

Synthesis, Biological Evaluation, and Molecular Modeling Investigation of Chiral Phenoxyacetic Acid Analogues with PPAR α and PPAR γ Agonist Activity

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that govern lipid and glucose homeostasis, and play a central role in cardiovascular disease, obesity, and diabetes. Thus, there is significant interest in developing new and specific agonists for these receptors. Herein we present screening results for a series of chiral phenoxyacetic acid analogues, some of which are potent PPAR α agonists as well as

PPAR γ agonists. The stereochemistry of these compounds plays an important role in determining their activity; the S isomers were observed to be more active than the corresponding R isomers. Interestingly, for one of these analogues, the stereoselectivity toward PPAR α was reversed, and for this reason docking experiments were performed to rationalize this peculiar behavior.

Introduction

Type 2 diabetes is a complex metabolic disorder that affects between 6 and 20% of the population in Western industrialized societies, with an estimated worldwide prevalence of 150 million people in 2000; this number is expected to increase to 220 million people by 2010.^[1] Type 2 diabetes is characterized by hyperglycemia, insulin resistance and defects in insulin secretion, and is usually associated with the so-called metabolic syndrome that includes dyslipidemia, hypertension, and obesity. Several drugs are currently available for the treatment of type 2 diabetes, including various insulin formulations, sulfonylureas, biguanides, glinides, and α -glucosidase inhibitors. Among the many approaches being evaluated for the discovery of new agents,^[2-4] one of the most promising is in the exploitation of peroxisome proliferator-activated receptor (PPAR) ligands.

PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily.^[5] PPARs are activated by a wide range of naturally occurring or metabolized lipids derived from the diet or from intracellular signaling pathways. These include saturated and unsaturated fatty acids and fatty acid derivatives such as prostaglandins and leukotrienes.^[6,7] Activation of PPARs leads to the formation of heterodimers with retinoid-X receptors (RXRs), and the resulting complex interacts with specific DNA response elements within promoter regions of target genes. When activated by the binding of an agonist, this heterodimer complex recruits transcription co-activators and regulates the transcription of genes involved in the control of lipid and carbohydrate metabolism.

There are three PPAR subtypes that are the products of distinct genes and are commonly designated PPAR α , PPAR γ , and

PPAR δ . Fibrates are a class of drugs that decrease serum triglycerides and increase HDL cholesterol through the activation of PPAR α , which is expressed predominantly in the liver.^[8] This receptor activation has also been shown to produce anti-inflammatory effects in vascular cells with possible beneficial effects in the prevention of atherosclerosis.^[9] Thiazolidine-2,4-diones (TZDs or glitazones), on the other hand, are antidiabetic agents that improve the blood glucose level in cases of type 2 diabetes through an insulin-sensitizing mechanism related to a selective activation of the PPAR γ subtype.^[10]

Given the importance of controlling both glucose and lipid levels in type 2 diabetes, the concept of identifying ligands that bind and activate both PPAR α and PPAR γ represents a logical continuation in the field of PPAR research. So far, therefore, a relatively high number of dual PPAR α and PPAR γ agonists have been described.^[11-22]

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Recently,^[23] we evaluated the effects of some chiral 2-aryloxy-3-phenylpropanoic acids on both PPAR α and PPAR γ and reported that the *S* isomers of the compounds containing an electron-withdrawing substituent on the aromatic ring of the phenoxy group (Figure 1, R=Cl, CF₃, Ph, 2-thienyl) present promising dual agonist activity. With the goal of improving the potency and efficacy of these compounds, we decided to fur-

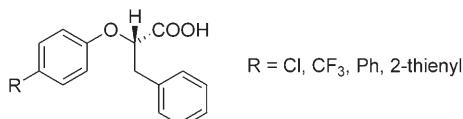


Figure 1. *S*-configured 2-aryloxy-3-phenylpropanoic acids with electron-withdrawing groups (R) shown to have promising dual agonist activity toward PPAR α and PPAR γ .

ther investigate this series by focusing our attention on the phenolic oxygen atom and the benzylic methylene group. Therefore, we report herein the synthesis and biological activity of a set of analogues (Table 1) in which the phenolic oxygen atom has been substituted by an isosteric sulfur or amino

group and the side chain on the stereogenic center has been lengthened or conformationally constrained, while maintaining the chlorine atom in the *para* position of the phenoxy moiety. The elongation of the side chain was carried out by introduction of one or more methylene units; in some cases, the methylene chain was linked to the aromatic ring by an ether group to give the corresponding bisphenoxylic derivatives for which PPAR activity had not yet been reported, although such compounds were recently claimed as hypolipidemic agents in a patent.^[24] Most compounds, moreover, had an additional chlorine atom in the *para* position of the side chain benzene ring.

Considering the high degree of stereoselectivity generally displayed by the PPAR ligands, a special emphasis was put on the influence of absolute configuration of our chiral compounds on PPAR α and PPAR γ activity. For this reason, when synthetically feasible, the preparation of racemates and separate enantiomers was accomplished (compounds **5**, **7**, **8**, **11**, and **12**), except compound **2**, for which only the optically active forms were prepared. For a correct evaluation of the biological activity of the racemates, we also synthesized and tested (*R,S*)-2-(4-chlorophenoxy)-3-phenylpropanoic acid **1** and its chloro derivative **4**, the effects of which on PPARs has not

been previously reported. The PPAR α and PPAR γ activity of all derivatives was evaluated by the transactivation assay, a powerful and widely used assay that is generally accepted to correlate well with *in vivo* activity.

Table 1. Structure and physical properties of compounds **1–16**.

Compd	A	B	R	Formula ^[a]	mp [°C]	Recrystallization Solvent	[α] _D ^[b]	1–15		16
								16		
1	O	CH ₂	H	C ₁₅ H ₁₃ ClO ₃	114–115	<i>n</i> -hexane	–			
(<i>R</i>)- 2	NH	CH ₂	H	C ₁₅ H ₁₄ ClNO ₂	172–173	<i>n</i> -hexane/CHCl ₃	–19			
(<i>S</i>)- 2	NH	CH ₂	H	C ₁₅ H ₁₄ ClNO ₂	175–176	<i>n</i> -hexane/CHCl ₃	+20			
3	S	CH ₂	H	C ₁₅ H ₁₃ ClO ₂ S	107–108	<i>n</i> -hexane	–			
4	O	CH ₂	Cl	C ₁₅ H ₁₂ Cl ₂ O ₃	130–131	<i>n</i> -hexane/CHCl ₃	–			
5	O	(CH ₂) ₂	H	C ₁₆ H ₁₅ ClO ₃	99–101	<i>n</i> -hexane/CHCl ₃	–			
(<i>R</i>)- 5	O	(CH ₂) ₂	H	C ₁₆ H ₁₅ ClO ₃	123–124	<i>n</i> -hexane/CHCl ₃	+58			
(<i>S</i>)- 5	O	(CH ₂) ₂	H	C ₁₆ H ₁₅ ClO ₃	123–124	<i>n</i> -hexane/CHCl ₃	–58			
6	O	(CH ₂) ₂	Cl	C ₁₆ H ₁₄ Cl ₂ O ₃	130–132	<i>n</i> -hexane/CHCl ₃	–			
7	O	(CH ₂) ₃	H	C ₁₇ H ₁₇ ClO ₃	100–101	<i>n</i> -hexane/CHCl ₃	–			
(<i>R</i>)- 7	O	(CH ₂) ₃	H	C ₁₇ H ₁₇ ClO ₃	104–105	<i>n</i> -hexane	+10			
(<i>S</i>)- 7	O	(CH ₂) ₃	H	C ₁₇ H ₁₇ ClO ₃	104–105	<i>n</i> -hexane	–7			
8	O	(CH ₂) ₃	Cl	C ₁₇ H ₁₆ Cl ₂ O ₃	97–98	<i>n</i> -hexane/CHCl ₃	–			
(<i>R</i>)- 8	O	(CH ₂) ₃	Cl	C ₁₇ H ₁₆ Cl ₂ O ₃	98–99	<i>n</i> -hexane/CHCl ₃	+5			
(<i>S</i>)- 8	O	(CH ₂) ₃	Cl	C ₁₇ H ₁₆ Cl ₂ O ₃	96–97	<i>n</i> -hexane/CHCl ₃	–5			
9	O	(CH ₂) ₄	H	C ₁₈ H ₁₉ ClO ₃	100–101	<i>n</i> -hexane	–			
10	O	(CH ₂) ₄	Cl	C ₁₈ H ₁₈ Cl ₂ O ₃	104–105	<i>n</i> -hexane	–			
11 ^[c]	O	CH ₂ O	Cl							
(<i>R</i>)- 11	O	CH ₂ O	Cl	C ₁₅ H ₁₂ Cl ₂ O ₄	151–152	<i>n</i> -hexane/CHCl ₃	–31			
(<i>S</i>)- 11	O	CH ₂ O	Cl	C ₁₅ H ₁₂ Cl ₂ O ₄	150–151	<i>n</i> -hexane/CHCl ₃	+30			
12 ^[c]	O	(CH ₂) ₂ O	Cl							
(<i>R</i>)- 12	O	(CH ₂) ₂ O	Cl	C ₁₆ H ₁₄ Cl ₂ O ₄	94–95	<i>n</i> -hexane/CHCl ₃	+22			
(<i>S</i>)- 12	O	(CH ₂) ₂ O	Cl	C ₁₆ H ₁₄ Cl ₂ O ₄	93–94	<i>n</i> -hexane/CHCl ₃	–21			
13 ^[c]	O	(CH ₂) ₃ O	Cl							
14 ^[c]	O	(CH ₂) ₄ O	Cl							
15 ^[c]	O	(CH ₂) ₅ O	Cl							
16				C ₁₅ H ₉ ClO ₃	254–255	<i>n</i> -hexane/CHCl ₃	–			

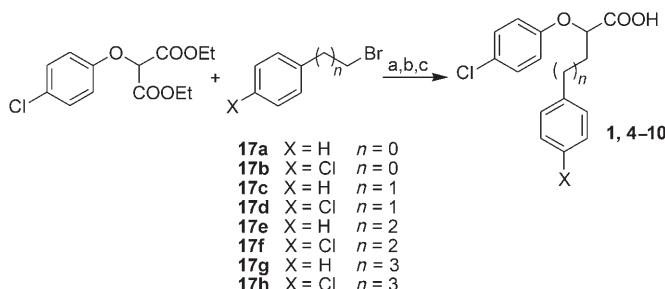
[a] Elemental analyses for C, H, and N or S were within $\pm 0.4\%$ of the theoretical values for the formulas given.

[b] $c=1.0$ in MeOH. [c] See reference [25].

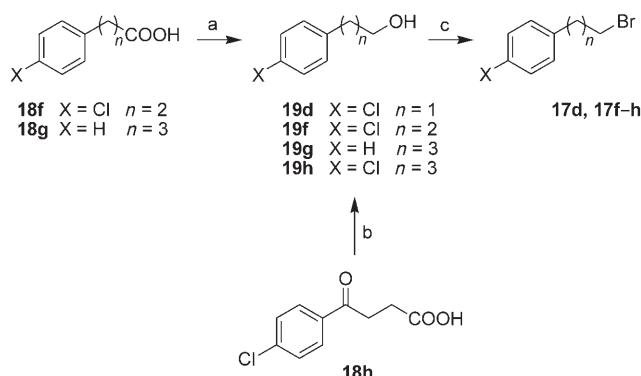
Results and Discussion

Chemistry

Racemates **1** and **4–10** were prepared by condensation of diethyl 4-chlorophenoxy malonate^[25] with aryl-alkyl bromides **17a–h** in the presence of NaH (Scheme 1) followed by alkaline hydrolysis and thermal decarboxylation at 160 °C. Bromides **17a–c** and **17e** are commercially available, whereas **17d** and **17f–h** were derived from treatment of the corresponding alcohols **19d** and **19f–h** with PBr₃ (Scheme 2). Except commercially available **19d**, these alcohols were obtained by reducing the aryl-alkyl acids **18f–g** with borane–methyl sulfide complex (BMS), or the keto acid **18h** with *tert*-butylaminoborane in the presence of AlCl₃.^[26] Stereoisomers of compound **5** were obtained by Mitsunobu conden-

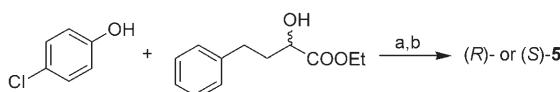


Scheme 1. a) NaH 95% powder, anhyd DMF; b) 1 N NaOH, EtOH 95%; c) de-carboxylation at 160 °C.

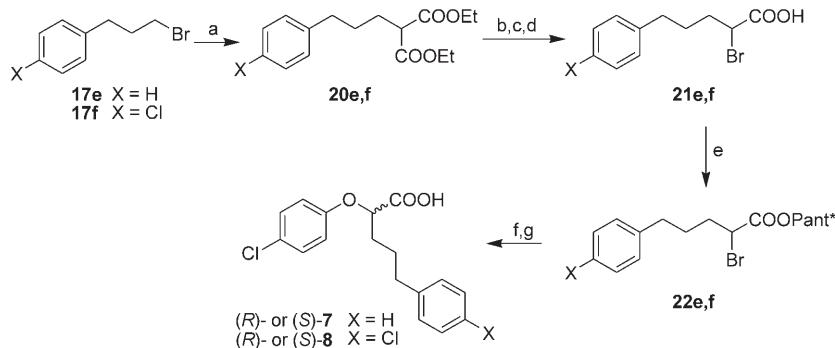


Scheme 2. a) BMS, anhyd THF; b) AlCl₃, tBu-NH₂·BH₃; c) PBr₃.

sation of 4-chlorophenol with commercially available (R)- or (S)-ethyl-2-hydroxy-4-phenylbutanoate. This reaction, which is known to occur with inversion of configuration,^[27] provided, after hydrolysis, (S)-5 and (R)-5, respectively (Scheme 3). The alternative pathway followed to prepare the enantiomers of **7** and **8** is reported in Scheme 4. The condensation of diethyl



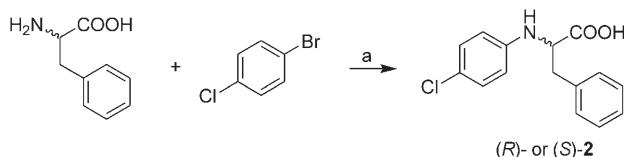
Scheme 3. a) Ph₃P, DIAD, anhyd toluene; b) 1 N NaOH/THF 1:1.



