Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supporting Information (ESI)

for

A Modular Scaffold for Triggerable and Tunable Nitroxyl (HNO) Generation with a Fluorescence Reporter

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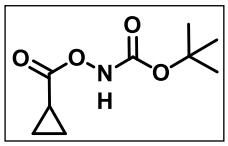
1. General methods:

All the chemicals and solvents were purchased from commercial sources and used as received unless stated otherwise. Column chromatography was performed using silica gel-Rankem (60–120 mesh) as stationary phase. Preparative high performance liquid chromatography (HPLC) was done using Combiflash EZ prep UV using a Kromasil®C-18 preparative column (250 mm × 21.2 mm, 5 μm). ^{1}H and ^{13}C spectra were recorded on a JEOL 400 MHz (or 100 MHz for ^{13}C) or a Bruker 400 MHz (or 100 MHz for ^{13}C) spectrometer unless otherwise specified using either residual solvent signals ($CDCl_3$ δH = 7.26 ppm, δC = 77.2 ppm), (CD_3OD δH = 3.31 ppm, δC = 49.0 ppm) or as an internal tetramethylsilane (δH = 0.00, δC = 0.0). Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The following abbreviations are used: m (multiplet), s (singlet), d (doublet), t (triplet) and dd (doublet of doublets). High-resolution mass spectra were obtained from HRMS-ESI-Q-Time of Flight LC/MS. FT-IR spectra were recorded using BRUKER-ALPHA FT-IR spectrometer and reported in cm^{-1}. All measurements were done using a LC/MS method in the positive ion mode using high resolution multiple reaction monitoring (MRM-HR) analysis on a Sciex X500R quadrupole time-of flight (QTOF) mass spectrometer fitted with an Exion UHPLC system. Photometric measurements were performed using an Ensight Multimode Plate Reader (PerkinElmer). Fluorometric measurements were performed using an and Thermo Scientific Varioscan microplate reader and HORIBA Scientific Fluoromax-4 spectrofluorometer.

2. Synthesis and characterization:

Synthesis of *tert*-butyl ((cyclopropanecarbonyl)oxy)carbamate (5): From cyclopropylcarbonyl chloride, *tert*-butyl ((cyclopropanecarbonyl)oxy)carbamate (**5**) was synthesized by using reported protocol.[1]

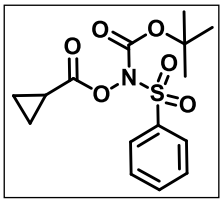


N-Boc hydroxylamine (764 mg, 5.74 mmol, 1.0 eq.) was dissolved in DCM (4 mL) and NEt_3 (0.74 mL, 7.31 mmol, 1.1 eq.) was added. At 0 ˚C, cyclopropanecarbonyl chloride (600 mg, 5.74 mmol, 1.0 eq.) was added dropwise and the reaction mixture was warmed to reach room temperature. After 2 h of stirring, the reaction mixture was vacuum filtered and the residue was washed with DCM. The filtrate was washed with water (20 mL), satd. $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated to yield the corresponding carbamate **5** as a colourless crystalline solid (800 mg, 70%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.93 (s, 1H), 1.81 - 1.75 (m, 1H), 1.49 (s, 9H), 1.16 - 1.12 (m, 2H), 1.05 - 1.0 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 174.5, 155.7, 83.3, 28.2, 11.1, 9.6; HRMS (ESI-TOF) for $[C_9H_{15}NO_4 + H]^+$: Calcd., 202.1078, Found, 202.1083.

General procedure for the synthesis of *N*-sulfonyl-*N*-(cyclopropanecarbonyl)oxy-*tert*-butyl-carbamate (2): From *tert*-butyl ((cyclopropanecarbonyl)oxy)carbamate (**5**), *N*-sulfonyl-*N*-(cyclopropanecarbonyl)oxy-*tert*-butyl-carbamate (**2**) was synthesized by using reported protocol.[2]

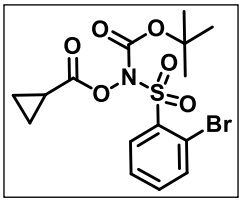
tert-butyl ((cyclopropanecarbonyl)oxy) carbamate (**5**) was dissolved in anhydrous THF. To this solution 2.5 eq. of NaH was added and the reaction stirred for 5 minutes until the completion of gas evolution. To this solution, 1 eq. of the sulfonyl chloride derivatives ($ArSO_2Cl$) was added. Upon completion of reaction (TLC analysis) the solvent was evaporated under reduced pressure to yield crude product, which was further purified using silica gel column chromatography to yield an *N*-sulfonyl-*N*-(cyclopropanecarbonyl)oxy-*tert*-butyl-carbamate (**2**).

***tert*-butyl ((cyclopropanecarbonyl)oxy)(phenylsulfonyl)carbamate (2a):**



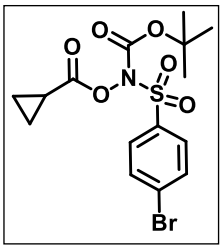
Yield (380 mg, 56%); FT-IR (υ_{max}, cm^{-1}): 1736, 1669; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.99 – 7.97 (m, 2H), 7.63 – 7.59 (m, 1H), 7.51 – 7.47 (m, 2H), 1.81 – 1.74 (m, 1H), 1.32 (s, 9H), 1.19 – 1.13 (m, 2H), 1.04 – 1.01 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 170.7, 147.6, 136.9, 133.3, 127.9, 127.8, 85.4, 26.8, 9.4, 8.8; HRMS (ESI-TOF) for $[C_{15}H_{19}NO_6S + Na]^+$: Calcd., 364.0800, Found, 364.0839.

***tert*-butyl ((2-bromo-phenyl)sulfonyl)((cyclopropanecarbonyl)oxy)carbamate (2b):**



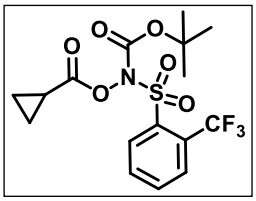
Yield (533 mg, 64%); FT-IR (υ_{max}, cm^{-1}): 1794, 1761; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.29 – 8.26 (m, 1H), 7.79 – 7.77 (m, 1H), 7.53 – 7.46 (m, 2H), 1.91 – 1.85 (m, 1H), 1.36 (s, 9H), 1.27 – 1.23 (m ,2H), 1.11 – 1.09 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.9, 148.2, 138.1, 135.8, 134.9, 133.6, 127.5, 121.0, 86.6, 27.9, 10.5, 10.0; HRMS (ESI-TOF) for $[C_{15}H_{18}BrNO_6S + Na)]^+$: Calcd., 441.9936, Found, 441.9940.

***tert*-butyl ((4-bromophenyl)sulfonyl)((cyclopropanecarbonyl)oxy)carbamate (2c):**



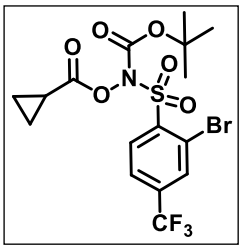
Yield (305 mg, 37%); FT-IR (υ_{max}, cm^{-1}): 1748; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.92 – 7.90 (m, 2H), 7.71 – 7.68 (m, 2H), 1.86 – 1.80 (m, 1H), 1.41 (s, 9H), 1.23 – 1.19 (m, 2H), 1.12 – 1.09 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.9, 148.6, 137.0, 132.3, 130.7, 129.9, 86.8, 28, 10.6, 10.0; HRMS (ESI-TOF) for $[C_{15}H_{18}BrNO_6S + Na]^+$: Calcd., 441.9936, Found, 441.9940.

***tert*-butyl ((cyclopropanecarbonyl)oxy)((2-(trifluoromethyl)phenyl)sulfonyl)carbamate (2d):**



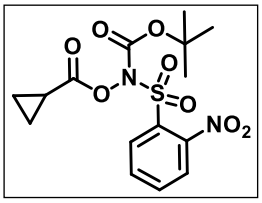
Yield (340 mg, 41%); FT-IR (υ_{max}, cm^{-1}): 1717; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.42 – 8.39 (m, 1H), 7.78 – 7.84 (m, 1H), 7.74 – 7.67 (m, 2H), 1.82 – 1.75 (m, 1H), 1.31 (s, 9H), 1.18 – 1.14 (m, 2H), 1.05 – 1.00 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 170.8, 147.1, 136.5, 133.0, 132.3, 131.2, 127.6, 127.2 (q, $J_{C,F}$ = 5.9 Hz), 119.8, 85.7, 26.7, 9.4, 8.8; HRMS (ESI-TOF) for $[C_{16}H_{18}F_3NO_6S + Na]^+$: Calcd., 432.0697, Found, 432.0708.

***tert*-butyl ((2-bromo-4-(trifluoromethyl)phenyl)sulfonyl)((cyclopropanecarbonyl)oxy)carbamate (2e):**



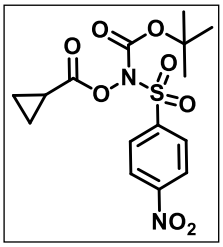
Yield (573 mg, 59%); FT-IR (υ_{max}, cm^{-1}): 1799, 1765; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.34 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 0.8 Hz, 1H), 7.69 (dd, J = 8.3 Hz, 1.1 Hz, 1H), 1.84 – 1.77 (m, 1H), 1.32 (s, 9H), 1.21 – 1.16 (m, 2H), 1.07 – 1.04 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 170.8, 146.9, 140.4, 135.4, 135.1, 132.9, 131.7 (q, $J_{C,F}$ = 3.7 Hz), 123.3, 120.6, 86.1, 26.8, 9.3, 9.0; HRMS (ESI-TOF) for $[C_{16}H_{17}BrF_3NO_6S + H]^+$: Calcd., 486.9900, Found, 486.8889.

***tert*-butyl ((cyclopropylcarbonyl)oxy)((2-nitrophenyl)sulfonyl)carbamate (2f):**



Yield (364 mg, 48%); FT-IR (υ_{max}, cm^{-1}): 1793, 1762; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.39 – 8.37 (m, 1H), 7.79 – 7.77 (m, 3H), 1.92 – 1.85 (m, 1H), 1.43 (s, 9H), 1.27 – 1.23 (m, 2H), 1.14 – 1.10 (m ,2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.9, 148.2, 148.0, 135.1, 132.8, 132.0, 131.7, 124.6, 87.1, 27.9, 10.5, 10.0; HRMS (ESI-TOF) for $[C_{15}H_{18}N_2O_8S + Na]^+$: Calcd., 409.0697, Found, 409.0683.

***tert*-butyl ((cyclopropanecarbonyl)oxy)((4-nitrophenyl)sulfonyl)carbamate (2g):**

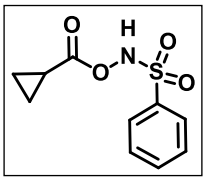


Yield (300 mg, 48%); FT-IR (υ_{max}, cm^{-1}): 1794, 1762; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.40 – 8.36 (m, 2H), 8.25 – 8.22 (m, 2H), 1.87 – 1.81 (m, 1H), 1.40 (s, 9H), 1.24 – 1.20 (m, 2H), 1.15 – 1.12 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.0, 151.0, 148.2, 143.5, 130.7, 124.1, 87.4, 28.0, 10.5, 10.2; HRMS (ESI-TOF) for $[C_{15}H_{18}N_2O_8S + H]^+$: Calcd., 387.0900, Found, 387.0720.

General procedure for the synthesis of *N*-cyclopropanecarbonyloxy-sulfonamide (1): From *N*-sulfonyl-*N*-(cyclopropanecarbonyl)oxy-*tert*-butyl-carbamate (**2**), *N*-cyclopropanecarbonyloxy-sulfonamide (**1**) was synthesized by using reported protocol.[2]

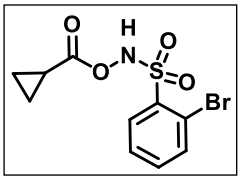
The *N*-sulfonyl-*N*-(cyclopropanecarbonyl)oxy-*tert*-butyl-carbamate (**2**), 5 eq. of trifluoroacetic acid was added and the mixture was stirred for 2 – 3 h. Upon completion of the reaction (TLC analysis), the mixture was washed with hexane several times and the resultant solvent mixture was evaporated under reduced pressure to yield crude product, which was further purified using silica gel column chromatography to yield an *N*-(cyclopropanecarbonyl)oxy-sulfonamide (**1**).

***N*-((cyclopropanecarbonyl)oxy)benzenesulfonamide (1a):**



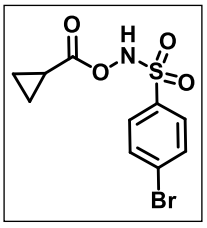
Yield (380 mg, 56%); FT-IR (υ_{max}, cm^{-1}): 1757; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.95 (s, 1H), 7.90 – 7.88 (m, 2H), 7.64 – 7.60 (m, 1H), 7.52 – 7.48 (m, 2H), 1.59 – 1.51 (m, 1H), 0.89 – 0.86 (m, 2H), 0.81 – 0.78 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.4, 134.3, 133.4, 128.2, 127.9, 9.7, 8.7; HRMS (ESI-TOF) for $[C_{10}H_{11}NO_4S + H]^+$: Calcd., 242.0478, Found, 242.0466.

2-bromo-*N*-((cyclopropanecarbonyl)oxy)benzenesulfonamide (1b):



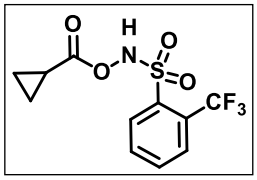
Yield (80 mg, 53%); FT-IR (υ_{max}, cm^{-1}): 1714; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.45 (s, 1H), 8.14 – 8.10 (m, 1H), 7.73 – 7.69 (m, 1H), 7.47 – 7.41 (m, 2H), 1.53 – 1.46 (m, 1H), 0.86 – 0.81 (m, 2H), 0.80 – 0.76 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.9, 134.6, 134.1, 133.9, 132.5, 126.7, 120.3, 9.6, 8.7; HRMS (ESI-TOF) for $[C_{10}H_{10}BrNO_4S + Na]^+$: Calcd., 343.9400, Found, 343.9407.

4-bromo-*N*-((cyclopropanecarbonyl)oxy)benzenesulfonamide (1c):



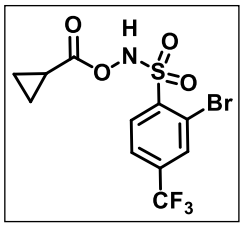
Yield (140 mg, 92%); FT-IR (υ_{max}, cm^{-1}): 1750; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.0 (s, 1H), 7.82 – 7.79 (m, 2H), 7.73 – 7.69 (m, 2H), 1.67 – 1.59 (m, 1H), 1.02 – 0.96 (m, 2H), 0.94 – 0.90 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 173.5, 134.5, 132.7, 130.4, 130.0, 10.8, 10.0; HRMS (ESI-TOF) for $[C_{10}H_{10}BrNO_4S + H]^+$: Calcd., 319.9592, Found, 319.9637.

***N*-((cyclopropanecarbonyl)oxy)-2-(trifluoromethyl)benzenesulfonamide (1d):**



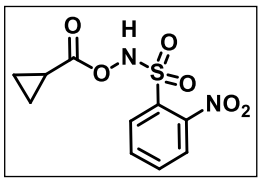
Yield (340 mg, 41%); FT-IR (υ_{max}, cm^{-1}): 1756; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.20 (s, 1H), 8.27 (d, J = 7.5 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.82 – 7.74 (m, 2H), 1.62 – 1.56 (m, 1H), 0.95 – 0.88 (m, 2H), 0.82 – 0.78 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 173.4, 134.5, 134.1, 133.9, 132.1, 129.3, 129.0 (q, J_{C-F} = 6.3 Hz), 123.8, 10.6, 9.9; HRMS (ESI-TOF) for $[C_{11}H_{10}F_3NO_4S + Na]^+$: Calcd., 332.0197, Found, 332.0191.

2-bromo-*N*-((cyclopropanecarbonyl)oxy)-4-(trifluoromethyl)benzenesulfonamide (1e):



Yield (60 mg, 40%); FT-IR (υ_{max}, cm^{-1}): 1759; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.40 (s, 1H), 8.25 (d, J = 8.24 Hz, 1H), 7.97 (s, 1H), 7.71 (d, J = 7.68 Hz, 1H), 1.54 – 1.48 (m, 1H), 0.89 – 0.81 (m, 4H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.0, 137.7, 135.9, 135.5, 132.9, 131.6 (q, J_{C-F} = 3.5 Hz), 122.6, 121.2, 119.8, 9.5, 8.9; HRMS (ESI-TOF) for $[C_{11}H_9BrF_3NO_4S + Na]^+$: Calcd., 409.9297, Found, 409.9260.

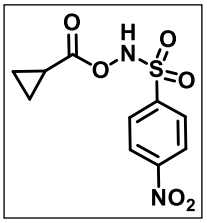
***N*-((cyclopropanecarbonyl)oxy)-2-nitrobenzenesulfonamide (1f):**



Yield (205 mg, 95%); FT-IR (υ_{max}, cm^{-1}): 1760; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.90 (s, 1H), 8.20 (dd, J = 7.6, 1.5 Hz, 1H), 7.98 (d, J = 7.6 Hz, 2H), 7.88 – 7.81 (m, 2H), 1.66 (br s, 1H), 0.99 – 0.93 (m, 2H), 0.90 – 0.86 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 173.1, 148.4, 135.5,

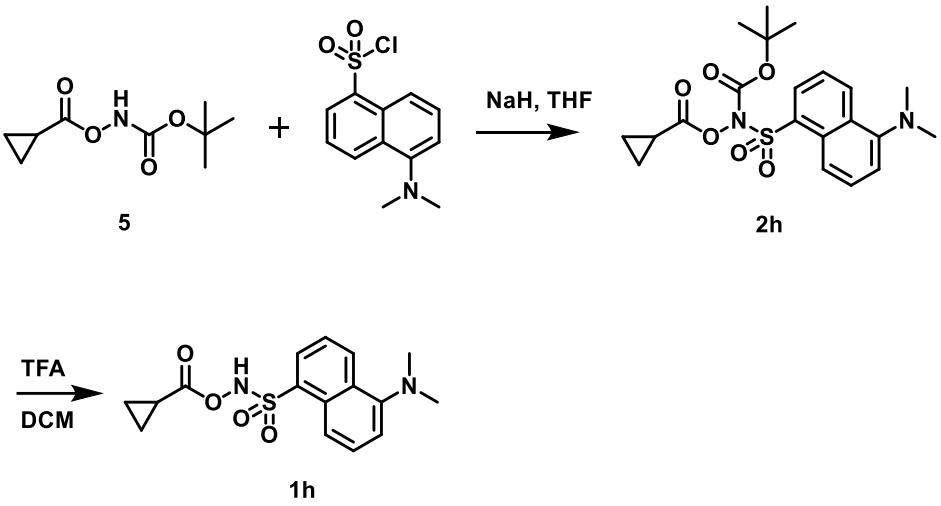
133.0, 130.2, 126.3, 10.7, 10.0; HRMS (ESI-TOF) for $[C_{10}H_{10}N_2O_6S + H]^+$: Calcd., 287.0300, Found, 287.0327.

***N*-((cyclopropanecarbonyl)oxy)-4-nitrobenzenesulfonamide (1g):**



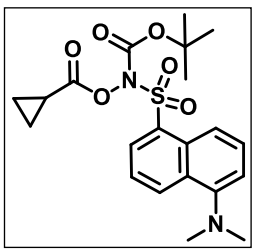
Yield (90 mg, 61%); FT-IR (υ_{max}, cm^{-1}): 1759; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.05 (s, 1H), 8.41 (d, *J* = 8.8 Hz, 2H), 8.16 (d, *J* = 8.8 Hz, 2H), 1.69 – 1.62 (m, 1H), 1.06 – 0.99 (m, 2H), 0.97 – 0.93 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.4, 150.1, 140.4, 129.2, 123.3, 9.7, 9.1; HRMS (ESI-TOF) for $[C_{10}H_{10}N_2O_6S + H]^+$: Calcd., 287.0300, Found, 287.0335.

Synthesis of 5-(dimethylamino)naphthalene-1-sulfinic acid (4h):



Scheme S1: Synthesis of **1h** and **2h.**

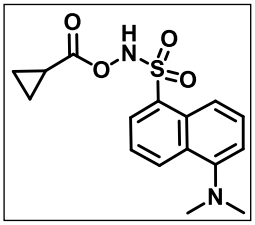
***tert*-butyl ((cyclopropanecarbonyl)oxy)((5-(dimethylamino)naphthalen-1-yl)sulfonyl)carbamate (2h):** From dansyl chloride, *tert*-butyl ((cyclopropanecarbonyl)oxy)((5-(dimethylamino)naphthalen-1-yl)sulfonyl)carbamate **(2h)** was synthesized by using general procedure for the synthesis of **2**.



Yield (220 mg, 34%); FT-IR (υ_{max}, cm^{-1}): 1795, 1756; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.57 (m, 1H), 8.37 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.22 (m, 1H), 7.52 (m, 2H), 7.14 (d, *J* = 7.1 Hz, 1H), 2.80 (s, 6H), 1.87 – 1.81 (m, 1H), 1.20 (s, 9H), 1.19 – 1.16 (m, 2H), 1.06 – 1.01 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.8, 152.0, 148.8, 133.6, 132.6, 132.3, 130.1, 129.9,

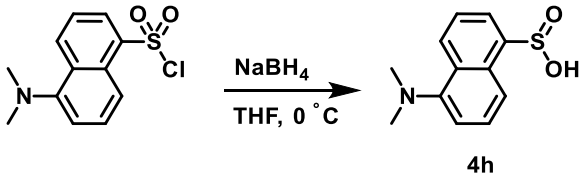
129.1, 123.0, 119.1, 115.7, 86.4, 45.6, 27.9, 10.6, 10.0; HRMS (ESI-TOF) for $[C_{21}H_{26}N_2O_6S + Na]^+$: Calcd., 435.1590, Found, 435.1583.

***N*-((cyclopropanecarbonyl)oxy)-5-(dimethylamino)naphthalene-1-sulfonamide (1h):**



From compound **2h**, *N*-((cyclopropanecarbonyl)oxy)-5-(dimethylamino)naphthalene-1-sulfonamide **(1h)** was synthesized by using general procedure for the synthesis of **1**. Yield (70 mg, 91%); FT-IR (υ_{max}, cm^{-1}): 1756; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 9.11 (s, 1H), 8.58 (d, *J* = 8.3 Hz, 1H), 8.35 (d, *J* = 8.6 Hz, 1H), 8.28 (d, *J* = 7 Hz, 1H), 7.56 – 7.46 (m, 2H), 7.14 (d, *J* = 7.4 Hz, 1H), 2.82 (s, 6H), 1.43 (s, 1H), 0.76 – 0.68 (m, 4H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.4, 150.9, 131.4, 131.2, 129.6, 129.4, 128.8, 128.0, 122.0, 117.9, 114.6, 44.4, 9.6, 8.5; HRMS (ESI-TOF) for $[C_{16}H_{18}N_2O_4S + H]^+$: Calcd., 335.1066, Found, 335.1057.

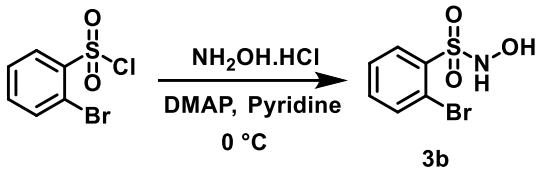
Synthesis of 5-(dimethylamino)naphthalene-1-sulfinic acid (4h): From dansyl chloride, 5-(dimethylamino)naphthalene-1-sulfinic acid (**4h**) was synthesized by using reported protocol.[3]



Scheme S2: Synthesis of **4h.**

To a solution of dansyl chloride (50 mg, 0.185 mmol) in THF (5 mL) was added $NaBH_4$ (35 mg, 0.926 mmol) in portions at 0 ˚C. Upon completion of reaction (TLC analysis), the solvent was evaporated under reduced pressure to afford crude product which was further purified by prep-HPLC (H_2O:MeOH) to give 5-(dimethylamino)naphthalene-1-sulfinic acid **4h** as a white solid. Yield (20 mg, 47%). FT-IR (υ_{max}, cm^{-1}): 3372; ^{1}H-NMR (Methanol-d4, 400 MHz): δ 8.37 (d, *J* = 8.4 Hz, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.01 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.55 (dd, *J* = 8.4, 7.1 Hz, 1H), 7.45 (dd, *J* = 8.5, 7.7 Hz, 1H), 7.16 (d, *J* = 7.1 Hz, 1H), 2.86 (s, 6H); ^{13}C-NMR (Methanol-d4, 100 MHz): δ 152.4, 132.8, 130.5, 127.1, 126.9, 125.4, 121.2, 119.8, 115.2, 47.5; HRMS (ESI-TOF) for $[C_{12}H_{13}NO_2S + H]^+$: Calcd., 236.0745, Found, 236.0739.

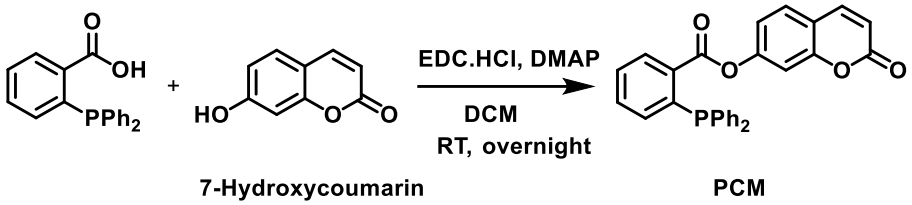
Synthesis of 2-Bromo-*N*-hydroxybenzenesulfonamide (3b): From 2-bromobenzenesulfonyl chloride, 2-Bromo-*N*-hydroxybenzenesulfonamide (**3b**) was synthesized by using reported protocol.[3]



Scheme S3: Synthesis of **3b.**

To a mixture of hydroxylamine hydrochloride (164 mg, 1.17 mmol) and DMAP (245 mg, 2.34 mmol) in pyridine (20 mL) was added 2-bromobenzenesulfonyl chloride (300 mg, 1.17 mmol) on an ice-bath. Then, the mixture was stirred for 5 min at room temperature. The resulting suspension was poured into EtOAc (100 mL) and 1 N HCl aq. (100 mL). The EtOAc layer was separated, washed with brine (100 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated. The crude product was purified by prep-HPLC (ACN:H_2O) to give 2-bromo-*N*-hydroxybenzenesulfonamide **3b** as a light yellow solid. Yield (100 mg, 34%). The analytical data is consistent with previously reported values.

Synthesis of PCM probe (6): From 2-(diphenylphosphino) benzoic acid, PCM probe (**6**) was synthesized by using reported protocol.[4]

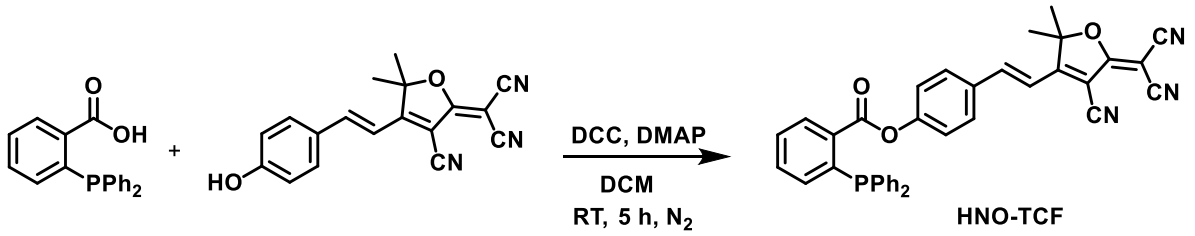


Scheme S4: Synthesis of PCM **6**

2-(diphenylphosphino) benzoic acid (306 mg, 0.33 mmol) was dissolved in 15 mL of anhydrous CH_2Cl_2 under N_2 atmosphere. 4-(dimethylamino) pyridine (3 mg, 0.01 mmol) and 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride (64 mg, 0.36 mmol) were added at 0 °C and the reaction mixture was stirred for 30 min. 7-hydroxycoumarin (65 mg, 0.33 mmol) was added and the resulting mixture was warmed to room temperature and stirred overnight. Upon completion of the reaction (TLC analysis), the solvent was evaporated under reduced pressure to yield the crude product, which was purified by prep-HPLC (ACN:H_2O) to

afford compound **6** as a faint yellow solid. Yield (55 mg, 37%). The analytical data is consistent with previously reported values.

