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Synthesis and anti-inflammatory activity of new arylidene-thiazolidine-2,4-diones as PPARγ ligands

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ABSTRACT

Eight new 5-arylidene-3-benzyl-thiazolidine-2,4-diones with halide groups on their benzyl rings were synthesized and assayed in vivo to investigate their anti-inflammatory activities. These compounds showed considerable biological efficacy when compared to rosiglitazone, a potent and well-known agonist of PPARγ, which was used as a reference drug. This suggests that the substituted 5-arylidene and 3-benzylidene groups play important roles in the anti-inflammatory properties of this class of compounds. Docking studies with these compounds indicated that they exhibit specific interactions with key residues located in the site of the PPARγ structure, which corroborates the hypothesis that these molecules are potential ligands of PPARγ. In addition, competition binding assays showed that four of these compounds bound directly to the ligand-binding domain of PPARγ, with reduced affinity when compared to rosiglitazone. An important trend was observed between the docking scores and the anti-inflammatory activities of this set of molecules. The analysis of the docking results, which takes into account the hydrophilic and hydrophobic interactions between the ligands and the target, explained why the 3-(2-bromobenzyl)-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2,4-dione compound had the best activity and the best docking score. Almost all of the stronger hydrophilic interactions occurred between the substituted 5-arylidene group of this compound and the residues of the binding site.

1. Introduction

The correct function of a tissue is indispensable for the proper function of the body. When tissues are injured through physical damage or are infected by exogenous microbial organisms, local and systemic responses are activated with the primary goals of eliminating the offending factors as fast as possible, restoring the tissue integrity, and retaining information about the offending agent to facilitate recognition and elimination on a future encounter. The outcome of these responses is a rapid physiological response of the body to damage and infection, that is, inflammation.[1]

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily which are ligand-activated transcription factors. So far, three PPAR isotypes have been reported: PPARα, PPARβ, and PPARγ. Originally, PPAR activity was thought to be limited to lipid metabolism and glucose homeostasis. Later studies showed that PPAR activation regulates inflammatory responses, cell proliferation and differentiation, as well as apoptosis.[2,3] The involvement of PPARγ in inflammatory processes was first suggested by the antagonism between the activities of proinflammatory cytokines and PPARγ. Additionally, macrophage activation is inhibited by several PPARγ agonists.[4] Therefore, this receptor is an attractive target for the development of anti-inflammatory agents due to its key roles at various stages in the inflammatory process.

Thiazolidine-2,4-dione activates PPARγ and is used as an antidiabetic drug in the treatment of type 2 diabetes.[5,6] Thiazolidines, such as rosiglitazone and pioglitazone (Fig. 1), are synthetic compounds that bind and activate PPARγ. The two thiazolidines share a common thiazolidine-2,4-dione structure that is responsible for the majority of their pharmacological effects, including anti-inflammatory effects.[7,8]

Recently, arylidene-thiazolidinediones were evaluated in the alloxan-induced hyperglycemia mice model, where the biomolecular

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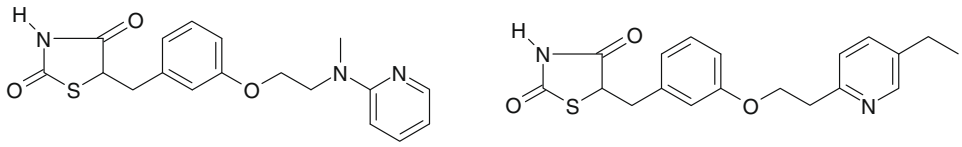


Rosiglitazone **Pioglitazone**

Figure 1. Rosiglitazone and pioglitazone structures that bind PPARγ with a high affinity.

target considered to be responsible for the process was nuclear PPARγ.[9] The present work describes the synthesis, anti-inflammatory activity and structural characteristics of compounds derived from the thiazolidine-2,4-diones substituted with an arylidene and a benzyl group, as shown in Scheme 1. The arylidene was included at position 5 with the substituents Br, Cl, OCH_3, SO_2CH_3, and C_6H_5, and the benzyl was included at position 3 with the substituents Cl, F, and Br. These selected substituents have been the main subject of previous SAR investigations on thiazolidinones, which were based upon the structural similarity between these molecules and the Coxib derivatives.[10,11] The effects of these substitutions on the biological response were analyzed and docking studies were performed to investigate the binding patterns with the PPARγ structure as a tool to support the hypothesis that these compounds are anti-inflammatory agents and act as PPARγ ligands.

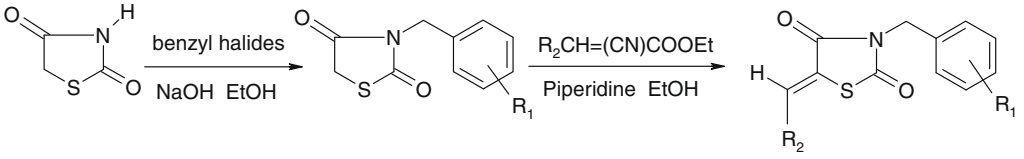
2. Results and discussion

The arylidene-thiazolidinediones **5**–**12** (Scheme 1) were tested for anti-inflammatory activity in an air-pouch assay in which they were evaluated for the ability to inhibit leukocyte migration from blood circulation, as illustrated in Table 1. The arylidene-thiazolidinediones **6**, **7, 9** and **11** were the most active anti-inflammatory agents; in particular, ligand **11** had an anti-inflammatory activity of 73.3%, which was slightly higher than that rosiglitazone (72.0%). It should be noted that **6**, **7** and **9** contain a chlorine atom at position 3 of the benzyl ring and compound **11** contains a bromine atom in position 2 of the benzyl ring. On the other hand, compounds **5** and **8**, which have two substituents in the benzyl ring, only showed moderate activity when compared to rosiglitazone.

After optimization of the arylidene-thiazolidinediones using the AM1 method,[12] agreement was observed with the previous results obtained by Leite et al.,[9] confirming that the *Z* isomer is the most stable for all of the compounds.

