Supplementary electronic information

PPARα agonists based on the stilbene and its bioisosters: biological evaluation and docking studies

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CONTENTS

Chemistry

General. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Infrared spectra were recorded on a FT-IR 1600 Perkin–Elmer spectrometer. NMR spectra were run at 300 MHz on a Varian instrument; chemical shifts (δ) are reported in ppm. Microanalyses were carried out with an Eurovector Euro EA 3000 model analyser and the analytical results were within 0.4% of the theoretical values. Commercial reagents were used as received from Aldrich or Fluka.

General procedure for the preparation of phenols 3a-l and 3n-p. A stirred mixture of piperidine (1.01 mL, 10.23 mmol), 4-hydroxybenzaldeyde (500 mg, 4.09 mmol), and properly phenylacetic acid **2a-l** or **2n-p** (4.91 mmol) in CH_2Cl_2 (4 mL) was heated gradually to 130 °C and the CH_2Cl_2 allowed to boil off. After 4-24 h, the residue was cooled at room temperature and partitioned between EtOAc (3 x 30 mL) and H_2O (20 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to yield the crude product that was purified by column chromatography (eluent CH_2Cl_2/MeOH 95:5 or cyclohexane/ethylacetate 6:4) giving the pure phenol **3a-l** and **3n-p**.

4-[(*E*)-2-(4-chlorophenyl)ethenyl]phenol (3a): white solid, 160 mg, 56% yield, mp 184-185 °C. ^{1}H NMR (CD_3OD) δ 6.83 (d, J = 9.0 Hz, 2H, CH_{Ar}), 6.95 (q, J = 20.7 Hz, 2H, C*H*=C*H*), 7.28 (d, J = 9.0 Hz, 2H, CH_{Ar}), 7.38 (d, J = 9.0 Hz, 2H, CH_{Ar}), 7.40 (d, J = 9.0 Hz, 2H, CH_{Ar}); ^{13}C NMR (CD_3OD) δ 115.33 (C_{Ar}), 127.36 (C_{Ar}), 120.37 (*C*H=CH), 124.11 (CH=*C*H), 136.93 (C_{Ar}CH), 132.22 (C_{Ar}Cl), 157.54 (C_{Ar}OH); IR (neat) 3267, 2728, 1606, 1513, 1245 cm^{-1}. Anal. Calc. (for $C_{14}H_{11}ClO$): C, 72.89; H, 4.81; Found: C, 72.66; H, 4.80.

4-[(*E*)-2-(4-methylphenyl)ethenyl]phenol (3b): white amorphous solid, 61 mg, 58% yield. ^{1}H NMR (Acetone-*d6*) δ 2.3 (3H, s, CH_3), 6.91 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.05 (q, J = 10.8 Hz, 2H, C*H*=C*H*), 7.15 (d, J = 7.8 Hz, 2H, CH_{Ar}CCH_{Ar}), 7.35 (d, J = 8.4 Hz, 2H, CH_{Ar}CCH_{Ar}), 7.42 (d, J = 7.2 Hz, 2H, CH_{Ar}CCH_{Ar}); ^{13}C NMR (Acetone-*d6*) δ 25.99 (*C*H_3), 116.09 (*C*H_{Ar}C), 126.22 (*C*H_{Ar}), 126.88 (*C*H=CH), 126.95 (*C*H_{Ar}), 127.72 (CH=*C*H), 128.77 (*C*H_{Ar}), 129.45 (*C*H_{Ar}), 135.46 (*C*H_{Ar}), 136.75(*C*H_{Ar}), 158.38 (*C*H_{Ar}); IR (neat) 3436, 2932, 2853, 1603, 1497, 1263 cm^{-1}. Anal. Calc. (for $C_{15}H_{14}O$): C, 85.68; H, 6.71; Found: C, 85.38; H, 6.72.

4-[(*E*)-2-(4-isopropylphenyl)vinyl]phenol (3c): white solid, 46 mg, 49% yield, mp 159-161 °C. ^{1}H NMR (MeOD) δ 1.23 (d, J = 6.6 Hz, 6H, CH_3), 2.84-2.92 (m, 1H, C*H*), 6.76 (d, J = 8.4 Hz, 2H, CH_{Ar}COH), 6.96 (q, J = 14.4 Hz, 2H, C*H*=C*H*), 7.16 (d, J = 7.5 Hz, 2H, CH_{Ar}CCH), 7.37 (d, J = 2.1 Hz, 4H, CH_{Ar}CCH); ^{13}C NMR (MeOD) δ 23.21(*C*H_3),

33.95 (*C*H), 115.29 (*C*H$_{Ar}$COH), 125.52 (*C*H=CH), 126.00 (*C*H$_{Ar}$CCH), 126.42 (*C*H$_{Ar}$), 127.47(CH=*C*H), 127.52 (*C*H$_{Ar}$), 129.44 (*C*$_{Ar}$), 135.73 (*C*$_{Ar}$), 147.82 (*C*$_{Ar}$), 157.08 (*C*$_{Ar}$OH); IR (neat) 3437, 2935, 2853, 1603, 1497, 1263cm^{-1}. Anal. Calc. (for $C_{17}H_{18}O$): C, 85.67 ; H, 6.61; Found: C, 85.99; H, 6.59.

4-{(*E*)-2-[4-(trifluoromethyl)phenyl]ethenyl}phenol (3d): white solid, 316 mg, 23% yield, mp 161-163 °C. ^{1}H NMR (Acetone-*d6*) δ 6.87 (d, J = 8.4 Hz, 2H, C*H*$_{Ar}$), 7.13 (d, J = 16.2 Hz, 1H, C*H*=CH), 7.34 (d, J = 16.5 Hz, 1H, CH=C*H*), 7.50 (d, J = 8.1 Hz, 2H, C*H*$_{Ar}$), 7.66 (d, J = 8.4 Hz, 2H, C*H*$_{Ar}$), 7.75 (d, J = 7.8 Hz, 2H, C*H*$_{Ar}$); ^{13}C NMR (Acetone-*d6*) δ 115.85 (*C*$_{Ar}$), 124.08 (*C*H=CH), 125.65 (q, *C*$_{Ar}$), 126.69 (*C*$_{Ar}$), 128.00 (*C*$_{Ar}$), 128.60 (*C*$_{Ar}$), 128.70, 142.27, 158.13 (*C*$_{Ar}$); IR (neat) 3614, 3419, 3271, 1599, 1511, 1327, 1254, 1181, 836 cm^{-1}. Anal. Calc. (for $C_{15}H_{11}F_3O$): C, 68.18; H, 4.20; Found: C, 68.35; H, 4.22.

4-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzonitrile (3e): green solid, 182 mg, 44% yield, mp 218-220 °C. ^{1}H NMR (Acetone-*d6*) δ 6.87 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 7.1 (q, J = 49.5 Hz, 2H, C*H*=C*H*), 7.38 (d, J = 9.0 Hz, 2H, C*H*$_{Ar}$), 7.51 (q, J = 12.9 Hz, 4H, C*H*$_{Ar}$); ^{13}C NMR (Acetone-*d6*) δ 114.0 (*C*$_{Ar}$CN), 115.02 (*C*H$_{Ar}$), 119.41 (*C*N), 126.76 (*C*H$_{Ar}$), 128.48 (*C*H$_{Ar}$), 129.15 (C*H*=CH), 129.69 (C*H*=CH), 130.37 (*C*H$_{Ar}$), 132.69 (*C*H$_{Ar}$), 142.47 (*C*H$_{Ar}$), 159.74 (*C*H$_{Ar}$), 179.0 (CO); IR (neat) 3313, 2234, 1595, 1209 cm^{-1}; Anal. Calc. (for $C_{15}H_{11}NO$): C, 81.43; H, 5.01; N, 6.33; Found: C, 81.15; H, 5.00; N, 6.34.

4-[(*E*)-2-(4-methoxyphenyl)ethenyl]phenol (3f): yellow solid, 112 mg, 47% yield, mp 206-207 °C. ^{1}H NMR (Acetone-*d6*) δ 3.79 (s, 3H, C*H*$_3$), 6.83 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 6.91 (d, J = 9.0 Hz, 2H, C*H*$_{Ar}$), 7.00 (s, 2H, C*H*=C*H*), 7.41 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 7.48 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 8.44 (s, 1H, O*H*); ^{13}C NMR (Acetone-*d6*) δ 54.88 (*C*H$_3$), 114.23 (*C*$_{Ar}$), 115.72 (*C*$_{Ar}$), 125.47 (*C*H=CH), 126.48 (CH=*C*H), 127.52 (*C*$_{Ar}$), 127.75 (*C*$_{Ar}$), 129.7 (*C*$_{Ar}$), 130.8 (*C*$_{Ar}$), 157.2 (*C*$_{Ar}$), 159.3 (*C*$_{Ar}$OCH$_3$); IR (neat): 3423, 3019, 2955, 2838, 2360, 1607, 1514, 1250 cm^{-1}. Anal. Calc. (for $C_{15}H_{14}O_2$): C, 79.62; H, 6.24; Found: C, 79.35; H, 6.25.

