Pharmacophore 2014, Vol. 5 (4), 509-516 USA CODEN: PHARM7 ISSN 2229-5402

Pharmacophore

(An International Research Journal)

Available online at http://www.pharmacophorejournal.com/

Original Research Paper

MOLECULAR DOCKING STUDIES OF *WATTAKAKA VOLUBILIS* FLAVONIDS AS INSULIN RECEPTOR TYROSIN KINASE ACTIVATOR AS CURE FOR DIABETES MELLITUS

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ABSTRACT

The studies on molecular docking have emerged during last three decades and now are becoming the integral part in drug discovery and development. In silico approach was used to access the use flavonids compounds of nutritionally enriched plant *Wattakaka volubilis*. Flavonids are potential agents that could act as insulin receptor tyrosin kinase activators and reduce the harmful effects of diabetes. Insulin binds to its receptors that are present on different cells of the body and mediates the absorption of glucose into the cell and they belong to the class of tyrosine kinase receptor. The three dimensional structure of Insulin receptor was obtained from PDB database and biocomponents of isoflovones and flavonones of the *Wattakaka volubilis* were downloaded from USDA database. Docking studies of Insulin receptor with *Wattakaka volubilis* biocomponents for isoflovones and flavonones were performed using HEX 6.3 software. Analysis of the docking results suggested that the flavonones sub category compound hesperidins can act as a potent insulin receptor activator than the other component.

Keywords: Diabetes**,** Insulin receptor, Isoflovones, Flavonones, *Wattakaka volubilis*, Docking HEX 6.3.

INTRODUCTION

Diabetes

Diabetes is a metabolic disorder in which blood sugar level rises due to lack of insulin. It is characterized by insulin resistance and relative deficiency of Insulin. It is one of the major diseases around the world which can result in diabetic retinopathy, neuropathy and many other conditions that may lead to death. Insulin receptor belongs to the class of tyrosine kinase. The binding of insulin to receptor causes conformational changes in the receptor leading to the activation of beta subunit of tyrosine kinase. Insulin is responsible for phosphorylation of insulin receptor that leads to glucose uptake by the cells. Most cells of the body have insulin receptors which bind the insulin and initiate a cascade of events that mediates the absorption of glucose from the blood into the cell.

Wattakaka volubilis

The plant *Wattakaka volubilis* Linn belongs to family Asclepiadaceae, is a traditional medicinal plant used to treat various diseases. The plant material is used in folk medicine for diabetes, analgesic and inflammation. Maruthupandian *et al.*, 2008; reported the presence of potent phytochemicals like flavonoids, terpenoids, tannins, glycosides, sterols, phenols and saponins in *W.volubilis leaf*. Rhemann *et al.*, 1989; reported that flavonoids, sterols, terpenoids, phenolic acids are known to be bioactive antidiabetic principles. Flavonoids are known to

regenerate the damaged beta cells in the alloxan diabetic rats (Chakravarthy *et al*., 1980).

Flavonoids

Flavonoids are well known for their multi-directional biological activities including anti-diabetic efficacy. The flavonoids are the largest class of polyphenols with a common structure of diphenypropanes (C6-C3-C6), (Ross *et al.*, 2002).

Docking Studies

Molecular docking is the technique employed for predicting and analyzing the interactions between protein receptors and ligands. It provides most detailed possible view of drug receptor interactions and also has created a new rational approach to drug design. Considering the high incidence of diseases, there is always a demand to find molecules for treatment. Diabetes, the third leading cause of death in the world, has many treatment regimens including insulin injections and oral hypoglycemic drugs (Bothara *et al*., 1998). The present study focused to dock the *Wattakaka volubilis* flavonoids (Isoflavones and Flavonones subcatogries) with insulin receptor tyrosine kinase to understand the interactions.

Data Banks

Protein Data Bank (PDB)

Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and acids. The data typically obtained by X-ray crystallography or NMR spectroscopy are submitted by biologists and biochemists around the world. PDB is considered as primary database and secondary database of structural genomics.

Pubchem

Pubchem is a database of chemical molecules. The system is maintained by the national center for biotechnology information (NCBI), which is part of the national library medicine of the National Institutes of Health (NIH). Pubchem gives description of substance and small modules with 1000 atoms and 1000 bonds. More than 80 database vendors contribute to the growing pubchem database.