The docking solutions (poses) of the arylidene-thiazolidinedione compounds in the PPARγ structure were compared to the position of the rosiglitazone docked in this receptor, as shown in Figure 2. The docking solutions for the eight ligands were positioned within the well-characterized site of the PPARγ structure. A closer examination of the interactions of these ligands with the site shows that some key residues are involved in important hydrophilic interactions (hydrogen bonds) with the arylidene-thiazolidinediones and with rosiglitazone. This can be seen in Figure 3, where compound **11** that had the best activity and the best docking score in the series (-23.45 kJ mol^{-1}) was superposed with the co-crystallized rosiglitazone in the presence of important residues of the site. It is important to note that compound **6** has essentially the same binding mode in comparison to **11** and presents the same interactions, which is confirmed by the very similar docking scores in Table 1, which were -22.83 and -23.45 kJ mol^{-1} for **6** and **11**, respectively.

In Table 2, the hydrophilic and hydrophobic interactions between the ligands **11**, **12** and rosiglitazone and the residues of the PPARγ site are listed. It is possible to remark that ligand **11**



1 **2, 3 or 4** **5 to 12**

Compound	R_1	R_2
5	3-Cl	5-Br, 2-$OCH_3C_6H_3$
6	3-Cl	4-$SO_2CH_3C_6H_4$
7	3-Cl	4-$C_6H_5C_6H_4$
8	3-Cl	3-Br, 4-$OCH_3C_6H_3$
9	3-Cl	2-Fluorene
10	3-Cl	4-$OCH_3C_6H_4$
11	2-Br	4-$SO_2CH_3C_6H_4$
12	2-Cl, 6-F	2,4-$Cl_2C_6H_3$

Scheme 1. Synthetic route for 5-arylidene-3-benzyl-thiazolidine-2,4-diones.

Table 1
Oral anti-inflammatory activity of the eight arylidene-thiazolidinediones using the air-pouch model and the respective docking scores

Compound	R_1	R_2	Dose (mg/kg)	PMNL count[a] (10^5/mL)	% Inhibition[b] (PMNL)	Docking score (kJ mol^{-1})
5	3-Cl	5-Br, 2-$OCH_3C_6H_3$	3	20.1 ± 2.7	47.4	−9.99
6	3-Cl	4-$SO_2CH_3C_6H_4$	3	13.8 ± 1.8	63.9	−22.83
7	3-Cl	4-$C_6H_5C_6H_4$	3	12.6 ± 0.7	67.0	−19.72
8	3-Cl	3-Br, 4-$OCH_3C_6H_3$	3	18.6 ± 1.2	51.5	−16.70
9	3-Cl	2-Fluorene	3	13.3 ± 1.8	65.2	−18.54
10	3-Cl	4-$OCH_3C_6H_4$	3	22.2 ± 1.3	42.0	−18.82
11	2-Br	4-$SO_2CH_3C_6H_4$	3	10.2 ± 1.7	73.3	−23.45
12	2-Cl, 6-F	2,4-$Cl_2C_6H_3$	3	22.4 ± 1.8	41.6	−13.98
Rosiglitazone	—	—	3	10.7 ± 2.0	72.0	−34.03
Control	—	—	—	38.3 ± 2.1	—	—

[a] PMNL is the cell infiltration by polymorphonuclear leukocytes. Data points represent the mean PMNL count number per animal group with the corresponding standard error. The presented values are significant for the 95% confidence interval (ANOVA, Bonferroni test).
[b] The inhibition was determined compared to a control group (untreated).

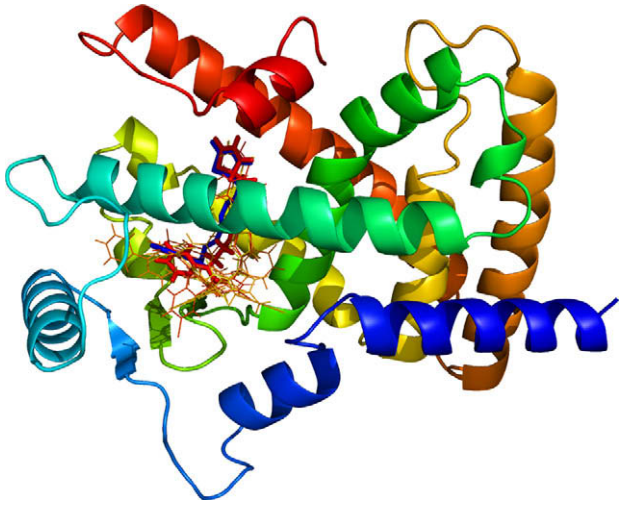


Figure 2. Superposition of the docking solutions for the eight arylidene-thiazolidinediones (**5**–**12**—Scheme 1) (line models in several colors) and the rosiglitazone (stick model in red) for the PPARγ receptor (cartoon model). The co-crystallized (experimental) rosiglitazone is also represented (stick model in blue). Figures 2 and 3 were generated using PyMOL v0.99.[19]

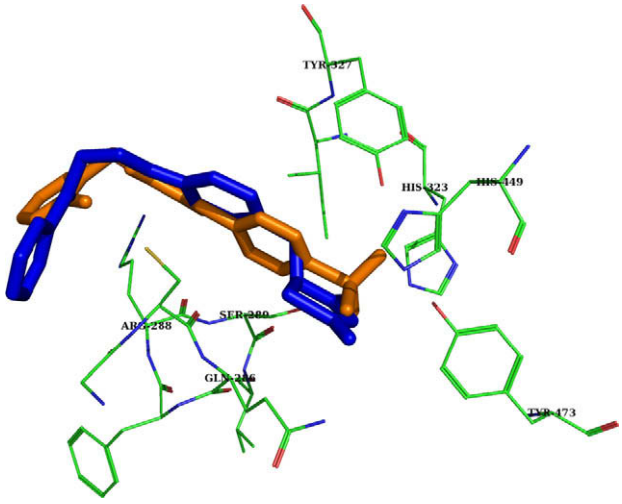


Figure 3. Docking results for the 3-(2-bromo-benzyl)-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2,4-dione (**11**; stick model in orange) and the co-crystallized rosiglitazone (stick model in blue) interacting with important labeled residues of the PPARγ receptor site. Compound **6**, not shown in this figure for clarity reasons, had essentially the same binding mode as **11**, which can be confirmed by the similar docking scores in Table 1, −22.83 and −23.45 kJ mol^{-1} for **6** and **11**, respectively.

