SUPPLEMENTAL DATA

DISCOVERY OF THE FIRST IRREVERSIBLE SMALL MOLECULE INHIBITORS OF THE INTERACTION BETWEEN THE VITAMIN D RECEPTOR AND COACTIVATORS[#]

Premchendar Nandhikonda[1,^], Wen Z. Lynt[1,^], Megan M. McCallum[1], Tahniyath Ara[1], Athena M. Baranowski[1], Nina Y. Yuan[1], Dana Pearson[1], Daniel D. Bikle[2], R. Kiplin Guy[3], Leggy A. Arnold[1*]

[1]Department of Chemistry and Biochemistry, University of Wisconsin Milwaukee, WI 53211, USA, [2]Endocrine Research Unit, Department of Medicine, Veterans Affairs Medical Center, San Francisco, CA 94121, USA, [3]Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN 38105, USA.

Running title: Vitamin D Receptor–Coactivator Inhibitors

[*]To whom correspondence should be addressed: Department of Chemistry and Biochemistry, University of Wisconsin Milwaukee, 3210 N. Cramer Street, Milwaukee WI 53211-3029, USA. Tel (414) 229 2612; Fax (414) 229 5530; Email: arnold2@uwm.edu.

[^]Both authors contributed equally to this work.

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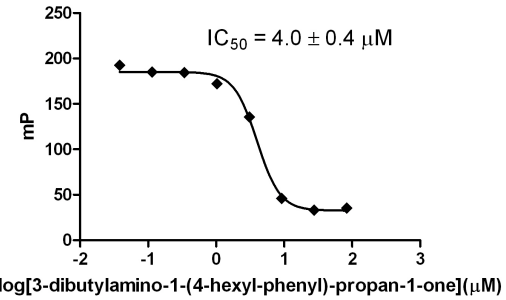
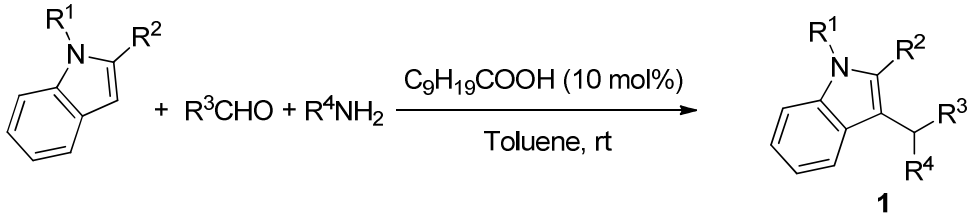
INHIBITION OF VDR-SRC2-3 BINDING BY 3-DIBUTYLAMINO-1-(4-HEXYL-PHENYL)-PROPAN-1-ONE



Determination of Inhibition:
The FP assay was conducted in 384-well black polystyrene microplates (Corning, #3573). The assay solution contained buffer (25 mM PIPES (pH 6.75), 50 mM NaCl, 0.01% NP-40, and 2% DMSO), VDR-LBD protein (1 μM), LG190178 (5 μM), and Alexa Fluor labeled SRC2-3 (7.5 nM). 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one transfer into 20 μl assay solution was accomplished using a stainless steel pin tool (V&P Scientific) delivering 100 nl of compound at different concentrations. Inhibition of binding was detected by fluorescence polarization using a M1000, Tecan reader at excitation/emission wavelength of 630/685 nm. IC50 value (μM) was calculated using the following non-linear regression equation: Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)) using three independent experiments in quadruplet.

SYNTHESIS OF 3-INDOLYL-METHANAMINES



SCHEME 1: **Synthesis of 3-indolyl-methanamines.**

General procedure for the aza-Friedel-Crafts reaction. In a dry flask, aniline (2 mmol) and aldehyde (2 mmol) were dissolved in toluene (2 ml) and stirred for 1h. Then indole (2 mmol) and decanoic acid (0.2 mmol, 10 mol%) (*1*) were added slowly as a solution in toluene (2 ml). The reaction mixture was stirred at room temperature and monitored by TLC. After the reaction was completed, saturated $NaHCO_3$ (6 ml) was added. The mixture was extracted with dichloromethane (3 x 10 ml). The organic layer was combined, washed with brine (10 mL), and dried over anhydrous Na_2SO_4. The solvents were removed under reduced pressure and the residue was purified by recrystallization or chromatography through Biotage SP1 flash system.
***N*-((2-methyl-1*H*-indol-3-yl)(phenyl)methyl)aniline (30a)**. R_f = 0.3 (EtOAc/hexanes = ¼). 230 mg white solid, 37% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.34 (s, 3H), 4.34 (s, 1H), 5.76 (s, 1H), 6.59 (dd, *J* = 1.8, 4.8 Hz, 2H), 6.69-6.73 (m, 1H), 6.69-7.05 (m, 1H), 7.08-7.18 (m, 4H), 7.26-7.29 (m, 3H), 7.38 (d, *J* = 7.5 Hz, 2H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.82 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.35, 54.40, 110.8, 112.45, 113.28, 116.12, 118.76, 119.10, 120.43, 126.79, 128.58, 143.9, 144.1, 148.88,

148.96; Anal. Calcd. for $C_{22}H_{20}N_2$: C 84.58, H 6.45, N 8.97 found C 84.2027, H 6.72, N 8.66.

