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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1547–1551
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and biological activity of novel PPARγ ligands based on rosiglitazone and 15d-PGJ_2

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Received 25 December 2004; revised 30 January 2005; accepted 31 January 2005

Abstract—To develop novel PPARγ ligands, we synthesized thirteen 3-{4-(2-aminoethoxy)phenyl}propanoic acid derivatives, which are designed based on the structures of rosiglitazone and 15d-PGJ_2. Among these compounds, compound **9** was found to be as potent as rosiglitazone in a binding assay and a preadipocyte differentiation test. Molecular modeling suggested that the nonyl group of **9** interacted with hydrophobic amino acid residues constructing the hydrophobic region of PPARγ protein where the alkyl chain of 15d-PGJ_2 is expected to be located.

Peroxisome proliferator-activated receptors (PPARα, PPARγ, and PPARδ) belong to the nuclear receptor superfamily and act as ligand-activated transcription factors.[1–3] These receptors play a pivotal roles in regulating the expression of a large number of genes involved in lipid metabolism and energy balance.[4] Many studies on PPARs have been performed, and these efforts led to the discovery of the clinically useful thiazolidinedione (TZD) class of insulin sensitizers such as rosiglitazone[5] and pioglitazone[6] (Fig. 1), which are potent PPARγ agonists used in the treatment of Type 2 diabetes. However, the use of TZDs has been limited because of their poor safety profiles. For example, troglitazone, which came out to the market first, disappeared from the market due to its severe hepatic toxicity in 1999,[7] and rosiglitazone is reported to be associated with liver, cardiovascular, and hematological toxicities.[8] We therefore initiated a search for non-TZD PPARγ ligands with the goal of finding novel insulin sensitizers. In this letter, we report the design, synthesis, and biological activity of non-TZD PPARγ ligands based on the structure of rosiglitazone and 15-deoxy-Δ-12,14-prostaglandin J_2 (15d-PGJ_2).

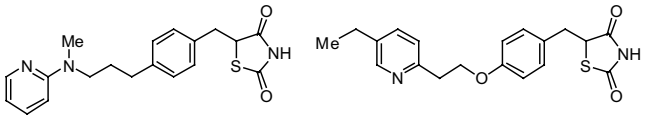

Figure 1. Structures of rosiglitazone (GSK) and pioglitazone (Takeda).

The compounds prepared for this study are shown in Figure 2, and the routes used for their synthesis are illustrated in Schemes 1–4. Scheme 1 shows the preparation of *N*-(pyridin-2-yl)-*N*-alkyl derivatives **1**–**3** bearing a methyl, ethyl, and propyl group, respectively, on their nitrogen atom as an alkyl chain.[9] The protection of dialkylamine **14a**–**c** by $(Boc)_2O$ gave **15a**–**c**.[10] The Mitsunobu reaction[11] was applied to the conversion of **15a**–**c** into 3-{4-(2-aminoethoxy)phenyl}propanoic acid derivatives **16a**–**c**: treatment of **15a**–**c** with diethylazodicarboxylate, PPh_3, and 3-(4-hydroxyphenyl)propanoic acid methyl ester **21** gave ethers **16a**–**c**. The *N*-Boc groups of **16a**–**c** were removed with trifluoroacetic acid to give amines **17a**–**c**. Treatment of **17a**–**c** with 2-fluoropyridine, or 2-chloropyridine gave *N*-(pyridin-2-yl)-*N*-alkyl compounds **18a**–**c** via nucleophilic aromatic substitution, and subsequent hydrolysis afforded carboxylic acids **1**–**3**.

