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In Silico Molecular Interaction Analysis and Pharmacokinetic Profiling of Flavonoids from *Catharanthus roseus* (Flower) Against TXNIP Protein

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Abstract

Catharanthus roseus is a flowering plant whose flowers have been used in traditional medicine to treat diabetes mellitus. Some of the flavonoids present in these flowers, namely, quercetin, petunidin, malvidin, kaempferol, and hirsutidin were utilized for studying molecular interaction analysis. Diabetes mellitus which is a metabolic disorder caused by the depletion in the secretion of insulin, which regulates the blood glucose levels by facilitating the metabolism of glucose. Reduction in insulin secretion is often caused by the loss of beta cells of the pancreas. Thioredoxin-interacting protein (TXNIP) is an alpha arrestin which inhibits the production of thioredoxin. It can regulate the beta cells and its inhibition can be advantageous. Reduction in insulin secretion is often caused by the loss of beta cells of the pancreas. Studies have shown that elevated levels of TXNIP can induce apoptosis in beta cells while deficiency of TXNIP leads to protection against Type I & II diabetes due to beta cell survival. To study molecular interactions, flavonoids from the flower of *C. roseus* and control drug glibenclamide were subjected to docking against 3D structure of TXNIP protein using Autodock 4.2 and their molecular interactions were visualized using a Biovia discovery studio visualizer. Docking interactions and ADMET studies of the bioactive compounds signified the application of *C. roseus* as a natural therapeutic agent to combat diabetes.

Keywords: Diabetes mellitus, TXNIP, *Catharanthus roseus*, Flavonoids, Molecular docking

Introduction

Diabetes Mellitus is a term used to denote a group of metabolic disorders marked by a rise in blood glucose levels occurring due to defects in the secretion of insulin or defect in its action [1]. Diabetes Mellitus can be differentiated into 3 different categories based on its pathophysiology [2]. Type1 Diabetes Mellitus is caused by an auto-immune reaction [3]. Gestational diabetes mellitus is another sub type seen in the period of pregnancy in the mother.

Type2 Diabetes Mellitus is caused by deterioration in insulin secretion from the beta cells and insulin sensitivity [4]. There are various documented evidences presents that confirm the role of pancreatic beta cell death in the development of diabetes [5]. Thioredoxin-interacting protein (TXNIP) which stands for Thioredoxin interacting protein is a metabolism regulating protein that is found in important sites like adipose tissues, skeletal muscles and liver. TXNIP are proteins belonging to alpha arrestin family [6]. TXNIP interacts with Thioredoxin (TXN1 & TXN2) which leads to negative modulation of their activity. TXNIP plays an important role in glucose regulation and lipid metabolism by regulating beta cell function, peripheral glucose uptake, adipogenesis etc. TXNIP upon overexpression leads to pancreatic beta cell apoptosis and reduction in insulin sensitivity [5]. TXNIP protein binds with TXN1 which affects the ability of TXN1 to reduce oxidized protein and results in oxidative stress and heightened chances of apoptosis. TXNIP migrates to the mitochondria where it binds with TXN2 by competing with Apoptosis signal regulating kinase 1 (ASK-1) which leads to the release of ASK-1. ASK-1 usually binds with TXN2 which leads to the inhibition of its action [4]. Due to the TXNIP action the released ASK-1 gets phosphorylated and activated, leading to the initiation of apoptosis signaling cascade in pancreatic beta cells. Beta cell impairment and reduction in the production of insulin are the major causes of diabetes [6].

Catharanthus roseus is a traditional medicinal plant that has been used to treat diabetes in various parts of India. Different parts of the plants such as a leaf, flower, stems are administered to the patients to treat Diabetes mellitus [7]. Flower of *C. roseus*, according to Ayurvedic studies is considered to treat diabetes. Various experiments done in the laboratory show hypoglycemic activity of *C. roseus* extracts obtained from different plant parts such as root, leaf, flower and stem [8]. Flowers of *C. roseus* contain several flavonoids like quercetin, petunidin, malvidin, kaempferol and hirsutidin and their activities were reported in **Table 1**. Flavonoids is a group of natural substances with variable polyphenolic structures is a good anti-oxidant, anti-diabetic activity and anti-ageing activity [9]. Quercetin controls the body glucose homeostasis by interacting with many molecular targets in places such as small intestine, pancreas, skeletal muscle, adipose tissue, liver etc., [10]. Other phenolic compounds such as petunidin and malvidin are dietary polyphenols that help in preventing and managing Type2 Diabetes Mellitus by protecting pancreatic beta cells, initiating their proliferation, reducing beta cell apoptosis, and limiting oxidative stress [11]. Studies have suggested that phytocompounds like kaempferol might be concerned with the alleviation of diabetes [12]. The current investigation was done to study the inhibitory effect of the flavonoids from the flowers of *C. roseus* against TXNIP protein using bioinformatics tools and evaluating the docking score by AutoDock Tools.

Table 1 Flavonoid from *C. roseus* and reported bioactivity review.

Bioactive compound from *C. roseus*	Reported activities	References
Kaempferol	Antidiabetic, Antimicrobial, Antioxidant	[13-15]
Quercetin	Antidiabetic, Antioxidant, Anti-inflammatory, Anti Mutagenic	[10, 16-18]
Malvidin	Antioxidant,	[19]
Petunidin	Antioxidant	[20]
Hirsutidin	Hypoglycemic	[21]

Material and methods

Retrieval of TXNIP protein and active site prediction

TXNIP proteins crystal structure was retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB; www.rcsb.org/pdb). To the retrieved protein structure Hydrogen bonds and Kollman charges were added using ADT. The active site of TXNIP protein was determined using Computed Atlas of Surface Topography of Proteins (castP) 3.0 [22].

Ligand preparation

The structures of quercetin, malvidin, hirsutidin, petunidin, kaempferol, and the control drug glibenclamide were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) in the form of canonical SMILES. Using the software ChemSketch the 3-dimensional (3D) structures were generated and optimized. The compound structures were then saved in MDL mol file (.mol). The MDL mol files were then converted to PDB format using Open Babel molecular converter [23].

