Supporting Information for

Cefsulodin inspired potent and selective inhibitors of mPTPB, a virulent phosphatase from Mycobacterium tuberculosis

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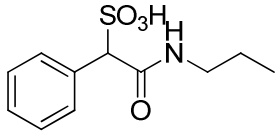
Materials and General Procedures

Reagents were used as purchased from Sigma-Aldrich and Fisher Scientific. ^{1}H and ^{13}C NMR spectra were obtained on a BrukerAvance II 500 MHz NMR spectrometer with TMS or residual solvent as standard. Mass spectra were obtained using an Agilent Technologies 6130 quadrupole LC/MS. HPLC purification was carried out on a Waters Delta 600 equipped with a Sunfire Prep C18 OBD column (30 mm/150 mm, 5 μm) with methanol water (both containing 0.1% TFA) as mobile phase (gradient: 50-100% methanol, flow 10 mL/min). The purity of all final tested compounds was established to be>95% by Agilent Technologies 6130 quadrupole LC/MS by using methanol water (both containing 0.1% TFA) as the mobile phase (gradient: 0-100% methanol, flow 1.0 mL/min), with UV monitoring at the fixed wavelength of 254 nm.

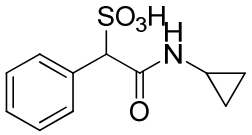
Representative Procedure for the Synthesis of Products

To α-Sulfophenyl acetyl chloride(0.234 g, 1 mmol) and DIEA (0.522 mL, 3 mmol) in DMF (2 mL) was added propyl amine (0.09 mL, 1.1mmol), the mixture was stirred at rt for 1 h. After quenching with water, it was subjected to HPLC purification, and product **1**was obtained as colorless oil (93% yield, >95% purity).

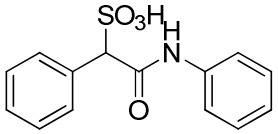
Characterizations of Compounds 1 to 9



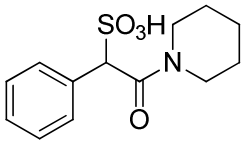
1:^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.45-7.44 (m, 2H), 7.27-7.20 (m, 3H), 4.42 (s, 1H), 3.11-3.02 (m, 2 H), 1.46-1.39 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H);^{13}C NMR (500 MHz, $CDCl_3$) δ 167.3, 135.7, 129.6, 127.4, 126.8, 71.5, 40.4, 22.3, 11.4. ESI-HRMS Cacld.for C11H16NO4S ($M+H^+$): m/z 258.0795; found 258.0799.



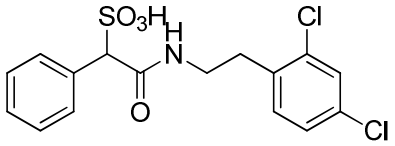
2:[1]H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.44-7.42 (m, 2H), 7.26-7.21 (m, 3H), 4.41 (s, 1H), 2.65-2.60 (m, 1 H), 0.64-0.61 (m, 2H), 0.39-0.38 (m, 2H); [13]C NMR ($CDCl_3$) δ 168.5, 135.5, 129.7, 127.4, 126.8, 71.2, 40.4, 22.6, 5.92, 5.90. ESI-HRMS Cacld.for C11H14NO4S ($M+H^+$): m/z 256.0638; found 256.0633.



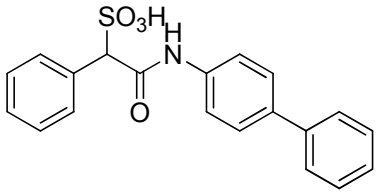
3: [1]H NMR (500 MHz, $CDCl_3$) δ 10.23 (s, 1H), 7.56-7.54 (m, 4H), 7.30-7.25 (m, 5H), 7.03 (t, *J* = 7.4 Hz, 1H), 4.76 (s, 1H); [13]C NMR ($CDCl_3$) δ 166.2, 139.2, 135.2, 130.1, 129.1, 127.8, 127.4, 123.7, 119.3, 72.2. ESI-HRMS Cacld.for C14H13NO4S ($M+H^+$): m/z 292.0638; found 292.0645.



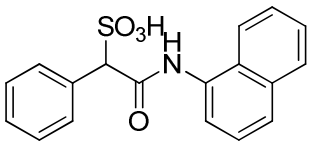
4:[1]H NMR (500 MHz, $CDCl_3$) δ 7.61-7.59 (m, 2H), 7.20-7.19 (m, 3H), 5.07 (s, 1H), 2.63-2.36 (m, 4H), 1.59-1.19 (m, 6H). ESI-HRMS Cacld. for C13H18NO4S ($M+H^+$): m/z 284.0951; found 284.0955.



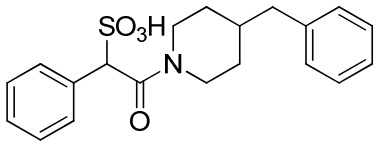
5:[1]H NMR (500 MHz, $CDCl_3$) δ 8.31 (t, *J* = 5.5 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 7.40-7.34 (m, 3H), 7.28-7.22 (m, 4H), 4.43 (s, 1H), 3.40-3.29 (m, 2H), 2.83 (t, , *J* = 7.0 Hz, 2H); [13]C NMR ($CDCl_3$) δ 167.5, 135.8, 135.5, 134.0, 132.7, 131.7, 129.6, 128.5, 127.3, 127.3, 126.8. ESI-HRMS Cacld.for C16H16Cl2NO4S ($M+H^+$): m/z 388.0172; found 388.0174.



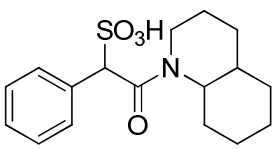
6:[1]H NMR (500 MHz, $CDCl_3$) δ 10.4 (s, 1H), 7.69-7.58 (m, 8H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.33-7.24 (m, 4H), 4.80 (s, 1H); [13]C NMR ($CDCl_3$) δ 166.0, 139.7, 138.6, 135.2, 134.8, 129.9, 128.9, 127.4, 127.0, 127.0, 126.6, 126.2, 119.3, 71.8. ESI-HRMS Cacld.for C20H18NO4S ($M+H^+$): m/z 368.0951; found 368.0947.



7:[1]H NMR (500 MHz, $CDCl_3$) δ 10.9 (s, 1H), 8.25 (d, *J* = 8.3 Hz, 1H), 8.03 (d, *J* = 7.2 Hz, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.62-7.55 (m, 4H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.33-7.26 (m, 3H), 4.80 (s, 1H); [13]C NMR ($CDCl_3$) δ 166.4, 135.3, 133.7, 133.5, 129.7, 128.4, 127.7, 127.1, 126.2, 126.1, 126.1, 125.7, 124.3, 121.5, 118.2, 72.1. ESI-HRMS Cacld.for C18H15NO4S ($M+H^+$): m/z 342.0795; found 342.0799.

