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Identification of a Series of PPARγ/δ Dual Agonists Via Solid-Phase Parallel Synthesis

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Abstract—We have developed a general solid-phase synthesis for identification of PPAR ligands. Synthesis of a 480-member library led to the identification of a potent PPARγ/δ dual agonist **23**. Compound **23** showed good plasma exposure in rats and demonstrated antihyperglycemic and antihyperlipidemic efficacy in diabetic fatty Zucker rats.

The metabolic syndrome X, which consists of a clustering of several metabolic risk factors in a single patient, contributes significantly to increased mortality.[1,2] The major components of the metabolic syndrome X include dyslipidemia (characterized by the lipid triad of low levels of HDL-c and high levels of triglycerides and LDL-c), insulin resistance, obesity, and hypertension. Although established therapies are available to treat these risk factors individually (e.g., statins and fibrates for dyslipidemia and metformin and glitazones for insulin resistance), no single drug is yet available to treat the multiple risk factors of the metabolic syndrome X.

Compelling evidence suggests that the hyperlipidemic effects of the fibrate drugs and the antidiabetic effects of the glitazones are due to activation of the α and γ subtypes, respectively, of the peroxisome proliferator-activated receptor (PPAR).[3–7] The PPARs are orphan receptors that belong to the nuclear receptor superfamily of ligand-activated transcription factors. Identification and development of PPARα/γ dual agonists for treatment of the metabolic syndrome X has therefore become one of the major efforts in the pharmaceutical industry.[8–10] PPARδ—the third subtype in the PPAR family—has not been identified as a receptor for any known class of drug molecules. However, we recently reported a potent and subtype-selective PPARδ agonist, GW501516, which has therapeutic potential for treatment of dyslipidemia.[11] In insulin-resistant obese rhesus monkeys, GW501516 increased HDL-c, decreased fasting triglycerides, and decreased VLDL in a dose-dependent manner over the treatment period, relative to vehicle-treated animals. These data suggest that PPARδ is a new molecular target for treatment of dyslipidemia. Hence, PPARγ/δ dual agonists may provide an efficient treatment for metabolic syndrome X by providing dual control of glucose and lipid metabolism. As part of our ongoing research in the identification of structurally novel nuclear receptor ligands, we report the identification of a PPARγ/δ dual agonist through solid-phase parallel synthesis of a 480-member library of lipophilic carboxylic acids.

The general solid-phase synthesis of target molecules **1** and **2** is depicted in Scheme 1. The molecules have three points of diversity: R^1, R^2, and the headgroup. The headgroups have a carboxylic acid group, central phenyl ring, and a linker to the urea or amide tail. This general solid-phase synthesis is a modified version of our previously published synthesis.[12] In our previous strategy, the R^1 was introduced by borane reduction of the amides. In this new approach, R^1 is introduced under milder and more convenient solid-phase Mitsunobu reaction conditions using Fukuyama's 2,4-dini-

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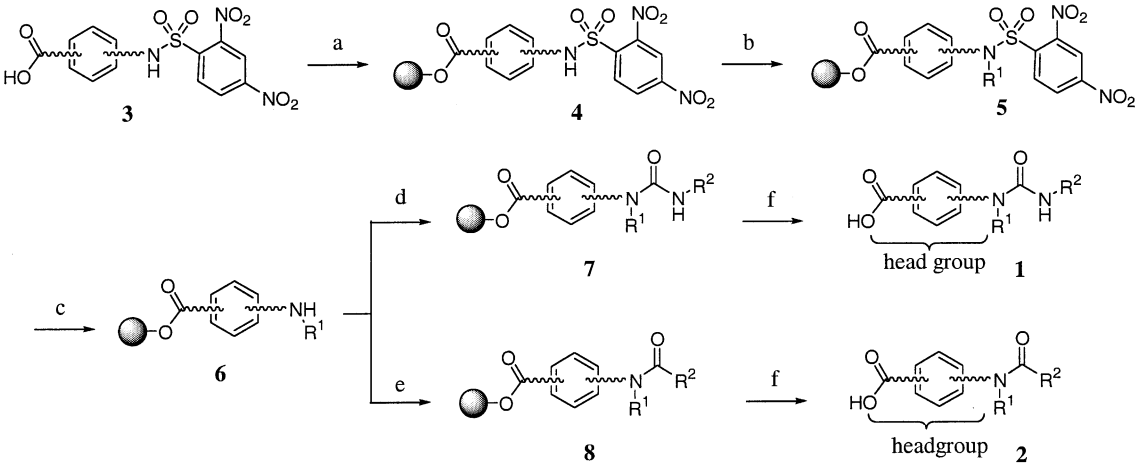


