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# Synthesis of new carbo- and heterocyclic analogues of 8-HETE and evaluation of their activity towards the PPARs

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**Abstract**—A new class of dual PPARs α and γ agonists was developed. These compounds are structural analogues of the arachidonic acid metabolite, the 8-(*S*)-HETE. A versatile strategy has been introduced to prepare the target molecules having different carbo- and heterocyclic cores and to modulate the unsaturations on the side chains. Their affinity towards the PPARs α and γ receptors is reported, together with their transactivation percentage. Most of these derivatives have a good activity as dual agonists but the quinoline-derived products appear as the most promising compounds.


The peroxisome proliferator activated receptors (PPARs) were discovered by I. Issemann and S. Green in 1990.[1] They are a subfamily of nuclear receptors also comprising steroids, thyroids, retinoid acid and vitamin $D_3$ receptors. Few years after their discovery, three subtypes were identified α, β (or δ) and γ.[2] The PPARα is highly expressed in tissues that efficiently harvest energy from lipids, including liver and skeletal muscle. In these tissues, PPARα regulates the expression of numerous genes involved in lipid uptake, catabolism and homeostasis. The PPARβ has a ubiquitous expression but its function is not fully understood yet. The PPARγ is highly expressed in adipocytes and it has been shown to regulate the expression of genes that mediate adipocyte differentiation, energy metabolism and insulin action, as well as those encoding a variety of secreted adipokines.[3] Recent studies have indicated that dual agonists of PPARs α/γ could be of much interest in medicinal chemistry. For instance in insulin resistant animal models, dual PPARα/γ agonists decreased the free triglyceride (TG) plasma concentration,[4] which can be associated with an increase in the activity of adipocyte LPL and an increase of β-oxidation. In addition, dual PPARα/γ agonists increase plasma HDL concentration.[5,6] These effects on glycaemia and insulin sensitivity are comparable to those of TZDs, which are known as excellent PPARγ agonists. This phenomenon is associated with an increase of insulin sensitivity in the rodent, but this should be a consequence of the decrease in free fatty acid concentration. Another interest for the PPARα/γ co-activation is the potent limitation of side effects observed in the actual treatment by TZDs, such as increase of weight and/or oedema.

Our goal was to develop a new family of dual PPAR agonists with a better activity on PPARα than on PPARγ. Many fatty acids and corresponding metabolites are known to activate the PPAR receptors.[7] Among them, the 8-(*S*)-HETE appeared particularly attractive since this natural product is a strong activator of subtype α ($EC_{50}$ = 100 nM) and it also exhibits a partial activity on subtype γ.[8,9] Furthermore, the 8-(*R*) enantiomer was found to be inactive.[10] Therefore, this arachi-

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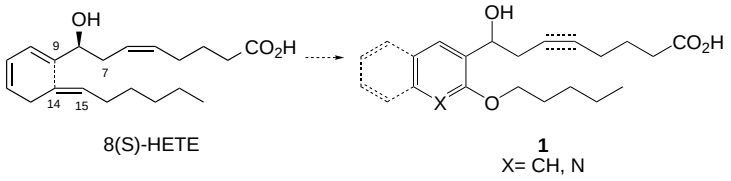



**Figure 1.** Design for the analogues of 8-(*S*)-HETE.

donic acid metabolite was selected as the starting model for the design of new analogues (Fig. 1). In a classical manner, we replaced the *E*,*Z*-conjugated diene by aromatic or heteroaromatic rings (benzene, pyridine, naphthalene and quinoline) to obtain more stable products. Simultaneously, we replaced the $sp^2$ carbon in position 15 by an oxygen atom. We have maintained the same chain lengths as in the natural eicosanoid but our design should allow further modulations at the level of the unsaturation in positions 5–6, affording alkyne, alkene, as well as saturated derivatives.