***N*-((4-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)aniline (30b)**. R_f = 0.3 (EtOAc/hexanes = ¼). 260 mg white solid, 36% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.41 (s, 3H), 4.35 (s, 1H), 5.73 (d, *J* = 2.4 Hz, 1H), 6.57 (dd, *J* = 1.5, 7.8 Hz, 2H), 6.74-6.78 (m, 2H), 6.99-7.05 (m, 1H), 7.11-7.19 (m, 3H), 7.26-7.31 (m, 3H), 7.43 (dd, *J* = 6.9, 7.5 Hz, 2H), 7.87 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.13, 53.75, 111.01, 112.12, 113.23, 114.33, 116.22, 118.99, 120.87, 126.71, 129.43, 132.72, 142.90, 148.72; Anal. Calcd. for $C_{22}H_{19}ClN_2$: C 76.18, H 5.52, N 8.08 found C 76.0612, H 5.82, N 7.87.

***N*-((4-methoxyphenyl)(2-methyl-1*H*-indol-3-yl)methyl)aniline (30c)**. R_f = 0.3 (EtOAc/hexanes = ¼). 266 mg white solid, 38% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.34 (s, 3H), 3.76 (s, 3H), 4.32 (s, 1H), 5.72 (s, 1H), 6.56 (d, *J* = 9.0 Hz, 2H), 6.68 (dd, *J* = 6.3, 7.2 Hz, 1H), 6.67-6.87 (m, 1H), 6.96-7.02 (m, 3H), 7.05-7.15 (m, 2H), 7.22-7.25 (m, 1H), 7.37 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.76 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$, TMS):12.17, 54.02, 55.11, 110.67, 112.53, 113.10, 113.62, 116.29, 119.14, 120.38, 127.28, 128.45, 129.15, 135.64, 148.74, 158.45; Anal. Calcd. for $C_{22}H_{19}ClN_2$: C 80.67, H 6.48, N 8.18 found C 78.99, H 6.59, N 7.71

***N*-((2-methyl-1*H*-indol-3-yl)(*p*-tolyl)methyl)aniline (30d)**. R_f = 0.3 (EtOAc/hexanes = ¼). 460 mg light yellow oil, 69% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.30 (s, 3H), 2.39 (s, 3H), 4.32 (s, 1H), 5.73 (s, 1H), 6.57 (dd, *J* = 0.6, 8.7 Hz, 2H), 6.69 (dd, *J* = 0.9, 7.2 Hz, 1H), 6.69-7.02 (m, 2H), 7.06-7.16 (m, 3H), 7.24-7.27 (m, 2H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.79 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS):12.46, 21.28, 55.36, 110.70, 112.38, 113.23, 115.95, 118.67, 120.35, 127.22, 129.17, 135.51, 141.28, 148.73.

***N*,*N*-dimethyl-4-((2-methyl-1*H*-indol-3-yl)(phenylamino)methyl)aniline (30e)**. R_f = 0.2 (EtOAc/hexanes = ¼). 368 mg light yellow oil, 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.10 (s, 3H), 2.93 (s, 6H), 5.94 (s, 1H), 6.68 (d, *J* = 9.0 Hz, 2H), 6.87 (ddd, *J* = 1.2, 7.5, 7.5 Hz, 2H), 7.04 (dd, *J* = 7.2, 7.5 Hz, 4H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.24 (dd, *J* = 0.6, 0.9 Hz, 1H), 7.26 (d, *J* = 1.2 Hz, 1H), 7.28-7.32 (m, 1H), 7.71 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.14, 52.89, 55.94, 110.18, 111.01, 113.74, 115.11, 118.68, 120.35, 126.98, 128.58, 135.84, 140.44, 142.14, 151.45 ; Anal. Calcd. for $C_{24}H_{25}N_3$: C 81.09, H 7.09, N 11.82 found C 82.11, H 7.02, N 10.45

