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Design, synthesis, and evaluation of novel aryl-tetrahydropyridine PPARα/γ dual agonists

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ABSTRACT

Aryl-tetrahydropyridine derivatives were prepared and their PPARα/γ dual agonistic activities were evaluated. Among them, compound **(S)-5b** was identified as a potent PPARα/γ dual agonist with an EC_{50} of 1.73 and 0.64 μM in hPPARα and γ, respectively. In diabetic (db/db) mice, compound **(S)-5b** showed good glucose lowering efficacy and favorable pharmacokinetic properties.

Type II diabetes mellitus accounts for 90–95% of all cases of diabetes and its worldwide frequency is expected to increase by 6% per annum, potentially reaching a total of 200–300 million cases in 2010.[1] Currently, the treatments for type II diabetes rely mainly on a variety of approaches intended to reduce the hyperglycemia itself. However, these therapies have significant mechanism-based side effects, such as weight gain and atherosclerotic cardiovascular disease, as well as limited efficacy and tolerability. Therefore, therapeutic approaches that not only lower the glucose level, but also specifically address the diabetic dyslipidemia and atherosclerotic cardiovascular disease complications are needed.[1] Among the many targets that have been investigated as possible cures for type 2 diabetes, peroxisome proliferators-activated receptors (PPARs) are known to be good targets for lowering the plasma glucose level and improving metabolic syndrome.[2] The PPARs are members of the nuclear receptor superfamily of ligand-modulated transcription factors that play a key role in regulating the storage and catabolism of dietary fat. There are three PPAR subtypes, which are the products of distinct genes and are commonly designated PPARα, PPARγ, and PPARδ.[3] PPARα agonists, such as fenofibrate, have been reported to decrease the serum triglycerides levels and increase the HDL cholesterol concentration.[4] PPARγ agonists have been studied most intensively for their ability to enhance insulin sensitivity, glucose homeostasis, and fat storage. There are some PPARγ agonists currently being marketed as antidiabetic agents, for example, rosiglitazone (Fig. 1). Although these agents have a glucose lowering effect and an improved lipid profile, for example, TG, LDL cholesterol, and HDL cholesterol, crucial side effects,[5] including hypertension, inflammation, congestive heart failure, edema, fluid retention, and weight gain, still remain unsolved.[3] Designing compounds with both PPARα and PPARγ activity may offer improved alternatives toward the control of hyperglycemia and hypertriglyceridemia in type II diabetic patients.[6] Therefore, a large number of PPARα/γ dual agonists, such as muraglitazar and tesaglitazar (Fig. 1), have been investigated by many research groups. Even though the development of these successful PPARα/γ dual agonists has been discontinued, PPARα/γ still would be a suitable target for the treatment of type II diabetes, which provided reduced side effects.

In order to find novel PPARα/γ dual agonists, the hydrophobic tailpiece was designed based on the receptor–ligand docking structure and was then prepared. First, we attempted to change the hydrophobic tailpiece of tesaglitazar (Fig. 1) into biaryl or any other Ring1–Ring2 type moieties to obtain improved efficacy and property. As a result, in order to improve the activity, it might be necessary that the direct covalent-bonded carbons between Ring1 and Ring2 have sp2 character. Interestingly, the introduction of aryl-tetrahydropyridine moieties at the hydrophobic tailpiece showed potential as an efficacious PPAR agonist. Therefore, a