Keywords: PPARγ ligand; 15d-PGJ_2; Insulin sensitizer; Agonist.
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doi:10.1016/j.bmcl.2005.01.074

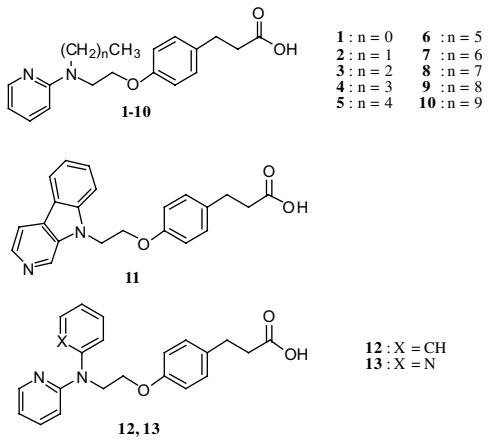
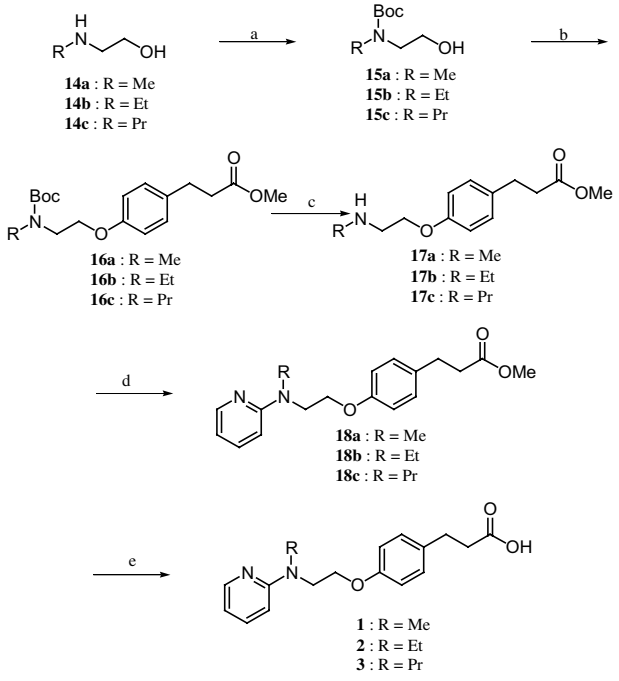


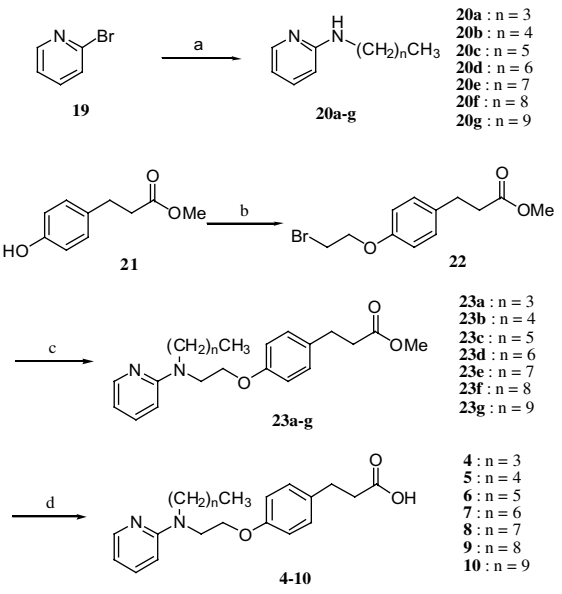
Figure 2. Structures of compounds **1**–**13**.



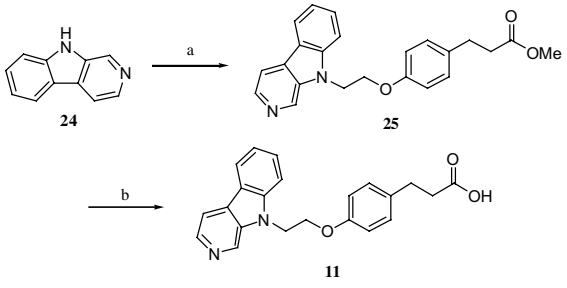
Scheme 1. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, 0 °C to rt, 91–100%; (b) DEAD, PPh_3, 3-(4-hydroxyphenyl)propanoic acid methyl ester **21**, THF, 0 °C to rt, 49–63%; (c) TFA, CH_2Cl_2, 0 °C to rt, 81–94%; (d) 2-fluoropyridine or 2-chloropyridine, DMF, reflux, 7–38%; (e) aq NaOH, THF/MeOH, rt, 78–92%.

