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Identification of a Subtype Selective Human PPARα Agonist Through Parallel-Array Synthesis

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Abstract—Using solid-phase, parallel-array synthesis, a series of urea-substituted thioisobutyric acids was synthesized and assayed for activity on the human PPAR subtypes. GW7647 (**3**) was identified as a potent human PPARα agonist with ~200-fold selectivity over PPARγ and PPARδ, and potent lipid-lowering activity in animal models of dyslipidemia. GW7647 (**3**) will be a valuable chemical tool for studying the biology of PPARα in human cells and animal models of disease. © 2001 Elsevier Science Ltd. All rights reserved.

The peroxisome proliferator activated receptors (PPARs) are members of the nuclear receptor gene family of ligand activated transcription factors.[1] The function of the PPARα subtype in the regulation of hepatic lipid metabolism was uncovered by its association with the fibrate class of lipid-lowering drugs.[2] PPARα is also expressed in extra-hepatic tissues, and it has been proposed that this subtype may be involved in the regulation of inflammatory processes[3] and macrophage signaling.[4] Although fibrates are often selective activators of the hepatic PPARα in rodents in vivo, they show only modest selectivity over the other PPAR subtypes in cell-based assays. For example, fenofibric acid and Wy-14643 show <10-fold selectivity for activation of human PPARα compared to PPARγ and/or PPARδ (Table 1).[1] Remarkably, despite the decades of use of fibrates in humans, there are no known subtype selective ligands for human PPARα that can be used to study the biology of this receptor in human cells.

We recently reported the identification of GW9578 (**1**),[5] a urea-substituted thioisobutyric acid (TiBA) that is a potent and subtype selective murine PPARα agonist. Although **1** was a useful chemical tool for studying the biology of PPARα in rodents, it shows only 20-fold selectivity on the human receptors (Table 1). Compound **1** also exists as a viscous oil or a foam, which leads to difficulties in dispensing aliquots of the compound on a routine basis. In order to identify a chemical tool for PPARα with improved selectivity and physical properties, we decided to use the solid-phase array synthesis that was employed in the identification of the ureidofibrate PPARδ agonist GW2433 (**2**).[6] This approach has led to the identification of the first subtype selective human PPARα agonist, GW7647 (**3**) (Fig. 1).

Synthesis of analogues of **1** required loading of the FMOC-substituted TiBA **4** onto solid phase (Scheme 1). Notably, the increased electrophilicity of the TiBA allowed the use of DIC/DMAP to load the FMOC acid **4** onto SASRIN resin, whereas this approach had failed in the oxygen substituted series.[7] Unreacted sites were capped with isovaleric anhydride. The resulting resin had a loading of 0.47 mmol/g by FMOC analysis. The remainder of the synthesis proceeded as previously described for the analogous ureidofibrates.[7] Deprotection of the FMOC group (piperazine/DMF) followed by coupling with a carboxylic acid afforded the intermediate resin-bound amide, which was reduced in situ with borane to generate the resin-bound secondary amine **5**. Reaction of the amine with isocyanates afforded the ureido-TiBA **6** in 70–90% purity after cleavage with 10% TFA/CH_2Cl_2. Library production proceeded in Robbins blocks using 25 mg of resin per well. Based on our knowledge of PPARα structure–activity relationships gained from earlier work on ureidofibrates,[6] an initial

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Table 1. Activity of ureido-TiBAs on the human PPAR subtypes in vitro and in fat-fed hamsters in vivo

Compound	R^1-CO_2H[a]	R^2-NCO[a]	EC_{50} (μM)[b]			Triglycerides (%)[c]
			Human PPARα	Human PPARγ	Human PPARδ	
Fenofibric acid			30	300	>100	—
Wy-14643			5.0	60	35	—
GW9578 (**1**)	**c**	**b**	0.05	1.0	1.4	−88
6a	**n**	**c**	0.005	0.32	1.1	n.a.
6b	**g**	**d**	0.017	8.5	>10	n.a.
6c	**g**	**o**	0.008	2.4	5.0	−70
6d	**g**	**v**	0.009	1.35	>10	n.a.
6e	**g**	**z**	0.10	10	>10	−33
6f	**g**	**d**	0.02	1.4	2.0	n.a.
6g	**g**	**bb**	0.05	2	>10	n.a.
6h	**g**	**jj**	0.006	1.2	4.0	n.a.
6i	**n**	**bb**	0.001	1.0	>10	n.a.
6j	**n**	**hh**	0.02	2.0	>10	−78
6k	**n**	**ii**	0.009	1.0	>10	−69
GW7647 (**3**)	**n**	**o**	0.006	1.1	6.2	−93

[a]Monomers are listed in the footnote.
[b]Concentration for half maximal activation of the GAL4-PPAR ligand binding domain; all compounds were full agonists; data are the mean of three to six determinations ±15%.
[c]Triglyceride lowering in fat-fed hamsters; compounds were administered at 3 mg/kg po bid for 7 days; — = not tested; n.a. = not active.

