Necrosulfonamide Inhibits Necroptosis by Selectively Targeting the Mixed Lineage Kinase Domain-like Protein

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Supporting Information

Ⅰ) Experimental Section

1) General information

^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer at ambient temperature with $CDCl_3$ as the solvent unless otherwise stated. ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer (with complete proton decoupling) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (^{1}H, δ 7.26; ^{13}C, δ 77.00). Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants. Infrared spectra were recorded on a Thermo Fisher FT-IR200 spectrophotometer. High-resolution mass spectra were obtained at Peking University Mass Spectrometry Laboratory using a Bruker APEX Flash chromatography. The samples were analyzed by HPLC/MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 2767 Sample Manager, and 2998 Photodiode Array (PDA) Detector). The system was equipped with a Waters C18 5μm SunFire separation column (150*4.6 mm), equilibrated with HPLC grade water (solvent A) and HPLC grade methanol (solvent B) with a flow rate of 1.0 mL/min at room temperature. Analytical thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Microwave reactions were carried out on CEM Explorer 24 microwave reactor. Flash chromatography was performed using 200-400 mesh silica gel. Yields refer to chromatographically and spectroscopically pure materials, unless otherwise stated. All reagents were used as supplied by Sigma-Aldrich, J&K and Alfa Aesar Chemicals, anti-MLKL polyclonal antibody was purchased from Sigma-Aldrich. Methylene chloride, toluene, 1, 2-dichloroethane were distilled from calcium hydride; tetrahydrofuran were distilled from sodium/benzophenone ketyl prior to use. All reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise noted.

Screen Assay Design

On day one, 2,000 HT-29 cells were split into each well of a 384-well assay plate. On day two, necrosis was induced by adding final concentrations of 20 ng/ml TNF-a (T), 100 nM Smac mimetic (S), and 20 mM z-VAD (Z) to the well. Identical concentrations of these necrosis-inducing agents were used in subsequent experiments unless otherwise stated. Concurrently, individual compounds from a chemical library of ~200,000 compounds were delivered into each well at a final concentration of 10 mM. Cell viability in this and subsequent panels was determined by measuring ATP levels by Cell Titer-Glo assay after 24 hrs.

Cell Culture and Stable Cell Lines

HT-29 cells were cultured in McCoy's 5A culture medium (Invitrogen). FADD-null Jurkat cells were grown in RPMI-1640 medium (Hyclone). All media were supplemented with 10% FBS (Invitrogen) and 100 units/ml penicillin/streptomycin (Hyclone). The cells were transfected using Lipofectamine2000 (Invitrogen) according to the manufacturer's instructions. Stable HeLa cell lines expressing the Tet repressor (HeLa-TetR cells) were selected with 10 mg/ml blasticidin after being transfected with pcDNA6/TR (Invitrogen). HeLa-TetR cells were transfected with a pcDNA3.1 plasmid encoding HA-3xFlag-RIP3 and were selected with 1 mg/ml G418 to establish the RIP3-HeLa cell line.

Cell Survival Assay

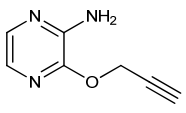
Cell survival assay was performed using the Cell Titer-Glo Luminescent Cell Viability Assay kit. A CellTiter-Glo assay (Promega) was performed according to the manufacturer's instructions. Luminescence was recorded with a Tecan GENios Pro plate reader.

Biotinylated Necrosulfonamide Precipitation

The cells were plated on 10-cm dishes and grown to confluence for one day. On day two, the cells were transfected with the indicated siRNA or cDNA. The cells were harvested and lysed in lysis buffer as described above 24 or 48 hours after transfection with cDNA or siRNA, respectively. Biotinylated necrosulfonamide (20 nmol) was preincubated with 20 ml streptavidin agarose (Invitrogen) for 2 hrs at 4 ℃. The beads were the washed two times in lysis buffer. The necrosulfonamide-bound agarose were then incubated with cell lysates overnight at 4 ℃. Non-biotinylated necrosulfonamide (or its derivatives) was included in the lysates as a binding competitor. The following day, the beads were washed four times with lysis buffer, then directly boiled in 1 x SDS loading buffer.

2) Synthesis of Sulfonamide analogues

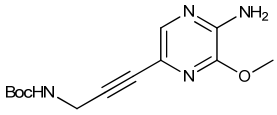
Pyrazine 2: To a solution of sodium hydride (1.7 eq.) suspended in DMF was added ROH (1.5 eq.). Stirring was continued at room temperature for 1.5 h. The aryl chloride **1** (1.0 eq.) was added rapidly. The reaction mixture was heated at 80 ℃ for 2 h. After all starting material had been consumed, as monitored by TLC, the mixture was allowed to cool to room temperature and the volatiles were removed *in vacuo*. The residue obtained was dissolved in DCM, washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude material was purified by silca gel chromatography eluting with petrol ether: ethyl acetate (1:1) to afford the pyrazine (51%~95%).



2b

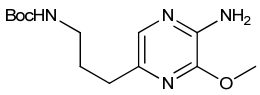
3-(prop-2-yn-1-yloxy)pyrazin-2-amine (2b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (t, *J* = 2.4 Hz, 1H), 4.84 (br, 2H), 5.00 (d, *J* = 2.4 Hz, 2H), 7.42 (d, *J* = 3.2 Hz, 2H), 7.59 (d, *J* = 3.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.9, 145.3, 134.5, 128.8, 78.4, 74.9, 53.5; mp. 98-100 ^{o}C; IR (neat) νmax: 3484, 3257, 3061, 2935, 2129, 1636, 1489 cm^{-1};HRMS (ESI) [M + H$^+$] calculated for $C_7H_8N_3O$: 150.06619, found: 150.06594

Bromide 3: N-Bromosuccinimide (1.03 eq.) was added portion wise over 30 minutes to a solution of 3-methoxypyrazin-2-amine**2** (1 eq.) in DCM at 0 ℃. After 45 minutes at 0 ℃, and 2 hours at room temperature, the mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The brown residue was purified by silica gel chromatography, eluting with petrol ether: ethyl acetate (6:1) to afford the aryl bromide (54%~91%) as a white solid.



11

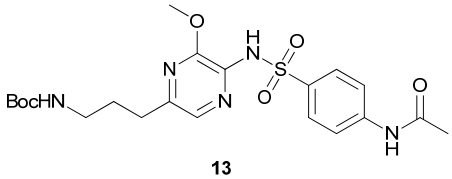
tert-butyl(3-(5-amino-6-methoxypyrazin-2-yl)prop-2-yn-1-yl)carbamate (11): To a degassed solution of aryl bromide **3** (1.0 eq.) in triethylamine (8 mL) were added $PdCl_2(PPh_3)_2$ (0.04 eq.), CuI (0.06 eq.) and the alkyne (1.2 eq.). The mixture was stirred at 80 ℃ for 3h. After removal of the solvent, the residue was filtered, concentrated, and purified by silca gel chromatography on silca gel eluting with petroleum ether: ethyl acetate (5:1~2:1) to afford the alkyne **11** (78%~86%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 4.00 (s, 3H), 4.17 (d, *J* = 2.8 Hz, 2H), 5.01 (br, 2H), 7.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3, 147.5, 145.1, 137.7, 122.7, 85.1, 80.6, 80.0, 53.8, 31.2, 29.6, 28.3; mp. 109-110 ℃; IR (neat) νmax: 3338, 2977, 2225, 1698, 1616, 1488 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{13}H_{18}N_4O_3$: 279.14517, found: 279.14518.



12

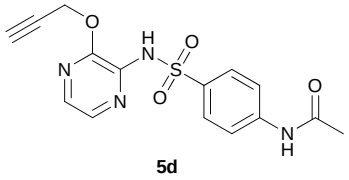
tert-butyl (3-(5-amino-6-methoxypyrazin-2-yl)propyl)carbamate (12): The alkyne **11** (1.0 eq.) was dissolved in 6mL of methanol and treated with 5% Pd/C (0.05 eq.). Then the reaction vessel was evacuated and back-filled with hydrogen (H_2 balloon). The reaction mixture was stirred under hydrogen at room temperature for overnight and then filtered over a plug of silica gel topped with Celite (MeOH eluent) to afford the desired product **12** (99%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.83 (m, 2H), 2.57 (t, *J* = 7.6 Hz, 2H), 3.16 (m, 2H), 3.95 (s, 3H), 4.69 (br, 2H), 4.86 (br, 1H), 7.35 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 148.0, 143.5, 140.8, 131.1, 79.0, 53.2, 40.1, 30.9, 29.4, 28.4; mp. 107-109 ℃; IR (neat) νmax: 3326, 2975, 1688, 1618, 1477 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{13}H_{23}N_4O_3$: 283.17647, found: 283.17641.

Sulfonamide 5a-5b, **13:** The aniline (1.0 eq.) was dissolved in pyridine and the resulting solution was cooled in an ice bath. The sulfonyl chloride (1.2 eq.) was added slowly to the solution. The reaction mixture was warmed to 60℃ for overnight. After all starting material had been consumed, monitored by TLC, the mixture was allowed to cool to room temperature and then saturated aqueous $NaHCO_3$ was added. The mixture was extracted with ethyl acetate (3 times), and the organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silca gel chromatography eluting with petroleum ether: ethyl acetate (1:1~1:3) to afford the desired sulfonamide (77%).



13

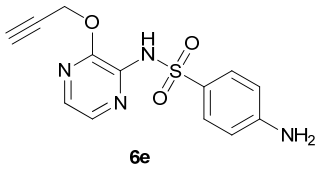
tert-butyl (3-(5-(4-acetamidophenylsul-fonamido)-6-methoxy-pyrazin-2-yl)propyl)carbamate (13): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.79 (m, 2H), 2.14 (s, 3H), 2.56 (t, *J* = 7.6 Hz, 2H), 3.10 (m, 2H), 3.91 (s, 3H), 4.81 (br, 1H), 7.50 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.72 (s, 1H), 7.99 (d, *J* = 8.8 Hz, 2H), 8.41 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 156.1, 148.1, 146.5, 142.9, 135.3, 133.7, 131.5, 129.4, 118.9, 79.2, 53.8, 40.0, 31.0, 29.1, 28.3, 24.5; mp. 105-106 ℃; IR (neat) νmax: 2976, 1684, 1532, 1469 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{21}H_{30}N_5O_6S$: 480.19113, found: 480.19156.



5d

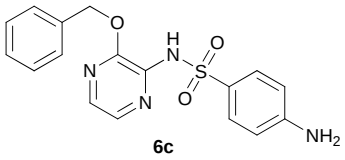
N-(4-(N-(3-(prop-2-yn-1-yloxy)pyrazin-2-yl)sulfamoyl)phenyl)acetamide (5d): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.07 (s, 3H), 3.57 (t, *J* = 2.4 Hz, 2H), 5.00 (d, *J* = 2.4 Hz, 2H), 7.74 (d, *J* = 4.4 Hz, 2H), 7.78 (s, 2H), 7.93 (d, *J* = 4.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.0, 152.6, 143.1, 141.4, 134.2, 133.9, 130.6, 128.7, 118.2, 78.8, 78.0, 53.8, 24.1; mp. 196-198 ℃; IR (neat) νmax: 3257, 3107, 2853, 2124, 1696, 1538 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{15}H_{15}N_4O_4S$: 347.08085, found: 347.08048

Aniline 6a-6e, 14: A suspension of acetyl amine **5a-5b** or **13** (1.0 eq.) in 3N of NaOH was heated to 105 ℃ to reflux for 4 h. Then the solution was cooled to 60~70 ℃, acidified to pH = 5~6. After cooling to room temperature, the precipitate was collected and washed with water (3 times) and petrol ether (3 times) to afford the desired product (80%~87%).



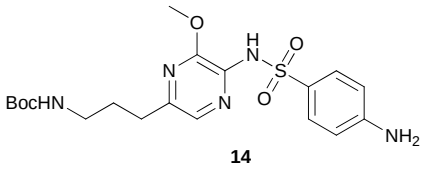
6e

4-amino-N-(3-(prop-2-yn-1-yloxy)pyrazin-2-yl)benzenesulfonamide (6e): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.52 (t, *J* = 2.4 Hz, 1H), 4.19 (br, 2H), 4.97 (d, *J* = 2.4 Hz, 2H), 6.65 (m, 2H), 7.65 (d, *J* = 2.8 Hz, 1H), 7.78 (d, *J* = 2.8 Hz, 1H), 7.91 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.2, 146.8, 137.9, 134.5, 133.3, 130.8, 113.5, 77.6, 75.7, 54.2; mp. 161-163 ℃; IR (neat) νmax: 3472, 3379, 3281, 3068, 2126, 1672, 1595, 1461 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{13}H_{13}N_4O_3S$: 305.07029, found: 305.07003.



6c

4-amino-*N*-(3-(benzyloxy)pyrazin-2-yl)benzenesulfonamide (6c): ^{1}H NMR (400 MHz, Acetone-d_6) δ 5.39 (s, 2H), 5.51 (br, 2H), 6.71 (d, *J* = 8.8 Hz, 2H), 7.35 (m, 3H), 7.47 (m, 2H),7.67 (d, *J* = 2.8 Hz, 1H), 7.73 (d, *J* = 2.8 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 9.17 (br, 1H);^{13}C NMR (100 MHz, Acetone-d^6) δ 153.57, 149.18, 139.16, 136.89, 133.86, 133.62, 130.94, 128.96, 138.85, 128.56, 126.88, 113.0668.52; mp. 173-174 ℃; IR (neat) νmax: 3476, 3373, 3240, 3064, 1625, 1445 cm^{-1}; HRMS (ESI) [M + H] $^+$ calculated for $C_{17}H_{17}N_4O_3S$: 357.10159, found: 357.10113.

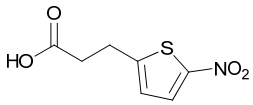


14

tert-butyl (3-(5-(4-aminophenylsulfonam-ido)-6-methoxypyrazin-2-yl)propyl)carbamate (14): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.81 (m, 2H), 2.59 (t, *J* = 7.6 Hz, 2H), 3.13 (m, 2H), 3.94 (s, 3H), 4.16 (s, 1H), 4.69 (br, 1H), 6.64 (m, 2H), 7.48 (br, 1H), 7.57 (s, 1H), 7.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 151.4, 147.9, 145.8, 135.7, 131.5, 130.3, 126.8, 113.4, 79.0, 53.6, 39.9, 31.0, 29.1, 28.3; mp. 102-104 ℃; IR (neat) νmax: 3364, 3242, 2977, 1691, 1594, 1454 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{19}H_{28}N_5O_5S$: 438.18057, found: 438.18062.

Aniline 6d: The benzyl ether **6c** (50 mg, 0.14 mmol) was dissolved in 2 mL of methanol and treated with 5% Pd/C (10 mg). Then the reaction vessel was evacuated and back-filled with hydrogen (H_2 balloon). The reaction mixture was stirred under hydrogen at room temperature for 2 h and then filtered over a plug of silica gel topped with Celite (MeOH eluent) to afford 17 mg (45%) of the desirred product as a white solid.

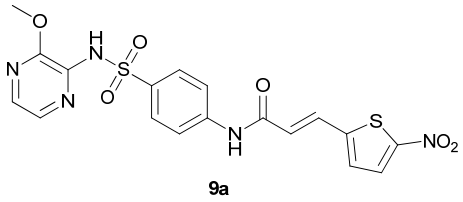
Acid 7[1]



7a

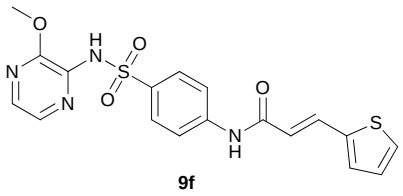
3-(5-nitrothiophen-2-yl)propanoic acid 7a:^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 (t, *J* = 7.2 Hz, 2H), 3.17 (t, *J* = 7.2 Hz, 2H), 6.84 (d, *J* = 4.4 Hz, 1H), 7.77 (d, *J* = 4.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 151.9, 128.8, 125.0, 34.9, 29.7, 25.8, ; mp. 69-71 °C; IR (neat) νmax: 3108, 2919, 2851, 1712, 1497 cm^{-1}; HRMS (ESI) [M + H^+] calculated for $C_7H_6NO_4S$: 200.00230, found: 200.00172

Sulfonamide 9a-9l, **15a**, **15b:** $SOCl_2$ (10 eq.) was added to the carboxylic acid (1.5 eq.) in toluene with stirring. Then the reaction mixture was warmed to 110 ℃ to reflux for 5 h. After removal of the solvent, the acyl chloride **8a-8h** was dissolved in toluene and added dropwise to the aniline **6a-6e** or **14** (1 eq.) dissolved in DMF containing $NaHCO_3$ (5 eq.) and 4 Å MS at 0℃. Stirring was continued at room temperature for overnight. After all aniline had been consumed, monitored by TLC, the mixture was quenched with ice-water. The mixture was extracted with ethyl acetate, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silca gel chromatography eluting with petrol ether: ethyl acetate (1:2) to afford the amide (75%~93%).



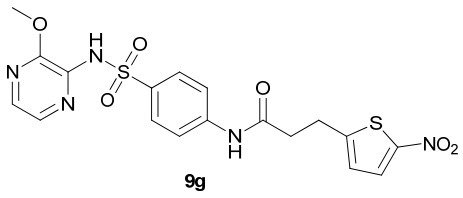
9a

(*E*)-*N*-(4-(*N*-(3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophen-2-yl)acrylamide (9a): ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (s, 3H), 6.86 (d, *J* = 15.6 Hz, 1H), 7.59 (d, *J* = 4.4 Hz, 1H), 7.74 (d, *J* = 4.8 Hz, 1H), 7.75 (s, 1H), 7.77 (s, 1H), 7.81 (d, *J* = 15.6 Hz, 2H), 7.85 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 8.15 (d, *J* = 4.4 Hz, 1H), 10.72 (s, 1H), 10.83 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.73, 150.64, 149.83, 146.44, 142.68, 137.92, 134.94, 134.22, 133.17, 132.50, 130.80, 130.42, 128.86, 125.50, 118.70, 53.68; mp. 288-289 ℃; IR (neat) νmax: 3331, 3104, 1689, 1591, 1538 cm^{-1}; HRMS (ESI) [M + H^+] calculated for $C_{18}H_{16}N_5O_6S_2$: 462.05365, found: 462.05357.



9f

(*E*)-*N*-(4-(*N*-(3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(thiophen-2-yl)acrylamide (9f): ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.90 (s, 3H), 6.59 (d, *J* = 15.6 Hz, 1H), 7.14 (t, *J* = 4.2 Hz, 1H), 7.48 (d, *J* = 3.0 Hz, 1H), 7.68 (d, *J* = 4.8 Hz, 1H), 7.73 (br, 1H), 7.75 (br, 1H), 7.79 (d, *J* = 15.6 Hz, 1H), 7.85 (d, *J* = 9.0 Hz, 2H), 7.97 (d, *J* = 9.0 Hz, 1H), 10.55 (s, 1H), 10.80 (br, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.68, 143.03, 139.42, 134.06, 131.72, 128.82, 128.79, 128.72, 128.71, 128.68, 128.45, 120.06, 118.37, 53.62; mp. 224-226 ℃; IR (neat) νmax: 3251, 3106, 1678, 1619, 1591 cm^{-1}; HRMS (ESI) [M + H^+] calculated for $C_{18}H_{17}N_4O_4S_2$: 417.06857, found: 417.06977.

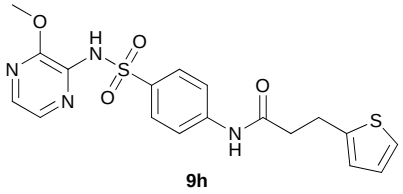


9g

***N*-(4-(*N*-(3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophen-2-yl)propanamide (9g)**: ^{1}H NMR (400 MHz, Ketone-d_6) δ 2.89 (t, *J* = 7.2 Hz, 3H), 3.28 (t, *J* = 7.2 Hz, 3H), 3.92 (s, 3H), 7.03 (d, *J* = 4.0 Hz, 1H), 7.69 (s, 1H), 7.70 (s, 1H), 7.82 (d, *J* = 7.2 Hz, 2H), 7.86 (d, *J* = 4.0 Hz, 1H), 8.06 (d, *J* = 7.2 Hz, 2H), 9.38 (br, 1H), 9.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.43, 154.21, 150.06, 143.86, 138.83, 135.11, 134.43, 133.68, 130.00, 129.72, 126.21, 118.82, 53.78, 37.86, 26.03; mp. 164-167 ℃; IR (neat) νmax: 3108, 2923, 2852, 1696, 1592 cm^{-1}; HRMS (ESI) [M + H^+]

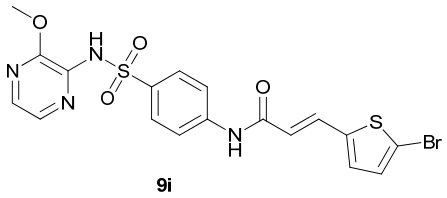
[1] Y. Taniguchi, H. Kato, *Chem. Pharm. Bull.*, **1973**, *21(9)*, 2070

calculated for $C_{18}H_{18}N_5O_6S_2$: 464.06930, found: 464.06876.



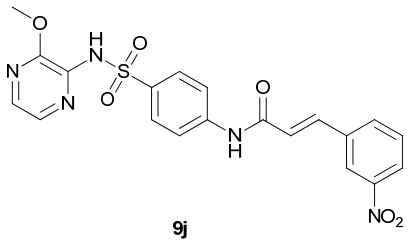
9h

***N*-(4-(*N*-(3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(thiophen-2-yl)propanamide (9h)**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.71 (t, *J* = 7.2 Hz, 3H), 3.12 (t, *J* = 7.2 Hz, 3H), 3.89 (s, 3H), 6.88 (m, 1H), 6.92 (m, 1H), 7.29 (m, 1H), 7.72 (m, 1H), 7.75 (d, *J* = 8.8 Hz, 2H), 7.93 (d, *J* = 8.8 Hz, 2H), 10.35 (s, 1H), 1078 9.38 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.58, 143.35, 142.98, 137.98, 134.30, 134.13, 133.16, 128.73, 126.85, 124.67, 123.80, 118.29, 118.20, 53.68, 38.09, 24.73; mp. 234-236 ℃; IR (neat) νmax: 3187, 2926, 1690, 1589, 1467 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{18}H_{19}N_4O_4S_2$: 419.08422, found: 419.08481



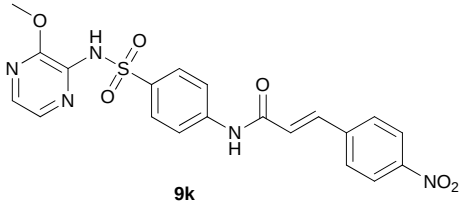
9i

(*E*)-3-(5-bromothiophen-2-yl)-*N*-(4-(*N*-(3-methoxypyrazin-2-yl)sulfamoyl)phenyl)acrylamide (9i): 1H NMR (400 MHz, Acetone-d_6) δ 3.93 (s, 3H), 6.54 (d, *J* = 15.6 Hz, 1H), 7.20 (d, *J* = 4.0 Hz 1H), 7.26 (d, *J* = 4.0 Hz, 1H), 7.70 (d, *J* = 4.4 Hz, 1H), 7.72 (d, *J* = 4.4 Hz, 1H), 7.76 (d, *J* = 15.6 Hz, 2H), 7.92 (d, *J*= 7.2 Hz, 2H), 8.08 (d, *J* = 7.2 Hz, 2H), 9.35 (br, 1H), 9.72 (br, 1H); ^{13}C NMR (100 MHz, Acetone-d^6) δ 163.4, 143.5, 141.8, 133.9, 133.6, 133.1, 131.8, 131.7, 129.5, 129.4, 120.7, 118.5, 118.4, 114.2, 53.2; IR (neat) νmax: 3234, 2954, 1670, 1591, 1532 cm^{-1}; mp. 219-221 ℃. HRMS (ESI) [M + H]$^+$ calculated for $C_{18}H_{16}BrN_4O_4S_2$: 494.97909, found: 494.97956.



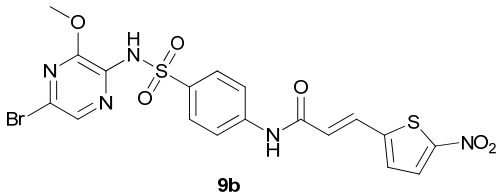
9j

(E)-N-(4-(N-(3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(2-nitrophenyl)acrylamide (9j): 1H NMR (400 MHz, DMSO-d_6) δ 3.91 (s, 3H), 7.02 (d, *J* = 15.6 Hz, 1H), 7.71-7.77 (m, 4H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.99 (d, *J* = 8.8 Hz, 2H), 8.07 (d, *J* = 7.6 Hz, 1H), 8.23 (d, *J* = 8.4 Hz, 2H), 8.47 (s, 1H), 10.66 (br, 1H), 10.84 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 163.4, 148.3, 142.9, 138.6, 136.3, 134.2, 130.6, 128.8, 124.6, 124.2, 121.8, 118.6, 53.7; mp. 280-282 ℃. IR (neat) νmax: 3336, 3207, 1671, 1592, 1527 cm^{-1}; HRMS (ESI) [M + H]$^+$ calculated for $C_{20}H_{18}N_5O_6S$: 456.09723, found: 456.09684.



9k

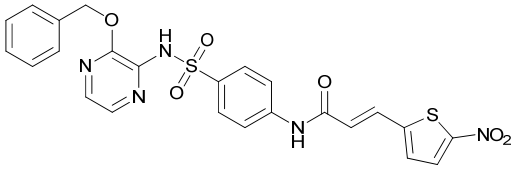
(*E*)-*N*-(4-(*N*-(3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(4-nitrophenyl)acrylamide (9k): 1H NMR (400 MHz, DMSO-d_6) δ 3.91 (s, 3H), 7.02 (d, *J* = 15.6 Hz, 1H), 7.74 (d, *J* = 15.6 Hz, 1H), 7.74 (br, 2H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 8.31 (d, J= 8.8 Hz, 2H), 10.74 (br, 1H), 10.86 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 163.4, 147.8, 142.8, 141.0, 138.6, 128.9, 126.0, 124.2, 117.7, 53.7; mp. 279-281 ℃. IR (neat) νmax: 2234, 2916, 1684, 1518 cm^{-1}; HRMS (ESI) [M + H]$^+$ calculated for $C_{20}H_{18}N_5O_6S$: 456.09723, found: 456.09705.



9b

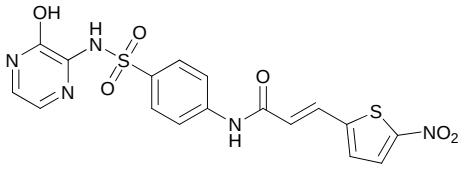
(E)-N-(4-(N-(6-bromo-3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophen-2-yl)acrylamide (9b): ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (s, 3H), 7.16 (d, *J* = 15.6 Hz, 1H), 7.56 (d, *J* = 4.4 Hz, 1H), 7.79 (d, *J* = 15.6 Hz,

1H), 7.91 (s, 1H), 7.93 (s, 4H), 8.13 (d, *J* = 4.4 Hz, 1H), 11.08 (br, 1H), 11.40 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 162.9, 150.6, 149.3, 146.7, 143.0, 134.0, 132.1, 130.8, 130.2, 128.7, 126.0, 118.6, 54.7; mp. 282-283 ℃. IR (neat) νmax: 3286, 3102, 2932, 1686, 1535, 1332 cm^{-1}; HRMS (ESI) [M + H]$^+$ calculated for $C_{20}H_{13}BrN_5O_6S_2$: 561.94851, found: 561.94755.