Molecular property assessment of the compounds

Physiochemical and Pharmacological properties of the compounds were assessed using Molinsipiration Server (https://molinspiration.com) [24]. Properties such as molecular size, rotatable bond, logP, hydrogen bond donor and acceptor characteristics were estimated. Membrane permeability, bioavailability, distribution, metabolism, adsorption (Lipinski's rule of 5) of the selected ligands were evaluated [25].

Ligands ADMET prediction studies

PkCSM (unimelb.edu.au) is a web server used to predict pharmacokinetic properties of compounds [26]. It anticipates different parameters based on certain characteristics. These parameters include absorption, distribution, metabolism and excretion that help us to determine the pharmacokinetics of a drug. Absorption of a drug can be predicted by predicting its characteristics such as CaCO2 & skin permeability, intestinal absorption, P-glycoprotein substrate or inhibitor. Characteristics such as volume distribution (VD) and permeability of central nervous system (CNS) and blood brain barrier (BBB) were utilized to

predict the distribution of the drug. For the determination of the Metabolism Cytochrome P450 model was used. Excretion of the drug was determined by Total clearance and Renal substrate.

Molecular docking

Docking analysis of flavonoids, quercetin, malvidin, petunidin, kaempferol, hirsutidin from *C.* roseus and the control glibenclamide against TXNIP protein was done using AutoDock Tool to make out the affinity [27]. To the 3D TXNIP macromolecular structure, hydrogen atoms (polar) and kollman charges were added. In the ligand preparation, centre node and rotatable bonds were selected and saved in PDBQT format. Many intermediate steps such as making pdbqt files of protein and ligand, grid preparation (grid size 72×72×72 Å) and grid map using the grid box were performed. Generation of active site was done using the data gathered from castP 3.0. Genetic algorithm was used as a search parameter and Lamarckian GA was the output selected. The parameters of genetic algorithm were set to 10 runs with 150 population size; the number of evaluations were set to 25,000,000; generation numbers to 27,000 with default mutation rate and crossover rate of (0.02) and (0.8) respectively. The protein-ligand interaction between quercetin, malvidin, hirsutidin, petunidin, kaempferol and the control glibenclamide against TXNIP protein was visualized using Biovia Discovery Studio Visualizer software [28].

Results and discussion

Diabetes mellitus refers to a widespread heterogenous group of metabolic disorders that can result in chronic illness and death. Despite the fact that the origin and development of diabetes mellitus can be due to various other mechanisms, TXNIP (Thioredoxin interacting protein) plays a major role in the pathogenesis of diabetes mellitus [6]. TXNIP proteins are from alpha arrestin family inhibiting TXN and leading to oxidative stress. Pancreatic beta cells are vulnerable to oxidative stress as it leads to apoptosis in them. So, TXNIP protein can be considered to be a target for bioactive compounds in order to control diabetes mellitus.

Protein structure preparation

Crystal structure of TXNIP from *Homo sapiens* was downloaded from PDB database and its structure id was 4LL1. Water molecules were removed and hydrogen atoms merger to the receptor molecule was carried out. RasMol application was used to visualize the 3D structure of TXNIP protein. The structure is depicted in **Figure 1**. Active sites of TXNIP protein were determined using castP 3.0. The amino acid sequences of the active sites were documented as GLY24, LEU47, TYR69, LEU70, ARG71, TYR72, GLU73, PHE103, GLU104, LEU105, PRO106, GLN107, PRO109, LEU222, ALA223, ASN224, GLY225, GLN226, THR227, LEU270, ARG271.

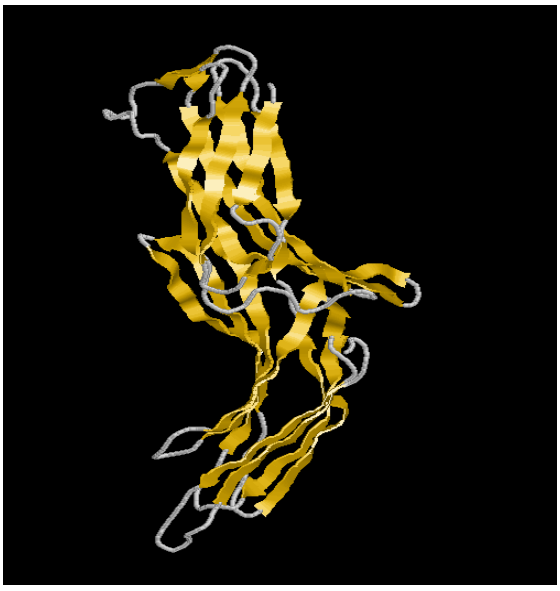

Figure 1 TXNIP protein structure visualization using RASMOL. yellow colour indicates betasheets and white colour indicates turns.

Ligand preparation

Bioactive compounds from the flowers of *C. roseus* are rich in flavonoids and alkaloids and are known for their hypoglycemic activity. Molecular Docking analysis was used to find the best fit of the bioactive compounds with TXNIP protein.

The compounds of quercetin, malvidin, petunidin, hirsutidin, kaempferol and the control glibenclamide were drawn using ACD ChemSketch, optimized and saved in MDL-mol format. Conversion to PDB format using OpenBabel molecular converter tool was carried out later. The pubchem id and SMILES format of all the compounds are shown in **Table 2**.

Table 2 Pubchem id and SMILES format of flavonoids from *C. roseus* and glibenclamide.

