Synthesis and biological evaluation of novel pyrrolidine acid analogs as potent dual PPARα/γ agonists

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · FEBRUARY 2015

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2015.01.066 · Source: PubMed

Bioorganic & Medicinal Chemistry Letters 25 (2015) 1196–1205

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of novel pyrrolidine acid analogs as potent dual PPARα/γ agonists

Hao Zhang [a,*], Charles Z. Ding [a], Zhi Lai [a], Sean S. Chen [a], Pratik Devasthale [a], Tim Herpin [a], George Morton [a], Fucheng Qu [a], Denis Ryono [a], Rebecca Smirk [a], Wei Wang [a], Shung Wu [a], Xiang-Xang Ye [a], Yi-Xin Li [f], Atsu Apedo [f], Dennis Farrelly [b], Tao Wang [c], Liqun Gu [b], Nathan Morgan [b], Neil Flynn [b], Cuixia Chu [b], Lori Kunselman [b], Jonathan Lippy [c], Kenneth Locke [c], Kevin O'Malley [c], Thomas Harrity [b], Michael Cap [b], Lisa Zhang [d], Vinayak Hosagrahara [d], Pathanjali Kadiyala [d], Carrie Xu [d], Arthur M. Doweyko [e], Robert Zahler [a], Narayanan Hariharan [b], Peter T. W. Cheng [a,*]

[a] Metabolic Diseases Chemistry, Bristol-Myers Squibb Research and Development (R&D), Princeton, NJ 08543-5400, USA
[b] Metabolic Diseases Biology, Bristol-Myers Squibb R&D, Princeton, NJ 08543-5400, USA
[c] Lead Evaluation, Bristol-Myers Squibb R&D, Princeton, NJ 08543-5400, USA
[d] Metabolism and Pharmacokinetics, Bristol-Myers Squibb R&D, Princeton, NJ 08543-5400, USA
[e] Computer-Assisted Drug Design, Bristol-Myers Squibb R&D, Princeton, NJ 08543-5400, USA
[f] Discovery Analytical Sciences, Bristol-Myers Squibb R&D, Princeton, NJ 08543-5400, USA

ARTICLE INFO

Article history:
Received 28 November 2014
Revised 27 January 2015
Accepted 29 January 2015
Available online 4 February 2015

Keywords:
Peroxisome Proliferator-Activated Receptor
Dual agonists
Pyrrolidine acid
Type 2 diabetes

ABSTRACT

The design, synthesis and structure–activity relationships of a novel series of 3,4-disubstituted pyrrolidine acid analogs as PPAR ligands is outlined. In both the 1,3- and 1,4-oxybenzyl pyrrolidine acid series, the preferred stereochemistry was shown to be the *cis*-3*R*,4*S* isomer, as exemplified by the potent dual PPARα/γ agonists **3k** and **4i**. The *N*-4-trifluoromethyl-pyrimidinyl pyrrolidine acid analog **4i** was efficacious in lowering fasting glucose and triglyceride levels in diabetic *db*/*db* mice.

The PPARs (Peroxisome Proliferator-Activated Receptors) are a group of nuclear hormone receptors which function as transcription factors in the regulation of genes involved in glucose and lipid fatty acid metabolism as well as vessel wall function.[1] Three PPAR subtypes have been identified: PPARα, γ and δ. PPARα is predominantly expressed in catabolically active tissues such as liver, heart, kidney, and muscle. PPARα has been shown to be intimately involved in the uptake and β-oxidation of free fatty acids as well as lipoprotein metabolism.[2] The clinically used PPARα agonists comprise the fibrate class of drugs (including fenofibrate[3] and gemfibrozil[4]), which elevate HDL cholesterol and lower triglyceride and LDL cholesterol levels. PPARγ, which is mainly expressed in adipose tissue, regulates insulin sensitivity, glucose and free fatty acid utilization as well as adipocyte differentiation.[5] The clinically used PPARγ agonists encompass the thiazolidinedione (TZD) class of antidiabetic drugs such as rosiglitazone[6] and pioglitazone.[7]

An attractive hypothesis has been that combining PPARγ and PPARα agonist activities in a single compound would result in an excellent anti-diabetic agent based on the projected synergistic improvements in insulin sensitivity and normalization of glucose metabolism as well as the amelioration of the characteristic dyslipidemia associated with type 2 diabetes.

As previously described, the initial structure–activity relationship (SAR) exploration of the oxybenzylglycine series of PPARα/γ dual agonists resulted in the discovery of the Phase III clinical compound muraglitazar **I**.[8] Compound **I** has good in vitro functional activity at both human PPARα and PPARγ (αEC_{50} = 0.32 μM and γ EC_{50} = 0.11 μM in CV-1 cells; αEC_{50} = 1.41 μM & γ EC_{50} = 0.035 μM in HEK293 cells) and demonstrated excellent efficacy in animal models of type 2 diabetes and the associated dyslipidemia. Our subsequent goal in the program was to discover backup PPARα/γ dual agonists with significantly differentiated in vitro/in vivo profiles from **I** (while retaining in vivo efficacy and safety). We initially investigated structurally differentiated analogs of muraglitazar by conformationally constraining the 'head-piece' *N*-carbamoyl

* Corresponding authors.

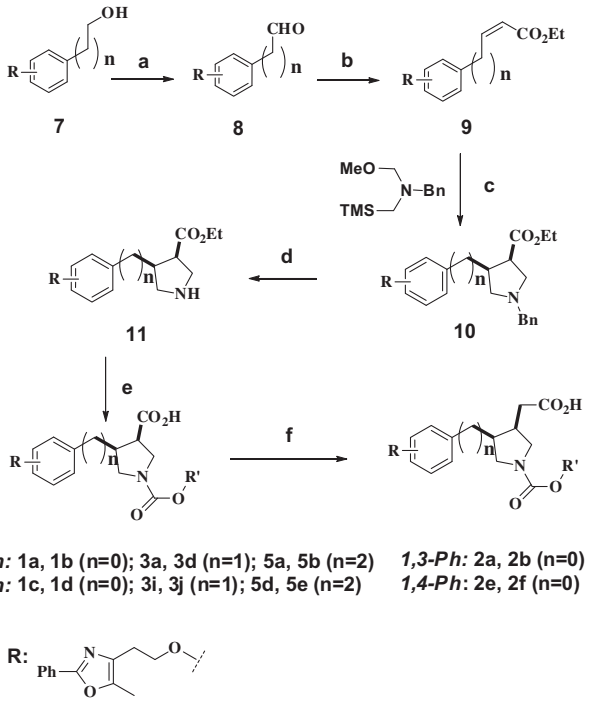
http://dx.doi.org/10.1016/j.bmcl.2015.01.066
0960-894X/

glycine moiety of **I**. This included the structural motif where the benzylic methylene is cyclized onto the glycine α-carbon to form a 2,5-disubstituted pyrrolidine acid, as exemplified by **II**, which is a relatively potent and highly selective PPARγ agonist; analogs from this series have demonstrated good activity both in vitro and in vivo. To further explore the scope of the SAR of this interesting pyrrolidine acid series, we decided to investigate the effect of shifting both the 5-aryl and 2-carboxylic acid substituents of the pyrrolidine ring of **II** into the corresponding 4- and 3-positions (i.e., structure **III**). We report here the exploration of the SAR of this 3,4-disubstituted pyrrolidine acid series (analogs **1a**–**6c**, Fig. 1). This effort resulted in the discovery of a number of analogs which have potent dual PPARα and PPARγ agonist in vitro activity (both in binding and functional assays).

