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Studies towards the conception of new selective PPARβ/δ ligands

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Abstract—In order to define new PPARβ/δ ligands, SAR study on the selective PPARβ/δ activator L-165,041 led to the identification of one key functional group for selective PPARβ/δ activation. Furthermore, taking advantage of SAR studies done elsewhere on the most selective PPARβ/δ ligand GW501516, the conception of new ligands showing good affinity for PPARβ/δ is reported. Finally, synthesis and biological evaluation of pyridine analogues have shown the benefical effect of the pyridine ring on the PPARβ/δ subtype selectivity.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptors, which include steroid, thyroid or retinoid receptors.[1] These receptors are ligand-dependent transcription factors. Upon ligand binding, the activated PPARs heterodimerize with another nuclear receptor, the 9-*cis*-retinoic acid receptor (RXR), and alter the transcription of the target genes after binding to specific peroxisome proliferator response elements (PPREs).[2] Three subtypes of PPARs, termed PPARα, PPARδ (also known as PPARβ) and PPARγ, have been identified so far in various species, including humans.[3] Whereas many studies have been carried out on the PPAR subtypes α and γ, the fact that PPARβ/δ is ubiquitously expressed, and that there are a few potent selective ligands available, has hampered research in determining its biological function.[1,4] Only very recently, design and synthesis of PPARα/δ dual agonists as well as a highly selective PPARβ/δ has been reported.[5]

In the course of our studies on PPAR ligands, we were interested in selective PPARβ/δ ligands. Indeed, the first highly selective PPARβ/δ ligand (GW501516) was reported in a GlaxoSmithKline patent and only recently published[6–8] (Fig. 1). Further synthetic route improvements and modulations were reported.[9] When we started on this project, the Merck-developed ligand L-165,041 was described (Fig. 1), at this time, as the most selective PPARβ/δ ligand with a 15- and 8-fold selectivity for human PPARβ/δ over human PPARα and PPARγ, respectively (EC_{50}).[3] L-165,041 is about 122-fold selective for PPARβ/δ binding over PPARγ (K_i) and was found to raise HDLc in db/db mice in contrast to the effect of PPARα ligands.[10]

Our first objective was to run some structure–activity relationship study on compound L-165,041 and, in parallel, to evaluate the potency of pyridine analogues. At this stage, to set out new potent and selective ligands for PPARβ/δ subtype, we combined our SAR results to those reported by GlaxoSmithKline on GW5015016.[6]

In order to determine whether the three functional groups (hydroxyl, acetyl and propyl) of ligand L-165,041 had a key role in the activity towards the PPARβ/δ receptor, compounds **6**–**9** were prepared as shown in Scheme 1. *p*-Benzyloxyphenol was reacted with 1,3-dibromopropane with K_2CO_3 in refluxing water using phase transfer catalysis.[11] After hydrogenolysis of the benzyl group and further alkylation with

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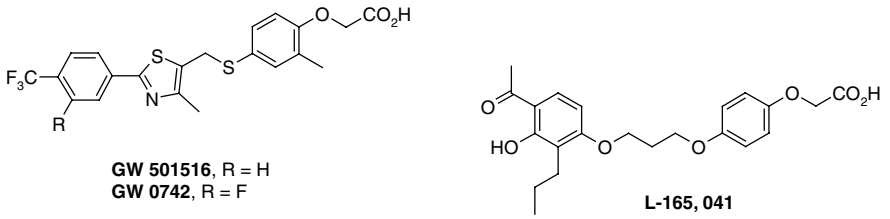
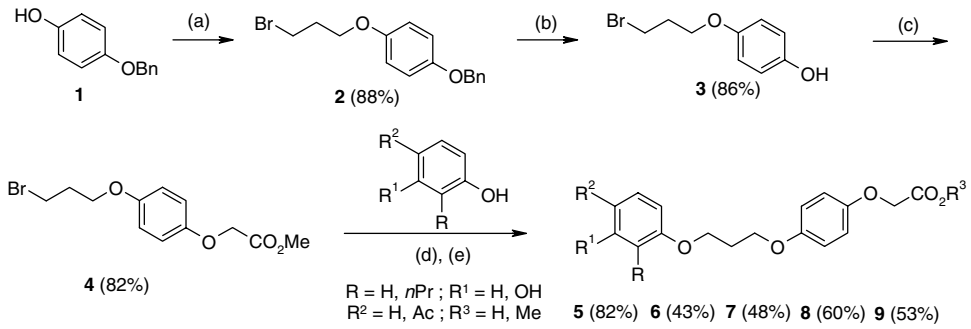


Figure 1. Compounds L-165,041, GW501516 and GW0742, respectively, selective and highly selective PPARβ/δ ligands.



