

Supporting Information

Synthesis, antiproliferative effect and topoisomerase II inhibitory activity of 3-methyl-2-phenyl-1*H*-indoles

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General Information – Chemistry

Synthetic Procedures and Analytical Data

¹H and ¹³C Spectra of the Representative Compounds

Materials and methods - Biological studies

Figure 1SI. Effect of quercetin on the relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II (topo II)

References

General Information – Chemistry. Chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) and TCI Europe N.V. (Zwijndrecht, Belgium) and used without further purification. Analytical TLC was performed on silica gel Merck 60 F₂₅₄ plates (0.25 mm), using visualization with UV light and spray reagents. Column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). HPLC analyses were performed on a Thermo Scientific Dionex Ultimate 3000 Binary Rapid Separation LC System (Thermo Fisher Scientific, Waltham, MA, USA) with an autosampler, a binary pump system and a photodiode array detector. We used an Agilent Eclipse C18 column (5 μ m, 4.6 \times 150 mm), with a flow rate of 1.0 mL/min. The eluent consisted of trifluoroacetic acid (0.1% in water) as solvent A and acetonitrile as solvent B; gradient: 30 to 90% B in 20 min; flow rate 1.0 mL/min; injection volume: 10 μ L. Melting points were determined on a Reichert hot stage microscope and are uncorrected. ¹H and ¹³C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, MA, USA) in DMSO-*d*₆, acetone-*d*₆ or CDCl₃ solutions, with TMS as the internal standard. Mass spectra were obtained using a VG Analytical Autospec Q mass spectrometer (Fisons, VG Analytical, Manchester, UK). Synthesis of compounds **4-6**, **8**, **9**, **11**, **12**, **22**, **23**, **34** and **35** has been reported previously.¹ All tested compounds were \geq 95% pure by HPLC.

Synthetic Procedures and Analytical Data. 2-(4-(Benzyloxy)phenyl)-3-methyl-1H-indole (7). To a solution of 1-(4-(benzyloxy)phenyl)propan-1-one (**3**) (2.00 g, 8.4 mmol) and phenylhydrazine (0.82 mL, 8.4 mmol) in MeOH (40 mL) H₂SO₄ (0.20 mL, 4.2 mmol) was added dropwise. Reaction mixture was stirred under reflux for 24 h. The solvent was evaporated under reduced pressure, crude product suspended in water (100 mL) and extracted with ethyl acetate (2 \times 100 mL). Combined organic phases were successively washed with saturated aqueous NaHCO₃ solution (100 mL) and brine (100 mL), dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. Crude product was purified by column chromatography using hexane/ethyl acetate (4:1) as eluent. Yield 0.656 g (25.2%); brown solid; mp 103 – 106 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.47 (s, 3H, Ar-CH₃), 5.16 (s, 2H, Ar-CH₂O), 7.10 – 7.14 (m, 1H, Ar-H), 7.20 (m, 2H, 2 \times Ar-H), 7.36 – 7.41 (m, 3H, 3 \times Ar-H), 7.42 – 7.47 (m, 2H, 2 \times Ar-H), 7.48 – 7.52 (m, 2H, 2 \times Ar-H), 7.52 – 7.56 (m, 2H, 2 \times Ar-H), 7.60 – 7.63 (m, 1H, Ar-H), 7.97 (s, 1H, NH) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 10.00, 70.51, 107.28, 111.61, 115.95, 119.14, 119.62, 122.25, 127.21, 128.49, 128.71, 129.35, 129.84, 130.99, 134.93, 137.15, 138.34, 159.02 ppm. MS (ESI) *m/z* = 313.1 ([M+H]⁺).

General procedure A. Synthesis of compounds 10, 36 and 37. To the solution of *O*-benzylphenol (1.0 mmol) in a mixture of absolute ethanol (25 mL) and THF (25 mL) was added 10% Pd/C (20 wt%) and the

reaction mixture was stirred under hydrogen atmosphere for 18 h. The catalyst was filtered off and the solvent was removed under reduced pressure.

4-(3-Methyl-1H-indol-2-yl)phenol (10). Prepared from **7** (160 mg, 0.51 mmol) according to general procedure A. The crude product was purified by column chromatography with dichloromethane as an eluent to afford deprotected phenol. Yield: 114 mg (100%); brown oil; ^1H NMR (400 MHz, CDCl_3) δ 2.45 (s, 3H, Ar- CH_3), 5.06 (s, 1H, OH), 6.95 – 6.99 (m, 2H, 2 \times Ar- $\underline{\text{H}}$), 7.15 – 7.19 (m, 1H, Ar- $\underline{\text{H}}$), 7.20 – 7.24 (m, 1H, Ar- $\underline{\text{H}}$), 7.38 (d, 1H, $J = 7.9$ Hz, Ar-H), 7.47 – 7.50 (m, 2 H, 2 \times Ar- $\underline{\text{H}}$), 7.61 (dd, 1H, $J_1 = 7.7$ Hz, $J_2 = 0.6$ Hz, Ar- $\underline{\text{H}}$), 7.97 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 9.98, 106.80, 111.54, 116.42, 119.02, 119.53, 122.05, 125.87, 129.94, 131.03, 135.34, 137.06, 157.68 ppm; HRMS m/z for $\text{C}_{15}\text{H}_{12}\text{NO}$ ($[\text{M}-\text{H}]^-$): calcd 222.0919; found 222.0917; HPLC: t_r 8.633 min (95.01% at 254 nm, 95.55% at 280 nm).

4-(3-Methyl-1H-indol-2-yl)phenyl 4-methylthiazole-5-carboxylate (13). To a suspension of compound **10** (85 mg, 0.38 mmol) in anhydrous dichloromethane (10 mL) at 0 °C 4-dimethylaminopyridine (3.0 mg, 0.024 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (109 mg, 0.57 mmol) and 4-methyl-thiazole-5-carboxylic acid (83 mg, 0.58 mmol) were added. Reaction mixture was stirred at room temperature overnight and then successively washed with 10% citric acid (20 mL), saturated aqueous NaHCO_3 solution (20 mL) and brine (20 mL), dried over Na_2SO_4 , filtered and the solvent removed under reduced pressure. Crude product was purified by column chromatography with ethyl acetate/hexane (1:2) as an eluent. Yield 59 mg (44.7%); beige solid; mp 166 – 168 °C; ^1H NMR (400 MHz, CDCl_3) δ 2.50 (s, 3H, indole- CH_3), 2.90 (d, 3H, $J = 0.4$ Hz, thiazole- CH_3), 7.19 (ddd, 1H, $J_1 = 1.1$ Hz, $J_2 = 7.1$ Hz, $J_3 = 8.1$ Hz, Ar- $\underline{\text{H}}$), 7.25 (ddd, 1H, $J_1 = 1.3$ Hz, $J_2 = 7.0$ Hz, $J_3 = 8.1$ Hz, Ar- $\underline{\text{H}}$), 7.32 – 7.38 (m, 2H, 2 \times Ar- $\underline{\text{H}}$), 7.41 (dt, 1H, $J_1 = 1.0$ Hz, $J_2 = 8.0$ Hz, Ar- $\underline{\text{H}}$), 7.60 – 7.73 (m, 3H, 3 \times Ar- $\underline{\text{H}}$), 8.04 (s, 1H, Ar- $\underline{\text{H}}$), 8.92 (d, 1H, $J = 0.5$ Hz, NH) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 10.01, 17.65, 108.66, 111.83, 119.48, 119.83, 121.91, 122.81, 123.09, 129.63, 130.84, 132.53, 134.08, 137.38, 150.36, 158.18, 161.21, 162.84 ppm. HRMS m/z for $\text{C}_{20}\text{H}_{17}\text{N}_2\text{O}_2\text{S}$ ($[\text{M}+\text{H}]^+$): calcd 349.1011; found 349.1019; HPLC: t_r 12.623 min (95.12% at 254 nm, 95.02% at 280 nm).

4-(3-Methyl-1H-indol-2-yl)phenyl 1H-imidazole-4-carboxylate (14). To a solution of compound **10** (70 mg, 0.31 mmol) in anhydrous *N,N*-dimethylformamide (10 mL) at 0 °C 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium tetrafluoroborate (TBTU) (265 mg, 0.83 mmol) and 4-imidazole carboxylic acid (66 mg, 0.59 mmol) were added. The pH of reaction mixture was adjusted to 8 using *N*-methylmorpholine. Reaction mixture was stirred at room temperature overnight. Ethyl acetate was added (80 mL) and the solution successively washed with 10% citric acid (80 mL), saturated aqueous NaHCO_3

solution (80 mL) and brine (80 mL), dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Crude product was purified by column chromatography with dichloromethane/methanol (9:1) as an eluent. Yield 37 mg (37.0%); white solid; mp 222 – 223 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.44 (s, 3H, ArCH₃), 6.97 – 7.07 (m, 1H, Ar-H), 7.08 – 7.16 (m, 1H, Ar-H), 7.31 – 7.45 (m, 3H, 3 × Ar-H), 7.55 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.69 – 7.79 (m, 2H, 2 × Ar-H), 7.92 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 11.21 (s, 1H, NH), 12.86 (s, 1H, NH) ppm; HRMS *m/z* for C₁₉H₁₆N₃O₂ ([M+H]⁺): calcd 318.1243; found 318.1240; HPLC: *t*_r 5.617 min (95.00% at 254 nm, 95.09% at 280 nm).

General procedure B. Synthesis of compounds 15, 16, 24 and 25. Indole (1.00 mmol) was dissolved in dry DMF (7 mL) and cooled to 0 °C under argon atmosphere. 95% Sodium hydride (1.50 mmol) was added and the solution was stirred for 1 h at room temperature. Reaction mixture was cooled to 0 °C and corresponding alkylhalide (1.5 mmol) in dry DMF (3 mL) was added dropwise. The mixture was stirred for 2 h at room temperature, diluted with ethyl acetate (100 mL) and washed with water (75 mL), 10% citric acid (2 × 75 mL), brine (75 mL) and dried over Na₂SO₄, filtered and the solvent removed under reduced pressure.

2-(4-(Benzyloxy)phenyl)-3-methyl-1-propyl-1H-indole (15). Prepared from **7** (0.157 g, 0.50 mmol) and 1-bromopropane (0.070 mL 0.78 mmol) according to general procedure B. Yield 0.175 g (98.3%); yellow solid; mp 85 – 87 °C; ¹H NMR (400 MHz, CDCl₃) δ 0.78 (t, 3H, *J* = 7.4 Hz, CH₂CH₃), 1.60 – 1.74 (m, 2H, CH₂CH₂CH₃), 2.25 (s, 3H, Ar-CH₃), 3.96 – 4.04 (m, 2H, CH₂CH₂CH₃), 5.17 (s, 2H, CH₂Ph), 7.08 – 7.21 (m, 3H, 3 × Ar-H), 7.25 (ddd, 1H, *J*₁ = 1.3 Hz, *J*₂ = 7.0 Hz, *J*₃ = 8.2 Hz, Ar-H), 7.29 – 7.44 (m, 4H, 4 × Ar-H), 7.41 – 7.50 (m, 2H, 2 × Ar-H), 7.52 (ddt, 2H, *J*₁ = 0.7 Hz, *J*₂ = 1.4 Hz, *J*₃ = 7.5 Hz, 2 × Ar-H), 7.61 (ddd, 1H, *J*₁ = 0.8 Hz, *J*₂ = 1.3 Hz, *J*₃ = 7.8 Hz, Ar-H) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 9.48, 11.49, 23.98, 45.87, 70.59, 108.69, 110.57, 115.64, 119.33, 119.59, 122.07, 125.71, 128.63, 128.77, 129.37, 129.60, 132.65, 137.41, 138.03, 138.27, 159.62 ppm; MS (ESI) *m/z* = 356.3 ([M+H]⁺).

Ethyl 2-(2-(4-(benzyloxy)phenyl)-3-methyl-1H-indol-1-yl)acetate (16). Prepared from **7** (0.215 g, 0.69 mmol) and ethyl bromoacetate (115 μL, 1.03 mmol) according to general procedure B. Crude product was purified by column chromatography with dichloromethane as an eluent. Yield 0.115 g (42.0%); yellow solid; mp 90 – 91 °C; ¹H NMR (400 MHz, CDCl₃) δ 1.25 (t, 3H, *J* = 7.1 Hz, CH₂CH₃), 2.28 (d, 3H, *J* = 1.4 Hz, Ar-CH₃), 4.20 (q, 2H, *J* = 7.1 Hz, CH₂CH₃), 4.70 (s, 2H, NCH₂), 5.16 (s, 2H, CH₂Ph), 7.07 – 7.14 (m, 2H, 2 × Ar-H), 7.17 – 7.27 (m, 3H, 3 × Ar-H), 7.32 – 7.54 (m, 7H, 7 × Ar-H), 7.62 (dt, 1H, *J*₁ = 1.1 Hz, *J*₂ = 7.7 Hz, Ar-H) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 9.50, 14.48, 46.28, 61.72, 70.58, 109.36, 110.19, 115.74, 119.40, 120.24, 122.58, 124.82, 128.60, 128.78, 129.37, 129.80, 132.64, 138.11, 138.16, 138.22, 159.83, 169.93 ppm; MS (ESI) *m/z* = 400.2 ([M+H]⁺).

2-(4-(Benzyloxy)phenyl)-3-methyl-1-(4-phenoxybutyl)-1H-indole (17). Prepared from **7** (0.151 g, 0.48 mmol) and (4-bromobutoxy)benzene (0.165 g, 0.72 mmol) according to general procedure B. The crude product **17** was used in the next step without further purification. Yield 0.215 g (95.0%); orange oil; ^1H NMR (400 MHz, CDCl_3) δ 1.59 – 1.66 (m, 2H, CH_2), 1.82 (q, 2H, $J = 7.4$ Hz, CH_2), 2.26 (s, 3H, CH_3), 3.76 (t, 2H, $J = 6.2$ Hz, CH_2), 4.14 (t, 2H, $J = 7.3$ Hz, CH_2), 5.10 (s, 2H, CH_2), 6.81 (d, 2H, $J = 8.7$ Hz, $2 \times \text{Ar-H}$), 6.93 – 6.97 (m, 1H, Ar-H), 7.07 (d, 2H, $J = 8.7$ Hz, $2 \times \text{Ar-H}$), 7.14 – 7.19 (m, 1H, Ar-H), 7.22 – 7.30 (m, 3H, $3 \times \text{Ar-H}$), 7.31 – 7.35 (m, 2H, $2 \times \text{Ar-H}$), 7.36 – 7.41 (m, 2H, $2 \times \text{Ar-H}$), 7.43 – 7.48 (m, 2H, $2 \times \text{Ar-H}$), 7.49 – 7.53 (m, 2H, $2 \times \text{Ar-H}$), 7.62 (d, 1H, $J = 7.7$ Hz, Ar-H) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 9.50, 27.01, 27.23, 43.81, 67.58, 70.56, 108.82, 110.61, 115.26, 115.67, 119.40, 119.68, 121.26, 122.17, 125.60, 128.59, 128.77, 129.37, 129.67, 130.22, 132.67, 137.38, 137.98, 138.25, 159.62, 159.96 ppm.

5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-propyl-1H-indole (18). Prepared from **8** (0.150 g, 0.36 mmol) and 1-bromopropane (97 μL , 1.07 mmol) according to general procedure B. Crude product was purified by column chromatography with dichloromethane/hexane (3:7) as an eluent. Yield 51 mg (30.9%); white solid; mp 138 – 142 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 0.76 (t, 3H, $J = 7.4$ Hz, CH_2CH_3), 1.64 (h, $J = 7.4$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.21 (s, 3H, Ar-CH_3), 3.92 – 4.00 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 5.16 (2 \times s, 4H, $2 \times \text{CH}_2\text{Ph}$), 6.98 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.8$ Hz, Ar-H), 7.11 (d, 2H, $J = 8.7$ Hz, $2 \times \text{Ar-H}$), 7.14 (d, 1H, $J = 2.4$ Hz, Ar-H), 7.26 (d, 1H, $J = 8.8$ Hz, Ar-H), 7.30 – 7.48 (m, 8H, $8 \times \text{Ar-H}$), 7.49 – 7.57 (m, 4H, $4 \times \text{Ar-H}$) ppm; ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 9.27, 11.03, 22.86, 44.78, 69.30, 69.78, 101.99, 106.99, 110.67, 111.62, 114.74, 124.20, 127.58, 127.89, 127.93, 128.31, 128.33, 128.47, 131.41, 136.92, 137.57, 137.82, 152.28, 158.00 (two signals missing) ppm. MS (ESI) $m/z = 462.3$ ($[\text{M}+\text{H}]^+$).

5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-(2-phenoxyethyl)-1H-indole (19). Prepared from **8** (0.300 g, 0.72 mmol) and (2-bromoethoxy)benzene (0.217 g; 1.08 mmol) according to general procedure B. Crude product was purified by column chromatography with ethyl acetate/hexane/triethylamine (1:7:1) as an eluent. Yield 0.159 g (41.2%); yellow solid; mp 92 – 96 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 2.21 (s, 3H, Ar-CH_3), 4.06 (t, 2H, $J = 6.5$ Hz, CH_2), 4.41 (t, 2H, $J = 6.5$ Hz, CH_2), 5.17 (2 \times s, 4H, $2 \times \text{CH}_2\text{Ph}$), 6.71 (dt, 2H, $J_1 = 1.1$ Hz, $J_2 = 7.9$ Hz, $2 \times \text{Ar-H}$), 6.89 – 6.96 (m, 1H, Ar-H), 7.01 (dd, 1H, $J_1 = 2.5$ Hz, $J_2 = 8.8$ Hz, Ar-H), 7.10 – 7.17 (m, 3H, $3 \times \text{Ar-H}$), 7.18 – 7.25 (m, 2H, $2 \times \text{Ar-H}$), 7.33 – 7.48 (m, 9H, $9 \times \text{Ar-H}$), 7.49 – 7.56 (m, 4H, $4 \times \text{Ar-H}$) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 9.60, 43.86, 67.13, 70.59, 71.19, 103.14, 109.00, 111.56, 112.87, 115.11, 115.64, 121.55, 125.50, 128.39, 128.62, 128.77, 129.22, 129.38, 130.17, 130.23, 132.96, 133.05, 138.29, 139.10, 139.24, 154.18, 159.40, 159.72 (one signal missing) ppm; MS (ESI) $m/z = 540.3$ ($[\text{M}+\text{H}]^+$).

5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-(3-phenoxypropyl)-1H-indole (20). Prepared from **8** (0.240 g, 0.57 mmol) and (3-bromopropoxy)benzene (0.249 g, 1.16 mmol) according to general procedure B. Crude product was purified by column chromatography with hexane/toluene (1:1) as an eluent. Yield 0.148 g (46.7%); beige solid; mp 98 – 101 °C; ¹H NMR (400 MHz, CDCl₃) δ 2.07 (p, 2H, *J* = 6.3 Hz, CH₂), 2.21 (s, 3H, Ar-CH₃), 3.74 (t, 2H, *J* = 5.8 Hz, CH₂), 4.28 (t, 2H, *J* = 6.8 Hz, CH₂), 5.11 (s, 2H, CH₂Ph), 5.17 (s, 2H, CH₂Ph), 6.74 (dd, 2H, *J*₁ = 1.1 Hz, *J*₂ = 8.7 Hz, 2 × Ar-H), 6.92 – 6.99 (m, 2H, 2 × Ar-H), 7.01 – 7.06 (m, 2H, 2 × Ar-H), 7.16 (dd, 1H, *J*₁ = 0.5 Hz, *J*₂ = 2.5 Hz, Ar-H), 7.20 – 7.26 (m, 2H, 2 × Ar-H), 7.29 – 7.49 (m, 9H, 9 × Ar-H), 7.51 – 7.55 (m, 4H, 4 × Ar-H) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 9.61, 30.57, 41.20, 65.22, 70.54, 71.18, 103.13, 108.69, 111.32, 112.83, 115.20, 115.31, 115.60, 121.29, 125.50, 128.39, 128.58, 128.76, 129.21, 129.38, 130.08, 130.16, 130.29, 132.57, 132.83, 138.31, 138.84, 139.25, 154.04, 159.58, 159.69 ppm; MS (ESI) *m/z* = 554.3 ([M+H]⁺).

5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-(4-phenoxybutyl)-1H-indole (21). Prepared from **8** (0.350 g, 0.83 mmol) and (4-bromobutoxy)benzene (0.250 g, 1.09 mmol) according to general procedure B. The crude product **21** was used in the next step without further purification.

5-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-3-methyl-1-(3-phenoxypropyl)-1H-indole (24). Prepared from **9** (0.525 g, 1.00 mmol) and (3-bromopropyl)benzene (0.320 g, 1.50 mmol) according to general procedure B. The crude product **24** was used in the next step without further purification.

5-(Benzyloxy)-2-(3,4-bis(benzyloxy)phenyl)-3-methyl-1-(4-phenoxybutyl)-1H-indole (25). Prepared from **9** (0.525 g, 1.00 mmol) and (4-bromobutoxy)benzene (0.344 g, 1.50 mmol) according to general procedure B. The crude product **25** was used in the next step without further purification.

4-(3-Methyl-1-propyl-1H-indol-2-yl)phenol (26). Prepared from **15** (0.148 g, 0.42 mmol) according to general procedure A. Yield 98 mg (89.1%); grey oil; ¹H NMR (400 MHz, CDCl₃) δ 0.77 (t, *J* = 7.4 Hz, 3H, CH₂CH₂CH₃), 1.57 – 1.73 (m, 2H, CH₂CH₂CH₃), 2.24 (s, 3H, Ar-CH₃), 3.94 – 4.03 (m, 2H, CH₂CH₂CH₃), 6.94 – 6.99 (m, 2H, 2 × Ar-H), 7.15 (ddd, 1H, *J*₁ = 1.0 Hz, *J*₂ = 7.0 Hz, *J*₃ = 7.9 Hz, Ar-H), 7.24 (ddd, 1H, *J*₁ = 1.3 Hz, *J*₂ = 7.1 Hz, *J*₃ = 8.2 Hz, Ar-H), 7.27 – 7.30 (m, 2H, 2 × Ar-H), 7.36 (dt, 1H, *J*₁ = 0.9 Hz, *J*₂ = 8.1 Hz, Ar-H), 7.61 (ddd, 1H, *J*₁ = 0.7 Hz, *J*₂ = 1.2 Hz, *J*₃ = 7.8 Hz, Ar-H) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 9.48, 11.49, 23.97, 45.83, 108.42, 110.51, 116.17, 119.26, 119.51, 121.93, 124.29, 129.63, 132.69, 137.34, 138.41, 158.15 ppm; HRMS *m/z* for C₁₈H₂₀NO ([M+H]⁺): calcd 266.1545; found 266.1542; HPLC: *t*_r 12.593 min (95.26% at 254 nm, 95.32% at 280 nm).

