Chlorocyclinones A−D, Chlorinated Angucyclinones from *Streptomyces* sp. Strongly Antagonizing Rosiglitazone-Induced PPAR-γ Activation

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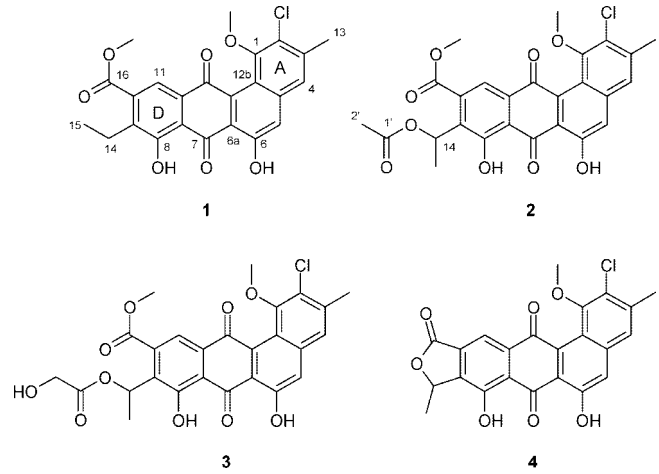
In the course of our screening to identify novel PPAR-γ modulators for the potential treatment of type 2 diabetes, four new chlorinated angucyclinones, chlorocyclinones A−D (**1**−**4**), were isolated from the mycelium of *Streptomyces* sp. strain DSM 17045. Their structures were established by spectroscopic methods. Chlorocyclinones antagonize rosiglitazone-induced peroxisome proliferator-activated receptor gamma (PPAR-γ) activation with IC_{50}'s < 0.4 μM in vitro using an AlphaScreen assay and are able to displace rosiglitazone from the PPAR-γ ligand-binding domain (LBD) in a scintillation proximity assay (SPA). The compounds proved to be active in a cell-based reporter gene assay as well, antagonizing rosiglitazone-induced PPAR-γ activity with IC_{50} values between 0.60 and 7.0 μM. Chlorocyclinone C (**3**) exhibited the most potent activity in all assays.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. The PPAR-γ2 isoform is highly expressed in adipose tissue and is a central regulator of adipocyte differentiation.[1] PPAR-γ2 activity controls the expression of various downstream target genes that are involved in, for example, the regulation of glucose homeostasis. While the positive effects of PPAR-γ activation on the development and progression of type 2 diabetes by the thiazolidinedione (TZD) class of compounds (e.g., rosiglitazone) have been shown in the clinic, these compounds are associated with a number of mechanism-related side-effects: Patients gain weight as the process of adipocytes differentiation is accelerated, and PPAR-γ activation leads to fluid retention. An increasing number of publications point toward an elevated risk of bone fractures on PPAR-γ therapy.[2] Finally, TZDs may cause or exacerbate congestive heart failure in some patients, resulting in a recent black box warning by the FDA for this class of compounds, and in a recent meta-analysis rosiglitazone has been linked to an increased risk of myocardial infarction.[3,4]

In order to retain the positive effects of PPAR-γ activation while avoiding the associated side-effects, a number of pharmaceutical companies are trying to develop PPAR-γ modulators, partial PPAR-γ agonists, or even PPAR-γ antagonists, as such compounds could provide an attractive therapeutic option to patients in need of effective alternative antidiabetic therapies.[5]

In the course of our HTS-supported lead-finding process for PPAR-γ modulators, more than 80 000 natural extracts from plant and microbial origin were tested. An extract from a culture broth of *Streptomyces* sp. (DSM 17045) displayed remarkable antagonistic activity in an AlphaScreen assay. The extract was subjected to a dereplication process integrating physicochemical data with biological information.[6,7] Comparative analysis of the LC/MS-UV data set and the bioactivity plot of the fractionated extract indicated that the activity of the extract was due to a group of quinonic pigments. We report here on the isolation and the chemical and biological characterization of these compounds, which were shown to be new chlorinated angucyclinone derivatives.