Scheme 1. Reagents and conditions: (a) 1,3-dimethyl-2-fluoropyridinium 4-toluenesulfonate (3 equiv relative to the resin), 2,6-lutidine (9 equiv relative to the resin), SASRIN resin (0.89 mmol/g), CH_2Cl_2, rt, overnight; (b) R^1OH (10 equiv), PPh_3 (10 equiv), diisopropyl azodicarboxylate (DIAD, 10 equiv), THF, rt, overnight; (c) mercaptoacetic acid (10 equiv), NEt_3 (10 equiv), CH_2Cl_2, rt, 1.5 h; (d) R^2NCO (10 equiv), DMF, rt, overnight; (e) R^2COOH (10 equiv), *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU, 10 equiv), iPr_2NEt (10 equiv), rt, overnight; (f) 10% TFA/CH_2Cl_2 (v/v), rt, 30 min.

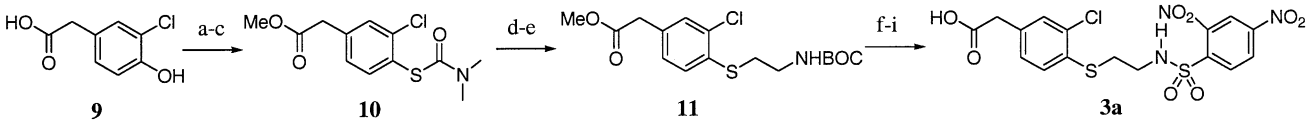
trobenzenesulfonamide chemistry.[13] The synthesis starts with loading of the 2,4-dinitrobenenesulfonyl-derivatized headgroup (vide infra) onto SASRIN resin (0.89 mmol/g) with Mukaiyama's reagent (1,3-dimethyl-2-fluoropyridinium 4-toluenesulfonate)[14] to provide **4**. Quantitative loading was achieved when more than three equivalents of the 2,4-dinitrobenzenesulfonyl-derivatized headgroup was used. Several other reaction conditions (e.g., carbodiimides) for loading the starting material to the support were also explored, but all these conditions failed to provide high yields.

The efficient loading is critical in order to ensure high purity of the final products **1** and **2** because it was found that the free hydroxyl groups left on the solid support could react [15] with the sulfonamide moiety at other sites on the resin during the subsequent Mitsunobu reaction, which ultimately leads to inseparable impurities after cleavage from the resin. Mitsunobu reaction between **4** and the desired alcohols provided **5** with different R^1 functionality. After removal of the 2,4-dinitrobenzenesulfonyl group with mecaptoacetic acid, the secondary amines **6** were then reacted with either isocyanates or carboxylic acids to provide support-bound compounds **7** and support-bound compounds **8**, respectively, which upon cleavage with TFA/CH_2Cl_2 furnished the targeted compounds **1** and **2**.

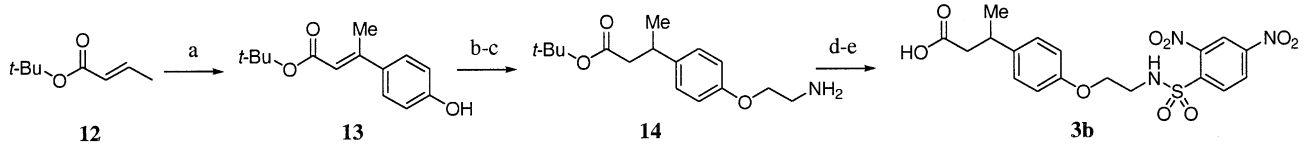
The previous SAR of PPAR ligands has shown that the structure of the headgroup is critical in order to achieve good affinity to all three PPAR subtypes, with PPARδ being the most difficult one to achieve. The headgroups **3a–c** (Schemes 2–4) chosen for the library production have been shown or hypothesized to provide good potency on PPARδ based on previous SAR, crystal structures, and molecular modeling.[16] Synthesis of the 2,4-dinitrobenzenesulfonyl-derivatized headgroups is depicted in Schemes 2–4. The alcohol, isocyanate, and carboxylic acid monomers[17] selected for the library were all commercially available except the alcohol monomer 2-cyclopropyl-4-hydroxymethylphenyl-1,2-4-oxadiazole (monomers: R^1OH, **f**). These monomers were selected based on previous SAR, molecular modeling, and synthetic compatibility.

