Journal of Applied Pharmaceutical Science Vol. 3 (02), pp. 127-131, February, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.30222
ISSN 2231-3354

Molecular docking studies of guggultetrol from *Nymphaea pubescens* with target glucokinase (GK) related to type-II Diabetes

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ARTICLE INFO

Article history:
Received on: 07/12/2012
Revised on: 31/12/2012
Accepted on: 07/02/2013
Available online: 28/02/2013

Key words:
Diabetes mellitus, *Nymphaea pubescens*, Guggultetrol, Glucokinase, Docking,

ABSTRACT

Diabetes prevalence is one of the life threatening diseases in India. In this work we address a specific suitable ligand for diabetes mellitus. A large focus has been on structure based drug designing. Guggultetrol isolated from *Nymphaea pubescens*was taken as ligand for molecular docking. A theoretical docking study, the evaluation of guggultetrol as inhibitor of Glucokinase (PDB ID: 1V4S) a validated drug target enzyme of the Type-II diabetes, was taken up. Guggultetrol was found to bind at active site of glucokinase with lowest binding energy and RMSD values to be -9.45Kcal/Mol and 2.0 Å respectively. Docking analysis of 1V4S with ligand enabled us to identify specific residues viz. Thr-168, Glu-290, Glu-51, Ser-411, Gly-410, Asn-254, Thr-206, Arg-155 and Asp-205 within the 1V4S binding pocket to play an important role in ligand binding affinity. The docking studies of the Guggultetrol with target protein showed that this is a suitable molecule which docks well with target related to diabetes mellitus. This compound has shown promising biological activity in preliminary studies by targeting multiple signaling pathways. Thus on the basis of our *in silico* studies we hypothesize that this compound into guggultetrol can be inhibitory effect on against diabetes. We concluded that the natural products with interesting biological properties and structural diversity have often served as valuable lead drug candidates for the treatment of human diseases.

INTRODUCTION

Nymphaea pubescens is an aquatic fresh water rhizomatous herb; leaves long petioled, peltate, orbicular, sagitate when young. The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative. They are useful in vitiated conditions of pita, dipsia, diarrhea and dermatopathy (Muthulingam, 2010). The whole plant or the parts of the plant used in siddha and folk medicine for treating diabetes, bleeding piles, dyspepsia, jaundice and eye disorders (Selvakumari *et al.*, 2010). The root stock and leaves are cooling, bitter, sweet, aromatic, alterant, diuretic and tonic. From the same genus Studies of antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats (Rajagopal *et al.*, 2008) were done. Nearly 20 antioxidants isolated from the *Nymphaea* flowers, including two 2S,3S,4S-trihydroxypentanoic acid and myricetin 3-O-(3"-O-acetyl)-alpha-l-rhamnoside (Zhang *et al.*, 2003).The plant has to possess antidiabetic (Selvakumari *et al.*, 2010 ; Karthiyayini *et al.*, 2011; Shajeela *et al.*, 2012) antihepatotoxic (Muthulingam, 2010), hypolipedemic (Shajeela *et al.*, 2012) activities. Diabetes mellitus is a quick prevalence of disorders with different etiologies. Diabetes claims a life every 10 seconds and in two decades time, 350 million people will be diabetic, if no action is taken (http://www.eatlas.idf.org/Cost). Type-2 diabetes mellitus is a heterogeneous metabolic disorder characterized by the impairment of insulin secretion from pancreatic beta cells and insulin resistance in peripheral tissues such as liver, skeletal muscle, and adipose tissue (Yoon *et al.*, 2007). It is associated with high risk of atherosclerosis and renal, nervous system and ocular damage (Habif *et al.*, 1997). Management of diabetes without any side effects is still a challenge to the medical system.Having being termed the silent epidemic, diabetes continues to ignite and sustain motivation in finding a cure. This leads to increasing demand for natural products with antidiabetic activity with fewer side effects. However, the efficacy of the medicinal plants, interaction with other medication, dosage requirements and possible toxicity needs extensive further research. There is also a possibility that medicinal plants used for diabetes may reveal another way of treatingthe disease that may even be more efficient than current treatments. Bioinformatics tools have become very important to pinpoint the targets for different ligands.

