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Discovery of a novel class of 1,3-dioxane-2-carboxylic acid derivatives as subtype-selective peroxisome proliferator-activated receptor α (PPARα) agonists

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Abstract—A new series of 1,3-dioxane-2-carboxylic acid derivatives was synthesized and evaluated for agonist activity at human peroxisome proliferator-activated receptor (PPAR) subtypes. Structure-activity relationship studies led to the identification of 2-methyl-*c*-5-[4-(5-methyl-2-phenyl-1,3-oxazol-4-yl)butyl]-1,3-dioxane-*r*-2-carboxylic acid **4b** as a potent PPARα agonist with high subtype selectivity at human receptor subtypes. This compound exhibited a substantial hypolipidemic effect in type 2 diabetic KK-A^y mice.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors and members of the thyroid/steroid hormone nuclear receptor superfamily.[1] Three subtypes, PPARα, PPARγ and PPARδ, exhibit different tissue distributions and play important roles in lipid, lipoprotein and glucose homeostasis. PPARα, abundant in metabolically active tissues such as liver, heart, kidney and muscle, regulates fatty acid catabolism.[2] Hypolipidemic fibrate drugs (fibrates), an important class of PPARα ligands, lower serum triglyceride levels and moderately raise high-density lipoprotein (HDL) cholesterol levels in patients with hyperlipidemia. However, recent molecular-pharmacological studies demonstrate that fibrates, such as fenofibrate **1a** and bezafibrate **2** (Fig. 1), show significant cross-reactivity with other PPAR subtypes.[3] For example, fenofibric acid **1b**, the active metabolite of fenofibrate **1a**, is a dual agonist for PPARα and PPARγ, while bezafibrate **2** activates all three PPAR subtypes at comparable doses. In addition, the agonist activities of these two compounds at PPARα are weak, and the clinical doses required to treat hyperlipidemia are relatively high (200–400 mg/day in Japan). Therefore, more potent and selective agonists for PPARα are expected to have superior therapeutic efficacy in treating these conditions.[4] Although some potent and selective PPARα agonists have already been reported,[5–8] they are not yet in clinical use.

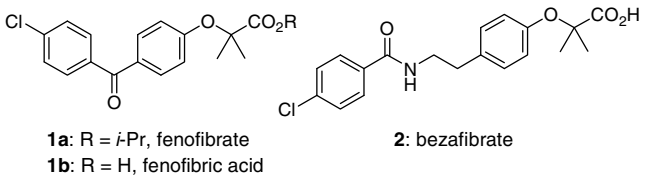

Figure 1. Chemical structures of fibrates.

During our efforts to identify compounds with lipid-lowering effects in KK-A^y mice, we found 2-methyl-2-[6-(5-methyl-2-phenyl-4-oxazolyl)hexyloxy]propionic acid **3** (Fig. 2) to have potent lipid-lowering activity. (KK-A^y mice display severe hyperglycemia, hyperinsulinemia, hypertriglyceridemia and obesity, the typical symptoms of type 2 diabetes, and are widely used as animal models of type 2 diabetes.[9]) When orally administered at a dose of 1 mg/kg once a day for 4 days, this compound lowered plasma triglyceride levels by 32%. The compound has a flexible conformation due to the long alkylene

