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Design and synthesis of highly potent and selective human peroxisome proliferator-activated receptor α agonists

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Abstract—A combination of benzoxazole, phenoxyalkyl side chain, and phenoxybutyric acids was identified as a highly potent and selective human peroxisome proliferator-activated receptor α (PPARα) agonist. The synthesis, structure–activity relationship (SAR) studies, and in vivo activities of the representative compounds are described.

Fibrates are used in the treatment of lipid abnormalities to decrease triglyceride (TG) levels and increase HDL-cholesterol (HDL-C) level moderately.[1] In many intervention studies, fibrates showed beneficial effects in preventing the progression of atherosclerotic lesions and cardiovascular events in both non-diabetic and diabetic patients.[2] These effects are attributed to PPARα activation, which enhances lipid catabolism, decreases TG by lowering apoC-III synthesis, and increases HDL-C by induction of apoA-I, A-II.[3] In more recent studies, PPARα activation was found to exert anti-inflammatory effects in blood vessels.[4]

Although fibrates have been shown to have clinical benefits, their PPARα agonistic activity and subtype-selectivity are poor.[5]

We postulated that highly potent and selective PPARα agonists would be useful drugs for dyslipidemia and atherosclerosis without body weight gain, edema, or other adverse effects observed with PPARγ activation.[6]

PPARα-selective agonists have been reported previously.[7] The common structure is composed of an aromatic ring and carboxylic acid with various spacers, as shown in Chart 1. The aromatic ring of the ligands interacts with the hydrophobic binding pocket and the acidic moiety forms hydrogen bonds with amino acids on the AF-2 region[8] in a roughly Y-shaped ligand binding site.

On the other hand, some natural fatty acids exhibit different subtype selectivity, which is dependent on the degree of saturation and chain length.

These observations suggest the possibility of identifying chemical structures capable of exhibiting high affinity for PPARα by introducing several hydrocarbon chains in our drug design.

We designed a Y-shaped structure as described in Chart 1. We selected the fibric acid as a common structure in fibrates. To enhance PPARα activity and selectivity, we selected a 2-aminobenzoxazole ring as a bioisostere of *N*-phenylurea in GW9578 and connected several alkyl and alkenyl chains to the nitrogen atom. In a study to explore adequate side chains, we found a phenoxyalkyl structure with high PPARα activity and selectivity.

Keywords: PPARα; Design; Synthesis; Structure–activity relationship; PPARα-selective; Fibrates; Lipid abnormalities; Triglyceride; HDL-cholesterol; Nuclear receptor.
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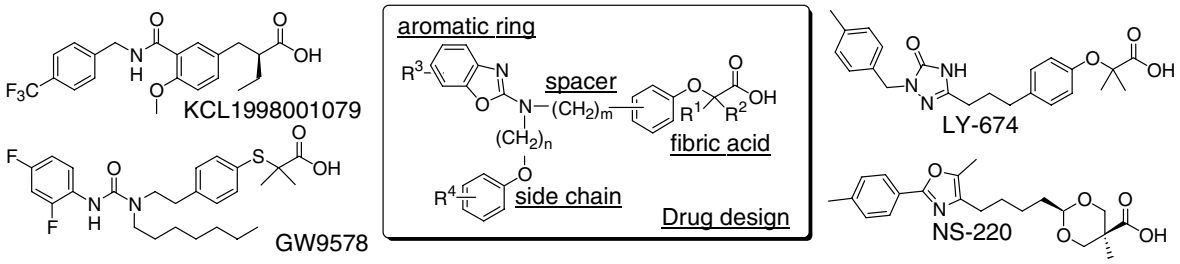
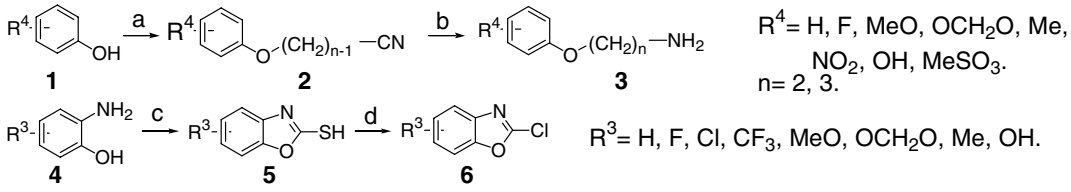


Chart 1. Structure of PPARα agonists and our drug design.

