# A Ureido-Thioisobutyric Acid (GW9578) Is a Subtype-Selective PPARα Agonist with Potent Lipid-Lowering Activity

Peter J. Brown,*,† Deborah A. Winegar,‡
Kelli D. Plunket,§ Linda B. Moore,§
Michael C. Lewis,‡ Joan G. Wilson,‡
Scott S. Sundseth,‡ Cecilia S. Koble,† Zhengdong Wu,∥
James M. Chapman,∥ Jürgen M. Lehmann,§
Steven A. Kliewer,§ and Timothy M. Willson†

*Departments of Medicinal Chemistry, Metabolic Diseases, and Molecular Endocrinology, Glaxo Wellcome Research & Development, Research Triangle Park, North Carolina 27709, and College of Pharmacy, University of South Carolina, Columbia, South Carolina 29208*

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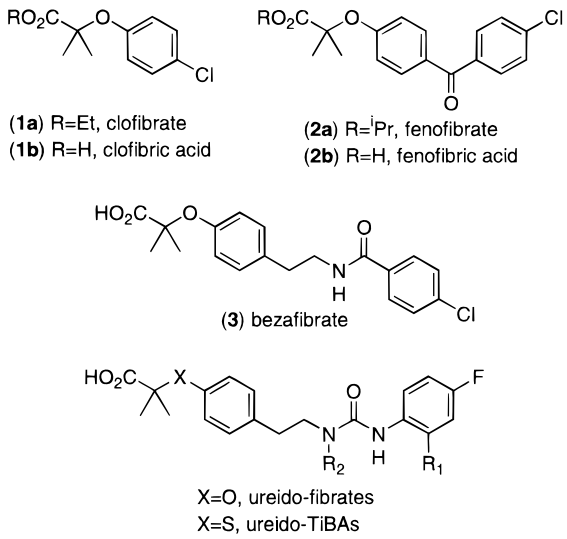
Hypercholesterolemia and hypertriglyceridemia are associated with an increased incidence of coronary heart disease,[1] the leading cause of death in the western world. Drug therapy with fibrates, such as clofibrate (**1a**), fenofibrate (**2a**), and bezafibrate (**3**) (Chart 1), is effective at lowering serum triglycerides and low-density lipoprotein (LDL) cholesterol and raising high-density lipoprotein cholesterol in humans.[2] These drugs have been shown to slow the progression of atherosclerosis and reduce the number of coronary events in high-risk patients.[3−5] Fibrates mediate their clinical effects primarily through increased clearance of circulating triglyceride-rich very low-density lipoproteins (VLDL),[2] leading to a reduction in the number of atherogenic particles. Apolipoprotein C-III (apoC-III) is a 79-amino acid glycoprotein which resides primarily on the surface of VLDL particles and inhibits their breakdown by lipoprotein lipase. In humans, fibrates lower serum levels of apoC-III,[6] increasing catabolism of VLDL to smaller particles which can be removed from the circulation by receptor-mediated uptake into the liver.[7]

The clinically used fibrate drugs were developed without knowledge of their cellular target.[8] In 1990, Issemann and Green reported the cloning of an orphan member of the nuclear receptor superfamily, designated the peroxisome proliferator-activated receptor α (PPARα), which was activated by the known fibrate drugs.[9] Adding to the significance of this discovery was the identification of a PPAR binding site in the proximal promoter of the *apoC-III* gene,[10] through which fibrates were shown to transrepress its expression.[10,11] Rodent pharmacology studies support the hypothesis that fibrates mediate their lipid-lowering activity through PPAR-mediated repression of hepatic *apoC-III* expression,[11,12] which in turn leads to lower circulating levels of apoC-III and increased lipoprotein catabolism.[7]

