Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1517–1521

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Selective Small Molecule Agonists for Peroxisome Proliferator-Activated Receptor δ (PPARδ)—Synthesis and Biological Activity

Marcos L. Sznaidman,[a,†] Curt D. Haffner,[a] Patrick R. Maloney,[a] Adam Fivush,[a] Esther Chao,[a] Donna Goreham,[a] Michael L. Sierra,[b] Christelle LeGrumelec,[b] H. Eric Xu,[a] Valerie G. Montana,[a] Millard H. Lambert,[a] Timothy M. Willson,[a] William R. Oliver, Jr.[a] and Daniel D. Sternbach[a,*]

[a]*GlaxoSmithKline, Five Moore Drive, Research Triangle Park, NC 27709-3398, USA*
[b]*GlaxoSmithKline, Centre de Recherches, 25 Avenue du Québec, 91951 Les Ulis Cedex, France*

Received 7 January 2003; revised 14 February 2003; accepted 27 February 2003

Abstract—We report the synthesis and biological activity of a new series of small molecule agonists of the human Peroxisome Proliferator-Activated Receptor δ (PPARδ). Several hits were identified from our original libraries containing lipophilic carboxylic acids. Optimization of these hits by structure-guided design led to **7k** (GW501516) and **7l** (GW0742), which shows an EC_{50} of 1.1 nM against PPARδ with 1000-fold selectivity over the other human subtypes.

The Peroxisome Proliferator-Activated Receptors (PPARs) comprise a family of ligand-activated transcription factors belonging to the nuclear receptor gene superfamily.[1] Three mammalian PPAR subtypes, commonly known as PPARα, PPARγ and PPARδ were originally cloned as orphan receptors.[2–4] Each subtype contains a signature DNA binding domain (DBD) and a ligand-binding domain (LBD), both of which are necessary for ligand activated gene transcription. The PPARs function through heterodimers with the 9-*cis*-retinoic acid receptor (RXR) to regulate lipid and carbohydrate metabolism. The role of PPARα in the regulation of hepatic lipid metabolism was uncovered when it was shown to be the target receptor for the fibrate class of antihyperlipidemic drug molecules.[2] Likewise, the role of PPARγ in the regulation of insulin sensitivity was discovered when it was shown to function as the cellular receptor of the glitazone class of antidiabetic drugs.[5] In contrast, PPARδ does not appear to be a receptor for any known classes of drugs.[6] As such, the biological role of this subtype has remained elusive due, in part, to its broad tissue expression and the lack of good chemical tools with which to study its pharmacology. Thus, part of the challenge in determining the function of PPARδ has been the identification of potent and selective ligands for use as chemical tools.[7] It is important to note that all of the ligands published to date either have low affinity for PPARδ or lack selectivity over the other PPARs.[8–12]

To elucidate the function of PPARδ we set out to identify potent and selective ligands for this subtype using combinatorial chemistry and structure-based drug design.

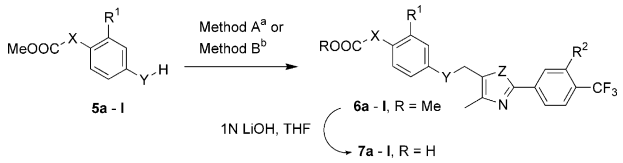
Chemistry

The general synthesis of compounds **7a–l** is depicted in Table 1. First, the corresponding phenoxy or thiophenoxy methyl ester (**5a–l**) was coupled to the appropriate chloromethyl (**4a–c**, Scheme 1) or hydroxymethyl (**3a–b**, Scheme 1) derivative by using either method A or B, respectively. Method A is the Williamson synthesis of an ether (or thioether) bond by displacement of the

*Corresponding author. Tel.: +1-919-483-1424; fax: +1-919-315-5668; e-mail: dds3687@gsk.com
†Current address: Norak Biosciences, PO Box 14769, Research Triangle Park, NC 27709-3398, USA

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00207-5

Table 1. General synthetic route[a,b]



Compd	X	R^1	R^2	Y	Z	Method	Overall yield (%)
7a	CH_2	H	H	O	S	A	38
7b	CH_2CH_2	H	H	O	S	B	17
7c	CH=CH	Me	H	O	S	A	25
7d	CH_2CH_2	Me	H	O	S	A	38
7e	CH_2CH_2	H	H	O	O	B	51
7f	—	H	H	O	O	A	50
7g	CH_2O	H	H	O	O	B	14
7h	CH_2O	Me	H	O	O	A	63
7i	CH_2O	H	H	S	O	A	10
7j	CH_2O	Me	H	S	O	A	57
7k GW501516	CH_2O	Me	H	S	S	A	52
7l GW0742	CH_2O	Me	F	S	S	A	50

