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Discovery of highly potent and selective benzyloxybenzyl-based peroxisome proliferator-activator receptor (PPAR) δ agonists

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Abstract—A series of 1,4-benzyloxybenzylsulfanylaryl carboxylic acids were prepared and their activities for PPAR receptor subtypes (α, δ, and γ) with potential indications for the treatment of dyslipidemia were investigated. Analog **13a** displayed the greatest binding affinity (IC_{50} = 10 nM) and selectivity (120-fold) for PPARδ over PPARα. Many of the analogs investigated were found to be highly selective for PPARδ and were dependent on the point of attachment of the substituent. In the 1,4-series, analog **28e** was found to be the most potent (IC_{50} = 1.7 nM) and selective (>1000-fold) compound for PPARδ. None of the compounds tested showed appreciable binding affinity for PPARγ.

The major risk factors correlated with the development of atherosclerosis include elevated LDL-c and triglycerides, and low HDL-c plasma levels. HMG CoA reductase inhibitors have commonly been used as therapy to lower LDL-c levels, while HDL-c are modulated with some members of the fibrate family.[1] Little progress, however, has been made with elevating HDL-c, and studies suggest that the involvement of peroxisome proliferator-activated receptors in mitochondrial fatty acid catabolism and increases in cellular cholesterol efflux can both contribute to the treatment of this dyslipidemia.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear transcription factors involved in the regulation of dietary fat storage and catabolism.[2] PPARs, upon ligand activation, heterodimerize with the retinoid X receptor (RXR) and subsequently bind to the PPAR response elements (PPRE). The RXR: PPAR complex, in the presence of co-activators, initiates the transcription process of the target genes. PPARs play a crucial role in cellular processes including lipid metabolism, cell proliferation, differentiation, adipogenesis, and inflammatory signaling. Three PPAR isoforms (α, δ, and γ) are known, each involved in different mechanisms of lipid homeostasis and all with diverse tissue distributions. PPARα is highly expressed in liver, where it controls peroxisomal and mitochondrial fatty acid catabolism, whereas PPARγ is concentrated in adipose tissues and acts as a transcriptional factor for adipogenesis. PPARδ is expressed ubiquitously at low levels.

Therapeutic agents acting as agonists of PPARα, such as fenofibrates, are indicated for the treatment of elevated triglyceride levels (Fig. 1), whereas PPARγ ligands, such as the thiazolidinedione class, are directed towards the treatment of type-2 diabetes by increasing insulin sensitivity. Pharmacologies of PPARδ receptor agonists, though relatively obscure, have increasingly been studied and recently reported to elevate HDL-c and lower triglyceride plasma levels in obese insulin resistant rhesus monkeys.[2] Herein, we report the preparation and structure–activity relationships (SAR) of series of benzyloxybenzyl-sulfonylaryl carboxylic acid ligands as potent and selective agonists of the PPARδ subtype receptor with potential indications for the treatment of dyslipidemia.

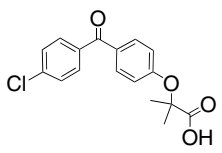

Figure 1. Fenofibric acid.

Keywords: Syndrome X; Metabolic syndrome; PPARβ; PPARδ; Peroxime proliferator activated receptor.
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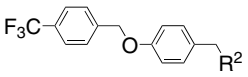
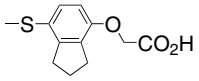
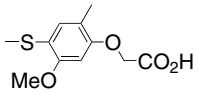
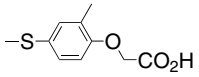
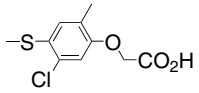
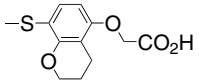
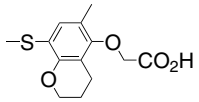
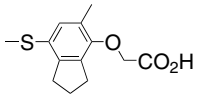
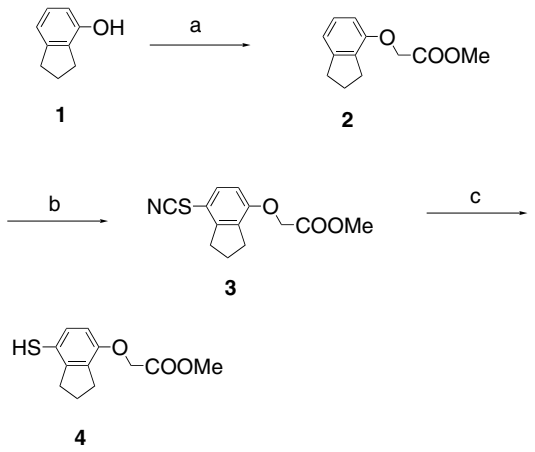
Table 1. PPAR receptor binding and cellular functional activities