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Many studies have indicated that computational approaches, such as structural bioinformatics (Chou *et al.,* 2004; Chou *et al.,* 2004) molecular docking (Wang *et al.,* 2011; Chou *et al.,* 2003) pharmacophore modeling (Sirois *et al.,* 2004) are best choice. Using bioinformatics tools we tried to evaluate whether Guggultetrol (Sukh dev, 1989) is a good ligand of the target protein related to diabetes such as glucokinase. Glucokinase (GK) is a one of the hepatic enzymes.

The liver removes a considerable portion of glucose from the blood and thus is especially suited to metabolize large quantities of carbohydrates. Hexokinase IV otherwise known as glucokinase (GK) is about half the molecular mass of the other hexokinases (I, II and III) (Postic *et al.,* 2001). GK is the chief glucose-phosphorylating enzyme in the liver; its abundance is regulated at a variety of levels, including transcriptionally by insulin and glucagon, and post-translationally by the GKRP. Hence GK initiates glycolysis unlike hexokinase (Collier *et al.,* 2004).

GK displays sigmoidal kinetics and its activity is not altered significantly by physiological concentrations of G6P (Postic *et al.,* 2001), however small changes in GK concentration are significant as they have an impact on the rate of glucose stimulated insulin secretion as well as the rate of glucose metabolism. GK also has a significant role in glucose utilization and glycogen synthesis (Postic *et al.,* 2001) and GK activity increases and decreases parallel to changes in blood glucose levels within the physiological range. Glucose metabolism proceeds with phosphorylation of the hexose and its subsequent metabolism via various pathways (Collier *et al.,* 2004).

GK plays a vital role in glucose disposal (Agius, 2008) and GK activity is highly correlated with blood glucose concentration and is thus considered to be a hepatic "glucose-sensor" (Collier *et al.,* 2004) for insulin secretion in the β cells of the pancreas (Matschinsky *et al.,* 2006). The inhibitory protein; glucokinase regulatory protein (GKRP) regulates GK. At basal glucose concentrations (5mmol/l) GKRP binds to GK with high affinity, blood glucose homeostasis is maintained by the liver by glucose production via glycogenolysis from glycogen and via gluconeogenesis from lactate and other gluconeogenic substrates. During the postprandial state, hyperglycemia (glucose concentrations >5 mmol/l) causes dissociation of GK from GKRP and translocation of GK into the cytoplasm; this results in increased glucose phosphorylation to G6P and conversion to glycogen, lactate, and triglyceride (TG). If TG production via glycolysis to pyruvate and synthesis of fatty acids exceeds the very low density lipids (VLDL) secretion capacity, TG accumulates in the hepatocyte (Agius *et al.,* 2009).

In high fat fed rats with hepatic insulin resistance, the activities of G6Pase and GK are dramatically increased and decreased respectively. During insulin resistance, the increased G6Pase activity contributes to increased hepatic glucose production (Minassian *et al.,* 1998). GK activators can alleviate the hyperglycemia associated with type-II Diabetes by increasing hepatic glycogen synthesis and glucose stimulated pancreatic insulin release (Castelhano *et al.,* 2005). The above mentioned target was subjected to *in silico* docking for the first time; we have isolated Guggultetrol from *Nymphaea pubescens* and established its antidiabetic property.

MATERIALS AND METHODS

Python 2.7 - language was downloaded from www.python.com, Cygwin 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.

The docking of Guggultetrol into the binding site of the 1V4S protein was explored using Autodock software, which has been shown to be powerful tools for molecular recognition. To validate the molecular modeling programs, we first evaluated the docking accuracies of Autodock by docking with known 1V4S inhibitor, Guggultetrol into the binding site.

