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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1673–1678

Design and synthesis of potent and subtype-selective PPARα agonists

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Received 16 November 2005; revised 2 December 2005; accepted 2 December 2005
Available online 27 December 2005

Abstract—Beginning with a moderately potent PPARγ agonist **9**, a series of potent and highly subtype-selective PPARα agonists was identified through a systematic SAR study. Based on the results of the efficacy studies in the hamster and dog models of dyslipidemia and the desired pharmacokinetic data, the optimized compound **39** was selected for further profiling.

Coronary heart disease (CHD) is the leading cause of death in the US and much of the developed world. An estimated 13 million Americans have CHD. The direct and indirect costs for CHD in US are quite staggering approaching more than $130 billion per year.[1] Among the risk factors for CHD, two of the most significant ones are elevated LDL cholesterol and low HDL cholesterol. In addition, hypertriglyceridemia is also a likely contributor to CHD risk. The statin class of HMG-CoA reductase inhibitors (Zocor, Lipitor) and the fibrate class of antidyslipidemic drugs such as fenofibrate or clofibrate (Fig. 1) have found wide acceptance for the clinical management of dyscholesterolemia and dyslipidemia. Statins effectively lower serum LDL-c levels but their HDL-raising effect is marginal. Fibrates, on the other hand, are quite effective at lowering serum triglycerides, LDL-c and also raise HDL cholesterol levels. The triglycerides-lowering and HDL-raising effects of fibrates are attributed to the activation of PPARα receptors. This results in an increase in lipoprotein lipase gene expression and transrepression of apoC-III thereby increasing lipoprotein catabolism.[2]

The HDL-cholesterol elevation observed with fibrates arises in part from the up-regulation of apoA-I and apoA-II.[3] Though effective, for dyslipidemia, fibrates are weak PPARα agonists and their subtype selectivity is poor. Therefore, a potent and subtype-selective human PPARα agonist could offer a superior alternative for the management of dyslipidemia. The first reported examples of selective PPARα agonists were GW9578 **3**[4] and GW7647 **4**.[5] This was followed by publications from researchers at Kyorin **5**[6] and Lilly **6**.[7] The PPARα selectivity of compounds **3**–**6** ranged from 20- to 200-fold over human PPARγ and PPARδ receptors. Recently, researchers from these laboratories have reported examples of potent and highly subtype-selective PPARα agonists as exemplified with cyclic fibrates **7** and **8**.[8,9] We have earlier disclosed work in the PPARα/γ dual agonist area[10,11] and as an extension of that work described below are the results of our efforts in identifying potent and highly subtype-selective (>1000-fold) PPARα agonists.

With the objective of looking at the outcome of replacing a TZD headpiece on a moderately potent PPARγ agonist **9** with propionic acid as found in classical fibrates such as fenofibrate, we synthesized compound **10**. In the binding assay, this analog displayed affinity for all three human PPAR receptors.

Keyword: PPARα agonist.
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doi:10.1016/j.bmcl.2005.12.022

