SUPPORTING INFORMATION

Liganding functional tyrosine sites on proteins using sulfur-triazole exchange chemistry

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CONTENTS:

1. SUPPORTING FIGURES

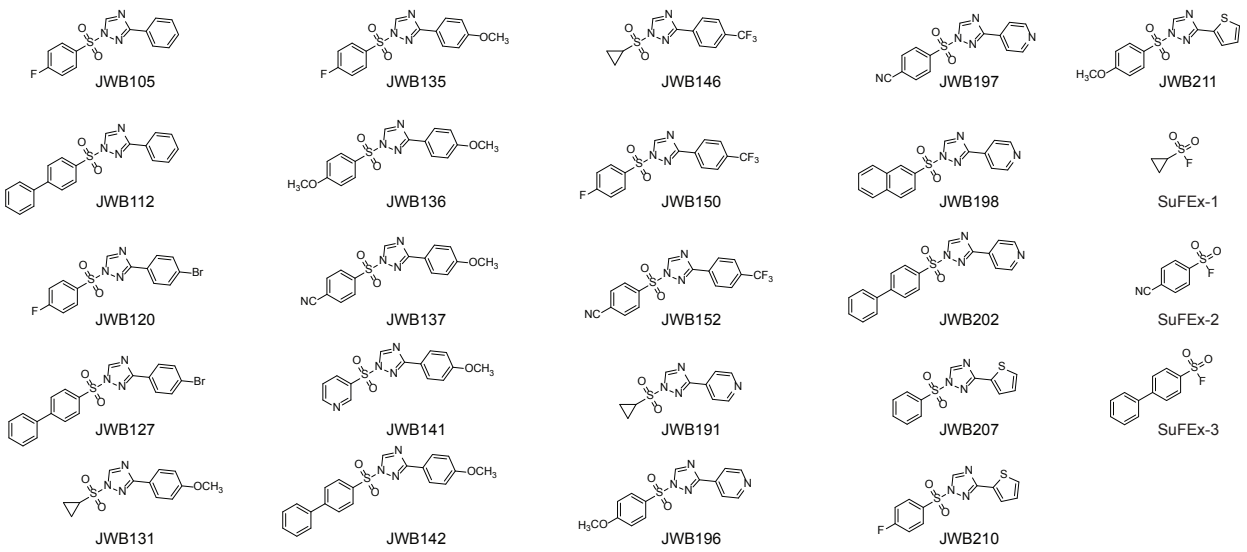


Figure S1. **Chemical structures of a SuTEx fragment library displaying adduct- (AG) and leaving-group (LG) diversity**.

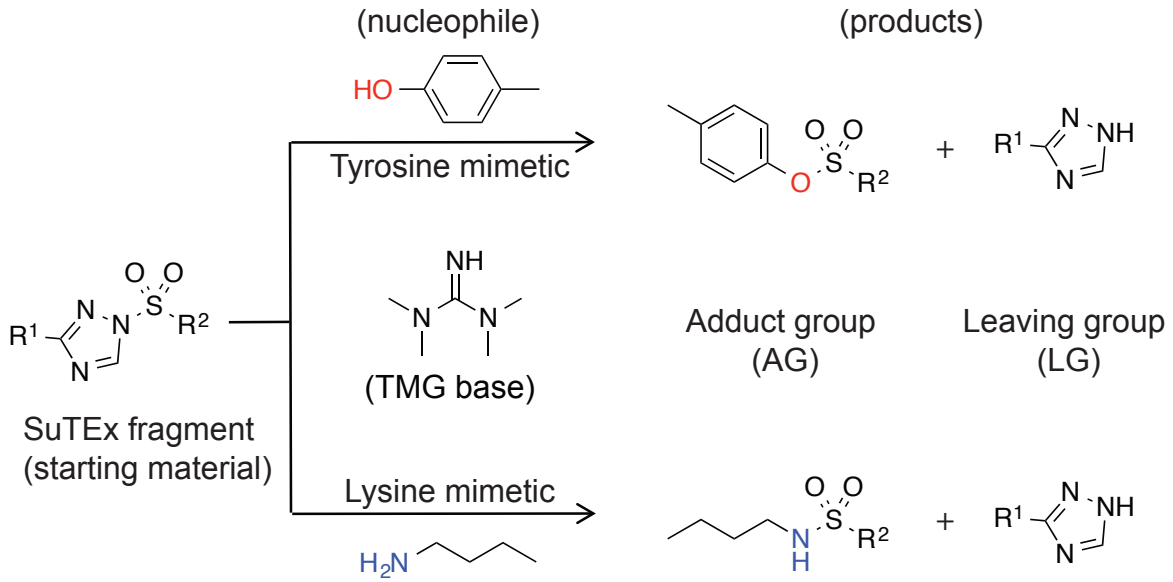


Figure S2. **Schematic of HPLC assay for measuring SuTEx fragment solution reactivity**. SuTEx fragments are incubated with *p*-cresol or *n*-butylamine in the presence of tetramethylguanidine (TMG) base and time-dependent covalent reaction monitored by the reduction of corresponding SuTEx fragment signal.

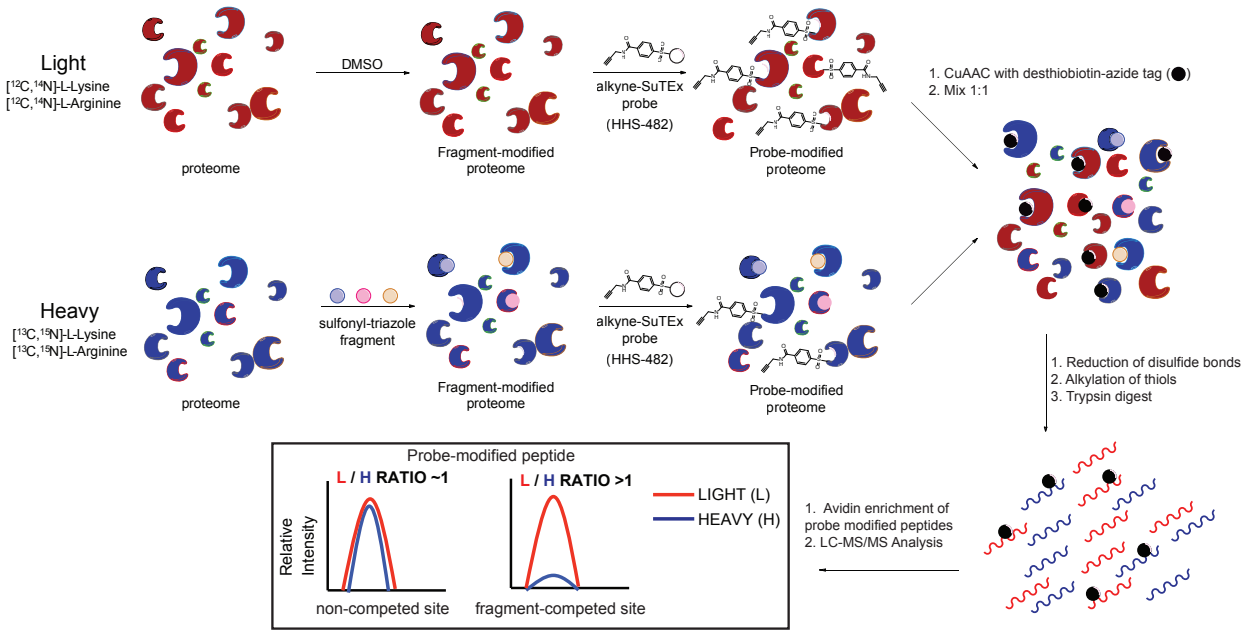


Figure S3. **Quantitative chemical proteomics for evaluating proteome-wide activity of SuTEx fragments**. Experimental workflow for quantitative chemical proteomics to evaluate fragment reactivity and specificity in proteomes. DM93 cells were cultured in SILAC media supplemented with either "light" ^{12}C, ^{14}N-labeled lysine and arginine (denoted in red) or "heavy" ^{13}C, ^{15}N-labeled lysine and arginine (denoted in blue). Light and heavy DM93 proteomes were treated with DMSO vehicle or SuTEx fragment (50 μM, 37 °C, 30 min), respectively, followed by HHS-482 probe labeling using the same reaction conditions. The resulting SILAC ratios (SR) were quantified using the area under the curve of MS1 extracted ion chromatograms. Non-liganded tyrosines are expected to show equivalent probe labeling intensity in vehicle (L)- and fragment (H)-treated conditions (left MS1, SR~1). Fragment-competed tyrosines (i.e. liganded tyrosine) are identified by sites showing substantial reduction in peptide abundance (due to competition of HHS-482 probe labeling) in fragment-treated compared with vehicle control samples (right MS1, SR >>1).

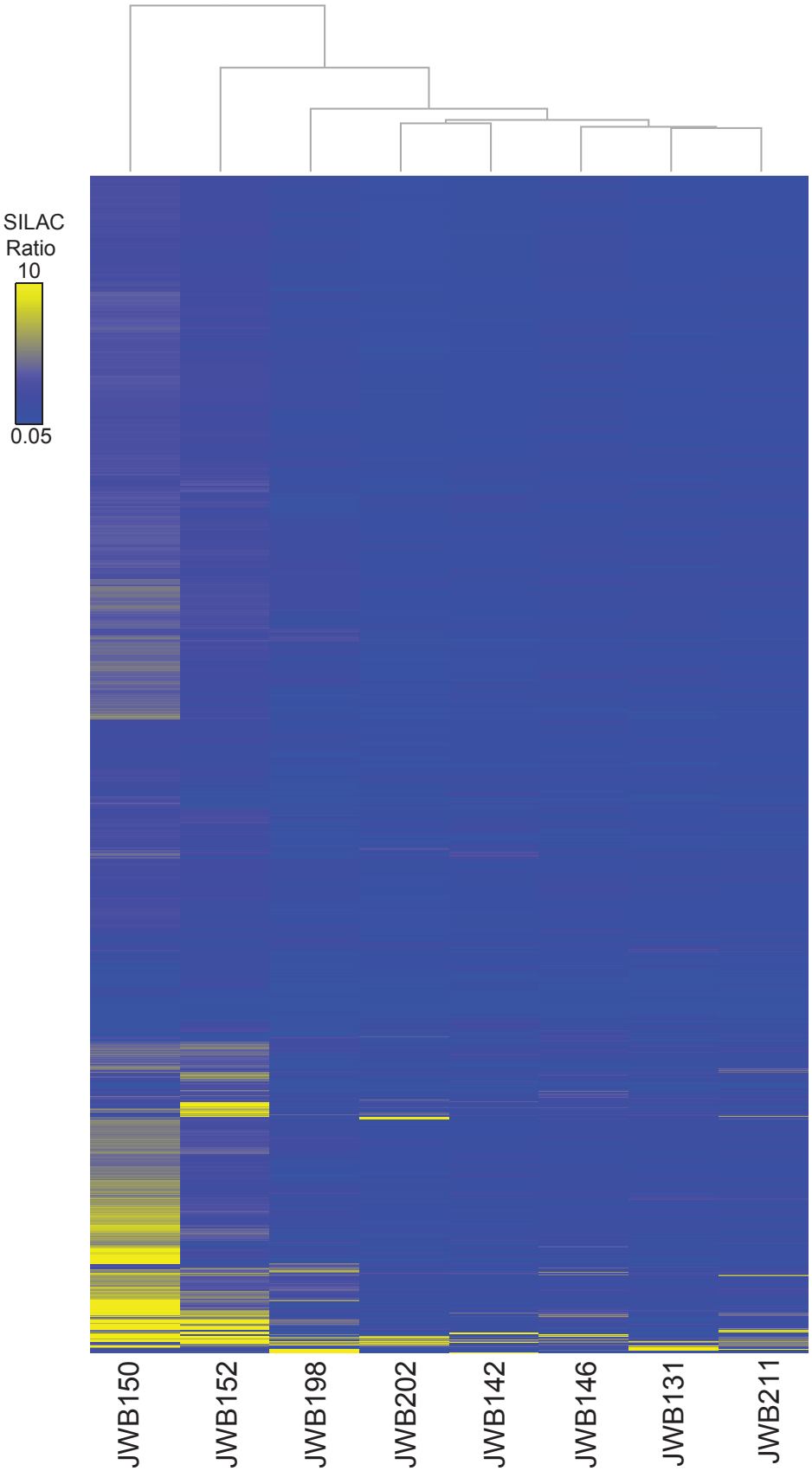

SILAC
Ratio
10
0.05
JWB150
JWB152
JWB198
JWB202
JWB142
JWB146
JWB131
JWB211

Figure S4. **Fragment-based ligand discovery using SuTEx (all tyrosine sites)**. Heat map showing SILAC ratios (SR) of all quantified tyrosines in chemical proteomic studies configured for evaluating ligand competition (see Figure S3). Fragments and liganded tyrosine sites are organized by hierarchical clustering. Data shown are representative of n = 2-3 biologically independent experiments.

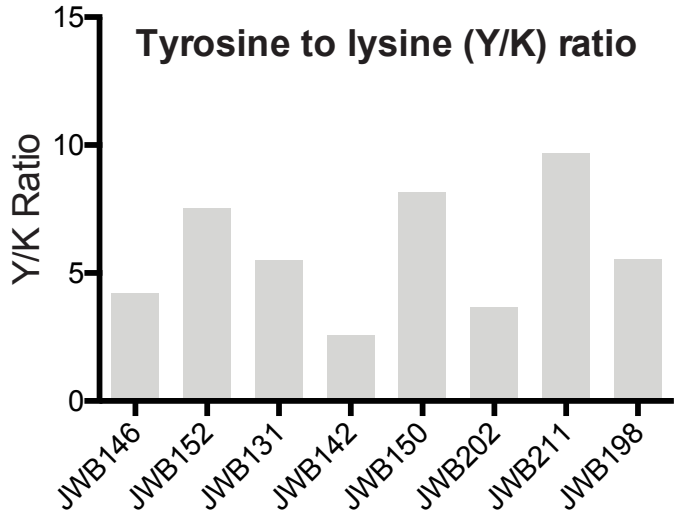


Figure S5. **Chemoselectivity of SuTEx fragments**. Ratio of the number of tyrosine to lysine sites liganded (SR >2) by each respective fragment. Data shown are representative of n = 2-3 biologically independent experiments.

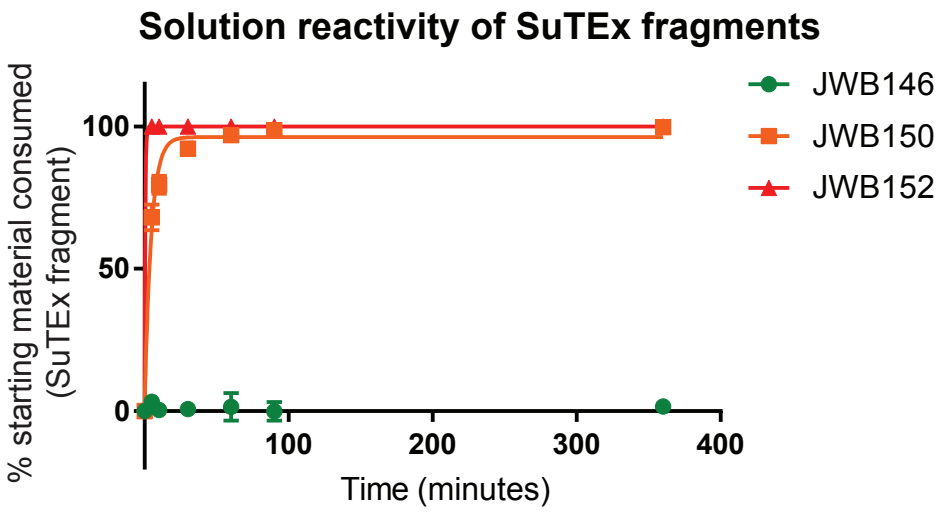


Figure S6. **Evaluating reactivity of fragment electrophiles in solution**. JWB150 and JWB152 fragment electrophiles showed comparable reaction with *p*-cresol nucleophile while JWB146 was largely unreactive as judged by HPLC assay. See Supporting Information for additional details of HPLC solution assay. Data shown are normalized to an internal standard (caffeine) and representative of n = 3 independent experiments.

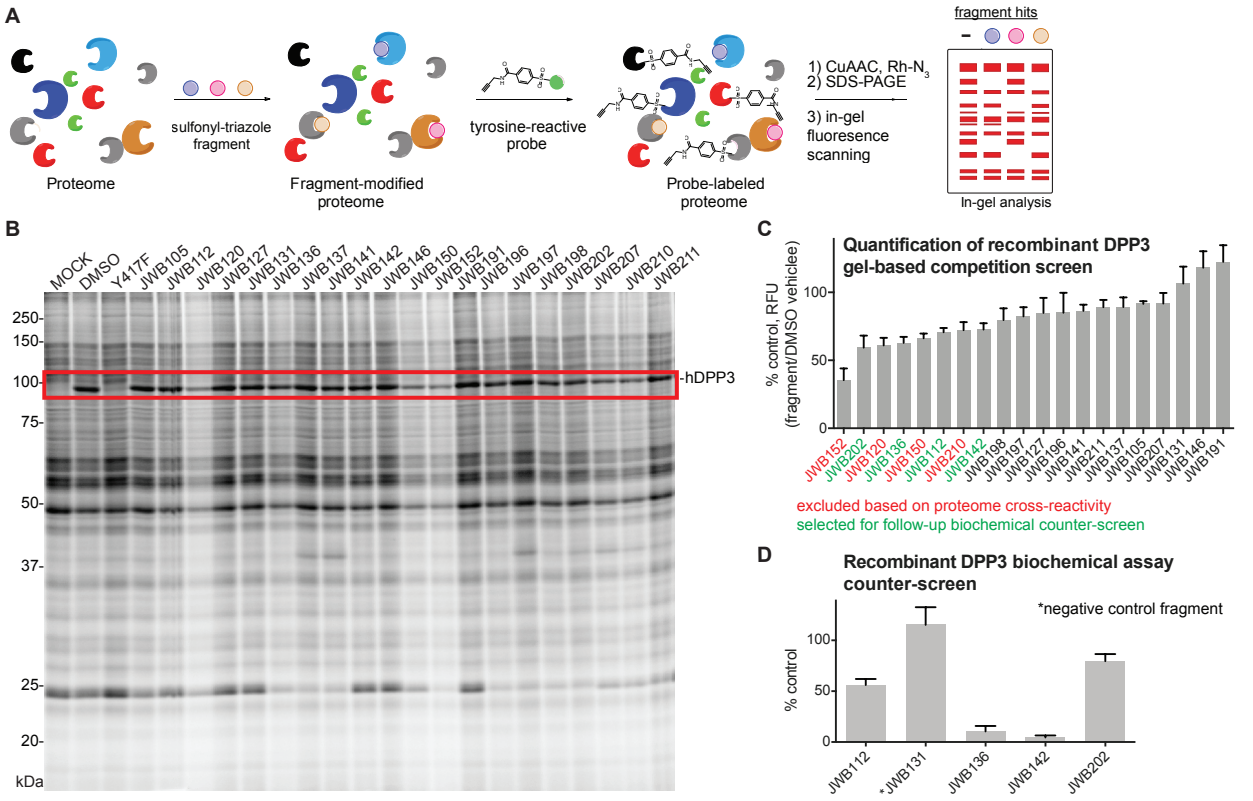


Figure S7. **Gel-based chemical proteomic screen for DPP3 fragment ligands**. (A) Schematic of gel-based competitive chemical proteomic assay to identify lead SuTEx fragments as ligands for perturbing human DPP3 (hDPP3) function. Proteomes were screened against the SuTEx fragment library shown in Figure S1. (B) Recombinant hDPP3-HEK293T soluble proteomes (1 mg/mL) were pretreated with indicated fragment (100 µM, 30 min, 37 °C) followed by labeling with HHS-482 (50 µM, 30 min, 37 °C). DPP3 Y417F mutant showed near-complete loss of HHS-482 labeling, which supports single site labeling at this tyrosine site as previously reported[1]. (C) Integrated band intensities from gel-based competitive screens identified lead DPP3 ligands. Compounds with broad activity in proteomes (highlighted in red) were excluded for further studies. The top 4 compounds (highlighted in green) were further evaluated by the DPP3 biochemical assay (25 µM fragment, D). From this counter-screen, JWB142 was selected as the lead DPP3 inhibitor because of its inhibitory activity and improved selectivity compared with JWB136 as determined by lack of activity against a ~23 kDa off-target protein band from gel-based screens in panel B. Data shown are representative of n = 3 biologically independent experiments.

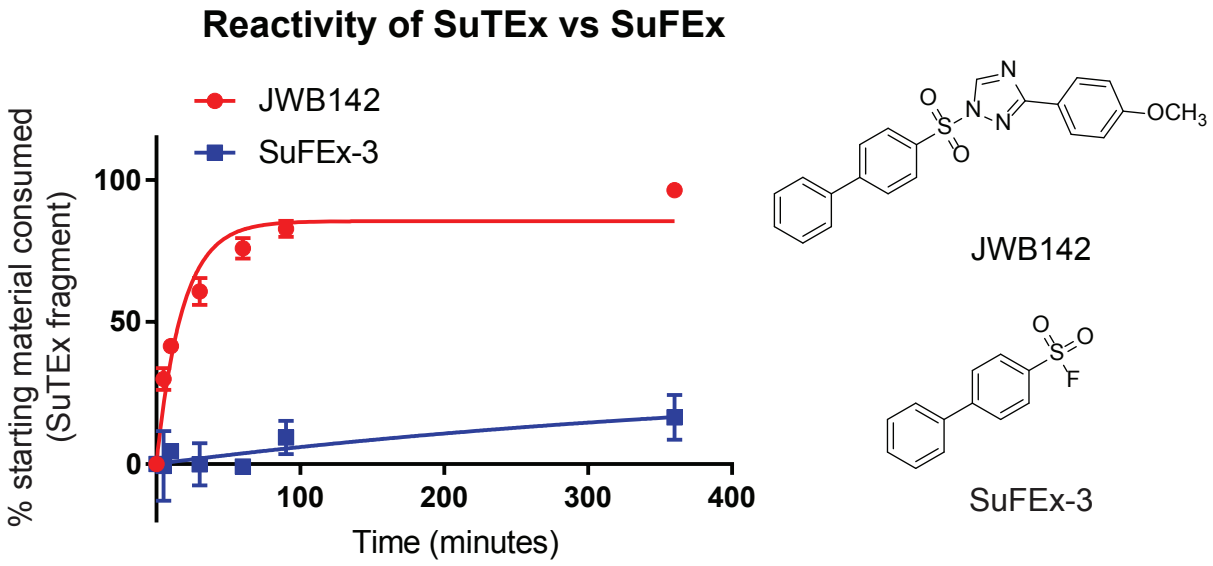


Figure S8. **Comparison of SuTEx and SuFEx reactivity in solution**. JWB142 fragment electrophile shows enhanced reaction with *p*-cresol nucleophile compared with the SuFEx analog SuFEx-3. Data shown are normalized to an internal standard (caffeine) and representative of n = 3 independent experiments.

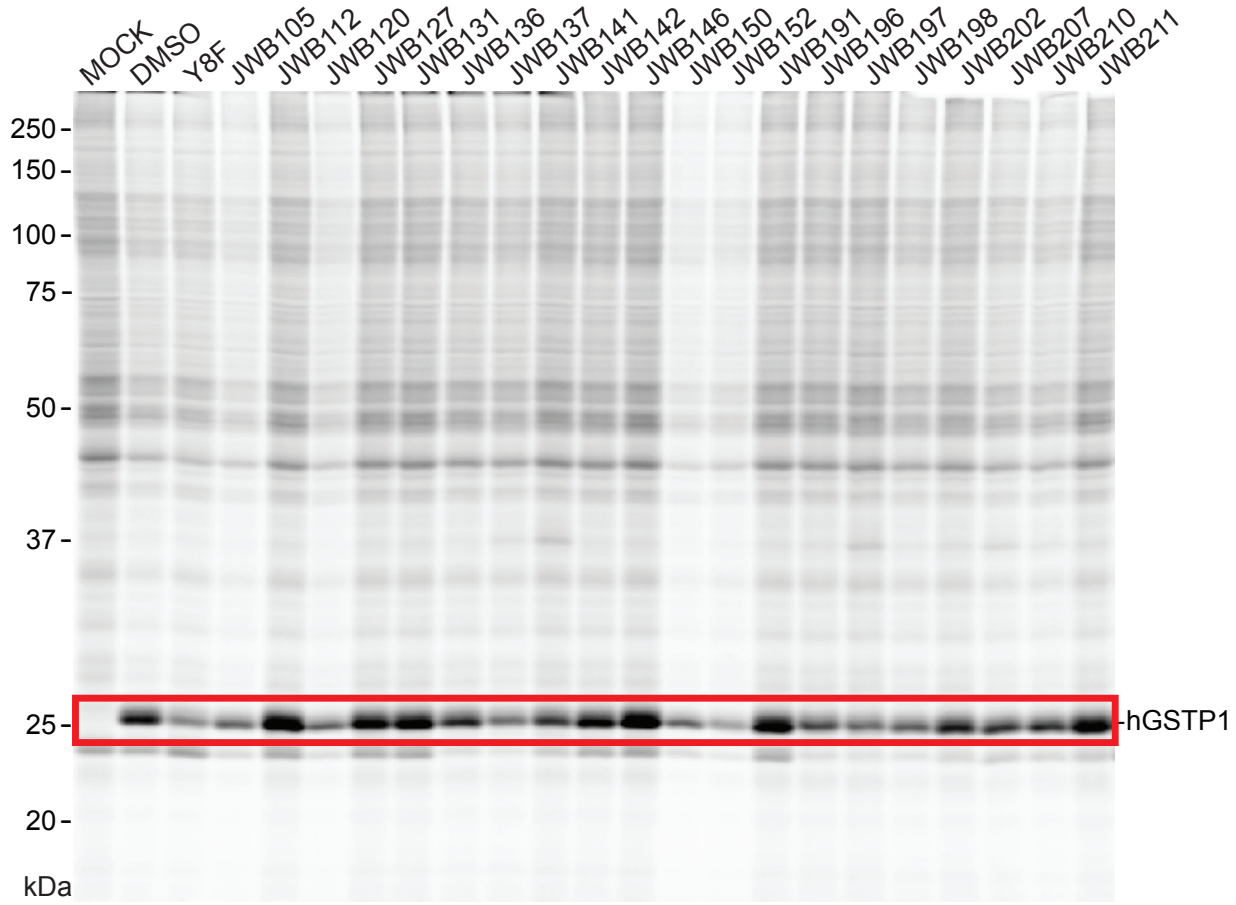


Figure S9. **Gel-based chemical proteomic screen for human GSTP1 (hGSTP1) fragment ligands**. Recombinant hGSTP1-HEK293T soluble proteomes (1 mg/mL) were pretreated with indicated fragment (100 μM, 30 min, 37 °C) followed by labeling with HHS-482 (50 μM, 30 min, 37 °C). GSTP1 Y8F mutant showed near-complete loss of HHS-482 labeling, which supports site selective labeling at this tyrosine site. Gel-based chemical proteomics was performed as shown in Figure S7A. Data shown are representative of n = 3 biologically independent experiments.

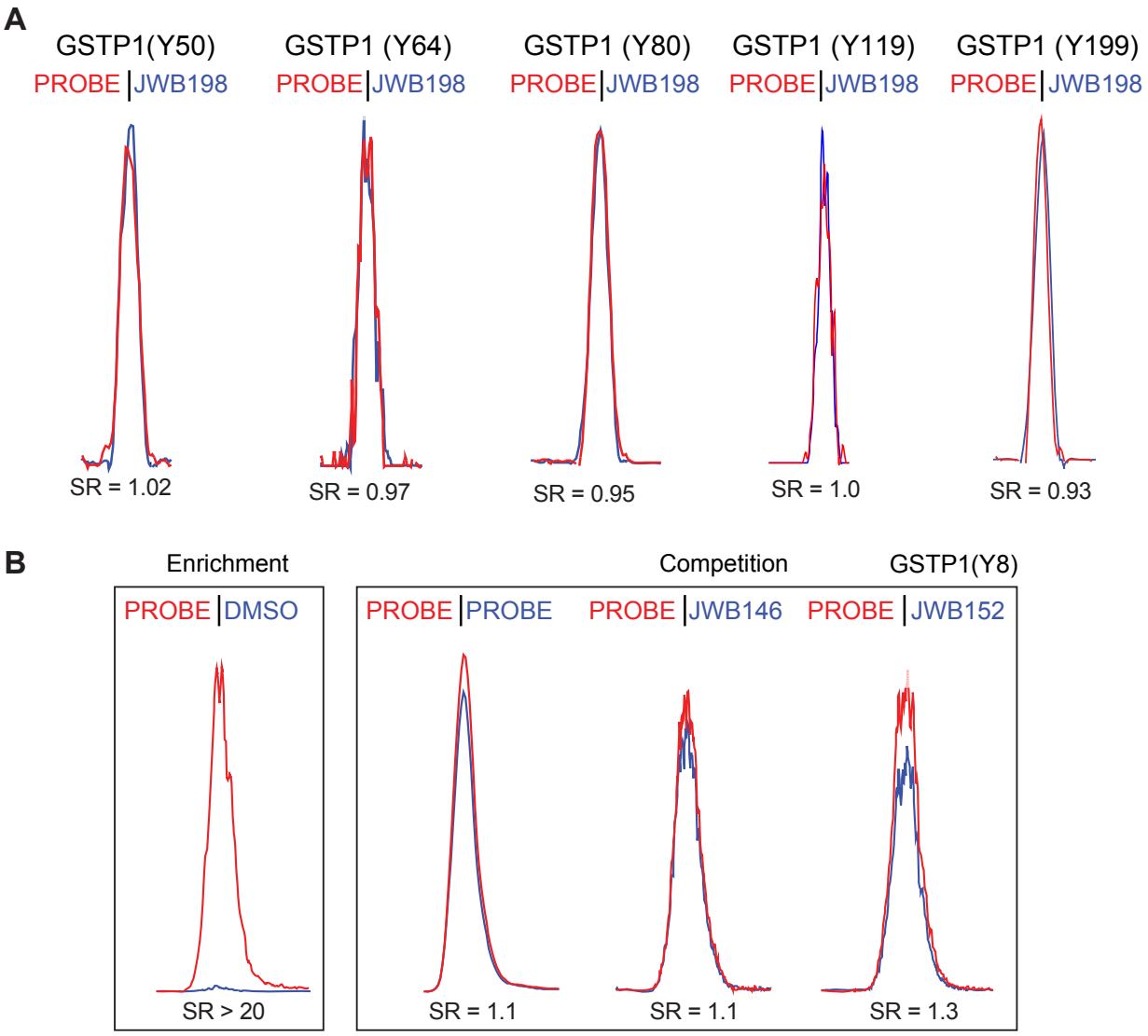


Figure S10. **Liganding GSTP1 Y8 with site specificity using SuTEx fragments**. Representative MS1 EICs of endogenous GSTP1 tyrosine sites from quantitative LC-MS chemical proteomic analyses of live DM93 cells treated with fragment electrophiles. (A) Liganding GSTP1 Y8 by JWB198 (Figure 6C and D) was site specific as evidenced by lack of competition at other quantified tyrosine sites (Y50, Y64, Y80, Y119, and Y199) in live cell studies. (B) GSTP1 Y8 was mildly liganded by JWB152 but not JWB146 in live cell competition studies. A probe (light) /DMSO (heavy) control was included to demonstrate specific enrichment of quantified tyrosine. Data shown are representative of n = 2 biologically independent experiments.

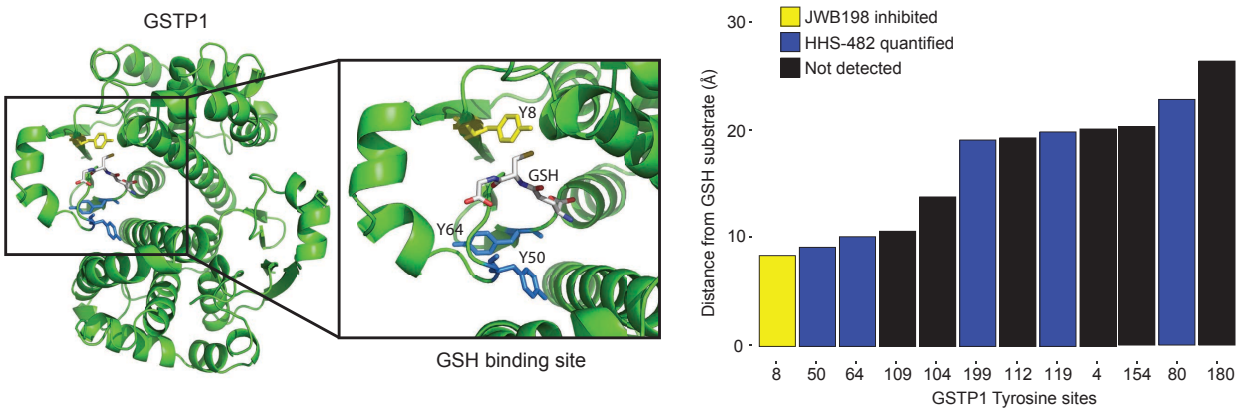


Figure S11. **Proximity of quantified tyrosine sites to GSH substrate in GSTP1**. (A) Crystal structure of GSTP1 with GSH ligand (PDB ID: 5GSS). Inset shows the GSH binding site and tyrosines in proximity to GSH that are liganded by JWB198 (yellow), non-liganded but quantified by HHS-482 probe labeling (blue), or not detected by chemical proteomics. Bar graph shows tyrosine sites organized by their Euclidean distance from GSH substrate.

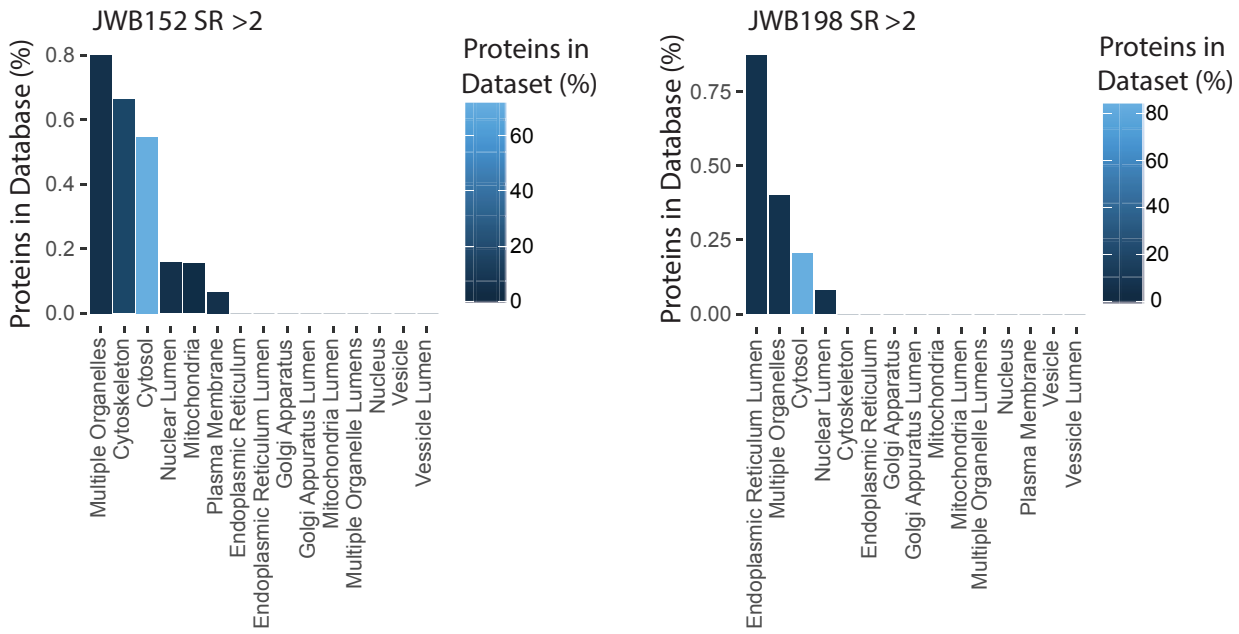


Figure S12. **Subcellular location analysis of liganded proteins from DM93 live cell chemical proteomics**. Proteins containing a liganded tyrosine site (SR >2) were grouped based on subcellular location using a subcellular location analysis (SLA) algorithm. Data shown are from DM93 cells treated with JWB152 (left panel) or JWB198 (right panel) SuTEx fragment at 50 µM for 30 min. The number of liganded proteins compared with the number of proteins from the Swiss-Prot database for each subcellular compartment (x-axis) using SLA analyses are shown (Proteins in Database, y-axis). The colored bars depict the percentage of liganded proteins from each subcellular compartment compared with all liganded proteins quantified in datasets. See Methods section for detailed explanation of SLA algorithm. All data shown are representative of n = 2 biologically independent experiments.

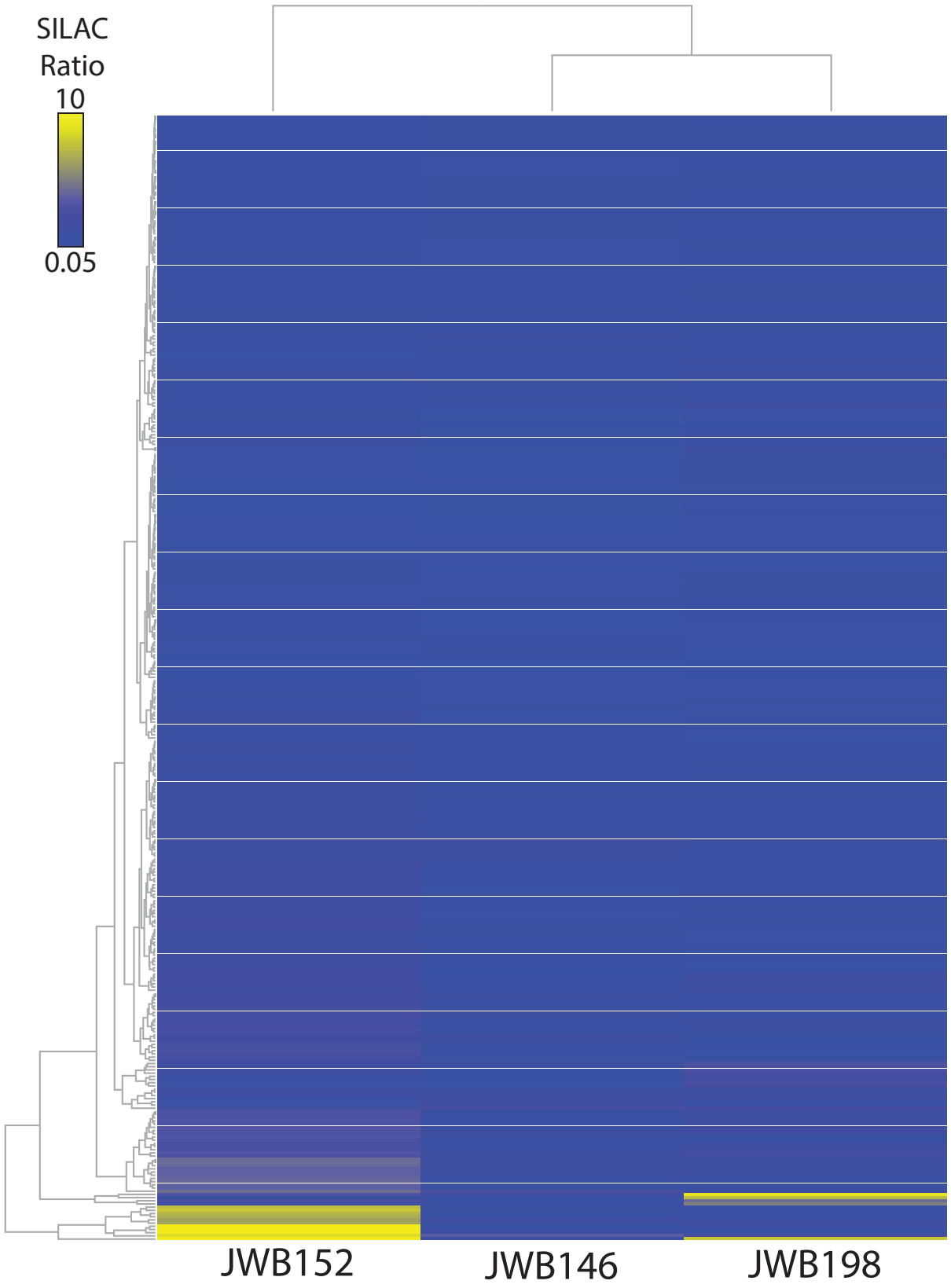

SILAC
Ratio
10
0.05
JWB152
JWB146
JWB198

Figure S13. **Proteome-wide activity of GSTP1 SuTEx fragments in live DM93 cells**. Heat map showing SILAC ratios (SR) of all quantified tyrosine sites competed by fragments in live cells and organized by hierarchical clustering. Fragment competition at tyrosine sites was quantified by chemical proteomics (Figure S3) using the area under the curve of MS1 extracted ion chromatograms (EIC) from HHS-482-labelled peptides in DMSO (light) versus fragment-treated (heavy) soluble proteomes from DM93 cellular experiments. All data shown are representative of n = 2 biologically independent experiments.

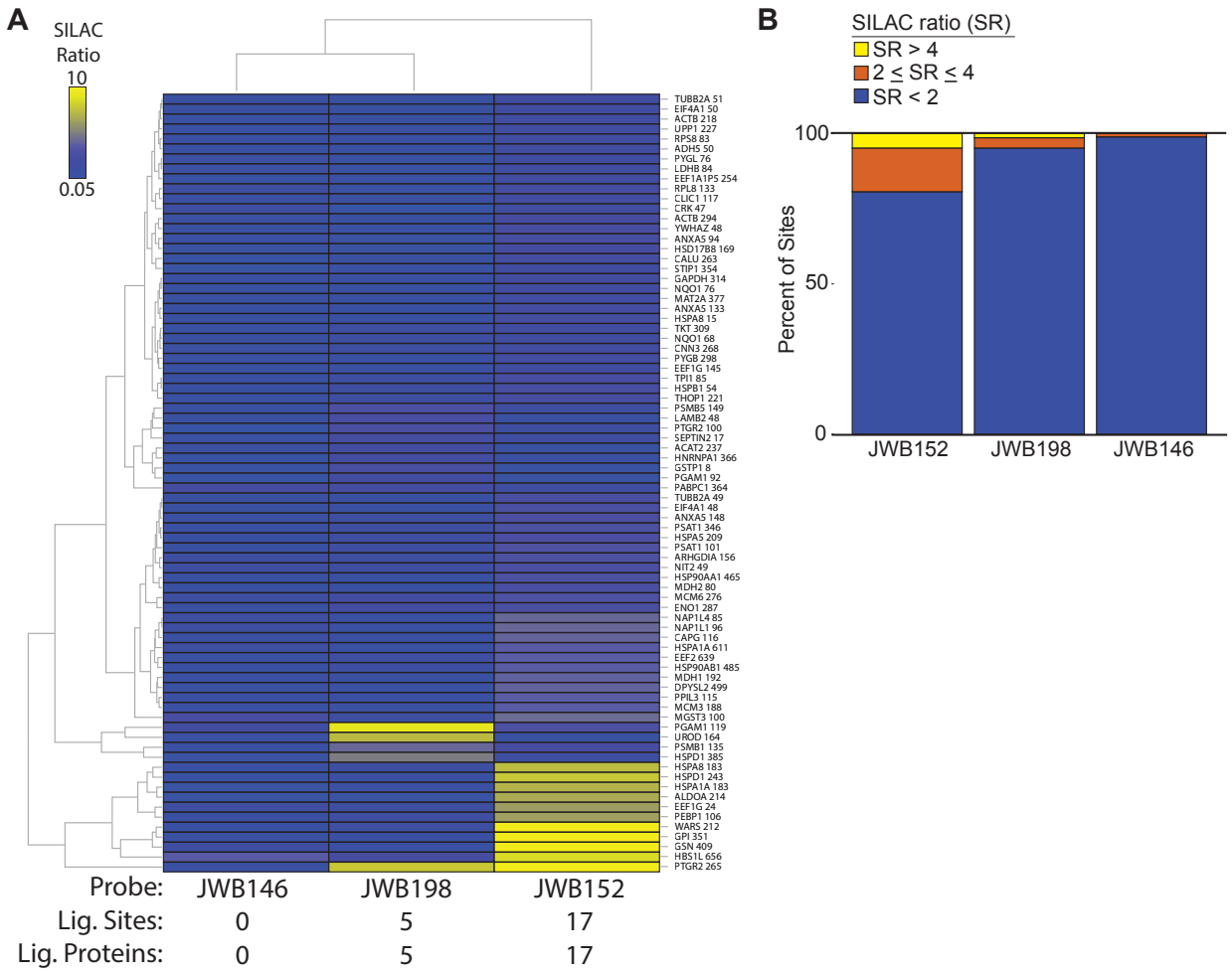


Figure S14. **Liganded tyrosine sites of GSTP1 SuTEx fragments in live DM93 cells**. (A) Heat map showing SILAC ratios (SR) of selected tyrosine sites competed by fragments in live cells and organized by hierarchical clustering. Fragment competition at tyrosine sites was quantified as described in Figure S13. Tyrosine sites shown were liganded (SR > 2) by at least 1 fragment with the number of liganded sites and proteins listed for each SuTEx molecule. Y-axis lists the protein name and quantified tyrosine site. (B) Reactivity of fragments from live cell studies was assessed by comparing the fraction of tyrosine sites competed: SR <2 (blue), 2≤ SR ≤4 (orange), and SR >4 (yellow). All data shown are representative of n = 2 biologically independent experiments.

2. CHEMICAL SYNTHESIS

General Information

Chemicals used were all reagent grade and used as supplied, except where noted. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV-irradiation. Analytical HPLC chromatograms were recorded on a Shimadzu 1100 Series spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 600 (600MHZ) or Bruker Avance III (800MHz) spectrometers in $CDCl_3$, Acetone-d_6, or DMSO-d_6 with chemical shifts referenced to internal standards ($CDCl_3$: 7.26 ppm ^{1}H, 77.16 ppm ^{13}C; $(CD_3)_2CO$: 2.05 ppm ^{1}H, 29.84 and 206.26 ppm ^{13}C; $(CD_3)_2SO$: 2.50 ppm ^{1}H, 39.52 ppm ^{13}C) unless stated otherwise. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet for 1H-NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hz. High resolution mass spectral (HRMS) data were obtained using an Agilent 6545B LC/Q-TOF (Agilent Technologies, Santa Clara, CA, USA).

Chemical Suppliers:

The chemicals listed below were purchased commercially and showed ≥ 95% purity. Solvents purchased commercially were LC-MS grade:

Fisher Scientific: *N,N*-diisopropylethylamine, hydrazine hydrate, *N,N*-dimethylformamide dimethyl acetal, acetic acid (HOAc, Optima LC/MS grade), water (HPLC grade), and acetonitrile (ACN, Optima LC/MS grade),

Combi-Blocks: 4-methoxybenzenesulfonyl chloride, *p*-cresol, *n*-butylamine

Acros: 1,1,3,3-tetramethylguanidine (99%), 2-naphthalenesulfonyl chloride, 4-fluorobenzenesulfonyl chloride, 4-biphenylsulfonyl chloride, 4-methoxyphenyl sulfonyl chloride,

benzenesulfonyl chloride, 4-bromobenzenesulfonyl chloride, 4-cyanobenzenesulfonyl chloride, 4-bromobenzamide, 4-methoxybenzamide,4-(trifluoromethyl)benzamide, pyridine-3-sulfonyl chloride

Alfa Aesar: caffeine, benzamide

Oakwood chemical: cyclopropanesulfonyl chloride, thiophene-2-carboxamide

Decon Laboratories: 200 proof ethanol

AK Scientific: isonicotinamide

HPLC assay for profiling solution reactivity and stability of sulfonyl triazole fragments

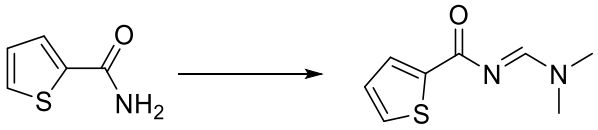
The following reagents were prepared and stored on ice prior to use. 0.1 M solution of caffeine in acetonitrile, 1.0 M solution of *n*-butylamine, *p*-cresol, tetramethylguanidine (TMF), 1 M HOAc in ACN and 10 mM solution of SuTEx fragment in DMF-ACN mixture (10:90, v/v). 500 µL of fragment solution was transferred to a dram vial on ice. To the mixture, 5.5 µL of tetramethylguanidine (TMG) and 5.5 µL of *p*-cresol or *n*-butylamine were added and solutions were stirred on ice for 6 h. To monitor reactivity, 50 µL aliquots were removed at indicated time points and quenched with 10 µL of a 1:1 mixture of caffeine and HOAc. Samples were injected (1 µL) and analyzed by reverse-phase HPLC on a Shimadzu 1100 Series spectrometer with UV detection at 254 nm. Reaction progress was evaluated by monitoring consumption of starting material (SuTEx fragment) normalized to caffeine standard. Chromatographic separation was performed using a Phenomenex Kinetex C18 column (2.6 µm, 50 x 4.6 mm). Mobile phases A and B were composed of H_2O + 0.1% HOAc and ACN + 0.1% HOAc, respectively. Samples were analyzed using the following analytical conditions: using a flow rate of 0.8 ml min^{-1}, the gradient was as follows: 0–0.5 min, 15% B; 0.5–6.5 min 85% B; 6.5–7 min 100% B; 7–8.5 min 100% B;

8.5–9 min 15% B; 9–9.8 min 15% B. The amount of SuTEx fragment consumed was calculated using the area under the curve (AUC) for the fragment peak at time (t) = experimental / t = 0. All SuTEx fragment peak AUCs used for calculations were normalized to caffeine standard AUCs at respective time points to account for run to run variations by HPLC. The amount of SuTEx fragment consumed (% starting material) was plotted as a function of time and the half-life for consumption was calculated by non-linear regression (one phase decay) in Graphpad Prism.

General protocol for synthesis of 1,2,4-sulfonyl-triazole fragment library

To a solution of sulfonyl chloride (1.0 mmol, 1.0 eq.) in anhydrous ethanol (1.9 mL, 0.2 M) was added the corresponding triazole (1.0 mmol, 1.0 eq.) and triethylamine (124 µL, 1.1 mmol, 1.1 eq.) at room temperature. The reaction mixture was stirred at room temperature for 2 h until product precipitated out of solution. Filtrate was removed and product was washed several times with cold ethanol. Product was dried under high-vacuum.

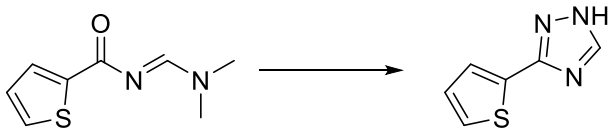
(E)-N-((Dimethylamino)methylene)thiophene-2-carboxamide



Thiophene-2-carboxamide (10.0 g, 78.6 mmol) was dissolved in 25 mL of DMF-DMA in a 100 mL round bottom flask. The reaction was allowed to stir at 120 °C for 30 min. Reaction was allowed to cool back to room temperature and 25 mL of anhydrous ether was added to reaction flask and the flask was placed on ice. Reaction was filtered and product was washed with 1:1 ether/hexanes. **Yield**: 68.8%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 8.63 (s, 1H), 7.87 (d, J = 4.8 Hz,

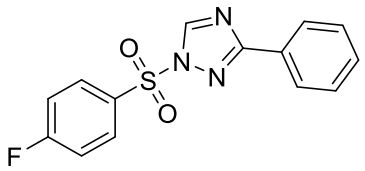
1H), 7.72 (d, J = 4.9 Hz, 1H), 7.22 – 7.16 (m, 1H), 3.34 (s, 3H), 3.25 (s, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 172.17, 161.39, 161.22, 144.74, 132.11, 131.98, 131.90, 128.50, 128.33, 41.55, 41.43, 41.31, 35.33. **ESI-TOF** (HRMS) m/z $[M+H]^+$ calculated for $C_8H_{11}N_2OS^+$ 183.0587, found 183.0582

3-(Thiophen-2-yl)-1H-1,2,4-triazole



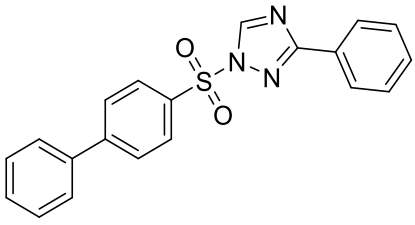
11.84 g (64.9 mmoL) of (E)-N-((dimethylamino)methylene)thiophen-2-carboxamide was dissolved in 50 mL of acetic acid. The solution was heated to 90 °C and hydrazine hydrate monohydrate (1.1 eq.) was added dropwise to reaction and allowed to stir for 90 min. The reaction was cooled to room temperature and concentrated via roto-evaporator. The reaction was diluted in ether and the pH was neutralized with saturated $NaHCO_3$. The aqueous and organic layers were separated and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The organic fraction was dried via roto-evaporator to provide the product. **Yield**: 78:4%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 8.40 (s, 1H), 7.70 (dd, J = 3.6, 1.2 Hz, 1H), 7.52 (dd, J = 5.0, 1.2 Hz, 1H), 7.14 (dd, J = 5.0, 3.6 Hz, 1H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 127.76, 127.59, 126.68, 126.55, 125.91, 125.82**. ESI-TOF** (HRMS) m/z $[M+H]^+$ calculated for $C_6H_6N_3S^+$ 152.0277, found 152.0281.

1-((4-Fluorophenyl)sulfonyl)-3-phenyl-1H-1,2,4-triazole, JWB105



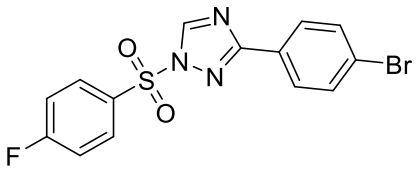
Yield: 56.4%, **^{1}H NMR** (600 MHz, $(CD_3)_2SO$) δ 9.47 (s, 1H), 8.26 (dd, *J* = 9.0, 4.9 Hz, 2H), 7.99 (d, *J* = 7.8 Hz, 2H), 7.60 (t, *J* = 8.8 Hz, 2H), 7.53 – 7.48 (m, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2SO$) δ 162.96, 161.74, 145.42, 130.07, 129.35, 128.31, 128.27, 126.46, 114.85, 114.75. **^{19}F NMR** (564 MHz, $(CD_3)_2SO$) δ -100.45. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{11}FN_3O_2S^+$ 304.0551, found 304.0539.

1-([1,1'-Biphenyl]-4-ylsulfonyl)-3-phenyl-1H-1,2,4-triazole, JWB112



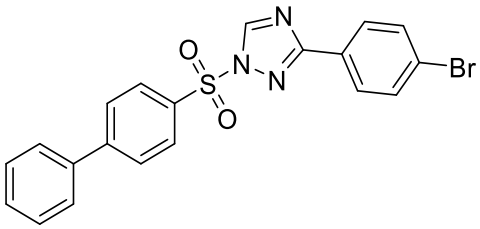
Yield: 47.5**%, ^{1}H NMR** (800 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.19 – 8.16 (m, 2H), 8.01 – 7.97 (m, 2H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.60 – 7.55 (m, 5H), 7.48 (t, *J* = 7.4 Hz, 2H), 7.44 (t, *J* = 7.3 Hz, 1H).**^{13}C NMR** (201 MHz, $CDCl_3$) δ 165.22, 148.48, 145.35, 138.59, 134.47, 130.47, 129.24, 129.16, 129.15, 129.08, 128.61, 128.22, 127.42, 127.11. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{20}H_{16}N_3O_2S^+$ 362.0958, found 362.0979.

3-(4-Bromophenyl)-1-((4-fluorophenyl)sulfonyl)-1H-1,2,4-triazole, JWB120



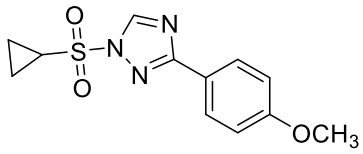
Yield: 72.2%, **^{1}H NMR** (600 MHz, $(CD_3)_2SO$) δ 9.49 (s, 1H), 8.26 (dd, *J* = 9.0, 4.9 Hz, 2H), 7.95 – 7.91 (m, 2H), 7.73 – 7.68 (m, 2H), 7.60 (t, *J* = 8.8 Hz, 2H). **^{13}C NMR** (201 MHz, $(CD_3)_2SO$) δ 162.97, 161.76, 145.38, 132.34, 128.37, 128.32, 128.28, 123.13, 114.87, 114.76. **^{19}F NMR** (564 MHz, $(CD_3)_2SO$) δ -100.30. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{10}BrFN_3O_2S^+$ 381.9656, found 381.9659.

1-([1,1'-Biphenyl]-4-ylsulfonyl)-3-(4-bromophenyl)-1H-1,2,4-triazole, JWB127



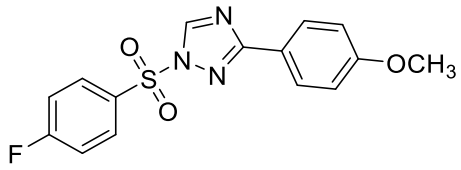
Yield: 63.8%. ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 9.54 (s, 1H), 8.22 (d, *J* = 8.7 Hz, 2H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 7.2 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.51 (d, *J* = 7.7 Hz, 2H), 7.48 – 7.43 (m, 1H). **^{13}C NMR** (151 MHz, $(CD_3)_2SO$) δ 183.25, 163.63, 148.35, 147.86, 138.08, 134.05, 132.56, 129.65, 129.46, 128.95, 128.83, 128.31, 127.81, 124.71. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{20}H_{15}BrN_3O_2S^+$ 440.0063, found 440.0061

1-(Cyclopropylsulfonyl)-3-(4-methoxyphenyl)-1H-1,2,4-triazole, JWB131



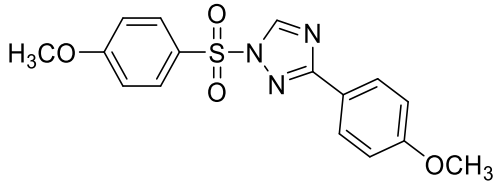
Yield: 17.3%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 8.90 (s, 1H), 8.12 – 8.08 (m, 2H), 7.09 – 7.06 (m, 2H), 3.88 (s, 3H), 3.19 (m, 1H), 1.50 – 1.47 (m, 2H), 1.36 (dd, *J* = 7.9, 2.7 Hz, 2H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 165.16, 162.53, 147.21, 129.19, 123.08, 115.05, 55.76, 31.96, 7.73. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{12}H_{14}N_3O_3S^+$ 280.0750, found 280.0749.

1-((4-Fluorophenyl)sulfonyl)-3-(4-methoxyphenyl)-1H-1,2,4-triazole, JWB135



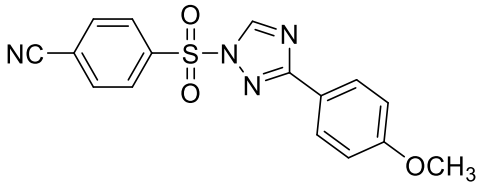
Yield: 51.0%, **^{1}H NMR** (600 MHz, $(CD_3)_2SO$) δ 9.41 (s, 1H), 8.24 (dd, *J* = 9.0, 4.9 Hz, 2H), 7.94 – 7.91 (m, 2H), 7.59 (t, *J* = 8.8 Hz, 2H), 7.06 – 7.03 (m, 2H), 3.80 (s, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2SO$) δ 162.97, 161.75, 161.27, 145.41, 145.39, 128.32, 128.28, 114.93, 114.86, 114.75, 55.83. **^{19}F NMR** (564 MHz, $(CD_3)_2SO$) δ -100.59. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{15}H_{13}FN_3O_3S^+$ 334.0656 , found 334.0658.

3-(4-Methoxyphenyl)-1-((4-methoxyphenyl)sulfonyl)-1H-1,2,4-triazole, JWB136



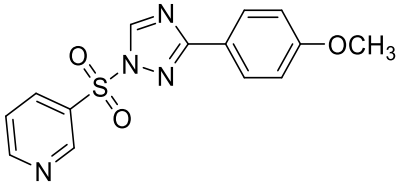
Yield: 49.1%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 9.04 (s, 1H), 8.13 – 8.09 (m, 2H), 8.02 – 7.98 (m, 2H), 7.22 – 7.18 (m, 2H), 7.04 – 6.99 (m, 2H), 3.92 (s, 3H), 3.84 (s, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 166.25, 165.36, 162.51, 147.03, 131.92, 129.13, 128.05, 122.91, 116.04, 114.99, 56.52, 55.73. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{16}H_{16}N_3O_4S^+$ 346.0856, found 346.0858

4-((3-(4-Methoxyphenyl)-1H-1,2,4-triazol-1-yl)sulfonyl)benzonitrile, JWB137



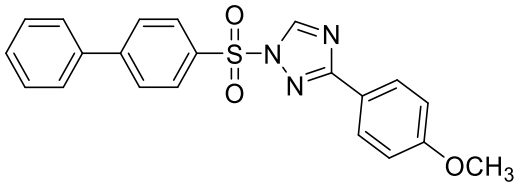
Yield: 60.7%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 9.15 (s, 1H), 8.40 – 8.38 (m, 2H), 8.17 – 8.15 (m, 2H), 8.02 – 8.00 (m, 2H), 7.04 – 7.02 (m, 2H), 3.85 (s, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 165.98, 162.76, 147.91, 140.94, 134.75, 130.12, 129.32, 122.45, 119.65, 117.64, 115.06, 55.77. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{16}H_{13}N_4O_3S^+$ 341.0703, found 341.0718.

3-((3-(4-Methoxyphenyl)-1H-1,2,4-triazol-1-yl)sulfonyl)pyridine, JWB141



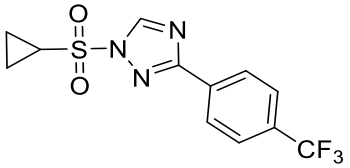
Yield: 44.9%, **^{1}H NMR** (800 MHz, $CDCl_3$) δ 9.32 (d, *J* = 2.4 Hz, 1H), 8.92 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.73 (s, 1H), 8.40 (m, 1H), 8.06 – 8.01 (m, 2H), 7.54 (dd, *J* = 8.9, 4.8 Hz, 1H), 6.96 – 6.93 (m, 2H), 3.85 (s, 3H). **^{13}C NMR** (201 MHz, $CDCl_3$) δ 165.58, 161.73, 155.48, 149.19, 145.35, 136.17, 133.36, 128.75, 123.99, 121.45, 114.12, 55.34. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{13}N_4O_3S^+$ 317.0703, found 317.0702.

1-([1,1'-Biphenyl]-4-ylsulfonyl)-3-(4-methoxyphenyl)-1H-1,2,4-triazole, JWB142



Yield: 60.4%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 9.13 (s, 1H), 8.25 (d, *J* = 8.7 Hz, 2H), 8.01 (dd, *J* = 17.9, 8.8 Hz, 4H), 7.75 (d, *J* = 7.2 Hz, 2H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 1H), 7.03 (d, *J* = 8.9 Hz, 2H), 3.85 (s, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 165.62, 162.61, 148.94, 147.46, 139.34, 135.66, 130.06, 130.00, 129.96, 129.22, 129.18, 128.33, 122.77, 115.03, 55.75. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{21}H_{18}N_3O_3S^+$ 392.1063, found 392.1072

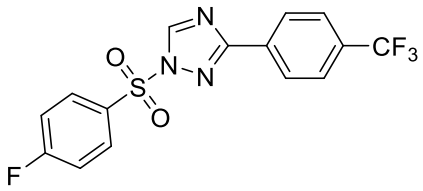
1-(Cyclopropylsulfonyl)-3-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole, JWB146



Yield: 14.6%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 9.04 (s, 1H), 8.37 (d, *J* = 8.6 Hz, 2H), 7.89 (d, *J* = 8.6 Hz, 2H), 3.26 (m, 1H), 1.55 – 1.52 (m, 2H), 1.42 – 1.39 (m, 2H). **^{13}C NMR** (201 MHz,

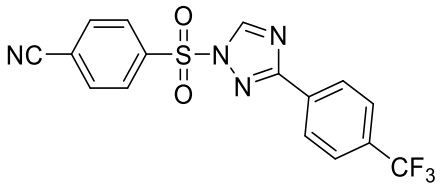
$(CD_3)_2CO$) δ 162.97, 146.87, 133.44, 131.65, 131.49, 131.33, 131.17, 31.17, 7.06. **^{19}F NMR** (201 MHz, $(CD_3)_2CO$) δ -63.38. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{12}H_{11}F_3N_3O_2S^+$ 318.0519, found 318.0533.