Software and Tools

Open Babel

Open Babel is also a complete programmer's toolkit in developing chemistry software. Babel is a cross-platform program designed to interconvert many file formats used in molecular modeling and computational chemistry and related areas. Lipinski's Rule of Five is used to evaluate drug likeness of a chemical compound with a certain pharmacological or biological activity. The rule describes the drugs pharmacokinetics in the human body (Lipinski, 2001).

CASTp

CASTp (computed altas of surface topography of proteins) server uses the weighted Delaunay triangulation and the alpha complex for shape measurements. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities for proteins and other molecules. It measures the area and volume of each pocket and cavity, both in solvent accessible surface and molecular surface.

HEX

HEX (Haematopoietically Expressed Homeobox) is an interactive protein docking and molecular superposition program. HEX understands protein and DNA structures in PDB format, and it can also read small-molecule SDF files. To study the nature of interactions, binding mode and selectivity of insulin receptor protein with individual Isoflavones and Flavonones, docking was carried out by HEX 6.3 software.

MATERIALS AND METHODS

Plant Material

Qualitative tests for detecting the presence of various types of bioactive compounds was carried out using hot water and acetone extracts of *Wattaakaka volubilis*. The organic and hot water extract of leaves was subjected to preliminary phytochemical screening. TLC was performed to determine the number of compound present in the leaf extract. The hot water and acetone extracts responded positively for alkaloid, flavonoid, carbohydrate, tannin, phytosterol, triterpenoid, phenol and negative to glycosides and saponin. Both the extracts showed the positive results to

alkaline reagent test for flavonoid, major flavonids identified are isoflavones and flavonones. It has been demonstrated that flavonoids can act per as insulin mimetic, by influencing the pleiotropic mechanisms (Goutam, Brahmachari; 2011).The table (1) show the active compound of isoflavonos and flavonones were obtained from USDA Database.

PDB

The three dimensional structure of the insulin protein complexed with peptide substrate receptor of homo sepians was obtained from PDB database.

Ligands

The CID files of the ligands were obtained from PUBCHEM database. (http://pubchem.ncbi.nlm.nih.gov/) PubChem is a database of chemical molecules. The chemical compound structures of SDF (structure data format) form was convert into the PDB form discovers by Open Babel. (http://www.openbable.org/) Lipinski's rule considers a compound as a drug if it satisfies Lipinski's Rule of Five probable ligands were selected (http://www.scfbioiitd.res.in/utility/Lipinskifilters.jsp/).

Active Sites Analysis

The exact binding pocket should be predicted from the active site of 1IR3 receptor using CASTp (http://www.sts.bioengr.uic.edu/castp/) an online tool. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities for proteins and other molecules.

Docking Studies

HEX 6.3 is an interactive molecular graphics program for calculating and displaying feasible docking modes of molecular pairs. HEX can also calculate protein-ligand docking, assuming the ligand is rigid. The present study attempted to dock the *Wattakaka volubilis* flavonoids with insulin receptor tyrosine kinase to understand the interactions based on drug designing approaches. (http:\\ www.hexcuda 6.3.1.org)

RESULTS AND DISCUSSION

Mol Inspiration an online tool, is used to identify important molecular properties of ligands such as logP, molecular weight, H bond donors, H bond acceptors, number of atoms were obtained and then analyzed using Lipinski's Rule of Five, then applied to select probable ligands of daidzein, glycetein, hesperidins, naringenin, eridictyol, homoeridictyol and genistein. The filter values of compounds are shown in table (2).

The retrieved 1IR3 protein receptor had some binding sites and active sites and their site was predicted from CASTp an online tools, the most probable binding sites residues are located in binding pocket 44. Annotated residues were active site residue that was displayed in red color exposure and binding residues were displayed in green color balls.

