Small Molecule-Mediated Gene Regulation Caused by Vitamin D Receptor –Coactivator Binding Inhibition.

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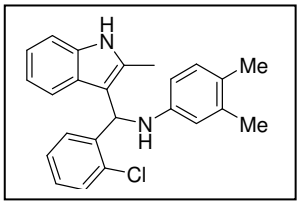
Contents

Experimental Section

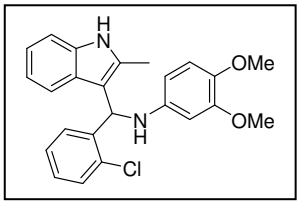
Chemistry. All chemicals and solvents were purchased from commercial suppliers and were used without any further purification. Solvents were handled under anhydrous condition using syringe technique. All glassware was dried overnight at 70°C before use. Thin layer chromatography was performed on pre-coated silica gel 60 F254 plates (Fisher Scientific). Purification of newly synthesized compounds was carried by normal phase automated flash chromatography (Biotage SP1, Silica gel 230-400 mesh size), concentrated under vacuum and dried at high vacuum overnight. All pure compounds were stored as solids at -20°C. For biochemical evaluation, compounds were dissolved in DMSO at a stock concentration of 10 mM. The purity of compounds was determined by LC-MS (Surveyor & MSQ) using a C18 column. All compounds had a purity of >95%. NMR spectra were recorded on a Bruker™ 400 MHZ using $CDCl_3$ (δ 7.26 ppm for ^1H and δ 77 ppm for ^{13}C as reference.

General Procedure for the Aza Friedel-Crafts Reaction. In a dry microwave vial (10 mL), aniline derivative (2 mmol) and benzaldehyde derivative (2 mmol) were either dissolved in toluene (1 mL) or used neat and stirred under microwave irradiation at 100 °C for 10-30 minutes. After the formation of the imine, indole derivative (2 mmol) and decanoic acid (0.2 mmol, 10 mol %) were added slowly as a solution in toluene (1 mL). The reaction mixture was stirred at room temperature and reaction progress was monitored using TLC (using ethyl acetate:hexanes). The reaction was quenched by adding saturated aqueous $NaHCO_3$ (2 mL). The mixture was extracted between brine (30 mL) and dichloromethane (3x30 mL) and dried over anhydrous sodium sulfate. The organic layer were combined and dried under vacuum, and residue was purified either by a recrystalization (toluene) or a normal phase flash chromatography using a Biotage SP1 automated flash system.

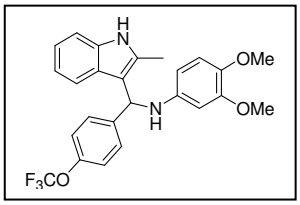
NMR



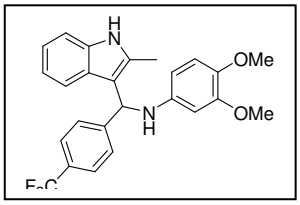
1; 1H (CDCl3, 300 MHz) δ 2.15 (s, 3H), 2.16 (s, 3H), 2.38 (s, 3H), 4.19 (s, 1H), 5.91 (s, 1H), 6.24 (dd, J=5.7, 2.4 Hz, 1H), 6.38 (d, J=1.8 Hz, 1H), 6.90 (d, J=8.1 Hz, 1H), 6.98 (t, J=7.2 Hz, 1H), 7.10 (t, J=7.5 Hz, 1H), 7.20 (t, J=7.5 Hz, 1H), 7.26 (m, 2H), 7.34 (d, J=7.8 Hz, 1H), 7.43 (d, J=7.8 Hz, 1H), 7.84 (s, 1H), 7.95 (d, J=7.8 Hz, 1H).13C (CDCl3, 300 MHz) δ 12.50, 18.70, 20.07, 53.33, 110.25, 111.32, 115.02, 118.89, 119.54, 121.16, 125.30, 127.08, 128.30, 129.96, 130.21, 132.81, 133.27, 135.07, 137.22, 139.34, 145.58. ESI HRMS (+ve) m/z calcd. for C24H23ClN2 [(M-H)-] 373.15, found 373.27 [(M-H)-].



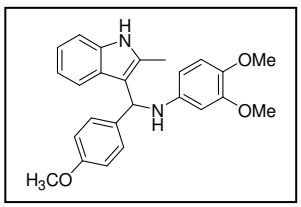
2; 1H (CDCl3, 300 MHz) δ δ 2.41 (s, 3H), 3.74 (s, 3H), 3.83 (s, 3H), 4.60 (s, 1H), 5.79 (s, 1H), 6.50 (s, 1H), 6.97 (d, J=8.4 Hz, 1H), 7.03 (t, J=7.5 Hz, 1H), 7.13 (t, J=8.4 Hz, 1H), 7.31 (m, 2H), 7.50 (m, 3H), 7.80 (s, 1H), 7.83 (dd, J=5.7, 1.8 Hz, 1H). 13C (CDCl3, 300 MHz) δ 12.55, 53.49, 55.39, 55.71, 99.06, 103.48, 110.16, 110.63, 111.40, 119.06, 119.46, 121.02, 126.63, 127.31, 128.03, 129.07, 129.92, 131.80, 132.88, 135.04, 139.48, 147.70. ESI HRMS (+ve) m/z calcd. for C24H23ClN2O2 [(M-H)-)] 405.14, found 405.30 [(M-H)-].



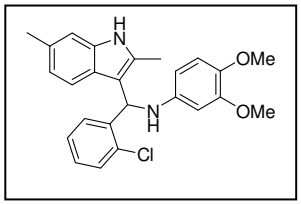
3; 1H (CDCl3, 300 MHz) δ 2.40 (s, 3H), 3.76 (s, 3H), 3.81 (s, 3H), 4.64 (s, 1H), 5.69 (s, 1H), 6.34 (s, 2H), 6.50 (s, 1H), 7.04 (t, J=7.2 Hz, 1H), 7.14 (t, J=8.4 Hz, 1H), 7.29 (d, J=7.2 Hz, 1H), 7.50 (m, 3H), 7.85 (s, 1H). 13C (CDCl3, 300 MHz) δ 12.53, 55.27, 55.45, 55.64, 99.10, 103.52, 110.29, 110.94, 113.73, 118.97, 119.67, 120.80, 121.35, 126.98, 128.28, 131.78, 132.23, 135.15, 142.16, 147.82, 151.96. ESI HRMS (+ve) m/z calcd. for C25H23F3N2O3 [(M-H)-)] 455.17, found 455.90 [(M-H)-].



