Supporting Information

S-Aroylthiooximes: A Facile Route to Hydrogen Sulfide-Releasing Compounds with Structure-Dependent Release Kinetics

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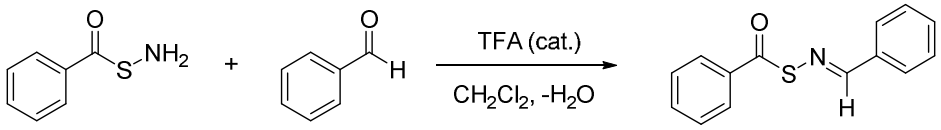
Materials and Methods:

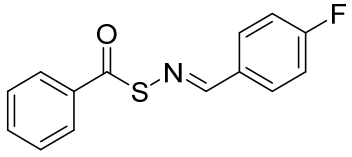
All reagents were obtained from commercial vendors and used as received unless otherwise stated. Thiobenzoic acid was purified via vacuum distillation. All other thioacids and *S*-aroylthiohydroxylamines were prepared as previously described.[1-3] NMR spectra were measured on Agilent 400 MHz or Bruker 500 MHz spectrometers. ^{1}H and ^{13}C NMR chemical shifts are reported in ppm relative to internal solvent resonances. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Infrared spectra were obtained on a Varian 670-IR spectrometer. HPLC was carried out on an Agilent 1220 system using water and CH_3CN as mobile phases with each containing 0.1% NH_4OH or 0.1% trifluoroacetic acid (TFA). Flow was maintained at 20 mL/min over gradients described for each purification on an Agilent PLRP-S column (100 Å, 10 µm, 25 x 150 mm). Fractions were analyzed by mass spectrometry (Advion Expression Compact Mass Spectrometer), and product-containing fractions were combined, rotovapped to remove CH_3CH, and lyophilized (LabConco). UV-Vis absorbance spectra were recorded on a Cary 5000 UV-Vis (Agilent) from 450 to 220 nm or on a Spectramax M2 plate reader (Molecular Devices). Reactions were performed in screw-cap scintillation vials over 3 Å molecular sieves.

Thiooxime synthesis representative procedure:



1a

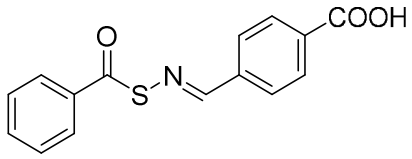
S-Benzoylthiohydroxylamine (SBTHA) (200 mg, 1.3 mmol) was dissolved in 3 mL of CH_2Cl_2 in a scintillation vial charged with molecular sieves. To the vial was added benzaldehyde (135 µL, 1.3 mmol) followed by 10 µL of TFA. The vial was sealed, and the mixture was allowed to stand for 1-5 h at rt. The reaction mixture was filtered and the solvent was removed under reduced pressure to give the pure product as a white powder (309 mg, 98% yield). ^{1}H NMR ($CDCl_3$): δ 7.47 (m, 5H), 7.61 (t, 1H), 7.85 (d, 2H), 7.94 (d, 2H), 8.81 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 189.12, 164.40, 135.79, 135.73, 133.92, 131.83, 129.05, 128.88, 128.49, 127.05. IR (ATR crystal) (cm^{-1}): 1673, 1203, 898, 751, 685, 641. HR-MS: $[M + H]^+$ calculated 242.0634; found 242.0640.



1b

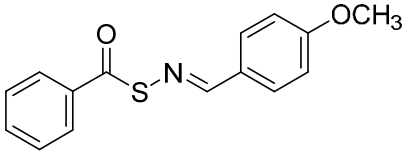
Compound **1b** was prepared from SBTHA (50 mg, 0.33 mmol) and 4-fluorobenzaldehyde (35 µL, 0.33 mmol) using the same procedure as **1a**. The product was isolated as an off-white powder (81 mg, 96%

yield). ^{1}H NMR ($CDCl_3$): δ 7.13 (t, 2H), 749 (t, 2H), 7.61 (t, 1H), 7.84 (t, 2H), 7.93 (d, 2H), 8.77 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 189.07, 166.18, 163.73, 162.96, 135.77, 133.98, 132.21, 132.18, 130.60, 130.51, 129.08, 127.05, 116.26, 116.04. IR (ATR crystal) (cm^{-1}): 1675, 1597, 1505, 1228, 1201, 898, 767, 688, 640. HR-MS: $[M + H]^+$ calculated 260.0540; found 260.0557.



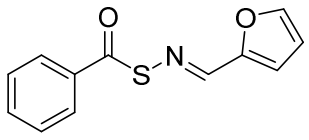
1c

Compound **1c** was prepared from SBTHA (100 mg, 0.65 mmol) and 4-formylbenzoic acid (107 mg, 0.71 mmol) using the same procedure as **1a**. The crude product was recrystallized from EtOAc to afford the pure product as a white powder (133 mg, 71.4% yield). ^{1}H NMR (DMSO-d_6): δ 7.61 (t, 2H), 7.74 (t, 1H), 7.92 (t, 4H), 8.07 (d, 2H), 9.07 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 187.77, 166.92, 164.44, 138.65, 134.95, 134.39, 133.96, 129.79, 129.44, 128.00, 126.58. IR (ATR crystal) (cm^{-1}): 2950, 2811, 2657, 1677, 1284, 1198, 896, 764, 680, 638. HR-MS: $[M + H]^+$ calculated 286.0532; found 286.0531.



1d

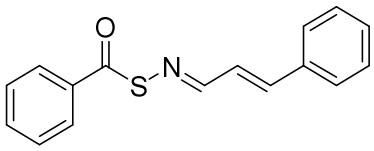
Compound **1d** was prepared from SBTHA (50 mg, 0.33 mmol) and anisaldehyde (40 μL, 0.33 mmol) using the same procedure as **1a**. The product was isolated as a white powder (79 mg, 89% yield). ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 3H), 6.95 (d, 2H), 7.48 (t, 3H), 7.60 (t, 1H), 7.79 (d, 2H), 7.93 (d, 2H), 8.71 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 189.63, 164.42, 162.68, 135.95, 133.78, 130.35, 129.01, 128.88, 126.97, 114.32, 55.55. IR (ATR crystal) (cm^{-1}): 2930, 2834, 1674, 1592, 1556, 1590, 1251, 1202, 1169, 1024, 898, 825, 788, 684, 639. HR-MS: $[M + H]^+$ calculated 272.0740; found 272.0760.



1e

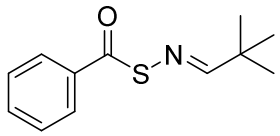
Compound **1e** was prepared from SBTHA (50 mg, 0.33 mmol) and furfural (27 μL, 0.33 mmol) using the same procedure as **1a**. The product was isolated as a white powder as a mixture of cis/trans isomers (71 mg, 94% yield in a ratio of 65:35). ^{1}H NMR ($CDCl_3$): δ 6.53 (dd, 0.65H, J = 2Hz), 6.62 (dd, 0.35H, J = 2Hz), 6.94 (d, 0.65H, J = 4Hz), 7.10 (d, 0.35H, J = 4Hz), 7.49 (q, 2H, J = 8Hz), 7.60 (t, 1.7H, J = 8Hz), 7.67 (d, 0.3H, J = 2Hz), 7.90 (d, 1.3H, J = 8Hz), 8.00 (d, 0.7H, J = 8Hz), 8.47 (s, 0.35H), 8.62 (s, 0.65H). ^{13}C NMR ($CDCl_3$): δ 188.78, 152.48, 151.31, 149.64, 147.15, 146.26, 145.95, 135.83, 135.68, 134.04,

133.94, 129.06, 127.37, 127.03, 118.48, 116.06, 112.88, 112.36. IR (ATR crystal) (cm^{-1}): 1669, 1596, 1204, 900, 769, 747, 681, 641. HR-MS: $[M + Na]^+$ calculated 254.0246; found 254.0259.



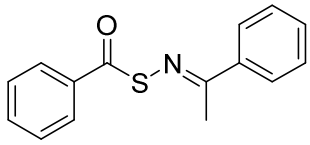
1f

Compound **1f** was prepared from SBTHA (176 mg, 1.15 mmol) and *trans*-cinnamaldehyde (145 µL, 1.15 mmol) using the same procedure as **1a**. The product was purified by HPLC (gradient of 30% to 90% CH_3CN) to afford the pure product as a light yellow powder as a mixture of cis/trans isomers (224 mg, 79% yield in a ratio of 65:35). ^1H NMR ($CDCl_3$): δ 6.98 (t, 0.35H), 7.02 (t, 0.65H), 7.10 (d 0.2H, J = 15 Hz), 7.15 (d, 0.5H, J = 5 Hz), 7.19 (d, 0.3H, J = 10 Hz), 7.39 (m, 3H), 7.49 (m, 3.6H), 7.57 (m, 0.4H), 7.62 (m, 1H), 7.93 (d, 1.5H, J = 5 Hz), 7.99 (d, 0.5H, J = 10 Hz), 8.34 (d, 0.2H, J = 10 Hz), 8.56 (d, 0.8H, J = 5 Hz). ^{13}C NMR ($CDCl_3$): δ 188.92, 165.63, 161.90, 144.96, 143.32, 135.79, 135.60, 135.24, 134.85, 133.78, 130.41, 129.80, 128.99, 128.94, 128.89, 128.87, 128.00, 127.89, 127.61, 127.14, 126.93, 121.72. IR (ATR crystal) (cm^{-1}): 1676, 1592, 1446, 1200, 899, 682, 642, HR-MS: $[M + H]^+$ calculated 268.0791; found 268.0788.



1g

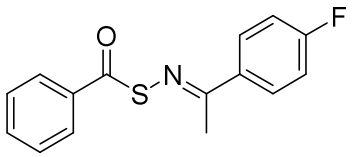
Compound **1g** was prepared from SBTHA (156 mg, 1.02 mmol) and pivalaldehyde (140 µL, 1.29 mmol) using the same procedure as **1a**. The crude product was purified on a silica gel column (0.5% NEt_3 + 10% EtOAc in hexanes) to afford the pure product as an off-white powder (84 mg, 37% yield). ^1H NMR ($CDCl_3$): δ 1.18 (s, 9H), 7.45 (t, 2H), 7.57 (t, 1H), 7.86 (d, 2H), 8.14 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 189.66, 179.07, 135.91, 133.68, 128.94, 126.89, 39.68, 26.57. IR (ATR crystal) (cm^{-1}): 2963, 1667, 1603, 1200, 899, 776, 688, 644. HR-MS: $[M + H]^+$ calculated 222.0947; found 222.0954.



1h

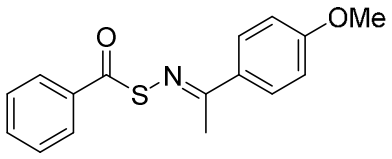
Compound **1h** was prepared from SBTHA (200 mg, 1.31 mmol) and acetophenone (152 µL, 1.31 mmol) using the same procedure as **1a**. The crude product was dried under vacuum overnight and then purified on a silica gel column (0.5% NEt_3 in CH_2Cl_2) to afford the pure product as an off-white white powder (180 mg, 54% yield). ^1H NMR ($CDCl_3$): δ 2.54 (s, 3H), 7.43 (d, 2H), 7.50 (t, 2H), 7.62 (t, 1H), 7.94 (m, 2H), 7.99 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 188.13, 167.41, 139.05, 136.29, 133.76, 130.56, 129.02, 128.58,

127.27, 127.08, 77.16, 21.57. IR (ATR crystal) (cm^{-1}): 1679, 1563, 1446, 1369, 1201, 896, 754, 677, 637. HR-MS: $[M + H]^+$ calculated 256.0791; found 256.0803.



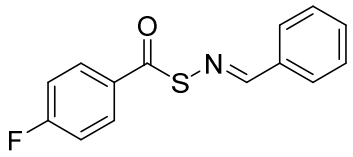
1i

Compound **1i** was prepared from SBTHA (200 mg, 1.31 mmol) and 4-fluoroacetophenone (158 µL, 1.31 mmol) using the same procedure as **1a.** The crude product was purified via recrystallization from hexanes to afford the pure product as a white powder (188 mg, 53% yield). ^1H NMR ($CDCl_3$): δ 2.51 (s, 3H), 7.09 (t, 2H), 7.50 (t, 2H), 7.61 (t, 1H), 7.94 (m, 2H), 7.96 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 188.03, 166.03, 165.61, 163.11, 136.17, 135.30, 135.27, 133.81, 129.23, 129.14, 129.02, 127.23, 115.65, 115.43, 21.47. IR (ATR crystal) (cm^{-1}): 1683, 1592, 1562, 1501, 1203, 896, 830, 768, 683, 641. HR-MS: $[M + H]^+$ calculated 274.0696; found 274.0714.



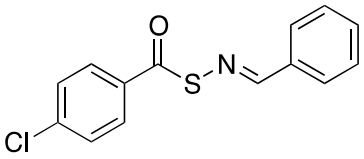
1j

Compound **1j** was prepared from SBTHA (100 mg, 0.65 mmol) and 4-methoxyacetophenone (98 mg, 0.65 mmol) using the same procedure as **1a**. The crude product was purified via HPLC (30% to 90% CH_3CN with 0.1% NH_4OH) to afford the pure product as an off-white white powder (40 mg, 22% yield). ^1H NMR ($CDCl_3$): δ 2.50 (s, 3H), 3.85 (s, 3H), 6.92 (d, 2H), 7.49 (t, 2H), 7.60 (t, 1H), 7.92 (d, 2H), 7.99 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 188.53, 166.88, 161.65, 136.35, 133.66, 131.90, 128.97, 128.78, 127.18, 113.81, 55.50, 21.28. IR (ATR crystal) (cm^{-1}): 1672, 1582, 1554, 1506, 1254, 1200, 1172, 900, 683, 645. HR-MS: $[M + H]^+$ calculated 286.0896; found 286.0910.



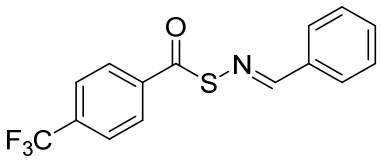
1k

Compound **1k** was prepared from *S*-(4-fluorobenzoyl)thiohydroxylamine (105 mg, 0.61 mmol) and benzaldehyde (63 µL, 0.61 mmol) using the same procedure as **1a**. The product was isolated as an off-white powder (78 mg, 91% yield). ^1H NMR ($CDCl_3$): δ 7.17 (t, 2H), 7.47 (m, 3H), 7.84 (d, 2H), 7.97 (d, 2H), 8.80 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 187.75, 167.49, 164.98, 164.64, 135.71, 132.18, 132.15, 131.93, 129.71, 129.62, 128.93, 128.52, 116.42, 116.20. IR (ATR crystal) (cm^{-1}): 1676, 1593, 1196, 904, 833, 752, 693, 630. HR-MS: $[M + H]^+$ calculated 260.0540; found 260.0564.



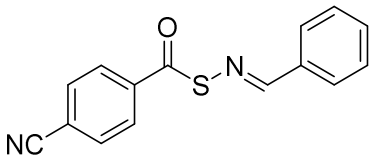
1l

Compound **1l** was prepared from *S*-(4-clorobenzoyl)thiohydroxylamine (100 mg, 0.53 mmol) and benzaldehyde (55 µL, 0.53 mmol) using the same procedure as **1a**. The product was isolated as a white powder (142 mg, 97% yield). ^{1}H NMR ($CDCl_3$): δ 7.47 (m, 5H), 7.84 (d, 2H), 7.88 (d, 2H), 8.80 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 188.07, 164.80, 140.32, 135.70, 134.17, 131.99, 129.43, 128.95, 128.56, 128.44. IR (ATR crystal) (cm^{-1}): 1674, 1586, 1481, 1395, 1195, 1081, 893, 858, 829, 751, 692, 629. HR-MS: $[M + H]^+$ calculated 276.0244; found 276.0249.



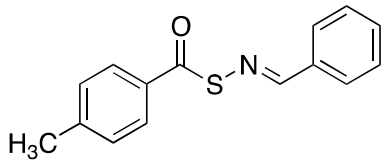
1m

Compound **1m** was prepared from *S*-(4-trifluoromethylbenzoyl)thiohydroxylamine (75 mg, 0.34 mmol) and benzaldehyde (35 µL, 0.34 mmol) using the same procedure as **1a**. The crude product was purified on a silica gel column (10% EtOAc + 0.5% NEt_3 in hexanes) to give the pure product as a white powder (78 mg, 74.4% yield). ^{1}H NMR ($CDCl_3$): δ 7.48 (m, 3H), 7.76 (d, 2H), 7.85 (d, 2H), 8.04 (d, 2H), 8.81 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 188.38, 165.24, 138.66, 135.58, 135.35, 135.03, 134.70, 132.14, 128.98, 128.61, 127.47, 126.22, 126.11, 124.93, 122.21. IR (ATR crystal) (cm^{-1}): 1671, 1319, 1109, 1063, 906, 842, 750, 689, 647. HR-MS: $[M + H]^+$ calculated 310.0508; found 310.0518.



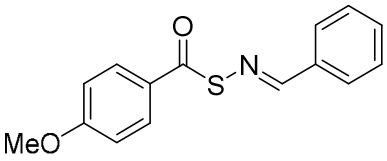
1n

Compound **1n** was prepared from 4-((aminothio)carbonyl)benzonitrile (100 mg, 0.56 mmol) and benzaldehyde (57 µL, 0.56 mmol) using the same procedure as **1a**. The product was isolated as an off-white powder (143 mg, 96% yield). ^{1}H NMR ($CDCl_3$): δ 7.47 (m, 3H), 7.79 (d, 2H), 7.83 (d, 2H), 8.01 (d, 2H), 8.79 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 188.07, 165.53, 138.99, 135.46, 132.90, 132.24, 129.00, 128.62, 127.56, 117.80, 117.12. IR (ATR crystal) (cm^{-1}): 1664, 1587, 1561, 1372, 903, 761, 696, 629. HR-MS: $[M + H]^+$ calculated 267.0587; found 267.0592.



1o

Compound **1o** was prepared from *S*-(4-methylbenzoyl)thiohydroxylamine (100 mg, 0.60 mmol) and benzaldehyde (61 μL, 0.60 mmol) using the same procedure as **1a**. The product was isolated as a white powder (139 mg, 91% yield). ^{1}H NMR ($CDCl_3$): δ 2.43 (s, 3H), 7.29 (d, 2H), 7.45 (m, 3H), 7.84 (d, 4H), 8.81 (s, 1H). ^{13}C NMR ($CDCl_3$): 188.72, 164.21, 144.91, 135.87, 133.30, 131.76, 129.73, 128.89, 128.49, 127.15, 21.91. IR (ATR crystal) (cm^{-1}): 1674, 1603, 1565, 1204, 1169, 900, 815, 751, 686, 639. $[M + H]^+$ calculated 256.0791; found 256.0804.



