Design and Synthesis of a Potent and Selective Triazolone-Based Peroxisome Proliferator-Activated Receptor α Agonist

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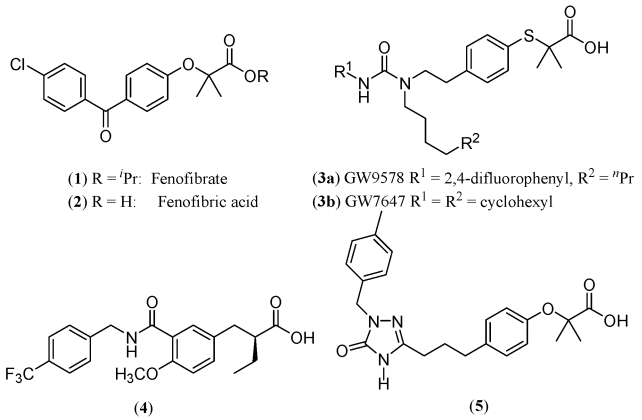
Abstract: A new series of hPPARα agonists containing a 2,4-dihydro-3*H*-1,2,4-triazol-3-one (triazolone) core is described leading to the discovery of **5** (LY518674), a highly potent and selective PPARα agonist.

Introduction. Coronary heart disease (CHD) remains the leading cause of death in the developed world and is linked to a number of associated risk factors including hypertriglyceridemia and hypercholesterolemia. As members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, the peroxisome proliferator-activated receptors (PPARs) regulate a multitude of cellular processes including the storage and catabolism of dietary fats and carbohydrates.[1] Three mammalian PPAR isotypes have been identified (PPARα, PPARγ, and PPARδ), and each receptor displays distinct tissue-selective expression patterns, ligand selectivity, and biological actions.[2] Following activation of the receptor by ligand binding, PPARs heterodimerize with the 9-*cis*-retinoic acid receptor (RXR).[3] Upon binding of the heterodimer receptor complex to peroxisome proliferator response elements (PPREs) located in the regulatory regions of the target genes and cofactor recruitment, gene transcription of proteins involved in lipid metabolism and homeostasis is stimulated.[4,5] PPREs have been identified in the promoter regions of several genes which encode proteins involved in lipid and lipoprotein metabolism, such as acyl-CoA oxidase (AOX),[6] liver-fatty acid-binding protein (L-FABP),[7] apolipoprotein C−III (apo C−III),[8] and lipoprotein lipase (LPL).[9]

For several years, the fibrates (Figure 1, e.g., fenofibrate, **1**) have been broadly utilized for the clinical treatment of dyslipidemia and remain the current treatment of choice for patients with severe hypertriglyceridemia.[10,11] These agents can effectively decrease serum triglycerides and elicit increases in HDL-cholesterol concentrations in humans.[10] Several studies have provided evidence that the hypolipidemic effect of this class of drugs is caused by their agonist at the PPARα receptor.[12] In humans, the triglyceride-lowering effect of fibrates is attributed to the activation of PPARα which in turn effects an increase in lipoprotein lipase gene expression and transrepression of apoC−III.[13] PPARα-mediated elevation of HDL-cholesterol levels observed with fibrates arises in part from the transcriptional induction of the major HDL apolipoproteins, apoA-I and apoA-II.[13] Although fibrates are ligands for the PPARα receptor, their activity and subtype selectivity is moderate. Therefore, a need exists for more potent and subtype-selective human PPARα agonists. Such agents could provide a superior clinical profile for the therapeutic intervention of dyslipidemia. Recent disclosures of highly selective and potent human PPARα agonists attests to the heightened level of interest in this arena. For example, GW9578 (**3a**)[14a] and GW7647 (**3b**) (Chart 1),[14b] selective hPPARα agonists, were found to possess potent lipid-lowering activities in the cholesterol/cholic acid-fed rat. Additionally, the Kyorin group has reported a nonfibrate phenylpropanoic acid derivative **(4)**[15] that is a potent hPPARα agonist in vitro with selectivity for PPARα over PPARγ. As part of our research directed toward the development of subtype-selective modulators of the PPAR receptor family, we have discovered a series of novel hPPARα agonists possessing a 2,4-dihydro-3*H*-1,2,4-triazol-3-one (triazolone) core that display potent and selective binding affinity and functional activity on the hPPARα receptor subtype and which exhibit highly potent in vivo efficacy in a human apoA-I transgenic mouse model. This account describes the synthesis and structure−activity relationships of this series of hPPARα agonists which culminated in the discovery of **5**.