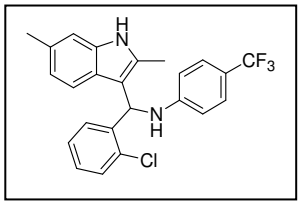
4; 1H (CDCl3, 300 MHz) δ 2.42 (s, 3H), 3.76 (s, 1H), 3.81 (s, 1H), 4.54 (s, 1H), 5.72 (s, 1H), 6.30 (d, J=4.2 Hz, 2H), 6.50 (d, J=2.1 Hz, 1H), 7.04 (t, J=7.5 Hz, 1H), 7.14 (t, J=7.5 Hz, 1H), 7.29 (d, J=7.5 Hz, 1H), 7.49 (d, J=7.2 Hz, 1H), 7.56 (d, J=8.1 Hz, 2H), 7.63 (d, J=8.1 Hz, 2H), 7.88 (s, 1H). 13C (CDCl3, 300 MHz) δ 12.39, 55.45, 55.62, 55.74, 99.13, 103.48, 110.33, 110.94, 113.56, 118.91, 119.74, 121.43, 125.31, 125.36, 126.85, 127.21, 131.83, 132.12, 135.15, 147.83, 152.04.



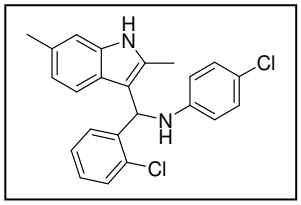
5; 1H (CDCl3, 300 MHz) δ 2.39 (s, 3H), 3.78 (s, 3H), 3.80 (s, 6H), 5.67 (s, 1H), 6.32 (d, J=2.4 Hz, 1H), 6.38 (d, J=8.2 Hz, 1H), 6.47 (d, J=2.4 Hz, 1H), 6.85 (d, *J*=8.7 Hz, 3H), 7.03 (t, *J*=7.2 Hz, 2H), 7.10 (t, *J*=7.2 Hz, 2H), 7.26 (d, *J*=7.2 Hz, 1H), 7.03 (t, *J*=7.2 Hz, 2H), 7.27 (d, *J*=7.2 Hz, 2H), 7.41 (d, *J*=8.7 Hz, 2H), 7.52 (d, *J*=7.8 Hz, 2H), 7.81 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.38, 55.22, 55.26, 55.44, 55.75, 99.00, 103.59, 110.14, 110.93, 113.53, 113.71, 114.18, 119.16, 119.47, 121.09, 127.30, 127.82, 128.06, 131.63, 132.60, 135.14, 135.53, 147.73, 151.63, 158.25.



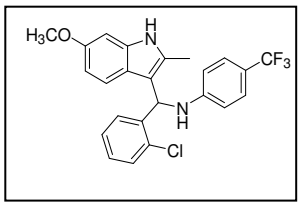
6; ^{1}H ($CDCl_3$, 300 MHz) δ 2.24 (s, 3H), 3.73 (s, 3H), 3.81 (s, 3H), 5.84 (s, 1H), 6.20 (d, *J*=6.3 Hz, 1H), 6.26 (d, *J*=2.4 Hz, 1H), 6.47 (s, 1H), 6.60 (s, 1H), 6.70 (d, *J*=8.4 Hz, 1H), 6.90 (m, 2H), 7.26 (m, 3H), 7.40 (t, *J*=7.2 Hz, 1H), 7.78 (m, 1H), 7.93 (m, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.43, 21.46, 53.46, 56.78, 56.97, 98.87, 106.74, 112.65, 115.67, 121.34, 122.34, 126.59, 127.89, 128.49, 129.12, 129.49, 130.38, 133.48, 136.78, 137.29, 139.76, 142.89, 148.39.



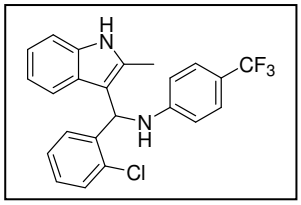
7; ^{1}H ($CDCl_3$, 300 MHz) δ 2.33 (s, 1H), 2.37 (s, 3H), 4.63 (d, *J*=3.3 Hz, 1H), 5.99 (d, *J*=3.3 Hz, 1H), 6.97 (d, *J*=8.4 Hz, 1H), 7.18 (s, 1H), 7.20 (d, *J*=2.7 Hz, 1H), 7.24 (d, *J*=1.8 Hz, 1H), 7,27 (d, *J*=1.8 Hz, 1H), 7.37-7.41 (m, 3H), 7.80 (s, 1H), 7.83 (dd, *J*=5.7, 1.8 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.53, 21.60, 52.95, 109.73, 110.10, 112.41, 118.26, 122.92, 126.52, 126.57, 126.75, 127.19, 128.53, 128.63, 129.01, 130.24, 133.14, 133.36, 133.46, 138.16, 149.66. ESI HRMS (+ve) m/z calcd. for $C_{24}H_{20}ClF_3N_2$ [(M-H)$^-$)] 427.13, found 427.27 [(M-H)$^-$].



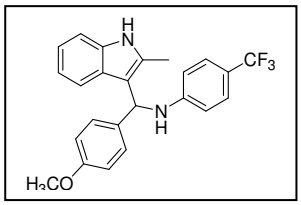
8; ^{1}H ($CDCl_3$, 300 MHz) δ 2.33 (s, 1H), 2.33 (s, 3H), 2.37 (s, 3H), 5.90 (s, 1H), 6.42 (d, *J*=5.7 Hz, 2H), 6.93 (d, *J*=8.1 Hz, 1H), 7.08 (d, *J*=8.7 Hz, 2H), 7.20 (m, 2H), 7.30 (d, *J*=6.0 Hz, 1H), 7.37 (d, *J*=5.7 Hz, 1H), 7.76 (s, 1H), 7.87 (d, *J*=6.0 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.47, 21.78, 53.47, 112.87, 113.79, 114.12, 121.49, 122.28, 123.76, 126.57, 128.11, 128.51, 128.91, 128.12, 128.91, 130.11, 133.45, 135.76, 138.12, 142.65, 144.58. ESI HRMS (+ve) m/z calcd. for $C_{23}H_{20}C_{l2}N_2$ [(M-H)$^-$)] 393.10, found 393.40 [(M-H)$^-$].



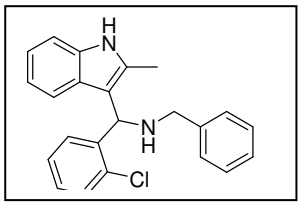
9; ^{1}H ($CDCl_3$, 300 MHz) δ 2.36 (s, 3H), 3.71 (s, 3H), 5.87 (s, 1H), 6.42 (d, *J*=8.7 Hz, 2H), 6.75 (m, 2H), 7.09 (d, *J*=8.7 Hz, 2H), 7.17 (d, *J*=8.7 Hz, 1H), 7.23 (m, 1H), 7.30 (m, 1H), 7.35 (dd, *J*=6.6, 1.2 Hz, 1H), 7.76 (s, 1H), 7.86 (dd, *J*=6.0, 1.5 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.54, 53.36, 55.71, 101.15, 110.45, 110.72, 110.95, 114.24, 122.18, 126.61, 127.43, 128.44, 128.74, 129.02, 130.11, 130.24, 133.48, 133.90, 138.53, 145.86, 153.89. ESI HRMS (+ve) m/z calcd. for $C_{23}H_{20}Cl_2N_2O$ [(M-H)$^-$] 409.10, found 409.23 [(M-H)$^-$].



