Docking of a *Cyperus rotundus* compound ‘15-Hydroxy-4-oxo-10-pentadecynoic acid lactone’ with antidiabetic drug targets: A comparative study

Lydia J*1,2* and Sudarsanam D3

1Research and Development Centre, Bharathiar University, Coimbatore-46. 2Department of Advanced Zoology, Biotechnology and Bioinformatics, Loyola College, Chennai-34, India

Abstract

**Background and Objective:** *Cyperus rotundus* L. or Mustaka or Motha is a medicinal herb that grows as a turf grass in the sandy or loamy soils of the tropical and subtropical parts of the world. It belongs to the plant family Cyperaceae. The grass is widely known for its antioxidant, anti-inflammatory, anti-diabetic and immune modulating properties. **Methodology:** The present study explores the antidiabetic potential of a particular compound, ‘15-Hydroxy-4-oxo-10-pentadecynoic acid lactone’ obtained by GCMS study, via a series of docking experiments, and ADMET studies. **Results:** It unravels the several ligand-target interactions that should possibly contribute to this property, and systematically compares it with the binding energy scores of commercial antidiabetic compounds such as metformin and gliclazide.

**Keywords:** *Cyperus rotundus* L. 15-Hydroxy-4-oxo-10-pentadecynoic acid lactone, metformin and Gliclazide

1. Introduction

*Cyperus rotundus* L. is a common weed that is widely used in the ancient Ayurvedic and Chinese traditional medicine. In the earlier investigations it has been primarily studied for its antioxidant properties. The medical uses of *Cyperus rotundus* L. have been exploited for thousands of years, especially in Chinese traditional medicine. The parts of *Cyperasrotundus* used include rhizome, leaves, seeds and oil. Ancient sages of India have considered it to be an appetizer, digestant, anti-diarrhoeal, anti-saturative, thirst relieving, anti-pruritic, reducing and lactodepurant herb. It is also well known for its diaphoretic properties. Earlier studies have documented the plant’s antioxidant12, hypolipidaemic2, anti-diabetic3, anti-inflammatory3, anti-bacterial4, antipyretic2, analgesic3 and tranquilizing activity5.

Diabetes is a disease that is prevalent in every nook and corner of today’s world. Almost every household has a diabetic patient and the disease has not spared rich or poor, old or young. There exist a vast community of medicinal plants that are found to have anti-diabetic property, and possess a rich number of antidiabetic compounds, but very few of these have been exploited for this purpose. Abdella et al explored computational tools to report 21 genes as ominous link with obesity associated diabetes6. This study suggests a novel agent with antidiabetic potential, which is a huge step forward to understand and compare Drug-Target interactions.7. Authors have used the Schrödinger Suite 2011 to perform the interaction studies (docking) and ADMET studies in the present investigation.

2. Methodology

The whole parts of *C. rotundus* L. plant at flowering stage were collected from Cholayil-Velagapuram farm, Chennai, India. An authenticated herbarium specimen of *Cyperus rotundus* L. (F. No: 3251) was deposited at the Herbarium, Sri Paramakalyani Center for Environmental Sciences Herbarium (SPKCESH), Alwarkurichi, Tamil Nadu, India.

The dried and powdered whole plant parts (which includes roots, rhizomes, flowers and leaves) of *C. rotundus* L. (500g) were incubated in Hexane, (1:3 ratio) for 48 hours in a shaker. The extract was collected using Whatman No. 1 filter paper and the above procedure was repeated. The hexane treated residual mixture was extracted with Methanol in 1:4 ratios and collected using Whatman No. 1 filter paper and evaporated below 40°C. The above procedure was repeated, and the extract was used for further analysis.

The rapidly increasing number of bioactive small molecules available from plant sources creates a unique opportunity to employ comparative modelling and docking to provide valuable insight into the function and ligand binding determinants of novel receptors, to assist in virtual screening and to design and optimize drug candidates. However, there are challenges which include, low sequence identity between receptors, conformational flexibility, and chemical diversity of ligands present an enormous challenge to molecular modelling approaches.