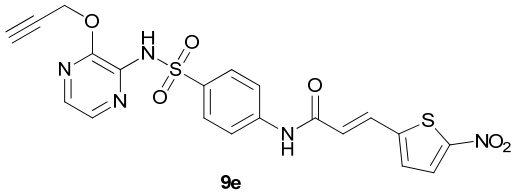
9c

(E)-N-(4-(N-(3-(benzyloxy)pyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophen-2-yl)acrylamide (9c): 1H NMR (400 MHz, DMSO-d_6) δ 5.40 (s, 2H), 6.86 (d, *J* = 15.6 Hz, 1H), 7.32-7.42 (m, 3H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.59 (d, *J* = 4.4 Hz, 1H), 7.78 (br, 2H), 7.82 (d, J = 15.6 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.99 (d, J = 8.8 Hz, 2H), 8.15 (d, J =4.4 Hz, 1H), 10.72 (s, 1H), 10.91 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d^6) δ 162.7, 150.6, 149.3, 146.4, 142.7, 137.9, 136.3, 135.0, 134.3, 133.4, 132.5, 130.8, 130.4, 128.8, 128.3, 128.1, 127.9, 125.5, 118.7, 67.7; mp. 238-239 ℃; IR (neat) νmax: 3322, 3089, 1683, 1589, 1534 cm^{-1}; HRMS (ESI) [M + H]$^+$ calculated for $C_{24}H_{20}N_5O_6S_2$: 538.08495, found: 538.08398.



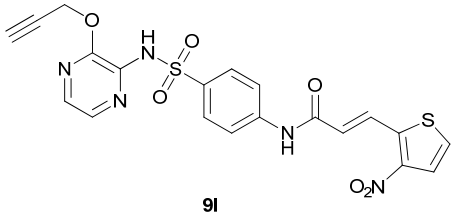
9d

(*E*)-*N*-(4-(*N*-(3-hydroxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophen-2-yl)acrylamide (9d): ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.85 (s, 1H), 6.88 (s, 1H), 6.97 (br, 1H), 7.59 (d, *J* = 4.4 Hz, 1H), 7.80 (s, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.96 (br, 2H), 8.15 (d, *J* = 4.4 Hz, 1H), 10.66 (br, 1H), 10.73 (br, 1H);^{13}C NMR (100 MHz, DMSO-d^6) δ 162.73, 150.64, 146.46, 142.67, 132.49, 130.81, 130.41, 128.78, 125.54, 118.69; mp .264-270 ℃; IR (neat) νmax: 3184, 3108, 2917, 2848, 1668, 1591 cm^{-1}; HRMS (ESI) [M + H]$^+$ calculated for $C_{17}H_{14}N_5O_6S_2$: 448.03800, found: 448.03792.



9e

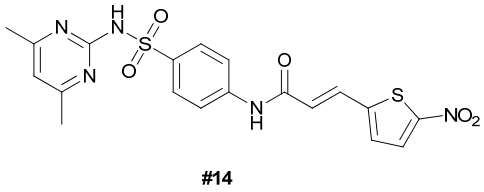
(E)-3-(5-nitrothiophen-2-yl)-N-(4-(N-(3-(prop-2-yn-1-yloxy)pyrazin-2-yl)sulfamoyl)phenyl)acrylamide (9e): ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.57 (t, *J* = 2.4 Hz, 1H), 5.01 (d, *J* = 2.4 Hz, 2H), 6.86 (d, *J* = 15.6 Hz, 1H), 7.59 (d, *J* = 4.0 Hz, 1H), 7.78-7.86 (m, 4H), 8.16 (d, *J* = 4.0 Hz, 1H), 10.72 (s, 1H), 11.00 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.7, 150.6, 146.4, 142.6, 132.5, 130.8, 130.4, 128.8, 125.5, 118.7, 78.8, 77.9, 53.8; mp. 205-207 ℃; IR (neat) νmax: 3284, 3106, 2924, 2852, 2126, 1687, 1538 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{20}H_{16}N_5O_6S_2$: 486.05365, found: 486.05326.



9l

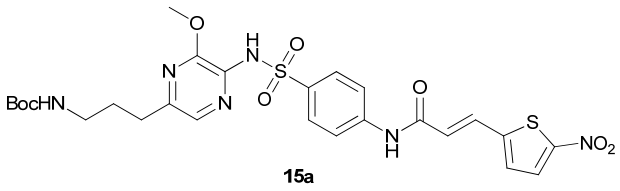
(E)-3-(3-nitrothiophen-2-yl)-N-(4-(N-(3-(prop-2-yn-1-yloxy)pyrazin-2-yl)sulfamoyl)phenyl)acrylamide (9l): ^{1}H NMR (400 MHz, Acetone-d_6) δ 3.08 (t, *J* = 2.4 Hz, 1H), 5.02 (d, *J* = 2.4 Hz, 1H), 6.92 (d, *J* = 15.6 Hz, 1H), 7.72 (m, 2H), 7.73 (d, *J* = 3.2 Hz, 1H), 7.79 (d, *J* = 3.2 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 2H), 8.12 (d, *J* = 8.8 Hz, 2H), 8.49 7.99 (d, *J* = 15.6 Hz, 1H), 9.90 (br, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 163.1, 143.8, 140.5, 131.3, 130.1, 127.8, 127.0, 125.9, 119.2, 76.4, 54.6, 54.3; mp.

235-237 ℃; IR (neat) νmax: 3354, 2922, 1661, 1592, 1537 cm^{-1}; HRMS (ESI) $[M + H]^+$ calculated for $C_{20}H_{15}N_5O_6S_2$: 486.05365, found: 486.05374.



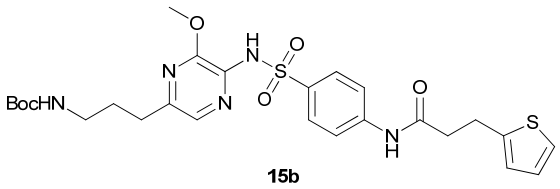
#14

***(E)*-*N*-(4-(*N*-(4, 6-dimethylpyrimidin-2-yl)sulfamoyl) phenyl)-3-(5-nitrothiophen-2-yl)-acrylamide (#14)**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 6H), 6.75 (s, 1H), 6.85 (d, *J* = 15.6 Hz, 1H), 7.58 (d, *J* = 4.8 Hz, 1H), 7.81 (d, *J* = 15.6 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 2H), 8.14 (d, *J* = 4.4 Hz, 1H), 10.70 (s, 1H), 11.64 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.70, 156.18, 150.64, 146.50, 142.41, 135.05, 132.44, 130.85, 130.45, 129.41, 125.57, 118.34, 113.50, 54.94, 22.89; mp. 288-290 ℃; IR (neat) νmax: 3257, 3104, 1686, 1592, 1536 cm^{-1}; HRMS (ESI) $[M + H^+]$ calculated for $C_{19}H_{18}N_5O_5S_2$: 460.07439, found: 460.07490.



15a

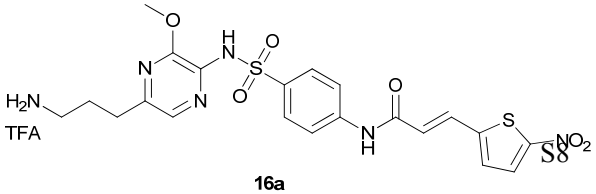
***(E)*-tert-butyl (3-(6-meth-oxy-5-(4-(3-(5-nitrothiophen-2-yl)acrylamido)phenylsulfonamido)pyrazin-2-yl)propyl)carbamate (15a)**: ^{1}H NMR (400 MHz, Acetone-d_6) δ 1.38 (s, 9H), 1.84 (m, 2H), 2.63 (t, *J* = 7.6 Hz, 2H), 3.12 (m, 2H), 3.94 (s, 3H), 5.96 (br, 1H), 6.96 (d, *J* = 15.6 Hz, 2H), 7.51 (d, *J* = 4.4 Hz, 1H), 7.62 (s, 1H), 7.83 (d, *J* = 15.6 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 2H), 8.01 (d, *J* = 4.4 Hz, 1H), 8.08 (d, *J* = 8.8 Hz, 2H), 10.01 (br, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 164.2, 157.3, 152.9, 150.4, 148.3, 147.9, 144.6, 136.9, 134.2, 132.9, 131.3, 131.2, 130.9, 126.9, 126.8, 120.2, 120.1, 79.0, 54.6, 41.2, 32.4, 29.2; mp. 205-207 ℃; IR (neat) νmax: 1684, 1592, 1538, 1331 cm^{-1};HRMS (ESI) $[M + H^+]$ calculated for $C_{26}H_{31}N_6O_8S_2$: 619.16393, found: 619.16467.



15b

***tert*-butyl (3-(6-methoxy-5-(4-(3-(thiophen-2-yl) propanamido)phenylsulfonamido)pyrazin-2-yl)propyl)carbamate (15b)**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.81 (m, *J* = 7.2 Hz, 2H), 2.58 (t, *J* = 7.6 Hz, 2H), 2.73 (t, *J* = 7.6 Hz, 2H), 3.11 (q, *J* = 6.4 Hz, 2H), 3.24 (t, *J* = 7.2 Hz, 2H), 3.94 (s, 3H), 4.71 (br, 1H), 6.83 (m, 1H), 6.91 (dd, J_1 = 5.2 Hz, J_2 =3.2 Hz, 1H), 7.13 (dd, J_1 = 5.2 Hz, J_2 = 1.2 Hz, 1H), 7.53 (br, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.71 (s, 1H), 8.02 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 156.0, 148.0, 146.5, 142.8, 142.4, 135.3, 134.0, 131.7, 129.6, 127.0, 125.0, 123.7, 119.0, 113.5, 79.2, 53.9, 40.0, 39.4, 31.1, 29.4, 28.4, 25.4; colorless oil; IR (neat) νmax: 2977, 1681, 1592, 1530, 1166 cm^{-1}; HRMS (ESI) $[M + H^+]$ calculated for $C_{26}H_{34}N_5O_6S_2$: 576.19450, found: 576.19448.

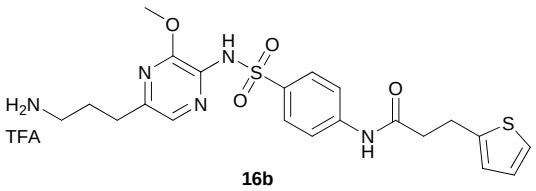
Amine 16a, 16b: The *N*-BOC amine **15a-15b** was treated with 10% (v/v) trifluoroacetic acid (TFA) in DCM in a round-bottomed flask under an argon atmosphere at 0 ℃. The reaction mixture was stirred at room temperature for 1.5 h. After all aniline had been consumed, monitored by TLC, the solvent was removed *in vacuo* and dried ether was added. The resulting yellow powder was filtered and collected to afford the amine (quant.) as a TFA salt.



16a

***(E)*-*N*-(4-(*N*-(5-(3-aminopropyl)-3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophen-2-yl)acrylamide (16a)**: ^{1}H NMR (400 MHz,

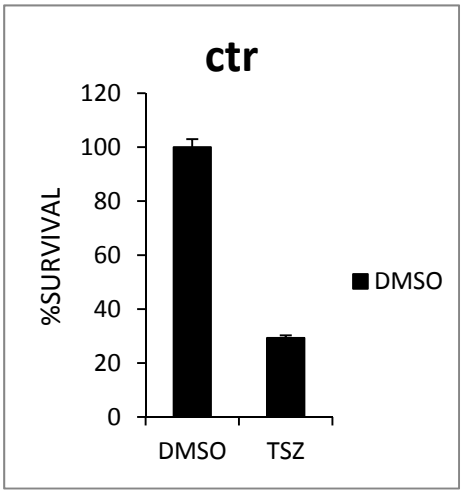
DMSO-d_6) δ 1.88 (m, 2H), 2.63 (t, J = 7.6 Hz, 2H), 2.81 (m, 2H), 3.90 (s, 3H), 6.88 (m, 2H), 7.59 (d, J = 4.0 Hz, 1H), 7.65 (s, 1H), 7.69 (br, 2H), 7.79 (s, 1H), 7.84 (m, 2H), 7.96 (m, 2H), 8.15 (d, J = 4.0 Hz, 2H), 10.72 (br, 1H), 10.78 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 150.7, 149.4, 146.4, 145.7, 142.6, 135.9, 135.2, 132.5, 131.4, 130.9, 130.5, 128.8, 125.5, 118.7, 53.5, 38.3, 29.9, 26.3; mp. 195-197 ℃; IR (neat) νmax: 3110, 1673, 1625, 1537, 1169 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{21}H_{23}N_6O_6S_2$: 519.11150, found: 519.11179

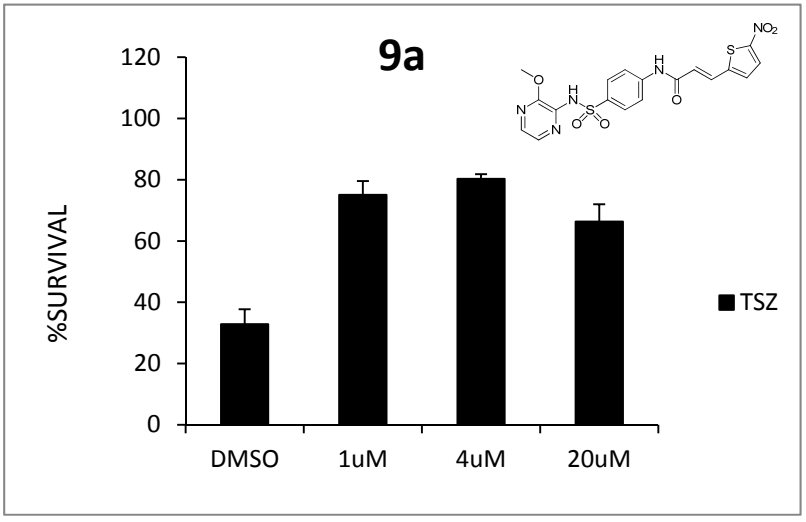


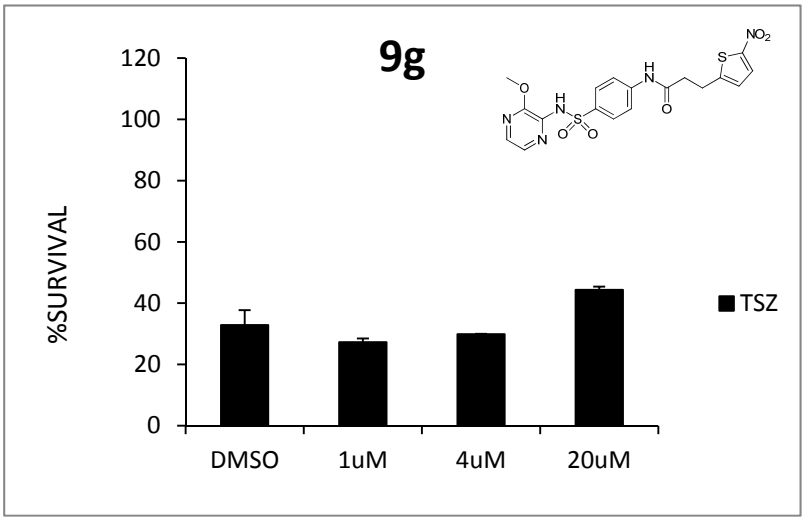
16b

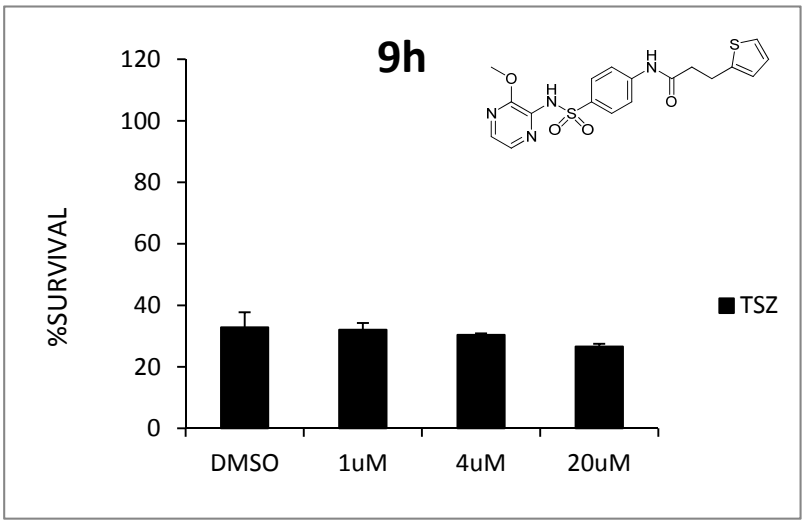
***N*-(4-(*N*-(5-(3-aminopropyl)-3-methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(thiophen-2-yl)propanamide (16b)**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.91 (m, J = 7.2 Hz, 2H), 2.42 (t, J = 6.4 Hz, 2H), 2.67 (t, J = 7.6 Hz, 2H), 2.71 (m, 2H), 3.11 (t, J = 7.6 Hz, 2H), 3.32 (s, 3H), 6.88 (dd, J_1 = 3.6 Hz, J_2 = 1.2 Hz, 1H), 6.92 (dd, J_1 = 5.2 Hz, J_2 = 3.6 Hz, 1H), 7.17 (s, 1H), 7.30 (dd, J_1 = 5.2 Hz, J_2 = 1.2 Hz, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H), 7.95 (br, 2H), 10.06 (s,1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 150.8, 145.4, 143.5, 140.5, 140.1, 136.4, 130.9, 127.7, 126.8, 124.6, 123.8, 117.6, 51.7, 38.1, 29.6, 26.4, 24.9; mp. 194-197 ℃; IR (neat) νmax: 2955, 2916, 2848, 1654, 1557, 1459 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{21}H_{26}N_5O_4S_2$: 476.14207, found: 476.14212.

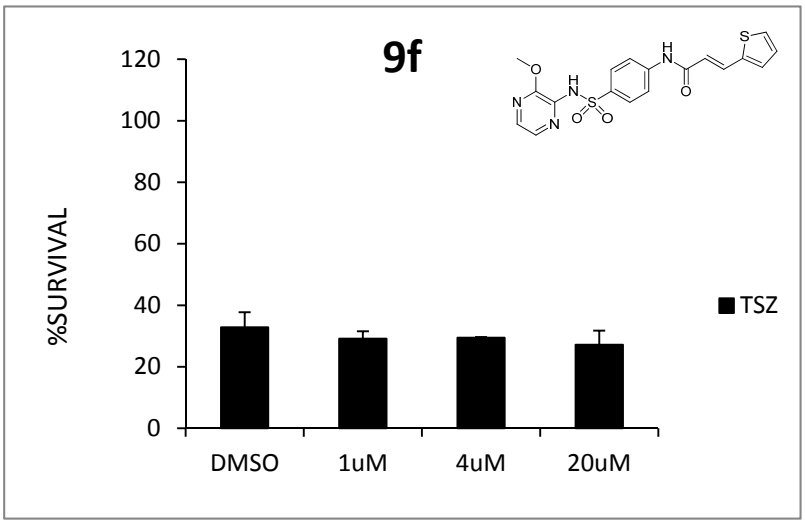
3) Biological evaluation of sulfonamide analogues

