Supporting Information

Novel zafirlukast derivatives exhibit selective antibacterial activity against *Porphyromonas gingivalis*

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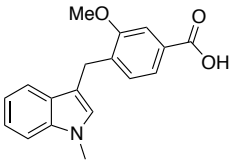
CHEMISTRY

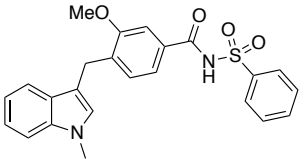
Materials and instrumentations for chemistry. All chemicals were purchased from Sigma Aldrich (St. Louis, MO), Alfa Aesar (Ward Hill, MA), and AK scientific (Union City, CA), and used without further purification. Chemical reactions were monitored by thin layer

chromatography (TLC) using Merck, Silica gel 60 F_{250} plates. Visualization was achieved using UV light and a ceric molybdate stain (5 g $(NH_4)_2Ce(NO_3)_6$, 120 g $(NH_4)_6Mo_7O_{24}•4H_2O$, 80 mL H_2SO_4, 720 mL H_2O). ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Varian 400 MHz spectrometer, using the indicated deuterated solvents. Chemical shifts (δ) are given in parts per million (ppm). Coupling constants (*J*) are given in Hertz (Hz), and conventional abbreviations used for signal shape are as follows: s = singlet; d = doublet; t = triplet; m = multiplet; dd = doublet of doublets; ddd = doublet of doublet of doublets; br s = broad singlet; dt = doublet of triplets. Liquid chromatography-mass spectrometry (LCMS) was carried out using an Agilent 1200 series Quaternary LC system equipped with a diode array detector, and Eclipse XDB-C18 column (250 mm x 4.6 mm, 5 μm), and an Agilent 6120 Quadrupole MSD mass spectrometer (Agilent Technologies, Santa Clara, CA). LCMS $[M+H]^+$ signals were consistent with the expected molecular weights for all of the reported compounds. Purity of the compound was further confirmed to be ≥95% by RP-HPLC by using one of the following methods: Method A: Flow rate = 0.5 mL/min; λ = 254 nm; column = Vydac 201SP^{TM} C18, 250 x 4.6 mm, 90A 5 μm; eluents: A = H_2O + 0.1% TFA, B = MeCN; gradient profile: starting from 5% B, increasing from 5% to 100% B over 10 min, holding at 100% for 15 min, decreasing from 100% to 5% over 12 min. Prior to each injection, the HPLC column was equilibrated for 13 min with 5% B; Method B: Flow rate = 0.5 mL/min; λ = 254 nm; column = Vydac 201SP^{TM} C18, 250 x 4.6 mm, 90A 5 μm; eluents: A = H_2O + 0.1% TFA, B = MeCN; gradient profile: starting from 5% B, increasing from 5% to 100% B over 17 min, holding at 100% for 5 min, decreasing from 100% to 5% over 3 min. Prior to each injection, the HPLC column was equilibrated for 5 min with 5% B.

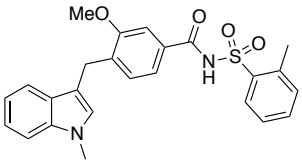


Synthesis of compound 3. A mixture of 1-methylindole (1.0 mL, 7.7 mmol), methyl 4-(bromomethyl)-3-methoxybenzoate (1.0 g, 3.9 mmol), and Ag_2O (1.3 g, 5.8 mmol) in dioxane was stirred at 60 °C overnight. The reaction mixture was filtered through a bed of Celite®, and eluted with EtOAc. The filtrate was concentrated and purified by column chromatography (SiO_2 gel, pure Hexanes to Hexanes:EtOAc/9:1, R_f 0.26 in Hexanes:EtOAc/9:1) to afford the *C*-3 substituted indole. The latter was then dissolved in MeOH:THF:H_2O/5:1:1 (14 mL total), treated with KOH pellets (573 mg, 1.9 mmol) and stirred at room temperature overnight. After completion of the reaction, the organic solvents were removed *in vacuo*. The resulting mixture was diluted with H_2O, acidified to pH 1

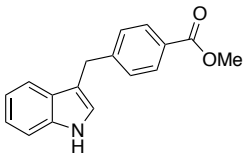
with 1 N aqueous HCl, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.21 in CH_2Cl_2:MeOH/19:1) to afford compound **3** (322 mg, 29%) as an off-white solid: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, Fig. S1) δ 10.60 (very br s, 1H, $CO_2\underline{H}$), 7.57 (d, J = 1.6 Hz, 1H, aromatic), 7.52 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, aromatic), 7.49 (d, J = 8.0 Hz, 1H, aromatic), 7.32 (d, J = 8.0 Hz, 1H, aromatic), 7.20 (d, J = 8.0 Hz, 1H, aromatic), 7.13 (ddd, J_1 = 8.0 Hz, J_2 = 7.2 Hz, J_3 = 1.2 Hz, 1H, aromatic), 7.00 (s, 1H, aromatic), 6.98 (ddd, J_1 = 8.0 Hz, J_2 = 7.2 Hz, J_3 = 1.2 Hz, 1H, aromatic), 4.09 (s, 2H, $C\underline{H}_2Ar$), 3.94 (s, 3H, $OC\underline{H}_3$), 3.75 (s, 3H, $NC\underline{H}_3$); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, Fig. S2) δ 166.7, 157.2, 137.3, 135.5, 129.6, 129.5, 128.0, 127.6, 121.9, 121.2, 118.8, 118.5, 112.2, 110.9, 109.2, 55.0, 31.7, 24.8; *m/z* calcd for $C_{18}H_{17}NO_3$ 295.1; found 296.1 $[M+H]^+$.



Synthesis of compound 4a. A mixture of compound **3** (100 mg, 0.34 mmol), benzenesulfonamide (59 mg, 0.37 mmol), EDC•HCl (84 mg, 0.44 mmol), and DMAP (62 mg, 0.51 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at room temperature overnight. After completion of the reaction, the solvents were removed and the crude product obtained was purified by column chromatography (SiO_2 gel, pure Hexanes to Hexanes:EtOAc/1:1, R_f 0.50 in CH_2Cl_2:MeOH/19:1) to afford compound **4a** (124 mg, 84%) as an off-white solid: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, Fig. S3) δ 10.85 (s, 1H, O=CN$\underline{H}$), 8.08 (d, J = 8.4 Hz, 2H, aromatic), 7.70 (t, J = 7.6 Hz, 1H, aromatic), 7.61 (t, J = 7.6 Hz, 2H, aromatic), 7.48 (s, 1H, aromatic), 7.44 (d, J = 8.8 Hz, 1H, aromatic), 7.41 (d, J = 9.2 Hz, 1H, aromatic), 7.32 (d, J = 7.6 Hz, 1H, aromatic), 7.17 (d, J = 7.6 Hz, 1H, aromatic), 7.12 (t, J = 8.4 Hz, 1H, aromatic), 6.99 (s, 1H, aromatic), 6.96 (t, J = 7.6 Hz, 1H, aromatic), 4.06 (s, 2H, $C\underline{H}_2Ar$), 3.92 (s, 3H, $OC\underline{H}_3$), 3.75 (s, 3H, $NC\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S4) δ 164.2, 157.5, 138.4, 137.0, 136.6, 134.0, 129.9, 129.7, 129.2, 129.0, 128.5, 127.8, 127.4, 126.4, 121.6, 119.5, 119.1, 118.8, 112.0, 109.4, 109.2, 55.6, 32.6, 25.2; *m/z* calcd for $C_{24}H_{22}N_2O_4S$ 434.1; found 435.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method B: R_t = 24.93 min (95%; Fig. S5).

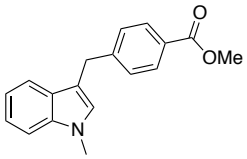


Synthesis of compound 4b. A mixture of compound **3** (100 mg, 0.34 mmol), *o*-toluenesulfonamide (64 mg, 0.37 mmol), EDC•HCl (84 mg, 0.44 mmol), and DMAP (62 mg, 0.51 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at room temperature overnight. After completion of the reaction, the solvents were removed and the crude product obtained was purified by column chromatography (SiO_2 gel, pure Hexanes to Hexanes:EtOAc/1:1, R_f 0.47 in CH_2Cl_2:MeOH/19:1) followed by recrystallization in EtOH to afford compound **4b** (90 mg, 59%) as an off-white solid: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, Fig. S6) δ 10.94 (s, 1H, O=CN<u>H</u>), 8.16 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.56 (td, $J_1 = J_2$ = 7.6 Hz, J_3 = 1.2 Hz, 1H, aromatic), 7.48 (s, 1H, aromatic), 7.49 (d, J = 8.4 Hz, 1H, aromatic), 7.44 (td, $J_1 = J_2$ = 7.6 Hz, J_3 = 1.2 Hz, 1H, aromatic), 7.43 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.38 (d, J = 7.6 Hz, 1H, aromatic), 7.32 (d, J = 8.4 Hz, 1H, aromatic), 7.18 (d, J = 7.6 Hz, 1H, aromatic), 7.12 (t, J = 8.0 Hz, 1H, aromatic), 7.01 (s, 1H, aromatic), 6.97 (t, J = 8.0 Hz, 1H, aromatic), 4.07 (s, 2H, C<u>H</u>$_2$Ar), 3.92 (s, 3H, OC<u>H</u>$_3$), 3.75 (s, 3H, NC<u>H</u>$_3$), 2.64 (s, 3H, ArC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, Fig. S7) δ 164.4, 157.3, 137.9, 137.5, 136.0, 133.5, 132.3, 131.2, 130.6, 129.7, 127.9, 127.6, 127.3, 126.0, 121.2, 120.2, 118.7, 118.5, 112.0, 109.7, 109.2, 55.1, 31.7, 24.9, 19.4; *m/z* calcd for $C_{25}H_{24}N_2O_4S$ 448.2; found 449.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method B: R_t = 25.35 min (96%; Fig. S8).

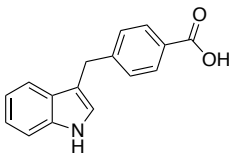


Synthesis of compound 7. A solution of indole (0.50 g, 4.3 mmol) and methyl 4-formylbenzoate (0.70 g, 4.3 mmol) in anhydrous CH_2Cl_2 was cooled down to 0 °C in an ice-H_2O bath. Et_3SiH (2.0 mL, 12.8 mmol) was then added, followed by TFA (0.65 mL, 8.5 mmol). The resulting mixture was stirred at 0 °C for 10 min before allowing it to warm up to room temperature overnight. The reaction was quenched with H_2O, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, Hexanes:CH_2Cl_2/1:1, R_f 0.23 in Hexanes:CH_2Cl_2/1:2) to afford compound **7** (0.26 g, 23%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S9) δ 8.05 (br s, 1H, N<u>H</u>), 7.95 (d, J = 8.4 Hz, 2H, aromatic), 7.46 (d, J = 7.6 Hz, 1H, aromatic), 7.35 (d, J = 8.0 Hz, 1H, aromatic), 7.34 (d, J = 8.4 Hz, 2H, aromatic), 7.19 (t, J = 8.0 Hz, 1H, aromatic), 7.08 (t, J = 7.6 Hz, 1H, aromatic), 6.92 (s, 1H, aromatic), 4.16 (s, 2H,

C<u>H</u>$_2$Ar), 3.89 (s, 3H, ArCO$_2$C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S10) δ 167.3, 146.8, 136.4, 129.7 (2 carbons), 128.7 (2 carbons), 127.9, 127.2, 122.5, 122.2, 119.5, 119.0, 114.7, 111.2, 52.0, 31.7; *m/z* calcd for $C_{17}H_{15}NO_2$ 265.1; found 266.1 $[M+H]^+$.

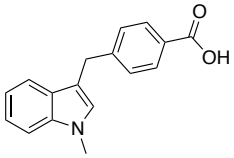


Synthesis of compound 8. A solution of 1-methylindole (0.50 g, 3.8 mmol) and methyl 4-formylbenzoate (0.63 g, 3.8 mmol) in anhydrous CH_2Cl_2 was cooled down to 0 °C in an ice-H_2O bath. Et_3SiH (1.8 mL, 11.4 mmol) was then added, followed by TFA (0.58 mL, 7.6 mmol). The resulting mixture was stirred at 0 °C for 10 min before allowing it to warm up to room temperature overnight. The reaction was quenched with H_2O, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, Hexanes:CH_2Cl_2/1:1, R_f 0.35 in Hexanes:CH_2Cl_2/1:1) to afford compound **8** (0.60 g, 56%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S11) δ 7.95 (d, *J* = 8.0 Hz, 2H, aromatic), 7.46 (d, *J* = 7.6 Hz, 1H, aromatic), 7.35 (d, *J* = 8.0 Hz, 2H, aromatic), 7.30 (d, *J* = 8.4 Hz, 1H, aromatic), 7.22 (t, *J* = 8.0 Hz, 1H, aromatic), 7.07 (t, *J* = 8.0 Hz, 1H, aromatic), 6.77 (s, 1H, aromatic), 4.15 (s, 2H, C<u>H</u>$_2$Ar), 3.89 (s, 3H, ArCO$_2$C<u>H</u>$_3$), 3.73 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S12) δ 167.2, 147.0, 137.2, 129.7 (2 carbons), 128.7 (2 carbons), 127.9, 127.6, 127.2, 121.7, 119.1, 118.9, 113.2, 109.2, 52.0, 32.6, 31.6; *m/z* calcd for $C_{18}H_{17}NO_2$ 279.1; found 280.1 $[M+H]^+$.

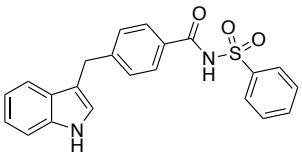


Synthesis of compound 9. A solution of compound **7** (186 mg, 0.63 mmol) in MeOH:THF:H_2O/5:1:1 (14 mL total) was treated with KOH pellets (247 mg, 4.4 mmol), and the mixture was stirred at room temperature overnight. After completion of the reaction, the organic solvents were removed *in vacuo*. The resulting mixture was diluted with H_2O, and acidified to pH 1 with 1 N aqueous HCl. The solid formed was filtered off, washed with H_2O, and air-dried to afford compound **9** (132 mg, 84%) as an off-white solid: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, Fig. S13) δ 10.10 (br s, 1H, CO$_2$<u>H</u>), 7.93 (d, *J* = 8.4 Hz, 2H, aromatic), 7.44 (d, *J* = 7.6 Hz, 1H, aromatic), 7.42 (d, *J* = 8.0 Hz, 2H, aromatic), 7.37 (d, *J* = 8.4 Hz, 2H, aromatic), 7.17 (d, *J* = 2.0 Hz, 1H, aromatic), 7.07 (t, *J* = 8.4 Hz, 1H, aromatic), 6.95 (t, *J* = 7.6 Hz, 1H, aromatic), 4.17 (s, 2H, C<u>H</u>$_2$Ar), 3.19 (very br s, 1H, N<u>H</u>); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, Fig. S14) δ 166.8, 147.5, 137.0, 129.6 (2 carbons), 128.6 (2 carbons), 128.1, 127.4,

123.1, 121.3, 118.65, 118.59, 113.7, 111.3, 31.3; *m/z* calcd for $C_{16}H_{13}NO_2$ 251.1; found 250.2 [M-H]$^-$.

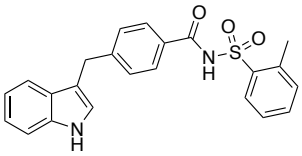


Synthesis of compound 10. A solution of compound **8** (200 mg, 0.71 mmol) in MeOH:THF:H_2O/5:1:1 (14 mL total) was treated with KOH pellets (254 mg, 6.4 mmol), and the mixture was stirred at room temperature overnight. After completion of the reaction, the organic solvents were removed *in vacuo*. The resulting mixture was diluted with H_2O, and acidified to pH 1 with 1 N aqueous HCl. The solid formed was filtered off, redissolved in CH_2Cl_2 and purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.47 in CH_2Cl_2:MeOH/19:1) to afford compound **10** (158 mg, 83%) as an off-white solid: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, Fig. S15) δ 7.92 (d, *J* = 8.0 Hz, 2H, aromatic), 7.44 (d, *J* = 8.0 Hz, 1H, aromatic), 7.41 (d, *J* = 8.0 Hz, 2H, aromatic), 7.33 (d, *J* = 8.4 Hz, 1H, aromatic), 7.13 (t, *J* = 8.0 Hz, 1H, aromatic), 7.04 (s, 1H, aromatic), 6.97 (t, *J* = 8.0 Hz, 1H, aromatic), 4.15 (s, 2H, C$\underline{H}_2$Ar), 3.77 (s, 3H, NC$\underline{H}_3$); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, Fig. S16) δ 166.8, 147.4, 137.4, 129.6 (2 carbons), 128.6 (2 carbons), 128.2, 127.7, 127.5, 121.3, 118.8, 118.5, 112.9, 109.3, 31.8, 31.1; *m/z* calcd for $C_{18}H_{17}NO_3$ 265.1; found 264.2 [M-H]$^-$.

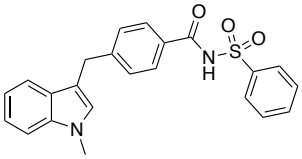


Synthesis of compound 11a. A mixture of compound **9** (40 mg, 0.16 mmol), benzenesulfonamide (28 mg, 0.18 mmol), EDC•HCl (40 mg, 0.21 mmol), and DMAP (29 mg, 0.24 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.43 in CH_2Cl_2:MeOH/19:1) to afford compound **11a** (36 mg, 58%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S17) δ 8.70 (s, 1H, O=CN$\underline{H}$), 8.13 (d, *J* = 8.0 Hz, 2H, aromatic), 8.01 (s, 1H, N$\underline{H}$), 7.64 (d, *J* = 8.0 Hz, 2H, aromatic), 7.63 (t, *J* = 7.6 Hz, 1H, aromatic), 7.53 (t, *J* = 7.6 Hz, 2H, aromatic), 7.36 (t, *J* = 8.0 Hz, 2H, aromatic), 7.32 (d, *J* = 8.0 Hz, 2H, aromatic), 7.17 (t, *J* = 7.6 Hz, 1H, aromatic), 7.04 (t, *J* = 7.6 Hz, 1H, aromatic), 6.92 (s, 1H, aromatic), 4.13 (s, 2H, C$\underline{H}_2$Ar); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S18) δ 164.2, 147.9, 138.4, 136.4, 134.0, 129.1 (2 carbons), 129.0 (2 carbons), 128.6, 128.5 (2 carbons), 127.9 (2 carbons), 127.1, 122.6, 122.2, 119.5, 118.8, 114.1, 111.3, 31.6; *m/z* calcd for $C_{22}H_{18}N_2O_3S$ 390.1; found 391.1 [M+H]$^+$. Purity of the

compound was further confirmed by RP-HPLC by using method A: R_t = 19.67 min (97%; Fig. S19).

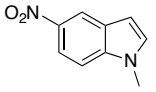


Synthesis of compound 11b. A mixture of compound **9** (40 mg, 0.16 mmol), *o*-toluenesulfonamide (30 mg, 0.18 mmol), EDC•HCl (40 mg, 0.21 mmol), and DMAP (29 mg, 0.24 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.46 in CH_2Cl_2:MeOH/19:1) to afford compound **11b** (67 mg, quant.) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S20) δ 9.36 (s, 1H, O=CN<u>H</u>), 8.24 (d, *J* = 8.0 Hz, 1H, aromatic), 8.08 (s, 1H, N<u>H</u>), 7.67 (d, *J* = 8.0 Hz, 2H, aromatic), 7.48 (t, *J* = 7.6 Hz, 1H, aromatic), 7.38 (t, *J* = 7.6 Hz, 2H, aromatic), 7.34 (d, *J* = 7.6 Hz, 1H, aromatic), 7.30-7.24 (m, 3H, aromatic), 7.16 (t, *J* = 7.6 Hz, 1H, aromatic), 7.04 (t, *J* = 7.6 Hz, 1H, aromatic), 6.89 (s, 1H, aromatic), 4.10 (s, 2H, C<u>H</u>$_2$Ar), 2.66 (s, 3H, ArC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S21) δ 164.2, 147.8, 137.6, 136.5, 136.4, 134.0, 132.5, 131.5, 129.2 (2 carbons), 128.5, 128.0 (2 carbons), 127.1, 126.4, 122.6, 122.2, 119.5, 118.8, 114.1, 111.3, 31.6, 20.4; *m/z* calcd for $C_{23}H_{20}N_2O_3S$ 404.1; found 403.1 $[M-H]^-$. Purity of the compound was further confirmed by RP-HPLC by using method B: R_t = 23.58 min (97%; Fig. S22).

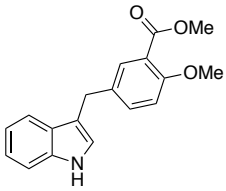


Synthesis of compound 12a. A mixture of compound **10** (40 mg, 0.15 mmol), benzenesulfonamide (23 mg, 0.15 mmol), EDC•HCl (34 mg, 0.18 mmol), and DMAP (25 mg, 0.20 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.51 in CH_2Cl_2:MeOH/19:1) to afford compound **12a** (59 mg, 97%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S23) δ 9.12 (s, 1H, O=CN<u>H</u>), 8.13 (d, *J* = 8.0 Hz, 2H, aromatic), 7.67 (d, *J* = 8.4 Hz, 2H, aromatic), 7.61 (t, *J* = 7.2 Hz, 1H, aromatic), 7.51 (t, *J* = 8.0 Hz, 2H, aromatic), 7.37 (d, *J* = 8.0 Hz, 1H, aromatic), 7.30 (d, *J* = 8.4 Hz, 2H, aromatic), 7.28 (d, *J* = 8.4 Hz, 1H, aromatic), 7.20 (t, *J* = 8.0 Hz, 1H, aromatic), 7.03 (t, *J* = 7.2 Hz, 1H, aromatic), 6.74 (s, 1H, aromatic), 4.09 (s, 2H, C<u>H</u>$_2$Ar), 3.71 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S24) δ 164.1, 148.0, 138.5, 137.1, 134.0, 129.2 (2 carbons), 129.0 (2 carbons), 128.61, 128.56 (2 carbons), 127.9 (2 carbons),

127.5, 127.3, 121.8, 119.0, 118.9, 112.6, 109.3, 32.6, 31.5; *m/z* calcd for $C_{23}H_{20}N_2O_3S$ 404.1; found 405.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method A: R_t = 25.00 min (99%; Fig. S25).

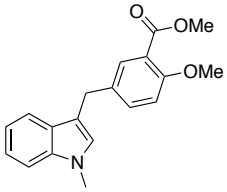


Synthesis of compound 13. A solution of 5-nitro indole (1.0 g, 6.17 mmol) in anhydrous DMF (20 mL) was treated with NaH (60% in mineral oil, 0.27 g, 6.78 mmol), and the mixture was stirred at room temperature for 1 h. Iodomethane (0.6 mL, 9.25 mmol) was then slowly added, and the resulting mixture was stirred at room temperature overnight. The reaction was quenched by pouring onto ice and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was triturated in hexanes and filtered to give the known compound **13**[1] (0.78 g, 72%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$, which matches the lit.,[1] Fig. S26) δ 8.57 (d, J = 2.0 Hz, 1H, aromatic), 8.12 (dd, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 1H, aromatic), 7.32 (d, J = 8.8 Hz, 1H, aromatic), 7.19 (d, J = 3.2 Hz, 1H, aromatic), 6.66 (d, J = 2.4 Hz, 1H, aromatic), 3.85 (s, 3H, NC<u>H</u>$_3$).

