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In Silico Molecular Modelling and Docking Studies of *Sophora flavescens* Derived Flavonoids against SGLT2 for Type 2 Diabetes Mellitus

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ABSTRACT

Sophora flavescens (S. flavescens), which is used in traditional Chinese medicine, It showed potent Na+-glucose co-transporter (SGLT) interruption activity. SGLT interruption have therapeutic potential inhibitor for diabetes mellitus. *S. flavescens* have diverse substantial activities for anti-oxidant, anti-diabetic, anti-bacterial, anti-asthmatic, anti-pyretic, anti-ulcerative, anti-arrhythmic, anti-inflammatory, and anti-neoplastic effects. Sophora species are widely used in the therapy of many diseases such as cancer, asthma, ulcer, diabetes and it's also used for oilments. Sophora is rich in alkaloids, flavonoids, triglycosides, isoprenylated flavonoids, isoflavonones, saponins, triterpenoid glycosides, phospholipids and polysaccharides. *S.flavescens* is derived compounds such as Maackiain, Variabilin, Pterocarpin, Sophoraflavone G and Formononetin etc. NIDDM is a chronic disease associated with the metabolic impairment of insulin actions, dominant to the evolution of serious complications. SGLT-2 inhibitors provide a unique way to treat the diabetes mellitus by decreasing hyperglycemia through increased glucosuria. This explosion decreases renal glucose tubular constriction in the proximal renal tubules imparting an insulin independent mechanism to curtail blood glucose. Homology modeling was performed using Schrodinger Glide 11.9 Advanced Homology Modelling to anticipate the 3D Complex of SGLT-2. In this present study, we established a novel SGLT2 inhibitor by means of docking. Docking was carried out using GLIDE software. The ligand Formononetin with the Glide score -5.074, shows the binding affinity with the amino acid (AA) residues TYR263, GLN428, GLN69, TYR87 and GLU88. These residues are acting as a conclusive pocket for the potential ligand. Hence, it has been concluded Formononetin as a potent inhibitor for SGLT2 Protein in Type 2 Diabetes.

Keywords: *Sophora flavescens (S.flavescens)*, Flavonoids, SGLT2, Formononetin, Type 2 Diabetes, Glide 11.9 and Homology Modelling.

Type 2 diabetes mellitus affects approximately 300 million peoples in worldwide, including more than a quarter of elderly population living in developed countries[1]. According to the American Diabetes Association (ADA), diabetes affects more than 20 million Americans about 8 percent of the US population. In India it is estimated that the total number of people with diabetes in 2010 to be around 50.8 million, rising to 87.0 million by 2030. Unfortunately, the rate of new cases and the death rate due to diabetes has been rising. Diabetes is characterized by chronically elevated serum glucose levels resulting in damage of several tissues (e.g. retina, kidney, nerves) due to higher protein glycation, retardation of wound healing, impaired insulin secretion, enhanced insulin resistance, cell apoptosis, and increased oxidative stress. Type 2 diabetes Mellitus (T2DM), representing 90-95 % of all diabetic cases, is a multi-factorial disease. The pathogenesis of type 2 diabetes is complex involving

progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic beta cells leading to overt hyperglycemia[2]. Sodium glucose co-transporter 2 (SGLT2) inhibitors are compounds with a new approach which is different from the currently available therapies. The mechanism action of SGLT2 is to interfere with sodium glucose co-transporter in the S1 segment of the proximal convoluted tubule. This class of drugs target insulin resistance and insulin deficiency, providing glucose dependent and insulin-independent pathway to control hyperglycemia[3]. This class of drugs have unique property of inducing weight loss and also useful in the treatment of type1 diabetes as its mechanism is insulin independent. SGLTs inhibitors are the agents which inhibit the membrane protein sodium glucose co-transporter, play an important role in the reabsorption of glucose[4]. Six isoforms of SGLTs, SGLT1 to SGLT6[5,6]. Among these only one isoforms SGLT2 are well investigated. SGLT2 is specially expressed in renal uriniferous tubules, a low-affinity, high capacity transporter. It plays critical role in renal glucose absorption[7]. Approximately 90-99% blood glucose is filtered through glomeruli and reabsorbed via SGLT in the renal uriniferous tubules. SGLT inhibitors work on urinary sugar excretory mechanism. Inhibition of SGLT leads to decrease glucose reabsorption, results in the urinary sugar excretion and normalize the blood glucose level without severe side effect[8]. SGLT2 is thought to be effective way for diabetes treatment[9]. Few SGLT2 inhibitors are in phase III clinical trials from which Dapagliflozin, a C-glycoside derivative and the first SGLT2 inhibitor came to the market[10].

