Supplementary Information

Non-natural 2*H*-azirine-2-carboxylic acids: an expedient synthesis and antimicrobial activity

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1 General information

Melting points were determined on a hot stage microscope and are uncorrected. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE 400 spectrometer in solvent indicated below. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane. Electrospray ionization (ESI) mass spectra were recorded on a Bruker MaXis mass spectrometer. IR spectra were recorded on a Shimadzu IR Affinity-1 spectrophotometer in tablets of KBr. Thermogravimetric analysis (TGA) was performed at 10 °C/min under argon using a NETZSCH TG 209 F1 Libra equipment. Differential scanning calorimetry (DSC) was performed using a Netzsch DSC 204 F1 Phoenix apparatus under nitrogen atmosphere in the temperature range from 30 to 220 °C, and at a heating rate of 20 °C/min. Single crystal X-ray data for **3a** were collected by means of a Xcalibur, Eos diffractometer at 100 K using monochromated MoKα radiation. Crystallographic data for the structure **3a** (CCDC 1956437) have been deposited with the Cambridge Crystallographic Data Centre. Thin-layer chromatography (TLC) was conducted on aluminum sheets precoated with SiO_2 ALUGRAM SIL G/UV254. Column chromatography was performed on Macherey-Nagel silica gel 60 M (0.04–0.063 mm). All solvents were distilled and dried prior to use. Acetonitrile was distilled from P_2O_5, then from anhydrous K_2CO_3 and stored over anhydrous K_2CO_3. Fresh commercially available $FeCl_2$ was used.

2 Synthesis of 5-Chloroisoxazoles

Chloroisoxazole **1e** was prepared by the reported procedure.[1]

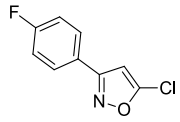
General procedure for the synthesis of 5-chloroisoxazoles 1a−d,f−t

Triethylamine (253 mg, 2.5 mmol, 0.35 mL) was added dropwise at 0 °C to a stirring suspension of isoxazol-5(4*H*)-one (3 mmol) in $POCl_3$ (4 mL). The mixture was stirred at 70 °C for several days (until starting material was consumed), poured into ice (300 g), and extracted with EtOAc. The organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the product was purified by column chromatography (petroleum ether–EtOAc, 10:1) to give chloroisoxazole **1**.

5-Chloro-3-phenylisoxazole (1a)[2]

Yield 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (s, 1H), 7.45–7.47 (m, 3H), 7.74–7.77 (m, 2H).

5-Chloro-3-(4-fluorophenyl)isoxazole (1b)



Light yellow solid (552 mg, yield 93%). Mp: 75–76 °C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (s, 1H), 7.16–7.20 (m, 2H), 7.75–7.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 99.5, 116.2 (d, J = 22.0 Hz), 124.4 (d, J = 3.4 Hz), 128.6 (d, J = 8.5 Hz), 155.3, 163.2, 164.1 (d, J = 251.1 Hz); HRMS−ESI: $[M + H]^+$ calcd for $C_9H_6{}^{35}ClFNO^+$, 198.0016; found, 198.0017.

5-Chloro-3-(4-chlorophenyl)isoxazole (1c)[2]

Yield 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (s, 1H), 7.45–7.48 (m, 2H), 7.70–7.73 (m, 2H).

3-(4-Bromophenyl)-5-chloroisoxazole (1d)[2]

Yield 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (s, 1H), 7.62–7.67 (m, 4H).

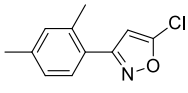
5-Chloro-3-(4-methylphenyl)isoxazole (1f)[2]

Yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 6.47 (s, 1H), 7.29 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H).

3-(4-*tert*-Butylphenyl)-5-chloro-isoxazole (1g)[3]

Yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 9H), 6.49 (s, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H).

5-Chloro-3-(2,4-dimethylphenyl)isoxazole (1h)



Light yellow solid (372 mg, yield 60%). Mp: 38–39 °C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H), 2.47 (s, 3H), 6.36 (s, 1H), 7.12 (d, J = 7.8 Hz, 1H), 7.15 (s, 1H), 7.39 (d, J = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.9, 21.2, 102.0, 125.0, 126.8, 129.2, 132.0, 136.7, 140.0, 154.2, 164.9.

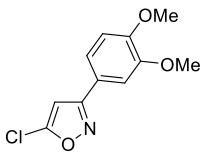
5-Chloro-3-(4-methoxyphenyl)isoxazole (1i)[2]

Yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.44 (s, 1H), 6.99 (d, J = 8.9 Hz, 2H), 7.71 (d, J = 8.9 Hz, 2H).

5-Chloro-3-(2,4-dimethoxyphenyl)isoxazole (1j)[3]

Yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H), 3.90 (s, 3H), 6.56 (d, J = 2.2 Hz, 1H), 6.60 (dd, J = 8.6 and 2.2 Hz, 1H), 6.67 (s, 1H), 7.83 (d, J = 8.6 Hz, 1H).

5-Chloro-3-(3,4-dimethoxyphenyl)isoxazole (1k)



Colorless solid (520 mg, yield 89%). Mp: 42−47 °C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (s, 1H), 6.93–6.95 (m, 1H), 7.24–7.26 (m, 1H), 7.37–7.38 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.9, 56.0, 99.4, 108.9, 111.1, 119.9, 120.8, 149.4, 151.1, 154.8, 163.9. HRMS−ESI: $[M + Na]^+$ calcd for $C_{11}H_{10}{}^{35}ClNNaO_3^+$, 262.0241; found, 262.0234.

5-Chloro-3-(furan-2-yl)isoxazole (1l)[3]

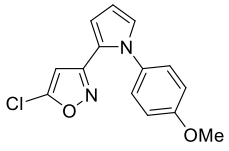
Yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.55 (dd, *J* = 3.4 and 1.8 Hz, 1H), 6.94 (dd, *J* = 3.5 and 0.4 Hz, 1H), 7.58 (dd, *J* = 1.7 and 0.6 Hz, 1H).

5-Chloro-3-(thiophen-2-yl)isoxazole (1m)[3]

Yield 40%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.44 (s, 1H), 7.14 (dd, *J* = 4.9 and 3.8 Hz, 1H), 7.46−7.48 (m, 2H).

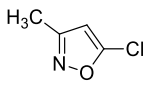
5-Chloro-3-[1-(4-methoxyphenyl)-1*H*-pyrrol-2-yl]isoxazole (1n)

Colorless solid (460 mg, yield 56%). Mp: 74−76 °C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89



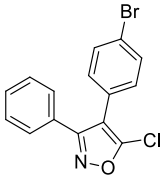
(s, 3H), 5.70 (s, 1H), 6.37–7.38 (m, 1H), 6.80–6.81 (m, 1H), 6.94–6.99 (m, 3H), 7.22–7.26 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.5, 100.3, 109.5, 112.9 114.2, 121.7, 127.1, 127.5, 132.6, 153.6, 157.7, 159.3; HRMS−ESI: $[M + Na]^+$ calcd for $C_{14}H_{11}{}^{35}ClN_2NaO_2^+$, 297.0401; found, 297.0402.

5-Chloro-3-methylisoxazole (1o)



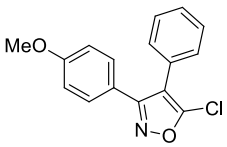
5-Chloro-3-methylisoxazole was purified by distillation at atmospheric pressure. Bp: 137–139 °C. Light yellow oil (246 mg, yield 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H), 6.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.7, 101.9, 154.3, 161.9; HRMS−ESI: $[M + H]^+$ calcd for $C_4H_5{}^{35}ClNO^+$, 118.0054; found, 118.0059.

4-(4-Bromophenyl)-5-chloro-3-phenylisoxazole (1p)



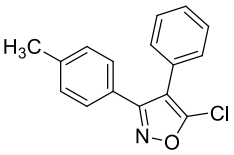
Light yellow solid (821 mg, yield 82%). Mp: 65–68°C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15–7.17 (m, 2H), 7.37–7.41 (m, 2H), 7.44–7.48 (m, 3H), 7.53–7.56 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 113.6, 122.8, 126.7, 127.9, 128.3, 128.8, 130.2, 131.1, 132.1, 152.3, 162.7; HRMS−ESI: $[M + H]^+$ calcd for $C_{15}H_{10}{}^{79}Br^{35}ClNO^+$, 333.9629; found, 333.9622.

5-Chloro-3-(4-methoxyphenyl)-4-phenylisoxazole (1q)



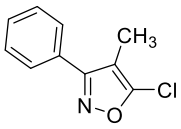
Light yellow solid (729 mg, yield 85%). Mp: 105–106°C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 3H), 6.87–6.89 (m, 2H), 7.28–7.31 (m, 2H), 7.39–7.43 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.3, 114.1, 114.4, 120.5, 128.0, 128.4, 128.7, 129.6, 129.7, 152.0, 161.0, 162.5; HRMS−ESI: $[M + H]^+$ calcd for $C_{16}H_{13}{}^{35}ClNO_2^+$, 286.0629; found, 286.0634.

5-Chloro-3-(4-methylphenyl)-4-phenylisoxazole (1r)



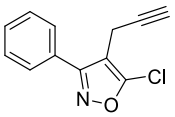
Light yellow solid (645 mg, yield 80%). Mp: 73–74°C (hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (s, 3H), 7.18 (d, J = 8.0 Hz, 2H), 7.28–7.31 (m, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.41–7.43 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.4, 114.5, 125.3, 127.9, 128.2, 128.4, 128.7, 129.3, 129.6, 140.2, 152.0, 162.8; HRMS−ESI: $[M + H]^+$ calcd for $C_{16}H_{13}{}^{35}ClNO^+$, 270.0680; found, 270.0685.

5-Chloro-4-methyl-3-phenylisoxazole (1s)



Light yellow oil (524 mg, yield 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.15 (s, 3H), 7.50–7.52 (m, 3H), 7.66–7.69 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.0, 108.4, 127.8, 128.8, 128.9, 129.9, 151.9, 163.8; HRMS−ESI: $[M + H]^+$ calcd for $C_{10}H_9{}^{35}ClNO^+$, 194.0367; found, 194.0365.

5-Chloro-3-phenyl-4-(prop-2-yn-1-yl)isoxazole (1t)



Light yellow oil (488 mg, yield 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12 (t, J = 2.7 Hz, 1H), 3.43 (d, J = 2.7 Hz, 2H), 7.52–7.54 (m, 3H), 7.74–7.78 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.9, 70.2, 79.0, 108.5, 128.1 (2C), 129.0, 130.3, 153.0, 163.6; HRMS−ESI: $[M + H]^+$ calcd for $C_{12}H_9{}^{35}ClNO^+$, 218.0367; found, 218.0372.

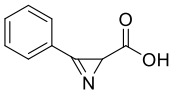
3 Synthesis of 2*H*-Azirine-2-carboxylic acid

General Procedure for the synthesis of 2*H*-azirine-2-carboxylic acid

Anhydrous $FeCl_2$ (50 mg, 0.2 equiv) was added to a solution of 5-chloroisoxazole **1** (2 mmol) in dry acetonitrile (25 mL) under Ar atmosphere. The mixture was stirred at room temperature for 1.5 h until 5-chloroisoxazole **1** was consumed (monitoring by TLC). Water (25 mL) was added and the mixture was stirred at room temperature for 15 min. The 2*H*-azirine-2-carboxylic acid was extracted with EtOAc (325 mL), washed with water (25 mL), brine (10 mL) and dried over

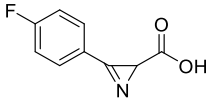
Na_2SO_4. The solvent was evaporated under reduced pressure and unless otherwise stated the residue was washed with hexane–Et_2O mixture (10 : 1) to give 2*H*-azirine-2-carboxylic acid **3**.