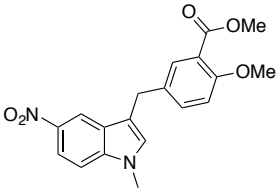


Synthesis of compound 15. A solution of indole (0.30 g, 2.6 mmol) and methyl 5-formyl-2-methoxybenzoate (0.50 g, 2.6 mmol) in anhydrous CH_2Cl_2 was cooled down to 0 °C in an ice-H_2O bath. Et_3SiH (1.2 mL, 7.2 mmol) was then added, followed by TFA (0.4 mL, 5.1 mmol). The resulting mixture was stirred at 0 °C for 10 min before allowing it to warm up to room temperature overnight. The reaction was quenched with H_2O, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, pure Hexanes to Hexanes:EtOAc/3:1, R_f 0.25 in Hexanes:EtOAc/3:1) to afford compound **15** (0.34 g, 43%) as a brown oil: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S27) δ 8.29 (s, 1H), 7.79 (d, J = 2.4 Hz, 1H, aromatic), 7.51 (d, J = 8.4 Hz, 1H, aromatic), 7.36 (dd, J_1 = 8.0 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.32 (d, J = 7.6 Hz, 1H, aromatic), 7.19 (td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.10 (td, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H, aromatic), 6.86 (d, J = 8.4 Hz, 1H, aromatic), 6.84 (s, 1H, aromatic), 4.08 (s, 2H, C<u>H</u>$_2$Ar), 3.89 (s, 3H, ArCO$_2$C<u>H</u>$_3$), 3.84 (s, 3H, ArOC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S28)

δ 167.1, 157.5, 136.6, 133.8, 133.2, 131.7, 127.3, 122.6, 122.0, 119.6, 119.3, 119.0, 115.3, 112.2, 111.3, 56.1, 52.1, 30.6; *m/z* calcd for $C_{18}H_{17}NO_3$ 295.1; found 296.1 $[M+H]^+$.

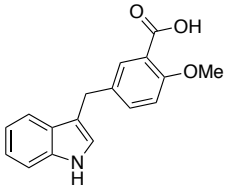


Synthesis of compound 16. A solution of 1-methylindole (0.32 mL, 2.6 mmol) and methyl 5-formyl-2-methoxybenzoate (0.50 g, 2.6 mmol) in anhydrous CH_2Cl_2 was cooled down to 0 °C in an ice-H_2O bath. Et_3SiH (1.2 mL, 7.2 mmol) was then added, followed by TFA (0.4 mL, 5.1 mmol). The resulting mixture was stirred at 0 °C for 10 min before allowing it to warm up to room temperature overnight. The reaction was quenched with H_2O, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, pure Hexanes to Hexanes:EtOAc/3:1, R_f 0.36 in Hexanes:EtOAc/3:1) to afford compound **16** (0.71 g, 89%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S29) δ 7.72 (d, J = 2.4 Hz, 1H, aromatic), 7.47 (dt, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.34 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.27 (d, J = 8.0 Hz, 1H, aromatic), 7.20 (td, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H, aromatic), 7.05 (td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 6.87 (d, J = 8.8 Hz, 1H, aromatic), 6.72 (s, 1H, aromatic), 4.04 (s, 2H, C$\underline{H}_2$Ar), 3.86 (s, 6H, ArCO$_2$C$\underline{H}_3$, ArOC$\underline{H}_3$), 3.72 (s, 3H, NC$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S30) δ 166.9, 157.4, 137.2, 133.6, 133.2, 131.7, 127.6, 127.1, 121.6, 119.8, 119.1, 118.8, 114.1, 112.1, 109.2, 56.1, 52.0, 32.6, 30.5; *m/z* calcd for $C_{19}H_{19}NO_3$ 309.1; found 310.1 $[M+H]^+$.

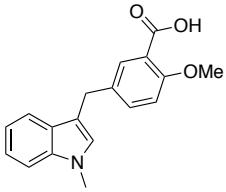


Synthesis of compound 17. A solution of 5-nitroindole (1.00 g, 5.1 mmol) and methyl 5-formyl-2-methoxybenzoate (0.84 g, 5.1 mmol) in anhydrous CH_2Cl_2 was cooled down to 0 °C in an ice-H_2O bath. Et_3SiH (2.3 mL, 14.4 mmol) was then added, followed by TFA (0.8 mL, 10.3 mmol). The resulting mixture was stirred at 0 °C for 10 min before allowing it to warm up to room temperature overnight. The reaction was quenched with H_2O, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, Hexanes:EtOAc/3:1 to Hexanes:EtOAc/1:1, R_f 0.32 in Hexanes:EtOAc/1:1) to afford the *C*-3 alkylated indole (0.50 g, 29%) as a yellow solid. A solution of this *C*-3 alkylated indole (250 mg,

0.73 mmol) in anhydrous DMF (10 mL) was treated with NaH (60% in mineral oil, 59 mg, 1.47 mmol), and the mixture was stirred at room temperature for 1 h. Iodomethane (0.07 mL, 1.10 mmol) was then added, and the resulting mixture was stirred at room temperature overnight. The reaction was quenched by pouring onto ice and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, Hexanes:EtOAc/2:1 to pure EtOAc, R_f 0.41 in Hexanes:EtOAc/1:1) to afford compound **17** (147 mg, 57%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S31) δ 8.46 (d, J = 2.0 Hz, 1H, aromatic), 8.10 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.68 (d, J = 2.4 Hz, 1H, aromatic), 7.35 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, aromatic), 7.27 (d, J = 8.8 Hz, 1H, aromatic), 6.91 (d, J = 8.4 Hz, 1H, aromatic), 6.84 (s, 1H, aromatic), 4.05 (s, 2H, C<u>H</u>$_2$Ar), 3.87 (s, 3H, ArOC<u>H</u>$_3$), 3.85 (s, 3H, ArCO$_2$C<u>H</u>$_3$), 3.77 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S32) δ 166.8, 157.7, 141.2, 139.9, 133.5, 131.8, 131.7, 130.1, 126.9, 120.1, 117.5, 117.4, 116.5, 112.3, 109.1, 56.1, 52.0, 33.1, 30.1; *m/z* calcd for $C_{19}H_{18}N_2O_5$ 354.1; found 355.1 $[M+H]^+$.

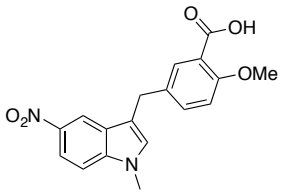


Synthesis of compound 18. A solution of compound **15** (115 mg, 0.29 mmol) in MeOH/THF/H_2O (5 mL/1 mL/1 mL) was treated with KOH pellets (114 mg, 2.04 mmol), and the mixture was refluxed at 65 °C for 2 h. After completion of the reaction, the reaction mixture was concentrated to dryness to afford compound **18** (68 mg, 93%) as an off-white solid: ^{1}H NMR (400 MHz, CD_3OD, Fig. S33) δ 8.52 (s, 1H), 7.37 (d, J = 7.6 Hz, 1H, aromatic), 7.30 (d, J = 2.4 Hz, 1H, aromatic), 7.27 (d, J = 7.6 Hz, 1H, aromatic), 7.13 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.01 (t, J = 7.6 Hz, 1H, aromatic), 6.94 (s, 1H, aromatic), 6.88 (t, J = 7.6 Hz, 1H, aromatic), 6.83 (d, J = 8.8 Hz, 1H, aromatic), 3.98 (s, 2H, C<u>H</u>$_2$Ar), 3.75 (s, 3H, ArOC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $(CD_3)_2SO$, Fig. S34) δ 167.8, 156.7, 136.8, 133.8, 133.3, 130.8, 127.3, 123.6, 121.4, 121.3, 118.9, 118.7, 114.2, 112.8, 111.8, 56.2, 30.3; *m/z* calcd for $C_{17}H_{15}NO_3$ 281.1; found 282.1 $[M+H]^+$.

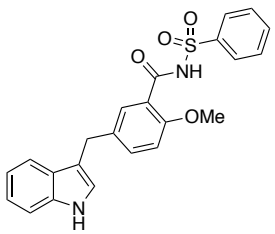


Synthesis of compound 19. A solution of compound **16** (250 mg, 0.81 mmol) in MeOH/THF/H_2O (5 mL/1 mL/1 mL) was treated with KOH pellets (317 mg, 5.66 mmol), and the mixture was refluxed at 65 °C for 2 h. After completion of the reaction, the organic solvents were removed *in vacuo*. The

resulting mixture was acidified to pH 1 with 1 N aqueous HCl and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to afford compound **19** (223 mg, 93%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S35) δ 8.13 (d, J = 2.4 Hz, 1H, aromatic), 7.45-7.41 (m, 2H, aromatic), 7.27 (d, J = 8.0 Hz, 1H, aromatic), 7.19 (td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.04 (td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 6.93 (d, J = 8.4 Hz, 1H, aromatic), 6.79 (s, 1H, aromatic), 4.07 (s, 2H, C$\underline{H}_2$Ar), 4.02 (s, 3H, ArOC$\underline{H}_3$), 3.73 (s, 3H, NC$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S36) δ 165.9, 156.5, 137.2, 135.6, 135.2, 133.5, 127.5, 127.2, 121.7, 119.0, 118.9, 117.2, 113.5, 111.8, 109.3, 56.7, 32.6, 30.5; *m/z* calcd for $C_{18}H_{16}N_2O_5$ 295.1; found 296.1 $[M+H]^+$.

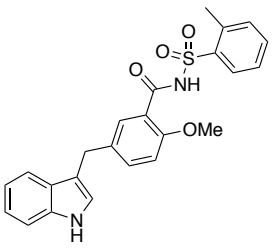


Synthesis of compound 20. A solution of compound **17** (112 mg, 0.32 mmol) in MeOH/THF/H_2O (3 mL/1 mL/0.6 mL) was treated with KOH pellets (124 mg, 2.21 mmol), and the mixture was refluxed at 65 °C for 2 h. After completion of the reaction, the organic solvents were removed *in vacuo*. The resulting mixture was acidified to pH 1 with 1 N aqueous HCl. The precipitate was filtered and eluted with H_2O to afford compound **20** (94 mg, 87%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S37) δ 10.7 (very br s, 1H, $CO_2\underline{H}$), 8.38 (d, J = 2.0 Hz, 1H, aromatic), 8.10 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1H, aromatic), 8.04 (d, J = 2.4 Hz, 1H, aromatic), 7.49 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, aromatic), 7.28 (d, J = 9.2 Hz, 1H, aromatic), 7.00 (d, J = 8.0 Hz, 1H, aromatic), 6.94 (s, 1H, aromatic), 4.09 (s, 2H, C$\underline{H}_2$Ar), 4.05 (s, 3H, ArOC$\underline{H}_3$), 3.79 (s, 3H, NC$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S38) δ 165.2, 156.5, 141.2, 140.0, 135.0, 134.4, 133.6, 130.3, 126.8, 117.6, 117.5, 116.5, 116.4, 111.9, 109.2, 56.8, 33.2, 30.1; *m/z* calcd for $C_{18}H_{16}N_2O_5$ 340.1; found 323.1 $[M-OH]^+$.

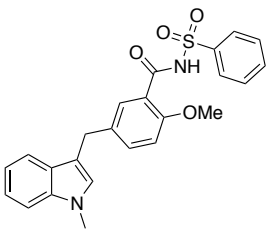


Synthesis of compound 21a. A mixture of compound **18** (30 mg, 0.11 mmol), benzenesulfonamide (20 mg, 0.13 mmol), EDC•HCl (33 mg, 0.17 mmol), and DMAP (23 mg, 0.19 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, pure CH_2Cl_2, R_f

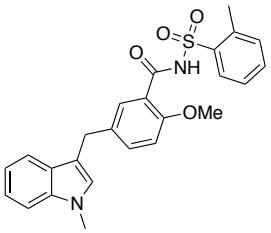
0.26 in pure CH_2Cl_2) to afford compound **21a** (9 mg, 20%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S39) δ 10.51 (br s, 1H, N<u>H</u>SO_2), 10.02 (br s, 1H, N<u>H</u>), 8.08 (d, J = 7.6 Hz, 2H, aromatic), 7.77 (s, 1H, aromatic), 7.69 (t, J = 7.2 Hz, 1H, aromatic), 7.60 (t, J = 7.2 Hz, 2H, aromatic), 7.50 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.38 (d, J = 8.0 Hz, 1H, aromatic), 7.34 (d, J = 8.4 Hz, 1H, aromatic), 7.12 (d, J = 2.0 Hz, 1H, aromatic), 7.10 (s, 1H, aromatic), 7.04 (t, J = 7.6 Hz, 1H, aromatic), 6.91 (t, J = 7.6 Hz, 1H, aromatic), 4.05 (s, 2H, C<u>H</u>$_2$Ar), 4.02 (s, 3H, ArOC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, Fig. S40) δ 162.5, 156.3, 139.7, 137.0, 135.1, 134.9, 133.6, 131.2, 128.8, 128.3, 127.3, 123.0, 122.8, 121.3, 119.0, 118.6, 118.5, 114.2, 112.2, 111.3, 111.2, 56.1, 30.0; *m/z* calcd for $C_{23}H_{20}N_2O_4S$ 420.1; found 421.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method A: R_t = 19.92 min (98%; Fig. S41).



Synthesis of compound 21b. A mixture of compound **18** (50 mg, 0.18 mmol), *o*-toluenesulfonamide (33 mg, 0.20 mmol), EDC•HCl (44 mg, 0.23 mmol), and DMAP (33 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.50 in Hexanes:EtOAc/1:1) to afford compound **21b** (73 mg, 95%) as an off-white solid: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, Fig. S42) δ 10.60 (s, 1H, N<u>H</u>SO_2), 10.01 (s, 1H, N<u>H</u>), 8.14 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, aromatic), 7.73 (d, J = 2.0 Hz, 1H, aromatic), 7.54 (td, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, aromatic), 7.51 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, aromatic), 7.44-7.35 (m, 3H, aromatic), 7.34 (dd, J_1 = 8.4 Hz, J_2 = 0.8 Hz, 1H, aromatic), 7.13 (d, J = 2.4 Hz, 1H, aromatic), 7.12 (d, J = 8.4 Hz, 1H, aromatic), 7.04 (app. t, J_1 = 7.2 Hz, 1H, aromatic), 6.91 (app. t, J_1 = 7.2 Hz, 1H, aromatic), 4.054 (s, 2H, C<u>H</u>$_2$Ar), 4.048 (s, 3H, OC<u>H</u>$_3$), 2.66 (s, 3H, ArC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, Fig. S43) δ 162.4, 156.1, 137.7, 137.5, 137.0, 135.1, 134.8, 133.6, 132.3, 131.2, 131.1, 127.2, 126.0, 122.9, 121.3, 119.2, 118.6, 118.5, 114.2, 112.2, 111.3, 56.1, 30.0, 19.3; *m/z* calcd for $C_{24}H_{22}N_2O_4S$ 434.1; found 435.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method A: R_t = 20.50 min (98%; Fig. S44).

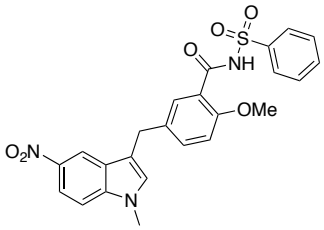


Synthesis of compound 22a. A mixture of compound **19** (30 mg, 0.10 mmol), benzenesulfonamide (19 mg, 0.12 mmol), EDC•HCl (31 mg, 0.16 mmol), and DMAP (22 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, pure Hexanes to Hexanes:EtOAc/1:1, R_f 0.47 in Hexanes:EtOAc/1:1) to afford compound **22a** (41 mg, 93%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S45) δ 10.42 (s, 1H, N<u>H</u>), 8.15 (m, 2H, aromatic), 8.01 (d, J = 2.0 Hz, 1H, aromatic), 7.60 (tt, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.54-7.50 (m, 2H, aromatic), 7.40-7.37 (m, 2H, aromatic), 7.25 (d, J = 8.4 Hz, 1H, aromatic), 7.17 (td, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.01 (td, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H, aromatic), 6.87 (d, J = 8.8 Hz, 1H, aromatic), 6.73 (s, 1H, aromatic), 4.00 (s, 2H, C<u>H</u>$_2$Ar), 3.98 (s, 3H, ArOC<u>H</u>$_3$), 3.69 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S46) δ 162.4, 156.1, 139.0, 137.2, 135.3, 135.2, 133.7, 132.5, 128.8 (2 carbons), 128.6 (2 carbons), 127.5, 127.1, 121.6, 118.9, 118.8, 118.3, 113.4, 111.8, 109.2, 56.5, 32.6, 30.4; *m/z* calcd for $C_{24}H_{22}N_2O_4S$ 434.1; found 435.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method B: R_t = 25.26 min (96%; Fig. S47).

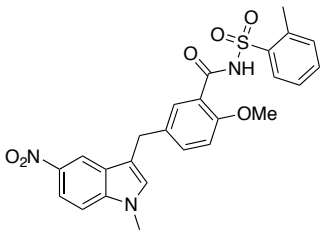


Synthesis of compound 22b. A mixture of compound **19** (30 mg, 0.10 mmol), *o*-toluenesulfonamide (21 mg, 0.12 mmol), EDC•HCl (31 mg, 0.16 mmol), and DMAP (22 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, pure Hexanes to Hexanes:EtOAc/1:1, R_f 0.47 in Hexanes:EtOAc/1:1) to afford compound **22b** (41 mg, 89%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S48) δ 10.42 (s, 1H, N<u>H</u>), 8.24 (dd, J_1 = 8.0 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.95 (d, J = 2.4 Hz, 1H, aromatic), 7.46 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H, aromatic), 7.40 (d, J = 2.8 Hz, 1H, aromatic), 7.38-7.35 (m, 2H, aromatic), 7.26-7.22 (m, 2H, aromatic), 7.16 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H, aromatic), 6.99 (td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H,

aromatic), 6.88 (d, J = 8.8 Hz, 1H, aromatic), 6.72 (s, 1H, aromatic), 4.00 (s, 3H, ArOC<u>H</u>$_3$), 3.98 (s, 2H, C<u>H</u>$_2$Ar), 3.68 (s, 3H, NC<u>H</u>$_3$), 2.66 (s, 3H, ArC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S49) δ 162.4, 156.1, 137.4, 137.2, 137.0, 135.3, 135.2, 133.7, 132.5, 132.7, 132.5, 132.4, 132.3, 131.4, 128.1, 127.4, 127.1, 126.3, 126.2, 121.6, 118.9, 118.8, 118.4, 113.4, 111.8, 109.2, 56.5, 32.6, 30.4, 20.2; *m/z* calcd for $C_{25}H_{24}N_2O_4S$ 448.2; found 449.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method B: R_t = 25.71 min (95%; Fig. S50).



Synthesis of compound 23a. A mixture of compound **20** (25 mg, 0.073 mmol), benzenesulfonamide (14 mg, 0.088 mmol), EDC•HCl (22 mg, 0.11 mmol), and DMAP (16 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.64 in CH_2Cl_2:MeOH/19:1) to afford compound **23a** (24 mg, 69%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S51) δ 10.38 (s, 1H, N<u>H</u>), 8.32 (d, J = 2.4 Hz, 1H, aromatic), 8.13-8.10 (m, 2H, aromatic), 8.07 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.91 (d, J = 2.4 Hz, 1H, aromatic), 7.59 (tt, J_1 = 7.6 Hz, J_2 = 2.0 Hz, 1H, aromatic), 7.53-7.48 (m, 2H, aromatic), 7.43 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.25 (d, J = 9.6 Hz, 1H, aromatic), 6.93 (d, J = 8.8 Hz, 1H, aromatic), 6.86 (s, 1H, aromatic), 4.01 (s, 5H, ArOC<u>H</u>$_3$, C<u>H</u>$_2$Ar), 3.75 (s, 3H, NC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S52) δ 162.3, 156.4, 141.2, 139.9, 138.9, 135.1, 133.9, 133.7, 132.5, 130.2, 128.8 (2 carbons), 128.5 (2 carbons), 126.7, 118.7, 117.5, 116.5, 116.3, 111.9, 109.2, 56.6, 33.1, 30.0; *m/z* calcd for $C_{24}H_{21}N_3O_6S$ 479.1; found 480.1 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method B: R_t = 24.48 min (97%; Fig. S53).



Synthesis of compound 23b. A mixture of compound **20** (25 mg, 0.073 mmol), *o*-toluenesulfonamide (15 mg, 0.088 mmol), EDC•HCl (22 mg, 0.11 mmol), and DMAP (16 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature overnight. Upon completion, the reaction mixture was diluted with H_2O and extracted

with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product obtained was purified by column chromatography (SiO_2 gel, CH_2Cl_2:MeOH/49:1, R_f 0.66 in CH_2Cl_2:MeOH/19:1) to afford compound **23b** (26 mg, 72%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S54) δ 10.39 (s, 1H, NH), 8.30 (d, J = 2.4 Hz, 1H, aromatic), 8.21 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, aromatic), 8.06 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H, aromatic), 7.86 (d, J = 2.4 Hz, 1H, aromatic), 7.47-7.41 (m, 2H, aromatic), 7.35 (t, J = 7.2 Hz, 1H, aromatic), 7.24 (d, J = 8.8 Hz, 2H, aromatic), 6.95 (d, J = 8.4 Hz, 1H, aromatic), 6.85 (s, 1H, aromatic), 4.03 (s, 3H, ArOCH$_3$), 4.00 (s, 2H, CH$_2$Ar), 3.74 (s, 3H, NCH$_3$), 2.65 (s, 3H, ArCH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, Fig. S55) δ 162.2, 156.3, 141.1, 139.9, 137.4, 137.0, 135.1, 134.0, 133.8, 132.4, 132.3, 131.4, 130.3, 126.7, 126.3, 118.7, 117.4, 116.4, 116.3, 112.0, 109.2, 56.6, 33.1, 30.0, 20.1; *m/z* calcd for $C_{25}H_{23}N_3O_6S$ 493.1; found 494.0 $[M+H]^+$. Purity of the compound was further confirmed by RP-HPLC by using method B: R_t = 25.04 min (98%; Fig. S56).

BIOLOGICAL EXPERIMENTS

Bacterial strains and growth conditions. The following bacterial strains were used in these studies: *Porphyromonas gingivalis* 381, *Actinomyces naeslundii* ATCC 49340, *Streptococcus sanguinis* ATCC 10556, *Veillonella parvula* ATCC 10790, *Aggregatibacter actinomycetemcomitans* JP2, and *Fusobacterium nucleatum* ATCC 25586. All bacterial strains were initially grown on blood agar plates (BBL, Becton Dickinson, Sparks, MD, USA) from a frozen stock and incubated in appropriate aerobic (for *S. sanguinis*) or anaerobic (for *P. gingivalis*, *A. naeslundii*, *V. parvula*, *A. actinomycetemcomitans*, and *F. nucleatum*) conditions at 37 °C for 24 h or 3 days, respectively. Then, a liquid culture was started for each strain in 3 mL of Brain Hearth infusion (BHI) broth alone or supplemented with 5 μg/mL of hemin and 1 μg/mL of menadione in the case of *P. gingivalis*. Overnight cultures were inoculated in fresh broth and allowed to reach logarithmic growth for 3-4 h, time at which antimicrobial activity of different treatments was evaluated.

Effect of zafirlukast derivatives on bacterial viability. Initial screening for testing the antimicrobial effect of zafirlukast derivatives (**4a**, **4b**, **11a**, **11b**, **12a**, **21a**, **21b**, **22a**, **22b**, **23a**, and **23b**) was performed using the colorimetric water-soluble tetrazolium-1 (WST-1) assay, which

serves as a surrogate marker for cell proliferation and viability. Bacterial strains (10^6/well) grown logarithmically in broth (90 μL) using 96-wells plates, were exposed to different treatments for 2 h and then 15 μL of WST-1 reagent (Roche, Manheim, Germany) were added to each well. Plates were incubated for 2 h under appropriate culture conditions and then absorbance was measured at 450 nm, with a reference wavelength of 600 nm, using SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, CA, USA). The percentage of inhibitory effect (*i.e.*, % growth inhibition) was calculated using the optical densities (ODs) of bacterial metabolic activity, under different experimental conditions according to the following formula: 100 × [OD (control) - OD (experimental) / OD (control)]. For *P. gingivalis*, control cultures were treated only with medium and experimental cultures treated with DMSO (vehicle for zafirlukast and its derivatives) and 1 μg/mL of the antibiotic tetracycline. These data are presented in Fig. 2. When tested against *A. naeslundii*, *A. actinomycetemcomitans*, *S. sanguinis*, *V. parvula*, and *F. nucleatum* control cultures were treated only with medium and experimental cultures were treated with DMSO (vehicle for zafirlukast and its derivatives), antibiotic penicillin/streptomycin -1X (100 U/mL of penicillin and 100 μg/mL of streptomycin) for Gram-positives and 1 μg/mL of tetracycline for Gram-negative anaerobic bacteria. These data are presented in Fig. 3.