Study of Molecular Docking

Protein Preparation for Docking

The 3D structure of Glucokinase (PDB ID: 1V4S) was downloaded from Protein Data Bank (PDB) (http: //www.pdb.org/pdb/home/home.do), before initiating the docking simulations, all non-protein molecules were removed from 1V4S; for any alternative atom locations only the first location was retained. All the docking calculations were performed by using Autodock 4.0. Glucokinase was modified by adding polar hydrogens and then kept rigid in the docking process, whereas all the torsional bonds of ligands were set free by Ligand module in Autodock Tools. Various inhibitors provide an excellent basic for using structure-based approaches for the discovery of new inhibitors.

Ligand Preparation for Docking

The steroid ligand like Guggultetrol was built using Chemsketch and optimized using “Prepare Ligands” in the AutoDock 4.2 for docking studies. The optimized ligand molecules were docked into refined Glucokinase model using “LigandFit” in the AutoDock 4.2.

Molecular docking

AutoDock 4.2 suite was used as molecular-docking tool in order to carry out the docking simulations. The Auto Dock 4.2 program was used to investigate ligand binding to structurally refined 1V4S model using a grid spacing of 2.0 Å and the grid points in X, Y and Z axis were set to 60 × 60 × 60. The grid center was placed in the active site pocket center. The grid boxes included the entire binding site of the enzyme and provided enough space for the ligand translational and rotational walk. At first dock pdb.qt files for protein and ligands were prepared. The search was based on the Lamarckian genetic algorithm and the results were analyzed using binding energy. For each ligand, a

docking experiment consisting of 100 stimulations was performed and the analysis was based on binding free energies and root mean square deviation (RMSD) values, and the ligand molecules were then ranked in the order of increasing docking energies. The binding energy of each cluster is the mean binding energy of all the conformations present within the cluster, the cluster with the lowest binding energy and higher number of conformations within it was selected as the docked pose of that particular ligand. The clusters were ranked by the lowest-energy representative of each binding mode.

The rest of the parameters were set as default values. At the end of a docking experiment with multiple runs, a cluster analysis was performed. Substrate docking with natural plant substrate sterol family of guggultetrol was performed on to 1V4S model with same parameters and PMV 1.4.5 viewer was then used to observe the interactions of the docked compound to the 1V4S model.

RESULT AND DISCUSSION

A detailed literature survey yielded that the inhibitory pathway has to be considered as a potential drug target against the anti-diabetic using bioinformatics tools. We can hypothesize that guggultetrol may be considered to play an important role in inhibiting the 1V4S activity and found as the most active compound in the respective target site. This guggultetrol can be promising candidate for the development towards the design of one of the key targets for Type 2 diabetes drug as therapeutic compound.

In silico docking of guggultetrol for 1V4S inhibition

Molecular docking methods are commonly used for predicting binding modes to proteins and energies of ligands (Bikadi *et al.*, 2009).

Docking was accomplished employing Autodock 4.0 which is a suite of automated docking tools was used to predict the affinity, activity, binding orientation of guggultetrol to our target protein molecule 1V4S. Analysis was based on Etotal or free energy of binding, lowest docked energy, and calculated RMSD values. For each approach, the number of hits, the RMSD value of the best hit (with the lowest RMSD) based on shape complementarity are listed in Table 1. Guggultetrol was found to bind at active site of 1V4S with lowest binding energy and RMSD values to be -9.45Kcal/Mol and 2.0 Å respectively (Table. 1).

Free energy of binding is calculated as a sum of four energy terms of intermolecular energy (vanderwaal, hydrogen bond, desolvation energy and electrostatic energy), total internal energy, torsional free energy and unbound system energy. From the results it has been clearly observed that guggultetrol formed nine hydrogen bond interactions with glucokinase was shown in Fig. 1.

