Bioorganic & Medicinal Chemistry Letters 18 (2008) 6251–6254

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The discovery of equipotent PPARα/γ dual activators

Paul Martres [a,*], Nicolas Faucher [a], Alain Laroze [a], Olivier Pineau [a], Marie Helene Fouchet [a], Florent Potvain [b], Didier Grillot[b], Veronique Beneton [c]

[a] Department of Medicinal Chemistry, Laboratoire GlaxoSmithKline, Centre de Recherches, 25-27 avenue du Québec, 91951 Les Ulis, France
[b] Department of Biology, Laboratoire GlaxoSmithKline, Centre de Recherches, 25-27 avenue du Québec, 91951 Les Ulis, France
[c] Department of DMPK, Laboratoire GlaxoSmithKline, Centre de Recherches, 25-27 avenue du Québec, 91951 Les Ulis, France

ARTICLE INFO

Article history:
Received 5 September 2008
Revised 24 September 2008
Accepted 26 September 2008
Available online 1 October 2008

Keywords:
PPARα
PPARγ
Dual
Agonist

ABSTRACT

We report the design and synthesis of equipotent PPARα/γ dual agonists starting from selective PPAR alpha agonist **1**. In vivo data for **7** in the Zucker fa/fa rat are presented.

© 2008 Elsevier Ltd. All rights reserved.

Elevated circulating levels of Low Density Lipoprotein cholesterol (LDLc) constitute a major risk-factor for coronary artery disease and there exists a wealth of clinical data supporting the use of the LDLc-lowering statins in an increasingly wide range of patients.[1] The contribution of low circulating levels of High Density Lipoprotein cholesterol (HDLc) to cardiovascular risk has been clearly demonstrated. Indeed each 1 mg/dL increment in HDL-chol is associated with a 2–3% decrease in cardiovascular risk .[2]

The fibrates are one of the two drug classes currently marketed which have demonstrated the ability to increase HDL levels by up to 20% and to decrease TG levels by up to 40%.[3] The beneficial pharmacological effects of fibrates are thought to be mediated in part by PPARα activation[4] although they are not particularly potent (high micromolar concentrations are needed to activate PPARα). Consequently, in humans, fibrates must be used at very high doses (about 300–1200 mg/day) to achieve a sufficient lipid-lowering effect.

The glitazones (rosiglitazone, pioglitazone) are used in the clinic to treat type II diabetes and work by activating PPARγ, leading to insulin sensitization and decreased glucose levels.[5] In addition, pioglitazone gives a moderate decrease of TG levels (15–20%), and both pioglitazone and rosiglitazone increase HDL levels (7–13%).[3] The glitazones induce weight gain as a major side effect and in rare cases promote fluid retention.[6]

Over the past decades, the fibrates have demonstrated an excellent tolerability profile, and clinical studies have demonstrated that they produce a weak but significant improvement in glucose homeostasis and insulin[7] although in the Field study no significant effect was observed on HbA1c.[8] The pharmacological activation of both PPARα and PPARγ by a single molecule could be of great interest for the simultaneous treatment of dyslipidemia and metabolic syndrome, since they would be expected to show additive effects on the lipid profile and a possible superior therapeutic index compared to the glitazones. Clinical studies with dual agonists that are more potent on PPARγ relative to PPARα have shown a profile in which the PPARγ-mediated undesired effects observed in humans were still observed.[9] Therefore, a more balanced profile might be of interest and compounds displaying a similar potency on the alpha and gamma isoforms, as determined in a transactivation assay, could potentially deliver the benefits of the synergistic effect on lipids whilst maximizing the therapeutic window.

Thus we have targeted compounds showing equipotent activation of both PPARα and γ isoforms whilst showing a 100-fold selectivity window towards PPARδ.

Recently we have reported the discovery of a new potent selective PPARα agonist, **1**.[10] Starting from the structure of **1**, we have designed a new class of PPARα/γ dual agonists.