Bactericidal effect of zafirlukast derivatives on *P. gingivalis*. The bactericidal effect of zafirlukast derivatives **22b**, **23a**, and **23b** (which exhibited the best antimicrobial potential at lower concentrations determined by WST-1) was evaluated by determination of colony forming units per milliliter (CFUs/mL) after different treatments. *P. gingivalis* (10^6/well) grown in appropriate medium and anaerobic conditions was exposed to zafirlukast derivatives dissolved in DMSO for 24 h and further 50 μL of bacterial culture dilutions (1:400) were spread onto blood agar plates. Numbers of CFUs were obtained after 7 days of incubation in appropriate anaerobic conditions. Bacteria exposed to only medium or DMSO were used as a negative controls and bacteria exposed to 2.25 μM of tetracycline (equivalent to 1 μg/mL, a standard concentration usually used for this control) and zafirlukast at 25 and 50 μM diluted in DMSO (concentrations that previously showed antimicrobial effect against *P. gingivalis*) were also used as positive controls.[2] These data are presented in Fig. 4.

Cytotoxic effect of zafirlukast derivatives in oral epithelial cells. The immortalized oral keratinocyte cell line OKF6/hTERT (OKF6) was used for viability assays in response to zafirlukast derivatives **22b**, **23a**, and **23b** as previously reported.[3, 4] Cells were cultured in keratinocyte-serum free medium (SFM) supplemented with bovine pituitary extract (25 μg/mL) and recombinant epidermal growth factor (0.2 ng/mL) (Ker-SFM). Cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

For viability assays, cells (10^5 cells/well) were seeded in 96-wells plates overnight in Ker-SFM and further exposed to zafirlukast at 25 μM or its derivatives at 1 and 10 μM dissolved in DMSO for 24 h. Cells were then harvested by trypsinization, washed with DMEM medium supplemented with 5% fetal bovine serum at 1100 rpm and resuspended in fresh Ker-SFM. Equal volumes of cell suspension and trypan blue were mixed and 10 μL evaluated in the automated cell counter (Countess II FL) (Life Technologies, Singapure) to determine percentage of alive and death cells. These experiments were performed in two independent experiments by duplicate. These data are presented in Fig. 5 and Table S1. To further determine the IC_{50} values for zafirlukast derivatives **22b**, **23a**, and **23b**, the same assay was performed in triplicate using the following concentrations of compounds: 0, 1, 2.5, 5, 10, 15, 20, 25, 50, and 100 μM. These data are presented in Fig. 6 and Table S2. The IC_{50} values were calculated by using the Quest Graph™ IC_{50} calculator (*ATT Bioquest, Inc*, https://www.aatbio.com/tools/ic50-calculator).

Cytotoxic effects of zafirlukast derivatives were confirmed by flow cytometry analysis (FACS). After different treatments, attached OKF6 cells were harvested by trypsinization and mixed with cells that were previously rescued from supernatants. Cells were washed with PBS at 1100 rpm for 5 min and further labeled with 5 μL of FITC-Annexin V and 5 μL of propidium iodide (BD Pharmingen, San Jose, CA) for 15 min at room temperature. Labeled cells were analyzed by FACS, with at least 10,000 events read at wavelengths averaging 488 nm for excitation and 530 nm for emission in a flow cytometer FACSCalibur (Becton Dickinson, San Jose, CA). These data are presented in Fig. 7 and Table S3.

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Figures S1-S56:

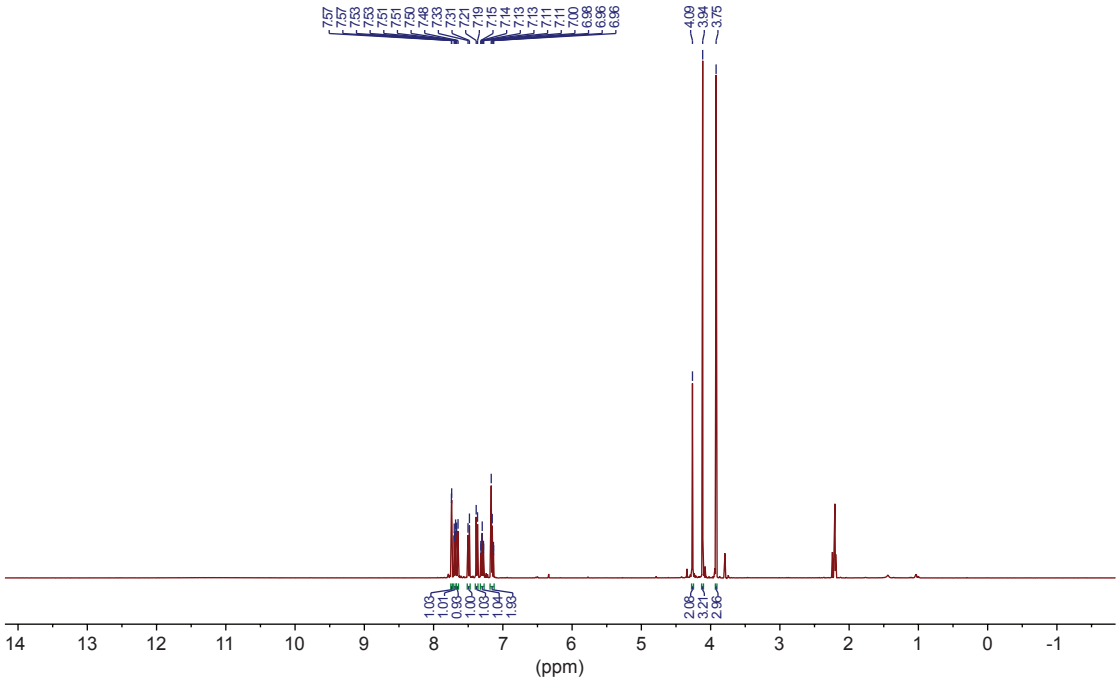

Fig. S1: ^{1}H NMR spectrum for compound **3** in $(CD_3)_2CO$ (400 MHz).

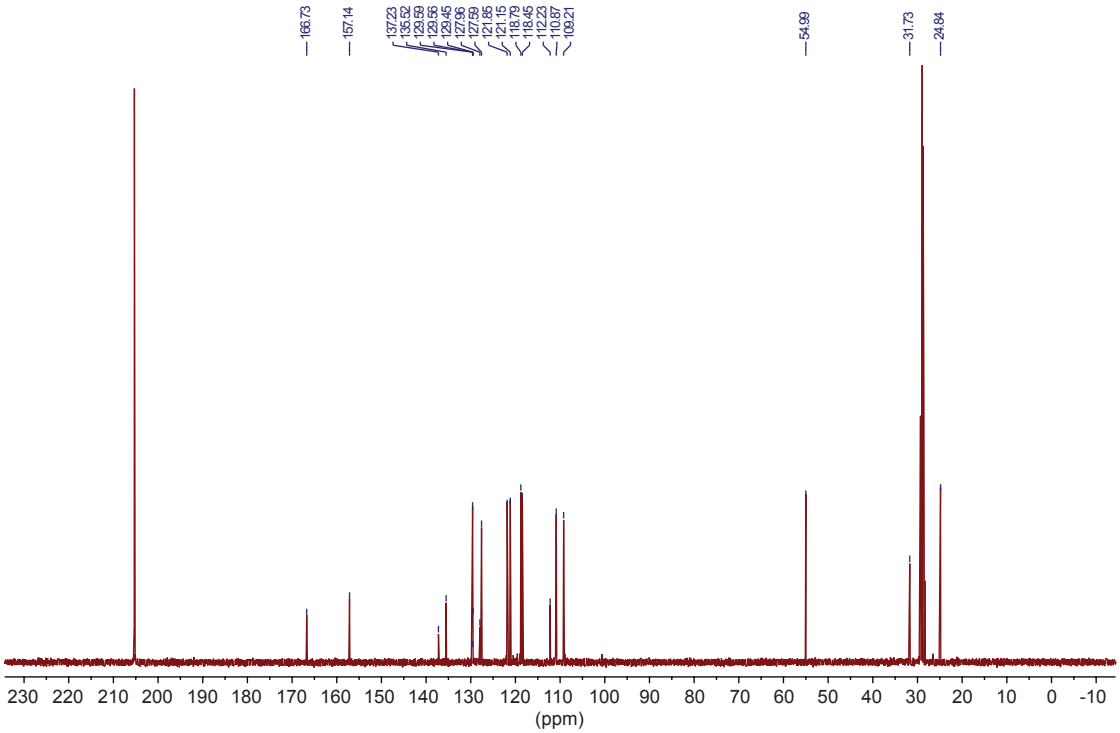

Fig. S2: ^{13}C NMR spectrum for compound **3** in $(CD_3)_2CO$ (100 MHz).

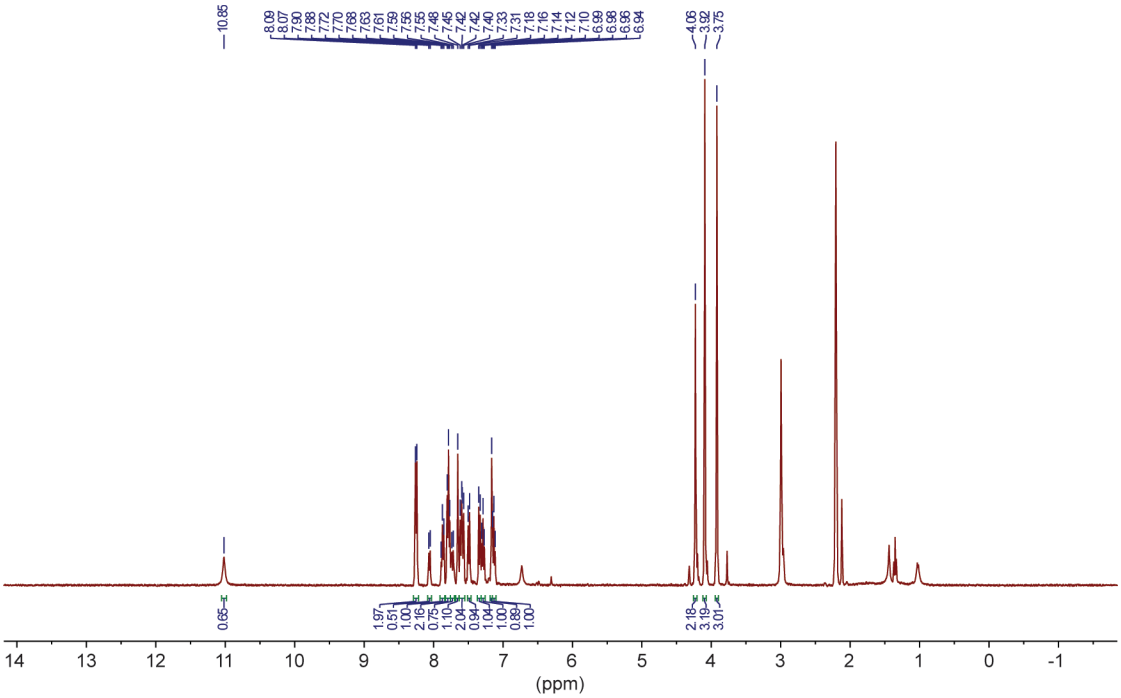

Fig. S3: ^{1}H NMR spectrum for compound **4a** in $(CD_3)_2CO$ (400 MHz).

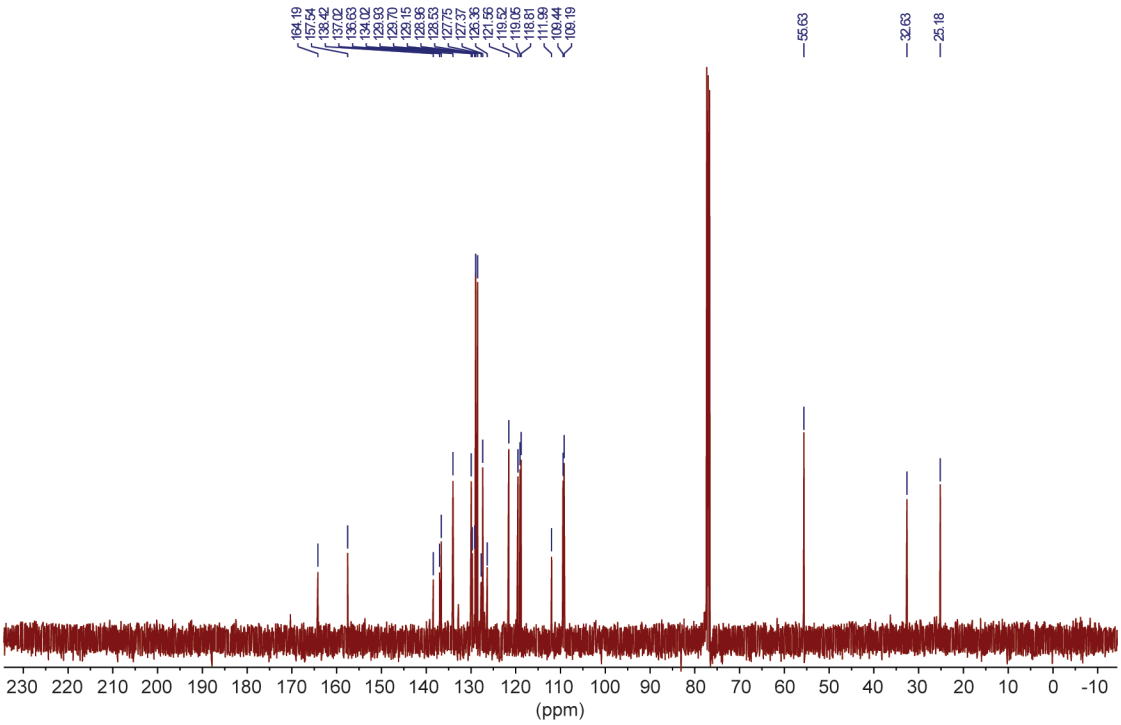

Fig. S4: ^{13}C NMR spectrum for compound **4a** in $CDCl_3$ (100 MHz).

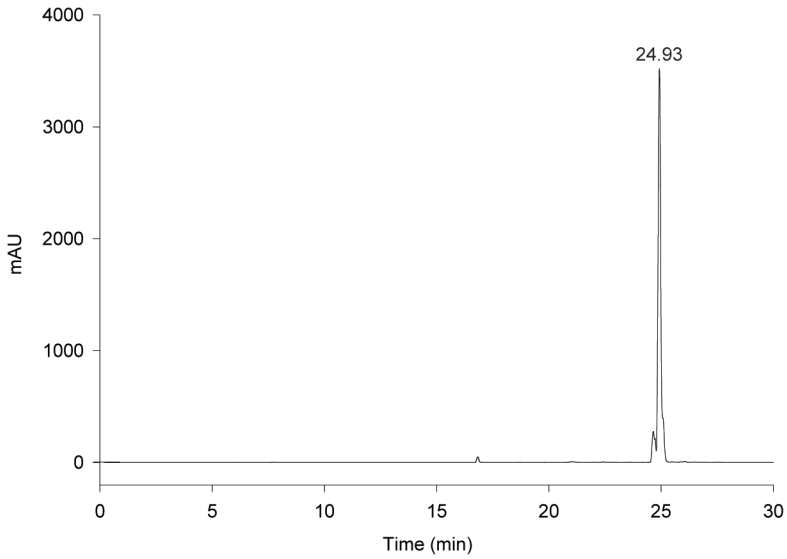

Fig. S5: HPLC trace for compound **4a** (R_t = 24.93 min).

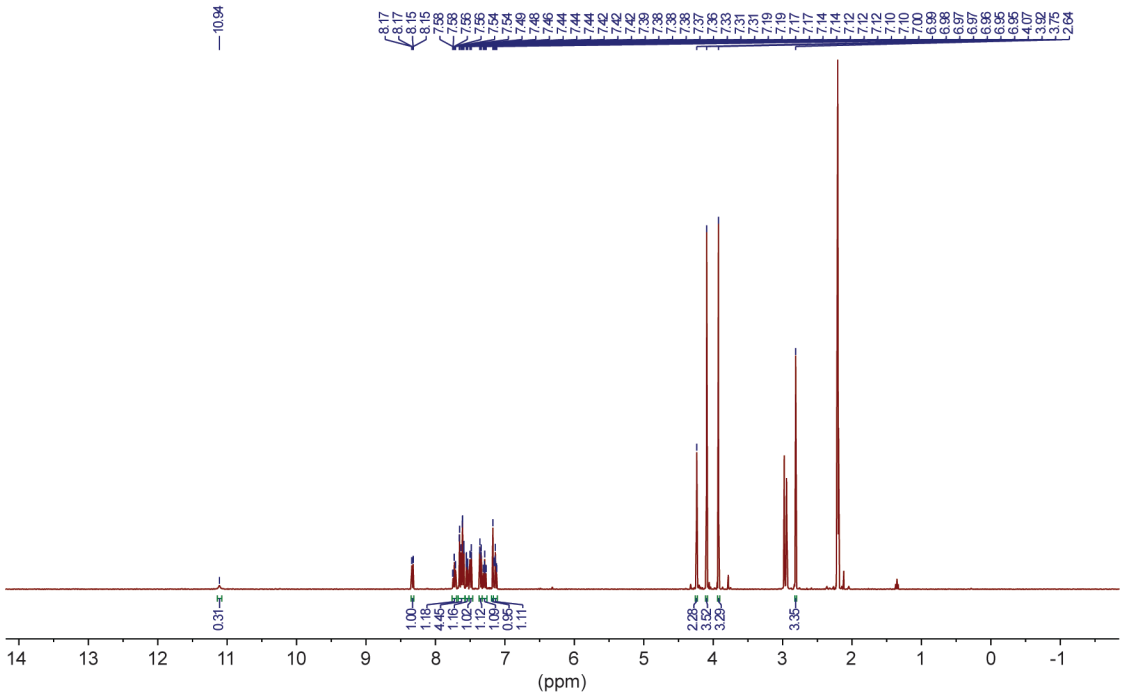

Fig. S6: ^{1}H NMR spectrum for compound **4b** in $(CD_3)_2CO$ (400 MHz).

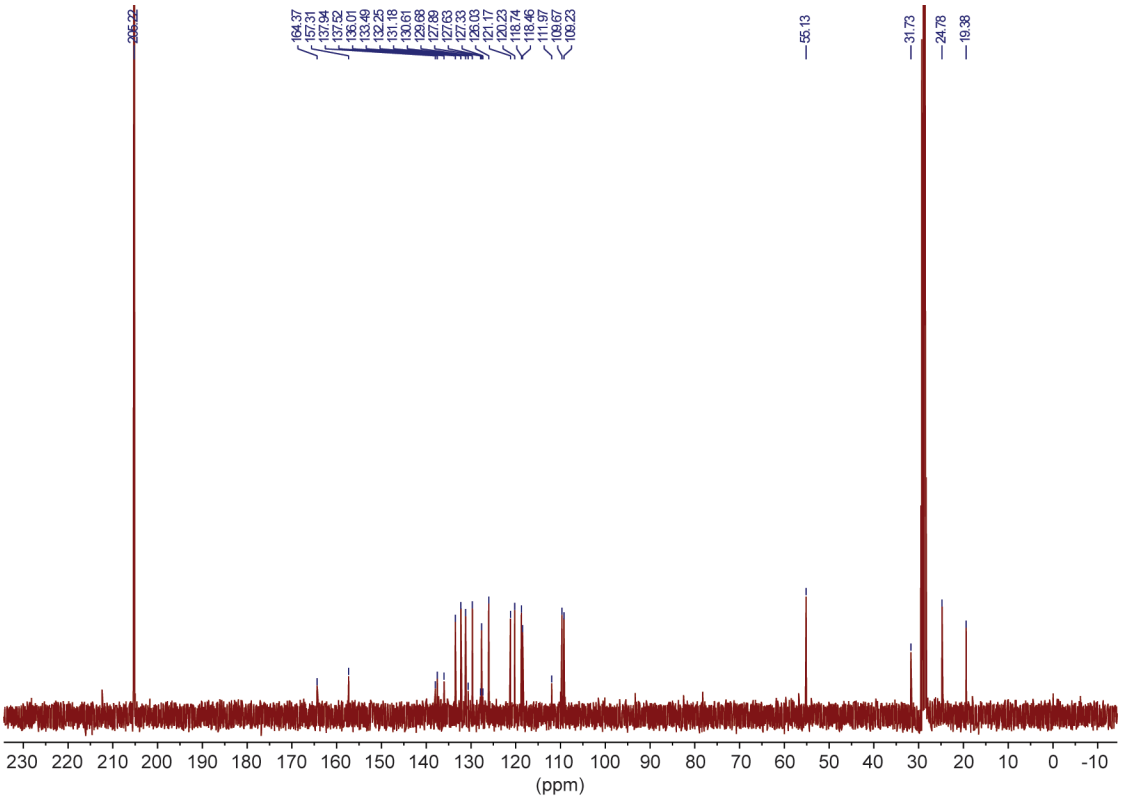

Fig. S7: ^{13}C NMR spectrum for compound **4b** in $(CD_3)_2CO$ (100 MHz).

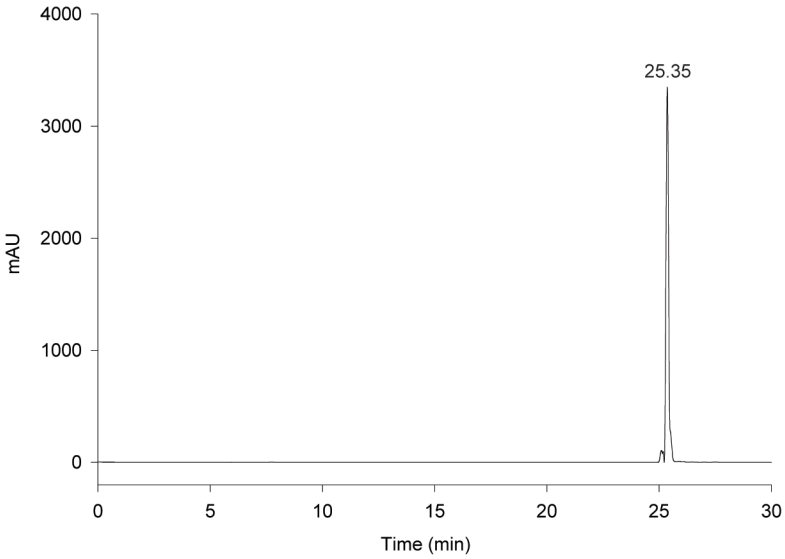

Fig. S8: HPLC trace for compound **4b** (R_t = 25.35 min).

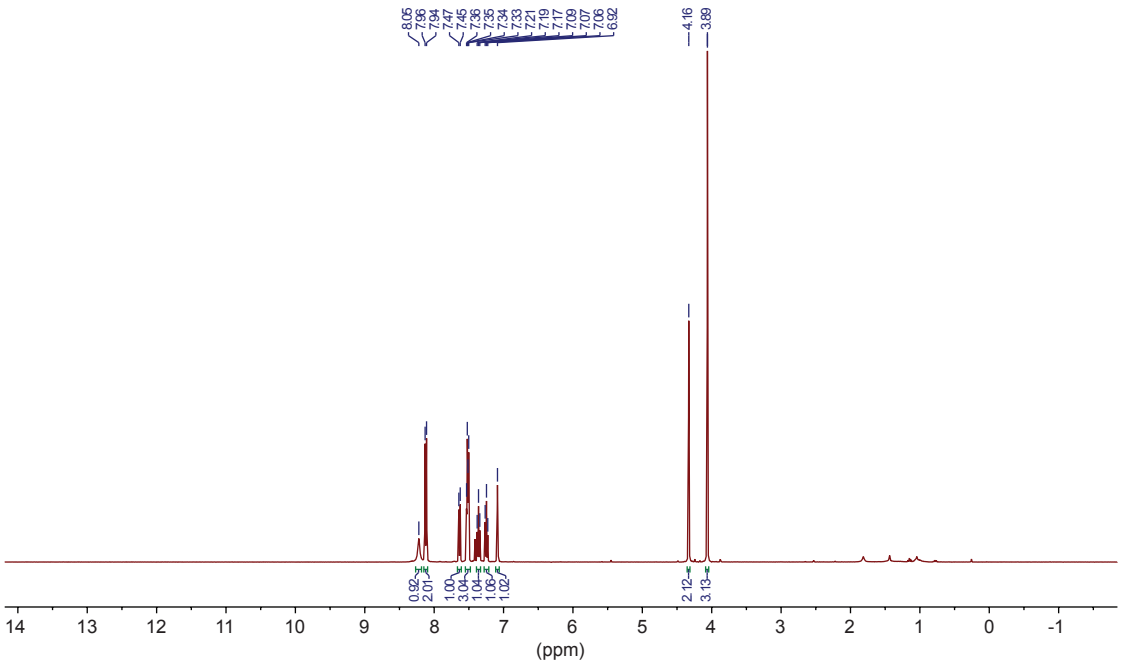

Fig. S9: ^{1}H NMR spectrum for compound **7** in $CDCl_3$ (400 MHz).