Compound	R^2	Selectivity (α/δ)	IC_{50}[a,c] (nM)			PPARδ EC_{50}[b,c] (nM)
			PPARδ	PPARα	PPARγ	
13a		120	10	1200	NT	251
14		2.8	1.7	4.7	23,600	13
15		30	19	578	>11,000	252
16		6	20	128	9120	374
17		5.8	136	791	5560	295
18		1.4	313	428	33,300	NA
19		0.5	586	311	21,600	NA

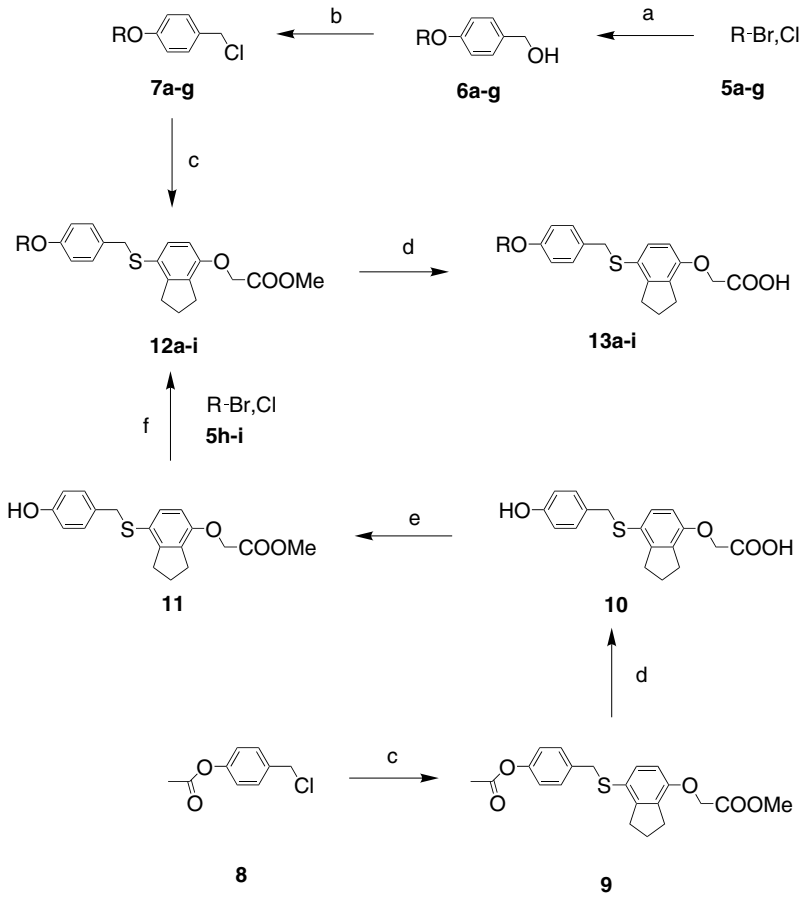
NA, IC_{50} > 10 μM; NT, not tested.
[a] Concentration that inhibits 50% of the interaction between the PPAR LBD and the radiolabeled ligand.
[b] Concentration of test compound which produced 50% of the maximal reporter activity.
[c] The results are based on at least three experiments, each dose done in triplicate (SD = 10%).

The general synthetic route used for the preparation of substituted aryl thiols as precursors in the synthesis of targets **13a** and **14–19** (Table 1) is illustrated in Scheme 1 using the formation of the indane moiety as an example. Alkylation of hydroxyindane **1** with methylbromoacetate under basic conditions gave the indane ester **2**, followed by treatment with sodium thiocyanate and bromine which provided the thiocyanate indane intermediate **3**.[3] Intermediate **3** was reduced with sodium borohydride to afford the mercaptoindane **4** in an overall yield of 91%. During the reduction process, sodium borohydride was added to cleave the disulfide dimer, a common side product under those reaction conditions.



