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Research Article

IN SILICO EVALUATION OF POTENT FOR PPARγ AGONIST OF LIGNAN DERIVATIVES FROM *Myristicafragrans* HOUTT SEEDS

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ABSTRACT

Objective: Peroxisome proliferator-activated receptors gamma (PPARγ) is a clinically established target for treatment of insulin resistance and has a significant effect to improve the hyperglycemia and insulin resistance condition. In this investigation, lignan derivatives from nutmeg seeds (*Myristicafragrans*) was evaluated by *in silico* to know the potency of these compounds.

Methods: Molecular docking simulation was performed to screen out that the compounds had potent for PPARγ agonist. Autodock 3.0.5 software was employed to dock all ligand against PPARγ and the all parameters of dcoking was validated by re-docking co-crystal ligand of (2S)-2-(4-benzylphenoxy)-3-phenylpropanoic acid (PDB id : 3HOD) against to PPARγ.

Results: Twenty compounds were favourably docked against PPARγ agonist (PDB id: 3HOD). The tail of hydrophobic of lignan compounds also favorable located in "diphenyl pocket" as well as TZD.

Conclusion: Macelignan and dihydro-di-isoegeunol (FEB -11.07 and -10.25 kkal/mol, respectively) could compete as agonist PPARγ by connecting to network hydrogen bond of His323, Tyr379, and Hist449, also formed hydrogen bond with Ser289 as mention thiazolidinediones (TZD) interacted with PPARγ, thus the both compounds might potent as agonist PPARγ.

Keywords: Antidiabetes, Agonist, *Myristicafragrans* extract, PPARγ

INTRODUCTION

WHO declares that Indonesia has become the fourth country that has the most diabetic patients in the world by following India, China and US [1]. WHO predicts this pandemic of the prevalence of diabetic in Indonesia that increasing from 8.4 million in 2000 to rise over 21.3 million in 2030.

More than 80 % of all diabetes is T2DM patients [2]. Based on epidemiology research, there are increasing the incidence and prevalence of T2DM due to ageing population structures in developed countries and increasing obesity globally[3]. Furthermore, the WHO warns that T2DM diabetes is global pandemic [4].

T2DM is the multi factorial and multi genetic disease which occurs as combination metabolic disorder; insulin resistance and beta pancreas cell insufficiency[5]. The both insulin resistance and β-pancreas cell insufficiency are caused by happening obesity and genetic factor [3, 6].

PPARγ agonists have drawn great concern in the therapy management of T2DM [7]. Sulfonylureas, metformin, acarbose, and thiazolidinones (TZDs) are current therapies for reducing plasma glucose [8, 9].

The antidiabetic effects of TZDs is due to the activation of the peroxisome proliferator-activated receptors (PPARγ) [9]. TZD is high-affinity ligand for agonists of PPARγ and this phenomenon was first reported by Lehman et al. [10]. However, this medicine gives side effect that may arise during treatment; TZDs also have side effects that 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. For example, the treatment of TZD drugs such as rosiglitazone and pioglitazone, require monitoring to reduce the risk of adverse side effects, even troglitazone which is TZDs derivatives compounds have been withdrawn from the market because it showed an increased incidence of hepatitis induced by the drug [11, 12].

Based on the side effect story of TZD and derivatives, the discovery of the other class drugs that selective into PPARγ and PPARα agonist increases to reduce the risk of fatal side effect of the drugs. The focus of this study is to development of new PPARγ agonists concentrate on structure-based design [13-15]. To date, there have been many crystal structures of PPARγ complexes available in Protein Data Bank (PDB) (www.rcsb.org).

Frachiolla et al. (2009) reported the design and synthesis of a novel class of PPARγ/α dual agonists, analogs of 2-aryloxy-3-phenyl-propanoic acids compounds, and they published the crystal structure (PDB id : 3HOD) that declares the compounds are active in nanomolar PPARγ agonist [16].

Besides finding of novel synthetic compounds, the effort to explore alternative therapies using natural materials has been doing frequently in the community[17], because the materials relatively inexpensive and easily available as well as empirically shows efficacy for antidiabetic. However, the research that revealed the molecular mechanism of action of natural product antidiabetic has not much done, thus causing natural products potentially as antidiabetic cannot legally be used in treatment of diabetes.

