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Minor structural modifications convert a selective PPARα agonist into a potent, highly selective PPARδ agonist

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Abstract—We report the solid-phase synthesis and pharmacological evaluation of a new series of small-molecule agonists of the human peroxisome proliferator-activated receptor δ (PPARδ) based on a lead structure from our PPARα program. Compound **33** showed good pharmacokinetics.

The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptor superfamily and consist of the three members: PPARα, PPARγ, and PPARδ. Many of the PPAR-regulated genes are involved in fatty-acid metabolism and play a central role in regulating the storage and catabolism of dietary fats. Consequently, subtype selective PPAR agonists are regarded as promising leads for novel drugs used for the treatment of metabolic syndrome, diabetes, obesity, dyslipidemia, and atherosclerosis.[1,2] Unlike for PPARα (fibrates) and PPARγ (glitazones), there are no known drugs that have been identified as working through PPARδ.[3] However, oral application of 3 mg/kg GW501516 (**1**, Fig. 1),[4] the first PPARδ-selective agonist in clinical trials, is reported to increase HDLc by 80%, while reducing LDL-cholesterol by 29% in a primate model of dyslipidemia.[5]

Another series of potent PPAR agonists with up to 25-fold selectivity for PPARδ over PPARα and PPARγ, respectively, has been identified recently, starting from a phenylacetic acid lead.[6]

As part of our ongoing research in the identification of novel nuclear receptor ligands, we report the identification of highly selective PPARδ agonists[7] through solid-phase parallel synthesis. The general lead structure **2**, specified by example **3**, from our in-house PPARα program served as the starting point for optimization towards δ-selectivity (Fig. 2).[8]

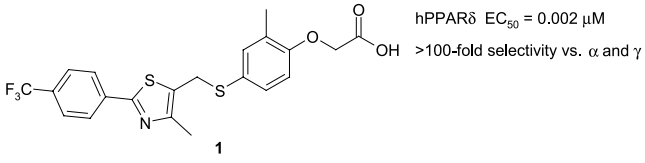

Figure 1. PPARδ-selective agonist GW501516.

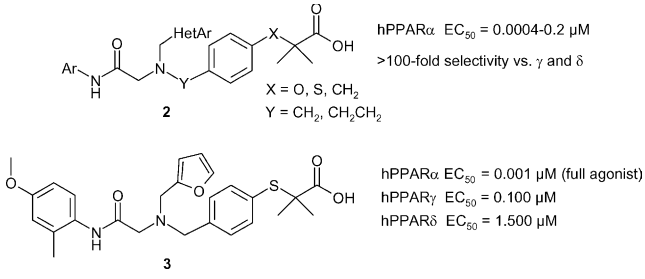

Figure 2. Lead structures from in-house PPARα agonist program.

The general synthesis leading to compounds **17**–**35** is depicted in Scheme 1. First, the corresponding α-bromoacetic acid derivatives **4** were attached to Wang resin (*p*-benzyloxybenzyl alcohol resin). Resin bound α-bromo-

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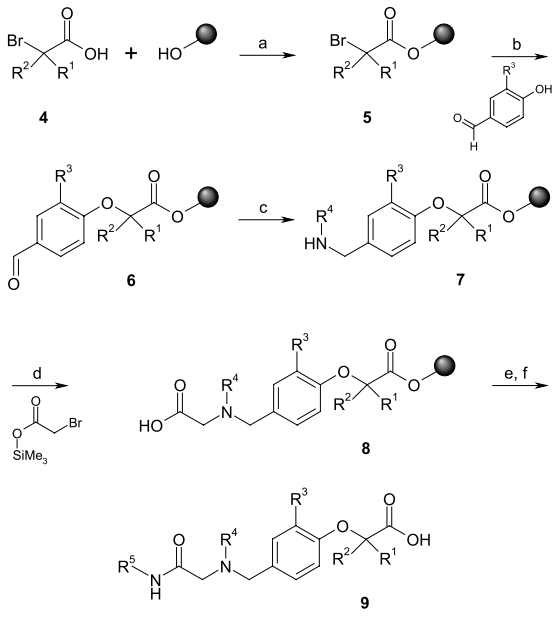