Results and Discussion

Strain DSM 17045 was cultivated on a 6 L scale in Erlenmeyer flasks containing a glucose−oatmeal medium. After 4 days, the mycelium was separated from the culture broth by filtration and extracted with EtOAc−MeOH, 9:1. Fractionation of the crude extract by preparative HPLC chromatography afforded compounds **1**–**4** as reddish-brown to brown, amorphous solids.

The UV spectra of **1**−**4** were very similar, showing absorption up to 650 nm. Three maxima (235, 307, and 430 nm in **1**) and a shoulder around 525 nm, or a fourth maximum at 504 nm in **4**, strongly suggested an angucyclinone chromophore.[8] The isotopic pattern in the ESI mass spectra with a typical $[M + H]^+/[M + H + 2]^+$ ratio of approximately 100:35 revealed the presence of one chlorine atom in all four compounds.

Compound **1** had the molecular formula $C_{24}H_{19}ClO_7$, deduced from the quasimolecular ion in the high-resolution ESIMS (455.0892, $[M + H]^+$, calcd 455.0897). The ^1H and ^{13}C NMR data confirmed the angucyclinone skeleton and showed the presence of two phenolic groups, a methyl group, a methoxy group, an ethyl chain, and a carbomethoxy function. The substitution pattern was deduced mainly from the HMBC and ROESY spectra (Table 1). The phenolic proton HO-6 shows a strong ROE correlation to H-5, and from there further ROEs lead to H-4 and Me-13. The chlorine atom was located at C-2 on the basis of the HMBC correlations between C-2 and H-4 and Me-13. The position of the methoxy group was assigned by HMBC correlations of C-1 with MeO-1 and H-4.

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Table 1. Key HMBC and ROESY Correlations in **1**[a]

	HMBC	ROESY
H-4	C-1 (w), C-2 (w), C-4a (w), C-5 (s), C-12b (s), C-13 (s)	H-5 (s), H_3-13 (s)
H-5	C-4 (s), C-6 (s), C-6a (s), C-7 (w), C-12a (w), C-12b (s)	H-4 (s), HO-6 (w)
H-11	C-7a (w), C-9 (s), C-12 (s), C-16 (s)	H_3CO-1 (w), H_3CO-16 (w)
H_3-13	C-2 (s), C-3 (s), C-4 (s), C-4a (w),	H-4 (s)
H_2-14	C-8 (s), C-9 (s), C-10 (s), C-15 (s)	
H_3-15	C-9 (s), C-14 (s)	
H_3CO-1	C-1 (s)	H-11 (w)
H_3CO-16	C-16 (s)	H-11 (w)
HO-6	C-4a (w), C-5 (s), C-6 (s), C-6a (w)	H-5 (w)
HO-8	C-7a (w), C-8 (s), C-9 (s)	

[a] s: strong; w: weak.

Regarding the substitution on ring D, H-11 was assigned on the basis of HMBC correlations with the carbonyl C-12 and C-7a, as well as an ROE correlation to MeO-16. H-11 showed no ROE correlations with other aromatic protons. The ethyl chain was placed at C-9 on the basis of HMBC correlations between C-9 and HO-8, H-11, and the methylene protons H_2-14. Finally, the position of the remaining methoxycarbonyl group at C-10 was confirmed by the above-mentioned ROE cross-peak with H-11. Thus, compound **1**, named chlorocyclinone A, is methyl 2-chloro-9-ethyl-6,8-dihydroxy-1-methoxy-3-methyl-7,12-dioxo-7,12-dihydrotetraphene-10 carboxylate.

The high-resolution MS analysis of **2** gave the molecular formula $C_{26}H_{21}ClO_9$ (513.0939, $[M + H]^+$, calcd 513.0952). The ^1H and ^{13}C NMR data indicated that **2** differed from **1** only in the presence of an acetoxy group located on the C_2 chain at C-9. Thus, compared to **1**, the methylene signal at δ 3.04 was replaced by a quadruplet integrating for 1H at δ 6.41 in the ^1H NMR spectrum of **2**. Moreover H_3C-15 now appears as a doublet at δ 1.73. The carbonyl of the acetyl group showed long-range correlations to the methyl protons and the methine proton H-14, thus establishing the structure of the side chain. Additional HMBC correlations were observed between H-14 and C-8, C-9, and C-10. Thus, compound **2**, named chlorocyclinone B, was assigned the structure methyl 9-[1-(acetyloxyethyl)]-2-chloro-6,8-dihydroxy-1-methoxy-3-methyl-7,12-dioxo-7,12-dihydrotetraphene-10 carboxylate.