Sophora is a genus of the Fabaceae family, contains about 52 species, nineteen varieties, and seven forms that are widely distributed in Asia, Oceanica, and the Pacific islands[11]. In the last decades the use of this genus in traditional Chinese drugs has led to rapid increase in the information available on active components and reported to posses various pharmacological/therapeutic properties, in particular Sophora alkaloids have been found to be their chief active chemical constituents including matrine, oxymatrine, sophocarpine, sophoramine, sophoridine and others[12-17]; along with flavonoids , iso flavonoids isoprenylated flavonoids[18], isoflavonones, flavones, flavonols and their glycosides, coumarochromones[19], saponins, triterpene glycosides, phospholipids, polysaccharides, oligostilbenes and fatty acids[20]. A number of quinoline alkaloids, prenylated flavonoids and oligostilbenes, were used as chemotaxonomic markers[21]. Several phytochemical researches, in-vivo and in-vitro experiments and clinical practices have demonstrated that Sophora constitutes many phyto-constituents possessing wide-reaching pharmacological actions, including anti-oxidant, anticancer, anti-diabetic, anti-asthamatic, anti-neoplastic, antimicrobial, antiviral, antipyretic, cardiotonic, antinflammatory, diuretic and in the treatment of skin diseases like eczema, colpitis and psoriasis. *S.flavescens* is derived compounds such as Maackiain, Variabilin, Pterocarpin, Sophoraflavone G and Formononetin etc.[22].

DATABASE AND METHODOLOGY

Ligand Preparation

Ligprep module of the Maestro was used for the development of ligands. It involves various steps perform conversions, apply corrections to the structures, generate variations on structures, remove unwanted structures and optimize them which was controlled by selecting options in the ligand preparation panel by specifying command line options.

Molecular Modeling

This was done by using GLIDE (Grid- based Ligand Docking with Energetics) software developed by Schrodinger Maestro 11.9 Graphical User Interface (GUI) was used for the ligand preparation, protein preparation and docking.

Docking by Glide

Docking was performed with GLIDE XP. The prepared ligands were docked into the binding pocket of the

protein SGLT-2 (3DH4). Scoring grids were placed at the center of crystal structure of compounds during docking. Glide XP (Extra precision) was used to perform docking calculations. The docked images with least glide score were selected because the perfect binding interactions are given by the most negative glide score.

Induced Fit Docking (IFD)

IFD of GLIDE 11.9 from Schrodinger was used to conduct the prepared ligand molecules with the prepared protein 3DH4. Here, the ligand and the receptor are flexible, which helps to bind the ligand at different sites on the receptor and generate various poses of the receptor- ligand complex, each ligand having unique structural conformations and Glide score (G-score). The derivatives were docked at the active site of 3DH4 individually. The poses generated were ranked on the basis of G score. From this Glide score, the best docked complex can be identified[23,24].

G-score is determined by considering the parameters like Hydrogen bonding (H bond) Hydrophobic contacts (Lipo), Vander Waals (vdW), Columbic (Coul), Polar interactions in the binding site (site), Metal binding term (Metal) and the penalty for buried polar group (Bury P) and freezing rotatble bonds (Rot B)[25].

Visualization and Analysis

Study of hydrogen bond, hydrophobic and pi-pi interactions was shown by Glide 11.9.

RESULTS AND DISCUSSION

Homology Modeling

The Primary Sequence of SGLT2 to be modeled in Glide 11.9 Advanced Homology Modelling Tool and searched against Protein Data Bank to identify the template. The structure with the higher sequence similarity to the modeled sequence has been selected as a template. The template for the Homology Modeling MFS (Major facilitator superfamily) transporter in complex with inhibitor (3DH4.PDB) was Identified (Fig. 1). Using the template which is downloaded from PDB and the primary sequence, the SGLT2 structure is generated by the Glide 11.9 software.

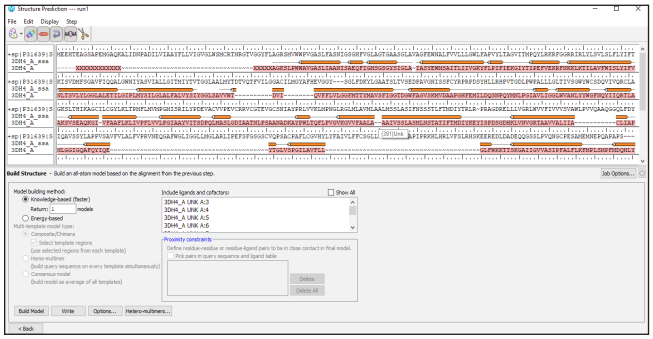

Fig. 1. **The pair-wise sequence alignment between SGLT; PDB_ ID: 3DH4 and SGLT2 used for homology modeling**

Structure Validation

Protein Structure validation is done to evaluate the SGLT2 inhibitor which is modeled in Glide 11.9. Ramachandran plot shows the phi-psi torsion angles for all residues in the structure. The coloring/shading on the plot represents different regions labelling of residues in disallowed regions can be switched off or alternatively can be extended into other region (Fig. 2 and 3).

