Sandra Megantara et al /J. Pharm. Sci. & Res. Vol. 9(9), 2017, 1478-1482

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Insilico Study of Thymoquinone as Peroxisome Proliferator Activated Receptor Gamma Agonist in the Treatment of Type 2 Diabetes Mellitus

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Abstract

Objective: The purpose of this study was to find the most advantageous binding sites for thymoquinone in the receptor ligand binding domain, predict binding modes and also compared effectiveness with well-known famous pioglitazone.

Methods:

Preparation of receptor and ligand. The former by downloaded crystal structure PPAR-γ in complex with the agonist pioglitazone, interaction analysis and protein target repair and separation. The latter by making two and three dimensions molecular modeling. Program validation by redocking and ligand structure overlay. The third step was docking process in which pioglitazone as standard drug and thymoquinone as test molecular. The last step was data interpretation.

Results: The results of receptor analysis of PPAR-ɣ by using Q-Site Finder found 10 binding pocket consisting of two binding pocket on chain A (2, 4), 3 binding pocket in chain B (1, 5, 9) and 5 binding pocket between the chain A and B (3, 6, and 7, 8, 10). Binding pocket in chain B was the most active site on PPAR-ɣ receptors and plays an important role on the pharmacological activity with the highest binding affinity. The procedures and docking programs were valid based on RMSD values of with standard ligand that was 0.4117 (RMSD <2).

Conclusion: These results predicted that pioglitazone had better binding interactions to PPAR-ɣ than thymoquinone with binding affinity and inhibition constant values Ei = -9.4 Kcal / mole; Ki = 0.13 uM (pioglitazone) and Ei = -7.0 Kcal / mole; Ki = 7, 43 uM (thymoquinone). However thymoquinone still potentially be developed as a PPAR-ɣ agonist compound due to the possibility of natural products safety was predicted better than synthetic compounds, such as cardiotoxicity of pioglitazone.

Keywords: Thymoquinone, PPAR-γ, in silico, pioglitazone, docking

INTRODUCTION

The prevalence of type 2 diabetes mellitus continues to rise. In 2020, the number of patients with type 2 diabetes is expected to reach 250 people around the world [1] Indonesia ranks 9th in the estimation of epidemiological diabetes mellitus world in 2010 with 7 million cases and will continue to rise to the rank-5 in 2030 to 20 million cases[2]. The understanding of pathophysiology and molecular modulation in type 2 diabetes treatment is still a major concern in the investigation of potent drugs for type 2 diabetes mellitus. One of the receptor target in type 2 diabetes mellitus is peroxisome proliferator-activated receptor gamma (PPAR-ɣ). PPAR-ɣ agonist has been most extensively studied for their ability to enhance the sensitivity of target tissues to the effect of insulin both in synthetic and natural compounds[3]. Thymoquinone, the natural compound that is existing in Arabic plant Nigella sativa Lorr have been proved effective in reducing hyperglycemia by inhibition of gluconeogenesis[4]. According to Woo *et al*.[5,6], thymoquinone was able to increase PPAR-ɣ activity in breast cancer cells. The increase of PPAR-ɣ activity was prevented in the presence of PPAR-ɣ specific inhibitor and PPAR-ɣ dominant negative plasmid. It is suggesting that thymoquinone may act as a ligand of PPAR-ɣ. Based on these finding, this paper reports the investigation of the interaction mode between thymoquinone and PPAR-ɣ. The interaction mode was consist of identification of the favorable binding site of the PPAR-ɣ receptor, predicted the binding mode and also the binding affinity of thymoquinone to PPAR-ɣ receptor. This prediction of interaction mode can be useful for drug design development based on thymoquinone structure.

MATERIALS AND METHODS

Methods were carried out by modification of Mustarichie *et al*.(7-9) methods.

Tools:

Hardware used for the calculations, molecular modeling, and molecular docking involves a personal computer, MacBook Pro (13-inch, Mid 2012), with macOS Sierra which Processor 2.5 GHz Intel Core i5, completed with Graphics Intel HD Graphics 4000 1536 MB, and memory of 16 GB 1600 MHz DDR3

The software used was as follows:

(1) MarvinSketch 17.11.0 (Academic License)
(2) LigandScout 4.1.4 (Universitas Padjadjaran License)
(3) Autodock Vina 1.1
(4) MacPyMOL: PyMOL 1.7.4.5 Edu
(5) Portable Hyper Chem Program Release 8.0.7 (by Hypercube Incorporation of 2007 downloaded from http://www.hyper.com).
(6) SwissPDBViewer v.4.01 program package (Glaxo Smith Kline R & D, downloaded from http://www.expasy.org).