(best activity) had many more interactions with the target then ligand **12** (worst activity). This explains, at a molecular level, why ligand **11** had a high docking score while ligand **12** had a low docking score (−13.98 kJ mol^{-1}). The interactions with the co-crystallized rosiglitazone were also included for comparison.

In Figure 4, the anti-inflammatory activity measured as the percent of inhibition (using the PMNL count) and the docking scores were plotted together, which exhibited an important trend. This means that greater stability (the most negative docking score values in kJ mol^{-1}) of the complex between the ligand and the PPARγ receptor is related to greater anti-inflammatory activity or the percent of inhibition of the PMNL count. This important result corroborates the hypothesis that these molecules act through the PPARγ target.

To determine whether the study compounds bind to the ligand-binding domain of human PPARγ (LBD-PPARγ), we assessed the ability of saturated solutions of the compounds in DMSO to displace [^{3}H]rosiglitazone bound to His-LBD-hPPARγ. As shown

Table 2
Hydrophilic and hydrophobic interactions between the docked ligands **11** and **12** and the co-crystallized rosiglitazone with respect to the residues of the PPARγ site. Residues emphasized (bold format and *) in this table are the same interactions observed with the co-crystallized rosiglitazone

Co-crystallized rosiglitazone		Ligand **11** (best activity)		Ligand **12** (worst activity)	
Hydrophilic	Hydrophobic	Hydrophilic	Hydrophobic	Hydrophilic	Hydrophobic
GLN286	ILE281	ARG288	***CYS285***	GLU291	***CYS285***
SER289	***GLY284***	***SER289***	ARG288	GLU343	***GLY284***
HIS323	***CYS285***	***HIS323***	***SER289***		ARG288
HIS449	GLN286	TYR327	LEU330		SER342
TYR473	SER289	***HIS449***	LEU333		GLU343
	ILE341	***TYR473***	***ILE341***		
	MET348		SER342		
	HIS449		GLU343		
			HIS449		

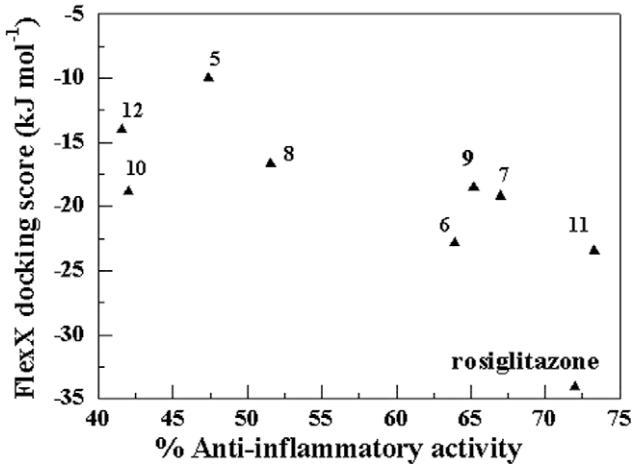


Figure 4. Trend between the docking results of the eight arylidene-thiazolidinediones (**5**–**12**—Scheme 1) and rosiglitazone ligands in the PPARγ receptor and the anti-inflammatory activities measured as the percent of inhibition with respect to the PMNL count.

in Figure 5A, unlabeled **6**, **7**, **11**, and **12** but not **9** bound directly to His-LBD-hPPARγ. A concentration-dependent dissociation of radiolabeled rosiglitazone was observed for **6, 7, 11**, and **12** with K_i values of 43 nM, 43.6 μM, 7.3 μM, and 4.0 μM, respectively (Fig. 5B). Taken together, these data indicate that **6**, **7**, **11**, and **12** have the ability to directly interact with the ligand-binding domain of PPARγ and are low affinity ligands for the receptor. We cannot rule out the possibility that these compounds bind to the other PPAR isotypes, since it has been shown that other thiazolidinediones, including pioglitazone[13] and rosiglitazone,[14] bind and act as partial agonists for PPARα and -δ.

3. Conclusion

In summary, arylidene-thiazolidinediones were synthesized and tested for their anti-inflammatory activities, and 3-(2-bromo-benzyl)-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2,4-dione, compound **11**, showed higher activity than the rosiglitazone reference drug. The binding patterns observed for the docked compounds strongly support the idea that they are possible ligands of the PPARγ receptor. Competition binding assays using purified His-LBD-PPARγ confirmed that **6**, **7**, **11** and **12** directly bind to LBD-hPPARγ in vitro and are low affinity ligands for the receptor. Although compound **11** was shown to have higher anti-inflammatory activity than rosiglitazone, it bound PPARγ with 200-fold lower affinity than this reference ligand. In fact, previous studies have characterized low affinity ligands for PPARγ with potent insulin-