Ethyl 2-(2-(4-hydroxyphenyl)-3-methyl-1H-indol-1-yl)acetate (27). Prepared from **16** (0.101 g, 0.26 mmol) according to general procedure A. Yield 49 mg (61.7%); beige solid; mp 158 – 160 °C; ¹H NMR

(400 MHz, DMSO- d_6) δ 1.13 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 2.17 (s, 3H, Ar- CH_3), 4.07 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 4.82 (s, 2H, NCH_2COO), 6.87 – 6.93 (m, 2H, Ar- H), 7.05 – 7.11 (m, 1H, Ar- H), 7.20 – 7.12 (m, 3H, $3 \times$ Ar- H), 7.36 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 4.9$ Hz, Ar- H), 7.53 (ddd, 1H, $J_1 = 0.7$ Hz, $J_2 = 1.2$ Hz, $J_3 = 7.7$ Hz, Ar- H), 9.74 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 9.51, 14.47, 46.24, 61.70, 109.07, 110.14, 116.33, 119.34, 120.17, 122.44, 123.35, 129.83, 132.67, 138.08, 138.49, 158.51, 169.98 ppm; HRMS m/z for $\text{C}_{19}\text{H}_{20}\text{NO}_3$ ($[\text{M}+\text{H}]^+$): calcd 310.1443; found 310.1438; HPLC: t_r 10.390 min (97.98% at 254 nm, 97.76% at 280 nm).

2-(2-(4-Hydroxyphenyl)-3-methyl-1H-indol-1-yl)acetic acid (28). To a solution of compound **27** (20 mg, 0.064 mmol) in ethanol (3 mL) 1 M NaOH (128 μL , 0.13 mmol) was added and reaction mixture stirred overnight at room temperature. Ethanol was removed under reduced pressure, water (3 mL) was added and solution acidified to pH 2 with 1 M HCl. The precipitate was filtered off and dried to obtain 14 mg (72.2%) of yellow solid. mp 158 – 160 $^\circ\text{C}$; ^1H NMR (400 MHz, DMSO- d_6) δ 2.17 (s, 3H, Ar- CH_3), 4.72 (s, 2H, NCH_2COO), 6.95 – 6.86 (m, 2H, $2 \times$ Ar- H), 7.07 (ddd, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.1$ Hz, $J_3 = 8.0$ Hz, Ar- H), 7.12 – 7.16 (m, 1H, Ar- H), 7.16 – 7.20 (m, 2H, $2 \times$ Ar- H), 7.34 (d, 1H, $J = 8.0$ Hz, Ar- H), 7.52 (ddd, 1H, $J_1 = 0.7$ Hz, $J_2 = 1.2$ Hz, $J_3 = 7.7$ Hz, Ar- H), 9.74 (s, 1H, OH), 12.87 (s, 1H, COOH) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 9.50, 45.83, 110.15, 116.32, 116.52, 119.29, 119.60, 120.10, 122.38, 125.03, 125.58, 132.65, 133.37, 160.85, 170.81 ppm; HRMS m/z for $\text{C}_{17}\text{H}_{16}\text{NO}_3$ ($[\text{M}+\text{H}]^+$): calcd 282.1130; found 282.1125; HPLC: t_r 9.417 min (95.10% at 254 nm, 95.04% at 280 nm).

4-(3-Methyl-1-(4-phenoxybutyl)-1H-indol-2-yl)phenol (29). Prepared from **17** (0.165 g, 0.26 mmol) according to general procedure A. The crude product was purified by column chromatography with ethyl acetate/hexane (1:2) as an eluent. Yield 0.070 g (51.5%); orange oil; ^1H NMR (400 MHz, CDCl_3) δ 1.58 – 1.65 (m, 2H, CH_2), 1.74 – 1.89 (m, 2H, CH_2), 2.25 (s, 3H, Ar- CH_3), 3.76 (t, 2H, $J = 6.2$ Hz, CH_2), 4.13 (t, 2H, $J = 7.4$ Hz, CH_2), 4.89 (s, 1H, OH), 6.77 – 6.85 (m, 2H, $2 \times$ Ar- H), 6.91 (d, 2H, $J = 8.6$ Hz, $2 \times$ Ar- H), 6.96 (td, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.4$ Hz, Ar- H), 7.14 – 7.20 (m, 1H, Ar- H), 7.22 – 7.33 (m, 5H, $5 \times$ Ar- H), 7.38 (d, 1H, $J = 8.1$ Hz, Ar- H), 7.62 (d, 1H, $J = 7.8$ Hz, Ar- H) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 9.49, 27.08, 27.26, 43.82, 67.69, 108.59, 110.56, 115.27, 116.29, 119.31, 119.59, 121.24, 122.02, 124.20, 129.68, 130.21, 132.72, 137.32, 138.35, 158.27, 159.95 ppm; HRMS m/z for $\text{C}_{25}\text{H}_{26}\text{NO}_2$ ($[\text{M}+\text{H}]^+$): calcd 372.1964; found 372.1956; HPLC: t_r 14.897 min (95.00% at 280 nm).

2-(4-Hydroxyphenyl)-3-methyl-1-propyl-1H-indol-5-ol (30). Prepared from **18** (0.196 g, 0.43 mmol) according to general procedure A. The crude product was purified by column chromatography with dichloromethane/acetone (40:1) as an eluent. Yield 98 mg (81.7%); beige solid; mp 139 – 140 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 0.75 (t, 3H, $J = 7.4$ Hz, CH_2CH_3), 1.64 (dt, 2H, $J_1 = 7.5$ Hz, $J_2 = 15.0$ Hz,

CH₂CH₂CH₃), 2.18 (s, 3H, Ar-CH₃), 3.88 – 3.97 (m, 2H, CH₂CH₂CH₃), 4.49 (s, 1H, OH), 4.93 (s, 1H, OH), 6.81 (dd, 1H, *J*₁ = 2.5 Hz, *J*₂ = 8.6 Hz, Ar-H), 6.98 – 6.94 (m, 2H, 2 × Ar-H), 7.00 (dd, 1H, *J*₁ = 0.5 Hz, *J*₂ = 2.5 Hz, Ar-H), 7.21 (dd, 1H, *J*₁ = 0.5 Hz, *J*₂ = 8.7 Hz, Ar-H), 7.25 – 7.28 (m, 2H, 2 × Ar-H) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 9.57, 11.49, 24.01, 45.96, 103.57, 107.53, 110.97, 111.85, 116.16, 124.57, 130.38, 132.29, 132.58, 139.05, 151.71, 158.08 ppm; HRMS *m/z* for C₁₈H₂₀NO₂ ([M+H]⁺): calcd 282.1494; found 282.1498; HPLC: *t*_r 6.973 min (96.72% at 254 nm, 96.58% at 280 nm).

2-(4-Hydroxyphenyl)-3-methyl-1-(2-phenoxyethyl)-1H-indol-5-ol (31). Prepared from **19** (0.135 g, 0.25 mmol) according to general procedure A. The crude product was purified by column chromatography with dichloromethane/acetone (40:1) as an eluent. Yield 90 mg (100%); grey oil; ¹H NMR (400 MHz, CDCl₃) δ 2.18 (s, 3H, Ar-CH₃), 4.05 (t, 2H, *J* = 6.5 Hz, CH₂), 4.39 (t, 2H, *J* = 6.5 Hz, CH₂), 4.67 (s, 1H, OH), 5.26 (s, 1H, OH), 6.68 – 6.73 (m, 2H, 2 × Ar-H), 6.84 (dd, 1H, *J*₁ = 2.5 Hz, *J*₂ = 8.7 Hz, Ar-H), 6.90 – 6.95 (m, 1H, Ar-H), 6.96 – 7.00 (m, 2H, 2 × Ar-H), 7.00 – 7.02 (m, 1H, Ar-H), 7.20 – 7.25 (m, 2H, 2 × Ar-H), 7.32 (dt, 3H, *J*₁ = 2.7 Hz, *J*₂ = 5.1 Hz, 3 × Ar-H) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 9.59, 43.75, 67.09, 103.66, 108.20, 111.20, 112.07, 115.11, 116.25, 121.51, 124.23, 130.22, 130.59, 132.48, 132.95, 139.26, 151.99, 158.27, 159.44 ppm; HRMS *m/z* for C₂₃H₂₂NO₃ ([M+H]⁺): calcd 360.1600; found 360.1592; HPLC: *t*_r 8.247 min (96.07% at 254 nm, 97.32% at 280 nm).

2-(4-Hydroxyphenyl)-3-methyl-1-(3-phenoxypropyl)-1H-indol-5-ol (32). Prepared from **20** (0.110 g, 0.19 mmol) according to general procedure A. The crude product was purified by column chromatography with dichloromethane/acetone (50:1) as an eluent. Yield 27 mg (38.3%); white solid; mp 152 – 154 °C; ¹H NMR (400 MHz, CDCl₃) δ 1.99 – 2.11 (m, 2H, CH₂), 2.17 (s, 3H, Ar-CH₃), 3.73 (t, 2H, *J* = 5.8 Hz, CH₂), 4.24 (t, 2H, *J* = 6.9 Hz, CH₂), 4.49 (s, 1H, OH), 4.84 (s, 1H, OH), 6.74 (d, 2H, *J* = 8.4 Hz, 2 × Ar-H), 6.77 (d, 1H, *J* = 10.6 Hz, Ar-H), 6.87 (d, 2H, *J* = 8.3 Hz, 2 × Ar-H), 6.94 (t, 1H, *J* = 7.3 Hz, Ar-H), 7.00 (s, 1H, Ar-H), 7.19 – 7.28 (m, 5H, Ph) ppm; ¹³C NMR (100 MHz, acetone-*d*₆) δ 8.69, 29.70, 40.31, 64.56, 102.75, 106.98, 110.07, 111.08, 114.33, 115.21, 115.30, 120.40, 123.39, 129.26, 129.59, 131.39, 138.12, 150.93, 157.23, 158.84 ppm; HRMS *m/z* for C₂₄H₂₄NO₃ ([M+H]⁺): calcd 374.1756; found 374.1746; HPLC: *t*_r 8.997 min (97.68% at 254 nm, 98.46% at 280 nm).

2-(4-Hydroxyphenyl)-3-methyl-1-(4-phenoxybutyl)-1H-indol-5-ol (33). Prepared from **21** (0.125 g, 0.22 mmol) according to general procedure A. The crude product was purified by column chromatography with dichloromethane/acetone (50:1) as an eluent. Yield 33 mg (38.8%); grey oil; ¹H NMR (400 MHz, CDCl₃) δ 1.55 – 1.64 (m, 2H, CH₂), 1.74 – 1.84 (m, 2H, CH₂), 2.18 (s, 3H, Ar-CH₃), 3.75 (t, 2H, *J* = 6.2 Hz, CH₂), 4.04 – 4.11 (m, 2H, CH₂), 4.52 (s, 1H, OH), 4.88 (s, 1H, OH), 6.78 – 6.84 (m, 3H, 3 × Ar-H), 6.88 – 6.92 (m, 2H, 2 × Ar-H), 6.93 – 6.98 (m, 1H, Ar-H), 7.00 (dd, 1H, *J*₁ = 0.5 Hz,

$J_2 = 2.5$ Hz, Ar-H), 7.21 – 7.31 (m, 5H, Ph) ppm; ^{13}C NMR (100 MHz, acetone- d_6) δ 9.60, 27.09, 27.28, 43.93, 67.71, 103.64, 107.72, 111.03, 111.95, 115.27, 116.24, 121.23, 124.48, 130.21, 130.45, 132.27, 132.61, 139.00, 151.77, 158.14, 159.96 ppm; HRMS m/z for $\text{C}_{25}\text{H}_{26}\text{NO}_3$ ($[\text{M}+\text{H}]^+$): calcd 388.1913; found 388.1905; HPLC: t_r 9.630 min (96.56% at 254 nm, 95.00% at 280 nm).

4-(5-Hydroxy-3-methyl-1-(3-phenoxypropyl)-1H-indol-2-yl)benzene-1,2-diol (36). Prepared from **24** (1.00 mmol) according to general procedure A. The crude product was purified by column chromatography with ethyl acetate/hexane (1:1) as an eluent to afford deprotected phenol. Yield over 2 steps: 332 mg (85%); brown solid; mp 43 – 46 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 1.90 (p, 2H, $J = 6.2$ Hz, CH_2), 2.08 (s, 3H, Ar- CH_3), 3.74 (t, 2H, $J = 6.2$ Hz, CH_2), 4.15 (t, 2H, $J = 7.0$ Hz, CH_2), 6.59 – 6.68 (m, 2H, 2 \times Ar-H), 6.76 – 6.82 (m, 4H, 4 \times Ar-H), 6.84 – 6.94 (m, 2H, 2 \times Ar-H), 7.20 – 7.29 (m, 3H, 3 \times Ar-H), 8.70 (br s, 1H, OH), 9.15 (br s, 2H, 2 \times OH) ppm; ^{13}C NMR (100 MHz, DMSO- d_6) δ 9.36, 29.42, 33.13, 64.57, 102.40, 106.07, 110.10, 111.04, 114.31, 115.62, 117.42, 120.45, 121.37, 122.63, 128.86, 129.37, 130.53, 137.92, 145.10, 145.21, 150.62, 158.24 ppm; HRMS m/z for $\text{C}_{24}\text{H}_{22}\text{NO}_4$ ($[\text{M}-\text{H}]^+$): calcd; 388.1554 found; 388.1556; HPLC: t_r 7.727 min (95.30% at 254 nm, 95.07% at 280 nm).

4-(5-Hydroxy-3-methyl-1-(4-phenoxybutyl)-1H-indol-2-yl)benzene-1,2-diol (37). Prepared from **25** (1.00 mmol) according to general procedure A. The crude product was purified by column chromatography with ethyl acetate/hexane (1:1) as an eluent to afford deprotected phenol. Yield over 2 steps: 307 mg (76%); yellow solid; mp 47 – 50 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 1.42 – 1.50 (m, 2H, CH_2), 1.57 – 1.66 (m, 2H, CH_2), 2.07 (s, 3H, Ar- CH_3), 3.74 (t, 2H, $J = 6.4$ Hz, CH_2), 4.03 (t, 2H, $J = 7.1$ Hz, CH_2), 6.63 – 6.68 (m, 2H, 2 \times Ar-H), 6.76-6.95 (m, 6H, 6 \times Ar-H), 7.22-7.29 (m, 3H, 3 \times Ar-H), 8.69 (br s, 1H, OH), 9.16 (br s, 2H, 2 \times OH) ppm; ^{13}C NMR (100 MHz, DMSO- d_6) δ 9.35, 25.78, 26.09, 30.38, 66.63, 102.36, 105.88, 110.25, 111.00, 114.33, 115.63, 117.41, 120.35, 121.34, 122.79, 128.83, 129.38, 130.50, 137.97, 145.08, 145.17, 150.57, 158.43 ppm. HRMS m/z for $\text{C}_{25}\text{H}_{24}\text{NO}_4$ ($[\text{M}-\text{H}]^+$): calcd 402.1711; found 402.1712; HPLC: t_r 8.380 min (95.94% at 254 nm, 95.66% at 280 nm).

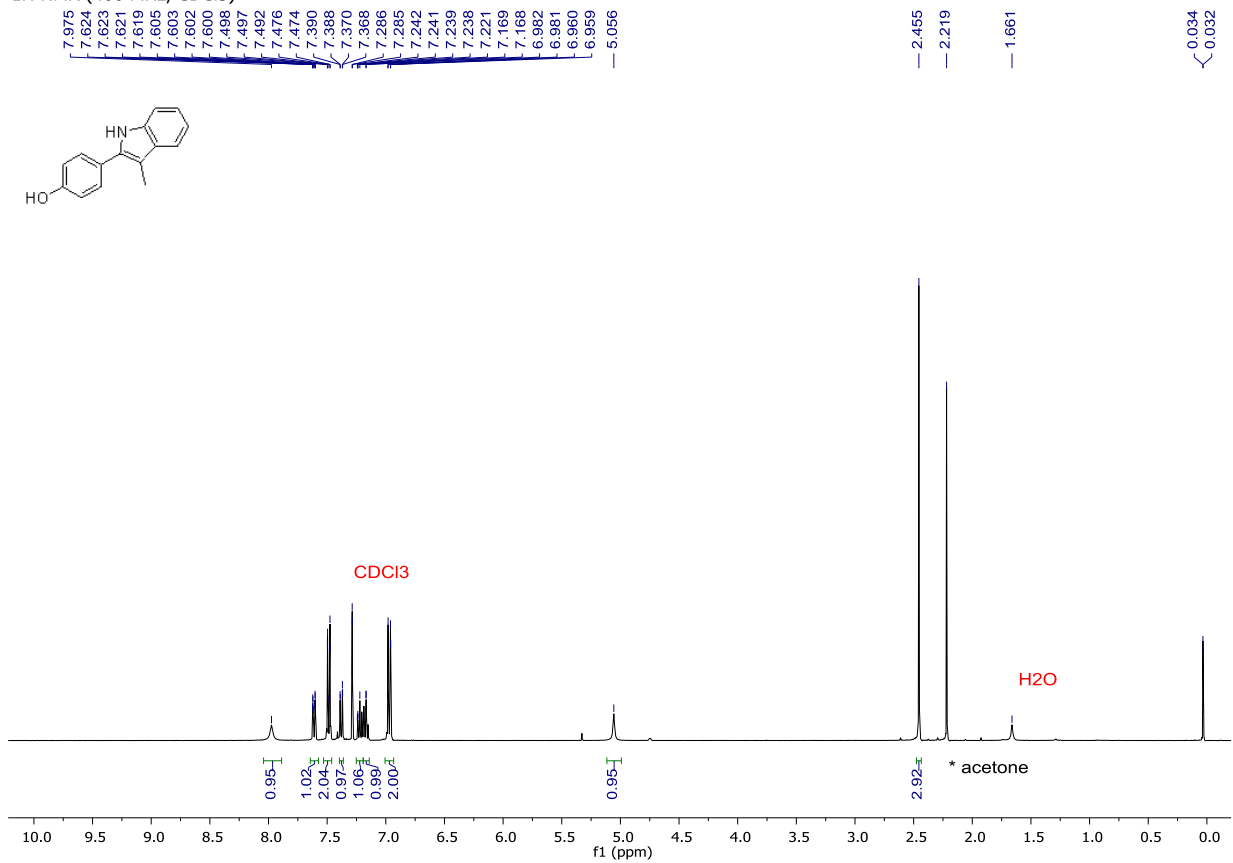
2-(3,4-Dimethoxyphenyl)-5-methoxy-3-methyl-1-(4-phenoxybutyl)-1H-indole (38). To the solution of 4-(5-hydroxy-3-methyl-1-(4-phenoxybutyl)-1H-indol-2-yl)benzene-1,2-diol (**37**) (61 mg, 0.15 mmol) in acetone (3 mL) were added Cs_2CO_3 (195 mg, 0.60 mmol) and methyl iodide (46.5 μL , 0.75 mmol). The mixture was refluxed for 48 h, solvent was removed under reduced pressure and the residue was suspended in ethyl acetate (20 mL). The suspension was then washed with water (1 \times 20 mL), 10% citric acid (2 \times 20 mL) and brine (1 \times 20 mL). Organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography with ethyl acetate/hexane (1:4) as an eluent to yield **38** as yellow solidified oil. Yield: 44 mg (66%); ^1H NMR (400 MHz, DMSO-

d_6) δ 1.43 – 1.52 (m, 2H, $\underline{\text{CH}_2}$), 1.60 – 1.69 (m, 2H, $\underline{\text{CH}_2}$), 2.15 (s, 3H, Ar- $\underline{\text{CH}_3}$), 3.73 (t, 2H, $J = 6.3$ Hz, $\underline{\text{CH}_2}$), 3.78 (s, 3H, O- $\underline{\text{CH}_3}$), 3.80 (s, 3H, O- $\underline{\text{CH}_3}$), 3.81 (s, 3H, O- $\underline{\text{CH}_3}$), 4.10 (t, 2H, $J = 7.1$ Hz, $\underline{\text{CH}_2}$), 6.77 – 6.82 (m, 3H, $3 \times$ Ar- $\underline{\text{H}}$), 6.87 – 6.97 (m, 3H, $3 \times$ Ar- $\underline{\text{H}}$), 7.01 (d, 1H, $J = 2.4$ Hz, Ar- $\underline{\text{H}}$), 7.04 (d, 1H, $J = 8.2$ Hz, Ar- $\underline{\text{H}}$), 7.21 – 7.27 (m, 2H, $2 \times$ Ar- $\underline{\text{H}}$), 7.39 (d, 1H, $J = 8.8$ Hz, Ar- $\underline{\text{H}}$) ppm; ^{13}C NMR (100 MHz, DMSO- d_6) δ 9.34, 25.75, 26.17, 42.88, 55.37 (2C), 55.54, 66.52, 100.35, 107.10, 110.67, 111.05, 111.56, 113.55, 114.26, 120.35, 122.65, 124.08, 128.40, 129.37, 131.20, 137.70, 148.45, 148.48, 153.33, 158.42 ppm; HRMS m/z for $\text{C}_{28}\text{H}_{32}\text{NO}_4$ ($[\text{M}+\text{H}]^+$): calcd 446.2326; found 446.2322; HPLC: t_r 15.777 min (95.12% at 254 nm, 95.91% at 280 nm).

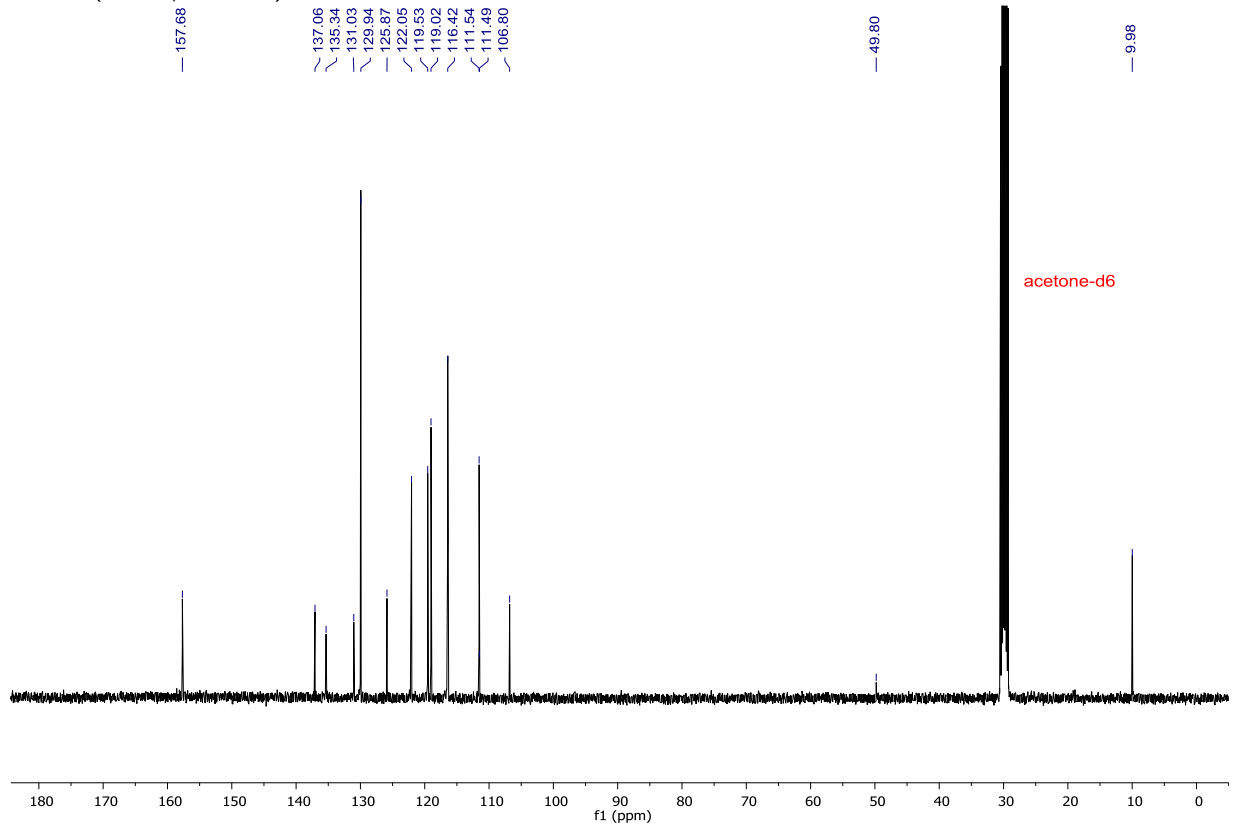
^1H and ^{13}C NMR spectra of the representative compounds

4-(3-Methyl-1*H*-indol-2-yl)phenol (10).