In this study, the Schrödinger Suite 2011 Induced Fit Docking...
protocol\textsuperscript{1,2,4}. Glide version 5.7, Prime version 3.0, Schrödinger and LigPrep, version 2.5, Schrödinger, LLC, New York, NY, 2011, were utilized to score the ligand-protein pair wise interaction energies.

Gas Chromatography- Mass Spectrometry (GC-MS) study was carried out at the South India Textile Research Association, Coimbatore - 641 014. The equipment used for the purpose was Thermo GC - Trace Ultra Version: 5.0, Thermo MS DSQ II. TR 5 - MS Capillary Standard Non - Polar Column of the following specification was used: Dimension: 30 mts, Id: 0.25 mm, Film: 0.25 mm. Carrier Gas Helium was used to flow at the rate of 1 ml per minute.

Four Protein targets were selected for the docking studies. These include the crystal structure of glycogen phosphorylase B complexed with glucose and cp320626, structure of DPPIV in complex with an inhibitor, the crystal structure of the murine class IIA PI 3-kinase p110delta in complex with IC87114 and the crystal structure of the complex of human interleukin-7 with glycosylated human interleukin-7 receptor alpha ectodomain.

The muscle glycogen phosphorylase is a protein with a single polymer with a length of 842aa. The 1.76 Astronm resolution crystal structure of glycogen phosphorylase B complexed with glucose and cp320626, a potential antidiabetic drug was downloaded from the Protein Data Bank, bearing the ID: 1HSU.\textsuperscript{15}Crystalllographic studies indicate, however, that selectivity between glycogen phosphorylase in skeletal muscle and liver is unlikely to be achieved\textsuperscript{15}. Therefore muscle glycogen phosphorylase was selected as a primary target for this study.

Dipeptidyl-peptidase IV (DPP-4) inhibitors are of immense use in treating Type 2 Diabetes. They inhibit the degradation of the incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) and thus help in lowering the blood glucose levels\textsuperscript{17}. The Structure of DPPIV in complex with an inhibitor\textsuperscript{18} was retrieved from the Protein Data Bank, bearing the ID:2RIP, and used for the docking studies.

Blocking of Phosphoinositide 3-kinase p110delta in a therapeutic treatment mode, with IC87114 treatment conferred prolonged protection from progression to overt diabetes in a number of animals. This suggests that PI3Kδ inhibitors could be useful for managing Type-1 diabetes\textsuperscript{18}. Also, numerous pathologies including cancer, diabetes, thrombosis, rheumatoid arthritis and asthma have found to be associated with the deregulation of the phosphoinositide-3-OH kinase (PI(3)K) pathway calling for the development of selective small-molecule inhibitors. The protein target phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta isoform with the PDB ID: 2X38 (The crystal structure of the murine class IIA PI 3 -Kinase P110delta in complex with IC87114) of length 940 aa\textsuperscript{19} was employed for the present docking studies.

Anti-IL-7 receptor-a have been proven to reverse established Type 1 Diabetes in nonobese diabetic mice by modulating effector T-cell function\textsuperscript{21}. Hence we employ the Crystal structure of the complex of human interleukin-7 with glycosylated human interleukin-7 receptor alpha ectodomain obtained from the PDB ID: 3DI3\textsuperscript{22} for the docking experiments.

Ligands used in the docking studies include metformin, one of the oldest drugs used to treat diabetes which is considered to be the best option\textsuperscript{23} and Gliclazide. The structure of metformin, a biguanideantihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM) was obtained from the Drug Bank DB00331\textsuperscript{24}. Metformin(C\textsubscript{4}H\textsubscript{11}N\textsubscript{5}), being the only oral antihyperglycemic agent that is not associated with weight gain, improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin (Figure I) may induce weight loss and is the drug of choice for obese NIDDM patients. Metformin decreases fasting plasma glucose, postprandial blood glucose and glycosolatedhemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control.