Compounds	Pubchem id	SMILES
Kaempferol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
Quercetin	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
Malvidin	159287	COC1=CC(=CC(=C1O)OC)C2=[O+]C3=CC(=CC(=C3C=C2O)O)O
Petunidin	73386	COC1=CC(=CC(=C1O)O)C2=[O+]C3=CC(=CC(=C3C=C2O)O)O.[Cl-]
Hirsutidin	441694	COC1=CC(=C2C=C(C(=[O+]C2=C1)C3=CC(=C(C(=C3)OC)O)OC)O)O
Glibenclamide	3488	COC1=C(C=C(C=C1)Cl)C(=O)NCCC2=CC=C(C=C2)S(=O)(=O)NC(=O)NC3CCCCC3

Molecular property assessment of the compounds

Bioactivity score prediction of quercetin, malvidin, petunidin, hirsutidin, kaempferol and the control glibenclamide is recorded and tabulated based on their drug likeliness in **Tables 3** and **4**. Lipinski's rule of 5 was followed while assessing the molecular properties. All the compounds chosen for the study adhere to Lipinski's rule [25]. Greater absorption and favorable oral bioavailability was predicted as topological polar surface area (TPSA) was < 140 Å for all of the compounds. From the bioactivity score prediction, the compound glibenclamide demonstrated good activity (> 0.20) towards G-protein coupled receptor (GPCR) ligand. Quercetin and kaempferol had a good bioactivity score against kinase receptors, whereas quercetin and kaempferol good bioactivity (> 0.20). Glibenclamide depicted a good bioactivity against protease inhibitor, whereas quercetin was found to be a strong enzyme inhibitor when compared to kaempferol and glibenclamide.

Table 3 Properties of flavonoids from *C. roseus* and glibenclamide predicted using molinspiration.

Compounds	M.wt	Hydrogen bond donor	Hydrogen bond acceptor	miLogP	Rotatable bonds	nViolations	TPSA (Å)	Volume	N atoms
Kaempferol	286.24	4	6	2.17	1	0	111.12	232.07	21
Quercetin	302.24	5	7	1.68	1	0	131.35	240.08	22
Malvidin	331.30	4	7	−0.42	3	0	110.55	277.88	24
Petunidin	317.27	5	7	−0.73	2	0	121.54	260.36	23
Hirsutidin	345.33	3	7	0.11	4	0	99.56	295.41	25
Glibenclamide	494.01	3	8	4.77	8	0	113.60	424.74	33

Table 4 Drug likeliness properties of flavonoids from *C. roseus* and glibenclamide predicted using molinspiration.

Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Kaempferol	−0.10	−0.21	0.21*	0.32*	−0.27	0.26*
Quercetin	−0.06	−0.19	0.28*	0.36*	−0.25	0.28*
Malvidin	−0.15	−0.17	0.02	0.01	−0.25	−0.03
Petunidin	−0.15	−0.17	0.03	0.01	−0.29	−0.01
Hirsutidin	−0.18	−0.19	−0.02	−0.03	−0.24	−0.05
Glibenclamide	0.20*	−0.05	−0.27	−0.33	0.24*	0.05

*Compounds with value greater than > 0.2 has good bioactivity against respective target

Molinspiration studies predicting the molecular properties such as hydrophobicity, membrane permeability, bioavailability are linked to molecular descriptors like log P, log S, the number of hydrogen donors or acceptors and molecular weight. These are associated with the designing of new drugs [29]. Molinspiration results were very favorable in the current study for quercetin, malvidin, petunidin, hirsutidin and kaempferol. TPSA of a molecule is a useful descriptor in molinspiration analysis as it helps to characterize the drug absorption and bioavailability. The values of TPSA and OH-NH interaction display the ability of the ligands to smoothly and efficiently bind to the target protein. Meanwhile, TPSA value of > 140 Å for a drug molecule depicts low absorption with lipophilicity and is crucial to estimate the oral bioavailability of the drug. Based on the previous statement it can be affirmed that flavonoids show good bioavailability when compared with glibenclamide which shows TPSA value greater than 140 Å.

Ligand ADMET characteristics analysis

ADME of the selected ligands was determined using pkCSM. The finding from the study are documented in **Table 5**. The TPSA value of all the ligands were < 100 which signify that the ligands show good membrane permeability. The AlogP value of the selected ligands turned out to be < 5 which is in correspondence with an ideal ligand AlogP value representing greater lipophilicity.

Table 5 ADMET prediction of ligands using PkCSM server.

Parameters	Kaempferol	Quercetin	Malvidin	Petunidin	Hirsutidin	Glibenclamide
TPSA	117.213	122.108	136.109	141.57	142.793	198.674
AlogP	2.2824	1.988	3.2205	−0.0785	3.5235	3.6417
Absorption						
Water solubility	−3.04	−2.925	−3.07	−2.938	−3.583	−4.582
CaCo2 permeability	0.032	−0.229	−0.381	−0.192	0.041	0.709
Intestinal absorption	74.29	77.207	88.785	68.964	84.27	71.775
Skin permeability	−2.735	−2.735	−2.735	−2.735	−2.735	−2.773
P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes	Yes
P-glycoprotein inhibitor1	No	No	No	No	No	Yes
P-glycoprotein inhibitor2	No	No	No	No	Yes	Yes
Distribution						
VDss(human)	1.274	1.559	0.763	0.94	−0.215	−0.218
Fraction unbound	0.178	0.206	0.137	0.206	0.054	0
BBB permeability	−0.939	−1.098	−1.335	−1.076	−1.292	−1.01
CNS permeability	−2.228	−3.065	−3.374	−3.88	−3.018	−2.674
Metabolism						
CYP2D6 substrate	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	Yes	Yes

Parameters	Kaempferol	Quercetin	Malvidin	Petunidin	Hirsutidin	Glibenclamide
CYP1A2 inhibitor	Yes	Yes	Yes	No	Yes	No
CYP2C19 inhibitor	No	No	No	No	Yes	No
CYP2C9 inhibitor	No	No	No	No	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	Yes
Excretion						
Total clearance	0.477	0.407	0.678	0.641	0.746	−0.155
Renal substrate	No	No	No	No	No	No
Toxicity						
AMES toxicity	No	No	No	No	No	No
Maximum tolerated Dose	0.531	0.499	0.554	0.528	0.657	−0.026
Herg-1 inhibition	No	No	No	No	No	No
Herg-2 inhibitor	No	No	No	No	No	No
Oral rat acute toxicity (LD50)	2.449	2.471	2.346	2.453	2.32	1.701
Oral Rat chronic toxicity	2.505	2.612	2.412	2.611	1.68	1.81
Hepatotoxicity	No	No	No	No	No	Yes
Skin sensitization	No	No	No	No	No	No
T. pyriformis toxicity	0.312	0.288	0.327	0.293	0.341	0.333
Minnow toxicity	2.885	3.721	1.224	3.002	1.626	0.173