1p

Compound **1p** was prepared from *S*-(4-methoxybenzoyl)thiohydroxylamine (21 mg, 0.12 mmol) and benzaldehyde (12 μL, 0.12 mmol) using the same procedure as **1a**. The product was isolated as a white powder (29 mg, 94% yield). ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H), 6.96 (d, 2H), 7.45 (m, 3H), 7.83 (d, 2H), 7.92 (d, 2H), 8.80 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 187.58, 164.22, 163.96, 135.89, 131.68, 129.26, 128.86, 128.59, 128.43, 114.28, 55.68. IR (ATR crystal) (cm^{-1}): 1670, 1596, 1262, 1211, 1164, 900, 832, 755, 690. 641. HR-MS: $[M + H]^+$ calculated 272.0740; found 272.0750.

Calibration of H_2S Selective Probe

An EDTA solution was prepared at 154 μM by dissolving 1.43 mg of EDTA in 25 mL of DI water in a volumetric flask. The solution was purged vigorously with nitrogen for 20 min. 7.7 mg of anhydrous Na_2S was added to a vial under inert atmosphere, followed by 20 mL of the EDTA solution (to make 5 mM H_2S). A small stir bar was added to a scintillation vial containing 20 mL of 1X PBS buffer (pH = 7.4). The vial was placed on a stir plate. The H_2S sensor was immersed in the solution and the background current was allowed to stabilize for several minutes. Five aliquots of the H_2S solution were injected sequentially into the vial (20 μL, 40 μL, 60 μL, 80 μL, 100 μL). The current increased rapidly after each injection before reaching a plateau. The second aliquot was injected as soon as the current had stabilized. The other aliquots were injected similarly. The recorded data was used to construct a linear calibration curve of concentration vs. current.

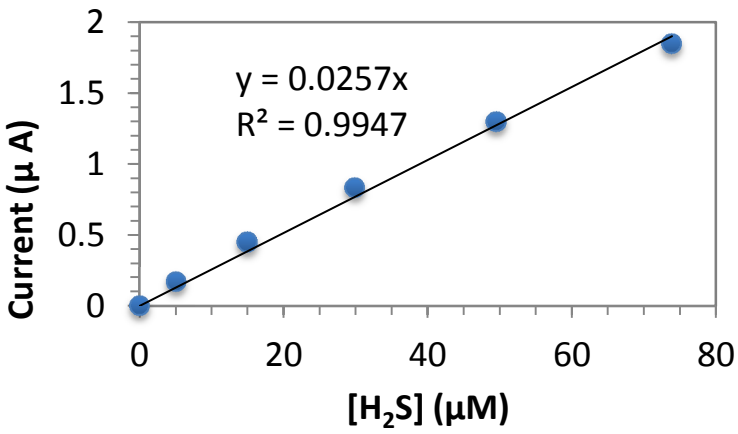


Fig S1. Standard curve for H_2S release in PBS buffer.

H_2S Release in the Presence of Cysteine and other Additives

A stock solution of cysteine or other additive (lysine, *N*-acetylcysteine, serine, glutathione, or none) was prepared in PBS buffer at 400 mM. 50 μL of this solution was added to a vigorously stirred vial containing 20 mL of PBS buffer. The current was allowed to equilibrate for several minutes. Once a stable current was observed, an aliquot of *S*-aroylthiooxime stock solution (100 μL, 8 mM in THF) was added rapidly via pipette. The current was monitored over a period of approximately 1h. A plot of H_2S concentration vs. time was constructed using the calibration curve. No background subtraction was performed. H_2S release was only observed upon addition of compounds containing a thiol functionality.

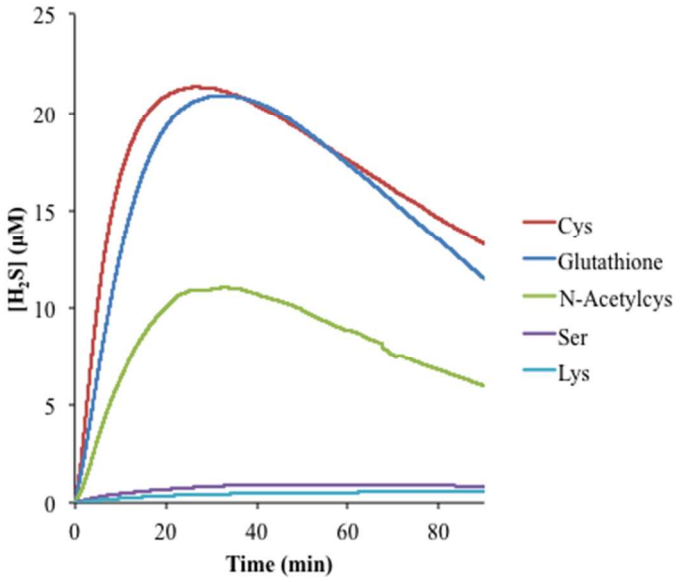


Fig S2. H_2S release in the presence of various nucleophiles.

H_2S Release as a Function of Cysteine Concentration

A stock solution of cysteine was prepared in PBS buffer at 400 mM. 12.5, 50, or 100 µL of this solution was added to a vigorously stirred vial containing 20 mL of PBS buffer. The current was allowed to equilibrate for several minutes. Once a stable current was observed, an aliquot of a **1a** stock solution (100 µL, 8 mM in THF) was added rapidly via pipette. The current was monitored over a period of approximately 1 h. A plot of H_2S concentration vs. time was constructed using the calibration curve. No background subtraction was performed.

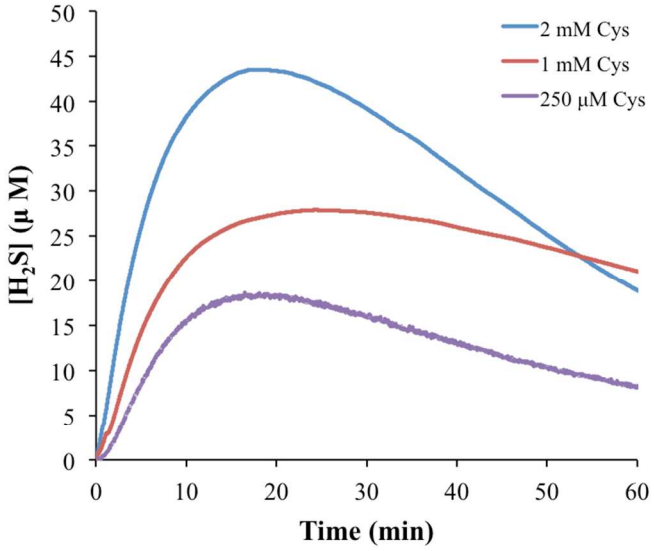


Fig S3. Effect of [cysteine] on H_2S release profile.

Calibration of H_2S Selective Probe in Bovine Plasma

A 100 mL of an 0.05 mg/mL EDTA solution was prepared and purged vigorously with N_2 for 20 min. Na_2S was then added to make 1 mM Na_2S. A small stir bar was added to a scintillation vial containing 15 mL of deionized water and 5 mL of bovine plasma. The vial was placed on a stir plate. The H_2S sensor was immersed in the solution and the background current was allowed to stabilize for several minutes. Three aliquots of the H_2S solution were injected sequentially into the vial (20 µL, 40 µL, 80 µL). The current increased rapidly after each injection before reaching a plateau. The second aliquot was injected as soon as the current had stabilized. The other aliquots were injected similarly. The recorded data was used to construct a linear calibration curve of concentration vs. current.

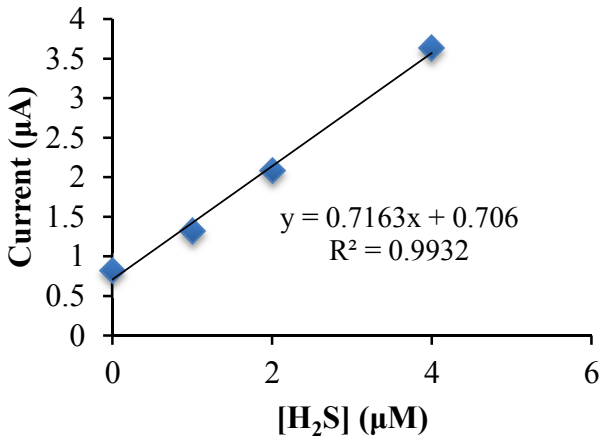


Fig S4. Standard curve for H_2S release in 25% v/v plasma.

H_2S release from Plasma

H_2S release from **1a** was conducted in bovine plasma. 5 μL of an 8 mM solution of **1a** in THF was added to 20 mL of a 25% v/v mixture of bovine plasma/deionized water. Thiols are known to be unstable in isolated plasma, having half-lives of disappearance on the order of minutes due to autooxidation and mixed disulfide formation reactions.[4-6] It has been reported that human plasma contains an average reduced cysteine concentration of 10 μM.[7] Therefore, we supplemented the commercial plasma with 10 μM cysteine before each run. The H_2S release profile of **1a** in plasma with 10 μM added cysteine is shown below (Fig S5 blue trace). As expected based on thiol autoxidation noted above, H_2S release was not observed in plasma in the absence of added cysteine (Fig S5 red trace). These results indicate that thiooximes would release H_2S in vivo where reduced cysteine is present.

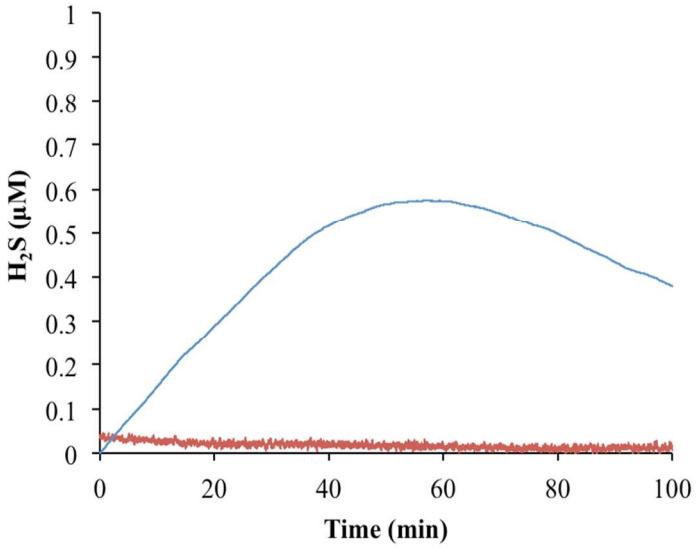


Fig S5. H_2S release in plasma. Blue curve shows H_2S evolution from 2 μM **1a** in bovine plasma supplemented with 10 μM cysteine. Red curve shows the same experiment with no cysteine supplement.

H_2S Release Kinetics via Methylene Blue Method

Reactions for kinetics were run in triplicate, with each reaction vial containing 1.296 mL PBS, 200 μL thiooxime solution (1 mM in THF), 400 μL THF, 100 μL $Zn(OAc)_2$ solution (40 mM in H_2O), and 4 μL cysteine solution (500 mM in PBS). Final concentrations were 100 μM thiooxime, 2 mM ZnOAc, and 1 mM cysteine. A control solution was also run for each experiment using lysine in place of cysteine at the same concentration. At predetermined timepoints, 100 μL was removed from each vial. Each 100 μL aliquot was diluted with 100 μL $FeCl_3$ solution (30 mM in 1.2 M HCl) followed by 100 μL *N*,*N*-dimethyl-*p*-phenylenediamine (20 mM in 7.2 M HCl). Aliquots were stored until 3-5 h after the final aliquot had been taken. A spectrum of each aliquot was collected from 500 to 800 nm on a plate reader. A background solution was also made using THF in place of the substrate solution. Three aliquots of this background solution were diluted with $FeCl_3$ and *N*,*N*-dimethyl-*p*-phenylenediamine as described above. Kinetic analysis was done by subtracting the absorbance of the background solution from the average absorbance at each timepoint at 676 nm. First-order half-life of H_2S release was determined by plotting time vs. ln(1/(1-% released), with $t_{1/2}$=ln(2)/slope.

Hydrolysis kinetics

Solutions for hydrolysis kinetics were prepared at 50 μM in *S*-aroylthiooxime in 20% CH_3CN in PBS. Spectra were taken at timepoints over the course of several days from 450 nm to 220 nm on a UV-Vis spectrophotometer. A background spectrum of 20% CH_3CN in PBS was subtracted from each sample spectrum, and all spectra were normalized at 450 nm, where absorbance was negligible for all samples.

Kinetic analysis was done by comparing the absorbance peak of the thiooxime (usually around 310-340 nm) to an isosbestic point for each hydrolysis experiment. The following equation was used to calculate % hydrolysis.[8]

$$\frac{A_{peak}}{A_{iso}} = \frac{\varepsilon_{SBTHApeak}[SBTHA] + \varepsilon_{spentpeak}[Spent]}{\varepsilon_{iso}([SBTHA]+[Spent])} = \frac{\varepsilon_{SBTHApeak}}{\varepsilon_{iso}} + \left(\frac{[SBTHA]}{([SBTHA]+[Spent])}\right)\left(\frac{\varepsilon_{SBTHApeak}-\varepsilon_{spentpeak}}{\varepsilon_{iso}}\right)$$

Therefore,

$$\%\ hydroylsis = \frac{[SBTHA]}{([SBTHA]+[Spent])} = \left(\frac{A_{peak}}{A_{iso}} - \frac{\varepsilon_{SBTHApeak}}{\varepsilon_{iso}}\right)\left(\frac{\varepsilon_{iso}}{\varepsilon_{SBTHApeak} - \varepsilon_{spentpeak}}\right)$$

Where

$\varepsilon_{SBTHApeak}$ = extinction coefficient of thiooxime at peak absorbance
$\varepsilon_{spentpeak}$ = extinction coefficient of the SBTHA/carbonyl mixture at equilibrium
ε_{iso} = extinction coefficient at the isosbestic point
A_{peak} = absorbance at the peak maximum at a given timepoint
A_{iso} = absorbance at the isosbestic point at a given timepoint

The extinction coefficient at the peak SBTHA absorbance of the SBTHA/carbonyl mixture was determined by making a sample of each SBTHA/carbonyl mixture matching the components of each *S*-aroylthiooxime. These were prepared at 50 μM SBTHA and 50 μM carbonyl (ketone or aldehyde) in 20% CH_3CN in PBS. Samples were allowed to sit for 3 d to ensure that equilibrium had been reached before taking spectra.

Fitting to a first-order rate was accomplished by plotting time versus ln(1/(1-% hydrolysis) with with $t_{1/2} = \ln(2)/\text{slope}$.

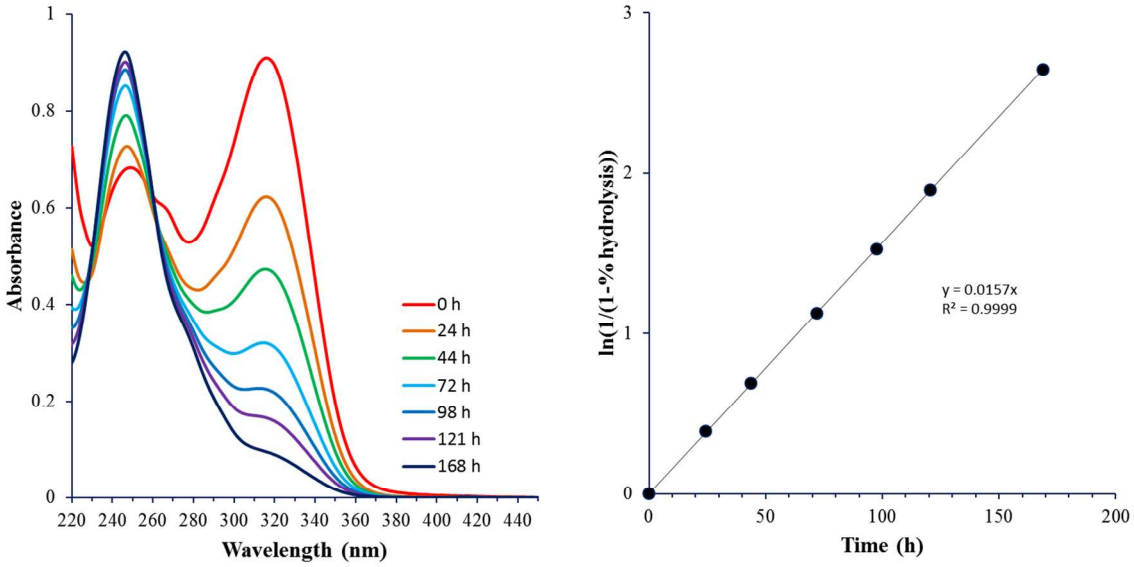


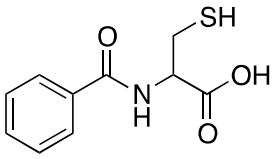
Fig S6. Example hydrolysis spectra and kinetic data for compound **1a**. A_{peak} and A_{iso} for this compound were 316 and 262 nm, respectively.

H_2S Release Mechanism (Scheme 3)

The proposed mechanism is supported by several pieces of evidence. Figure S2 shows the H_2S release profile of **1a** in the presence of various nucleophiles. H_2S release was not observed in the absence of thiol functionality. Additionally, the rate of H_2S release is slower for the reaction of **1a** with *N*-acetylcysteine compared with cysteine (peaking time of 33 min vs. 24 min). The acetyl group of *N*-acetylcysteine prohibits S → N acyl transfer. The apparent dependence of the H_2S release rate on the ability of the *S*-benzoylcysteine byproduct to undergo S → N acyl transfer, coupled with the need of thiol reactivity to promote reversible thiol exchange, provides evidence for the proposed mechanism.

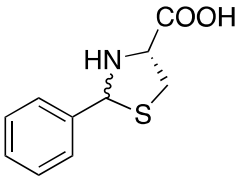
Product Analysis (Scheme 3)

Compound **1a** (40 mg, 0.16 mmol) in 10 mL of CH_3CN was added to a stirred solution of cysteine (250 mg, 2.06 mmol) in 10 mL of PBS buffer (pH = 7.4) in a round bottom flask. The solution was stirred at room temperature overnight. The following day, the solution was filtered and concentrated to remove the organic solvent. The resulting aqueous solution was purified via preparative HPLC (gradient of 2% to 90% CH_3CN with 0.5% NH_4OH). The isolated products were characterized by NMR and HRMS as shown below.



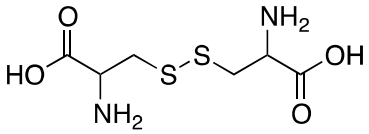
***N*-benzoylcysteine**

***N*-benzoylcysteine** was isolated by HPLC. The resulting solid was found to contain a small quantity of the disulfide dimer 3,3'-disulfanediylbis(2-benzamidopropanoic acid). ^{1}H NMR (D_2O): δ 3.10 (qd, 2H), 3.43 (dd, 0.2H), 4.66 (m, 1H), 4.76 (m, 0.2H), 7.48 (t, 0.4H), 7.58 (t, 2H), 7.86 (t, 1H), 7.76 (d, 0.4H), 7.88 (d, 2H). ^{13}C NMR (D_2O): δ 176.12, 170.36, 133.32, 132.21, 128.71, 127.15, 57.07, 26.23. $[M - H]^-$ calculated 224.0387; found 224.0396.



