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Discovery of a novel class of 2-mercaptohexanoic acid derivatives as highly active PPARα agonists

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ABSTRACT

A novel and robust scaffold for highly active PPARα agonists based on the 2-mercaptohexanoic acid substructure is presented. Systematic structural variation of the substitution pattern of the phenolic backbone yielded detailed SAR especially of *ortho* and *meta* substituents. We corroborated the importance of the sulfur atom as well as of the *n*-butyl chain for PPARα activity in the 2-mercaptohexanoic acid head group by preparation of carbon analogs and α-unsubstituted derivatives. Compound **10** represents a low nano molar active PPARα activator with excellent selectivity towards PPARγ.

The peroxisome proliferator-activated receptors (PPARs) are among today's most prominent targets for antidiabetic and antilipidemic drugs. Activation of different PPAR subtypes (α, β, γ) leads to elevated expression of apolipoproteins and cellular glucose transporters, resulting in enhanced clearance of lipids and glucose from the blood. PPARα-selective fibrates are well established drugs for the treatment of dyslipidemias, whereas PPARγ-selective glitazones have been proven as valuable drugs for the treatment of type 2 diabetes.[1]

PPARα is mainly expressed in tissues with a high rate of fatty acid catabolism such as liver and skeletal muscle. Its activation by fibrates has been proven to be efficient in lowering triglyceride levels accompanied by a moderate elevation of HDL–cholesterol. The clinical benefits are nevertheless limited, as several large clinical trials failed to show a reduction in cardiovascular mortality.[2,3] In respect to their pharmacological profiles, these drugs have some significant detrimental properties: Exhibiting EC_{50} values in the micro molar range, they are neither very potent PPARα agonists nor highly selective versus other PPAR subtypes.[1] Thus, the question remains if highly active and selective PPARα agonists would provide better clinical prospects.[4] State-of-the-art compounds, such as the recently reported 1,3-dioxane-2-carboxylic acid derivative NS220, display nano molar PPARα activity in vitro and very promising hypolipidemic properties in mice models.[5]

Synthetic PPAR agonists commonly share a general structure mimicking those of fatty acids, which serve as endogenous ligands.[6] In general, the structural features include an acidic head group combined with a large lipophilic backbone. Subtype selectivity may be influenced by two means: either by the introduction of a lipophilic α-substituent or by varying size and branching of the backbone of the molecule (Fig. 1).[7]

In previous studies, we used pirinixic acid (WY14,643; **1**) as a scaffold for structural optimization. By introducing alkyl chains in α-position of the carboxylic acid we achieved an enhanced activity for both PPARα and PPARγ and identified an optimal chain length of 4–6 carbons, represented by *n*-butyl-substituted derivative **2**.[8]

For this study, we replaced the central pyrimidine ring of **2** by benzene and the amine linker by a larger propane-1,3-diol moiety. We maintained the 2-mercaptohexanoic acid substructure as the acidic head group for the first series (Fig. 2), and systematically varied the cyclic tail of the molecule in order to evaluate the SAR of various alkyl and alkoxy substituents in *ortho* and *meta* positions. The 2,3-dimethylphenyl-moiety has been retained in the second series (Fig. 3) to evaluate the influence of the α-*n*-butyl-chain (by preparing the α-unsubstituted analogs) and of the sulfur (by replacement with a methylene group).

The 2-mercaptohexanoic acid derivatives **6**–**21** were synthesized starting with 4-mercaptophenol and ethyl 2-bromohexanoate as shown in Scheme 1. Ethyl 2-bromoacetate was used as the ester component for the preparation of **21**. Nucleophilic substitution with the α-bromoester first yielded the thioether derivatives **3a** and **3b**.[9] Two equal synthetic pathways could then be used to

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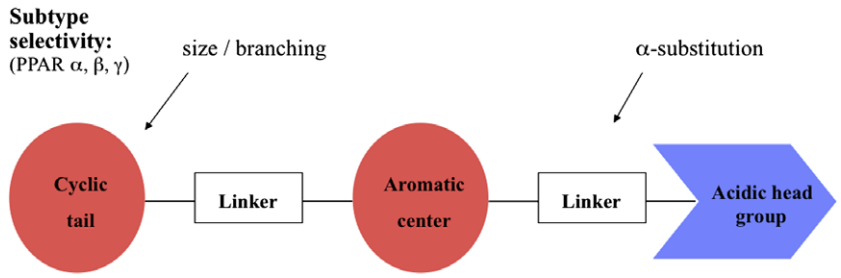


Figure 1. General structure of synthetic PPAR agonists.