^1H NMR (400 MHz, CDCl_3)

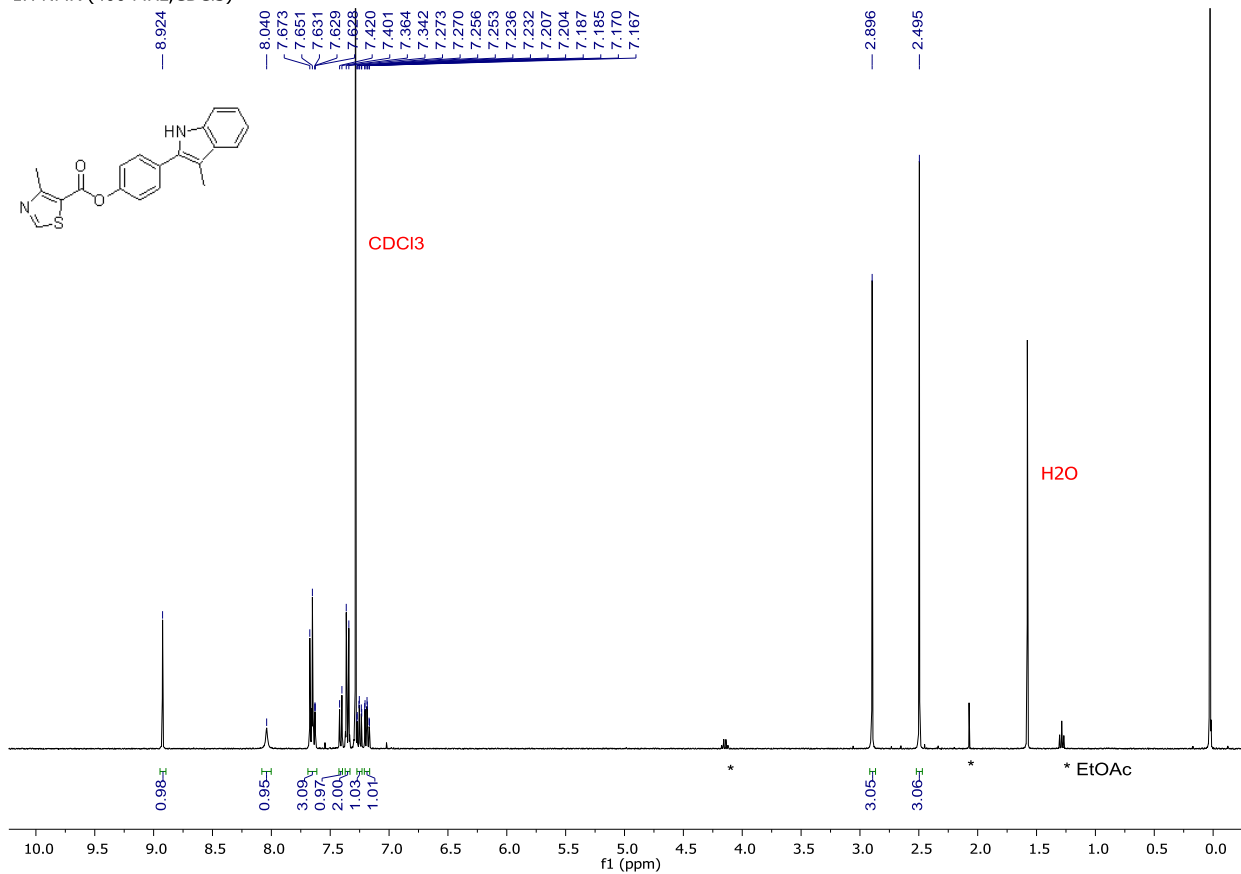


^{13}C NMR (100 MHz, acetone-d_6)

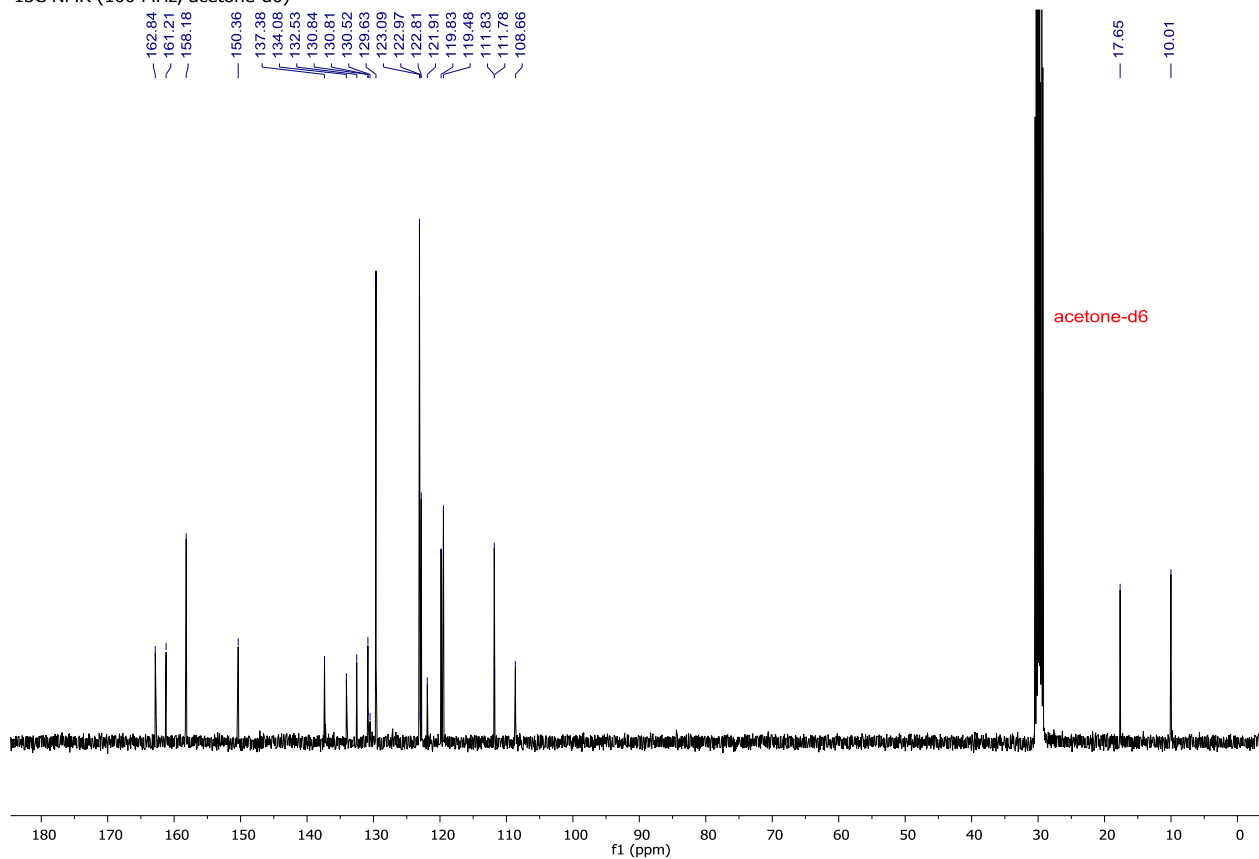


4-(3-Methyl-1H-indol-2-yl)phenyl 4-methylthiazole-5-carboxylate (13).

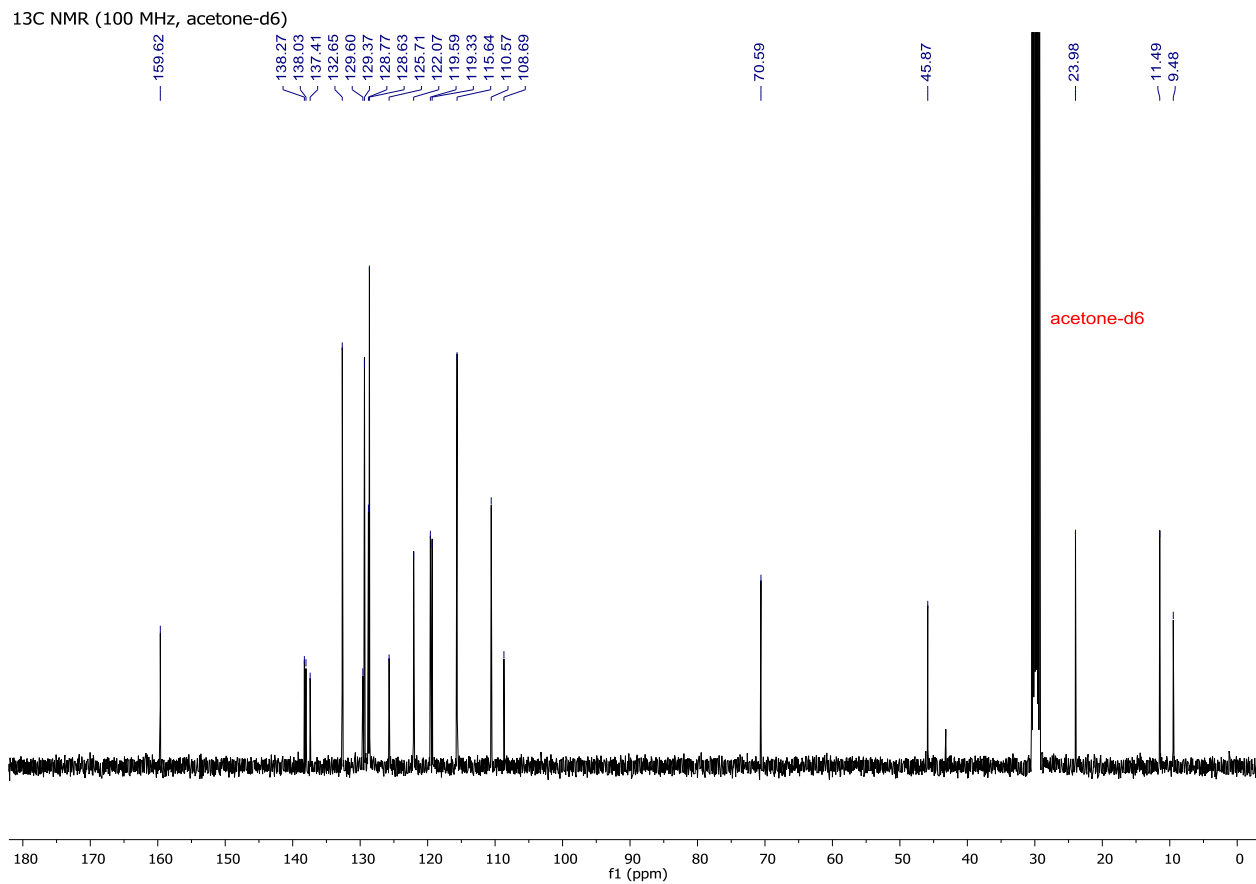
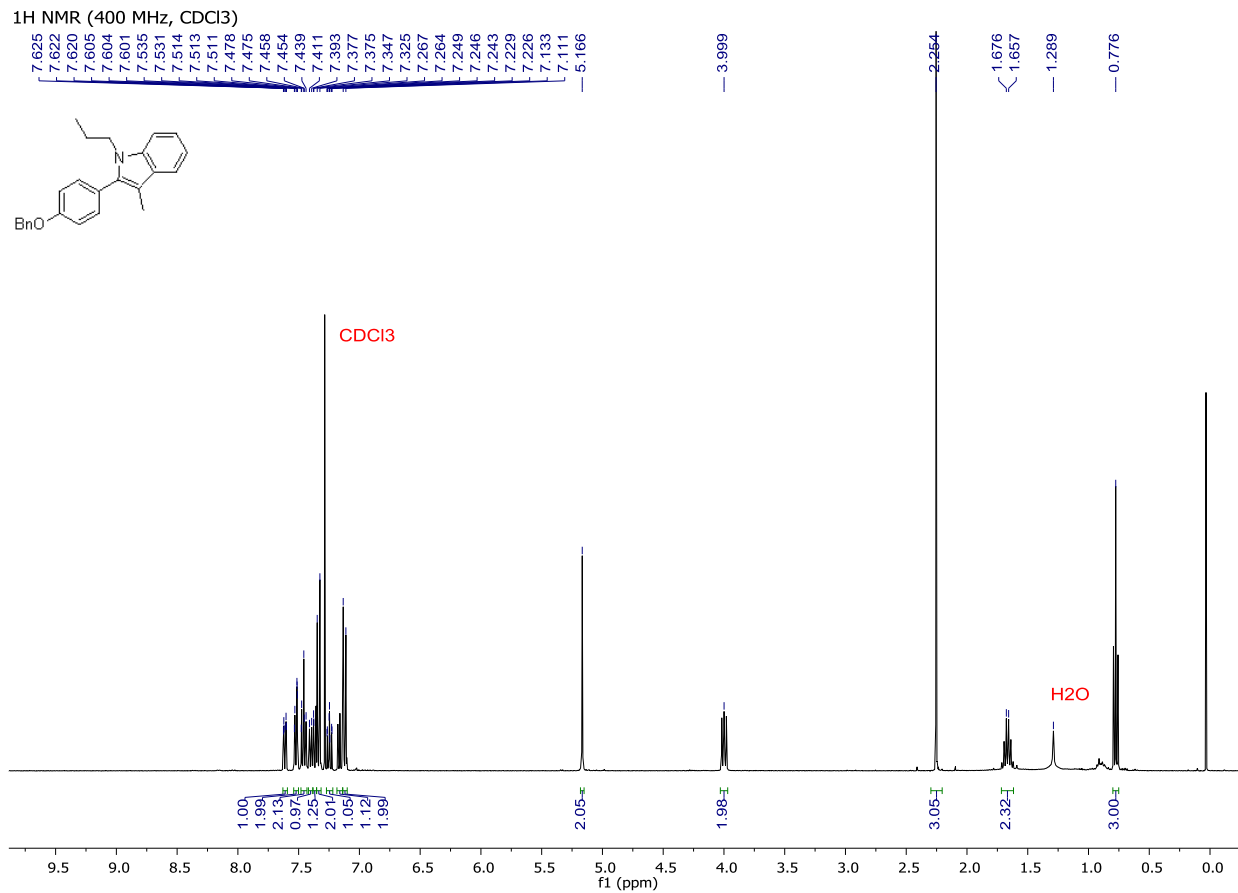
¹H NMR (400 MHz, CDCl₃)



¹³C NMR (100 MHz, acetone-d₆)

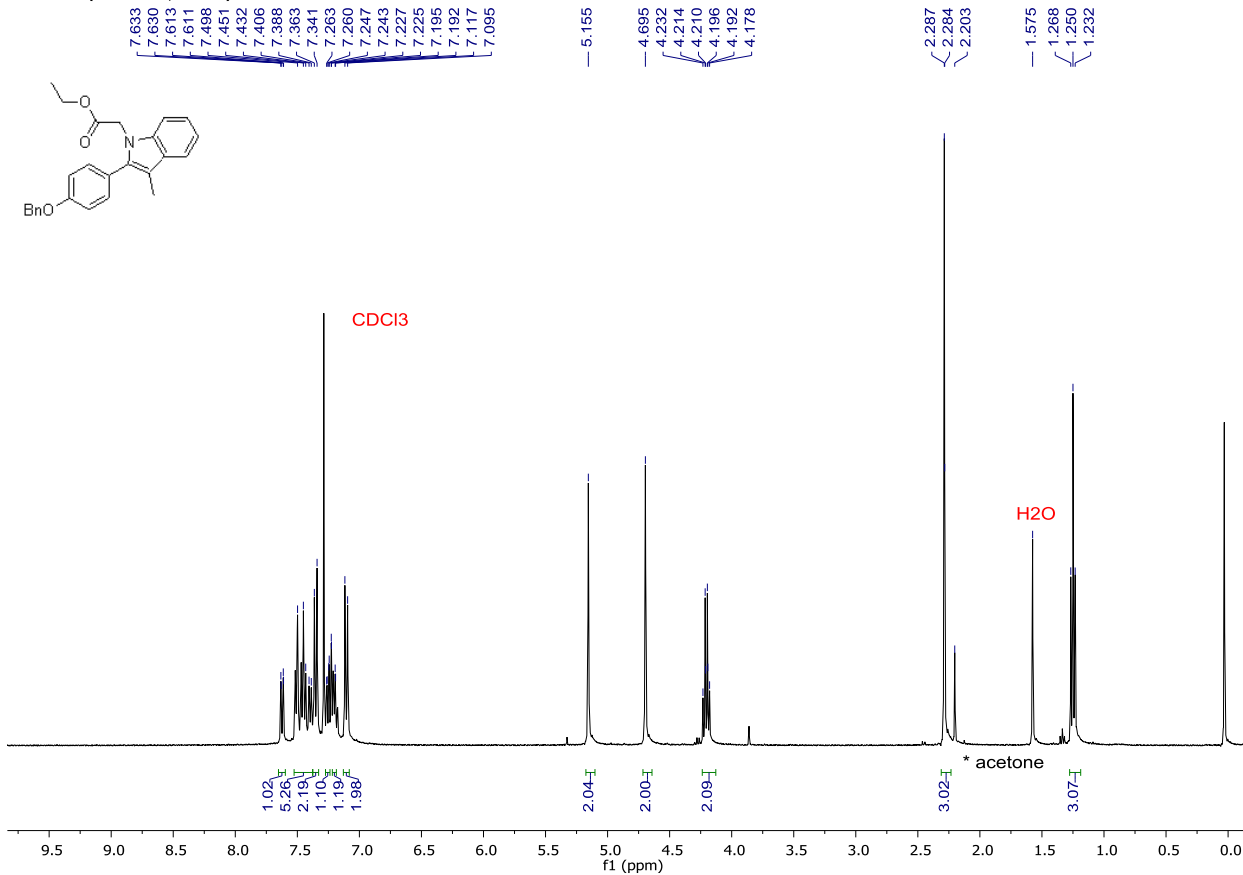


2-(4-(Benzyloxy)phenyl)-3-methyl-1-propyl-1H-indole (15).

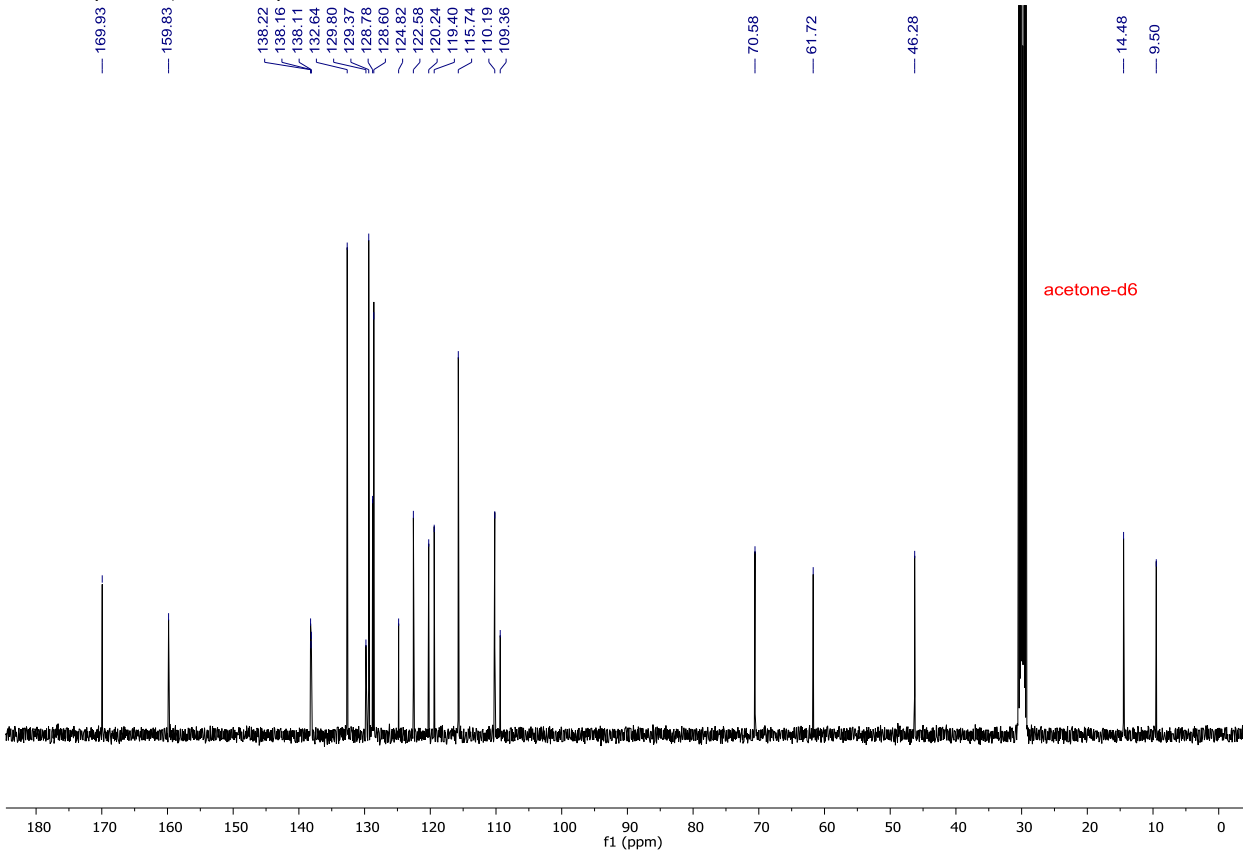


Ethyl 2-(2-(4-(benzyloxy)phenyl)-3-methyl-1H-indol-1-yl)acetate (16).