***N*-((2-methyl-1*H*-indol-3-yl)(4-nitrophenyl)methyl)aniline (30f)**. R_f = 0.2 (EtOAc/hexanes = ¼). 700 mg light yellow oil, 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.43 (s, 3H), 4.40 (s, 1H), 5.78 (d, *J* = 2.4 Hz, 1H), 6.52 (dd, *J* = 0.9, 8.1 Hz, 2H), 6.76 (dd, *J* = 7.2, 7.5 Hz, 1H), 7.01 (dd, *J* = 1.2, 8.1 Hz, 1H), 7.10-7.19 (m, 3H), 7.28 (ddd, *J* = 0.6, 0.6, 7.8 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.95 (s, 1H), 8.15 (d, *J* = 9.0 Hz, 2H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.40, 55.36, 111.08, 111.75, 113.38, 114.22, 116.12, 116.4, 118.65, 120.72, 123.90, 126.88, 128.80, 133.75, 135.68, 146.74, 148.10, 151.75.

***N*-(1-(2-methyl-1*H*-indol-3-yl)ethyl)aniline (30g)**. R_f = 0.3 (EtOAc/hexanes = ¼). 448 mg yellow solid, 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 1.93 (d, *J* = 7.8 Hz, 3H), 2.30 (s, 3H), 4.69 (q, *J* = 4.8 Hz, 1H), 6.96 (ddd, *J* = 0.9, 1.2, 8.1 Hz, 2H), 7.05 (ddd, *J* = 0.9, 1.2, 7.8 Hz, 2H), 7.22 (s, 1H), 7.24 (s, 1H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.64 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS):12.84, 21.81, 28.75, 110.85, 114.79, 118.47, 119.84, 128.29, 130.63, 135.25; Anal. Calcd. for $C_{17}H_{18}N_2$: C81.56, H 7.25, N 11.19 found C 81.6702, H 7.2919, N 9.1870.

***N*-((2-methyl-1*H*-indol-3-yl)(naphthalen-2-yl)methyl)aniline (30h)**. R_f = 0.3 (EtOAc/hexanes = ¼). 546 mg orange oil, 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.38 (d, *J* = 3.3 Hz, 3H), 3.89 (t, *J* = 6.0 Hz, 2H), 5.90 (s, 1H), 6.59-6.62 (m, 2H), 6.94-6.99 (m, 1H), 7.05-7.15 (m, 3H), 7.18-7.27 (m, 1H), 7.41-7.53 (m, 4H), 7.74-7.83 (m, 3H), 8.01 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.24, 54.41, 110.87, 112.19, 113.19, 114.12, 116.45, 118.84, 119.79, 124.99, 126.31, 135.59, 141.56, 148.76.

3-(4-((2-methyl-1*H*-indol-3-yl)(phenylamino)methyl)phenoxy)propan-1-ol (30i). R_f = 0.3 (EtOAc/hexanes = ¼). 431 mg red oil, 56% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.05-2.10 (m, 5H), 3.89 (t, *J* = 6.0 Hz, 2H), 4.20 (t, *J* = 6.3 Hz, 2H), 5.94 (s, 1H), 6.83 (dd, *J* = 9.0, 13.5 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 7.15-7.26 (m, 3H), 7.36-7.41 (m, 2H), 7.74(s, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 8.38 (s, 1H).

***N*-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)aniline (31a)**. R_f = 0.3 (EtOAc/hexanes = ¼). 520

mg white solid, 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.33 (s, 3H), 4.30 (s, 1H), 5.93 (s, 1H), 6.48 (d, J = 7.8 Hz, 2H), 6.69 (dd, J = 7.2, 7.5 Hz, 1H), 6.97 (ddd, J = 1.2, 6.6, 8.4 Hz, 1H), 7.05-7.34 (m, 7H), 7.41 (d, J = 8.1 Hz, 1H), 7.75 (s, 1H), 7.92 (dd, J = 1.5, 7.8 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.55, 51.76, 109.53, 110.54, 112.79, 116.13, 118.77, 120.99, 127.31, 129.64, 139.19, 147.42. Anal. Calcd. for $C_{22}H_{19}ClN_2$: C 76.18, H 5.52, N 8.08 found C 75.6379, H 5.4467, N 7.96.

4-chloro-*N*-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)aniline (31b). R_f = 0.3 (EtOAc/hexanes = ¼). 560 mg light pink solid, 74% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.39 (s, 3H), 4.34 (d, J = 3.0 Hz, 1H), 5.92 (d, J = 3.3 Hz, 1H), 6.42 (dd, J = 2.1, 8.7 Hz, 1H), 6.97-7.14 (m, 4H), 7.22-7.43 (m, 6H), 7.87 (dd, J = 2.1, 7.8 Hz, 2H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.55, 51.76, 109.22, 110.85, 111.16, 114.11, 119.08, 127.30, 132.90, 135.23, 146.80. Anal. Calcd. for $C_{22}H_{18}Cl_2N_2$: C 69.30, H 4.76, N 7.35 found C 69.602, H 4.85, N 7.24.

