Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3916–3920
Bioorganic & Medicinal Chemistry Letters

Co-crystal structure guided array synthesis of PPARγ inverse agonists

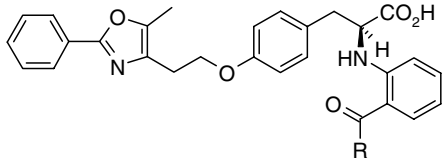
Ryan P. Trump, Jeffrey E. Cobb, Barry G. Shearer, Millard H. Lambert, Robert T. Nolte, Timothy M. Willson, Richard G. Buckholz, Sumin M. Zhao, Lisa M. Leesnitzer, Marie A. Iannone, Kenneth H. Pearce, Andrew N. Billin and William J. Hoekstra*

Drug Discovery, GlaxoSmithKline, Research Triangle Park, NC 27709, USA

Received 8 March 2007; revised 27 April 2007; accepted 30 April 2007
Available online 18 May 2007

Abstract—PPARγ-activating thiazolidinediones and carboxylic acids such as farglitazar exert their anti-diabetic effects in part in PPARγ rich adipose. Both pro- and anti-adipogenic PPARγ ligands promote glucose and lipid lowering in animal models of diabetes. Herein, we disclose representatives of an array of 160 farglitazar analogues with atypical inverse agonism of PPARγ in mature adipocytes.

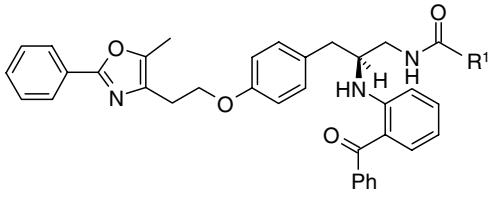
Peroxisome proliferator-activated receptor-gamma (PPARγ) agonists such as the thiazolidinedione (TZD) drugs and related carboxylic acids such as farglitazar (GI262570) are effective insulin sensitizers in patients with type 2 diabetes mellitus.[1,2] Insulin resistance is an early marker of this type of adult onset diabetes. Oral administration of PPARγ agonists to these patients reverses hyperglycemia and hyperinsulinemia by increasing glucose metabolism in muscle and reducing glucose biosynthesis in the liver. PPARγ also plays a critical role in adipocyte function. PPARγ agonists promote differentiation of pre-adipocytes to mature adipocytes as measured by lipid accumulation, changes in cell morphology, and the expression of adipocyte-specific genes.[3]



R = Ph **Farglitazar (GI262570)**

R = OMe **GW7845**

Adipogenesis involves a complex interplay between PPARγ and a variety of genes, notably fatty acid binding protein-4 (FABP4).[4] Gene knockout studies have demonstrated that PPARγ is essential for adipocyte differentiation in vivo.[5,6] Paradoxically, the PPARγ antagonist GW9662 exerts a modest insulin sensitizing effect but, unlike agonists, decreases weight gain in obese mice.[7] These results are consistent with knockout of adipocytic PPARγ in a mouse study.[8] Despite these reports, little is known about the interruption of PPARγ-induced target gene expression.



2

To date, ligands have been identified primarily for their propensity to stimulate or inhibit adipogenesis, or recruit co-activators to PPARγ.[2,9] TZDs and carboxylic acids (e.g., farglitazar) form a network of hydrogen bonds with Tyr-473, His-323, His-449, and Ser-289 adjacent to the AF2 helix.[1,10] Herein, we have devised and synthesized a mini-library of 160 diverse analogues **2**

Keywords: PPARγ; Inverse agonist.
* Corresponding author. Fax: +1 919 315 0430; e-mail: william.j.hoekstra@gsk.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.111

of the PPARγ agonist farglitazar to attempt to exert differential effects at the AF-2 helix domain, thus leading to some atypical cofactor peptide interactions.[11,12] These compounds differentially recruited a set of 49 coactivator or corepressor-derived peptides to PPARγ ligand binding domain (LBD) in a multiplexed fluorescent microsphere based binding assay.[11,13] We determined the functional profiles of these PPARγ ligands in both a GAL4-based reporter assay and mature adipocytes. The synthetic approaches to these ligands and biological results are revealed below.