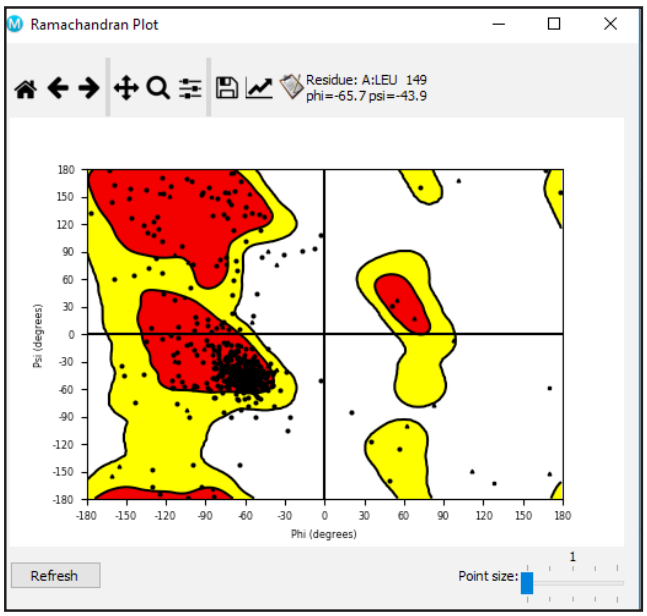

Fig. 2: Ramachandran plot

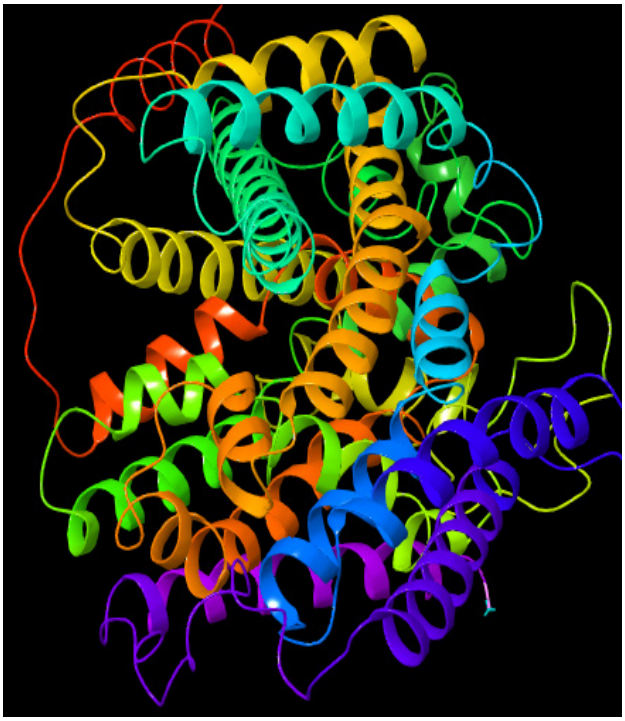

Fig. 3: Crystal structure of SGLT2

Molecular Docking Studies

The molecular docking studies of the designed ligands with protein active sites were performed by an advanced molecular docking program Schrodinger Maestro 11.9 version to determine the various binding affinities of the compounds. Molecular Docking is an effective and competent tool for *In silico* screening. Molecular docking may be defined as an optimization problem which would describe the best-fit orientation of ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or molecules. In modern drug designing molecular docking is routinely used for understanding drug-receptor interaction[26]. The designed compounds are docked towards the SGLT2 (3DH4) inhibition activity. The compounds Formononetin (Figure 4) showed good affinity to the receptor. The compounds Maackiain, Variabilin, and Formononetin have more Glide scores. This is due to more lipophilic evidence and hydrogen bonding. The results are summarized in the Table 1. The best affinity modes of the top one docked compound (Formononetin) with SGLT2 having good Glide score are shown in Figure 4 [27].

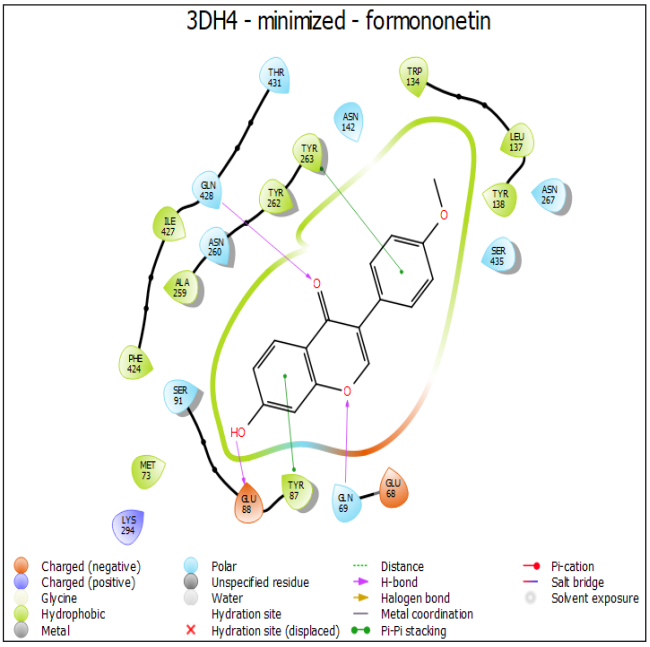

Fig. 4: Schematic 2D representation of Formononetin in the binding pocket of 3DH4

