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Development of a new class of benzoylpyrrole-based PPARα/γ activators

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ABSTRACT

Starting with a subtle blood glucose-lowering effect of a TGF-β inhibitor, we designed and synthesized a series of benzoylpyrrole-based carboxylic acids as PPARs activators. Among these compounds, **10sNa** exhibited favorable blood glucose-lowering effect without body weight gain. We assume that the beneficial effect of **10sNa** is attributed to not only its compound PPARα agonistic activity but also its PPARγ partial agonistic activity.

Type 2 diabetes is a metabolic disorder that affects approximately 150 million people worldwide with projections of 300 million people by the year 2025.[1] Current drugs used for the treatment of type 2 diabetes are selected from biguanides, sulfonylureas, insulin formulations, glinides, α-glucosidase inhibitors,[2] dipeptidyl peptidase IV inhibitors,[3] Glucagon-like peptide (GLP)-1 analogs[4] and peroxisome proliferator activated receptor (PPAR)γ agonists. Among these classes, PPARγ agonists (Fig. 1), such as rosiglitazone

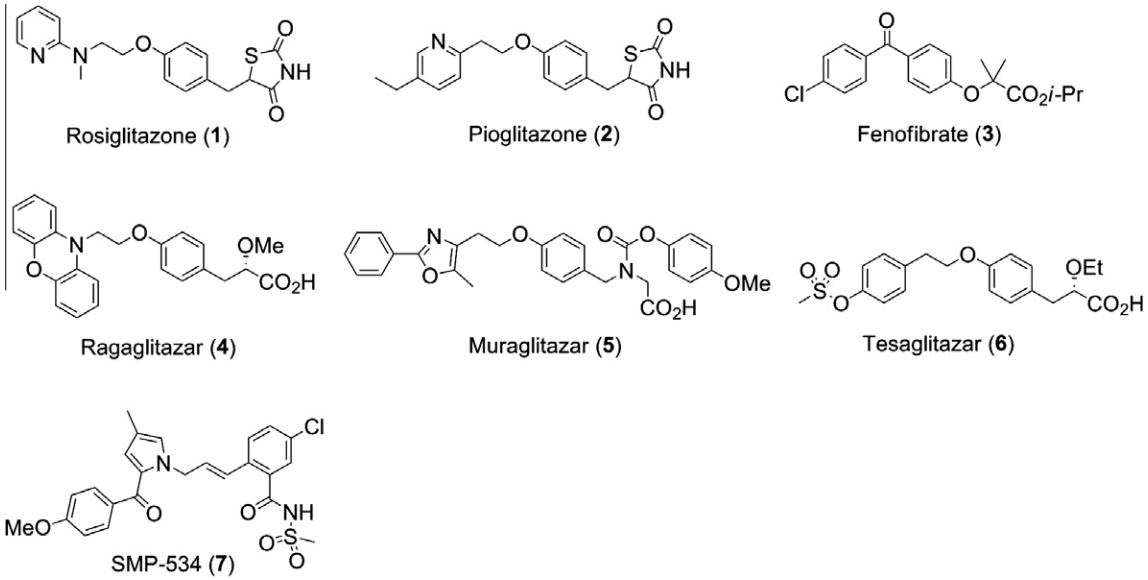

Figure 1. Chemical structures of selected PPAR agonists and SMP-534 **(7)**.