The synthesis of the racemic *cis*-3,4-disubstituted pyrrolidine acid analogs is shown in Scheme 1.[9] Oxidation of alcohol **7**[10] with pyridinium chlorochromate (PCC) gave the aldehyde **8**, which was subjected to reaction with a bis-trifluoroethyl phosphonate Horner–Emmons reagent to give preferentially the *cis*-alkenyl ester **9**.[11] The *cis*-alkenyl ester **9** was then reacted with *N*-trimethylsilylmethyl *N*-methoxymethyl benzylamine under acidic conditions in a 1,3-dipolar cycloaddition to give the corresponding *cis*-*N*-benzyl pyrrolidine ester **10**.[12]

Hydrogenolysis of the *N*-benzyl group gave the free amine pyrrolidine ester **11**. Reaction of pyrrolidine ester **11** with appropriate chloroformates provided the corresponding pyrrolidine carbamates. Acid-mediated deprotection of the ethyl ester then provided the desired *cis*-substituted pyrrolidine-carbamate acid analogs (**1a**–**d**; **3a**, **3d**, **3i**, **3j**; **5a**, **5b**, **5d**, **5e**). The *cis* pyrrolidine acetic acid analogs **2a**, **2b**, **2e** and **2f** were synthesized by carrying corresponding pyrrolidine acids through a standard Arndt–Eistert homologation sequence.

Alternatively, standard Horner–Emmons reaction of aldehyde **8** gave the *trans*-alkenyl ester **12** after chromatography.[13] The *trans*-alkenyl ester **12** was subjected to the same acid-mediated 1,3 dipolar cycloaddition with *N*-TMS-methyl *N*-methoxymethyl benzylamine as in Scheme 1 to give the corresponding *trans*-substituted *N*-benzyl pyrrolidine-ester **13**. Analogous to the sequence described in Scheme 1, hydrogenolysis of the *N*-benzyl pyrrolidine ester **13** gave the pyrrolidine-ester **14**, which was reacted with the appropriate chloroformates, then deprotected under acidic conditions to provide the *trans*-substituted pyrrolidine-carbamate acids



Scheme 1. Reagents and conditions: (a) PCC; (b) $(CF_3CH_2O)_2P(O)CH_2CO_2Et$, KHMDS, 18-crown-6, THF, −78 °C; (c) TFA; (d) H_2, 10% Pd/C, acetic acid; (e) (i) R′OCOCl/$NaHCO_3$, (ii) HCl; (f) (i) $(COCl)_2$, (ii) CH_2N_2, (iii) $PhCO_2Ag$, $(C_2H_5)_3N$, (iv) LiOH/THF.

(**1e**, **1f**; **3g**, **3h**; **5c**, **5f**, **5g**). The *trans* pyrrolidine acetic acids (**2c**, **2d**; **6a**, **6b**) were synthesized from corresponding pyrrolidine acids by Arndt–Eistert homologation (Scheme 2).

The synthesis of the corresponding heteroaryl-substituted pyrrolidine acids is shown in Scheme 3. Copper-mediated *N*-arylation of the pyrrolidine ester **11** with an appropriate heteroaryl halide furnished the corresponding *N*-heteroaryl pyrrolidine ester.[14]

Deprotection of this ester under acidic conditions (necessitated due to partial epimerization of the stereochemistry of the α-carbon of the ester under basic hydrolytic conditions) then provided the racemic *cis*-aryl-substituted pyrrolidine acids (**4a**, **4m**, **4n**).

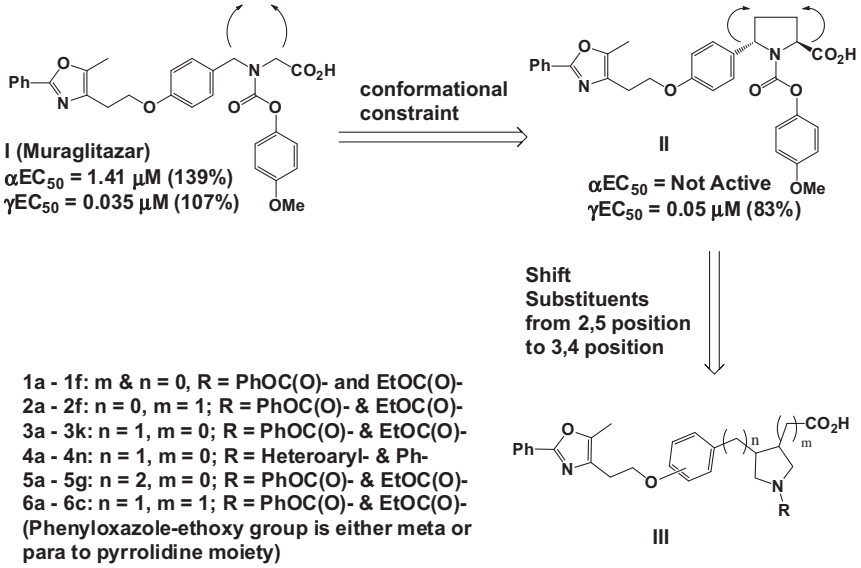
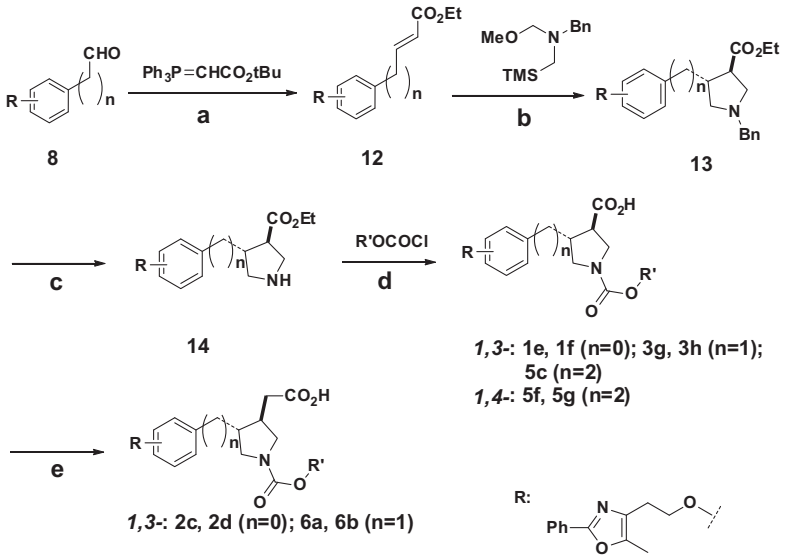
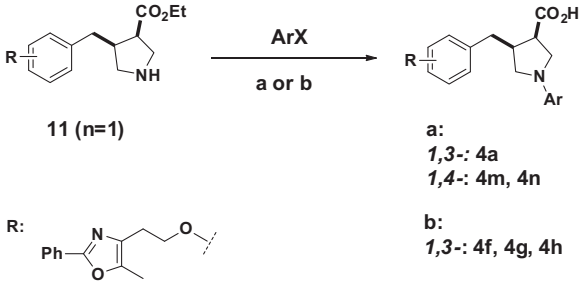


Figure 1. Design of 3,4-disubstituted pyrrolidine acids as PPARα/γ agonists.



Scheme 2. Reagents and conditions: (a) toluene, reflux; (b) TFA; (c) H_2, 10% Pd/C, acetic acid; (d) (i) $NaHCO_3$, (ii) HCl; (e) (i) $(COCl)_2$, (ii) CH_2N_2, (iii) $PhCO_2Ag$, $(C_2H_5)_3N$, (iv) LiOH/THF.



Scheme 3. Reagents and conditions: (a) (i) CuI, K_3PO_4, 2,6-lutidine, (ii) HCl; (b) (i) DIPEA, (ii) HCl.