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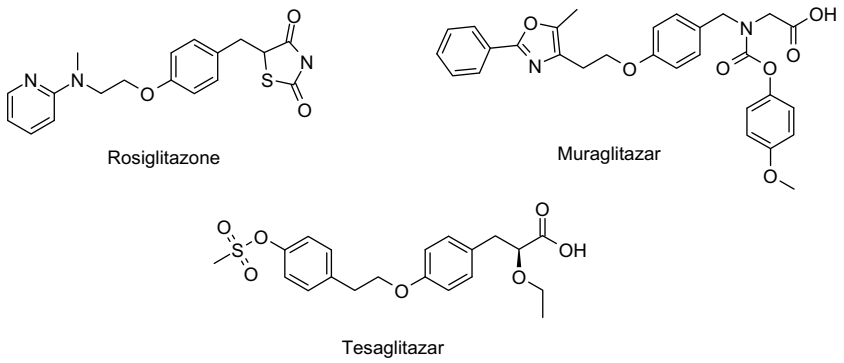
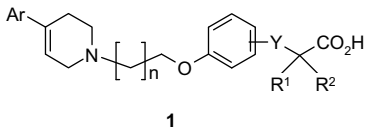


Figure 1. Structure of PPAR agonists.

structurally novel series of aryl-tetrahydropyridine PPARα/γ dual agonist **1** was studied. This letter reports the synthesis, structure–activity relationships (SARs), and biological evaluation of these new antidiabetic agents (**1**, Fig. 2).

Scheme 1 describes the synthesis of the aryl-tetrahydropyridines. The key racemic intermediate **2** was synthesized from the commercially available triethyl phosphonoacetate.[7]

The diazo transformation of phosphonoacetate and subsequent coupling with ethanol in the presence of rhodium acetate provided ethoxyphosphono-acetate. The Wadsworth–Emmons reaction of ethoxyphosphono-acetate with 4-benzyloxybenzaldehyde,[8] followed by hydrogenation of the resulting olefin, provided racemic compound **2**. Alkylation of phenol **2** with ethylene carbonate ($n = 1$) or 3-bromo-1-propanol ($n = 2$) and the following reaction with methanesulfonyl chloride afforded mesylate **3**.[9] N-Alkylation of the aryl-tetrahydropyridine moieties **4** with mesylate **3** was carried out by heating with K_2CO_3 in DMF to furnish the esters in good yield. The esters were hydrolyzed with lithium hydroxide in aqueous THF to give the acids **5**. The optically active aryl-tetrahydropyridine analogs **(S)-5** were prepared using the commercially available 2-(*S*)-ethoxy-3-(4-hydroxy-phenyl)-propanoic acid ethyl ester instead of the racemic 2-ethoxy-3-(4-hydroxy-phenyl)-propanoic acid ethyl ester **2** through the same procedure in Scheme 1.



1

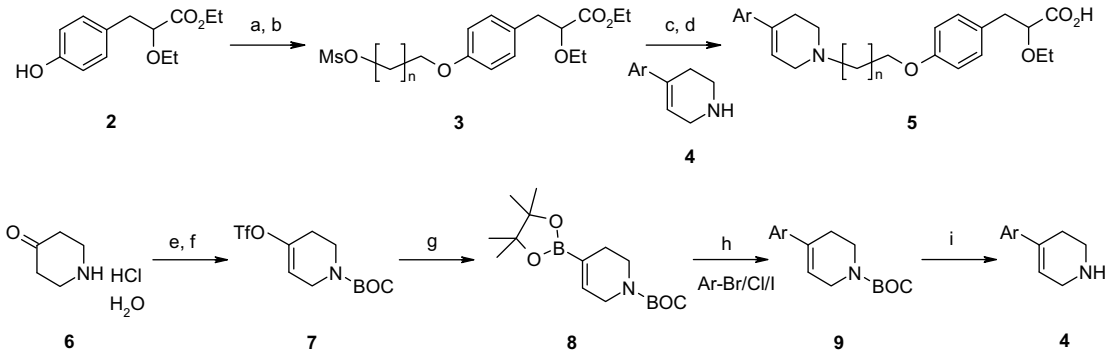
Ar = Ph, heteroAr
n = 1, 2
Y = CH_2, O
R^1, R^2 = H, OEt, Me, Et, OPh, respectively

Figure 2. Design concept of aryl-tetrahydropyridine.