4-[(*E*)-2-(4-nitrophenyl)ethenyl]phenol (3g): red solid, 296 mg, 40% yield, mp 201 °C dec. ^{1}H NMR (Acetone-*d6*) δ 6.89 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 7.19 (d, J = 16.5 Hz, 1H, C*H*=CH), 7.44 (d, J = 16.5 Hz, 1H, CH=C*H*), 7.54 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 7.79 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 8.20 (d, J = 9.0 Hz, 2H, C*H*$_{Ar}$); ^{13}C NMR (Acetone-*d6*) δ 115.96 (*C*$_{Ar}$), 123.49 (*C*H=CH), 124.14 (*C*$_{Ar}$), 126.87 (*C*$_{Ar}$), 128.45 (*C*$_{Ar}$), 128.97 (*C*$_{Ar}$), 133.59 (*C*H=CH), 145.13 (*C*$_{Ar}$), 163.26 (*C*$_{Ar}$), 190.40 (*C*$_{Ar}$NO$_2$); IR (KBr) 3422 (broad), 1585, 1504, 1336, 1108 cm^{-1}. Anal. Calc. (for $C_{14}H_{11}NO_3$): C, 69.70; H, 4.60; N, 5.81; Found: C, 69.87; H, 4.60; N, 5.80.

4-[(*E*)-2-(2-naphthyl)vinyl]phenol (3h): green solid, 188 mg, 45% yield, mp 212-213 °C. ^{1}H NMR (Acetone-*d6*) δ 6.87 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.28 (q, J = 15.3 Hz, 2H, C*H*=C*H*), 7.41-7.52 (m, 2H, CH_{Ar}), 7.52 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.85 (d, J = 6.0 Hz, 4H, CH_{Ar}), 7.92 (s, 1H, CH_{Ar}), 8.53 (s, O*H*); ^{13}C NMR (Acetone-*d6*) δ 115.82 (*C*H_{Ar}COH), 123.65 (*C*H_{Ar}CCH), 125.80 (*C*H_{Ar}), 125.83 (*C*H_{Ar}), 126.05 (*C*H_{Ar}CCH), 126.50 (*C*H_{Ar}), 127.83 (*C*H=CH), 128.02 (CH=*C*H), 128.19 (*C*H_{Ar}CCH), 128.39 (*C*H_{Ar}C), 129.24 (*C*H_{Ar}C), 129.34 (C_{Ar}CH), 133.12 (C_{Ar}), 134.19 (C_{Ar}CH), 135.82 (C_{Ar}), 157.69 (C_{Ar}); IR (neat) 3395, 3051, 1597, 1510, 1250 cm^{-1}. Anal. Calc. (for $C_{18}H_{14}O$): C, 87.77; H, 5.73; Found: C, 88.99; H, 5.71.

4-[(*E*)-2-thien-3-ylvinyl]phenol (3i): white solid, 188 mg, 35% yield, mp 199-200 °C; ^{1}H NMR ($CDCl_3$) δ 6.8 (d, J = 9.0 Hz, 2H, *C*H_{Ar}), 6.93 (q, J = 15.3 Hz, 2H, C*H*=C*H*), 7.20-7.21 (m, 1H, CH_{Th}), 7.31 (2H, t, CH_{Th}), 7.36 (d, J = 8.4 Hz, 2H, *C*H_{Ar}); ^{13}C NMR ($CDCl_3$) δ 115.82 (*C*H_{Ar}), 121.23 (*C*H=CH), 121.84 (*C*H_{Th}), 125.06 (*C*H_{Th}), 126.33 (CH=*C*H), 127.89 (*C*H_{Ar}), 128.33 (*C*H_{Th}), 130.23 (C_{Ar}), 140.6 (C_{Th}), 158.82 (C_{Ar}); IR (KBr) 3430, 3092, 1601, 1504, 1249 cm^{-1}. Anal. Calc. (for $C_{12}H_{10}OS$): C, 71.25; H, 4.98; S, 15.85; Found: C, 71.45; H, 4.98; S, 15.87.

4-[(*E*)-2-(pyridin-4-yl)ethenyl]phenol (3l): yellow powder, 131 mg, 44% yield, mp 267-268 °C. ^{1}H NMR (MeOD) δ 6.79 (d, J = 8.4 Hz, 2H, CH_{Ar}), 6.96 (d, J = 16.5 Hz, 1H, C*H*=CH), 7.41 (d, J = 16.8 Hz, 1H, CH=C*H*), 7.46 (d, J = 8.4 Hz, 2H, CH_{Ar}), 7.51 (d, J = 4.5 Hz, 2H, CH_{Ar}), 8.41 (d, J = 4.5 Hz, 2H, CH_{Ar}); ^{13}C NMR (DMSO) δ 116.34 (*C*H_{Ar}), 121.16 (*C*H_{Ar}), 123.12 (*C*H=CH), 127.86 (C_{Ar}), 129.35 (*C*H_{Ar}), 133.72 (CH=*C*H), 145.48 (C_{Ar}), 150.58 (*C*H_{Ar}), 158.89 (C_{Ar}); IR (neat) 3532, 299, 1586, 1464, 1256 cm^{-1}. Anal. Calc. (for $C_{13}H_{11}NO$): C, 79.16; H, 5.62; N, 7.10; Found: C, 78.88; H, 5.61; N, 7.12.

2-Chloro-4-[(*E*)-2-phenylvinyl]phenol (3n): brown solid, 99 mg, 42 % yield, mp 126-127 °C. ^{1}H NMR ($CDCl_3$) δ 7.03 (d, J = 9.0 Hz, 1H, CH_{Ar}OH), 7.48-7.50 (m, 3H, CH_{Ar}), 7.25-7.39 (m, 5H, CH_{Ar} + C*H*=C*H*); ^{13}C NMR ($CDCl_3$) δ 116.68 (C_{Ar}), 120.53 (C_{Ar}Cl), 126.65 (C_{Ar}H), 126.91 (C_{Ar}H), 127.04 (C_{Ar}H), 127.12 (C_{Ar}H), 127.88 (*C*H), 128.19 (*C*H), 128.97 (C_{Ar}H), 131.60 (C_{Ar}OH); IR (neat) 3513, 3435, 3022, 1588, 1497 cm^{-1}. Anal. Calc. (for $C_{14}H_{11}ClO$): C, 72.89; H, 4.81; Found: C, 72.61; H, 4.80.

4-[(*E*)-2-(2,4-dichlorophenyl)vinyl]phenol (3o): yellow solid, 58 mg, 23% yield, mp 119-120°C. ^{1}H NMR ($CDCl_3$) δ 6.86 (d, J =8.7 Hz, 2H, CH_{Ar}OH), 7.08 (d, J = 16.2 Hz, 1H, C*H*=CH,), 7.22 (m, 2H, CH_{Ar}), 7.29 (d, J = 14.1 Hz, 2H, CH=C*H*,), 7.39-7.46 (m, 2H, CH_{Ar}), 7.59 (d, J = 8.4 Hz, 1H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 115.96 (C_{Ar}H), 121.77 (*C*H=CH), 127.15 (CH=*C*H), 127.50 (C_{Ar}H), 128.50 (C_{Ar}H), 128.63 (C_{Ar}H), 129.75 (C_{Ar}), 131.37 (C_{Ar}H), 133.23 (C_{Ar}),

133.83 (C_{Ar}Cl), 134.51 (C_{Ar}Cl); IR (neat) 3240 (broad), 1592, 1443, 1231 cm^{-1}. Anal. Calc. ($C_{14}H_{10}Cl_2O$): C, 63.42; H, 3.80; Found: C, 63.40; H, 3.81.

2-Chloro-4-[(*E*)-2-(2,4-dichlorophenyl)vinyl]phenol (3p): brown solid, 121 mg, 41 % yield, mp 135-136 °C; ^{1}H NMR ($CDCl_3$) δ 6.91 (d, J = 16.2 Hz, 1H, C*H*=CH,), 7.02 (d, J = 8.4 Hz, 1H, CH_{Ar}), 7.24-7.25 (n, 3H, CH_{Ar}), 7.32 (d, J = 15.4 Hz, 1H, CH=C*H*), 7.39 (d, J = 2.1 Hz, 1H, CH_{Ar}), 7.50 (d, J = 2.4 Hz, 1H, CH_{Ar}), 7.56 (d, J = 8.7 Hz, 1H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 116.72 (C_{Ar}H), 120.58 (C_{Ar}Cl), 123.03 (*C*H=CH), 127.22 (C_{Ar}H), 127.33 (C_{Ar}H), 127.38 (C_{Ar}H), 127.55 (C_{Ar}), 129.81 (CH=*C*H), 130.12 (C_{Ar}), 130.98 (C_{Ar}), 133.9 (C_{Ar}Cl), 150.19 (C_{Ar}OH); IR (neat) 3507 (broad), 1595, 1467, 1262 cm^{-1}. Anal. Calc. ($C_{14}H_9Cl_3O$): C, 56.13; H, 3.03; Found: C, 56.28; H, 3.04.

Synthesis and data of 4-[(*E*)-2-(4-aminophenyl)ethenyl]phenol (3m): $SnCl_2$ (4.4 g, 6.4 mmol) was added to a solution of **3g** (3.0 g, 1.24 mmol) in EtOH (10 mL), followed by the addition of conc HCl (0.6 mL). The solution was brought to reflux for 3.5 h and cooled to room temperature stirring overnight. Aqueous NaOH 2 N was added to adjust the pH to 7. After standard workup with CH_2Cl_2 (30 ml), crude product **3m** was obtained and was used in the following step without further purification. Brown solid, 156 mg, 60% yield, mp 250 °C dec. ^{1}H NMR ($CDCl_3$) δ 6.64 (d, J = 8.4 Hz, 2H, CH_{Ar}), 6.82 (d, J = 8.7 Hz, 2H, CH_{Ar}), 6.85 (s, 2H, C*H*=C*H*), 7.28 (d, J = 8.4 Hz, 2H, CH_{Ar}), 7.36 (d, J = 8.7 Hz, 2H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 114.82 (*C*H_{Ar}), 115.46 (*C*H_{Ar}), 124.92 (*C*H=CH), 126.78 (CH=*C*H), 127.42 (*C*H_{Ar}), 127.63 (*C*H_{Ar}), 128.57 (C_{Ar}), 130.87 (C_{Ar}), 146.02 (C_{Ar}), 158.40 (C_{Ar}); IR (neat) 3437, 1614, 1512, 1250 cm^{-1}. Anal. Calc. (for $C_{14}H_{13}NO$) C, 79.59; H, 6.20; N, 6.63; Found: C,79.29; H, 6.21; N, 6.61.

