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Insilico docking study of compounds elucidated from *helicteres isora* fruits with ampkinase- insulin receptor

Subramanium Vennila*, Giridharan Bupesh*, Krishnan Saravanamurali, Viajayan SenthilKumar, Ramalingam SenthilRaja, Natarajan Saran & Sachidanandam Magesh

King Institute of Preventive Medicine and Research, Guindy, Chennai-600032; Giridharan Bupesh – Email: bupeshgiri@gmail.com; Subramanium Vennila – Email: Venajay1974@gmail.com *Corresponding authors

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Abstract:
Insulin receptor (IR) proteins were essential intracellular signaling peptides in the insulin action cascade. Insulin receptor substrate proteins (IRS-1and IRS-2) serve and regulate the insulin level in the normal insulin action. The broad role of IRS-1 and IRS-2 in cell growth and survival reveals a common regulatory pathway linking development, somatic growth, fertility, neuronal proliferation, and aging to the core mechanisms used by vertebrates for nutrient sensing. Such type of proteins were cyclic adenosine monophosphate-activated protein kinase, this proteins play a key role in the insulin response and regulation. Type -2 Diabetes mellitus occurs during prolonged periods of peripheral insulin resistance due to inactivation of IRS proteins. The compounds isolated from the medicinal plants were safer than synthetic drugs and possess high bio activity. In the present study, four compounds were elucidated from fruits of *Helicteres isora*. The elucidated compounds were evaluated for the antidiabetic activity using *Insilico* docking study. The receptor was analyzed for the active site and pocket finder tools. The aminoacids such as Phenylalanine, Lysine, Glutamic acid and Asparigine were predicted as active site binding residues. Docking studies were done through Autodock 4 software. All the compounds from fruits of *Helicteres isora* showed good docking profiles with AMP Kinase, except compound-3 (1,2,3,4-tetrahydro-1,5,6,8-tetramethyl-7-(2-methylprop-1-enylnaphthalene-4-ylpivalate). Finally the result from the study demonstrates that the HS-1, HS-2 and HS-4 posses potent anti diabetic activity against type-2 diabetes mellitus through drug action on AMP kinase cascade system.

Key words: *Helicteres isora*, Q-site finder, Autodock, Insulin receptor substrate proteins, Antidiabetic activity, Protein-drug docking.

Background:
Drug lead screening has been an active area of research for many years. Due to the tedious and expensive nature of experimental screening procedures, computational compound screening has been pursued extensively in recent years **[1, 2].** Receptors are macromolecules involved in chemical signaling between and within cells. They may be located on the cell surface membrane or within the cytoplasm. Activated receptors directly or indirectly regulate cellular biochemical processes (eg. ion conductance, protein phosphorylation, DNA transcription, enzymatic activity). Molecules such as drugs, hormones and neurotransmitters which bind to a receptor are called ligands. A ligand may activate or inactivate a receptor; activation may either increase or decrease a particular cell function. A high throughput virtual screening by molecular docking can be used nowadays in screening millions of compounds rapidly, reliably and cost effectively. Screening of pure compounds by *insilico* will be evaluated for the drug likeliness and ADME properties. Further it is also depending on structural complexity, functional group modified, the charges and interactions on the surfaces, can take from a few minutes to hours on a standard personal computer, which means screening all compounds in a single database can take years. Computation time can be reduced very significantly with a large grid gathering thousands of computers **[3, 4].** Fragment-based discovery attempts to identify such molecular anchors providing attractive starting points for inhibitor design. This strategy has gained increasing interest in primary screening **[5, 6].** In the present study the Identified pure

compounds from the *H. Isora* extracts (data were not given) were docking with Insulin receptor proteins to find out the antidiabetic activity by the Docking parameters.

