Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4376–4380
Bioorganic & Medicinal Chemistry Letters

3,4,5-Trisubstituted isoxazoles as novel PPARδ agonists: Part 1

Robert Epple,[a,*] Ross Russo,[a] Mihai Azimioara,[a] Christopher Cow,[a] Yongping Xie,[a] Xing Wang,[a] John Wityak,[a] Don Karanewsky,[a] Andrea Gerken,[b] Maya Iskandar,[b] Enrique Saez,[b] H. Martin Seidel[b] and Shin-Shay Tian[b]

[a]*Department of Medicinal Chemistry, The Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA*
[b]*Department of Lead Discovery, The Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA*

Received 27 March 2006; accepted 16 May 2006
Available online 5 June 2006

Abstract—We report the identification of a novel series of trisubstituted isoxazoles as PPAR activators from a high-throughput screen. A series of structural optimizations led to improved efficacy and excellent functional receptor selectivity for PPARδ. The isoxazoles represent a series of agonists which display a scaffold that lies outside the typical PPAR agonist motif.

The metabolic syndrome is defined by the presence of conditions associated with lipid and/or glucose homeostasis abnormalities. These conditions comprise dyslipidemia, obesity, elevated blood pressure, insulin resistance, and a pro-inflammatory state. All of the above are recognized risk factors for cardiovascular disease and type 2 diabetes.[1–4]

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors involved in the regulation of lipid and glucose homeostasis. Dyslipidemia and insulin resistance, two major components of the metabolic syndrome, are commonly treated with fibrates and TZDs, drugs that target PPARα and PPARγ, respectively. The pharmacological role of these receptors has been elucidated by the availability of these compound classes.[5,6] The synthetic ligands for the PPARα and PPARγ subtypes have been reviewed extensively.[7–11]

A third subtype, PPARδ, is the least well understood, and is believed to also play a role in energy homeostasis.[12] To date only a few reports claim the synthesis of PPARδ agonists.[13–16] GW501516 and L-165461

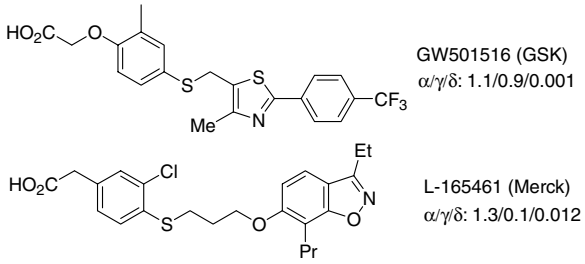


Figure 1. Early PPARδ agonists with reported EC_{50} values for transactivation of the three PPAR subtypes in micromolar.

(Fig. 1) have been used for in vivo studies in rodents and NHPs,[12,17–21] and GW501516 is currently in phase II clinical trials for its antihyperlipidemic properties. However, the considerable crossactivity of these compounds to other PPAR subtypes makes it difficult to assign their pharmacological effects solely to PPARδ. Herein, we report a compound class with novel structural features and improved selectivity to further investigate the role of PPARδ in energy metabolism.

The PPARδ agonists reported to date were discovered using several strategies: GW501516 was optimized from a library of hydrophobic carboxylates,[13] L165461 was derived from an in silico approach to find novel PPARγ agonists using TZDs as structural templates.[14] Recently, the selective Bayer compound 33 was identified from a PPARα agonist program,[15] and we reported the emer-

Keywords: PPARδ agonists; Metabolic syndrome; Energy homeostasis; Isoxazoles.
* Corresponding author. Tel.: +1 858 812 1720; fax: +1 858 812 1848; e-mail: repple@gnf.org

