May – June 2020
ISSN: 0193-4120 Page No. 5485-5490

# Detection of Selective Glucose Transporter Activators for Type 2 Diabetes by Artificial Intelligence Modules

Vani Mathakalal[1], Ritesh Siddartha Reddy[2], Uma Maheswari Devi Palempalli[3]
[1,3]Department of Applied Microbiology & BioChemistry,
Sri Padmavati Mahila Visvavidyalayam, Tirupati
[1]vanibio456@gmail.com, umadevi66@gmail.com (Corresponding Author)
[2]Sri Venkateswara Medical College, Tirupati

***Article Info***
***Volume 83***
***Page Number: 5485 - 5490***
***Publication Issue:***
***May - June 2020***

***Article History***
***Article Received:*** *19 November 2019*
***Revised:*** *27 January 2020*
***Accepted:*** *24 February 2020*
***Publication:*** *17 May 2020*

**Abstract**

Type 2 diabetes (T2D),a global pandemic disease, is characterized with high blood glucose levels and dysregulation in glucose metabolism. These conditions are attributed to insulin resistance which leads to the failure in the translocation of glucose transporter protein (GLUT4). During the dearth of insulin, GLUT4 is confined to storage vesicles within the cells and less than 5% of the transporter is present on the cell membranes. In this context, we intent to detect the effect of seagrass metabolites on the regulation of GLUT4 transport protein which is an hallmark of type 2 diabetes. In order to detect the novel selective activators of GLUT4 protein, artificial intelligence model was adopted to dock natural ligands with the transporter protein. Due to non availability of GLUT4 crystal structure, a homology model was constructed based on the experimental data available on GLUT1. The homology model represented glucose transport channel along with the substrate interacting residues. Among the six metabolites of seagrass extract, Cis-9 Oleic acid, hexadecanoic acid, beta-sitosterol and β-1,4 dicarboxylic acid were seem to be potential activators with high docking energy value and H-bond interactions. Thus, the in-silico data supplements the natural activators of GLUT4 in designing the anti-diabetic gents.

***Keywords:*** *Diabetes, Glucose transporters, Natural ligands, Structure based screening*

## I. INTRODUCTION

The global prevalence of diabetes is very high and may cross 300 million people by 2025[1]. Based on etiology, diabetes is classified into two types, i.e., Insulin dependent diabetes (T1D) and Non insulin dependent diabetes (T2D). T1D is caused by destruction of insulin-producing pancreatic β-cell where as T2D is a life style disorder, caused either by insulin resistance mechanism or impairment in insulin secretion. Insulin is a central mediator in the regulation of Glucose levels through intensifying the glucose uptake triggered by glucose transporter protein -4 (GLUT-4) in muscle and adipose tissues [2].

Glucose undergoes glycolytic pathway and it provides energy to the human body and the dietary glucose is supplied to the body by Skeletal muscle. Pancreas releases Insulin and the insulin induces the transport of glucose into various tissues, like brain, skeletal muscle, and adipocytes, which are sensitive towards insulin [3&4]. Insulin resistance mainly occurs in skeletal muscle due to inadequate exocytic translocation of glucose transporter to the membrane than normal GLUT4 mobilization [5]. Thus to resolve insulin resistance, there is need to determine the dynamic strategies to enhance the glucose influx into the insulin resistant tissues by targeting the regulation of GLUT4. The novel strategies are useful to unravel the resistance of the cells towards insulin and design the selective drugs for increasing the sensitivity of the cells towards insulin.

The Glucose transporters, are a group of thirteen transporter proteins distributed throughout the body on different tissues [6]. In skeletal muscle, the

*May – June 2020*
*ISSN: 0193-4120 Page No. 5485-5490*

glucose uptake is mediated by the major transporter namely Glucose transporter type 4 (GLUT4). The GLUT4 residing in the vesicles is translocated from the cytoplasm to plasma membrane immediately after binding insulin to the receptor. The inhibition of translocation leads to Insulin resistance in type 2 diabetes [7, 8]. Currently, glucose tranporter-4, is identified as specific protein involved in the translocation process only identified transport protein in insulin-regulated vesicular traffic and its activation may improve the sensitivity of vesicular cells to insulin. Thus the current study is formulated to identify the potential activators of GLUT4 from sea grass.