Keywords: PPARα agonist; 1,3-Dioxane carboxylic acid; Metabolic disorder.
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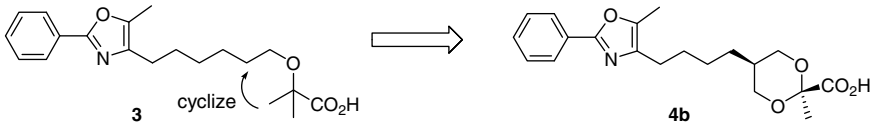
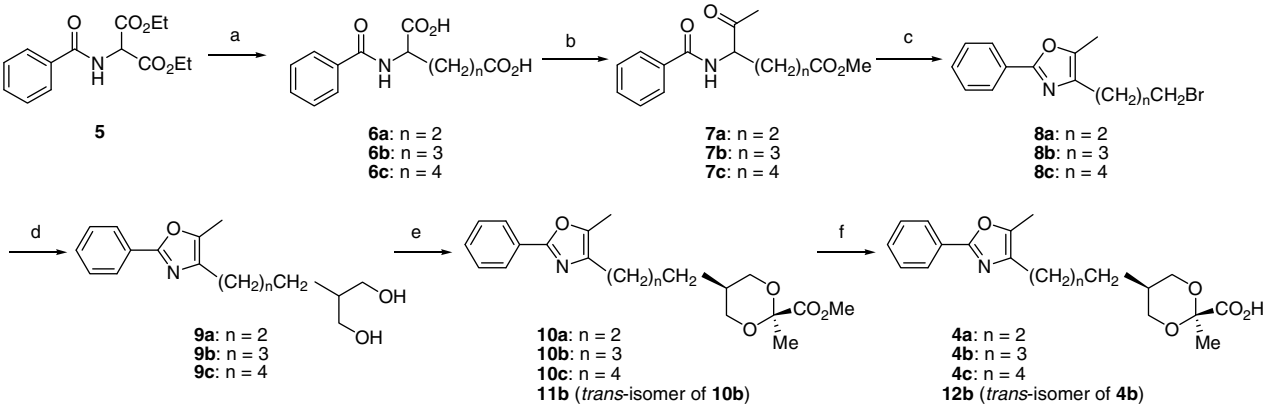


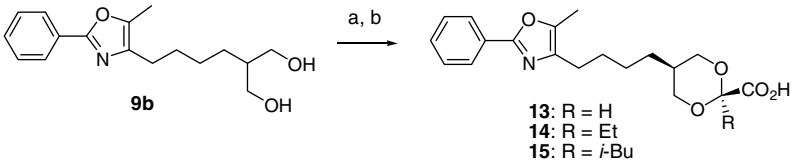
Figure 2. Structural modification of **3**.

chain linking the oxazole ring and carboxyl group, and it seemed to us that the potency of the compound could be enhanced by restricting its conformation. To test this hypothesis, we designed and synthesized 1,3-dioxane-2-carboxylic acid derivative **4b** (Fig. 2), whose conformation is significantly restricted by cyclization of the carboxylic acid tail of **3**. As expected, this compound exhibited superior in vivo pharmacological activity. During our investigation of their lipid-lowering activity, we found that some 1,3-dioxane-2-carboxylic acid derivatives had strong and highly subtype-selective agonist activity at human PPARα. To gain a better understanding of their pharmacological characteristics, we investigated the effect of the structure of the linker and of the dioxane moiety on their PPAR subtype agonist activities.

The general method for the synthesis of compounds **4a**–**c** and **12b** with an alkylene tether between the oxazole ring and the dioxane ring is shown in Scheme 1. Diethyl 2-(benzoylamino)malonate **5**[10] was alkylated with one of three commercially available ethyl bromoalkylcarboxylates, and subsequent alkaline hydrolysis and decarboxylation gave the dicarboxylic acids **6a**–**c**. After Dakin–West conversion[11] of compounds **6a**–**c** to the corresponding methyl ketones, esterification of the remaining carboxyl group afforded the methyl esters **7a**–**c**. Cyclodehydration[12] of **7a**–**c** by treatment with phosphorus oxychloride gave the corresponding oxazoles, which after reduction with lithium aluminium hydride followed by bromination of the resulting alcohols gave the bromides **8a**–**c**. Alkylation of diethyl malonate with **8a**–**c** yielded the diesters, which were converted to the dialcohols **9a**–**c** by reduction with lithium aluminium hydride. Formation of the dioxane ring from the diols **9a**–**c** with methyl pyruvate on refluxing with boron trifluoride in acetonitrile yielded a mixture of geometrical isomers, which was resolved by column chromatography to afford the *cis*-isomers **10a**–**c** and one *trans*-isomer **11b**. The geometrical isomers **10b** and **11b** have different chemical-shift patterns of the methylene protons at the 4- and 6-positions and the methine proton at the 5-position of the 1,3-dioxane ring moiety, which are identical to those observed by Harabe et al.[13] The configurations of the protons were also confirmed by NOE measurements.[14] The ratio of *cis* to *trans* isomers was approximately 3:1 by gas chromatographic analysis. Finally, the target compounds **4a**–**c** and **12b** were obtained by alkaline hydrolysis of the esters **10a**–**c** and **11b**. Compounds **13**–**15**, which have different substituents at the 2-position of the 1,3-dioxane ring, were prepared from **9b** and the requisite α-ketoesters as outlined in Scheme 2. The configuration of the compounds was determined to be *cis* from the chemical-shift patterns