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.05.055

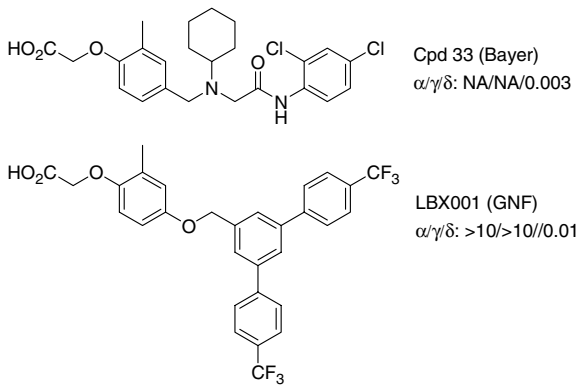


Figure 2. Recent PPARδ agonists with reported EC_{50} values for transactivation of the three PPAR subtypes in micromolar.

gence of LBX001 from computer-aided drug design (Fig. 2).[16]

In order to discover PPARδ selective agonists on novel structural scaffolds, we performed a high throughput screen (HTS) on an unbiased library of ~1 million compounds based on a cell-based transactivation assay using a Gal4-PPARδ LBD chimeric construct. A number of hits sharing a common isoxazole motif such as compound **1** were identified (Fig. 3).

A typical PPAR pharmacophore consists of three parts, namely a head group (HG), a linker (L), and a tail group (TG).[8] The HG usually consists of a phenyl ring bearing a carboxylate (or surrogate) functionality, which enables the molecule to form the key hydrogen bonding interaction with the AF2 helix of the receptor and holds the receptor in an active conformation. A flexible linker L connects the HG to a hydrophobic TG (usually a heteroaromatic system), which fills the rest of the large and predominantly hydrophobic binding pocket.

The isoxazole hits from our HTS did not display a carboxylate moiety or a surrogate commonly seen in PPAR agonists (except for the *ortho* nitro group in the phenol substituent), which may explain their low efficacy in the

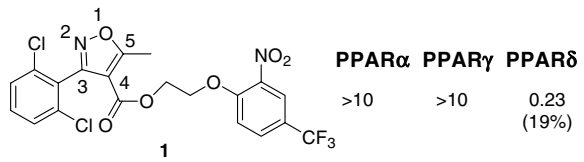
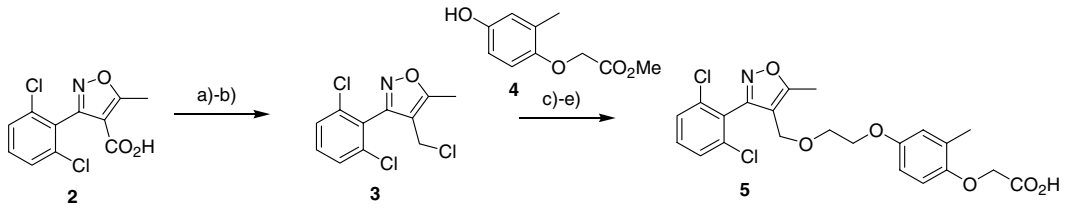


Figure 3. An isoxazole HTS hit from the PPAR screen with EC_{50} (μM) and efficacy values for PPAR transactivation.

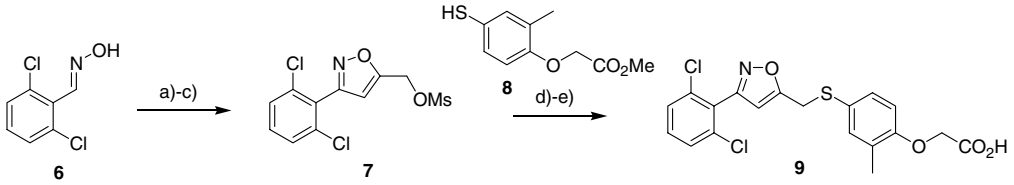
in vitro PPARδ transactivation assays (<20% efficacy compared to GW501516). To increase activity to a desired full agonist, we first tried to incorporate a carboxylate into the isoxazole scaffold. Based on a comparison of compound **1** with the above mentioned common agonist motifs and a recent communication of an isoxazole agonist for the FXR nuclear receptor,[22] we hypothesized that the *p*-CF_3-*o*-NO_2-phenyl group in position 4 of the isoxazole ring could be replaced by a HG typical for PPAR activation. The commercially available isoxazole **2** was reduced and chlorinated to intermediate **3**, the chloride was then displaced in neat ethylene glycol (Scheme 1). Mitsunobu coupling with the HG intermediate **4** followed by saponification gave compound **5**. Surprisingly, neither compound **5** nor any other analogs displaying various L and HG in position 4 showed activity for the PPARs.