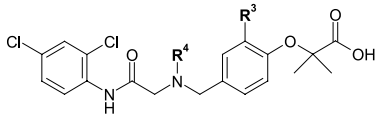
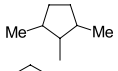
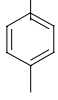
Scheme 1. Synthesis of solid-phase parallel library: Reagents and conditions: (a) Wang resin, diisopropylcarbodiimide, DMPA, Et_3N, CH_2Cl_2, rt; (b) Cs_2CO_3, dioxane/DMF (1:1), rt; (c) R^4NH_2, trimethylorthoformate/DMF (1:1), rt; Bu_4NBH_4, AcOH, DMF, rt; (d) Et_3N, dioxane, 60 °C; Bu_4NF, dioxane, rt; (e) R^5NH_2, HATU, pyr/DMF (2:1), rt; (f) CF_3COOH, CH_2Cl_2, rt.

acetic acid ester **5** was coupled with 4-hydroxy benzaldehydes in a Williamson ether synthesis. Reductive amination lead to the secondary amine **7**, which upon treatment with trimethylsilyl protected α-bromoacetic acid, followed by fluoride-induced ester cleavage, afforded the intermediate **8**. Amide coupling of **8** with various amines and subsequent cleavage from the resin with trifluoro acetic acid delivered the desired library of phenoxyacetic acids of the general formula **9**.

The combinatorial library was synthesized using a split-and-pool IRORI Kan method.[9] All new compounds gave satisfactory spectral and analytical data including ^1H NMR.[10]

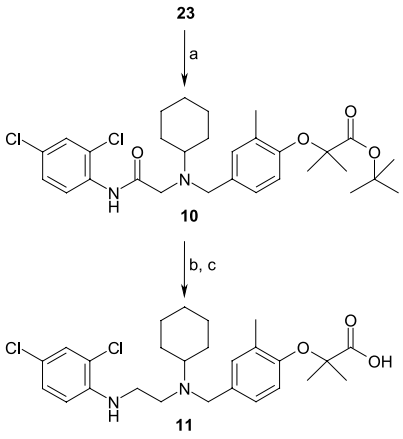
In addition to the depicted solid-phase approach, several structural features were addressed using solution chemistry: compound **23** (see Table 1), obtained via solid-phase chemistry according to Scheme 1, was esterified with isobutylene in acidic media.[11] Subsequent reduction of the amide bond with borane dimethyl sulfide complex and acid catalyzed hydrolysis of the *tert*-butyl ester yielded the aminoethyl substituted phenoxy acetic acid **11** (Scheme 2). The harsh reaction conditions required for amide reduction were incompatible with our general solid-phase approach.

Table 1. Activity[a] of compounds **17**–**25** against human PPAR receptors

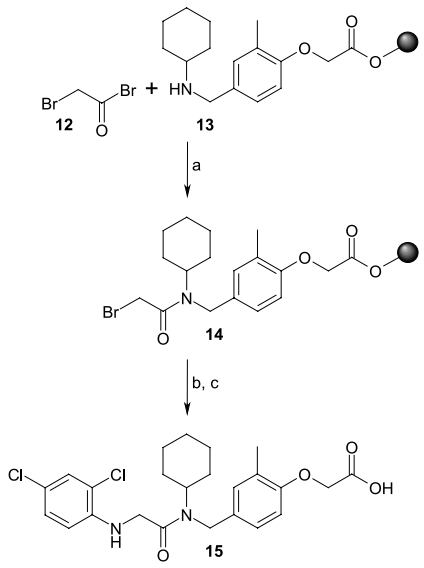
Compound	Structures[a] **16**		Human PPARδ EC_{50} (μM)[14]	Human PPARα EC_{50} (μM)	Human PPARγ EC_{50} (μM)
					
	R^4	R^3			
17		H	0.08	0.25	1.0
18		Me	0.04	0.17	0.8
19		Me	0.1	⩾1.5[b]	⩾ 1.5
20		Me	0.15	⩾2.5	⩾2.5
21		Me	0.02	0.5	⩾5
22		Me	0.003	>10	>10
23		Me	0.003	⩾1	0.3
24		H	0.06	⩾4	n.d.
25		Me	0.007	>10	0.015

[a] Cell-based transactivation assay.[12]

[b] ⩾ Means no saturation of dose–response curve at concentration.



