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Original article

Synthesis and biological evaluation of novel (−)-cercosporamide derivatives as potent selective PPARγ modulators

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ABSTRACT

Selective peroxisome proliferator-activated receptor gamma (PPARγ) modulators are expected to be a novel class of drugs improving plasma glucose levels without PPARγ-related adverse effects. As a continuation of our studies for (−)-Cercosporamide derivatives as selective PPARγ modulators, we synthesized substituted naphthalene type compounds and identified the most potent compound **15** (EC_{50} = 0.94 nM, E_{max} = 38%). Compound **15** selectively activated PPARγ transcription and did not activate PPARα and PPARδ. The potassium salt of compound **15** showed a high solubility and a good oral bioavailability (58%). Oral administration of the potassium salt remarkably improved the plasma glucose levels of female Zucker diabetic fatty rats at 1 mg/kg. Moreover, it did not cause a plasma volume increase or a cardiac enlargement in Wistar–Imamichi rats, even at 100 mg/kg.

1. Introduction

Diabetes is one of the most serious health problems in the world. The International Diabetes Federation (IDF) estimated that the number of people living with diabetes has soared to 366 million, representing 8.3% of the global adult population. This number is projected to increase to 552 million people by 2030, or 9.9% of adults [1]. Type 2 diabetes, which is the more common type of diabetes, usually develops in adulthood and is related to obesity, lack of physical activity, and unhealthy diets. For treatment of type 2 diabetes, lifestyle changes and weight loss are recommended. If a sufficient control of plasma glucose level cannot be achieved, several oral medications are available, such as metofolmin, sulfonylureas, DPP-IV inhibitors and peroxisome proliferator-activated receptor gamma (PPARγ) agonists.

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of a large family of ligand-activated nuclear hormone receptors, and is known to mediate adipocyte differentiation and improve plasma glucose levels effectively [2]. Pioglitazone and rosiglitazone are the PPARγ full agonists used in clinical settings. In spite of their beneficial effects, the use of these drugs has been limited because of adverse effects such as weight gain, edema, and anemia [3]. Furthermore, additional concerns that the use of PPARγ full agonists elevates the risk of cardiovascular events [4] and fractures [5] have been raised.

The PPARγ is associated with transcriptions of various genes as well as glycometabolism related genes. It is believed that some of the genes are related to the adverse effects. Therefore, the selective PPARγ modulators (SPPARMs), which partially activate the PPARγ transcriptional activity, have been considered to be ideal drug candidates. They are expected to improve the insulin resistance of type 2 diabetes with attenuated PPARγ related adverse effects [6]. Some SPPARMs, such as Fmoc-L-Leucine [7], FK614 [8], T2384 [9], INT-131 [10], MBX-102 [11], MK-0533 [12], and benzimidazolones [13] were reported to improve plasma glucose levels in hyperglycemic rodent models without several undesired side effects. Thus, the development of novel SPPARMs as drug candidates has drawn considerable attention.

We also reported that (−)-Cercosporamide derivative **2** (Scheme 1) was a novel SPPARM and it showed ideal pharmacological effects in hyperglycemic db/db mice [14]. (−)-Cercosporamide was originally isolated in 1991 as an antifungal agent and phytotoxin from

Abbreviations: PPARγ, peroxisome proliferator-activated receptor gamma; SPPARM, selective peroxisome proliferator-activated receptor gamma modulator.

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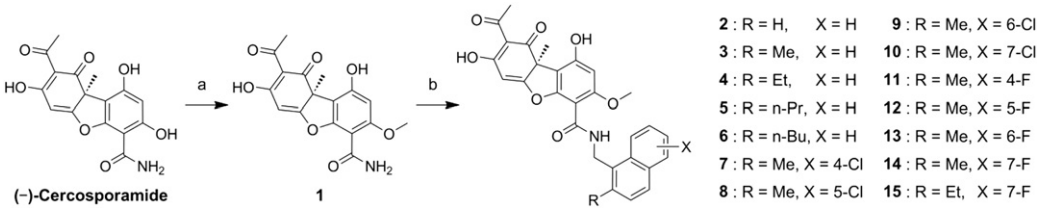
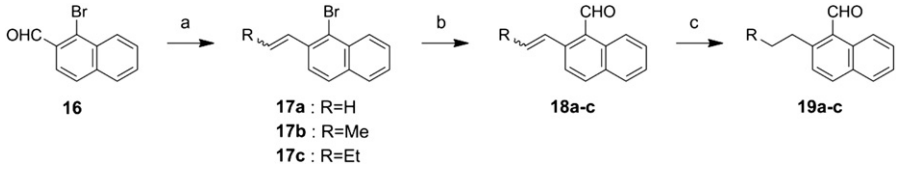


Scheme 1. Reagents and conditions: (a) MeI, K_2CO_3, DMF, r.t., 24 h; (b) substituted naphthaldehyde, Et_3SiH, TFA, CH_3CN, r.t., 1–12d.



Scheme 2. Reagents and conditions: (a) RCH_2PPh_3Br, NaH, THF, 0 °C to r.t., 1.5 h; (b) DMF, n-BuLi, THF, −78 °C to r.t., 1.5 h; (c) Pd–C, EtOAc, r.t., 1 h.

a fungal plant pathogen of cassava, *Cercosporidium henningsii* [15]. In our exploratory campaign for a novel antihyperglycemic agent, we discovered that (−)-Cercosporamide had a plasma glucose-lowering effect in hyperglycemic KK/Ta mice [16]. We tried to synthesize more potent (−)-Cercosporamide derivatives and to clarify the mechanism of action. In the research program, we identified a potent compound **2** as a novel SPPARM.

Here, we report further efforts for enhancing the potency of compound **2**. Compound **15** was found to be the most potent compound in a full-length human PPARγ reporter gene assay. The detailed pharmacological evaluations of compound **15** revealed strong *in vivo* efficacies and attenuated adverse effects.

2. Results and discussions

2.1. Chemistry

The X-ray crystal structure analysis of the complex of PPARγ-LBD (ligand binding domain) and compound **2** [14] suggested that there is a hydrophobic space in front of the naphthalene C2 position. Thus, we introduced alkyl substituents at the naphthalene C2 position of compound **2**, expecting the alkyl substituents to fill the space and make the derivatives more potent. We also tested incorporating halogen substituents into the naphthalene ring.

All of the (−)-Cercosporamide derivatives **2**–**15** were synthesized by reductive N-alkylation [17] of amide **1** using trifluoroacetic acid and triethylsilane with substituted naphthaldehydes as alkylating agents in moderate to high yield (61%–89%) as shown in Scheme 1.

2-Alkylated naphthaldehydes **19a**–**c** were synthesized from compound **16** as shown in Scheme 2. Alkyl groups were introduced by Wittig reaction. Following conversion of the 1-bromo group to the formyl group and hydrogenation of the double bond gave naphthaldehydes **19a**–**c**.

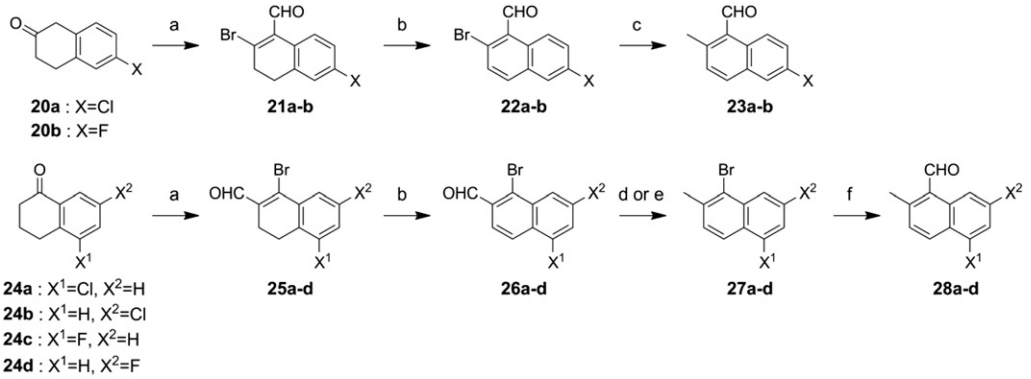
Syntheses of 6-halo-2-methylnaphthaldehydes **23a**–**b** and 5- or 7-halo-2-methylnaphthaldehydes **28a**–**d** were performed as shown in Scheme 3. β-Tetralones **20a**–**b** were converted to compound **21a**–**b** via Vilsmeier–Haack type reaction [18], followed by dehydrogenation to give compound **22a**–**b**. The subsequent Migita–Kosugi–Stille couplings afforded the naphthaldehydes **23a**–**b**. α-Tetralones **24a**–**d** were converted to compound **26a**–**d** in the same way as β-tetralones. Subsequent reductions, lithiations, and formylations gave naphthaldehydes **28a**–**d**.

Syntheses of 4-halo-2-alkylnaphthaldehydes were achieved as shown in Scheme 4. Compound **29** was formylated to give naphthaldehyde **30**. The *ortho* lithiation of compound **31** mediated by *N*,*N*,*N*′-trimethylethylenediamine [19] and successive treatment with iodomethane yielded naphthaldehyde **32**. The bromination of lithiated **31** and subsequent Suzuki-Miyaura coupling afforded naphthaldehyde **34**.

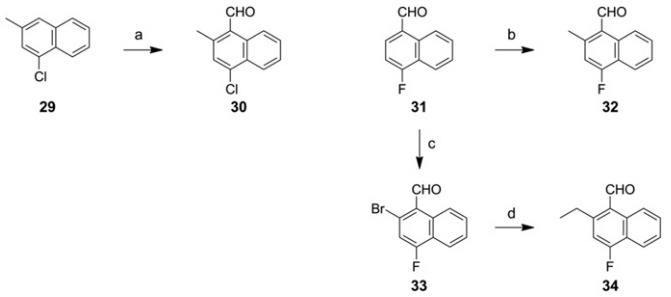
2.2. In vitro pharmacology

The effects of these derivatives **2**–**15** were tested in a full-length human PPARγ reporter gene assay using MG-63 cells. The results are summarized in Table 1.

Alkyl substituents on the naphthalene C2 position strengthened the derivatives without large changes of the maximum efficacy. In



Scheme 3. Reagents and conditions: (a) DMF, PBr_3, $CHCl_3$, reflux, 2 h; (b) DDQ, toluene, reflux, 2–4d; (c) Me_4Sn, $Pd(PPh_3)_4$, toluene, reflux, 9 h; (d) N_2H_4–H_2O, KOH, diethylene glycol, 180 °C, 1.5 h; (e) $B(C_6F_5)_3$, Et_3SiH, CH_2Cl_2, r.t., 1–4d; (f) DMF, n-BuLi, THF, −78 °C to r.t., 1 h.



Scheme 4. Reagents and conditions: (a) $MeOCHCl_2$, $TiCl_4$, CH_2Cl_2 0 °C to r.t., 4 h.; (b) MeI, *N,N,N′*-trimethylethylenediamine, n-BuLi, THF, −78 °C to r.t., 26 h.; (c) $BrCF_2CF_2Br$, *N,N,N′*-trimethylethylenediamine, n-BuLi, THF, −78 °C to r.t., 19 h.; (c) Et_2BOMe, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_2CO_3, DMF, 70 °C, 2.5 h.