Scheme 1. Reagents: (a) i—$Br(CH_2)_nCO_2Et$, NaOEt, EtOH, ii—aq NaOH, EtOH, iii—AcOEt, xylene; (b) i—Ac_2O, pyridine, ii—H_2O, iii—H_2SO_4, MeOH; (c) i—$POCl_3$, toluene, ii—$LiAlH_4$, Et_2O, iii—CBr_4, Et_2O; (d) i—$CH_2(CO_2Et)_2$, NaH, THF, ii—$LiAlH_4$, Et_2O; (e) i—$MeCOCO_2Me$, $BF_3{\cdot}Et_2O$, MeCN; (f) aq NaOH, MeOH.



Scheme 2. Reagents: (a) i—$RCOCO_2Me$, $BF_3{\cdot}Et_2O$, MeCN, (b) aq NaOH, MeOH.

of the 1,3-dioxane ring moiety of the corresponding esters or carboxylic acids, judging from their similarity to the ^{1}H NMR spectrum of **10b** and the observations of Harabe et al.[13]

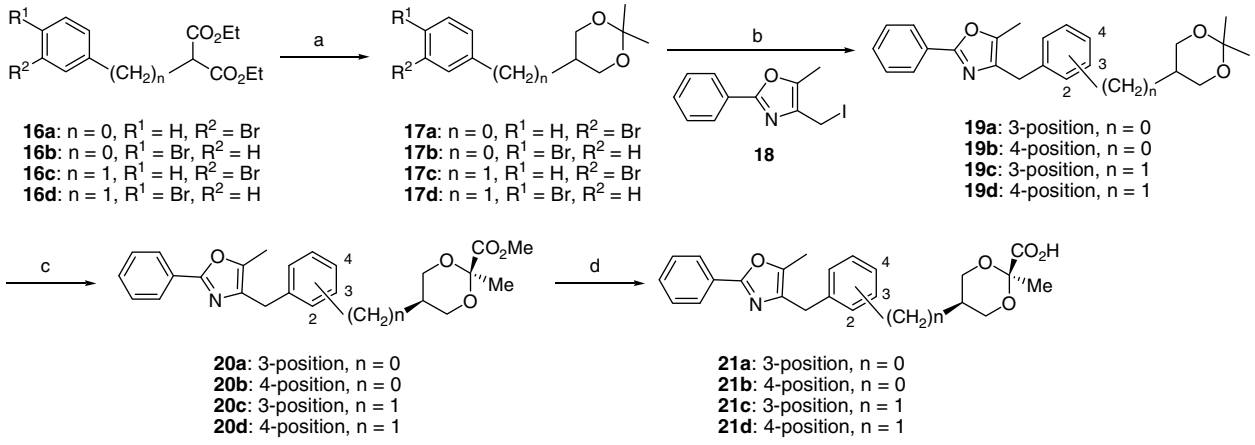
The synthesis of compounds **21a–d**, in which a phenyl ring is included within the methylene linker, is shown in Scheme 3. The starting materials **16a**,**b** and **16c**,**d** were prepared from ethyl 3- or 4-bromophenylacetate and 3- or 4-bromobenzylbromide, respectively, according to a known method.[15,16] Compounds **16a–d** were reduced with diisobutylaluminium hydride and then protected by acetonide formation to give the dioxanes **17a–d**. The oxazole **18** was easily derived from 4-chloromethyl-5-methyl-2-phenyloxazole[17] by refluxing with sodium iodide in acetone. The coupling of **17a–d** and **18** was accomplished by a procedure similar to that previously outlined[18,19] to give **19a–d**. After deprotection by cleavage of the acetonides **19a–d** with pyridinium *p*-toluenesulfonate, the final compounds **21a–d** were prepared through **20a–d** in a stepwise manner similar to that described for the preparation of **4a–c**.