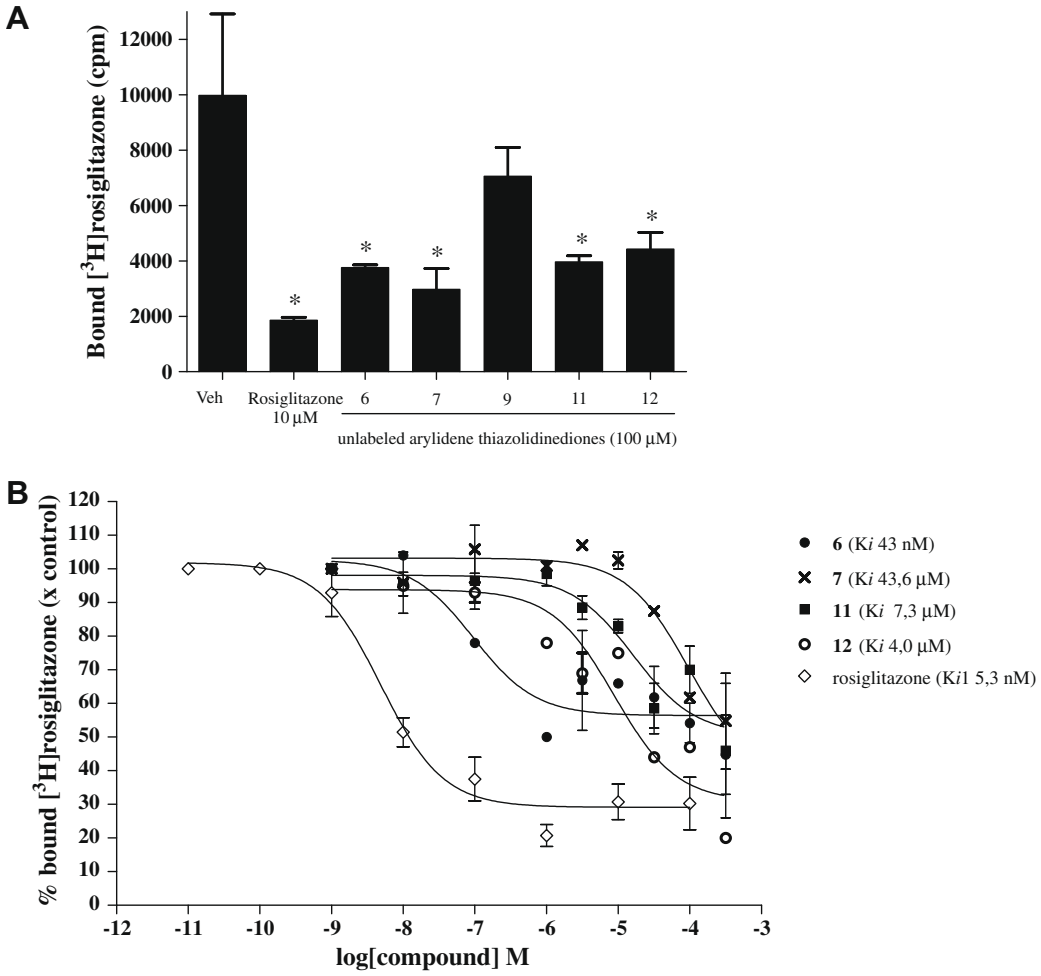


Figure 5. Competition of unlabeled arylidene-thiazolidinediones **6**, **7**, **9**, **11**, and **12** for the ligand-binding domain of PPARγ bound to [^{3}H]rosiglitazone. (A) Competition binding assay using a saturating concentration of unlabeled rosiglitazone (10 μM) and the highest concentration at which the study compounds were soluble in DMSO (100 μM) revealed that arylidene-thiazolidinediones **6**, **7**, **11**, and **12** displaced [^{3}H]rosiglitazone from LBD-PPARγ. (B) Concentration-dependent displacement of [^{3}H]rosiglitazone by unlabeled rosiglitazone and arylidene-thiazolidinediones **6**, **7**, **11** and **12**. Values are expressed as the means ± SEM. * Significantly different (p <0.05) from vehicle control using one-way analysis of variance. Data are representative of at least two independent experiments.

sensitizing activity.[15,16] Since improvement of insulin resistance by PPARγ agonist has been related to the anti-inflammatory effects of the activated receptor,[17] it is possible that the anti-inflammatory activity of PPARγ ligands is not directly correlated to their affinity for the receptor.

The arylidene-thiazolidinedione compound **9**, however, was not characterized as a PPARγ ligand by the competition binding assay, although it had active anti-inflammatory properties in vivo, and the results of the docking studies suggested that it interacts with key residues in the site of the receptor.

Additionally, an important in vivo versus in silico trend between the anti-inflammatory activities and the docking scores was found, showing the appropriate choice of docking model used for this study. This trend corroborates the hypothesis that arylidene-thiazolidinediones act against the inflammation response through a mechanism mediated by PPARγ. This hypothesis was also reinforced by the structural similarity between rosiglitazone and the arylidene-thiazolidinediones evaluated in this study.

Finally, the comparison between the hydrophilic and hydrophobic interactions observed in the docking results for ligands **11** (best activity), **12** (worst activity) and rosiglitazone revealed the intermolecular reasons for the superior docking score and anti-inflammatory activity of compound **11**.

4. Methods

4.1. Synthesis

Thiazolidine-2,4-dione (**1**) was N-(3)-alkylated in the presence of sodium hydroxide, which allows the thiazolidine sodium salt to react with the benzyl halide in a hot ethanol medium, yielding intermediates **2, 3** and **4** as described in Scheme 1. The 5-arylidene-3-benzyl-thiazolidine-2,4-diones were prepared by a nucleophilic Michael addition of the 3-benzyl-thiazolidine-2,4-dione **2**, **3** or **4** and the respective aryl-substituted ethyl-(2-cyano-3-phenyl)-acrylates[20] to obtain the arylidene-thiazolidine-2,4-diones **5**–**12** (Scheme 1). After cooling, the precipitates were purified by column chromatography or crystallized in suitable solvents. The ethyl-(2-cyano-3-phenyl)-acrylates were prepared by Knoevenagel condensation of ethyl cyanoacetic ester and substituted benzaldehydes in the presence of piperidine. The arylidene-thiazolidine-2,4-diones were isolated in a single isomeric form, which was verified by TLC and NMR analysis. X-ray crystallographic studies and ^{13}C NMR have demonstrated a preference for the *Z* configuration for 5-arylidenethiazolidinones[21–23] (Guarda et al., 2003; Tan et al., 1986; Albuquerque et al., 1995).

4.1.1. General procedures

3-Benzyl-thiazolidine-2,4-diones (**2**–**4**) general procedure: An equimolar solution of sodium hydroxide in an ethanol/water mixture (6:4) was added dropwise with stirring to a suspension of thiazolidine-2,4-dione in the same ethanol/water mixture. A few minutes later, the substituted benzyl chloride was added and the mixture was stirred for a few minutes before being refluxed for 24 h. After cooling at room temperature, the expected compound was precipitated by addition of crushed ice before purification by flash chromatography on silica with chloroform/methanol (92:8) as the eluent. The published chemical data on 3-chloro-benzyl-thiazolidine-2,4-dione[24] **2** and 2-bromo-benzyl-thiazolidine-2,4-dione[25] **3** are not reported here.