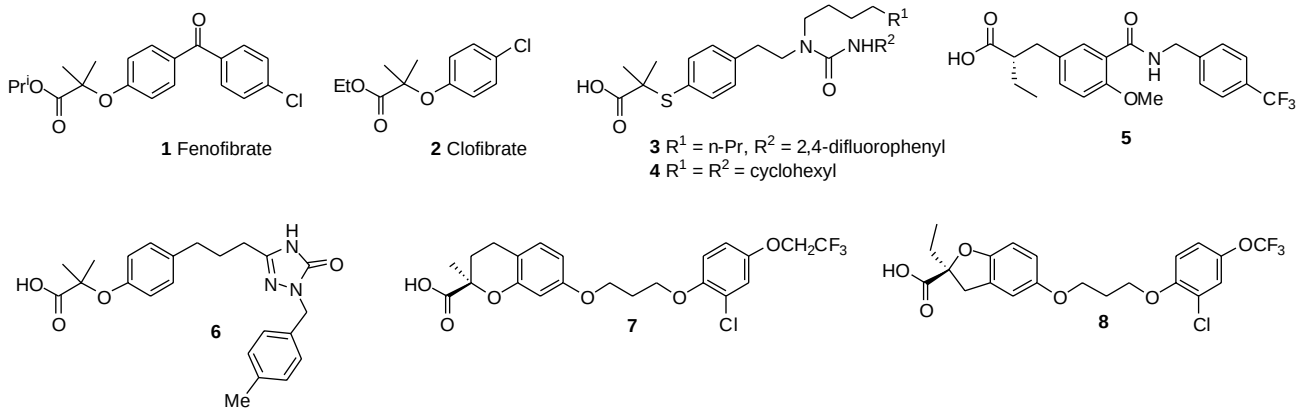


Figure 1. PPARα agonists.

Substituting the propyl chain in **10** with a chloro substituent as in compound **11** showed a 4-fold improvement in PPARα potency,[12] but more importantly, in the functional transactivation (TA) assay this analog displayed only PPARα activity (Fig. 2). Encouraged by these results, we initiated an SAR study to investigate the role of the methylene spacer and the orientation of the propionic acid side chain. As seen from the TA data in Figure 3, extending the methylene bridge by an extra unit results in PPARα,γ dual agonist **12**, whereas reducing the methylene spacer by one carbon (compound **13**) leaves it unchanged when compared to **11**. Similarly, the meta-oriented propionic acid analog **14** is indistinguishable from **11**, whereas the corresponding ortho-linked derivative **15** was devoid of useful activity. Considering the potential for the metabolic oxidation at the para carbon atom in the meta-linked analog **14**, we elected to pursue only the para-linked series. Next, we probed the effects of alkyl group substitutions on the propionic acid side chain to optimize the substituents (Table 1). In the case of mono-substituted analogs **16**–**20**, there was not much variance in binding activity. The Me/Et-substituted analog **21** was found to be the most potent analog displaying around 250-fold PPARα selectivity in the binding assay. The diethyl-substituted analog **22** was essentially indistinguishable from **21**. However, due to synthetic ease in making **21**, we pursued only this substitution pattern for further SAR. Having established the optimum substituents, the next endeavor was to separate the enantiomers and determine if there was a difference in the activity. This was conveniently achieved by a chiral resolution of the starting phenol **46** using Chiracel OJ column and converting the enantiomers to the final targets **24** and **25**. The absolute

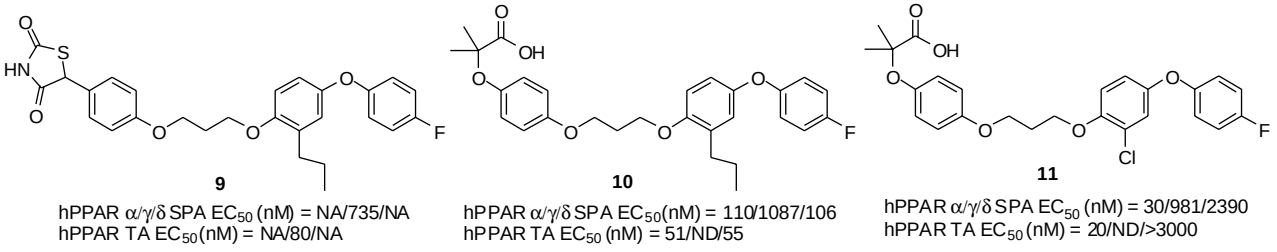


Figure 2. Lead development.

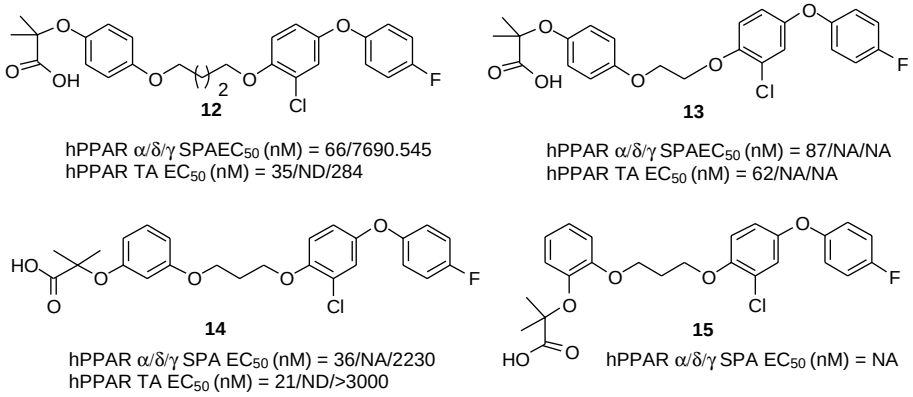
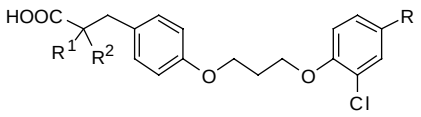