Scheme 4. a) NaOEt, abs EtOH, diethylmalonate, reflux; b) 2 N KOH, EtOH 95%, reflux; c) Br₂, CH₂Cl₂, 37 °C; d) de-carboxylation at 140 °C; e) (R)- or (S)-pantolactone, DCC, DMAP, anhyd THF; f) 4-chlorophenol, NaH 95% powder, n-tetrpentylammonium iodide, anhyd THF, -10 °C; g) LiOH, H₂O₂ 35%, THF/H₂O 4:1. *Pant = (R)- or (S)-pantolactone.

malonate with aryl-alkyl bromides **17e-f** in the presence of NaOEt gave diethyl esters **20e-f**, which were hydrolyzed, brominated, and thermally decarboxylated to give the bromo acids **21e-f**. Condensation with (R)- or (S)-pantolactone afforded esters **22e-f** which, after reaction with 4-chlorophenol under the diastereoselective conditions reported by Koh and Durst,^[28] were hydrolyzed to give the desired stereoisomers. The absolute configuration of these enantiomers was determined on the basis of circular dichroism analysis; the *R* configuration was assigned to the dextrorotatory isomers of **7** and **8**, whose CD curves show positive Cotton effects around 280 nm and in the range between 230 and 235 nm, and a negative Cotton effect around 220 nm. These effects in CD are also present for the stereochemically ascertained *R* isomers of homologues **1**^[23] and **5**.

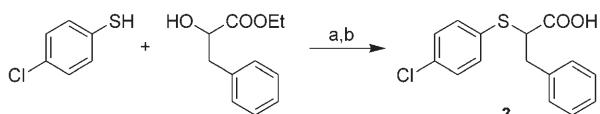
The optically active forms of amino compound **2** were prepared according to a published procedure^[29,30] (Scheme 5), by



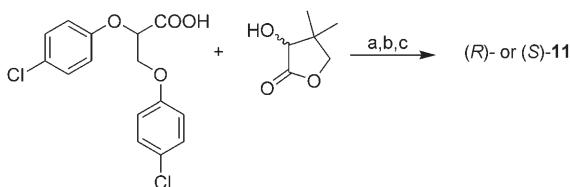
Scheme 5. a) K₂CO₃, Cul, anhyd DMF.

condensation of the corresponding (R)- or (S)-phenylalanine with 4-bromochlorobenzene in the presence of Cul and K₂CO₃ in dry *N,N*-dimethylformamide (DMF). The sulfur analogue **3** was synthesized as a racemate by hydrolysis of the ester obtained from (R,S)-ethyl phenyllactate and 4-chlorothiophenol under Mitsunobu conditions (Scheme 6). The attempt to prepare the enantiomers by the same procedure starting from (R)- or (S)-phenyllactate failed because of the extensive racemization occurring in the hydrolytic process leading to the final acid. This was in accordance with a previously reported racemization of chiral thio analogues of clofibrate acid.^[31]

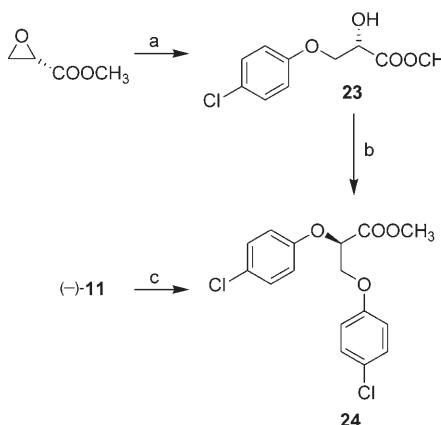
Racemates **11-15** were prepared as previously reported.^[25] Stereoisomers of compound **11** were obtained by fractional crystallization followed by hydrolysis of the diastereomeric esters through condensation with (R)- or (S)-pantolactone (Scheme 7). The absolute configuration was established by chemical correlation as depicted in Scheme 8. Commercially available (S)-ethyl glycidate was condensed with 4-chlorophenol to afford the (S)-hydroxy ester **23**, which was treated once again with 4-chlorophenol under Mitsunobu conditions to give the bisphenoxy compound **24** with the opposite configuration. This compound was also



Scheme 6. a) Ph₃P, DIAD, anhyd toluene; b) 1 N NaOH/THF 1:1.



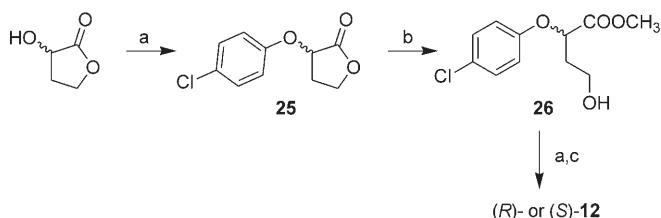
Scheme 7. a) DCC, DMAP; b) crystallization from *n*-hexane/CHCl₃; c) LiOH, H₂O₂ 35%, THF/H₂O 4:1.



Scheme 8. a) 4-chlorophenol, 95% NaH powder, anhyd MeOH; b) 4-chlorophenol, Ph₃P, DIAD, anhyd toluene; c) CH₂N₂.

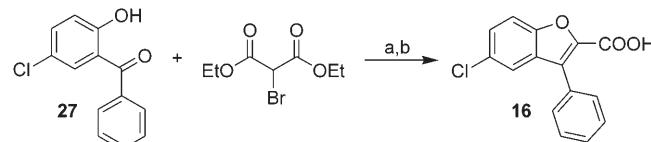
obtained from the levorotatory enantiomer of **11** by esterification with diazomethane, thus allowing assignment of the *R* configuration to this isomer, in contrast to what had been erroneously reported in our previous paper.^[32]

An alternate procedure was followed to obtain the enantiomers of **12** (Scheme 9) via intermediate **25** which was achieved by a Mitsunobu reaction between 4-chlorophenol and (*R*)- or (*S*)-2-hydroxybutyrolactone. The acid-catalyzed hydrolysis of **25** in MeOH gave the hydroxy ester **26**, which underwent a second condensation with 4-chlorophenol under Mitsunobu conditions and a final hydrolysis to give (*R*)- and (*S*)-**12**. The desired acids had the opposite absolute configuration of the



Scheme 9. a) 4-chlorophenol, Ph₃P, DIAD, anhyd toluene; b) MeOH, H₂SO₄ concd; c) 1 N NaOH/THF 1:1.

starting (*R*)- or (*S*)-2-hydroxybutyrolactone as assigned on the basis of the known stereochemical course of the Mitsunobu reaction. The known compound **16** was prepared differently from the published procedure^[33] that in our hands, gave a very low yield. Therefore, we followed the pathway^[34] illustrated in Scheme 10, in which benzophenone **27** was treated with diethyl bromomalonate in the presence of K₂CO₃ in boiling acetone to afford an intermediate ester the alkaline hydrolysis of which provided the desired compound.



Scheme 10. a) K₂CO₃, acetone; b) 1 N NaOH/THF 1:1.

PPAR activity

Compounds **1–16** were evaluated for their agonist activity toward the human PPAR α (hPPAR α) and PPAR γ (hPPAR γ) subtypes. For this purpose, GAL4-PPAR chimeric receptors were expressed in transiently transfected HepG2 cells according to a previously reported procedure.^[35] The results obtained were compared with corresponding data for Wy-14,643 and rosiglitazone used as reference compounds in the PPAR α and PPAR γ transactivation assays, respectively (Table 2). Maximum obtained fold induction with the reference agonist was defined as 100%.

Racemate **1** and its isosteres **2** and **3** were examined first. As expected, (*R,S*)-**1** displayed a weaker activity on PPAR α and PPAR γ than its previously tested *S* isomer.^[23] Surprisingly, both enantiomers of **2** were completely inactive on PPAR α and PPAR γ , allowing us to hypothesize that the presence of the basic amino group and the carboxylic function gives rise to the possible formation of a zwitterion with different physicochemical properties from those of the oxygenated isostere. In contrast, substitution of the amino group in **2** with the much more lipophilic sulfur atom gave **3**, which behaved differently toward the two receptor subtypes, showing good potency and high efficacy on PPAR α with low activity on PPAR γ .

Lengthening the methylenic bridge between the stereogenic center and the aromatic ring in compound **1** increased the potency and efficacy toward PPAR α , with the exception of the most hydrophobic analogues of the series, compounds **9** and **10**. The same behavior was found on PPAR γ , but in this case, the exceptions were the compounds with two methylenic units (compounds **5** and **6**). In all cases, the introduction of the second chlorine atom as a substituent of the side chain aromatic ring afforded more potent compounds with almost unchanged efficacy. Stereochemistry also played a significant role; the *S* isomers were more active than the corresponding *R* isomers. Surprisingly, this rule was partially reversed for compound **8**, whose *R* isomer turned out to be the most active derivative of the whole series regarding the PPAR α isoform. It was fivefold more potent than Wy-14,643 with a much higher

Table 2. Activity of compounds tested in the cell-based transactivation assay.

Compd	PPAR α		PPAR γ	
	EC ₅₀ [μ M]	Efficacy [%]	EC ₅₀ [μ M]	Efficacy [%]
1	7.94 \pm 1.70	82 \pm 4	14.46 \pm 3.46	48 \pm 4
(<i>R</i>)- 2	i.a. ^[a]	i.a.	i.a.	i.a.
(<i>S</i>)- 2	i.a.	i.a.	i.a.	i.a.
3	5.56 \pm 1.52	149 \pm 28	25.81 \pm 2.84	30 \pm 6
4	3.16 \pm 0.21	77 \pm 6	2.23 \pm 0.23	49 \pm 1
5	3.69 \pm 0.68	119 \pm 12	16.45 \pm 4.60	33 \pm 4
(<i>R</i>)- 5	8.13 \pm 2.22	115 \pm 16	23.32 \pm 3.87	30 \pm 24
(<i>S</i>)- 5	2.39 \pm 0.30	114 \pm 3	7.03 \pm 4.62	48 \pm 17
6	1.36 \pm 0.46	105 \pm 5	5.05 \pm 1.34	29 \pm 6
7	0.51 \pm 0.02	132 \pm 46	1.02 \pm 0.35	56 \pm 14
(<i>R</i>)- 7	1.07 \pm 0.16	117 \pm 1	n.c. ^[b]	11 \pm 1
(<i>S</i>)- 7	0.59 \pm 0.30	134 \pm 1	0.91 \pm 0.33	46 \pm 6
8	0.35 \pm 0.18	152 \pm 49	n.c.	64 \pm 11
(<i>R</i>)- 8	0.33 \pm 0.07	161 \pm 2	n.c.	26 \pm 1
(<i>S</i>)- 8	0.63 \pm 0.10	130 \pm 6	0.32 \pm 0.01	66 \pm 4
9	1.59 \pm 0.92	99 \pm 12	1.28 \pm 0.34	86 \pm 14
10	0.56 \pm 0.22	68 \pm 7	0.61 \pm 0.25	72 \pm 10
11	2.91 \pm 0.81	74 \pm 6	2.51 \pm 0.21	34 \pm 2
(<i>R</i>)- 11	i.a.	i.a.	i.a.	i.a.
(<i>S</i>)- 11	2.49 \pm 1.80	87 \pm 7	1.71 \pm 0.40	40 \pm 7
12	2.13 \pm 0.29	80 \pm 2	2.63 \pm 0.62	39 \pm 5
(<i>R</i>)- 12	6.74 \pm 1.35	35 \pm 8	n.c.	12 \pm 2
(<i>S</i>)- 12	3.00 \pm 0.14	59 \pm 4	1.25 \pm 0.56	46 \pm 6
13	4.68 \pm 2.60	62 \pm 13	1.24 \pm 0.53	66 \pm 5
14	1.06 \pm 0.65	45 \pm 6	0.14 \pm 0.05	82 \pm 7
15	0.22 \pm 0.12	74 \pm 19	n.c.	47 \pm 8
16	i.a.	i.a.	i.a.	i.a.
Wy-14,643	1.6 \pm 0.3	100 \pm 9.71	i.a.	i.a.
rosiglitazone	i.a.	i.a.	0.039 \pm 0.003	100 \pm 9.06

[a] i.a.: Inactive at tested concentrations. Efficacy values were calculated as the percentage of the maximum obtained fold induction with the reference compounds (Wy-14,643 for PPAR α ; rosiglitazone for PPAR γ).
[b] n.c.: Not computable; in fact, the activity increases with increasing concentrations up to 10 μ M, above which the activity begins to decrease.

efficacy and twice as potent as the *S* isomer (0.33 μ M versus 0.63 μ M, $p < 0.05$). Moreover, the racemates of **7** and **8** were shown to be as active as their most active isomers, suggesting some kind of synergistic effect from the optical isomers.

The introduction of a second ether oxygen atom between the methylenic chain and the aromatic ring of the side chain linked to the stereogenic center gave the bisphenoxy derivatives **11**–**15**. The analogues of this series did not show a well-defined structure–activity relationship on both PPAR α and PPAR γ even though the potency was basically increased by the elongation of the methylenic chain (with some exceptions). These compounds displayed low to moderate efficacy and on the whole turned out to be less active than the corresponding methylenic isosteres (**12** versus **8** and **13** versus **10**), allowing us to hypothesize the involvement of the oxygen atom in some polar interaction unfavorable for receptor activation. Nonetheless, derivative **14** was the most potent and efficacious PPAR γ agonist of the whole series of analogues reported herein. Stereochemistry also exerted a strong influence in this subset of compounds, with the *S* isomers more active than the corresponding *R* isomers, one of which ((*R*)-**11**) turned out to

be completely inactive on both PPAR α and PPAR γ isoforms. The same lack of activity was also found with compound **16**, in which the conformational constraint imposed by the benzofuran system gave low flexibility and a high degree of planarity that was detrimental for receptor activation.