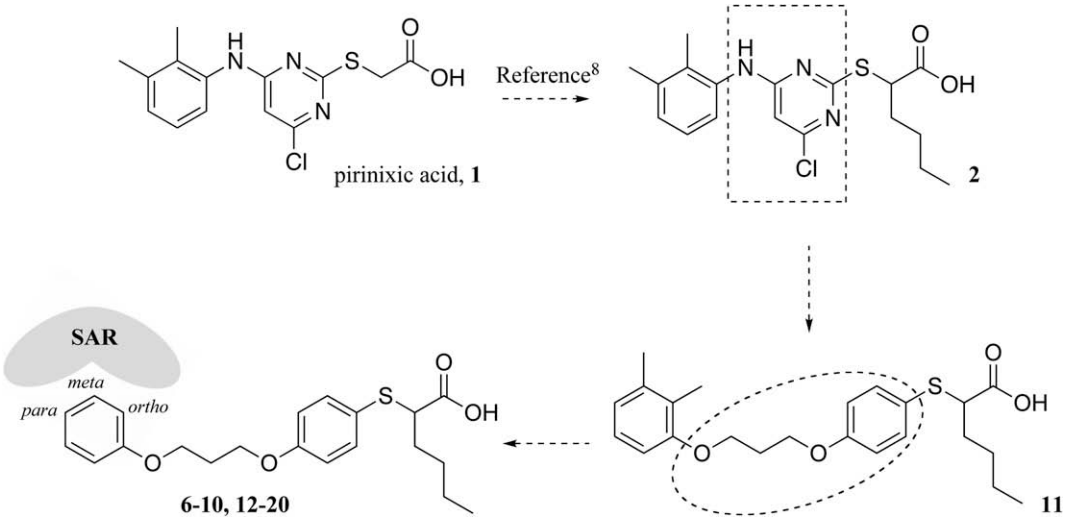


Figure 2. Structural development of presented compounds according to previous studies.[8,15]

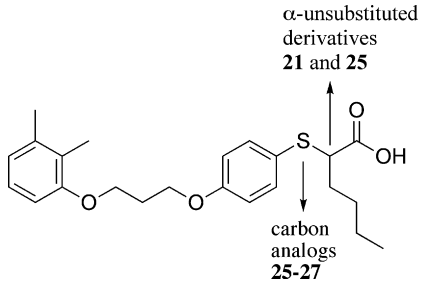
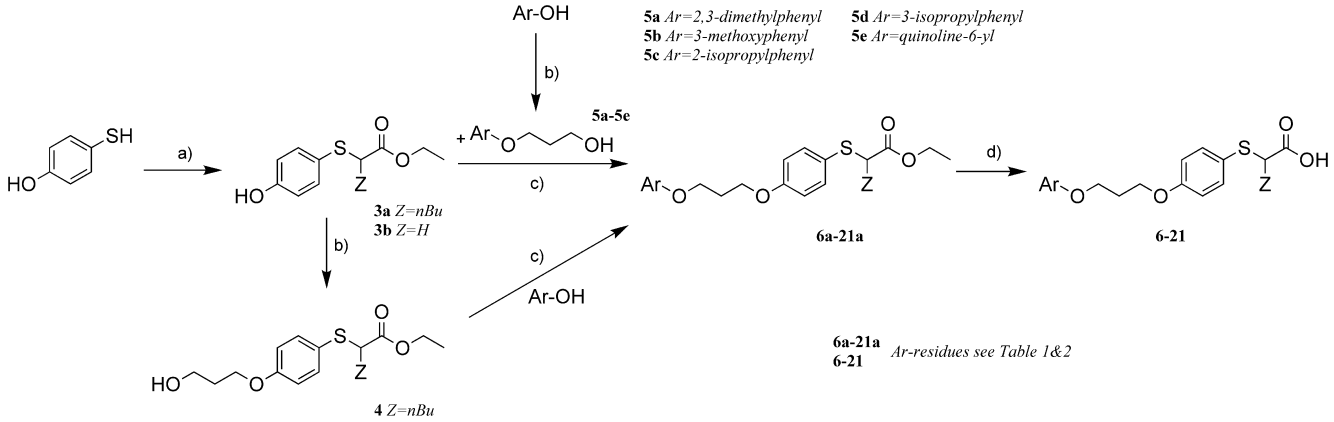


