Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3329–3334

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NOVEL SERIES OF INDOLE-DERIVED PPARγ AGONISTS

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Received 13 September 1999; accepted 25 October 1999

Abstract: The synthesis and structure–activity relationships of a novel series of indole 5-carboxylic acids that bind and activate peroxisome proliferator-activated receptor gamma (PPARγ) are reported. These new analogs are selective for PPARγ vs the other PPAR subtypes, and the most potent compounds in this series are comparable to in vitro potencies at PPARγ reported for the thiazolidinedione-based antidiabetic drugs currently in clinical use.

Introduction

Peroxisome Proliferator-Activated Receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors, a family that includes the receptors for steroid hormones, retinoids, thyroid hormone, and vitamin D. Three mammalian PPARs have been identified to date, termed PPARα, PPARγ, and PPARδ. The physiological role of the PPARs as regulators of lipid and glucose metabolism has been the subject of several recent reviews.[1–5] The PPARγ subtype is predominantly expressed in adipose tissue and plays a pivotal role in adipocyte differentiation in vitro, suggesting that the PPARγ is an important component in the adipogenic signaling cascade and in lipid storage and utilization.[6] A number of naturally occurring fatty acids, eicosanoids, prostaglandins, and their metabolites have been shown to activate PPARγ, consistent with the hypothesis that fatty acids or their metabolites may be naturally occurring PPARγ ligands.[2] A group of synthetic compounds, the thiazolidinediones (TZDs), have also been discovered to function as high affinity PPARγ agonists.[7,8] These TZDs are potent antidiabetic agents with the ability to enhance the action of endogenous insulin and normalize elevated plasma glucose, insulin and lipid levels in rodent models of type 2 diabetes. Three TZDs—troglitazone,[9] pioglitazone,[10] and rosiglitazone[11] (Figure 1) are now approved for use in humans as antihyperglycemic agents in the treatment of type 2 diabetes.

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00603-4

In addition to the TZDs, other structurally diverse synthetic PPARγ agonists have been identified. NSAIDS such as indomethacin (Figure 1) and ibuprofen have been shown to activate PPARγ at micromolar concentrations.[12] Several series of α-substituted-β-phenylpropanoic acids have also been reported to act as PPARγ agonists.[13–15] We recently reported a series of L-tyrosine derivatives, exemplified by GW1929 (Figure 1), which function as potent, selective PPARγ agonists.[16–18] As part of our ongoing program in the identification of novel activators of the PPAR family we have discovered a series of 2,3-disubstituted indole 5-acetic acids (e.g., **13**, Figure 1) which selectively bind to and activate PPARγ. In this paper we report the synthesis and initial structure-activity relationships of these novel PPARγ agonists.