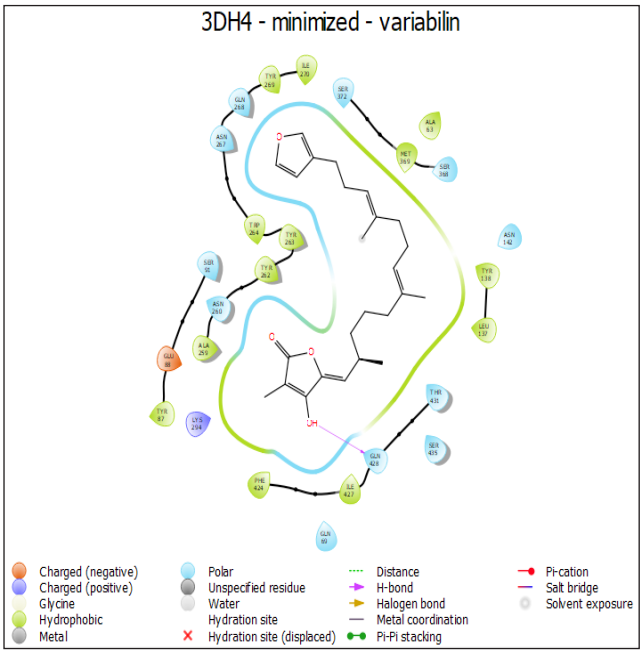

Fig. 5: Schematic 2D representation of Variabilin in the binding pocket of 3DH4

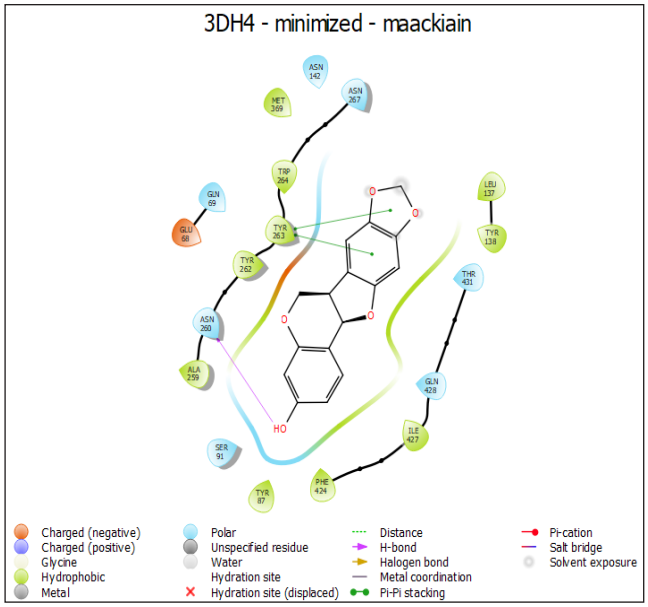

Fig. 6: Schematic 2D representation of Maackiain in the binding pocket of 3DH4

Table 1: Results of Docking analysis of *S.flavescens* against modelled protein of 3DH4.

Title	Docking Score	Glide Score	Xp Score	Mmgbsa Dg Bind
Maackiain	-5.848	-5.848	-5.848	-26.36
Variabilin	-5.438	-5.438	-5.438	-32.37
Formononetin	-5.074	-5.074	-5.074	-18.3

CONCLUSION

Formononetin compound are eco-friendly, safer and cheaper for the remedy of Diabetes Mellitus. The intention of this study is focused to examine the comparative molecular docking studies on the target modelled protein of SGLT2 (3DH4) which is responsible for Diabetes with the ligand of Maackiain, Variabilin, and Formononetin. The comparative docking studies was done by "Schrodinger Maestro 11.9". Formononetin is having best binding score (-5.074 Kcal/mol) than the other compounds. The ligand Formononetin with the Glide score -5.074, shows the binding affinity with the amino acid (AA) residues TYR263, GLN428, GLN69, TYR87 and GLU88. These residues are acting as a conclusive pocket for the potential ligand. Hence it has been concluded Formononetin as a potent inhibitor for SGLT2 Protein in Type 2 Diabetes.

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