Absorption nature of the selected ligands was studied using parameters such as CaCo2 permeability, skin permeability, and absorption by intestine [30]. Compound with Papp value > 8×10 − 6 cm/s and predicted value > 0.90 signifies high CaCo2 permeability. None of the selected ligands showed predicted value of > 0.90 except the control glibenclamide indicating low CaCO2 permeability [31]. Intestinal absorption is a parameter which signifies the absorption of a molecule in the intestine. Molecule with < 30 % absorption is considered to be poorly absorbed [32]. The results indicated that all the selected ligands showed greater intestinal absorption (i.e) > 30 %. Malvidin showed the highest intestinal absorption rate with 88.758 %. P-glycoprotein belonging to ATP binding cassette protein family acts in stopping the xenobiotics and toxins from entering our system [33]. P-glycoprotein substrate model was used to analyse whether the compound is a substrate of pgp or not [34]. From the current study, it can be determined that all the selected ligands act as P-glycoprotein substrate. Log Kp is used to signify the skin permeability of a compound. > −2.5 Log Kp value signifies low skin permeability. Results from the current study illustrate that all the selected ligands have Log Kp value < −2.5 indicating favorable skin permeability. P-glycoprotein inhibitors help in inhibiting P-glycoprotein and help in raising drug bioavailability. P-glycoprotein inhibitors are of 2 types class 1 and 2, and this model helps in determining whether the selected compounds are P-glycoprotein 1 and 2 inhibitors [35]. Current studies suggest that all the selected ligands except the control glibenclamide are not Class 1 P-glycoprotein inhibitor. Meanwhile, only hirsutidin and the control glibenclamide act as inhibitors for class 2.

VD is one of the characteristics that can be used to calculate the rate of distribution of a drug [35]. If the VD value of a drug is > 0.45, then it is considered to have a high distribution rate. Current study suggests that from the selected compounds kaempferol, petunidin, malvidin show greater VD meanwhile hirsutidin and the control glibenclamide displayed low VD. The BBB protects the brain from exogenous molecules by stopping them from reaching CNS [34]. BBB permeability can be used to study the distribution of a drug in the brain. Compounds with LogBB value > 0.3 will easily cross the blood brain barrier while permeability of those with a LogBB value of < −1 is considered poor. All selected ligands from the current study display poor BBB permeability.

Cytochrome P450 is an enzyme that is found in the liver. It plays a major role in detoxifying xenobiotics in our body [36]. It is also involved in the activation and inactivation of drug molecules in our body. It is necessary to examine the Cytochrome P450 inhibiting capability of the selected ligands. In this study different models of isoforms (CYP1A2,CYP2C19,CYP2C9,CYP2D6, CYP3A4) were used to predict the Cytochrome P450 inhibitory capabilities of a compound [37]. Current studies suggest that from the

selected ligands kaempferol, quercetin and malvidin show inhibitory effect on (CYP1A2) while hirsutidin shows inhibitory effect on (CYP1A2, CYP2C19, CYP2C9), petunidin shows no inhibitory effect while the control glibenclamide exhibits an inhibitory effect on (CYP2C9,CYP3A4). CYP2D6 & CYP3A4 are 2 isoforms of Cytochrome P450 substrate which are used to assess the metabolism of the compound [37]. The current study suggests that of the selected ligands only hirsutidin and the control glibenclamide were capable of acting as CY3A4 substrate.

The selected ligands showed no toxicity in the current study. Toxicity studies, like AMES study, skin sensitivity, cardiotoxicity, Herg 1 and 2 inhibition revealed negative results except for glibenclamide which displayed hepatotoxicity [34,38]. Determination of the pharmacokinetic property of a possible drug is extremely vital. PkCSM is a web serving platform which uses machine-learning to predict the pharmacokinetics properties of small molecules. PkCSM studies were done to determine the ADMET properties of the selected ligands, parameters such as CaCO2 permeability, intestinal absorption, BBB permeability, CNS permeability, Cytochrome P450 model study and various toxicity studies such as AMES toxicity, cardiotoxicity and hepatotoxicity [32]. For the current study, were deduct that all the ligands exhibit poor CaCO2 permeability and P-glycoprotein inhibitors except hirsutidin and the control glibenclamide. Meanwhile skin permeability and intestinal absorption was good for all of the ligands. Distribution of the selected ligands was found to be favorable. All of the selected ligands exhibited greater VD except hirsutidin and glibenclamide. BBB permeability was very poor for all the selected ligands. To assess the metabolism of the selected ligands a study was done using Cytochrome P450 model. Cytochrome P450 model had 2 isoforms CYP2D6 and CYP3A4. The current study showed that of the selected ligands only hirsutidin and glibenclamide were capable of acting as substrate (CYP3A4 substrate) indicating the lower metabolism in the liver. From the toxicity studies of our selected ligands only glibenclamide was found to be hepatotoxic. Present studies showed that all the flavonoids display good intestinal absorption, good bioavailability when compared with glibenclamide, they are incapable of crossing the BBB and unable to penetrate the CNS. For a successful drug development, it is important that the lead molecule should exhibit maximal potency against the drug target, be safe as well as have appropriate pharmacokinetic properties.

Molecular docking analysis

ADT used in this study, is an automated docking tool consisting of software that helps in modelling flexible small molecules like drug molecule binding to receptor protein [27]. Ten conformations of protein-ligand complex can be generated in ADT 2.0 or higher version. It displays conformations from lowest binding energy to highest binding energy. Prediction of relative binding affinity of TXNIP with quercetin, malvidin, petunidin, kaempferol, hirsutidin and the control glibenclamide was done using a computational docking algorithm which helped in observing the structure-inhibitory action relationship. The best fit was determined according to the number of hydrogen bonds with the enthalpic gain due to water molecules [39]. In the present analysis, the docking of quercetin, malvidin, petunidin, kaempferol, hirsutidin, and the control glibenclamide against TXNIP developed favorable receptor-ligand complex. There are 2 important steps associated with docking analysis where the prognosis of the identical orientation of the conformers in the best active site pocket known as pose and scoring was utilized to get the strength of target-ligand binding interactions [40].

