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# Design and Synthesis of Oxime Ethers of β-Oxo-γ-phenylbutanoic Acids as PPAR α and -γ Dual Agonists

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Oxime ethers of β-oxo-γ-phenylbutanoic acids were prepared to develop more effective PPAR α and γ dual agonists. Among them, compound **11k** exhibited potent *in vitro* activities with $EC_{50}$ of 2.5 nM and 3.3 nM in PPAR α and γ, respectively. It showed better glucose lowering effects than rosiglitazone **1** and improved the lipid profile like plasma triglyceride in db/db mice model.

**Key Words :** PPAR α and γ dual agonist, Diabetes, Oxime ethers, β-Oxo-γ-phenylbutanoic acid

## Introduction

Peroxisome proliferator-activated receptors (PPARs) are categorized as a subfamily of the nuclear receptor family and three isotypes, *i.e.* PPAR α, -β/δ, and -γ have been identified.[1,7] PPAR α which was the first discovered PPAR is widely expressed in liver, kidney, heart, skeletal muscle, and adipocytes, while PPAR γ is distributed in the intestinal mucosa, kidney, pancreas, spleen, macrophages, and adipocytes.[3,8] PPARs can be activated by fatty acids and eicosanoids leading to formation of PPAR heterodimers with retinoid-X receptors (RXRs). In turn, the dimers bind to peroxisome proliferator-response elements (PPREs) at the promoter region of various target genes regulating different metabolic and endocrine functions.[1-3] In particular, impaired lipid and glucose metabolism can be normalized by pharmacological modulation of PPAR activity, which was a trigger of extensive research on development of PPAR agonists to treat type II diabetes mellitus (DM).[1]

There are several therapeutic targets recently emerging in type II DM treatment, which include dipeptidyl peptidase IV[3] (DPP IV), glucokinase[6] as well as PPARs.

Currently, at least six different pharmacologic classes of oral agents to treat type II DM, *i.e.* sulfonylureas, biguanides, α-glucosidase inhibitors, metiglitinides, DPP IV inhibitors, and thiazolidinediones (TZDs), are available in market.[4,5] Among them, TZDs are PPAR γ agonists and two TZDs are rosiglitazone **1** (Avandia®, GlaxoSmithKline) and pioglitazone **2** (Actos®, Takeda).[5,9]

Type II diabetes has been reported to increase in most countries these days. The increasing prevalence of type II diabetes is partly accounted for increased obesity and physical inactivity.[22] High glucose level in the blood causes hyperglycemia and insulin resistance, which result in the deterioration of the pancreatic β-cell function.[1] Hyperglycemia should be controlled with careful manners, otherwise higher morbidity and mortality have been found in the related diseases such as retinopathy, nephropathy, neuropathy, dyslipidemia, coronary heart disease, hypertension, and obesity.[2] In adipocyte tissue, PPAR γ promotes the differentiation and the lipid storage.[23] PPAR γ agonists like rosiglitazone **1** and pioglitazone **2** ameliorate insulin sensitivity.[24] Activation of PPAR α induces fatty acid oxidation in the liver, and reduces serum triglyceride (TG) and increases high-density lipoprotein (HDL) cholesterol.[23]

Three-year clinical study indicated that pioglitazone reduced the risk of myocardial infarction or stroke significantly.[10] However, long clinical treatment (3-4 months) of TZDs is required to reach their full glucose reduction effect.[11]