The binding mode and interactions between 1IR3 receptor and daidzein, glycetein, genistein, hesperetin, naringenin, eriodictyol and homo eriodictyol of the each selected drugs were analyzed according to the binding energies obtained by the HEX software that is illustrated in Figure 2, 3. The binding energies and their binding sites are tabulated in table (3), indicate that Daidzein energy value from HEX was found to be Emin value -197.43, Daidzein was found to interacted with 1IR3 binding site residue GLY 990, SER 1067, LEU 1062, THR 1072, ASN 1046, PHE 1151,ASP 1150 by means of hydrogen bond. The Glycetein energy value of from HEX was found to be Emin value -185.03, Glycetein found to interacted with 1IR3 binding site residue GLU 990, SER 1067, LEU 1062, GLU 1047,ALN 1046, ILE 1148, PHE 1154, GLU 1043, ARG 1039 by means of hydrogen bond. The energy value of Genistein from HEX was found to be Emin value -189. Genistein found to interacted with 1IR3 binding site residue TRP 989, GLU 990, LEU 1062, VAL 1065, SER 1067, LEU 1045, VAL 1059, PHE 1151, THR 1154, LEU 1133 by means of hydrogen bond. The energy value of Hesperetin from HEX was found to be Emin value -212.43, Hesperetin found to interact with 1IR3 binding site residue PHE 1151, ILE 1148, VAL 1059, GLU 1047 by

hydrogen bond interactions. The energy value of Naringenin from HEX was found to be Emin value -199.87, Naringenin was seen to interact with 1IR3 binding site residues LEU 1063, LEU 1062, ARG 1061, GLY 1064, GLU 1047, PHE 1151, VAL 1059, GLY 1149, HIS 1130, PHE 1151, LEU 1135 by means of hydrogen bond interaction. The energy value of Eriodictyol from HEX was found to be Emin value -203.86. Eriodictyol found to interacted with 1IR3 binding site residue LEU 1063, GLU 988, GLU 990, LEU 1062, ARG 1061, GLU 1047, ILE 1148, VAL 1059, GLY 1152, CYS 1138, GLU 1043 by means of hydrogen bond. The energy value of Homo eriodictyol from HEX was found to be Emin value -1 so the Homo eriodictyol was eliminated and there inhibitory action is failed in 1IR3 receptor.

DISCUSSION

The plant *Wattakaka volubilis* is a traditional medicinal plant used to treat various diseases. Particularly, the plant material is used in folk medicine for diabetes, analgesic and inflammatory activity. (Maruthupandian, *et al.*; 2010) reported the presence of potent phytochemicals like flavonoids, terpenoids, tannins, glycosides, sterols, phenols and saponins in *W. volubilis* leaf Rhemann, et al. 1989 reported that flavonoids, sterols or terpenoids, phenolic acids are known to be bioactive antidiabetic components.

This study was performed to find the phytochemicals present in the plant extract and to find the docking nature of the component to the tyrosine kinase receptor which in turn help in control of the sugar in the blood and in the study it was found that flavonoid compound was present in the sample extract which was also reported by other researchers. The subcategories of isoflavone or flavnones such as daidzein, glycetein, genistein, hesperetin, naringenin, eriodictyol and homo eriodictyol were obtained from USDA database, similarly flavonoids component of banana flower from USDA database by previous researchers (Aashish *et al.*, 2012). Marles, *et al.*, 1995; Gray **et al**., 1997; reported that many plants have been traditionally used in the treatment of diabetes. Polyphenols contained in these plants have various therapeutic activities. Sabitha, V *et al.*; 2013 performed insilico study to find the interaction of polyphenols with insulin receptor tyrosine kinase has been analysed. But insilico study was performed by using the sub-categories compounds of isoflavone or flavnones of *W. volubilis* leaf.

Ganugapati, J *et al.*, 2011; performed docking studies of insulin receptor 1IR3 with flavonoids from green tea and reported flavone luteolin docked well into the active site of 1IR3. The isoflavones and flavonones bioactive compounds of the plant *Wattakaka voubilis* were subjected to the docking study with the insulin receptor tyrosine kinase in the present study. The three dimensional structure of Insulin Receptor of Homo sapiens was downloaded from RSCB Protein Databank (PDB) and PDB ID: 1IR3 the protein structure was having 546 residues, 3159 atoms, heterogen atoms 45 and solvent atoms 202 selected for the docking analysis. In the study Lipinski's Rule of Five, were applied to select probable ligands of daidzein, glycetein, hesperidins, naringenin, eridictyol, homoeridictyol and genistein. All the ligands were considered as drug like molecules as per lipinski's rules.