The most representative compounds of the series (**1**–**4**, **6**–**8**, **10**, **12**, and **16**) were also tested with the PPAR δ subtype using the known agonist L-165,041 as a reference compound, but in this case no activation was observed (data not shown), suggesting that these molecules are PPAR α / γ -selective ligands, as expected for 2-aryloxyacetic acids with a bulky substituent situated alpha to the carboxylic group.^[23]

To get a better understanding of the different behavior of both *R* and *S* isomers of **7** and **8** toward PPAR α at a molecular level and to propose a binding mode that explains the SAR data, docking experiments were performed by using the crystallographic coordinates of hPPAR α in complex with GW409544 as a reference^[36] (PDB code: 1K7L). Flexible ligand docking was performed with the help of DOCK 6.0 (University of California, San Francisco; <http://dock.compbio.ucsf.edu>), an automated molecular docking and database screening program developed by Kuntz and co-workers.^[37,38] The core of the DOCK searching and scoring algorithm is to superimpose the ligand atoms onto predefined site-points that map out the negative image of the protein binding site and evaluate the complementarity between the two.^[39]

The binding site of GW409544, a potent full agonist of both PPAR α and PPAR γ , on the surface of hPPAR α was represented as a cluster of 169 site-points which were generated by the sphere-generation accessory program, SPHGEN, integrated into the DOCK program suite. The identified binding site was then analyzed by the program GRID, which saves information about the steric and electrostatic environment at each point on a grid. The conformation of each compound was searched and evaluated based on an energy score function, a measurement of the extent of van der Waals and electrostatic interactions between the ligand and the protein.

As a preliminary test of the docking method, GW409544 was docked into the hPPAR α crystal structure. The docking test indicated that the top scoring solution reproduced the crystallographic binding mode of GW409544 to hPPAR α very closely. The hydrogen bond network predicted by DOCK was virtually identical to that found in the crystal structure. This docking test provided validation for using this program to perform docking studies of our ligands to hPPAR α .

Docking of both *R* and *S* isomers of **7** and **8** into the hPPAR α binding site revealed a very clear preference for a single binding position. Interestingly, the top scoring solutions of the *S* isomers of **7** and **8** were found to bind hPPAR α in an orientation very similar to that previously described for structurally similar compounds.^[23] Surprisingly, a similar binding pose was also found for *R* isomers, although the phenoxy and phenylpropyl moieties occupied the hydrophobic pockets in a reversed manner. Figure 2a–c depicts the energy-minimized (*R*)-**7**–PPAR α , (*S*)-**7**–PPAR α , (*R*)-**8**–PPAR α and (*S*)-**8**–PPAR α complexes, in which only the amino acids located within a distance of 4 \AA from any atom of the bound ligand are displayed.

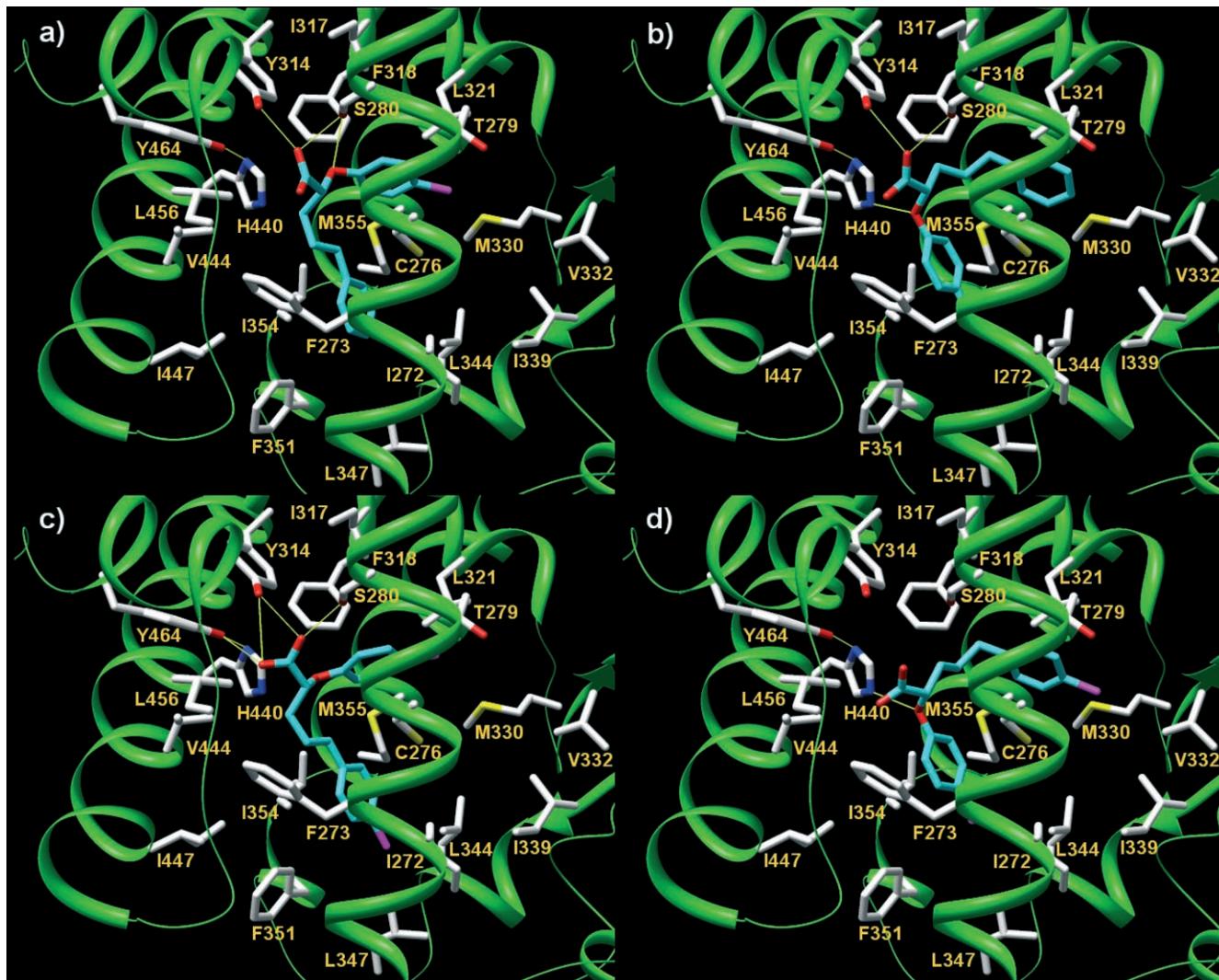


Figure 2. Compounds a) (R)-7, b) (S)-7, c) (R)-8, and d) (S)-8 are shown docked into the hPPAR α binding site. Only amino acids located within 4 Å of the bound ligand are displayed and labeled. The ligand atoms are shown in cyan; hydrogen bonds discussed in the text are depicted as yellow lines.

The ligands adopt a U-shaped conformation that allows the carboxylate group to form H bonds with Y314 and S280, with the exception of (S)-8. H440, the N $^{\delta}$ atom of which is hydrogen bonded to O $^{\alpha}$ of Y464, forms H bonds solely with the phenoxylic oxygen atom of S isomers through its N $^{\epsilon}H$ group (see Figure 2b and d). Interestingly, only compound (R)-8 makes a direct H bond with Y464 on the C-terminal activation function 2 (AF-2) helix (Figure 2c), which has been proven to stabilize the receptor in the active conformation,^[36] thus explaining the high efficacy of this isomer toward hPPAR α . The above-mentioned hydrogen bonding pattern is a conserved feature in all agonist-PPAR α complexes and is expected to be essential for the formation of a tight binding ligand complex that stabilizes a charge clamp^[40] between the C-terminal AF-2 helix and a conserved lysine residue on the surface of the receptor to permit co-activator recruitment.^[41,42]

The phenylpropyl moiety of both (S)-7 and (S)-8 is bound in a hydrophobic cavity formed by T279, I317, F318, L321, M330, V332, I339, L344, and M355 side chains. The V332 side chain appears in a suitable orientation to make lipophilic inter-

actions with the *p*-chlorine atom of (S)-8. The *p*-chlorophenoxy ring of both enantiomers points to a large hydrophobic cleft lined by residues I272, F273, L344, L347, F351, I354, M355, and I447. In particular, the electron-rich benzene ring of Phe273 appears to be optimally oriented for a favorable T-shaped π - π interaction with that of the ligand (made electron-deficient by the *p*-chlorine atom). Notably, the Cys276 side chain is in close contact with both aromatic rings of the ligand, making additional hydrophobic interactions.

From a visual inspection of the ligands complexed with hPPAR α , it seems clear that the optimal length of the bridge between the stereogenic center and the aromatic ring is that of three methylene units. In fact, the introduction of an additional methylene unit, as in compounds **9** and **10**, increases the steric hindrance inside the binding cavity and changes the optimal binding mode of the ligands, slightly decreasing the relative stability of the complex.

As shown in Figure 2a and c, (R)-7 and (R)-8 assume a binding mode in which the carboxylate group still interacts with Tyr314 and Ser280 side chains, even if the phenoxy and phe-

nylpropyl groups appear to occupy the above-described hydrophobic pockets in a reversed way. In particular, the *p*-chlorophenoxy ring of both enantiomers is hosted by a hydrophobic pocket made up by residues T279, I317, F318, L321, M330, V332, I339, L344, and M355. In particular, F318 interacts with the ligand's aromatic ring by a T-shaped interaction, while the chlorine at the *para* position makes favorable hydrophobic contacts with the L321 side chain. On the other hand, the phenylpropyl moiety is inserted in a lipophilic pocket formed by residues I272, F273, L344, L347, F351, I354, M355, and I447. Within this pocket, the benzene rings of Phe351 and Phe273 make favorable charge-transfer interactions with the aromatic ring of the two enantiomers. From the docking model illustrated in Figure 2c, it may also be deduced that the *p*-chlorine atom of (R)-8 makes favorable hydrophobic interactions with the side chains of I272, L344, and L347. Such interactions are in consonance with the activity trend of these compounds, showing that the introduction of a second chlorine atom as a substituent of the side chain aromatic ring increases potency.

To understand the low activity toward hPPAR γ , both *R* and *S* isomers of 7 and 8 were also docked into the hPPAR γ receptor binding domain.^[40] Surprisingly, DOCK calculations did not converge towards a single binding position but predicted several binding modes, all located in regions different from the co-crystallized bound agonist rosiglitazone, which is known to form H bonds with residues Ser289, His323, His449, and Tyr473. Many reports have suggested that the interactions with this conserved hydrogen-bond-rich area are important for the activities of PPAR γ agonists, as this H bonding network could stabilize the AF-2 helix in a conformation favoring the binding of co-activators to PPAR γ and, consequently, enhance their recruitment.^[40] Hence, the absence of these critical H bonding interactions with the protein might provide the structural basis for the much weaker transactivation activity (efficacy <70%) of 7, 8, and related compounds for PPAR γ .

In conclusion, we prepared and tested a new series of chiral 4-chlorophenoxyacetic acid analogues, some of which are potent agonists of PPAR α and PPAR γ . The stereochemistry of these compounds played an important role in determining their activity; the *S* isomers, in fact, were more active than the corresponding *R* isomers, with the exception of (R)-8, which turned out to be the most active derivative of the whole series toward PPAR α . Docking experiments were performed to rationalize this peculiar behavior, allowing us to hypothesize only for this stereoisomer the possibility to form a direct H bond with Y464 on the C-terminal AF-2 helix.

Experimental Section

Biological methods: Media, other cell culture reagents, and Wy-14,643 were purchased from Sigma (Milan, Italy). BRL 49653 (rosiglitazone) was obtained from Hefei Scenery Chemical Co. (Hefei, Anhui, PR China).

Plasmids: The expression vectors for the chimeric receptors containing the yeast GAL4 DNA binding domain fused to the human PPAR α , PPAR γ , or PPAR δ ligand binding domain (LBD) and the reporter plasmid for these GAL4 chimeric receptors (pGAL5TKpGL3)

containing five repeats of the GAL4 response elements upstream of a minimal thymidine kinase promoter that is adjacent to the luciferase gene were described previously.^[43] These plasmids were kindly donated by Dr. Krister Bamberg (AstraZeneca, Mölndal, Sweden).

Cell culture and transfections: Human hepatoblastoma cell line HepG2 (Interlab Cell Line Collection, Genoa, Italy) was cultured in minimum essential medium (MEM) containing heat-inactivated fetal bovine serum (FBS, 10%), penicillin G (100 U mL⁻¹), and streptomycin sulfate (100 μ g mL⁻¹) at 37 °C in a humidified atmosphere of 5% CO₂. For transactivation assays, 105 cells per well were seeded in a 24-well plate in triplicate, and transfections were performed after 24 h, with CAPHOS (Sigma, Milan, Italy), a calcium-phosphate method, according to the manufacturer's guidelines. Cells were transfected with expression plasmids encoding the fusion protein GAL4-PPAR α LBD or GAL4-PPAR γ LBD (30 ng), pGAL5TKpGL3 (100 ng), pCMV β gal (250 ng). After transfection, cells were treated for 20 h with the indicated ligands. Luciferase activity in cell extracts was then determined by a luminometer (VICTOR³ V Multilabel Reader, PerkinElmer). β -Galactosidase activity was determined using β -D-galactopyranoside (Sigma, Milan, Italy) as described previously.^[44] All transfection experiments were repeated at least twice. The Student *t* test was used to assess the statistical significance of the difference between means calculated for compounds (R)-8 and (S)-8.

