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Design and synthesis of substituted phenylpropanoic acid derivatives as human peroxisome proliferator-activated receptor α/δ dual agonists

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Abstract—A series of phenylpropanoic acids was prepared as candidate dual agonists of peroxisome proliferator-activated receptors (PPAR) α and δ. Structure–activity relationship studies indicated that the shape of the linker moiety and the nature of the substituent at the distal benzene ring play key roles in determining the potency and selectivity of PPAR subtype transactivation. Optically active α-ethylphenylpropanoic acid derivatives were identified as potent human PPAR α and δ dual agonists with potential for the treatment of metabolic syndrome.

Identification of the molecular targets of the transducer proteins critically involved in metabolic pathways is crucial for understanding energy homeostasis in humans, and also for developing new therapeutic agents for the treatment of altered metabolic homeostasis, such as atherosclerosis, diabetes, and obesity. Metabolic nuclear receptors are particularly attractive target molecules, since they play a central role in maintaining cellular and whole-body glucose and lipid homeostasis. These nuclear receptors are activated by a wide variety of physiological ligands, including dietary fatty acids and cholesterol metabolites, and xenobiotic compounds, thereby modulating the transcriptional network of the target response genes. Among these receptors, special attention has been paid for more than a decade to the members of the peroxisome proliferator-activated receptor (PPAR) family.

PPARs are members of the nuclear receptor superfamily and are activated by endogenous saturated and unsaturated fatty acids and their metabolites, as well as synthetic ligands.[1] PPARs are heterogeneous and three subtypes have been identified to date; PPARα [officially NR1C1], PPARδ/β [NR1C2], and PPARγ [NR1C3].[2] Each PPAR subtype appears to be differentially expressed in a tissue-specific manner and to play a pivotal role in lipid, lipoprotein, and glucose homeostasis.[3] PPARα is mostly expressed in the tissues involved in lipid oxidation, such as liver, kidney, skeletal, cardiac muscle, and adrenal glands. PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles. In contrast to the specific distribution of PPARα and PPARγ, PPARδ is ubiquitously expressed in almost all mammalian tissues. PPARs heterodimerize with another nuclear receptor partner, retinoid X receptor (RXR), and the heterodimers regulate gene expression by binding to a specific consensus DNA sequence, termed PPRE (peroxisome proliferator responsive element), which is a direct repeat of the hexameric AGGTCA recognition motif separated by a single nucleotide (DR1), present in the promoter region of the target genes.[4]

PPARγ was first identified as a master regulator of adipocyte differentiation, but more recent molecular-biological studies have indicated that PPARγ activation is also linked to the expression of many important genes that affect energy metabolism, such as the TNF-α, leptin, and adiponectin genes.[5]

PPARα regulates the expression of genes encoding for proteins involved in lipid and lipoprotein homeostasis.[6] For example, it regulates genes involved in fatty acid uptake (such as fatty acid binding protein, FABP), β-oxidation (acyl-CoA oxidase), and ω-oxidation (cyto-

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chrome P450). It down-regulates apolipoprotein C-III, a protein that inhibits triglyceride hydrolysis by lipoprotein lipase,[6] and it also regulates genes involved in reverse cholesterol transport, such as apolipoprotein A-I and apolipoprotein A-II.[6]

Based on the findings that antidiabetic thiazolidine-2,4-diones (glitazones) and antidyslipidemic fibrates are ligands of PPARγ and PPARα, respectively, much research has been focused on these metabolic nuclear receptor subtypes as therapeutic targets for the treatment of metabolic syndrome (also known as insulin resistance syndrome, visceral fat syndrome, syndrome X, and deadly quartet), such as GW-9578 (**1**) and KCL (**2**) (Fig. 1).

Although PPARδ is ubiquitously distributed in a wide range of tissues and cells, research interest in PPARδ has been limited. However, after 2001, the availability of PPARδ-knockout animals and selective ligands, such as GW-501516 (**4**) and L-165041 (**5**), prompted us to examine the roles of PPARδ in fatty acid metabolism, reverse cholesterol transport, and other disease states.[7,8] Considering the positive contributions of both PPARα and PPARδ to lipid, lipoprotein, and cholesterol homeostasis, dual agonists of both PPARα and PPARδ might be other candidates for the treatment of metabolic disease, but there have been very limited reports on such agonists, such as GW-2433 (**3**) (Fig. 1), and the activity is moderate.

Previously, we have reported the design and synthesis of substituted phenylpropanoic acid derivatives as human PPARα-selective agonists.[9] As part of our continuing research directed toward the structural development of subtype-selective PPAR agonists, we report here potent novel substituted phenylpropanoic acid dual PPARα/δ agonists. To develop structurally new human PPARα/δ dual agonists, we selected **2** (KCL) (Fig. 1), a human PPARα-selective agonist, as a lead compound,[9] and performed further structural modification focusing on the distal hydrophobic tail part and the linker moiety.