Ethyl-(2-cyano-3-phenyl)-acrylates general procedure: An equimolar (23 mMol) mixture of aldehyde and ethyl cyanoacetate, in the presence of piperidine (three drops) and benzene (20 mL), was heated at 110–120 °C for 8–10 h. After cooling, the mixture was caught in mass. The solid phase was recrystallized from an ethanol–water mixture. The published chemical data on ethyl-3-(5-bromo-2-methoxi-phenyl)-2-cyanoacrylate, ethyl-3-biphenyl-4-yl-2-cyanoacrylate, ethyl-3-(3-bromo-4-methoxi-phenyl)-2-cyanoacrylate, ethyl-3-(4-methoxi-phenyl)-2-cyanoacrylate,[26] and ethyl-3-(2,4-dichloro-phenyl)-2-cyanoacrylate[27] are not reported here.

5-Arylidene-3-benzyl-thiazolidine-2,4-diones (**5**–**12**) general procedure: An equimolar (0.83 mMol) mixture of 3-benzyl-thiazolidine-2,4-dione **2–4** and (2-cyano-3-phenyl)-ethyl acrylates dissolved in ethanol (10 mL) with piperidine (250 μL) was heated at 50 °C for 2–3 h. After cooling, the precipitated product was recrystallized from an ethanol–water mixture or purified by flash column chromatography. The melting points were measured in a capillary tube on a Buchi (or Quimis) apparatus. Thin layer chromatography was performed on silica gel plates (Merck 60F254). Infrared spectra of 1% KBr pellets were recorded on a Bruker IFS66 spectrometer. ^1H NMR spectra were recorded on a Bruker AC 300 P spectrophotometer in DMSO-d_6 as the solvent, with tetramethylsilane as the internal standard. Mass spectra were recorded on an HCTultra Bruker Daltonics spectrometer on the ESI positive ion polarity.

4.1.2. 3-(2-Chloro-6-fluoro-benzyl)-thiazolidine-2,4-dione; 4

$C_{10}H_7ClFNO_2S$. Yield: 27%. Mp: 90 °C. TLC (*n*-hexane/ethyl acetate, 9:1) R_f 0.4. IR (KBr, cm^{-1}): 1755, 1685, 1600, 1341, 971, 784. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 4.22 (s, 2H, CH_2), 4.81 (s, 2H, NCH_2), 7.19–7.26 (m, 1H, pos. 5 benzyl), 7.3–7.34 (m, 1H, pos. 4 benzyl), 7.35–7.43 (m, 1H, pos. 3 benzyl). MS, ESI^+: *m*/*z* 260 $[M+H]^+$, 262 $[M+H+2]^+$, 277 $[M+NH_4]^+$, 279 $[M+NH_4+2]^+$, 282 $[M+Na]^+$, 284 $[M+Na+2]^+$, 298 $[M+K]^+$, 300 $[M+K+2]^+$, 219, 143, 113.

4.1.3. Ethyl-3-(4-methanesulfonyl-phenyl)-2-cyanoacrylate

$C_{13}H_{13}NO_4S$. Yield: 70%. Mp: 125–126 °C. TLC (benzene/ethyl acetate, 1:1) R_f 0.76. IR (KBr, cm^{-1}): 2222, 1728, 1607, 1301, 1264, 1197, 766. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 1.32 (t, 3H, CH_3 ester, J = 6.9 Hz), 4.35 (q, 2H, CH_2 ester, J = 6.9 Hz), 3.3 (s, 3H, SO_2CH_3), 8.12 (d, 2H, pos. 2, 6 phenyl, J = 8.7 Hz), 8.23 (d, 2H, pos. 3, 5 phenyl), 8.53 (s, 1H, ethylene).

4.1.4. Ethyl-3-(9*H*-fluoren-2-yl)-2-cyanoacrylate

$C_{19}H_{15}NO_2$. Yield: 98%. Mp: 145–146 °C. TLC (*n*-hexane/ethyl acetate, 7:3) R_f 0.65. IR (KBr, cm^{-1}): 2217, 1717, 1598, 1268, 1241, 1214, 1119. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 1.32 (t, 3H, CH_3 ester, J = 7.2 Hz), 4.06 (s, 2H, CH_2 fluorene), 4.33 (q, 2H, CH_2 ester, J = 6.9 Hz), 7.4–7.5 (m, 2H, pos. 6, 7 fluorenylidene), 7.64–7.69 (m, 1H, pos. 3 fluorenylidene), 8.03–8.07 (m, 1H, pos. 4 fluorenylidene), 8.1–8.17 (m, 2H, pos. 1, 8 fluorenylidene), 8.32–8.35 (m, 1H, pos. 5 fluorenylidene), 8.47 (s, 1H, ethylene).

4.1.5. 5-(5-Bromo-2-methoxy-benzylidene)-3-(3-chloro-benzyl) -thiazolidine-2,4-dione; 5

$C_{18}H_{13}BrClNO_3S$. Yield: 99%. Mp: 139–140 °C. TLC (*n*-hexane/ethyl acetate, 7:3) R_f 0.68. IR (KBr, cm^{-1}): 1336, 1382, 1479, 1598, 1691, 1744. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 3.9 (s, 3H, OCH_3), 4.83 (s, 2H, CH_2), 7.24–7.29 (m, 1H, pos. 5 benzyl), 7.39 (1H, pos. 2 benzyl), 7.39 (dd, 2H, pos. 4, 6 benzyl, J = 4.5 and 1.2 Hz), 7.15 (d, 1H, pos. 3 benzylidene, J = 9.3 Hz), 7.55 (d, 1H, pos. 6 benzylidene, J = 2.4 Hz), 7.67 (dd, 1H, pos. 4 benzylidene, J = 8.7 and 2.4 Hz), 7.97 (s, 1H, ethylene). MS, ESI^+: *m*/*z* 438 $[M+H]^+$, 440 $[M+H+2]^+$, 460 $[M+Na]^+$, 462 $[M+Na+2]^+$, 268, 122.