***N*-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)-4-methoxyaniline (31c)**. R_f = 0.3 (EtOAc/hexanes = ¼). 266 mg white solid, 38% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.32 (s, 3H), 3.70 (s, 3H), 4.08 (s, 1H), 5.86 (s, 1H), 6.43 (dd, J = 2.1, 6.6 Hz, 2H), 6.72 (dd, J = 2.4, 7.2 Hz, 2H), 6.82-6.99 (m, 1H), 7.03-7.08 (m, 1H), 7.16-7.33 (m, 4H), 7.44 (d, J = 7.8 Hz, 1H), 7.78 (s, 1H), 7.93 (dd, J = 1.8, 7.2 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 11.63, 55.93, 109.8, 111.13, 115.25, 118.7, 120.1, 127.2, 133.6, 140.6, 142.5, 151.2. Anal. Calcd. for $C_{23}H_{21}ClN_2O$: C 73.30, H 5.62, N 7.43 found C 73.24, H 5.75, N 7.2355.

***N*-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)-4-methylaniline (31d)**. R_f = 0.3 (EtOAc/hexanes = ¼). 420 mg light pink solid, 58% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.39 (s, 3H), 2.44 (s, 3H), 4.24 (s, 1H), 5.93 (s, 1H), 6.43 (dd, J = 2.1, 6.6 Hz, 2H), 6.95-7.02 (m, 3H), 7.11 (dd, J = 1.2, 7.8 Hz, 1H), 7.20-7.23 (m, 1H), 7.27-7.34 (m, 2H), 7.35 (dd, J = 1.5, 7.8 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.85 (s, 1H), 7.96 (dd, J = 1.8, 7.8 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.15, 20.17, 52.37, 110.18, 111.28, 112.90, 118.98, 124.76, 127.99, 129.66, 140.18, 146.21. Anal. Calcd. for $C_{23}H_{21}ClN_2$: C 76.55, H 5.87, N 7.76 found C 76.55, H 5.98, N 7.5355.

***N*^{1}-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)-*N*^{4},*N*^{4}-dimethylbenzene-1,4-diamine (31e)**. R_f = 0.2 (EtOAc/hexanes = ½). 160 mg yellow solid, 21% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.25 (s, 3H), 2.80 (s, 6H), 5.27 (s, 1H), 5.95 (s, 1H), 6.51-6.55 (m, 2H), 6.77 (d, J = 5.7 Hz, 2H), 6.97-7.17 (m, 4H), 7.22-7.28 (m, 1H), 7.31-7.34 (m, 1H), 7.51 (d, J = 7.8 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 8.31 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 11.61, 37.22, 111.02, 118.15, 119.77, 126.96, 128.31, 129.69, 131.11, 135.20, 142.13. Anal. Calcd. for $C_{24}H_{24}ClN_3$: C 73.93, H 6.20, N 10.78 found C 77.40, H 5.54, N 7.11.

***N*-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)-4-nitroaniline (31f)**. R_f = 0.2 (EtOAc/hexanes = ¼). 433 mg yellow solid, 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.38 (s, 3H), 5.06 (s, 1H), 6.08 (d, J = 4.5 Hz, 1H), 6.45 (dd, J = 2.1, 7.2 Hz, 2H), 6.97-7.02 (m, 1H), 7.09-7.15 (m, 1H), 7.24-7.41 (m, 5H), 7.71 (dd, J = 2.1, 6.9 Hz, 1H), 7.96 (s, 1H), 8.04 (dd, J = 2.1, 7.2 Hz, 2H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.24, 51.76, 108.21, 111.15, 117.77, 118.77, 120.70, 128.94, 130.27, 133.29, 134.22, 134.92, 135.93, 138.87, 153.10. Anal. Calcd. for $C_{22}H_{18}ClN_3O_2$: C 67.43, H 4.63, N 10.72 found 67.4615, H 4.7347, N 10.4091.