Scheme 1. General scheme for preparation of substituted thiols with preparation of the mercaptoindane as an example. Reagents and conditions: (a) Cs_2CO_3, methylbromoacetate, acetonitrile, 25 °C, 100%; (b) NaSCN, NaBr, Br_2, MeOH, 0 °C, 99%; (c) dithiothreitol, $NaBH_4$, MeOH, 0 °C, 91%.

The formation of the 1,4-benzyloxybenzylsulfanyl indane carboxylic acid analogs **13a–g** was achieved in four steps starting with commercially available substituted benzyl halides **5a–g** (Scheme 2).[4] These electrophiles reacted with 4-hydroxymethylphenol to provide the benzyloxybenzyl alcohol derivatives **6a–g** and then converted to their respective benzyloxybenzyl chlorides **7a–g**. The chloride intermediates were prepared by an in situ formation of the mesylates, followed by displacement of the mesyl group with chloride using methanesulfonyl chloride and triethylamine. Coupling of mercaptoindane **4** with substituted benzyloxybenzyl chlorides **7a–g** afforded indane esters **12a–g**, followed by saponification with sodium hydroxide and then acidification with hydrochloric acid which gave the target compounds **13a–g** in an overall average yield of 35% over four steps. Likewise, the analogs **14–19** illustrated



Scheme 2. General scheme for the preparation of substituted 1,4-benzyloxybenzylsulfanyl indane carboxylic acid derivatives. Reagents and conditions: (a) Cs_2CO_3, 4-hydroxymethylphenol, acetonitrile, 25 °C, 72–100%; (b) methanesulfonyl chloride, DCM, Et_3N, 0 °C, 53–73%; (c) Cs_2CO_3, mercaptoindane **4**, acetonitrile, 25 °C, 80–100%; (d) 1—LiOH·H_2O, aq THF; 2—aq HCl, 25 °C, 72–89%; (e) 2,2-dimethoxypropane, HCl (concd), 0–25 °C, 18 h, 70%; (f) substituted benzyl halide, Cs_2CO_3, acetonitrile, 25 °C, 77–88%.

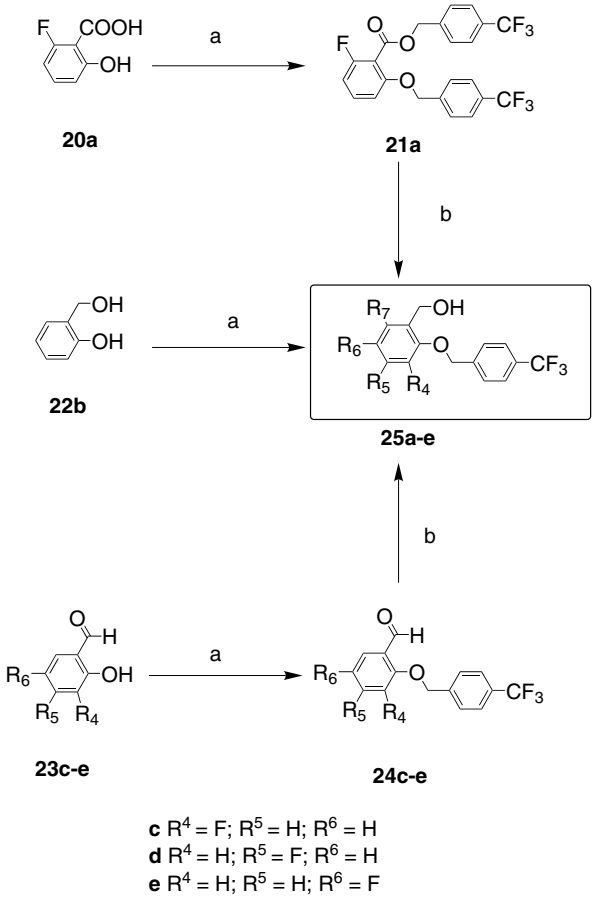
in Table 1 were prepared under the same reaction conditions from their respective substituted aryl thiols.