Synthetic routes to the phenylpropanoic acids prepared in this study are outlined in Schemes 1 and 2.

Compounds (**10a**–**10d**) were prepared from a benzaldehyde derivative (**6a** or **6b**) in 4 steps. Compound **6a** or **6b**, which was prepared from 5-formylsalicylic acid, was treated with Emmons reagents, followed by hydrogenolysis, condensation with substituted benzylamine, and alkaline hydrolysis to afford the desired products (Scheme 1).

Optically active α-ethyl-substituted phenylpropanoic acid derivatives (**16a**, **16b**) were prepared by means of Evans' asymmetric alkylation procedure as a key step. Acylation of 4-(*R*)-benzyloxazolidinone provided **11**, which was treated with benzyl 5-bromomethyl-2-methoxybenzoate under Evans's asymmetric alkylation protocol, followed by hydrogenolysis to afford the benzoic acid intermediate **12**. This was reduced with BH_3, then protection of the hydroxyl group with a *tert*-butyldimethylsilyl group (TBDMS), treatment with benzyl lithium and deprotection of the TBDMS, afforded **13**. **13** was brominated, and treated with potassium phthalimide, then deprotection of the phthaloyl group afforded the key aminomethyl intermediate **14**. **14** was reacted with benzoyl chloride derivatives, and subsequent removal of the chiral oxazolidinone moiety afforded the desired (*S*) form of compounds **16a** and **16b** (97% ee) (Scheme 2). The antipodal (*S*)-isomer (**19**) was prepared by the use of 4-(*S*)-benzyloxazolidinone as a starting material. Racemic **2**, **17**, and **18** were prepared by the reported methods.[9]

The transactivation activity of the present series of compounds toward PPARs is summarized in Table 1, together with the results for racemic KCL, a human PPARα-selective agonist. First, we investigated the effect of the introduction of a fluorine atom in racemic KCL, because we expected that a fluorinated compound would bind more tightly to PPAR by effectively excluding the water molecule located in the hydrophobic binding pocket of PPAR. Compound (**10a**), which has a fluorine atom on the central benzene ring, instead of a methoxyl group, showed considerably decreased PPARα, PPARδ, and PPARγ transactivation activity as compared with racemic KCL, that is, this compound did not show apparent activity at the concentration of 1 μM. This result clearly indicated the importance of the methoxyl group located at the *ortho* position of the amide carbonyl group; it may be involved in a hydrogen bonding interaction that restricts the conformation of the central benzene part of the molecule. On the other hand, introduction of a fluorine atom at the distal benzene ring (**10b**, **10c**) enhanced the PPARα transactivation activity to some extent.

As regards PPARδ transactivation activity, introduction of a fluorine atom at the distal benzene ring (**10b**, **10c**)

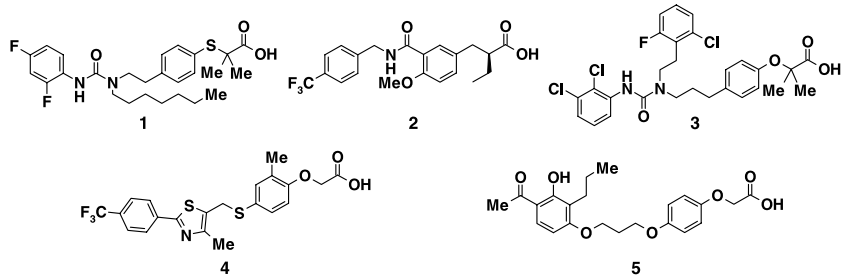
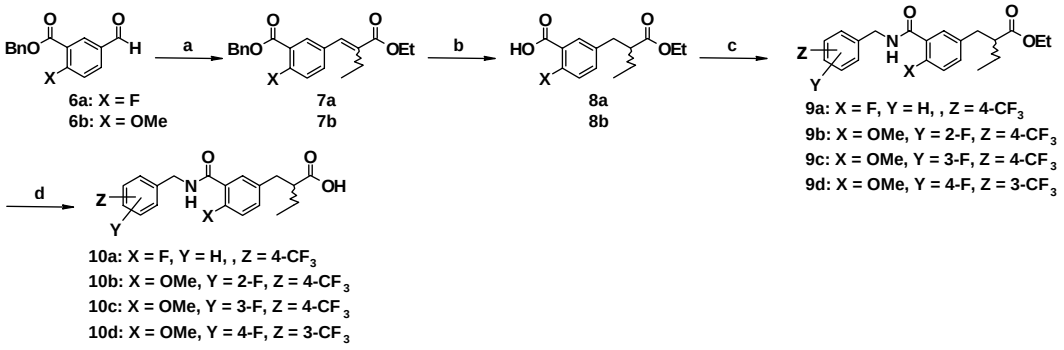
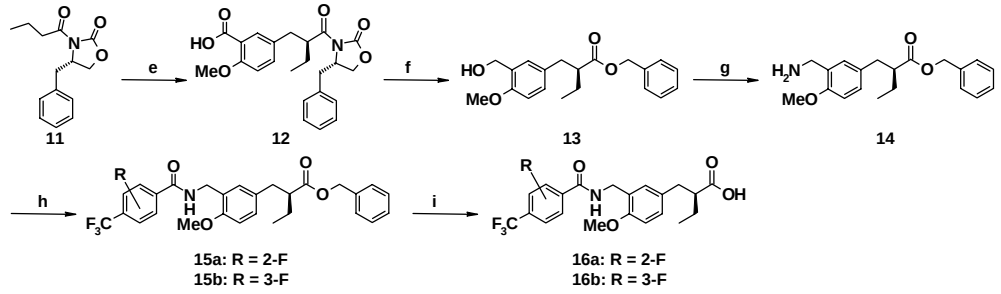