Compound **3** possessed the molecular formula $C_{26}H_{21}ClO_{10}$, revealed by the quasimolecular ion (529.0902, $[M + H]^+$, calcd 529.0901) in the high-resolution ESIMS. The ^{1}NMR and ^{13}C NMR spectra indicated that compound **3** differed from **2** only in the hydroxylation of the acetyl group. Thus, the methyl group of the acetyl function was replaced by an oxymethylene (δ_C 60.6, δ_H 4.14 and 4.21, 2d). The structure of **3**, named chlorocyclinone C, is methyl 2-chloro-6,8-dihydroxy-9-{1-[(hydroxyacetyl)oxy]ethyl}-1-methoxy-3-methyl-7,12-dioxo-7,12-dihydrotetraphene-10 carboxylate.

The high-resolution ESIMS of **4** exhibited a quasimolecular ion at 439.0598 ($[M + H]^+$, calcd 439.0584), indicating the molecular formula $C_{23}H_{15}ClO_7$. ^1H and ^{13}C NMR spectra showed that compound **4** differed from **1**−**3** only in the substitution of ring D. Compared to the latter, the methyl ester group was absent. This, together with the downfield resonance of C-14 (δ_C 77.3), suggested the presence of a lactone ring. HMBC correlations from the carbonyl C-16 to H-11 and H-14 confirmed the ring structure. Additional correlations were observed between C-9 and HO-8, H-11, H-14, and H_3-15. Thus compound **4**, named chlorocyclinone D, was assigned the structure 2-chloro-6,8-dihydroxy-1-methoxy-3,9-dimethyltetrapheno[10,9-*c*]furan-7,11,13(9*H*)-trione. The absolute configuration of compounds **2**–**4** has not been established.

Inhibitory activity of compounds **1**–**4** on rosiglitazone-induced PPAR-γ activation was assessed in AlphaScreen and cell-based reporter assays. Displacement of rosiglitazone from the ligand-binding domain of PPAR-γ was measured in a scintillation proximity assay.

In the AlphaScreen assay, compounds **1**–**4** showed a dose-dependent antagonism of the rosiglitazone effect (Figure 1). The

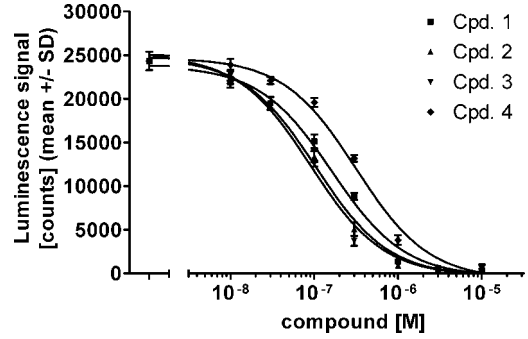

Figure 1. Inhibition of rosiglitazone-induced PPAR-γ activation in an AlphaScreen assay. IC_{50} (μM): 0.16 ± 0.03 (**1**), 0.10 ± 0.01 (**2**), 0.090 ± 0.016 (**3**), 0.32 ± 0.06 (**4**) (mean ± SD).

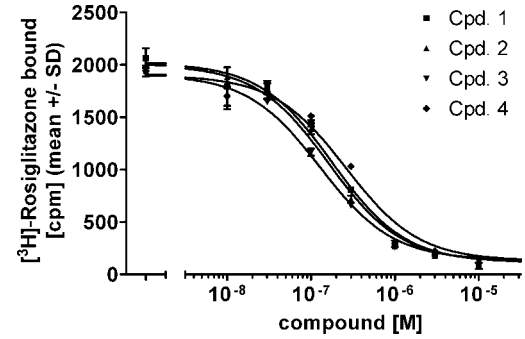

Figure 2. Displacement of rosiglitazone from the PPAR-γ LBD in a scintillation proximity assay (SPA). EC_{50} (μM): 0.18 ± 0.05 (**1**), 0.16 ± 0.04 (**2**), 0.13 ± 0.03 (**3**), 0.26 ± 0.09 (**4**) (mean ± SD).