1-((4-Fluorophenyl)sulfonyl)-3-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole, JWB150



Yield: 51.6%, **^{1}H NMR** (800 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.31 (m, 2H), 8.29 – 8.25 (m, 2H), 7.79 – 7.77 (m, 2H), 7.41 – 7.37 (m, 2H). **^{13}C NMR** (201 MHz, $CDCl_3$) δ 167.52, 166.23, 164.08, 145.47, 131.88, 131.83, 127.39, 125.65, 125.63, 117.36, 117.25. **^{19}F NMR** (564 MHz, $(CD_3)_2SO$) δ -61.47, -85.01. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{15}H_{10}F_4N_3O_2S^+$ 372.0424, found 372.0426.

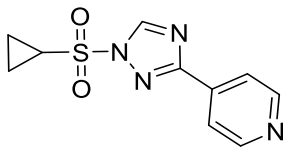
4-((3-(4-(Trifluoromethyl)phenyl)-1H-1,2,4-triazol-1-yl)sulfonyl)benzonitrile, JWB152



Yield: 57.2%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 9.29 (s, 1H), 8.44 – 8.42 (m, 2H), 8.30 – 8.28 (m, 2H), 8.20 – 8.18 (m, 2H), 7.86 (d, J = 8.7 Hz, 2H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 164.68, 148.40, 140.60, 134.83, 133.79, 132.63, 132.47, 130.31, 128.35, 126.75, 119.89, 117.59. **^{19}F**

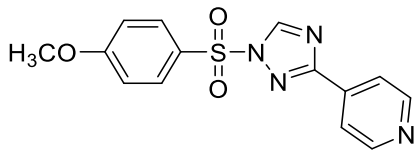
NMR (564 MHz, $(CD_3)_2CO$) δ -63.47. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{16}H_{10}F_3N_4O_2S^+$ 379.0471, found 379.0484

4-(1-(Cyclopropylsulfonyl)-1H-1,2,4-triazol-3-yl)pyridine, JWB191



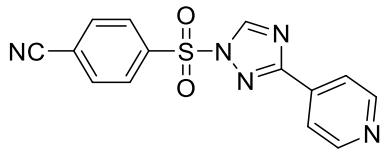
Yield 13.1%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 9.06 (s, 1H), 8.78 – 8.74 (m, 2H), 8.05 – 8.02 (m, 2H), 3.26 (m, 1H), 1.55 – 1.52 (m, 2H), 1.42 – 1.39 (m, 2H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 163.26, 151.56, 147.89, 137.63, 121.44, 32.07, 8.00. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{10}H_{11}N_4O_2S^+$ 251.0597, found 251.0606.

4-(1-((4-Methoxyphenyl)sulfonyl)-1H-1,2,4-triazol-3-yl)pyridine, JWB196



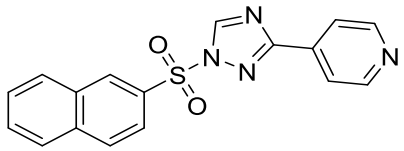
Yield: 22.6%, **^{1}H NMR** (800 MHz, $(CD_3)_2SO$) δ 9.54 (d, *J* = 2.1 Hz, 1H), 8.76 – 8.71 (m, 2H), 8.15 – 8.07 (m, 2H), 7.95 – 7.90 (m, 2H), 7.24 (dd, *J* = 9.0, 1.9 Hz, 2H), 3.87 (d, *J* = 1.7 Hz, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2SO$) δ 165.17, 161.72, 150.14, 147.80, 136.48, 131.14, 125.63, 120.69, 115.63, 56.18. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{13}N_4O_3S^+$ 317.0703, found 317.0709.

4-((3-(Pyridin-4-yl)-1H-1,2,4-triazol-1-yl)sulfonyl)benzonitrile, JWB197



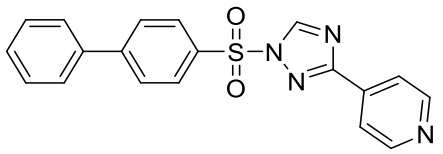
Yield: 34.8%, **^{1}H NMR** (800 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.74 – 8.72 (m, 2H), 8.28 – 8.26 (m, 2H), 7.97 – 7.95 (m, 2H), 7.94 – 7.92 (m, 2H). **^{13}C NMR** (201 MHz, $CDCl_3$) δ 163.84, 150.55, 146.09, 139.73, 136.36, 133.64, 129.54, 121.14, 119.59, 116.56. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{10}N_5O_2S^+$ 312.0550, found 312.0551.

4-(1-(Naphthalen-2-ylsulfonyl)-1H-1,2,4-triazol-3-yl)pyridine, JWB198



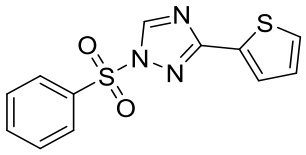
Yield: 52.6%, **^{1}H NMR** (600 MHz, $(CD_3)_2SO$) δ 9.63 (s, 1H), 9.01 – 8.98 (m, 1H), 8.71 – 8.67 (m, 2H), 8.32 (d, *J* = 8.2 Hz, 1H), 8.25 (d, *J* = 8.9 Hz, 1H), 8.10 (d, *J* = 8.1 Hz, 1H), 8.06 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.90 – 7.87 (m, 2H), 7.82 (m, 1H), 7.76 (m, 1H). **^{13}C NMR** (201 MHz, $(CD_3)_2SO$) δ 147.04, 146.02, 145.51, 144.19, 133.18, 132.61, 128.91, 127.91, 127.78, 126.89, 126.76, 124.51, 124.43, 122.93, 99.98. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{17}H_{13}N_4O_2S^+$ 337.0754, found 337.077.

4-(1-([1,1'-Biphenyl]-4-ylsulfonyl)-1H-1,2,4-triazol-3-yl)pyridine, JWB202



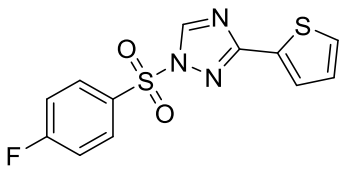
Yield: 31.8%, **^{1}H NMR** (800 MHz, $(CD_3)_2SO$) δ 9.63 (s, 1H), 8.72 (d, *J* = 5.2 Hz, 2H), 8.24 (d, *J* = 8.2 Hz, 2H), 8.03 (d, *J* = 8.3 Hz, 2H), 7.91 (d, *J* = 5.2 Hz, 2H), 7.75 (d, *J* = 7.7 Hz, 2H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.46 (t, *J* = 7.3 Hz, 1H). **^{13}C NMR** (201 MHz, $(CD_3)_2SO$) δ 163.64, 152.13, 149.73, 149.07, 139.12, 137.40, 134.93, 130.78, 130.74, 130.64, 129.95, 128.90, 122.07. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{19}H_{15}N_4O_2S^+$ 363.0910, found 363.0925.

1-(Phenylsulfonyl)-3-(thiophen-2-yl)-1H-1,2,4-triazole, JWB207



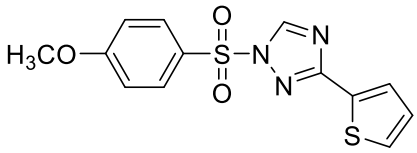
Yield: 66.3%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.12 (dd, *J* = 8.5, 1.1 Hz, 2H), 7.77 – 7.69 (m, 2H), 7.64 – 7.58 (m, 2H), 7.41 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.09 (dd, *J* = 5.0, 3.7 Hz, 1H). **^{13}C NMR** (201 MHz, $CDCl_3$) δ 161.24, 145.36, 136.13, 135.37, 131.76, 129.68, 128.61, 128.38, 128.34, 127.82. **ESI-TOF (HRMS**) m/z $[M+H]^+$ calculated for $C_{12}H_{10}N_3O_2S_2^+$ 292.0209, found 292.0211.

1-((4-Fluorophenyl)sulfonyl)-3-(thiophen-2-yl)-1H-1,2,4-triazole, JWB210



Yield: 35.5%, **^{1}H NMR** (800 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.26 – 8.23 (m, 2H), 7.84 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.50 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.38 – 7.35 (m, 2H), 7.18 (dd, *J* = 5.0, 3.7 Hz, 1H). **^{13}C NMR** (201 MHz, $CDCl_3$) δ 167.43, 166.13, 161.38, 145.27, 131.81, 131.76, 128.48, 127.86, 117.27, 117.16. **^{19}F NMR** (564 MHz, $(CD_3)_2SO$) δ -100.34. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{12}H_9FN_3O_2S_2^+$ 310.0115, found 310.0114.

1-((4-Methoxyphenyl)sulfonyl)-3-(thiophen-2-yl)-1H-1,2,4-triazole, JWB211



Yield: 36.2%, **^{1}H NMR** (800 MHz, $(CD_3)_2CO$) δ 9.06 (s, 1H), 8.10 (d, *J* = 9.1 Hz, 2H), 7.72 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.62 (d, *J* = 1.2 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.16 (dd, *J* = 5.0, 3.6 Hz, 1H), 3.94 (s, 3H). **^{13}C NMR** (201 MHz, $(CD_3)_2CO$) δ 166.37, 161.58, 147.22, 132.85, 132.00, 129.44, 128.99, 128.84, 127.76, 116.11, 56.55. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{13}H_{12}N_3O_3S_2^+$ 322.0315, found 322.0327.

General synthesis of sulfonamide adducts

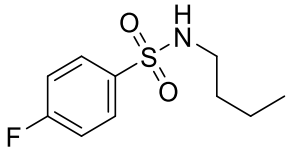
To a solution of sulfonyl chloride (0.50 mmol, 1.0 eq) in anhydrous DCM (5 mL, 100 mM) was added n-butylamine (1.1 eq) and DIPEA (1.1 eq). The reaction was stirred at room temperature for

1.5 hours. The reaction mixture was poured into 1 M HCl aqueous solution (5.0 mL) and extracted with DCM (5.0 mL) 3 times. The organic phase was combined and washed with saturated $NaHCO_3$ (10.0 mL) and brine (10.0 mL). The solution was dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified using silica gel flash column chromatography (ethyl acetate/hexane =1:2 to 1:1).

General synthesis of sulfonate adducts

To a solution of sulfonyl chloride (0.5 mmol, 1.0 eq.) in anhydrous DCM (5.0 mL, 100 mM) was added *p*-cresol (0.9 eq.), DIPEA (1.0 eq) and DMAP (0.2 eq). The reaction was stirred at room temperature for 1 hour. The reaction mixture was poured into 1 M HCl aqueous solution (5.0 mL) and extracted with DCM (5.0 mL) 3 times. The organic phase was combined and washed with saturated $NaHCO_3$ (5.0 mL) and brine (5.0 mL). The solution was dried over $MgSO_4$ and evaporated under reduced pressure. The purification was carried out using silica gel flash column chromatography (ethyl acetate/hexane =1:3 to 1:1) to afford product.

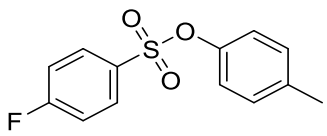
N-Butyl-4-fluorobenzenesulfonamide, KY-347-BA



Yield: 66.2%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.90 – 7.85 (m, 2H), 7.21 – 7.15 (m, 2H), 4.35 (s, 1H), 2.95 (q, 2H), 1.48 – 1.40 (m, 2H), 1.33 – 1.23 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 165.80, 164.11, 136.02, 129.75, 116.15, 42.87, 31.45, 19.60, 13.43. **^{19}F**

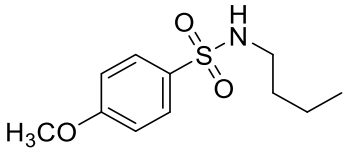
NMR (564 MHz, $CDCl_3$) δ -105.55. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{10}H_{15}FNO_2S^+$ 232.0802, found 232.0802

p-Tolyl 4-fluorobenzenesulfonate, KY-347-CA



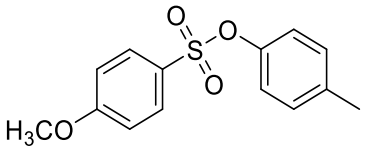
Yield: 82.7%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.88 – 7.78 (m, 2H), 7.21 – 7.15 (m, 2H), 7.09 – 7.05 (m, 2H), 6.86 – 6.82 (m, 2H), 2.30 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.79, 165.08, 147.29, 137.21, 131.34, 130.18, 121.95, 116.38, 20.85. **^{19}F NMR** (564 MHz, $CDCl_3$) δ -102.39. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{13}H_{12}FO_3S^+$ 267.0486, found 267.0480.

N-Butyl-4-methoxybenzenesulfonamide, KY-348-BA



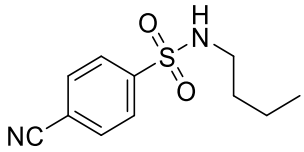
Yield: 90%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.81 – 7.76 (m, 2H), 6.99 – 6.94 (m, 2H), 3.86 (s, 3H), 2.91 (t, J = 7.1 Hz, 2H), 1.46 – 1.38 (m, 2H), 1.32 – 1.24 (m, 2H), 0.84 (t, J = 7.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 162.75, 131.53, 129.16, 114.16, 55.55, 42.83, 31.48, 19.65, 13.48. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{11}H_{18}NO_3S^+$ 244.1002, found 244.0998.

p-Tolyl 4-methoxybenzenesulfonate, KY-348-CA



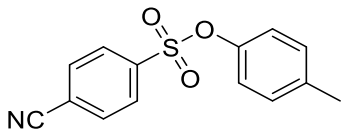
Yield: 39.5%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.75 – 7.71 (m, 2H), 7.08 – 7.03 (m, 2H), 6.98 – 6.92 (m, 2H), 6.87 – 6.82 (m, 2H), 3.87 (s, 3H), 2.30 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 163.96, 147.45, 136.84, 130.69, 130.00, 126.73, 122.06, 114.20, 55.65, 20.81. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{15}O_4S^+$ 279.0686, found 279.0682.

N-Butyl-4-cyanobenzenesulfonamide, KY-349-BA



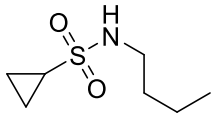
Yield: 70.9%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.00 – 7.94 (m, 2H), 7.84 – 7.78 (m, 2H), 4.43 (s, 1H), 2.99 (td, *J* = 7.1, 6.1 Hz, 2H), 1.49 – 1.41 (m, 2H), 1.33 – 1.24 (m, 2H), 0.86 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 144.43, 132.95, 127.65, 117.33, 116.26, 43.02, 31.58, 19.58, 13.45. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{11}H_{15}N_2O_2S^+$ 239.0849, found 239.0849.

p-Tolyl 4-cyanobenzenesulfonate, KY-349-CA



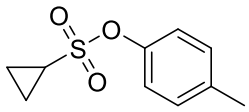
Yield: 49.4%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.97 – 7.90 (m, 2H), 7.84 – 7.78 (m, 2H), 7.12 – 7.06 (m, 2H), 6.86 – 6.80 (m, 2H), 2.31 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 146.99, 139.48, 137.59, 132.79, 130.33, 129.08, 121.68, 117.82, 116.88, 20.80. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{12}NO_3S^+$ 274.0532, found 274.0526.

N-Butylcyclopropanesulfonamide, KY-350-BA



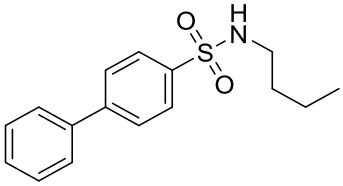
Yield: 78.2%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.15 (s, 1H), 3.14 (t, *J* = 7.1 Hz, 2H), 2.38 (tt, *J* = 8.0, 4.8 Hz, 1H), 1.57 – 1.50 (m, 2H), 1.41 – 1.31 (m, 2H), 1.17 – 1.13 (m, 2H), 0.99 – 0.95 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 43.08, 32.25, 29.89, 19.69, 13.57, 5.20. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_7H_{16}NO_2S^+$ 178.0896, found 178.0895.

p-Tolyl cyclopropanesulfonate, KY-350-CA



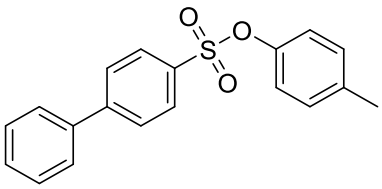
Yield: 51.8%, **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.16 (s, 4H), 2.55 (tt, *J* = 8.0, 4.7 Hz, 1H), 2.34 (s, 3H), 1.27 – 1.19 (m, 2H), 1.12 – 1.04 (m, 2H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 147.47, 137.01, 130.23, 121.93, 27.52, 20.83, 6.11. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{10}H_{13}O_3S^+$ 213.0580, found 213.0576.

N-Butyl-[1,1'-biphenyl]-4-sulfonamide, KY-351-BA



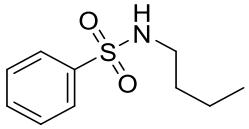
Yield: 82.9%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 5.2 Hz, 2H), 7.61 (d, *J* = 8.2 Hz, 2H), 7.52 – 7.45 (m, 2H), 7.45 – 7.39 (m, 1H), 4.67 (s, 1H), 3.00 (t, *J* = 7.1 Hz, 2H), 1.48 (p, *J* = 7.3 Hz, 2H), 1.31 (m, 2H), 0.86 (t, *J* = 7.3 Hz, 1.0 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 145.50, 139.32, 138.54, 129.03, 128.44, 127.69, 127.57, 127.29, 42.98, 31.63, 19.68, 13.52. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{16}H_{20}NO_2S^+$ 290.1209, found 290.1207.

p-Tolyl [1,1'-biphenyl]-4-sulfonate, KY-351-CA



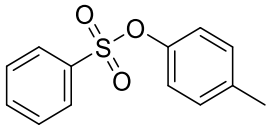
Yield: 34.6%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.5 Hz, 2H), 7.75 – 7.70 (m, 2H), 7.65 – 7.60 (m, 2H), 7.53 – 7.47 (m, 2H), 7.47 – 7.42 (m, 1H), 7.11 – 7.06 (m, 2H), 6.92 – 6.87 (m, 2H), 2.31 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 147.44, 146.95, 138.87, 137.03, 133.97, 130.13, 129.11, 129.02, 128.80, 127.57, 127.35, 122.05, 20.87. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{19}H_{17}O_3S^+$ 325.0893, found 325.0881.

N-Butylbenzenesulfonamide, KY-352-BA



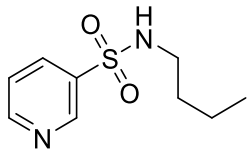
Yield: 81.7%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.88 – 7.84 (m, 2H), 7.59 – 7.55 (m, 1H), 7.54 – 7.49 (m, 2H), 4.37 (s, 1H), 2.95 (q, *J* = 6.5 Hz, 2H), 1.48 – 1.39 (m, 2H), 1.34 – 1.23 (m, 2H), 0.84 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 139.96, 132.53, 129.05, 127.01, 42.91, 31.54, 19.63, 13.48. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{10}H_{16}NO_2S^+$ 214.0896, found 214.0895.

p-Tolyl benzenesulfonate, KY-352-CA



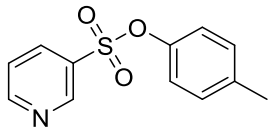
Yield: 32.9%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.85 – 7.80 (m, 2H), 7.67 – 7.64 (m, 1H), 7.54 – 7.49 (m, 2H), 7.08 – 7.04 (m, 2H), 6.86 – 6.82 (m, 2H), 2.30 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 147.37, 137.01, 135.42, 134.07, 130.06, 129.03, 128.45, 121.96, 20.81. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{13}H_{13}O_3S^+$ 249.0580, found 249.0577.

N-Butylpyridine-3-sulfonamide, KY-353-BA



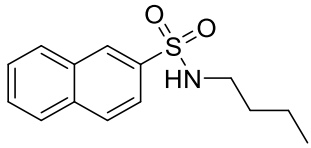
Yield: 34.6%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.79 (d, *J* = 5.1 Hz, 1H), 8.15 (m, 1H), 7.47 (dd, *J* = 8.1, 4.8 Hz, 1H), 5.08 (t, *J* = 6.2 Hz, 1H), 2.99 (q, *J* = 6.7, 6.1 Hz, 2H), 1.50 – 1.41 (m, 2H), 1.28 (dt, *J* = 15.1, 7.4 Hz, 2H), 0.84 (td, *J* = 7.3, 1.1 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 152.88, 147.80, 136.93, 134.87, 123.80, 42.94, 31.59, 19.61, 13.44. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_9H_{15}N_2O_2S^+$ 215.0849, found 215.0854.

p-Tolyl pyridine-3-sulfonate, KY-353-CA



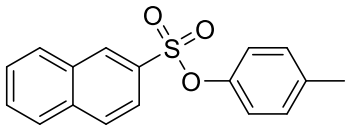
Yield: 40.8%, **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.98 (dd, *J* = 2.3, 0.8 Hz, 1H), 8.86 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.09 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.47 (m, 1H), 7.11 – 7.05 (m, 2H), 6.88 – 6.83 (m, 2H), 2.29 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 154.53, 149.07, 147.02, 137.55, 136.09, 132.26, 130.36, 123.69, 121.84, 20.83. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{12}H_{12}NO_3S^+$ 250.0532, found 250.0538

N-Butylnaphthalene-2-sulfonamide, JWB-195-BA



Yield: 59.2%. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.85 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.67 – 7.58 (m, 2H), 4.70 (s, 1H), 2.98 (t, *J* = 7.2 Hz, 2H), 1.49 – 1.40 (m, 2H), 1.28 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 136.75, 134.76, 132.15, 129.45, 129.20, 128.71, 128.40, 127.88, 127.50, 122.32, 42.97, 31.59, 19.65, 13.47. **ESI-TOF (HRMS)** m/z $[M+H]^+$ calculated for $C_{14}H_{18}NO_2S^+$ 264.1053, found 264.1050.

p-Tolyl naphthalene-2-sulfonate, JWB-195-CA



Yield: 57.1%. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.37 (d, *J* = 2.1 Hz, 1H), 7.98 (d, *J* = 8.9 Hz, 1H), 7.96 – 7.90 (m, 2H), 7.84 (dt, *J* = 8.7, 1.8 Hz, 1H), 7.69 (m, 1H), 7.62 (m, 1H), 7.03 (d, *J* = 8.6 Hz, 2H), 6.89 – 6.83 (m, 2H), 2.28 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 147.45, 137.00, 135.36, 132.37, 131.77, 130.41, 130.10, 129.49, 129.42, 129.41, 127.97, 127.76, 122.95, 121.99, 20.81. **ESI-TOF (HRMS)** m/z $[M+Na]^+$ calculated for $C_{17}H_{14}NaO_3S^+$ 321.0556, found 321.0553.

3. BIOLOGICAL METHODS

Cell culture

All cell lines were cultured at 37 °C with 5% CO_2 using manufacturer recommended media supplemented with 10% fetal bovine serum (US Source, Omega Scientific) and 1% L-glutamine (Fisher Scientific): HEK293T, DMEM; DM93, RPMI. Cells were seeded at 440,000 cells per plate and collected for experimental use when they reached 90 % confluency. Media was aspirated and cells washed with cold PBS (2X) before scraping from plates. Cells were transferred to microfuge tubes and pelleted by centrifugation at 500 x *g* for 5 min, and snap-frozen using liquid N_2 and stored -80 °C until further use.

SILAC cell culture

SILAC cells were cultured at 37 °C with 5% CO_2 in either 'light' or 'heavy' media supplemented with 10% dialyzed fetal bovine serum (Omega Scientific), 1% L-glutamine (Fisher Scientific), and isotopically labeled amino acids. Light media was supplemented with 100 μg ml^{-1} L-arginine and 100 μg ml^{-1} L-lysine. Heavy media was supplemented with 100 μg ml^{-1} [$^{13}C_6{}^{15}N_4$] L-arginine and 100 μg ml^{-1} [$^{13}C_6{}^{15}N_2$] L-lysine. Labelled amino acids were incorporated for at least five passages before utilizing SILAC cells for experiments.

Transient Transfection

Recombinant proteins were produced by transient transfection of HEK293T as previously described[2]. The following plasmid constructs for expressing human proteins were purchased from GenScript: pcDNA3.1-GSTP1-FLAG, pcDNA3.1-DPP3-FLAG. Site-directed mutagenesis was used to generate mutant plasmids as previously described[1]: pcDNA3.1-GSTP1(Y8F)-FLAG, pcDNA3.1-DPP3 (Y417F)-FLAG.

Western blot analysis

Western blot analysis of recombinant protein expression was performed as previously described[2].

Gel-based chemical proteomic assay

Cell pellets were lysed in PBS and fractionated (100,000 x *g*, 45 min, 4 °C) to generate soluble and membrane fractions. Protein concentrations were determined using Bio-Rad DC protein assay and adjusted to 1 mg ml^{-1} in PBS. Proteome samples (49 μL aliquots) were treated with DMSO vehicle or indicated concentration of SuTEx fragment (1 μL, 50X stock in DMSO) for 30 min at 37 °C. Samples were then treated with HHS-482 probe (1 μL, 2.5 mM stock in DMSO) for 30 min at 37 °C. Probe labeled samples were conjugated to rhodamine-azide (1 μL, 1.25 mM stock; final concentration of 25 μM) by copper-catalyzed azide-alkyne cycloadditions (CuAAC) for 1 h at room temperature followed by SDS-PAGE and in-gel fluorescence scanning as previously described[1].

Live cell evaluation of sulfonyl-triazole fragments

Cells grown to ~90% confluency in 10 cm plates were treated with DMSO vehicle or SuTEx fragment (10 µl of 1,000X stock) in serum-free media for the indicated concentrations and times at 37 °C with 5% CO_2. After treatment, cells were washed with cold PBS twice before collection and preparation for gel-based chemical proteomic evaluation as described above. For LC–MS studies, protein concentrations were normalized to 2.3 mg ml^{-1} and 432 µl (for 1 mg final protein amount) were used for sample preparation as detailed below.

Preparation of SILAC proteomes for liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis

Heavy and light proteomes (432 µl of each) were diluted to 2.3 mg ml^{-1} in PBS and sample aliquots (432 µl) were treated with SuTEx fragment at the indicated concentrations (5 µl, 100X stock in DMSO), mixed gently and incubated for 30 min at 37 °C. Samples were then treated with HHS-482 (5 µL, 5 mM stock in DMSO) for 30 min at 37 °C. Probe-modified proteomes were conjugated to desthiobiotin-PEG3-azide followed by enrichment of probe-modified peptides for nano-electrospray ionization–LC–MS/MS analyses as previously described[1].

LC–MS/MS data analysis

Identification of peptides and proteins from tandem mass spectrometry analyses was accomplished using bioinformatics software and quality control criteria as previously described[1]. The only

exception was evaluation of GSTP1 Y119 probe-modified peptide that had a Byonic score <300 and peptide sequence <6 amino acids.

GSTP1 biochemical substrate assay

Recombinant GSTP1-HEK293T soluble cell proteomes were diluted to 1 mg ml^{-1} in assay buffer (100 mM NaH_2PO_4, pH 7.0). Samples were treated with inhibitor at indicated concentrations for 30 min at 37 °C. GSH stock solution (250 mM in water) was diluted to 4 mM in assay buffer and 25 µl of diluted GSH solution was added to each sample. A substrate stock solution of 75 mM 1-bromo-2,4-dinitrobenzene (DBNB) in ethanol was diluted to 2 mM in assay buffer. Samples (25 µl) were aliquoted into a 96-well plate and spun briefly via centrifuge. Next, 50 µl of 2 mM BDNB was added to each well and the reaction was monitored in kinetic mode by measuring absorbance at 340 nm for 10 min on a BMG Labtech CLARIOstar plate reader.

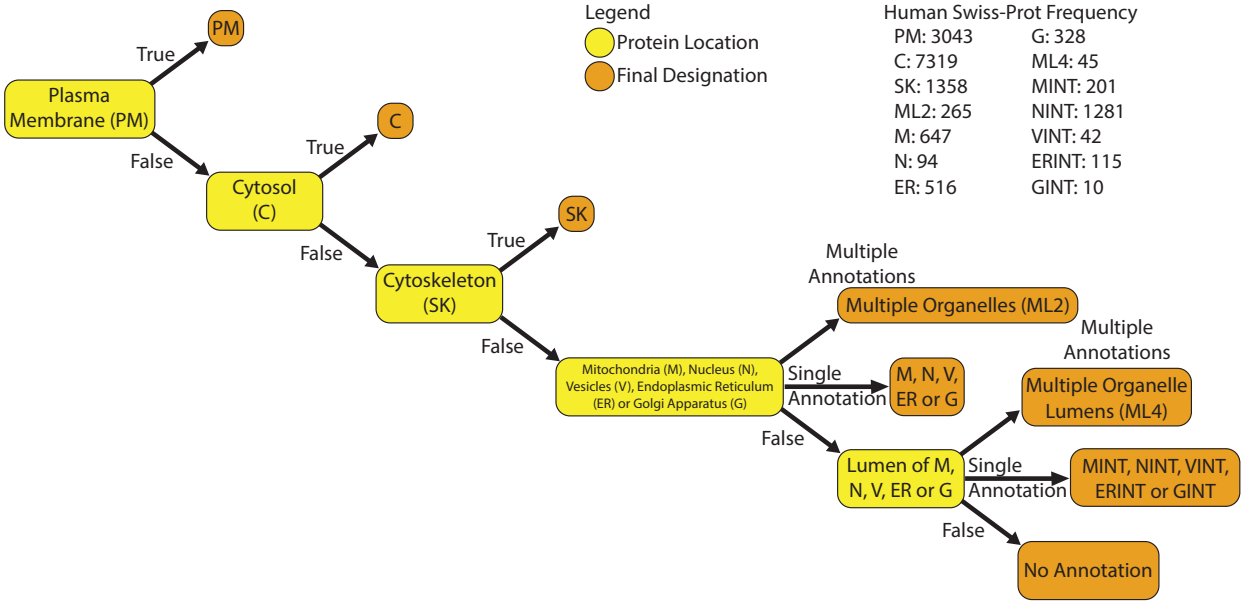
DPP3 biochemical substrate assay

Substrate assays were performed using purified DPP3 protein diluted to 5 ng μL^{-1} in assay buffer. DPP3 samples (10 µl) were diluted to 85 µl with assay buffer and transferred to a black 96-well plate. Samples were treated with a 50X stock of SuTEx-fragment at indication concentration for 30 min at 37 °C. A stock solution of DPP3 substrate (Arg-Arg β-naphthylamide trihydrochloride, 0.5 mM; Sigma-Aldrich) was diluted to 100 µM in assay buffer. Substrate solution (5 µl) was added to each sample. Samples were mixed briefly by shaking and reaction monitored in kinetic

mode by measuring fluorescence at 450 nm for 10 min on a BMG Labtech CLARIOstar plate reader.

Subcellular location analysis of SuTEx probe-modified proteins

Cellular Component Gene Ontology (CCGO) terms were counted for all human proteins in Swiss-Prot database (02/26/2020 Version). The 156 most frequent CCGO terms in the human database were manually assigned to a specific cellular compartment. Subcellular location of proteins with multiple annotated locations were ultimately assigned using the following decision tree algorithm:



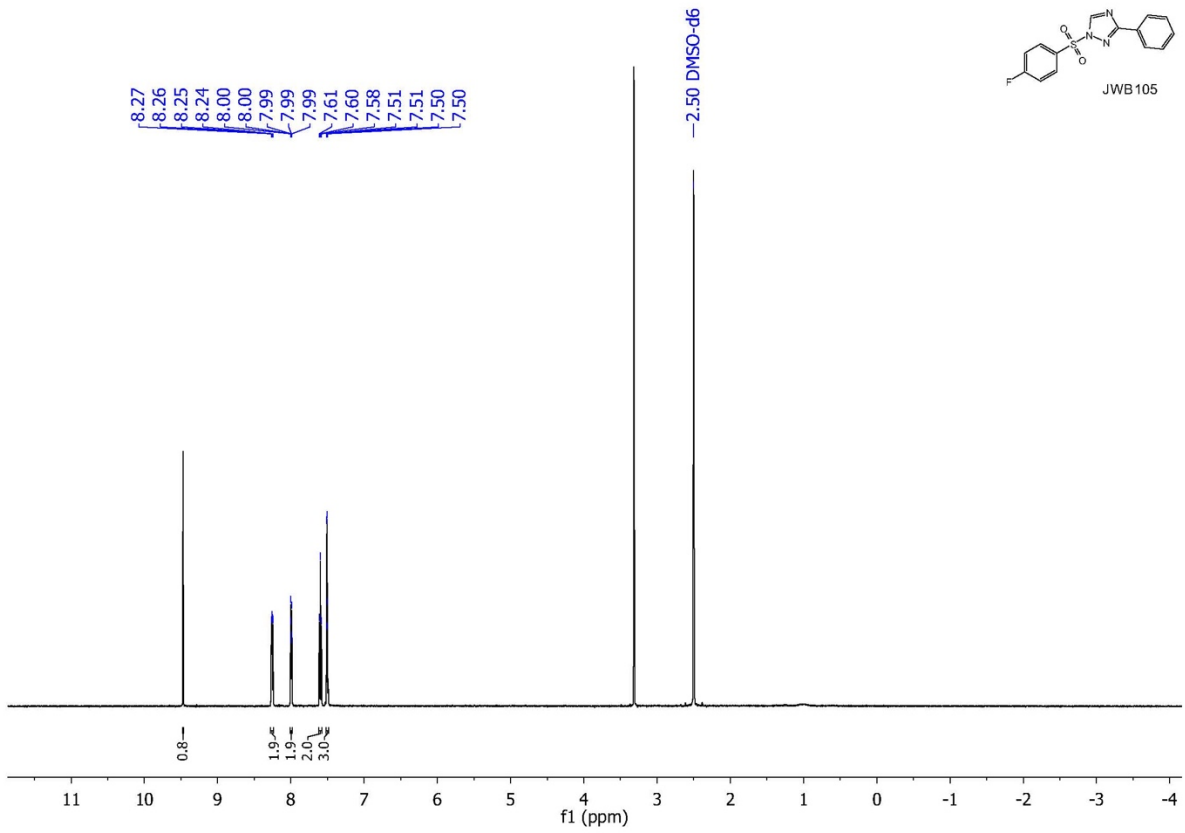
The algorithm is based on the premise that a probe or inhibitor will likely interact with proteins in cellular compartments that represent the path where fewer physical barriers (e.g. lipid bilayers or protective protein barriers like the nuclear lamina) must be crossed. A protein that can be detected in various compartments would receive a location assignment matching the most accessible location. For example, MAPK1 has been shown to translocate from the plasma membrane through

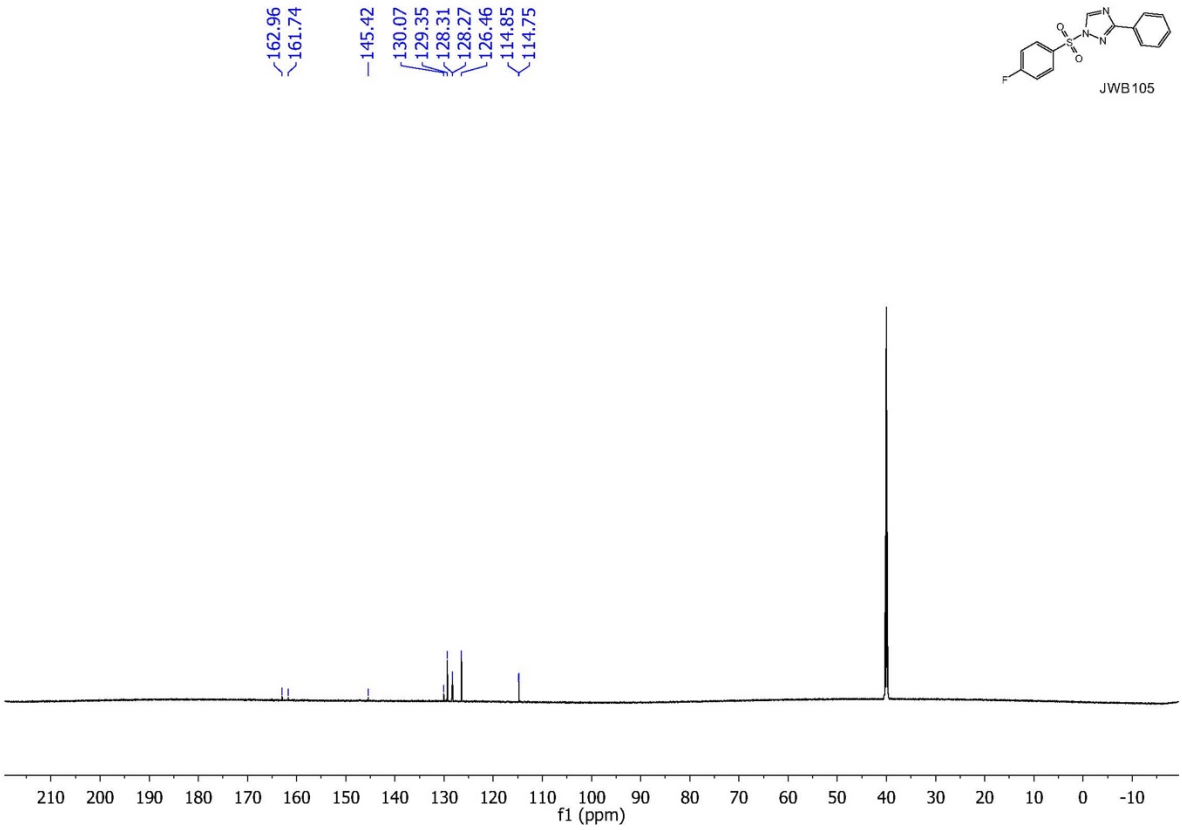
the cytosol to the nucleus[3]. MAPK1 would be considered a plasma membrane protein using our subcellular location analysis algorithm. Membranes of organelles are grouped into respective organelles because a molecule can interact with membrane proteins without necessarily passing through membranes. A protein is excluded from analyses if it does not have a CCGO annotation. The subcellular compartments used for analyses are as follows: PM: Plasma Membrane, C: Cytosol, SK: Cytoskeleton, M: Mitochondria, N: Nucleus, V: Vesicle, ER: Endoplasmic Reticulum, G: Golgi Apparatus, ML2: Multiple Organelles, MINT: Mitochondrial Lumen, NINT: Nuclear Lumen, VINT: Vesicle Lumen, ERINT: Endoplasmic Reticulum Lumen, GINT: Golgi Apparatus Lumen, ML4: Multiple Organelle Lumens.

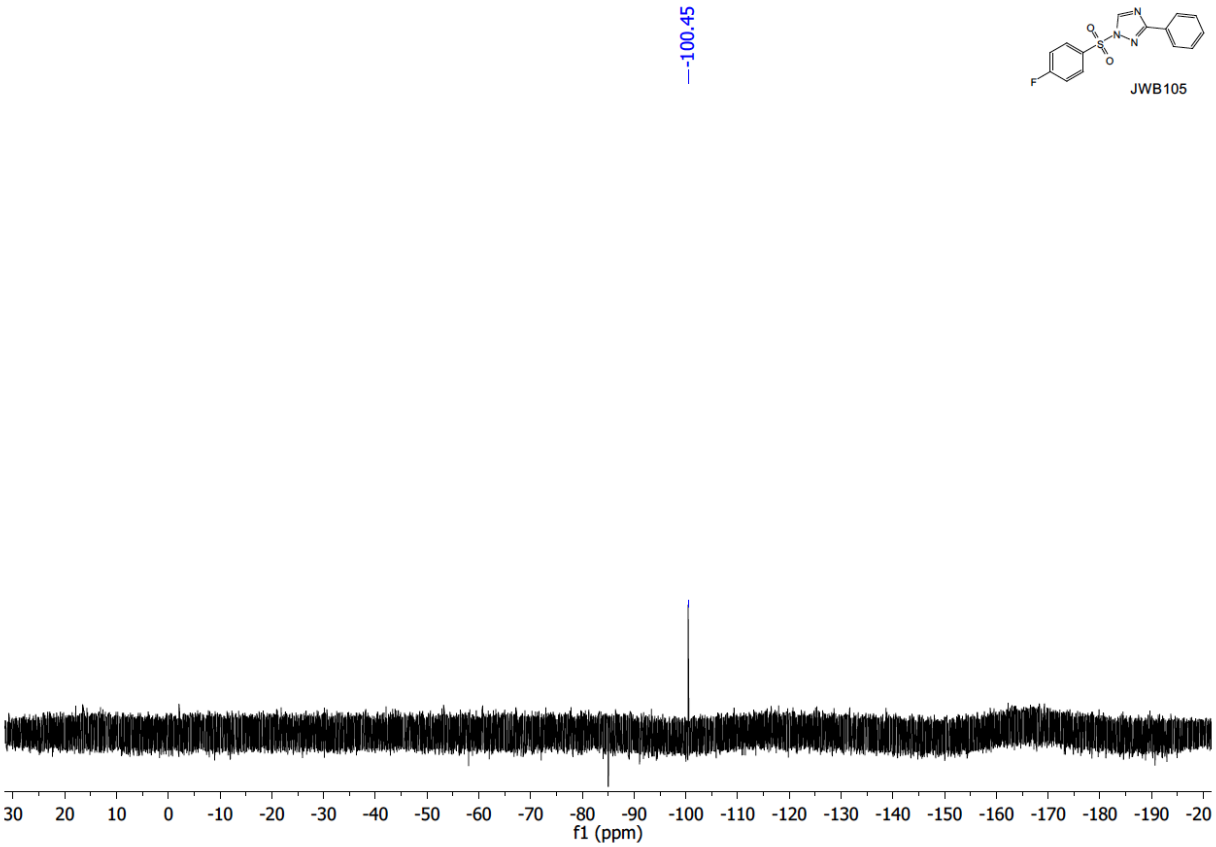
4. REFERENCES

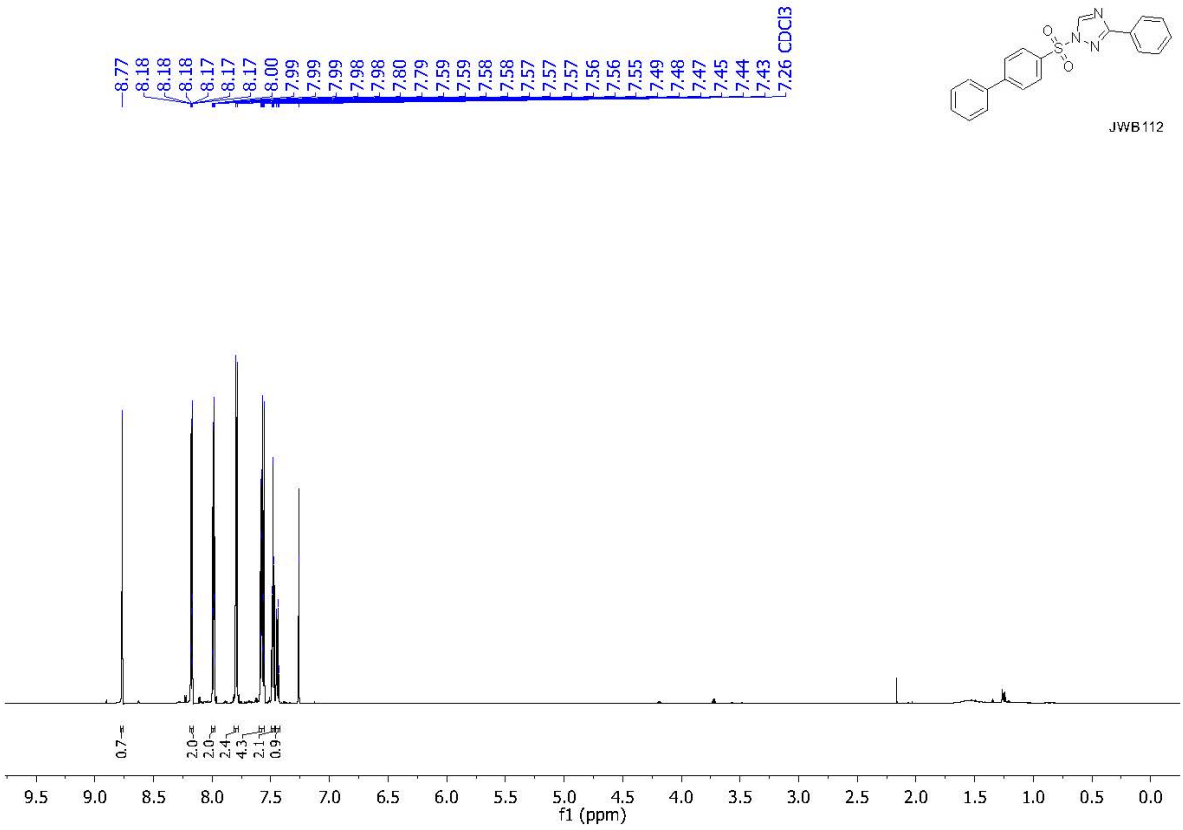
1. Hahm, H. S.; Toroitich, E. K.; Borne, A. L.; Brulet, J. W.; Libby, A. H.; Yuan, K.; Ware, T. B.; McCloud, R. L.; Ciancone, A. M.; Hsu, K. L., Global targeting of functional tyrosines using sulfur-triazole exchange chemistry. *Nat Chem Biol* **2020,** *16* (2), 150-159.
2. Ware, T. B.; Franks, C. E.; Granade, M. E.; Zhang, M.; Kim, K. B.; Park, K. S.; Gahlmann, A.; Harris, T. E.; Hsu, K. L., Reprogramming fatty acyl specificity of lipid kinases via C1 domain engineering. *Nat Chem Biol* **2020,** *16* (2), 170-178.
3. Zehorai, E.; Yao, Z.; Plotnikov, A.; Seger, R., The subcellular localization of MEK and ERK--a novel nuclear translocation signal (NTS) paves a way to the nucleus. *Mol Cell Endocrinol* **2010,** *314* (2), 213-20.

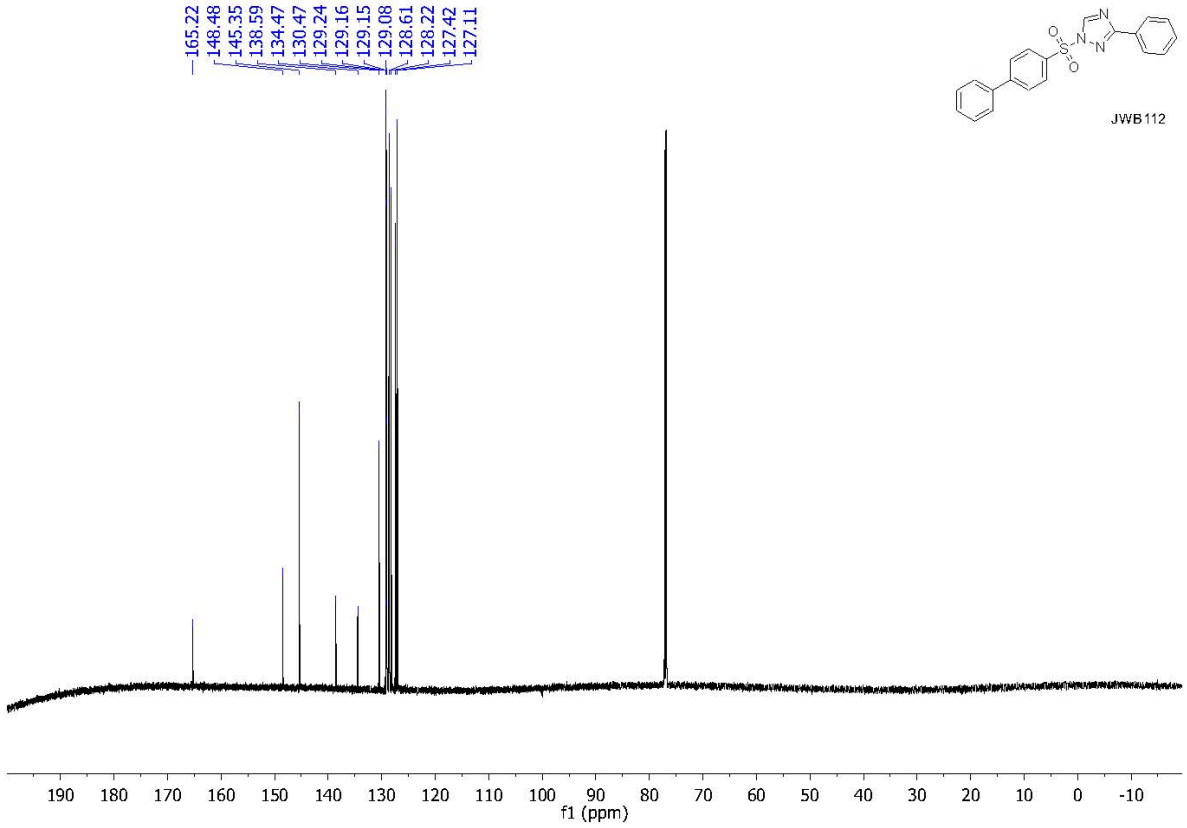
5. APPENDIX
5.1 NMR Spectra

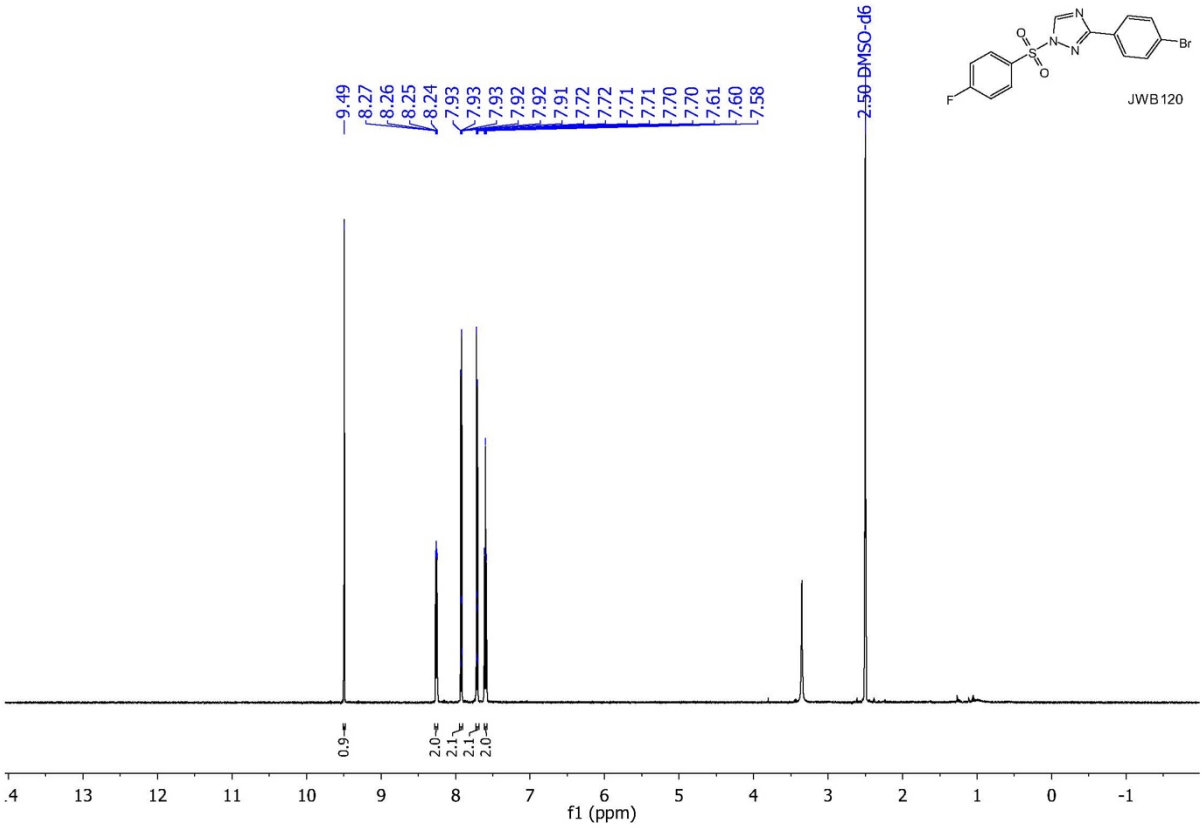


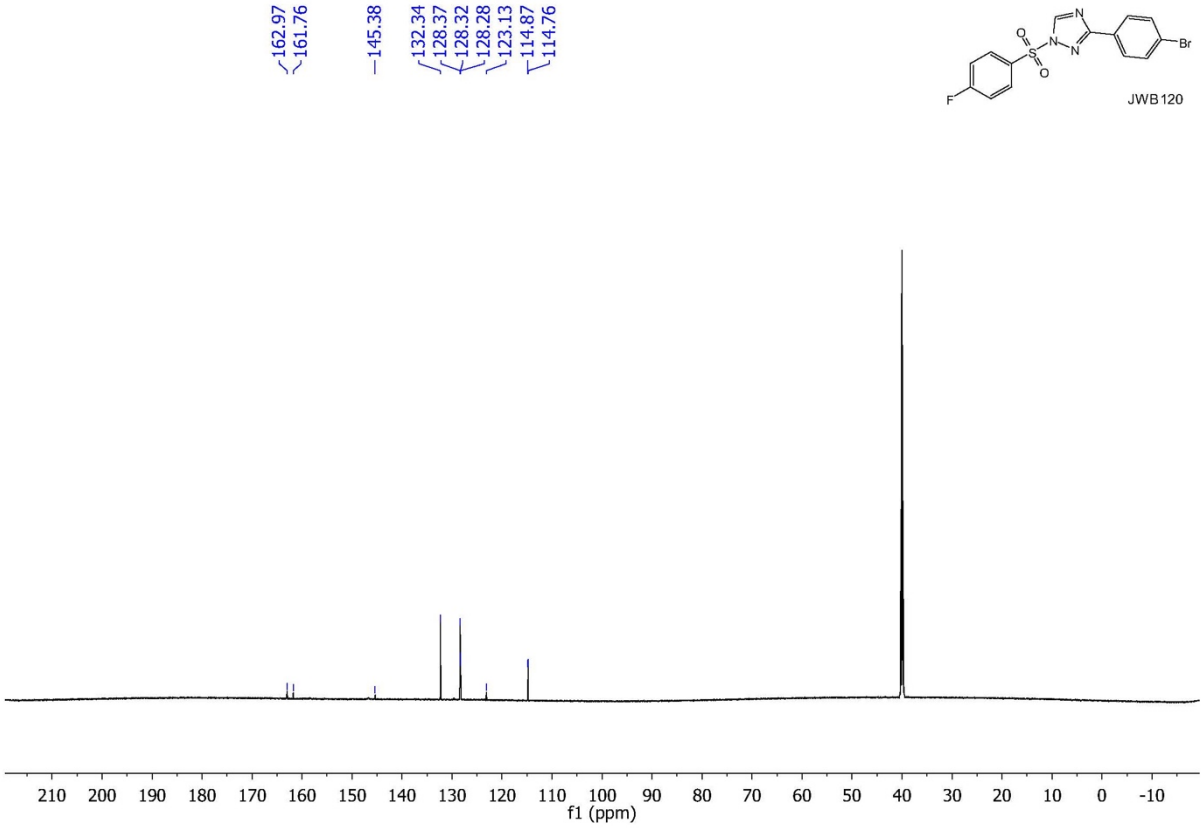

162.96
161.74
145.42
130.07
129.35
128.31
128.27
126.46
114.85
114.75
JWB105
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

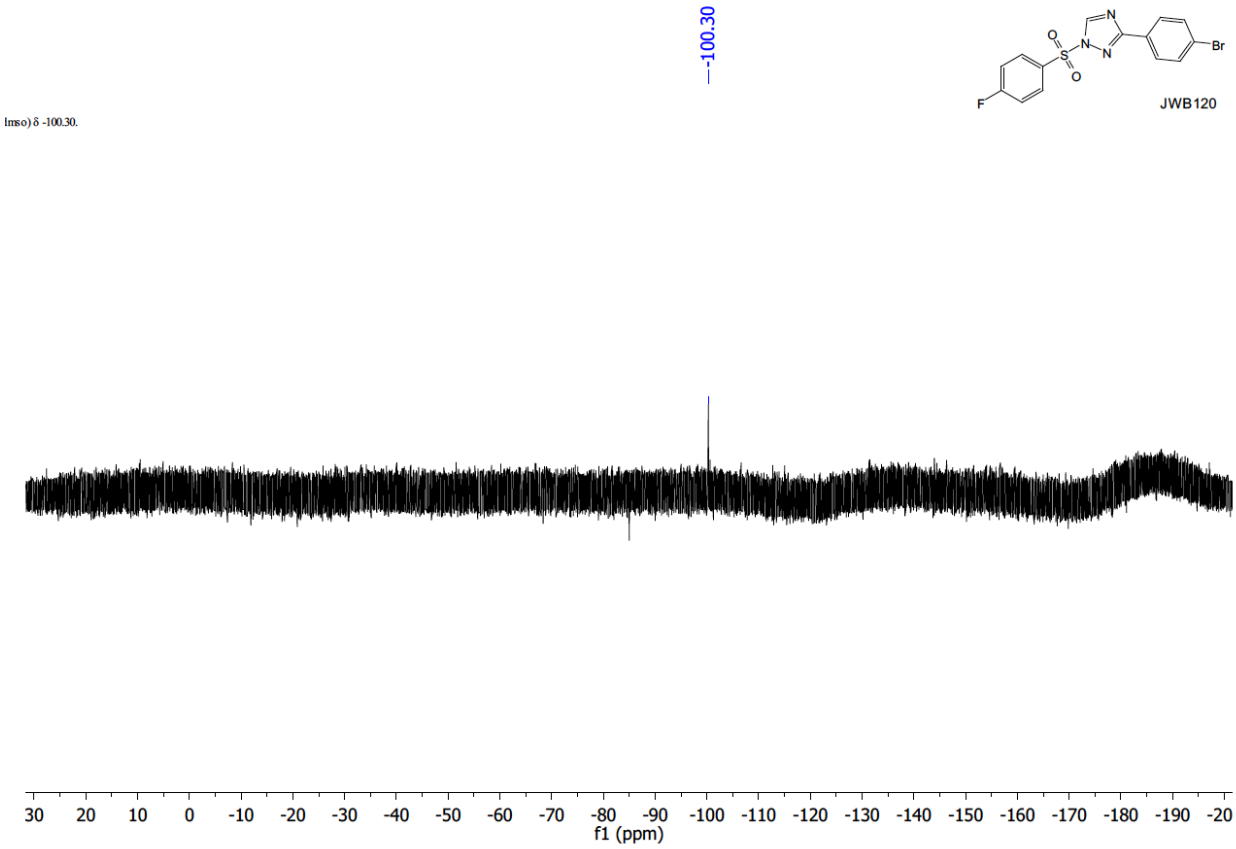

-100.45
JWB105
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -20
f1 (ppm)

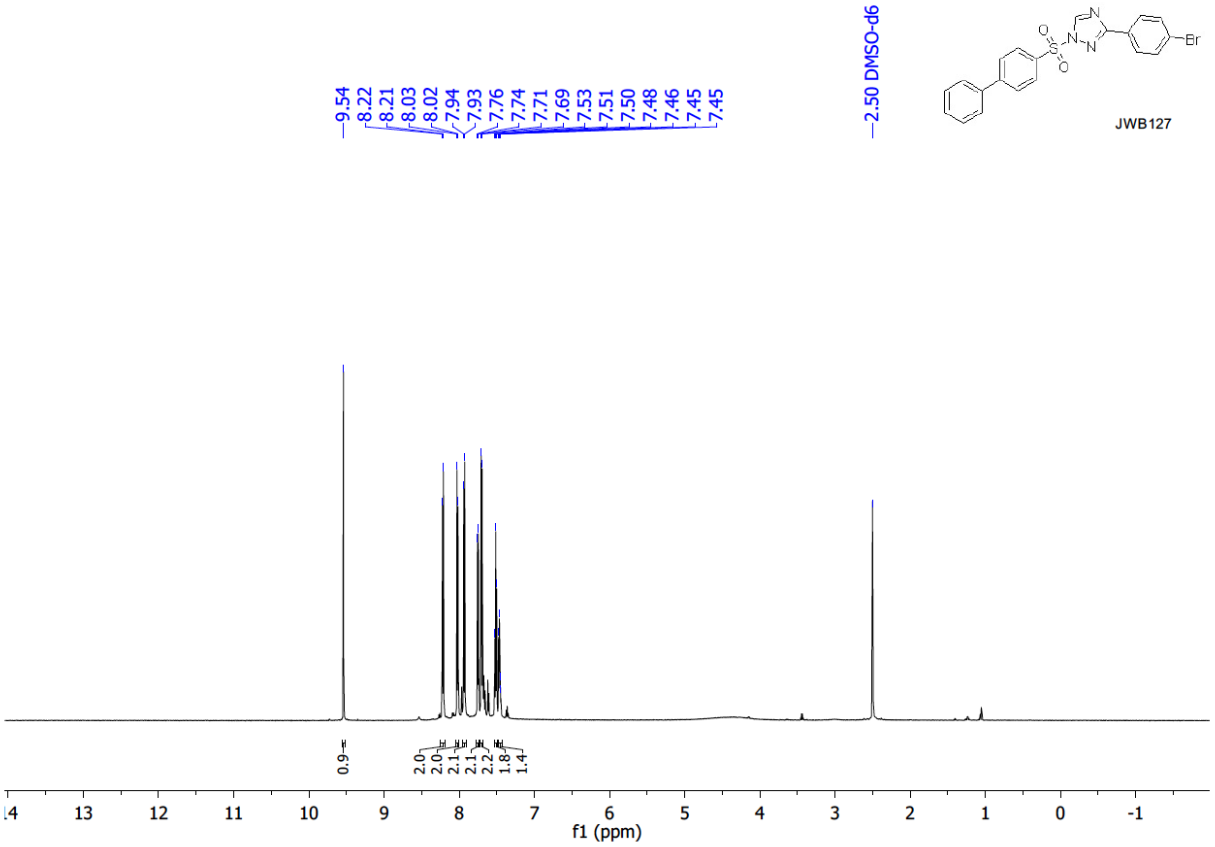

8.77
8.18
8.18
8.18
8.17
8.17
8.17
8.00
7.99
7.99
7.99
7.98
7.98
7.80
7.79
7.59
7.59
7.58
7.58
7.57
7.57
7.57
7.56
7.56
7.55
7.49
7.48
7.47
7.45
7.44
7.43
7.26 CDCl3
JWB112
0.7
2.0
2.0
2.4
4.3
2.1
0.9
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

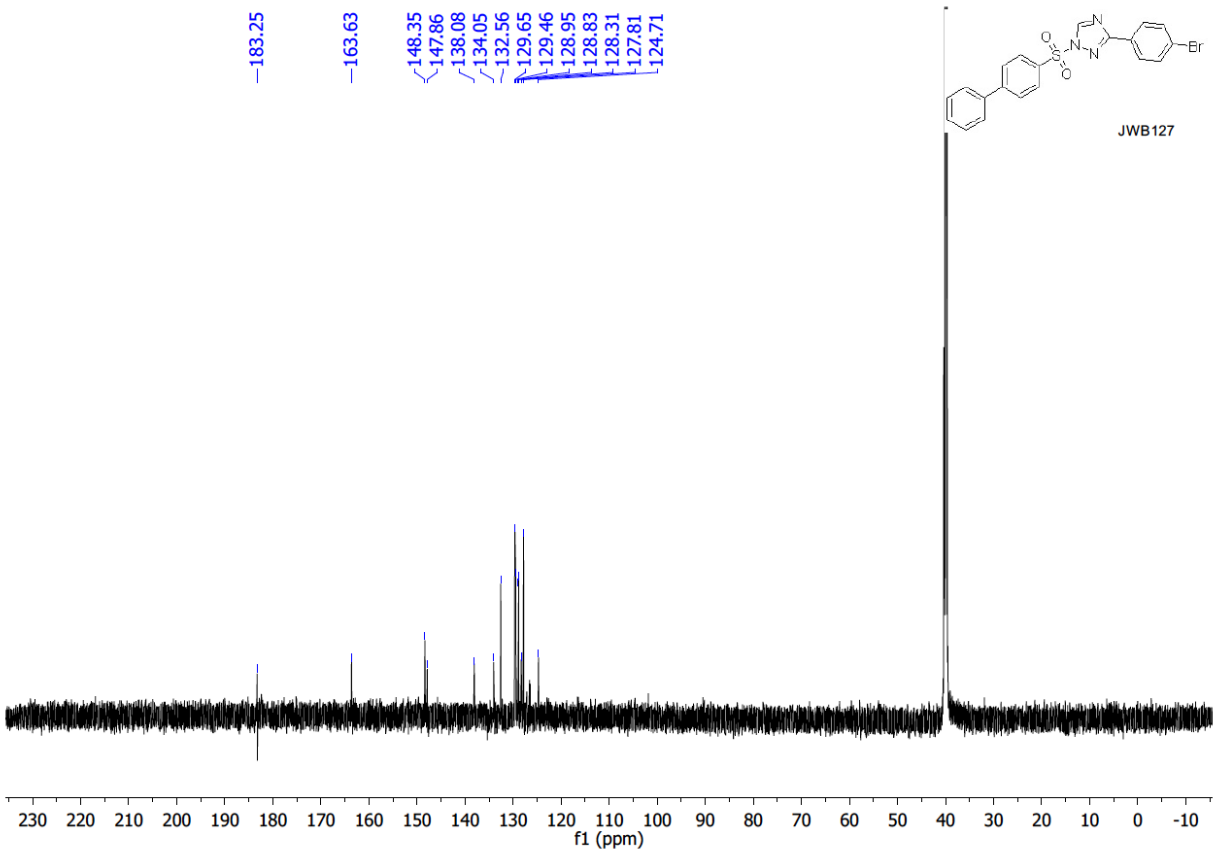

165.22
148.48
145.35
138.59
134.47
130.47
129.24
129.16
129.15
129.08
128.61
128.22
127.42
127.11
JWB112
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