2-Phenylthiazolidine-4-carboxylic acid

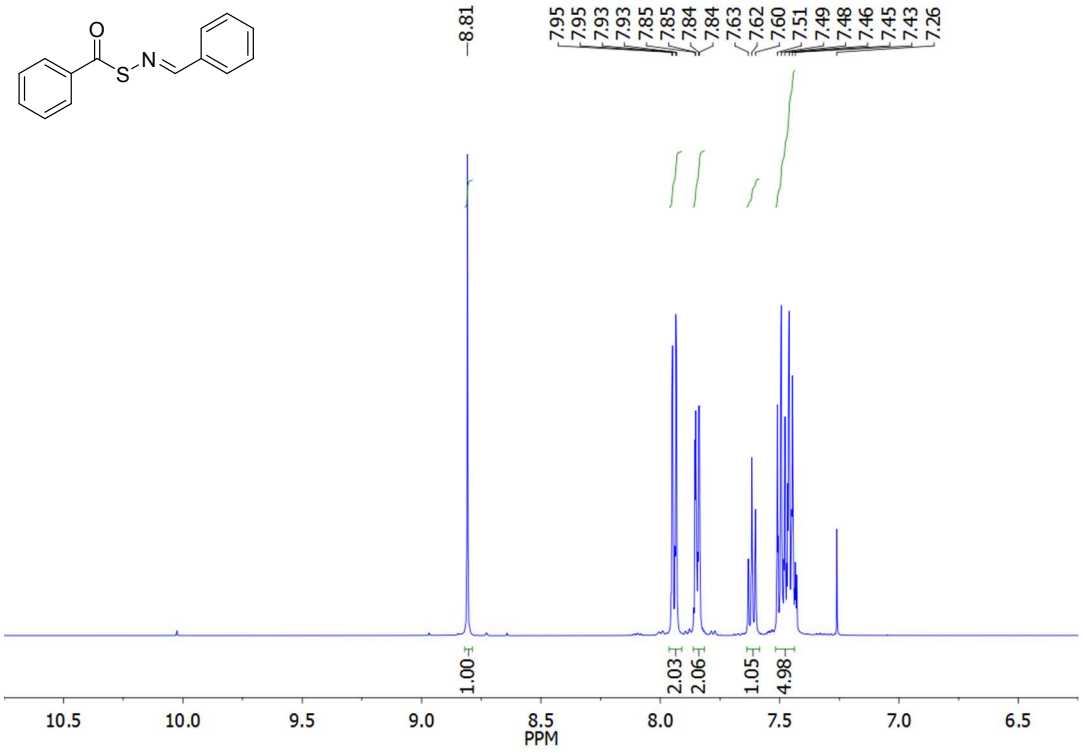
2-Phenylthiazolidine-4-carboxylic acid was isolated by HPLC, a product of reaction of benzaldehyde with excess cysteine.[9] The resulting solid was a 50:50 mixture of diastereomers. ^{1}H NMR (D_2O): δ 3.02 (dd, 0.5H), 3.09 (dd, 0.5H), 3.28 (dd, 0.5H), 3.35 (dd, 0.5H), 3.78 (dd, 0.5H), 4.14 (dd, 0.5H), 5.48 (s, 0.5H), 5.70 (s, 0.5H), 7.25 (t, 0.5 H), 7.32 (m, 1.5H), 7.36 (t, 1H), 7.42 (d, 1H), 7.50 (d, 1H). ^{13}C NMR (D_2O): δ 173.22, 172.85, 141.96, 139.24, 128.50, 128.22, 128.17, 127.36, 127.22, 126.79, 72.10, 71.10, 66.65, 65.45, 59.76, 38.37, 20.78, 14.10. $[M + H]^+$ calculated 210.0583; found 210.0596.



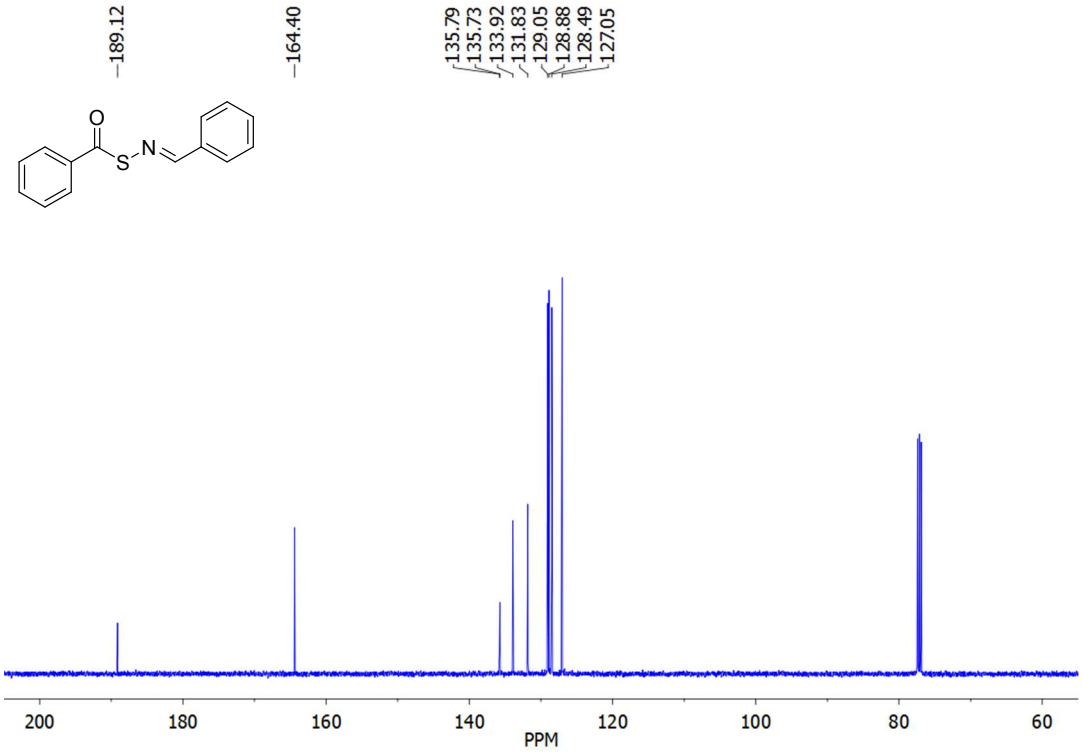
Cystine

Cystine was isolated as the HCl salt via filtration from the product analysis reaction mixture and subsequent purification by HPLC (gradient of 2% to 90% CH_3CN with 0.1% TFA). ^{1}H NMR (D_2O): δ 3.13 (m, 2H), 3.31 (dd, 2H), 4.26 (m, 1H). ^{13}C NMR (D_2O): δ 170.84, 51.93, 36.46. $[M + H]^+$ calculated 241.03; found 240.96 (LRMS).

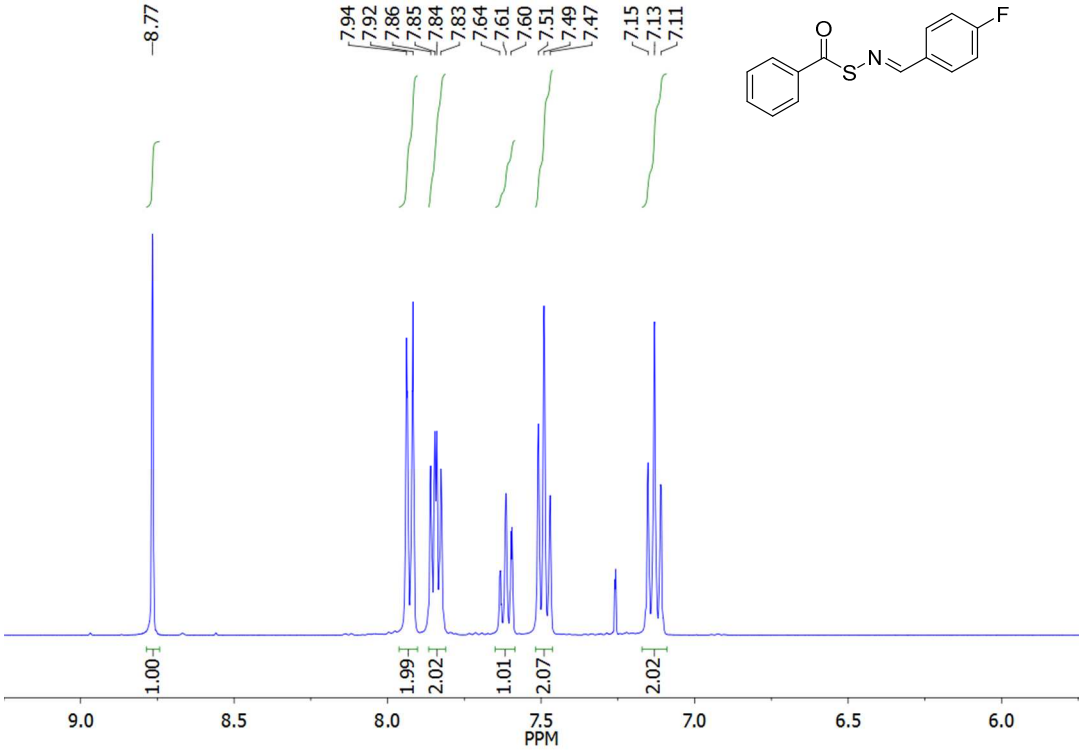
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1a**



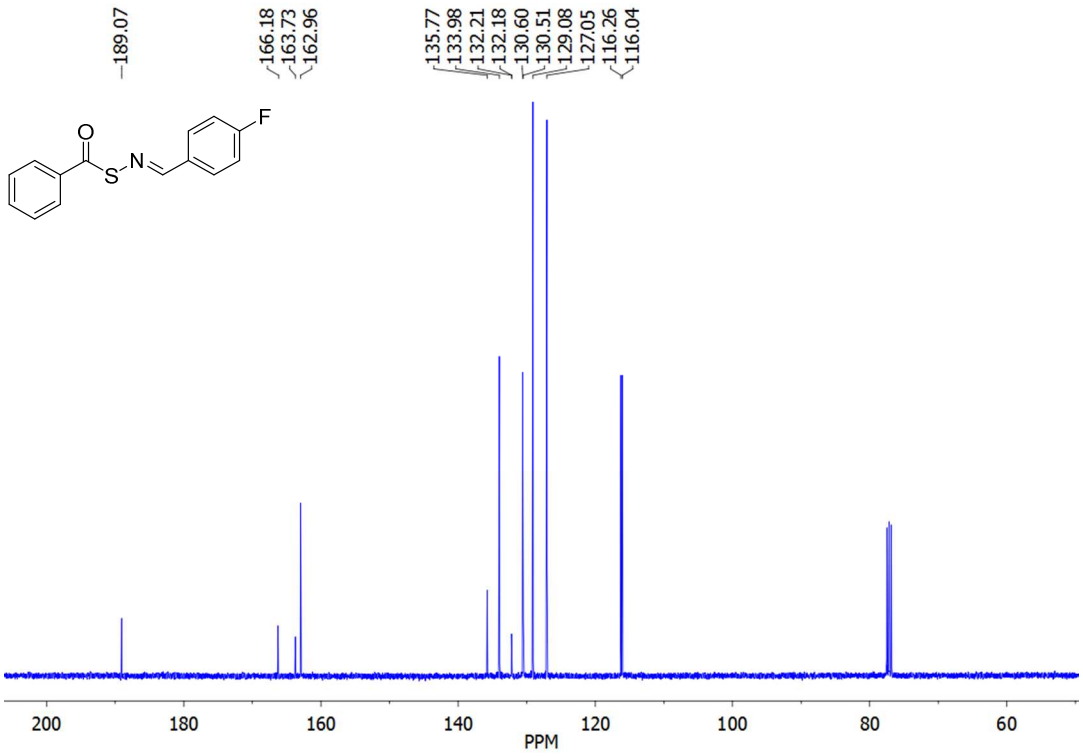
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1a**



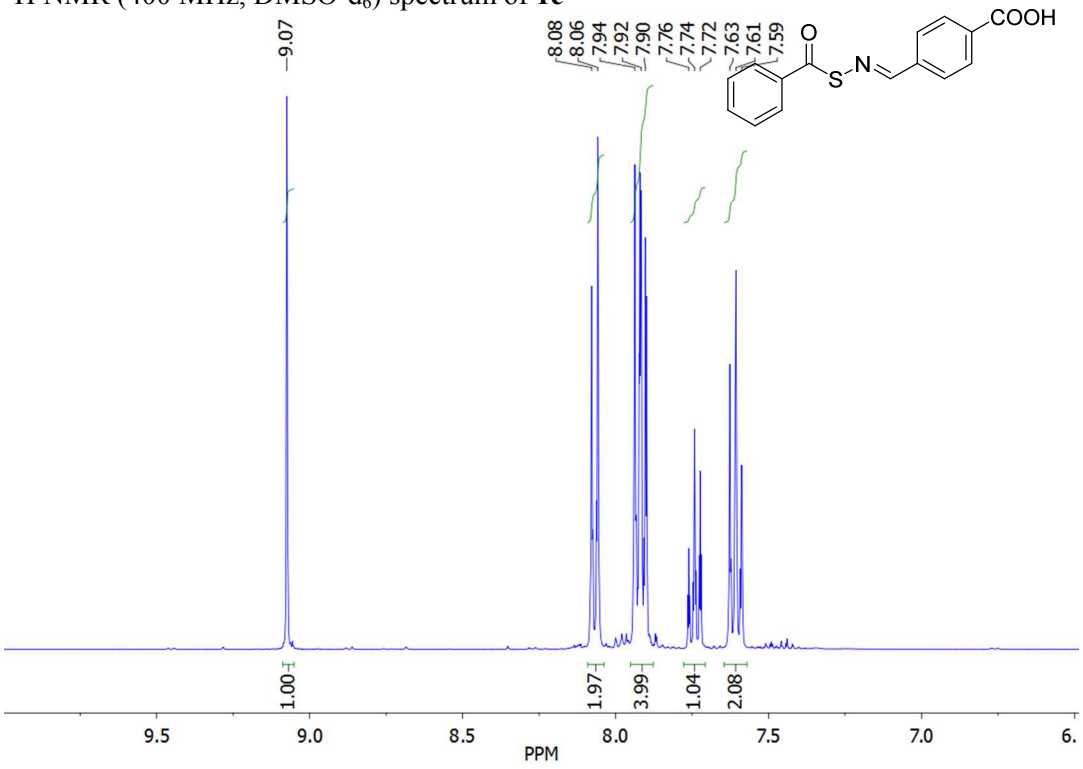
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1b**



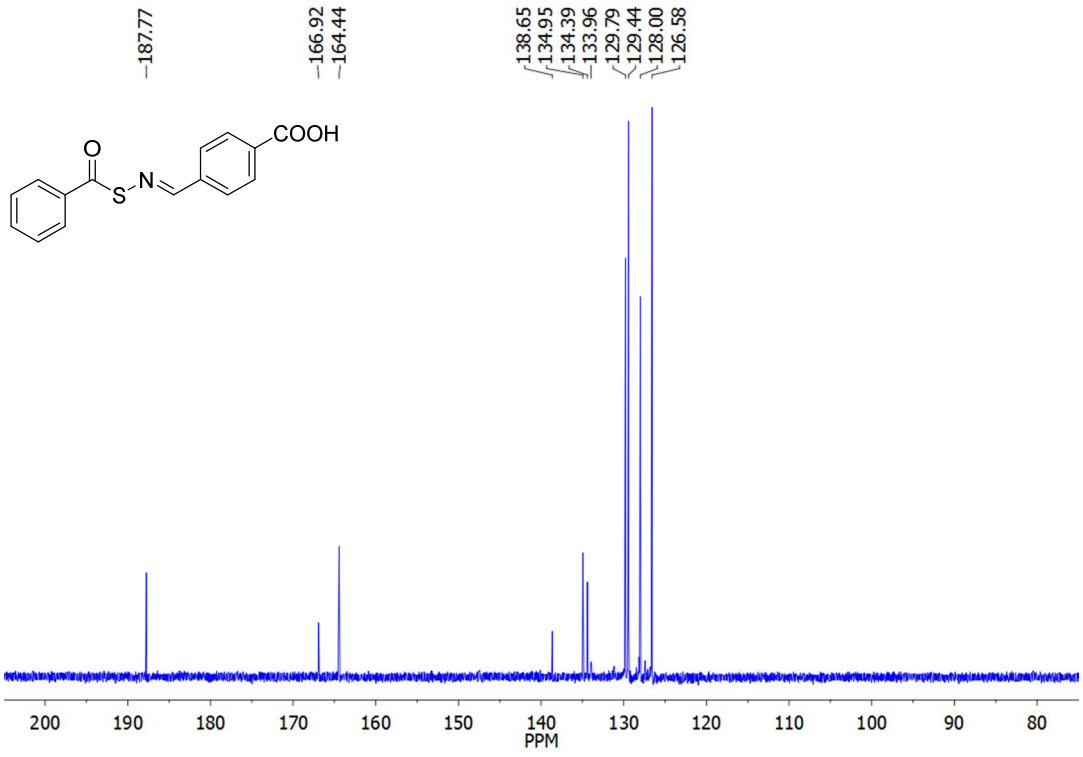
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1b**



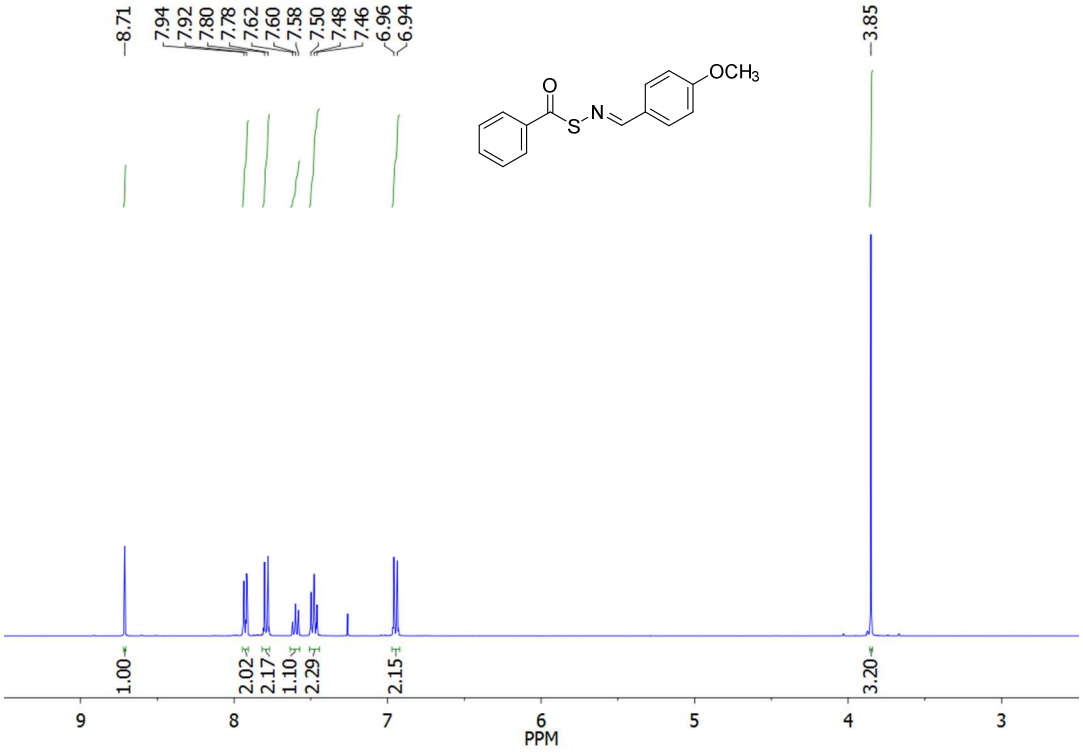
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **1c**