Scheme 1. Reagents and conditions: (a) dibromopropane, K_2CO_3, Bu_4NBr, H_2O, reflux; (b) Pd/C, H_2 (55 psi), MeOH, rt; (c) methyl bromoacetate, K_2CO_3, acetone, reflux; (d) commercially available phenols, K_2CO_3, DMF, rt; (e) further saponification for compounds **8** and **9**: NaOH (1 M), THF, rt.

methyl bromoacetate, the common bromide intermediate **4** was isolated in 82% yield. Williamson reaction in refluxing acetone with the commercially available 2-*n*-propylphenol, resorcinol, 4-hydroxyacetophenone and 2,4-dihydroxyacetophenone allows us to, respectively, isolate the desired compounds **6**–**9**. Due to synthetic facilities, we choose to evaluate the biological activities on the ester moieties for compounds **6** and **7**. For these reasons and to be able to compare biological activities, we also synthesized the L-165,041 methyl ester **5** by reacting 2,4-dihydroxy-3-propylacetophenone with the bromide **4**.

Compounds were screened for functional potency in a transient transfection assay performed on Cos7 cells where a previously established chimeric receptor system was used to allow comparison on the relative transcriptional activity on the same target gene. Cos-7 cells were transiently transfected with luciferase reporter plasmid (pG5-TK-pGL3) in the presence of pGal4hPPARγ, pGAL4hPPARα or pGAL4hPPARβ/δ (these vectors expressed chimeric proteins containing the Gal4 DNA-binding domain fused to the human PPARγ, PPARα or PPARβ/δ ligand binding domain coding sequence) expression vectors. Plasmids pGal4-hPPARs, and pG5-TK-pGL3 were constructed as described previously.[12] Cells were seeded in 60-mm dishes at a density of 5.5×10^5 cells/dish in DMEM supplemented with 10% FCS and incubated at 37 °C for 24 h prior to transfection. Cells were transfected in OptiMEM without FCS for 3 h at 37 °C, using polyethylenimine (PEI), with reporter and expression plasmids. The plasmid pBluescript (Stratagene, La Jolla, CA) was used as carrier DNA to set the final amount of DNA to 5.5 μg/dish. The pCMV-β-galactosidase expression plasmid was cotransfected as a control for transfection efficiency. Transfection was stopped by addition of DMEM supplemented with 10% FCS and cells were then incubated at 37 °C. After 16 h, cells were trypsinised and seeded in 96-well plates at the density of 2×10^4 cells/well and incubated for 6 h in 10% FCS containing DMEM. Cells were then incubated for 16 h in DMEM containing 0.2% FCS and increasing concentrations of the compound tested (10 μM–10 nM) or vehicle (DMSO). At the end of the experiment, cells were washed once with ice-cold PBS and the luciferase activity was measured and normalized to internal control β-galactosidase activity as described previously.[12] Compounds which elicited on average at least 80% activation of PPAR(s) versus rosiglitazone (PPARγ), WY14.643 (PPARα) or MWI 66 (PPARβ/δ) (GAL4) (positive controls) were considered full agonists. EC_{50} were estimated using Prism software (GraphPad). All transfection experiments were performed at least three times.

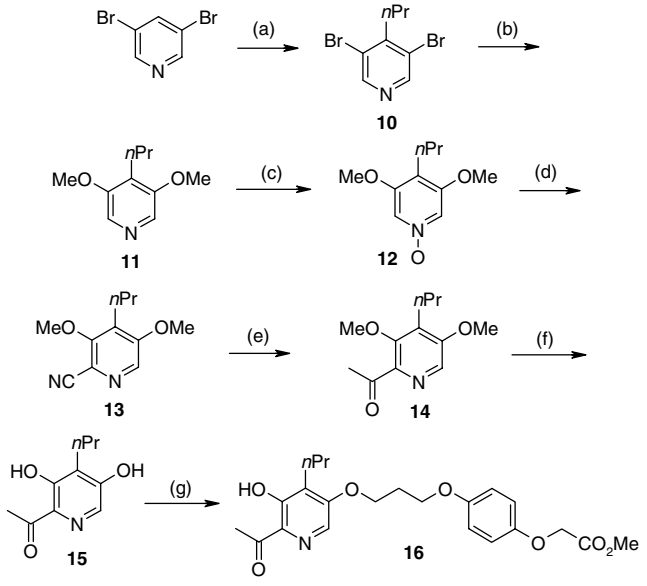
Table 1 shows the EC_{50} for compounds **5**–**9** in the transient transactivation assay against the three human PPAR subtypes: α, γ and δ. First, we can notice that ligand L-165,041 and its methyl ester **5** have comparable activities towards the PPARβ/δ subtype. Compounds **7** and **8** bearing only the hydroxyl and acetyl function, respectively, were completely inactive towards all three PPAR subtypes. On the other hand, compound **6** bearing only the propyl function proved to be active (EC_{50} = 1049 nM, activity = 129%) and highly selective towards PPARβ/δ. Those results were confirmed with compound **9**, indeed, this L-165,041 analogue devoid of the propyl group was completely inactive. From those results, it is clear that the propyl chain (which was already present in the original screening hit)[4b] has a crucial impact for PPARβ/δ activity in this series.

