Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

IN-SILICO DOCKING STUDIES OF SELECTED N-GLYCOSIDE BEARING TETRAZOLE RING IN THE TREATMENT OF HYPERGLYCEMIA SHOWING INHIBITORY ACTIVITY ON SGLT

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Received: 06 Mar 2013, Revised and Accepted: 29 Apr 2013

ABSTRACT

Objective: Sodium glucose co transporter (SGLT) inhibitor is a novel approach which is different from the available antidiabetic therapies. This class of drugs targets insulin resistance and insulin deficiency, providing a glucose dependent and insulin-independent pathway to control hyperglycemia. SGLT inhibitors work on urinary sugar excretory mechanism. The current study is based on *in-silico* ligand (tetrazole derivative) protein (2XQ2) interaction to evaluate hypoglycemic activity.

Method: Molecular docking software AutoDock 4.2 was used to dock the prepared ligand in the binding site of the crystal structure of protein.

Result: Docking results are based on the least binding energy of the test (prepared ligand) and standard. Top ten compounds showed binding energy in the range of -11.31 kcal/mol to -5.64 kcal/mol when compared with that of standard (-8.40 kcal/mol).

Conclusion: Among the proposed tetrazole derivatives some compounds showed good inhibitory activity and further work may help to develop a compound as an active therapeutic agent for the treatment of hyperglycemia.

Keywords: Anti-diabetic, Dapagliflozin, SGLT2, Molecular docking, AutoDock 4.2

INTRODUCTION

Type 2 diabetes affects approximately 300 million people 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. The rate of new cases rose by more than 90 percent among adults over the last 10 years, according to a 2008 study by the US Centers for Disease Control and prevention. 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 multifactorial 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.

There are several distinct classes of hypoglycemic agents that are available for monotherapy or for combination therapy to treat hyperglycemia, such as biguanides, sulfonylureas, meglitinides, thiazolidinediones, α-glucosidase inhibitors, incretin mimetic and DPP-4 inhibitors [2]. However, United Kingdom Prevention of Diabetes Study report says, only 25-50% of T2DM patients are effectively treated by current available oral hypoglycemic agent. Therefore, to treat resistance or uncontrolled hyperglycemia immediately, continuous exploration for alternative target is being made involving the maintenance of glucose homeostasis [3]. Sodium glucose cotransport 2 (SGLT2) inhibitors are compounds with a new approach which is different from the currently available therapies. The mechanism of action of SGLT2 is to interfere with sodium glucose cotransport 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 [4]. 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 [5]. Six isoforms of SGLTs (SGLT1 to SGLT6) are known [6, 7]. Among these only two isoforms SGLT1 and SGLT2 are well investigated. SGLT1 is high affinity, low capacity transporter and highly expressed in the small intestine and in kidney. In contrast, SGLT2 is specially expressed in renal uriniferous tubules, a low-affinity, high capacity transporter. It plays critical role in renal glucose absorption while SGLT1 helps in absorption of dietary glucose in small intestine [8, 9]. 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 [10]. It has been reported that inhibition of SGLT1 is associated with sever gastrointestinal discomfort, thus selective inhibition of SGLT2 is thought to be effective way for diabetes treatment [11]. 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. Present study is based on *in-silico* protein ligand interaction. The AutoDock offers different types of search algorithms to search the conformational space. Among these, the Genetic Algorithm is the most modern and sophisticated algorithm. Genetic Algorithms are a family of powerful mathematical functions derived from the concepts of language of molecular genetics. Other types of search algorithms in Auto Dock include Simulated Annealing and Local Search. In our research work, the structural models of the ligand in the sodium glucose co transporter protein binding sites have been carried out, which may facilitate further development of more potent anti diabetic agents [12].

MATERIALS AND METHODS

Software used

ChemSketch was downloaded from www.acdlabs.com. Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, Python 2.7 - language was downloaded from www.python.com, Cygwin (a data storage) c:\program and Python 2.5, Spd Viewer and Chimera.