3-Phenyl-2*H*-azirine-2-carboxylic acid (3a)[4]



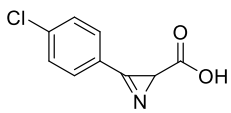
Light yellow solid (315 mg, yield 98%). Mp: 105–107 °C (Et_2O–hexane, dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (s, 1H), 7.58–7.62 (m, 2H), 7.65–7.69 (m, 1H), 7.90–7.92 (m, 2H), 11.06 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.1, 121.7, 129.4, 130.6, 134.1, 157.7, 178.1; IR ν_{max} (KBr) cm^{-1}: 1781, 1721.

3-(4-Fluorophenyl)-2*H*-azirine-2-carboxylic acid (3b)



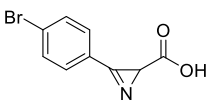
Light yellow solid (340 mg, yield 95%). Mp: 115–117 °C (Et_2O–hexane, dec.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (s, 1H), 7.31 (t, *J* = 8.5 Hz, 2H), 7.92–7.96 (m, 2H), 11.15 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.2, 117.0 (d, *J* = 22.6 Hz), 118.1 (d, *J* = 3.1 Hz), 133.1 (d, *J* = 9.5 Hz), 156.7, 166.2 (d, *J* = 257.7 Hz), 177.9; HRMS-ESI $[M + H]^+$ calcd for $C_9H_7FNO_2^+$, 180.0455; found, 180.0448.

3-(4-Chlorophenyl)-2*H*-azirine-2-carboxylic acid (3c)



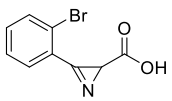
Light yellow solid (382 mg, yield 98%). Mp: 116–117 °C (Et_2O–hexane, dec.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.82 (s, 1H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 2H), 12.82 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.6, 121.5, 130.4, 132.3, 139.4, 158.6, 173.1; IR ν_{max} (KBr) cm^{-1}: 1770, 1687; HRMS-ESI $[M + Na]^+$ calcd for $C_9H_6{}^{35}ClNNaO_2^+$, 217.9979; found, 217.9980.

3-(4-Bromophenyl)-2*H*-azirine-2-carboxylic acid (3d)



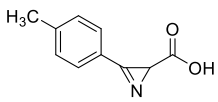
Light yellow solid (468 mg, yield 98%). Mp: 137–138 °C (Et_2O–hexane, dec.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.82 (s, 1H), 7.87 (s, 4H), 12.84 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.6, 121.8, 128.5, 132.3, 133.4, 158.8, 173.1; HRMS-ESI $[M - H]^-$ calcd for $C_9H_5{}^{79}BrNO_2^-$, 237.9509; found, 237.9505.

3-(2-Bromophenyl)-2*H*-azirine-2-carboxylic acid (3e)



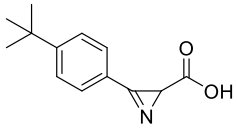
Colorless solid (454 mg, yield 95%). Mp: 122–123 °C (Et_2O–hexane, dec.). ^{1}H NMR (400 MHz, mixture of $CDCl_3$ and DMSO-d_6) δ 2.66 (s, 1H), 7.35–7.36 (m, 2H), 7.57–7.67 (m, 2H), 10.03 (br.s, 1H); ^{13}C NMR (100 MHz, mixture of $CDCl_3$ and DMSO-d_6) δ 29.5, 121.7, 124.2, 127.1, 132.3, 133.0, 133.7, 158.3, 172.2; HRMS-ESI $[M - H]^-$ calcd for $C_9H_5{}^{79}BrNO_2^-$, 237.9509; found, 237.9496.

3-(4-Methylphenyl)-2*H*-azirine-2-carboxylic acid (3f)



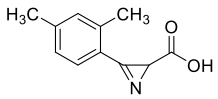
Light yellow solid (343 mg, yield 98%). Mp: 109–110 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 2.46 (s, 3H), 2.82 (s, 1H), 7.39 (d, *J* = 7.8 Hz, 2H), 7.79 (d, *J* = 7.8 Hz, 2H), 11.86 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.8, 28.9, 118.8, 130.0, 130.5, 145.2, 157.1, 178.2; HRMS-ESI $[M + Na]^+$ calcd for $C_{10}H_9NNaO_2^+$, 198.0525; found, 198.0528.

3-(4-(*tert*-Butyl)phenyl)-2*H*-azirine-2-carboxylic acid (3g)



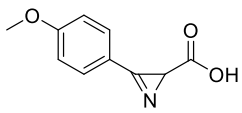
Light yellow solid (386 mg, yield 89%). Mp: 121–123 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9H), 7.51–7.52 (m, 2H), 7.27–7.28 (m, 2H), 10.29 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.0, 35.2, 49.6, 119.5, 126.2, 130.4, 157.6, 158.5, 177.8; HRMS-ESI $[M - H]^-$ calcd for $C_{13}H_{14}NO_2^-$, 216.1030; found, 216.1035.

3-(2,4-Dimethylphenyl)-2*H*-azirine-2-carboxylic acid (3h)



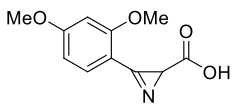
Light yellow solid (370 mg, yield 98%). Mp: 117–119 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, DMSO-d_6) δ 2.37 (s, 3H), 2.57 (s, 3H), 2.66 (s, 1H), 7.25–7.29 (m, 2H), 7.55 (d, *J* = 7.6 Hz, 1H), 10.96 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.6, 21.7, 27.8, 118.7, 127.9, 132.2, 132.5, 141.0, 144.5, 157.7, 173.9; HRMS-ESI $[M + Na]^+$ calcd for $C_{11}H_{11}NNaO_2^+$, 212.0682; found, 212.0685.

3-(4-Methoxyphenyl)-2*H*-azirine-2-carboxylic acid (3i)



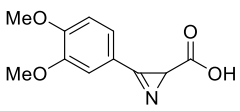
Light yellow solid (351 mg, yield 92%). Mp: 92–93 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 2.78 (s, 1H), 3.89 (s, 3H), 7.06 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.8 Hz, 2H), 8.22 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.9, 55.5, 113.9, 114.9, 132.6, 156.3, 164.1, 177.7; IR ν_{max} (KBr) cm^{-1}: 1763, 1696; HRMS-ESI $[M + Na]^+$ calcd for $C_{10}H_9NNaO_3^+$, 214.0475; found, 214.0485.

3-(2,4-Dimethoxyphenyl)-2*H*-azirine-2-carboxylic acid (3j)



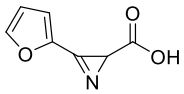
Light yellow oil (398 mg, yield 90%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.65 (s, 1H), 3.89 (s, 3H), 3.95 (s, 3H), 6.53 (s, 1H), 6.62 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 10.54 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 55.7, 55.9, 98.5, 103.6, 105.7, 134.6, 152.9, 161.9, 166.1, 178.6; HRMS-ESI $[M - H]^-$ calcd for $C_{11}H_{10}NO_4^-$, 220.0615; found, 220.0620.

3-(3,4-Dimethoxyphenyl)-2*H*-azirine-2-carboxylic acid (3k)



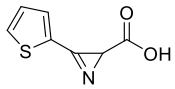
Beige solid (384 mg, yield 87%). Mp: 142−144 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, DMSO-d_6): δ 2.72 (s, 1H), 3.88 (s, 3H), 3.89 (s, 3H), 7.21–7.24 (m, 1H), 7.44–7.46 (m, 2H), 12.64 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 29.3, 56.3, 56.4, 112.0, 112.5, 114.6, 125.5, 149.9, 154.0, 157.8, 173.6; HRMS−ESI: $[M + Na]^+$ calcd for $C_{11}H_{11}NNaO_4^+$, 244.0580; found, 244.0586.

3-(Furan-2-yl)-2*H*-azirine-2-carboxylic acid (3l)



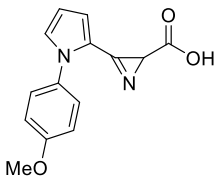
Light yellow solid (242 mg, yield 80%). Mp: 98–99 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 2.86 (s, 1H), 6.66–6.77 (m, 1H), 7.31–7.32 (m, 1H), 7.82–7.90 (m, 1H), 9.62 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.9, 113.1, 122.1, 138.9, 147.6, 149.4, 177.2; HRMS-ESI $[M + Na]^+$ calcd for $C_7H_5NNaO_3^+$, 174.0162; found, 174.0170.

3-(Thiophen-2-yl)-2*H*-azirine-2-carboxylic acid (3m)



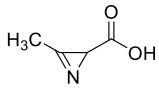
Light yellow solid (274 mg, yield 82%). Mp: 112–113 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 2.89 (s, 1H), 7.29–7.31 (m, 1H), 7.75 (d, J = 3.3 Hz, 1H), 7.91 (d, J = 4.8 Hz, 1H), 10.88 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.9, 124.0, 128.6, 135.8, 135.9, 151.0, 177.5; IR ν_{max}(KBr) cm^{-1}: 1777, 1713; HRMS-ESI $[M + Na]^+$ calcd for $C_7H_5NNaO_2S^+$, 189.9933; found, 189.9936.

3-[1-(4-Methoxyphenyl)-1*H*-pyrrol-2-yl]-2*H*-azirine-2-carboxylic acid (3n)



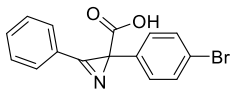
Eluent for chromatography: from hexane−EtOAc 3:1, through hexane−EtOAc−MeOH 3:1:0.15, to MeOH. Beige solid (359 mg, yield 70%). Mp: 130−132 °C (hexane−EtOAc−MeOH, dec.). ^1H NMR (400 MHz, DMSO-d_6): δ 2.45 (s, 1H), 3.83 (s, 3H), 6.54–6.56 (m, 1H), 7.05–7.09 (m, 2H), 7.11–7.13 (m, 1H), 7.41–7.45 (m, 2H), 7.60–7.61 (m, 1H), 12.51 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 27.6, 56.0, 112.1, 114.9, 116.6, 121.9, 127.0, 131.4, 132.4, 148.1, 159.4, 173.5; HRMS−ESI: $[M + Na]^+$ calcd for $C_{14}H_{12}N_2NaO_3^+$, 279.0740; found, 279.0753.

3-Methyl-2*H*-azirine-2-carboxylic acid (3o)[5]



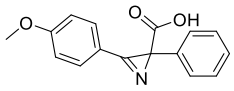
Light yellow oil (182 mg, yield 92%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.10 (s, 1H), 2.27 (s, 3H), 11.32 (br.s, 1H); ^{13}C NMR (100 MHz, mixture of $CDCl_3$ and DMSO-d_6) δ 11.8, 27.9, 158.8, 173.4.

2-(4-Bromophenyl)-3-phenyl-2*H*-azirine-2-carboxylic acid (3p)



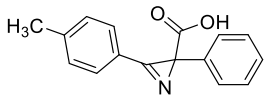
Light yellow solid (472 mg, yield 75%). Mp: 107–108 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.6 Hz, 2H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.59–7.63 (m, 2H), 7.67-7.70 (m, 1H), 7.92–7.94 (m, 2H), 11.15 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 121.1, 122.1, 129.6, 129.9, 130.6, 131.4, 134.3, 134.4, 159.7, 177.1; HRMS-ESI $[M - H]^-$ calcd for $C_{15}H_9{}^{79}BrNO_2^-$, 313.9822; found, 313.9812.

3-(4-Methoxyphenyl)-2-phenyl-2*H*-azirine-2-carboxylic acid (3q)



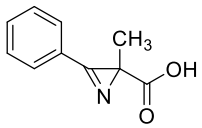
Light yellow solid (320 mg, yield 60%). Mp: 112–113 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, 3H), 7.06 (d, *J* = 8.0 Hz, 2H), 7.30–7.32 (m, 2H), 7.50–7.52 (m, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 10.70 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.5, 55.6, 113.7, 115.0, 127.7, 128.1, 128.3, 132.6, 136.0, 158.7, 164.1, 177.4; HRMS-ESI $[M - H]^-$ calcd for $C_{16}H_{12}NO_3^-$, 266.0823; found, 266.0819.