We are encourage to develop nutmeg seeds as PPAR γ antagonist, it contains chemical compounds derived of 2-aryloxy-3-phenyl-propanoic acids that proven as antagonist PPAR γ [18] such as lignan and neolignan derivatives even macelignan is established and patented by Jae-Kwang et al [19, 20].

Here we screened nutmeg seeds lignan compounds-derived by using *in silico* by molecular docking simulation. Nutmeg seeds has been used traditionally as a spice and for medicinal purposes in Indonesia and other Asian countries [21-24]. In this study, evaluation of lignan derivatives against PPARγmay have not published yet, however one of active compound of nutmeg seeds published by Jae-Kwang co-worker [19].

MATERIAL AND METHODS

Material

This research used LBD (ligand binding domain) of peroxisome proliferator-activated receptor gamma or PPARγ(PDB id : 3HOD) [18] structure with 2.1 Å resolution. In this crystal structure (3HOD),

the co-crystal ligand of (2S)-2-(4-benzylphenoxy)-3-phenylpropanoic acid is complexed with PPARγof homo sapiens that expressed in *E. Coli*. The crystal structures are selected should have best resolution or lower resolution value, and also have R-free and R-value lower than 0.25 [25, 26]. The 3D structures of lignan derivatives compounds were constructed using Hyperchem 7, then were optimized using Austin Model 1 (AM1).

Molecular Docking Simulation

MGL tools program package 1.5.4. (Molecular Graphics Laboratory, The Scripps Research Institute) was used to prepare protein structures, ligand structures, grid parameter file and docking parameter file; furthermore, the AutoGrid v 3.05 program (The Scripps Research Institute) is used to prepare the grid, the Autodock 3.05 (http://autodock.scripps.edu) was employed to simulate the docking process under Linux program. As proposed by Brown and Ramaswamy (2007), qualified crystal structures should have the best resolution or lower resolution value, and also have R-free and R-value lower than 0.25 [25].

The chemical structures for the lignan derivatives of nutmeg seeds were obtained from literatures [27-30]. Twenty lignan compounds that contained in nutmeg seeds had been virtually screened via molecular docking (Autodock 3.0.5) [31].

The ligands and proteins were prepared by AutoDockTools (ADT). Molecular docking was carried out on PPARγ, PDB ID: 3HOD [18]. Ligand and protein available in the PDB structure were converted to PDBQ and PDBQS format by adding charges, hydrogens and assigning ligand fexibility. Kollman charges and solvation parameter were assigned using default value to the protein while Gasteiger charges were added to each ligand. A grid box of 60 x 60 x 60 points, with a spacing of 0.375 Å and a precise coordinate -29.305, 12.570, -20.539 along the x, y and z axes pertaining the centre of the active site was built around the binding region. Population size of 50 and 250 000 energy evaluations were used for 100 search runs via Lamarckian Genetic Algorithm (LGA) [26]. Docking result were analyzed based on the lowest free energy binding chosen from the most populated cluster and saved in dlg file for visualizationn. TZD was used as control docking that imposed against PPARγ(PDB ID:3HOD)[18].

RESULTS AND DISCUSSION

Validation of Docking Method

Interaction TZDs as Control Ligand Against of PPARγ

The protein target of PPARγthat used in this study has "diphenyl pocket". This pocket is new L-shaped region of the PPARγ that formed by forming several favourable hydrophobic interactions. This pocket increases the stabilization of the helix H3, inducing a conformation of the LBD less favourable to the recruitment of co-activators required for full activation of PPARγ [18]. As shown in Fig. 1.a, the tail of aryloxy phenyl-propanoic acids (co-crystal ligand) and TZD occupied the "diphenyl pocket" in PPARR assume a different slope into the cavity in order to maintain the carboxylate H-bond network because of the longer protrusion of the Y314 side chain (H323 in PPARγ). PPARγformsstrong hydrophobic contacts with several lipophilic residues such as Cys285, Leu330, Ile341, Met348 and Met364.

In the bottom of PPAR γ binding site (blue colored helix in Fig. 1), there are the loop 11/12 and is contoured sidewise by H3 and H11. This loop stabilize "diphenyl pocket" that located between H3 and loop 11/12.

