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Docking studies of 3,5-disubstituted thiazolidinedione chalcones as PPAR-γ agonist

***Fajeelath Fathima, Abitha Haridas, Baskar Lakshmanan**

Department of Pharmaceutical Chemistry, Grace College of Pharmacy, Kodunthirapully, Palakkad-678004, Kerala, India

Abstract: PPARs play crucial role in the regulation of cellular differentiation, development and metabolism of carbohydrates, lipids and proteins in human, of which PPAR- γ has pivotal role in glucose homeostasis. In modern drug designing, molecular docking is routinely used for understanding drug receptor interaction. In the present study molecular docking were performed on a diverse set of 3,5-disubstituted thiazolidinedione chalcone derivatives that demonstrate antidiabetic activity by stimulating PPAR- γ. Among the designed analogues, **e3**, **a3, b3** and **c3** showed significant binding free energy of -12.29, -12.04, -11.53 and -11.45 kcal/mol with predicted inhibitory constant values of 987.38 pM, 1.5, 3.53 and 4.04 nM respectively and all the selected compounds were compared with standard drug Rosiglitazone.

Keywords: AutoDock 4.2; Docking; PPARγ; Thiazolidinedione chalcones

1. Introduction

Diabetes mellitus is one of the very common chronic diseases across the world and the number of diabetic patients is on the rise. The World Health Organization (WHO) estimates that about 200 million people all over the globe are suffering from diabetes and this figure is likely to be doubled by 2030. WHO says that about 80% of the deaths occur every year due to diabetes in middle-income countries[1]. Type 2 diabetes mellitus (T2DM) is a genetically heterogeneous, polygenic disease with a complex inheritance pattern and is caused by genetic predisposition and environmental factors[2]. The disease is characterized by altered expression of many genes and their products in several tissue types[3,4]. The recently published Indian council for medical research-India diabetes (ICMR-INDIAB) national study reported that there are 62.4 million people with T2DMand 77 million people with pre-diabetes in India[5]. This will be increased to 100 million by 2030. Thiazolidinedione (TZD) is a powerful insulin sensitizer in the treatment of T2DM.[6] It acts as a ligand to the nuclear receptor PPAR-γ and induces transcription of PPAR-γ responsive genes. Derivatives of TZD, such as rosiglitazone and pioglitazone are more powerful than metformin or berberine in insulin sensitization. Although they have common side effects such that they increase the risk of heart attack and angina, fluid retention, weigh gain, and cardiac failure, thus TZDs use should be selective in diabetic patients who are not impaired liver and heart failure.[7,8,9]

Based on the side effect story of TZD and derivatives, it is an effort to minimize the side effects by selectivity in to ligand binding domain of PPAR-γ. Ligand-binding site is a large T-shaped cavity that extends from the C-terminal helix to the β-sheet lying between helices H3 and H6. This domain is mainly hydrophobic and is buried within the bottom half of the ligand binding domain. The surface around the entry site comprises several hydrophilic side chain amino acids namely, ASP243, GLU290, ARG288 and GLU295. The newly designed PPAR-γ analogues occupies roughly 40% of the ligand-binding site in the ternary complex. In general the ligand is in a U-shaped conformation and can makes several specific interactions with amino acids LEU333, ARG288, SER289, GLN286, CYS285 and can be changed with each conformation. The carbonyl groups of the TZD form hydrogen bonds with LEU228 and HIS449.The partly negatively charged Nitrogen of the TZD head group is within hydrogen-bonding distance of the TYR473 side chain carboxyl group. All of these primary and secondary hydrogen bonds result in a fixed conformation of the TZD head group and of the participating amino acids. Next to the head group, the sulphur atom of the TZD ring is positioned in a hydrophobic region of the PPAR-γ ligand-binding pocket formed by PHE363, GLN286, PHE282 and LEU469.[10,11] The CH=CH-C=O group between the N-substituted benzene ring (linker) and the benzene ring (tail) provides vital geometry for the compounds. Moreover Ligand-dependent transcriptional activation by nuclear receptors probably requires the recruitment of co-activator proteins such as **steroid receptor co-activating factor-1 (**SRC1). These co-activator proteins contain one or more copies of the LXXLL motif (where X

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*corresponding author: FF Tel: 8943583780; E-mail: fathufaji14@gmail.com

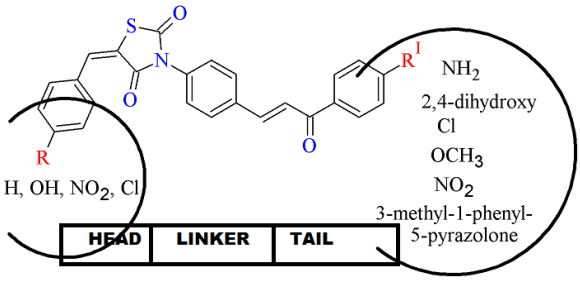


Figure 1. Design of PPAR-γ agonists

is any amino acid) interacting with these overcome the side effects associated with PPAR-γ agonists.[12,13,14] Our study reveals the interaction of analogues specifically with co-activator proteins getting the highest dock score. The design of a ligand-based approach is outlined in (Figure 1).