3-(4-Methylphenyl)-2-phenyl-2*H*-azirine-2-carboxylic acid (3r)



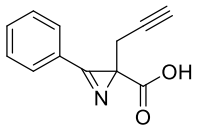
Light yellow oil (351 mg, yield 70%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 7.14 (br.m, 5H), 7.39 (br.m, 2H), 7.64 (br.m, 2H), 11.53 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.8, 41.7, 119.3, 126.9, 127.8, 128.4, 129.8, 130.6, 137.2, 144.2, 160.3, 176.7; HRMS-ESI $[M - H]^-$ calcd for $C_{16}H_{12}NO_2^-$, 250.0874; found, 250.0867.

2-Methyl-3-phenyl-2*H*-azirine-2-carboxylic acid (3s)



Light yellow solid (315 mg, yield 90%). Mp: 135–136 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, $CDCl_3$) δ 1.64 (s, 3H), 7.57–7.61 (m, 2H), 7.64–7.66 (m, 1H), 7.86–7.87 (m, 2H), 11.49 (br.s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1, 35.1, 122.0, 129.4, 130.2, 133.8, 162.7, 179.4; IR ν_{max} (KBr) cm^{-1}: 1769, 1723; HRMS-ESI $[M + Na]^+$ calcd for $C_{10}H_9NNaO_2^+$, 198.0525; found, 198.0527.

3-Phenyl-2-(prop-2-yn-1-yl)-2*H*-azirine-2-carboxylic acid (3t)

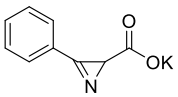


Light yellow solid (239 mg, yield 60%). Mp: 112–113 °C (Et_2O–hexane, dec.). ^1H NMR (400 MHz, DMSO-d_6) δ 2.78 (t, *J* = 2.5 Hz, 1H), 2.88 (dd, *J* = 18.1 and 2.6 Hz, 1H), 3.21 (dd, *J* = 18.1 and 2.6 Hz, 1H), 7.66–7.76 (m, 3H), 7.95 (d, *J* = 7.0 Hz, 2H), 12.92 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.4, 37.9, 74.4, 80.8, 122.8, 130.1, 130.5, 134.5, 162.7, 173.3; HRMS-ESI $[M - H]^-$ calcd for $C_{12}H_8NO_2^-$, 198.0561; found, 198.0558.

4 Synthesis of salt 4

Potassium 3-phenyl-2*H*-azirine-2-carboxylate (4)

t-BuOK (1 mmol) was added portionwise at 0 °C to a stirred solution of 3-phenyl-2*H*-azirine-2-carboxylic acid **3a** (1.1 mmol) in MeOH (10 mL). The resulting reaction mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure, and the solid residue was washed with diethyl ether and dried under vacuum to give the potassium salt **4**.



Light yellow solid (189 mg, yield 95%). Mp: 210–212 °C (Et_2O–MeOH). ^{1}H NMR (400 MHz, D_2O) δ 2.74 (s, 1H), 7.58–7.62 (m, 2H), 7.66–7.70 (m, 1H), 7.87 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, D_2O) δ 31.6, 122.4, 129.5, 130.3, 134.3, 161.5, 179.6.

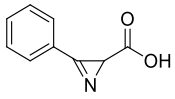
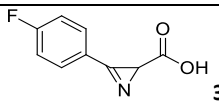
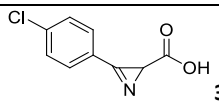
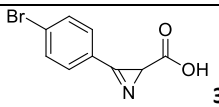
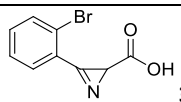
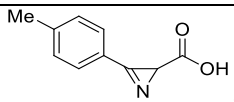
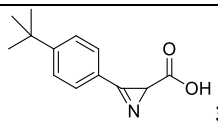
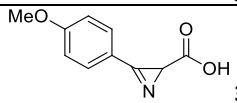
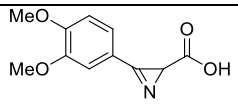
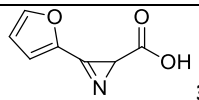
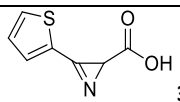
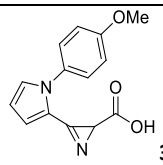
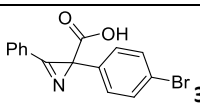
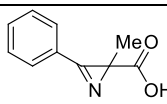
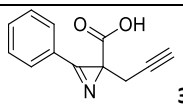
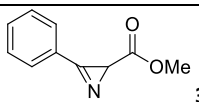
5 Determination of antibacterial activity *in vitro*

ESKAPE bacterial strains used for experiments: *Enterococcus faecium* (ATCC 19434, NCTC 7171), *Staphylococcus aureus* (ATCC 29213, NCTC 12973), *Klebsiella pneumonia* (ATCC 13883, NCTC 9633), *Acinetobacter baumannii* (ATCC 19606, NCTC 12156), *Pseudomonas aeruginosa* (ATCC 27853, NCTC 12903), *Enterobacter aerogenes* (NCTC 9735). Full information about these strains is presented on the websites: https://www.lgcstandards-atcc.org/, https://www.phe-culturecollections.org.uk/ https://www.ncbi.nlm.nih.gov/gene

Disk diffusion method

Testing of susceptibility of microorganisms to compounds **3a**–**g,i,k**–**n,p,s,t,3a'** as well as Sulfamethoxazole (positive control) was performed using the conventional Kirby-Bauer disk diffusion test[6] under the Standard Operating Procedure of The European Committee on Antimicrobial Susceptibility Testing (EUCAST).[7] Disks containing 5 μg of Sulfamethoxazole were used. The tested compound (1 mg) was dissolved in dimethyl sulfoxide (10 μL) and diluted to a volume of 1 mL with deionized water. To a Petri dish containing Muller-Hilton agar inoculated with a bacterial suspension (McFarland OD = 0.5) 5 μL of this solution were added. After drying of the compound solution, the Petri dish was incubated at 37 °C for 24 h. The susceptibility to a drug was assessed by measuring the bacterial growth inhibition zone diameter around the disc with Sulfamethoxazole or the tested compound dried solution circular spot.

Table S-1. Growth inhibition zones (mm) for compounds 3a,b,d–g,i,k–n,p.

Acid	*E. faecium*	*P. aeruginosa*	*A. baumanii*	*K. pneumoniae*	*S. aureus*	*E. cloacae*
 **3a**	17	18	14	18	19	8
 **3b**	11	0	0	8	13	0
 **3c**	0	0	0	7	7	0
 **3d**	0	0	0	10	15	0
 **3e**	5	0	0	10	15	0
 **3f**	0	5	0	7	7	0
 **3g**	5	0	6	7	9	6
 **3i**	7	5	0	7	5	0
 **3k**	0	0	6	7	5	9
 **3l**	0	0	12	5	10	5
 **3m**	21	0	12	18	18	16
 **3n**	6	0	5	3	6	8
 **3p**	9	0	4	6	5	7
 **3s**	0	0	0	7	0	0
 **3t**	8	0	0	0	5	0
 **3a'**	0	0	0	7	0	0

Determination of the minimum inhibitory concentration (MIC)

The test was performed under the Standard Operating Procedure of The European Committee on Antimicrobial Susceptibility Testing (EUCAST)[8] at a final volume of 0.2 mL in a 96-well sterile immunology plate with sterile lids. The nutrient medium for this method is the Muller – Hinton medium. A standard microbial suspension equivalent to 0.5 according to the Mcfarland standard was used for inoculation, diluted 100 times on a nutrient broth, after which the concentration of the microorganism in it was approximately 10^6 CFU/ml.

The working solution of the antibiotic compound was prepared from the basic solution using a liquid nutrient medium. The first concentration was the maximum. In all wells of the plate 100 μL of nutrient medium were placed. Then 100 μL of the maximum concentration solution of the compound were placed in the first well of the horizontal row of the plate. The contents of the well were mixed and 100 μL from the first well of the first horizontal row was transferred to the second well of the first horizontal row. So it was continued to the well number 10, from which 100 μL of the contents were removed after mixing. Thus, a number of wells with a solution of antibacterial compound was obtained, the concentrations of which differed in neighboring tubes by 2 times. Then, in the first 10 wells 100 μL of the prepared suspension of bacteria was placed. Wells 11 and 12 were controls. Well 11 was a control of bacteria, it contained 100 μL of the nutrient medium and 100 μL of the bacteria suspension, which was used in the first horizontal row. Well 12 was a control of the broth, it contained 200 μL of the nutrient medium.

Each horizontal row of the plate corresponded to a separate antibacterial compound or a separate microorganism. One or two rows of the plate were used to establish controls of the respective antibiotics selected as reference for the compound under study with each microorganism.

The plates were incubated at +37 °C in the thermostat during 18–24 h. The results were taken into account visually, comparing the growth of microorganisms in the presence of an antibacterial compound with the growth of culture in the cell without it. The minimum concentration providing complete suppression of the visible growth of the studied strain was used for MIC value.

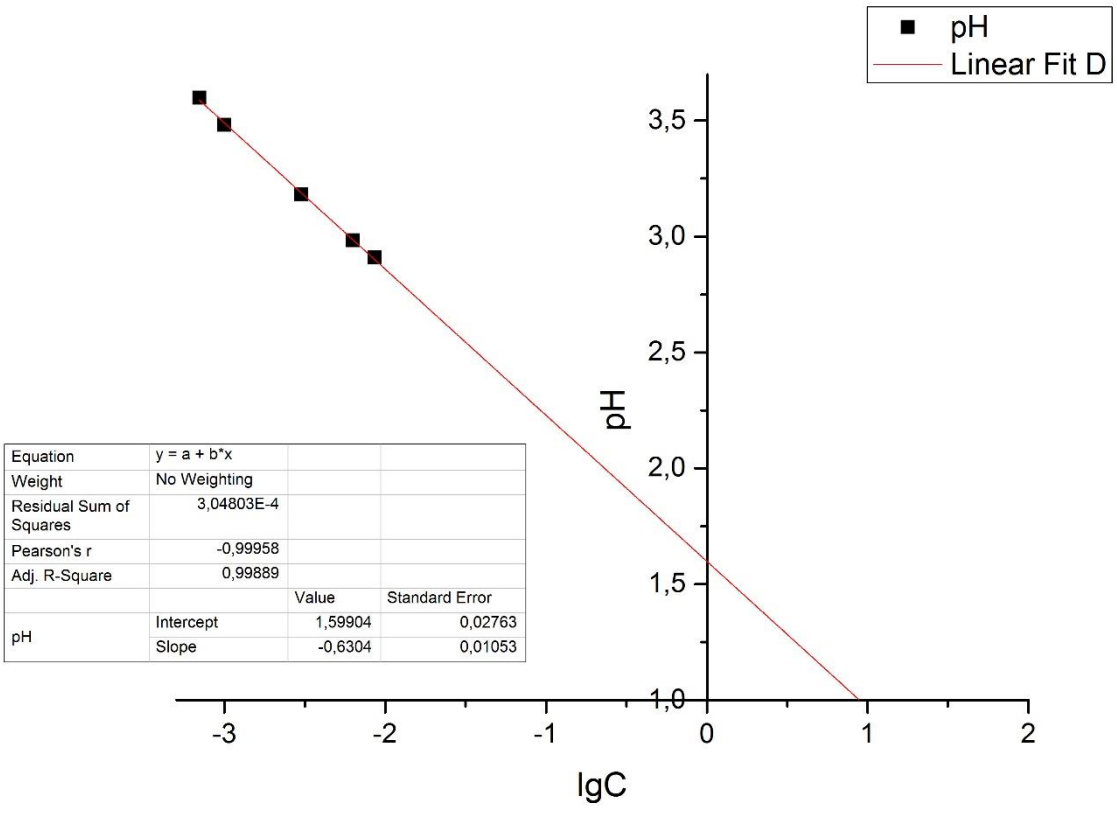
6 Cell viability assay

HEK293 (human epithelial kidney cells) were obtained from Russian Cell Culture Collection (Institute of Cytology RAS). ARPE-19 (ATCC® CRL-2302™ retinal pigment epithelial) cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA). HEK293 and ARPE-19 cells were cultured in DMEM-F12 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, at +37 °C in a humidified atmosphere containing 95% air and 5% CO_2. The cytotoxicity of tested compounds was studied using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay as previously has been described.[9] MTT-formazan crystals formed by metabolically active cells were dissolved in dimethyl sulfoxide and absorbance was measured at 540 nm and 690 nm in Varioskan™ LUX Multimode Microplate Reader (Thermo Scientific, USA). Values measured at 540 nm were subtracted for background values at 690 nm, and the data were shown as a percent of control untreated samples.