Gliclazide (C\textsubscript{15}H\textsubscript{21}N\textsubscript{3}O\textsubscript{3}S) is also an oral antihyperglycemic agent used for the treatment of NIDDM. It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Gliclazide (Figure I) has been shown to decrease fasting plasma glucose,

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FigureI.png}
\caption{Structure showing Metformin (left) and Gliclazide (right)}
\end{figure}

The Glide Energy (Table I) for the docking was -32.83. Residues other than the six water molecules that envelope the ligand include arginine 60, histidine 37, threonine 38, Proline 188, tryptophan 189, lysine 191, arginine 193, tryptophan 67 and valine 64.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{SL} & \textbf{RSI} & \textbf{Compound Name} & \textbf{Molecular Formula} & \textbf{Molecular Weight} & \textbf{Area %} \\
\hline
110 & 967 & 15-Hydroxy-4-oxo-10 -pentadecyanoic acid lactone & C\textsubscript{15}H\textsubscript{22}O\textsubscript{3} & 250 & 0.50 \\
\hline
\end{tabular}
\caption{Table I: GC-MS data of isolated compound}
\end{table}

\section*{2.1 Statistical analysis:}
Data collected was entered into MS-Excel and Survey data was analyzed using SPSS (Statistical Package for Social Sciences) version 11.5 software. Statistical tests chi squared was also used. p value < 0.05 is statistically significant.

\section*{3. Results}
Induced Fit Docking was done using the Schrodinger software. GC-MS results are shown in the Table I/Figure II.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FigureII.png}
\caption{Figure II: Structure showing Metformin (left) and Gliclazide (right)}
\end{figure}

They were studied elaborately and the best output was selected for the discussion. These findings offer specific guidelines which may lead to increased success in determining receptor-ligand complexes.

The ligand binding site for the Docking of Glycogen Phosphorylase B involved the centroid of the ligand, CHN205-Cloro-1H-Indole-2-Carboxylic Acid and a pocket formed by at least 6 water molecules namely, HOH 2017, 2018, 2101, 2102, 2349 and 2099.

Docking of Glycogen Phosphorylase B with 15HOPL, yielded an IFD score of -1903.49, with a corresponding glide score of -5.82271 and Prime energy, 37953.4. The Glide Energy for the docking was -32.83. Residues other than the six water molecules that envelope the ligand include arginine 60, histidine 37, threonine 38, Proline 188, tryptophan 189, lysine 191, arginine 193, tryptophan 67 and valine 64.

Docking of Glycogen Phosphorylase B with Metformin, yielded an IFD score of -1901.2, with a corresponding glide score of -3.2998 and Prime energy, -37958.

The Glide Energy (Table I) for the docking was -21.39. The pocket formed by at least 7 water molecules namely, HOH 2017, 2018, 2101, 2102, 2349, 2099 and 2103.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{SL} & \textbf{RSI} & \textbf{Compound Name} & \textbf{Molecular Formula} & \textbf{Molecular Weight} & \textbf{Area %} \\
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110 & 967 & 15-Hydroxy-4-oxo-10 -pentadecyanoic acid lactone & C\textsubscript{15}H\textsubscript{22}O\textsubscript{3} & 250 & 0.50 \\
\hline
\end{tabular}
\caption{Table I: GC-MS data of isolated compound}
\end{table}
The binding site for the Docking of DPP IV is the centroid of the ligand 34Q: (3R,4R)-4-(pyrrolidin-1-ylcarbonyl)-1-(quinolin-2-ylcarbonyl)pyrrolidin-3-amine. The docking of DPP IV with 15HOPL generated an IFD score of -1629.88, with a corresponding glide score of -5.76367 and Prime energy, -32483.2. The Glide Energy for the docking was -26.92. As shown in Figure III, a single H bond side chain was formed with the O atom of the ligand and Aspartagine 710 of the protein.

These findings demonstrate that, for the target 2RIP, 15HOPL would be the best ligand with highest the IFD score and Glide scores, though the glide score and the H bond score are the second highest. Long-term treatment with isoleucine thiazolidide, a Dipeptidyl peptidase IV inhibitor has been found to stimulate beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats suggesting that DPP IV inhibitors may be useful also, for managing Type-1 diabetes.28

Docking of DPP IV with Metformin returned an IFD score of -1619.1, with a corresponding glide score of -3.5767 and Prime energy, -32310. Three H bond side chains were formed with N2 of ligand and Tyrosine 547, NH of ligand and Tyrosine 666 and with N5 of ligand and Tyrosine 662.