Synthesis of HNO-TCF probe (7): From 2-(diphenylphosphino) benzoic acid, HNO-TCF probe (**7**) was synthesized by using reported protocol.[5]



Scheme S5: Synthesis of HNO-TCF **7.**

A mixture of 2-(diphenylphosphino) benzoic acid (149 mg, 0.489 mmol), 4-dimethylaminopyridine (DMAP, 41 mg, 0.326 mmol) and dicyclohexylcarbodiimide (DCC, 137 mg, 0.652 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at 0 °C for 30 min in nitrogen atmosphere. To the mixture added 1-(3-cyano-2-dicyanomethylen-5,5-dimethyl-2,5-dihydrofuran-4-yl)-2-(4-hydroxylphenyl) ethane (100 mg, 0.326 mmol) and reaction mixture was allowed to stir at RT for 4 h. Upon completion of reaction (TLC analysis) the solvent was evaporated under reduced pressure to yield crude product, which was purified by prep-HPLC (ACN:H_2O) to afford compound **7** as an orange solid. Yield (50 mg, 26%). The analytical data is consistent with previously reported values.

3. Experimental protocols

HNO detection by using PCM 6: Stock solutions of **PCM 6** (1 mM), **1a-1h** (5 mM)**, 2a-2h** (5 mM) in DMSO and esterase (1 U/mL) in phosphate buffer saline (10 mM, pH 7.4) were prepared. The reaction mixture was prepared by adding 10 μM **PCM 6** (2 μL, 1 mM), 50 μM **1a-1h, 2a-2h** (2 μL, 5 mM) along with 0.5 U/mL esterase (100 μL, 1 U/mL stock) and the volume was adjusted to 200 μL using phosphate buffer saline (10 mM, pH 7.4) in 96-well plate and then incubated for 30 min at 37 ˚C. The fluorescence (excitation at 370 nm; emission at 460 nm) was measured using an Ensight Multimode Plate Reader (PerkinElmer). (Figure S1 and figure S2)

Stock solutions of PMSF (100 mM) in isopropanol, esterase (1 U/mL) in phosphate buffer saline (PBS), pH 7.4 were prepared. The reaction mixture was prepared by adding 1 mM PMSF (7.5 μL, 100 mM) along with 0.5 U/mL esterase (100 μL, 1 U/mL stock) and the volume was

adjusted to 750 μL using phosphate buffer saline (10 mM, pH 7.4) in 96-well plate. Measurements were carried out after incubation for 30 min at 37 ˚C.

Stock solutions of **PCM 6** (1 mM), **1h** and **2h** (5 mM) in DMSO and esterase (1 U/mL, pre-treated with PMSF) in phosphate buffer saline (10 mM, pH 7.4) were prepared independently. Measurements were carried out by using above-described protocol. (Figure 3B and figure S6A).

Measurement of absorbance:

Stock solutions of **4h** (10 mM) in DMSO was prepared. The solution was prepared by adding 100 μM **4h** (10 μL, 10 mM) with 990 μL phosphate buffer saline (10 mM, pH 7.4) into a cuvette. UV/vis spectrum were recorded by using SHIMADZU, UV-2600 UV-Vis spectrophotometer at room temperature. (Figure S3A).

Measurement of fluorescence:

Stock solutions of **4h** (10 mM) in DMSO was prepared. The solution was prepared by adding 100 μM **4h** (10 μL, 10 mM) with 990 μL phosphate buffer saline (10 mM, pH 7.4) into a cuvette. Fluorescence spectrum were recorded by using HORIBA Scientific Fluoromax-4 spectrofluorometer at room temperature. (Figure S3B).

Stock solutions of **4h** (10 mM) in DMSO was prepared. The solution was prepared by adding 50 μM **4h** (5 μL, 10 mM) with 990 μL phosphate buffer saline (10 mM, pH 8.0, 7.4, 6.0 and 5.0) independently into a cuvette. Fluorescence spectrum were recorded by using HORIBA Scientific Fluoromax-4 spectrofluorometer at room temperature. (Figure S4A).

Stock solutions of **4h** (5 mM) in DMSO was prepared. The solution was prepared by adding 50 μM **4h** (2 μL, 5 mM) with 198 μL phosphate buffer saline (10 mM, pH 7.4) in 96-well plate then incubated for 12 h at 37 ˚C. The stability of fluorescence intensity (excitation at 370 nm; emission at 460 nm) was measured using an Ensight Multimode Plate Reader (PerkinElmer). (Figure S4B)

Time-resolved photoluminescence (PL) measurements:[6]

Time-resolved PL studies were performed using Horiba Jobin Yvon Flurolog-3 spectrofluorometer (HORIBA Scientific) Time-Correlated Single Photon Counting (TCSPC) system using a 292 nm nanoLED as the excitation source with a time to amplitude converter (TAC) range of 200 ns for 10,000 counts. The average lifetime (τ_{avg}) was calculated using the following equation.

$$\tau_{avg} = \frac{\alpha_1\tau_1 + \alpha_2\tau_2}{\alpha_1 + \alpha_2}$$

α = Relative amplitude

τ = Excited state

Stock solution of **4h** (10 mM) in DMSO was prepared. The reaction was prepared by adding 10 μM (1 μL, 10 mM) and volume was adjusted to 1 mL using phosphate buffer saline (10 mM, pH 7.4) in cuvette. The lifetime of excited state of **4h** was measured by using above mentioned protocol. (Figure S5).

Detection of 4h:

Stock solutions of **4h** (5 mM), **1h** and **2h** (1 mM, 2.5 mM and 5 mM) in DMSO and esterase (1 U/mL) in phosphate buffer saline (10 mM, pH 7.4) were prepared. The reaction mixture was prepared by adding 10 μM, 25 μM and 50 μM **1h** or **2h** (2 μL, 1 mM, 2.5 mM and 5 mM) independently along with 0.5 U/mL esterase (100 μL, 1 U/mL stock) and the volume was adjusted to 200 μL using phosphate buffer saline (10 mM, pH 7.4) in 96-well plate and then incubated for 120 min at 37 ˚C. The fluorescence corresponding to **4h** release (excitation at 308 nm; emission at 497 nm) was measured using an Ensight Multimode Plate Reader (PerkinElmer). (Figure S7).

Stock solutions of **4h** (5 mM), **1h** and **2h** (5 mM) in DMSO and esterase (1 U/mL, pre-treated with PMSF) in phosphate buffer saline (10 mM, pH 7.4) were prepared. Measurements were carried out by using above-described protocol. (Figure S7).

Griess assay for nitrite detection:

Stock solutions of $NaNO_2$ (1 mM), **1a-1h** (5 mM), **2a-2h** (5 mM) in DMSO and esterase (1 U/mL) in phosphate buffer saline (10 mM, pH 7.4) were prepared. The reaction mixture was prepared by adding 50 μM **1a-1h** and **2a-2h** (2 μL, 5 mM) independently along with 0.5 U/mL esterase (100 μL, 1 U/mL stock) and the volume was adjusted to 200 μL using phosphate buffer saline (10 mM, pH 7.4) in 96-well plate and then incubated for 30 min at 37 ˚C. After 30 min incubation, Griess reagent (14 μL) was added to each well and further incubated for 30 min at 37 ˚C in an incubator. The absorbance (at 540 nm) was measured using an Ensight Multimode Plate Reader (PerkinElmer). (Figure S8).

Stability of 2h in PBS pH 7.4:

Stock solution of **2h** (10 mM) were prepared in DMSO and stock solution of esterase (50 U/mL) was prepared in PBS pH 7.4. The reaction mixture was prepared by adding 50 μM **2h** (2.5 μL, 10 mM) independently with and without 0.5 U/mL esterase (5 μL, 50 U/mL stock) and the volume was adjusted to 0.5 mL using phosphate buffer saline (10 mM, pH 7.4) in an eppendorf then incubated for 10 min at 37 ˚C on thermomixer (300 rpm). The reaction was quenched by adding 100 μL of ethyl acetate. The TLC was run in 30% ethyl acetate in hexane as mobile phase. (Figure S9).

Decomposition of 1h and 2h using LC-MS:

Stock solutions of **1h** (1 mM), **2h** (1 mM) and **4h** (1 mM) were prepared in DMSO. Stock solution of esterase (2 U/mL) was prepared in PBS pH 7.4. The reaction mixture was prepared by adding 10 μM **1h** or **2h** or **4h** (2 μL, 1 mM) independently along with 0.1 U/mL esterase (50 μL, 2 U/mL stock) and the volume was adjusted to 1 mL using phosphate buffer saline (10 mM, pH 7.4) in an eppendorf then incubated for 5 h at 37 ˚C on thermomixer (300 rpm). 100 μL aliquots of the reaction mixture were taken at pre-determined time points and the reaction was quenched by adding 100 μL of methanol. The samples were centrifuged at 10000g for 4 min at 4 ˚C, the supernatant was collected and assessed thereafter by LC-MS. All measurements were done using the following protocol: 0.1% formic acid in methanol (A) and 0.1% formic acid in water (B) was used as the mobile phase. A multistep gradient was used with the flow rate of 0.3 mL/min starting with 75:25 → 0 min, 75:25 to 60:40 → 0 - 3 min, 60:40 to 40:60 → 3 - 7 min, 40:60 to 30:70 → 7 - 9 min, 30:70 to 0:100 → 9 - 12 min, 0:100 to 0:100 → 12 - 14 min, 0:100 to 75:25 → 14 - 18 min, 75:25 to 75:25 → 18 - 20 min.

Measurements were carried out in the positive ion mode using high resolution multiple reaction monitoring (MRM-HR) analysis on a Sciex X500R quadrupole time-of flight (QTOF) mass spectrometer fitted with an Exion UHPLC system using a Kinetex 2.6 mm hydrophilic interaction liquid chromatography (HILIC) column with 100 Å particle size, 150 mm length and 3 mm internal diameter (Phenomenex). Nitrogen was the nebulizer gas, with the nebulizer pressure set at 50 psi, declustering potential =80 V, entrance potential = 10 V, collision energy = 20 V, and collision exit potential = 5 V. The MRM-HR mass spectrometry parameters for measuring compounds are: m/z precursor ion mass ($M + H^+$) 435.1582 (**2h**), 367.1332 (**Intermediate I**), 335.1060 (**1h**), 267.0802 (**3h**), 236.0744 (**4h**). (Figure S10-S14).

Polysulfide measurement using LC/MS:

Stock solutions of **1h** (5 mM), **2h** (5 mM), **3b** (5 mM) and HPE-IAM (100 mM) were prepared in DMSO. Stock solution of Na_2S (20 mM) was prepared in DI water. Stock solution of esterase (50 U/mL) was prepared in PBS pH 7.4. The reaction mixture was prepared by adding 50 μM **1h** or **2h** or **3b** (10 μL, 5 mM) independently with 200 μM Na_2S (10 μL, 20 mM) along with (1 U/mL esterase (20 μL, 50 U/mL stock) and the volume was adjusted to 1 mL using phosphate buffer saline (10 mM, pH 7.4) in an eppendorf then incubated for 15 min at 37 ˚C on thermomixer (400 rpm). 100 μL aliquots of the reaction mixture were taken and then incubated with HPE-IAM (2 μL, 100 mM) for further 15 min. The reaction was quenched by adding 100 μL of acetonitrile. The samples were centrifuged at 10000g for 10 min at 4˚C, the supernatant was collected and assessed thereafter by LC-MS. All measurements were done using the following protocol: Acetonitrile (A) and 0.1% formic acid in water (B) was used as the mobile phase. A gradient was used with the flow rate of 0.3 mL/min starting with 100:0 → 0 min, 100:0 to 0:100 → 0 - 30 min. The IDA-HR mass spectrometry parameters for measuring compounds are: m/z precursor ion mass ($M + H^+$) 335.1060 (**1h**), 435.1582 (**2h**), 305.9985 (HPE-IAM), 215.0740 (HS-HPE-AM), 389.1530 (Bis-S-HPE-AM), 421.1250 (Bis-SS-HPE-AM), 453.0971 (Bis-SSS-HPE-AM). (Figure S15-S21).

Detection of 4h release in A549 cell lysate:

Lung carcinoma cells (A549) was cultured in a 10 cm plates in complete DMEM medium supplemented with 5% FBS (fetal bovine serum) and 1% antibiotic solution in an atmosphere of 5% CO_2 at 37 ˚C. When the cells were 70% confluent, old media was removed and the cells were washed with serum free DMEM media. The cells were trypsinized and subsequently resuspended in DMEM. The cells were harvested by centrifugation at 1000 rpm/min at 4 ˚C. Pellets were washed twice with PBS (1x), resuspended in PBS (1x, 2 mL) and transferred to a microcentrifuge tube. Cells were lysed by sonication using (130 W ultrasonic processor, VX 130W) stepped microtip for 2 min (with 3 sec. ON and 3 sec. OFF pulse, 60% amplitude) under ice cold conditions. The total protein concentration of the whole cell lysate was determined by Bradford assay and further adjusted to 1 mg/mL with PBS (1x).

Stock solutions of **4h** (2.5 mM), **1h, 2h** (0.5 mM, 1.25 mM, 2.5 mM) independently in DMSO and 1 mg/mL stock solution of cell lysate in phosphate buffer saline (10 mM, pH 7.4) were prepared. The reaction mixture was prepared by adding 10 μM, 25 μM and 50 μM **1h** or **2h** (2 μL from 0.5 mM, 1.25 mM and 2.5 mM) independently along with cell lysate (98 μL, 1 mg/mL) in 96-well plate and incubated at 37 ˚C. The fluorescence corresponding to **4h** release

(excitation at 308 nm; emission at 497 nm) was measured for 3 h using an Ensight Multimode Plate Reader (PerkinElmer).

Stock solutions of PMSF (100 mM) in isopropanol and cell lysate (1 mg/mL) in phosphate buffer saline (10 mM, pH 7.4) were prepared. The reaction mixture was prepared by adding 5 mM PMSF (50 μL, 100 mM) along with cell lysate (1 mL, 1 mg/mL) in 96-well plate and incubated at 37 ˚C on thermomixer (300 rpm).

Stock solutions of **4h** (2.5 mM), **1h, 2h** (2.5 mM) independently in DMSO and 1 mg/mL stock solution of cell lysate (pre-treated with PMSF) in phosphate buffer saline (10 mM, pH 7.4) were prepared and DANSA **4h** release was measured by above-described protocol (Figure S24).

Cell viability Assay:

Mouse embryonic fibroblasts (MEF) cells, Lung carcinoma cells (A549) and MCF-7 cells were seeded at a concentration of 1×10^4 cells/well overnight in a 96-well plate in complete DMEM medium supplemented with 5% FBS (fetal bovine serum) and 1% antibiotic solution in an atmosphere of 5% CO_2 at 37 ˚C. Cells were exposed to varying concentrations of the test compounds prepared as a DMSO stock solution so that the final concentration of DMSO was 0.5%. The cells were incubated for 24 h at 37 ˚C. A 0.5 mg/mL stock solution of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was prepared in DMEM and 100 μL of the resulting solution was added to each well. After 4 h incubation, the media was removed carefully and 100 μL of DMSO was added. Spectrophotometric analysis of each well using a microplate reader (Thermo Scientific Varioscan) at 570 nm was carried out to estimate cell viability (Figure S25-S27).

Fluorescence microscopy protocol:

HNO detection by using HNO-TCF (7) probe and 4h detection in MCF-7 cells:

MCF-7 cells were seeded at 1×10^5 cells/well in 6-well Corning plate (on cover slip) for overnight in DMEM medium supplemented with 10% FBS (fetal bovine serum) and 1% antibiotic solution in an atmosphere of 5% CO_2 at 37 ˚C. After incubation, media was removed and the cells were washed with 1 mL of PBS. Then 1 mL of fresh DMEM media was added along with 20 μM of HNO-TCF probe for 30 min at 37 ˚C. After 30 min, 1 mL of fresh DMEM media was added along with test compounds (200 μM). The cells were incubated for 2 h at 37 ˚C. After 2 h, old media was removed, cells were washed with 1 mL of PBS and then imaged on Carl Zeiss LSM710 laser scanning confocal microscope with anisotropy with 63x oil filter.

The cells were imaged in two different channels (RED channel-Alexa fluor 568 for HNO detection and DAPI channel for **4h** detection) (Figure S22).

Table S1: Calculated pK_a values of Ar-SO_2H[7]

Entry	Ar-SO_2H	pK_a
1	Ph-SO_2H	1.36
2	2-Br-Ph-SO_2H	0.53
3	4-Br-Ph-SO_2H	1.33
4	2-CF_3-Ph-SO_2H	0.40
5	2-Br-4-CF_3-Ph-SO_2H	0.51
6	2-NO_2-Ph-SO_2H	-1.56
7	4-NO_2-Ph-SO_2H	0.59
8	Dansyl-SO_2H	0.90

Table S2: Calculated pK_a values of **3** and intermediate **II**[7]

Entry	**Ar**	**Compd No.**	**pK_a**	**Compd No.**	**pK_a**
1	Ph	**3a**	9.61	**IIa**	8.36
2	2-Br-Ph	**3b**	8.66	**IIb**	8.36
3	4-Br-Ph	**3c**	9.11	**IIc**	8.36
4	2-CF_3-Ph	**3d**	8.79	**IId**	8.36
5	2-Br-4-CF_3-Ph	**3e**	8.60	**IIe**	8.36
6	2-NO_2-Ph	**3f**	8.07	**IIf**	8.36
7	4-NO_2-Ph	**3g**	9.00	**IIg**	8.36
8	Dansyl	**3h**	9.41	**IIh**	8.86

Supplementary figures:

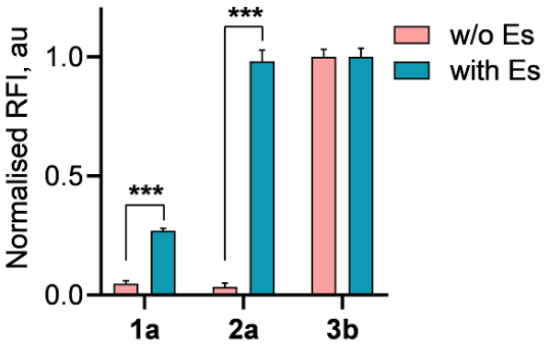


Figure S1: HNO detection using PCM (**6**): Compounds **1a**, **2a** and **3b** incubated with **6**, with and w/o esterase (0.5 U/mL) in PBS (pH 7.4) at 37 ˚C for 30 min and fluorescence was measured (excitation, 370 nm and emission, 460 nm). **3b** refers to 2-bromopiloty's acid.

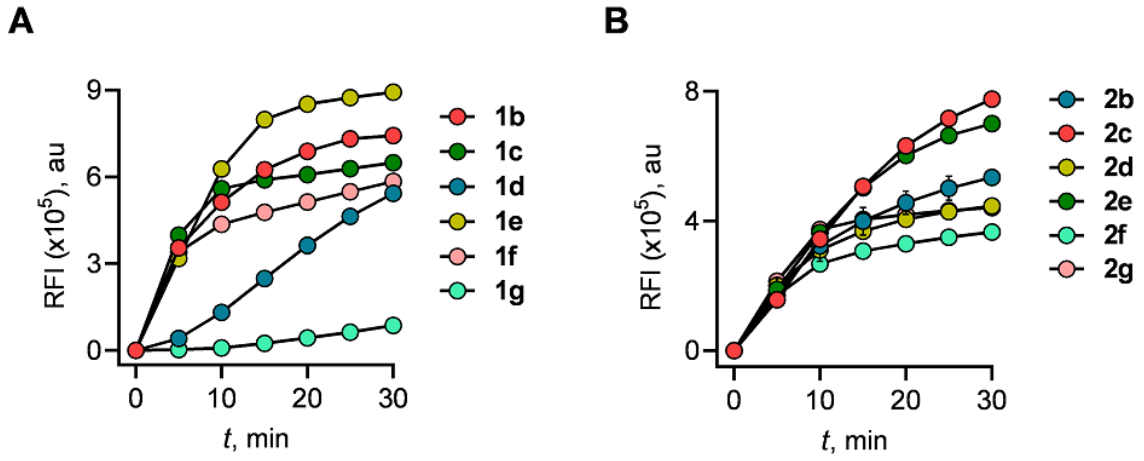


Figure S2: Time dependent HNO detection using PCM (**6**): (A) Compounds **1b-1g**; (B) **2b-2g** were incubated with **6** and esterase (0.5 U/mL) in PBS (pH 7.4) at 37 ˚C for 30 min. Fluorescence was measured at excitation, 370 nm and emission, 460 nm.

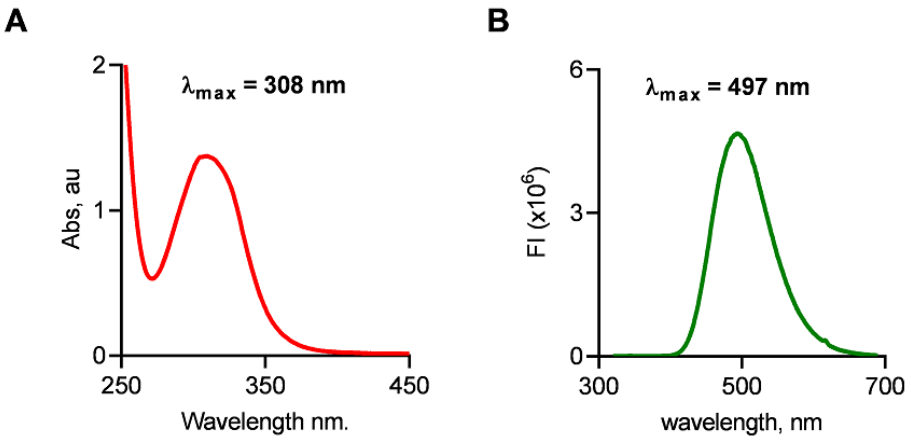


Figure S3: (A) Absorbance spectrum for compound **4h** (100 µM) were measured in PBS (pH 7.4); (B) Fluorescence spectrum for compound **4h** (100 µM) were measured in PBS (pH 7.4); (λ_{ex} = 308 nm; λ_{em} = 497 nm); Stokes shift is 189 nm.

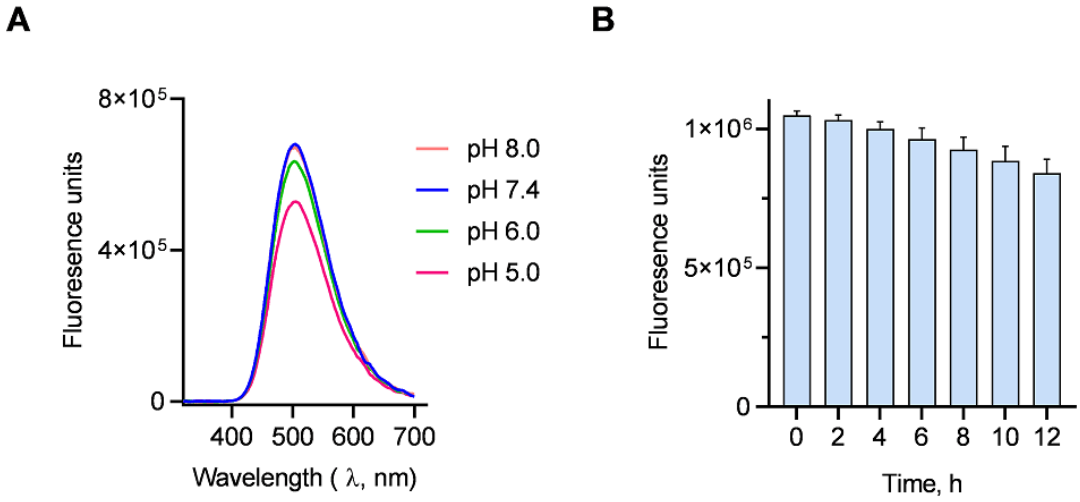
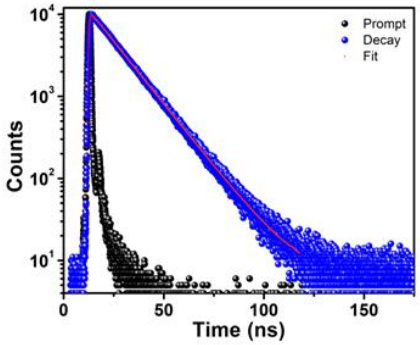


Figure S4: (A) Fluorescence of **4h** was measured with varying the pH (8.0 – 5.0); (B) Time course of fluorescence intensity of **4h** was measured in PBS pH 7.4; (λ_{ex} = 308 nm; λ_{em} = 320 - 700 nm).



sample	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	τ_{avg} (ns)	CHISQ
4h (10 µM)	6.8	4.01	14.12	95.99	**13.82**	1.07256

Figure S5: Time-resolved (lifetime) PL studies of **4h** (10 µM) was measured by TCSPC (Time Correlated Single Photon Counting) in PBS pH 7.4 for 200 ns. The data was fitted by using second exponential and the τ_{avg} was observed **13.82** ns.

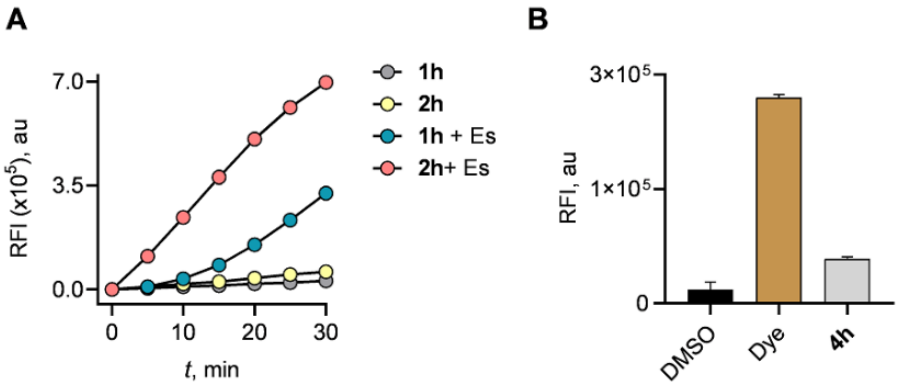


Figure S6: HNO detection using PCM (**6**): (A) Time dependent HNO release from compounds **1h** and **2h** upon incubated with and w/o esterase (0.5 U/mL) in PBS (pH 7.4) at 37 ˚C for 30 min; (B) Relative fluorescence intensity of dye and **4h** was measured at excitation, 370 nm and emission, 460 nm. Dye refers to PCM (**6**); **4h** refers to dansylsulfinic acid.