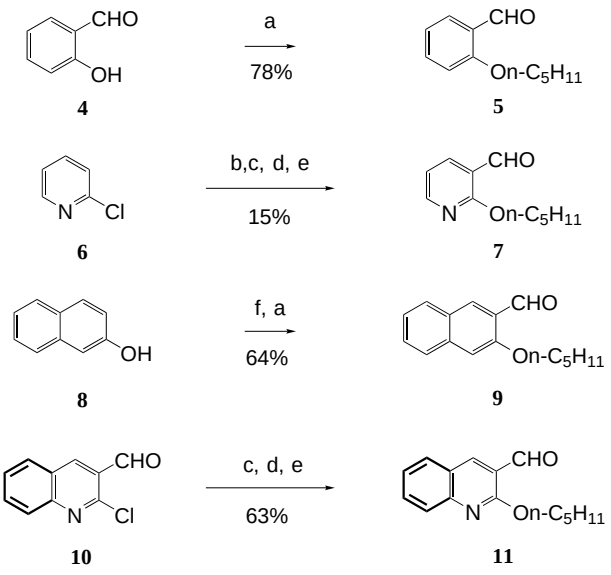
Our retrosynthesis is described in Scheme 1: the various target molecules **1** should be accessible by hydrogenation of propargylic derivatives **2**. The latter compounds should be prepared by alkylation of type **3** homopropargylic alcohols. Finally, those derivatives should be easily obtained by Grignard reactions on type **4** aldehydes.

Therefore, our first intermediates were the different aromatic derivatives with a pentoxy group in position 1 and an aldehyde in position 2. Their synthesis is shown in Scheme 2. In the case of the benzene ring, the intermediate **5** was easily obtained in good yield by O-alkylation of salicylaldehyde. For the pyridine, the first step was a formylation of 2-chloropyridine.[11] Then, after protection of the carbonyl as a ketal, the substitution of chloride by a pentoxy was performed. A final deprotection gave the desired formyl pyridine in 15% overall yield from **6**. In the case of the naphthalene ring, the 2-naphthol was first formylated and then alkylated to give **9**. Finally, in the case of the quinoline, a sequence of reactions similar to the pyridine case allowed the preparation of **11** in 63% overall yield from the commercially available chloroquinoline **10**.

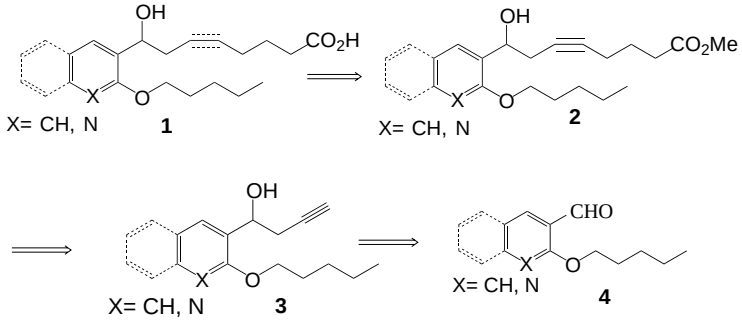
The preparation of the key propargylic intermediates is reported in Scheme 3 for the derivatives with the benzene and the pyridine cores. The first step was a propargyl bromide-derived Grignard reaction on aldehydes **5** and **7**, affording **12a** and **12b** in good yields. After protection as silyl ethers **13a** and **13b**, these derivatives were alkylated by trimethyl 4-bromo-orthobutyrate.

This reaction was found to require a strict control of the temperature, otherwise elimination products can be formed. While **14a** could be obtained in good yield, only low yields were obtained for **14b**.