¹H NMR (400 MHz, CDCl₃)

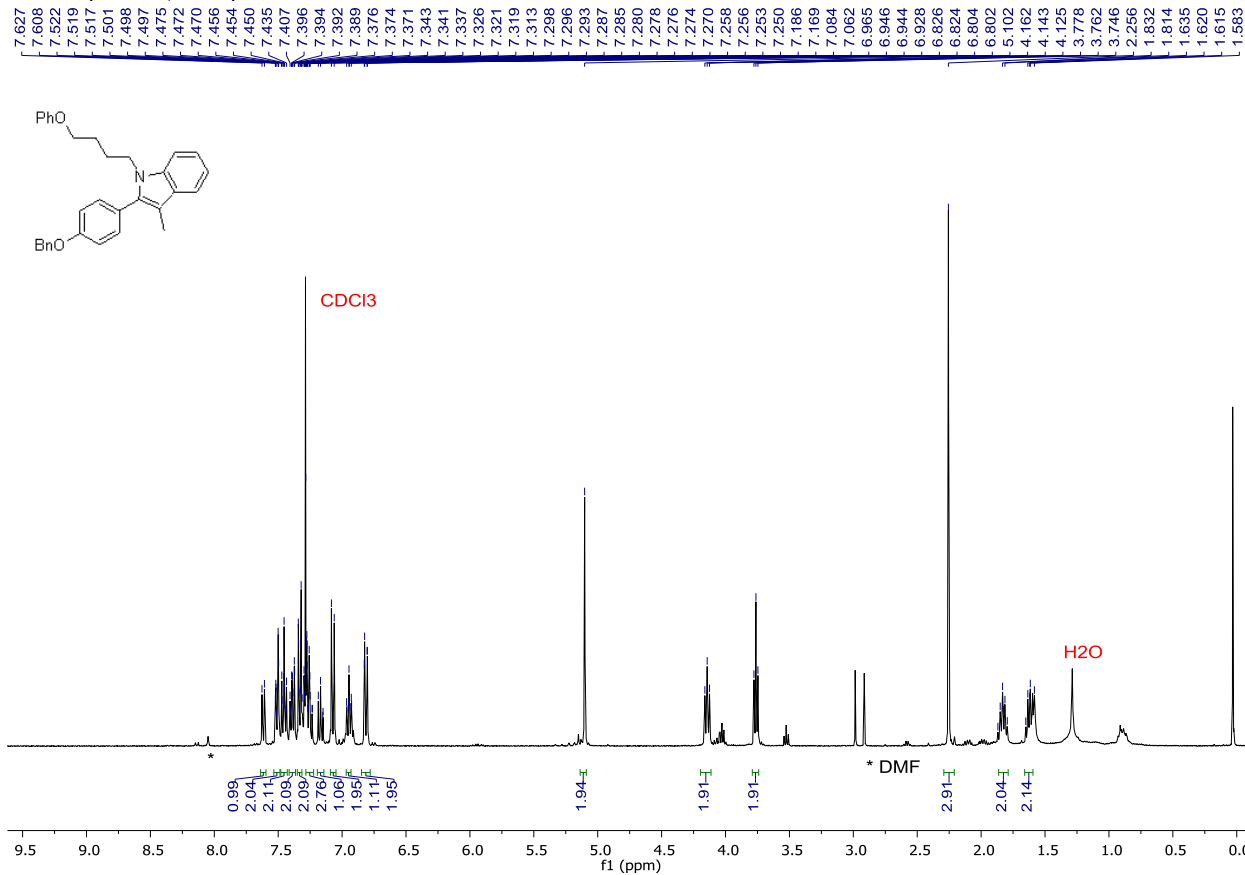


¹³C NMR (100 MHz, acetone-d₆)

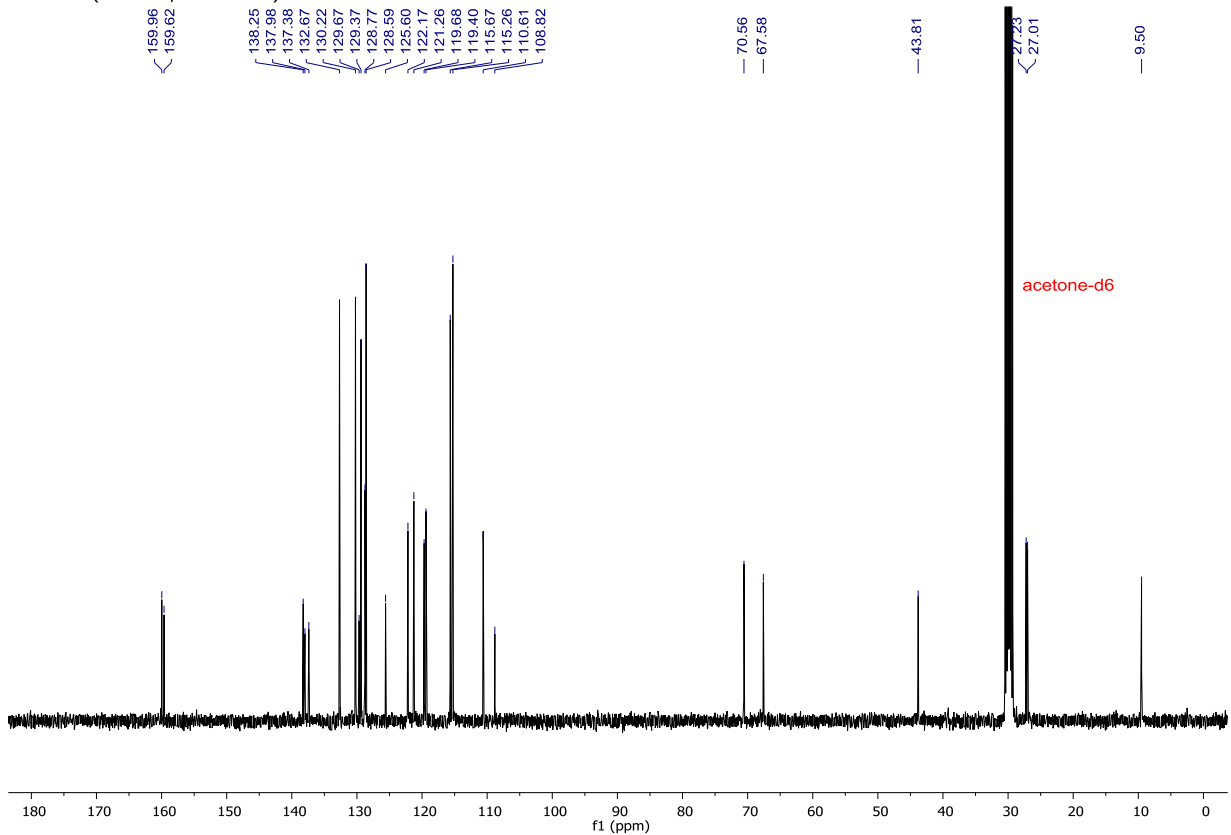


2-(4-(Benzyloxy)phenyl)-3-methyl-1-(4-phenoxybutyl)-1H-indole (17).

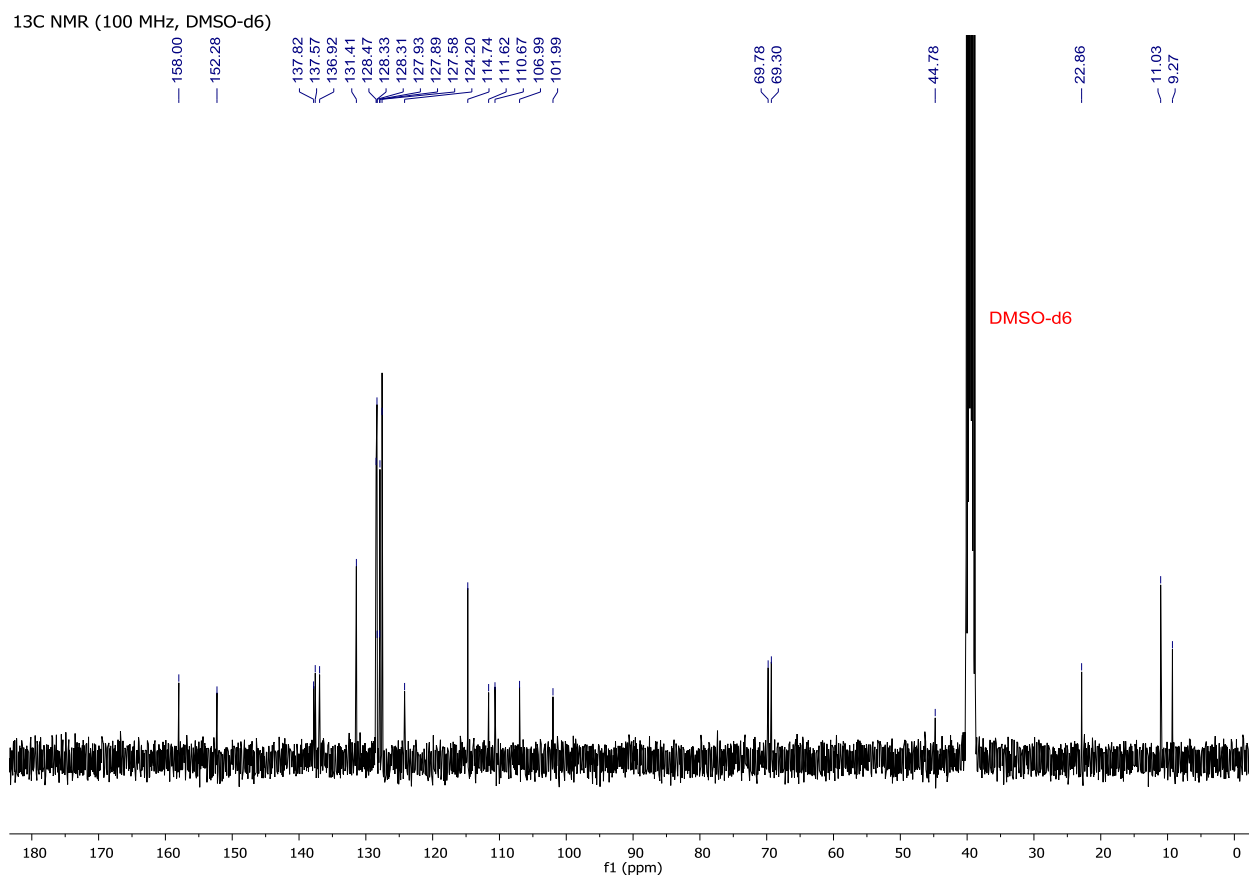
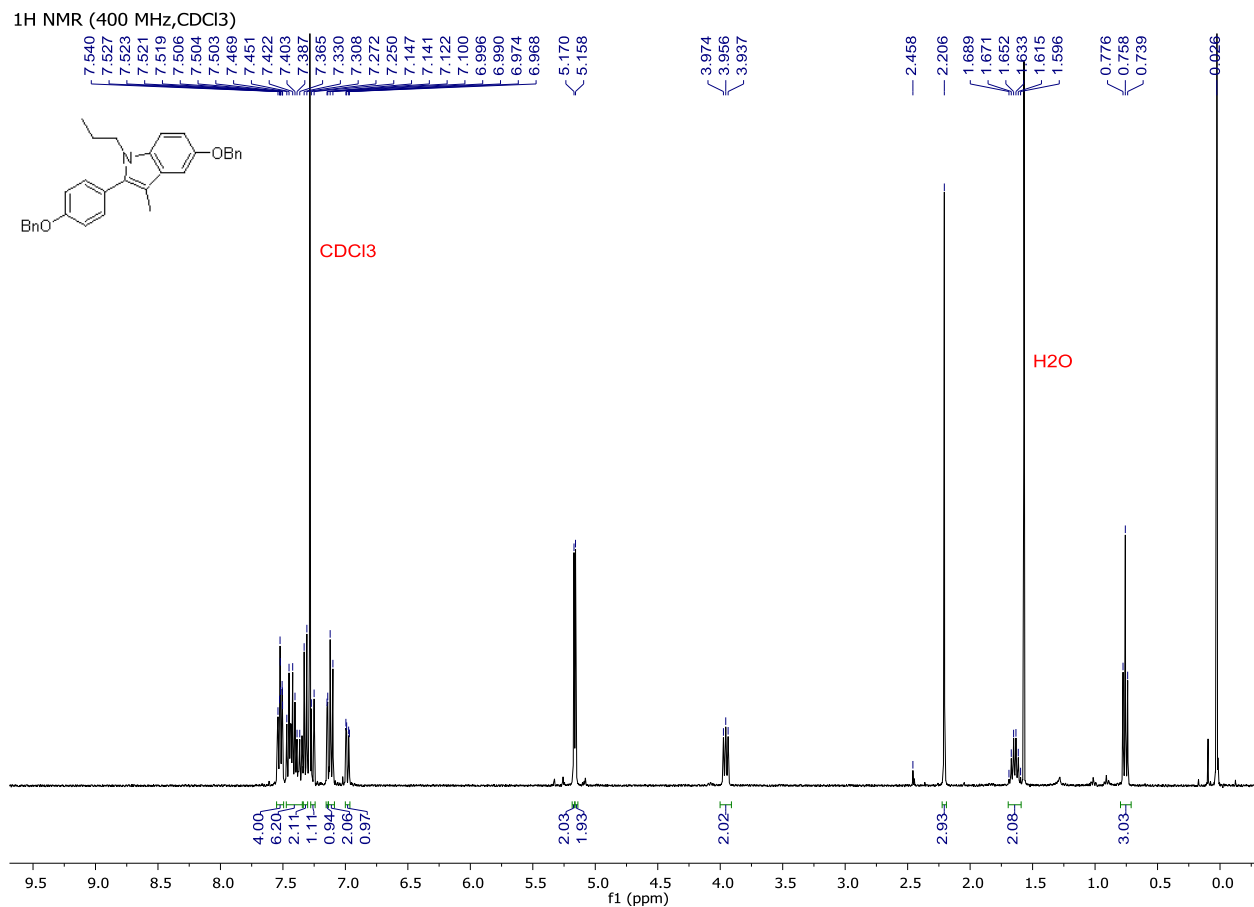
¹H NMR (400 MHz, CDCl₃)



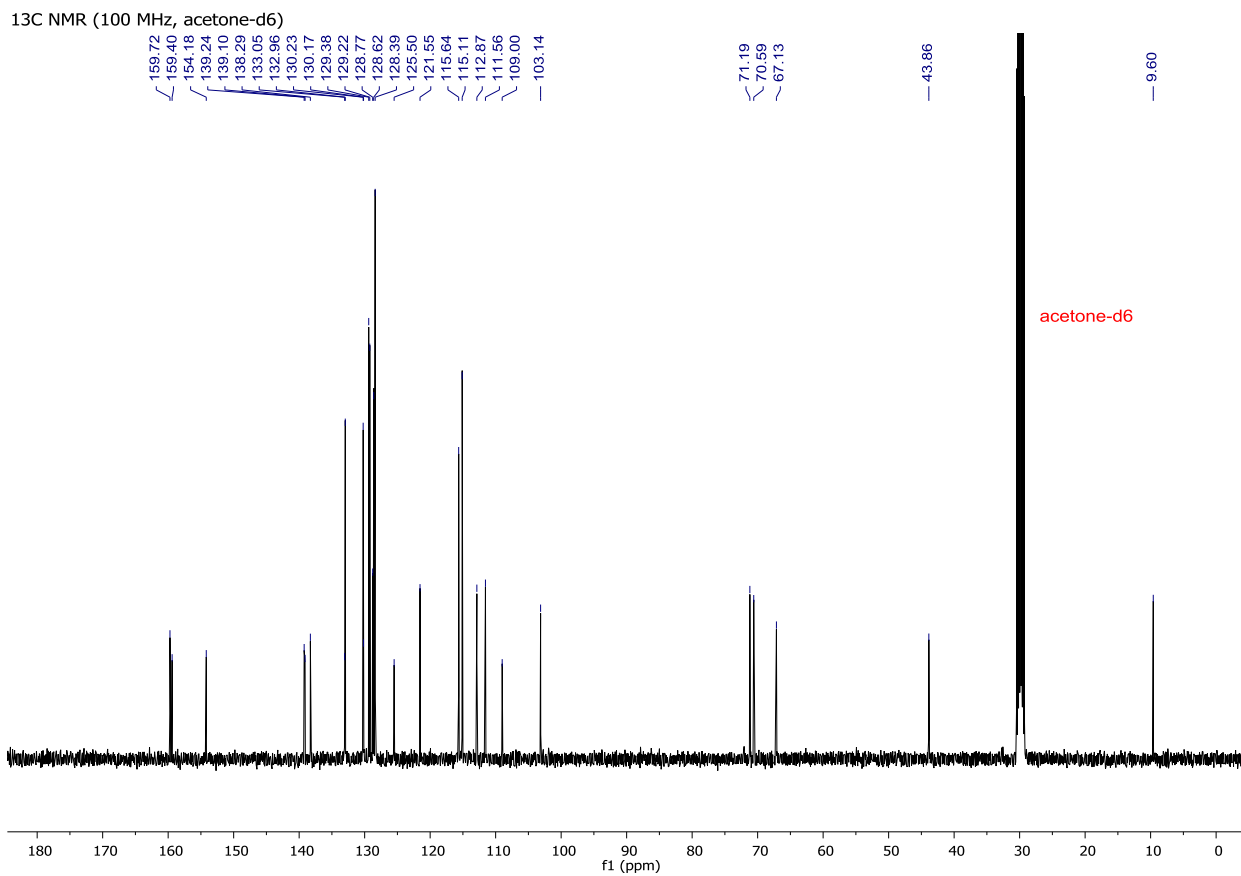
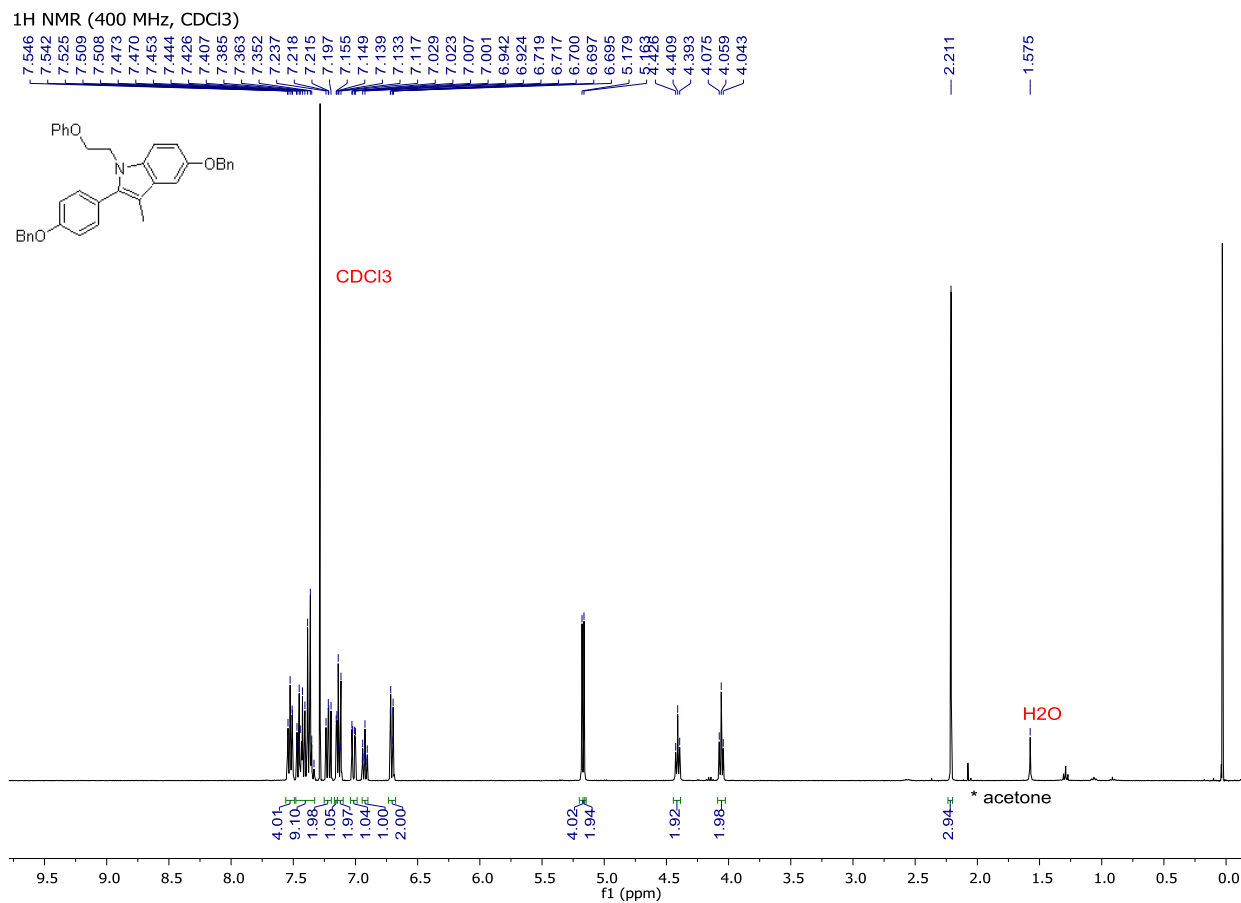
¹³C NMR (100 MHz, acetone-d₆)



5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-propyl-1H-indole (18).

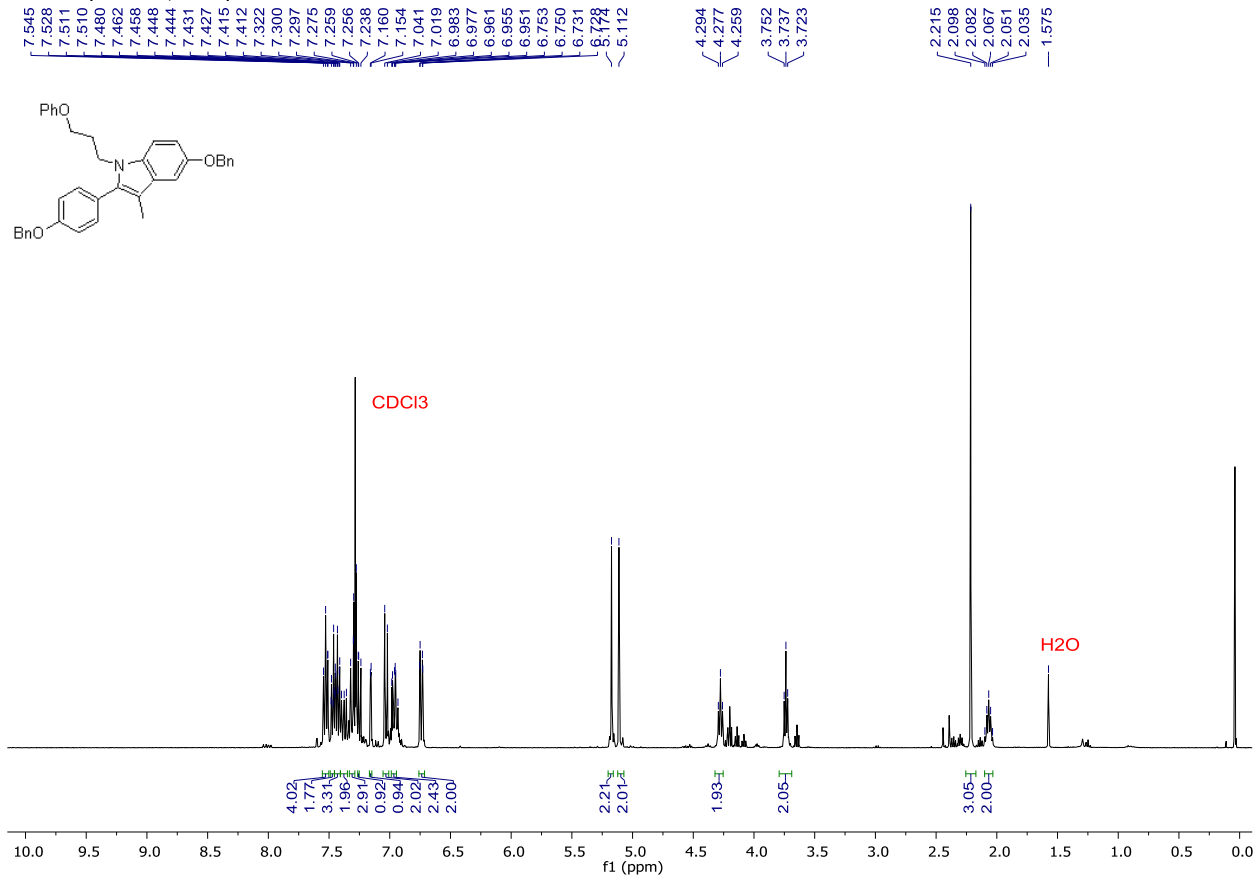


5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-(2-phenoxyethyl)-1H-indole (19).