Chart 1. Structures of PPARα Agonists



Chemistry. Two different synthetic approaches were utilized as shown in Scheme 1. Demethylation of ether **6b** followed by alkylation with methyl or *tert*-butyl bromoisobutyrate provided a diester which upon treatment with hydrazine hydrate afforded hydrazide **7**. Installation of the R1 group using the appropriate isocyanates afforded acylsemicarbazide **8**. Ring construction under KOH/MeOH led to the triazolone core

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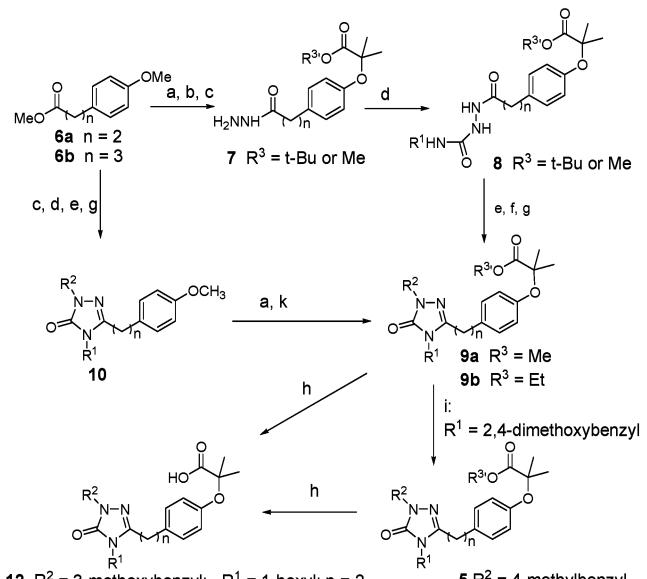
Scheme 1[a]



[a] Reagents and conditions: (a) BBr_3, CH_2Cl_2, 0 °C; (b) $BrC(Me)_2CO_2tBu$, K_2CO_3, $MgSO_4$, DMF, 75 °C; (c) hydrazine hydrate, CH_3OH, rt; (d) R^1NCO, THF, rt; (e) KOH, CH_3OH, reflux; (f) CH_3OH, H_2SO_4, rt; (g) R^2Br or R^2Cl, K_2CO_3, DMF, 45 °C; (h) 2N NaOH, CH_3OH, rt; (i) HBr/HOAC, rt; CH_3OH, H_2SO_4, rt. (j) R^1I, or R^1Br, K_2CO_3, DMF, 45 °C; (k) $BrC(Me)_2CO_2Et$, K_2CO_3, $MgSO_4$, DMF, 75 °C.

with concomitant hydrolysis of the methyl or *tert*-butyl ester.[16,17,18] The acid was re-esterified with methanol and sulfuric acid. Triazolone **9a** (R^2 = H) was employed as a late stage intermediate for introduction of various alkyl groups at the N^2 position. Regioselective N^2 alkylation of **9a** occurred by treatment with appropriate benzyl or alkyl halides in the presence of K_2CO_3 in DMF.[19] Final products **14**−**19** were prepared by ester hydrolysis of the appropriately substituted ester intermediate **9a**. This method served a convergent approach to the analogues that allowed us to explore the receptor pocket at R2 region.

To explore various R1 groups exemplified by compound **5**, **20**, and **21**, an N^4-dimethoxybenzyl (DMB) group was employed on triazolone **9a** as an acid-labile protecting group. Using the same reaction sequence described above, N^4-2,4-DMB-protected triazolone **9** was prepared from 2,4-dimethoxybenzyl isocynate. An acid-mediated cleavage of the N^4-2,4-DMB group was accomplished by using HBr/HOAc with concomitant ester hydrolysis to afford the carboxylic acid **5**. To explore various R1 groups, acid **5** was re-esterified under Fisher esterification conditions to afford the N^4-unsubstituted triazolone **11a**. Ester **11a** was regioselectively alkylated at the triazolone N^4 position by treatment with appropriately substituted alkyl bromides in the presence of K_2CO_3 in DMF to afford intermediate **11b.**[16] Saponification of the ester **11b** provided the desired products (**20** and **21**).

Products can also be synthesized via intermediate **10** as shown in Scheme 1. Thus, methyl 3-methoxyphenylpropionate **6a** was treated with hydrazine hydrate, followed by the appropriate isocyanate to give acylsemicarbazides. Ring cyclization was achieved under KOH/MeOH led to the triazolone core. Demethylation of **10** with BBr_3 afforded the phenol, which was alkylated using ethyl bromoisobutyrate to give triazolone ester **9b**. This latter intermediate provided acids **12** and **13** upon alkaline hydrolysis.