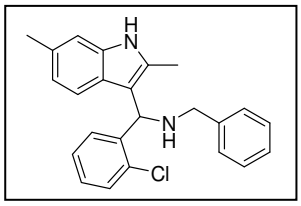
10; ^{1}H ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H), 4.64 (d, *J*=3.3 Hz, 1H), 6.01 (d, *J*=3.3 Hz, 1H), 6.51 (d, *J*=8.7 Hz, 2H), 7.01 (t, *J*=7.5 Hz, 1H), 7.11 (t, *J*=7.5 Hz, 1H), 7.28 (m, 2H), 7.39 (m, 4H), 7.84 (d, *J*=6 Hz, 1H), 7.90 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.45, 52.97, 110.22, 110.47, 112.42, 118.56, 119.80, 121.43, 126.54, 126.60, 126.84, 126.88, 128.60, 130.26, 133.13, 133.43, 135.12, 138.13, 149.63. ESI HRMS (+ve) m/z calcd. for $C_{23}H_{18}ClF_3N_2$ [(M+H)$^+$)] 415.11, found 415.19 (M+H)$^+$.



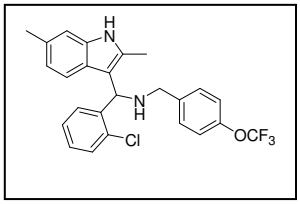
PS121912; ^{1}H ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H), 3.80 (s, 3H), 5.79 (s, 1H), 6.59 (d, *J*=8.1 Hz, 2H), 6.86 (d, *J*=8.7 Hz, 2H), 7.02 (t, *J*=7.2 Hz, 1H), 7.13 (t, *J*=7.2 Hz, 1H), 7.31 (s, 1H), 7.38 (dd, *J*=9.3, 2.1 Hz, 4H), 7.90 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.23, 54.47, 55.25, 110.40, 112.42, 112.92, 113.97, 114.16, 118.72, 119.78, 121.49, 126.41, 126.46, 126.51, 126.87, 127.97, 131.92, 133.81, 135.18, 150.26, 158.61. ESI HRMS (+ve) m/z calcd. for $C_{24}H_{21}F_3N_2O$ [(M-H)$^-$)] 410.16, found 409.27 [(M-H)$^-$].



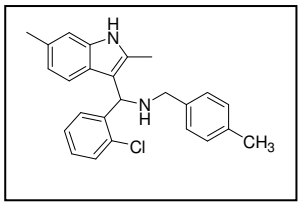
12; ^{1}H ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H), 3.85 (s, 2H), 5.54 (s, 1H), 7.05-7.20 (m, 3H), 7.25-7.32 (m, 3H), 7.36 (d, *J*=4.5 Hz, 1H), 7.79 (s, 1H), 7.82 (d, *J*=7.5 Hz, 1H), 8.07 (d, *J*=7.5 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.55, 52.34, 55.74, 110.18, 11.61, 119.37, 119.74, 120.94, 126.71, 126.88, 127.89, 128.27, 128.35, 129.44, , 129.66, 132.71, 133.66, 135.23, 140.63, 140.72. ESI HRMS (+ve) m/z calcd. for $C_{23}H_{21}ClN_2$ [(M-H)$^-$] 359.14, found 359.29 [(M-H)$^-$].



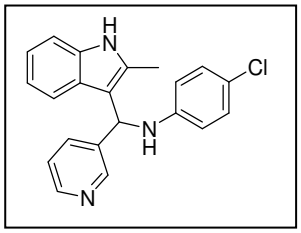
13; ^{1}H ($CDCl_3$, 300 MHz) δ 2.33 (s, 3H), 2.44 (s, 3H), 3.84 (s, 2H), 5.51 (s, 1H), 6.96 (d, *J*=8.1 Hz, 1H), 7.14-7.21 (m, 2H), 7.28-7.37 (m, 4H), 7.57 (s, 1H), 7.68 (s, 1H), 8.04 (d, *J*=6.3 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.57, 21.68, 52.36, 55.66, 109.80, 11.08, 119.39, 122.44, 126.67, 126.87, 127.86, 128.33, 129.52, 129.64, 132.82, 133.48, 133.68, 140.66, 140.78. ESI HRMS (+ve) m/z calcd. for $C_{24}H_{23}ClN_2$ [(M-H)$^-$)] 373.15, found 373.31 [(M-H)$^-$].



14; ^{1}H ($CDCl_3$, 300 MHz) δ 2.34 (s, 3H), 2.43 (s, 3H), 3.88 (s, 2H), 5.48 (s, 1H), 6.95 (d, J=8.1 Hz, 1H), 7.16 (d, J=8.1 Hz, 3H), 7.30 (m, 1H), 7.45 (d, J=8.1 Hz, 2H), 7.57 (m , 3H), 7.71 (s, 1H), 7.95 (d, J=6.6 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.60, 21.65, 51.82, 55.80, 109.85, 110.74, 119.32, 122.55, 125.15, 125.20, 126.74, 128.01, 128.43, 128.88, 129.40, 129.68, 132.84, 133.49, 133.59, 140.54, 144.74. ESI HRMS (+ve) m/z calcd. for $C_{25}H_{22}ClF_3N_2$ [(M-H)$^-$)] 441.14, found 441.29 [(M-H)$^-$].

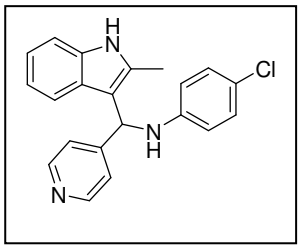


15; ^{1}H ($CDCl_3$, 300 MHz) δ 2.33 (s, 3H), 2.36 (s, 3H), 2.41 (s, 3H), 3.77 (s, 2H), 5.48 (s, 1H), 6.93 (d, *J*=8.4 Hz, 1H), 7.13 (m, 3H), 7.22 (m, 2H), 7.29 (m, 2H), 7.51 (s, 1H), 7.67 (s, 1H), 8.00 (dd, *J*=1.2, 8.4 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.61, 21.12, 21.64, 52.06, 55.62, 109.71, 111.17, 119.38, 122.39, 126.61, 127.78, 128.07, 128.23, 128.43, 128.98, 129.49, 129.59, 132.75, 133.44, 133.67, 136.35, 137.61, 140.82. ESI HRMS (+ve) m/z calcd. for $C_{25}H_{25}ClN_2$ [(M-H)$^-$)] 396.17, found 396.27 [(M-H)$^-$].