We hypothesised to detect the new marine metabolites from seagrass collected from Pulicat lake. The seagrass was characterised as *Halophila beccarii* [9] and used to prepare methanol extract by solvent extraction method. Different bioactive metabolites were identified from crude seagrass extract through GC-MS analysis such as Beta sitosterol, Oleic acid, Hexadecanoic acid, Methyl Hexadecanoic acid, Isocoumarin and 1,2 Benezene di carboxylic acid. Keeping in view the importance of marine natural matabolites, artificial intelligence models such as structure based virtual screening, protein –ligand interaction were adopted to design the protein and selection of ligand repectively.seagrass metabolites and GLUT4 protein along with standard antidiabetic drugs Glibenclamide and metformin were considered for docking experiments.

## II. METHODOLOGY

### Chemical data libraries

Six compounds were identified from seagrass and the chemical structures of these compounds were sketched by using chemsketch software and the structures were validated with pubchem structures. Due to non availability of three dimensional structures for Glucose transporter protein -4, structure based homology modelling was utilised to construct the structure of the tranporter protein.

.

### Molecular modeling of Glucose Transporter- 4

The modeling software (i.e. MODELER) was used to predict the full length structure of GLUT4. PROCHECK and ProSa web portals were utilized to crosscheck the geometry of predicted model.The X-ray structure of GLUT4 was generated with ProSa web serve.

### Template Search and Sequence Alignment

To predict the full length structure of human glucose transporter (GLUT4) the protein sequences containing 509 amino acids was download from UniProt web site (Entry ID: P14672). To find out the suitable sequence for modeling, all were subjected to BLASTp program to obtain their homologue structures from Protein Data Bank.After BLASTp analysis, 4PYP was selected as templates for modeling of GLUT4 structure as it shares 65% identity and 97% query coverage with GLUT4 sequence and template protein. The template sequences were aligned with their respective target GLUT4 sequences using CLUSTALW module.

### Molecular Modeling of GLUT4 Protein

The aligned sequences were used to build model structures using MODELERE program (https://salilab.org/modeller/). The crystal strucuture of 4PYP was used to build the three dimensional strucuture od Glucose transporter -4 as per the protocol of Homology modeling . Nearly10 structures were framed for each target for GLUT4. Subsequently, the produced models were scored and ranked based on their mol pdf scores. Further, their geometry reliability was verified using PROCHECK and ProSa web servers and the best reliable structure of GLUT4 was chosen for further study.

### Structural Assessment of Model Protein

The main properties of the protein such as length of the back bone , Phi angle, Psi angle, amino acid propensity, side chain residues, hydrophobicity, preponderance of hydrophilic amino acids , planarity as well as distorted angles were analyzed to validate the predicted protein[10].

May – June 2020
ISSN: 0193-4120 Page No. 5485-5490

**Optimization of Ligands**

Ligands ( compounds identified from seagrass extract), and reference compounds (metformin-4091, glibenclamide-3488) were prepared using 'Prepare Ligand' module of DS. While preparation, hydrogen atoms were added and charged groups were neutralized. The state of ionization, cis-trans isomers, chiral carbons s and energy dependant stable conformations were optimized for ligands. The energy of ligands is minimized out using CHARMm force field. The chemical stucture of selected ligands namely Beta-sitosterol (222284), Methyl palmitate (8181), 1,2 benzene di carboxylic acid (1017), Cis-9 Octadecenoic acid (445639), Hexadecanoic acid (985), Methyl Hexadecanoic acid was retrieved as SD file from Pubchem database.

**Molecular Docking**

Molecular docking carried out using firedock solutions. During docking analysis, the prepared ligands were docked at the defined binding site of GLUT4 structure by employing default parameters. After docking, the generated ligand poses were considered for measuring docking energy by adopting "Calculate Binding Energy Module". The generated ligand poses were stratified to perform binding mode analysis.