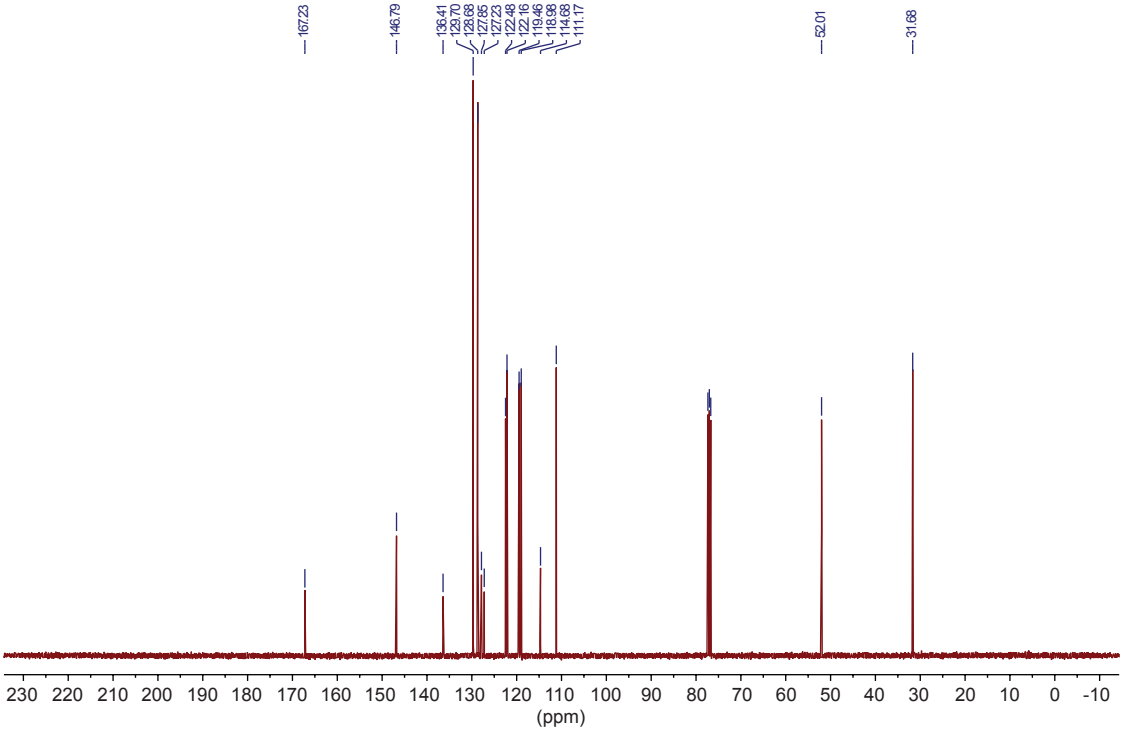

Fig. S10: ^{13}C NMR spectrum for compound **7** in $CDCl_3$ (100 MHz).

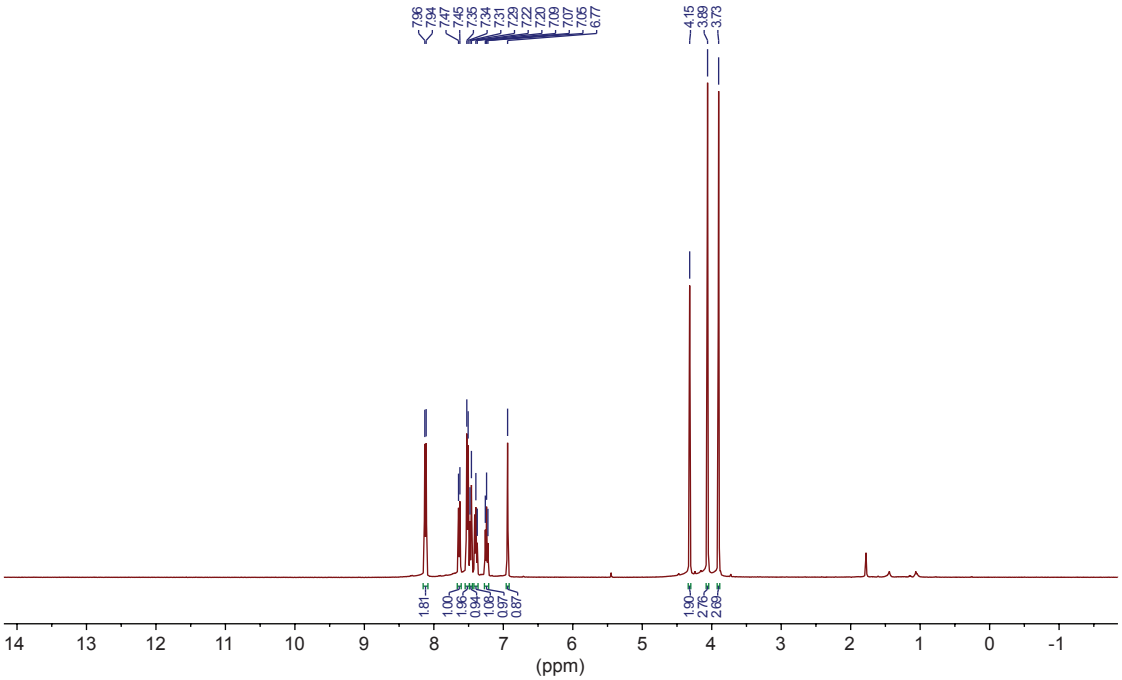

Fig. S11: ^{1}H NMR spectrum for compound **8** in $CDCl_3$ (400 MHz).

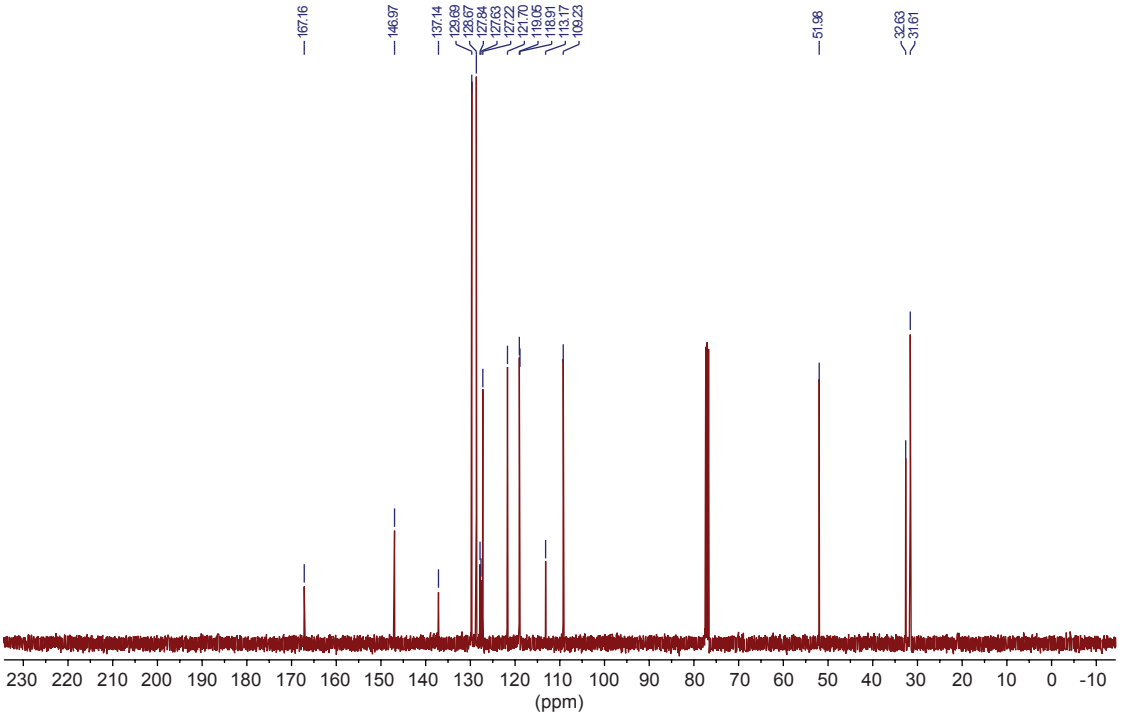

Fig. S12: ^{13}C NMR spectrum for compound **8** in $CDCl_3$ (100 MHz).

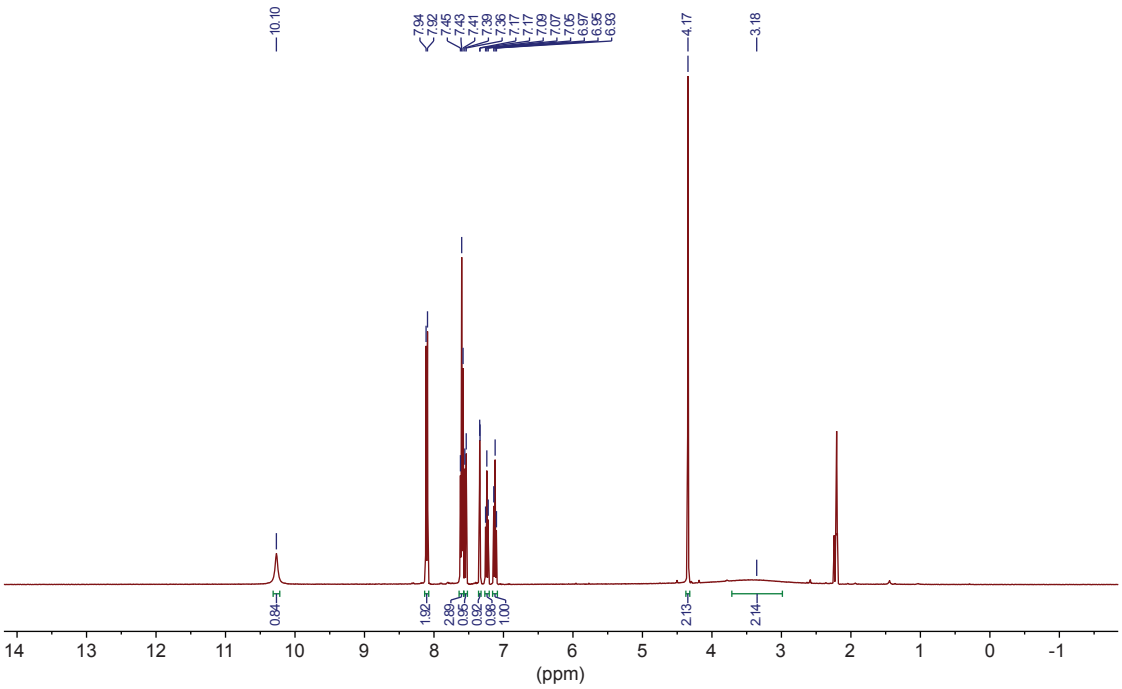

Fig. S13: ^{1}H NMR spectrum for compound **9** in $(CD_3)_2CO$ (400 MHz).

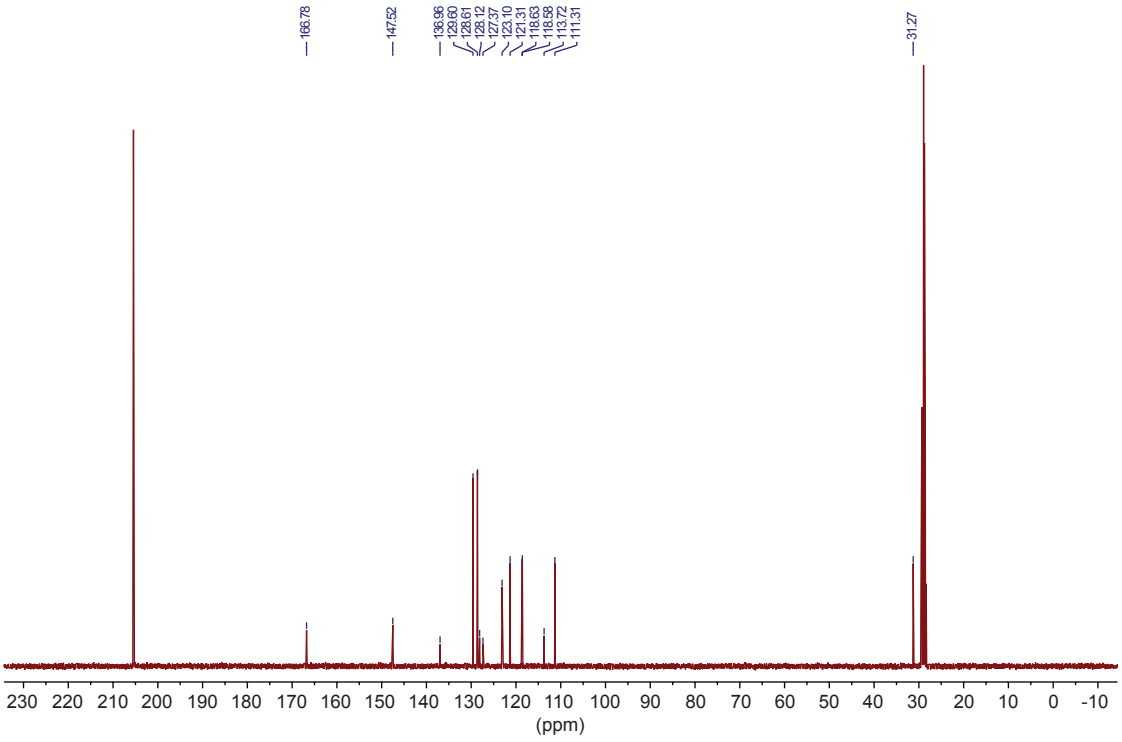

Fig. S14: ^{13}C NMR spectrum for compound **9** in $(CD_3)_2CO$ (100 MHz).

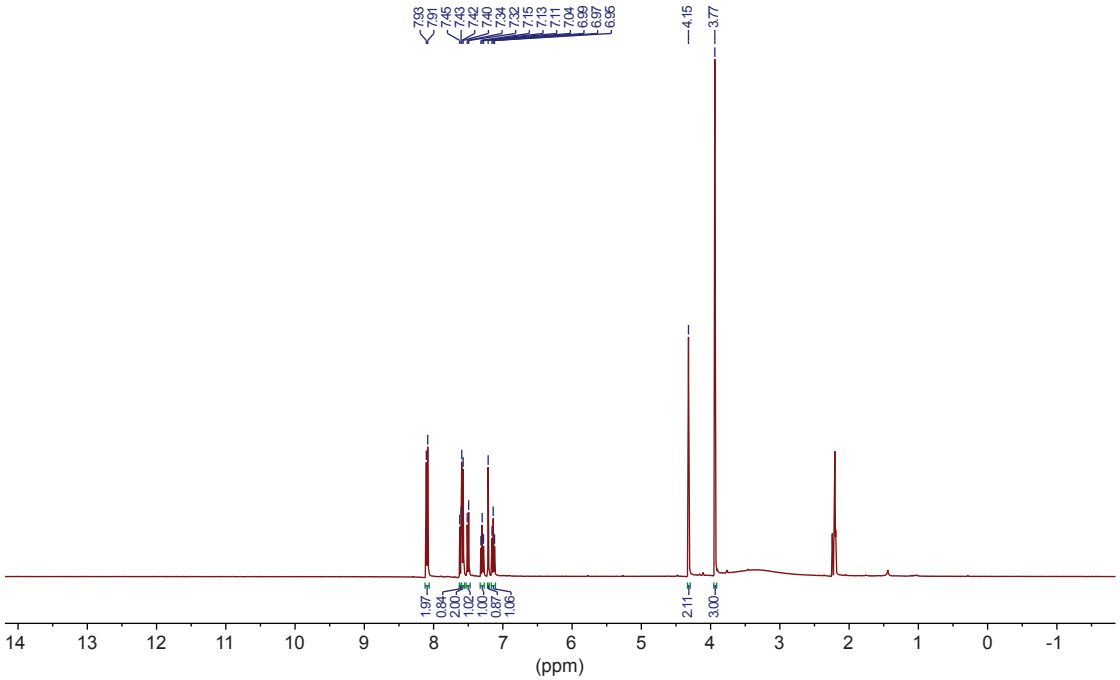

Fig. S15: ^{1}H NMR spectrum for compound **10** in $(CD_3)_2CO$ (400 MHz).

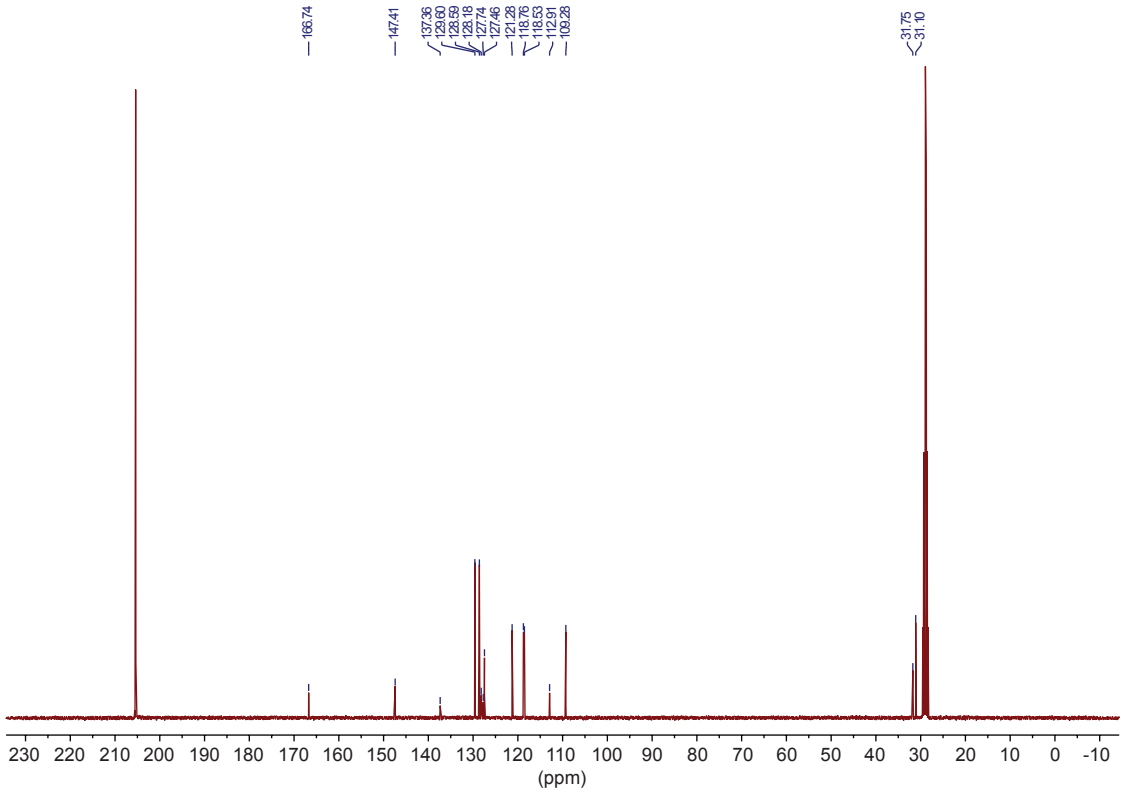

Fig. S16: ^{13}C NMR spectrum for compound **10** in $(CD_3)_2CO$ (100 MHz).

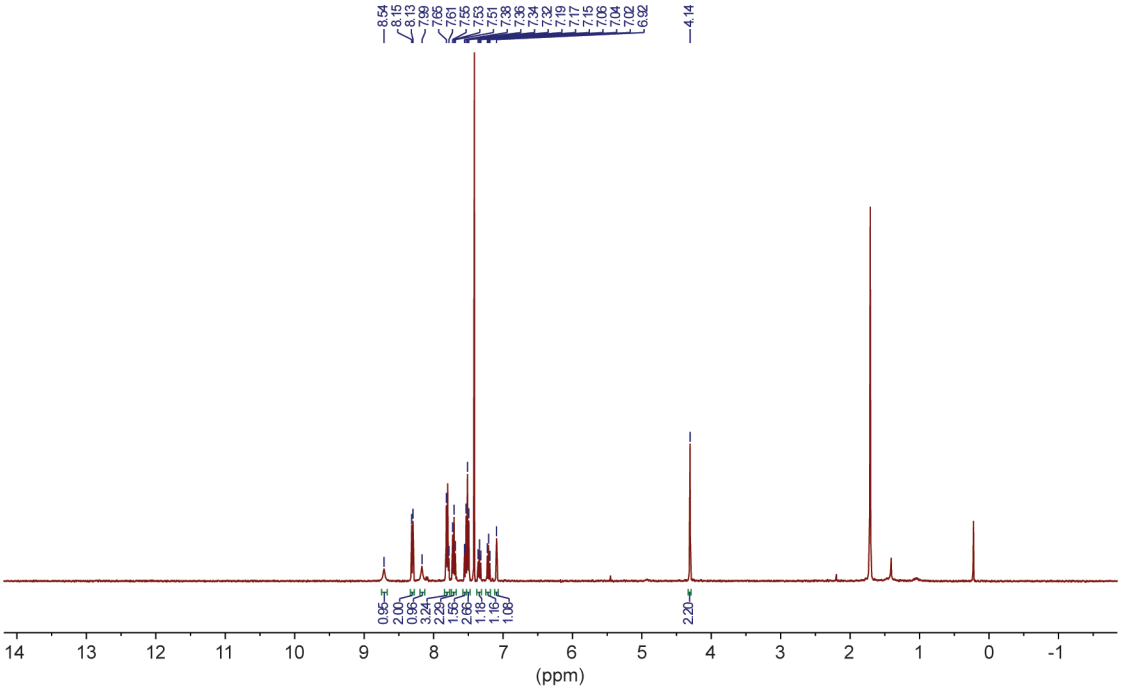

Fig. S17: ^{1}H NMR spectrum for compound **11a** in $CDCl_3$ (400 MHz).

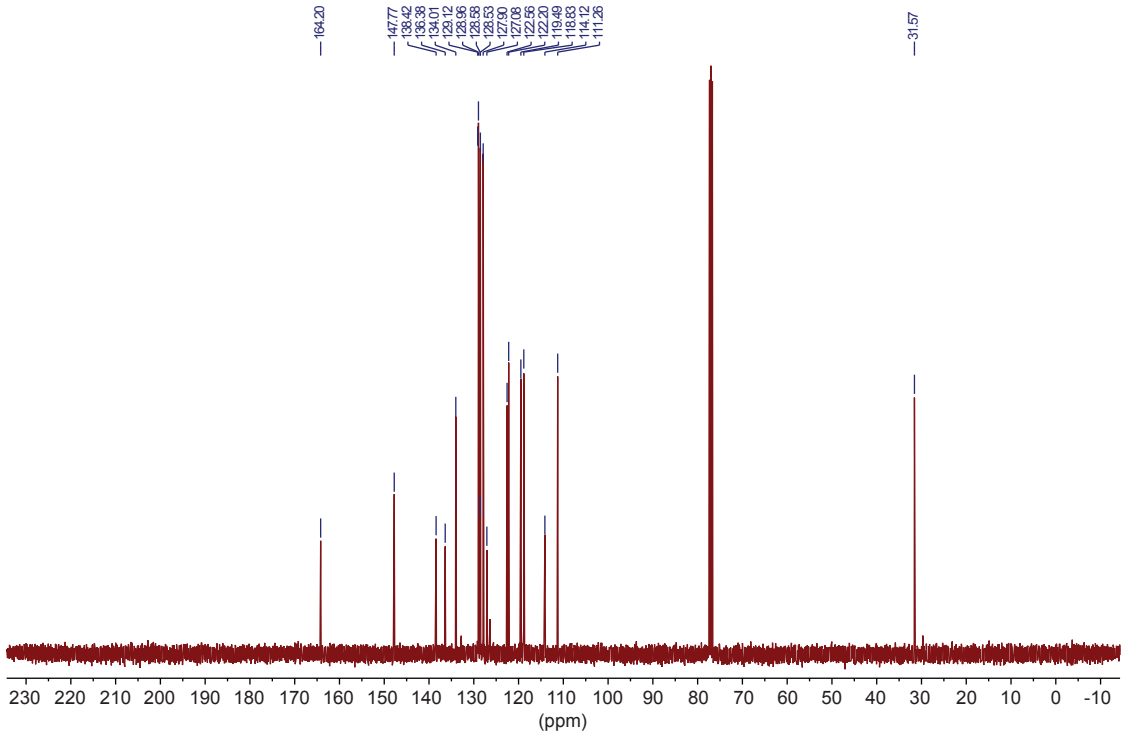

Fig. S18: ^{13}C NMR spectrum for compound **11a** in $CDCl_3$ (100 MHz).

