Supporting Information

Sulfonimide and Amide Derivatives as Novel PPARα Antagonists: Synthesis, Antiproliferative Activity and Docking Studies

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Figure S1. Chemical structures of reference compounds Wy-14,643, Rosiglitazone, and GW6471.
S1. **Chemical Procedures and Characterisation of Synthesized Compounds**

Melting points were determined with a Buchi Melting Point B-450 and were uncorrected. NMR spectra were recorded on a Varian Mercury 300 spectrometer with $^1$H at 300.060 MHz and $^{13}$C at 75.475 MHz. Proton chemical shifts were referenced to the TMS internal standard. Chemical shifts are reported in parts per million (ppm, $\delta$ units). Coupling constants are reported in units of Hertz (Hz). Splitting patterns are designed as s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; m, multiplet; b, broad. Infrared spectra were recorded on a FT-IR 1600 Perkin Elmer. Microanalyses were carried out with an Eurovector Euro EA 3000 model analyzer. Analyses were within ± 0.4 % of the theoretical values.

All commercial chemicals and solvents were reagent grade and were purchased from Sigma Aldrich; they are used without further purification, unless otherwise specified. Reactions were monitored by thin layer chromatography on silica gel plates (60F-254, Sigma Aldrich) and the analysis of the plates was carried out using a UV lamp 254/365 nm. Flash chromatography was performed on silica gel 60 (Merk). The following solvents and reagents have been abbreviated: acetonitrile (ACN), dichloromethane (DCM), diethyl ether (Et$_2$O), dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF). All reactions were carried out with the use of the standard techniques.

The anhydrous THF was prepared from commercial THF reagent grade with the following procedure: THF was refluxed over sodium for 4 hours under nitrogen atmosphere. The distillate was refluxed again over sodium and benzophenone, under nitrogen atmosphere, until the solution turned blue and then collected by distillation.

The synthesis of Lead compound I and II (LC1 and LC2) was previously reported [1-2].

**General procedure for the synthesis of sulfonimides (1-2-3-10e-13e)**

To a stirred solution of acids LC1, LC2, 9, 12 (1.0 eq) in 5-8 mL of dry DCM in a two-neck, round bottomed flask fitted with a nitrogen inlet and addition funnel at 0°C, were added 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC, 1.0 eq) and 4-dimethylaminopyridine (DMAP, 1.0 eq). After 15-25 min, the $p$-substituted benzenesulfonamide (1.1 eq) was added to the reaction mixture and the cold water/ice bath was removed. The reaction mixture was allowed to stir at room temperature for 24 h, diluted with DCM (15 mL), washed with 2N HCl (3 x 20 mL), dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. Crude products were purified by column chromatography (silica gel, DCM/MeOH 95:5).

**2,2-Dimethyl-N-(methylsulfonyl)-5-{4-[(E)-2-phenylvinyl]phenoxy}pentanamide (1a)**
White needles, 44% yield; m.p. 166-168 °C; IR (KBr) 1712, 1600, 1334, 1172 cm⁻¹; ¹H NMR (CDCl₃) δ 1.26 (s, 6H, C(CH₃)₂), 1.73-1.79 (m, 4H, OCH₂CH₂CH₂), 3.28 (s, 3H, CH₃SO₂), 3.97 (s, 2H, OCH₂), 6.89 (d, 2H, J 8.7 Hz, CH₈), 6.96 (d, 1H, J 16.2 Hz, CH=CH), 7.05 (d, 1H, J 16.2 Hz, CH=CH), 7.21-7.50 (m, 7H, CH₈), 8.26 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 25.0, 25.1, 36.8, 41.7, 43.8, 67.6, 114.8, 126.4, 126.9, 127.4, 127.9, 128.3, 128.8, 130.5, 137.7, 158.6, 176.8. Anal. Calcd. for C₂₂H₂₈NO₄S: C, 65.81; H, 6.78; N, 3.49; Found: C, 65.93; H, 6.77; N, 3.48.

2,2-Dimethyl-N-(phenylsulfonyl)-5-{4-[(E)-2-phenylvinyl]phenoxy}pentanamide (1b)
White solid, 53% yield; m.p. 175-177 °C; IR (KBr) 1709, 1599, 1341, 1175 cm⁻¹; ¹H NMR (CDCl₃) δ 1.17 (s, 6H, C(CH₃)₂), 1.49-1.64 (m, 4H, OCH₂CH₂CH₂), 3.85 (t, 2H, J 5.7 Hz, OCH₂CH₂CH₂), 6.88 (d, 2H, J 8.4 Hz, CH₈), 6.98 (d, 1H, J 16.2 Hz, CH=CH), 7.07 (d, 1H, J 16.2 Hz, CH=CH), 7.21-7.64 (m, 10H, CH₈), 8.07 (d, 2H, J 8.4 Hz, CH₈), 8.48 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 24.8, 25.0, 36.9, 43.6, 67.5, 114.8, 126.4, 126.9, 127.4, 127.9, 128.3, 128.6, 128.8, 129.1, 130.5, 134.2, 137.8, 138.5, 158.6, 175.4. Anal. Calcd. for C₂₇H₂₉NO₄S: C, 69.95; H, 6.31; N, 3.02; Found: C, 69.86; H, 6.32; N, 3.01.

2-Methyl-N-(methylsulfonyl)-2-[4-(E)-phenyldiazenyl]phenoxyethyl]phenoxy]propanamide (2a)
Pale orange needles, 47% yield; m.p. 62-64 °C; IR (KBr) 3247, 2934, 1718, 1599, 1504, 1396, 1167 cm⁻¹; ¹H NMR (CDCl₃) δ 1.53 (s, 6H, C(CH₃)₂), 3.10 (t, 2H, J 6.9 Hz, CH₂Ph), 3.34 (s, 3H, SO₂CH₃), 4.24 (t, 2H, J 6.9 Hz, OCH₂), 6.88 (d, 2H, J 8.7 Hz, CH₈), 7.00 (d, 2H, J 8.7 Hz, CH₈), 7.25 (d, 2H, J 7.8 Hz, CH₈), 7.40-7.53 (m, 3H, CH₈), 7.85-7.92 (m, 4H, CH₈), 9.05 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 24.6, 35.1, 41.5, 69.0, 82.0, 114.9, 122.1, 122.7, 124.9, 129.2, 130.3, 130.6, 134.5, 147.2, 151.7, 152.9, 161.4, 174.2. Anal. Calcd. for C₂₅H₂₇N₃O₅S: C, 62.35; H, 5.65; N, 8.73; Found: C, 62.40; H, 5.65; N, 8.75.