Retrieval of 3D Structure of macromolecules

Crystal structure of the protein was downloaded from RCSB, Protein Databank (PDB, http://www.pdb.org). The PDB ID of the selected protein was found to be 2XQ2 and refined by Spd viewer. Polar hydrogen, charges and salvation parameters were added orderly to

generate optimized protein structure. The Protein was having 538 no. of groups, 8363 no. of atoms and 8468 no. of bonds.

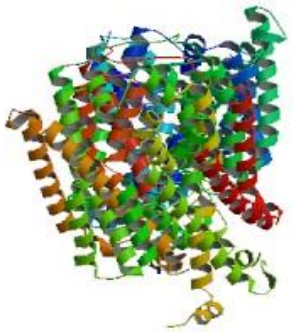


Fig. 1: It shows 3D structure of 2XQ2 receptor

Modelling of SGLT2 [13].

Template PDB code: 2XQ2 (The mechanism of sodium and substrate release from the binding pocket of vSGLT. Watanabe et.al Nature 2010)

Resolution: 2.73Å

Organism: *Vibrio parahaemolyticus*
Sequence identity: 33%
rmsd = 0.26Å

Structural Assessment of the Protein

The protein was sent for structural assessment to Exome Horizon. The Ramchandran Plot for all residue types are given in **Fig.2**, Chi1-Chi2 plots, Main-chain parameters, Side-chain parameters, Residue properties, Main-chain bond length, and Main-chain bond angles; RMS distances from planarity and distorted geometry are analyzed for input atom only [14].

Ligand preparation and docking

Ligand were prepared by using Chem draw ultra 8.0 in 2D (MDL mol format) and converted in to pdb file format using open Babel 2.0.2 before submission in AutoDock 4.2. The optimized ligand molecules were docked in to refined sodium glucose co transporter protein (SGLT). To the refined protein 2XQ2 all the hydrogen were added by AutoDock tools. For each atom of the molecules Kollman charge was generated in AutoDock 4.2 [15]. For docking all prepared ligand was input as PDBQT file format. The centre of active site in refined protein was chosen as grid map values for preparation of the grids. Three dimensional grid of size 60×60×60 ^{0}A with 0.375 spacing were generated [16].

