Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2437–2440
Bioorganic & Medicinal Chemistry Letters

Selective PPARγ modulators with improved pharmacological profiles

Kun Liu, Regina M. Black, John J. Acton, III, Ralph Mosley, Sheryl Debenham, Ramon Abola, Meng Yang, Richard Tschirret-Guth, Lawrence Colwell, Cherrie Liu, Margaret Wu, Chuanlin F. Wang, Karen L. MacNaul, Margaret E. McCann, David E. Moller, Joel P. Berger, Peter T. Meinke, A. Brian Jones and Harold B. Wood*

Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA

Received 25 February 2005; revised 21 March 2005; accepted 23 March 2005
Available online 16 April 2005

Abstract—A series of metabolically robust *N*-benzyl-indole selective PPARγ modulators with either a 3-benzoyl or 3-benzisoxazoyl moiety have been identified. In vitro, these compounds are partial agonists and exhibit reduced adipogenesis in human adipocytes. In vivo, these SPPARγMs result in potent glucose lowering in db/db mice and attenuate increases in heart weight and brown adipose tissue that is typically observed in rats upon treatment with PPARγ full agonists.

The peroxisome proliferator-activated receptor gamma (PPARγ) is member of a large family of ligand activated nuclear transcription factors. Both rosiglitazone and pioglitazone are full agonists that specifically activate PPARγ and are used to treat type 2 diabetes (T2DM).[1] However, their efficacy is limited due to mechanism-based adverse events (AEs). In humans, AE's associated with PPARγ activation include weight gain, edema, and anemia, while in rodents cardiac hypertrophy is also observed.[2] Although the underlying causes for these AEs are poorly understood, a PPARγ ligand that ameliorates these AEs would be desirable. In search of PPAR ligands devoid of these AEs, new classes of selective PPARγ modulators (SPPARγMs)[3] have been reported.[4] We recently reported the discovery of SPPARγMs that exhibit potent glucose lowering in the db/db mouse model for T2DM.[5] Interestingly, these indole derived SPPARγMs did not cause cardiac hypertrophy (CH) and attenuated brown adipose tissue (BAT) increases in S–D rats.

During preclinical evaluation of **1** in rats, loss of the *p*-anisoyl group was observed (Fig. 1). Although the unsubstituted indole **1a** was inactive on all three PPAR isoforms (α, δ, γ) in vitro, it was a major circulating metabolite in plasma which accumulated on multiple dosing (10% of parent). Unfortunately, **1a** was a potent inhibitor of CYP2C9 (IC_{50} = 50 nM), a liability which precluded further studies. Herein, we describe the identification and evaluation of second generation indole-based SPPARγMs with improved metabolic profiles that are inactive on the α and δ PPAR isoforms.

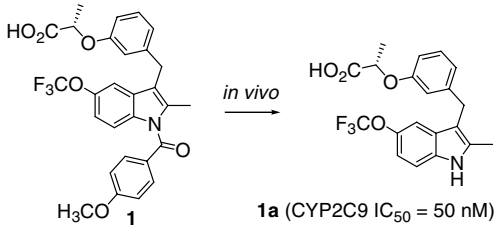


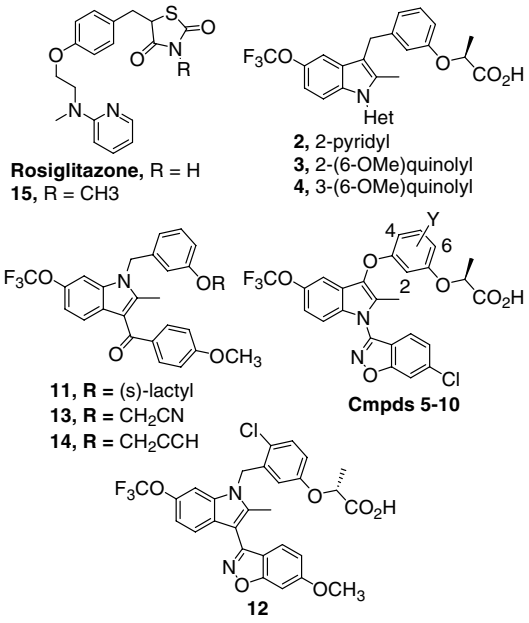
Figure 1. In vivo loss of the *N*-benzoyl group.

Alternative replacements for the *N*-benzoyl group that would be less susceptible to metabolism were investigated. Considering the importance of the benzoyl carbonyl for activity, both pyridyl and quinolyl analogs (**2**–**4**) with a nitrogen positioned to replace the carbonyl of the *p*-anisoyl group were appended onto the nitrogen of the indole. Unfortunately, these derivatives exhibited diminished in vitro potency (Table 1).