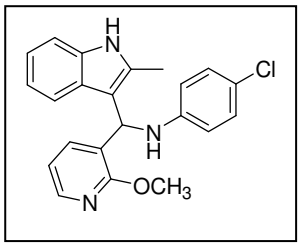


16; ^{1}H ($CDCl_3$, 300 MHz) δ 2.41 (s, 3H), 5.75 (s, 1H), 6.49 (d, *J*=8.7 Hz, 1H), 7.02 (t, *J*= 4.2 Hz, 1H), 7.14 (t, *J*= 7.2 Hz, 2H), 7.25 (m, 3H), 7.38 (d, *J*= 7.8 Hz, 1H), 7.82 (d, *J*= 7.8 Hz, 1H), 8.31 (s, 1H), 8.49 (d, *J*= 1.2 Hz, 1H), 8.68 (d, *J*= 1.2 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.43, 56.35, 112.12, 113.10, 113.98, 115.08, 119.46, 120.38, 121.97, 122.87, 123.38, 129.15, 130.67, 131.56, 133.48, 135.78, 136.39, 138.28, 141.62,

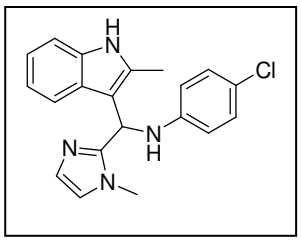
148.47, 151.28. ESI HRMS (+ve) m/z calcd. for $C_{21}H_{18}ClN_3$ [(M-H)$^-$)] 346.12, found 346.24 [(M-H)$^-$].



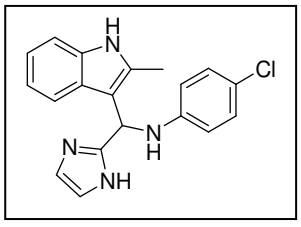
17; ^{1}H ($CDCl_3$, 300 MHz) δ 2.43 (s, 3H), 4.44 (s, 1H), 5.68 (s, 1H), 6.47 (d, *J*=8.7 Hz, 2H), 7.03 (t, J=7.2 Hz, 1H), 7.13 (d, J=8.7 Hz, 3H), 7.29 (d, J=7.8 Hz, 1H), 7.37 (d, J=7.8 Hz, 1H), 7.44 (d, J=5.4 Hz, 2H), 8.55 (d, J=6.0 Hz, 2H), 8.71 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.30, 54.76, 110.73, 111.86, 114.45, 118.38, 119.90, 121.69, 122.24, 122.68, 126.28, 129.13, 132.45, 135.36, 146.06, 149.59, 152.27. ESI HRMS (+ve) m/z calcd. for $C_{21}H_{18}ClN_3$ [(M-H)$^-$)] 346.12, found 346.24 [(M-H)$^-$].



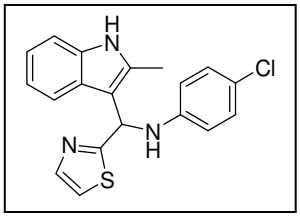
18; ^{1}H ($CDCl_3$, 300 MHz) δ 2.40 (s, 3H), 3.97 (s, 3H), 4.85 (d, *J*=2.7 Hz, 1H), 5.94 (d, *J*=2.7 Hz, 1H), 6.42 (d, *J*=7.5 Hz, 1H), 6.67 (dd, *J*=5.1, 2.7 Hz, 1H), 7.01 (t, *J*=8.2 Hz, 1H), 7.12 (t, *J*=8.2 Hz, 1H), 7.24 (m, 1H), 7.27 (m, 1H), 7.37 (d, *J*=7.5 Hz, 1H), 7.42 (d, *J*=8.2 Hz, 1H), 7.50 (d, *J*=2.4 Hz, 1H), 7.83 (d, *J*=8.2 Hz, 1H), 7.91 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.50, 52.66, 53.21, 110.33, 110.47, 115.60, 117.35, 118.79, 119.66, 121.23, 126.76, 127.13, 128.41, 128.74, 130.12, 131.95, 132.90, 133.09, 133.43, 135.08, 138.32, 152.38. ESI HRMS (+ve) m/z calcd. for $C_{25}H_{20}ClN_3$ [(M-H)$^-$] 377.13, found 377.10 [(M-H)$^-$].



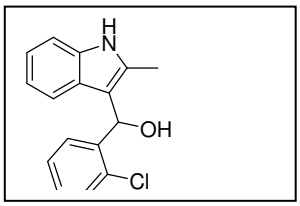
19; ^{1}H ($CDCl_3$, 300 MHz) δ 2.32 (s, 3H), 3.43 (s, 3H), 5.87 (s, 1H), 6.59 (d, *J*=8.2 Hz, 2H), 6.81 (s, 1H), 7.04 *J*=4.8 Hz, 2H), 7.38 (d, *J*=8.2 Hz, 2H), 7.55 (d, *J*=4.8 Hz, 1H), 8.04 (s, 1H), 8.55 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.48, 28.81, 56.48, 111.38, 113.29, 119.86, 120.28, 121.29, 124.19, 126.87, 128.29, 128.39, 128.69, 129.39, 131.39, 133.29, 136.49, 139.39, 141.29, 144.38. ESI HRMS (+ve) m/z calcd. for $C_{19}H_{18}ClN_4$ [(M-H)$^-$)] 349.14, found 349.32 [(M-H)$^-$].



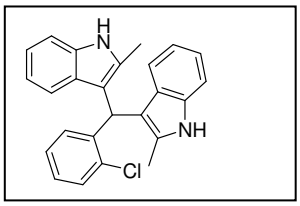
20; ^{1}H ($CDCl_3$, 300 MHz) δ 2.34 (s, 3H), 5.82 (s, 1H), 6.53 (d, *J*=8.2 Hz, 2H), 6.91 (s, 1H), 7.03 *J*=4.8 Hz, 2H), 7.41 (d, *J*=8.2 Hz, 2H), 7.59 (d, *J*=4.8 Hz, 1H), 8.045(s, 1H), 8.58 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.41, 28.81, 111.48, 113.21, 119.66, 121.28, 121.99, 124.10, 126.87, 127.29, 128.39, 129.69, 130.39, 132.39, 134.29, 136.49, 140.39, 142.29, 145.38.