Table 1. Activity of compounds **5**–**9** in cell-based transactivation assay against human PPAR

Compound	R	R^1	R^2	R^3	Transactivation EC_{50}[a] nM (% activity)[b]		
					hPPARβ/δ	hPPARα	hPPARγ
L-165,041	*n*-Pr	OH	Ac	H	125 (51)	977 (97)	1824 (79)
5	*n*-Pr	OH	Ac	Me	457 (80)	1068 (32)	288 (90)
6	*n*-Pr	H	H	Me	1046 (129)	na	NA
7	H	OH	H	Me	Na	NA	NA
8	H	H	Ac	H	Na	NA	NA
9	H	OH	Ac	H	10,000 (6)	NA	NA

[a] Values are means of three experiments (NA, not active).
[b] Refer to maximal activity obtained with each compound expressed in percentage of maximal activity of MWI 66 at 10^{-6} M for PPARβ/δ; of rosiglitazone at 10^{-6} M for PPARγ; of WY 14.643 at 10^{-5} M for PPARα.

In order to increase interactions (hydrogen bonds, electronic effect,...) with the ligand binding domain, we planed to evaluate what would be the effect of a pyridine ring instead of a benzene ring. Thus, the synthesis of L-165,041 pyridine analogue **16** was envisaged and is reported in Scheme 2. Metallation of 3,5-dibromo-pyridine with LDA and subsequent quenching with iodopropane gave compound **10**.[13] Substitution of the bromines using sodium methanolate in the presence of CuBr furnished the 3,5-dimethoxy-4-propylpyridine **11**. The cyanation in position 2 of the pyridine ring was realized by the Vorbrüggen method.[14] *N*-Oxide pyridine **12** was isolated after treatment with *m*CPBA and then was condensed with trimethylsilyl cyanide to afford the cyanopyridine **13**. Addition of MeMgBr on the nitrile in the presence of trimethylsilyl chloride followed by the deprotection of the hydroxyl functions with $AlCl_3$ afforded the tetrasubstituted pyridine **15**. The pyridine analogue **16** was obtained by the Williamson synthesis of an ether bond between the bromide **4** and the pyridol **15**.



Scheme 2. Reagents and conditions: (a) 1—LDA (1.6 equiv), THF, −78 °C; 2—*n*-PrI (1.5 equiv), 16 h (74%); (b) MeONa, CuBr, MeOH, 150 °C, sealed tube, 24 h (57%); (c) *m*CPBA, $CHCl_3$ (98%); (d) TMSCN, Et_3N, CH_3CN, reflux (63%); (e) MeMgBr, TMSCl, Et_2O, rt (76%); (f) $AlCl_3$, CH_2Cl_2, reflux, 16 h (66%); (g) **4**, K_2CO_3, KI, DMF, rt (63%).

Table 2. Activity of compound **16** in cell-based transactivation against human PPAR

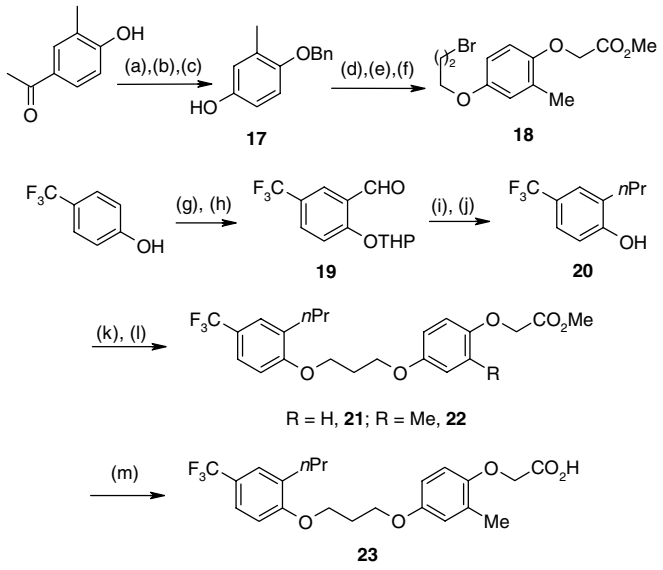
Compound	Transactivation EC_{50}[a] nM (% activity)[b]		
	hPPARδ	hPPARα	hPPARγ
L-165,041	125 (51)	977 (97)	1824 (79)
5	457 (80)	1068 (32)	288 (90)
16	82 (94)	8391 (64)	757 (105)