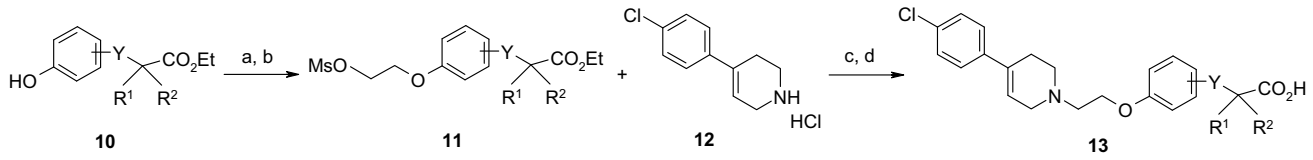
Scheme 1 also shows the synthetic route to aryl-tetrahydropyridine moieties **4**. Tetrahydropyridine triflate **7** was conveniently prepared by the BOC-protection of commercial 4-piperidone **6** to *N*-BOC-4-piperidone, followed by deprotonation of the *N*-BOC-4-piperidone and quenching the resulting enolate with *N*-phenyltrifluoromethanesulfonimide.[10] The desired boronate **8** was synthesized in high yield from the triflate **7** via palladium-mediated cross-coupling with bis(pinacolato)diborone. The aryl-tetrahydropyridine **9** was prepared by Suzuki coupling of the corresponding intermediate **8** with a variety of aryl halides. The desired aryl-tetrahydropyridine moieties **4** ready for further functionalization on nitrogen were easily prepared using the standard deprotection method.[11]

In an effort to examine the effects of substitution on the acid part, the acid **11** could also be prepared using various acid parts **3** by the procedure shown in Scheme 2 similar to that in Scheme 1.

The final compounds were tested for PPAR receptor transactivation (EC_{50}) activities (Tables 1 and 2).[12] The synthesis of compound **5b** was simply initiated using commercially available 4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine·HCl, and it was found to be a potent PPARα and γ agonists. The results were so encouraging that a decision was made to examine the effects of substitution on the tailpiece tetrahydropyridine aryl group of compound **5** (Table 1). As shown in Table 1, there were some interesting trends in the data. First, the introduction of a functional group on the phenyl ring adjacent to the tetrahydropyridine was generally effective for PPARα/γ activity in the order of *para* > *meta* > *ortho* (**5b**–**5l**).

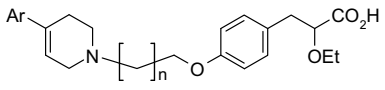


Scheme 1. Reagents and conditions: (a) ethylene carbonate ($n = 1$), 3-bromo-1-propanol ($n = 2$), K_2CO_3, DMF, 80 °C, 80% ($n = 1$), 50% ($n = 2$); (b) MsCl, Et_3N, CH_2Cl_2, −15 °C to 0 °C, 85%; (c) K_2CO_3, NaI, DMF, 60 °C; (d) LiOH·H_2O, THF, rt; (e) $(BOC)_2O$, $NaHCO_3$, H_2O/CH_3CN, rt, 62%; (f) LiHMDS, Tf_2NPh, THF, −78 °C to rt, 34%; (g) bis(pinacolato)diborone, $PdCl_2$(dppf), KOAc, 1,4-dioxane, 80 °C, 80%; (h) Na_2CO_3/H_2O, $PdCl_2$(dppf), DMF, 80 °C; (i) TFA, CH_2Cl_2, 0 °C to rt.



Scheme 2. Reagents and conditions: (a) ethylene carbonate, K_2CO_3, DMF, 80 °C; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C; (c) K_2CO_3, NaI, DMF, 60 °C; (d) LiOH·H_2O, EtOH, rt.