Alternatively, reaction of pyrrolidine ester **11** with activated halo-heteroaryls such as 2-chloro-4-(trifluoromethyl) pyrimidines in the presence of Hunig's base followed by acid-mediated ester hydrolysis provided the pyrimidine substituted pyrrolidine acid analogs (**4f**, **4g**, and **4h**).

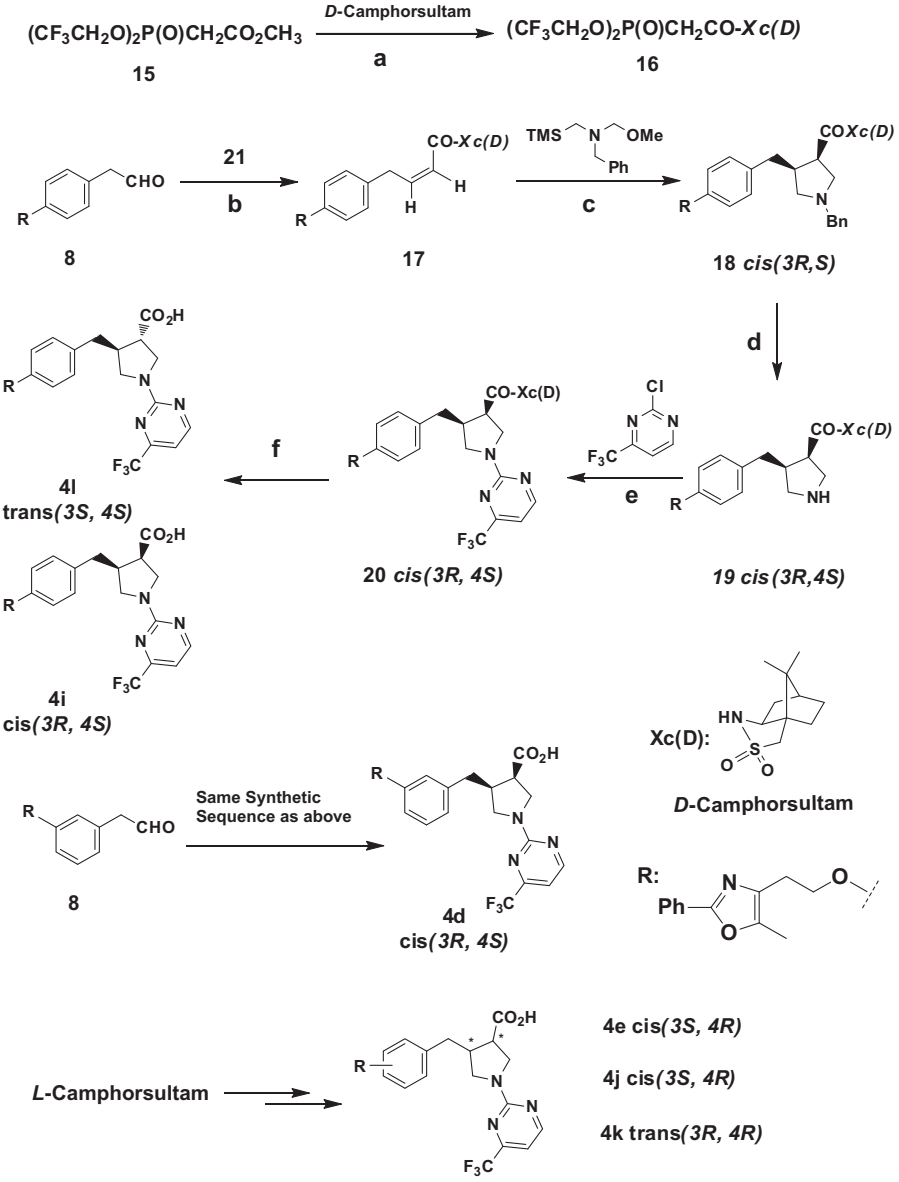
The absolute stereochemistry of the enantiomers of the 1,4-phenyl *cis*-pyrrolidine acid analogs **4j** (*cis 3S,4R*) or **4i** (*cis 3R,4S*) and **4l** (*trans 3S,4S*) or **4k** (*trans 3R,4R*) was determined by asymmetric synthesis as shown in Scheme 4. The key step in the synthesis is a chiral 1,3-dipolar cycloaddition using D-camphorsultam as the chiral auxiliary (X_c).[15] Me_3Al-mediated reaction of methyl 2-(bis(2,2,2-trifluoroethoxy)phosphoryl)acetate **15** with D-camphorsultam gave the chiral Horner–Emmons reagent **16**, which was reacted with aldehyde **8** to give the predominantly (*Z*)-alkene ester **17**. TFA-mediated 1,3-dipolar cycloaddition of chiral alkene-ester **17** with *N*-trimethylsilylmethyl *N*-methoxymethyl benzylamine provided a ~4:1 mixture of diastereoisomers, as determined by HPLC analysis. The major product, the chiral *cis*-pyrrolidine ester **18**, was readily isolated by column chromatography in 73% yield. The predicted absolute stereochemistry of the 2 chiral centers of **18** is (3*R*,4*S*), based on the 1,3-dipolar cycloaddition proceeding via the accepted transition state for the cycloaddition reaction of D-camphorsultam derivatives.[15] Hydrogenolysis of **18** gave the pyrrolidine ester **19**, which was then reacted with 2-chloro-4-(trifluoromethyl)pyrimidine in the presence of Hunig's base to provide the *N*-pyrimidinyl pyrrolidine ester **20**.

Deprotection was deliberately carried out under basic conditions in order to induce partial epimerization of C-3 on the pyrrolidine ring. This protocol provided a mixture of the chiral *cis* pyrrolidine-acid **4i** (3*R*,4*S*) and the corresponding *trans* pyrrolidine-acid **4l** (3*S*,4*S*) which were separated by preparative HPLC; their structures were fully characterized by extensive 1D and 2D NMR studies.

The corresponding *cis* (3*R*,4*S*) pyrrolidine-acid analog **4d** (in the 1,3-oxybenzyl series) was prepared by the same synthetic sequence as for **4i** except that acidic hydrolysis was used as the last step to avoid epimerization. Using this general methodology, we have also synthesized the corresponding reference enantiomers **4e** (*cis 3S,4R*), **4j** (*cis 3S,4R*), and **4k** (*trans 3R,4R*) by simply using the opposite L-camphorsultam chiral auxiliary.

Alternatively, the racemic pyrrolidine-ester **11** was also separated by chiral HPLC (on a Chiralpak AD column [5 cm × 50 cm, 20 μm] using an isocratic eluting system of 20% MeOH/EtOH(1:1) + 80% heptanes + 0.1% Et_2NH) to provide the two individual enantiomers **21** and **22**. Reaction of optically pure pyrrolidines **21** and **22** with chloroformates followed by acid-mediated ester hydrolysis provided the optically pure pyrrolidine-carbamate acid analogs **3b**, **3c**, **3e**, **3f**, **and 3k**, respectively (Scheme 5). The absolute configurations of the individual enantiomers from the chiral separation were determined by correlation with the enantiomers of known absolute stereochemistry obtained via asymmetric synthesis as described above. The homologated *cis* chiral pyrrolidine acetic acids **6c** was also synthesized by Arndt–Eistert reaction with **3b**. The absolute stereochemistry of analog **3e** (*cis 3R,4S*) was also separately confirmed by Vibrational Circular Dichroism (VCD).[16]