7 Determination of pK_a of acid 3a

The determination of pK_a of acid **3a** was performed by the potentiometric method.[10] Glass pH-metric electrode (ES-10603, Russia) was chosen as working electrode, the silver chloride electrode was chosen as the reference electrode. During the potential measurement the cell was thermostated externally at 25 ± 0.1 ºC. In the first step, glass electrode was calibrated in 5 buffer solutions and the E-pH curve was plotted. For determination of pK_a value of acid **3a**, 5 solutions of acid **3a** (0.0086, 0.0063, 0.00300, 0.0010, 0.0007 mol/L) in aqueous 0.01 M KCl were prepared. Then the potentials of these solutions were measured and the potential values were used to calculate the pH of the solutions using the calibration curve. A plot of pH versus lg*C* was plotted. Using ehe Henderson–Hasselbalch equation with some assumptions (pH = 1/2*pKa* – 1/2lg*C*) acidity constant can be found from the lg*C* – pH plot. The segment (1.599) cut off by a straight line on the ordinate axis corresponds to 1/2*pKa*.

pKa (**3a**) = 3.198 ± 0.055



Equation	y = a + b*x		
Weight	No Weighting		
Residual Sum of Squares	3,04803E-4		
Pearson's r	-0,99958		
Adj. R-Square	0,99889		
		Value	Standard Error
pH	Intercept	1,59904	0,02763
	Slope	-0,6304	0,01053

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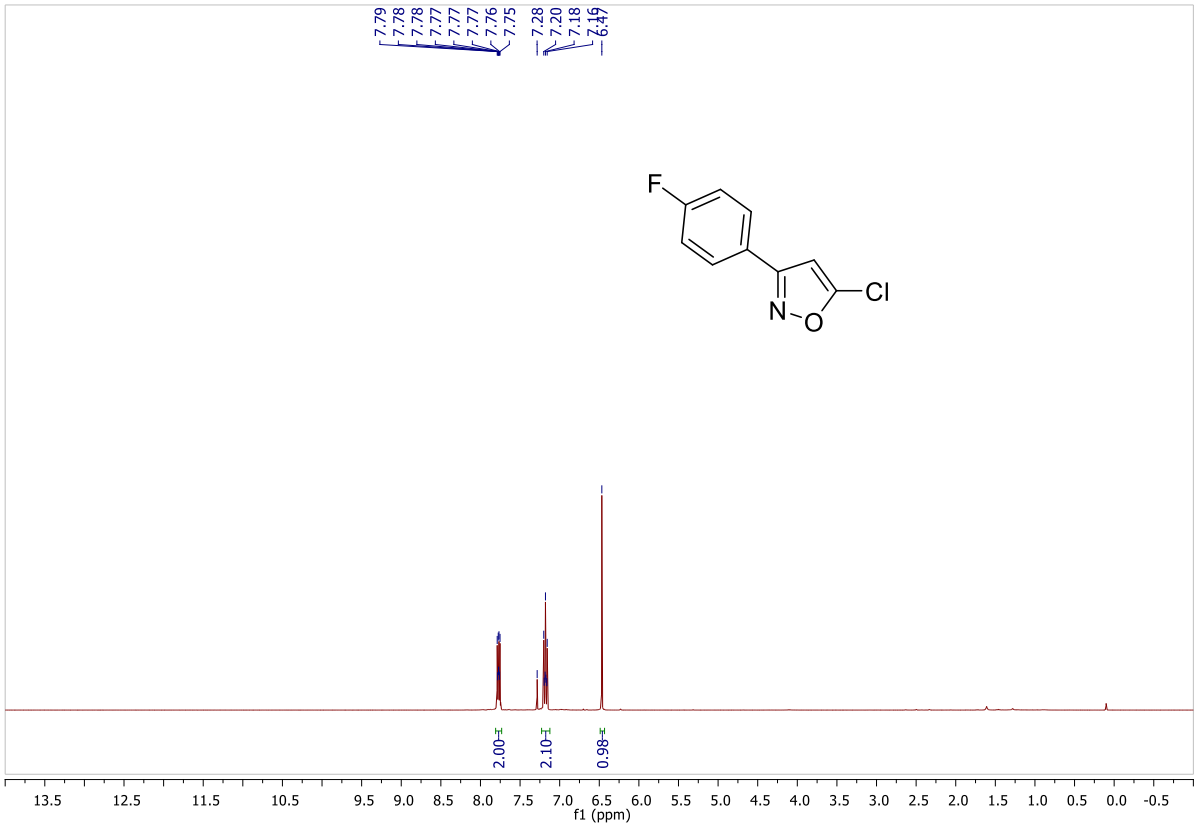
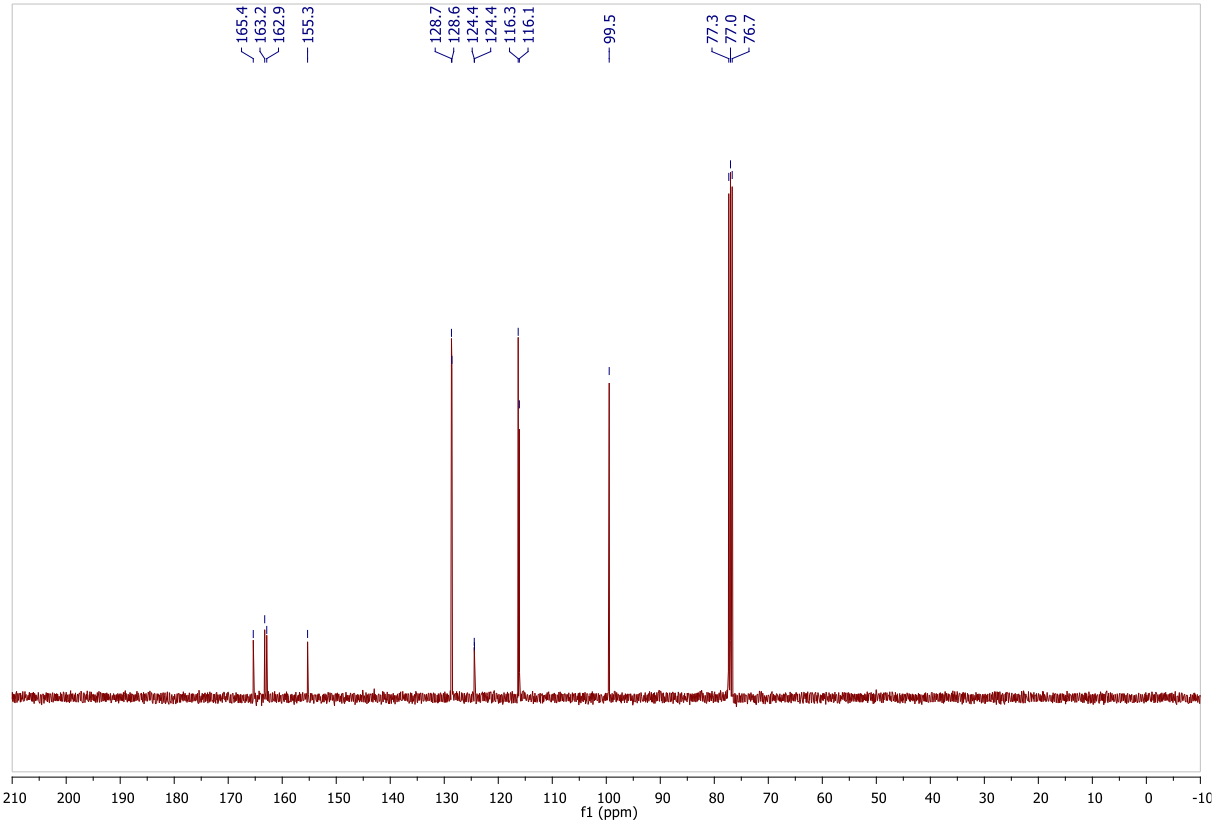
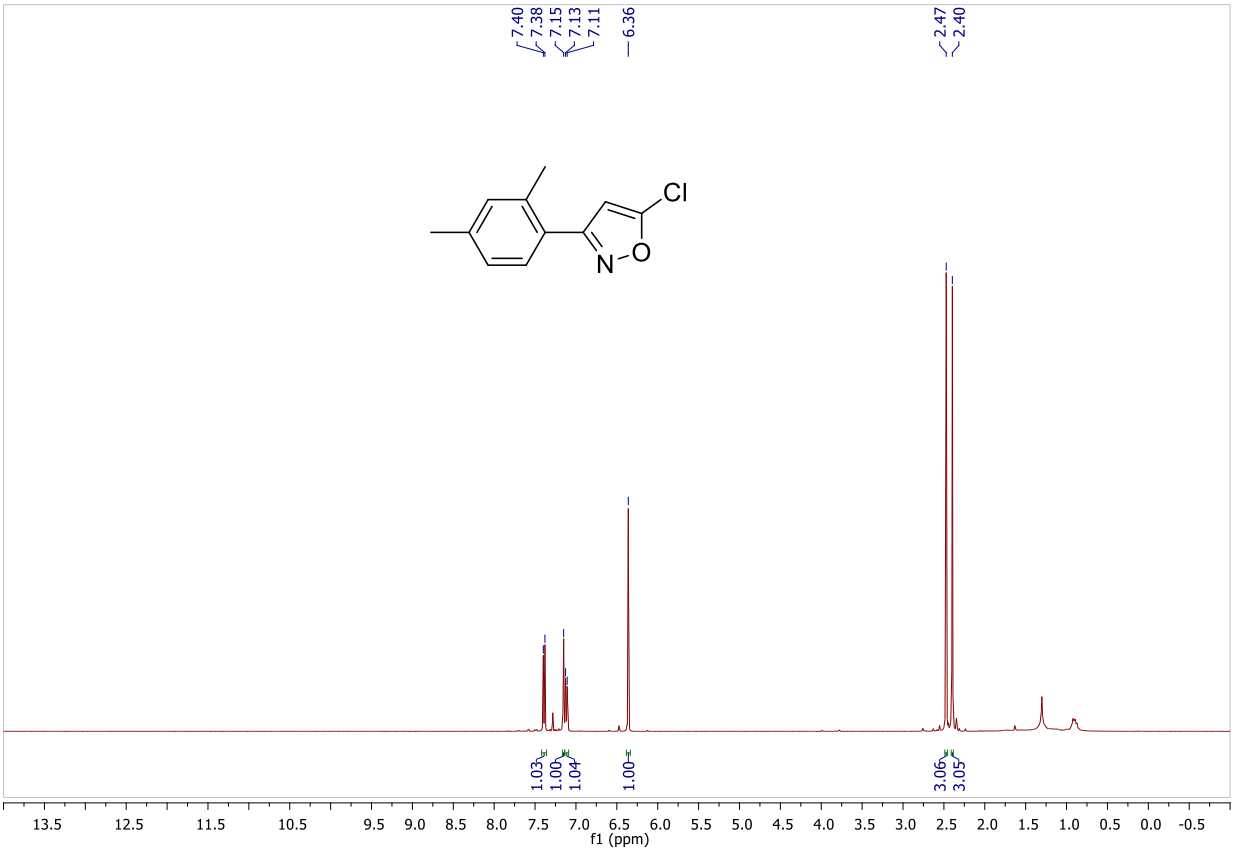
9 ^{1}H and ^{13}C NMR spectra of chloroisoxazoles