(7) Argus Lab program v.4.0.1 (by Mark Thompson and Planaria Software downloaded from http://www.arguslab.com).
(8) Open Babel program v2.2.3.

RESEARCH METHODS:

A. Preparation of ligand Pioglitazone and Thymoquinone

(1) Manufacture of two-and three-dimensional structure with MarinSketch and LigandScout program package.

(2) Geometry optimization with Portable software LigandScout.

(3) Analysis of molecular properties of Pioglitazone and Thymoquinone with LigandScout

B. PPAR-γ receptor preparation obtained from Protein Data Bank website (http://www.pdb.org/pdb/explore/explore.do?structureId=2XKW).

3D visualization and study of the structure of the receptor LigandScout software, AutoDock Vina program 1.1.

C. Determination of binding pocket using Q-Finder site.

D. Validation program AutoDock by calculating the standard deviation of binding free energy with docking simulation results from AutoDock VinaTools program 1.1.

E. Pioglitazone and Thymoquinone docking simulations with PPAR-γ.

F. Interpretation of data from docking simulations

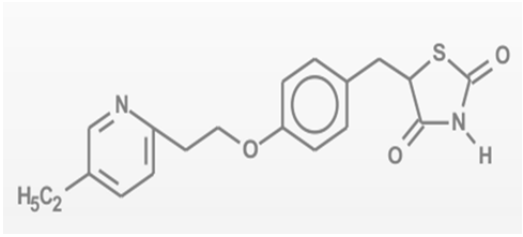
RESULTS AND DISCUSSION

The first step in the preparation of the ligand was making a two-dimensional structure which was then converted into three-dimensional structures with MarvinSketch Software as shown in Figure 1.

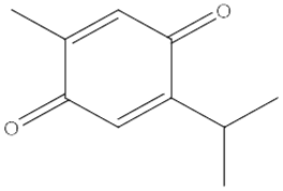
The three-dimensional structure then optimized geometry to obtain the most stable conformation.

Conformational changes and specifications of the 15 ligands were shown by LigandScout's window in Fig. 2.

Lipinsky rule of five [11] was predicted by ChemPropPro as shown in Table1.



(a)



(b)

Figure 1. The 2D structure of Pioglitazone (a) and Thymoquinone (b)

A hydrogen bond is a bond between the H atom that has a partial positive charge with other atoms that are electronegative and has a lone pair with octets complete, such as O, N, and F [13]. It should be noted that ring aromatic donating properties of non-polar to a compound. Interaction energy or free energy is the energy required for a ligand can enter into binding pockets and interact with the receptor. The negative sign indicates that the compounds can interact spontaneously with the receptor. According to Siswandono and Soekardjo [14], an amino acid which is about 4 - 6A will form van der Waals interactions. Although the van der Waals bonds are weak, the sum of van der Waals bonding is a substantial binding factor, especially for compounds with high molecular weight. Van der Waals interactions also give effect to the lipid solubility of the ligand. The more the van der Waals interaction that happens it will be easier ligand is soluble in lipid ligands that can penetrate the cell membrane to be able to bind to the receptor.

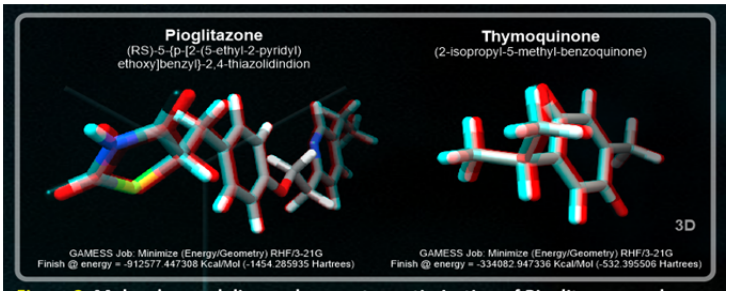


Figure 2. Molecular modeling and geometry optimization of Pioglitazone and Thymoquinone

Table 1. Lipinski's rule of five prediction

Parameter	Pioglitazone	Thymoquinone	Criteria
Molecular Weight (Da)	356.119	164.084	Less than 500 Daltons
Number of H-Bond Acceptors	4	2	Not more than 10
Number of H-Bond Donors	1	0	Not more than 5
Log P (Octanol – Water)	3.533	2.174	Not greater than 5

Low Ki value indicates that the ligand has a higher strength than the Ki values were higher (weaker bond strength). A good values range of Ki is 10-6-10-12 M. When ligands bound to the receptor too powerful, the ligand will be difficult excreted from the body and the ability to cause an activity is inhibited, but when the ligand binds weakly to the receptors, the ligands will be easily removed before generating an activity. The absence of hydrogen bonds in the interaction suggests that the ability to bind to receptor weaker compared to compounds which have hydrogen binding affinity.