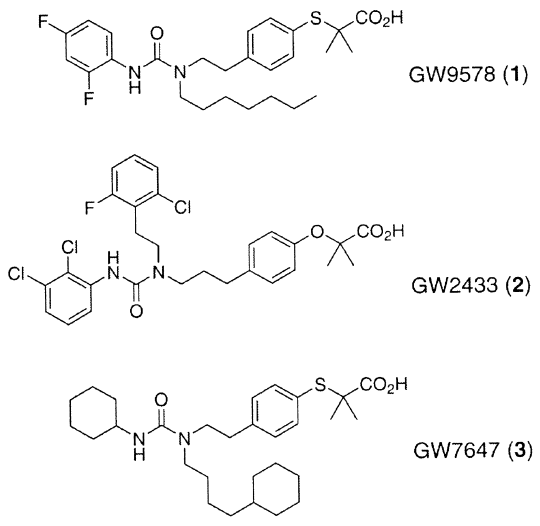


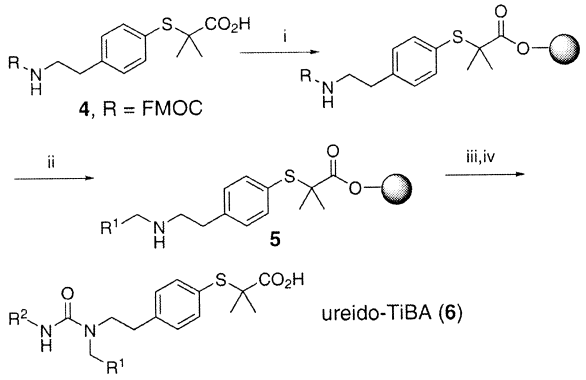
Figure 1. Ureido-TiBA and ureidofibrate PPAR agonists.

set of eight arylisocyanates (R^1-NCO, **a**–**h**[†]) was selected along with 20 carboxylic acids (R^2-CO_2H, **a**–**r**[†]).

The resulting array was screened for activity against each of the three human PPAR subtypes using an established cell-based reporter assay in CV-1 cells.[6] Full dose–response curves were obtained for all 160 compounds. Two ureido-TiBAs (**6a** and **6b**, Table 1) were identified which initially showed >100-fold selectivity for human PPARα over PPARγ and PPARδ. **6a** and **6b** were resynthesized in solution, using the route developed for **1**,[5] and purified to homogeneity. Upon retest, both compounds were potent human PPARα agonists, with **6a** showing >60-fold selectivity and **6b** >500-fold selectivity over PPARγ and PPARδ (Table 1). Unfortunately, both compounds demonstrated poor oral bioavailability (data not shown) and neither **6a** or **6b** lowered triglycerides in fat-fed hamsters[8] following oral dosing at 3 mg/kg (Table 1).

Using **6a** and **6b** as leads, a second library was synthesized in which R^1 (Scheme 1) was derived from 4-biphenylacetic acid or cyclohexylbutanoic acid (R^1-CO_2H, **g** and **n**[1]) and R^2 was varied using 33 aromatic and aliphatic isocyanates (R^2-NCO, **e**–**jj**[†]). Screening of this array identified 10 compounds with activity on human PPARα <100 nM and selectivity >100-fold over PPARγ and PPARδ. These 10 compounds (**6c**–**6k** and **3**) were resynthesized in solution, purified to homogeneity and retested on the three human PPAR subtypes (Table 1). Six of the ureido-TiBAs (**6c**, **6d**, **6h**, **6i**, **6k**, and **3**) showed EC_{50} <10 nM on human PPARα with >100-fold selectivity over PPARγ and PPARδ. Ureido-TiBAs (**6c**–**6k** and **3**) were also assayed for triglyceride lowering activity in fat-fed hamsters. The compounds were administered at a dose of 3 mg/kg po

