Supporting Information

Synthesis and Structural Characterization of Novel Trihalo-sulfone Inhibitors of WNK1

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1. General Chemical Methods

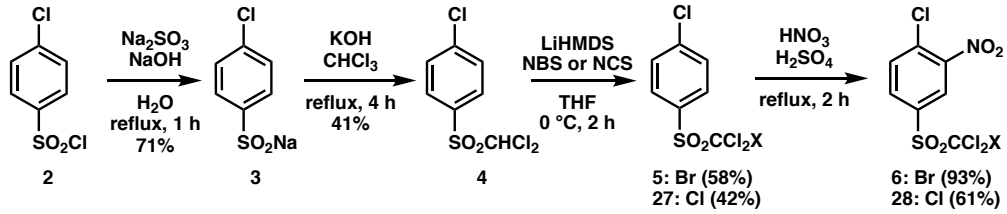
All NMR data was recorded on Agilent 400-MR DD2 or Bruker AVANCE NEO Nano 400 NMR spectrometers and processed via TopSpin 3.2 software. Data for ^{1}H-NMR and spectra are reported relative to deuterated chloroform or methanol as an internal standard (7.26 ppm and 3.31 ppm respectively) and are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Data for ^{13}C-NMR spectra are reported relative to chloroform or methanol as an internal standard (77.2 ppm and 49.00 ppm respectively) and are reported in terms of chemical shift (δ ppm). For reference the following abbreviations were used: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublets, brm = broad multiplet, brs = broad singlet. The following solvents and reagents have been abbreviated: dichloromethane (DCM), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), chloroform (CHCl3), and dimethylformamide (DMF). All reactions were monitored by thin-layer chromatography with E. Merck silica gel 60 F254 pre-coated plates (0.25 mm). All column chromatography was carried out with silica gel (particle size 0.032 - 0.063 mm) purchased from SiliCycle and Preparatory TLC purification performed using E. Merck silica gel 60 F254 pre-coated plates (0.5 mm). Solvents were dried by passage through an activated alumina column under argon and were evaporated using a standard Heidolph rotovapor and high vacuum. Commercially obtained reagents were used as received. Liquids and solutions were transferred via syringe. All non-aqueous reactions were performed in ovendried glassware and under argon atmosphere unless otherwise stated. Reactions involving lower temperatures were cooled to 0 °C under an ice bath. Reactions carried out at room temperature were performed at 20–25 °C. Reactions involving higher temperatures were heated using a Heidolph MR Hei-Tec magnetic stirrer/heat plate. X-ray diffraction structural data was obtained by Dr. Vincent Lynch at the X-ray Diffraction Lab at The University of Texas at Austin.

All mass spectrometry samples and HRMS spectra were acquired via high resolution electrospray (ESI) technique using a SciexTriple Quad 6500+ System At UT Southwestern.

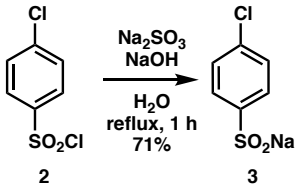
Safety Statement: No unexpected or unusually high safety hazards were encountered during the research. All scientists at UT Southwestern are properly trained in the most essential laboratory safety practices.

2. Synthesis and Characterization of Precursors

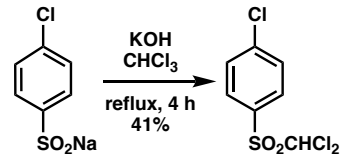
Following a modified reported procedure,[1] the synthetic sequence was executed as follows:



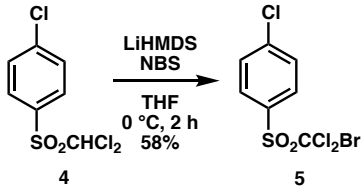
Synthesis of sodium 4-chlorobenzenesulfinate **3**:



Sulfonyl chloride **2** (4.5378g, 21.5 mmol, 1 equiv) was added portionwise to a solution of Na_2SO_3 (1.89 g, 15 mmol, 0.7 equiv) in water (8 mL). The mixture was stirred at 90 ˚C for 1 h, while adding a 50% NaOH (w/v) aqueous solution to maintain pH= 8-9 (tested with pH paper). Upon completion, the ***hot*** mixture was filtered and the filtrate was allowed to cool to room temperature. Crystal formation began immediately. The white crystalline product was washed with ***cold*** water and dried under vacuum at room temperature. Product **3** was obtained in 71% yield (3.06 g). Spectral data matched the reported literature values.

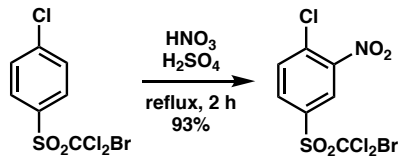


A solution of KOH (0.672 g, 12 mmol, 2 equiv) in water (4.2 mL) was added to a suspension of **3** (1.2 g, 6 mmol, 1 equiv) in chloroform (6 mL). The reaction mixture was heated to reflux and intensely stirred for 4 h. After cooling, the organic layer was separated and the water phase was extracted with chloroform (x 3). The combined organic phase was washed with water, dried over anhydrous Na_2SO_4, filtered, and the solvent was removed in a rotary evaporator. The product **4** was purified via column chromatography using a 10% EtOAC/hexanes eluent (prod R_f 0.4) and obtained as a white crystallic substance (640 mg, 41% yield). Spectral data matched the reported literature values. **^{1}H NMR** (400MHz, $CDCl_3$) δ: 7.98-7.96 (d, *J*=8.8 Hz, 2H), 7.62-7.60 (d, *J*=8.8 Hz, 2H), 6.27 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 142.81, 132.65, 130.35, 129.72, 79.79



To a flame dried, argon cooled flask equipped with a stirbar, the solids: sulfone **4** (640 mg, 2.48 mmol, 1 equiv) and NBS (2.195g, 12.40 mmol, 5 equiv) were added. The flask was evacuated and refilled 3 times to maintain inert atmosphere. Dry THF (9.33ml) was added and the reaction mixture was cooled to 0 ˚C under an ice bath. Upon cooling, LiHMDS (12.4ml) was added ***slowly*** via syringe under a protected argon atmosphere. The reaction was stirred at 0 ˚C for 2 h. Upon completion, the reaction was quenched using $NH_4^+Cl^-$ and extracted with EtOAc (x 3). The organic phase was dried using Na_2SO_4, filtered, and concentrated under vacuum. The oily dark brown

residue was purified using 5% EtOAc/hexanes to afford the product **5** as a white crystal (486.78mg, 58%). Spectral data matched the reported literature values. **^{1}H NMR** (400MHz, $CDCl_3$) δ: 8.11-8.07 (m, 2H), 7.64 – 7.60 (m, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 143.28, 134.26, 129.58, 127.65, 88.15.



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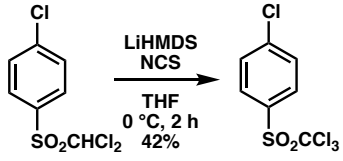
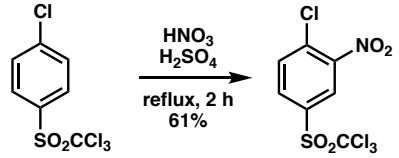
Sulfone **5** (288 mg, 0.8510 mmol) was dissolved in concentrated sulfuric acid 1.07 mL). Concentrated nitric acid (44.5µL, 1.06 mmol HNO_3) was added slowly to avoid violent fumes. When the addition was completed, the mixture was heated at 80°C for 2 h. After the 2 h, crushed ice was poured into the hot mixture. The precipitate was filtered off, washed with water, and dried under vacuum. The product nitrophenyl sulfone **6** was obtained as a white solid (301.4 mg, 93%). Spectral data matched the reported literature values. **^{1}H NMR,** 400MHz, ($CDCl_3$) δ: 8.64-8.63 (d, *J*=1.2 Hz, 1H), 8.29-8.27 (dd, *J*=1.6 Hz, 1H) 7.87- 7.85 (d, *J*=5.6 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ: 147.84, 136.40, 135.70, 135.05, 129.92, 129.66, 87.31.



4 27

To a flame dried, argon cooled flask equipped with a stirbar, the solids: sulfone **4** (60 mg, 0.2325 mmol, 1 equiv) and NCS (178.51, 1.1627 mmol, 5 equiv) were added. The flask was evacuated and refilled 3 times to maintain inert atmosphere. Dry THF (0.930 ml) was added and the reaction mixture was cooled to 0 ˚C under an ice bath. Upon cooling, LiHMDS (0.1946 ml) was added

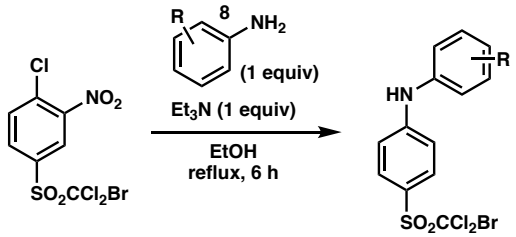
slowly via syringe under a protected argon atmosphere. The reaction was stirred at 0 ˚C for 2 h. Upon completion, the reaction was quenched using $NH_4^+Cl^-$ and extracted with EtOAc (x 3). The organic phase was dried using Na_2SO_4, filtered, and concentrated under vacuum. The oily dark brown residue was purified using 5% EtOAc/hexanes to afford the product **27** as a white crystal (32.6 mg, 48%).



27 28

Sulfone **27** was converted to nitrophenyl sulfone **28** using the same procedure employed for the conversion of sulfone **5** was converted to nitrophenyl sulfone **6**.

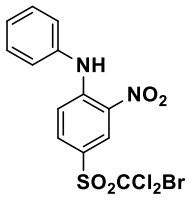
3. Synthesis and Characterization of Inhibitor with Aromatic Amine



6 21

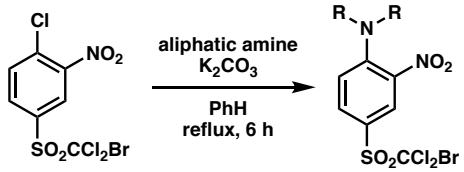
Following a modified literature procedure, to a reaction vial equipped with a stirbar, chlorinated nitrosulfone **6** (1 equiv), aromatic amine (1 equiv) and Et_3N (1 equiv) were added and further dissolved in ethanol (0.4 M). The resulting bright-yellow mixture was vigorously refluxed for 6 h. Upon completion, the reaction mixture was allowed to cool to room temperature and the EtOH was removed via rotary evaporation. The solids were dissolved in CH_2Cl_2, and the mixture was washed with a 5% solution of HCl, water, sodium bicarbonate, and water. The organic layer

was dried over sodium sulfate, filtered, and concentrated under vacuum. The products were purified via preparatory thin layer chromatography (Prep-TLC) using 30% EtOAc/hexanes as eluent.



21: (18.4 mg, 47%). **TLC** R_f = 0.6 (hexanes/EtOAc = 7:3, v/v). **^{1}H-NMR** (600MHz), $CDCl_3$, δ: 10.06 (s, 1H), 9.00-8.98 (dd, *J*=8.4, 2.4 Hz, 1H), 7.95-7.91 (m, 1H), 7.53-7.51 (t, *J*=7.8 Hz, 2H), 7.41-7.39 (t, *J*=7.8 Hz, 1H), 7.33-7.32 (m, 2H), 7.22-7.20 (m, 1H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 147.95, 137.73, 137.51, 136.35, 133.71, 133.54, 130.34, 128.04,128.02, 125.71, 116.08, 88.45. ESI-HRMS calcd for $[C_{13}H_9BrCl_2N_2O_4S + H]^+$ 438.8916, found 438.8920

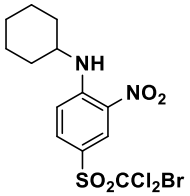
4. Synthesis and Characterization of Inhibitors with Aliphatic Amines



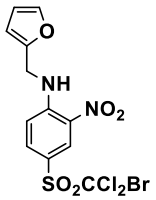
6 7

Following a modified literature procedure,[1] to a reaction vial equipped with a stirbar, chlorinated nitrosulfone **6** (1 equiv), aliphatic amine (1 equiv) and K_2CO_3 (1 equiv) were added and further dissolved in benzene (0.4 M). The bright-yellow mixture was vigorously refluxed for 6 h. Upon completion the reaction mixture was allowed to cool to room temperature and the benzene was removed via rotary evaporation. The solids were dissolved in EtOAc, and the mixture was washed with a 5% solution of HCl, water, sodium bicarbonate, and water. The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum. The products were

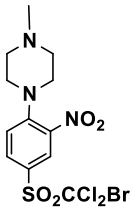
purified via preparatory thin layer chromatography (Prep-TLC) using 30% EtOAc/hexanes as eluent.



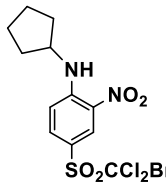
1: (39 mg, 68%). **TLC** R_f = 0.4 (hexanes/EtOAc = 7:3, v/v). **^{1}H NMR** (400MHz), $CDCl_3$, δ: 8.95-8.93(dd, *J*=7.5, 2.3 Hz, 1H), 8.69-8.67 (d, *J* = 7.2 Hz, 1H), 8.01-7.98 (m, 1H), 7.02-6.99 (dd, *J*=7.6 ,2 Hz, 1H), 3.62-3.61 (t, *J* = 3.2 Hz, 1H), 2.10-2.07 (brm, 2H), 1.87-1.84 (brm, 2H), 1.72-1.69 (brm, 1H), 1.498-1.41 (m, 5H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 148.05, 137.79, 134.22, 131.10, 131.05, 114.35, 112.96, 88.68, 51.93, 32.38, 25.27, 24.36. ESI-HRMS calcd for $[C_{13}H_{15}BrCl_2N_2O_4S+ H]^+$ 444.9386, found 444.9387.



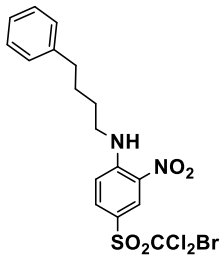
22: (26 mg, 56 %). **TLC** R_f = 0.4 (hexanes/EtOAc = 7:3, v/v). **^{1}H NMR** (400MHz), $CDCl_3$, δ: 8.97-8.96 (d, *J* = 2.4 Hz, 1H), 8.94 (brs, 1H), 8.10-8.05 (ddt, *J*=7.08, 1.72, 0.52 Hz, 1H), 7.43-7.42 (dd, *J*=0.96, 0.8 Hz, 1H), 7.17-7.14 (dd, *J*=6.84, 2.4 Hz, 1H), 6.40-6.36 (m, 2H), 4.64-4.62 (d, *J*=5.64 Hz, 2H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 148.63, 143.21, 138.15, 133.67, 131.71, 115.11, 114.51, 110.76, 108.56, 88.52, 40.55. ESI-HRMS calcd for $[C_{12}H_9BrCl_2N_2O_5S+H]^+$ 442.8865, found 442.8854.