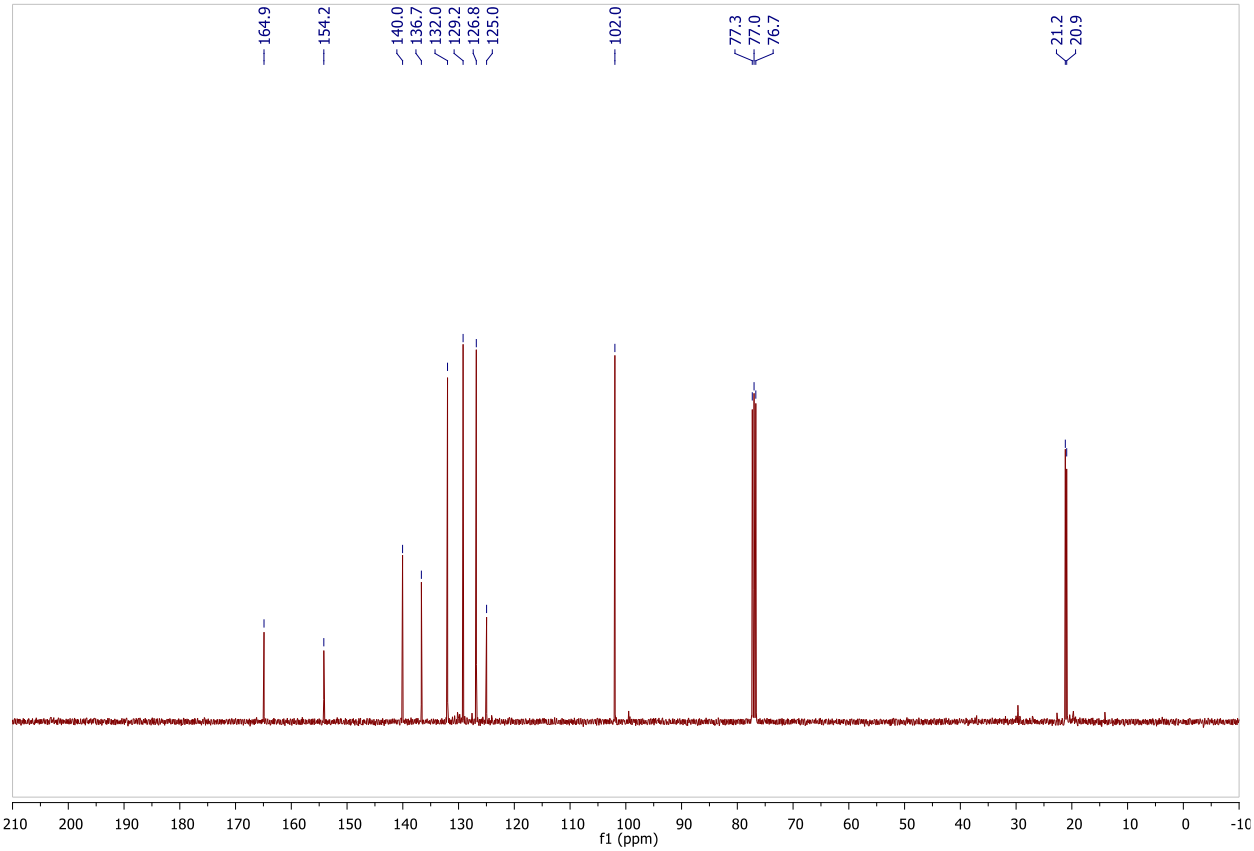
^{1}H and ^{13}C NMR spectra of compound **1b**



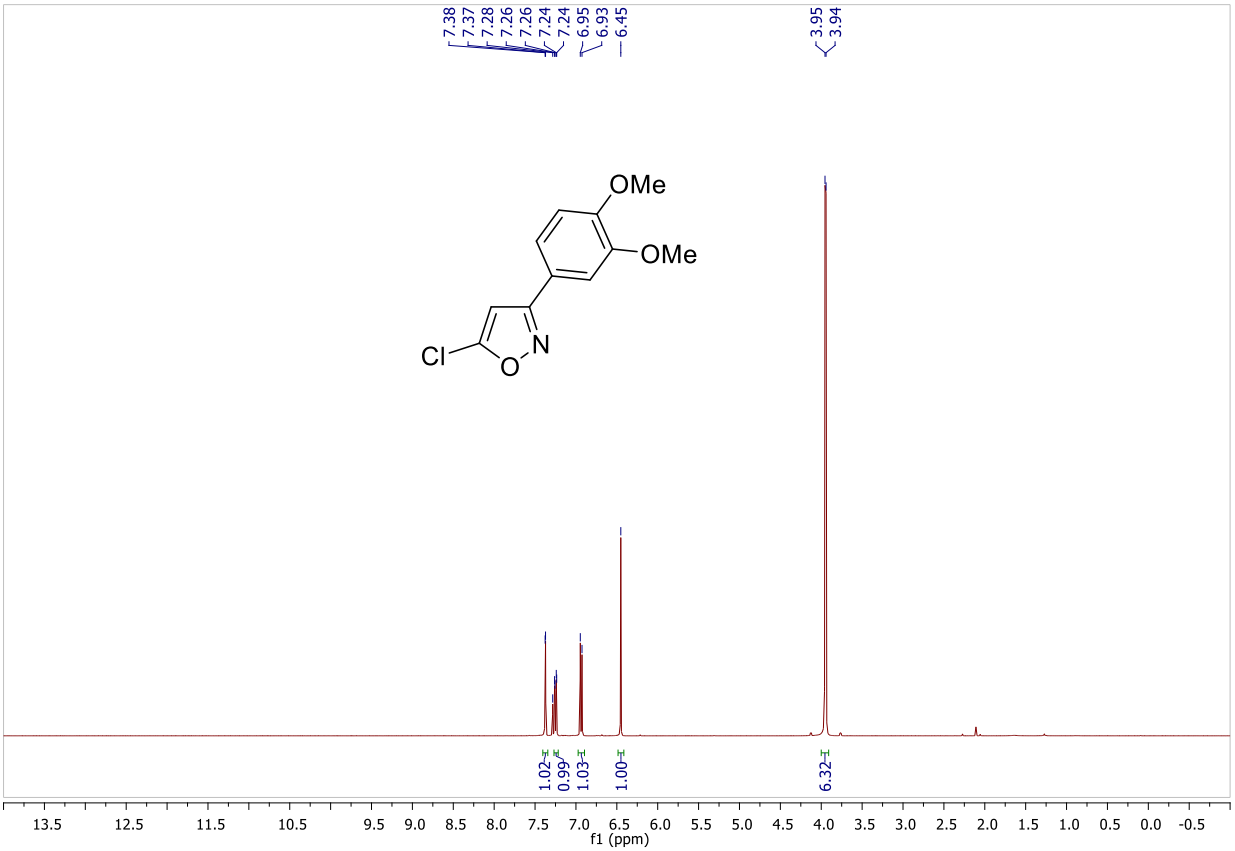


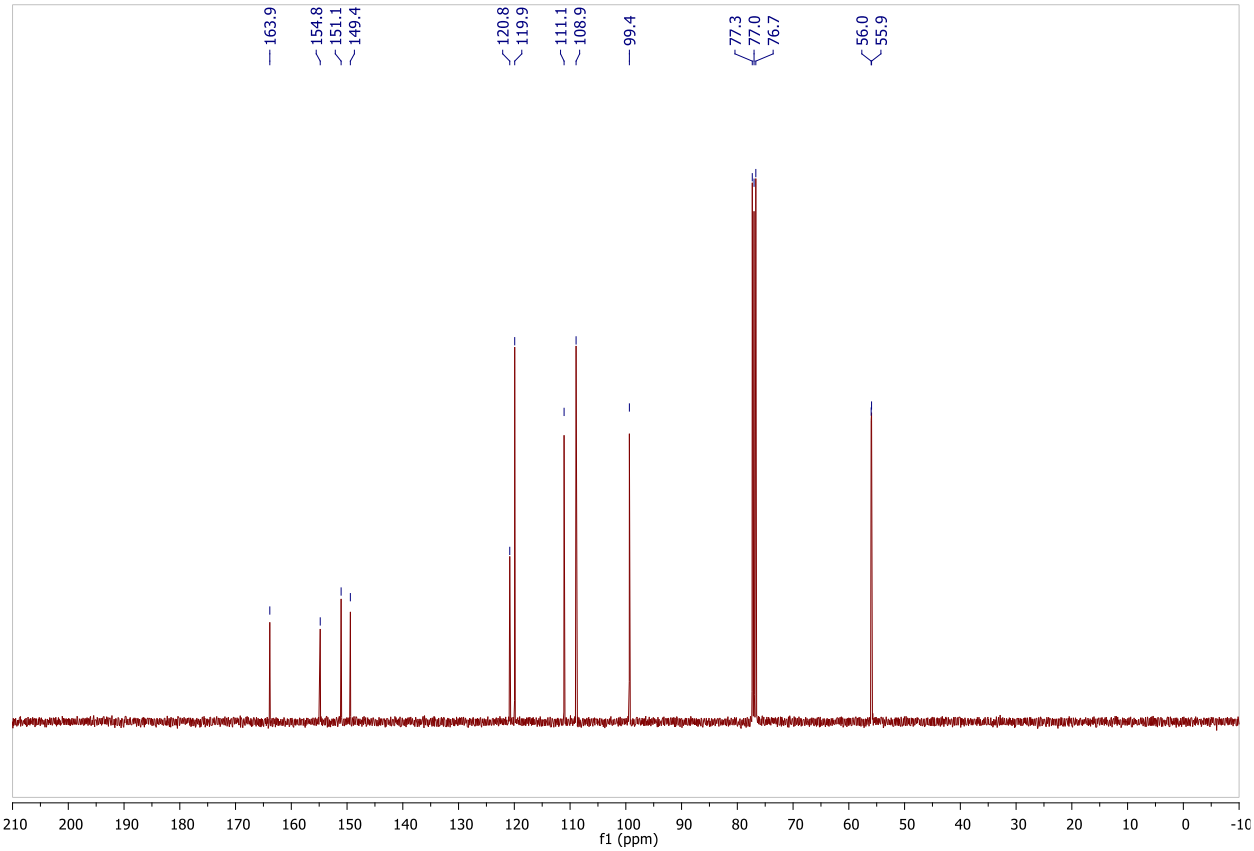
^{1}H and ^{13}C NMR spectra of compound **1h**