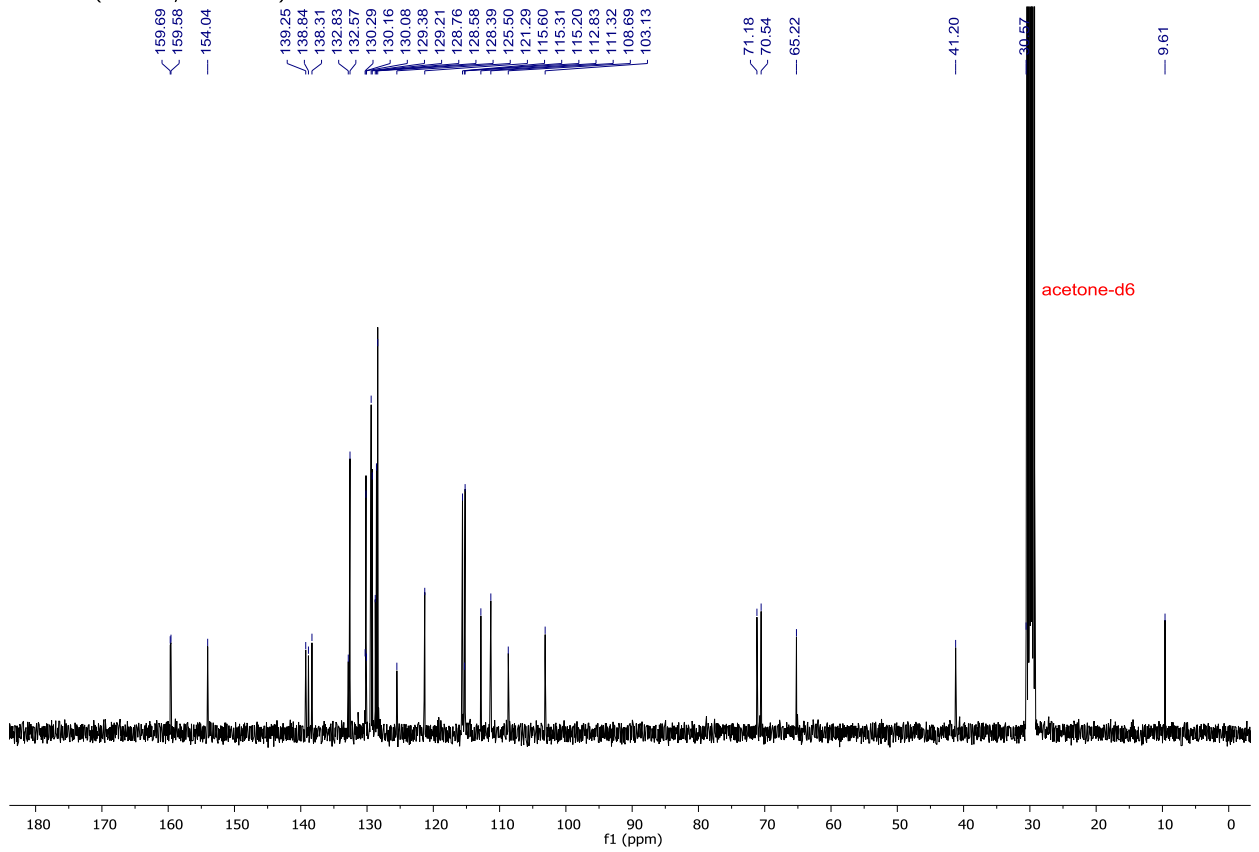


5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-(3-phenoxypropyl)-1H-indole (20).

1H NMR (400 MHz, CDCl₃)

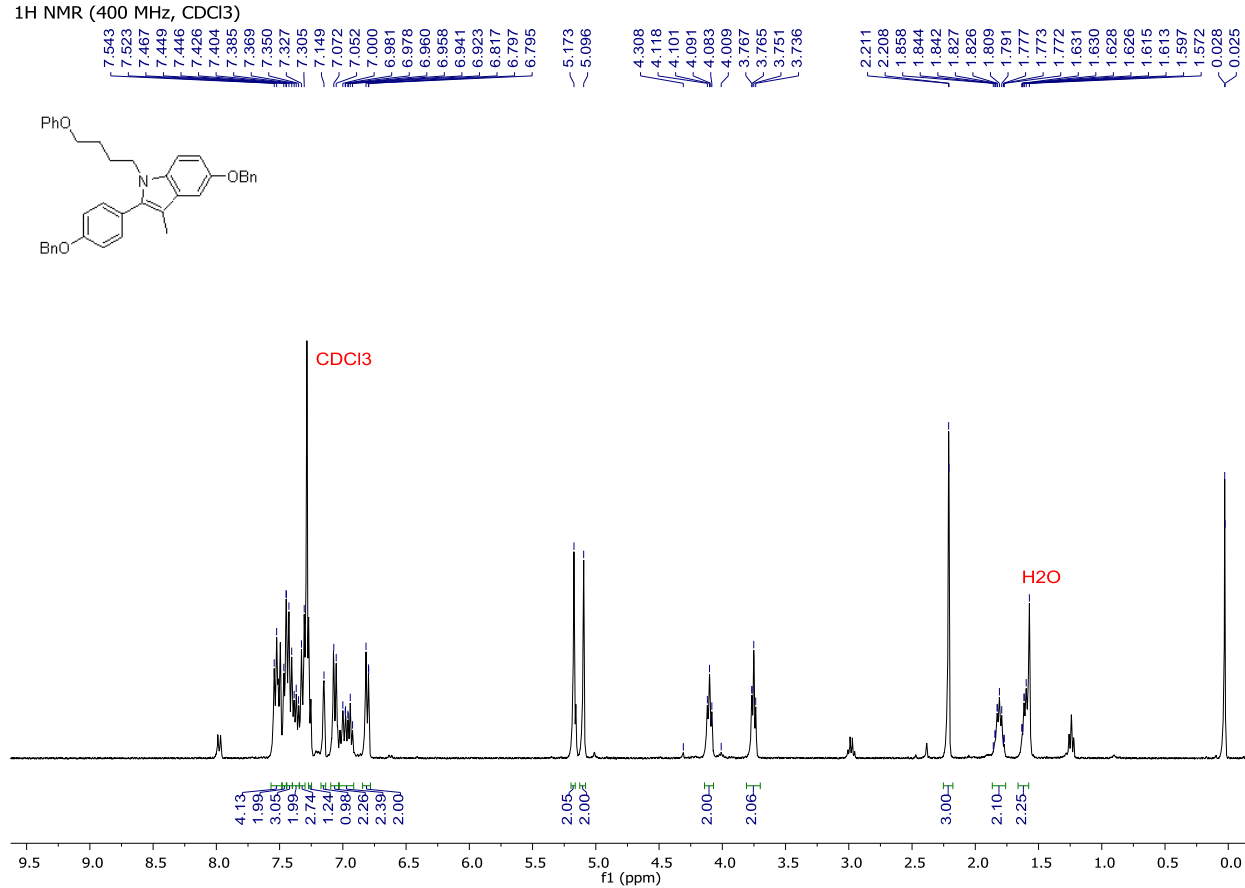
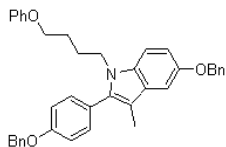


13C NMR (100 MHz, acetone-d₆)

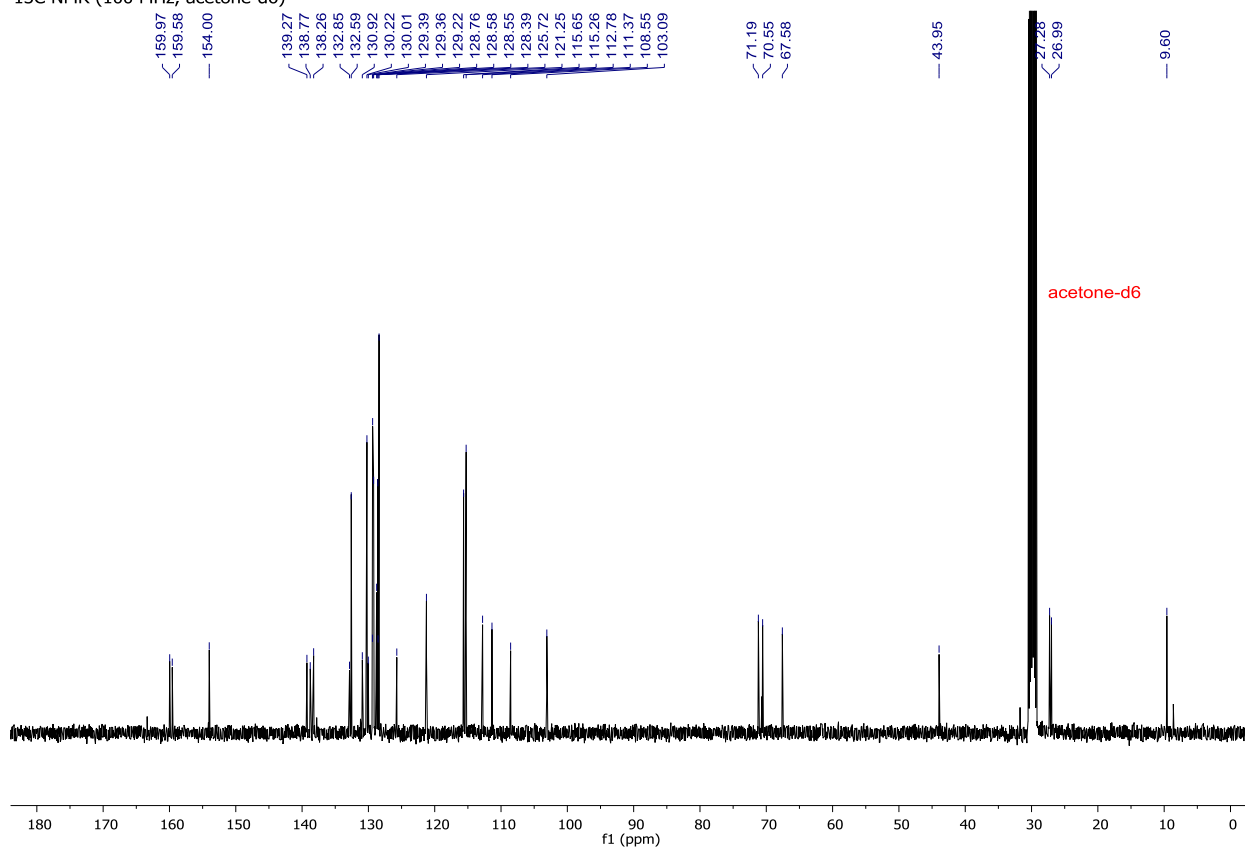


5-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-3-methyl-1-(4-phenoxybutyl)-1H-indole (21).

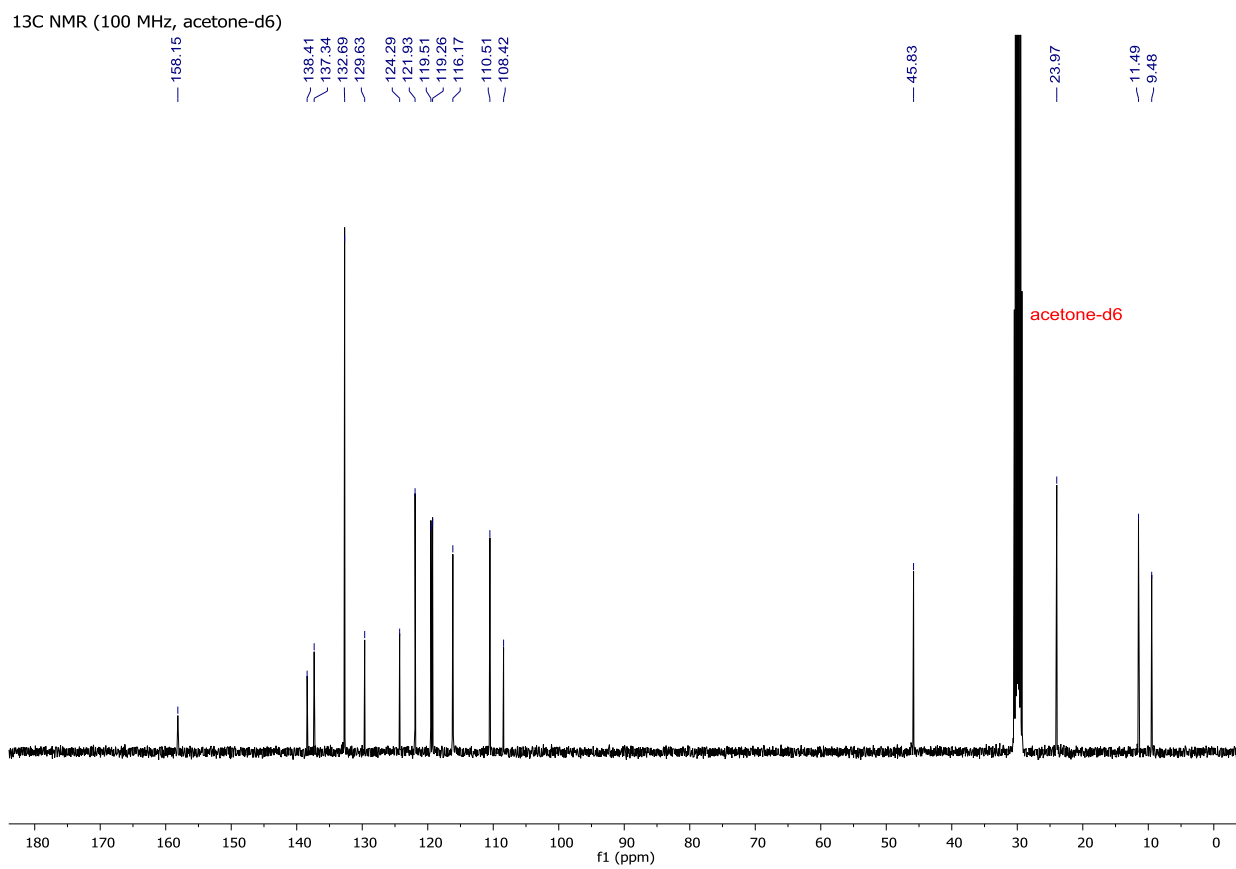
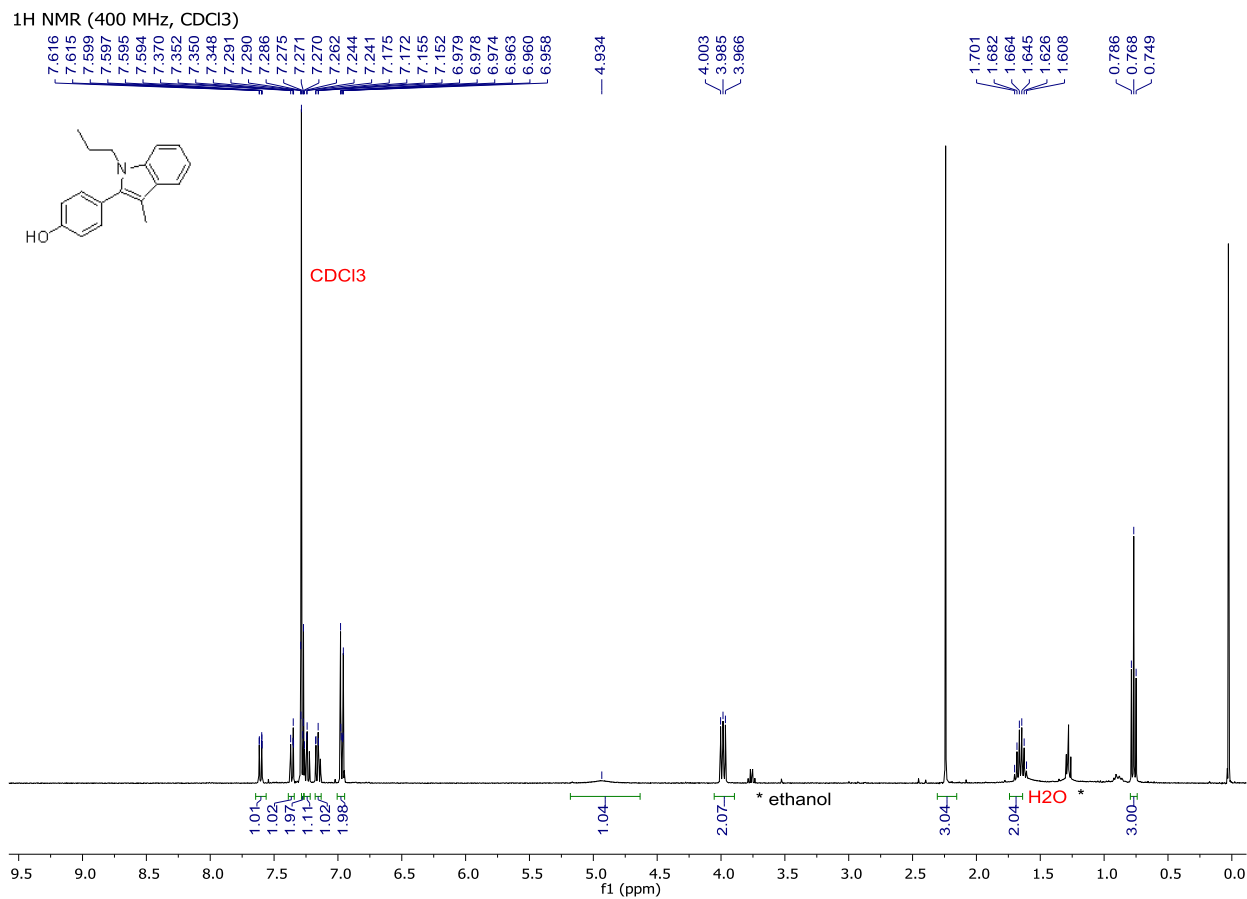
¹H NMR (400 MHz, CDCl₃)



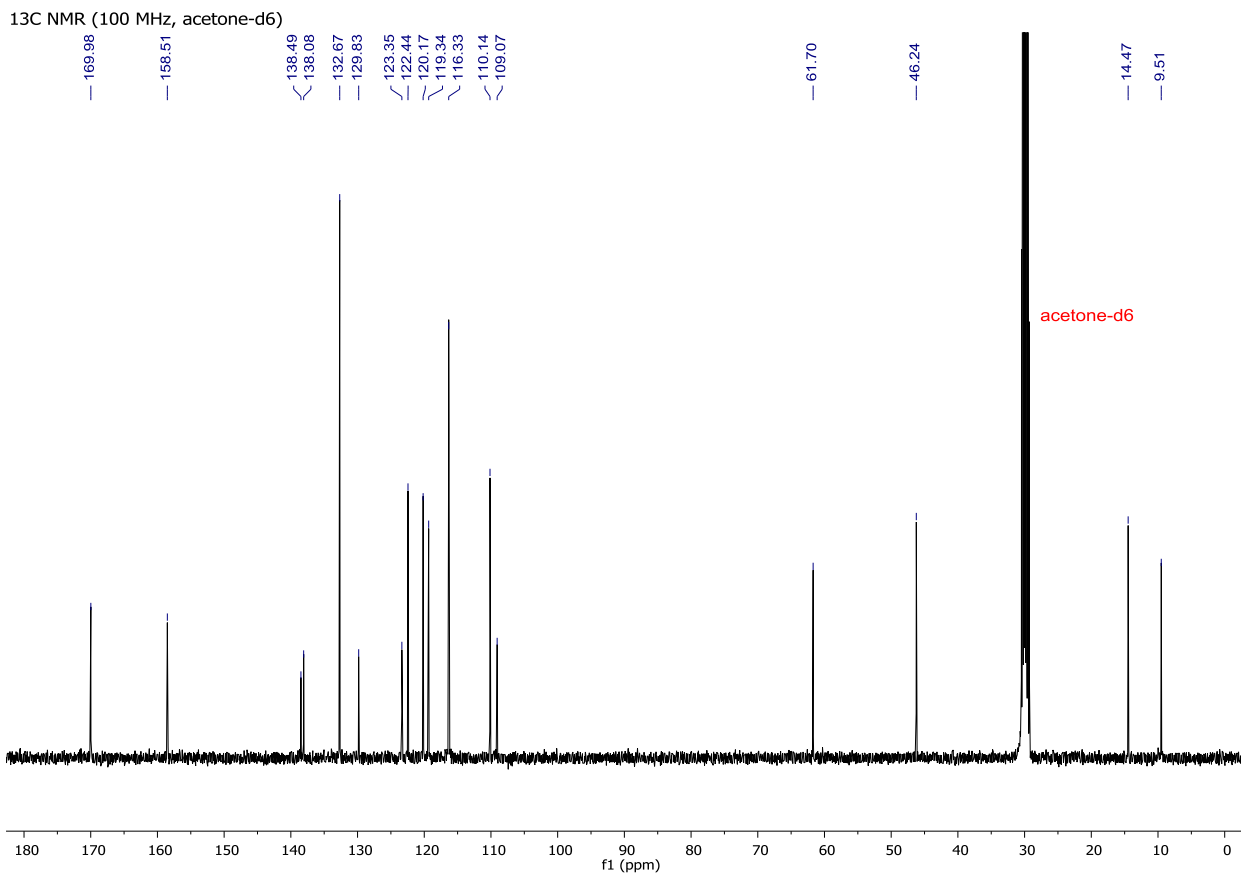
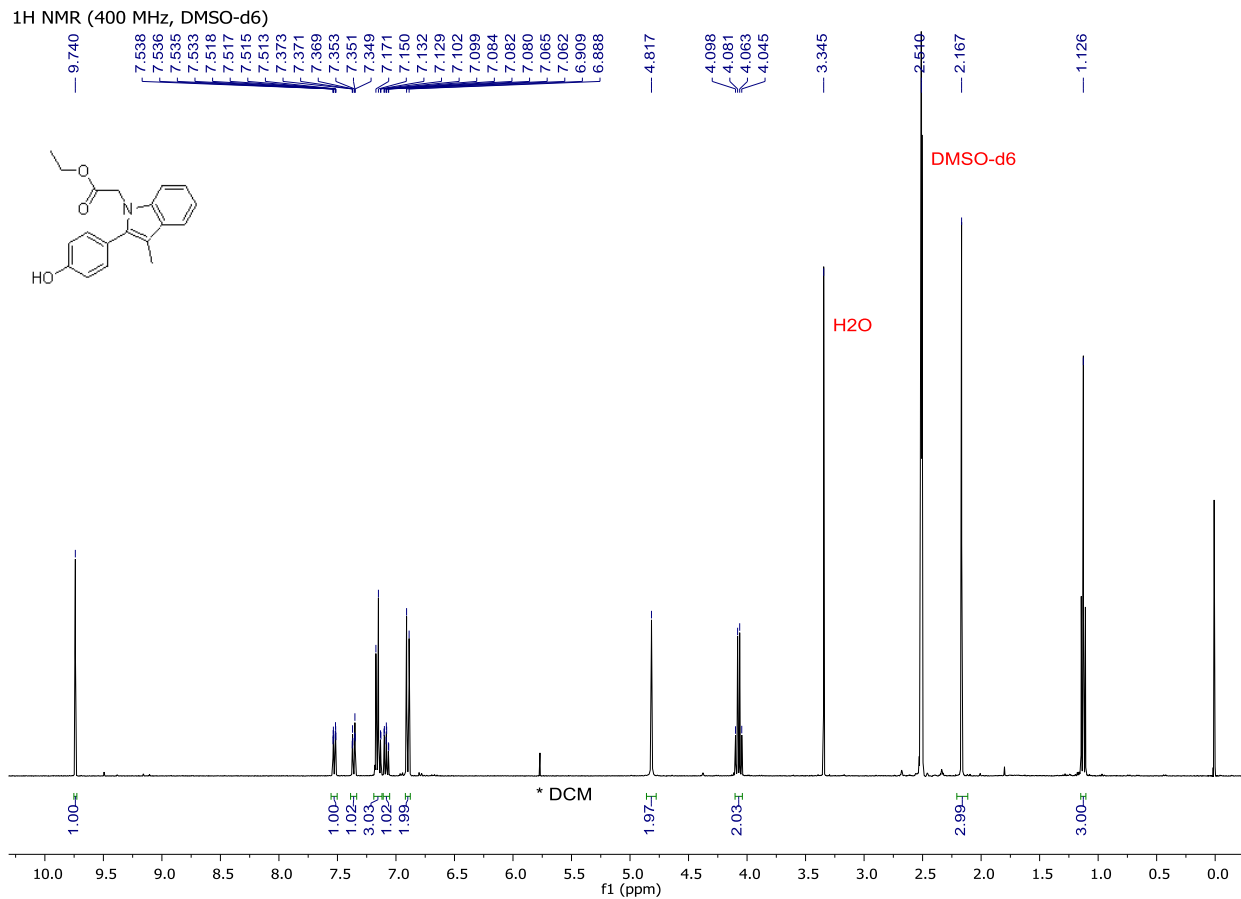
¹³C NMR (100 MHz, acetone-d₆)



4-(3-Methyl-1-propyl-1H-indol-2-yl)phenol (26).