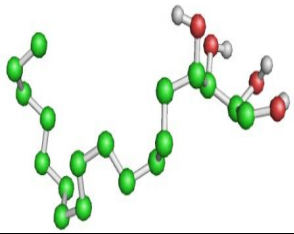
Docking analysis of 1V4S with ligand enabled us to identify specific residues viz. Thr-168, Glu-290, Glu-51, Ser-411, Gly-410, Asn-254, Thr-206, Arg-155 and Asp-205 within the 1V4S binding pocket to play an important role in ligand binding affinity. The docking of 1V4S and guggultetrol is shown in Fig. 2. Our *in silico* experiments demonstrate that guggultetrol binds 1V4S, and also is itself inhibits its function and thus may act as a drug.

CONCLUSION

Natural compounds have played an important role in treating and preventing human diseases. Here, based on the above *in silico* study, Here, we focused that the binding of naturally occurring molecules were seated properly on the particular position and the hydrogen and hydrophobic interactions involves in the position of Thr-168, Glu-290, Glu-51, Ser-411, Gly-410, Asn-254, Thr-206, Arg-155 and Asp-205 within the 1V4S, residues.

Hence, the proposed drug is presented to the scientific community for further investigational confirmation. The results of the present study clearly demonstrated the *in silico* molecular docking studies of guggultetrol with Glucokinase enzyme exhibited binding interactions and warrants further studies needed for the development of potent glucokinase inhibitor for the treatment of Type-II diabetes.

Table. 1: Docking results of Guggultetrol molecules docked on to Glucokinase (1v4s) model.

S. No	Lead Molecule	Cluster Rank	Run	RMSD from reference structure (Å)	Estimated Free Energy of Binding (kcal/mol)	Docked energy (kcal/mol)	Estimated Inhibition Constant, Ki uM or mM (micromolar/millimolar) [Temp= 98.15K]
1.	**Guggultetrol**	1	44	0.252	-9.45	-9.25	01.89 Mm
2.		2	15	0. 876	-5.43	-7.74	105.21 Mm
3.		3	8	0.773	-3.72	-8.02	10.77 Mm
4.		4	42	0.925	-6.27	-7.34	13.37 Mm
5.		5	28	0.526	-5.73	-7.55	48.94 Mm
6.		6	50	0.487	-4.86	-6.53	38.56 Mm

Number of distinct conformational clusters found = 06, out of 50 runs,
Using an RMSD -tolerance of 2.0 Å

