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Synthesis and Pharmacological Evaluation of a New Class of Peroxisome Proliferator-Activated Receptor Modulators

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Abstract—A series of 5-substituted 2-benzoylaminobenzoic acids has been synthesized and assayed for PPARα/γ activity. Both dual activators and selective PPARγ agonists have been identified. This class of compounds was shown to activate the PPARγ receptor through interaction with a novel binding site.

The peroxisome proliferator-activated receptors (PPARα, PPARδ and PPARγ) belong to the nuclear receptor superfamily and function as ligand-activated transcription factors.[1] These receptors play a key role in various tissues controlling the expression of genes involved in lipid and carbohydrate metabolism. Interest in the PPARs has increased with the discovery of the clinically useful thiazolidinedione (TZD) class of insulin sensitizers (e.g., rosiglitazone and troglitazone) which are potent and selective PPARγ agonists used in the treatment of type II diabetes. However, the success of these drugs has been hampered by cases of liver toxicity and side-effects such as fluid retention and weight gain.[2] This has prompted the search for non-TZD ligands with different characteristics compared to the glitazones.

Type II diabetes is almost invariably accompanied by a dyslipidemia which is highly atherogenic and thus represents a major risk factor for the development of premature atherosclerosis and coronary artery disease. The finding that the established hypolipidemic fibrate drugs (e.g., bezafibrate and clofibrate) most probably exert their effect by activation of the PPARα receptor further demonstrates that these targets are suitable for therapeutic intervention.[3] Accordingly, a dual action drug which binds and activates the PPARα/γ receptors is hypothesized to mechanistically target the two major metabolic abnormalities observed in type II diabetic patients, insulin resistance and dyslipidemia.

We herein report the synthesis and initial pharmacological evaluation of a new series of 5-substituted 2-benzoylaminobenzoic acids as PPARα/γ modulators. BVT.142 (Fig. 1) was identified in a cell based reporter gene assay as being a dual activator of the PPARα/γ receptors. In the course of expanding the chemistry around BVT.142 more potent dual activators, as well as selective PPARγ agonists and antagonists, have been identified through variations at the 5-position. We have been able to obtain co-crystals of the PPARγ receptor and a number of analogues within this series. X-ray experiments show that these compounds activate the receptor through interactions with a novel binding site.

The Mitsunobu reaction[4] and Suzuki coupling[5] were identified as suitable synthetic methods for variations at the 5-position of BVT.142. The synthesis of aryl ethers utilizing the Mitsunobu reaction is outlined in Scheme 1.

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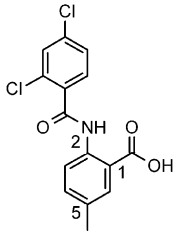


Figure 1. The dual PPARα/γ activator BVT.142.

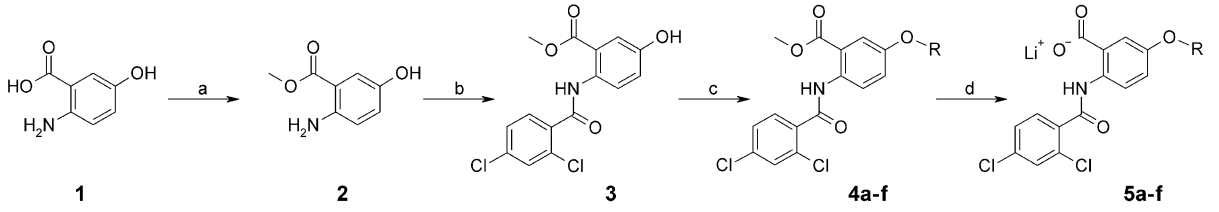
Starting from commercially available 2-amino-5-hydroxy benzoic acid **1** the corresponding methyl ester **2** was formed by treatment with concentrated sulphuric acid in methanol. Subsequent coupling of **2** with 2,4-dichlorobenzoyl chloride provided the amide **3**. The key intermediate **3** was reacted with a set of alcohols in the presence of 1,1′ - azo - bis(*N,N* - dimethylformamide) (TMAD) and polymer supported triphenylphosphine in a mixture of dichloromethane and tetrahydrofuran to give the adducts 4**a**–**f**. Ester hydrolysis, using 1 M aqueous lithium hydroxide, afforded the target compounds **5a**–**f** as lithium salts.

The synthesis of biaryl analogues was performed as shown in Scheme 2. Coupling of the commercially available methyl 2-amino-5-iodobenzoate **6** with 2,4-dichlorobenzoyl chloride provided the intermediate **7**. Palladium-catalyzed cross-coupling of **7** with an array of aryl or heteroaryl boronic acids afforded biaryls **8a**–**e** or a mixture of **8a**–**e** and the corresponding cyclized benzoxazine equivalent. Subsequent ester hydrolysis gave the target compounds **9a**–**e**.