General procedure for the preparation of esters 4a-f and 4h-p. K_2CO_3 (4.4 mmol, 619 mg) was added, at room temperature, to a solution of phenol **3a-f** and **3h-p** (1.77 mmol) in DMF (5 mL). The mixture was heated gradually to 60 °C for 30 min and isobutyl 5-chloro-2,2-dimethylpentanoate (1.77 mmol, 355 mg) in DMF (5 ml) was added. After the mixture was stirred for 5-12 h at 60 °C, the solvent was removed under reduced pressure. The residue was partitioned between EtOAc (10 mL) and 2N NaOH (10 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The solid was purified by column chromatography on silica gel (eluent cyclohexane/ethylacetate, 9:1) or recrystallized from *n*-exane, giving the desired ester **4a-f** and **4h-p**.

Isobutyl 5-{3-[(*E*)-2-(4-chlorophenyl)vinyl]phenoxy}-2,2-dimethylpentanoate (4a): white solid, 176 mg, 50% yield, mp 95-96 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 6H, CH_3), 1.23 (s, 6H, CH_3), 1.69-1.72 (m, 4H, CH_2CH_2), 1.91-1.95 (m, 1H, C*H*), 3.84 (d, 2H, CH_2), 3.95 (t, 2H, CH_2), 6.86 (d, J = 8.7 Hz, 2H, CH_{Ar}), 6.96 (q, 2H, C*H*=C*H*), 7.3 (d, 2H,CH_{Ar}),

7.39-7.43 (m, 4H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 19.36 (*C*H_3), 25.25 (*C*H_2), 25.44 (*C*H_3), 28.0 (*C*H), 37.2 (*C*H_2), 42.4 (*C*), 68.35 (*C*H_2), 70.77 (*C*H_2), 114.9 (C_{Ar}), 125.4 (*C*H=CH), 127.6 (C_{Ar}), 127.99 (CH=*C*H), 129.0 (C_{Ar}), 129.07 (C_{Ar}), 129.8 (C_{Ar}), 132.8 (C_{Ar}), 136.4 (C_{Ar}), 159.1 (C_{Ar}), 178.0 (*C*O); IR (neat) 3439, 2960, 1727, 1603, 1512, 1250, 833 cm^{-1}. Anal. Calc. (for $C_{25}H_{31}ClO_3$): C, 72.90; H, 7.53; Found: C, 72.09; H, 7.54.

Isobutyl 2,2-dimethyl-4-[(*E*)-2-(4-methylphenyl)ethenyl]phenoxypentanoate (4b): white amorphous solid, 103 mg, 51% yield. ^{1}H NMR (Acetone-*d6*) δ 0.94 (d, J = 6.9 Hz, 6H, CH_3), 1.20 (s, 6H, CH_3), 1.73-1.70 (m, 4H, CH_2CH_2), 1.87-1.96 (m, 1H, C*H*), 2.30 (s, 3H, CH_3), 3.83 (d, J = 6.3 Hz, 2H, CH_2), 3.99-4.00 (m, 2H, CH_2), 6.91 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.09 (q, J = 10.8 Hz, 2H, C*H*=C*H*), 7.16 (d, J = 7.8 Hz, 2H, CH_{Ar}), 7.44 (d, J = 7.8 Hz, 2H, CH_{Ar}), 7.5 (d, J = 9.0 Hz, 2H, CH_{Ar}); ^{13}C NMR (Acetone-*d6*) δ 18.66 (*C*H_3), 25.25 (*C*H_2), 25.44 (*C*H_3), 25.99 (*C*H_3), 28.0 (*C*H), 37.88 (*C*H_2), 45.39 (*C*), 68.09 (*C*H_2), 70.22 (*C*H_2), 114.82 (*C*H_{Ar}), 126.84 (*C*H_{Ar}), 127.39 (*C*H=CH), 127.80 (*C*H_{Ar}), 127.72 (CH=*C*H), 128.77 (C_{Ar}), 129.46 (*C*H_{Ar}), 130.18 (C_{Ar}), 136.97 (C_{Ar}), 158.38 (C_{Ar}), 176.74 (CO); IR (neat) 3430, 2963, 1724, 1604, 1515, 1247 cm^{-1}. Anal. Calc. (for $C_{26}H_{34}O_3$): C, 79.15; H, 8.69; Found: C, 78.37; H, 8.68.

Isobutyl 5-{4-[(*E*)-2-(4-isopropylphenyl)vinyl]phenoxy}-2,2-dimethylpentanoate (4c): white solid, 105 mg, 53% yield, mp 66-67 °C. ^{1}H NMR (Acetone-*d6*) δ 0.94 (d, J = 8.7 Hz, 6H, CH_3), 1.20 (s, 6H, CH_3), 1.23 (d, J = 6.9 Hz, 6H, CH_3), 1.73-1.70 (m, 4H, CH_2CH_2), 1.85-1.96 (m, 1H, C*H*), 2.85-2.94 (m, 1H, C*H*), 3.83 (d, J = 7.2 Hz, 2H, CH_2), 3.99-4.00 (m, 2H, CH_2), 6.91 (d, J = 8.4 Hz, 2H, CH_{Ar}), 7.10 (q, J = 9.6 Hz, 2H, C*H*=C*H*), 7.23 (d, J = 8.1 Hz, 2H, CH_{Ar}), 7.49 (t, 4H, CH_{Ar}); ^{13}C NMR (Acetone-*d6*) δ 18.71 (*C*H_3), 23.60 (*C*H_3), 24.85 (*C*H_3), 25.13 (*C*H_2), 27.93(*C*H), 33.91 (*C*H), 37.05 (*C*H_2), 42.04 (*C*), 68.10 (*C*H_2), 70.18 (*C*H_2), 114.83 (*C*H_{Ar}), 127.41 (*C*H=CH), 126.45 (*C*H_{Ar}), 126.82 (*C*H_{Ar}), 127.50 (CH=*C*H), 127.82 (*C*H_{Ar}), 130.45 (C_{Ar}), 135.72 (C_{Ar}), 148.09 (C_{Ar}), 159.00 (C_{Ar}), 176.88 (CO); IR (KBr) 3435, 2961, 1725, 1604, 1513, 1247 cm^{-1}. Anal. Calc. (for $C_{28}H_{38}O_3$): C, 79.58; H, 9.06; Found: C,79.78; H, 9.03.

Isobutyl 2,2-dimethyl-4-[(*E*)-2-(4-trifluoromethylphenyl)ethenyl]phenoxypentanoate (4d): white amorphous solid, 134 mg, 47% yield. ^{1}H NMR (Acetone-*d6*) δ 0.94 (d, J = 6.9 Hz, 6 H, CH_3), 1.20 (s, 6H, CH_3), 1.73-1.70 (m, 4H, CH_2CH_2), 1.96-1.87 (m, 1H, C*H*), 3.82 (d, J = 7.2 Hz, 2H, CH_2), 4.01 (m, 2H, CH_2), 6.87 (d, J = 8.4 Hz, 2H, CH_{Ar}), 7.13 (d, J = 16.2 Hz, 1H, C*H*=CH), 7.34 (d, J = 16.5 Hz, 1H, CH=C*H*), 7.50 (d, J = 8.1 Hz, 2H, CH_{Ar}), 7.66 (d, J =8.4 Hz, 2H, CH_{Ar}), 7.75 (d, J =7.8 Hz, 2H, CH_{Ar}); ^{13}C NMR (Acetone-*d6*) δ 18.68 (*C*H_3), 24.83 (*C*H_3), 25.08 (*C*H_2), 27.92 (*C*H), 37.02 (*C*H_2), 42.03 (*C*), 68.15 (*C*H_2), 70.17 (*C*H_2), 115.85 (C_{Ar}), 124.08 (*C*H=CH), 125.65 (q, C_{Ar}), 126.69 (C_{Ar}),

128.00 (C_{Ar}), 128.60 (C_{Ar}), 128.70, 131.34 (CH=*C*H), 134.00 (CF_3), 158.13 (C_{Ar}), 185.64 (CO); IR (neat) 3445, 2965, 1728, 1604, 1328, 1126 cm^{-1}. Anal. Calc. (for $C_{26}H_{31}F_3O_3$): C, 69.62; H, 6.97; Found: C,69.65; H, 6.97.