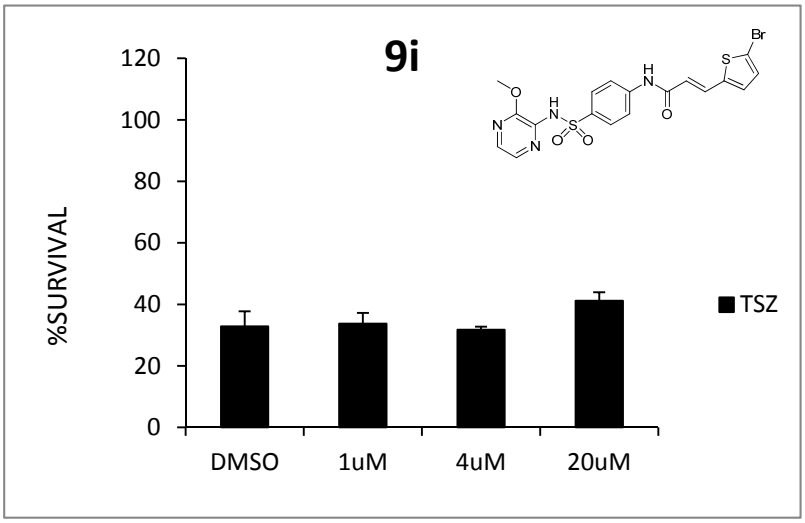


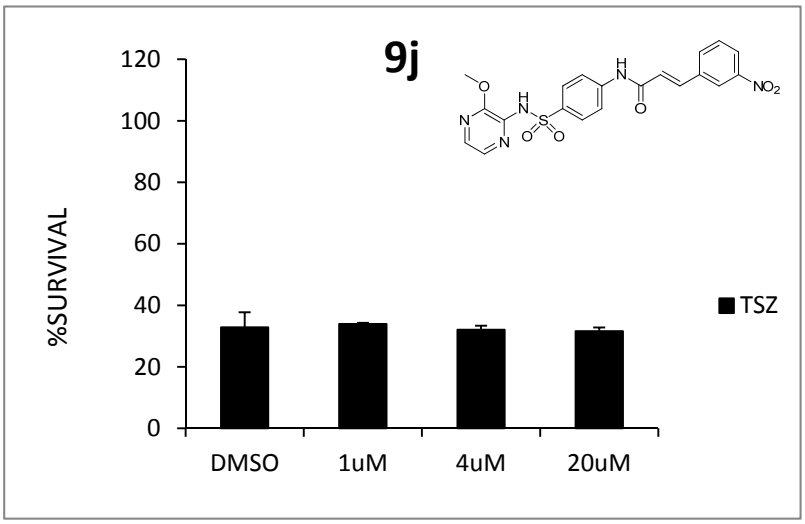


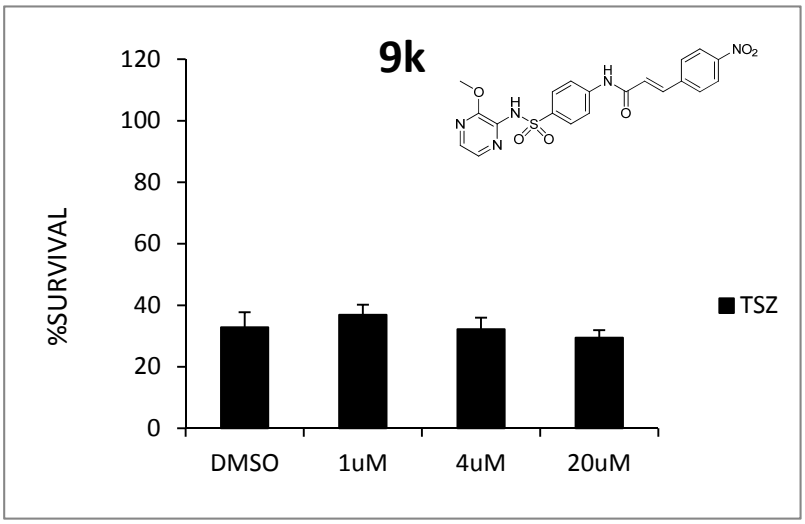


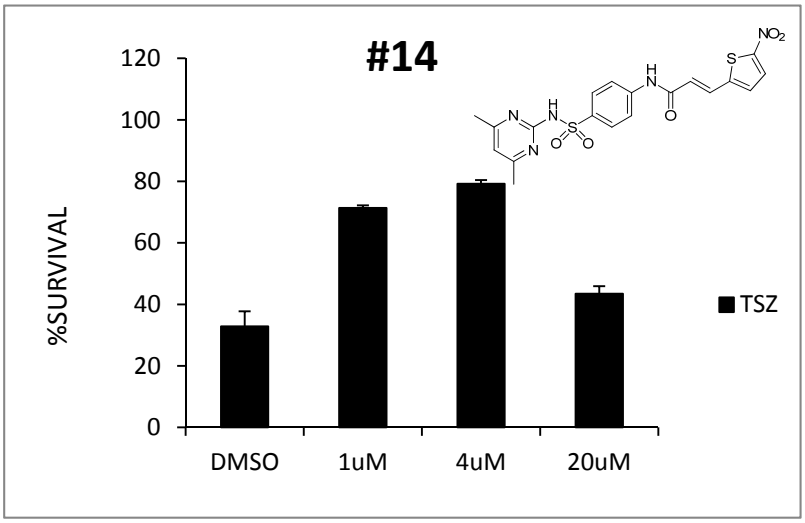


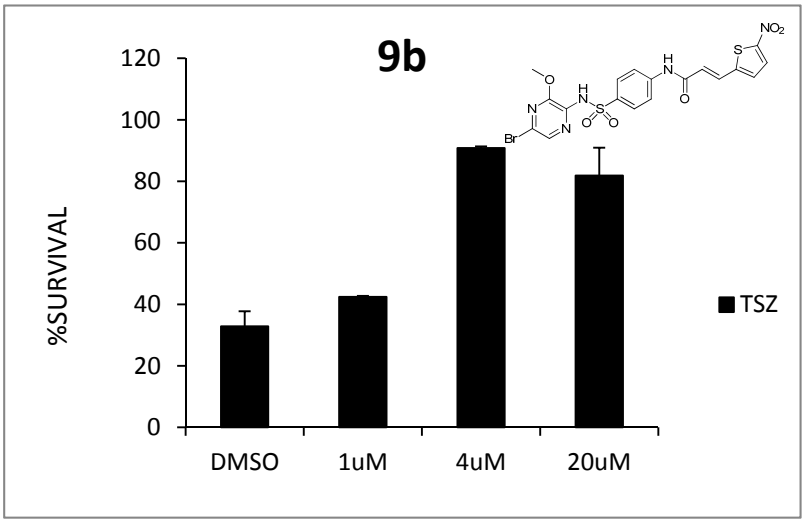


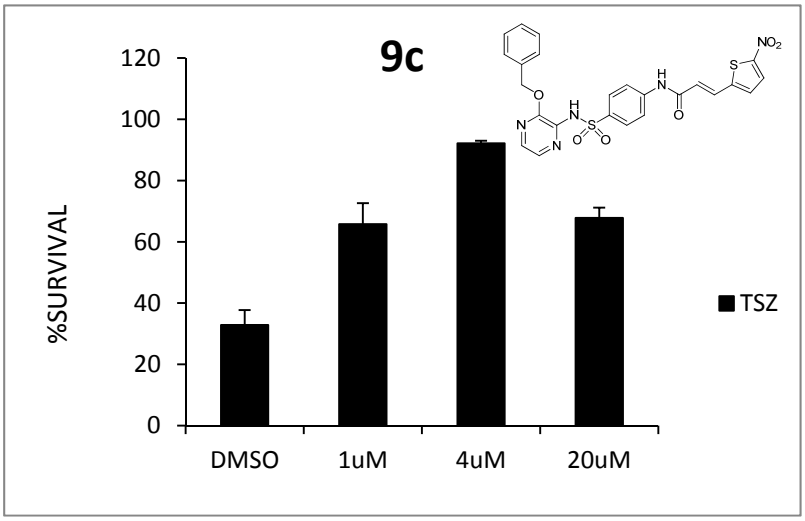


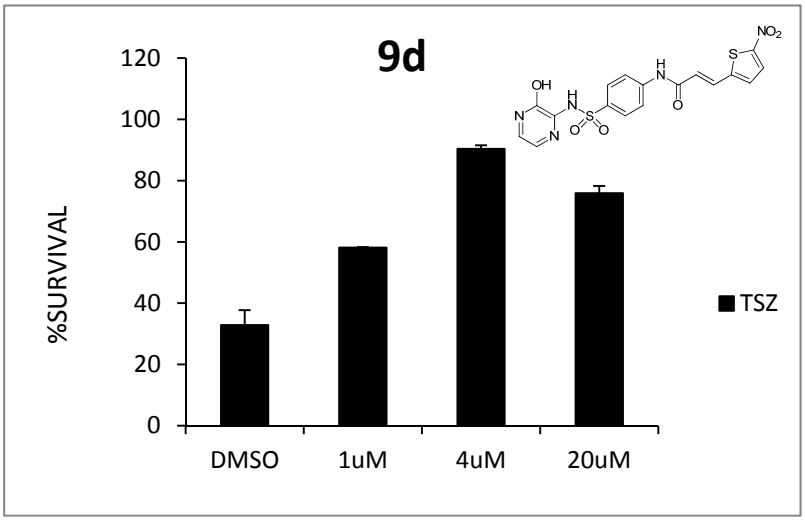


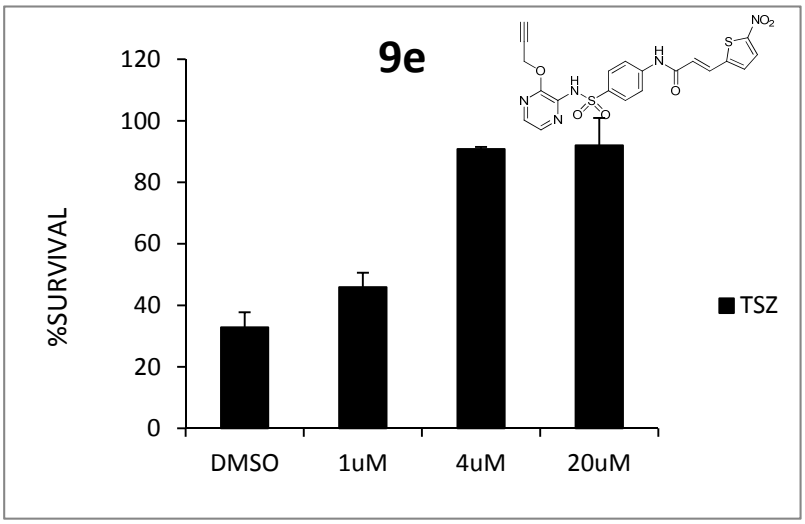


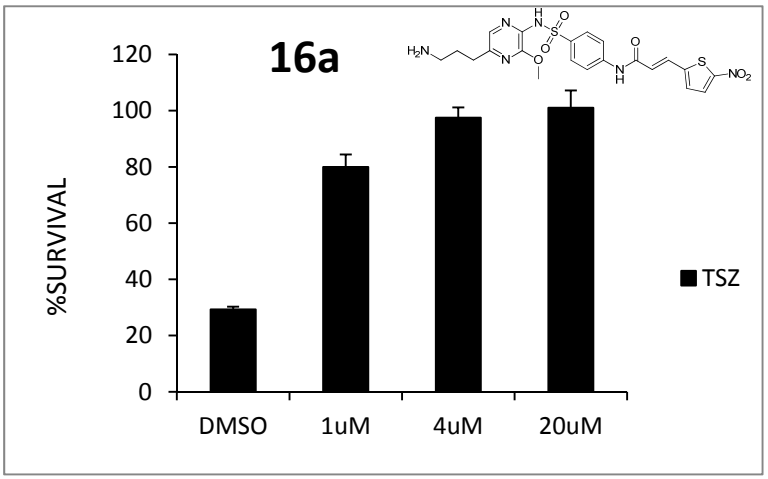


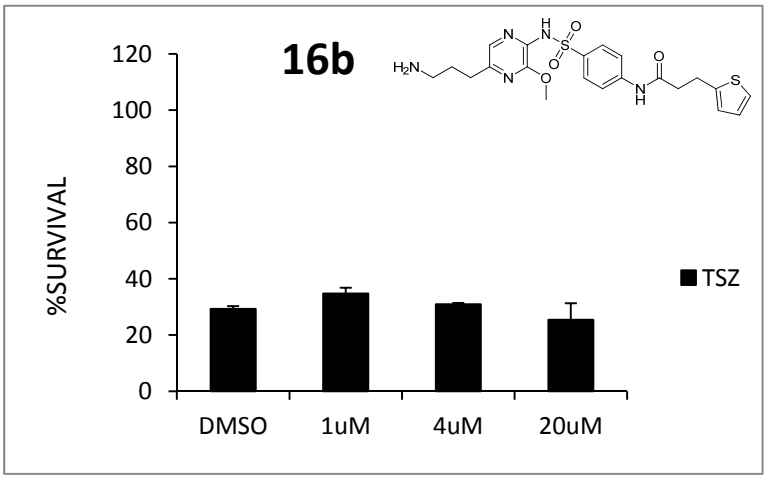








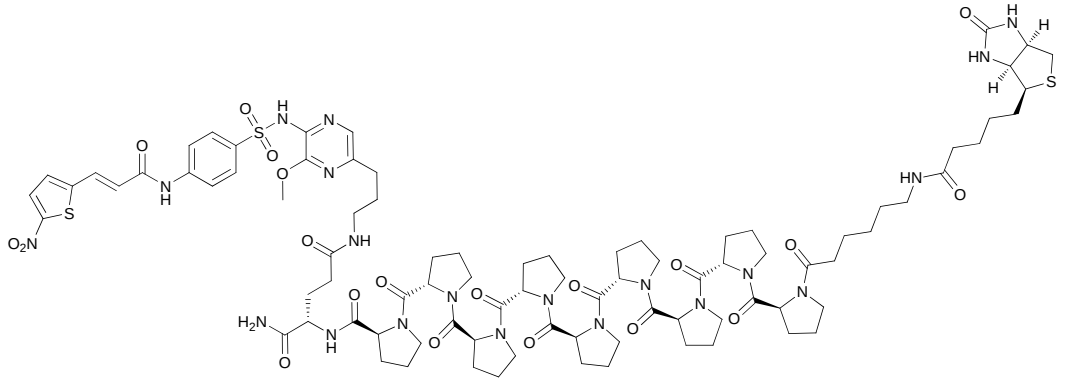




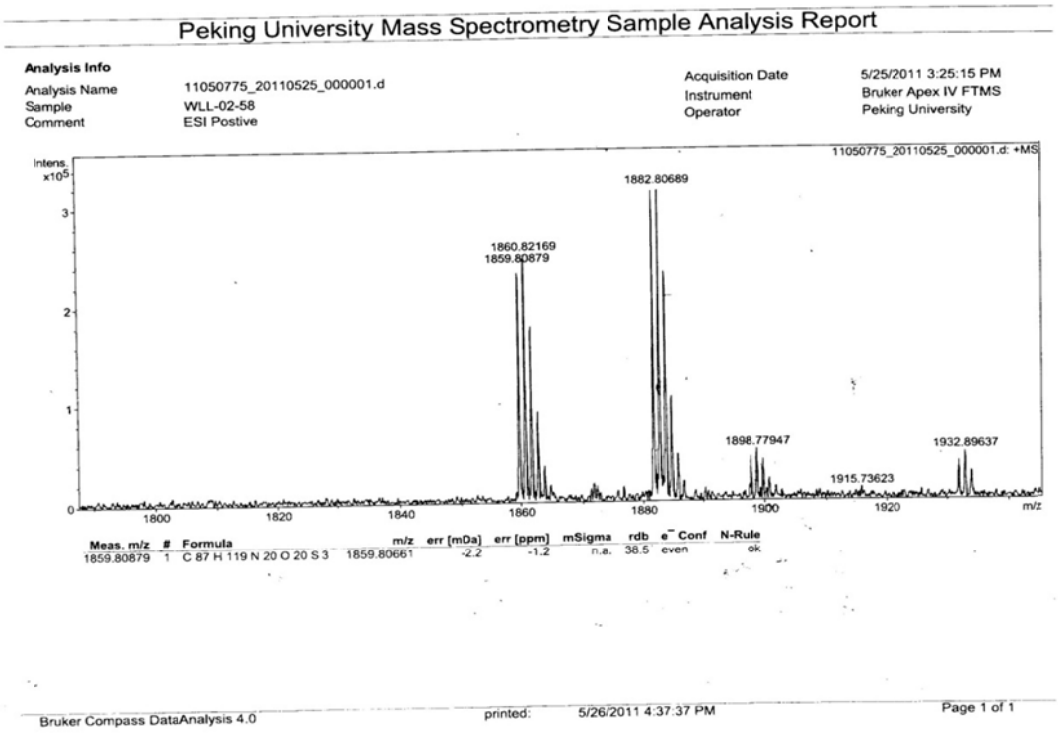
4) Synthesis and biological evaluation of the chemical probes

Synthesis of the chemical probes:

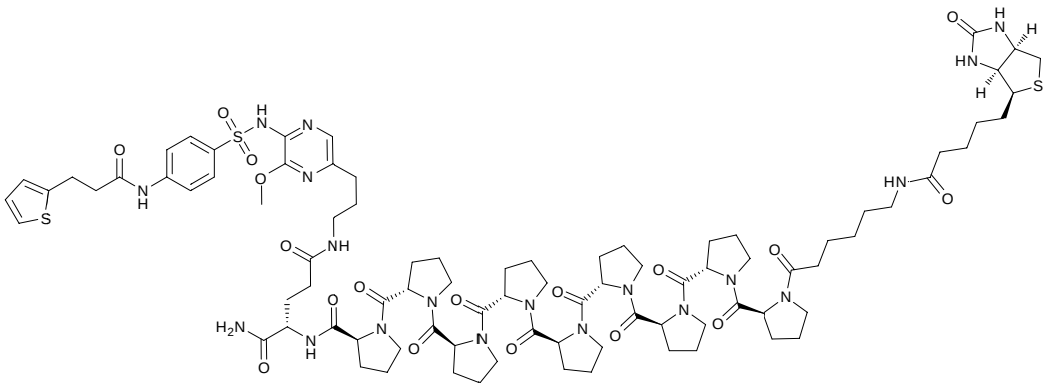
Biotin-LC-proline acid **17** (14 mg, 0.01 mmol) and primary amine **16a** or **16b** (16 mg, 0.03 mmol) were dissolved in 1 mL of DMF at room temperature under the argon atmosphere. DIPEA(17 μl, 0.1 mmol) was added to the solution, and followed by HATU(12 mg, 0.03 mmol). The resulting solution was stirred at room temperature for 18 hours. The solvents were removed under high vacuum and the residue was purified by reversed-phase HPLC [X-brige C18, 4.6x150 mm, UV 377 nm, CH3CN/0.5%TFA in H_2O gradient 10:90~30:70 (0-3 min) 30:70~45:55(12-15min) 45:55~100:0 (15-20 min), 1.0 mL/min] to afford the desired probe as a colorless oil (6 mg , yield 32%).



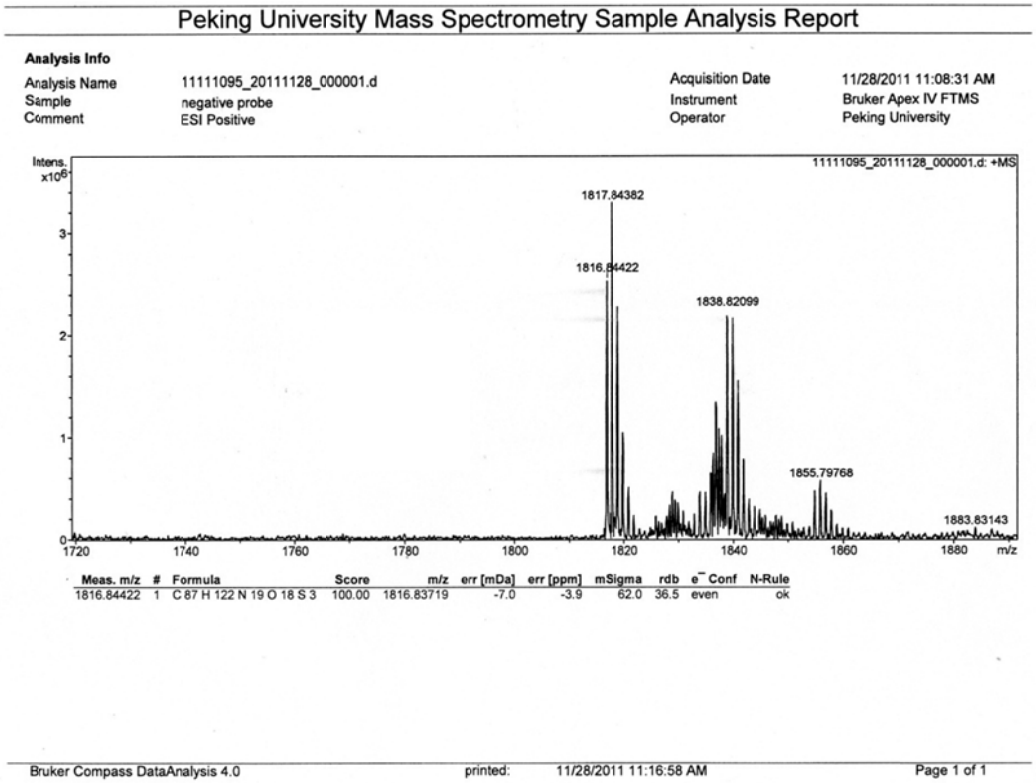
18a



HRMS (ESI) [M + H^+] calculated for $C_{87}H_{119}N_{20}O_{20}S_3$: 1859.80661, found: 1859.80879

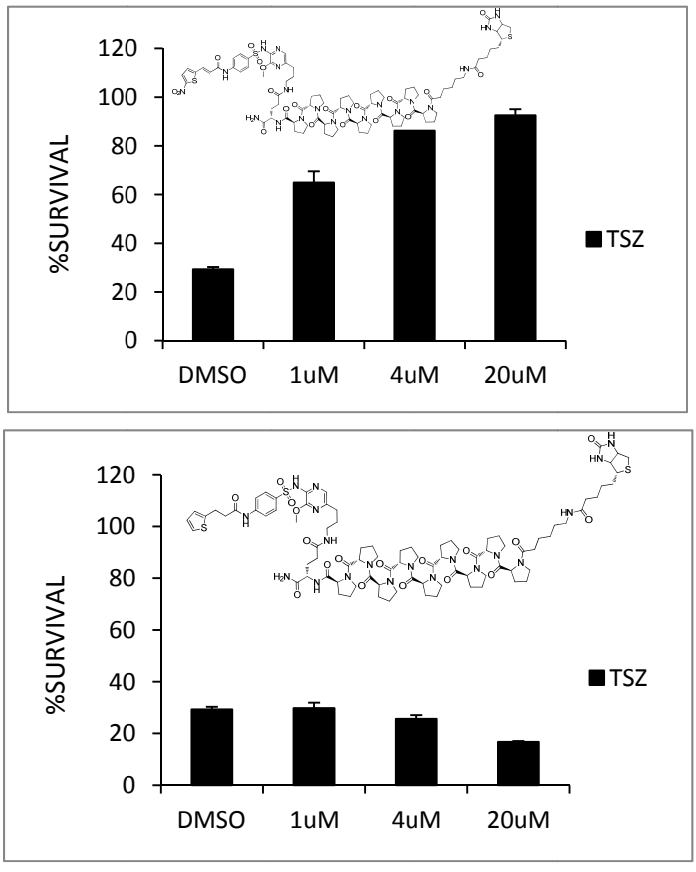


18b

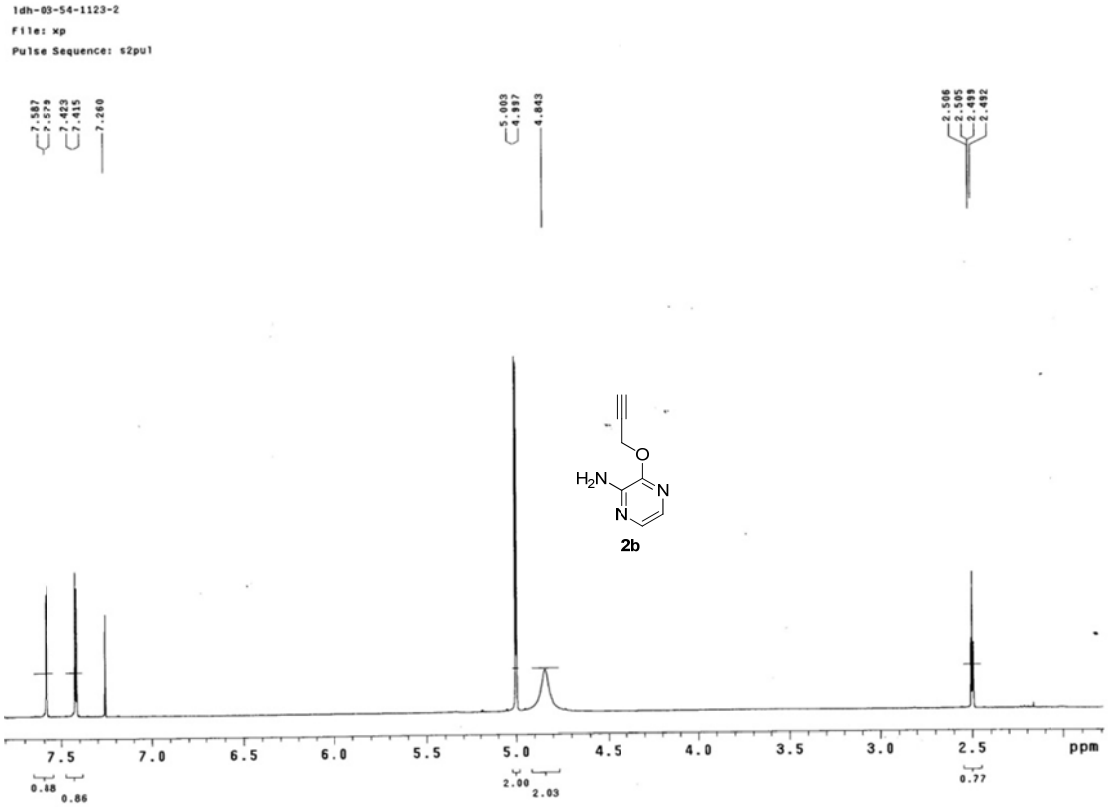


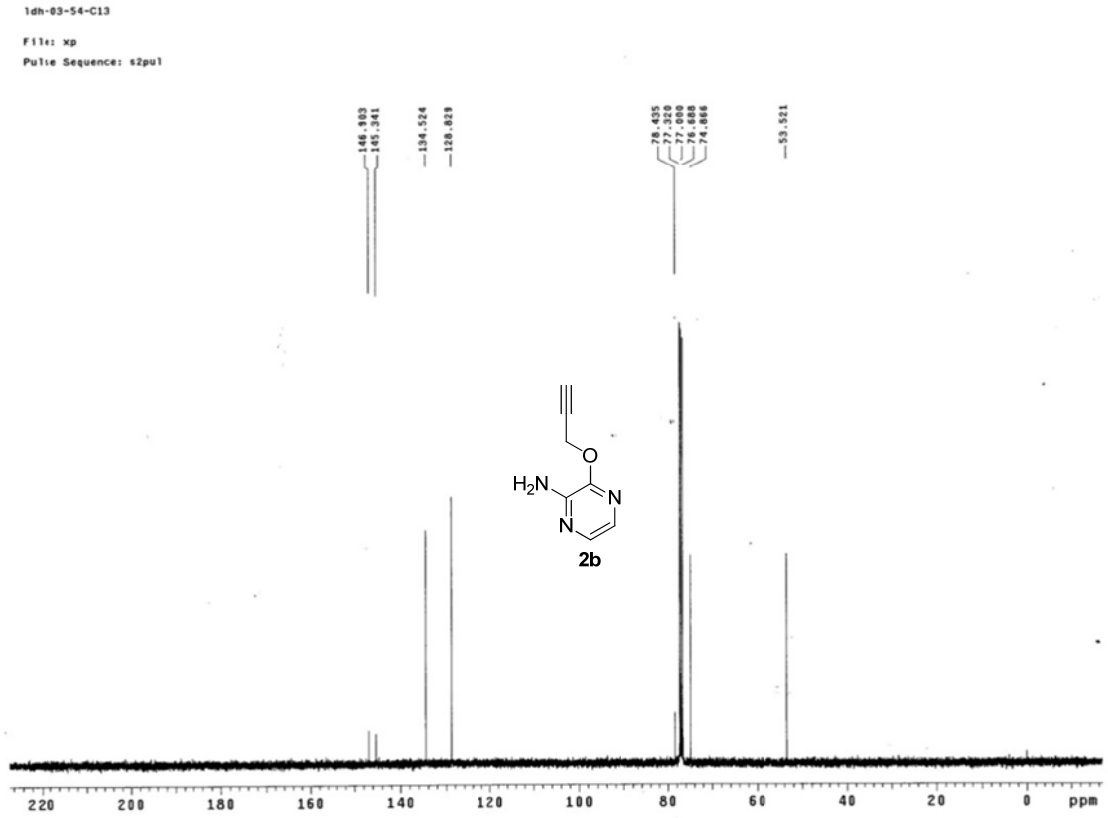
HRMS (ESI) $[M + H^+]$ calculated for $C_{87}H_{122}N_{19}O_{18}S_3$: 1816.83719, found: 1816.84422

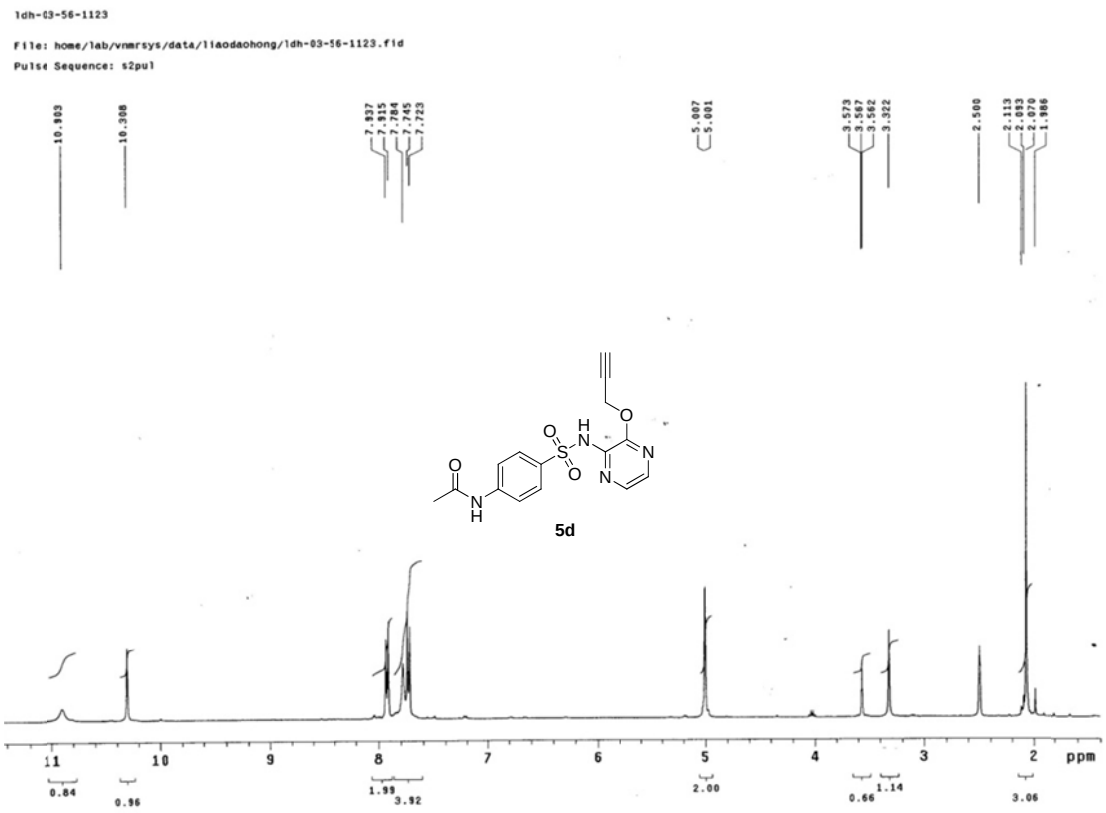
Biological evaluation of the chemical probes:



^{1}H and ^{13}C NMR spectra



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7.937
7.915
7.784
7.745
7.723
5.007
5.001
3.573
3.567
3.562
3.322
2.560
2.113
2.083
2.070
1.986
5d
11
10
9
8
7
6
5
4
3
2
ppm
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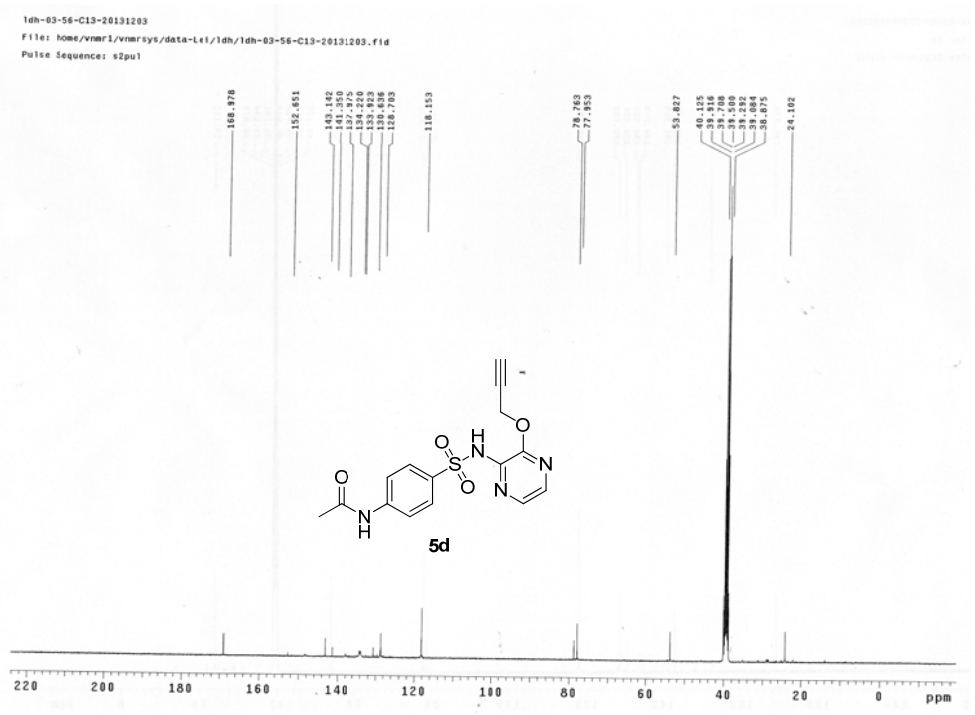
0.96
1.98
3.92
2.00
0.66
1.14
3.06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141.350
137.975
134.220
133.925
130.636
128.703
118.153
79.763
77.953
53.827
40.125
39.916
39.708
39.500
39.292
39.084
38.875
24.102
5d
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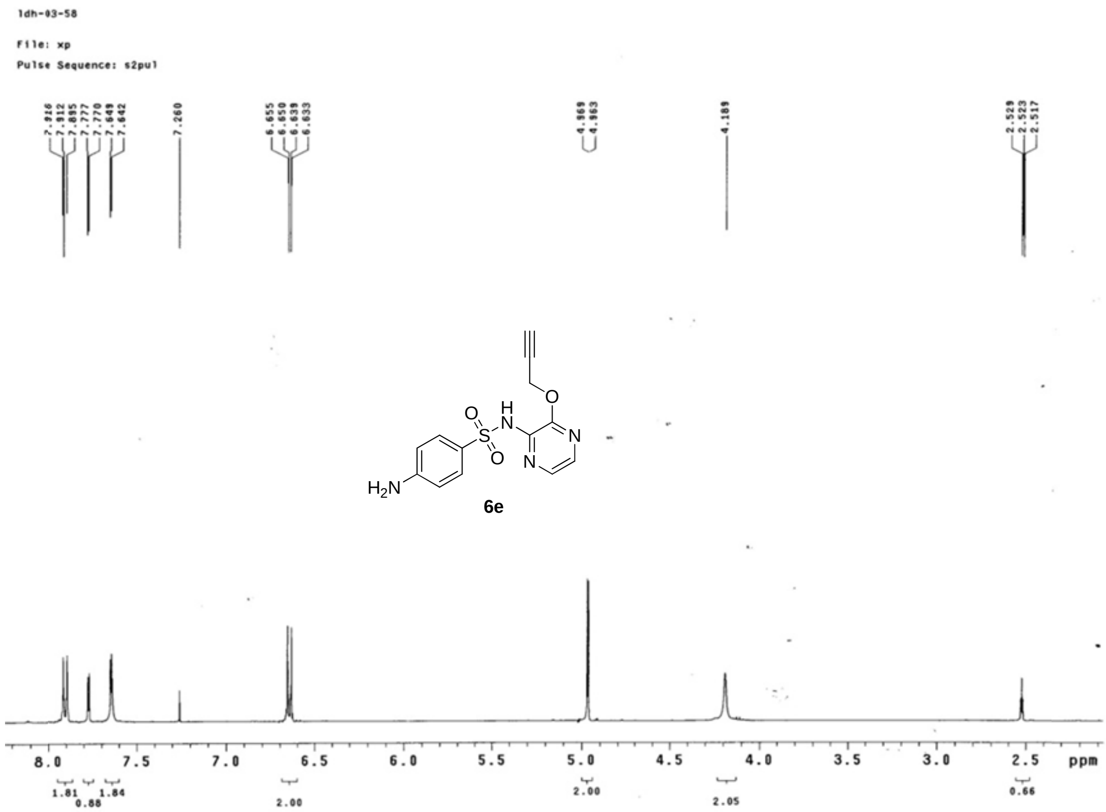
200
180
160
140
120
100
80
60
40
20
0
ppm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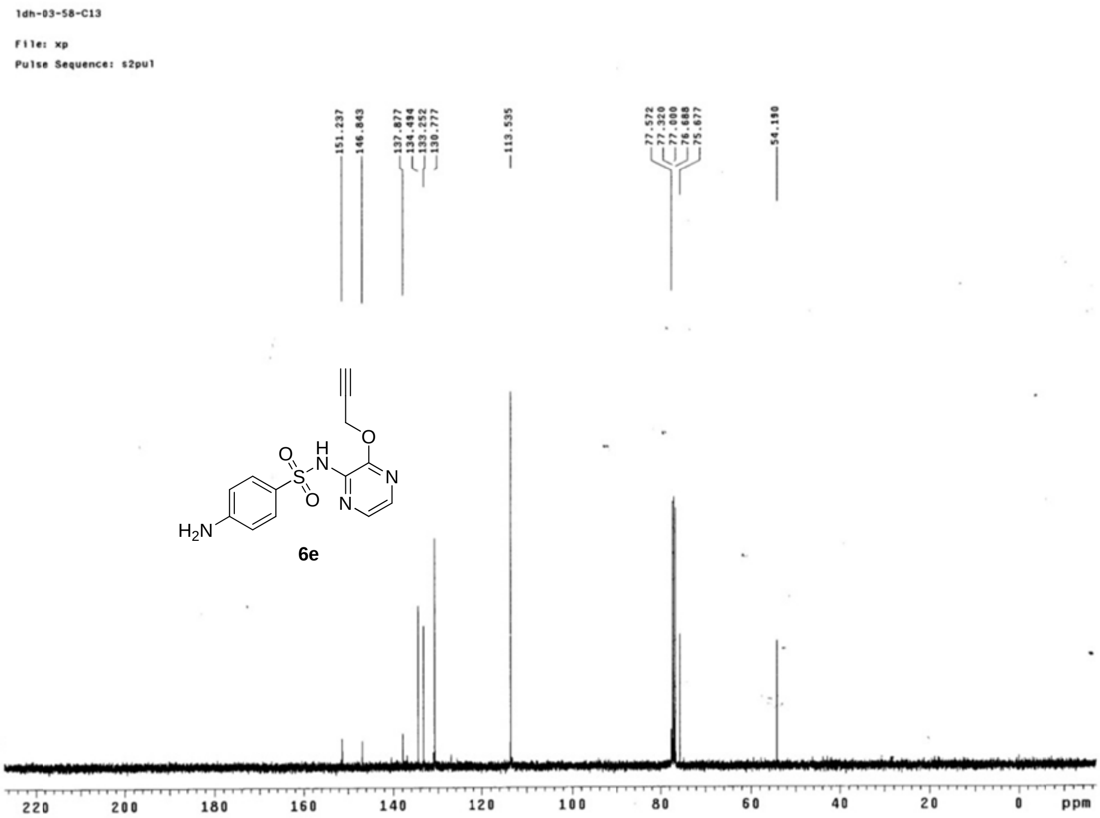
ppm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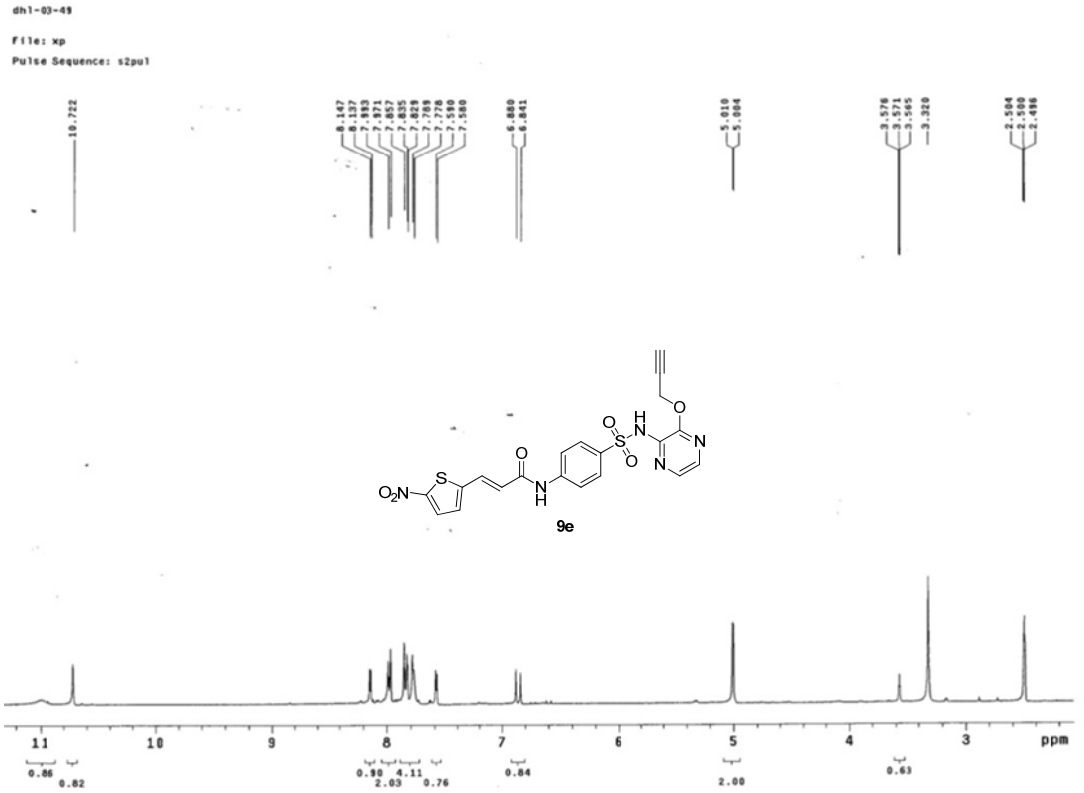
H2N
ppm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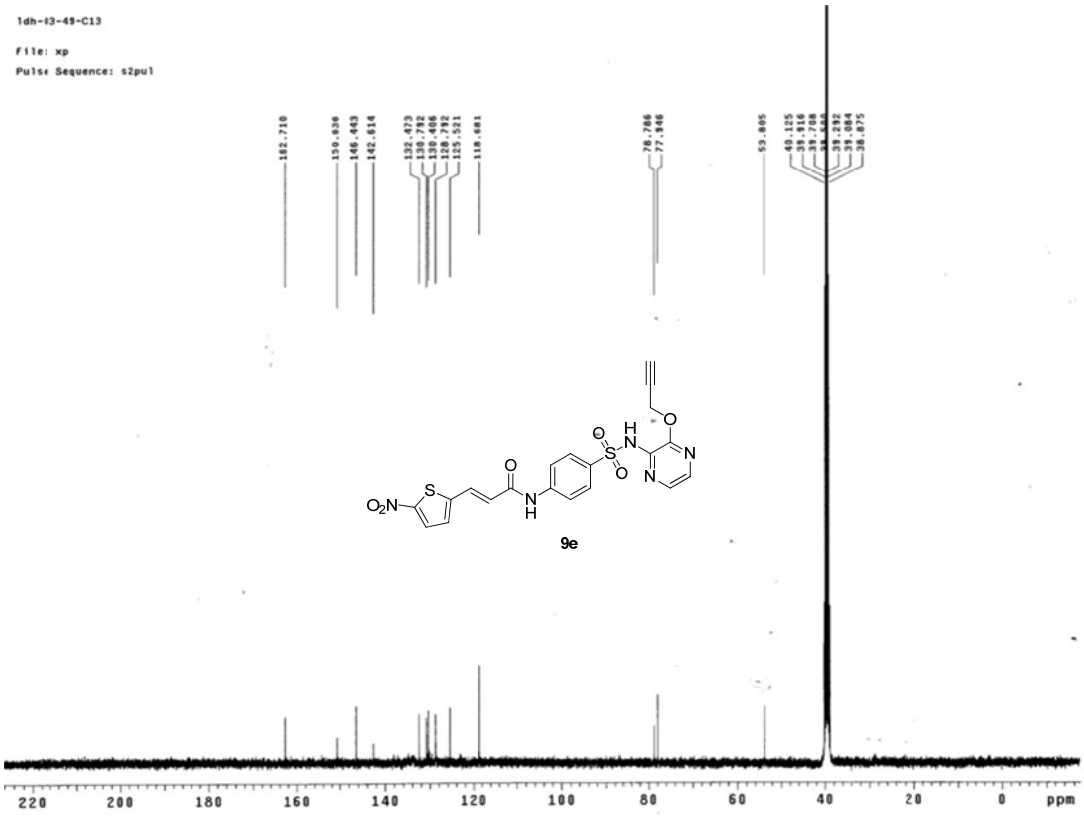
8.137
7.793
7.671
7.657
7.635
7.629
7.589
7.576
7.560
7.560
6.880
6.841
5.010
5.004
3.576
3.571
3.565
3.320
2.504
2.500
2.496
9e
11
10
9
8
7
6
5
4
3
ppm
0.86
0.82
0.90
4.11
2.03
0.76
0.84
2.00
0.63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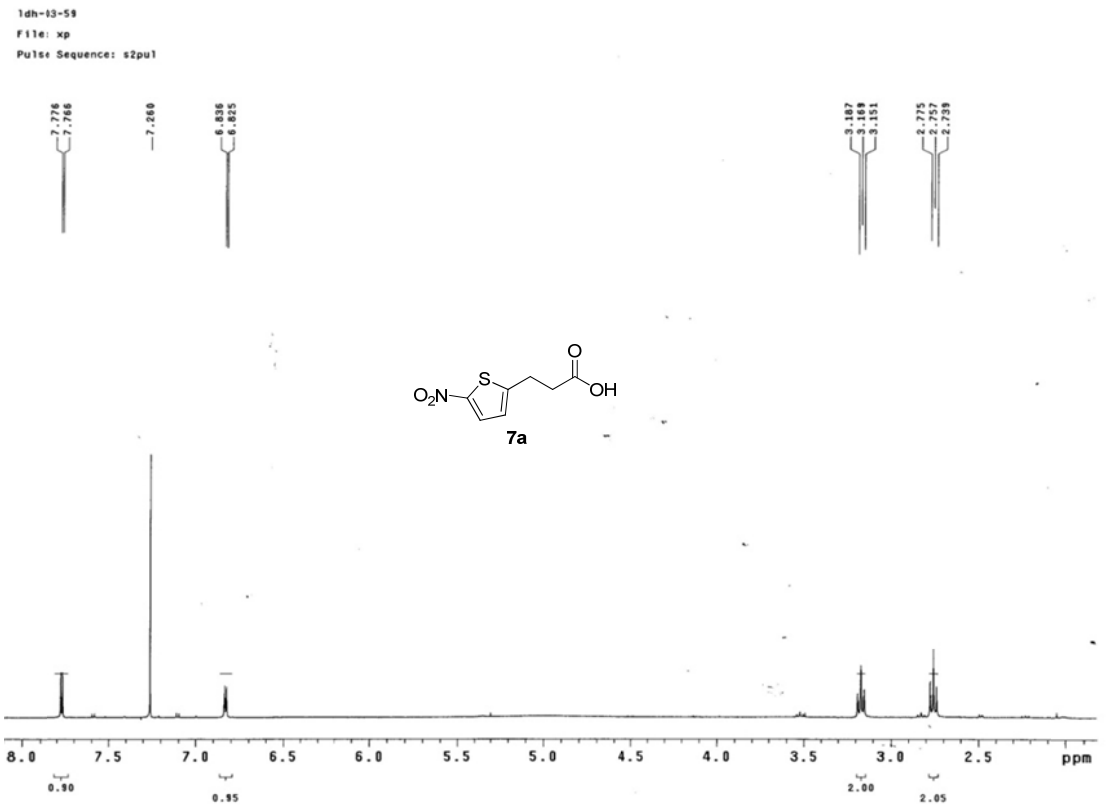
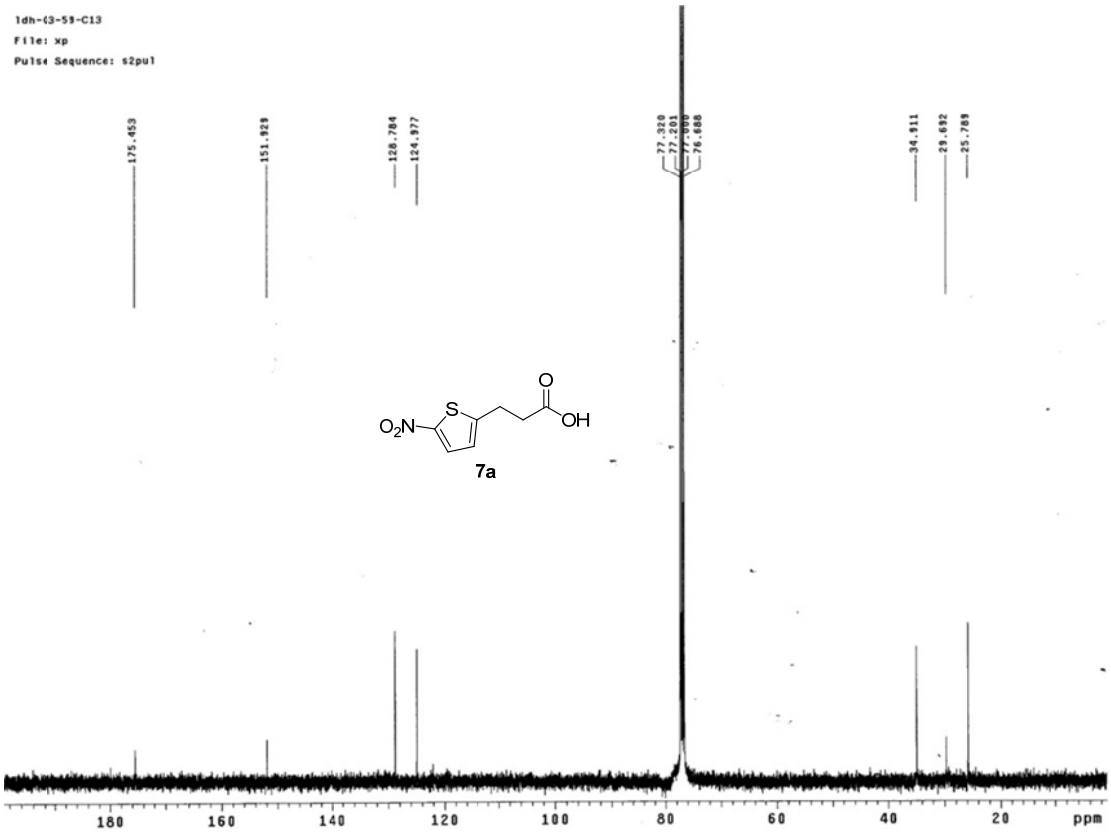
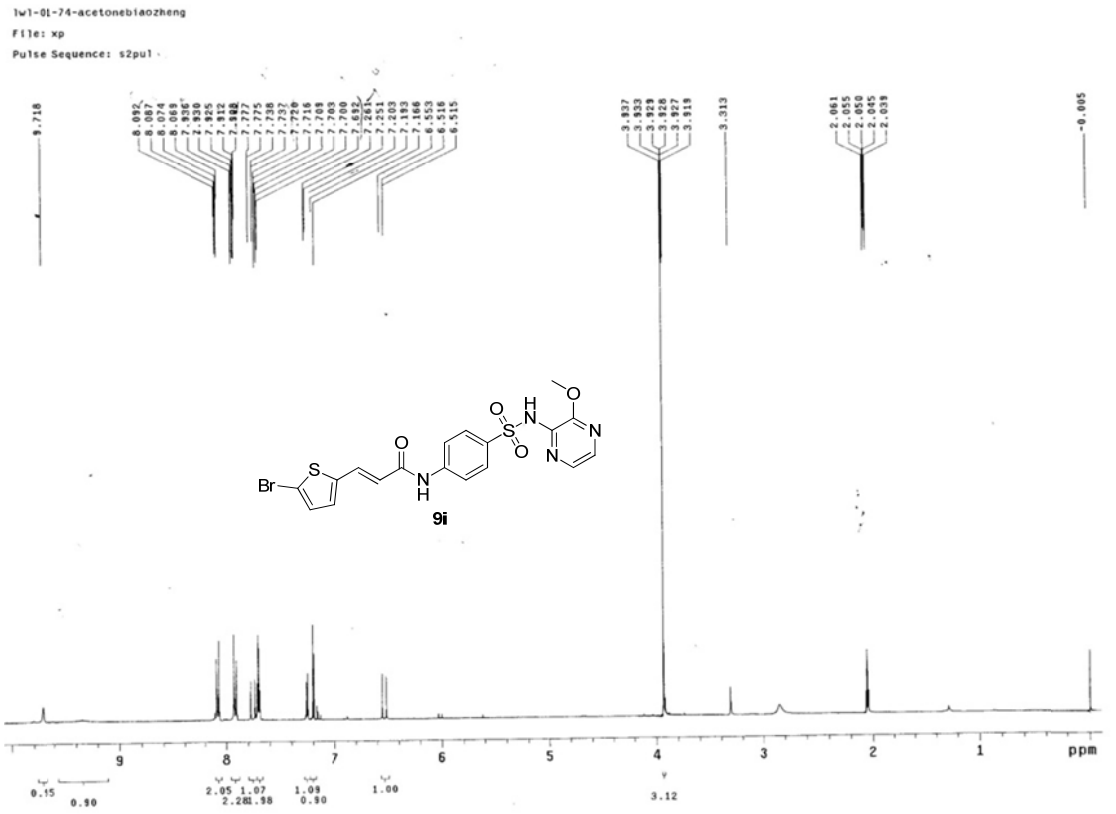
118.681
78.786
77.946
53.805
40.125
39.916
39.708
39.500
39.292
39.084
38.875
9e
220
200
180
160
140
120
100
80
60
40
20
0
ppm