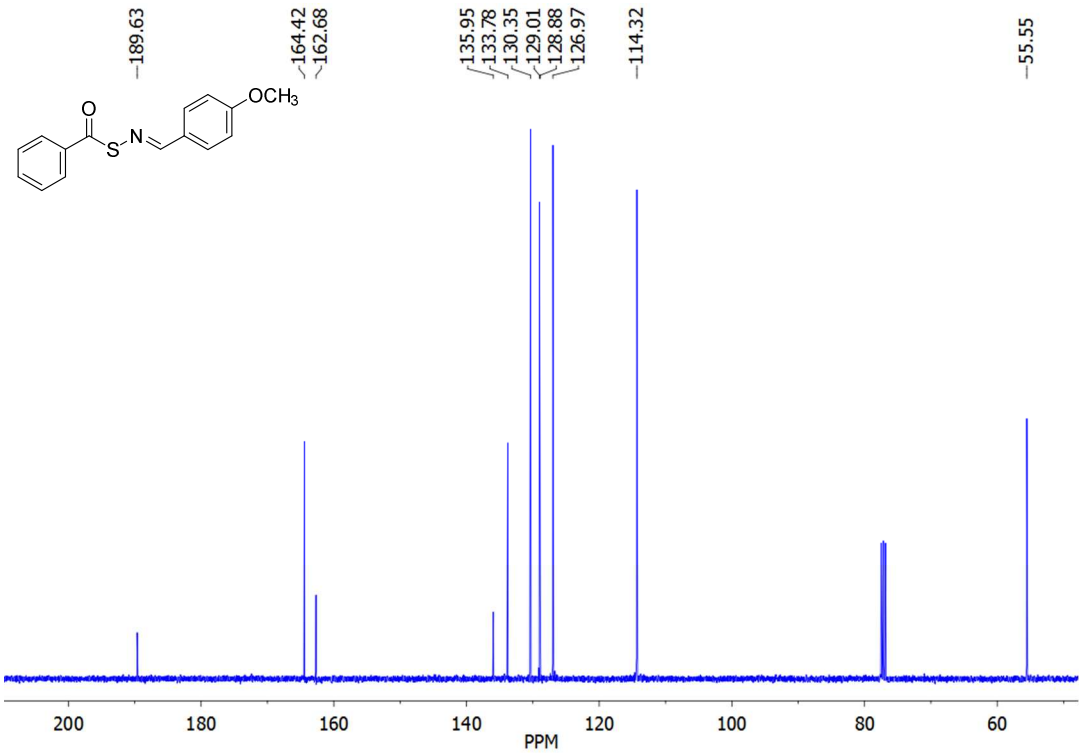
^{13}C NMR (400 MHz, DMSO-d_6) spectrum of **1c**



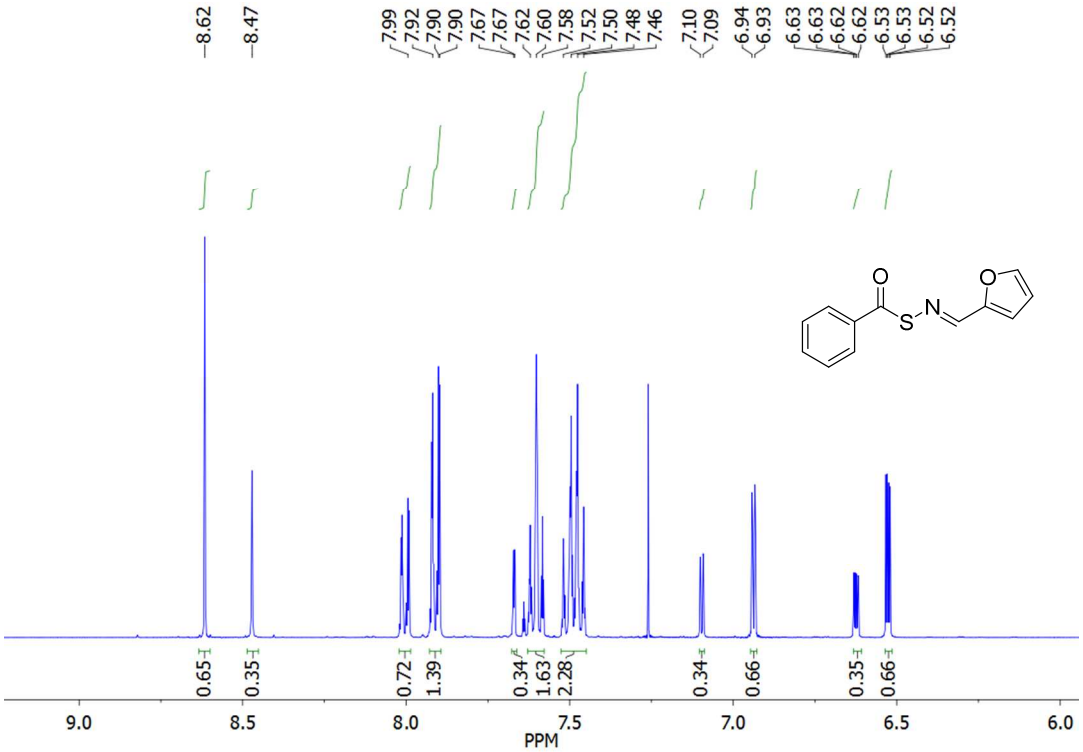
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1d**



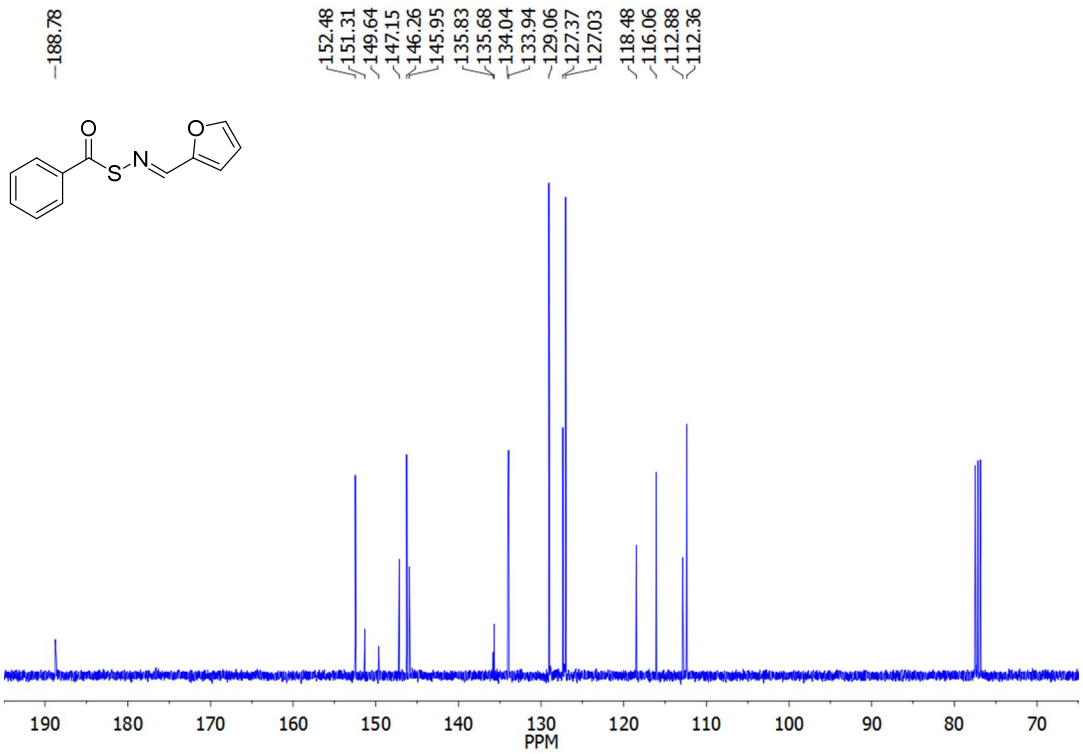
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1d**



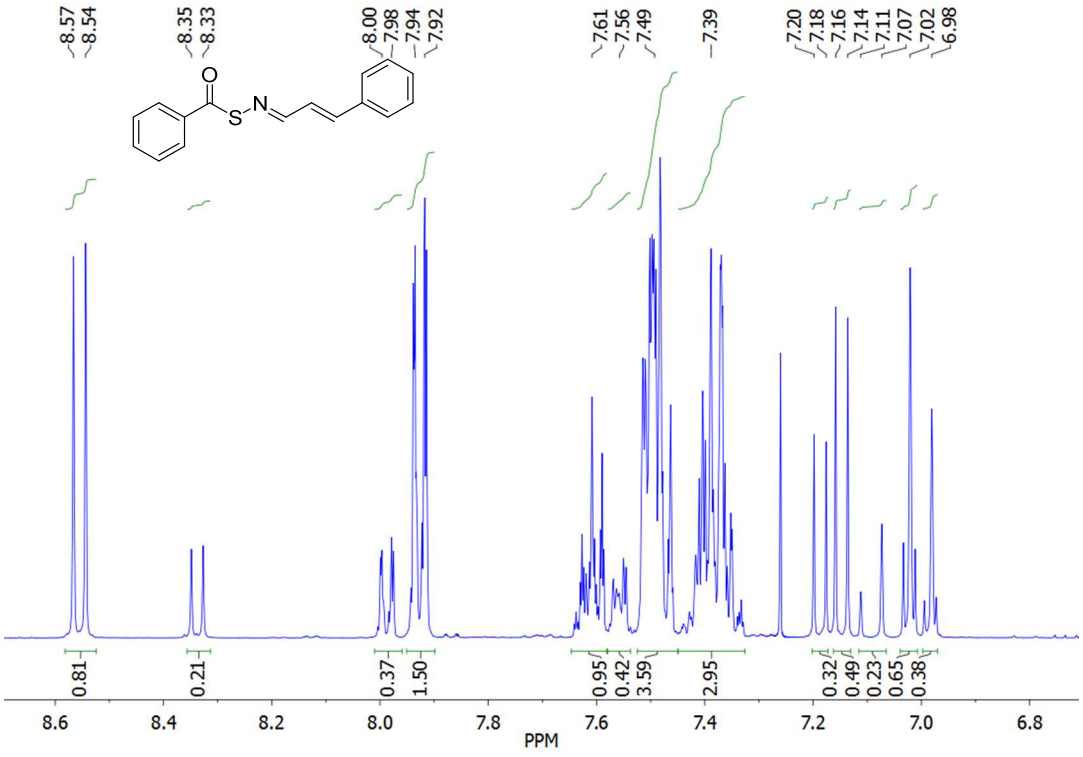
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1e**



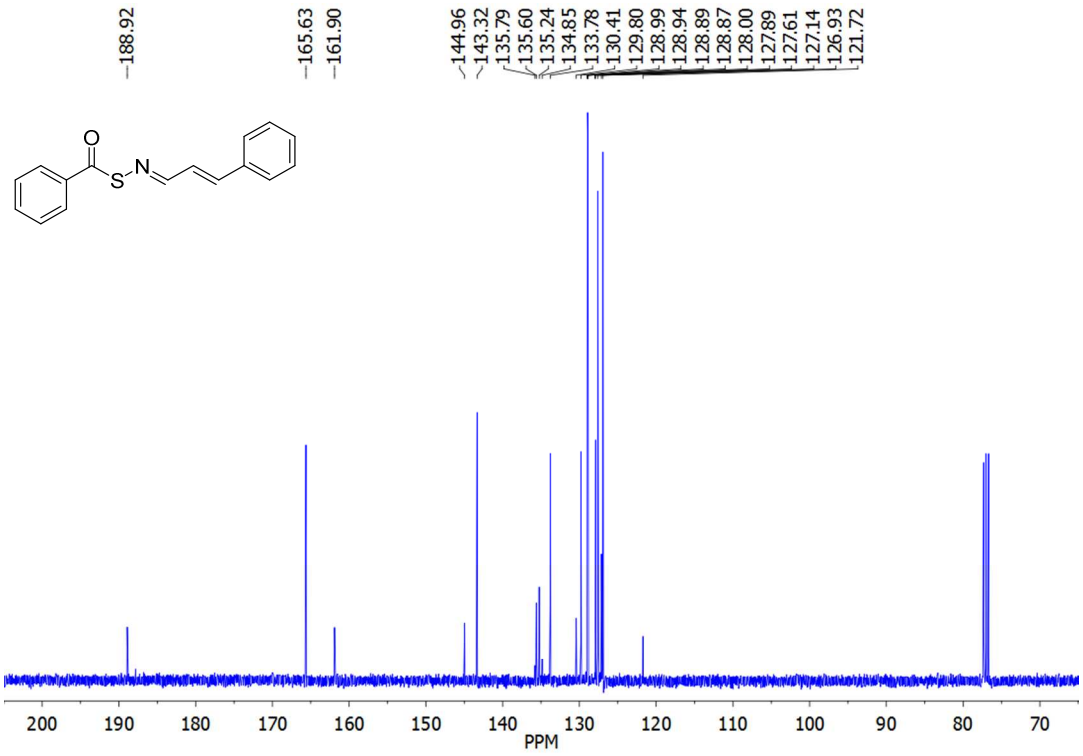
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1e**



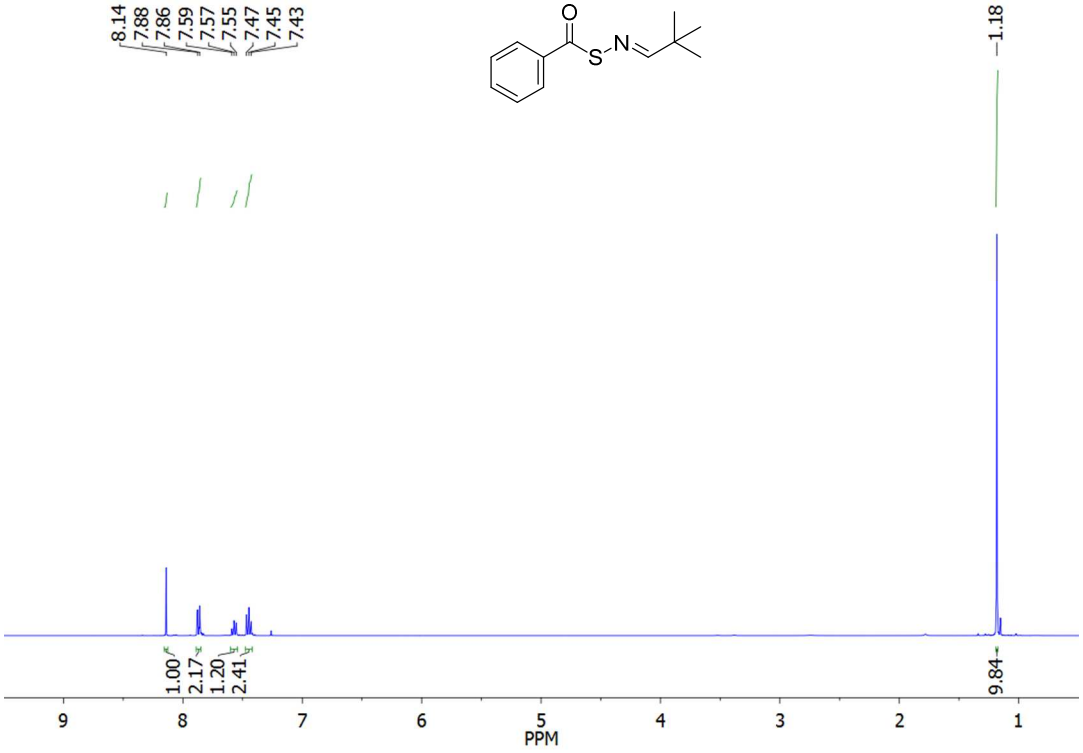
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1f**



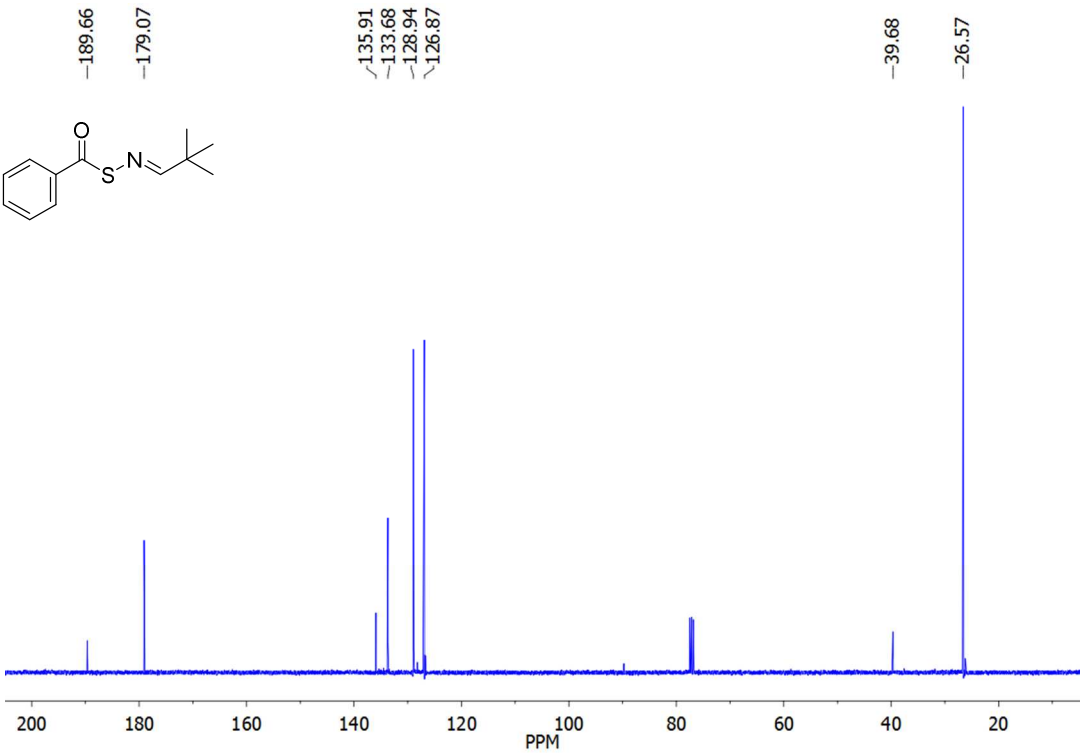
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1f**