Interaction TZDs as Control Ligand Against of PPARγ

Binding interaction ofTZD against PPARγ explained well by some literature [32-34]. In this study, TZD was used as control ligand, three-dimension of TZD structure was built by modelling, further docked into PPARγ(3HOD). TZD imposed against co-crystal ligand (2S)-2-(4-benzylphenoxy)-3-phenylpropanoic acid (PDB id: 3HOD) as shown in Fig. 2. The hydrogen bond network of His323, His449, and Tyr473 interacted with the polar head of TZD as shown Fig. 2.

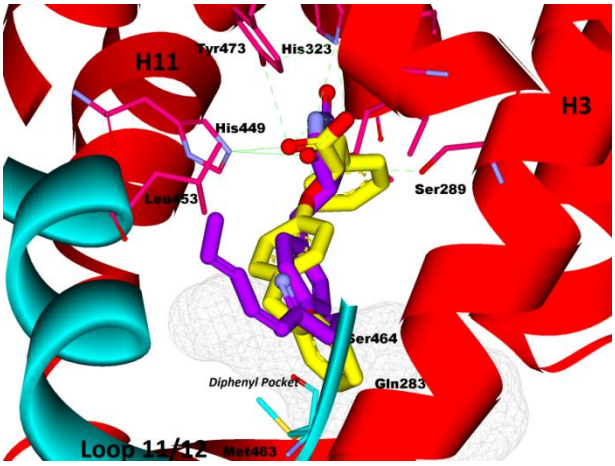

Fig. 1: Binding interaction pattern of TZD docked (purple) and imposed against co-crystal ligand (aryloxy phenyl-propanoic acids-yellow) with H3 and H11 in complex with PPARγ. Blue light colored is Loop 11/12, and wire-grey colored is "diphenyl pocket".

Yu et al. (2003) analysed that the polar moiety of TZD (troglitazone) form five hydrogen bonds with Gln286, His449, Tyr473, His323 and Ser289, whereas the tails of the hydrophobic of TZD are located in the hydrophobic pocket of PPARγ[35]. The electrostatic interaction appeared through pi-pi cation interaction (line orange colored in Fig. 2) between Phe282 of PPARγand aromatic ring of TZD.

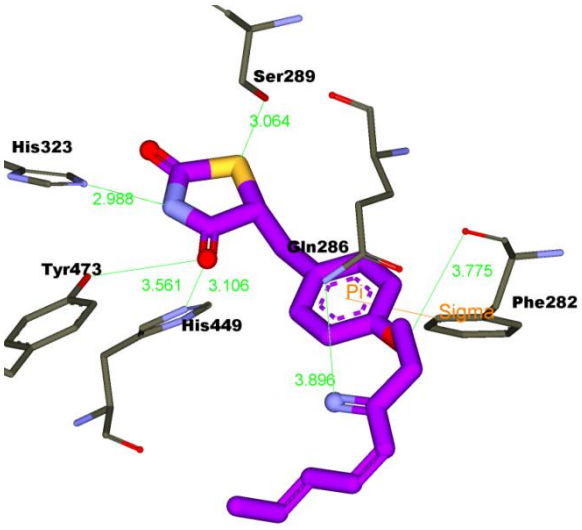

Fig. 2: Hydrogen bond network (His323, His444, and Tyr473) and hydrophobic interaction of TZD docked in complex with PPARγ. Green colored carbon is hydrophobic residue.

Binding Interaction Prediction of Lignan derivatives of Nutmeg Seeds of Agonist PPARγ

As in our previous study [36], we studied that nutmeg seeds extract might have potential as antidiabetic agent from natural product. The nutmeg seeds extract gave increasing PPARγ-dependent luciferase activity, however this is not as good compared to TZD in enhancing the activity of PPARγ-dependent luciferase.

Base on that results, we hypothesized that lignan derivatives compounds in nutmeg seeds containing might play role in the activity to increase PPARγ-dependent luciferase. Macelignan is one of lignan derivatives have been published that has same mechanism [20]. Macelignan significantly improves glucose and insulin tolerance in mice, and without altering food intake, their body weights were slightly reduced while weights of troglitazone-treated mice increased [20]. Besides maceligan, there were others twenty

lignan compounds in nutmeg as reported by Hattori et al [27], Orabi et al. [29], Miyazawa et al. [37], Yang [38], and [39], prompted us to explore of the potency of these compounds as PPARγagonist.