Chemical methods: Column chromatography was performed on ICN silica gel 60 Å (63–200 μ m) as a stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus and are uncorrected. Mass spectra were recorded with an HP GC-MS 6890–5973 MSD spectrometer, electron impact 70 eV, equipped with HP chemstation. ¹H NMR spectra were recorded in CDCl₃ (the use of [D₆]DMSO as a solvent is specified) on a Varian-Mercury 300 (300 MHz) spectrometer at room temperature (20 °C). Chemical shifts are expressed as parts per million (δ). For optical isomers, MS and NMR spectra are reported only for the racemate or one of the two enantiomers. Microanalyses of solid compounds were carried out with a Eurovector Euro EA 3000 model analyzer; the analytical results are within \pm 0.4% of theoretical values. Optical rotations were measured with a PerkinElmer 341 polarimeter at room temperature (20 °C); concentrations are expressed as g(100 mL)⁻¹. The CD curves were registered on a J-810 model JASCO spectropolarimeter. The enantiomeric excesses of acids were determined by HPLC analysis of their methyl esters, obtained by reaction with a solution of diazomethane in ether, on Chiralcel OD or AD columns (4.6 mm i.d. \times 250 mm, Daicel Chemical Industries, Ltd., Tokyo, Japan). Analytical liquid chromatography was performed on a PE chromatograph equipped with a Rheodyne 7725i model injector, a 785 A model UV/Vis detector, a series 200 model pump, and NCI 900 model interface. Chemicals were obtained from Aldrich (Milan, Italy), Lancaster (Milan, Italy), or Across (Milan, Italy), and were used without any further purification.

Preparation of aryl-alkyl bromides 17d and 17f–h; general procedure: PBr₃ (11 mmol) was carefully added to the appropriate aryl-alkyl alcohol (19d, 19f–h) (10 mmol) at 0 °C. The reaction mixture was stirred for 1–2 h at 0 °C and for 4–8 h at room temperature, then poured onto ice and extracted with Et₂O. The organic layer was washed with brine, dried over Na₂SO₄, and the solvent was evaporated in vacuo to give a pale-yellow oily residue, which was used in the next step without any further purification or after chromatography on a silica gel column (petroleum ether/ethyl acetate 95:5 or 90:10 as eluents).

2-(4-Chlorophenyl)ethyl bromide (17d): 90% yield; GC-MS m/z (%): 222 (7) [$M^+ + 4$], 220 (28) [$M^+ + 2$], 218 (22) [M^+], 125 (100) [$C_7H_6Cl^+$].

3-(4-Chlorophenyl)propyl bromide (17f): 92% yield; GC-MS m/z (%): 236 (10) [$M^+ + 4$], 234 (36) [$M^+ + 2$], 232 (28) [M^+], 125 (100) [$C_7H_6Cl^+$].

4-Phenylbutyl bromide (17g): 94% yield; GC-MS m/z (%): 214 (18) [$M^+ + 2$], 212 (18) [M^+], 91 (100) [$C_7H_7^+$].

4-(4-Chlorophenyl)butyl bromide (17h): 87% yield; GC-MS m/z (%): 250 (7) [$M^+ + 4$], 248 (25) [$M^+ + 2$], 246 (20) [M^+], 125 (100) [$C_7H_6Cl^+$].

Preparation of alcohols 19f,g: Borane–methyl sulfide complex (BMS, 45 mmol) was carefully added dropwise, under N_2 atmosphere, to a stirred and cooled (0°C) solution of **18f** or **18g** (15 mmol) in anhyd THF (70 mL). The reaction mixture was stirred for 15 h at room temperature, cooled to 0°C , and CH_3OH (30 mL, 0.5 h) was carefully added dropwise to destroy excess BMS. After distilling off the organic solvents, the mixture was dissolved in Et_2O , and the resulting solution was washed with 2 N NaOH and brine. The organic layer was dried over Na_2SO_4 and filtered. Evaporation of the solvent in vacuo afforded the desired compound as colorless oils in quantitative yield.

3-(4-Chlorophenyl)-1-propanol (19f): GC-MS m/z (%): 172 (12) [$M^+ + 2$], 170 (35) [M^+], 117 (100) [$C_9H_9^+$].

4-Phenyl-1-butanol (19g): GC-MS m/z (%): 150 (29) [M^+], 104 (100) [$C_8H_8^+$], 91 (77) [$C_7H_7^+$].

4-(4-Chlorophenyl)-1-butanol (19h): Prepared according published methods.^[26]

Preparation of diethyl 2-(4-chlorophenoxy)-2-aryl-alkyl malonates; general procedure (Scheme 1): A solution of diethyl 2-(4-chlorophenoxy)malonate^[25] (10 mmol) in anhyd DMF (25 mL) was added dropwise to a suspension of NaH (95% powder, 18 mmol) in anhyd DMF (20 mL) at 0°C . After stirring at room temperature for 20 min, a solution of the suitable aryl-alkyl bromide (**17a–h**) (12 mmol) in anhyd DMF (15 mL) was added dropwise and the resulting reaction mixture was stirred at 60°C for 15–20 h. The solvent was removed under reduced pressure, and the residue was poured into water and extracted with diethyl ether. The organic layer was washed with saturated ammonium chloride, dried over Na_2SO_4 , and the solvent was evaporated in vacuo to give an oily residue, which was separated on a silica gel column (petroleum ether/ethyl acetate 9:1 as eluent). The title compounds were obtained as pale-yellow oils in 42–78% yield.

Diethyl 2-(4-chlorophenoxy)-2-benzyl malonate: 64% yield; GC-MS m/z (%): 378 (28) [$M^+ + 2$], 376 (85) [M^+], 202 (100) [$C_{12}H_{10}O_3^+$]; ^1H NMR: δ = 1.15 (t, 6 H, 2 CH_3), 3.55 (s, 2 H, ArCH_2), 4.16 (q, 4 H, 2 CH_2O), 6.84–7.26 ppm (m, 9 H, aromatics).

Diethyl 2-(4-chlorophenoxy)-2-(4-chlorobenzyl)malonate: 44% yield; GC-MS m/z (%): 414 (13) [$M^+ + 4$], 412 (58) [$M^+ + 2$], 410 (92) [M^+], 237 (100) [$C_{12}H_{10}ClO_3^+$]; ^1H NMR: δ = 1.15 (t, 6 H, 2 CH_3O), 3.55 (s, 2 H, ArCH_2), 4.16 (q, 4 H, 2 CH_2O), 6.84–7.26 ppm (m, 8 H, aromatics).

Diethyl 2-(4-chlorophenoxy)-2-(2-phenylethyl)malonate: 56% yield; GC-MS m/z (%): 392 (2) [$M^+ + 2$], 390 (6) [M^+], 286 (100) [$C_{13}H_{15}ClO_3^+$]; ^1H NMR: δ = 1.21 (t, 6 H, 2 CH_3), 2.47–2.56 (m, 2 H, ArCH_2), 2.60–2.71 (m, 2 H, ArCH_2CH_2), 4.21 (q, 4 H, 2 CH_2O), 6.71–7.25 ppm (m, 9 H, aromatics).

Diethyl 2-(4-chlorophenoxy)-2-[2-(4-chlorophenyl)ethyl]malonate: 63% yield; GC-MS m/z (%): 424 (2) [M^+], 286 (100) [$C_{13}H_{15}ClO_3^+$]; ^1H NMR: δ = 1.22 (t, 6 H, 2 CH_3), 2.46–2.54 (m, 2 H, ArCH_2), 2.62–2.72 (m, 2 H, ArCH_2CH_2), 4.22 (q, 4 H, 2 CH_2O), 6.88–7.25 ppm (m, 8 H, aromatics).

Diethyl 2-(4-chlorophenoxy)-2-(3-phenylpropyl)malonate: 78% yield; GC-MS m/z (%): 406 (9) [$M^+ + 2$], 404 (25) [M^+], 129 (100); ^1H NMR: δ = 1.19 (t, 6 H, 2 CH_3), 1.62–1.78 (m, 2 H, $\text{ArCH}_2\text{CH}_2\text{CH}_2$), 2.18–2.30 (m, 2 H, $\text{Ar}(\text{CH}_2)_2\text{CH}_2$), 2.59 (t, 2 H, ArCH_2), 4.21 (q, 4 H, 2 CH_2O), 6.79–7.31 ppm (m, 9 H, aromatics).

Diethyl 2-(4-chlorophenoxy)-2-[3-(4-chlorophenyl)propyl]malonate: 67% yield; GC-MS m/z (%): 442 (3) [$M^+ + 4$], 440 (16) [$M^+ + 2$], 438 (23) [M^+], 125 (100) [$C_7H_6Cl^+$]; ^1H NMR: δ = 1.22 (t, 6 H, 2 CH_3), 1.61–1.77 (m, 2 H, $\text{ArCH}_2\text{CH}_2\text{CH}_2$), 2.17–2.30 (m, 2 H, $\text{ArCH}_2\text{CH}_2\text{CH}_2$), 2.58 (t, 2 H, ArCH_2), 4.23 (q, 4 H, 2 CH_2O), 6.81–7.33 ppm (m, 8 H, aromatics).

Diethyl 2-(4-chlorophenoxy)-2-(4-phenylbutyl)malonate: 42% yield; GC-MS m/z (%): 420 (8) [$M^+ + 2$], 418 (21) [M^+], 91 (100) [$C_7H_7^+$]; ^1H NMR: δ = 1.19 (t, 6 H, 2 CH_3), 1.34–1.47 (m, 2 H, $\text{ArCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.55–1.67 (m, 2 H, $\text{ArCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.20–2.28 (m, 2 H, $\text{Ar}(\text{CH}_2)_3\text{CH}_2$), 2.57 (t, 2 H, ArCH_2), 4.21 (q, 4 H, 2 CH_2O), 6.81–7.29 ppm (m, 9 H, aromatics).

Diethyl 2-(4-chlorophenoxy)-2-[4-(4-chlorophenyl)butyl]malonate: 60% yield; GC-MS m/z (%): 456 (4) [$M^+ + 4$], 454 (18) [$M^+ + 2$], 452 (28) [M^+], 125 (100) [$C_7H_6Cl^+$]; ^1H NMR: δ = 1.19 (t, 6 H, 2 CH_3), 1.31–1.42 (m, 2 H, $\text{ArCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.52–1.64 (m, 2 H, $\text{ArCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.18–2.28 (m, 2 H, $\text{Ar}(\text{CH}_2)_3\text{CH}_2$), 2.54 (t, 2 H, ArCH_2), 4.22 (q, 4 H, 2 CH_2O), 6.81–6.88, 7.01–7.06 and 7.15–7.24 ppm (m, 8 H, aromatics).

Preparation of acids 1 and 4–10; general procedure: The suitable diethyl malonate (3 mmol), obtained from the reaction described above, was stirred at reflux with 1 N NaOH (3 mL) in 95% EtOH (12 mL) for 4–6 h. The organic solvent was distilled off under reduced pressure and the remaining aqueous phase was washed with Et_2O , acidified to pH 2 with 6 N HCl, and extracted with Et_2O . The combined organic extracts were dried over sodium sulfate, and the solvent was removed under reduced pressure. The resulting products were heated at 160°C for 2 h to afford the desired acids as white solids, which were purified by recrystallization from *n*-hexane/CHCl₃.

2-(4-Chlorophenoxy)-3-phenylpropanoic acid (1): 56% yield; GC-MS (methyl ester) m/z (%): 292 (20) [$M^+ + 2$], 290 (56) [M^+], 121 (100) [$C_8H_9O^+$]; ^1H NMR: δ = 3.21–3.28 (m, 2 H, CH_2), 4.76–4.83 (m, 1 H, CH), 6.74–7.34 ppm (m, 10 H, aromatics + COOH, D₂O exchanged); anal.: calcd for C₁₅H₁₃ClO₃: C 65.11%, H 4.74%, found: C 65.21%, H 4.82%.

2-(4-Chlorophenoxy)-3-(4-chlorophenyl)propanoic acid (4): 51% yield; GC-MS (methyl ester) m/z (%): 328 (5) [$M^+ + 4$], 326 (23) [$M^+ + 2$], 324 (33) [M^+], 155 (100) [$C_8H_8ClO^+$]; ^1H NMR: δ = 3.16–3.31 (m, 2 H, CH_2), 4.76 (dd, 1 H, CH), 6.72–6.79 and 7.18–7.30 ppm (m, 9 H, aromatics + COOH, D₂O exchanged); anal.: calcd for C₁₅H₁₂Cl₂O₃: C 57.90%, H 3.89%, found: C 57.82%, H 3.89%.

2-(4-Chlorophenoxy)-4-phenylbutanoic acid (5): 33% yield; GC-MS (methyl ester) m/z (%): 306 (13) [$M^+ + 2$], 304 (40) [M^+], 91 (100) [$C_7H_7^+$]; ^1H NMR: δ = 2.26–2.31 (m, 2 H, ArCH_2CH_2), 2.84–2.87 (m, 2 H, ArCH_2), 4.58 (dd, 1 H, CH), 6.78–6.81 and 7.15–7.30 ppm (m, 10 H, aromatics + COOH, D₂O exchanged); anal.: calcd for C₁₆H₁₅ClO₃: C 66.10%, H 5.20%, found: C 66.13%, H 5.27%.

2-(4-Chlorophenoxy)-4-(4-chlorophenyl)butanoic acid (6): 25% yield; GC-MS (methyl ester) m/z (%): 342 (5) [M^++4], 340 (23) [M^++2], 338 (34) [M^+], 125 (100) [$C_7H_6Cl^+$]; 1H NMR: δ = 2.20–2.32 (m, 2H, ArCH₂CH₂), 2.75–2.90 (m, 2H, ArCH₂), 4.54 (dd, 1H, CH), 7.06–7.12 and 7.20–7.30 ppm (m, 9H, aromatics + COOH, D₂O exchanged); anal.: calcd for $C_{16}H_{14}Cl_2O_3$: C 59.10%, H 4.34%, found: C 59.22%, H 4.38%.