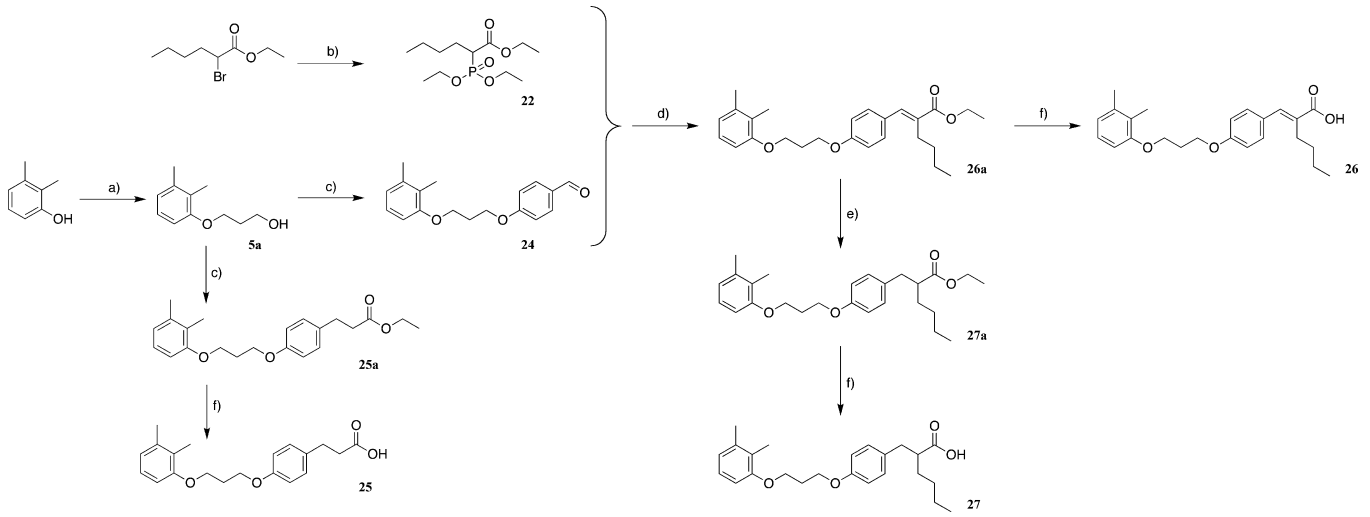
Figure 3. SAR-investigation of the 2-mercaptohexanoic acid head group based on compound **11**.

introduce the propylene spacer via a *Williamson*-like ether synthesis with 3-bromopropan-1-ol (step b): either by direct coupling to **3a** to yield **4** or by reaction with the commercially available phenol derivatives to yield the precursors **5a**–**5e**. Subsequent arrangement of the second ether function was achieved by a *Mitsunobu* reaction (**6a**–**21a**).[10,11] Finally, hydrolysis with lithium hydroxide gave the desired carboxylic acids **6**–**21**.

The carbon analogs of **11** were prepared starting with the conversion of ethyl 2-bromohexanoate into its phosphonate derivative **22** by an *Arbuzov* reaction (Scheme 2).[12] This was coupled via a *Wittig–Horner* reaction to the aldehyde group of the precursor **24**, which was synthesized based on 4-hydroxybenzaldehyde.[13] The resulting ester **26a** could be directly hydrolyzed to yield **26** or



Scheme 1. Reagents and conditions: (a) ethyl-2-bromoacetate ($Z = H$) or ethyl-2-bromohexanoate ($Z = nBu$), Et_3N, $CHCl_3$, reflux, 1 h; (b) 3-bromopropanol, K_2CO_3, ACN, reflux, 24-48 h; (c) DEAD or ADDP, TPP, THF, rt, 4–48 h; (d) LiOH, MeOH, H_2O, 40 °C, 2 h.



Scheme 2. Reagents and conditions: (a) 3-bromopropanol, K_2CO_3, ACN, reflux, 24 h; (b) triethylphosphite, reflux, overnight; (c) 4-hydroxybenzaldehyde or ethyl 3-(4-hydroxyphenyl)propanoate, DEAD, TPP, THF, rt, 4 h; (d) NaH, THF, rt, 24 h; (e) Pd/C, H_2, EtOH, rt, 24 h; (f) LiOH, MeOH, H_2O, reflux, 8–20 h.

hydrogenated and afterwards hydrolyzed to yield **27**. Only two steps (*Mitsunobu* condensation and hydrolysis) were needed for the preparation of the α-unsubstituted carbon analog **25** as the precursor molecule ethyl 3-(4-hydroxyphenyl)propanoate was commercially available.