The library was produced in a spatially-separated format using Robins plates with 50 mg of resin in each well. For each headgroup, 10 alcohols and eight isocyanates or eight carboxylic acids were used. Totally, 480 compounds (240 compounds **1** and 240 compounds **2**) were synthesized via this methodology. All the final compounds were analyzed by LC–MS and the average purity was greater than 70%.

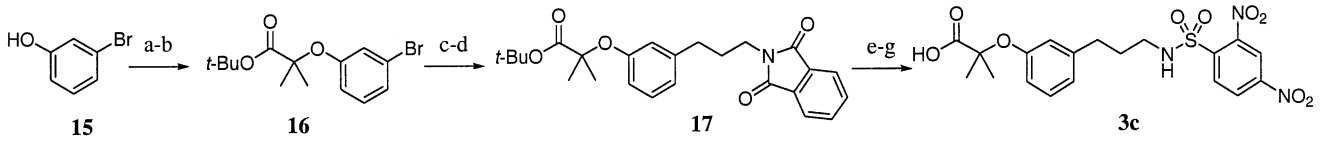
The library was screened against all three human PPAR subtypes in a SPA binding assay.[18] Based on the binding assay results, 23 compounds were identified with $>50\%$ displacement of radioligand [^{3}H]-GW2433 at 1 μM in the PPARδ binding assay. Following screening of these compounds in a cell-based assay,[19] a subset of six compounds was remade in solution phase for confirmation of activity and the functional data is depicted in Table 1. From this effort we identified a potent and selective dual PPARγ/δ dual agonist **23** (Fig. 1). Compound **23** has



Scheme 2. Reagents and conditions: (a) CH_3COCl, MeOH, reflux, overnight; (b) dimethylthiocarbamoyl chloride, 4-dimethylamino pyridine (DMAP), NEt_3, 1,4-dioxane, reflux, 4 h; (c) tetradecane, 250 °C, 5 h, 83% (3 steps); (d) NaOMe, MeOH; (e) *tert*-butyl *N*-(2-(*p*-tosyloxy)ethyl)-carbamate (prepared from *tert*-butyl *N*-(2-hydroxyethyl)-carbamate and tosyl chloride), MeOH, rt, overnight, 77% (2 steps); (f) 4 M HCl/1,4-dioxane, rt, 2 h; (g) Na_2CO_3; (h) 2,4-dinitrobenzenesulfonyl chloride, NEt_3, CH_2Cl_2, 0 °C, 1 h, 52% (3 steps); (i) LiOH, THF/H_2O/MeOH (4:1:0.4), 95%.



Scheme 3. Reagents and conditions: (a) 4-iodophenol, $Pd_2(dba)_3$, tri-*O*-tolylphospine, NEt_3, pyridine, 55%; (b) benzyl *N*-(2-(*p*-tosyloxy)ethyl)-carbamate (prepared from benzyl *N*-(2-hydroxyethyl)-carbamate and tosyl chloride), Cs_2CO_3, DMF, rt, overnight, 50%; (c) H_2, 10% Pd/C, 98%; (d) 2,4-dinitrobenzenesulfonyl chloride, NEt_3, CH_2Cl_2, 0 °C, 1 h, 60%; (e) TFA/CH_2Cl_2, rt, 2 h, 100%.



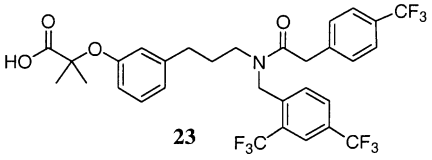
Scheme 4. Reagents and conditions: (a) 1-methyl-1-trichloromethylethanol, NaOH, acetone, 99%; (b) isobutylene, H_2SO_4, CH_2Cl_2, 70%; (c) *N*-allylphthalimide, $Pd(OAc)_2$, tri-*O*-tolylphospine, iPr_2NEt, CH_3CN, 60%; (d) H_2, 10% Pd/C, 95%; (e) EtOH, NH_2NH_2, reflux, 88%; (f) 2,4-dinitrobenzenesulfonyl chloride, NEt_3, CH_2Cl_2, 0 °C, 1 h, 62%; (g) TFA/CH_2Cl_2, rt, 2 h, 100%.