Results and Discussion. The 2,4-dihydro-3*H*-1,2,4-triazol-3-ones were evaluated for in vitro potency and selectivity by direct receptor binding (expressed as IC_{50} for displacement of radio-labeled reference compound) as well as by cell-based assays to determine their functional activity. The assays are transient transfection assays in which the gene for the nuclear receptor(s), as well as a plasmid containing a response element upstream of a luciferase cDNA, are transfected into CV-1 cells. Luciferase transcription occurs after activation of the nuclear receptor(s) by binding of an agonist to the receptor. For each compound, we determine an EC_{50} as well as a percent maximum efficacy relative to a reference compound.

The original screening lead **12** possessed a disubstituted triazolone tethered by a two-carbon atom alkyl linker to a phenoxyisobutyrate (fibrate) moiety. This compound possessed μM activity (EC_{50}) on both hPPARα and hPPARγ receptors. Investigation of the substituent effects on the triazolone N^2 and N^4 positions on this two-atom linker ($n = 2$) lead series did not lead to improvements in vitro (cf. **13**). However a dramatic 450-fold increase in hPPARα agonist activity (EC_{50}) was observed by homologating the linker length to three carbons (see **15**, $n = 3$ vs **13**, $n = 2$). Compound **15** retains significant selectivity over hPPARγ and δ in terms of both binding affinity and agonist activity. Upon the basis of this result, efforts were concentrated on the three carbon linker series. An early indication of the critical requirement for substitution at the triazolone N^2 position within this series was provided by the observation of a complete loss of binding affinity and functional activity across all three receptor subtypes when this position was unsubstituted (R^2 = H) or substituted by small alkyl groups such as n-Pr, regardless of the substitutents on N^4 (data not shown). In general, an N^2-benzyl substituent was consistent with activity. Furthermore, activity was maintained or increased by utilizing relatively nonpolar substituents at the 3- or 4-positions of the benzyl moiety (c.f. **14**, **15**, **18**, and **19**). The chain-extended phenethyl analogue **16** afforded a more than 9-fold reduction in both hPPARα binding affinity and transactivation potency relative to compound **15**, however remained devoid of cellular activity at concentrations up to 10 μM on the other PPAR receptor subtypes. The cyclohexylmethyl analogue **17** resulted in a similar reduction in activity over the substituted benzyl derivatives **14** or **15**. Compound **18** (R^2 = *p*-*tert*-butylbenzyl, R^1 = Me) displayed the greatest hPPARα functional potency (EC_{50} = 2 nM) and was >700-fold more selective for hPPARα over the other subtypes from a functional perspective (EC_{50}). Substituent effects at the N^4 position were less pronounced compared to those at the N^2 position. For example,

Table 1. Binding IC_{50}[a] and Cotransfection EC_{50}[b] Data[c] on Human and PPAR Receptor Subtypes

	hPPARα[f]		hPPARγ[g]		hPPARδ[f]	
no.	IC_{50} (nM)	EC_{50} (nM)[d]	IC_{50} (nM)	EC_{50} (nM)	IC_{50} (nM)	EC_{50} (nM)[d]
2	24000 ± 290	13000 ± 260	n.b.	n.a.	n.b.	n.a.
3a	120 ± 11	340 ± 130	550 ± 55	1900 ± 160	[e]	[e]
12	[e]	2200	[e]	2600	[e]	[e]
13	[e]	2800 ± 61	[e]	2900 ± 78	[e]	[e]
14	19 ± 2	31 ± 6	6200 ± 480	n.a.	2000 ± 220	n.a.
15	16 ± 2	6 ± 1	4900 ± 1000	2700 ± 63	1200 ± 120	3000 ± 19
16	220 ± 19	220 ± 15	6100 ± 720	n.a.	1500 ± 200	n.a.
17	250 ± 52	56 ± 7	1700 ± 120	2600 ± 47	880 ± 31	3000 ± 20
18	15 ± 1	2 ± 0	520 ± 27	1500 ± 130	1700 ± 15	3000 ± 19
19	12 ± 1	4 ± 1	1700 ± 190	2300 ± 80	740 ± 70	2900 ± 8
20	27 ± 2	88 ± 26	6100 ± 350	n.a.	4100 ± 330	n.a.
21	960 ± 48	2700 ± 36	7900 ± 540	n.a.	n.b.	n.a.
5	24 ± 3	42 ± 5	6500 ± 686	n.a.	3800 ± 1000	n.a.