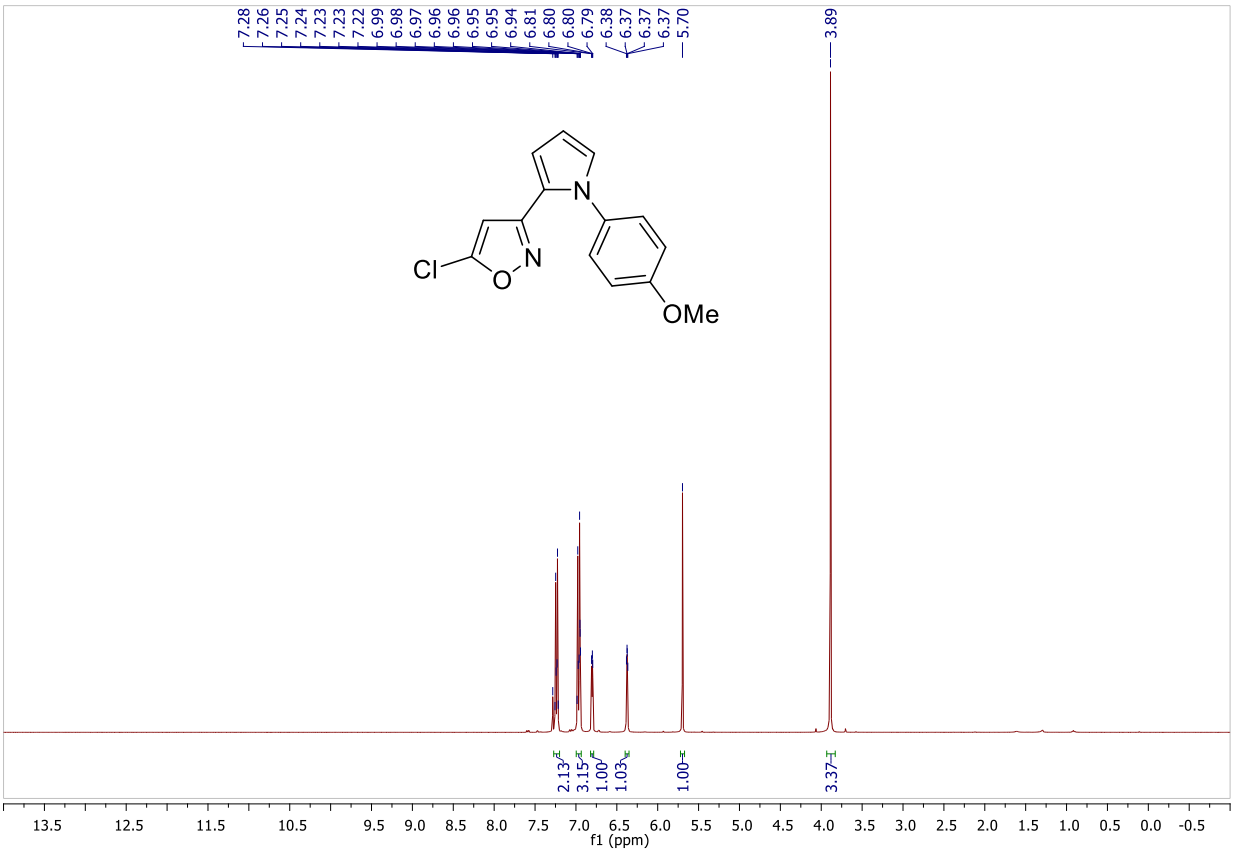


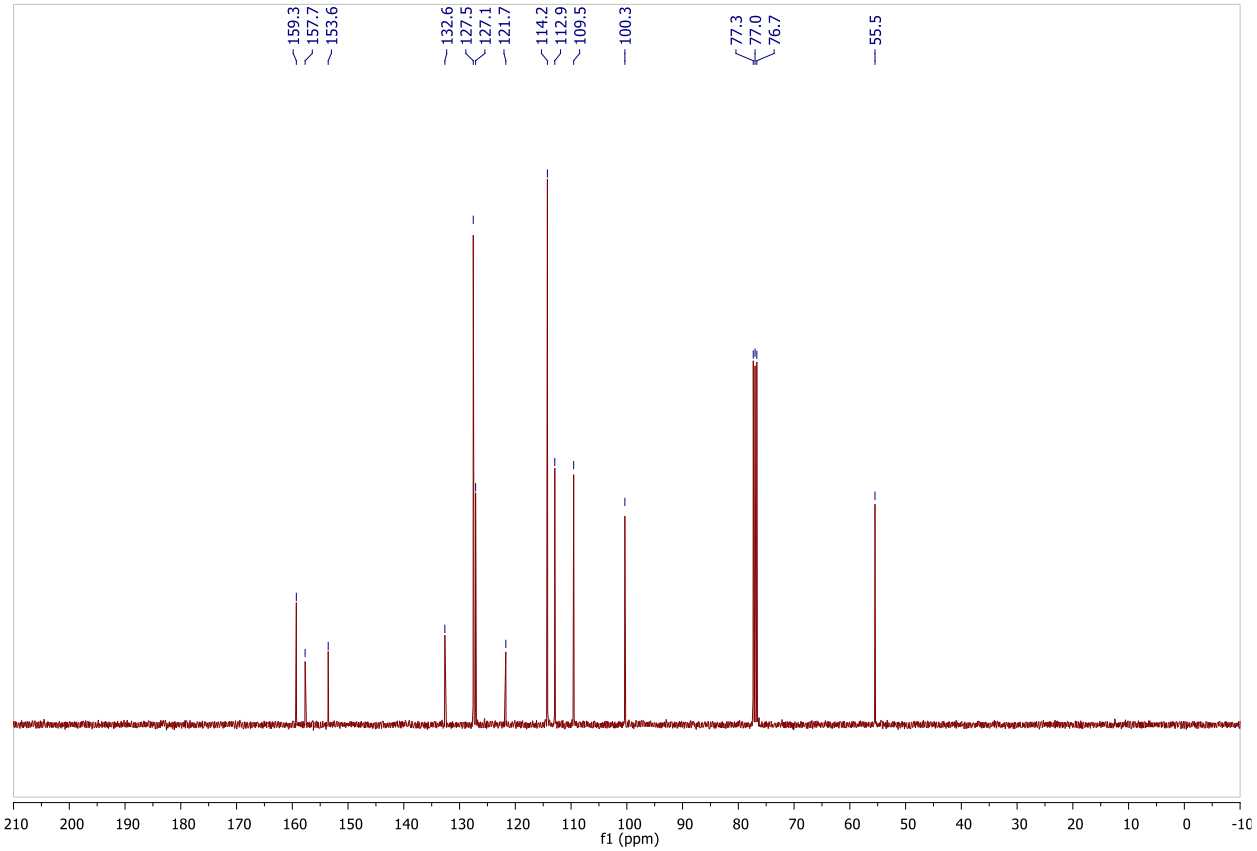
^{1}H and ^{13}C NMR spectra of compound **1k**



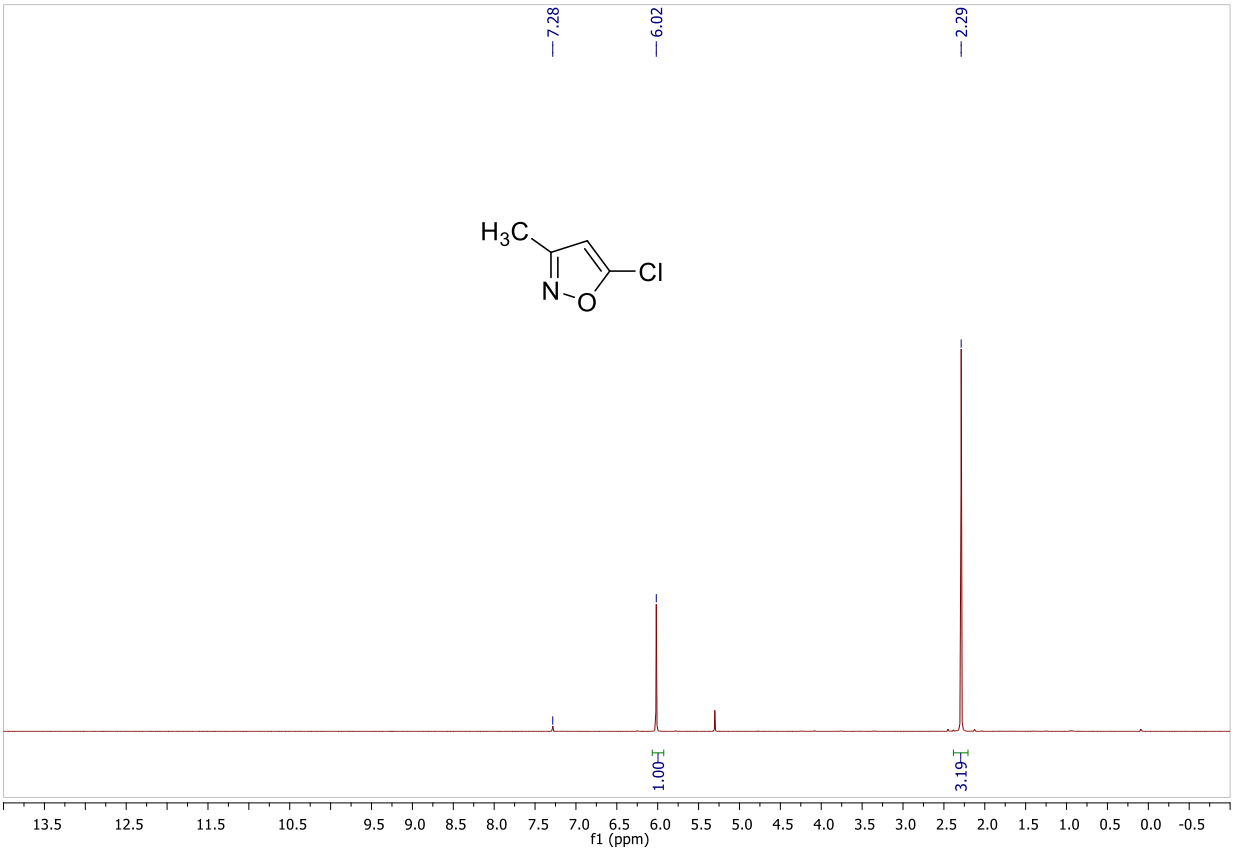


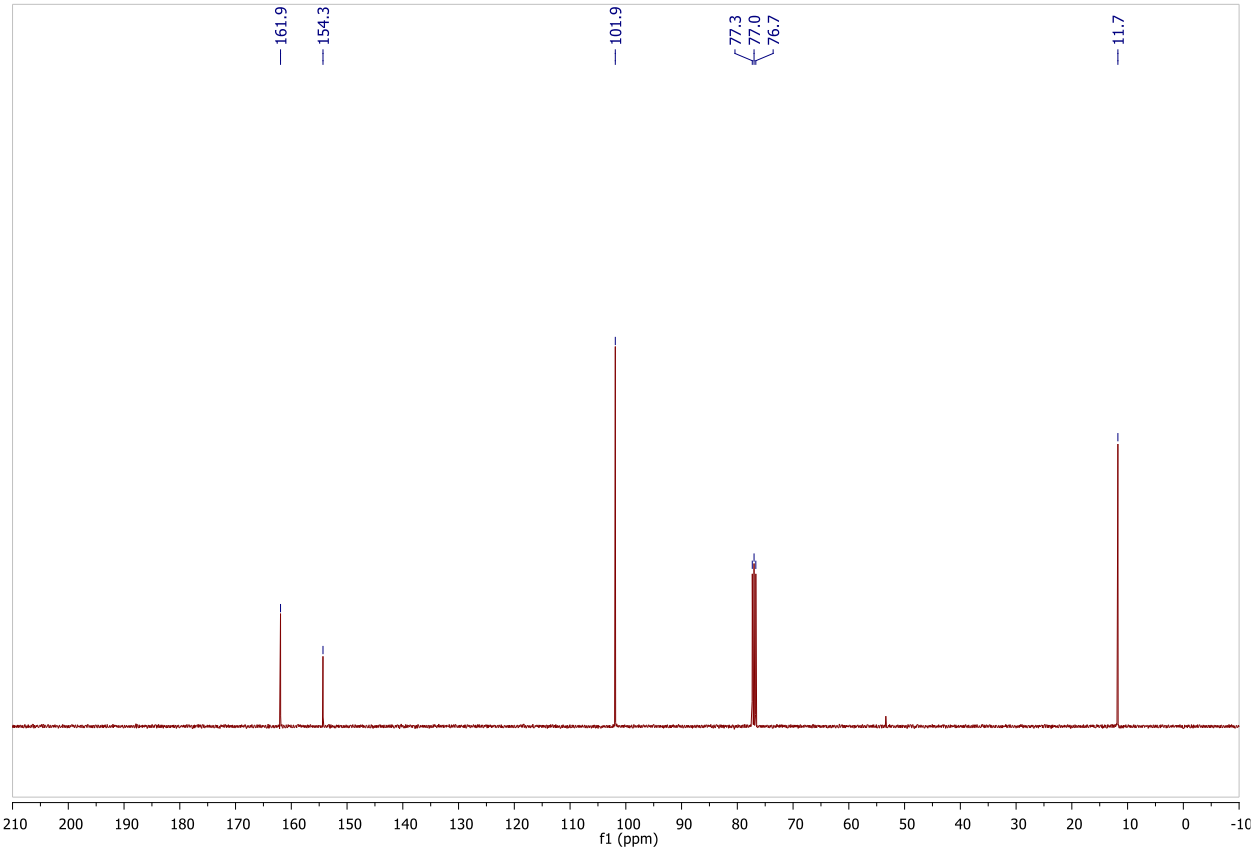
^{1}H and ^{13}C NMR spectra of compound **1n**