Molecular docking simulation was employed to predict the potency these compounds. Autodock 3.0.5 was employed in this study [40]. Protein structure with PDB ID of 3HOD was selected as representing PPARγ ligand binding domain (LBD) and complexes with aryloxypropanoic acid as ligand. TZD was became control ligand in this methods. Subsequently, TZD and co-crystal ligand of 2-aryloxy-3-phenyl-propanoic acids were docked to 1F8B thus produced RMSD less than 2.0 Å.

This resultsshowed that Lamarckian Genetic Algorithm using in AutoDock 3.0.5 was efficient and effective to predict true binding modes of TZD in grid dimension, which cover all important residues with a proper algorithm run amount, besidesthat, in all variations, RMSD values are equal or less than 2 Å, then Autodock was valid in docking simulation[41], even all the lowest crystallographic RMSD values were 0.89 Å or less, indicating that low-energy structures found by the force field were very similar to the corresponding crystal structure [42].

All lignan derivatives from nutmeg seeds was favorably docked against PPAR γ(3HOD). Interestingly, macelignan gave the smallest of binding free energies (-11.07 kcal/mol), while neolignan had the highest free energy (FEB) (-8.00) kcal/mol (Table 1). This fact might be connected with the previous findings that macelignan has important role in the activity to increase PPARγ-dependent luciferase [19].

However, the FEB of co-crystal ligand (PDB id: 3HOD) (-12.02 kcal/mol) was less than macelignan, even TZD had lower than them (-12.65 kcal/mol). The all lignan derivatives had good binding interaction against PPARγas shown in Fig. 3 and Table 1. The lignan compounds (grey colored carbon in Fig 3.) might imposed into TZD (purple colored carbon) in same position. The tail hydrophobic of lignan derivative interacted against hydrophobic pocket and occupied “diphenyl pocket” as well as TZD.

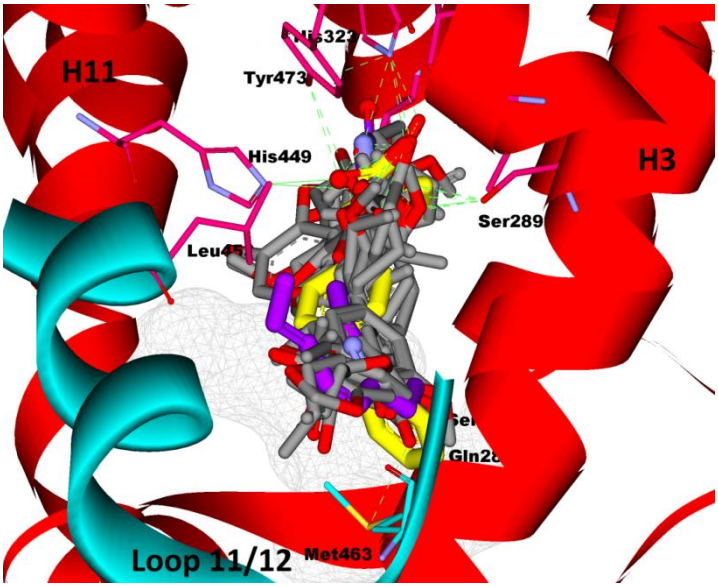

Fig. 3: Imposition of 20 lignan derivatives compounds (grey colored carbons), ariloxypropanoic acid (yellow colored carbon) and TZD (purple colored carbon)of nutmeg seeds against PPARγ.

Table 1: Molecular Docking results of Lignan Derivatives Compounds of Nutmeg Seeds and Compared than TZD and co-crystal ligand.

S. No.	Compounds Name	Free Energy Binding (kkal/mol)	Interaction with network H-bond (His323, Tyr379, His449)	Diphenyl pocket (hydrophobic pocket)
1.	TZD	-12.65	Yes	Yes
2.	(2S)-2-(4-benzylphenoxy)-3-phenylpropanoic acid	-12.02	Yes	Yes
3.	Macelignan	-11.07	Yes	Yes
4.	Verrucosin	-10.45	Yes	Yes
5.	Nectandrin B	-10.37	Yes	Yes
6.	Fragransin B1	-10.33	Yes	Yes
7.	Dihydro-di-isoegeunol	-10.25	Yes	Yes
8.	Fragransin C1	-10.23	Yes	Yes
9.	Malabaricone B	-10.12	Yes	Yes
10.	Fragransin A2	-9.92	Yes	Yes
11.	Malabaricone C	-9.11	Yes	Yes
12.	Myrisligan	-9.07	Yes	Yes
13.	Neolignan	-8.78	Yes	Yes
14.	Myristic acid	-8.00	Yes	No
15.	Tridecanoic acid	-7.65	Yes	No
16.	Isoeugenol acetate	-6.71	Yes	No
17.	Myristicin	-6.30	No	No
18.	Elimicin	-5.91	No	No
19.	Sarole	-5.79	No	No
20.	Trimyristicin	-4.83	No	Yes