[a] Concentration of test compound required to displace 50% of tritiated ligand. [b] Concentration of test compound which produced 50% of the maximal reporter activity. [c] $n = 3-6$; n.b. = no binding; n.a. = efficacy relative to control was less that 20% at 10 μM. [d] Gal4-hPPARα was used to eliminate interference by endogenous PPARγ receptors in CV-1 cells. [e] Not determined. [f] Tritium-labeled PPARα/PPARδ agonist, 2-(4-{2-[3-(2,4-difluorophenyl)-1-heptylureido]ethyl}phenoxy)-2-methylbutyric acid, was used as radioligand for generating displacement curves and IC_{50} values. [g] Tritium-labeled PPARγ agonist, 5-{4-[2-(methylpyridin-2-ylamino)ethoxy]benzyl}thiazolidine-2,4-dione, was used as radioligand for generating displacement curves and IC_{50} values.

compound **20** (R^1 = hydroxyethyl) suffered a minor loss in hPPARα functional potency compared to compound **15**; however, incorporation of an acetic acid moiety at this position (**21**) resulted in a sharp loss in hPPARα affinity and potency. The unsubstituted analogue **5** was slightly less active on hPPARα (EC_{50} = 42 nM) when compared to the N^4-propyl analogue **15** (EC_{50} = 6 nM). Triazolones **5** and **15** demonstrated sufficient potency and selectivity for the hPPARα receptor over other hPPAR isoforms to warrant further investigation.

Further selectivity of compounds **5** and **15** was examined using a panel of nuclear transcription factors. These compounds either failed to bind or displayed poor affinity (K_i > 10 μM) to the retinoic acid receptor (RARα, RARβ, RARγ), retinoic acid X receptor (RXRα, RXRβ, RXRγ), glucocorticoid receptor, and thyroid receptor (TRα and TRβ). Furthermore, cotransfection assays for RARα and RXRα demonstrated a lack of agonist activity for **5** and **15** at these receptors.

Triazolones **5** and **15**, as well as fenofibrate **1**, were evaluated in human apoA-I transgenic mice for their ability to alter serum triglyceride and HDL-cholesterol levels. Introduction of the promoter and transgene for human apolipoprotein A−I into the mouse represents a more humanlike model in which PPARα agonists cause induction, rather than repression, of apolipoprotein A−I, thus elevating serum HDL-cholesterol.[20] Since this mouse model relies on endogenous PPARα to interact with the human apoA-1 promoter, the in vitro activity at the murine PPARα receptor was determined.[21] In cotransfection assays containing the murine PPARα receptor, compounds **5** and **15** exhibited EC_{50} values of 1052 ± 30 and 390 ± 63 nM, respectively. After oral administration once daily at 3 mg/kg for one week, compound **5** produced a 208% elevation in HDL-cholesterol (ED_{50} = 0.3 mg/kg) and a 96% decrease in serum triglycerides (ED_{50} = 0.10 mg/kg), relative to control levels. Compound **15** showed similar efficacy and potency in this model. The ED_{50}'s for both HDL-cholesterol elevation and triglyceride reduction for **5** are 2−3 orders of magnitude more potent than the corresponding values for fenofibrate **1**. Pharmacokinetic studies conducted in Beagle dogs and F344 rats with **5** and **15** indicated that while both compounds had good oral bioavailability (>50%), **5** had higher exposure following oral dosing and a longer plasma half-life than compound **15** in both species. These pharmacokinetic differences between **5** and compound **15** have been attributed primarily to the higher clearance observed for compound **15** in Beagle dogs and F344 rats.

Table 2. Efficacy in Human ApoA-I Transgenic Mice[a]

	HDL-c elevation		triglycerides lowering
no.	ED_{50} (mg/kg)	max. (% increase)	ED_{50} (mg/kg)
1	110 ± 76	120 ± 15	79 ± 21
5	0.30 ± 0.1	210 ± 15	0.10 ± 0.04
15	0.70 ± 0.2	200 ± 17	0.50 ± 0.21

[a] Dosed 7 days q.d. by gavage, serum parameters determined 3 h after the last dose. ED_{50}s and maximal % increase in HDL-c were obtained by nonlinear regression analysis of dose−response curves.

In summary, we have identified a novel series of 2,4-dihydro-3*H*-1,2,4-triazol-3-ones as potent and selective hPPARα agonists. This work led to the discovery of LY518674 (**5**), which has been selected for clinical studies. A full account of the structure−activity relationships within this series will be published in due course.

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Supporting Information Available: Comparative pharmacokinetic data, experimental details for the binding assays, cotransfection assays, and the synthetic procedures for intermediates and final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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