An alternative route for the asymmetric synthesis of pyrrolidine acids is shown in Scheme 6. Reaction of maleic anhydride **23** with (*R*)-methylbenzylamine and $(COCl)_2/Et_3N$ in CH_2Cl_2 provided maleimide **24** in 55%. This chiral maleimide underwent 1,3-dipolar cycloaddition with *N*-trimethylsilylmethyl-*N*-methoxymethyl benzylamine to give the corresponding *N*-benzyl pyrrolidine-ester **25**. Stereoselective reaction of *meso*-imide **25** (from the less-hindered face of the imide) with a protected 4-oxyaryl Grignard reagent gives an intermediate hydroxy lactam **26**,[17] which is immediately reduced with $NaBH_4$ followed by thermally-induced intramolecular cyclization to give the lactone **27** in 61% yield (98% ee). Deprotection of the THP group of **27** provided the phenol–lactone **27**,



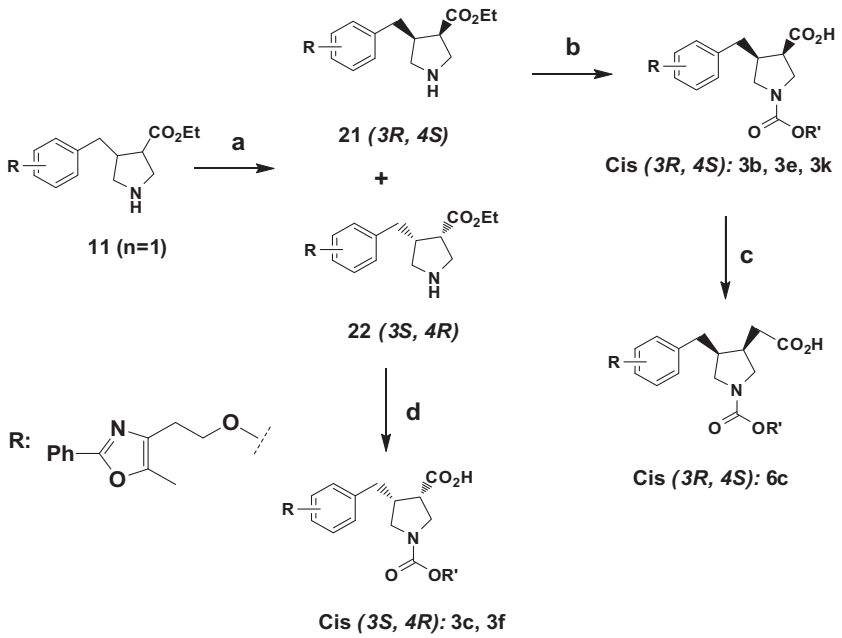
Scheme 4. Reagents and conditions: (a) $AlMe_3$; (b) KHMDS, 18-crown-6, THF, −78 °C; (c) TFA; (d) H_2, 10% Pd/C, acetic acid; (e) DIPEA; (f) LiOH/THF.

followed by base-mediated alkylation of the phenol **27** with phenyloxazole-ethanol mesylate **28** to provide the alkylated phenol lactone **28**. Hydrogenolysis of the *N*-benzyl group with concomitant cleavage of the benzylic lactone C–O bond provided the chiral pyrrolidine acid **29**. Subsequent reaction of **29** with 2-chloro-4-(trifluoromethyl)pyrimidine provided the chiral *N*-pyrimidinyl pyrrolidine acid **4i**;[18] the absolute stereochemistry of **4i** was correlated with material from the alternative asymmetric synthesis described above.

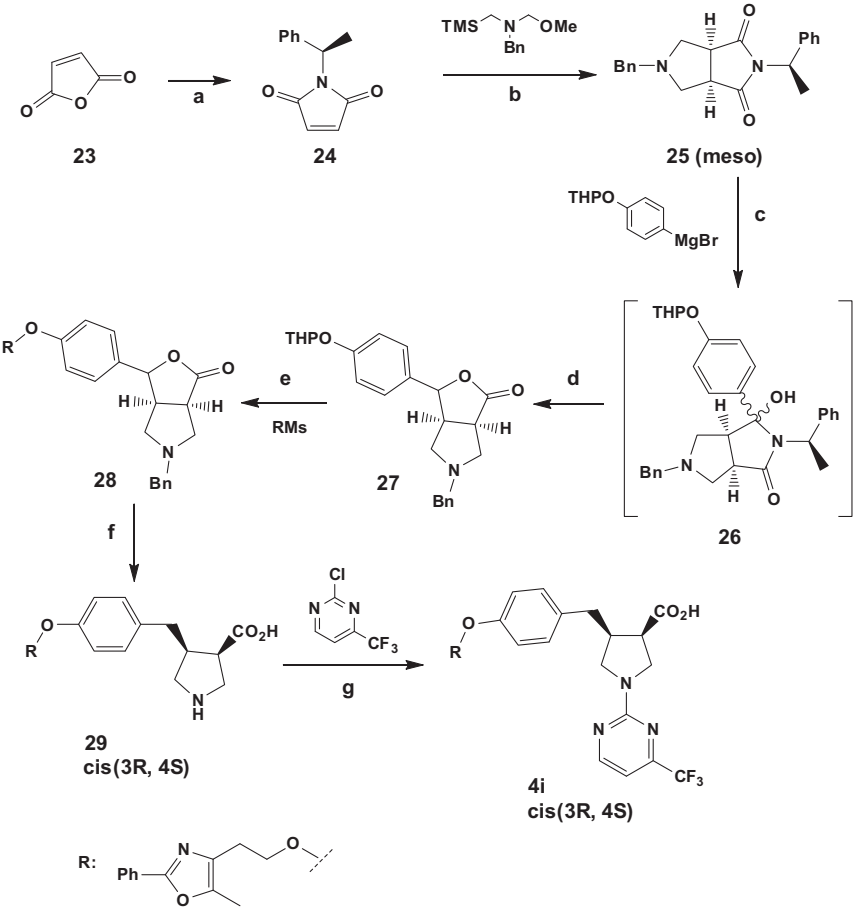
The PPARα and PPARγ in vitro activities (binding affinity[19] as well as transactivation activity[20]) of representative analogs from these two novel pyrrolidine-acid series are shown in Tables 1–5. In an earlier SAR study[21] on a structurally analogous azole acid series (derived from constraining the carbamate moiety of 1), we showed that PPARα/γ activities were modulated by: 1) the linker length between the central phenyl ring and the azole core and 2) 1,3 versus 1,4-substitution on the central phenyl ring. We sought to determine the importance of these structural features in the pyrrolidine acid series.

Our initial SAR studies with pyrrolidine acid analogs **1a**–**1f** are shown in Table 1. Comparison of the relative PPAR activities of both the 1,3- & 1,4-oxyphenyl series of racemic *cis*- or *trans*-pyrrolidine carbamate-acids (**1a**–**1e**) showed that they displayed very weak in vitro activities at both PPARα and PPARγ. The only compound to show significant PPARγ agonist activity was the racemic *trans*-pyrrolidine phenyl carbamate acid analog **1f** in the 1,4-oxyphenyl series. The analogs in this series have their two key pharmacophores (oxyphenyl & carboxylic acid) in fixed conformations at the 3- and 4-positions of the pyrrolidine ring, and are most likely not in optimal position in the PPARα or γ binding sites in the ligand-binding domain. We postulated that the introduction of additional flexibility into these two key substituents on the pyrrolidine ring would be necessary to improve their PPARα/γ in vitro activity.

Addition of a methylene linker between the pyrrolidine ring and the carboxylic acid, as illustrated by analogs **2a**–**2f**, resulted in dramatic changes in their PPAR in vitro activities (Table 2). In the 1,3-oxyphenyl series, the racemic homologated *cis* pyrrolidine

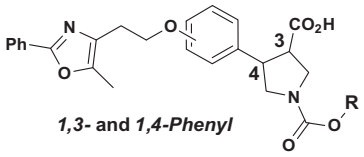


Scheme 5. Reagents and conditions: (a) chiral separation using AD column, 20% (MeOH/EtOH) in heptane + 0.1% DEA; (b) (i) R′OC(O)Cl/$NaHCO_3$, (ii) 20% HCl/HOAc; (c) (i) $(COCl)_2$, (ii) CH_2N_2, (iii) $PhCO_2Ag$, $(C_2H_5)_3N$, (iv) LiOH/THF; (d) (i) R′OC(O)Cl/$NaHCO_3$, (ii) 20% HCl/HOAc.