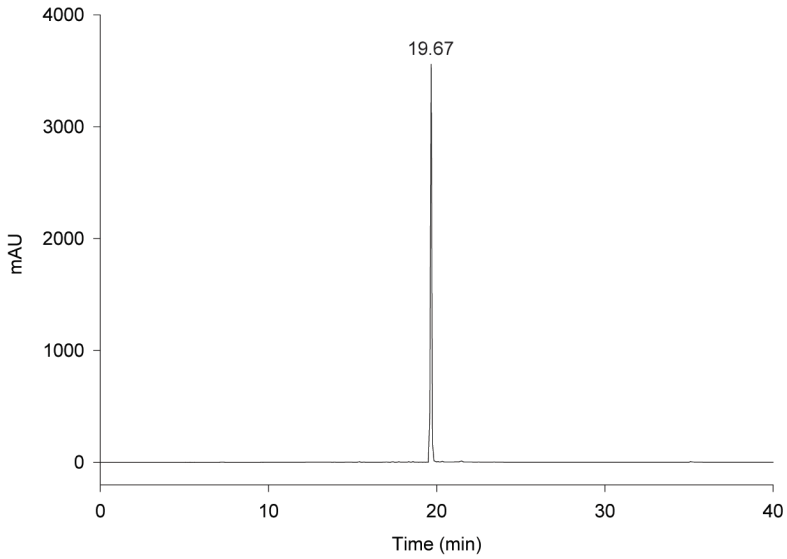

Fig. S19: HPLC trace for compound **11a** (R_t = 19.67 min).

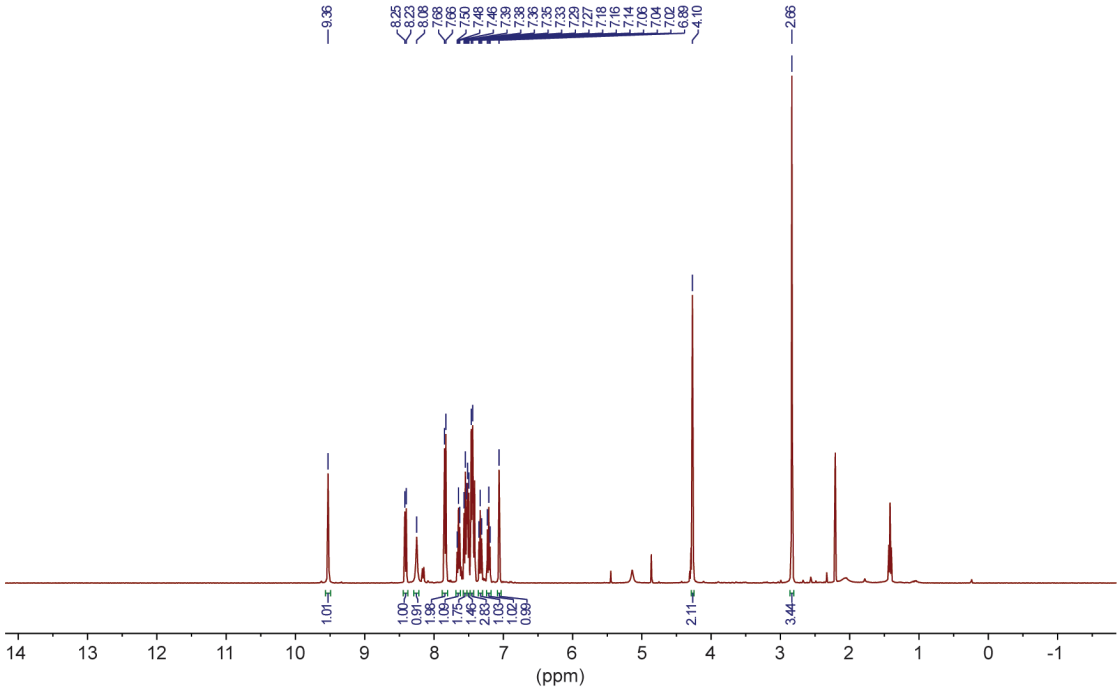

Fig. S20: ^{1}H NMR spectrum for compound **11b** in $CDCl_3$ (400 MHz).

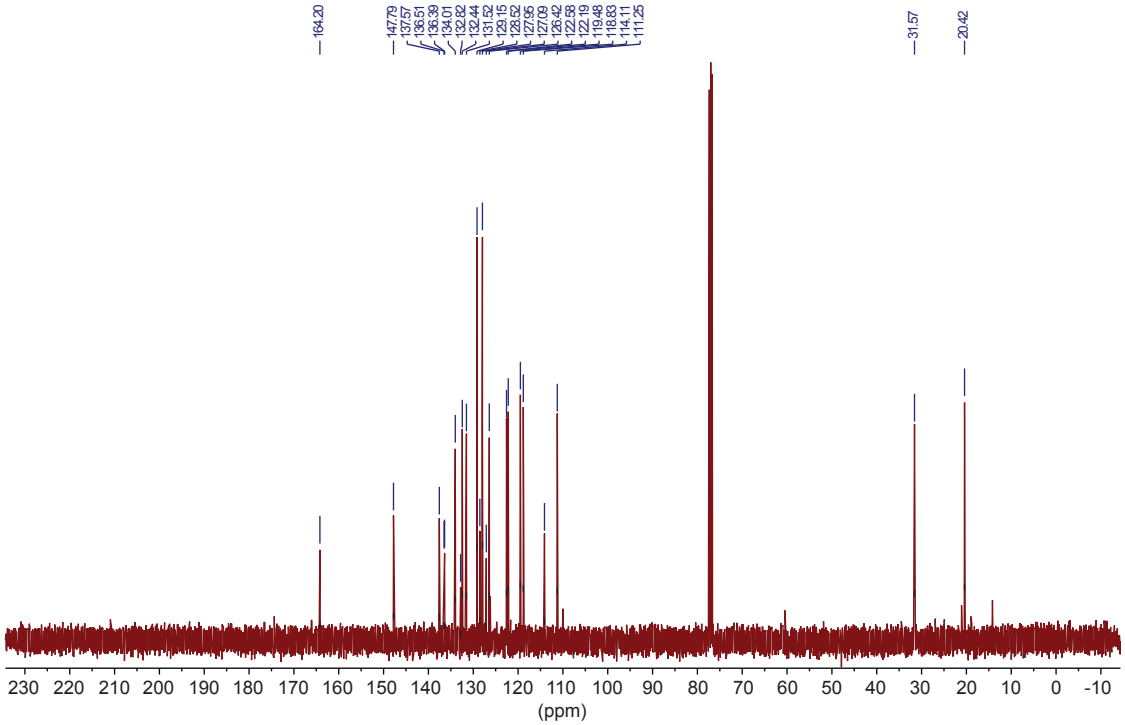

Fig. S21: ^{13}C NMR spectrum for compound **11b** in $CDCl_3$ (100 MHz).

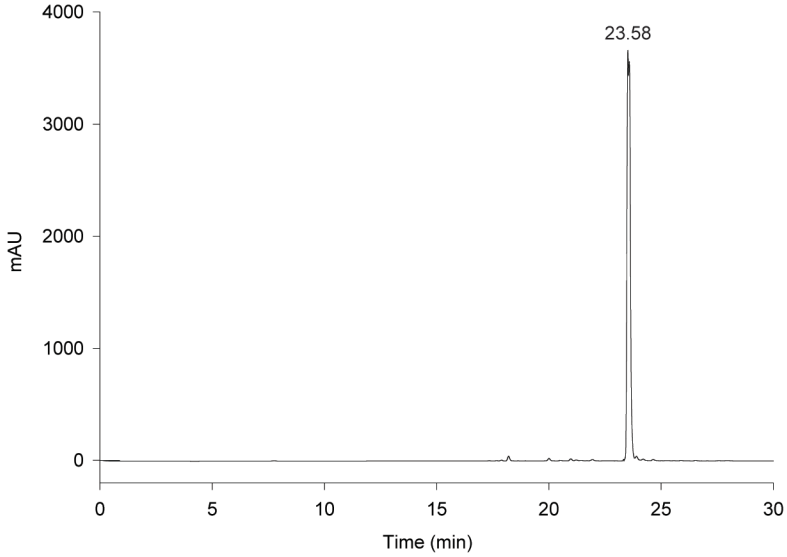

Fig. S22: HPLC trace for compound **11b** (R_t = 23.58 min).

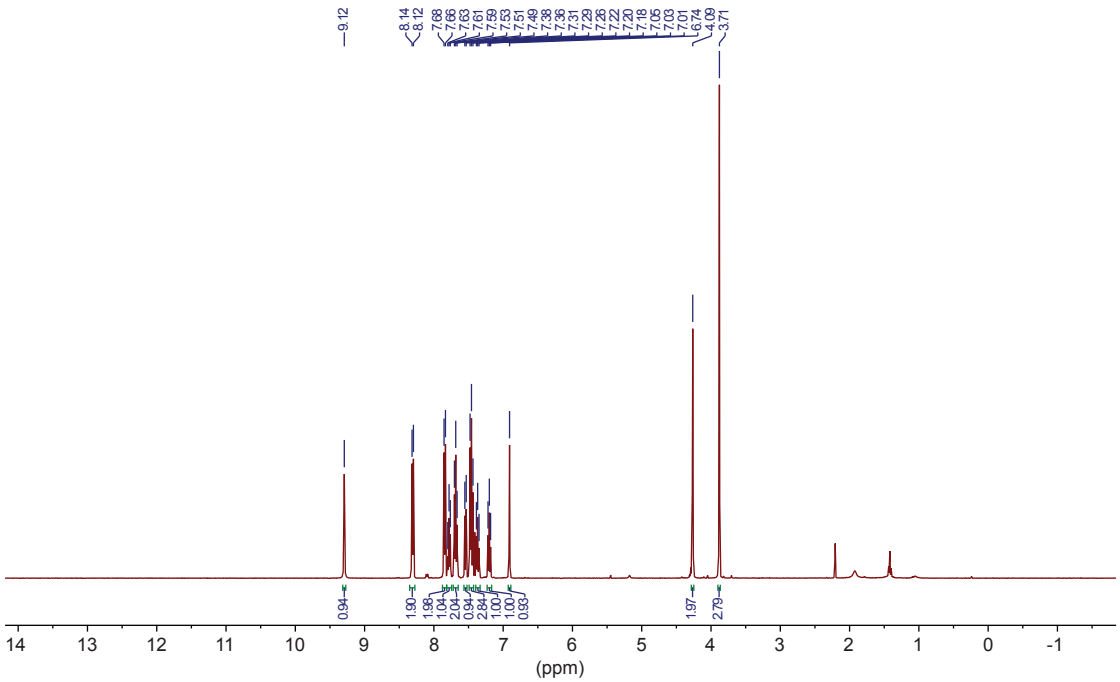

Fig. S23: ^{1}H NMR spectrum for compound **12a** in $CDCl_3$ (400 MHz).

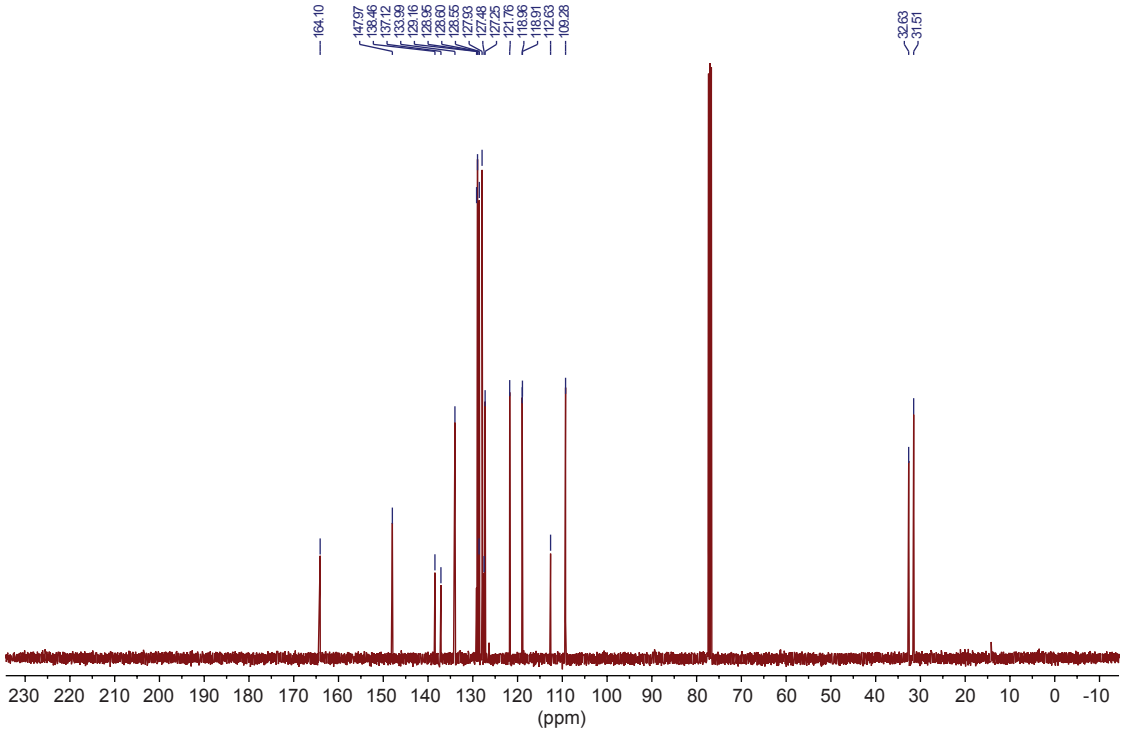

Fig. S24: ^{13}C NMR spectrum for compound **12a** in $CDCl_3$ (100 MHz).

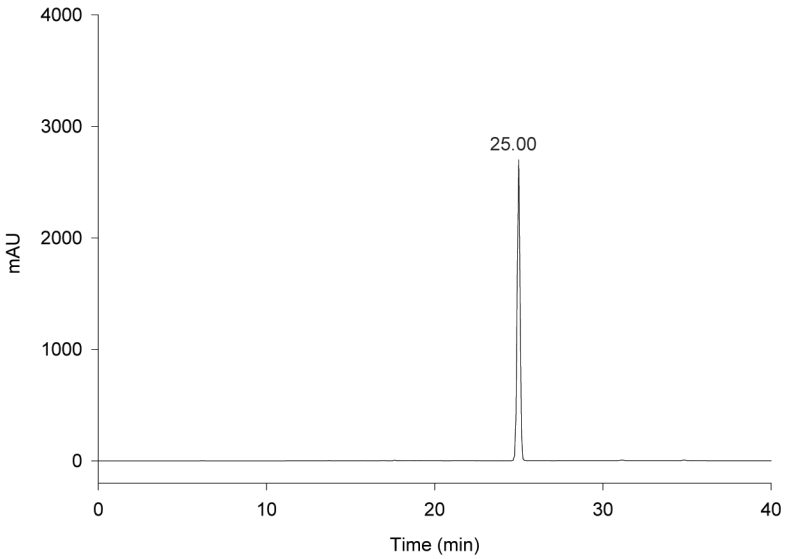

Fig. S25: HPLC trace for compound **12a** (R_t = 25.00 min).

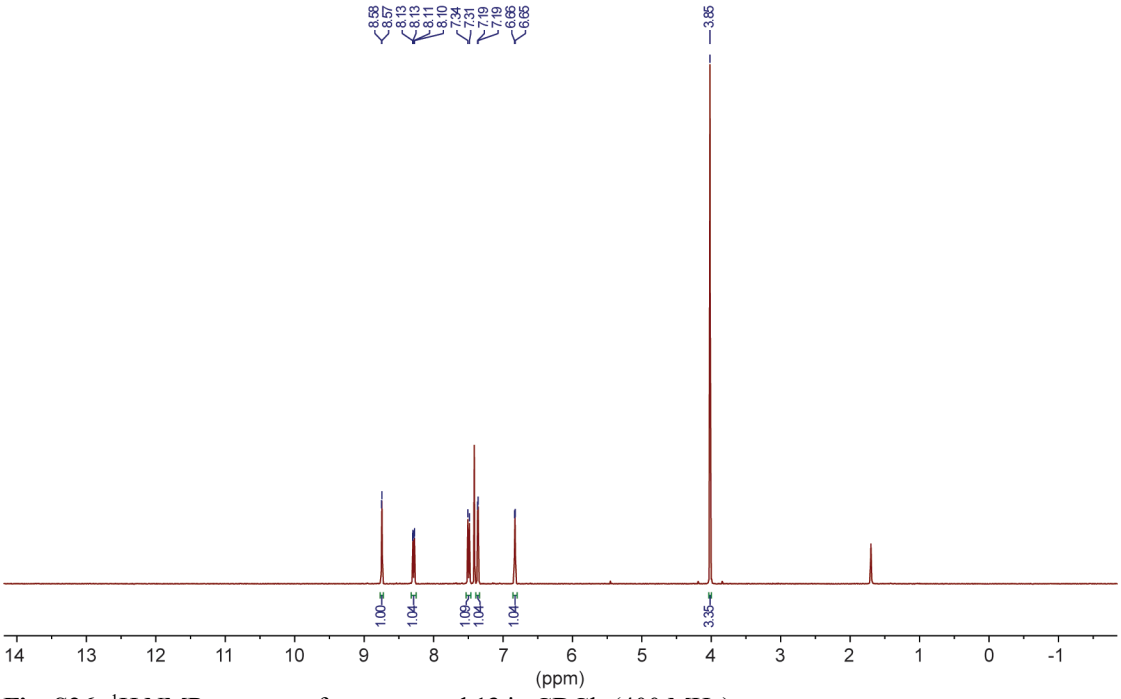

Fig. S26: ^{1}H NMR spectrum for compound **13** in $CDCl_3$ (400 MHz).

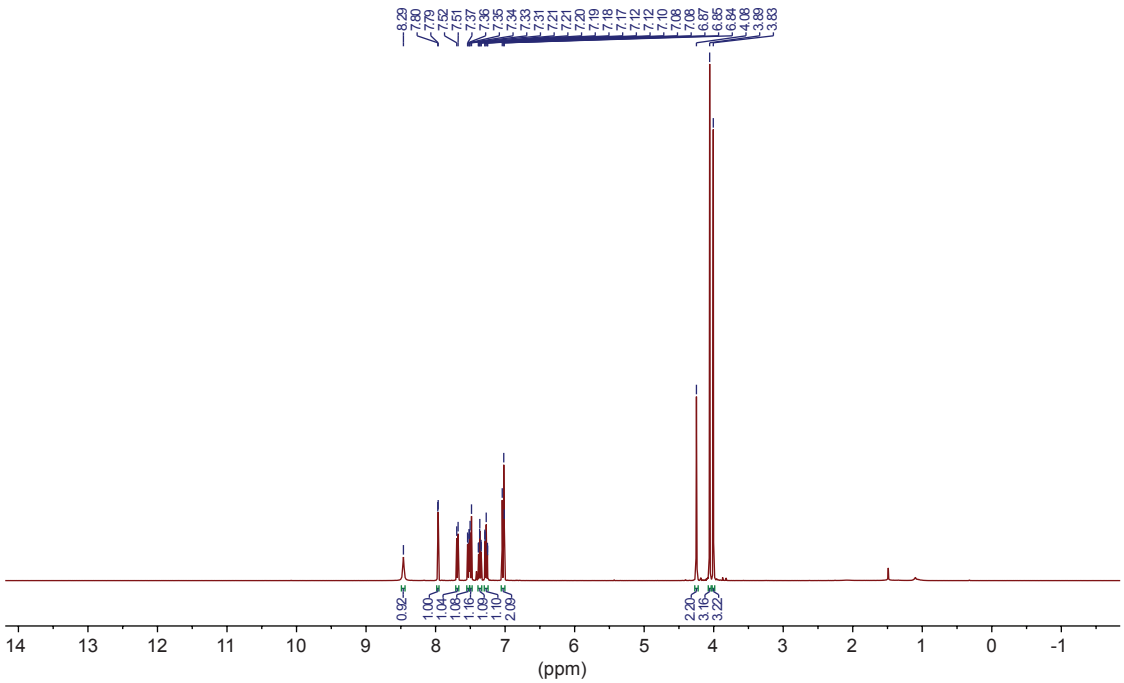

Fig. S27: ^{1}H NMR spectrum for compound **15** in $CDCl_3$ (400 MHz).

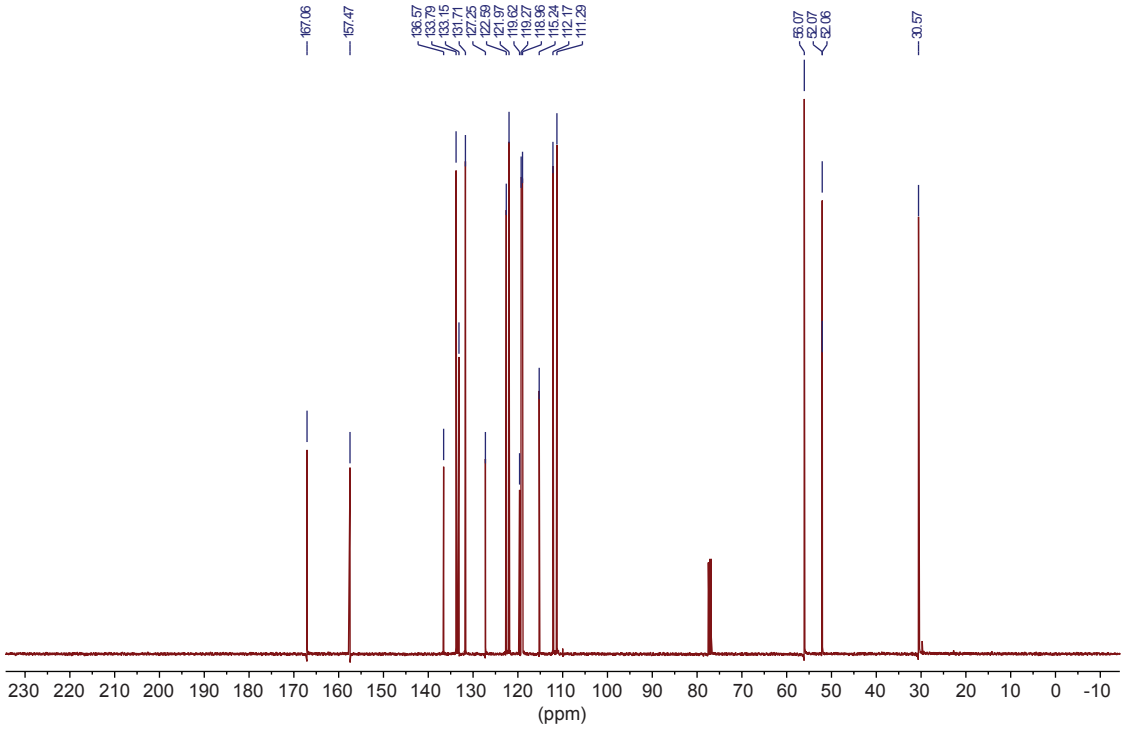

Fig. S28: ^{13}C NMR spectrum for compound **15** in $CDCl_3$ (100 MHz).