The preparation of the other *N*-(pyridin-2-yl)-*N*-alkyl derivatives **4**–**10** is outlined in Scheme 2. The preparation of 2-alkylamino pyridine **20a**–**g** was achieved by the method of Buchwald:[12] treatment of **19** with *n*-alkylamine, $Pd_2(DBA)_3$, BINAP, and *t*-BuONa in toluene under reflux. Propanoic acid methyl ester **21** was allowed to react with 1,2-dibromoethane to give ether **22**. Coupling between amines **20a**–**g** and ether **22** afforded *N*-(pyridin-2-yl)-*N*-alkyl compounds **23a**–**g**, and subsequent hydrolysis afforded carboxylic acids **4**–**10**.

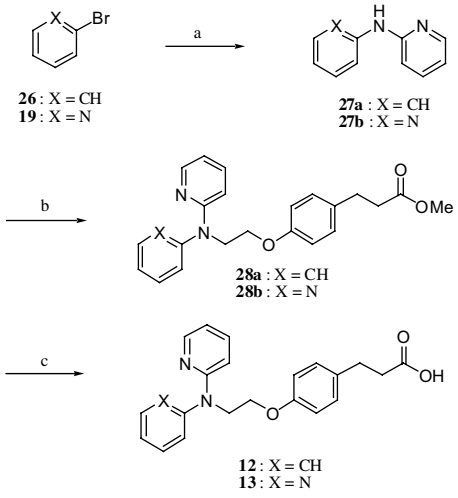
N-(2-Pyridin-2-yl)-*N*-aryl derivatives **11**–**13** were prepared by the procedure outlined in Schemes 3 and 4.



Scheme 2. Reagents and conditions: (a) $CH_3(CH_2)_nNH_2$, $Pd_2(DBA)_3$, BINAP, *t*-BuOH, toluene, 80 °C, 7–53%; (b) 1,2-dibromoethane, K_2CO_3, THF, 115 °C, 25%; (c) **20a**–**g**, Et_3N, KI, THF, 120 °C, 2–25%; (d) aq NaOH, THF, rt, 81–96%.



Scheme 3. Reagents and conditions: (a) (i) NaH, DMF, rt; (ii) **22**, KI, 90 °C, 72%; (b) aq NaOH, THF, rt, 89%.



Scheme 4. Reagents and conditions: (a) 2-aminopyridine, $Pd_2(DBA)_3$, BINAP, *t*-BuONa, toluene, 80 °C, 70–88%; (b) (i) NaH, DMF, rt, (ii) **22**, KI, 90 °C, 18–30%; (c) aq NaOH, THF, rt, 70–80%.

Norharman **24** was reacted with bromide **22** in the presence of sodium hydride in DMF to give 9*H*-β-carboline compound **25**, and subsequent hydrolysis gave compound **11** (Scheme 3). Compounds **27a** and **b** were prepared in the same way as 2-alkylamino pyridines **20a**–**g** (Scheme 4). Compounds **27a** and **b** were allowed to react with bromide **22** in the presence of sodium hydride in DMF to give compounds **28a** and **b**. Treatment of **28a** and **b** with aqueous NaOH gave *N*-(pyridin-2-yl)-*N*-phenyl derivative **12** and dipyridinyl derivative **13**.

The binding affinity of the compounds for PPARγ was evaluated with a CoA–BAP system (Microsystems).[13] In this system, the alkaline phosphatase (AP) activity is directly proportional to the PPARγ-binding affinity of the ligands.