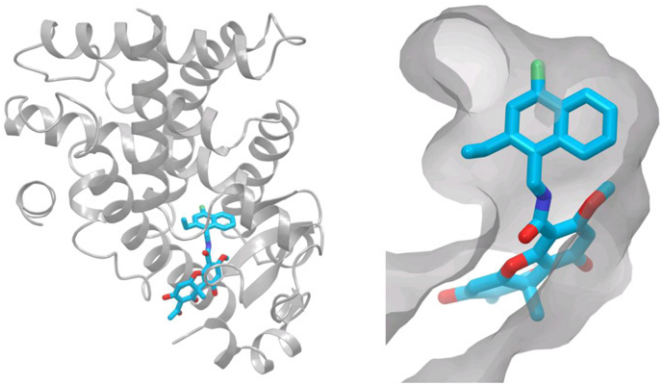


Fig. 1. Crystal structure of the complex of PPARγ-LBD and compound **15**.

comparison with non-substituted compound **2** (EC_{50} = 180 nM, E_{max} = 47%), methyl-substituted compound **3** (EC_{50} = 28 nM, E_{max} = 47%) was six times more potent and ethyl-substituted compound **4** (EC_{50} = 3.7 nM, E_{max} = 39%) was fifty times more potent. Compounds **5** and **6**, n-propyl and n-butyl substituents, also showed enhanced potencies, but were inferior to compound **3**. Interestingly, compound **6** was found to have a lower maximal efficacy (E_{max} = 22%) than the other alkyl derivatives **3**–**5**. The reason why the large alkyl substituent decreased the E_{max} value is not clear, and is now under investigation.

Regarding the effect of the halogen substituents, we started our SAR study from 2-methyl compound **3** due to synthetic feasibility. 7-Chloro compound **10** (EC_{50} = 4.9 nM, E_{max} = 44%) was the most potent among the chloro-substituted compounds **7**–**10**. 4-Fluoro compound **11** (EC_{50} = 4.8 nM, E_{max} = 50%) was the most potent among the fluoro-substituted compounds **11**–**14**. These compounds were five times more potent than compound **3**. We combined the most potent alkyl substituent of 2-ethyl and the most potent halogen substituent of 4-fluoro. The resulting 2-ethyl-4-fluoro compound **15** showed an extreme potency (EC_{50} = 0.94 nM, E_{max} = 38%), which was almost two hundred times more potent than non-substituted compound **2**.

X-ray crystal structure analysis of the complexes of PPARγ–LBD with compound **15** showed that 2-ethyl group sufficiently filled the hydrophobic space (Fig. 1, PDB ID: 4F9M). 4-Fluoro substituent was located near the side chain of Lys367 causing a bigger van der Waals force than that of compound **4**. We speculated that 4-fluoro substituent would change the electrostatic potential of the naphthalene ring to strengthen the interaction with PPARγ. We considered these effects made compound **15** two hundred times more potent than compound **2**.

The selectivity on PPAR subtypes of compound **15** was tested in GAL4-PPAR-LBD reporter gene assays using COS7 cells (Fig. 2). The compound **15** potently induced PPARγ transactivation (EC_{50} = 12 nM, E_{max} = 64%). On the other hand, only slight and no induction was observed even at 10 μM for the PPARα and PPARδ transactivation, respectively. These results suggested that compound **15** is a highly selective PPARγ partial agonist.

Table 1
Full-length human PPARγ reporter gene assay.

Compound	*R*	*X*	Transactivation	
			EC_{50} (nM)	E_{max} (%)
2	H	–	180	47
3	Me	–	28	47
4	Et	–	3.7[a]	39[a]
5	n-Pr	–	43	37
6	n-Bu	–	59	22
7	Me	4-Cl	22	57
8	Me	5-Cl	170	59
9	Me	6-Cl	56	26
10	Me	7-Cl	4.9	44
11	Me	4-F	4.8	50
12	Me	5-F	16	39
13	Me	6-F	27	28
14	Me	7-F	65	40
15	Et	4-F	0.94	38
Rosiglitazone			11	106

[a] The PPARγ transcriptional activity in the presence of compound **4** is slightly different from the previously reported values (EC_{50} = 3.5 nM, E_{max} = 27%) [20] because of a variant assay system.

2.3. In vivo pharmacology

2.3.1. Pharmacokinetics

In consideration of the excellent *in vitro* profiles, we expected that compound **15** would show ideal pharmacological effects *in vivo*. Prior to detailed *in vivo* evaluations, we tested the pharmacokinetic profiles in rats. Since compound **15** had a low solubility (23 μg/mL in JP2 at pH6.8), we tried to administer it as a salt form. Compound **15** was readily converted to potassium salt by treatment with aqueous potassium hydroxide in a co-solvent of ethanol and dichloromethane. The potassium salt **35** showed an improved solubility (910 μg/mL in JP2 at pH6.8). The potassium salt **35** was administered to Fischer rats at 10 mg/kg orally and intravenously. Compound **15** was metabolically stable in rats: the elimination terminal half-life after intravenous administration was estimated to be 4.0 h and the total clearance to be 6.98 mL/min/kg. Sufficient plasma concentration of compound **15** was observed after oral administration (Fig. 3), and the bioavailability was estimated to be 58%. These good pharmacokinetic profiles of potassium salt **35** prompted us to conduct detailed *in vivo* evaluations.

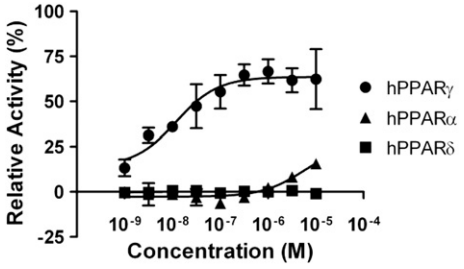


Fig. 2. PPAR subtypes selectivity Footnote: Data are represented as mean ± S.E.M. (n = 3).

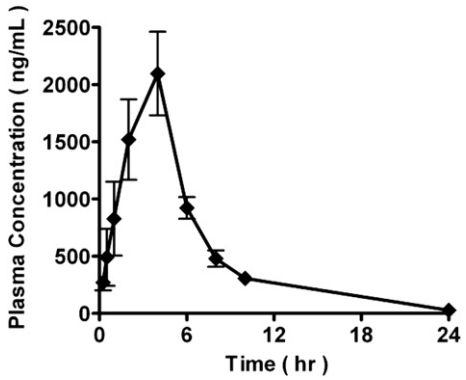


Fig. 3. Plasma concentrations of compound **15** in Fischer rats. Footnote: Data are represented as mean ± S.D. ($n = 4$) after oral administration of compound **35** at 10 mg/kg.

2.3.2. Pharmacological effects in type 2 diabetes model

We evaluated the pharmacological effects of compound **35** in female Zucker diabetic fatty (ZDF) rats by comparing the marketed PPARγ agonist rosiglitazone and vehicle treated groups. Both compounds were suspended in 0.5% (w/v) methylcellulose solution and administered once daily by oral gavage for 12 days. The measured parameters are shown in Fig. 4. Both compounds significantly lowered the non-fasting plasma glucose level below 200 mg/dL at over 1 mg/kg ($p < 0.01$). Both compounds also dose-dependently improved plasma lipids profiles. Compound **35** significantly decreased the triglyceride at 100 mg/kg ($p < 0.05$) and free fatty acids at over 1 mg/kg ($p < 0.01$). Rosiglitazone significantly decreased the triglyceride at 1 mg/kg ($p < 0.05$), 10 and 100 mg/kg ($p < 0.01$) and free fatty acids at over 1 mg/kg ($p < 0.01$). Body weight increments were induced in the plasma glucose lowered groups ($p < 0.01$) and the slight body weight increase was also recognized in the rosiglitazone 0.1 mg/kg group ($p < 0.05$).

Compound **35** showed almost an equal potency to rosiglitazone in glucose-lowering effects. Although body weight gains were increased at the pharmacologically effective doses (over 1 mg/kg), the degree of body weight gain of compound **35** inclined to be small in comparison to the same dose of rosiglitazone. These results indicated that compound **35** had sufficient anti-diabetic effects on type 2 diabetes at clinically acceptable doses.

2.3.3. PPARγ agonist-related adverse effects

The PPARγ agonists are known to induce several adverse effects such as body weight increase, edema, anemia, and cardiac hypertrophy. We tried to evaluate these adverse effects by measuring body weight gain, plasma volume, and heart weight in normal Wistar–Imamichi rats. Compound **35** (100 mg/kg/day), rosiglitazone (30, 100 and 300 mg/kg/day), and vehicle were administered for 14 days under the same condition as ZDF rats. The results are shown in Fig. 5. In comparison with the vehicle treated group, the compound **35** and rosiglitazone 30 and 100 mg/kg treated groups were inclined to increase body weight gain without statistically significant difference. On the other hand, the rosiglitazone 300 mg/kg treated group did not show an increase of body weight gain. Thus, we considered that rosiglitazone 300 mg/kg was likely to be over-dosing. Compound **35** did not increase plasma volume or heart weight even at 100 mg/kg, although rosiglitazone significantly increased plasma volume ($p < 0.01$) and heart weight ($p < 0.01$) in a dose dependent manner at 30 and 100 mg/kg.

Although compound **35** at 100 mg/kg slightly affected the body weight gain of Wistar–Imamichi rats without statistical significance, it did not cause an increase of plasma volume or cardiac hypertrophy. On the other hand, rosiglitazone 30 and 100 mg/kg significantly increased plasma volume and heart weight in a dose dependent manner. The increase of plasma volume was associated with edema and anemia. The increased plasma volume also stressed the heart and resulted in cardiac enlargement. These results indicated that compound **35** was safer than marketed PPARγ full agonist rosiglitazone, in terms of the cardiovascular risks.

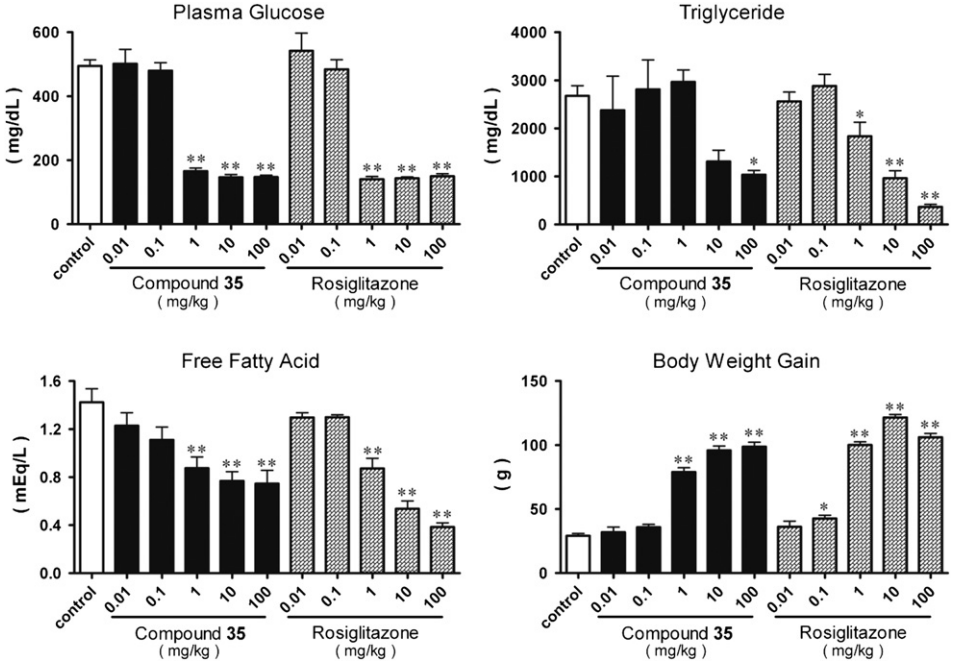


Fig. 4. Evaluation of pharmacological effects of compound **35** in Zucker diabetic fatty rats. Footnote: Each group of rats were orally administered (dose indicated in mg/kg/day) for 12 days with compound **35**, rosiglitazone, or vehicle. Data are represented as mean ± S.E.M. ($n = 5$). Statistical significances of differences between the control and treated groups were analyzed by one-way ANOVA with Dunnett's multiple comparison test (*$P < 0.05$, **$P < 0.01$).

