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Design, synthesis, and evaluation of 2-alkoxydihydrocinnamates as PPAR agonists

Ying Lu, Zongru Guo,* Yanshen Guo, Jun Feng and Fengming Chu

Department of Synthetic Medicinal Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, PR China

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Abstract—A series of 2-alkoxydihydrocinnamates were synthesized as PPARγ and PPARα dual agonists. In vitro studies in cell model showed that these compounds were efficacious. Compound **1g** was found to be a potent PPARα/γ dual agonist and will be further evaluated for the treatment of type II diabetes.

Type II diabetes is a complex, metabolic disorder characterized by hyperglycemia and subsequent chronic complications leading to renal failure, blindness, and coronary artery disease.[1] Hyperglycemia in type II diabetes is caused by increased insulin resistance and impaired insulin secretion from the pancreas.[2] The conventional approach to treat type II diabetes focuses on the control of blood glucose level in order to reduce the incidence of the microvascular and macrovascular complication associated with high blood glucose level.[3] The $PPAR_S$ (peroxisome proliferator-activated receptors) are members of a super family of nuclear receptors that include steroid, retinoid, and thyroid hormone receptors.[4] These receptors play a pivotal role in regulating the expression of a large number of genes involved in lipid metabolism and energy balance.[5] It has been shown that PPARγ located in nucleus is able to increase the insulin sensitivity, promote the differentiation of lipocytes, and retard the occurrence of complications. Although some PPARγ modulators, such as rosiglitazone and pioglitazone (Fig. 1), which have been marketed as antidiabetes, may cause weight gain, and in some cases, edema.[6] Unsatisfactory efficacy and safety profile restrict their application, while PPARα agonists have exhibited lipid lowering activity and reduce weight gain. Therefore, we were prompted to search for new PPARγ and PPARα dual agonists as novel insulin sensitizers.

By scrutinizing the binding mode of PPARγ and PPARα to the dual agonist AZ-242 (the PDB deposition numbers were 1I7I and 1I7G) with docking operation and molecular simulation, and analyzing the 3D-QSAR of PPARγ and PPARα dual agonists, a common U-shaped pharmacophore model was derived, which was featured by the presence of an acidic moiety responsible for hydrogen bonding network on one terminal, a hydrophobic and bulky fragment on the other end, and a flat and narrow linker between the two terminals (Fig. 2).[7]

Guided by the mutual pharmacophore, the PPARγ/α dual agonists can be divided into three regions: A is the acidic head group, B is the linker part, and C is the hydrophobic tail group (Fig. 3). A novel virtual library of the dual agonists was constructed based on a combinatorial strategy. The building blocks A–C were selected from commercially available compounds with the criteria of drug-like properties, pharmacophoric requirement, and chemical feasibility. Accordingly, a virtual library with the capacity of over 1000 compounds was automatically generated from the building blocks with the Project Library program. The compounds in the resultant virtual library were matched with the binding pocket of PPARγ and PPARα with the program DOCK5.0[8] and virtual compounds with potentially high binding affinity were selected as 'hits' based on the scoring system of shape and energies. In the subsequent synthesis and evaluation of the hit compounds, we found that the *para*-ethyl biphenoxy 2-methoxydihydrocinnamic acid **1g** was a high affinity ligand

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* Corresponding author. Tel.: +86 10 631 65249; fax: +86 10 831 55752; e-mail: zrguo@imm.ac.cn

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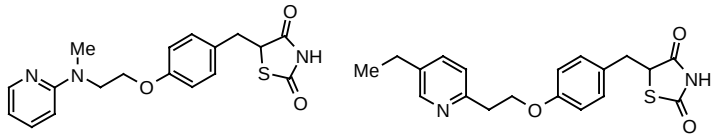


Rosiglitazone Pioglitazone

Figure 1. Structures of rosiglitazone and pioglitazone.