## III. RESULT AND DISCUSSION

**Homology Modeling of GLUT4 Protein**

Homology modeling was adopted to predict protein structure based on structure of the known protein. The 3D structure of the protein GLUT4 and their X-ray structure provide a significant information about its function and catalytic activity . This information is useful to analyze the interaction of GLUT4 with either substrate molecules or agents which inhibits its activity. The modeled structure of GLUT4 appears like a typical barrel shape transporter. The structural validation results approved the reliable structural geometry for docking study .The predicted structures of GLUT4 were represented in Figure.1

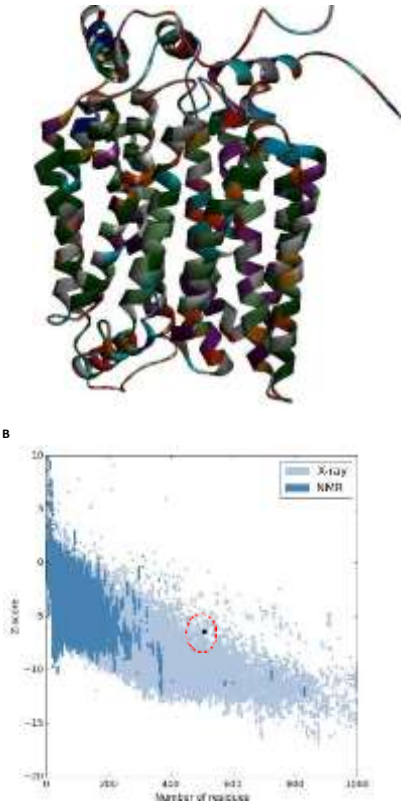


**Fig 1.** (a) Advanced structure of GLUT4 and (b) Schematic representation of model quality using ProSa web server GLUT4 model

**Secondary structure validation of GLUT4** The secondary structure of Glucose tranporter-4 was authenticated with Ramachandran plot. As per the PROCHEK results , GLUT4 structure contains 97.4% residues in favorable region and the remaining 3.0% residues were spread in additional allowed region and disallowed region with 2.4 % and 0.2 % respectively (Figure-2)

**Prediction of Catalytic Site**

The "q site finder module" was used to analyze the amino acid profile of active site of Glucose transporter -4.The data portrayed the presence of VAL 94, TYR 110, PRO 111, VAL 112 amino acids in the catalytic region.

May – June 2020
ISSN: 0193-4120 Page No. 5485-5490

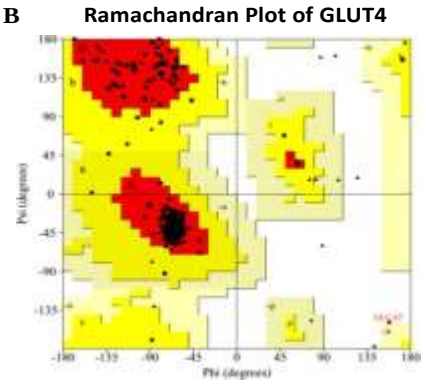


**Fig 2.** Schematic representation of Ramachandran plot of GLUT4

**Molecular docking of phytochemicals with GLUT4**

To detect the mode of ligand binding and interaction mechanism of prepared ligands with GLUT4, docking was carried out by using PATCH DOCK analysis. The results displayed the role of several conserved amino acid residues in structural confirmation as well as in active binding site of GLUT4 protein. Hydrogen bonds play significant role in maintaining the secondary structure of the protein which in turn function of the protein. In addition to magnitude the features of H bonding pattern, the docking interaction between the seagrass ligands and glucose transporter protein-4 was measured by Lig Plot analysis. The figure.3 represents the most promising protein-ligand interaction along with the details of H-bond interaction of seagrass ligands with Glucose transporter-4.

**Table 1.** Docking energy values and interactions of seagrass bioactive metabolites and standard antidiabetic with GLUT4 protein