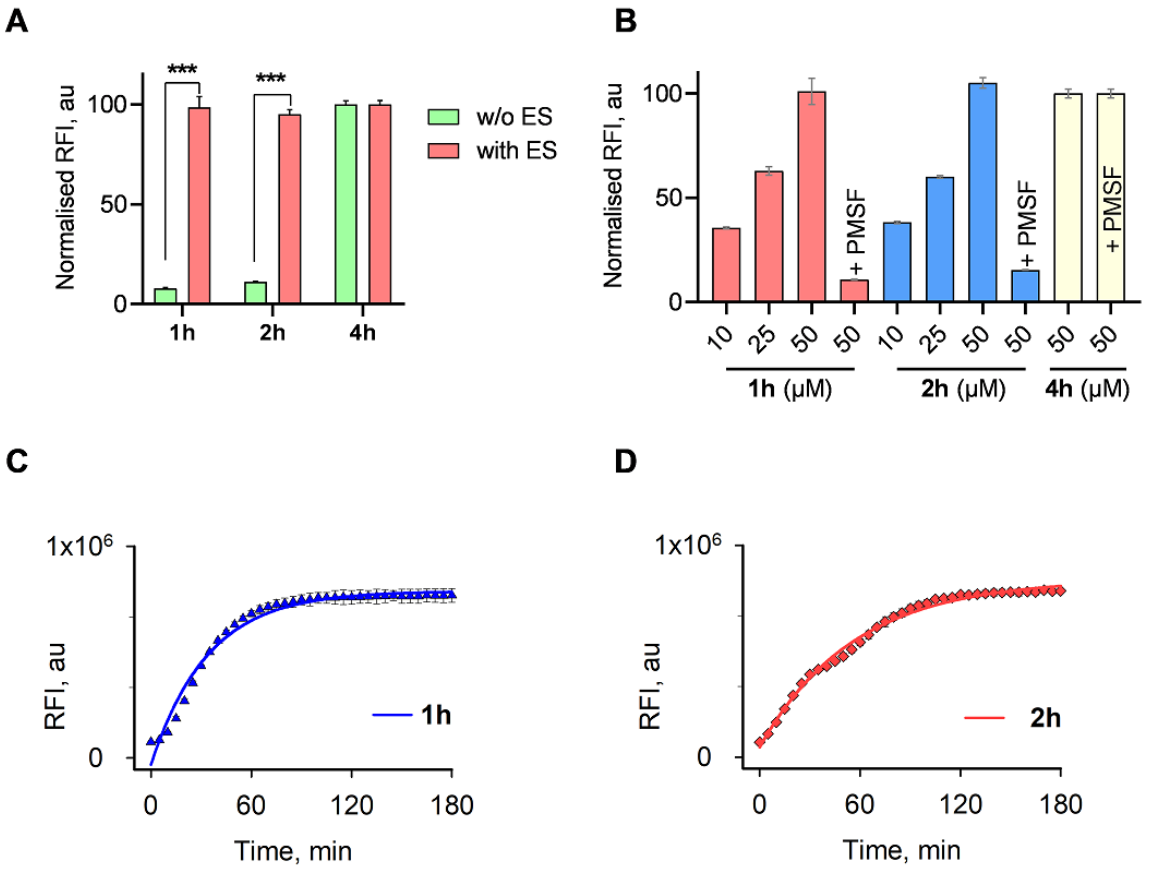


Figure S7: Detection of **4h**: (A) Compounds **1h** and **2h** were incubated with and w/o esterase (0.5 U/mL) in PBS (pH 7.4) at 37 ˚C for 120 min; **4h** refers to dansylsulfinic acid; (B) Varying concentration of compounds **1h** and **2h** were incubated with esterase (0.5 U/mL) in presence and absence of PMSF (esterase inhibitor) in PBS (pH 7.4) at 37 ˚C for 120 min; + PMSF refers to esterase was pre-treated with PMSF for 30 min at 37 ˚C. Fluorescence measurement was carried out at λ_{ex} = 308 nm; λ_{em} = 497 nm; (C) Time dependent release of **4h** from **1h** in presence of esterase (0.5 U/mL) in PBS (pH 7.4) at 37 ˚C for 180 min (k = 1.77 $\pm$ 0.010 h^{-1}); (D) Time dependent release of **4h** from **2h** in presence of esterase (0.5 U/mL) in PBS (pH 7.4) at 37 ˚C for 180 min (k = 1.06 $\pm$ 0.05 h^{-1}). Data were fitted to pseudo-first order kinetics.

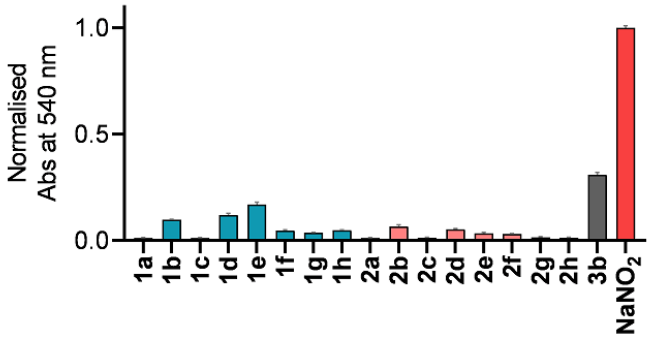


Figure S8: NO detection using Griess assay: Compounds **1a-1h, 2a-2h, 3b** and $NaNO_2$ incubated with esterase (0.5 U/mL) in PBS (pH 7.4) at 37 ˚C for 30 min. **3b** refers to 2-bromopiloty's acid. Absorbance at 540 nm was normalised w.r.t. $NaNO_2$.

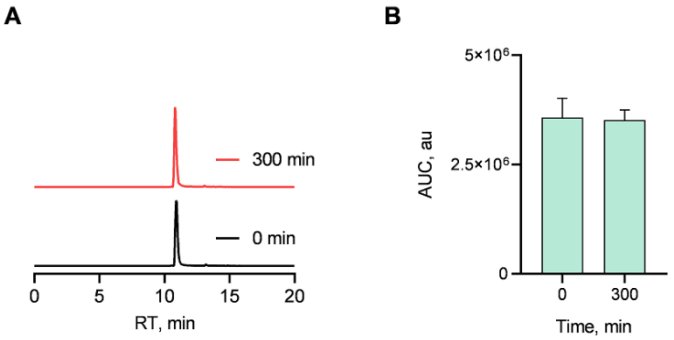


Figure S9: Stability of **1h** in PBS pH 7.4 was estimated by LC/MS analysis. Compound **1h** was incubated in PBS pH 7.4 for 5 h.

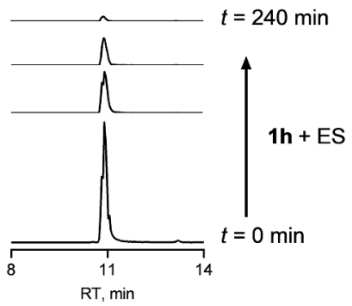


Figure S10: Extracted ion chromatograms from an LC/MS analysis of decomposition of **1h** upon incubation with esterase (0.5 U/mL) in PBS (pH 7.4). The time points considered were 0 min, 60 min, 120 min and 240 min.

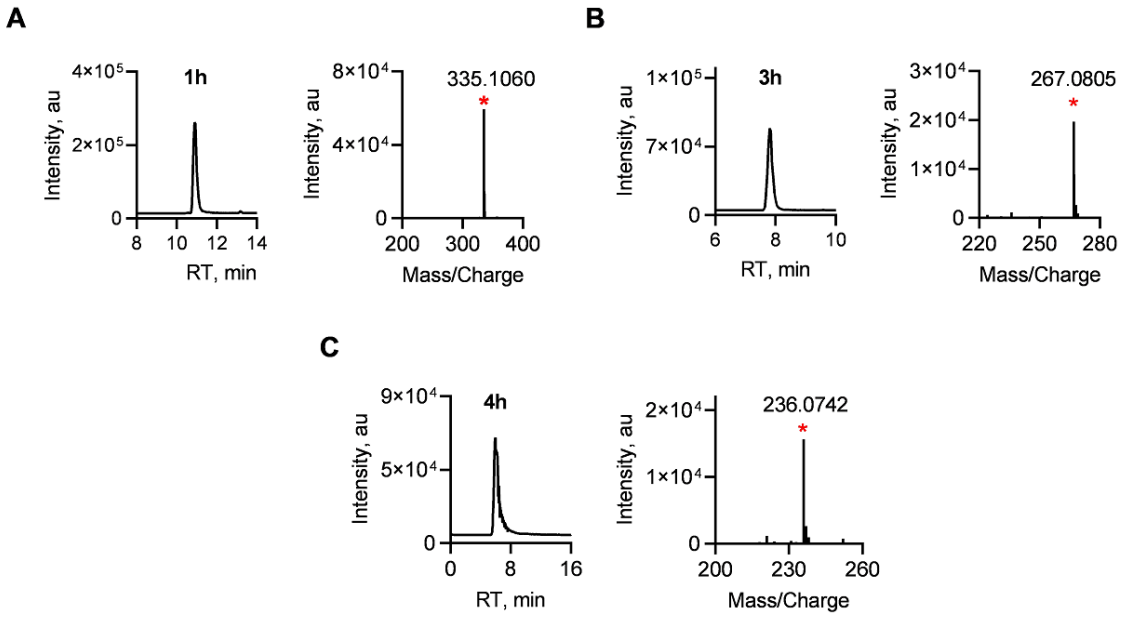


Figure S11: (A) Extracted ion chromatograms from an LC/MS analysis of **1h** along with the mass (expected, m/z = 335.1058 $[M + H]^+$; observed, m/z = 335.1060); (B) Extracted ion chromatograms from an LC/MS analysis of **3h** along with the mass (expected, m/z = 267.0798 $[M + H]^+$; observed, m/z = 267.0805); (C) Extracted ion chromatograms from an LC/MS analysis of **4h** along with the mass (expected, m/z = 236.0739 $[M + H]^+$; observed, m/z = 236.0742).

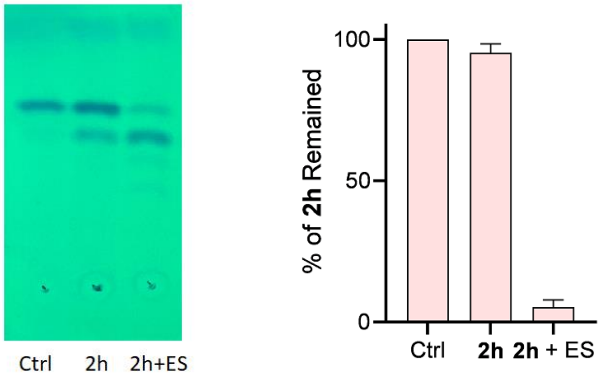


Figure S12: Stability of **2h** in PBS pH 7.4 estimated by TLC analysis. Compound **2h** was incubated in PBS pH 7.4 with and without esterase (0.5 U/mL) for 10 min. Ctrl refers to authentic compound **2h**; 2h refers to compound **2h** in PBS pH 7.4; 2h+ES refers to compound **2h** with esterase in PBS pH 7.4**.**

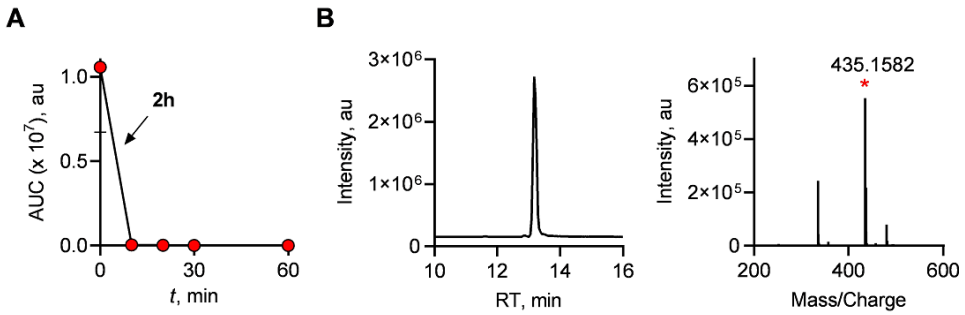


Figure S13: LC/MS study. (A) Decomposition of **2h** upon incubation with esterase (0.5 U/mL) as monitored by LC/MS. (B) Extracted ion chromatograms from an LC/MS analysis of **2h** along with the mass (expected, m/z = 435.1584 $[M + H]^+$; observed, m/z = 435.1582).

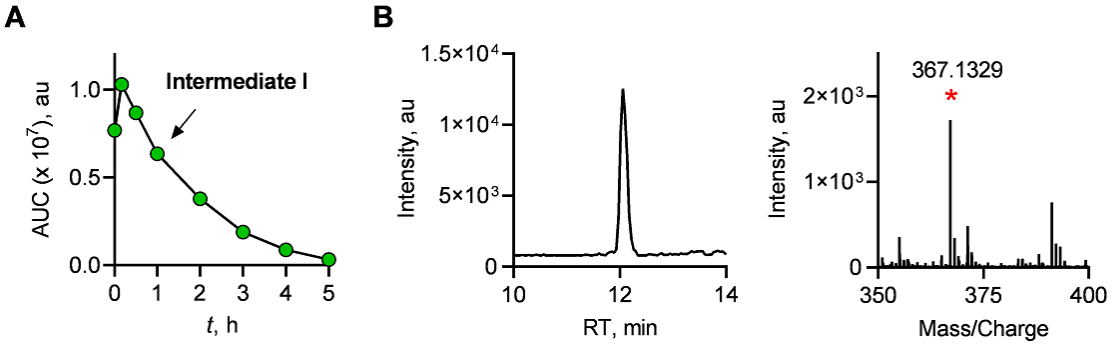


Figure S14: LC/MS study. (A) Formation of intermediate **I** (or **II**) during the decomposition of **2h** upon incubation with esterase (0.5 U/mL) as monitored by LC/MS. (B) Extracted ion chromatograms from an LC/MS analysis of intermediate **I** along with the mass (expected, m/z = 367.1322 $[M + H]^+$; observed, m/z = 367.1329).

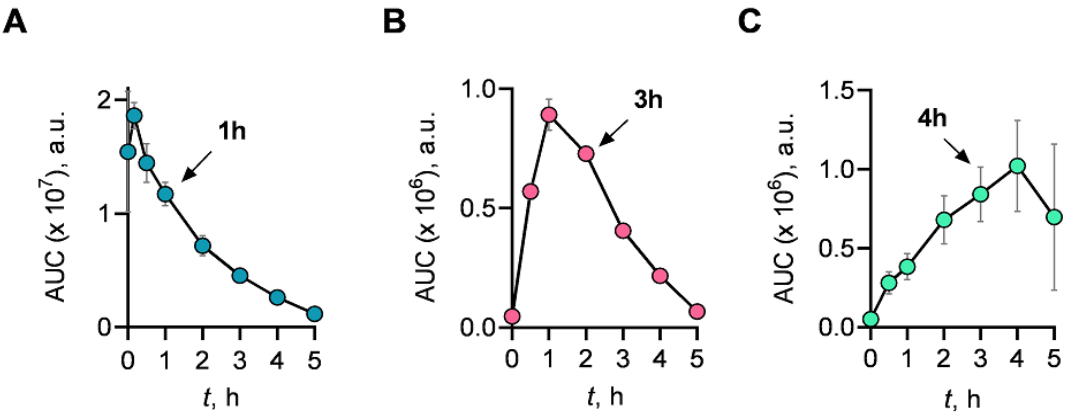
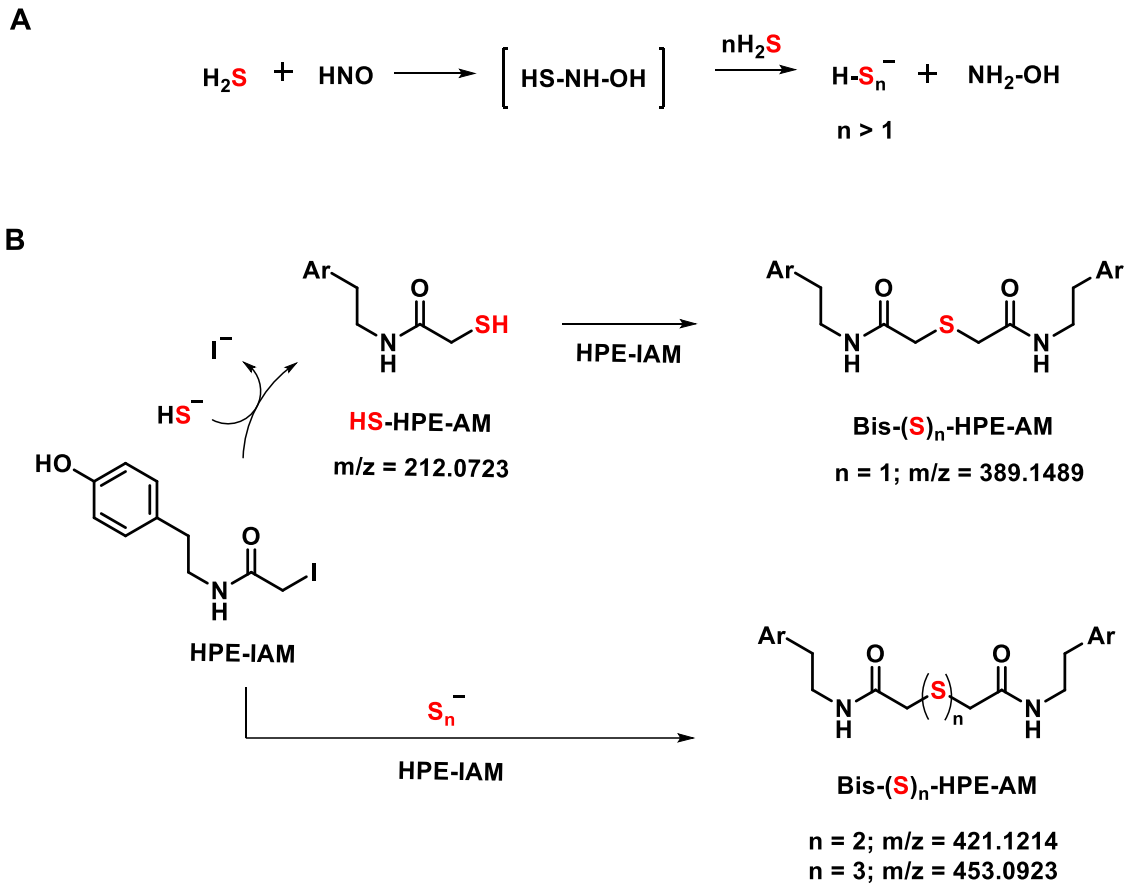


Figure S15: LC/MS study. (A) Formation of **1h** during the decomposition of **2h** upon incubation with esterase (0.5 U/mL) as monitored by LC/MS. (B) Formation of **3h** during the decomposition of **2h; (C)** Formation of **4h** during the decomposition of **2h.**

Note: Respective ion chromatograms and the mass spectra's for **1h**, **3h** and **4h** have shown in **Figure S11**.



Scheme S6: LC/MS study. (A) Proposed mechanism of HNO reaction with H_2S; (B) Proposed mechanism of polysulfides formation; Ar refers to 4-hydroxyphenyl.

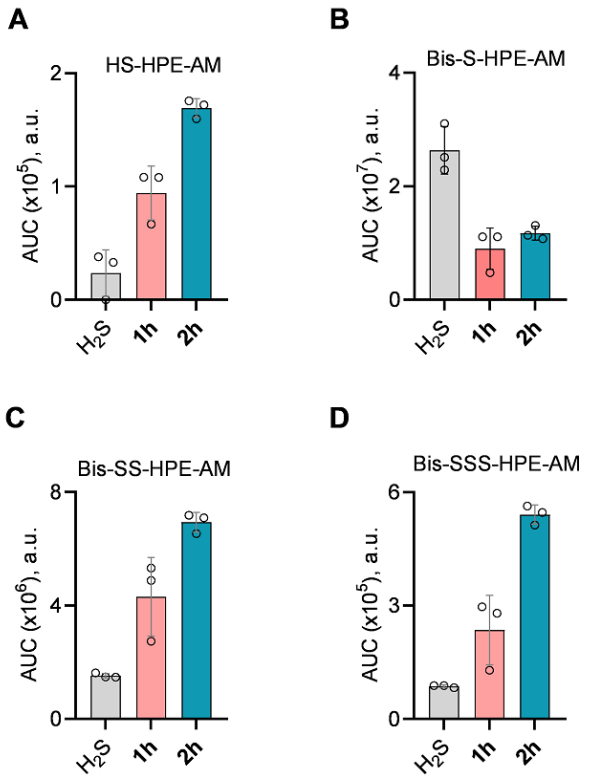


Figure S16: LC/MS study. Polysulfide formation was measured by detection of trapped HPE-IAM species from Na_2S, **1h** and **2h** upon incubation with esterase in PBS (pH 7.4) for at 37 ˚C for 15 min, Ar refers to 4-hydroxyphenyl; (A) Formation of HS-HPE-AM (expected, m/z = 212.0740 $[M + H]^+$; observed, m/z = 212.0719); (B) Formation of Bis-S-HPE-AM (expected, m/z = 389.1530 $[M + H]^+$; observed, m/z = 389.1489); (C) Formation of Bis-SS-HPE-AM (expected, m/z = 421.1250 $[M + H]^+$; observed, m/z = 421.1205); (D) Formation of Bis-SSS-HPE-AM (expected, m/z = 453.0971 $[M + H]^+$; observed, m/z = 453.0927).

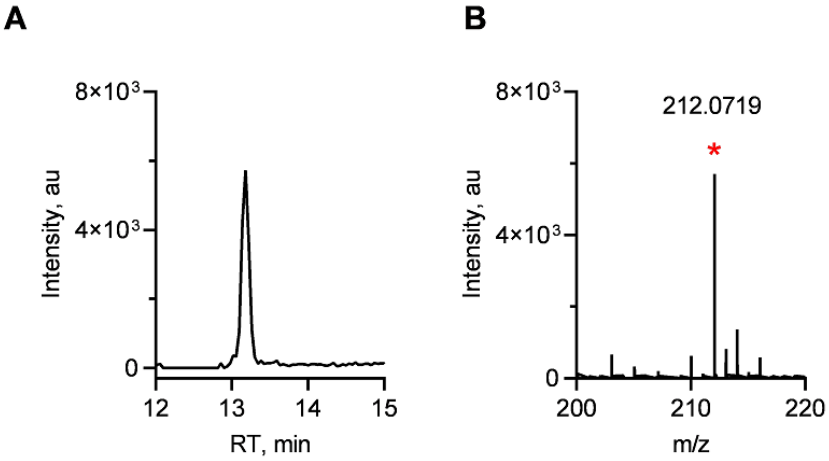


Figure S17: (A) Representative LC trace; (B) Mass spectra for **HS-HPE-AM** adduct from Na_2S with HPE-IAM (expected, m/z = 212.0740 $[M + H]^+$; observed, m/z = 212.0719).

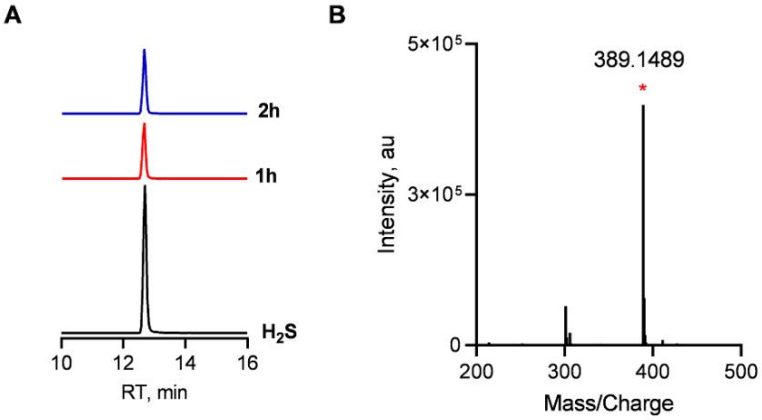


Figure S18: (A) Extracted ion chromatograms from an LC/MS analysis of **Bis-S-HPE-AM** formation from Na_2S, **1h** and **2h**; (B) Mass spectra for **Bis-S-HPE-AM** (expected, m/z = 389.1530 $[M + H]^+$; observed, m/z = 389.1489).

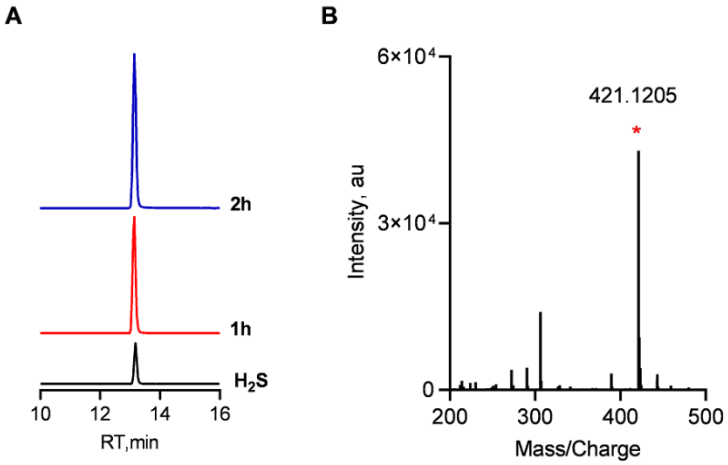


Figure S19: (A) Extracted ion chromatograms from an LC/MS analysis of **Bis-SS-HPE-AM** formation from Na_2S, **1h** and **2h**; (B) Mass spectra for **Bis-SS-HPE-AM** (expected, m/z = 421.1250 $[M + H]^+$; observed, m/z = 421.1205).

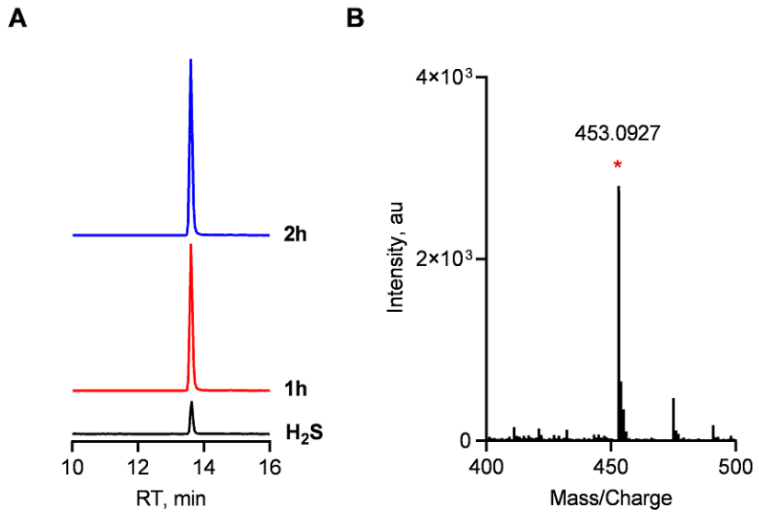


Figure S20: (A) Extracted ion chromatograms from an LC/MS analysis of **Bis-SSS-HPE-AM** formation from Na_2S, **1h** and **2h**; (B) Mass spectra for **Bis-SSS-HPE-AM** (expected, m/z = 453.0971 $[M + H]^+$; observed, m/z = 453.0927).

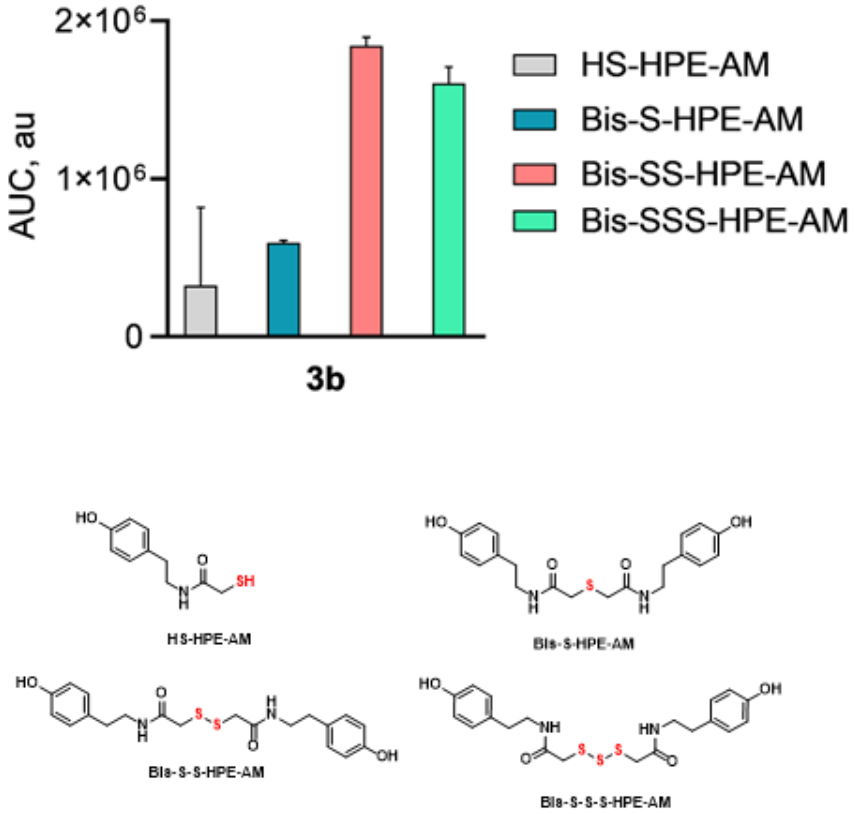


Figure S21: A comparison of sulfur species measured by detection of trapped HPE-AM species from H_2S with **3b** and structures of polysulfide adduct with HPE-IAM. **HS-HPE-AM** (expected, m/z = 212.0740 $[M + H]^+$; observed, m/z = 212.0720); **Bis-S-HPE-AM** (expected, m/z = 389.1530 $[M + H]^+$; observed, m/z = 389.1489); **Bis-SS-HPE-AM** (expected, m/z =

421.1250 $[M + H]^+$; observed, m/z = 421.1203): **Bis-SSS-HPE-AM** (expected, m/z = 453.0971 $[M + H]^+$; observed, m/z = 453.0923).