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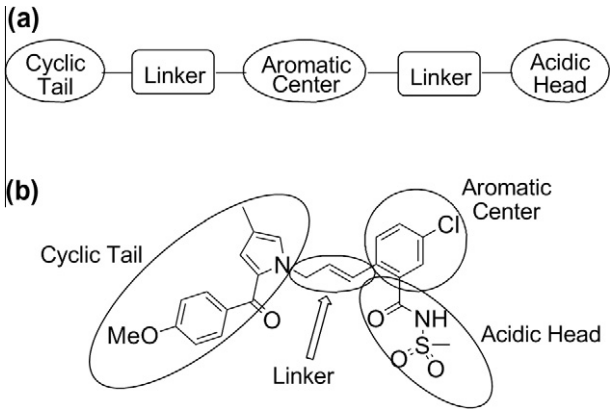
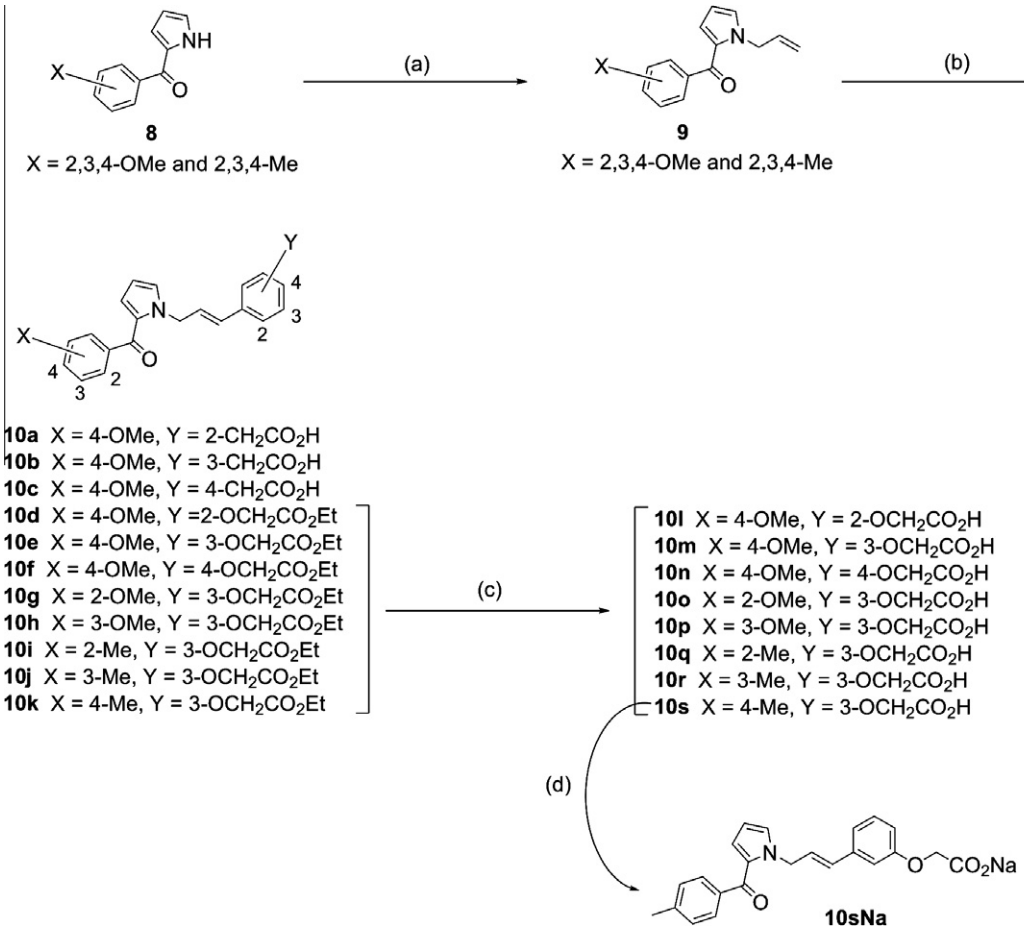


Figure 2. (a) Simplified topology of typical synthetic PPAR agonists (b) Schematic representation of SMP-534 **(7).**

(**1**) and pioglitazone (**2**), which belong to the thiazolidinediones (TZDs) class of anti-diabetics, have achieved profitable sales amounting to 2.4 and 3.6 billion dollars, respectively, in 2007.[5] However, these drugs are reported to have side effects, including body weight gain and fluid retention, that limit their wider use.[6] Indeed, TZDs cannot be used to treat type 2 diabetes patients with risk for congestive heart failure.[7]

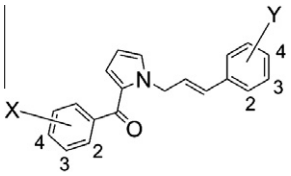
PPARs, which belong to a nuclear hormone receptor superfamily, act as transcription factors in the regulation of genes involved in glucose and lipid homeostasis.[8] Up to date, three PPAR subtypes, PPARα, PPARγ and PPARδ have been identified. Activation of PPARα by fibrates, such as fenofibrate (**3**), is known to be useful in the treatment of dyslipidemia. On the other hand, compounds that act on both PPARα and γ including ragaglitazar (**4**), muraglitazar (**5**) and tesaglitazar (**6**) have been identified as very attractive candidates in the treatment of dyslipidemic type 2 diabetes.[8a] Although such dual agonists have shown promising efficacy both in rodent models and in human clinical trials, almost all have failed the late stages of clinical trial with no PPARα/γ agonist being marketed so far. For example, the clinical development of tesaglitazar (**6**) was discontinued in phase III studies due to potential kidney toxicity. Thus, development of novel, efficacious, and yet safe PPARα/γ agonists is considered of great therapeutic value.