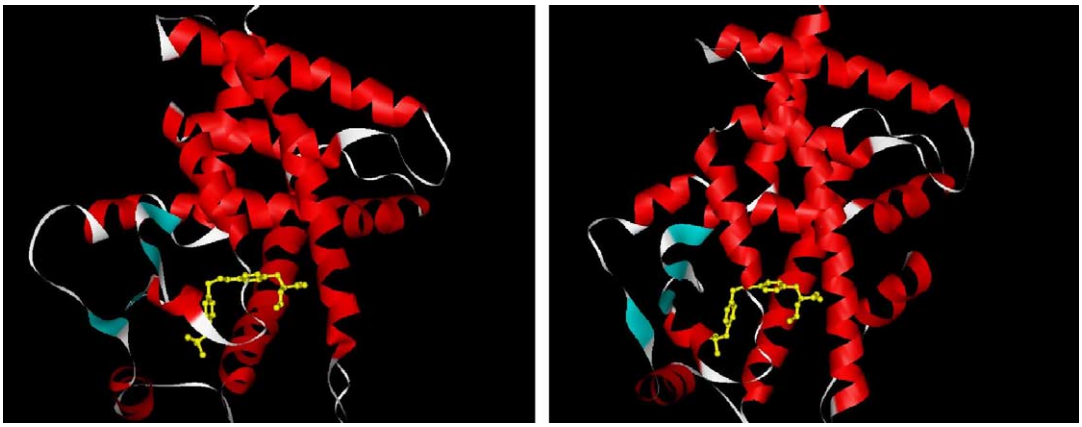


Figure 2. Crystal structure of dual agonist AZ-242 and PPARγ/α complex.

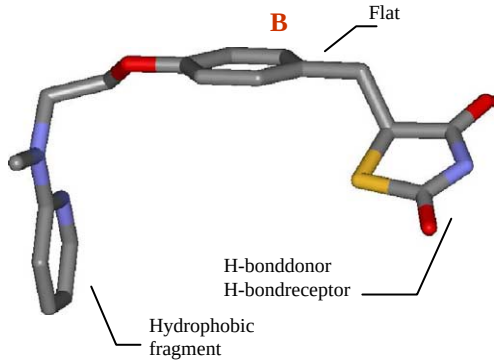


Figure 3. The pharmacophore of PPARγ and PPARα dual agonists.

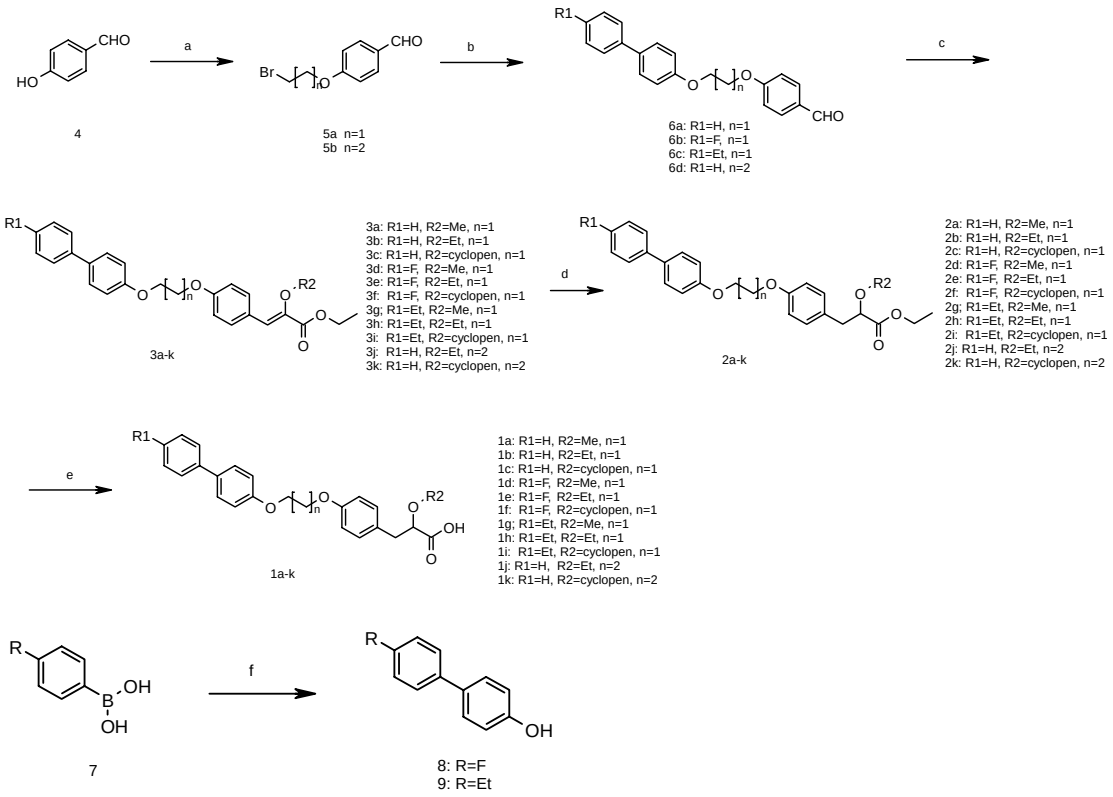
for PPARγ, and furthermore, it showed modest PPARα agonist activity in the cell-based assay.