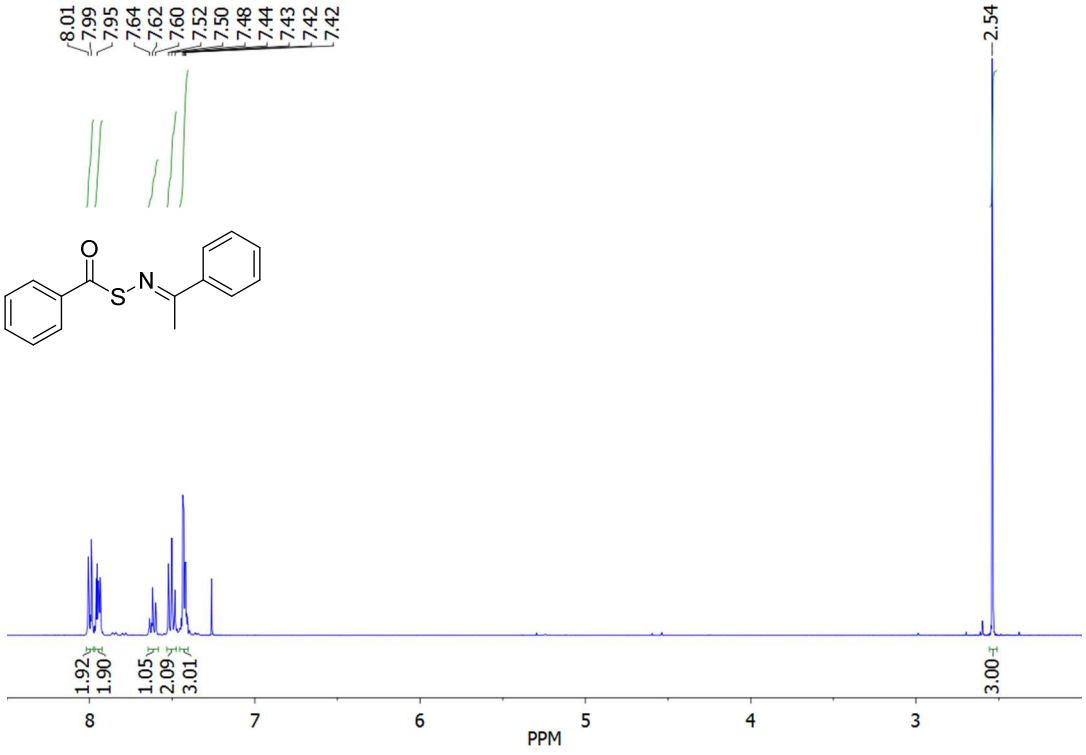
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1g**



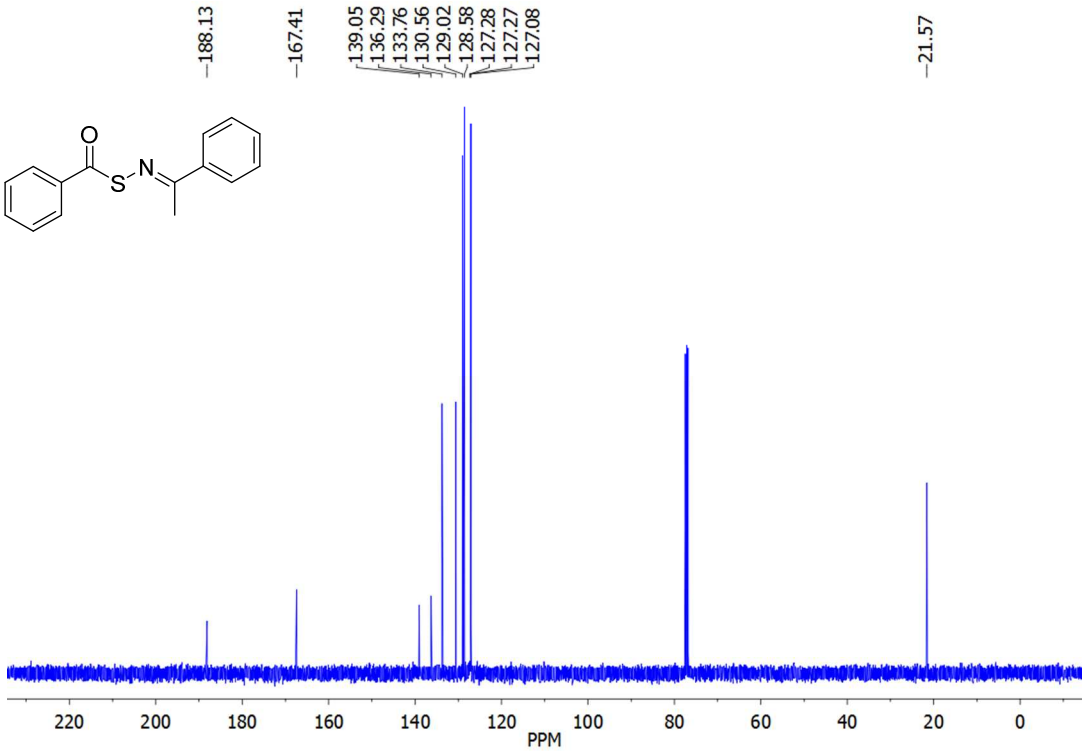
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1g**



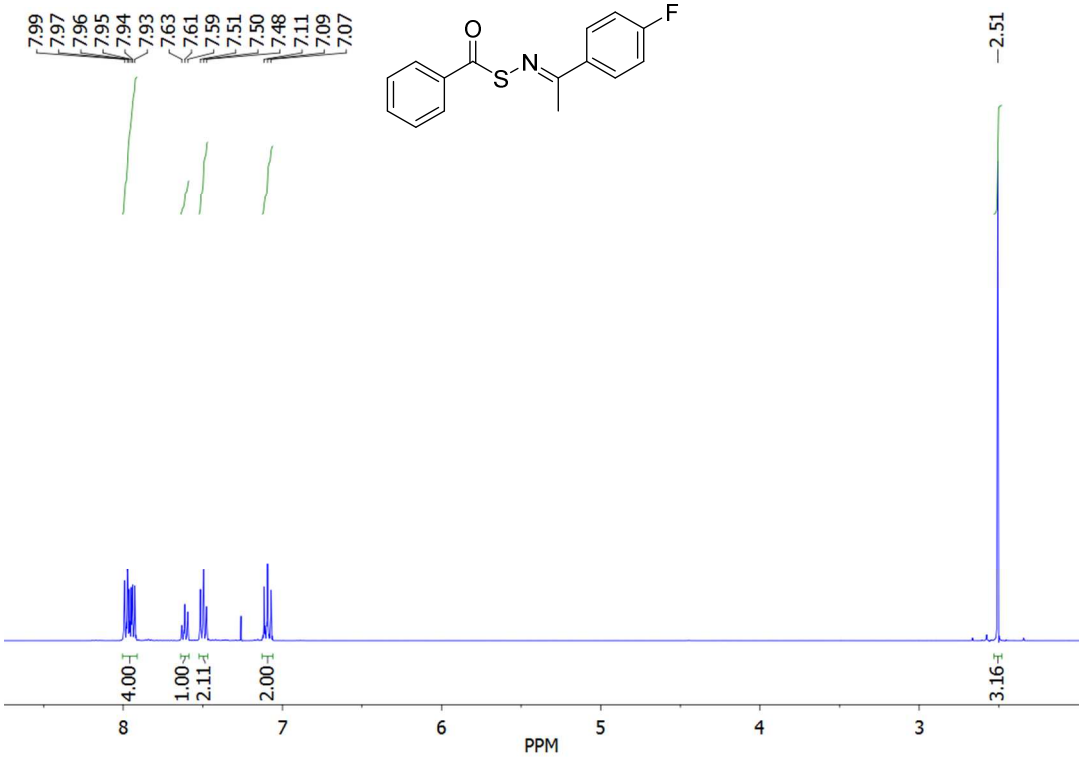
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1h**



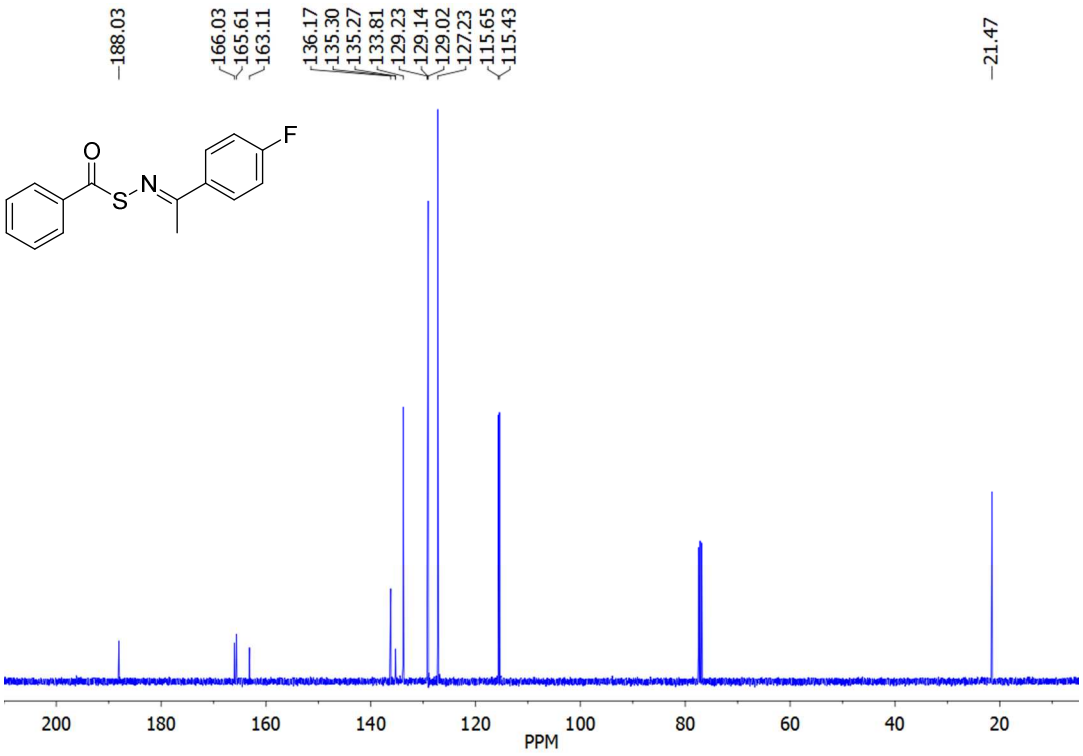
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1h**



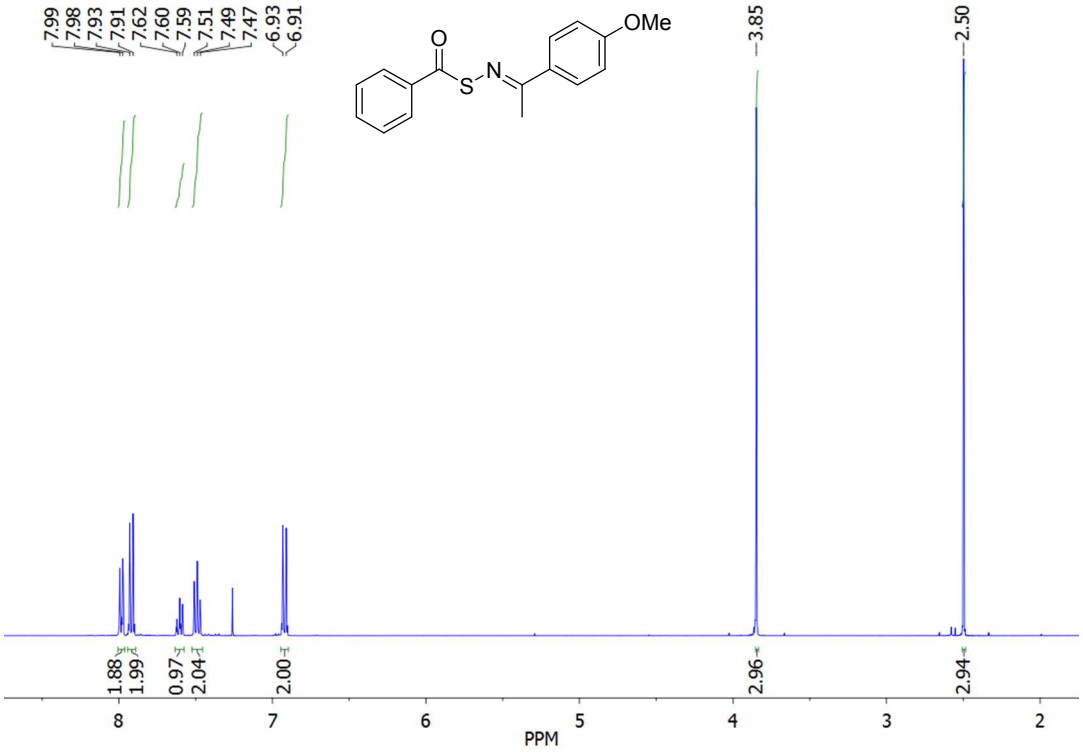
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1i**



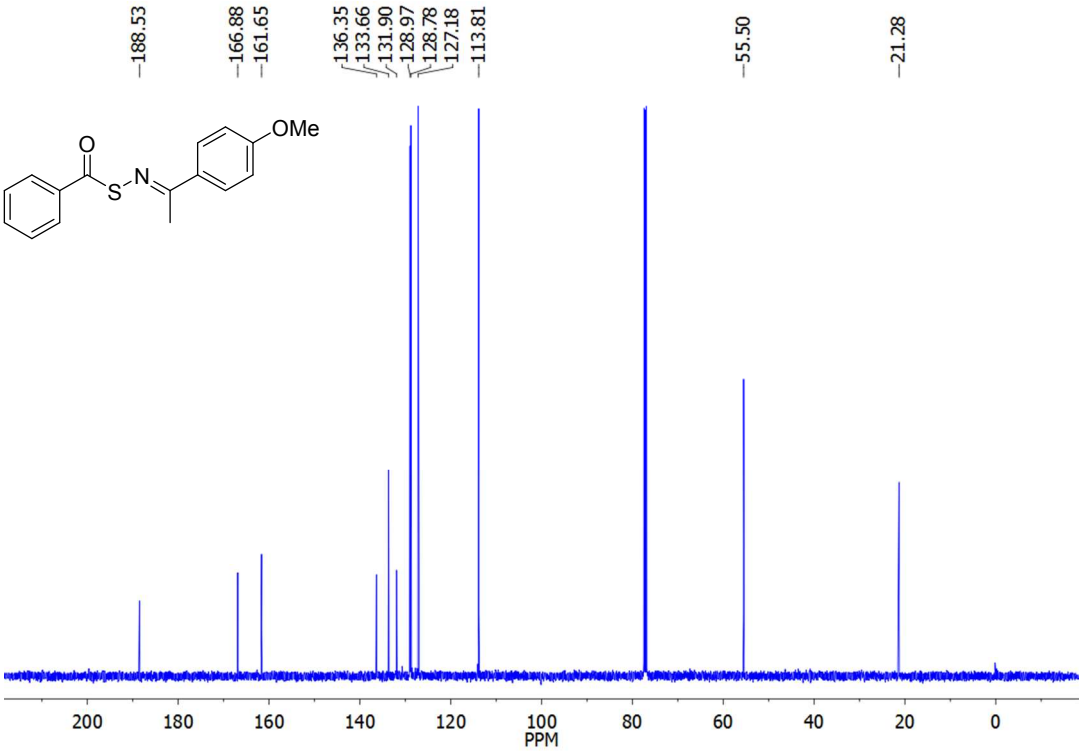
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1i**



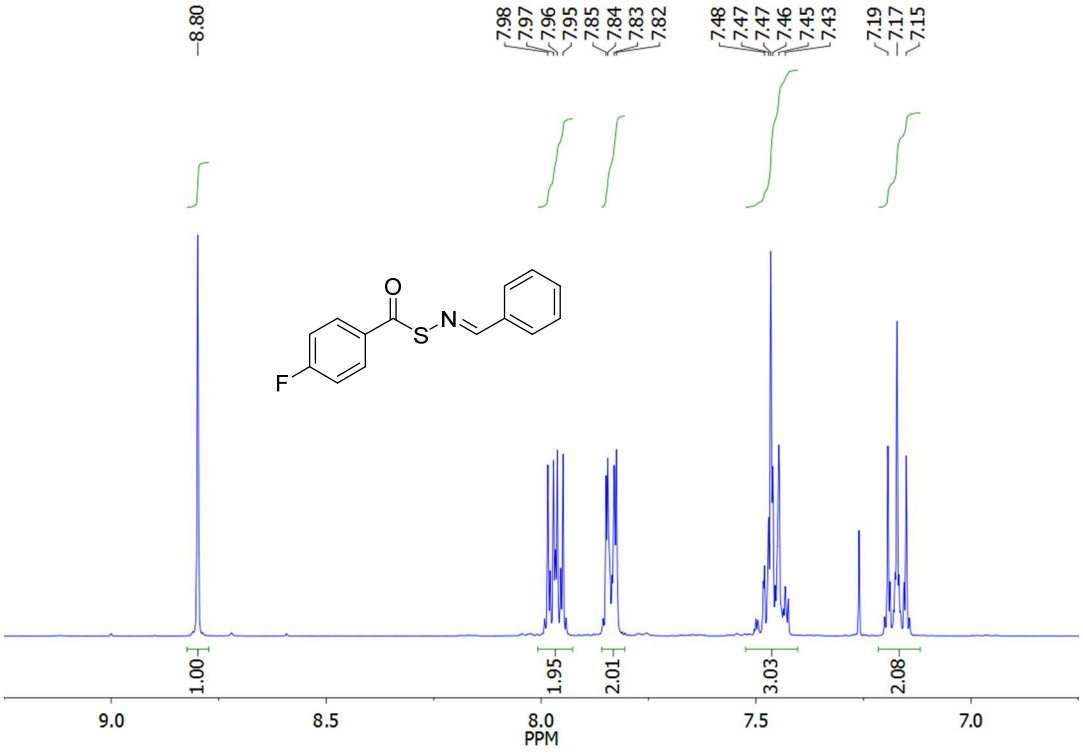
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1j**