4.1.6. 3-(3-Chloro-benzyl)-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2,4-dione; 6

$C_{18}H_{14}ClNO_4S_2$. Yield: 79%. Mp: 196–197 °C. TLC (*n*-hexane/ethyl acetate 6:4) R_f 0.66. IR (KBr, cm^{-1}): 1151, 1383, 1607, 1697, 1762. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 3.28 (s, 3H, SO_2CH_3), 4.86 (s, 2H, CH_2), 7.27–7.31 (m, 1H, pos. 5 benzyl), 7.38 (d, 1H,

pos. 2 benzyl, J = 1.5 Hz), 7.39–7.42 (m, 2H, pos. 4, 6 benzyl), 7.89 (d, 2H, pos. 2, 6 benzylidene, J = 8.7 Hz), 8.07 (d, 2H, pos. 3, 5 benzylidene, J = 8.4 Hz), 8.06 (s, 1H, ethylene). MS, ESI$^+$: m/z 408 $[M+H]^+$, 410, 425 $[M+NH_4]^+$, 427 $[M+H+2]^+$, 430 $[M+Na]^+$, 432 $[M+Na+2]^+$, 446 $[M+K]^+$, 448 $[M+K+2]^+$, 268, 154, 122.

4.1.7. 5-Bipheny-4-ylmethylene-3-(3-chloro-benzyl)-thiazolidine-2,4-dione; 7

$C_{23}H_{16}ClNO_2S$. Yield: 73%. Mp: 169–170 °C. TLC (n-hexane/ethyl acetate; 7:3) R_f 0.77. IR (KBr, cm^{-1}): 1148, 1383, 1481, 1595, 1670, 1740. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 4.86 (s, 2H, CH_2), 7.27–7.31 (m, 1H, pos. 5 benzyl), 7.39–7.42 (m, 2H, pos. 4, 6 benzyl), 7.39 (d, H, pos. 2 benzyl, J = 1.2 Hz), 7.44 (t, 1H, pos. 4 phenyl, J = 1.2 Hz), 7.51 (t, 2H, pos. 3 phenyl, J = 7.5 Hz), 7.77 (dd, 2H, pos. 2 phenyl, J = 6.3 and 1.8 Hz), 7.74 (d, 2H, pos. 2 benzylidene, J = 8.4 Hz), 7.88 (d, 2H, pos. 3 benzylidene, J = 8.4 Hz), 8.03 (s, 1H, ethylene). MS, ESI$^+$: m/z 406 $[M+H]^+$, 408 $[M+H+2]^+$, 428 $[M+Na]^+$, 430 $[M+Na+2]^+$, 268, 122.

4.1.8. 5-(3-Bromo-4-methoxy-benzylidene)-3-(3-chloro-benzyl)-thiazolidine-2,4-dione; 8

$C_{18}H_{13}BrClNO_3S$. Yield: 71%. Mp: 153–154 °C. TLC (n-hexane/ethyl acetate, 6:4) R_f 0.83. IR (KBr, cm^{-1}): 1268, 1327, 1376, 1495, 1587, 1684, 1742. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 3.93 (s, 3H, OCH_3), 4.84 (s, 2H, CH_2), 7.24–7.28 (m, 1H, pos. 5 benzyl), 7.38 (dd, 2H, pos. 4, 6 benzyl, J = 4.8 and 1.2 Hz), 7.39 (1H, hidden, pos. 2 benzyl), 7.30 (d, 1H, pos. 3 benzylidene, J = 9 Hz), 7.64 (dd, 1H, pos. 2 benzylidene, J = 9 and 2.4 Hz), 7.91 (d, 1H, pos. 2 benzylidene, J = 2.4 Hz), 7.93 (s, 1H, ethylene). MS, ESI$^+$: m/z 438 $[M+H]^+$, 440 $[M+H+2]^+$, 460 $[M+Na]^+$, 462 $[M+Na+2]^+$, 309, 268, 122.

4.1.9. 3-(3-Chloro-benzyl)-5-(9*H*-fluoren-2-yl-methylene)-thiazolidine-2,4-dione; 9

$C_{24}H_{16}ClNO_2S$. Yield: 81%. Mp: 185–186 °C. TLC (n-hexane/ethyl acetate, 7:3) R_f 0.74. IR (KBr, cm^{-1}): 1331, 1380, 1595, 1683, 1733. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 4.03 (s, 2H, CH_2), 4.86 (s, 2H, NCH_2), 7.27–7.31 (m, 1H, pos. 5 benzyl), 7.39 (d, H, pos. 2 benzyl, J = 1.2 Hz), 7.4 (dd, 2H, pos. 4, 6 benzyl, J = 7.5 and 1.8 Hz), 7.39–7.45 (m, 2H, pos. 6, 7 fluorenylidene), 7.64 (d, 1H, pos. 3 fluorenylidene, J = 6.9 Hz), 7.69 (d, 1H, pos. 5 fluorenylidene, J = 7.8 Hz), 7.86 (s, 1H, pos. 1 fluorenylidene), 7.99 (d, 1H, pos. 8 fluorenylidene, J = 7.5 Hz), 8.08 (d, 1H, pos. 4 fluorenylidene, J = 8.1 Hz), 8.06 (s, 1H, ethylene). MS, ESI$^+$: m/z 418$[M+H]^+$, 420 $[M+H+2]^+$, 440 $[M+Na]^+$, 442 $[M+Na+2]^+$, 304, 282, 168.

4.1.10. 3-(3-Chloro-benzyl)-5-(4-methoxy-benzylidene)-thiazolidine-2,4-dione; 10

$C_{18}H_{14}ClNO_3S$. Yield: 72%. Mp: 125–126 °C. TLC (n-hexane/ethyl acetate, 7:3) R_f 0.8. IR (KBr, cm^{-1}): 1258, 1325, 1374, 1509, 1590, 1673, 1733. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 3.84 (s, 3H, OCH_3), 4.84 (s, 2H, CH_2), 7.24–7.3 (m, 1H, pos. 5 benzyl), 7.39 (d, 1H, pos. 2 benzyl, J = 1.8 Hz), 7.39 (dd, 2H, pos. 4, 6 benzyl, J = 5.1 and 1.2 Hz), 7.12 (d, 2H, pos. 3, 5 benzylidene, J = 8.7 Hz), 7.61 (d, 2H, pos. 2, 6 benzylidene, J = 8.7 Hz), 7.93 (s, 1H, ethylene). MS, ESI$^+$: m/z 360 $[M+H]^+$, 362 $[M+H+2]^+$, 382 $[M+Na]^+$, 384 $[M+Na+2]^+$, 332, 282.