Figure 3. Role of methylene tether and orientation of acid side chain.

Table 1. In vitro human PPAR activities of compounds **16–25**



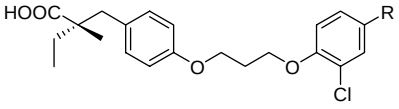
Compound	R^1, R^2	Binding IC_{50} (nM)[17]			Transactivation EC_{50} (nM)[16]		
		α	δ	γ	α	δ	γ
16	H, H	675	>50	6440			
17	H, Me	208	4070	NA			
18	H, Et	156	1170	NA			
19	H, Pr	130	1130	NA	454		
20	H, Ph	262	1340	NA			
11	Me, Me	30	981	2390	20	ND	>3000
21	Me, Et	5	NA	1240	2.5	ND	NA
22	Et, Et	22	NA	NA	2.7	ND	NA
23	Cyclobutyl	116	1350	NA	15	ND	NA
24	Me, Et *R*	6	1090	NA	3	ND	NA
25	Me, Et *S*	101	2414	NA	20	ND	>3000

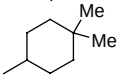
NA, not active; ND, not determined.

configuration of the slower eluting isomer was determined to be (*S*) based on the X-ray crystallographic data on the corresponding (2*R*)-2-phenyloxazoline amide derivative (Scheme 2). As seen from the data, the (*R*) enantiomer **24** is around 7-fold more potent that the corresponding (*S*) isomer **25**.

Having established the optimum substituents on the propionic acid side chain as well as identifying the desired (*R*) enantiomer as the preferred isomer, the stage was now set for the further expansion of SAR on the eastern phenols. Earlier, we had identified the 4 position as a suitable site for substituent introduction. Accordingly, a variety of substituents were introduced at the 4 position and as seen from the data in Table 2, with the exception of compounds **26–27** and **30** which showed PPARα/δ agonism in the binding assay, all other analogs displayed potent PPARα activity and an excellent

Table 2. In vitro human PPAR activities of compounds **26–33**



Compound	R	Binding IC_{50} (nM)[17]			Transactivation EC_{50} (nM)[16]		
		α	δ	γ	α	δ	γ
26	H	42	4630	851	54.1	ND	NA
27	OCF_3	3	742	NA	2.1	ND	NA
28	OCH_2CF_3	10	NA	NA	7.8	ND	NA
29	OSO_2Me	30	NA	NA	11.4	ND	NA
30	CF_3	3	603	NA	2.9	ND	NA
31	CH_2CF_3	<1	>70000	>6000	<1	ND	NA
32		2	NA	1851	1	ND	>3000
33		22	NA	3610	27.5	ND	>3000

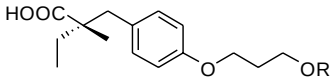
NA, not active; ND, not determined.

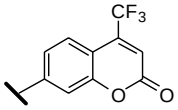
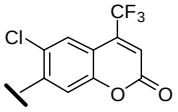
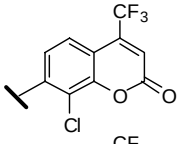
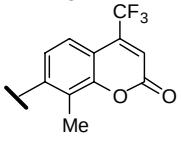
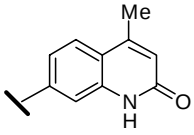
subtype selectivity. In particular, the trifluoromethylethyl containing analog **31** displayed subnanomolar binding affinity for the PPARα receptor and >5000-fold subtype selectivity making it perhaps the *most* potent agonist reported to date. Unfortunately, **31** was also found to be a potent inhibitor of Cyp2C9 (IC_{50} < 0.05 μM), thus precluding it from further evaluation. In general, all compounds in Table 2 suffered to a varying degree from P450 enzyme inhibition issues making it necessary to explore different phenolic ring systems as coupling partners with the western phenols containing 1,3-propylidene linker.