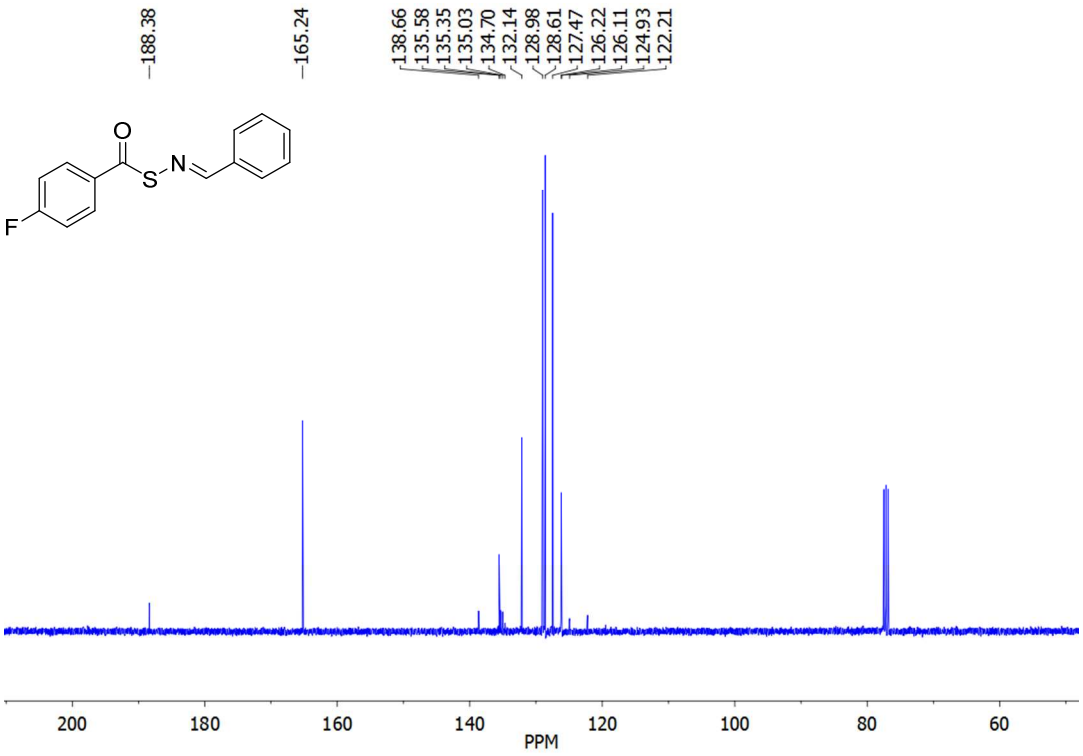
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1j**



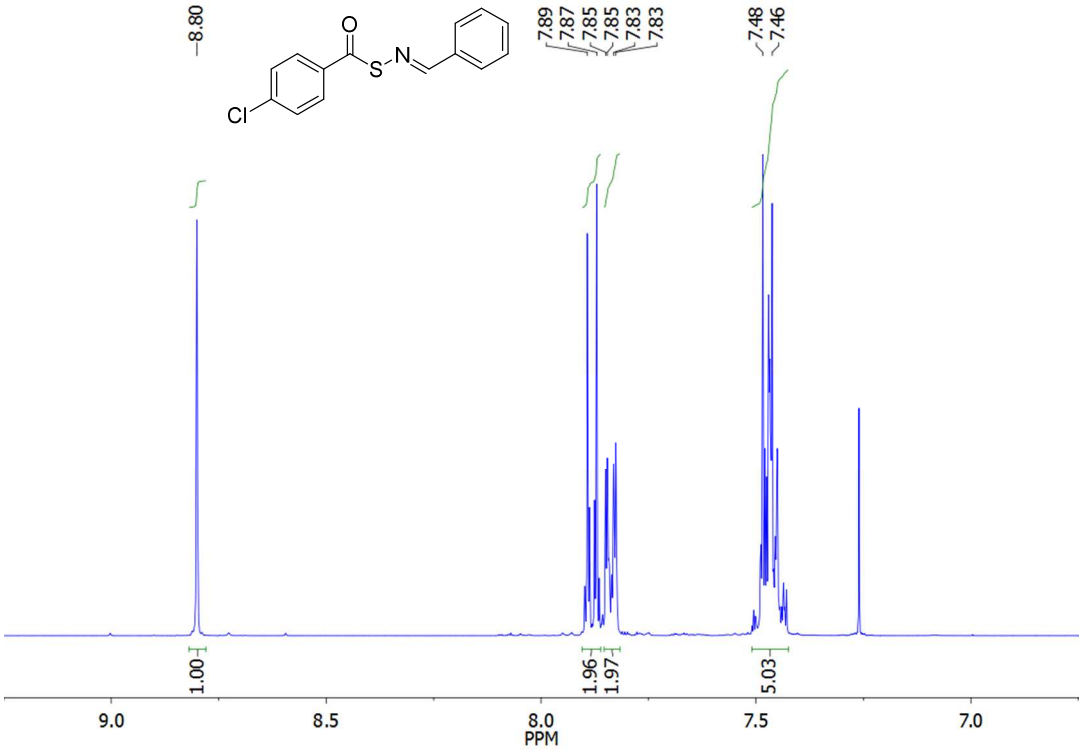
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1k**



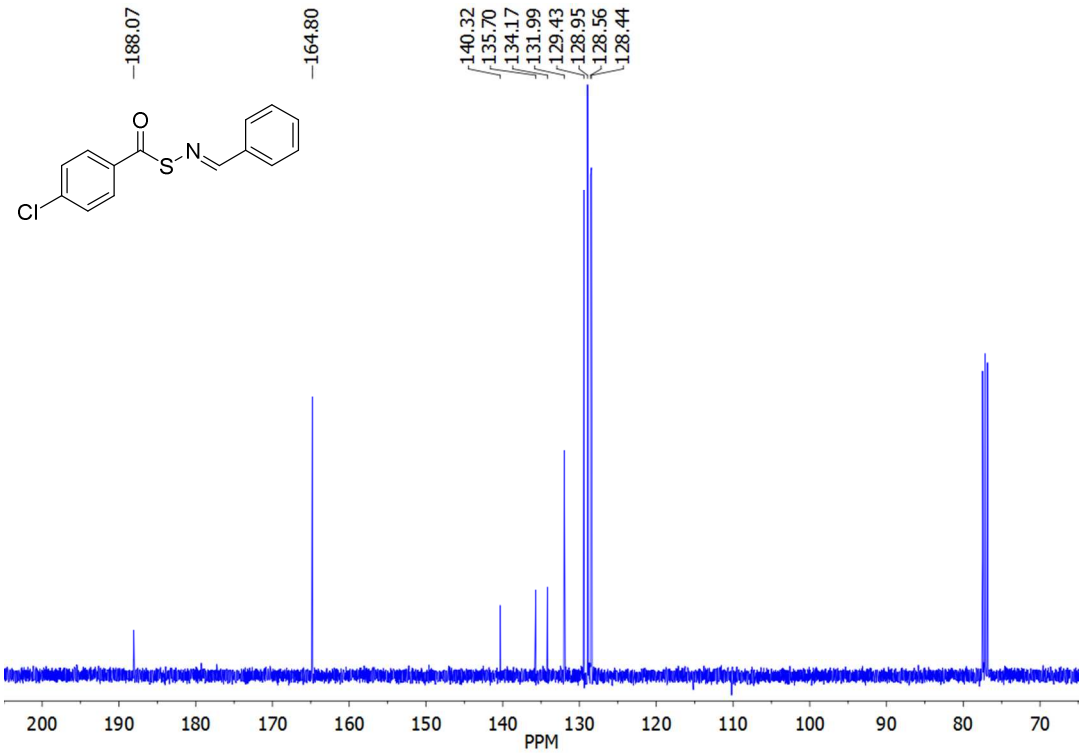
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1k**



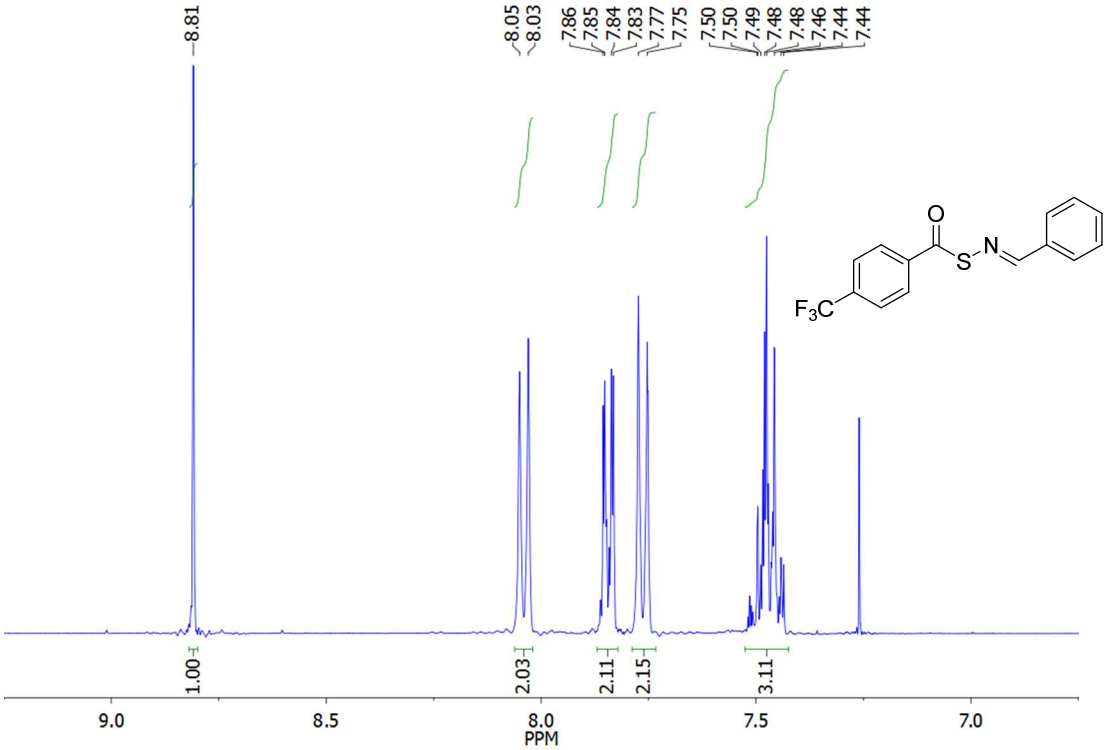
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1l**



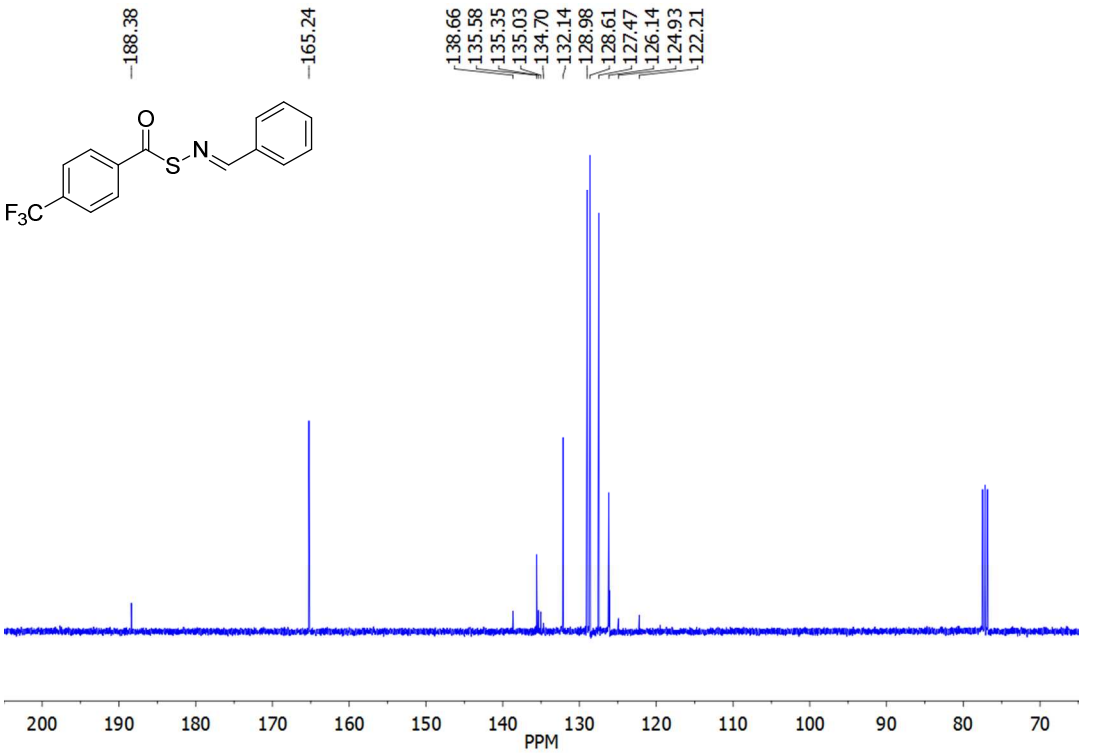
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1l**



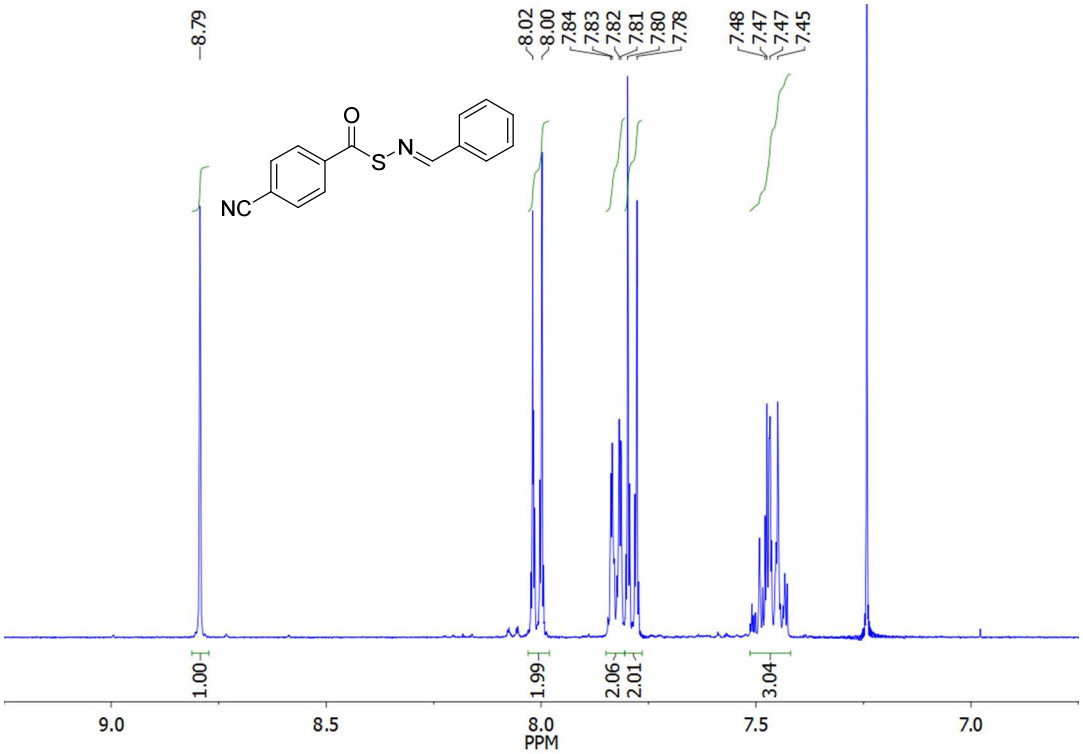
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1m**



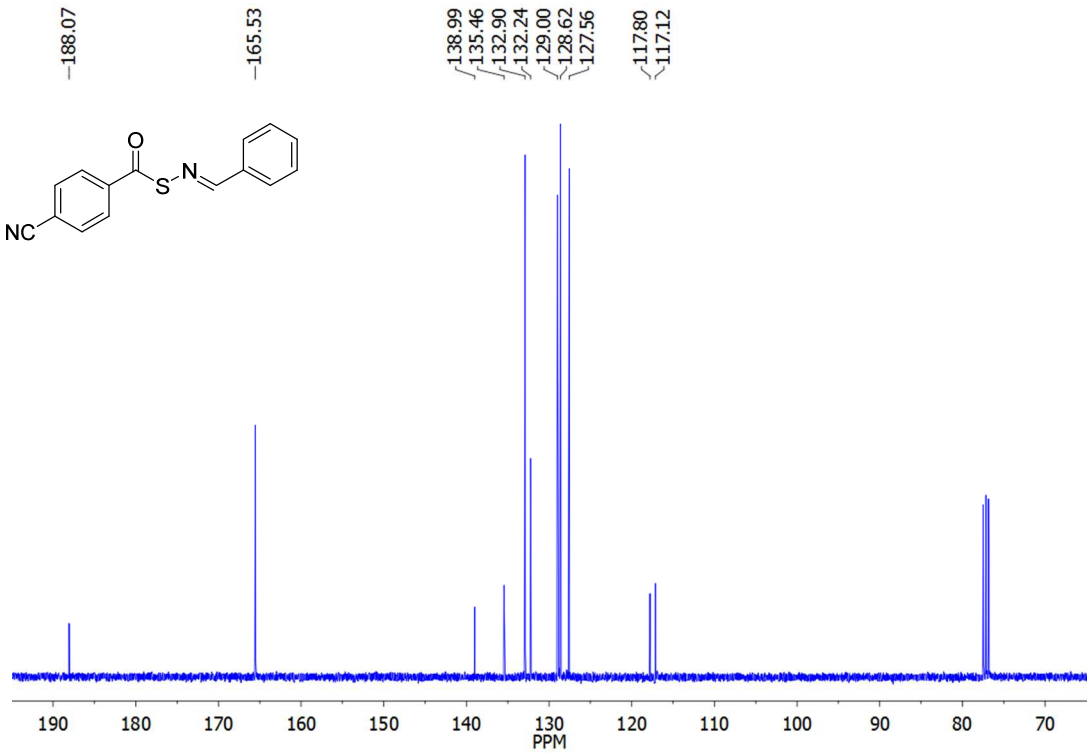
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1m**