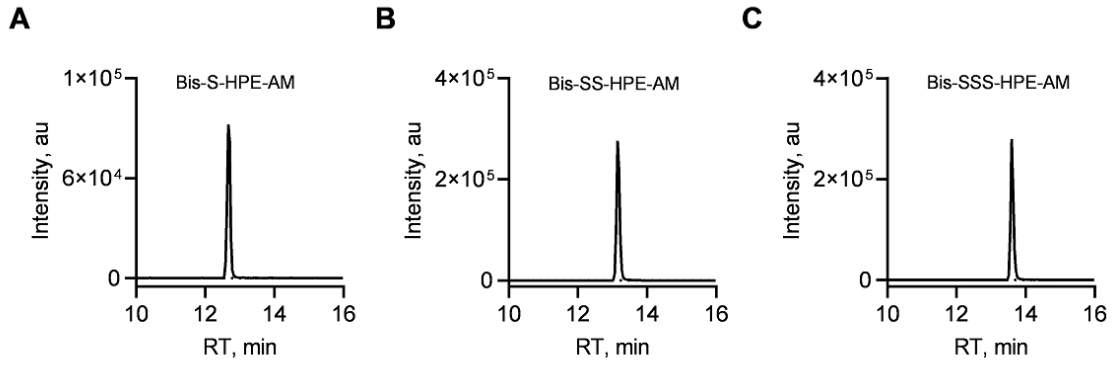


Figure S22: LC traces for the **Bis-S-HPE-AM, Bis-SS-HPE-AM, Bis-SSS-HPE-AM** formation from compound **3b**.

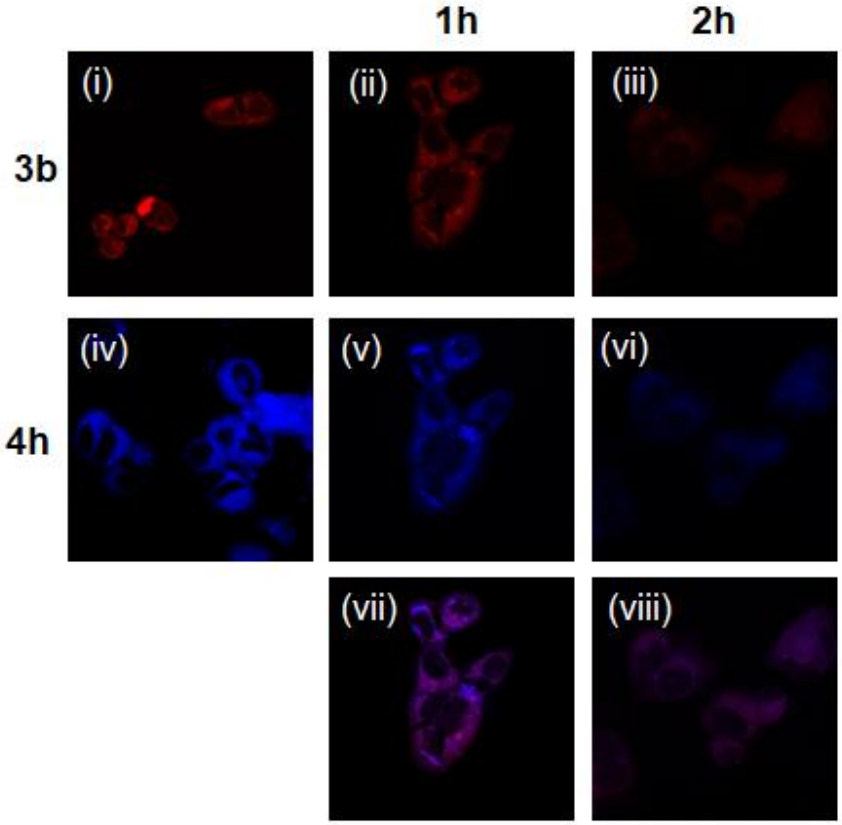


Figure S23: Confocal microscopy images of HNO and **4h** in breast cancer cells (MCF-7).

HNO detection: MCF-7 cells were pre-incubated with HNO-TCF dye **7 (**20 μM) for 30 min followed by treatment of (i) **3b**; (ii) **1h** (iii) **2h** (200 μM) independently for 2 hours. The cells were imaged on Carl Ziess LSM710 laser scanning confocal microscope with anisotropy. The cells were imaged in the RED channel (Alexa fluor 568 for HNO detection). (iv) **4h** (200 μM);

(v) **1h** (vi) **2h**. The cells were imaged in the DAPI channel. (vii) Merged image of (ii and v); (viii) Merged image of (iii and vi).

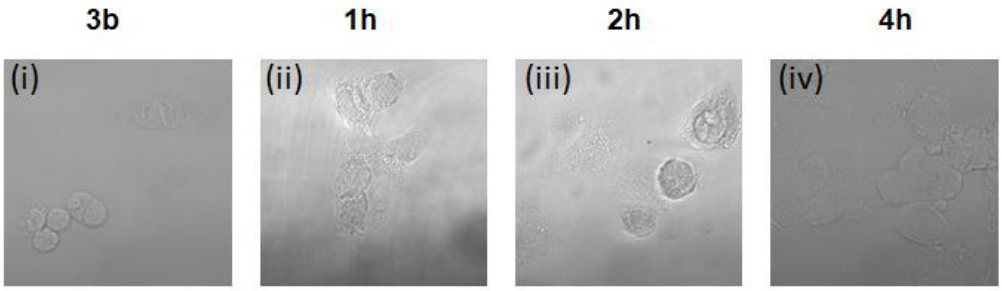


Figure S24: Bright filed images: (i) **3b**; (ii) **1h**; (iii) **2h** and (iv) **4h** were imaged on Carl Ziess LSM710 laser scanning confocal microscope with anisotropy.

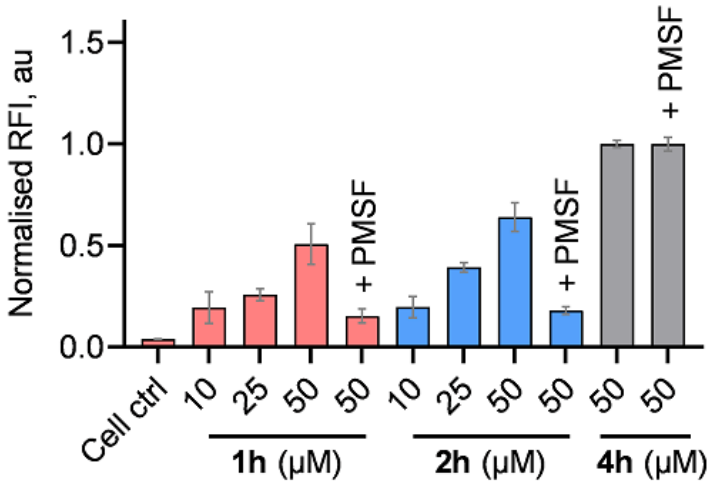


Figure S25: Compounds **1h, 2h** and **4h** were incubated in A549 cell lysate (1 mg/mL) in presence and absence of PMSF (esterase inhibitor) for 3 hours; cell ctrl refers to untreated cell lysate; + PMSF refers to cell lysate were preincubated with PMSF for 30 mins. Fluorescence measurement (λ_{ex} = 308 nm; λ_{em} = 497 nm) was carried out by varying concentration of **1h** and **2h.**

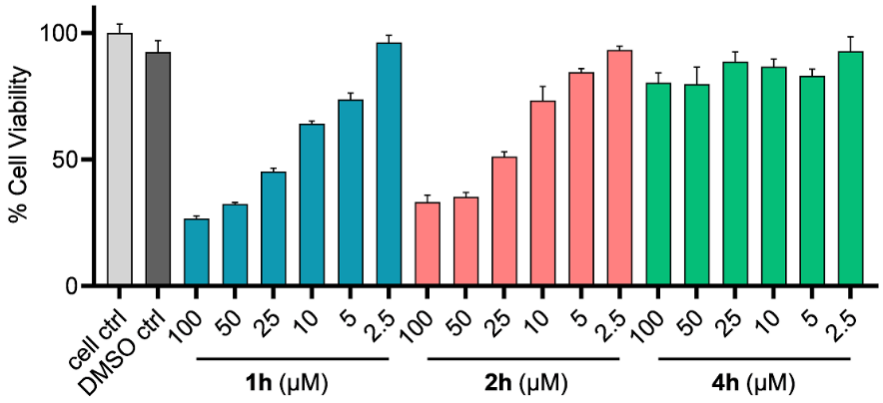


Figure S26: Cell viability assay carried out with varying concentration of compounds **1h**, **2h** and **4h** on MEF cells for 24 h. All data are presented as mean ± SD (n = 3/group).

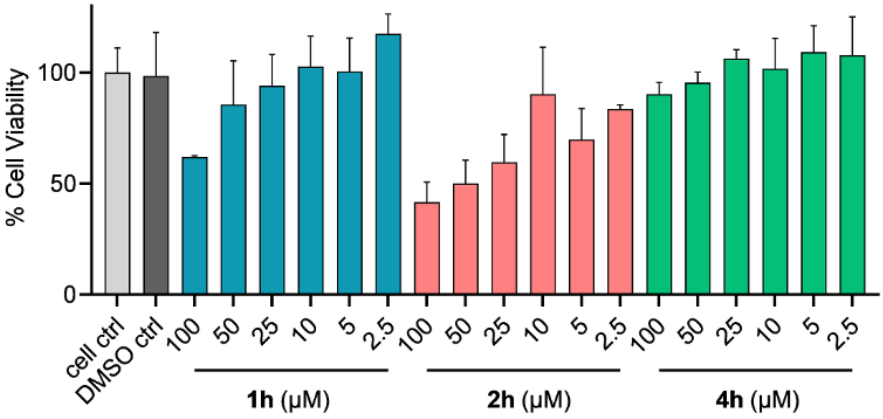


Figure S27: Cell viability assay carried out with varying concentration of compounds **1h**, **2h** and **4h** on A549 cells for 24 h. All data are presented as mean ± SD (n = 3/group).

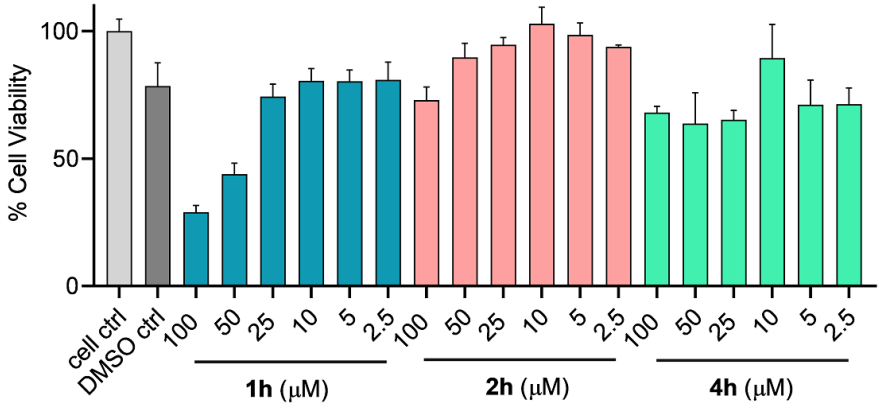


Figure S28: Cell viability assay carried out with varying concentration of compounds **1h**, **2h** and **4h** on MCF-7 cells for 24 h. All data are presented as mean ± SD (n = 3/group).

References:

1 J. T. M. Correia, G. Piva da Silva, E. André and M. W. Paixão, *Adv. Synth. Catal.*, 2019, **361**, 5558–5564.

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6. I. N. Chakraborty, S. Roy, G. Devatha, A. Rao and P. P. Pillai, *Chem. Mater.*, 2019, **31**, 2258–2262.

7. Marvin 5.4.0.1, http://www.chemaxon.com.

NMR spectra:

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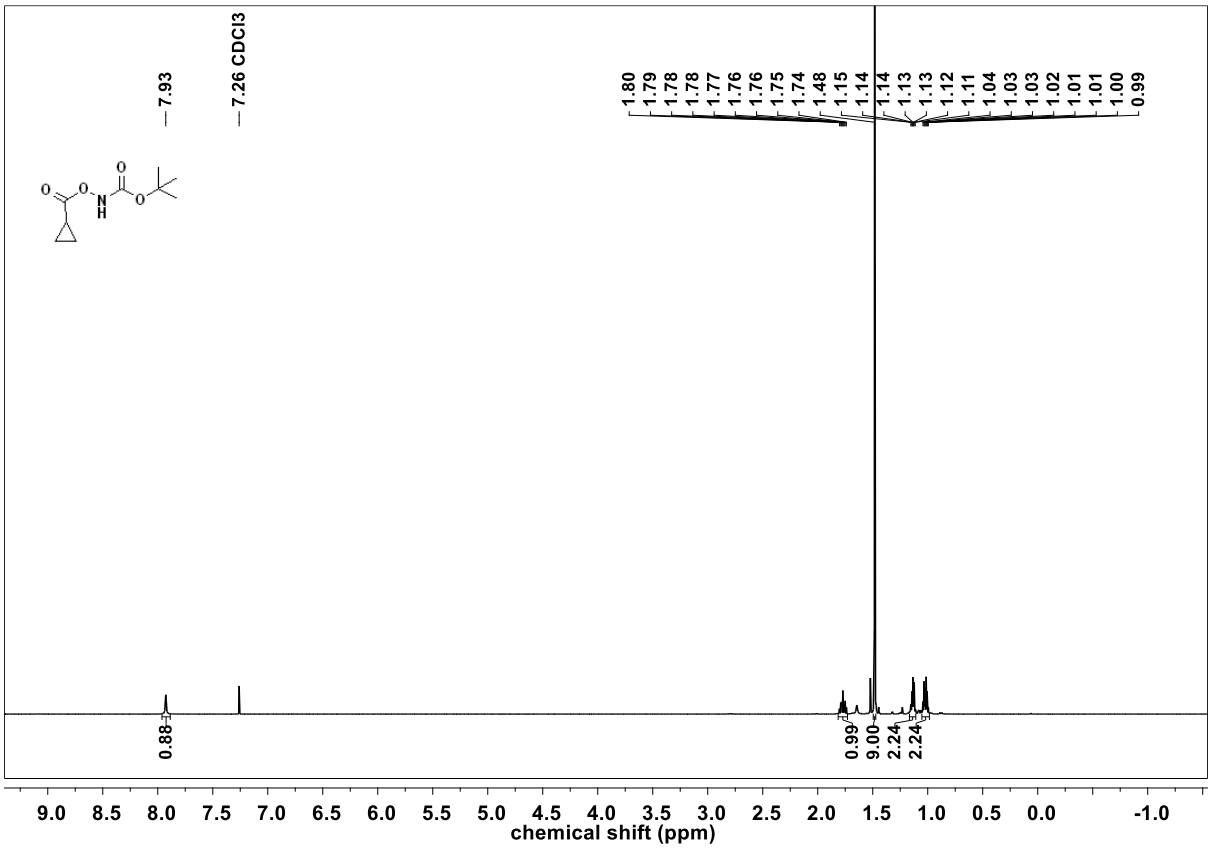

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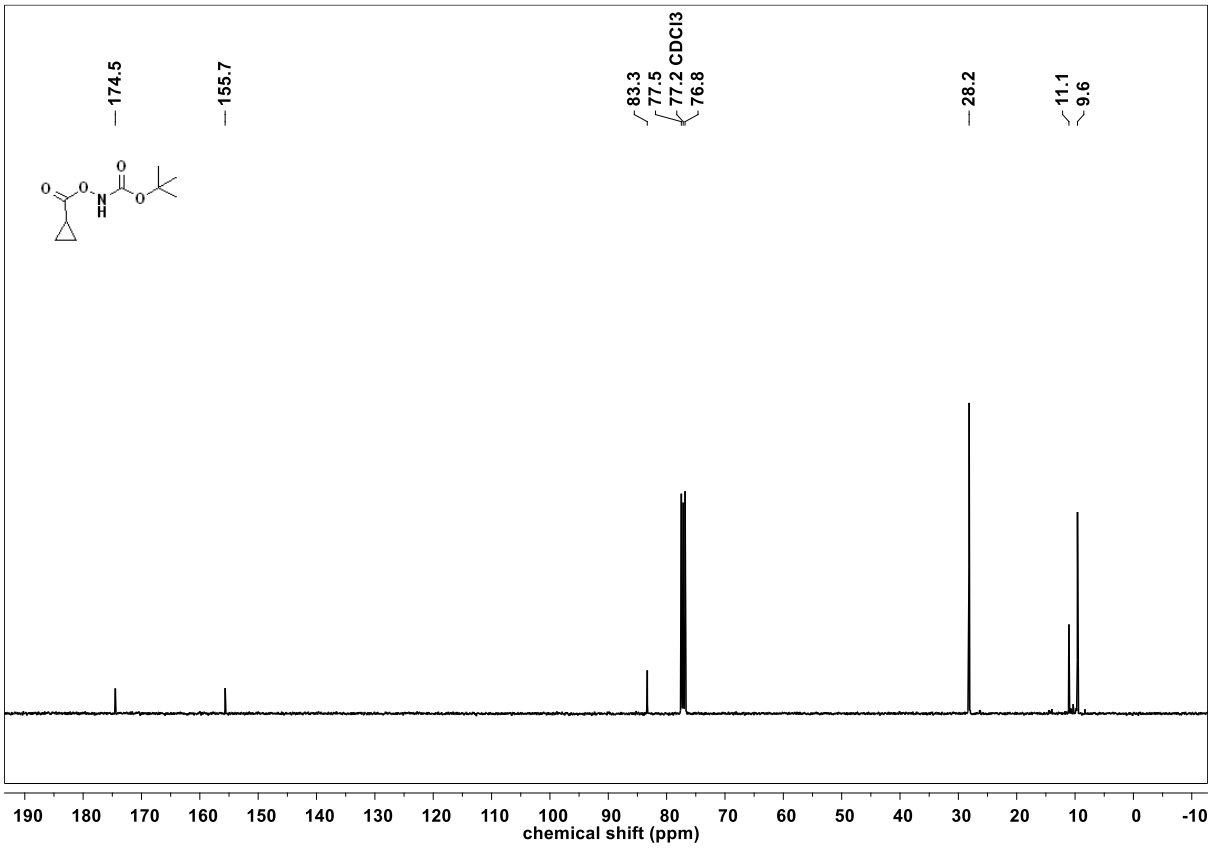

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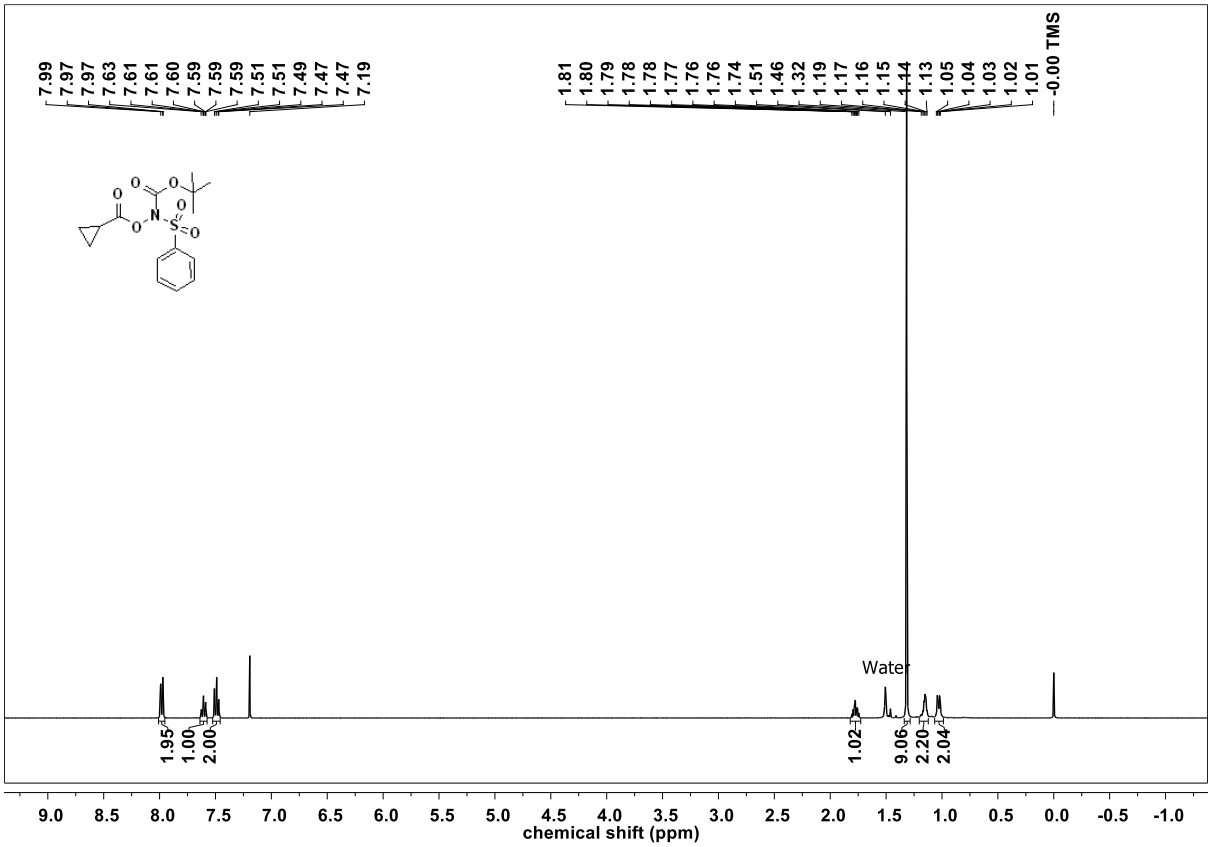

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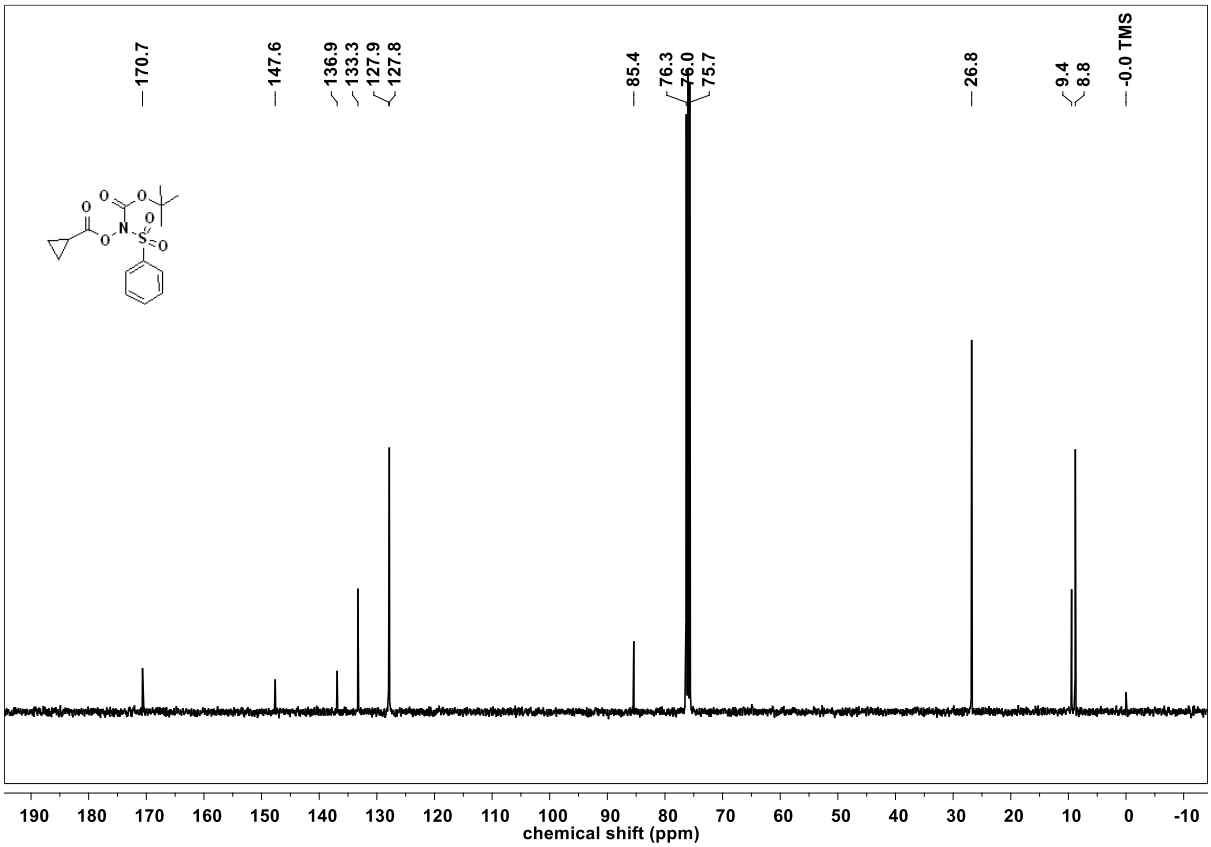

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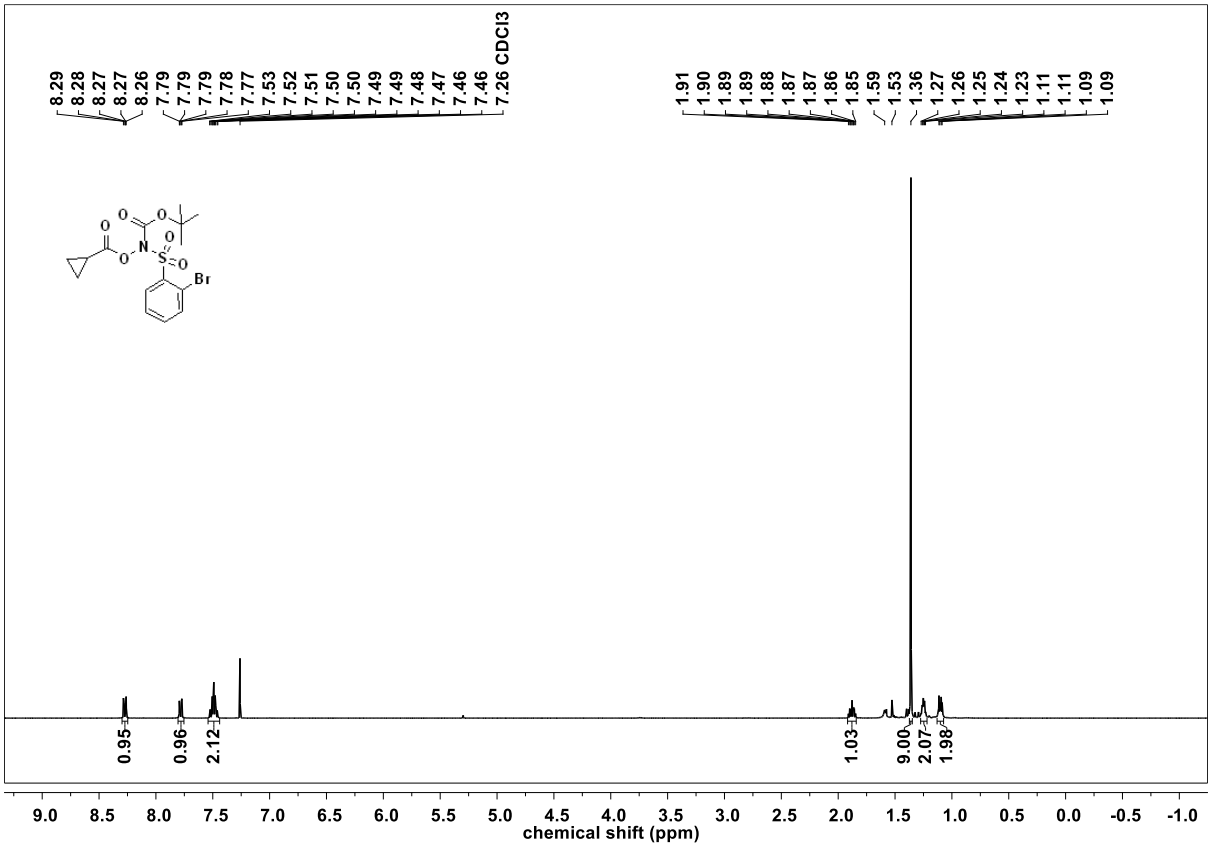