Keywords: PPAR; Selective modulator; Partial agonists.
* Corresponding author. Tel.: +1 732 594 7592; fax: +1 732 594 9556; e-mail: blair_wood@merck.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.03.092

Table 1. Human PPARγ binding (SPA) IC_{50}s and transactivation EC_{50}s[6]



Compds	Y	SPA IC_{50}, nM[a]	Transactivation EC_{50}, nM[b]	% Max activation
Rosiglitazone	—	250	20	100
1	—	1	3	21
2	—	466	—	—
3	—	135	—	—
4	—	29	49	32
5	H	33	195	26
6	2-*n*Pr	641	—	—
7	4-Cl	5	3	31
8	5-Cl	109	114	24
9	6-*n*Pr	20	39	33
10	6-Cl	1	3	21
11	—	2	1	28
12	—	2	3	24
13	—	42	16	22
14	—	62	31	19
15	—	>50,000	>3000	—

[a] IC_{50} is measured by the complete displacement of a radio-labeled full agonist indicating competitive binding to the receptor.
[b] EC_{50} is the compound concentration at which 50% of a given compound's intrinsic maximal response has been reached.

However, the use of an *N*-(1-benzisoxazole, BZX) to mimic the carbonyl group yielded analogs with the desired activity but with slightly reduced potency in vitro. It was possible to regain potency comparable to the *N*-benzoyl indoles, if appropriate substitution was introduced onto the aromatic ring at the indole 3-position. The most potent derivatives were those substituted in either the 4- or 6-position of this aromatic ring, while substitution at the 2-position afforded a reduction in binding potency (compounds **5**–**10**, Table 1). Like the *N*-benzoyl analogs, loss of the *N*-(1-BZX) group occurred in vivo in rats and the parent indole was detected in plasma, albeit at significantly reduced levels (1–3% of parent). In this case, in vitro studies suggested that loss of the *N*-(1-BZX) occurred via reductive metabolism at the BZX moiety.[7] Despite the improved metabolic stability of the *N*-(1-BZX) indole analogs, given the potent CYP2C9 inhibition and the propensity for the unsubstituted indole metabolite to accumulate on multiple dosing in rat, analogs completely devoid of this liability were desired.

To this end, metabolically more stable analogs were prepared by repositioning the substituents on the indole nucleus. Transposing the attachment of the benzyl moiety from the 3-position to the nitrogen of the indole provided compounds with the desired features. With these transposed *N*-benzyl indoles attaching the benzoyl or the 1-BZX to the indole 3-position returned analogs that were potent SPPARγMs (**11**, **12**, Table 1). The SAR of the transposed indole analogs tracked identically with the *N*-benzoyl indoles reported previously.

Given the unique differences in the transactivation assay with these SPPARγMs relative to full agonists, further investigation of these SPPARγMs was undertaken. The acidic TZD head group present in rosiglitazone is crucial for its binding and functional activity. For example, *N*-methyl rosiglitazone (**15**) was devoid of both binding and functional activity. An X-ray co-crystal structure with rosiglitazone bound to PPARγ has shown that the acidic TZD moiety interacts with Tyr-473 on helix 12 (AF-2).[8] However, the carboxylic acid in the indole SPPARγMs could be replaced by a nitrile (**13**) and, most surprisingly, an acetylenic group (**14**). Unlike full agonists, both of these analogs retained in vitro activity despite the absence of an acidic group in the molecule (Table 1).[9]

Compound **12** was selected to examine the effects of these SPPARγMs on adipocyte differentiation. The level of adipose fatty acid binding protein (aP2) mRNA expression was measured in differentiating human preadipocytes.[10] SPPARγM **12** was found to induce only 15% of the maximal level of aP2 mRNA, a marker of adipogenesis as compared to rosiglitazone treatment. Attenuated lipid accumulation was observed in human adipocytes upon treatment with **12** versus rosiglitazone at similar concentrations as judged by oil red O staining (Fig. 2).[11]

Based upon its in vitro profile, compound **12** was selected for evaluation in vivo. This analog was metabolically stable and no metabolites were produced in detectable quantities in rodents that were inhibitors of CYP2C9. In db/db mice, SPPARγM **12** afforded dose dependent glucose lowering comparable to that

