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Structure-based de novo design, synthesis, and biological evaluation of the indole-based PPARγ ligands (I)

Xiaochun Dong,[a] Zhenshan Zhang,[b] Ren Wen,[a,*] Jianhua Shen,[b] Xu Shen[b] and Hualiang Jiang[b]

[a]*Department of Medicinal Chemistry, Fudan University, Shanghai 200032, China*
[b]*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

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Abstract—MCSS and LeapFrog, two de novo drug design programs, were used for the novel indole-based PPARγ ligands' study. The designed compounds were synthesized and tested for the PPARγ protein binding activities in vitro. Out of the compounds that were synthesized, two molecules (compounds **14d** and **7d**) possessed potent PPARγ protein binding activity close to rosiglitazone in vitro.

The peroxisome proliferator-activated receptor (PPAR) belongs to the family of nuclear receptors, which play an important role in regulating the expression of a large number of genes involved in lipid metabolism and energy balance.[1] Synthetic PPARγ agonists for the treatment of type II diabetes have been proved successful for glucose control, for example, the marketed drugs rosiglitazone (**1**) and pioglitazone (**2**). They belong to the class of thiazolidinedione (TZD) antidiabetic agents (Fig. 1) and control the blood glucose level in type II diabetes by an insulin sensitizing mechanism.[2,3]

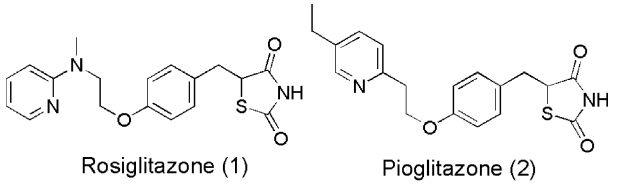

Figure 1. Structures of marketed PPARγ agonists.

Recently more compounds in clinical and preclinical research have been reviewed.[4] In addition to the TZDs, other structurally diverse synthetic PPARγ agonists have been identified such as α-alkoxy-β-phenyl propanoic acids (ragaglitazar, **3**[5]) and tyrosine derivatives (farglitazar, **4**[6]) (Fig. 2). A typical PPARγ agonist consists of an acidic head attached to an aromatic scaffold, a linker, and a hydrophobic tail.

Lately several indole-based compounds have been reported to be potent PPARγ agonists like **5** and **6** (Fig. 2).[7,8] It is shown that indole ring perhaps improves the binding activity for these compounds.

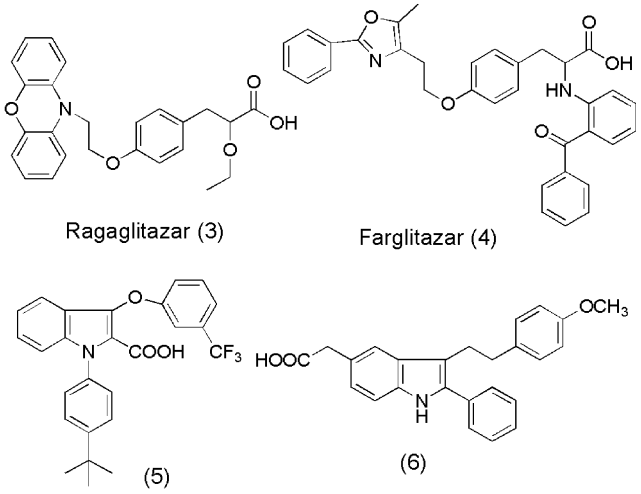

Figure 2. Structures of some PPARγ agonists in clinical and preclinical research.

Keywords: De novo drug design; PPARγ ligand; Type II diabetes; Indole compounds.
* Corresponding author. Tel.: +86 021 54237560; fax: +86 021 64389178; e-mail: rwen@shmu.edu.cn

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In this letter, we report the structure-based de novo design[9] by MCSS and LeapFrog programs, synthesis, and biological evaluation of new indole-based PPARγ ligands.

The X-ray crystal structure of the human apo-PPARγ ligand-binding domain (LBD) was first reported in 1998.[10] Up to now, there are six different co-crystal structures of PPARγ with the synthetic agonists. The protein model used in our work was constructed according to the crystal structure of PPARγ–LBD–ragaglitazar complex from the Brookhaven Protein Data Bank (PDB), entry 1NYX.[11]

The multiple copy simultaneous search (MCSS) program[12] was employed to calculate the energetically favorable position and the orientation of indole nucleus in the active site of the receptor. The functional group chosen for the MCSS calculation was trpr (tryptophan side chain: 3-methyl indole). Replicas of the given functional group were randomly distributed inside the binding site and then simultaneously and independently energy-minimized.