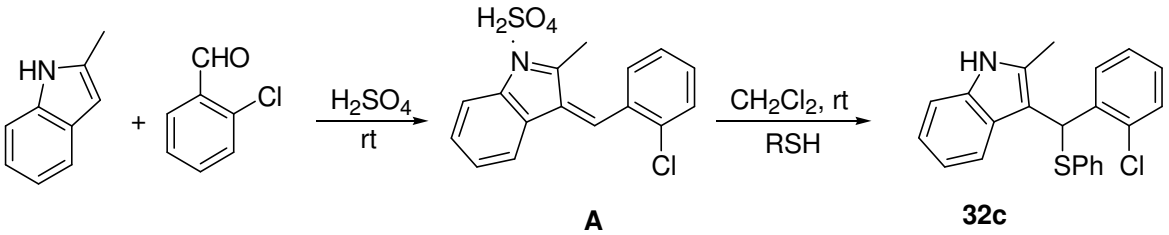
***N*-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)(phenyl)methyl)-4-(trifluoromethyl)aniline (31g).** R_f = 0.3 (EtOAc/hexanes = ¼). 320 mg red solid, 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.17 (s, 3H), 4.84 (s, 1H), 5.94 (s, 1H), 6.69 (d, J = 7.8 Hz, 2H), 6.99 (d, J = 7.8 Hz, 2H), 7.04-7.09 (m, 1H), 7.13-7.33 (m, 3H), 7.30-7.40 (m, 4H), 7.41 (d, J = 8.4 Hz, 1H), 7.42-7.58 (m, 1H), 7.84 (s, 1H), 7.88 (dd, J = 2.1, 7.8 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 11.0, 61.07, 110.4, 112.6, 116.9, 119.4, 122.1, 126.1, 127.2, 128.2, 132.9, 136.8, 141.4.

***N*-((2-chlorophenyl)(2-methyl-1*H*-indol-3-yl)methyl)-2-methoxyaniline(31h)**. R_f = 0.3 (EtOAc/hexanes = ¼). 486 mg white solid, 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.33 (s, 3H), 3.78 (s, 3H), 4.89 (s, 1H), 5.94 (s, 1H), 6.29 (dd, J = 1.5, 7.8 Hz, 1H), 6.62-6.79 (m, 4H), 6.93-6.98 (m, 1H), 7.04-7.09 (m, 1H), 7.13-7.33 (m, 3H), 7.41 (d, J = 8.4 Hz, 1H), 7.84 (s, 1H), 7.88 (dd, J = 2.1, 7.8 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.24, 52.71, 55.41, 109.54, 110.16, 111.17, 117.15, 121.44, 121.44, 127.0, 127.32, 129.0, 130.30, 132.61, 136.58, 146.56. Anal. Calcd. for $C_{23}H_{21}ClN_2O$: C

73.30, H 5.62, N 7.43 found C 73.0355, H 5.707, N 7.45.

***N*-((2-chlorophenyl)(1-methyl-1*H*-indol-3-yl)methyl)aniline (32a).** R_f = 0.3 (EtOAc/hexanes = ¼). 266 mg white solid, 38% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 3.70 (s, 3H), 4.38 (d, J = 2.4 Hz, 1H), 6.19 (d, J = 3.0 Hz, 1H), 6.51-6.55 (m, 3H), 6.69 (dd, J = 7.2, 7.5 Hz, 1H), 7.09-7.16 (m, 3H), 7.20-7.33 (m, 4H), 7.39-7.42 (m, 1H), 7.66 (s, 1H), 7.68-7.72 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 32.98, 50.83, 110.17, 113.18, 114.81, 115.86, 119.06, 121.69, 126.95, 128.91, 137.59, 140.22, 147.42. Anal. Calcd. for $C_{22}H_{19}ClN_2$: C 76.18, H 5.52, N 8.08 found C 76.1473, H 5.6148, N 7.7124.

***N*-((2-chlorophenyl)(1*H*-indol-3-yl)methyl)aniline (32b)**. R_f = 0.3 (EtOAc/hexanes = ¼). 146 mg light yellow oil, 22% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 6.21 (s, 1H), 6.55 (d, J = 7.8 Hz, 2H), 6.68-6.73 (m, 2H), 7.10-7.16 (m, 4H), 7.21-7.28 (m, 4H), 7.37-7.42 (m, 2H), 7.66-7.71 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 51.14, 112.18, 114.12, 115.14, 119.9, 121.12, 123.68, 126.69, 127.6, 129.0, 132.56, 136.57, 140.53, 148.44.



SCHEME 2: **Synthesis of 32c.**

Synthesis of compound 32c.

Concentrated sulfuric acid was added slowly on a stirring rod to 0.5 g of 2-methylindole dissolved in a 3 ml 2-chlorobenzaldehyde, until the entire mass was almost solid. The reaction product was washed thoroughly with toluene and ether. It formed orange-colored solid complex **A**, 1.3g, yield 97%.

To a solution of complex **A** (48 mg, 0.14 mmol) in acetone (1 mL) was added benzenethiol (0.28 mmol) dropwise at room temperature. After stirring for 30 minutes, the orange precipate disappeared, and a homogenous solution was formed. The reaction mixture was washed with $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated and purified by SP1 system to obtain pure product.

