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Selective peroxisome proliferator-activated receptor δ isosteric selenium agonists as potent anti-atherogenic agents in vivo

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ABSTRACT

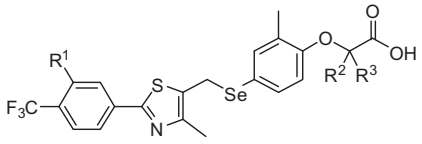
We report the synthesis and in vivo activity of a novel anti-atherogenic agent, isosteric selenium PPARδ-selective ligand. This ligand did not cause significant body or liver weight changes and did not have obvious adverse effects on intestinal polyp formation. Our overall results clearly demonstrate that PPARδ is a viable drug candidate for targeting and treating atherosclerosis.

Atherosclerosis is a disease characterized by enlarged arterial blood vessels that are induced by the dysfunction of lipid metabolism. This disease progresses via chronic inflammation resulting from the interaction between lesional lipoproteins and immune cells.[1] The peroxisome proliferator-activated receptors (PPARs) are transcription factors that are activated by their cognate ligands and act as master regulators in mammalian physiology and development.[2] Among the three different isoforms (PPAR-α, -γ, and -δ), PPARδ regulates lipid homeostasis and inflammation in the human body, and the impairment of these processes can lead to atherosclerosis.[3] Several studies have shown that the activation of PPARδ promotes fatty acid catabolism and reverses cholesterol transport and represses inflammatory gene expression. These effects suggest a beneficial role of PPARδ-selective agonists in the treatment of atherosclerosis.[4–6]

Prior research has shown that the activation of PPARδ by GW0742,[7] a PPARδ agonist, increases the level of high-density lipoprotein-cholesterol (HDL-c) in DBA/1 mice.[8] Considering the atheroprotective role of HDL-c,[9] it was expected that the PPARδ-selective agonist would have anti-atherogenic potential.

However, recent studies have produced incompatible results regarding this prediction. In a study by Li et al., there was no obvious inhibitory anti-atherogenic effect of the PPARδ agonist, independent of the PPARα and PPARγ agonists, in *LDLR*$^{-/-}$ mice.[10] In contrast, Graham et al. demonstrated the anti-atherogenic potential of the PPARδ agonist in *LDLR*$^{-/-}$ mice.[11] However, the inhibitory effect was only observed with a high dose regimen (60 mg/kg) or long-term treatment (16 weeks) and was associated with the adverse effect of increased liver weight. The compound was reported to have no selectivity for PPARδ above 1 μM over the other isotypes.[7] Considering the serum concentration of GW0742 (21 μM at 60 mg/kg) in the study by Graham et al., the anti-atherogenic activity might have been mediated through PPARα or γ activation. In this regard, it still unclear whether PPARδ-selective agonists have anti-atherogenic effects.[12]

We developed a series of novel selenium-containing PPARδ-selective ligands to resolve this issue (Fig. 1).[13] Isosterism is a useful strategy for molecular modification and a rational approach in drug design.[14] Isosteric analogs possess an equally well-established biological potency in protein–receptor interaction.[15] Our



1f R^1 = H, R^2 = H, R^3 = H **2f** R^1 = F, R^2 = H, R^3 = H
1g R^1 = H, R^2 = CH_3, R^3 = H **2g** R^1 = F, R^2 = CH_3, R^3 = H
1h R^1 = H, R^2 = CH_2CH_3, R^3 = H **2h** R^1 = F, R^2 = CH_2CH_3, R^3 = H
1i R^1 = H, R^2 = CH_3, R^3 = CH_3 **2i** R^1 = F, R^2 = CH_3, R^3 = CH_3

Figure 1. Structures of selenoether-containing PPARδ agonists.

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research interest therefore lay on the discovery of more potent and selective analogs of any known PPARδ ligand, viz GW501516 or GW0742. As a proof-of concept, the sulfur–selenium bioisosterism[16] was applied to GW501516 and GW0742 series because molecular modeling study suggested a bulkier element at the sulfur fit the receptor better. α-Substituted phenylpropanoic acid was a versatile template for the design of PPAR subtype-specific agonists.[17] Further modification had thus been applied to the α-position of the carboxylic acid region and its alkyl substituent.

A series of novel selenoether-containing PPARδ ligands were efficiently prepared via a simple one-pot lithium exchange reaction.[18] First, we prepared the key intermediate selenides **1a** and **2a** (86% and 85% yields, respectively) from 4-iodo-2-methylphenol via a one-pot reaction. This reaction included the in situ protection of phenol with isopropylmagnesium chloride, lithium–halogen exchange by *tert*-butyllithium, selenolate formation, and quenching the resultant selenolates with the corresponding chloromethyl thiazole (**1** or **2**). In the second step, treatment of **1a** or **1b** with K_2CO_3 and bromoester in acetone produced ester intermediates **1b–2d** with quantitative yields. In the final step, ester hydrolysis with 2 M LiOH afforded the target compounds **1f–1h** and **2f–2h** with greater than 90% yields. Notably, compounds **1i** and **2i** were synthesized by refluxing procedures (Scheme 1, see Supplementary data for detailed experimental procedures).