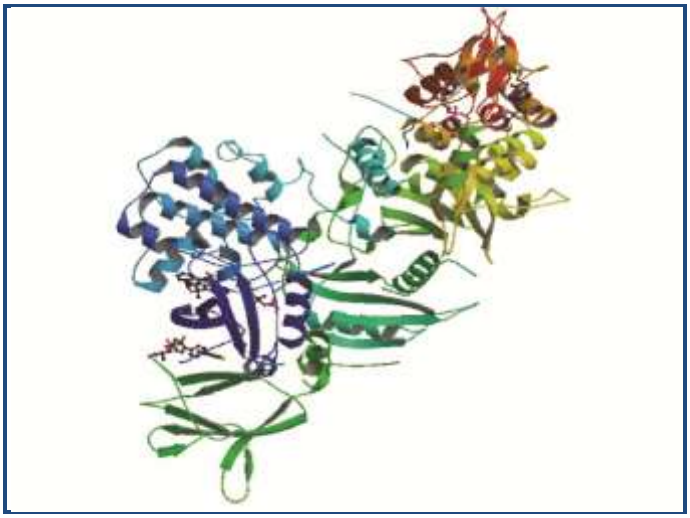

Figure 1: 3D strucutre of 5'-AMP-Activated Protein Kinase Catalytic Subunit Alpha-1 Pdb.Id.4CFF

Methodology:

Preparation of Receptor for docking

The Insulin receptors 5'-AMP-Activated Protein Kinase Catalytic Subunit Alpha-1 Pdb.Id.4CFF, was imported from the (http://.rcsb.org/pdb/home/home.do) shown in **Figure 1.** The receptor crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were connected using alternate conformations and valence monitor options. Further the protein was subjected to energy minimization using the CHARMm Force field.

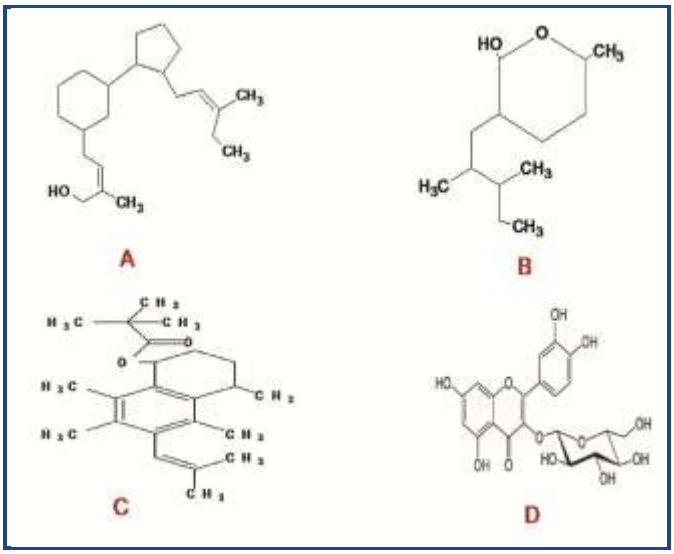

Figure 2: Structure of the identified ligands from *H.Isora:* **A)** ((2z)-2-methyl -4-(3-2((z) -3-methylpent-2-enyl)cyclopendyl) cyclohexyl) but-2-en-1-ol) **B**) Tetra hydro-6-methyl-3-(2,3-dimethylpentyl)-2H-pyran-2-ol; **C)** 1,2,3,4-tetra hydro -1,5,6,8-tetramethyl-7-(2-methylprop-1-enyl naphthalene-4-ylpivalate; **D)** 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxychromen-4-one.