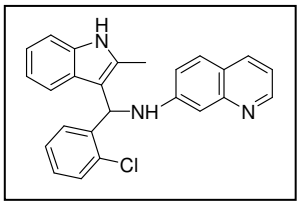
21; ^{1}H ($CDCl_3$, 300 MHz) δ 2.37 (s, 3H), 4.96 (s, 1H), 6.01 (s, 1H), 6.61 (d, *C*), 7.08-7.14 (m, 4H), 7.25-7.29 (m, 2H), 7.61 (d, *J*=8.1 Hz, 1H), 7.76 (d, *J*=3.3 Hz, 1H), 8.16 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.14, 54.83, 110.68, 114.84, 118.62, 119.30, 119.95, 121.63, 126.11, 129.05, 132.84, 135.35, 142.81, 145.83, 175.21. ESI HRMS (+ve) m/z calcd. for $C_{19}H_{16}ClN_3S$ [(M-H)$^-$] 352.08, found 352.84 [(M-H)$^-$].



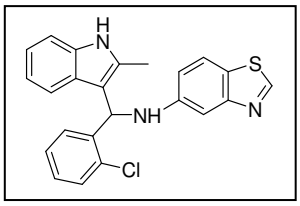
22; ^{1}H ($CDCl_3$, 300 MHz) δ 2.23 (s, 1H), 2.43 (s, 3H), 6.36 (s, 1H), 7.02 (t, *J*=7.2 Hz, 1H), 7.09 (t, *J*=7.2 Hz, 1H), 7.24-7.28 (m, 2H), 7.32-7.37 (m, 2H), 7.48 (d, *J*=7.2 Hz, 1H), 7.93 (s, 1H), 8.03 (d, *J*=2.4 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.38, 67.21, 110.29, 111.95, 119.13, 119.70, 121.27, 126.59, 126.78, 128.07, 128.33, 129.62, 132.46, 133.31, 135.18, 140.60. ESI HRMS (+ve) m/z calcd. for $C_{16}H_{14}ClNO$ [$(M-H)^-$] 270.08, found 270.25 [$(M-H)^-$].



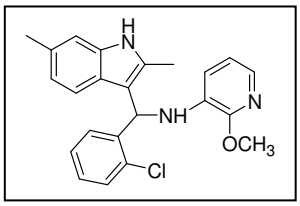
23; ^{1}H ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H), 6.22 (s, 1H), 6.86 (t, *J*=6.9 Hz, 2H), 6.96 (d, *J*=6.9 Hz, 2H), 7.05 (t, *J*=6.9 Hz, 2H), 7.14 (m, 4H), 7.29 (m, 3H), 7.39 (d, *J*=7.3 Hz, 1H), 7.75 (s, 2H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.23, 37.36, 109.97, 112.01, 118.95, 119.17, 120.66, 125.30, 126.43, 127.59, 128.23, 129.04, 129.49, 131.02, 131.95, 135.00, 141.37. ESI HRMS (+ve) m/z calcd. for $C_{25}H_{21}ClN_2$ [$(M+H)^+$)] 385.14, found 385.25 $(M+Na)^+$.



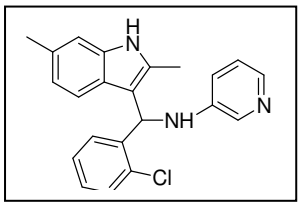
24; ^{1}H ($CDCl_3$, 300 MHz) δ 2.40 (s, 3H), 5.47 (d, *J*=5.4 Hz, 1H), 6.61 (d, *J*=8.7 Hz, 2H), 6.98 (t, *J*=7.2 Hz, 1H), 7.10 (t, *J*=7.2 Hz, 1H), 7.21 (m, 4H), 7.38 (d, *J*=6.0 Hz, 1H), 7.42 (d, *J*=8.4 Hz, 1H), 7.50 (t, *J*=8.7 Hz, 1H), 7.58 (d, *J*=6.0 Hz, 1H), 7.68 (d, *J*=7.2 Hz, 1H), 7.80 (m, 2H), 7.98 (s, 1H) . ^{13}C ($CDCl_3$, 300 MHz) δ 12.50, 50.97, 110.38, 110.44, 118.64, 119.61, 121.19, 122.18, 123.60, 126.45, 126.59, 127.03, 127.42, 128.23, 128.36, 128.42, 129.54, 130.05, 133.12, 133.53, 135.17, 137.67, 139.37, 148.05, 156.00. ESI HRMS (+ve) m/z calcd. for $C_{25}H_{20}ClN_3$ [$(M-H)^-$] 396.13, found 396.27 [$(M-H)^-$].



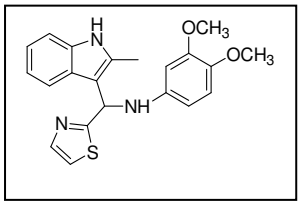
25; ^{1}H ($CDCl_3$, 300 MHz) δ 2.36 (s, 3H), 2.77 9s, 3H), 4.47 (d, *J*=2.7 Hz, 1H), 6.01 (d, *J*=2.7 Hz, 1H), 6.70 (dd, *J*=2.4, 6.3 Hz, 1H), 7.01 (m, 2H), 7.09 (t, *J*=5.2 Hz, 1H), 7.20 (m, 1H), 7.27 (m, 2H), 7.35 (d, *J*=6.3 Hz, 1H), 7.37 (d, *J*=2.4 Hz, 1H), 7.46 (d, *J*=2.4 Hz, 1H), 7.54 (d, *J*=6.3 Hz, 1H), 7.95 (d, *J*=5.2 Hz, 1H), 8.01 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.43, 20.07, 53.63, 104.69, 110.40, 110.81, 113.72, 118.76, 119.62, 121.22, 121.45, 124.25, 126.72, 127.07, 128.37, 128.74, 130.21, 133.09, 133.56, 135.14, 138.59, 146.54, 154.85, 167.56. ESI HRMS (+ve) m/z calcd. for $C_{24}H_{20}ClN_3S$ [$(M-H)^-$] 416.11, found 416.11 [$(M-H)^-$].



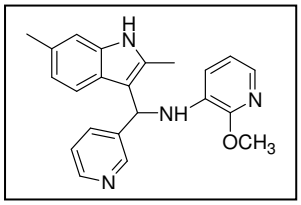
26; ^{1}H ($CDCl_3$, 300 MHz) δ 2.34 (s, 3H), 2.40 (s, 3H), 4.00 (s, 3H), 4.88 (s, 1H), 5.96 (s, 1H), 6.46 (d, *J*=7.5 Hz, 1H), 6.70 (dd, *J*=5.1, 2.7 Hz, 1H), 6.97 (d, *J*=8.1 Hz, 1H), 7.18 (d, *J*=8.1 Hz, 1H), 7.29 (d, *J*=8.1 Hz, 1H), 7.39 (d, *J*=7.8 Hz, 1H), 7.54 (d, *J*=5.1 Hz, 1H), 7.86 (d, *J*=7.8, 1H), 7.98 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.50, 21.66, 52.71, 53.29, 53.33, 109.87, 110.06, 115.70, 117.44, 118.47, 122.70, 126.72, 127.48, 128.41, 128.81, 130.15, 132.09, 132.80, 133.29, 133.40, 133.49, 138.43, 152.43. ESI HRMS (+ve) m/z calcd. for $C_{23}H_{22}ClN_3O$ [$(M+H)^+$)] 392.15, found 392.17 $(M+Na)^+$.