2-Methyl-2-[4-(2-[4-[(E)-phenyldiazenyl]phenoxy]ethyl]phenoxy]-N-(phenylsulfonyl)propanamide (2b)
Orange solid, 65% yield; m.p. 115-117 °C; IR (KBr) 3440, 3253, 1723, 1598, 1503, 1405, 1176 cm⁻¹; ¹H NMR (CDCl₃) δ 1.42 (s, 6H, CH₃C), 3.08 (t, 2H, J 6.9 Hz, CH₂C₆), 4.23 (t, 2H, J 6.9 Hz, CH₂O), 6.71 (d, 2H, J 8.7 Hz, CH₈), 7.00 (d, 2H, J 9.0 Hz, CH₈), 7.14 (d, 2H, J 9.0 Hz, CH₈), 7.43-7.59 (m, 4H, CH₈), 7.67 (d, 2H, J 7.2 Hz, CH₈), 7.87 (d, 2H, J 7.2 Hz, CH₈), 7.91 (d, 2H, J 8.7 Hz, CH₈), 8.07 (d, 2H, J 8.7 Hz, CH₈), 9.10 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 24.5, 35.1, 69.0, 81.6, 114.9, 121.3, 122.7, 124.9, 128.7, 129.2, 129.2, 130.2, 130.6, 134.0, 134.3, 138.3, 147.2,
2-Methyl-N-[(4-methylphenyl)sulfonyl]-2-[4-(2-[4-[(E)-phenyldiazenyl]phenoxy]ethyl)phenoxy]propanamide (3a)

Orange solid, 80% yield; m.p. 135-136 °C; IR (KBr) 3441, 3229, 1717, 1601, 1503, 1417, 1356, 1172 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.41 (s, 6H, \(CH_3C\)), 2.46 (s, 3H, \(CH_3\)), 3.08 (t, 2H, \(J\ 6.9\ Hz, CH_2C\)), 4.23 (t, 2H, \(J\ 6.9\ Hz, CH_2O\)), 6.73 (d, 2H, \(J\ 8.7\ Hz, CH_A\)), 7.00 (d, 2H, \(J\ 9.3\ Hz, CH_A\)), 7.15 (d, 2H, \(J\ 8.7\ Hz, CH_A\)), 7.35 (d, 2H, \(J\ 8.1\ Hz, CH_A\)), 7.43-7.52 (m, 3H, \(CH_A\)), 7.86-7.96 (m, 6H, \(CH_A\)), 9.11 (bs, 1H, NH); \(^13\)C NMR (CDCl\(_3\)) \(\delta\) 21.7, 24.3, 34.8, 68.8, 81.4, 114.7, 121.2, 122.5, 124.7, 128.5, 129.0, 129.6, 130.0, 130.4, 133.7, 135.1, 145.2, 147.0, 151.8, 152.7, 161.2, 172.6. Anal. Calcd. for C\(_{31}\)H\(_{31}\)N\(_5\)O\(_8\): C, 66.77; H, 5.60; N, 7.54; Found: C, 66.89; H, 5.61; N, 7.52.

2-Methyl-N-[(4-methoxyphenyl)sulfonyl]-2-methyl-2-[4-(2-[4-[(E)-phenyldiazenyl]phenoxy]ethyl)phenoxy]propanamide (3b)

Bright orange solid, 58% yield; m.p. 179-180 °C; IR (KBr) 3420, 3245, 1718, 1597, 1501, 1411, 1340, 1161 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.41 (s, 6H, \(CH_3C\)), 3.08 (t, 2H, \(J\ 6.9\ Hz, CH_2C\)), 3.89 (s, 3H, \(OCH_3\)), 4.23 (t, 2H, \(J\ 6.9\ Hz, CH_2O\)), 6.72 (d, 2H, \(J\ 8.7\ Hz, CH_A\)), 7.00 (d, 4H, \(J\ 8.7\ Hz, CH_A\)), 7.14 (d, 2H, \(J\ 8.7\ Hz, CH_A\)), 7.43-7.53 (m, 3H, \(CH_A\)), 7.86-7.93 (m, 4H, \(CH_A\)), 8.00 (d, 2H, \(J\ 9.0\ Hz, CH_A\)), 9.10 (bs, 1H, NH); \(^13\)C NMR (CDCl\(_3\)) \(\delta\) 24.3, 34.8, 55.7, 68.8, 81.4, 114.1, 114.7, 121.1, 122.5, 124.7, 129.0, 129.4, 130.0, 130.4, 133.7, 146.9, 151.8, 152.6, 161.2, 164.0, 172.7. Anal. Calcd. for C\(_{31}\)H\(_{31}\)N\(_5\)O\(_8\): C, 64.90; H, 5.45; N, 7.32; Found: C, 64.79; H, 5.47; N, 7.31.

2-Methyl-N-[(4-chlorophenyl)sulfonyl]-2-methyl-2-[4-(2-[4-[(E)-phenyldiazenyl]phenoxy]ethyl)phenoxy]propanamide (3c)

Dark yellow solid, 64% yield; m.p. 151-152 °C; IR (KBr) 3430, 3236, 1721, 1603, 1503, 1415, 1358, 1182 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.42 (s, 6H, \(CH_3C\)), 3.08 (t, 2H, \(J\ 6.9\ Hz, CH_2C\)), 4.22 (t, 2H, \(J\ 6.9\ Hz, CH_2O\)), 6.72 (d, 2H, \(J\ 8.7\ Hz, CH_A\)), 7.00 (d, 2H, \(J\ 9.3\ Hz, CH_A\)), 7.14 (d, 2H, \(J\ 8.7\ Hz, CH_A\)), 7.43-7.53 (m, 5H, \(CH_A\)), 7.86-7.93 (m, 4H, \(CH_A\)), 8.01 (d, 2H, \(J\ 8.7\ Hz, CH_A\)), 9.23 (bs, 1H, NH); \(^13\)C NMR (CDCl\(_3\)) \(\delta\) 24.2, 34.8, 68.7, 81.4, 114.7, 121.1, 122.5, 124.7, 129.0, 129.3, 130.0, 130.1, 133.9, 136.5, 140.8, 147.0, 151.7, 152.7, 161.2, 172.8. Anal. Calcd. for C\(_{30}\)H\(_{29}\)ClN\(_5\)O\(_8\): C, 62.33; H, 4.88; N, 7.27; Found: C, 62.42; H, 4.87; N, 7.27.
2-Methyl-N-[(4-nitrophenyl)sulfonyl]-2-[4-(2-[(E)-phenyldiazenyl]phenoxy]ethyl)phenoxy]propanamide (3d)

Yellow solid, 59% yield; m.p. 190-191 °C; IR (KBr) 3435, 3290, 1726, 1602, 1530, 1406, 1350, 1179 cm⁻¹; ¹H NMR (CDCl₃) δ 1.42 (s, 6H, CH₃C), 3.09 (t, 2H, J 6.7 Hz, CH₂C₆H₄), 4.23 (t, 2H, J 6.7 Hz, CH₂O), 6.75 (d, 2H, J 8.7 Hz, CH₆H₂), 6.70 (d, 2H, J 9.0 Hz, CH₆C₆H₄), 7.17 (d, 2H, J 8.7 Hz, CH₆C₆H₄), 7.43-7.52 (m, 3H, CH₆C₆H₄), 7.85-7.92 (m, 4H, CH₆C₆H₄), 8.27 (d, 2H, J 8.7 Hz, CH₆C₆H₄), 8.39 (d, 2H, J 8.7 Hz, CH₆C₆H₄), 9.29 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 24.2, 24.8, 25.3, 34.8, 68.7, 81.6, 114.7, 121.5, 122.5, 124.2, 124.7, 129.0, 130.0, 130.1, 130.4, 134.3, 143.5, 147.0, 150.8, 151.4, 152.6, 161.1, 172.8. Anal. Calcd. for C₃₀H₂₈N₄O₇S: C, 61.21; H, 4.79; N, 9.32; Found: C, 60.99; H, 4.80; N, 9.53.