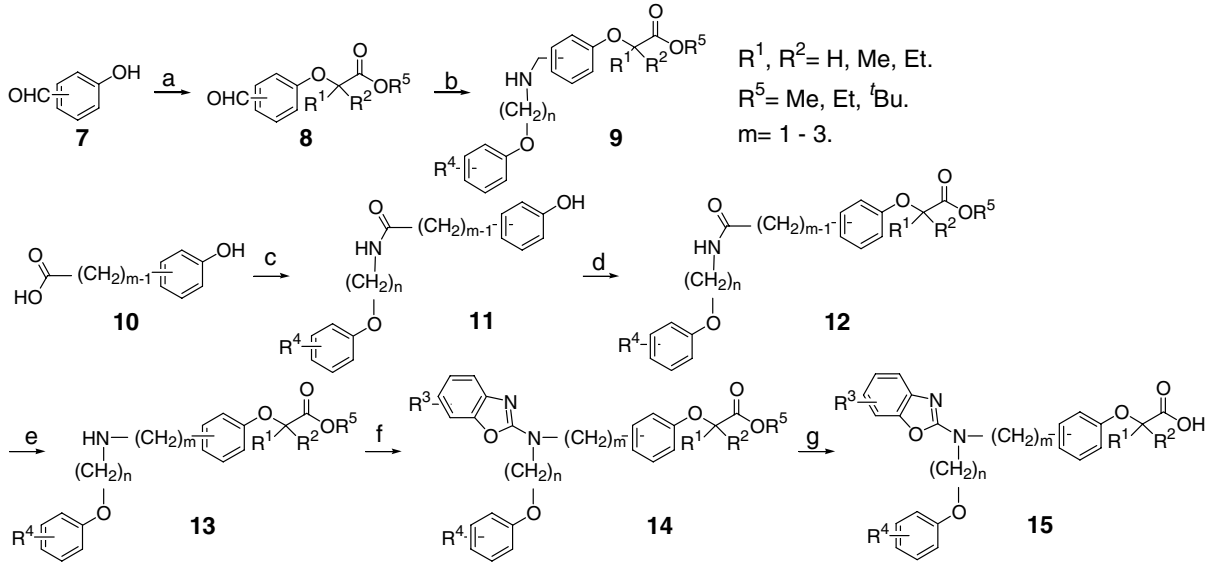
The syntheses of phenoxyalkylamine **3** and 2-chlorobenzoxazole **6** are illustrated in Scheme 1. Phenol **1** was alkylated with bromoacetonitrile ($n = 2$) or acrylonitrile ($n = 3$) to give phenoxyalkylcyanide **2**, followed by reduction with borane-THF to provide phenoxyalkylamine **3**. Substituted 2-aminophenol **4** was cyclized by potassium xanthogenate to give 2-mercaptobenzoxazole **5**, followed by chlorination to give 2-chlorobenzoxazole **6**.

The synthetic routes of all compounds were planned to allow flexibility of substituents on the benzoxazole ring, length of spacer, and side chain in the preparation of analogs for SAR studies (Scheme 2). When the number of methylene carbons in the spacer was $m = 1$, hydroxybenzaldehyde **7** was alkylated with 2-bromoacetate to give **8**, followed by reductive amination, which afforded the secondary amine **9**. When the number of methylene carbons of the spacer was $m = 2$ and 3, the hydroxyphenylalkanoic acid **10** was converted to amide **11**, then by alkylation with 2-bromoacetate to give **12**, followed by reduction, which afforded secondary amine **13**. The resulting secondary amines **9** and **13** were reacted with 2-chlorobenzoxazole **6** to afford compound **14**, which was converted to the acid **15**.