binding of rosiglitazone, a well-known PPAR-γ agonist, leads to the recruitment of the CBP-derived LXXLL cofactor peptide, thereby increasing the AlphaScreen signal. By preventing the rosiglitazone effect on the PPAR-γ ligand-binding domain (LBD), a subsequent conformational change of the LBD into the antagonized state leads to a decreased recruitment of the LXXLL peptide and thereby decreases the AlphaScreen signal. Chlorocyclinone C (**3**), the most active compound, was able to inhibit rosiglitazone activity with an IC_{50} of 0.090 μM. Compounds **1**–**4** were free of agonistic properties in the absence of rosiglitazone in this assay (data not shown).

In a scintillation proximity assay, compounds **1**–**4** were able to displace radioactively labeled rosiglitazone from the PPAR-γ LBD in a dose-dependent manner (Figure 2). Not only were the compounds able to antagonize the rosiglitazone-induced PPAR-γ activation but due to overlapping binding sites, they precluded rosiglitazone from binding. Chlorocyclinone C (**3**) was the most active and displaced [^{3}H]-rosiglitazone with an EC_{50} of 0.13 μM.

Cellular activity was assessed using a PPAR-γ-driven luciferase reporter cell line. Rosiglitazone as a cellularly active PPAR-γ agonist increased luciferase activity, while compounds **1**–**4** inhibited

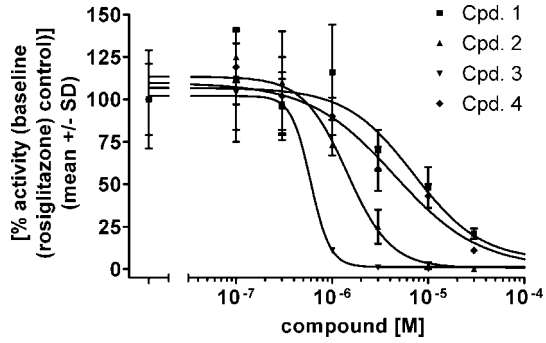

Figure 3. Inhibition of rosiglitazone-induced PPAR-γ activation in a cell-based reporter assay. IC_{50} (μM): 7.0 ± 3.6; –1.235 (**1**), 1.4 ± 0.3; –1.900 (**2**), 0.60 ± 0.04; –4.315 (**3**), 4.4 ± 2.0; –0.981 (**4**) (mean ± SD; Hill coefficient). Baseline (100%) activity corresponds to the experiment performed with rosiglitazone alone.

in a dose-dependent manner the rosiglitazone-induced PPAR-γ activation and subsequently decreased luciferase protein expression and activity (Figure 3). Compounds **1**–**4** were all cellularly active and displayed no signs of cytotoxicity during the course of the experiment. Chlorocyclinone C (**3**), the most active compound, antagonized rosiglitazone with an IC_{50} of 0.60 μM.

Numerous angucyclinones have been described from Actinomycetes.[9,10] However, only a few chlorinated angucyclinones have been found in nature. The antibiotic BE 45985A1, which has been recently patented as an antitumor agent, differs from chlorocyclinone A by the presence of a methyl group instead of a methoxy at C-1.[11] A further representative, BE-2354, contains a chlorine atom at C-9 and a carboxy group at C-2 instead of the typical methyl group at C-3.[12]

Several natural products, including isoflavones,[13] polyketides,[14] furanoditerpenes,[15] and anthraquinones,[16] have been found to activate PPAR-γ. However, chlorocyclinones A−D are the first angucyclinones reported to modulate the activity of the nuclear hormone receptor PPAR-γ in a dose-dependent manner. Moreover, chlorocyclinones A−D are the first PPAR-γ antagonists of natural origin. The compounds were active in a reporter cell line, demonstrating their ability to enter cells and to interfere with PPAR-γ activation. Among the compounds investigated, chlorocyclinone C (**3**) exhibited the most potent activity in all assays applied. Chlorocyclinone C constitutes a new scaffold from which more potent PPAR-γ antagonists could be designed as potential therapeutic agents for the treatment of type 2 diabetes.