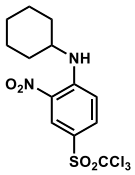
25: (42 mg, 72 %). **TLC** R_f = 0.3 (hexanes/EtOAc = 7:3, v/v). **^{1}H NMR** (400MHz), CD_3OD, δ: 8.60-8.58 (dd, *J*=7.2, 2.4 Hz, 1H), 8.26-8.22 (dt, *J*=6.8, 2 Hz, 1H), 7.60-7.57 (dd, *J*=6, 2.8, 1H), 3.63 (brs, 4H), 3.48 (brs, 4H), 2.99 (s, 3H). **^{13}C NMR** (150 MHz) CD_3OD, δ: 149.27, 139.40, 137.01, 131.92, 131.79, 121.18, 119.85, 87.25, 52.76, 42.31, 29.36. ESI-HRMS calcd for $[C_{12}H_{14}BrCl_2N_3O_4S+H]^+$ 445.9338, found 445.9341.



24: (30 mg, 61%). **TLC** R_f = 0.4 (hexanes/EtOAc = 7:3, v/v). **^{1}H-NMR** (400MHz), $CDCl_3$, δ: 8.95-8.93 (dd, *J*=5.6, 2 Hz, 1H), 8.67-8.66 (d, *J* = 5.2 Hz, 1H), 8.01-7.98 (dt, *J*=1.52, 0.8 Hz, 1H), 7.04-7.01 (d, *J* = 7.36, 1.96 Hz, 1H), 4.07-4.03 (m, 1H), 2.18-2.15 (m, 2H), 1.84-1.54 (m, 6H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 148.48, 137.75, 133.96, 131.21, 114.83, 113.16, 88.66, 54.75, 33.43, 24.03. ESI-HRMS calcd for $[C_{12}H_{13}BrCl_2N_2O_4S+H]^+$ 430.9229, found 430.9233.

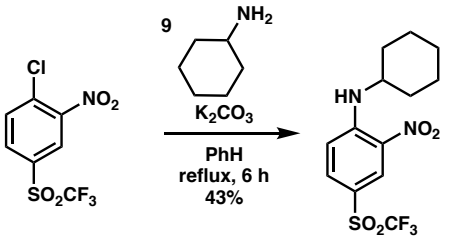


23: (44 mg, 68 %). **TLC** R_f = 0.5 (hexanes/EtOAc = 7:3, v/v). **^{1}H NMR** (600MHz), $CDCl_3$, δ: 8.94-8.93 (d, *J*=2.4 Hz, 1H), 8.62 (brs, 1H), 8.02-7.99 (dd, *J*=7.02, 2.2 Hz, 1H), 7.31-7.26 (t, *J*=7.2, 2H), 7.22-7.19 (t, *J*=5.4, 3H), 6.95-6.94 (d, *J*=9 Hz, 1H), 3.43-3.41 (q, *J*=6 Hz, 2H), 2.72-2.70 (t, *J*=6.6 Hz, 2H), 1.83-1.80 (m, 4H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 148.94, 141.34, 137.99, 137.97, 133.88, 133.84, 131.20, 128.54, 128.39, 126.17, 114.10, 113.39, 88.64, 43.43, 35.32, 28.51, 28.08. ESI-HRMS calcd for $[C_{17}H_{17}BrCl_2N_2O_4S+H]^+$ 494.9542, found 494.9548.



29: (8mg, 63%). **TLC** R_f = 0.4 (hexanes/EtOAc = 6:4, v/v). **^{1}H NMR** (600MHz), $CDCl_3$, δ: 8.94-8.93 (d, *J*=2.4 Hz, 1H), 8.68 (brs, 1H), 7.98-7.96 (m, 1H), 7.02-6.99 (d, *J*=9.6, 2H), 3.61 (t, *J*=3 Hz, 1H), 2.08 (s, 2H), 1.84 (s, 2H), 1.71-1.69 (d, *J*=12.6, 1H), 1.47-1.44 (t, *J*= 10.2, 5H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 148.09, 137.58, 134.06, 131.10, 114.44, 113.58, 104.66, 51.93, 32.37, 29.73, 25.27, 24.36. ESI-HRMS calcd for $[C_{13}H_{15}Cl_3N_2O_4S+ H]^+$ 400.9891, found 400.9893.

Alternate Synthetic Routes:



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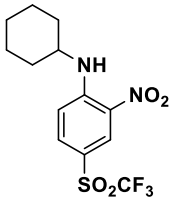
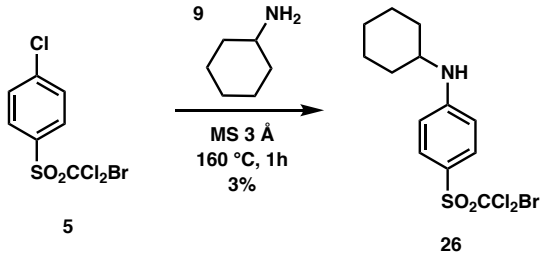


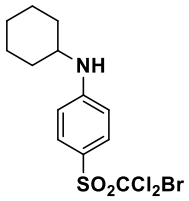
19: (25.6 mg, 43%). **TLC** R_f = 0.5 (hexanes/EtOAc = 5:1, v/v). **^{1}H NMR** (600MHz), $CDCl_3$, δ: 8.84 (d, *J*=1.8 Hz, 1H), 8.71-8.70 (d, *J*=6 Hz, 1H), 7.86-7.84 (dd, *J*=7.2, 1.8Hz, 1H), 7.05-7.03 (d, *J*=9.6 Hz, 1H), 3.62 (brs, 1H), 2.07 (s, 2H), 1.85-1.83 (d, *J*=4.2 Hz, 2H), 1.71-1.69 (d, *J*=12.6 Hz, 1H), 1.48-1.45 (t, *J*=11.4 Hz, 4H), 1.35 (brs, 1H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 148.39, 135.51, 132.50, 115.43, 51.97, 32.33, 25.24, 24.32. ESI-HRMS calcd for $[C_{13}H_{15}F_3N_2O_4S\text{-}H]^-$ 351.0632, found 351.0612



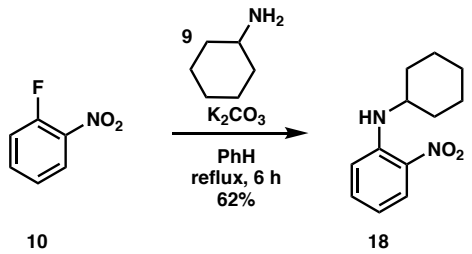
5 26

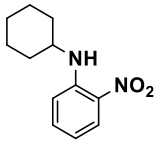
To a flame-dried and Argon cooled small vial equipped with a stirrer, sulfone **6** was added (100mg, 0.2954 mmol, 1 equiv) followed by addition of molecular sieves 3Å (20 mg). Lastly, cyclohexylamine (33.78µL, 0.2954mmol, 1 equiv) was added and the heterogeneous mixture was stirred at 150˚ C for 1 hour. After the first five minutes of stirring the heterogeneous mixture turned homogeneous. After 1 hour, the brown residue was re-suspended in DCM and purified via

preparatory thin layer chromatography (Prep-TLC) using 30% EtOAc/hexanes as eluent to reveal a clear oily residue of **26** in 3% yield.

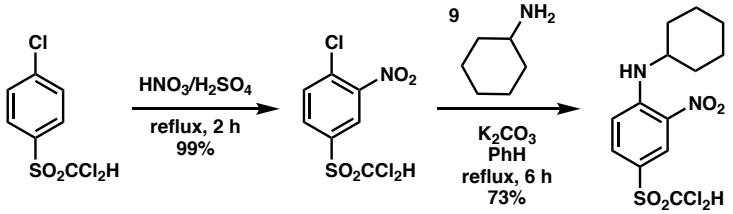


26: (3.9mg, 3.3%). **TLC** R_f = 0.2 (hexanes/EtOAc = 6:4, v/v). **^{1}H NMR** (400MHz), $CDCl_3$, δ: 7.80-7.79 (d, *J* = 6 Hz, 2H), 7.47-7.45 (d, *J* = 6 Hz, 2H), 4.39-4.38 (d, *J* = 5.2 Hz, 1H), 3.15-3.11 (m, 1H), 1.75-1.73 (m, 2H), 1.64-1.60 (m, 2H), 1.55-1.50 (m, 1H), 1.25-1.19 (m, 6H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 140.04, 138.89, 52.79, 34.02, 29.73, 25.09, 24.64. ESI-HRMS calcd for $[C_{13}H_{16}BrCl_2NO_2S]$ 398.9462, found 398.9466.

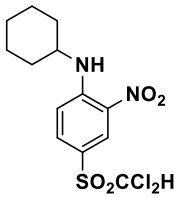




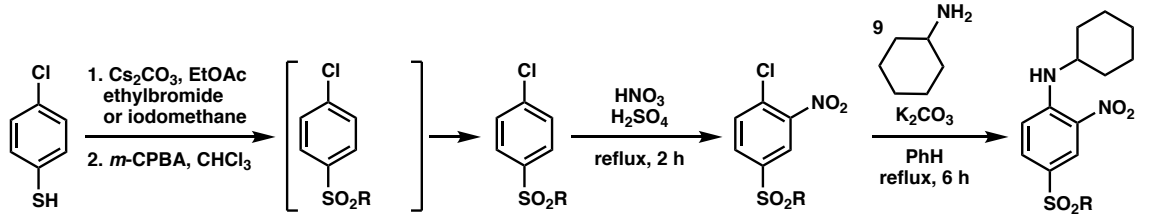
18: (182 mg, 62%). **TLC** R_f = 0.3 (hexanes/EtOAc = 19:1, v/v). **^{1}H NMR** (400MHz), $CDCl_3$, δ: 8.17-8.14 (dd, *J*=7.2, 1.6 Hz, 2H), 7.41-7.37 (dt, *J*= 6.8,1.6 Hz, 1H), 6.87-6.85 (d, *J*=8.4 Hz, 1H), 6.60-6.56 (dt, *J*=7.32, 0.88 Hz, 1H), 3.52-3.50 (t, *J*=3.92 Hz, 1H), 2.06-2.04 (m, 2H), 1.82-1.79 (m, 2H), 1.68-1.64 (m, 1H), 1.45-1.34 (m, 5H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 144.80, 136.06, 127.10, 114.77, 114.22, 50.98, 32.75, 25.61, 24.57. ESI-HRMS calcd for $[C_{12}H_{16}N_2O_2+H]^+$ 221.1285, found 221.1277.



4 12 20



20: (61 mg, 73%). **TLC** R_f = 0.5 (hexanes/EtOAc = 4:1, v/v). **^{1}H NMR** (600MHz), $CDCl_3$, δ: 8.84.-8.83 (d, *J* = 2.4 Hz, 1H), 8.65-8.64 (d, *J* = 6.6 Hz, 1H), 7.91-7.89 (dd, *J*=7.02, 1.8 Hz, 1H), 7.02-7.00 (d, *J* = 9 Hz, 1H), 6.25 (s, 1H), 3.62-3.61 (t, *J* = 3.54 Hz, 1H), 2.09-2.07 (m, 2H), 1.86-1.82 (m, 2H), 1.71-1.69 (m, 1H), 1.49-1.44 (t, *J*=9.3 Hz, 4H), 1.42-1.35 (m, 1H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ: 147.93, 136.56, 132.57, 131.03, 116.19, 114.60, 80.07, 51.85, 32.39, 29.72, 25.29, 24.37. ESI-HRMS calcd for $[C_{13}H_{16}Cl_2N_2O_4S+H]^+$ 367.0281, found 367.0282.



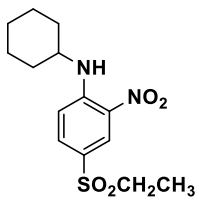
13

14: CH_2CH_3
31: CH_3

15: CH_2CH_3 (52%)
32: CH_3 (47%)

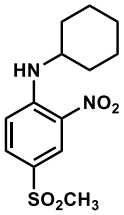
16: CH_2CH_3 (93%)
33: CH_3 (95%)

17: CH_2CH_3 (72%)
30: CH_3 (69%)



17: (90 mg, 72%). **TLC** R_f = 0.4 (hexanes/EtOAc = 1:1, v/v). **^{1}H NMR** (600MHz), $CDCl_3$, δ: 8.71-8.70 (d, *J* = 3 Hz, 1H), 8.48-8.47 (d, *J* = 10.8 Hz, 1H), 7.81-7.78 (dd, *J* =10.8, 3 Hz, 1H), 6.99-6.96 (d, *J* =13.8 Hz, 1H), 3.59-3.57 (m, 1H), 3.13-3.07 (q, *J* = 10.8 Hz, 2H), 2.07-2.05 (m, 2H), 1.84-1.81 (m, 2H), 1.70-1.67 (m, 1H), 1.47-1.41 (m, 5H), 1.39-1.37 (t, *J* = 3.6 Hz, 3H). **^{13}C NMR** (150 MHz) $CDCl_3$, δ:146.98, 134.15,

130.84, 129.17, 123.99, 114.87, 51.59, 50.84, 32.45, 25.36, 24.38, 7.61. ESI-HRMS calcd for $[C_{14}H_{20}N_2O_4S\text{-}H]^-$ 311.1071, found 311.1060.



30: (33 mg, 69%). **TLC** R_f = 0.6 (hexanes/EtOAc = 1:1, v/v). **^{1}H NMR** (600MHz), $CDCl_3$, δ: ^{1}H NMR (600 MHz, CDCl3) δ 8.77 (d, J = 2.3 Hz, 1H), 7.85 (ddd, J = 9.1, 2.3, 0.7 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 3.13 (s, 1H), 3.05 (s, 3H), 2.09 – 2.04 (m, 2H), 1.83 (dd, J = 9.5, 4.5 Hz, 2H), 1.49 – 1.40 (m, 4H), 1.25 (s, 1H). ^{13}C NMR (151 MHz, CDCl3) δ 146.99, 133.43, 128.51, 126.11, 115.00, 77.25, 77.04, 76.83, 51.59, 44.81, 32.44, 25.36, 24.39. $[C_{13}H_{17}N_2O_4S\text{-}H]^-$ 297.0915, found 297.0921.

5. Biochemical Reagents

A plasmid encoding a substrate peptide derived from Oxidative Stress Response kinase 1, OSR1 (GST-TEV-GAM-^{314}RAKKVRRVPGSSGRLHKTEDGGWEWSDDEF^{344}) (GST-OSR1) was donated from Melanie Cobb (unpublished). The reagent Kinase-Glo® luminescence was from Promega Inc., Cat No: V6711; [γ-^{32}P] ATP was obtained from PerkinElmer (Cat No: NEG035C005MC). Immunoblot assay components were Anti-phospho-SPAK Antibody (Ser373) / phospho-OSR1 Antibody (Ser325) (Sigma Aldrich, 07-2273), Anti-OXSR1 Mouse Monoclonal Antibody [clone: S1] (VWR, 10624-616), IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody (926-32211), and IRDye® 680RD Goat anti-Mouse IgG Secondary Antibody (926-68070). The reagents Dulbecco's modified Eagle's medium (DMEM; Gibco, 11965-092) and fetal bovine serum (FBS; Genesee Scientific, 25-514) were used. MDAMB 231 cells were gifted by Melanie Cobb. Pierce™ IP Lysis Buffer buffer (87787) Protease and phosphatase inhibitors were from Fisher Sci. (Cat. Nos. PIA32963 and PIA32965). Precast gels used were Mini-PROTEAN gels (4568096). LI-COR Intercept (TBS) blocking buffer was from Fisher Sci. (Cat. No. NC22420).