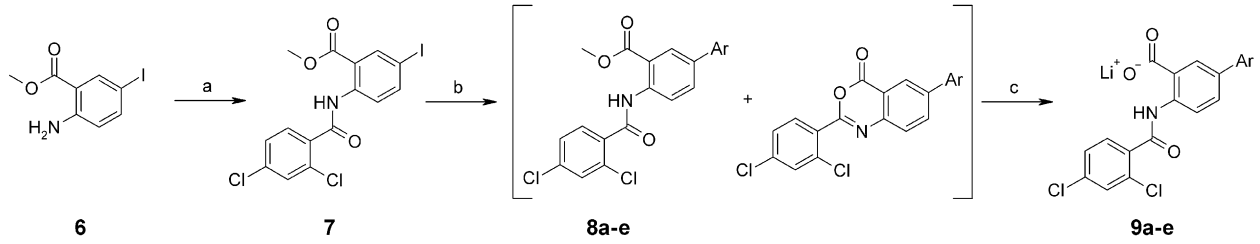
Utilizing the intermediate **3**, a number of diaryl ethers have also been prepared as outlined in Scheme 3. Simple nucleophilic aromatic substitution reactions of 2-chloro nitrogen containing heterocycles yielded, after hydrolysis, compounds **10a**–**d**.

The three different series of compounds have been screened in a PPARα/γ competitive binding assay and a cell-based reporter gene efficacy assay. The latter is based on an expression vector containing the ligand binding domain of the human PPARα/γ receptor and the yeast transcription factor GAL4. Data for some selected analogues is presented in Table 1.

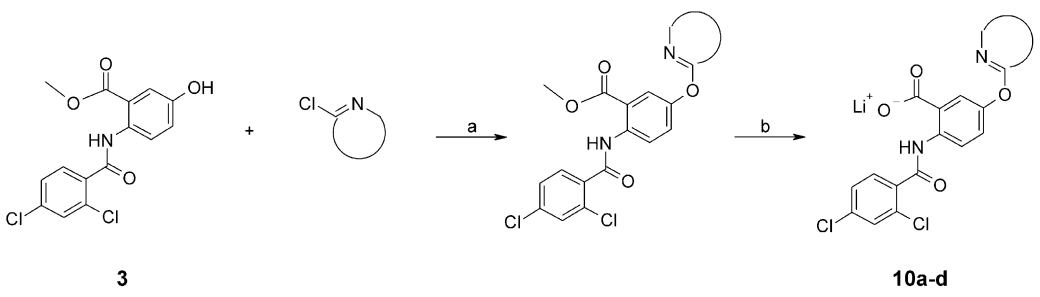
The introduction of different substituents in the 5-position of the BVT.142 scaffold allowed us to identify compounds with an improved potency at the PPARα and PPARγ receptors. In general, it seems easier to gain activity at PPARγ within these three series although dual activators are found in both the Mitsunobu and Suzuki arrays. Aryl ethers **5a** and **5b** are significantly more active on both receptors compared to the original



Scheme 1. Preparation of aryl ethers **5a**–**f** utilizing the Mitsunobu reaction. Reagents: (a) H_2SO_4, MeOH; (b) 2,4-dichlorobenzoyl chloride, pyridine; (c) R-OH, TMAD, polymer supported PPh_3, THF/DCM (1:1); (d) 1 M LiOH, THF.



Scheme 2. Preparation of biaryls **9a**–**e** utilizing the Suzuki coupling. Reagents: (a) 2,4-dichlorobenzoyl chloride, diisopropylethylamine, THF; (b) $Pd(PPh_3)_4$, Ar-$B(OH)_2$, 2N Na_2CO_3, DME; (c) 1 M LiOH, dioxane.



Scheme 3. Preparation of diaryl ethers **10a**–**d**. Reagents: (a) K_2CO_3, DMF, heat; (b) 1 M LiOH, THF.

Table 1. In vitro binding affinity (K_i) and potency (EC_{50}) for selected compounds

Compd	5-Substituent	K_i (μM)[a,c]		EC_{50} (μM)[b,c,d]	
		PPARα	PPARγ	PPARα	PPARγ
BVT.142	Methyl	30	2.2	9.5	1.6
5a	2-Thienylmethoxy	25	0.4	5	0.3
5b	2-(3-Thienyl)ethoxy	20	0.6	3.8	0.8
5c	2-(2-Thienyl)ethoxy	>100	1	>100	0.7
5d	$OCH_2CH_2SCH_3$	19	1.6	2.5	0.6
5e	4-Ethoxybenzyloxy	>100	3.6	>100	0.7
5f	2-(2-Pyridinyl)ethoxy	>100	1.5	n.d.	3.2
9a	3-Thienyl	28	0.9	12	0.7
9b	2-Furyl	22	0.5	10	1
9c	3-Ethoxyphenyl	>100	1.2	>100	0.3
9d	8-Quinolinyl	>100	0.7	>100	0.3
9e	3-Carboxyphenyl	67	1.4	>100	>100
10a	5-Nitro-2-pyridinyloxy	>100	0.18	>100	0.4
10b	3-Nitro-2-pyridinyloxy	>100	0.17	>100	0.4
10c	2-Pyrimidinyloxy	>100	0.1	>100	1.3
10d	6-Chloro-2-pyrazinyloxy	21	0.16	>100	0.45