The present studies focused the active site analysis of the Insulin Receptor using CASTp software. The binding pocket of the1IR3 receptor containing LEU 1062, GLU 1047, PHE 1151 residues was identified as the most favorable binding site for all the ligands, so LEU 1062, GLU 1047, PHE 1151 active site residues can be used as the major target site for the anti-diabetic compounds and Ganugapati.J et al., 2011 reported that active site contains SER 1006, LYS 1030, GLU 1077, ASP 1083, ASN 1137, and ASP 1150, MET 1079. Docking results indicated that majority of the compounds bind to insulin receptor tyrosine kinase.

In the present study binding energy of the ligand hesperidins was calculated by HEX and hesperidins was found to be the most active compound having minimum binding energy (-

212.43) and interacted with probable binding sites of LEU 1062:A, PHE 1151:A,ILE 1148:A, GLU 1047:A by means of hydrogen bond. From this result, hesperidin was found to be a potent inhibitor of 1IR3 receptor in the present study and can be used as the major anti-diabetic compound from Wattakaka volubilis and in previous study (Ganugapati, J *et al.*; 2011). Autodock was used to calculate binding energies and hypothesized that Hesperitin triacetate, Naringenin, Naringenin pelargonidin and naringinen flavonone are potent activators of IR tyrosine.

CONCLUSION

The field of molecular docking is a one of the part in drug discovery and development. Insilico approach is used to assess the use of flavonoids compounds of nutritionally enriched plant *Wattakaka volubilis* which act as potential agents that could act as insulin receptor tyrosine kinase activators and reduce the harmful effects of diabetes. From the docking result the flavonones sub-categories compound hesperidins was found to be the most active compound and having minimum binding energy (-212.43) interacted with probable binding sites of LEU 1062: A, PHE 1151:A, ILE:A by means of hydrogen bond. These result shows that hesperidins is a potent inhibitor of 1IR3 receptor and can be used as the major anti-diabetic compound. The predicted information is hoped to help for understanding the mechanisms of these medicinal plant based anti-diabetic compounds for the treatment of diabetics cost effectively. These studies give a better advancement into the drug development studies in future.

ACKNOWLEDGEMENTS

We wish to thank the Management of Arignar Anna College for providing the necessary facilities to carry out this work. We render our heartiest thanks to Dr. M. Subbiah, M.A. (Eng), M.A. (JMC), M.A. (Political Sci), B. L., M. Phil., Ph.D, Principal, Arignar Anna College, Krishnagiri, for having given us an opportunity to carry out this work. Words find no bounds to express our heartful and sincere thanks to Dr. D. Sathish Sekar, M.Sc., M.Phil., Ph.D., Head, Department of Biotechnology, Arignar Anna College, Krishnagiri, who inspired and turned us into exponents in the broad field of our research work.

Table 1: Identified active constituents in *Wattakaka volubilis* flavonoids obtained from USDA Database

S. No.	ISO Flavones	Flavonones
1.	Daidzein	Hesperetin
2.	Genistein	Naringenin
3.	Glycitein	Eriodictyol
4.	----------	Homo eriodictyol

Table 2: The Lipinski Filter Values of compounds

Compound	Compound ID	Mol. Weight g/mol)	Mol. Formula	X-Log P	H-Bond Donor	H-Bond Acceptor	Molare Refractivity
Glycitein	5317750	254.00	$C_{16}H_{12}O_5$	2.714	2	4	69.148
Daidzein	5281708	284.00	$C_{15}H_{10}O_4$	2.726	2	5	75.700
Genistein	5250961	270.00	$C_{15}H_{10}O_5$	2.420	3	5	70.812
Hesperetin	72281	302.27	$C_{16}H_{14}O_6$	2.400	3	6	71.690
Naringenin	439246	272.00	$C_{15}H_{12}O_5$	2.510	3	2	66.320
Eriodictyol	440735	302.00	$C_{15}H_{12}O_6$	2.522	3	6	71.690
Homo eriodictyol	73635	288.00	$C_{16}H_{14}O_6$	2.215	4	6	67.161