Experimental Section

General Experimental Procedures. UV spectra: Perkin-Elmer lambda 2S. CD spectra: Jasco J-715 spectropolarimeter. IR spectra: ThermoNicolet ATR-FTIR Avatar 370 system. 1D and 2D NMR spectra were recorded at 293 K in $CDCl_3$, on DPX400 and DRX400 spectrometers (Bruker, Karlsruhe). For all 2D experiments standard pulse sequences contained in the software release XWINNMR 3.1 PL11 (Bruker) were used. TMS was used for referencing. ESIHRMS: LCT time-of-flight mass spectrometer (Micromass Waters) in the positive ion mode. The mass spectrometer was coupled to a Waters 2795 Alliance HPLC system. Preparative HPLC: Waters purification system consisting of a Delta Prep 600 pump, a 2996 PDA detector, and a 2767 sample manager.

Organism. *Streptomyces* sp. DSM 17045 was isolated from a soil sample collected in 1997 in Monument Valley, UT/AZ. The strain has been deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Germany. Taxonomical characterization has been carried out at the DSMZ. The strain was assigned to the genus *Streptomyces* because of its morphological appearance and characteristic chemotaxonomic features, such as LL-diaminopimelic acid in the peptidoglycan and the typical pattern of iso/anteiso-branched fatty acids. Spore chains are spirals. Aerial mycelium is gray; substrate mycelium gray-brown. Melanin is not produced. D-Glucose, L-arabinose, D-xylose, raffinose, D-mannitol, inositol, and D-fructose are utilized. Phylogenetic data were based on the comparison of the partial sequences of the 16S rRNA gene (500 first nucleotides). The sequence of DSM 17045 was compared to all *Streptomyces* sequences available at the DSMZ and in the EMBL database. Only low similarity was observed with known *Streptomyces* sequences. The nearest phylogenetic neighbors of strain DSM 17045 were found to be *S. aurantiacus* DSM 40795 and *S. ascidiscabies* DSM 41668^T, both showing sequence similarity of 97.7%. No concordance was observed with the RiboPrint[17] patterns stored in the DSMZ RiboPrint database. Combining the phylogenetic data with morphological data[18] and a physiological test[19] did not enable assigning strain DSM 17045 to a known *Streptomyces* species. The data above strongly suggest that strain DSM 17045 is a representative of *Streptomyces* species not described hitherto.

Fermentation. *Streptomyces* sp. DSM 17045 was cultivated on a 6 L scale in 1 L Erlenmeyer flasks each containing 250 mL of a medium consisting of glucose (2%), oatmeal (2%), yeast extract (0.3%), NaCl (0.3%), $CaCO_3$ (0.3%), $FeSO_4 \cdot 7H_2O$ (0.054%), and $MnCl_2 \cdot 4H_2O$ (0.06%). Prior to sterilization, the pH of the medium was adjusted to 6.0 with 8 N NaOH. Each flask was inoculated with 20 mL of a 48 h old preculture (medium: soybean meal (1.5%), glucose (1.5%), NaCl (0.5%), $CaCO_3$ (0.1%), KH_2PO_4 (0.03%), pH 6.9). The fermentation was carried out on a rotary shaker (160 rpm) for 96 h at 28 °C.

Isolation of 1–4. The mycelium and the culture filtrate were separated by filtration. After freeze-drying, the mycelium (105 g) was extracted at rt twice with EtOAc−MeOH, 9:1 (1 L overnight, followed by 600 mL for 8 h). After evaporation to dryness, the combined extracts (7.3 g) were dissolved in a mixture of MeOH (15 mL), DMSO (4 mL), and THF (2 mL) and separated by preparative HPLC on a C18 Nova-Pack column (Waters, 6 mm, 2.5 × 10 cm, i.d.) equipped with a precolumn (2.5 × 1.0 cm i.d.). Elution was carried out with a gradient of MeCN in 1 mM aqueous NH_4OAc (pH 4) (65% isocratic for 9 min, then to 100% in 11 min); flow rate was 20 mL/min; UV detection was at 426 nm. The mixture was injected in 38 portions to afford compounds **1** (t_R 17.4 min, crude, 29 mg), **2** (t_R 13.0 min, 191 mg), **3** (t_R 6.6 min, 280 mg), and **4** (t_R 9.8 min, crude, 57 mg). Crude compound **1** was washed with cold *n*-hexane (5 mL) to give pure **1** (17 mg). Crude compound **4** was further purified on Sephadex LH-20 with MeOH containing 0.01% 1 N HCl to give 26 mg of pure **4**.