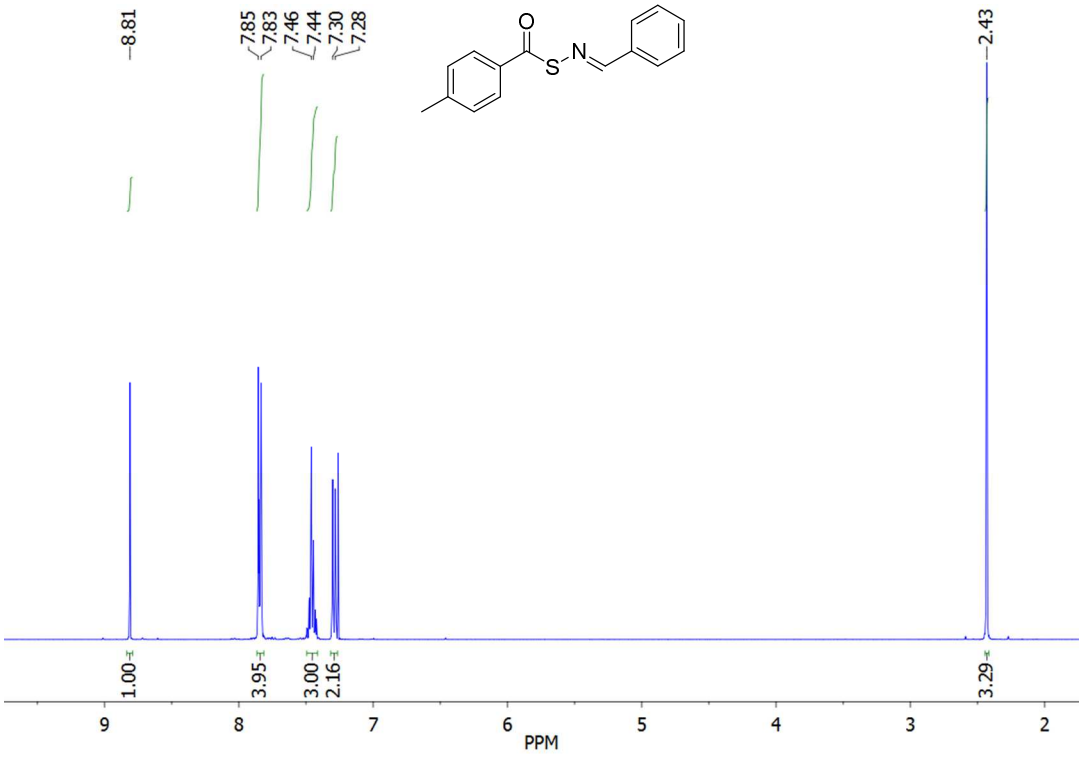
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1n**



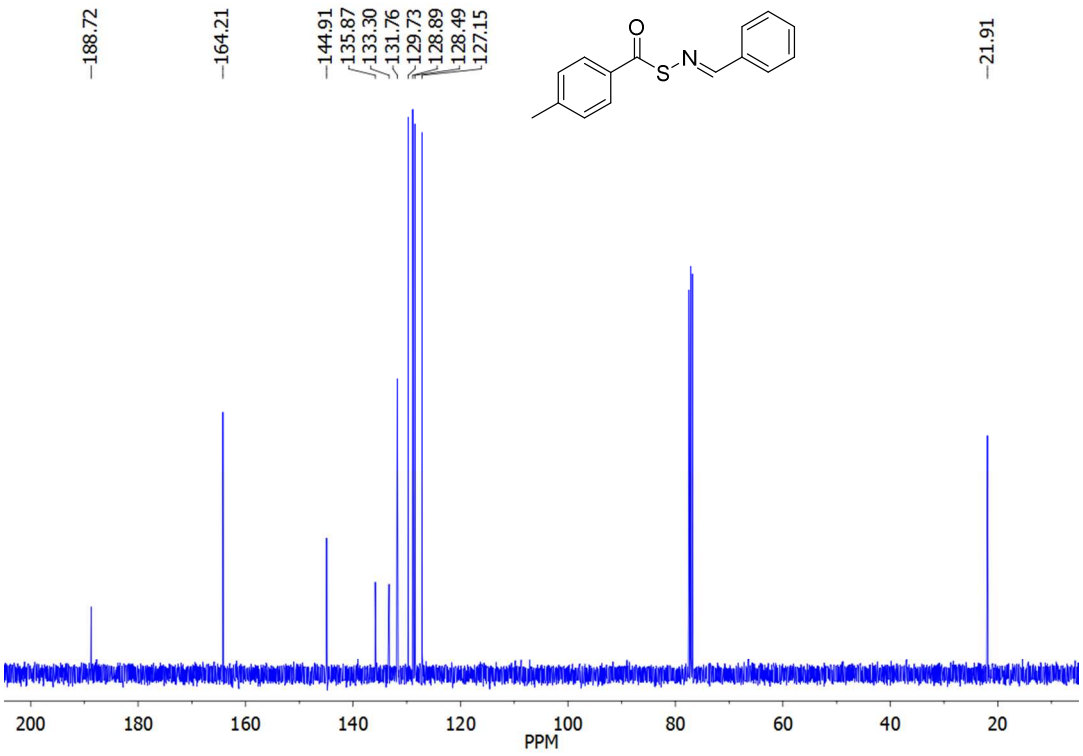
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1n**



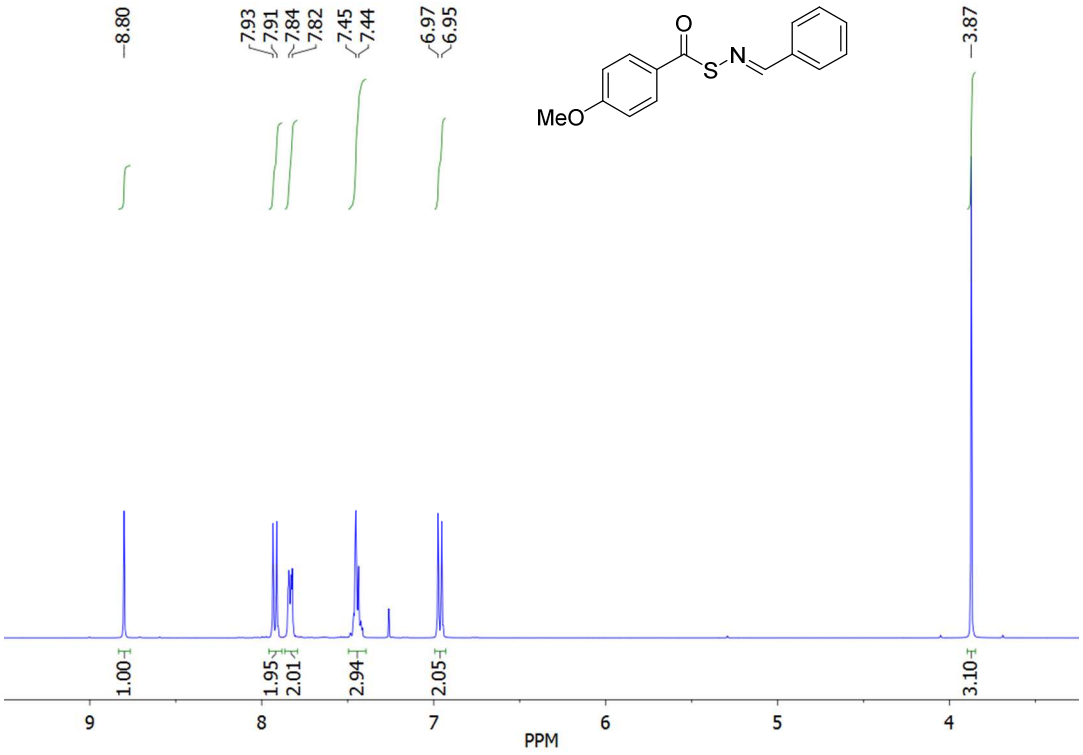
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1o**



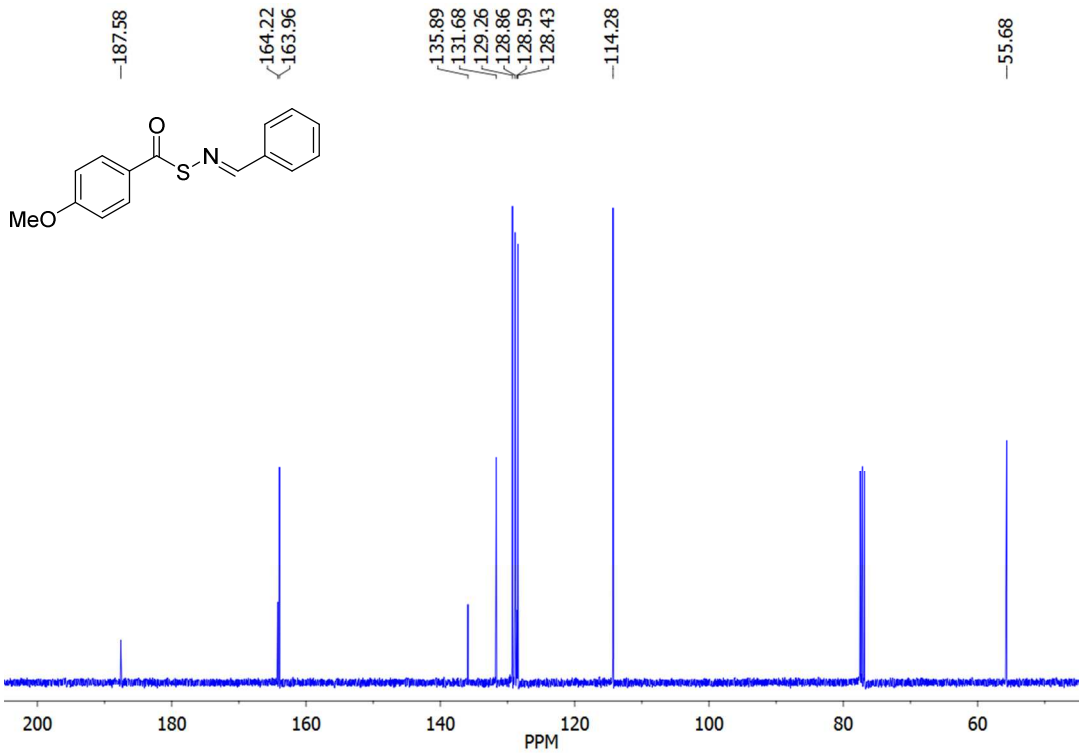
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1o**



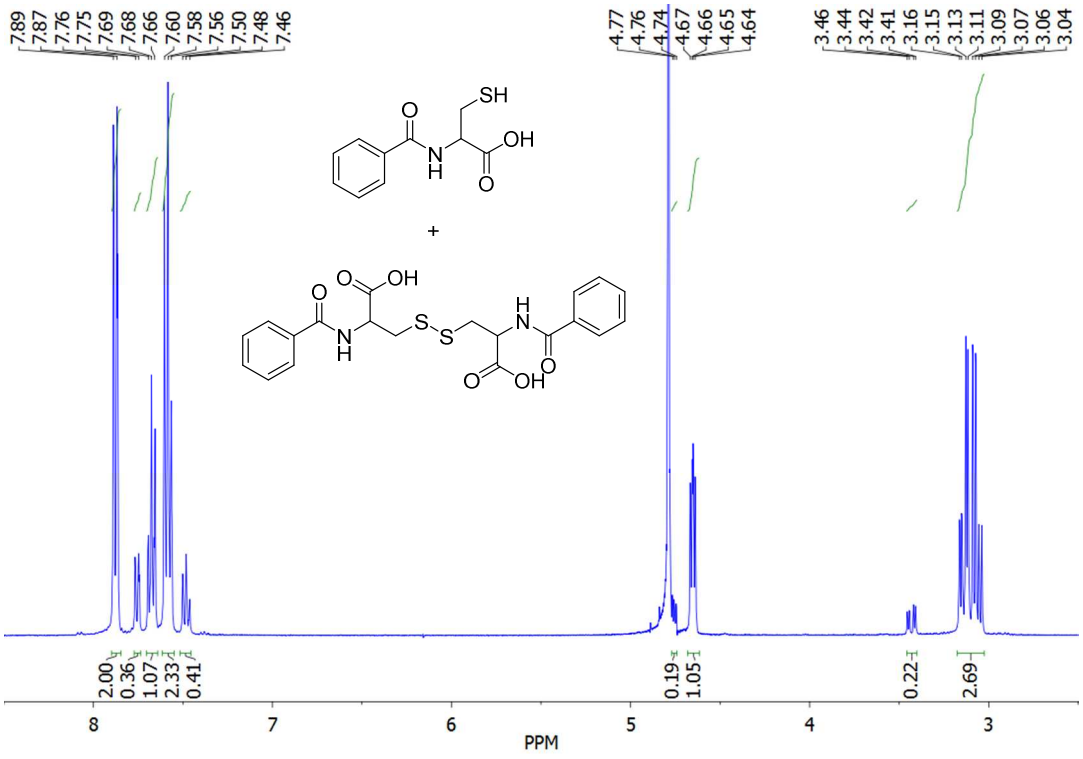
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1p**



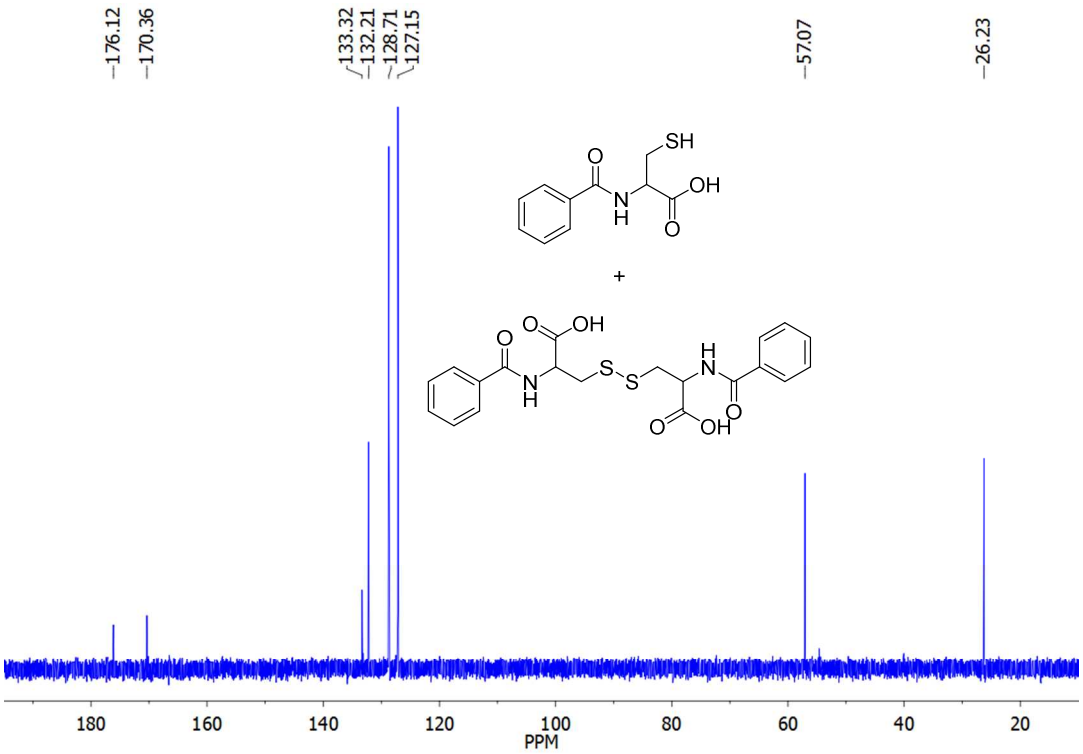
^{13}C NMR (400 MHz, $CDCl_3$) spectrum of **1p**



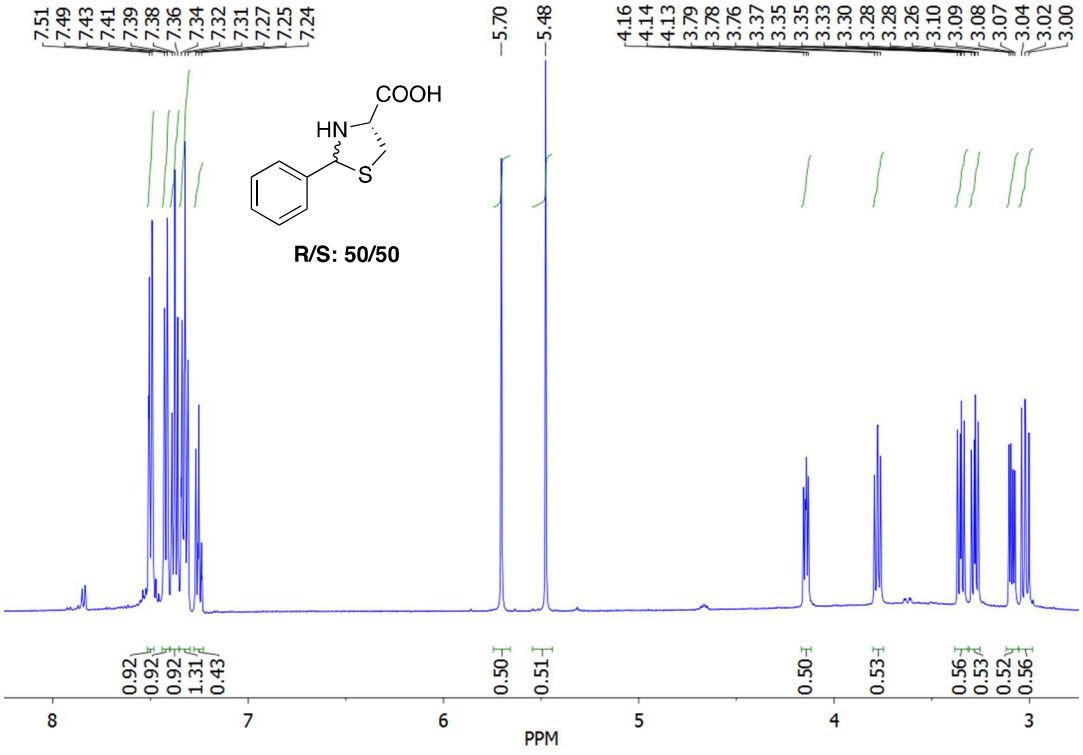
^{1}H NMR (400 MHz, D_2O) spectrum of ***N*-benzoylcysteine.**



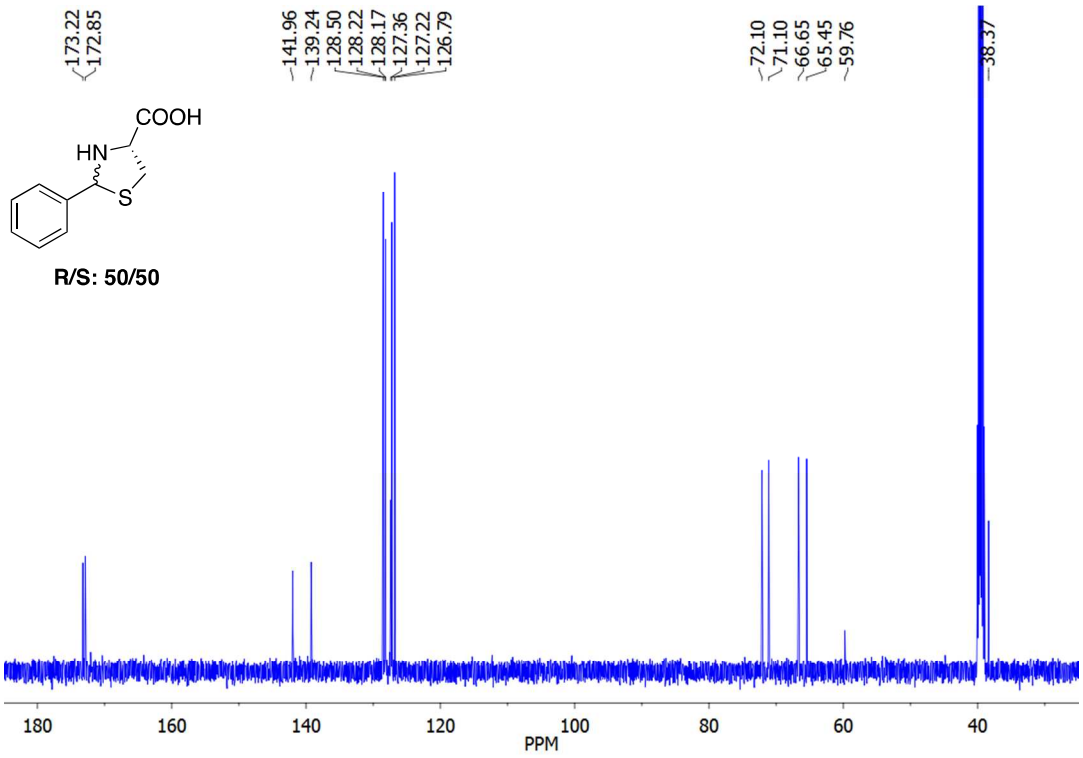
^{13}C NMR (400 MHz, D_2O) spectrum of ***N*-benzoylcysteine.**