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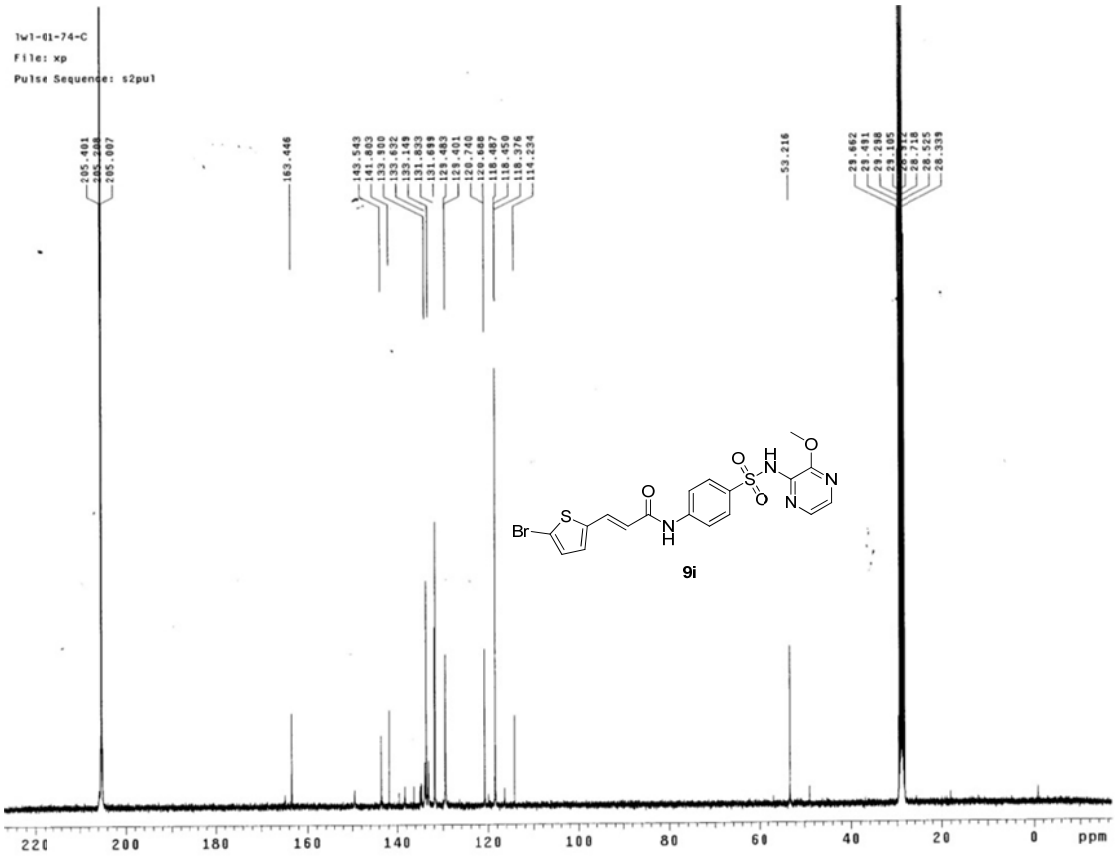
9i
ppm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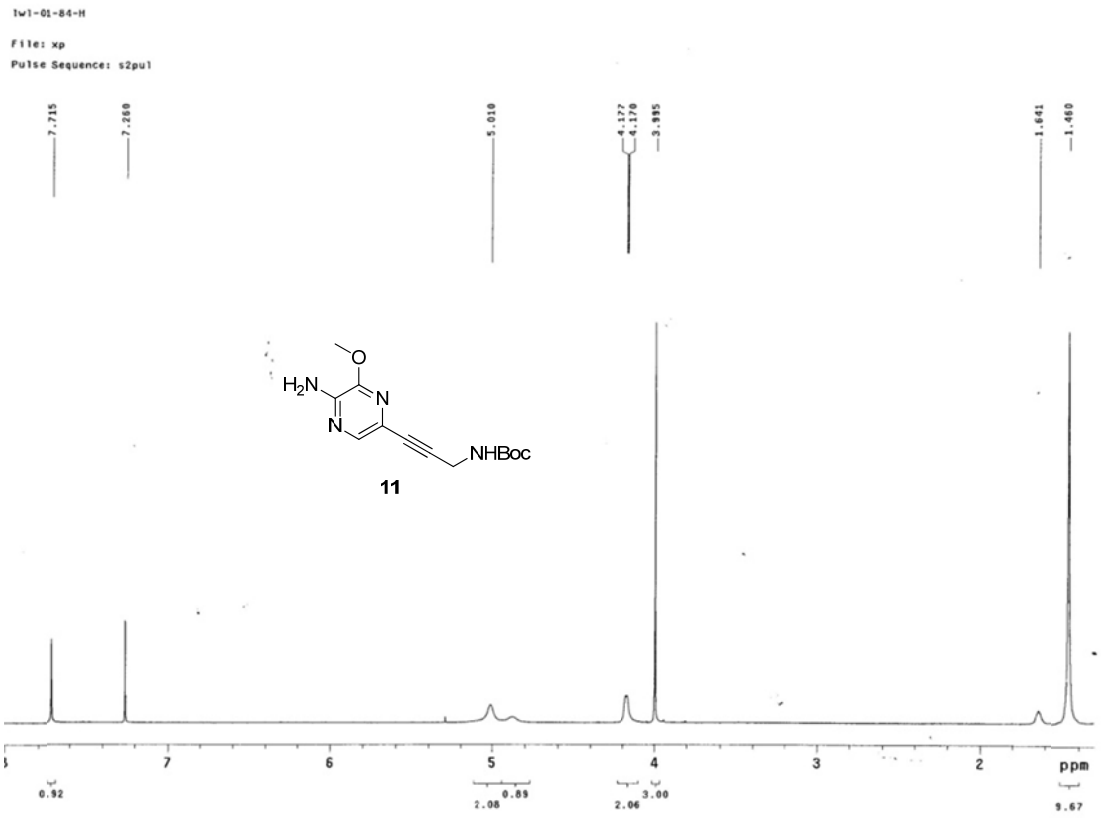
9i
ppm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5.010
4.177
4.170
3.995
1.641
1.460
O
H2N
N
N
NHBoc
11
7
6
5
4
3
2
ppm
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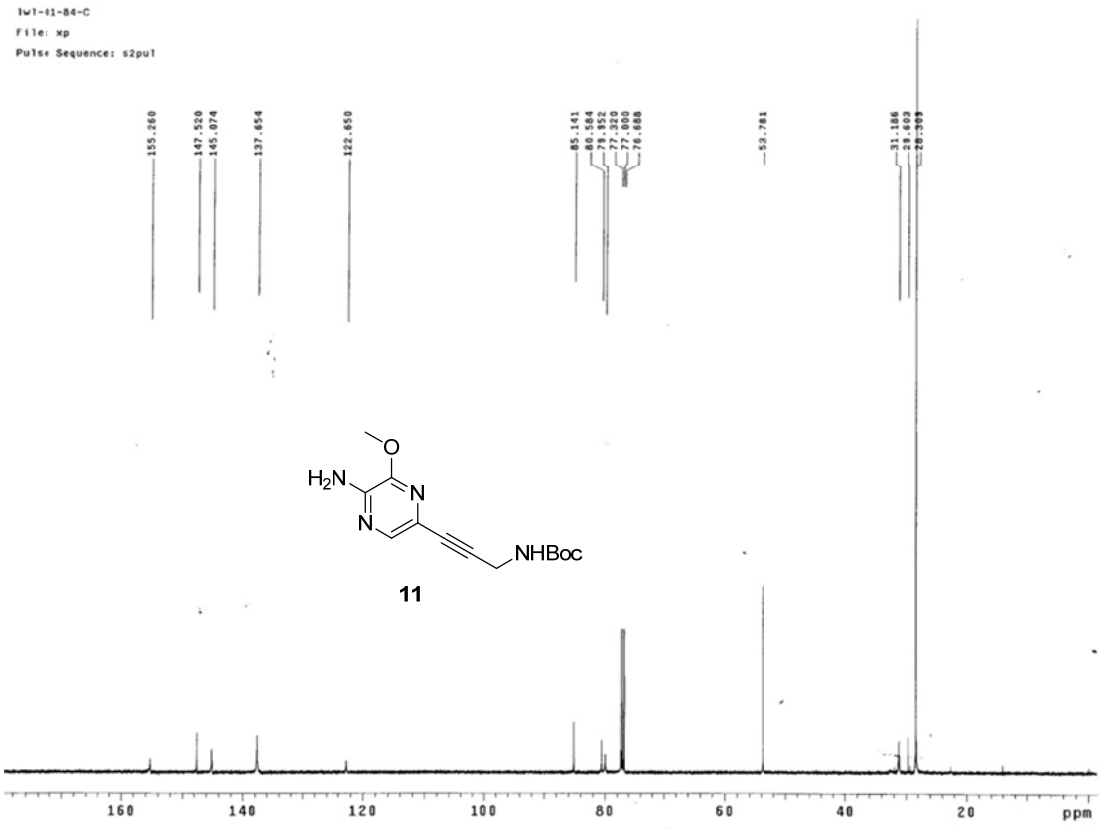
2.08
0.89
2.06
3.00
9.67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Pulse Sequence: s2pul
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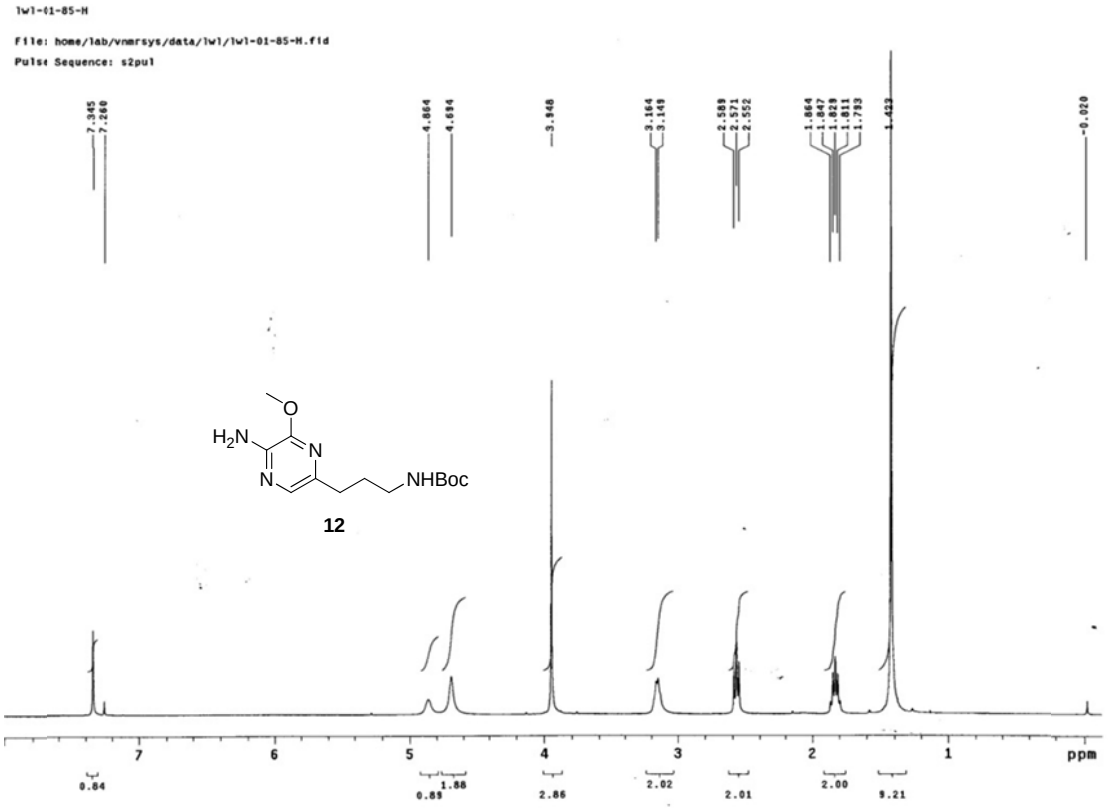
145.074
137.654
122.650
85.141
80.584
79.152
77.320
77.000
76.688
53.781
31.186
28.603
28.369
O
H2N
N
N
NHBoc
11
160
140
120
100
80
60
40
20
ppm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4.694
3.948
3.164
3.149
2.589
2.571
2.552
1.864
1.847
1.829
1.811
1.793
1.422
-0.020
O
H2N
N
N
NHBoc
12
7
6
5
4
3
2
1
ppm
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1.88
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2.86
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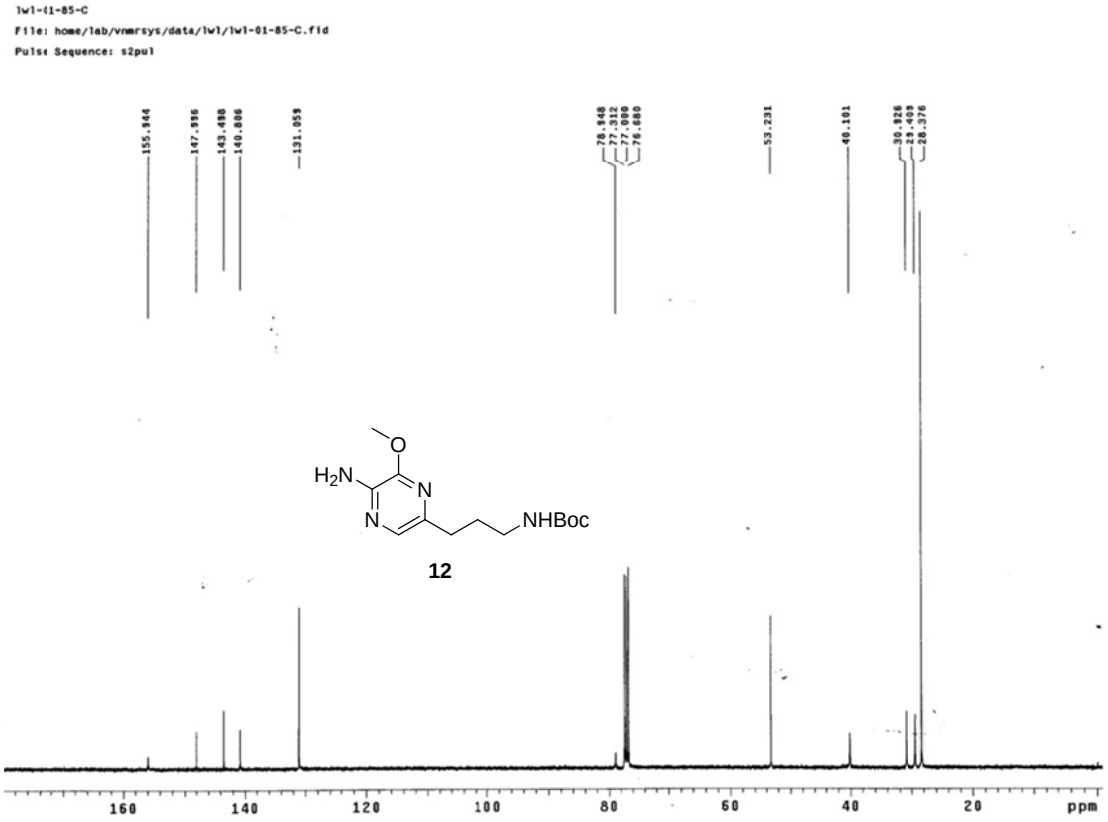
2.01
2.00
9.21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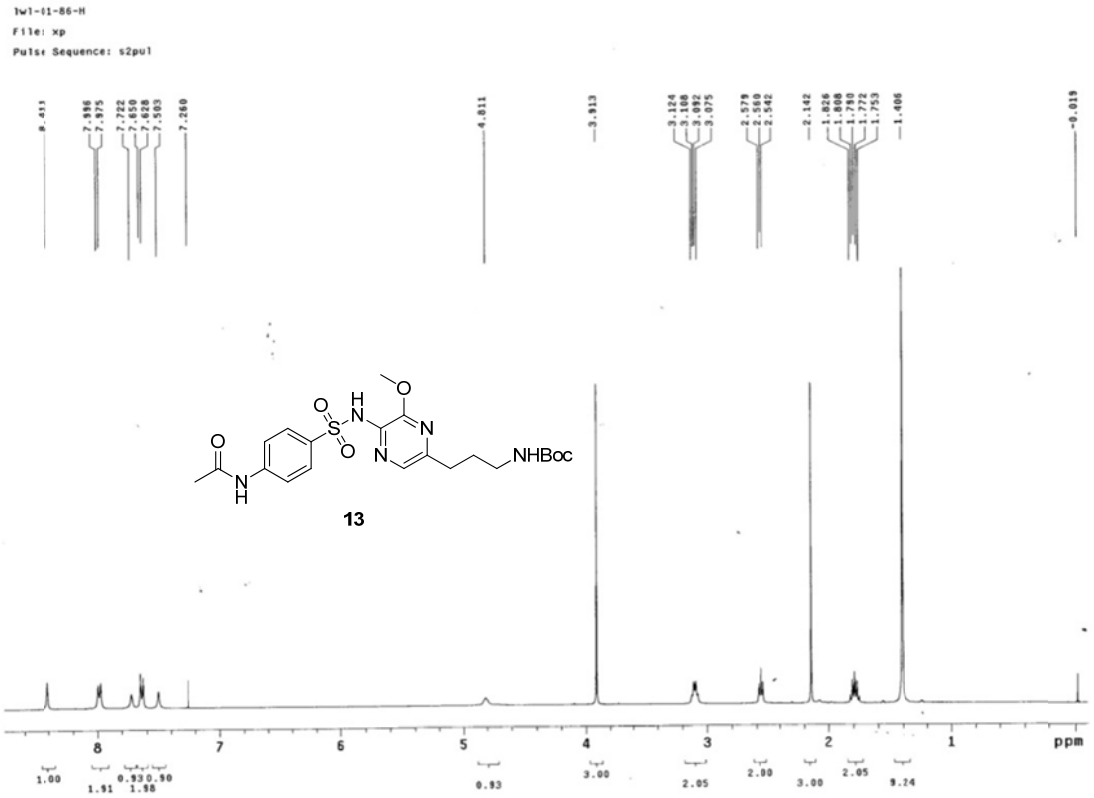
143.498
140.866
131.059
78.848
77.312
77.000
76.680
53.231
40.161
30.926
28.498
28.376
O
H2N
N
N
NHBoc
12
160
140
120
100
80
60
40
20
ppm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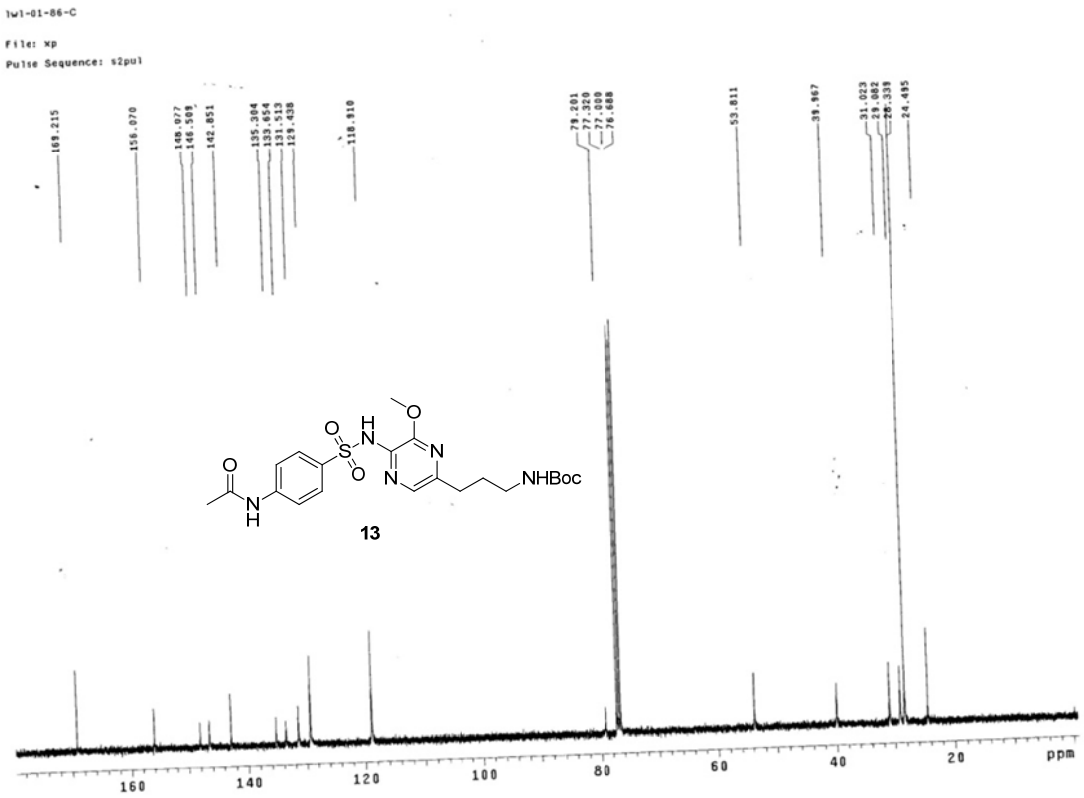
NHBoc
13
ppm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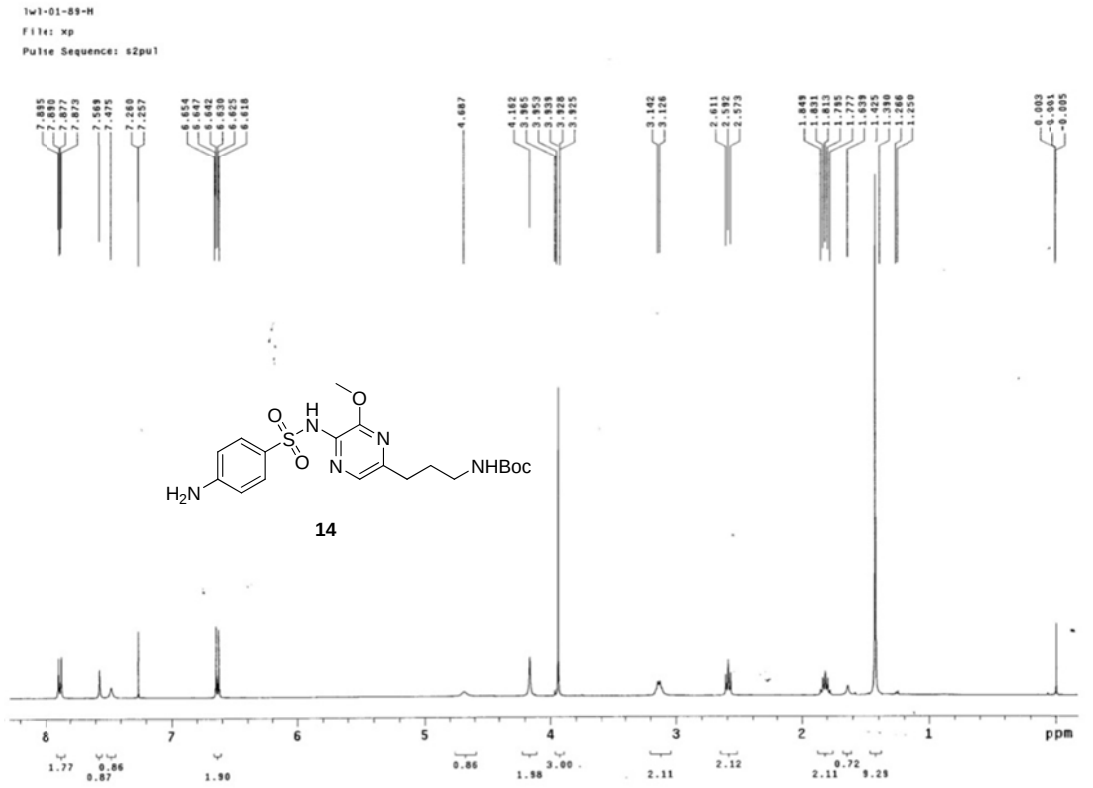
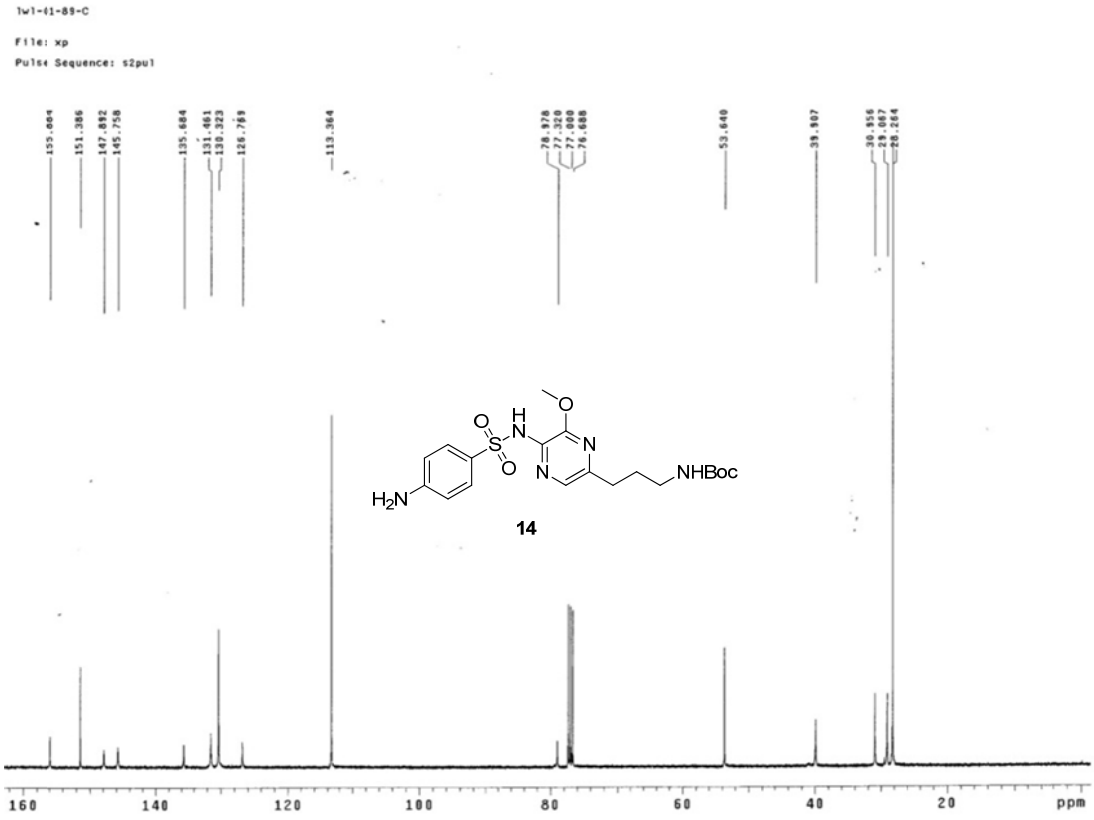
Pulse Sequence: s2pul
NHBoc
13
ppm


1w1-01-89-H
File: xp
Pulse Sequence: s2pul
NHBoc
H_2N
14
ppm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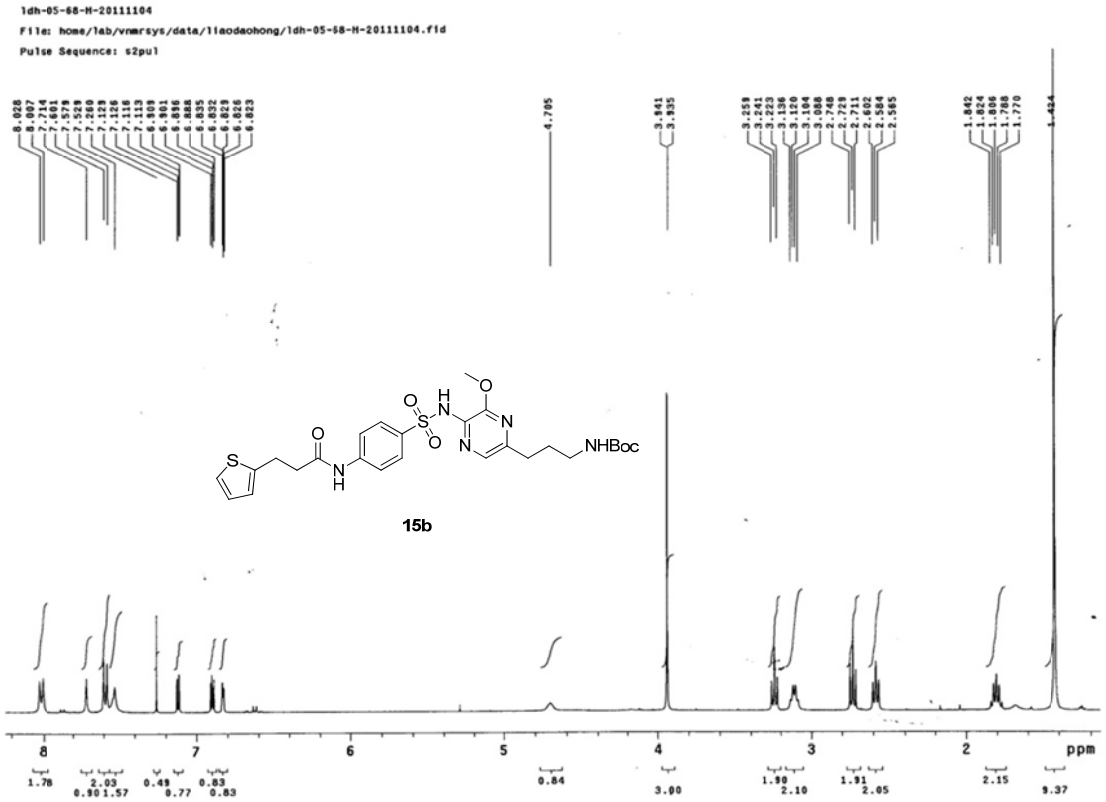
File: xp
Pulse Sequence: s2pul
NHBoc
H_2N
14
ppm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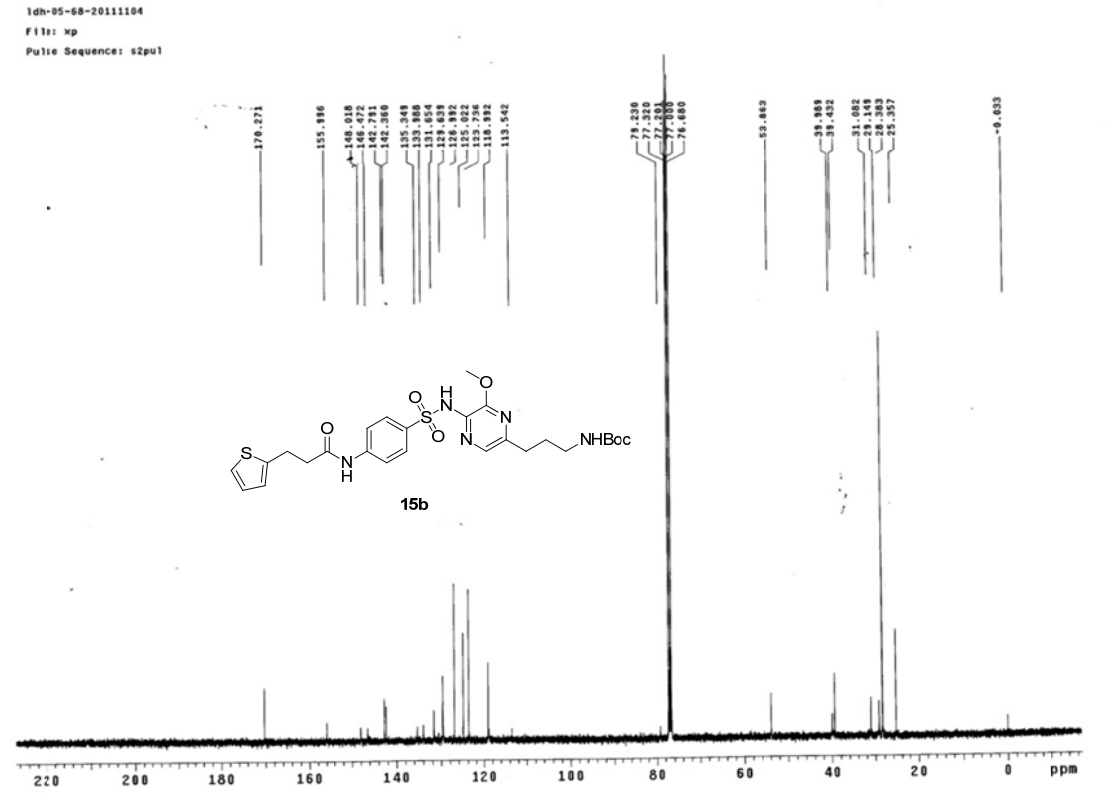
NHBoc
15b
ppm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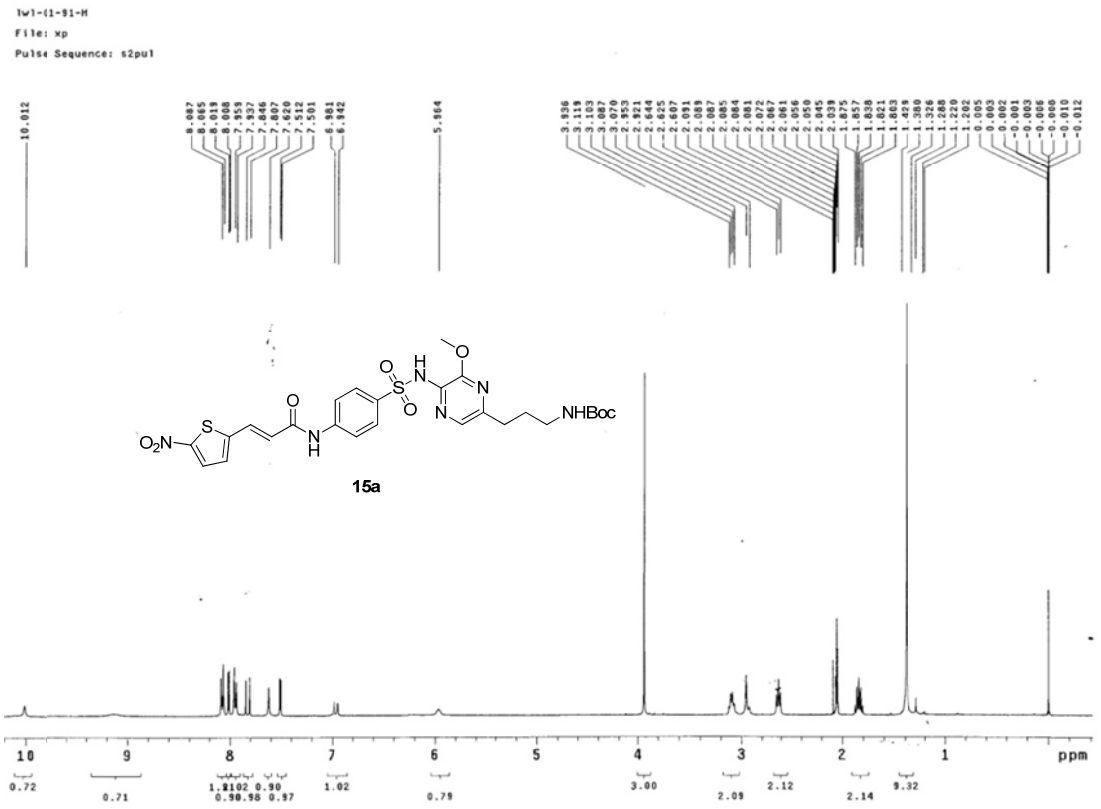
15b
ppm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8.008
7.959
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6.981
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3.136
3.119
3.103
3.087
3.070
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2.921
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1.875
1.857
1.838
1.821
1.805
1.429
1.380
1.326
1.288
1.220
1.202
0.085
0.003
0.002
0.001
-0.003
-0.006
-0.008
-0.010
-0.012
O2N
S
O
N
H
O
S
O
N
H
N
N
O
NHBoc
15a
10
9
8
7
6
5
4
3
2
1
ppm
0.72
0.71
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0.90.98
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1.02
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3.00
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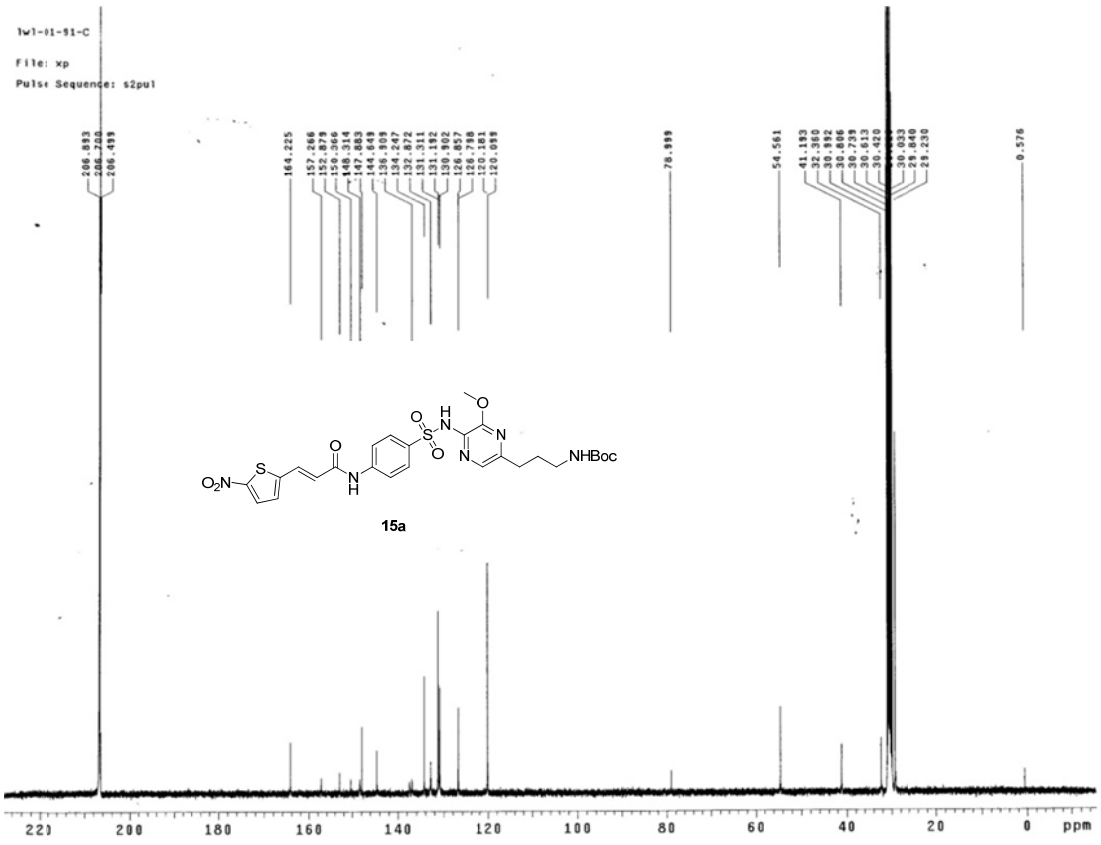
2.12
2.14
9.32