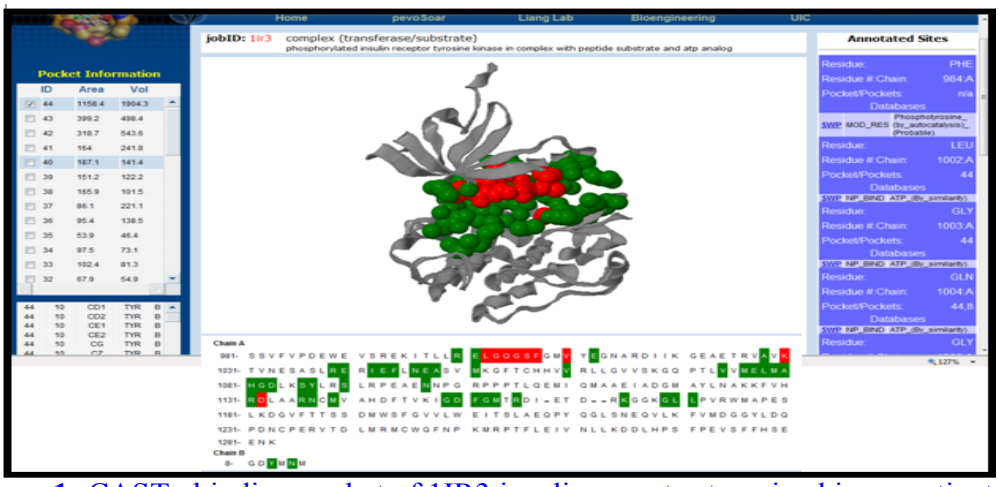

Figure1: CASTp binding pocket of 1IR3 insulin receptor tyrosine kinase activator

Table 3: The Hex Results of Docked Molecules and Binding sites of Molecules

S. No	Protein	Drug (Ligand)	Energy Range: Emin	Energy Range: Emax	Binding Site Residues
1	1IR3	Daidzein	-171.43	-87.47	GLU990,SER1067,LEU1062, THR1072,ASN1046,PHE1151,ASP1150.
2	1IR3	Glycetein	-185.03	-109.80	GLU990,SER1067,LEU1062,GLU1047, ASN1046,ILE1148,PHE1154,GLU1043,ARG1039.
3	1IR3	Genistein	-189	-110.71	TRP989,GLU990,LEU1062,VAL1065,LEU1045, VAL109,PHE1151,THR11154,LEU 1133.
4	1IR3	Hesperidins	-212.43	-118.49	LEU1062,PHE1151,ILE1148,VAL1059,GLU1047.
5	1IR3	Naringenin	-199.87	-115.80	LEU1063,LEU1062,ARG1061,GLY1064,GLU1047,PHE1 151,VAL1059,GLY1149,HIS1130,PHE1151,LEU 1135.
6	1IR3	Eriodictyol	-203.86	-118.03	LEU1063,GLU988,ILE1062,ARG1061,GLU1047,LEU114 8, VAL 1059,GLY 1152, CYS 1138, GLU 1043.
7	1IR3	Homo Eriodictyol	-1	-1	-------

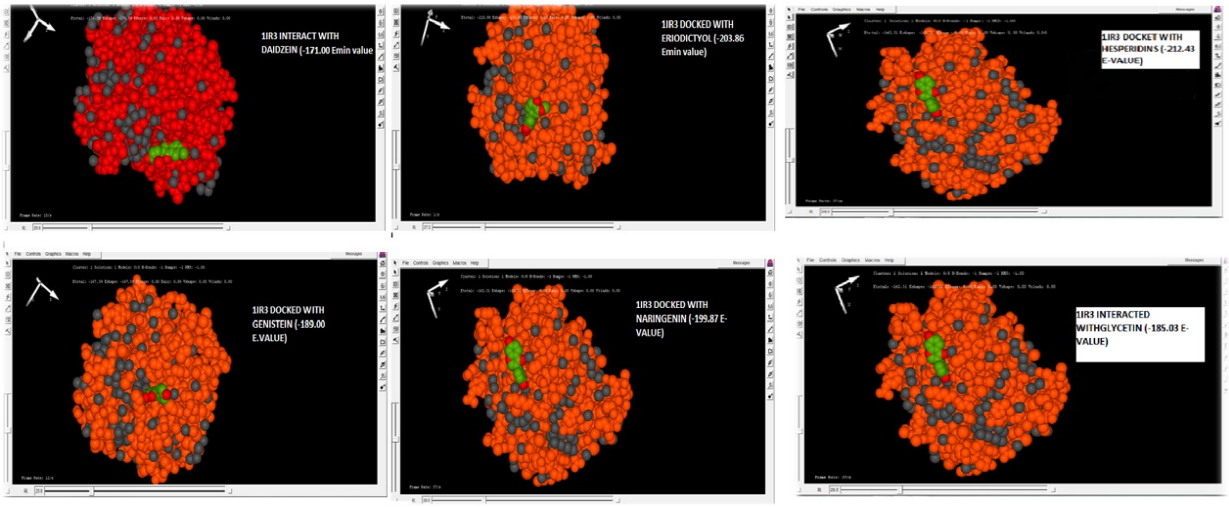

Figure 2: Illustrate that the HEX software can sucessfully dock 1IR3 insulin receptor tyrosin kinase activator with ligands (daidzein,genistein,naringenin,hesperitine and eriodictyol)

