In-silico ANALYSIS AND MOLECULAR DOCKING STUDIES OF NOVEL THIAZOLIDINEDIONE DERIVATIVES AGAINST PPAR-γ

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ABSTRACT
Type 2 Diabetes mellitus (T2DM) is one of the immunometabolic disorders that are distinguished by hyperglycemia persisting over a period of time. Thiazolidinediones are the drugs that activate the Peroxisome proliferator-activated receptors (PPARs). Materials and Methods: A sequence of new Thiazoline 2,4-dione derivatives was designed with ChemDraw software. In silico predictions like PASS prediction, Molinspiration, and Acute rat toxicity prediction with the help of GUSAR software were done. Results: Based on the results, the designed derivatives were docked with the target protein Peroxisome Proliferator-Activated Receptor Gama (PPARγ) (Pdb Id: 7AWC). Conclusion: Compounds T6, T5, T7, T9, T63, and T11 showed the maximum potency and binding affinity to the PPAR-γ receptor when compared to the rosiglitazone as standard. From the docking score, it is quite clear that the substitution of the fluorene ring to the thiazolidine 2,4-dione nucleus increases the antidiabetic activity.

Keywords: Anti-diabetic, PASS, PPARγ, Docking, and Thiazolidinedione.

INTRODUCTION
Type 2 Diabetes mellitus (T2DM) is one among the immunometabolic disorders that are distinguished by hyperglycemia persisting over a period of time.1,2 Polyurea, polydipsia, and polyphagia are some manifestations of the disease.3 Diabetes majorly affects fatality and mortality rates and is considered one of the most alarming disorders worldwide.4,5 Currently available antidiabetic medications include sulfonylurea, biguanides, α-glucosidase inhibitors, thiazolidine-2,4-diones (TZDs), and DPP IV inhibitors, of which thiazolidinediones, also known as glitazones plays a major role as insulin-sensitizing agents.6-9 These drugs act upon the PPAR-γ thereby exerting its therapeutic action. Peroxisome proliferator-activated receptors (PPARs) are transcription factors, the activity of which is regulated by binding with ligands and comes under nuclear receptors. They are prominently found in adipose tissues and are divided into 3 subtypes which include PPARα, PPARβ/δ, and PPAR-γ. PPARs, when bound to PPAR-responsive regulatory elements (PPER), are responsible for regulating specific gene expressions that are associated with adipogenesis, lipid metabolism, and maintenance of metabolic homeostasis.10-13 In type II diabetes mellitus, the subtype PPAR-γ plays a vital role as a glitazone receptor and is considered the primary biochemical target for thiazolidinediones.14,15 Pioglitazone and Rosiglitazone are notable members of the thiazolidinedione class. Despite its efficacy, in 2010, Rosiglitazone was directed to include the black box warning by FDA, due to the increased risk of heart attacks.16 In our current study, we have utilized the CADD techniques to design and analyze new chemical structures for the already existing drug, rosiglitazone. Thus, keeping in mind the toxicity of this compound, the physicochemical properties, toxicity studies, and docking studies were performed for newly designed thiazolidinedione analogs, in order to bring compounds with increased efficacy and decreased toxicity.

EXPERIMENTAL
Software and Hardware
The computer system with Intel® Core ™ i5 9th Gen CPU processor having 4GB RAM and 500 GB hard disk with Windows 10 as the operating system was used. All the computational studies were carried out in

http://doi.org/10.31788/RJC.2021.1526797

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various software such as Schrodinger (2020-3) – Maestro, version 12.4 (Paid version), ChemDraw Ultra version 8.0, and Discovery studio Visualiser v21.1.0. Online tools like PASS, Molinspiration, GUSAR, and Chemical Databases such as Protein Data Bank were used (Open Sources).

**Target Selection**
In our current study, we've attempted to locate PPAR-γ modulators that can serve as modern beneficial antidiabetic agents. In this case, the three-dimensional (3D) crystal structure of Peroxisome proliferator-activated receptor gamma (PDB ID: 7AWC) was obtained from the Protein Data Bank (www.rcsb.org/pdb) in pdb format. The Crystal structure of PPAR-γ contains chain A with 2,4-thiazolidinedione, 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-(9CL), and glycerol.