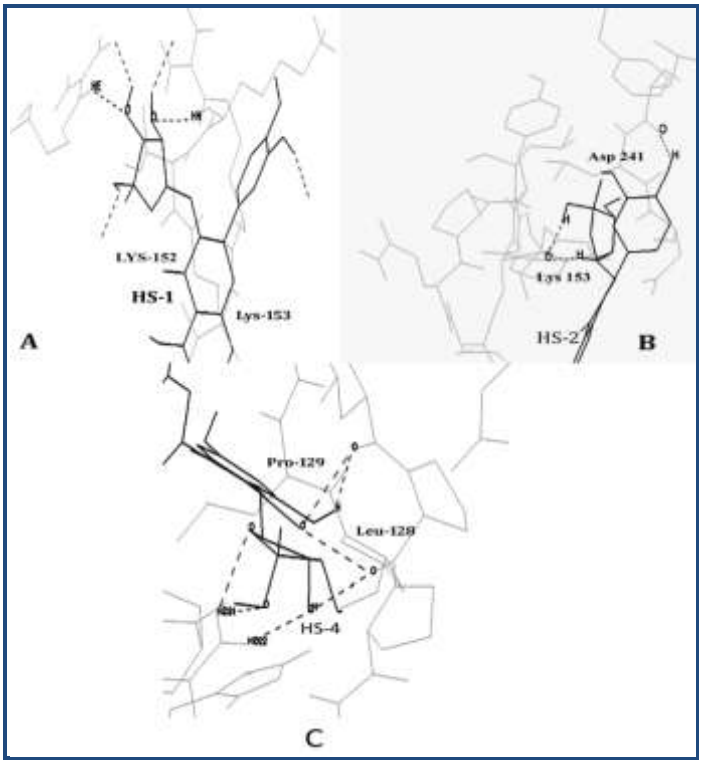

Figure 3: **A)** Docking of ((2z)-2-methyl -4-(3-2((z)-3-methylpent-2-enyl)cyclopendyl) cyclohexyl) but-2-en-1-ol) with AMP kinase; **B)** Docking of Tetra hydro-6-methyl-3-(2,3-dimethylpentyl)-2H-pyran-2-ol with AMP kinase; **C)** Docking of 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxychromen-4-one with AMP kinase.

Design of small molecules (Ligand)

The Efficacy of the enzyme inhibition with the identified four compounds (NMR data not given) from the *H.Isora* were docked with the Amp kinase Insulin receptor. The compounds were 1. ((2z)-2-methyl -4-(3-2((z)-3-methylpent-2-enyl) cyclopendyl) cyclohexyl) but-2-en-1-ol) 2.Tetra hydro-6-methyl-3-(2,3-dimethylpentyl)-2H-pyran-2-ol 3.1,2,3,4-tetra hydro -1,5,6,8-tetramethyl-7-(2-methylprop-1-enyl naphthalene-4-ylpivalate 4. 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxychromen-4-one. The above ligands were screened for antidiabetic activity against insulin receptors. The structure of these ligands was retrieved from NCBI –PubChem Compound database (http //pubchem.ncbi.nlm.nih.gov/) **[7].** The structures of these compounds were shown in **Figure 2.**

Active site finder

The active site was predicted by using the Qsite finder (www. bioinformatics.leeds.ac.uk./ qsitefinder/). This tool predicts the active atoms in the ligand binding site.

Molecular Docking studies by Autodock

AutoDock is docking software predicting the interaction of ligands with Biomacro molecular targets. AutoDock Tools software prepares the receptor by adding all hydrogen atoms to the macromolecule, to correct calculation of partial atomic charges. Gasteiger charges are calculated for each atom of the AMP kinase receptor molecule in AutoDock 4.2 instead of Kollman charges which were used in the previous versions of

the program. 3D- affinity grids of size 277 × 277 × 277 Å with 0.6 Å spacing were centered on the geometric center of the target protein and were calculated for each of the following atom types: HD, C, A, N, OA, and SA, representing all possible atom types in a protein. Additionally, an electrostatic map and a desolvation map were also calculated **[8].** The dimensional affinity grid was fixed based upon the necessity to run the AutoGrid file. The selected important docking parameters for the LGA as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and randominitial positions and conformations. The probability of performing local search on an individual in the population was set to 0.06. AMP kinase is a Insulin receptor which play a important role in the type 2 diabetes mellitus. This receptor was chosen and evaluated for the antidiabetic activity by *insilico* tool. The Compounds identified from the *H.isora* mentioned in the ligands were docked with the AMP kinase receptor using Autodock 4.2 software with above mentioned procedure. The drug score, binding frequencies, active residues interacted and energy minimizations were simulated.

