

Selective PPAR γ modulators with improved pharmacological profiles

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Abstract—A series of metabolically robust *N*-benzyl-indole selective PPAR γ modulators with either a 3-benzoyl or 3-benzisoxazolyl moiety have been identified. In vitro, these compounds are partial agonists and exhibit reduced adipogenesis in human adipocytes. In vivo, these SPPAR γ Ms 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 PPAR γ full agonists.

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The peroxisome proliferator-activated receptor gamma (PPAR γ) is member of a large family of ligand activated nuclear transcription factors. Both rosiglitazone and pioglitazone are full agonists that specifically activate PPAR γ 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 PPAR γ 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 PPAR γ ligand that ameliorates these AEs would be desirable. In search of PPAR ligands devoid of these AEs, new classes of selective PPAR γ modulators (SPPAR γ Ms)³ have been reported.⁴ We recently reported the discovery of SPPAR γ Ms that exhibit potent glucose lowering in the db/db mouse model for T2DM.⁵ Interestingly, these indole derived SPPAR γ Ms 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

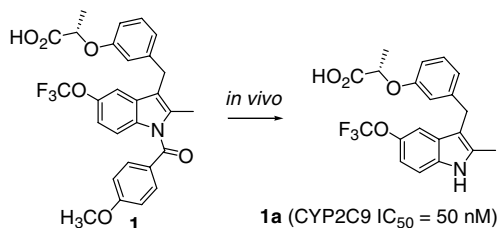


Figure 1. In vivo loss of the *N*-benzoyl group.

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 (IC₅₀ = 50 nM), a liability which precluded further studies. Herein, we describe the identification and evaluation of second generation indole-based SPPAR γ Ms with improved metabolic profiles that are inactive on the α and δ PPAR isoforms.

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.

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Table 1. Human PPAR γ binding (SPA) IC₅₀s and transactivation EC₅₀s^a

Rosiglitazone , R = H 15 , R = CH ₃				
2 , 2-pyridyl 3 , 2-(6-OMe)quinolyl 4 , 3-(6-OMe)quinolyl				
11 , R = (s)-lactyl 13 , R = CH ₂ CN 14 , R = CH ₂ CCH				
Cmpds 5-10				
12				
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- <i>n</i> Pr	641	—	—
7	4-Cl	5	3	31
8	5-Cl	109	114	24
9	6- <i>n</i> 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 IC₅₀ is measured by the complete displacement of a radio-labeled full agonist indicating competitive binding to the receptor.

^b EC₅₀ 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 inhibi-

tion 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 SPPAR γ Ms (**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 SPPAR γ Ms relative to full agonists, further investigation of these SPPAR γ Ms 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 PPAR γ has shown that the acidic TZD moiety interacts with Tyr-473 on helix 12 (AF-2).⁸ However, the carboxylic acid in the indole SPPAR γ Ms 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 SPPAR γ Ms on adipocyte differentiation. The level of adipose fatty acid binding protein (aP2) mRNA expression was measured in differentiating human pre-adipocytes.¹⁰ SPPAR γ M **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, SPPAR γ M **12** afforded dose dependent glucose lowering comparable to that

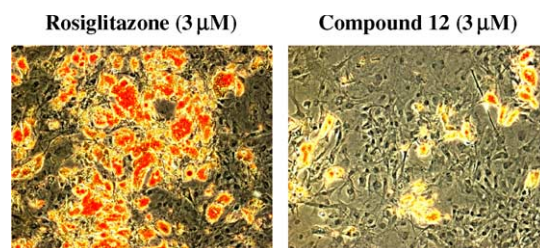


Figure 2. Visualization of lipid accumulation in human adipocytes by oil red O staining.

