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Design, synthesis and evaluation of novel zwitterionic compounds as PPARα/γ dual agonists (1)

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ABSTRACT

We describe here the design, syntheses and structure–activity relationships (SAR) of novel zwitterionic compounds as non-thiazolidinedion (TZD) based peroxisome proliferator activated receptor (PPAR) α/γ dual agonists. We commenced the medicinal research with compound **1** originated by Eli Lilly, which was reported to possess PPAR α/γ dual agonist activity. We incorporated an amine linker and optimized it on the nitrogen of the linker, thereby envisioning the enhancement of the PPAR α/γ dual agonist activity together with altering the physicochemical properties. As a result, we could generate compounds showing the PPAR α/γ dual activity, especially among which compound **22e** had a franylmethyl group on the linker and 2,6-dimethyl phenyl ring at the carboxylic acid head group furnishing a highly potent dual agonist activity, together with a great glucose lowering effect. Moreover, it remedied the lipid profile, that is, triglyceride without body weight gain in the *db/db* mice model.

Recently, it has been generally recognized that obesity, dyslipidemia and hypertension, so-called metabolic syndrome, lead to increased myocardial infarction, cerebral stroke or arteriosclerosis obliterans.[1] One of the general factors of metabolic syndrome is insulin resistance, which is also known as a basic etiological factor for type 2 diabetes.[2] Pioglitazone belonging to the thiazolidinedione (TZD) class of compound, which targets an insulin sensitizing mechanism and now on the market, is currently used for the treatment of type 2 diabetes. The molecular mechanism of TZD is an activation of peroxisome proliferator-activated receptor γ (PPARγ), a member of a family of ligand activated nuclear hormone receptors. PPARγ is mainly expressed in insulin sensitive tissue such as adipocytes and plays a central role in adipogenesis and glucose homeostasis. Activation of PPARγ by TZD lead to mitigated insulin resistance by promoting hypertrophied adipocyte differentiation and improving balance of physiological active substance such as adiponectin, TNF-α or leptin. However their activation has been plagued by side effects including weight gain, fluid retention and edema caused by differentiation induction to a mature adipocyte from an undifferentiated one. PPARα, on the other hand, is predominantly expressed in the liver and regulates lipid homeostasis via fatty acid catabolism including fatty acid binding, uptake and oxidation as well as lipoprotein assembly and transport.[3] PPARα activation has been identified to mediate the lipid-lowering activity in the study with the fibrate class of hypolipidemic drugs. The agonistic effects of these receptors have been shown to reduce serum triglycerides, increase high-density lipoprotein cholesterol (HDL-C) levels while lowering the low-density lipoprotein cholesterol level to a variable extent and improve cardiovascular outcomes.[4] Furthermore, some agonists have been reported to reduce weight gain in rodents without effect on food intake.[5] Therefore, adding the PPARα activation to the one for PPARγ was expected to improve lipid parameters as well as showing lower side effects, that is, weight gain caused by PPARγ activation.

We herein report the design, syntheses and structure–activity relationship of the PPAR α/γ dual agonist.

Our tactics of drug design for generating PPAR α/γ dual agonist is shown in Figure 1. The typical structure of PPAR agonists is comprised of a carboxylic acid head group, a linker and a lipophilic tail. The representative PPAR α/γ dual agonists thus far reported include compounds **1a,b** (reported by Eli Lilly),[6] Muraglitazar (**2**),[7] Tesaglitazar (**3**),[8] Naveglitazar (**4**),[9] Ragaglitazar (**5**),[10] and KRP-297 (**6**).[11] There are a variety of different substructures both for the carboxylic acid head group and the lipophilic tail, whilst the linker to connect those two moieties has a simple structure bearing an ether or an amide without substituents. The zwitterionic compounds, on the other hand, was already reported in the past, however, these compounds did not show PPAR α/γ dual activity but either selective PPARα[12] or PPARδ[13] activity.