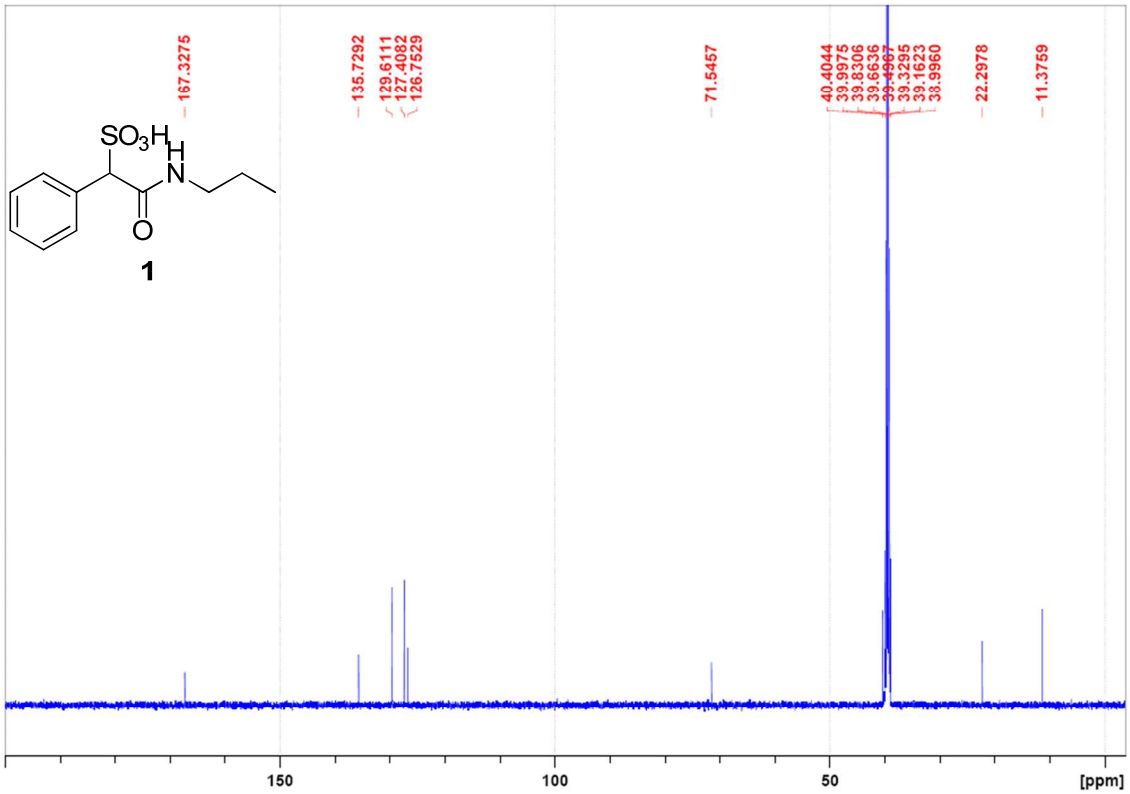


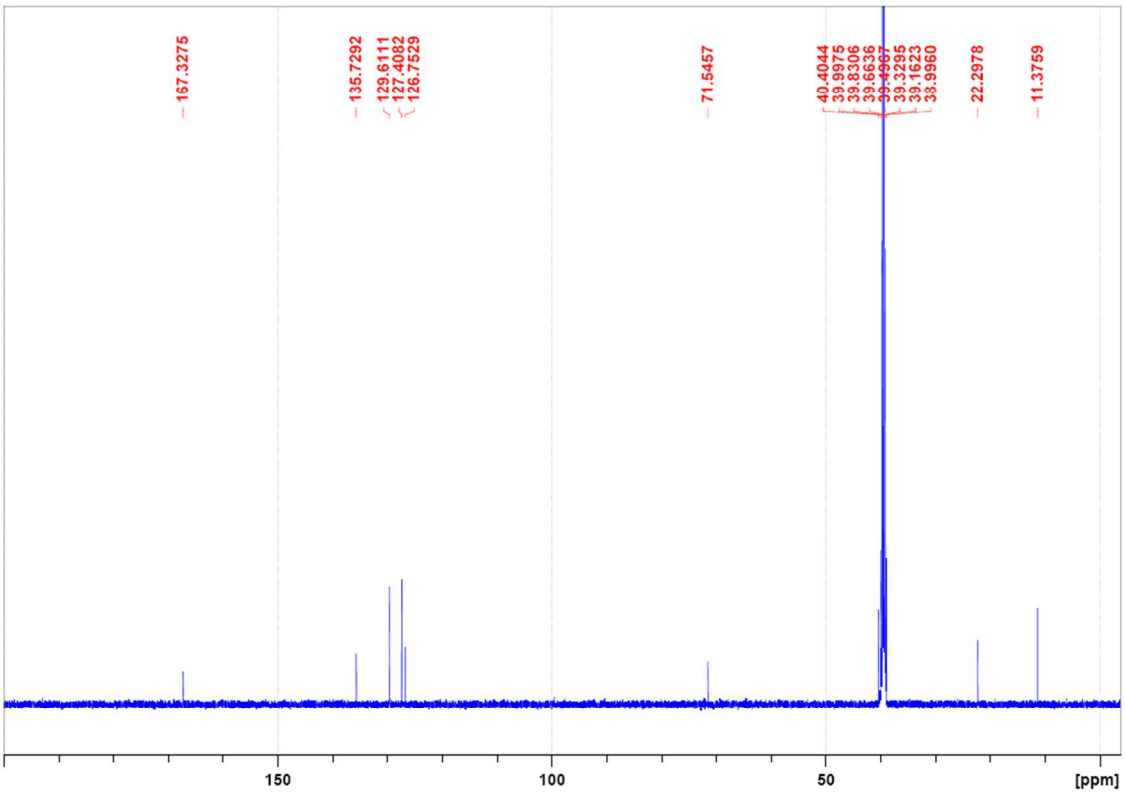
8:[1]H NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1H),7.60 (s, 1H), 7.28-7.10 (m, 8H),5.15 (s, 1H), 4.35 (d, *J* = 12.1 Hz, 1H), 4.23 (t, *J* = 14.5 Hz, 1H), 2.95 (t, *J* = 12.9 Hz, 0.5H), 2.86 (t, *J* = 11.6 Hz, 0.5H), 2.49-2.49 (m, 2H), 1.71 (br, 1H), 1.51-1.48 (br, 2H), 1.36 (q, *J* = 10.8 Hz, 0.5H), 1.02 (q, *J* = 10.7 Hz, 0.5H), 0.84-0.74 (m, 1H); [13]C NMR ($CDCl_3$) δ 165.4, 158.5, 158.2, 140.2, 140.1, 135.8, 130.6, 129.0, 128.2, 127.0, 127.0, 126.6, 125.8, 116.1, 113.9, 99.5, 65.5, 46.6, 46.0, 42.2, 42.1, 41.9, 41.6, 37.5, 32.5, 31.8, 31.5, 31.4. ESI-HRMS Cacld.for C20H24NO4S ($M+H^+$): m/z 374.1421; found 374.1428.

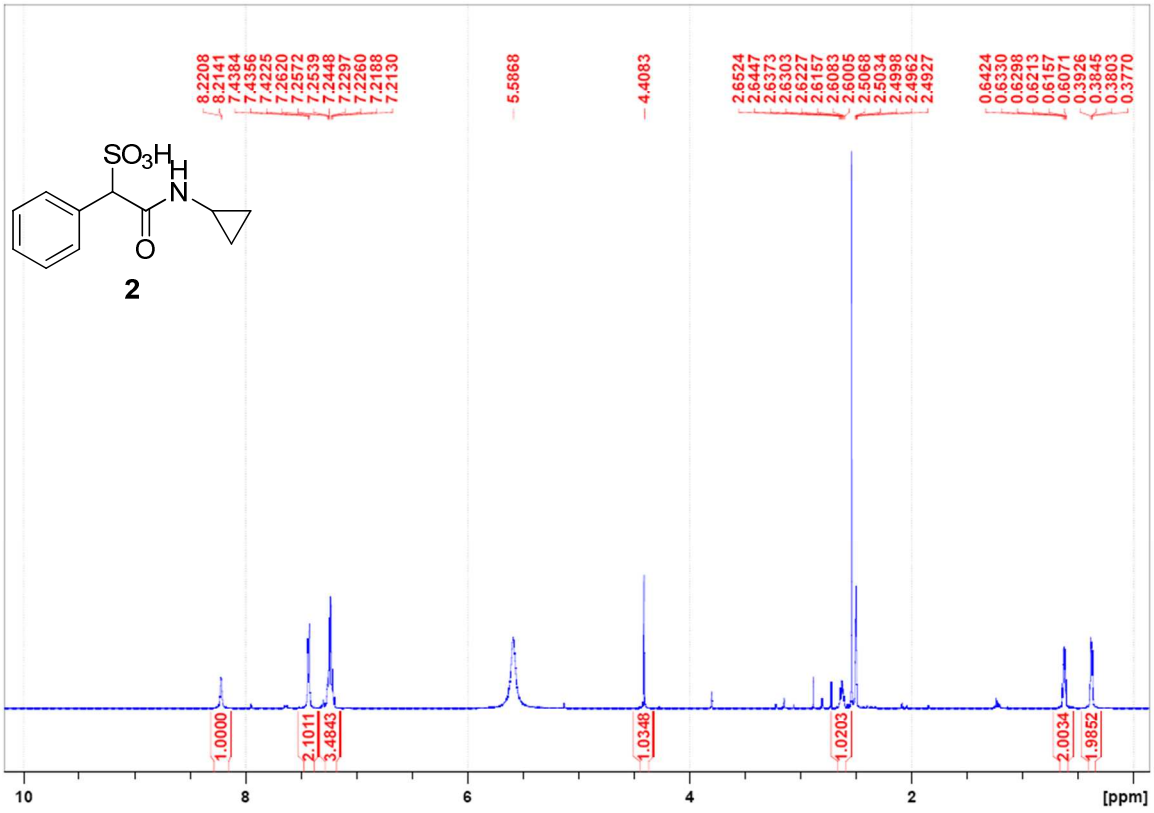


9: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (d, 2H), 7.24-7.20 (m, 3H), 5.02 (s, 0.5H), 4.95 (s, 0.5H), 3.92-3.90 (m, 1H), 3.19-3.08 (m, 1H), 1.94-0.92 (m, 14H); ^{13}C NMR ($CDCl_3$) δ166.6, 158.5, 158.2, 135.7, 135.7, 130.5, 130.4, 127.2, 127.1, 126.7, 116.1, 113.9, 99.5, 66.4, 61.1, 60.9, 37.6, 32.8, 32.6, 25.8, 25.8, 25.6, 25.1, 22.3. ESI-HRMS Cacld. for C17H24NO4S ($M+H^+$): m/z 338.1421; found 338.1426.