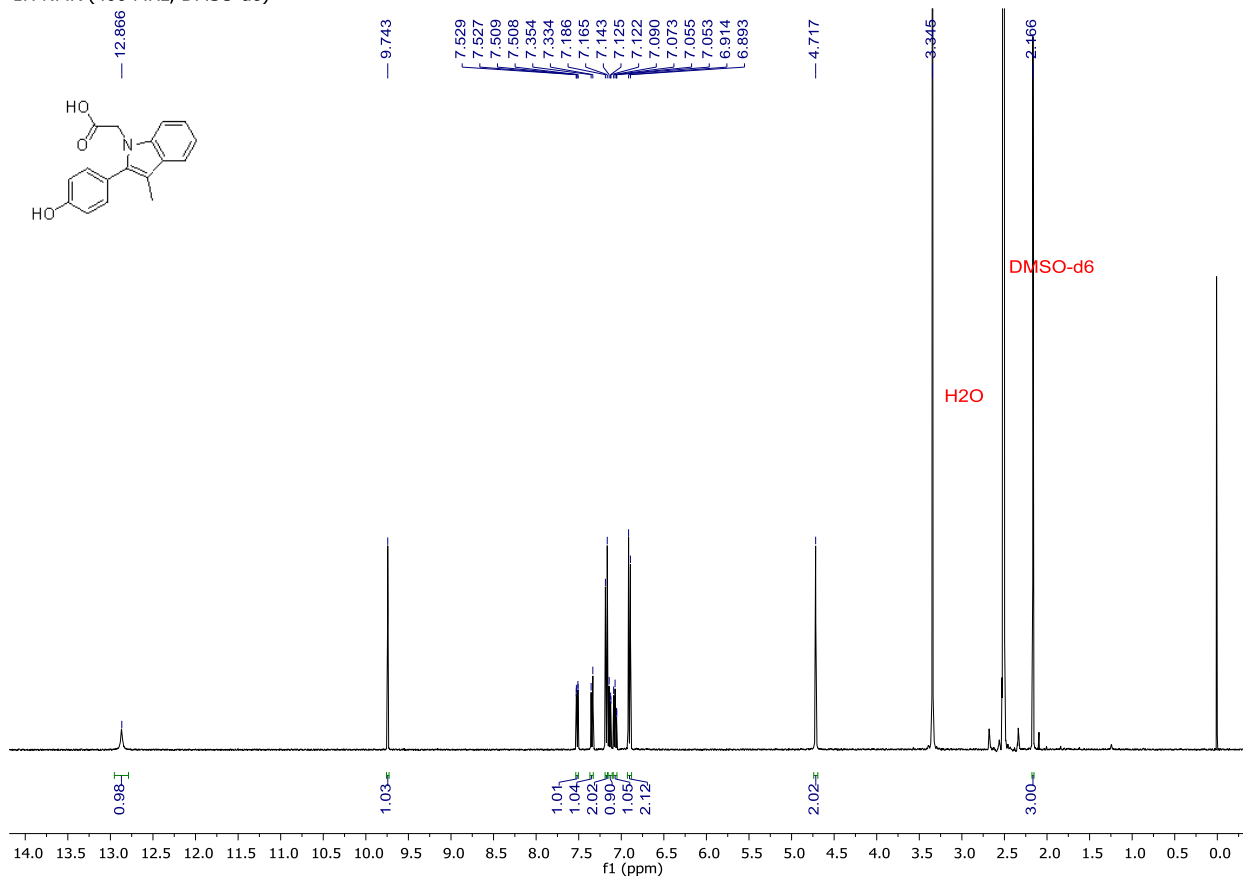


Ethyl 2-(2-(4-hydroxyphenyl)-3-methyl-1H-indol-1-yl)acetate (27).

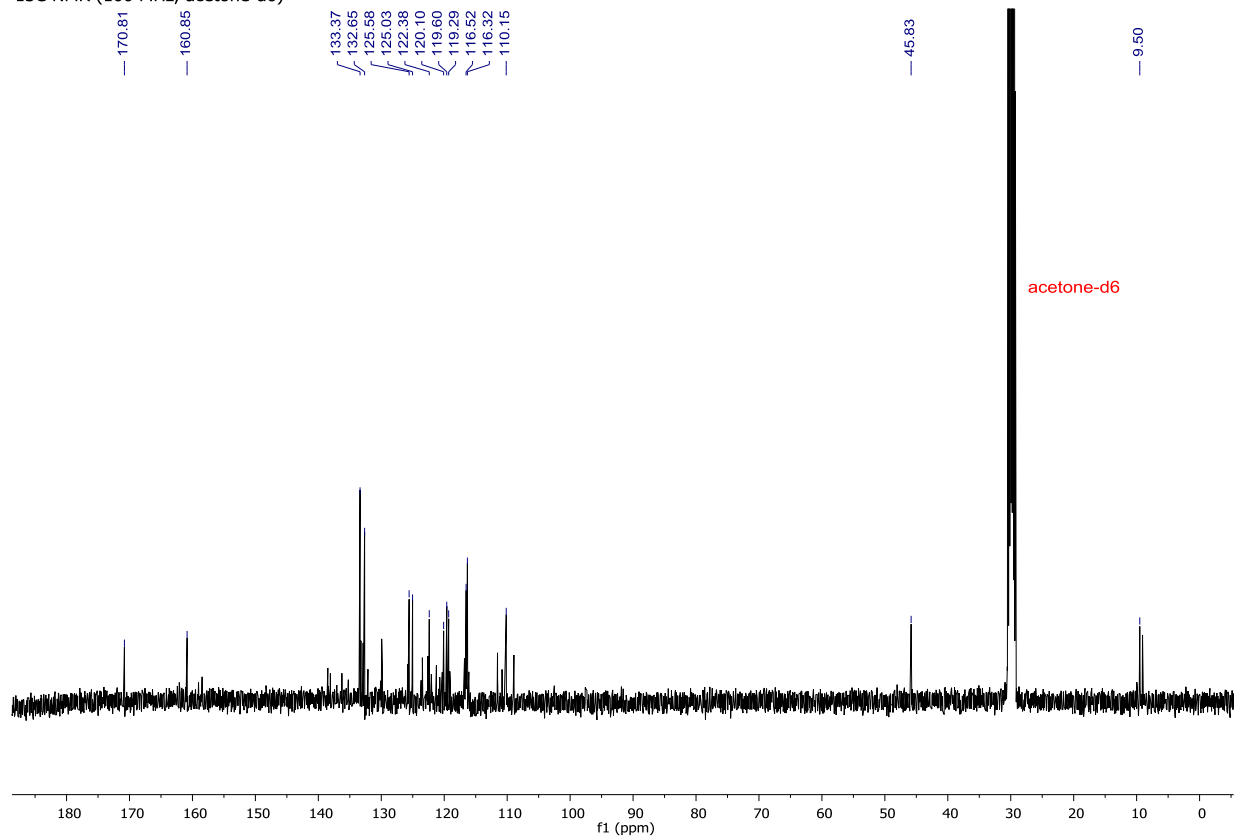


2-(2-(4-Hydroxyphenyl)-3-methyl-1H-indol-1-yl)acetic acid (28).

¹H NMR (400 MHz, DMSO-d₆)

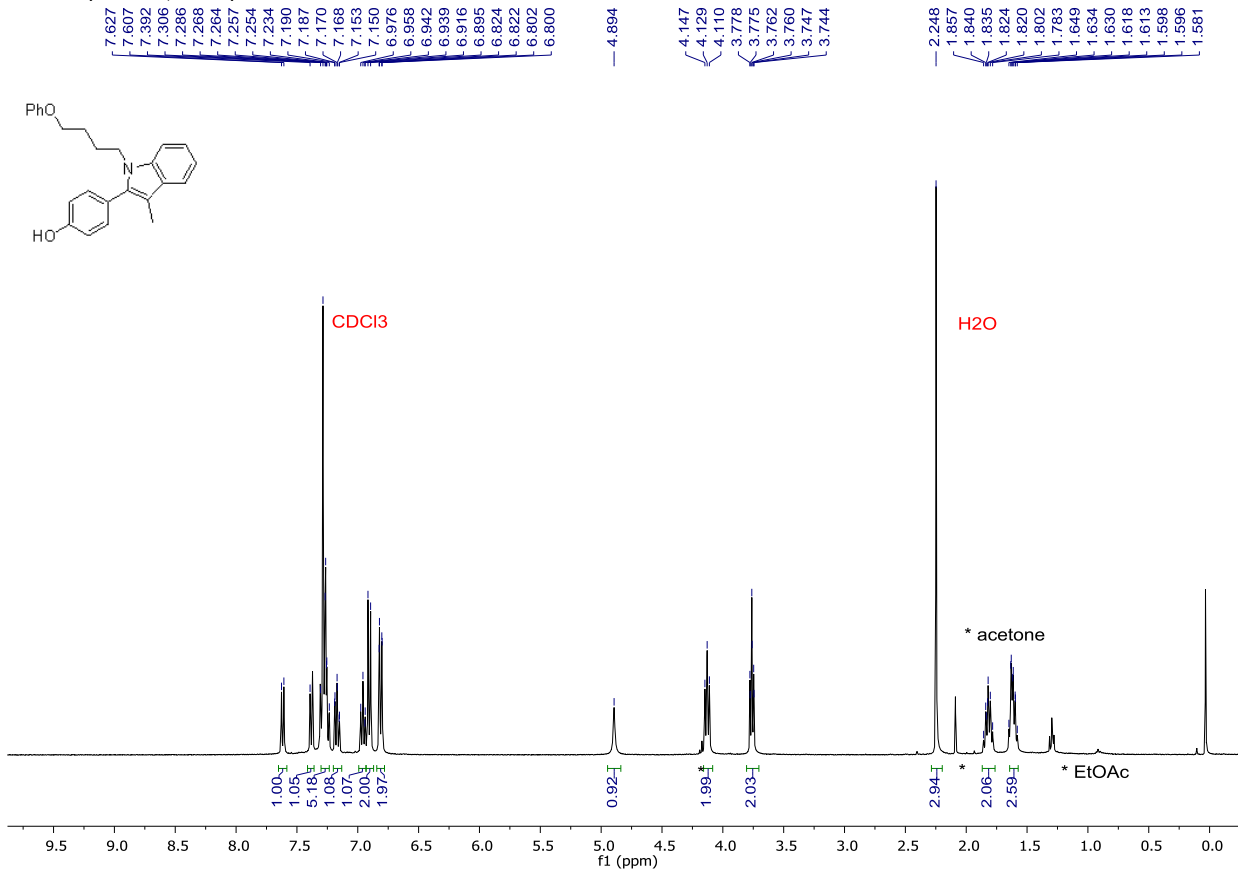


¹³C NMR (100 MHz, acetone-d₆)

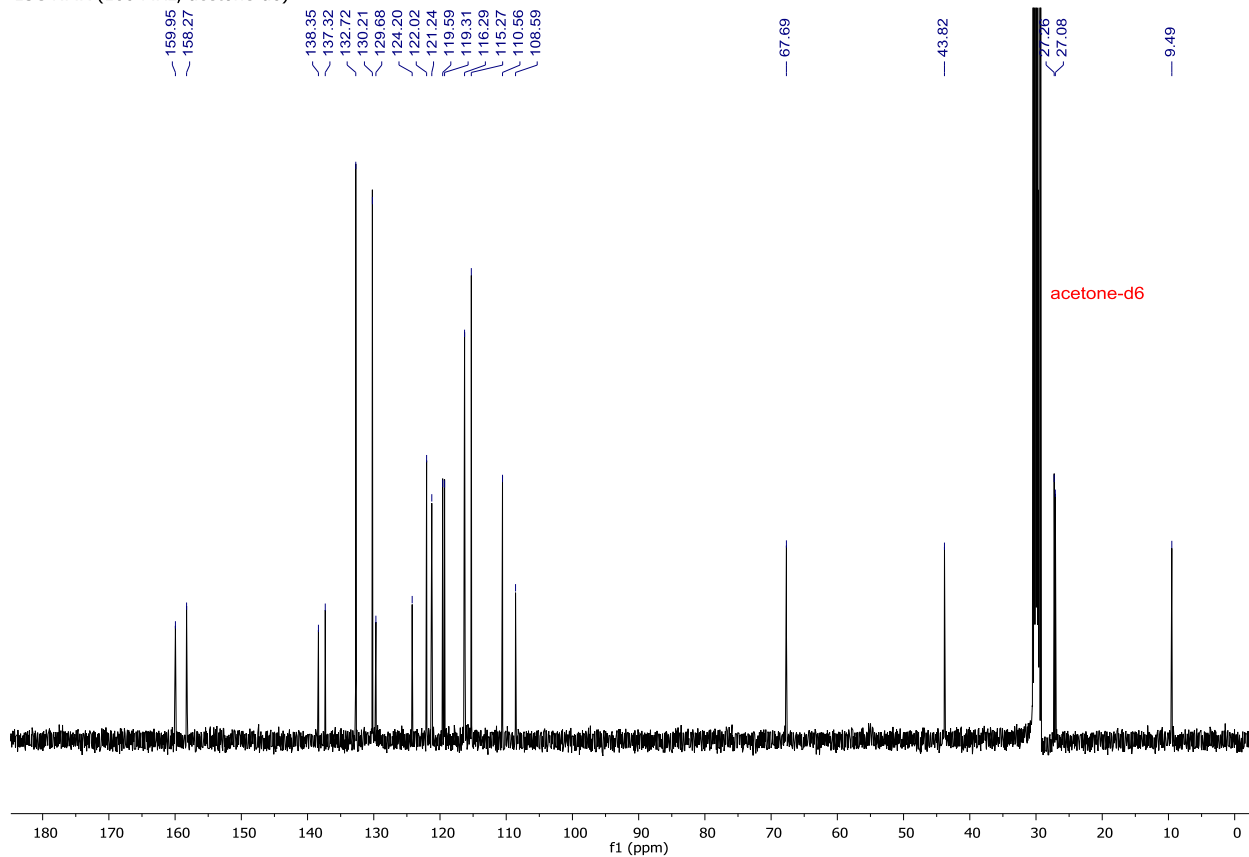


4-(3-Methyl-1-(4-phenoxybutyl)-1H-indol-2-yl)phenol (29).

¹H NMR (400 MHz, CDCl₃)

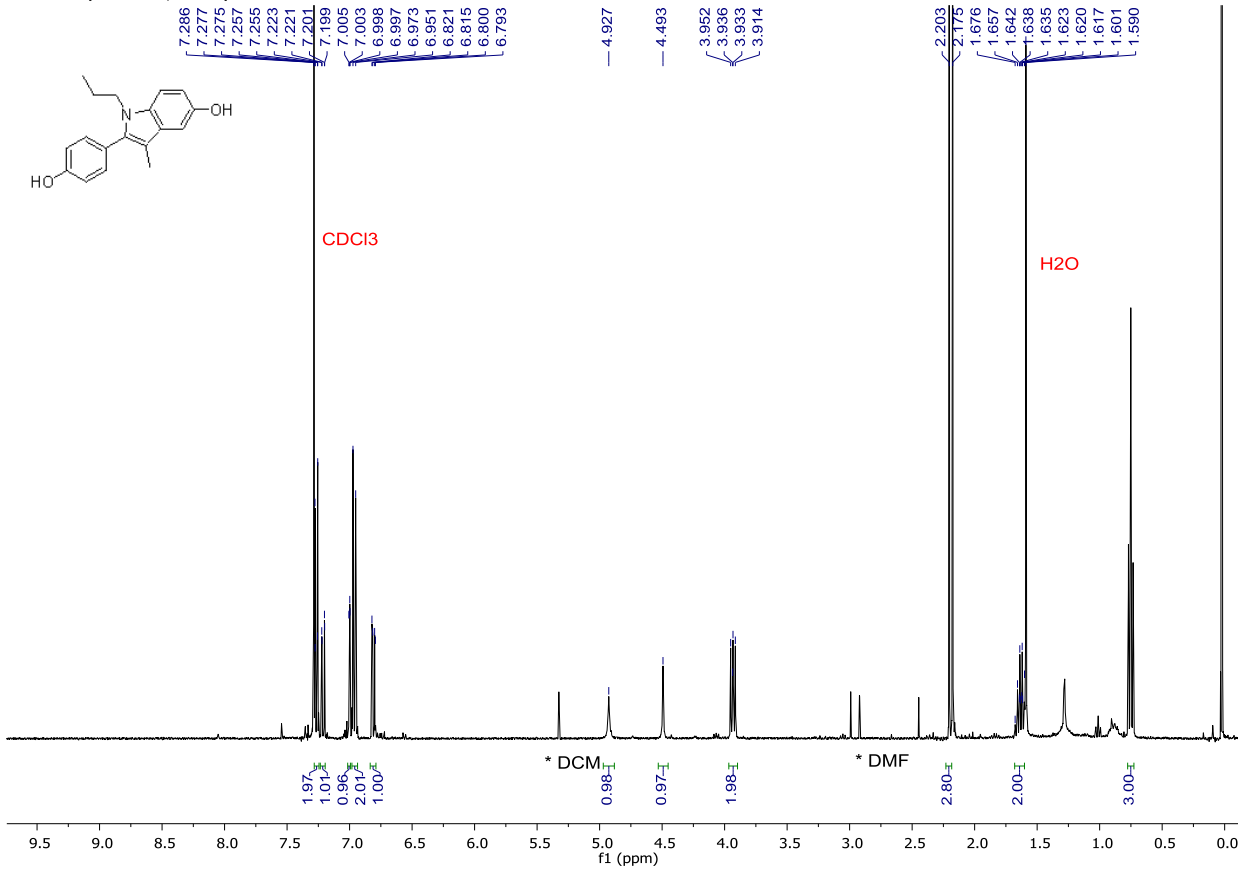


¹³C NMR (100 MHz, acetone-d₆)

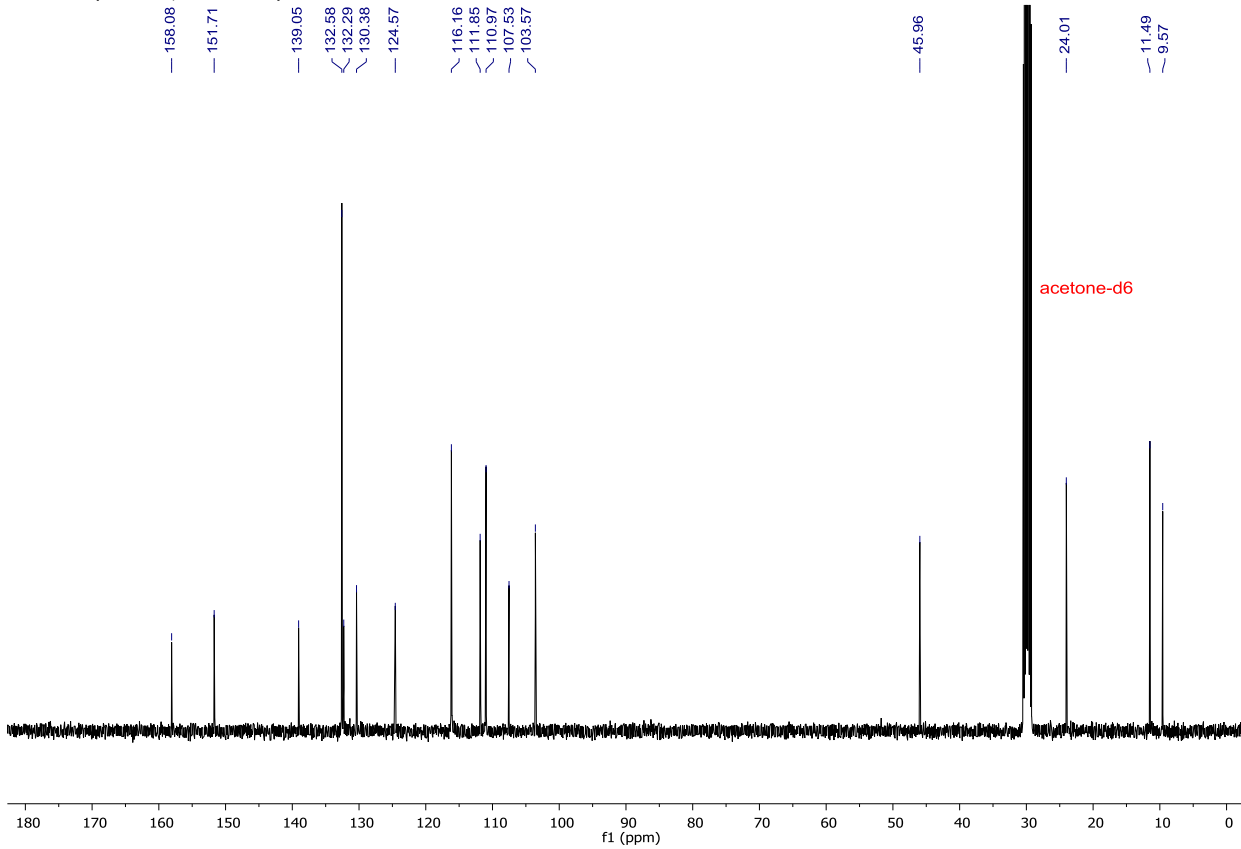


2-(4-Hydroxyphenyl)-3-methyl-1-propyl-1H-indol-5-ol (30).