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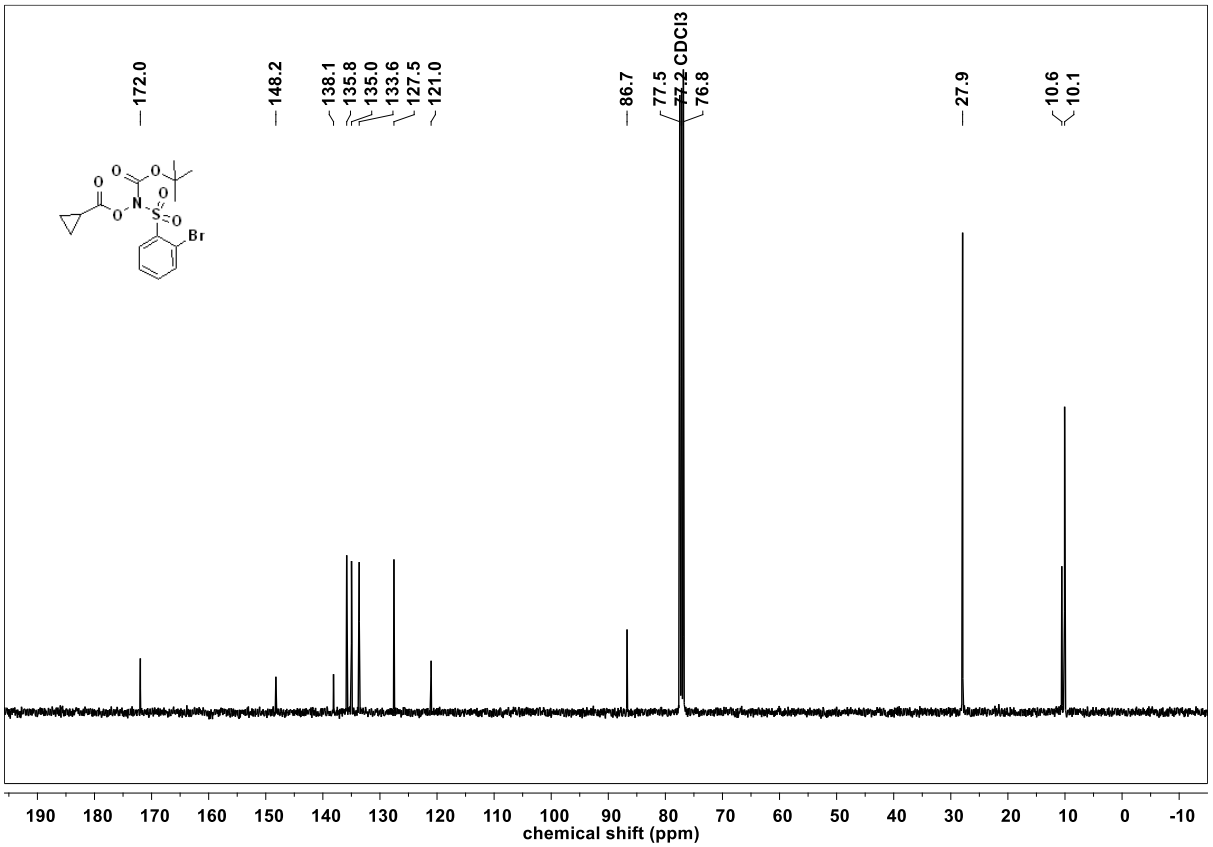

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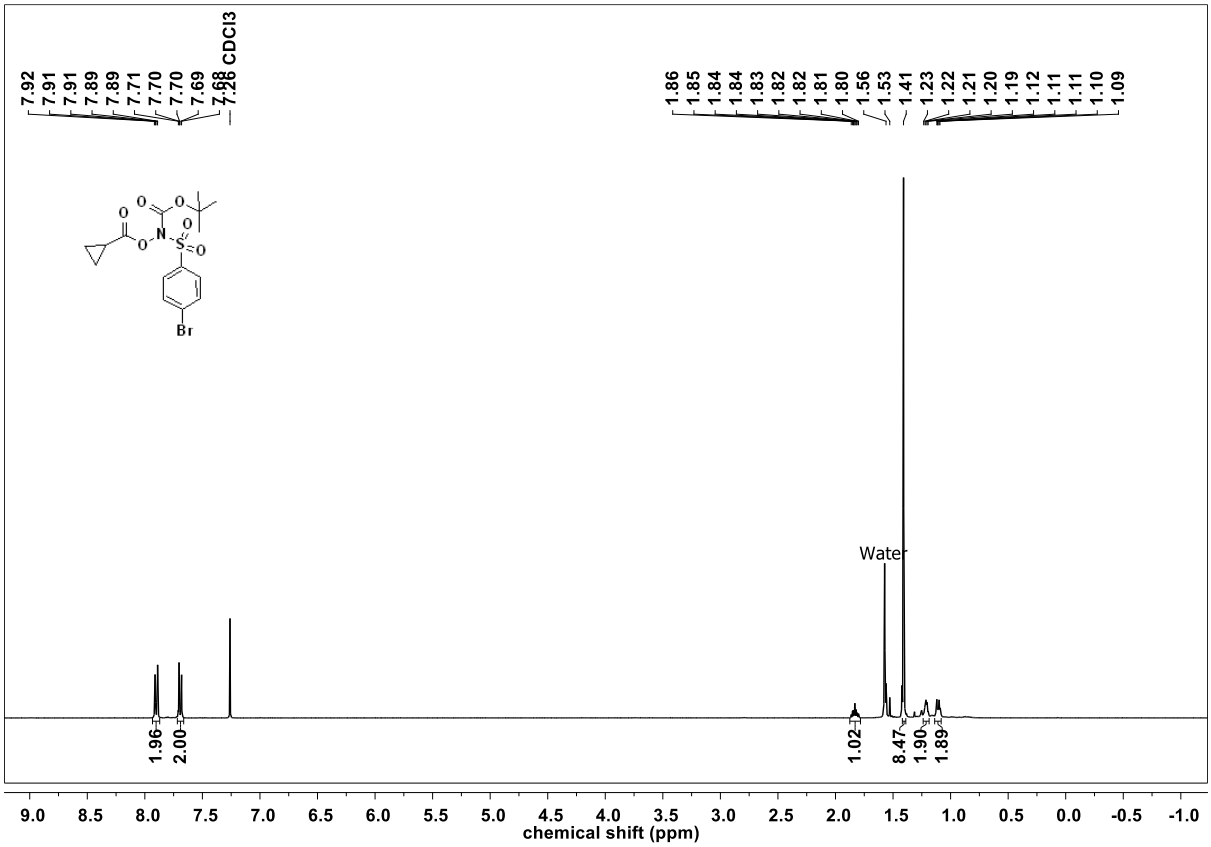

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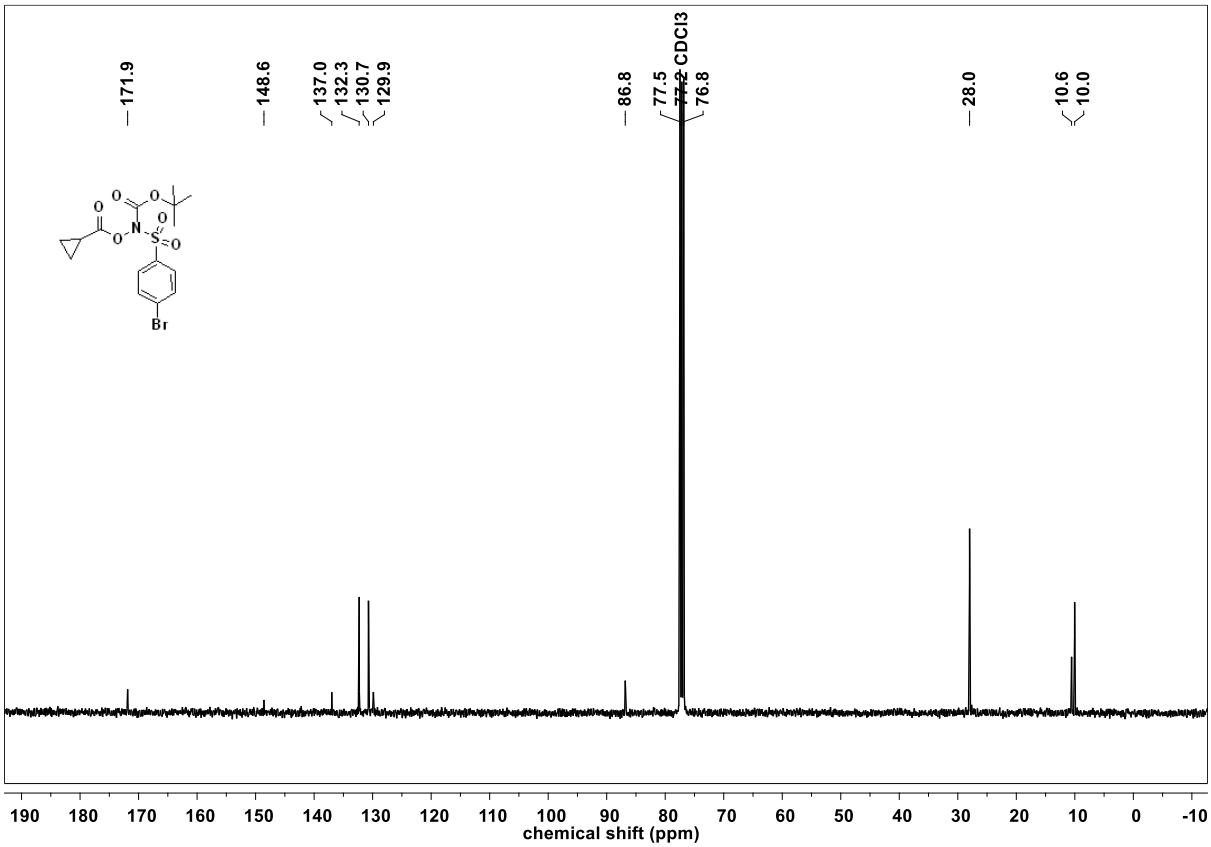

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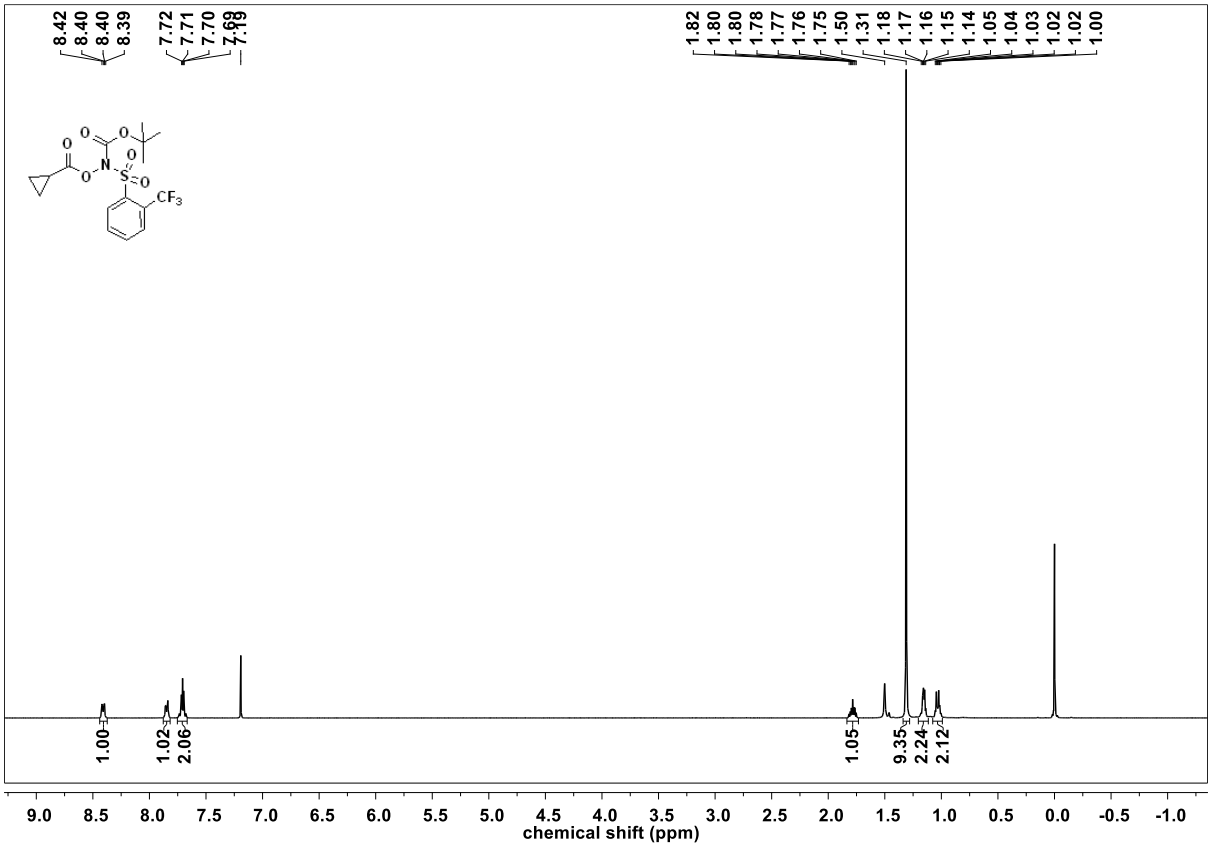

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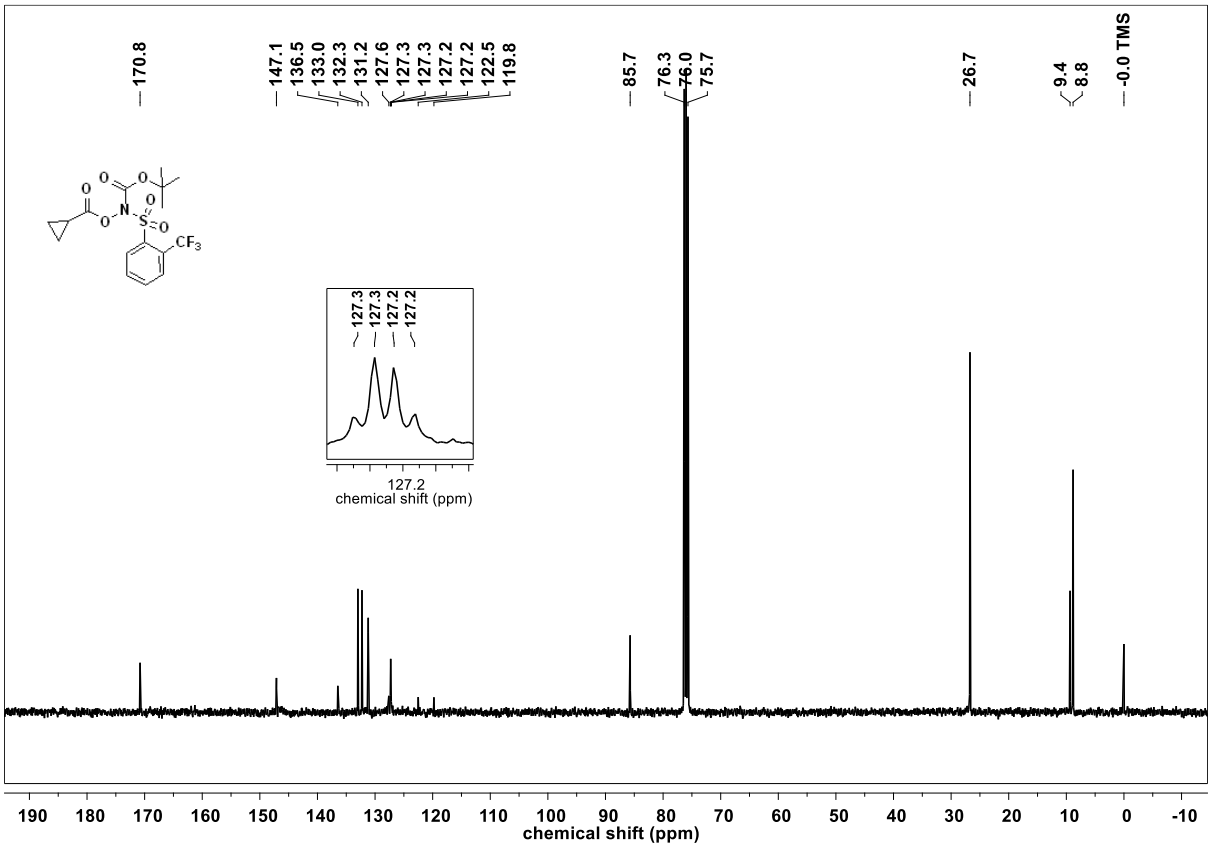

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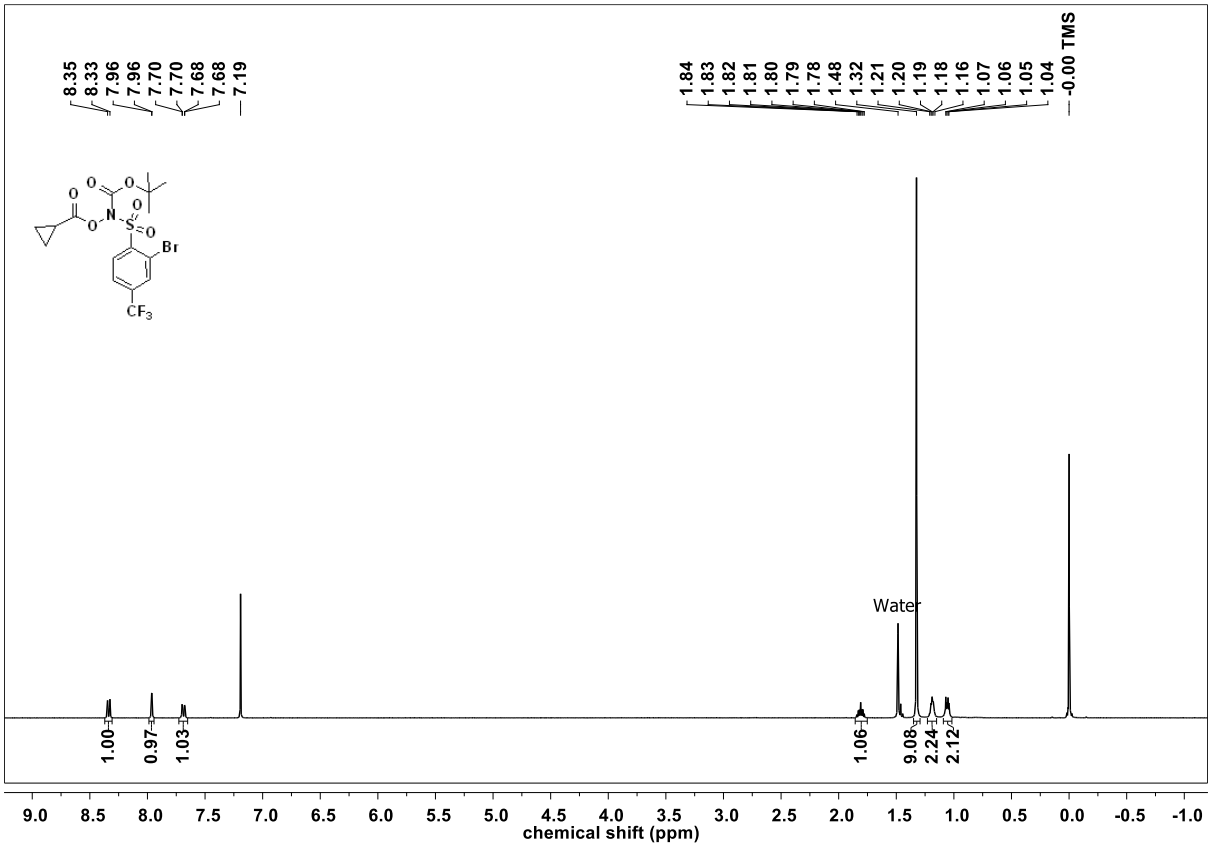

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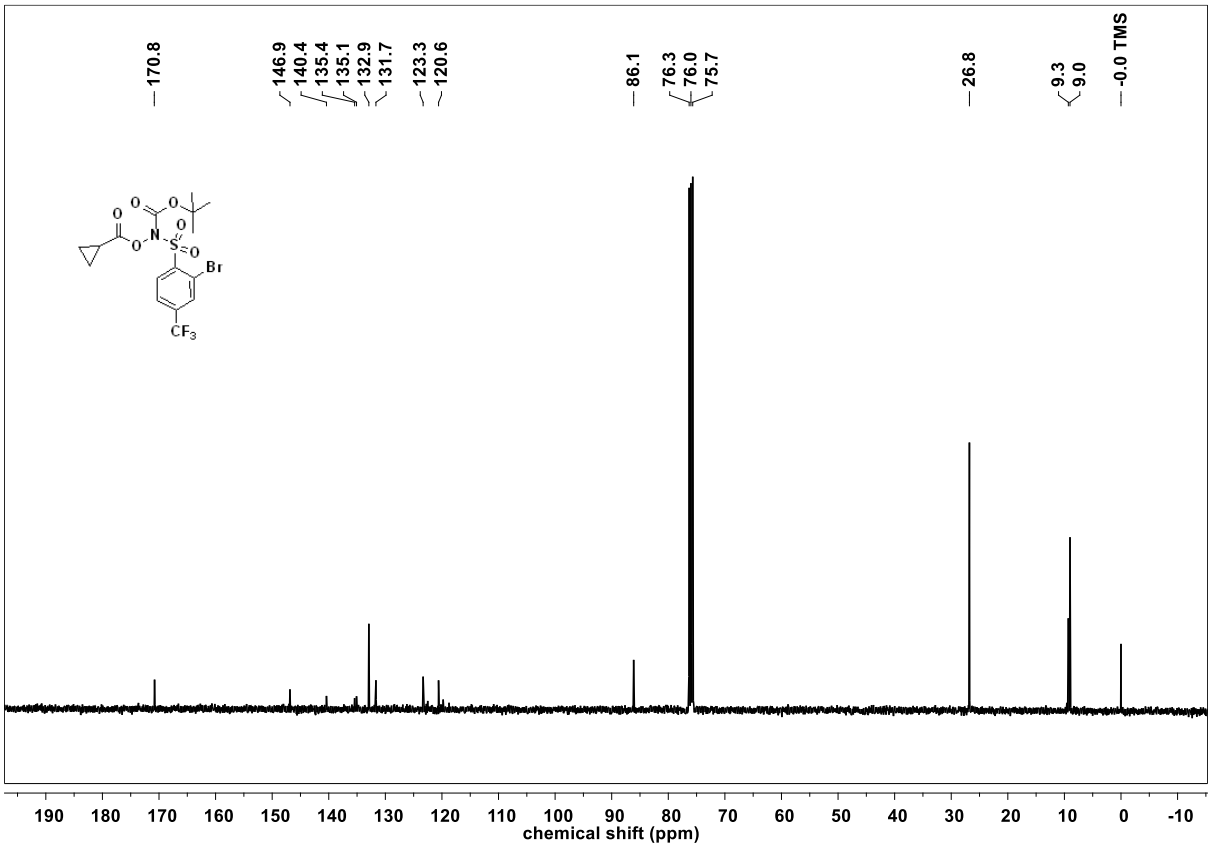