Toward that end, drawing from our earlier experience with PPARγ agonists,[13] we first synthesized compound **34**, a trifluoromethyl-substituted coumarin derivative (Table 3). Though not as potent as some of the analogs described earlier, **34** nonetheless maintained the PPARα subtype selectivity. Introduction of the chloro substituent at the 6 position (**35**) improved potency almost 20-fold furnishing a 28 nM PPARα-selective agonist. Interestingly, moving chlorine to 8 position as in **36** caused an almost 6-fold drop in the PPARα potency and also introduced some binding activity for PPARδ when compared to **34**. Unlike coumarin derivatives **34**, the corresponding lactam analog **38** was found to be inactive at the PPAR receptors, thus highlighting the need for the oxygen atom for PPAR activity. Based on the superior in vitro potency, compound **35** was selected for in vivo evaluation of hypolipidemic efficacy. As shown in Table 5, oral administration of compound **35** at a dose of 1 mpk for 9 days lowered serum triglycerides and cholesterol by 35% and 41%, respectively. For comparison, fenofibrate achieved 30% and 31% reduction of triglycerides and cholesterol at a much higher dose of 100 mpk. Compound **35** was also evaluated in a dog model to assess its lipid-lowering profile. The dog model was chosen because dogs exhibit strong lipid lowering in response to fibrates and statins, and have been used as the principal preclinical species during the development of simvastatin (Zocor).[12,13] Compound **35** was orally dosed at 1 mpk for 14 days. For comparison purposes, in this study simvastatin and fenofibrate were dosed po at 4 and 50 mpk, respectively. As seen from the data in Table 6, average serum cholesterol was reduced by 22%, whereas simvastatin showed an average decrease of 19%. Interestingly, an additive effect of cholesterol lowering was observed when **35** was co-dosed with simvastatin. A combined dose of **35** at 3 mpk and simvastatin at 4 mpk lowered cholesterol by 32% which is greater than by either **35** or simvastatin at the corresponding dose (Table 6). Based on the results of superior in vivo efficacy in the two animal models, **35** was characterized in pharmacokinetic studies in three preclinical animal species (Table 7).

Overall, **35** exhibited low plasma clearance, good oral bioavailability, and systemic exposure across the species Encouraged by these results, we looked at the Pan Labs assay[18] for the off-target activity for **35**. No significant off-target activity was observed for compound **35**. Unfortunately, the results for the stability studies of **35** indicated the lactone ring stability issues. It was

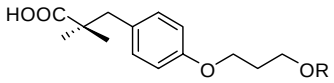
Table 3. In vitro human PPAR activities of compounds **34–38**

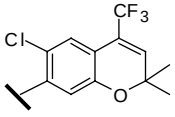
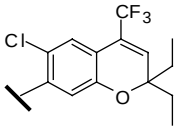
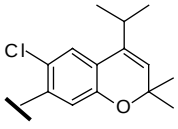
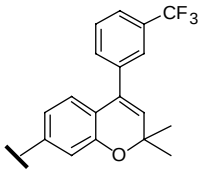


Compound	R	Binding IC_{50} (nM)[17]			Transactivation EC_{50} (nM)[16]		
		α	δ	γ	α	δ	γ
34		537	NA	NA	ND	ND	ND
35		28	NA	NA	44	ND	>3000
36		157	3270	NA	ND	ND	ND
37		49	134	NA	10	ND	ND
38		6090	NA	NA	ND	ND	ND

NA, not active; ND, not determined.

Table 4. In vitro human PPAR activities of compounds **39–42**



Compound	R	Binding IC_{50} (nM)[17]			Transactivation EC_{50} (nM)[16]		
		α	δ	γ	α	δ	γ
39		20	NA	NA	7.3	ND	NA
40		125	NA	NA	ND	ND	ND
41		19	NA	1845	ND	ND	ND
42		NA	NA	NA	10	ND	ND

NA, not active; ND, not determined.