Macelignan had the lowest FEB than others lignan compounds. As mention TZD, macelignan also formed hydrogen bond with network hydrogen bond (His323, Tyr473, and His449), and also interact with Ser389. Aryloxy of diphenyl moiety of macelignan interacted with Ser464 through hydrogen bond interaction.

The diphenyl tail of macelignan also fitted into the bottom of the cavity of the Met463 side-chain of the loop 11/12 (light blue colored). This loop accommodated of the benzyl-phenoxy and phenethyl-phenoxy groups faces of the terminal end. This interaction made strong interactions between the methyl and the p-cloud (aromatic ring). There were pi-pi interaction that indicated the electrostatic interactions occurred between aromatic ring and the ether group (-C-O-) of the Gln283 side chain. In the bottom cavity of PPARγ, the Gln286 side chain is also engaged in such interactions. The residues of Met463 and Gln283 contacted well with the aromatic rings of hydrophobic tail of some lignan compounds. It was evidence that macelignan was active as agonist PPARγ [19].

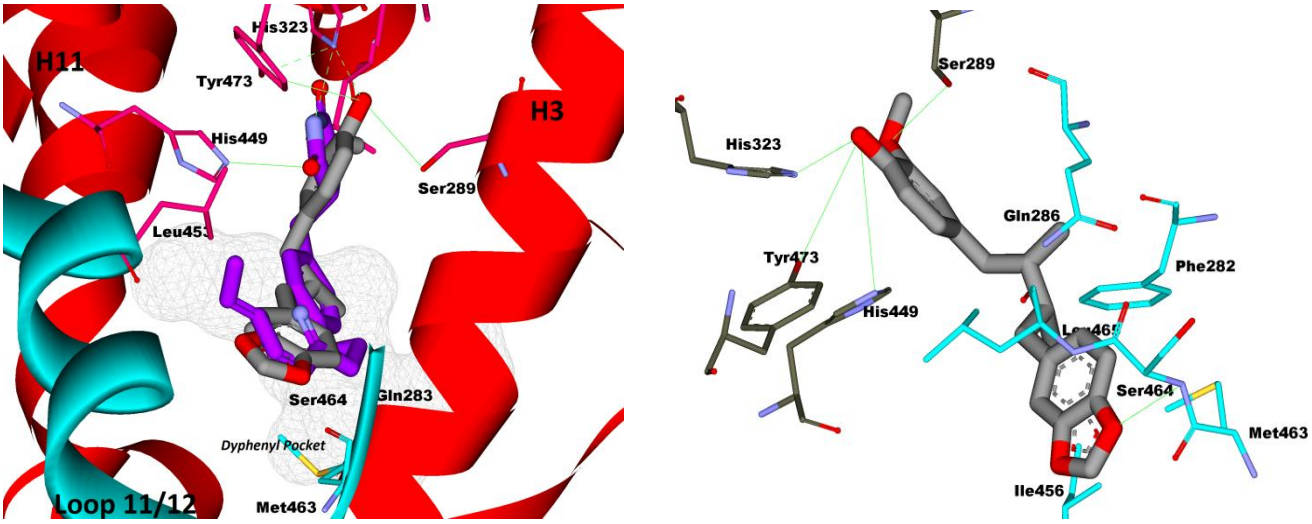

Fig. 4: Binding interaction of macelignaninti PPARγ. (a) Macelignan imposed against TZD in same position, (b) hydrogen bond interaction (green colored line) of macelignan and hydrogen bond network of PPARγ(His323, Tyr473, His449, and Sr289) and hydrophobic interaction of macelignan tail and hydrophobic pocket.