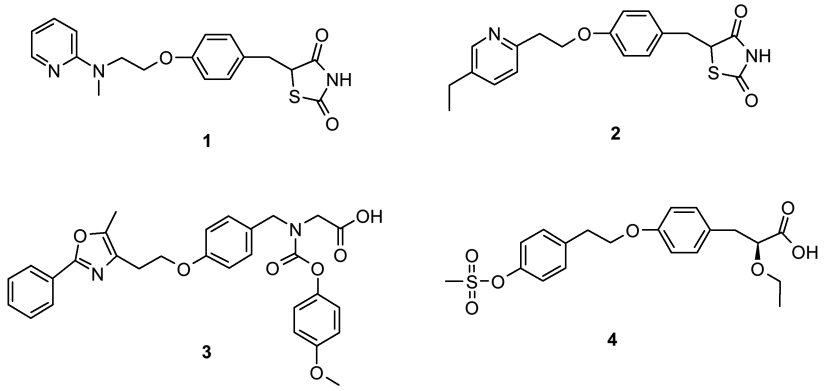



**Figure 1.** Structures of PPAR agonists.

Although these agents have lowering glucose effect and improve the lipid profile like TG, low-density lipoprotein (LDL) cholesterol and HDL cholesterol, the crucial side effects like congestive heart failure, edema, fluid retention and weight gain still remain unsolved.[12] Until now, a large number of PPAR α and γ dual agonists have been investigated in many research groups, but safe and effective PPAR α and γ dual agonists including tesaglitazar **4** (AstraZeneca) and muraglitazr **3** (BMS-Merck) have not been marketed.[13] Nevertheless, PPAR α and γ dual agonist will be well suited for treatment of diabetes and cardiovascular disease as long as the aforementioned side effect can be reduced.

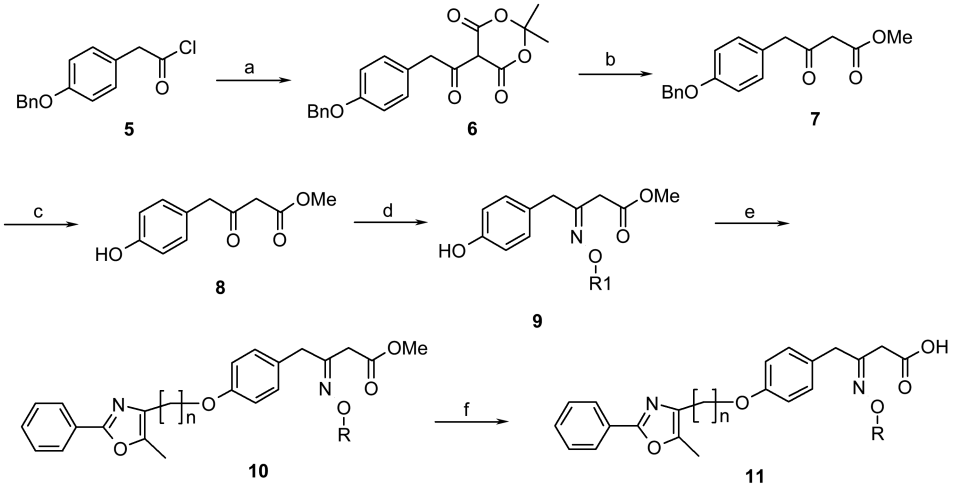
## Results and Discussion

Many PPAR α and γ dual agonists contain α-alkoxy acids, α-carbamate acids or thiazolidinediones as shown in tesaglitazar **4**, muraglitazar **3**, rosiglitazone **1** and pioglitazone **2**. Acid group in PPAR agonists is known to play a key role in interacting with the dual agonism because the acidic moiety forms a conserved hydrogen-bonding network with several residues in PPAR ligand binding domain (LBD).[4b] Search for the new PPAR agonist led us to investigate the effect of β-substituent in phenylbutanoic acids. Therefore, we designed a series of new phenylbutanoic acids containing the oxime functionality as PPAR α and γ dual agonists.[21] The synthesis of phenylbutanoic acids having the oxime group is described in Scheme 1.

Acid chloride **5** was reacted with 2,2-dimethyl-1,3-dioxane-4,6-dione in the presence of pyridine in dichloromethane to give **6**. Methanolysis of **6** at 70 °C afforded the β-keto ester **7**. This method enabled us to prepare the β-keto ester with ease.[14] Hydrogenation of **7** and the following coupling of *O*-alkyl hydroxyl amine furnished the *para*-substituted phenols **9**. The generated geometric (*E*) and (*Z*)-isomers were not isolated in this step but separated in the final step using HPLC. The corresponding *meta*-substituted phenols were be also synthesized by using the same procedure as the above. Coupling of phenols **9** with the commonly used oxazoles **15** or **18** was carried out by refluxing with $Cs_2CO_3$ in acetonitrile to furnish the esters **10** in good yields.