2. Result and Discussion

To evaluate the accuracy of AutoDock 4.2 as an appropriate docking tool for our current study, the co-crystalized ligand rosiglitazone for 2PRG.pdb was redocked within the binding cavity of PPAR-γ by maintaining the SRC-1 as flexible residue. As mentioned in the many articles, the interaction of rosiglitazone towards the active pocket of PPAR-γ mainly stabilized by two hydrogen bonding with the SER289 to the carbonyl group of ligand and a covalent ternary adduct with the HIS323. Interestingly the same hydrogen bonding interaction could generate the current type of flexible molecular docking study with rosiglitazone (Figure 2). So the same docking methodology proceeded with the remaining designed PPAR-γ agonists.

Previously, many studies revealed that PPAR-γ analogues showed antidiabetic activity and also used in the treatment of hyperlipidemia. Keeping in view the chemical structure of thiazolidinedione and its crucial role in the treatment of diabetes, we designed a new series of thiazolidinediones incorporated with various acetophenones. Almost all the designed structures showed better binding energy towards the active pocket of PPAR-γ than the standard rosiglitazone. The details of the binding score and calculated inhibition constant were shown in the Table 1. The values of predi

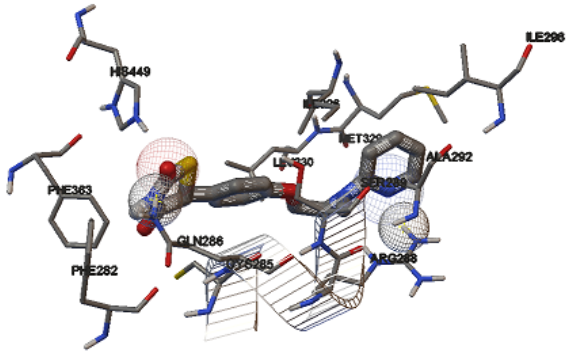
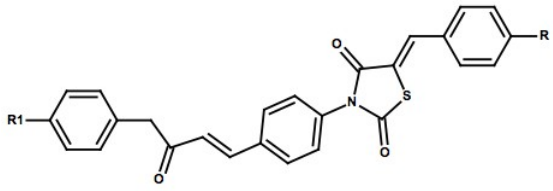
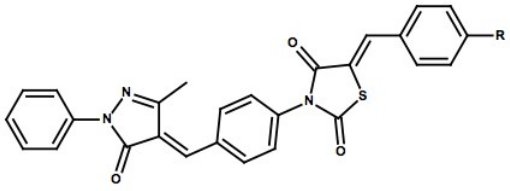


Figure 2. Flexible type of docking of Rosiglitazone

Table 1. Docking results of designed chalcones of substituted thiazolidinediones towards PPAR-gamma



a1-a5 to d1-d5



e1-e5

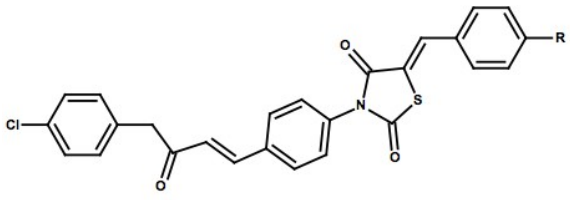
SI. No.	Code	R	R¹	Binding energy (Kcal/mole)	Calculated Inhibition constant(nM)	No. of H- bonds	Rank
1.	a1	NO_2	NH_2	-10.54	18.77	1	16
2.	a2	NO_2	OH	-9.59	93.51	2	23
3.	a3	NO_2	Cl	-12.04	1.5	1	2
4.	a4	NO_2	OCH_3	-10.05	43.01	1	20
5.	a5	NO_2	NO_2	-10.76	12.97	2	11
6.	b1	OH	NH_2	-10.22	32.03	1	18
7.	b2	OH	OH	-10.43	22.78	1	17
8.	b3	OH	Cl	-11.53	3.53	1	3
9.	b4	OH	OCH_3	-11.47	3.92	3	4
10.	b5	OH	NO_2	-10.09	40.21	-	19
11.	c1	H	NH_2	-11.06	7.84	1	8
12.	c2	H	OH	-10.72	13.99	2	13
13.	c3	H	Cl	-11.45	4.04	-	5
14.	c4	H	OCH_3	-11.15	6.76	-	7
15.	c5	H	NO_2	-10.58	17.62	-	15
16.	d1	Cl	NH_2	-10.75	13.22	-	12
17.	d2	Cl	OH	-10.86	10.9	2	10
18.	d3	Cl	Cl	-9.91	54.14	-	21
19.	d4	Cl	OCH_3	-8.99	258.84	-	24
20	d5	C	NO_2	-10.64	15.82	1	14
21.	e1	NO_2		-9.82	63.76	2	22
22.	e2	OH		-11.38	4.59	2	6
23.	**e3**	**H**		**-12.29**	**0.99 (987.38pM)**	**-**	**1**
24.	e4	Cl		-10.88	10.52	2	9
25.	Rosiglitazone	-	-	-8.93	286.79	2	25