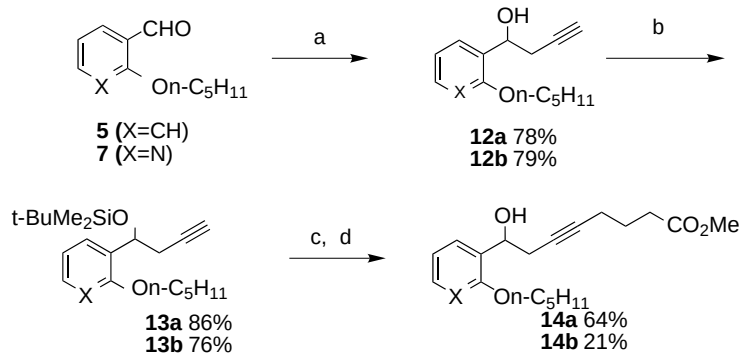
The synthesis of the first target molecules is reported in Scheme 4. The two *Z* alkenes **16a** and **16b** were obtained in good to excellent yields by semi-hydrogenation, using



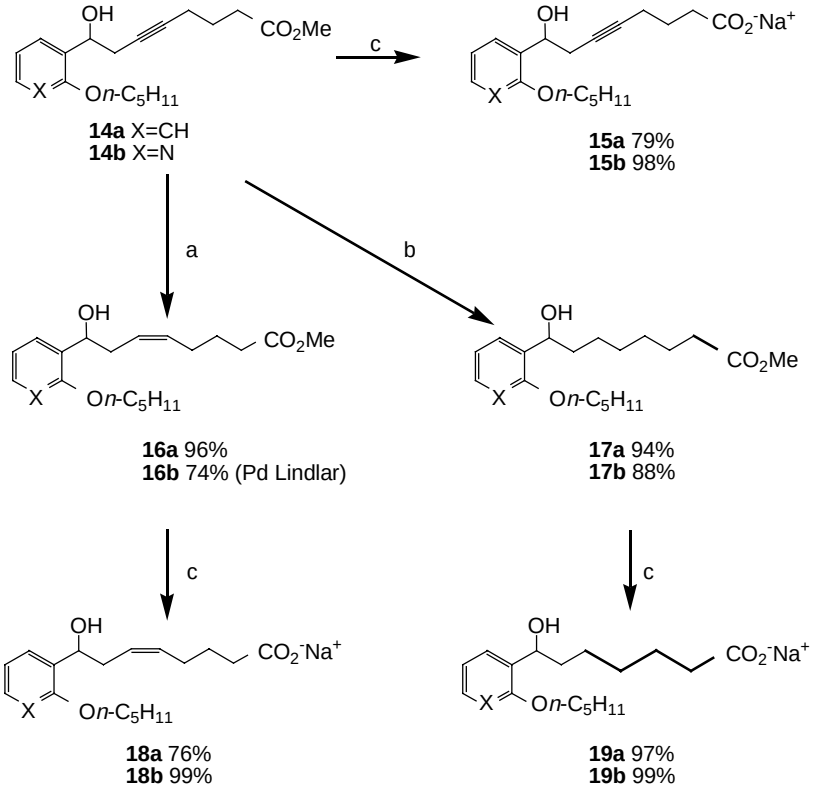

**Scheme 2.** Reagents and conditions: (a) $C_5H_{11}I$, KOH, EtOH/$H_2O$, 0 °C; (b) PhLi, $iPr_2NH$ cat, *N*-formylpiperidine, THF, −78 °C; (c) $HC(OMe)_3$, $NH_4NO_3$(cat), MeOH; (d) $C_5H_{11}OH$/NaH, NMP; (e) APTS, THF/$H_2O$; (f) $^t$BuLi, DMF, THF, −80 °C.




**Scheme 1.** Retrosynthesis.




**Scheme 3.** Reagents and conditions: (a) Propargyl bromide, Mg, $Et_2O$, −80 °C, 20 min; (b) $^t$BuMe$_2$SiCl, Im., DMF, 0 °C and rt, 20 h; (c) *n*-BuLi/THF, −80 °C, Br(CH$_2$)$_3$C(OMe)$_3$/HMPA, $NH_4Cl_{aq}$ (work-up); (d) *n*-$Bu_4NF$, THF, 45 °C, 3 h.




**Scheme 4.** Reagents and conditions: (a) $H_2$, Ni(OAc)$_2$, $NaBH_4$, EtOH, 5 h; (b) Pd/C, $H_2$, MeOH; (c) LiOH, MeOH/$H_2O$, $(CO_2H)_2$, then NaOH.