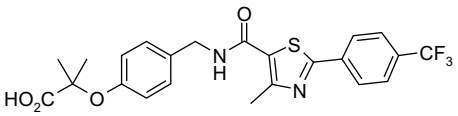
SAR studies around **1** have demonstrated that the para substitution of the phenyl ring anchored to the thiazole moiety could modulate potency on the PPARγ isoform.[10] The *p*-trifluoromethyl analogue **1** is weakly active on PPARγ whereas the *p*-*tert*-butyl **2** displays significant PPARγ activity while being as potent as **1** on PPARα. (Table 1)

In addition, molecular modeling studies of the alpha and gamma binding modes have been performed on the generic scaffold

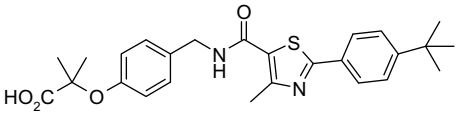
* Corresponding author.
E-mail address: paul.martres@gsk.com (P. Martres).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.09.094

Table 1
In vitro potencies of PPARα agonists.[a]



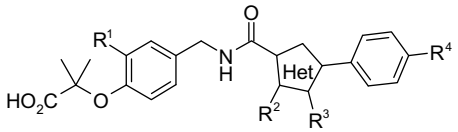
1



2

Isoform	EC_{50} (μM)	
	1	**2**
h-PPARα	0.004	0.004
h-PPARδ	2.83	10
h-PPARγ	9.71	0.8

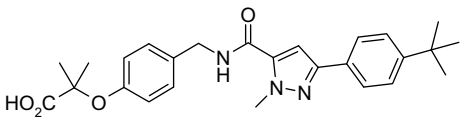
[a] Data generated using cell based transient transfection assays[12]; compounds behave as full agonists.



3

Figure 1. Generic scaffold of PPARα/γ agonists.

Table 2
In vitro potencies of compound **4**.[a]



4

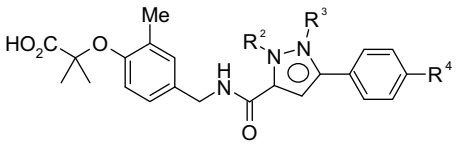
Isoform	EC_{50} (μM)
h-PPARα	0.16
h-PPARδ	10
h-PPARγ	1.0

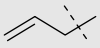
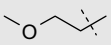
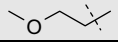
[a] Data generated using cell based transient transfection assays[12]; compounds behave as full agonists.

3 depicted in Figure 1. The model has raised the hypothesis that aliphatic substitution at R^1 and R^2/R^3 on **3** could lead to an increase in potency on PPARγ. According to the crystal structure of PPARs ligand binding domain,[11] two hydrophobic pockets exist on α and γ subunits which could be accommodated by R^1 groups for the first pocket and R^3/R^3 groups for the second. The pyrazole core was selected as a versatile ring which would be synthetically amenable to the rapid variation of substituents at the R^2/R^3 positions. *p*-*tert*-Butyl derivative **4**, the first example synthesized, maintained potency on the gamma isoform with a significant decrease in potency on the alpha isoform when compared to **2** (Table 2).

Despite the reduced PPARα potency, **4** displayed a more balanced PPARα/γ ratio. An SAR study was conducted retaining pyrazole as the central core heterocycle. Compounds bearing a bulky aliphatic group at R^4 were synthesized with variation at R^1, R^2 and R^3. The choice of R^1 was driven by our knowledge of the binding mode in the alpha and gamma subunit. Although the PPARα and PPARγ binding pockets are very similar in size and shape, one key determinant for subtype selectivity is the size of the R^1 group. Larger R^1 groups generally give higher PPARγ potency[11] therefore we hypothesized that smaller groups such as methyl and methoxy should experience diminished steric interactions and therefore could be more suitable for PPARα/γ dual compounds.[9]

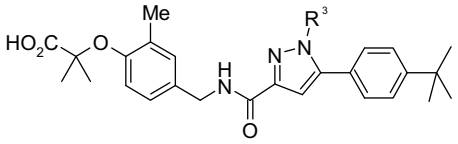
Table 3
In vitro potencies of pyrazoles **5-17**[a] (R^1 = Me).

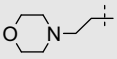


Compound	R^2	R^3	R^4	EC_{50} (μM) h-PPARα	EC_{50} (μM) h-PPARδ	EC_{50} (μM) h-PPARγ
5		H	*t*-Bu	0.383	>25	0.270
6	Me		*t*-Bu	0.050	2.165	0.03
7		Me	*t*-Bu	0.01	>10	0.006
8	Me		*i*-Pr	0.020	1.360	0.05
9		Me	*i*-Pr	0.004	4.850	0.008
10	Me		*i*-Bu	0.045	2.870	0.05
11		Me	*i*-Bu	0.002	2.060	0.008
12	Et		*t*-Bu	0.050	2.700	0.030
13		Et	*t*-Bu	0.010	>25	0.015
14			*t*-Bu	0.045	1.150	0.06
15			*t*-Bu	0.032	>25	0.069
16			*t*-Bu	0.066	2.05	0.303
17			*t*-Bu	0.042	>25	0.478

[a] Data generated using cell based transient transfection assays[12]; compounds behave as full agonists.