N-[(4-acetylamino)phenyl]sulfonyl]-2-methyl-2-[4-(2-[(E)-phenyldiazenyl]phenoxy]ethyl)phenoxy]propanamide (3e)

Orange solid, 34% yield; m.p. 175-176 °C; IR (KBr) 3460, 3353, 1691, 1594, 1539, 1406, 1356, 1169 cm⁻¹; ¹H NMR (CDCl₃) δ 1.41 (s, 6H, CH₃C), 2.21 (s, 3H, COCH₃), 3.08 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 4.23 (t, 2H, J 6.9 Hz, CH₂O), 6.72 (d, 2H, J 8.1 Hz, CH₆C₆H₄), 7.00 (d, 2H, J 8.7 Hz, CH₆C₆H₄), 7.15 (d, 2H, J 9.0 Hz, CH₆C₆H₄), 7.43-7.51 (m, 3H, CH₆C₆H₄), 7.69 (d, 2H, J 8.7 Hz, CH₆C₆H₄), 7.85 (s, 1H, NH), 7.86-7.99 (m, 6H, CH₆C₆H₄), 9.17 (s, 1H, NH); ¹³C NMR (CDCl₃) δ 24.3, 24.7, 34.8, 68.7, 81.4, 114.7, 118.9, 121.2, 122.5, 124.7, 129.0, 129.9, 130.0, 130.4, 132.3, 133.8, 143.2, 146.9, 151.7, 152.6, 161.2, 168.2, 172.6. Anal. Calcd. for C₃₂H₃₂N₄O₆S: C, 63.98; H, 5.37; N, 9.33; Found: C, 64.01; H, 5.36; N, 9.37.

N-[(2-methyl-2-[4-(2-[(E)-phenyldiazenyl]phenoxy]ethyl)phenoxy]propanoyl]amino)sulfonyl]phenyl]benzamide (3f)

Yellowish solid, 21% yield; m.p. 203-205 °C; IR (KBr) 3347, 1666, 1593, 1533, 1426, 1360, 1172 cm⁻¹; ¹H NMR (CDCl₃) δ 1.42 (s, 6H, CH₃C), 3.09 (t, 2H, J 6.7 Hz, CH₂C₆H₄), 4.23 (t, 2H, J 6.7 Hz, CH₂O), 6.72 (d, 2H, J 8.7 Hz, CH₆C₆H₄), 7.00 (d, 2H, J 9.0 Hz, CH₆C₆H₄), 7.16 (d, 2H, J 8.4 Hz, CH₆C₆H₄), 7.43-7.60 (m, 6H, CH₆C₆H₄), 7.86-7.92 (m, 6H, CH₆C₆H₄), 8.07 (d, 2H, J 9.3 Hz, CH₆C₆H₄), 8.11 (s, 1H, NH), 9.13 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 24.3, 34.8, 68.7, 81.4, 114.7, 119.4, 121.1, 122.5, 124.7, 127.1, 129.0, 130.0, 130.1, 130.4, 132.5, 132.7, 133.8, 134.0, 143.2, 146.9, 151.7, 152.6, 161.2, 165.9, 172.8. Anal. Calcd. for C₃₇H₃₄N₄O₆S: C, 67.05; H, 5.17; N, 8.45; Found: C, 66.97; H, 5.18; N, 8.47.
2-Methyl-N-\{(4-\{(phenylacetyl)amino\}phenyl)sulfonyl\}-2-\{(4\{(E\}phenyldiazenyl)phenox y\}ethyl)phenoxy\}propenamide (3g)
Orange solid, 61% yield; m.p. 142-143 °C; IR (KBr) 3467, 3329, 1699, 1596, 1502, 1403, 1349, 1167 cm⁻¹; ¹H NMR (CDCl₃) δ 1.39 (s, 6H, CH₃), 3.07 (t, 2H, J 6.7 Hz, CH₂C₆H₄), 3.77 (s, 2H, CH₂CONH), 4.22 (t, 2H, J 6.7 Hz, CH₂O), 6.71 (d, 2H, J 9.0 Hz, CH₆), 7.00 (d, 2H, J 9.3 Hz, CH₆), 7.14 (d, 2H, J 8.7 Hz, CH₆), 7.32-7.50 (m, 8H, CH₆), 7.62 (d, 2H, J 9.6 Hz, CH₆), 7.86-7.97 (m, 6H, CH₆ and NH), 9.14 (s, 1H, NH); ¹³C NMR (CDCl₃) δ 24.2, 34.8, 44.9, 68.8, 81.4, 114.7, 119.0, 121.1, 122.5, 124.9, 128.0, 129.0, 129.4, 129.9, 130.0, 130.4, 132.7, 133.6, 133.8, 142.8, 146.8, 151.7, 152.5, 161.3, 169.4, 172.7. Anal. Calcd. for C₃₈H₃₆N₄O₆S: C, 67.44; H, 5.36; N, 8.28; Found: C, 67.51; H, 5.38; N, 8.26.

N-\{4-\{(2-1,1-dimethyl-2-oxo-2\{(phenylsulfonyl)amino\}ethoxy\}phenyl\}ethoxy\}phenyl benzamide (10e)
White needles, 34% yield; m.p. 65-66 °C; IR (KBr) 3363, 3265, 3062, 1721, 1650, 1510, 1411, 1229, 1173 cm⁻¹; ¹H NMR (CDCl₃) δ 1.41 (s, 6H, C(CH₃)₂), 3.03 (t, 2H, J 7.2 Hz, CH₂C₆H₄), 4.14 (t, 2H, J 6.9 Hz, CH₂O), 6.68 (d, 2H, J 8.7 Hz, CH₆), 6.88 (d, 2H, J 8.7 Hz, CH₆), 7.11 (d, 2H, J 8.1 Hz, CH₆), 7.44-7.58 (m, 7H, CH₆), 7.75-7.71 (m, 1H, CH₆), 7.78 (bs, 1H, NH), 7.83-7.91 (m, 2H, CH₆), 8.05 (d, 2H, J 6.9 Hz, CH₆), 9.16 (bs, 1H, NHCO); ¹³C NMR (CDCl₃) δ 24.2, 34.9, 68.8, 81.3, 114.7, 121.0, 122.1, 126.4, 126.9, 128.4, 128.7, 128.9, 129.1, 130.0, 131.0, 131.7, 134.1, 138.0, 151.7, 155.7, 167.9, 172.7. Anal. Calcd. for C₃₁H₃₀N₂O₆S: C, 66.65; H, 5.41; N, 5.01; Found: C, 66.63; H, 5.40; N, 5.03.

2-\{(2-4\{(Anilinocarbonyl)amino\}phenoxy\}ethyl\}phenoxy-2-methyl-N-(phenylsulfonyl) propanamide (13e)
White solid, 64% yield; m.p. 75-76 °C; IR (KBr) 3351, 2360, 2340, 1719, 1656, 1598, 1550, 1509, 1225 cm⁻¹; ¹H NMR (CDCl₃) δ 1.39 (s, 6H, C(CH₃)₂), 2.97 (t, 2H, J 7.2 Hz, CH₂C₆H₄), 4.02 (t, 2H, J 7.2 Hz, CH₂O), 6.67 (d, 2H, J 8.4 Hz, CH₆), 6.75 (d, 2H, J 9 Hz, CH₆), 7.85-7.27 (m, 9H, CH₆ and NH), 7.46-7.68 (m, 4H, CH₆), 7.90-7.93 (m, 1H, CH₆), 7.99 (s, 1H, NH), 7.02-8.05 (m, 1H, CH₆), 9.33 (s, 1H, NH); ¹³C NMR (CDCl₃) δ 24.2, 34.9, 68.7, 81.3, 115.0, 120.4, 123.5, 123.6, 126.3, 128.4, 129.0, 129.9, 130.8, 132.7, 133.9, 134.1, 138.0, 138.2, 151.7, 154.4, 155.7, 172.9. Anal. Calcd. for C₃₁H₃₁N₃O₆S: C, 64.90; H, 5.45; N, 7.32; Found: C, 64.81; H, 5.46; N, 7.34.
General procedure for the synthesis of amides 4a-d, 10a-d, 13a-d

To a stirred solution of acids LC2, 9, 12 (1.0 eq) in DMF (2 mL) at 0 °C N,N'-dicyclohexylcarbodiimide (DCC, 1.0 eq) and 1-hydroxybenzotriazole hydrate (HOBt, 1.0 eq) were added. After 15 min, N-methylmorpholine (NMM, 1.0 eq) and the selected amine (1.0 eq) were added in sequence to the reaction mixture. The solution was allowed to rise the room temperature, stirred overnight, and concentrated at rotary evaporator. The resulting residue was dissolved in DCM (15 mL), washed with NaHCO₃ (3 x 15 mL) and brine (15 mL). The combined organic layers were dried over sodium sulfate and concentrated under vacuum to provide the crude products, purified by column chromatography or crystallization.