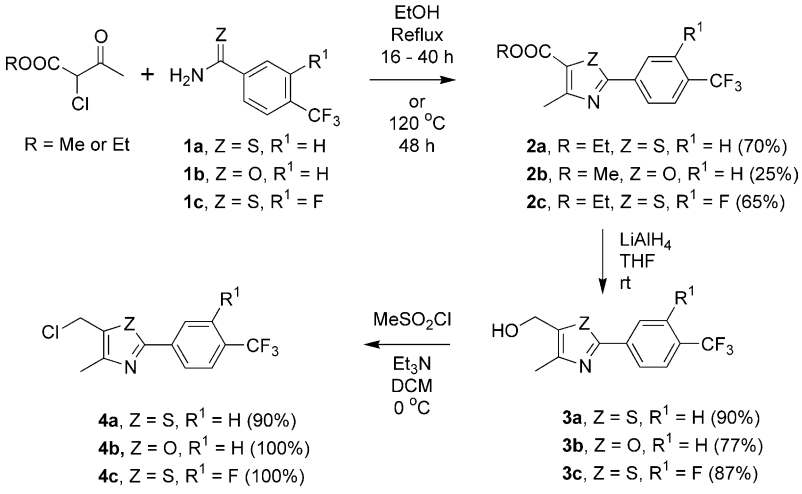
[a]Method A: Cs_2CO_3, CH_3CN, 16 h, rt.
[b]Method B: Ph_3P, DEAD, THF, 48 h, rt.

reactive chlorine of **4a–c** with a phenoxide (or thiophenoxide) salt and method B is the Mitsunobu condensation of an alcohol (**3a–b**) with an acidic phenol or thiophenol. The intermediate esters (**6a–l**) were then hydrolyzed to the corresponding carboxylic acids **7a–l**.