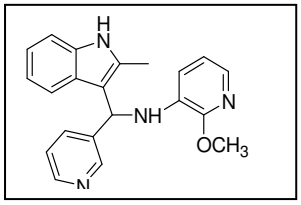
27; ^{1}H ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H), 5.22 (d, *J*=4.5 Hz, 1H), 6.22 (s, 1H), 6.24 (d, *J*=3.6 Hz, 1H), 6.58-6.63 (m, 1H), 6.96 (t, *J*=6.9 Hz, 1H), 7.09 (t, *J*=6.9 Hz, 1H), 7.22 (m, 2H), 7.28-7.36 (m, 2H), 7.86 (d, *J*=6.0 Hz, 1H), 7.93 (s, 1H), 8.11 (d, *J*=3.9 Hz, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.48, 21.39, 53.29, 109.29, 111.67, 113.89, 114.29, 121.29, 123.39, 126.13, 127.87, 128.19, 128.39, 129.07, 129.59, 133.29, 135.69, 136.67, 138.29, 142.78, 148.96, 162.39.



28; ^{1}H ($CDCl_3$, 300 MHz) δ 2.47 (s, 3H), 3.75 (s, 3H), 3.81 (s, 1H), 5.11 (s, 1H), 5.98 (s, 1H), 6.34 (dd, *J*=2.4, 6.0 Hz, 1H), 6.49 (m, 2H), 7.10 (m, 2H), 7.23 (d, *J*=3.3 Hz, 1H), 7.29 (d, *J*=6.6 Hz, 1H), 7.70 (d, *J*=7.5 Hz, 1H), 7.75 (d, *J*=3.3 Hz, 1H), 7.95 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.30, 55.52, 55.59, 55.69, 99.19, 103.47, 110.42, 111.33, 111.73, 118.91, 119.14, 119.81, 121.41, 126.39, 131.75, 132.47, 135.28, 142.85, 148.17, 152.58, 176.57. ESI HRMS (+ve) m/z calcd. for $C_{21}H_{21}N_3O_2S$ [$(M-H)^-$)] 378.14, found 378.29 [$(M-H)^-$].



29; ^{1}H ($CDCl_3$, 300 MHz) δ 2.42 (s, 3H), 3.98 (s, 3H), 4.92 (s, 1H), 5.77 (s, 1H), 6.54 (d, J=7.8 Hz, 1H), 6.70 (dd, J=5.1, 2.4 Hz, 1H), 7.04 (t, J=7.5 Hz, 1H), 7.13 (t, J=7.5 Hz, 1H), 7.27 (m, 2H), 7.44 (d, J=7.8 Hz, 1H), 7.53 (d, J=5.1, 1H), 7.82 (d, J=7.8 Hz, 1H), 8.53 (m, 2H), 8.70 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.43, 21.79, 56.79, 56.05, 111.38, 111.87, 112.89, 120.39, 122.57, 122.89, 123.68, 128.46, 128.97, 132.58, 135.34, 135.96, 136.20, 136.84, 137.56, 148.49, 152.38, 153.29. ESI HRMS (+ve) m/z calcd. for $C_{22}H_{22}N_4O$ [$(M+Na)^+$)] 380.18, found 380.43 $(M+Na)^+$.



30; ^{1}H ($CDCl_3$, 300 MHz) δ 2.42 (s, 3H), 3.98 (s, 3H), 4.92 (s, 1H), 5.77 (s, 1H), 6.54 (d, *J*=7.5 Hz, 1H), 6.70 (dd, *J*=5.4, 2.4 Hz, 1H), 7.04 (t, *J*=7.5 Hz, 1H), 7.13 (t, *J*=8.1 Hz, 1H), 7.28 (m, 2H), 7.44 (d, *J*=8.1 Hz, 1H), 7.54 (d, *J*=4.8 Hz, 1H), 7.81 (d, *J*=8.1 Hz, 1H), 8.51 (dd, *J*=9.9, 4.8 Hz, 1H), 8.70 (s, 1H). ^{13}C ($CDCl_3$, 300 MHz) δ 12.32, 53.32, 110.58, 112.00, 115.80, 117.31, 118.53, 119.84, 121.53, 123.49, 126.58, 132.21, 133.30, 134.50, 135.34, 137.89, 148.15, 148.91, 152.46. ESI HRMS (+ve) m/z calcd. for $C_{21}H_{20}N_4O$ [$(M-H)^-$)] 343.16, found 343.32 [$(M-H)^-$].

Reagents, Protein and Peptides. GW7647, Rosiglitazone, GW0742, Bexarotene, T3, and estradiol were purchased from Sigma. $1,25(OH)_2D_3$ was purchased from Endotherm, Germany. LG190178 was synthesized following a published procedure.[40] Peptides SRC1-3 (CESKDHQLLRYLLDKDEKDL), SRC2-3 (CKKKENALLRYLLDKDDTKD), SRC2-2 (CLKEKHKILHRLLQDSSSPV), SRC3-3 (CKKENNALLRYLLDRDDPSD) and DRIP2 (CNTKNHPMLMNLLKDNPAQD) were purchased and labeled with cysteine-reactive fluorophores, such as Alexa Fluor 647 maleimide and Fluorescein maleimide. The labeled peptides were purified by reverse phase quantitative HPLC using a C18 column and stored at -20°C. The VDR-LBD was produced as described before.[24]

VDR-SRC2-3 fluorescence polarization assay. The FP assay was conducted in 384- well black polystyrene microplates (Corning). The assay buffer was composed of 25 mM PIPES, 50 mM NaCl, 0.01% NP-40, 2% DMSO, VDR-LBD (1 μM), LG190178 (5 μM), and Alexa Fluor 647-labeled SRC2-3 (7.5 nM). 10 mM stock solutions of synthesized compound were made in DMSO. Each compound was serially diluted, and four 20 μL aliquots of each compound concentration were added to a 384-well polypropylene plate. Using 50H hydrophobic coated pin tool (V&P Scientific), 100 nL of each compound well was transferred into a 384-well black assay plate. Fluorescence polarization was detected after 2 hours at an excitation/emission wavelength of 650/665 nm (Alexa Fluor) and 495/520 nm (Fluorescein). A positive control 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one and a negative control DMSO were measured within each plate to determine the assay plate quality and to enable data normalization. Three independent experiments were carried out in quadruplicate, and data was analyzed using nonlinear regression with a variable slope (GraphPrism).