Amplified Luminescent Proximity Homogeneous Assay (Alpha-Screen). The assay was performed as previously described[20] in 384-well plates. The assay buffer consisted of 25 mmol/L Hepes pH 7.4, 100 mmol/L NaCl, 1 mmol/L DTT, 0.1% (v/v) Tween-20, and 0.1% (w/v) BSA. For testing, 3 nmol/L GST-hPPAR-γ LBD-fusion protein, 15 nmol/L biotinylated LXXLL-peptide of the cofactor CBP,[16] 10 μg/mL anti-GST acceptor beads, and 10 μg/mL streptavidin-donor beads (Applied Biosystem) were incubated for 4 h at rt in a final volume of 12.5 μL with 100 nmol/L rosiglitazone and various concentrations of the substances to be tested. Compounds to be tested were dissolved in DMSO. The final concentration of DMSO in the assay was 1% (v/v) in all cases. Data were read out on a Packard-Fusion microplate reader. Control experiments were performed with rosiglitazone alone and in the absence of rosiglitazone, respectively. Activity is measured as luminescence signal (counts). Measurements were made in triplicate.

Scintillation Proximity Assay (SPA). The assay was performed as previously described[21] in 96-well plates. The assay buffer consists of 20 mmol/L Tris pH 7.5, 25 mmol/L KCl, 10 mmol/L DTT, and 0.2% (v/v) Triton X-100. For testing, 30 nmol/L GST-hPPAR-γ LBD fusion protein, 30 nmol/L His-hRXR alpha LBD fusion protein, anti-GST antibody (1:600, Amersham Pharmacia), 0.25 mg of protein A SPA PVT antibody-binding beads (Amersham Pharmacia), and 30 nmol/L [^{3}H]-labeled rosiglitazone (Amersham) were incubated together with the substances to be tested in a total volume of 100 μL for 5 h at rt. Following the incubation, the plates were centrifuged for 5 min at 2000 rpm using a Hettich Universal 30Rf centrifuge and subsequently measured using a Packard TopCount NXT. As a baseline control, 10 μmol/L unlabeled rosiglitazone was used. DMSO alone was used as a negative control. Activity is measured as cpm. Measurements were performed in triplicate.

Luciferase Reporter Gene Assay. Human PPAR-γ2 (aa205–505) was subcloned via *Bam*HI and *Hin*dIII in frame with a Gal4 DNA binding domain into the multiple cloning site of the pFA-CMV vector

Table 2. ^{13}C NMR Spectroscopic Data (100 MHz, $CDCl_3$) for Compounds **1**–**4**[a]

	1	2	3	4
position	δ_C, mult	δ_C, mult	δ_C, mult	δ_C, mult
1	154.0, qC	153.9, qC	153.8, qC	153.9, qC
2	127.0, qC	127.1, qC	127.1, qC	127.1, qC
3	141.4, qC	141.5, qC	141.6, qC	142.0, qC
4	123.0, CH	123.0, CH	123.0, CH	123.0, CH
4a	138.9, qC[b]	138.9, qC[b]	138.9, qC[b]	139.1, qC[b]
5	117.9, CH	118.2, CH,	118.2, CH	118.2, CH
6	156.6, qC	156.6, qC	156.5, qC	156.6, qC
6a	119.5, qC	119.3, qC	119.2, qC	119.2, qC
7	193.2, qC	193.0, qC	192.9, qC	193.4, qC
7a	115.7, qC	115.6, qC	115.8, qC	118.3, qC
8	160.6, qC	159.0, qC	159.1, qC	156.7, qC
9	140.8, qC	134.3, qC	133.4, qC	143.3, qC
10	138.1, qC	139.4, qC	139.3, qC	134.0, qC
11	119.2, CH	117. 9, CH	117.9, CH	115.0, CH
11a	133.5, qC[b]	135.2, qC[b]	135.5, qC[b]	138.7, qC[b]
12	183.8, qC	183.3, qC	183.1, qC	183.6, qC
12a	137.2, qC[b]	136.8, qC[b]	136.7, qC[b]	137.3, qC[b]
12b	119.6, qC	119.7, qC	119.7, qC	120.1, qC
13	21.3, CH_3	21.3, CH_3	21.3, CH_3	21.4, CH_3
14	20.6, CH_2	66.8, CH	68.2, CH,	77.3, CH
15	13.9, CH_3	19.4, CH_3	19.3, CH_3	18.8, CH_3
16	166.7, qC	168.1, qC	167.9, qC	168.2, qC
MeO-C-1	61.5, CH_3	61.5, CH_3	61.5, CH_3	61.6, CH_3
MeO-C-16	52.7, CH_3	52.8, CH_3	53.0, CH_3	
1′ (Ac)		169.9, qC	172.3, qC	
2′ (Ac)		20.9, CH_3	60.6, CH_2	