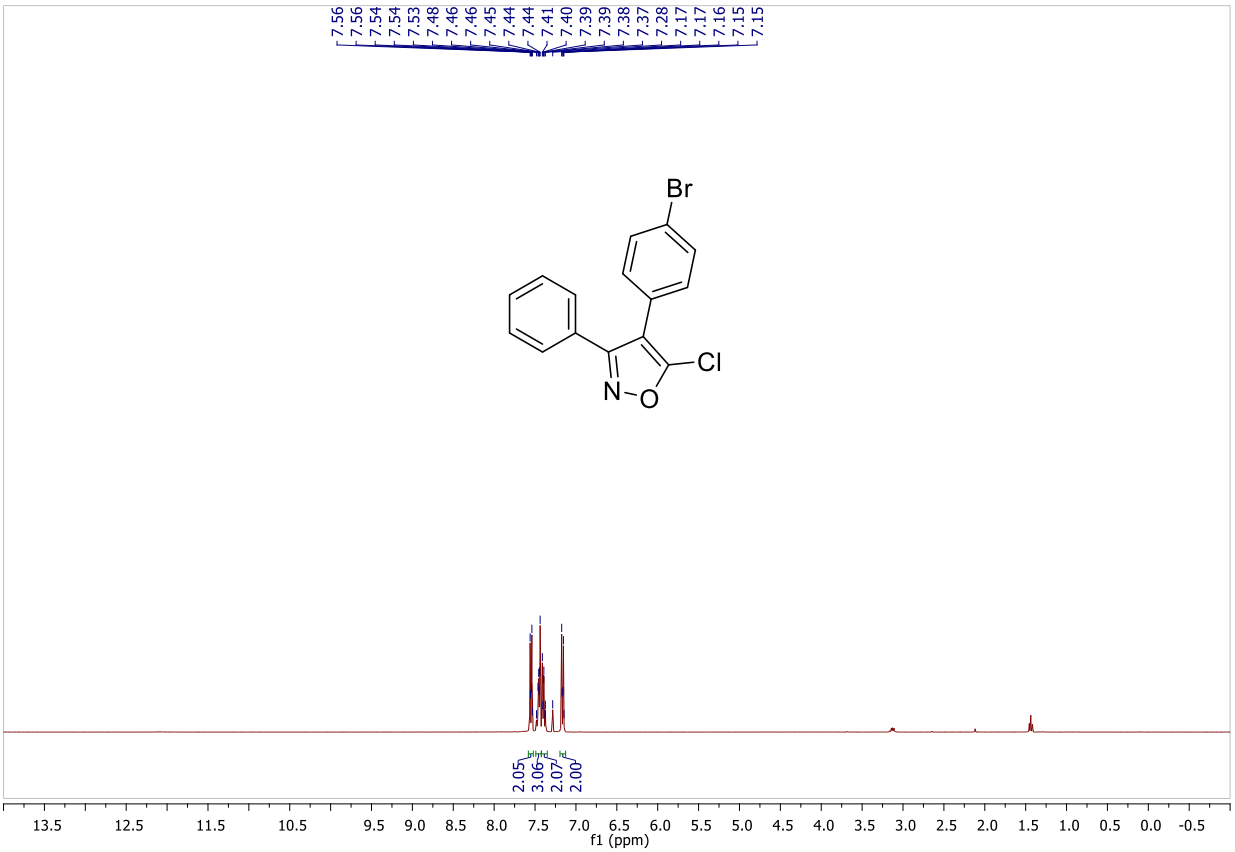


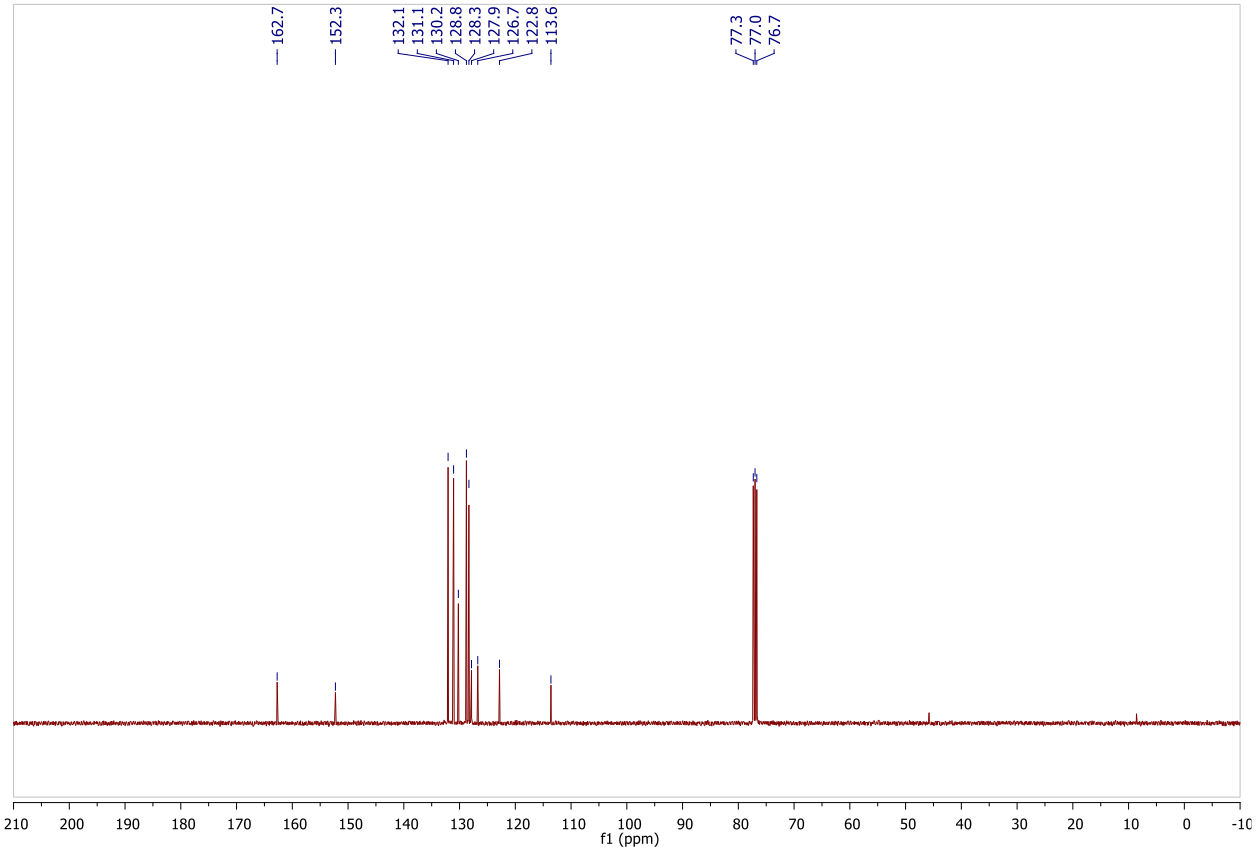
^{1}H and ^{13}C NMR spectra of compound **1o**



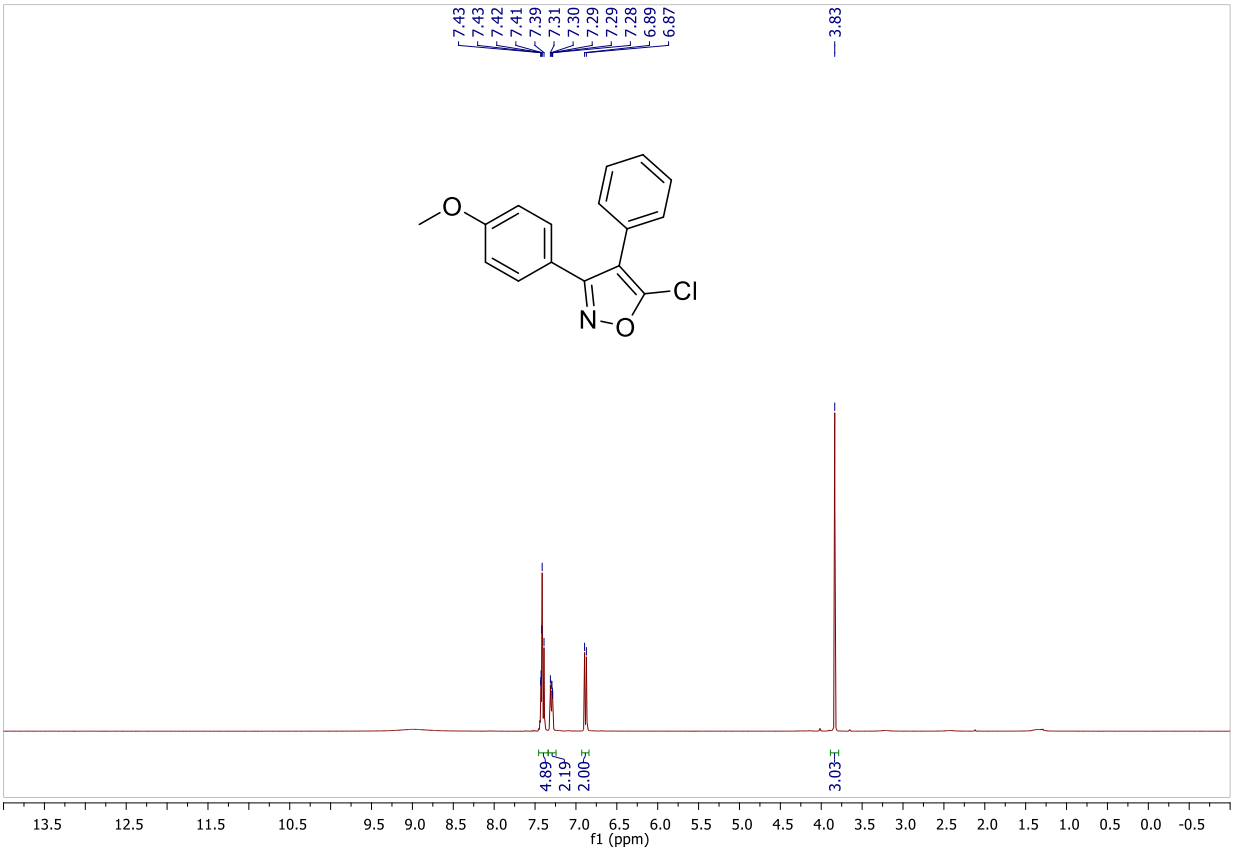


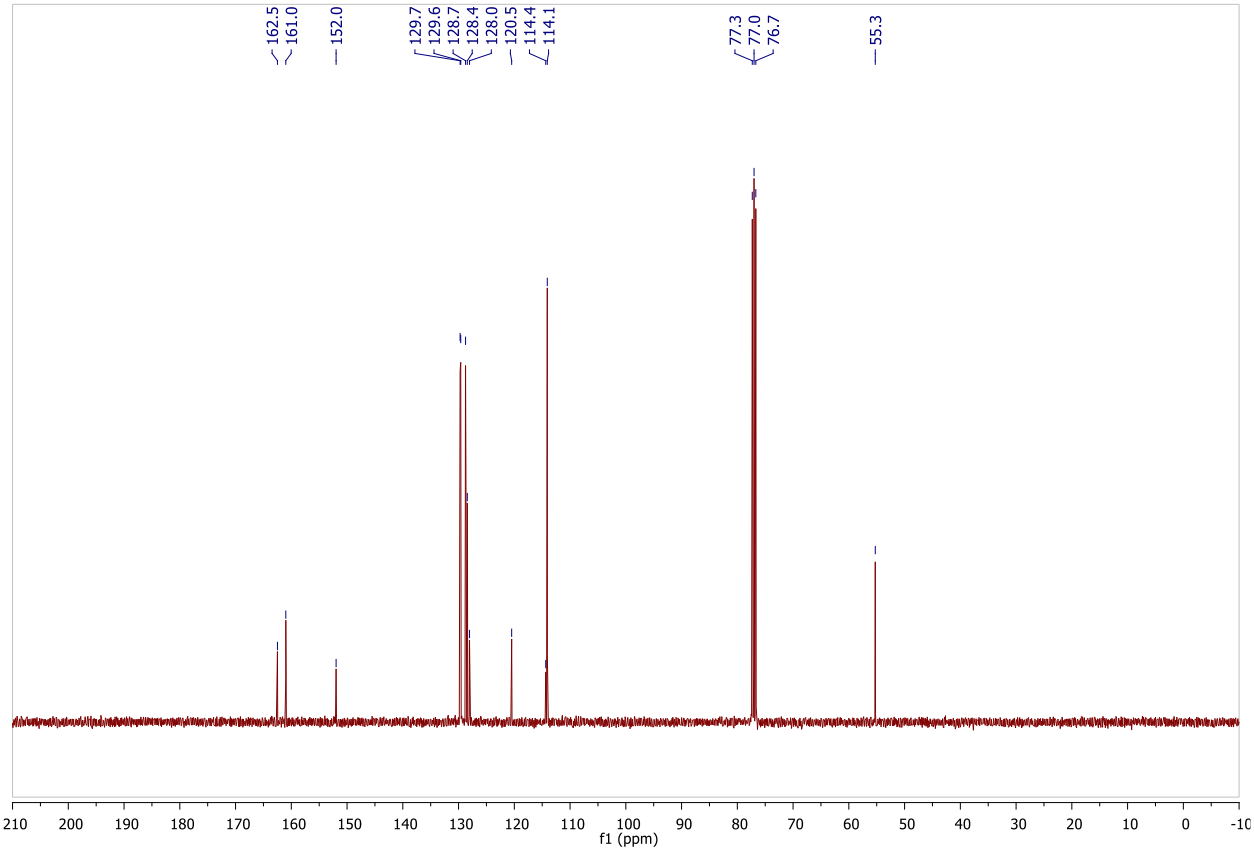
^{1}H and ^{13}C NMR spectra of compound **1p**