Above the acidic PPAR α/γ dual agonists were shown to have a moderate glucose lowering effect with weight gain.[6–11] We assumed that the moderate activity in vivo owed to poor pharmacokinetic property due to its high lipophilicity and acidity, and the

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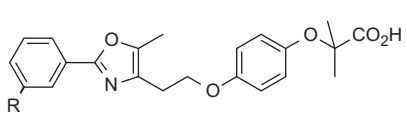
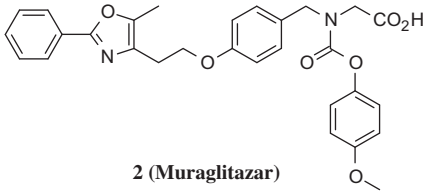


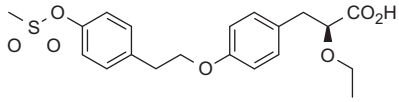
1a: R = H (Ely Lilly)
1b: R = Br (Ely Lilly)

1α; α : EC_{50} = 0.7 uM, γ: EC_{50} = 2.1 uM [6]



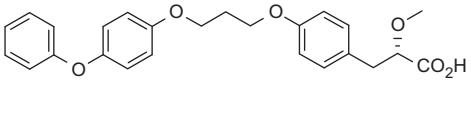
2 (Muraglitazar)

α: EC_{50} = 0.3 uM, γ: EC_{50} = 0.1 uM [7]



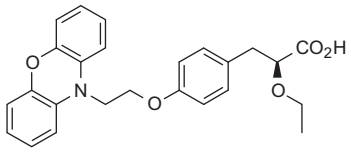
3 (Tesaglitazar)

α: EC_{50} = 1.2 uM, γ: EC_{50} = 1.3 uM [8]



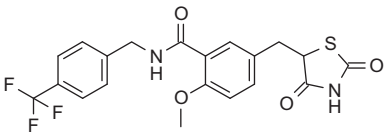
4 (Naveglitazar)

α: EC_{50} = 2.8 uM, γ: EC_{50} = 0.4 uM [9]



5 (Ragaglitazar)

α: EC_{50} = 3.2 uM, γ: EC_{50} = 0.6 uM [10]



6 (KRP-297)

α: EC_{50} = 0.8 uM, γ: EC_{50} = 1.0 uM [11]

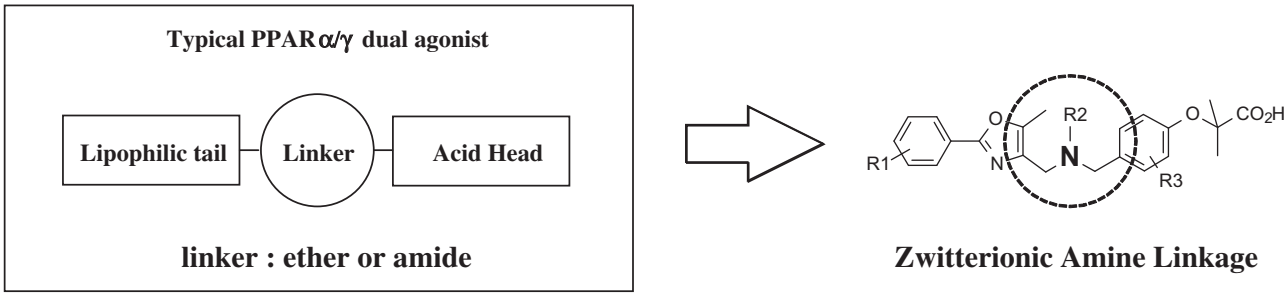
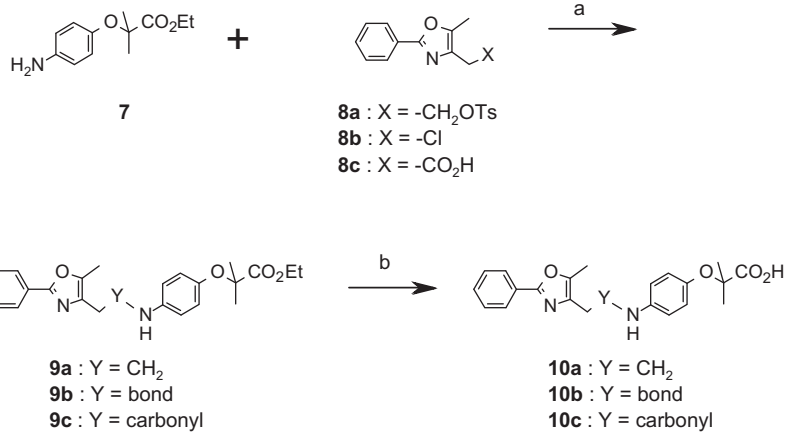