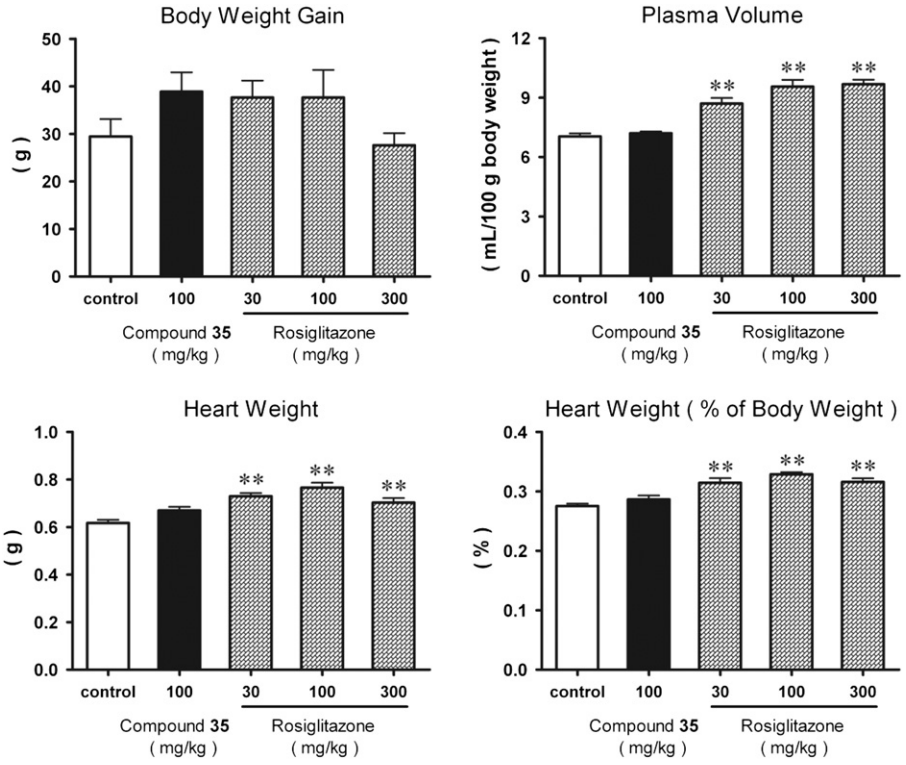


Fig. 5. Evaluation of PPARγ agonist-related adverse effects of compound **35** in Wistar–Imamichi rats. Footnote: Each group of rats were orally administered (dose indicated in mg/kg/day) for 14 days with compound **35**, rosiglitazone, or vehicle. Data are represented as mean ± S.E.M. ($n = 5$). There was no statistical significance of difference between the control and the compound **35** treated group analyzed by an unpaired, two-tailed *t*-test. Statistical significances of differences between the control and rosiglitazone-treated groups were analyzed by one-way ANOVA with Dunnett's multiple comparison test ($^{**}P < 0.01$).

3. Conclusion

For enhancing the potency of compound **2** as a selective PPARγ modulator, we introduced alkyl substituents at C2 position of the naphthalene ring and halogen substituents at the C4, C5, C6, or C7 position. The most potent 2-ethyl-4-fluoro substituted compound **15** was two hundred times more potent than compound **2** in a full-length human PPARγ transactivation reporter assay ($EC_{50} = 0.94$ nM, $E_{max} = 38\%$). Compound **15** selectively induced PPARγ transactivation and did not affect PPARα and PPARδ in GAL4-PPAR-LBD reporter gene assays. Although compound **15** has a low solubility, the potassium salt **35** has a high solubility (910 μg/mL in JP2 at pH6.8) and a good oral bioavailability (58%) at a dose of 10 mg/kg in Fischer rats. *In vivo* pharmacological evaluation of compound **35** demonstrated potent anti-diabetic effects on type 2 diabetes models of ZDF rats, which were almost equivalent to rosiglitazone. Moreover, compound **35** did not induce PPARγ-related adverse effects such as a plasma volume increase or a cardiac enlargement in Wistar–Imamichi rats. These results support potassium salt **35** as a promising anti-diabetic drug candidate.

4. Experimental section

4.1. Chemistry

All reactions were performed under a nitrogen atmosphere, unless otherwise indicated. Commercially available chemicals were used without further purification. Thin-layer chromatography (TLC) performed on Merck TLC Silica gel 60 F_{254} glass plates was used routinely to monitor the progress of the reactions and the purity of the compounds. For the flash column chromatography, silica gel 60 (Merck, 230–400) was employed. Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on Varian Mercury 400, Varian Mercury 500, or JEOL JNM-ECA500 with tetramethylsilane as an internal reference. Mass spectra were recorded on JEOL JMS-GCmate, JEOL JMS-600H, JEOL JMS-Lcmate, JEOL JMS-T100LC, or Waters Xevo Qtof.

4.1.1. *(9aS)-8-acetyl-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**1**)*

(−)-Cercosporamide (10.49 g, 31.7 mmol) was dissolved in DMF (50 mL). Iodomethane (5.0 mL, 80.3 mmol) and K_2CO_3 (9.03 g, 65.3 mmol) were added, and the mixture was stirred at room temperature for 36 h. K_2CO_3 was separated by filtration from the reaction mixture and 0.1 N hydrochloric acid was added, followed by extraction with EtOAc. The organic layer was washed with brine, then dried over Na_2SO_4. The solvent was removed under reduced pressure, and the resulting residue was recrystallized from EtOAc to afford 7.19 g of compound **1** (66% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.76 (3H, s), 2.65 (3H, s), 3.94 (3H, s), 5.76 (1H, brs), 6.04 (1H, s), 6.36 (1H,s), 7.10 (1H, brs), 10.79 (1H, s), 18.83 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 28.0, 32.1, 56.7, 58.7, 96.6, 98.3, 101.6, 105.2, 106.5, 156.1, 157.9, 160.5, 164.8, 180.3, 191.9, 198.0, 201.7. MS (ESI) *m/z*: 346 $(M + H)^+$. HRMS (ESI) *m/z*: 346.0929 (calcd for $C_{17}H_{16}NO_7$: 346.0927). Anal. Calcd for $C_{17}H_{15}NO_7$: C, 59.13; H, 4.38; N, 4.06; O, 32.43. Found: C, 59.03; H, 4.42; N, 4.14; O, 32.31.

4.1.2. *General procedure to synthesize PPARγ modulators (**2**–**15**)*

Compound **1** was suspended in CH_3CN. Substituted naphthaldehyde (1–3eq.), triethylsilane (2–3eq.), and trifluoroacetic acid (2–3eq.) were added, and then the mixture was stirred at room temperature for 1–12 days. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/1 to 50/1) to afford the desired product. The product was further purified by

reverse phase column chromatography (COSMOSIL 75C_{18}-PREP, CH_3CN/H_2O = 1/1 to 4/1) when necessary.

*4.1.2.1. (9aS)-8-acetyl-1,7-dihydroxy-3-methoxy-9a-methyl-N-(naphthalen-1-ylmethyl)-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**2**).* In accordance with the general procedure, compound **1** (5.04 g, 14.6 mmol) and 1-naphthaldehyde (4.00 mL, 29.5 mmol) provided 5.46 g of compound **2** (77% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.75 (3H, s), 2.64 (3H, s), 3.71 (3H, s), 5.06 (1H, dd, J = 14.7, 5.2 Hz), 5.12 (1H, dd, J = 14.7, 5.2 Hz), 6.00 (1H, s), 6.27 (1H, s), 7.29 (1H, brs), 7.43–7.59 (4H, m), 7.83 (1H, d, J = 8.3 Hz), 7.90 (1H, d, J = 8.3 Hz), 8.12 (1H, d, J = 8.3 Hz), 10.66 (1H, s), 18.82 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 28.0, 32.0, 41.9, 56.6, 58.8, 96.5, 98.2, 102.3, 105.2, 106.3, 123.6, 125.5, 125.9, 126.4, 126.6, 128.5, 128.8, 131.4, 133.6, 133.9, 155.7, 157.4, 160.1, 162.4, 180.4, 191.9, 198.0, 201.6. MS (FAB) *m/z*: 486 (M + H)$^+$. HRMS (FAB) *m/z*: 486.1551 (calcd for $C_{28}H_{24}NO_7$: 486.1553). Anal. Calcd for $C_{28}H_{23}NO_7$: C, 69.27; H, 4.78; N, 2.89; O, 23.07. Found: C, 68.95; H, 4.80; N, 2.91; O, 23.00.

*4.1.2.2. (9aS)-8-acetyl-1,7-dihydroxy-3-methoxy-9a-methyl-N-[(2-methylnaphthalen-1-yl)methyl]-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**3**).* In accordance with the general procedure, compound **1** (304 mg, 0.880 mmol) and 2-methyl-1-naphthaldehyde (448 mg, 2.63 mmol) provided 379 mg of compound **3** (86% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.74 (3H, s), 2.63 (3H, s), 2.64 (3H, s), 3.62 (3H, s), 5.05 (1H, dd, J = 14.4, 4.6 Hz), 5.12 (1H, dd, J = 14.4, 4.6 Hz), 6.00 (1H, s), 6.23 (1H, s), 7.02 (1H, brs), 7.35 (1H, d, J = 8.3 Hz), 7.44–7.48 (1H, m), 7.53–7.57 (1H, m), 7.75 (1H, d, J = 8.8 Hz), 7.84 (1H, d, J = 7.8 Hz), 8.12 (1H, d, J = 8.3 Hz), 10.63 (1H, s), 18.82 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.2, 28.0, 32.0, 37.4, 56.5, 58.8, 96.4, 98.2, 102.4, 105.2, 106.3, 123.5, 125.0, 126.6, 128.2, 128.6, 129.2, 130.3, 132.3, 132.6, 135.1, 155.6, 157.3, 160.0, 162.5, 180.4, 191.9, 198.0, 201.6. MS (FAB) *m/z*: 500 (M + H)$^+$. HRMS (FAB) *m/z*: 500.1711 (calcd for $C_{29}H_{26}NO_7$: 500.1709). Anal. Calcd for $C_{29}H_{25}NO_7$: C, 69.73; H, 5.04; N, 2.80; O, 22.42. Found: C, 69.41; H, 5.02; N, 2.98; O, 22.57.