The newly synthesized compounds were screened for activity against each of the human PPAR subtypes by using an established cell-based transactivation assay in CV-1 fibroblast cells as described previously.[20] The initial lead compound **3** was found to be a potent dual agonist of PPARα (EC_{50} = 0.3 μM) and PPARγ EC_{50} = 0.3 μM) with 100-fold selectivity over PPARδ (EC_{50} = 30 μM). Unexpectedly, the dioxane **4b** derived from **3** had even higher agonist activity at PPARα, whereas it activated PPARγ and PPARδ hardly at all. Compound **4b** is a potent and selective agonist of human PPARα with more than 300-fold selectivity over the γ and δ subtypes. The *trans*-isomer **12b** was 10-fold less potent as an agonist of PPARα, a characteristic which reduces its PPARα selectivity.

We next turned our attention to the conformationally flexible alkylene linker of **4b** and investigated the effects of its length and rigidity (Table 1). Reducing the length of the alkylene linker of **4b** by one methylene unit gave a compound, **4a**, which was inactive at all PPAR subtypes. In contrast, increasing the length of the linker gave a compound, **4c**, whose PPARα agonist activity was enhanced threefold compared to **4b**. Compound **4c** was found to be an essentially equipotent dual PPARα/PPARγ agonist with activation characteristics more similar to **3** than to **4b**, indicating that the length of the linker in this series has a dramatic effect on PPARγ agonist activity. We also synthesized four compounds **21a–d** in which a phenyl ring is inserted into the side-chain tether of **4b** to restrict its conformational flexibility. Although **21a**, with its *meta*-substituted phenyl linker, showed PPARα selectivity, its PPARα agonist activity was 10-fold lower than that of **4b**. On the other hand, *para*-substituted variant **21b** had substantially less activity at all PPAR subtypes. Increasing the length of the linker in **21a**,**b** by one methylene unit led to **21c**,**d**, which moderately activated both the α and the γ subtypes. These results strongly support the notion that the potency and selectivity for PPARα are highly dependent on the spatial relationship between the phenyloxazole moiety and the carboxyl group.

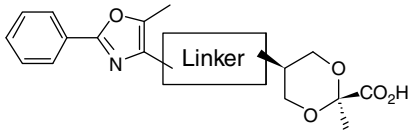
To confirm the requirement for a substituent at the 2-position of the 1,3-dioxane ring, we next explored a limited number of compounds **13–15** (Table 2). Compounds **14** (R = Et) and **l5** (R = *i*-Bu) retained potent PPARα activity and selectivity, with 10 times the potency of **4b** and equal PPARα selectivity, so that medium-sized alkyl groups were well tolerated. In contrast, replacement of the 2-position methyl group with hydrogen afforded a compound, **13**, that was inactive at all three PPAR subtypes. These data indicate that an alkyl group with suitable bulk at this position is indispensable for the display of potent PPARα agonist activity.