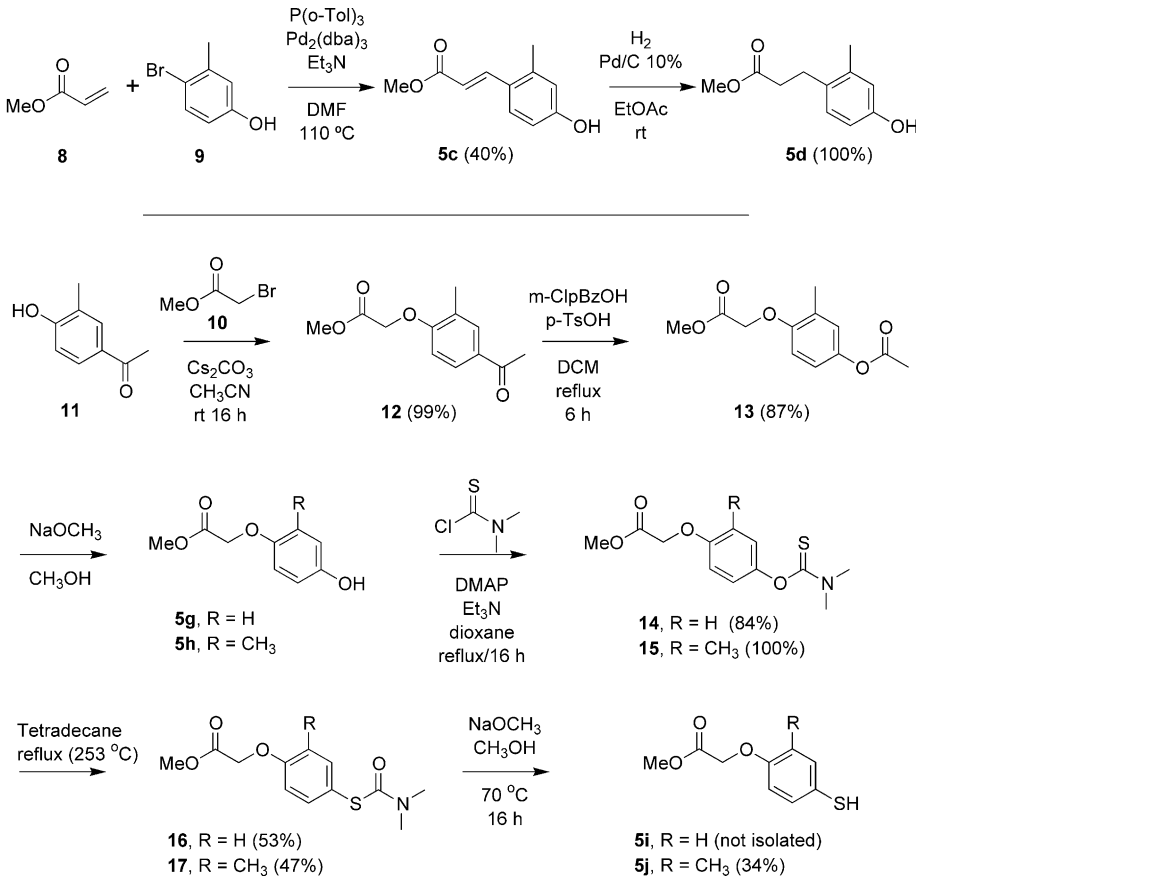
Synthesis of intermediates **3a–b** and **4a–c** is depicted in Scheme 1. The ethyl α-chloro-β-keto ester was condensed with the thioamide **1a** to form the substituted thiazole **2a**. Reduction with $LiAlH_4$, afforded the alcohol **3a**, which upon treatment with methansulfonyl chloride afforded the chloromethyl thiazole **4a**. In a similar fashion the oxazoles **3b** and **4b**, and thiazoles **3c** and **4c** were synthesized. Scheme 2 shows the synthesis of the non-commercially available phenoxy (**5c**, **5d** and **5h**) and thiophenoxy (**5i** and **5j**) esters. Compound **5c** was obtained by Heck coupling of methyl acrylate (**8**) and bromophenol **9**. Hydrogenation of the double bond of **5c** afforded compound **5d** in quantitative yield. Phenoxy ester **5h** was obtained in three steps. First, condensation of α-bromoacetic ester **10** with hydroxyacetophenone **11** afforded ester **12**. Baeyer–Villiger oxidation of the ketone to an ester afforded **13**, which after removal of the acetate group afforded the phenoxy ester **5h**. Thiophenoxy esters **5i** and **5j** were synthesized as follows: first, either phenoxyacid **5g** or **5h** was condensed with dimethylthiocarbamoyl chloride to afford the protected phenoxy esters **14** or **15**, respectively. Rearrangement at high temperatures afforded the protected thiophenoxy esters **16** or **17**, and final deprotection with sodium methoxide afforded the final thiophenoxy esters **5i** (not isolated, used in situ in the next step) and **5j**, respectively.

SAR Studies

Table 2 shows the EC_{50} for compounds **7a–l** in the transient transactivation assay against the three human PPAR subtypes: α, γ and δ; and binding IC_{50} against the human PPARδ receptor. Since the PPARs were known to bind lipophilic carboxylic acids[13] we decided to screen existing libraries with these structural properties.[14] As a result, several PPARδ agonists were discovered, compounds **7b** and **7c** being the most potent with single digit nM activity in the PPARδ cell based reporter assay. The selectivity versus PPARα and PPARγ in this assay was 35- and 80-fold respectively for **7b** and greater than 100-fold on both receptors for **7c**. While the binding data was in good agreement with the cell based reporter assay for **7b**, the cinnamic acid derivative (**7c**) showed an anomalous low binding affinity for PPARδ (IC_{50} = 1.0 μM, Table 2). The cocrystal structure of **7c** with the human PPARδ ligand-binding domain provided a possible explanation for the divergent assay results. Although **7c** was synthesized as the pure *E*-cinnamic acid, the X-ray structure (Fig. 1) showed that the double bind had isomerized to the Z-configuration during the crystallization process. Since **7b** is a propionic acid derivative it could readily assume the s-*cis* geometry needed for high affinity binding to PPARδ while **7c** could not. However, within the time course of the cell-based assay it is possible that **7c** could isomerize to the *Z*-isomer, which was observed in the



Scheme 1. Synthesis of intermediates **3a**, **3b**, **3c**, **4a**, **4b** and **4c**.



Scheme 2. Synthesis of phenoxy esters **5c**, **5d** and **5h**, and thiophenoxy esters **5i** and **5j**.