¹H NMR (400 MHz, CDCl₃)

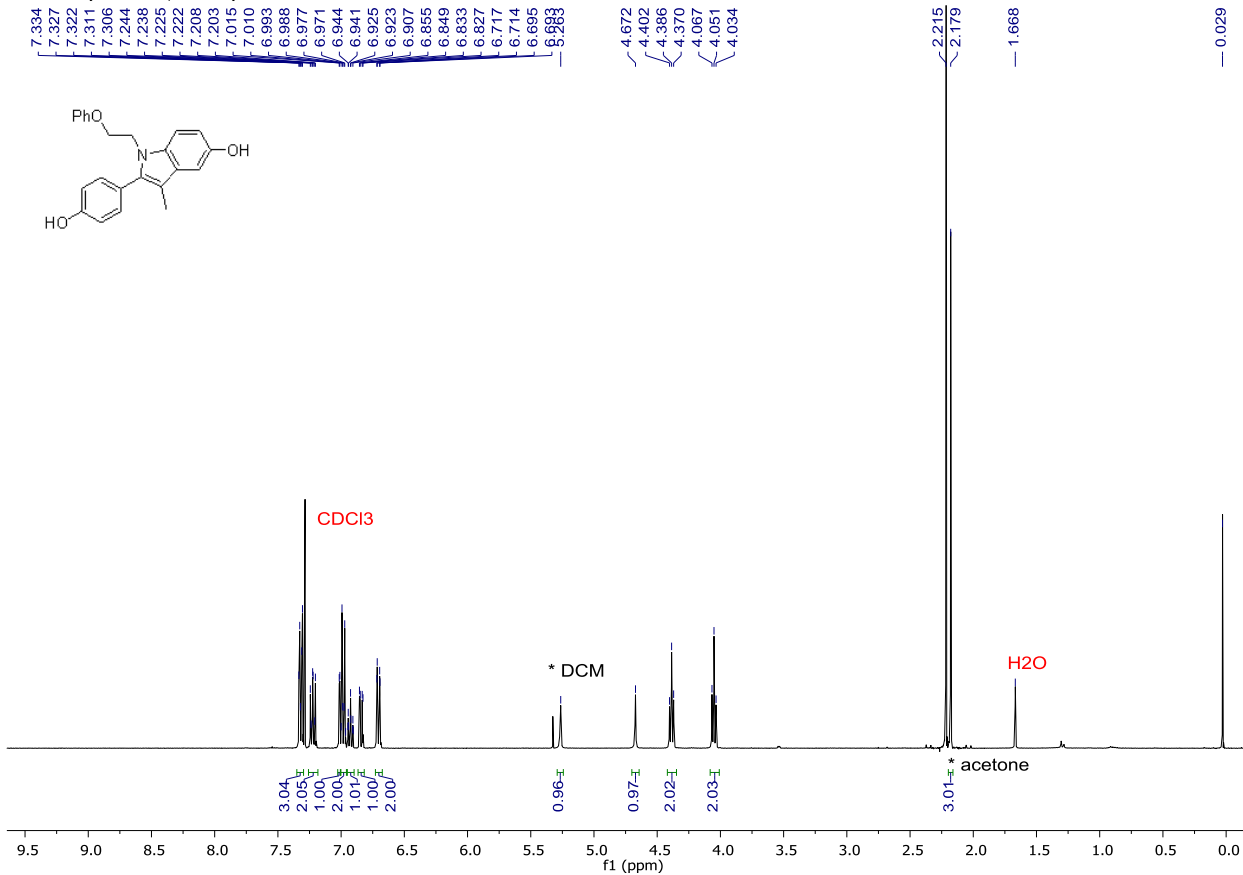


¹³C NMR (100 MHz, acetone-d₆)

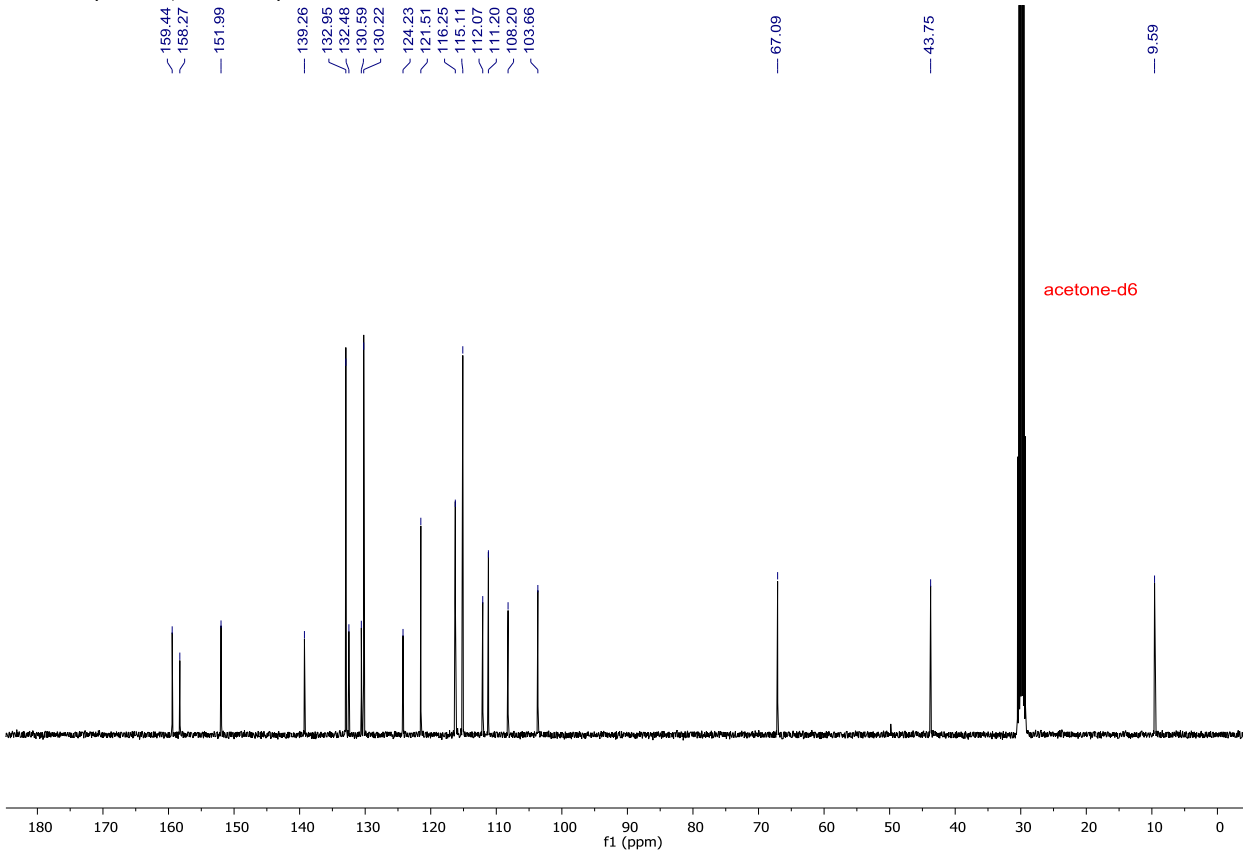


2-(4-Hydroxyphenyl)-3-methyl-1-(2-phenoxyethyl)-1H-indol-5-ol (31).

1H NMR (400 MHz, CDCl₃)

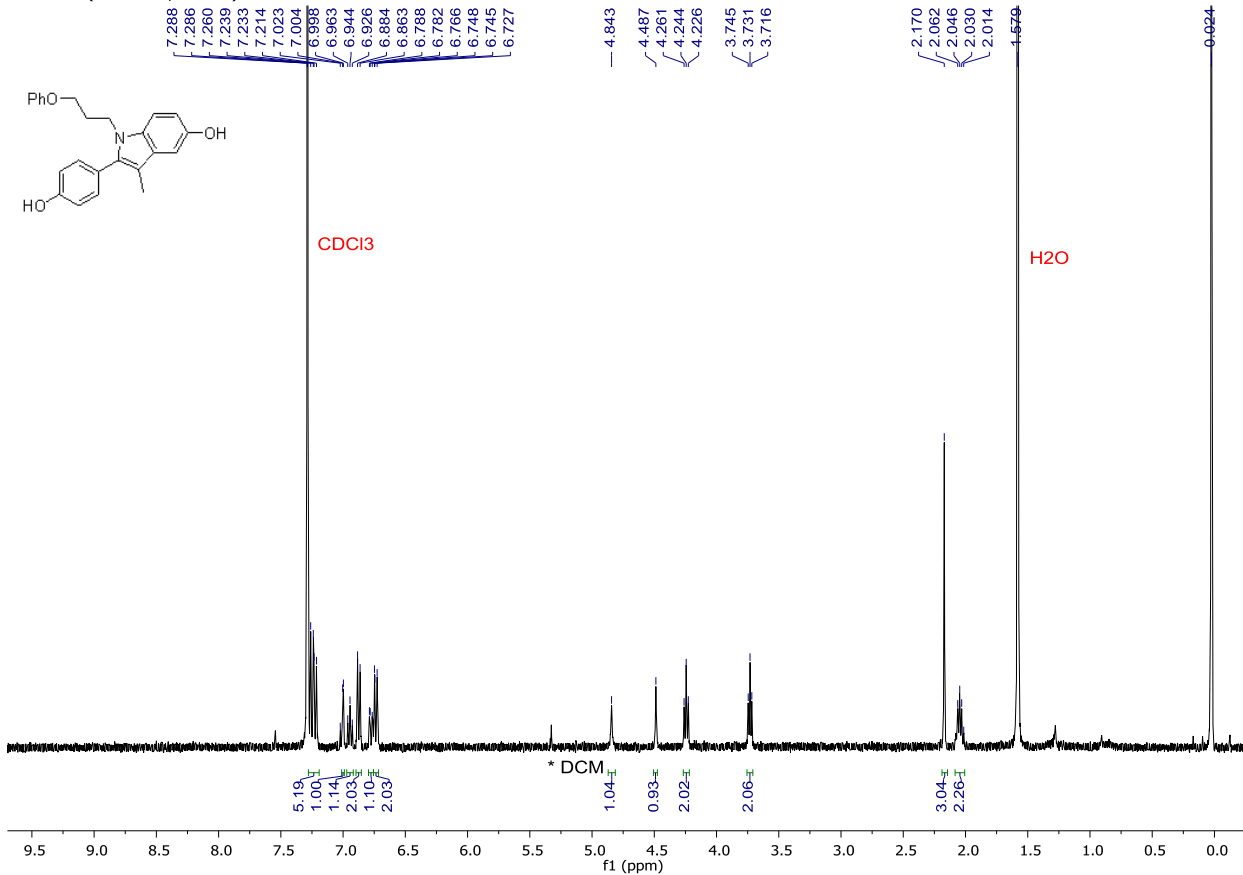


13C NMR (100 MHz, acetone-d₆)

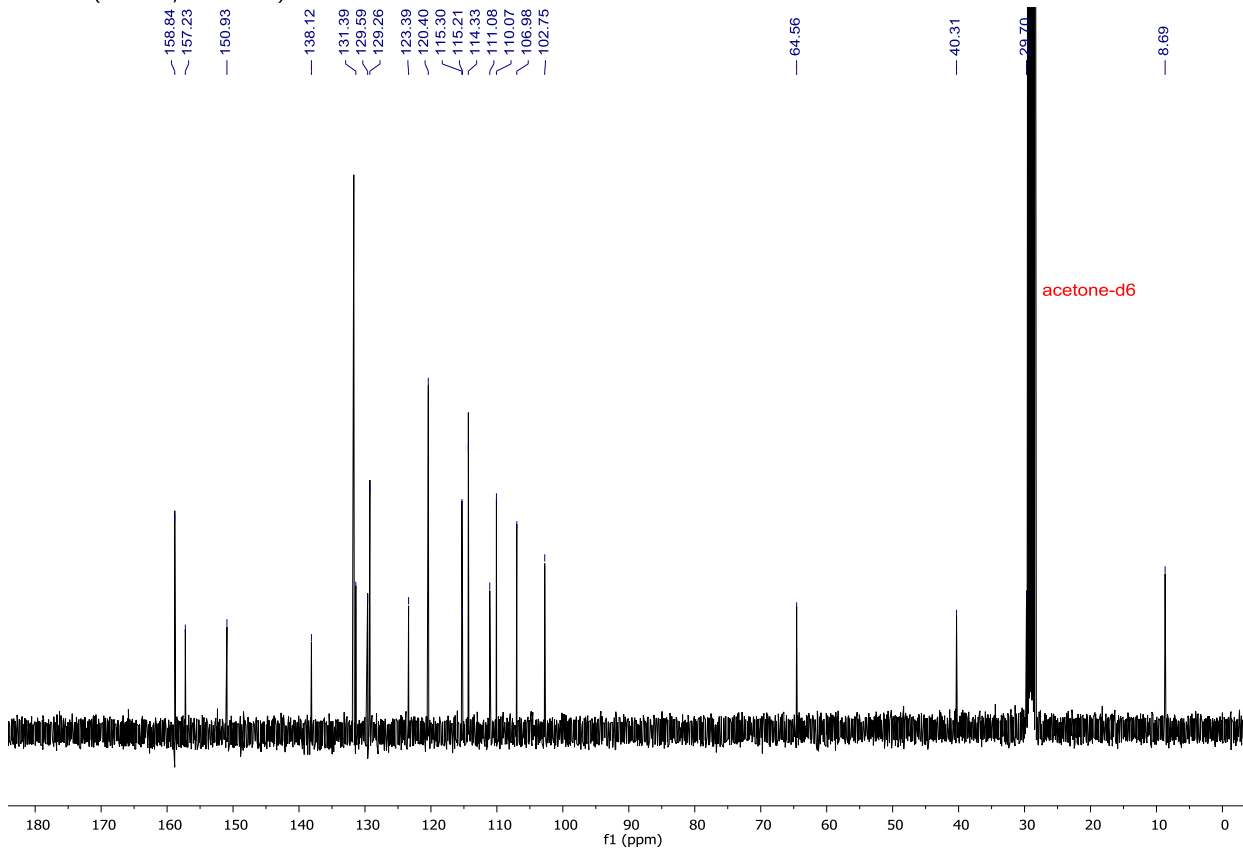


2-(4-Hydroxyphenyl)-3-methyl-1-(3-phenoxypropyl)-1H-indol-5-ol (32).

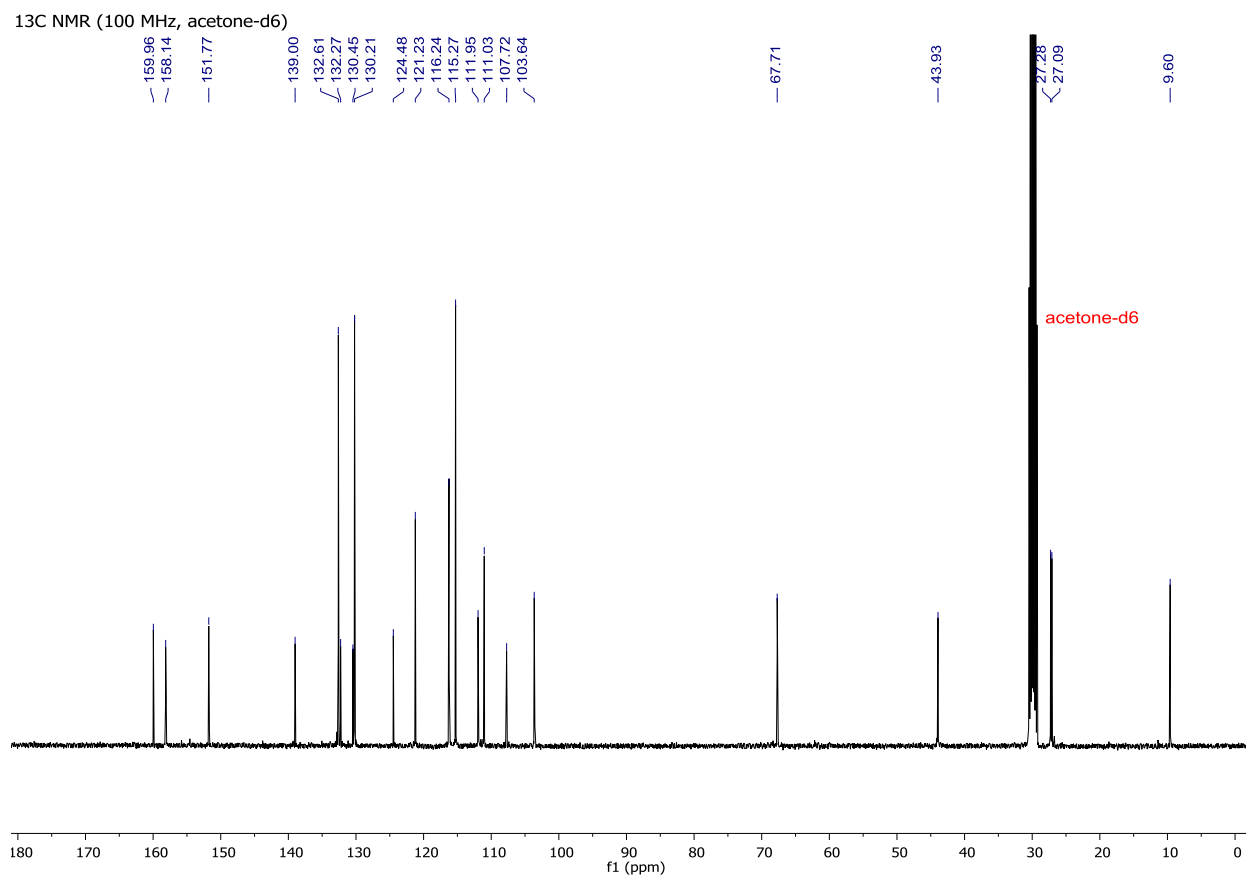
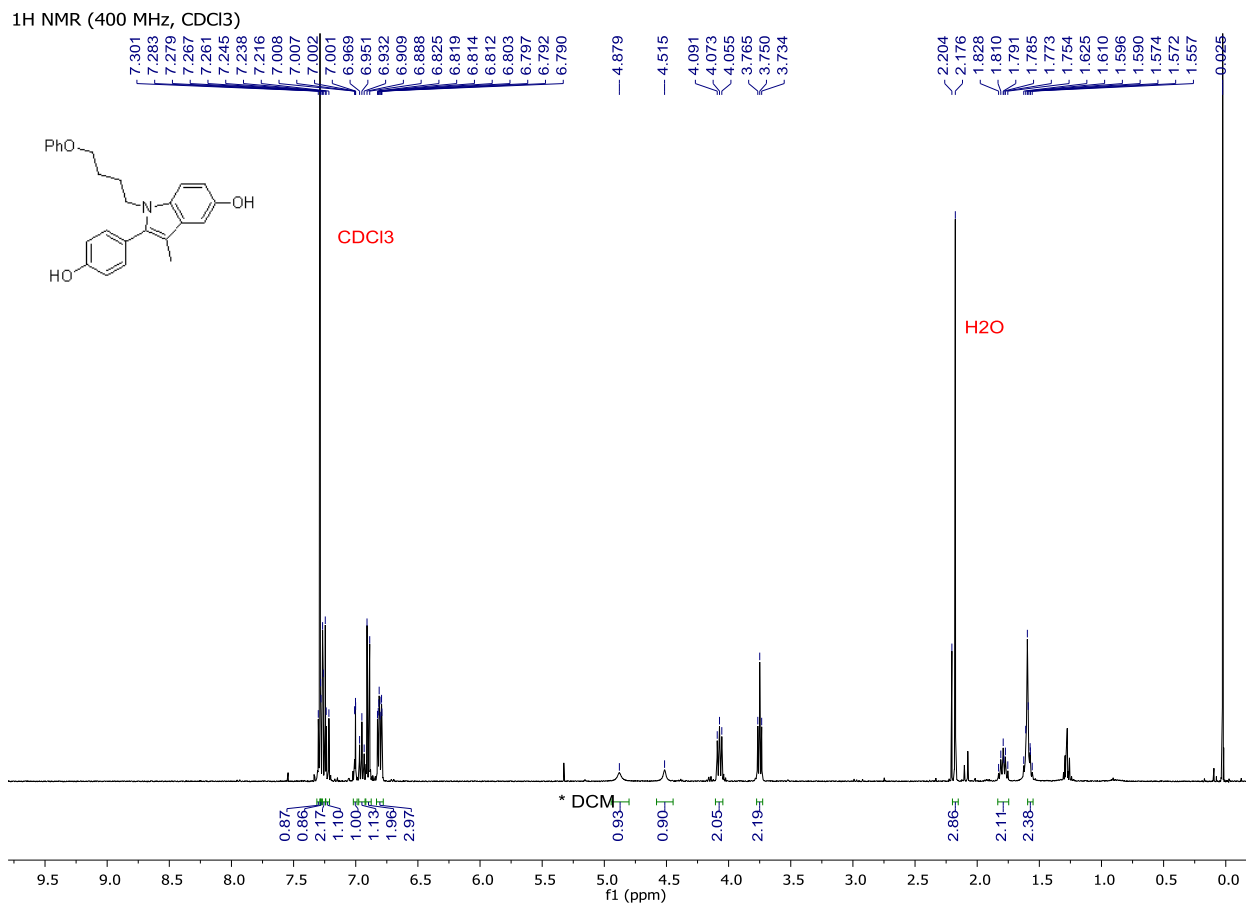
1H NMR (400 MHz, CDCl₃)



13C NMR (100 MHz, acetone-d₆)

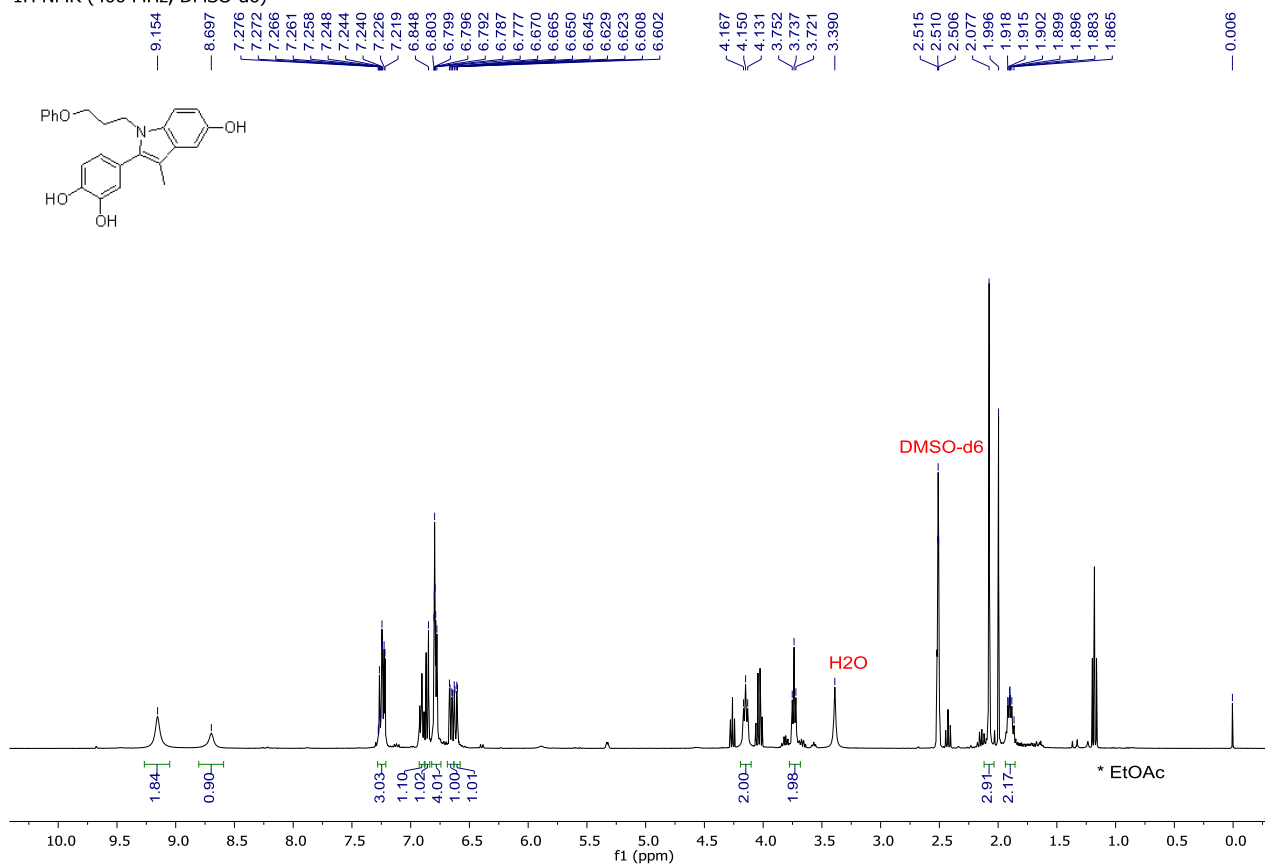
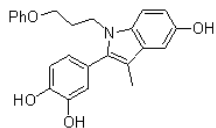


2-(4-Hydroxyphenyl)-3-methyl-1-(4-phenoxybutyl)-1H-indol-5-ol (33).