2-Methyl-2-[4-(2-{[E]-phenyldiazenyl}phenoxy)ethyl]phenoxy|propanamide (4a)

Orange crystals (silica gel, DCM), 67% yield; m.p. 136-137 °C; IR (KBr) 3470, 3167, 1690, 1600, 1504, 1244, 1227, 1149 cm⁻¹; ¹H NMR (CDCl₃) δ 1.52 (s, 6H, C(CH₃)₂), 3.08 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 4.22 (t, 2H, J 6.9 Hz, CH₂O), 5.65 (s, 1H, NH), 6.67 (s, 1H, NH), 6.90 (d, 2H, J 12 Hz, CH₃), 6.99 (d, 2H, J 9.6 Hz, CH₃), 7.21 (d, 2H, J 9 Hz, CH₃), 7.42-7.52 (m, 3H, CH₃), 7.86-7.92 (m, 4H, CH₃); ¹³C NMR (CDCl₃) δ 24.9, 34.8, 68.9, 81.2, 114.7, 121.3, 122.5, 124.7, 129.0, 129.8, 130.4, 132.9, 146.9, 152.6, 153.1, 161.2, 177.7. Anal. Calcd. for C₂₄H₂₈N₃O₃: C, 71.44; H, 6.25; N, 10.41; Found: C, 71.58; H, 6.26; N, 10.38.

N-butyl-2-methyl-2-[4-(2-{[E]-phenyldiazenyl}phenoxy)ethyl]phenoxy|propenamide (4b)

Orange solid (silica gel, DCM/MeOH 98:2), 90% yield; m.p. 91-92 °C; IR (KBr) 3374, 2927, 2867, 1657, 1600, 1505, 1261, 1234, 1150 cm⁻¹; ¹H NMR (CDCl₃) δ 0.90 (t, 3H, J 6.9 Hz, CH₂CH₂CH₂CH₂CH₃), 1.25-1.38 (m, 2H, CH₂CH₂CH₂CH₂CH₃), 1.43-1.56 (m, 8H, C(CH₃)₂, CH₂CH₂CH₂CH₂CH₃), 3.08 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 3.29 (m, 2H, CH₂CH₂CH₂CH₂CH₃), 4.22 (t, 2H, J 6.9 Hz, CH₂O), 6.72 (bs, 1H, NH), 6.87 (d, 2H, J 8.4 Hz, CH₃), 6.99 (d, 2H, J 9.6 Hz, CH₃), 7.20 (d, 2H, J 8.4 Hz, CH₃), 7.40-7.52 (m, 3H, CH₃), 7.85-7.92 (m, 4H, CH₃); ¹³C NMR (CDCl₃) δ 13.7, 20.0, 25.1, 31.5, 34.8, 39.0, 68.9, 81.4, 114.7, 121.3, 122.5, 124.8, 129.0, 129.7, 130.4, 132.8, 146.9, 152.6, 152.9, 161.2, 174.7. Anal. Calcd. for C₂₈H₃₃N₃O₃: C, 73.18; H, 7.24; N, 9.14; Found: C, 73.31; H, 7.22; N, 9.15.

2-Methyl-N-phenyl-2-[4-(2-{[E]-phenyldiazenyl}phenoxy)ethyl]phenoxy|propanamide (4c)

Dark orange solid (silica gel, DCM), 80% yield; m.p. 118-119 °C; IR (KBr) 3370, 2924, 2868, 1681, 1600, 1503, 1258, 1235, 1143 cm⁻¹; ¹H NMR (CDCl₃) δ 1.58 (s, 6H, C(CH₃)₂), 3.09 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 4.23 (t, 2H, J 6.9 Hz, CH₂O), 6.95 (d, 2H, J 9 Hz, CH₃), 6.99 (d, 2H, J 9 Hz,
N-benzyl-2-methyl-2-[4-(2-[4-(E)-phenyl diazenyl][phenoxy]ethyl)phenoxy]propanamide (4d)

Dark orange oil (silica gel, DCM), 70% yield; IR (neat) 3421, 2932, 2871, 1671, 1600, 1503, 1248, 1228, 1149 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.53 (s, 6H, C(CH\(_3\))\(_2\)), 3.08 (t, 2H, J 6.9 Hz, CH\(_2\)CAr), 4.21 (t, 2H, J 6.9 Hz, CH\(_2\)O), 4.51 (2H, J 6 Hz, CH\(_2\)CH\(_2\)Ar), 6.86 (d, 2H, J 8.1 Hz, CH\(_2\)Ar), 7.00 (d, 2H, J 9 Hz, CH\(_2\)Ar), 7.11 (t, 1H, J 4.9 Hz, NH), 7.18 (d, 2H, CH\(_2\)Ar), 7.25-7.35 (m, 5H, CH\(_2\)Ar), 7.41-7.53 (m, 3H, CH\(_2\)Ar), 7.87-7.93 (m, 4H, CH\(_2\)Ar); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 24.1, 34.9, 43.4, 68.9, 81.5, 114.7, 121.5, 122.5, 124.8, 127.4, 127.7, 128.7, 129.0, 129.8, 130.4, 132.9, 138.1, 146.9, 152.6, 152.7, 161.2, 174.7. Anal. Calcd. for C\(_{36}\)H\(_{29}\)N\(_3\)O\(_3\): C, 75.13; H, 6.10; N, 8.76; Found: C, 75.02; H, 6.11; N, 8.78.

N-(4-[2-[4-(2-amino,1,1-dimethyl-2-oxoethoxy)phenyl]ethoxy]phenyl)benzamide (10a)

White crystals (silica gel, DCM/MeOH 98:2), 50% yield; m.p. 169-170 °C; IR (KBr) 3439, 3355, 1662, 1648, 1512, 1232 cm\(^{-1}\); \(^1\)H NMR (CD\(_3\)OD) \(\delta\) 1.47 (s, 6H, (C(CH\(_3\))\(_2\)), 3.01 (t, 2H, J 7.2 Hz, CH\(_2\)CAr), 4.15 (t, 2H, J 7.2 Hz, CH\(_2\)O), 6.88-6.93 (m, 2H, CH\(_2\)Ar), 7.22 (d, 2H, J 8.1 Hz, CH\(_2\)Ar), 7.55-7.46 (m, 5H, CH\(_2\)Ar and NH), 7.88-7.91 (m, 2H, CH\(_2\)Ar); \(^{13}\)C NMR (CD\(_3\)OD) \(\delta\) 24.0, 34.5, 68.7, 80.2, 110.0, 114.2, 120.7, 122.6, 127.1, 128.1, 128.2, 129.3, 131.3, 133.1, 134.8, 153.2, 156.0, 167.0, 170.0. Anal. Calcd. for C\(_{25}\)H\(_{28}\)N\(_2\)O\(_2\): C, 71.75; H, 6.26; N, 6.69; Found: C, 71.90; H, 6.25; N, 6.67.