Copies of ^{1}H and ^{13}C NMR Spectra of Compounds 1 to 9



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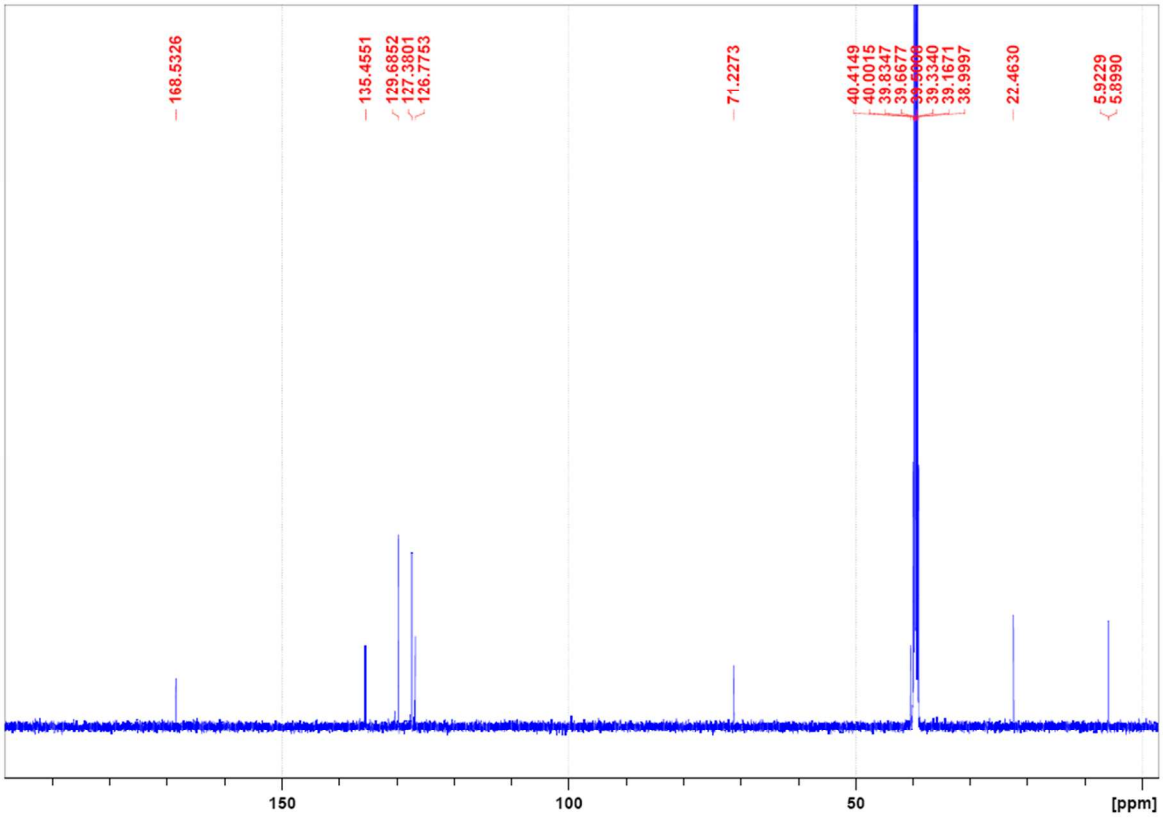
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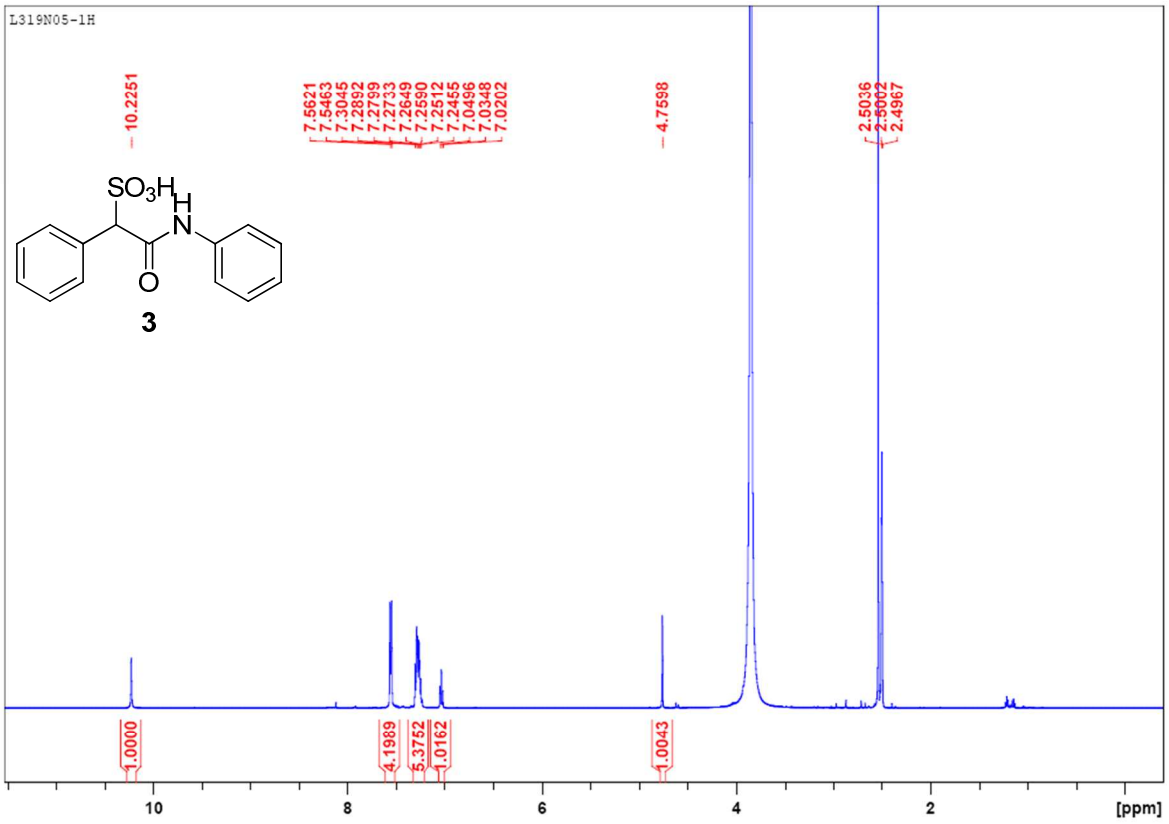
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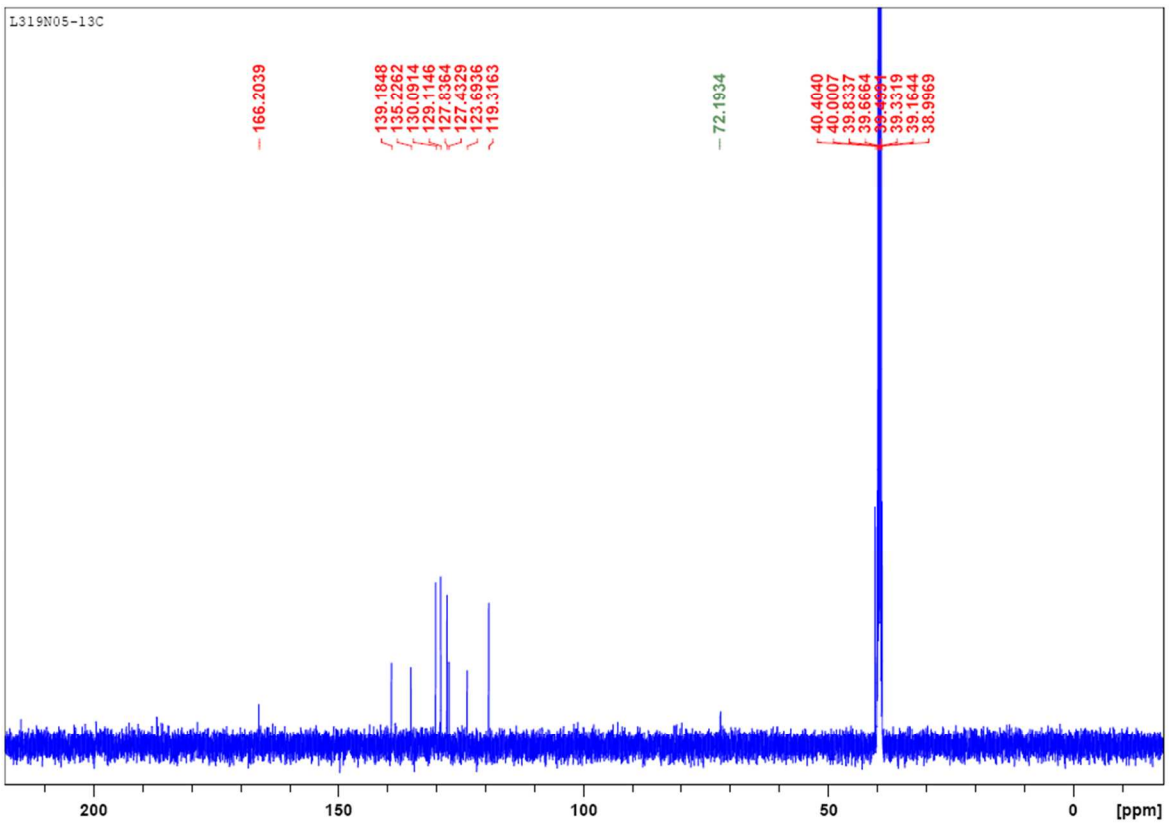
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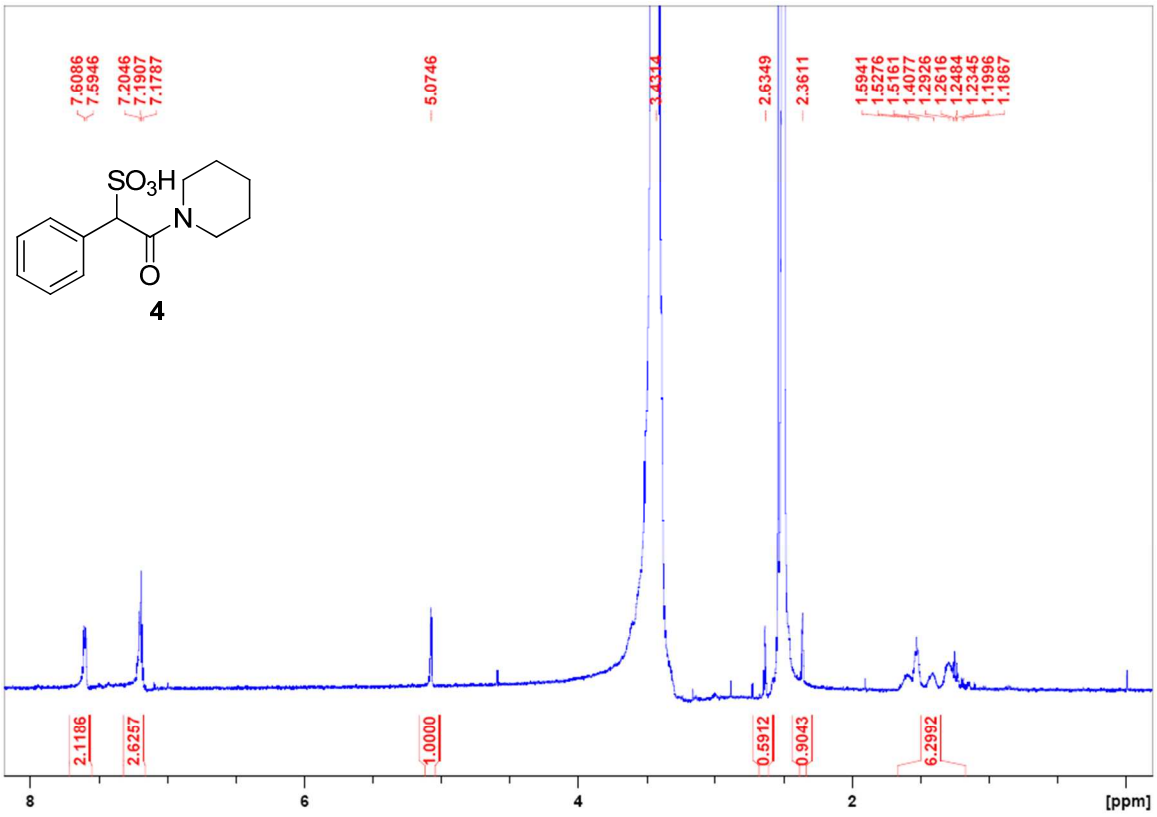