Table 1. Transient transfection[a] data of the library remakes

Compd	Headgroup	R^1OH	R^2NCO	R^2COOH	EC_{50} (nM) hPPARγ	EC_{50} (nM) hPPARδ	EC_{50} (nM) hPPARα
18	**3c**	i	c		0.5	160	230
19	**3c**	i		d	30	93	710
20	**3c**	f		e	81	430	620
21	**3c**	f		d	170	1000	230
22	**3c**	f		a	320	1000	59
23	**3c**	i		e	4	19	620

[a]Compounds were assayed for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected CV-1 cells as described;[19] EC_{50} = the concentration of test compound that gave 50% of the maximal reporter activity. All data ±15% ($n=3$).

sub-nanomolar potency against PPARγ and PPARδ and has greater than 30-fold selectivity versus PPARα in both binding and functional assays. Compound **23** was also tested against the three murine PPAR subtypes in the functional assay and showed EC_{50} of 28 nM, 19 nM, and >1000 nM on murine PPARγ, PPARδ, and PPARα, respectively. The in vivo exposure of **23** was evaluated in rats ($n=3$) with an oral dose of 3 mg/kg. At 0.5, 1.0, and 2.0 h, the mean plasma concentrations of **23** were found to be 373, 722, and 524 ng/mL, respectively. The in vivo antihyperglycemic and antihyperlipidemic efficacy of **23** were then evaluated in male ZDF rats ($n=6$). When dosed orally at 30 mg/kg bid, the compound reduced plasma glucose by 47% and serum triglyerides by 51% of the vehicle animals after 7 days of administration. During the same period of administration, compound **23** increased the HDL-c by 24% compared to the vehicle animals.



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	hPPARγ	hPPARδ	hPPARα
K_i (nM)	10	5	420
EC_{50} (nM)	4	19	620

Figure 1. A PPARγ/δ dual agonist.

In summary, we have developed a general solid-phase synthesis of lipophilic carboxylic acids that led to the identification of a potent PPARγ/δ dual agonist. Compounds with this dual receptor activity may provide a new approach to the development of drugs for the metabolic syndrome X.

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17. Monomers: R^1OH: **a**, 4-biphenylmethanol [3597-91-9]; **b**, 4-(dimethylamino)phenethyl alcohol [50438-75-0]; **c**, 1-heptanol [111-70-6]; **d**, 6-methyl-2-pyridinemethanol [1122-71-0]; **e**, 4-isopropylbenzyl alcohol [536-60-7]; **f**, 2-cyclopropyl-4-hydoxymethylphenyl-1,2-4-oxadiazole; **g**, 4-(trifluoromethyl)-benzyl alcohol [349-95-1]; **h**, methyl 4-(hydroxymethyl)-benzoate [6908-41-4]; **i**, 2,4-bis(trifluoromethyl)benzyl alcohol [143158-15-0]; **j**, piperonyl alcohol [495-76-1]. R^2NCO: **a**, trans-2-phenylcyclopropyl isocyanate [63009-74-5]; **b**, 2-(trifluoromethyl)phenyl isocyanate [2285-12-3]; **c**, ethyl 4-isocyanatobenzoate [30806-83-8]; **d**, 4-(trifluoromethyl)phenyl isocyanate [1548-13-6]; **e**, cyclohexyl isocyanate [3173-53-3]; **f**, benzyl isocyanate [3173-56-6]; **g**, 4-isopropylphenyl isocyanate [31027-31-3]; **h**, 4-(trifluoromethylthio)phenyl isocyanate [24032-84-6]. R^2COOH: **a**, 3,5-bis(trifluoromethyl)benzoic acid [725-89-3]; **b**, 1-(4-chlorophenyl)-1-cyclopropanecarboxylic acid [72934-37-3]; **c**, 3-(3,4-dimethoxyphenyl)propionic acid [2107-70-2]; **d**, 3,4-dichlorophenoxyacetic acid [588-22-7]; **e**, 4-(trifluoromethyl)phenylacetic acid [32857-62-8]; **f**, 4-(2-thienyl)butyric acid [4653-11-6]; **g**, 3-(3-pyridyl)acrylic acid [1126-74-5]; **h**, (5-methyl-2-phenyloxazol-4-yl)acetic acid.
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