WNK1, WNK3 and GST-OSR1 Expression and Purification

Cloning, expression, and purification of WNK1 (194-483) and WNK3 (118-409) were performed as described previously [1, 2]. Slight modifications include use of benzonase nuclease and Protease Inhibitor Cocktail (PIC) (Sigma) in advance of breaking cells in an EmulsiFlex-C5 cell disrupter (Avestin). The purified WNK1 and WNK3 proteins are phosphorylated on Ser382 and Ser308, respectively as confirmed by mass spectrometry (pWNK1, pWNK3) as described previously [2]. The GST-OSR1(314-344) peptide plasmid was expressed in Rosetta (DE3) pLys S competent E. coli cells and was purified using glutathione beads (GE Healthcare).

6. *In Vitro* Kinase Assays

IC_{50} of compounds were determined by Kinase Glo® (Promega, Inc.) assay as an initial screening method. A subset of compounds was retested using a [γ-^{32}P] ATP-labelled radiometric assay necessary for determining sub-micromolar IC50's [3].

Kinase Glo® Luminescent Assay (Promega, Inc.)

WNK1 (194-483) phosphorylation activity toward GST OSR1 (314-344) was determined from the residual ATP using the Kinase Glo® reagent according to the manufacturer's protocol. Assays were conducted for 2 hours at 25 ^{o}C in 96 well plates. Final concentrations of reagents were 0.25 mM purified WNK1, 10 mM GST OSR1 (314-344), 50 mM HEPES, pH 7.4, 30 mM $MgCl_2$, 4mM ATP in 25 mL reaction volumes. The inhibitor concentrations were 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 25, 50, 100 mM. The positive control was DMSO (di-methyl sulfoxide) (8%) in place of WNK1;

the negative control lacked ATP. Kinase reactions were stopped by adding 15mL Kinase Gloâ reagent. Plates were centrifuged and luminescence was recorded by using CLARIOstar Plus® micro plate reader (BMG Labtech). Data was analyzed by using “nonlinear fit, log(inhibitor) vs. response (three parameters)” analysis in GraphPad Prism (Graphpad Inc., CA).

Radiometric Assay

IC50’s toward WNK1 or WNK3 of good compounds were determined to greater accuracy using radiometric assays as described previously [3]. In brief, final concentration of reagents were 5 nM WNK1 or WNK3, 10 mM GST OSR1 (314-344), 10 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 50mM “cold” ATP and 1 mL of g-^{32}P labelled ATP (0.01mCi/mL) in 25 mL reaction volumes. The inhibitor concentrations were similar to those used above. The negative control was DMSO (8%) in place of WNK1. Reactions were incubated for 30 min at 30°C and stopped by adding Laemmli SDS buffer. Samples were resolved in 4-15% Bio-Rad precast gels (Cat no: 4561086). Gels were dried and ^{32}P incorporation into the OSR1 fragment was quantified by liquid scintillation counting. Data was analyzed by using “nonlinear fit, log(inhibitor) vs. response (three parameters)” analysis in GraphPad Prism (Graphpad Inc., CA).

7. Eurofins Kinase Profiling by Radiometry

The Eurofins panel contains 50 kinases spanning all five classes of protein kinases defined by Manning[4]. Incorporation of ^{33}P from - g^{33}P ATP into native substrate or myelin basic protein (MBP) at 0.33 mg/ml was measured in duplicate. The buffer used is 8 mM MOPS, pH 7.0, 0.2 mM EDTA, 10 mM $Mg(OAC)_2$. The compound concentration used was 10 μM.

8. Cell Based Assays

Cell-based assays of trihalo-sulfone 1 were performed in the MDAMB231 breast-cancer cell line. Activation of the endogenous substrate OSR1 was measured by western blotting using antibodies to total OSR1 and to pOSR1. MDAMB231 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C in a 5% CO_2 incubator. MDAMB231 cells were grown to 60% confluence. Cells were treated with different concentrations of compound 1 (0.2, 04, 0.8, 1.6, 3.2, 6.4, 12. 5 μM) for 24 hours. To prepare whole-cell extracts, cells were washed twice and harvested with ice-cold phosphate-buffered saline (PBS). Cell pellets were resuspended in lysis buffer supplemented with protease and phosphatase inhibitors and incubated for 15 min at 4°C with gentle rotation. Lysates were syringed through a 25-gauge needle 10 times and centrifuged at 21,000 g for 10 min at 4°C. A 30 microgram of lysate was loaded onto 4–20% gradient gels. Proteins were transferred on to a nitrocellulose membrane at 750 mA constant current for 2 hrs. Membrane was blocked with LI-COR Intercept (TBS) blocking buffer for 1 hour and incubated with antibodies for pOSR1 and OSR1 (1:1000 dilution) overnight. Membrane was probed with fluorescent secondary antibodies for 1 hour and scanned by using Licor-Odyssey imaging system. Experiments were triplicated. For the quantification, infrared fluorescent signal of pOSR1 to OSR1 was calculated and percentage phosphorylation was calculated in relation to DMSO only control.

9. X-ray Crystallography

Crystals were obtained for non-phosphorylatable WNK1 lacking the primary activating phosphorylation site serine, WNK1-KDm/ (194–483, S382A) (uWNK1/SA). Crystals were grown

complexed with compounds SW004356 and SW393183 in 50 mM NaCl, 50 mM Hepes (pH 8.0), 1mM EDTA and 1 mM (2-carboxyethyl) phosphine hydrochloride (TCEP). A 1:1 ratio of protein and precipitant solution was mixed (2 mL each). Precipitant solution was 20% PEG 3350, 0.2 M sodium formate. Crystals were grown at 16°C and were cryoprotected in 20% glycerol. 2.7 Å resolution data for SW004356 and 2.4 Å data for SW393183 were collected at the APS 19-ID beamline. Integration and scaling were performed with the HKL3000 software suite (HKL Research, Charlottesville VA). The structure was solved using molecular replacement with 3FPQ [1] or [5] 5DRB a starting model. The structure was refined using REFMAC in CCP4 suite [6]. Model-building was performed in Coot using the ligand docking function[7] .The coordinates and structure factors have been deposited in the protein data bank (PDB files (**1:** 7UOS, **23**: 7UOU).

10. Table S1. Statistics of Crystallographic Data and Refinement

	uWNK1/1	**uWNK1/23**
X-ray Data	PDB 7UOS	PDB 7UOU
Space group	P1 2_1 1	P1 2_1 1
Unit cell dimensions (Å)	*a,b,c*=37.85,57.53,65.66	*a,b,c*=37.69, 57.77, 65.49
Angles (°)	α,β,γ=90, 90.6,90	α,β,γ=90, 91.3,90
Wavelength (Å)	0.9795	0.9795
Resolution (Å)	37.85-2.9	40.0-2.7
Unique reflections (last shell)	4894	7214
Completeness (%) (last shell)	86(47)	97(89)
I/σ (last shell)	10.4(2.0)	15.3(6)
Rsym, Rpim (last shell)[a]	0.12,0.06(0.49,0.28)	0.16, 0.07 (0.64,0.30)
Redundancy (last shell)	4.6(2.9)	5.0(3.8)
CC1/2 (last shell)	0.95(0.81)	1.00(0.82)
Wilson B factor	26.3	45
Structure		
Rwork/Rfree[b]	0.222/0.294	0.218/0.278
Non-H protein atoms	2283	2288
Waters	70	54
RMSD in bond length (Å)[c]	0.008	0.008
RMSD in bond angles(°)[c]	1.7	1.6
Average B-values (Å^2)	50	45
Ramachandran plot stats. (%)		
Most favored region	98.5	97.8
Disallowed region	1.5	2.2
Molprobity Score	2.8	3.1
Residues missing from the model	None	230-233 261-264 294-295 375-379

[a] $R_{sym} = \sum | I_{avg} - I_j | / \sum I_j$.

[b] $R_{factor} = \sum | F_o - F_c | / \sum F_o$, where F_o and F_c are observed and calculated structure factors, respectively, R_{free} was calculated from a randomly chosen 5% of reflections excluded from the refinement, and R_{factor} was calculated from the remaining 95% of reflections.

[c] r.m.s.d. is the root-mean-square deviation from ideal geometry.

11. Table S2. Trihalo-sulfone 1 and 23 inhibition strengths against WNK1 and 3

WNK isoform	IC50, uM (Trihalo-sulfone 1)	IC50, uM (Trihalo-sulfone 23)
WNK1	4	71
WNK3	43	20

12. Supplementary Figures

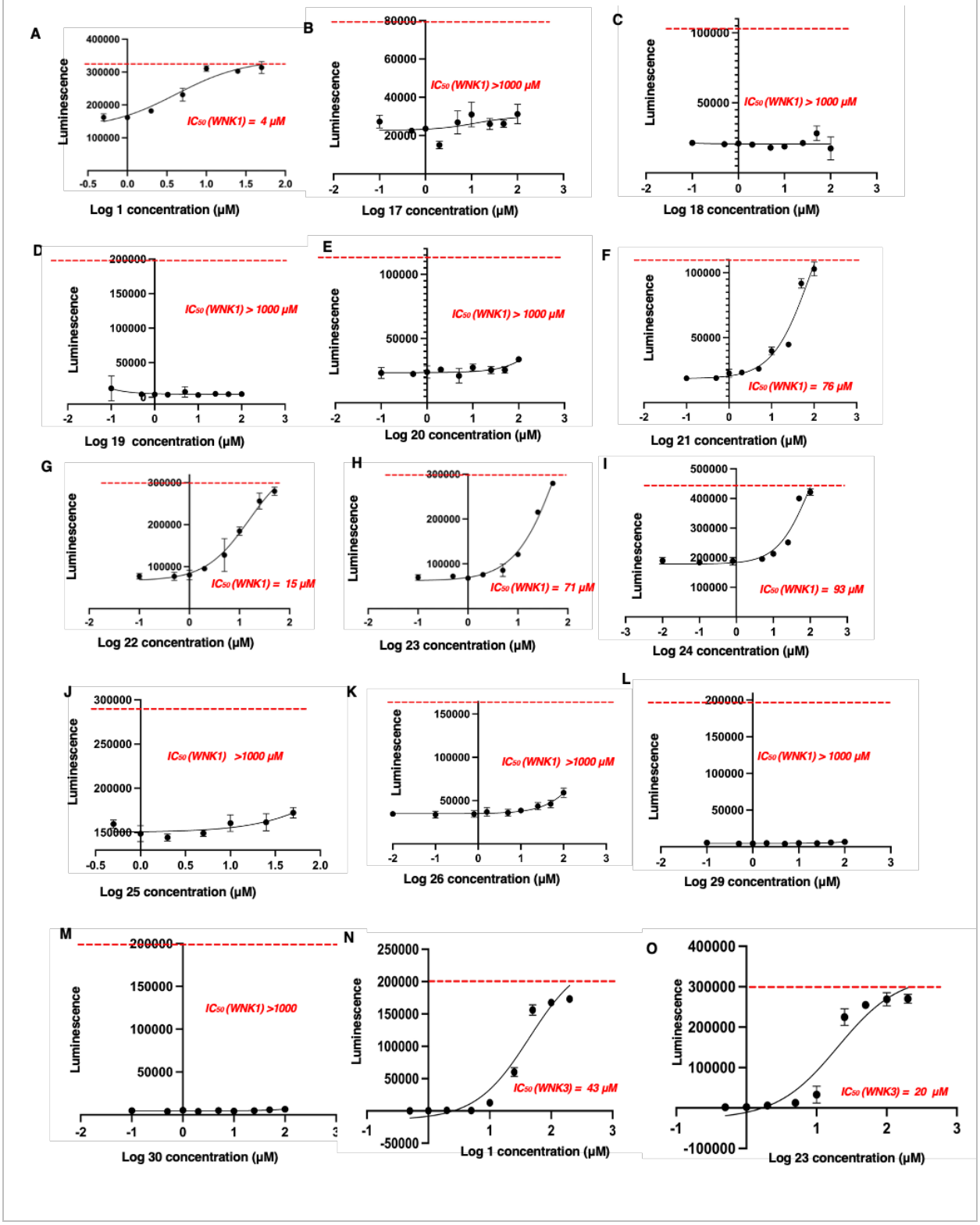


Figure S1. Kinase-Glo® derived dose response curves for trihalo-sulfone **1** and analogs. *In vitro* phosphorylation of GST-OSR1 (314-344) by WNK1 (A-M) and WNK3 (N-O) in the presence of inhibitors

was quantified by Kinase-Glo® Luminescent assay Each graph is from independent three experiments. Data was analyzed and IC_{50} was calculated by using GraphPad version 8.0.

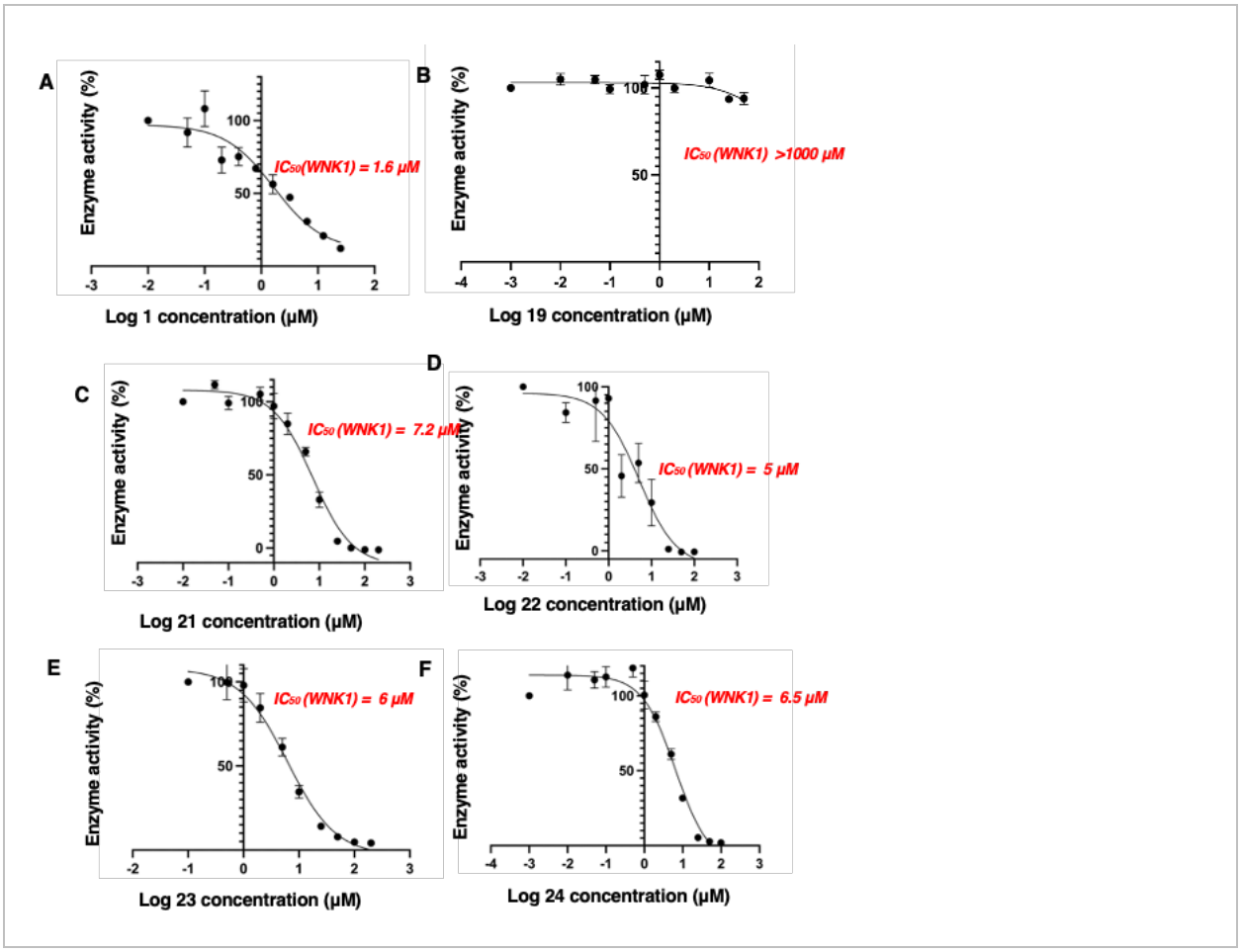


Figure S2. Radiometrically-measured dose response curves for trihalo-sulfones **1**, 19, **22**, **23**, **24** and **21**. *In vitro* phosphorylation of GST-OSR1 (314-344) by WNK1 in the presence of inhibitors was quantified by radiometric assay. Data was analyzed and IC_{50} was calculated by using GraphPad Prism version 8.0.