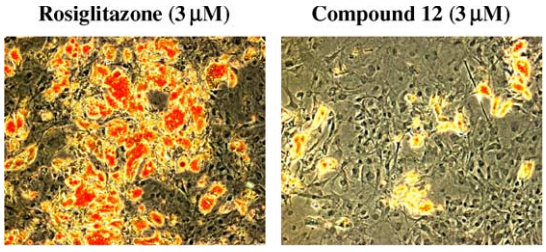


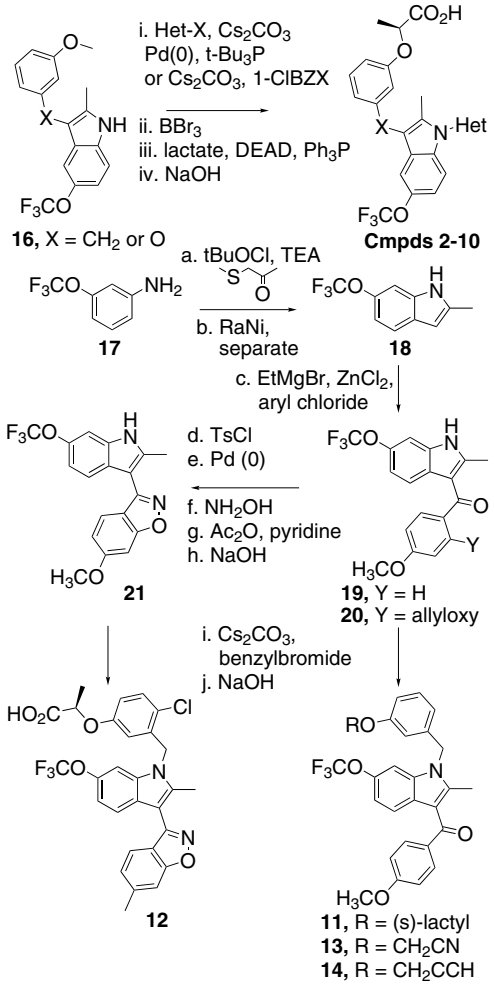
Figure 2. Visualization of lipid accumulation in human adipocytes by oil red O staining.

Table 2. Comparison of **12** and rosiglitazone in male db/db mice and Sprague–Dawley rats

Compds	Db/db mice		S–D rat[a]		
	Dose (mpk)	Gluc. Corr. (%)	Dose (mpk)	HW[c] (g)	BAT (g)
Rosiglitazone	10	77	150	*1.26 ± 0.06*[b]	*1027 ± 466*[b]
12	10	62	100	1.04 ± 0.05	462 ± 45
12	30	78			
Vehicle	—	—	—	1.04 ± 0.08	316 ± 40

[a] Values are means of six animals/group ± standard deviation.
[b] For italics values $P < 0.05$ compared to vehicle (Dunnett's *t*-test).
[c] HW = heart weight.

of rosiglitazone (Table 2) after once daily oral dosing for 11 days.[12] Similar plasma exposures were obtained for compound **12** at 30 mg/kg and rosiglitazone at 10 mg/kg (AUC = 660 and 250–700 μM h,[13] respectively). Compound **12** was also studied in male Sprague–Dawley rats. After once daily dosing for two weeks, **12** (AUC = 1030 μM h, ~1.6–10× efficacy exposure) resulted in less CH and BAT increases as compared to that of rosiglitazone (AUC = 2700 μM h, ~4–10× efficacy exposure), which was included as an internal positive control (Table 2).[14]



Scheme 1. Indole syntheses.

The preparation of the indole core (**16**) for compounds **1**–**10** (Scheme 1) was accomplished via Fischer's method from commercially available starting materials and has been described previously.[5] *N*-Arylation of **16** was achieved by either of two complementary methods: method one utilized a Pd(0) catalyzed reaction with a heteroaryl halide[15] while method two involved a base mediated reaction with a 1-chlorobenzisoxazole (1-Cl BZX)[16] (compds **2**–**10**, Scheme 1). Preparation of the 3-benzoyl (**11**) and 3-(1-BZX) (**12**) indole analogs was accomplished in the following manner. Sequential treatment of commercially available 3-trifluoromethoxy aniline **17** with *t*-butyl hypochlorite, thiomethyl acetone, and triethyl amine in methylene chloride at 0 °C provided a 3:1 mixture of isomeric 3-thiomethyl indoles.[17] The crude mixture was treated with RaNi in ethanol to afford the desired 6-trifluoromethoxy indole **18** in 50–60% yield after separation of the isomers. Reaction of **18** and $ZnCl_2$ with EtMgBr in THF followed by the addition of a benzoyl chloride resulted in the selective aroylation at the indole 3-position to give **19** and **20** in 75–85% yield.[18] The 3-(1-BZX) indole **21** was synthesized from **20** in five steps and 10–15% overall yield. *N*-Benzylation of the indole nitrogen with the appropriate benzyl bromide was achieved in high yields with Cs_2CO_3 in DMF to afford **13** and **14**. After *N*-benzylation, base hydrolysis provided **11** and **12** (Scheme 1). The requisite benzyl bromides were readily accessible from commercially available starting materials.[19]