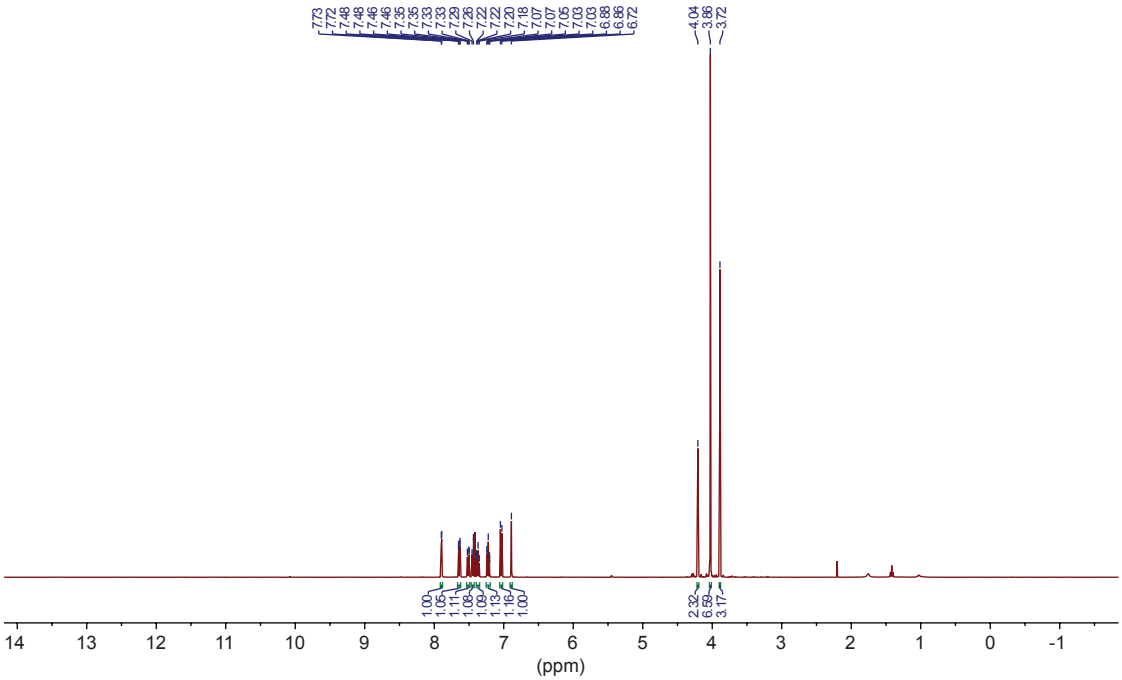

Fig. S29: ^{1}H NMR spectrum for compound **16** in $CDCl_3$ (400 MHz).

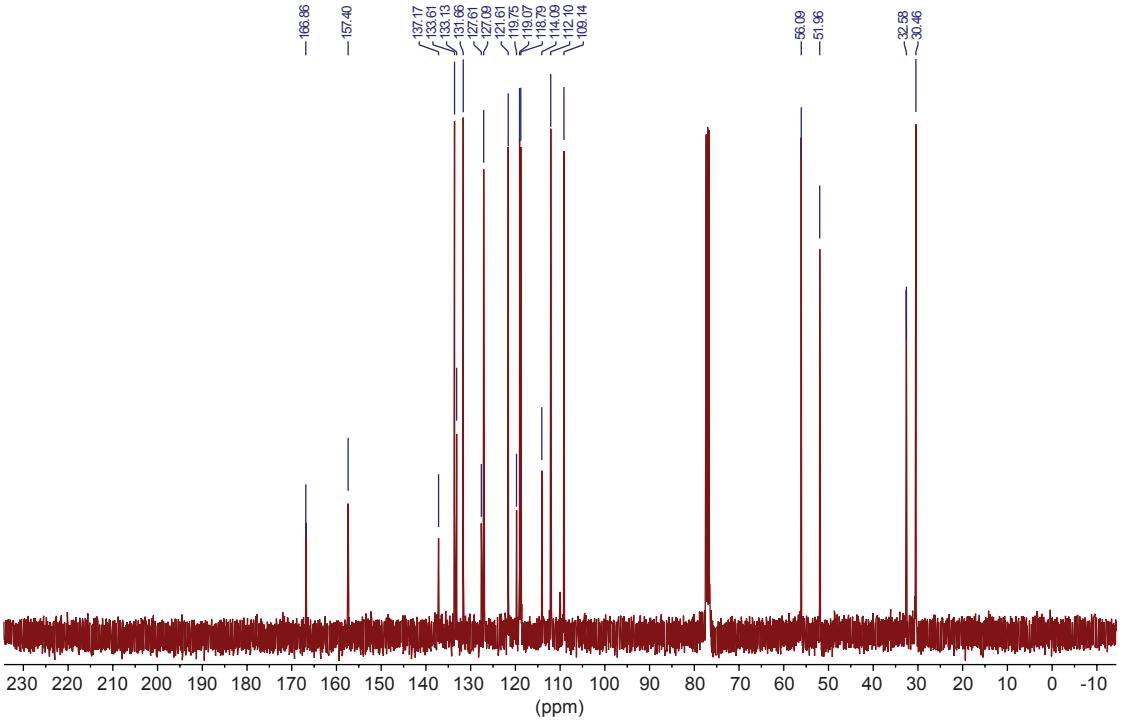

Fig. S30: ^{13}C NMR spectrum for compound **16** in $CDCl_3$ (100 MHz).

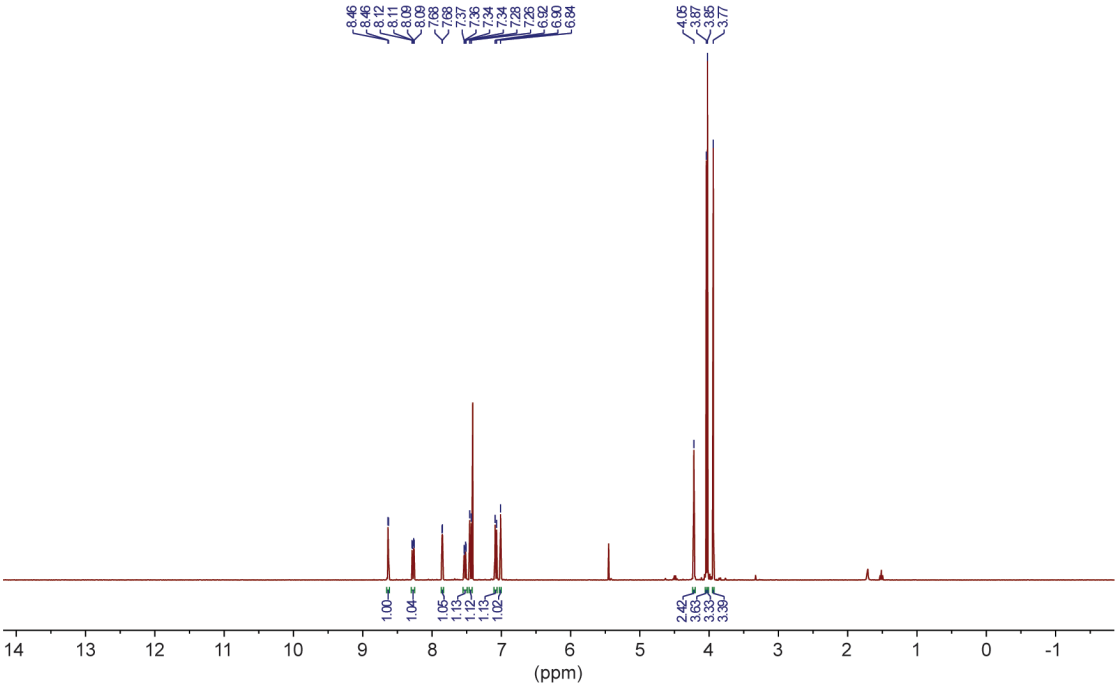

Fig. S31: ^{1}H NMR spectrum for compound **17** in $CDCl_3$ (400 MHz).

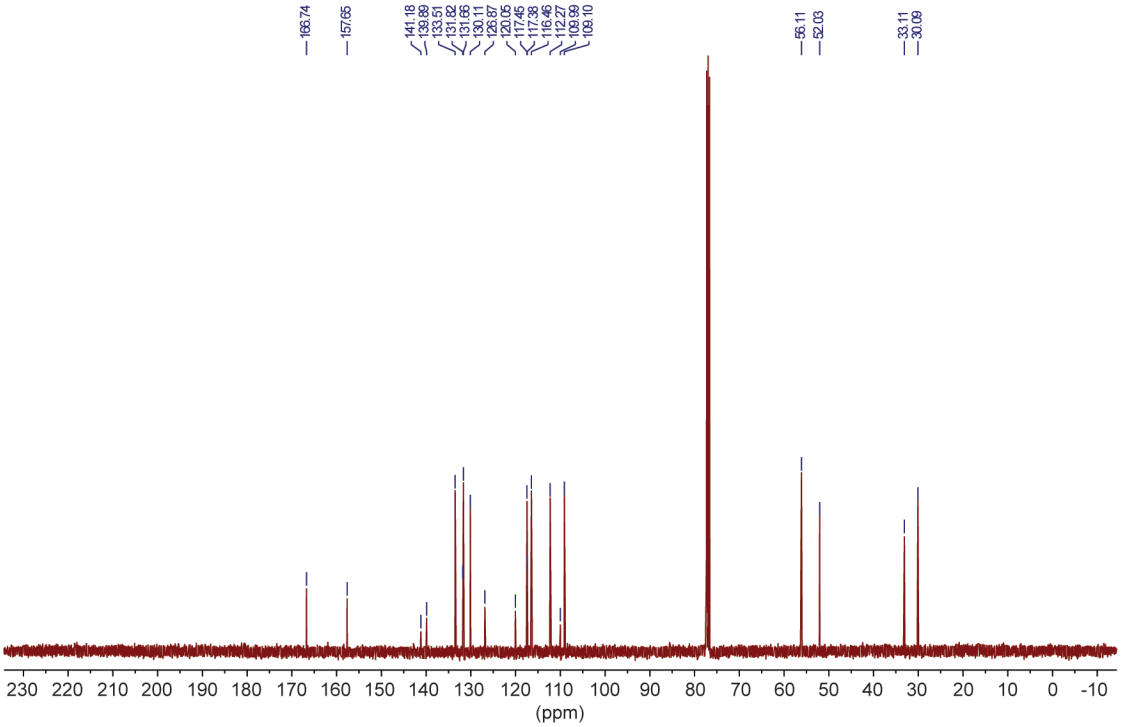

Fig. S32: ^{13}C NMR spectrum for compound **17** in $CDCl_3$ (100 MHz).

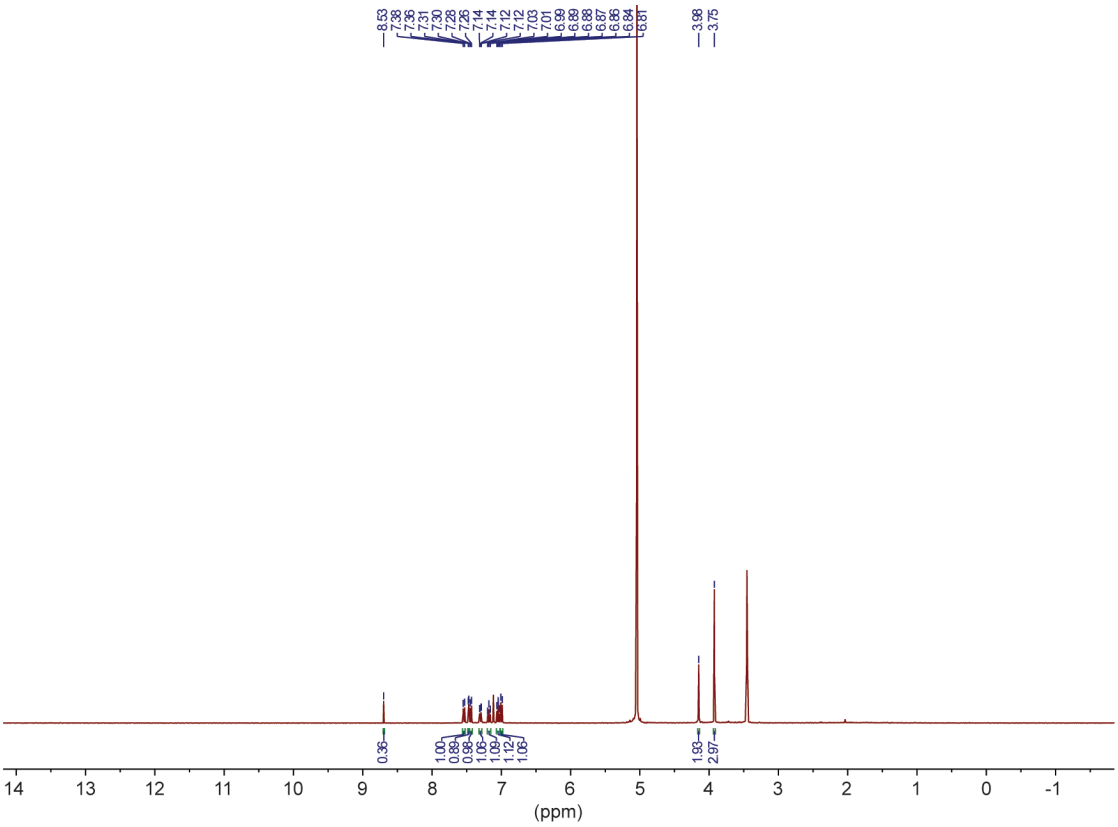

Fig. S33: ^{1}H NMR spectrum for compound **18** in CD_3OD (400 MHz).

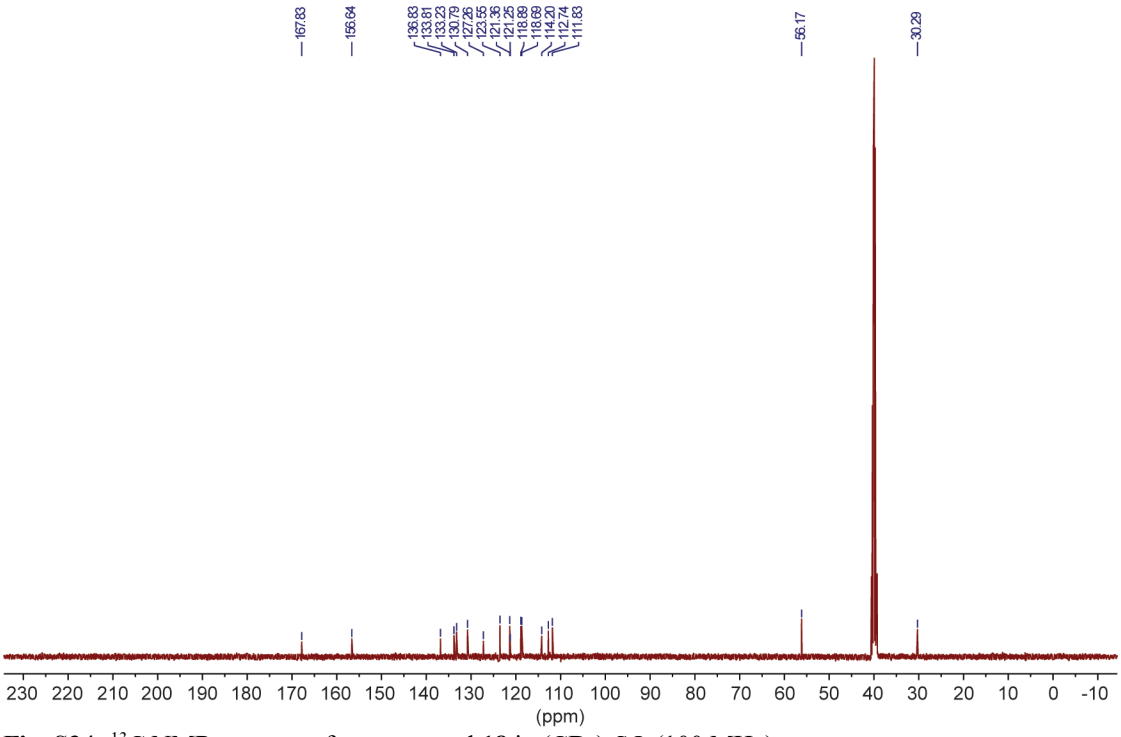

Fig. S34: ^{13}C NMR spectrum for compound **18** in $(CD_3)_2SO$ (100 MHz).

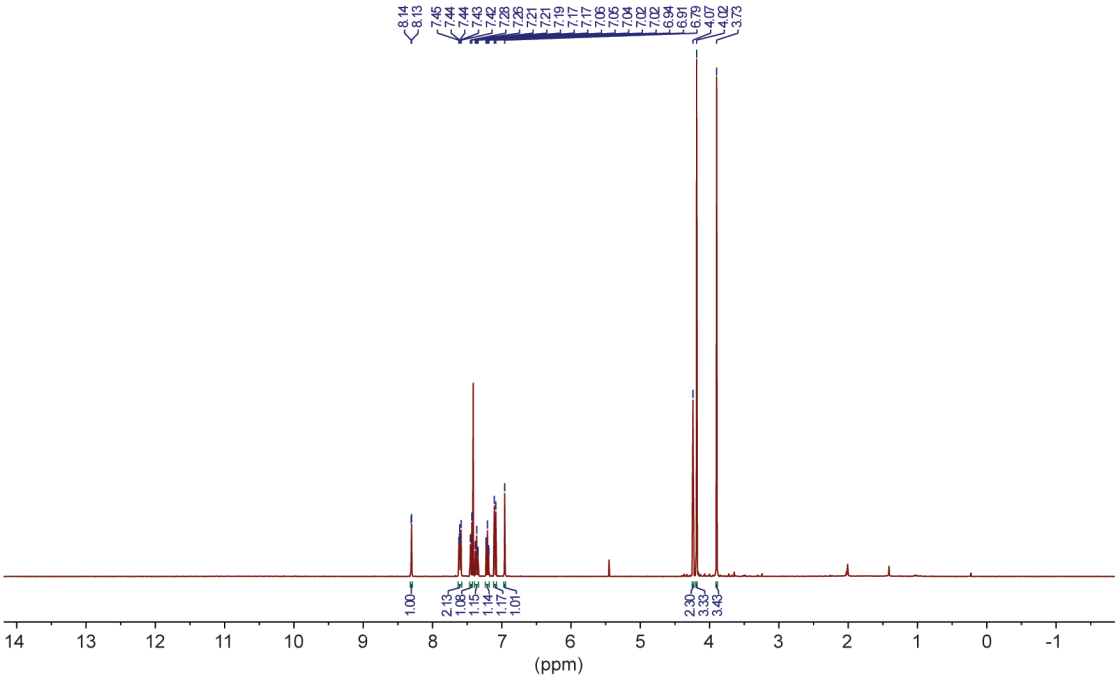

Fig. S35: ^{1}H NMR spectrum for compound **19** in $CDCl_3$ (400 MHz).

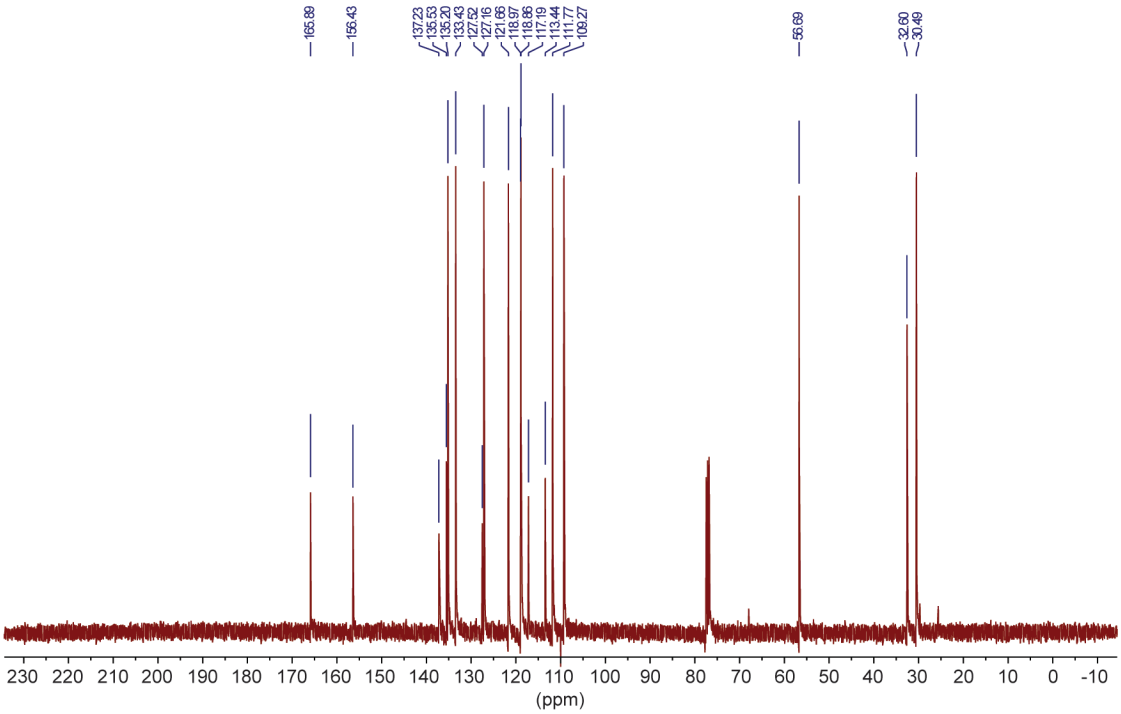

Fig. S36: ^{13}C NMR spectrum for compound **19** in $CDCl_3$ (100 MHz).

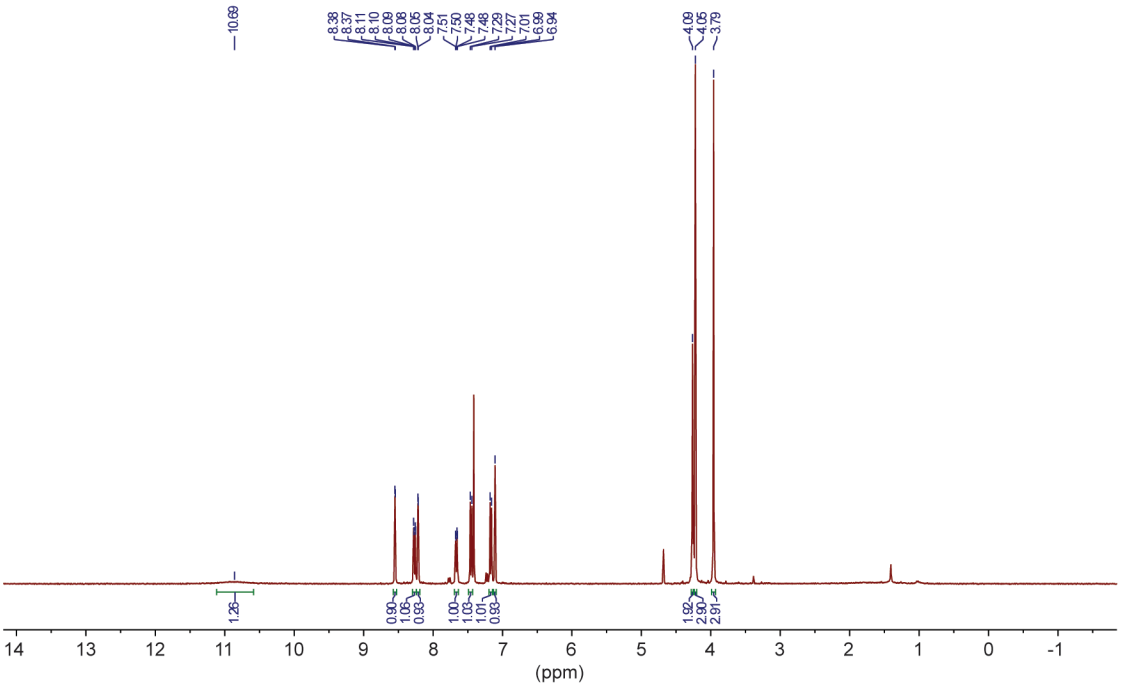

Fig. S37: ^{1}H NMR spectrum for compound **20** in $CDCl_3$ (400 MHz).