The final compounds were tested in vitro for PPAR transactivation (EC_{50} values are given in Tables 1 and 2).[14] The structural reference used as the starting point was the phenyl-unsubstituted 2-(4-(3-phenoxypropoxy)-phenylthio)hexanoic acid **6** (Table 1). This compound itself is already a nano molar active PPARα-agonist with an EC_{50} of 0.29 μM and a ninefold selectivity versus PPARγ. This compound and all other 2-mercaptohexanoic acid derivatives presented are PPARβ-inactive. The *ortho*(**7**)-, *meta*(**8**)- and *para*(**9**)-methyl analogs were prepared based on **6**. SAR revealed a significant improvement for the *meta* and *para*-methyl substituted compounds shifting EC_{50} values to the double-digit nanomolar range (PPARα **8**: EC_{50} 0.09 μM, **9**: EC_{50} 0.085 μM), whereas no improvement could be observed for the *ortho*-methyl derivative. Comparison of the selectivity profile of *meta* and *para*-methyl derivatives (PPARγ **8**: EC_{50} 3.1 μM, **9**: EC_{50} 0.93 μM) revealed that *meta*-methyl substitution was able to selectively enhance PPARα activity whereas *para*-methyl substitution enhanced PPARα and PPARγ activity equally.

Two vicinal methyl groups were introduced next, yielding the 3,4-dimethyl (**10**) and 2,3-dimethyl (**11**) derivatives. Both compounds were able to further enhance PPARα activity compared with the mono methyl-substituted analogs. As indicated by the activity of the *meta* and *para*-methyl analogs, the combination 3,4-dimethyl represents the most active racemic compound within this series (PPARα **10**: EC_{50} 0.021 μM). It was noted with interest that the 2,3-dimethyl moiety also showed significant improvement of PPARα activity (EC_{50} 0.056 μM). In regard to PPARγ, selectivity increased by the introduction of two methyl groups from factor nine for unsubstituted phenyl (**6**) to factor 54 for compound **11**. According to previous studies,[8] this substructure is of particular interest (see Fig. 2). We thus maintained **11** as a scaffold for the investigation of the stereochemistry (**(*R*)-11** and **(*S*)-11**) as well as for the SAR of the acidic head group of our series (**21** and **25–27**).

Bridging of the 2,3-dimethyl substructure of **11** yielded the tetrahydronaphthyl derivative **12**, which showed a slight decrease in PPARα activity compared to **11**. We decided to further elucidate SAR of the *ortho* and *meta* position by introducing larger substituents based on the various methyl analogs. We thus prepared methoxy and isopropyl *ortho* and *meta* analogs. Both *ortho*-substituted compounds (**13** and **15**) showed decreased PPARα activity. The most significant loss was observed for the largest substituent (**15**; isopropyl). *meta*-Methoxy substituted **14** was able to further enhance PPARα activity (EC_{50} 0.049 μM) compared to *meta*-methyl substituted **8** whereas the *meta*-isopropyl substitution (**16**) did not cause any further improvement.

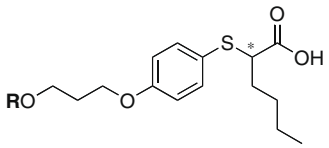
As the *meta*-substitution turned out to be the most eligible tool to selectively improve PPARα activity, we replaced the methyl group of **8** by a trifluoromethyl to yield compound **17**. This modification was the most successful structural modification of the *meta*-position with a PPARα EC_{50} of 0.025 μM and excellent selectivity towards PPARγ (EC_{50} 4.6 μM). It was noted with interest that the additional *para*-chloro substituent of **18** decreased both PPARα and PPARγ activity.

Compounds **19** and **20** represent a larger structural modification and could be described as PPARα-preferential dual PPARα/γ agonists. Biphenyl-substituted **19** is the most potent synthesized compound in regard to PPARγ (EC_{50} 0.54 μM) with an approx. fivefold remaining selectivity for PPARα. Compound **20**, which bears a quinoline-6-yl moiety, shows a very promising PPARγ modulating profile (EC_{50} 1.3 μM, maximal relative activation of 49%) combined with a sixfold selectivity for PPARα.