*4.1.2.3. (9aS)-8-acetyl-1,7-dihydroxy-3-methoxy-9a-methyl-N-[(2-ethylnaphthalen-1-yl)methyl]-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**4**).* In accordance with the general procedure, compound **1** (273 mg, 0.791 mmol) and aldehyde **19a** (280 mg, 1.52 mmol) provided 323 mg of compound **4** (80% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.31 (3H, t, J = 7.6 Hz), 1.74 (3H, s), 2.64 (3H, s), 2.96 (2H, q, J = 7.6 Hz), 3.61 (3H, s), 5.06 (1H, dd, J = 14.4, 4.6 Hz), 5.12 (1H, dd, J = 14.4, 4.6 Hz), 6.01 (1H, s), 6.22 (1H, s), 7.03 (1H, brs), 7.38 (1H, d, J = 8.8 Hz), 7.45–7.48 (1H, m), 7.53–7.57 (1H, m), 7.79 (1H, d, J = 8.3 Hz), 7.84 (1H, d, J = 8.3 Hz), 8.11 (1H, d, J = 8.8 Hz), 10.63 (1H, s), 18.83 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 16.5, 27.1, 28.0, 32.0, 37.1, 56.5, 58.8, 96.4, 98.3, 102.3, 105.2, 106.3, 123.7, 125.1, 126.6, 128.0, 128.60, 128.63, 129.4, 132.4, 132.6, 141.5, 155.7, 157.3, 160.1, 162.3, 180.5, 191.9, 198.0, 201.6. MS (FAB) *m/z*: 514 (M + H)$^+$. HRMS (FAB) *m/z*: 514.1848 (calcd for $C_{30}H_{28}NO_7$: 514.1866). Anal. Calcd for $C_{30}H_{27}NO_7$: C, 70.16; H, 5.30; N, 2.73; O, 21.81. Found: C, 70.04; H, 5.23; N, 2.82; O, 21.68.

*4.1.2.4. (9aS)-8-acetyl-1,7-dihydroxy-3-methoxy-9a-methyl-N-[(2-propylnaphthalen-1-yl)methyl]-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**5**).* In accordance with the general procedure, compound **1** (304 mg, 0.880 mmol) and aldehyde **19b** (523 mg, 2.64 mmol) provided 415 mg of compound **5** (89% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.02 (3H, t, J = 7.3 Hz), 1.71 (2H, q, J = 7.8 Hz), 1.75 (3H, s), 2.64 (3H, s), 2.91 (2H, td, J = 7.8, 2.8 Hz), 3.60 (3H, s), 5.06 (1H, dd, J = 14.4, 4.6 Hz), 5.12 (1H, dd, J = 14.2, 4.4 Hz), 6.01 (1H, s), 6.22 (1H,s), 7.01 (1H, brs), 7.36 (1H, d, J = 8.3 Hz), 7.45–7.48 (1H, m), 7.53–7.56 (1H, m), 7.77 (1H, d, J = 8.3 Hz), 7.84 (1H, d, J = 7.8 Hz), 8.11 (1H, d, J = 8.8 Hz), 10.63 (1H, s), 18.83 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 14.1, 25.3, 28.0, 32.0, 35.9, 37.2, 56.4, 58.8, 96.4, 98.3, 102.4, 105.2, 106.3, 123.9, 125.1, 126.6, 128.4, 128.58, 128.62, 129.7, 132.4, 132.6, 139.8, 155.7, 157.4, 160.1, 162.3, 180.5, 191.9, 198.0, 201.6. MS (FAB) *m/z*: 528 (M + H)$^+$. HRMS (FAB) *m/z*: 528.2048 (calcd for $C_{31}H_{30}NO_7$: 528.2022).

*4.1.2.5. (9aS)-8-acetyl-N-[(2-butylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**6**).* In accordance with the general procedure, compound **1** (303 mg, 0.877 mmol) and aldehyde **19c** (509 mg, 2.40 mmol) provided 391 mg of compound **6** (82% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.95 (3H, t, J = 7.3 Hz), 1.41–1.48 (2H, m), 1.62–1.68 (2H, m), 1.75 (3H, s), 2.64 (3H, s), 2.92 (2H, td, J = 7.8, 2.8 Hz), 3.60 (3H, s), 5.06 (1H, dd, J = 14.2, 4.4 Hz), 5.11 (1H, dd, J = 14.4, 4.6 Hz), 6.01 (1H, s), 6.22 (1H, s), 7.00 (1H, brs), 7.36 (1H, d, J = 8.8 Hz), 7.44–7.48 (1H, m), 7.52–7.56 (1H, m), 7.77 (1H, d, J = 8.3 Hz), 7.84 (1H, d, J = 7.8 Hz), 8.11 (1H, d, J = 8.8 Hz), 10.63 (1H, s), 18.83 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 14.1, 22.8, 28.0, 32.0, 33.8, 34.4, 37.2, 56.5, 58.8, 96.4, 98.3, 102.4, 105.2, 106.3, 123.8, 125.1, 126.6, 128.4, 128.6 (×2), 129.6, 132.4, 132.6, 140.1, 155.7, 157.4, 160.1, 162.3, 180.5, 191.9, 198.0, 201.6. MS (FAB) *m/z*: 542 (M + H)$^+$. HRMS (FAB) *m/z*: 542.2185 (calcd for $C_{32}H_{32}NO_7$: 542.2179). Anal. Calcd for $C_{32}H_{31}NO_7$: C, 70.97; H, 5.77; N, 2.59; O, 20.68. Found: C, 70.69; H, 5.62; N, 2.66; O, 20.62.

*4.1.2.6. (9aS)-8-acetyl-N-[(4-chloro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**7**).* In accordance with the general procedure, compound **1** (250 mg, 0.724 mmol) and aldehyde **30** (150 mg, 0.733 mmol) provided 298 mg of compound **7** (77% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.73 (3H, s), 2.61 (3H, s), 2.64 (3H, s), 3.62 (3H, s), 5.01 (1H, dd, J = 14.6, 3.9 Hz), 5.08 (1H, dd, J = 14.4, 4.2 Hz), 6.00 (1H, s), 6.23 (1H, s), 7.07 (1H, brs), 7.48 (1H, s), 7.56–7.62 (2H, m), 8.13 (1H, d, J = 8.3 Hz), 8.29 (1H, d, J = 8.3 Hz), 10.65 (1H, s), 18.82 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.0, 28.0, 32.0, 37.2, 56.5, 58.8, 96.5, 98.3, 102.1, 105.2, 106.4, 123.9, 125.1, 126.1, 127.4, 129.2, 129.76, 129.81, 131.8, 133.4, 135.5, 155.8, 157.4, 160.0, 162.5, 180.3, 191.9, 198.0, 201.6. MS (ESI) *m/z*: 534 (M + H)$^+$. HRMS (ESI) *m/z*: 534.12937 (calcd for $C_{29}H_{25}ClNO_7$: 534.13195).

*4.1.2.7. (9aS)-8-acetyl-N-[(5-chloro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**8**).* In accordance with the general procedure, compound **1** (210 mg, 0.608 mmol) and aldehyde **28a** (125 mg, 0.611 mmol) provided 259 mg of compound **8** (80% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.74 (3H, s), 2.64 (3H, s), 2.64 (3H, s), 3.62 (3H, s), 5.04 (1H, dd, J = 14.4, 4.2 Hz), 5.11 (1H, dd, J = 14.2, 4.9 Hz), 6.01 (1H, s), 6.23 (1H, s), 7.06 (1H, brs), 7.43–7.48 (2H, m), 7.55 (1H, d, J = 6.8 Hz), 8.06 (1H, d, J = 8.3 Hz), 8.21 (1H, d, J = 8.8 Hz), 10.65 (1H, s), 18.82 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.2, 28.0, 32.0, 37.5, 56.5, 58.8, 96.5, 98.3, 102.1, 105.2, 106.4, 122.7, 124.6, 125.4, 126.4, 129.9, 130.3, 130.8, 132.7, 133.6, 136.1, 155.8, 157.4, 160.0, 162.5, 180.4, 191.9, 198.0, 201.6. MS (ESI) *m/z*: 534 (M + H)$^+$. HRMS (ESI) *m/z*: 534.12980 (calcd for $C_{29}H_{25}ClNO_7$: 534.13195).

*4.1.2.8. (9aS)-8-acetyl-N-[(6-chloro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**9**).* In accordance with the general procedure, compound **1** (470 mg, 1.36 mmol) and aldehyde **23a** (283 mg, 1.38 mmol) provided 445 mg of compound **9** (61% yield) as

a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.74 (3H, s), 2.61 (3H, s), 2.63 (3H, s), 3.63 (3H, s), 5.00 (1H, dd, J = 14.5, 4.6 Hz), 5.08 (1H, dd, J = 14.5, 5.0 Hz), 6.00 (1H, s), 6.22 (1H, s), 7.02 (1H, brs), 7.36 (1H, d, J = 8.7 Hz), 7.45 (1H, dd, J = 9.1, 2.4 Hz), 7.64 (1H, d, J = 8.7 Hz), 7.79 (1H, d, J = 2.4 Hz), 8.05 (1H, d, J = 9.1 Hz), 10.63 (1H, s), 18.78 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.1, 28.0, 32.0, 37.3, 56.6, 58.8, 96.5, 98.3, 102.1, 105.2, 106.4, 125.4, 127.2, 127.31, 127.35, 130.4, 130.6 (×2), 130.8, 133.3, 135.5, 155.8, 157.4, 160.0, 162.5, 180.4, 191.9, 198.0, 201.6. MS (ESI) m/z: 534 (M + H)$^+$. HRMS (ESI) m/z: 534.13247 (calcd for $C_{29}H_{25}ClNO_7$: 534.13195).

*4.1.2.9. (9aS)-8-acetyl-N-[(7-chloro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**10**).* In accordance with the general procedure, compound **1** (450 mg, 1.30 mmol) and aldehyde **28b** (330 mg, 1.61 mmol) provided 540 mg of compound **10** (78% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.73 (3H, s), 2.63 (6H, s), 3.68 (3H, s), 4.94–5.04 (2H, m), 5.99 (1H, s), 6.22 (1H, s), 7.04 (1H, brs), 7.32 (1H, d, J = 8.3 Hz), 7.38 (1H, d, J = 8.7 Hz), 7.68 (1H, d, J = 8.3 Hz), 7.75 (1H, d, J = 8.7 Hz), 8.05 (1H, s), 10.61 (1H, s), 18.77 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.3, 28.0, 32.0, 37.5, 56.5, 58.8, 96.4, 98.3, 102.3, 105.2, 106.3, 122.5, 125.9, 128.0, 129.6, 129.7, 130.3, 130.8, 132.6, 133.1, 136.5, 155.7, 157.3, 160.1, 162.6, 180.4, 191.9, 198.0, 201.6. MS (ESI) m/z: 534 (M + H)$^+$. HRMS (ESI) m/z: 534.12981 (calcd for $C_{29}H_{25}ClNO_7$: 534.13195).