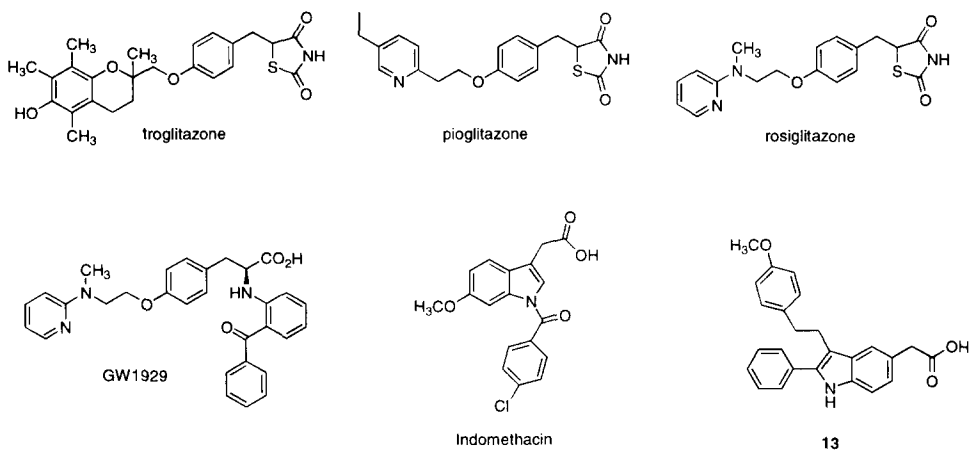


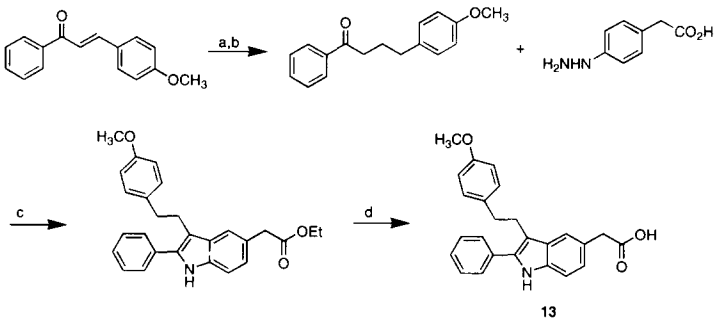
Figure 1. Representative PPARγ agonists

Chemistry

The general preparation of most analogs listed in Table 1 utilized a Fischer indole synthesis[19] of the appropriately substituted ketone and aryl hydrazine, followed by simple functional group transformation if needed. The starting ketones, if not commercially available, were prepared in a few steps via known methodology. The synthesis of compound **13** (Scheme 1) serves as a representative example. Commercially available 2*E*-3-(4-methoxyphenyl)-1-phenylpropenone was converted to the cyclopropyl derivative via the methodology of Corey[20] and then reductively cleaved with Zn-$ZnCl_2$ in ethanol[21] to provide the requisite ketone. Treatment of a mixture of this ketone and 4-hydrazinophenylacetic acid[22] with $ZnCl_2$ in refluxing ethanol afforded the desired indole in good yield as the corresponding ethyl ester. Hydrolysis of the ester using 1 M NaOH in ethanol then provided compound **13**.

Biology

Compounds **1–13** were first tested for their ability to bind to human PPARγ ligand binding domain using a Scintillation Proximity Assay (SPA). The details of this assay have been reported elsewhere.[23] Compounds were also screened for functional potency in a transient transfection assay in CV-1 cells for their ability to activate the three human PPAR subtypes (transactivation assay). A previously established chimeric receptor system[7] was utilized to allow comparison of the relative transcriptional activity of the three receptor subtypes on the same target gene and to prevent endogenous receptor activation from complicating the interpretation of results. Rosiglitazone (BRL 49653) was used as a positive control in all PPARγ transactivation assays, and compounds that elicited at least 70% activation of the receptor vs. rosiglitazone and were considered full agonists. Details of the transactivation assay protocols and controls for the other two PPAR subtypes have been described previously.[24]

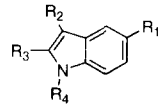


Scheme 1. Reagents and conditions: (a) NaH, $(CH_3)_3SOI$, DMSO, rt, 72%. (b) Zn/$ZnCl_2$, EtOH, reflux, 85%. (c) $ZnCl_2$, EtOH, reflux, 74%. (d) 1 M NaOH, EtOH, rt, 92%.

Results and Discussion

Table 1 summarizes the affinity and potency of activation at PPARγ for a series of indole-5-acetic acid derivatives **1–13**. *Importantly, at the highest concentrations tested (10 μM) all compounds were devoid of affinity and functional activity at the other two PPAR subtypes, PPARα and PPARδ* (data not shown). The original screening hit **1** contained an acetic acid moiety at the 5 position of the indole ring and was substituted by a phenyl ring and a propyl chain at indole carbons 2 and 3, respectively. This compound displays only micromolar affinity and potency of activation at PPARγ. Removal of the methylene spacer to provide the indole 5-carboxylic acid derivative **2** resulted in no increase in affinity and a drop in cell-based potency. However, replacement of the propyl side chain with phenethyl to afford compound **3** resulted in greater than 10-fold increase in both binding affinity and potency of activation at PPARγ. Attempts to modify the acetic acid within this 3-phenethyl-substituted series by chain extension with a methylene (**4**) or oxygen (**5**) resulted in a loss of

Table 1. In Vitro Profile of Indole-based PPARγ Agonists **1–13**.



	Structure[a]				Binding[b]	Transactivation[c]
no.	R_1	R_2	R_3	R_4	PPARγ pK_i	PPARγ pEC_{50}
1	$-CH_2CO_2H$	$-(CH_2)_2-CH_3$	Ph	H	5.41 ± 0.22 (3)	5.15 ± 0.08 (3)
2	$-CO_2H$	$-(CH_2)_2-CH_3$	Ph	H	5.43 ± 0.21 (3)	<5.00 (3)
3	$-CH_2CO_2H$	$-(CH_2)_2-Ph$	Ph	H	6.83 ± 0.05 (3)	6.51 ± 0.24 (3)
4	$-(CH_2)_2-CO_2H$	$-(CH_2)_2-Ph$	Ph	H	5.14 ± 0.13 (3)	5.52 (1)
5	$-OCH_2CO_2H$	$-(CH_2)_2-Ph$	Ph	CH_3	6.67 ± 0.06 (3)	<5.00 (3)
6	$-CH_2$-TZD[d]	$-(CH_2)_2-Ph$	Ph	H	5.72 ± 0.06 (3)	<5.00 (3)
7	$-CH_2CO_2H$	$-CH_2Ph$	Ph	H	IA[e]	<5.00 (3)
8	$-CH_2CO_2H$	-Ph	Ph	H	<5.00 (3)	<5.00 (3)
9	$-CH_2CO_2H$	H	Ph	H	5.35 ± 0.30 (3)	IA[e]
10	$-CH_2CO_2H$	$-(CH_2)_2-Ph$	H	H	<5.00 (3)	<5.00 (3)
11	$-CH_2CO_2H$	$-(CH_2)_2-Ph$	Ph	CH_3	6.26 ± 0.05 (3)	5.25 ± 0.27 (3)
12	$-CH_2CO_2H$	$-(CH_2)_2-Ph(p-F)$	(*p*-F)Ph	H	6.31 ± 0.06 (3)	6.47 ± 0.19 (3)
13	$-CH_2CO_2H$	$-(CH_2)_2-Ph(p-OCH_3)$	Ph	H	7.32 ± 0.07 (3)	7.36 ± 0.21 (10)
Troglitazone[f]		------	-----	---	6.52 ± 0.06 (3)	6.27 ± 0.07 (3)
Pioglitazone[f]		------	-----	---	5.91 ± 0.02 (3)	6.23 ± 0.07 (3)
Rosiglitazone[f]		------	-----	---	7.33 ± 0.02 (3)	7.05 ± 0.07 (14)

