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(*S*)-3-(4-(2-(5-Methyl-2-phenyloxazol-4-yl)ethoxy)phenyl)-2-(piperazin-1-yl)-propanoic acid compounds: Synthesis and biological evaluation of dual PPARα/γ agonists

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ABSTRACT

A series of novel, potent PPARα/γ dual agonists were synthesized and appraised. The most potent analogue, compound 2b demonstrated EC_{50} value of 0.012 ± 0.002 and 0.032 ± 0.01 μM, respectively, for hPPARα and hPPARγ in transactivation assay. Additionally, compound **2b** demonstrated good glucose and lipid lowering effect in genetic diabetic (*db*/*db*) mice.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, belonging to the nuclear receptor gene family.[1,2] Three closely related isoforms, PPARα, γ, and δ, have been identified. PPARα agonists, for example, fenofibrate, primarily decrease serum triglyceride levels and increase HDL cholesterol (HDLc) levels.[3] Furthermore, fibrates had also been reported to reduce weight gain in rodents without effects on food intake.[4] PPARγ agonists, for example, the thiazolidinedione (TZD) class of rosiglitazone (Fig. 1), have been used for the treatment of type 2 diabetes (T 2DM). However, recently TZDs were observed to produce side effects of weight gain, fluid retention, and chronic heart failure, etc. It also had been proved that the side effect of weight gain is associated with both adipogenesis and fluid retention. And combination therapy with fibrate was shown to be able to antagonize both of them.[5] Therefore, our aim was to design and synthesize a series of novel dual PPARα/γ agonists which could combine the beneficial effects seen with insulin sensitizers and fibrates, yet without the unwanted effects mentioned above.

The TZDs contain an asymmetric center, of which only the (*S*)-enantiomers binding to the receptor with high affinity, but they have been developed as racemates because they undergo racemization in vivo.[6] To solve this problem many novel compounds that are less prone to racemization have been developed, such as L-tyrosine derivatives represented by PPARα/γ agonist GW409544 (Fig. 1) and PPARγ agonist farglitazar (Fig. 1). These two series are chiral compounds containing *S*-configuration, which are conveniently synthesized from naturally-occurring L-tyrosine. The X-ray crystal structures of GW409544 binding to both PPARγ and PPARα show that the tyrosine–NH and vinylogous amide C=O form an intramolecular hydrogen bond, which are equival to generate a six-membered ring, and then the group was inserted into a lipophilic pocket.[7] So we designed, synthesized a series of 2-(piperazin-1-yl)propanoic acid derivatives containing piperazine-ring (**1a**–**1e**, **2a**–**2c**) (Fig. 1).

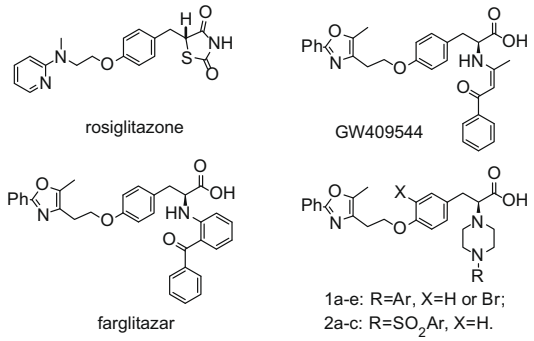

Figure 1. Structure of PPAR agonists.

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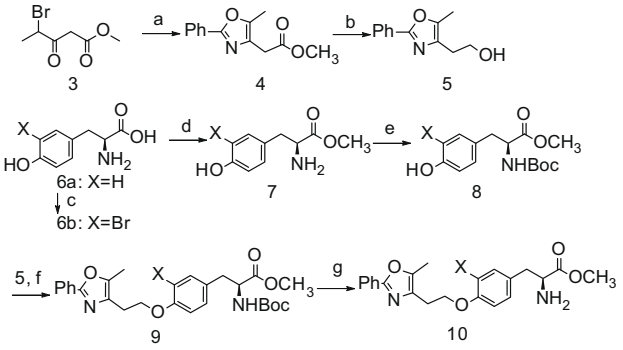
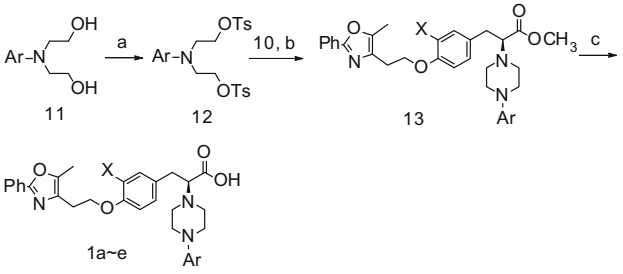
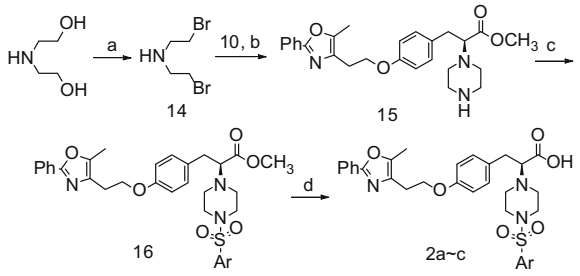