*4.1.2.10. (9aS)-8-acetyl-N-[(4-fluoro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**11**).* In accordance with the general procedure, compound **1** (250 mg, 0.724 mmol) and aldehyde **32** (137 mg, 0.728 mmol) provided 295 mg of compound **11** (79% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.74 (3H, s), 2.61 (3H, s), 2.64 (3H, s), 3.62 (3H, s), 4.99 (1H, dd, J = 14.5, 4.6 Hz), 5.07 (1H, dd, J = 14.7, 4.8 Hz), 6.01 (1H, s), 6.23 (1H, s), 7.03–06 (2H, m), 7.52 (1H, t, J = 7.5 Hz), 7.60 (1H, t, J = 7.5 Hz), 8.11 (2H, d, J = 8.3 Hz), 10.66 (1H, s), 18. 83 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.2, 28.0, 32.0, 37.0, 56.5, 58.8, 96.4, 98.3, 102.2, 105.2, 106.3, 112.4 (d, J_{CF} = 19 Hz), 121.1 (d, J_{CF} = 5 Hz), 122.7 (d, J_{CF} = 6 Hz), 123.5, 125.3, 126.4 (d, J_{CF} = 4 Hz), 127.6, 133.6 (d, J_{CF} = 5 Hz), 135.7 (d, J_{CF} = 8 Hz), 155.7, 157.4, 158.2 (d, J_{CF} = 253 Hz), 160.0, 162.5, 180.4, 191.9, 198.0, 201.6. MS (ESI) m/z: 518 (M + H)$^+$. HRMS (ESI) m/z: 518.16034 (calcd for $C_{29}H_{25}FNO_7$: 518.16151).

*4.1.2.11. (9aS)-8-acetyl-N-[(5-fluoro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**12**).* In accordance with the general procedure, compound **1** (330 mg, 0.956 mmol) and aldehyde **28c** (181 mg, 0.962 mmol) provided 400 mg of compound **12** (81% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.74 (3H, s), 2.64 (6H, s), 3.63 (3H, s), 5.03 (1H, dd, J = 14.3, 4.4 Hz), 5.11 (1H, dd, J = 14.3, 4.8 Hz), 6.02 (1H, s), 6.24 (1H, s), 7.06–7.15 (2H, m), 7.41–7.49 (2H, m), 7.90 (1H, d, J = 8.7 Hz), 8.03 (1H, d, J = 8.7 Hz), 10.66 (1H, s), 18.83 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.3, 28.0, 32.0, 37.5, 56.5, 58.8, 96.5, 98.3, 102.2, 105.2, 106.4, 108.6 (d, J_{CF} = 19 Hz), 119.3 (d, J_{CF} = 4 Hz), 120.6 (d, J_{CF} = 6 Hz), 122.8 (d, J_{CF} = 17 Hz), 126.3 (d, J_{CF} = 10 Hz), 129.5, 130.4, 134.0 (d, J_{CF} = 4 Hz), 136.2, 155.7, 157.4, 159.2 (d, J_{CF} = 252 Hz), 160.0, 162.5, 180.4, 191.9, 198.0, 201.6. MS (ESI) m/z: 518 (M + H)$^+$. HRMS (ESI) m/z: 518.15972 (calcd for $C_{29}H_{25}FNO_7$: 518.16151).

*4.1.2.12. (9aS)-8-acetyl-N-[(6-fluoro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**13**).* In accordance with the general procedure, compound **1** (420 mg, 1.22 mmol) and aldehyde **23b** (230 mg, 1.22 mmol) provided 495 mg of compound **13** (78% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.74 (3H, s), 2.61 (3H, s), 2.64 (3H, s), 3.63 (3H, s), 5.02 (1H, dd, J = 14.3, 4.4 Hz), 5.10 (1H, dd, J = 14.5, 4.6 Hz), 6.01 (1H, s), 6.23 (1H, s), 7.04 (1H, brs), 7.29–7.34 (1H, m), 7.37 (1H, d, J = 8.3 Hz), 7.44 (1H, d, J = 9.1 Hz), 7.68 (1H, d, J = 8.3 Hz), 8.13 (1H, dd J = 9.1, 5.2 Hz), 10.65 (1H, s), 18.82 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.0, 28.0, 32.0, 37.5, 56.5, 58.8, 96.5, 98.3, 102.2, 105.2, 106.4, 111.5 (d, J_{CF} = 20 Hz), 116.6 (d, J_{CF} = 25 Hz), 126.1 (d, J_{CF} = 8 Hz), 127.5 (d, J_{CF} = 5 Hz), 129.3, 130.4, 130.7, 133.4 (d, J_{CF} = 8 Hz), 134.3, 155.8, 157.4, 160.0 (d, J_{CF} = 247 Hz), 160.0, 162.5, 180.4, 191.9, 198.0, 201.6. MS (ESI) m/z: 540 (M + Na)$^+$. HRMS (ESI) m/z: 540.14208 (calcd for $C_{29}H_{24}FNNaO_7$: 540.14345).

*4.1.2.13. (9aS)-8-acetyl-N-[(7-fluoro-2-methylnaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**14**).* In accordance with the general procedure, compound **1** (265 mg, 0.767 mmol) and aldehyde **28d** (147 mg, 0.781 mmol) provided 242 mg of compound **14** (61% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.74 (3H, s), 2.62 (3H, s), 2.63 (3H, s), 3.67 (3H, s), 4.97 (1H, dd, J = 14.1, 4.6 Hz), 5.03 (1H, dd, J = 14.3, 4.8 Hz), 6.00 (1H, s), 6.23 (1H, s), 6.97 (1H, brs), 7.21 (1H, dd, J = 8.7, 2.4 Hz), 7.30 (1H, d, J = 8.3 Hz), 7.69–7.72 (2H, m), 7.81 (1H, dd, J = 8.7, 5.9 Hz), 10.61 (1H, s), 18.78 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 20.3, 28.0, 32.0, 37.7, 56.5, 58.8, 96.4, 98.3, 102.3, 105.2, 106.3, 107.3 (d, J_{CF} = 22 Hz), 115.2 (d, J_{CF} = 25 Hz), 128.1, 128.5, 129.6, 129.8 (d, J_{CF} = 6 Hz), 131.0 (d, J_{CF} = 8 Hz), 133.4 (d, J_{CF} = 8 Hz), 136.4, 155.7, 157.3, 160.0, 161.3 (d, J_{CF} = 246 Hz), 162.6, 180.4, 191.9, 198.0, 201.7. MS (ESI) m/z: 518 (M + H)$^+$. HRMS (ESI) m/z: 518.15792 (calcd for $C_{29}H_{25}FNO_7$: 518.16151).

*4.1.2.14. (9aS)-8-acetyl-N-[(2-ethyl-4-fluoronaphthalen-1-yl)methyl]-1,7-dihydroxy-3-methoxy-9a-methyl-9-oxo-9,9a-dihydrodibenzo[b,d]furan-4-carboxamide (**15**).* In accordance with the general procedure, compound **1** (700 mg, 2.03 mmol) and aldehyde **34** (410 mg, 2.03 mmol) provided 782 mg of compound **15** (72% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.31 (3H, t, J = 7.7 Hz), 1.75 (3H, s), 2.64 (3H, s), 2.94 (2H, q, J = 7.4 Hz), 3.61 (3H, s), 5.01 (1H, dd, J = 14.5, 4.6 Hz), 5.07 (1H, dd, J = 14.5, 4.6 Hz), 6.02 (1H, s), 6.23 (1H, s), 7.00 (1H, brs), 7.07 (1H, d, J = 11.5 Hz), 7.51–7.55 (1H, m), 7.58–7.63 (1H, m), 8.10–8.13 (2H, m), 10.66 (1H, s), 18.83 (1H, s). ^{13}C NMR ($CDCl_3$, 500 MHz) δ: 16.2, 27.1, 28.0, 32.0, 36.7, 56.5, 58.8, 96.4, 98.3, 102.2, 105.2, 106.4, 111.0 (d, J_{CF} = 19 Hz), 121.0 (d, J_{CF} = 5 Hz), 122.8 (d, J_{CF} = 17 Hz), 123.8 (d, J_{CF} = 2 Hz), 125.4, 125.5 (d, J_{CF} = 5 Hz), 127.5, 133.8 (d, J_{CF} = 4 Hz), 142.1 (d, J_{CF} = 7 Hz), 155.7, 157.4, 158.5 (d, J_{CF} = 252 Hz), 160.0, 162.4, 180.4, 191.9, 198.0, 201.6. MS (ESI) m/z: 532 (M + H)$^+$. HRMS (ESI) m/z: 532.17768 (calcd for $C_{30}H_{27}FNO_7$: 532.17716). Anal. Calcd for $C_{30}H_{26}FNO_7$: C, 67.79; H, 4.93; N, 2.64; F, 3.57. Found: C, 67.74; H, 4.92; N, 2.83; F, 3.68.

*4.1.3. 1-Bromo-2-ethenylnaphthalene (**17a**)*

To a suspension of sodium hydride (>55%, 970 mg, 22.2 mmol) in THF (200 mL), methyltriphenylphosphonium bromide (7.66 g, 21.4 mmol) was slowly added at 0 °C. The mixture was stirred at 0 °C for 30 min, then compound **16** (2.01 g, 8.55 mmol) was added. The mixture was stirred at room temperature for 1.5 h. A saturated aqueous NH_4Cl was added to the reaction mixture. The aqueous layer was extracted with hexane. The combined organic layer was washed with brine, and then dried over Na_2SO_4. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane) to give 1.18 g of compound **17a** (59% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.49 (1H, dd, J = 10.7, 1.0 Hz), 5.83 (1H, dd, J = 17.1, 1.0 Hz), 7.39 (1H, dd, J = 17.3, 11.0 Hz), 749–7.52 (1H, m), 7.57–7.60 (1H, m), 7.67 (1H, d, J = 8.8 Hz), 7.76 (1H, d, J = 8.3 Hz), 7.80 (1H, d, J = 8.8 Hz), 8.35 (1H, d, J = 8.8 Hz).

*4.1.4. 1-Bromo-2-(prop-1-en-1-yl)naphthalene (**17b**)*

Compound **17b** was prepared in a similar manner to as described for **17a** as a colorless oil (94% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.81 (1.5H, dd, J = 7.0, 1.5 Hz), 2.00 (1.5H, dd, J = 6.6, 1.6 Hz), 5.92–6.01 (0.5H, m), 6.29–6.38 (0.5H, m), 6.70 (0.5H, d, J = 11.3 Hz), 7.06 (0.5H, d, J = 15.6 Hz), 7.41–7.62 (3H, m), 7.71–7.83 (2H, m),8.33 (0.5H, d, J = 8.2 Hz), 8.34 (0.5H, d, J = 8.2 Hz).

*4.1.5. 1-Bromo-2-(but-1-en-1-yl)naphthalene (**17c**)*

Compound **17c** was prepared in a similar manner to as described for **17a** as a colorless oil (quant.). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.05 (1.5H, t, J = 7.3 Hz), 1.17 (1.5H, t, J = 7.3 Hz), 2.18–2.24 (1H, m), 2.32–2.38 (1H, m), 5.84 (0.5H, dt, J = 11.2, 7.3 Hz), 6.36 (0.5H, dt, J = 15.6, 6.3 Hz)., 6.64 (0.5H, d, J = 11.2 Hz), 7.04 (0.5H, d, J = 16.1 Hz), 7.38–7.63 (3H, m), 7.71–7.82 (2H, m), 8.34–8.32 (1H, m).