Lamarckian Genetic Algorithm was used in the current study to see the binding conformational landscape of quercetin, malvidin, petunidin, kaempferol, hirsutidin and the control glibenclamide against TXNIP. Docking scores on TXNIP is a clear indication of a direct relationship between energy of the binding affinity and stability. Considering the previous statement, apart from the binding energy, torsional energy, and intramolecular energy were higher for petunidin followed by kaempferol and quercetin. The quercetin forms 5 hydrogen bonds with ARG271, TYR69, THR227, ARG71 and 2 hydrophobic interactions with PRO106, THR227 and kaempferol forms 5 hydrogen bonds with ARG71, ARG271, TYR69 and GLN107 which is similar to the position of glibenclamide which forms 5 hydrogen bonds with ARG271**,** THR227 and GLN107 and hydrophobic interactions with PRO106, LEU270, LEU222. Glibenclamide utilizes binding energy of −6.91 kcal/mol whereas quercetin and kaempferol utilizes little higher energy value of −6.59 and −6.56 kcal/mol. From the docking results, Quercetin and kaempferol found to inhibit TXNIP in the same position as glibenclamide. Hence among the screen flavonoids, Quercetin and kaempferol found to inhibit TXNIP. Quercetin and kaempferol when compared with glibenclamide have good bioavailability value, pharmacokinetics parameter, drug likeliness property and inhibitor activity against kinase receptors, enzymes and nuclear receptor ligand that was confirmed by molinspiration results. Elevated levels of TXNIP levels were found to induce β-cell death and causes

diabetes [6]. and hence the compounds Quercetin and kaempferol from *C. roseus* prevents type -I and type-II diabetes by inhibiting the activity of TXNIP.

The compounds quercetin, malvidin, petunidin, hirsutidin, kaempferol and the control glibenclamide were subjected to molecular docking against TXNIP. The best conformer was selected based on the binding energies. The bond interaction between quercetin, malvidin, petunidin, hirsutidin, kaempferol and the control glibenclamide against TXNIP in stick model using Biovia Discovery studio visualization tool is presented in **Figures 2(a) - 2(f)**. The bioactive compound Petunidin binding with amino acids of TXNIP displayed lowest estimated binding free energy of −7.29 kcal/mol and forms 1 hydrogen bond interaction with GLU104. Malvidin interacted with TXNIP with the binding energy of −6.63 kcal/mol and hydrogen bond with THR227, GLN107, LEU70 and ARG71 GLU104 and hydrophobic interaction with PRO106 and LEU70. Kaempferol exhibited a binding energy of −6.59 kcal/mol, 5 hydrogen bond interactions with ARG71, ARG271, TYR69 and GLN107 and hydrophobic interaction with LEU270 and PRO106. Quercetin displayed a binding energy of −6.56 kcal/mol with 5 hydrogen bond interactions, namely ARG271, ARG271, TYR69, THR227 and ARG71, whereas PRO106 and THR227 formed hydrophobic interactions. Hirsutidin showed a binding energy of −6.42 kcal/mol and 3 hydrogen bond interactions with GLU104, ARG71, and ARG271 while the control Glibenclamide displayed binding energy of −6.91 kcal/mol and 6 hydrogen bond interactions with ARG271, THR227 and GLN107. **Table 6** displays the Ligand efficiency and docking scores between bioactive compounds and TXNIP. The 2D diagram depicting Hydrogen and hydrophobic interactions are shown in **Figures 3(a) - 3(f)** and residues forming interactions are tabulated in **Table 7**.