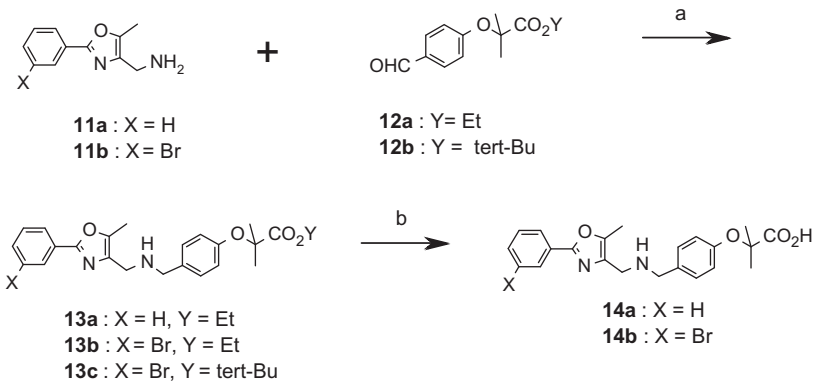
Figure 1. Drug design for novel PPARα/γ dual agonists by incorporating amine linkage.

weight gain was caused by the weak activity of PPARα. Thus, we started a medicinal study with compound **1a,b**, which possessed more potency for PPARα than for PPARγ, and designed compounds bearing amino linkage, which should reduce lipophilicity and acidity, thereby altering the physicochemical property and pharmacokinetics, expecting both the improvement of PPARα activity and potency in vivo.

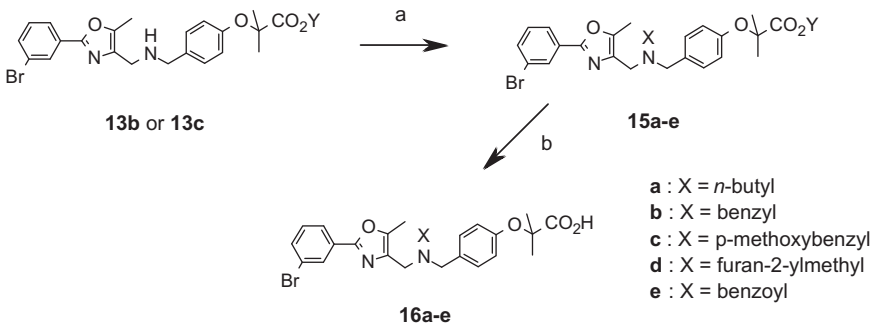
First, secondary amine compounds **10a**, **10b** and **10c** were designed and synthesized as depicted in Scheme 1. Aniline **7**[14] was coupled with tailpiece tosylate **8a**[15] or chloride **8b**[15] using cesium carbonate in DMF, or with carboxylic acid **3c** using EDCl and HOBt in DMF, followed by hydrolysis to provide **10a**, **10b** and **10c**, respectively. The compounds **14a** and **14b**, whose nitrogen was displaced into β-position, were synthesized as shown in Scheme 2. Reductive amination of primary amine **11a**[16] or **11b**[16] with corresponding aldehyde **12a**[17] or **12b**[17] in the presence of sodium triacetoxyborohydride provided esters **13a–c**, followed by hydrolysis to give **14a** and **14b**. Tertiary amine compounds **16a–d** and amide compound **16e** were synthesized as displayed in Scheme 3, which basically utilized the same procedure for **14a**,**b**. Amide **16e** was prepared by the benzoylation of **13b**, followed by hydrolysis. Synthesis of compounds **22a–g** bearing various



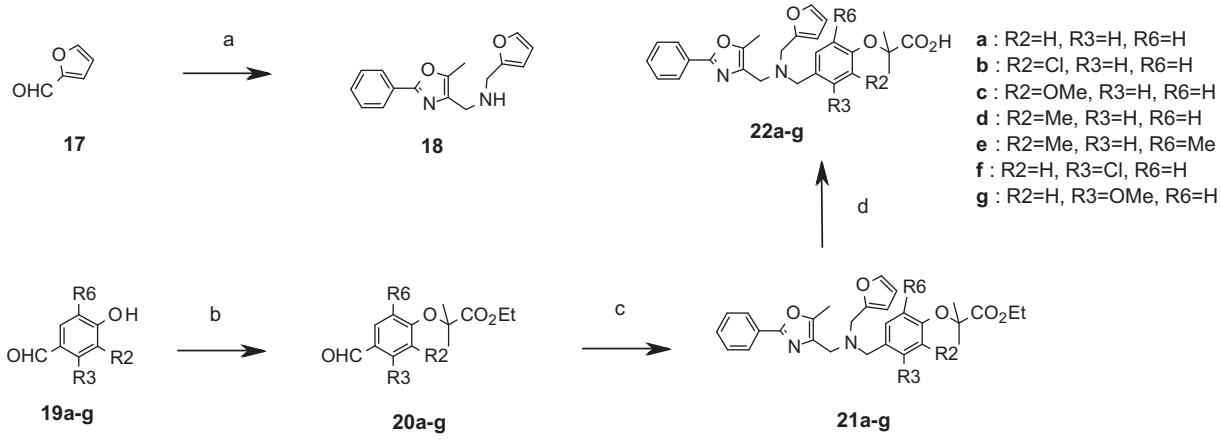
Scheme 1. Reagents and conditions: (a) Cs_2CO_3, DMF or EDCl, HOBt, DMF; (b) NaOH, MeOH.