Next, we rationally designed compound **9** from a superposition of compound **1** with GW501516, which places the HG in position 5 of the isoxazole ring (Scheme 2). The oxime **6** was in situ transformed into the nitrile oxide by NCS chlorination and regioselectively cyclized with propargyl alcohol. The hydroxyl functionality was mesylated to yield isoxazole **7**. Substitution of intermediate **8** with HG **4** and saponification of the corresponding methyl ester gave isoxazole **9**. Again, compound **9** was inactive against all three PPAR subtypes.

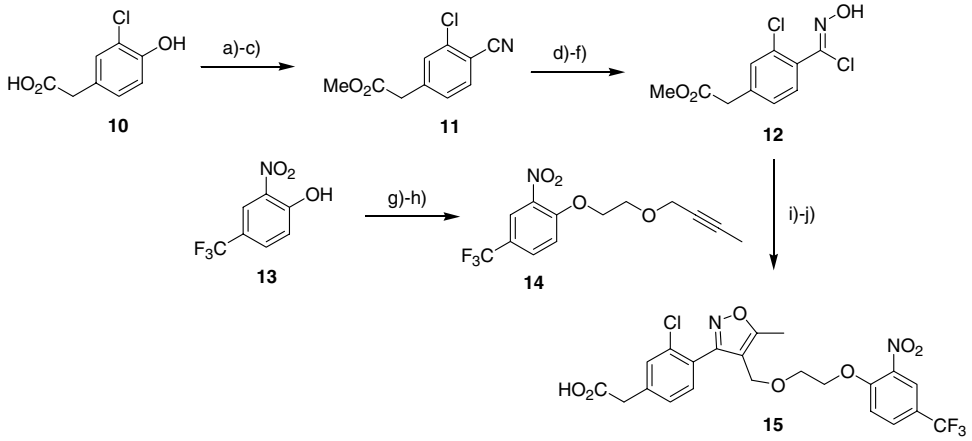
Finally, the docking results of compound **1** with the structure of the PPARδ ligand-binding domain co-crystallized with GW2433[23] revealed best scores when substituting the aryl ring in position 3 of the isoxazole with a carboxylate bearing HG. Starting from 3-chloro-4-hydroxy-phenylacetic acid **10** esterification, triflation, and palladium-mediated cyanide displacement gave intermediate **11** (Scheme 3). The cyanide of intermediate **11** was reduced to the aldehyde with Raney-Nickel alloy, reacted to the oxime with hydroxylamine, and activated to the chloroxime **12** by NCS chlorination. The phenol of intermediate **13** was alkylated with excess 1,2-dibromoethane, then reacted with 2-butyne-1-ol to give alkyne **14**. Cyclization of intermediates **12** and **14** under basic conditions gave a mixture of two regioisomeric isoxazoles, which could be chromatographically separated. Saponification of the regioisomer with the methyl group in position 5 gave the isoxazole **15**. Compound **15** displayed improved potency and efficacy for activation of PPARδ, but was also active on the PPARα subtype (Table 1). The other regioisomer was inactive (data not shown).



Scheme 1. Reagents and conditions: (a) LAH, THF, rt, 12 h (92%); (b) $SOCl_2$, DCM, rt, 1 h (91%); (c) ethyleneglycol, NaH, 80 °C, 4 h (65%); (d) PPh_3, DEAD, DCM, rt, 5 h (70%); (e) 1 N LiOH, dioxane, 60 °C, 5 h (95%).

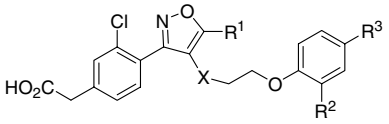


Scheme 2. Reagents and conditions: (a) NCS, DMF, rt, 1 h; (b) propargyl alcohol, NEt_3, DCM, rt, 5 h (90%); (c) MsCl, NEt_3, DCM, 0 °C, 30 min (85%); (d) Cs_2CO_3, CH_3CN, rt, 18 h (95%); (e) 1 N LiOH, THF, rt, 12 h (95%).