-cted binding and docked energies are the sum of the intermolecular energy and the torsional free-energy penalty, and the ligand's internal docking energy, respectively. The inhibition constant (*Ki*) is calculated in AutoDock4.2 as

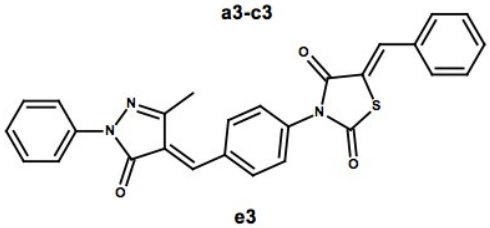
$$Ki = \exp(\Delta G \times 1000/Rcal \times TK)) \ldots\ldots\text{Eq 1.}$$

Where ΔG is the docking energy, *Rcal* is 1.98719, and TK is 298.15.[21] The low inhibition constant values indicated the efficiency of the compounds to stimulate the enzyme and prove its greater affinity towards the catalytic site of the enzyme. In the current docking study, for target enzyme PPAR-γ , binding energy and calculated inhibition constant values ranges from -8.99 to -12.29 kcal/mol and 987.38pM to 258.84nM respectively.

Among the designed analogues 5-benzylidene-3-{4-[(3-methyl-5-oxo-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl]phenyl}-1,3-thiazolidine-2,4-dione (Figure 3) **(e3)**, 3-{4-[-3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl]phenyl}-5-[(4-nitrophenyl)methylidene]-1,3-thiazolidine-2,4-dione (Figure 3) **(a3),** 3-{4-[-3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl]phenyl}-5-(4-hydroxybenzylidene)-1,3-thiazolidine-2,4-dione (Figure 3) **(b3)** and 5-benzylidene-3-{4-[-3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl]phenyl}-1,3-thiazolidine-2,4-dione (Figure 3) **(c3)** showed significant binding free energy of -12.29,-12.04 -11.53, and -11.45 Kcal/mol with predicted inhibition constant values of **987.38pM**, 1.5, 3.53,and 4.04nM respectively. Interestingly, it has been noted that all the top ranked molecules have significant hydrogen bonding interaction with NH2 of LEU228, SER289 and ARG288. In addition the binding energy of **e3** may be enhanced by its selectiveness to SRC-1. For the detailed conformational pattern analysis of e3, its structure can be divided into three fragments, namely, (i) 5-benzylidene thiazolidinedione (hydrophobic head), (ii) N-phenyl group (linker) and (iii) 3-methyl-1-phenyl-5-pyrazolone moiety (tail). It can be assumed that the significantly high binding energy of this PPARγ analogues is mainly due to its selectiveness with SRC1.



a3-c3



e3

Code	R	Estimated Binding Energy (Kcal/mol)	Estimated Ki (nM)
a3	-	-12.04	1.5
b3	-NO_2	-11.53	3.53
c3	-OH	-11.45	4.04
e3	-H	-12.29	0.99

Figure 3. Structure of identified HITs **a3**, **b3**, **c3** and **e3**

The presence of an pyrazolone moiety as a tail causes its to adopt a non coplanar conformation with SRC-1 and makes the fitness to PPAR-γ. We also claim that during best docking pose of **e3**, the pyrazolone substituted part in the structure resides in the accessory- binding pocket of PPAR-γ surrounded by GLN286, CYS285 and GLN286 (Figure 7).