3-((2-chlorophenyl)(phenylthio)methyl)-2-methyl-1*H*-indole (32c). R_f = 0.3 (EtOAc/hexanes = ¼). 41 mg white solid, 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.29 (s, 3H), 6.15 (s, 1H), 7.02-7.33 (m, 11H), 7.79 (d, J = 7.8 Hz, 2H), 8.11 (dd, J = 2.1, 8.1 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, TMS): 12.46, 46.18, 107.43, 111.13, 118.97, 120.59, 126.85, 127.07, 127.28, 129.14, 130.28, 130.77, 133.32, 133.98, 135.60, 135.8, 137.95 . Anal. Calcd. for $C_{22}H_{18}ClNS$: C 72.61, H 4.99, N 3.85 found C 73.1803, H 5. 48, N 3.724.

DETERMINATION OF 3-INDOLYL-METHANAMINES RATE CONSTANTS

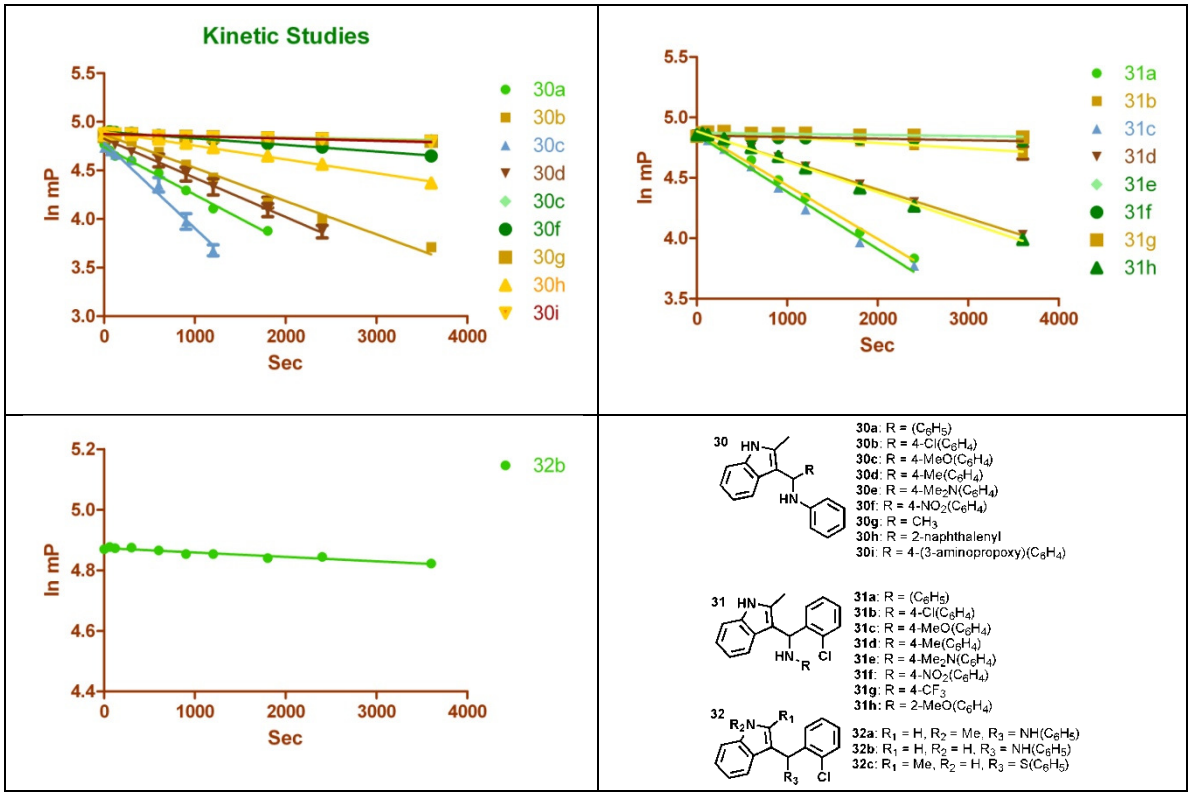


FIGURE 1. **Determination of 3-indolyl-methamines rate constants.**

Determination of 3-indolyl-methamines rate constants.

The FP assay was conducted in 384-well black polystyrene microplates (Corning, #3573). The assay solution contained buffer (25 mM PIPES (pH 6.75), 50 mM NaCl, 0.01% NP-40, and 2% DMSO), VDR-LBD protein (1 µM), LG190178 (5 µM), and Alexa Fluor labeled SRC2-3 (7.5 nM). Small molecule transfer into 20 µl assay solution was accomplished using a stainless steel pin tool (V&P Scientific) delivering 100 nl of compound at different concentrations. Inhibition of binding was detected by fluorescence polarization using a M1000, Tecan reader at excitation/emission wavelength of 630/685 nm. The mP values (FP) were recorded at different time points. Natural log transformed mP values (ln mP) mwere plotted against time (s). Linear regression resulted in negative reaction rate values, which describes the kinetic dissociation of the VDR-SRC2-3 complex in the presence of small molecules.