We have recently reported the pharmacological profile of SMP-534 (**7**), a TGF-β signaling pathway inhibitor and potential candidate for the treatment of diabetic nephropathy.[9] During our pharmacological evaluation of **7** and its related compounds, we have found that repetitive administration of one of **7** derivatives slightly lowered blood glucose level in a rodent model of diabetic nephropathy. Since SMP-534 itself did not affect the blood glucose levels, we assumed that inhibition of the TGF-β signaling pathway[9a] would be independent of hypoglycemic action.[9b] It then occurred to us that chemical modification of this derivative could provide us with a new class of antidiabetic drugs possessing affinity for PPARs, since the chemical structure of **7** resembles a typical pharmacophore chemotype of PPARs agonists as depicted in Figure 2. In this Letter, we report the synthesis, structure–activity relation-



Scheme 1. Reagents and conditions: (a) KO*tert*-Bu, allyl bromide, THF, 40 °C, 85–96%; (b) iodophenyl acetic acid or ethyl iodophenoxy acetate, $Pd(OAc)_2$, Cy_2NMe, Et_3NBnCl, DMF, 70 °C, 69–83%, (c) NaOH aq, THF, MeOH, rt, quant.; (d) NaOH aq, EtOH, rt, 86%.

Table 1
PPAR transactivation activity of benzoylpyrrole-based derivatives



Compound	X	Y	hPPAR transactivation				
			α (10 μM)[a]	α EC_{50} (μM) (% effect)[b]	γ (10 μM)[a]	γ EC_{50} (μM) (% effect)[b]	α:γ ratio
Pioglitazone **(2)**			n.d.	n.d.	7.8	0.39 (100%)[c]	n.d.
Fenofibric acid			5.6	12 (100%)[d]	n.d.	n.d.	n.d.
SMP-534 (**7**)	4-OMe	2-$CONHSO_2Me$	0.9	>90	1.5	12 (19%)	>7.5
10a	4-OMe	2-CH_2CO_2H	1.0	n.d.[e]	2.8	n.d.	n.d.
10b	4-OMe	3-CH_2CO_2H	1.6	21 (43%)	3.6	2.0 (37%)	11
10c	4-OMe	4-CH_2CO_2H	0.8	n.d.	1.5	n.d.	n.d.
101	4-OMe	2-OCH_2CO_2H	0.8	n.d.	1.2	n.d.	n.d.
10m	4-OMe	3-OCH_2CO_2H	5.8	3.7 (42%)	2.2	1.9 (16%)	1.9
10n	4-OMe	4-OCH_2CO_2H	3.5	n.d.	1.9	n.d.	n.d.
10o	2-OMe	3-OCH_2CO_2H	0.9	n.d.	1.1	n.d.	n.d.
10p	3-OMe	3-OCH_2CO_2H	1.4	n.d.	1.3	n.d.	n.d.
10q	2-Me	3-OCH_2CO_2H	1.1	n.d.	1.2	n.d.	n.d.
10r	3-Me	3-OCH_2CO_2H	1.9	n.d.	1.2	n.d.	n.d.
10s[f]	4-Me	3-OCH_2CO_2H	5.2	1.8 (37%)	2.0	1.2 (17%)	1.5

[a] Activities are presented as fold induction of PPARα and γ activation.
[b] EC_{50} value is the molar concentration of the test compound that affords 50% of the maximal reporter activity.
[c] The maximum efficacy of PPARγ activation of pioglitazone was defined as 100%.
[d] The maximum efficacy of PPARα activation of fenofibric acid was defined as 100%.
[e] n.d. = not done.
[f] Compound **10s** was also tested in mouse PPARα assay. EC_{50} = 4.9 μM (25%).

Table 2
Pharmakokinetic properties of **10sNa** in rats

	po	iv
Dose (mg/kg)	10	1
AUC (μg h/mL)	59	14
C_{max} (μg/mL)	4.3	
T_{max} (h)	4.0	
$T_{1/2}$ (h)		3.2
CL (mL/min/kg)		1.2
V_{dss} (L/kg)		0.3
BA (%)	42	

ships (SARs) and pharmacological profile of a new class of benzoylpyrrole-based PPARα/γ activators.