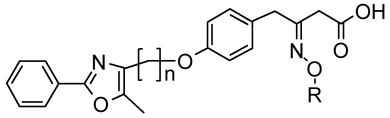
Oxazoles **15** or **18** could be prepared by using the published procedures.[15] Scheme showed a simple synthetic route to the oxazoles. Butane-2,3-dione mono-oxime **13** was reacted with benzaldehyde **12** and hydrochloric acid in dioxane to afford oxazole *N*-oxide **14**. Treatment of *N*-oxide **14** with phosphorus oxychloride in chloroform gave chloride **15**. For the one carbon elongation, chloride **15** was subjected to sodium cyanide in DMF at 80 °C and the subsequent alkaline hydrolysis with KOH in aqueous THF to furnish the corresponding acid. Reduction of the acid with boranedimethylsulfide complex and the following reaction with methanesulfonyl chloride afforded mesylate **18**. Esters **10** were hydrolyzed with sodium hydroxide in aqueous THF and methanol, and then the (*E*) and (*Z*)- isomers were separated with HPLC to give the pure acid **11**.[16]

Compounds **11** in our hand were screened for PPAR α and γ agonist activity on PPAR-GAL4 chimeric receptor in transfected HepG2 cells.[17] Rosiglitazone **1**, muraglitazar **3** and GW9578[18] were used as PPAR γ, PPAR α/γ and PPAR α agonist controls. Table 1 summarized functional transactivation data for all the compounds. **11a** and **11b** were found to show a potent activities for PPAR γ and moderate activities for PPAR α. (*E*)-Isomer **11a** had higher activity for PPAR γ than (*Z*)-Isomer **11b**. Oxime ethers of β-oxo-γ-phenylbutanoic acids provide a working scaffold of PPAR α and γ dual agonist. It was so encouraging that we decided to investigate the influence of R groups. Modification of the chain in R group indicated that the optimal substitute was *n*-propyl group in this study. The binding pocket of oxime group is likely to be sensitive to the size of the alkyl chain. For **11e** and **11f** with shorter linkers, the (*Z*)-configuration was 5-6 times as potent as (*E*)-configuration, suggesting that oxazole with a shorter linker matches well with (*Z*)-con-




**Scheme 1.** Reagents and conditions: (a) 2,2-dimethyl-1,3-dioxane-4,6-dione, pyridine, $CH_2Cl_2$; (b) MeOH, 70 °C, 26%; (c) 10% Pd/C, $H_2$, MeOH, 49%; (d) NaOAc, HCl·$H_2$N-OR, MeOH, 80-90%; (e) 15 or 18, $Cs_2CO_3$, $CH_3CN$, 80 °C, 60-90%; (f) THF:MeOH:1 N NaOH=1:1:1, 90%; (g) 4 M HCl in dioxane, rt, 99%; (h) $CHCl_3$, $POCl_3$, reflux, 99%; (i)NaCN, DMF, 80 °C, 95%; (j) KOH, aq. THF, reflux; (k) $BH_3$·$SMe_2$, THF, rt, 68%; (l) $MeSO_2Cl$, $Et_3N$, $CH_2Cl_2$, 0 °C, 99%.