The obtained compounds were evaluated for in vitro potency and subtype selectivity in cell-based transactivation assays[9] using hPPAR-GAL-4 chimeric receptors and the results are expressed as EC_{50}, defined as the con-



Scheme 1. Reagents: (a) $n = 1$: bromoacetonitrile, K_2CO_3, DMF or MeCN; $n = 2$: acrylonitrile, Triton B, MeCN; (b) BH_3–THF, THF; (c) potassium xanthogenate, EtOH; (d) $SOCl_2$, DMF, toluene.



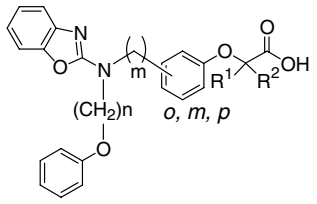
Scheme 2. Reagents: (a) $BrR^1R^2CCO_2R^5$, K_2CO_3, DMF or MeCN; (b) **3**, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane; (c) **3**, WSC–HCl, CH_2Cl_2; (d) $BrR^1R^2CCO_2R^5$, K_2CO_3, DMF or MeCN; (e) BH_3–THF , THF; (f) **6**, $^iPr_2NEt$, MeCN; (g) R^5 = Me or Et; NaOH, MeOH, $R^5 = {}^t$Bu; TFA, CH_2Cl_2.

centration of test compounds to produce 50% of maximal reporter activity.

These results are summarized in Tables 1 and 2. First, we studied the effects of position and length of spacer, length of phenoxyalkyl chain, and α-substituents of carboxylic acid (Table 1). With regard to the effect of the position of the spacer, the *meta*-position (**18**) showed high PPARα activity. Surprisingly, *para*-(**17**) and *ortho*-positions (**16**) clearly lost PPARα activity and did not activate any subtype at 1 μM. A two-methylene spacer (*m* = 2, **19**) showed the highest PPARα activity and subtype selectivity when R^1 and R^2 were methyl groups.

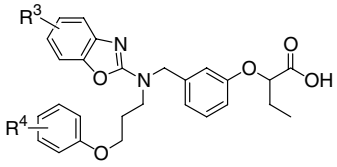
Although a three-methylene spacer (*m* = 3, **20**) showed higher PPARα activity than the one-methylene (*m* = 1, **18**) spacer, there was a tendency to decrease PPARα

Table 1. In vitro h-PPAR transactivation activity of **16**–**26**



Compound	R^1	R^2	*m*	*n*	*o*, *m*, *p*	h-α EC_{50} (μM)	h-γ EC_{50} (μM)	h-δ EC_{50} (μM)
16	Me	Me	1	2	*o*	i.a.	i.a.	i.a.
17	Me	Me	1	2	*p*	i.a.	i.a.	i.a.
18	Me	Me	1	2	*m*	0.63	2.1	n.d.
19	Me	Me	2	2	*m*	0.03	1.1	n.d.
20	Me	Me	3	2	*m*	0.1	0.1	0.8
21	Me	Me	1	3	*m*	0.19	5	1.4
22	H	H	1	3	*m*	1.2	n.d.	n.d.
23	Me	H	1	3	*m*	0.01	1.2	2.7
24	Et	H	1	3	*m*	0.009	>10	2.0
25	Me	H	2	3	*m*	0.01	1.8	2.3
26	Et	H	2	3	*m*	0.05	1.5	1.4