Scheme 1. Reagents and conditions: (a) $PhCONH_2$, $PhCH_3$, reflx, 22%; (b) LAH, Et_2O, rt, 87%; (c) Br_2, HAc, 0–25 °C; (d) $SOCl_2$, MeOH, reflx; (e) BOC_2O, Et_3N, CH_3CN, rt, 19% (three steps); (f) PPh_3, DIAD, PCH_3, rt, 70%; (g) CF_3COOH, CH_2Cl_2, 70%.



Scheme 2. Reagents and conditions: (a) TsCl, Et_3N, CH_2Cl_2, 0 °C, 35%; (b) HMPA, $NaHCO_3$, 140 °C; (c) LiOH, THF–H_2O, rt, 22–38%(two steps).

The synthesis of compounds **1a–e** and **2a–c** is depicted in Scheme 1–3. The oxazole alcohol for **5** (Scheme 1) was synthesized following exposure of benzamide to methyl 4-bromo-3-oxopentanoate1 **3**[8] in hot toluene followed by ester reduction with LAH in THF. The intermediates **8a,b** were prepared from compounds **6a,b**, respectively, followed by amidation with BOC_2O. Mitsunobu reaction between the intermediates **8** and **5** using triphenylphosphine and diisopropylazodi-carboxylate in toluene was followed by removal of the BOC protecting group with CF_3COOH in dichloromethane to afford the amine **10**.

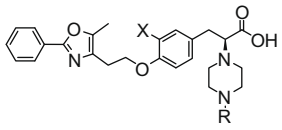


Scheme 3. Reagents and conditions: (a) 48% HBr, reflx, 100%; (b) Na_2CO_3, EtOH, reflx, 34%; (c) $ArSO_2Cl$, Et_3N, rt; (d) LiOH, THF–H_2O, rt, 23–35% (two steps).

Intermediate **10** is derivatized on the tyrosine nitrogen to provide the targeted set of analogues following saponification with LiOH (Schemes 2 and 3). Reaction of the amine **10** with **12** synthesized from **11** with $NaHCO_3$ provided the 4-phenyl-piperazine compound **13**. Saponification with aqueous LiOH in THF provided the target compounds **1a–e** (Scheme 2). Reaction of the amine **10** with **14** synthesized from 2-(2-hydroxy-ethylamino)-ethanol in the presence of Na_2CO_3 provided the **15**, followed by amidation with a variety of arylsulfonyl chloride affords **16**. Saponification with aqueous LiOH in THF provided the target compounds **2a–c** (Scheme 3).

All compounds were screened for the agonist activity on hPPAR-GAL4 chimeric receptors in transiently transfected HEK-293 cells (human embryonic kidney cells). The results are summarized in Table 1. All tested compounds have no agonistic activity for hPPAR δ. Generally, compounds **2a–c** which are substituted by sulfonyl-piperazine exhibited higher agonistic activity than compounds **1a–e** which are substituted by phenylpiperazine in both PPARα and PPARγ transactivation assays. For compounds **1a–e**, compounds replaced by the bromine group, such as **1e** and **1b**, showed significantly lower activity. Compound **1a** substituted by methyl group at the *m*-position of phenyl ring showed better PPAR subtype selectivity than other compounds. For sulfonylpiperazine substituted compounds **2a–c**, 2b containing the fluoride as the substituent at the *o*-position of phenyl ring showed better PPAR subtype selectivity than **2c** containing the fluoride at the *p*-position. According to the result, compound **2b** (α, EC_{50} = 0.012 μM; γ, EC_{50} = 0.032 μM) was the most potent compound, which pos-

Table 1
In vitro PPAR transactivation activity of compounds **1a–e**, **2a–c** and rosiglitazone