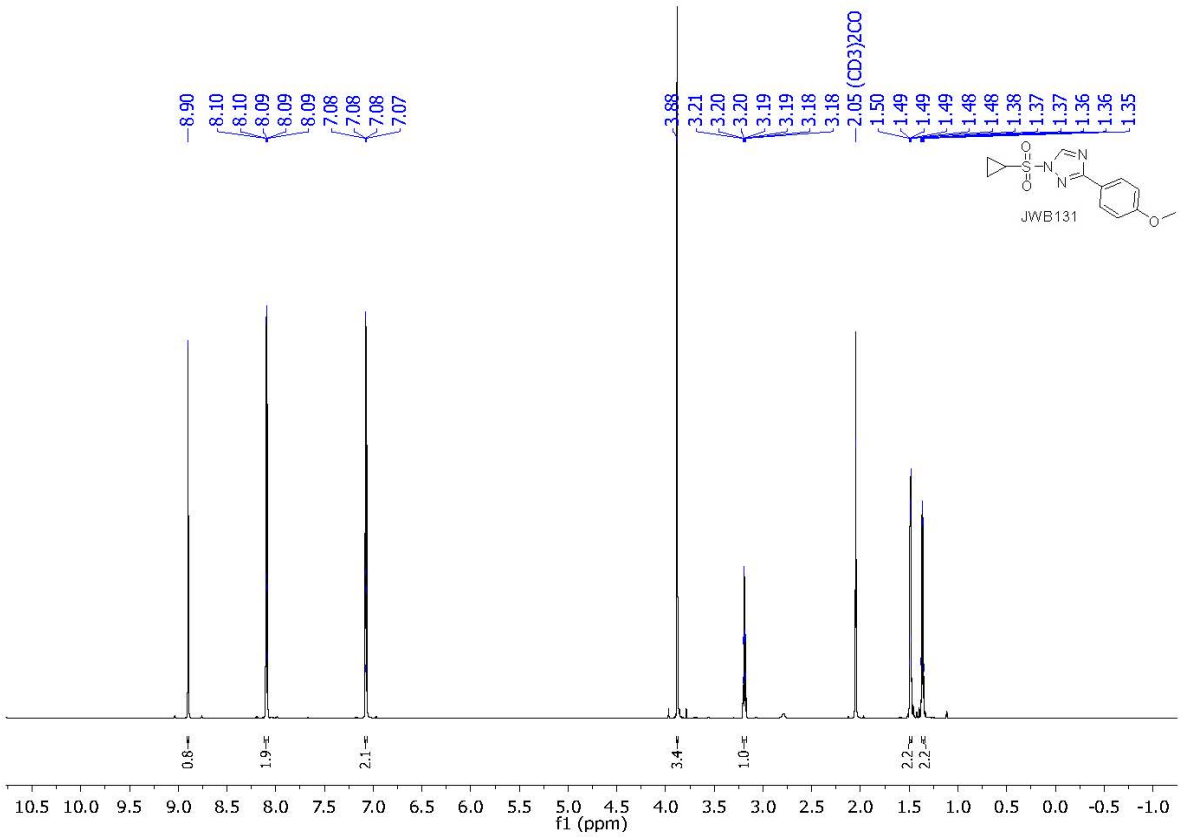

9.49
8.27
8.26
8.25
8.24
7.93
7.93
7.92
7.92
7.91
7.72
7.72
7.71
7.71
7.70
7.70
7.61
7.60
7.58
2.50 DMSO-d6
JWB120
0.9
2.0
2.1
2.1
2.0
f1 (ppm)

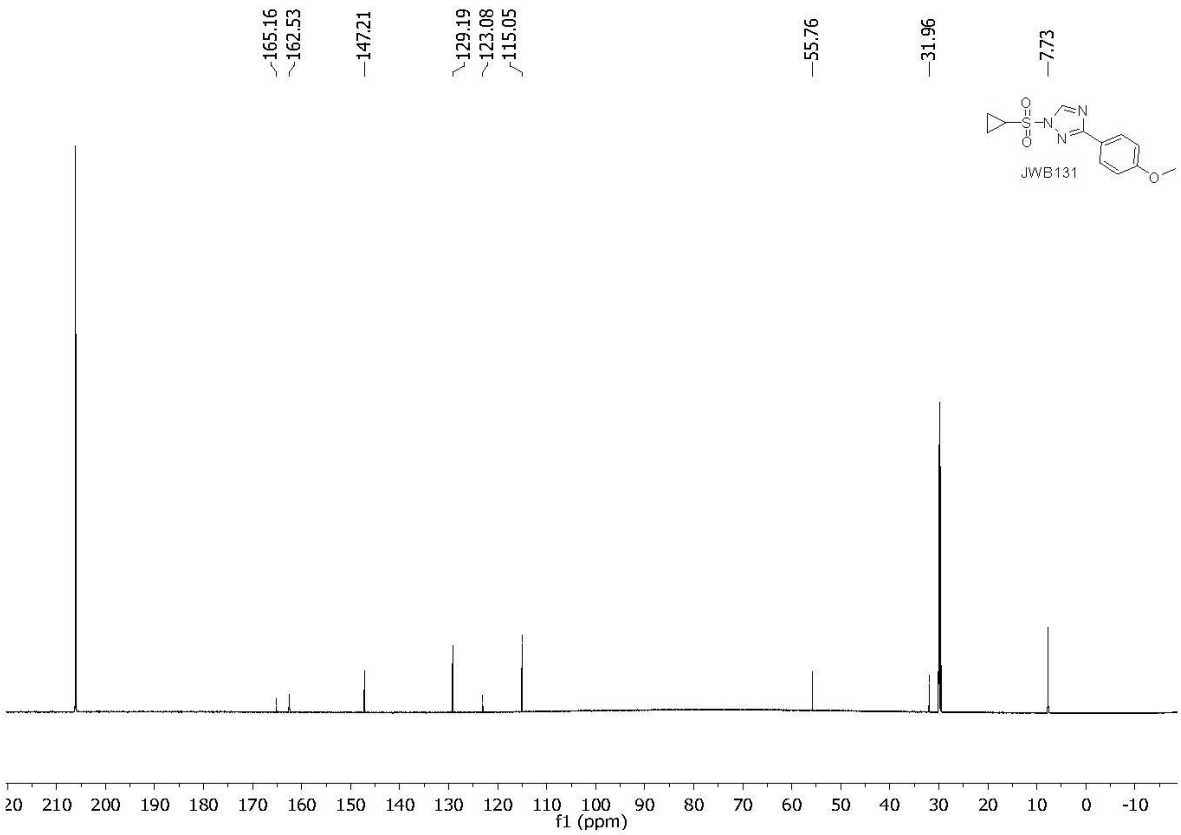

162.97
161.76
145.38
132.34
128.37
128.32
128.28
123.13
114.87
114.76
JWB120
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

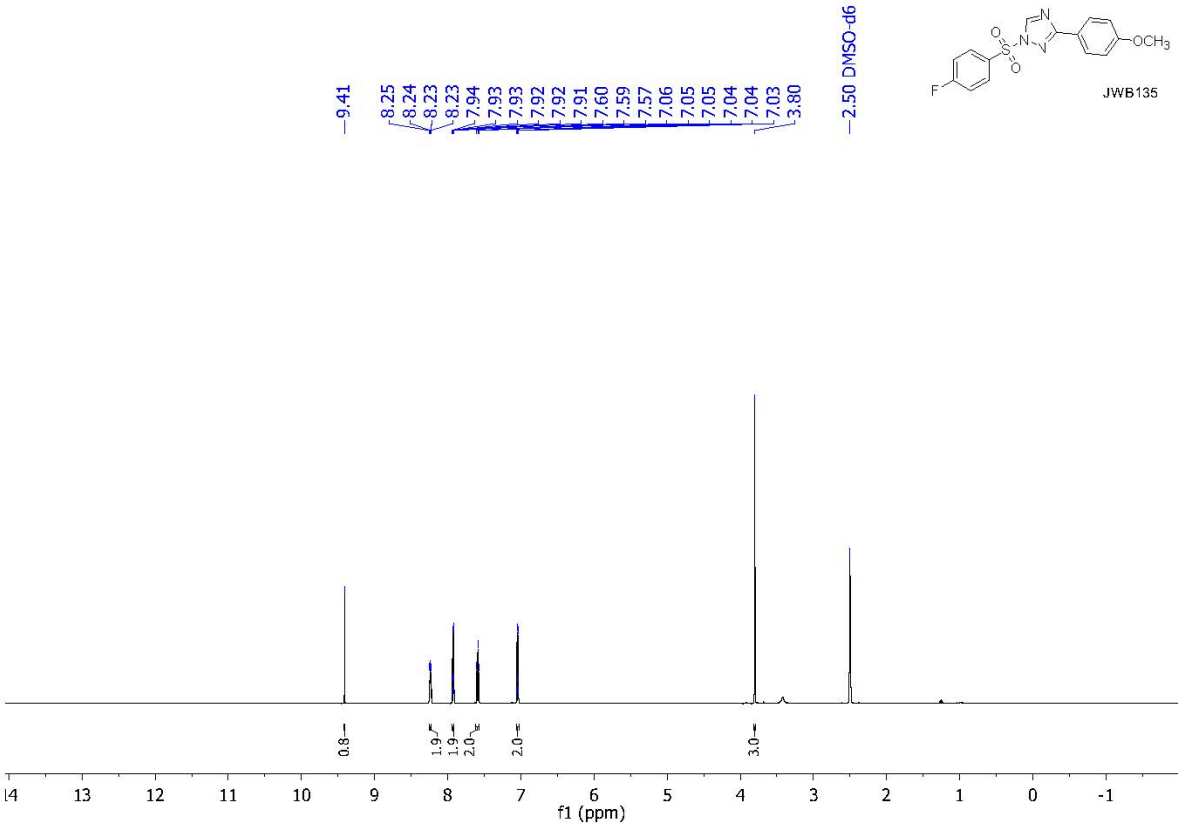

-100.30
JWB120
lmso) δ -100.30.
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -20
f1 (ppm)

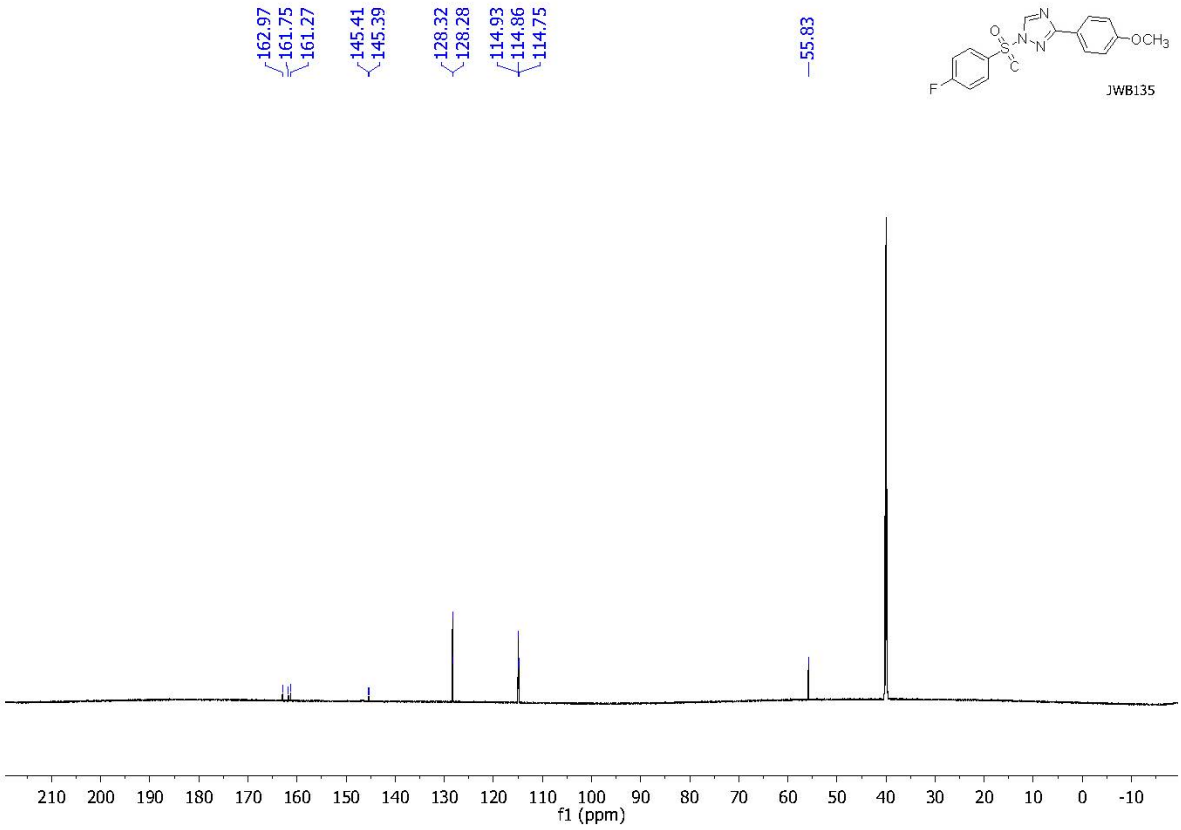

9.54
8.22
8.21
8.03
8.02
7.94
7.93
7.76
7.74
7.71
7.69
7.53
7.51
7.50
7.48
7.46
7.45
7.45
2.50 DMSO-d6
Br
JWB127
0.9
2.0
2.0
2.1
2.1
2.2
1.8
1.4
f1 (ppm)

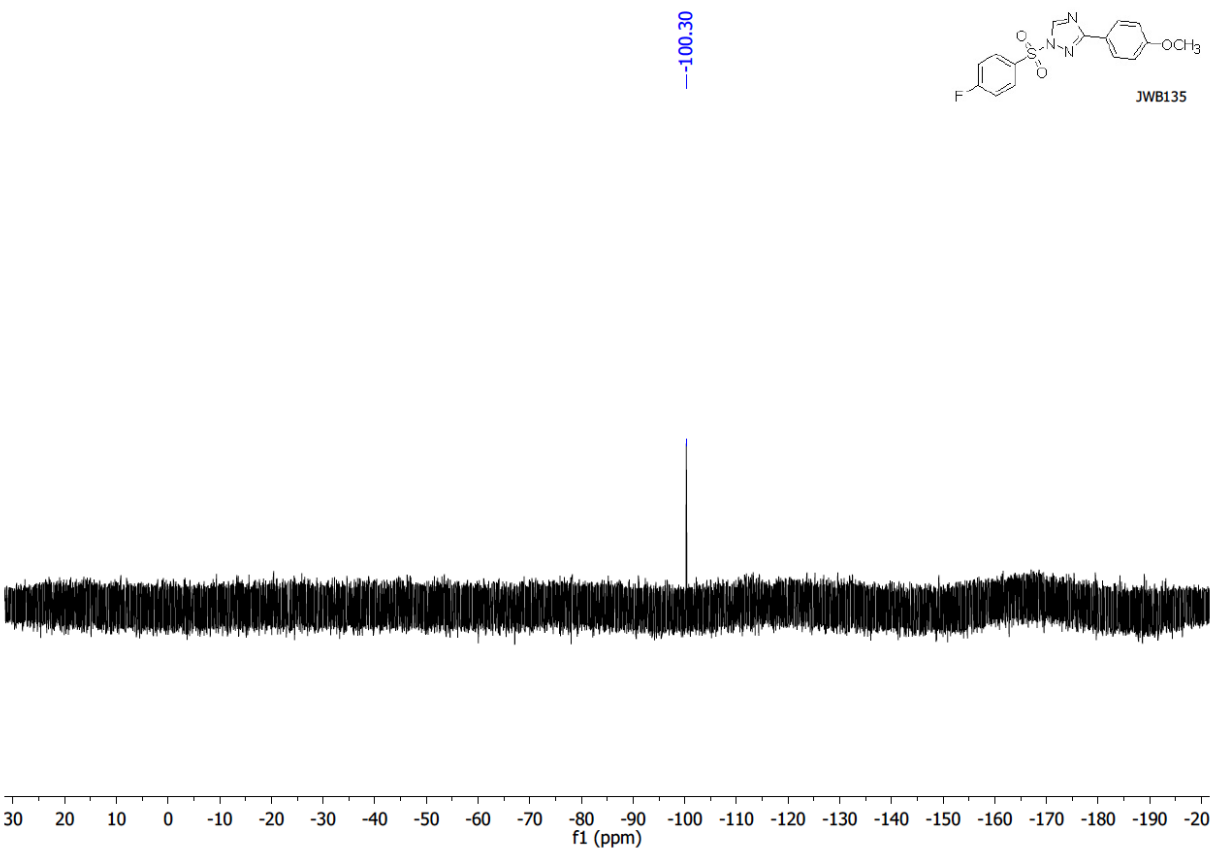

183.25
163.63
148.35
147.86
138.08
134.05
132.56
129.65
129.46
128.95
128.83
128.31
127.81
124.71
JWB127
Br
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

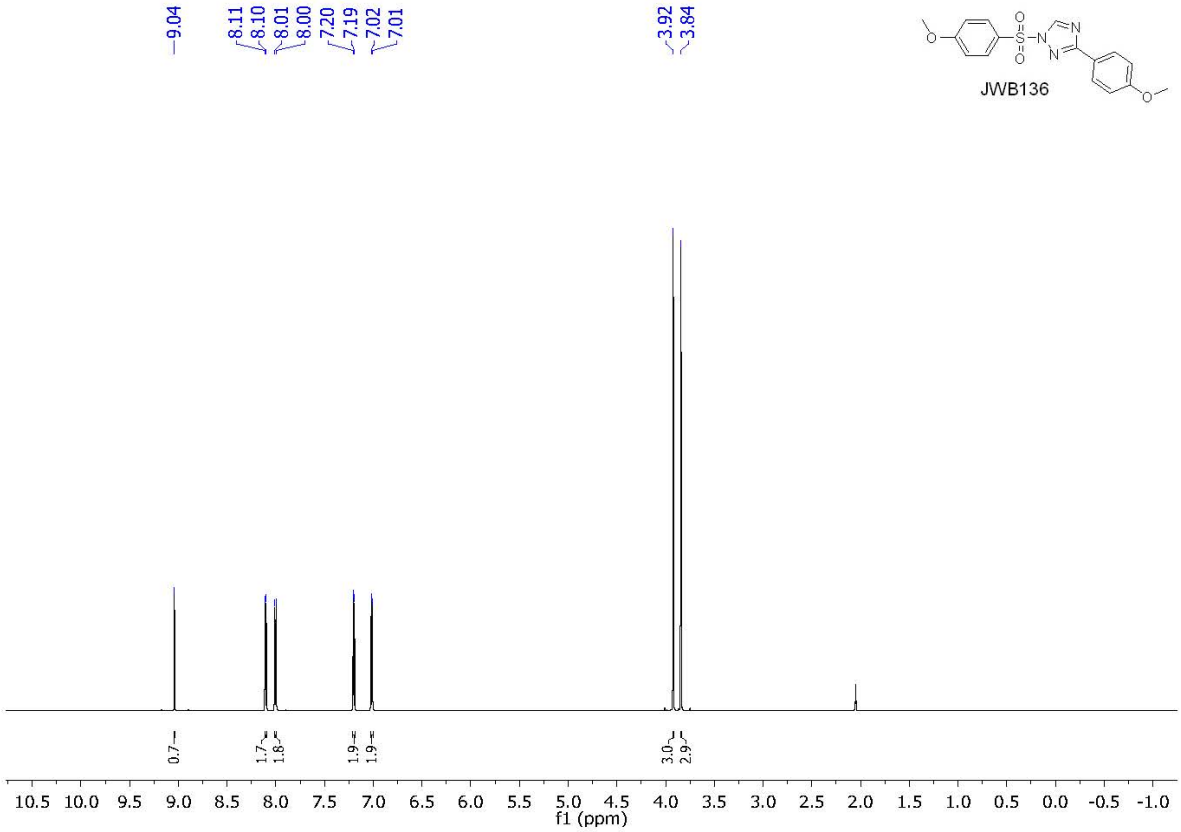

8.90
8.10
8.10
8.09
8.09
8.09
7.08
7.08
7.08
7.07
3.88
3.21
3.20
3.20
3.19
3.19
3.18
2.05 (CD3)2CO
1.50
1.49
1.49
1.49
1.48
1.48
1.38
1.37
1.37
1.36
1.36
1.35
JWB131
0.8
1.9
2.1
3.4
1.0
2.2
2.2
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

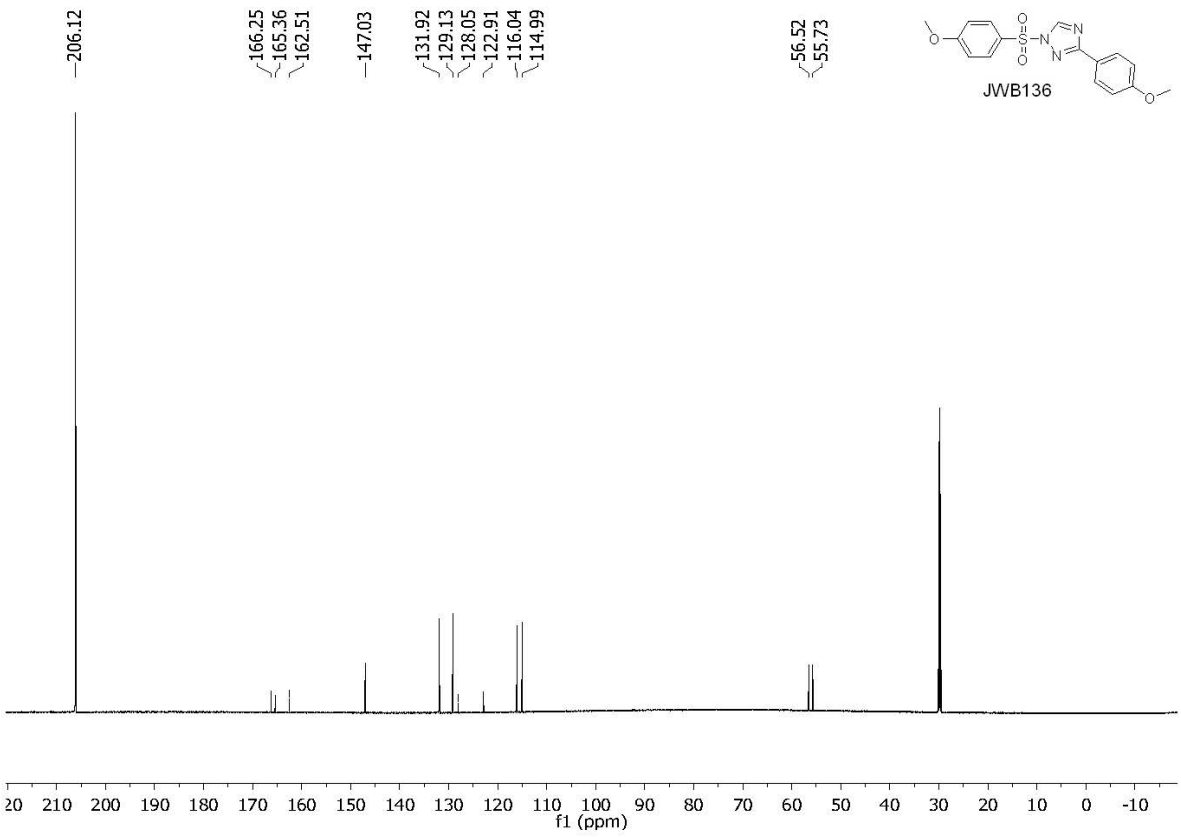

165.16
162.53
147.21
129.19
123.08
115.05
55.76
31.96
7.73
JWB131
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

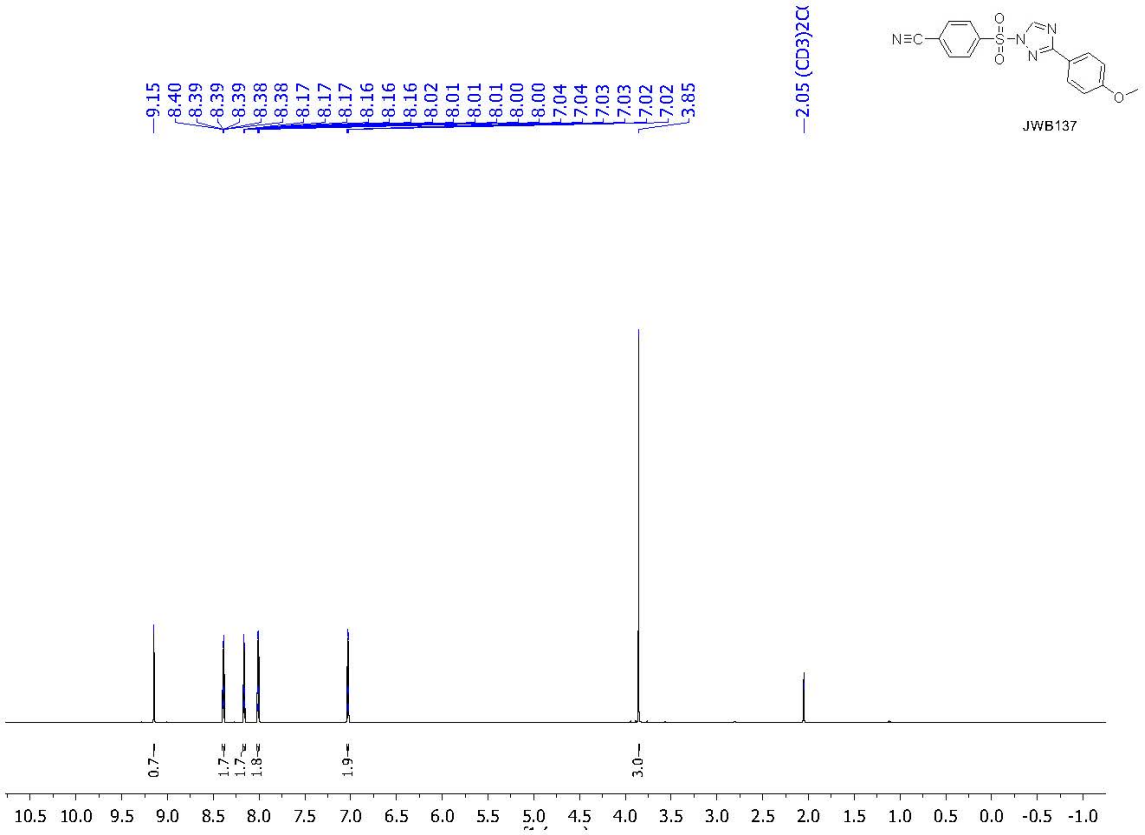

9.41
8.25
8.24
8.23
8.23
7.94
7.93
7.93
7.92
7.92
7.91
7.60
7.59
7.57
7.06
7.05
7.05
7.04
7.04
7.03
3.80
2.50 DMSO-d6
OCH3
F
JWB135
0.8
1.9
1.9
2.0
2.0
3.0
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

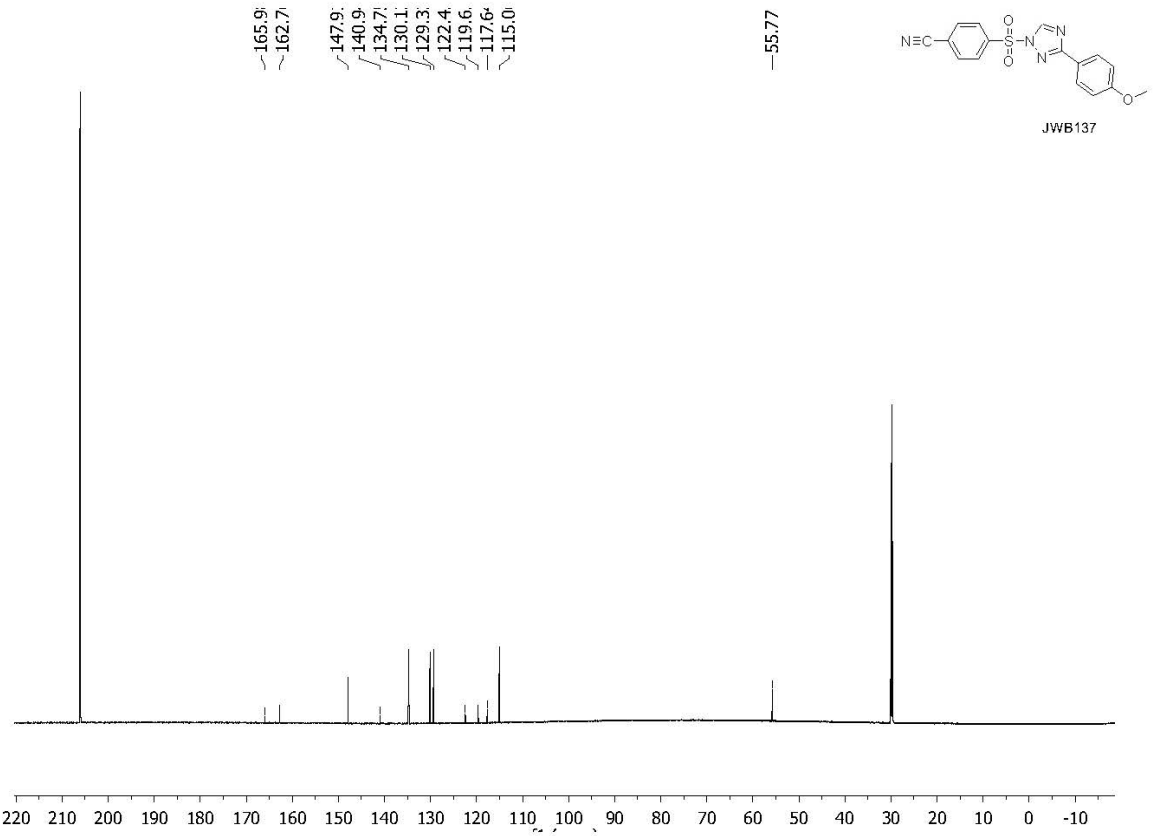

162.97
161.75
161.27
145.41
145.39
128.32
128.28
114.93
114.86
114.75
55.83
JWB135
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

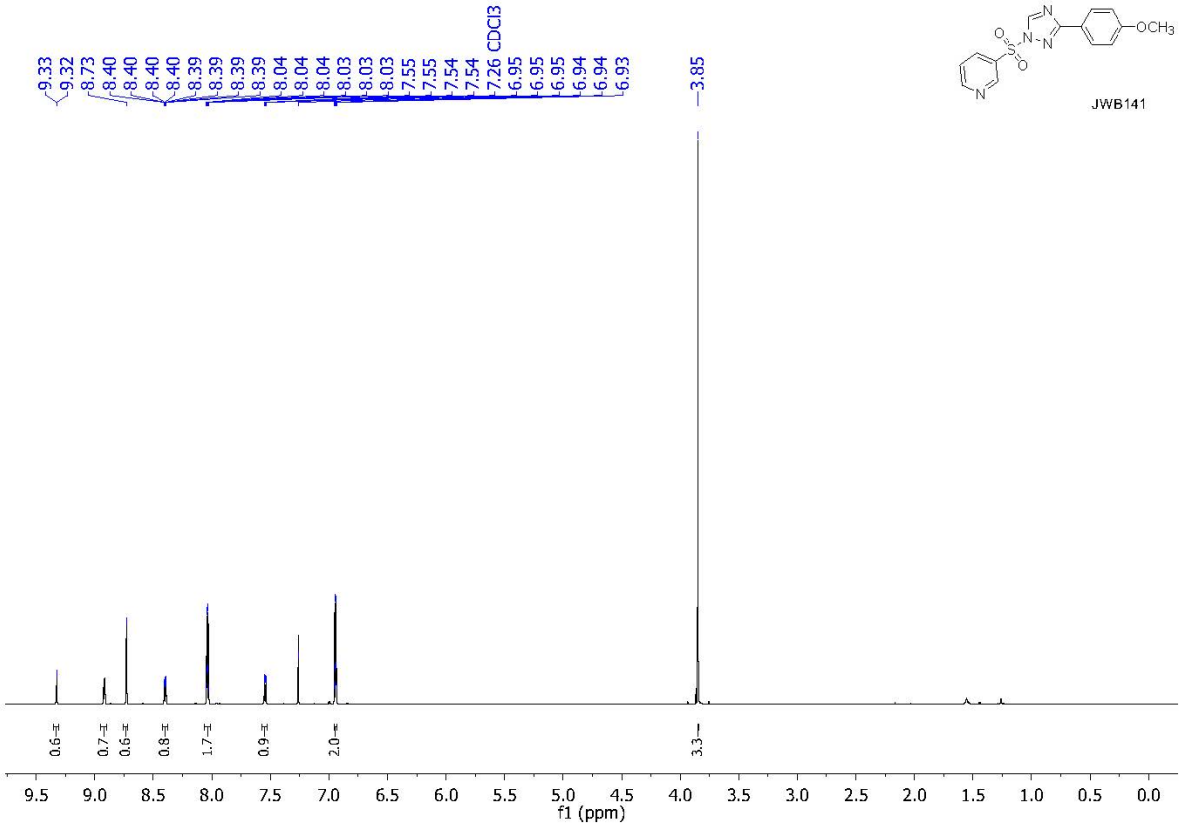

-100.30
JWB135
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -20
f1 (ppm)

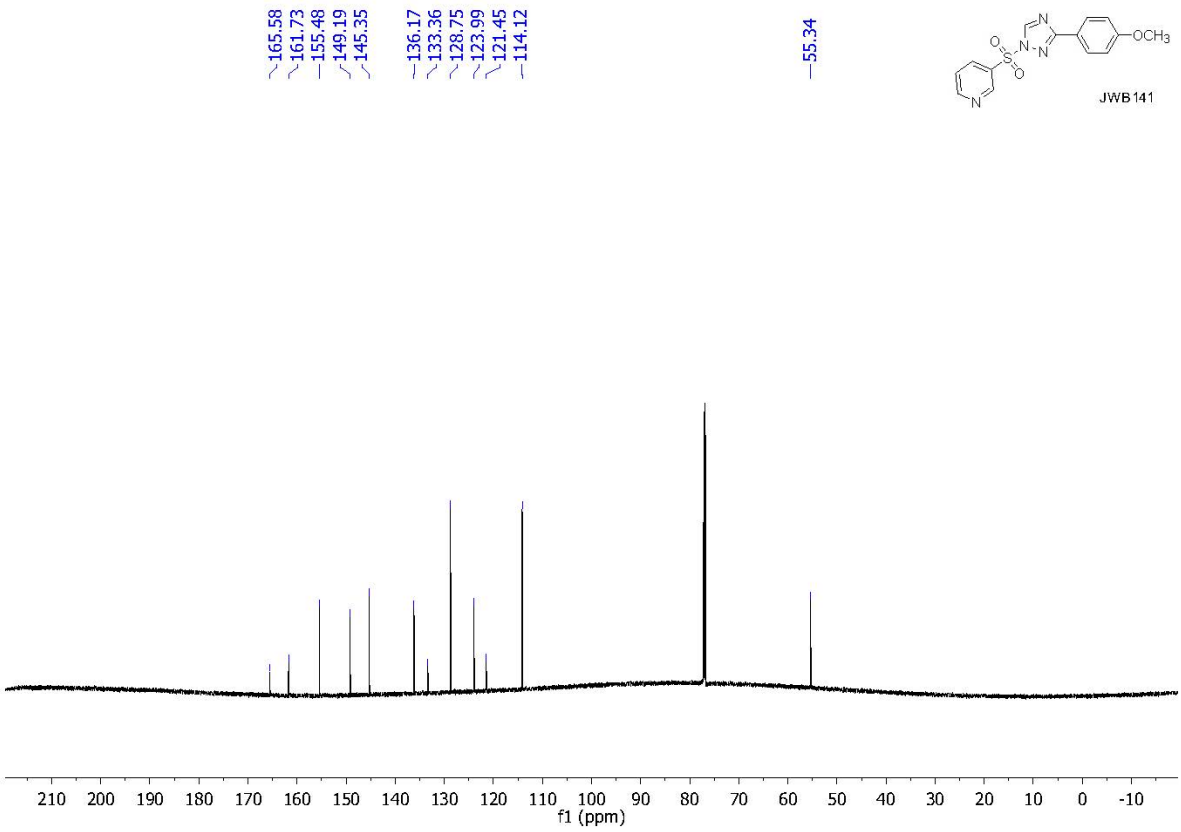

9.04
8.11
8.10
8.01
8.00
7.20
7.19
7.02
7.01
3.92
3.84
JWB136
0.7
1.7
1.8
1.9
1.9
3.0
2.9
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

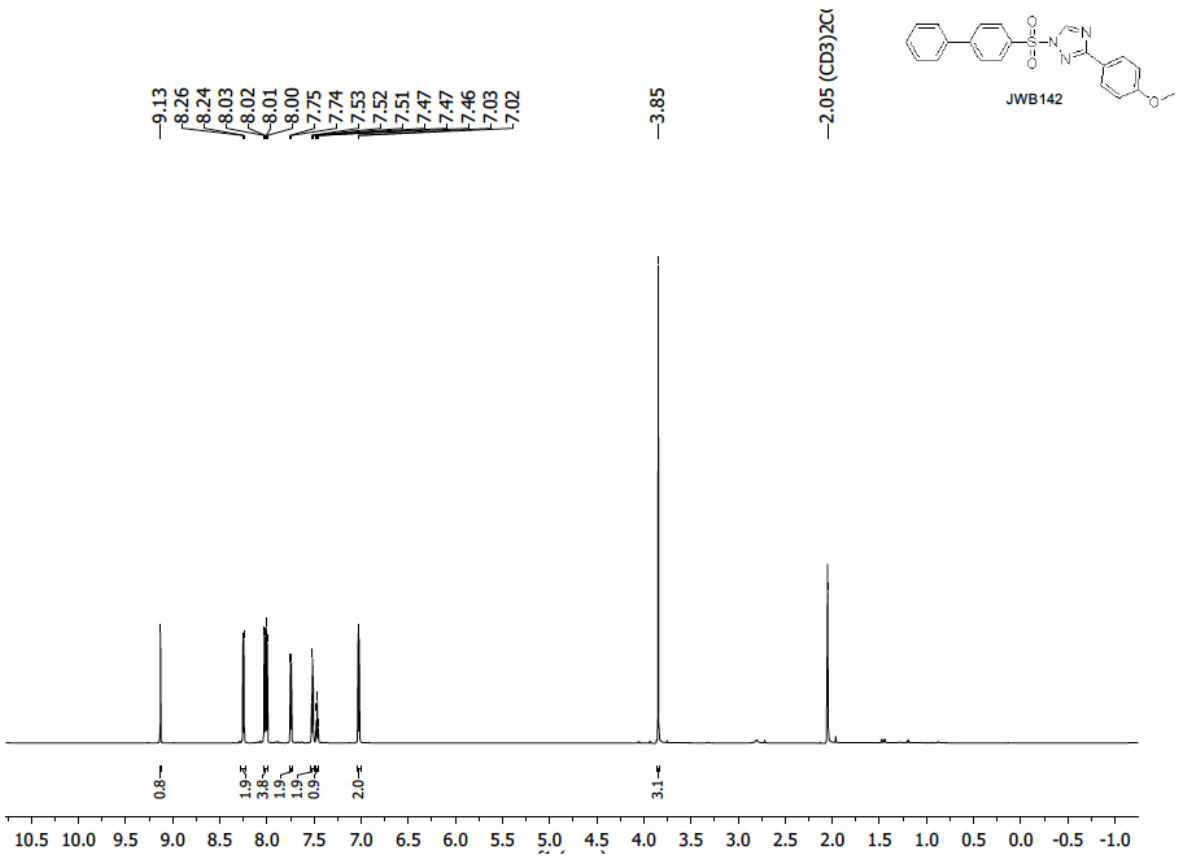

206.12
166.25
165.36
162.51
147.03
131.92
129.13
128.05
122.91
116.04
114.99
56.52
55.73
JWB136
f1 (ppm)

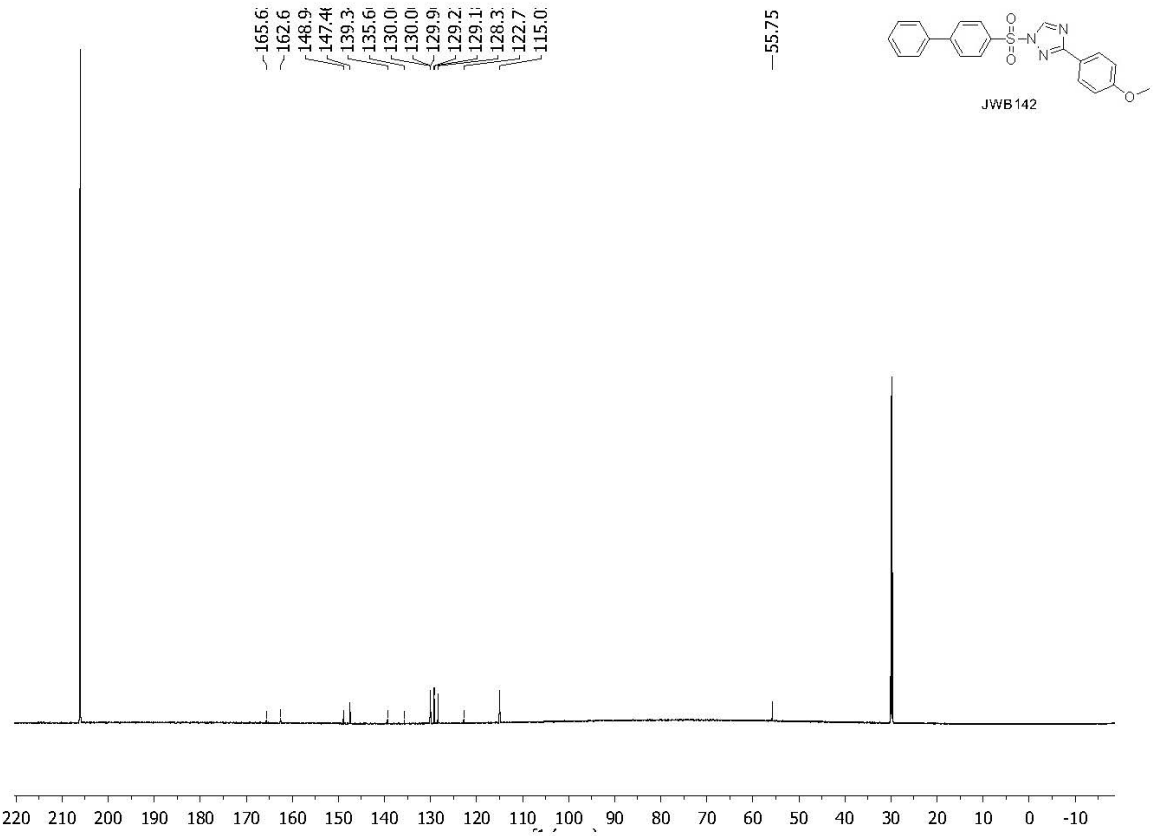

9.15
8.40
8.39
8.39
8.39
8.38
8.38
8.17
8.17
8.17
8.16
8.16
8.16
8.02
8.01
8.01
8.01
8.00
8.00
7.04
7.04
7.03
7.03
7.02
7.02
3.85
2.05 (CD3)2C(
JWB137
0.7
1.7
1.7
1.8
1.9
3.0
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

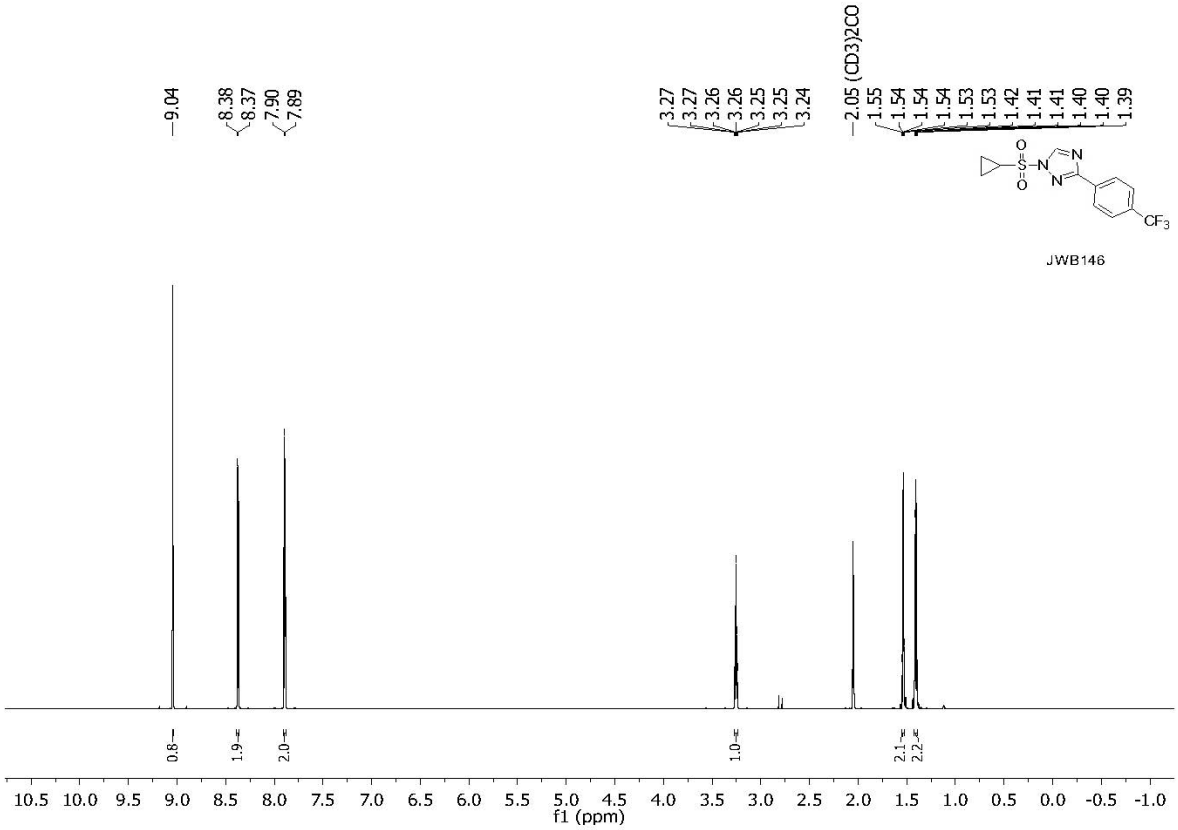

165.9
162.7
147.9
140.9
134.7
130.1
129.3
122.4
119.6
117.6
115.0
55.77
JWB137
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

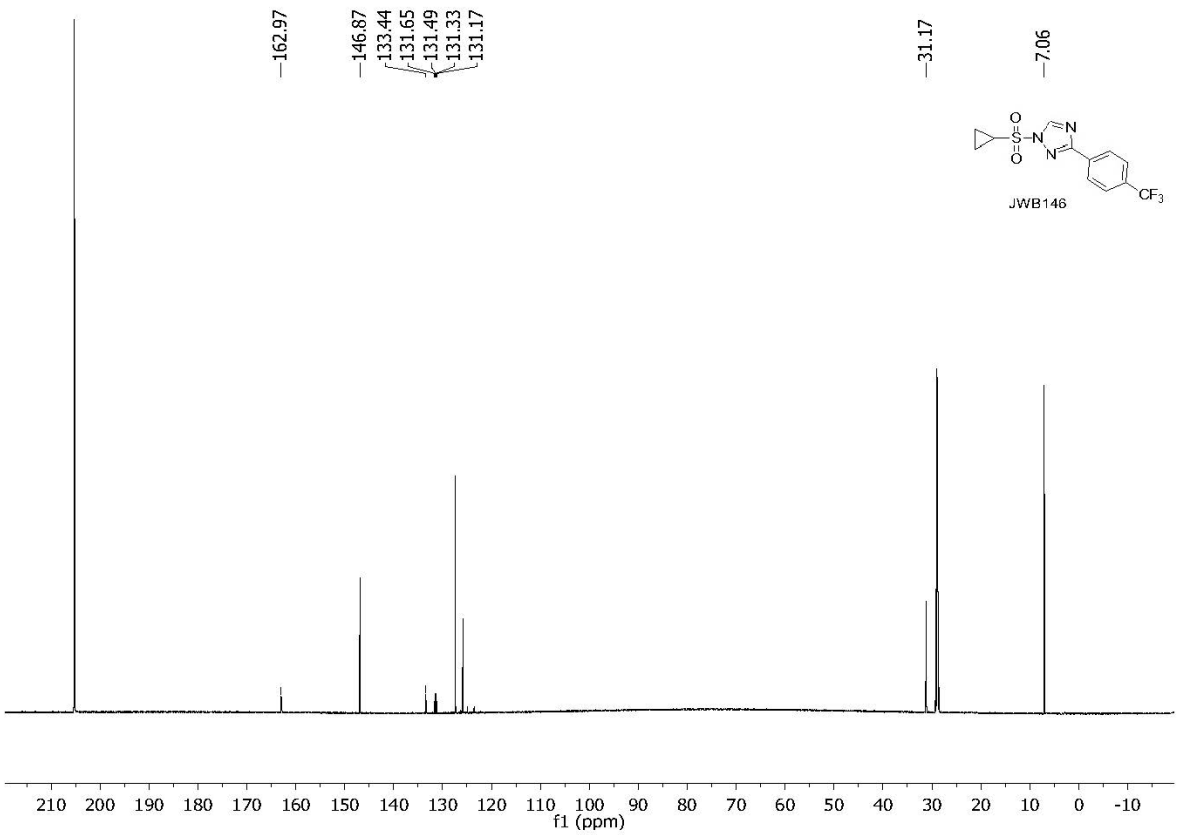

9.33
9.32
8.73
8.40
8.40
8.40
8.40
8.39
8.39
8.39
8.39
8.04
8.04
8.04
8.03
8.03
8.03
7.55
7.55
7.54
7.54
7.26 CDCl3
6.95
6.95
6.95
6.94
6.94
6.93
3.85
OCH3
JWB141
0.6
0.7
0.6
0.8
1.7
0.9
2.0
3.3
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

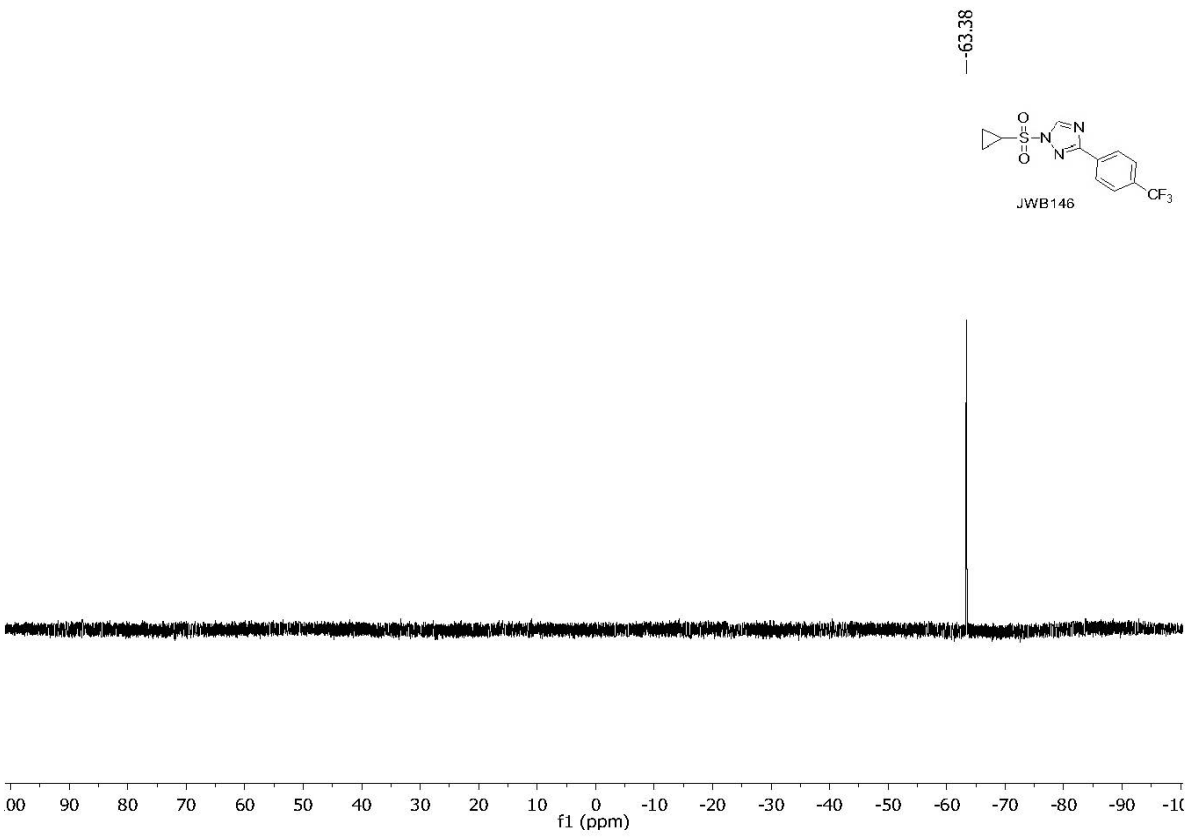

165.58
161.73
155.48
149.19
145.35
136.17
133.36
128.75
123.99
121.45
114.12
55.34
OCH3
JWB 141
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

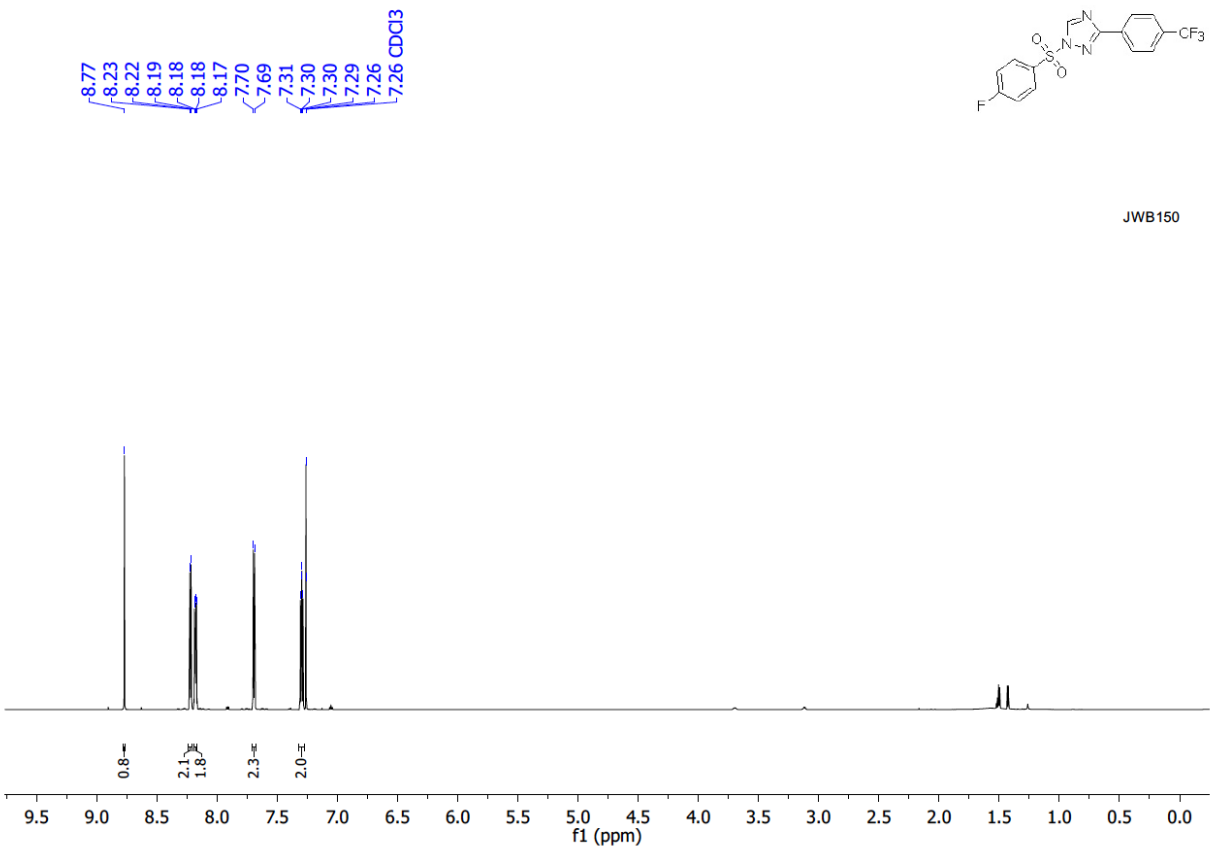

9.13
8.26
8.24
8.03
8.02
8.01
8.00
7.75
7.74
7.53
7.52
7.51
7.47
7.47
7.46
7.03
7.02
3.85
2.05 (CD3)2C(
JWB142
0.8
1.9
3.8
1.9
0.9
2.0
3.1
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

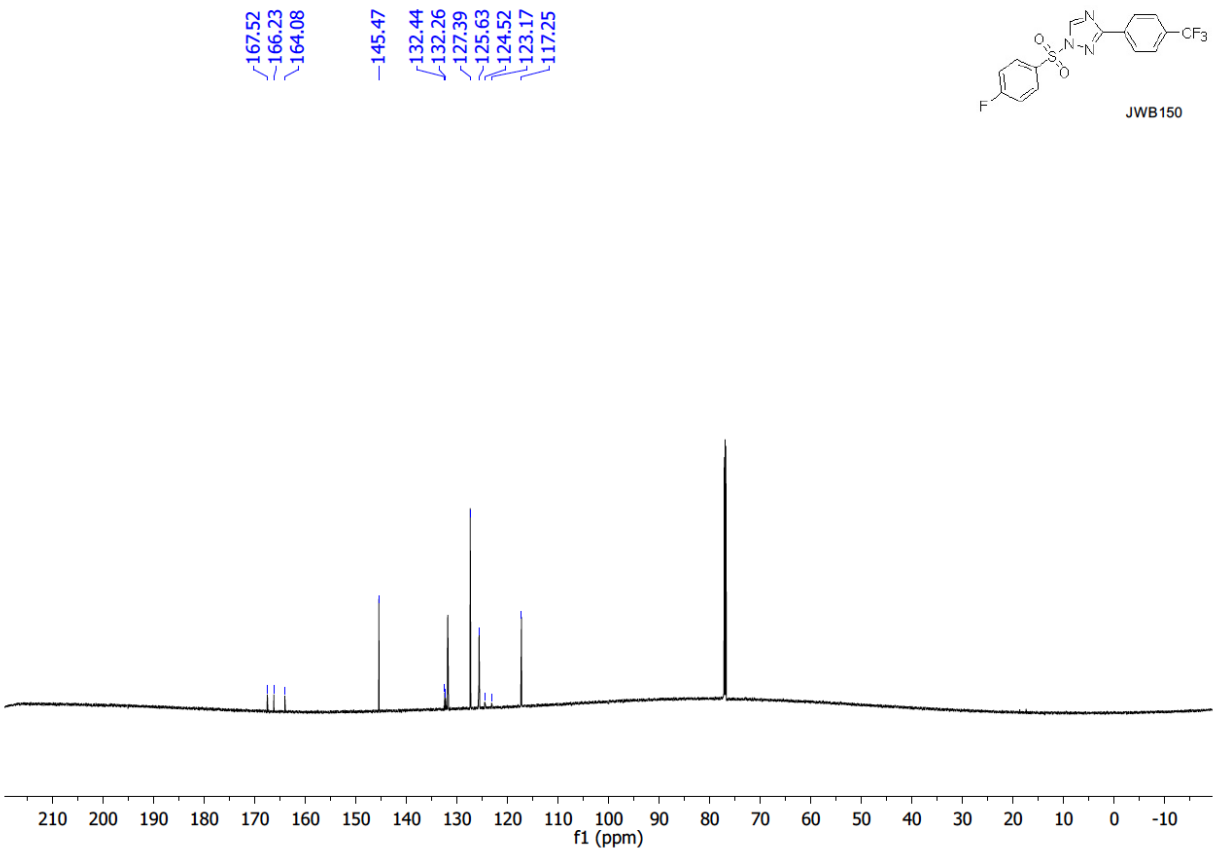

165.6
162.6
148.9
147.4
139.3
135.6
130.0
130.0
129.9
129.2
129.1
128.3
122.7
115.0
55.75
JWB142
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

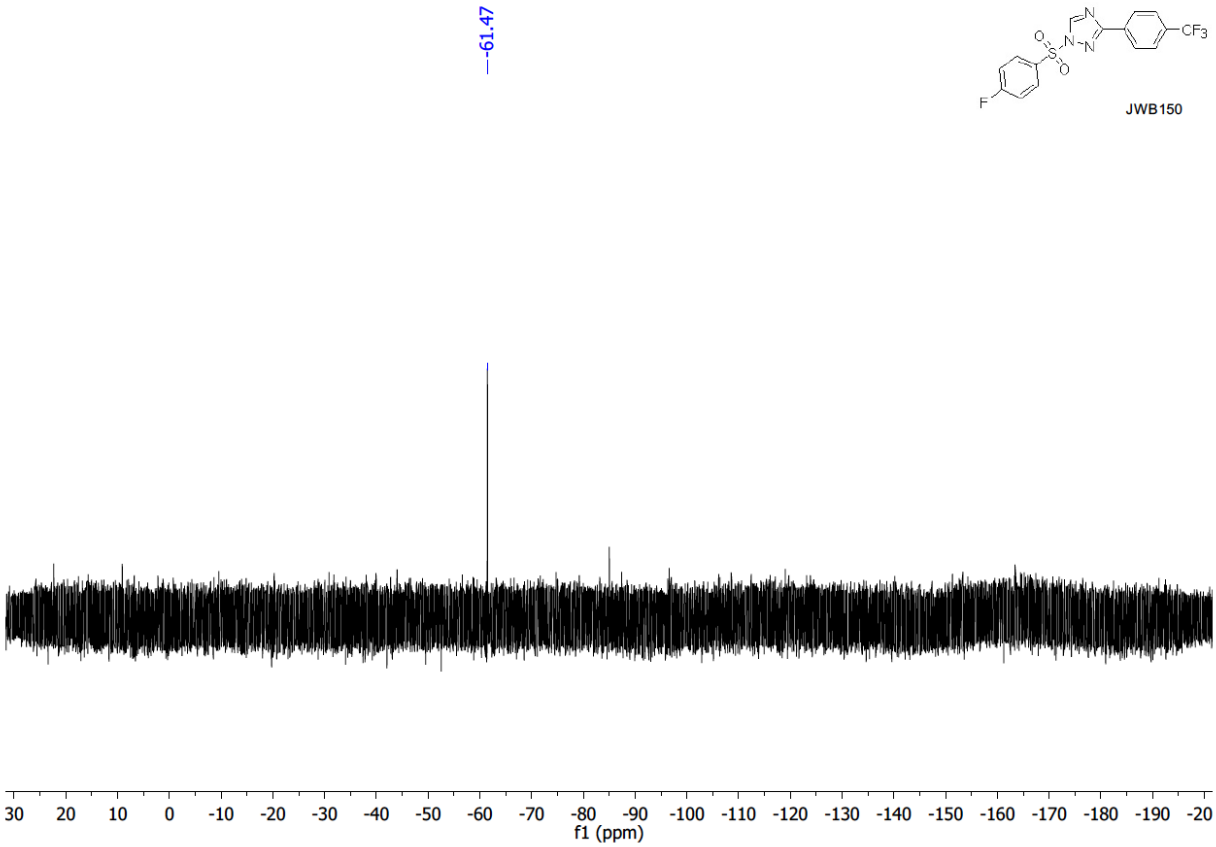

9.04
8.38
8.37
7.90
7.89
3.27
3.27
3.26
3.26
3.25
3.25
3.24
2.05 (CD3)2CO
1.55
1.54
1.54
1.54
1.53
1.53
1.42
1.41
1.41
1.40
1.40
1.39
JWB146
0.8
1.9
2.0
1.0
2.1
2.2
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