N-[4-(2-[4-(2-(butylamino),1,1-dimethyl-2-oxoethoxy)phenyl]ethoxy]phenyl]benzamide (10b)

White amorphous solid (silica gel, DCM/MeOH 98:2), 74% yield; m.p. 152-153 °C; IR (KBr) 3348, 2924, 1650, 1519, 1232 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 0.89 (t, 3H, J 6.9, CH\(_2\)CH\(_2\)), 1.32 (sext, 2H, J 6.9 Hz, CH\(_3\)CH\(_2\)CH\(_2\)), 1.47 (s, 6H, (C(CH\(_3\))\(_2\)), 1.43-1.53 (m, 2H, CH\(_2\)CH\(_2\)NH), 3.03 (t, 2H, J 6.9 Hz, CH\(_2\)CAr), 3.27 (q, 2H, J 6 Hz, CH\(_2\)CH\(_2\)NH), 4.12 (t, 2H, J 6.9 Hz, CH\(_2\)O), 6.74 (bs, 1H, NH), 6.83-6.88 (m, 2H, CH\(_2\)Ar), 7.17 (d, 2H, J 7.2 Hz, CH\(_2\)Ar), 7.41-7.53 (m, 5H, CH\(_2\)Ar), 7.85 (d, 2H, J 8.1 Hz, CH\(_2\)Ar), 7.95 (bs, 1H, NH); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 13.7, 20.0, 25.1, 31.5, 34.9, 39.0, 68.9, 81.4, 114.8, 121.3, 122.1, 127.0, 128.6, 129.7, 131.1, 131.6, 133.1, 134.9, 152.8, 155.7, 174.7, 176.2. Anal. Calcd. for C\(_{39}\)H\(_{34}\)N\(_2\)O\(_4\): C, 73.39; H, 7.22; N, 5.90; Found: C, 73.48; H, 7.24; N, 5.88.
**N-(4-{2-[4-(2-anilino-1,1-dimethyl-2-oxoethoxy)phenyl][ethoxy]phenyl}benzamide (10c)**

White crystals (from MeOH), 36% yield; m.p. 188-189 °C; IR (KBr) 3354, 2918, 1674, 1646, 1516, 1233 cm⁻¹; ¹H NMR (CDCl₃) δ 1.56 (s, 6H, (C(CH₃)₂), 3.05 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 4.14 (t, 2H, J 6.9 Hz, CH₂O), 6.87-6.95 (m, 4H, CH₂Ar), 7.10-7.15 (m, 1H, CHAr), 7.20 (d, 2H, J 9 Hz, CH₂Ar), 7.34 (t, 2H, J 8.4 Hz, CHAr), 7.44-7.60 (m, 5H, CHAr), 7.74 (bs, 1H, NH), 7.84 (d, 2H, J 8.1 Hz, CH₂Ar), 8.59 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 24.9, 34.8, 39.0, 68.8, 81.4, 114.9, 119.7, 121.9, 122.0, 124.4, 126.9, 128.7, 129.0, 129.9, 131.0, 132.3, 133.8, 137.4, 145.7, 152.4, 164.1, 169.5, 173.0.

Anal. Calcd. for C₃₁H₃₀N₂O₄: C, 75.28; H, 6.11; N, 5.66; Found: C, 75.09; H, 6.12; N, 5.67.

**N-[4-{2-[4-{2-(benzylamino)-1,1-dimethyl-2-oxoethoxy]phenyl}[ethoxy]phenyl] benzamide (10d)**

Light yellow needles (from MeOH), 73% yield; m.p. 150-151 °C; IR (KBr) 3346, 2934, 1655, 1647, 1522, 1512, 1230 cm⁻¹; ¹H NMR (CDCl₃) δ 1.52 (s, 6H, CH₂C₆H₄), 3.03 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 4.12 (t, 2H, J 6.9 Hz, CH₂O), 4.48 (d, 2H, J 5.7 Hz, NHCH₂C₆H₄), 6.82-6.88 (m, 4H, CH₂Ar), 7.09 (bs, 1H, NH), 7.15 (d, 2H, J 8.1 Hz, CH₂Ar), 7.26-7.33 (m, 5H, CH₂Ar), 7.42-7.54 (m, 5H, CH₂Ar), 7.85 (d, 2H, J 6.9 Hz, CH₂Ar), 7.92 (bs, 1H, NH); ¹³C NMR (CDCl₃) δ 25.1, 34.9, 43.3, 68.9, 81.5, 114.8, 121.5, 122.0, 127.0, 127.4, 127.6, 128.6, 129.7, 131.1, 131.7, 131.2, 134.9, 138.1, 152.6, 154.1, 155.7, 165.5, 174.8. Anal. Calcd. for C₃₂H₃₂N₂O₄: C, 75.57; H, 6.34; N, 5.51; Found: C, 75.43; H, 6.35; N, 5.52.

**2-[4-{2-[4-{[(Anilinocarbonyl)amino]phenoxy}ethyl]phenoxy]-2-methylpropanamide (13a)**

White crystals (silica gel, DCM/MeOH 98:2), 42% yield; m.p. 151-152 °C; IR (KBr) 3452, 3337, 3198, 1661, 1600, 1555, 1509, 1232 cm⁻¹; ¹H NMR (CDCl₃) δ 1.52 (s, 6H, (C(CH₃)₂), 3.02 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 4.10 (t, 2H, J 6.9 Hz, CH₂O), 5.55 (bs, 1H, NH₂), 6.68 (bs, 1H, NH₂), 6.82-6.91 (m, 4H, CH₂Ar), 7.04-7.08 (m, 1H, CH₂Ar), 7.16-7.21 (m, 4H, CH₂Ar), 7.25-7.32 (m, 6H, CH₂Ar and NH₂); ¹³C NMR (CDCl₃) δ 24.9, 34.9, 68.9, 81.2, 115.2, 120.7, 121.2, 123.9, 124.9, 129.2, 129.8, 130.2, 133.1, 138.0, 152.7, 154.0, 156.3, 177.7. Anal. Calcd. for C₂₃H₂₇N₃O₄: C, 69.27; H, 6.28; N, 9.69; Found: C, 69.40; H, 6.26; N, 9.70.

**2-[4-{2-[4-{[(Anilinocarbonyl)amino]phenoxy}ethyl]phenoxy]-N-butyl-2-methylpropanamide (13b)**

Pearl white crystals (from MeOH), 98% yield; m.p. 151-152 °C; IR (KBr) 3295, 2956, 2936, 1639, 1597, 1563, 1509, 1231 cm⁻¹; ¹H NMR (CDCl₃) δ 0.89 (t, 3H, J 7.2 Hz, CH₃CH₂), 1.31 (sext, 2H, J 7.2 Hz, CH₃CH₂CH₂), 1.49 (s, 6H, (C(CH₃)₂), 1.42-1.55 (m, 2H, CH₂CH₂NH), 2.99 (t, 2H, J 7.2
The reaction mixture was allowed to warm up to room temperature and stirred overnight.

To a solution of 4-aminophenol (recrystallized from water, 18.3 mmol) in DMF (20 mL) at 0 °C under nitrogen atmosphere, triethylamine (27.5 mmol) was added. The reaction mixture was stirred for 10 min before the slowly addition of benzoyl chloride (22 mmol) over a period of 3 min. The mixture was allowed to warm up to room temperature and stirred overnight. DMF was removed.
under reduced pressure and the crude product treated with water (50 mL) and extracted with EtOAc (3 x 50 mL). The organic layers were dried over sodium sulfate, filtered and concentrated, obtaining a light yellow solid. The crude product was crystallized from EtOAc, affording the pure product as white crystals.

