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

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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, Gln-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, dipsa, diarrhoea 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-trihydroxyxypentanoic 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; Shajeeja et al., 2012) antihepatotoxic (Muthulingam, 2010), hypolipidemic (Shajeeja 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.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 treating the 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., 2005) and molecular docking (Wang et al., 2011; Chou et al., 2003) 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 Chemskech 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 pdbqt 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
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.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Lead Molecule</th>
<th>Cluster Rank</th>
<th>Run</th>
<th>RMSD from reference structure (Å)</th>
<th>Estimated Free Energy of Binding (kcal/mol)</th>
<th>Docked energy (kcal/mol)</th>
<th>Estimated Inhibition Constant, Ki (μM or mM) [Temp= 98.15K]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Guggultetrol</td>
<td>1</td>
<td>44</td>
<td>0.252</td>
<td>-9.45</td>
<td>-9.25</td>
<td>01.89 Mm</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>2</td>
<td>15</td>
<td>0.876</td>
<td>-5.43</td>
<td>-7.74</td>
<td>105.21 Mm</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>3</td>
<td>8</td>
<td>0.773</td>
<td>-3.72</td>
<td>-8.02</td>
<td>10.77 Mm</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>4</td>
<td>42</td>
<td>0.925</td>
<td>-6.27</td>
<td>-7.34</td>
<td>13.37 Mm</td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td>5</td>
<td>28</td>
<td>0.526</td>
<td>-5.73</td>
<td>-7.55</td>
<td>48.94 Mm</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>6</td>
<td>50</td>
<td>0.487</td>
<td>-4.86</td>
<td>-6.53</td>
<td>38.56 Mm</td>
</tr>
</tbody>
</table>

*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.

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