The compounds were synthesized as shown in Scheme 1.[10] Allylation of 2-benzoylpyrrole derivatives **8**[11] with allyl bromide in the presence of KO*tert*-Bu gave allyl pyrroles **9**. Heck coupling of **9** with iodophenyl acetic acid or ethyl iodophenoxy acetate in the presence of a catalytic amount of $Pd(OAc)_2$ gave carboxylic acids **10a–c** or esters **10d–k**. Standard hydrolysis of esters **10d–k** afforded carboxylic acids **10l–s**. Compound **10s** was converted to its sodium salt **10sNa** to increase its solubility in water for in vivo study.

The compounds synthesized in this study were evaluated in a transactivation assay using GAL4-hPPARγ and GAL4-hPPARα.[12] A selected compound was also evaluated using GAL4-mPPARα. The results of these assays are given in Table 1, where activity is reported as fold induction of PPARα/γ activation as well as EC_{50} values. SMP-534 (**7**) showed slight activity at PPARγ but was inactive at PPARα (EC_{50} >90 μM).

First of all, we planned to introduce a carboxylic acid as a bioisostere of acyl sulfonamide of SMP-534 (**7**), because most PPARs agonists possess a carboxylic acid as an acidic head group.[13] Additionally, we examined the effects of inserting a linker between the carboxylic group and the right-hand benzene ring, because appropriate linkers between a carboxylic group and an aromatic center had been used extensively in PPAR drug discovery research (Fig. 2). We also examined the effects of changing the substituted positions on the right-hand benzene ring. According to these strategies, we prepared compounds **10a–c** and **10l–s**. As shown in Table 1, introduction of a carboxylic acid moiety at the *meta*-position on the right-hand benzene ring increased PPARα/γ activity compared to the *ortho*- or *para*-position (**10b** vs **10a** and **10c**, **10m** vs **10l** and **10n**) in terms of fold induction at 10 μM of the test compounds, whereas the antifibrotic activity was totally abolished (data not shown). Among compounds **10a–c** and **10l–n** listed in Table 1, the *meta*-phenoxy acetic acid **10m** displayed potent and balanced dual PPARα/γ activity. Efficacy of compounds to activate PPARγ was next examined. Compounds **10b** and **10m**, which activated PPARγ with a maximal efficacy of 37% and 16%, respectively, as compared to that of pioglitazone (defined as 100%), were considered as PPARγ partial agonists.

Based on the results of this initial SAR survey, we systematically examined the effects of the substituent on the left-hand benzene ring of **10m**. According to this plan, we prepared compounds **10o–s**. Moving the *para*-substituent to the *ortho*- or *meta*-position on the left-hand benzene ring resulted in a decrease in PPARα/γ activity (**10m** vs **10o** and **10p**, **10s** vs **10q** and **10r**). Importantly, converting the *para*-methoxy group to the *para*-methyl group gave a modest increase in the activity for both receptor subtypes (**10m** vs **10s**) in terms of the fold induction. The representative compound **10s** showed well-balanced PPARα and γ activity with EC_{50} values of 1.8 and 1.2 μM, respectively. Moreover, compound **10s** showed partial agonistic activity for PPARγ, as indicated by 17% of the maximal activation obtained with pioglitazone (**2**). Furthermore, compound **10s** exhibited species-dependent PPARα activity with EC_{50} values of 1.8 and 4.9 μM for human and mouse PPARα, respectively.

Since the next step in this investigation was to perform studies in a diabetic rodent model, the pharmacokinetic properties of the sodium salt of **10s (10sNa)** were first evaluated in rats to determine whether this compound would be suitable for oral administration. Bioavailability, distribution volume, plasma clearance and