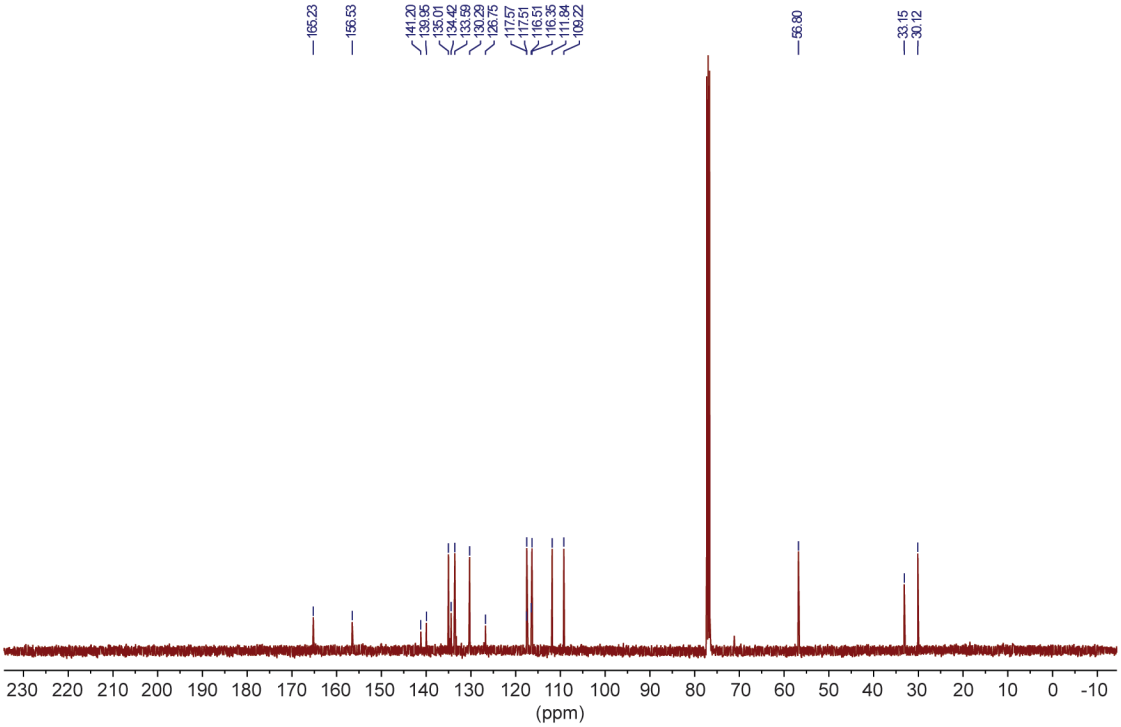

Fig. S38: ^{13}C NMR spectrum for compound **20** in $CDCl_3$ (100 MHz).

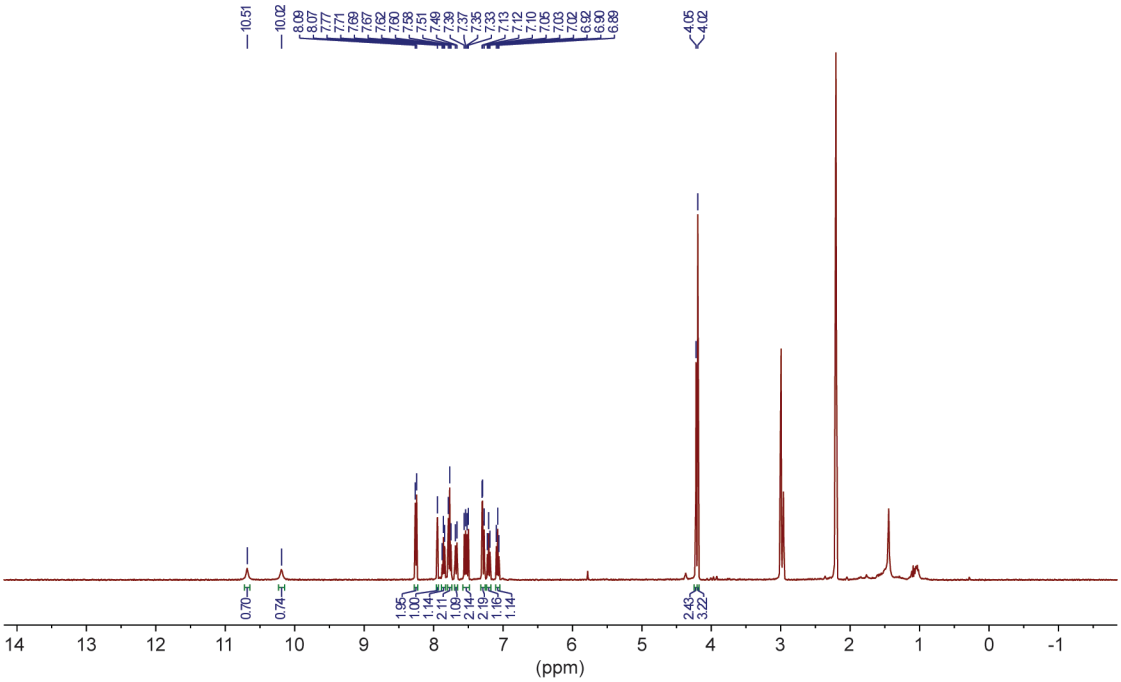

Fig. S39: ^{1}H NMR spectrum for compound **21a** in $CDCl_3$ (400 MHz).

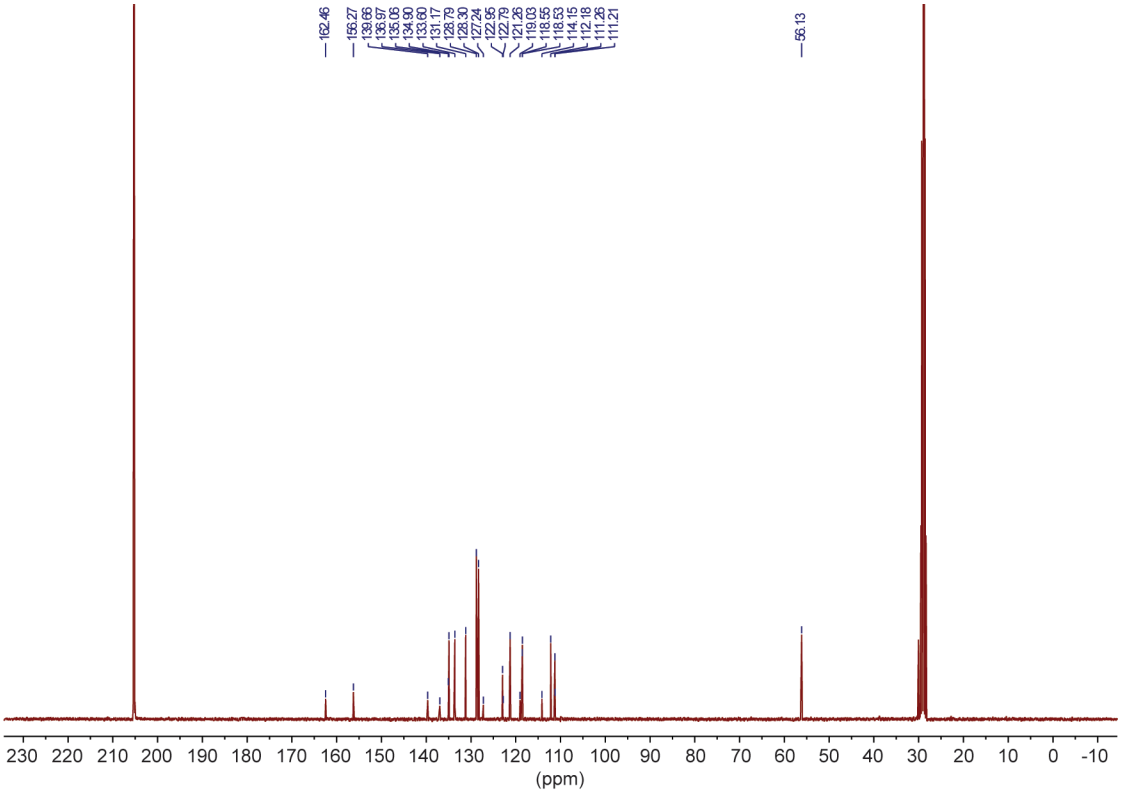

Fig. S40: ^{13}C NMR spectrum for compound **21a** in $(CD_3)_2CO$ (100 MHz).

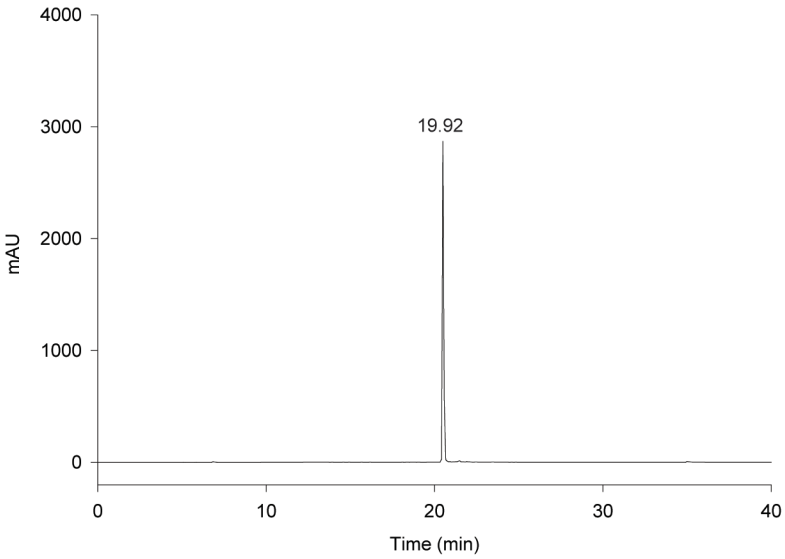

Fig. S41: HPLC trace for compound **21a** (R_t = 19.92 min).

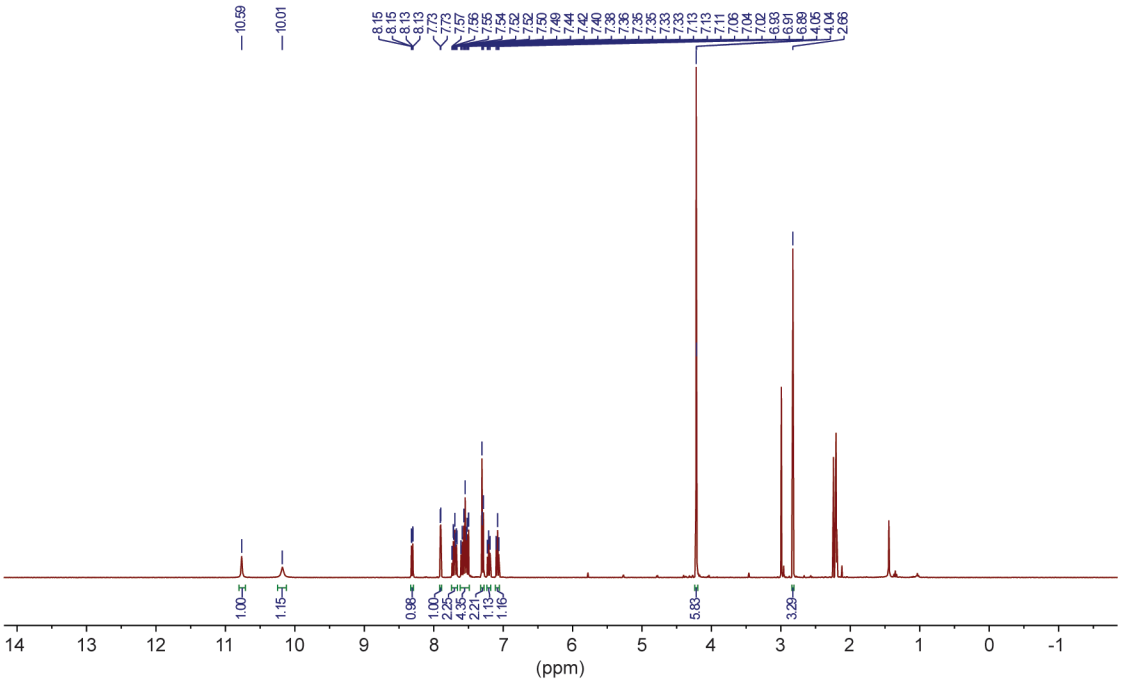

Fig. S42: ^{1}H NMR spectrum for compound **21b** in $(CD_3)_2CO$ (400 MHz).

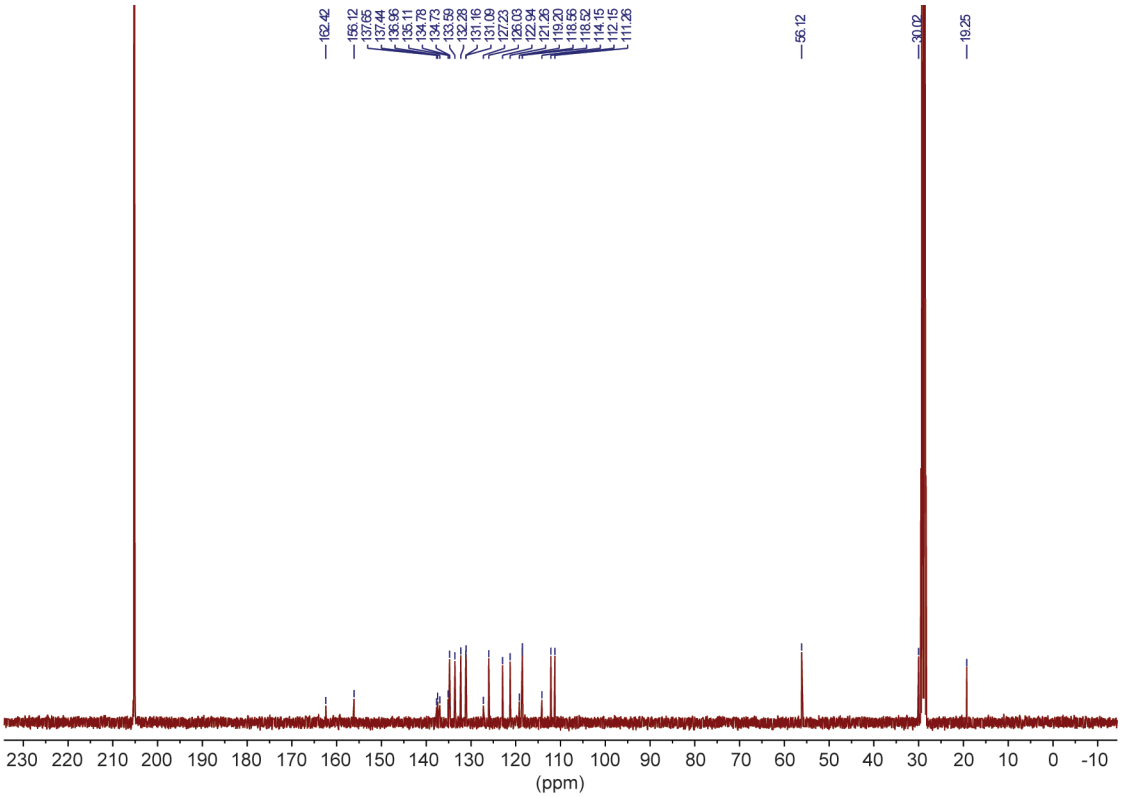

Fig. S43: ^{13}C NMR spectrum for compound **21b** in $(CD_3)_2CO$ (100 MHz).

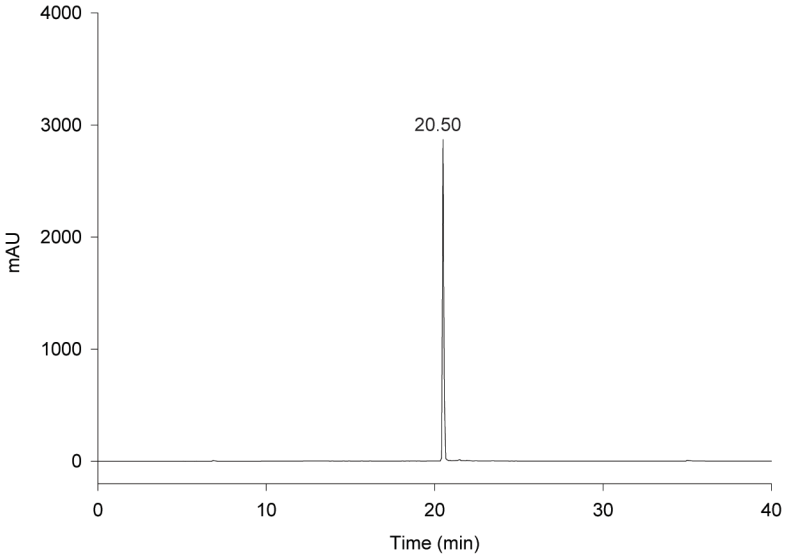

Fig. S44: HPLC trace for compound **21b** (R_t = 20.50 min).

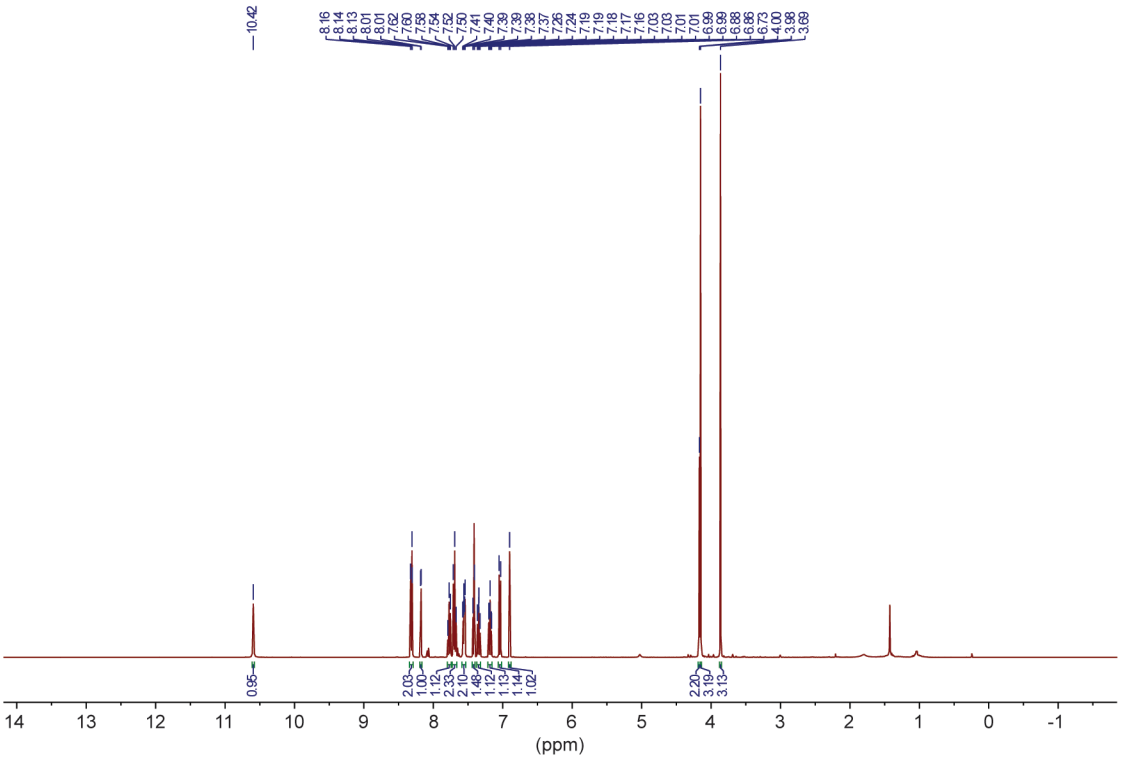

Fig. S45: ^{1}H NMR spectrum for compound **22a** in $CDCl_3$ (400 MHz).

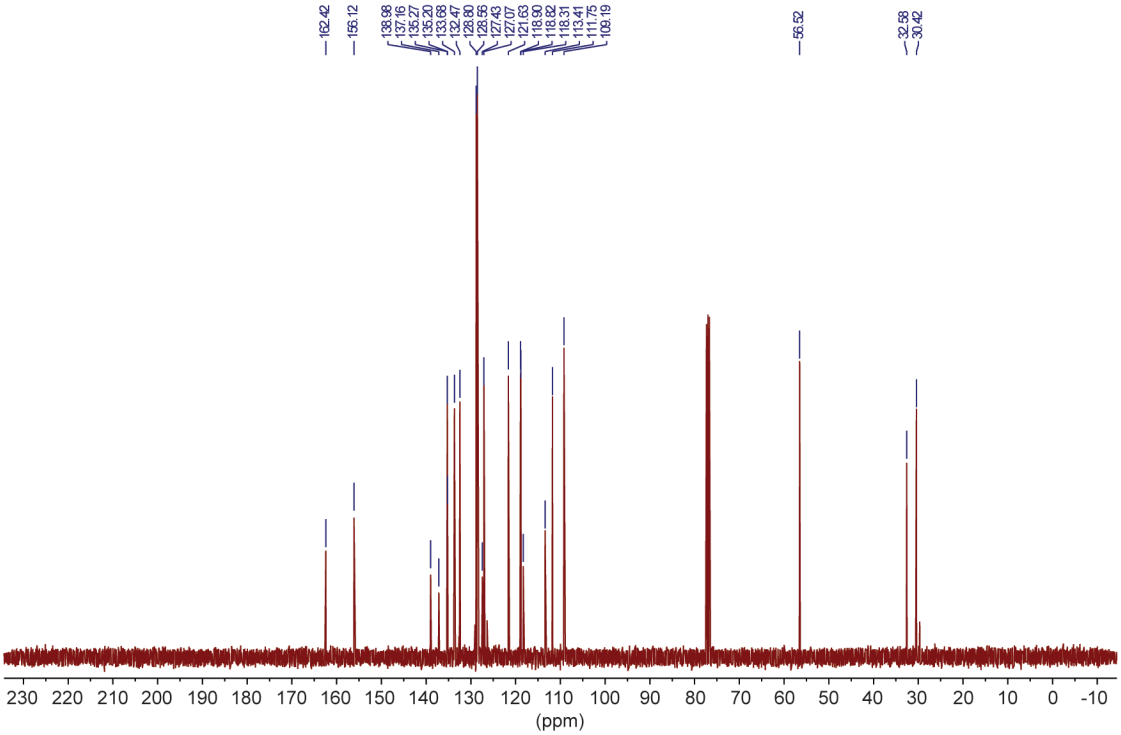

Fig. S46: ^{13}C NMR spectrum for compound **22a** in $CDCl_3$ (100 MHz).

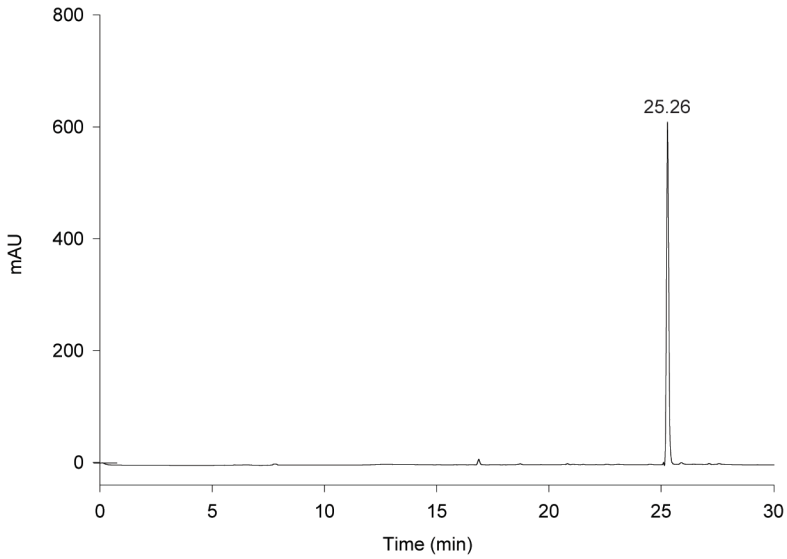

Fig. S47: HPLC trace for compound **22a** (R_t = 25.26 min).