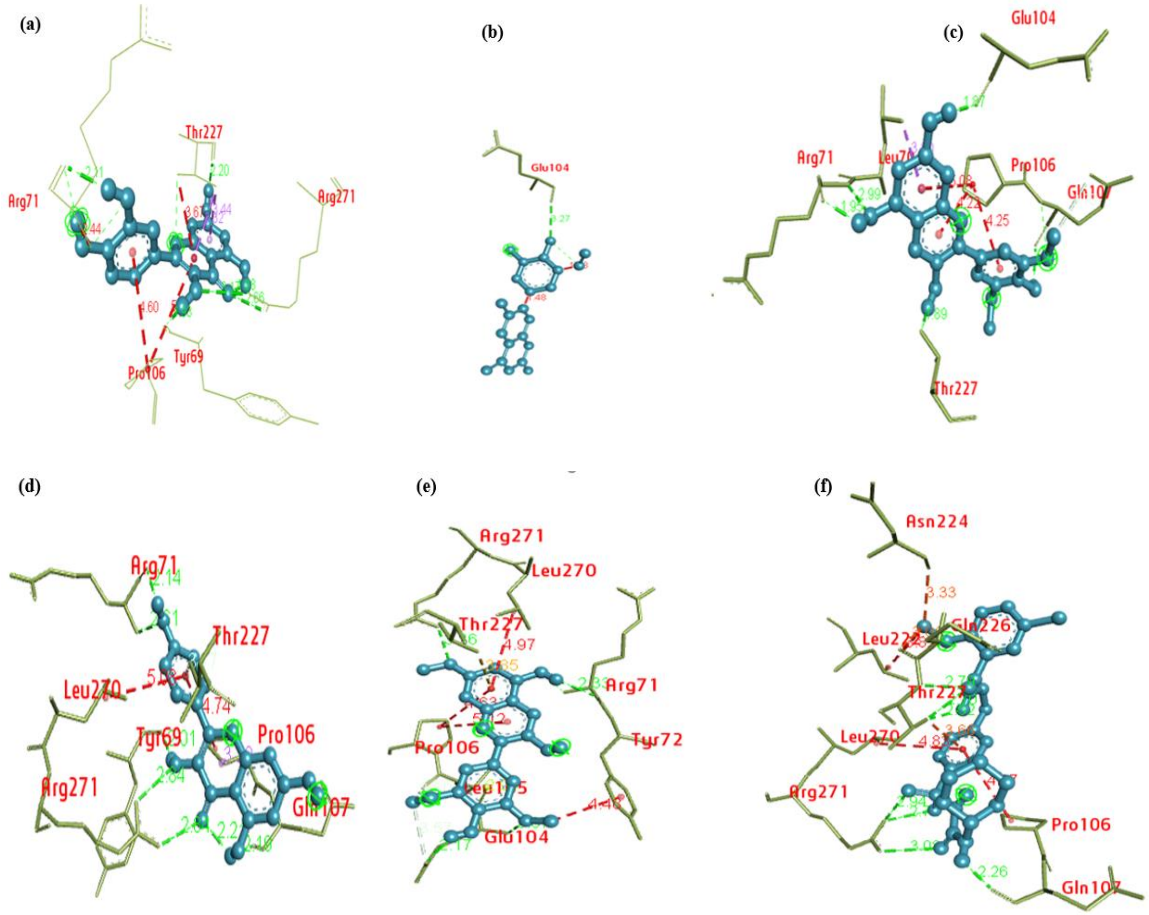

Figure 2 TXNIP (depicted in light green stick model) with compounds (shown in scaled ball and stick model) (a) quercetin; b) Petunidin; c) Malvidin; d) kaempferol; e) hirsutidin; f) glibenclamide interaction visualized by Biovia Discovery Visualizer. green colour dotted lines indicates hydrogen bond.

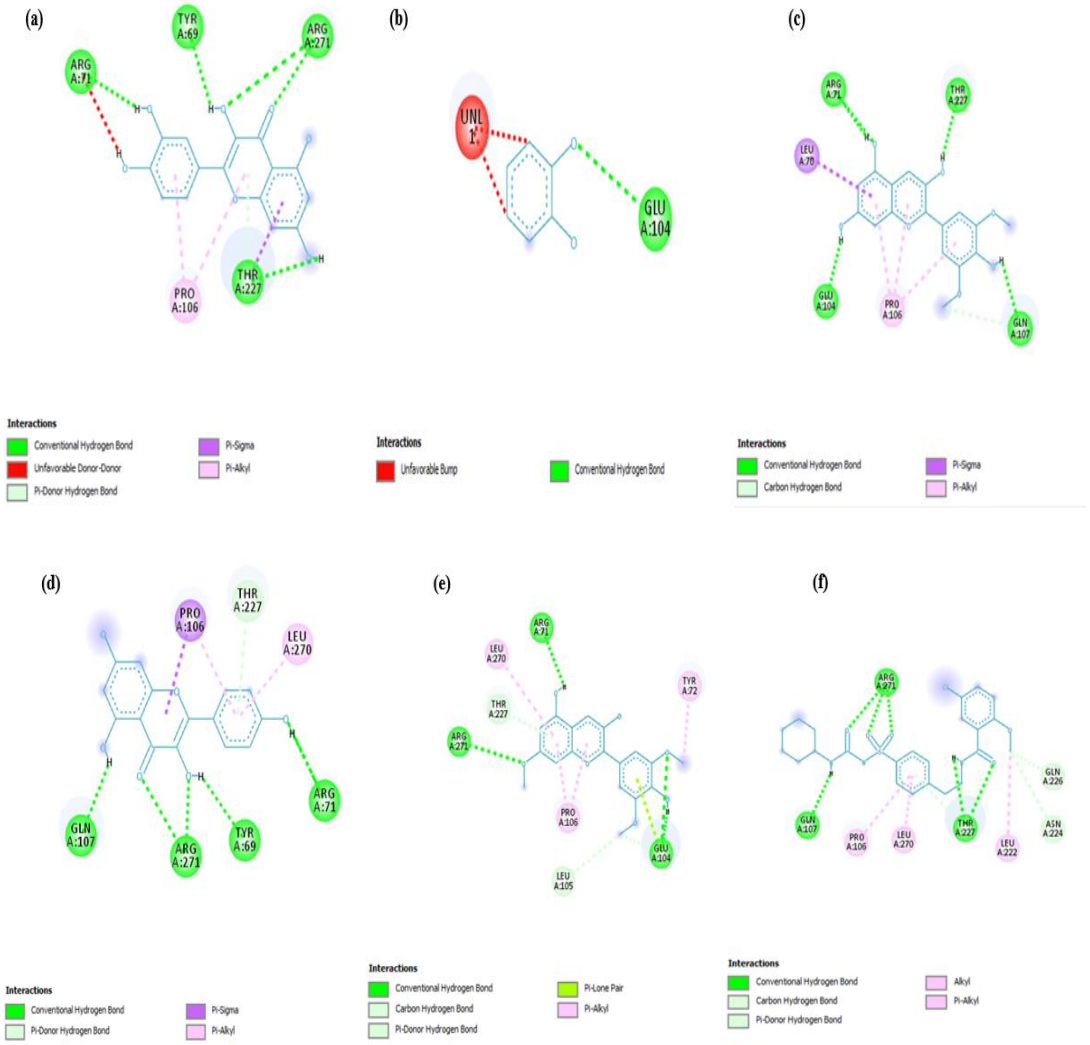

Figure 3 Two dimensional interaction of TXNIP with (a) quercetin; (b) Petunidin; (c) malvidin; (d) kaempferol; (e) hirsutidin; (f) glibenclamide visualized using Biovia Discovery studio visualizer showing hydrogen and hydrophobic interactions.

Table 6 Binding energies and ligand efficiency of flavonoids from *C. roseus* and glibenclamide and TXNIP predicted using autodock 4.2

TXNIP docking with compounds	Number of hydrogen bonds	Binding energy	Ligand Efficiency	Intermolecular energy	VdW + Hbond + Desolv energy	Electrostaic energy	Torsional energy	Total internal unbound
Quercetin	5	−6.56	−0.3	−8.35	−7.98	−0.38	1.79	−1.81
Petunidin	1	−7.26	−0.3	−7.86	−7.74	−0.12	0.6	−0.2
Malvidin	4	−6.63	−0.28	−8.27	−8.4	−0.32	2.09	−0.7
Kaempferol	5	−6.59	−0.31	−8.08	−7.72	−0.36	1.49	−0.99
Hirsutidin	3	−6.42	−0.26	−8.51	−8.19	−0.32	2.09	−0.75
Glibenclamide	6	−6.91	−0.27	−11.24	−10.84	−0.4	2.39	−1.36

Note: Unit for all energy values is Kcal/mol.