Table 1. LeapFrog scores and AutoDock calculated binding free energy for the complexes of the lead compounds

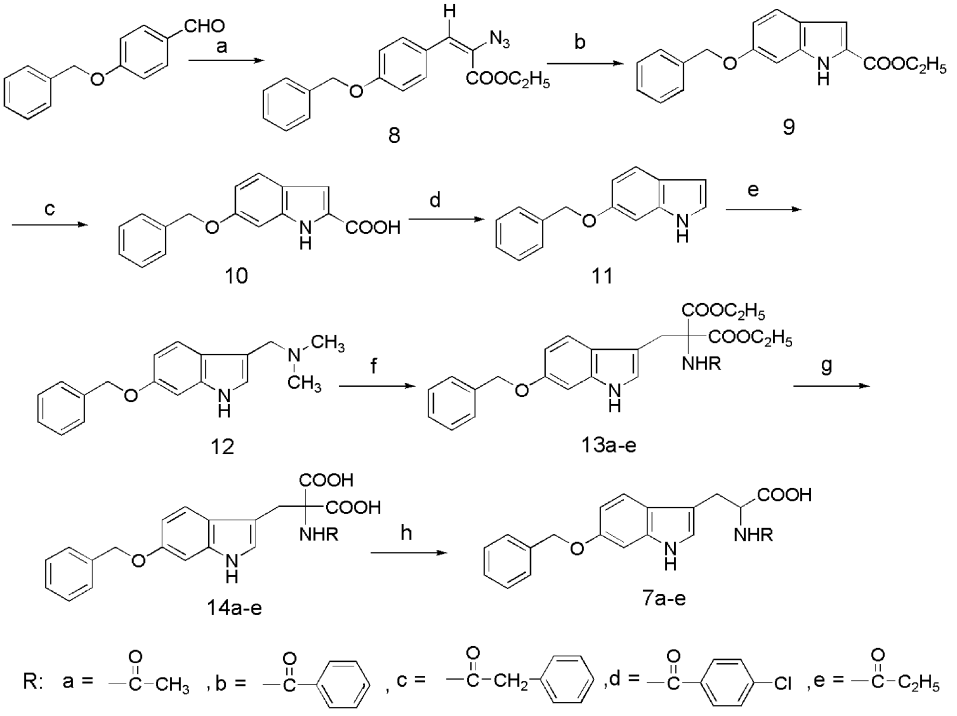
Compounds	LeapFrog score	Binding free energy (Kcal/mol)
7a	−15.13	−7.94
7b	−21.02	−10.06
7c	−24.67	−10.58
7d	−27.09	−11.07
Ragaglitazar	na	−10.43

Thus, the suitable position of 3-methyl indole obtained by MCSS calculations was used along with the JOIN move in LeapFrog program to generate the novel ligands. The new ligands were checked for alternative orientations using FLY move and were completely minimized using TWIST move to evaluate the binding energy for the minimum energy conformation of the ligand. The process was repeated to generate the different ligands.

The MCSS and LeapFrog design result indicated that 3-(6-benzyloxy-1*H*-indol-3-yl)-2-acylaminopropionic acid **7a–d** were nicely accommodated by PPARγ ligand-binding pocket. Then the advanced docking program AutoDock 3.0[13] was used to determine the lowest energy position in the active site for the above lead molecules. The calculated binding free energy and LeapFrog score of each molecule are given in Table 1.

Table 2. Biological activity of compounds **14a–e** and **7a–e** compared to the marketed compound rosiglitazone

Compounds	RU (10^{-5}mol/L)	K_D (mol/L)
14a	9.36	na
7a	9.20	na
14b	16.23	na
7b	14.16	na
14c	13.46	na
7c	20.00	na
14d	35.29	8.69×10^{-6}
7d	73.42	6.86×10^{-6}
14e	11.58	na
7e	16.69	na
Rosiglitazone	na	4.98×10^{-6}



Scheme 1. Reagents and conditions: (a) $N_3CH_2COOC_2H_5$, C_2H_5ONa, C_2H_5OH, 85%; (b) xylene, reflux, 80%; (c) 2 N NaOH, EtOH, reflux, 91%; (d) Cu, quinoline, reflux, 82%; (e) $(CH_3)_2NH$, HCHO, CH_3COOH, 91%; (f) $RNHCH(COOC_2H_5)_2$, NaOH, toluene, reflux, 31–62%; (g) 10% NaOH, reflux, 73–86%; (h) H_2O, reflux, 72–83%.