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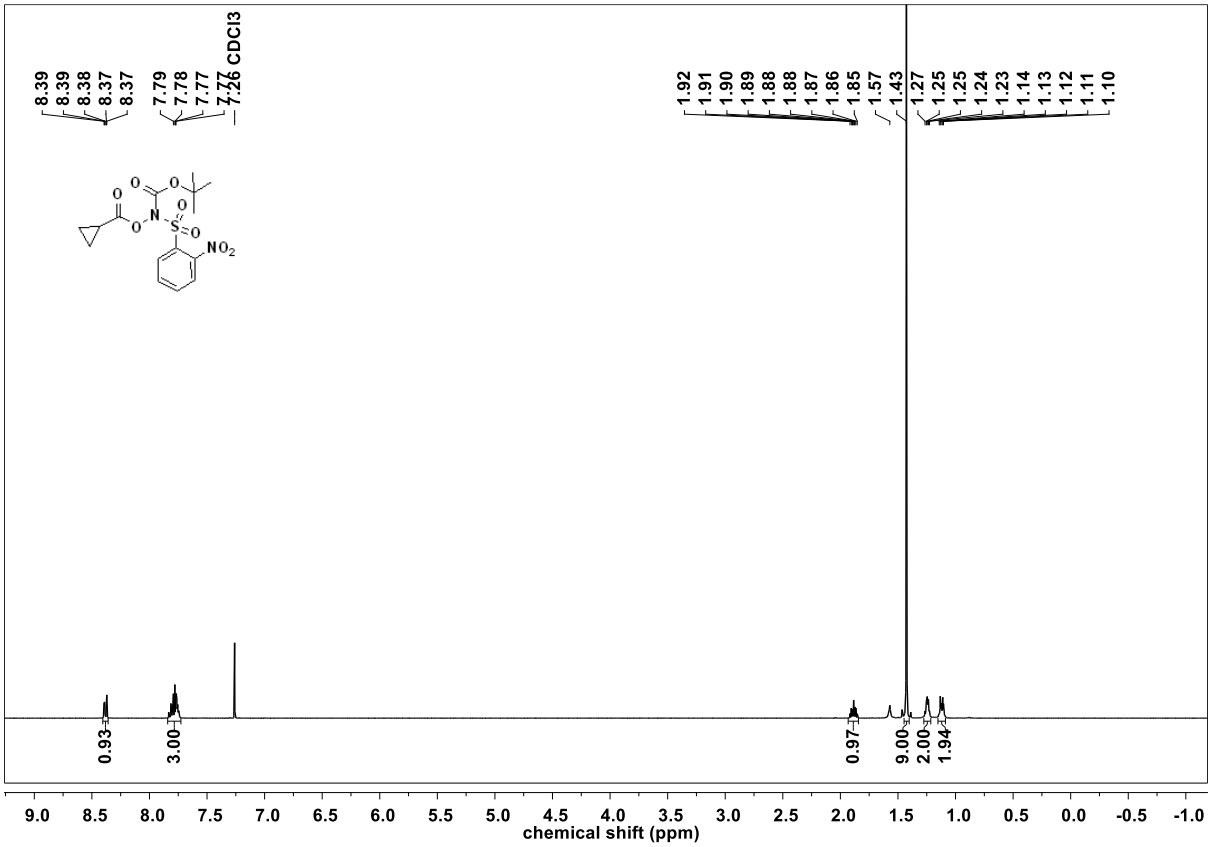
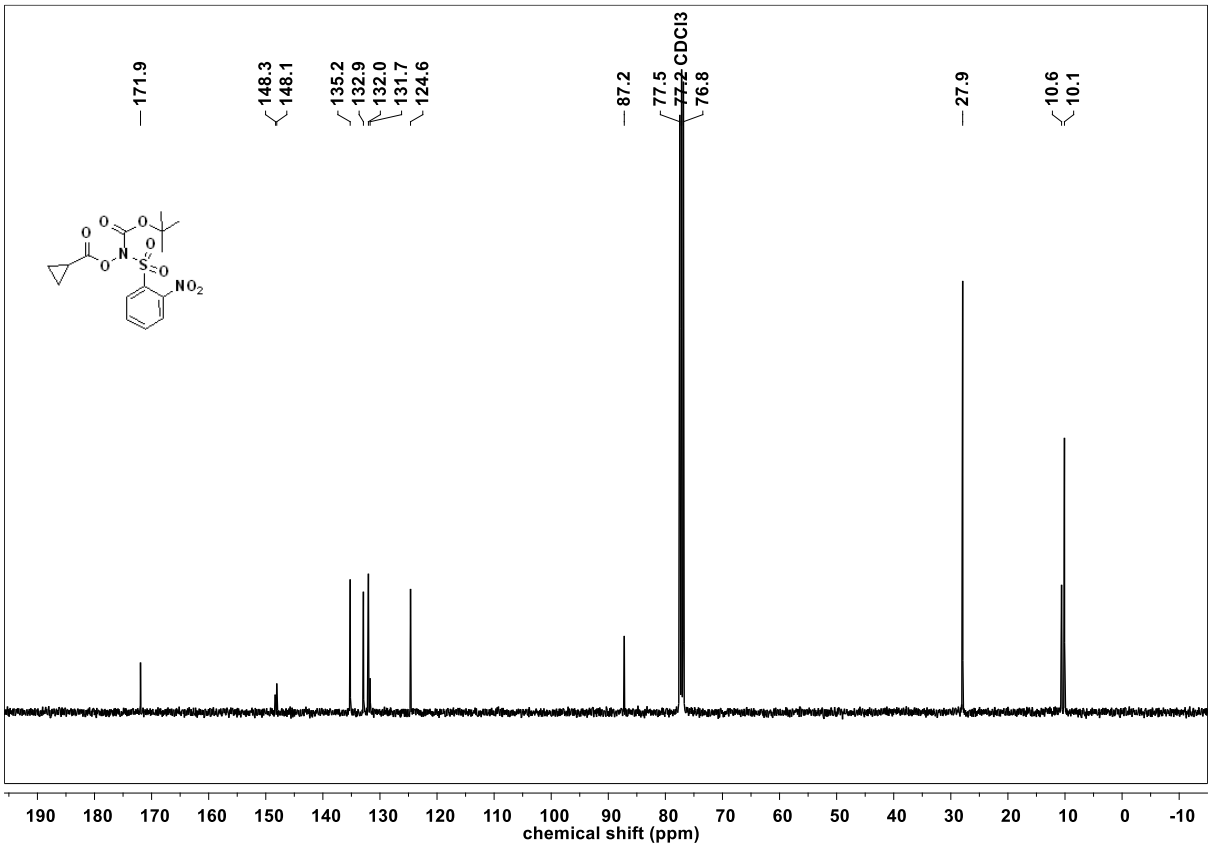
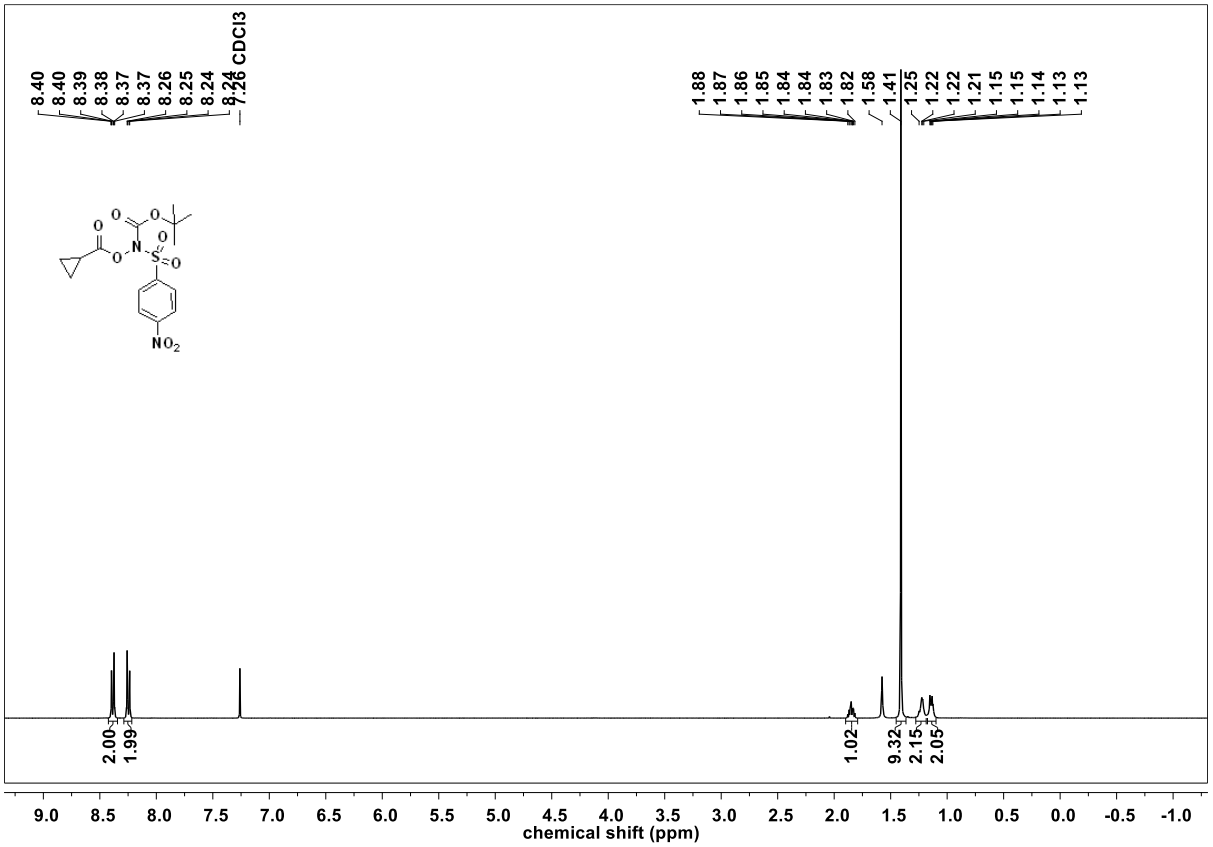