**Chart 1.** Chemical Structures of Fibrate Compounds




Three PPAR subtypes have been identified in humans and rodents:[13] PPARα is found primarily in the liver; PPARγ, the target for the glucose- and lipid-lowering action of the thiazolidinedione (TZD) drugs,[14,15] is found at high levels in adipose tissue and at lower levels in the spleen and liver; and PPARδ (also known as PPARβ, NUCI, and FAAR) is expressed in most tissues. Although all three subtypes are found in the liver, the level of PPARα expression is generally higher, and it has been widely assumed that this is the subtype through which fibrates mediate their lipid-lowering activity.[2,16] However, the fibrates that are used in the clinic have not been rigorously characterized for their activities on the three PPAR subtypes. We profiled clofibric acid (**1b**), fenofibric acid (**2b**), and bezafibrate (**3**) for their PPAR agonist activity on the human and murine receptors (Table 1). In agreement with earlier reports,[9,16] these fibric acids activated PPARα at high micromolar concentrations. Interestingly, all three compounds showed significant cross-reactivity with the other PPAR subtypes. Clofibric acid (**1b**) and fenofibric acid (**2b**) were dual activators of PPARα and PPARγ, with ∼10-fold selectivity for PPARα, while bezafibrate (**3**) activated all three PPAR subtypes at comparable doses. The PPAR selectivity data is consistent with the proposal that PPARα mediates the lipid-lowering activity of the fibrate drugs,[2,16] although their cross-reactivity with PPARγ may contribute to some of the observed pharmacology.[17]

Since the fibrates (**1**−**3**) are relatively weak PPARα agonists, we were interested in profiling compounds with increased potency and selectivity for PPARα as lipid-lowering drugs. We have previously described a series of ureido-fibrates (Chart 1) with either potent PPAR activity[18,19] or potent lipid-lowering activity in hyperlipidemic rats.[20] Four of the compounds (**4**−**7**) with good in vivo activity were assayed for their PPAR agonist activity (Table 1). These ureido-fibrates (**4**−**7**) were potent agonists of murine PPARα. However, like fibrates (**1**−**3**), they showed only moderate levels of subtype selectivity. In addition, all four ureido-fibrates

* Address correspondence to Peter J. Brown, Department of Medicinal Chemistry, NTH-M1108, Glaxo Wellcome Research & Development, P.O. Box 13398, 5 Moore Dr., Research Triangle Park, NC 27709-3398. Tel: (919) 483-1195. Fax: (919) 315-3900. E-mail: pjb5890@glaxowellcome.com.
† Department of Medicinal Chemistry.
‡ Department of Metabolic Diseases.
§ Department of Molecular Endocrinology.
∥ College of Pharmacy.

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**Table 1.** PPAR and Lipid-Lowering Activity of Fibrates

| compd[a] | | | | murine receptor activity[b] $EC_{50}$ ($\mu$M) | | | human receptor activity[b] $EC_{50}$ ($\mu$M) | | | MED[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| no. | X | $R_1$ | $R_2$ | PPARα | PPARγ | PPARδ | PPARα | PPARγ | PPARδ | (mg/kg) |
| **1b** | | | clofibric acid | 50 | >300 | ia | 55 | >300 | ia | nd |
| **2b** | | | fenofibric acid | 18 | 250 | ia | 30 | 300 | ia | 50 |
| **3** | | | bezafibrate | 90 | 55 | 110 | 50 | 60 | 20 | 50 |
| **4** | O | F | $cC_7H_{13}$ | 0.12 | 0.012 | 1.4 | 1.4 | 0.006 | 0.79 | 1.0 |
| **5** | O | H | $nC_7H_{15}$ | 0.033 | 0.87 | 5.5 | 0.41 | 0.28 | 3.1 | 0.1 |
| **6** | O | H | $(CH_2)_5cC_6H_{11}$ | 0.053 | 1.0 | 23 | 0.15 | 1.6 | nd | 1.0 |
| **7** | O | F | $nC_7H_{15}$ | 0.010 | 0.40 | 3.2 | 0.79 | 0.20 | nd | 0.1 |
| **8** | S | F | $nC_7H_{15}$ | 0.005 | 1.5 | 2.6 | 0.05 | 1.0 | 1.4 | 0.2 |