*4.1.6. 2-Ethenylnaphthalene-1-carbaldehyde (**18a**)*

To a solution of compound **17a** (1.17 g, 5.02 mmol) in THF (25 mL), n-butyllithium (2.44 mol/L in hexane, 2.50 mL, 6.10 mmol) was added dropwise at −78 °C. The mixture was stirred at −78 °C for 30 min. DMF (0.78 mL, 10.0 mmol) was slowly added, and the mixture was stirred with warming to room temperature for 1 h. A saturated aqueous NH_4Cl was added to the reaction mixture. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 30/1 to 10/1) to give 475 mg of compound **18a** (52% yield) as a light yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.69 (1H, d, J = 10.9 Hz), 5.74 (1H, d, J = 17.2 Hz), 7.47 (1H, dd, J = 17.2, 10.9 Hz), 7.52–7.67 (3H, m), 7.86 (1H, d, J = 8.2 Hz), 8.02 (1H, d, J = 8.6 Hz), 8.97 (1H, d, J = 8.2 Hz), 10.86 (1H, s).

*4.1.7. 2-(Prop-1-en-1-yl)naphthalene-1-carbaldehyde (**18b**)*

Compound **18b** was prepared in a similar manner to as described for **18a** as a light yellow oil (88% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.66 (1.5H, dd, J = 6.8, 1.8 Hz), 2.02 (1.5H, dd, J = 6.6, 1.6 Hz), 6.10–6.22 (1H, m), 6.91 (0.5H, d, J = 11.3 Hz), 7.10 (0. 5H, d, J = 15.6 Hz), 7.34 (0.5H, d, J = 8.6 Hz), 7.49–7.67 (2.5H, m), 7.84 (1H, t, J = 9.8 Hz), 7.96 (0. 5H, d, J = 8.6 Hz), 8.01 (0.5H, d, J = 8.2 Hz), 9.00 (0.5H, d, J = 8.6 Hz), 9.24 (0.5H, d, J = 8.6 Hz), 10.62 (0.5H, s), 10.77 (0.5H, s).

*4.1.8. 2-(But-1-en-1-yl)naphthalene-1-carbaldehyde (**18c**)*

Compound **18c** was prepared in a similar manner to as described for **18a** as a light yellow oil (81% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.98 (1.5H, t, J = 7.6 Hz), 1.17 (1.5H, t, J = 7.3 Hz), 2.01–2.07 (1H, m), 2.34–2.40 (1H, m), 6.02 (0.5H, dt, J = 11.7, 7.3 Hz), 6.21 (0.5H, dt, J = 15.6, 6.3 Hz), 6.86 (0.5H, d, J = 11.7 Hz), 7.08 (0.5H, d, J = 15.6 Hz), 7.33 (0.5H, d, J = 8.3 Hz), 7.50–7.56 (1.5H, m), 7.60–7.67 (1H, m), 7.83 (0.5H, d, J = 8.3 Hz), 7.85 (0.5H, d, J = 8.3 Hz), 7.96 (0.5H, d, J = 8.8 Hz), 8.01 (0.5H, d, J = 8.8 Hz), 9.01 (0.5H, d, J = 8.3 Hz), 9.24 (0.5H, d, J = 8.3 Hz), 10.66 (0.5H, s), 10.77 (0.5H, s).

*4.1.9. 2-Ethylnaphthalene-1-carbaldehyde (**19a**)*

To a solution of compound **18a** (228 mg, 1.25 mmol) in EtOAc (12 mL), 10% palladium-carbon (58 mg) was added. The mixture was stirred under a hydrogen (1 atm) atmosphere for 1 h. Palladium–carbon was separated by filtration. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 30/1 to 10/1) to give 222 mg of compound **19a** (96% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.37 (3H, t, J = 7.6 Hz), 3.16 (2H, q, J = 7.6 Hz), 7.38 (1H, d, J = 8.8 Hz), 7.50–7.53 (1H, m), 7.61–7.64 (1H, m), 7.84 (1H, d, J = 7.8 Hz), 7.99 (1H, d, J = 8.8 Hz), 9.00 (1H, d, J = 8.8 Hz), 10.93 (1H, s). MS (EI) m/z: 184 (M^+).

*4.1.10. 2-Propylnaphthalene-1-carbaldehyde (**19b**)*

Compound **19b** was prepared in a similar manner to as described for **19a** as a colorless oil (94% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.03 (3H, t, J = 7.3 Hz), 1.72–1.79 (2H, m), 3.10 (2H, t, J = 7.8 Hz), 7.36 (1H, d, J = 8.8 Hz), 7.49–7.52 (1H, m), 7.60–7.64 (1H, m), 7.83 (1H, d, J = 8.3 Hz), 7.97 (1H, d, J = 8.3 Hz), 9.02 (1H, d, J = 8.8 Hz), 10.91 (1H, s). MS (EI) m/z: 198 (M^+).

*4.1.11. 2-Butylnaphthalene-1-carbaldehyde (**19c**)*

Compound **19c** was prepared in a similar manner to as described for **19a** as a colorless oil (68% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.96 (3H, t, J = 7.3 Hz), 1.41–1.48 (2H, m), 1.67–1.73 (2H, m), 3.12 (2H, t, J = 8.1 Hz), 7.36 (1H, d, J = 8.3 Hz), 7.50 (1H, t, J = 7.6 Hz), 7.60–7.63 (1H, m), 7.83 (1H, d, J = 7.8 Hz), 7.96 (1H, d, J = 8.8 Hz), 9.02 (1H, d, J = 8.8 Hz), 10.91 (1H, s). MS (EI) m/z: 212 (M^+).

*4.1.12. 2-Bromo-6-chloro-3,4-dihydronaphthalene-1-carbaldehyde (**21a**)*

To a solution of phosphorus tribromide (1.35 mL, 14.2 mmol) in $CHCl_3$ (30 mL), DMF (1.30 mL, 16.7 mmol) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 2 h. Then, a solution of compound **20a** (1.00 g, 5.54 mmol) in $CHCl_3$ (10 mL) was added to the mixture at 0 °C. The resulting mixture was stirred with heating to reflux for 2 h. A saturated aqueous $NaHCO_3$ was added to the reaction mixture. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting light yellow oil (1.11 g) was directly used for the next reaction.

*4.1.13. 2-Bromo-6-chloronaphthalene-1-carbaldehyde (**22a**)*

To a solution of compound **21a** (1.11 g) in toluene (30 mL), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.80 g, 12.3 mmol) was added. The mixture was stirred with heating to reflux for 3 days. The reaction mixture was filtered through Celite, and the solvent was evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/toluene = 10/1) to give 410 mg of compound **22a** (27% yield, two steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, dd, J = 9.2, 1.4 Hz), 7.69–7.80 (3H, m), 9.04 (1H, d, J = 9.0 Hz), 10.69 (1H, s).

*4.1.14. 2-Bromo-6-fluoronaphthalene-1-carbaldehyde (**22b**)*

Compound **22b** was prepared in a similar manner to as described for **22a** as a white solid (34% yield, two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.38–7.45 (2H, m), 7.70 (1H, d, J = 9.0 Hz), 7.79 (1H, d, J = 8.6 Hz), 9.12 (1H, dd, J = 9.4, 5.5 Hz), 10.70 (1H, d, J = 1.2 Hz).

*4.1.15. 6-Chloro-2-methylnaphthalene-1-carbaldehyde (**23a**)*

To a solution of **22a** (405 mg, 1.50 mmol) in toluene (15 mL), Me_4Sn (0.625 mL, 4.51 mmol) and $Pd(PPh_3)_4$ (52 mg, 0.045 mmol) were added. The mixture was stirred with heating to reflux for 9 h. The reaction mixture was filtered through Celite, and the solvent was evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/toluene = 10/1) to give 285 mg of compound **23a** (93% yield) as a light yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 2.80 (3H, s), 7.36 (1H, d, J = 8.3 Hz), 7.53 (1H, dd, J = 9.3, 2.0 Hz), 7.78 (1H, s), 7.83 (1H, d, J = 8.3 Hz), 8.96 (1H, d, J = 9.3 Hz), 10.89 (1H, s). MS (EI) m/z: 204 (M^+).

*4.1.16. 6-Fluoro-2-methylnaphthalene-1-carbaldehyde (**23b**)*

Compound **23b** was prepared in a similar manner to as described for **23a** as a light yellow solid (95% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 2.80 (3H, s), 7.35–7.43 (3H, m), 7.86 (1H, d, J = 8.3 Hz), 9.04 (1H, dd, J = 9.3, 5.9 Hz), 10.90 (1H, s). MS (EI) *m/z*: 188 (M^+).

*4.1.17. 1-Bromo-5-chloronaphthalene-2-carbaldehyde (**26a**)*

Compound **26a** was prepared in a similar manner to as described for **22a** as a white solid (15% yield, two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, dd, J = 8.6, 7.4 Hz), 7.76 (1H, d, J = 7.4 Hz), 8.01 (1H, d, J = 8.6 Hz), 8.33 (1H, d, J = 9.0 Hz), 8.46 (1H, d, J = 8.2 Hz), 10.64 (1H, s).

*4.1.18. 1-Bromo-7-chloronaphthalene-2-carbaldehyde (**26b**)*

Compound **26b** was prepared in a similar manner to as described for **22a** as a white solid (30% yield, two steps). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.63 (1H, dd, J = 8.5, 2.2 Hz), 7.83 (1H, d, J = 8.3 Hz), 7.85 (1H, d, J = 8.3 Hz), 7.94 (1H, d, J = 8.8 Hz), 8.51 (1H, d, J = 2.0 Hz), 10.65 (1H, s).

*4.1.19. 1-Bromo-5-fluoronaphthalene-2-carbaldehyde (**26c**)*

Compound **26c** was prepared in a similar manner to as described for **22a** as a white solid (19% yield, two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.36 (1H, ddd, J = 9.8, 7.8, 0.8 Hz), 7.58–7.64 (1H, m), 7.96 (1H, d, J = 8.6 Hz), 8.13 (1H, d, J = 8.2 Hz), 8.29 (1H, d, J = 8.6 Hz), 10.63 (1H, d, J = 0.8 Hz).

*4.1.20. 1-Bromo-7-fluoronaphthalene-2-carbaldehyde (**26d**)*

Compound **26d** was prepared in a similar manner to as described for **22a** as a white solid (26% yield, two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46 (1H, td, J = 8.5, 2.5 Hz), 7.86–7.92 (3H, m), 8.16 (1H, dd, J = 10.6, 2.3 Hz), 10.66 (1H, s).

*4.1.21. 1-Bromo-5-chloro-2-methylnaphthalene (**27a**)*

To a solution of compound **26a** (546 mg, 2.01 mmol) in diethylene glycol (20 mL), hydrazine monohydrate (0.28 mL, 5.14 mmol) and KOH (340 mg, 6.06 mmol) were added. The mixture was stirred at 180 °C for 1.5 h. After the reaction solution was cooled to room temperature, water was added. The aqueous layer was extracted with Et_2O. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane) to give 340 mg of compound **27a** (66% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.63 (3H, s), 7.42–7.46 (2H, m), 7.54 (1H, dd, J = 7.4, 0.8 Hz), 8.14 (1H, d, J = 8.6 Hz), 8.23 (1H, d, J = 8.6 Hz).