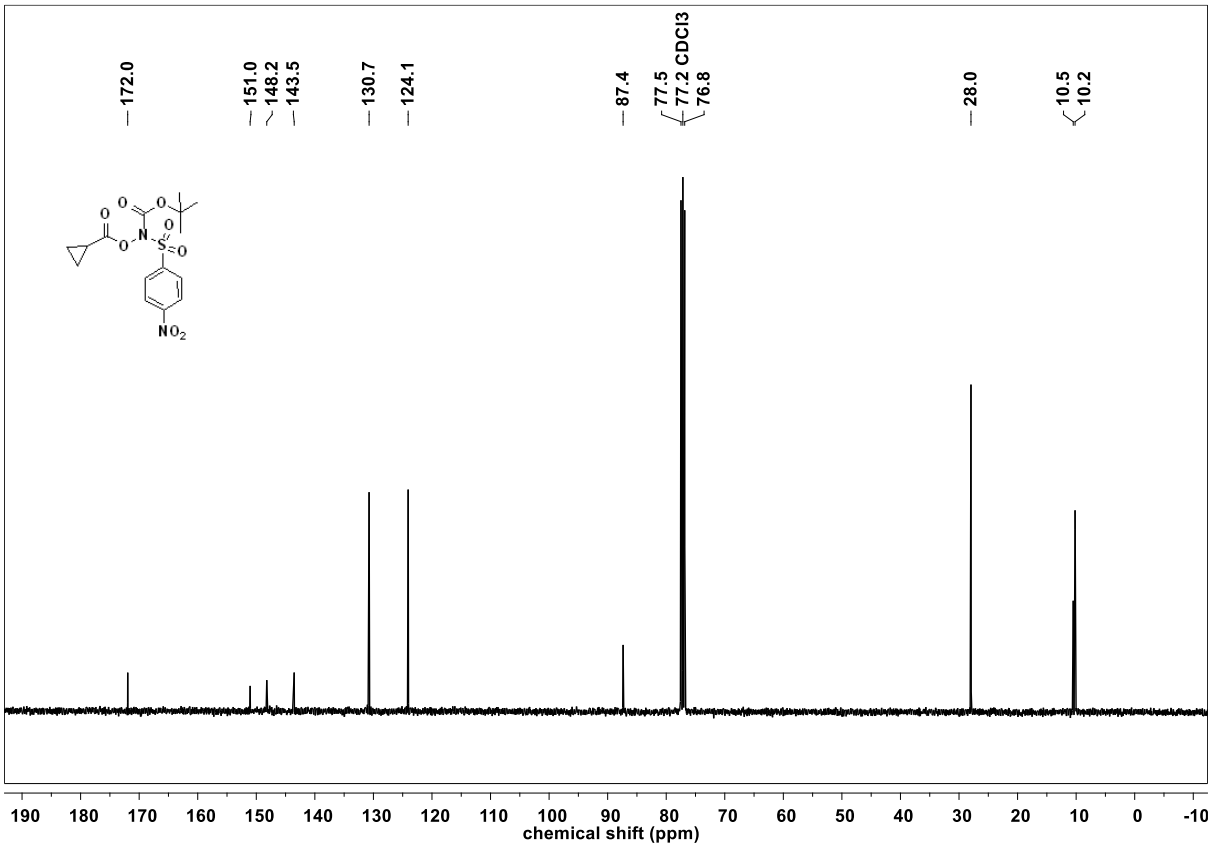
^{13}C NMR spectra of **2f:**



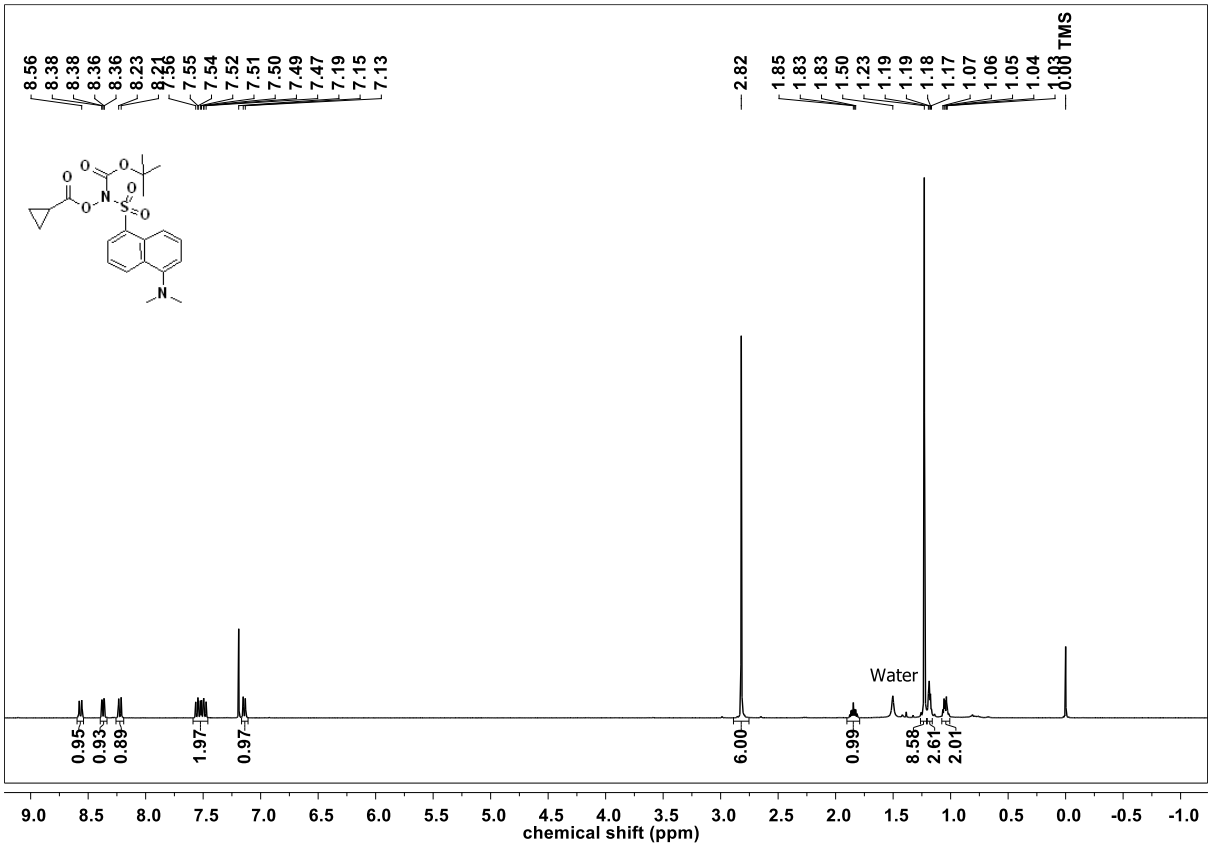
^{1}H NMR spectra of **2g:**



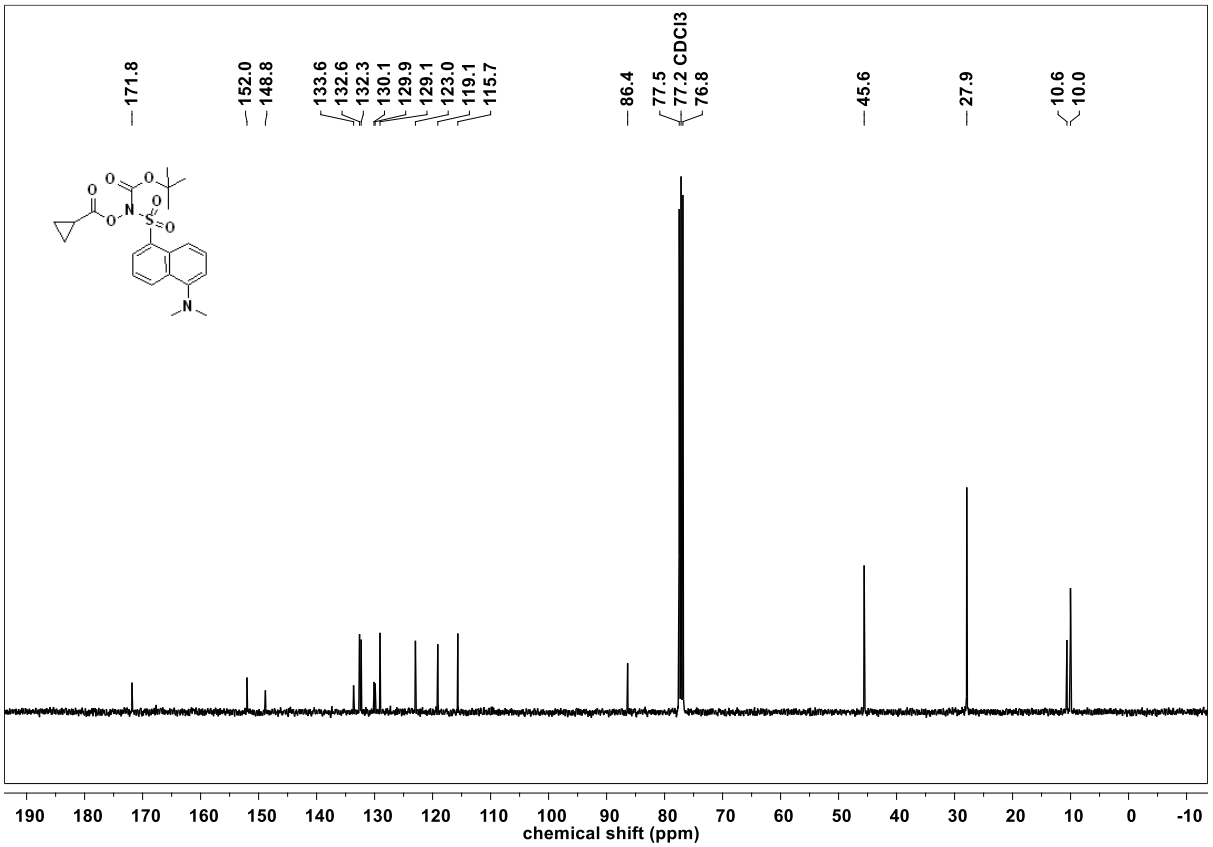
^{13}C NMR spectra of **2g:**



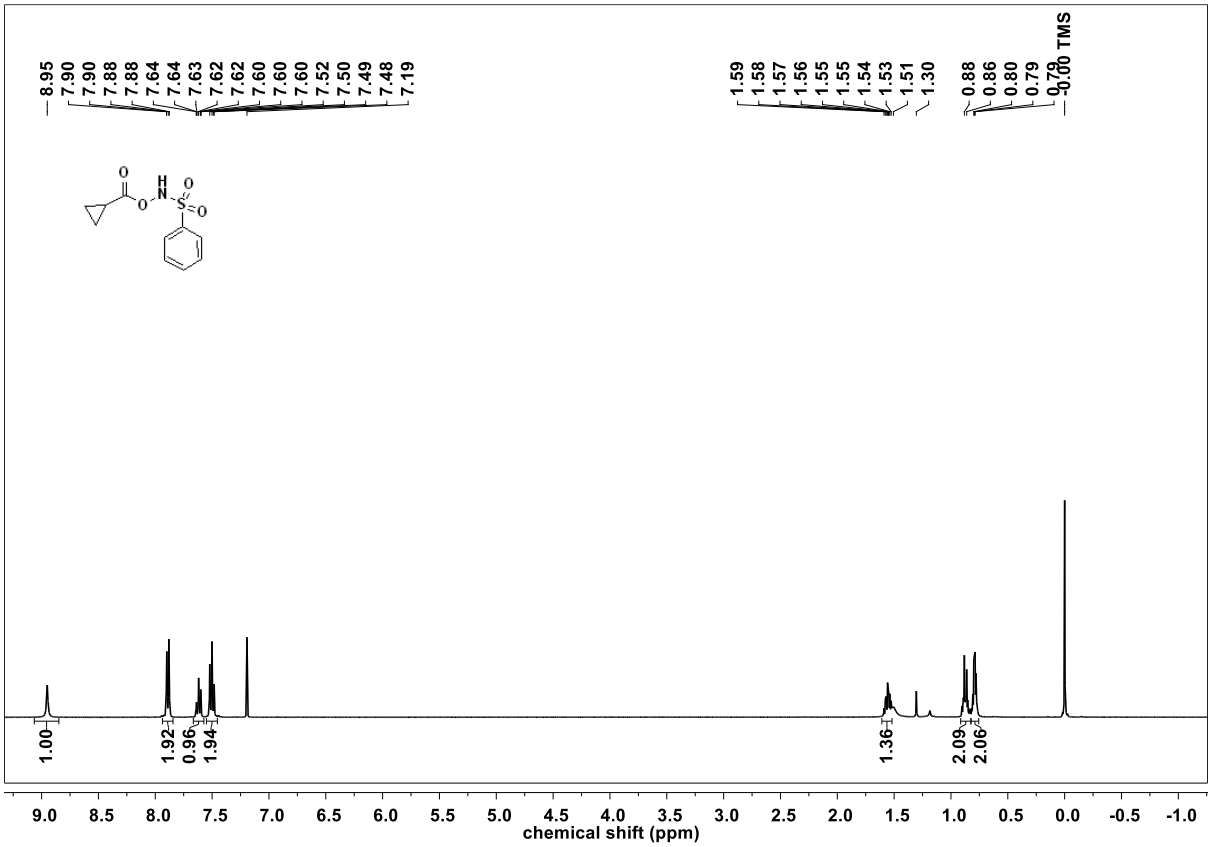
^{1}H NMR spectra of **2h:**



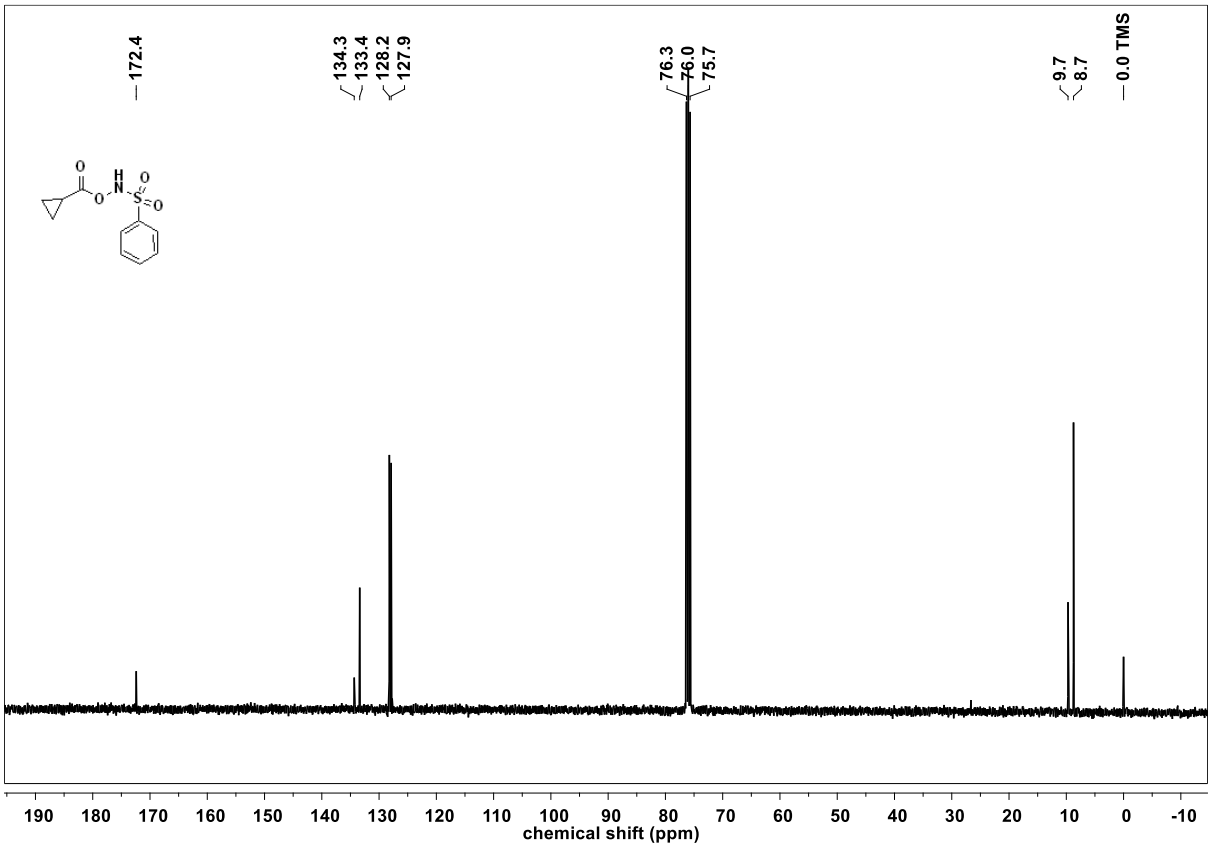
^{13}C NMR spectra of **2h:**



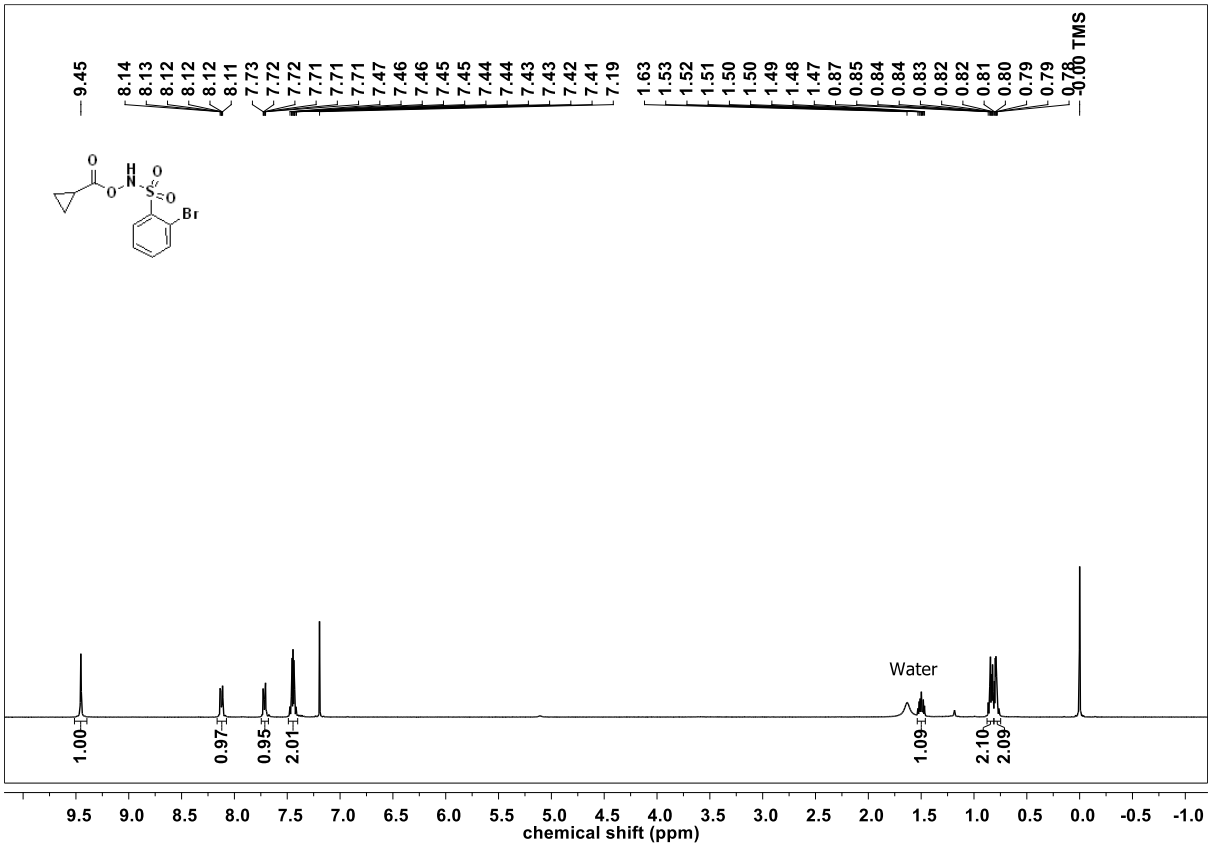
^{1}H NMR spectra of **1a:**



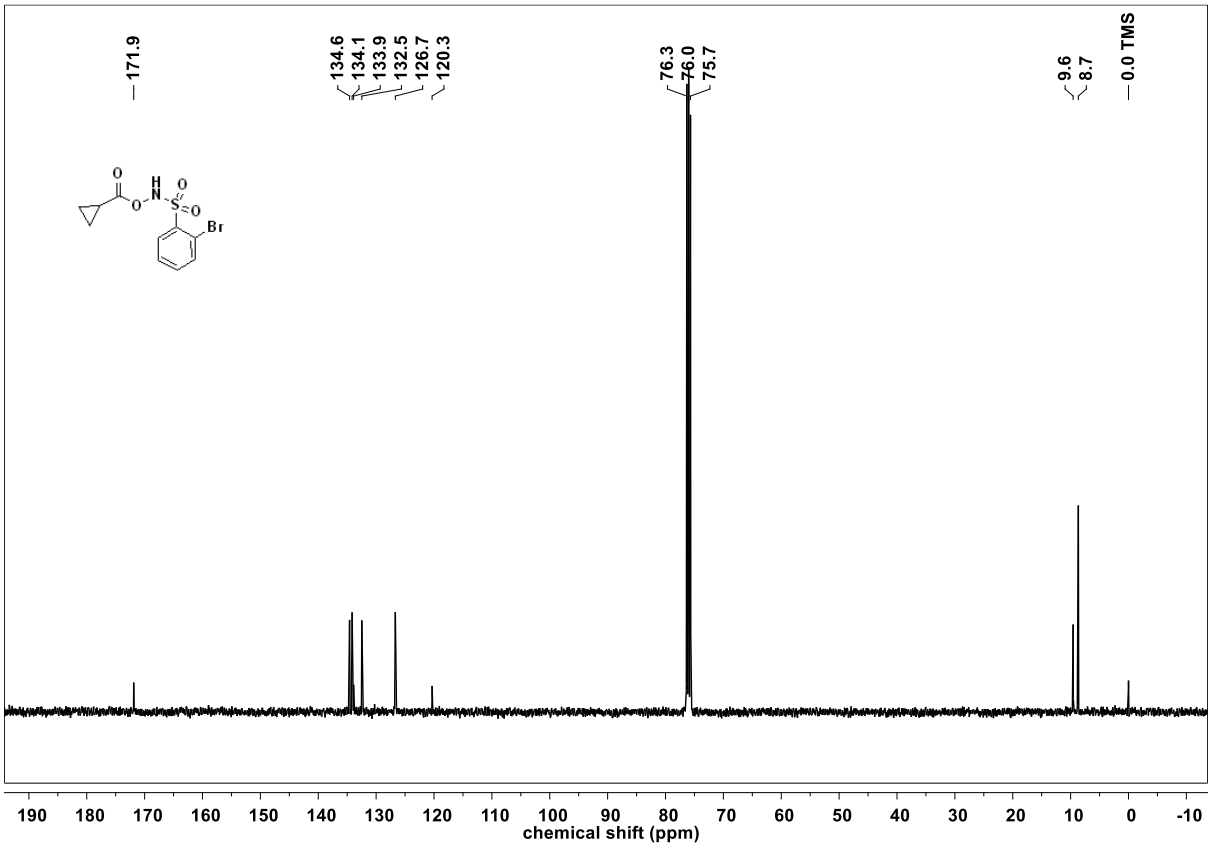
^{13}C NMR spectra of **1a:**



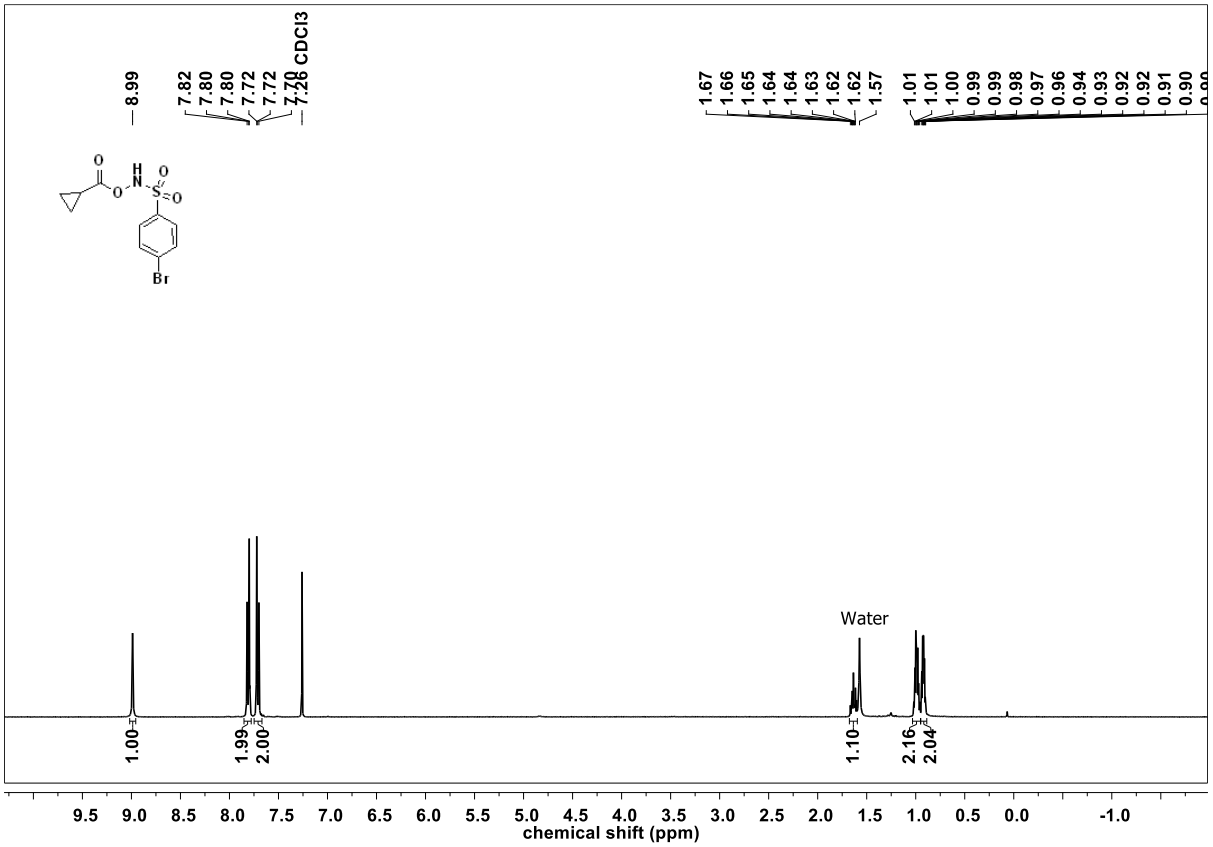
^{1}H NMR spectra of **1b:**



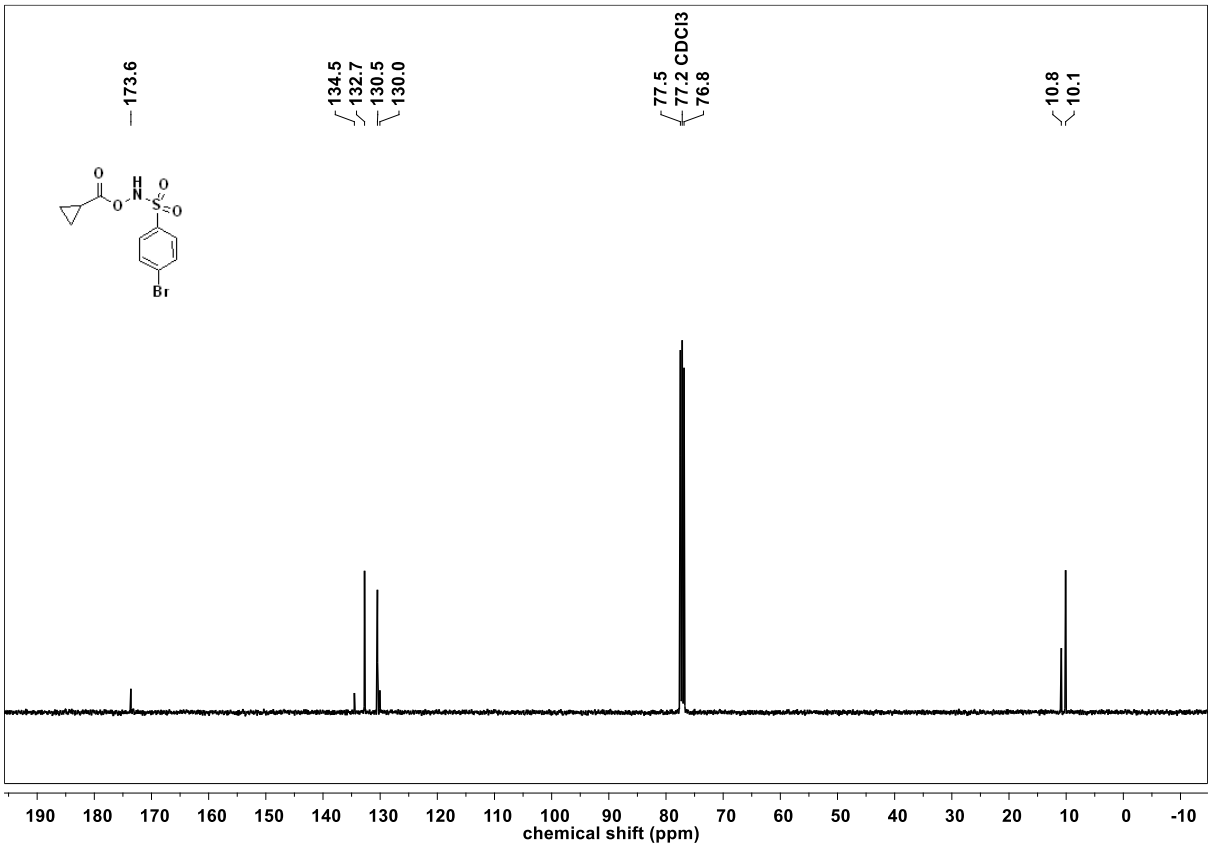
^{13}C NMR spectra of **1b:**



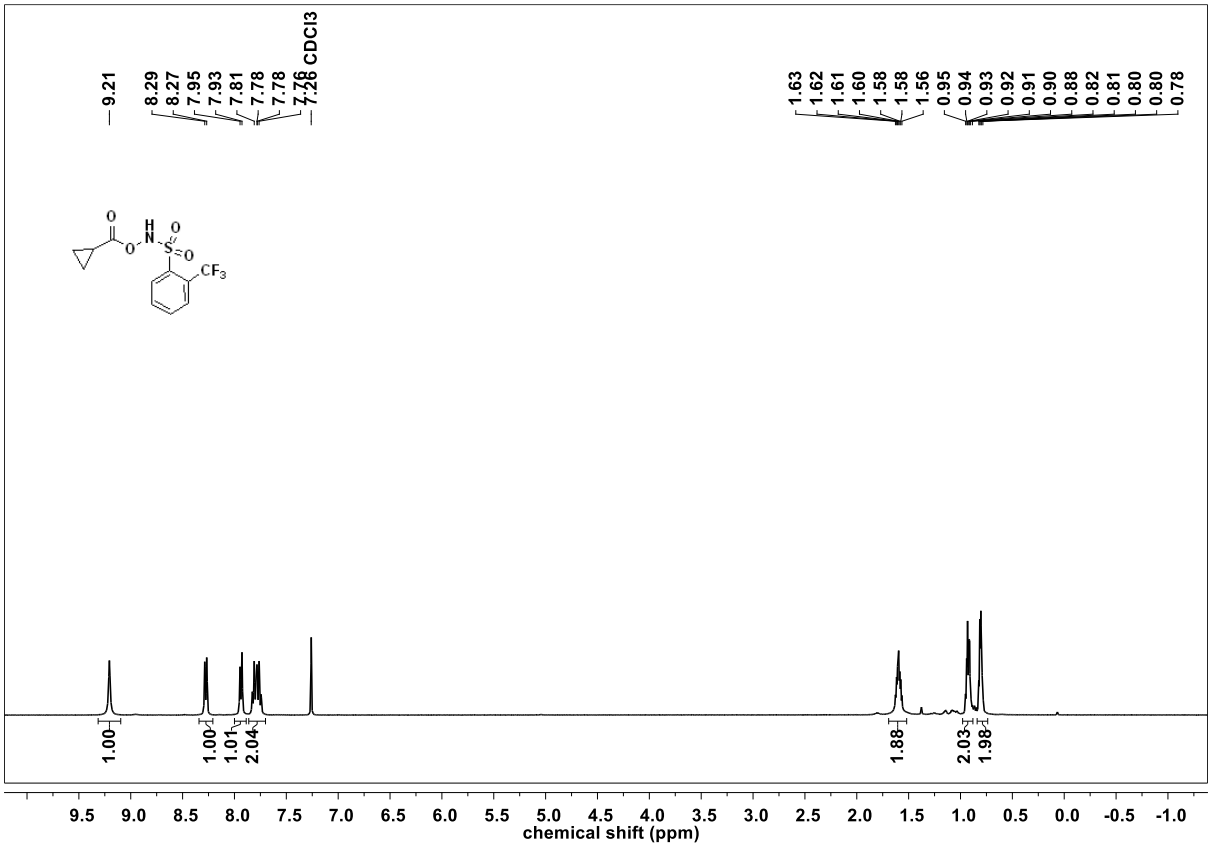
^{1}H NMR spectra of **1c:**



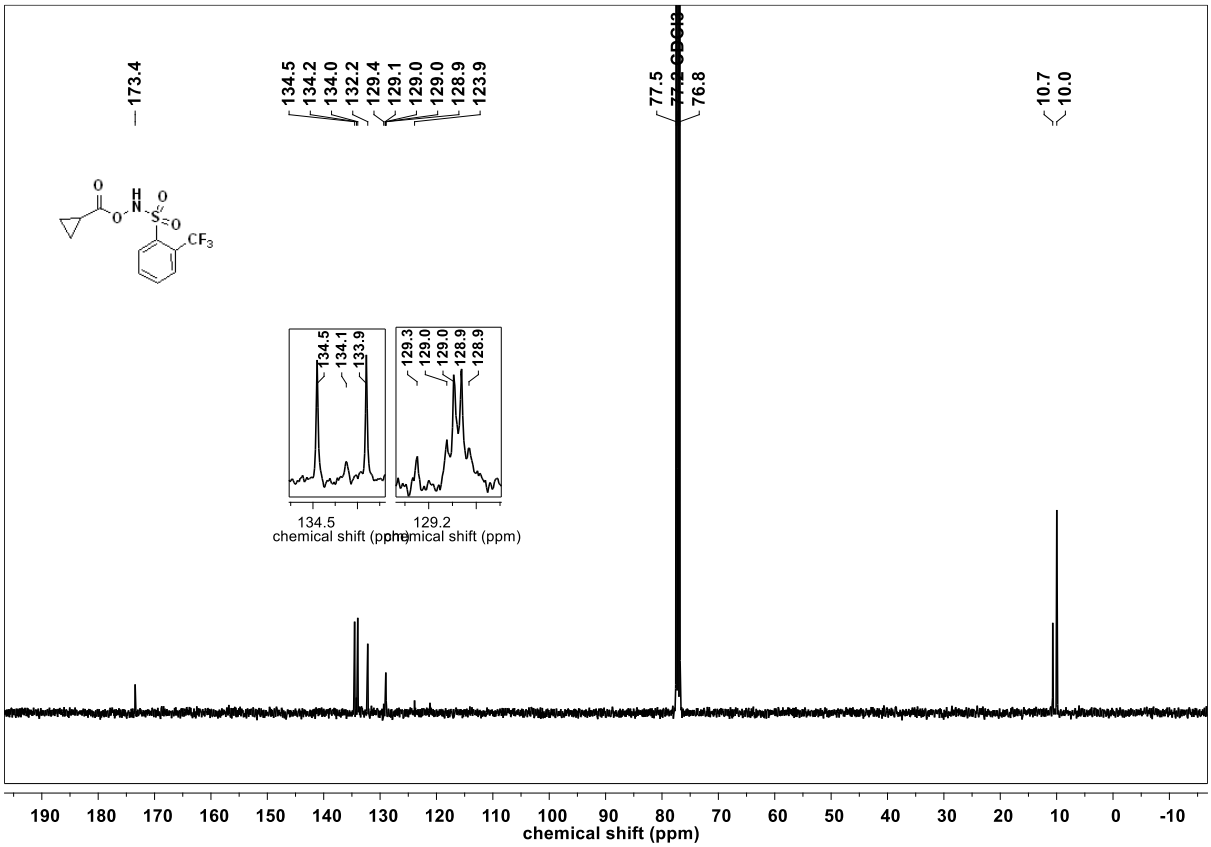
^{13}C NMR spectra of **1c:**



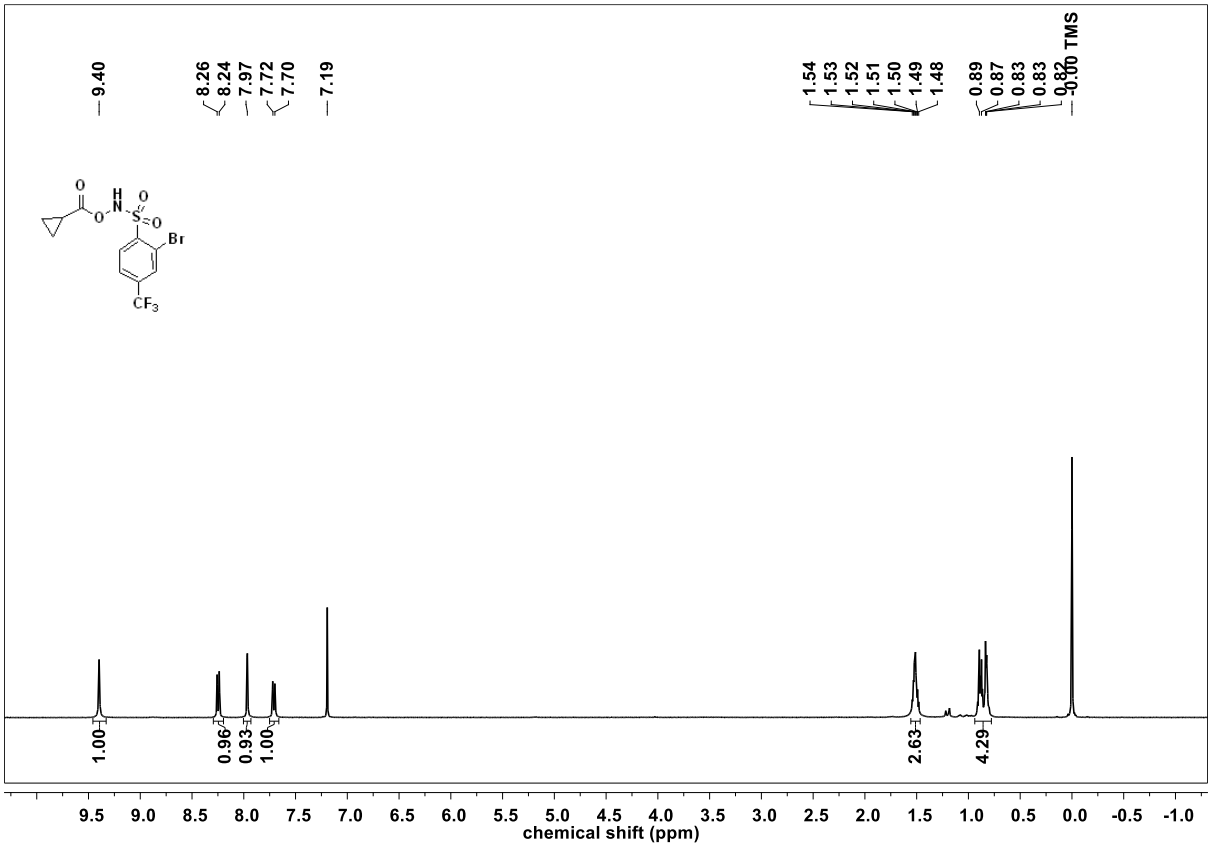
^{1}H NMR spectra of **1d:**



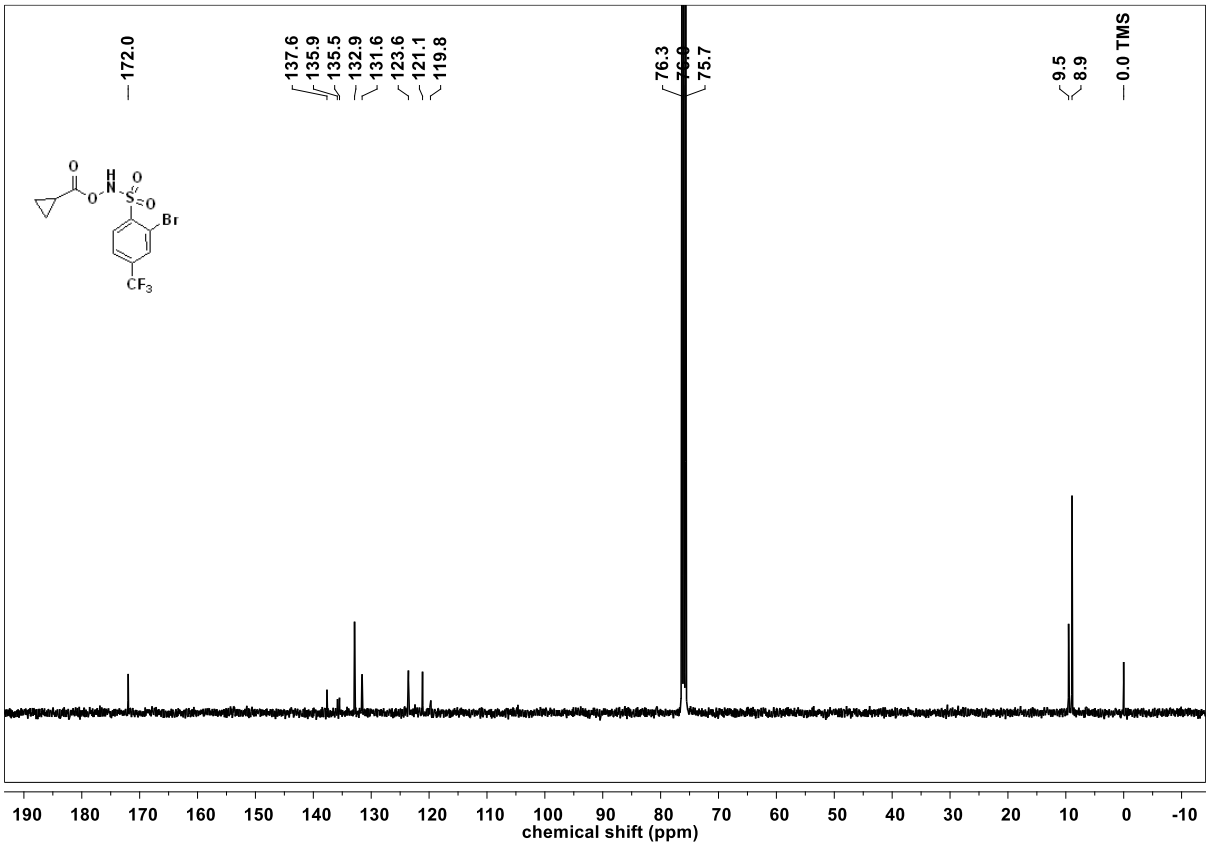