[†]Monomers. R^1-CO_2H: **a**, 2-phenoxypropionic acid; **b**, phenylacetic acid; **c**, heptanoic acid; **d**, 3,3-diphenylpropionic acid; **e**, 2,4-dichlorophenoxybutanoic acid; **f**, 1-naphthyloxyacetic acid; **g**, 4-biphenylacetic acid; **h**, thien-2-ylbutanoic acid; **i**, 3,4-methylenedioxyphenylpropionic acid; **j**, 2-naphthylthiopropionic acid; **k**, 4-tolylsulfonylacetic acid; **l**, benzyloxycarbonylaminobutanoic acid; **m**, 2′-trifluoromethyl-2-chlorobiphenylacetic acid; **n**, cyclohexylbutanoic acid; **o**, 4-chloro-2-methylphenyloxybutanoic acid; **p**, 4-(benzyloxy)phenoxyacetic acid; **q**, 4,4,4-trifluoro-3-methylbutanoic acid; **r**, 3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-ylpropionic acid; **s**, methylthiopropionic acid; **t.** adamantylacetic acid. R2-NCO: **a**, 4-fluorophenylisocyanate; **b**, 2,4-difluorophenylisocyanate; **c**, 2-methoxyphenylisocyanate; **d**, 4-isopropylphenylisocyanate; **e**, 3,5-bis(trifluoromethyl)phenylisocyanate; **f**, 4-biphenylisocyanate; **g**, 4-acetylphenylisocyanate; **h**, 2-nitrophenylisocyanate; **i**, 2,6-diethylphenylisocyanate; **j**, 2-bromophenylisocyanate; **k**, 2-ethylphenylisocyanate; **l**, 3-ethoxycarbonylphenylisocyanate; **m**, 4-butoxycarbonylphenylisocyanate; **n**, 2,6-diisopropylphenylisocyanate; **o**, 3-methoxyphenylisocyanate; **n**, phenethylisocyanate; **o**, cyclohexylisocyanate; **p**, 1-ethoxycarbonyl-3-methylthiopropylisocyanate; **q**, a-methylbenzylisocyanate; **r**, 4-butylphenylisocyanate; **s**, 3-fluoro-4-methylphenylisocyanate; **t**, 2,4-dimethylphenylisocyanate; **u**, 4-ethylphenylisocyanate; **v**, 2,4,5-trimethylphenylisocyanate; **w**, 2,5-dichlorophenylisocyanate; **x**, 2,4,5-trichlorophenylisocyanate; **y**, 3,4-dichlorophenylisocyanate; **z**, 2-methoxy-5-methylphenylisocyanate; **aa**, 3-iodophenylisocyanate; **bb**, 3,5-dichlorophenylisocyanate; **cc**, 2,4-diethoxyphenylisocyanate; **dd**, 2,4,6-trimethylphenylisocyanate; **ee**, 3-chloro-4-methylphenylisocyanate; **ff**, 4-trifluoromethylphenylisocyanate; **gg**, 3,4-dimethylphenylisocyanate; **hh**, 3-nitro-4-methylphenylisocyanate; **ii**, 3-cyanophenylisocyanate; **jj**, 2,4-dimethoxyphenylisocyanate.



Scheme 1. Reagents: (i) SASRIN resin; DIC, DMAP; (ii) piperidine, DMF; R^1CO_2H, DIC, HOBT; BH_3·THF; (iii) R^2NCO, CH_2Cl_2; (iv) 10% TFA, CH_2Cl_2.

bid for 7 days. Ureido-TiBAs **6c**, **6e**, **6j**, **6k**, and **3** showed significant lowering of triglycerides. However, only **3** was as effective as GW9578 (**2**) in this animal model of fibrate activity.[8]

GW7647 (**3**) is a potent human PPARα agonist with ~200-fold selectivity over the other subtypes and in vivo lipid-lowering activity. To further characterize this compound, it was assayed against the three murine PPAR subtypes. Compound **3** showed $EC_{50} = 0.001$, 1.3, and 2.9 μM on murine PPARα, PPARγ, and PPARδ, respectively. Administration of **3** (3 mg/kg po bid) to cholesterol/cholic acid-fed rats[5] for 4 days resulted in a 60% increase in HDL-cholesterol, a 60% decrease in triglycerides, and a 40% decrease in serum apolipoprotein CIII.

Finally, in contrast to **1**,[5] which has poor physical properties, **3** is a white powder with mp 153–154 °C. Thus, GW7647 (**3**) will be a valuable chemical tool for studying PPARα action in human cells as well as rodent models of disease.

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