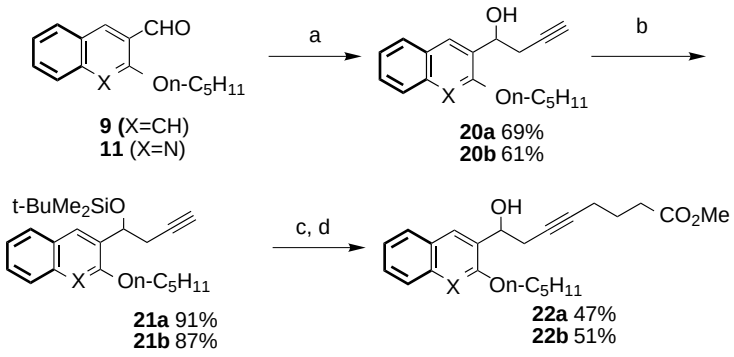
Ni/$P_2$ catalyst in the case of **16a** and Lindlar catalyst for pyridine **16b**. The hydrogenation using palladium on carbon gave the saturated compounds **17a** and **17b**. All methyl esters were saponified and the corresponding salts **15a**, **15b**, **18a**, **18b**, **19a** and **19b** were submitted to biological tests.

The same route was followed for the other series, bearing naphthalene and quinoline cores (Scheme 5).

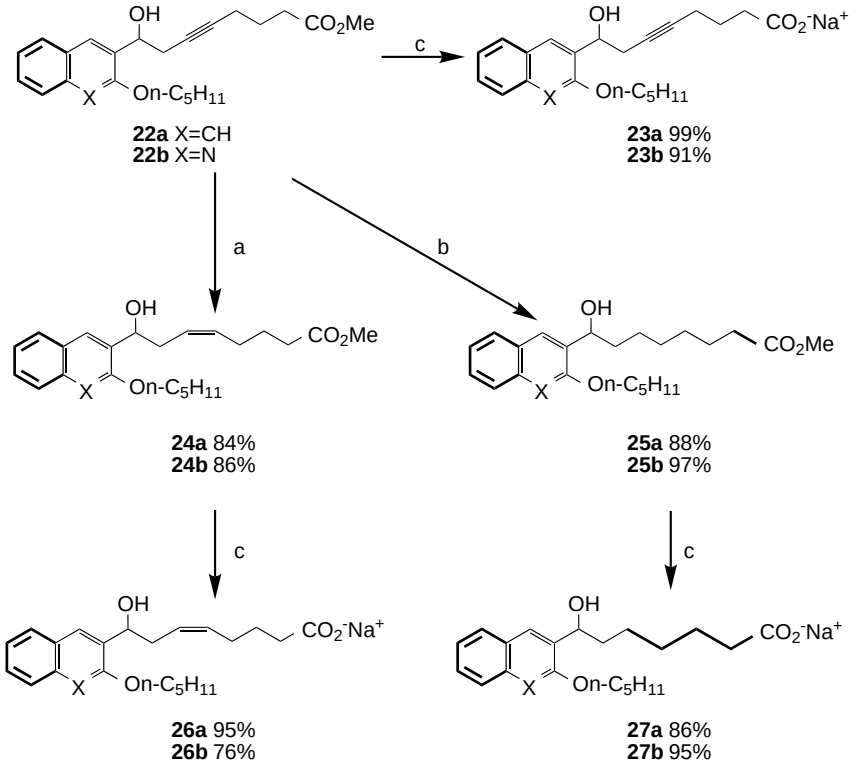
Starting from **9** or **11**, Grignard addition, followed by protection of the alcohol function, afforded **21a** and **21b**. Here again, a careful control of the reaction conditions was necessary to obtain the key intermediates **22a** and **22b** in fair yields.