Targets **13h** and **13i** were synthesized by a different route in an effort to prepare a common intermediate two steps away from the desired targets and to facilitate purification in a parallel chemistry strategy (Scheme 2). The alternative route began with ester chloride **8**, which reacted with **4** under basic conditions in acetonitrile to form the diester **9** and then hydrolyzed with excess base in aqueous THF, followed by acidification, to provide the acid phenol **10** in an overall yield of 80% over two steps. The methyl ester was regenerated using 2,2-dimethoxypropane and concentrated hydrochloric acid, a mild condition for preparing methyl esters, to afford the common ester phenol intermediate **11** in 70% yield. Finally, alkylation of **11** with organohalides **5h–i** provided the target compounds in 77–88% yields.

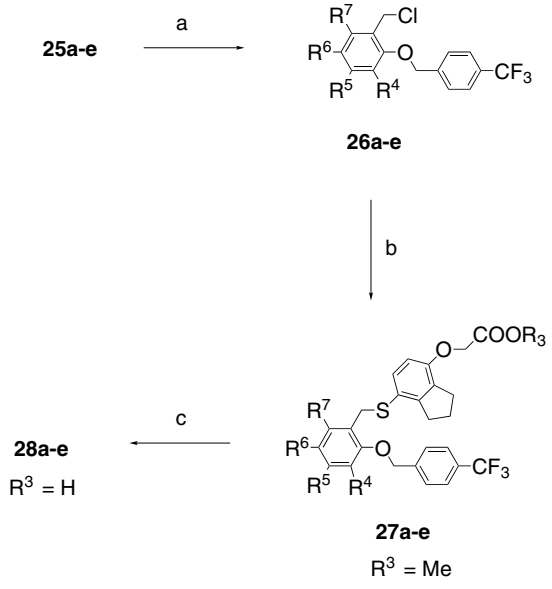
For the synthesis of the 1,2-benzyloxybenzylsulfanyl indane carboxylic acid analogs **28a–e**, the reaction pathway utilized was dependent on the availability of commercial starting material (Scheme 3). For example, the benzyl alcohol intermediate **25a** was prepared in two steps from the carboxylic acid phenol **20a** using an excess of the electrophile and base to provide the dialkylated intermediate **21a,** followed by reduction with lithium aluminum hydride in tetrahydrofuran (Scheme 3).[5] Similarly, benzyl alcohols **25c–e** were synthesized from the salicaldehydes **23c–e** via alkylation of the phenols to form the aldehyde ethers **24c–e** and then reduction under the same conditions to give the intermediates. In contrast to the two reaction pathways previously mentioned, benzyl alcohol **25b** was formed in one step in 81% yield from **22b** using the same alkylation conditions. The final targets **28a–e** were prepared from their respective benzyl alcohol intermediates **25a–e** by the reaction conditions described for the preparation of the 1,4-substituted analogs **13a–g** in yields ranging from 47 to 80% (Scheme 4).

Potent and selective PPARδ agonists are desirable compounds and are believed to increase the transcription of factors leading to the production of desirable HDL-c seen lacking in certain dyslipidemias.

Directed SAR was derived from known PPAR agonists and activities were determined in a manner similar to known methods.[6–8] The requisite acidic motif was required for activity and was included in all examples. Through a parallel synthetic strategy combining various phenoxyacetic acid thiol monomers with hydrophobic



Scheme 3. Preparation of substituted 1,2-benzyloxybenzyl alcohols, Reagents and conditions: (a) Cs_2CO_3, 1-bromomethyl-4-trifluoromethylbenzene, acetonitrile, 25 °C, 81–98%; (b) LAH, THF, reflux, 39–65%.



Scheme 4. Preparation of substituted 1,2-benzyloxy-benzylsulfanyl indane carboxylic acid derivatives. Reagents and conditions: (a) methanesulfonyl chloride, DCM, Et_3N, 0 °C, 86–91%; (b) Cs_2CO_3, mercaptoindane 4, acetonitrile, 25 °C, 78–100%; (c) 1—LiOH:H_2O, aq. THF; 2—aq HCl, 25 °C, 47–80%.