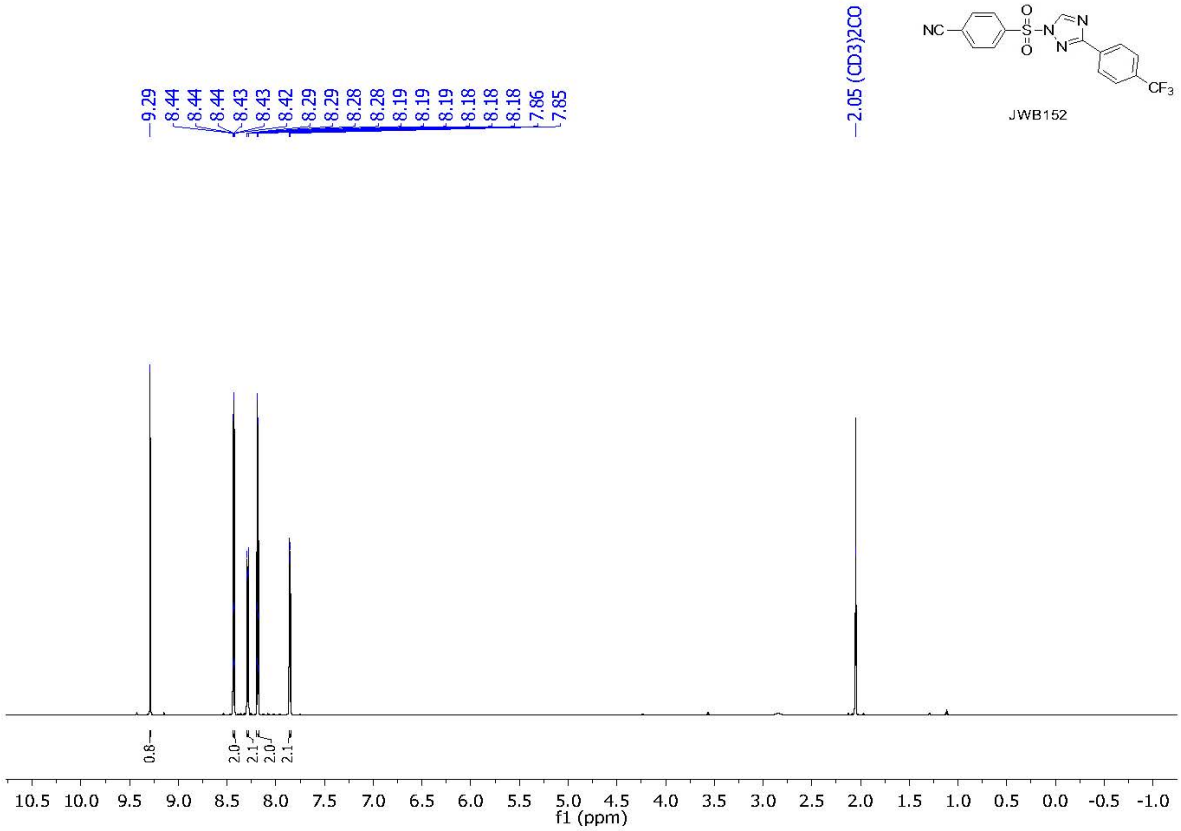

162.97
146.87
133.44
131.65
131.49
131.33
131.17
31.17
7.06
JWB146
CF3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

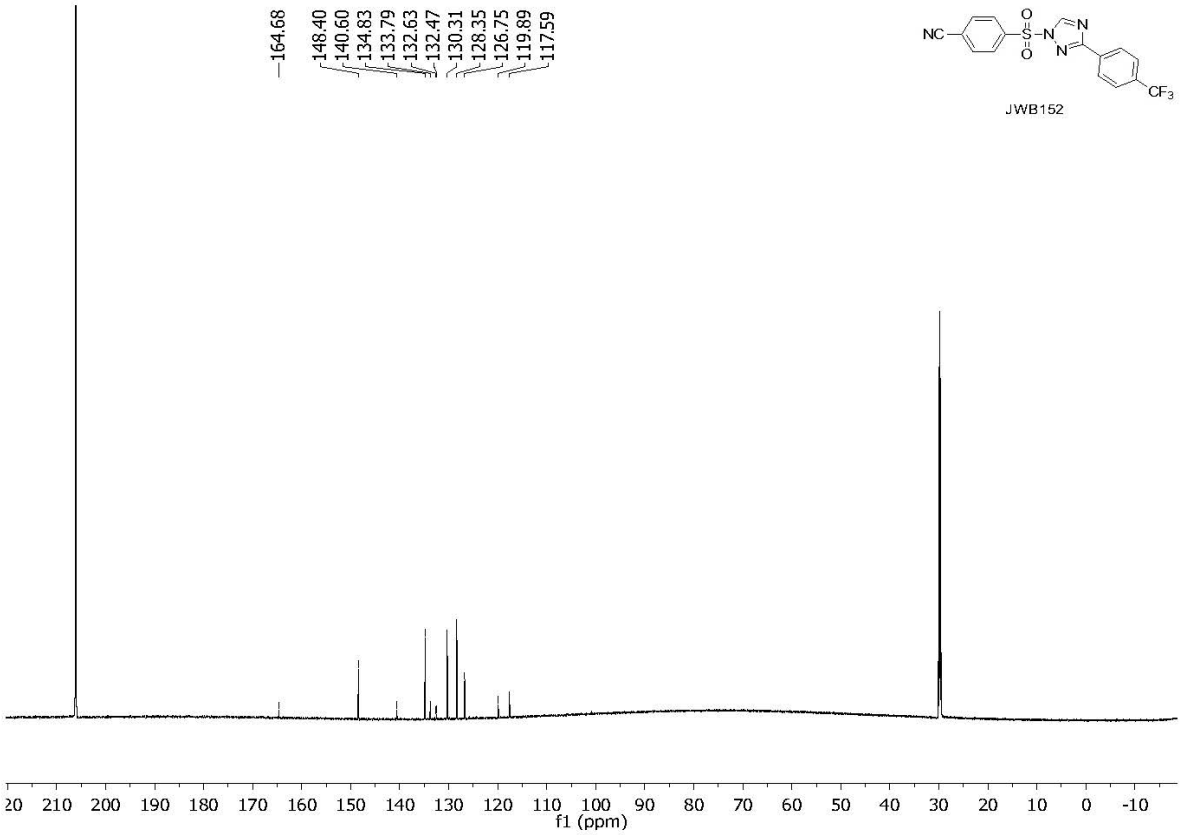

-63.38
JWB146
f1 (ppm)

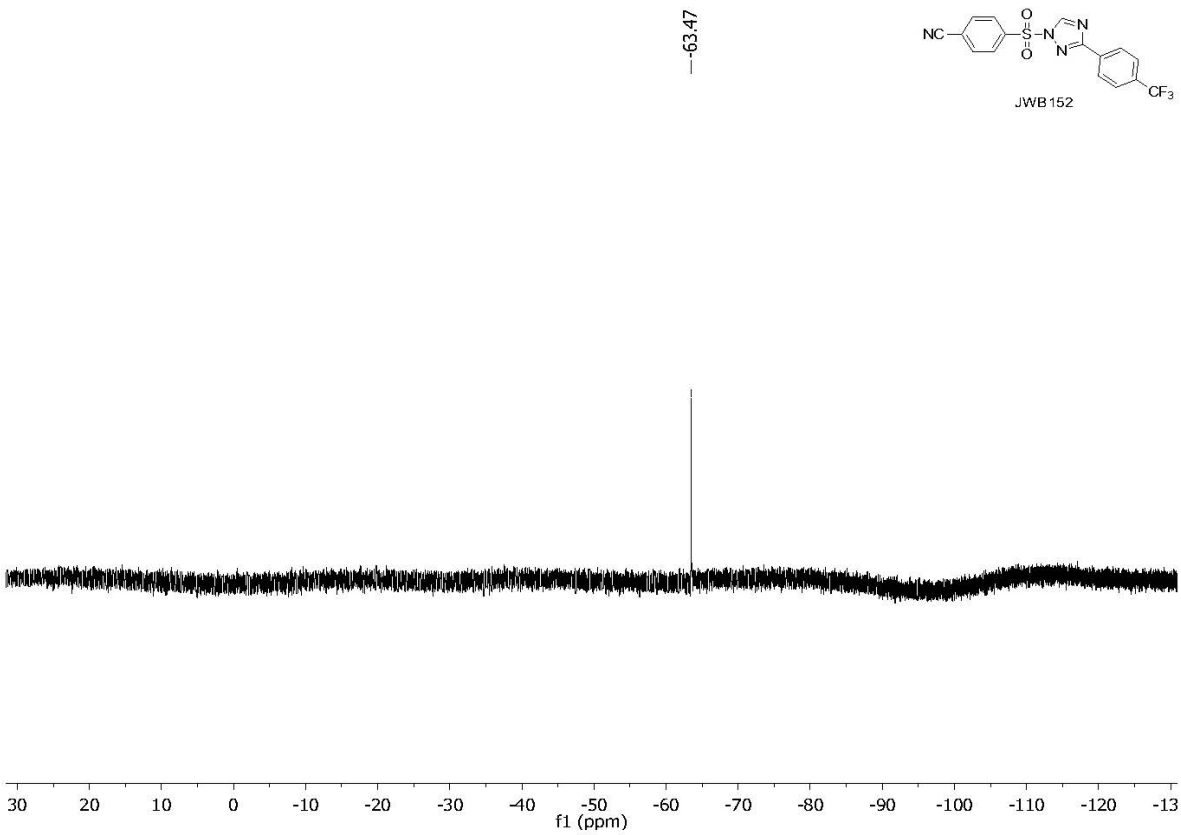

8.77
8.23
8.22
8.19
8.18
8.18
8.17
7.70
7.69
7.31
7.30
7.30
7.29
7.26
7.26 CDCl3
JWB150
0.8
2.1
1.8
2.3
2.0
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

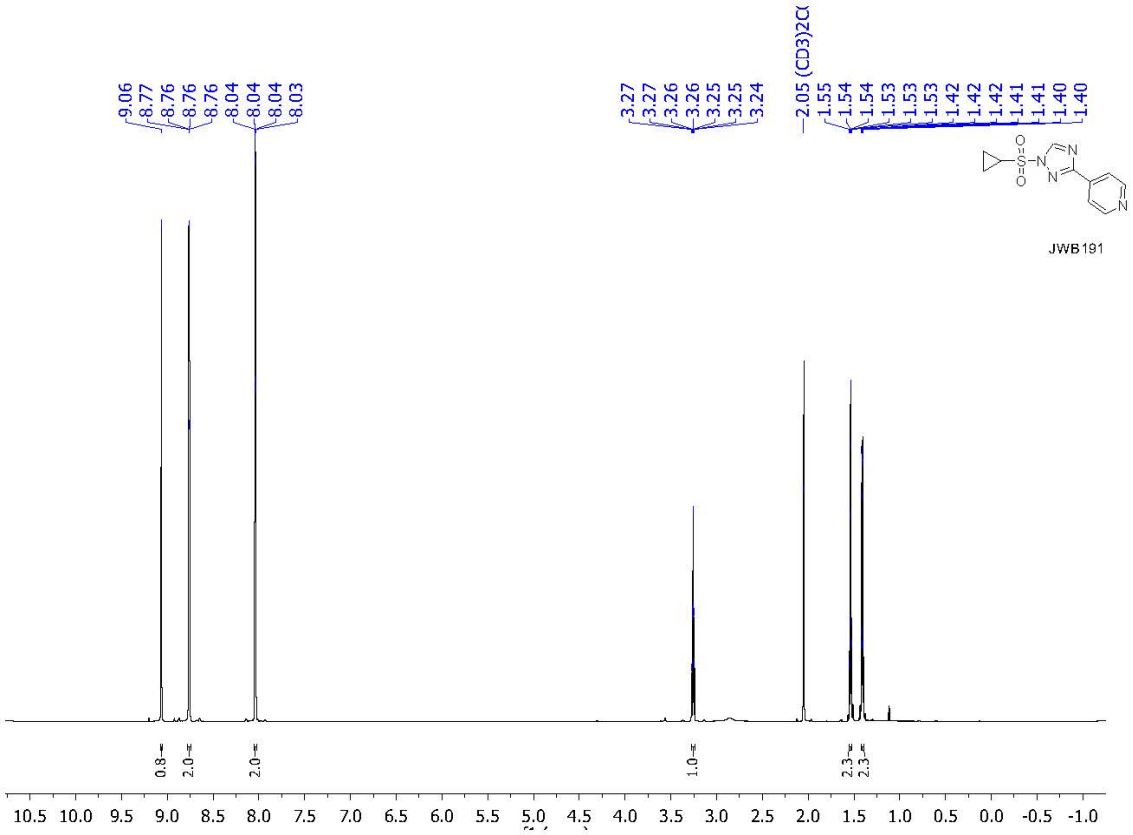

167.52
166.23
164.08
145.47
132.44
132.26
127.39
125.63
124.52
123.17
117.25
F
O
S
O
N
N
N
CF3
JWB150
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

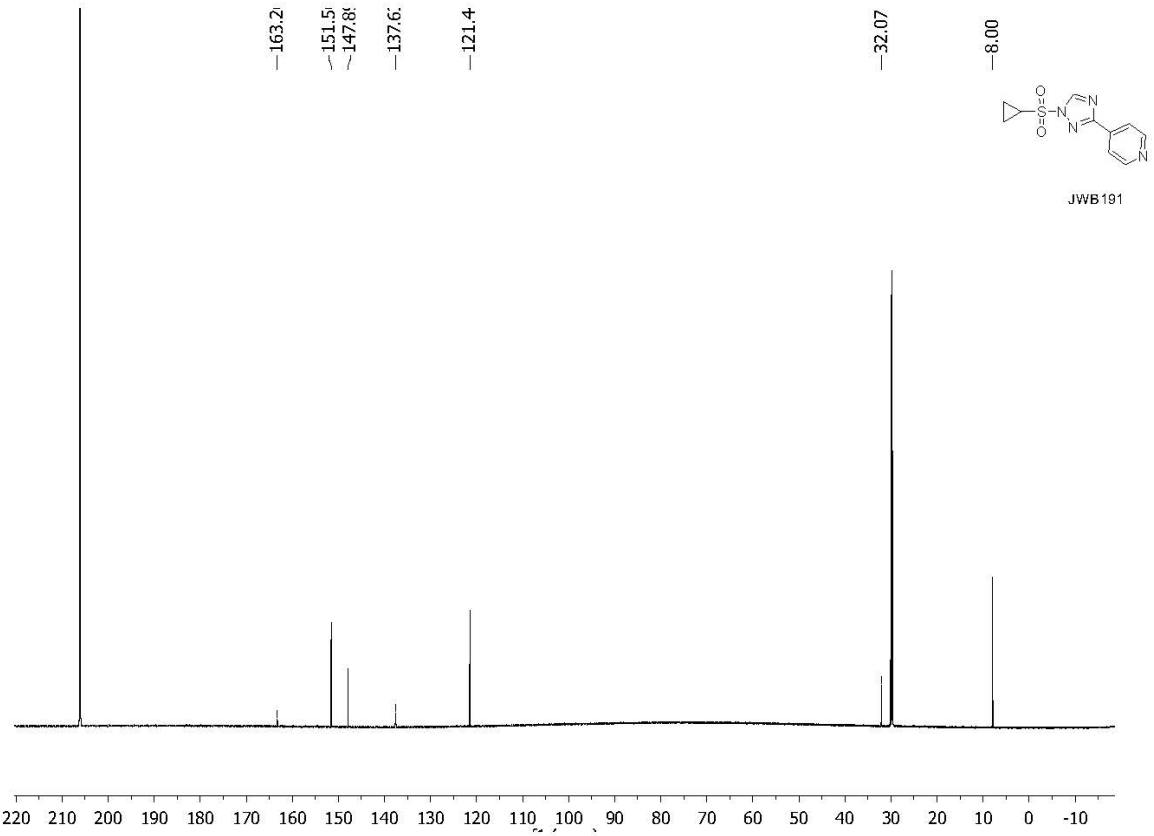

-61.47
JWB150
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -20
f1 (ppm)

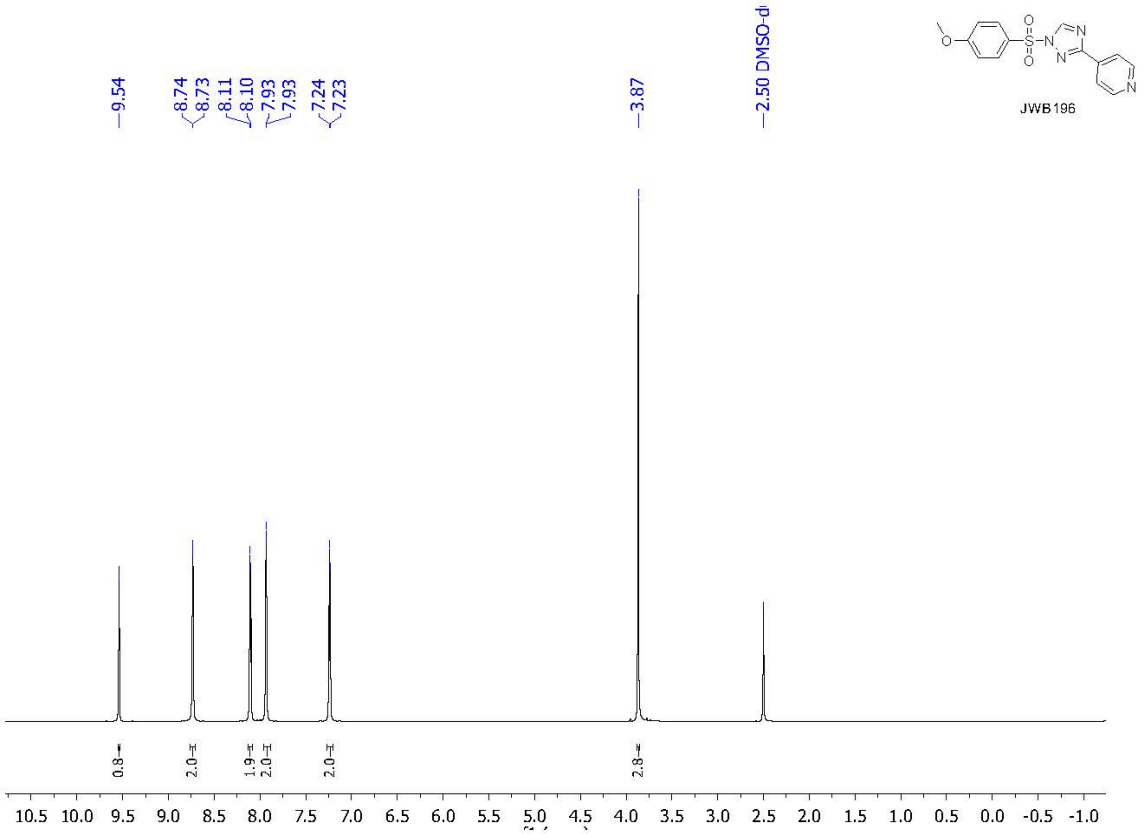

9.29
8.44
8.44
8.44
8.43
8.43
8.42
8.29
8.29
8.28
8.28
8.19
8.19
8.19
8.18
8.18
8.18
7.86
7.85
2.05 (CD3)2CO
NC
O
S
O
N
N
N
CF3
JWB152
0.8
2.0
2.1
2.0
2.1
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

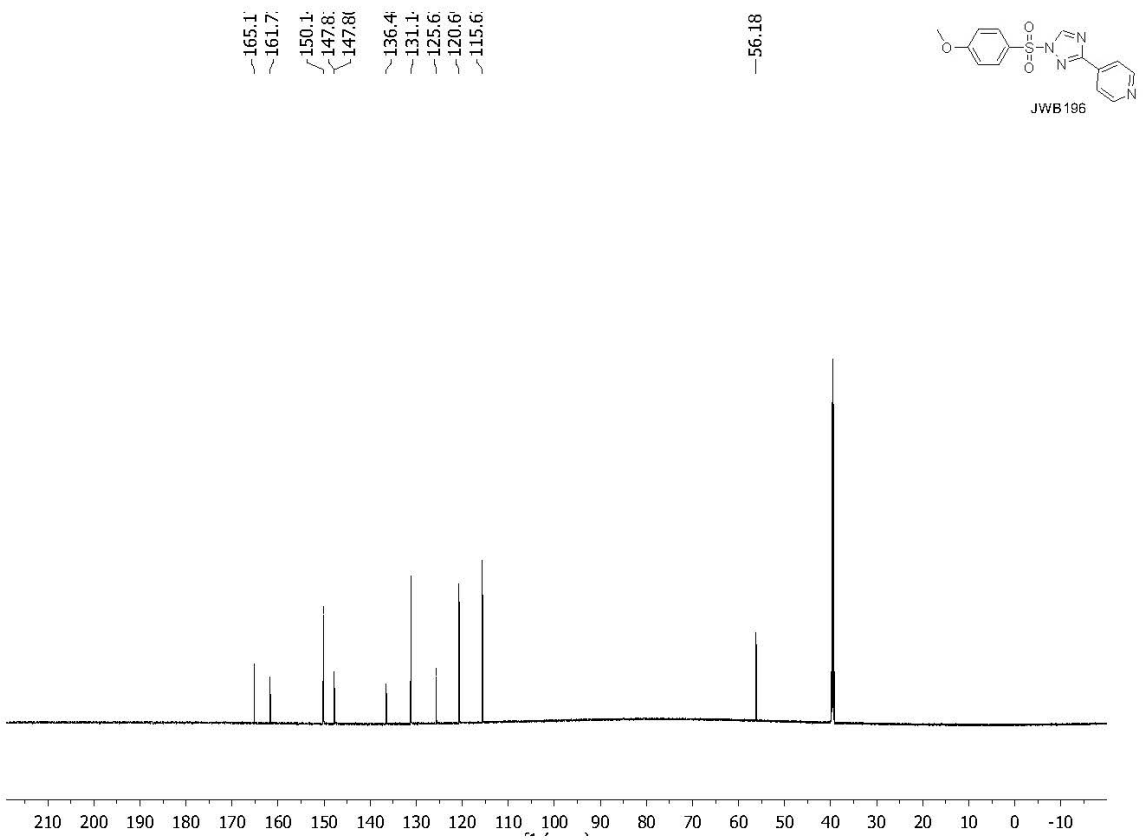

164.68
148.40
140.60
134.83
133.79
132.63
132.47
130.31
128.35
126.75
119.89
117.59
NC
O
S
O
N
N
N
CF3
JWB152
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

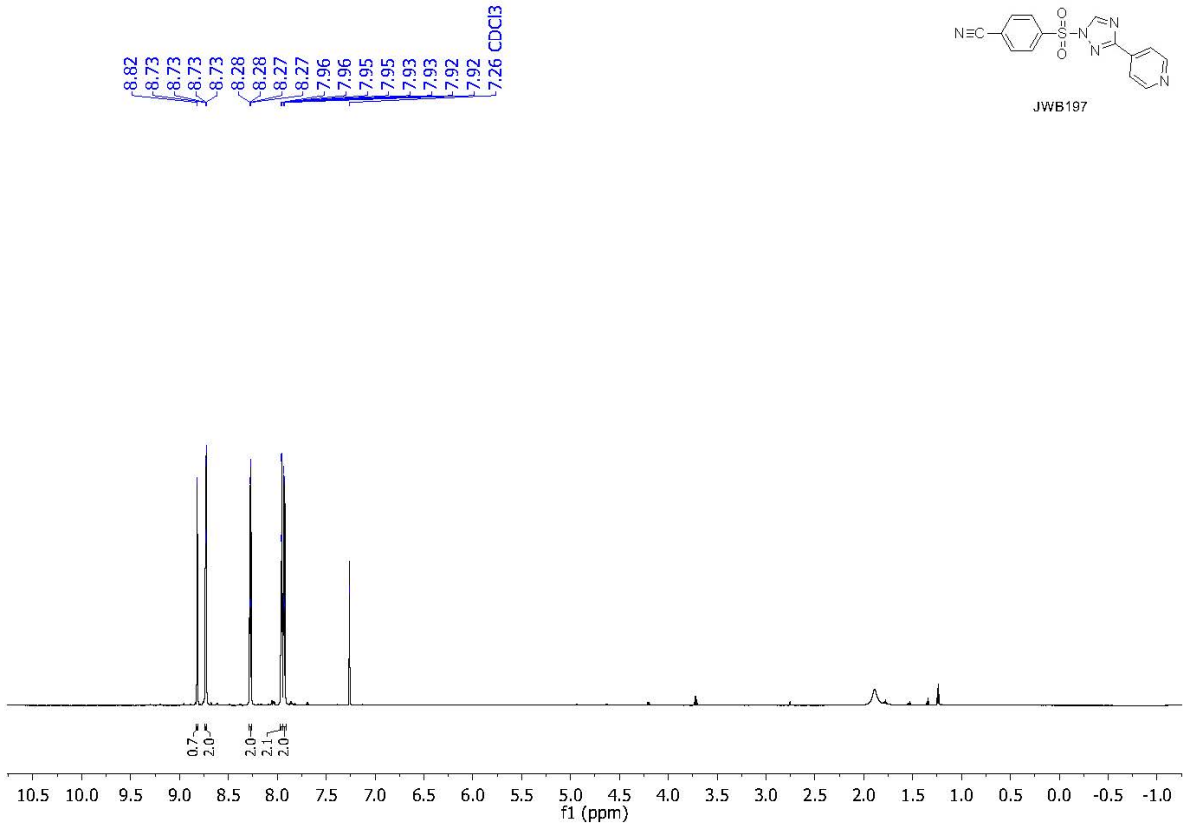

-63.47
NC
O
S
O
N
N
N
CF3
JWB152
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-13
f1 (ppm)

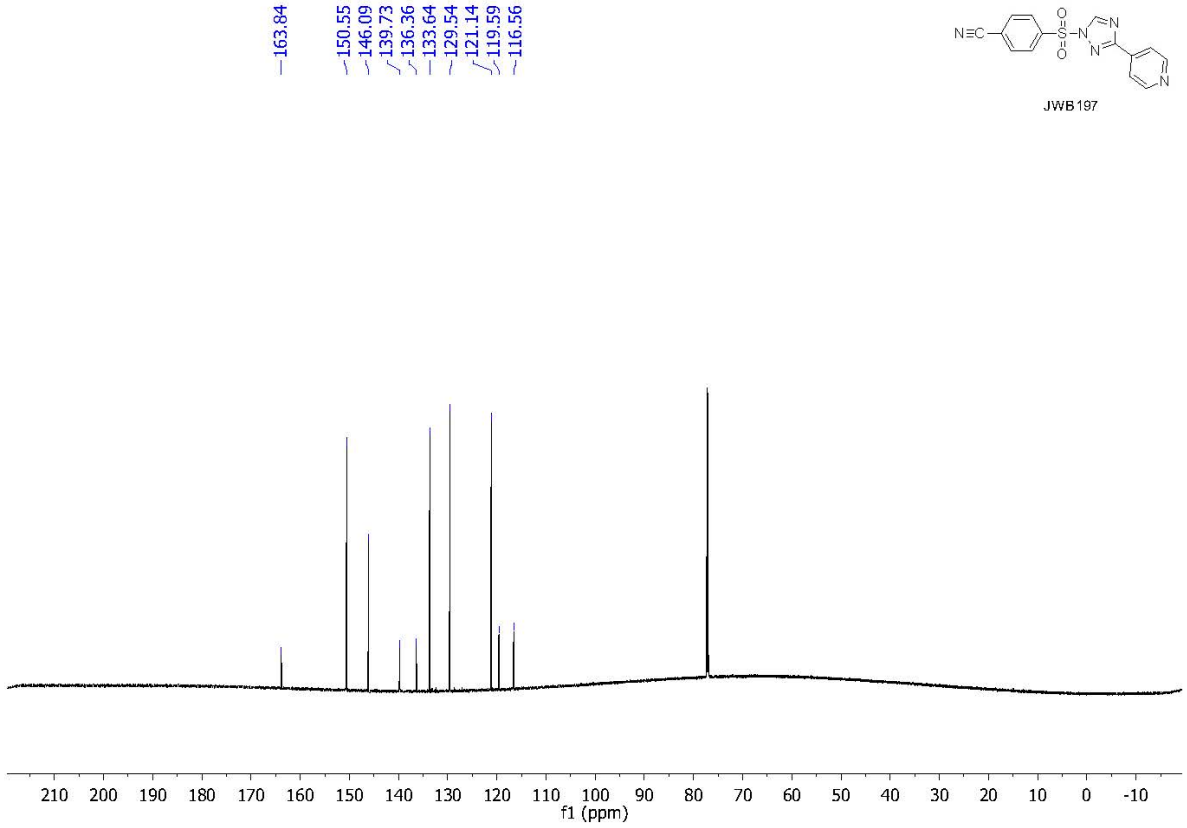

9.06
8.77
8.76
8.76
8.76
8.04
8.04
8.04
8.03
3.27
3.27
3.26
3.26
3.25
3.25
3.24
2.05 (CD3)2C(
1.55
1.54
1.54
1.53
1.53
1.53
1.42
1.42
1.42
1.41
1.41
1.40
1.40
JWB191
0.8
2.0
2.0
1.0
2.3
2.3
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

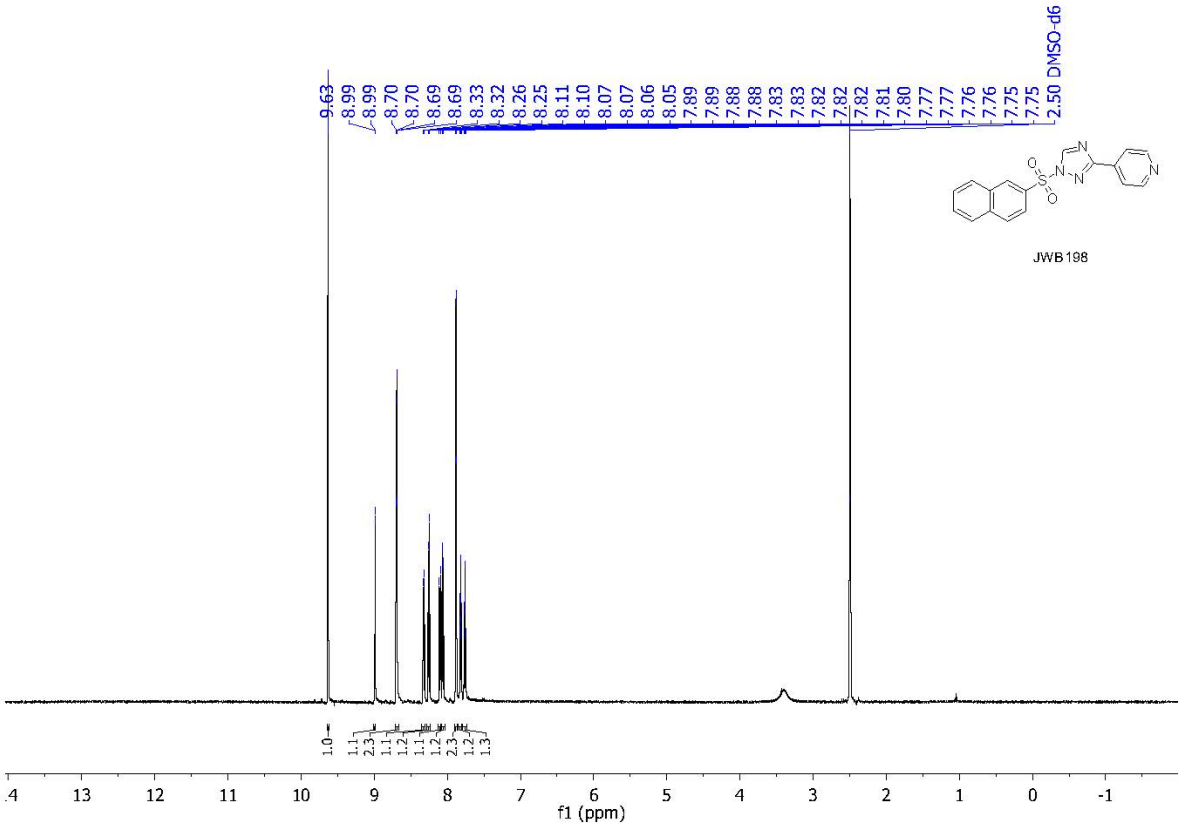

163.2
151.5
147.8
137.6
121.4
32.07
8.00
JWB191
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

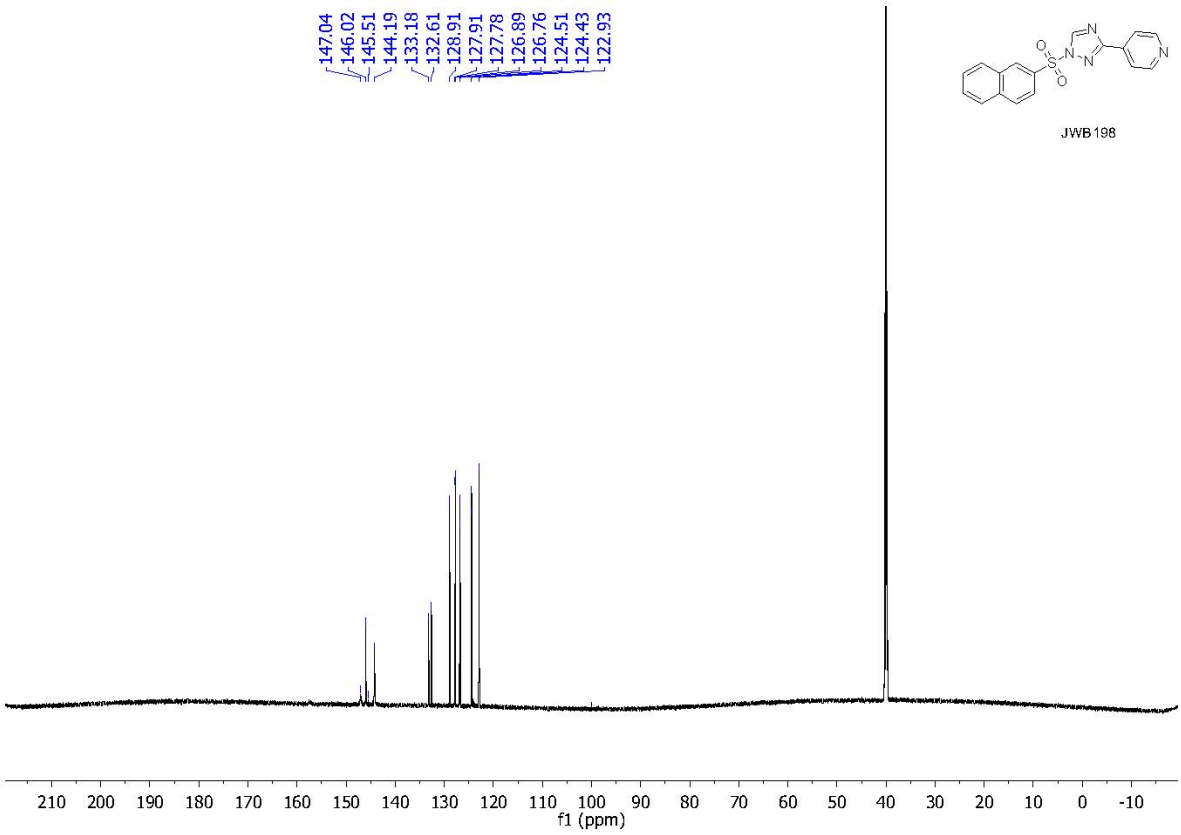

9.54
8.74
8.73
8.11
8.10
7.93
7.93
7.24
7.23
3.87
2.50 DMSO-d
JWB196
0.8
2.0
1.9
2.0
2.0
2.8
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

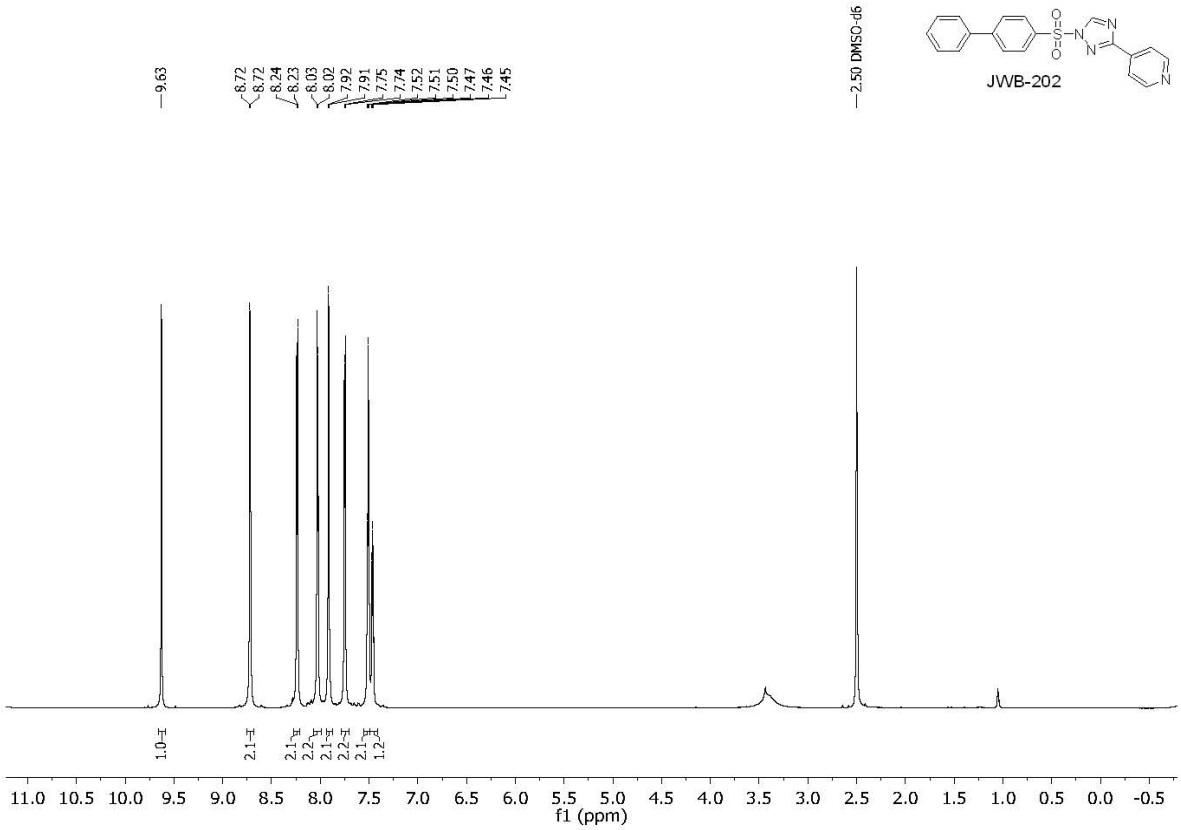

165.1
161.7
150.1
147.8
147.8
136.4
131.1
125.6
120.6
115.6
56.18
JWB196
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

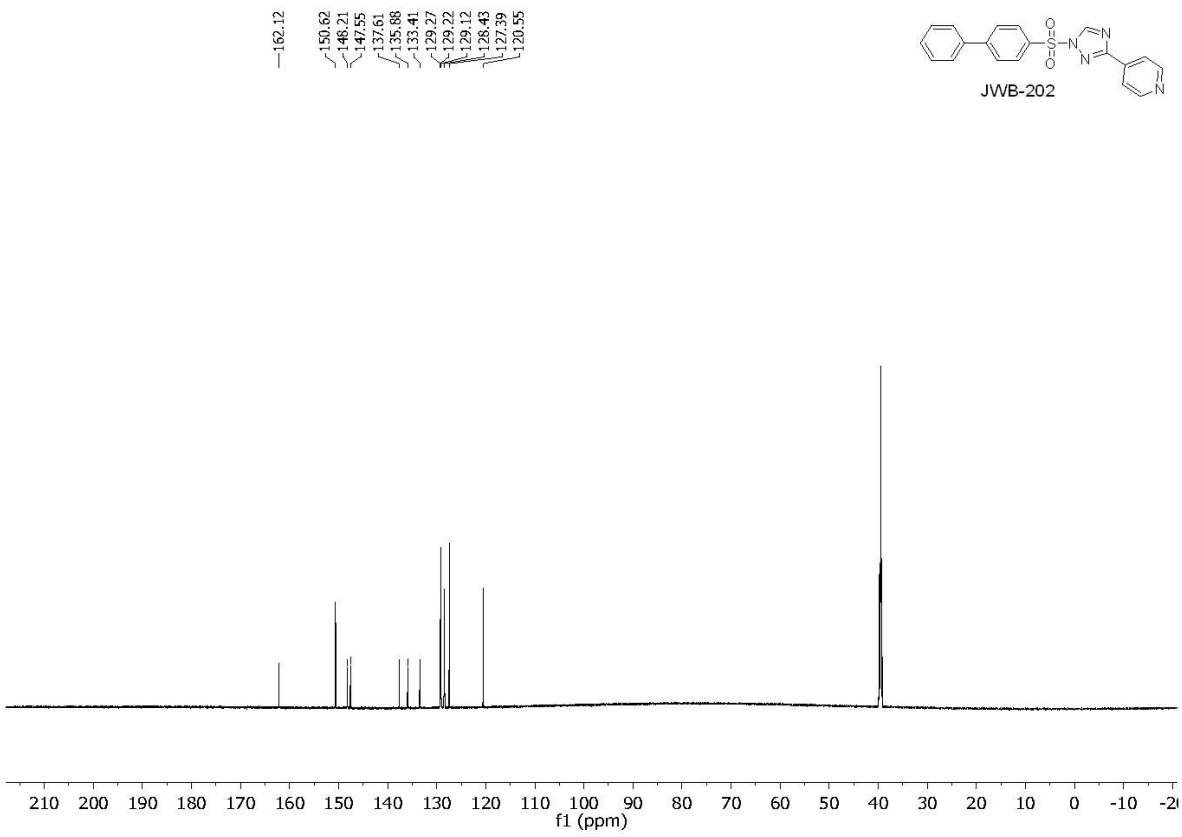

8.82
8.73
8.73
8.73
8.73
8.28
8.28
8.27
8.27
7.96
7.96
7.95
7.95
7.93
7.93
7.92
7.92
7.26 CDCl3
JWB197
0.7
2.0
2.0
2.1
2.0
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

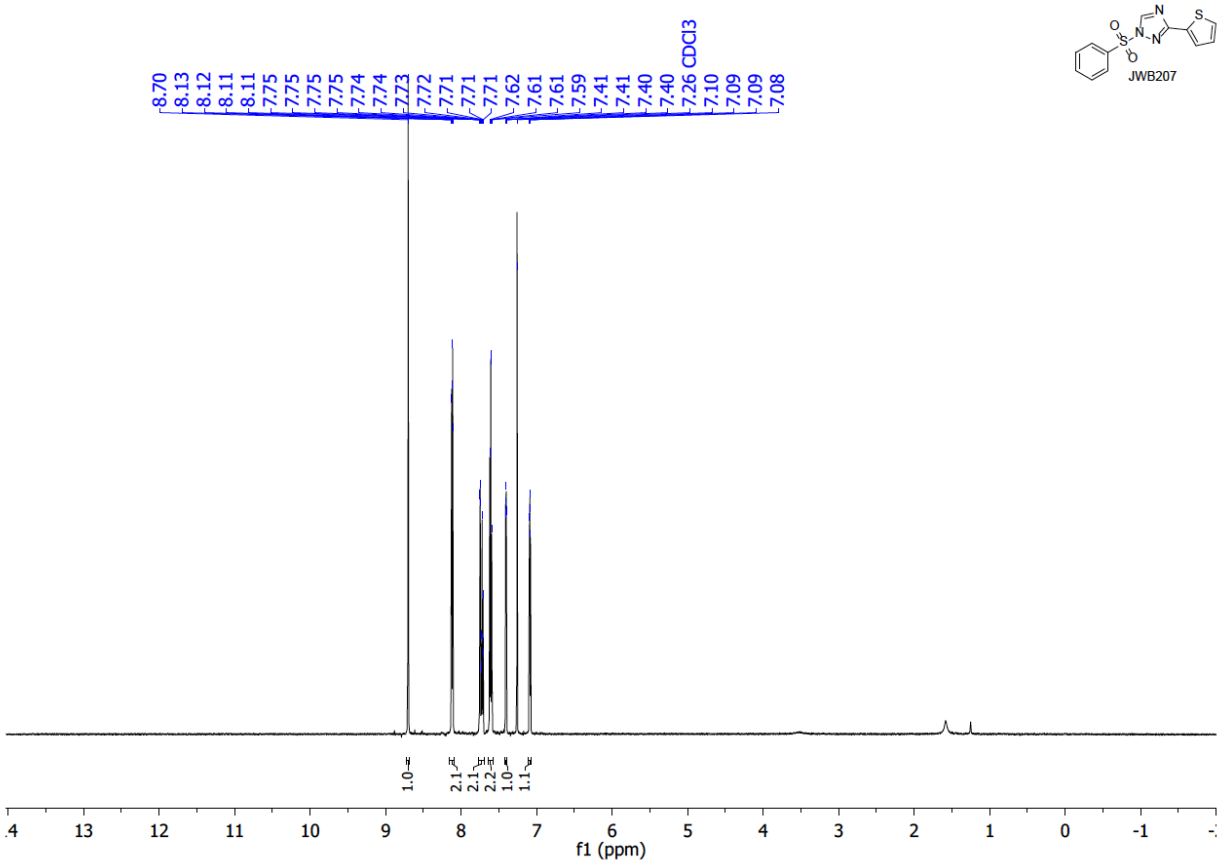

163.84
150.55
146.09
139.73
136.36
133.64
129.54
121.14
119.59
116.56
JWB197
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

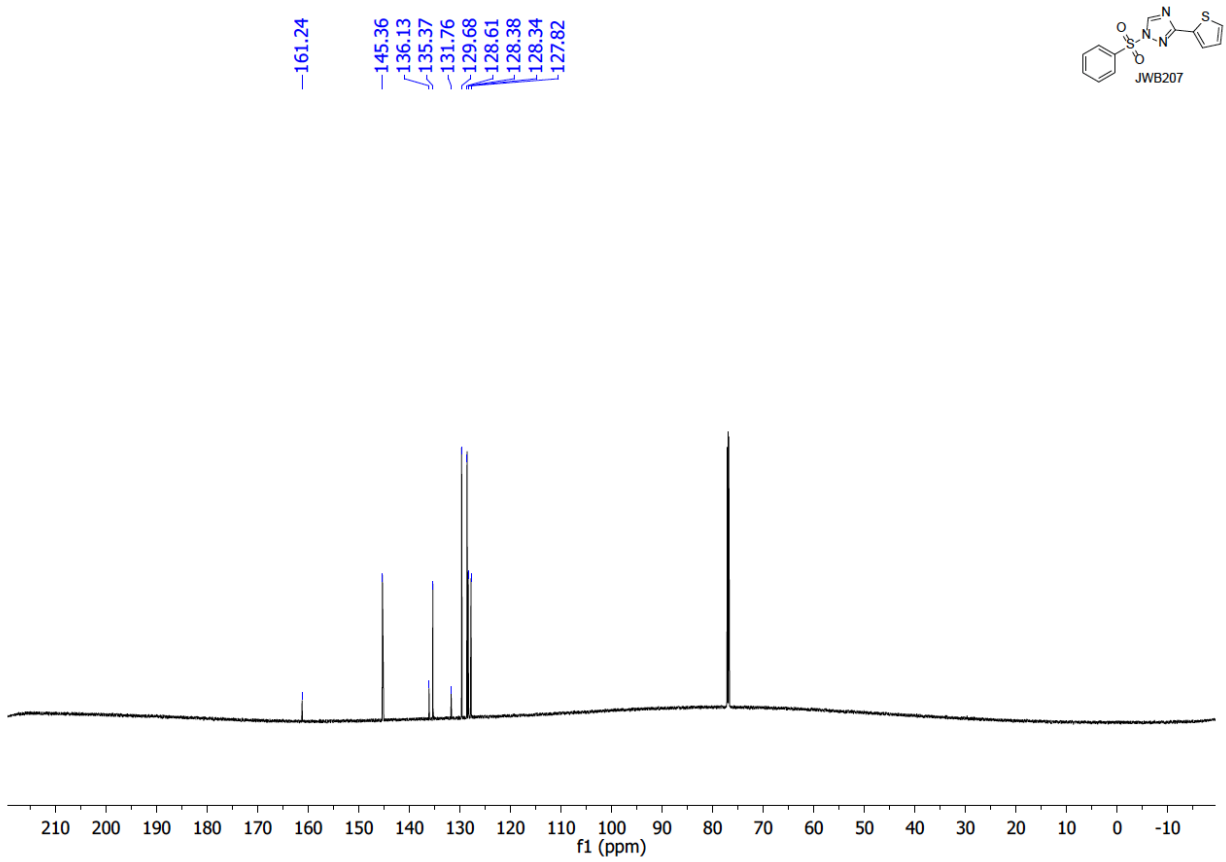

9.63
8.99
8.99
8.70
8.70
8.69
8.69
8.33
8.32
8.26
8.25
8.11
8.10
8.07
8.07
8.06
8.05
7.89
7.89
7.88
7.88
7.83
7.83
7.82
7.82
7.81
7.80
7.77
7.77
7.76
7.76
7.75
7.75
2.50 DMSO-d6
JWB198
1.0
1.1
2.3
1.1
1.2
1.1
1.2
2.3
1.2
1.3
f1 (ppm)

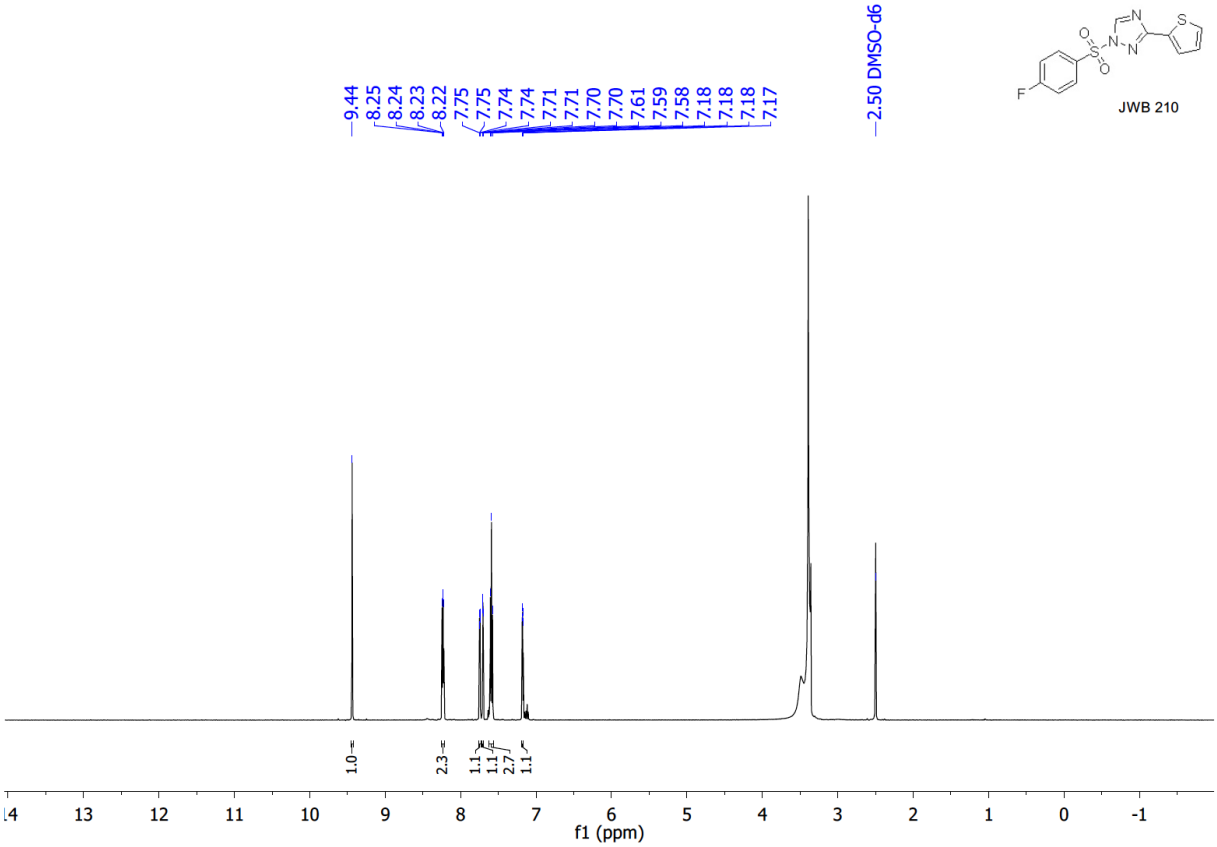

147.04
146.02
145.51
144.19
133.18
132.61
128.91
127.91
127.78
126.89
126.76
124.51
124.43
122.93
JWB198
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

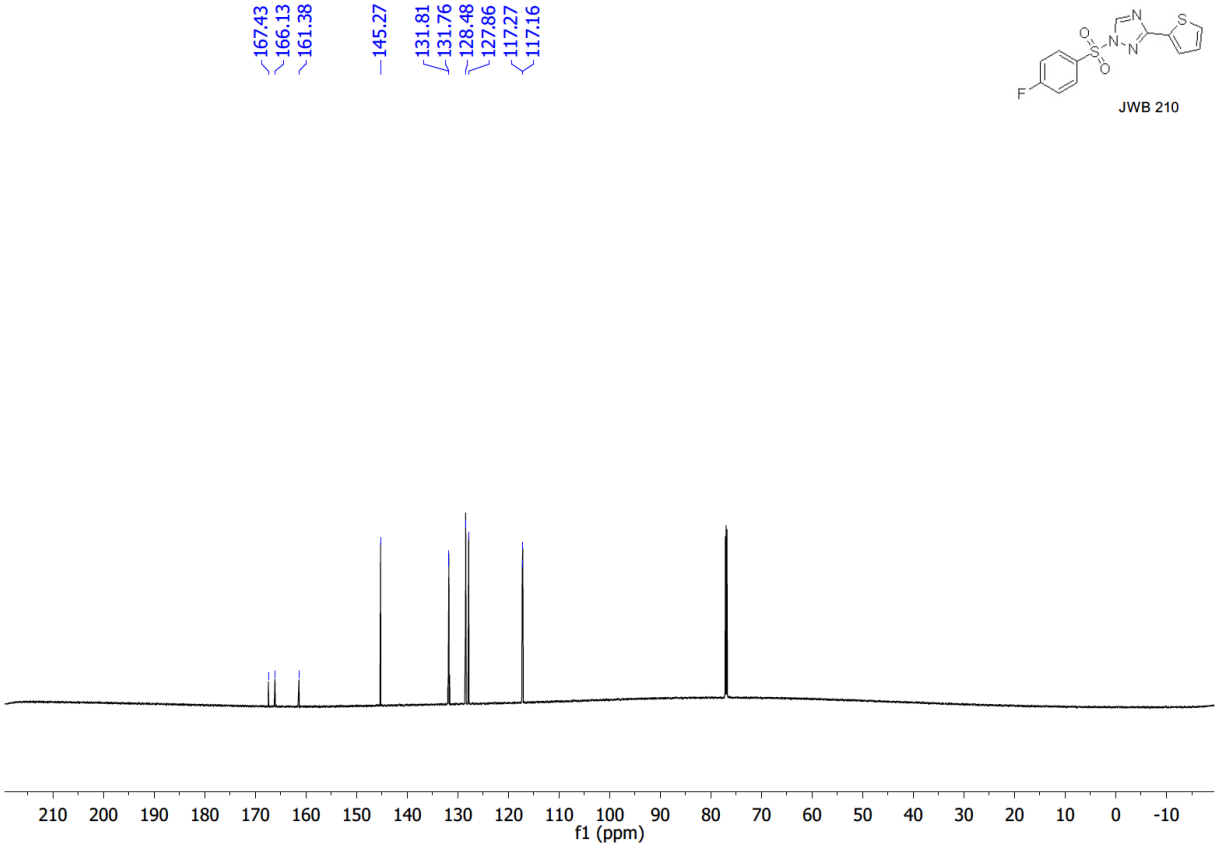

9.63
8.72
8.72
8.24
8.23
8.03
8.02
7.92
7.91
7.75
7.74
7.52
7.51
7.50
7.47
7.46
7.45
2.50 DMSO-d6
JWB-202
1.0
2.1
2.1
2.2
2.1
2.2
2.1
1.2
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

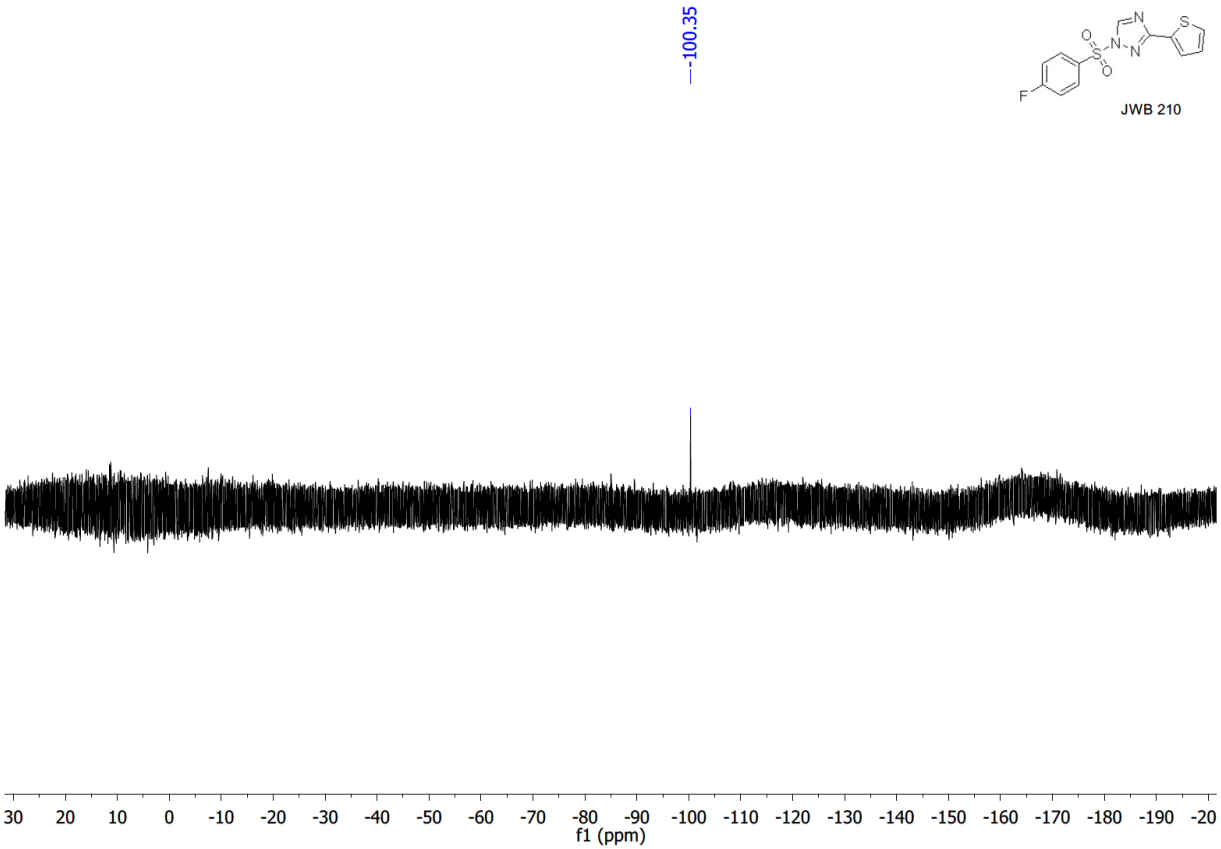

162.12
150.62
148.21
147.55
137.61
135.88
133.41
129.27
129.22
129.12
128.43
127.39
120.55
JWB-202
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

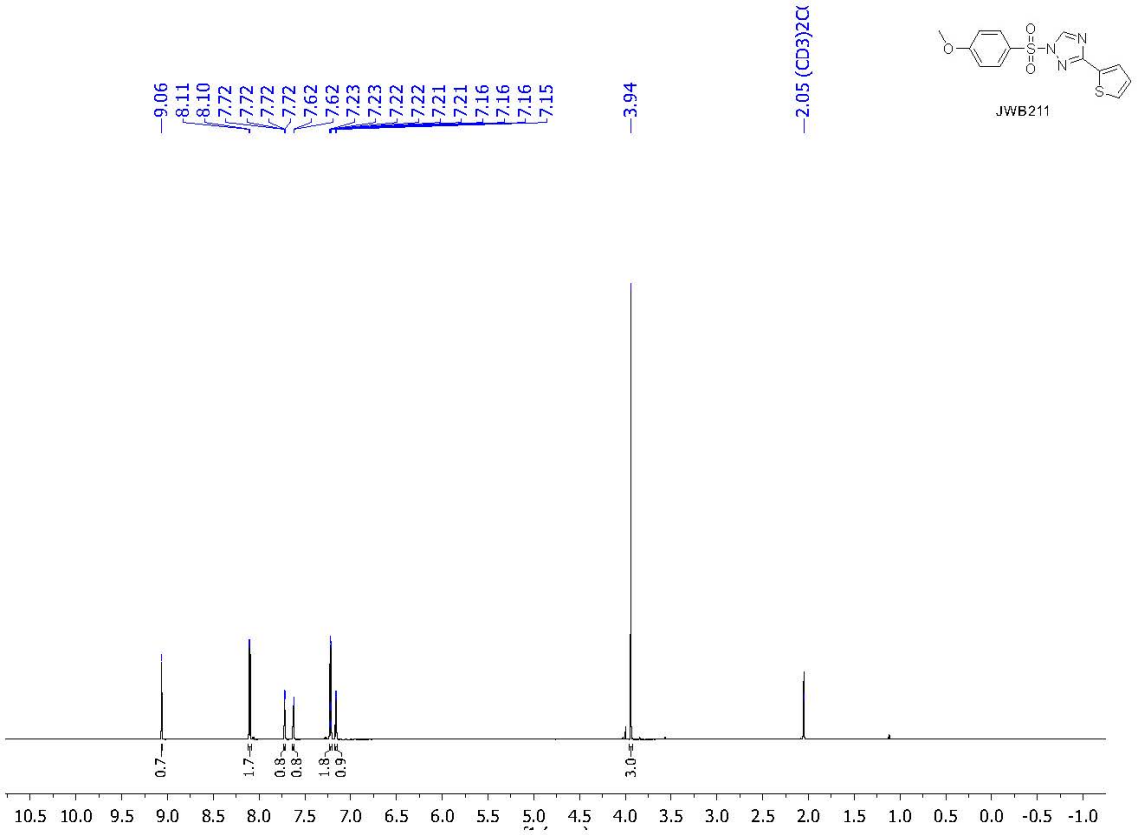

JWB207
8.70
8.13
8.12
8.11
8.11
7.75
7.75
7.75
7.75
7.74
7.74
7.73
7.72
7.71
7.71
7.71
7.62
7.61
7.61
7.59
7.41
7.41
7.40
7.40
7.26 CDCl3
7.10
7.09
7.09
7.08
1.0
2.1
2.1
2.2
1.0
1.1
f1 (ppm)

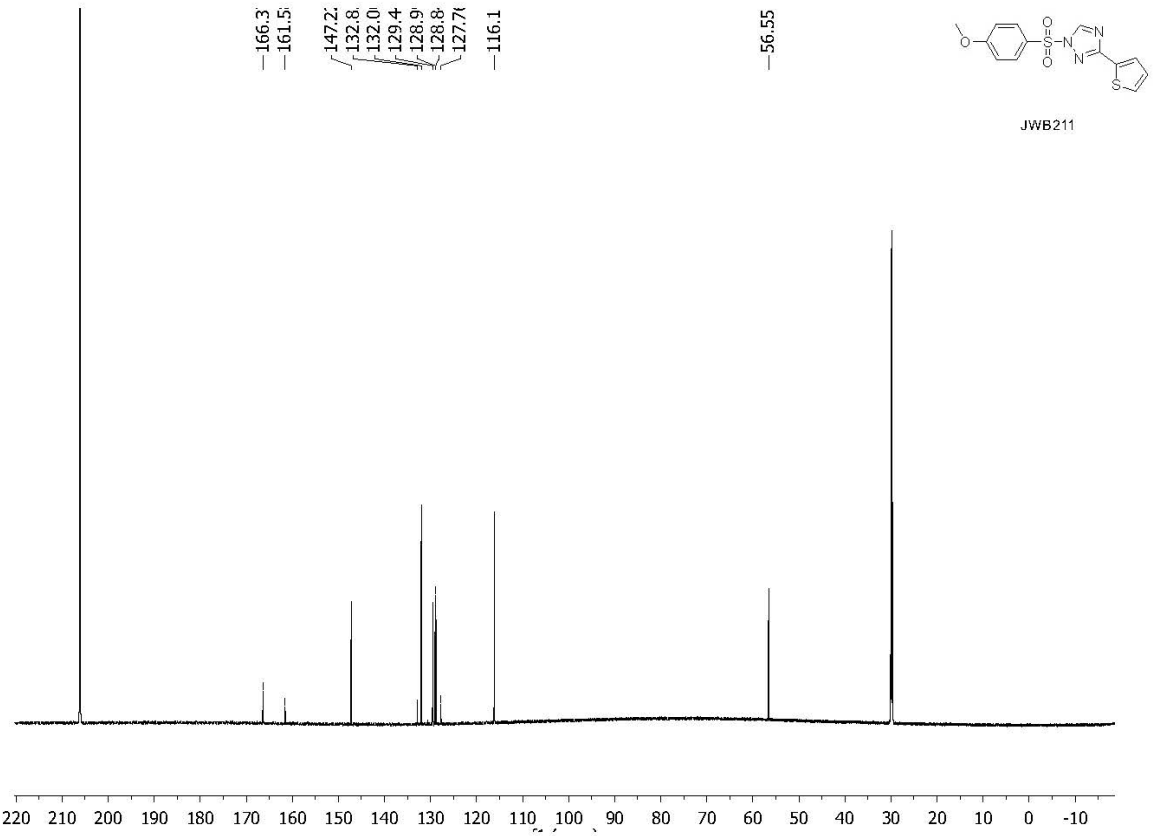

161.24
145.36
136.13
135.37
131.76
129.68
128.61
128.38
128.34
127.82
JWB207
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