Table 4
In vitro potencies of pyrazoles **18-19**[a] (R^1 = Me, R^4 = *t*-Bu).



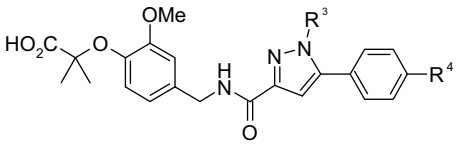
Compound	R^3	EC_{50} (μM) h-PPARα	EC_{50} (μM) h-PPARδ	EC_{50} (μM) h-PPARγ
18		0.57	>25	>25
19	Bn	0.20	>25	1.7

[a] Data generated using cell based transient transfection assays[12]; compounds behave as full agonists.

Methyl, ethyl, allyl and methoxyethyl groups in R^2 or R^3 position (compounds **6** to **17**, Table 3) increased the transactivation activity on the alpha isoform compared to the non-substituted pyrazole **5** and methyl, ethyl and allyl substitution at R^3 delivered the most promising overall profile. Substitution at R^2 appeared to decrease the level of selectivity over the delta isoform (Table 3).

Larger groups introduced at the R^3 position (compounds **18** and **19**, Table 4) have delivered a less attractive profile on both the human alpha and gamma isoforms.

Table 5
In vitro potencies of pyrazoles **20-22**[a] (R^1 = OMe).



Compound	R^3	R^4	EC_{50} (μM) h-PPARα	EC_{50} (μM) h-PPARδ	EC_{50} (μM) h-PPARγ
20	Et	*t*-Bu	>25	>25	0.190
21	Me	*t*-Bu	>25	>25	0.176
22	Me	*i*-Bu	>25	>25	0.130

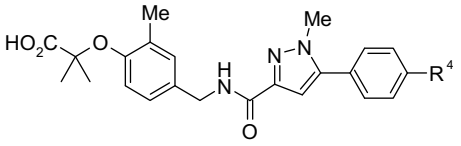
[a] Data generated using cell based transient transfection assays[12]; compounds behave as full agonists.

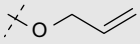
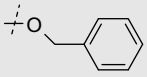
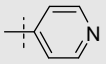
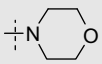
Surprisingly, moving from a methyl group to a methoxy group for R^1 appeared to be less favorable leading to a general decrease in activity on PPAR alpha (compounds **20** to **22**, Table 5).

It clearly emerged from transactivation data that the best substitution combinations were R^1 = Me; R^3 = Me or Et and R^4 = bulky aliphatic group. With those results in hand, the stage was set for introduction of diversity at R^4 (Table 6). Among R^4 substitutions depicted in Table 6, only the 2-tolyl **26** and the 4-pyridyl **28** derivatives achieved our selectivity criteria and acceptable in vitro potencies. *N*-Pyrolidine **30** displayed good in vitro potency; however its delta selectivity was insufficient.

Several compounds achieved our goal of equipotent activation of h-PPARα and h-PPARγ isoforms in the transactivation assay

Table 6
In vitro potencies of pyrazoles **23–30**[a] (R^1 = R^2 = Me).



Compound	R^4	EC_{50} (μM) h-PPARα	EC_{50} (μM) h-PPARδ	EC_{50} (μM) h-PPARγ
23		0.094	>25	0.028
24		>25	>25	0.080
25		0.020	1.550	0.025
26		0.090	>25	0.03
27		0.004	1.63	0.030
28		0.730	>25	0.310
29		0.085	0.035	0.110
30		0.008	1.060	0.020

[a] Data generated using cell based transient transfection assays[12]; compounds behave as full agonists.

Table 7
In vitro potencies of selected pyrazoles on murine PPARs.[a]

Compound	EC_{50} (μM) m-PPARα	EC_{50} (μM) m-PPARδ	EC_{50} (μM) m-PPARγ
7	0.64	>25	0.011
9	0.28	7.1	0.016
13	2.26	>25	0.036
15	6	>25	0.15
26	1.8	>25	0.06

[a] Data generated using cell based transient transfection assays[12]; compounds behave as full agonists.

Table 8
Pharmacokinetic data for compound **7** in CD rat.[a]

F %	Cl (mL/min/kg)	C_{max} (ng/mL)	Vd_{ss} (L/kg)	$T_{1/2}$ (h)	AUC_{po} (ng h/mL)
22	17	1791	0.30	0.83	1130

[a] IV dose: 2.5 mg/kg (DMSO/cyclodextrine 20% (1/99 v/v)); PO dose: 5 mg/kg (0.5%HPMC K100/0.1% Tween 80).