*4.1.22. 5-Chloro-2-methylnaphthalene-1-carbaldehyde (**28a**)*

To a solution of compound **27a** (340 mg, 1.33 mmol) in THF (20 mL), n-butyllithium (2.71 mol/L in hexane, 0.80 mL, 2.17 mmol) was added dropwise at −78 °C. The mixture was stirred at −78 °C for 30 min. DMF (0.42 mL, 5.40 mmol) was slowly added, and the mixture was stirred with warming to room temperature for 1 h. A saturated aqueous NH_4Cl was added to the reaction mixture. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 10/1 to 5/1) to give 126 mg of compound **28a** (46% yield) as a light yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.83 (3H, s), 7.44–7.52 (2H, m), 7.58 (1H, dd, J = 7.4, 0.8 Hz), 8.42 (1H, d, J = 8.6 Hz), 8.87 (1H, d, J = 9.0 Hz), 10.92 (1H, s). MS (EI) *m/z*: 204 (M^+).

*4.1.23. 7-Chloro-2-methylnaphthalene-1-carbaldehyde (**28b**)*

Compound **28b** was prepared in a similar manner to as described for **28a** as a yellow solid (33% yield, two steps) ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.82 (3H, s), 7.34 (1H, d, J = 8.6 Hz), 7.45 (1H, dd, J = 8.6, 2.0 Hz), 7.75 (1H, d, J = 8.6 Hz), 7.91 (1H, d, J = 8.2 Hz), 9.10 (1H, d, J = 2.3 Hz), 10.88 (1H, s). MS (EI) *m/z*: 204 (M^+).

*4.1.24. 1-Bromo-5-fluoro-2-methylnaphthalene (**27c**)*

To a solution of compound **26c** (925 mg, 3.66 mmol) in CH_2Cl_2 (40 mL), tris(pentafluorophenyl)borane (2.06 g, 4.02 mmol) and triethylsilane (5.90 mL, 36.5 mmol) were added. The mixture was stirred at room temperature for 4 days. The reaction solution was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane) to give 428 mg of compound **27c** (49% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.63 (3H, s), 7.12 (1H, dd, J = 10.4, 7.6 Hz), 7.38 (1H, d, J = 8.6 Hz), 7.43–7.48 (1H, m), 7.96 (1H, d, J = 8.2 Hz), 8.05 (1H, d, J = 8.6 Hz).

*4.1.25. 5-Fluoro-2-methylnaphthalene-1-carbaldehyde (**28c**)*

To a solution of compound **27c** (428 mg, 1.79 mmol) in THF (20 mL), n-butyllithium (2.71 mol/L in hexane, 1.00 mL, 2.71 mmol) was added dropwise at −78 °C. The mixture was stirred at −78 °C for 30 min. DMF (0.70 mL, 9.04 mmol) was slowly added, and the mixture was stirred with warming to room temperature for 1 h. A saturated aqueous NH_4Cl was added to the reaction mixture. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 10/1 to 5/1) to give 181 mg of compound **28c** (54% yield) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 2.79 (3H, s), 7.14 (1H, dd, J = 10.3, 7.8 Hz), 7.36 (1H, d, J = 8.8 Hz), 7.49–7.53 (1H, m), 8.19 (1H, d, J = 8.8 Hz), 8.70 (1H, d, J = 8.8 Hz), 10.90 (1H, s). MS (EI) *m/z*: 188 (M^+).

*4.1.26. 7-Fluoro-2-methylnaphthalene-1-carbaldehyde (**28d**)*

Compound **28d** was prepared in a similar manner to as described for **28c** as a yellow solid (21% yield, two steps) ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.83 (3H, s), 7.25 (1H, dd, J = 11.0, 8.6 Hz), 7.28 (1H, d, J = 8.2 Hz), 7.79 (1H, dd, J = 9.0, 5.9 Hz), 7.91 (1H, d, J = 8.2 Hz), 8.78 (1H, dd, J = 12.1, 2.3 Hz), 10.85 (1H, s). MS (EI) *m/z*: 188 (M^+).

*4.1.27. 4-Chloro-2-methylnaphthalene-1-carbaldehyde (**30**)*

To a solution of compound **29** (460 mg, 2.60 mmol) and dichloromethyl methyl ether (0.29 mL, 3.20 mmol) in CH_2Cl_2 (30 mL), titanium tetrachloride (0.35 mL, 3.19 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature for 4 h. Water was added to the reaction mixture. The aqueous layer was extracted with Et_2O. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 10/1 to 5/1) to give 385 mg of compound **30** (72% yield) as a brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.79 (3H, s), 7.47 (1H, s), 7.57–7.61 (1H, m), 7.64–7.68 (1H, m), 8.29 (1H, d, J = 8.2 Hz), 8.96 (1H, d, J = 8.6 Hz), 10.88 (1H, s).

*4.1.28. 4-Fluoro-2-methylnaphthalene-1-carbaldehyde (**32**)*

To a solution of N,N,N′-trimethylethylenediamine (0.78 mL, 6.00 mmol) in THF (40 mL), n-butyllithium (2.71 mol/L in hexane, 2.20 mL, 5.96 mmol) was added dropwise at −20 °C and the mixture was stirred for 15 min. Compound **31** (800 mg, 4.59 mmol) was added to the mixture at −20 °C, and the mixture was stirred for 30 min. After cooling to −78 °C, n-butyllithium (2.71 mol/L in hexane, 5.10 mL, 13.8 mmol) was added dropwise, and the mixture

was stirred with warming to 0 °C for 6 h. Iodomethane (2.30 mL, 36.9 mmol) was further added at 0 °C, and the mixture was warmed to room temperature and stirred for 20 h 1 N hydrochloric acid was added to the reaction mixture. The aqueous layer was extracted with Et_2O. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 10/1). Then, the resulting crude purified product was recrystallized from hexane to give 146 mg of compound **32** (17% yield) as white needles. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.82 (3H, s), 7.02 (1H, d, J = 10.6 Hz), 7.53–7.57 (1H, m), 7.64–7.69 (1H, m), 8.09 (1H, d, J = 8.2 Hz), 9.04 (1H, d, J = 8.6 Hz), 10.85 (1H, s). MS (EI) m/z: 188 (M^+).

4.1.29. 2-Bromo-4-fluoronaphthalene-1-carbaldehyde (**33**)

To a solution of N,N,N′-trimethylethylenediamine (2.90 mL, 22.3 mmol) in THF (150 mL), n-butyllithium (2.67 mol/L in hexane, 8.40 mL, 22.4 mmol) was added dropwise at −30 °C and the mixture was stirred for 30 min. Compound **31** (3.00 g, 17.2 mmol) was added to the mixture at −30 °C, and the mixture was stirred for 30 min. After cooling to −78 °C, n-butyllithium (2.67 mol/L in hexane, 16.0 mL, 42.7 mmol) was added dropwise, and the mixture was stirred with warming to 0 °C for 3 h 1,2-Dibromo-1,1,2,2-tetrafluoroethane (8.30 mL, 69.0 mol) was further added at 0 °C, and the mixture was warmed to room temperature and stirred for 16 h 1 N hydrochloric acid was added to the reaction mixture. The aqueous layer was extracted with Et_2O. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 20/1 to 10/1) to give 2.35 g of compound **33** (54% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.44 (1H, d, J = 9.4 Hz), 7.65 (1H, t, J = 7.6 Hz), 7.72–7.76 (1H, m), 8.12 (1H, d, J = 8.2 Hz), 9.22 (1H, d, J = 8.6 Hz), 10.70 (1H, s).

4.1.30. 2-Ethyl-4-fluoronaphthalene-1-carbaldehyde (**34**)

To a solution of compound **33** (38.0 g, 150 mmol) in DMF (500 mL), diethylmethoxyborane (30.0 mL, 228 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (3.70 g, 4.53 mmol), and K_2CO_3 (52.0 g, 376 mmol) were added. The mixture was stirred at 70 °C for 2.5 h 2 N hydrochloric acid was added to the reaction mixture. The aqueous layer was extracted with Et_2O. The combined organic layer was washed with brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc = 10/1) to give 27.4 g of compound **34** (90% yield) as a light yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.38 (3H, t, J = 7.6, Hz), 3.16 (2H, q, J = 7.6 Hz), 7.06 (1H, dd, J = 10.9, Hz), 7.55–7.59 (1H, m), 7.66–7.71 (1H, m), 8.12 (1H, d, J = 8.2 Hz), 9.07–9.10 (1H, m), 10.83 (1H, s). MS (EI) m/z: 202 (M^+).

4.1.31. Potassium (9bS)-2-acetyl-6-{[(2-ethyl-4-fluoronaphthalen-1-yl)methyl]carbamoyl}-9-hydroxy-7-methoxy-9b-methyl-1-oxo-1,9b-dihydrodibenzo[b,d]furan-3-olate (**35**)

To a solution of compound **15** (8.17 g, 15.4 mmol) in CH_2Cl_2–EtOH (1:1, 150 mL), 1 N aqueous potassium hydroxide (15.4 mL, 15.4 mmol) was added slowly. The mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure, and the resulting residue was purified by recrystallization from EtOAc/toluene to afford 4.10 g of compound **35** (47% yield) as a light yellow solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 1.26 (3H, t, J = 7.6 Hz), 1.46 (3H, s), 2.17 (3H, s), 2.93 (2H, q, J = 7.6 Hz), 3.60 (3H, s), 4.81 (2H, d, J = 4.9 Hz), 5.23 (1H, s), 6.07 (1H, s), 7.26 (1H, d, J = 12.2 Hz), 7.60 (1H, t, J = 7.3 Hz), 7.67 (1H, t, J = 7.6 Hz), 8.03 (1H, d, J = 8.3 Hz), 8.18 (1H, d, J = 8.3 Hz), 8.38 (1H, t, J = 4.9 Hz), 13.32 (1H, s). ^{13}C NMR (DMSO-d_6, 500 MHz) δ: 15.7, 26.1, 31.8, 32.5, 35.9, 54.4, 55.7, 94.2, 102.1, 102.7, 106.6, 110.1, 110.7, (d, J_{CF} = 18 Hz), 119.8 (d, J_{CF} = 6 Hz), 121.6 (d, J_{CF} = 17 Hz), 124.8, 125.5, 126.6 (d, J_{CF} = 4 Hz), 127.1, 133.6 (d, J_{CF} = 4 Hz), 141.7 (d, J_{CF} = 7 Hz), 154.6, 155.2, 157.3 (d, J_{CF} = 250 Hz), 158.6, 162.4, 172.4, 185.7, 191.3, 196.3. Anal. Calcd for $C_{30}H_{25}FNKO_7$: C, 63.26; H, 4.42; N, 2.46; F, 3.34; K, 6.86. Found: C, 63.27; H, 4.39; N, 2.55; F, 3.33; K, 7.14.

4.2. Full-length human PPARγ reporter gene assay

MG-63 cells culture and preparation of plasmids were described in a previous report [20].