Scheme 6. Reagents and conditions: (a) (i) (*R*)-α-methyl benzyl amine, (ii) $(COCl)_2/CH_2Cl_2$′, (iii) TEA; (b) TFA; (c) THF; (d) (i) $NaBH_4$, (ii) toluene, reflux; (e) (i) *p*-TsOH in DCM, (ii) K_2CO_3 in CH_3CN; (f) H_2, 10% Pd/C, acetic acid; (g) DIPEA.

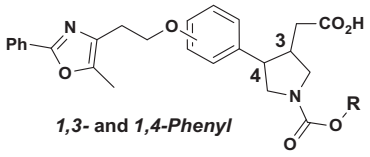
Table 1
In vitro PPARα and γ activities of phenyl pyrrolidine acid analogs **1**



Compd	Oxybenzyl substitution	3,4-Stereochemistry	R	γIC$_{50}$ (μM)	γEC$_{50}$ (μM) IA (%)	αIC$_{50}$ (μM)	αEC$_{50}$ (μM) IA (%)
1a	1,3-	±*cis*	-Et	>10	24 (58%)	>10	20 (52%)
1b	1,3-	±*cis*	-Ph	>10	5.65 (35%)	>10	1.65 (54%)
1c	1,4-	±*cis*	-Et	>10	>32	>10	>15
1d	1,4-	±*cis*	-Ph	4.47	4.18 (33%)	>10	>15
1e	1,4-	±*trans*	-Et	>10	>32	>10	>15
1f	1,4-	±*trans*	-Ph	5.86	0.01 (99%)	2.86	2.58 (58%)

IA = intrinsic activity.

Table 2
In vitro PPARα and γ activities of phenyl pyrrolidine-acetic acid analogs **2**



Compd	Oxybenzyl substitution	3,4-Stereochemistry	R	γIC$_{50}$ (μM)	γEC$_{50}$ (μM) IA (%)	αIC$_{50}$ (μM)	αEC$_{50}$ (μM) IA (%)
2a	1,3-	±*cis*	-Et	0.91	0.02 (85%)	0.99	1.13 (70%)
2b	1,3-	±*cis*	-Ph	0.24	4.80 (7%)	4.02	3.55 (18%)
2c	1,3-	±*trans*	-Et	5.46	9.68 (104%)	>10	8.10 (88%)
2d	1,3-	±*trans*	-Ph	1.82	14.6 (51%)	>10	11.7 (17%)
2e	1,4-	±*cis*	-Et	>10		>10	
2f	1,4-	±*cis*	-Ph	8.31	4.18 (33%)	>15	>15

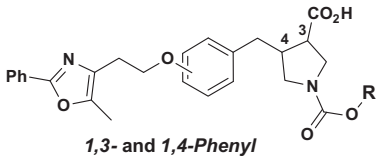
carbamate-acids (**2a** & **2b**) showed ⩾10-fold increases in potency at both PPARα & γ versus the corresponding pyrrolidine acid analogs (e.g., **1a** and **1b**). However, the *trans* pyrrolidine analogs **2c** & **2d** only showed modest PPARα and γ agonist activities. In the 1,3-oxyphenyl series, the homologated racemic *cis* pyrrolidine carbamate analogs (**2e** & **2f**) also did not show any improvement in PPARα and γ agonist activities versus **1c** and **1d**.

We next explored the effects of inserting a methylene spacer between the oxyphenyl substituent and the pyrrolidine ring (analogs **3a**–**3k** in Table 3). In the 1,3-oxyphenyl series, the corresponding oxybenzyl analogs (i.e., **3a** vs **1a**, **3d** vs **1b**) showed that increasing the distance not only dramatically improved PPARα/γ binding affinities, but also resulted in more potent functional activity at both receptors (from 5- to 30-fold at PPARγ and >1500-fold at PPARα for these 2 pairs of analogs). We then examined the effects of the relative & absolute stereochemistry of the 3- and 4-substituents on the pyrrolidine ring on PPAR potency. The (3*R*,4*S*) enantiomers (**3b** & **3e**) in the 1,3-oxybenzyl *cis*-pyrrolidine carbamate-acid series are very potent PPARα agonists (EC$_{50}$ = 3–4 nM), but the ethyl carbamate **3b** is a much more potent PPARγ agonist (γEC$_{50}$ = 9 nM), than the phenyl carbamate **3e** (γEC$_{50}$ = 2.0 μM). Both of the *cis* (3*R*,4*S*) enantiomers (**3b** & **3e**) are much more potent at PPARα/γ than the corresponding *cis* (3*S*,4*R*) enantiomers **3c** & **3f** (**3f** shows activity only at PPARα).

The SAR in the 1,4-oxybenzyl series correlates with the 1,3-oxybenzyl series; the racemic *cis* pyrrolidine carbamates **3i** and **3j** have moderate PPARγ activity (EC$_{50}$ = 0.30–0.64 μM), but are very potent PPARα agonists (EC$_{50}$ = 1–6 nM; >50-fold α vs γ selectivity). Of the two enantiomers of the *cis* pyrrolidine carbamate-acid **3i**, the preferred (3*R*,4*S*) analog **3k**, as expected, is a very potent PPARα/γ dual agonist (γ EC$_{50}$ = 30 nM; αEC$_{50}$ = 5 nM). Based on these results, for this pyrrolidine acid series, the *cis*-(3*R*,4*S*) absolute stereochemistry is established to be the preferred absolute stereochemistry for both binding affinity and optimal functional activity at PPARα and PPARγ.

In this oxybenzyl pyrrolidine acid series, we also replaced the *N*-carbamoyl moiety with various *N*-aryl substituents (which had provided very potent analogs in the related triazole acid series[21]) (**4a**–**4n**; Table 4). In the 1,3-oxybenzyl series, the racemic *cis* *N*-phenyl pyrrolidine acid **4a** is a potent PPARα agonist (EC$_{50}$ = 20 nM), but is >170-fold less active at PPARγ (EC$_{50}$ = 3.44 μM). As observed previously with the *N*-carbamoyl-substituted pyrrolidine

Table 3
In vitro PPARα and γ activities of benzyl pyrrolidine-acid analogs **3**



1,3- and *1,4-Phenyl*

Compd	Oxybenzyl substitution	3,4-Stereochemistry	R	γIC$_{50}$ (μM)	γEC$_{50}$ (μM) IA (%)	αIC$_{50}$ (μM)	αEC$_{50}$ (μM) IA (%)
3a	1,3-	±*cis*	Et	1.90	0.75 (58%)	0.17	0.01 (170%)
3b	1,3-	*cis* (3*R*,4*S*)	Et	1.51	0.009 (75%)	0.26	0.003 (127%)
3c	1,3-	*cis* (3*S*,4*R*)	Et	4.28	>10	>10	10.9 (27%)
3d	1,3-	±*cis*	Ph	1.33	1.25 (27%)	0.22	0.01 (165%)
3e	1,3-	*cis* (3*R*,4*S*)	Ph	0.49	2.0 (44%)	0.07	0.004 (130%)
3f	1,3-	*cis* (3*S*,4*R*)	Ph	0.59	13.3 (9%)	0.66	0.95 (93%)
3g	1,3-	±*trans*	Et	8.11	2.85 (105%)	2.12	0.15 (96%)
3h	1,3-	±*trans*	Ph	3.63	6.17 (59%)	3.09	2.59 (74%)
3i	1,4-	±*cis*	Et	2.68	0.30 (76%)	2.02	0.006 (96%)
3j	1,4-	±*cis*	Ph	0.08	0.64 (105%)	0.61	0.001 (125%)
3k	1,4-	*cis* (3*R*,4*S*)	Ph	0.08	0.03 (93%)	0.18	0.005 (98%)