Table 2. Activity of compounds **7a–l** in cell based transactivation assay[a] and binding assay[b] against human PPAR receptors

Compd	Transactivation EC_{50} (μM)[a]			Binding IC_{50} (μM)[b]
	hPPARα	hPPARγ	HPPARδ	hPPARδ
7a	6.9±1.7	7.9±1.3	1.9±0.39	1.0±0.07
7b	3.0±1.5	7.04±3.6	0.09±0.02	0.03±0.002
7c	3.2±7.3	2.7±3.3	0.02±0.006	1.0±0.03
7d	1.7±0.17	4.2±2.2	0.03±0.03	0.06[c]
7e	0.28±0.089	3.3±1.9	0.06±0.02	0.09±0.003
7f	Inactive	Inactive	5.9±5.5	5.5±0.28
7g	Inactive	9.8±8.0	0.47±0.18	0.34±0.008
7h	3.2±1.7	Inactive	0.03±0.002	0.03±0.001
7i	Inactive	6.4±3.9	0.42±0.18	0.09±0.002
7j	2.5±1.6	1.61±2.6	0.01±0.001	0.003±0.0001
7k GW501516	1.1±0.15	0.85±0.29	0.001±0001	0.001±0.0001
7l GW0742	1.1±0.109	2.0±1.3	0.001±0002	0.001±0.00001

[a]EC_{50}, the concentration of test compound required to induce 50% of the maximum alkaline phosphatase activity (SEM).
[b]K_i, the concentration of test compound required to achieve an apparent K_i value according to the equation $K_i = IC_{50}/(1+[L]/K_d)$, where IC_{50} is the concentration of test compound required to inhibit 50% of the specific binding of the radioligand, [L] is the concentration of the radioligand used, and K_d is the dissociation constant for the radioligand at the receptor.
[c]Average of two measurements.

crystal structure. Further confirmation of this hypothesis resulted from the reduction of the double bond in **7c** to yield propionic acid **7d**, which had comparable activity to **7c** in the cell-based assay and importantly, showed 37-fold higher affinity than **7c** for PPARδ in the binding assay.

Changing the heterocycle in the propionic acid **7b** (thiazole) to an oxazole (cf. **7e** in Table 2) had little effect on PPARδ binding activity however the PPARδ selectivity versus PPARα in the cellular assay dropped from 80-fold to 5-fold. Pharmacokinetic measurements with **7e** in mice revealed that it had a very short half-life (<10 min) due to rapid and extensive metabolism. The inactive benzoic acid **7f** was identified (by mass spec) as the primary metabolite, presumably resulting from β-oxidative degradation of the propionic acid side chain. Thus, although compounds **7b**, **7c**, **7d** and **7e**

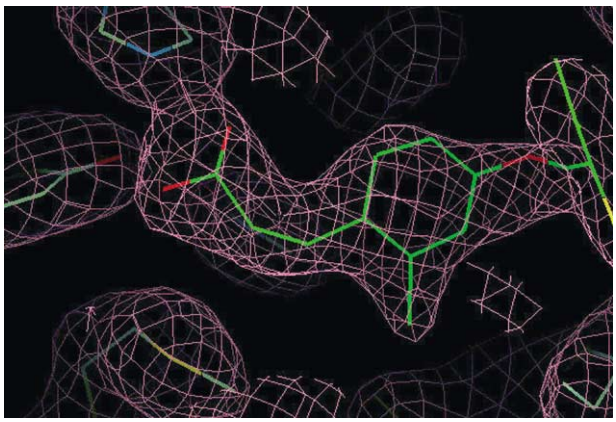


Figure 1. X-ray data showing the binding interactions of cinnamic acid **7c** to the hPPARδ receptor.

were potent PPARδ agonists in vitro, they were unsuitable for use as chemical tools in vivo.

The phenyl acetic acid derivative **7a** is unable to undergo β-oxidation but its weak PPARδ activity ($EC_{50}=1.77$ μM) and the fact that crystallographic studies[13] indicated that a two-atom spacer between the carboxylic acid and the aryl ring would be preferred led us to explore other alternatives. Replacement of the propionic side chain of **7e** with an isosteric oxyacetic acid side chain in **7g** would also serve to block β-oxidation. Unfortunately, **7g** was significantly less potent on PPARδ (0.47 μM) and showed no selectivity over PPARγ.

Early modeling studies,[15] later confirmed by crystallographic studies,[13] showed that there is a lipophilic pocket in the PPARδ ligand-binding domain that could accommodate small substituents at the *ortho* position of the aromatic ring. The enhancement of PPARδ activity by methyl substitution at the *ortho* position can be seen by comparing **7d** with **7b** (3-fold enhancement) and **7h** with **7g** (15-fold enhancement). Comparable effects were observed in the binding assay. Notably, the selectivity versus PPARα and PPARγ remained high.