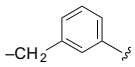
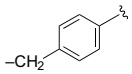
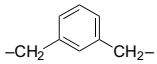
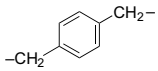
We examined in type 2 diabetic KK-A^y mice the pharmacological effects of two compounds, **4b** and **14**, with excellent human PPARα agonist activity and subtype selectivity (Table 3). Even though they activated mouse PPARα threefold less potently than they did human PPARα, they had sufficient in vitro activity (**4b**, EC_{50} = 1 μM; **14**, EC_{50} = 0.1 μM) with high selectivity



Scheme 3. Reagents: (a) i—diisobutylaluminium hydride, toluene, ii—acetone, TsOH, benzene; (b) *n*-BuLi, *n*-Bu_3P·CuI, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, THF; (c) i—pyridinium *p*-toluenesulfonate, EtOH, ii—$MeCOCO_2Me$, BF_3·Et_2O, MeCN; (d) aq NaOH, MeOH.

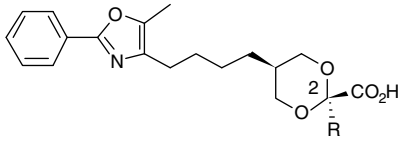
Table 1. Effects of linker variations in *cis*-1,3-dioxane-2-carboxylic acid derivatives on human PPAR agonist activity



Compound	Linker	Human PPAR subtype (EC_{50}, μM)[a,b]		
		α	γ	δ
3	(Lead compound)	0.3	0.3	30
4a	$–(CH_2)_3–$	(8%)[c]	(2%)	(12%)
4b	$–(CH_2)_4–$	0.3	100	(0%)
4c	$–(CH_2)_5–$	0.1	0.3	(34%)
21a		3	(12%)	(7%)
21b		(5%)	(10%)	(0%)
21c		3	1	(28%)
21d		3	3	(38%)
12b	$–(CH_2)_4–$ (*trans*-1,3-dioxane)	3	30	(10%)
1b	(Fenofibric acid)	30	>100	>100
2	(Bezafibrate)	30	100	30

[a] The agonist activity of each PPAR subtype was measured by the corresponding transactivation assay in transiently transfected CV-1 cells ($n = 2$).
[b] EC_{50}, the concentration of test compounds that gave 50% of the maximum transactivation activity of each positive control.
[c] Less than 50% of maximum activity at 10 μM. The percentage of maximum activation observed at 10 μM is shown in parentheses.

Table 2. Effects of substituents at the 2-position of the 1,3-dioxane ring in 1,3-dioxane-2-carboxylic acid derivatives on human PPAR agonist activity



Compound	R	Human PPAR subtype (EC_{50}, μM)[a,b]		
		α	γ	δ
4b	Me	0.3	100	(0%)[c]
13	H	(8%)	(0%)	(3%)
14	Et	0.03	10	(19%)
15	*i*-Bu	0.03	10	(6%)

[a,b,c] See corresponding footnotes to Table 1.

Table 3. Hypolipidemic and hypoglycemic effects of 1,3-dioxane-2-carboxylic acid derivatives **4b** and **14** in diabetic KK-A^y mice

Compound	Dose (mg/kg)	% Change			
		TG	PG	(V)LDL-C	HDL-C
4b	1	−46	−12	−29	36
	3	−56	−13	−49	55
14	3	−52	−18	−26	5
Fenofibrate	300	−40	−25	−31	17

Compounds were orally administered to male KK-A^y mice (aged 10 weeks, $n = 5$) once a day for 4 days at the indicated dose.
Each value represents the percentage change of the mean value from the control group. TG, plasma triglyceride; PG, plasma glucose; (V)LDL-C, very-low-density plus low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

for mouse PPARα over mouse PPARγ and δ to justify in vivo evaluation of their potential benefit (Table 4). Each compound was orally administered to 10-week-old male KK-A^y mice once a day for 4 days at 1–3 mg/kg. Both compounds markedly lowered plasma triglyceride levels (TG) and very-low-density plus low-density lipoprotein cholesterol levels ((V)LDL-C)), while they raised HDL cholesterol levels (HDL-C). These compounds also decreased plasma glucose (PG) levels. Compound **4b** in particular, at a dose of 1 mg/kg, lowered TG to 46% of the levels of vehicle-treated animals, although it was less potent in vitro than **14**. The reason for the apparent discrepancy between the in vitro and in vivo results for these compounds is unclear, but it may be due, for example, to differences in distribution or metabolism between the two compounds. By contrast, fenofibrate **1a** showed similar effects only at a dosage as high as 300 mg/kg. These results provide clear evidence that potent PPARα agonists can have high efficacy in the treatment of hyperlipidemia and hyperglycemia.