Since it has been revealed that the TZD ring can be replaced by a carboxyl group,[14] we initially examined the binding affinity for PPARγ of compound **1**, in which the TZD group of rosiglitazone is replaced by a carboxyl group. Although compound **1** did not show any activity at 0.1 and 1 μM, a certain level of activity was observed at 10 μM (Table 1, line 1). For the further design of PPARγ ligands, we focused on the alkyl chain of 15d-PGJ_2,[15,16] an endogenous ligand of PPARγ. Since certain fatty acids with a long alkyl chain are known to be natural PPARγ ligands,[17] the hydrophobic moiety is assumed to be critical for the binding affinity for PPARγ. Our study regarding the binding mode of 15d-PGJ_2 in PPARγ protein (PDB code 1FM6) by computer calculation (Macromodel 8.1)[18] also suggested that the alkyl chain of 15d-PGJ_2 is located in the wide hydrophobic region of the PPARγ ligand-binding cavity (Fig. 3). However, the crystal structure of a PPARγ/rosiglitazone complex[19] revealed that rosiglitazone does not have any hydrophobic groups interacting with the hydrophobic amino acid residues of PPARγ. We hypothesized that the introduction of a hydrophobic group into compound **1** may increase the affinity for PPARγ (Fig. 4). We therefore designed compounds **2**–**10** in which alkyl groups of various lengths were introduced at the 2-aminopyridinyl moiety of compound **1**, and evaluated their ability to bind PPARγ. It was found that the affinity of compounds **1**–**10** was closely related to chain length, and the most potent compounds were heptyl **7**, octyl **8**, and nonyl **9**. In addition, *N*,*N*-diaryl compounds **11**–**13** exhibited weak activity compared with compounds **7**–**9** (Table 1, lines 11–13). We next compared the binding affinity of compounds **7**–**9** with that of rosiglitazone at lower concentrations. As shown in Figure 5, compound **9** showed the highest activity among the three, and had only slightly less affinity for PPARγ than did rosiglitazone.

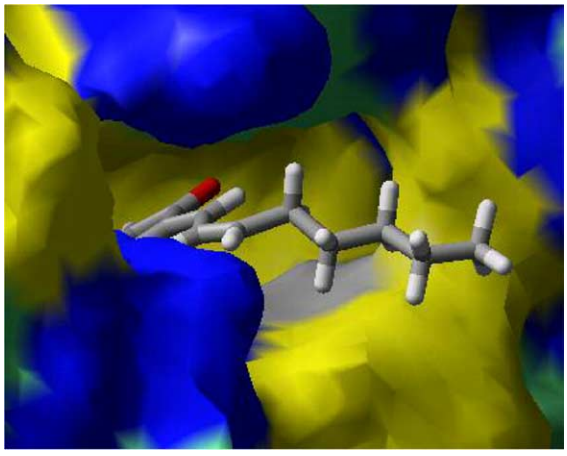


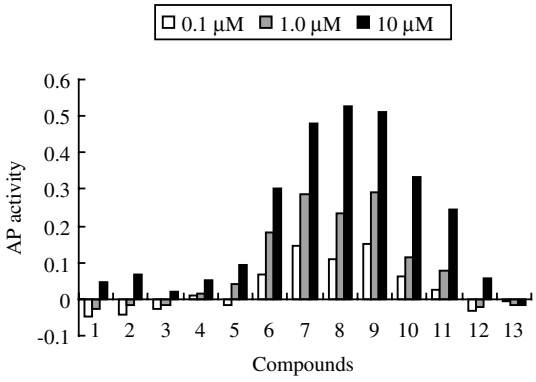
Figure 3. View of the conformation of 15d-PGJ_2 (tube) docked in PPARγ. The hydrophobic and hydrophilic regions are shown in yellow and blue, respectively.

As compound **9** was most active in our study, we used it for further evaluation. Since it has been reported that activation of PPARγ enhances adipocyte differentiation[20] and increases insulin sensitivity, compound **9** was subjected to a rat abdominal preadipocyte differentiation test.[21,22] The accumulation of neutral fat in the cells was observed after the administration of compound **9** at concentrations of 1, 2.5, and 5 μM, and the activity of compound **9** was found to be comparable to that of rosiglitazone (Fig. 6).

Since *N*-nonyl carboxylic acid **9** had a high level of activity, we studied its mode of binding to PPARγ. A low energy conformation was calculated when **9** was docked in a model based on the crystal structure of PPARγ using Macromodel 8.1 software.[18] An inspection of the simulated PPARγ/**9** complex suggested that oxygen atoms of compound **9** form hydrogen bonds with Ser 289, Tyr 327, and Tyr 473 (Fig. 7). In addition, it was shown that the nonyl group of **9** is located in the hydrophobic region formed by Phe 287, Gly 284, Ile 281, Ile 341, and Met 348 (Fig. 8) where the alkyl chain of 15d-PGJ_2 is calculated to be located (Fig. 3).