Isobutyl 2,2-dimethyl-4-[(E)-2-(4-nitrilephenyl)ethenyl]phenoxypentanoate (4e): white amorphous solid, 152 mg, 65% yield. ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 6.6 Hz, 6H, CH_3), 1.22 (s, 6H, CH_3), 1.73-1.70 (m, 4H, CH_2CH_2), 1.90-1.96 (m, 1H, C*H*), 3.84 (d, J = 6.6 Hz, 2H, CH_2), 3.94-3.98 (m, 2H, CH_2), 6.88 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.04 (q, J = 49.5 Hz, 2H, C*H*=C*H*), 7.45 (d, J = 9.0 Hz, 2H, CH_{Ar}), 7.44 (q, J = 12.9 Hz, 4H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 19.35 (*C*H_3), 25.22 (*C*H_2), 25.44 (*C*H_3), 28.0 (*C*H), 37.18 (*C*H_2C), 42.40 (*C*), 68.40 (O*C*H_2), 70.77 (*C*H_2OCO), 114.0 (C_{Ar}CN), 115.02 (*C*H_{Ar}), 119.41 (*C*N), 126.76 (*C*H_{Ar}), 128.48 (*C*H_{Ar}), 129.15 (C*H*=CH), 129.69 (C*H*=CH), 130.37 (*C*H_{Ar}), 132.69 (*C*H_{Ar}), 142.47 (*C*H_{Ar}), 159.74(*C*H_{Ar}), 179.0 (CO); IR (neat) 3423, 2959, 2220, 1721, 1598, 1157cm^{-1}. Anal. Calc. (for $C_{26}H_{31}NO_3$): C, 77.01; H, 7.71; N, 3.45; Found: C, 77.30; H, 7.71; N, 3.44.

Isobutyl 5-{3-[(*E*)-2-(4-methoxyphenyl)vinyl]phenoxy}-2,2-dimethylpentanoate (4f): white solid, 97 mg, 58% yield, mp 102 °C dec. ^{1}H NMR ($CDCl_3$) δ 0.94 (d, J = 6.9 Hz, 6H, CH_3), 1.18 (s, 6H, CH_3), 1.58-1.71 (m, 4H, CH_2CH_2), 1.91-1.98 (m, 1H, C*H*), 3.82 (s, 3H, CH_3), 3.94 (t, 2H, OCH_2), 6.84-6.92 (m, 6H, CH_{Ar}), 7.39-7.43 (m, 4H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 19.36 (*C*H_3), 25.25 (*C*H_2), 25.44 (*C*H_3), 28.0 (*C*H), 37.2 (*C*H_2), 42.4 (*C*), 54.88 (*C*H_3), 68.38 (*C*H_2), 70.77 (*C*H_2), 114.9 (C_{Ar}), 126.4 (*C*H=CH), 127.6 (C_{Ar}), 127.99 (CH=*C*H), 129.0 (C_{Ar}), 129.07 (C_{Ar}), 129.8 (C_{Ar}), 132.8 (C_{Ar}), 136.4 (C_{Ar}), 159.1 (C_{Ar}O),178.0 (*C*O); IR (neat) 3439, 2960, 1727, 1602, 1512, 1250, 833 cm^{-1}. Anal. Calc. (for $C_{26}H_{34}O_4$): C, 76.06; H, 8.35; Found: C, 75.86; H, 8.35.

Isobutyl 2,2-dimethyl-5-{4-[(*E*)-2-(2-naphthyl)vinyl]phenoxy}pentanoate (4h): white solid, 95 mg, 95% yield, mp 113-115 °C. ^{1}H NMR ($CDCl_3$) δ 0.94 (d, J = 6.9 Hz, 6H, CH_3), 1.23 (s, 6H, CH_3), 1.70-1.75 (m, 4H, CH_2CH_2), 1.91-1.98 (m, 1H, C*H*), 3.85 (d, J = 6.3 Hz, 2H, CH_2), 3.96 (t, J = 5.7 Hz, 2H, CH_2), 6.94 (d, J = 9.0 Hz, 2H, CH_{Ar}), 7.16 (q, J = 5.4 Hz, 2H, C*H*=C*H*), 7.39-7.45 (m, 2H, CH_{Ar}), 7.48 (d, J = 9.0 Hz, 2H, CH_{Ar}), 7.70-7.82 (m, 5H,CH_{Ar}); ^{13}CNMR ($CDCl_3$) δ 19.36 (*C*H_3), 25.27 (*C*H), 25.44 (*C*H_3), 28.02 (*C*H_2), 37.21 (*C*H_2), 42.42 (*C*), 68.38 (*C*H_2), 70.77 (*C*H_2), 114.94 (*C*H_{Ar}), 123.69 (*C*H_{Ar}), 125.90 (*C*H_{Ar}), 126.31 (*C*H_{Ar}), 126.50 (*C*H_{Ar}), 126.80 (*C*H_{Ar}), 127.90 (*C*H=CH), 127.96 (*C*H_{Ar}), 128.12 (CH=*C*H), 128.46 (*C*H_{Ar}), 128.83 (*C*H_{Ar}), 130.27 (C_{Ar}), 133.06 (C_{Ar}), 135.40 (C_{Ar}), 159.02 (C_{Ar}), 178.00 (*C*O); IR (KBr) 2955, 1724, 1603, 1508 cm^{-1}. Anal. Calc. (for $C_{29}H_{34}O_3$): C, 80.89; H, 7.96; Found: C, 81.00; H, 7.96.

Isobutyl 2,2-dimethyl-5-{4-[(E)-2-thien-3-ylvinyl]phenoxy}pentanoate (4i): white solid, 261 mg, 74% yield, mp 70-72 °C. ^{1}H NMR($CDCl_3$) δ 0.94 (d, J = 6.9 Hz, 6H, CH_3), 1.22 (s, 6H, CH_3), 1.58-1.71 (m, 4H, CH_2CH_2), 1.91-1.98 (m,

1H, C*H*), 3.84 (d, J = 6.3 Hz, 2H, CH_2), 3.96 (t, J = 5.7 Hz, 2H, CH_2), 6.85 (d, J = 6.9 Hz, *C*H$_{Ar}$), 6.94 (q, J = 14.4 Hz, 2H, C*H*=C*H*), 7.20-7.21 (m, 1H, CH_{Th}), 7.31 (t, J = 3.0 Hz, 2H, CH_{Th}), 7.36 (d, J = 8.4 Hz, 2H, *C*H$_{Ar}$); ^{13}C NMR ($CDCl_3$) δ 19.36 (*C*H$_3$), 25.27 (*C*H), 25.44 (*C*H$_3$), 28.02 (*C*H$_2$), 37.20 (*C*H$_2$), 42.41 (*C*), 68.34 (*C*H$_2$), 70.77 (*C*H$_2$), 114.87 (*C*H$_{Ar}$), 121.04 (*C*H=CH), 121.72 (*C*H$_{Th}$), 125.06 (*C*H$_{Th}$), 126.29 (CH=*C*H), 127.66 (*C*H$_{Ar}$), 128.47 (*C*H$_{Th}$), 130.23 (C_{Ar}), 140.61 (C_{Th}), 158.82 (C_{Ar}O), 178.02 (CO); IR (KBr) 2955, 1724, 1603, 1508 cm^{-1}. Anal. Calc. (for $C_{23}H_{30}O_3S$): C, 71.46; H, 7.82; S, 8.30; Found: C, 71.66; H, 7.00; S, 8.31.

Isobutyl 2,2-dimethyl-4-[(*E*)-2-(pyridin-4-yl)ethenyl]phenoxypentanoate (4l): orange solid, 111 mg, 48% yield, mp 62-63 °C. ^{1}H NMR($CDCl_3$) δ 0.93 (d, J = 6.9 Hz, 6H, C*H*$_3$), 1.22 (s, 6H, C*H*$_3$), 1.58-1.71 (m, 4H, C*H*$_2$), 1.91-1.98 (m, 1H, C*H*), 3.84 (d, J = 6.3 Hz, 2H, C*H*$_2$), 3.96 (t, 2H, C*H*$_2$), 6.88 (d, J = 8.4 Hz, 2H, *C*H$_{Ar}$), 6.89 (d, J = 16.2 Hz, 1H, C*H*=CH), 7.24 (d, J = 16.2 Hz, 1H, C*H*=CH), 7.32 (d, J = 6.3 Hz, 2H, *C*H$_{Ar}$), 7.46 (d, J = 8.7 Hz, 2H, *C*H$_{Ar}$), 7.51 (d, J = 4.5 Hz, 2H, C*H*$_{Ar}$), 8.54 (d, J = 6.0 Hz, 2H, *C*H$_{Ar}$); ^{13}C NMR ($CDCl_3$) δ 19.36 (*C*H$_3$), 25.22 (*C*H), 25.44 (*C*H$_3$), 28.17 (*C*H$_2$), 37.18 (*C*H$_2$), 42.40 (*C*), 68.38 (*C*H$_2$), 70.78 (*C*H$_2$), 114.99 (*C*H$_{Ar}$), 120.85 (*C*H$_{Ar}$), 123.84 (*C*H=CH), 128.60 (*C*H$_{Ar}$), 128.98 (C_{Ar}), 132.95 (CH=*C*H), 145.22 (C_{Ar}), 150.34 (*C*H$_{Ar}$), 159.81 (C_{Ar}), 177.97 (CO); IR (KBr) 2957, 1723, 1594, 1252 cm^{-1}. Anal. Calc. (for $C_{24}H_{31}NO_3$): C, 75.56; H, 8.19; N, 3.67; Found: C, 75.36; H, 8.20; N, 3.68.