Scheme 2. Amide reduction: Reagents and conditions: (a) isobutylene, H_2SO_4, CH_2Cl_2, rt, 65%; (b) BH3*SMe_2, toluene, 110 °C, 55%; (c) CF_3COOH, CH_2Cl_2, rt, 79%.



Scheme 3. Synthesis of glycyl amino acetic acid **15**: Reagents and conditions: (a) Et_3N, CH_2Cl_2, rt; (b) 2,4-dichloro aniline, Et*i*Pr_2N, DMF, 80 °C; (c) CF_3COOH, CH_2Cl_2, rt, 55% over all steps.

The importance of the position of the carbonyl group was further elucidated: glycylamino acetic acid **15** was obtained by amid formation between benzylic amine **13**—an intermediate of our general solid-phase approach—and bromoacetyl bromide (**12**), followed by the introduction of 2,4-dichloro aniline under basic conditions and final release of the free acid from the resin (Scheme 3).

Table 1 shows the EC_{50} for compounds **17**–**25** in the transient transactivation assay against the three human PPAR subtypes: δ, α, and γ, respectively.[12,13] Compound **17** has already been synthesized in the course of our PPARα program.[8] Due to its reversed selectivity profile compared to the PPARα agonist **3**, we chose **17** as the starting point for our optimization program towards δ-selectivity. Simple introduction of an *ortho*-methyl substituent, as in **18**, increased the potency by the factor 2, while retaining the same selectivity profile. Variation of the substituent R^4 at the secondary amide, while keeping the rest of the molecule unchanged compared to **18**, led to compounds **19**–**25**. Introduction of lipophilic substituents with an *n*-alkyl (not shown) or β-branched cycloalkyl chain (**19**, **20**) did not lead to higher δ-selectivity. However, direct attachment of sterically demanding cycloalkyl groups as in compounds **21**–**23** resulted in a significant increase in potency at the δ receptor while decreasing the activity at α and γ receptors. Exchanging the methyl group at R^3 for hydrogen (**24**) proves the initial hypothesis that potency increases with the introduction of substituents at that position.

Surprisingly, exchanging the cyclohexyl group in **23** by a phenyl moiety led to the PPARδ/γ nonselective agonist **25**, thus paving the way to compounds with a dual mode of action for further mechanistic studies.

Similarly, compound **11** (Scheme 2) derived from **23** via reduction of the amide bond shows considerable activity on the δ and γ receptor (0.01 and 0.35 μM) while keeping the selectivity profile against the α receptor (>10 μM).

Cyclohexyl substituted phenoxy acetic acid **23** served as the prototype for further optimization, as discussed in Table 2.

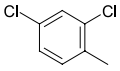
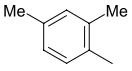
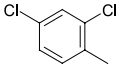
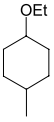
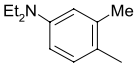
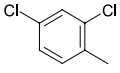
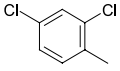
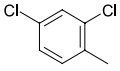
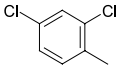
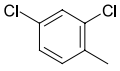
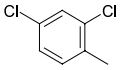
Introduction of substituents in the 2- (**26**) and 3-position (**27**) of the cyclohexyl ring reduced the activity at the δ receptor. In contrast, derivatization at the 4-position as in ethoxy substituted cyclohexyl derivative **28**, combined high potency with >1000-fold selectivity against the other PPAR receptors. Modification of the aryl group R^5 confirmed results from previous studies:[8] 2,4-disubstituted anilines are superior to other substitution patterns. In addition, introduction of polar substituents on the aryl ring as in **29** are not tolerated.