Table 7 Hydrogen bond interactions, hydrophobic and other interactions between TXNIP and flavonoids from *C. roseus* visualized using biovia discovery studio visualizer.

TXNIP with ligand interactions	Hydrogen bond interactions		Hydrophobic interactions	Other interactions
	Amino acid (Atom···Ligand atom)	Distance (Å)		
TXNIP + Quercetin	ARG271(NH1···O) ARG271(NH2···O) TYR69(O···H) THR227(O···H) ARG71(O···H)	3.17 2.66 1.93 2.20 2.11	**Alkyl/pi-alkyl** PRO106 **Pi-Sigma** THR227	NIL
TXNIP + Petunidin	GLU104 (O···O)	3.27	NIL	NIL
TXNIP + Malvidin	THR227(OG1···H) GLN107(O···H) LEU70(N···H) ARG71(O···H)	1.89 2.13 2.99 1.95	**Alkyl/pi-alkyl** PRO106 **Pi-Sigma** Leu70	NIL
TXNIP + Kaempferol	ARG71(O···H) ARG271(NH2···O) ARG271(NH1···O) TYR69(O···H) GLN107(O···H)	2.14 2.84 2.84 2.01 2.19	**Alkyl/pi-alkyl** LEU270 PRO106 **Pi-Sigma** PRO106	**Pi-Donor H-Bond** THR227
TXNIP + Hirsutidin	GLU104 (OE2···H) ARG71(O···H) ARG271(NH1···O)	2.17 2.33 3.36	**Alkyl/pi-alkyl** LEU270 PRO106 TYR72	**Pi-Lone Pair** GLU104 **Carbon hydrogen bond** LEU105 **Pi-Donor H-Bond** THR227
TXNIP + Glibenclamide	ARG271(NH2···O) ARG271(NH1···O) ARG271(NH1···O) THR227(OG1···H) GLN107(O···H)	3.08 2.94 2.72 2.52 2.26	**Alkyl/pi-alkyl** PRO106 LEU270 LEU222	**Pi-Donor H-Bond** GLN226 ASN224

Conclusions

Diabetes mellitus is the third biggest cause of early deaths worldwide and beta cell apoptosis is a very important characteristic of this disease. TXNIP protein can influence beta cell apoptosis. As a result, selecting an inhibitor against TXNIP target protein is a necessity, keeping in mind the approaching diabetes epidemic. This can be achieved by *in silico* molecular mechanism prediction. The current study, in docking proposes that bioactive compounds quercetin and kaempferol from the flowers of *C. roseus*, and act as a favorable candidate against TXNIP by downregulating its activity that in turn by promotes β-cell survival that combat type-2 diabetes. Quercetin and kaempferol may serve as a potential lead compound for as a promising anti-diabetic agent for further *in vivo* and *in vitro* studies.

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References

[1] MA Ganie and S Kotwal. Recent advances in management of diabetes mellitus. *J. Int. Med. Sci. Acad*. 2012; **25**, 171-5.

[2] International Diabetes Federation. *Diabetes atlas*. International Diabetes Federation. Brussels, Belgium, 2013.

[3] CY Liu, YN Hao, F Yin, YL Zhang and J Liu. Geniposide accelerates proteasome degradation of Txnip to inhibit insulin secretion in pancreatic β-cells. *J. Endocrinol. Investig*. 2017; **40**, 505-12.

[4] E Yoshihara, S Masaki, Y Matsuo, Z Chen, H Tian and J. Yodoi. Thioredoxin/TXNIP: Redoxisome, as a redox switch for the pathogenesis of diseases. *Front. Immunol.* 2014; **4**, 514.

[5] NM Alhawiti, S Al-Mahri, MA Aziz, SS Malik and S. Mohammad. TXNIP in metabolic regulation: Physiological role and therapeutic outlook. *Curr. Drug Targets* 2017; **18**, 1095-103.

[6] DZ Wondafrash, AT Nire'a, GG Tafere, DM Desta, DA Berhe and KA Zewdie. Thioredoxin-interacting protein as a novel potential therapeutic target in diabetes mellitus and its underlying complications. *Diabetes Metab. Syndrome Obes.* 2020; **13**, 43-51.

[7] M Jayanthi, N Sowbala, G Rajalakshmi, U Kanagavalli and V Sivakumar. Study of anti hyperglycemic effect of *Catharanthus roseus* in alloxan induced diabetic rats. *Int. J. Pharm. Pharmaceut. Sci.* 2010; **2**, 114-6.

[8] E Vega-Ávila, JL Cano-Velasco, FJ Alarcón-Aguilar, C Fajardo OrtízMdel, JC Almanza-Pérez and R Román-Ramos. Hypoglycemic activity of aqueous extracts from *Catharanthus roseus. Evid. Base. Compl. Alternative Med.* 2012; **2012**, 934258.

[9] N Saul, K Pietsch, R Menzel, SR Stürzenbaum and CE Steinberg. Catechin induced longevity in C. elegans: From key regulator genes to disposable soma. *Mech. Ageing Dev.* 2009; **130**, 477-86.

[10] HM Eid and PS Haddad. The antidiabetic potential of quercetin: Underlying mechanisms. *Curr. Med. Chem.* 2017; **24**, 355-64.

[11] C Sun, C Zhao, E Capanoglu, P Paoli, J Simal-Gandara, K Ramkumar, W Shengpeng, B Florina, P Ana, T Vladiana, D Georgiana, D Simona, T Merve, K Washim, W Mingfu, D Dominique, PP Maria, D Parsa, C Lei and J Xiao. Dietary polyphenols as antidiabetic agents: Advances and opportunities. *Food Front.* 2020; **1**, 18-44.

[12] Y Zang, H Sato, and K Igarashi. Anti-diabetic effects of a kaempferol glycoside-rich fraction from unripe soybean (Edamame, Glycine max L. Merrill. 'Jindai') Leaves on KK-Ay Mice. *Biosci. Biotechnol. Biochem.* 2011; **75**, 1677-84.