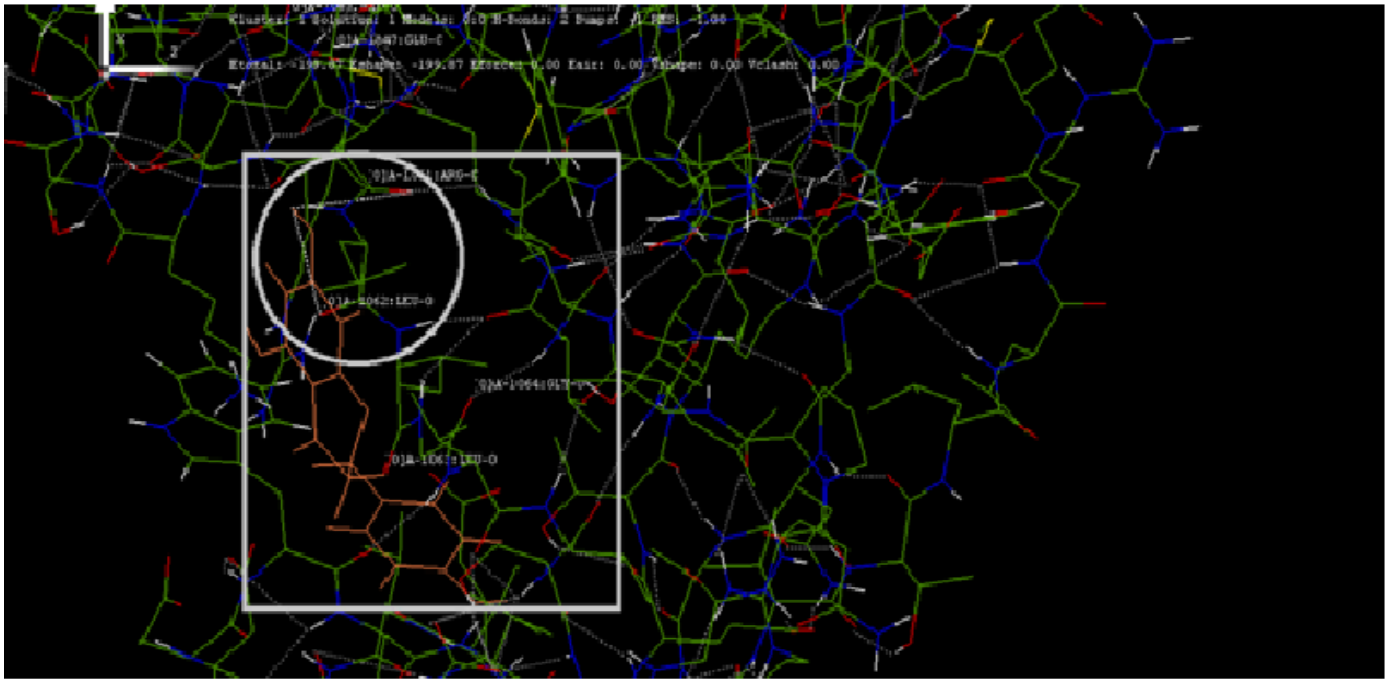

Figure 3: Illustrate that the HEX software can sucessfully dock 1IR3 insulin receptor tyrosin kinase activator with ligands that intraction in active site by means of hydrogen bond (square box- docked ligands (hesperidins) and circle ring- ligand hydrogen interact with receptor hydrogen bond in force of electrostatisical.

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Cite This Article: M, Priya and J, Caroline Rose (2014), "Molecular docking studies of *Wattakaka volubilis* flavonids as insulin receptor tyrosin kinase activator as cure for diabetes mellitus", ***Pharmacophore***, Vol. 5 (4), 509-516.