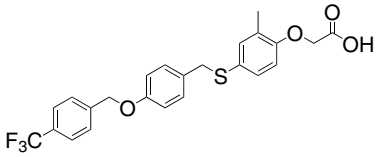


Figure 2. Analog **15**.

fragments, a library of potential PPAR ligands was produced. A lead compound **15**, containing a 4-benzyloxybenzyl substituent, showed high affinity for PPARδ (IC_{50} = 19 nM) and moderate affinity for PPARα (IC_{50} = 578 nM) (Fig. 2). Modifications of the phenoxyacetic acid core with bulky hydrophobic substituents led to increased affinity for PPARδ. The highest selectivity was seen with the indane core, whereas smaller groups showed diminished affinity for PPARδ (Table 1).

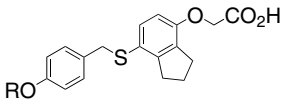
The inclusion of an oxygen atom showed a marked reduction in selectivity. This was observed in both cyclic and acyclic ethers. Interestingly, addition of a methyl group opposite a larger fused ring decreased the binding to PPARδ. However, this weaker binding affect was not observed with small non-polar groups para to the methyl group. This observation may be due to a sterically confined space around the phenoxyacetic acid.

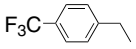
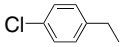
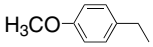
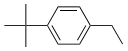
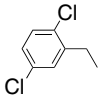
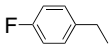
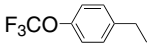
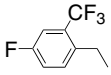
In contrast, the hydrophobic 'tail' portion of this series was more amenable to substitution. Structure activity relationships on the benzyl portion of **13a** were further explored (Table 2). Changes at the 2- and 5-positions on the benzyl ring gave an unclear trend in structure–activity relations. Electron donating and withdrawing groups did not have significant impact on the binding affinities with both PPARα and δ. Hydrophobicity and steric interaction were slightly more important considerations in this portion of the receptor. The PPARδ receptor showed a greater affinity for multiple fluorine containing substituents when attached at the 4-position of the ring. Groups of larger and smaller size showed diminished binding affinity. Functionality attached at the 2-position indicated restricted space in PPARα, but was relatively well accommodated.

The benzyloxy substitution pattern imparts significant changes to both binding and selectivity (Table 3). Although the 1,4-substituted benzyloxybenzyl example **13a** demonstrated some PPARα activity, modification to a 1,2 substitution pattern was devoid of measurable activity at the same receptor. Additional fluorine substitutions *ortho*, *meta*, and *para* to the oxygen linker further altered binding affinity to PPARδ. This trend of increasing PPARα binding activity runs slightly counter to the decreasing PPARδ binding activity. While the contribution and positioning of the monofluoro substituent is unclear, it was a requisite member of the most selective compounds.

All members of the benzyloxybenzyl series showed low binding affinity for PPARγ. Although potentially a promiscuous receptor, significant selectivity was seen for PPARα and PPARδ, with IC_{50}'s for PPARγ ranging from 5.4 to 22 μM.

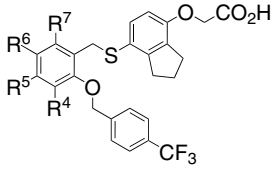
Table 2. PPAR receptor binding and cellular functional activities



Compound **13**	R	Selectivity (α/δ)	IC_{50}[a,c] (nM)			PPARδ EC_{50}[b,c] (nM)
			PPARα	PPARγ	PPARδ	
a		120	10	1200	NA	251
b		44	89	3910	NT	933
c		41	120	4950	13,800	1970
d		10	148	1540	NT	267
e		>1000	125	NA	7570	2210
f		69	69	4190	NT	585
g		>1000	191	NA	NT	13,600
h		9	76	672	<11,100	530
i		10	356	3481	<11,100	1720

NA, IC_{50} > 10 μM, NT, not tested.
[a] Concentration that inhibits 50% of the interaction between the PPAR LBD and the radiolabeled ligand.
[b] Concentration of test compound which produced 50% of the maximal reporter activity.
[c] The results are based on at least two experiments, each dose done in triplicate (SD = 10%).