**Table 1.** *In vitro* activity of oxime ethers of β-oxo-γ-phenylbutanoic acids **11**[a]



| Compounds | n | Geometric isomer | Substitution | R | $EC_{50}$ (nM)[b] of hPPAR γ | $EC_{50}$ (nM)[b] of hPPAR α |
|---|---|---|---|---|---|---|
| **11a** | 2 | *E* | *para* | $CH_2CH_2F$ | 42 | 518 |
| **11b** | 2 | *Z* | *para* | $CH_2CH_2F$ | 177 | 237 |
| **11c** | 2 | *E* | *para* | $CH_2CH_2CH_3$ | 12 | 30 |
| **11d** | 2 | *Z* | *para* | $CH_2CH_2CH_3$ | 158 | 138 |
| **11e** | 1 | *E* | *para* | $CH_2CH_2CH_3$ | 35 | 39 |
| **11f** | 1 | *Z* | *para* | $CH_2CH_2CH_3$ | 6.1 | 5.9 |
| **11g** | 2 | *E* | *para* | $CH(CH_3)_2$ | 103 | 497 |
| **11h** | 2 | *Z* | *para* | $CH(CH_3)_2$ | 757 | 987 |
| **11i** | 2 | *E* | *para* | $CH_2$-cyclopropyl | 21 | 69 |
| **11j** | 2 | *Z* | *para* | $CH_2$-cyclopropyl | 149 | 78 |
| **11k** | 2 | *E* | *meta* | $CH_2CH_3$ | 3.3 | 2.5 |
| **11l** | 2 | *Z* | *meta* | $CH_2CH_3$ | 12 | 16 |
| **11m** | 2 | *E* | *meta* | $CH_2CH_2CH_3$ | 3.3 | 1 |
| **11n** | 2 | *Z* | *meta* | $CH_2CH_2CH_3$ | 9.6 | 8 |
| **11o** | 1 | *E*/*Z* = 1.3/1 | *meta* | $CH_2CH_2CH_3$ | 252 | 10 |
| **11p** | 2 | *E* | *meta* | $CH_2$-cyclopropyl | 3.3 | 1 |
| **11q** | 2 | *Z* | *meta* | $CH_2$-cyclopropyl | 14 | 4 |
| **Rosiglitazone 1** | | | | | 82 | |
| **Muraglitazar 3** | | | | | 363 | 2000 |
| **GW9578** | | | | | | 78 |

[a]Agonist activities were measured in human PPAR-GAL4 chimeric HepG2 cells using the published procedure.[16] [b]Concentration of the test compounds which produced 50% of the maximal reporter activity.

figuration. Relocation of butanoic acid from *para* to *meta* position resulted in improved activities for PPAR α and γ. Especially, compounds **11k**, **11m** and **11p** with (*E*)-configuration exhibited well balanced and potent activities in PPAR α and γ. In the case of *meta* substituted phenyl ethers, varioud R group of the oxime seemed to be well tolerated.

In general, a series of oxime ethers of β-oxo-γ-phenylbutanoic acids **11** exhibited excellent PPAR α and γ activities to provide a useful scaffold for PPAR α and γ dual agonist. Finally, X-ray crystallographic data of **11k** was obtained to confirm the geometry of the oxime group and verify how well PPAR protein binds with it. The compound **11k** was found a potent PPAR agonist as shown in Figure 2.

To investigate the interaction of these compounds with the protein, the compound **11k** was chosen to co-crystallize with PPARα LBD and the co-activator peptide fragment (SRC-1).

The U-shaped compound **11k** binds to the PPARα LBD using four hydrogen bonds and many van der Waals contacts. The carboxylate group of the compound **11k** forms hydrogen bonds with Ser280 on helix 3, Tyr314 on helix 5, and Tyr464 on helix 12 as in the structure of tesaglitazar.[19] Its oxime group is located in the so-called "benzophenone" pocket and makes contacts with Phe273, Cys276, Met355 and Ile354. The linker benzene ring is rotated about twenty degrees compared to that of tesaglitarzar. The phenyl oxazole group is positioned in a hydrophobic pocket formed by helix 2'; helix 3 and the β sheet. In the case of *para*-substituted compounds, the additional X-ray study revealed that the overall binding structure is similar except the position of the linker benzene ring.

Based on the cell-based activities and the phamacokinetic studies in rats, **11k** was selected to adjust the *in vivo* experiment in male db/db mice for the further evaluation. Its pharmocokinetic behavior in Sprague-Dawley (SD) rats was

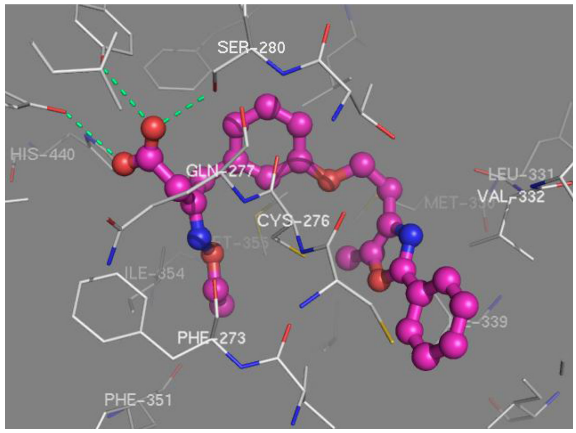



**Figure 2.** Crystal structure of the PPARα LBD in complex with compound **11k**. Compound **11k** is shown in purple and all the residues within 4 Å from the compound are labeled.