CYP24A1 promoter transcription assays. The cell-based transcription assay was performed using HEK 293T cells (ATTC) which were cultured in 75 cm^2 flasks using DMEM/High Glucose (Hyclone, #SH3024301), non-essential amino acids, HEPES (10 mM), penicillin and streptomycin, and 10% of dialyzed FBS (Invitrogen, #26400-044). At 70-80 percent confluency, 2 mL of untreated DMEM containing 0.7 μg of VDR-CMV plasmid, 16 μg of a CYP24A1-luciferase reporter gene, Lipofectamine™ LTX (75 μl), and PLUS™ reagent (25 μl) was added. After 16 hours, the cells were harvested with 0.05% Trypsin (3 mL) (Hyclone, #SH3023601), added to 15 ml of DMEM high glucose (Hyclone, #SH3028401), non-essential amino acids, sodium pyruvate (1 mM), HEPES (10 mM), penicillin and streptomycin, and 2% percent charcoal treated FBS (Invitrogen, #12676-011), and spun down for 2 minutes at 1000 rpm. The cell were resuspended in the same media and plated in sterile cell culture treated black 384-well plates with optical bottom (Nunc, #142761) at a concentration of 15,000 cells per well, which were treated with a 0.25% solution of Matrigel (BD Bioscience, #354234) beforehand. After 2 hours, plated cells were treated with $1,25(OH)_2D_3$ (10 nM) and small molecules in vehicle DMSO, followed by a 16 hours incubation time. The inhibition of transcription was determined using Bright-Glo™ Luciferase Assay Kit (Promega, Madison, WI). Controls for cell viability were 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one (100 μM in DMSO) (positive) and DMSO (negative). Three independent experiments were performed in quadruplicate and data was analyzed using nonlinear regression with variable slope (GraphPrism).

Two-hybrid assay. The cell-based assay was performed using HEK 293T cells (ATTC) which were cultured in 75 cm^2 flasks using DMEM/High Glucose (Hyclone, #SH3024301), non-essential amino acids, HEPES (10 mM), penicillin and streptomycin, and 10% of dialyzed FBS (Invitrogen, #26400-044). At 70-80 percent confluency, 2 mL of untreated DMEM containing 5.0 μg of VP16-VDR-LBD plasmid, 4.0 μg of SRC1-GAL4 plasmid, 16 μg of a luciferase reporter plasmid, Lipofectamine™ LTX (75 μl), and PLUS™ reagent (25 μl) was added. After 16 hours, the cells were harvested with 0.05% Trypsin (3 mL) (Hyclone, #SH3023601), added to 15 ml of DMEM high glucose (Hyclone, #SH3028401), non-essential amino acids, sodium pyruvate (1 mM), HEPES (10 mM), penicillin and streptomycin, and 2% percent charcoal treated FBS (Invitrogen, #12676-011), and spun down for 2 minutes at 1000 rpm. The cell were resuspended in the same media and plated in sterile cell culture treated black 384-well plates with optical bottom (Nunc, #142761) at a concentration of 15,000 cells per well, which were

treated with a 0.25% solution of Matrigel (BD Bioscience, #354234) beforehand. After 2 hours, plated cells were treated with $1,25(OH)_2D_3$ (10 nM) and small molecules in vehicle DMSO, followed by a 16 hours incubation time. The inhibition of transcription was determined using Bright-Glo™ Luciferase Assay Kit (Promega, Madison, WI). Controls for cell viability were 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one (100 μM in DMSO) (positive) and DMSO (negative). Three independent experiments were performed in quadruplicate and data was analyzed using nonlinear regression with variable slope (GraphPrism).

Solubility assay. In a 384 UV plate (Corning #3675), 16 compounds were serial diluted in quadruplet starting from a 10 mM compound stock solution in DMSO. Therefore, buffer (90 mM ethanolamine, 90 mM KH_2PO_4, 90 mM potassium acetate, and 30 mM KCl (pH 7.4)) containing 20 percent acetonitrile was used. The plate was sealed (Corning #6570), sonicated for 1 minute, and agitated for an additional 5 minutes before scanning from 230-800 nm at 5 minutes increments. A calibration plot was prepared for each compound for the maximal absorbance using background-subtracted values. A 384 well filter plate (Pall # 5037) was pre-wetted with 20 percent acetonitrile/buffer, and filled with buffer (47.5 μl) and 10 mM of compound in DMSO (2.5 μl). The final DMSO concentration was 5 percent. After sonication (1 minute) and agitation (12 hours), the mixtures were filtered and 30 μl of each well was transferred into a 384 well UV plate, together with the addition of 20 μl of acetonitrile. The plate was agitated for 5 minutes and scanned from 230-800 nm at 5 minutes increments. The solubility was determined using background-subtracted values and the following equation: sol=absorbance at λmax/slope*(5/3). Each plate had the following solubility standards: 4,5-diphenylimidazole (67.3 ± 3.7 μM), β-estradiol (43.0 ± 2.3 μM), diethylstilbestrol (108.3 ± 5.4 μM), ketoconazole (134.5 ± 2.4 μM), and 3-phenylazo-2,6-diaminopyridine (357.7 ± 7.0 μM). All experiments were conducted in quadruplet.

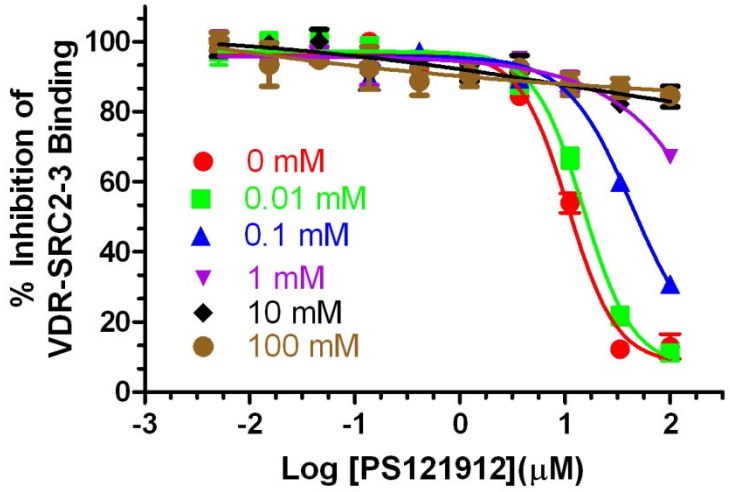
Permeability assay. This assay was carried out using Millipore's MultiscreenTM protocol, AN1725EN00. Each plate had the following standards with the following permeability values ($logP_e$): Ranitidine (-8.02 ± 0.074 cm/s) represents low permeability, carbamazepine (-6.81 ± 0.0011 cm/s) represents medium permeability, and verapamil (-5.93 ± 0.015 cm/s) represents high permeability. All experiments were conducted in triplet.