Table 9
In vivo potencies of pyrazole **7**.[a]

Dose (mg/kg)	Total TG (p)[b]	Insulin (p)[b]	Liver weight (p)[b]
10 mg/kg	−57 ± 3% *	−10 ± 18%	+9 ± 4% *
30 mg/kg	−45 ± 4% *	−35 ± 4% *	+14 ± 3% *

[a] Five days chronic bid Zucker Fatty rats.
[b] * indicates values of $p < 0.05$, as determined by a one-way-ANOVA.

(see Tables 3–6). However, a 20-fold decrease in potency was generally observed in the transactivation assay when moving from human to murine PPARα. We anticipated that compounds having an EC_{50} higher than 4 μM on m-PPARα would not be capable of delivering a significant effect on lipid parameters in our in vivo model. Consequently only compounds **7, 9, 13, 15** and **26**, which showed EC_{50} < 0.2 μM on h-PPAR alpha, were selected for profiling against m-PPARα, δ and γ isoforms (Table 7).

Of these, compounds **13, 15** and **26** were only weakly active weak at the m-PPARα isoform and so were not progressed further.

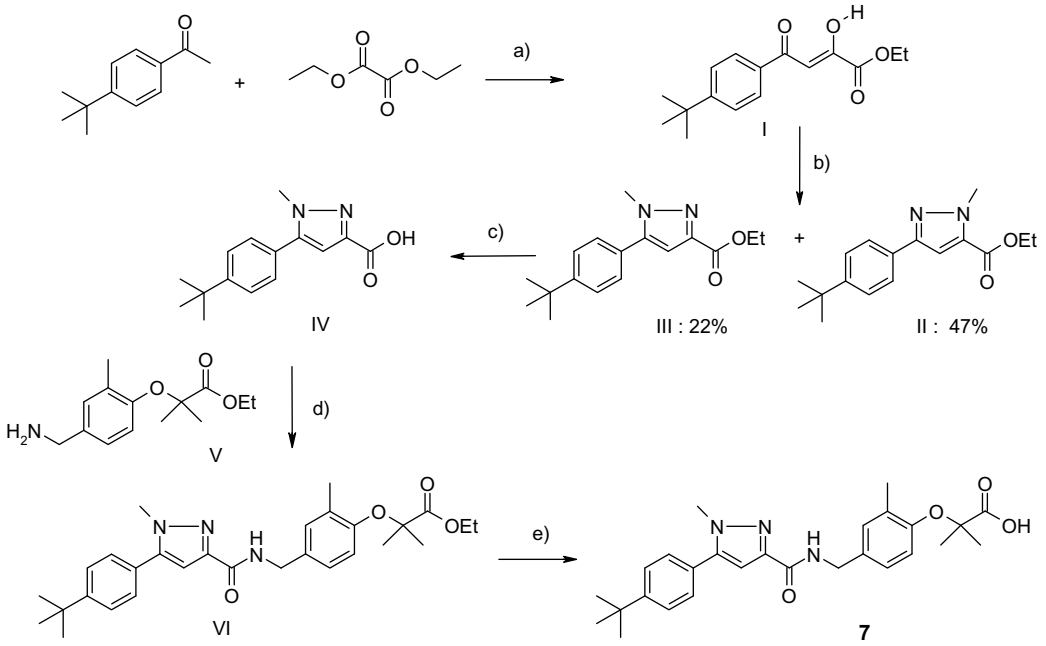
The pharmacokinetic properties of compound **7** were evaluated in the CD rat and found to be suitable for chronic administration (Table 8). Total plasma clearance was medium (17 mL/min/kg) with a half-life of 0.83 h. Following a single oral dose of compound **7** at 5 mg/kg, the maximum concentration of compound in the plasma was 1130 ng/mL and the bioavailability was 22%.

Compound **7** was evaluated in a five days chronic experiment in the Zucker fa/fa rat (Table 9).

As explained in the introduction, one argument in favor of an equipotent PPARα/γ dual agonist is the potential superior therapeutic index compared to glitazones. However, in contrast to its human PPAR profile, **7** does not profile as an equipotent PPARα/γ dual agonist in rodent therefore the in vivo experiment was performed purely to assess pharmacodynamic activity.