Isobutyl 2,2-dimethyl-4-[(*E*)-2-(4-aminophenyl)ethenyl]phenoxypentanoate (4m): orange solid, 186 mg, 49% yield. ^{1}HNMR ($CDCl_3$) δ 0.94 (d, J = 6.9 Hz, 6H, C*H*$_3$), 1.18 (s, 6H, C*H*$_3$), 1.58-1.71 (m, 4H, C*H*$_2$C*H*$_2$), 1.91-1.98 (m, 1H, C*H*), 3.82 (d, J = 5.7 Hz, 2H, C*H*$_2$), 6.64 (d, J = 8.4 Hz, 2H, C*H*$_{Ar}$), 6.82 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$), 6.85 (s, 2H, C*H*=C*H*), 7.28 (d, J = 8.4 Hz, 2H, C*H*$_{Ar}$), 7.36 (d, J = 8.7 Hz, 2H, C*H*$_{Ar}$); ^{13}C NMR ($CDCl_3$) δ 19.32 (*C*H$_3$), 25.27 (*C*H), 25.44 (*C*H$_3$), 28.63 (*C*H$_2$), 37.19 (*C*H$_2$), 42.40 (*C*), 68.32 (*C*H$_2$), 70.76 (*C*H$_2$), 114.82 (*C*H$_{Ar}$), 115.46 (*C*H$_{Ar}$), 127.42 (*C*H$_{Ar}$), 127.63 (C_{Ar}), 124.92 (*C*H=CH), 126.78 (CH=*C*H), 128.57 (C_{Ar}), 130.87 (C_{Ar}), 146.02 (C_{Ar}), 158.40 (C_{Ar}), 178.01 (CO); IR (neat) 2963, 1723, 1613, 1193 cm^{-1}. Anal. Calc. (for $C_{25}H_{33}NO_3$): C, 75.91; H, 8.41; N, 3.54; Found: C, 76.18; H, 8.39; N, 3.54.

Isobutyl 5-{2-chloro-4-[(*E*)-2-phenylvinyl]phenoxy}-2,2-dimethylpentanoate (4n): brown oil, 89 mg, 98% yield. ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 6.9 Hz, 6H, C*H*$_3$), 1.22 (s, 6H, C*H*$_3$), 1.58-1.71 (m, 4H, C*H*$_2$C*H*$_2$C), 1.91-1.98 (m, 1H, C*H*), 3.84 (d, J = 6.3 Hz, 2H, C*H*$_2$O), 3.96 (t, 2H, OC*H*$_2$), 7.03 (d, J = 9.0 Hz, 1H, C*H*$_{Ar}$OH), 7.48-7.50 (m, 3H, C*H*$_{Ar}$), 7.25-7.39 (m, 5H, C*H*$_{Ar}$ + C*H*=C*H*); ^{13}C NMR ($CDCl_3$) δ 19.36 (*C*H$_3$CH*C*H$_3$), 25.22 (CH$_3$*C*HCH$_3$), 25.44 (*C*H$_3$C*C*H$_3$), 28.17 (CH$_2$*C*H$_2$CH$_2$), 37.18 (CH$_2$*C*H$_2$C), 42.40 (CH$_3$*C*CH$_3$), 68.38 (O*C*H$_2$CH$_2$), 70.78 (*C*H$_2$COO), 116.68 (C_{Ar}), 120.53 (C_{Ar}Cl), 126.65 (C_{Ar}H), 126.91 (C_{Ar}H), 127.04 (C_{Ar}H), 127.12 (C_{Ar}H), 127.88 (*C*H), 128.19 (*C*H), 128.97 (C_{Ar}H),

131.60 (C_{Ar}OH); IR (neat) 3440, 2965, 1726, 1468 cm^{-1}. Anal. Calc. (for $C_{25}H_{31}ClO_3$): C, 72.36; H, 7.53; Found: C, 72.15; H, 7.54.

Isobutyl 5-{4-[(*E*)-2-(2,4-dichlorophenyl)vinyl]phenoxy}-2,2-dimethylpentanoate (4o): brown oil, 58 mg, 82% yield. ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 6.9 Hz, 6H, CH_3), 1.22 (s, 6H, CH_3), 1.58-1.71 (m, 4H, CH_2CH_2C), 1.91-1.98 (m, 1H, C*H*), 3.84 (d, J = 6.3 Hz, 2H, CH_2O), 3.96 (t, 2H, OCH_2), 6.86 (d, J = 8.7 Hz, 2H, CH_{Ar}OH), 7.08 (d, J = 16.2 Hz, 1H, C*H*=CH,), 7.22 (m, 2H, CH_{Ar}), 7.29 (d, 2H, CH=C*H*, J = 14.1 Hz), 7.39-7.46 (m, 2H, CH_{Ar}), 7.59 (d, 1H, CH_{Ar}, J=8.4); ^{13}C NMR ($CDCl_3$) δ 19.36 (CH_3CHCH_3), 25.22 (CH_3CHCH_3), 25.44 (CH_3CCH_3), 28.17 ($CH_2CH_2CH_2$), 37.18 (CH_2CH_2C), 42.40 (CH_3CCH_3), 68.38 (OCH_2CH_2), 70.78 (CH_2COO), 115.96 ($C_{Ar}H$), 121.77 (*C*H=CH), 127.15 (CH=*C*H), 127.50 ($C_{Ar}H$), 128.50 ($C_{Ar}H$), 128.63 ($C_{Ar}H$), 129.75 (C_{Ar}), 131.37 ($C_{Ar}H$), 133.23 (C_{Ar}), 133.83 ($C_{Ar}Cl$), 134.51 ($C_{Ar}Cl$). IR (neat) 3441, 2965, 1726, 1468 cm^{-1}. Anal. Calc. (for $C_{25}H_{30}Cl_2O_3$): C, 66.81; H, 6.73; Found: C, 66.98; H, 6.75.

Isobutyl 5-{2-chloro-4-[(*E*)-2-(2,4-dichlorophenyl)vinyl]phenoxy}-2,2-dimethylpentanoate (4p): colorless oil, 60 mg, 46% yield; ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 6.9 Hz, 6H, CH_3), 1.22 (s, 6H, CH_3), 1.58-1.71 (m, 4H, CH_2CH_2C), 1.91-1.98 (m, 1H, C*H*), 3.84 (d, J = 6.3 Hz, 2H, CH_2O), 3.96 (t, 2H, OCH_2), 6.9 (d, J = 16.2 Hz, 1H, C*H*=CH,), 7.02 (d, J = 8.4 Hz, 1H, CH_{Ar}), 7.24-7.25 (m, 3H, CH_{Ar}), 7.32 (d, J = 15.4 Hz, 1H, CH=C*H*), 7.39 (d, J = 2.1 Hz, 1H, CH_{Ar}), 7.50 (d, J = 2.4 Hz, 1H, CH_{Ar}), 7.56 (d, J = 8.7 Hz, 1H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 19.36 (CH_3CHCH_3), 25.22 (CH_3CHCH_3), 25.44 (CH_3CCH_3), 28.17 ($CH_2CH_2CH_2$), 37.18 (CH_2CH_2C), 42.40 (CH_3CCH_3), 68.38 (OCH_2CH_2), 70.78 (CH_2COO), 116.72 ($C_{Ar}H$), 120.58 ($C_{Ar}Cl$), 123.03 (*C*H=CH), 127.22 ($C_{Ar}H$), 127.33 ($C_{Ar}H$), 127.38 ($C_{Ar}H$), 127.55 (C_{Ar}), 129.81 (CH=*C*H), 130.12 (C_{Ar}), 130.98 (C_{Ar}), 133.9 ($C_{Ar}Cl$), 150.19 ($C_{Ar}OH$); IR (neat) 3440, 2965, 1726, 1468 cm^{-1}. Anal. Calc. (for $C_{25}H_{29}Cl_3O_3$): C, 62.06; H, 6.04; Found: C, 62.28; H, 6.05.

General procedure for the preparation of acids 5a-f and 5h-p. A solution of NaOH 1% in absolute EtOH (3.61 mmol, 144.0 mg) was added to ester **4a-f** and **4h-p** (0.18 mmol) in absolute EtOH (5 mL), and the mixture was stirred at reflux for 8-12 h. The solvent was removed under reduced pressure and the residue was poured into H_2O (10 mL) and acidified with 2N HCl. The aqueous layer was extracted with AcOEt (3 x 25 mL) and then the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by crystallization in cyclohexane or by flash chromatography eluent cyclohexane:ethylacetate 8:2) to give the acid **5a-f** and **5h-p**.

5-{4-[(*E*)-2-(4-chlorophenyl)ethenyl]phenoxy}-2,2-dimethylpentanoic acid (5a): white solid, 25 mg, 39% yield, mp 193-194 °C. ^{1}HNMR ($CDCl_3$) δ 1.95 (s, 6H, CH_3), 1.63-1.77 (m, 4H, CH_2CH_2), 3.94 (t, 2H, CH_2), 6.93 (q, 2H, C*H*=C*H*), 6.84 (d, 2H, CH_{Ar}), 7.27 (d, 2H, CH_{Ar}), 7.36-7.41 (m, 4H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 25.25 (*C*H_2), 25.44 (*C*H_3), 37.2 (*C*H_2), 42.4 (*C*), 68.35 (*C*H_2), 114.9 (*C*$_{Ar}$), 125.4 (*C*H=CH), 127.6 (*C*$_{Ar}$), 127.99 (CH=*C*H), 129.0 (*C*$_{Ar}$), 129.07 (*C*$_{Ar}$), 129.8 (*C*$_{Ar}$), 132.8 (*C*$_{Ar}$), 136.4 (*C*$_{Ar}$), 159.1 (*C*$_{Ar}$O), 178.1 (*C*O); IR (neat) 3430, 3023, 2950, 1693, 1605, 1511 cm^{-1}. Anal. Calc. (for $C_{21}H_{23}ClO_3$): C, 70.29; H, 6.46; Found: C, 70.53; H, 6.47.