In this paper, we describe our exploration around the hydrophobic tailpiece of the 2-alkoxydihydrocinnamate class, which has led to potent PPAR agonists in vitro.

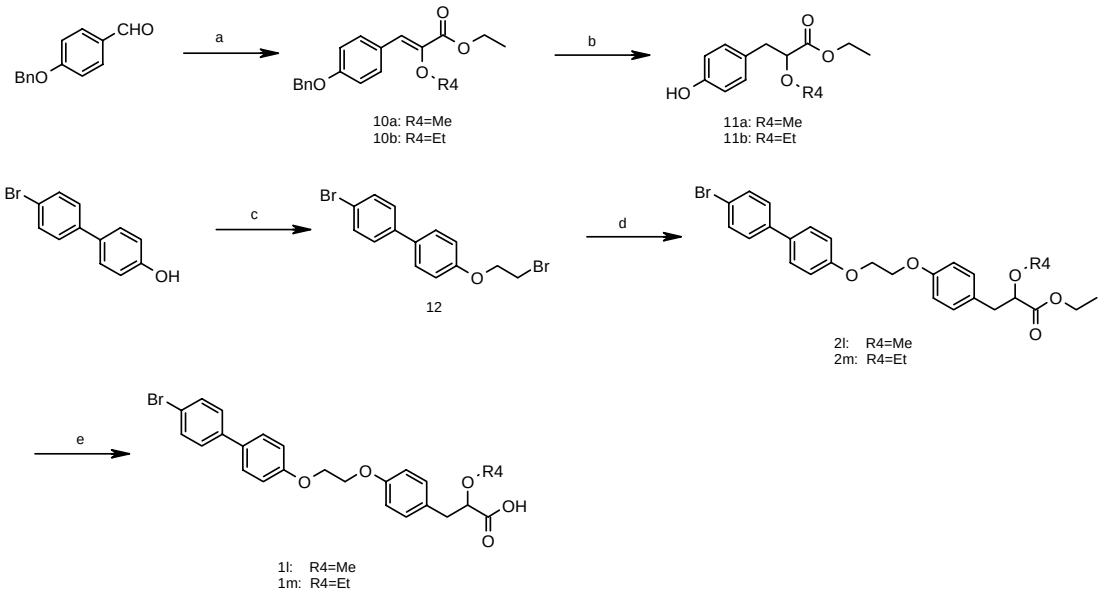
The compounds were synthesized as described in Schemes 1 and 2. The aldol condensation of *para*-substituted biphenol **6a**–**d** and ethyl alkoxyacetate derivatives gave the corresponding unsaturated acetic ester **3a**–**k**. Exhaustive hydrogenolysis gave the racemic headpiece **2a**–**k**. Subsequent hydrolysis afforded **1a**–**k** (Scheme 1).[9] Tailpiece **5a** and **b** were prepared using the phenol **4** and 1,2-dibromoethane or 1,3-dibromopropane. The coupling of 4-substituted phenylboronic acid **7** and 4-iodophenol gave the substituted biphenol **8** and **9**. Compounds **11a** and **b** were allowed to react with bromide **12** in the presence of potassium carbonate in DMF to give **2l** and **m**. Treatment of **2l** and **m** with aqueous NaOH gave **1lm** (Scheme 2).