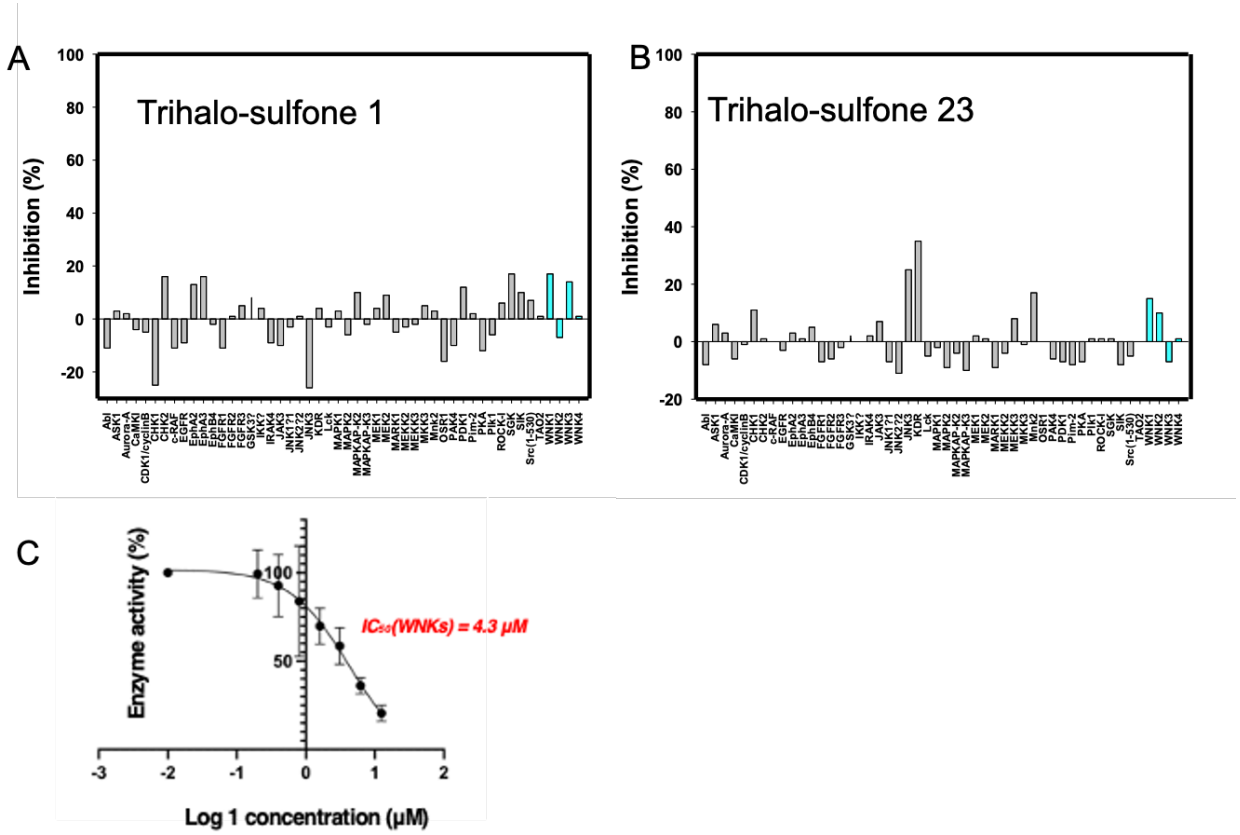


Figure S3. Eurofins kinase profiling against 50 kinases for trihalo-sulfone **1** (A) and trihalo-sulfone **23** (B) by radiometry. Blue lines represent four WNK isoforms. (C) Dose response curve for trihalo-sulfone **1** in MDAMB231 cells. Phosphorylation of endogenous OSR 1 (Ser) 325 by WNKs was measured in the presence of trihalo-sulfone **1**. Data was analyzed and IC_{50} was calculated by using GraphPad Prism version 8.0.

13. References

(1) Min, X. S.; Lee, B. H.; Cobb, M. H.; Goldsmith, E. J. Crystal structure of the kinase domain of WNK1, a kinase that causes a hereditary form of hypertension. *Structure* **2004**, *12* (7), 1303-1311, Journal Article.
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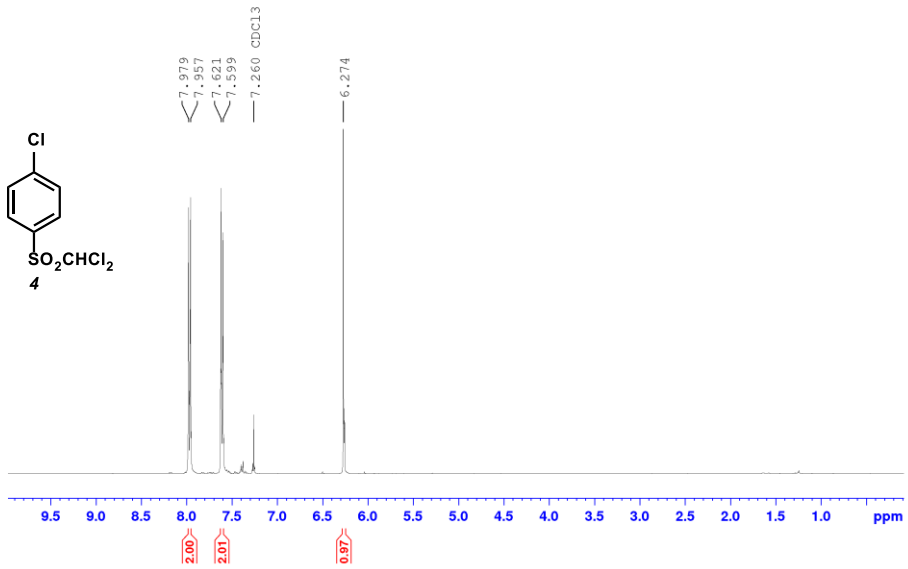
(3) Karra, A. S.; Stippec, S.; Cobb, M. H. Assaying Protein Kinase Activity with Radiolabeled ATP. *J Vis Exp* **2017**, (123). DOI: 10.3791/55504.
(4) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The protein kinase complement of the human genome. *Science* **2002**, *298* (5600), 1912-1934, Journal Article.
(5) Yamada, K.; Park, H. M.; Rigel, D. F.; DiPetrillo, K.; Whalen, E. J.; Anisowicz, A.; Beil, M.; Berstler, J.; Brocklehurst, C. E.; Burdick, D. A.; et al. Small-molecule WNK inhibition regulates cardiovascular and renal function. *Nat Chem Biol* **2016**, *12* (11), 896-898. DOI: 10.1038/nchembio.2168.

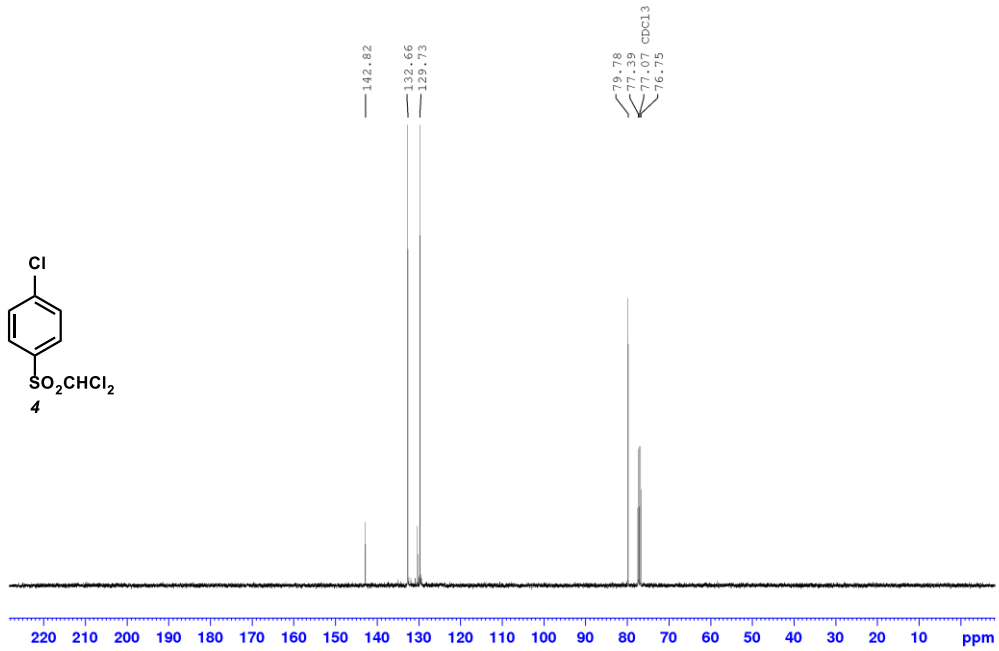
(6) Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G.; McCoy, A.; et al. Overview of the CCP4 suite and current developments. *Acta Crystallogr D Biol Crystallogr* **2011**, *67* (Pt 4), 235-242. DOI: 10.1107/S0907444910045749.
(7) Emsley, P. Tools for ligand validation in Coot. *Acta Crystallogr D Struct Biol* **2017**, *73* (Pt 3), 203-210. DOI: 10.1107/S2059798317003382.

14. NMR Spectra

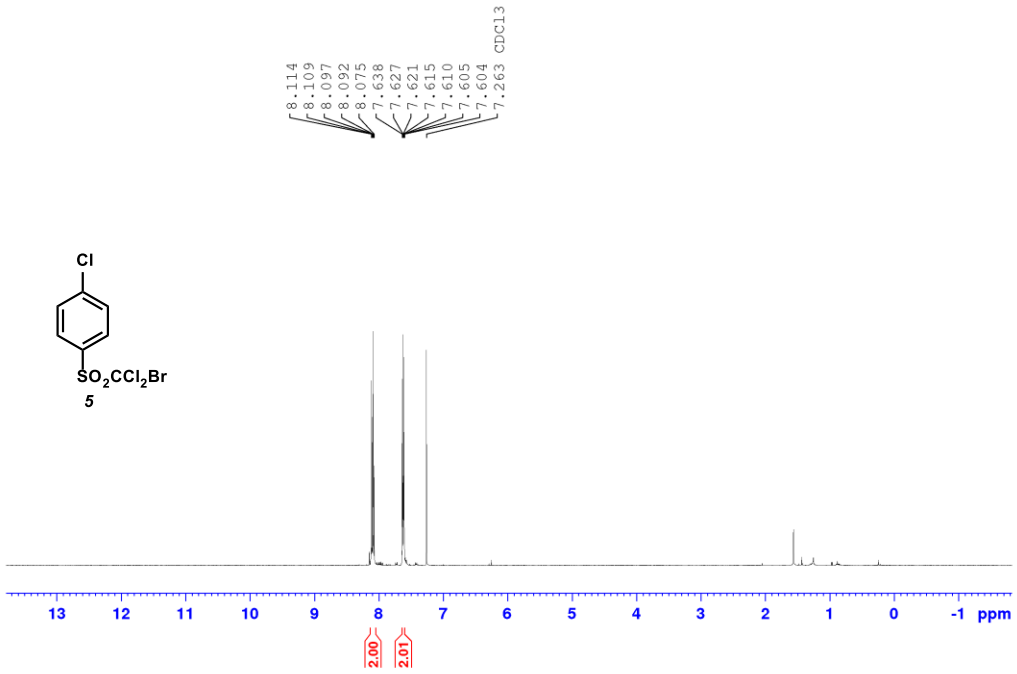
^{1}H NMR (400 MHz, $CDCl_3$)



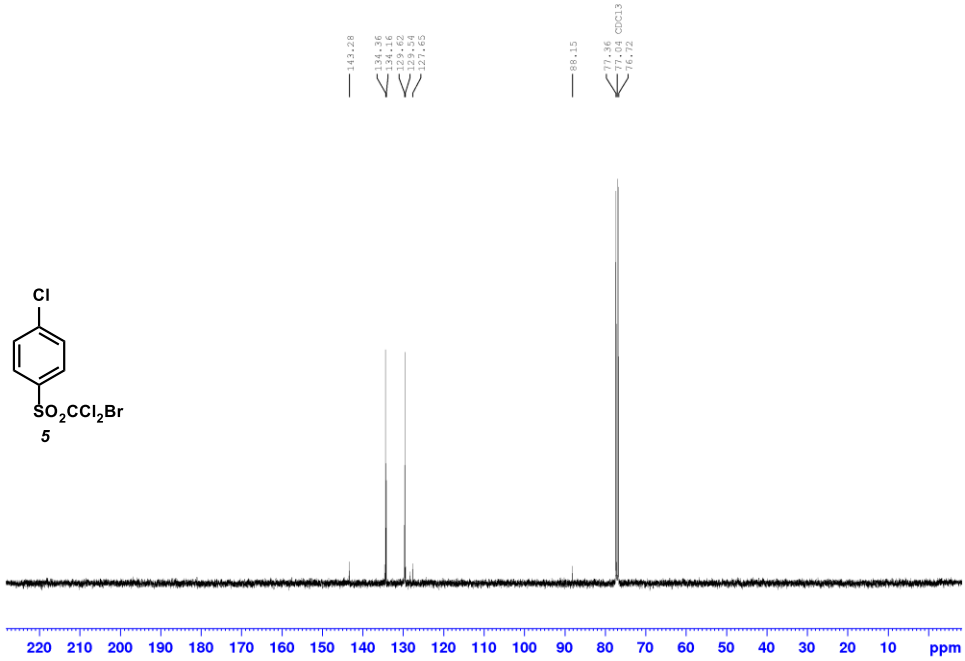
^{13}C NMR (100 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100 MHz, $CDCl_3$)