2-(4-Chlorophenoxy)-5-phenylpentanoic acid (7): 48% yield; GC-MS (methyl ester) m/z (%): 320 (8) [M^++2], 318 (22) [M^+], 131 (100) [$C_{10}H_{11}^+$]; 1H NMR: δ = 1.82–2.12 (m, 4H, ArCH₂CH₂CH₂), 2.68 (t, 2H, ArCH₂), 4.59 (t, 1H, CH), 6.65–6.75 and 7.12–7.31 (m, 9H, aromatics), 9.12 ppm (bs, 1H, COOH, D₂O exchanged); anal.: calcd for $C_{17}H_{17}ClO_3$: C 67.00%, H 5.62%, found: C 66.88%, H 5.59%.

2-(4-Chlorophenoxy)-5-(4-chlorophenyl)pentanoic acid (8): 59% yield; GC-MS (methyl ester) m/z (%): 356 (3) [M^++4], 354 (16) [M^++2], 352 (22) [M^+], 165 (100) [$C_{10}H_{10}Cl^+$]; 1H NMR: δ = 1.74–2.08 (m, 4H, ArCH₂CH₂CH₂), 2.64 (t, 2H, ArCH₂), 4.59 (t, 1H, CH), 5.42 (bs, 1H, COOH, D₂O exchanged), 6.76–6.82 and 7.05–7.28 ppm (m, 8H, aromatics); anal.: calcd for $C_{17}H_{16}Cl_2O_3$: C 60.19%, H 4.75%, found: C 60.52%, H 5.03%.

2-(4-Chlorophenoxy)-6-phenylhexanoic acid (9): 66% yield; GC-MS (methyl ester) m/z (%): 334 (15) [M^++2], 332 (42) [M^+], 91 (100) [$C_7H_7^+$]; 1H NMR: δ = 1.48–1.75 (m, 4H, ArCH₂(CH₂)₂CH₂), 1.85–2.05 (m, 2H, Ar(CH₂)₃CH₂), 2.62 (t, 2H, ArCH₂), 4.58 (t, 1H, CH), 6.72–6.83 and 7.12–7.30 (m, 9H, aromatics), 7.98 ppm (bs, 1H, COOH, D₂O exchanged); anal.: calcd for $C_{18}H_{19}ClO_3$: C 67.82%, H 6.01%, found: C 67.57%, H 6.02%.

2-(4-Chlorophenoxy)-6-(4-chlorophenyl)hexanoic acid (10): 19% yield; GC-MS (methyl ester) m/z (%): 370 (8) [M^++4], 368 (39) [M^++2], 366 (56) [M^+], 125 (100) [$C_7H_6Cl^+$]; 1H NMR: δ = 1.48–1.72 (m, 4H, ArCH₂(CH₂)₂CH₂), 1.85–2.05 (m, 2H, Ar(CH₂)₃CH₂), 2.58 (dd, 2H, ArCH₂), 4.59 (t, 1H, CH), 6.77–6.83, 7.04–7.09 and 7.21–7.27 (m, 8H, aromatics), 8.02 ppm (bs, 1H, COOH, D₂O exchanged); anal.: calcd for $C_{18}H_{18}Cl_2O_3$: C 61.20%, H 5.14%, found: C 60.87%, H 5.07%.

Preparation of (R)- and (S)-ethyl 2-(4-chlorophenoxy)-4-phenylbutanoates (Scheme 3): A solution of diisopropylazodicarboxylate (DIAD, 10 mmol) in anhyd toluene (15 mL) was added dropwise to an ice-bath-cooled mixture of (S)- or (R)-ethyl 2-hydroxy-4-phenylbutanoate (10 mmol), 4-chlorophenol (10 mmol), and triphenylphosphine (10 mmol) in anhyd toluene (45 mL). The reaction mixture was stirred at room temperature overnight under N₂ atmosphere. The solvent was evaporated in vacuo, and a mixture of Et₂O/hexane (40 mL, 1:1) was added to the residue. The resulting precipitate was filtered, and the filtrate was evaporated to dryness. The residue was separated on a silica gel column (petroleum ether/ethyl acetate 98:2 as eluent), to afford the desired compounds as oils.

(R)-Ethyl 2-(4-chlorophenoxy)-4-phenylbutanoate: Colorless oil; 83% yield; GC-MS m/z (%): 320 (24) [M^++2], 318 (63) [M^+], 91 (100) [$C_7H_7^+$]; 1H NMR: δ = 1.23 (t, 3H, CH₃), 2.12–2.36 (m, 2H, CH₂CH), 2.74–2.94 (m, 2H, ArCH₂), 4.18 (q, 2H, CH₂O), 4.52 (dd, 1H, CH), 6.75–6.82 and 7.23–7.42 ppm (m, 9H, aromatics); $[\alpha]_D$ = +63 (c = 0.5 in MeOH).

(S)-Ethyl 2-(4-chlorophenoxy)-4-phenylbutanoate: Colorless oil; 80% yield; $[\alpha]_D$ = -62 (c = 0.5 in MeOH).

Preparation of (R)- and (S)-5: A solution of the corresponding ethyl esters (5 mmol) in THF (30 mL) and 1N NaOH (30 mL) was stirred at room temperature for 4 h. The organic layer was removed under reduced pressure, and the residue was acidified with

6N HCl and extracted with Et₂O. The organic layer was dried over Na₂SO₄ and evaporated to dryness to afford the final acids in quantitative yield as white solids, which were recrystallized from *n*-hexane/CHCl₃.

(R)-2-(4-Chlorophenoxy)-4-phenylbutanoic acid [(R)-5]: 78% yield; ee = 99% (Chiralcel AD column, *n*-hexane/isopropanol/TFA 80:20:0.01 as a mobile phase, flow rate: 0.5 mL min⁻¹, detection: 280 nm); GC-MS (methyl ester) m/z (%): 306 (26) [M^++2], 304 (70) [M^+], 91 (100) [$C_7H_7^+$]; 1H NMR: δ = 2.19–2.37 (m, 2H, CH₂CH), 2.78–2.95 (m, 2H, PhCH₂), 4.55 (dd, 1H, CH), 6.77–6.83 and 7.12–7.32 (m, 9H, aromatics), 8.35 ppm (bs, 1H, COOH, D₂O exchanged); anal.: calcd for $C_{16}H_{15}ClO_3$: C 66.10%, H 5.20%, found: C 66.45%, H 5.25%.

(S)-2-(4-Chlorophenoxy)-4-phenylbutanoic acid [(S)-5]: 86% yield; ee = 99% (Chiralcel AD column, *n*-hexane/isopropanol/TFA 80:20:0.01 as a mobile phase, flow rate: 0.5 mL min⁻¹, detection: 280 nm); anal.: calcd for $C_{16}H_{15}ClO_3$: C 66.10%, H 5.20%, found: C 66.11%, H 5.24%.

Preparation of diethyl 2-(3-aryl-propyl)malonates 20e,f: A solution of diethyl malonate (30 mmol) in abs EtOH (5 mL) was added to a solution of sodium ethoxide (30 mmol) in abs EtOH (100 mL). After 0.5 h a solution of 17e,f (56 mmol) in abs EtOH (5 mL) was added, and the resulting mixture was held at reflux for 5 h. The organic solvent was distilled off, the oily residue was dissolved with Et₂O, washed with brine, and the organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure, and the title compounds were obtained as pale-yellow oils after chromatography on a silica gel column using petroleum ether/ethyl acetate 95:5 as eluent.

Diethyl 2-(3-phenylpropyl)malonate (20e): 72% yield; GC-MS m/z (%): 278 (35) [M^+], 158 (100) [$C_{11}H_{10}O^+$]; 1H NMR: δ = 1.18 (t, 6H, 2CH₃), 1.61–1.75 (m, 2H, ArCH₂CH₂CH₂), 1.84–1.97 (m, 2H, Ar(CH₂)₂CH₂), 2.60 (t, 2H, ArCH₂), 3.31–3.38 (dd, 1H, CHCOOEt), 4.22 (q, 4H, 2CH₂O), 6.79–7.31 ppm (m, 5H, aromatics).

Diethyl 2-[3-(4-chlorophenyl)propyl]malonate (20f): 54% yield; GC-MS m/z (%): 314 (11) [M^++2], 312 (32) [M^+], 192 (100) [$C_{11}H_9ClO^+$]; 1H NMR: δ = 1.25 (t, 6H, 2CH₃), 1.58–1.68 (m, 2H, ArCH₂CH₂CH₂), 1.85–1.98 (m, 2H, Ar(CH₂)₂CH₂), 2.61 (t, 2H, ArCH₂), 3.29–3.37 (dd, 1H, CHCOOEt), 4.19 (q, 4H, 2CH₂O), 7.02–7.26 ppm (m, 4H, aromatics).

Preparation of 2-(3-aryl-propyl)malonic acids: A solution of 20e,f (10 mmol) in 95% EtOH (10 mL) was added to 2N KOH (20 mL), and the resulting mixture was held at reflux for 5 h. The organic solvent was distilled off, and the residue was diluted with distilled water (10 mL). The solution was acidified to pH 1 with 6N HCl and extracted with Et₂O (5 × 20 mL); the collected organic phase was washed with brine and dried over sodium sulfate. The solvent was removed under reduced pressure to afford the desired malonic acids in quantitative yield as white solids, which were used in the next step without further purification.

2-(3-Phenylpropyl)malonic acid: GC-MS (dimethyl ester) m/z (%): 250 (26) [M^+], 104 (100) [$C_8H_8^+$]; 1H NMR: δ = 1.65–1.82 (m, 2H, ArCH₂CH₂CH₂), 1.90–2.05 (m, 2H, Ar(CH₂)₂CH₂), 2.64 (t, 2H, ArCH₂), 3.44 (t, 1H, CH), 6.83–7.80 ppm (m, 7H, aromatics + 2COOH, D₂O exchanged).

2-[3-(4-Chlorophenyl)propyl]malonic acid: GC-MS (dimethyl ester) m/z (%): 286 (12) [M^++2], 284 (34) [M^+], 138 (100) [$C_8H_7Cl^+$]; 1H NMR ($[D_6]DMSO$): δ = 1.38–1.62 (m, 2H, ArCH₂CH₂CH₂), 1.62–1.87 (m, 2H, Ar(CH₂)₂CH₂), 2.58 (t, 2H, ArCH₂), 3.20 (t, 1H, CH), 7.15–7.38

(m, 4 H, aromatics), 12.20–13.00 ppm (bs, 2 H, 2COOH, D₂O exchanged).

Preparation of 2-bromo-2-(3-aryl-propyl)malonic acids: A solution of Br₂ (11.80 mmol, 0.6 mL) in CH₂Cl₂ (3 mL) was carefully added to a suspension of the suitable aryl-propylmalonic acid (9 mmol) in CH₂Cl₂ (18 mL). The resulting mixture was stirred at 35 °C for 6 h and poured into cold water. The organic layer was separated, and the aqueous phase was extracted four times with Et₂O; the collected organic phase was washed twice with a saturated solution of sodium thiosulfate and with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure to afford the desired acids in quantitative yield as pale-yellow oils which solidified after treatment with cold *n*-hexane.

2-Bromo-2-(3-phenylpropyl)malonic acid: GC-MS (dimethyl ester) *m/z* (%): 330 (1) [M⁺+2], 328 (1) [M⁺], 145 (100) [C₆H₉O₄⁺]; ¹H NMR: δ = 1.75–1.79 (m, 2 H, ArCH₂CH₂CH₂), 2.35 (t, 2 H, Ar(CH₂)₂CH₂), 2.71 (t, 2 H, ArCH₂), 6.91 (bs, 2 H, 2COOH, D₂O exchanged), 7.14–7.32 ppm (m, 5 H, aromatics).

2-Bromo-2-[3-(4-chlorophenyl)propyl]malonic acid: GC-MS (dimethyl ester) *m/z* (%): 364 (1) [M⁺+2], 362 (1) [M⁺], 145 (100) [C₆H₉O₄⁺]; ¹H NMR ([D₆]DMSO): δ = 1.52–1.71 (m, 2 H, ArCH₂CH₂CH₂), 2.18 (t, 2 H, Ar(CH₂)₂CH₂), 2.62 (t, 2 H, ArCH₂), 3.60 (bs, 2 H, 2COOH, D₂O exchanged), 7.16–7.36 ppm (m, 4 H, aromatics).

Preparation of 2-bromo-5-aryl-pentanoic acids 21e,f: The compounds reported above were heated at 150 °C for 30–50 min to afford the title acids as dark solids, which were purified by recrystallization from *n*-hexane.

2-Bromo-5-phenylpentanoic acid (21e): 73% yield; GC-MS (methyl ester) *m/z* (%): 272 (7) [M⁺+2], 270 (7) [M⁺], 104 (100) [C₈H₈⁺], 91 (95) [C₇H₇⁺]; ¹H NMR: δ = 1.67–1.94 (m, 2 H, ArCH₂CH₂CH₂), 1.98–2.20 (m, 2 H, Ar(CH₂)₂CH₂), 2.68 (t, 2 H, ArCH₂), 4.25 (dd, 1 H, CH), 7.14–7.32 (m, 5 H, aromatics), 7.47 ppm (bs, 1 H, COOH, D₂O exchanged).

2-Bromo-5-(4-chlorophenyl)pentanoic acid (21f): 95% yield; GC-MS (methyl ester) *m/z* (%): 308 (5) [M⁺+4], 306 (17) [M⁺+2], 304 (13) [M⁺], 138 (100) [C₈H₇Cl⁺], 125 (81) [C₇H₅Cl⁺]; ¹H NMR: δ = 1.60–1.91 (m, 2 H, ArCH₂CH₂CH₂), 1.98–2.18 (m, 2 H, Ar(CH₂)₂CH₂), 2.61 (t, 2 H, ArCH₂), 4.22 (t, 1 H, CH), 7.06–7.30 (m, 4 H, aromatics), 7.78 ppm (bs, 1 H, COOH, D₂O exchanged).