Remarkably, compounds **1f**, **2f**, and **2i** displayed excellent potency for PPARδ (EC_{50} = 0.9, 1.0, and 1.8 nM, respectively) in a cell-based co-transfection assay. Compound **1f** exhibited the most potent and selective agonistic activity for PPARδ over the other subtypes PPARα and γ (Table 1 and Fig. 2). Time-resolved fluorescence resonance energy transfer (TR-FRET) was conducted to investigate the direct binding of **1f** and PPARδ. The dose-dependent sigmoidal curve showed that **1f** facilitated direct interaction between the fluorescein-conjugated copeptide and hPPARδ protein, indicating the direct binding of **1f** to the receptor protein (Fig. 2, see also Supplementary data).

Table 1
Selectivity of PPARδ agonists

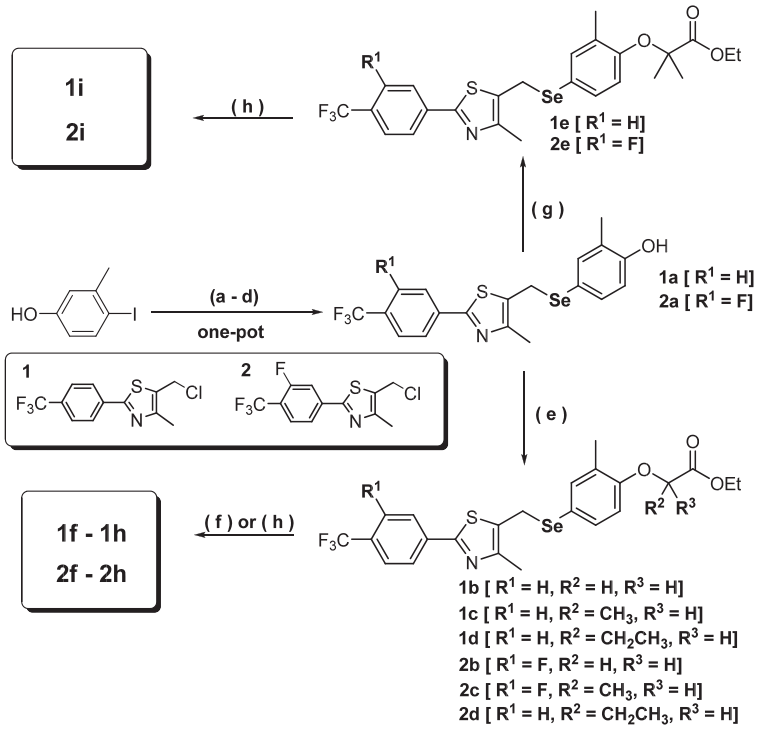
Compd	hPPARα (EC_{50}, nM)	hPPARδ (EC_{50}, nM)	hPPARγ (EC_{50}, nM)
1f	i.a.[a]	0.9	i.a.[a]
1g	120.0	11.0	i.a.[a]
1h	83.1	6.2	i.a.[a]
1i	15.2	4.5	100.2
2f	350.3	1.0	i.a.[a]
2g	39.4	8.5	i.a.[a]
2h	i.a.[a]	7.0	270.6
2i	24.6	1.8	290.3

[a] EC_{50} values are higher than 1 μM.

Compounds **1g** and **2g** (methyl group at R^2) serve as α/δ dual agonists. Compound **1h** (ethyl group at R^2) also behaved as an α/δ dual agonist. Yet compounds **2h** (ethyl group at R^2) acts as a γ/δ dual agonist. Compounds **1i** and **2i** (dimethyl side chains at R^2 and R^3) were pan-agonists for PPARs (Table 1).

We fed *apolipoprotein E*-deficient mice ($Apoe^{-/-}$ HC/HF diet (1.25% cholesterol, 21% fat) for 14 weeks and treated them with one of the most potent and selective compounds (**1f**) at 2 mg/kg/day for the last 4 weeks. The newly developed PPARδ ligand attenuated atherosclerosis, resulting in an increase in HDL-c and the suppression of pro-atherogenic molecules. The results, along with our previous study using the well-known agonist GW501516,[19] present clear evidence of the anti-atherogenic potential of PPARδ.