analogs, the (3*R*,4*S*) enantiomer **4b** is ~13-fold more potent at PPARα than the corresponding (3*S*,4*R*) enantiomer **4c**. The enantiomeric pair of 4-trifluoromethyl pyrimidine analogs **4d** and **4e** also showed very similar PPAR activities to **4b** and **4c**. The more active (3*R*,4*S*) enantiomer **4d** is a potent PPARα selective dual agonist (αEC$_{50}$ = 14 nM; α/γ selectivity = >170-fold). In the racemic *cis* 1,4-oxybenzyl pyrrolidine acid series , the effect of the 4-pyrimidinyl substituent on PPAR activity was examined. The parent unsubstituted pyrimidine analog **4f** showed modest activity at both PPARα (EC$_{50}$ = 2.91 μM) and γ (EC$_{50}$ = 1.06 μM). Addition of a 4-methyl group on the pyrimidine (**4g**) improved PPARα activity (EC$_{50}$ = 170 nM; 17-fold vs **4f**) but PPARγ activity was relatively unchanged. The most potent analog in this series was the 4-trifluoromethyl pyrimidine **4h**, which is a potent, balanced PPARα (EC$_{50}$ = 30 nM) and γ (EC$_{50}$ = 50 nM) dual agonist. As expected, the (3*R*,4*S*) enantiomer **4i** retains all the PPARα/γ agonist activity of **4h**, whereas the (3*S*,4*R*) enantiomer **4j** was inactive at both PPARα and γ. Interestingly, in the corresponding *trans* 1,4-oxybenzyl pyrrolidine acid series, both the (3*R*,4*R*) and (3*S*,4*S*) enantiomers **4k** and **4l** showed only weak PPAR functional activities. Other *N*-heteroaryl substituents, such as *N*-2-benzoxazole and 4-trifluoromethyl oxazole, also provided very weakly active analogs (**4m** and **4n**, respectively).

Based on the improved PPARα/γ activities of the oxybenzyl pyrrolidine acid series versus the oxyphenyl pyrrolidine acids, we then examined the effect of further extending the linker between the oxyphenyl group and the pyrrolidine ring. Insertion of another methylene group resulted in a series of oxyphenethyl pyrrolidine-acid analogs as shown in Table 5.

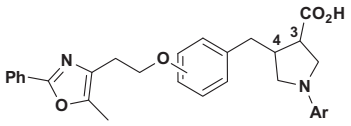
Overall both the *cis* 1,3 and 1,4-oxyphenylethyl pyrrolidine analogs have very comparable PPARα/γ activities to the corresponding analogs in the oxybenzyl pyrrolidine series (e.g., compare **5a**, **5b**, **5d**, and **5e** vs **3a**, **3d**, **3i**, **3j**, respectively). These analogs are generally potent PPARα agonists, but are significantly weaker (3- to 20-fold) versus PPARγ. In contrast, analogs in the corresponding *trans*-oxyphenethyl series (e.g., **5c**) show significantly reduced (300-fold) PPARα agonist activity. Significantly, in the *trans*-(1,4-oxyphenethyl) pyrrolidine acid series, we were able to identify the *N*-carbamate analogs **5f** and **5g** as potent, highly selective PPARα agonists (without significant PPARγ functional activity).

Finally, we explored the effects of inserting a methylene linker between the pyrrolidine ring and the carboxylic acid in the 1,3-oxybenzyl-pyrrolidine acid series (Table 6). In the *trans*-oxybenzyl pyrrolidine acetic acid series, the ethyl carbamate analog **6a** is a very potent, highly selective PPARα full agonist (EC$_{50}$ = 6 nM). However, the corresponding phenyl carbamate analog **6b** is >10-fold less potent (EC$_{50}$ = 0.85 μM) and is, moreover, a partial PPARα agonist. Both **6a** and **6b** show minimal PPARγ agonist functional activity. In the *cis*-oxybenzyl series, even the preferred (3*R*,4*S*) enantiomer **6c** only showed weak, partial PPARα agonist activity (EC$_{50}$ = 2.87 μM; intrinsic activity = 32%). Overall, we were unable to identify any balanced PPARα/γ dual agonists in this oxybenzyl pyrrolidine acetic acid series. Interestingly, in our structurally related triazole acid series of PPARα/γ agonists,[20] the optimal compounds were oxybenzyl triazole acetic acids (analogous in structure to **6**), whereas in the pyrrolidine acid series, the optimal structures are oxybenzyl pyrrolidine acids **3** & **4**; this is a rather striking divergence in the SAR between these two series.

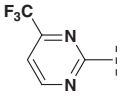
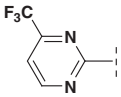
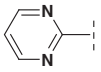
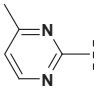
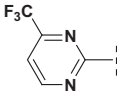
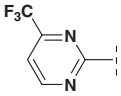
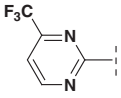
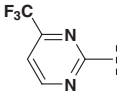
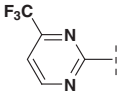
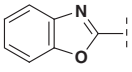
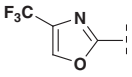
Among these compounds, we further evaluated the *N*-phenyl carbamate analog **3k** and the 4-trifluoromethyl *N*-pyrimidine analog **4i** (from the 1,4-oxybenzyl *cis*-3R,4S-pyrrolidine acid series), both of which have significantly improved in vitro potency at PPARγ (>10-fold for both) and at PPARα (>10-fold for **3k** and >100-fold for **4i**) versus muraglitazar **I** (PPARα EC$_{50}$ = 1.41 μM, and PPARγ EC$_{50}$ = 35 nM in HEK293 cells).

The in vivo antidiabetic and lipid-lowering activities of **3k** and **4i** were characterized in a 14-day study in female *db*/*db* mice.[22] The data are shown in Table 7. The *N*-4-trifluoromethyl pyrimidinyl pyrrolidine acid **4i** (administered orally at a 10 mg/kg dose once daily) showed excellent efficacy in this diabetic animal

Table 4
In vitro PPARα and γ activities of benzyl pyrrolidine-acid analogs **4**



1,3-* and *1,4-Phenyl

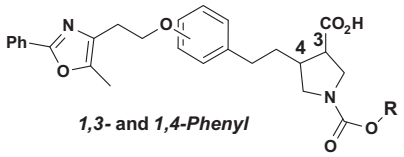
Compd	Oxybenzyl substitution	3,4-Stereochemistry	Ar	γIC$_{50}$ (μM)	γEC$_{50}$ (μM) IA (%)	αIC$_{50}$ (μM)	αEC$_{50}$ (μM) IA (%)
4a	1,3-	±*cis*	Ph	0.22	3.41 (24%)	0.04	0.02 (107%)
4b	1,3-	*cis* (3*R*,4*S*)	Ph	0.08	3.44 (29%)	0.07	0.05 (152%)
4c	1,3-	*cis* (3*S*,4*R*)	Ph	0.14	>10	0.32	0.68 (75%)
4d	1,3-	*cis* (3*R*,4*S*)		0.04	2.50 (75%)	0.02	0.014 (107%)
4e	1,3-	*cis* (3*S*,4*R*)		0.11	>10	1.12	>10
4f	1,4-	±*cis*		2.04	1.06 (67%)	>10	2.91 (58%)
4g	1,4-	±*cis*		0.45	0.63 (49%)	3.98	0.17 (93%)
4h	1,4-	±*cis*		0.03	0.05 (107%)	0.74	0.03 (110%)
4i	1,4-	*cis* (3*R*,4*S*)		0.02	0.03 (85%)	0.96	0.09 (108%)
4j	1,4-	*cis* (3*S*,4*R*)		1.53	>10	>10	>10
4k	1,4-	*trans* (3*R*,4*R*)		3.57	>30	1.12	1.0 (21%)
4l	1,4-	*trans* (3*S*,4*S*)		1.16	>30	>50	4.41 (21%)
4m	1,4-	±*cis*		0.59	2.08 (52%)	2.19	2.48 (44%)
4n	1,4-	±*cis*		0.05	>30	5.01	1.47 (20%)