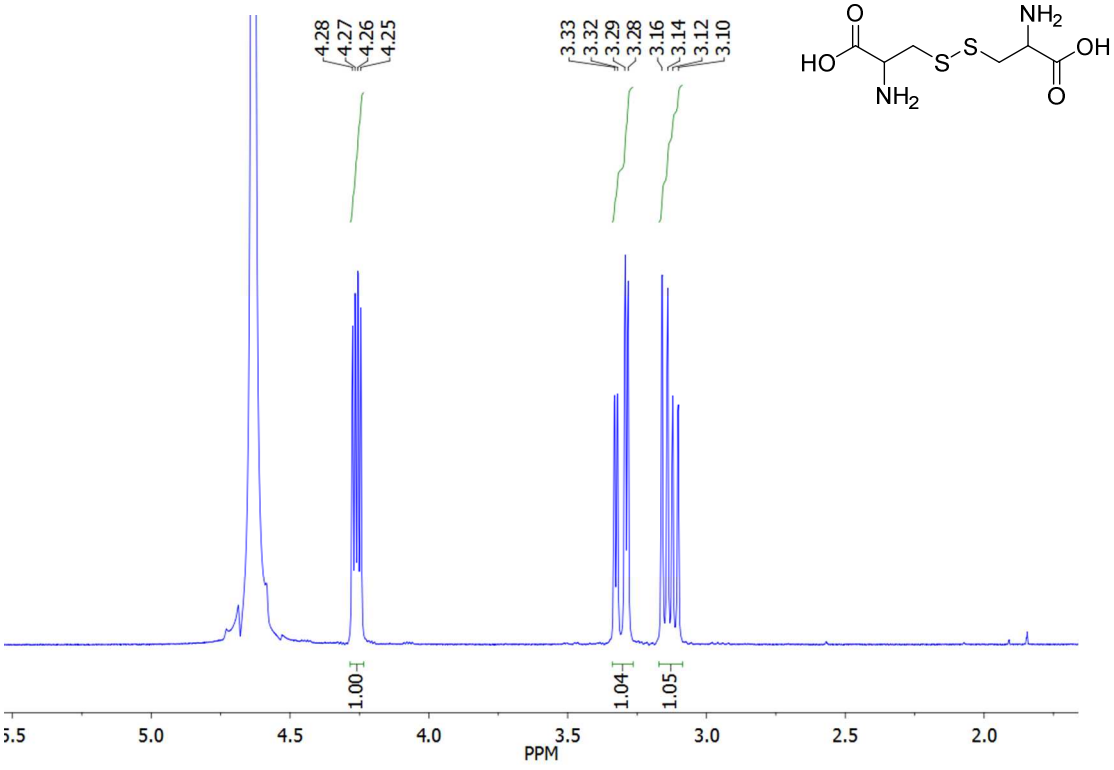
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **2-Phenylthiazolidine-4-carboxylic acid.**



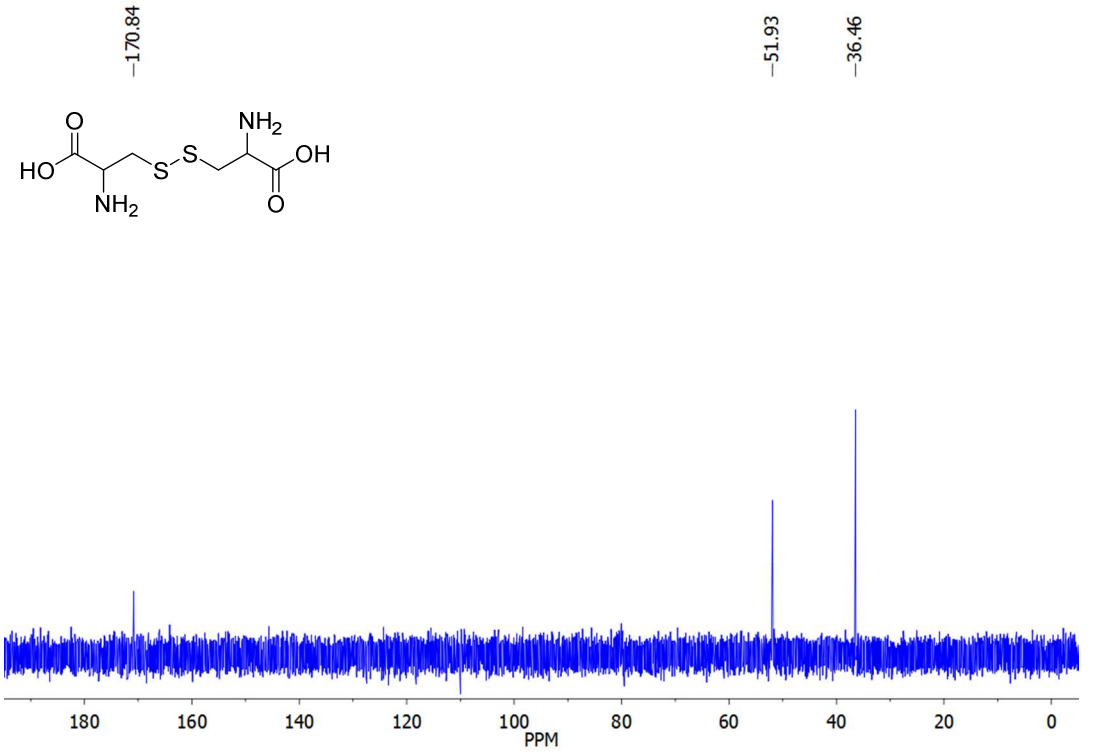
^{13}C NMR (400 MHz, DMSO-d_6) spectrum of **2-Phenylthiazolidine-4-carboxylic acid.**



^{1}H NMR (400 MHz, D_2O) spectrum of **cystine** (impure).



^{13}C NMR (400 MHz, D_2O) spectrum of **cystine** (impure).



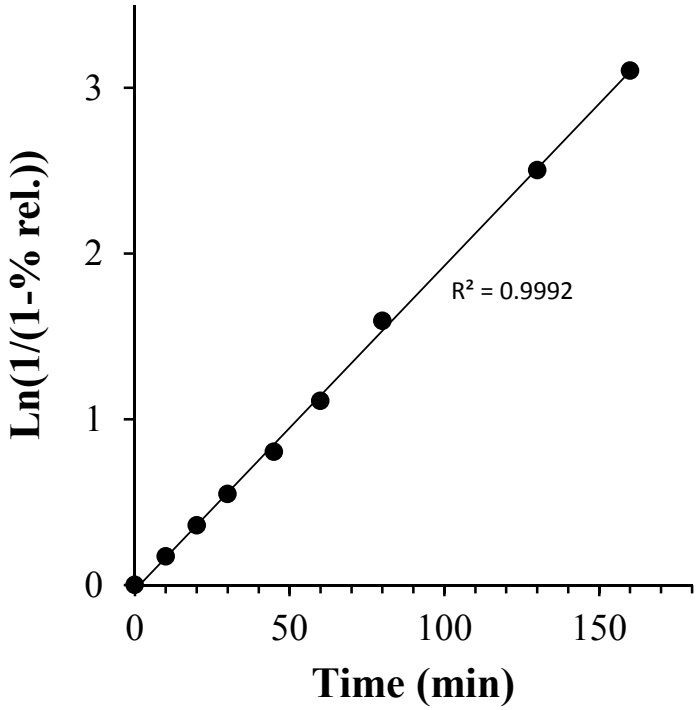


Fig S7. Methylene Blue Kinetics for Compound **1a** (R_2 = H).

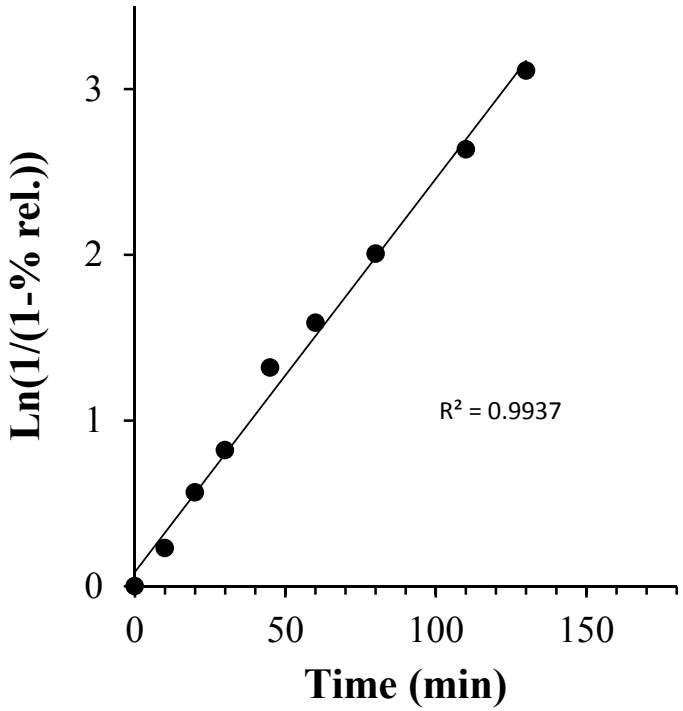


Fig S8. Methylene Blue Kinetics for Compound **1k** (R_2 = F)**.**

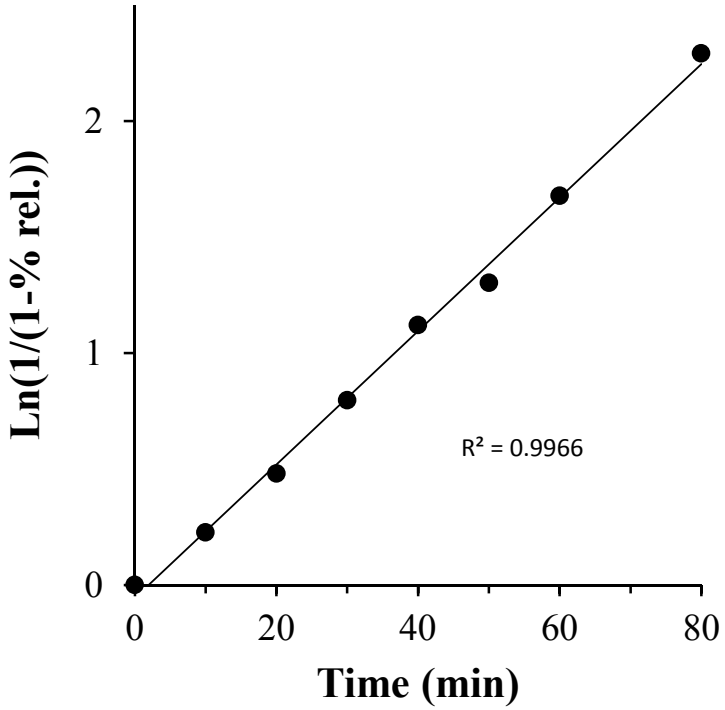


Fig S9. Methylene Blue Kinetics for Compound **1l** (R_2 = Cl).

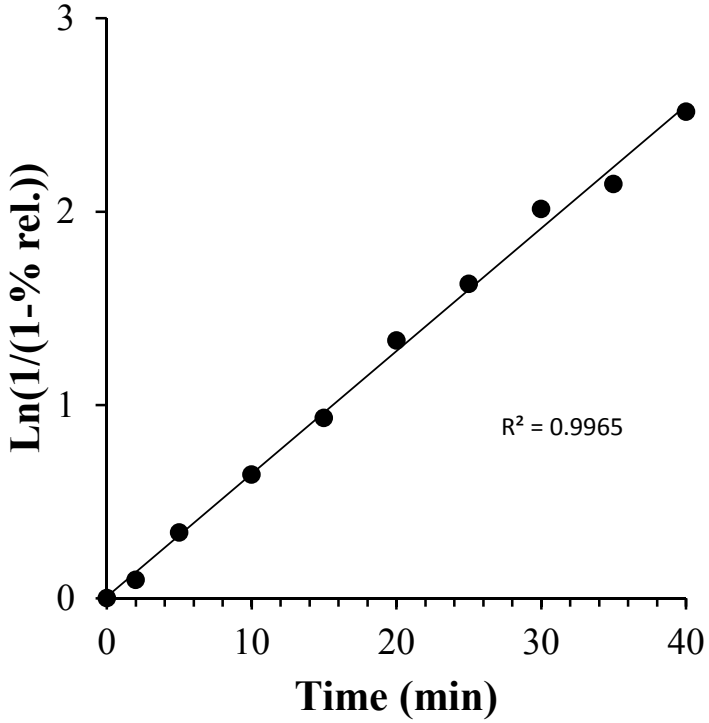


Fig S10. Methylene Blue Kinetics for Compound **1m** (R_2 = CF_3).

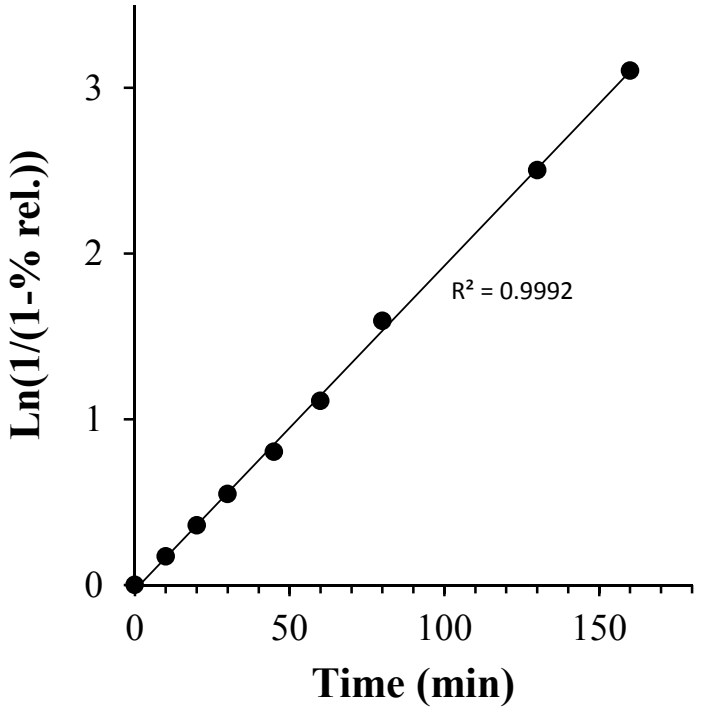


Fig S11. Methylene Blue Kinetics for Compound **1n** (R_2 = CN).

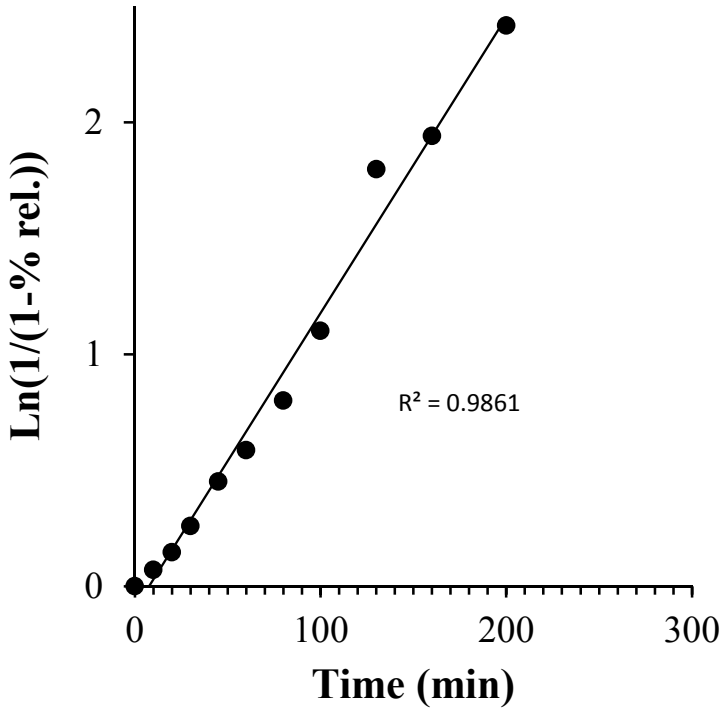


Fig S12. Methylene Blue Kinetics for Compound **1o** (R_2 = CH_3).

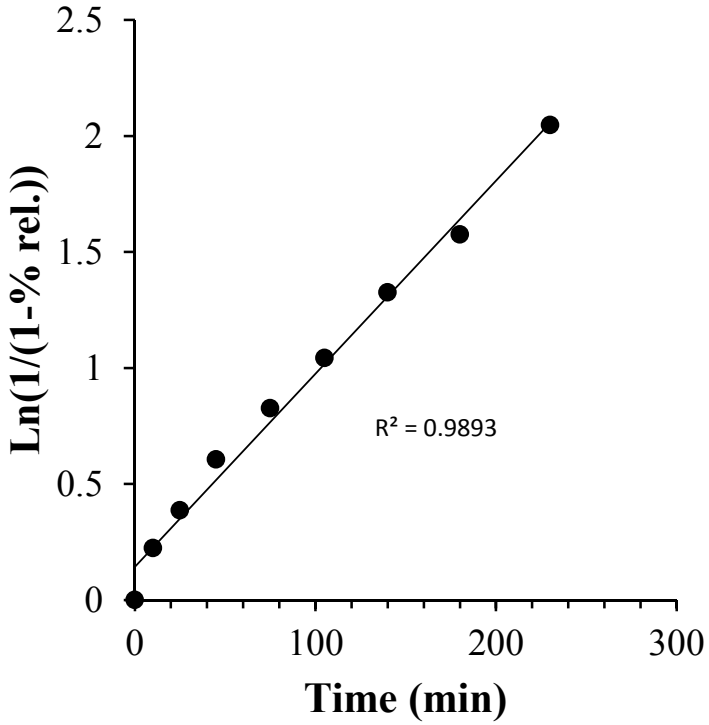


Fig S13. Methylene Blue Kinetics for Compound **1p** ($R_2 = CH_3$).

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