Table 5. In vivo efficacy of compounds **35** and **39** on serum cholesterol and triglycerides in hamster[14]

Compound	Dose (mpk)	Cholesterol (%)	Triglyceride (%)
35	1	−41 ± 5	−35 ± 6
39	0.3	−21 ± 3	−14 ± 6
Fenofibrate	100	−31 ± 3	−30 ± 4

Table 6. Cholesterol lowering by compounds **35**, **39**, fenofibrate, and simvastatin in male beagle dogs[15]

Compound	Dose (mpk)	Cholesterol (%)
35	1	−22 ± 4
39	0.3	−19 ± 3
Fenofibrate	50	−18 ± 4
Simvastatin	4	−19 ± 2
35 + simvastatin	3 + 4	−32 ± 2
39 + simvastatin	0.3 + 4	−26 ± 5

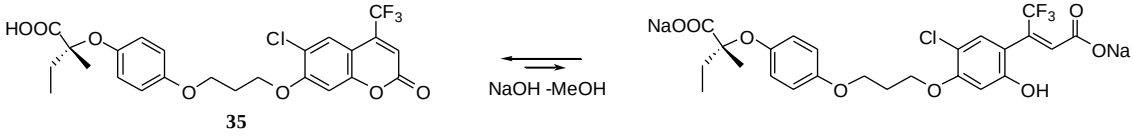
Table 7. Pharmacokinetic data for compounds **35** and **39**

Compound	Species	Cl (mL min^{-1} kg^{-1})	AUC (po) (μM h/mL)	$t_{1/2}$ (h)	F (%)
35	Rat	1.6	17	2.3	84
	Dog	1.45	30.6	3.6	99
	Monkey	9.45	3.95	2.5	39
39	Rat	2.3	25.4	2.4	94
	Dog	1.5	8.7	7.7	35
	Monkey	7.8	3.95	5.2	24

Doses used were 0.5 mg/kg, iv and 2 mg/kg, po (n = 3, except monkey, where, n = 2).

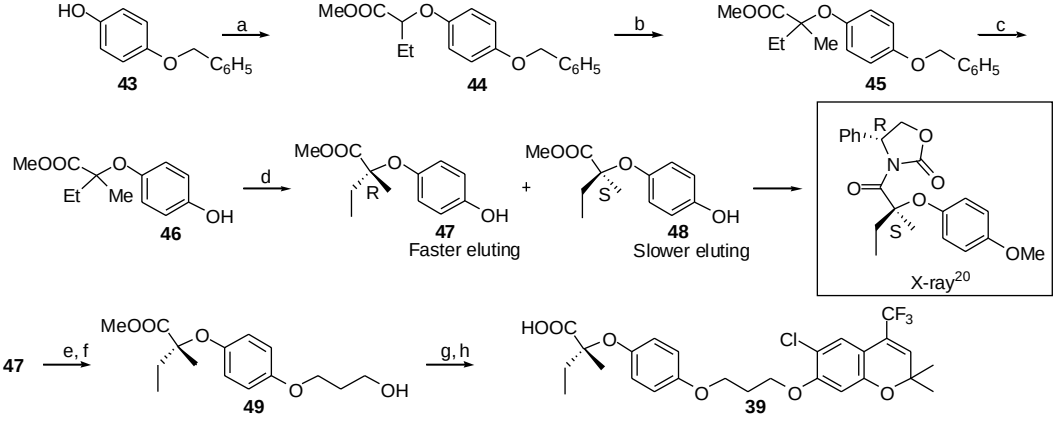
observed that at pH 7.5, **35** exists as an equilibrium mixture of the closed and the open forms (Scheme 1). This posed a significant developmental problem. To address the instability issue, we transformed the lactone carbonyl into a gem-dialkyl-substituted derivative. **39** (Table 4). To our delight, **39** not only retained the potent PPARα-binding activity but also showed 6-fold potency improvement in the functional TA assay. The corresponding gem-diethyl analog **40** gave up some potency, whereas analog **41** displayed binding activity at the PPARα and PPARδ receptors. In the in vivo efficacy studies, **39** showed robust lowering of serum triglycerides and cholesterol in the two animal models, hamster and dog, at a dose of 0.3 mpk. Also, like its predecessor **35**, compound **39** showed an additive cholesterol-lowering effect when co-administered with simvastatin (Table 6). Compound **39** was also characterized in pharmacokinetic studies in three preclinical animal species and exhibited low plasma clearance, good oral bioavailability and systemic exposure across the species (Table 7). No significant off-target activity was observed for **39** in the Pan Labs counterscreens.