4.1.11. 3-(2-Bromo-benzyl)-5-(4-methanesulfonyl-benzylidene)-thiazolidine-2,4-dione; 11

$C_{18}H_{14}BrNO_4S_2$. Yield: 56%. Mp: 59–60 °C. TLC (n-hexane/ethyl acetate, 1:1) R_f 0.69. IR (KBr, cm^{-1}): 1149, 1306, 1383, 1603, 1678, 1748. ^1H NMR 300 MHz (δ ppm, DMSO-d_6): 3.25 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 7.24 (dd, 1H, pos. 6 benzyl, J = 7.8 and 1.2 Hz), 7.27 (dt, 1H, pos. 4 benzyl, J = 7.8 and 1.2 Hz), 7.36 (dt, 1H, pos. 5 benzyl, J = 7.5 and 1.2 Hz), 7.67 (dd, 1H, pos. 3 benzyl, J = 7.8 and 1.2 Hz), 7.91 (d, 2H, pos. 2, 6 benzylidene, J = 8.1 Hz), 8.09 (d, 2H, pos. 3, 5 benzylidene, J = 8.4 Hz), 8.08 (s, 1H ethylene). MS, ESI$^+$: m/z 452 $[M+H]^+$, 409, 382, 296, 169.

4.1.12. 3-(2-Chloro-6-fluoro-benzyl)-5-(2,4-dichloro-benzylidene)-thiazolidine-2,4-dione; 12

$C_{17}H_9Cl_3FNO_2S$. Yield: 76%. Mp: 169–170 °C. TLC (n-hexane/ethyl acetate, 7:3) R_f 0.8. IR (KBr, cm^{-1}): 1335, 1377, 1601, 1689, 1742. ^1H NMR MHz (δ ppm, DMSO-d_6): 4.99 (s, 2H, CH_2), 7.23–7.29 (m, 1H pos. 5 benzyl), 7.33–7.38 (m, 1H, pos. 4 benzyl), 7.39–7.47 (m, 1H, pos. 3 benzyl), 7.6 (d, 1H, pos. 5 benzylidene, J = 8.7 Hz), 7.63 (d, 1H, pos. 6 benzylidene, J = 8.7 Hz), 7.86–7.88 (m, 1H, pos. 3 benzylidene), 7.95 (s, 1H, ethylene). MS, ESI$^+$: m/z 416 $[M+H]^+$, 418 $[M+H+2]^+$, 438 $[M+Na]^+$, 440 $[M+Na+2]^+$, 302, 268.

4.2. Biological tests

The anti-inflammatory effect was tested by the production of air pouches on the dorsal cervical region of mice of 25–30 g by a subcutaneous injection of 2.5 mL of sterile air on day 0, followed by a second injection of 2.5 mL of sterile air 3 days later. On day 6, the 1 mL of 1% (w/v) carrageenan solution was injected into the cavity. The arylidene-thiazolidinedione compounds (**5**–**12**) and the reference drug rosiglitazone were administered orally 1 h before the injection of carrageenan. After 6 h, the mice were sacrificed by ether exposure, and the pouches were washed with 3 mL of saline solution containing 3 μM of EDTA. The number of migrated neutrophils were determined by staining with Turk's solution (0.01% crystal violet in 3% acetic acid) and counted using a Neubauer hemocytometer.

4.3. Docking

The structures of the arylidene-thiazolidinediones **5**–**12** shown in Scheme 1 were initially optimized using the AM1 method[12] implemented with the BioMedCache program[28] with the default values for the convergence criteria. The preference for the Z configuration of the exocyclic double bond of the 5-arylidenethiazolidinones was confirmed. Before the docking calculation, the geometries of the arylidene-thiazolidinediones **5**–**12** were subsequently optimized using the Tripos force field available in the SYBYL package.[18]

The potential affinities of those compounds with respect to the PPARγ structure were evaluated through docking studies using the enzyme Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) co-crystallized with 5-[4-(2-[methyl(pyridin-2-yl)amino] ethoxy)benzyl]thiazolidine-2,4-dione (rosiglitazone-BRL) as the target. This complex was obtained from the RCSB Protein Data Bank[29] under the PDB code 2PRG. The FlexX 7.2 Program[16] was used for these computations because it takes into account the ligand flexibility (main torsions) during the calculations.[30] The structure of the 'A' monomer of the homodimer was chosen as the target for docking studies. The site was defined as all atoms within a radius of 6.5Å from the co-crystallized rosiglitazone ligand. Additionally, the rosiglitazone ligand was re-docked to test the program protocol. The theoretical binding profile proposed for the thiazolidinedione ligands was determined as the highest (most negative) score among 30 possible solutions generated according to the FlexX[16] scoring function and protocol. Therefore, the docking results presented here are just the highest score for each molecule studied.

4.4. PPARγ-competition binding assay

Polyhistidine-tagged human PPARγ ligand-binding domain (His-LBD-hPPARγ) was overexpressed in *Escherichia coli* BL21 cells using the pET28a expression plasmid (the details of this construct

have been described previously[31]) and purified by immobilized metal ion affinity chromatography on a Co^{2+}-loaded HiTrap chelating column (Clontech).