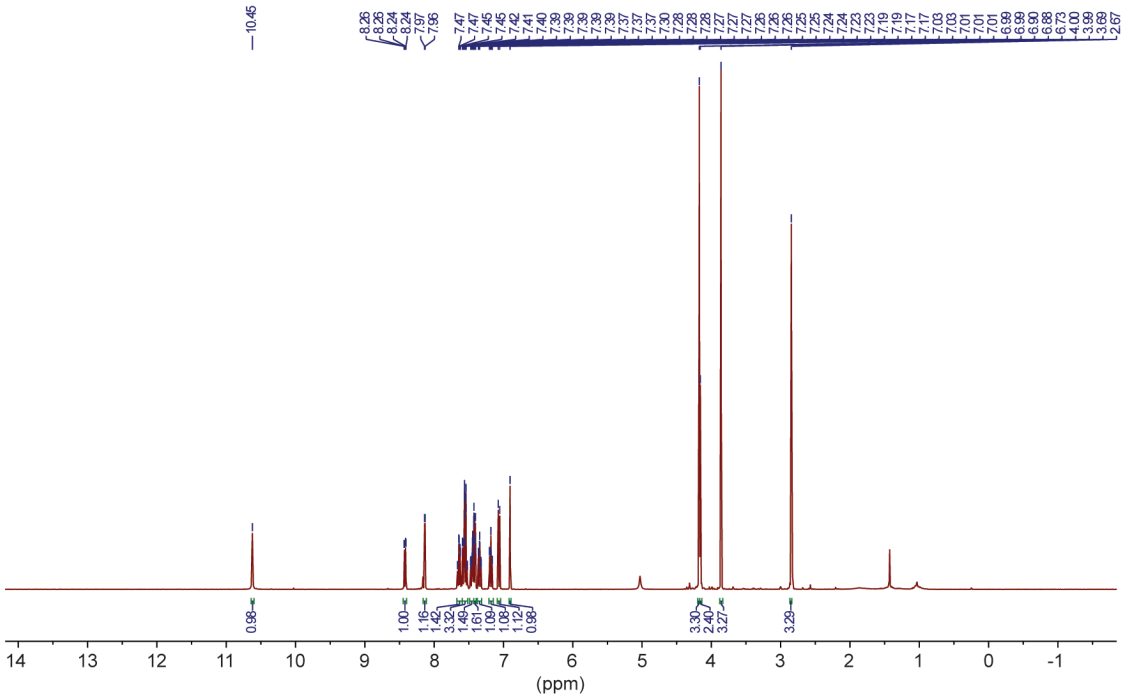

Fig. S48: ^{1}H NMR spectrum for compound **22b** in $CDCl_3$ (400 MHz).

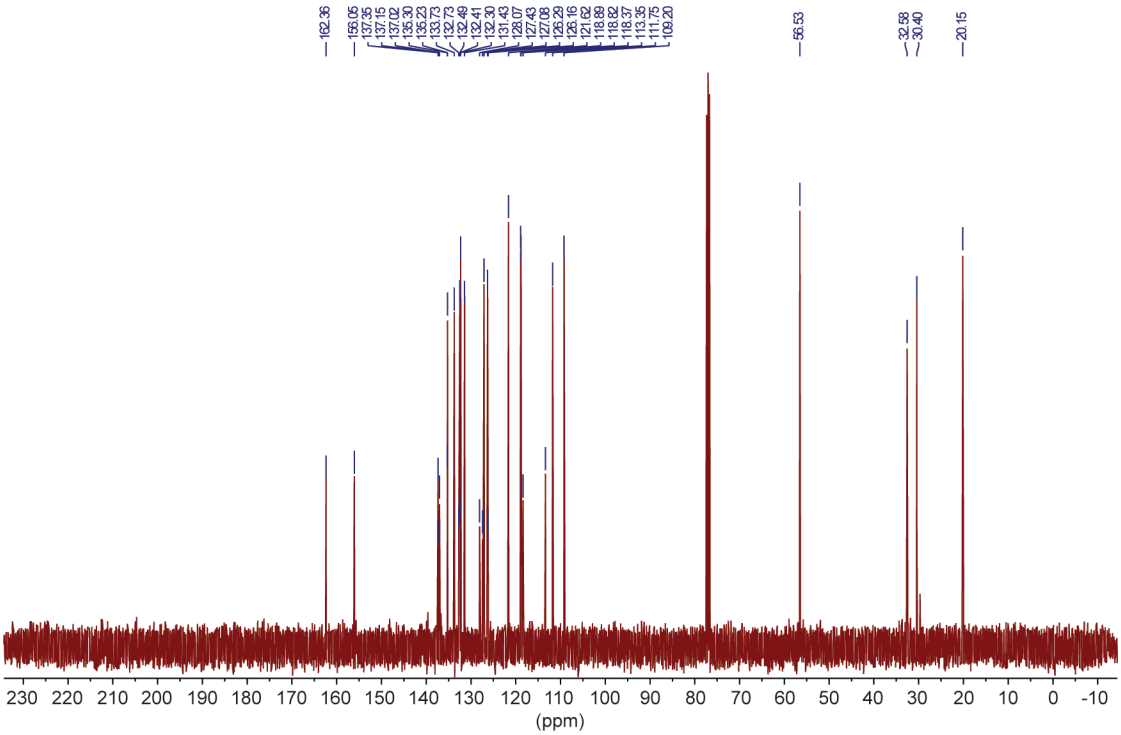

Fig. S49: ^{13}C NMR spectrum for compound **22b** in $CDCl_3$ (100 MHz).

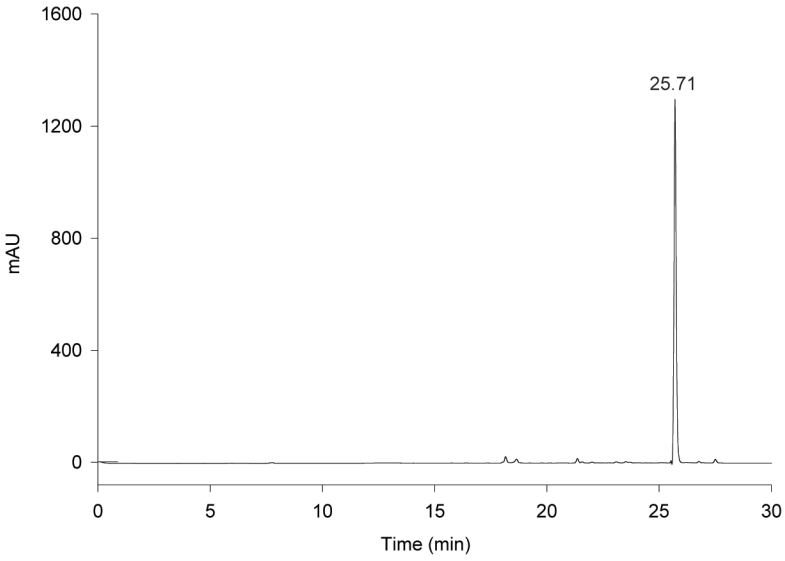

Fig. S50: HPLC trace for compound **22b** (R_t = 25.71 min).

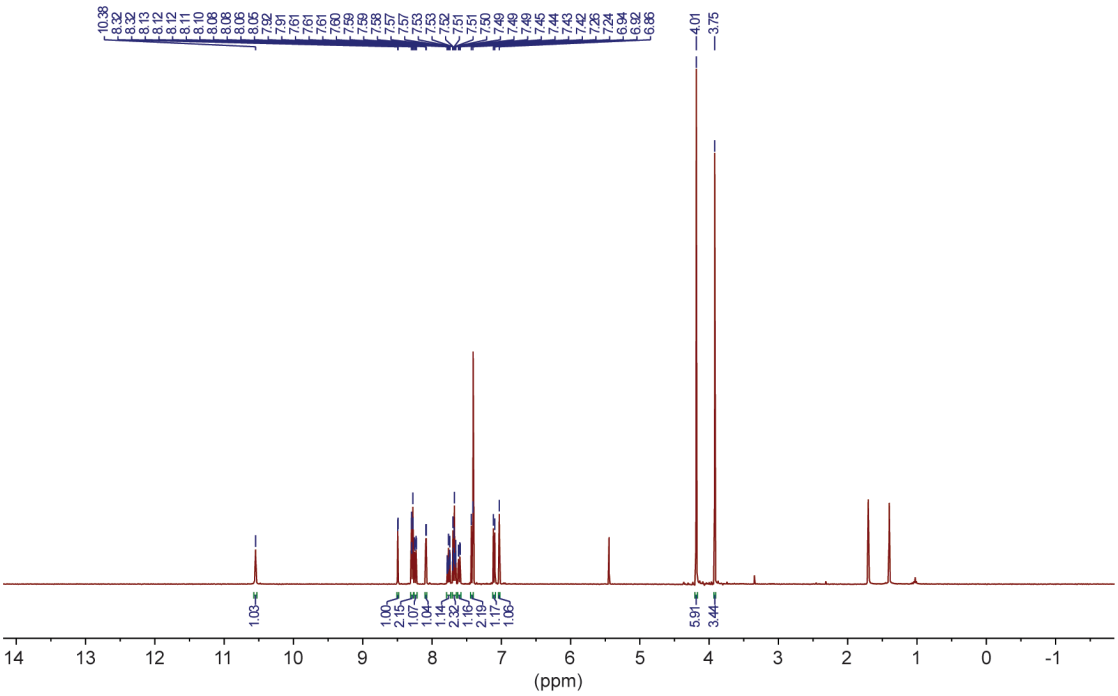

Fig. S51: ^{1}H NMR spectrum for compound **23a** in $CDCl_3$ (400 MHz).

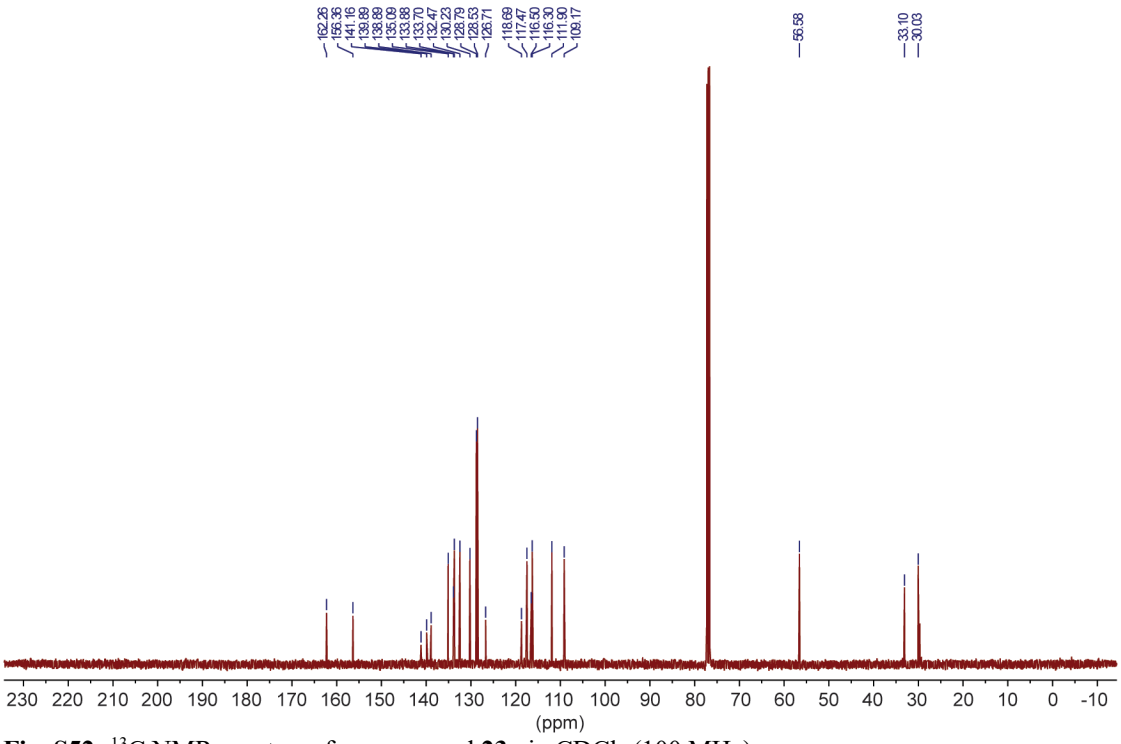

Fig. S52: ^{13}C NMR spectrum for compound **23a** in $CDCl_3$ (100 MHz).

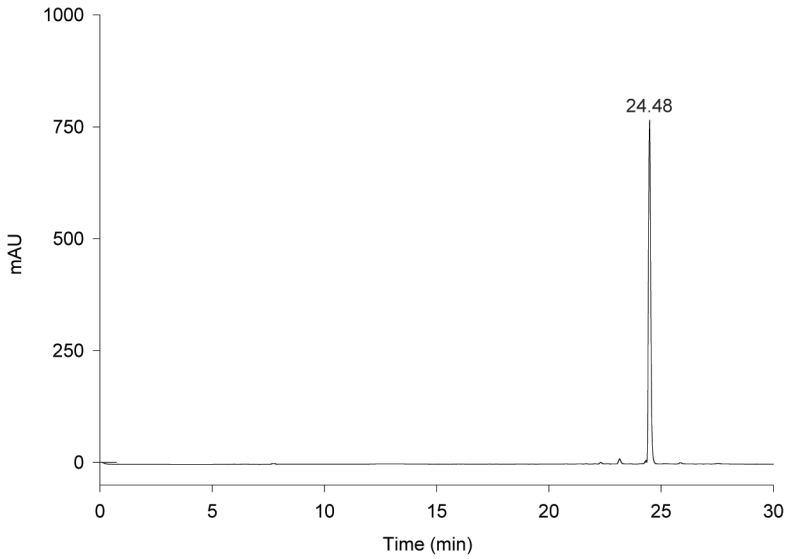

Fig. S53: HPLC trace for compound **23a** (R_t = 24.48 min).

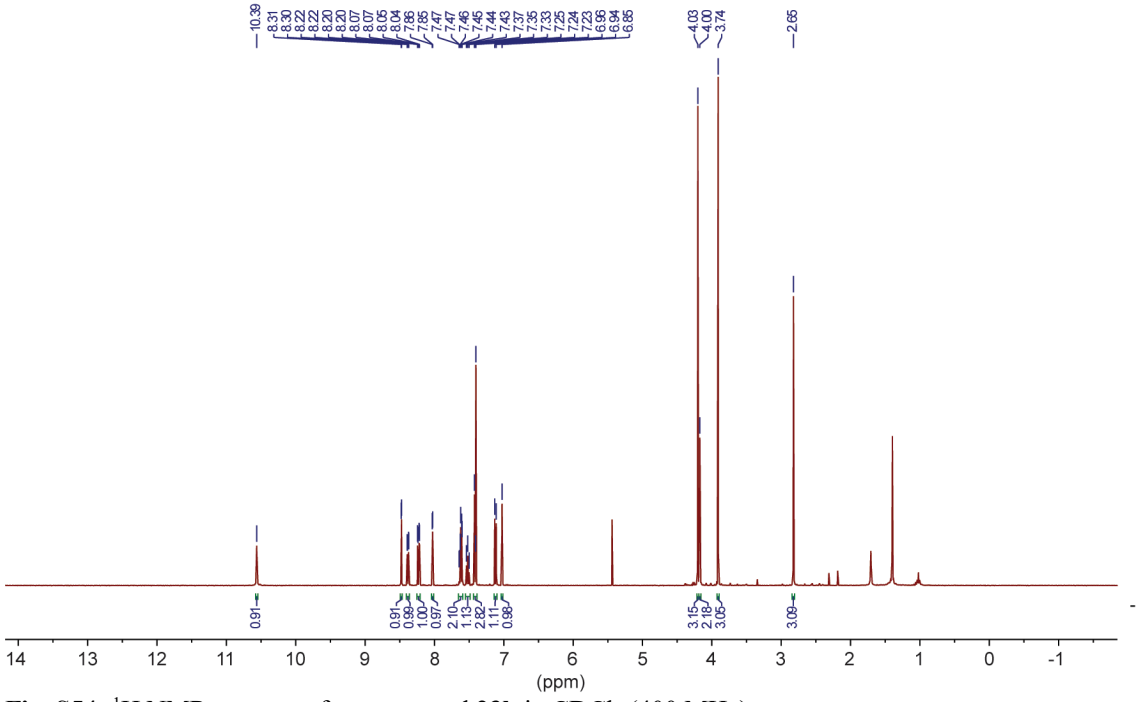

Fig. S54: ^{1}H NMR spectrum for compound **23b** in $CDCl_3$ (400 MHz).

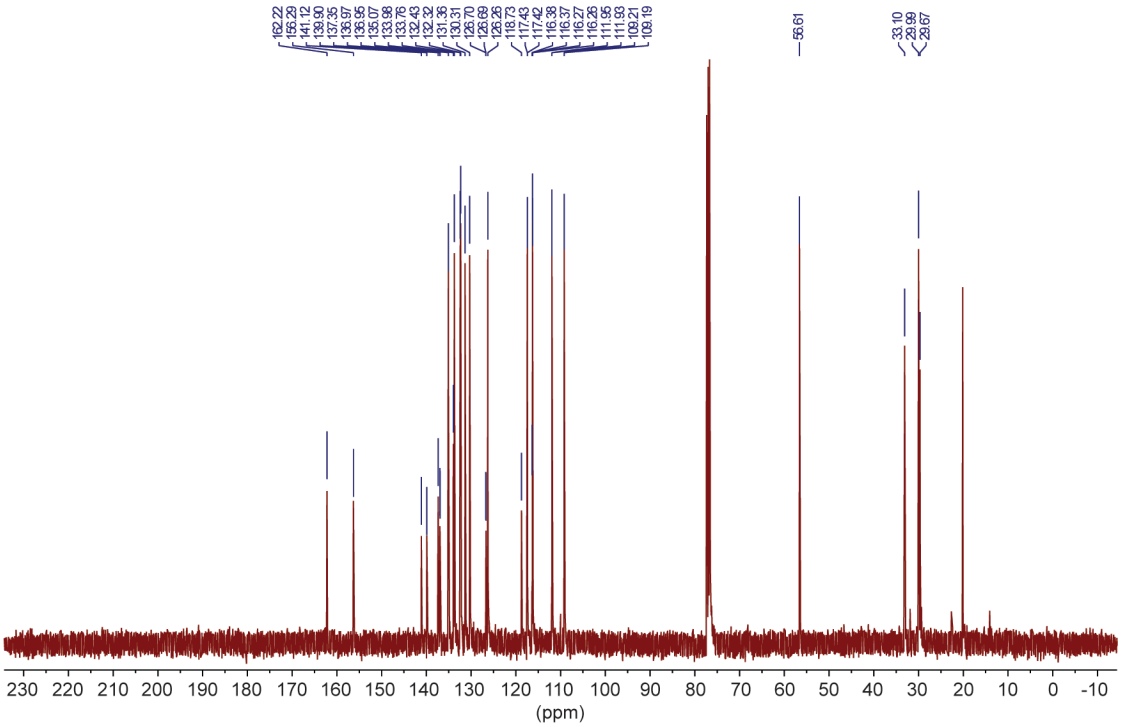

Fig. S55: ^{13}C NMR spectrum for compound **23b** in $CDCl_3$ (100 MHz).

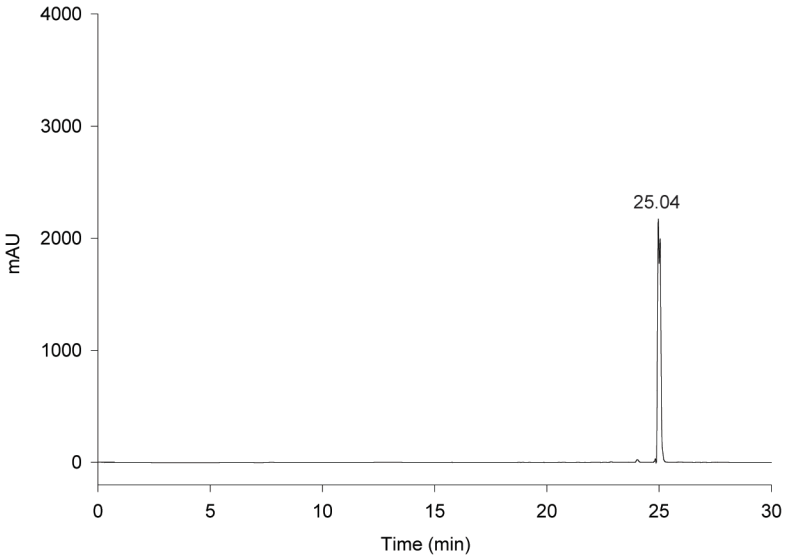

Fig. S56: HPLC trace for compound **23b** (R_t = 25.04 min).

Table S1: Percentage of OKF6 oral epithelial cells alive and dead after 24 h of exposure to compounds **22b**, **23a**, and **23b**.[a]		
Cpd and concentration used	**Alive (%)**	**Dead (%)**
Medium	83.0 ± 2.6	17.0 ± 2.6
DMSO	85.3 ± 4.6	14.8 ± 4.6
Zafirlukast 25 μM	17.3 ± 5.2	82.8 ± 5.2
22b 1 μM	91.5 ± 1.7	8.5 ± 1.7
22b 10 μM	40.8 ± 16.6	59.3 ± 16.6
23a 1 μM	79.5 ± 16.4	20.5 ±16.4
23a 10 μM	73.8 ± 4.1	26.3 ± 4.1
23b 1 μM	82.8 ± 9.5	17.3 ± 9.5
23b 10 μM	48.3 ± 17.5	51.8 ± 17.5
[a] The values presented were used to generate Fig. 5.		

Table S2: Percentage of OKF6 oral epithelial cells alive after 24 h of exposure to compounds **22b**, **23a**, and **23b**.[a]			
Cpd and concentration used	**22b**	**23a**	**23b**
Medium	78.7 ± 4.0	86.0 ± 7.0	93.3 ± 1.2
Zafirlukast 25 μM	5.3 ± 3.1	11.7 ± 10.8	9.3 ± 1.5
0 μM	77.7 ± 17.5	70.3 ± 12.5	89.3 ± 5.5
1 μM	69.3 ± 9.6	68.0 ± 5.0	83.7 ± 9.1
2.5 μM	41.0 ± 1.0	70.7 ± 10.3	87.7 ± 3.8
5 μM	66.0 ± 5.6	55.3 ± 8.1	87.0 ± 2.6
10 μM	38.3 ± 4.9	49.0 ± 16.1	83.0 ± 5.3
15 μM	41.0 ± 17.3	50.3 ± 9.5	79.0 ± 5.3
20 μM	22.7 ± 11.7	48.0 ± 6.1	75.3 ± 3.8
25 μM	47.0 ± 20.0	54.0 ± 7.8	77.0 ± 2.6
50 μM	33.0 ± 15.1	45.3 ± 13.1	69.0 ± 7.5
100 μM	12.3 ± 11.0	15.7 ± 7.1	58.7 ± 4.2
[a] The values presented were used to generate Fig. 6.			

Table S3: Percentage of OKF6 oral epithelial cells alive and dead (apoptosis and necrosis) after 24 h of exposure to compounds **22b**, **23a**, and **23b**.[a]			
Cpd and concentration used	**Viable**	**Apoptosis (%)**	**Necrosis (%)**
DMSO	85.9 ± 2.2	10.3 ± 6.9	3.73 ± 5.92
Zafirlukast 25 μM	8.53 ± 3.08	90.8 ± 2.5	0.65 ± 0.58
22b 1 μM	74.1 ± 18.1	23.7 ± 16.6	2.16 ± 1.62
22b 10 μM	69.1 ± 18.9	29.6 ± 19.1	1.37 ± 0.39
23a 1 μM	60.9 ± 27.4	38.5 ± 27.5	0.69 ± 0.46
23a 10 μM	53.2 ± 29.0	44.2 ± 26.9	2.81 ± 2.31
23b 1 μM	74.4 ± 13.8	24.8 ± 13.9	1.08 ± 0.17
23b 10 μM	61.7 ± 22.2	35.8 ± 21.3	2.23 ± 1.18
[a] The values presented were used to generate Fig. 7.			