NUCLEAR RECEPTOR–COACTIVATOR BINDING STUDIES IN THE PRESENCE OF 3-INDOLYL-METHANAMINES USING FLUORESCENCE POLARIZATION

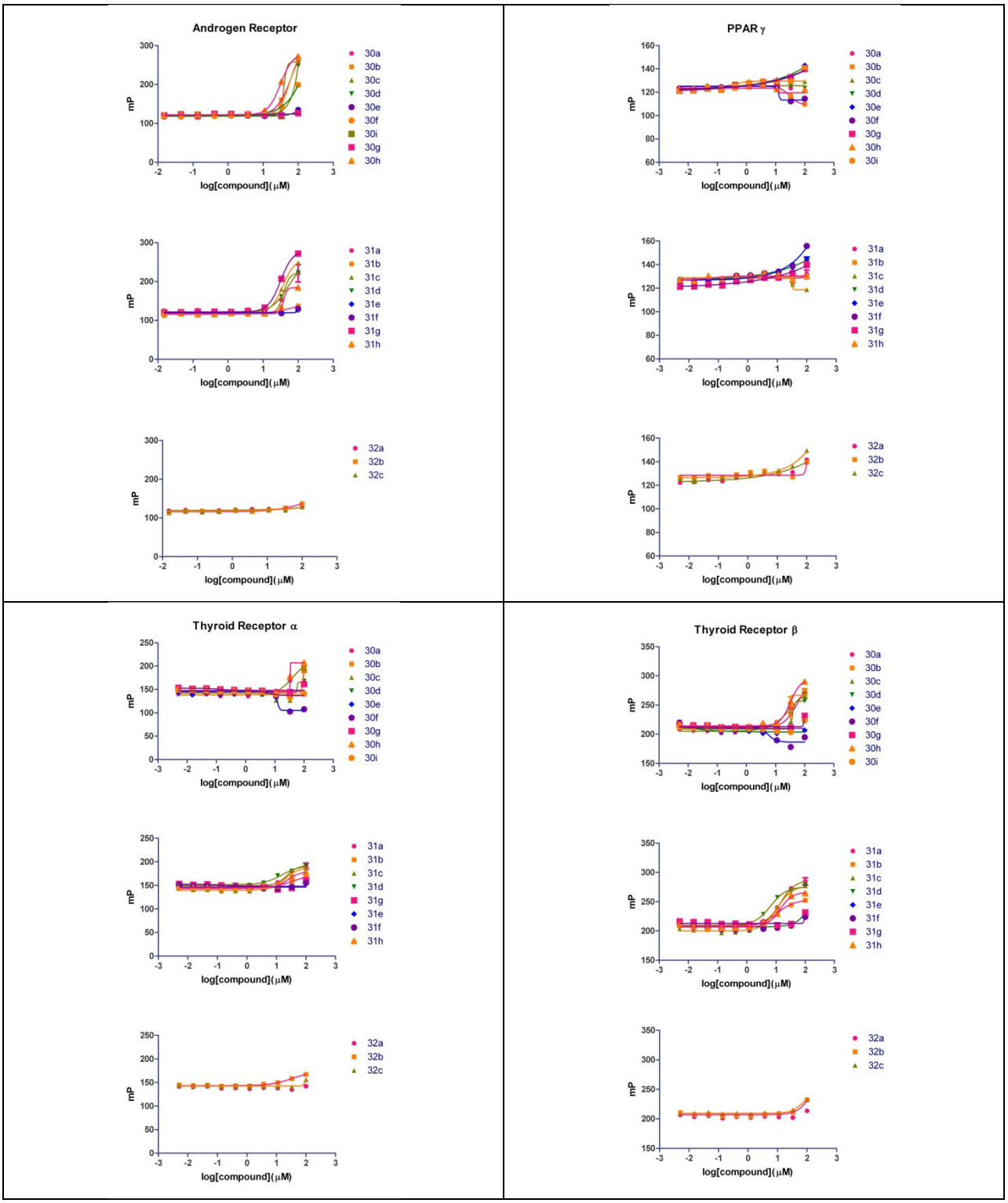


FIGURE 2. **Nuclear receptor–coactivator binding studies in the presence of 3-indolyl-methanamines using fluorescence polarization.**