**Designing of Compounds**
The Structural Activity Relationship (SAR) of thiazolidinediones was studied in detail through various articles and the new compounds were designed accordingly. As said earlier, Rosiglitazone was chosen as the standard drug, and taking into account the importance of thiazolidine 2,4-dione as the basic nucleus, 68 structures were accurately drawn using ChemDraw Ultra 8.0 to this basic scaffold to increase the biological activity. These structures were exported in mol2 format for further docking purposes.

**Biological Activity Prediction**
To predict the biological activity spectrum of the designed compounds, we’ve used the PASS (Prediction of Activity Spectra for Substances) online tool (http://way2drug.com/PassOnline/). The pharmacological actions and their underlying mechanisms can be simultaneously predicted for several compounds using this web-based tool. After prediction, the results were tabulated showing the biological activity with their probability values namely Pa (Probability of Activity) Pi (Probability of Inactivity).

- If $Pa > 0.7$, the substance exhibits the activity and is likely to be an analogue of a known drug.
- If $0.5 < Pa < 0.7$, the substance may or may not exhibit activity, with the probability being less, and is not an analogue of any known drug.
- If $Pa < 0.5$, the substance either exhibits no activity or might be a new chemical entity exhibiting activity.

**Acute Toxicity Prediction**
Acute toxicity refers to all those adverse effects that occurs after a one-time introduction to a substance, within a given time. LD50 value is one of the key attributes of acute toxicity to quantify the short-term acute toxicity of a material. It indicates the dose that may cause 50% of population death within 24 hours of administration. For acute toxicity assessment, it is important to predict the oral, intraperitoneal, and intravenous acute rodent toxicity. Owing to the abundance of data available, these studies are most commonly performed in mice and rats. The importance of computational toxicity assessment paved the way for the development of various methods. The toxicity classes with the LD50 values are given in
Table-3. In our study, we’ve used the web-based software GUSAR (General Unrestricted Structure-Activity Relationships) for predicting acute rat toxicity.

**In Silico Evaluation of Physicochemical Properties**

**Drug Likeness**

Drug likeness refers to the compatibility of a compound with different molecular properties and structural features to most of the known drugs.\(^{26,27}\)

**Lipinski’s Rule**

Lipinski's rule of five, also known as Pfizer's rule of five, was developed by Christopher A. Lipinski in 1997. It is a traditional method to evaluate the drug-likeness of a compound.\(^{28-30}\) The rule basically estimates the pharmacokinetic properties of the drug without predicting its biological activity.\(^{26}\) “According to Lipinski’s rule, a drug-like compound should not violate more than one of the following criteria:

- The molecular weight of less than 500 g/mol,
- A log P value of less than 5 represents its hydrophobicity,
- No more than 5 hydrogen bond donors (HBD), and
- No more than 10 hydrogen bond acceptor (HBA) sites”.\(^{28}\)

If more than one parameter violates the rule, the compound may produce poor absorption or permeability.\(^{28,29}\)

**Molinspiration**

The physicochemical properties and drug-likeness of the designed set of compounds were predicted using the online tool Molinspiration (http://www.molinspiration.com) to optimize their design and therapeutic activity.\(^{31,32}\) The properties were evaluated based on Lipinski’s rule, to predict whether the compounds comply with the criteria of drug likeness. The molecular properties such as molecular weight, partition coefficient, number of hydrogen bond donors, number of hydrogen bond acceptors, and polar surface area were calculated.

**Molecular Docking**

Molecular docking is the computational prediction of the favored orientation of a ligand to its target molecule when complexed with each other.\(^{33}\) By knowing the optimized conformation and orientation through molecular docking, one can determine the binding affinity using scoring functions.\(^{34}\) The docking can be: (i) Rigid body docking (ii) Flexible ligand docking and (iii) Flexible docking, the most common method being the flexible ligand docking wherein the ligand is free to move and the protein is kept stationary. Different software is available for docking namely: Glide, AutoDock, AutoDock vina, Gold, FlexX, etc. In our study, we’ve used the Glide software to estimate the binding energies of the compounds.

(i) **Protein Preparation**

The target protein with the PDB ID: 7AWC was imported into Maestro and some typical operations were performed which may include: (i) addition of hydrogen atoms (ii) removal of heteroatoms, water molecules, and unwanted chains (iii) adding protons, and (iv) optimizing protonation states. This was followed by optimization and minimization of the H bond network, which completes the protein preparation step.