[13] JS Park, HS Rho, DH Kim and IS Chang. Enzymatic preparation of kaempferol from green tea seed and its antioxidant activity. *J. Agr. Food Chem.* 2006; **54**, 2951-6.

[14] XK Fang, J Gao and DN Zhu. Kaempferol and quercetin isolated from euonymus alatus improve glucose uptake of 3T3-L1 cells without adipogenesis activity. *Life Sci.* 2008; **82**, 615-22.

[15] SJN Tatsimo, JDD Tamokou, L Havyarimana, D Csupor, P Forgo, J Hohmann, JR Kuiate and P Tane. Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from Bryophyllumpinnatum. *BMC Res. Notes* 2012; **5**, 158.

[16] M Miyazawa, Y Okuno, S Nakamura and H Kosaka. Antimutagenic activity of flavonoids from Pogostemoncablin. *J. Agr. Food Chem.* 2000; **48**, 642-7.

[17] RJ Williams, PE Spencer and C Rice-Evans. Flavonoids: antioxidants or signalling molecules? *Free Radic. Biol. Med*. 2004; **36**, 838-49.

[18] HK Obied, MS Allen, NR Bedgood, PD Prenzler, K Robards and R Stockman. Bioactivity and analysis of biofenols recovered from olive mill waste. *J. Agr. Food Chem.* 2005, **53**, 823-37.

[19] CA Rice-Evans and NJ Miller. Antioxidant activities of flavonoids as bioactive components of food. *Biochem. Soc. Trans.* 1996; **24**, 790-5.

[20] S Zheng, Z Deng, F Chen, L Zheng, Y Pan, Q Xing, R Tsao and H Li. Synergistic antioxidant effects of petunidin and lycopene in H9c2 cells submitted to hydrogen peroxide: Role of Akt/Nrf2 pathway. *J. Food Sci*. 2020, **85**, 1752-63.

[21] P Tolambiya and S Mathur. A study on potential phytopharmaceuticals assets in catharanthus roseus L.(Alba). *Int. J. Life Sci. Biotechnol. Pharma Res.* 2016; **5**, 1-6.

[22] W Tian, C Chen, X Lei, J Zhao and J Liang. CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Res.* 2018; **46**, W363-7.

[23] NMO Boyle, M Banck, CA James, C Morley, T Vandermeersch and GR Hutchison. Open babel: An open chemical toolbox. *J. Cheminformatics* 2011; **3**, 33.

[24] A Jarrahpour, J Fathi, M Mimouni, MH Youssoufi, M Mimouni, ZH Chohan and TB Hadda. Petra, osiris and molinspiration (POM) together as a successful support in drug design: Antibacterial activity and biopharmaceutical characterization of some azo Schiff bases. *Med. Chem. Res.* 2012; **21**, 1984-90.
[25] CA Lipinski, F Lombardo, BW Dominy and PJ Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 2001; **46**, 3-26.
[26] DE Pires, TL Blundell and DB Ascher. pkCSM: Predicting small-molecule pharmacokinetic properties using graph-based signatures. *J. Med. Chem.* 2015; **58**, 4066-72.
[27] GM Morris, DS Goodsell, RS Halliday, R Huey, WE Hart, RK Belew and AJ Olson. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* 1998; **19**, 1639-62.
[28] L Design. *Pharmacophore and ligand-based design with Biovia Discovery Studio®*. BIOVIA. California, 2014.
[29] KC Cheng, WA Korfmacher, RE White and FG Njoroge. Lead optimization in discovery drug metabolism and pharmacokinetics/case study: The hepatitis C virus (HCV) protease inhibitor SCH 503034. *Perspect. Med. Chem.* 2007; **1**, 1-9.
[30] MP Gleeson. Generation of a set of simple, interpretable ADMET rules of thumb. *J. Med. Chem.* 2008; **51**, 817-34.
[31] AN Nordqvist, T Tuulikki Lindmark, A Eriksson, P Garberg and K Mats. A general model for prediction of Caco-2 cell permeability. *QSAR Combin. Sci.* 2004; **23**, 303-10.
[32] DEV Pires, LM Kaminskas and DB Ascher. Prediction and optimization of pharmacokinetic and toxicity properties of the ligand. *Meth. Mol. Biol.* 2018; **1762**, 271-84.
[33] D Lagorce, D Douguet, MA Miteva and BO Villoutreix. *In ADMET for medicinal chemists: A Practical guide (eds K. Tsaioun & S. A. Kates)*. John Wiley & Sons, Inc. New Jersey, 2010.
[34] D Lagorce, D Douguet, MA Miteva and BO Villoutreix. Computational analysis of calculated physicochemical and ADMET properties of protein-protein interaction inhibitors. *Sci. Rep.* 2017; **7**, 1-15.
[35] LM Kaminskas, VM McLeod, DB Ascher, GM Ryan, S Jones, JM Haynes, NL Trevaskis, LJ Chan, EK Sloan, BA Finnin, M Williamson, T Velkov, ED Williams, BD Kelly, DJ Owen and CJ Porter. Methotrexate-conjugated PEGylated dendrimers show differential patterns of deposition and activity in tumorburdened lymph nodes after intravenous and subcutaneous administration in rats. *Mol. Pharm*. 2015; **12**, 432-43.
[36] A Isvoran, M Louet, DL Vladoiu, D Craciun, MA Loriot, BO Villoutreix and MA Miteva. Pharmacogenomics of the cytochrome P450 2C family: Impacts of amino acid variations on drug metabolism. *Drug Discov. Today* 2017; **22**, 366-76.
[37] Y Han, J Zhang, CQ Hu, X Zhang, B Ma and P Zhang. *In silico* ADME and toxicity prediction of ceftazidime and its impurities. *Front. Pharmacol.* 2019; **10**, 434.
[38] pkCSM: Theory - how to interpret pkCSM results, Available at: http://biosig.unimelb.edu.au/pkcsm/theory, accessed June 2021.
[39] P Leeson and B Springthorpe. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* 2007; **6**, 881-90.
[40] R Wang, Y Lu and S Wang. Comparative evaluation of 11 scoring functions for molecular docking. *J. Med. Chem.* 2003; **46**, 2287-303.