Transiently transfected MG-63 cells were prepared using Lipofectamine™ (Invitrogen Corporation) reagent according to the manufacturer's protocol. The transfection mixture for each 500 cm^2-cell tray (Sumitomo Bakelite Co., Ltd.) containing 0.0504 mg of pSG5-hPPARγ, 0.0504 mg of pGV-P2-PPRE, and Lipofectamine reagent at a ratio of 1.4 (ml of reagent/mg of DNA) was exposed for 4 h in serum-, antibiotics-free medium. The cells were trypsinized and replated onto 96-well plates at 40,000 cells/well. After 24 h of incubation and the replacement of the medium, various concentrations of the test compound were added to the culture medium of the transfected cells for 20 h. A luciferase assay was performed using a Picagene LT 2.0 Luminescence Reagent (TOYO INK, Co., Ltd.). The luciferase activity was measured with a luminometer Analyst (Molecular Devices, Inc.).

The median effective concentration values (EC_{50}) of partial agonists were defined as follows: The transcriptional activity in the presence of 3.3 nM in-house thiazolidinedione compound, 5-(4-{[6-(4-hydroxy-3,5-dimethylphenoxy)-1-methyl-1*H*-benzimidazol-2-yl]methoxy}benzyl)-1,3-thiazolidine-2,4-dione, was defined as 100%, while that in the vehicle alone was defined as zero. The maximum transcriptional activity of the test compound alone was defined as the maximum efficacy (E_{max}, %). The concentration of the test compound indicating a half value of E_{max} was defined as the EC_{50} value in the transcriptional activity. Values of each parameter were determined by nonlinear curve fitting using GraphPad Prism 4.0 (GraphPad Software Inc.).

4.3. Evaluations of PPAR subtypes selectivity

GAL4-human PPARγ chimera receptor expression vector, pM-hPPARγ, expressed the ligand-binding domain of PPARγ as a fusion protein with the DNA-binding domain of yeast transcription factor GAL4. The pG5luc vector (included in CheckMate Mammalian Two-Hybrid System, Promega Corporation), which contains five tandem repeats of a GAL4-binding DNA sequence upstream of minimal TATA to facilitate the detection of firefly luciferase activity induced by a GAL4-hPPARγ fusion protein, was used as a GAL4-dependent reporter vector. Dulbecco's Modified Eagle Medium (DMEM, Invitrogen Corporation) containing 10% (v/v) heat-inactivated fetal bovine serum (FBS, Invitrogen Corporation) was prepared as a culture medium. The COS7 cells were cultured in the culture medium at 37 °C with 5% CO_2 (5% CO_2, 95% air). The COS7 cells were cultured to the confluent in 75 cm^2 culture flasks. Cells were transfected with 4.8 μg of the pM-hPPARγ and 19.2 μg of pG5luc using Lipofectamine 2000 (Invitrogen Corporation) in Opti-MEM I reduced serum medium (Opti-MEM I, Invitrogen Corporation) according to the manufacturer's instruction. After the transfection, the COS7 cells were harvested and re-seeded in 96-well white plates and cultured for about 24 h in a CO_2 incubator. The pM-PPARα and pM-PPARδ expression vectors were used for the GAL4-PPARα-LBD and GAL4-PPARδ-LBD reporter gene assays, respectively.

The serial dilutions of the test compounds and the control solution (0.1% DMSO) were added into the individual wells in the 96-well plates and the cells were further incubated for about 24 h in a CO_2 incubator. A luciferase assay was performed using a Picagene LT 2.0 Luminescence Reagent (TOYO INK, Co., Ltd.) according to the manufacturer's instruction. The light intensity in each well was measured using a Multimode Microplate Reader (Analyst GT, Molecular Devices, Inc.). Rosiglitazone, 2-(4-tert-Butylphenoxy)-3-[4-[2-[(4-pyridin-2-ylbenzoyl)amino]ethoxy]phenyl]propionic acid [21], and GW501516 [22] (Enzo Life Sciences, Inc.) were used as positive references for the PPARγ, PPARα, and PPARδ tests, respectively. The maximum transcriptional activity of the test compound alone was defined as the maximum efficacy (E_{max}, %). The concentration of the test compound indicating a half value of E_{max} was defined as the EC_{50} value. Values of each parameter were determined by nonlinear curve fitting using GraphPad Prism 4.0 (GraphPad Software Inc.).

4.4. X-ray crystallography

Crystals of PPARγ-LBD complexed with a peptide derived from SRC-1(RHKILHRLLQEGSPS) and compound **15** were obtained using hanging drop vapor diffusion at 22 °C. The well solution contained 24% (w/v) PEG4000, 0.2 M sodium thiocyanate, and 0.1 M Tris–HCl (pH 8.5). The crystal was transferred to the well solution containing an additional 8% PEG400 as a cryoprotectant, and was flash frozen. X-ray diffraction data were collected using in-house X-ray generator FR-E with detector R-AXIS VII (RIGAKU Corporation) and processed with HKL2000 (HKL Research, Inc.) [23]. The structure of PPARγ-LBD derived from 3LMP.pdb was used as an initial model. Several rounds of manual rebuilding with O [24] followed by refinement with CNX (Accerlys K.K.) [25] were carried out.

4.5. Pharmacokinetics

Male F344/DuCrlCrlj (Fischer) rats were purchased at 7 weeks old from Charles River Laboratories Japan, Inc. For acclimation, they were housed in stainless steel cages for 7–11 days in the controlled animal area. The rats were allowed free access to FR2 laboratory food (Funabashi Farm Co., Ltd.) and tap water.

NIKKOL HCO-60 (Nikko Chemicals Co., Ltd.) was melted at 60 °C and mixed well in saline to make a 20% (v/v) HCO-60 solution. A 5% (v/v) HCO-60 solution was prepared to dilute a 20% (v/v) HCO-60 solution with saline. Compound **35** was dissolved in a 20% (v/v) HCO-60 solution for oral administration and a 5% (v/v) HCO-60 solution for intravenous administration. For the administration, 2 mL/kg of the solution at a concentration of 5 mg/mL was used. The solutions of compound **35** were administered to male Fischer rats, after overnight fasting. A blood sample of approximately 0.2 mL was collected from the jugular vein with a heparinized syringe. The blood was centrifuged at 14,000 rpm for 3 min at 4 °C (himac CR15D, Hitachi Koki Co., Ltd. rotor: RT15A2) to obtain the plasma. The plasma was stored frozen at −20 °C until use for measurement of plasma concentration. The determination of the plasma concentration of compound **15** was performed by LC-MS/MS method using API 4000QTRAP (Applied Biosystems/MDS SCIEX). PK parameters were calculated using a non-compartmental model by the computer software WinNonlin Professional version 4.0.1. (Pharsight Corporation).

4.6. In vivo pharmacology

4.6.1. Animals

Five-week-old female ZDF rats and 7-week-old female Wistar–Imamichi rats were obtained from Charles River Laboratories Japan, Inc. and Institute of Animal Reproduction, respectively. The rats were acclimatized for over 1 week and provided with water and MR-DBT containing 10% lard (Nosan Corporation) for ZDF rats and FR2 (Funabashi Farms Co., Ltd.) for Wistar–Imamichi rats *ad libitum*, respectively. All experimental procedures were performed in accordance with the guideline of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

4.6.2. Pharmacological effects in type 2 diabetes model

Body weight (BW), plasma glucose (PG), insulin, hematocrit (Hct), hemoglobin (Hb), the number of red blood cells (RBC) and mean corpuscular volume (MCV) of ZDF rats were used for randomization into treatment groups on Day-1. Rats ($n = 5$ per treatment group) were treated once daily by oral gavages with vehicle (0.5% methylcellulose), compound **35** (0.01, 0.1, 1, 10, 100 mg/kg), or rosiglitazone (0.01, 0.1, 1, 10, 100 mg/kg) from Day 0 to Day 11 for a total of 12 days. Body weight was recorded daily. Blood was collected from a tail vein into a heparinized capillary tube on Day 12 and plasma samples were obtained after centrifugation at 11000 rpm for 5 min (MC-150 [rotor: TH-1, TH-2 or TH-11], Tomy Seiko Co., Ltd.).

PG (plasma glucose) (Glucoroder-GXT, Shino-Test Corporation), TG (triglyceride) (TG E-test Wako, Wako Pure Chemical Industries, Ltd.), Insulin (High range speedy Insulin ELISA, Morinaga Institute of Biomedical Science Inc.) and, FFA (free fatty acid) (NEFA C-test Wako, Wako Pure Chemical Industries, Ltd.) were determined according to manufacturer's protocol.

4.6.3. PPARγ agonist-related adverse effects

BW was used for randomization into treatment groups on Day-1. Rats ($n = 5$ per treatment group) were treated once daily by oral gavages with vehicle (0.5% methylcellulose), compound **35** (100 mg/kg) or rosiglitazone (30, 100, 300 mg/kg) from Day 0 to Day 13 for a total of 14 days. BW was recorded daily. On Day 14, after the animals were retained in a holder, 0.05% Evans blue (EB) solution prepared with physiological saline (OTSUKA NORMAL SALINE: Otsuka Pharmaceutical Industries) was administered to unanesthetized rats via a tail vein at a volume of 0.1 mL/100 g body weight using a syringe (1 mL: Terumo Corporation) and a wing-type needle (27G or 25G, Terumo Corporation). Seven minute later, the animals were anesthetized with ether. Blood was sampled from the abdominal aorta using a wing-type needle (19G, Terumo Corporation) at 10 min after the EB administration. The heart was removed and the weight was measured. The collected blood samples were placed in separating agent-containing spitz tubes (Coagulation Rapid Tube S: Shino-Test Corporation). The plasma samples were obtained by centrifuging the blood with a heparin sodium injection solution at 3000 rpm at 4 °C for 10 min with a refrigerated centrifuge (CF7D2: [rotar T3S6-A006] Hitachi Koki Co., Ltd.). To deproteinize, the obtained plasma samples were mixed and stirred with 100% (w/v) trichloroacetic acid solution (1:1), and stood at room temperature for 10 min or more. Furthermore, to release the EB bound with plasma proteins, the samples were sonicated (OUTPUT 2, DUTY CONT., TIME 2 min) on ice using a sonicator (UD-201: Tomy Seiko Co., Ltd.). Then, the samples were centrifuged at 3000 rpm at 4 °C for 10 min using the refrigerated centrifuge. The supernatant was used for the measurement. The absorbance (620 nm) of the obtained supernatant was measured using a spectrophotometer (DU7500: Beckman-Coulter Co., Ltd.). The circulating plasma volume (P.V.) was calculated from the following calibration curve: Concentrations of quality control (QC) samples: 0, 0.0001, 0.0002, 0.0004 and 0.0008% (w/v) EB solution (dissolved in saline) mixed and stirred with 100% (w/v) trichloroacetic acid solution (1:1).

Equation of calibration curve : $Y = aX + b$

where Y: absorbance at 620 nm, a: slope, b: *Y* intercept, X: concentration of EB(%).

P.V. (mL) = V (mL) × 0.05 (%)/[(Y − b)/a] (%) − V (mL), V: EB injection volume (mL).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.05.040. These data include MOL files and InChiKeys of the most important compounds described in this article.

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