In summary, in order to explore novel PPARγ ligands, we prepared several 3-{4-(2-aminoethoxy)phenyl}propanoic acid derivatives designed based on the structures of rosiglitazone and 15d-PGJ_2. Among them, *N*-(pyridin-2-yl)-*N*-nonyl compound **9** was found to be as

Table 1. Binding affinity for PPARγ of compounds **1**–**13** at 0.1, 1.0, and 10 μM.[a]



[a] Values are means of at least three experiments.

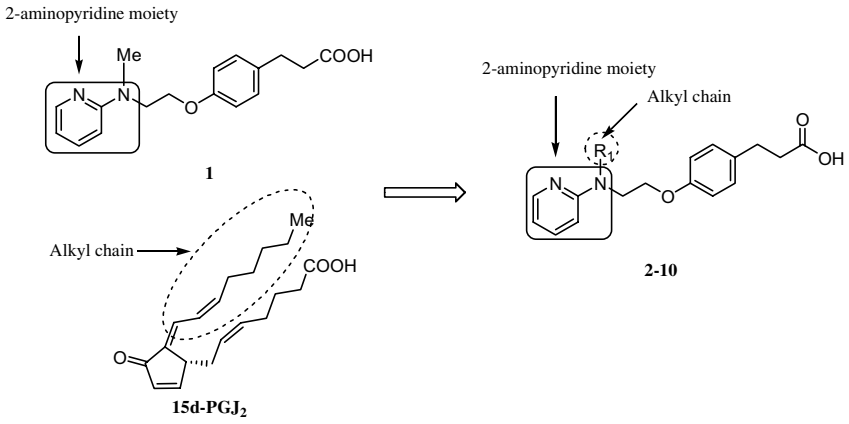


Figure 4. Structures of compounds **2**–**10** designed on the basis of the structure of 15d-PGJ_2 and rosiglitazone.

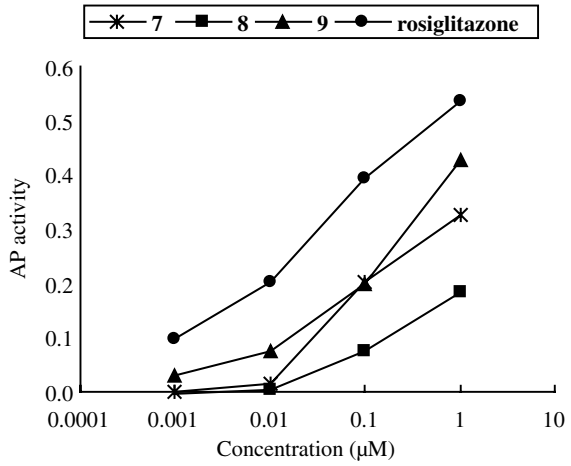


Figure 5. Binding affinity for PPARγ of rosiglitazone and compounds **7**–**9** at 0.001, 0.01, 0.1, and 1.0 μM. Values are means of at least three experiments.

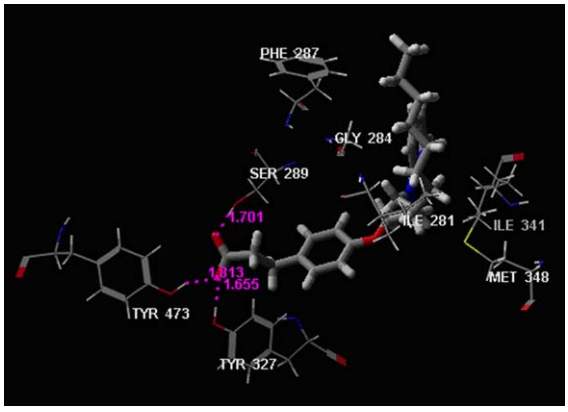


Figure 7. View of the conformation of **9** (tube) docked in PPARγ. Residues around compound **9** and hydrogen bonds are displayed as wires, and dotted lines, respectively. Figures represent distances in angstroms.