Scheme 2. Reagents and conditions: (a) $NaBH(OAc)_3$, TEA, DCM; (b) NaOH, MeOH or TFA, DCM.



Scheme 3. Reagents and conditions: (a) RCHO, $NaBH(OAc)_3$, DCM or benzoic acid, EDCI, HOBt, DCM; (b) NaOH, MeOH or TFA, DCM.



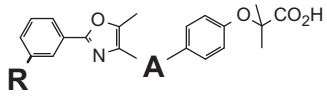
Scheme 4. Reagents and conditions: (a) **11a**, $NaBH_4$, EtOH; (b) ethyl 2-bromo-2-methylpropanoate, Cs_2CO_3, DMF, 80 °C; (c) **18**, $NaBH(OAc)_3$, DCM; (d) NaOH, MeOH.

substituents on the phenyl ring at the carboxylic acid head group is shown in Scheme 4. Imine was generated by heating the primary amine **11a** with 2-furaldehyde (**17**) in ethanol, followed by reduction with sodium borohydride to provide secondary amine **18**. Compounds **20a–g** were synthesized under a conventional condition from 4-hydroxybenzaldehydes **19a–g** possessing various substituents, that is, R2, R3 or R6, on the phenyl ring and commercially available ethyl 2-bromo-2-methylpropanoate. Reductive amination of the amine **18** with aldehyde **20a–g** in the presence of sodium triacetoxyborohydride provided ester **21a–g**, followed by hydrolysis to give **22a–g**, respectively.

The novel zwitterionic compounds were evaluated in cell-based transcription assay using GAL4-PPAR chimeric receptors and pFA-SEAP as reporter vector, and the activities are reported as the EC_{50} value. The results of the secondary amino compounds and the amide variant are presented in Table 1. The compound **10a**, whose oxygen was substituted to nitrogen**,** possessed higher potency for PPARα than for PPARγ as we envisaged although which was weaker than that of compound **1a.** Besides, the same magnitude of activity was observed with the compound **10b** and the compound **14a**. On the other hand, incorporation of bromine to the lipophilic tail (**14b**) led to enhancement of both PPARα and PPARγ potency, albeit 10 times less potent than compound **1b**. The amide compound **10c** resulted in the disappearance of both PPAR α/γ activities.

Next, the effects of substituents on nitrogen were examined. The results are summarized in Table 2. Since 3-bromophenyl variants on the lipophilic tail gave more potency, we thought that the

Table 1
In vitro trans activation activities of various linkage compounds



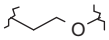
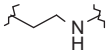
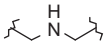
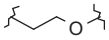
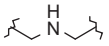
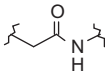
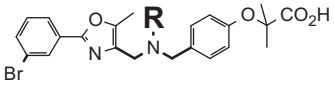
Compd	R	A	PPARα EC_{50} (nM)	PPARγ EC_{50} (nM)
1a	H		500	>25,000
10a	H		5200	16,000
10b	H		7200	8800
14a	H		10,000	>25,000
1b	Br		81	800
14b	Br		670	7700
14c	H		>25,000	>25,000

Table 2
In vitro trans activation activities of compounds **16a–e**



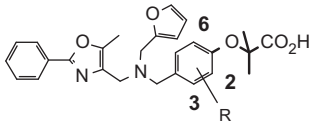
Compd	R	PPARα EC_{50} (nM)	PPARγ EC_{50} (nM)
16a	*n*-Butyl	500	1300
16b	Benzyl	56	2200
16c	*p*-Methoxybenzyl	26	700
16d	Furan-2-ylmethyl	8.1	310
16e	Benzoyl	>25,000	>25,000

SAR can be comprehensible by utilizing the compounds. Thus, compounds **16a–e** were synthesized aiming to investigate the capacity around nitrogen in the linker. As a result, it was found to be possible to introduce some side chains on nitrogen with holding activities. Moreover, the arylmethyl group furnished strong potency both for PPARα/γ, as exemplified in **16c** and **16d**. We surmised that the tri-benzyl-like structure was important to express high potency for PPARα. Especially, furan variant **16d** showed very potent PPARα activity along with moderate PPARγ activity. Meanwhile, an amide variant **16e** was devoid of potency both for PPARα and PPARγ.