A number of hydrogen bonds formed in the ligand-receptor interaction depends on the presence of atomic H, O, N or S ligands which are located around the H atom acts as a hydrogen bond donor, while O atoms, N, and S acts as a hydrogen bond acceptor. According to Bohm and Schneider [15], the range of hydrogen bond distances are good docking simulation results are 1.72-2.85 Å. The four compounds had bond distances in the range, in other words, that the four compounds had a hydrogen bond distance corresponding requirements so that they could interact with either the tyrosinase enzyme through hydrogen bonding. Curcumin formed van der Waals bond with the amino acids in α-MSH with DPN4 (4,645 Å) and Cys7 (4,571 Å). Van der Waals interaction is the force of attraction between molecules or atoms are not charged and is located adjacent to the bond strength of 0.5-1 kcal/mol [11]. According to Siswandono and Soekardjo [14,16], amino acids that are about 4-6A will form van der Waals interactions. Although the van der Waals bonds are weak, the sum of van der Waals bonding is a substantial binding factor, especially for compounds with high molecular weight. Van der Waals interactions also give effect to the lipid solubility of the ligand. The more the van der Waals interaction that happens it will be easier ligand is soluble in lipid ligands that can penetrate the cell membrane to be able to bind to the receptor. Our result showed that the standard ligand (pioglitazone) and the test ligand (thymoquinone) all met the criteria of Lipinski's Rule of Five, this can be stated that in general, both had orally active drug.

Data virtual of PPAR-γ can be seen from figure 3.

The binding pocket analysis of this receptor by Q –Site Finder showed there was two chain (A and B) of 2XKW wich favorable interaction with pioglitazone ligand. Both of them consist of ten binding sites with diverse two active binding site in A chain (2; 4), three binding sites in B chain (1;5;9) and five binding sites between A and B chain (3; 6; 7; 8; 10). The table of this binding site is shown in Table 2.

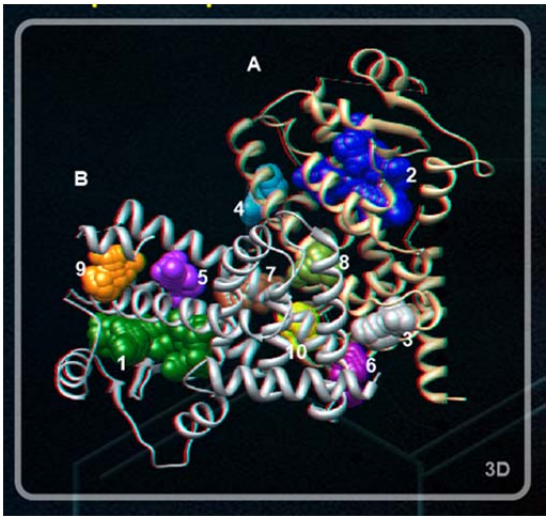


Figure 3. Virtual receptor target of PPAR gamma from Protein Data Bank website (http://www.pdb.org/pdb/explore/explore.do?structureId=2XKW)

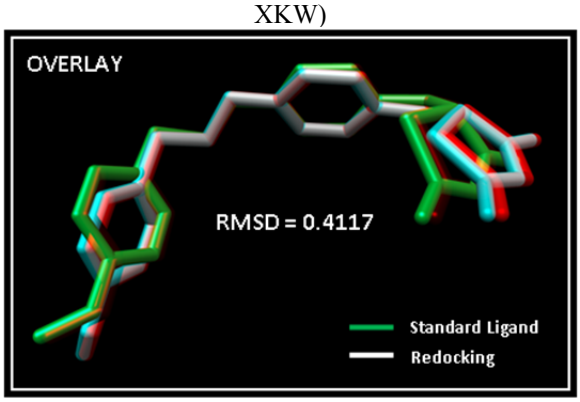


Figure 4. Overlay of natural ligand and standard ligand of pioglitazone.