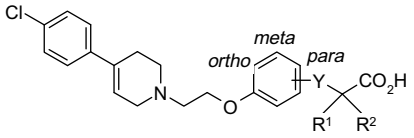
Table 1
In vitro activity of the synthesized compounds **5**[a]



Compound	Ar	*n*	EC_{50}[b] (μM) of hPPARα	EC_{50} (μM) of hPPARγ
Rosiglitazone			3.46	0.033
Muraglitazar			4.11	0.153
Tesaglitazar			3.12	0.704
5a	Ph	1	5.98	2.15
5b	4-Cl-Ph	1	7.07	2.06
(S)-5b	4-Cl-Ph	1	1.73	0.639
5c	3-Cl-Ph	1	3.24	1.67
5d	2-Cl-Ph	1	7.72	5.83
5f	4-CF_3-Ph	1	3.40	1.89
5g	3-CF_3-Ph	1	3.12	2.23
5h	2-CF_3-Ph	1	0.542	7.56
5i	4-F-Ph	1	2.23	3.72
5j	4-MeO-Ph	1	11.3	0.396
5k	3-MeO-Ph	1	17.3	2.38
5l	2-MeO-Ph	1	18.1	3.93
5m	4-Et-Ph	1	7.58	0.689
5n	4-*n*-Pr-Ph	1	2.08	0.174
5o	2,3-DiCl-Ph	1	0.064	0.661
5p	2,4-DiCl-Ph	1	1.12	0.706
5q	2,5-DiCl-Ph	1	1.12	1.44
5r	3,4-DiCl-Ph	1	1.15	0.670
(S)-5r	3,4-DiCl-Ph	1	0.615	0.862
5s	3,5-DiCl-Ph	1	2.99	1.42
5t	2-Pyridine	1	10.2	2.30
5u	2-Thiophene	1	8.58	3.30
5v	2-Pyrazine	1	3.67	12.6
5w	2-Pyrimidine	1	7.67	4.60
5x	4-Cl-Ph	2	9.80	2.32
5y	4-CF_3-Ph	2	9.79	0.504
5z	3,4-DiCl-Ph	2	1.70	0.728

[a] Agonistic activities in cell-based GAL4-PPARα/γ transactivation assay.
[b] Concentration at which a given compound shows 50% of the intrinsic maximal response.

Table 2
In vitro activity of the synthesized compounds **13**[a]



Compound	Position	Y	R^1	R^2	EC_{50}[b] (μM) of hPPARα	EC_{50} (μM) of hPPARγ
13a	*para*	CH_2	Et	H	3.86	5.29
13b	*para*	CH_2	OPh	Me	25.48	4.88
13c	*para*	O	Me	Me	0.868	22.83
13d	*meta*	O	Me	Me	0.331	18.76

[a] Agonist activities in cell-based GAL4-PPARα/γ transactivation assay.
[b] Concentration at which a given compound shows 50% of the intrinsic maximal response.

The introduction of an electron-donating group such as OMe, Et, and *n*-Pr (**5j**, **5m**, **5n**) showed better PPARγ activity than that of electron-withdrawing groups such as Cl, CF_3, and F (**5b**, **5f**, **5i**). However, the OMe-substituted compounds anywhere on the phenyl ring (**5j**–**5l**) showed significantly reduced PPARα functional activity. Compounds **5o**–**5s** with a disubstituted phenyl ring showed more potent PPARα and γ agonist activity than the monosubstituted compounds **5b**–**5d**. In the case of all mono- and disubstituted compounds, compound **5o** showed the most potent activity for both PPARα and γ. Increasing the size of the alkyl substituents on the phenyl ring improves the PPARα/γ activity (**5a, 5m, 5n**). Compound **5n**, bearing a 4-*n*-propylphenyl-tetrahydropyridine, was approximately 3- and 4-times more active than compound **5m** with the corresponding 4-ethylphenyl-tetrahydropyridine for PPARα and PPARγ, respectively. Replacement of the phenyl ring with an alternative hetero-aromatic ring generally decreased the activity, as shown in the activities of **5t**–**5w**.