NR transcription assays. The cell-based transcription assay was performed using HEK 293T cells (ATTC) which were cultured in a 6-well plate using DMEM/High Glucose (Hyclone, #SH3024301), non-essential amino acids, HEPES (10 mM), penicillin and streptomycin, and 10% of dialyzed FBS (Invitrogen, #26400-044). At 70-80 percent confluency, 300 ul of untreated DMEM containing 0.5 μg of NR-LBD-Gal4-DBD plasmid, 2.6 μg of a 5xGAL4-luciferase reporter gene, Lipofectamine™ LTX (12.5 μl), and PLUS™ reagent (4.3 μl) was added to each well. After 16 hours, the cells of each well were harvested with 0.05% Trypsin (0.3 mL) (Hyclone, #SH3023601), added to 5 ml of DMEM high glucose (Hyclone, #SH3028401), non-essential amino acids, sodium pyruvate (1 mM), HEPES (10 mM), penicillin and streptomycin, and 2% percent charcoal treated FBS (Invitrogen, #12676-011), and spun down for 2 minutes at 1000 rpm. The cell were resuspended in the same media and plated in sterile cell culture treated black 384-well plates with optical bottom (Nunc, #142761) at a concentration of 15,000 cells per well, which were treated with a 0.25% solution of Matrigel (BD

Bioscience, #354234) beforehand. After 2 hours, plated cells were treated with PS121912 in vehicle DMSO, followed by a 16 hours incubation time. The following NR agonists were used: PPARα GW7647 (30 nM), PPARγ Rosiglitazone (300 nM), PPARδ GW0742 (50 nM), AR DHT (10 nM), RxRα Bexarotene (200 nM), TR T3 (10 nM), and ER estradiol 10 nM). Transcription was determined using Bright-Glo™ Luciferase Assay Kit (Promega, Madison, WI). Controls for cell viability were 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one (100 µM in DMSO) (positive) and DMSO (negative). Three independent experiments were performed in quadruplicate and data was analyzed using nonlinear regression with variable slope (GraphPrism).

VDR–coactivator binding assay. The FP assay was conducted in 384-well black polystyrene microplates (Corning). The assay buffer composed of 25 mM PIPES, 50 mM NaCl, 0.01% NP-40, 2% DMSO, VDR-LBD (1-10 µM), LG190178 (5 µM), and 7.5 nM of Fluorescein SRC1-3, Alexa Fluor 647 SRC2-2, Alexa Fluor 647 SRC2-3, Fluorescein SRC3-3, or Alexa Fluor 647 DRIP2. Using 50H hydrophobic coated pin tool (V&P Scientific) 100 nL of each concentration of PS121912 was transferred into a 384-well black assay plate. Fluorescence polarization was detected after 2 hours at an excitation/emission wavelength of 495/520 (Fluorescein) or 650/665 (Alexa Fluor). A positive control 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one and a negative control DMSO were measured within each plate to determine the assay plate quality and to enable data normalization. Three independent experiments were carried out in quadruplicate and data was analyzed using nonlinear regression with a variable slope (GraphPrism).

VDR–coactivator binding in the presence of mercaptoethanol: The FP assay was carried out as described above. In addition, different concentrations of mercaptoethanol were added. A positive control 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one and a negative control DMSO were measured within each plate to determine the assay plate quality and to enable data normalization. Three independent experiments were carried out in quadruplicate and data was analyzed using nonlinear regression with a variable slope (GraphPrism).



The IC_{50} values of PS121912 increase with increasing concentrations of mercaptoethanol: 0 mM (10.5±1.2 μM), 0.01mM (14.4±1.5 μM), 0.1mM (46.3±5.4 μM), 1 mM, (>100 μM).

Semi-quantitative real time PCR. HL-60 cells (ATTC) were cultured in 75 cm^2 flasks using DMEM/High Glucose (Hyclone, #SH3024301) or RPMI-1640 (ATTC, #30-2001), non-essential amino acids, HEPES (10 mM), penicillin and streptomycin, and 10% dialyzed FBS (Invitrogen, #26400-044). Cells were incubated at 37 °C with either DMSO (0.03%) or inhibitor PS121912 (7.5 μM) in the presence or absence of $1,25(OH)_2D_3$ (10 nM) for 18 hours in a 6 well plate. The cells were harvested using 0.3 ml of 0.25% Trypsin (Hyclone, #SH3023601) and added to DMEM or RPMI media (1 mL). The cell suspension was spun down for 2 minutes at 1000 rpm, media was removed and the cell pellet was resuspended in RTL buffer (RNAeasy kit, Qiagen) with the addition of mercaptoethanol. The cells were lysed using a QIAshredder (Qiagen) and total RNA was isolated using RNAeasy kit (Qiagen). A QuantiFast SYBR Green RT-PCR Kit (Qiagen) was used for the real time PCR following manufacturer's recommendations. Primers used in these studies are as follows: GAPDH FP 5'- ACCACAGTCCATGCCATCAC-3', GAPDH RP 5'-TCCACCACCCTGTTGCTGTA-3'; CYP24A1 FP 5'-CTTTGCTTCCTTTTCCCAGAAT-3'; CYP24A1 RP 5'-CGCCGTAGATGTCACCAGTC-3'; CAMP FP 5'-GCTAACCTCTACCGCCTCCT-3'; CAMP RP 5'-GGTCACTGTCCCCATACACC-3'; Real-time rt-PCR was carried out on a Mastercycler (Eppendorf). We used the ΔΔCt method to measure the fold change in gene expression of target genes. Standard errors of mean were calculated from two biological independent experiments performed in triplicates.

Viability, toxicity, and apoptosis assay. HL-60 cells (ATTC) were cultured in 75 cm^2 flasks using RPMI-1640 (ATTC, #30-2001), non-essential amino acids, penicillin and streptomycin, and 10% dialyzed FBS (Invitrogen, #26400-044). The cells were dispensed into a 384-well plate with optical bottom (Nunc 142761) and treated with 50H hydrophobic coated pin tool (V&P Scientific) with different concentration of PS121912 for 18 hours. CellTiter-Glo (Promega), CellTiter-Fluor (Promga) and Caspase-Glo 3/7 (Promega) were used using the vendor's directions. Three independent experiments were conducted in quadruplicate and data was analyzed using nonlinear regression with variable slope (GraphPrism).