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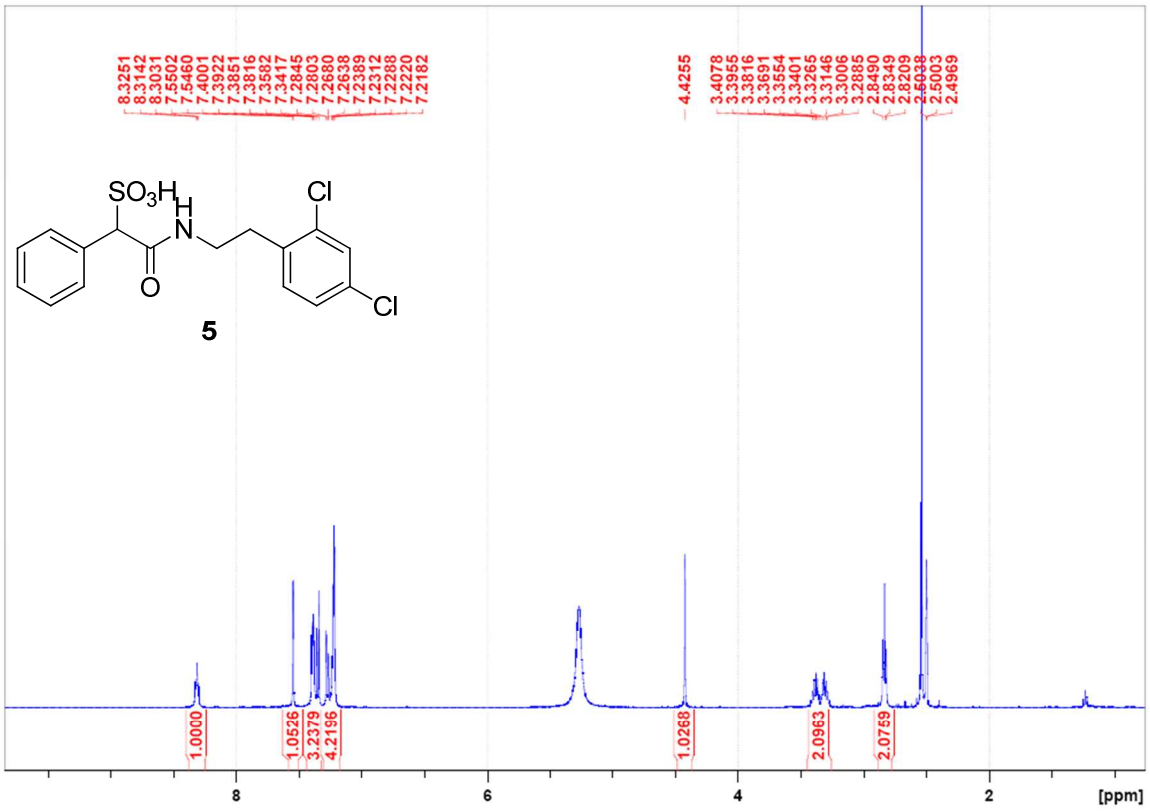
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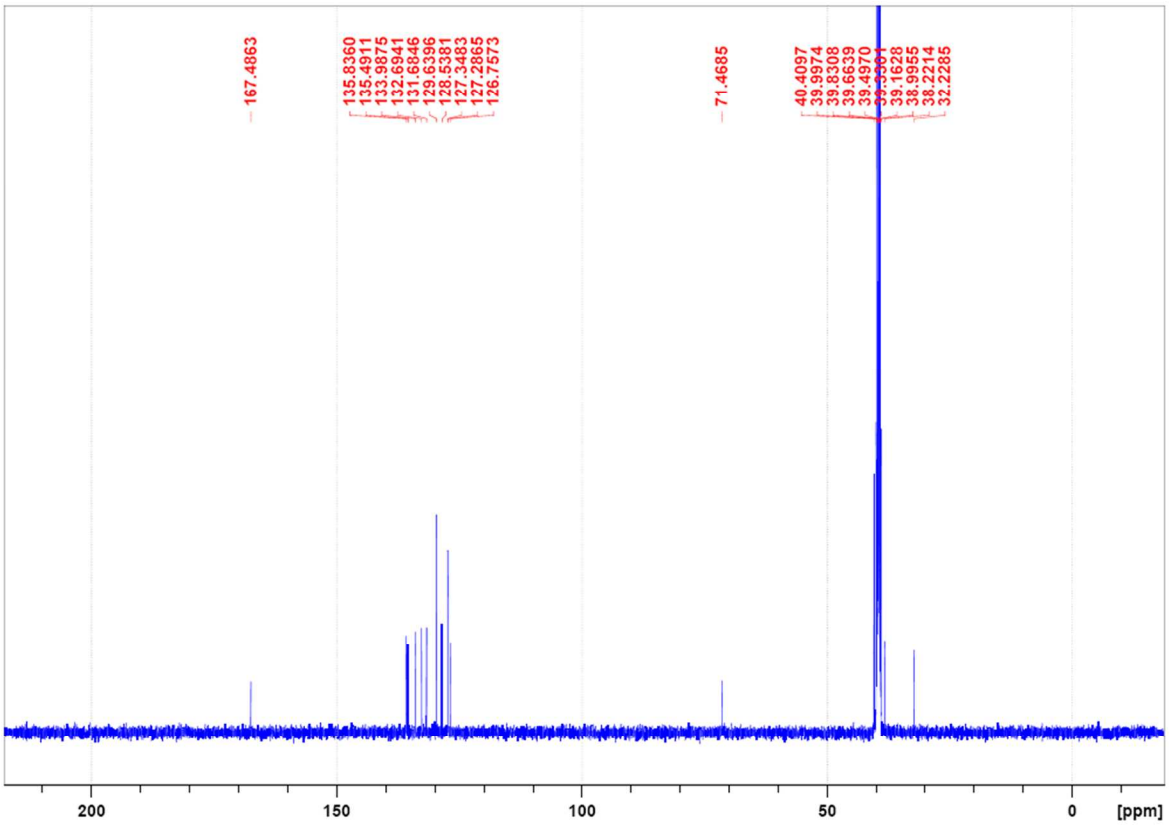
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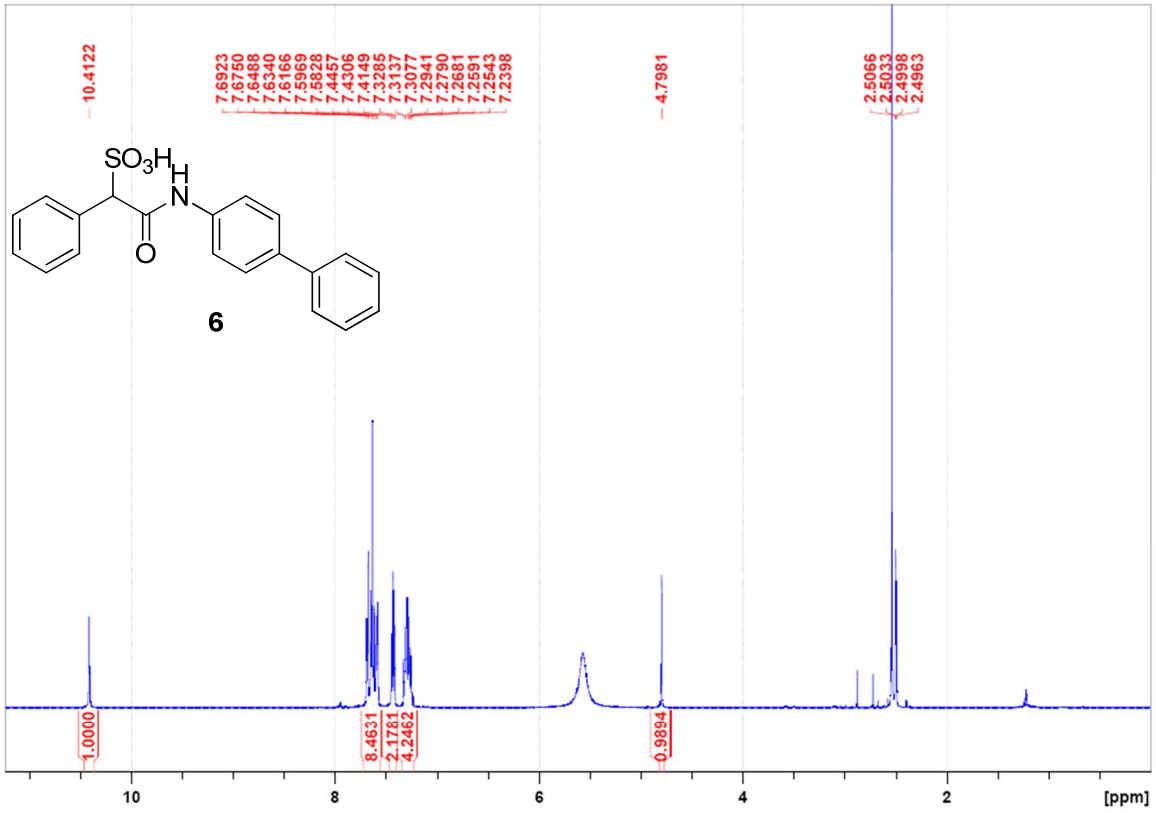
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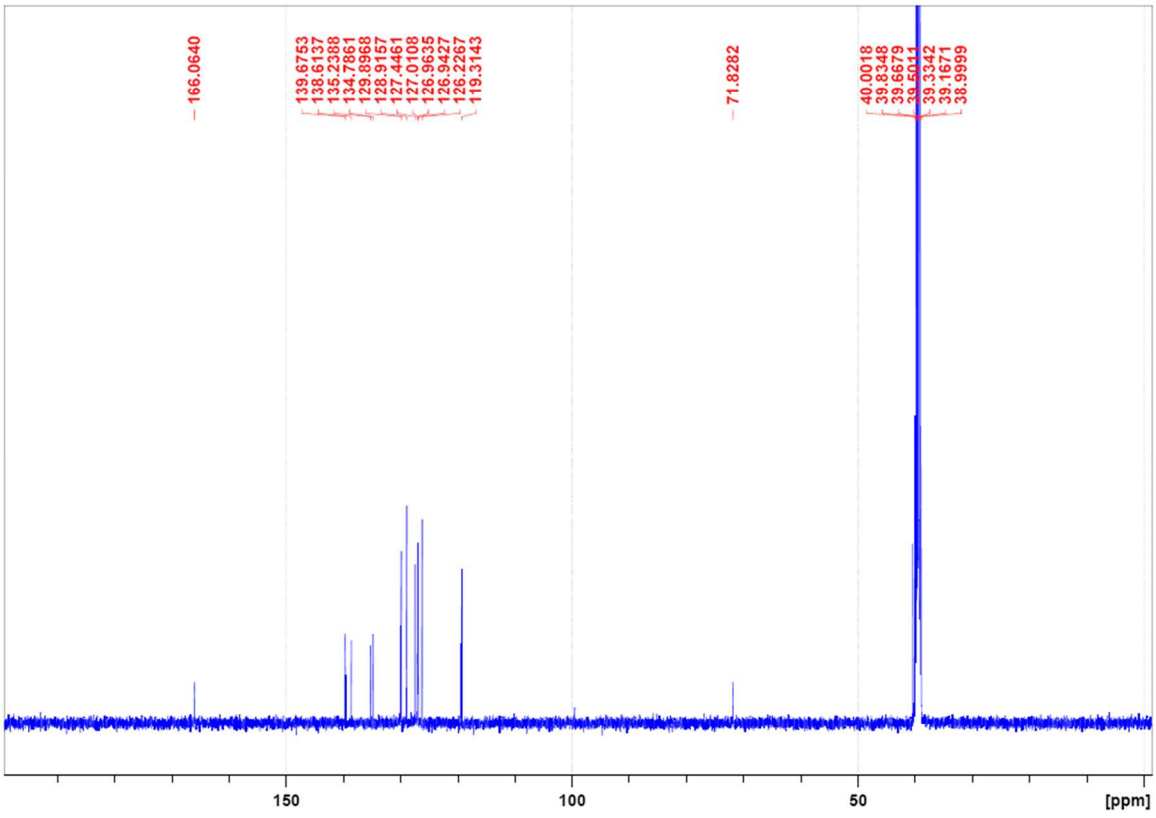
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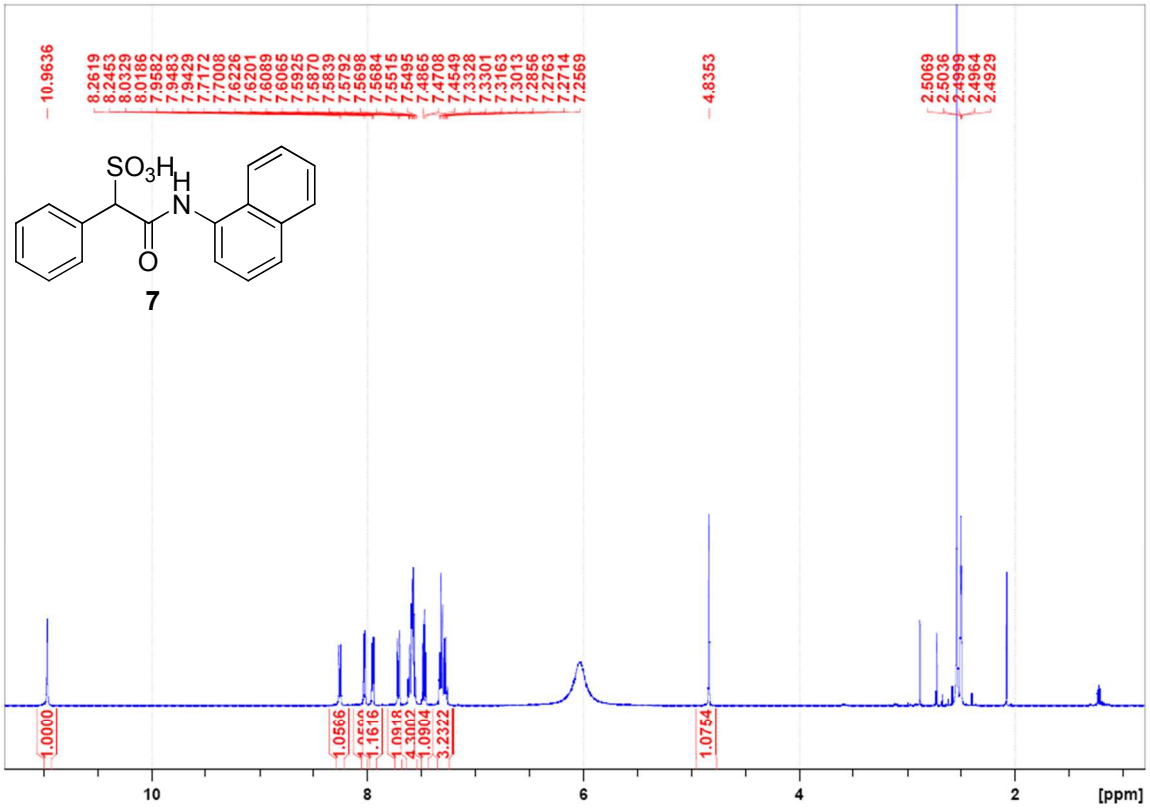