Scheme 3. Reagents and conditions: (a) MeOH, H_2SO_4, reflux, 8 h, (quant.); (b) Tf_2O, NEt_3, DCM, 0 °C, 1 h (95%); (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, 30 h (70%); (d) Raney–Nickel alloy, HCO_2H, 110 °C, 10 h (55%); (e) $H_2NOH{\cdot}HCl$, Na_2CO_3, H_2O, rt, 2 h, (90%); (f) NCS, DMF, HCl (g), rt, 30 min; (g) 1,2-dibromoethane, Cs_2CO_3, CH_3CN, 90 °C, 1 h (75%); (h) 2-butyne-1-ol, 4 M NaOH, μW (150 °C, 3 min) (30%) (i) NEt_3, DCE, 90 °C, 6 h (60%); (j) 1 N LiOH, THF, 60 °C, 36 h (95%).

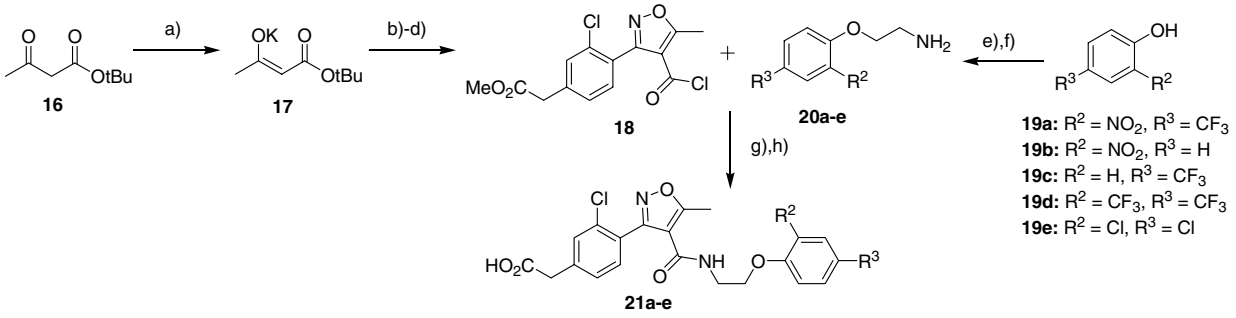
Table 1. In vitro activities for PPARs (subtypes α, γ, and δ) in cell-based transactivation assays (GAL4-PPAR LBD)



Compound	X	R^1	R^2	R^3	α (μM)	γ (μM)	δ (μM)	%eff
15	CH_2O	Me	NO_2	CF_3	0.22	>10	0.13	48
21a	CONH	Me	NO_2	CF_3	0.34	1.25	0.004	67
21b	CONH	Me	H	CF_3	>10	>10	>10	—
21c	CONH	Me	NO_2	H	>10	>10	>10	—
21d	CONH	Me	CF_3	CF_3	>10	>10	0.26	43
21e	CONH	Me	Cl	Cl	>10	>10	0.31	63
24a	CONH	Et	Cl	Cl	>10	>10	0.11	71
24b	CONH	*i*-Pr	Cl	Cl	7.11	>10	0.09	67
24c	CONH	*t*-Bu	Cl	Cl	>10	>10	2.59	26
24d	CONH	cyPr	Cl	Cl	1.67	3.23	0.03	68
24e	CONH	2Fur	Cl	Cl	>10	>10	0.01	72
24f	CONH	Ph	Cl	Cl	>10	>10	0.08	66
25	CONMe	Ph	Cl	Cl	>10	>10	3.67	19
26	CH_2O	Ph	Cl	Cl	>10	>10	0.19	44
29a	$COCH_2$	Ph	Cl	Cl	>10	>10	0.03	80
GW501516					1.23	>10	0.003	100

Efficacies are reported relative to GW501516 (%eff = 100%).