Amide **2t** (R^1 = Me) was prepared as a singleton to aid in design of the array. Following X-ray co-crystal analysis of **2t**, amides **2** were prepared as described below.[16] Optically pure farglitazar was converted to its corresponding activated ester with isobutyl chloroformate and reduced to the alcohol with sodium borohydride. This alcohol was treated with diphenylphosphoryl azide and subsequently reduced to its corresponding amine by hydrogenation over 10% palladium-on-carbon; the amine was protected in situ using di-*tert*-butyl-dicarbonate to afford shelf-stable **1**. *N*-Boc-protected amine **1** was treated with hydrochloric acid and then quickly acylated with a variety of polystyrene-bound activated hydroxybenzotriazole esters of carboxylic acids to furnish an array of 160 farglitazar inverse amide products **2**. For products containing terminal carboxylic acids or amines, *t*-butyl ester or *N*-*tert*-butoxycarbonyl protection, respectively, was employed and cleaved as a final step in a hydrochloric acid/dioxane medium.

Farglitazar analogues **2** were tested in PPAR scintillation proximity assay (SPA) binding and a transiently transfected PPARγ CV-1 cell GAL4 reporter assay (Tables 1 and 2).[15] Approximately two-thirds of the array members exhibited PPARγ $pK_i > 6.0$ (102/160). All compounds exhibited >5-fold binding selectivity for PPARγ (PPARα, PPARδ data not shown). Small hydrophobic amides furnished high PPARγ affinity (**2p**–**t**). Carboxylic acids exhibited somewhat lower affinity (**2k**–**o**). Small hydrophobic amides were devised to occupy minimal AF2 domain space. Thus, partial intrinsic efficacy was observed for this class of amides in the GAL4 reporter assay. Conversely, larger hydrogen-bonding amines and carboxylic acids were designed to displace the AF2 helix and occupy the coactivator cleft to interrupt coactivator binding (**2g**, **2i**, **2n**, and **2o**). Herein, these amides exhibited antagonist GAL4 activity (Fig. 1).

Table 2. PPARγ CV-1 cell GAL4 reporter activity of representative amides

	GAL4 PPARγ			
Compound	pEC_{50}[a]	%max	pIC_{50}[b]	%max
2g	<5.0	—	5.2	55
2i	<5.0	—	5.2	48
2n	<5.0	—	5.7	80
2o	<5.0	—	6.0	92
2q	6.2	29	5.6	59
2s	6.2	27	5.6	57
2t	6.0	37	5.8	54
GW7845	9.2	92	<5.0	—

[a] Assay measures transactivation of human PPARγ in CV-1 cells ($n \geqslant 2$, SD = 0.10).[15]

[b] Assay measures the inhibition of BRL-49653 stimulated (100 nM) transactivation of human PPARγ in CV-1 cells ($n \geqslant 2$, SD = 0.15).[9]

We also examined the co-regulator affinity signatures of the 102 array members with $pK_i > 6.0$ using a multiplexed interaction assay to determine PPARγ LBD interactions with 49 peptides derived from the binding motifs of known co-regulator proteins.[11,13] In the assay, mean fluorescence intensity units (MFI) correlate with affinity of peptide binding. TRAP-220 is a co-activator that plays a central role in PPARγ-mediated adipocyte gene expression pathways. While the farglitazar/PPARγ complex bound tightly to the TRAP-220 derived peptide (MFI = 499),[13] amide analogues **2i** (MFI = −99) and **2n** (MFI = −296) exhibited markedly reduced, atypical coactivator peptide binding relative to apo-PPARγ (MFI = 0) and a neutral effect on corepressor binding.[11] Based on these unusual PPARγ/co-regulator interaction

Table 1. Monomer examples (R^1CO_2H) selected from co-crystal structure analysis for acylation[a] of a primary amine analogue of farglitazar that furnished amides **2a**–**t** with PPARγ $pK_i > 6$[b]