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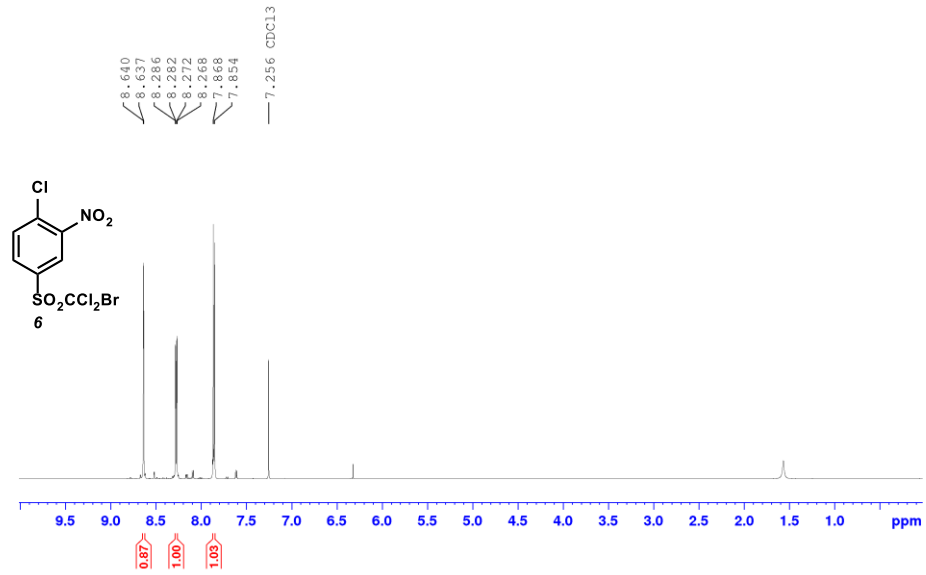

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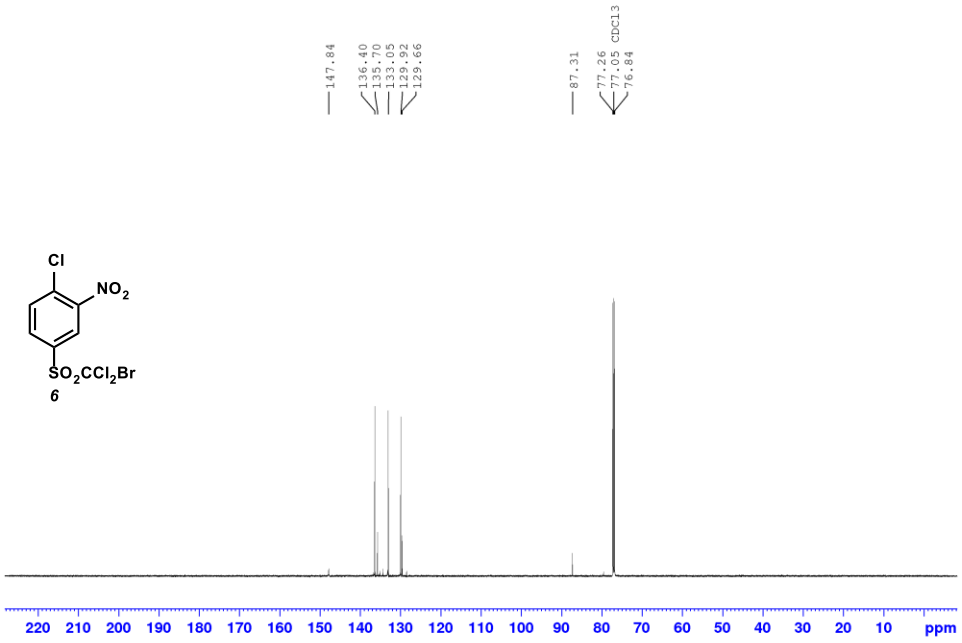
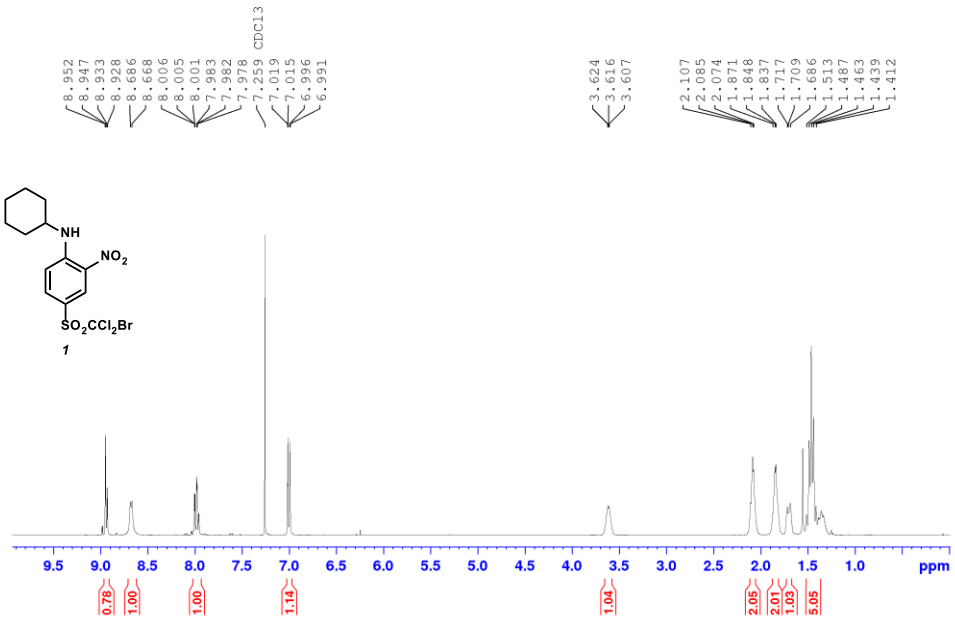
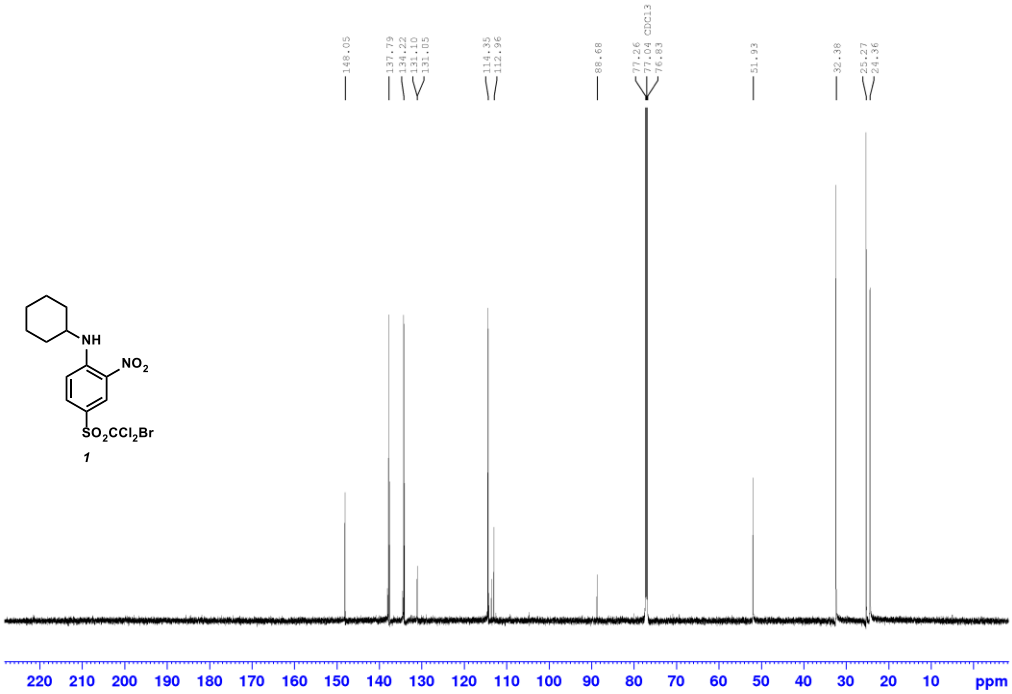


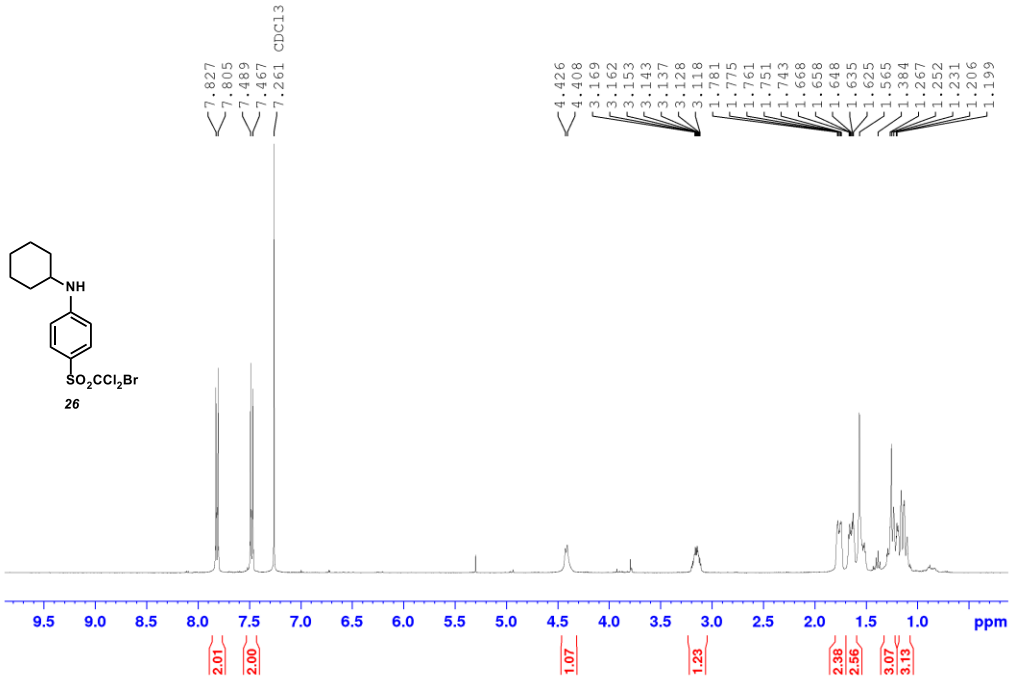
^{1}H NMR (400 MHz, $CDCl_3$)



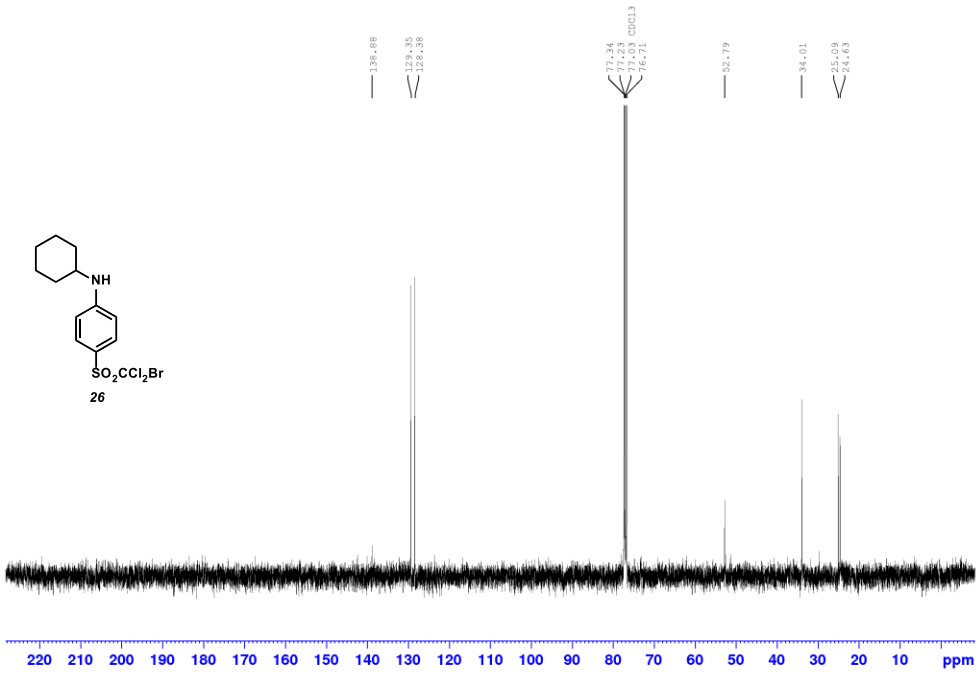
^{13}C NMR (150 MHz, $CDCl_3$)



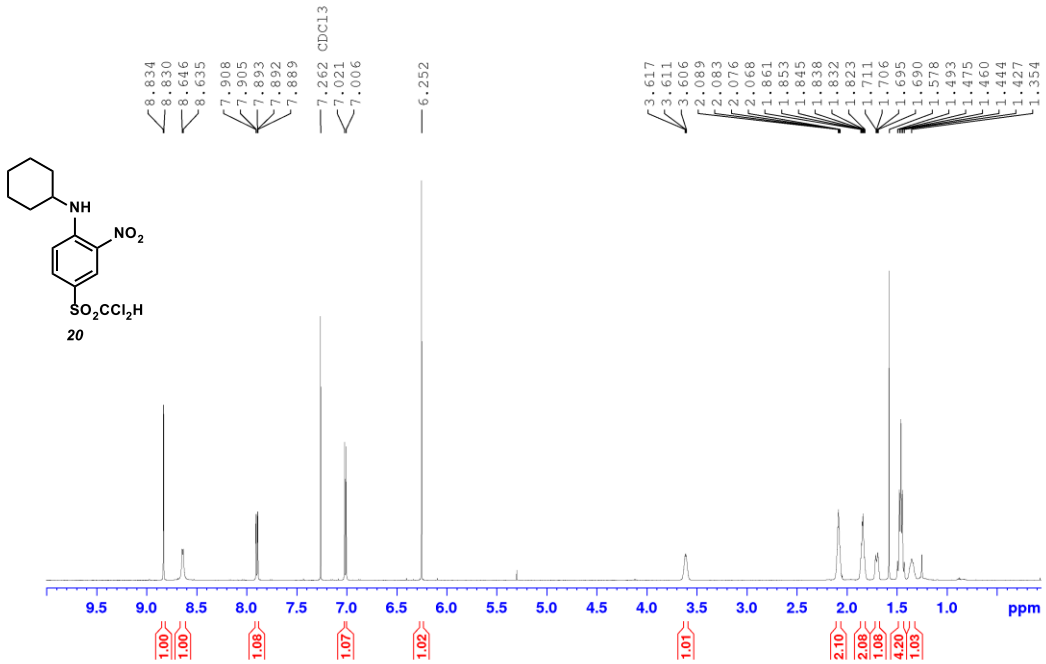
^{1}H NMR (400 MHz, $CDCl_3$)