Table 2. In vitro h-PPAR transactivation activities of **24**, **27**–**45**



Compound	R^3	R^4	h-α EC_{50} (μM)	h-γ EC_{50} (μM)	h-δ EC_{50} (μM)
24	H	H	0.009	>10	2.0
27	5-F	H	0.003	1.0	1.1
28	6-F	H	0.005	1.1	1.9
29	7-F	H	0.002	0.53	8.0
30	5-CF_3	H	0.023	0.40	0.16
31	5-Me	H	0.10	>10	5.0
32	5,6-OCH_2O	H	0.005	1.6	1.1
33	4-OH	H	0.16	2.0	1.9
34	5-OH	H	0.18	>10	0.14
35	H	4-F	0.008	>10	1.6
36	H	4-MeO	0.001	>10	1.7
37	H	3,4-OCH_2O	0.007	1.8	1.7
38	H	3-NO_2	0.024	>10	1.4
39	H	4-$MeSO_3$	0.12	>10	n.d.
40	H	4-Me	0.001	0.21	0.12
41	H	2,4-diMe	0.001	0.13	0.11
42	H	3,4-diMe	0.001	0.75	0.83
43	H	2,4-diF	0.011	2.1	6.5
44	H	3,4-diF	0.002	1.8	1.3
45	H	4-OH	0.26	1.4	0.003

selectivity. With regard to the length of the phenoxyalkyl chain, a three-methylene linkage ($n = 3$, **21**) showed 3-fold higher PPARα activity than the shorter linkage ($n = 2$, **18**). Next, we examined the effects of α-substituent based on the structure of **21**. Interestingly, mono-alkyl substitution (Me, **23**; Et, **24**) showed remarkable increases in PPARα activity and subtype selectivity. On the other hand, no-substitution at the α-position (**22**) showed weak PPARα activity. With regard to the effects of two-methylene spacer ($m = 2$), compounds (Me, **25**; Et, **26**) exhibited high PPARα activity but lost the selectivity in comparison with **24**. We selected **24** with higher PPARα activity (h-αEC_{50} = 0.009 μM) and subtype selectivity (more than 200-fold by EC_{50}), and further explored the SAR of a series of compounds.

Second, we studied the effects of substituents on the benzoxazole ring (Table 2). Although there were slight differences in the activity, trifluoromethyl (**30**) as an electron-withdrawing group and a fluorine atom (**27**, **28**, **29**) yielded higher PPARα activity than electron-donating groups (Me, **31**; OCH_2O, **32**).

Third, we studied the effects of substituents on the benzene ring of the side chain. A fluorine atom (**35**, **43**, **44**) and electron-donating groups (Me, **40**; MeO, **36**; OCH_2O, **37**) showed high PPARα activity. There were no significant differences in PPARα activity related to the degree of substituents (di-Me, **41**, **42**; di-F, **43**, **44**). Electron-withdrawing groups showed slight decreases in PPARα activity (NO_2, **38**; $MeSO_3$, **39**). Notably, the derivative with a hydroxy group (**45**) showed the lowest PPARα activity. Similar inactivation was observed in **33** and **34**. Due to the excellent PPARα activity and selectivity, we selected compounds **24**, **35**, and **36** for further evaluation.

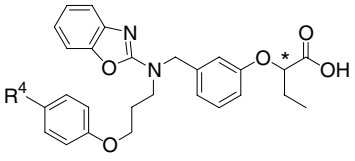
Next, we performed optical resolution to investigate the enantiodependency of the activities.[10] The results of in vitro experiments are summarized in Table 3. All *R*-enantiomers[11] showed more than 10-fold higher PPARα activity than the corresponding *S*-enantiomers. The selected compounds exhibited enantiodependent PPARα activity. Especially, (*R*)-**36** showed superior PPARα activity and subtype selectivity (h-αEC_{50} = 0.001 μM, h-γEC_{50} = 1.10 μM, h-δEC_{50} = 1.58 μM, >1000-fold subtype selectivity). The stereochemistry of the α-substituent of carboxylic acid is identical to that in KCL1998001079, tesaglitazar[12], and ragaglitazar.[13]

This suggests that the common steric feature can make favorable hydrogen bond interactions on the AF-2 region in PPARα ligand binding domain.