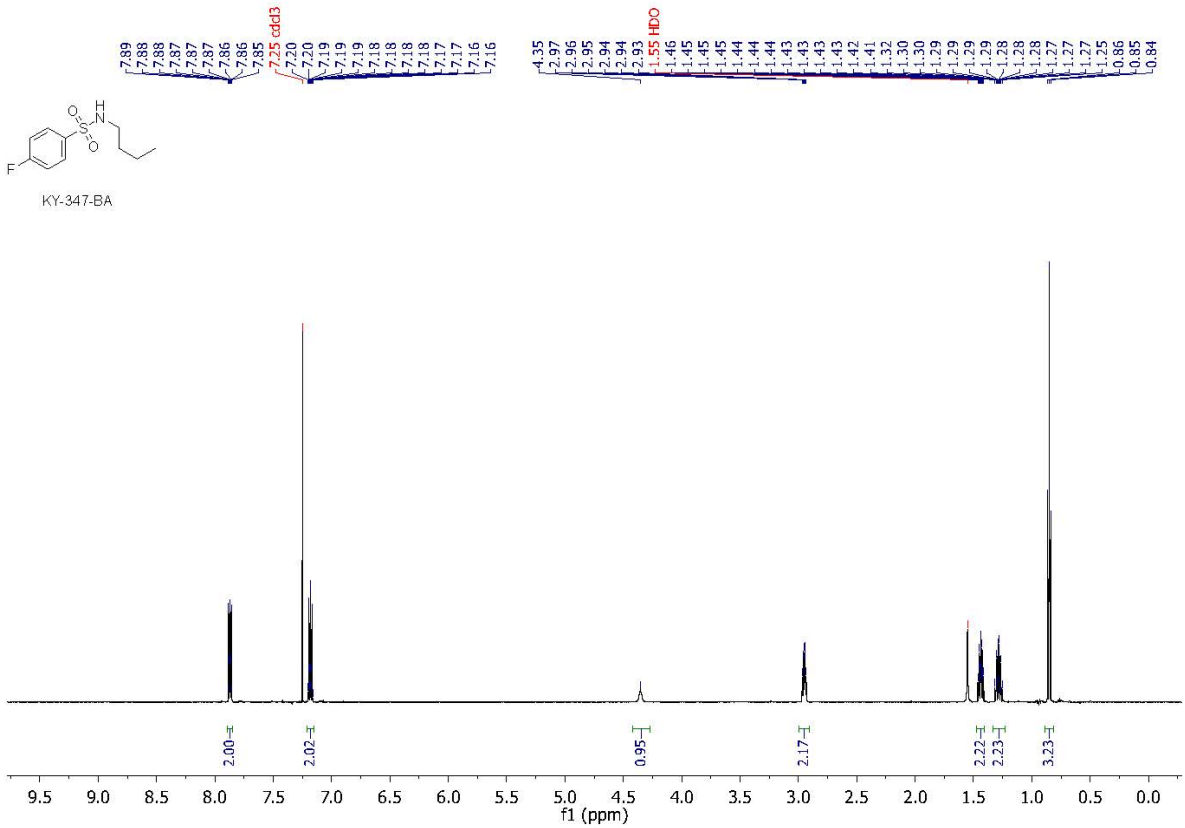

9.44
8.25
8.24
8.23
8.22
7.75
7.75
7.74
7.74
7.71
7.71
7.70
7.70
7.61
7.59
7.58
7.18
7.18
7.17
2.50 DMSO-d6
JWB 210
1.0
2.3
1.1
1.1
2.7
1.1
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

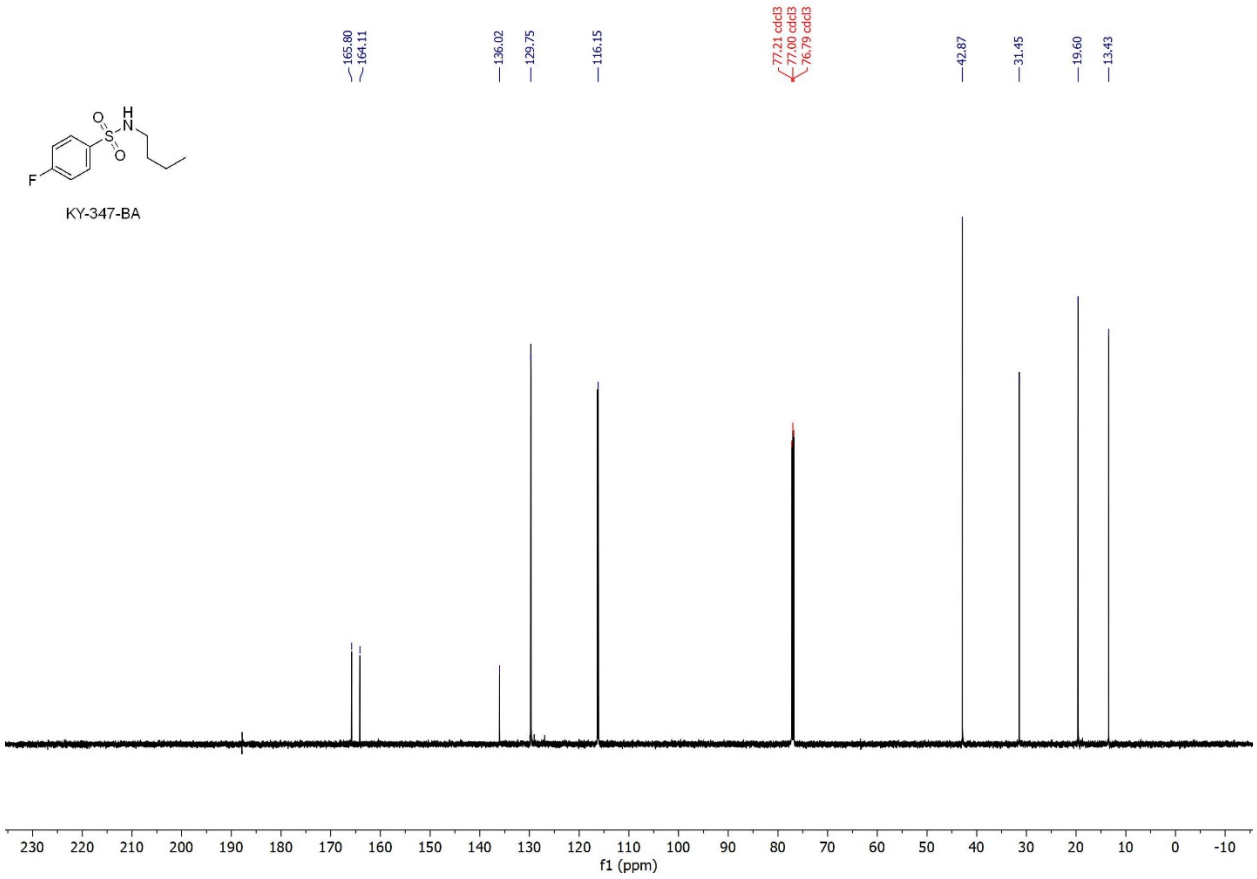

167.43
166.13
161.38
145.27
131.81
131.76
128.48
127.86
117.27
117.16
JWB 210
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

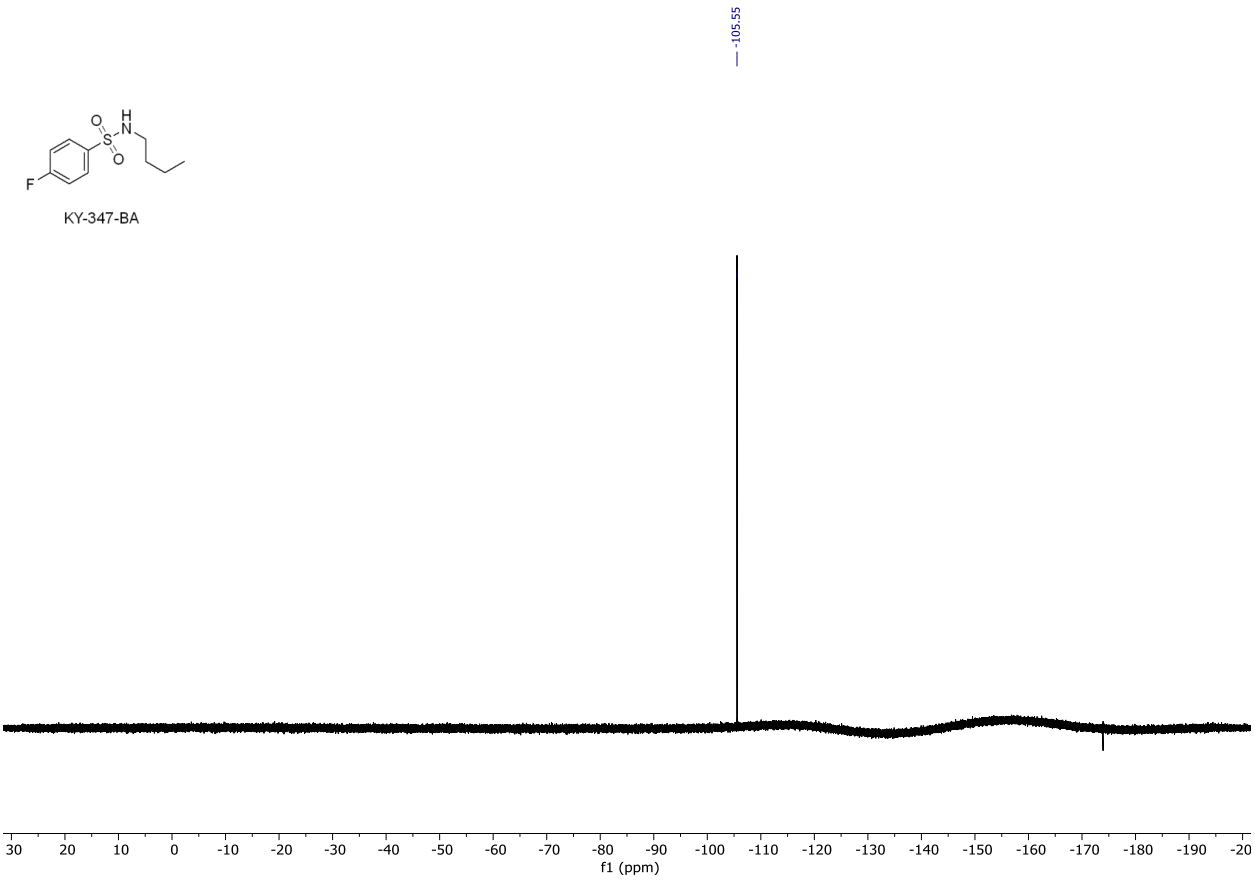

-100.35
JWB 210
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -20
f1 (ppm)

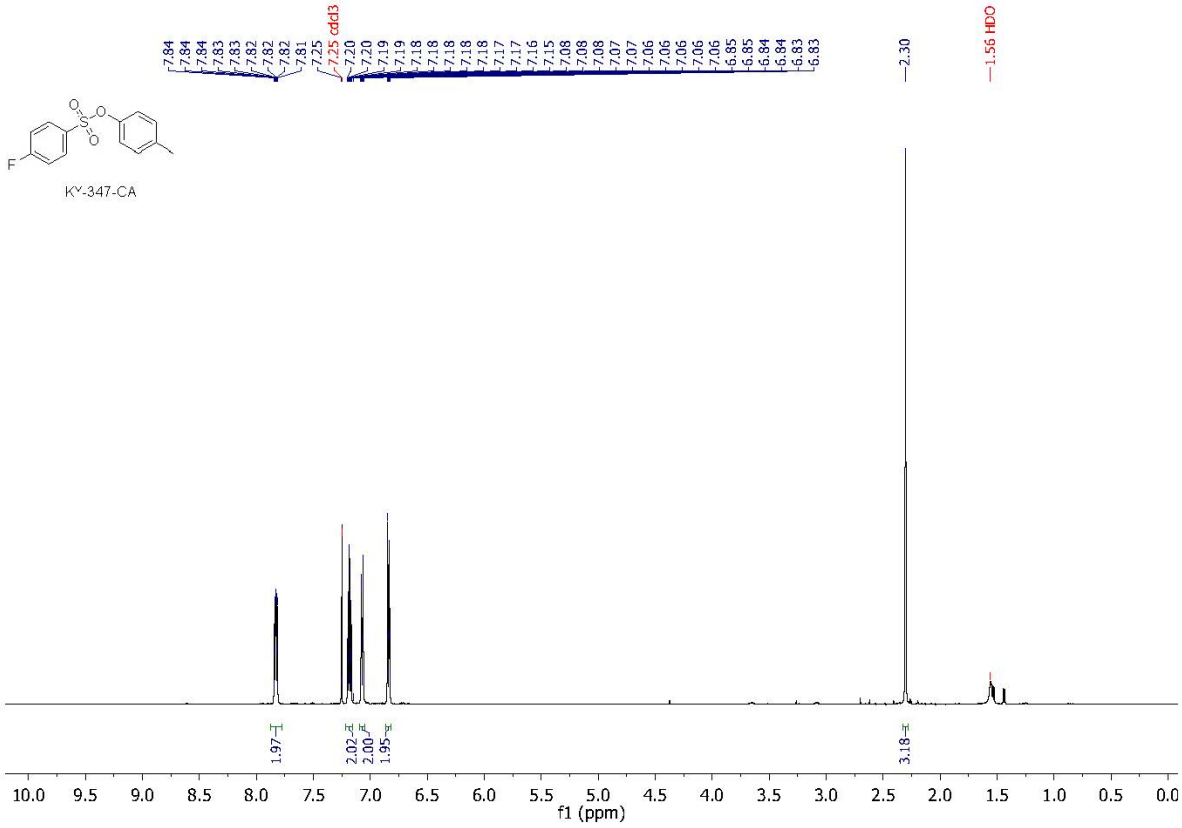

9.06
8.11
8.10
7.72
7.72
7.72
7.72
7.62
7.62
7.23
7.23
7.22
7.22
7.21
7.21
7.16
7.16
7.16
7.15
3.94
2.05 (CD3)2C(
JWB211
0.7
1.7
0.8
0.8
1.8
0.9
3.0
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

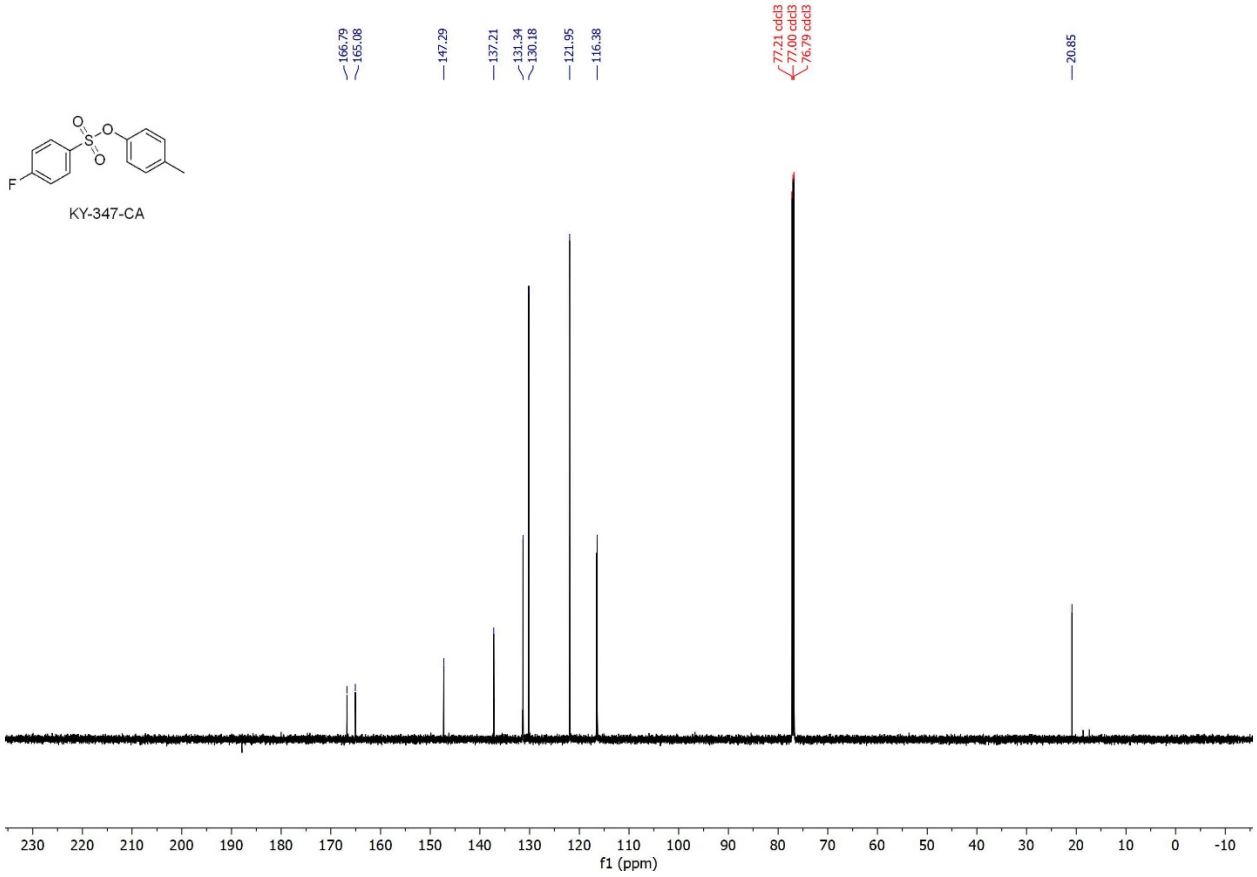

166.3
161.5
147.2
132.8
132.0
129.4
128.9
128.8
127.7
116.1
56.55
JWB211
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

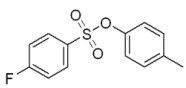

7.89 7.88 7.88 7.87 7.87 7.87 7.86 7.86 7.85 7.25 cdd3 7.20 7.20 7.19 7.19 7.19 7.18 7.18 7.18 7.17 7.17 7.16 7.16
4.35 2.97 2.96 2.95 2.94 2.94 2.93 1.55 HDO 1.46 1.45 1.45 1.45 1.44 1.44 1.44 1.43 1.43 1.43 1.42 1.41 1.32 1.30 1.30 1.29 1.29 1.29 1.29 1.28 1.28 1.28 1.27 1.27 1.27 1.25 0.86 0.85 0.84
KY-347-BA
2.00 2.02 0.95 2.17 2.22 2.23 3.23
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


165.80
164.11
136.02
129.75
116.15
77.21 cdcl3
77.00 cdcl3
76.79 cdcl3
42.87
31.45
19.60
13.43
KY-347-BA
f1 (ppm)


-105.55
KY-347-BA
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -20
f1 (ppm)

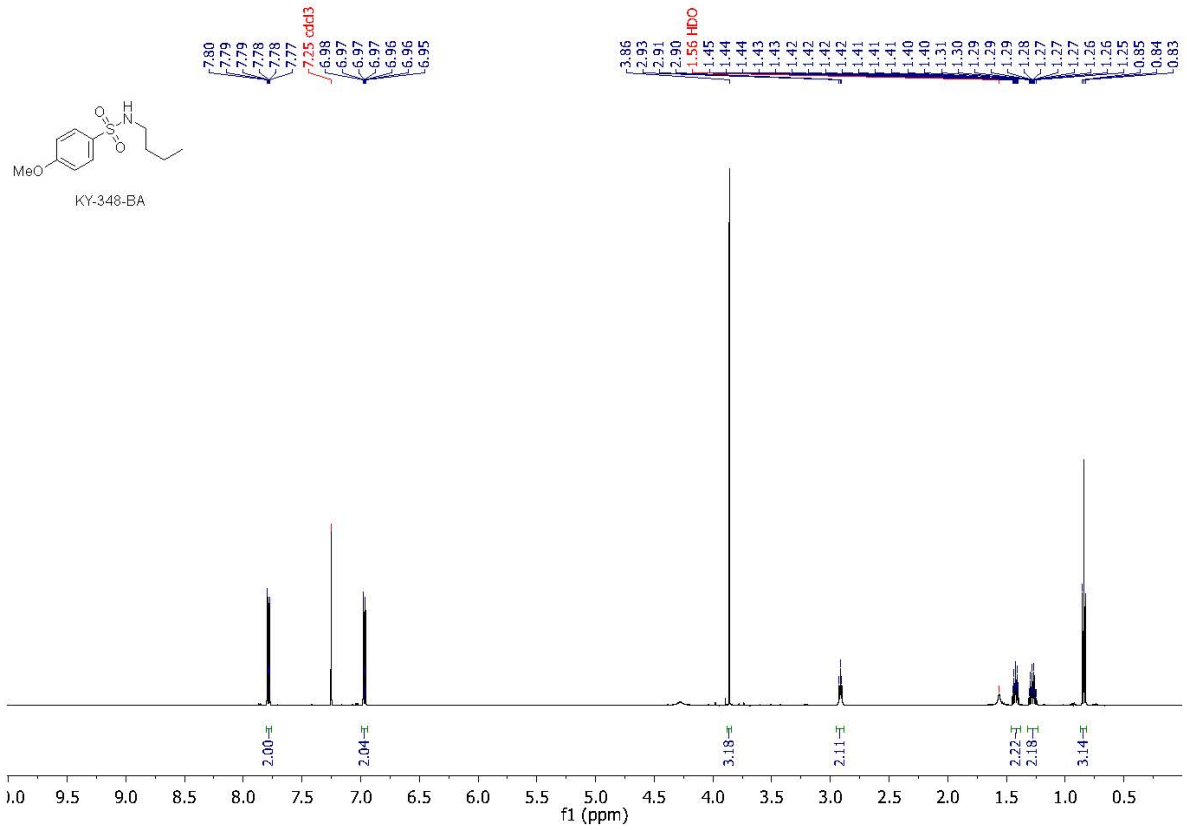

7.84
7.84
7.84
7.83
7.82
7.82
7.82
7.81
7.25
7.25 cdd3
7.20
7.20
7.19
7.19
7.18
7.18
7.18
7.18
7.17
7.17
7.16
7.15
7.08
7.08
7.08
7.07
7.07
7.06
7.06
7.06
7.06
7.06
6.85
6.85
6.84
6.84
6.83
6.83
2.30
1.56 HDO
F
KV-347-CA
1.97
2.02
2.00
1.95
3.18
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

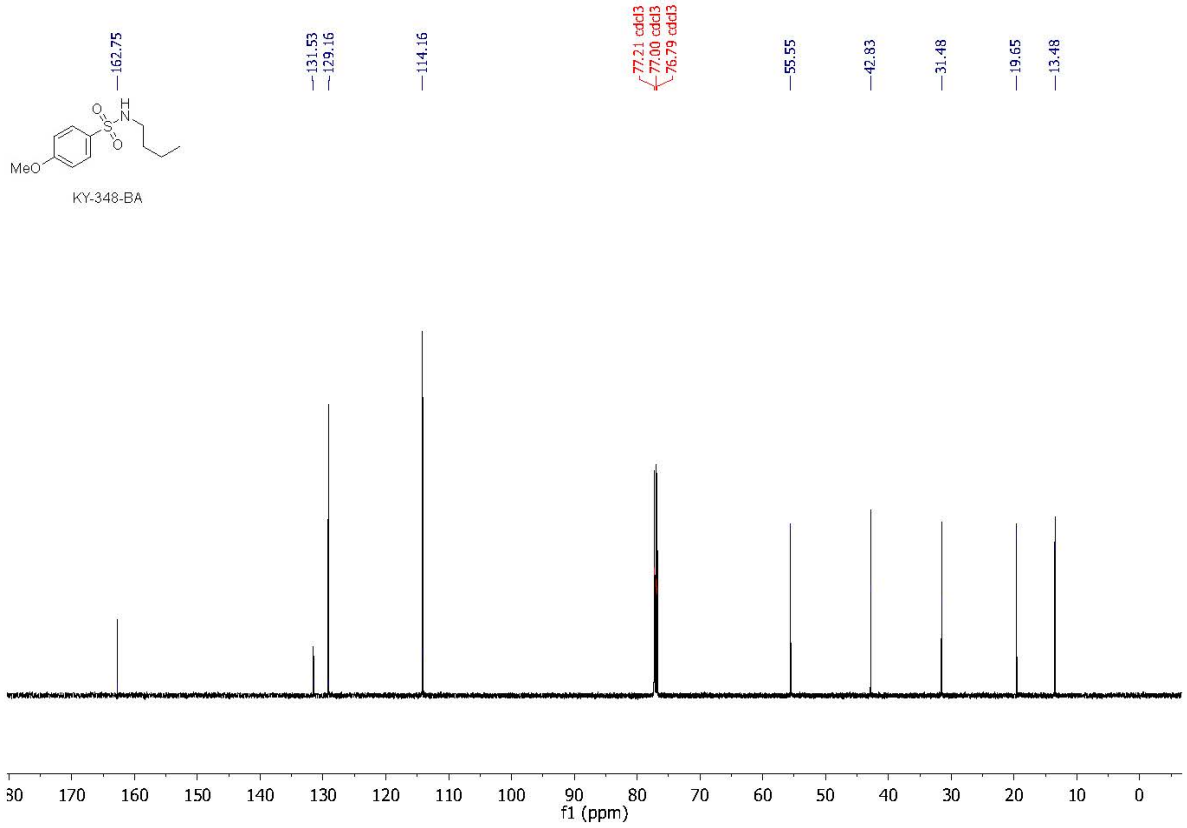

166.79
165.08
147.29
137.21
131.34
130.18
121.95
116.38
77.21 cdcl3
77.00 cdcl3
76.79 cdcl3
20.85
KY-347-CA
F
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

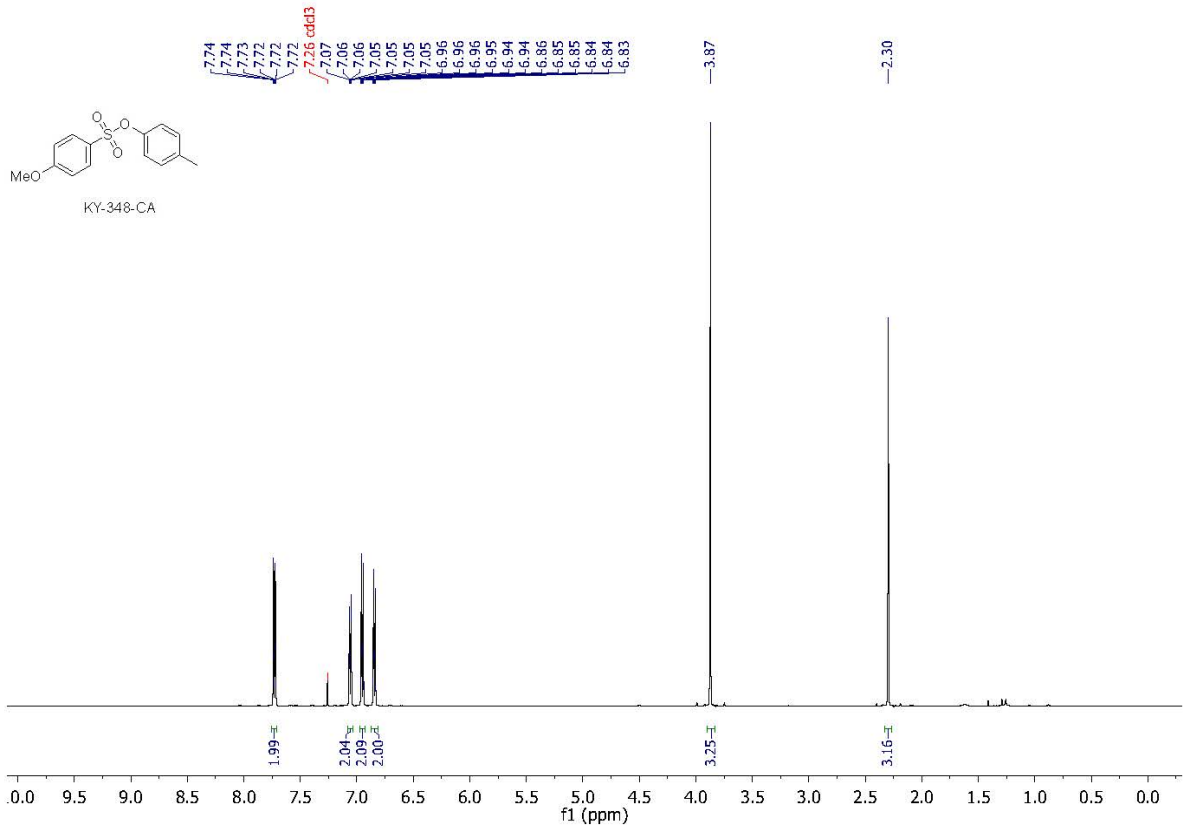

O
S
O
O
F

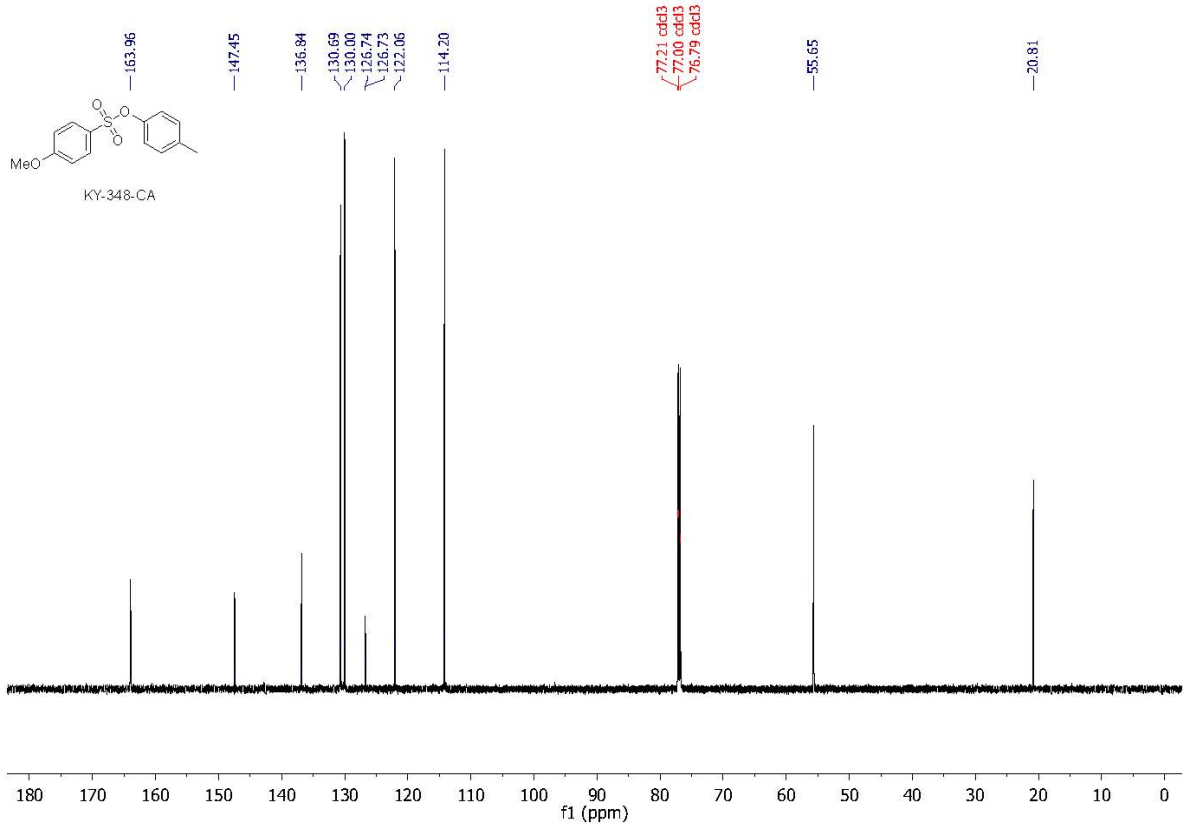
KY-347-CA

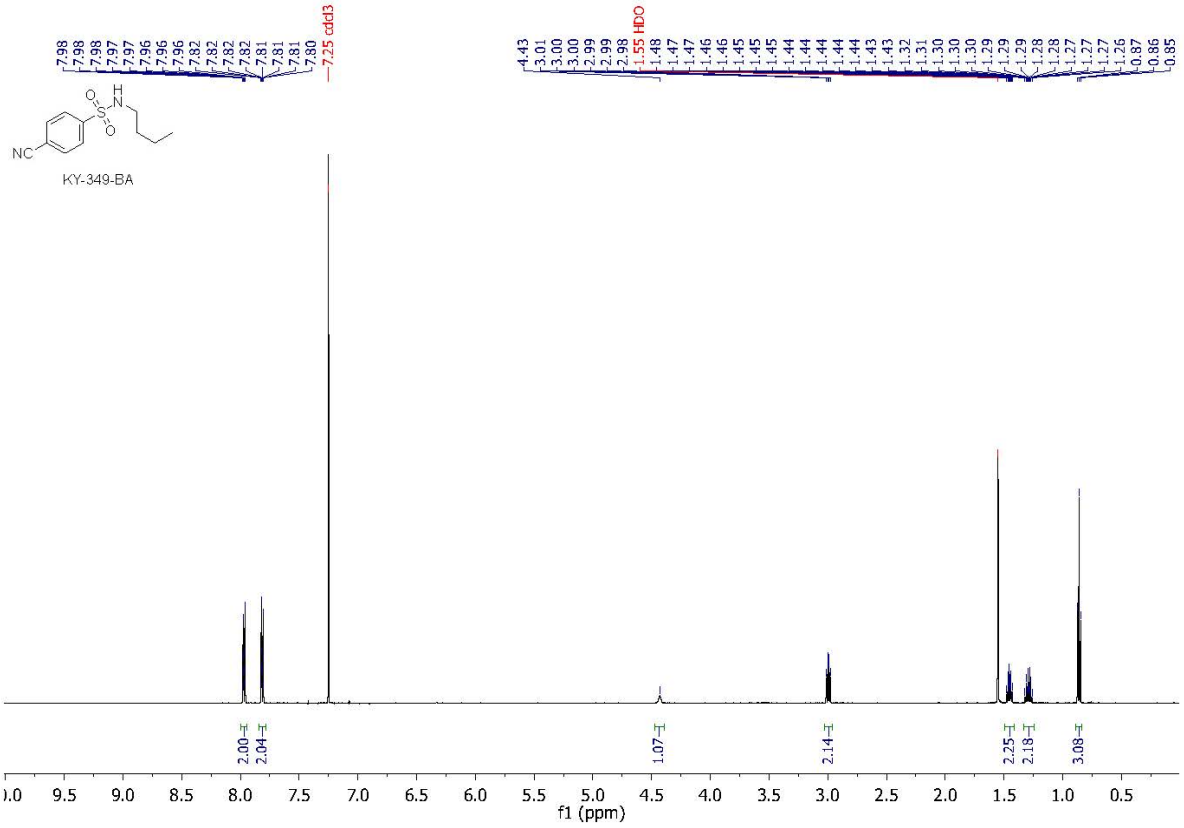
-102.39
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-20
f1 (ppm)

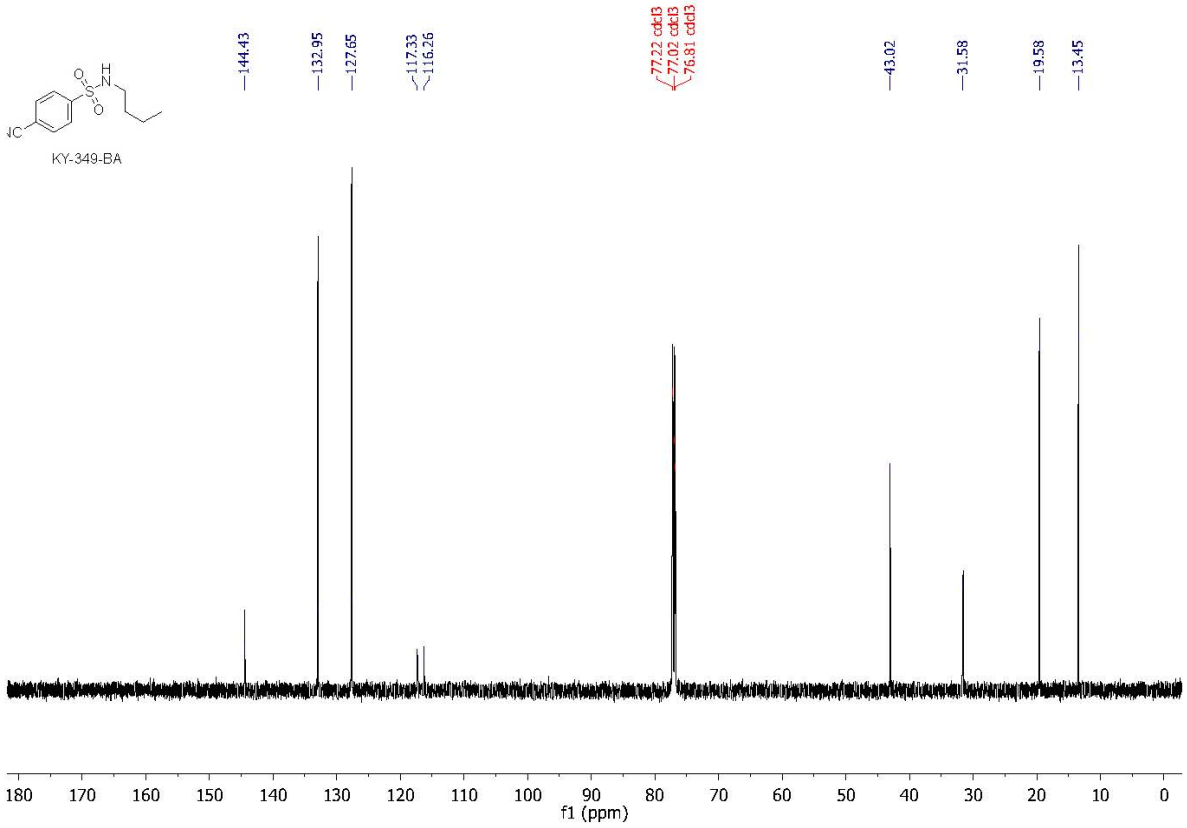

7.80
7.79
7.79
7.78
7.78
7.77
7.25 cdcl3
6.98
6.97
6.97
6.97
6.96
6.96
6.95
3.86
2.93
2.91
2.90
1.56 HDO
1.45
1.44
1.44
1.43
1.43
1.42
1.42
1.42
1.42
1.41
1.41
1.41
1.40
1.40
1.31
1.30
1.29
1.29
1.29
1.28
1.27
1.27
1.27
1.26
1.26
1.25
0.85
0.84
0.83
MeO
KY-348-BA
2.00
2.04
3.18
2.11
2.22
2.18
3.14
f1 (ppm)

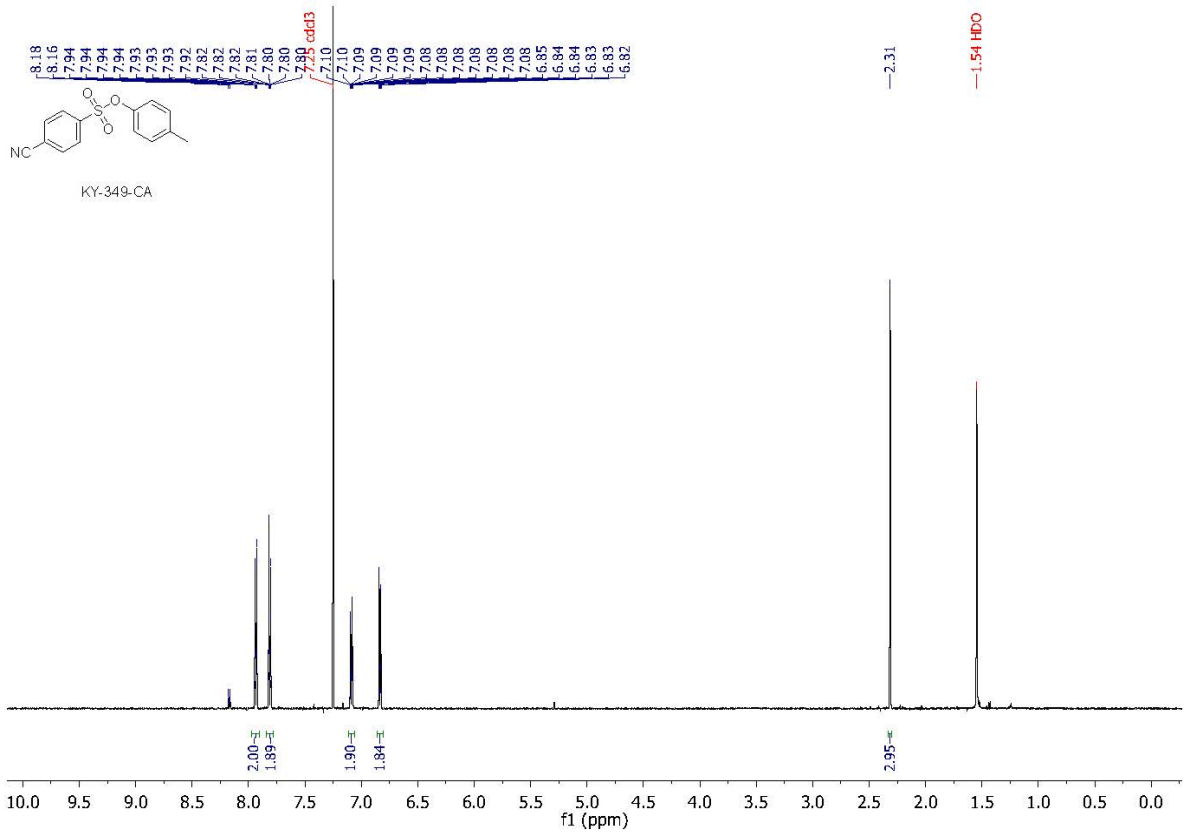

162.75
131.53
129.16
114.16
77.21 cdd3
77.00 cdd3
76.79 cdd3
55.55
42.83
31.48
19.65
13.48
MeO
KY-348-BA
f1 (ppm)

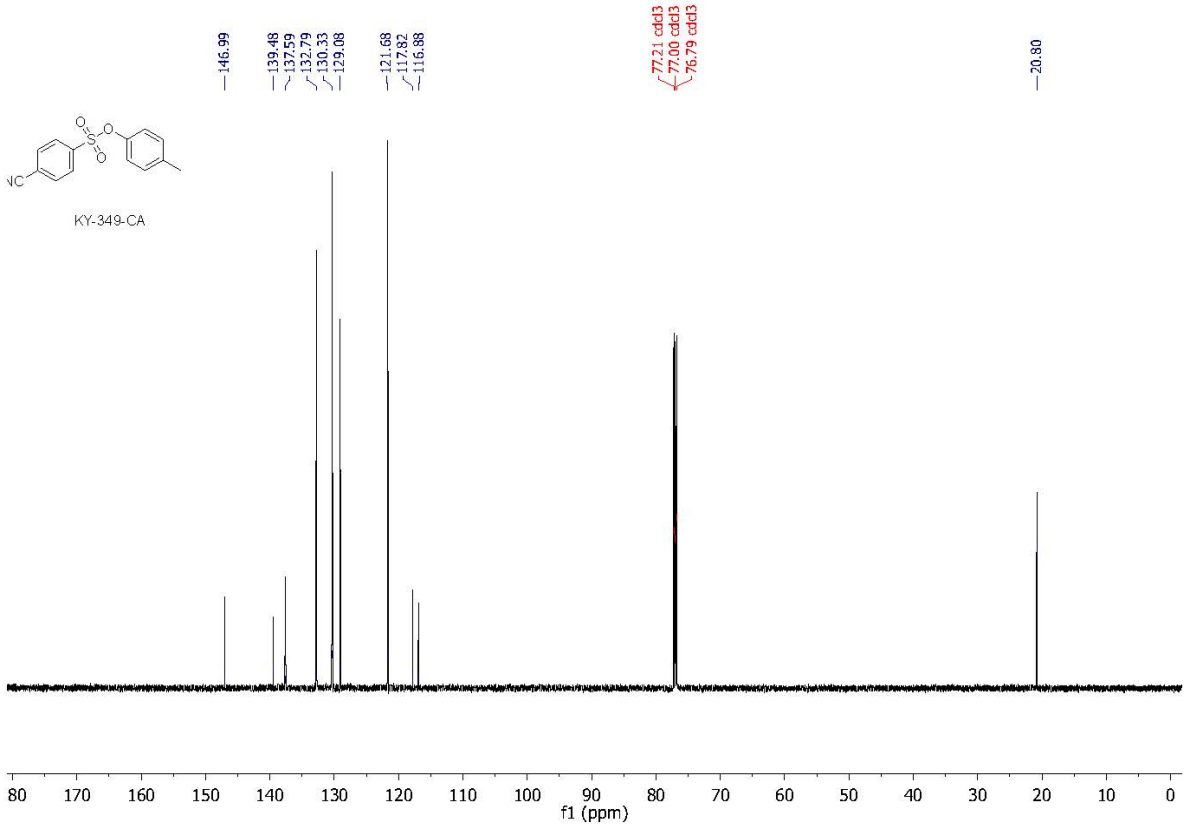

7.74
7.74
7.73
7.72
7.72
7.72
7.26 cdd3
7.07
7.06
7.06
7.05
7.05
7.05
7.05
6.96
6.96
6.96
6.95
6.94
6.94
6.86
6.85
6.85
6.84
6.84
6.83
3.87
2.30
MeO
KY-348-CA
1.99
2.04
2.09
2.00
3.25
3.16
f1 (ppm)

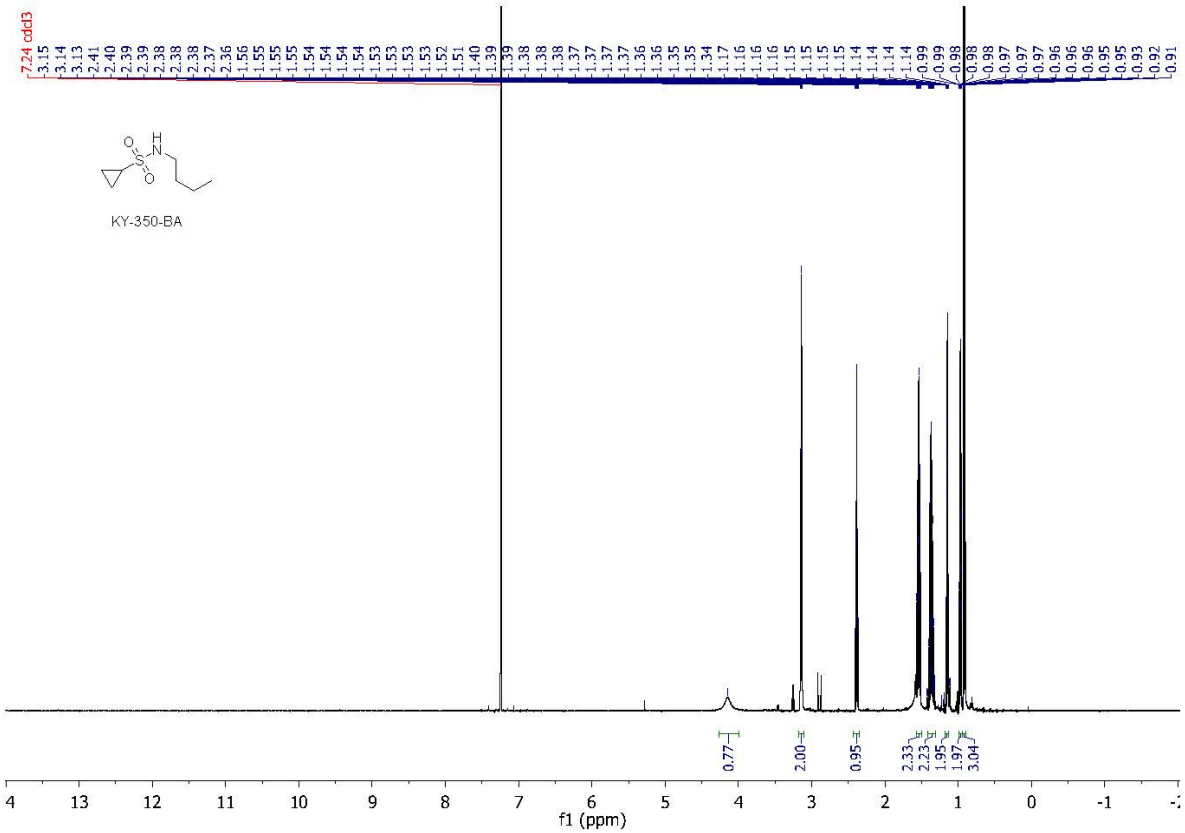

163.96
147.45
136.84
130.69
130.00
126.74
126.73
122.06
114.20
77.21 cdd3
77.00 cdd3
76.79 cdd3
55.65
20.81
MeO
KY-348-CA
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

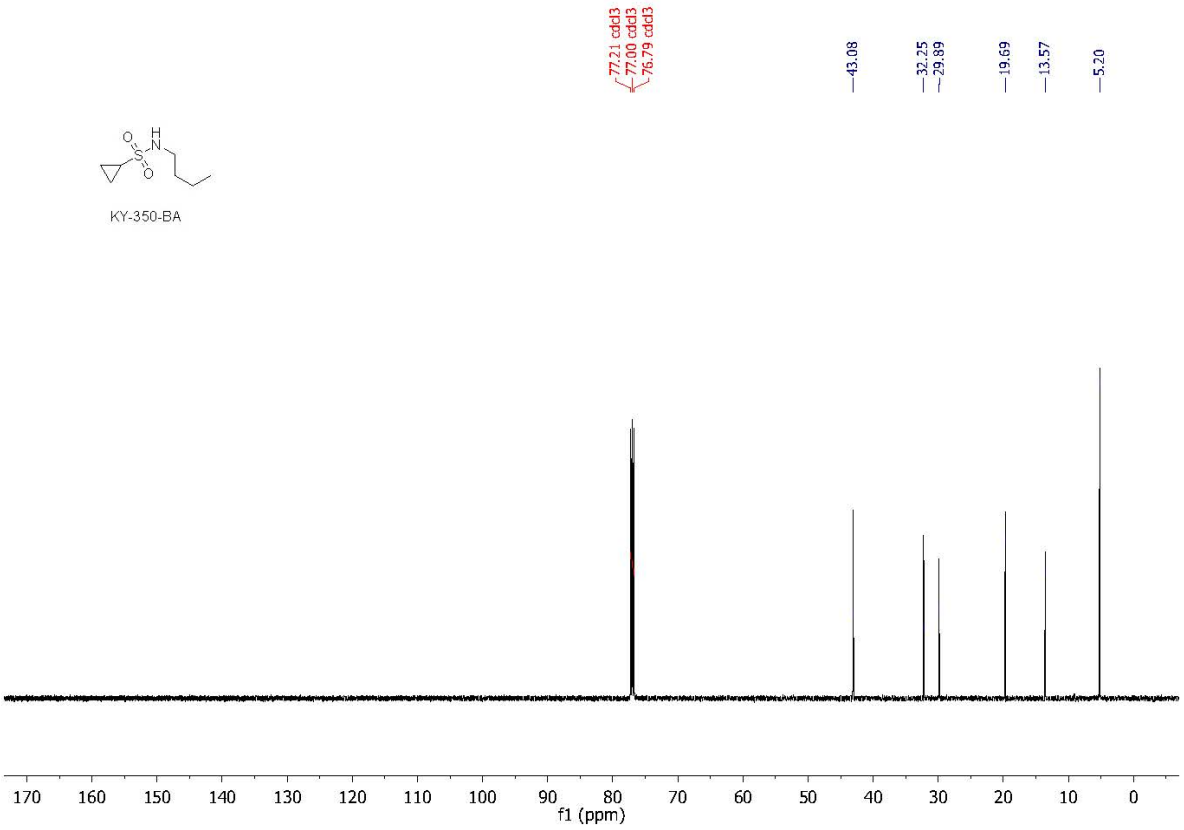

KY-349-BA
f1 (ppm)

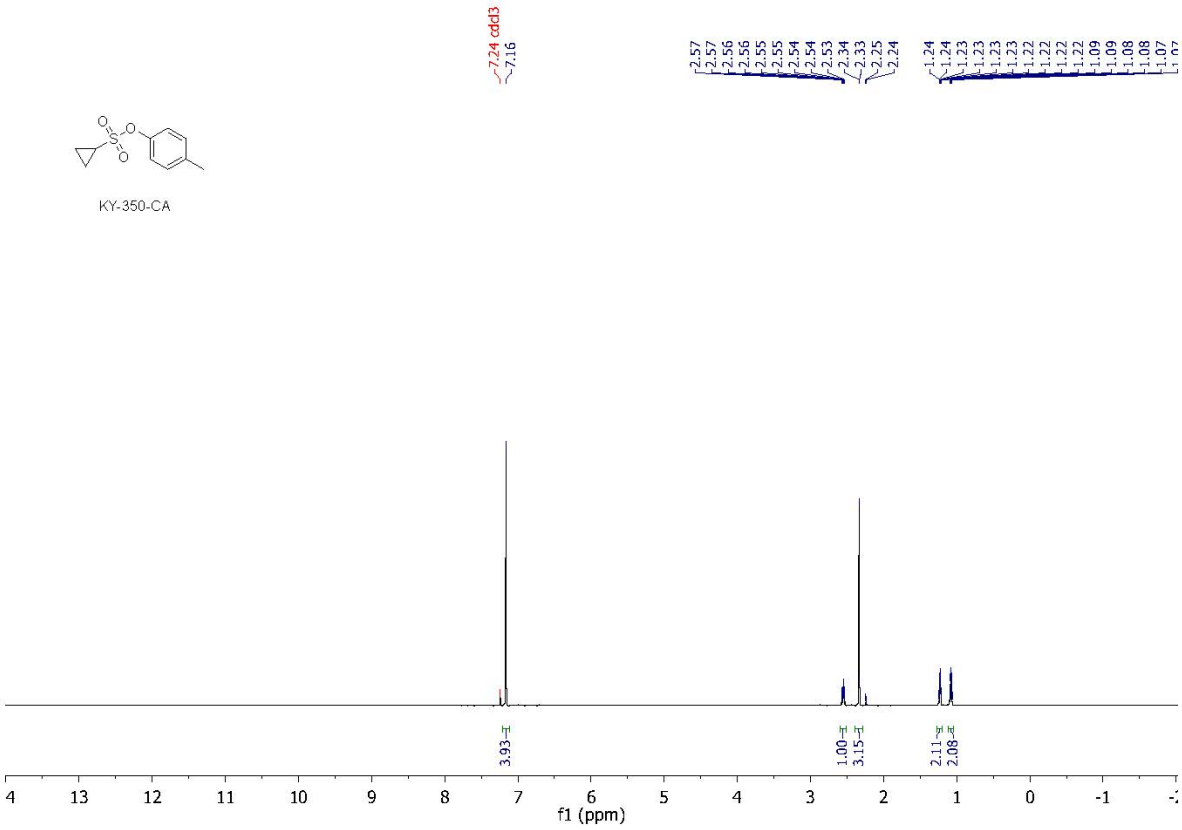

KY-349-BA
144.43
132.95
127.65
117.33
116.26
77.22 cdcl3
77.02 cdcl3
76.81 cdcl3
43.02
31.58
19.58
13.45
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

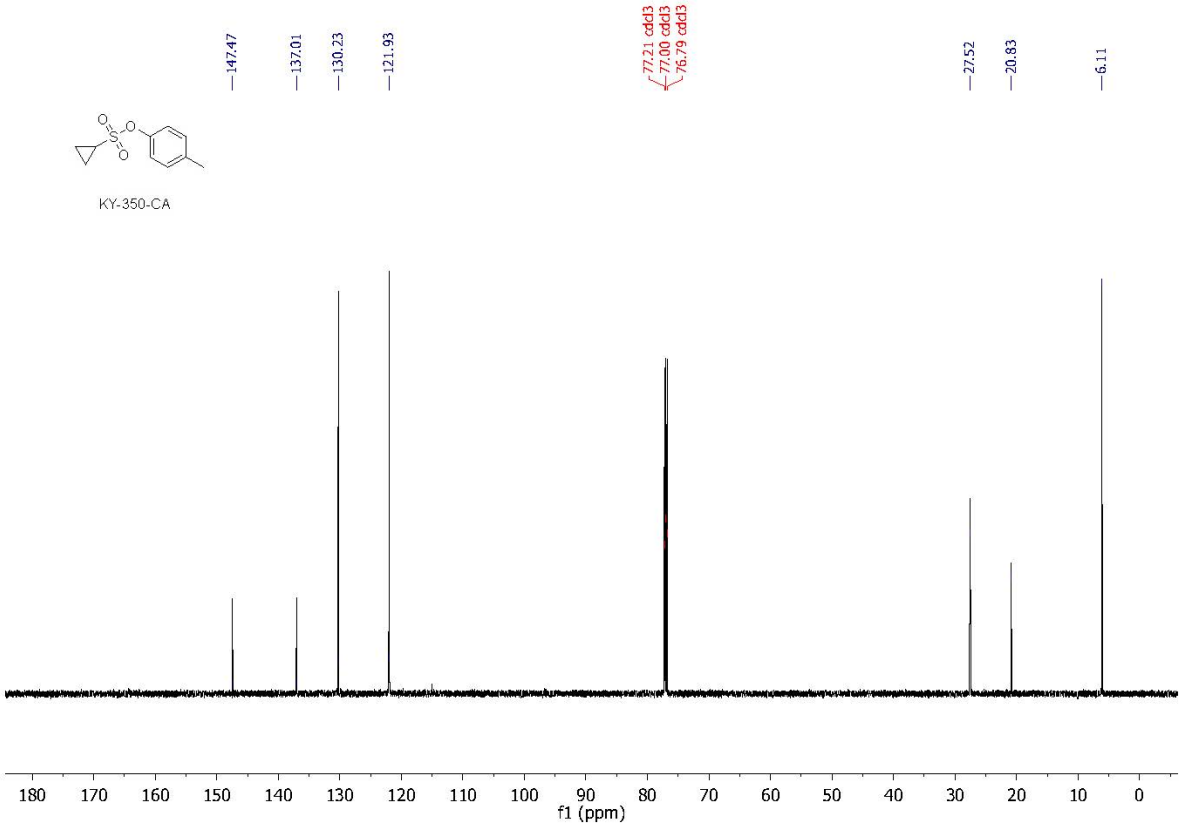

KY-349-CA
cdd3
HDO
2.31
1.54
2.00
1.89
1.90
1.84
2.95
f1 (ppm)

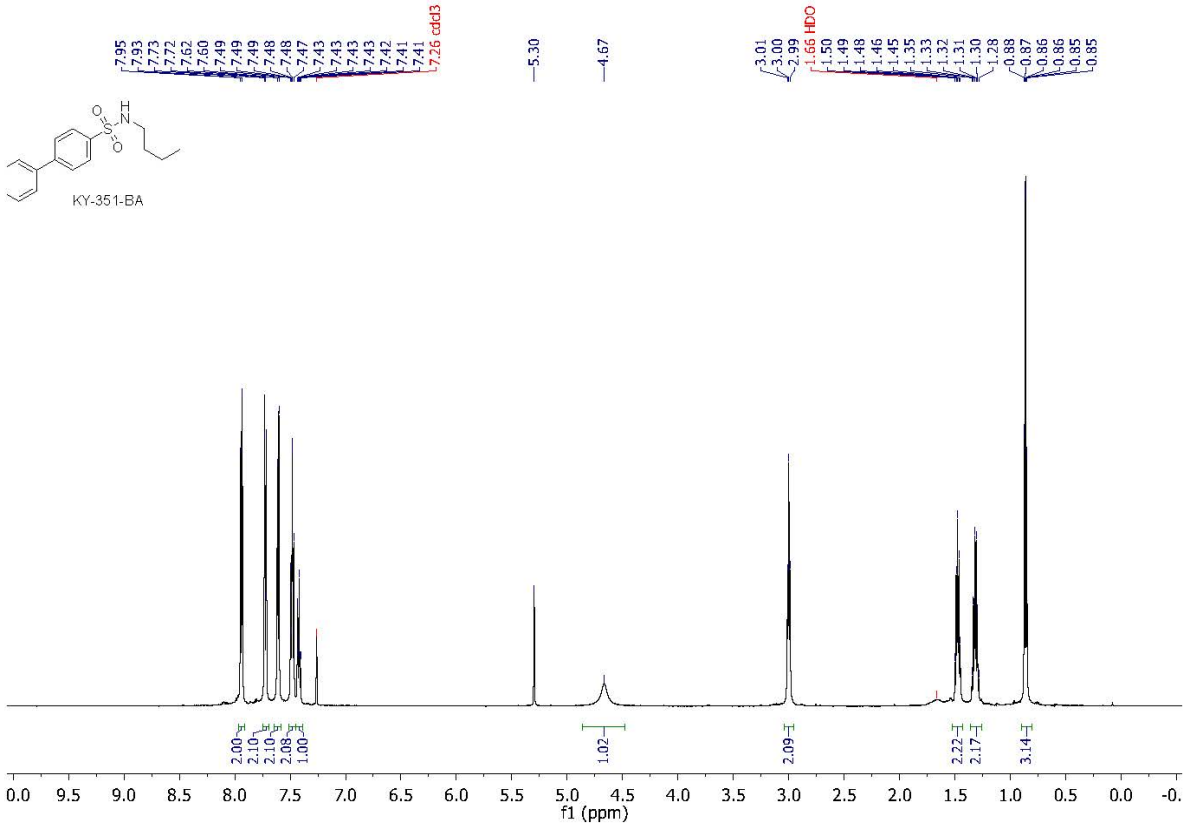

146.99
139.48
137.59
132.79
130.33
129.08
121.68
117.82
116.88
77.21 cdd3
77.00 cdd3
76.79 cdd3
20.80
NC
KY-349-CA
80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

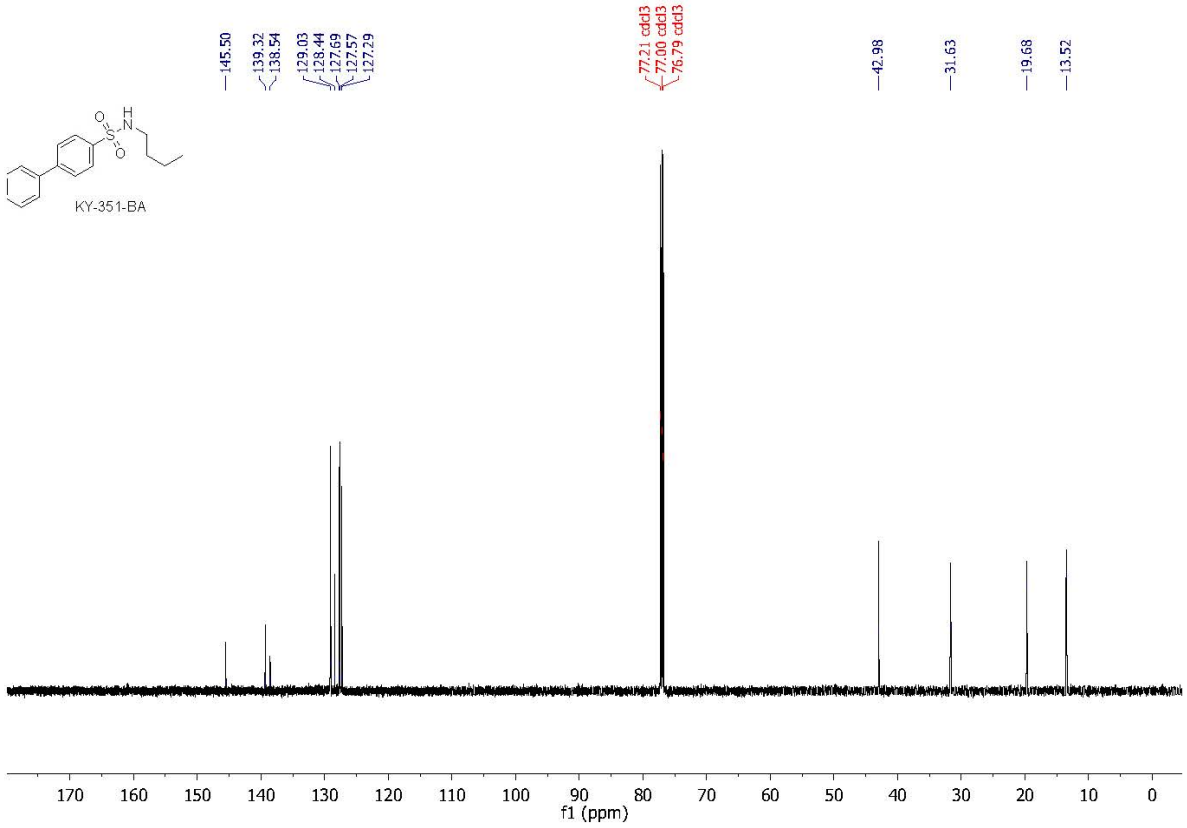

KY-350-BA
f1 (ppm)