Importantly, the agonistic activity of the aryl-tetrahydropyridine analogs changed sensitively according to the stereochemistry at the α-alkoxy-β-phenyl propanoic acid headpiece. It is generally known that the PPARα/γ agonistic activity depends on the stereochemistry of the α-substituted-β-phenyl propanoic acid headpiece, and the (*S*)-form is more potent than the (*R*)-antipode,[13–15] so we try to make the (*S*)-isomers of compounds **5**. The (*S*)-isomer of compound **5b** showed more potent PPARα and γ agonistic activity than the racemate **5b**. The (*S*)-isomer, **(S)-5r,** showed slightly more potent PPARα agonist activity than the racemate **5r**, while both exhibited the similar activity for PPARγ.[16] Extension of the linker between the aryl-tetrahydropyridine moiety and the α-ethoxy-β-phenyl propanoic acid adversely affected the potency of the PPARα more than PPARγ, but generally left the PPARγ activity unchanged (**5x**–**5z**).

A variation at the carboxylic acid headpiece influenced the PPAR activity of these ligands. Compounds **13a**–**13d** showed that both the PPAR agonistic activity and α/γ selectivity were sensitive to the type of acid headpiece. Replacement of the α-OEt/H (R^1/R^2) group in **5b** by α-Et/H[13] or α-OPh/Me[17] resulted in compounds **13a** and **13b**, which had a rather lower PPARγ agonist activity. On the other hand, compound **13b** had significantly reduced

Table 3
Pharmacokinetic data[a] of **(S)-5b**, rosiglitazone, muraglitazar in SD rats[b]

Parameters	**(S)-5b**	Rosiglitazone	Muraglitazar
Single intravenous			
$T_{1/2}$ (h)	23	1.05	14.0
$AUC_{0-24\ h}$ (μM h)	272.0	53.7	101
CL (mL/min/kg)	0.23	2.99	0.73
Vdss (L/kg)	0.44	0.25	0.57
Single oral			
T_{max} (h)	0.5	0.25	0.58
C_{max} (μM)	15.9	44.3	25.8
$AUC_{0-24\ h}$ (μM h)	270.1	94.2	98.8
BA, solubility			
Solubility[c] (mg/mL)	3.263	0.014	0.041
BA (%)	>100	>100	96.2

[a] Dose (mg/kg) = 3.
[b] *n* = 3, male SD rats.
[c] Solubility at pH = 6.8, 37 °C.

Table 4
In vivo data of compound **(S)-5b** in db/db mice[a]

Compound	Reduction of plasma glucose[b] (%)					ΔBW[c] (g)					ED_{30}
	0.03[d]	0.1	0.3	1.0	3.0	0.03	0.1	0.3	1.0	3.0	(mg/kg)
Rosiglitazone	—	17	37	56	68	—	+10.5	+11.1	+12.5	+13.9	0.210
Muraglitazar	—	4	11	54	78	—	+10.5	+12.7	+14.5	+16.1	0.411
(S)-5b	14	37	63	77	—	+11.2	+12.4	+12.6	+13.7	—	0.067
Lean			—				+2.4				—
db/db			—				+9.0				—

[a] Male db/db mice (7-week-old) and lean mice were dosed daily for 28 days by oral with the indicated doses of test compound.
[b] Reduction of plasma glucose was calculated as the percentage reduction with respect to the non-fasting glucose value of control after 28 days of oral dosing.
[c] Body weight change in db/db mice after 28 days of oral dosing.
[d] Dose in mg/kg unit.

PPARα activity. With the 2-dimethyl-phenoxy propanoic acid headpiece,[18] compounds **13c** and **13d** showed remarkably increased PPARα activity and significantly reduced PPARγ activity compared with α-ethoxy-β-phenyl propanoic acid headpiece-based **5b**.

After determining the in vitro properties of the aryl-tetrahydropyridine compounds, compound **(S)-5b** was selected for the pharmacokinetic (PK) study in rats, and the in vivo experiment in male db/db mice for a further evaluation.[19] The PK parameters of **(S)-5b** in Sprague–Dawley (SD) rats were satisfactory, as shown in Table 3.