**Table 2.** Pharmacokinetic data of **11k** in SD rats

| Pharmacokinetic parameters | p.o. | i.v. |
|---|---|---|
| Dose (mg/kg) | 1 | 1 |
| $AUC_{0\text{-}24h}$ (μg.min/mL) | 724 | 997 |
| Cmax (μg/mL) | 1.3 | |
| Tmax (min) | 19 | |
| T1/2 (min) | 611 | 345 |
| Clearance (mL/min/kg) | | 1.0 |
| Vd (ml/kg) | | 506 |
| Bioavailability (%) | | 73 |

**Table 3.** *In vivo* data of compound **11k** in db/db mice[20]

| Compound | Dose (mg/kg) | Plasma glucose[a] | Plasma TG[a] |
|---|---|---|---|
| Muraglitazar **3**[b] | 1 | 40% | 10% |
| | 10 | 76% | 49% |
| Rosiglitazone **1**[c] | 10 | 65% | 65% |
| **11k**[b] | 1 | 58% | 28% |
| | 10 | 76% | 55% |

[a]Reduction of plasma glucose and plasma TG were calculated as the percent of reduction and increase with respect to control value. [b]Blood samples were collected after 11 days. [c]Blood samples were collected after 14 days.

described in Table 2. When dosed orally at 1 and 10 mg/kg in db/db mice[20] for 14 days, compound **11k** exhibited a dose-dependent decrease in plasma glucose and plasma triglyceride (TG) as depicted in Table 3. Treatment of 10 mg/kg of **11k** showed a glucose reduction of 76% as compared with that of 65% and 76% at 10 mg/kg of rosiglitazone **1** and muraglitazar **3**, respectively. At the same time, **11k** reduced plasma TG by 55% at a dose of 10 mg/kg. Glucose lowering effect was also found at a dose of 1 mg/kg of **11k**. In respect to glucose and TG lowering effect, **11k** had better efficacy than rosiglitazone **1** and *in vivo* efficacy comparable with muraglitazar **3**.

## Conclusions

In conclusion, a series of compounds **11** turned out to have glucose lowering effect and ameliorate lipid profile like TG in db/db mice model. We found that a series of oxime ethers of β-oxo-γ-phenylbutanoic acids were potent PPAR α and γ agonists and exhibited good *in vivo* efficacy, which will be further developed.

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16. All compounds described in this paper gave consistent $^1$H-NMR, $^{13}$C-NMR and LC/MS data. Among them, spectra data for the representative **11k** were given below. Spectra for **11k**: $^1$H-NMR ($CDCl_3$) δ 7.90-7.88 (2H, m), 7.35-7.30 (3H, m), 6.96 (1H, t, $J$ = 15.9 Hz), 6.70 (1H, brs), 6.64 (1H, d, $J$ = 7.35 Hz), 6.58 (1H, dd, $J$ = 7.35 Hz, 1.85 Hz), 4.06 (2H, t, $J$ = 6.40 Hz), 3.95 (2H, q, $J$ = 6.92 Hz), 3.63 (1H, s), 3.93 (1H, s), 3.83 (1H, t, $J$ = 6.40 Hz), 2.24 (3H, s), 1.07 (3H, t, $J$ = 6.92 Hz); $^{13}$C-NMR ($CDCl_3$) δ 177.05, 159.45, 158.74, 157.65, 145.08, 138.12, 132.72, 129.83, 129.38, 128.71, 127.67, 125.92, 121.91, 115.54, 112.38, 68.91, 66.48, 42.28, 34.52, 26.25, 14.51, 10.23; Mass (ESI) 423 $(M+1)^+$. Synthetic experimental procedures and spectral data can be found in the patent WO 2006057505.
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