The effects of substituents on the phenyl ring at the carboxylic acid head group were then examined. The results are summarized in Table 3. In this investigation, phenyl variants were utilized aiming to avoid enhancing the lipophilicity. 2-Substituted compounds **22b–e** showed higher potency both for PPARα and PPARγ than that of non-substituted compound **22a** or 3-substituted compounds **22f** or **22g**. Especially for PPARγ, the potency was increased significantly. Furthermore, the tendency was markedly observed for 2,6-disubstituted compound **22e**, whose potency was improved more than 100-fold compared to compound **1a**.

With obtaining very potent compound **22e**, in vivo studies were carried out in *db/db* mice, obese animal models of type 2 diabetes characterized by severe insulin resistance and marked hypertriglyceridemia. Compound **22e** exhibited a significant decrease in

Table 3
In vitro trans activation activities of compounds **23a–g**



Compd	R on phenyl-ring	PPARα EC_{50} (nM)	PPARγ EC_{50} (nM)
22a	H	13	670
22b	2-Cl	5.1	71
22c	2-OCH_3	9.0	180
22d	2-CH_3	3.9	42
22e	2,6-CH_3	1.7	4.7
22f	3-Cl	36	890
22g	3-OCH_3	32	330

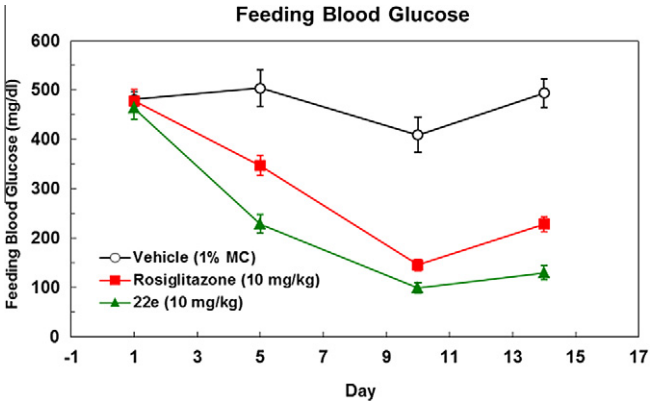


Figure 2. Plasma glucose decrease test in *db/db* mice. Plasma glucose decrease test was conducted with 14 days of treatment.

plasma glucose and plasma triglyceride when dosed orally at 10 mg/kg in *db/db* mice (six per group) for 14 days as depicted in Figure 2 and in Table 4. Treatment of 10 mg/kg of **22e** showed a glucose reduction of 74% as compared with that of 54% and 25% of Rosiglitazone (10 mg/kg) and compound **1a** (30 mg/kg), respectively. Furthermore, compound **22e** reduced plasma triglyceride by 88% at a dose of 10 mg/kg. It was noteworthy that weight gain as the side effect of PPARγ activation was not observed when these favorable effects were obtained.

We described the design, syntheses and evaluation of novel zwitterionic compounds as PPARα/γ dual agonists. The structure–activity relationship was established and confirmed on our lead, which possessed the nitrogen linker between the carboxylic acid head group and the lipophilic tail. The potency for PPARα was improved by the introduction of the arylmethyl group (e.g., furanylmethyl group) onto the nitrogen atom at the linker. The potency for PPARγ was significantly increased by the introduction of 2,6-disubsutituents on the phenyl ring. Compound **22e** exhibited a significant decrease in plasma glucose and plasma triglyceride levels without weight gain. Further pharmacological, pharmacokinetic or drug-metabolic studies on these compounds are now underway, and will be reported in due course.

Acknowledgments

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Table 4
Parameters in vivo study for compound **22e** on *db/db* mice

Exp.	Compds	Dose (mg/kg)	Plasma glucose[a] (mg/dl)	Change (%)	Plasma triglyceride[a] (mg/dl)	Change (%)	BW change (%)
1	Vehicle		494 ± 106		165 ± 116		
	22e	10	130 ± 51	−74[b]	20 ± 12	−88[b]	−7.6
	Rosiglitazone	10	228 ± 50	−54[b]	69 ± 24	−58[b]	+10.1[c]
2	Vehicle		487 ± 57		152 ± 41		
	1a	30	365 ± 13	−25[c]	67 ± 23	−56[d]	+8.4[b]

[a] Mean ± SD (n = 6).
[b] $p < 0.001$.
[c] $p < 0.01$.
[d] $p < 0.05$ versus vehicle control (t-test).

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