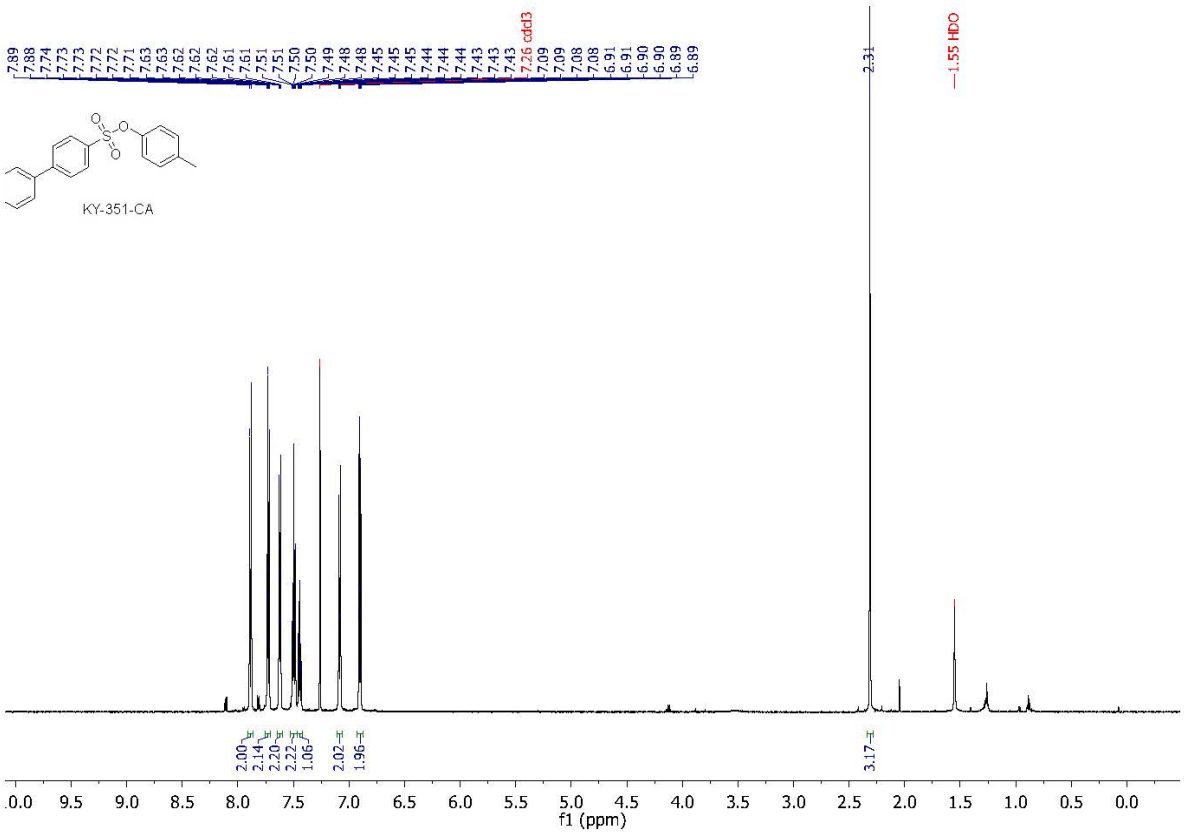

77.21 cdd3
77.00 cdd3
76.79 cdd3
43.08
32.25
29.89
19.69
13.57
5.20
KY-350-BA
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

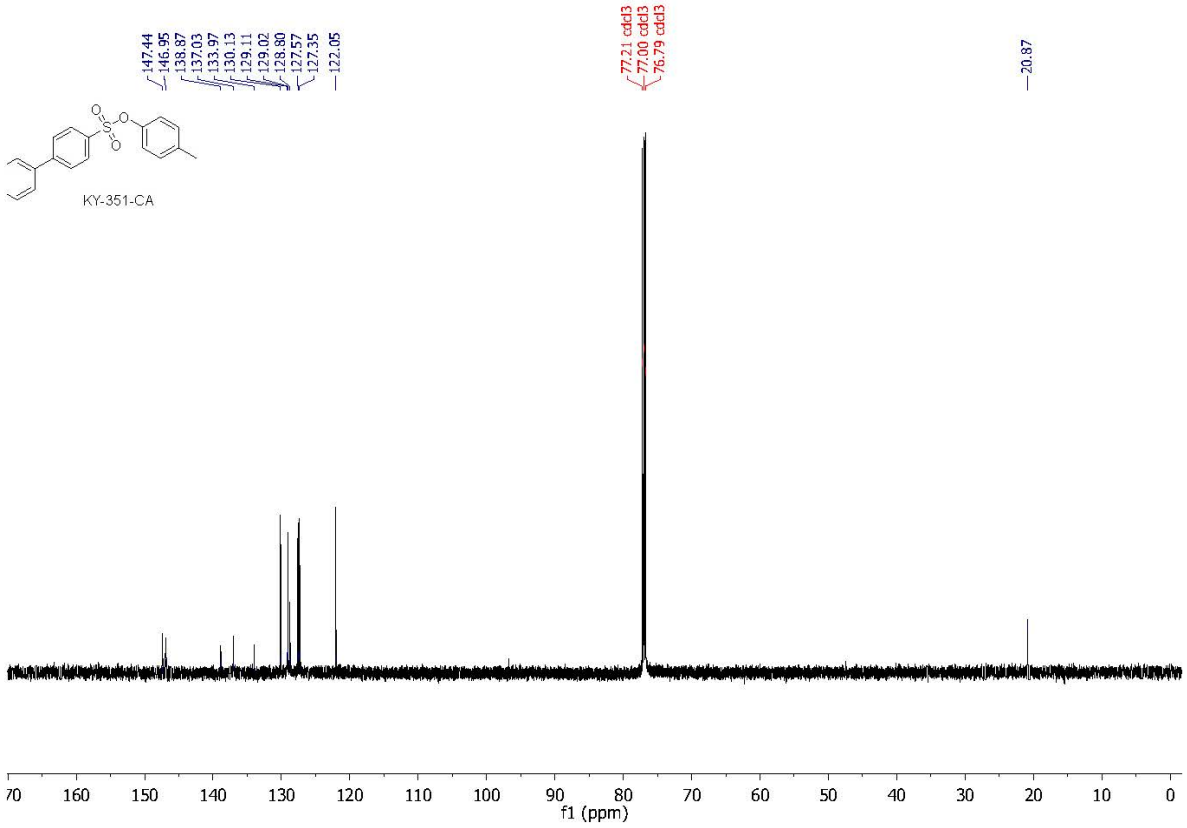

7.24 cdcl3
7.16
2.57
2.57
2.56
2.56
2.55
2.55
2.54
2.54
2.53
2.34
2.33
2.25
2.24
1.24
1.24
1.23
1.23
1.23
1.23
1.22
1.22
1.22
1.22
1.09
1.09
1.08
1.08
1.07
KY-350-CA
3.93
1.00
3.15
2.11
2.08
4
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

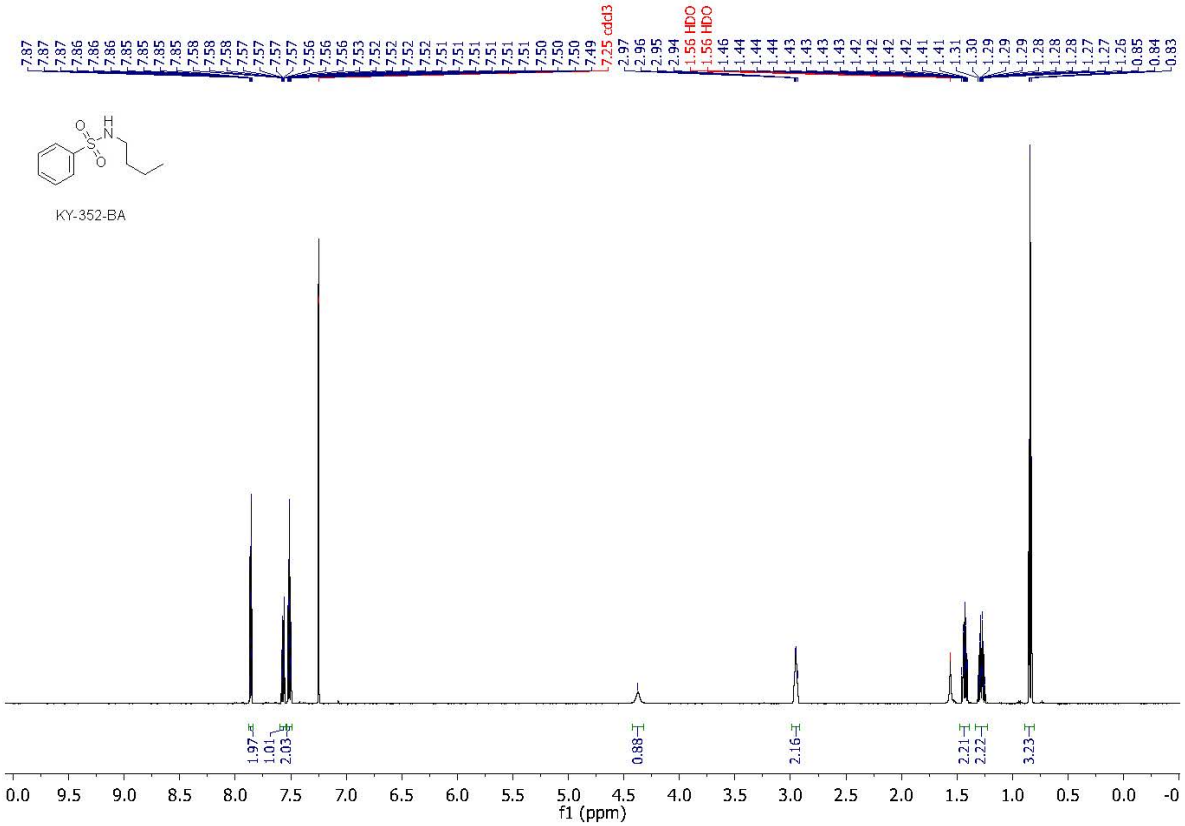

147.47
137.01
130.23
121.93
77.21 cdd3
77.00 cdd3
76.79 cdd3
27.52
20.83
6.11
KY-350-CA
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

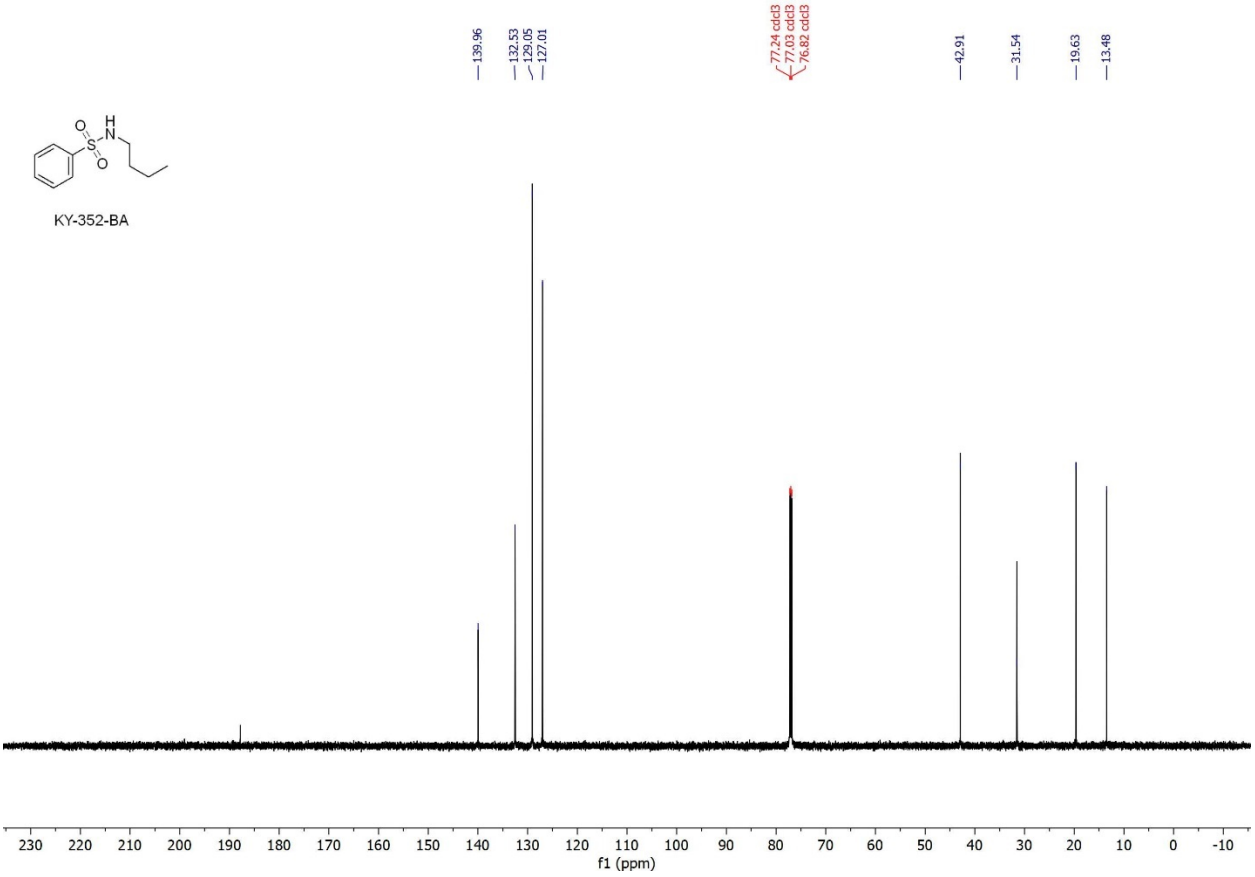

7.95
7.93
7.73
7.72
7.62
7.60
7.49
7.49
7.49
7.48
7.48
7.47
7.43
7.43
7.43
7.43
7.42
7.41
7.41
7.26 cdd3
5.30
4.67
3.01
3.00
2.99
1.66 HDO
1.50
1.49
1.48
1.46
1.45
1.35
1.33
1.32
1.31
1.30
1.28
0.88
0.87
0.86
0.86
0.85
0.85
KY-351-BA
2.00
2.10
2.10
2.08
1.00
1.02
2.09
2.22
2.17
3.14
f1 (ppm)

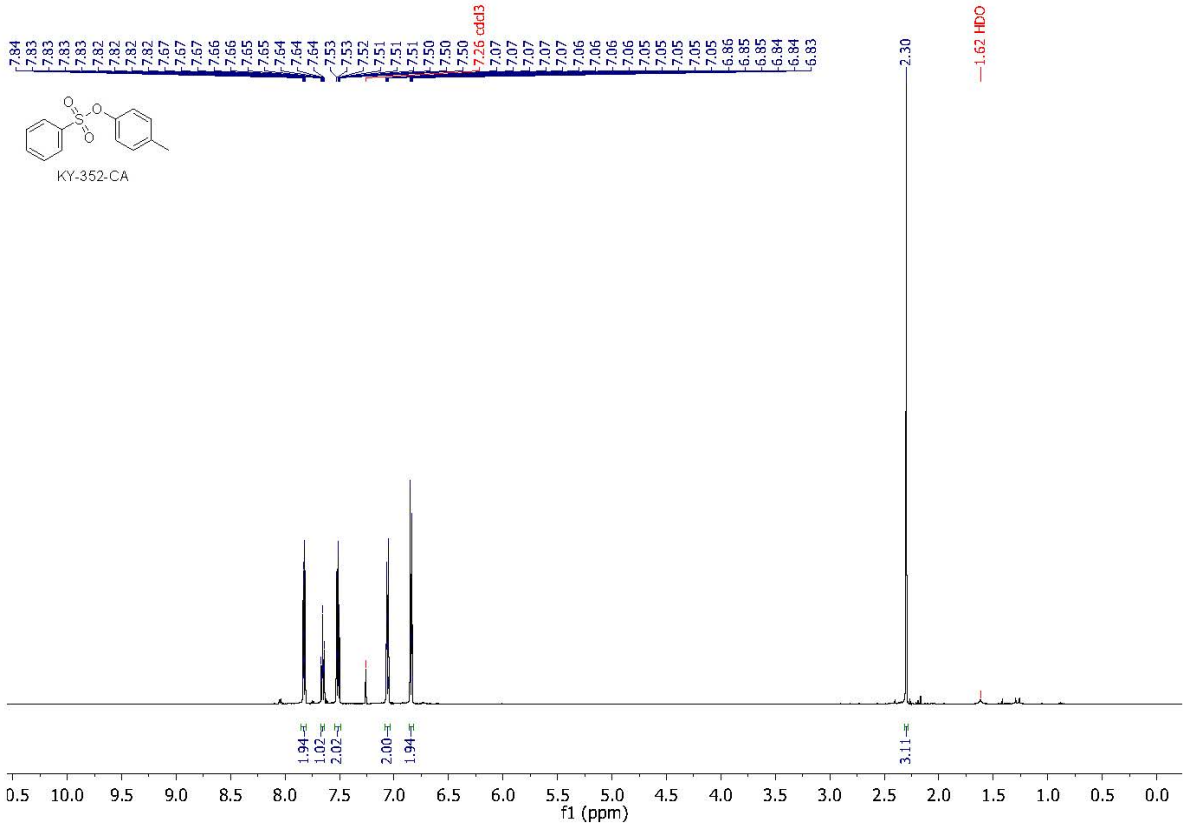

145.50
139.32
138.54
129.03
128.44
127.69
127.57
127.29
77.21 cdd3
77.00 cdd3
76.79 cdd3
42.98
31.63
19.68
13.52
KY-351-BA
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

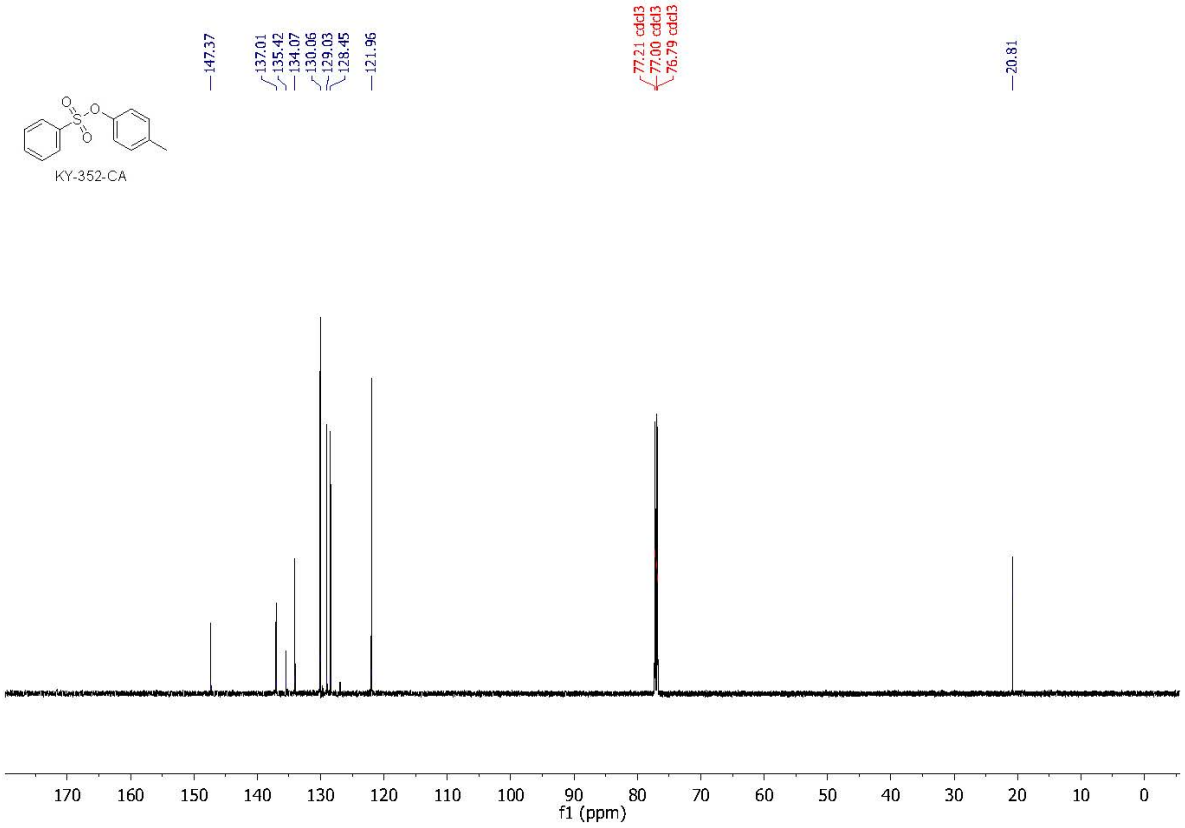

7.89
7.88
7.74
7.73
7.72
7.72
7.71
7.63
7.62
7.62
7.62
7.61
7.61
7.51
7.51
7.50
7.50
7.49
7.48
7.48
7.45
7.45
7.45
7.44
7.44
7.44
7.43
7.43
7.43
7.26 cdcl3
7.09
7.09
7.08
7.08
6.91
6.91
6.90
6.90
6.90
6.89
6.89
2.31
1.55 HDO
KY-351-CA
2.00
2.14
2.20
2.22
1.06
2.02
1.96
3.17
f1 (ppm)

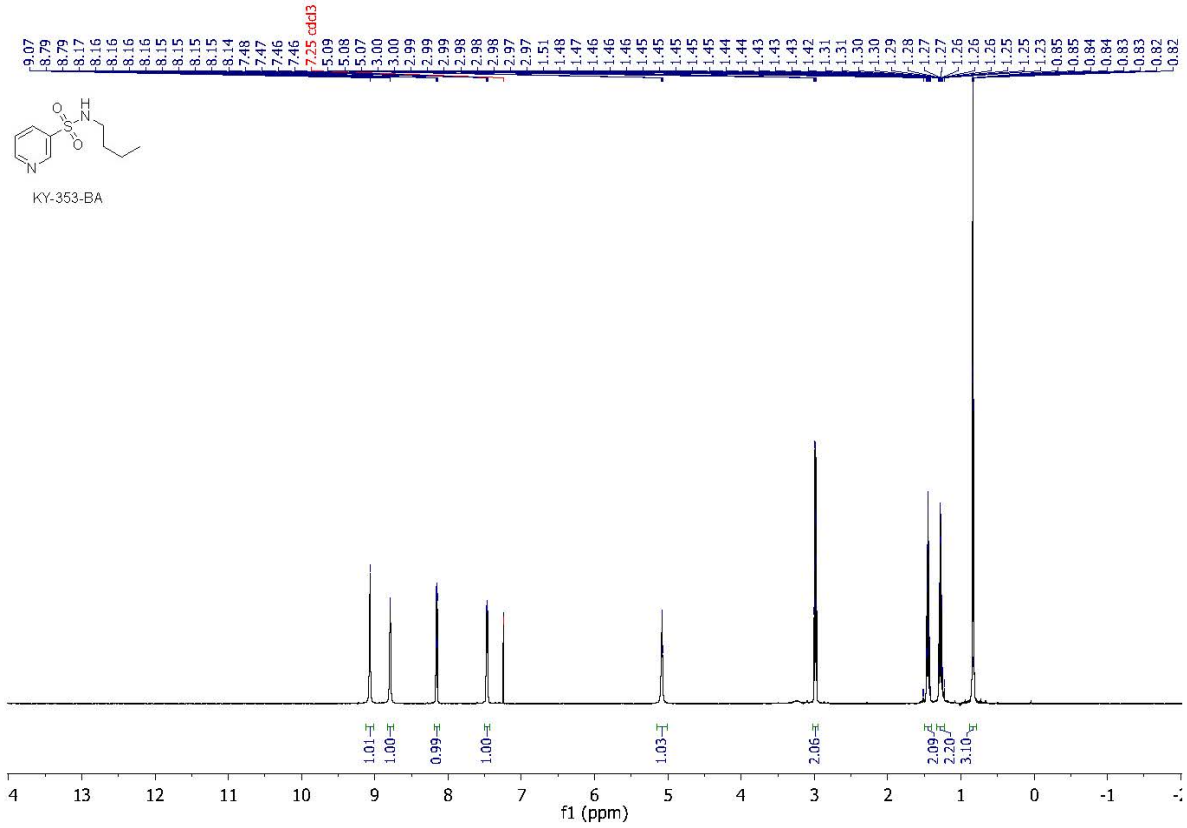

147.44
146.95
138.87
137.03
133.97
130.13
129.11
129.02
128.80
127.57
127.35
122.05
77.21 cdd3
77.00 cdd3
76.79 cdd3
20.87
KY-351-CA
70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

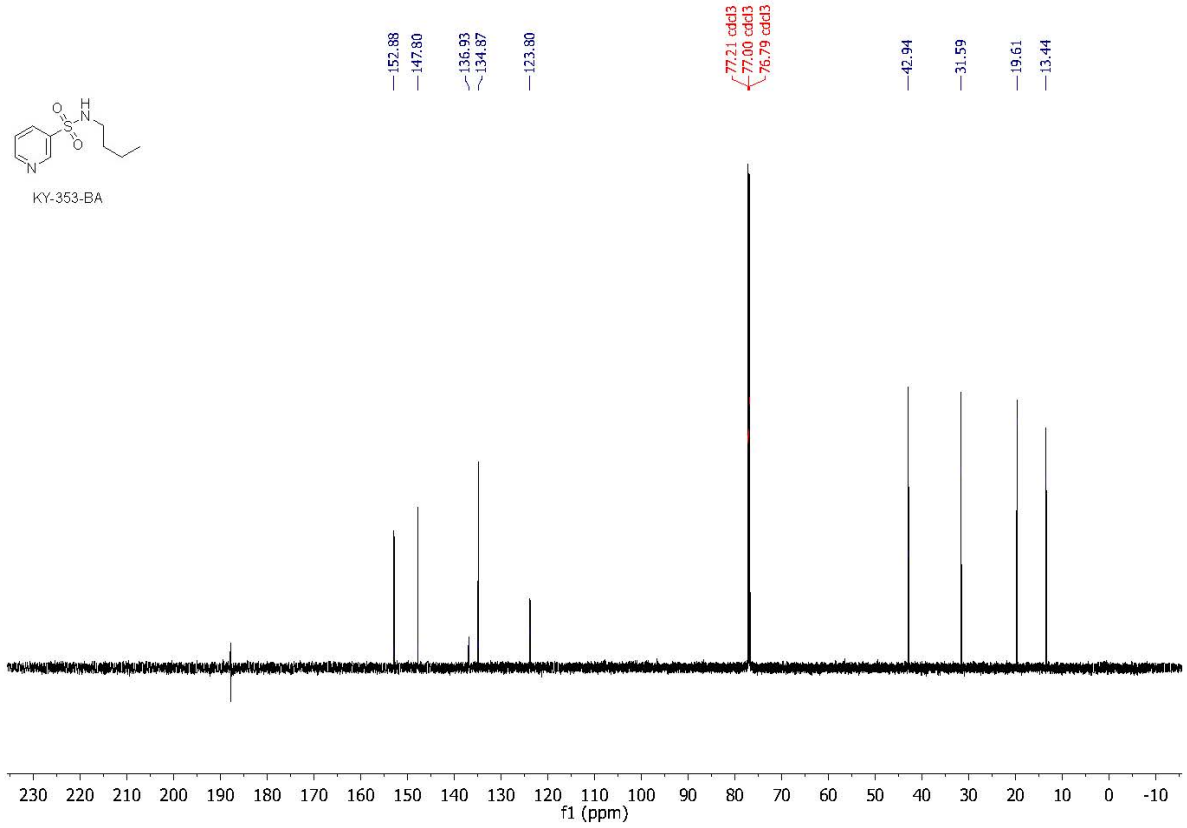

KY-352-BA
f1 (ppm)

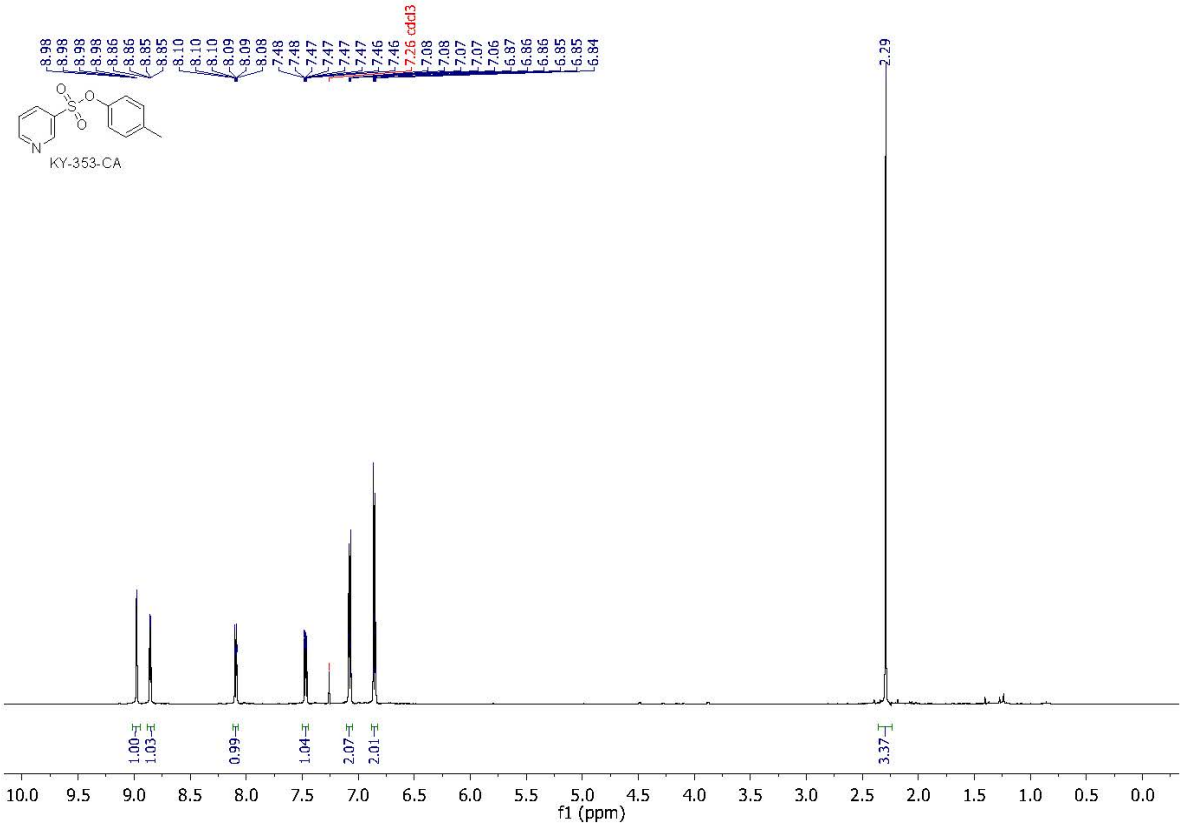

KY-352-BA
139.96
132.53
129.05
127.01
77.24 cdcl3
77.03 cdcl3
76.82 cdcl3
42.91
31.54
19.63
13.48
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

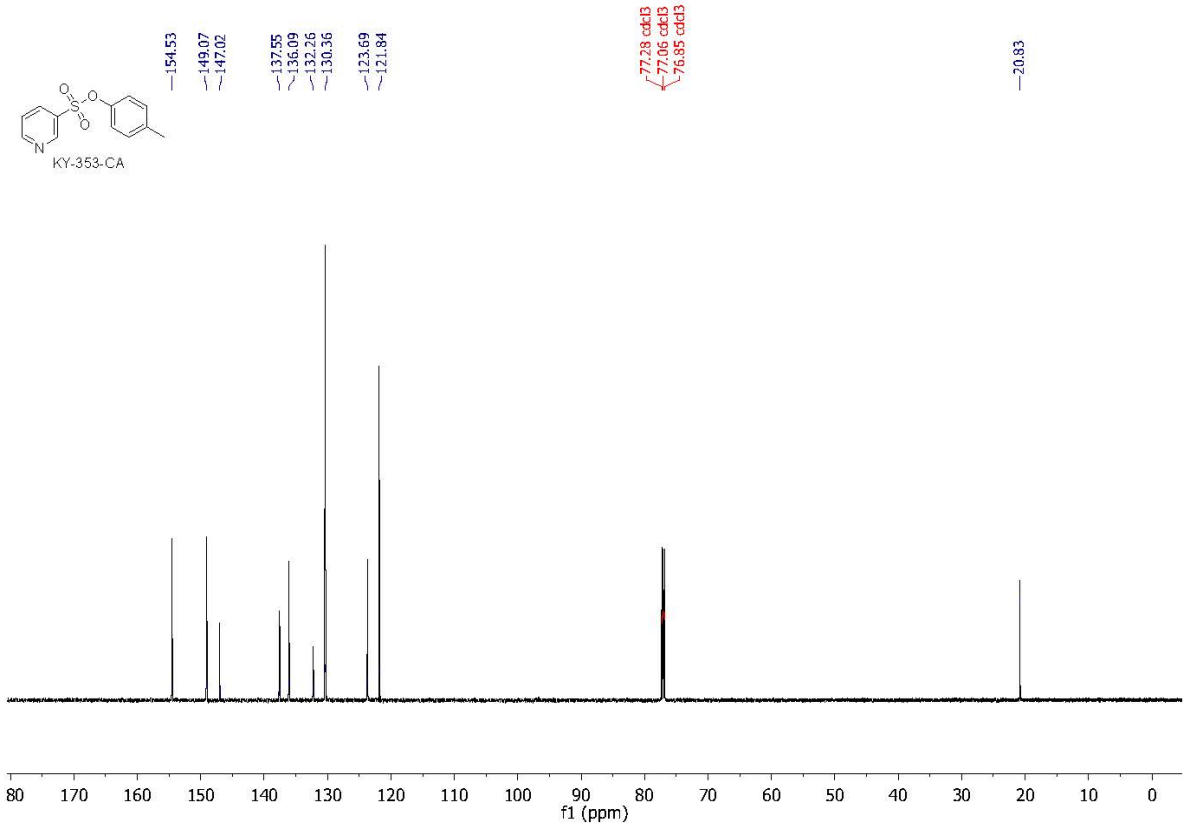

KY-352-CA
cdd3
HDO
1.94
1.02
2.02
2.00
1.94
3.11
f1 (ppm)

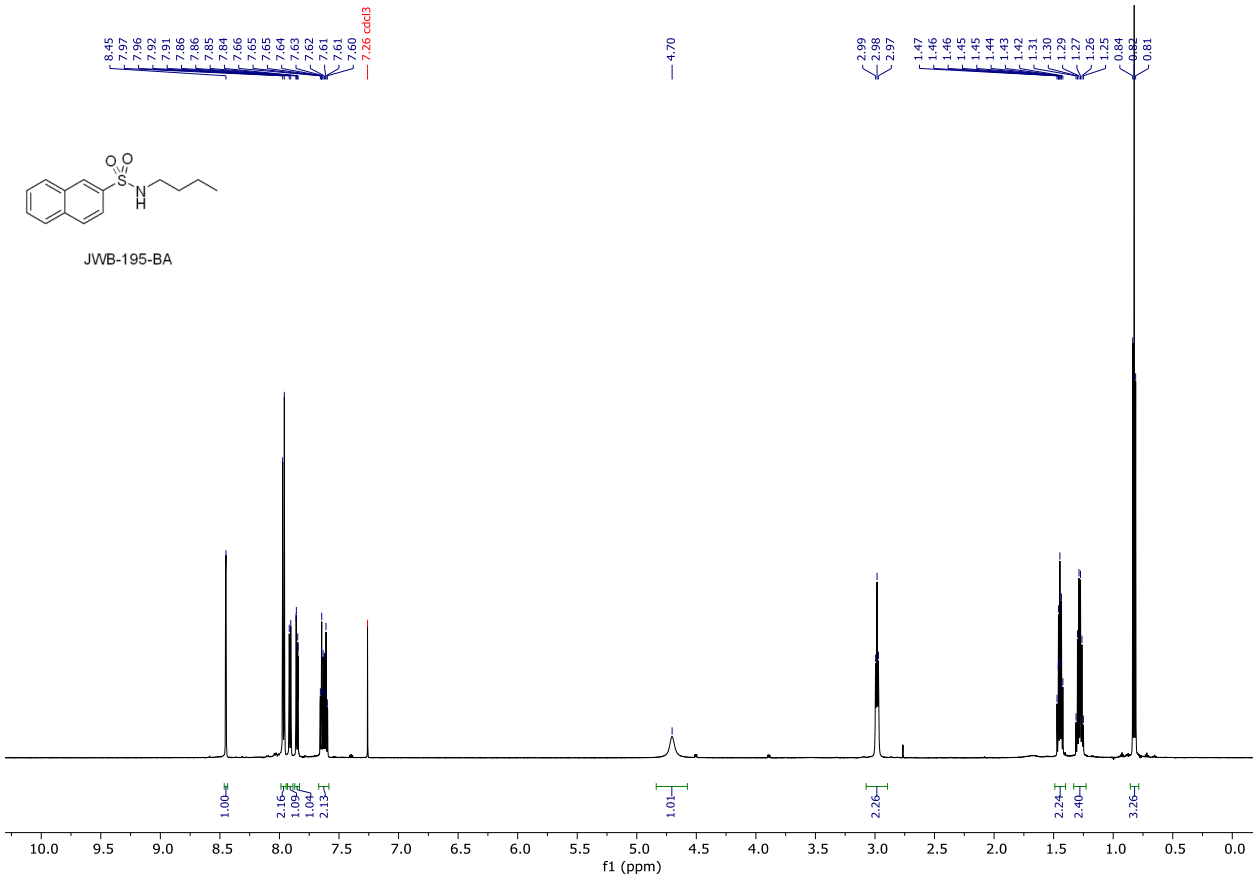

147.37
137.01
135.42
134.07
130.06
129.03
128.45
121.96
77.21 cdd3
77.00 cdd3
76.79 cdd3
20.81
KY-352-CA
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

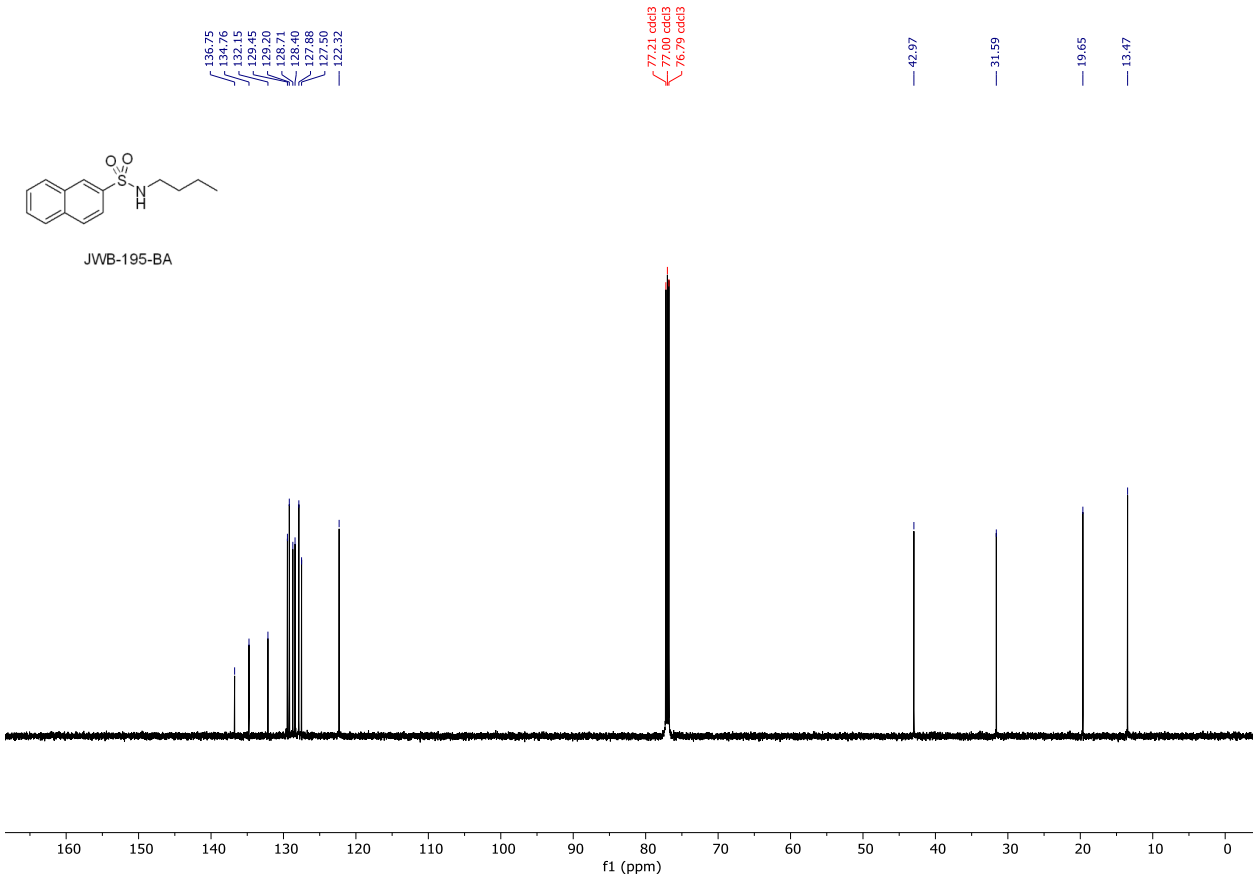

KY-353-BA
cdd3
1.01
1.00
0.99
1.00
1.03
2.06
2.09
2.20
3.10
f1 (ppm)

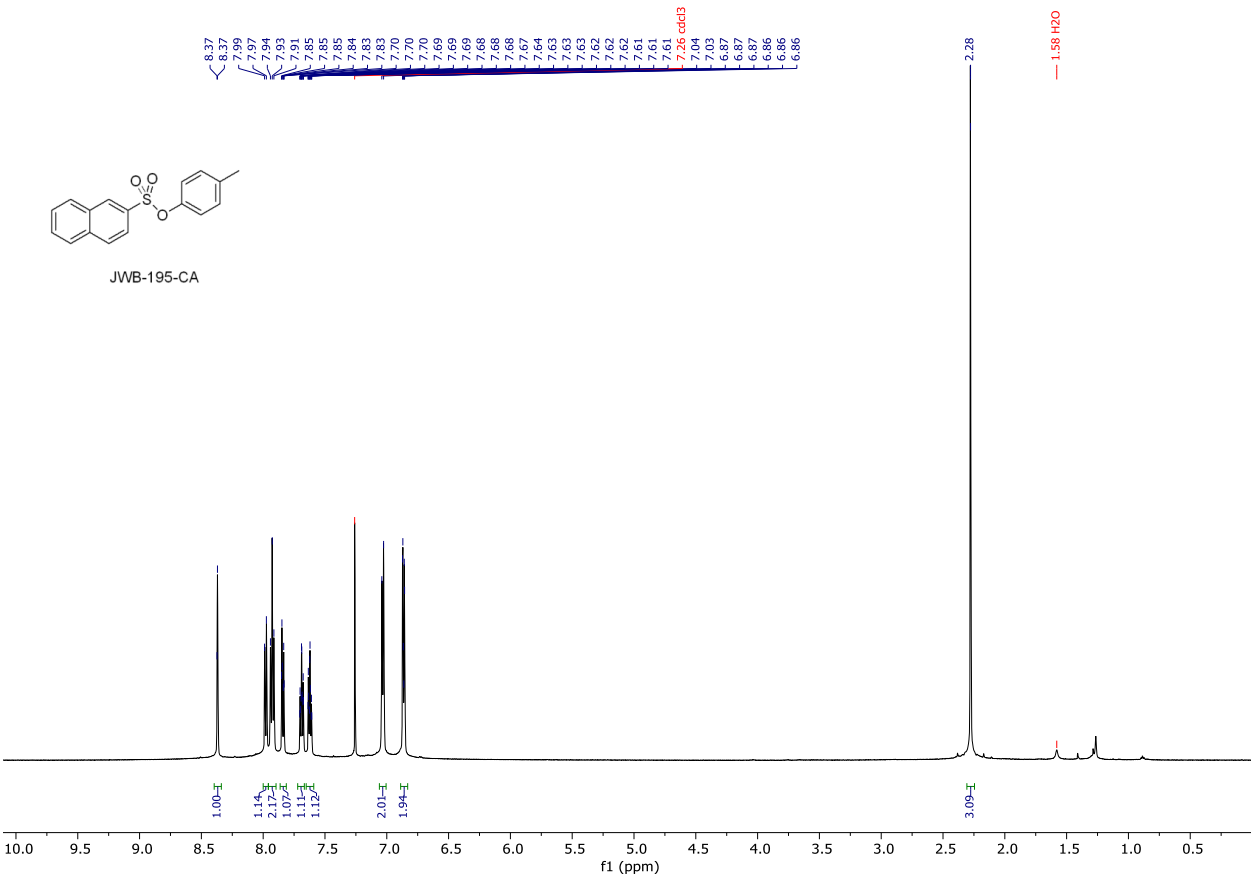

KY-353-BA
152.88
147.80
136.93
134.87
123.80
77.21 cdd3
77.00 cdd3
76.79 cdd3
42.94
31.59
19.61
13.44
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

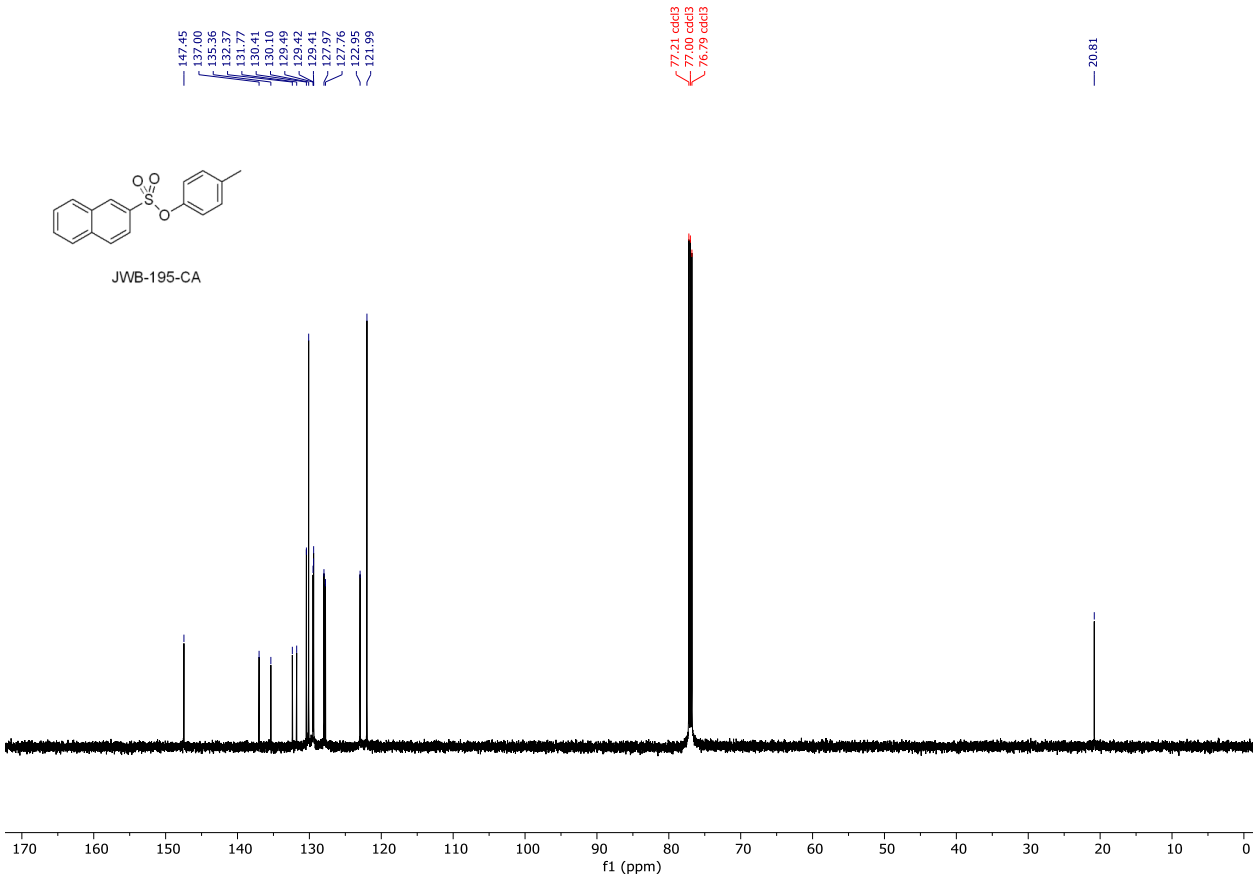

KY-353-CA
cdd3
2.29
1.00
1.03
0.99
1.04
2.07
2.01
3.37
f1 (ppm)


154.53
149.07
147.02
137.55
136.09
132.26
130.36
123.69
121.84
77.28 cdcl3
77.06 cdcl3
76.85 cdcl3
20.83
KY-353-CA
80
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

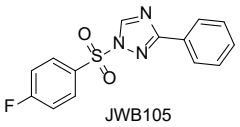

8.45
7.97
7.96
7.92
7.91
7.86
7.86
7.85
7.84
7.66
7.65
7.65
7.64
7.63
7.62
7.61
7.61
7.60
7.26 cdcl3
4.70
2.99
2.98
2.97
1.47
1.46
1.46
1.45
1.45
1.44
1.43
1.42
1.31
1.30
1.29
1.27
1.26
1.25
0.84
0.82
0.81
JWB-195-BA
1.00
2.16
1.09
1.04
2.13
1.01
2.26
2.24
2.40
3.26
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


136.75
134.76
132.15
129.45
129.20
128.71
128.40
127.88
127.50
122.32
77.21 cdcl3
77.00 cdcl3
76.79 cdcl3
42.97
31.59
19.65
13.47
JWB-195-BA
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

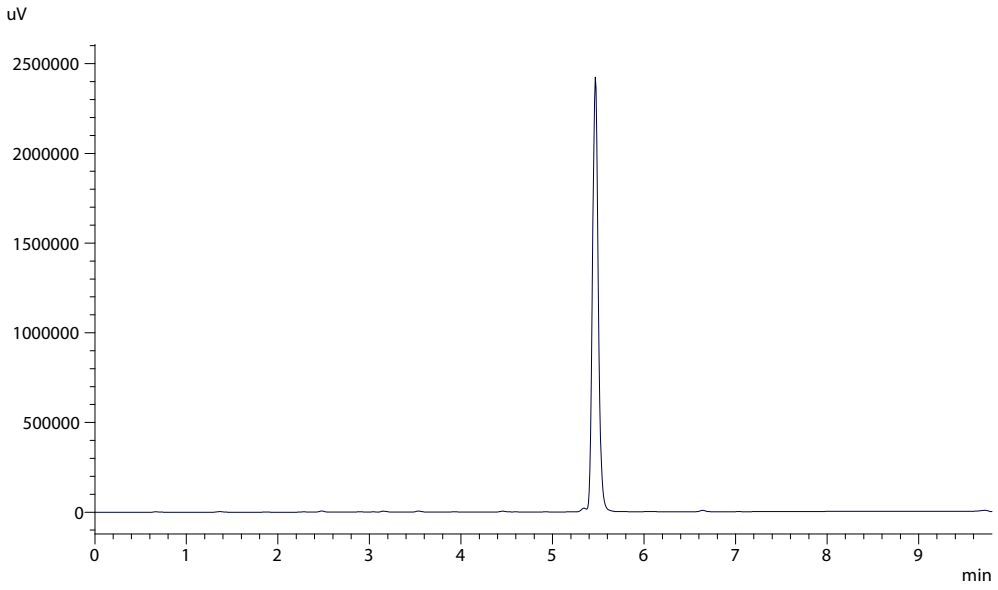

8.37
8.37
7.99
7.97
7.94
7.93
7.91
7.85
7.85
7.85
7.84
7.83
7.83
7.70
7.70
7.70
7.69
7.69
7.69
7.68
7.68
7.68
7.67
7.64
7.63
7.63
7.63
7.62
7.62
7.62
7.61
7.61
7.61
7.26 cdcl3
7.04
7.03
6.87
6.87
6.87
6.86
6.86
6.86
2.28
1.58 H2O
JWB-195-CA
1.00
1.14
2.17
1.07
1.11
1.12
2.01
1.94
3.09
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


147.45
137.00
135.36
132.37
131.77
130.41
130.10
129.49
129.42
129.41
127.97
127.76
122.95
121.99
77.21 cdcl3
77.00 cdcl3
76.79 cdcl3
20.81
JWB-195-CA
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

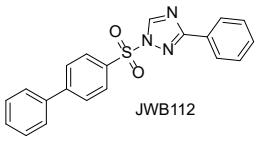
5.2 HPLC analysis of compound purity

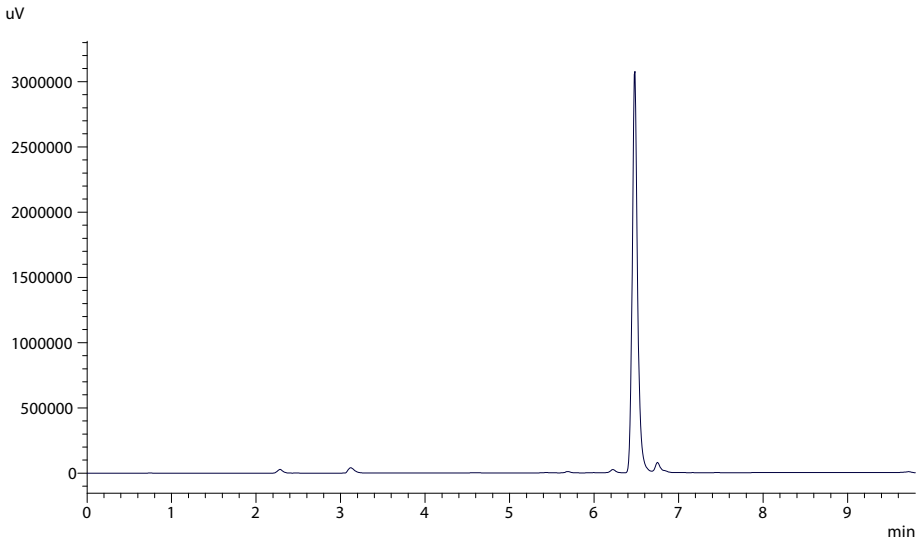


JWB105

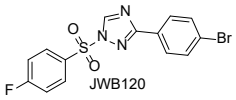


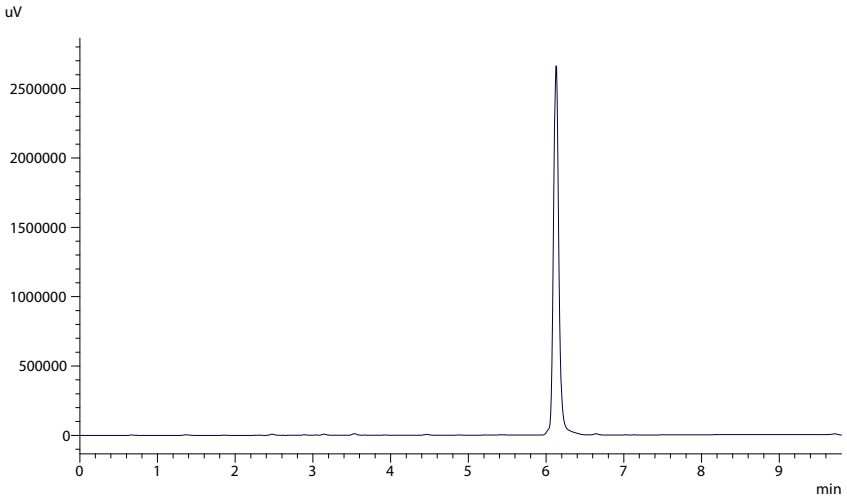
HPLC method A: chromatographic separation was performed using a Phenomenex Kinetex C18 column (2.6 μm, 50 x 4.6 mm). Mobile phases A and B were composed of H_2O + 0.1% HOAc and ACN + 0.1% HOAc, respectively. Samples were analyzed using the following analytical conditions: using a flow rate of 0.8 ml min^{-1}, the gradient was as follows: 0–0.5 min, 15% B; 0.5–6.5 min 85% B; 6.5–7 min 100% B; 7–8.5 min 100% B; 8.5–9 min 15% B; 9–9.8 min 15% B. JWB105 HPLC purity was determined to be >97% using this method.



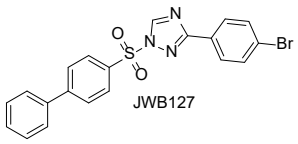


The purity of JWB112 was determined to be >95% using HPLC method A .

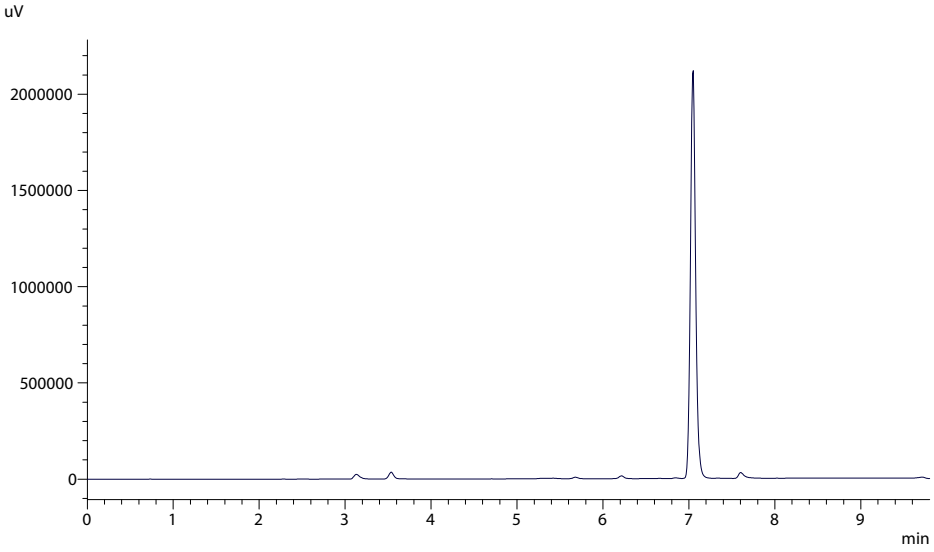




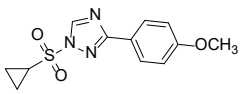
The purity of JWB120 was determined to be >97% using HPLC method A.



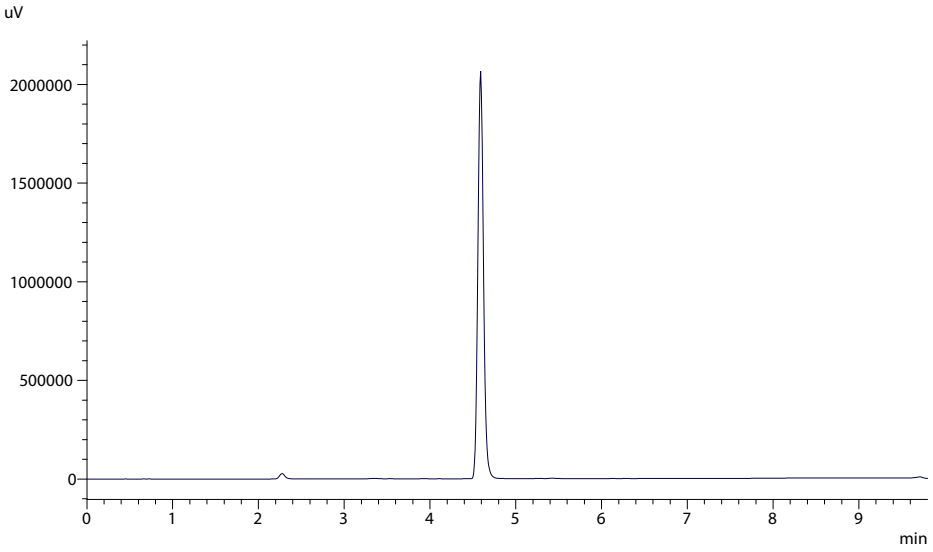
JWB127



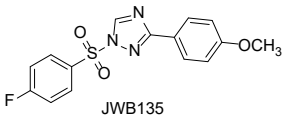
The purity of JWB127 was determined to be >95% using HPLC method A.



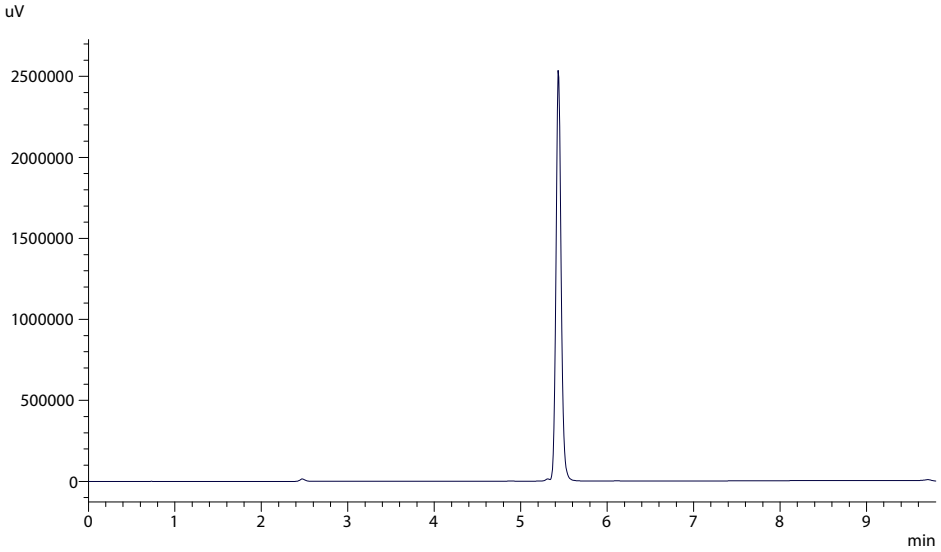
JWB131



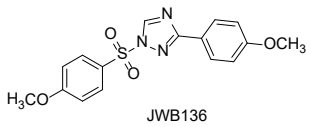
The purity of JWB131 was determined to be >96% by using HPLC method A.



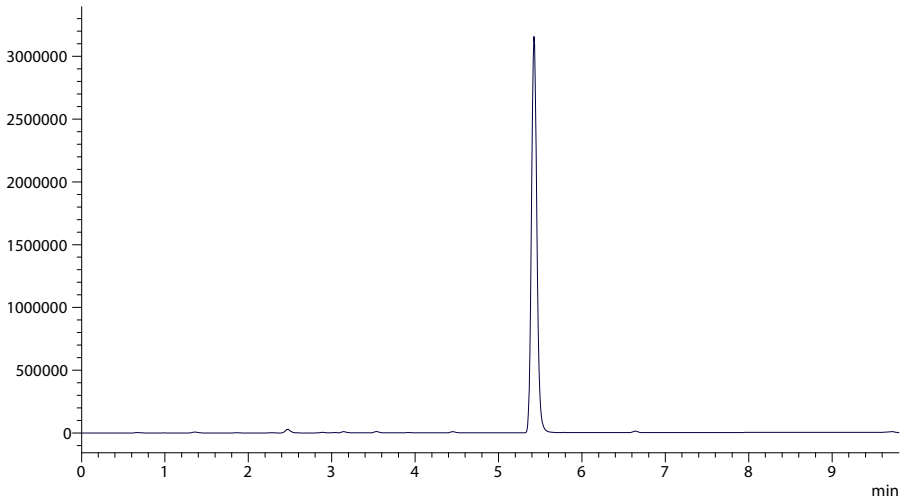
JWB135



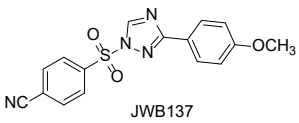
The purity of JWB135 was determined to be >98% by using HPLC method A.