After identification of the HG location in position 3 of the isoxazole core, we turned our attention to the substituent in position 4. In order to investigate different substituents in position 4, we replaced the ether with an amide functionality. The synthesis shown in Scheme 4 gave not only better access to various analogs, but also implemented a higher yielding, regioselective cyclization step. *t*-Butyl acetoacetate **16** was deprotonated

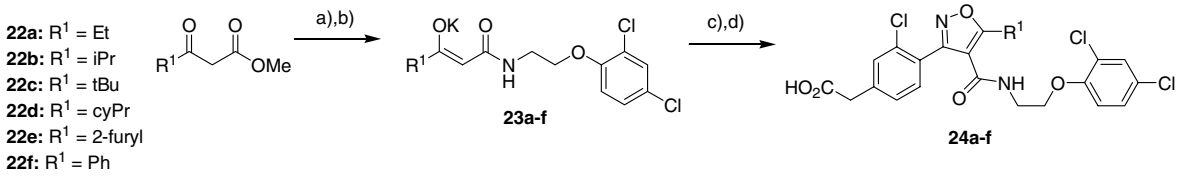


Scheme 4. Reagents and conditions: (a) $KN(TMS)_2$, Et_2O, rt, 1 h; (b) **12**, CH_3CN, 0 °C, 3 h (85%); (c) TFA, rt, 4 h (quant.); (d) $SOCl_2$, toluene, reflux, 3 h (90%); (e) $BrCH_2CH_2NHBoc$, Cs_2CO_3, CH_3CN, 90 °C, 6 h, (50–90%); (f) DCM/TFA 95:5, rt, 1 h (quant.); (g) NEt_3, DCM, rt, 30 min (60%); (h) 1 N LiOH, THF, 60 °C, rt (95%).

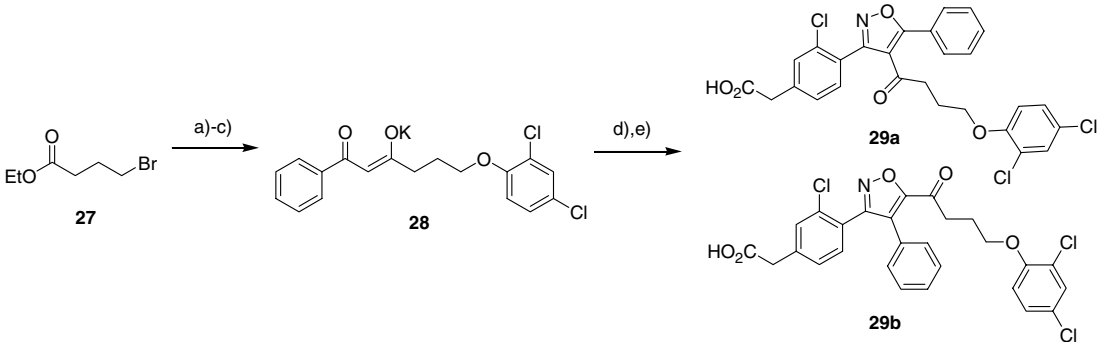
to intermediate **17** using potassium bis(trimethylsilyl)amide. Intermediate **12** was in situ transformed into the nitrile oxide with triethylamine, then cyclized with enolate **17** at low temperature to give selectively the correct isoxazole regioisomer. Selective saponification of the *t*-butyl protected ester in TFA and activation of the carboxylate to the acid chloride intermediate **18** was followed by amide formation with the corresponding amines such as **20a**–**e**. Saponification of the head group methyl ester gave final products **21a**–**e**.

Switching from the ether of compound **15** to the amide of compound **21a** is not only tolerated, but also improves both potency (4 nM) and selectivity for PPARδ activation (Table 1). We quickly realized that many amines employed as part of a first round broad survey in the amide formation gave inactive compounds (data not shown). The narrowness of the SAR in position 4 was best exemplified by the fact that omitting either the nitro (**21b**) or the trifluoromethyl group (**21c**) in otherwise identical molecules compared to compound **21a** led to complete loss of activity. Since a nitro group presented a potential toxicological liability, we continued to search for a replacement. A 2,4-bistrifluoromethylphenyl (**21d**) or a 2,4-dichlorophenyl group (**21e**) were the only replacements that maintained some PPARδ activity, although it dropped by a factor of 100.