The atherosclerosis model mice,[20] $apoE^{-/-}$ mice, were treated with **1f** to examine its anti-atherogenic potential. Compound **1f** was used at a lower concentration (2 mg/kg) than in previous studies[10,11] (5 mg/kg by Li et al.; 6 and 60 mg/kg by Graham et al.) to minimize its cross-reactivity toward the other two subtype receptors. The dose regimen also allowed us to directly assess the effect of the PPARδ ligand on atherosclerosis because a higher dose treatment of this compound led to weight loss. The ligand decreased the extent of lesion fatty streak throughout the aorta by 25% (vehicle:



Scheme 1. Reagents and conditions: (a) isopropylmagnesium chloride, 0 °C, 10 min, anhydrous THF; (b) *tert*-butyllithium, −78 °C, 0.5 h; (c) selenium power, −78 to 0 °C, 1 h; (d) compound **1** or **2**, 0.5 h; (e) K_2CO_3, bromoesters; (f) 2 M LiOH, 0.5 M $NaHSO_4$; (g) Cs_2CO_3, DMF, ethyl 2-bromoisobutyrate; (h) 5 N NaOH, EtOH, reflux (see Supplementary data for detailed experimental procedures and yields).

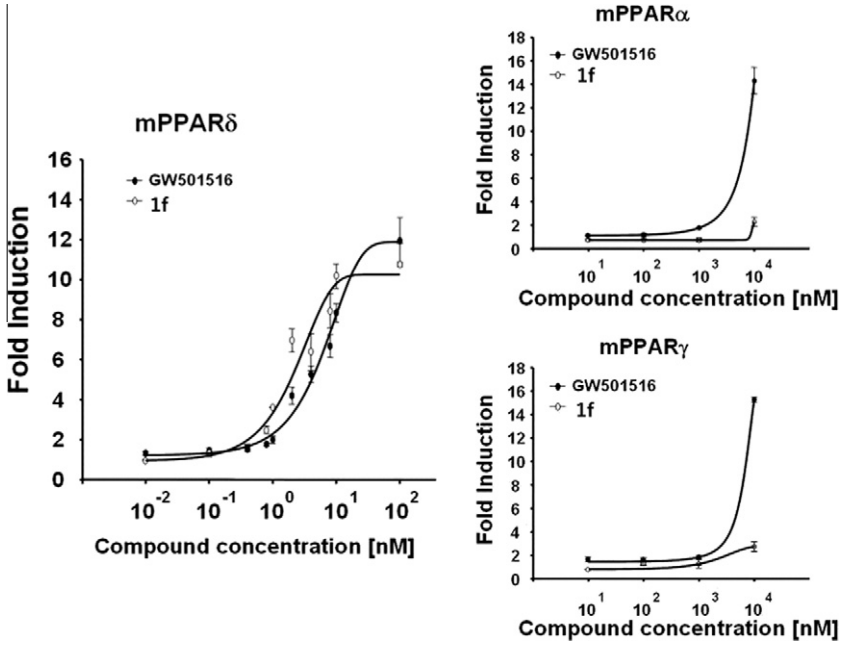


Figure 2. Co-transfection assay was conducted to confirm the selectivity and potency of **1f** against mouse PPARs for the following in vivo experiments. The **1f** compound showed more potent agonistic activity to mouse PPARδ than GW501516, currently known as the most potent and selective PPARδ agonist (EC_{50} = 2.3 and 5.8 nM for **1f** and GW501516, respectively). The other PPAR subtypes were barely activated by **1f**, in contrast to GW501516, which robustly activated these receptors at micro-molar concentrations.

17.07 ± 2.20: **1f**: 12.87 ± 2.32%, $p = 0.004$, Fig. 3). Plasma HDL-c level, a key protective factor against atherosclerosis,[9] was significantly increased by **1f**, and this effect accounted for the reduced formation of lesions (Fig. 4).

Quantitative real-time PCR analysis showed that endothelial cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), were down-regulated by **1f** in the aortic lesion. The expression level of the pro-atherogenic cytokine Interleukin-6 (IL-6) was also greatly reduced by **1f**. The results indicate that the PPARδ agonist alleviated the inflammatory response in the lesion. However, the expression of genes involving cholesterol homeostasis remained unchanged by **1f** (see supporting Information). During experimental periods, *Apoe*$^{-/-}$ mice were weighed every week. There were no significant changes in body weight between the treated and the control groups, suggesting that the anti-atherogenic effect of **1f** was not caused by ligand-induced weight reduction (see Supplementary data).[21] In addition, liver weight was not adversely increased, a finding in contrast to the study done by Graham et al. (see Supplementary data).[11]

Several studies have suggested that PPARδ has carcinogenic potential in colonic tumorigenesis.[22] Many groups have reported mixed results, indicating that the issue has yet to be resolved.[23] Thus, **1f** was given to the spontaneous intestinal adenoma model, adenomatous polyposis coli multiple intestinal neoplasia mice[24] (*APC*$^{Min/+}$ mice), to assess its carcinogenicity. The results demonstrated that the ligand did not have any significant detrimental effect on intestinal polyp formation (Fig. 5). In addition, the plasma HDL-c level was also significantly increased by **1f** in *APC*$^{Min/+}$ mice, which is the mechanism by which PPARδ agonists protect mice from atherosclerosis (see Supplementary data).