Results & discussion:

Helicteres isora is a traditional medicinal plant which was rich in secondary metabolites such as terpenes, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates,oxalate and anthrax- quinines **[9-10].** In the present study the antidiabetic activity was evaluated using the *Insilico* docking activity using autodock. The *H.isora* compounds methanolic fractions were elucidated by Nuclear magnetic resonance spectroscopy and the structure identified was simulated and presented in the **Figure 2**. The strucutres were evaluated and mined from the NCBI pubchem databases. Further the active site residues were identified using the Q-site finder and shown in the **Table 1 (see supplementary material).** The Active site residues Phenylalanine ans asparigine were predominantly predicted and the numbers from 139-155 position. The other residues Methionine, Proline, Leucine, Glutamic acid were predicted in the receptor. The isolated compounds were docked with modeled AMP kinase auto dock. Compound (HS1) and compound (HS2) formed one hydrogen bond with LYS, ASP amino acid at position 152 and 241 presented in the **Figure 3**. Whereas compound HS3 showed no bonding therefore the docking of the compound 3 is not shown in the figure. Compound 4 showed good docking energy with 2 hydrogen bonds with AMPK Proteins bind to many types of molecules using a wide variety of binding sites. They have binding sites used by natural ligands, e.g., enzyme active sites and allosteric regulatory sites, as well as ''novel'' binding sites at which artificial or non-natural ligands, such as drugs, bind. Proteins are often bound to cofactors or are post-translationally modified and these non-protein components can have an important influence on the protein binding sites **[11].** In the present study, docking of isolated compounds from the methanol fraction of *H. isora* with AMP kinase receptor indicated that the compounds (HS1 and HS2) had binding score value of -7.79 and -7.61 **Table 2 (see supplementary material)** good docking score than the other two compounds. The compounds got hydrogen bonding with LYS (155) amino acid in the target of AMP kinase. But there were no interaction of the compound (HS3) with the AMP kinse. Whereas compound HS4 had high binding energy of -8.84 with formation of two hydrogen bonds with LEU(128) and PRO 129.AMP Kinase as being important in control of fatty acid oxidation and glucose uptake in skeletal muscle and possibly in modulating insulin secretion by the pancreatic b-cell. It is recognized that Type 2 diabetes is not one disease but a syndrome with numerous possible etiologies. Winder and Hardie (1999) reported that the activation of AMPK control system either with exercise or by pharmacological manipulation may partially correct the metabolic perturbations of the forms of Type 2 diabetes resulting from defects in the insulin signaling cascade **[12].**

Conclusion:

The Insilico docking studies of 4 compounds identified and docked from *H.isora* with AMP kinase insulin receptor (Type 2 diabetic targets) demonstrates, all the three compounds were well docked with specific targets. The Drug score and energy minimization reveals that the active compounds 1, 2 and 4 have potent antidiabetic activity. This ultimately confirms the compounds induce the secretion of necessary proteins and activates the IRS-receptors in carbohydrate metabolism for maintenance of glucose homeostasis condition during Diabetes mellitus. Finally, this paper concludes that the compounds isolated from *Helicteres isora* have good receipting activity of the Insulin receptor substrate proteins. Therefore this study recommends that the methanolic extracts of identifying compounds would be taken for the Type 2 Diabetes mellitus and it can be profiled for further clinical studies for the therapeutics of Diabetes mellitus.

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Supplementary material:

Table 1: Active site residues of AMP kinase predicted by Q-site finder.

Active site residues	Atoms	Residues Position
Phe	CA,CB,CG,CD1,CD2,CE1	139-44
Phe	CZ,C,O	146-48
ASN	N,CA,CB,CG,OD1, ND2,C	149-55
MET	CA	161
LYS	CB	152
LEU	ND1	128
PRO	CG	129

Table 2: Insilico docking of compounds isolated from *H. isora* with AMP kinase.

Compound	Score	Binding energy (kcal/mol)	Number of hydrogen bonds	Amino acids
HS 1	7.79	-38.18	1	LYS152
HS 2	7.61	-37.84	1	LYS152
HS 3	-	-	-	-
HS 4	8.84	-40.08	2	LEU128, PRO129