lw1-01-91-C
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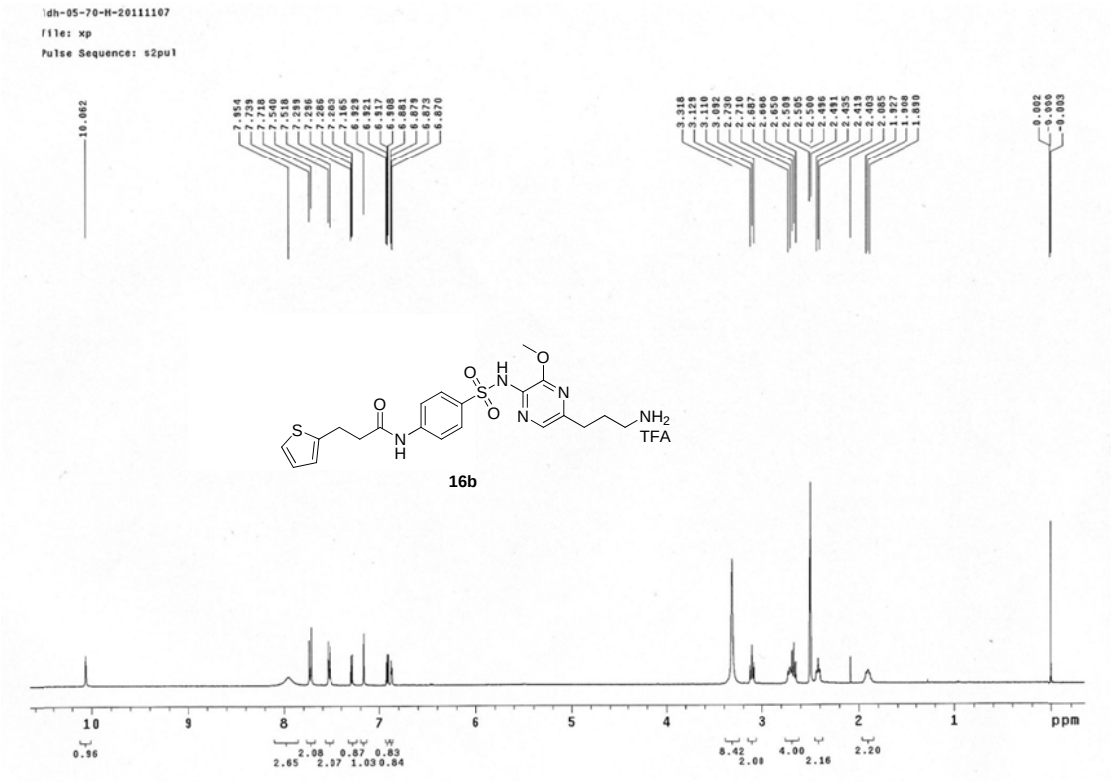
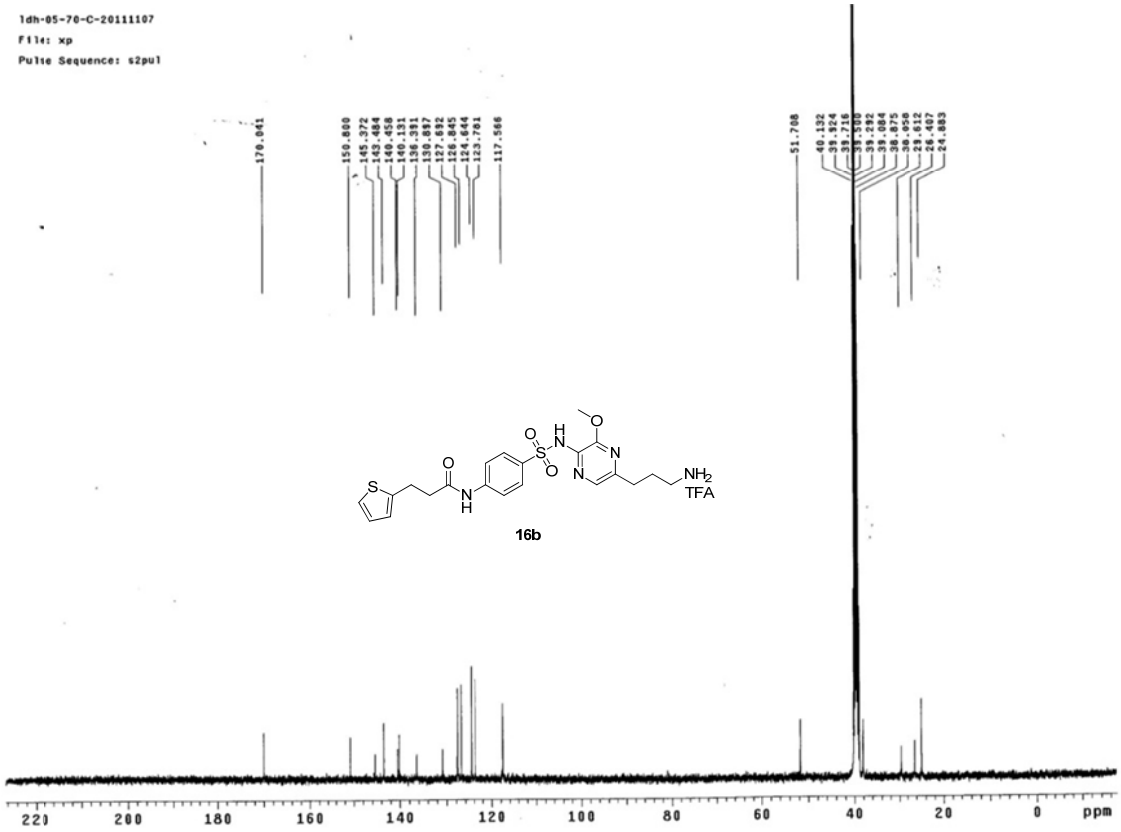
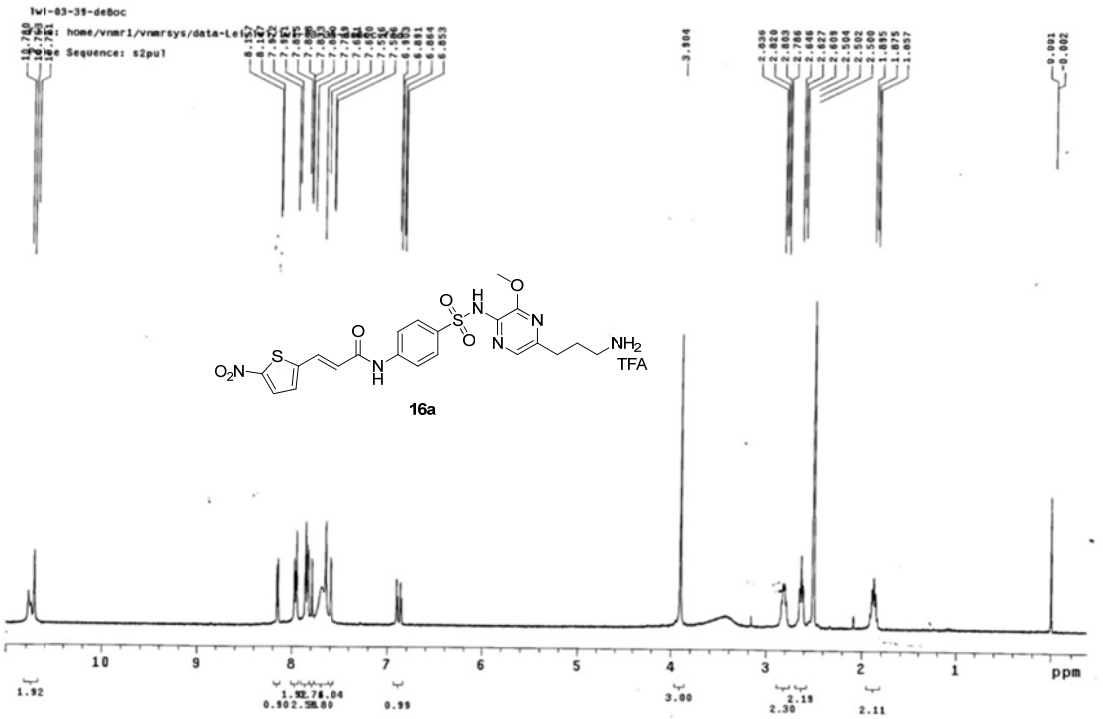
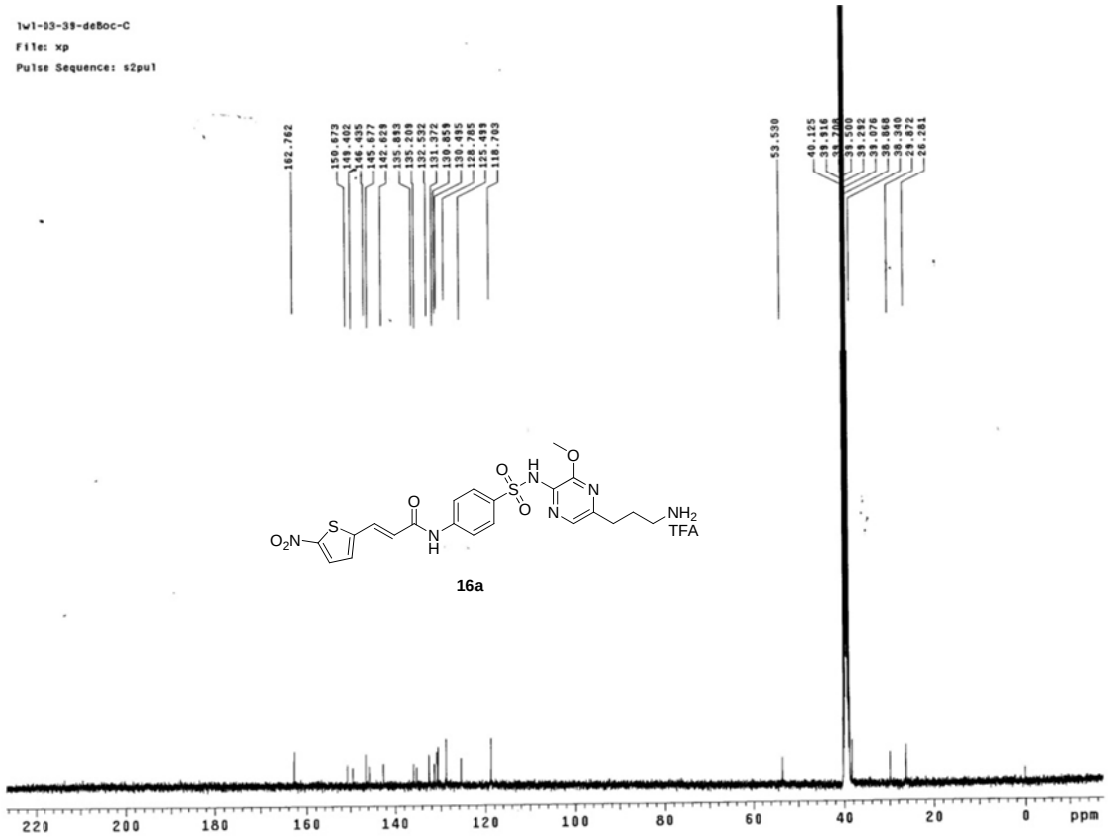
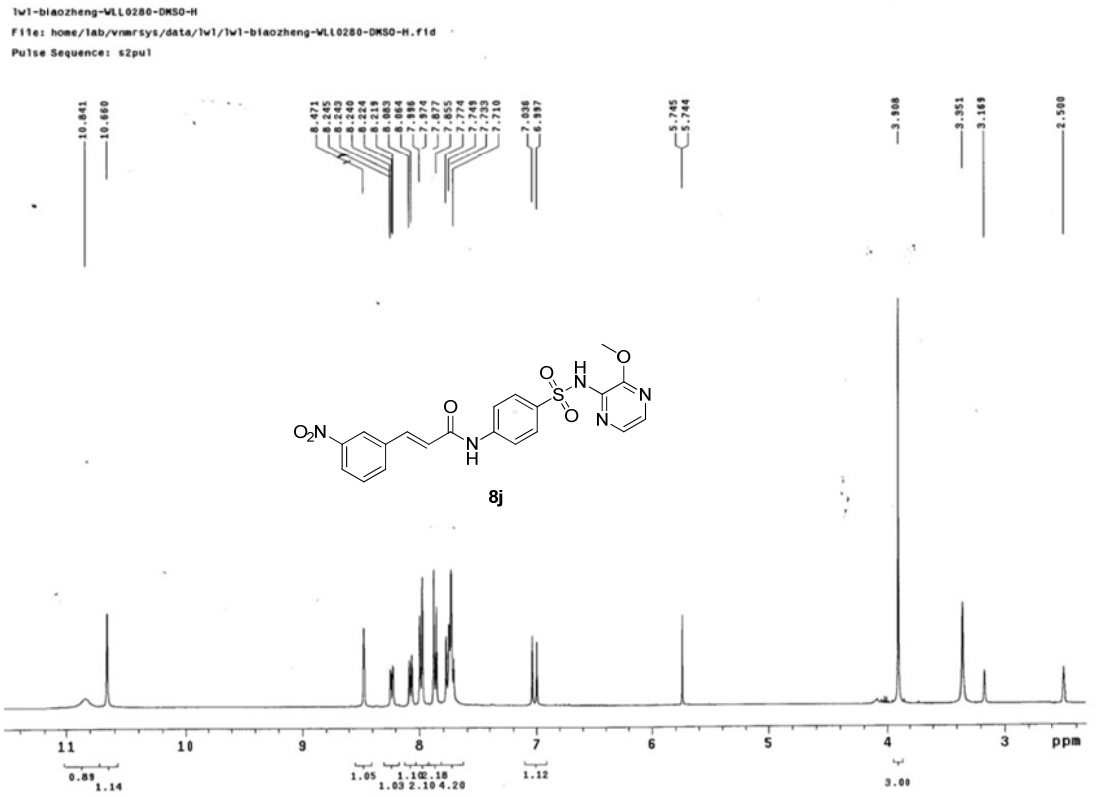
206.893
206.280
206.459
164.225
157.266
152.879
151.866
148.314
147.883
144.649
136.309
134.247
132.872
131.311
131.152
130.902
126.857
126.798
120.181
120.059
78.999
54.561
41.193
32.360
30.992
30.686
30.273
30.613
30.420
30.033
29.840
29.230
0.576
O2N
S
O
N
H
O
S
O
N
H
N
N
O
NHBoc
15a
220
200
180
160
140
120
100
80
60
40
20
0
ppm