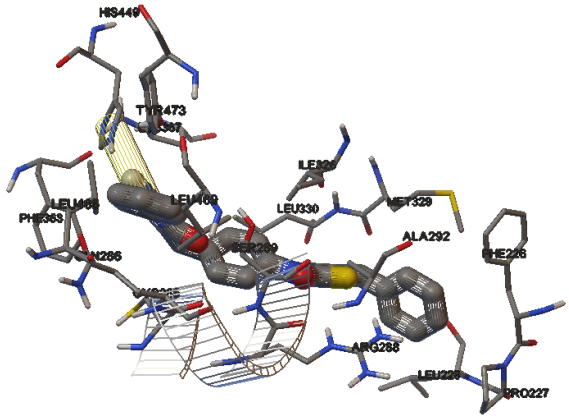


Figure 7. Docking pose of e3

The current virtual screening of designed scaffolds can generate many noteworthy findings for the design of potent PPAR-γ agonists. Placement of a electron donating, deactivating group such as Cl and F in the para position of tail showed promising results. On the othe rhand, combination of both the electron donating and withdrawing groups in the phenyl system also favour good binding energy. The presence of electron withdrawing NO_2 group in the hydrophobic head and tail does not provide any good binding interaction towards PPAR-γ. At the same time introduction of lipophilic 3-methyl-1-phenyl-5-pyrazolone as a tail produced tremendous increase in the binding energy.

3. Conclusion

The attractive feature of our design is based on 3,5-disubstitution of thiazolidinedione and the alteration done in the 3rd and 5th position with different substituents. The binding interaction of the designed scaffold can produce more steric and charge transfer interactions in the active pocket of PPAR-γ. The design can successfully initiate the discovery of new potent PPAR-γ agonists with good binding affinity and improved lipophilic character. The current design of novel chalcone based PPAR-γ analogues opens up a possibility for the contemporary rational design of new antidiabetic agents. Using ligand-based drug design, we found that electron donating phenyl-substituted chalcones of PPARγ could be perspective candidates of PPARγ agonists from this class. We must note that presented in this article inhibition constants are calculated using the computational assisted docking techniques. So more experimental data is needed to further confirm predictive power of such approach and thus facilitate the development of novel class of PPARγ analogues based on this chemical class.

4. Experimental

Materials and methods: In the present investigation, molecular docking methodology was implemented by AutoDock tools 1.4.6 and MGL tools 1.5.4 packages (The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA, 92037). Construction and energy minimization of ligands were done with Chem Draw Ultra 8.0 and Chem3D ultra 8.0 (Cambridge Soft.Com, 100 Cambridge park drive, Cambridge, MA 02140, USA) respectively. Missing residues of the enzyme were corrected by

PDB2PQR online software. In this study, AUTODOCK4.2 software was used to establish a ligand-based computer-modeling algorithm for the prediction of binding energy and calculation of inhibition constants of the designed chalcones with the PPARγ enzyme. The rosiglitazone was kept as the standard and SRC1 was included as flexible residue during docking calculations. The docking results provided the binding affinities and corresponding predicted inhibition constants (*Ki*) of the designed PPARγ analogues could be compared with standard rosiglitazone.

2.1. Preparation of Enzyme Structure

Crystallographic model of PPAR-γ (PDB code: 2PRG) was retrieved from www.pdb.org. Initially, the side chain C and the BRL49653 were extracted from the enzyme active site using Arguslab software.[15] Then, all bonds were modified automatically and missing hydrogen atoms were added using PDB2PQR with PARSE force field[16]. The resulted file is saved in *.pqr* and this format is compatible with AutoDock4.2.

2.2. Preparation of Ligands

All the designed ligands were built by ChemDraw Ultra 8.0 version and saved in mol formats. The saved mol format was further imported into the Chem3D Ultra 8.0 version and the energy minimization was done with molecular orbital package (MOPAC). The energy minimized structure was saved in the pdb format which is a compatible input file of AutoDock. The imported ligands gave partial atomic charges and a set of torsion angles. The final structure is saved in a *.pdbqt* format. [17]

2.3. Docking Methodology

In the current docking procedure, SRC-1 was included as a flexible residue for introducing conformational search of flexible side chains and all the rest of the amino acid residues in the macromolecule were treated as rigid. The torsional freedom of SRC-1 residue was determined and saved as "pdbqt" format of flexible residue. The receptor grids of both enzymes were developed by using 54 × 54 × 54 grid points in xyz with grid spacing of 0.375Å. The Lamarckian genetic algorithm was used for all molecular docking simulations. Population size of 150, mutation rate of 0.02 and crossover rate of 0.8 were set as the parameters. Simulations were performed using up to 2.5 million energy evaluations with a maximum of 27,000 generations. Each simulation was performed 50 times, yielding 50 docked conformations. AutoDock4.2 rank them according to their energies. The lesser the energy, the better the conformation, therefore best confirmation (i.e. least energy) was selected [18,19,20].

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