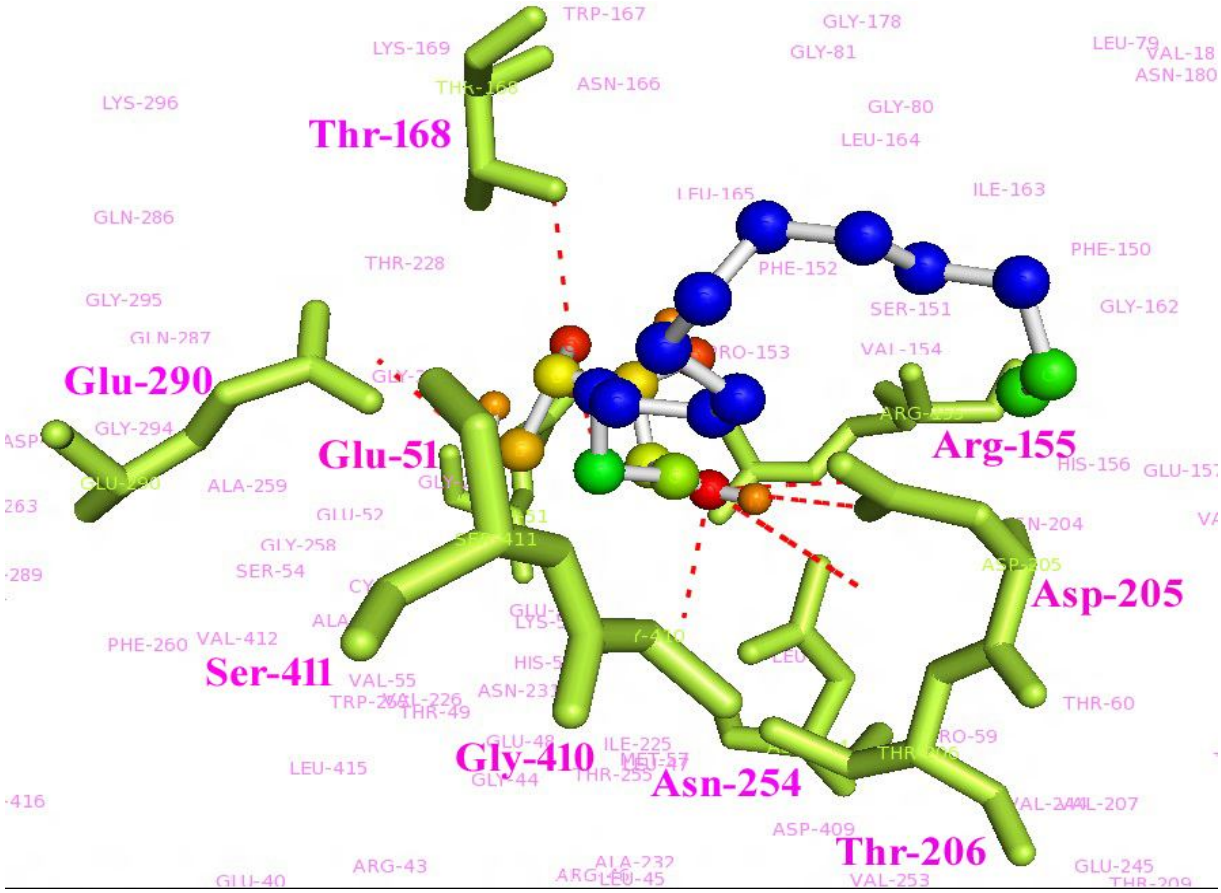


Fig. 1: The binding of guggultetrol with active site of glucokinase (1V4S) was having 7-H bond interaction. The hydrogen bond was formed between Thr-168, Glu-290, Glu-51, Ser-411, Gly-410, Asn-254, Thr-206, Arg-155 and Asp-205.