model, significantly reducing levels of both fasting plasma glucose (−37%) as well as fasting triglycerides (−48%). Interestingly, the structurally closely related *N*-phenyl carbamoyl pyrrolidine acid **3k** (also dosed orally at 10 mg/kg/day), which also lowering fasting glucose comparably (−30%), did not significantly lower fasting plasma triglyceride levels (−12%), in spite of being actually more potent at PPARα (EC$_{50}$ = 5 nM) than **4i** (EC$_{50}$ = 90 nM) in the functional assay.

Analog **4i** also showed an acceptable profile in a set of standard in vitro liabilities (metabolic stability in human/mouse/rat microsomes, hERG and other ion channels). In a panel of 6 standard Cytochrome P450 (CYP450) inhibition assays, **4i** only showed modest activity at CYP2C9 (IC$_{50}$ = 3.2 μM) and CYP2C19 (IC$_{50}$ = 8.8 μM). In a rat pharmacokinetics study, (dosed orally at 10 mg/kg in a solution of 40% polyethylene glycol, 10% Cremophor and 50% aqueous phosphate buffer), **4i** had good oral bioavailability (65%) and reasonable plasma/tissue distribution, as reflected by the V_{ss} (volume of distribution) of 0.47 L/kg. However, the compound had a relatively short half-life of 1.54 h.

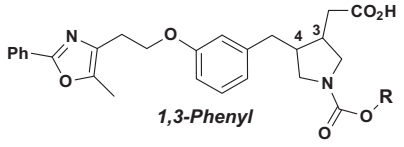
In conclusion, the structure–activity relationships of several series of novel 1,3- and 1,4-oxyphenyl 3,4-disubstituted pyrrolidine acids (analogs **1a**–**6c**) have been explored, resulting in the

Table 5
In vitro PPARα and γ activities of phenethyl-pyrrolidine-acid analogs **5**



Compd	Oxybenzyl substitution	3,4-Stereochemistry	R	γIC$_{50}$ (μM)	γEC$_{50}$ (μM) IA (%)	αIC$_{50}$ (μM)	αEC$_{50}$ (μM) IA (%)
5a	1,3-	±*cis*	Et	0.49	0.71 (26%)	2.69	0.20 (117%)
5b	1,3-	±*cis*	Ph	0.21	0.63 (126%)	1.06	0.008 (113%)
5c	1,3-	±*trans*	Ph	0.52	1.17 (45%)	>10	2.60 (30%)
5d	1,4-	±*cis*	Et	0.69	0.54 (64%)	3.27	0.02 (133%)
5e	1,4-	±*cis*	Ph	0.09	0.16 (62%)	0.48	0.01 (77%)
5f	1,4-	±*trans*	Et	1.59	>10	1.81	0.01 (81%)
5g	1,4-	±*trans*	Ph	0.34	>10	3.25	0.98 (89%)

Table 6
In vitro PPARα and γ activities of oxybenzyl-pyrrolidine-acetic acid analogs **6**



Compd	Oxybenzyl substitution	3,4-Stereochemistry	R	γIC$_{50}$ (μM)	γEC$_{50}$ (μM) IA (%)	αIC$_{50}$ (μM)	αEC$_{50}$ (μM) IA (%)
6a	1,3-	±*trans*	-Et	8.11	>10	2.12	0.006 (143%)
6b	1,3-	±*trans*	-Ph	0.21	>10	3.09	0.85 (44%)
6c	1,3-	*cis* (3*R*,4*S*)	-Et	2.83	>10	5.55	2.87 (32%)

Table 7
14-day In vivo efficacy study of **3k** & **4i** (10 mg/kg/day administered p.o.) in 8–10 week old female *db*/*db* mice

Treatment 10 mg/kg/day	Fasting glucose (mg/dL) after 14 days	Fasting triglycerides (mg/dL) after 14 days
Vehicle	382 ± 18	175 ± 5
4i	240 ± 18 (−37%)*	91 ± 6 (−48%)*
Vehicle	511 ± 11	143 ± 16
3k	355 ± 26 (−30%)*	127 ± 10 (−12%)

* p value <0.05.

discovery of analogs with a wide range of PPARγ and α agonist activities, in particular the potent PPARα/γ dual agonists **3a**–**3k** and **4a**–**4n**. From these SAR studies, we have found that: among the different permutations of spacing the oxyphenyl and carboxylic acid pharmacophores on the pyrrolidine ring: (1) the oxybenzyl pyrrolidine acid series overall provided the best balance of PPARα/γ functional activities and (2) the *cis*-substituted pyrrolidine acids are preferred versus the *trans* series, and (3) *N*-carbamoyl and *N*-aryl-substituted oxybenzyl pyrrolidine acid analogs provide potent, balanced PPARα/γ dual agonists. The analogs in the 1,3-oxybenzylglycine pyrrolidine acid series are relatively α-selective PPARα/γ agonists, whereas analogs in the 1,4-oxybenzylglycine pyrrolidine acid series have relatively more balanced PPARγ and PPARα agonist activities. In the 1,4-oxybenzylglycine 3,4-disubstituted pyrrolidine acid series, a set of *N*-pyrimidinyl & *N*-carbamoyl pyrrolidine analogs was identified as particularly promising PPARα/γ dual agonists. In both of these sub-series, the *cis* enantiomer (3*R*,4*S* absolute stereochemistry) was found to be preferred for optimal PPARα/γ functional activity and binding affinities. The *cis* enantiomer *N*-pyrimidine analog **4i** and the *N*-phenyl carbamate **3k** both display very potent, balanced PPARα/γ agonist activities in vitro and were characterized in chronic efficacy studies. The *N*-pyrimidinyl pyrrolidine acid analog **4i** showed efficacious glycemic & triglyceride lowering in a 14-day chronic study in *db*/*db* mice. Additional SAR studies will be needed to further optimize the in vitro liability/ADME profile of **4i**. The SAR of PPARα-selective agonists derived from the 1,3-oxybenzylglycine 3,4-disubstituted pyrrolidine acid series will be reported separately.

Acknowledgments

We thank the BMS Discovery Synthesis Group and the BMS Discovery Analytical Sciences Department for support. We thank Dr. Jeff Robl for careful proofreading of this manuscript.