White crystals, 70% yield; m.p. 226-227 °C; IR (KBr) 3316, 2920, 1638, 1513, 1412, 1247, 1100 cm⁻¹; ¹H NMR (DMSO-d6) δ 6.72 (d, 2H, J 9.3 Hz, CHAr), 7.45-7.54 (m, 5H, CHAr), 7.89-7.92 (m, 2H, CHAr), 9.25 (s, 1H, NH), 10.01 (s, 1H, OH); ¹³C NMR (DMSO-d6) δ 115.4, 122.6, 127.9, 128.7, 131.1, 131.7, 135.6, 154.1, 165.3.

Procedure for the synthesis of 1-(4-hydroxyphenyl)-3-phenylurea (6)
To a stirring solution of phenylisocyanate (freshly distilled, 4.2 mmol) in dry ACN (20 mL), 4-aminophenol (4.2 mmol) was added under nitrogen atmosphere. The reaction mixture was warmed up to reflux for 5 h, cooled and evaporated to dryness. The crude material was purified by crystallization (acetone/ACN), affording the desired product as a white solid.

White solid, 65% yield; m.p. 222-223 °C; IR (KBr) 3306, 1636, 1569, 1509, 1462, 1223 cm⁻¹; ¹H NMR (DMSO-d6) δ 6.66 (d, 2H, J 9.0 Hz, CHAr), 6.88-6.93 (m, 1H, CHAr), 7.17-7.26 (m, 4H, CHAr), 7.39-7.42 (m, 2H, CHAr), 8.30 (s, 1H, NH), 8.51 (s, 1H, NH), 9.05 (s, 1H, OH); ¹³C NMR (DMSO-d6) δ 115.6, 118.4, 120.8, 121.9, 129.1, 131.5, 140.4, 152.9, 153.2.

Ethyl 2-[4-(2-hydroxyethyl)phenoxy]-2-methylpropanoate (7)
A suspension of 4-(2-hydroxyethyl)phenol (15.9 mmol) and K₂CO₃ (159 mmol) in 45 mL of DMF was stirred at room temperature for 30 min, then ethyl 2-bromo-2-methylpropanoate (49.4 mmol) was added. The reaction mixture was refluxed for 4 hours and the solvent was evaporated to give dark yellow oil. The crude product was dissolved in water (30 mL) and extracted with DCM (4 x 30 mL); the organic layer was dried (Na₂SO₄), filtered, concentrated under vacuo and purified by column chromatography (silica gel; 3:2 ciclohexane/EtOAc).

Pale yellow oil, 75% yield; IR (KBr) 3416, 1733 cm⁻¹; ¹H NMR (CDCl₃) δ 1.24 (t, 3H, J 6.9 Hz, CH₃CH₂), 1.56 (s, 6H, CH₃C), 2.79 (t, 2H, J 6.9 Hz, CH₂C₂Ar), 3.79 (t, 2H, J 6.9 Hz, CH₂OH), 4.18-4.25 (m, 2H, CH₂O), 6.77 (d, 2H, J 8.7 Hz, CHAr), 7.07 (d, 2H, J 8.7 Hz, CHAr); ¹³C NMR (CDCl₃) δ 14.3, 25.5, 38.5, 61.6, 63.9, 79.2, 119.5, 129.9, 132.2, 154.2, 174.5.

General procedure for the synthesis of esters (8, 11)
A stirring solution of phenols (5,6) (1.0 eq), ethyl 2-[4-(2-hydroxyethyl)phenoxy]-2-methylpropanoate (7) (1.0 eq) and triphenylphosphine (1.5 eq) in dry THF (freshly distilled) at 0°C, was treated dropwise with a solution of diisopropyl azodicarboxylate (DIAD, 1.5 eq) in dry THF (freshly distilled). The reaction mixture was allowed to stir for 24 hours at room temperature, evaporated in vacuo and the crude products purified by column chromatography (silica gel, cyclohexane/EtOAc 9:1).

**Ethyl 2-(4-(2-(4-benzamidophenoxy)ethyl)phenoxy)-2-methylpropanoate (8)**

White crystals, 54% yield; m.p. 118-119 °C; IR (KBr) 3314, 2985, 2942, 1726, 1645, 1519, 1244 cm⁻¹; ¹H NMR (CDCl₃) δ 1.25-1.27 (m, 3H, C(H₃)₂), 1.58 (s, 6H, C(CH₃)₂), 3.02 (t, 2H, J 7.2 Hz, CH₂C₆H₄), 4.12 (t, 2H, J 7.2 Hz, CH₂O), 4.23 (q, 2H, J 7.2 Hz, CH₂O), 6.79 (d, 2H, J 9 Hz, CH₆H₄), 7.14 (d, 2H, J 8.1 Hz, CH₆H₄), 7.43-7.53 (m, 5H, CH₆H₄), 7.80-7.86 (m, 3H, CH₆H₄); ¹³C NMR (CDCl₃) δ 14.1, 25.3, 34.9, 61.4, 69.4, 79.0, 114.9, 119.2, 122.0, 126.9, 128.7, 129.6, 131.0, 131.7, 131.7, 135.0, 153.9, 155.8, 174.3.

**Ethyl 2-methyl-2-(4-(2-(4-phenylureido)phenoxy)ethyl)phenoxy)propanoate (11)**

White solid, 97% yield; m.p. 99-100 °C; IR (KBr) 3316, 2987, 2937, 1731, 1647, 1608, 1564, 1510, 1232 cm⁻¹; ¹H NMR (DMSO-d₆) δ 1.14 (t, 3H, J 6.9 Hz, C(H₃)₂), 2.92 (t, 2H, J 6.9 Hz, CH₂C₆H₄), 4.07 (t, 2H, J 6.9 Hz, CH₂O), 1.48 (s, 6H, C(CH₃)₂), 4.14 (q, 2H, J 6.9 Hz, CH₃CH₂O), 6.71 (d, 2H, J 8.7 Hz, CH₆H₄), 6.83 (d, 2H, J 9 Hz, CH₆H₄), 8.44 (s, 1H, NH), 8.55 (s, 1H, NH); ¹³C NMR (DMSO-d₆) δ 14.3, 25.4, 34.5, 61.4, 68.8, 78.9, 115.0, 118.4, 119.1, 120.3, 122.0, 129.1, 130.2, 132.3, 133.2, 140.3, 151.1, 153.9, 172.0, 173.7.

**General procedure for the synthesis of acids 9, 12**

A solution of esters (8, 11) (1.0 eq) in THF was treated with 2N NaOH (10 eq). The resulting mixture was stirred at reflux for 16-17 h, cooled at room temperature and concentrated under reduced pressure. The obtained oil was dissolved in water (100 mL), washed with Et₂O (3 x 100 mL) and acidified with 2N HCl at pH 2. The aqueous layer was extracted with DCM (3 x 100 mL). The organic solution was washed with brine (100 mL), dried over sodium sulfate and concentrated at reduced pressure.

**2-(4-(2-(4-Benzamidophenoxy)ethyl)phenoxy)-2-methylpropanoic acid (9)**


White crystals, 63% yield; m.p. 135-136 °C; IR (KBr) 3365, 2995, 2859, 1704, 1648, 1519, 1237 cm\(^{-1}\); \(^1\)H NMR (Acetone) \(\delta\) 1.56 (s, 6H, C(CH\(_3\))\(_2\)), 3.01 (t, 2H, J 7.2 Hz, CH\(_2\)Ar), 4.16 (t, 2H, J 7.2 Hz, CH\(_2\)O), 6.87 (d, 2H, J 9 Hz, CH\(_{Ar}\)), 7.25 (d, 2H, J 8.4 Hz, CH\(_{Ar}\)), 7.46-7.55 (m, 3H, CH\(_{Ar}\)), 7.73 (d, 2H, J 8.7 Hz, CH\(_{Ar}\)), 8.41 (s, 1H, NH); \(^{13}\)C NMR (Acetone) \(\delta\) 24.7, 34.6, 68.7, 78.5, 114.4, 119.1, 121.6, 127.3, 128.3, 129.6, 131.2, 132.5, 135.5, 154.2, 155.3, 165.0, 174.6.