[a] Assignments are supported by DEPT, COSY, ROESY, HSQC, and HMBC spectra. [b] Assignments within the same column can be reversed, although assignments shown are preferred.

(Strategene); the resulting plasmid was designated pFA-CMV/hPPAR-γ LBD. CHO-K1 cells were cotransfected with both pFA-CMV/hPPAR-γ LBD and pFR-Luc (Stratagene), and a stable cell line was generated according to standard procedures. For subsequent testing, 3×10^4 cells per well were seeded in a 96-well plate and cultivated overnight in Ham's F-12 medium (10% (v/v) FBS; 0.5 mg/mL G-418); subsequently medium without G-418 was used. Substances to be tested were diluted using DMSO. Final DMSO concentrations in the wells were 1% (v/v) in all cases. All wells contained 100 nmol/L rosiglitazone. As a negative control, DMSO instead of rosiglitazone was used. Baseline levels were obtained in the presence of rosiglitazone alone. Twenty-four hours post compound application, the supernatants were discarded and the cells were washed twice using 150 μL of washing buffer (25 mmol/L tricine, 16.3 mmol/L $MgSO_4$, pH 7.8). Following the washing steps, 50 μL of washing buffer and 150 μL of luciferase assay buffer (25 mmol/L tricine, 0.5 mmol/L EDTA, 0.54 mmol/L NaTPP, 16.3 mmol/L $MgSO_4$, 1.2 mmol/L ATP, 0.05 mmol/L luciferin, 56.8 mmol/L 2-mercaptoethanol, 0.1% (v/v) triton X-100, pH 7.8) were added. Following a 5 min incubation, the luminescence was measured using a Packard TopCount NXT. The luciferase activity was obtained by integration of the relative luciferase units (RLU) over the first 10 s of the measurement. Activity is expressed as percent of the baseline activity of the control containing rosiglitazone alone. Assays were performed in triplicate.

Activity Calculations. Curve fittings and IC_{50}/EC_{50} calculations were made using Prism 5 for Windows (GraphPad Software Inc.). For Figures 1 and 2, a one-site model with a constant slope was used for the curve fittings; for Figure 3 a one-site model with a variable slope was used.

Methyl 2-chloro-9-ethyl-6,8-dihydroxy-1-methoxy-3-methyl-7,12-dioxo-7,12-dihydrotetraphene-10 carboxylate (chlorocyclinone A, 1). brown, amorphous solid; UV (EtOH) λ_{max} (log ε) 235 (4.59), 307 (4.24) 430 (3.90), 525 (sh) nm; IR (neat) ν_{max} 3076, 2960, 2928, 2870, 1722, 1622, 1408, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 12.10 (1H, s, HO-8), 11.51 (1H, s, OH-6), 7.98 (1H, s, H-11), 7.43 (1H, s, H-5), 7.36 (1H, s, H-4), 3.99 (3H, s, MeO-16), 3.83 (3H, s, MeO-1), 3.04 (2H, q, $J = 7.5$ Hz, H_2-14), 2.54 (3H, s, H_3-13), 1.27 (3H, t, $J = 7.5$ Hz, H_3-15); ^{13}C NMR Table 2; HRESIMS m/z $[M + H]^+$ obsd 455.0892, calcd for $C_{24}H_{20}ClO_7$ 455.0897.