The target molecules with the naphthalene and quinoline cores were obtained in good yields using the same sequence of reactions as before: hydrogenation, followed by saponification (Scheme 6).

Using this strategy 24 compounds have been prepared and submitted to biological tests to obtain preliminary data on structure–activity relationships (SAR) in these series: of much interest was the effect of the nature of the aromatic core, as well as the degree of unsaturation of the side chain. The biological data were obtained on a chimera human PPAR/Gal$_4$ gene reporter luciferase system. The most important results are given in Table 1. Our purpose was to obtain partial agonists of PPAR$\gamma$ with a high activity on PPAR$\alpha$. Therefore, we report




**Scheme 5.** Reagents and conditions: (a) Propargyl bromide, Mg, $Et_2O$, −80 °C, 20 min; (b) $^tBuMe_2SiCl$, Im., DMF, 0 °C and RT, 20 h; (c) *n*-BuLi/THF, −80 °C, $Br(CH_2)_3C(OMe)_3$/HMPA, $NH_4Cl_{aq}$ (work-up); (d) *n*-$Bu_4NF$, THF, 45 °C, 3 h.




**Scheme 6.** Reagents and conditions: (a) $H_2$, $Ni(OAc)_2$, $NaBH_4$, EtOH, 5h; (b) Pd/C, $H_2$, MeOH; (c) LiOH, $MeOH/H_2O$, $(CO_2H)_2$, then NaOH.

only the results obtained with these two subtypes. However, each product was also tested on PPARβ and no activity was found on this subtype.

A first analysis shows that, except for **14b**, **16a** and **18a**, all compounds have similar $EC_{50}$ values on the two PPAR(s) subtypes or a higher activity on subtype α. Indeed, compounds, such as **23a** and **22b**, and even better **14a** and **23b**, have excellent affinities for PPARα (with $EC_{50}$ down to 114 nM). Compounds **14a** and **23a** showed partial agonist activity on human PPARα (with transactivations of 81% and 75%), whereas **14b** and **23b** were full agonists of this subtype (with transactivations of 148% and 287%). Furthermore, the partial activity on PPARγ observed for these compounds could be of interest to reduce side effects such as adipogenesis and weight gain, which are often observed with PPARγ full agonists. This is an interesting result since there are very few known compounds with a higher activity on subtype α, which were developed in clinical trials.[5,12,13]

The first structure–activity relationship was the comparison between the esters and the sodium salts. Our results showed no important differences, in most cases, between the esters and sodium salts for the same compound in terms of $EC_{50}$ values on the two subtypes PPAR(s).

In the second step, we compared the effect of the degree of saturation in position 5. In general, the saturated compounds are less active. The derivatives with the *Z* double bond demonstrate intermediate activity. For instance, **26a** and **26b** have $EC_{50}$ values around 1 μM

**Table 1.** In vitro activity of compounds in cell-based transactivation assay and binding assay against human PPARα/$Gal_4$ and PPARγ/$Gal_4$ receptors