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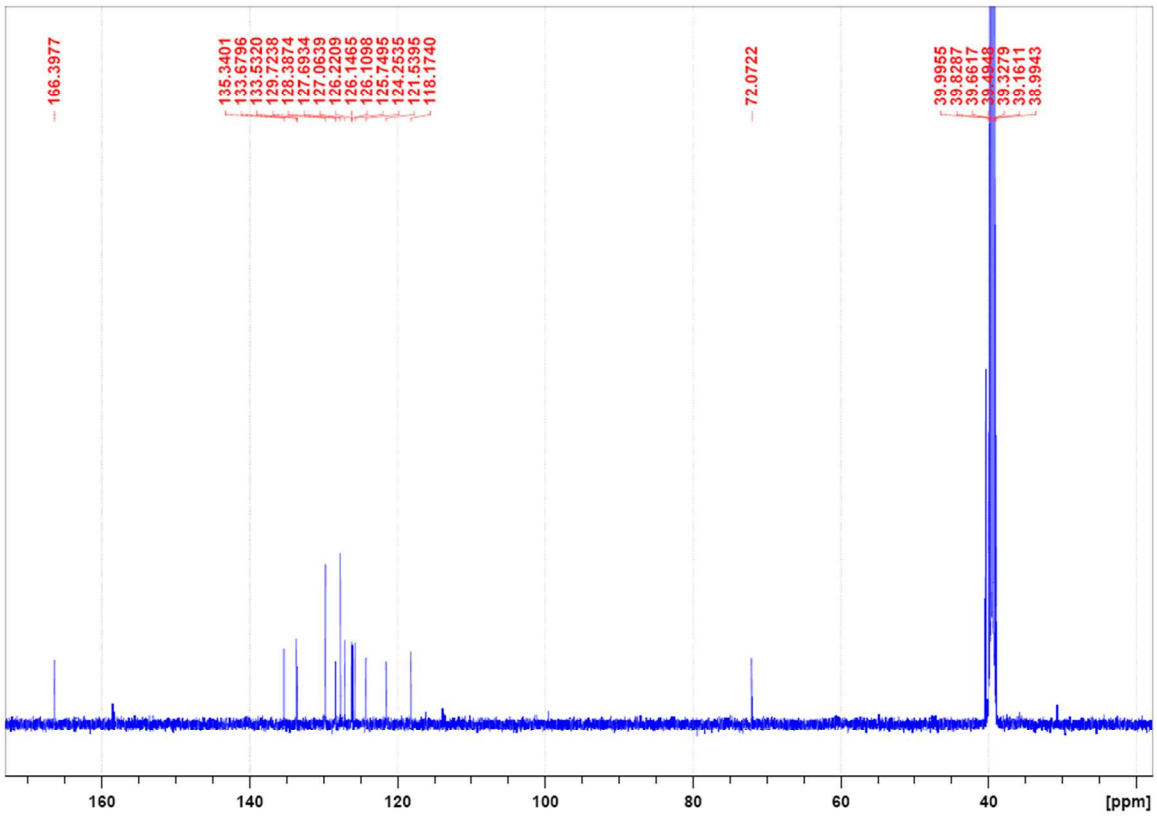
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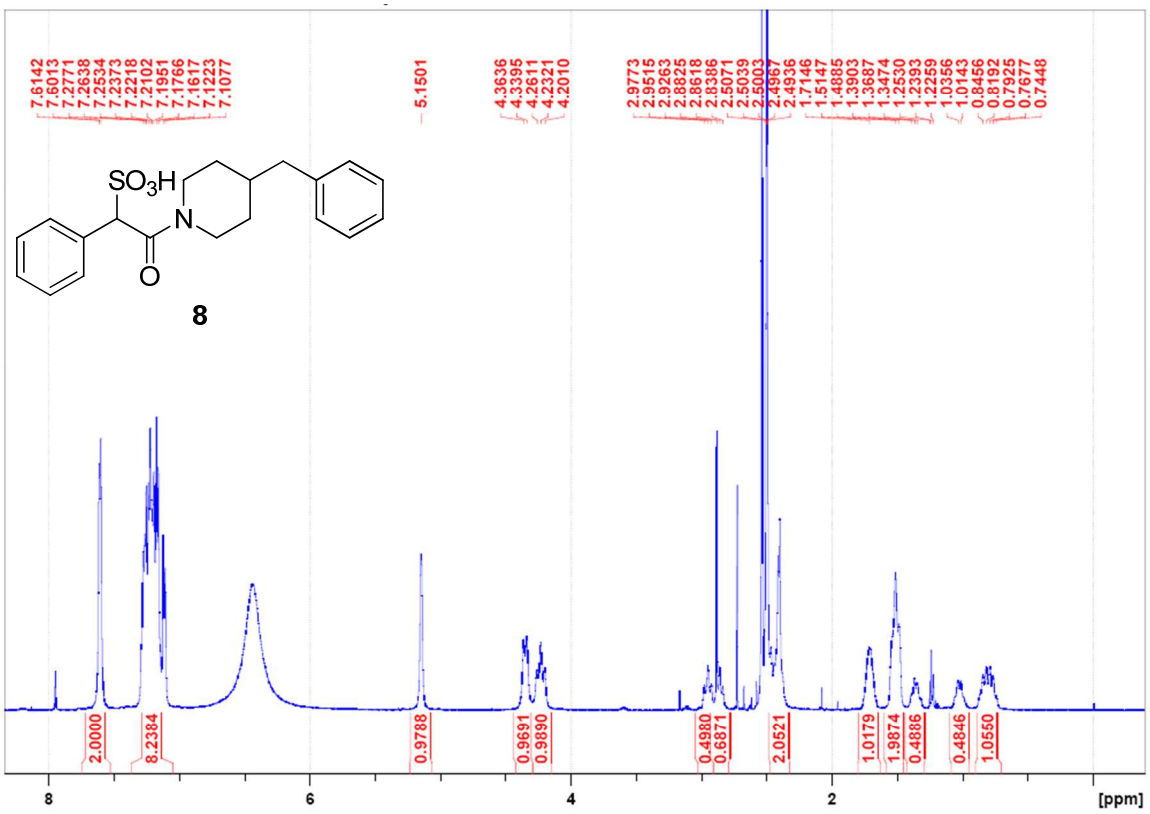
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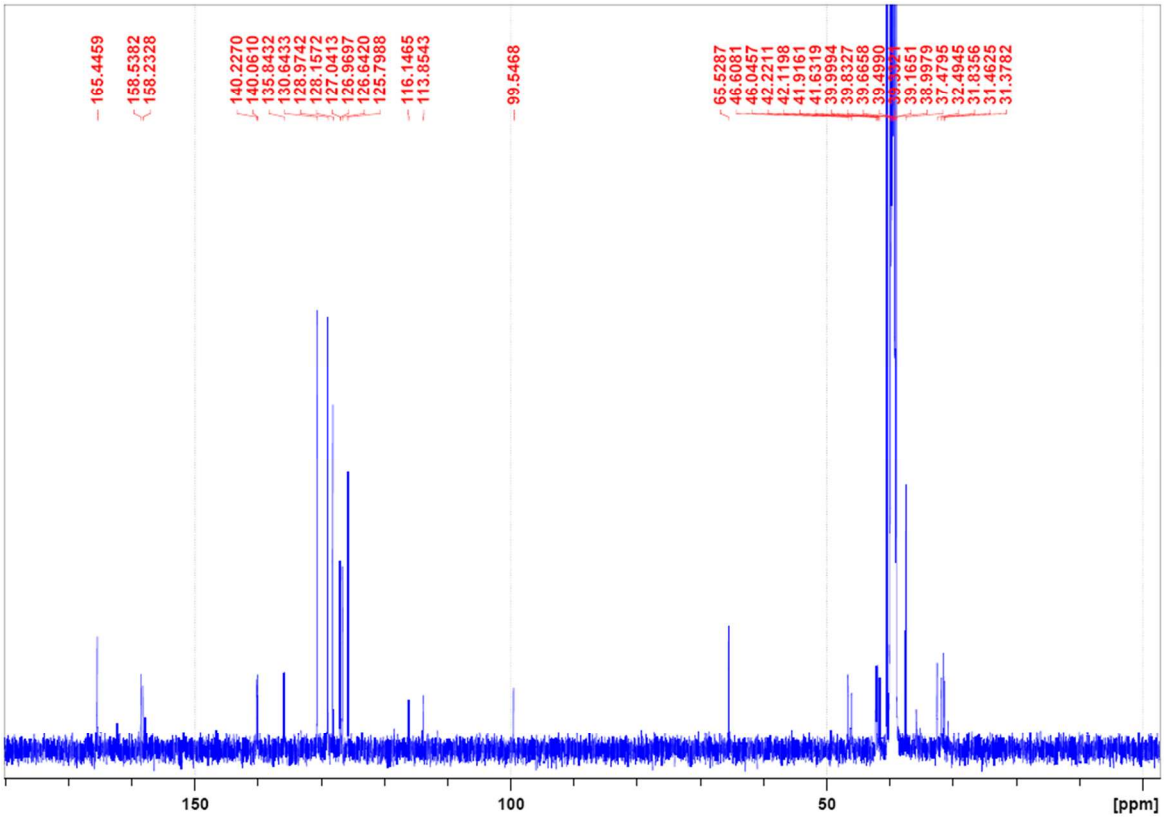
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113.8543
99.5468
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46.6081
46.0457
42.2211
42.1198
41.9161
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39.8327