In the PK study, compound **(S)-5b** exhibited an apparently longer half-life than rosiglitazone and muraglitazar (Fig. 1), and had good water solubility (3.26 mg/mL, pH = 6.8), which may contribute to the excellent oral bioavailability (~100%). Because the aryl-tetrahydropyridine compound **(S)-5b** has both acid (–COOH) and amino (–NRR′) functional groups in the molecule, it has possible zwitterionic character under neutral conditions (pH = 7.0). As a result, **(S)-5b** showed good water solubility and stability over all pH region, which probably is the reason for the satisfactory PK profile.

Compound **(S)-5b** was evaluated in the db/db mouse model, using rosiglitazone and muraglitazar (Fig. 1) for comparison.[20] When the db/db mice were treated orally by 0.03, 0.1, 0.3, and 1.0 mg/kg for 28 days, **(S)-5b** showed dose-dependent decrease of blood glucose level (Table 4). Treatment with 1.0 mg/kg of **(S)-5b** showed a glucose reduction of 77% compared with that of 68% with 3.0 mg/kg of rosiglitazone. A glucose lowering effect was also observed at a dose of 0.1 mg/kg of **(S)-5b**. The ED_{30} of **(S)-5b** was determined to be 0.067 mg/kg, which is significantly lower than the ED_{30} of rosiglitazone (0.210 mg/kg) and muraglitazar (0.411 mg/kg). Consequently, compound **(S)-5b** displayed a better glucose lowering effect than rosiglitazone and muraglitazar based on the oral dose, whereas it also caused a similar increase in body weight to muraglitazar.

In summary, this letter reported the synthesis and in vitro characterization of a novel series of potent and selective PPARα/γ dual agonists. Compound **(S)-5b** showed an excellent in vivo profile in the db/db mouse model of type II diabetes, and also shows desirable pharmacokinetic properties. A further evaluation of compound **(S)-5b** is currently underway.

Acknowledgment

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12. In vitro transient transactivation assays: the ligand binding domains (LBD) of the human PPARα/γ receptors were fused to the DNA binding domain (DBD) of the yeast transcription factor GAL4. The CV-1 cells were transiently transfected with an expression vector for the respective PPAR chimera along with a reporter construct containing five copies of the GAL4DNA binding site and pRL-TK as a control vector (Promega, WI). The test compounds were dissolved in DMSO and diluted 1:1000 in DMEM supplemented with 10% fetal bovine serum (FBS) and 0.1 mM MEM non-essential amino acid (MEAA). The cells were treated with the test compounds in triplicate for 24 h and subjected to a dual luciferase assay using the Dual-Glo luciferase reagent (Promega, WI). The increases in luminescence were measured using a microplate reader (Lmax II^{384}, Molecular Devices, CA). EC_{50} values were calculated using the Hill 4-parametric equation in SigmaPlo t 4.0 (SPSS, IL).
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16. The difference values of the PPARα/γ agonistic activity between the racemate **5r** and the (*S*)-isomer, **(S)-5r** were not enough significative to conclude as the latter was a better one. Even though we do not describe in this letter, another (*S*)-isomers of compounds **5** showed a tendency to have a better activity than the comparative racemates.
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19. *In vivo study:* It was evaluated using two reference substances, rosiglitazone, which is marketed, and muraglitazar, which has a different acid headpiece from compound **(S)-5b**. Tesaglitazar was used not in in vivo study but in in vitro assay as a reference substance.
20. In vivo db/db mice study: male homozygous db/db mice were obtained from Japan SLC, Inc. (Shizuoka Prefecture, Japan). In order to evaluate the effect of the test compound, 7-week-old animals were acclimated for 1 week, and allocated to the corresponding groups (n = 7/group) according to the plasma glucose level and weight. The mice were treated once daily by an oral gavage with the test compound, muraglitazar, rosiglitazone, or an equivalent amount of 0.5% MC for 28 days. The decreases in the plasma glucose levels were calculated as the percentage change from those of the controls.