References and notes

1. For general reviews on PPARs: (a) Sternbach, D. D. *Annu. Rep. Med. Chem.* **2003**, *38*, 71; Cheng, P. T. W.; Mukherjee, R. *Mini-Rev. Med. Chem.* **2005**, *5*, 741.
2. Duval, C.; Muller, M.; Kersten, S. *Biochim. Biophys. Acta* **2007**, *1771*, 961; Zandberg, F.; Plutzky, J. *Biochim. Biophys. Acta* **2007**, *1771*, 972.
3. Saurav, A.; Kaushik, M.; Mohiuddin, S. M. *Exp. Opin. Pharmacother.* **2012**, *13*, 717.
4. Todd, P. A.; Ward, A. *Drugs* **1988**, *36*, 314.
5. Tontonoz, P.; Spiegelman, B. M. *Annu. Rev. Biochem.* **2008**, *77*, 289.
6. Malinowski, J. M.; Bolesta, S. *Clin. Ther.* **2000**, *22*, 1151.
7. Derosa, G. *Drugs* **2010**, *70*, 1945.
8. (a) Devasthale, P. V.; Chen, S.; Jeon, Y.; Qu, F.; Shao, C.; Wang, W.; Zhang, H.; Farrelly, D.; Golla, R.; Grover, G.; Harrity, T.; Ma, Z.; Moore, L.; Ren, J.; Seethala, R.; Cheng, L.; Sleph, P.; Sun, W.; Tieman, A.; Wetterau, J. R.; Doweyko, A.; Chandrasena, G.; Chang, S. Y.; Humphreys, W. G.; Sasseville, V. G.; Biller, S. A.; Ryono, D. E.; Selan, F.; Hariharan, N.; Cheng, P. T. W. *J. Med. Chem.* **2005**, *48*, 2248; (b) Devasthale, P. V.; Chen, S.; Jeon, Y.; Qu, F.; Ryono, D. E.; Wang, W.; Zhang, H.; Cheng, L.; Farrelly, D.; Golla, R.; Grover, G.; Ma, Z.; Moore, L.; Seethala, R.; Sun, W.; Doweyko, A. M.; Chandrasena, G.; Sleph, P.; Hariharan, N.; Cheng, P. T. W. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2312; (c) Harrity, T.; Farrelly, D.; Tieman, A.; Chu, C.; Kunselman, L.; Gu, L.; Ponticiello, R.; Cap, M.; Qu, F.; Shao, C.; Wang, W.; Zhang, H.; Fenderson, W.; Chen, S.; Devasthale, P.; Jeon, Y.; Seethala, R.; Yang, W.-P.; Ren, J.; Zhou, M.; Ryono, D.; Biller, S.; Mookhtiar, K. A.; Wetterau, J.; Gregg, R.; Cheng, P. T.; Hariharan, N. *Diabetes* **2006**, *55*, 240; (d) Kendall, D. M.; Rubin, C. J.; Mohideen, P.; Ledeine, J.-M.; Belder, R.; Gross, J.; Norwood, P.; O'Mahony, M.; Sall, K.; Sloan, G.; Roberts, A.; Fiedorek, F. T.; DeFronzo, R. A. *Diabetes Care* **2006**, *29*, 1016.
9. Detailed experimental procedures for the synthesis of the compounds in this article can be found in WO 2004004665.
10. (a) Cheng, P. T.; Jeon, Y.; Wang, W. US Patent 7105556 (2006).; (b) Cheng, P. T.; Jeon, Y.; Wang, W. poster presented at the National Medicinal Chemistry Symposium 2006.; (c) Cheng, P. T.; Chen, S.; Devasthale, P.; Ding, C. Z.; Herpin, T. F.; Wu, S.; Zhang, H.; Wang, W.; Ye, X.-Y.; US Patent 7951793 (2011).
11. Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1985**, *24*, 4405.
12. Padwa, A.; Dent, W. *Org. Synth.* **1989**, *67*, 133.
13. Preparation of Alkenes, A Practical Approach. In *The Wittig Reaction and Related Methods*; Williams, J. M. J., Ed.; Oxford University Press: N.J. Lawrence, 1996. Chapter 2.
14. Klapars, Artis; Antilla, Jon C.; Huang, Xiaohua; Buchwald, Stephen L. *J. Am. Chem. Soc.* **2001**, *123*, 7727.
15. (a) Karlsson, S.; Hogberg, H.-E. *Tetrahedron: Asymmetry* **2001**, *12*, 1975; (b) Carey, J. S. *J. Org. Chem.* **2001**, *66*, 2526.
16. Freedman, T. B.; Cao, X.; Phillips, L. M.; Cheng, P. T. W.; Dalterio, R.; Shu, Y.-Z.; Zhang, H.; Zhao, N.; Shukla, R. B.; Tymiak, A.; Gozo, S. K.; Nafie, L. A.; Gougoutas, J. Z. *Chirality* **2006**, *18*, 746.
17. Mueller, R. H.; Wang, S.; Pansegrau, P. D.; Jannotti, J. Q.; Poss, M. A.; Thottathil, J. K.; Singh, J.; Humora, M. J.; Kissick, T. P.; Boyhan, B. *Org. Process. Res. Dev.* **1997**, *1*, 14.
18. $[M+H]^+$ = 553.2; $[\alpha](0.96w/v\%)^{589}_{MeOH} = -70.8°$; Analytical chiral HPLC: Chiralpak AD chiral column 4.6 × 250 mm, 10 μm; Isocratic solvent system: 3:7 A/B, where Solvent A = 100% IPA + 0.1% TFA; Solvent B = 100% heptane Detection = 254 nm; Flow rate = 2 mL/min; Retention time = 6.7 min. ^{1}H NMR (CD_3OD): δ 8.41 (m, 1H), 7.85 (m, 2H), 7.36 (m, 3H), 7.00 (d, J = 9 Hz, 2H), 6.76 (m, 3H), 4.13 (m, 2H), 3.81 (m, 1H), 3.62 (m, 1H), 3.51 (m, 2H), 3.18 (m, 1H), 2.87 (m, 2H), 2.73 (m, 2H), 2.41 (m, 1H), 2.28 (s, 3H).
19. Seethala, R.; Golla, R.; Ma, Z.; Cheng, L.; Zhang, H.; O'Malley, K.; Lippy, J.; Zhang, L.; Hariharan, N.; Cheng, P. T. W. *Anal. Biochem.* **2007**, *363*, 263.
20. In vitro PPAR agonist functional assays were performed by transiently transfecting GAL4-hPPARα-LBD or GAL4-hPPARγ-LBD constructs, respectively, into HEK293 cells stably expressing 5 copies of GAL4RE-Luciferase. Data were normalized for efficacy at 1 μM to known agonists (rosiglitazone for hPPARγ and GW-2331 for hPPARα). Agonist binding results in an increase in luciferase enzyme activity which can be monitored by measuring luminescence upon cell lysing and the addition of luciferin substrate. EC_{50} values (μM) for PPARα or γ agonist activity were calculated as the concentration of the test ligand (μM) required for the half-maximal fold induction of HEK293 cells. The 'intrinsic activity' of a test ligand is defined as its activity at 1 μM (expressed as a percentage) relative to the activity of the primary standards (GW2331 for PPARα and rosiglitazone for PPARγ, respectively, both tested at 1 μM).
21. Zhang, H.; Ryono, D. E.; Devasthale, P.; Wang, W.; O'Malley, K.; Farrelly, D.; Gu, L.; Harrity, T.; Cap, M.; Chu, C.; Kunselman, L.; Morgan, N.; Flynn, N.; Moore, L.; Hosagrahara, V.; Zhang, L.; Kadiyala, P.; Xu, C.; Doweyko, A. M.; Bell, A.; Chang, C.; Muckelbauer, J.; Zahler, R.; Ryono, D. E.; Hariharan, N.; Cheng, P. T. W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1451.
22. *db*/*db* mice, 8–10 weeks old, fed normal chow *ad lib*, were orally dosed with compound and/or vehicle (5% *N*-methyl pyrrolidinone, 20% PEG-400, 20 mM aqueous sodium phosphate buffer, pH = 8) once daily for 14 days. Blood samples were drawn from the tail vein on the 7th day after overnight fasting, and plasma analysis was performed on a COBAS automated analyzer. After 14 days, fasted animals were sacrificed. Blood and tissue samples were collected for clinical biochemistry analyses.