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

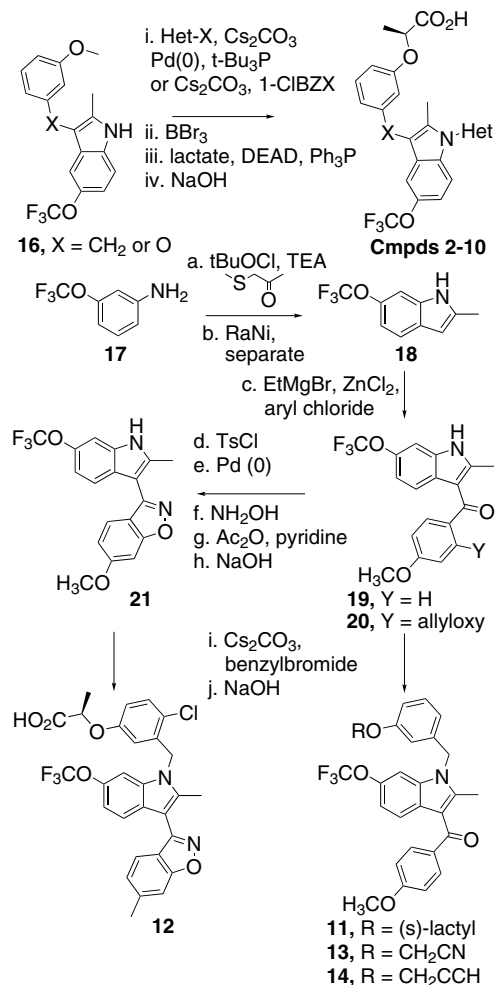
Compds	Db/db mice		S–D rat ^a		
	Dose (mpk)	Gluc. Corr. (%)	Dose (mpk)	HW ^c (g)	BAT (g)
Rosiglitazone	10	77	150	1.26 ± 0.06 ^b	1027 ± 466 ^b
12	10	62	100	1.04 ± 0.05	462 ± 45
12	30	78	—	—	—
Vehicle	—	—	—	1.04 ± 0.08	316 ± 40

^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 $\mu\text{M h}$,¹³ respectively). Compound **12** was also studied in male Sprague–Dawley rats. After once daily dosing for two weeks, **12** (AUC = 1030 $\mu\text{M h}$, ~ 1.6 – $10\times$ efficacy exposure) resulted in less CH and BAT increases as compared to that of rosiglitazone (AUC = 2700 $\mu\text{M h}$, ~ 4 – $10\times$ efficacy exposure), which was included as an internal positive control (Table 2).¹⁴

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 halide¹⁵ while method two involved a base mediated reaction with a 1-chlorobenzisoxazole (1-Cl BZX)¹⁶ (comps **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 ZnCl₂ with EtMgBr in THF followed by the addition of a benzoyl chloride resulted in the selective arylation 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 Cs₂CO₃ 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 SPPAR γ Ms. Interestingly, these compounds do not require an acidic head group which is integral for activity with traditional full agonists. This difference suggests that these SPPAR γ Ms 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 SPPAR γ Ms afforded reductions in glucose in db/db mice equal to that of rosiglitazone, in rats the SPPAR γ Ms elicit reduced heart and BAT weight increases relative to rosiglitazone. Gratifyingly, these SPPAR γ Ms have demonstrated an improvement in the AEs commonly observed in preclinical species. Further characterization of these SPPAR γ Ms continues in an effort to explore the possibility that compounds with these features may afford beneficial glycaemic control in humans with reduced AEs.

**Scheme 1.** Indole syntheses.

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References and notes

- Reviews on PPAR γ 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.
- (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.
- 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.
- (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.; Klier, 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**, *274*, 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.
- 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.
- 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.
- Tschirret-Guth, R. A.; Wood, H. B. *Drug Metab. Dispos.* **2003**, *31*, 1000–1004.
- 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.
- Ligand–receptor co-crystal structures in which the ligand does not make contact with Tyr-473 have been reported. See Refs. **4b** and **4f**.
- Experimental details Thompson, G. M.; Trainor, D.; Tanen, M.; Biswas, C.; LaCerte, C.; Berger, J. P.; Kelly, L. *J. Anal. Biochem.* **2004**, *330*, 21.
- Experimental details described in Ref. **4g**.
- 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.
- The AUC reported is the historical range of values obtained after dosing at 10 mkg.
- Male S–D rats were used as described in Ref. **5**.
- Hartwig, J. F.; Kawatsura, M.; Hauck, S. I.; Shaughnessy, K. H.; Alcazar-Roman, L. M. *J. Org. Chem.* **1999**, *64*, 5575–5580.
- Boshagen, H. *Chem. Ber.* **1967**, *100*, 3326–3330.
- Gassman, P. G.; van Bergen, T. J. *J. Org. Synth.* **1988**, 601–605, Coll. Vol. 6.
- Bergman, J.; Venemalm, L. *Tetrahedron* **1990**, *46*, 6061.
- All compounds gave satisfactory ^1H NMR and LC/MS data.