| Compound | hPPARα/$Gal_4$ | | hPPARγ/$Gal_4$ | | Binding Rosiglitazone |
|---|---|---|---|---|---|
| | $EC_{50}$ (nM) | % transactivation[a] | $EC_{50}$ (nM) | % transactivation[b] | $K_i$ (nM) |
| Rosiglitazone | >10,000 | 15 | 4 | 100 | 8 |
| WY 14,643 | >10,000 | 100 | >10,000 | 15 | |
| **14a** | 173 | 81 | 642 | 16 | 1,050 |
| **14b** | 1,632 | 148 | 549 | 45 | >10,000 |
| **15a** | >10,000 | 41 | >10,000 | 32 | >10,000 |
| **15b** | 1,543 | 183 | >10,000 | 25 | >10,000 |
| **16a** | >10,000 | 20 | 28 | 24 | 3,760 |
| **16b** | >10,000 | 121 | >10,000 | 19 | >10,000 |
| **17a** | >10,000 | 30 | >10,000 | 42 | 515 |
| **17b** | >10,000 | 187 | >10,000 | 13 | >10,000 |
| **18a** | >10,000 | 35 | 340 | 13 | >10,000 |
| **18b** | >10,000 | 155 | >10,000 | 31 | >10,000 |
| **19a** | >10,000 | 35 | >10,000 | 29 | 6,690 |
| **19b** | >10,000 | 204 | >10,000 | 27 | >10,000 |
| **22a** | 1,142 | 112 | >10,000 | 58 | 695 |
| **22b** | 262 | 114 | 1,413 | 42 | >10,000 |
| **23a** | 723 | 75 | >10,000 | 100 | 4,390 |
| **23b** | 114 | 287 | 617 | 72 | 947 |
| **24a** | 1,454 | 122 | >10,000 | 26 | 718 |
| **24b** | 1,300 | 278 | 1,546 | 141 | 1,330 |
| **25a** | 1,531 | 100 | 1,356 | 34 | 579 |
| **25b** | >10,000 | 149 | >10,000 | 41 | >10,000 |
| **26a** | 1,194 | 80 | 1,503 | 85 | 1,030 |
| **26b** | 1,086 | 186 | 1,195 | 62 | >10,000 |
| **27a** | 1,485 | 138 | >10,000 | 51 | 969 |
| **27b** | 1,399 | 410 | 1,566 | 85 | 2,860 |

[a] Maximal signal obtained by comparison to WY 14,643 $10^{-5}$ M.
[b] Maximal signal obtained at $10^{-5}$ M by comparison to Rosiglitazone $10^{-5}$ M.

on PPARα, and a similar activity on subtype γ. For transactivation, we showed that many of these new derivatives are more active than the reference compound (WY 14,643) on the PPARα transactivation test. Furthermore, the most active derivatives are the compounds with the triple bond, such as **14a**, **22b**, **23a** and **23b**.

If now we consider only the propargylic derivatives, as their sodium salts, we can study the effect of the nature of the aromatic core: we note that the benzene and pyridine derivatives (**15a**, **15b**) are essentially inactive as PPAR dual agonists. On the contrary, the compounds with the naphthalene core (**23a**) and even more the quinoline derivative (**23b**) gave excellent results. Therefore, as far as our goal is concerned, the best aromatic cores are the quinoline derivatives.

Furthermore, the quinoline-derived analogues, especially the derivatives **22b**, **23b** and **27b** (410% as compared to WY 14,463), have also the highest transactivation on subtype α. Among these analogues, three compounds, **14a**, **22b** and **23b**, appear to be particularly attractive. These products present a potent activity on PPARα, between 114 and 262 nM, and a good to excellent transactivation between 81% and 287%. Furthermore, these compounds have a partial activity on subtype γ with an $EC_{50}$ between 617 and 1413 nM, and a percentage of transactivation between 16% and 72% of the Rosiglitazone response. The most promising product, the derivative **23b**, has a high activity on PPARα, with an $EC_{50}$ value equal to 114 nM and 287% for transactivation response compared to the WY14,643 response; furthermore, its partial response on the subtype γ is characterized by an $EC_{50}$ at 617 nM and 72% for transactivation. Therefore, it was selected as the lead compound for future studies.

It is noteworthy that there was no correlation between $EC_{50}$ values obtained on transactivation PPARγ tests and binding tests, suggesting that these derivatives have a binding site different from the Rosiglitazone binding site. These results suggest a different capacity for these compounds on the co-activators'/co-repressors' recruitment on PPARγ, as compared to those observed with Rosiglitazone. Complementary experiments, especially in vivo tests, are under study to set up, with more precision, the impact of these new compounds in various pathologies, such as type 2 diabetes and dyslipidemia.