[a] Values are means of three experiments.
[b] Refer to maximal activity obtained with each compound expressed in percentage of maximal activity of MWI 66 at 10^{-6} M for PPARβ/δ; of rosiglitazone at 10^{-6} M for PPARγ; of WY 14.643 at 10^{-5} M for PPARα.

Transactivation tests for the pyridine analogue **16** are reported in Table 2. Interestingly, if we compare the activities for compound **16** with those of the reference L-165,041 methyl ester **5**, we can notice that the pyridine ring increases the PPARβ/δ activity (**16** EC_{50} = 82 nM vs **5** EC_{50} = 457 nM, EC_{50} PPARβ/δ ratio = 6) and improves the selectivity against PPARα (EC_{50} PPARα ratio = 8) and PPARγ (EC_{50} PPARγ ratio = 2.5). Moreover, the compound **16** activity is stronger on PPARβ/δ subtype. This effect is illustrated by the maximal transactivation percentage obtained (94%) which expresses full agonist activity. This is a major difference with reference compound L165.041 which is a partial agonist on PPARβ/δ transactivation test (51%).

The SAR studies done on compound GW501516[6] have shown the importance of the trifluoromethyl group as well as the enhancement of PPARβ/δ activity by methyl substitution at the *ortho* position on the phenoxy moiety. We decided to evaluate what would be the effect of those two substituents on our molecules. For this, we planed the synthesis of two new compounds bearing both a CF_3 substituent in *meta* position to the crucial *n*-propyl chain but differing by the methyl substituent on the phenoxy moiety (compounds **21** and **22**, and Scheme 3).

As reported in Scheme 3, the commercially available 4-trifluoromethylphenol was first protected as OTHP to allow direct ortho-metallation and subsequent formylation with DMF.[15] Wittig condensation followed by catalytic hydrogenation in acidic medium gave the phenol intermediate **20** in good yields. Direct alkylation with *n*-propyl iodide after the ortho-lithiation reaction gave only starting material. Williamson reaction in



Scheme 3. Reagents and conditions: (a) BnBr, Bu_4NBr (cat.), NaOH 10%, CH_2Cl_2, rt (99%); (b) *m*CPBA, PTSA, CH_2Cl_2, reflux 16 h (87%); (c) MeONa/MeOH, rt, 1 h (99%); (d) 1,3-dibromopropane, K_2CO_3, Bu_4NBr, H_2O, reflux (95%); (e) Pd/C 10%, H_2 (4 bar), MeOH, rt (76%); (f) methyl bromoacetate, K_2CO_3, acetone, reflux (86%); (g) DHP, PTSA, CH_2Cl_2, rt (83%); (h) BuLi, TMEDA, THF, −10 °C then DMF, −10 °C, 3 h (60%); (i) Ph_3PEtBr, BuLi, THF, rt (79%); (j) H_2 (4 bar), Pd/C (10%), MeOH, HCl (1 N), rt, 16 h (91%); (k) R=H, **4**, K_2CO_3, DMF, rt (50%); (l) R=Me, **18**, K_2CO_3, DMF, rt (76%); (m) R=Me, KOH, MeOH/H_2O/THF then AcOH (69%).

DMF with bromo derivatives **4** and **18** (obtained in a protection/Bayer–Villiger oxidation/saponification/alkylation/deprotection/alkylation sequence from 4-hydroxy-3-methylacetophenone, Scheme 3) at room temperature for 48 h in the presence of K_2CO_3 allows us to, respectively, isolate ligands **21** and **22** in 50% and 76% yields.

Transactivation tests are reported in Table 3. As expected, the presence of the CF_3 group had a crucial benefical effect on the affinity for PPARβ/δ. Furthermore, affinity for PPARα and PPARγ was also increased (compound **21**, Table 3 vs compound **6**, Table 1). On the other hand, compound **22**, with an extra methyl group, has shown much higher affinity for PPARβ/δ (6-fold enhancement), the affinity for the two other subtypes being similar (compound **22** vs compound **21** and Table 3).