Preparation of pantolactone esters 22e,f: (S)- or (R)-pantolactone (10 mmol), dimethylaminopyridine (DMAP; 0.1 mmol) and 1,3-dicyclohexylcarbodiimide (DCC; 10 mmol) were added, under N₂ atmosphere, to a stirred solution of the racemic acids 21e,f (10 mmol) in anhyd THF (40 mL). The reaction mixture was stirred at room temperature for 15 h, and afterwards the precipitate was filtered off, the organic phase was evaporated to dryness, dissolved in ethyl acetate (50 mL) and washed twice with H₂O, 3 N HCl, and brine. The organic layer was dried over Na₂SO₄ and evaporated to dryness to afford a yellow oil. The desired diastereomeric esters were obtained, as pale-yellow oils, by column chromatography on silica gel using petroleum ether/ethyl acetate 9:1 or 8:2 as eluent.

(R)- and (S)-2-Bromo-5-phenylpentanoic acid, (R)-pantolactone ester [(R)-22e]: 36% yield; GC-MS *m/z* (%): 370 (2) [M⁺+2], 368 (2) [M⁺], 104 (100) [C₈H₈⁺]; ¹H NMR: δ = 1.13, 1.16, 1.22 and 1.24 (4 s, 6 H, 2CH₃), 1.66–1.77 (m, 2 H, PhCH₂CH₂CH₂), 1.79–2.25 (m, 2 H, PhCH₂CH₂CH₂), 2.59–2.76 (m, 2 H, PhCH₂), 4.01–4.10 (2d, 2 H, OCH₂), 4.30–4.41 (m, 1 H, BrCH), 5.36 and 5.39 (2 s, 1 H, OCH), 7.14–7.34 ppm (m, 5 H, aromatics).

(R)- and (S)-2-Bromo-5-phenylpentanoic acid, (S)-pantolactone ester [(S)-22e]: 39% yield.

(R)- and (S)-2-Bromo-5-(4-chlorophenyl)pentanoic acid, (R)-pantolactone ester [(R)-22f]: 60% yield; GC-MS *m/z* (%): 404 (4) [M⁺+2], 402 (3) [M⁺], 138 (100); ¹H NMR: δ = 1.13, 1.16, 1.22 and 1.24 (4 s, 6 H, 2CH₃), 1.62–1.78 (m, 2 H, PhCH₂CH₂CH₂), 1.80–2.23 (m, 2 H, PhCH₂CH₂CH₂), 2.62–2.67 (m, 2 H, PhCH₂), 4.04–4.10 (2d, 2 H, OCH₂), 4.28–4.40 (m, 1 H, BrCH), 5.36 and 5.39 (2 s, 1 H, OCH), 7.08–7.30 ppm (m, 4 H, aromatics).

(R)- and (S)-2-Bromo-5-(4-chlorophenyl)pentanoic acid, (S)-pantolactone ester [(S)-22f]: 35% yield.

Preparation of pantolactone esters of (S)- or (R)-7 and (S)- or (R)-8: 4-Chlorophenol (5 mmol) was added, under N₂ atmosphere, to a stirred and cooled (0 °C) suspension of 95% NaH powder (5.2 mmol) in anhyd THF (20 mL). Stirring continued until evolution of hydrogen ceased. The resulting solution was then added dropwise, under N₂ atmosphere, to a stirred and cooled (–15 °C) anhyd THF solution (35 mL) of (R)- or (S)-pantolactone esters 22e,f (5 mmol) and *n*-tetrahexylammonium iodide (1 mmol). (R)-Pantolactone esters 22e,f were used to obtain the (S,R)-diastereomers; (S)-pantolactone esters 22e,f to obtain (R,S)-diastereomers. The reaction mixture was stirred between –15 and –5 °C for 8 h and –5 and +10 °C for 20 h, then it was quenched with a saturated solution of NaCl (15 mL). The organic layer was separated, and the aqueous phase was extracted with Et₂O. The combined organic layers were washed twice with brine, dried over Na₂SO₄, and evaporated to dryness to afford the crude esters as viscous colorless oils. Diastereomeric excesses were in the range 75–92% as determined by GC-MS of the crude reaction mixture and confirmed by ¹H NMR (300 MHz) after purification of the esters by column chromatography on silica gel using petroleum ether/ethyl acetate 95:5 as eluent.

(S)-2-(4-Chlorophenoxy)-5-phenylpentanoic acid, (R)-pantolactone ester: 25% yield; GC-MS *m/z* (%): 418 (7) [M⁺+2], 416 (20) [M⁺], 131 (100) [C₁₀H₁₁⁺]; ¹H NMR: δ = 0.89 and 1.02, (2 s, 6 H, 2CH₃, major diastereomer), 1.84–1.96 (m, 2 H, PhCH₂CH₂CH₂), 2.01–2.13 (m, 2 H, PhCH₂CH₂CH₂), 2.70 (t, 2 H, PhCH₂), 3.98–4.05 (2d, 2 H, OCH₂), 4.71–4.80 (dd, 1 H, 4-Cl-PhOCHCOO), 5.36 (s, 1 H, CHC(CH₃)₂), 6.79–6.86, 7.14–7.22 and 7.23–7.33 ppm (m, 9 H, aromatics); *de* = 75% as determined by comparing the integration of the methyl signals of the pantolactone moiety (δ = 1.06 and 1.16 for the minor diastereomer).

(R)-2-(4-Chlorophenoxy)-5-phenylpentanoic acid, (S)-pantolactone ester: 23% yield; *de* = 92% determined as reported above.

(S)-2-(4-Chlorophenoxy)-5-(4-chlorophenyl)pentanoic acid, (R)-pantolactone ester: 21% yield; GC-MS *m/z* (%): 454 (4) [M⁺+4], 452 (20) [M⁺+2], 450 (28) [M⁺], 165 (100) [C₁₀H₁₀Cl⁺]; ¹H NMR: δ = 0.88 and 1.01, (2 s, 6 H, 2CH₃, major diastereomer), 1.84–1.92 (m, 2 H, PhCH₂CH₂CH₂), 1.99–2.09 (m, 2 H, PhCH₂CH₂CH₂), 2.66 (t, 2 H, PhCH₂), 3.98–4.05 (2d, 2 H, OCH₂), 4.72–4.78 (dd, 1 H, 4-Cl-PhOCHCOO), 5.37 (s, 1 H, CHC(CH₃)₂), 6.78–6.85, 7.08–7.13 and 7.18–7.29 ppm (m, 8 H, aromatics); *de* = 92% as determined by comparing the integration of the methyl signals of the pantolactone moiety (δ = 1.08 and 1.16 for the minor diastereomer).

(R)-2-(4-Chlorophenoxy)-5-(4-chlorophenyl)pentanoic acid, (S)-pantolactone ester: 65% yield; *de* = 93% determined as reported above.

Preparation of (S)-7, (R)-7, (S)-8 and (R)-8: H₂O₂ (35% v/v, 0.22 mL) and a solution of LiOH·H₂O (1.32 mmol) in H₂O (1.5 mL) were added to a stirred and cooled (0 °C) suspension of the suita-

ble pantolactone ester (0.66 mmol) in THF/H₂O (4:1, 12.5 mL). The reaction mixture was stirred at 0 °C for 6 h. THF was evaporated in vacuo, and the aqueous phase was acidified with 6 N HCl and extracted with Et₂O. The combined organic layers were washed twice with brine, dried over Na₂SO₄, and evaporated to dryness to afford the desired acids as white solids, which were purified by recrystallization from suitable solvents.

(S)-2-(4-Chlorophenoxy)-5-phenylpentanoic acid [(S)-7]: 89% yield; ee = 75% (HPLC: Chiralcel OD column; *n*-hexane/isopropanol/TFA 98:2:0.05; flow rate 1 mL min⁻¹; detection 280 nm); anal.: calcd for C₁₇H₁₇ClO₃: C 67.00%, H 5.62%, found: C 66.96%, H 5.60%.

(R)-2-(4-Chlorophenoxy)-5-phenylpentanoic acid [(R)-7]: 70% yield; ee = 99% (HPLC: Chiralcel OD column; *n*-hexane/isopropanol/TFA 98:2:0.05; flow rate 1 mL min⁻¹; detection 280 nm); anal.: calcd for C₁₇H₁₇ClO₃: C 67.00%, H 5.62%, found: C 66.83%, H 5.58%.

(S)-2-(4-Chlorophenoxy)-5-(4-chlorophenyl)pentanoic acid [(S)-8]: 52% yield; ee = 99% (HPLC: Chiralcel OD column; *n*-hexane/isopropanol/TFA 99:1:0.1; flow rate 1 mL min⁻¹; detection 280 nm); anal.: calcd for C₁₇H₁₆Cl₂O₃: C 60.19%, H 4.75%, found: C 60.19%, H 4.81%.

(R)-2-(4-Chlorophenoxy)-5-(4-chlorophenyl)pentanoic acid [(R)-8]: 65% yield; ee = 98% (HPLC: Chiralcel OD column; *n*-hexane/isopropanol/TFA 99:1:0.1; flow rate 1 mL min⁻¹; detection 280 nm); anal.: calcd for C₁₇H₁₆Cl₂O₃: C 60.19%, H 4.75%, found: C 60.18%, H 4.80%.

Preparation of (S)- and (R)-2-(4-chlorophenylamino)-3-phenylpropanoic acid [(S)- and (R)-2]: A suspension of D- or L-phenylalanine (12 mmol), 4-chlorobromobenzene (12 mmol), K₂CO₃ (18 mmol), and Cul (1.5 mmol) in anhyd DMF (35 mL) was stirred at 90 °C for 24 h, added to ethyl acetate (30 mL) and water (15 mL), and acidified with 2 N HCl to pH 2. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine and dried over Na₂SO₄. The solvent was evaporated to dryness to give a yellow solid, which was recrystallized from *n*-hexane/CHCl₃ to afford the desired compounds as white solids in 30–35% yield.

(R)-2-(4-Chlorophenylamino)-3-phenylpropanoic acid [(R)-2]: 30% yield; ee = 96% (methyl ester, Chiralcel AD column, *n*-hexane/isopropanol 95:5 as a mobile phase, flow rate: 1.0 mL min⁻¹, detection: 280 nm); GC-MS (methyl ester) m/z (%): 291 (6) [M⁺ + 2], 289 (19) [M⁺], 198 (100) [C₉H₉CINO₂⁺]; ¹H NMR: δ = 2.88–3.14 (m, 2H, PhCH₂), 4.12 (t, 1H, CH), 4.20 (bs, 2H, NH + COOH, D₂O exchanged), 6.35–6.44 and 6.89–7.21 ppm (m, 9H, aromatics); anal.: calcd for C₁₅H₁₄CINO₂: C 65.34%, H 5.12%; N, 5.08%, found: C 65.48%, H 5.18%; N, 5.41%.

(S)-2-(4-Chlorophenylamino)-3-phenylpropanoic acid [(S)-2]: 35% yield; ee = 99% (methyl ester, Chiralcel AD column, *n*-hexane/isopropanol 95:5 as a mobile phase, flow rate: 1.0 mL min⁻¹, detection: 280 nm); anal.: calcd for C₁₅H₁₄CINO₂: C 65.34%, H 5.12%; N, 5.08%, found: C 64.97%, H 5.12%; N, 5.23%.

Ethyl 2-(4-chlorophenylthio)-3-phenylpropanoate (Scheme 6): This compound was prepared as reported for (R)- and (S)-ethyl 2-(4-chlorophenoxy)-4-phenylbutanoates starting from ethyl phenyl-lactate and 4-chlorothiophenol. The purification was carried out by column chromatography on silica gel using petroleum ether/ethyl acetate 8:2 as eluent. Yellow oil; 20% yield; GC-MS m/z (%): 322 (41) [M⁺ + 2], 320 (100) [M⁺], 177 (75) [C₁₁H₁₃O₂⁺]; ¹H NMR: δ = 1.19 (t, 3H, CH₃), 2.99–3.23 (m, 2H, CH₂CH), 3.81–3.87 (dd, 1H, CH₂CH), 4.04 (q, 2H, CH₂O), 7.13–7.38 ppm (m, 9H, aromatics).

2-(4-Chlorophenylthio)-3-phenylpropanoic acid (3): This compound was prepared as reported for (R)- and (S)-. 67% yield; ¹H NMR: δ = 2.99–3.24 (m, 2H, CH₂CH), 3.82 (dd, 1H, CH), 7.18–7.35 (m, 9H, aromatics), 7.81 ppm (bs, 1H, COOH, D₂O exchanged); anal.: calcd for C₁₅H₁₃ClO₂S: C 61.54%, H 4.48%; S, 10.95%, found: C 61.26%, H 4.51%; S, 11.01%.

Preparation of pantolactone esters of 11 (Scheme 7): (R)- or (S)-Pantolactone (10 mmol), DMAP (0.1 mmol), and DCC (10 mmol) were added, under N₂ atmosphere, to a stirred solution of the acid (R,S)-11 (10 mmol) in anhyd THF (40 mL). The reaction mixture was stirred at room temperature for 24 h; afterwards the precipitate was filtered off and the organic phase was evaporated to dryness, the residue was dissolved in ethyl acetate (50 mL) and washed twice with H₂O, 3 N HCl, and brine. The organic layer was dried over Na₂SO₄ and evaporated to dryness to afford a yellow oil. The desired esters were obtained by column chromatography on silica gel (petroleum ether/ethyl acetate 8:2 as eluent), as pale-yellow oils, which solidified on standing. The crystallization from *n*-hexane/CHCl₃ of the (R)-pantolactone esters of (R,S)-11 afforded the (R,S) diastereomer in 22% yield. In the same way, the (S,R) diastereomer was obtained from the crystallization of the (S)-pantolactone esters of (R,S)-11 in 24% yield. Diastereomeric excesses were 95% and 97% respectively, as determined by GC-MS and ¹H NMR (300 MHz) of the crystals.