[a]See Figure. [b]pK_i = -log apparent K_i. The values for K_i were obtained from least squares fit of the concentration response curves according to the equation $-b = -b_0 / 1 + [L]/K_i$ where b_0 = the counts bound in the absence of test compound and b = counts bound in the presence of test compound at concentration [L] ± standard deviation (number of determinations); [c]pEC_{50}, -log of the concentration of test compound required to induce 50% of the maximum alkaline phosphatase activity ± standard deviation (number of determinations); [d]TZD = 2,4,-thiazolidinedione;[e]IA = inactive at 10^{-4} M. [f]see reference 13.

cell potency. Interestingly, however, compound **5** showed similar receptor binding affinity to **3**. We hypothesize that the loss of cell potency with **5** is due to increased metabolic lability associated with the alkoxyacetic acid side chain. Replacement of the carboxylic acid moiety in **3** with a 2,4-thiazolidinedione (TZD) group to provide compound **6** resulted in a considerable loss of activity. This is not surprising since literature

reports suggest that replacement of the TZD group with a carboxylic acid seemed to require an additional methylene unit for optimal activity.[13,15,16] Thus it might be expected that the direct replacement with the TZD group is non-optimal and would best be attached directly to the indole ring; unfortunately, this compound was not readily accessible using the described synthetic methodology.

Removal of the phenyl group from the 2-position of the indole ring to provide compound **10** resulted in complete loss of biological activity. In addition, methylation of the indole nitrogen reduced both binding affinity and functional potency at PPARγ (compare entries **3** and **11**). No additional analogs were prepared at either of these two positions.

Modifications at the 3-position of the indole ring were also briefly evaluated. Removal of one or both methylene units to provide the benzyl- and phenyl-substituted analogs **7** and **8**, respectively, resulted in complete loss of biological activity. Not surprisingly, removal of the 3-substituent altogether (compound **9**) also led to a loss of activity. Substitution at the 4-position of the phenyl ring with fluorine to afford compound **12** maintained but did not enhance affinity and cell potency (compare entries **3** and **12**). Interestingly, substitution at the 4-position with a methoxy group (**13**) increased both binding affinity and functional potency. Compound **13** displays a K_i and an EC_{50} of 50 nM at human PPARγ, which is more potent than both troglitazone and pioglitazone and is equipotent with rosiglitazone in this assay.[16] In addition, this compound did not bind or activate either PPARα or PPARδ at concentrations up to 10^{-4} M, suggesting that **13** is quite selective for PPARγ over the other two receptor subtypes.

On the basis of its in vitro potency and selectivity at PPARγ, the pharmacokinetic profile of **13** was evaluated in the rat. Compound **13** displayed excellent pharmacokinetics in the rat, with a low mean total body clearance (CL_{TOT} = 3 mL/min/kg) and good half-life ($t_{1/2}$ = 3.8 h). The mean oral bioavailability of compound **13** in the rat was 78% dosed as a solution and 73% dosed as a suspension.

In summary, we have developed a novel series of 2,3-disubstituted indole 5-phenylacetic acid derivatives that function as selective PPARγ agonists in vitro. The most potent compound of this series, 2-{3-[2-(4-methoxyphenyl)ethyl]-2-phenylindol-5-yl} acetic acid (**13**) has an in vitro potency at PPARγ similar to or better than the three currently marketed thiazolidinedione antidiabetic agents. In addition, compound **13** displays an excellent pharmacokinetic profile in the rat, suggesting that it would be a suitable candidate for oral administration. The in vivo antidiabetic activity of this novel series of PPARγ agonists in animal models of insulin resistance is under investigation.

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