Nuclear receptor–coactivator binding studies in the presence of 3-indolyl-methamines.
These assays were conducted in 384-well black polystyrene microplates (Corning) using a general buffer (20 mM TRIS (pH 7.50), 100 mM NaCl, 0.01% NP-40, 2% DMSO) and analyzed with a M1000 reader (Tecan) to detect fluorescence polarization at excitation/emission wavelength of 595/615 nm. For the androgen receptor: AR-LBD (5 μM), Texas Red-labeled SRC2-3 (7 nM), and dihydrotestosterone (5 μM) were incubated in buffer with small molecule for 3h; for the thyroid receptor α: TRα-LBD (2 μM), Texas Red-labeled SRC2-2 (7 nM), and triiodothyronine (1 μM) were incubated with small molecule for 3h; for the thyroid receptor β: TRβ-LBD (0.8 μM), Texas Red-labeled SRC2-2 (7 nM), and triiodothyronine (1 μM) were incubated with small molecule for 3h; for the peroxisome proliferator-activated receptor γ: PPARγ-LBD (5 μM), Texas Red-labeled DRIP2 (7 nM), and rosiglitazone (5 μM) were incubated with small molecule for 3h; for the vitamin D receptor: VDR-LBD (1 μM), Texas Red-labeled SRC2-3 (7 nM), and LG190178 (5 μM) were incubated with small molecule for 3h. Two independent experiments were carried out in quadruplet.

VDR–LIGAND COMPETITION STUDY

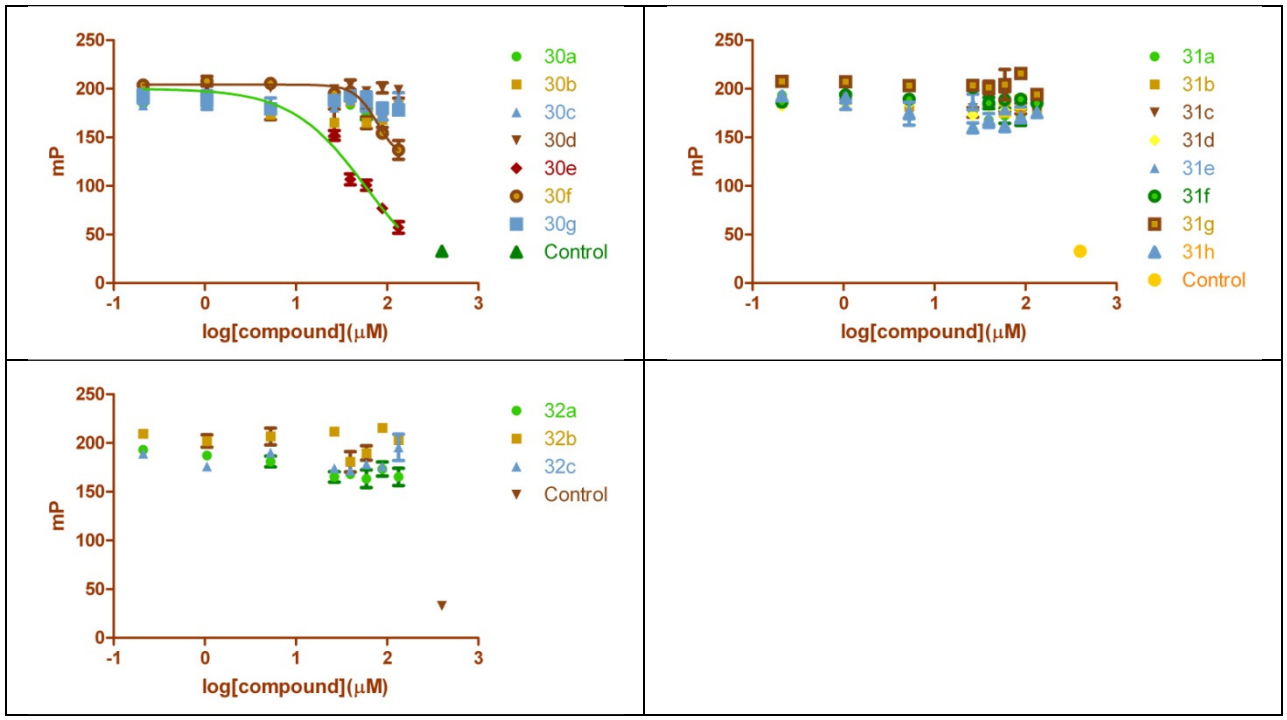


FIGURE 3. **VDR–ligand competition study.**

Ligand competition assay.
Ligand antagonism was excluded by using a fluorescence polarization assay (PolarScreen, Invitrogen), which employs a fluorescently labeled $1,25(OH)_2D_3$ analog. Two independent experiments were conducted in quadruplet.

REFERENCES

1. Shirakawa, S., and Kobayashi, S. (2006) Carboxylic acid catalyzed three-component aza-Friedel-Crafts reactions in water for the synthesis of 3-substituted indoles, *Organic Letters 8*, 4939-4942.