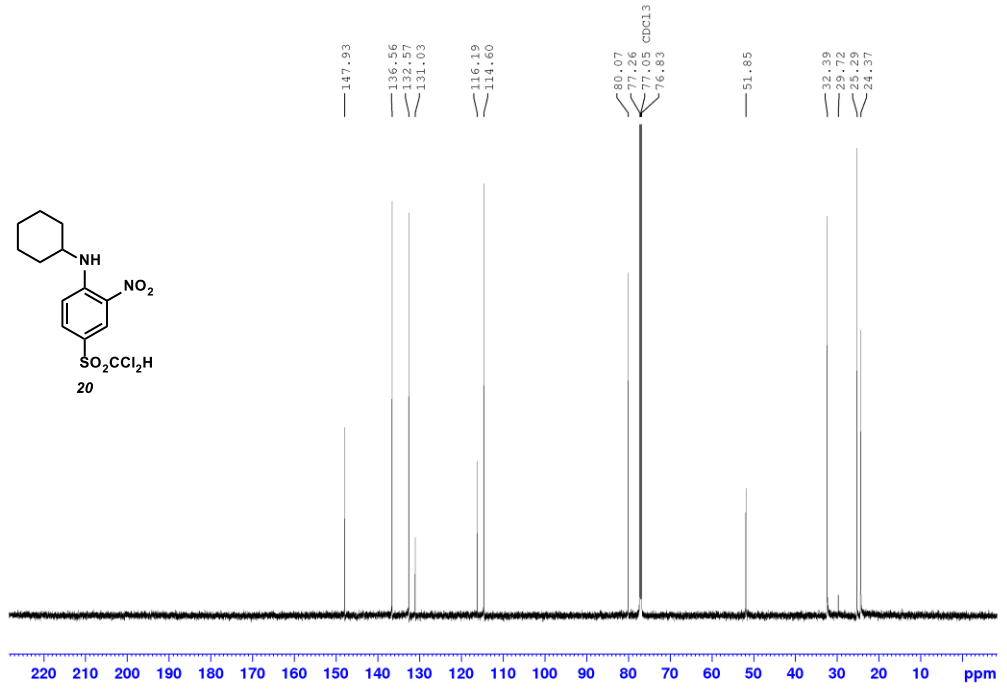
^{13}C NMR (150 MHz, $CDCl_3$)



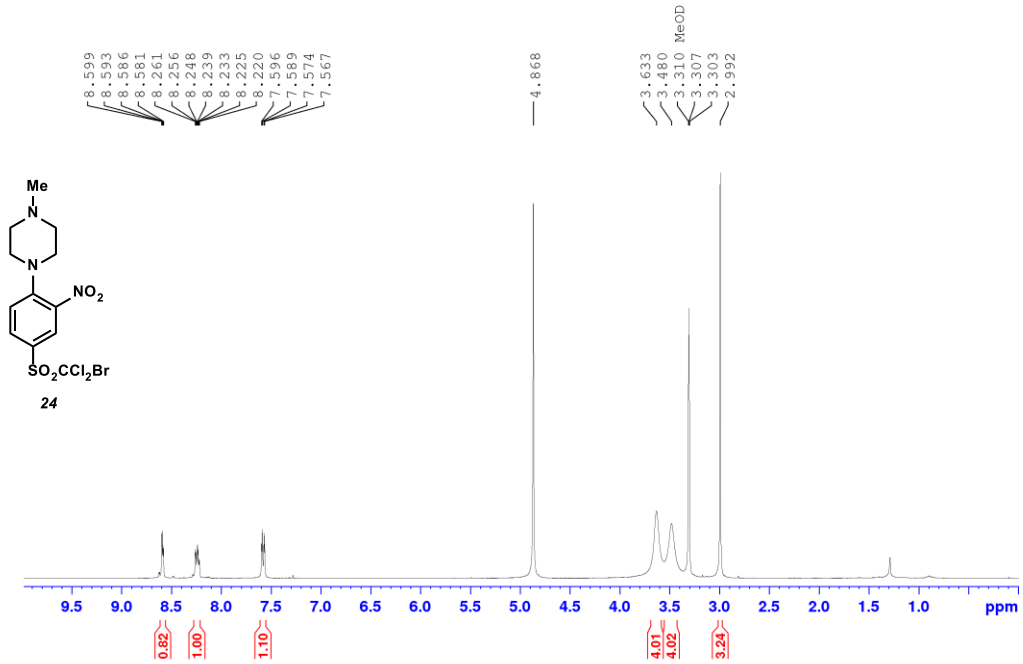
^{1}H NMR (600 MHz, $CDCl_3$)



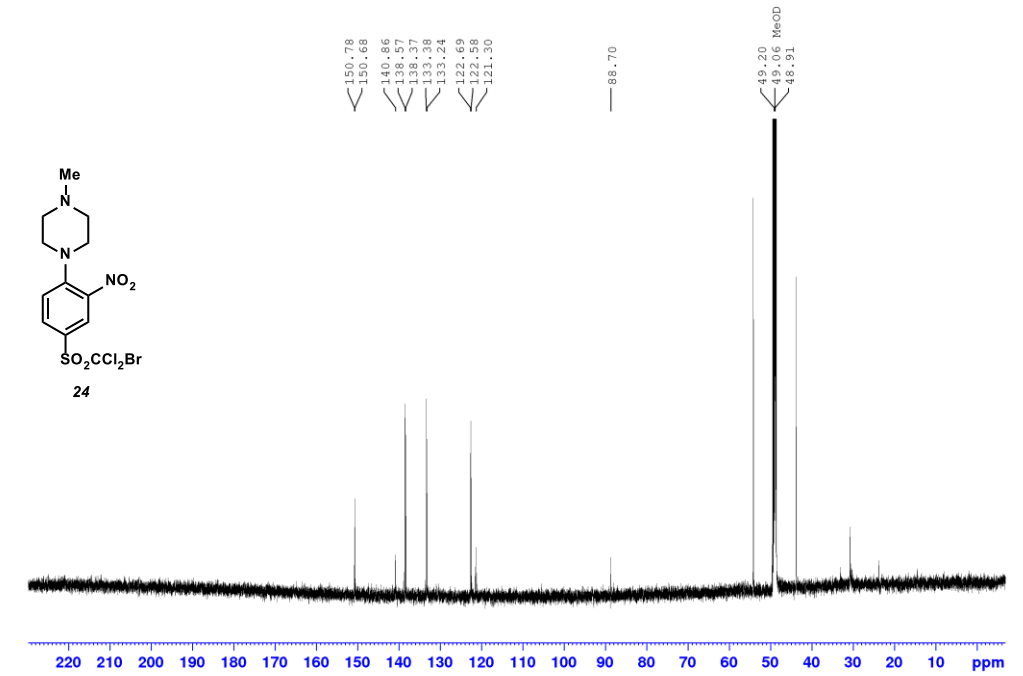
^{13}C NMR (150 MHz, $CDCl_3$)



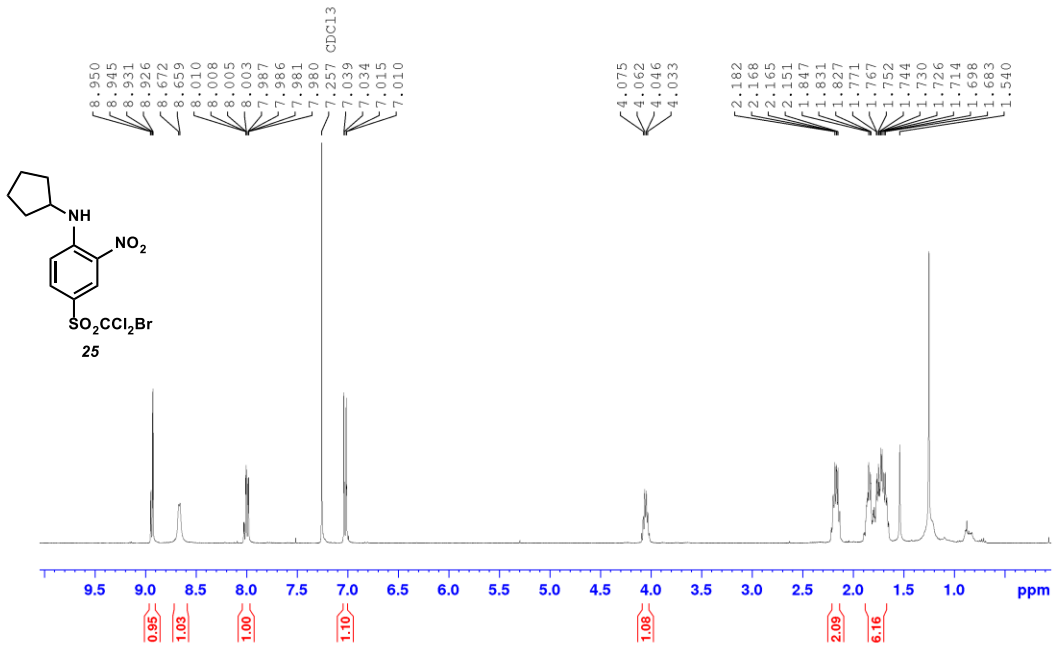
^{1}H NMR (400 MHz, CD_3OD)



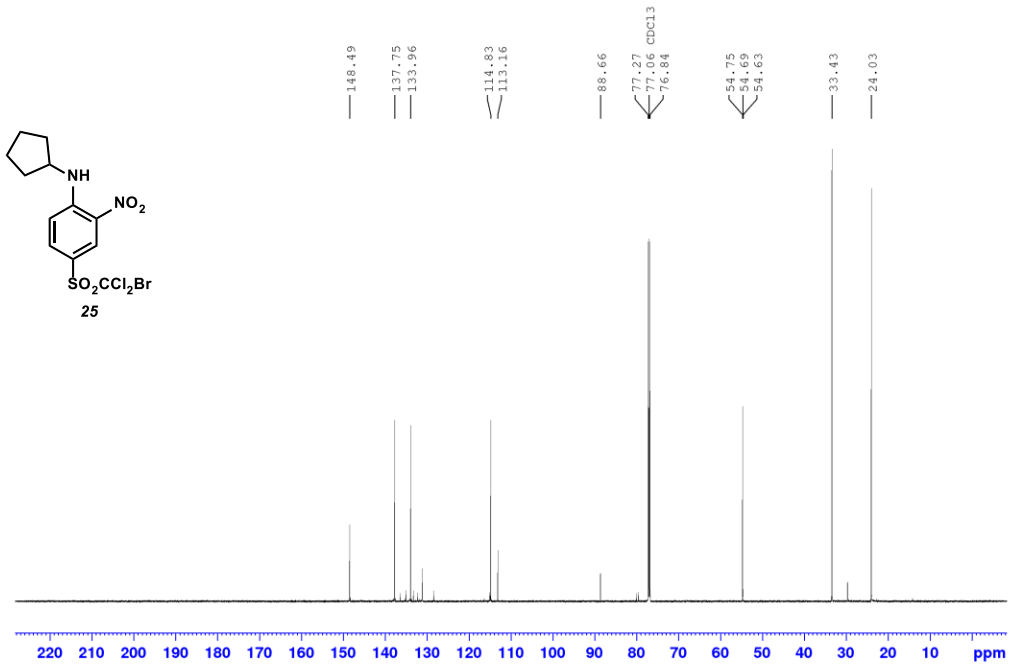
^{13}C NMR (150 MHz, CD_3OD)



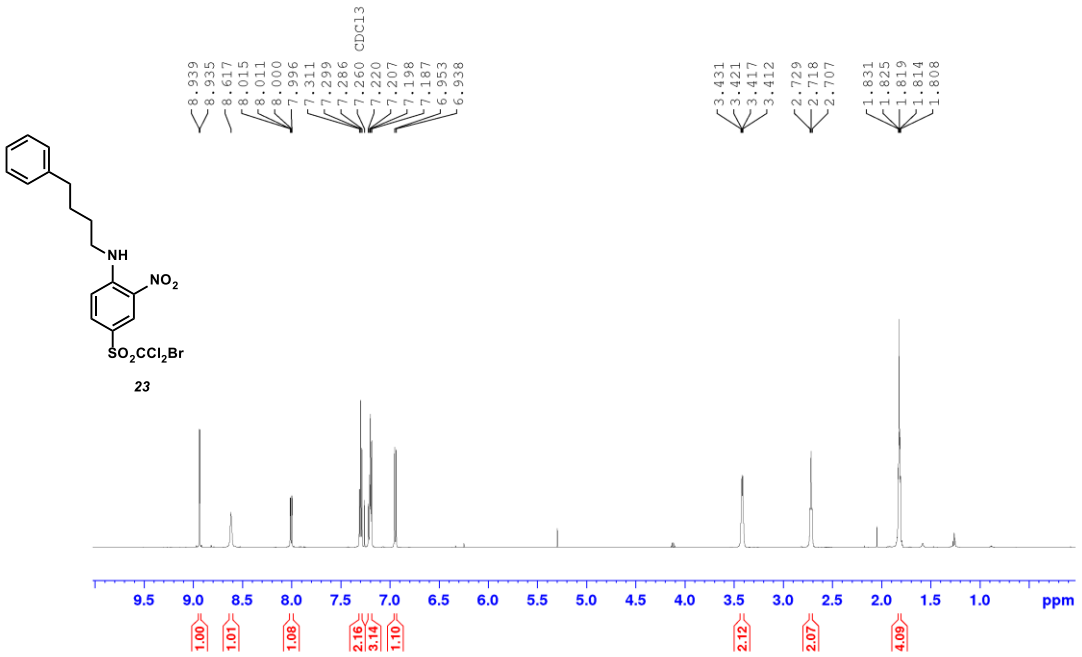
^{1}H NMR (400 MHz, $CDCl_3$)



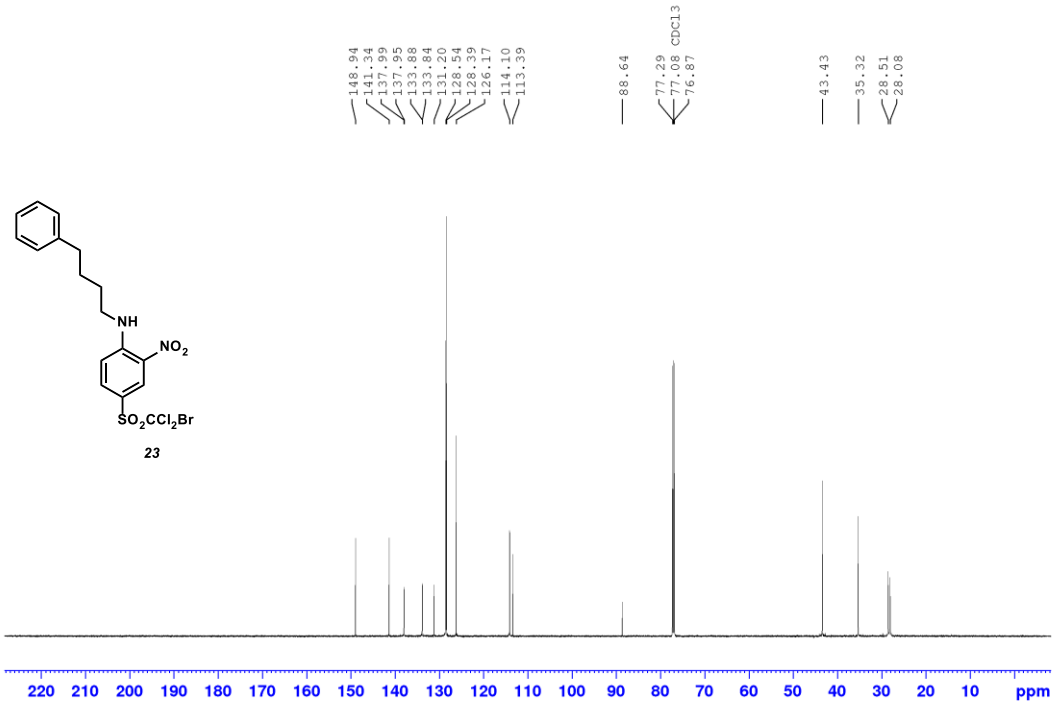
^{13}C NMR (100 MHz, $CDCl_3$)