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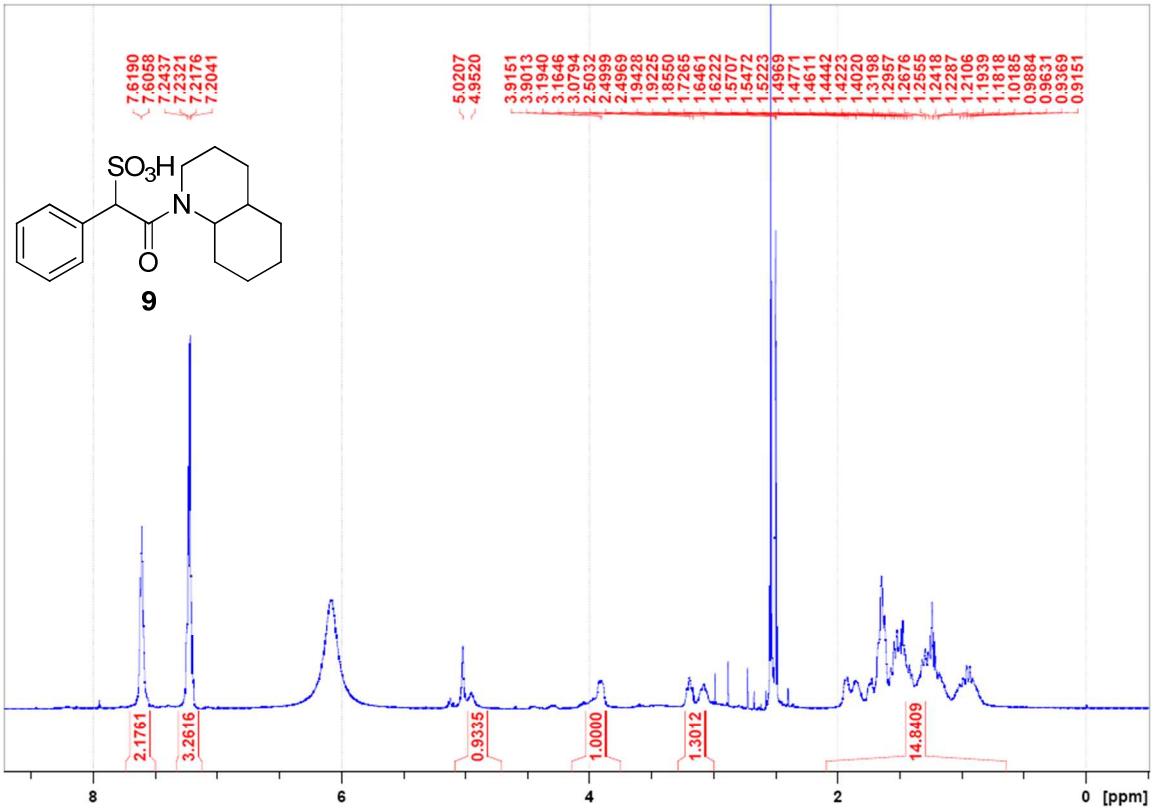
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SO3H
N
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4
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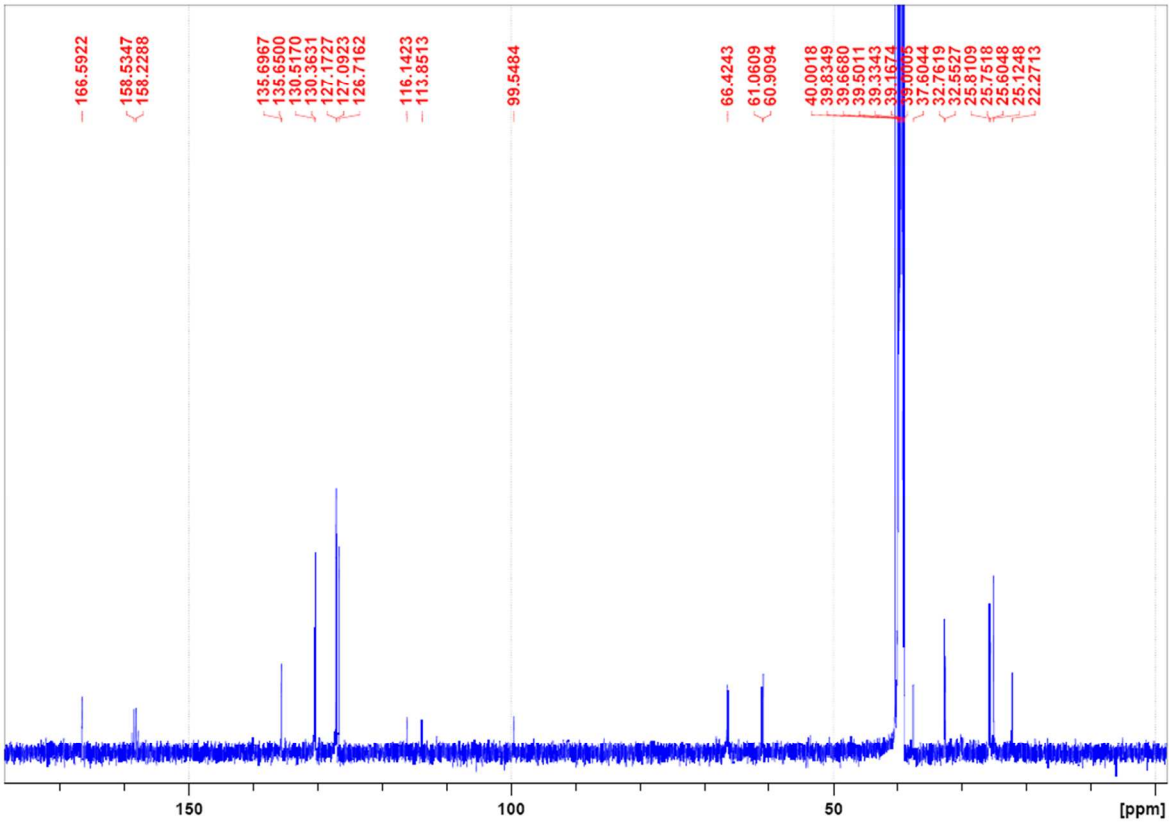
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100
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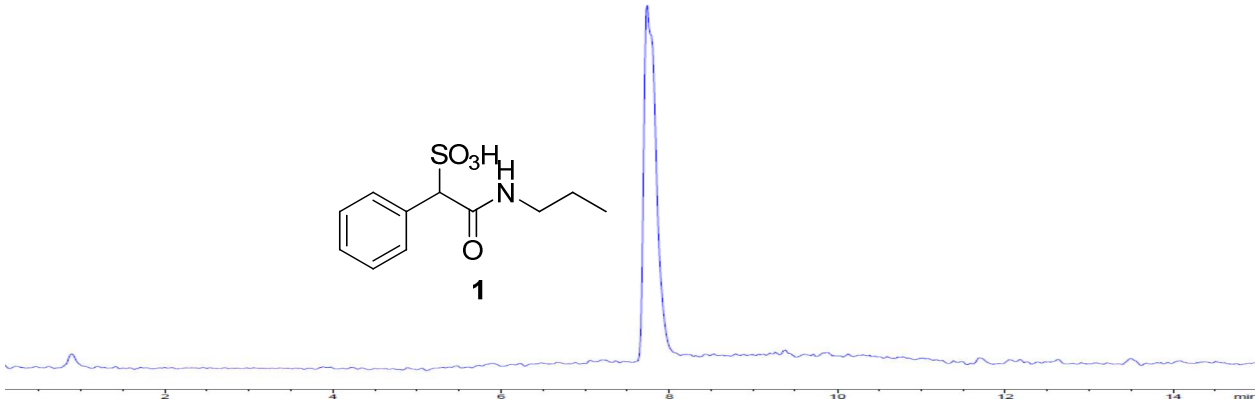
[ppm]