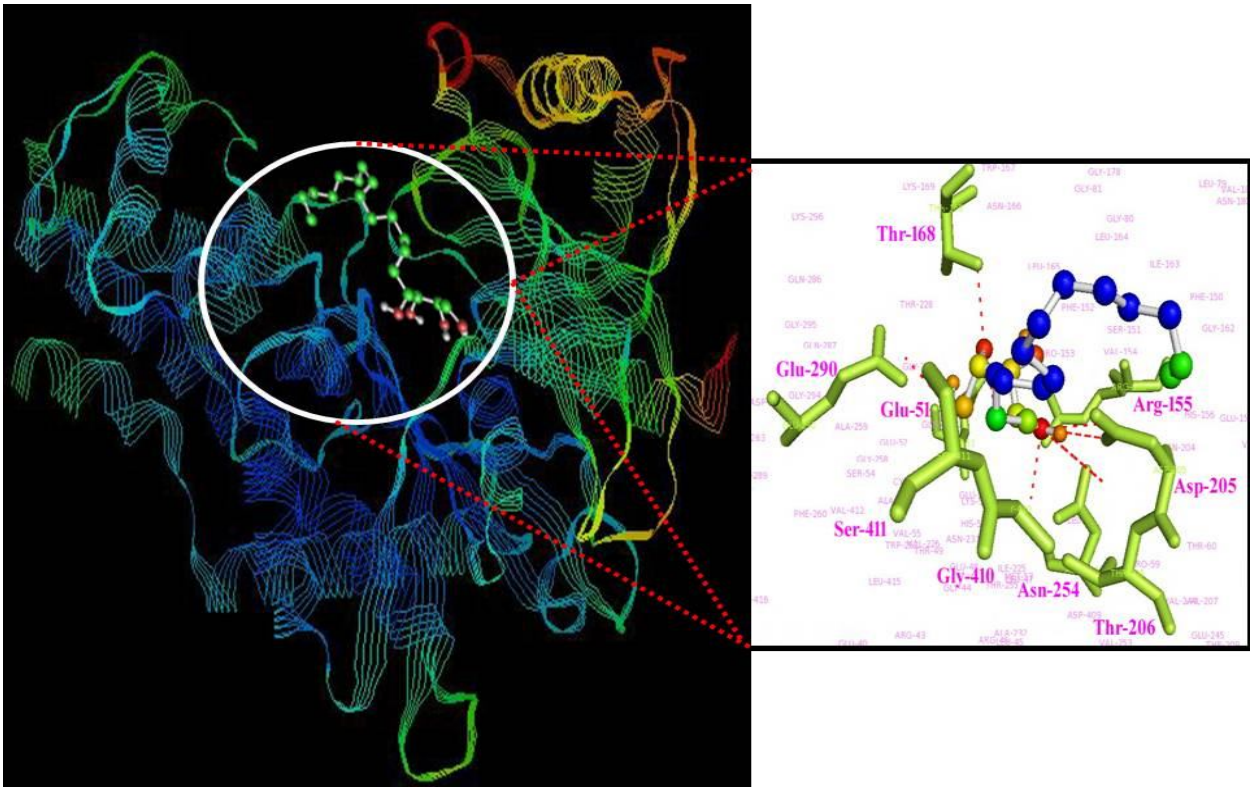


Fig. 2: *In silico* analysis of the interaction of 1V4S with guggultetrol. 1V4S amino acids Thr-168, Glu-290, Glu-51, Ser-411, Gly-410, Asn-254, Thr-206, Arg-155 and Asp-205 from hydrogen bonds with guggultetrol.

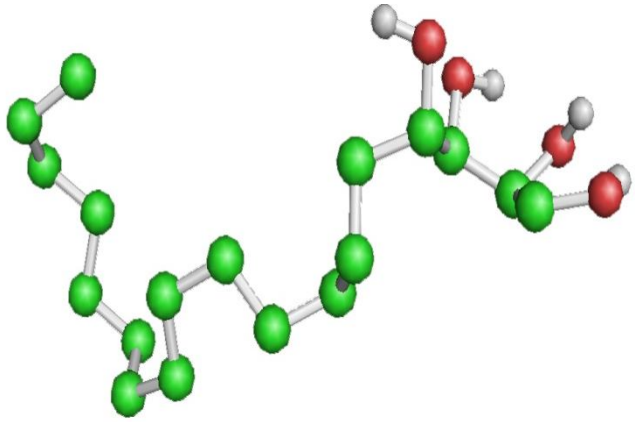


Fig. 3: Stick and boll structure of guggultetrol.

ACKNOWLEDGEMENTS

AKK gratefully acknowledges University Grant Commission for financial support. The encouragement given by Department of Biotechnology, Acharya Nagarjuna University, India was providing laboratory and technical support. RKG wants to thank D.S. Kothari Post-Doctoral Fellowship, University Grants Commission, Government of India for financial and the Molecular Biology Unit, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India for providing laboratory and technical support to our research work is gratefully acknowledged.

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How to cite this article:

Kiran Kumar Angadi, Ravi Kumar Gundampati, Medicherla V. Jagannadham, Ammani Kandru., Molecular docking studies of guggultetrol from Nymphaea pubescens with target glucokinase (GK) related to type-II Diabetes. J App Pharm Sci. 2013; 3 (02): 127-131.