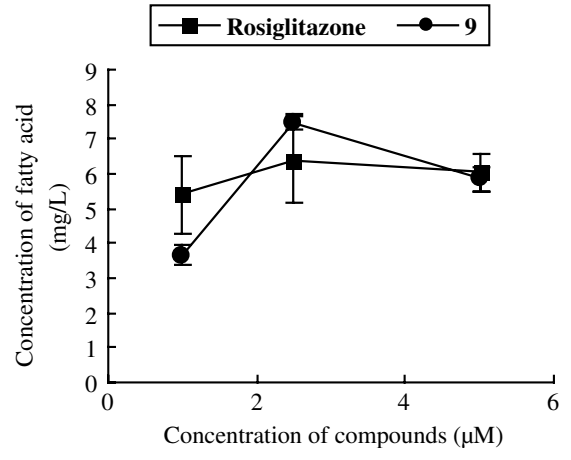


Figure 6. Accumulation of fatty acid in rat preadipocytes by rosiglitazone and compound **9**. Values are means of at least three experiments.

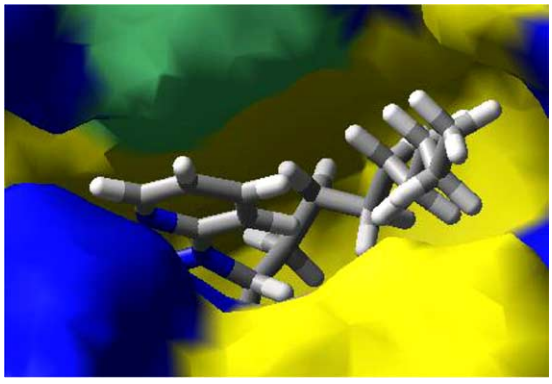


Figure 8. View of the conformation of **9** (tube) docked in PPARγ. The hydrophobic and hydrophilic regions are shown in yellow and blue, respectively.

potent as rosiglitazone in the binding assay and the preadipocyte differentiation test. Molecular modeling suggested that the carboxylate anion of **9** forms hydrogen bonds with some hydrophilic amino acid residues, and the nonyl group appropriately interacts with hydrophobic amino acid residues. The findings of this study will help provide an effective agent for Type 2 diabetes.

Currently, further detailed studies on compound **9** are under way.

Acknowledgements

This work was supported in part by grants from the Health Sciences Foundation of the Ministry of Health, Labor, and Welfare of Japan.

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18. Docking and subsequent scoring were performed using Macromodel 8.1 software. Coordinates of PPARγ complexed with rosiglitazone were taken from the Brookhaven Protein Data Bank (PDB code 1FM6) and hydrogen atoms were added computationally at appropriate positions. The structure of a ligand bound to PPARγ was constructed by molecular mechanics (MM) energy minimization. The starting position of a ligand was determined manually: its carboxyl group was superimposed onto the TZD ring of crystallographic rosiglitazone. The conformation of the ligand in the active site was minimized by a MM calculation based upon the OPLS-AA force field with each parameter set as follows; solvent: water, method: LBFGS, Max # Iterations: 10,000, converge on: gradient, convergence threshold: 0.05.
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22. In rat preadipocyte differentiation experiments, we basically followed the protocol of the preadipocyte Total Kit (TOYOBO. Co., Ltd, Osaka, Japan). Rat preadipocytes obtained from the abdominal tissue (Wistar, male, 8 weeks old) were cultured for 10 days in a humidified incubator at 37 °C and 5% CO_2 in a preadipocyte growth medium. The medium was renewed every other day. After the preadipocytes reached confluence, they were treated with preadipocyte differentiation medium containing compound **9** or rosiglitazone. The cells were cultured for 15 days with the differentiation medium renewed every three days. Accumulating neutral fat in the cells was measured as absorbance at 590 nm with a 1420 ARVO^{TM} multilabel-counter (PerkinElmer, Boston, MA, U.S.A.) after staining with lipidos liquid (TOYOBO. Co., Ltd, Osaka, Japan).