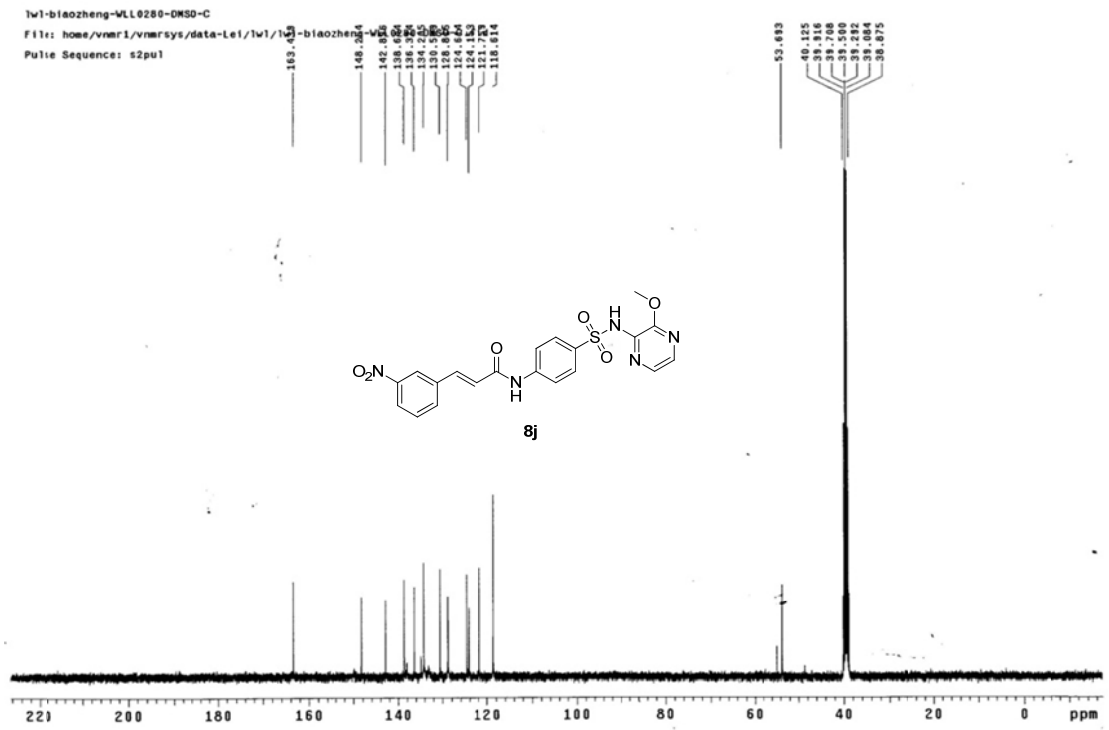

ldh-05-70-H-20111107
file: xp
Pulse Sequence: s2pul
16b
NH2
TFA
ppm

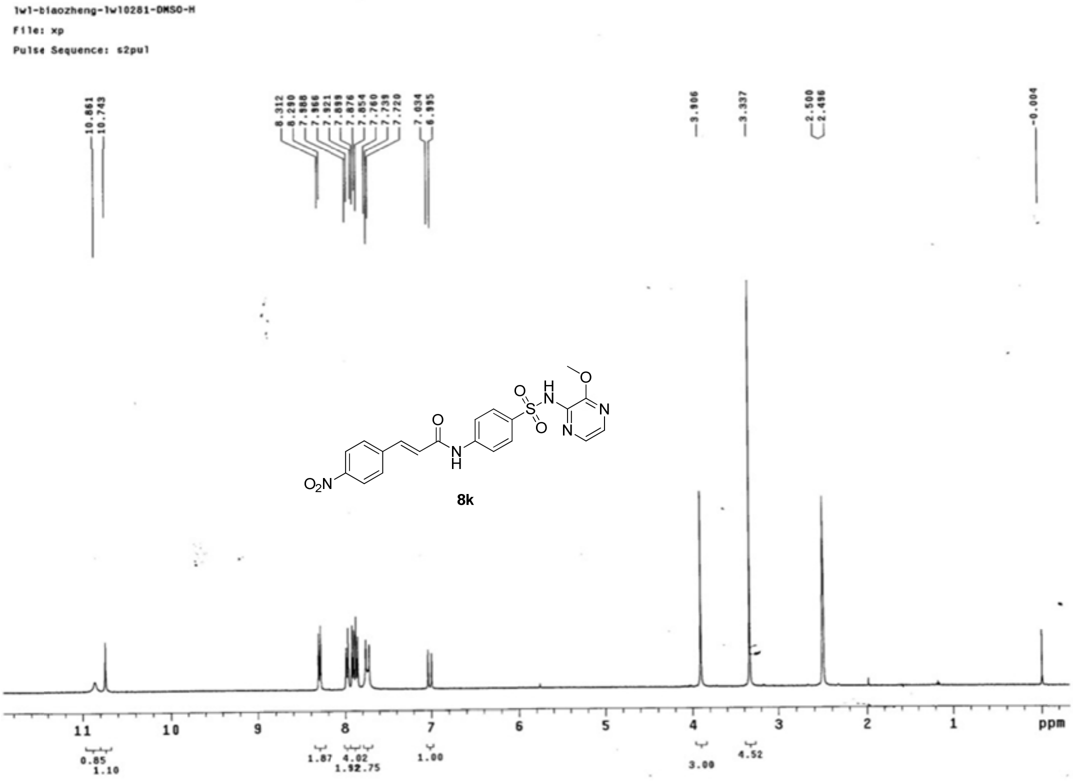

ldh-05-70-C-20111107
file: xp
Pulse Sequence: s2pul
16b
NH2
TFA
ppm

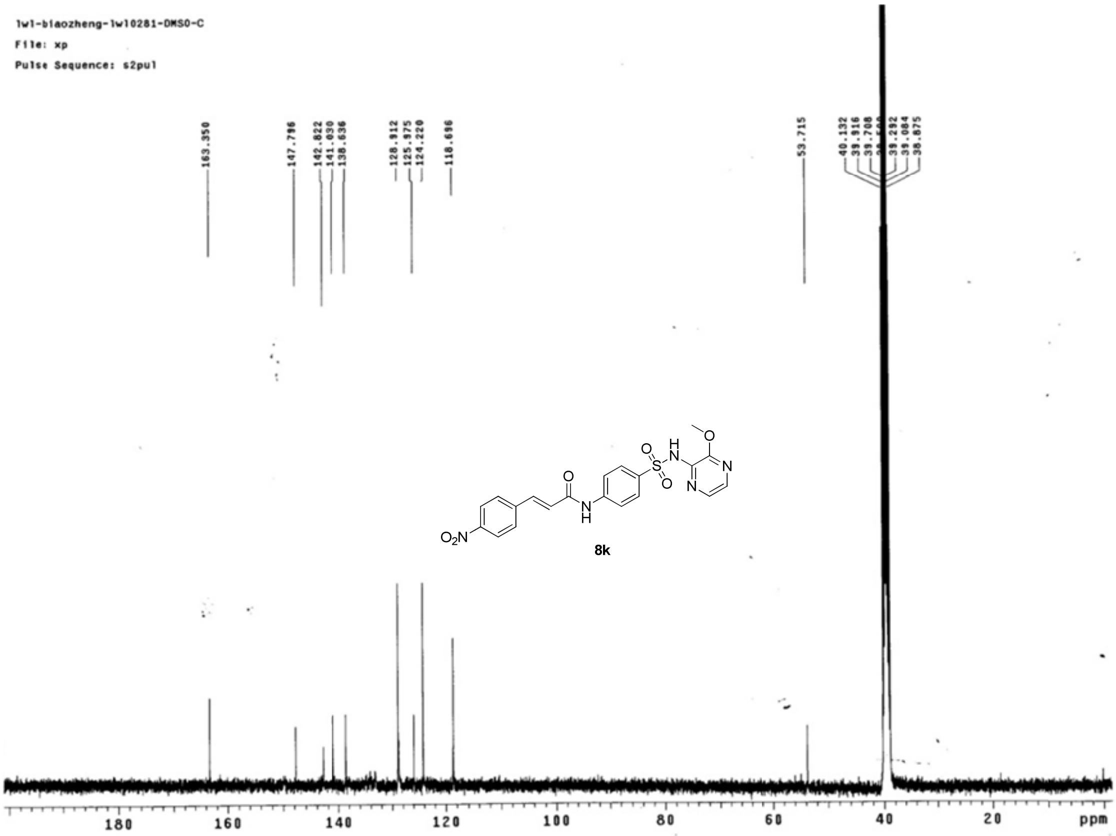

lwl-03-39-deBoc
Pulse Sequence: s2pul
NH2
TFA
16a
ppm

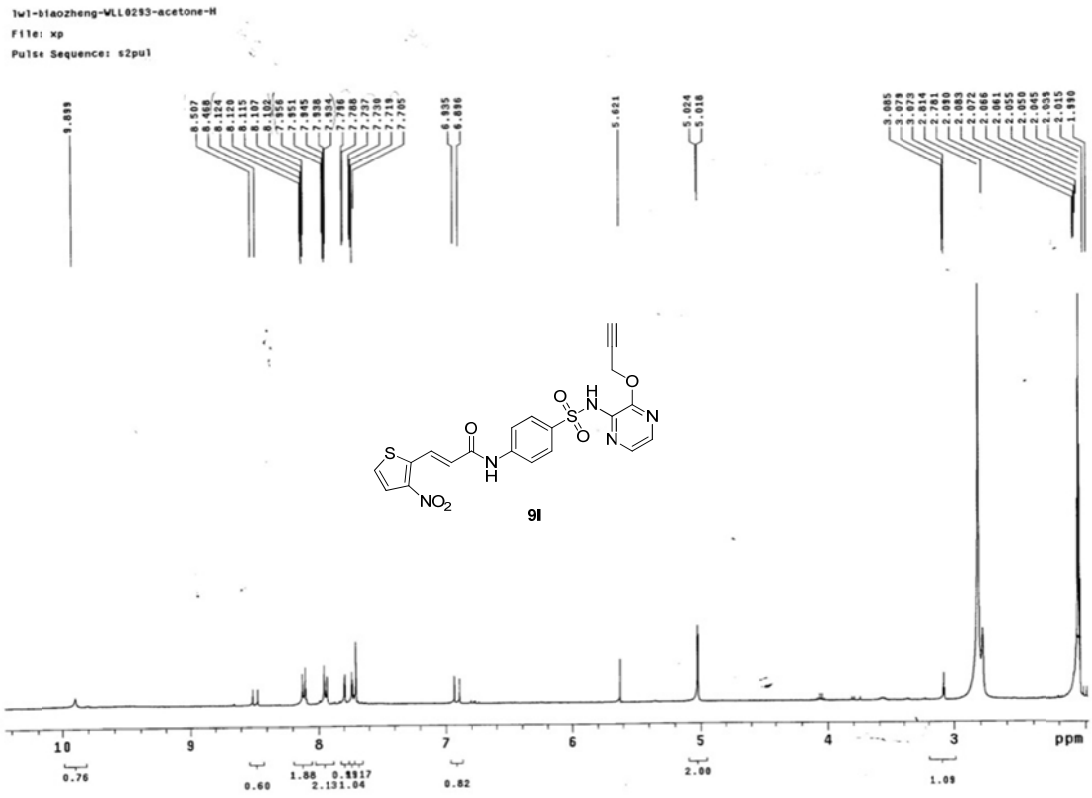

lwl-03-39-deBoc-C
File: xp
Pulse Sequence: s2pul
NH2
TFA
16a
ppm

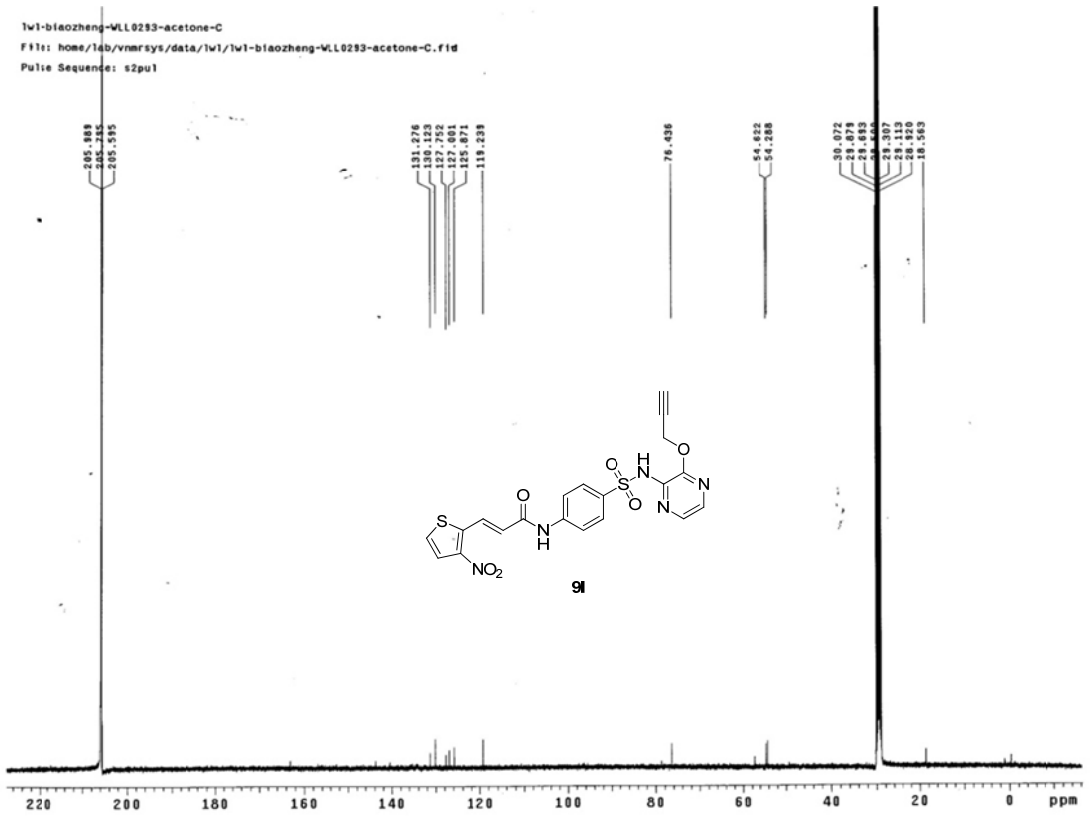

1w1-biaozheng-WLL0280-DMSO-H
File: home/lab/vnmrsys/data/1w1/1w1-biaozheng-WLL0280-DMSO-H.fid
Pulse Sequence: s2pul
10.841
10.660
8.471
8.245
8.243
8.240
8.224
8.218
8.083
8.064
7.996
7.974
7.877
7.855
7.774
7.749
7.733
7.710
7.036
6.997
5.745
5.744
3.808
3.351
3.169
2.500
8j
11
10
9
8
7
6
5
4
3
ppm
0.89
1.14
1.05
1.10
2.18
1.03
2.10
4.20
1.12
3.00

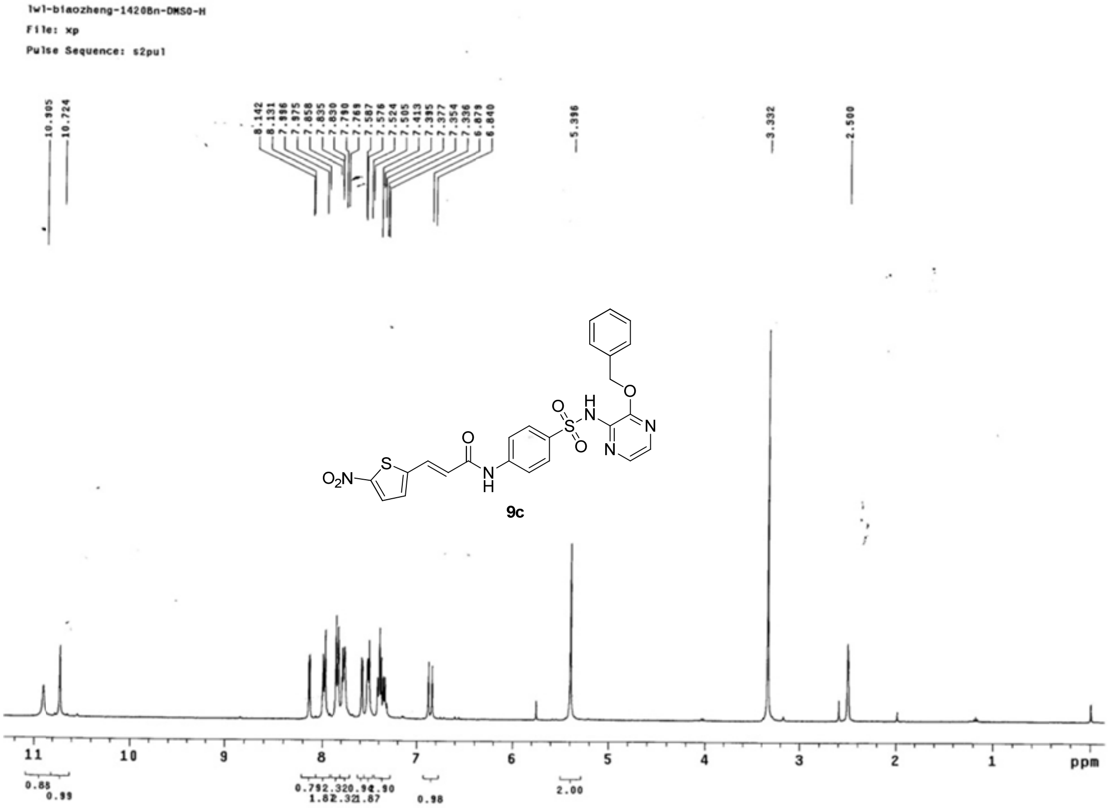

1w1-biaozheng-WLL0280-DMSO-C
Pulse Sequence: s2pul
53.693
40.125
39.916
39.708
39.500
39.292
39.084
38.875
8j
220
200
180
160
140
120
100
80
60
40
20
0
ppm

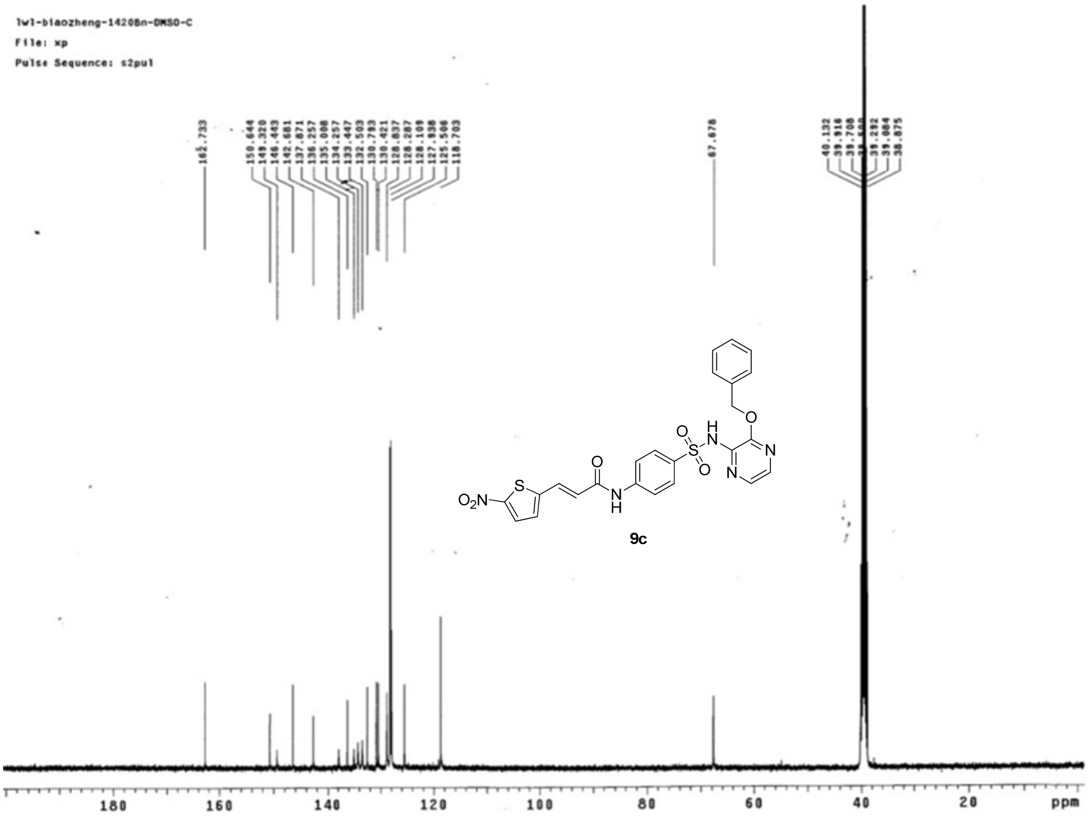


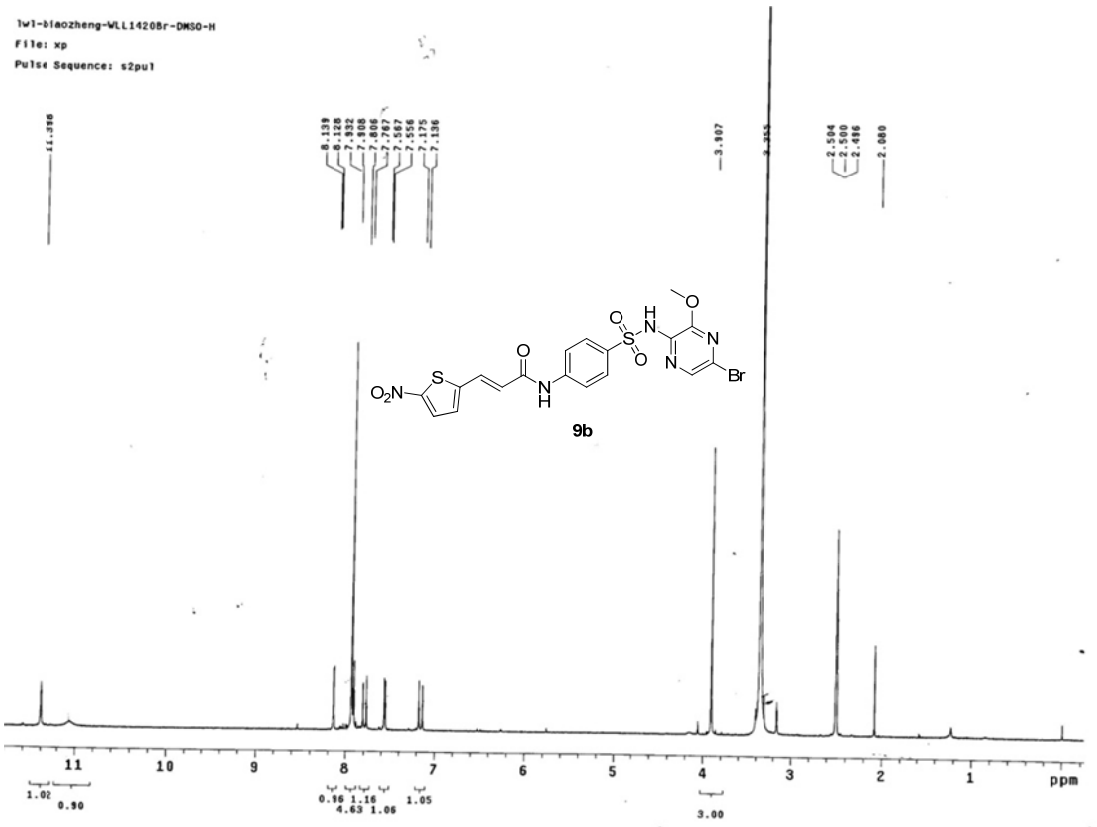


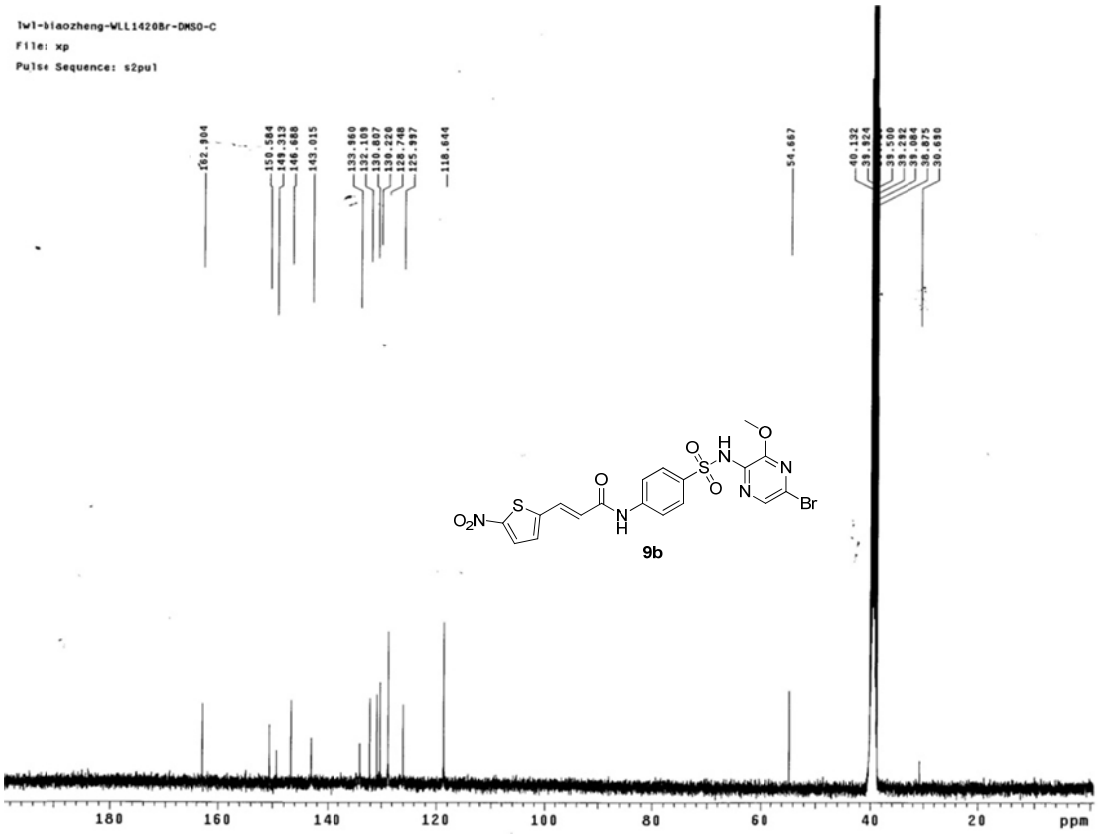

lwl-biaozheng-WLL0293-acetone-H
File: xp
Pulse Sequence: s2pul
9.889
8.507
8.468
8.124
8.115
8.107
8.105
7.956
7.951
7.845
7.838
7.768
7.737
7.730
7.719
7.705
6.835
6.826
5.621
5.024
5.018
3.085
3.079
3.073
2.814
2.781
2.069
2.066
2.072
2.066
2.061
2.055
2.050
2.045
2.035
2.015
1.990
O
H
N
O
S
O
N
N
O
N
H
S
NO2
9l
10
9
8
7
6
5
4
3
ppm
0.76
0.60
1.88
0.8917
2.131.04
0.82
2.00
1.09

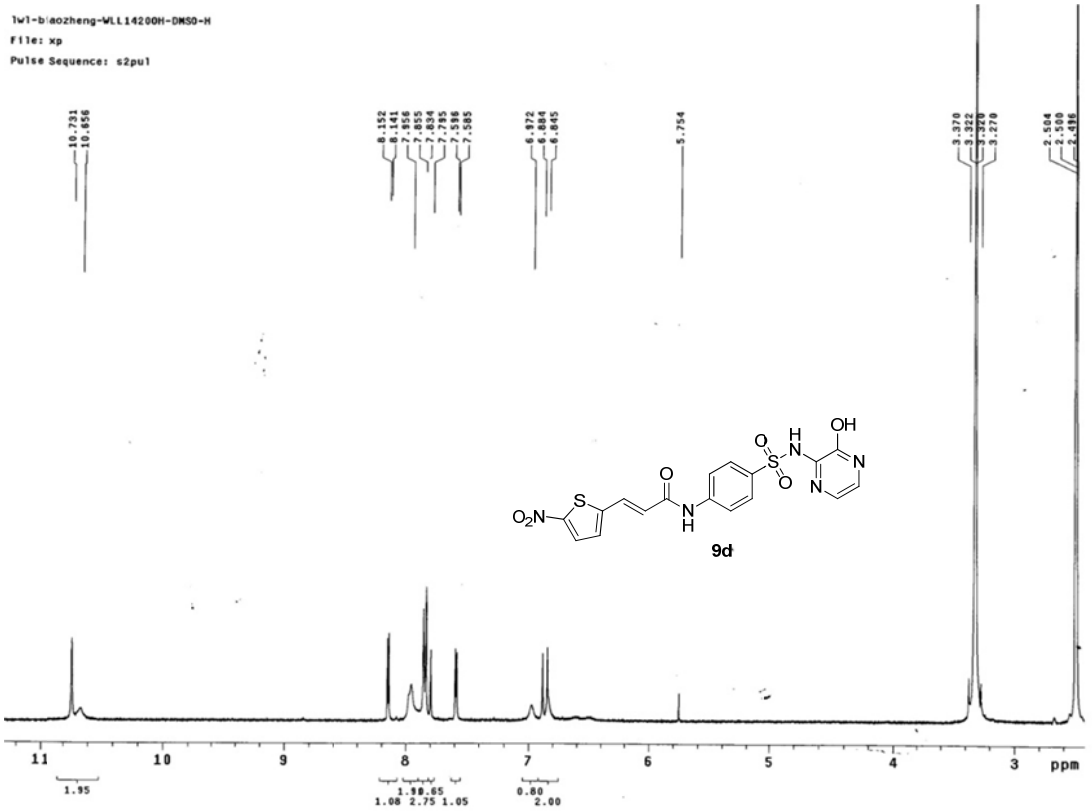

lwl-biaozheng-WLL0293-acetone-C
File: home/lab/vnmrsys/data/lwl/lwl-biaozheng-WLL0293-acetone-C.fid
Pulse Sequence: s2pul
205.989
205.745
205.595
131.276
130.253
127.752
127.601
125.871
119.239
76.436
54.622
54.288
30.072
29.879
29.683
29.483
29.307
29.113
28.920
18.563
O
H
N
O
S
O
N
N
O
N
H
S
NO2
9l
220
200
180
160
140
120
100
80
60
40
20
0
ppm

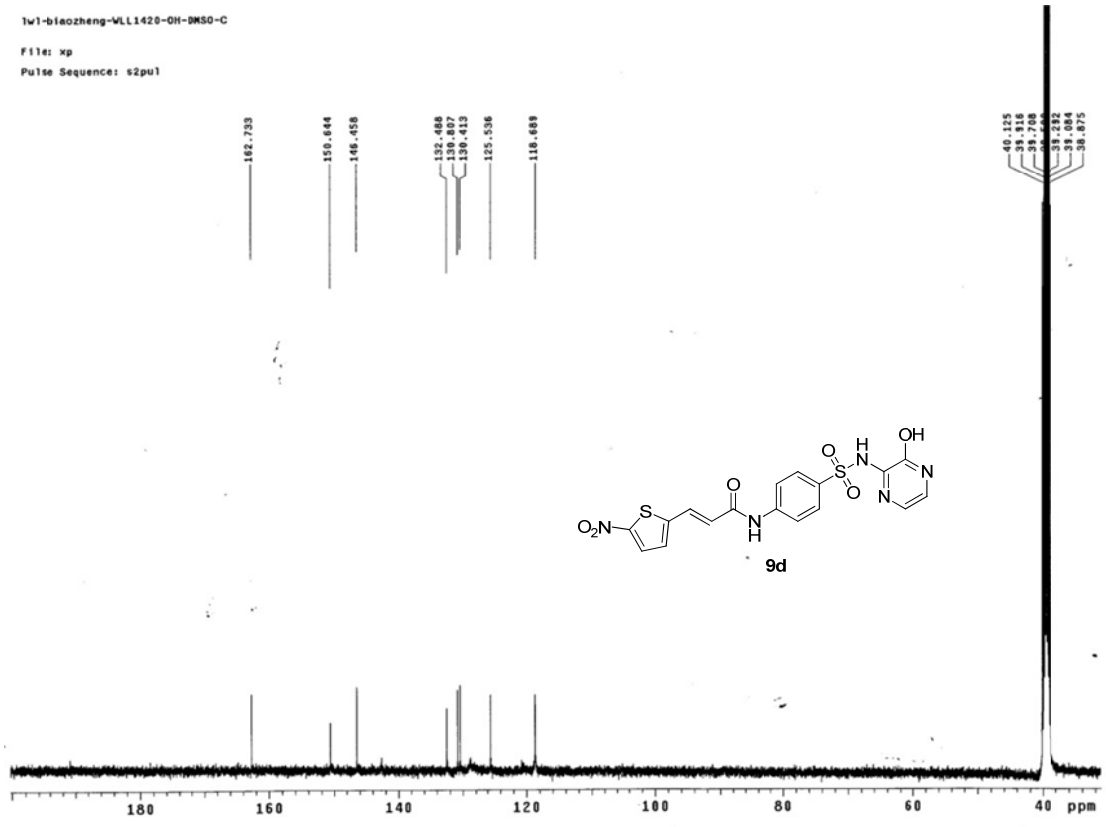

lwl-biaozheng-1420Bn-DMSO-H
File: xp
Pulse Sequence: s2pul
O$_2$N
S
O
N
H
O
S
O
H
N
O
N
N
9c
ppm

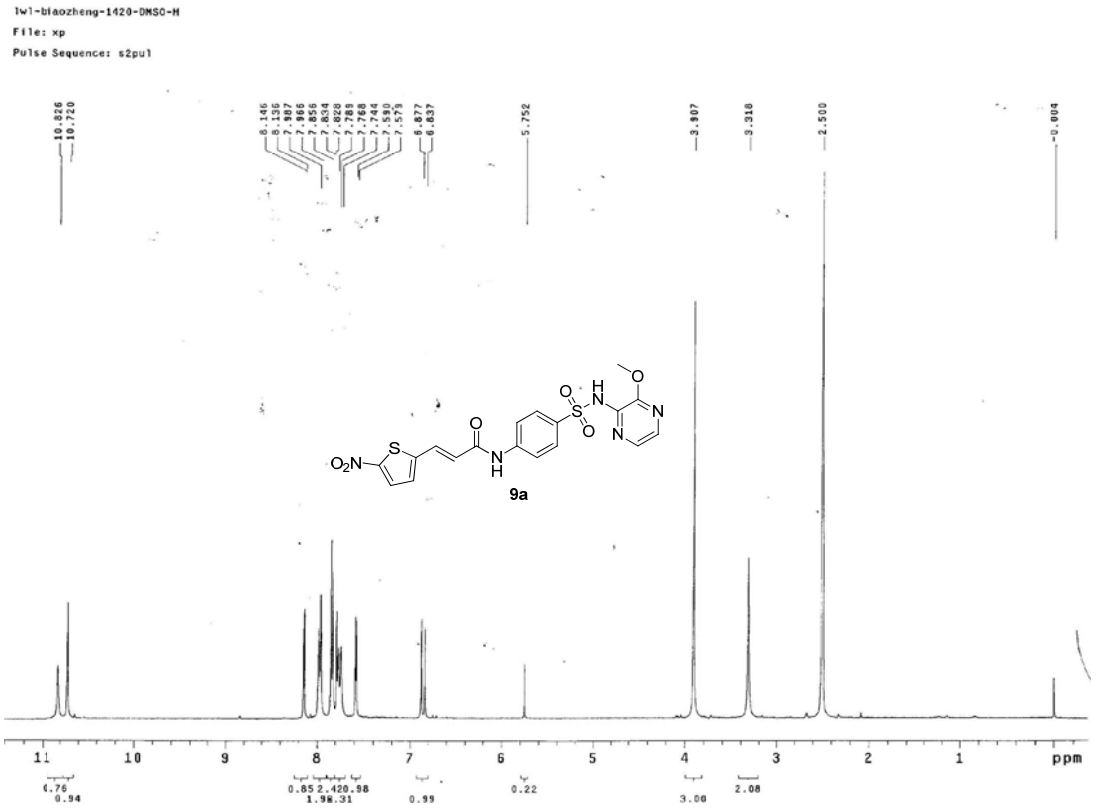

lwl-biaozheng-1420Bn-DMSO-C
File: xp
Pulse Sequence: s2pul
O$_2$N
S
O
N
H
O
S
O
H
N
O
N
N
9c
180
160
140
120
100
80
60
40
20
ppm