(ii) **Grid Generation**

After preparing the protein, the grid was generated by using the coordinates 40.3, 1.5, and 90.9, which were recognized from the position of the previously existing ligand in the protein.

(iii) **Ligand Preparation**

The previously drawn structures were imported into the LigPrep… window and the ligand preparation was started which took less than a minute to complete.

(iv) **Flexible Ligand Docking**

For docking, only the grid and the set of ligands prepared for screening were defined and not necessarily the receptor. The scoring function was chosen and docking was performed. We have used the SP (Standard-Precision) scoring function which is a quick way to evaluate the poses.
(v) Analysis of the results
In the end, the results were incorporated into the project table and the docked poses along with 2D ligand interactions were visualized using the pose viewer function of Maestro. Also, the hydrogen bond interactions were visualized using Discovery studio Visualiser v21.1.0.

RESULTS AND DISCUSSION
In the present study, the TZD analogues were evaluated through in silico analysis. Initially, the structure of these compounds was designed (see Table-1) and screened for their biological activity (see Table- 3). 35 compounds that showed the best activity was chosen and predicted for toxicity and physicochemical properties and the results are tabulated in Table-4 & 5. Finally, docking simulation between the protein 7AWC and the chosen 20 compounds was done to optimize the best fit for the ligand in the active site of the target and the results are shown in Table-6. From the final results it was observed that, a docking score between -13 and -12 showed the highest binding energy. With that being the case, the compound T6 showed the highest binding energy of -13.184 kcal/mol. The best 6 compounds were chosen based on the docking score and were visualized. The 3D docking poses, 2D ligand interactions and the hydrogen bond interactions are shown in Figs.-3, 4 and 5.

![Fig.-3. 3D Poses of Ligand Interaction with the Target](image-url)

a) T5

b) T6

c) T7

d) T9

e) T11

f) T63
Table-1: R-Group of Compounds Showing Best Activity

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound Code</th>
<th>R GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>T5</td>
<td>![T5 Diagram]</td>
</tr>
<tr>
<td>2.</td>
<td>T6</td>
<td>![T6 Diagram]</td>
</tr>
<tr>
<td>3.</td>
<td>T7</td>
<td>![T7 Diagram]</td>
</tr>
<tr>
<td>4.</td>
<td>T9</td>
<td>![T9 Diagram]</td>
</tr>
</tbody>
</table>

Fig.-4; Hydrogen Bond Interactions of Designed Ligands
Table- 2: Toxicity Chart with LD50 Values$^{19,25}$

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Deadly if ingested</td>
<td>≤ 5</td>
</tr>
<tr>
<td>Class II</td>
<td>Deadly if ingested</td>
<td>5 ≤ 50</td>
</tr>
<tr>
<td>Class III</td>
<td>Toxic if ingested</td>
<td>50 ≤ 300</td>
</tr>
<tr>
<td>Class IV</td>
<td>Harmful if ingested</td>
<td>300 ≤ 2000</td>
</tr>
<tr>
<td>Class V</td>
<td>Might be harmful if ingested</td>
<td>2000 ≤ 5000</td>
</tr>
<tr>
<td>Class VI</td>
<td>Not harmful</td>
<td>&gt; 5000</td>
</tr>
</tbody>
</table>