Methyl 9-[1-(acetyloxyethyl)]-2-chloro-6,8-dihydroxy-1-methoxy-3-methyl-7,12-dioxo-7,12-dihydrotetraphene-10 carboxylate (chlorocyclinone B, 2). brown, amorphous solid; UV (EtOH) λ_{max} (log ε) 234 (4.64), 306 (4.28), 424 (3.96), 525 (sh) nm; CD (MeCN) λ_{max} (θ) 233 (−8280), 241 (−7250), 255 (−10490), 288 (−4880) 318 (−7230) nm; IR (neat) ν_{max} 3115, 2954, 2855, 1733, 1679, 1629, 1351, 1217, 1083 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 12.17 (1H, s, OH-8), 11.42 (1H, s, OH-6), 7.68 (1H, s, H-11), 7.44 (1H, s, H-5), 7.36 (1H, s, H-4), 6.41 (1H, q, $J = 6.7$ Hz, H-14), 4.01 (3H, s, MeO-16), 3.80 (3H, s, MeO-1), 2.54 (3H, s, H_3-13), 2.06 (3H, s, H_3-2′), 1.73 (3H, d, $J = 6.7$ Hz, H_3-15); ^{13}C NMR Table 2; HRESIMS m/z $[M + H]^+$ obsd 513.0939, calcd for $C_{26}H_{22}ClO_9$ 513.0952.

Methyl 2-chloro-6,8-dihydroxy-9-{1-[(hydroxyacetyl)oxy]ethyl}-1-methoxy-3-methyl-7,12-dioxo-7,12-dihydrotetraphene-10 carboxylate (chlorocyclinone C, 3). reddish-brown, amorphous solid; UV (EtOH) λ_{max} (log ε) 235 (4.65), 307 (4.28), 425 (3.98), 525 (sh) nm; CD (MeCN) λ_{max} (θ) 233 (−8450), 240 (−6950), 254 (−11090), 284 (−5660), 318 (−8320) nm; IR (neat) ν_{max} 3482, 2984, 2950, 2852, 1733, 1677, 1629, 1241, 1218, 1079 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 12.18 (1H, s, HO-8), 11.34 (1H, s, HO-6), 7.70 (1H, s, H-11), 7.39 (1H, s, H-5), 7.32 (1H, s, H-4), 6.54 (1H, q, $J = 6.8$ Hz, H-14), 4.21 and 4.14 (2d, $J = 17.1$ Hz, H_2-2′), 4.01 (3H, s, MeO-16), 3.80 (3H, s, MeO-1), 2.52 (3H, s, H_3-13), 2.47 (1H, broad, HO-2′), 1.78 (3H, d, $J = 6.8$ Hz, H_3-15); ^{13}C NMR Table 2; HRESIMS m/z $[M + H]^+$ obsd 529.0902, calcd for $C_{26}H_{22}ClO_{10}$ 529.0901.

2-Chloro-6,8-dihydroxy-1-methoxy-3,9-dimethyltetrapheno[10,9-*c*]furan-7,11,13(9*H*)-trione (chlorocyclinone D, 4). brown, amorphous solid; UV (EtOH) λ_{max} (log ε) 238 (4.56), 322 (4.15), 417 (3.88), 504 (3.43) nm; CD (MeCN) λ_{max} (θ) 219 (−5460), 238 (6160), 280 (440), 294 (1250), 321 (−1060) nm; IR (neat) ν_{max} 3185, 2956, 2855, 1772, 1681, 1637, 1246, 1034 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 11.96 (1H, s, HO-8), 11.32 (1H, s, HO-6), 8.10 (1H, s, H-11), 7.46 (1H, s, H-5), 7.38 (1H, s, H-4), 5.76 (1H, q, $J = 6.7$ Hz, H-14), 3.90 (3H, s, MeO-1), 2.55 (3H, s, H_3-13), 1.80 (3H, d, $J = 6.7$ Hz, H_3-15); ^{13}C NMR Table 2; HRESIMS m/z $[M + H]^+$ obsd 439.0598, calcd for $C_{23}H_{16}ClO_7$ 439.0584.

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Supporting Information Available: IR (**1**−**4**) and CD spectra (**2**−**4**) of the isolated compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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