In summary, repositioning the 3-benzyl moiety to the indole nitrogen has resulted in a metabolically stable series of indole SPPARγMs. Interestingly, these compounds do not require an acidic head group which is integral for activity with traditional full agonists. This difference suggests that these SPPARγMs possibly bind to the receptor in a functionally active conformation distinct from full agonists. It may be the case that a unique binding conformation leads to an altered expression of genes. This may explain the attenuated adipogenic activity as compared to rosiglitazone in human adipocytes. While these SPPARγMs afforded reductions in glucose in db/db mice equal to that of rosiglitazone, in rats the SPPARγMs elicit reduced heart and BAT weight increases relative to rosiglitazone. Gratifyingly, these SPPARγMs have demonstrated an improvement in the AEs commonly observed in preclinical species. Further characterization of these SPPARγMs continues in an effort to explore the possibility that compounds with these features may afford beneficial glycaemic control in humans with reduced AEs.

Acknowledgements

We wish to acknowledge the skills and hard work of the many scientists who provided biological support including Thomas T. Doebber, John Ventre, Roger Meurer, Neelam Sharma, Chhabi Biswas, and Raul Alvaro.

References and notes

1. Reviews on PPARγ and insulin resistance: (a) Olefsky, J. M.; Saltiel, A. R. *Trends Endocrinol. Metab.* **2000**, *11*, 362; (b) Berger, J.; Wagner, J. A. *Diabetes Technol. Therap.* **2002**, *4*, 163; (c) Berger, J.; Moller, D. E. *Annu. Rev. Med.* **2002**, *53*, 409; (d) Knouff, C.; Auwerx, J. *Endocrine Rev.* **2004**, *25*, 899–918.
2. (a) Plosker, G. L.; Faulds, D. *Drugs* **1999**, *57*, 409–438; (b) Balfour, J. A.; Plosker, G. L. *Drugs* **1999**, *57*, 921–930; (c) Tugwoog, J. D.; Montague, C. T. *Hum. Exp. Toxicol.* **2002**, *21*, 429–437; (d) Nesto, R. W.; Bell, D.; Bonow, R. O.; Fonseca, V.; Grundy, S. M.; Horton, E. S.; Winter, M. L.; Porte, D.; Semenkovich, C. F.; Smith, S.; Young, L. H.; Kahn, R. *Diabetes Care* **2004**, *27*, 256–263.
3. SPPARM refers to a compound that is a partial agonist in a transcriptional assay and has demonstrated unique molecular and pharmacological properties relative to traditional agonists. It has been suggested that modulation of the PPARs may be the preferred strategy for drug design. Cock, T.-A.; Houten, S. M.; Auwerx, J. *EMBO Rep.* **2004**, *5*, 142–147.
4. (a) Reginato, M. J.; Bailey, S. T.; Krakow, S. L.; Minami, C.; Ishii, S.; Tanaka, H.; Lazar, M. A. *J. Biol. Chem.* **1998**, *273*, 32679–32684; (b) Oberfield, J. L.; Collins, J. L.; Holmes, C. P.; Goreham, D. P.; Cooper, J. P.; Cobb, J. E.; Lenhard, J. M.; Hull-Ryde, E. A.; Mohr, C. P.; Blanchard, S. G.; Parks, D. J.; Moore, L. B.; Lehmann, J. M.; Plunket, K.; Miller, A. B.; Milburn, M. V.; Kliewer, S. A.; Willson, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6102; (c) Elbrecht, A.; Chen, Y.; Adams, A.; Berger, J.; Griffin, P.; Klatt, T.; Zhang, B.; Menke, J.; Zhou, G.; Smith, R. G.; Moller, D. E. *J. Biol. Chem.* **1999**, *27*, 7913; (d) Shimaya, A.; Kurosaki, E.; Nakano, R.; Hirayama, R.; Shibasaki, M.; Shikama, H. *Metabolism* **2000**, *49*, 411–417; (e) Rocchi, S.; Picard, F.; Vamecq, J.; Gelman, L.; Potier, N.; Zeyer, D.; Dubuquoy, L.; Bac, P.; Champy, M.