Table -3: PASS Data for Anti-Diabetic Activity of Designed Compounds

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound Code</th>
<th>Pa</th>
<th>Pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>T5</td>
<td>0.985</td>
<td>0.003</td>
</tr>
<tr>
<td>2.</td>
<td>T6</td>
<td>0.987</td>
<td>0.003</td>
</tr>
<tr>
<td>3.</td>
<td>T7</td>
<td>0.991</td>
<td>0.003</td>
</tr>
<tr>
<td>4.</td>
<td>T9</td>
<td>0.990</td>
<td>0.003</td>
</tr>
<tr>
<td>5.</td>
<td>T11</td>
<td>0.983</td>
<td>0.003</td>
</tr>
<tr>
<td>6.</td>
<td>T63</td>
<td>0.974</td>
<td>0.003</td>
</tr>
<tr>
<td>7.</td>
<td>Rosiglitazone(Standard)</td>
<td>0.955</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table-4: Acute Toxicity Profile of Designed Compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound Code</th>
<th>Rat IP LD50 (mg/kg)</th>
<th>Rat IV LD50 (mg/kg)</th>
<th>Rat Oral LD50 (mg/kg)</th>
<th>Rat SC LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>T5</td>
<td>1366,000 in AD</td>
<td>204,400 in AD</td>
<td>1307,000 out of AD</td>
<td>3057,000 out of AD</td>
</tr>
<tr>
<td>2.</td>
<td>T6</td>
<td>1180,000 in AD</td>
<td>222,300 in AD</td>
<td>979,100 in AD</td>
<td>1451,000 out of AD</td>
</tr>
<tr>
<td>3.</td>
<td>T7</td>
<td>1184,000 in AD</td>
<td>340,200 in AD</td>
<td>585,300 in AD</td>
<td>684,100 in AD</td>
</tr>
<tr>
<td>4.</td>
<td>T9</td>
<td>1047,000 in AD</td>
<td>285,100 in AD</td>
<td>1655,000 out of AD</td>
<td>1317,000 in AD</td>
</tr>
<tr>
<td>5.</td>
<td>T11</td>
<td>1120,000 in AD</td>
<td>176,800 in AD</td>
<td>515,900 in AD</td>
<td>1286,000 out of AD</td>
</tr>
<tr>
<td>6.</td>
<td>T63</td>
<td>819,100 in AD</td>
<td>156,900 in AD</td>
<td>223,700 out of AD</td>
<td>1386,000 in AD</td>
</tr>
<tr>
<td>7.</td>
<td>Rosiglitazone(Standard)</td>
<td>619,700 out of AD</td>
<td>126,100 in AD</td>
<td>574,100 in AD</td>
<td>760,600 out of AD</td>
</tr>
</tbody>
</table>

Table-5: Predicted Physicochemical Properties and Drug Likeness of Designed Compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound Code</th>
<th>Mol. Wt.</th>
<th>HBD</th>
<th>HBA</th>
<th>logP</th>
<th>TPSA</th>
<th>Lipinski Rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>T5</td>
<td>421.90</td>
<td>1</td>
<td>3</td>
<td>5.64</td>
<td>59.31</td>
<td>Yes; 1 violation</td>
</tr>
<tr>
<td>2.</td>
<td>T6</td>
<td>437.96</td>
<td>1</td>
<td>2</td>
<td>6.28</td>
<td>46.17</td>
<td>Yes; 1 violation</td>
</tr>
<tr>
<td>3.</td>
<td>T7</td>
<td>403.52</td>
<td>1</td>
<td>2</td>
<td>5.67</td>
<td>46.17</td>
<td>Yes; 1 violation</td>
</tr>
<tr>
<td>4.</td>
<td>T9</td>
<td>386.47</td>
<td>2</td>
<td>2</td>
<td>4.93</td>
<td>61.96</td>
<td>Yes; 0 violation</td>
</tr>
<tr>
<td>5.</td>
<td>T11</td>
<td>389.47</td>
<td>2</td>
<td>2</td>
<td>3.71</td>
<td>61.43</td>
<td>Yes; 0 violation</td>
</tr>
<tr>
<td>6.</td>
<td>T63</td>
<td>428.50</td>
<td>1</td>
<td>4</td>
<td>4.56</td>
<td>68.30</td>
<td>Yes; 0 violation</td>
</tr>
<tr>
<td>7.</td>
<td>Rosiglitazone(Standard)</td>
<td>357.43</td>
<td>1</td>
<td>4</td>
<td>2.35</td>
<td>71.53</td>
<td>Yes; 0 violation</td>
</tr>
</tbody>
</table>
CONCLUSION

A series of thiazolidinedione analogs were designed and investigated for safety and efficacy in treating type II diabetes mellitus. For this, we have performed various studies predicting the biological activity (Pass prediction), toxicity prediction (GUSAR), physicochemical properties evaluation (Molinspiration), and molecular docking analysis (Glide). In conclusion, the compounds T6, T5, T7, T9, T63, and T11 showed the maximum potency and binding affinity to the PPAR-γ receptor with amino acids SER 499, TYR 473, HIE 323, and HIE 449. From the docking score, it is quite clear that the substitution of the fluorene ring to the thiazolidine 2,4-dione nucleus increases the antidiabetic activity.

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[RJC-6797/2021]