2-Methyl-2-(4-(2-(4-(3-phenylureido)phenoxy)ethyl)phenoxy)propanoic acid (12)
White solid, 57% yield; m.p. 101-102 °C; IR (KBr) 3299, 2989, 2937, 1701, 1633, 1611, 1557, 1506, 1443, 1230 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.59 (s, 6H, C(CH\(_3\))\(_2\)), 2.96 (t, 2H, J 6.9 Hz, CH\(_2\)Ar), 4.01 (t, 2H, J 6.9 Hz, CH\(_2\)O), 6.73 (d, 2H, J 8.7 Hz, CH\(_{Ar}\)), 6.84 (d, 2H, J 8.7 Hz, CH\(_{Ar}\)), 6.07-7.12 (m, 4H, CH\(_{Ar}\)), 7.22-7.25 (m, 4H, CH\(_{Ar}\)); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 25.1, 34.9, 68.9, 79.3, 115.2, 119.9, 121.0, 124.0, 124.7, 129.1, 129.7, 129.9, 132.4, 137.7, 153.3, 155.2, 156.3, 177.7.

S2. Cell Culture and Transfections
Reference compounds, the medium, and other cell culture reagents were purchased from Sigma-Aldrich (Milan, Italy) and Invitrogen (Carlsbad, CA). Human hepatoblastoma cell line HepG2 was cultured at 37 °C and in a humidified atmosphere of 5% CO\(_2\) in growth medium composed of Minimum Essential Medium (MEM) containing 10% of heat-inactivated FBS, 1% penicillin G/streptomycin, 1% MEM non-essential amino acid, and 1% glutamine. For transactivation assays, 105 cells/well were seeded in a 24-well plate and cultured until confluence. Cells were transiently transfected with 30 ng of expression plasmids encoding the fusion protein GAL4-PPAR\(\alpha\)-LBD or GAL4-PPAR\(\gamma\)-LBD, 100 ng of reporter plasmid pGAL5TKpGL3, and 150 ng of control plasmid pCMV\(\beta\)gal per well by CAPHOS\(^{\circledR}\), a calcium-phosphate coprecipitation method, according to the manufacturer’s guidelines. Four hours after transfection, medium was replaced with fresh serum-free medium supplemented with test compounds and reference compounds Wy14,643 (100 μM), rosiglitazone (2 μM), or DMSO 0.1%. After 20-22 h treatment, luciferase activity and β-galactosidase activity in cell extracts were determined by a Multilabel Plate Reader (VICTOR3 V, PerkinElmer). Luciferase activity was normalized to β-galactosidase activity to correct the transfection efficiencies. All transfection experiments were performed in triplicate and repeated at least twice.
S3. Quantitative Real-time PCR

HepG2 cell line was cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% FBS at 37 °C, 5% CO₂. Briefly, cells were seeded in 6-well plates at a density of 5 × 10⁵ cells/well grown for 24 hr. Then, cells were treated for 48 h in a serum free medium with control, with test compounds 3a-e, 10e and 13e (0.5 µM), and with PPARα agonist GW7647 (2 µM) alone or in combination with each compound. After treatment cells were washed with PBS and lysed for RNA extraction.

Total RNA was isolated by TRIzol reagent (Invitrogen, Carlsbad, CA), following the manufacturer’s instructions and it was treated with Turbo DNA-Free kit (Life Technologies). cDNA was synthesized retrotranscribing 0.5 µg of total RNA in a total volume of 10 µL using the High Capacity DNA Archive Kit (Applied Biosystems, Foster City, CA) and following the manufacturer’s instructions.

Expression of carnitine palmitoyltransferase-1A (CPT1A) and cyclophilin (PPIB) transcripts was analyzed performed by real-time quantitative PCR (RTqPCR), using the PowerUp SYBR Green Master Mix (Life Technologies) on the ABI 7900HT Real-Time PCR platform (Applied Biosystem) as previously described [3]. Relative quantification was performed using the DDCT method. cDNA obtained from cells treated with vehicle (DMSO) was used as a calibrator.

S4. Cell Lines and MTT Assay

Paraganglioma (PTJ86i and PTJ64i), pancreatic (AsPC-1, BxPC-3, Capan-2) and colorectal (HT-29, SW480) cancer cell lines were cultured as previously described [4]. Renal tumor cell line (A498) was cultured in DMEM supplemented with 10% FBS (Sigma-Aldrich, St. Louis, MO, USA). All compounds were dissolved in DMSO and then in culture media for the final working concentrations. The effect of treatments on cell viability was tested by MTT assay (Sigma-Aldrich, St. Louis, MO, USA), both for initial screening of all compounds and for dose-response curves of the most active molecules. MTT assay was carried out as described by Ammazzalorso et al. [4].

S5. Cytotoxic Concentration (CC₅₀), Selectivity Index (SI) and Statistical Analysis

CC₅₀ values were calculated by CompuSyn software [5]. Selectivity index values were calculated as the ratio between the CC₅₀ values of compounds on normal cells and the CC₅₀ values of compounds on cancer cell lines [6]. Comparisons of mean values were performed using an unpaired Student’s t-test. For multiple comparisons one-way ANOVA followed by Dunnett’s test were employed. A p-value <0.05 was estimated as statistically significant.
S5.1 Supplementary Table S1

Supplementary Table S1. CC$_{50}$ values were determined using CompuSyn software after 72 hour treatment of pancreatic (AsPC-1, BxPC-3, Capan-2), colorectal (HT-29, SW480) and renal (A498) tumor cell lines with 3c, 3d and 10e at concentrations ranging from 0 to 24 µM.

|        | AsPC-1 | BxPC-3 | Capan-2 | HT-29  | SW480 | A498  |
|--------|--------|--------|---------|--------|-------|-------|
| 3c     | >24    | >24    | >24     | >24    | >24   | >24   |
| 3d     | >24    | 16.99  | >24     | >24    | >24   | >24   |
| 10e    | 7.15   | 7.01   | 7.76    | 7.26   | >24   | 4.60  |

S6. Computational Chemistry

S6.1 Protein and Ligand Preparation

The crystal structure of PPAR$_{a}$ in complex with the antagonist GW6471 (PDB ID: 1KKQ) [7], recovered from Brookhaven Protein Database, was employed for the automated docking experiments. The protein was processed through the Protein Preparation Wizard in Maestro version 11.0 (Schrödinger, LLC, New York, NY, 2019). X-ray water molecules were removed, the appropriate bond orders as well as charges and atom types were assigned and the hydrogen atoms were added. The H-bond network was optimized by exhaustive sampling of rotamers, tautomers and protonation states of titratable amino acids at neutral pH. Imidazole ring of H440 was set as N$_{e}$ 2-H (N$_{tau}$-H) tautomeric state. Finally, the protein structure was relaxed with a restrained minimization using the Impref module with the OPLS3 force field by imposing a 0.3 Å rmsd limit from the initial coordinates as the constraint. The core structures of 3a and 10e were sketched using the Molecular Builder module in Maestro. The ligands were then preprocessed with LigPrep 3.3 (Schrödinger, LLC, New York, NY, 2019) and optimized by means of Macromodel 11.5 (Schrödinger, LLC, New York, NY, 2019), employing the MMFFs force field with 1000 steps of steepest descent; the resulting molecules were then submitted to 500 steps of truncated Newton conjugate gradient method. Partial atomic charges were assigned using the OPLS-AA force field.
S6.2 Docking

Docking studies were performed using the genetic algorithm (GA) implemented in GOLD software (CCDC Software Limited: Cambridge, U.K.). Each asymmetric unit of the crystal structure contained four PPARα LBDs (chains A, B, C, D), four SMRT corepressor peptides (chains E, F, G, H) and four GW6471 molecules. Re-docking experiments were run in order to select the most suitable protein structure among the four PPARα LBDs and to validate the accuracy of GOLD at reproducing the experimental binding mode of GW6471. Therefore, the co-crystal ligand GW6471 was removed and docked back into the binding site. The active-site radius was set equal to 10 Å. For each molecule tested the number of islands was set to 5, population size to 100, number of operations was 100,000 with a selective pressure of 1.1. The number of GA runs was set to 200 and the early termination option was switched off. The obtained docked poses were ranked according to the original ChemPLP scoring function and rescored with ChemScore [8].