2,2-Dimethyl-4-[(E)-2-(4-methylphenyl)ethenyl]phenoxypentanoic acid (5b): white solid, 25 mg, 43% yield. ^{1}H NMR (MeOD) δ 1.20 (s, 6H, CH_3), 1.71-1.78 (m, 4H, CH_2CH_2), 2.31 (3H, s, CH_3), 389-3.98 (m, 2H, CH_2), 6.87 (d, J = 9.0 Hz, 2H, CH_{Ar}), 6.99 (q, J = 9.6 Hz, 2H, C*H*=C*H*), 7.13 (d, J = 8.1 Hz, 2H, CH_{Ar}), 7.38 (d, J = 8.4 Hz, 2H, CH_{Ar}), 7.4 (d, J = 8.7 Hz, 2H, CH_{Ar}); ^{13}C NMR (MeOD) δ 20.03 (*C*H_3), 24.51 (*C*H_3), 25.03 (*C*H_2), 36.99 (*C*H_2), 41.63 (*C*), 68.09 (O*C*H_2), 114.50 (*C*H_{Ar}), 125.98 (*C*H_{Ar}), 126.18 (*C*H=CH), 127.10 (CH=*C*H), 127.39 (*C*H_{Ar}), 129.08 (*C*H_{Ar}), 129.93 (*C*H_{Ar}), 135.21 (*C*H_{Ar}), 136.85 (*C*H_{Ar}), 158.38 (*C*H_{Ar}), 183.43 (CO); IR (neat) 3432, 3026, 2963, 1725, 1610, 1193 cm^{-1}; Anal. Calc. (for $C_{22}H_{26}O_3$): C, 78.07; H, 7.74; Found: C, 78.05; H, 7.71.

5-{4-[(*E*)-2-(4-isopropylphenyl)vinyl]phenoxy}-2,2-dimethylpentanoic acid (5c): white solid, 67 mg, 95% yield, mp 174-176 °C. ^{1}H NMR (Acetone-*d6*) δ 1.24 (s, 2H, CH_3), 1.21 (d, J = 4.2 Hz, 6H, CH_3), 1.70-1.73 (m, 4H, CH_2CH_2), 2.85-2.94 (m, 1H, C*H*), 3.98-4.02 (m, 2H, CH_2), 6.92 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.10 (q, J = 9.6 Hz, 2H, C*H*=C*H*), 7.23 (d, J = 8.1 Hz, 2H, CH_{Ar}), 7.49 (t, 4H, CH_{Ar}); ^{13}C NMR (Acetone-*d6*) δ 24.85 (*C*H_3), 25.13 (*C*H_2), 33.91 (*C*H), 36.97 (*C*H_2), 68.23 (*C*H_2), 114.85 (*C*H_{Ar}), 126.39 (*C*H=CH), 126.45 (*C*H_{Ar}), 126.82 (*C*H_{Ar}), 127.51 (CH=*C*H), 127.82 (*C*H_{Ar}), 130.45 (*C*$_{Ar}$), 135.72 (*C*$_{Ar}$), 148.09 (*C*$_{Ar}$), 159.00 (*C*$_{Ar}$), 176.88 (CO); IR (neat) 3400, 3059, 2963, 1725, 1610, 1193 cm^{-1}. Anal. Calc. (for $C_{24}H_{30}O_3$): C, 78.65; H, 8.25; Found: C, 78.68; H, 8.22.

2,2-Dimethyl-4-[(*E*)-2-(4-trifluoromethylphenyl)ethenyl]phenoxypentanoic acid (5d): white solid, 39 mg, 56% yield, mp 173-174 °C. ^{1}H NMR (Ac_D) δ 1.20 (s, 6H, CH_3), 1.69-1.75 (m, 4H, CH_2CH_2), 2.85 (t, 2H, CH_2), 6.95 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.18 (d, J = 17.1 Hz, 1H, C*H*=CH), 7.37 (d, J = 16.5 Hz, 1H, CH=C*H*), 7.57 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.67 (d, J = 7.8 Hz, 2H, CH_{Ar}), 7.77 (d, J = 7.8 Hz, 2H, CH_{Ar}); ^{13}C NMR (Ac_D) δ 24.83 (*C*H_3), 25.08 (CH$_2$*C*H$_2$CH$_2$), 27.92 (*C*H), 37.02 (*C*H$_2$C), 42.03 (*C*(CH$_3$)$_2$), 68.15 (O*C*H$_2$), 115.85 (*C*$_{Ar}$), 124.08 (*C*H=CH), 125.65 (q, *C*$_{Ar}$), 126.69 (*C*$_{Ar}$), 128.00 (*C*$_{Ar}$), 128.60 (*C*$_{Ar}$), 128.70, 131.34 (CH=*C*H); 134.00 (CF_3), 158.13 (*C*$_{Ar}$), 185.64 (CO); IR (neat) 3438, 2919, 1702, 1602, 1325 cm^{-1}; Anal. Calc. (for $C_{22}H_{23}F_3O_3$): C, 67.34; H, 5.91; Found: C, 67.30; H, 5.90.

5-{4-[(*E*)-2-(4-cyanophenyl)ethenyl]phenoxy}-2,2-dimethylpentanoic acid (5e): yellow solid, 25 mg, 40% yield, mp 295 °C dec. ^{1}H NMR (Acetone-*d6*) δ 1.20 (s, 6H, CH_3), 1.60-1.76 (m, 4H, CH_2CH_2), 3.96 (t, 2H, CH_2O), 6.89 (d, J = 8.1 Hz, 2H, CH_{Ar}CCH_{Ar}), 7.05 (d, J = 15.9 Hz, 1H, C*H*=CH), 7.23 (d, J = 15.9 Hz, 1H, CH=C*H*), 7.49 (d, J = 7.8 Hz, 2H, CH_{Ar}), 7.60 (d, J = 8.1 Hz, 2H, CH_{Ar}), 7.84 (d, J = 7.8 Hz, 2H, CH_{Ar}); ^{13}C NMR (MeOD) δ 24.49 (*C*H_2), 24.11 (*C*H_3), 36.53 (*C*H_2C), 41.2 (*C*), 67.54 (*C*H_2O), 113.57 (C_{Ar}), 114.0 (C_{Ar}), 126.86 (*C*N), 126.94 (C_{Ar}), 128.19 (C_{Ar}), 129.6 (C_{Ar}), 129.50 (C_{Ar}), 130.97 (*C*H=*C*H), 141.26 (C_{Ar}), 158.25 (C_{Ar}), 184.0 (*C*O); IR (neat) 3390, 3198, 2960, 2359, 1647, 1535, 1404 cm^{-1}. Anal. Calc. (for $C_{22}H_{23}N\,O_3$): C, 75.62; H, 6.63; N, 4.01; Found: C, 75.68; H, 6.62; N, 4.00.

2,2-Dimethyl-4-[(*E*)-2-(4-methoxyphenyl)ethenyl]phenoxypentanoic acid (5f): white solid, 42 mg, 67% yield, mp 262 °C dec. ^{1}H NMR (MeOD) δ 1.14 (s, 6H, CH_3), 1.63 (t, 2H, CH_2C), 1.74-1.75 (m, 2H, CH_2), 3.79 (s, 3H, CH_3O), 3.94 (t, 2H, OCH_2), 6.41-6.93 (m, 6H, CH_{Ar}), 7.39-7.44 (m, 4H, CH_{Ar}); ^{13}C NMR (MeOD) δ 25.54 (*C*H_2), 25.62 (*C*H_3), 37.82 (*C*H_2C), 43.04 (*C*), 54.53 (*C*H_3O), 68.67 (*C*H_2O), 113.85 (C_{Ar}), 114.55 (C_{Ar}), 125.72 (C_{Ar}), 126.14 (C_{Ar}), 127.24 (C_{Ar}), 158.98 (C_{Ar}), 159.32 (C_{Ar}), 185.62 (*C*O); IR (neat) 3369, 2958, 2870, 1707, 1606, 1538, 1514 cm^{-1}. Anal. Calc. (for $C_{22}H_{26}O_4$): C, 74.55; H, 7.39; Found: C, 74.28; H, 7.40.

2,2-Dimethyl-5-{4-[(*E*)-2-(2-naphthyl)vinyl]phenoxy}pentanoic acid (5h): white solid, 32 mg, 48% yield, mp 208-210 °C. ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 6H, CH_3), 1.70-1.75 (m, 4H, CH_2CH_2C), 3.96 (t, J = 5.7 Hz, 2H, OCH_2), 6.94 (d, J = 9.0 Hz, 2H, CH_{Ar}COH), 7.16 (q, J = 5.4 Hz, 2H, C*H*=C*H*), 7.39-7.45 (m, 2H, CH_{Ar}), 7.48 (d, J = 9.0 Hz, 2H, CH_{Ar}CCH), 7.70-7.82 (m, 5H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) δ 25.44 (*C*H_3C*C*H_3), 28.02 (CH_2*C*$H_2$$CH_2$), 37.21 ($CH_2$*C*$H_2$C), 42.42 ($CH_3$*C*$CH_3$), 68.38 (O*C*$H_2$$CH_2$), 114.94 (*C*$H_{Ar}$CO), 123.69 (*C*$H_{Ar}$CCH), 125.90 (*C*$H_{Ar}$), 126.31 (*C*$H_{Ar}$), 126.50 (*C*$H_{Ar}$CCH), 126.80 (*C*$H_{Ar}$), 127.90 (*C*H=CH), 127.96 (*C*H_{Ar}), 128.12 (CH=*C*H), 128.46 (*C*H_{Ar}), 128.83 (*C*H_{Ar}), 130.27 (C_{Ar}), 133.06 (C_{Ar}), 135.40 (C_{Ar}), 159.02 (C_{Ar}), 178.00 (*C*O); IR (neat) 3436, 2966, 1694, 1599, 1251 cm^{-1}. Anal. Calc. (for $C_{25}H_{26}O_3$): C, 80.18; H, 7.00; Found: C, 80.22; H, 6.97.