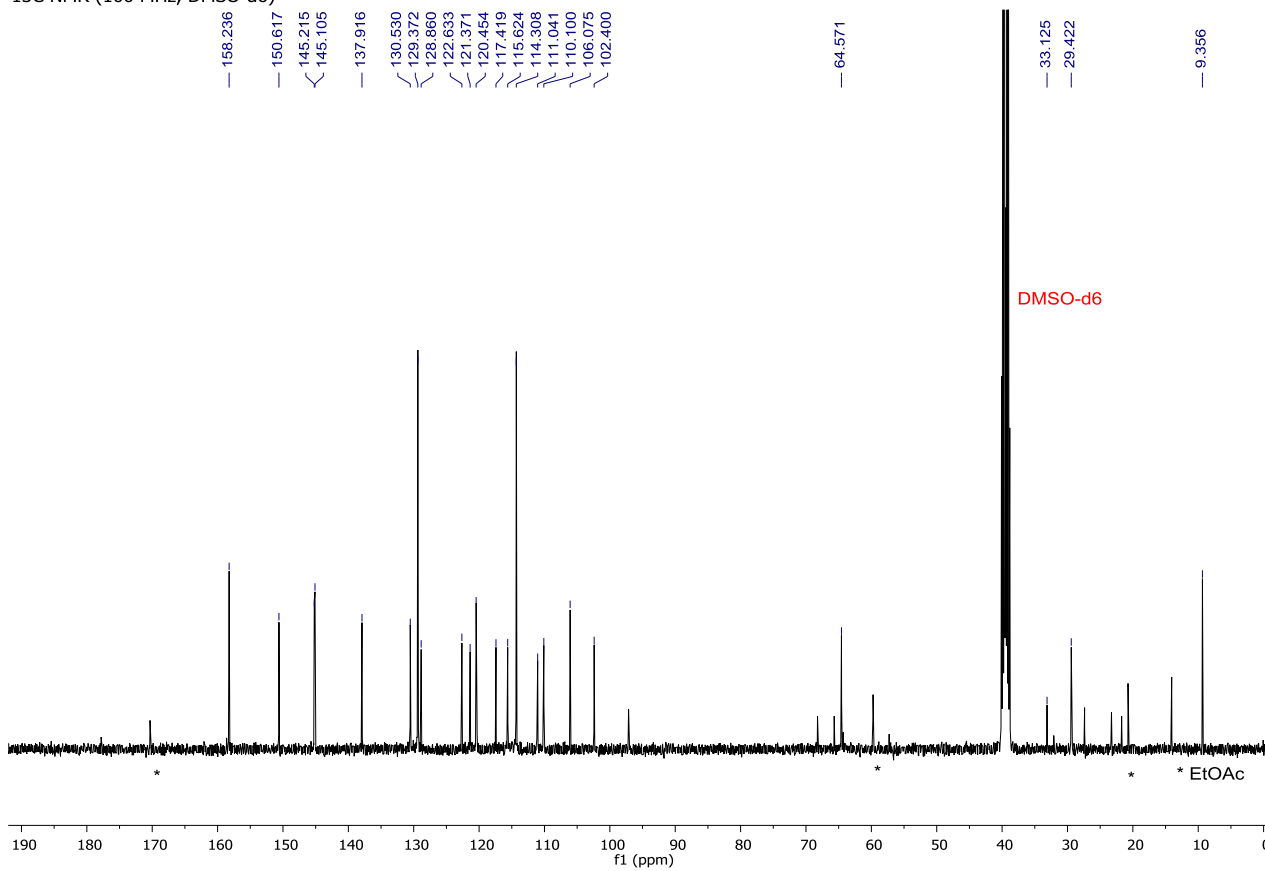


4-(5-Hydroxy-3-methyl-1-(3-phenoxypropyl)-1H-indol-2-yl)benzene-1,2-diol (36).

¹H NMR (400 MHz, DMSO-d₆)

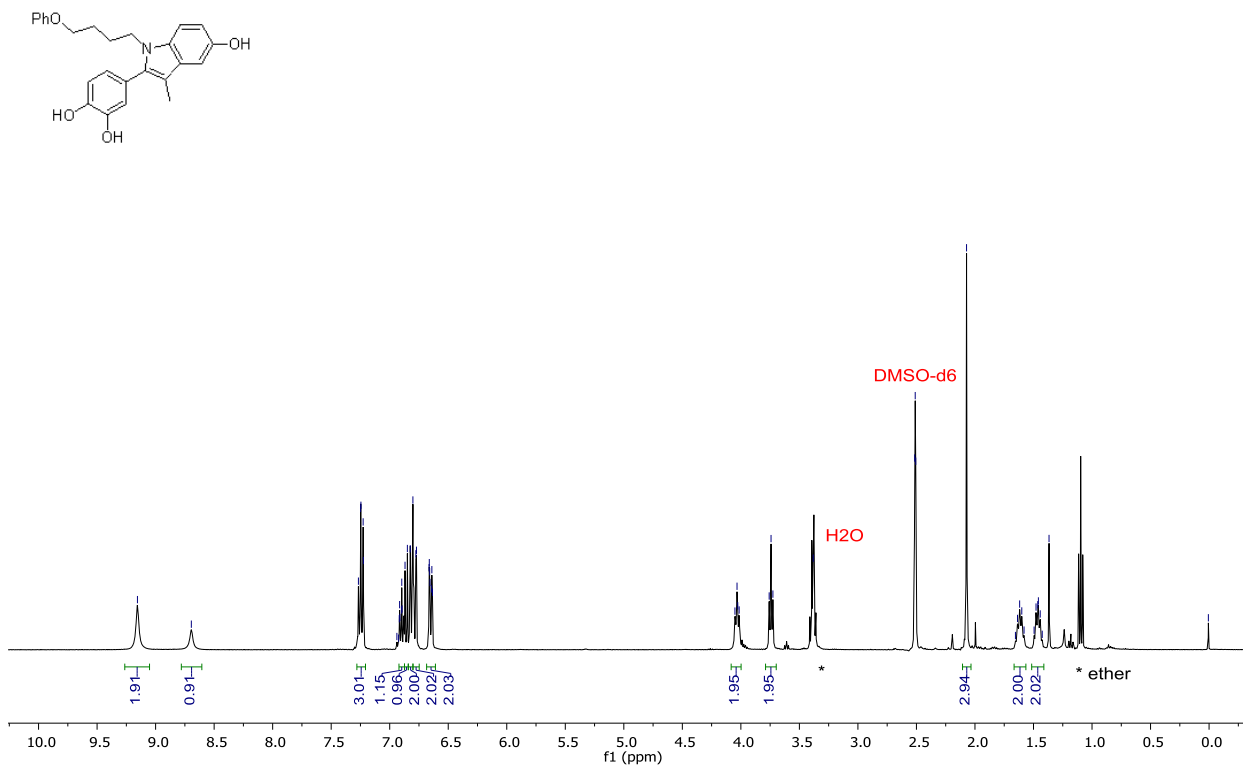


¹³C NMR (100 MHz, DMSO-d₆)

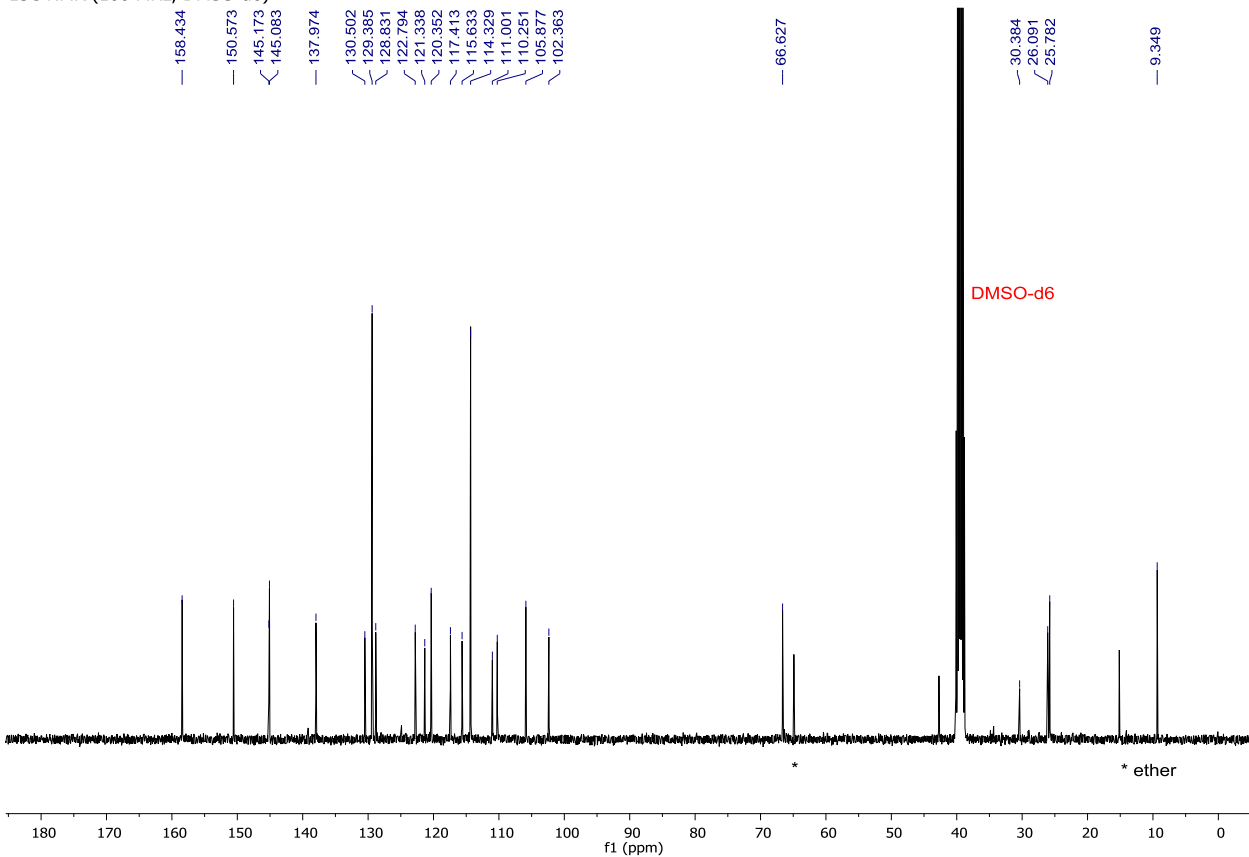


4-(5-Hydroxy-3-methyl-1-(4-phenoxybutyl)-1H-indol-2-yl)benzene-1,2-diol (37).

1H NMR (400 MHz, DMSO-d6)

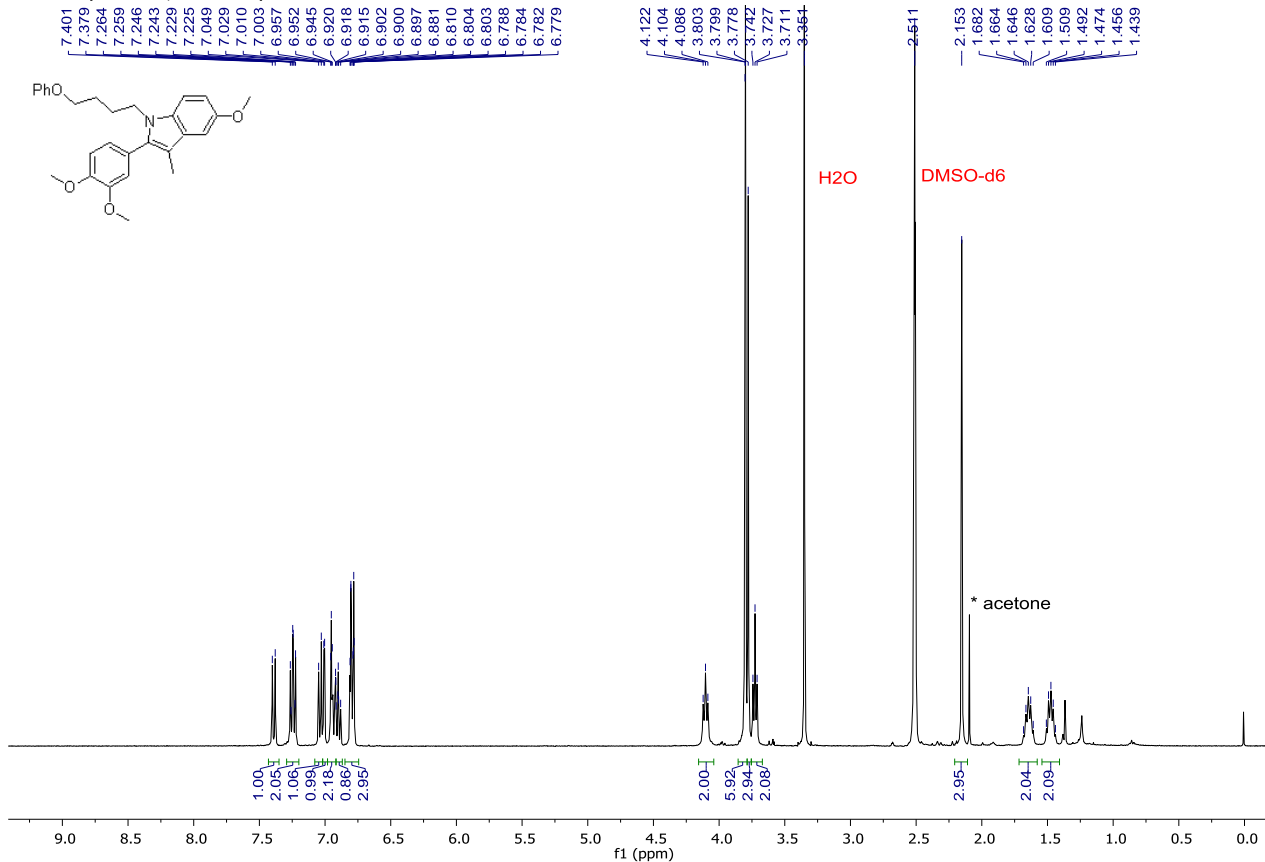


13C NMR (100 MHz, DMSO-d6)

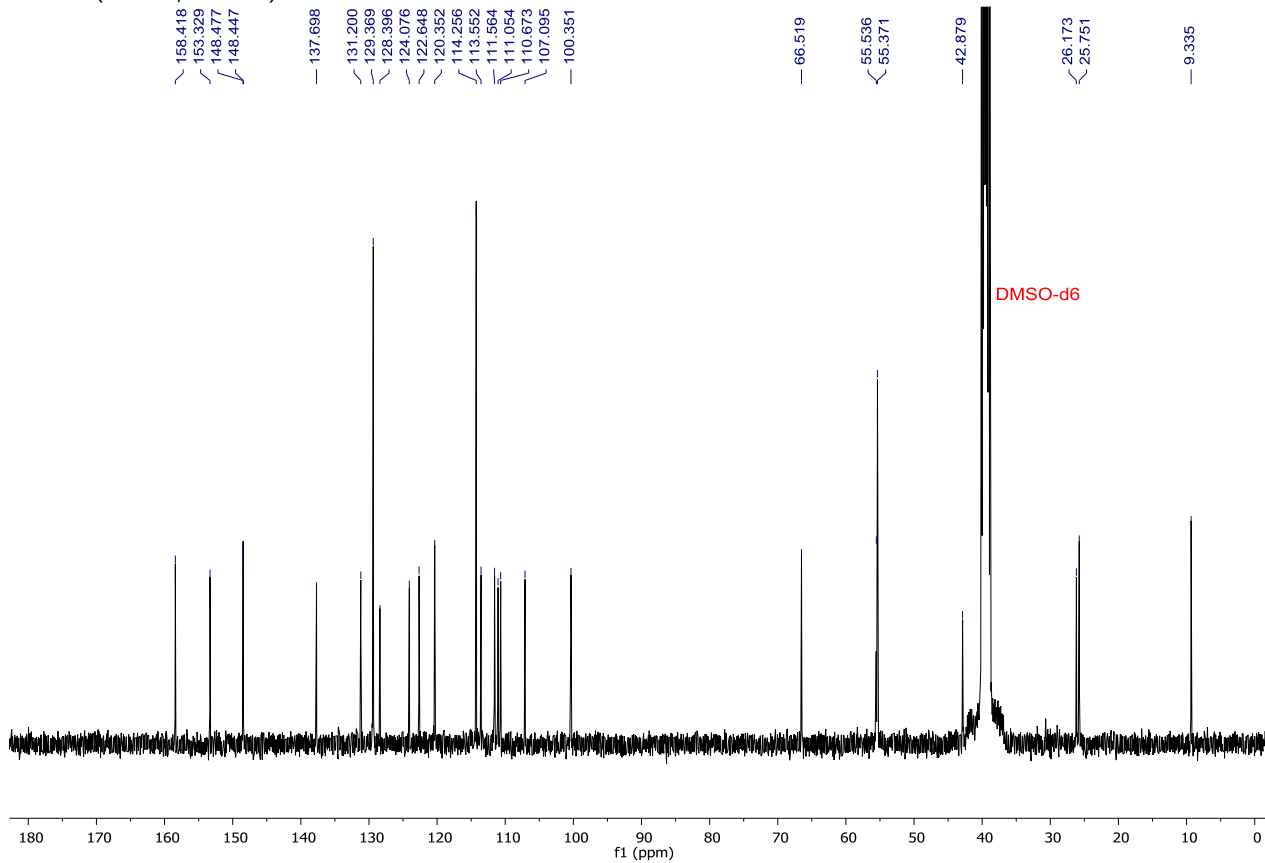


2-(3,4-Dimethoxyphenyl)-5-methoxy-3-methyl-1-(4-phenoxybutyl)-1H-indole (38).

¹H NMR (400 MHz, DMSO-d₆)



¹³C NMR (100 MHz, DMSO-d₆)



Materials and methods - Biological studies

Cell cultures. HeLa (human cervix adenocarcinoma cells) and A2780 (human ovarian carcinoma cells) were cultured in Nutrient Mixture F-12 [HAM] and RPMI 1640 (Sigma Chemical Co.), respectively. MSTO-211H (human biphasic mesothelioma cells) were grown in RPMI 1640 (Sigma Chemical Co.) additioned with 2.38 g/L Hepes, 0.11 g/L pyruvate sodium and 2.5 g/L glucose. All media were supplemented with 1.5 g/L NaHCO₃, 10% heat-inactivated fetal bovine serum (Biowest), 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 0.25 µg/mL amphotericin B (Sigma Chemical Co.). The cells were cultured as monolayers at 37 °C in a humidified atmosphere containing 5% CO₂ in air.

Inhibition growth assay. Cells were plated into 24-well cell culture plates (approximately 3 × 10⁴ cells/well) and incubated for 24 h. Test compounds were added to the cells at different concentrations and the cell viability was determined after 72 h of incubation by using the trypan blue exclusion test. The antiproliferative data were expressed as GI₅₀ values, i.e. the concentration (µM) of derivative that induces 50% reduction in cell number with respect to control cultures.

Topoisomerase II relaxation assay. The assay was carried out in a volume of 20 µL at 37 °C for 60 min. The incubation mixture contained supercoiled pBR322 plasmid DNA (0.25 µg, Fermentas Life Sciences), 1U topoisomerase II (human topoisomerase II alpha, Inspiralis) and the test compounds at the indicated concentrations in reaction buffer. Reactions were terminated by addition of 4 µL stop buffer (5% sodium dodecyl sulfate, 0.125% bromophenol blue, and 30% glycerol) and 50 µg/mL proteinase K (Sigma) for 30 min at 37 °C. The samples were electrophoresed in 1% agarose gel at room temperature for 90 min in TAE buffer (0.04 M Tris-acetate and 0.001 M EDTA). The gels were stained with 1 µg/mL ethidium bromide in TAE and visualized by a Bio-Rad Gel Doc XR apparatus.

Determination of mitochondrial membrane potential. The mitochondrial transmembrane potential was assayed by BD™ MitoScreen Kit (BD Pharmigen), according to Cossarizza et al.² Briefly, A2780 cells (3 × 10⁵) were seeded and incubated for 24 h. The test agents were added at the indicated concentrations and drug-treated cells were incubated for a further 28 h. The cells were then centrifuged, resuspended in JC-1 Working Solution and incubated for 30 min at 37 °C. Following incubation, cells were washed twice in Assay buffer, resuspended and immediately analyzed on a FACSCanto II flow cytometer (Becton-Dickinson, Mountain View, CA).

Analysis of DNA content. A2780 cells were seeded at a density of 3×10^5 in 60 mm cell culture plate and incubated for 24 h before addition of the drug. The test agents were then added to the complete medium at the indicated concentrations and the cells were incubated for a further 28 h. After treatment, 3×10^5 cells were fixed in 70% iced-cold ethanol at -20°C for 20 min and then washed with phosphate buffered saline (PBS). The obtained cell pellet was gently resuspended in PBS in the presence of 0.1 mg/mL RNase and 38 $\mu\text{g/mL}$ propidium iodide (PI). The mixed cells were incubated in the dark at room temperature for 20 min and kept in the dark at 4°C until measured. The analysis was performed using a FACSCanto II flow cytometer (Becton–Dickinson, Mountain View, CA).

Figure 1 SI.

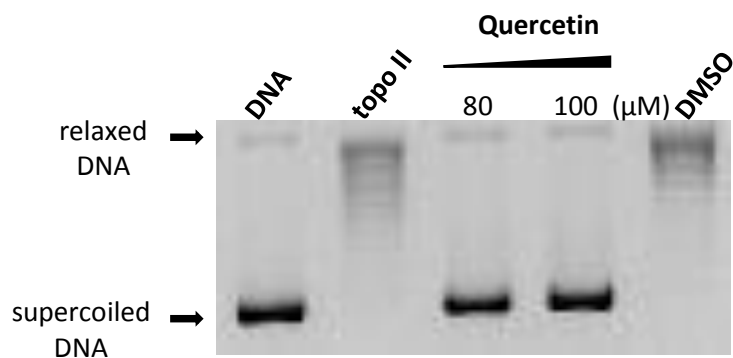


Figure 1 SI. Effect of quercetin on the relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated with topo II in the absence (topo II) and in the presence of quercetin at indicated concentrations.

References

- Hodnik, Z.; Masic, L. P.; Tomasic, T.; Smodis, D.; D'Amore, C.; Fiorucci, S.; Kikelj, D., Bazedoxifene-Scaffold-Based Mirnetics of Solomonsterols A and B as Novel Pregnane X Receptor Antagonists. *J Med Chem* **2014**, *57* (11), 4819-4833.
- Cossarizza, A.; Baccaranicontri, M.; Kalashnikova, G.; Franceschi, C. A new method for the cytofluorometric analysis of mitochondrial-membrane potential using the J-aggregate forming lipophilic cation 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1). *Biochem. Biophys. Res. Commun.* **1993**, *197* (1), 40-45.