In vitro transactivation assays: cDNAs for human PPARα and PPARγ were obtained by RTPCR from the human liver and adipose tissue, respectively. Amplified cDNAs were cloned into pcDNA3.1 expression vector and the inserts were confirmed by sequencing. U2OS cells were cultured in McCoy's 5A with 10% heat-inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. Cells were seeded in 96-well plates the day before transfection to give a confluence of 50–80% at transfection. A total of 60 ng of DNA containing 10 ng PPAR, 10 ng pCMV Gal, 10 ng of nuclear receptor expression vectors, and 30 ng of the corresponding reporters were co-transfected per well using FuGene6 transfection reagent according to the manufacturer's instructions. Following 24 h after transfection, cells were incubated with 10% charcoal-stripped FBS DMEM and were treated with the individual compound dissolved in DMSO. The final concentration of DMSO in culture medium was 0.1%. Cells were treated with compound for 24 h and then collected with cell culture lysis buffer. Luciferase activity was monitored using the luciferase assay kit according to the manufacturer's instructions. Light emission was read in a Labsystems Ascent Fluoroskan reader. To measure galactosidase activity to normalize the luciferase data, 50 μL of supernatant from each transfection lysate was transferred to a new microplate. Galactosidase assays were performed in the microwell plates using a kit from Promega and read in a microplate reader.

In our studies, we found that compound **1a** (Table 1), containing a *para*-biphenyloxy tailpiece, was a potent



Scheme 1. Reagents: (a) 1,2-dibromoethane or 1,3-dibromopropane, NaOH, H_2O, $Bu_4N^+Br^-$; (b) *para*-substituted biphenol, K_2CO_3, DMF; (c) ethyl 2-alkoxyacetate, *t*-BuOK/*t*-BuOH, DMF; (d) H_2, Pd/C, EtOH; (e) 1 mol/l NaOH, C_2H_5OH; (f) 4-iodophenol, K_2CO_3, Pd/C, H_2O.

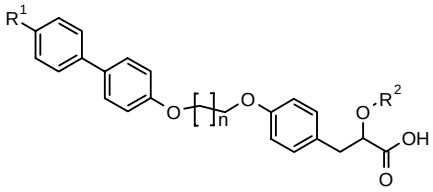


Scheme 2. Reagents: (a) ethyl 2-alkoxyacetate, *t*-BuOK/*t*-BuOH, DMF; (b) H_2, Pd/C, EtOH; (c) 1,2-dibromoethane, NaOH, H_2O, $Bu_4N^+Br^-$; (d) **11**, K_2CO_3, DMF; (e) 1 mol/l NaOH, C_2H_5OH.

PPARγ and PPARα dual agonist (EC_{50} PPARα/γ = 0.58 μM/0.89 μM, MAX % PPARα/γ = 46%/58%). Encouraged by this preliminary result, we synthesized a series of analogs of **1a** in order to optimize the activities for the two receptors. The results of the in vitro evaluation of the obtained compounds are summarized in Table 1.

Substitution of the methoxy group at C-2 position of the propanoic acid for an ethoxy (**1b**) gave a threefold decrease in both PPARγ and PPARα functional activity. Similar effects were observed when a methoxy group was changed to an ethoxy group (**1e** vs **1d**). The incorporation of a cyclopropentyloxy moiety (**1c** vs **1a** and **1f** vs **1d**) resulted in a great decrease

Table 1. Functional activity, in vitro data for compounds **1a**–**m**



Compound	R^1	R^2	*n*	PPARγ		PPARα	
				EC_{50} (μM)	% MAX[a]	EC_{50} (μM)	% MAX[a]
1a	H	Me	2	0.89	58	0.58	46
1b	H	Et	2	2.57	68	1.77	31
1c	H	Cyclopentyl	2	6.31	26	6.3	43
1d	F	Me	2	0.38	98	0.05	154
1e	F	Et	2	1.23	98	0.12	160
1f	F	Cyclopentyl	2	6.46	44	0.5	192
1g	Et	Me	2	0.14	132	0.1	131
1h	Et	Et	2	0.15	103	0.4	104
1i	Et	Cyclopentyl	2	2.24	87	1.12	83
1j	H	Et	3	1.58	96	5.88	52
1k	H	Cyclopentyl	3	3.8	65	3.8	45
1l	Br	Me	2	1.69	104	3.24	42
1m	Br	Et	2	0.76	82	2.19	28
Rosiglitazone[c]				0.03	100	ND[b]	ND
WY-14643[c]				ND[b]	ND	20	100

[a] The relative maximum efficacy to the percentage of the standards.
[b] No detection.
[c] The positive control samples were synthesized by Shenzhen Chipscreen Biosciences Ltd.