^{13}C NMR spectra of **1d:**



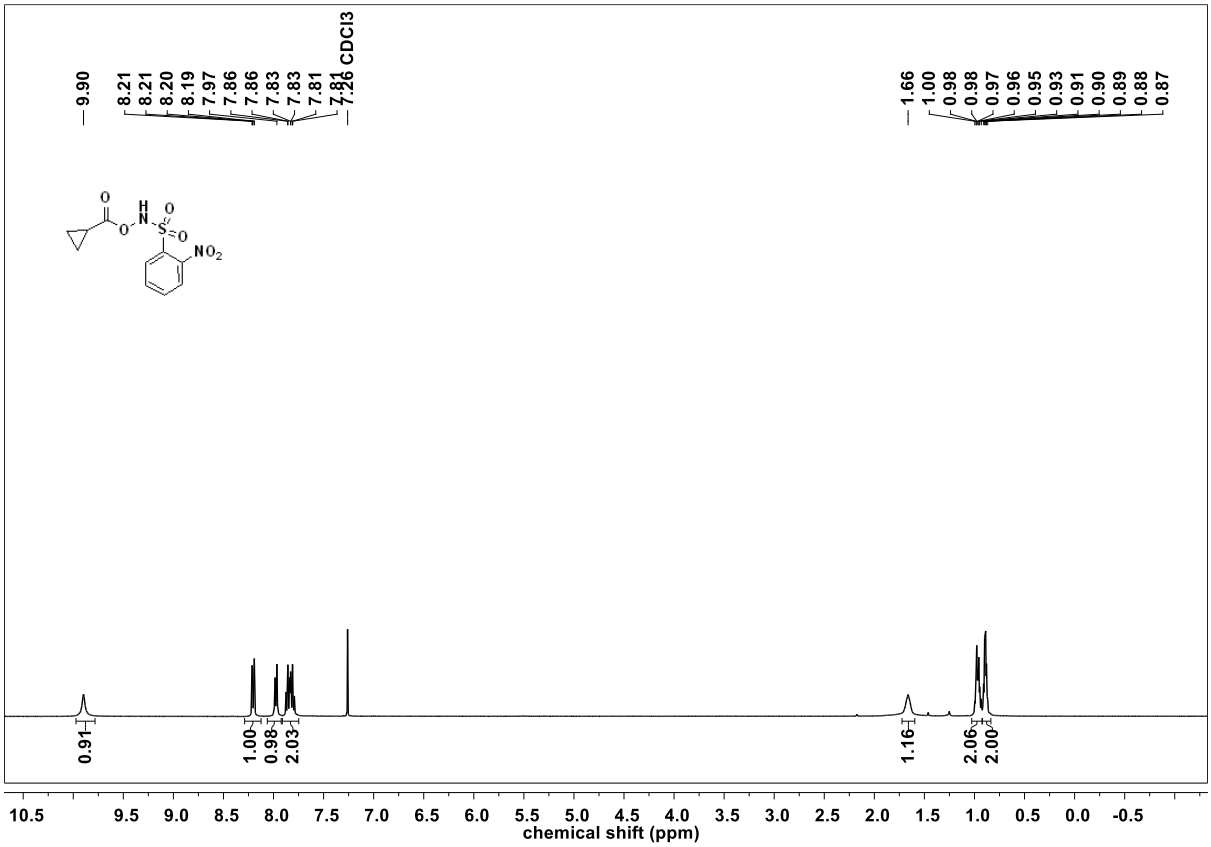
^{1}H NMR spectra of **1e:**



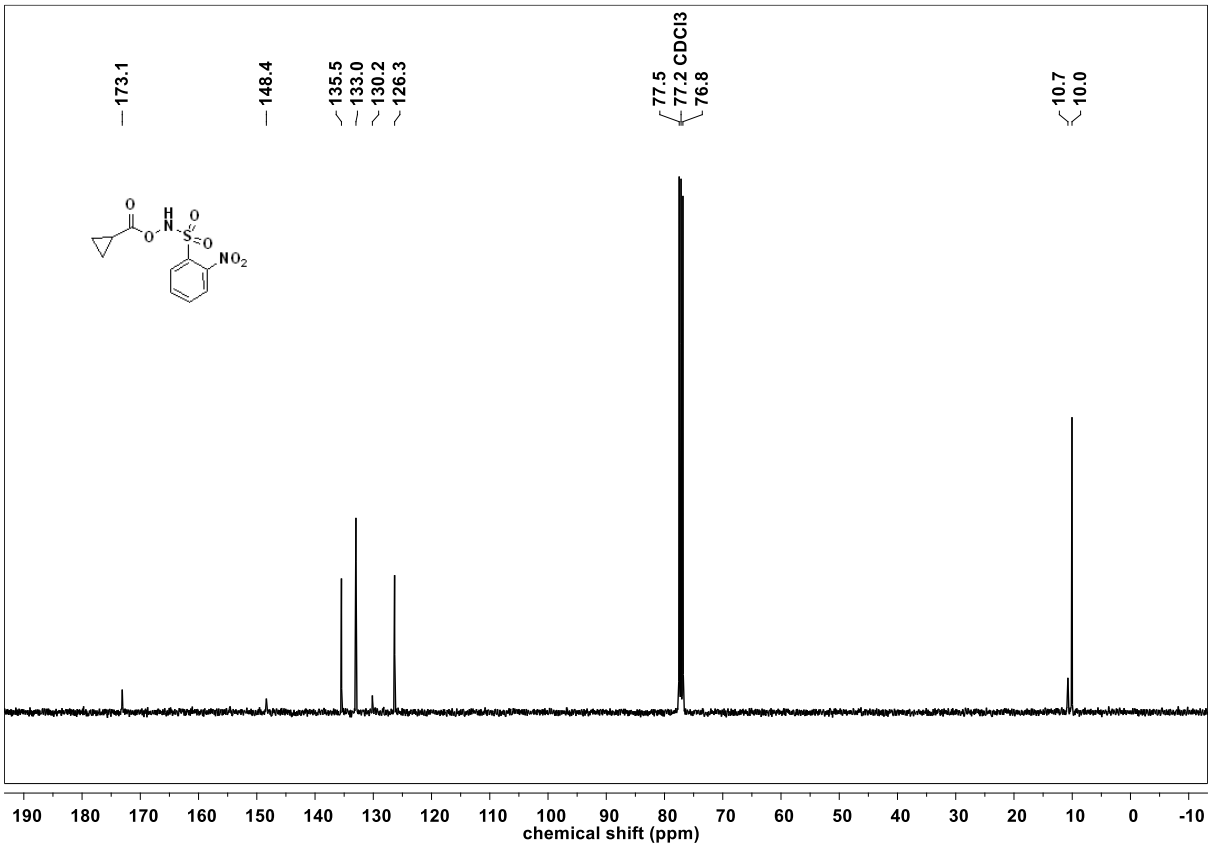
^{13}C NMR spectra of **1e:**



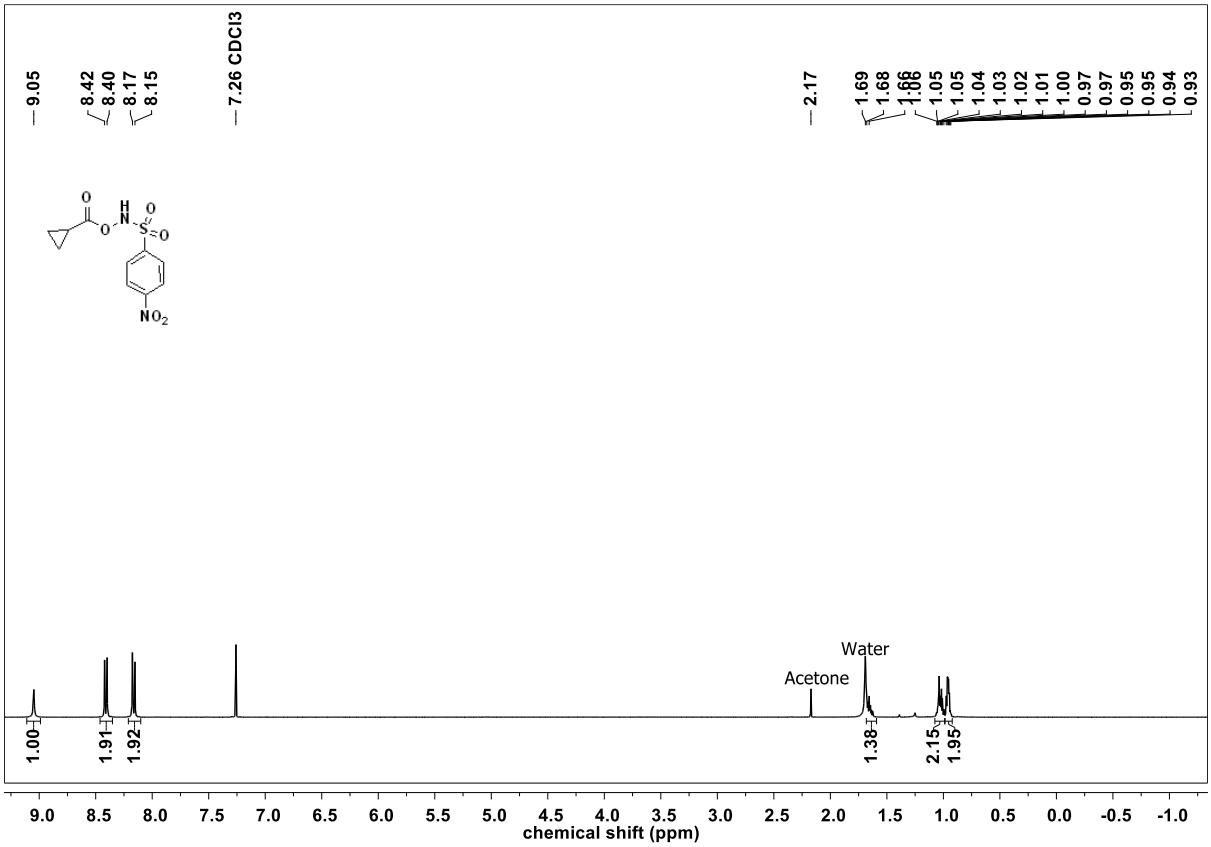
^{1}H NMR spectra of **1f:**



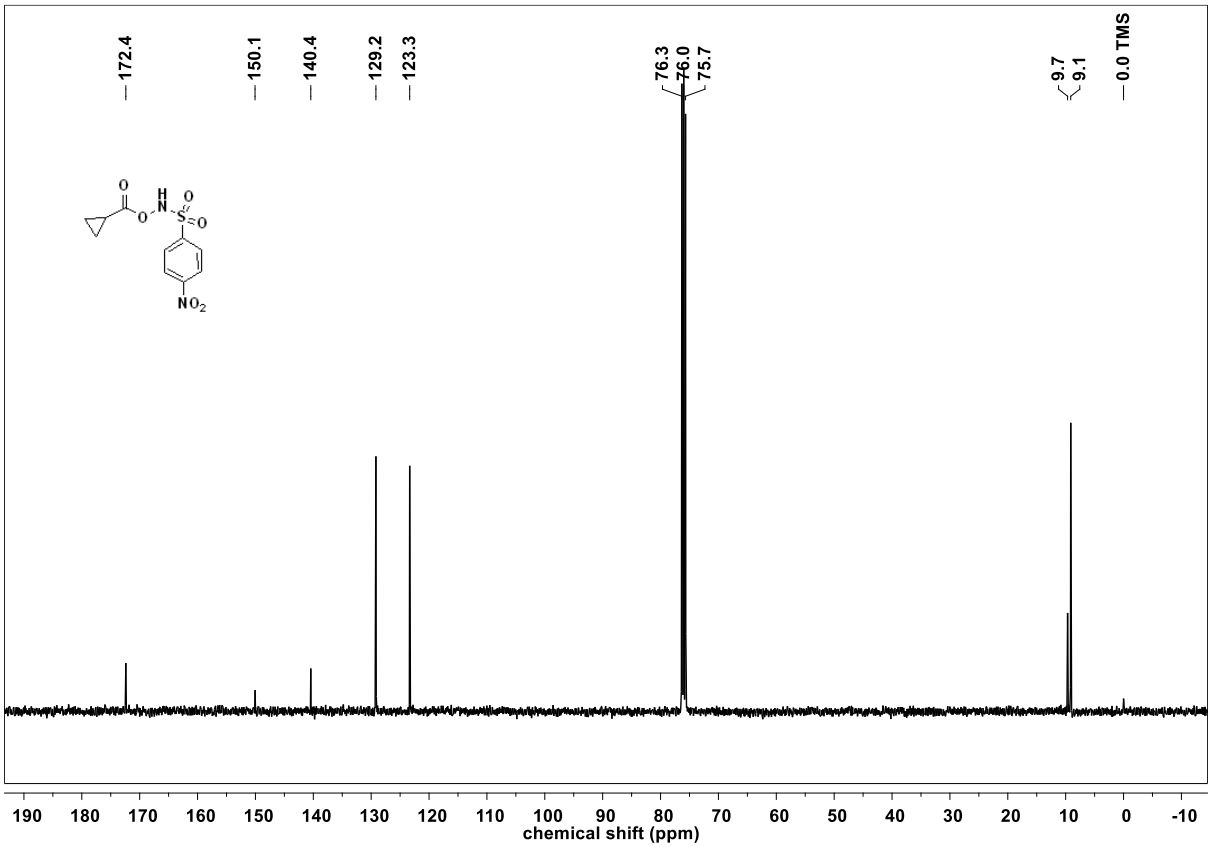
^{13}C NMR spectra of **1f:**



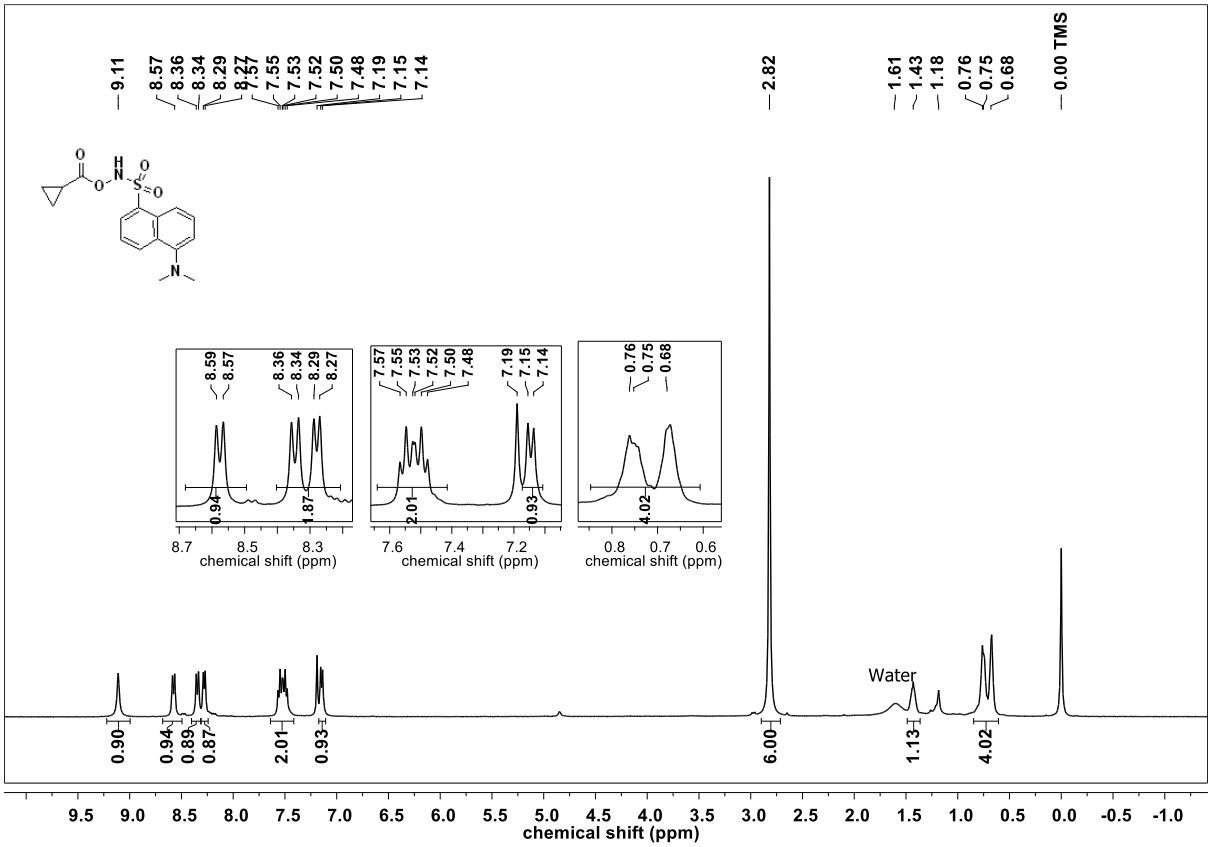
^{1}H NMR spectra of **1g:**



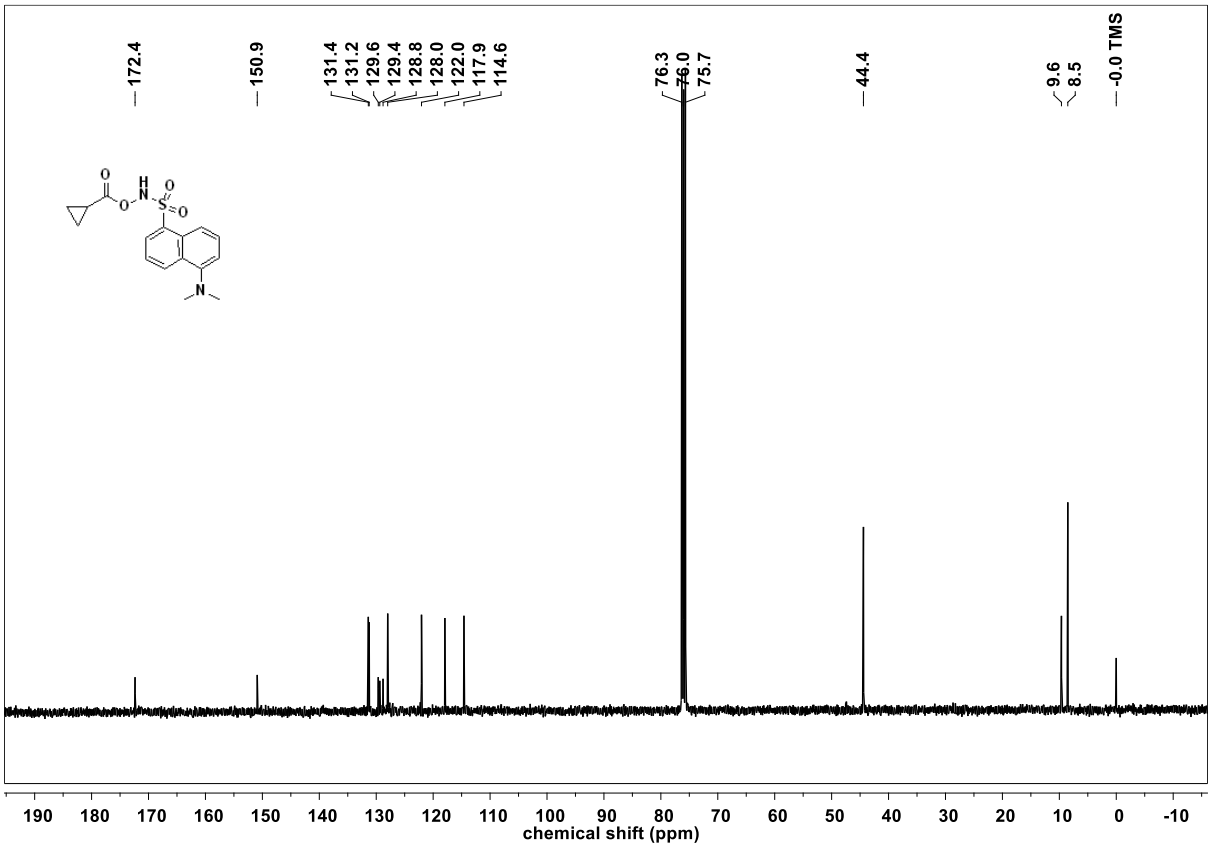
^{13}C NMR spectra of **1g:**



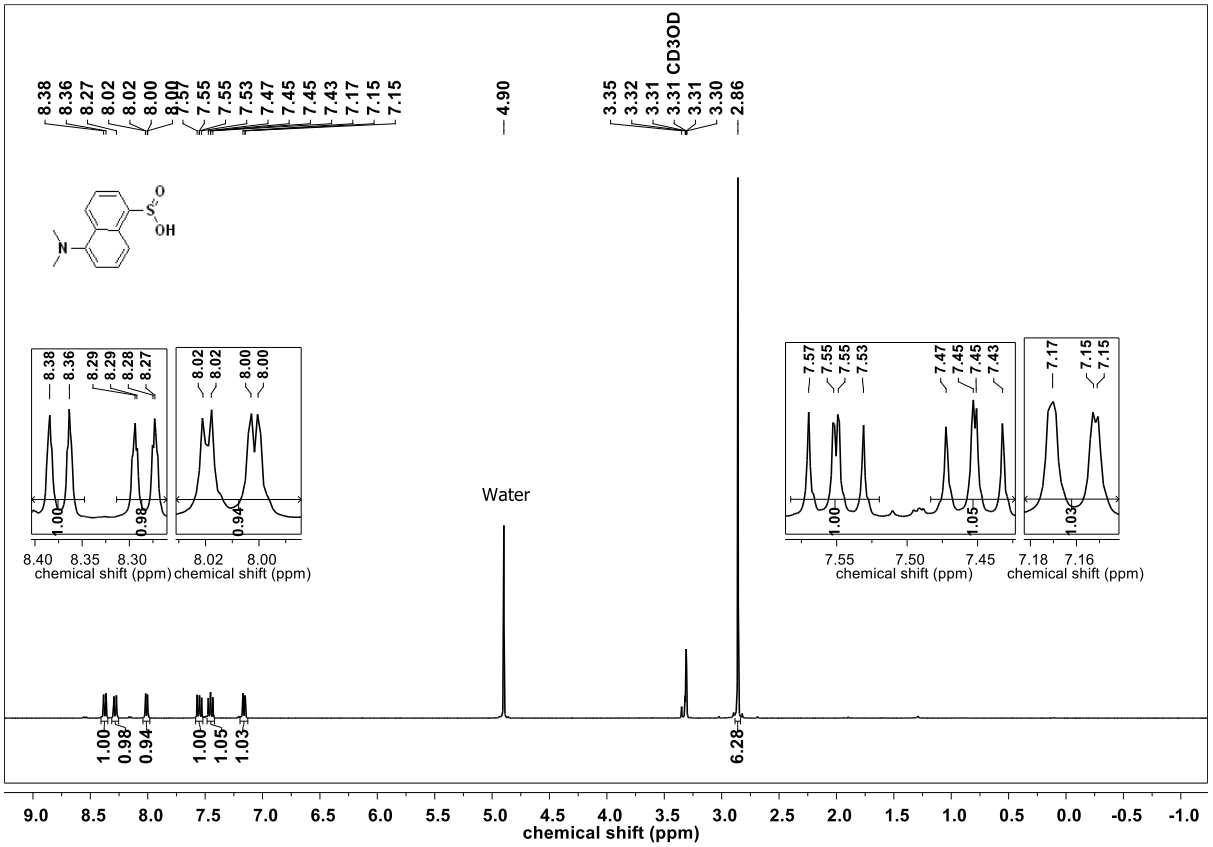
^{1}H NMR spectra of **1h:**



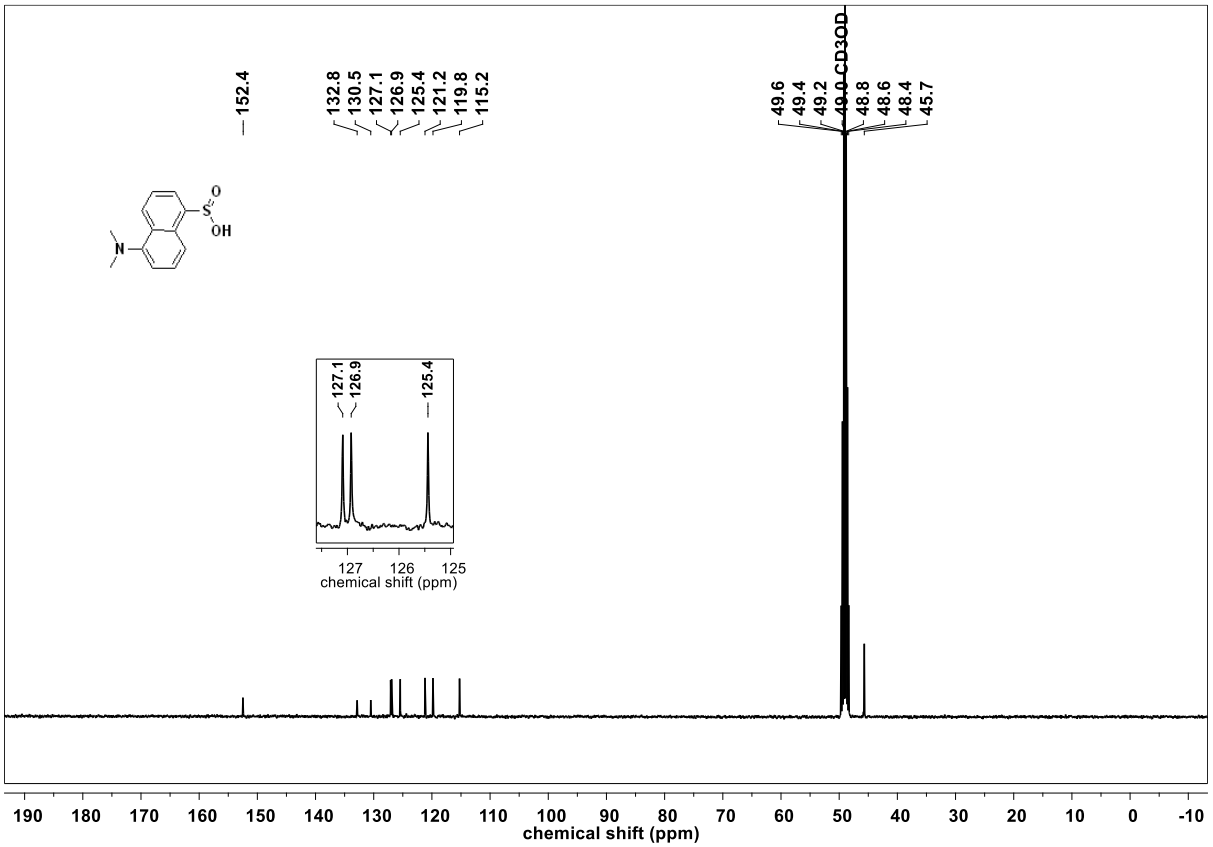
^{13}C NMR spectra of **1h:**



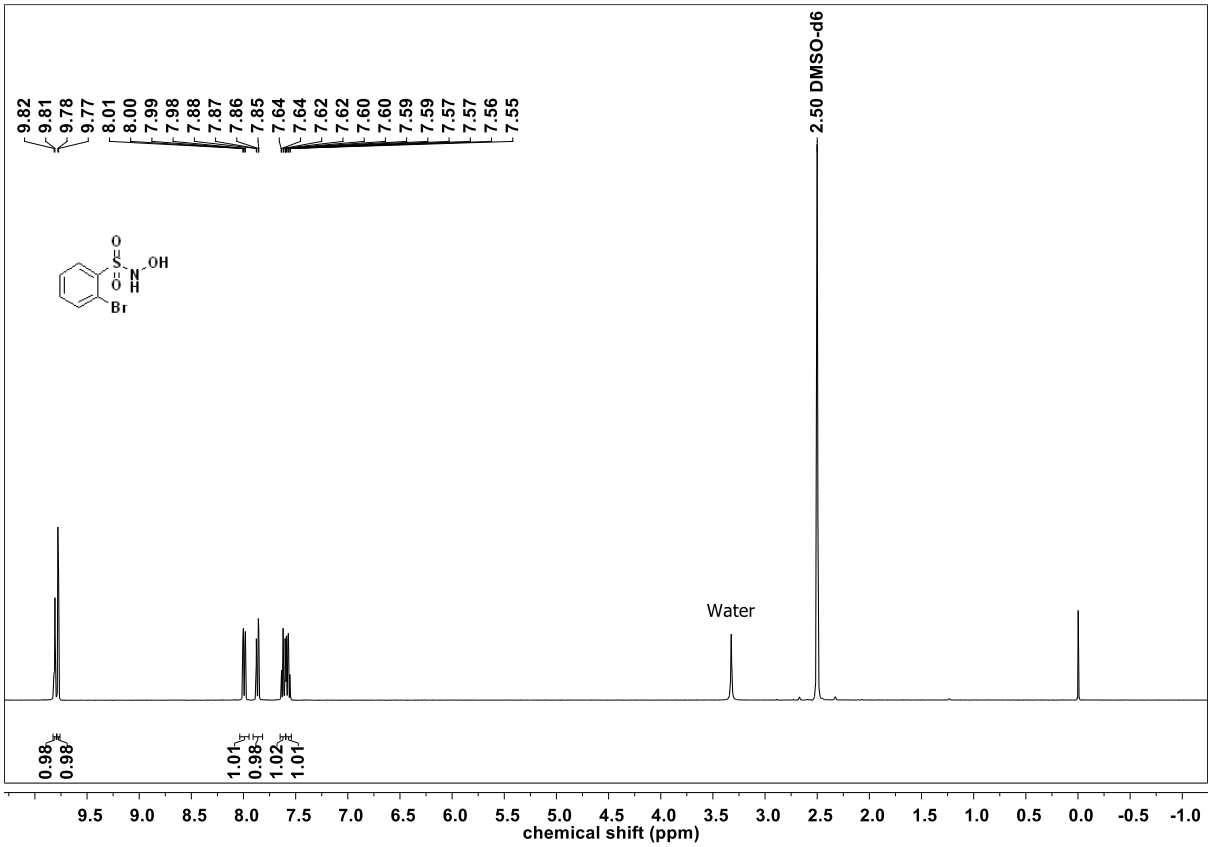
^{1}H NMR spectra of **4h:**



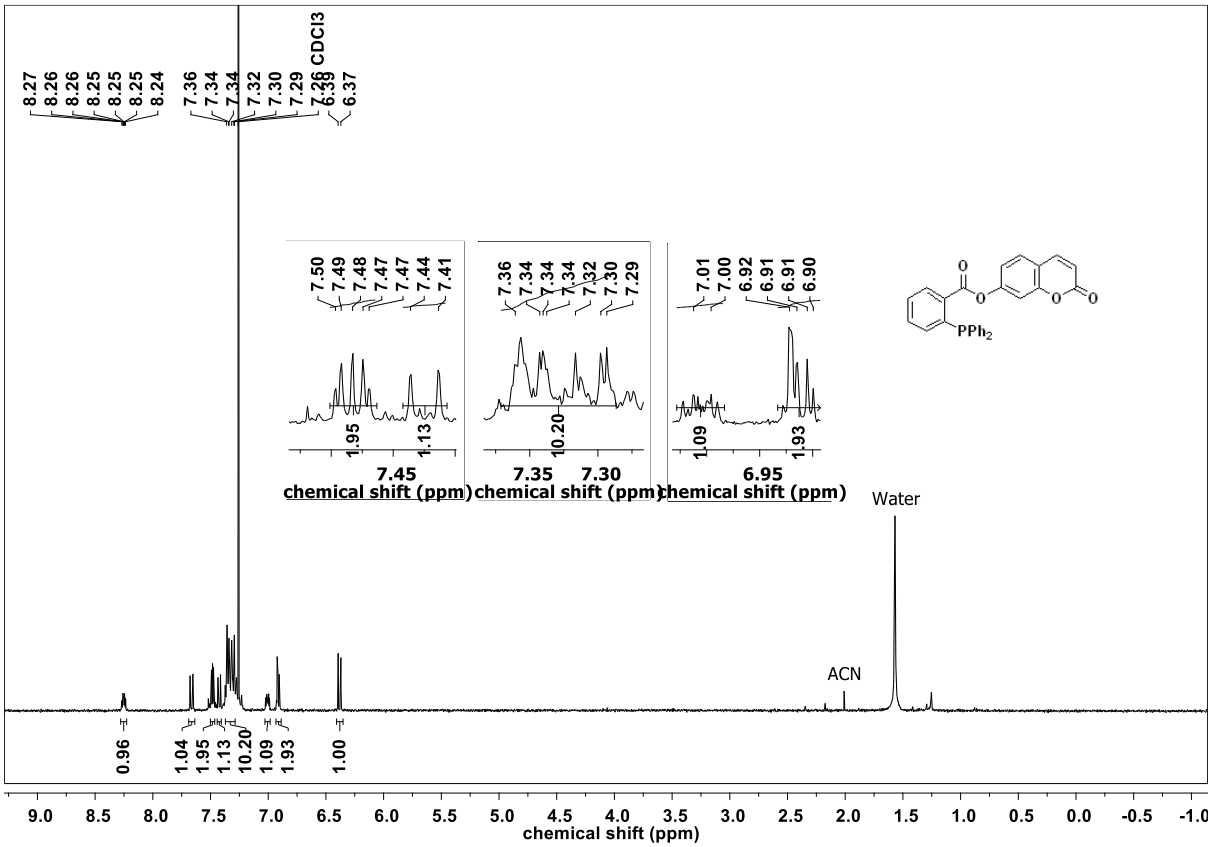
^{13}C NMR spectra of **4h:**



^{1}H NMR spectra for compound **3b:**



^{1}H NMR spectra for compound **6:**



^{1}H NMR spectra for compound **7:**