Figure 1. Structures of representative PPARα (**1** (GW-9578), **2** (KCL)), PPARδ (**4** (GW-501516), **5** (L-165041)), and dual PPARα/δ (**3** (GW-2433)) agonists.

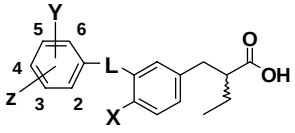


Scheme 1. Reagents: (a) $(EtO)_2POCH(Et)CO_2Et$, NaH, THF; (b) H_2, 10% Pd-C, EtOH; (c) substituted benzylamine, $ClCO_2Et$, TEA, CH_2Cl_2; (d) aq NaOH, EtOH.



Scheme 2. Reagents and conditions: (e) 1—LiHMDS, THF, benzyl 5-bromomethyl-2-methoxybenzoate, −40 °C–rt, 2—H_2, 10% Pd-C, AcOEt, rt; (f) 1—BH_3, THF, −10 °C, 2—TBDMSCl, imidazole, THF, rt, 3—benzyl alcohol, *n*-BuLi, THF, −40 °C–rt, 4—TBAF, THF, rt; (g) 1—$P(Ph)_3$-polymer, CBr_4, rt, 2—potassium phthalimide, DMF, 3—$NaBH_4$, *i*PrOH-H_2O, rt then AcOH, 80 °C; (h) RPhCOCl, TEA, CH_2Cl_2, rt; (i) H_2, 10% Pd-C, AcOEt, rt.

Table 1. In vitro functional PPAR transactivation activity of substituted phenylpropanoic acids



Compound	X	Y	Z	L	Stereo	EC_{50} (nM)[a]		
						PPARα	PPARδ	PPARγ
Racemic **2**	MeO	H	4-CF_3	CH_2NHCO	Racemic	70	700	3000
10a	F	H	4-CF_3	CH_2NHCO	Racemic	ia[b]	ia[b]	ia[b]
10b	MeO	2-F	4-CF_3	CH_2NHCO	Racemic	20	1000	500
10c	MeO	3-F	4-CF_3	CH_2NHCO	Racemic	25	500	1600
17	MeO	H	4-CF_3	$CONHCH_2$	Racemic	10	130	2000
18	MeO	4-F	3-CF_3	$CONHCH_2$	Racemic	250	2000	>10,000
16a	MeO	2-F	4-CF_3	$CONHCH_2$	*S*	10	12	1900
16b	MeO	3-F	4-CF_3	$CONHCH_2$	*S*	12	23	4900
19	MeO	3-F	4-CF_3	$CONHCH_2$	*R*	180	700	>10,000

[a] Compounds were screened for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected HEK-293 cells as described. EC_{50} value is the molar concentration of the test compound that affords 50% of the maximal reporter activity. $n = 3$.
[b] 'ia' means inactive (no apparent activity) at the concentration of 1 μM.

apparently did not influence the activity, but in the case of PPARγ, introduction of a fluorine atom at the 2-position increased the transactivation activity to some extent. These results might indicate that the shape and environment of the hydrophobic cavity hosting the distal benzene ring differ somewhat among the three PPAR isoforms.

Previously, we found that the 3-atom unit linker; $–CH_2–NH–CO–$ was very potent and PPARα selective, but shortening or lengthening of the linking group considerably decreased the activity.[9] We also found that a flexible linker, such as $–CH_2–CH_2–CH_2–$ or $–CH_2–CH_2–O–$, decreased PPARα transactivation activity, but caused PPARδ transactivation activity to appear.[9] Therefore,

we reinvestigated the effect of the –CO–NH–CH_2– linker (**17**) and found that this linker increased both PPARα and PPARδ transactivation activities, as compared to those of the –CH_2–NH–CO– linker compound (racemic **2**). We speculated that the introduction of a conformationally flexible group and/or atom, such as –CH_2– or –O–, next to the central benzene ring might favor both PPARα and PPARδ activity, while introduction of a more conformationally restricted group (–CO–) next to the central benzene ring might be unfavorable for PPARδ activity. These results might reflect differences of the shape and environment of the hydrophobic cavity hosting the distal benzene ring between PPARα and PPARδ.