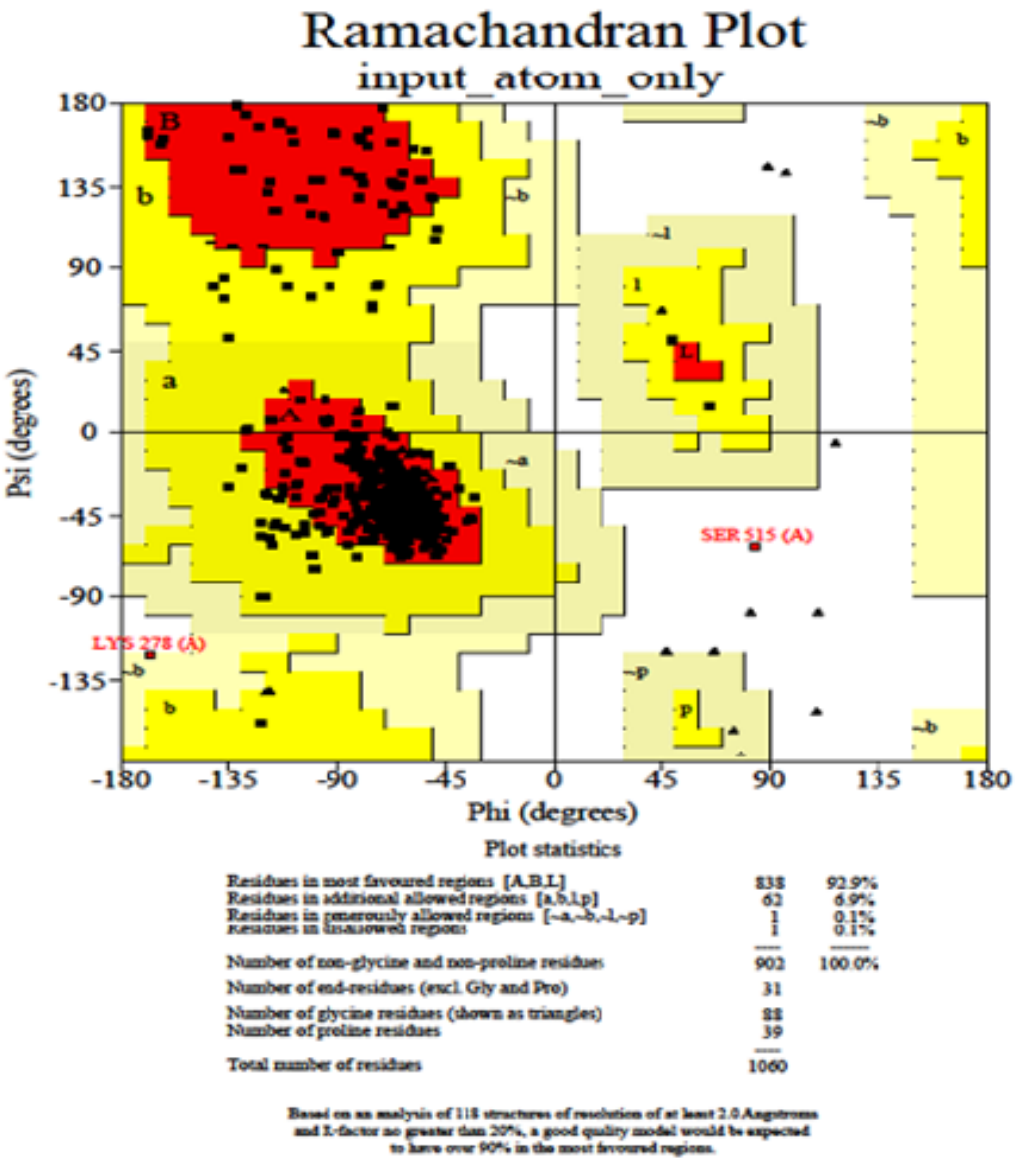
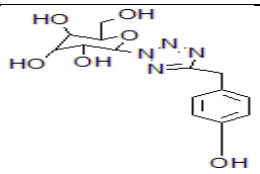
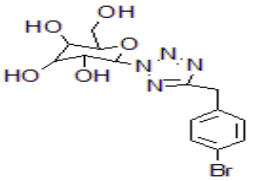
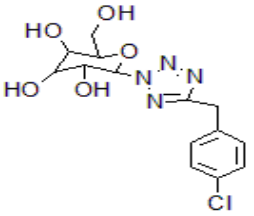
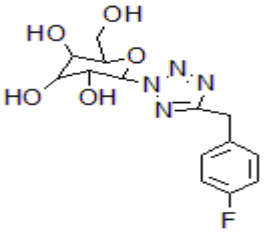
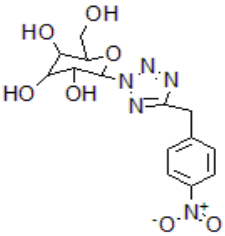
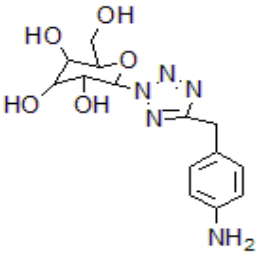
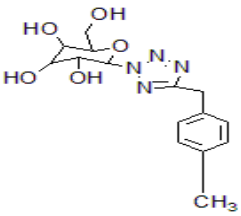
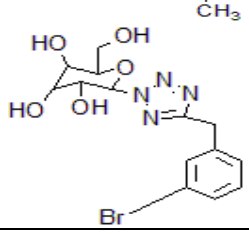
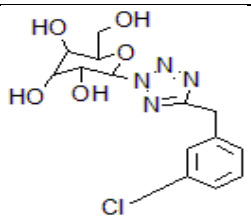
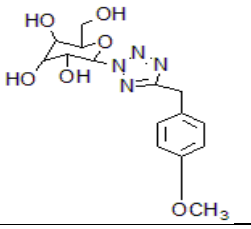