Table 2. The binding site properties of PPAR-γ

Binding Site	Site Volume (Å^3)	Grid Box Properties					
		Center Coordinate			Dimension (Å)		
		x	y	z	x	y	z
1	851	-3.7670	-27.3158	17.5661	16.4805	13.5867	17.0956
2	885	19.2057	2.8214	44.0029	16.2813	14.8589	14.2958
3	203	24.8634	-25.7316	40.9851	5.3477	4.4690	7.4542
4	195	8.7089	7.3401	30.5738	3.8366	5.8065	7.6711
5	236	-5.7580	-21.4786	25.9480	7.3304	6.2077	7.7926
6	183	26.3497	-27.5490	29.8827	5.5028	5.1034	6.5165
7	230	10.7373	-11.2520	23.3950	8.2254	5.9828	5.9533
8	171	19.4485	-15.8221	25.3889	6.2758	4.3589	4.8260
9	194	-15.5077	-21.5650	21.0915	6.6583	5.6476	6.6281
10	167	12.0988	-20.2074	39.7753	4.6353	5.8522	6.5204

Table 3. The Docking Result of Pioglitazone and Thymoquinone to PPAR-γ

Binding Site	Pioglitazone		Thymoquinone	
	Binding Affinity (Kcal/Mol)	Inhibition Constant Ki (µM)	Binding Affinity (Kcal/Mol)	Inhibition Constant Ki (µM)
1	-9.4	0.13	-7.0	7.43
2	-7.4	3.78	-5.6	78.85
3	-	-	-1.7	56,805.47
4	-	-	-4.5	504.43
5	-	-	-4.4	597.13
6	-	-	-	-
7	-	-	-4.9	256.87
8	-	-	-1.5	79,603.56
9	-	-	-5.0	216.99
10	-	-	-	-

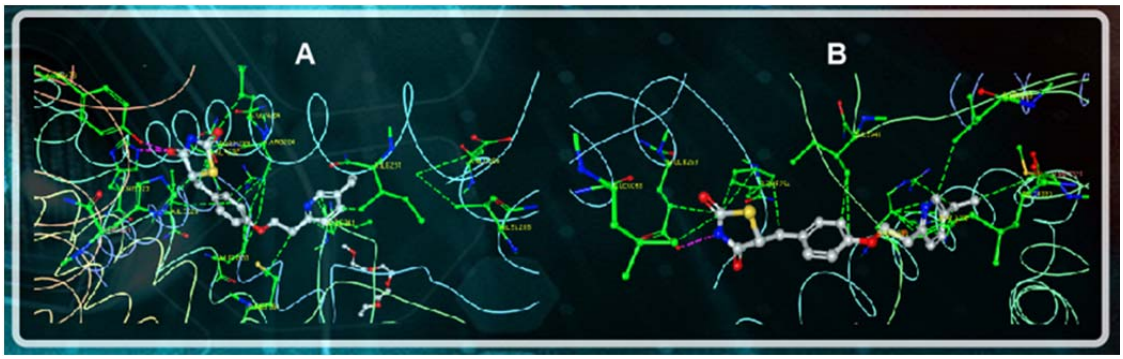


Figure 5. The ligand-binding pocket from the PDB complexed with 2XKW was visualized using the Ligand Explorer Viewer 4.1.0.

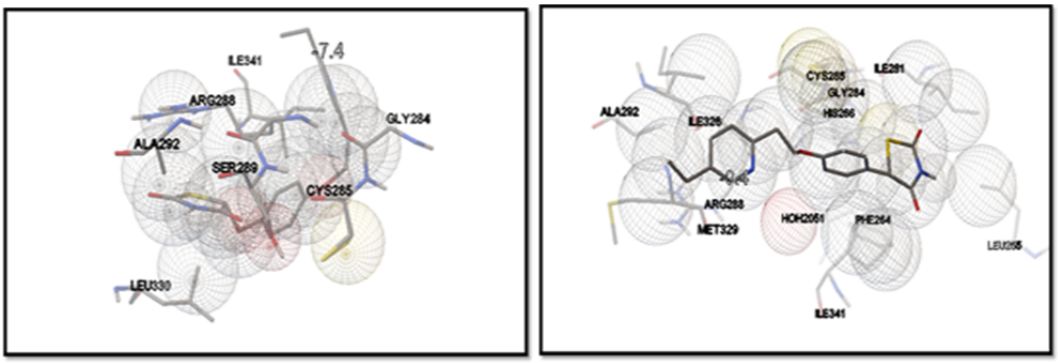


Fig. 6. PPAR-ɣ ligand binding pocket.