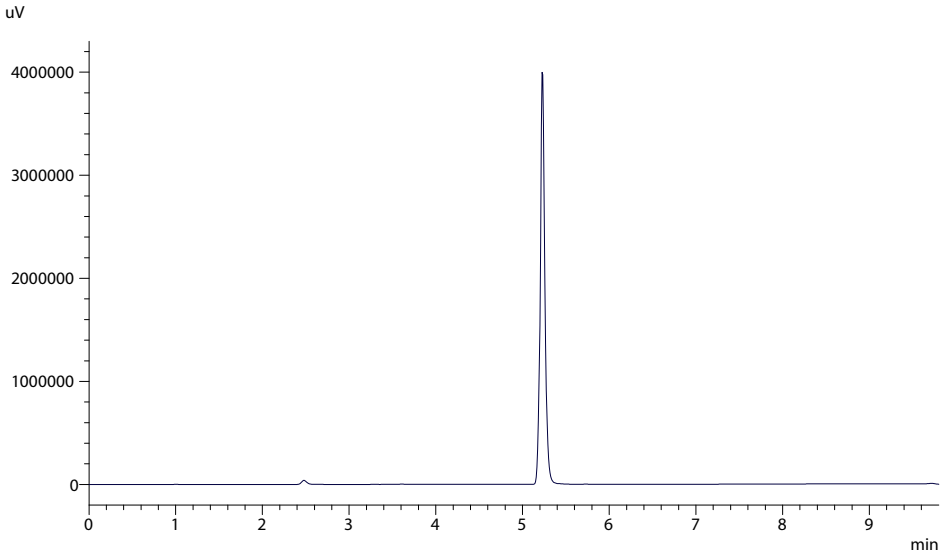
JWB136



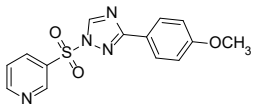
The purity of JWB136 was determined to be >96% by using HPLC method A.



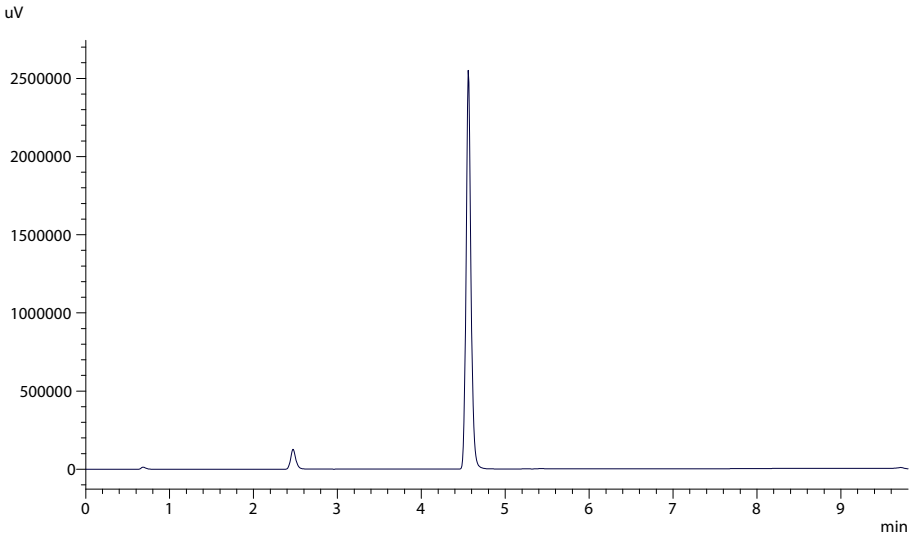
JWB137



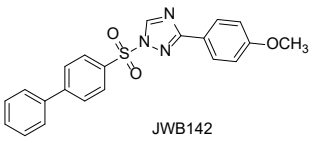
The purity of JWB137 was determined to be >98% by using HPLC method A.



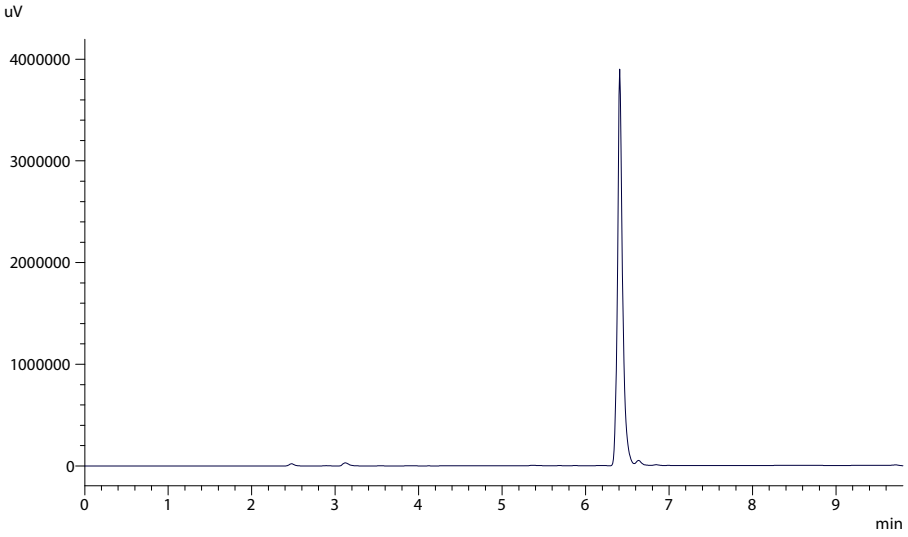
JWB141



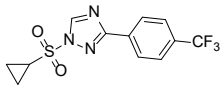
The purity of JWB141 was determined to be >95% by using HPLC method A.



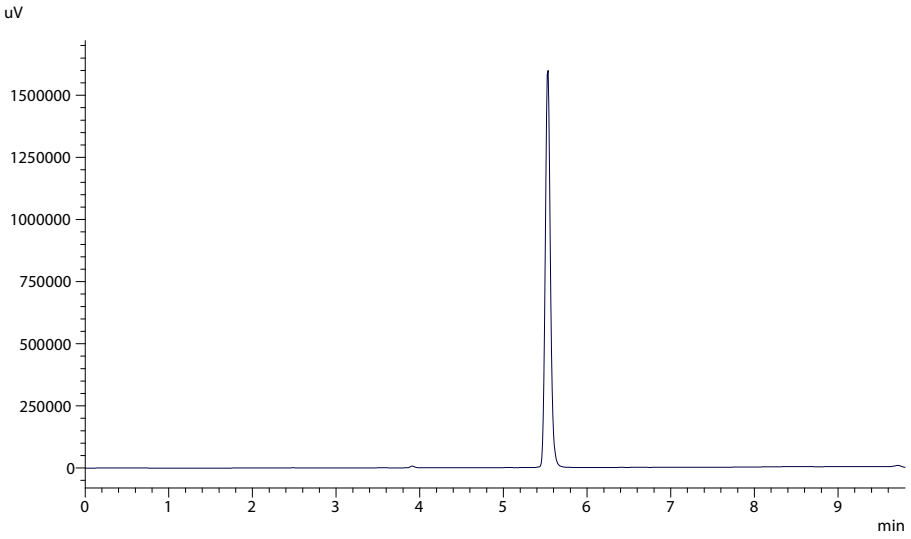
JWB142



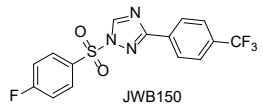
The purity of JWB142 was determined to be >96% by using HPLC method A.

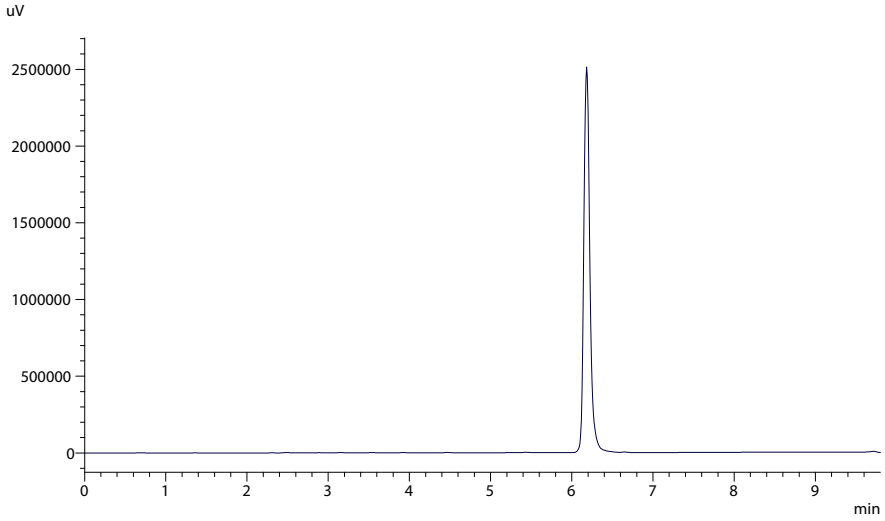


JWB146

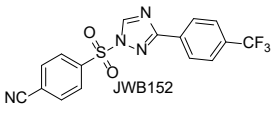


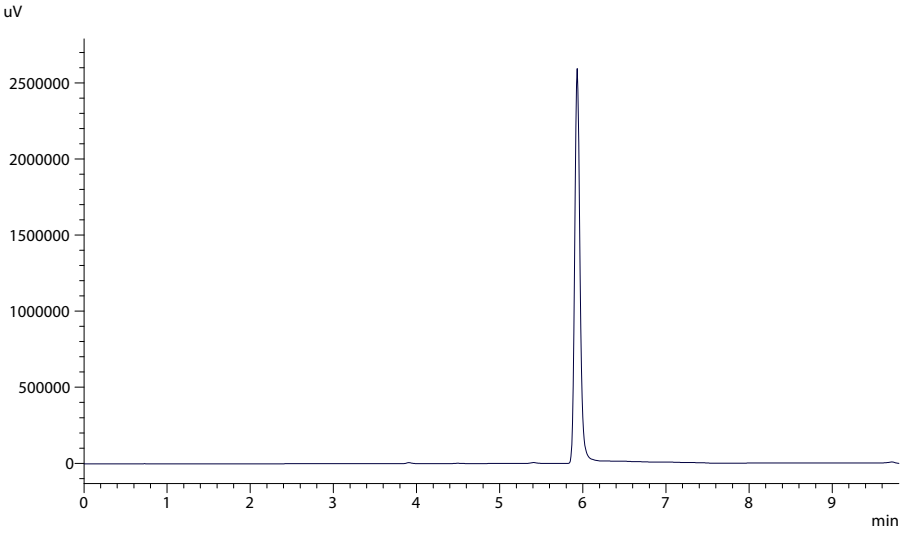
The purity of JWB146 was determined to be >96% by using HPLC method A.



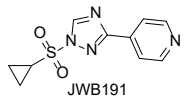


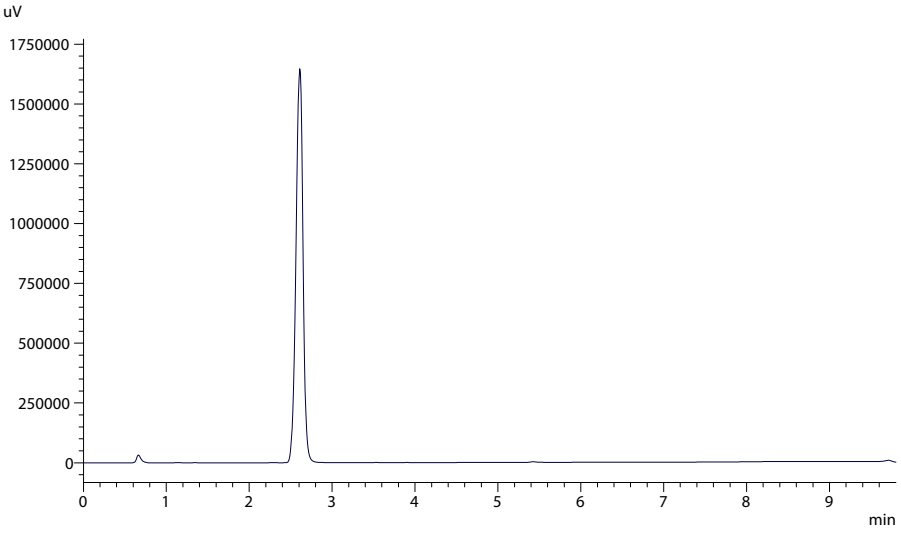
The purity of JWB150 was determined to be >98% by using HPLC method A.



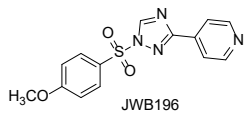


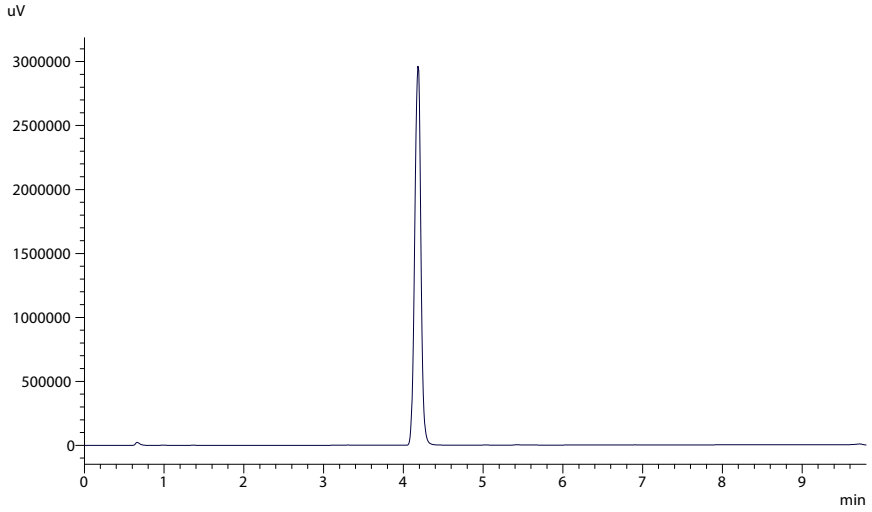
The purity of JWB152 was determined to be >98% by using HPLC method A.



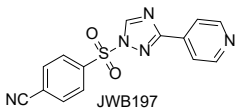


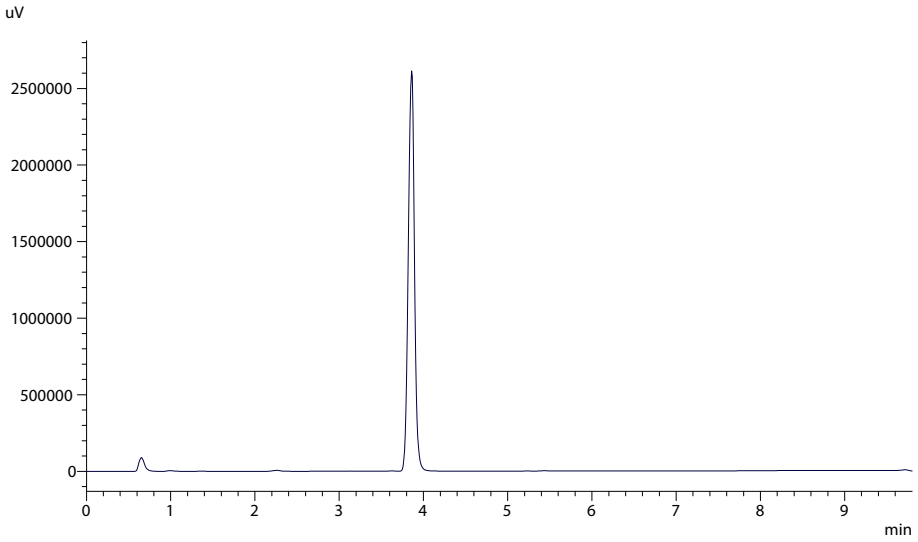
The purity of JWB191 was determined to be >97% by using HPLC method A.



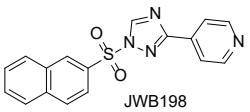


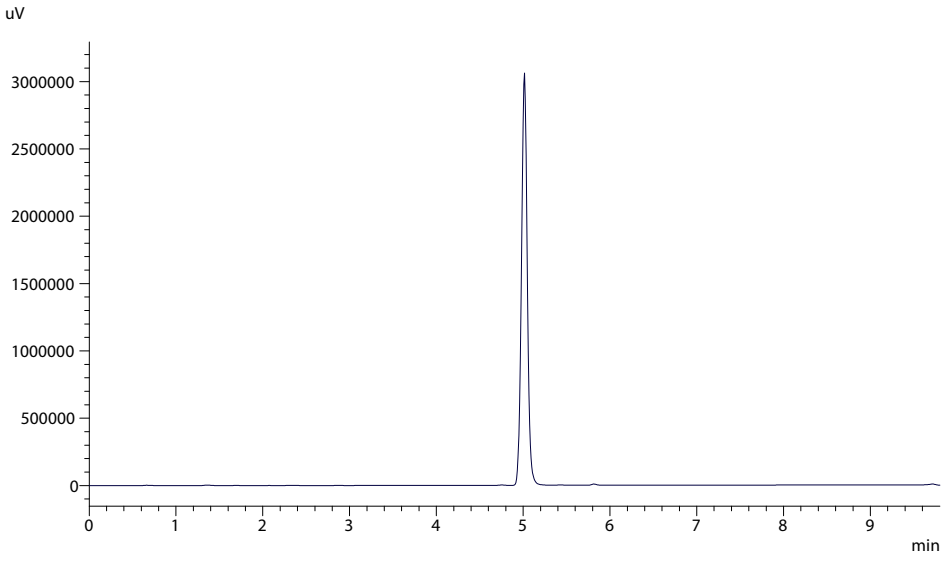
The purity of JWB196 was determined to be >98% by using HPLC method A.



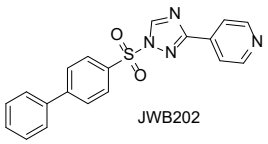


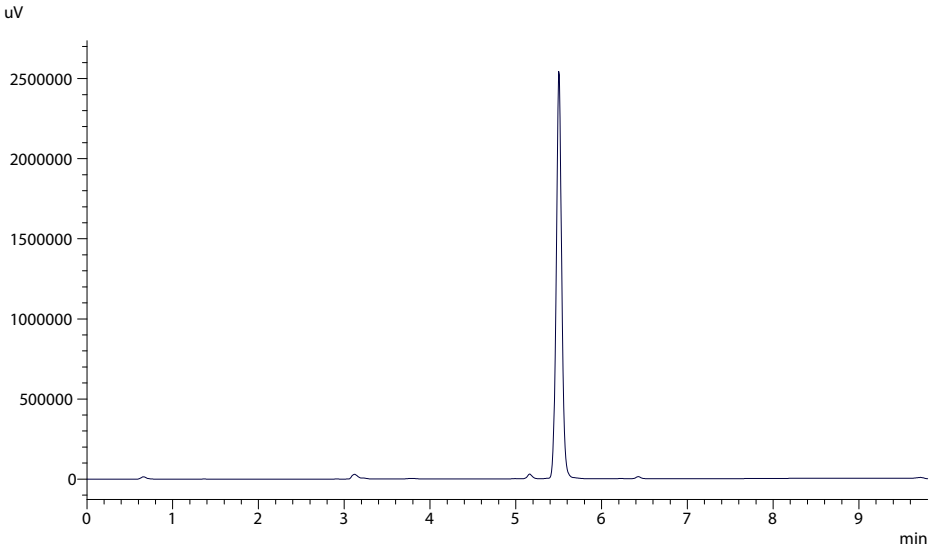
The purity of JWB197 was determined to be >96% by using HPLC method A.



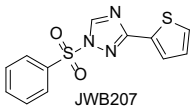


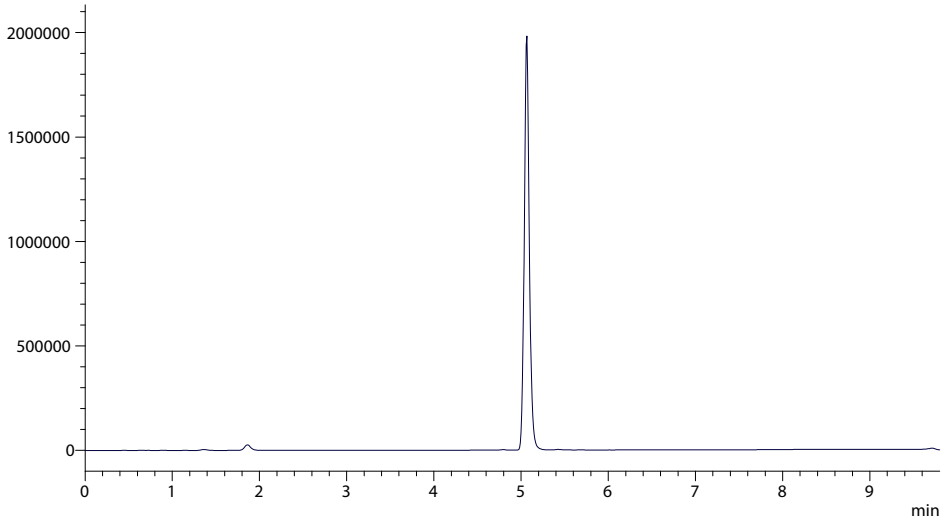
The purity of JWB198 was determined to be >98% by using HPLC method A.



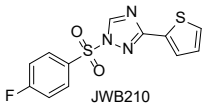


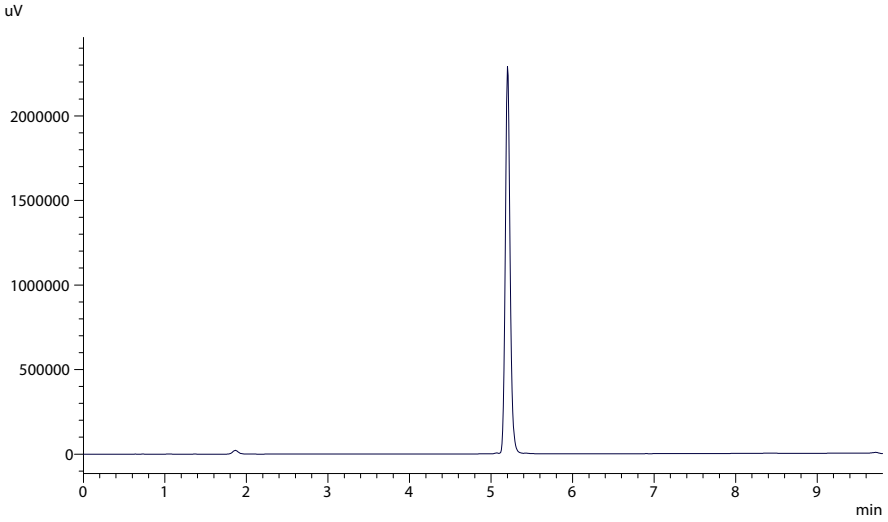
The purity of JWB202 was determined to be >95% by using HPLC method A.



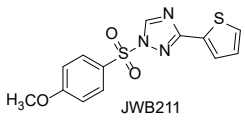


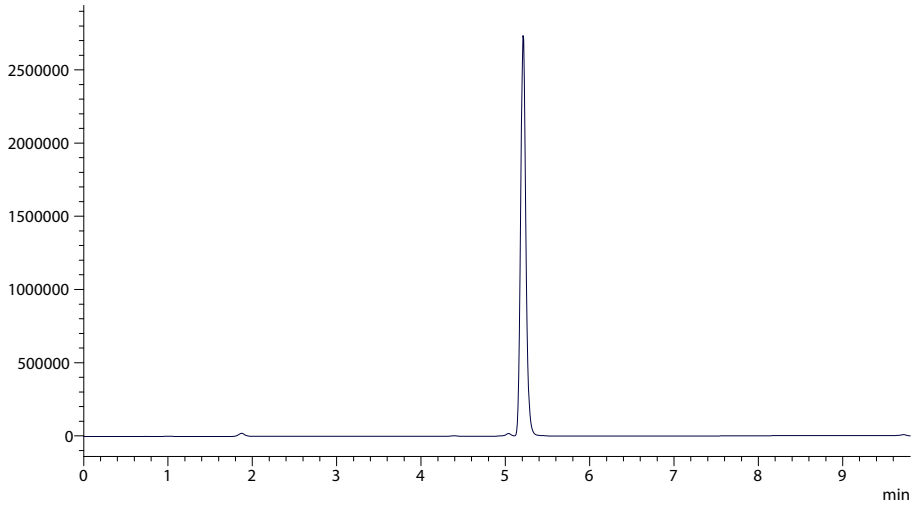
The purity of JWB207 was determined to be >96% by using HPLC method A.



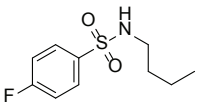


The purity of JWB210 was determined to be >97% by using HPLC method A.

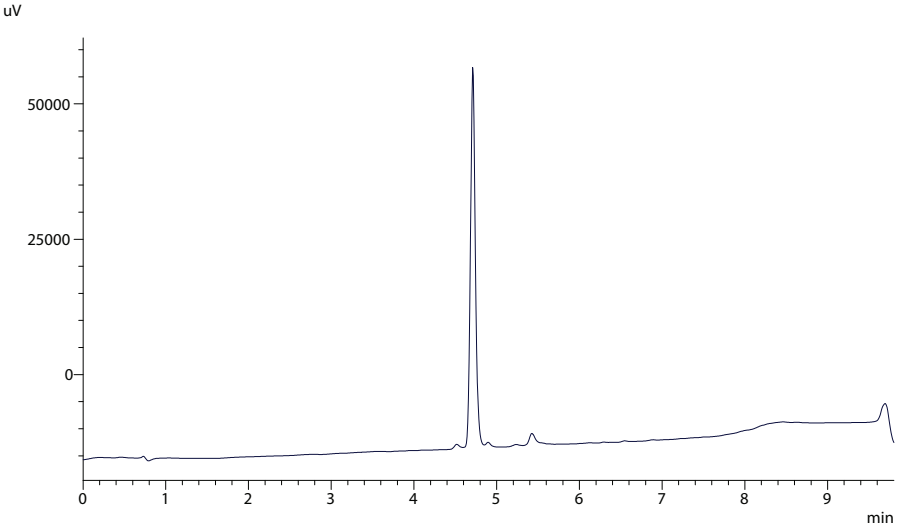




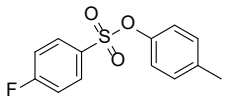
The purity of JWB211 was determined to be >97% by using HPLC method A.



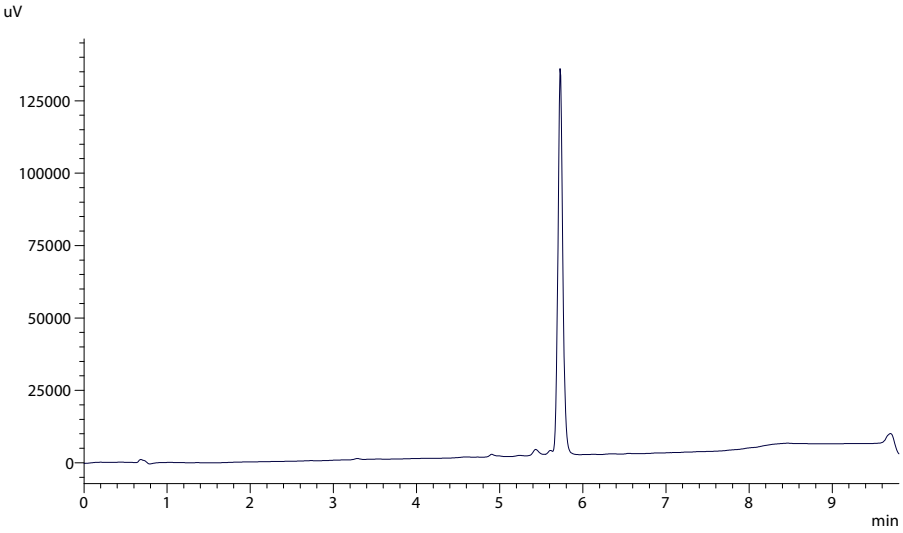
KY-347-BA



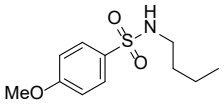
The purity of KY-347-BA was determined to be >95% by using HPLC method A.



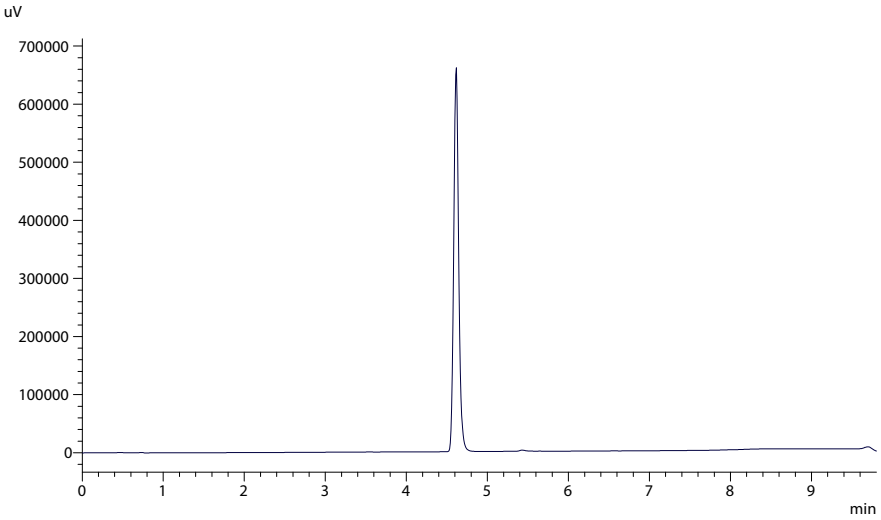
KY-347-CA



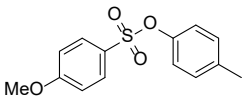
The purity of KY-347-CA was determined to be >95.0% by using HPLC method A.



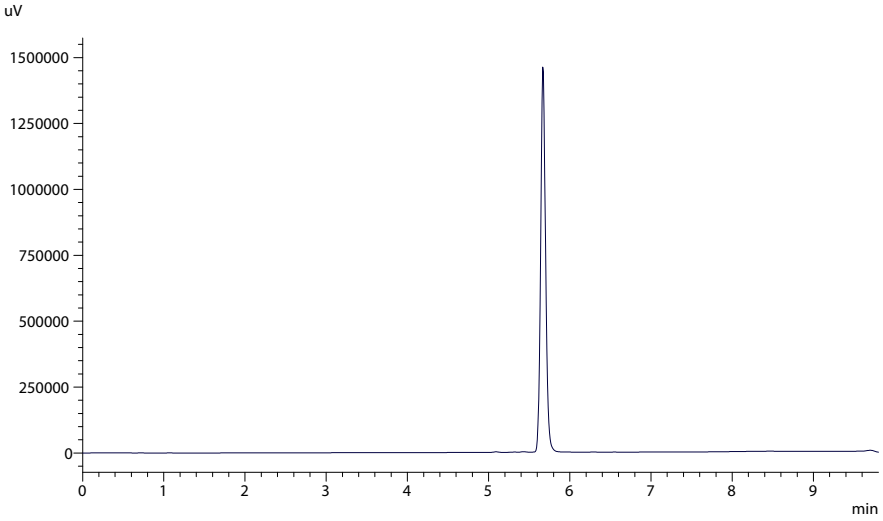
KY-348-BA



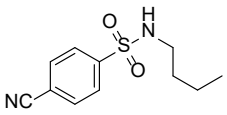
The purity of KY-348-BA was determined to be >97% by using HPLC method A.



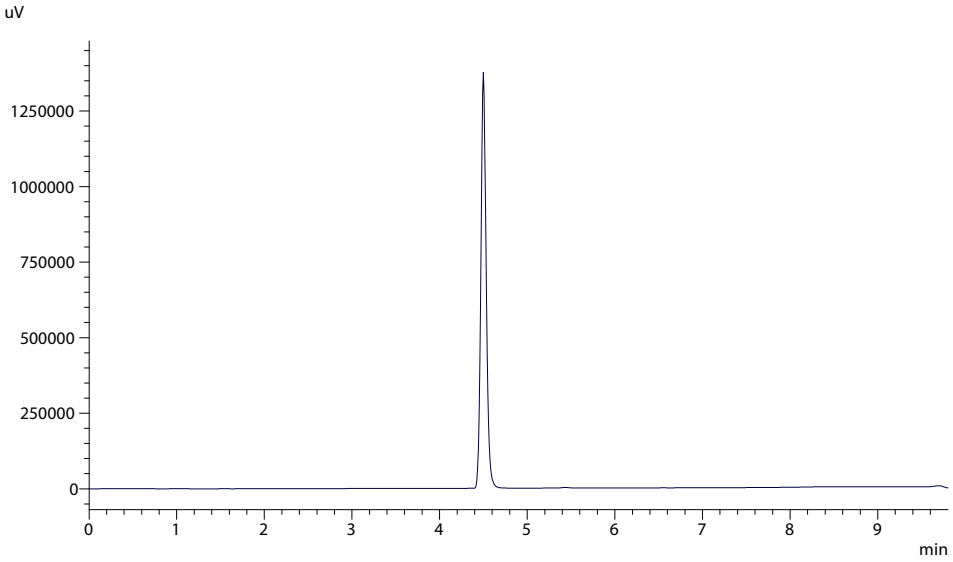
KY-348-CA



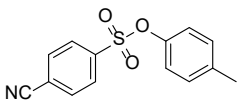
The purity of KY-348-CA was determined to be >97% by using HPLC method A.



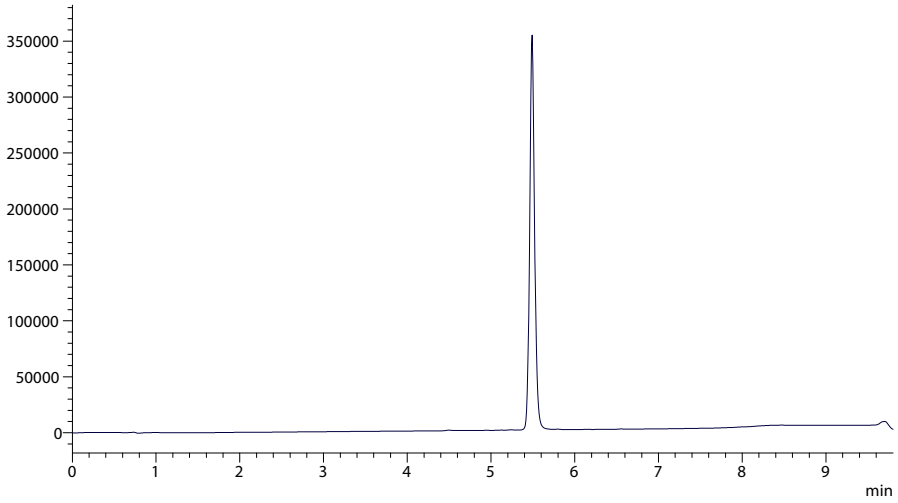
KY-349-BA



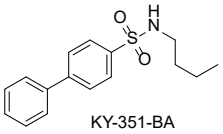
The purity of KY-349-BA was determined to be >97% by using HPLC method A.

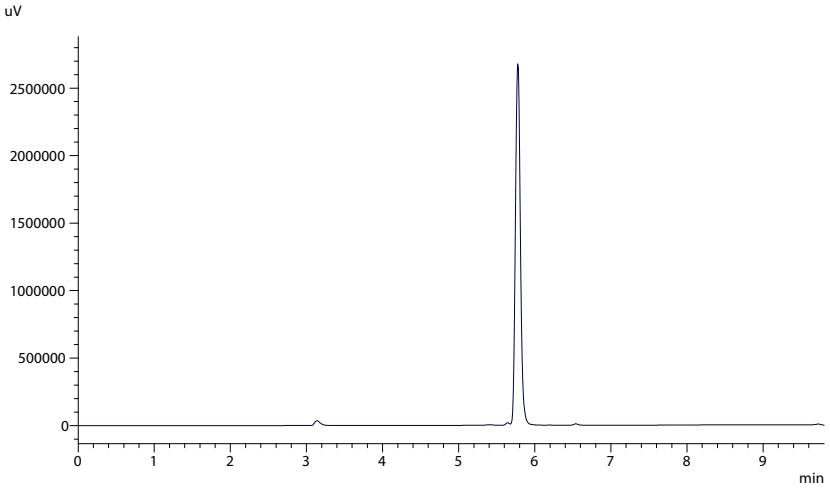


KY-349-CA

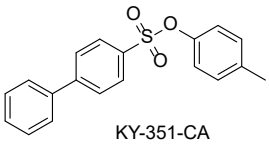


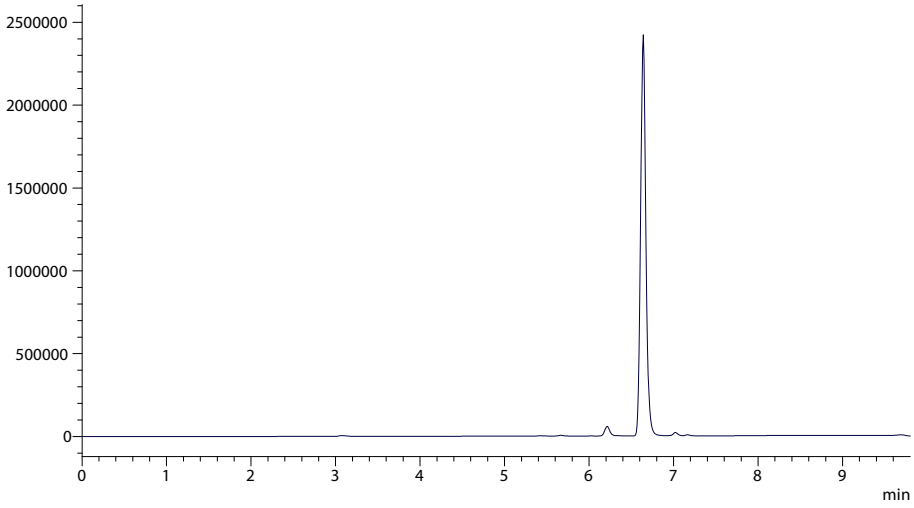
The purity of KY-349-CA was determined to be >96% by using HPLC method A.



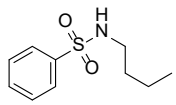


The purity of KY-351-BA was determined to be >96% by using HPLC method A.

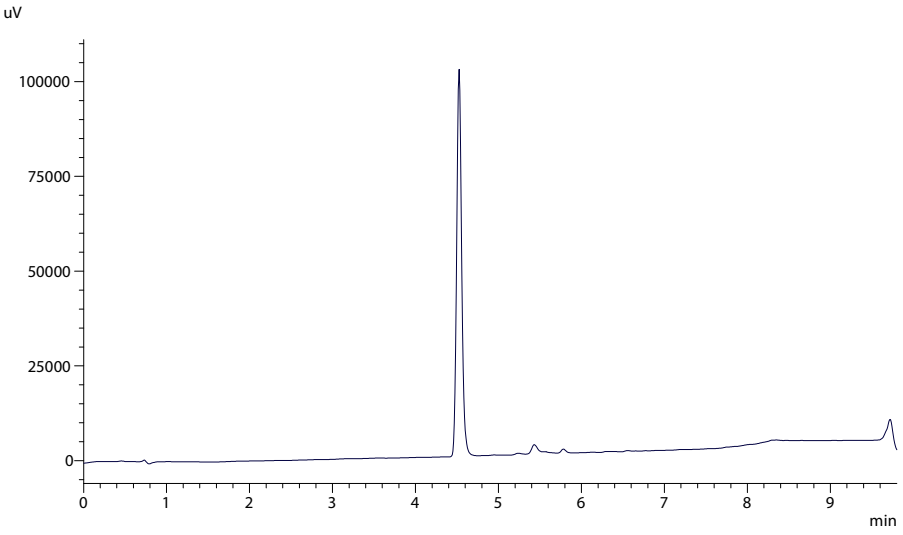




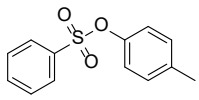
The purity of KY-351-CA was determined to be >97% by using HPLC method A.



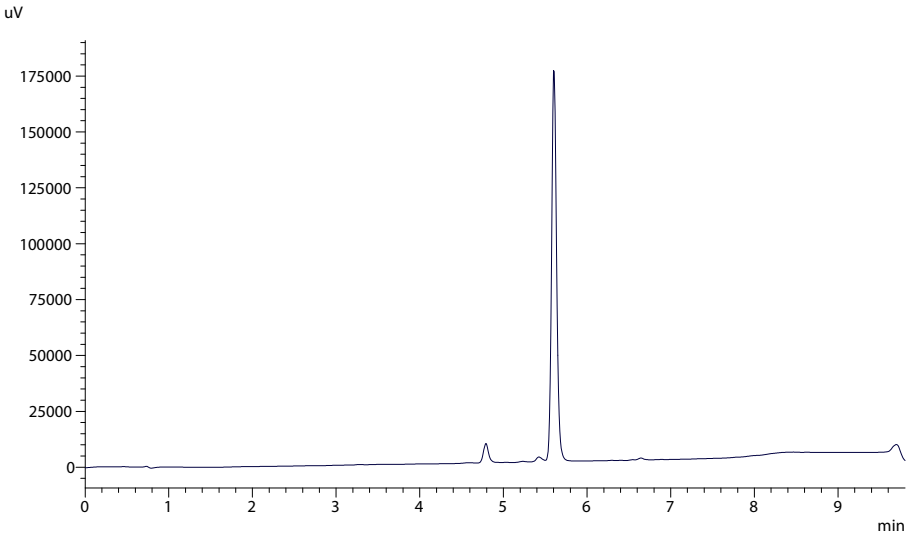
KY-352-BA



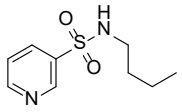
The purity of KY-352-BA was determined to be >95% by using HPLC method A.



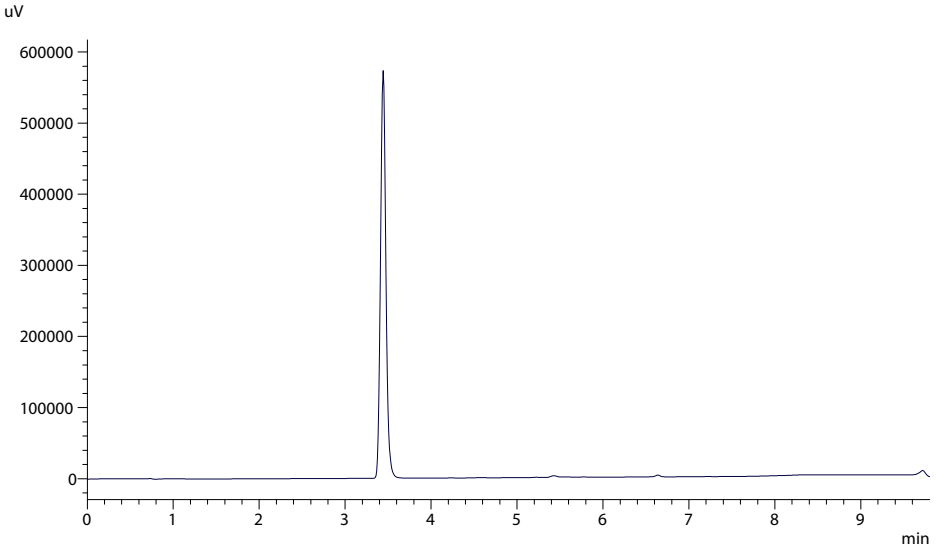
KY-352-CA



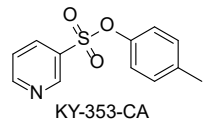
The purity of KY-352-CA was determined to be >95% by using HPLC method A.

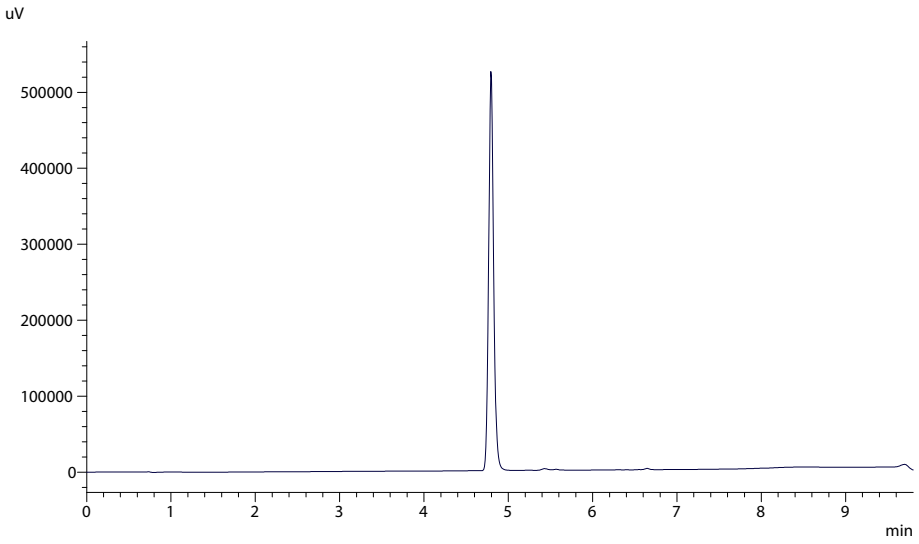


KY-353-BA

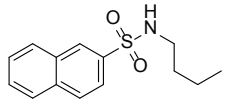


The purity of KY-353-BA was determined to be >97% by using HPLC method A.

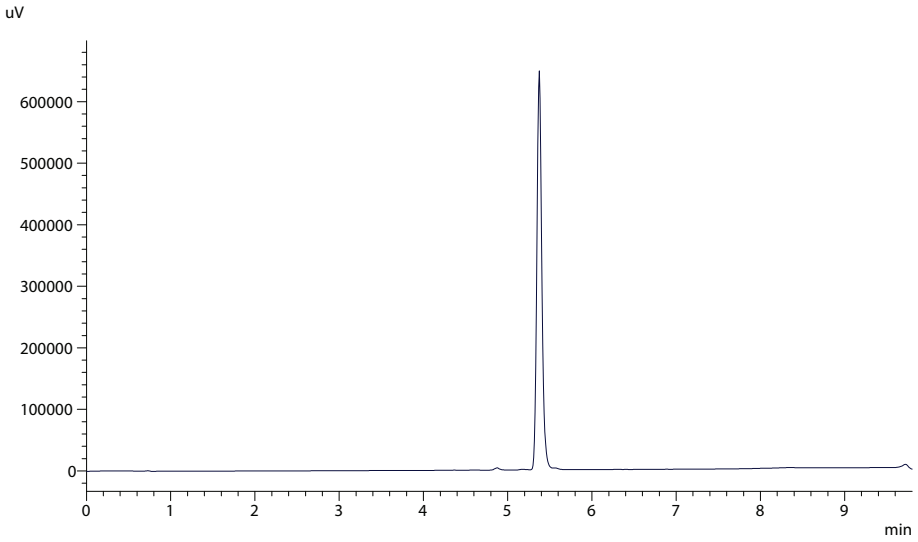




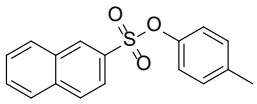
The purity of KY-353-CA was determined to be >97% by using HPLC method A.



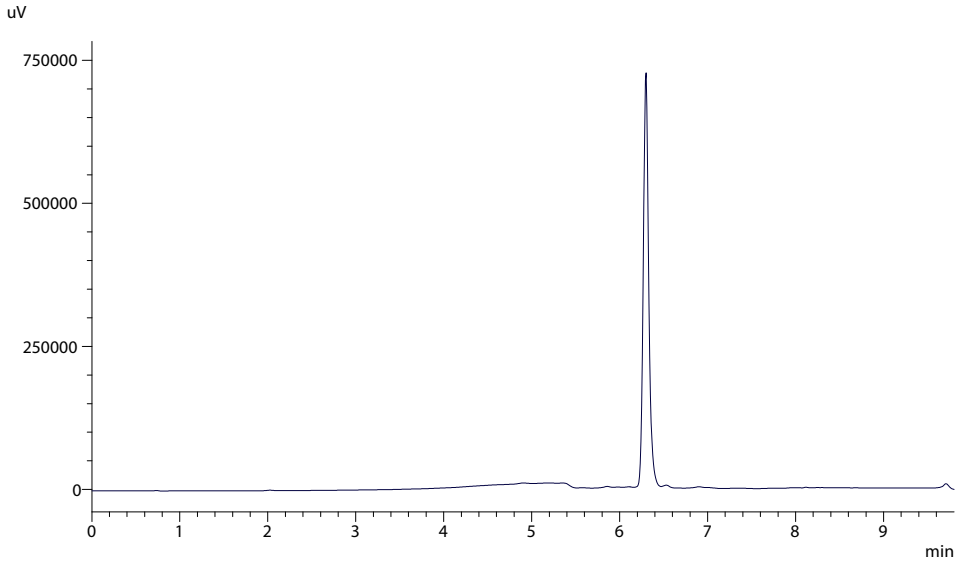
JWB-195-BA



The purity of JWB-195-BA was determined to be >98% by using HPLC method A.

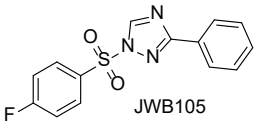


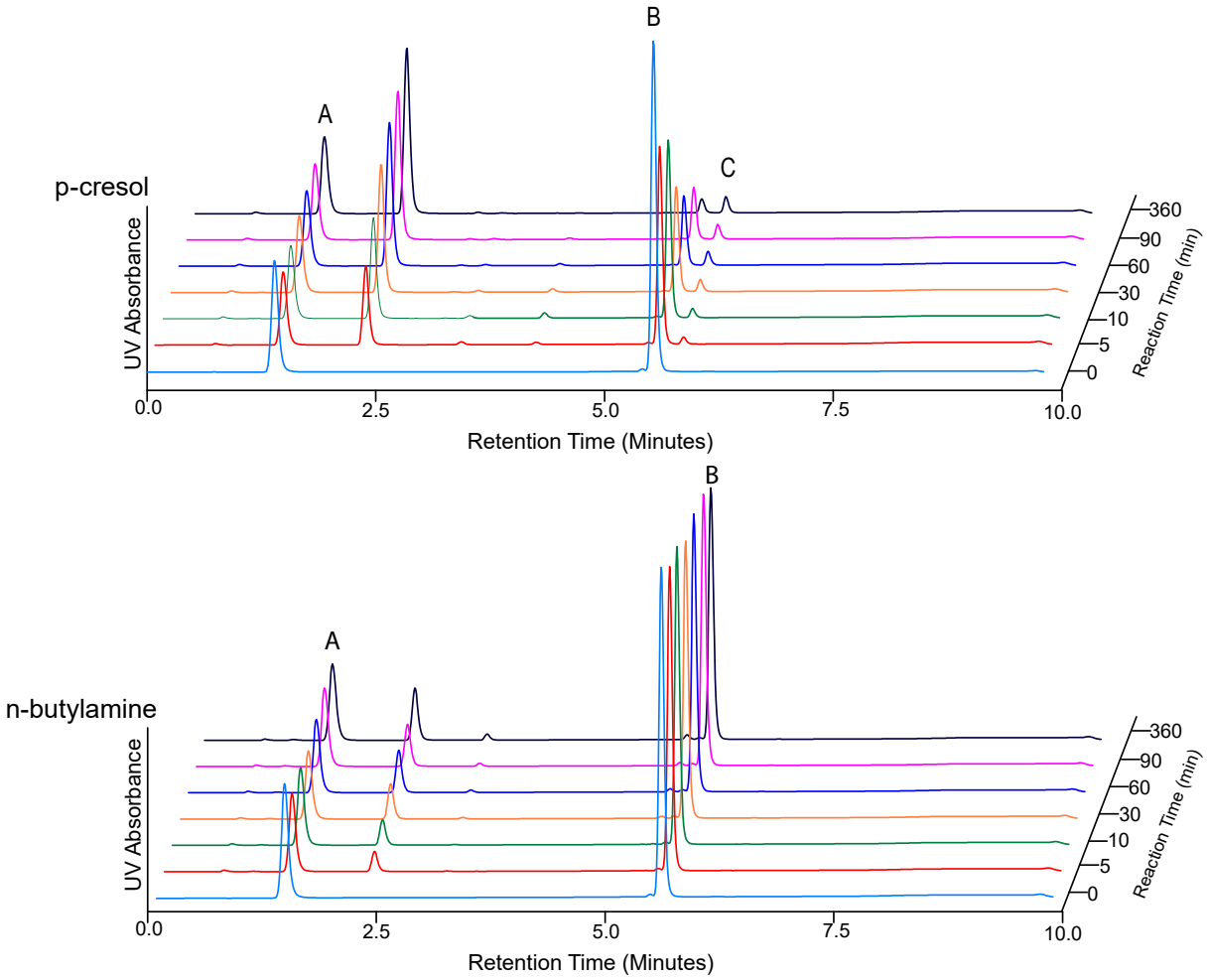
JWB-195-CA



The purity of JWB-195-CA was determined to be >96% by using HPLC method A.

5.3 HPLC reactivity assay



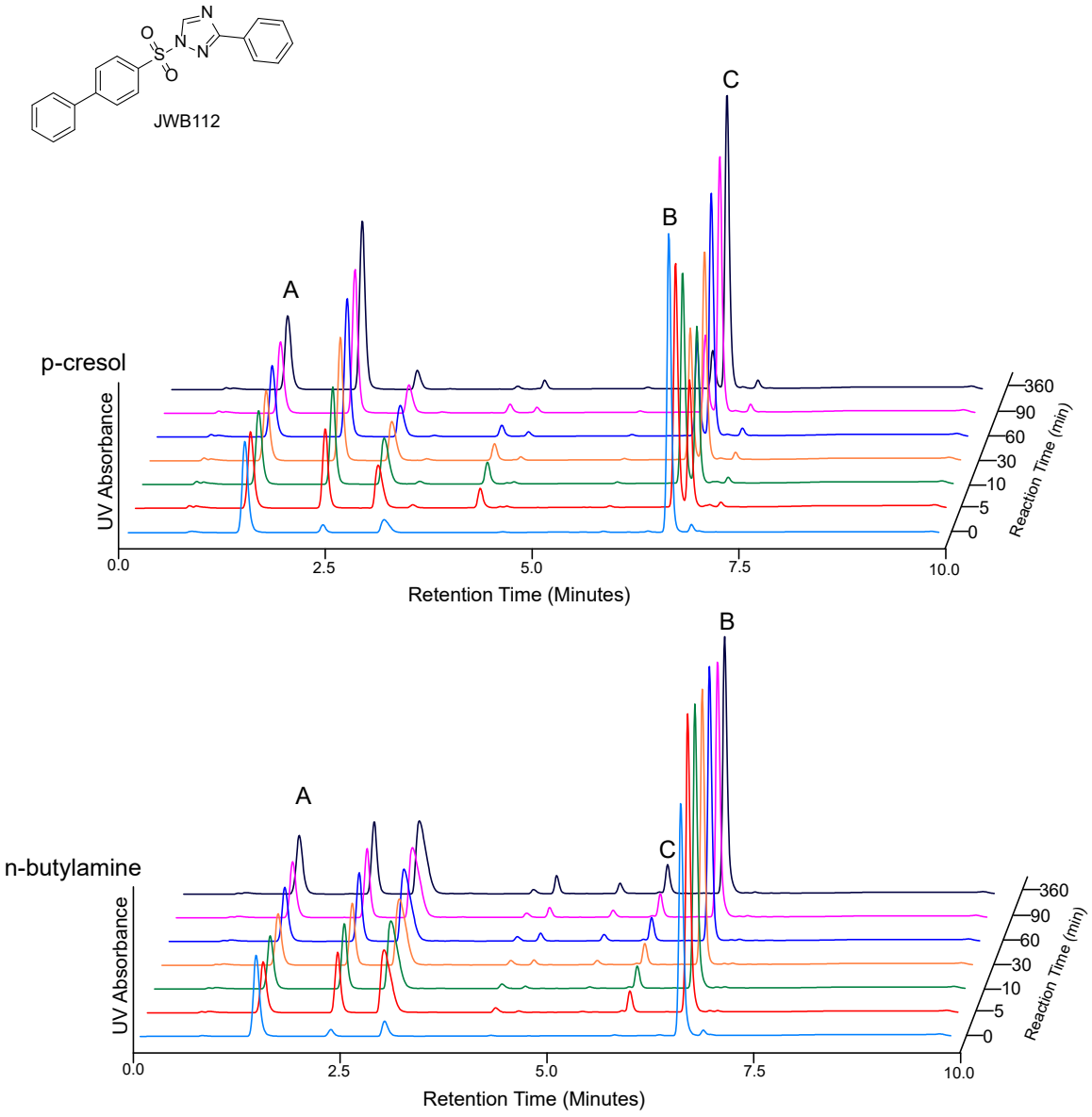


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

HPLC method B: SuTEx fragment was dissolved in 500 µL DMF-ACN solution and stirred on ice with TMG and the amino acid mimetics *p*-cresol or *n*-butylamine. At the indicated time point, a 50 µL aliquot was removed and quenched in a solution of acetic acid and caffeine. Solutions were analyzed by HPLC and consumption of SuTEx fragment was quantified as described in the Methods section. The half-life for JWB105 consumption ($T_{1/2\ cresol}$) was estimated to be 6.5 min using this method.

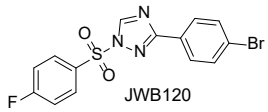


A = Internal Standard

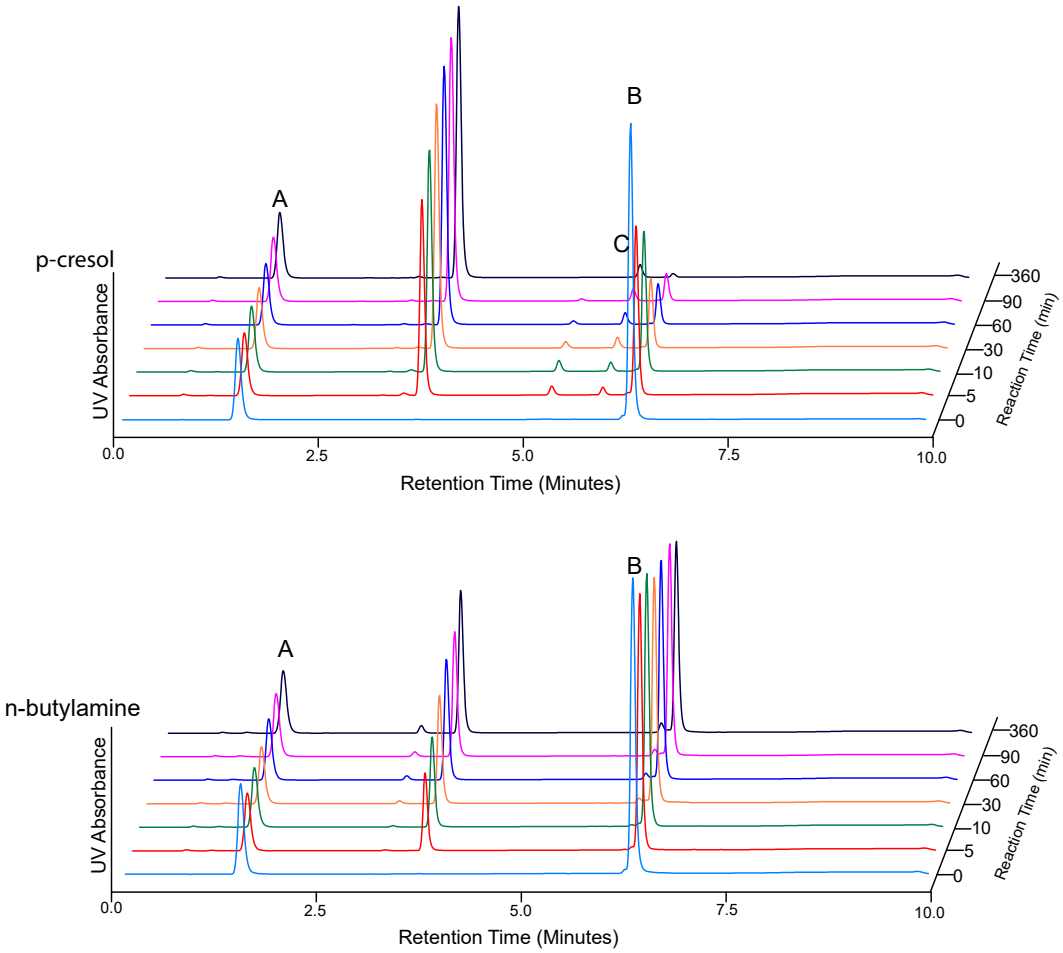
B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB112 consumption ($T_{1/2\ cresol}$) was estimated to be 18.7 min using HPLC method B.



JWB120

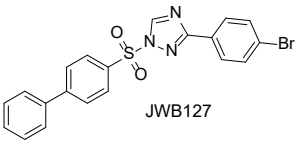


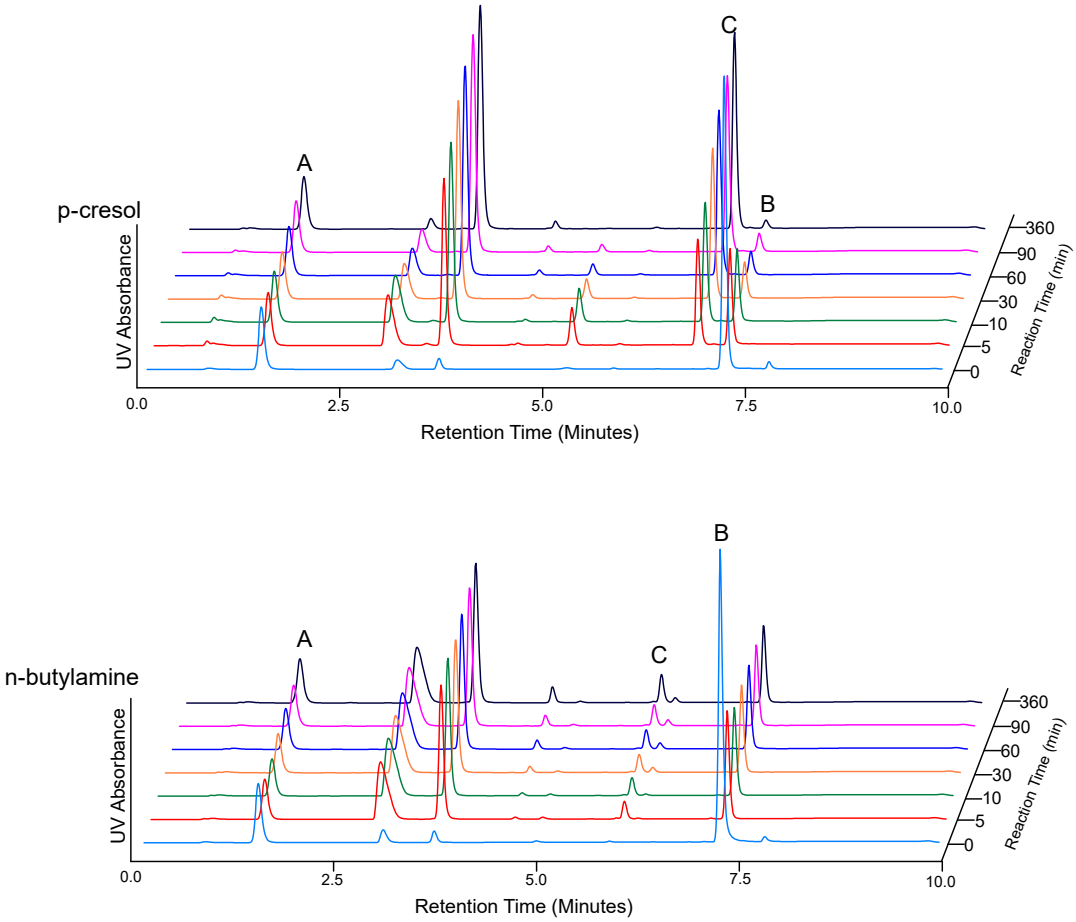
A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB120 consumption ($T_{1/2\ cresol}$) was estimated to be 6.6 min using HPLC method B.