Fig. 2: It shows Ramachandran plot analysis of 2XQ2 receptor

Table 1: It shows chemical properties of some tetrazole N-glycoside derivatives

Compound Code	Compound Structure	Compound Name	Molecular Weight	Molecular Formula
Bk1	$C_{14}H_{18}N_4O_6$	2-(5-(4-hydroxybenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	338.32	
Bk2	$C_{14}H_{17}BrN_4O_5$	2-(5-(4-bromobenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol 401.21	401.21	
Bk3	$C_{14}H_{17}ClN_4O_5$	2-(5-(4-chlorobenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	356.76	
Bk4	$C_{14}H_{17}FN_4O_5$	2-(5-(4-flurobenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	340.31	
BK5	$C_{14}H_{17}N_5O_7$	2-(5-(4-nitrobenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	367.11	
BK6	$C_{14}H_{19}N_5O_5$	2-(5-(4-aminobenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	37.33	
BK7	$C_{15}H_{20}N_4O_5$	2-(5-(4-methyl benzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	336.34	
BR(b)	$C_{14}H_{17}BrN_4O_5$	2-(5-(3-bromo benzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	401.21	

BR(c)	$C_{14}H_{17}ClN_4O_5$	2-(5-(3-chlorobenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	356.76	
BR(d)	$C_{15}H_{20}BrN_4O_6$	2-(5-(3-methoxybenzyl)-2H-tetrazol-2-yl)-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol	352.34	

Docking Methodology

Present docking study is based on conformational search for ligand. Lamarckian genetic algorithm (LGA) is used as default search function in AutoDock 4.2. LGA is a hybrid genetic algorithm with local optimization that uses a parameterized free energy scoring function to estimate binding energy [12]. To carry out ligand-receptor docking, software accepts macromolecules and ligand as input then utilizes the LGA to generate ligand position. AutoDock requires pre-calculated grid maps, one for each atom type present in the ligand being docked. These maps are calculated by AutoGrid [16, 17]. A grid map consists of a three dimensional lattice of regularly spaced points, surrounding (either entirely or partly) and centered on some region of interest of the macromolecule under study. The each docking comprised of several independent execution of the LGA [18]. AutoDock was run several times to get various docked conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates. AutoDock 4.2 uses a semi-empirical free energy force field to evaluate conformations during docking simulations. The force field was parameterized using a large number of protein-inhibitor complexes for which both structure and inhibition constants (Ki), are known [15, 17]. AutoDock requires: 1) grid maps for each atom type in the ligand, calculated by Auto Grid, 2) a PDBQT file for the ligand, and 3) a docking parameter file that specifies the files and parameters for the docking calculation. To generate final docking result, the individual LGA execution are clustered and ranked. LGA used some default parameter as follows: population of 150 individuals. 2500000 function evaluations, The GA run for at most 27000 generations. The 1 best will be preserved each generation. The mutation rate is 0.020000. The crossover rate is 0.800000.

RESULT AND DISCUSSION

Protein-Ligand Docking Studies are used to check the structure, position and orientation of a protein when it interacts with small molecules like ligand [12]. Binding energy, hydrogen bond interactions, π – π interactions orientation of the docked compound within the active site, and RMSD of active site residues, are some parameter to analyze the protein ligand interaction on the active site of the macromolecules [18]. Present docking result is based on two parameter binding energy. The DLG file provides docked conformations, orientations and the binding energies. The similarity of docked structures is measured by computing the root-mean-square deviation (RMSD) between the coordinates of selected molecular conformation with the molecular conformation having lowest interaction energy which is ranked on top. Clusters are created based on the comparison of conformations using RMSD values [19].