Table 4. In vitro activity of **4b** and **14** at mouse PPARα, β and δ subtypes

Compound	Mouse PPAR subtype (EC_{50}, μM)[a,b]		
	α	γ	δ
4b	1	(21%)[c]	(6%)
14	0.1	10	(7%)

[a,b,c]See corresponding footnotes to Table 1.

In conclusion, we have synthesized a series of novel 1,3-dioxane-2-carboxylic acid derivatives and evaluated their PPAR subtype agonist activities. Structure-activity relationship studies on the linker and the dioxane moiety of this series revealed that the biological activity and selectivity were sensitive to the length of the linker and the geometrical configuration of the 1,3-dioxane ring. This investigation led to the identification of several compounds as highly potent and selective human PPARα agonists, and the representative compound **4b** showed excellent hypolipidemic activity in diabetic KK-A^y mice. Our results suggest that the development of potent and selective PPARα agonists will be useful in the treatment of hyperlipidemia and metabolic disorders in type 2 diabetes.

Acknowledgments

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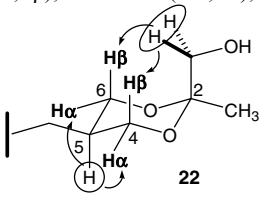
14. The configuration of the 1,3-dioxane ring moiety of **4b** was determined to be *cis* by ^{1}H NMR analysis of the corresponding alcohol **22**, which has protons that allow such a determination. The alcohol derivative **22** was synthesized by $LiAlH_4$ reduction of purified oxazole methyl ester **10b** free of *trans*-isomer. The α-protons at the 4- and 6-positions were clearly distinguished from the β-protons by the NOE correlation observed between the 5-α-and 4-α(6-α)-protons. The strong NOE from the 4,6-β-protons assigned above to the 2-hydroxymethyl protons shows conclusively that the configuration is *cis*. The strong NOE in **12b** between the 4,6-β- and the 2-methyl protons indicates a *trans*-configuration. ^{1}H NMR of **22** (pyridine-d_5, 300 MHz) δ1.25–1.30 (4H, m), 1.70 (2H, m), 1.71 (3H, s), 1.75 (1H, m, H-5α), 2.23 (3H, s), 2.48 (2H, t, J = 7.5 Hz), 3.72 (2H, dd, J = 11.7, 7.5 Hz, H-4,6β), 3.98 (2H, dd, J = 11.7, 4.5 Hz, H-4,6α), 4.07 (2H, d, J = 4.2 Hz, 2β-CH_2OH), 7.21–7.58 (3H, m), 8.16–8.20 (2H, m). ^{1}H NMR of **12b** (tetrahydrofuran-d_8, 300 MHz) δ 1.10–1.31 (2H, m), 1.36 (3H, s, 2β-CH_3), 1.38–1.46 (1H, m, H-5α), 1.65–1.77 (4H, m), 2.31 (3H, s), 2.49 (2H, t, J = 7.5 Hz), 3.67 (2H, dd, J = 11.7, 1.8 Hz, H-4,6α), 4.00 (2H, dd, J = 11.7, 1.5 Hz, H-4,6β), 7.35–7.43 (3H, m), 7.93–7.96 (2H, m).



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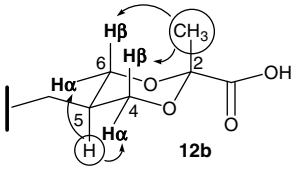

12b

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