| Compound name | Binding energy (kacl/mol) | Amino acids involved in interaction | Ligand atom involved in interaction | H-Bond distance | Attractive vander Waals energy | Repulsive vander Waals energy | Atomic contact energy |
|---|---|---|---|---|---|---|---|
| Metformin | -16.41 | Asn 427<br><br>Asn 431 | N1<br>N2<br>N3 | 2.21<br>2.71<br>2.40 | -5.73 | 0.33 | -5.61 |
| Glibenclamide | -54.78 | Trp 428<br>Ser 153 | N3<br>O4 | 2.59<br>3.13 | -21.62 | 3.38 | -16.52 |
| Beta sitosterol | -49.86 | Trp 428<br>Ser 96 | O<br>O | 2.48<br>3.05 | -21.72 | 8.31 | -14.99 |
| Cis-9 Oleic acid | -36.94 | Asn 176<br>Ser 153 | O2<br>O1 | 2.41<br>2.89 | -17.10 | 3.69 | -9.04 |
| Hexadecanoic acid | -34.42 | Thr 337 | O1<br>O1 | 3.15 | -13.87 | 2.63 | -10.23 |
| Methyl HDA | -33.71 | Arg 416 | O2 | 2.40 | -16.41 | 8.51 | -10.65 |
| Isocoumarin | -22.25 | Asn 304<br>Gln 299 | O2<br>O2 | 2.90<br>2.49 | -7.84 | 0.35 | -7.49 |
| 1,2 Benezene di carboxylic acid | -13.28 | Asn 431<br>Asn 304 | O2&O3<br>O3 | 2.10&2.28<br>2.99 | -9.94 | 1.85 | -0.63 |

The seagrass metabolites showed impending interaction with catalytic site of GLUT4 protein through several interactions namely hydrogen, attractive vander waals interactions . The seagrass ligands extended hydrogen interactions with amino acids such as Asn 427, Asn 431, Trp 428, Ser 96,Trp 428, ser 96, Asn 176, Ser 153, Thr 337, Arg 416, Asn 304, Gln 299, Asn 431 and Asn 304 of Glucose transporter-4 (Table-1).

In protein ligand interactions, the hydrogen bond interaction exhibit either induced or inhibitory activity between selected metabolite and protein. In the computational analysis, the hydrogen linkage between ligand and protein represented agonist activity towards GLUT4. Majority of the ligands, in

May – June 2020
ISSN: 0193-4120 Page No. 5485-5490

the docking analysis, showed < 3 H-bonds with GLUT4 protein. More than one H-bond interaction showed high affinity between ligand and protein. When compared the docking interaction of standard antidiabetic drugs Glibenclamide and metformin with GLUT4, the seagrass compounds showed similar index of interactions. The site of action of Cis -9 Oleic acid and beta sitosterol of sea grass is found to be Trp 428 of GLUT4 which is the same with Glibenclamide and metformin (Fig.3). In-silico analysis demonstrated that Asn 427, Trp 428, Ser 153, Ser 96, and Gln-299 residues are catalytically significant to comprehend the molecular activity of GLUT4 protein. Among the six compounds analysed, Cis-9 Oleic acid and Beta sitosterol seems to be good candidates in boosting the activity of GLUT4. Thus, these compounds may utilized to stimulate the translocation of GLUT4 storage vesicles (GSV) trafficking from cytoplasm to the outer membrane.