The chemistry used in the preparation of analogs **11**–**42** is illustrated with the synthesis of compound **39** (Scheme 2). Commercially available 4-benzyloxyphenol was first alkylated with methyl 2-bromobutyrate to give **44** which on alkylation with MeI followed by removal of the benzyl-protecting group provided the bis alkylated derivative **46**. Chiral resolution of **46** using Chiracel OJ column furnished enantiomers **47** and **48**. Alkylation of slower eluting *S* enantiomer with benzyl 3-bromopropylbromide followed by hydrogenolysis gave the desired alcohol derivative

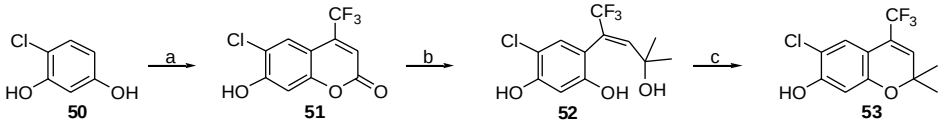


35

Scheme 1. Lactone ring stability of compound **35**.



Scheme 2. Reagents: (a) $COOMeCHBrCH_2CH_3$, Cs_2CO_3, CH_3CN; (b) LDA, MeI, THF, −78 °C; (c) Pd/C, H_2; (d) Chiracel OJ; (e) $Br(CH_2)_3OBn$, Cs_2CO_3, DMF; (f) Pd/C, H_2; (g) $(C_6H_5)_3P$, EtOOCN=NCOOEt, **53**, THF; (h) NaOH–MeOH. See above mentioned reference for further information.



Scheme 3. Reagents: (a) CF_3COCH_2COOMe, H_2SO_4; (b) MeMgBr; (c) pTSA.

49. Mitsunobu reaction of **49** with phenol **53** followed by hydrolysis of the methyl ester furnished the final target **39**. The preparation of phenol **53** is described in Scheme 3.[19]

Condensation of 4-chlororesorcinol with methyl trifluoroacetoacetate in the presence of sulfuric acid gave coumarin **51**, which on treatment with excess methyl magnesium bromide gave compound **52**. pTSA catalyzed ring closure of **52** furnished phenol **53**.

In summary, starting with a weak PPARγ lead, we have developed through a systematic SAR studies a series of potent and subtype-selective PPARα agonists. Based on its excellent in vivo efficacy as well as the desirable pharmacokinetic data, compound **39** was selected for further evaluation. Finally, the additive cholesterol-lowering effect seen on co-administering **39** with simvastatin in the dog model offers an interesting possibility of combining a potent PPARα agonist with statins to more effectively manage the lipid profile in high risk patients with chronic heart disease.

Acknowledgments

We thank Neelam Sharma, Marc Ippolito, Frank Xiaodong Gan, Melba Hernandez, Ying Li, and Raul Alvaro for the technical support. We also thank Nancy Tsou and Richard G. Ball for X-ray crystallography data. Thanks are also due to Dr. Timothy Blizzard for help with the manuscript preparation.