The overall results indicated that the binding conformation of GW6471 determined by GOLD matched well with that of the co-crystallized conformation in PPARα, with a root mean square deviation (rmsd) between the top ranked predicted conformation and the crystallographic conformation of 1.53 Å for chain C. These results are also in accordance with previous findings [3]. Therefore, chain C was selected for docking of compounds 3a and 10e, using the same protocol. Figures in the manuscript were rendered with Pymol 2.0 (Schrödinger, LLC, New York, NY, 2017). All computations were performed on a E4 Computer Engineering E1080 workstation provided of a Intel Core i7-930 Quad-Core processor.
6.3.  **Figure S2. 2D ligand-interaction diagram of compound 3a into the PPARα binding pocket.** Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino acids are represented with green circles. H-bonds are depicted with purple arrows–dashed arrows for H-bonds involving amino acid side chain and regular arrows for H-bonds involving amino acid backbone. Straight green lines represent π-stacking interactions.
S6.4. Figure S3. 2D ligand-interaction diagram of compound 10e into the PPARα binding pocket. Positively charged amino acids are represented with dark blue circles, negatively charged amino acids are represented with red circles, polar amino acids are represented with light blue circles and hydrophobic amino acids are represented with green circles. H-bonds are depicted with purple arrows—dashed arrows for H-bonds involving amino acid side chain and regular arrows for H-bonds involving amino acid backbone. Straight green lines represent π-stacking interactions.
S6.5. **Figure S4.** (A) Overlays of 3a (yellow sticks) and 10e (violet sticks) docked poses with the X-ray crystal pose of the antagonist GW6471 (cyan sticks) into PPARα LBD represented as green ribbon model (PDB ID: 1KKQ). (B) Overlay of 3a and 10e with the agonist bound conformation of PPARα LBD represented as slate ribbon model (PDB ID: 1K7L). The key residue Y464 is displayed (white sticks) and labeled. The bound agonist GW409544 was removed for clarity.
S6.6. **Figure S5.** Overlays of the docked poses of (A) 3g (aquamarine sticks), (B) 4c (salmon sticks) with 3a (yellow sticks) and (C) 10b (wheat sticks), (D) 13e (grey sticks) with 10e (violet sticks) bound to PPARα LBD represented as green ribbon model (PDB ID: 1KKQ). For clarity, only key amino acids surrounding the bound ligand are displayed (white sticks) and labeled.
S7. References

[1] De Filippis, B.; Giancristofaro, A.; Ammazzalorso, A.; D’Angelo, A.; Fantacuzzi, M.; Giampietro, L.; Maccallini, C.; Petruzelli, M.; Amoroso, R. Discovery of gemfibrozil analogues that activate PPARα and enhance the expression of gene CPT1A involved in fatty acids catabolism. Eur. J. Med. Chem. 2011, 46, 5218-5224.

[2] Giampietro, L.; D’Angelo, A.; Giancristofaro, A.; Ammazzalorso, A.; De Filippis, B.; Fantacuzzi, M.; Linciano, P.; Maccallini, C.; Amoroso, R. Synthesis and structure-activity relationships of fibrate-based analogues inside PPARs. Bioorg. Med. Chem. Lett. 2012, 22, 7662-7666.

[3] Ammazzalorso, A.; Carrieri, A.; Verginelli, F.; Bruno, I.; Carbonara, G.; D’Angelo, A.; De Filippis, B.; Fantacuzzi, M.; Florio, R.; Fracchiolla, G.; Giampietro, L.; Giancristofaro, A.; Maccallini, C.; Cama, A.; Amoroso, R. Synthesis, in vitro evaluation, and molecular modeling investigation of benzenesulfonimide Peroxisome Proliferator-Activated Receptors α antagonists. Eur. J. Med. Chem. 2016, 114, 191-200.

[4] Ammazzalorso, A.; De Lellis, L.; Florio, R.; Laghezza, A.; De Filippis, B.; Fantacuzzi, M.; Giampietro, L.; Maccallini, C.; Tortorella, P.; Veschi, S.; Loiodice, F.; Cama, A.; Amoroso, R. Synthesis of novel benzothiazole amides: evaluation of PPAR activity and anti-proliferative effects in paraganglioma, pancreatic and colorectal cancer cell lines. Bioorg Med Chem Lett 2019, 29, 2302-2306.

[5] Chou, T.C. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Research 2010, 70, 440-446.

[6] Villanueva, P. J.; Martinez, A.; Baca, S. T.; DeJesus, R. E.; Larragoity, M.; Contreras, L.; Gutierrez, D. A.; Varela-Ramirez, A.; Aguilera, R. J. Pyronaridine exerts potent cytotoxicity on human breast and hematological cancer cells through induction of apoptosis. PLoS One 2018, 13, e0206467.

[7] Xu, H. E.; Stanley, T. B.; Montana, V. G.; Lambert, M. H.; Shearer, B. G.; Cobb, J. E.; McKee, D. D.; Galardi, C. M.; Plunket, K.; Nolte, R. T.; Parks, D. J.; Moore, J. T.; Kliewer, S. A.; Willson, T. M.; Stimmel, J. B. Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARα. Nature 2002, 415, 813-817.

[8] Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved protein-ligand docking using GOLD. Proteins: Struct. Funct. Genet. 2003, 52, 609-623.

S8. 1H and 13C Spectra of Final Compounds 1a-b, 2a-b, 3a-g, 4a-d, 10a-e, 13a-e
CDCl3

ppm

1.98
6.84
9.99
3.66
6.64
6.97
5.97
3.73

Total time 6 min 45 sec
16 repeats

Sample: 40.33 \text{ mg}
Solvent: CDCl3
Temp: 25.0 \text{ C} / 298.1 \text{ K}

D雅nal colectec od: MR 7 与04
Solution: CDCl3

Spectral data:

Sample weight:
Acetone/CDCl3

Measurements:
1H NMR
300-MHz-NMR

Chemical shift:

Chiral analysis

1H NMR
TOTAL TIME 0 min 45 sec

NAME: PROTON

DATE COLLECTED: Apr 9 2015

SAMPLE: 40B

EXPERIMENT: PROTON

REFERENCE: 6.28 ppm

NMR: 400 MHz

Sample concentration: 500 mg/ml

Matrix: DMSO-d6

Detected by: Bruker

Institute:

Sample Name:

new experiment

\[
\text{Chemical Structure Image}
\]
new experiment

Sample Name: IB75B
Data Collected on: m300-mercury300
Archive directory: /export/home/chempack/vnmrsys/data
Sample directory:

FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Dec 9 2014

Operator: amoroso

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.706 sec
Width 4801.1 Hz
16 repetitions
OBSERVE M1, 300.1990802 MHz
DATA PROCESSING
FT size 16384
Total time 0 min 45 sec