The ligand binding site for the Docking of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta isoform is centroid of the ligandIC82028(2-[(6-amino-9H-purin-9-yl)methyl]-5-methyl-3-(2-methylphenyl)quinazolin-4(3H)-one). The docking of PI 3-Kinase P110delta with 15 HOPL yielded an IFD score of 1941.7, with a corresponding glide score of -7.11495 and Prime energy, -38691.7. The Glide Energy for the docking was -32.69. The ligand is bound by two water molecules, HOH 2040 and HOH 2036, which forms two H bond with the residue arginine 60, and N5 forms 3 H bond backbones with Proline 188 and Glutamate 190.

In the docking of Glycogen Phosphorylase B with Gliclazide, the IFD score was -1903, with a corresponding glide score of -5.7267 and Prime energy, 37945. The Glide Energy for the docking was -34.19. The ligand formed two H bonds with the residue Glutamate 190 and Tryptophan 189. The ligand pocket was bound by similar residues as the above dockings, including two water molecules.

The above experiments reveal that, for the target 1H5U, 15HOPL would be the best ligand with highest the IFD and Glide scores. Hepatic glucose output is elevated in type 2 diabetic patients and current evidence indicates that glycoenolysis (release of monomeric glucose from the glycogen polymer storage form) is an important contributor to the abnormally high production of glucose by the liver. Glycogen phosphorylase is the enzyme that catalyses this release and recent advances in new inhibitors of this structurally and kinetically well studied enzyme have enabled work which further delineate the pharmacological and physiological consequences of inhibiting glucose production by this pathway. Moreover, the agents that inhibit glycogen phosphorylase, also lower glucose in diabetic animal models, both acutely and chronically, affecting both gluconeogenic and glycogenolytic pathways and demonstrate potential for a beneficial effect on cardiovascular risk factors.27

### Table II: Results of Induced Fit Docking

<table>
<thead>
<tr>
<th>Docking</th>
<th>Glide Score</th>
<th>IFD Score</th>
<th>Prime Energy</th>
<th>Glide Energy</th>
<th>H-Bond Score</th>
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</thead>
<tbody>
<tr>
<td>3H5U- Metformin</td>
<td>-3.29</td>
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<td>-37958</td>
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<td>-37945</td>
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<tr>
<td>3H5U- 15HOPL</td>
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<td>-1903.5</td>
<td>-37953</td>
<td>-32.83</td>
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</tr>
<tr>
<td>2RIP- Metformin</td>
<td>-3.58</td>
<td>-1619.1</td>
<td>-32310</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2RIP - Gliclazide</td>
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<td>-</td>
<td>-</td>
<td>-38.06</td>
<td>-1.12</td>
</tr>
<tr>
<td>2RIP - 15HOPL</td>
<td>-5.73</td>
<td>-1629.9</td>
<td>-32483</td>
<td>-26.92</td>
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</tr>
<tr>
<td>3X38- Metformin</td>
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<td>-38702</td>
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</tr>
<tr>
<td>3X38- Gliclazide</td>
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<td>-38866</td>
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<tr>
<td>3X38- 15HOPL</td>
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<td>1941.7</td>
<td>-38691</td>
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<tr>
<td>3D13- Metformin</td>
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<td>-686.4</td>
<td>-13653</td>
<td>-28.71</td>
<td>-1.71</td>
</tr>
<tr>
<td>3D13- Gliclazide</td>
<td>-5.51</td>
<td>-686.1</td>
<td>-13612</td>
<td>-38.87</td>
<td>-1.2</td>
</tr>
<tr>
<td>3D13- 15HOPL</td>
<td>-3.50</td>
<td>-683.1</td>
<td>-13599</td>
<td>-29.21</td>
<td>-1</td>
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</tbody>
</table>
Docking of PI 3-Kinase P110delta with Gliclazide yielded an IFD score of -1942.4 and a glide score of -8.0932 and Prime energy, -38686. The Glide Energy for the docking was -49.63. The ligand forms two H bond side chains with Lysine 779 and HOH 2036.