Table 3. PPAR receptor binding and cellular functional activities



Compound **28**	R^4	R^5	R^6	R^7	Selectivity (α/δ)	IC_{50}[a,c] (nM)			PPARδ EC_{50}[b,c] (nM)
						PPARδ	PPARα	PPARγ	
e	H	H	F	H	>1000	1.7	NA	NA	70
b	H	H	H	H	>1000	10.4	NA	NA	230
d	H	F	H	H	50	62	3110	NT	NT
c	F	H	H	H	33	111	3670	NT	NT
a	H	H	H	F	>1000	286	NA	NT	2610

NA, IC_{50} > 10 μM, NT, not tested.
[a] Concentration that inhibits 50% of the interaction between the PPAR LBD and the radiolabeled ligand.
[b] Concentration of test compound which produced 50% of the maximal reporter activity.
[c] The results are based on at least three experiments, each dose done in triplicate (SD = 10%).

In the course of our study with these compounds, we found that there was not a clear correlation between the PPARδ binding affinity and the PPARδ functional activity. In the event, we constructed our structure–activity relationships with data derived from the receptor binding assay and assessed agonist activity using the cell based functional assay. Ultimately, compound **28e** showed greater than 1000-fold selectivity of PPARδ over PPARα with an EC_{50} of 59 nM and was thus selected for further study in vivo.