We recently showed the impact of the stereocentre in α-position of α-*n*-hexyl substituted pirinixic acid derivatives on PPAR activity emphasized by in silico studies.[15] Encouraged by these results and by examples found in literature,[16] we separated **11** by enantio-selective preparative HPLC into its distinct enantiomers.[15,17,18] In vitro data of **(*R*)-11** and **(*S*)-11** are in line with previous observations: **(*R*)-11** (PPARα EC_{50} 0.011 μM) displays superior biological activity and was shown to be approx. five times more potent than the racemic mixture and approx. 140 times more potent than the (*S*)-enantiomer. **(*R*)-11** is the most potent PPARα activator in this series with the highest selectivity versus PPARγ.

We again chose the 2,3-dimethyl substitution pattern of compound **11** for the investigation of the SAR (Table 2) of the linker between the acidic head group and the central aromatic ring (Fig. 1). We first prepared the α-unsubstituted analog **21**. This modification led to an attenuation of PPARα selectivity compared to **11**; nevertheless, it showed an interesting PPAR-*pan*-agonistic profile with a slight preference for PPARα. Obviously, PPARβ activity was gained by removal of the α-substitution. Finally, the sulfur atom was re-

Table 1
In vitro transactivation activities for compounds **6**–**20** on human PPARs



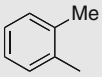
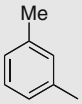
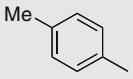
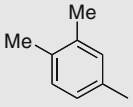
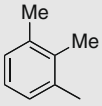
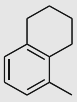
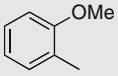
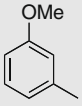
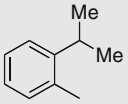
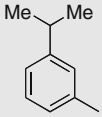
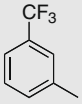
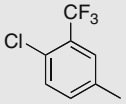
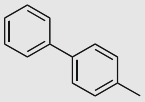
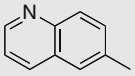
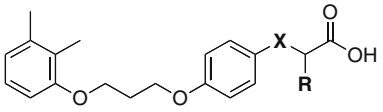
Compound	R	Transactivation EC_{50} (μM) ± SD (% activation compared to control)		
		PPARα	PPARβ	PPARγ
6		**0.29**$^{\pm 0.04}$ (76%)	ia	**2.6**$^{\pm 0.35}$ (83%)
7		**0.25**$^{\pm 0.05}$ (64%)	ia	**2.3**$^{\pm 0.5}$ (114%)
8		**0.09**$^{\pm 0.02}$ (80%)	ia	**3.0**$^{\pm 0.41}$ (96%)
9		**0.085**$^{\pm 0.03}$ (91%)	(31% at 10 μM)	**0.93**$^{\pm 0.12}$ (87%)
10		**0.021**$^{\pm 0.01}$ (72%)	ia	**3.5**$^{\pm 0.1}$ (61%)
11		**0.056**$^{\pm 0.019}$ (113%)	ia	**3.0**$^{\pm 0.22}$ (82%)
(***R***)-**11**		**0.011**$^{\pm 0.004}$ (115%)	ia	**3.9**$^{\pm 0.83}$ (125%)
(***S***)-**11**		**1.7**$^{\pm 0.18}$ (94%)	ia	**9.9**$^{\pm 1.44}$ (77%)
12		**0.19**$^{\pm 0.04}$ (101%)	ia	**8.1**$^{\pm 0.5}$ (107%)
13		**0.55**$^{\pm 0.03}$ (92%)	ia	**1.6**$^{\pm 0.41}$ (85%)
14		**0.049**$^{\pm 0.02}$ (85%)	ia	**1.2**$^{\pm 0.16}$ (85%)
15		**2.0**$^{\pm 0.43}$ (89%)	ia	**4.1**$^{\pm 0.32}$ (92%)
16		**0.15**$^{\pm 0.02}$ (98%)	ia	**5.3**$^{\pm 0.3}$ (91%)
17		**0.025**$^{\pm 0.02}$ (75%)	ia	**4.6**$^{\pm 0.19}$ (68%)

Table 1 (*continued*)

Compound	R	Transactivation EC_{50} (μM) ± SD (% activation compared to control)		
		PPARα	PPARβ	PPARγ
18		**0.39**$^{\pm 0.02}$ (65%)	ia	**7.8**$^{\pm 0.4}$ (96%)
19		**0.11**$^{\pm 0.01}$ (125%)	ia	**0.54**$^{\pm 0.06}$ (108%)
20		**0.22**$^{\pm 0.04}$ (95%)	ia	**1.3**$^{\pm 0.9}$ (49%)

EC_{50}/SD values were calculated using SigmaPlot2001 based on the mean values of at least three determinations. Values in brackets give the relative activation compared to the positive control. Positive controls were GW7647 for PPARα, pioglitazone for PPARγ and L165,041 for PPARβ, each at 1 μM; ia: inactive at 10 μM. Rand X are typed in bold as these entities display the varied substructure of the respective scaffold.