-F.; Plunket, K. D.; Leesnitzer, L. M.; Blanchard, S. G.; Desreumaux, P.; Moras, D.; Renaud, J.-P.; Auwerx, J. *Mol. Cell* **2001**, *8*, 743; (f) Thor, M.; Beierlein, K.; Dykes, G.; Gustovsson, A.-L.; Heidrich, J.; Jendeberg, L.; Lindqvist, B.; Pegurier, C.; Roussel, P.; Slater, M.; Svensson, S.; Sydow-Bachmann, M.; Thornstrom, U.; Uppenberg, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3565–3567; (g) Berger, J. P.; Petro, A. E.; McNaul, K. L.; Kelly, L. J.; Zhang, B. B.; Richards, K.; Elbrecht, A.; Johnson, B. A.; Zhou, G.; Doebber, T. W.; Biswas, C.; Parikh, M.; Sharma, N.; Tanen, M. R.; Thompson, G. M.; Ventre, J.; Adams, A. D.; Mosley, R.; Surwit, R. S.; Moller, D. E. *Mol. Endocrinol.* **2003**, *17*, 662; (h) Schupp, M.; Janke, J.; Clasen, R.; Unger, T.; Kintscher, U. *Circulation* **2004**, *109*(17), 2054–2057; (i) Minoura, H.; Takeshita, S.; Ita, M.; Hirosumi, J.; Mabuchi, M.; Kawamura, I.; Nakajima, S.; Nakayama, O.; Kayakiri, H.; Oku, T.; Ohkubo-Suzuki, A.; Fukagawa, M.; Kojo, H.; Hanioka, K.; Yamasaki, N.; Imoto, T.; Kobayashi, Y.; Mutoh, S. *Eur. J. Pharmacol.* **2004**, *494*, 273–281.
5. Acton, J. J.; Black, R. M.; Jones, A. B.; Moller, D. E.; Colwell, L.; Doebber, T. W.; MacNaul, K. L.; Berger, J.; Wood, H. B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 357–362.
6. Procedures for the SPA and transactivation assay are described in: Berger, J. P.; Petro, A. E.; MacNaul, K.; Kelly, L. E.; Zhang, B. B.; Richards, K.; Elbrecht, A.; Johnson, B.; Zhou, G.; Doebber, T. W.; Biswas, C.; Parikh, M.; Sharma, N.; Tanen, M. R.; Thompson, M.; Ventre, J.; Adams, A. D.; Mosley, R.; Surwit, R. S.; Moller, D. E. *Mol. Endocrinol.* **2003**, *17*, 662, and; Berger, J.; Liebowitz, M. D.; Doebber, T. W.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C. A.; Hayes, N. S.; Li, Y.; Tanen, M.; Ventre, J.; Wu, M. S.; Berger, G. D.; Mosley, R.; Marquis, R.; Santini, C.; Sahoo, S. P.; Tolman, R. L.; Smith, R. G.; Moller, D. E. *J. Biol. Chem.* **1999**, *274*, 6718.
7. Tschirret-Guth, R. A.; Wood, H. B. *Drug Metab. Dispos.* **2003**, *31*, 1000–1004.
8. Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. *Nature* **1998**, *395*, 137–143.
9. Ligand–receptor co-crystal structures in which the ligand does not make contact with Tyr-473 have been reported. See Refs. 4b and 4f.
10. Experimental details Thompson, G. M.; Trainor, D.; Tanen, M.; Biswas, C.; LaCerte, C.; Berger, J. P.; Kelly, L. *J. Anal. Biochem.* **2004**, *330*, 21.
11. Experimental details described in Ref. 4g.
12. db/db mice were used as described in: Berger, J.; Bailey, P.; Biswas, C.; Cullinan, C. A.; Doebber, T. W.; Hayes, N. S.; Saperstein, R.; Smith, R. G.; Liebowitz, M. D. *Endocrinology* **1996**, *137*, 4189.
13. The AUC reported is the historical range of values obtained after dosing at 10 mkd.
14. Male S–D rats were used as described in Ref. 5.
15. Hartwig, J. F.; Kawatsura, M.; Hauck, S. I.; Shaughnessy, K. H.; Alcazar-Roman, L. M. *J. Org. Chem.* **1999**, *64*, 5575–5580.
16. Boshagen, H. *Chem. Ber.* **1967**, *100*, 3326–3330.
17. Gassman, P. G.; van Bergen, T. J. *Org. Synth.* **1988**, 601–605, Coll. Vol. 6.
18. Bergman, J.; Venemalm, L. *Tetrahedron* **1990**, *46*, 6061.
19. All compounds gave satisfactory ^{1}H NMR and LC/MS data.