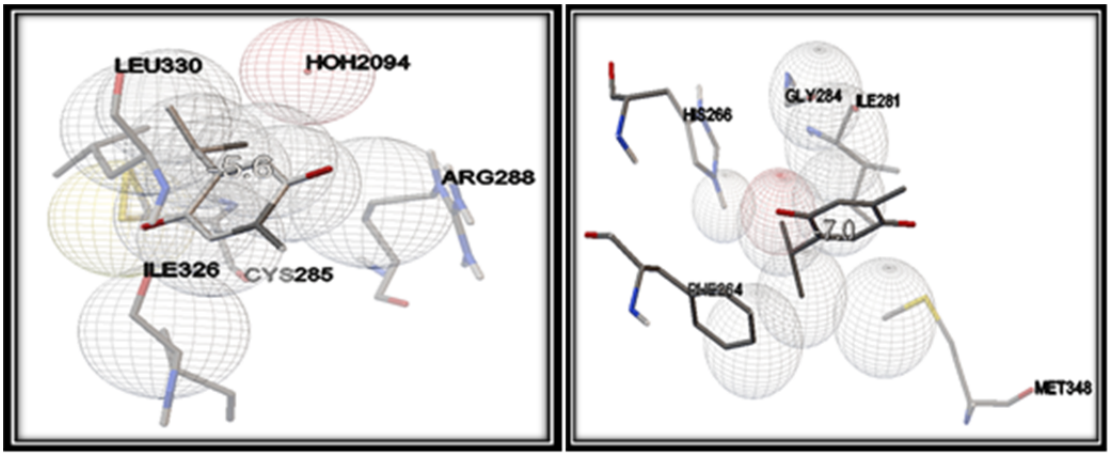


Figure 7. Docking simulation of thymoquinone into the binding site of PPAR-γ

It is necessary to overlay the studied ligand to determine the validity of the docking program [10-12]. It was found that the overlay of natural ligand and standard ligand of pioglitazone, its Root Mean Standard Deviation (RMSD) was smaller than 2, meaning that the procedure and program docking was valid.

Results of ligand-receptor binding by auto dock Vina can be seen in Figur 5,6 and 7.

Further detail of ligand-binding pocket from the PDB complexed with 2XKW are shown in Figure 6 and Figure 7.

The ligand-binding pocket from the PDB complexed with 2XKW was visualized using the Ligand Explorer Viewer 4.1.0. The amino acids residues involved in the binding site of PPAR gamma. A chain was Gly 284, Cys 285, Arg 288, Ser 289, Ala 292, Leu 330 and Ile 341. While in chain B were Phe 264, Leu 265, His 266, Ile 281, Gly 284, Cys 288, Arg 288, Ala 292, Ile 326, Met 329, Ile 341 and the ligand formed hydrogen bonding at 2051 (B chain). The analysis of ligand interaction showed that the amino acids residues in B chain that involved in the ligand interaction were higher than in A chain and also its binding activity [table 3]. It was indicated that ligand interaction in B chain was might be important than A chain for its pharmacological activity. There was no hydrogen bonding found in this PPAR-ɣ ligand binding pocket suggested the ligand was not soluble in water.

Similarity bonding was found in Docking simulation of thymoquinone into the binding site of PPAR gamma (see Figure 7.). The amino acids residues involved in the binding site of PPAR gamma. A chain was Cys 285, Arg 288, Ile 326, Leu 330. While in chain B were Phe 264, His 266, Ile 281, Gly 284, Met 348 and the ligand formed hydrogen bond at 2094 (A chain). The amino acids residues that involved in thymoquinone was lesser than in pioglitazone ligand interaction. The binding activity of both seems in the same phenomenon [table 3]. This finding indicated that pioglitazone has better interaction than thymoquinone to bind PPAR gamma and its correlated with its pharmacological activity.The thymoquinone was only soluble in lipid.

CONCLUSIONS

These results predicted that thymoquinone acts as the PPAR-ɣ agonist by interaction in binding pocket 1 of B chain and binding pocket 2 of A chain in the same interaction with pioglitazone. Although the binding affinity of thymoquinone is lower than pioglitazone to PPAR-ɣ, thymoquinone still potentially be developed as a PPAR-ɣ - agonist compound due to the possibility of natural products safety is predicted better than synthetic compounds, such as cardiotoxicity of pioglitazone.

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