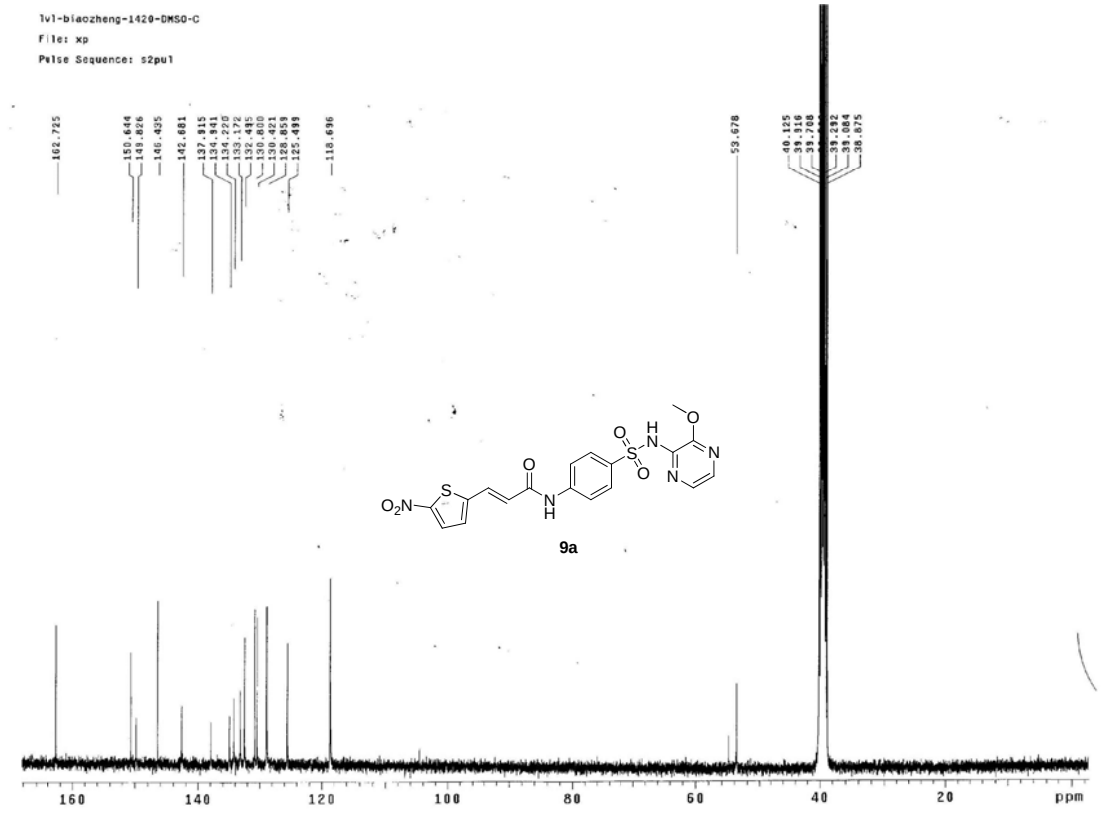

lwl-biaozheng-WLL1420Br-DMSO-H
File: xp
Pulse Sequence: s2pul
9b
ppm

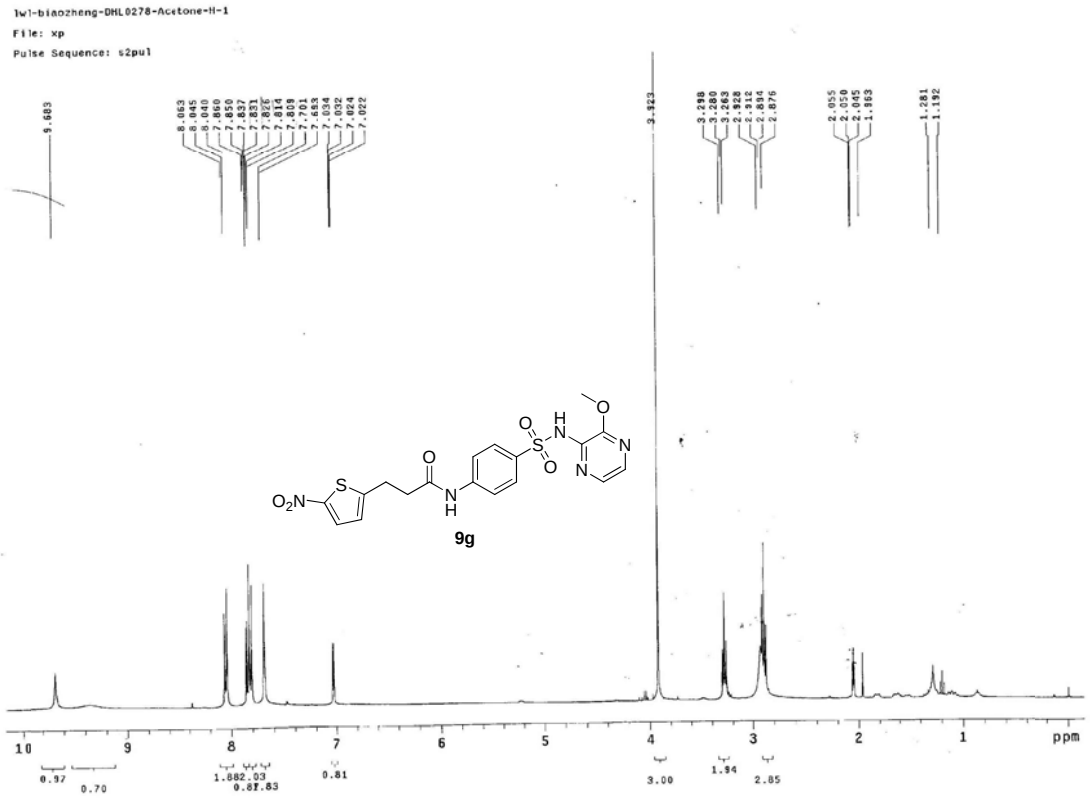

lwl-biaozheng-WLL1420Br-DMSO-C
File: xp
Pulse Sequence: s2pul
9b
ppm

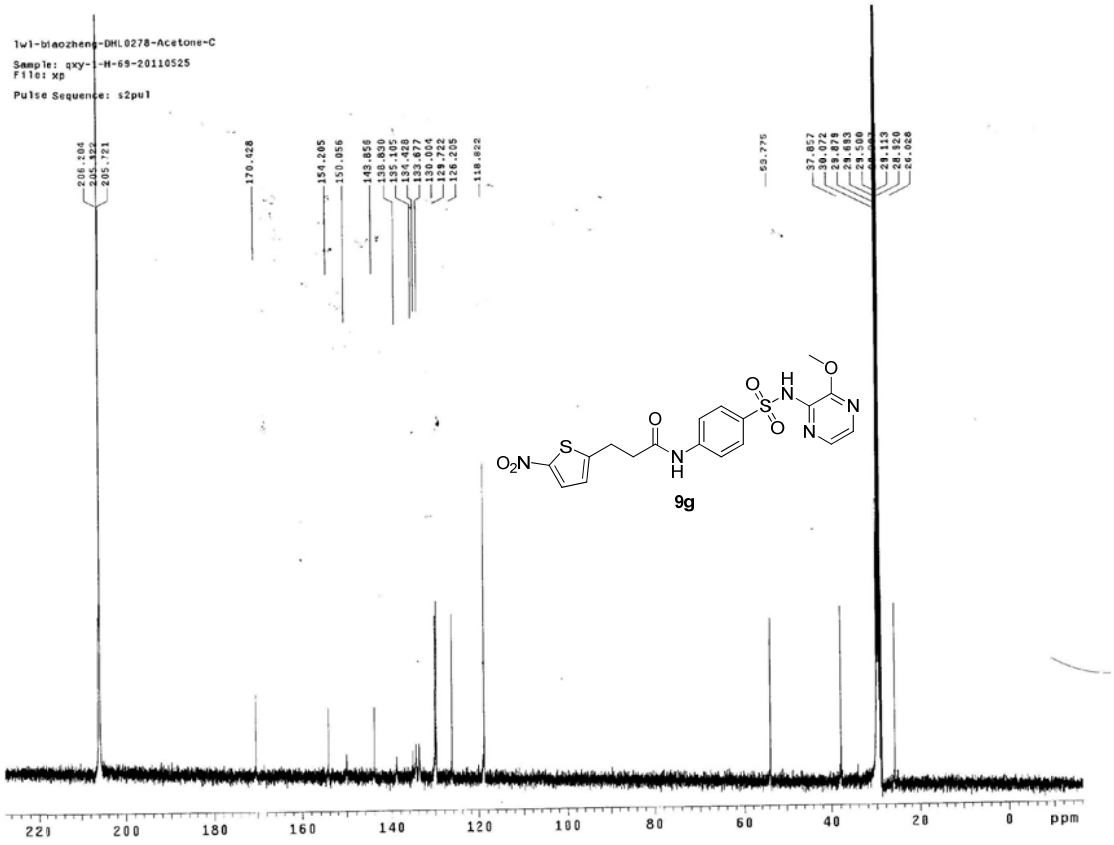

1w1-biaozheng-WLL1420OH-DMSO-H
File: xp
Pulse Sequence: s2pul
10.731
10.656
8.152
8.141
7.856
7.855
7.834
7.795
7.596
7.585
6.872
6.864
6.845
5.754
3.370
3.322
3.320
3.270
2.504
2.500
2.496
11
10
9
8
7
6
5
4
3
ppm
1.95
1.98
0.65
1.08
2.75
1.05
0.80
2.00
9d

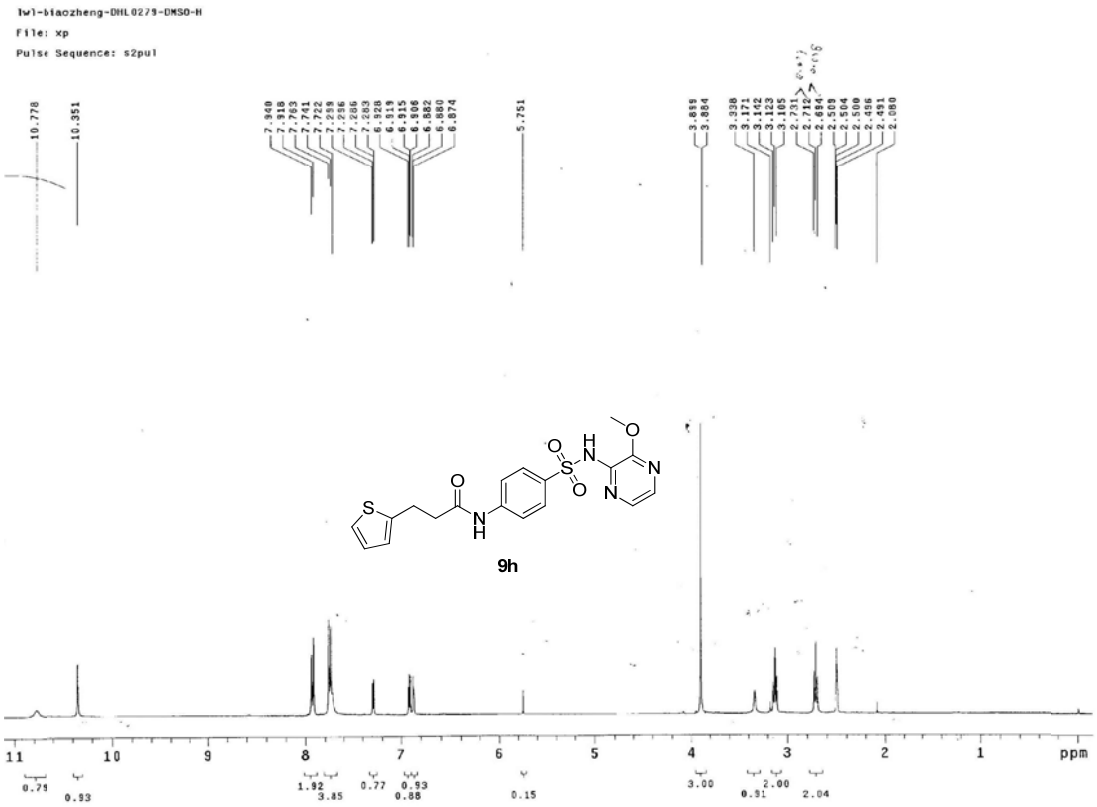

1w1-biaozheng-WLL1420-OH-DMSO-C
File: xp
Pulse Sequence: s2pul
162.733
150.644
146.458
132.488
130.807
130.413
125.536
118.689
40.125
39.916
39.708
39.292
39.084
38.875
180
160
140
120
100
80
60
40
ppm
9d

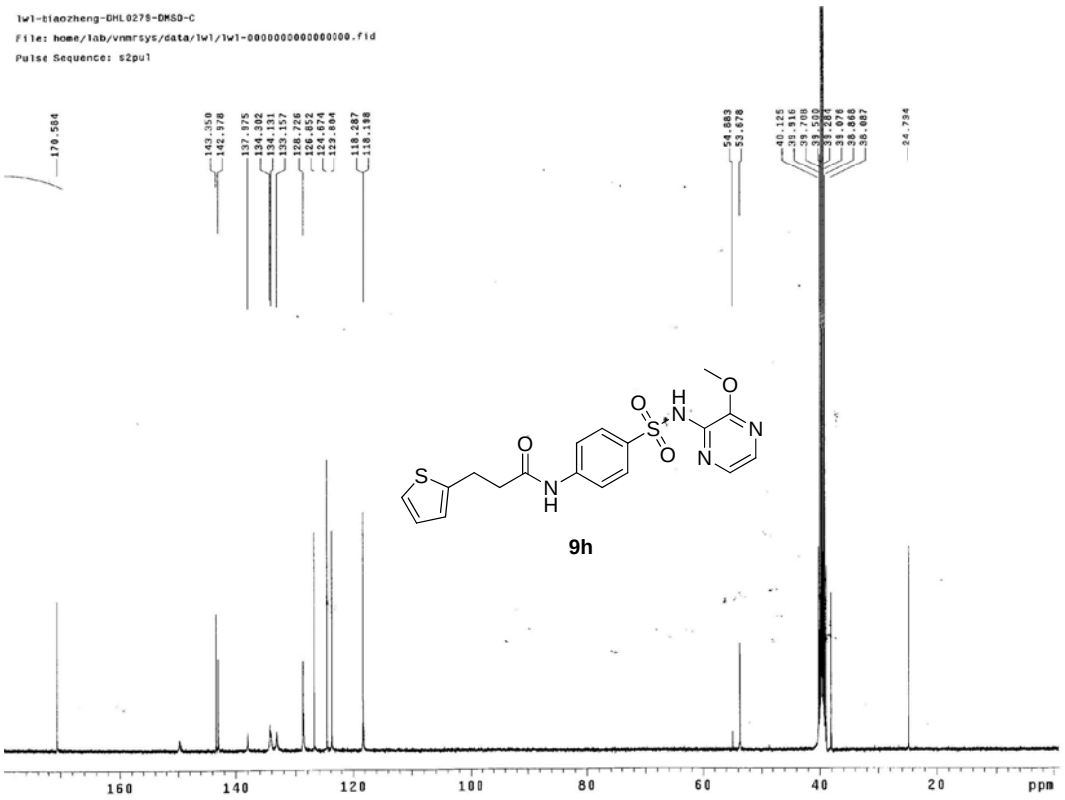

lvl-biaozheng-1420-DMSO-H
File: xp
Pulse Sequence: s2pul
10.826
10.720
8.146
8.136
7.987
7.966
7.856
7.834
7.828
7.788
7.766
7.744
7.590
7.579
6.877
6.837
5.752
3.907
3.318
2.500
-0.004
9a
11
10
9
8
7
6
5
4
3
2
1
ppm
0.76
0.94
0.85
2.42
0.98
1.98
0.31
0.99
0.22
3.00
2.08

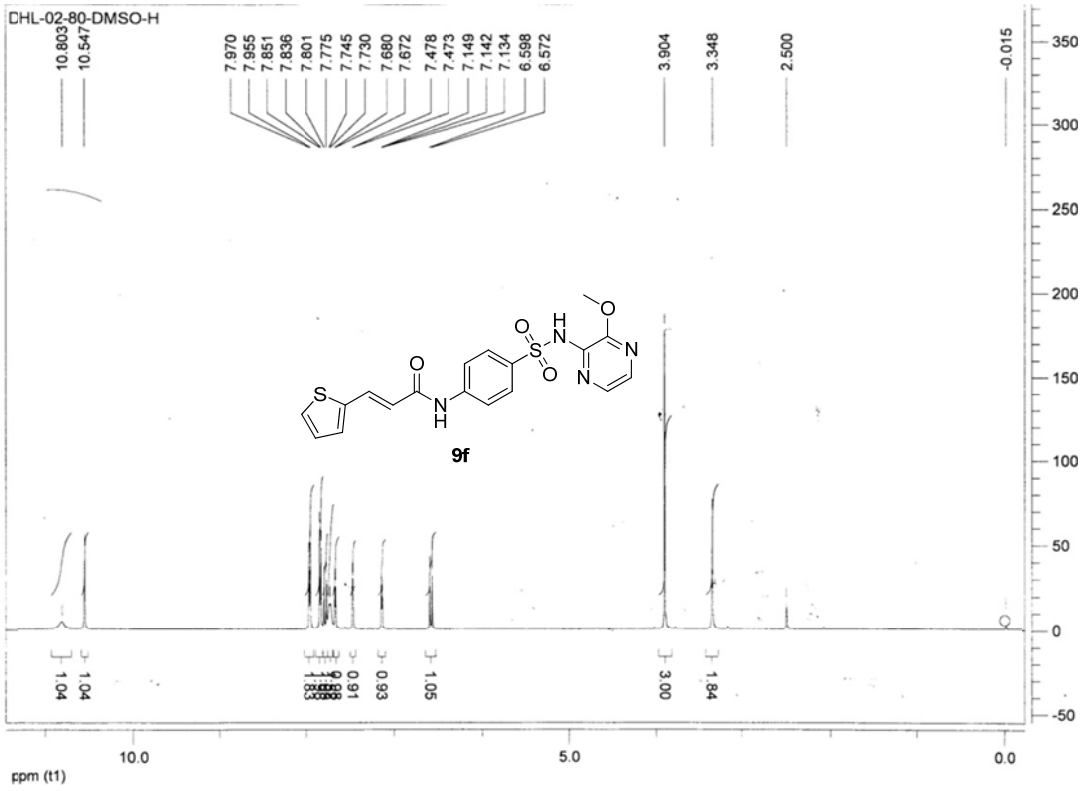

lvl-biaozheng-1420-DMSO-C
File: xp
Pulse Sequence: s2pul
162.725
150.644
149.826
146.435
142.681
137.915
134.941
134.220
133.772
132.485
130.800
130.421
128.859
125.499
118.696
53.678
40.125
39.916
39.708
39.292
39.084
38.875
9a
160
140
120
100
80
60
40
20
ppm


lwl-biaozheng-DHL0278-Acetone-H-1
File: xp
Pulse Sequence: s2pul
9g
ppm

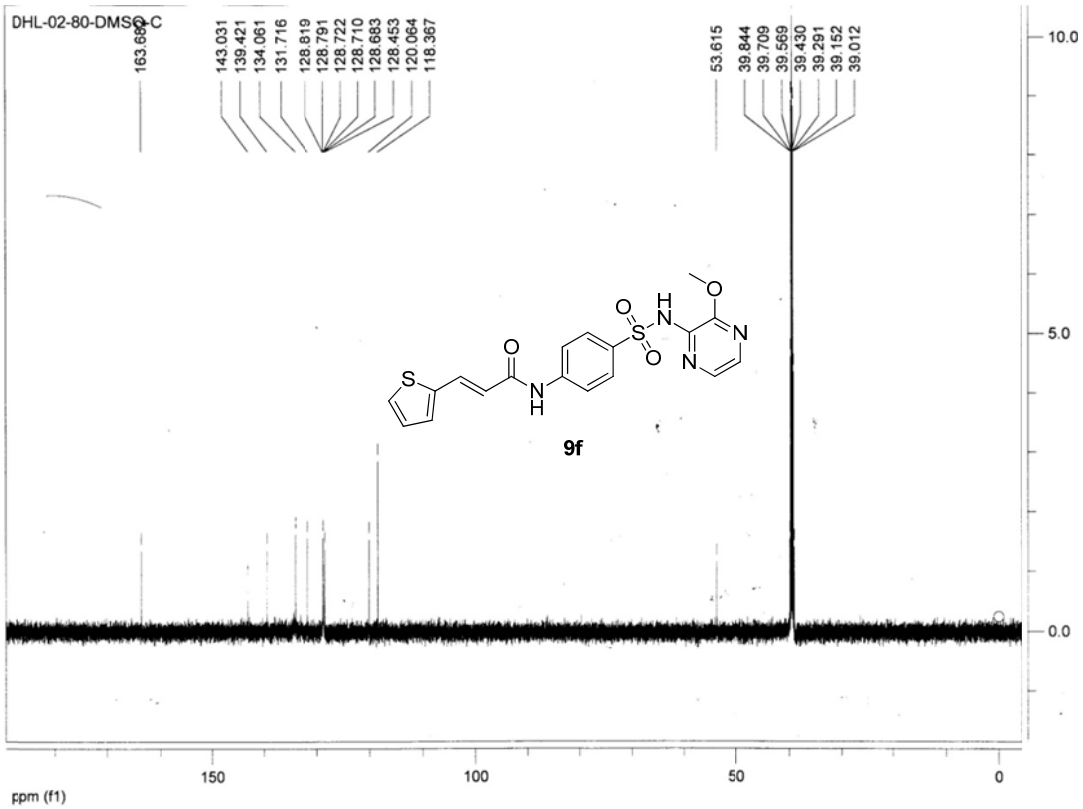

lwl-biaozheng-DHL0278-Acetone-C
Sample: qxy-1-H-69-20110525
File: xp
Pulse Sequence: s2pul
9g
ppm


lwl-biaozheng-DHL0279-DMSO-H
File: xp
Pulse Sequence: s2pul
9h

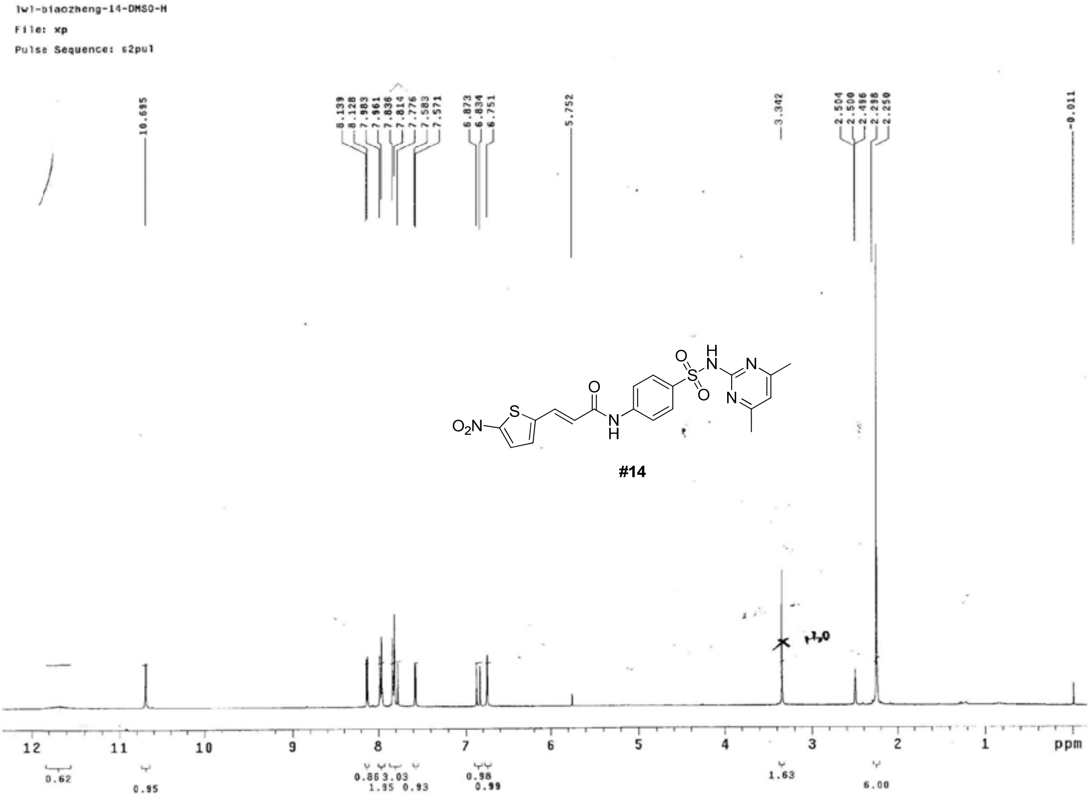

lwl-biaozheng-DHL0279-DMSO-C
Pulse Sequence: s2pul
9h

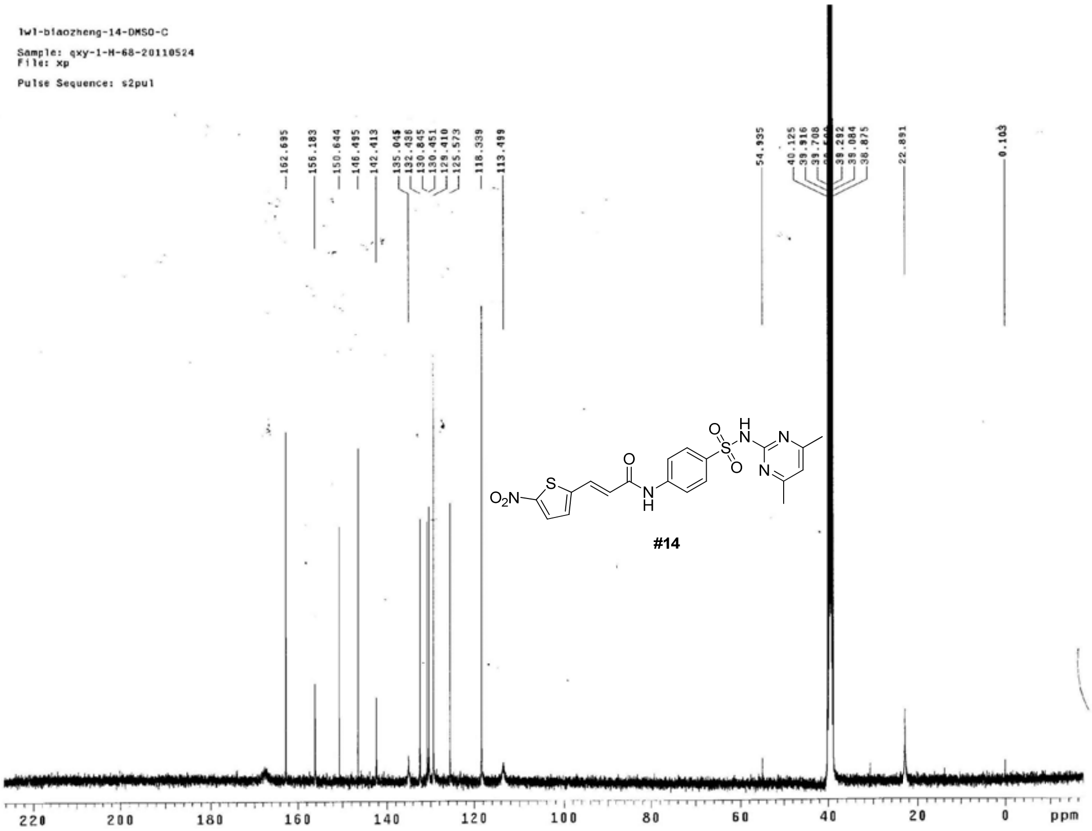


9f



9f


lwl-biaozheng-14-DMSO-H
File: xp
Pulse Sequence: s2pul
O₂N
S
O
N
H
O
S
O
H
N
N
N
#14
ppm


lwl-biaozheng-14-DMSO-C
Sample: qxy-1-H-68-20110524
File: xp
Pulse Sequence: s2pul
O₂N
S
O
N
H
O
S
O
H
N
N
N
#14
ppm