The above outcome demonstrates that, for this target, Gliclazide would be the best ligand with highest the IFD score, glide energy and glide score. 15 HOPL would be the second best ligand with the second highest scores. Selective PI 3-Kinase P110delta inhibitors are of immense use to treat Diabetes Mellitus that occurs especially due to autoimmune malfunction.

The ligand binding site for the Docking of IL-7Rα included the residues of Chain B:151, B:175, B:176, B:178, B:179, B:180, B:181, B:206, B:207, B:208.

The docking of IL-7Rα with 15 HOPL generated an IFD score of -683.11, with a corresponding glide score of -3.50 and Prime energy, -13599.7 (Figure V). The Glide Energy for the docking was -29.21. The O-atom of the ligand, as displayed in Figure 5, formed two H bond side chains with Lysine 157 and Tryptophan 181.

The docking of IL-7Rα with Metformin yielded an IFD score of -686.37, with a corresponding glide score of -3.7105 and Prime energy, -13653. The Glide Energy for the docking was -28.71. (Figure VI shows the various interactions of the ligand with this receptor.

Docking of IL-7Rα with Gliclazide yielded an IFD score of -668.11, a glide score of -5.5089 and Prime energy, -13612. The Glide Energy for the docking was -38.87. The O atom of the ligand formed two H bond side chains with Glutamine 171 and Tryptophan 181.

The above results demonstrate that, for the target IL-7Rα, Gliclazide would be the best ligand with highest the scores and, though the H bond score is the second highest. IL-7Rα expression is found to be mirrored by the dependency of aTregs on IL-7 for persistence. It has been suggested that IL-7 uniquely maintains FoxP3+ adaptive Treg cells that reverse diabetes in NOD mice via integrin-β7-dependent localization. IL-7Rα blockade altered the balance of regulatory T cells and T (E/M) cells, promoting T cell-extrinsic regulation and further increasing the threshold for diabetogenic T-cell activation. Blockade of IL-7 signals with anti-IL-7 receptor α (IL-7Rα) mAbs in NOD mice demonstrated that this treatment not only prevented the development of diabetes, but also reversed established disease. It appears that IL-7 contributes to the pathogenesis of autoimmune diabetes by enabling T(E/M) cells to remain in a functionally competent state which implies IL-7Rα blockade as a therapy for established T-cell-dependent autoimmune diseases.

The ADMET result of the ligand 15HOPL showed that it does not violate any of the Lipinski Rule of 5 and has good oral absorption and very low toxicity.

**Conclusion**

The plant, *Cyperus rotundus*, being a key ingredient of many traditional antidiabetic preparations, poses as an attractive target for diabetologists to explore the various phytochemicals, it comprises of, so as to arrive at the principal compounds involved in the antidiabetic activity. Parts of this plant is used in medicinal preparations such as Abana, Diarex, Evecare, Renalka, Diarex Vet, Himpyrin, Himpyrin Vet, HimROP Vet, Himfertin, Liv.52 HB and Bresol. This study shows a novel compound, 15-Hydroxy-4-oxo-10-pentadecynoic acid lactone, reported in this plant for the first time, to possess comparable scores as that of certain commercial compounds/drugs, being established by insilico studies. It has been demonstrate that 15HOPL is the best ligand when compared with metformin and Gliclazide, when docked to Glycogen Phosphorylase B and DPP IV. It was the second best ligand for the receptor, PI 3-Kinase P110delta, while Gliclazide would be the best ligand for the receptor IL-7Rα.

**Acknowledgement**

Authors acknowledge Dr. Sushil Kumar Middha, Assistant professor, Department of Biotechnology, DBT-BIF facility, Maharani Ammanni College for women, Bangalore for his valuable suggestions during study.
Conflict of Interest
Authors don’t have any conflict of interest.

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