By performing saturating binding studies with purified His-LBD-hPPARγ and increasing amounts of [^{3}H]rosiglitazone, we determined that the dissociation constant (*Kd*) of rosiglitazone for PPARγ was 33 nM, which is consistent with previously reported values.[32] For the competition binding assays, His-LBD-hPPARγ was incubated for 12 h at 4 °C with 40 nM [^{3}H]rosiglitazone (specific activity of 50 Ci/mmol) in buffer containing 10 mM Tris, pH 8.0, 50 mM KCl, and 10 mM dithiothreitol in a final volume of 100 μL. Vehicle (DMSO) or unlabeled ligand was then added. After incubation for an additional 12 h at 4 °C, bound radioactivity was separated from free radioactivity by gravity flow through a 1 mL Sephadex G-25 desalting column (Amersham) and quantitated using a liquid scintillation counter (Perkin–Elmer). Concentration-dependent experiment results were expressed as the percent [^{3}H]rosiglitazone bound compared to the assay tube in which vehicle was added. Binding curves were fit to a nonlinear regression, and IC_{50} values were obtained using a one-site competition equation using the GraphPad 4.0 software (GraphPad, San Diego, CA). Inhibition constant (K*i*) values were calculated by the equation of Cheng and Prussof.[33]

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References and notes

1. Bannenberg, G.; Makoto, A.; Nharles, N. *World J.* **2007**, *7*, 1440.
2. Houseknecht, K. L.; Cole, B. M.; Steele, C. P. *Domest. Anim. Endocrinol.* **2002**, *22*, 1.
3. Sung, B.; Park, S.; Yu, B. P.; Chung, H. Y. *J. Gerontol. A Biol. Sci. Med. Sci.* **2004**, *59*, 997.
4. Ricote, M.; Li, A. C.; Willson, T. M.; Kelly, J.; Glass, C. K. *Nature* **1998**, *391*, 79.
5. Petersen, K. F.; Krssak, M.; Inzucchi, S.; Cline, G. W.; Dufour, S.; Shulman, G. I. *Diabetes* **2000**, *49*, 827.
6. Plutzky, J. *Am. J. Cardiol.* **2003**, *92*, 34J.
7. Willson, T. M.; Wahli, W. *Curr. Opin. Chem. Biol.* **1997**, *1*, 235.
8. Komers, R. A.; Vrana, A. *Physiol. Res.* **1998**, *47*, 215.
9. Leite, L. F. C. C.; Mourão, R. H. V.; Lima, M. C. A.; Galdino, S. L.; Hernandes, M. Z.; Neves, F. A. R.; Vidal, S.; Barbe, J.; Pitta, I. R. *Eur. J. Med. Chem.* **2007**, *42*, 1263.
10. Lages, A. S.; Romeiro, N. C.; Fraga, C. A. M.; Barreiro, E. J. *Química Nova* **1998**, *21*, 761.
11. Flower, R. J. *Nat. Rev.* **2003**, *2*, 179.
12. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
13. Sakamoto, J.; Kimura, H.; Moriyama, S.; Odaka, H.; Momose, Y.; Sugiyama, Y.; Sawada, H. *Biophys. Res. Commun.* **2000**, *278*, 704.
14. Hall, J. M.; McDonnell, D. P. *Mol. Endocrinol.* **2007**, *21*, 1756.
15. Allen, T.; Zhang, F.; Moodie, S. A.; Clemens, L. E.; Smith, A.; Gregoire, F.; Bell, A.; Muscat, G. E. O.; Gustafson, T. A. *Diabetes* **2006**, *55*, 2523.
16. Reginato, M. J.; Bailey, S. T.; Krakow, S. L.; Minami, C.; Ishii, S.; Tanaka, H.; Lazar, M. A. *J. Biol. Chem.* **1998**, *273*, 32679.
17. Semple, R. K.; Chatterjee, V. K.; O'Rahilly, S. *J. Clin. Invest.* **2006**, *116*, 581.
18. SYBYL version 7.2, Tripos Associates Inc., St. Louis, Missouri, USES., http://www.tripos.com.
19. DeLano, W. L. The PyMOL Molecular Graphics System 2002, DeLano Scientific, San Carlos, CA (USA), http://pymol.sourceforge.net.
20. Mourão, R. H.; Silva, T. G.; Soares, A. L. M.; Vieira, E. S.; Santos, J. N.; Lima, M. C. A.; Lima, V. L. M.; Galdino, S. L.; Barbe, J.; Pitta, I. R. *Eur. J. Med. Chem.* **2005**, *40*, 1129.
21. Guarda, V. L. M.; Pereira, M. A.; De Simone, C. A.; Albuquerque, J. C.; Galdino, S. L.; Chantegrel, J.; Perrissin, M.; Beney, C.; Thomasson, F.; Pitta, I. R.; Luu-Duc, C. *Sulfur Lett.* **2003**, *26*, 17.
22. Tan, S. F.; Ang, K. P.; Fong, Y. F. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1941.
23. Albuquerque, J. F. C.; Albuquerque, A.; Azevedo, C. C.; Thomasson, F.; Galdino, S. L.; Chantegrel, J.; Catanho, M. T. J.; Pitta, I. R.; Luu-Duc, C. *Pharmazie* **1995**, *50*, 387.
24. Muto, S.; Kubo, A.; Itai, A.; Sotome, T.; Yamaguchi, Y. PCT Int. Appl. 2005, WO 2005026127.
25. Bozdag-Dundar, O.; Ceylan-Unlusoy, M.; Verspohl, E. J.; Ertan, R. *Arzneimittel-Forschung* **2006**, *56*, 621.
26. Santos, L. C.; Uchôa, F. T.; Cañas, A. R. P. A.; Sousa, I. A.; Moura, R. O.; Lima, M. C. A.; Galdino, S. L.; Pitta, I. R.; Barbe, J. *Heterocycl. Commun.* **2005**, *11*, 121.
27. Silva, T. G.; Barbosa, F. S. V.; Brandão, S. S. F.; Lima, M. C. A.; Galdino, S. L.; Pitta, I. R.; Barbe, J. *Heterocycl. Commun.* **2001**, *7*, 523.
28. BioMedCache version 6.1, Copyright© 2000–2003 Fujitsu Limited, Copyright© 1989–2000, Oxford Molecular Ltd, http://www.CACheSoftware.com.
29. RCSB Protein Data Bank, http://www.rcsb.org/pdb.
30. Rarey, M.; Kramer, B.; Lengauer, T.; Kléber, G. *J. Mol. Biol.* **1996**, *261*, 470.
31. Ambrosio, A. L.; Dias, S. M.; Polikarpov, I.; Zurier, R. B.; Burstein, S. H.; Garratt, R. C. *J. Biol. Chem.* **2007**, *282*, 18625.
32. Ferry, G.; Bruneau, V.; Beauverger, P.; Goussard, M.; Rodriguez, M.; Lamamy, V.; Dromaint, S.; Canet, E.; Galizzi, J. P.; Boutin, J. A. *Eur. J. Pharmacol.* **2001**, *417*, 77.
33. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.