	R^1 Groups		
Alcohols	pK_i[c]	Amines	pK_i[c]
a CH_2NHCO-3-$C_6H_4CH_2OH$	7.00	**f** 3-piperidinyl	7.29
b CH_2OH	6.95	**g** 3-C_6H_4-$O(CH_2)_2NMe_2$	7.00
c 4-$C_6H_4CH_2OH$	6.80	**h** 2-pyridyl	6.78
d CH_2NHCO-4-C_6H_4OH	6.76	**i** CH_2O-3-$C_6H_4NH_2$	6.72
e 4-*c*-$C_6H_{10}OH$	6.51	**j** 4-*c*-$C_6H_{10}CH_2NH_2$	6.71
Carboxylic acids	pK_i[c]	Hydrophobes	pK_i[c]
k 2-$C_6H_4CO_2H$	6.94	**p** 4-C_6H_4OMe	7.32
l $CH(Me)CH_2CH(Me)CO_2H$	6.84	**q** *c*-C_3H_5	7.26
m $(CH_2)_2CO_2H$	6.57	**r** $(CH_2)_5Me$	7.21
n $(CH_2)_2$-4-$C_6H_4SC(Me)_2CO_2H$	6.60	**s** $(CH_2)_2Me$	7.06
o $CH_2CH(Me)CO_2H$	6.04	**t** Me	6.86

[a] See conversion of **1**–**2** in Scheme 1.

[b] Listed in descending order of PPARγ affinity for each of the four functional classifications, $n = 2$.

[c] The scintillation proximity assay measures compound interaction with the ligand binding domain of PPARγ by displacement of tritiated BRL-49653 ($n \geqslant 2$, SD = 0.15).[15]

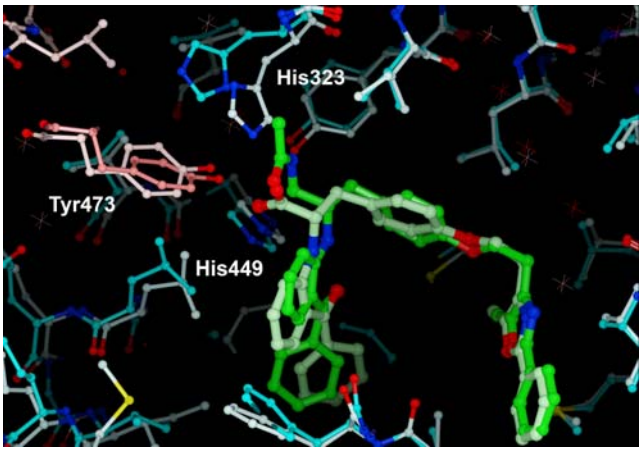


Figure 1. X-ray co-crystal structure of farglitazar bound to the PPARγ LBD overlaid on the co-crystal structure of **2t** (R^1 = Me) bound to the PPARγ LBD.[14] Whereas the farglitazar carboxylate forms a network of hydrogen bonds with His-323, His-449 (side chain above/behind label), and the AF2 helix's Tyr-473, the amide of **2t** merely approaches His-323 and Tyr-473, without actually making any strong hydrogen bonds. The AF2 helix is modestly unwound (not shown) consistent with some destabilization of the activated conformation of the AF2 helix.