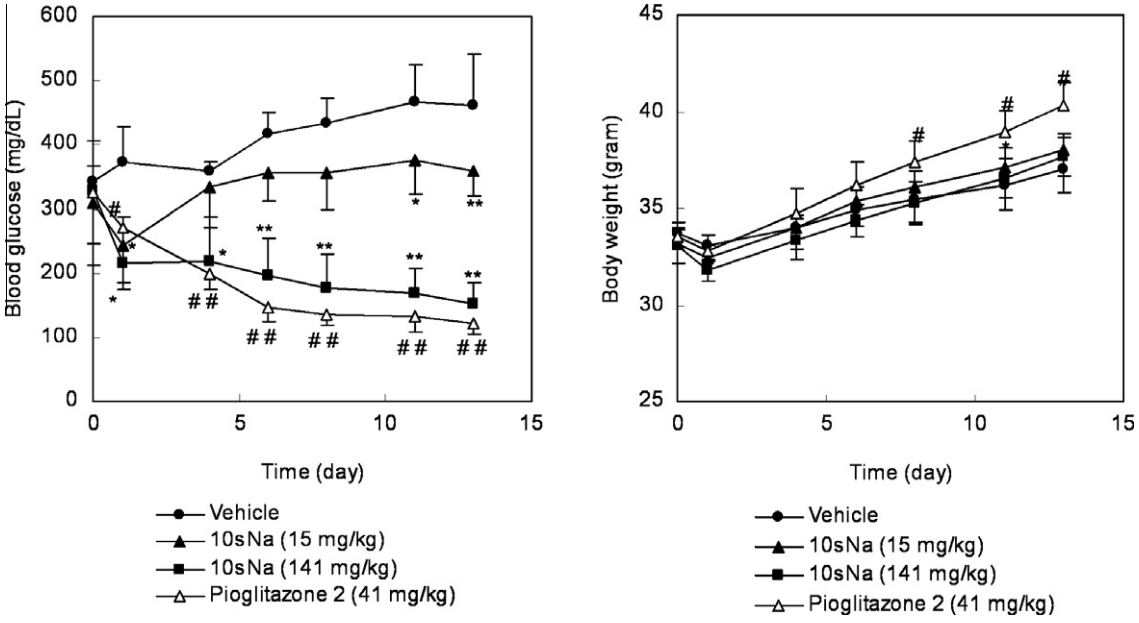


Figure 3. Evaluation of **10sNa** in *db*/*db* mice for 14 days. $^{*}P$ <0.05, $^{**}P$ <0.01 compared to vehicle control (Dunnett's test). $^{\#}P$ <0.05, $^{\#\#}P$ <0.01 compared to vehicle control (Student's test). Values are the means of 6 animals/group.

the measured half-life values of **10sNa** were all acceptable for in vivo study (Table 2).

Compound **10sNa** was evaluated in *db*/*db* mouse model showing both hyperglycemia and obesity.[14] Two-week-old male *db*/*db* mice were randomly assigned to four groups that received control rodent chow, compound **10sNa** (15 or 141 mg/kg/day) in chow, or pioglitazone (**2**) (41 mg/kg/day) in chow. The results of this in vivo study are described in Figure 3. Although compound **10sNa** at 15 mg/kg/day demonstrated a weaker blood glucose-lowering effect than pioglitazone (**2**) at 41 mg/kg/day, this effect was statistically-significant compared to that of the vehicle control. Compound **10sNa** at 141 mg/kg/day demonstrated favorable blood glucose-lowering effect similar to that observed with pioglitazone (**2**) at 41 mg/kg/day. Surprisingly, treatment with pioglitazone (**2**) caused a significant increase in body weight compared to the vehicle control, whereas treatment with **10sNa** did not cause body weight gain. No difference in mice food intake was observed between test drugs treated groups and the vehicle control group. While body weight gain is a well-known side effect of PPARγ full agonist,[15] body weight reduction in rodents has been reported with PPARα agonists, such as fenofibrate **3**[16] and other drugs.[17] It has also been reported that PPARγ partial agonists or modulators cause little change in body weight.[18] Compound **10sNa** showed 4-fold weaker activity for mouse PPARα than for human PPARγ, and displayed partial agonistic activity for PPARγ. We therefore assume that such beneficial profile of the blood glucose-lowering effect without body weight gain observed with **10sNa** is attributed to not only its PPARα agonistic activity but also its PPARγ partial agonistic activity.

In summary, starting with a subtle glucose-lowering effect of a TGF-β inhibitor, a new class of benzoylpyrrole-based carboxylic acids was found to show PPARα/γ activity. The SARs of these compounds were then examined, and *meta*-phenoxy acetic acid analogs were identified as potent PPARα/γ agonists. The selected compound **10sNa** showed an acceptable pharmacokinetic profile for in vivo study and a favorable blood glucose-lowering effect without body weight gain. Compound **10sNa** differs from other PPARα/γ agonists in that it is a PPARγ partial agonist. It is therefore believed that the analogs described in this study are attractive candidates for the treatment of type 2 diabetes. We are currently examining in detail the pharmacological mechanism of PPARγ partial agonists or modulators using **10sNa** and its derivatives.

Acknowledgments

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