Variation of R^1–R^3 on the phenoxyacetic acid head group while keeping R^4 (cyclohexyl) and R^5 (2,4-dichlorophenyl) constant (**30**–**35**) clearly shows that in order to achieve high δ-selectivity, it is mandatory to introduce a substituent *ortho* to the phenol while reducing the steric bulk adjacent to the acid functionality. Thus, unsubstituted acetic acid derivative **33** is a full agonist at the δ receptor with an EC_{50} of 3 nM with virtually no effect on the α and γ receptors up to greater than 100 μM.

Inverted introduction of the bromoacetyl bromide building block, as exemplified by compound **15** (Scheme 3), leads to a complete loss in activity on all the three receptor subtypes (>1 μM).

The PPAR subtypes show significant sequence variation in the residues that line the ligand-binding domain (LBD). However, several conserved hydrogen-bonding interactions of PPAR agonists with key amino acids of the LBD have been determined across all subtypes.[15] Despite the availability of crystal structures of all three PPAR LBDs with and without ligand,[15,16] it can only

Table 2. Further optimization of **23** by variation of R^1–R^5

Compound	Structures **9**				Human PPARδ EC_{50} (μM)[14]	Human PPARα EC_{50} (μM)	Human PPARγ EC_{50} (μM)
	R^5	R^4	R^3	R^1 R^2			
26			Me	Me Me	0.4	>10	⩾4
27			Me	Me Me	0.1	⩾4	⩾4
28			Me	Me Me	0.005	⩾5	⩾5
29			Me	Me Me	1.5	0.2	0.5
30			OMe	Me Me	0.03	>10	>10
31			OMe[a]	Me Me	0.03	⩾1	⩾2
32			Me	Me[b] H	0.01	1	>10
33			Me	H H	0.003	>100	>100
34			H	H H	0.05	>10	>10
35			Cl	H H	0.015	>10	>10

[a] *meta*-OMe instead of *ortho*-OMe.
[b] Racemic mixture.

be speculated about the nature of the selectivity of our novel PPARδ ligands.

Pharmacokinetics of **33** were evaluated in rats ($n = 2$) with an oral dose of 5 mg/kg. The maximum plasma concentration was found to be 1.24 μM, the half life being 1.3 h. Incubation of **33** with rat or human liver microsomes in vitro revealed that metabolism of the parent compound occurs mainly by hydroxylation of the cyclohexyl moiety. Consequently, introduction of polar or sterically demanding groups in this position should further improve half-life of novel congeners. Investigations in this direction as well as in-depth pharmacological studies are currently ongoing in our group.

Starting from a highly selective PPARα agonist, **3**, we were able to identify a series of potent and selective PPARδ agonists (e.g. **33**) by using combinatorial chemistry on solid support. These phenoxyacetic acid derivatives will be valuable tools for further elucidation of the biological role of the PPARδ receptor as well as new therapeutic agents for the treatment of metabolic diseases like dyslipidemia, obesity, and atherosclerosis.

Acknowledgments

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10. e.g. **33**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.10–1.40 (m, 3H), 1.55 (m, 2H), 1.70 (m, 1H), 1.93 (m, 2H), 2.14 (m, 2H), 2.16 (s, 3H), 3.41 (m, 1H), 4.09 (s, 2H), 4.21 (s, 2H), 4.62 (s, 2H), 6.57 (d, J = 8.3 Hz, 1H), 7.03 (dd, J = 1.9 and 8.2 Hz, 1H), 7.10 (d, J = 1.8 Hz, 1H), 7.17 (dd, J = 2.3 and 8.7 Hz, 1H), 7.31 (d, J = 2.3 Hz, 1H), 7.63 (d, J = 8.7 Hz, 1H), 9.57 (s, 1H).
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12. The different PPAR subtypes used in the assay are fusion proteins containing the ligand binding domain for PPARδ (aa 139–442), PPARα (aa 167–468) and PPARγ (aa 203–506), respectively, fused to the GAL4 DNA-binding domain (aa 1–147). The assay was performed as stated in literature.[7,8]
13. The results are based on two to three independent experiments each dose done in triplicate (SD = 20%).
14. All compounds (except **29**) are full agonists on PPARδ with >70% efficacy, e.g. **33**: PPARδ 2 nM, 75% efficacy; PPARα /γ >10 μM, 30% efficacy.
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