With this new potent ligand in hand, we evaluated the corresponding acid **23** obtained by saponification with KOH in MeOH in 69% yield (Scheme 3). We were very pleased to note that compound **23** had a nanomolar affinity for PPARβ/δ very close to the reference PPARβ/δ ligand GW501516. Unfortunately, this acid form was not selective as it has also shown a good affinity for PPARα and PPARγ (compound **23** and Table 3).

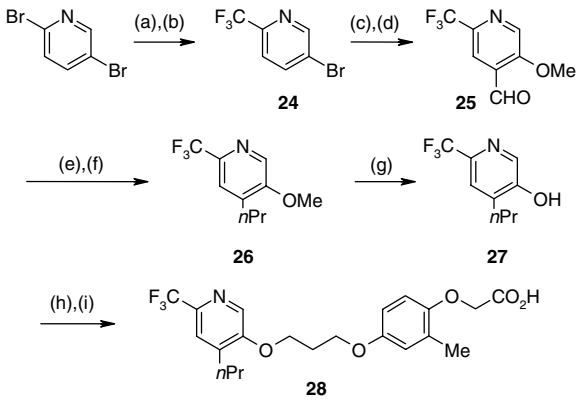
Nevertheless, encouraged by this result, we logically planed to take advantage of the higher affinity of CF_3 containing ligand **23** and the higher selectivity of pyridine analogue **16** for PPARβ/δ subtype. The synthesis is described in Scheme 4.

Table 3. Activity of CF_3 containing compounds in cell-based transactivation assay against human PPAR

Compound	Transactivation EC_{50}[a] nM (% activity)[b]		
	hPPARβ/δ	hPPARα	hPPARγ
GW5015016	1 (95)	10,000 (452)	10,000 (37)
L-165,041	125 (51)	977 (97)	1824 (79)
21	98 (93)	1401 (49)	1185 (71)
22	16 (114)	1010 (85)	1261 (65)
23	7 (100)	103 (40)	153 (100)
28	15 (110)	1330 (136)	1582 (62)

[a] Values are means of three experiments.

[b] Refer to maximal activity obtained with each compound expressed in percentage of maximal activity of MWI 66 at 10^{-6} M for PPARβ/δ; of rosiglitazone at 10^{-6} M for PPARγ; of WY 14.643 at 10^{-5} M for PPARα.



Scheme 4. Reagents and conditions: (a) HI 67%, 100 °C, 16 h then 170 °C 4 h then NaOH 40% (95%); (b) CuI, KF, $TMSCF_3$, NMP/DMF (1:1), rt, 16 h (87%); (c) MeONa, DMF, 65 °C, 2 h (90%); (d) *t*-BuLi (2 equiv), THF, −78 °C then DMF, −78 °C, 3 h (58%); (e) Ph_3PEtBr, BuLi, THF, rt, 65%; (f) Pd/C (20%), H_2 (1 bar), MeOH, rt, 24 h, 86%; (g) BCl_3, Bu_4NI, CH_2Cl_2, −78 °C to rt, 24 h (43%); (h) **18**, K_2CO_3, KI (10%), DMF, rt (86%); (i) LiOH, H_2O/THF then AcOH (87%).

Starting from the commercially available 2,5-dibromopyridine, the 5-bromo-2-trifluoromethyl-pyridine **24** was obtained following the synthesis described by Schlosser et al.[16] Nucleophilic substitution of the bromine by sodium methanolate followed by formylation at C-4 afforded aldehyde **25**. The Wittig/reduction sequence used previously to obtain the propyl chain was successfully applied. After deprotection of the alcohol function using the smooth conditions BCl_3, Bu_4NI in dichloromethane,[17] pyridinol **27** was engaged in a Williamson reaction with bromo derivative **18**. The resulting compound was saponified and the corresponding acid **28** was isolated in 86% yield. The transactivation tests, presented in Table 3, were consistent with our expectations. Indeed, new ligand **28** has a high affinity for PPARδ and is 100-fold more selective for human PPARδ over human PPARα and PPARγ.

In conclusion, on the basis of a structure-activity relationship study, we have shown the key role of the propyl chain in L-165,041-type ligands for a PPARδ activity. Using SAR evidence on GW501516, the affinity for PPARβ/δ was increased by adding a trifluoromethyl group and a methyl group on our structures. Finally, we have shown that the presence of the pyridine ring increases the selectivity for PPARβ/δ subtype by decreasing the affinity for PPARα and PPARγ subtypes.

Acknowledgments

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