Copies of LC Data of Compounds 1 to 9



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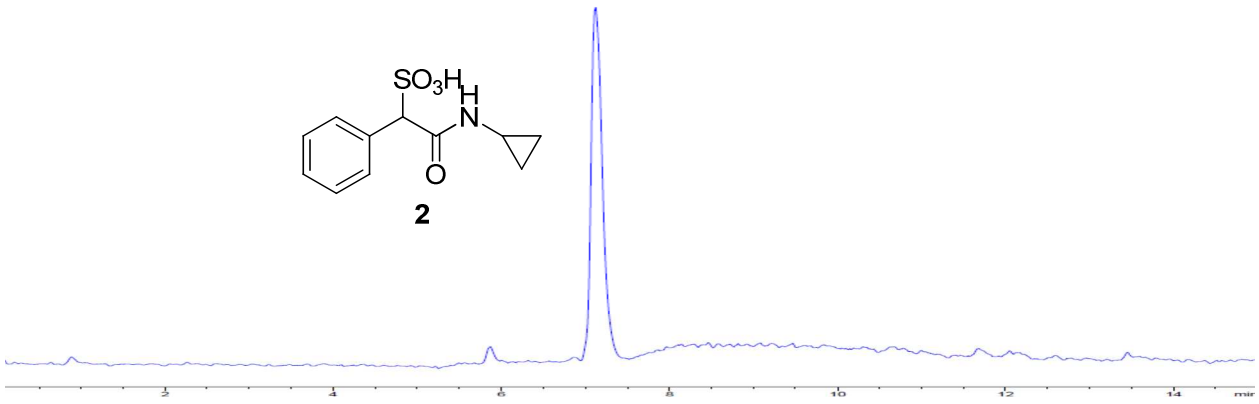

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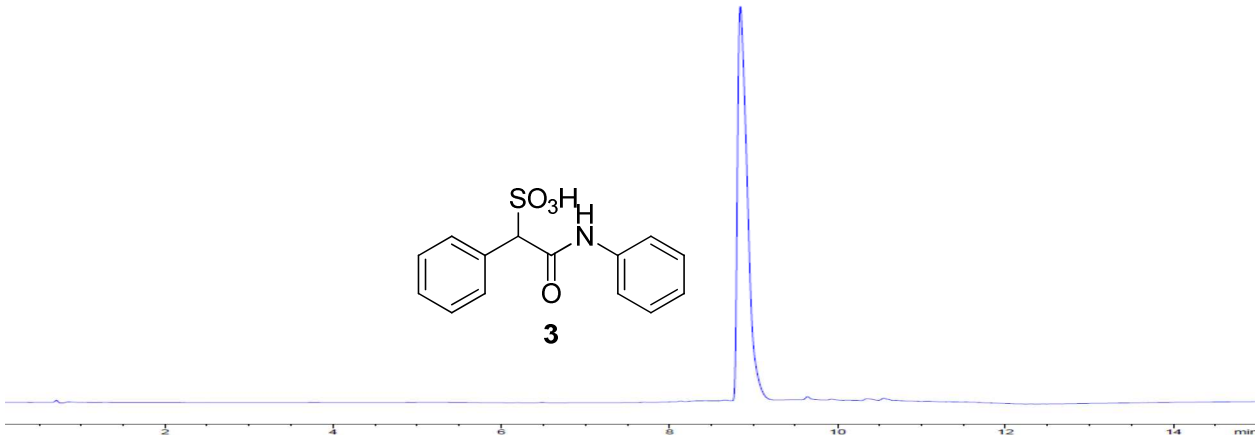

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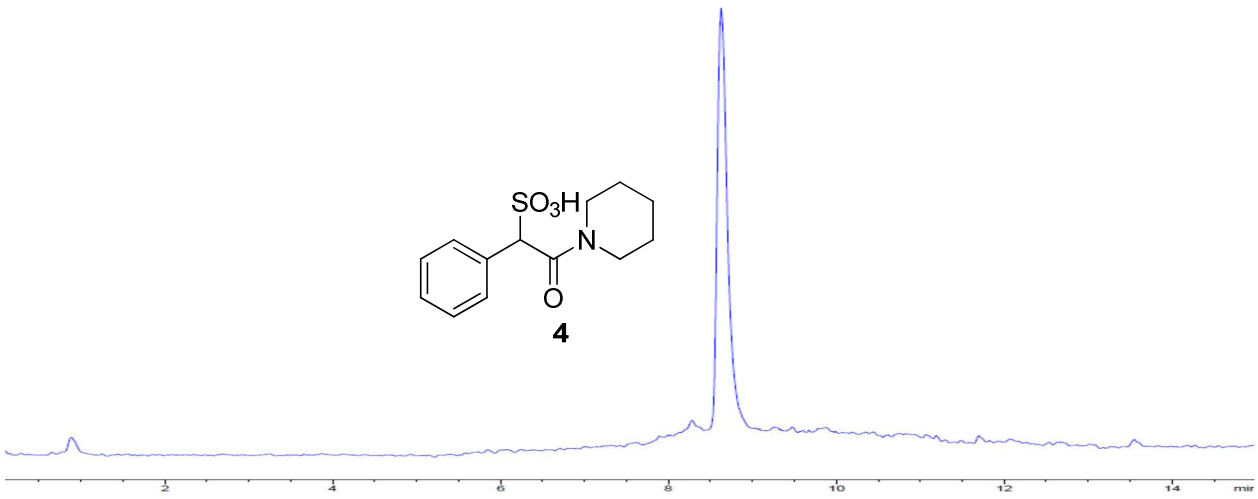
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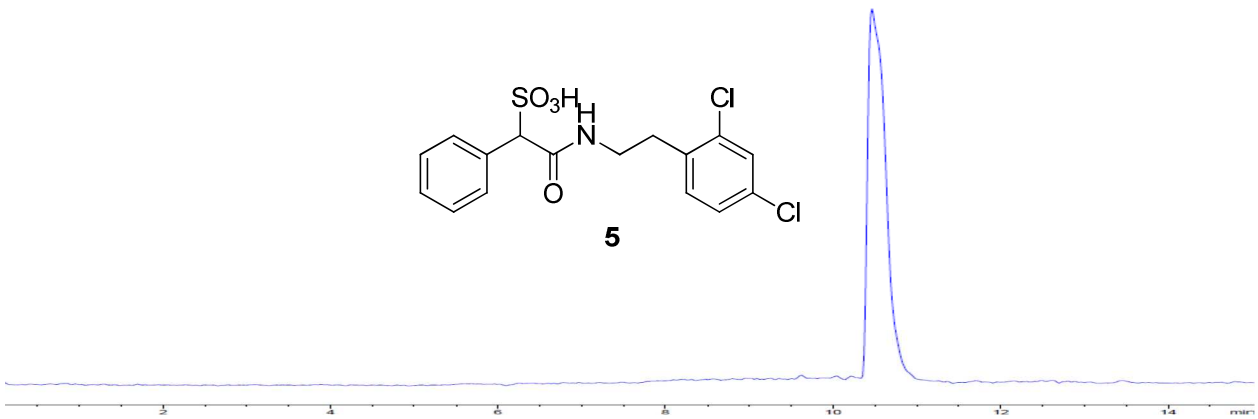
N
O
4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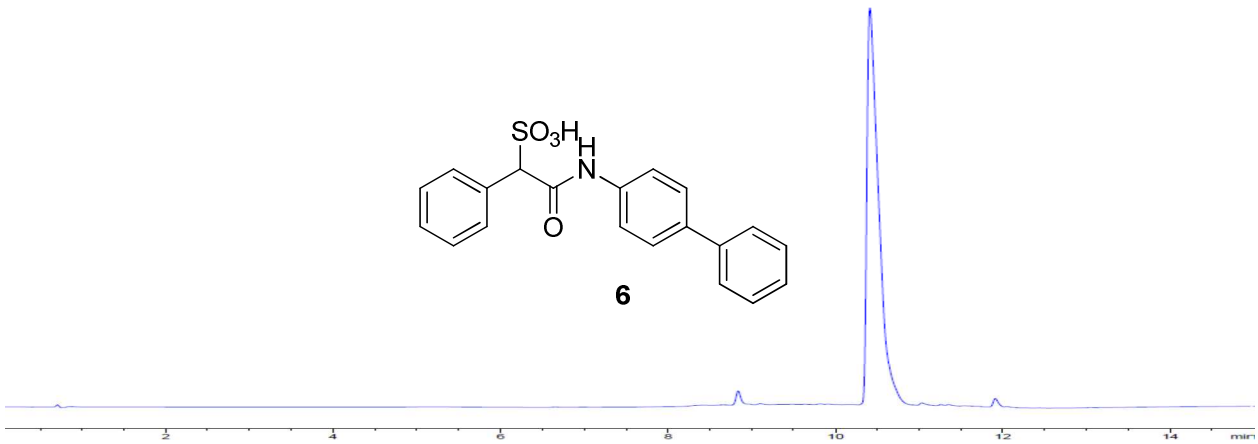
H
N
O
Cl
Cl
5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H
N
O
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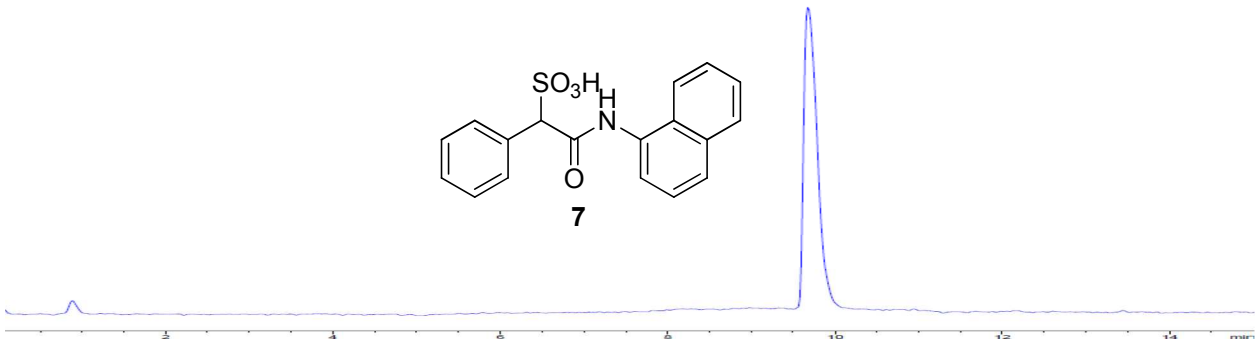