The sodium salts of (*R*)-**24**, (*R*)-**35**, and (*R*)-**36** were evaluated for TG lowering effects in normal rats after 1 week of treatment (Table 4). Fenofibrate was used as a reference compound. These *R*-enantiomers at 1 mg/kg showed much better TG lowering effects than fenofibrate at 100 mg/kg. In rats, PPARα agonists increase liver weight due to peroxisomal proliferation, a phenomenon that is well known in rats as the response to PPARα activation. Fenofibrate showed a 44% decrease in TG and 44% increase in liver weight. Interestingly, the *R*-enantiomers showed relatively small changes in liver weight despite high TG lowering effects.

Next, we evaluated (*R*)-**24**, (*R*)-**35**, and (*R*)-**36** in human apoA-I (h-apoA-I) transgenic mice for the ability to increase HDL-C.[14] The results of plasma h-apoA-I changes are summarized in Table 5. (*R*)-**35** at 3 mg/kg increased plasma h-apoA-I to an extent similar to fenofibrate at 300 mg/kg. Interestingly, (*R*)-**24** at 3 mg/kg and (*R*)-**36** at 1 mg/kg showed more pronounced increases in plasma h-apoA-I than fenofibrate at 300 mg/kg. The

Table 3. In vitro h-PPAR transactivation activity of chiral compounds



Compound	*R*/*S*	R^4	h-α EC_{50} (μM)	h-γ EC_{50} (μM)	h-δ EC_{50} (μM)
24	*R*	H	0.002	0.91	1.61
24	*S*	H	0.13	0.87	1.66
35	*R*	F	0.003	0.94	1.19
35	*S*	F	0.02	1.19	1.57
36	*R*	MeO	0.001	1.10	1.58
36	*S*	MeO	0.01	1.31	1.80

Table 4. In vivo efficacy of (*R*)-**24**, (*R*)-**35**, and (*R*)-**36** in normal rats

Compound	Dose (mg/kg)	Triglyceride[a] (%)	Liver weight[b] (%)
(*R*)-**24**	1	−51 ± 9[c]	19 ± 4[c]
(*R*)-**35**	1	−46 ± 9[c]	20 ± 9[c]
(*R*)-**36**	1	−49 ± 15[c]	25 ± 8[c]
Fenofibrate	100	−44 ± 16[c]	44 ± 8[c]

Male Sprague–Dawley rats (6 weeks old, $n = 6$) were fed normal chow with free access to water and received oral dosing with the sodium salts of the test compounds and fenofibrate by gavage with vehicle (0.5% methylcellulose) once a day for 7 days.

[a] Values are means ± SD of TG change.

[b] Values are means ± SD of liver weight (g/100 g body weight) change.

[c] $P < 0.05$ versus vehicle control by Student's *t* test.

Table 5. In vivo efficacy of (*R*)-**24**, (*R*)-**35**, and (*R*)-**36** in human apoA-I transgenic mice

Compound	Dose (mg/kg)	h- apoA-I (%)[a]
(*R*)-**24**	3	201 ± 30*
(*R*)-**35**	3	147 ± 43*
(*R*)-**36**	1	194 ± 19*
Fenofibrate	300	145 ± 26*

Heterozygous human apoA-I transgenic mice ($n = 5$–6) were fed normal chow with free access to water and received oral dosing of the sodium salts of the test compounds and fenofibrate by gavage with vehicle (0.5% methylcellulose) once a day for 14 days.

[a] Values are means ± SD of h-apoA-I change.

* $P < 0.05$ versus vehicle control by Dunnett's multiple comparison test.

changes in HDL-C were correlated with those of plasma h-apo-AI (data not shown).

In summary, we have identified highly potent and selective human PPARα agonists and demonstrated the excellent pharmacological effects of (*R*)-**36**. Based on the results of in vitro and in vivo studies, we expect that (*R*)-**36** would exhibit higher efficacy in patients with dyslipidemia than currently available fibrates.

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