Compound	Structure		TA EC_{50}[a] (μM)		
	R	X	hPPARα	hPPARγ	hPPARδ
1a	*m*-Tolyl	H	8.98 ± 1.14	1.14 ± 0.65	NO[b]
1b	Phenyl	H	11.13 ± 5.72	10.56 ± 2.18	NT
1c	*p*-Tolyl	H	>100	50.22 ± 4.12	NT[c]
1d	3-Chlorophenyl	H	>100	45.23 ± 3.61	NT
1e	Phenyl	Br	>100	>100	NT
2a	2-Nitrophenylsulfonyl	H	1.45 ± 0.28	2.16 ± 1.04	NO
2b	2-Fluorophenylsulfonyl	H	0.012 ± 0.002	0.032 ± 0.01	NO
2c	4-Fluorophenylsulfonyl	H	1.35 ± 0.04	2.81 ± 0.25	NO
Rosiglitazone	—	—	10.58 ± 1.36	0.035 ± 0.003	NO
GW409544	—	—	0.01 ± 0.003	0.0027 ± 0.0005	NT

[a] TA (transactivation assay).
[b] NO, no measurable activity against specified receptor when compound was studied at concentration of 30 μM (n = 3).
[c] NT = not tested.

Table 2
Cytotoxic assay results for some compounds

Compound	IC_{50}[a] (μM)
1c	45.0 ± 2.18
1d	44.4 ± 2.37
1e	66.3 ± 2.95
2a	134.6 ± 7.25
2b	260.7 ± 6.17
2c	260.7 ± 5.23
Rosiglitazone	156.6 ± 5.93
GW409544	108.6 ± 3.17

[a] Values are means of three independent experiments.

Table 3
Effect of **2b** on serum insulin and leptin in *db/db* mice[9]

Group	Dose (mg/kg)	Insulin (ng/ml)	Leptin (ng/ml)
Model	—	0.41 ± 0.06	10.55 ± 0.60
Rosiglitazone	10	0.28 ± 0.07*	7.15 ± 1.87*
2b	1	0.44 ± 0.13	11.77 ± 0.94
2b	2.5	0.28 ± 0.11*	8.11 ± 3.52
2b	10	0.35 ± 0.05	4.87 ± 2.57**

$n = 8$.
* $P < 0.05$.
** $P < 0.01$ versus control group.

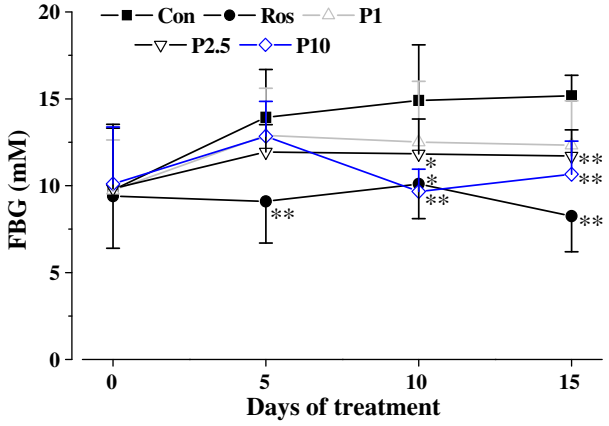


Figure 2. Effect of **2b** on blood glucose. Sequential monitoring of blood glucose in *db/db* mice were performed after overnight fasting. Con, control; Ros, rosiglitazone; P1, **2b** 1 mg kg-1; P2.5, **2b** 2.5 mg kg^{-1}; P10, **2b** 10 mg kg^{-1}.

sessed similar PPARγ agonist activity and better PPARα agonist activity compared with listed rosiglitazone, although exhibiting an order of magnitude lower PPARγ agonist activity compared with GW409544.

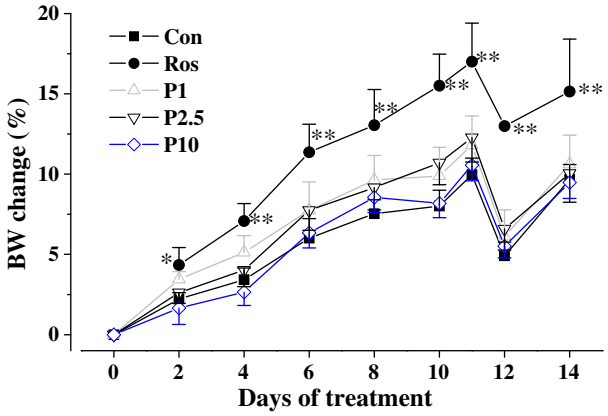


Figure 3. Effect of **2b** on body weight in *db/db* mice. Net body weight change in *db/db* mice were calculated for individual and then averaged. Con, control; Ros, rosiglitazone; P1, **2b** 1 mg kg^{-1}; P2.5, **2b** 2.5 mg kg^{-1}; P10, **2b** 10 mg kg^{-1};.Values are mean ± s.d. $n = 8$; *$P < 0.05$, **$P < 0.01$ versus control group.