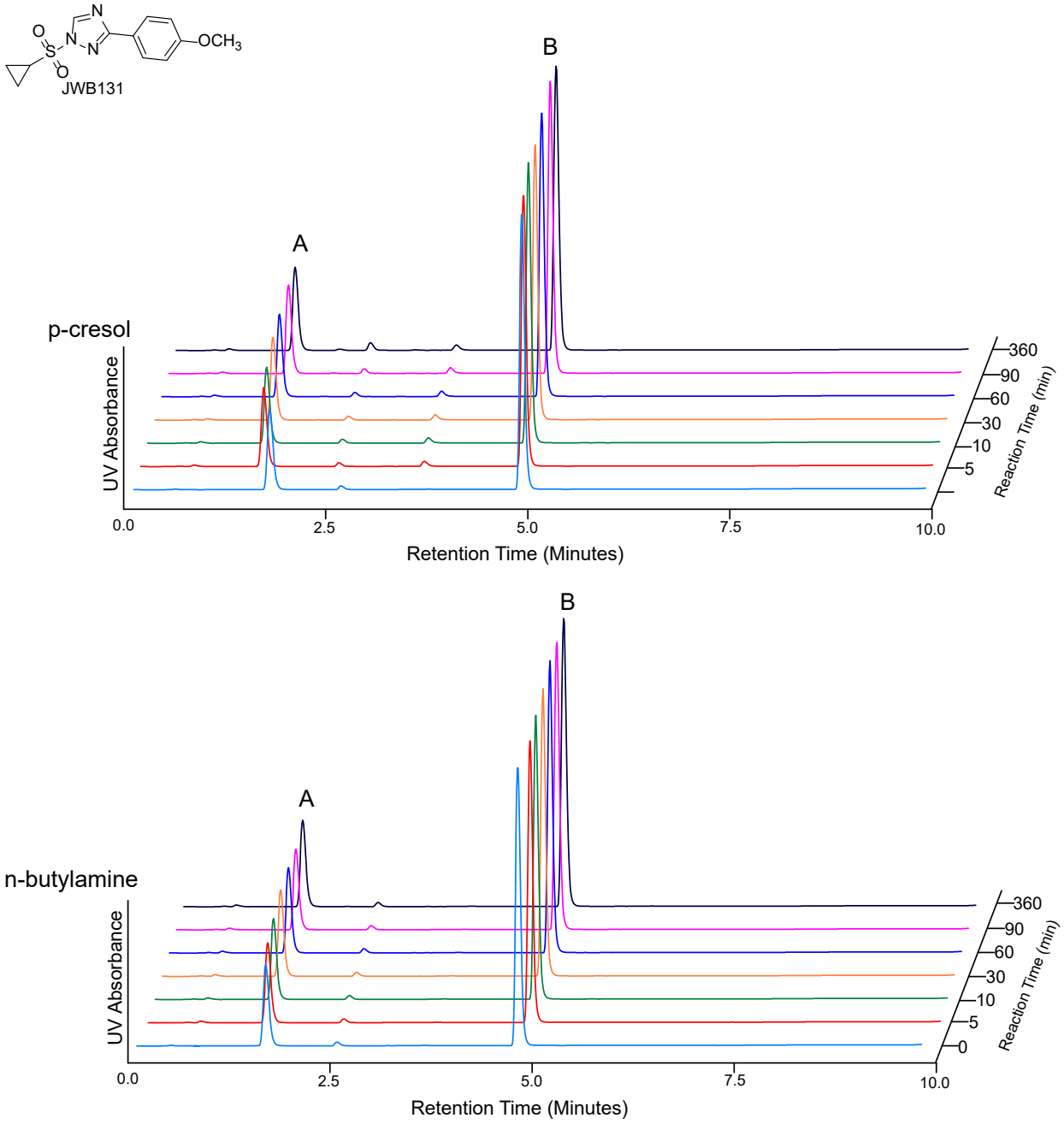


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB127 consumption ($T_{1/2\ cresol}$) was estimated to be 4.8 min using HPLC method B.

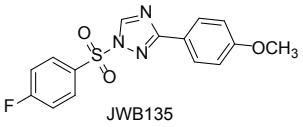


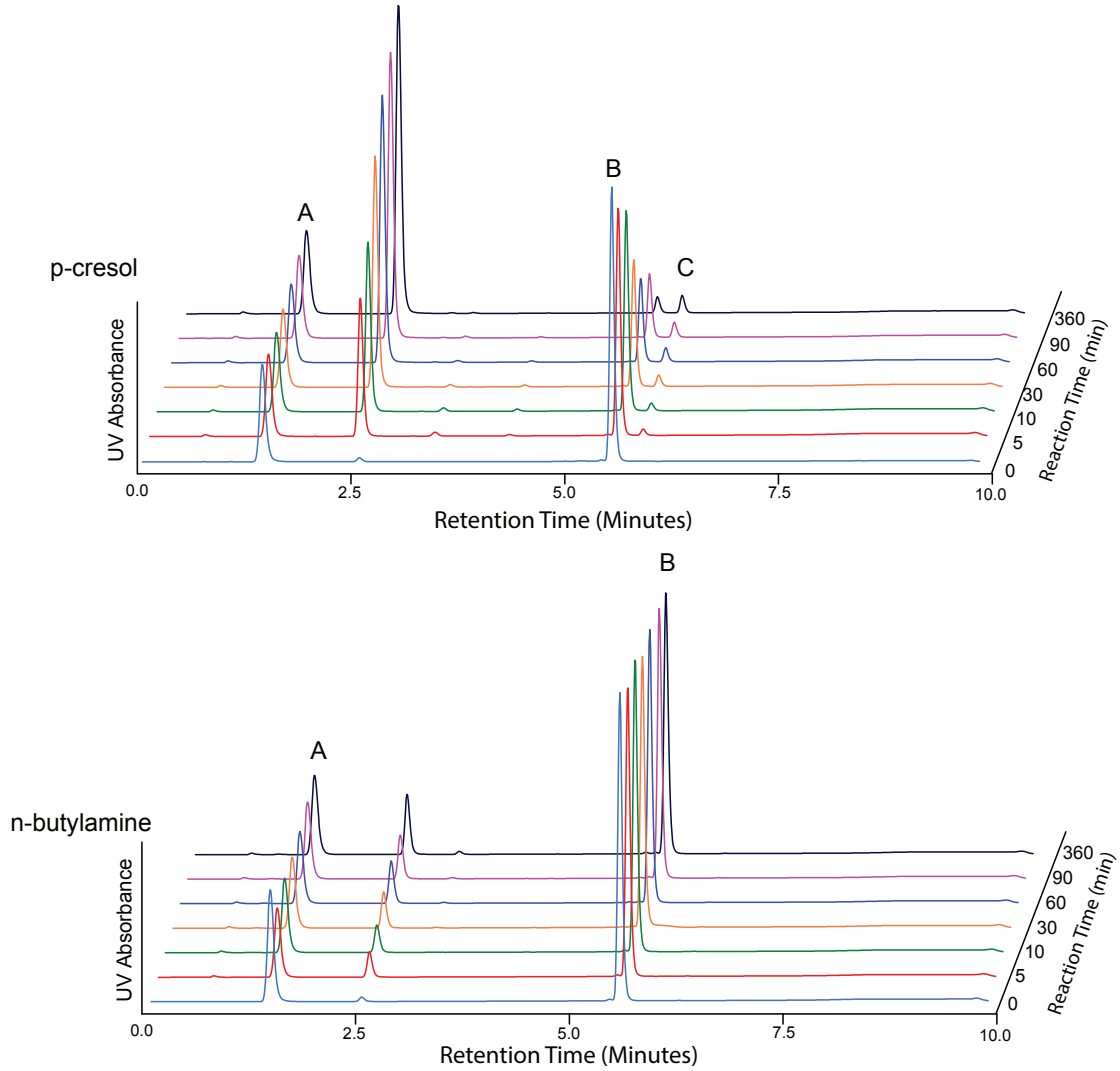
A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB131 consumption ($T_{1/2\ cresol}$) was estimated to be >360 min using HPLC method B.



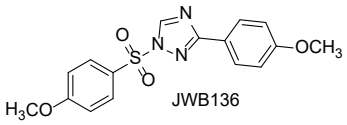


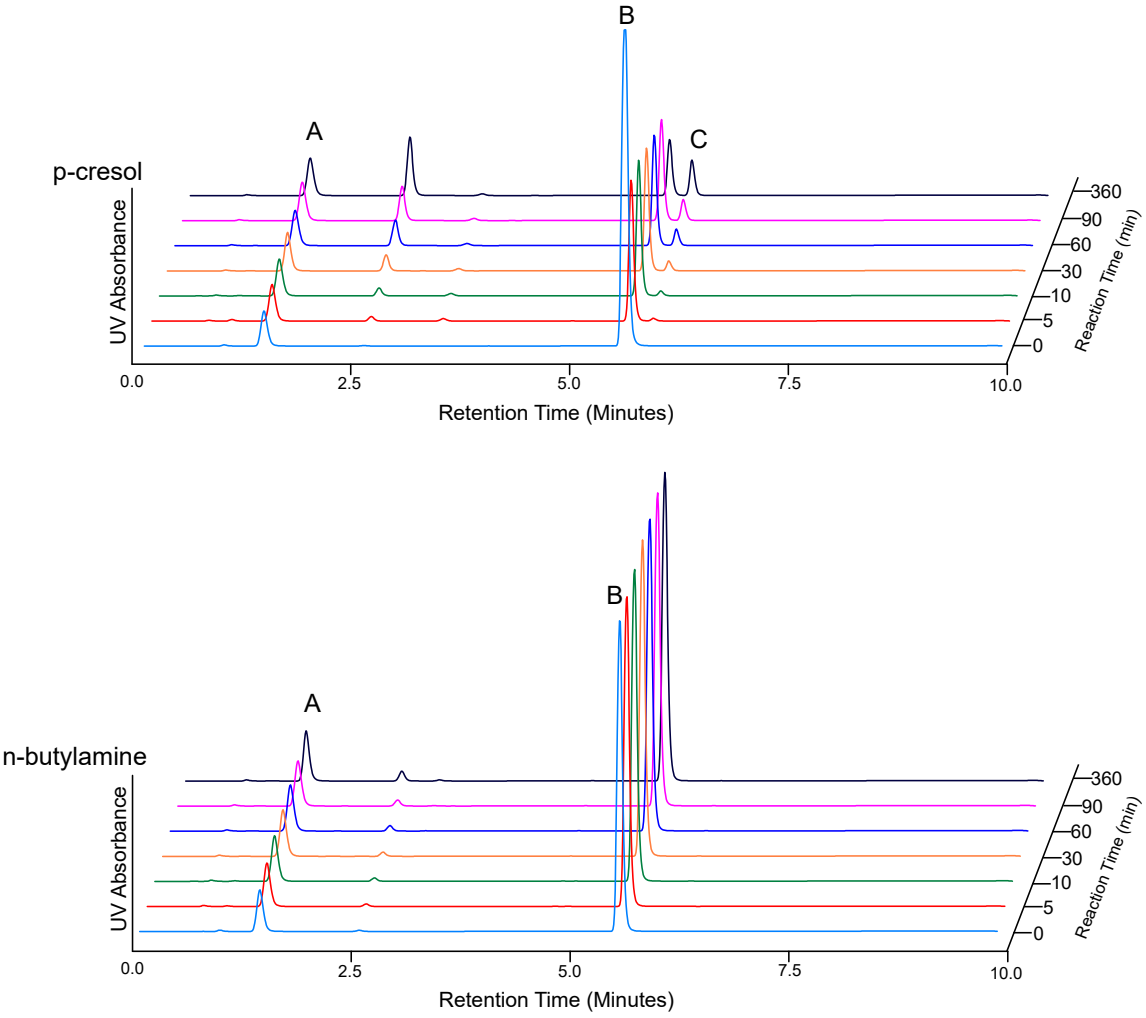
A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB135 consumption ($T_{1/2\ cresol}$) was estimated to be 14.2 min using HPLC method B.



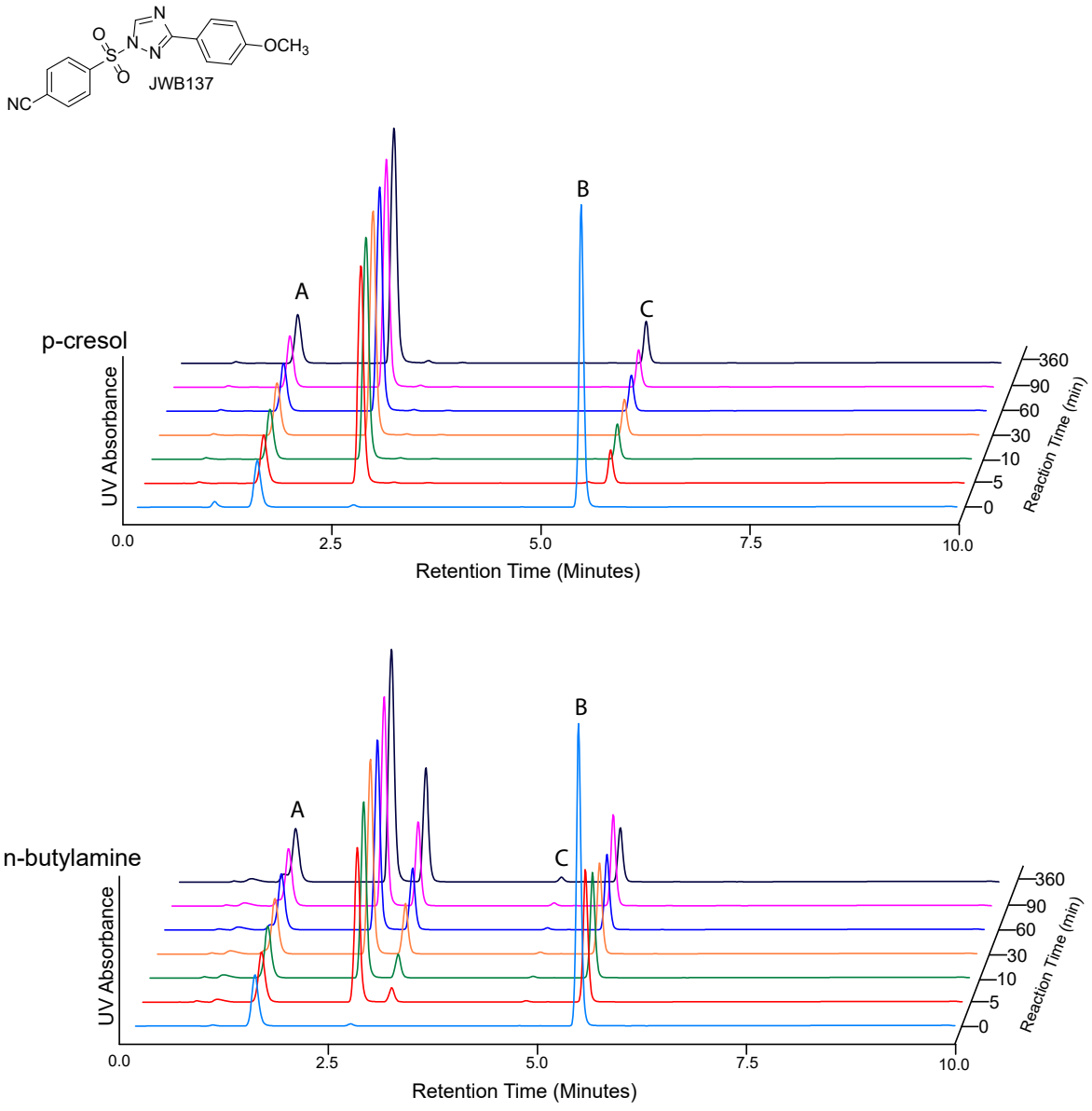


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB136 consumption ($T_{1/2\ cresol}$) was estimated to be 182.7 min using HPLC method B.

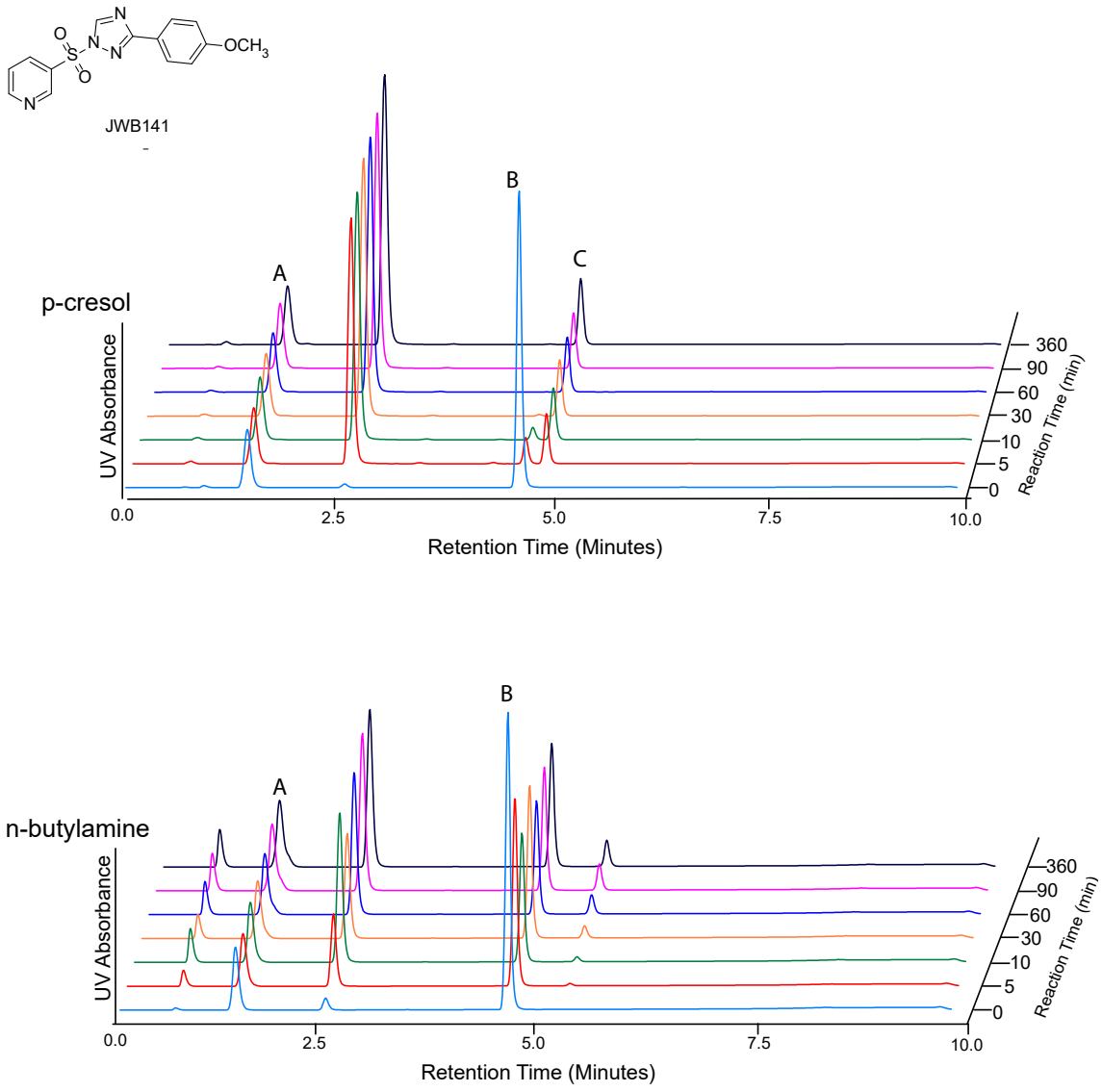


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB137 consumption ($T_{1/2\ cresol}$) was estimated to be 1.1 min using HPLC method B.

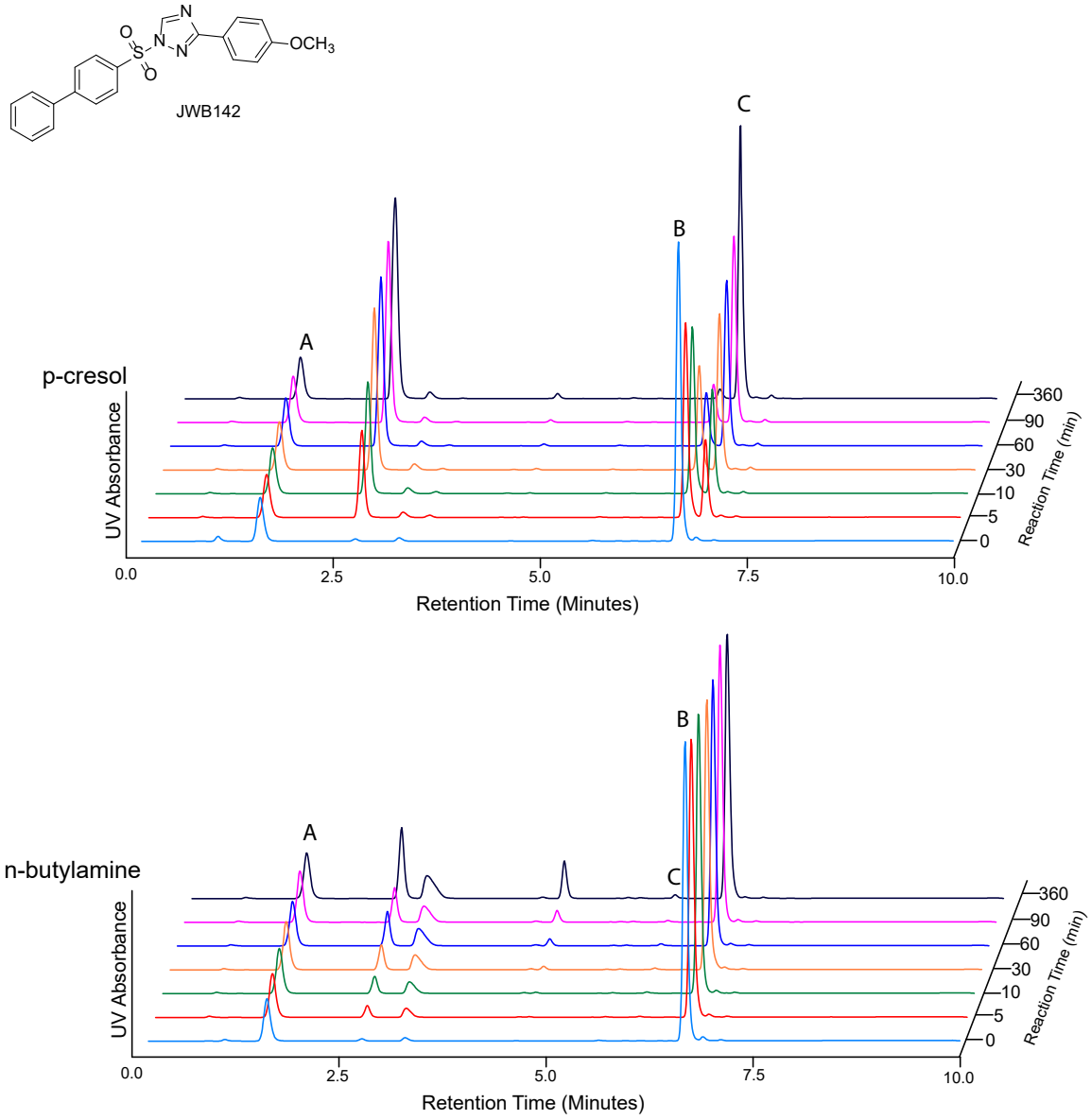


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB141 consumption ($T_{1/2\ cresol}$) was estimated to be 1.6 min using HPLC method B.

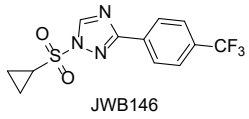


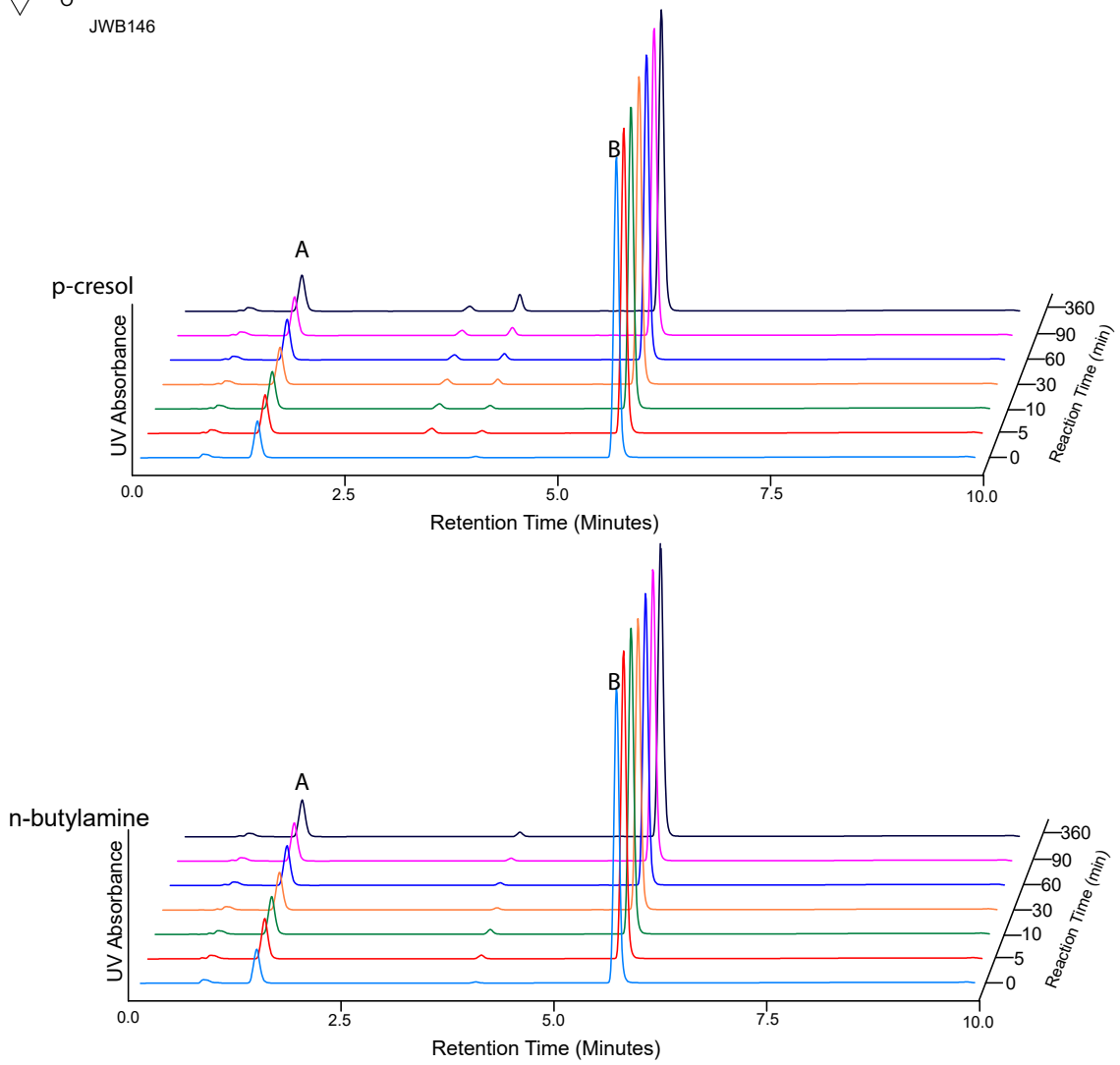
A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB142 consumption ($T_{1/2\ cresol}$) was estimated to be 17.3 min using HPLC method B.



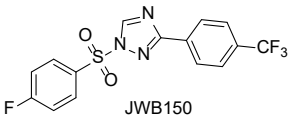


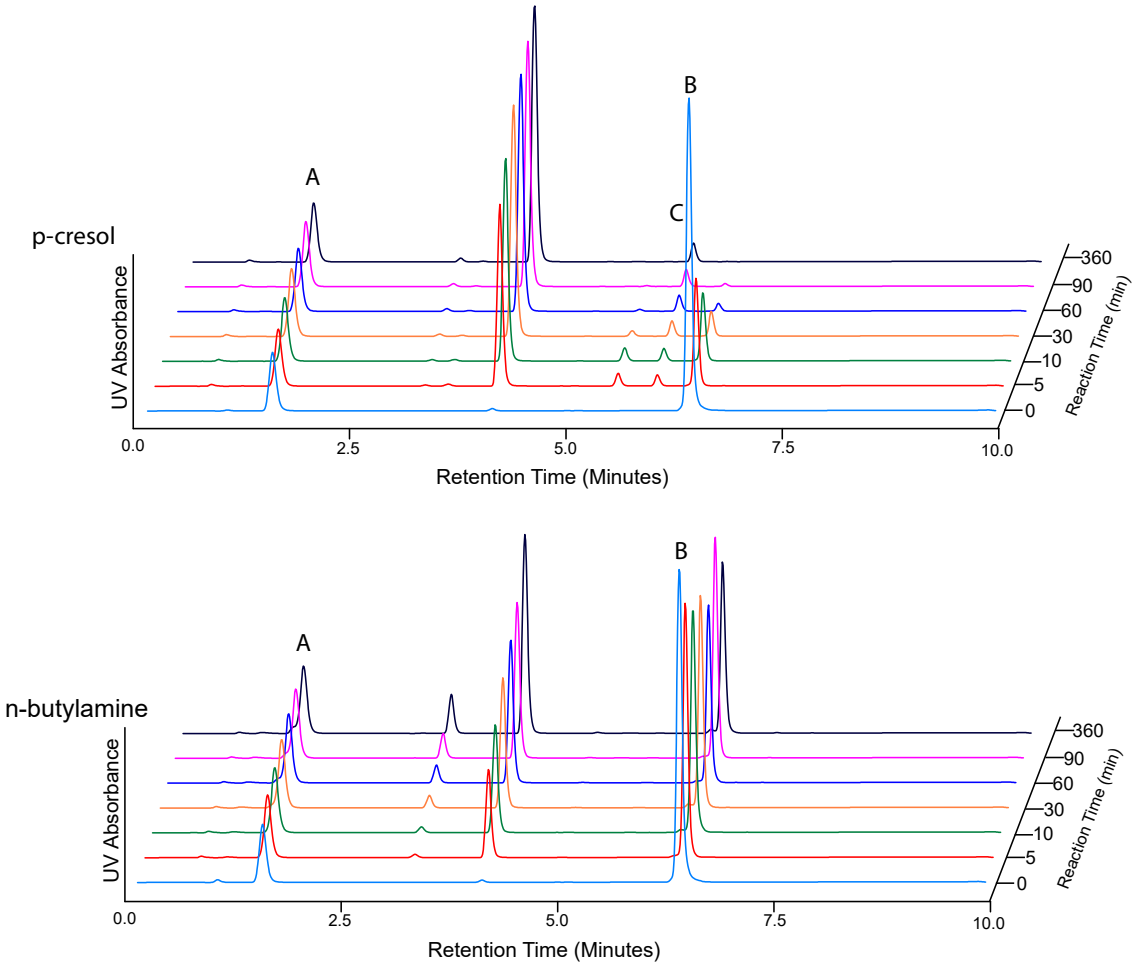
A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB146 consumption ($T_{1/2\ cresol}$) was estimated to be >360 min using HPLC method B.



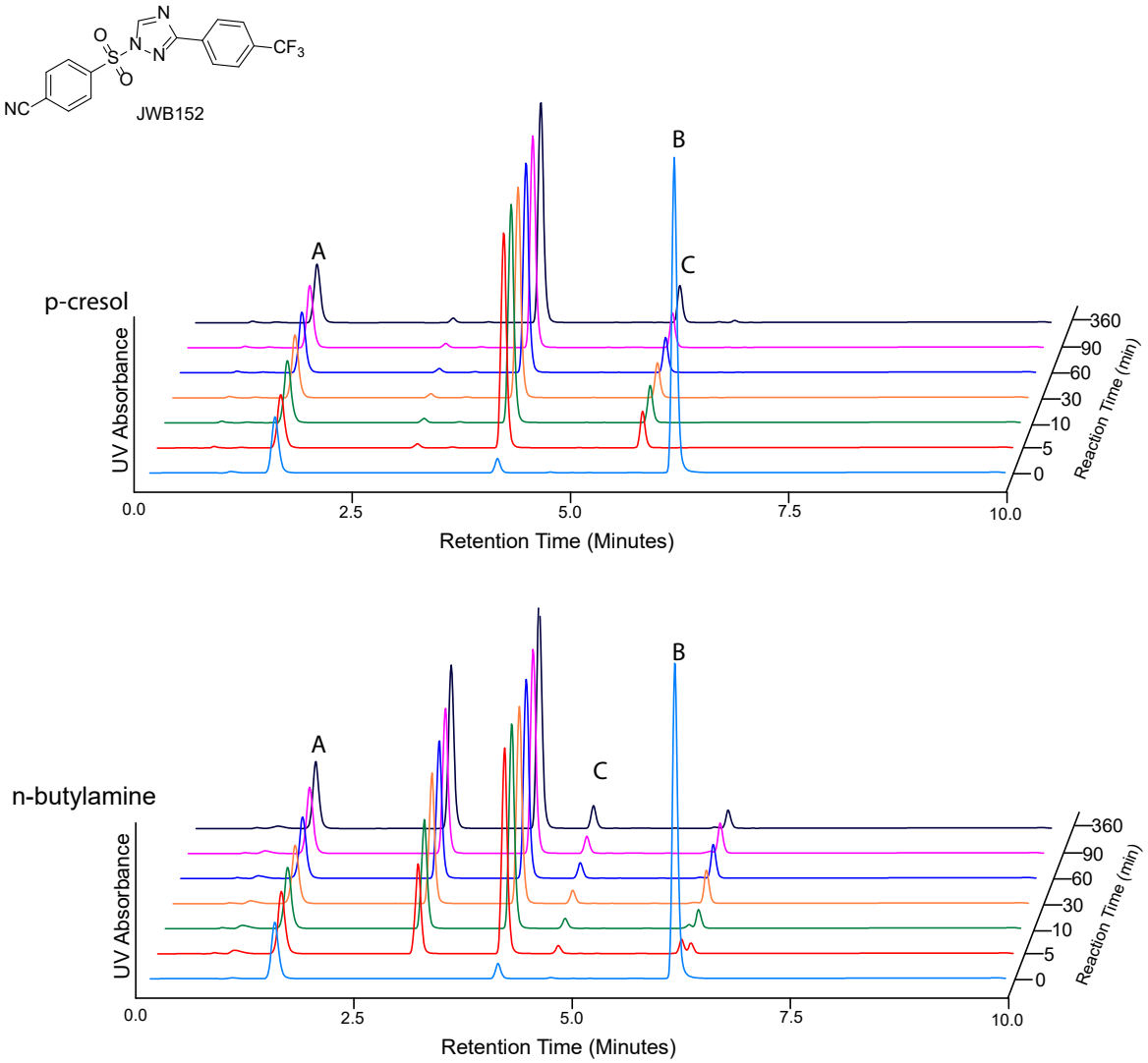


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB150 consumption ($T_{1/2\ cresol}$) was estimated to be 3.2 min using HPLC method B.

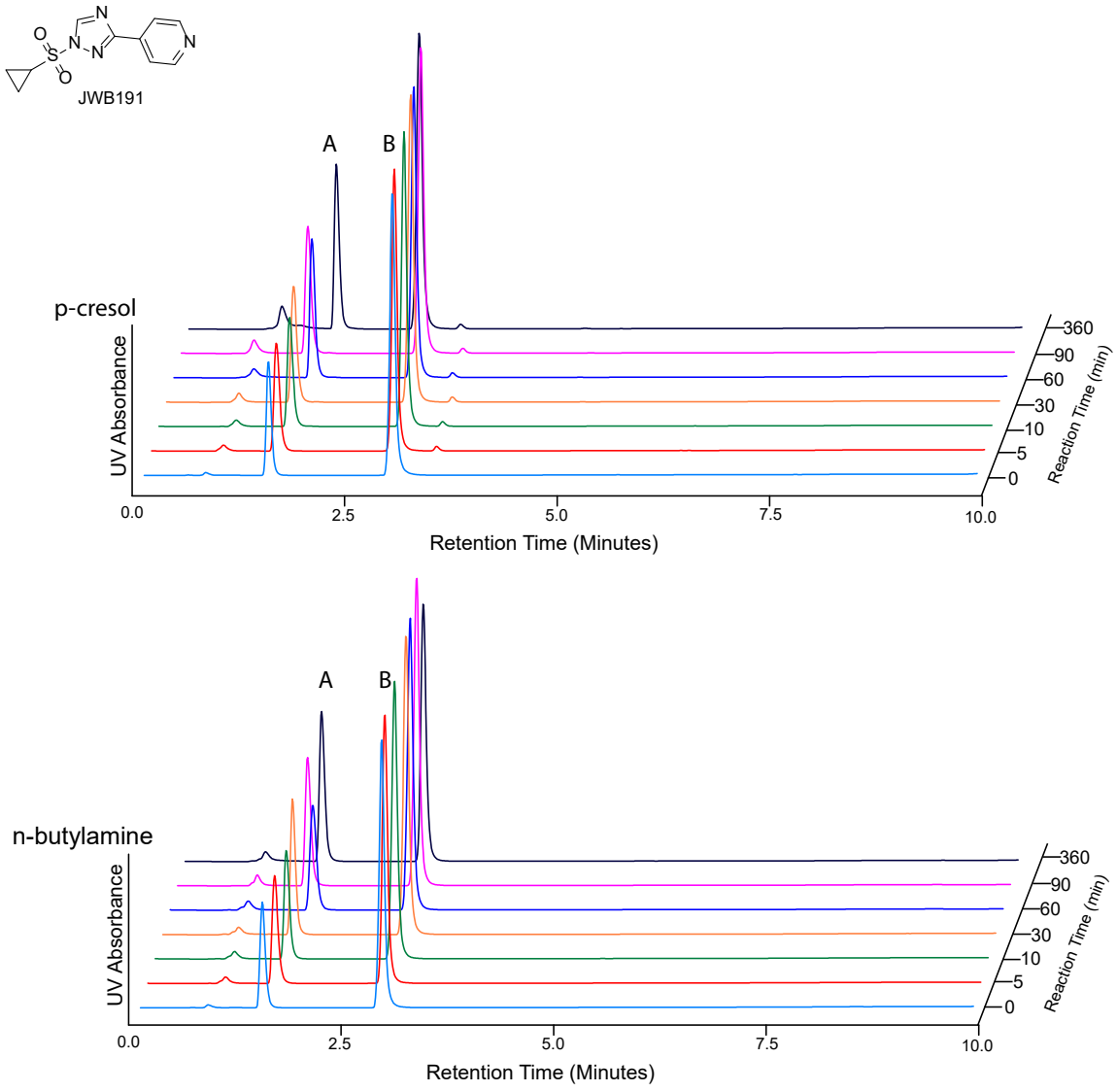


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB152 consumption ($T_{1/2\ cresol}$) was estimated to be 0.3 min using HPLC method B.

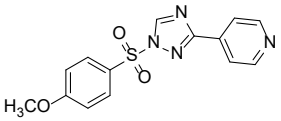


A = Internal Standard

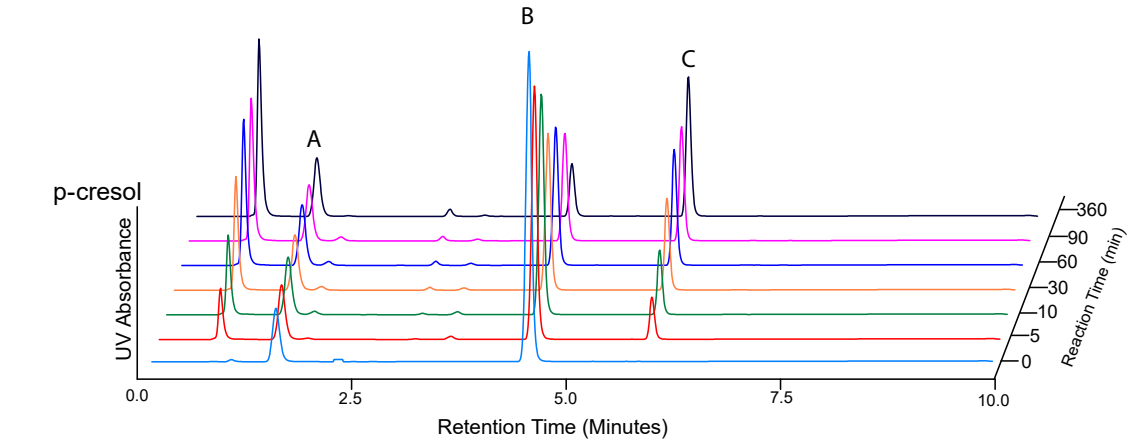
B = SuTEx Fragment

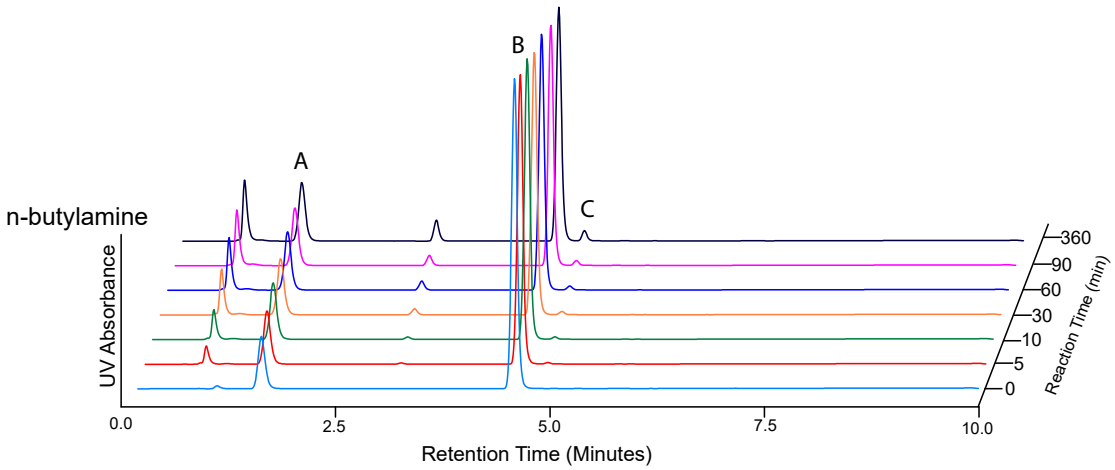
C = Sulfonate or Sulfonamide Adduct

The half-life for JWB191 consumption ($T_{1/2\ cresol}$) was estimated to be >360 min using HPLC method B.



JWB196



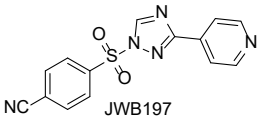


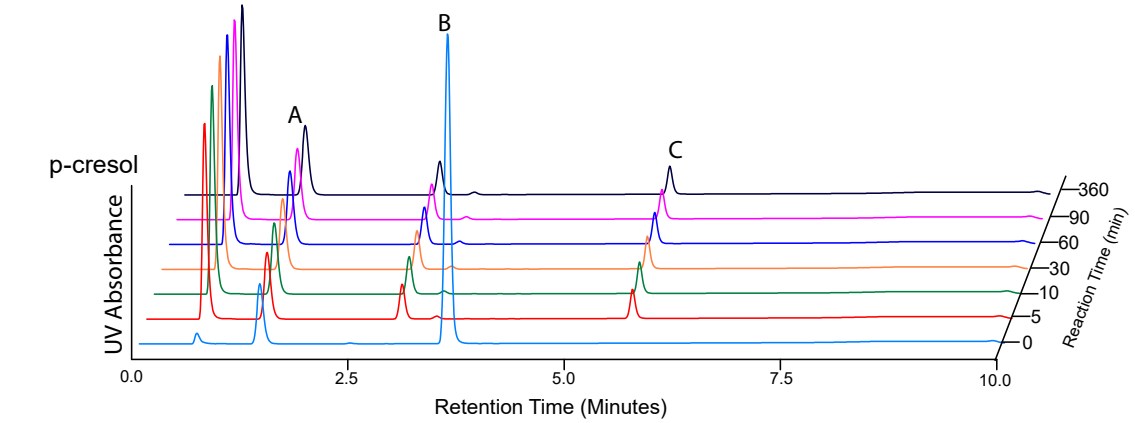
A = Internal Standard

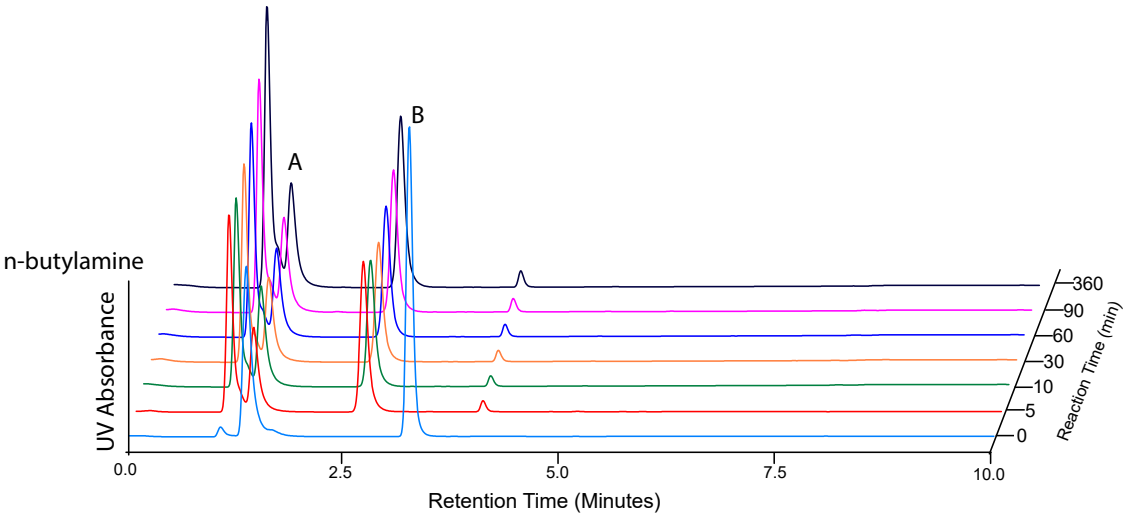
B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB196 consumption ($T_{1/2\ cresol}$) was estimated to be 15.4 min using HPLC method B.





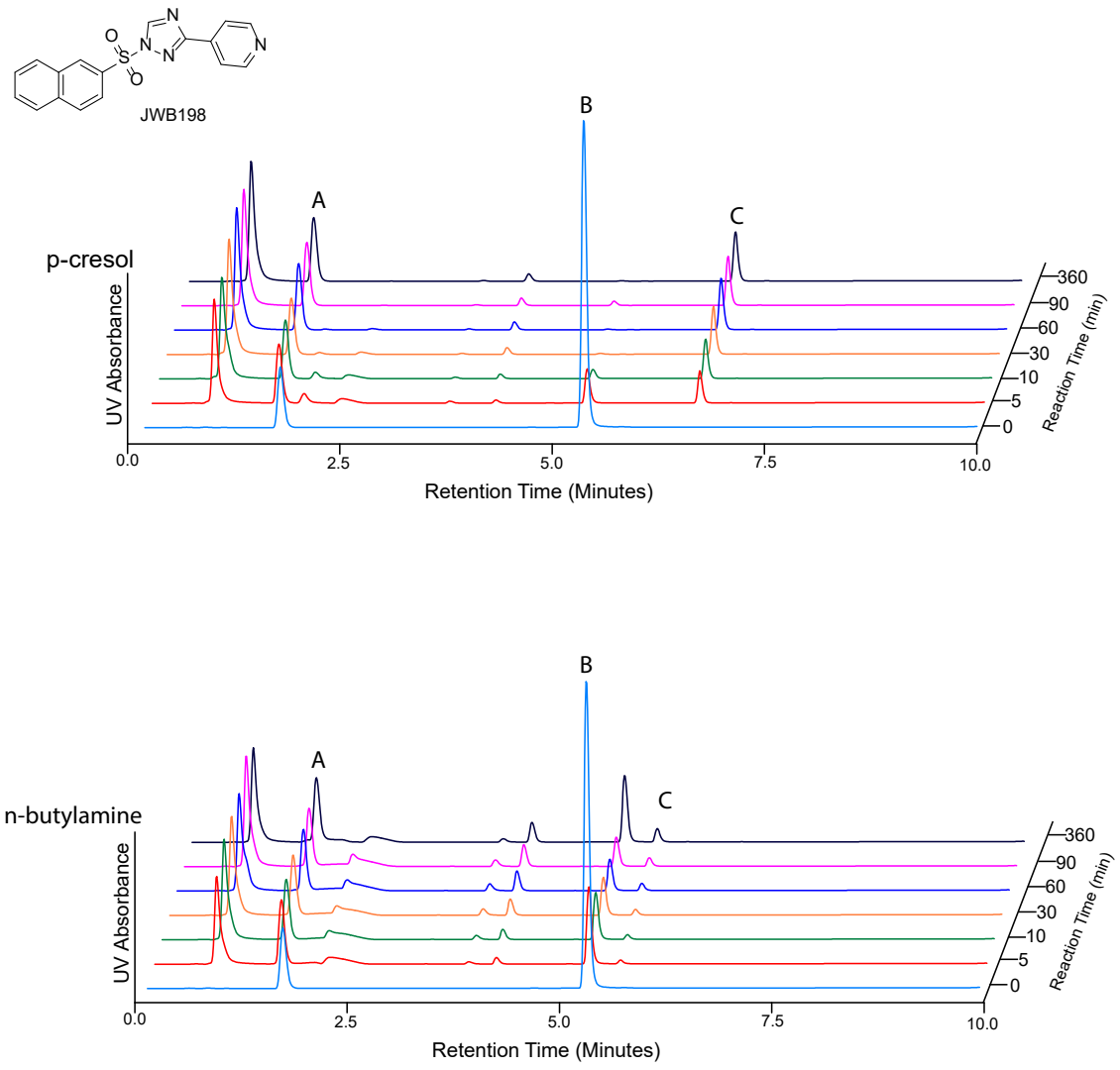


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB197 consumption ($T_{1/2\ cresol}$) was estimated to be 1.1 min using HPLC method B.

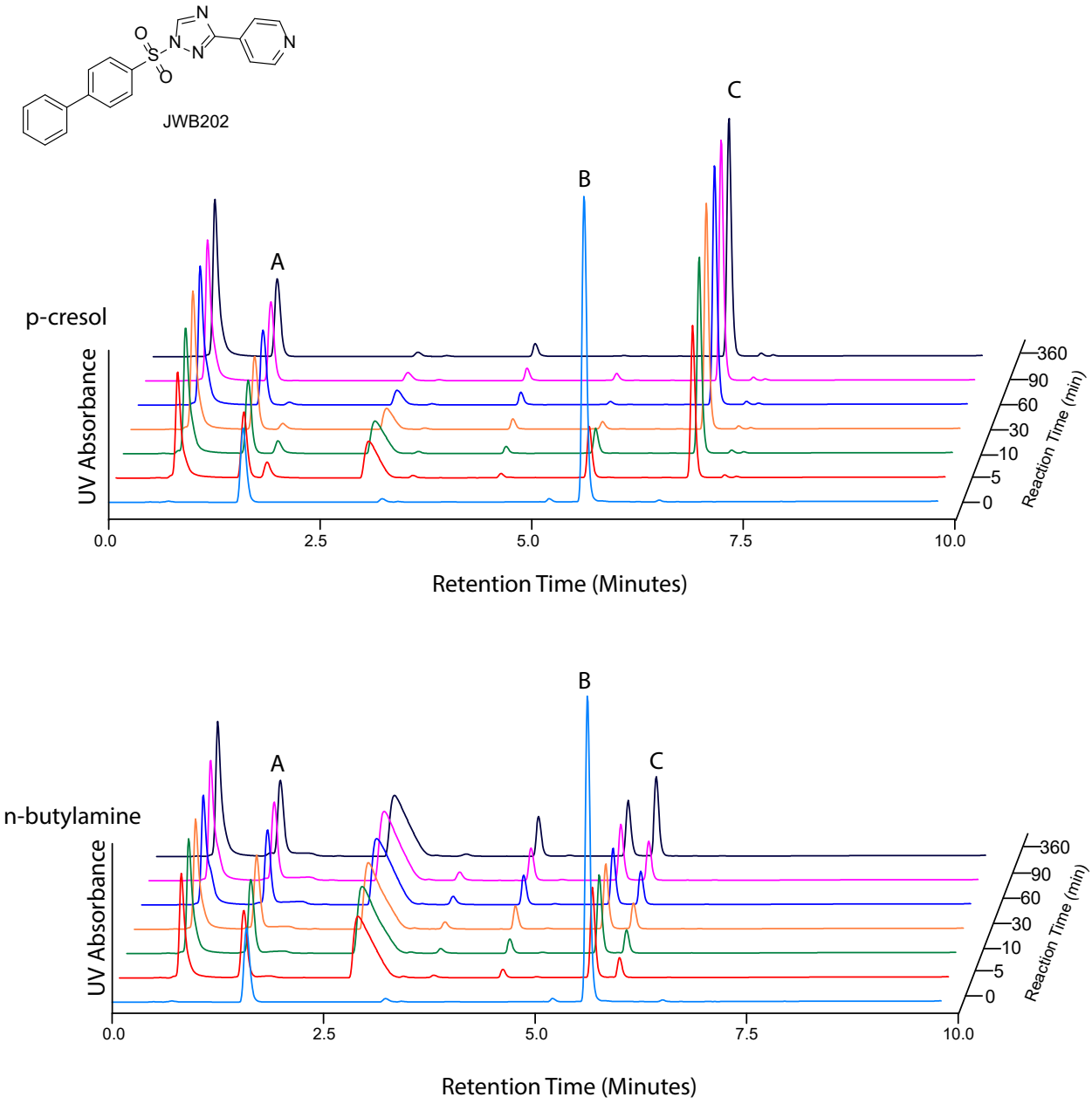


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB198 consumption ($T_{1/2\ cresol}$) was estimated to be 1.1 min using HPLC method B.

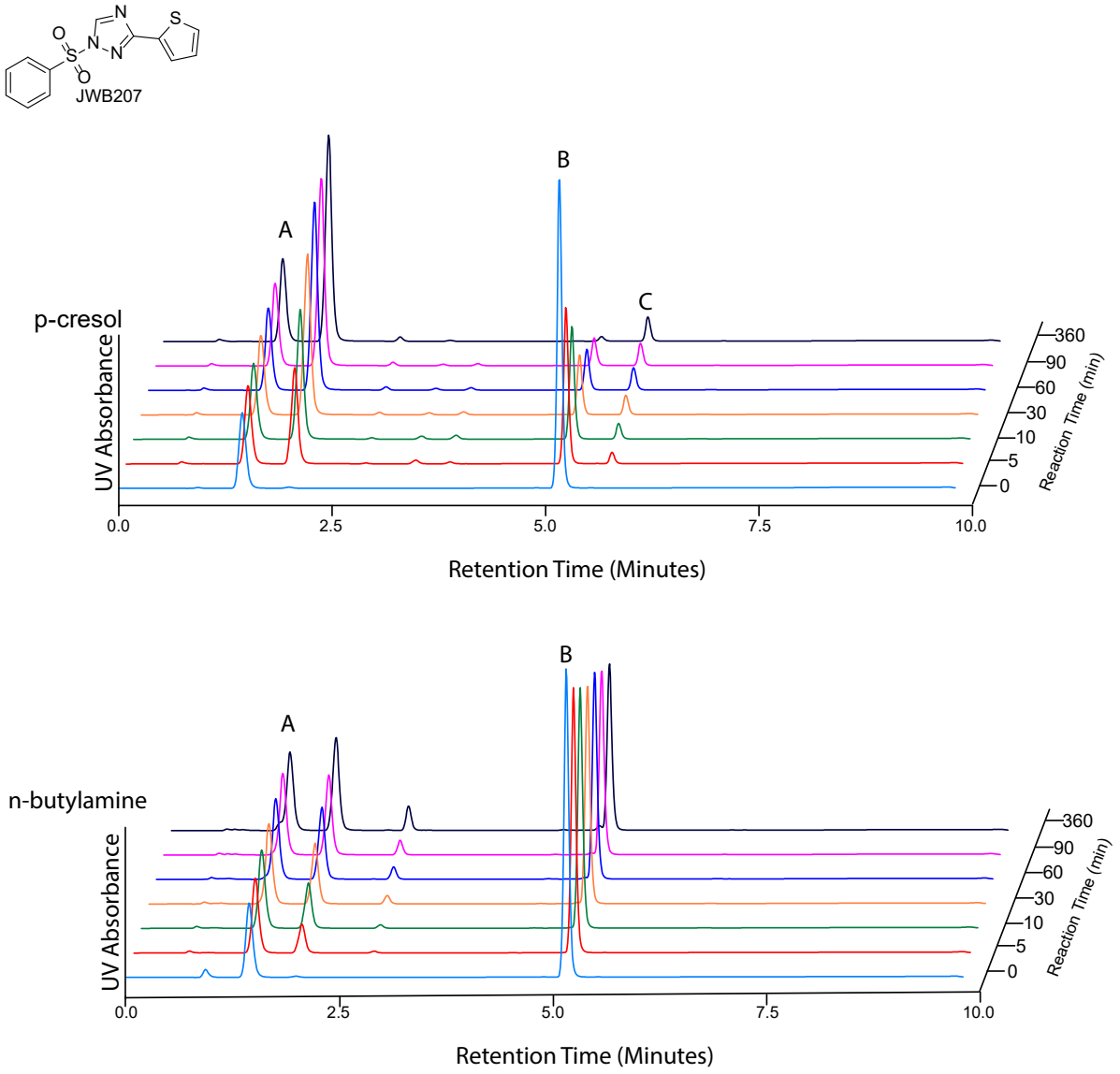


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB202 consumption ($T_{1/2\ cresol}$) was estimated to be 1.5 min using HPLC method B.

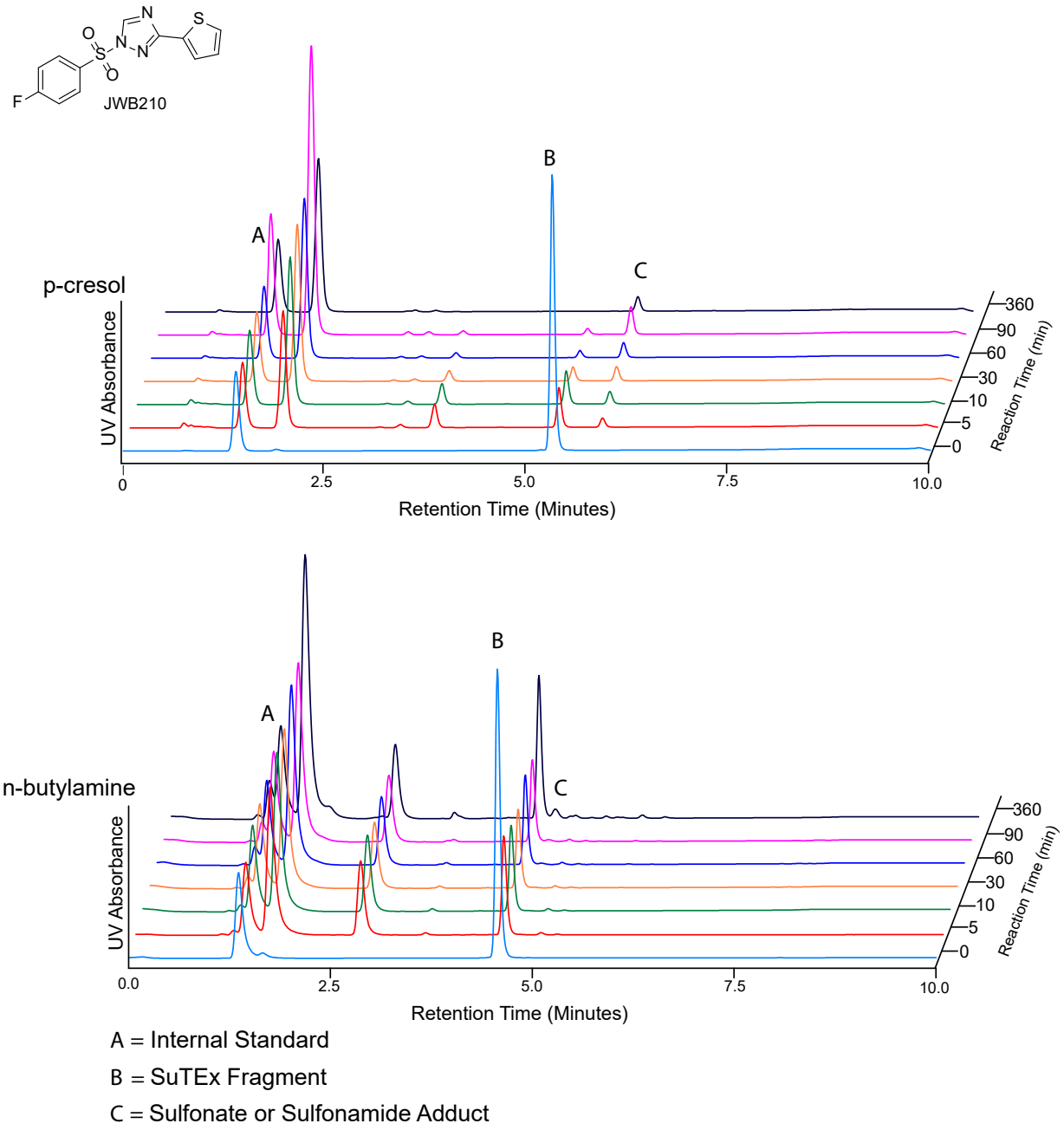


A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB207 consumption ($T_{1/2\ cresol}$) was estimated to be 4.9 min using HPLC method B.

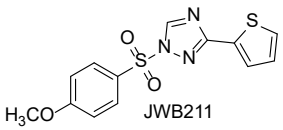


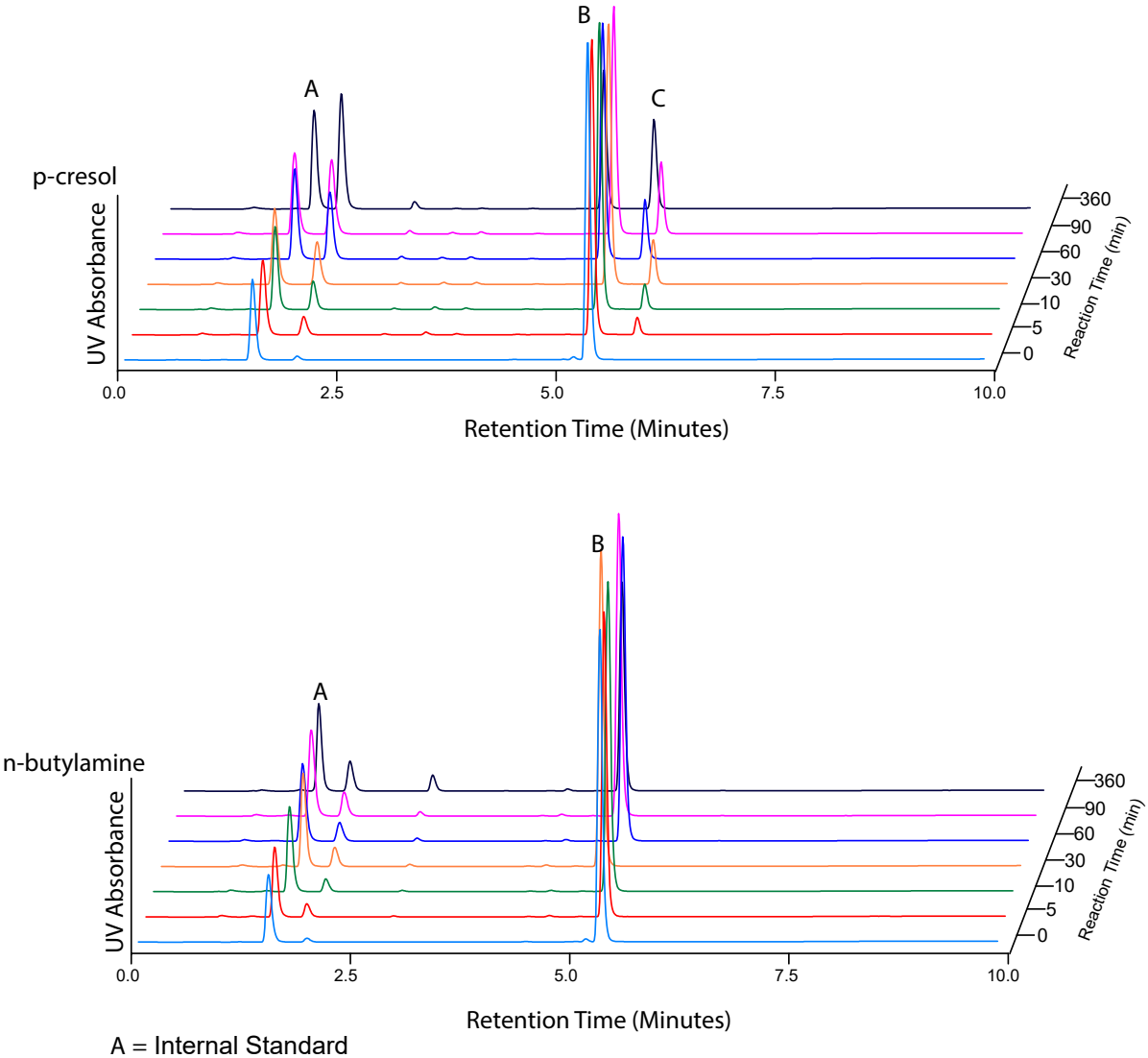
A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB210 consumption ($T_{1/2\ cresol}$) was estimated to be 3.6 min using HPLC method B.





A = Internal Standard

B = SuTEx Fragment

C = Sulfonate or Sulfonamide Adduct

The half-life for JWB211 consumption ($T_{1/2\ cresol}$) was estimated to be 80.2 min using HPLC method B.