References and notes

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2. Oliver, W. R., Jr.; Shenk, J. L.; Snaith, M. R. *Proc. Natl. Acad. Sci. U.S.A* **2001**, *98*, 5306.
3. Bordwell, F.; Boutan, J. *J. Am. Chem. Soc.* **1956**, *78*, 854.
4. Example for preparing 1,4-benzyloxy-benzylsulfanyl aryl carboxylic acids from substituted benzyl halides: 1,4-Benzyloxybenzyl alcohol **(6a)**: To a solution of 4-dihydroxymethylphenol (1.9 g, 15 mmol) in 53 mL of acetonitrile was added 1-bromomethyl-4-trifluoro-methylbenzene (4.0 g, 17 mmol), followed by cesium carbonate (7.4 g, 23 mmol). The reaction mixture was stirred at 25 °C for 18 h under nitrogen atmosphere. The reaction mixture was filtered and the filtrate was evaporated to afford a crude solid, which was purified by flash chromatography (silica gel, 30 % ethyl acetate in hexane) to provide, after drying, 3.27 g (76%) of a white solid.
   1,4-Benzyloxybenzyl chloride **(7a)**: To a cold (0 °C) solution of **6a** (3.0 g, 11 mmol) in 40 mL of dichloromethane was added 3.7 mL of triethylamine (2.7 g, 27 mmol), followed by 1.65 mL of methanesulfonyl chloride (2.4 g, 21 mmol). The reaction mixture was stirred at 0 °C for 2 h and then at 25 °C for 18 h. The reaction mixture was evaporated to give a crude orange oil, which was flash chromatographed (silica gel, 10% ethyl acetate in hexane) to afford, after drying, 2.3 g (72%) of an oil.
   1,4-Benzyloxybenzylsulfonyl indane ester **(12a)**: To a solution of **7a** (0.45 g, 1.5 mmol) in 9 mL of acetonitrile was added **4** (0.36 g, 1.5 mmol), followed by cesium carbonate (0.98 g, 3.0 mmol). The reaction mixture was stirred at 25 °C for 18 h under nitrogen atmosphere. The reaction mixture was filtered and the filtrate was evaporated to afford a crude solid, which was purified by flash chromatography (silica gel, 20% ethyl acetate in hexane) to provide, after drying, 699 mg (93%) of a white solid.
   1,4-Benzyloxybenzylsulfonyl indane carboxylic acid **(13a)**: To a solution of **12a** (0.60 g, 1.2 mmol) in a mixture of 10 mL of tetrahydrofuran and 2 mL of water was added lithium hydroxide monohydrate (0.15 g, 3.5 mmol). The reaction mixture was stirred at 25 °C for 3 h and then evaporated to give an oily residue. The residue was suspended in 50 mL of water and then acidified with 1 M hydrochloric acid to pH 2. The reaction mixture was filtered to collect a white solid, which was rinsed with water and dried to provide 514 mg (89%) of the title compound.
5. Kiong, L.; Tyman, J. *J. Chem. Soc. Perkin Trans.* **1981**, *1*, 1942.
6. Pawar, A.; Xu, J.; Jerks, E.; Mangelsdorf, D. J.; Jump, D. B. *J. Biol Chem* **2002**, *77*, 39243.
7. *PPAR receptor binding assay*: The human PPARδ and PPARα scintillation proximity assay (SPA) was used to measure the affinity of ligands for the respective human PPAR receptor. The hPPARδ LBD encoding amino acids 145–441 (GenBank Accession No. NM_006238), and hPPARα LBD encoding amino acids 196–468 (GenBank Accession No. L02932) were used. Volumes of 99 μL of buffer (50 mM Tris, 10 mM Na–Molybdate, 1 mM EDTA, and 10% Glycerol, pH 7.6) containing 50 nM of radiolabeled ligand (^{3}H-2-(4-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)propoxy)-phenoxy)acetic acid (34 Ci/mmol) for PPARδ and ^{3}H-2-(4-(2-(3-(2,4-difluorophenyl)-1-heptylureido)ethyl)-phenoxy)-2-methylbutanoic acid (86 Ci/mmol) for PPARα), 0.2 mg anti-rabbit beads (Amersham, RPN140), 0.24 μg rabbit anti-GST (Molecular Probes Inc., A5800), and 0.2 μg purified GST/PPARhLBD were placed into the wells of Corning 96-well tissue culture plates. Dimethylsulfoxide (DMSO) (1 μL) or 1 μL of DMSO containing a test compound at a concentration sufficient to give a final assay concentration of between 1 nM and 100 μM were added into each well. After incubation with shaking at room temperature for 30 min, radioactivity bound to the PPAR LBD-GST fusion protein/anti-GST/SPA antibody-binding bead complex was assessed using a Wallac MicroBeta plate reader. The potency of interaction of a compound with the respective PPAR LBD was determined as the concentration that inhibits 50% of the interaction between the respective PPAR LBD and the radiolabeled ligand.
   The human PPARγ scintillation proximity assay (SPA) was used to measure the affinity of ligands for the human PPARγ receptor. The hPPARγ LBD encoding amino acids 206–477 (GenBank Accession No. NM_138712.1) was used. Volumes of 168 μL of buffer (1X PBS, 12 mM β-mercapto ethanol, 0.002% Tween-20, and 9% Glycerol, pH 7.6) containing 40 nM ^{3}H-5-(4-(3-(5-methyl-2-phenyloxazol-4-yl)propanoyl)-benzyl)thiazolidine-2,4-dione (9.57 Ci/mmol), 0.3 mg polylysine-coated yttrium silicate beads (Amersham, RPNQ0010), and 10 nM purified His-tagged human PPARγ LBD were placed into the wells of a 96-well white assay plate (Corning 3604). 2 μL of dimethylsulfoxide (DMSO) or 2 μL of DMSO containing a test compound at a concentration sufficient to give a final assay concentration binding curve between 1 nM and 100 μM was added into each well. After incubation with shaking at room temperature for 2 h, radioactivity bound to the PPARγ LBD-HIS fusion protein/yttrium bead complex was assessed using a Wallac MicroBeta plate reader. The potency of interaction of a compound with the PPARγ LBD was determined as the concentration that inhibits 50% of the interaction between the PPARγ LBD and the radiolabeled ligand.
8. *PPARδ chimeric receptor assay (Functional Assay): Transient transfections assay using the HepG2 hepatoma cell line:* The GAL4 hPPARδLBD, chimeric receptor expression constructs containing the ligand binding domain for the human PPARδ LBD (encoding amino acids 145–441 GenBank Accession No. NM_006238), was used to cotransfect cells with GAL4-Luciferase reporter plasmid (p5Eb-Luc) and β-Gal plasmid. Briefly, HepG2 cells were seeded in a 100-mm cell culture dish containing 10 mL DMEM plus 10% serum. Transfection mix was prepared by combining 15 μg GAL4-Luc plasmid with 15 μg of GAL4-hPPARδLBD. β-Gal plasmid (1.5 μg) was also added to each as a control. LipofectAMINE 2000 reagent was used as suggested by the manufacturer (Invitrogen, Carlsbad, CA). For each well, 2.4 mL transfection mix was added and incubated at 37 °C overnight. The next day, transfected HepG2 cells were reseeded to a 96-well cell culture plate at the density of 3000 cells per well and compounds were subsequently added to each well. After 16 h incubation, cells were then harvested in a lysis buffer (Promega, Madison, Wisconsin) and luciferase activity was determined using a luminometer. Luciferase activity was then normalized with β-Gal activity.