(100- to 200-folds) of both PPARγ and PPARα activity, indicating that the bulky substituent conveys unfavorable effect on the activities. Surprisingly, while the hydrophobic group was *para*-bromine substituted biphenyl moiety, substitution of the methoxy group for an ethoxy gave a twofold increase in PPARγ activity (**1l** vs **1m**). The variation of the biphenyl moiety by introducing a substituent at the 4-position produced a notable improvement in the in vitro potency (**1d**,**g** vs **1a** and **1e**,**h** vs **1b**). Introduction of a bromine atom gave a slight increase in PPARγ functional activity (**1l** vs **1a** and **1m** vs **1b**).

The linker connecting the biphenyloxy and the central ring of the molecule was also examined. Variation of the length of the spacer could alter its in vitro functional activity profile without regularity. The compounds with three carbon spacer seem to be favorable for agonism of PPARγ over those with two carbon atoms. For example, compounds **1j** and **k** exhibited a slightly increased (twofold) PPARγ functional activity as compared to **1b** and **c**; however, the PPARα activity of compounds **1j** and **k** was maintained in the in vitro assay.

The series of 2-alkoxydihydrocinnamate derivatives described in this paper were proved to be potent PPARγ and PPARα dual agonists. The substituted biphenyl tailpiece was proved to be an effective scaffold. This work led to the discovery of compound **1g** exhibiting activity comparable to that of rosiglitazone in cellular assay.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.10.104.

References and notes

1. Cobb, J. E.; Blanchard, S. G.; Boswell, E.; John, M. N.; Allan, S. W. *Annu. Rep. Med. Chem.* **2000**, *35*, 211.
2. Shinkai, H. *Exp. Opin. Ther. Pat.* **2000**, *10*, 59.
3. Reaven, G. M. *Exp. Clin. Endocrinol. Diabetes* **2000**, *108*, S274.
4. (a) Issemann, I.; Green, S. *Nature* **1990**, *347*, 645; (b) Mangelsdorf, D. J.; Evans, R. M. *Cell* **1995**, *83*, 841; (c) Willson, T. M.; Brown, P. J.; Sterbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
5. Bogacka, I.; Xie, H.; Bray, G. A.; Smith, S. R. *Diabetes Care* **2004**, *27*, 1660.
6. Shinya, U.; Takayoshi, S.; Yoshifumi, H.; Kazuma, E.; Hiroki, F.; Makoto, N.; Masayoshi, I.; Hidehiko, N.; Kohfuku, K.; Naoki, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1547.

7. Cronet, P.; Petersen, J. F. W.; Folmer, R.; Blomberg, N.; Sjoblom, K.; Karlsson, U.; Lindstedt, E. L. *Structure* **2001**, *9*, 699.
8. Ewing, T. J. A.; Makino, S.; Skillman, A. G.; Kuntz, I. D. *J .Comput. Aided Mol. Des.* **2001**, *15*, 411.
9. General procedure: *t*-BuOK in *i*-butanol (1 mol/l) 5 ml was slowly dropped to a solution of 4-[2-(biphenyl-4-yloxy)-ethoxy]-benzaldehyde (5 mmol) and methoxy-acetic acid ethyl ester (5 mmol) in 20 ml dried DMF at 0 °C. The reaction mixture was allowed to reach room temperature. While the reaction was completed, the mixture was poured into water, acidified with hydrochloric acid, and extracted with ethyl ester and the organic phase was dried with sodium sulfate and evaporated in vacuo. The residue was purified by chromatography on silica gel using EA/PE (1/6) as eluents to give 0.505 g (yield 24.2%) of **2a**. Sodium hydroxide hydrate (1 mol/l) 5 ml was added to a solution of 2a (224 mg) in 30 ml ethanol, then the reaction mixture was refluxed for 3 h, evaporated and acidified with diluted hydrochloric acid to pH = 1, and filtered. The crude product was crystallized to give 135 mg (yield 64.6%) of **1a** as white crystals.