Last, we examined different substituents in position 5 of the isoxazole ring. We found that analogs were best accessible by reacting the appropriate β-ketoester with amine **20e** to give amides **23a**–**e** (Scheme 5), which were then cyclized and saponified to compounds **24a**–**e** in a similar fashion to earlier described procedures. The SAR in this part of the molecule turned out to be rather broad, with aromatic rings such as a 2-furanyl (compound **24e**) or a phenyl (compound **24f**) displaying best activity and selectivity. Other heteroaromatic systems or introducing substituents into the phenyl ring did not lead to any further improvement (data not shown).



Scheme 5. Reagents and conditions: (a) **20e**, toluene, μW (160 °C, 10 min) (40–60%); (b) $KN(TMS)_2$, Et_2O, rt, 1 h; (c) **12**, CH_3CN, rt, 3 h (60–80%); (d) 1 N LiOH, THF, rt, 12 h (95%).



Scheme 6. Reagents and conditions: (a) **19e**, Cs_2CO_3, CH_3CN, 50 °C, 7 h, (90%); (b) acetophenone, NaH, dioxane, reflux, 3 h; (c) $KN(TMS)_2$, CH_3CN, rt, 1 h; (d) **12**, CH_3CN, rt, 3 h (75%); (e) 1 N LiOH, THF, rt, 12 h (95%).

Methylation of the amide nitrogen (compound **25**; using MeI and NaH) or switching back to the ether linkage (compound **26**; synthetic procedure according to Scheme 3) resulted in a drastic reduction of activity, whereas a ketomethylene replacement of the amide (compound **29a**) slightly improved both potency and efficacy. Unfortunately, the depicted synthesis route to compound **29a** (Scheme 6) predominantly gave the undesired, inactive regioisomer **29b** (ratio **29a**/**29b** $\sim$ 1:7).

In summary, we were able to identify a novel isoxazole scaffold displaying PPAR activity based on hits derived from a high throughput screen. In an attempt to improve the activity to a full agonist profile, we designed and synthesized a number of analogs showing various degrees of potency, efficacy, and selectivity for the PPAR subtypes. Compounds **24e** and **29a** are potent and highly selective activators of the PPARδ receptor. Notably this series is different from the common PPAR agonist motifs reported in the literature. The compounds do not seem to require a flexible linker between functional head and hydrophobic tail, a unique feature distinct from most reported PPAR modulators. The isoxazoles presented may be used as tool compounds for further elucidation of the specific biological roles of PPARδ.

Acknowledgments

We gratefully acknowledge Drs. T.R. Vedananda, Sandra Teixeira, and Peter G. Schultz for their helpful discussions and support.