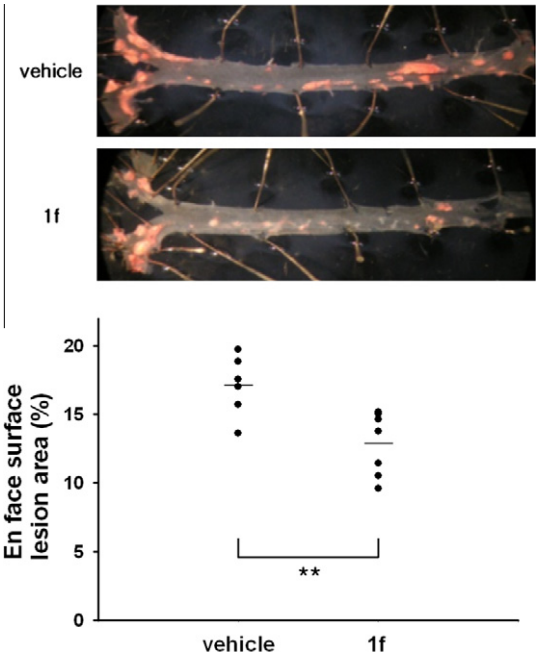


Figure 3. Effect of **1f** on atherosclerotic lesion progression. Eight-week-old *apoE*$^{-/-}$ mice were fed an atherogenic diet for 14 weeks and treated with either **1f** ($n = 7$) or vehicle ($n = 6$) for the last 4 weeks. The extent of atherosclerotic lesion was determined by the en face staining method (see Supplementary data). $^{**}p$ <0.005.

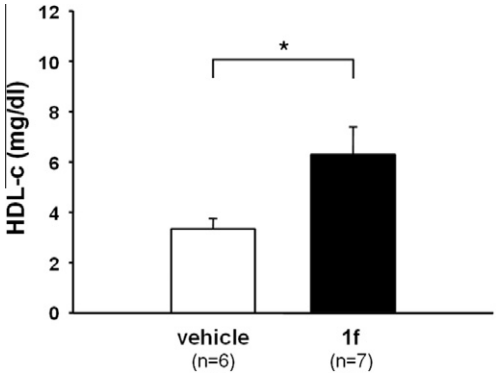


Figure 4. HDL-c-increasing effect of **1f** in *apoE*$^{-/-}$ mice. Data are expressed as means ± SEMs. $^{*}p$ <0.05.

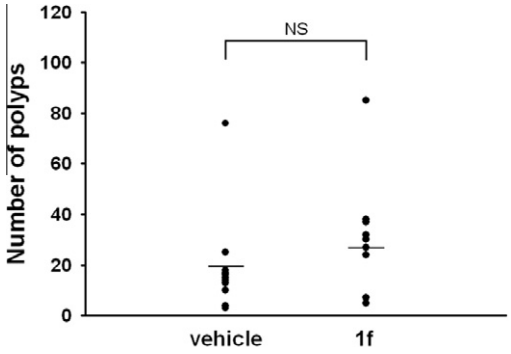


Figure 5. The **1f** compound has no effect on small intestinal polyp formation in $APC^{Min/+}$ mice. C57BL/6J $APC^{Min/+}$ mice were treated with 10 mg/kg of **1f** ($n = 13$) or vehicle control ($n = 13$) once a day for 6 weeks. The number of small intestinal polyps was determined by intestinal polyp assessment (see Supplementary data). p = NS, non-significant.

In conclusion, we developed a novel PPARδ-selective agonist (**1f**) with potent anti-atherogenic activity in vivo. Treatment of **1f** at a low dose (2 mg/kg) in $apoE^{-/-}$ mice (to minimize the cross-reactivity effects of PPARα and γ) greatly attenuated atherosclerotic lesion progression. Plasma HDL-c level was significantly increased by the ligand in both $apoE^{-/-}$ and $APC^{Min/+}$ mice, suggesting that the atheroprotective effect of **1f** was mediated through an HDL-c dependent pathway.[9] Furthermore, **1f** did not show significant adverse effects on intestinal polyp formation. These results clearly demonstrated that PPARδ is a good drug target for atherosclerosis and that our compound is a promising drug candidate to treat this disease.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.103.

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