Triglyceride effects can be ascribed to the activation of both the alpha and gamma isoforms. The moderate liver weight increase is a common readout of PPARα activation and the decreased insulin levels can be ascribed to PPARγ activation.

Starting from a selective PPARα agonist, we have generated compounds with a balanced profile on PPAR alpha and gamma. Pyrazole **7**, a representative compound, has demonstrated in vivo activity on Zucker fatty rat. It is noteworthy that **7** has also been tested in a rat seven days toxicological study and no safety issues



Scheme 1. Synthetic pathway. Reagents and conditions: (a) EtOH, Na 0 °C, rt 16 h, then 80 °C 16 h, 99%; (b) methyl-hydrazine, EtOH 90 °C, 16 h; silica gel column first eluted **II**; (c) NaOH 1 M, 4 h, 98%; (d) thionyl chloride, toluene, 80 °C, 3 h 30 min then **V** Et_3N, CH_2Cl_2, rt, 1 h (for preparation of **V** see Ref. 13), 96%; (e) NaOH, 80 °C, 4 h, 80%.

were raised that could preclude further development (results not shown) (Scheme 1).

Acknowledgments

We thank Dr. Andrew Brewster (GlaxoSmithKline) for proof-reading of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.094.

References and notes

1. Tobert, J. A. *Nat. Rev. Drug Discov.* **2003**, *2*, 517.
2. Brown, William M.; Chiacchia, Fabrizio S. *Drug Target Insights* **2008**, *3*, 45.
3. Rader, D. J. *Am. J. Cardiol.* **2003**, *91*, 18E.
4. Fruchart, J-C.; Duriez, P.; Staels, B. *Curr. Opin. Lipidol.* **1999**, *10*, 245.
5. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
6. (a) Goldberg, R. B.; Kendall, D. M.; Deeg, M. A.; Buse, J. B.; Zagar, A. J.; Pinaire, J. A.; Tan, M. H.; Khan, M. A.; Perez, A. T.; Jacober, S. J. *Diabetes Care* **2005**, *28*, 1547; (b) Elte, J. W. F.; Blicklé, J. F. *Eur. J. Int. Med.* **2007**, *18*, 18.
7. Chinetti-Gbaguidi, G.; Fruchart, J. C.; Staels, B. *Curr. Atheroscler. Rep.* **2005**, *7*, 396.
8. Keech, A.; Simes, R. J.; Barter, P.; Best, J.; Scott, R.; Taskinen, M. R.; Forder, P.; Pillai, A.; Davis, T.; Glasziou, P.; Drury, P.; Kesaniemi, Y. A.; Sullivan, D.; Hunt, D.; Colman, P.; d'Emden, M.; Whiting, M.; Ehnholm, C.; Laakso, M. *Lancet* **2005**, *366*, 1849.
9. Henke, B. R. *J. Med. Chem.* **2004**, *47*, 4118.
10. Sierra, M. L.; Beneton, V.; Boullay, A.-B.; Boyer, T.; Brewster, A. G.; Donche, F.; Forest, M.-C.; Fouchet, M.-H.; Gellibert, F. J.; Grillot, D. A.; Lambert, M. H.; Laroze, A.; Le Grumelec, C.; Linget, J.-M.; Montana, V. G.; Nguyen, V.-L.; Nicodeme, E.; Patel, V.; Penfornis, A.; Pineau, O.; Pohin, D.; Potvain, F.; Poulain, G.; Ruault, C.; Saunders, M.; Toum, J.; Xu, H. E.; Xu, R. X.; Pianetti, P. M. *J. Med. Chem.* **2007**, *50*, 685.
11. Xu, H. E.; Lambert, M. H.; Montana, V. G.; Plunket, K. D.; Moore, L. B.; Collins, J. L.; Oplinger, J. A.; Kliewer, S. A.; Gampe, R. T., Jr.; McKee, D. D.; Moore, J. T.; Willson, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*(24), 13919.
12. Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K.; Cobb, J. E.; Collins, J. L., ; Harrington, W. W., Jr.; Hashim, M. A.; Hull-Ryde, E. A.; Kaldor, I.; Kliewer, S. A.; Lake, D. H.; Leesnitzer, L. M.; Lehmann, J. M.; Lenhard, J. M.; Orband-Miller, L. A.; Miller, J. F.; Mook, R. A.; Noble, S. A.; Oliver, W.; Parks, D. J.; Plunket, K. D.; Szewczyk, J. R.; Willson, T. M. *J. Med. Chem.* **1998**, *41*, 5020.
13. Faucher, N.; Martres, P. WO 2005049578.