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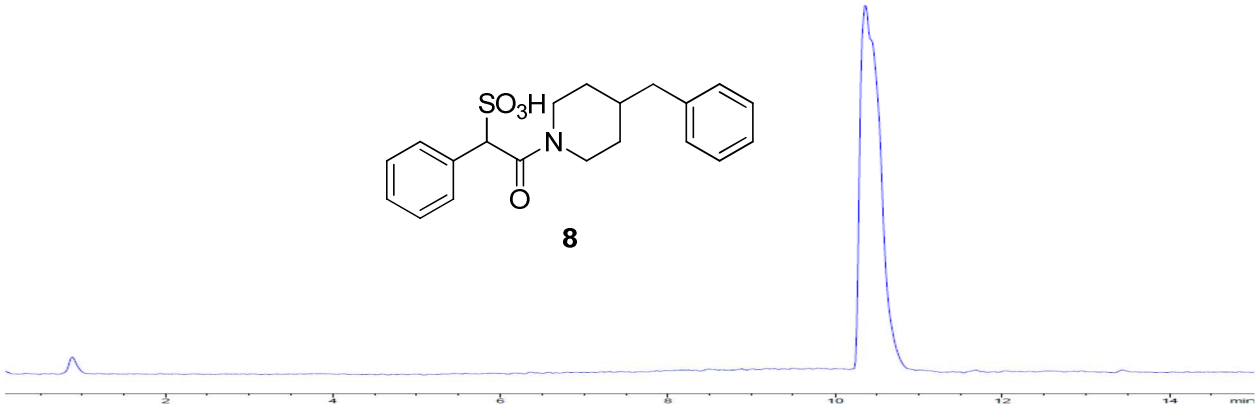
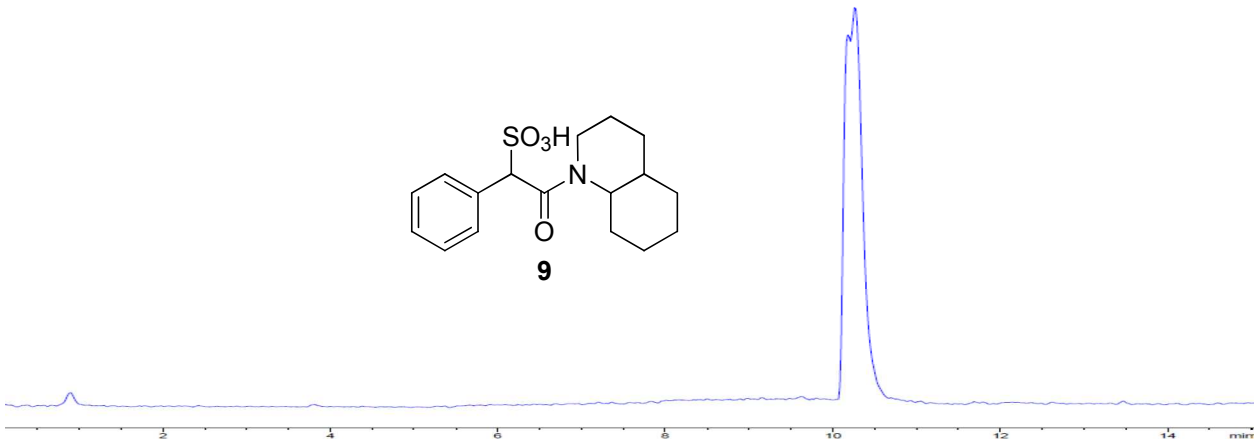


8



9

Expression and Purification of Recombinant mPTPB

pET28b-mPTPB (a generous gift from Dr. Christoph Grunder, University of California, Berkeley) was used to transform into E.coli BL21/DE3 and grown in LB medium containing 50 μg/ml kanamycin at 37°C to an OD600 of 0.5. Following the addition of IPTG to a final concentration of 20 μM, the culture was incubated at 20°C with shaking for additional 16 hr. The cells were harvested by centrifugation at 5000 rpm for 5 min at 4°C. The bacterial cell pellets were resuspended in 20 mM Tris, pH 7.9, 500 mM NaCl, 5 mM imidazole, and were lysed by passage through a French press cell at 1,200 p.s.i. twice. Cellular debris was removed by centrifugation at 16,000 rpm for 30 min at 4°C. The protein was purified form the supernatant using standard procedures of Ni-nitrilotriacetic acid-agarose (Qiagen) affinity purification. The protein eluted from Ni-NTA column was concentrated with an Amicon Ultra centrifugal filter device (Millipore) and the buffer was changed to 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA and 1 mM DTT. Protein concentration was determined using the Bradford dye binding assay (Bio-Rad) diluted according to the manufacturer's recommendations with bovine serum albumin as standard. The purified mPTPB were made to 20% glycerol and stored at -20°C.

Enzyme Kinetics and Inhibition Studies

The inhibition assays were performed at 25°C in 50 mM 3,3-dimethylglutarate buffer, pH 7.0, containing 1 mM EDTA with an ionic strength of 0.15M adjusted by NaCl. The reaction was started by the addition of 5 μl of the enzyme to 195 μl of reaction mixture containing 3.6 mM (the Km value) of pNPP and various concentrations of the inhibitor. The reaction was quenched after 5 min by the addition of 50 μl of 5N NaOH, and then 200 μl of reaction mixture was transferred to a 96-well plate. The absorbance at 405 nm was detected by a Spectra MAX340 microplate spectrophotometer (Molecular Devices). IC_{50} values were calculated by fitting the absorbance at 405 nm versus inhibitor concentration to the following equation:

$$A_I/A_0=IC_{50}/(IC_{50}+[I])$$

Where A_I is the absorbance at 405 nm of the sample in the presence of inhibitor; A_0 is the absorbance at 405 nm in the absence of inhibitor; and [I] is the concentration of the inhibitor.

For selectivity studies, the PTPs, including mPTPA, YopH, CD45, FAP-1, HePTP, Lyp, PTP1B, SHP1, SHP2, and VHX were expressed and purified from E. coli. The inhibition assay for these PTPs were performed under the same conditions as mPTPB except using a different *p*NPP concentration corresponding to the K_m of the PTP studied.

The inhibition constant (Ki) of the inhibitor for mPTPB was determined at pH 7.0 and 25°C. The mode of inhibition and Ki value were determined in the following manner. At various fixed concentrations of inhibitor (0-3 Ki), the initial rate at a series of pNPP concentrations was measured by following the production of p-nitrophenol as describe above, ranging from 0.2- to 5-fold the apparent Km values. The data were fitted to appropriate equations using SigmaPlot-Enzyme Kinetics to obtain the inhibition constant and to assess the mode of inhibition.

Molecular Modeling Studies

The 3D-structure of SPAA and compound **9** were modeled and energy-minimized in Chem3D program. The coordinates of mPTPB were taken from the Protein Data Bank (PDBID: 2OZ5.pdb) and the bound inhibitor OMTS was removed. Both ligand and receptor were respectively loaded into AutoDockTools1.5.6 software for pre-docking processing, such as merge non-polar hydrogens, add Gasteiger charges, set atom types, specify rotatable bond for ligand, and so on. The docking space was visually set around the catalytic active site, and the energy grid size was set to 46 × 60 × 46 points with 0.375Å spacing on each axis. The energy grid maps for each atom type in the ligands (i.e. A, C, HD, N, NA, OA and S), as well as the electrostatics and de-solvation maps were calculated using the AutoGrid4.2.6 program. The molecular docking were completed in AutoDock4.2.6 program, the optimal binding conformation was determined by LGALS (Lamarckian Genetic Algorithm with Local Search) algorithm with the following parameters during each docking run: energy evaluations of 2500000, population size of 100, mutation rate of 0.02, crossover rate of 0.8, Solis and Wets local search iterations of 300 with probability of 0.06. 1000 docking runs were performed and the resulted 1000 binding conformations were classified into different clusters and ranked

according to the calculated binding free energy. Finally, the binding mode analyses were performed in AutoDockTools1.5.6 software by visual inspections and energy comparisons.

Cell Culture and Immunoblot Analysis

Raw264.7 mouse macrophages were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (Invitrogen), penicillin (50 units/mL), and streptomycin (50 μg/mL) under a humidified atmosphere containing 5% CO_2 at 37°C. Transfected Raw264.7 cells (Vector, WT-mPTPB) were seeded in a 12-well plate at a density of 4 x 10^4 cells/well. The following day cells were treated with mPTPB inhibitor Compound **9** for 1 hr, then stimulated with IFN-γ (20 ng/ml) for 1 hr. Subsequently, the cells were washed with ice-cold phosphate buffered saline, and lysed with lysis buffer on ice for 30 min. Cell lysate was then cleared by centrifuging at 13,000 rpm for 15 min. The phosphorylation of ERK1/2, p38 and Akt, as well as total ERK1/2, p38 and Akt were detected by Western blotting. All antibodies were purchased from Cell Signaling Technology Inc.