References and notes

1. Anum, E. A.; Adera, T. *Ann. Epidemiol.* **2004**, *14*, 705.
2. Staels, B.; Auwerx, J. *Curr. Pharm. Des.* **1997**, *3*, 1.
3. Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J. C. *Circulation* **1998**, *89*, 2088.
4. Brown, P. J.; Winegar, D. A.; Plunket, K. D.; Moore, L. B.; Lewis, M. C.; Wilson, J. G.; Sundseth, S. S.; Koble, C. S.; Wu, Z.; Chapman, J. M.; Kliewer, S. A.; Wilson, T. M. *J. Med. Chem.* **1999**, *423*, 3785.
5. Brown, P. J.; Stuart, L. W.; Hurley, K. P.; Lewis, M. C.; Winegar, D. A.; Wilson, J. G.; Wilkinson, W. O.; Ittoop, O. R.; Wilson, T. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1225.
6. Nomura, M.; Tanase, T.; Ide, T.; Tsunoda, M.; Suzuki, M.; Uchiki, H.; Murakami, K.; Miyachi, H. *J. Med. Chem.* **2003**, *46*, 3581.
7. Xu, Y.; Mayhugh, D.; Saeed, A.; Wang, X.; Thompson, R. C.; Dominianni, S. J.; Kauffman, R. F.; Singh, J.; Bean, J. S.; Bensch, W. R.; Barr, R. W.; Osborne, J.; Montrose-Rafizadeh, C.; Zink, R. W.; Yumibe, N. P.; Huang, N.; Luffer-Atlas, D.; Rungta, D.; Maise, D. E.; Mantlo, N. B. *J. Med. Chem.* **2003**, *46*, 5121.
8. Koyam, H.; Boueres, J. K.; Miller, D. A.; Berger, J. P.; MacNaul, K. L.; Wang, P.; Ippolito, M. C.; Wright, D. D.; Agrawal, A. K.; Moller, D. A.; Sahoo, S. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3347.
9. Shi, G. Q.; Dropinski, J. F.; Zhang, Y.; Santini, C.; Sahoo, S. P.; Berger, J. P.; MacNaul, K. L.; Zhou, G.; Agrawal, A.; Alvaro, R.; Cai, T.; Hernandez, M.; Wright, S. D.; Moller, D. E.; Heck, J. V.; Meinke, P. T. *J. Med. Chem.* **2005**, *48*, 5589.
10. Desai, R. C.; Han, W.; Metzger, E. J.; Bergman, J. P.; Gratale, D. F.; MacNaul, K. L.; Berger, J. P.; Doebber, T. W.; Leung, K.; Moller, D. E.; Heck, J. V.; Sahoo, S. P. *Bioorg. Med. Chem. Lett.* **2005**, *13*, 2795.
11. Desai, R. C.; Gratale, D. F.; Han, W.; Koyama, H.; Metzger, E. J.; Lombardo, V. K.; MacNaul, K. L.; Berger, J. P.; Doebber, T. W.; Leung, K.; Franklin, R.; Moller, D. E.; Heck, J. V.; Sahoo, S. P. *Bioorg. Med. Chem. Lett.* **2005**, *13*, 3541.
12. We have consistently noted in several cases 4- to 10-fold improvements in PPARα potency by replacing propyl chain with chloro substituent.
13. Unpublished results from these laboratories. The coumarin containing analogs **34**–**37** did not have cytochrome P450 enzyme inhibition issues.
14. Male Golden Syrian hamsters (120–150 g weight, $n = 10$) were fed normal rodent chow with free access to water and received once-a-day oral dosing of the sodium salts of the tested compounds by gavage with vehicle (0.5% methylcellulose) for 9 days.
15. Male beagle dogs (12–18 kg weight, $n = 5$) were fed a cholesterol-free chow diet ad libitum with free access to water. Test compounds were suspended in 0.5% methylcellulose and gavaged daily for 14 days. Mean values are shown. Data at the final day were $p < 0.05$ against vehicle control.
16. Berger, J. P.; Leibowitz, M. D.; Doebber, T. W.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C. A.; Hayes, N. S.; Li, Y.; Tanem, M.; Venture, J.; Wu, S. M.; 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.
17. Adams, A. D.; Yuen, W.; Hu, Z.; Santini, C.; Jones, B. A.; MacNaul, K. L.; Berger, J. P.; Doebber, T. B.; Moller, D. E. *Bioorg. Med. Chem. Lett.* **2003**, *12*, 931.
18. These assays were performed by MDS Pharma Services.
19. The preparation of compound **39** has been described: Desai, R. C.; Sahoo, S. P. WO Patent 2004/010992 A1.
20. CCDC 291597 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.