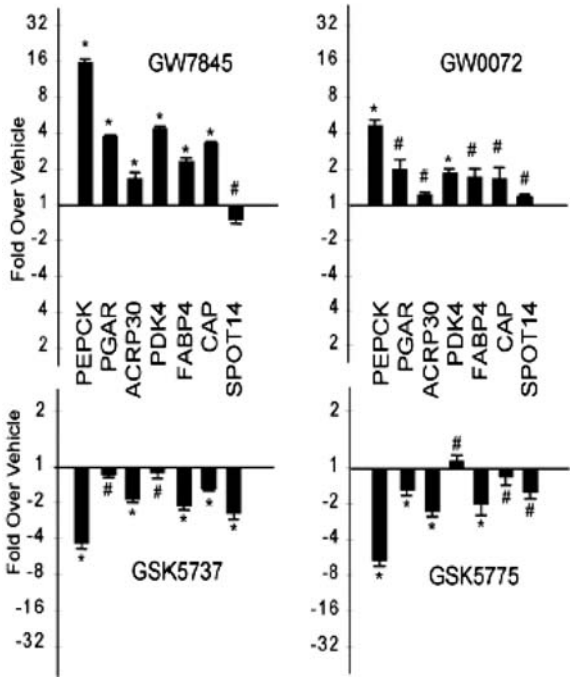
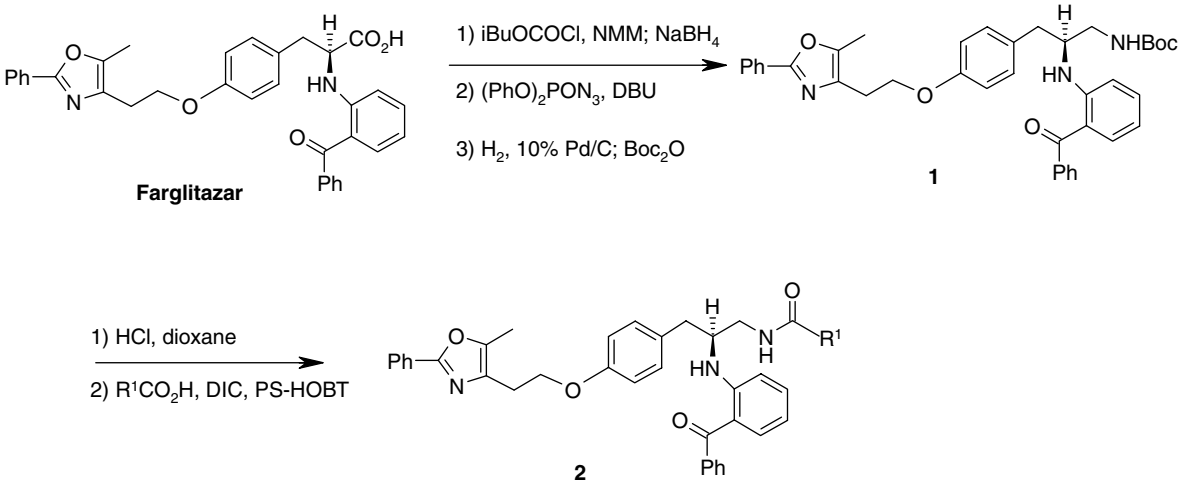


Figure 2. Gene expression by PPARγ agonist GW7845, partial agonist GW0072,[9] and inverse agonists GSK5737 (**2n**) and GSK5775 (**2i**) in mature 3T3L1 adipocytes.[18]

profiles,[13] the phenotypes of these ligands and other array members in Table 2 were characterized in a mature 3T3L1 adipocyte gene expression assay to compare them to ligands with known activities. The highly efficacious agonist GW7845 positively regulated six of seven marker genes of fatty acid synthesis, transport, storage, and oxidation (Fig. 2). [17] Partial activators regulated these same genes less efficaciously.[11] By contrast, inverse agonists GSK5737 (**2n**) and GSK5775 (**2i**) exhibited atypical activity in that genes were driven to expression levels below basal PPARγ.[11] For example, the PEPCK and FABP4 genes were down-regulated significantly (Scheme 1).

In summary, a synthetic array of farglitazar analogues was synthesized with guidance from X-ray co-crystal structures. These amides were evaluated in PPARγ binding and GAL4 reporter assays, and a mature 3T3L1 adipocyte gene expression assay. Strikingly, sterically bulky members of this array (**2i**, **2n**), designed to repress coactivator binding in the AF2 helix domain, suppressed induction of adipogenic genes relative to basal expression levels.[11] Low efficacy partial agonists (e.g., GW0072)[9] and antagonists (e.g., GW9662,[7] T0070907,[19] PD-068235)[20] of PPARγ transcription that inhibit PPARγ agonist-induced adipogenesis yet do not invert the PPARγ phenotype in adipocytes have been disclosed in the literature. In our adipocyte assay, the competitive antagonist GW9662 exhibited a neutral,



Scheme 1. Synthesis of inverse amide analogues of farglitazar.

basal-like effect on PPARγ target gene expression. Thus, inverse agonists of PPARγ-mediated activity (e.g., **2n**) herein appear to be uncommon. These amides are representatives of a new class of ligands that are available to evaluate co-activator destabilization and inverse agonism at PPARγ in biological models of diabetes, obesity, and other diseases.[21]

Acknowledgments

We thank Jeffrey D. Harless, Julie B. Stimmel, and James M. Way for helpful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.04.111.