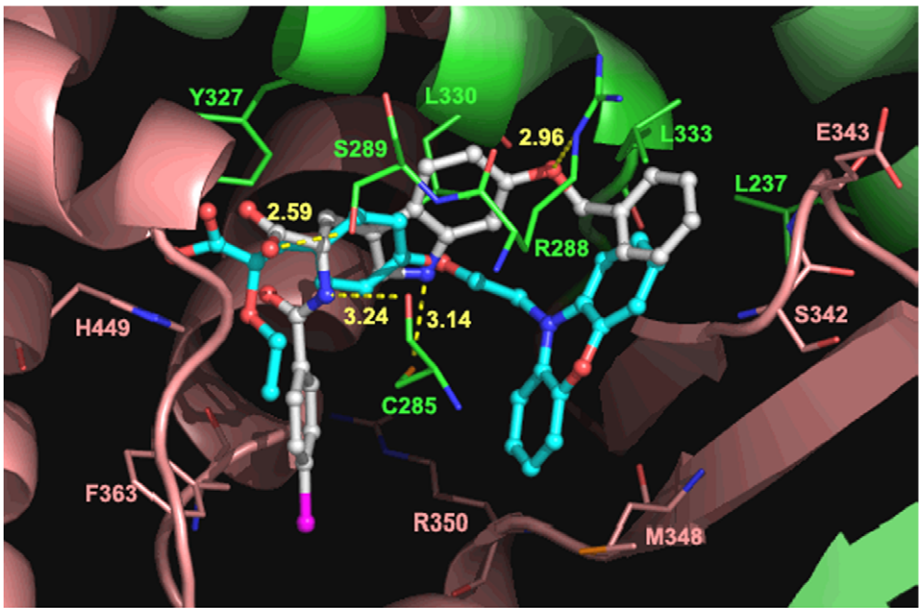


Figure 3. Superimposition of compound **7d** (white) on the PPARγ-bound conformation of ragaglitazar (blue). Residues involved in hydrogen bonding action to **7d** are also indicated.

The designed ligands were synthesized as shown in Scheme 1. 4-Benzyloxybenzaldehyde was condensed with ethyl azidoacetate in the presence of sodium ethoxide to obtain azidocinnamate **8**, which was heated in xylene to provide ester **9**. The subsequent hydrolysis and Cu/quinoline mediated decarboxylation afforded the 6-benzyloxyindole **11**. By the Mannich reaction, compound **11** was transformed to 3-(dimethylaminomethyl)-6-benzyloxyindole **12**, which acted with 2-acylamino malonic acid diethyl ester in the presence of sodium hydroxide and toluene to afford **13a**–**e**. The compounds **13a**–**e** were saponifed under basic condition to afford diacid **14a**–**e**, and decarboxylated to obtain target compounds **7a**–**e**.

The newly synthesized 3-(6-benzyloxy-1*H*-indol-3-yl)-2-acylaminopropionic acid **7a**–**e** and their synthetic precursors **14a**–**e** were tested through the receptor/ligand binding assay in vitro.[14] The result shows that (1) the Response Unit (RU) values of the compounds **14a**–**c**, **14e**, **7a**–**c**, and **7e** were bellow 20 at 10^{-5} mol/L concentration, which indicated that their binding activities to PPARγ were weak; (2) the compounds **14d** and **7d** exerted significant binding activities. As shown in \Table 2, the K_D values of **14d** and **7d** were close to that of the marketed drug rosiglitazone.

As our Docking study prediction, the compound **7d** was nicely accommodated by PPARγ-ligand binding pocket. The calculated binding free energy was −11.07 Kcal/mol, better than other designed compounds **7a**–**c**. As seen in Figure 3, the amino acid group of **7d** as the hydrophilic group head is involved in hydrogen bonds formation with Cys285 and Ser289. The indole heterocycle as the flat aromatic group also forms hydrogen bonds interaction with Cys285. Arg288 forms H-bond action with the O atom of the benzyloxy. The benzene ring of benzyloxy is situated in the hydrophobic pocket formed by Leu333, Glu343 and Ser342. Other residues with hydrophobic action include Phe282, Phe363, His449, Met364, Tyr327, Ile326 and Leu330.

In summary, MCSS and LeapFrog programs were used successfully to design novel indole-based PPARγ ligands. Among our designed and synthesized compounds, in fact two new indole molecules (compounds **7d** and **14d**) possessed potent PPARγ binding activity close to rosiglitazone in in vitro biological assay. Currently, further detailed study and in vivo pharmacological evaluation of these compounds are under way.

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11. The ligand-binding pocket of the receptor was defined as the collection of the amino acids enclosed within a sphere of 5 Å radius around the bound ligand.
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13. The docking parameters were set on as follow: not only the atom types but also the generations and the number of runs for the LGA algorithm were edited and properly assigned according to the requirement of the Amber force field. The number of generation, energy evaluation, and docking runs was set to 370,000, 1,500,000, and 10, respectively. The kinds of atomic charges were taken as Kollman-all-atom for PPARγ and Gasteiger–Huckel for ligands.
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