2,2-Dimethyl-5-{4-[(*E*)-2-thien-3-ylvinyl]phenoxy}pentanoic acid (5i): white solid, 30 mg, 51% yield, mp 172-173 °C. ^{1}H NMR($CDCl_3$) δ 1.25 (s, 6H, CH_3), 1.73-1.77 (m, 4H, CH_2CH_2), 3.96 (t, J = 5.7 Hz, 2H, OCH_2), 6.86 (d, J = 8.7 Hz, 2H, *C*H_{Ar}), 6.94 (q, J = 15.3 Hz, 2H, C*H*=C*H*), 7.20-7.21 (m, 1H, CH_{Th}), 7.31 (t, J = 3.0 Hz, 2H, CH_{Th}), 7.39 (d, J = 8.7 Hz, 2H, *C*H_{Ar}); ^{13}C NMR ($CDCl_3$) δ 25.44 (*C*H_3C*C*H_3), 28.02 (CH_2*C*$H_2$$CH_2$), 37.21 ($CH_2$*C*$H_2$C), 42.42 ($CH_3$*C*$CH_3$), 68.38 (O*C*$H_2$$CH_2$), 115.82 (*C*$H_{Ar}$), 121.23 (*C*H=CH), 121.84 (*C*H_{Th}), 125.06 (*C*H_{Th}), 126.33 (CH=*C*H), 127.89 (*C*H_{Ar}), 128.33 (*C*H_{Th}), 130.23 (C_{Ar}), 140.6 (C_{Th}), 158.82 (C_{Ar}), 178.00 (*C*O); IR (KBr) 2955, 1724, 1603, 1508 cm^{-1}. Anal. Calc. (for $C_{19}H_{22}O_3S$): C, 69.06; H, 6.71; S, 9.70; Found: C, 69.00; H, 6.74; S, 9.68.

2,2-Dimethyl-5-{4-[(*E*)-2-(pyridin-4-yl)ethenyl]phenoxy}pentanoic acid (5l): yellow solid, 28 mg, 47% yield, mp 213 °C dec. ^{1}H NMR (DMSO) δ 1.09 (s, 6H, CH_3), 1.55-1.62 (m, 4H, CH_2CH_2C), 3.95 (t, 2H, OCH_2), 6.93 (d, J = 8.7 Hz, 2H, CH_{Ar}), 7.06 (d, J = 16.5 Hz, 1H, C*H*=CH), 7.49 (q, 3H, C*H*=CH and CH_{Ar}), 7.56 (d, J = 8.7 Hz, 2H, CH_{Ar}), 8.48 (d, J = 6.0 Hz, 2H, CH_{Ar}); ^{13}C NMR (DMSO) δ 25.18 (CH$_2$CH_2CH$_2$), 25.64 (CH_3CCH$_3$), 41.67 (CH$_3$CCH$_3$), 68.25 (OCH$_2$CH$_2$), 115.42 (CH_{Ar}), 121.26 (CH_{Ar}), 123.83 (CH=CH), 129.22 (CH_{Ar}), 130.64 (C_{Ar}), 133.35 (CH=CH), 145.32 (C_{Ar}), 150.61 (CH_{Ar}), 159.67 (C_{Ar}), 177.97 (CO); IR (KBr) 3426, 1598, 1556, 1406 cm^{-1}. Anal. Calc. (for $C_{20}H_{23}NO_3$): C, 73.82; H, 7.12; N, 4.30; Found: C, 73.86; H, 7.10; N, 4.31.

5-{4-[(*E*)-2-(4-aminophenyl)ethenyl]phenoxy}-2,2-dimethylpentanoic acid (5m): yellow solid, 29 mg, 47% yield. ^{1}H NMR (CD$_3$OD) δ 1.19 (s, 6H, CH_3), 1.68-1.72 (m, 4H, CH_2CH_2C), 3.94 (d, J = 6.3 Hz, 2H, OCH_2), 6.78 (d, J = 7.5 Hz, 2H, CH_{Ar}), 6.85 (d, J = 7.5 Hz, 4H, CH_{Ar} and CH=C*H*), 7.26 (d, J = 7.5 Hz, 2H, CH_{Ar}), 7.37 (d, J = 7.5 Hz, 2H, CH_{Ar}); ^{13}C NMR (CD$_3$OD) δ 24.50 (CH_3CCH$_3$), 25.04 (CH$_2$CH_2CH$_2$), 36.97 (CH$_2$CH_2C), 41.63 (CH$_3$CCH$_3$), 68.10 (OCH$_2$CH$_2$), 114.49 (CH_{Ar}), 115.41 (CH_{Ar}), 124.06 (CH=CH), 126.64 (CH=CH), 126.96 (CH_{Ar}), 127.09(CH_{Ar}), 128.15 (C_{Ar}C), 131.07 (C_{Ar}), 147.05 (C_{Ar}), 158.40 (C_{Ar}), 185.64 (CO); IR (neat) 3366, 3000, 2963, 1723, 1613, 1193 cm^{-1}. Anal. Calc. (for $C_{21}H_{25}NO_3$): C, 74.31; H, 7.42; N, 4.13; Found: C, 74.55; H, 7.40; N, 4.11.

5-{2-Chloro-4-[(*E*)-2-phenylvinyl]phenoxy}-2,2-dimethylpentanoic acid (5n): yellowish solid**,** 21 mg, 45% yield, mp 121-122 °C; ^{1}H NMR (CDCl$_3$) δ 1.09 (s, 6H, CH_3), 1.55-1.62 (m, 4H, CH_2CH_2C), 3.95 (t, 2H, OCH_2), 7.03 (d, J = 9.0 Hz, 1H, CH_{Ar}OH,), 7.48-7.50 (m, 3H, CH_{Ar}), 7.25-7.39 (m, 5H, CH_{Ar} + CH=C*H*); ^{13}C-NMR (CDCl$_3$) δ 25.18 (CH$_2$CH_2CH$_2$), 25.64 (CH_3CCH$_3$), 41.67 (CH$_3$CCH$_3$), 68.25 (OCH$_2$CH$_2$), 116.68 (C_{Ar}), 120.53 (C_{Ar}Cl), 126.65 (C_{Ar}H), 126.91 (C_{Ar}H), 127.04 (C_{Ar}H), 127.12 (C_{Ar}H), 127.88 (CH), 128.19 (CH), 128.97 (C_{Ar}H), 131.60 (C_{Ar}OH); IR (neat) 3446, 2969, 1700, 1501 cm^{-1}. Anal. Calc. (for $C_{21}H_{23}ClO_3$): C, 70.29; H, 6.46; Found: C, 70.36; H, 6.46.

5-{4-[(*E*)-2-(2,4-dichlorophenyl)vinyl]phenoxy}-2,2-dimethylpentanoic acid (5o): yellow solid, 271 mg, 50% yield, mp 137-138 °C; ^{1}H NMR (CDCl$_3$) δ 1.09 (s, 6H, CH_3), 1.55-1.62 (m, 4H, CH_2CH_2C), 3.95 (t, 2H, OCH_2), 6.86 (d, J = 8.7 Hz, 2H, CH_{Ar}OH), 7.08 (d, J = 16.2 Hz, 1H, CH=CH,), 7.22 (m, 2H, CH_{Ar}), 7.29 (d, J = 14.1 Hz, 2H, CH=C*H*), 7.39-7.46 (m, 2H, CH_{Ar}), 7.59 (d, J = 8.4 Hz, 1H, CH_{Ar}); ^{13}C NMR (CDCl$_3$) δ 25.54 (CH_3CCH$_3$), 28.17 (CH$_2$CH_2CH$_2$), 37.18 (CH$_2$CH_2C), 42.40 (CH$_3$CCH$_3$), 68.40 (OCH$_2$CH$_2$), 115.96 (C_{Ar}H), 121.77 (CH=CH), 127.15 (CH=CH), 127.50 (C_{Ar}H), 128.50 (C_{Ar}H), 128.63 (C_{Ar}H), 129.75 (C_{Ar}), 131.37 (C_{Ar}H), 133.23 (C_{Ar}), 133.83 (C_{Ar}Cl), 134.51 (C_{Ar}Cl); IR (neat) 3437, 3060, 2960, 1700, 1470 cm^{-1}. Anal. Calc. (for $C_{21}H_{22}Cl_2O_3$): C, 64.13; H, 5.64; Found: C, 64.22; H, 5.63.

5-{2-Chloro-4-[(*E*)-2-(2,4-dichlorophenyl)vinyl]phenoxy}-2,2-dimethylpentanoic acid (**5p**): white solid, 30 mg, 57% yield, mp 126-127 °C; ^{1}H NMR ($CDCl_3$) δ 1.09 (s, 6H, CH_3), 1.55-1.62 (m, 4H, CH_2CH_2C), 3.95 (t, 2H, OCH_2), 6.9 (d, J = 16.2 Hz, 1H, C*H*=CH), 7.02 (d, J = 8.4 Hz, 1H, CH_{Ar}), 7.24-7.25 (m, 3H, CH_{Ar}), 7.32 (d, J = 15.4 Hz, 1H, CH=C*H*), 7.39 (d, J = 2.1 Hz, 1H, CH_{Ar}), 7.50 (d, J = 2.4 Hz, 1H, CH_{Ar}), 7.56 (d, J = 8.7 Hz, 1H, CH_{Ar}); ^{13}C NMR ($CDCl_3$) 25.44 (*C*H_3C*C*H_3), 28.17 (CH_2*C*$H_2$$CH_2$), 37.18 ($CH_2$*C*$H_2$C), 42.40 ($CH_3$*C*$CH_3$), 68.38 (O*C*$H_2$$CH_2$), 116.72 ($C_{Ar}$H), 120.58 ($C_{Ar}$Cl), 123.03 (*C*H=CH), 127.22 (C_{Ar}H), 127.33 (C_{Ar}H), 127.38 (C_{Ar}H), 127.55 (C_{Ar}), 129.81 (CH=*C*H), 130.12 (C_{Ar}), 130.98 (C_{Ar}), 133.9 (C_{Ar}Cl), 150.19 (C_{Ar}OH); IR (neat) 3437, 3060, 2960, 1700, 1470 cm^{-1}. Anal. Calc. (for $C_{21}H_{21}Cl_3O_3$): C, 58.97; H, 4.95; Found: C, 59.17; H, 4.93.