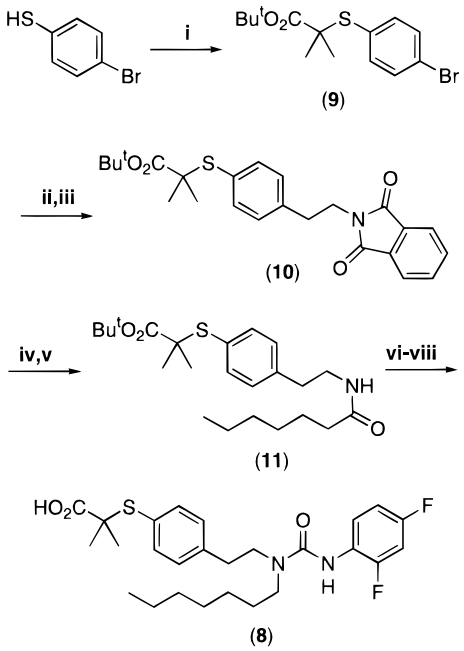
[a] Compound structures in Chart 1. [b] Compounds were assayed for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected CV-1 cells as described (refs 14, 15, and 19); $EC_{50}$ = the concentration of test compound that gave 50% of the maximal reporter activity ± 10%, $n$ = 4−6. All compounds were full agonists unless indicated; >300 = agonist activity observed only at 300 $\mu$M.; ia = inactive at 300 $\mu$M.; nd = not determined. [c] Lipid-lowering activity in male Sprague−Dawley rats fed a 1% cholesterol, 0.5% cholic acid diet. Animals ($n$ = 6/group) were dosed orally once daily for 3 days with either vehicle (5% bicarbonate) or doses of test compound ranging from 0.1 to 50 mg/kg as described (ref 20). Fenofibrate (**2a**) and the dicyclohexylamine salt of **8** were used for the in vivo studies; all other compounds (**3**−**7**) were the free acids. MED = minimum effective dose producing a 40−60% decrease in serum TLDL cholesterol relative to vehicle-treated controls, where TLDL cholesterol = (VLDL + LDL) cholesterol.

(**4**−**7**) were less potent as activators of human PPARα compared to murine PPARα, further eroding their PPARα selectivity on the human receptors. Ureido-fibrate **6** was the only analogue that showed moderate PPARα selectivity on both the murine and human receptors. Ureido-fibrates **5** and **7** were moderately PPARα-selective on the murine receptors, but dual PPARα/PPARγ agonists on the human receptors. Ureido-fibrate **4** was more potent on PPARγ than PPARα, especially on the human receptors where its profile is comparable to that of the TZD antidiabetic drugs.[13]

Previous studies[19] have shown that ureido-fibrate analogues (Chart 1, X = O), with modified urea substituents generated by solid-phase parallel synthesis,[21] do not show increased PPARα selectivity. However, through conventional analogue synthesis we discovered that modification of the fibrate headgroup to a thioisobutyric acid (TiBA; Chart 1, X = S) increased PPARα activity relative to PPARγ and PPARδ (Table 1, **7** vs **8**). Ureido-TiBA **8** (GW9578) is a potent PPARα agonist with 300-fold selectivity on the murine receptors and 20-fold selectivity on the human receptors. Scheme 1 shows the synthesis of the ureido-TiBA **8**. Briefly, 4-bromothiophenol was alkylated with *tert*-butyl bromoisobutyrate to give the bromo ester **9** in good yield. Heck reaction with vinylphthalimide and hydrogenation of the olefin gave intermediate **10**. Following phthalimide deprotection, the resulting primary amine was coupled with heptanoic acid to afford the amide **11**. Borane reduction furnished the secondary amine which was treated with 2,4-difluorophenyl isocyanate to give the ureido-TiBA **8** following TFA cleavage of the *tert*-butyl ester.

To assess the potential utility of **8** as a lipid-lowering drug, its activity was compared to that of fibrates **2**−**7** in the cholesterol/cholic acid-fed rat.[20] Reduction of total low-density lipoprotein (TLDL) cholesterol in this model has been shown to correlate with the antihyperlipidemic activity of fibrates in humans.[22] In this model,[20] fenofibrate (**2a**) and bezafibrate (**3**) produced a 40−60% decrease in TLDL cholesterol only at the highest dose tested, while the ureido-fibrates **4**−**7** and ureido-TiBA **8** were active at 50−500-fold lower doses (Table 1). Several lines of evidence suggest that the lipid-lowering activity of **8** is due to its potent PPARα activity. First, the analogues **5**, **7**, and **8** with the best PPARα activity

**Scheme 1**[a]




[a] Reagents: (i) $BrC(Me)_2CO_2tBu$, KOH, EtOH (80%); (ii) vinylphthalimide, $Pd(OAc)_2$, DIEA, $(o\text{-}Tol)_3P$, MeCN (84%); (iii) Wilkinson's catalyst, EtOH, $H_2$ (91%); (iv) hydrazine, EtOH (86%); (v) heptanoic acid, DIC, HOBT (66%); (vi) 1 M $BH_3$·THF (95%); (vii) 2,4-difluorophenyl isocyanate, $CH_2Cl_2$ (83%); (viii) 50% TFA/$CH_2Cl_2$ (90%).