Table 2
In vitro transactivation activities for compounds **21** and **25**–**27** on human PPARs



Compound	X	R	Transactivation EC_{50} (μM) ± SD (% activation compared to control)		
			PPARα	PPARβ	PPARγ
21	–S–	H	**0.60**$^{\pm 0.12}$ (81%)	**1.4**$^{\pm 0.38}$ (52%)	**3.8**$^{\pm 0.34}$ (77%)
25	$-CH_2-$	H	**13.8**$^{\pm 3.4}$ (63%)	**11.2**$^{\pm 0.45}$ (72%)	**15.7**$^{\pm 0.77}$ (54%)
26	–CH=	*n*-Bu	ia	ia	ia
27	$-CH_2-$	*n*-Bu	**1.5**$^{\pm 0.25}$ (73%)	ia	**4.2**$^{\pm 0.53}$ (120%)

EC_{50}/SD values were calculated using SigmaPlot2001 based on the mean values of at least three determinations. Values in brackets give the relative activation compared to the positive control. Positive controls were GW7647 for PPARα, pioglitazone for PPARγ and L165,041 for PPARβ, each at 1 μM; ia: inactive at 10 μM. Rand X are typed in bold as these entities display the varied substructure of the respective scaffold.

placed by a methylene group and the α-unsubstituted (**25**) as well as the α-*n*-butyl-substituted (**27**) analog was synthesized. Methin-analog **26** was obtained during synthesis. Interestingly, rigidization by the additional exocyclic double bond caused a complete loss of PPAR activity. Methylene analog **25** revealed an attenuated activity for all PPAR subtypes compared to **21**, whereas for *n*-butyl-substituted **27** an impressive loss could be observed only for PPARα activity. Regarding the 2-mercaptohexanoic acid substructure, these findings suggest a strong impact of the sulfur atom as well as of the α-*n*-butyl chain for PPARα but not for PPARγ activity.

In summary, we successfully established a novel and robust scaffold for highly active PPARα agonists based on the 2-mercaptohexanoic acid substructure. By systematic structural variation, we were able to cover a broad selectivity profile from PPARα-preferential dual PPARα/γ agonists to selective and nano molar active PPARα agonists. By preparation of carbon analogs and α-unsubstituted derivatives, we corroborated the importance of the sulfur atom as well as of the *n*-butyl chain of our 2-mercaptohexanoic acid head group for PPAR activity. Regarding the phenolic backbone of our scaffold, SAR revealed that especially substituents in *meta*-position (e.g., –Me, $-CF_3$, –OMe) were able to selectively improve PPARα activity. Encouraged by these promising in vitro results, we are planning further profiling of an eligible candidate in vivo in the near future.

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Supplementary data

Supplementary data (detailed synthetic procedure and analytical characterization of the compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.057.

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14. Cell culture, transfection and Gal4-transactivation-assay: Cos7 cells were grown in DMEM, supplemented with FCS, sodium pyruvate and penicillin/

streptomycine at 37 °C and 5% CO_2. The day before transfection, cells were seeded in 96-well plates in a density of 30,000 cells/well. Transfection was carried out by Lipofectamine™ 2000 reagent (Invitrogen) according to the manufacturer's protocol with pFR-Luc (Stratagene), pRL-SV40 (Promega) and the Gal4-fusion receptor plasmids (pFA-CMV-hPPAR-LBD) of the respective subtype. 5 h after transfection, medium was changed to DMEM without FCS, containing 0.1% DMSO and the respective concentrations of the test compounds. Following overnight incubation with the test compounds, cells were assayed for reportergene activity using Dual-Glo™ Luciferase Assay System (Promega) according to the manufacturer's protocol. Luminescence was measured with a GENios Pro luminometer (Tecan).

Each concentration of the compounds was tested in triplicate wells and each experiment was repeated independently at least three times.

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