(R)-Pantolactone ester of (S)-11 and (S)-Pantolactone ester of (R)-11: GC-MS m/z (%): 442 (8) [M⁺ + 4], 440 (42) [M⁺ + 2], 438 (64) [M⁺], 310 (48) [C₁₅H₁₂Cl₂O₃⁺], 181 (100); ¹H NMR: δ = 0.94 and 1.11 (2s, 6H, 2CH₃), 4.02 (s, 2H, CH₂OCO), 4.42–4.53 (m, 2H, ArOCH₂), 5.13 (t, 1H, CH₂CH), 5.46 (s, 1H, CHC(CH₃)₂), 6.81–6.94 and 7.20–7.31 ppm (m, 8H, aromatics).

Preparation of 2,3-bis(4-chlorophenoxy)propanoic acids [(S)- and (R)-11]: H₂O₂ (35% v/v, 1.1 mL) and a solution of LiOH-H₂O (5.6 mmol) in H₂O (15 mL) were added to a stirred and cooled (0 °C) suspension of the pantolactone esters of 11 (2.8 mmol) in THF/H₂O (4:1, 40 mL). The reaction mixture was stirred at 0 °C for 6 h. THF was evaporated under reduced pressure, and the aqueous phase was acidified with 6 N HCl and extracted with Et₂O. The combined organic layers were washed twice with brine, dried over Na₂SO₄ and evaporated to dryness to afford the desired acids as white solids, which were purified by recrystallization from *n*-hexane/CHCl₃.

(+)-(S)-2,3-Bis(4-chlorophenoxy)propanoic acid [(S)-11]: 61% yield; ee = 98% (HPLC: Chiralcel OD column; *n*-hexane/isopropanol/TFA 99:1:0.4; flow rate 1 mL min⁻¹; detection 280 nm); all the spectral data were in accordance with those reported in a previous work for the same compound in racemic form;^[25] anal.: calcd for C₁₅H₁₂Cl₂O₄: C 55.07%, H 3.70%, found: C 55.12%, H 3.72%.

(-)-(R)-2,3-Bis(4-chlorophenoxy)propanoic acid [(R)-11]: 60% yield; ee = 99% (HPLC: Chiralcel OD column; *n*-hexane/isopropanol/TFA 99:1:0.4; flow rate 1 mL min⁻¹; detection 280 nm); all the spectral data were in accordance with those reported in a previous work for the same compound in racemic form;^[25] anal.: calcd for C₁₅H₁₂Cl₂O₄: C 55.07%, H 3.70%, found: C 55.15%, H 3.76%.

(R)- and (S)-2-(4-Chlorophenoxy)-4-butyrolactones (25): These compounds were prepared as reported for (R)- or (S)-ethyl 2-(4-chlorophenoxy)-4-phenylbutanoate starting from 4-chlorophenol and (S)- or (R)-2-hydroxy-4-butyrolactone, respectively. The purification was carried out by column chromatography on silica gel using CH₂Cl₂/petroleum ether 85:15 as eluents. White solids in 61–63% yield; GC-MS m/z (%): 214 (30) [M⁺ + 2], 212 (90) [M⁺], 128 (100)

$[\text{C}_6\text{H}_5\text{ClO}^+]$; ^1H NMR: $\delta = 2.39\text{--}2.78$ (m, 2H, $\text{CH}_2\text{CH}_2\text{O}$), 4.30–4.56 (m, 2H, $\text{CH}_2\text{CH}_2\text{O}$), 4.89 (t, 1H, CH), 6.95–7.01 and 7.24–7.31 ppm (m, 4H, aromatics); $[\alpha]_D = +88$ or -85 ($c = 1.0$ in MeOH) for the *R* and *S* isomers, respectively.

(*R*)- and (*S*)-Methyl 2-(4-chlorophenoxy)-4-hydroxybutanoates (26): A solution of the corresponding lactones 25 (2.5 mmol) in MeOH (20 mL) and H_2SO_4 (0.01 mL) was held at reflux with stirring for 4 h. The solvent was removed under reduced pressure, the residue was added to a saturated solution of NaHCO_3 and extracted with Et_2O . The organic layer was dried over Na_2SO_4 and evaporated to dryness to afford the hydroxy esters 26 in quantitative yield. These compounds were immediately used in the next step without further purification. GC-MS m/z (%): 246 (4) [$M^+ + 2$], 244 (12) [M^+], 128 (100) $[\text{C}_6\text{H}_5\text{ClO}^+]$.

(*R*)- and (*S*)-Methyl 2,4-bis(4-chlorophenoxy)butanoates: Following the same procedure described for compound 25, the hydroxy esters 26 were treated with DIAD, 4-chlorophenol, and triphenylphosphine to give the title compounds after chromatography on a silica gel column (petroleum ether/ethyl acetate 8:2 as eluent) as colorless oils in 85–87% yield. GC-MS m/z (%): 358 (2) [$M^+ + 4$], 356 (10) [$M^+ + 2$], 354 (15) [M^+], 227 (100) $[\text{C}_{11}\text{H}_{12}\text{ClO}_3^+]$; ^1H NMR: $\delta = 2.31\text{--}2.51$ (m, 2H, ArOCH_2), 3.76 (s, 3H, CH_3), 4.10–4.20 (m, 2H, CH_2CH), 4.87 (dd, 1H, CH), 6.76–6.85 and 7.18–7.24 ppm (m, 8H, aromatics); $[\alpha]_D = +24$ or -29 ($c = 0.5$ in MeOH) for the *R* and *S* isomers, respectively.

Preparation of (*R*)- and (*S*)-12: These acids were prepared as reported for (*R*)- and (*S*)-5.

(+)-(*R*)-2,4-Bis(4-chlorophenoxy)butanoic acid [(*R*)-12]: 70% yield; $ee = 99\%$ (Chiralcel AD column, *n*-hexane/isopropanol/TFA 75:25:0.01 as a mobile phase, flow rate: 0.5 mL min $^{-1}$, detection: 280 nm); all the spectral data were in accordance with those reported in a previous work for the same compound in racemic form;^[25] anal.: calcd for $\text{C}_{16}\text{H}_{14}\text{Cl}_2\text{O}_4$: C 56.32%, H 4.14%, found: C 56.65%, H 4.43%.

(-)-(*S*)-2,4-Bis(4-chlorophenoxy)butanoic acid [(*S*)-12]: 71% yield; $ee = 99\%$ (Chiralcel AD column, *n*-hexane/isopropanol/TFA 75:25:0.01 as a mobile phase, flow rate: 0.5 mL min $^{-1}$, detection: 280 nm); all the spectral data were in accordance with those reported in a previous work for the same compound in racemic form;^[25] anal.: calcd for $\text{C}_{16}\text{H}_{14}\text{Cl}_2\text{O}_4$: C 56.32%, H 4.14%, found: C 56.38%, H 4.04%.

Ethyl 5-chloro-3-phenylbenzofuran-2-carboxylate (Scheme 10): Diethyl 2-bromomalonate (0.79 g, 3.31 mmol) was added, under N_2 atmosphere, to a solution of K_2CO_3 (4.56 g, 33 mmol) and 5-chloro-2-hydroxybenzophenone (0.77 g, 3.3 mmol) in anhyd acetone (70 mL). The mixture was held at reflux for 5 h, and the solvent was evaporated to dryness to give a solid residue. Cold water (40 mL) was added to the residue to afford a yellow solid, which was recrystallized from *n*-hexane/CHCl $_3$ to afford the desired compound in quantitative yield. GC-MS m/z (%): 302 (35) [$M^+ + 2$], 300 (100) [M^+]; ^1H NMR: $\delta = 1.27$ (t, 3H, CH_3), 4.35 (q, 2H, CH_2), 7.41–7.58 ppm (m, 8H, aromatics).

5-Chloro-3-phenylbenzofuran-2-carboxylic acid (16): This compound was prepared as reported for (*R*)- and (*S*)-5. 90% yield; GC-MS (methyl ester) m/z (%): 288 (35) [$M^+ + 2$], 286 (100) [M^+]; ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta = 7.41\text{--}7.82$ (m, 8H, aromatics), 13.62 ppm (bs, 1H, COOH, D_2O exchanged); anal.: calcd for $\text{C}_{15}\text{H}_9\text{ClO}_3$: C 66.07%, H 3.33%, found: C 66.31%, H 3.30%.

Assignment of the absolute configuration of acids 11 (Scheme 8):

(+)-(S)-Methyl 3-(4-chlorophenoxy)-2-hydroxypropanoate (23): A solution of commercially available (*S*)-methyl glycidate (1.51 g, 14.7 mmol) in anhyd MeOH (30 mL) was added, under N_2 atmosphere, to a solution of sodium phenoxide prepared by adding 95% NaH powder (0.71 g, 29.4 mmol) to a cooled (0 °C) solution of 4-chlorophenol (4.73 g, 36.7 mmol) in anhyd MeOH (30 mL). The resulting mixture was heated at 55 °C for 22 h, the solvent was evaporated to dryness, and the residue was dissolved in ethyl acetate. The organic phase was washed with 0.5 N NaOH and brine, dried over Na_2SO_4 , and evaporated to dryness to afford a yellow oil, which was separated on a silica gel column (petroleum ether/ethyl acetate from 8:2 to 6:4 as eluents) to afford the desired compound in 43% yield. GC-MS m/z (%): 232 (20) [$M^+ + 2$], 230 (57) [M^+], 128 (100) $[\text{C}_6\text{H}_5\text{ClO}^+]$; ^1H NMR: $\delta = 3.18$ (d, 1H, OH, D_2O exchanged), 3.82 (s, 3H, CH_3), 4.19–4.28 (m, 2H, CH_2), 4.51 (q, 1H, CH), 6.80–7.26 ppm (m, 4H, aromatics); $[\alpha]_D = +25$ ($c = 1.0$ in MeOH).

(-)-(R)-Methyl 2,3-bis(4-chlorophenoxy)propanoate (24): This compound was prepared in 15% yield following the same procedure described for the preparation of (*R*)- and (*S*)-ethyl 2-(4-chlorophenoxy)-4-phenylbutanoate starting from 4-chlorophenol and 23 in anhyd toluene. The title compound was obtained as a pale-yellow oil by chromatography on a silica gel column (petroleum ether/ethyl acetate 95:5 as eluent). GC-MS m/z (%): 344 (11) [$M^+ + 4$], 342 (66) [$M^+ + 2$], 340 (100) [M^+]; ^1H NMR: $\delta = 3.80$ (s, 3H, CH_3), 4.41 (d, 2H, CH_2), 4.97 (t, 1H, CH), 6.85–7.31 ppm (m, 8H, aromatics); $[\alpha]_D = -8$ ($c = 1.0$ in MeOH). This compound was also obtained by esterification with CH_2N_2 of the levorotatory acid 11, allowing assignment of the *R* configuration to this isomer.

Computational chemistry: Molecular modeling and graphics manipulations were performed using the SYBYL and UCSF CHIMERA software packages^[45,46] running on a Silicon Graphics R12000 workstation. Model building of both enantiomers of 7 and 8 was accomplished with the TRIPPOS force field^[47] available within SYBYL. Energy minimizations and molecular dynamics (MD) simulations were performed with the AMBER 8.0 program,^[48] using the parm99 force field.^[49]

Ligand and receptor preparation: Molecular models of enantiomers (*R*)-7, (*S*)-7, (*R*)-8 and (*S*)-8 were constructed using standard bond lengths and bond angles of the SYBYL fragment library. The carboxylate group was taken as dissociated. Geometry optimizations were realized with the SYBYL/MAXIMIN2 minimizer by applying the BFGS (Broyden, Fletcher, Goldfarb, and Shannon) algorithm^[50] and setting an rms gradient of the forces acting on each atom of 0.05 kcal mol $^{-1}$ Å $^{-1}$ as the convergence criterion. AM1-BCC charges were assigned to ligands by using the ANTECHAMBER module^[51] in AMBER. The crystal structures of hPPAR α in complex with GW409544 (PDB code: 1K7L)^[36] and hPPAR γ in complex with rosiglitazone (PDB code: 2PRG)^[40] were used in the docking experiments. Bound ligands and water molecules were removed. A correct atom assignment for Asn, Gln, and His residues was done, and hydrogen atoms were added using standard SYBYL geometries.

Docking simulations: Both isomers of 7 and 8 were docked into the active site of hPPAR α and hPPAR γ using the DOCK 6.0 program (DOCK 6.0 manual, 2006, <http://dock.compbio.ucsf.edu>). Briefly, a molecular surface of the target site was created using the MS algorithm.^[52] The program SPHGEN^[53] was used to create a negative image of the receptor site by filling the target region with overlapping spheres of varying sizes. To orient a ligand within the binding

site, some of the sphere centers were matched with ligand atoms.^[54] Ligands were docked taking ligand flexibility into account,^[55] using the grid-based energy scoring option for minimization after initial placement in the site. The box for the scoring grid was defined such that all spheres were enclosed with an extra 5.0 Å added in each dimension. Scoring grids for contact and energy scores were calculated with a grid spacing of 0.3 Å. The bump check was set such that compounds with atoms closer than half the sum of the van der Waals radii of the respective atoms were rejected. The energy cutoff was 99.0 Å. A 6-12 Lennard-Jones van der Waals potential was used, with a distance-dependent dielectric constant of 4r.

The flexible docking was performed as follows: 1) automatic selection and matching of an anchor fragment within a maximum of 500 orientations, 2) iterative growing of the ligand using at least 100 conformations (peripheral seeds) for seeding the next growing stage with assignment of energy-favored torsion angles, and 3) simultaneous relaxation of the base fragments as well as all peripheral segments and final relaxation of the entire molecule. Orientations and conformations were relaxed (energy score only) in 500 cycles of 500 simplex minimizations to a convergence of 0.1 kcal mol⁻¹. The top solution corresponding to the best Dock energy score (electrostatic + van der Waals interactions) for each ligand was then stored into a single multi mol2 file.