were active at doses as low as 0.1−0.2 mg/kg. Second, the ureido-fibrate **4**, which possesses potent activity on PPARγ in addition to PPARα, did not perform significantly better than analogues **5**−**8**. Third, comparison of the in vitro murine PPARα activity with the in vivo potency of **2**−**8** provided a strong correlation (Figure 1). Similar associations could not be drawn with either PPARγ or PPARδ (Table 1).

Finally, to confirm that the observed lipid-lowering activity in the cholesterol/cholic acid-fed rat was mediated through a clinically relevant mechanism,[2] the effects of fenofibrate (**2a**) and ureido-TiBA **8** on serum apoC-III and TLDL cholesterol were determined (Figure 2). A dose-dependent reduction in TLDL cholesterol was observed with both compounds, reaching 50% lowering at 30 mg/kg fenofibrate (**2a**) (Figure 2a) and 60%

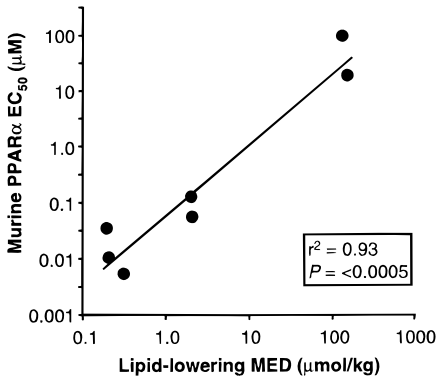



**Figure 1.** Correlation between PPARα and lipid-lowering activity.

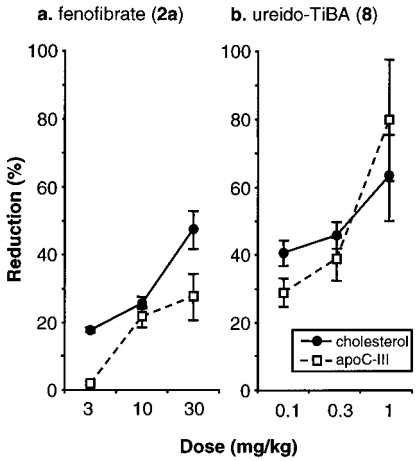



**Figure 2.** Effects of fenofibrate (**2a**) and ureido-TiBA **8** on serum apoC-III and TLDL cholesterol. Male Sprague−Dawley rats ($n$ = 6/group) fed a 1% cholesterol, 0.5% cholic acid diet were dosed orally twice daily for 4 days with either vehicle (0.5% methylcellulose) or increasing doses of fenofibrate (**2a**) or the dicyclohexylamine salt of **8**. Serum TLDL cholesterol was determined as described (ref 20). Serum apoC-III was quantitated by a noncompetitive ELISA using a polyclonal goat antibody against rat apoC-III. Data are expressed as percent reduction compared to vehicle-treated animals ± standard error.

lowering at 1.0 mg/kg **8** (Figure 2b). Analysis of serum apoC-III levels revealed a dose-dependent decrease with both compounds, reaching a maximal reduction of ∼30% with fenofibrate (**2a**) and ∼80% with **8**. Thus, activation of PPARα in the cholesterol/cholic acid-fed rat results in changes in serum apoC-III which are consistent with the pharmacology of fibrates in humans.[6] The increased efficacy of ureido-TiBA **8** compared to fenofibrate (**2a**) for reduction of serum apoC-III is likely due to its increased potency on PPARα.

In summary, the ureido-TiBA **8** is the most potent PPARα agonist reported to date. It exhibits excellent subtype selectivity on the murine receptors and moderate selectivity on the human receptors. In addition to its lipid-lowering activity, **8** prevents weight gain and the development of hyperinsulinemia in insulin-resistant rats.[23] Since hyperlipidemia, obesity, and insulin resistance are independent risk factors for coronary heart disease,[1] our results suggest that development of potent human PPARα-selective agonists may lead to improved drugs for primary prevention of cardiovascular mortality.[24]

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**Supporting Information Available:** Detailed experimental procedures for the synthesis of **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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