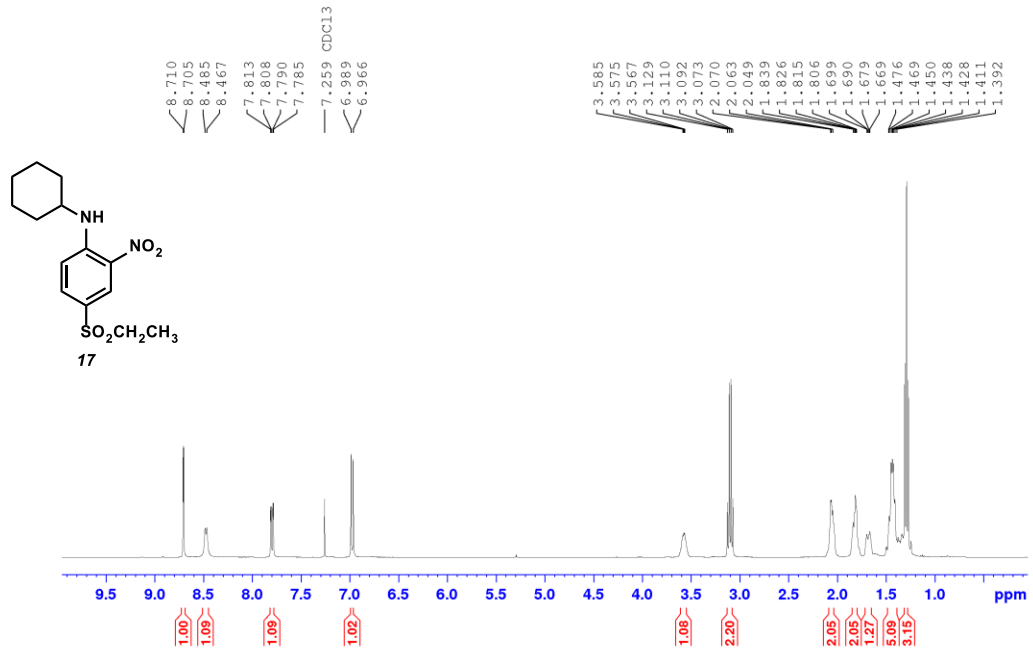
^{1}H NMR (600 MHz, $CDCl_3$)



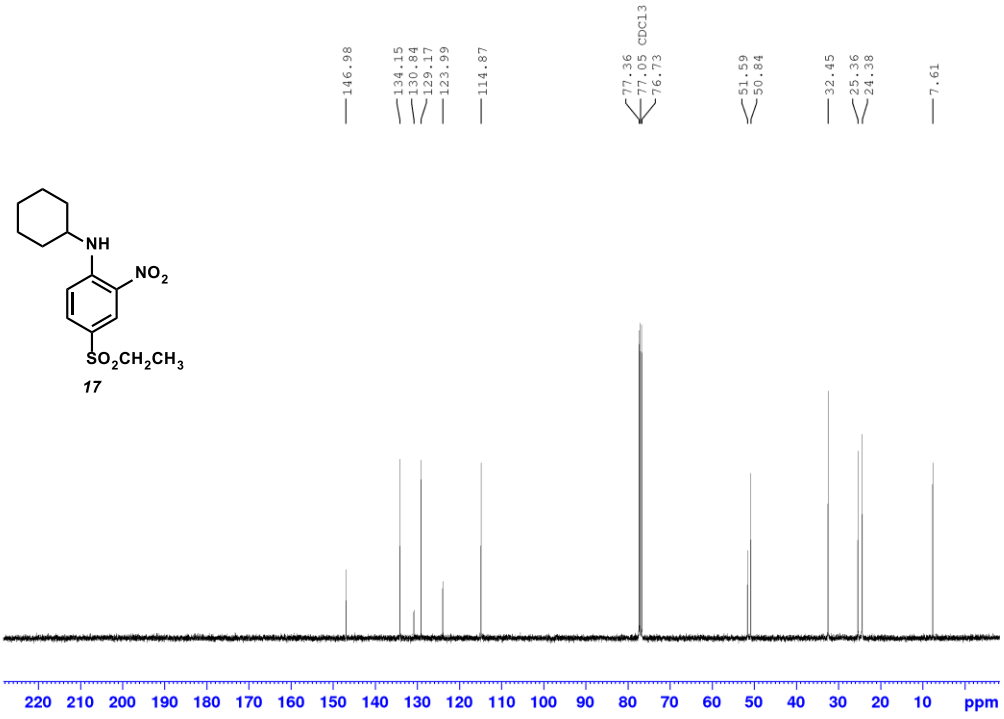
^{13}C NMR (150 MHz, $CDCl_3$)



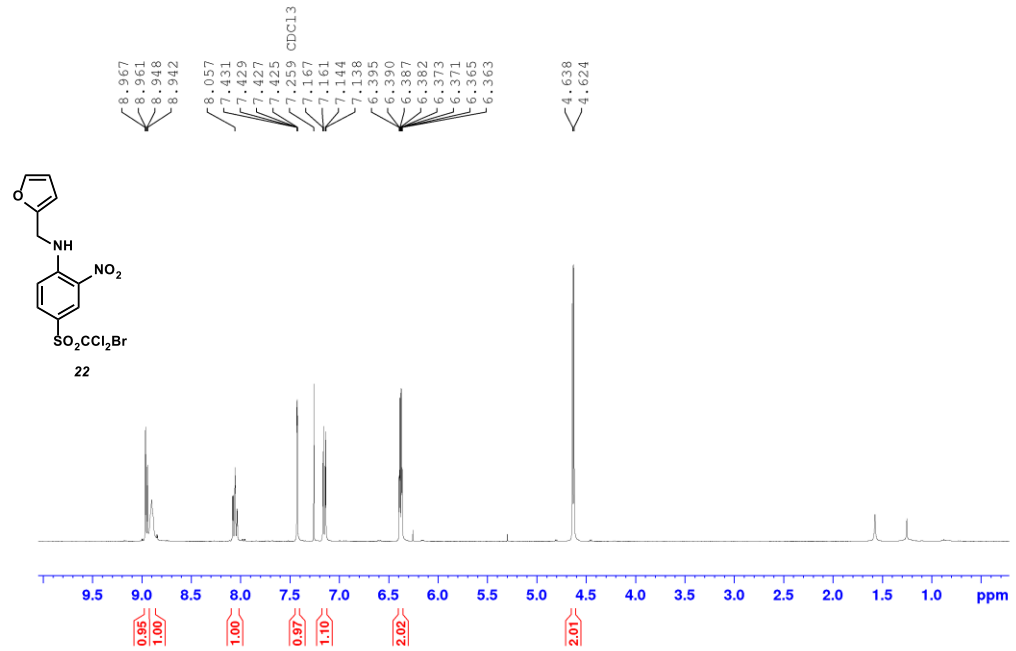
^{1}H NMR (600 MHz, $CDCl_3$)



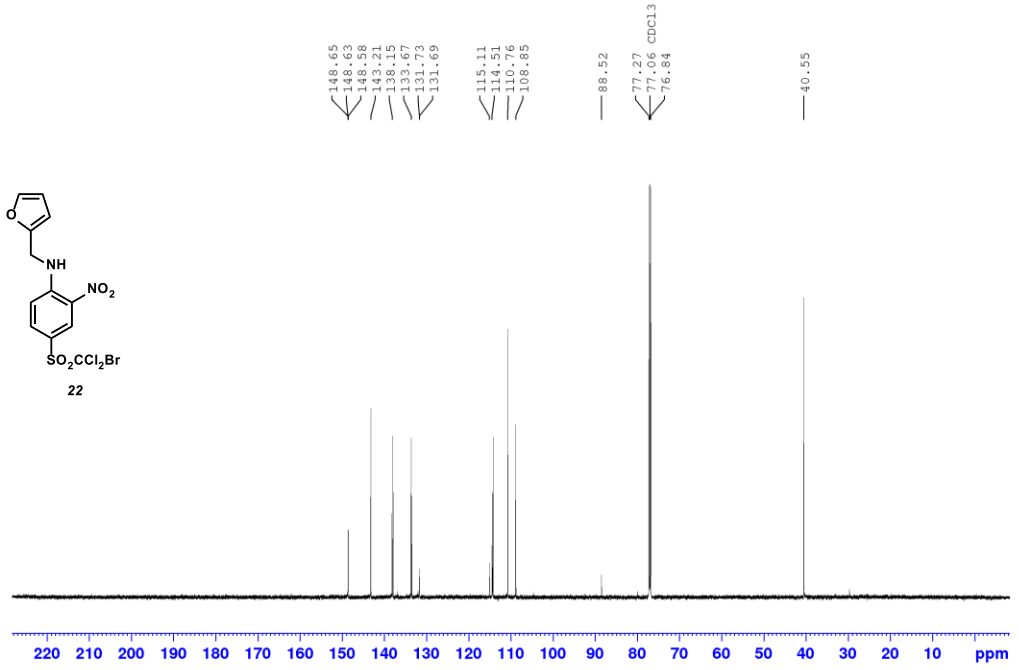
^{13}C NMR (150 MHz, $CDCl_3$)



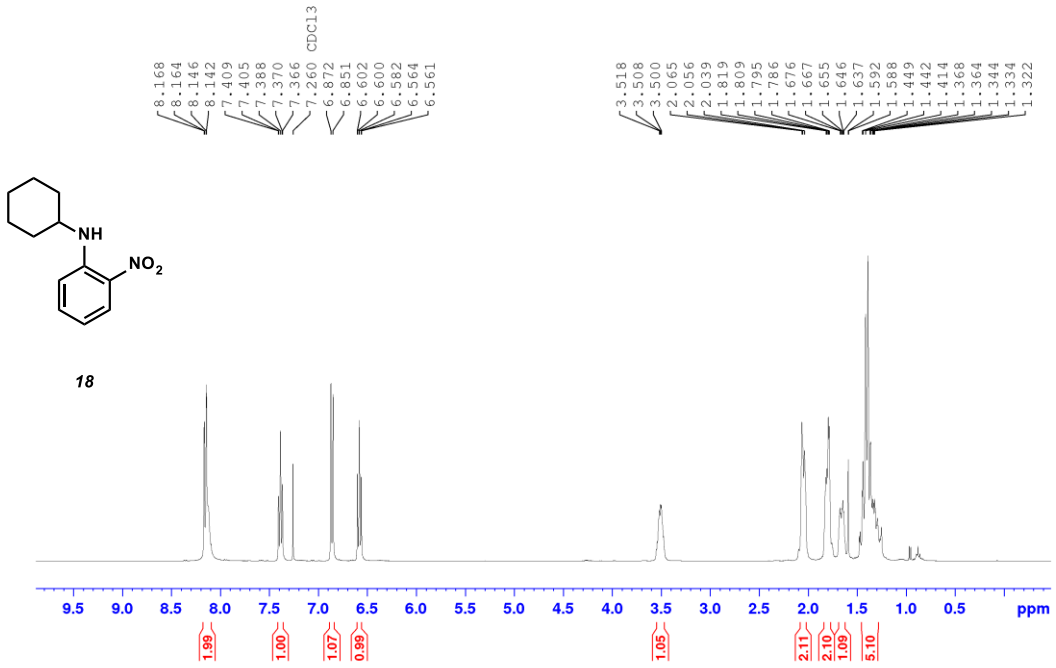
^{1}H NMR (400 MHz, $CDCl_3$)



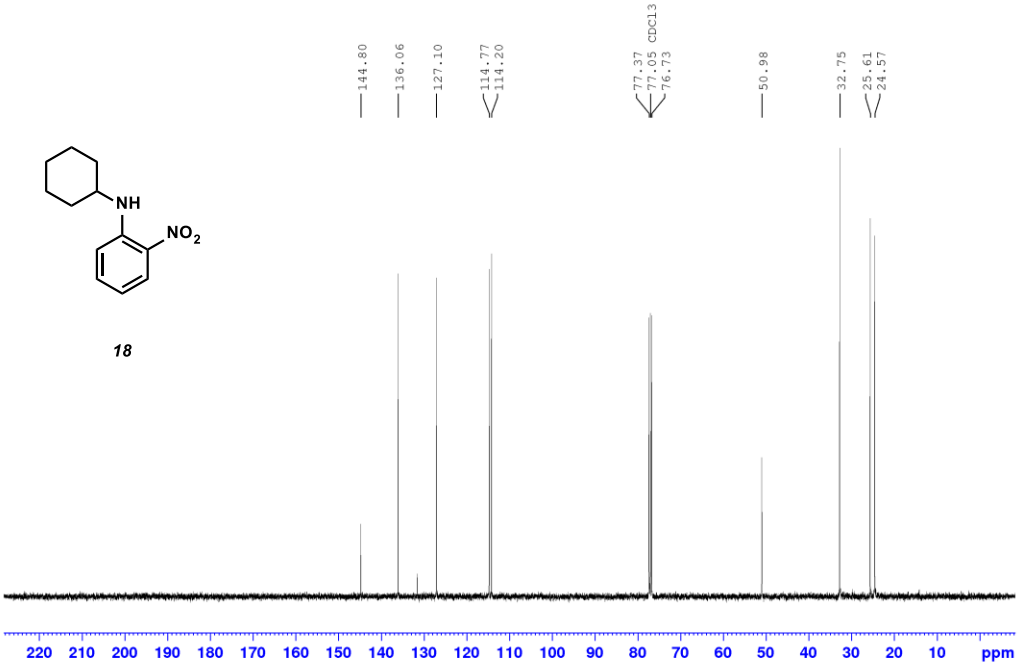
^{13}C NMR (150 MHz, $CDCl_3$)