The position of the substituents introduced onto the distal benzene ring is very important; compound **18**, which has a trifluoromethyl group and a fluorine atom at the 3- and 4-positions, exhibited a 10-fold lower PPARα transactivation activity. This is consistent with the previously obtained SAR result that steric bulkiness at the 4-position is an important factor for potent PPARα transactivation activity.

Considering the results obtained above, we then prepared optically active derivatives, **16a**, **16b**, and **19**. As can be seen from Table 1, a clear enantio-dependence of the transactivation activity toward PPARα and PPARδ isoforms was found. **16b**, which has (*S*) configuration, exhibited much more potent transactivation activity on both PPARα and PPARδ, while the antipodal (*R*) isomer **19** exhibited less potency. Therefore, we concluded that the activity resides almost exclusively in the (*S*)-enantiomer, and both **16a** and **16b** showed dual-agonist activity toward PPARα and PPARδ.

As previously described, some peroxisome proliferators show species-dependent transactivation characteristics for PPARα.[10] The classical PPARα agonist WY-14643 is more effective on rodent PPARα than on human PPARα. 5,8,11,14-Eicosatetraenoic acid (ETYA) showed the reverse preference, that is, it is 10-fold more effective on human PPARα than rodent PPARα. On the other hand, fenofibric acid, an active metabolite of the fibrate class antihyperlipidemic agent fenofibrate, did not show clear species differences.

Therefore, we evaluated the species-selectivity profile of PPARα transactivation by **16b**.

As can be seen from Table 2, **16b** activated human and mouse PPARα with EC_{50} values of 10 and 1000 nM, respectively. Thus, **16b** showed species preference for humans, and the transactivation activity of **16b** for PPARα was approximately 100-fold less potent in mice than that in humans.

Previously we have demonstrated that a single amino acid residue is responsible for this human-selective PPARα activation of **2**, that is, the human selectivity of KCL is primarily mediated through the specific contact of the hydrophobic tail part of KCL with amino acid residue 272, isoleucine (Ile272), which is located in the helix 3 region of the human PPARα LBD.[11,12] Therefore, we investigated the activity with I272F mutant. As can be seen from Table 2, **16b** exhibited about 100-fold less activity, which is comparable to that obtained with mouse PPARα. This result indicates that although the shape of the linking group is somewhat different from that of the normal amide linker derivative, such as KCL, the hydrophobic tail part of **16b** also interacts specifically with amino acid 272, isoleucine.

Table 2. In vitro functional PPAR transactivation activity of **16b** on hPPARα, mPPARα, hI272F, hPPARδ, and mPPARδ

EC50 (nM)[a]				
hPPARα	mPPARα	hI272F	hPPARδ	mPPARδ
10	1000	630	40	130

[a] Compounds were screened for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected HEK-293 cells as described. EC_{50} value is the molar concentration of the test compound that causes 50% of the maximal reporter activity. $n = 3$.

In contrast to PPARα, there is no report about species selectivity of PPARδ agonist. Therefore, we also evaluated the species-selectivity profile of PPARδ transactivation by **16b**. Table 2 also indicates that species selectivity of **16b** for the PPARδ subtype is not so marked; **16b** shows EC_{50} values against human and mouse PPARδ of 40 and 130 nM, respectively. In the case of PPARδ, 14 amino acids are different between the human and mouse PPARδ ligand binding domain, that is, 205Ala, 208Thr, 239Lys, 251Cys, 266Ser, 269Ser, 314Arg, 369Arg, 387Ala, 392Ala, 419Arg, 420Ile, 425Thr, and 428Ser of human PPARδ are replaced with 204Ser, 207Asn, 238Asn, 250Ser, 265Asn, 268Asn, 313His, 368Gln, 386Val, 391Ser, 418Trp, 419Leu, 424Ser, and 427Leu in the case of mouse PPARδ. But, based on a consideration of the X-ray crystallographic study of human PPARδ and GW-2433 complex and/or human PPARδ and eicosapentaenoic acid complex, none of these amino acids appears to be involved in specific contact with the agonist ligand. Therefore, compound **16b** did not show a large species selectivity between human and mouse PPARδ. As far as the authors know, this is the first report that discusses the species selectivity of PPARδ agonist.

In summary, we have developed the potent human dual PPARα/δ agonists **16a** and **16b**. Further cell-based assay and in vivo pharmacological evaluation of these compounds are under way.

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