Biology

Reporter plasmids and luciferase assays. Human embryonic kidney cells (HEK293) were grown in Dulbecco's Modified Eagles's Medium (DMEM) containing 10% of FCS, 1% penicillin/streptomycin, 1% MEM non-essential amino acid and 1% sodium pyruvate MEM 100 mM. HEK293 cells were incubated at 37 °C in 5% CO_2 incubator until they were 80% confluent. One day before the experiment, the cells were plated in 96-well plates. First, the cells were detached with 500 µL of trypsin-EDTA 0.25% which were inactivated with complete DMEM (3.5 mL), then are taken 500 µL of cell suspension and were resuspended in the culture medium and subsequently introduce 100 µL in each well. The next day, the culture medium was replaced by a fresh one without FCS and the transient transfection was conducted using the calcium phosphate method. Cells were transfected with expression plasmids encoding the fusion protein GAL4-PPARαLBD, GAL4-PPARγLBD or GAL4-PPARδLBD (30 ng), MH100 luciferase reporter plasmid (50 ng), renilla luciferase normalization vector (20 ng), and pGEM carrier DNA (40 ng) to make a total of 140 ng of DNA per well. After 8 h, cells were treated for 18 h with the indicated ligands. The two luciferase activities were measured using a dual luciferase assay kit (Promega) according to ther supplier's instructions on a microplate luminometer (Labsystems Ascent LuminoskanReader). All transfection experiments were repeated in at least three separate experiments.

Real-time quantitative PCR (RTqPCR). HepG2 cells were obtained from ATCC (ATCC-LGC Promochem, London, U.K.). Cells were maintained in growth medium composed of DMEM supplemented with 10% FCS-GOLD and 1% penicillin/streptomycin. One day before the treatment, HepG2 cells were seeded in six-well plates at a density of 5 x 10^5

cells/well and incubated at 37 °C, 5% CO_2. The next day, the cells were treated for 24 h with the indicated ligands dissolved in DMSO and diluted in the growth medium. After incubation, cells were washed with PBS and RNA was extracted. Total RNA was isolated by TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. To avoid possible DNA contamination, RNA was treated with DNAase-1 (Ambion, Foster City, CA). RNA purity was checked by spectrophotometer and RNA integrity by examination on agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized reverse-transcribing 4 μg of total RNA in a total volume of 100 μL using the High Capacity DNA Archive Kit (Applied Biosystems, Foster City, CA) and following the manufacturer's instructions.

RTqPCR primers were designed using Primer Express Software. RTqPCR assays were performed in 96 well optical reaction plates using the ABI 7500HT machine (Applied Biosystems) and were conducted in triplicate wells for each sample. Baseline values of amplification plots were set automatically, and threshold values were kept constant to obtain normalized cycle times and linear regression data. The following reaction mixture per well was used: 10 μL Power Syber Green (Applied Biosystem), 2.4 μL primer at the final concentration of 150 nM, 4.6 μL of RNAse free water, and 3 μL cDNA (60 ng). For all experiments the following conditions were used: denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, then at 60 °C for 60 s. Quantitative normalization of cDNA in each sample was performed using hGAPDH as internal control. Relative quantification was performed using the ΔΔCT method.

Validated primers for RTqPCR are listed below:

Human CPT1A: FW-5'TGCCATGGATCTGCTGTATATCC3',

RV-5'GCGTTGCCGGCTCTTG3'

Human GAPDH: FW-5'CAACTTTGGTATCGTGGAAGGAC3',

RV-5'ACAGTCTTCTGGGTGGCAGTG3'.

Docking

Docking protocol. All calculations were performed on a DELL T5500 workstation, equipped with two Intel® Xeon® E5630 2.53 GHz processors. All PPARα agonists were manually built in Maestro version 9.3.5, exploiting the Built facility. Ligands were submitted to Epik v. 2.3[1-3] to calculate the protonation state at neutral pH. All structures were minimized to a derivative convergence of 0.001 $kJ\text{Å}^{-1}mol^{-1}$, using the Truncated Newton Conjugate Gradient (TNCG) minimization algorithm, the OPLS2005 force field, and the GB/SA water solvation model implemented in MacroModel version 9.9.[1] Conformational searches, applying the Mixed torsional/Low-mode sampling and the automatic set-up

protocol, were carried out on all minimized ligand structures in order to obtain the global minimum geometry of each molecule, which was then used as the starting conformation for docking calculations with Glide version 5.8.[1,4-5] Three-dimensional coordinates of PPARα (PBD ID: 2P54 [6]) was downloaded from the Brookhaven Protein Data Bank.[7] PPARα 3D structure was submitted to the Protein Preparation routine in Maestro[1] that allows fixing the receptor structure, bond orders, adding hydrogen atoms, and ionizing charged residues. 11 water molecules were not removed as they occupy a pocket close to the binding region and are conserved in at least 6 over 12 X-ray complexes, namely WAT 474, 475, 477, 478, 488, 493, 497, 505, 517, 546, 608. Water molecules orientation was optimized, along with the overall hydrogen bond network, histidine tautomers and ionization states were predicted, 180° rotations of the terminal χ angle of Asn, Gln, and His residues were assigned, and hydroxyl and thiol hydrogen atoms were sampled. For each structure, a brief relaxation was performed using an all-atom constrained minimization carried out with the OPLS-2005 force field to reduce steric clashes that may exist in the original PDB structures. The minimization was terminated when the energy converged or the root mean square deviation (RMSD) reached a maximum cut-off of 0.30 Å. Glide energy grid was generated for the receptor structure using the crystallographic ligand as the centre of the grid as a reference. The size of the box was determined automatically on the basis of the ligand dimensions. The OH group of Tyr314, Tyr464 and Ser280 was considered rotatable during docking calculation. The global minimum geometry of each ligand was submitted to flexible docking in the previously prepared protein. The van der Waals radii for non-polar ligand atoms were scaled by a factor of 0.8, while receptor atoms were not scaled. A first docking run was carried out applying the Standard Precision settings of Glide, followed by the Extra Precision settings. Ten poses were saved and the best ranking pose for each ligand was submitted to a full minimization of the complexes applying the MM-GBSA module of Prime.[2] Resulting energy parameters were used to generate a custom scoring function using the Multiple Linear Regression method in Strike.[2] The automatic selection of the optimal parameters subset was allowed, using 3 factors. The resulting model was cross-validated using the Leave one out method. SiteMap [2] calculation was carried out evaluating the ligand binding site of the 2P54 structure, using a more restrictive definition of hydrophobicity, a standard grid and the OPLS-2005 force field.

Details on the setup of the docking protocol. All stilbene derivatives, in the deprotonated form were docked in the crystal structure of PPARα with PDB ID: 2P54. This X-ray structure was chosen among available crystallographic complexes because of its good resolution (1.79 Å) and the structural similarity between the co-crystallized ligand and the studied compounds. Glide docking protocol was validated by the reproduction of the crystallographic pose of X-ray ligands. This preliminary analysis allowed us to evaluate the ability of receptor structure to correctly reproduce the X-ray ligands binding mode. During docking calculations, conserved water molecules placed in a side pocket of PPARα

binding region, surrounded by residues Thr238, Ile317, Leu321, Met330, Val324, Ser280, were explicitly considered to avoid incorrect positioning of ligands: eliminating all water molecules, in fact, produced poses occupying mainly this pocket for most of the studied derivatives, probably because of conformational requirements of ligands. The reliability of predicted poses was assessed comparing them with available experimental structures and analysing properties of the binding site. The inspection of all available crystallographic ligands revealed as none of them binds in this side pocket. Moreover the SiteMap calculation performed on the receptor to better depict the binding region properties, highlighted that the side pocket is large and amphipathic, differently from the main binding site that is exclusively hydrophobic (**Figure 1**).

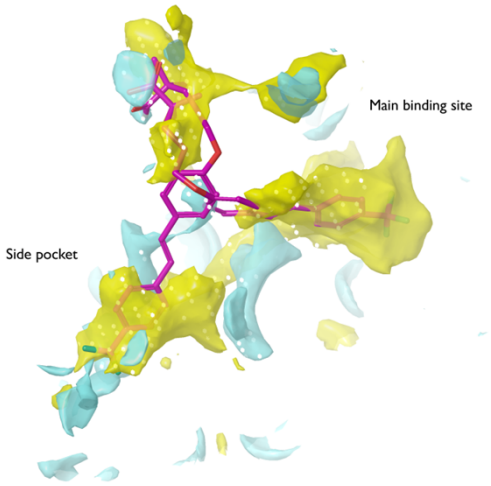

Figure 1. SiteMap analysis of the PPARα binding site. The surface indicate regions where interaction are preferred: yellow hydrophobic and cyan hydrophilic. Binding mode of the **5d** ligand in the main and side pocket is reported.

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