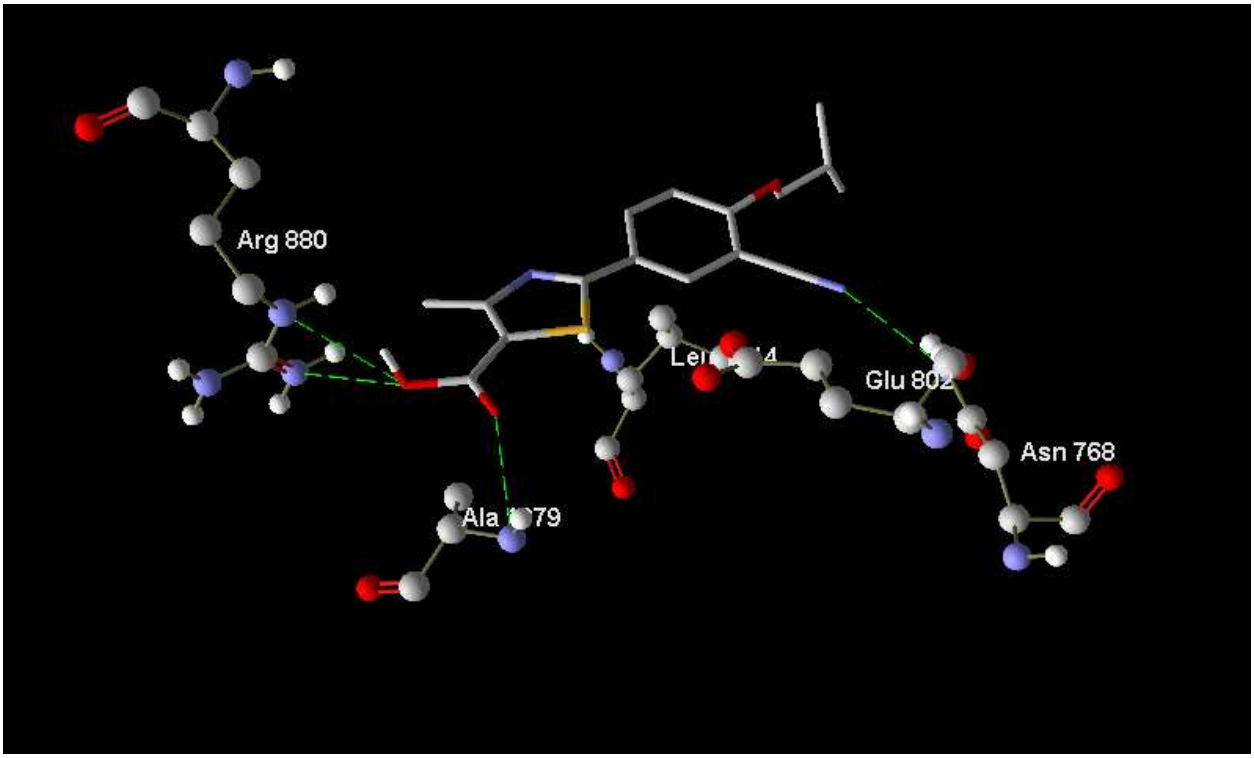

Fig. 4: The best docking solution of Febuxostat against Xanthine Oxidase (PDB ID 3NVY). (Amino acids in the active site are presented in ball and stick by element colour and docked febuxostat is presented in solid lines by element colour. Green lines shows hydrogen bonds)

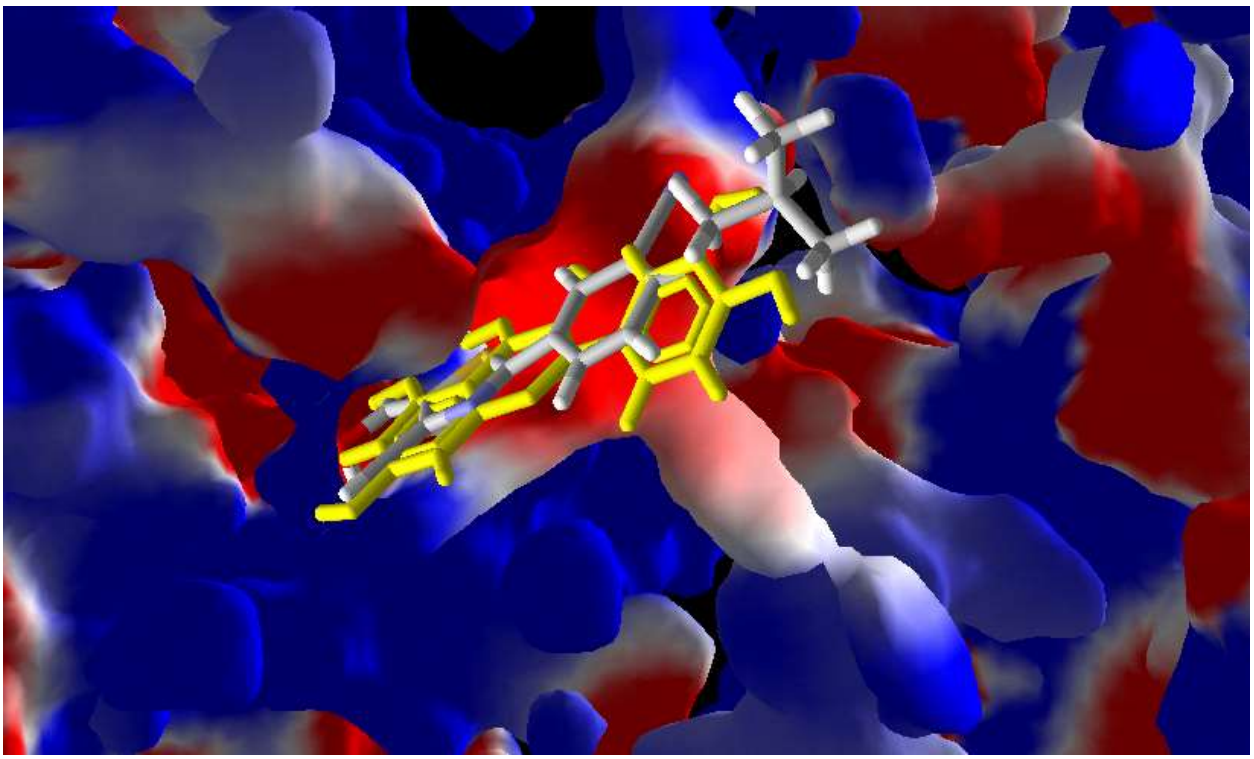

Fig. 5: Comparison of binding mode of quercetin (yellow colour) and docked febuxostat (by element colour) within the active site of XO (PDB ID: 3NVY)

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