Estimated Free Energy of Binding kcal/mol was calculated by using formula:

Binding energy = (1) + (2) + (3)-(4), Where (1) cited for Final Intermolecular Energy kcal/mol + vdW + Hbond + desolvation Energy + Electrostatic Energy kcal/mol. (2) Final Total Internal Energy kcal/mol (3) Torsional Free Energy kcal /mol (4) Unbound System's Energy, k cal/mol. Auto Dock 4.2 was run for several times to give rank wise binding energy individually for all compounds as shown in Table 2. The docking results consist of the PDBQT of the transformed 3D Cartesian coordinates of the ligand atoms as docked to the receptor molecule. The newly marketed SGLT2 inhibitor, Dapagliflozin was taken as standard and its least binding energy was calculated as -8.40 kcal/ mol and compared with series of tetrazole moiety (-11.31 kcal/mol to -5.64 kcal/mol).

Table 2: It shows rank wise binding energy of the docked compounds

Compound code	Binding energy (kcal/mol)of the compound based on their rank									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Bk1	-7.40	-7.31	-7.28	-6.48	-6.43	-6.37	-6.17	-6.15	-6.11	-5.77
Bk2	-9.63	-9.61	-9.60	-9.60	-9.59	-9.58	-9.57	-9.56	-9.52	-9.51
Bk3	-6.20	-6.18	-6.17	*-6.14*	-6.10	-6.08	-6.06	-6.05	-6.05	-6.05
Bk4	-6.00	-5.92	-5.80	-5.95	-5.94	-5.93	-5.90	-5.79	-5.72	-5.64
Bk5	-8.01	-7.79	-7.43	-7.83	-7.79	-7.75	-7.58	-7.40	-7.37	-7.35
Bk6	-9.03	-9.02	-8.96	-8.94	-8.93	-8.89	-8.89	-8.81	-8.77	-8.72
Bk7	-8.46	-8.45	-8.44	-8.44	-8.41	-8.38	-8.38	-8.36	-8.33	-8.29
BR (a)	-5.64	-5.61	-5.51	-5.49	-5.33	-5.32	-5.23	-5.21	-5.20	-4.94
BR (b)	-8.74	-8.53	-8.45	-5.54	-5.53	-5.52	-5.50	-5.47	-5.37	-5.27
BR (c)	-11.31	-11.30	-11.30	-11.29	-11.29	-11.29	-11.26	-11.26	-11.26	-11.25
BR (d)	-8.61	-8.56	-8.54	-8.53	-8.53	-8.47	-8.45	-7.89	-7.87	-7.85
Dapagliflozin	-8.40	-8.34	-7.95	-7.60	-7.57	-7.80	-7.22	-7.70	-7.67	-7.63

Binding energy of some of the enlisted compounds, Bk2, Bk6, Bk7, BR (b), BR (c), and BR (d) are less than the standard compound. It means that these compounds have higher inhibitory activity, as least binding energy is related with higher activity. The binding energy of the selected ligand are plotted in the graph and from the graph the binding energy of all the active sites are observed among which the best ligand which shows better activity in all the active site is found to be Bk2, Bk6, Bk7, BR (b), BR (c), BR (d).