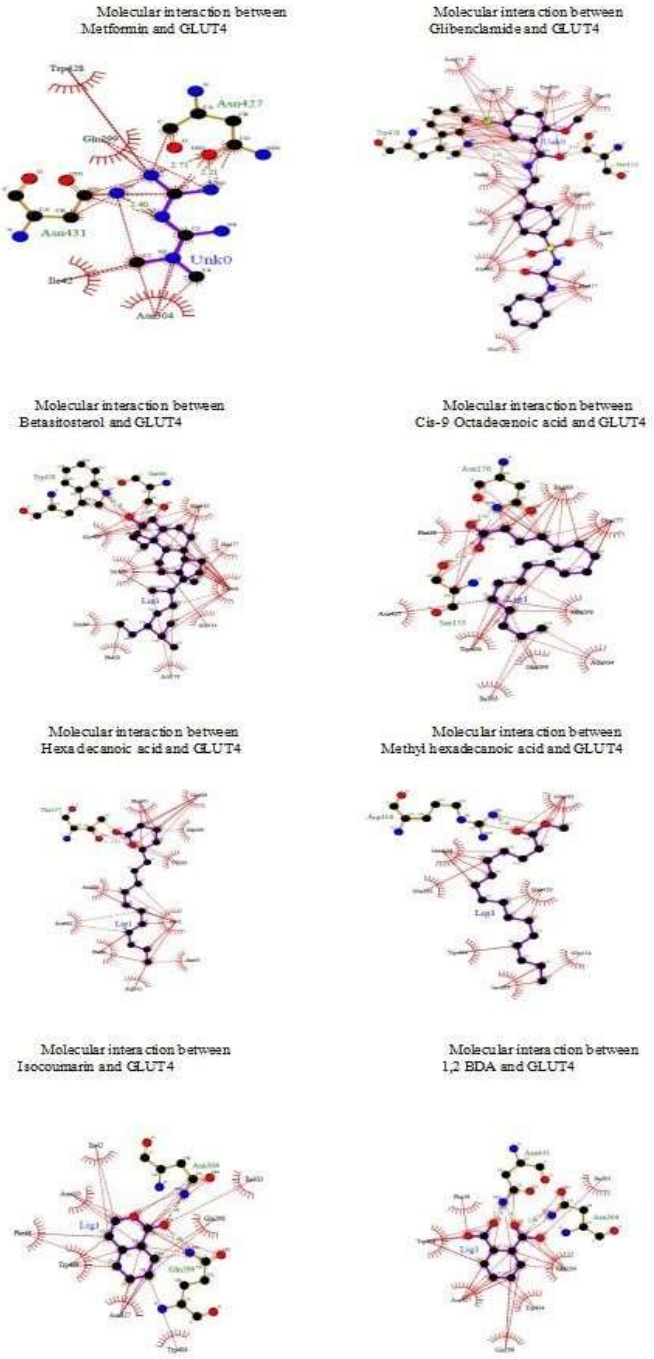


**Fig 3.** Molecular interactions between seagrass metabolites and GLUT4

## IV. CONCLUSION

Of six compounds of sea grass tested, the Cis-9 Oleic acid and Beta sitosterol were detected as activators of GLUT4 and these compounds may facilitate the active mobilization of Glucose transporter-4.

*May – June 2020*
*ISSN: 0193-4120 Page No. 5485-5490*

Further investigation on the in-vitro and in-vivo analysis is useful in developing anti-diabetic drugs targeting activation of GLUT4 protein. This activation may lead to sensitization of insulin resistant tissues of type2 diabetes.

## REFERENCES

[1] Zimmet P., Alberti K.G., Shaw J., Global and societal implications of the diabetes pidemic, Nature, 414, 782-787 (2001)

[2] Stuart, C. A., Howell, M. E. A., Zhang, Y., Yin D.: Insulin stimulated translocation of glucose transporter (GLUT) 12 parallels that of GLUT4 in normal muscle. J. of Cli. Endo.and Meta. 9, 3535– 3542 (2009).

[3] Pendergrass, M., Bertoldo, A., Bonadonna, R.: Mu scle glucose transport and phosphorylation in type 2 diabetic, obese nondiabetic, and genetically predisposed individuals. Am. J. Physiol. Endocrinol. Metab. **292,** E92–E100pmid:16896161 (2007).

[4] Hollenbeck, C.B., Chen. Y.D., Reaven. G.M.: A comparison of the relative effects of obesity and non-insulin-dependent diabetes mellitus on *in vivo* insulin-stimulated glucose utilization. Diabetes.**33**,622–626,pmid:6376218 (1984*).*

[5] Bjornholm M., Zierath J.R., Insulin, Signal Transduction in Humans Skeletal Muscle Identifying the Defect in Type II Diabetes, Biochem. Soc.

[6] Zhao F.Q., Keating A.F., Functional Properties and Genomics of Glucose Transporters, Curr. Genomics, 8, 113-128, (2007)

[7] Kahn, B.B.: Dietary regulation of glucose transporter gene expression: tissue specific effects in adipose cells and muscle. *J.Nutr.* 124, *(*Suppl.*)* 1289S–1295 Spmid: 8064377 (1994*).*

[8] Hansen, P.A., Han, D.H., Marshall, B.A.: A high fat diet impairs stimulation of glucose transport in muscle. Functional evaluation of potential mechanisms. J. Biol. Chem. 273, 26157–26163 pmid: 9748297 (1998).

[9] Vani M., Vasavi T., Uma Maheswari Devi P., Evaluation of *in vitro* antidiabetic activity of methanolic extract of seagrass *Halophila beccarii.* Asian. J. Pharm. Clin. Res. 8, 1-4 (2018).

[10] Ramachandra G.N., Ramakrishna C., Sasisekharn V., Stereochemistry of polypeptide chain configurations, In. J Mol. Biol., 7: 95-9 (1963)

TEST Engineering & Management

*May – June 2020*
*ISSN: 0193-4120 Page No. 5485-5490*