References and notes

1. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
2. Shearer, B. G.; Hoekstra, W. J. *Curr. Med. Chem.* **2003**, *10*, 267.
3. Tontonoz, P.; Hu, E.; Spiegelman, B. M. *Cell* **1994**, *79*, 1147.
4. Lowell, B. B. *Cell* **1999**, *99*, 239.
5. Barak, Y.; Nelson, M. C.; Ong, E. S.; Jones, Y. Z.; Ruiz-Lozano, P.; Chien, C. R.; Koder, A.; Evans, R. M. *Mol. Cell* **1999**, *4*, 585.
6. Rosen, E. D.; Sarraf, P.; Troy, A. E.; Bradwin, G.; Moore, K.; Milstone, D. S.; Spiegelman, B. M.; Mortensen, R. M. *Mol. Cell* **1999**, *4*, 611.
7. Nakano, R.; Kurosaki, E.; Yoshida, S.; Yokono, M.; Shimaya, A.; Maruyama, T.; Shibasaki, M. *Biochem. Pharm.* **2006**, *72*, 42.
8. Jones, J. R.; Barrick, C.; Kim, K.-A.; Lindner, J.; Blondeau, B.; Fujimoto, Y.; Shiota, M.; Kesterson, R. A.; Kahn, B. B.; Magnuson, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6207.
9. Oberfield, J.; Collins, J. L.; Holmes, C. P.; Goreham, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6102.
10. Xu, H. E.; Lambert, M. H.; Montana, V. G.; Plunket, K. D.; Moore, L. B.; Collins, J. L.; Oplinger, J.; Kliewer, S. A.; Gampe, R. T.; McKee, D. D.; Willson, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13919.
11. Buckholz, R. G.; Trump, R. P.; Zhao, S.; Iannone, M. A.; Leesnitzer, L. M.; Merrihew, R. V.; Lobe, D. C.; Winegar, D. A.; Lazar, M. A.; Cobb, J. E.; Willson, T. M.; Hoekstra, W. J.; Billin, A. B. *Biochem. Biophys. Res. Commun.*, submitted for publication; also see supplemental material.
12. Xu, H. E.; Stanley, T. B.; Montana, V. G.; Lambert, M. H. *Nature* **2002**, *415*, 813.
13. Iannone, M. A.; Simmons, C. A.; Kadwell, S. H.; Svoboda, D. L.; Vanderwall, D. E.; Deng, S. J.; Consler, T. G.; Shearin, J.; Gray, J. G.; Pearce, K. H. *Mol. Endocrinol.* **2004**, *18*, 1064.
14. Co-crystals of compound **2t** with the PPARγ LBD were grown from a complex containing 15 mg/ml protein (20 mM Tris, pH 8.0, 5 mM DTT, 100 mM NaCl, 2 mM EDTA) complexed at a 5:1 molar ratio with the compound (at 50 mM in DMSO) using the vapor phase diffusion method. The two co-crystals' PDB codes are 2pob (**2t**) and 1fm9 (farglitazar).
15. Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K. *J. Med. Chem.* **1998**, *41*, 5020.
16. Array members were prepared as described for compound **2t**: To a stirred, cooled (−15 °C) solution of farglitazar (compound **20** described in reference 15; 5.00 g, 9.15 mmol) in DME (50 mL) were added *N*-methylmorpholine (0.92 g, 9.15 mmol) and isobutyl chloroformate (1.26 g, 9.25 mmol). This suspension was stirred for 5 min, filtered, and cooled (−15 °C). To this solution was added a solution of $NaBH_4$ (0.35 g, 9.30 mmol) in water (5 mL). After 5 min the reaction mixture was diluted with water (250 mL) and warmed to RT. The mixture was poured into HCl (1 N; 250 mL) and extracted with DCM. The organic layer was washed with brine and dried over $MgSO_4$. Removal of solvent in vacuo and recrystallization of the resultant solid from EtOAc/hexane (1:1, 250 mL) provided the desired primary alcohol as a yellow solid (3.88 g, 80% yield). To a stirred solution of the yellow solid (0.50 g, 0.94 mmol) and diphenylphosphoryl azide (1.28 g, 4.64 mmol) in DMF (30 mL) was added DBU (0.76 g, 5.02 mmol). The mixture was heated at 90 °C for 18 h, cooled to rt, and the DMF removed in vacuo. The residue was partitioned between EtOAc and HCl (1 N), and the organic layer washed with water, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give a solid. The crude material was purified by silica gel chromatography eluting with 1:4 ethyl acetate/hexanes to give the azide as a yellow solid (0.48 g, 91% yield). The azide (0.39 g, 0.70 mol) was dissolved in THF (10 mL) under nitrogen, and di-*tert*-butyl-dicarbonate (0.46 g, 2.12 mmol) and 10% Pd/C (0.070 g) were added. The solution was stirred under one atmosphere of hydrogen for 18 h, filtered (Celite), and concentrated in vacuo to furnish crude, oily **1**. This oil was purified by silica gel chromatography (2:1 EtOAc/hexanes) to give intermediate **1** as a yellow solid (0.22 g, 50% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.6 (d, J = 8.5 Hz, 1H), 8.0 (m, 2H), 7.6 (m, 2H), 7.5–7.2 (m, 8H), 7.1 (m, 2H), 6.8 (m, 1H), 6.8 (m, 2H), 6.5 (t, J = 7.4, 1H), 4.8 (br s, 1H), 4.2 (t, J = 6.6, 2 H), 4.0 (m, 1H), 3.5 (m, 1H), 3.3 (m, 1H), 3.0 (t, J = 6.6, 2H), 2.8 (m, 2H), 2.4 (s, 3 H), 1.4 (s, 9H). MS (APCI) *m/z* 632 (M+1). Intermediate **1** (0.22 g, 0.35 mmol) was dissolved in HCl (4 M dioxane, 4.0 mL). The solution was stirred for 1 h and concentrated in vacuo to a yellow solid (0.20 g, 99% yield). Polystyrene-bound hydroxybenzotriazole (0.029 g, 0.030 mmol) was suspended in DMF (1 mL). Reagent monomer example acetic acid (0.0027 g, 0.045 mmol), dimethylaminopyridine (0.0022 g, 0.018 mmol), and diisopropylcarbodiimide (0.017 g, 0.135 mmol) were added, and the resulting suspension was shaken for 2 h. The resin was filtered, washed with DCM, and suspended in DCM (1 mL). The yellow, de-protected HCl salt of intermediate **1** (0.012 g, 0.021 mmol) and polystyrene-bound *N*-methylmorpholine (0.022 g, 0.09 mmol) were added to the suspension. After 2 h of rotation, the organic solution was collected by filtration and concentrated in vacuo to give a crude oil that was purified by silica gel chromatography to furnish **2t** as a yellow solid (0.009 g, 75% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.1 (d, J = 6.1, 2H), 7.5 (m, 2H), 7.4 (m, 6H), 7.3 (m, 1H), 7.2 (s, 3H), 7.0 (m, 2H), 6.8 (d, J = 8.6, 1H), 6.7 (d, J = 8.6, 2H), 4.2 (t, J = 6.1, 2H), 3.0 (d, J = 5.8, 2H), 2.8 (m, 1H), 2.4 (s, 3H), 2.0 (s, 1H), 1.9 (s, 3H), 1.8 (m, 2H), 1.2 (m, 1H). MS (APCI) *m/z* 574 (M+1).
17. For more information on these genes, see Way, J. M.; Harrington, W. W.; Brown, K. K.; Gottschalk, W. K.;