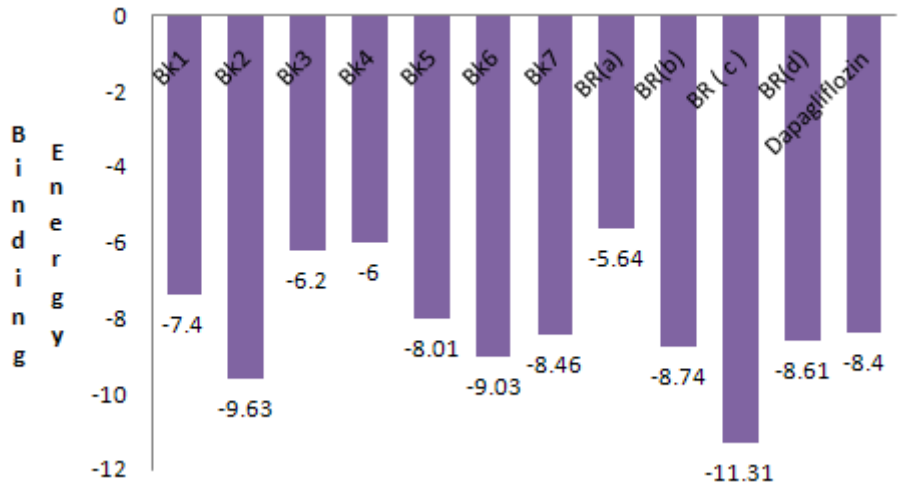
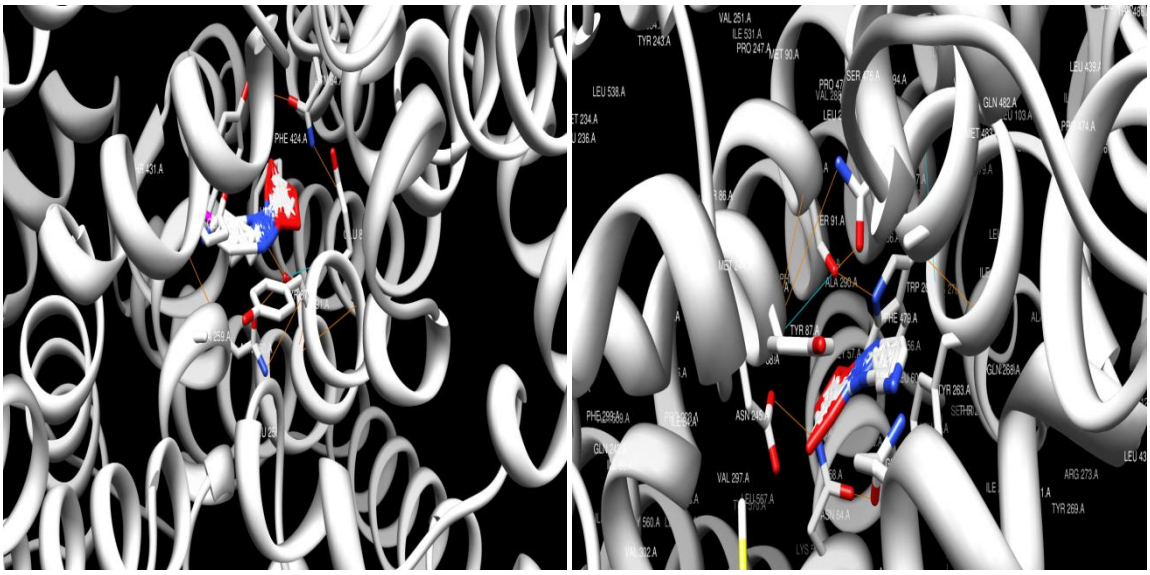
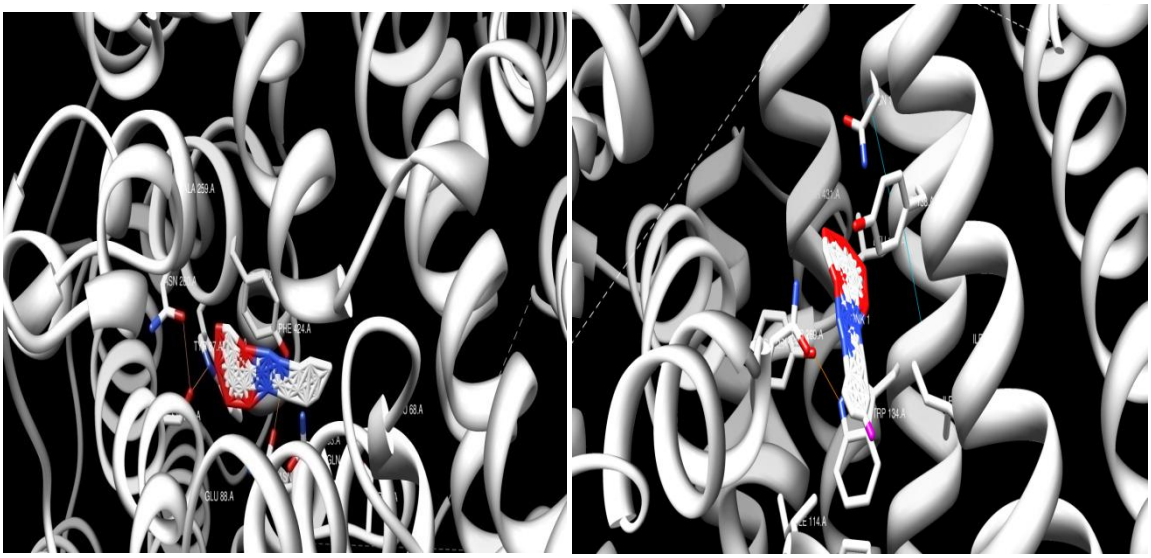