[a]Ligand binding affinities were determined by displacement of a tritiated tracer by the unlabeled compound to a GST-PPAR fusion-protein, for PPARα, containing residues 167–468 and ^{3}H-GW2331 as tracer, and for PPARγ, containing residues 204–477 and ^{3}H-Rosiglitazone as tracer.
[b]Transactivation potency was measured by luciferase activity in Caco-2/TC7 cells transiently co-transfected with an expression vector for the fusion-protein Gal4-PPARα (167–468) or Gal4-PPARγ (204–477), respectively, and a reporter vector containing 4xGAL4RE-luciferase, after 24 h of incubation with compounds.
[c]Values are the mean of at least two experiments, where $n \geq 2$, and Xlfit version 2.0 (Business Solutions Limited) was used for curve fitting analysis.
[d]n.d. = not determined.

hit. Interestingly, compound **5c**, which only differs from **5b** in the position of the sulfur in the thiophene ring, does not bind or activate the PPARα receptor but still retains PPARγ receptor activity. In general, analogues containing an aromatic portion in the 5-substituent are more active, however compound **5d** shows that aliphatic substituents could also be of interest. In the Suzuki biaryl series, compounds **9a** and **9b** are dual PPARα/γ agonists while compounds such as **9c** and **9d** are potent and highly selective PPARγ agonists. From the same series the PPARγ antagonist **9e** was also identified. The analogues showing the highest binding affinities are the diaryl ethers **10a–d**. Interestingly, all four compounds, prepared according to Scheme 3, are highly selective and potent PPARγ agonists. The data demonstrates the possibility to modify the effect on the PPARα and PPARγ receptors by variations in the 5-position of BVT.142.

It has been suggested that PPARγ and other nuclear receptors are activated by a common mechanism in which the ligand stabilizes helix 12.[6,7] This in turn allows for the recruitment of co-activator proteins and subsequent stimulation of transcriptional activity. We have been able to obtain the structure of human PPARγ LBD in complex with the PPARγ agonists **5a–b** and **9a**.[8]

The X-ray crystallographic data shows that these compounds bind to a site different from the reported binding site of the thiazolidinediones. In contrast to TZD ligands such as rosiglitazone, which appear to activate the PPARγ receptor through interactions with amino acids (e.g., His323, His449 and Tyr473) located deep into the ligand binding pocket, compound **5a** occupies a region in proximity with helix 3. In Figure 2 the key interaction between the carboxylic acid group of **5a** and the backbone nitrogen of Ser342, situated near the entrance of the PPARγ pocket, is shown.

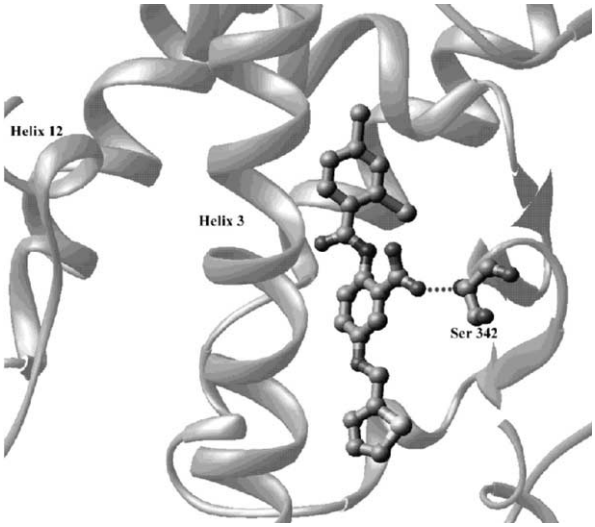


Figure 2. The binding site of **5a** in the PPARγ receptor.

Other variations around BVT.142, such as carboxylic acid isosters and various modifications of the 2,4-dichloro moiety, have also been investigated but are not within the scope of this paper.

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8. The ligand binding domain of hPPARγ was produced in *E. coli* and the protein was purified to homogeneity and concentrated to a final concentration of 10 mg/mL.[9] Crystals of PPARγ-LBD complexes were grown by the hanging drop diffusion method. A GRIP-1 derived co-activator peptide was co-crystallized with the receptor. All data were collected at room temperature using a Rigaku RU300 rotating anode and an Raxis4 image plate detector. The crystals diffracted to 2.9 Å resolution. A detailed description of the structure will be published elsewhere.
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