The inhibitions of HLF cells (human fibroblast cells) proliferation by compounds were assessed by MTT assay, and results were showed in Table 2. IC_{50} values for **2a**–2c were 134.6, 260.7, and 260.7 μM, respectively, comparable to that of rosiglitazone (156.6 μM) and GW409544 (108.6 μM), and which was probably correlated with the activities of the compounds, indicating that compounds **2** substituted by sulfonylpiperazine are low cytotoxic and excellent PPARα/γ dual agonists. Among them, compound **2b** is one of the most potential.

Compound **2b** (P633H) was chosen for further in vivo evaluation in the *db/db* mice, rosiglitazone were used for comparison. Compound **2b was** administered at 1–10 mg/kg daily by oral gavage for two weeks. The effects on glucose, insulin, Leptin, triglyceride, free fatty acids, and total cholesterol were determined at the end of the treatment (Tables 3 and 4).

After 14 days treatment, compound **2b** (2.5 and 10 mg kg^{-1} group) demonstrated glucose lowering effects, comparable to that of rosiglitazone (Fig. 2). Moreover, **2b** dose dependently decreased hyperleptinemia (Table 3), and is more effective than rosiglitazone at the same dose, which may contribute to the relievement of insulin resistance.[9]

Furthermore, compound **2b** produced a significant reduction on serum TG ($P < 0.01$ for 2.5 mg kg^{-1} group and $P < 0.05$ for 10 mg kg^{-1} group), TCHO ($P < 0.05$ for 2.5 and 10 mg kg^{-1} group), TCHO/HDLc ($P < 0.05$ for 2.5 mg kg^{-1} group), and FFA ($P < 0.05$ for 2.5 and 10 mg kg^{-1} group) (Table 4). However, rosiglitazone only significantly reduced the serum TG and FFA.[9]

All doses of compound **2b did not** show significantly impact to the body weight gain of *db/db* mice in the 14 days of treatment, as that observed in the rosiglitazone treatment group (Fig. 3).[9]

Table 4
Effects of **2b** on physical and metabolic parameters in *db/db* mice

		Control	Rosiglitazone	Compound **2b**		
				1 mg/kg	2.5 mg/kg	10 mg/kg
TG	$mMol^{-1}$	1.03 ± 0.29	0.59 ± 0.14**	1.04 ± 0.08	0.60 ± 0.23**	0.78 ± 0.09*
TCHO	$mMol^{-1}$	5.56 ± 0.60	4.45 ± 0.89	5.37 ± 0.43	4.89 ± 0.22*	4.69 ± 0.36*
HDL	$mMol^{-1}$	1.99 ± 0.13	1.46 ± 0.45	1.90 ± 0.20	2.05 ± 0.12	1.88 ± 0.19
TCHO/HDL		2.70 ± 0.11	2.90 ± 0.21	2.82 ± 0.10	2.50 ± 0.17*	2.66 ± 0.16
FFA	$mMol^{-1}$	2.17 ± 0.23	1.53 ± 0.15**	2.21 ± 0.30	1.71 ± 0.37*	1.87 ± 0.19*

Compounds were orally administered to mice once a day for at respective doses. Data are mean ± s.d. TG, triglyceride; TCHO, total cholesterol. $n = 8$.
* $P < 0.05$.
** $P < 0.01$ versus control group.

In conclusion, a series of novel 2-(piperazin-1-yl)propanoic acid derivatives were designed, synthesized and appraised. Compound **2b**, identified as a low cytotoxic and excellent PPARα/γ dual agonist, displayed comparable glucose lowering effect and better lipid lowering result than rosiglitazone in the *db/db* mouse model of type II diabetes, meanwhile it didn't cause similar side effects as that of rosiglitazone, such as increase in body weight and subcutaneous fat. Further investigations of compound **2b** are currently underway.

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