Replacement of the oxygen closest to the oxazole ring with sulfur, led to compound **7i** which showed similar cellular PPARδ activity (PPARδ $EC_{50}=0.42$ μM) and better selectivity against PPARγ and PPARα when compared to **7g** (PPARδ $EC_{50}=0.47$ μM). Combining both of these modifications led to compound **7j**, which showed a dramatic increase in PPARδ activity ($EC_{50}=0.006$ μM) and selectivity (~400-fold against PPARα and 250-fold against PPARγ). Replacing oxazole with thiazole (compound **7k**) showed further improvement in PPARδ activity ($EC_{50}=0.001$ μM) as well as selectivity (1100 against PPARα, and 800 against PPARγ). Compound **7k** also showed excellent selectivity on the murine receptors ($EC_{50}=2.5$, 1.0, 0.02 μM for PPARα, PPARγ, and PPARδ, respectively) and against other nuclear or non-nuclear receptors.[16] Finally compound **7l** demonstrated that addition of a fluorine *ortho* to the CF_3 group, maintained potency and selectivity on the human and murine receptors (murine PPAR $EC_{50}=8.8$, >10, 0.03, PPARα, PPARγ, and PPARδ, respectively).

When dosed to insulin-resistant middle-aged rhesus monkeys, **7k** causes a dramatic dose-dependent rise in serum HDLc while lowering the levels of LDLc, fasting triglycerides, and fasting insulin.[16] At the cellular level, compound **7k** increased expression of the reverse cholesterol transporter ATP-binding cassette A1 (ABCA1) and induced apolipoprotein A1-specific cholesterol efflux.

Conclusions

We were able to identify the first truly selective PPARδ agonists, GW501516 (**7k**) and GW0742 (**7l**), starting with high-throughput screening, then using combinatorial chemistry to develop small focused libraries, and finally using structure guided lead optimization. These compounds will be valuable tools for determining the biological activity of the PPARδ receptor and as potential therapeutic agents for the treatment of diseases associated with raised serum triglycerides and low levels of HDLc.

Acknowledgements

We gratefully acknowledge Kim Adkison, Kelli Plunket, Raymond Merrihew and Lisa Leesnitzer, for their help in pharmacokinetics, transient transfection and binding determinations.

References and Notes

1. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
2. Issemann, I.; Green, S. *Nature* **1990**, *347*, 645.
3. Kliewer, S. A.; Forman, B. M.; Blumberg, B.; Ong, E. S.; Borgmeyer, U.; Mangelsdorf, D. J.; Umesono, K.; Evans, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7355.
4. Schmidt, A.; Endo, N.; Rutledge, S. J.; Vogel, R.; Shinar, D.; Rodan, G. A. *Mol. Endocrinol.* **1992**, *6*, 1634.
5. Lehmann, J. M.; Moore, L. B.; Smith-Oliver, T. A.; Wilkison, W. O.; Willson, T. M.; Kliewer, S. A. *J. Biol. Chem.* **1995**, *270*, 12953.
6. Willson, T. M.; Wahli, W. *Curr. Opin. Chem. Biol.* **1997**, *1*, 235.
7. Kliewer, S. A.; Lehmann, J. M.; Willson, T. M. *Science* **1999**, *284*, 757.
8. Brown, P. J.; Smith-Oliver, T. A.; Charifson, P. S.; Tomkinson, N. C. O.; Fivush, A. M.; Sternbach, D. D.; Wade, L. E.; Orband-Miller, L.; Parks, D. J.; Blanchard, S. G.; Kliewer, S. A.; Lehmann, J. M.; Willson, T. M. *Chem. Biol.* **1997**, *4*, 909.
9. Berger, J.; Leibowitz, 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.
10. Johnson, T. E.; Holloway, M. K.; Vogel, R.; Rutledge, S. J.; Perkins, J. J.; Rodan, G. A.; Schmidt, A. *J. Steroid Biochem. Mol. Biol.* **1997**, *63*, 1.

11. Brooks, C. D. W.; Summers, J. B. *J. Med. Chem.* **1996**, *39*, 2629.
12. Vosper, H.; Patel, L.; Graham, T. L.; Khoudoli, G. A.; Hill, A.; Macphee, C. H.; Pinto, I.; Smith, S. A.; Suckling, K. E.; Wolf, C. R.; Palmer, C. N. A. *J. Biol. Chem.* **2001**, *276*, 44258.
13. 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.
14. 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.
15. Lambert, M. H., Unpublished data.
16. 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. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5306.