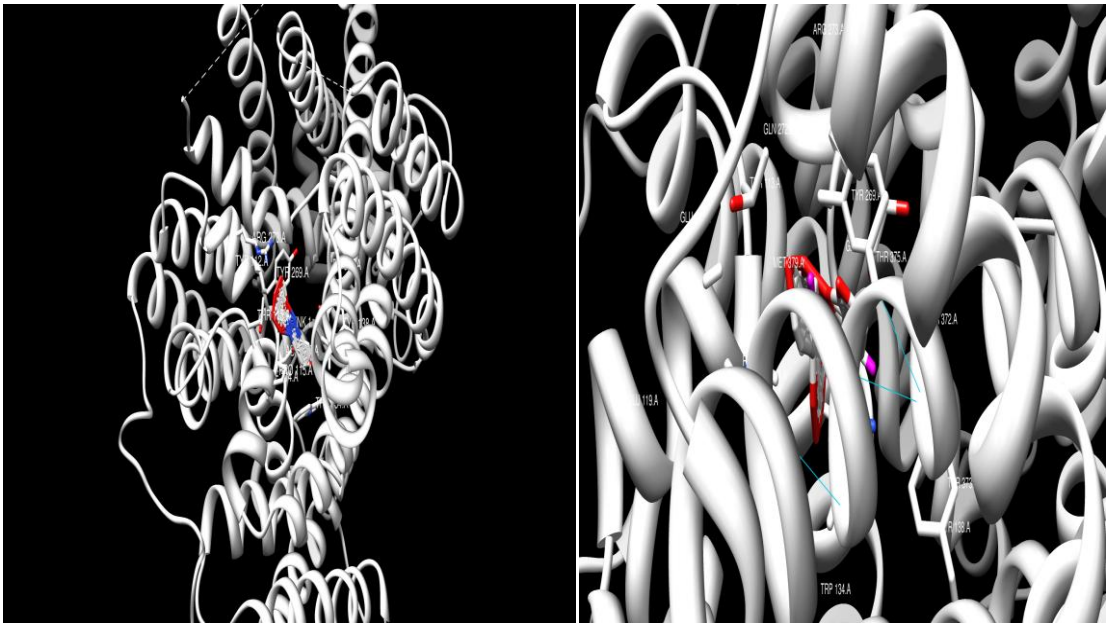
Fig. 3: It shows representation of Binding Energies of the drugs against the Receptor



a. Bk 2 **b. Bk 6**



c. Bk 7 **d. BR (b)**



e. BR(d) **f. Dapagliflozin**

Fig. 4: It shows interaction of drugs against the protein 2XQ2. The thin lines with colors represent interacting hydrogen bonds bet the macromolecules and the drugs.

CONCLUSION

The least binding energy is found to be -11.31 Kcal/mol corresponding to the compound BR(c) when compared to standard (-8.40 kcal/mol).). Further modification can be carried out to develop better hypoglycemic agent.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Pharmaceutical Sciences, Dibrugarh University and IIT-Guwahati.