MD simulations: Refinement of the ligand-receptor complexes was achieved by energy minimization with the SANDER module of AMBER (10 000 steps; distance-dependent dielectric function of $\epsilon = 4r$), by applying an energy penalty force constant of 5 kcal mol⁻¹ on the protein backbone atoms. After the minimizations, MD simulations were initiated without explicit water using the pairwise GB continuum solvent model of Hawkins and co-workers^[56,57] implemented in the SANDER module of AMBER. Simulations employed a 1-fs time step for 40010 steps corresponding to a total of 40.01 ps of GB-MD. The final desired temperature of 298 K was obtained by requesting a heating cycle from 0 to 298 K over the course of the first 5000 MD steps, with temperature regulation maintained by coupling to an external heat bath using the Berendsen scheme^[58] and a coupling time constant $\tau_{\text{autp}} = 1.0$ ps. Protein main chain atoms were lightly restrained using a weak harmonic force constant = 5.0 kcal mol⁻¹ Å², and the SHAKE algorithm^[59] was applied to constrain bonds involving hydrogen atoms. Dielectric constants of 1 (interior) and 80 (exterior) were employed in all GB-MD simulations. Average structures of each ligand-protein complex were computed using 201 snapshots from the last 20.01 ps of the MD trajectory and energy-minimized using the protocol as specified above.

Keywords: chirality • molecular modeling • peroxisome proliferator-activated receptors

- [1] P. Z. Zimmet, K. G. M. M. Alberti, J. Shaw, *Nature* **2001**, *414*, 782–787.
- [2] B. Hulin, *Prog. Med. Chem.* **1994**, *31*, 1–58.
- [3] M. H. Moneva, S. Dagogo-Jack, *Curr. Drug Targets* **2002**, *3*, 203–221.
- [4] J. S. Skyler, *J. Med. Chem.* **2004**, *47*, 4113–4117, and references therein.
- [5] Nuclear Receptor Nomenclature Committee, *Cell* **1999**, *97*, 161–163.
- [6] J. Berger, D. E. Moller, *Annu. Rev. Med.* **2002**, *53*, 409–435.
- [7] G. Krey, O. Braissant, F. L'Horset, E. Kalkhoven, M. Perroud, M. G. Parker, W. Wahli, *Mol. Endocrinol.* **1997**, *11*, 779–791.
- [8] B. Staels, J. Dallongeville, J. Auwerx, K. Schoonjans, E. Leitersdorf, J.-C. Fruchart, *Circulation* **1998**, *98*, 2088–2093.
- [9] J.-C. Fruchart, P. Duriez, B. Staels, *Curr. Opin. Lipidol.* **1999**, *10*, 245–257.

- [10] T. M. Willson, J. E. Cobb, D. J. Cowan, R. W. Wiethe, I. D. Correa, S. R. Prakash, K. D. Beck, L. B. Moore, S. A. Kliewer, J. M. Lehmann, *J. Med. Chem.* **1996**, *39*, 665–668.
- [11] P. Cronet, J. F. W. Petersen, R. Folmer, N. Blomberg, K. Sjöblom, U. Karlsson, E.-L. Lindstedt, K. Bamberg, *Structure* **2001**, *9*, 699–706.
- [12] D. A. Brooks, G. J. Etgen, C. J. Rito, A. Shuker, S. J. Dominian, A. M. Warshawsky, R. Ardecky, J. R. Paterniti, J. Tyhonas, D. S. Karanewsky, R. F. Kauffman, C. L. Broderick, B. A. Oldham, C. Montrose-Rafizadeh, L. L. Winneroski, M. M. Faul, J. R. McCarthy, *J. Med. Chem.* **2001**, *44*, 2061–2064.
- [13] B. B. Lohray, V. B. Lohray, A. C. Bajji, S. Kalchar, R. R. Poondra, S. Padakanti, R. Chakrabarti, R. K. Vikramadithyan, P. Misra, S. Juluri, N. V. S. R. Mamidi, R. Rajagopalan, *J. Med. Chem.* **2001**, *44*, 2675–2678.
- [14] P. Sauerberg, I. Pettersson, L. Jeppesen, P. S. Bury, J. P. Mogensen, K. Wassermann, C. L. Brand, J. Sturis, H. F. Wöldike, J. Fleckner, A.-S. T. Andersen, S. B. Mortensen, L. A. Svensson, H. B. Rasmussen, S. V. Lehmann, Z. Polivka, K. Sindelar, V. Panajotova, L. Ynddal, E. M. Wulff, *J. Med. Chem.* **2002**, *45*, 789–804.
- [15] G. J. Etgen, B. A. Oldham, W. T. Johnson, C. L. Broderick, C. R. Montrose, J. T. Brozinick, E. A. Misener, J. S. Bean, W. R. Bensch, D. A. Brooks, A. J. Shuker, C. A. Rito, J. R. McCarthy, R. A. Ardecky, J. S. Tyhonas, S. L. Dana, J. M. Bilakovics, J. R. Paterniti Jr., K. M. Ogilvie, S. Liu, R. F. Kauffman, *Diabetes* **2002**, *51*, 1083–1087.
- [16] S. Ebdrup, I. Pettersson, H. B. Rasmussen, H.-J. Deussen, A. F. Jensen, S. B. Mortensen, J. Fleckner, L. Pridal, L. Nygaard, P. Sauerberg, *J. Med. Chem.* **2003**, *46*, 1306–1317.
- [17] Y. Xu, C. J. Rito, G. J. Etgen, R. J. Ardecky, J. S. Bean, W. R. Bensch, J. R. Bosley, C. L. Broderick, D. A. Brooks, S. J. Dominian, P. J. Hahn, S. Liu, D. E. Mais, C. Montrose-Rafizadeh, K. M. Ogilvie, B. A. Oldham, M. Peters, D. K. Rungta, A. J. Shuker, G. A. Stephenson, A. E. Tripp, S. B. Wilson, L. L. Winneroski, R. Zink, R. F. Kauffman, J. R. McCarthy, *J. Med. Chem.* **2004**, *47*, 2422–2425.
- [18] H. Koyama, D. J. Miller, J. K. Boueres, R. C. Desai, B. Jones, J. P. Berger, K. L. MacNaul, L. J. Kelly, T. W. Doepper, M. S. Wu, G. Zhou, P. Wang, M. C. Ippolito, Y.-S. Chao, A. K. Agrawal, R. Franklin, J. V. Heck, S. D. Wright, D. E. Moller, S. P. Sahoo, *J. Med. Chem.* **2004**, *47*, 3255–3263.
- [19] M. Takamura, M. Sakurai, E. Yamada, S. Fujita, M. Yachi, T. Takagi, A. Isobe, Y. Hagiwara, T. Fujiwara, H. Yanagisawa, *Bioorg. Med. Chem.* **2004**, *12*, 2419–2439.
- [20] K. Murakami, K. Tobe, T. Ide, T. Mochizuki, M. Ohashi, Y. Akanuma, Y. Yazaki, T. Kadowaki, *Diabetes* **1998**, *47*, 1841–1847.
- [21] K. G. Liu, J. S. Smith, A. H. Ayscue, B. R. Henke, M. H. Lambert, L. M. Leesnitzer, K. D. Plunket, T. M. Willson, D. D. Sternbach, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2385–2388.
- [22] T. M. Willson, P. J. Brown, D. D. Sternbach, B. R. Henke, *J. Med. Chem.* **2000**, *43*, 527–550.
- [23] A. Pinelli, C. Godio, A. Laghezza, N. Mitro, G. Fracchiolla, V. Tortorella, A. Lavechia, E. Novellino, J.-C. Fruchart, B. Staels, M. Crestani, F. Loiodice, *J. Med. Chem.* **2005**, *48*, 5509–5519.
- [24] J. Shoichi, Y. Yoshiaki, O. Takayuki, O. Shinji (Jpn. Kokai Tokyo Koho), Jpn. Appl. 02193942, **1990** [Chem. Abstr. **1990**, *113*, 211579].
- [25] G. Carbonara, G. Fracchiolla, F. Loiodice, P. Tortorella, D. Conte-Camerino, A. M. De Luca, A. Liantonio, *Farmaco* **2001**, *56*, 749–754.
- [26] C. K. Lau, S. Tardif, C. Dufresne, J. Scheigetz, *J. Org. Chem.* **1989**, *54*, 491–494.
- [27] O. Mitsunobu, *Synthesis* **1981**, 1–28.
- [28] K. Koh, T. Durst, *J. Org. Chem.* **1994**, *59*, 4683–4686.
- [29] D. J. Reynolds, S. A. Hermitage, *Tetrahedron* **2001**, *57*, 7765–7770.
- [30] G. A. Doherty, T. Kamenecka, E. McCauley, G. Van Riper, R. A. Mumford, S. Tong, W. K. Hagmann, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 729–731.
- [31] S. Ferorelli, F. Loiodice, A. Longo, A. Molfetta, V. Tortorella, R. Amoroso, *Chirality* **2000**, *12*, 697–704.
- [32] A. Liantonio, A. De Luca, S. Pierro, M. P. Didonna, F. Loiodice, G. Fracchiolla, P. Tortorella, A. Laghezza, E. Bonerba, S. Traverso, L. Elia, A. Picollo, M. Pusch, D. Conte Camerino, *Br. J. Pharmacol.* **2003**, *139*, 1255–1264.
- [33] G. N. Walker, R. T. Smith, *J. Org. Chem.* **1971**, *36*, 305–308.
- [34] R. R. Soni, K. N. Trivedi, *Pharmazie* **1989**, *44*, 760–762.
- [35] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, S. A. Kliewer, *J. Biol. Chem.* **1995**, *270*, 12953–12956.

- [36] H. E. Xu, M. H. Lambert, V. G. Montana, K. D. Plunket, L. B. Moore, J. L. Collins, J. A. Oplinger, S. A. Kliewer, R. T. Gampe, Jr., D. D. McKee, J. T. Moore, T. M. Willson, J. B. Stimmel, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13919–13924.
- [37] R. L. DesJarlais, R. P. Sheridan, G. L. Seibel, J. S. Dixon, I. D. Kuntz, R. Venkataraghavan, *J. Med. Chem.* **1988**, *31*, 722–729.
- [38] D. A. Gschwend, A. C. Good, I. D. Kuntz, *J. Mol. Recognit.* **1996**, *9*, 175–186.
- [39] I. D. Kuntz, *Science* **1992**, *257*, 1078–1082.
- [40] R. T. Nolte, G. B. Wisely, S. Westin, J. E. Cobb, M. H. Lambert, R. Kurokawa, M. G. Rosenfeld, T. M. Willson, C. K. Glass, M. V. Milburn, *Nature* **1998**, *395*, 137–143.
- [41] R. T. Gampe, V. G. Montana, M. H. Lambert, A. B. Miller, R. K. Bledsoe, M. V. Milburn, S. A. Kliewer, T. M. Willson, H. E. Xu, *Mol. Cell* **2000**, *5*, 545–555.
- [42] J. Uppenberg, C. Svensson, M. Jaki, G. Bertilsson, L. Jendeberg, A. Berkenstam, *J. Biol. Chem.* **1998**, *273*, 31108–31112.
- [43] E. Raspe, L. Madsen, A.-M. Lefebvre, I. Leitersdorf, L. Gelman, J. Peinado-Onsurbe, J. Dallongeville, J.-C. Fruchart, R. Berge, B. Staels, *J. Lipid Res.* **1999**, *40*, 2099–2110.
- [44] T. Hollons, F. K. Yoshimura, *Anal. Biochem.* **1989**, *182*, 411–418.
- [45] SYBYL Molecular Modeling System (Version 7.1), TRIPOS Assoc., St. Louis, MO (USA).
- [46] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605–1612.
- [47] J. G. Vinter, A. Davis, M. R. Saunders, *J. Comput.-Aided Mol. Des.* **1987**, *1*, 31–55.
- [48] D. A. Case, T. A. Darden, T. E. Cheatham III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, K. M. Merz, B. Wang, D. A. Pearlman, M. Crowley, S. Brozell, V. Tsui, H. Gohlke, J. Mongan, V. Hornak, G. Cui, P. Beroza, C. Schafmeister, J. W. Caldwell, W. S. Ross, P. A. Kollman, AMBER 8; University of California, San Francisco, 2004.
- [49] W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz Jr., D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179–5197.
- [50] J. Head, M. C. Zerner, *Chem. Phys. Lett.* **1985**, *122*, 264–274.
- [51] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, *J. Comput. Chem.* **2004**, *25*, 1157–1174.
- [52] M. L. Connolly, *Science* **1983**, *221*, 709–713.
- [53] I. D. Kuntz, J. M. Blaney, S. J. Oatley, R. Langridge, T. E. Ferrin, *J. Mol. Biol.* **1982**, *161*, 269–288.
- [54] B. Shoichet, D. Bodian, I. D. Kuntz, *J. Comput. Chem.* **1992**, *13*, 380–397.
- [55] A. R. Leach, I. D. Kuntz, *J. Comput. Chem.* **1992**, *13*, 730–748.
- [56] G. D. Hawkins, C. J. Cramer, D. G. Truhlar, *Chem. Phys. Lett.* **1995**, *246*, 122–129.
- [57] G. D. Hawkins, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem.* **1996**, *100*, 19824–19839.
- [58] H. J. C. Berendsen, J. P. M. Postma, W. F. Vangunsteren, A. Dinola, J. R. Haak, *J. Chem. Phys.* **1984**, *81*, 3684–3690.
- [59] J. P. Ryckaert, G. Ciccotti, H. J. C. Berendsen, *J. Comput. Phys.* **1977**, *23*, 327–341.

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