Sundseth, S. S.; Mansfield, T. A.; Ramachandran, R. K.; Willson, T. M.; Kliewer, S. A. *Endocrinology* **2001**, *142*, 1269.

18. Adipocytes were treated with differentiation cocktail to generate mature adipocytes, and then dosed with test compounds at the indicated concentrations. GW7845 (1 μM) displays agonism on the tested genes, whereas GW0072 (1 μM) acts as a partial agonist. Contrast this with the inverse agonism exhibited for most of the genes by GSK5737 (10 μM) and GSK5775 (10 μM): (*) $p < 0.01$, (#) $p > 0.01$ versus vehicle.[11] Because only array members shown in Table 2 were run in this assay, results for other high MW array members with an arene/two-carbon tether motif are not available.
19. Lee, G.; Elwood, F.; McNally, J.; Weiszmann, J.; Lindstrom, M.; Amaral, K.; Nakamura, M.; Miao, S.; Cao, P.; Learned, R. M.; Chen, J.-L.; Li, Y. *J. Biol. Chem.* **2002**, *277*, 19649.
20. Camp, H. S.; Chaudhry, A.; Leff, T. *Endocrinology* **2001**, *142*, 3207.
21. The study of a PPARγ inverse agonist in a diabetic animal model will be disclosed in a future communication.