REFERENCES

1. Lorenzati B, Zucco C, Miglietta S, Lamberti F, Bruno G. Oral Hypoglycemic Drugs: Pathophysiological basis of their mechanism of action. Pharmaceuticals 2010; 3: 3005-3020.
2. Tang C, Zhu X. A specific pharmacophore model of sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors. J Mol Model. 2012; 18:2795–2804.
3. Wu J S, Peng YH, Wu J M, Hsieh C J, Wu S H, Coumar M S et al. Discovery of Non-Glycoside Sodium-Dependent Glucose Co-Transporter 2 (SGLT2) Inhibitors by Ligand-Based Virtual Screening. J. Med. Chem. 2010; 53(24):8770–8774.
4. Hale C Wang M. Development of 11beta-HSD1 inhibitors for the treatment of type 2 Diabetes; Mini Rev Med Chem 2008; 8:702-710.
5. Derdau V, Fey T, Atzrodt J. Synthesis of isotopically labelled SGLT inhibitors and their metabolites; Tetrahedron 2010 ; 66(7):1472–1482.
6. Rajesh R, Naren P, Vidyasagar S, Unnikrishnan, Pandey S, Varghese M et al. Sodium Glucose Co transporter 2 (SGLT2) Inhibitors: A New Sword for the Treatment of Type 2 Diabetes Mellitus. International Journal of Pharma Sciences and Research. 2010; 2:139-147.
7. Grempler R, Augustin R, Froehner S, Hildebrandt T, Simon E, Mark M et al. Functional characterisation of human SGLT-5 as a novel kidney-specific sodium-dependent sugar transporter. FEBS Letters. 2012; 586:248–253.
8. Zhanga W, Welihinda A, Mechanic J, Ding H, Zhu L, Lu Y et al. EGT1442, a potent and selective SGLT2 inhibitor, attenuates blood glucose and HbA1c levels in db/db mice and prolongs the survival of stroke- prone rats. Pharmacological Research 2011; 63:284–293.
9. Idris I, Donnelly R. Sodium-glucose co-transporter -2 inhibitors: An emerging new class of oral antidiabetic drug. Diabets obes Metab 2009; 11:79-88.
10. Ohsumi K, Matsueda H, Hatanaka T. Pyrazole-O-Glucosides as Novel Na+-Glucose Cotransporter (SGLT) Inhibitors; Bioorganic& Medicinal Chemistry Letters. 2003;13:2269–2272.
11. Kanai Y, Lee W S, You G, Brown D, Hediger M A, Brown D et al. The human kidney low affinity Na/ glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D- glucose. J Clin Invest. 1994; 93:397–404.
12. Morris G M, Goodsell D S, Halliday R S, Huey R, Hart W E, Belew R K et al. Automated Docking Using a Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function. J Comput Chem. 1998; 19(14):1639-1662.
13. Annapurna H V, Apoorva B, Ravichandran N, Arun K P, Brindha P, Swaminathan S et al. Isolation and in silico evaluation of antidiabetic molecules of Cynodon dactylon (L.). Journal of Molecular Graphics and Modelling. 2013; 3:987–97.
14. Ramachandran G N, Ramakrishnan C, Sasisekharan V. Stereochemistry of polypeptide chain configurations. In J Mol Biol. 1963; 7:95-9.
15. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS et al. Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility. J. Comput. Chem. 2009; 30: 2785-2791.
16. Goodsell DS, Morris GM, Olson AJ. Automated docking of flexible ligands: Applications of Autodock. J Mol Recog. 1996; 9: 1-5.
17. Cosconati S, Forli S, Perryman A L Harris R, Goodsell D S et al. Virtual screening with AutoDock: theory and practice. Expert. Opin. Drug Discovery 2010; 5(6):597-607.
18. Hetényi C Spoel DVD. Efficient docking of peptides to proteins without prior knowledge of the binding site. Protein Sci. 2002;11: 1729-1737.
19. Parida P, Yadav R N S. Comparative docking study of M1 protein (influenza virus) to check drug efficacy. International journal of pharmacy and pharmaceutical sciences 2012; 4(3):243-246.