The other interesting of lignan derivatives, the new potency of dihydro-di-isoegeunol as agonist PPARγwas shown in this study. Molecular docking result of dihydro-di-isoegeunol almost coincided with TZD and co-crytal ligand s shon in Fig. 4. All the important residue of PPARγ interacted well with dihydro-di-isoegeunol. The polar head of ortho-methoxyphenol of dihydro-di-isoegeunol imposed against azo group of TZD and connected with hydrogen bond network of His323, Tyr473, and His449.

The ortho-methoxyphenol group of dihydro-di-isoegeunol also formed hydrogen bond with Ser289 thus this group might important role in bioactivity as agonist PPARγ. There were electrostatic interactions between Met346 of Loop 11/12 with aromatic ring of dihydro-di-isoegeunol through pi-pi interaction (orange colored line) as shown in Fig. 5. The previous study, the connecting between the aromatic ring and Met463 is evidenced by continuous electron density between the two interacting groups [16].

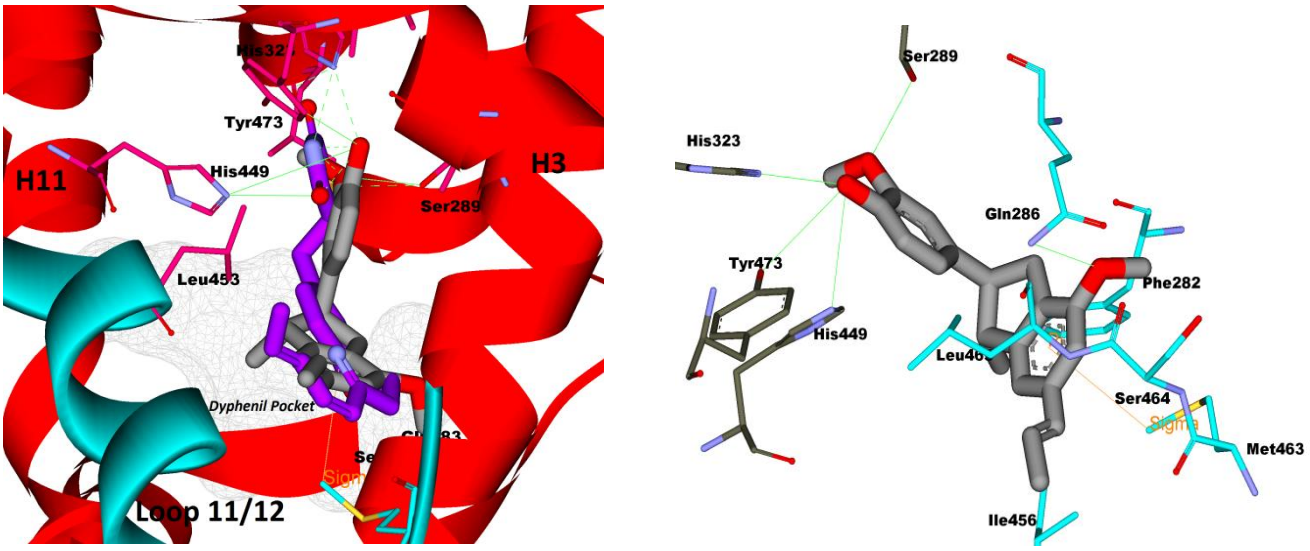

Fig. 5: (a) Dihydro-di-isoegeunol imposed against TZD in same position, (b) hydrogen bond interaction (green colored line) of macelignan and hydrogen bond network of PPARγ(His323, Tyr473, His449, and Sr289) and hydrophobic interaction of dihydro-di-isoegeunol tail and hydrophobic pocket.

Based on the molecular docking simulation results, the binding interaction of macelignan and dihydro-di-isoegeunol was similar compare than TZD thus the both macelignan and dihydro-di-isoegeunol had potent as PPARγagonist.

CONCLUSION

Lignan derivatives compounds of nutmeg seeds had favorably docked against PPARγ. The some lignan compounds interacted with important residues of PPARγLBD. Macelignan and dihydro-di-isoegeunol formed hydrogen bond network of His323, Tyr379, His449, and Ser489. The hydrophobic tail of macelignanand dihydro-di-isoegeunol fitted into "diphenyl pocket", thus the both compounds might potent as agonist PPARγ.

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