References and notes

1. Eckel, R. H.; Grundy, S. M.; Zimmet, P. Z. *Lancet* **2005**, *365*, 1415.
2. Roberts, W. C. *Am. J. Cardiol.* **2004**, *93*, 274.
3. Grundy, S. M.; Brewer, H. B., Jr.; Cleeman, J. I.; Smith, S. C., Jr.; Lenfant, C. *Circulation* **2004**, *109*, 433.
4. Haffner, S.; Cassells, H. B. *Diabetes Obes. Metab.* **2003**, 359.
5. Issemann, I.; Green, S. *Nature* **1990**, *347*, 645.
6. Lehmann, J. M.; Moore, L. B.; Simth-Oliver, T. A.; Wilkison, W. O.; Willson, T. M.; Kliewer, S. A. *J. Biol. Chem.* **1995**, *270*, 12953.
7. Berger, J. P.; Akiyama, T. E.; Meinke, P. T. *Trends Pharmacol. Sci.* **2005**, *26*, 244.
8. Cheng, P. T. W.; Mukherjee, R. *Mini-Rev. Med. Chem.* **2005**, *5*, 741.
9. Fernandez, A. Z. *Curr. Opin. Invest. Drugs* **2004**, *5*, 936.
10. Etgen, G. J.; Mantlo, N. *Curr. Top. Med. Chem.* **2003**, *3*, 1649.
11. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
12. Oliver, W. R., Jr.; Shenk, J. L.; Snaith, M. R.; Russell, C. S.; Plunket, K. D.; Bodkin, N. L.; Lewis, M. C.; Winegar, D. A.; Sznaidman, M. L.; Lambert, M. H.; Xu, H. E.; Sternbach, D. D.; Kliewer, S. A.; Hansen, B. C.; Willson, T. M. *Proc. Nat. Acad. Sci. U.S.A.* **2001**, *98*, 5306.
13. Sznaidman, M. L.; Haffner, C. D.; Maloney, P. R.; Fivush, A.; Chao, E.; Goreham, D.; Sierra, M. L.; LeGrumelec, C.; Xu, H. E.; Montana, V. G.; Lambert, M. H.; Willson, T. M.; Oliver, W. R.; Sternbach, D. D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1517.
14. Santini, C.; Berger, G. D.; Han, W.; Mosley, R.; MacNaul, K.; Berger, J.; Doebber, T.; Wu, M.; Moller, D. E.; Tolman, R. L.; Sahoo, S. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1277.
15. Weigand, S.; Bischoff, H.; Dittrich-Wengenroth, E.; Heckroth, H.; Lang, D.; Vaupel, A.; Woltering, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4619.
16. Epple, R.; Azimioara, M.; Russo, R.; Bursulaya, B.; Tian, S.-S.; Gerken, A.; Iskandar, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2969.
17. Wang, Y.-X.; Lee, C.-H.; Tiep, S.; Yu, R. T.; Ham, J.; Kang, H.; Evans, R. M. *Cell* **2003**, *113*, 159.
18. Wang, Y.-X.; Zhang, C.-L.; Yu, R. T.; Cho, H. K.; Nelson, M. C.; Bayuga-Ocampo, C. R.; Ham, J.; Kang, H.; Evans, R. M. *PLoS Biol.* **2004**, *2*, 1532.
19. Leibowitz, M. D.; Fievet, C.; Hennuyer, N.; Peinado-Onsurbe, J.; Duez, H.; Berger, J.; Cullinan, C. A.; Sparrow, C. P.; Baffic, J.; Berger, G. D.; Santini, C. J.; Marquis, R. W.; Tolman, R. L.; Smith, R. G.; Moller, D. E.; Auwerx, J. *FEBS Lett.* **2000**, *473*, 333.
20. Tanaka, T.; Yamamoto, J.; Iwasaki, S.; Asaba, H.; Hamura, H.; Ikeda, Y.; Watanabe, M.; Magoori, K.; Ioka, R. X.; Tachibana, K.; Watanabe, Y.; Uchiyama, Y.; Sumi, K.; Iguchi, H.; Ito, S.; Doi, T.; Hamakubo, T.; Naito, M.; Auwerx, J.; Yanagisawa, M.; Kodama, T.; Sakai, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 15924.
21. Graham, T. L.; Mookherjee, C.; Suckling, K. E.; Palmer, C. N. A.; Patel, L. *Atherosclerosis* **2005**, *181*, 29.
22. Maloney, P. R.; Parks, D. J.; Haffner, C. D.; Fivush, A. M.; Chandra, G.; Plunket, K. D.; Creech, K. L.; Moore, L. B.; Wilson, J. G.; Lewis, M. C.; Jones, S. A.; Willson, T. M. *J. Med. Chem.* **2000**, *43*, 2971.
23. Xu, H. E.; Lambert, M. H.; Montana, V. G.; Parks, D. J.; Blanchard, S. G.; Brown, P. J.; Sternbach, D. D.; Lehmann, J. M.; Wisely, G. B.; Willson, T. M.; Kliewer, S. A.; Milburn, M. V. *Mol. Cell* **1999**, *3*, 397.