In summary, we have reported an efficient and flexible strategy for the preparation of 8-HETE analogues. Some of these products exhibit a very promising activity as dual PPARα/γ agonists. Moreover, the most active compounds, such as **23b**, present a relatively unusual structural characteristic with the triple bond in positions 5–6. Indeed, the in vitro activities of **23b** or **14a** and **22b** are similar to, or better, than most of the reference products. Furthermore, this is a new class of dual agonists with a higher potency on subtype α and a partial agonist activity on PPARγ. Development of these new series of 8-HETE analogues, and especially the quinoline derivatives, is under active study in our laboratories.

Binding assays were performed in 96-well plate format, using a classical filtration assay with a human full-length PPARγ construct (GST-PPAR LBD (25 μg/ml)) expressed in bacteria with some modifications to the experimental conditions. The membrane-associated PPARγ was used as a biological source, as previously described. The binding buffer consisted of 10 mM Tris/HCl, pH 8.2, containing 50 mM KCl and 1 mM dithiothreitol. Membrane preparations (5 μg/mL) were incubated for 180 min at 4 °C in the presence of [$^3$H]Rosiglitazone [BRL49653, Amersham] (4 nM) and the tested compounds. Nonspecific binding was defined using an excess of unlabelled Rosiglitazone (10 μM). Incubation was terminated by the addition of ice-cold 50 mM Tris/HCl buffer, pH 7.4, followed by rapid filtration under reduced pressure through Whatman GF/C filter plates presoaked with ice-cold buffer, followed by three successive washes with the same buffer. Radioactivity was measured in a TopCount apparatus (Packard). The receptor preparation used during these experiments presented a $B_{max}$ of 49 pmol/mg proteins and a $K_d$ of 5.58 nM for [$^3$H]Rosiglitazone. The compounds were solubilized in pure DMSO and diluted to the appropriate working concentrations (100 μM to 0.1 nM). For each compound tested, plots of ligand concentration versus DPM of radioligand binding were constructed and apparent $K_i$ values were estimated from nonlinear least-squares fit of the data assuming simple competitive binding. The details of this assay have been reported elsewhere.[14]

Compounds were screened for functional potency in a transient transfection assay performed on Cos-7 cells where a previously established chimeric receptor system was used to allow comparison on the relative transcriptional activity on the same target gene. Cos-7 cells were transiently transfected with luciferase reporter plasmid (pG5-TK-pGL3) in the presence of pGal4hPPARγ or pGAL4hPPARα (these vectors expressed chimeric proteins containing the Gal4 DNA-binding domain fused to the human PPARγ or the PPARα ligand binding domain coding sequence) expression vectors. Plasmids pGal4-hPPARs and pG5-TK-pGL3 were constructed, as described previously.[15] Cells were seeded in 60 mm dishes at a density of $5.5 \times 10^5$ cells/dish in DMEM supplemented with 10% FCS and incubated at 37 °C for 24 h prior to transfection. Cells were transfected in Opti-MEM without FCS for 3 h at 37 °C, using polyethyleneimine (PEI), with reporter and expression plasmids. The plasmid pBluescript (Stratagene, La Jolla, CA) was used as carrier DNA to set the final amount of DNA to 5.5 μg/dish. The pCMV-β-galactosidase expression plasmid was cotransfected as a control for transfection efficiency. Transfection was stopped by the addition of DMEM supplemented with 10% FCS and cells were then incubated at 37 °C. After 16 h, cells were trypsinised and seeded in 96-well plates at a density of $2 \times 10^4$ cells/well and incubated for 6 h in 10% FCS containing DMEM. Cells were then incubated for 16 h in DMEM containing 0.2% FCS and increasing concentrations of the compound tested (10 μM to 10 nM) or vehicle (DMSO). At the end of the experiment, cells were washed once with ice-cold PBS and the luciferase activity was measured and normalized to internal control β-galactosidase activity, as described previously.[15] Compounds, which elicited on an average at least 80% activation of PPAR(s) versus Rosiglitazone (PPARγ) or WY14.643 (PPARα) (positive controls), were considered full agonists. $EC_{50}$ were estimated using Prism software (GraphPad). All transactivation and binding experiments were performed once. For each concentration tested, the measurements were made in triplicate.

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