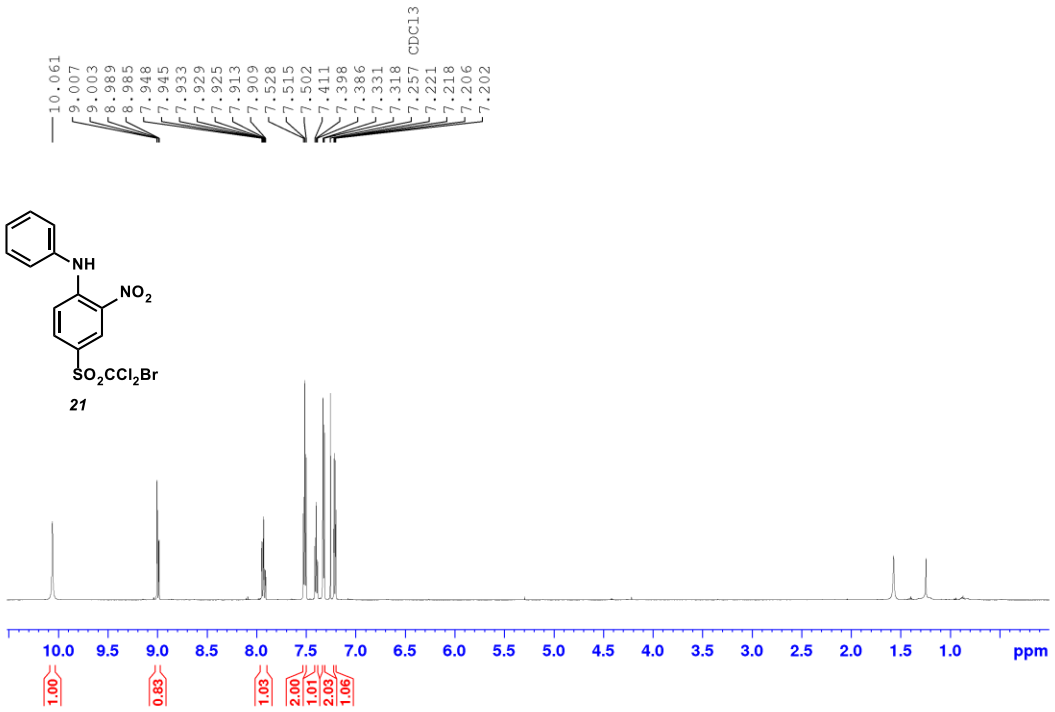
^{1}H NMR (400 MHz, $CDCl_3$)



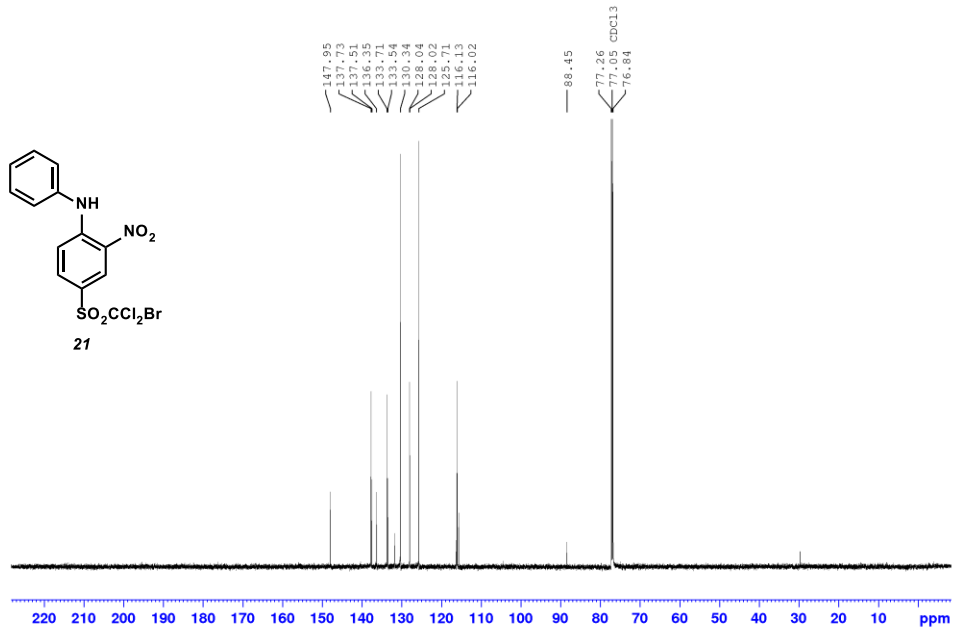
^{13}C NMR (100 MHz, $CDCl_3$)



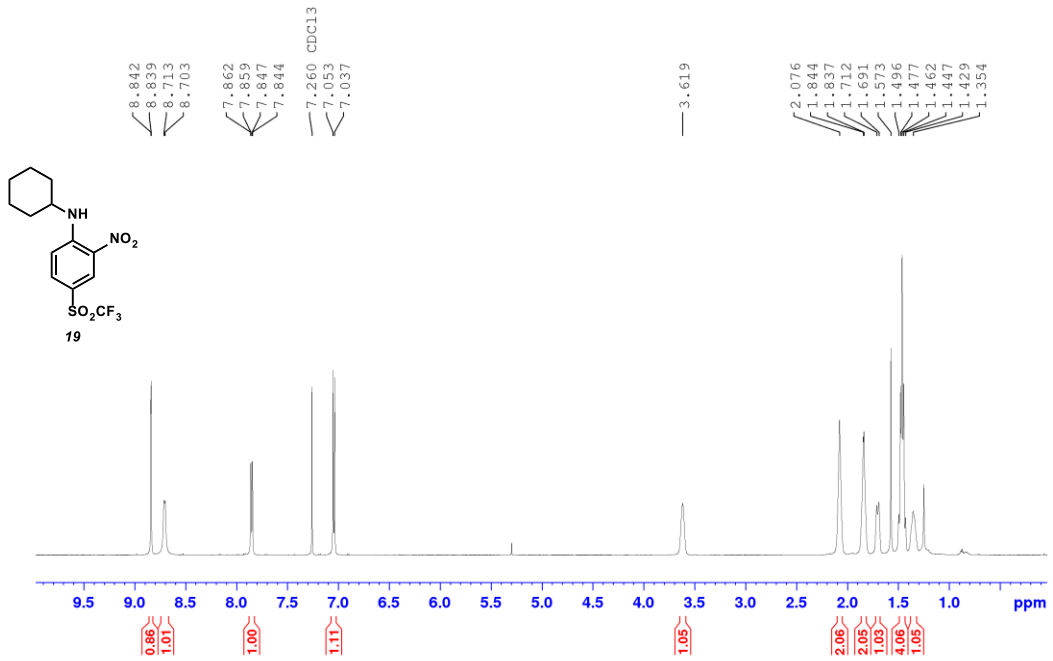
^{1}H NMR (600 MHz, $CDCl_3$)



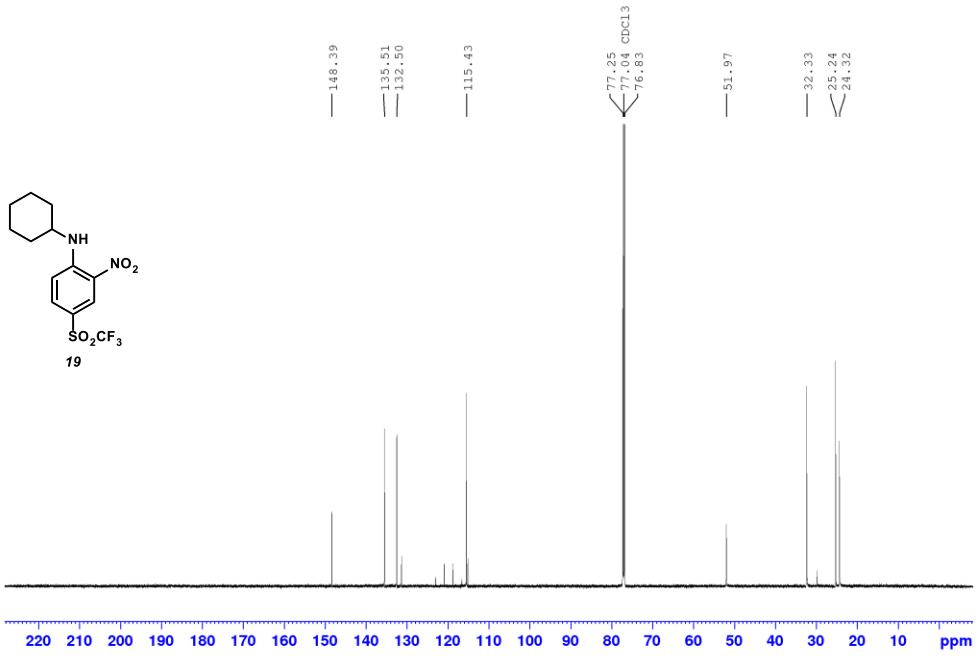
^{13}C NMR (150 MHz, $CDCl_3$)



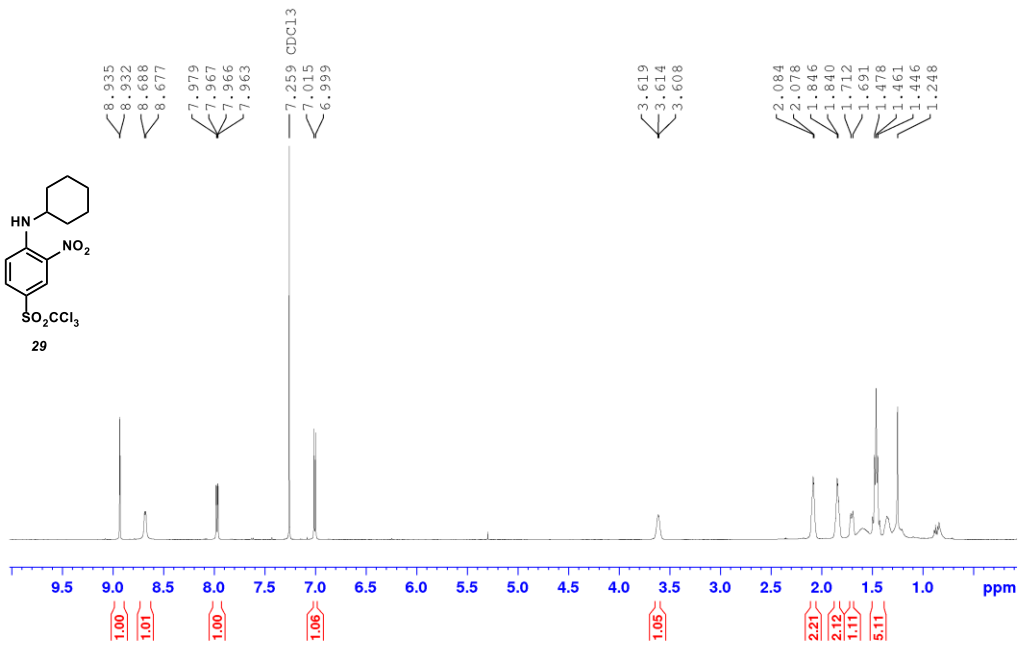
^{1}H NMR (600 MHz, $CDCl_3$)



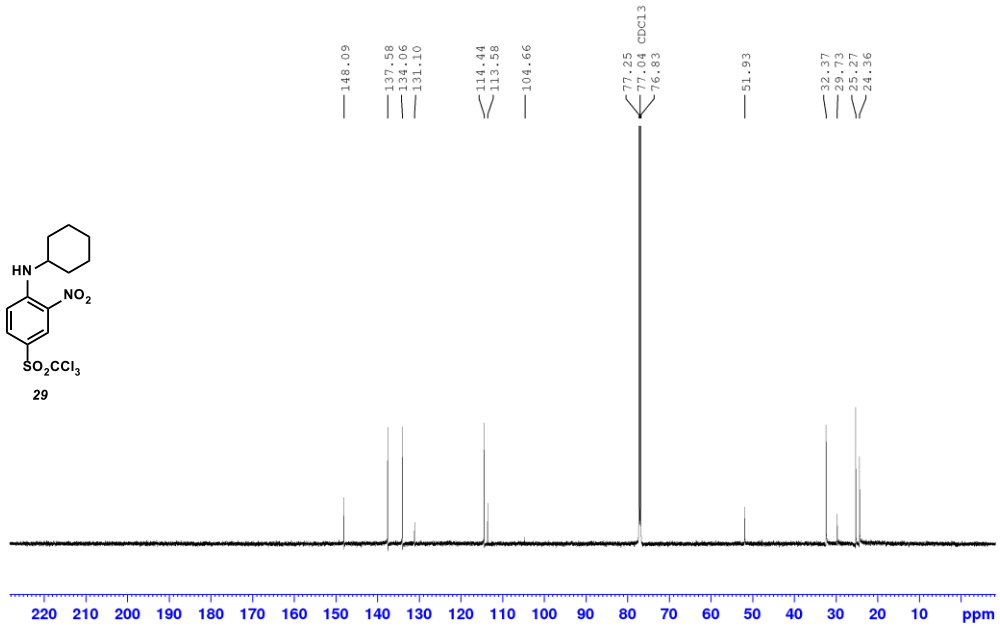
^{13}C NMR (150 MHz, $CDCl_3$)



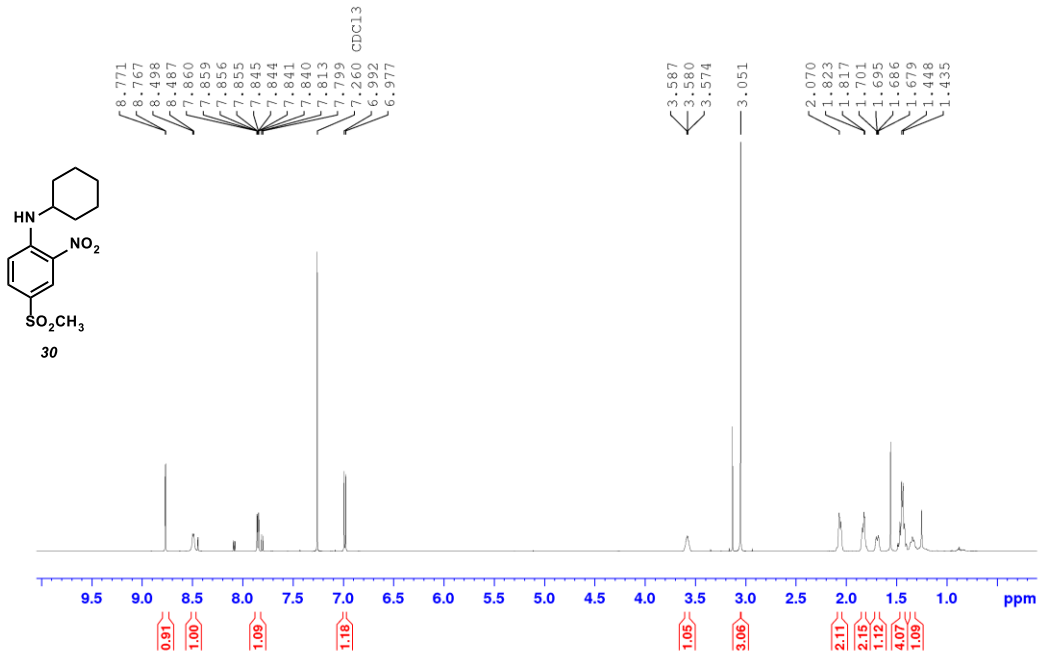
^{1}H NMR (600 MHz, $CDCl_3$)



^{13}C NMR (150 MHz, $CDCl_3$)



^{1}H NMR (600 MHz, $CDCl_3$)



^{13}C NMR (150 MHz, $CDCl_3$)