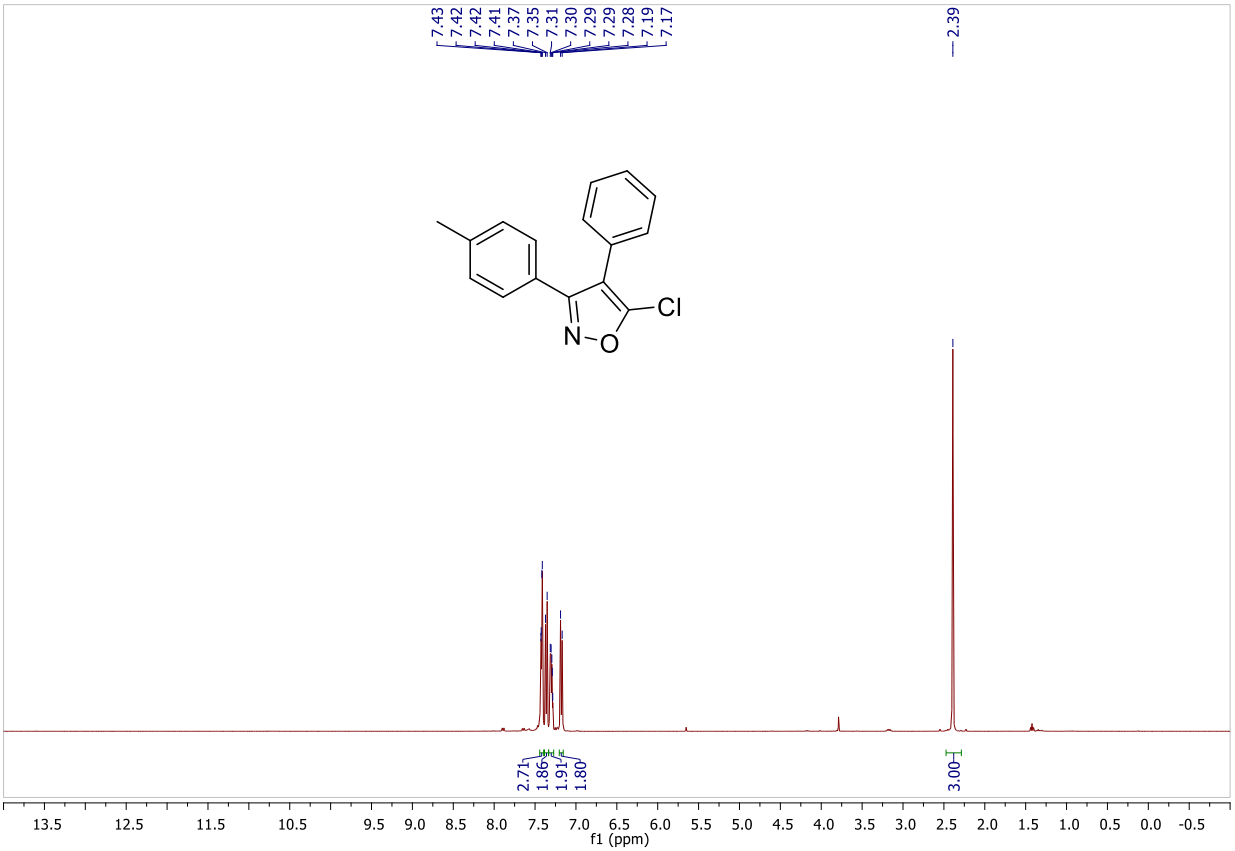


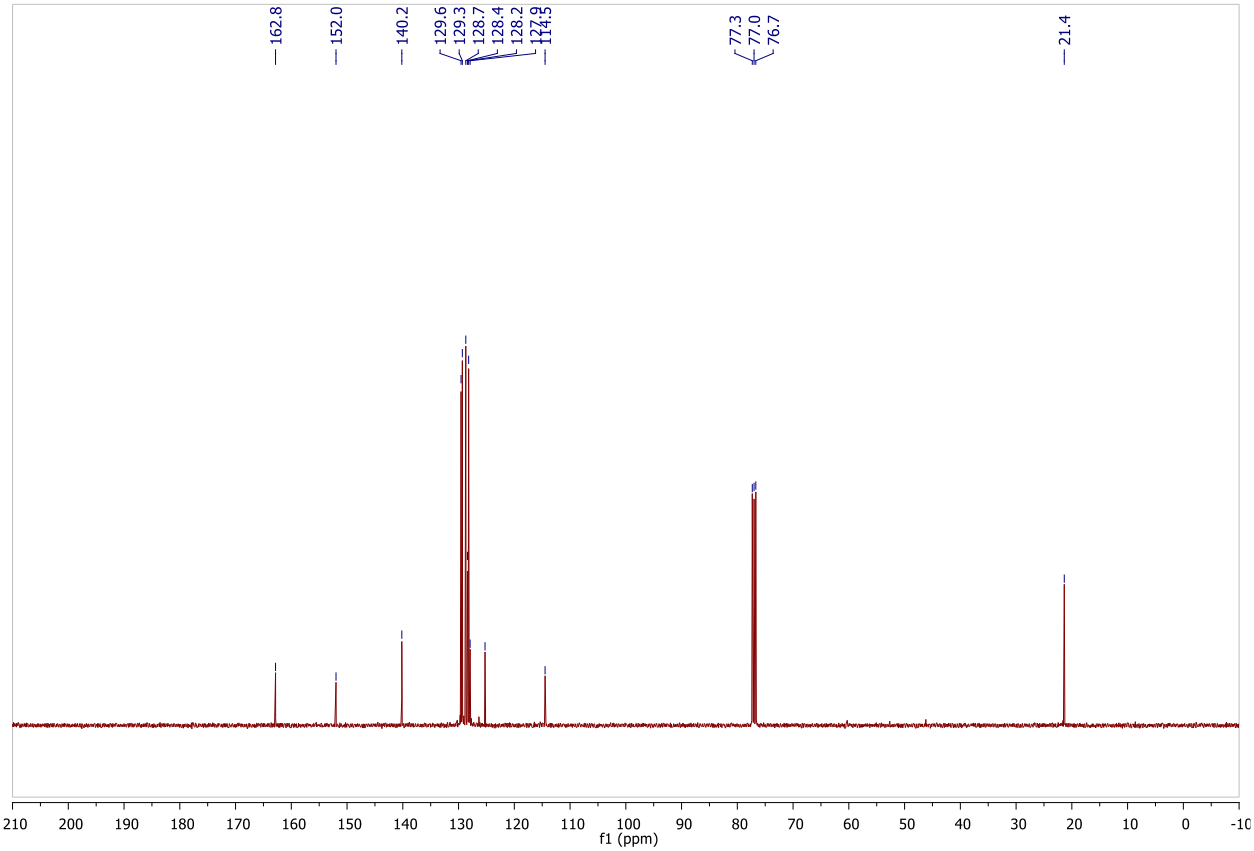
^{1}H and ^{13}C NMR spectra of compound **1q**



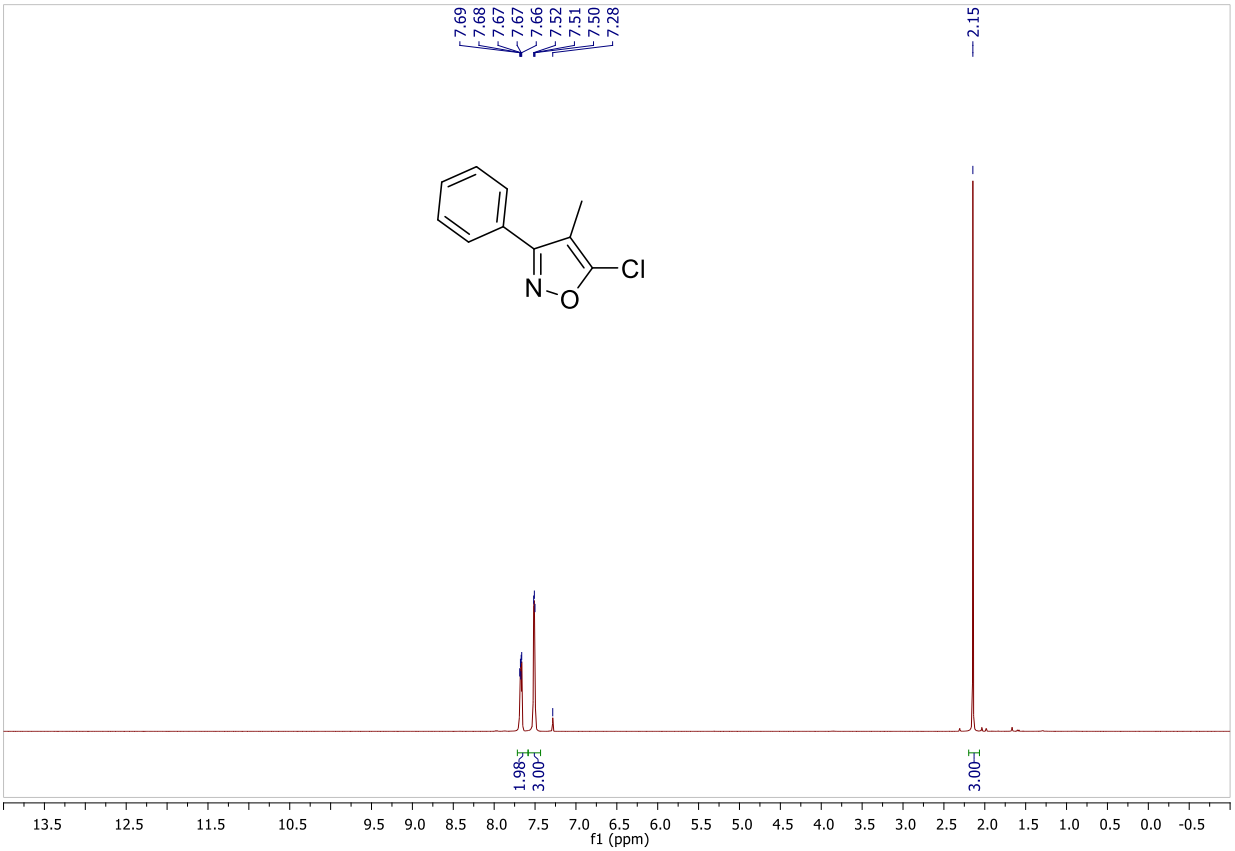


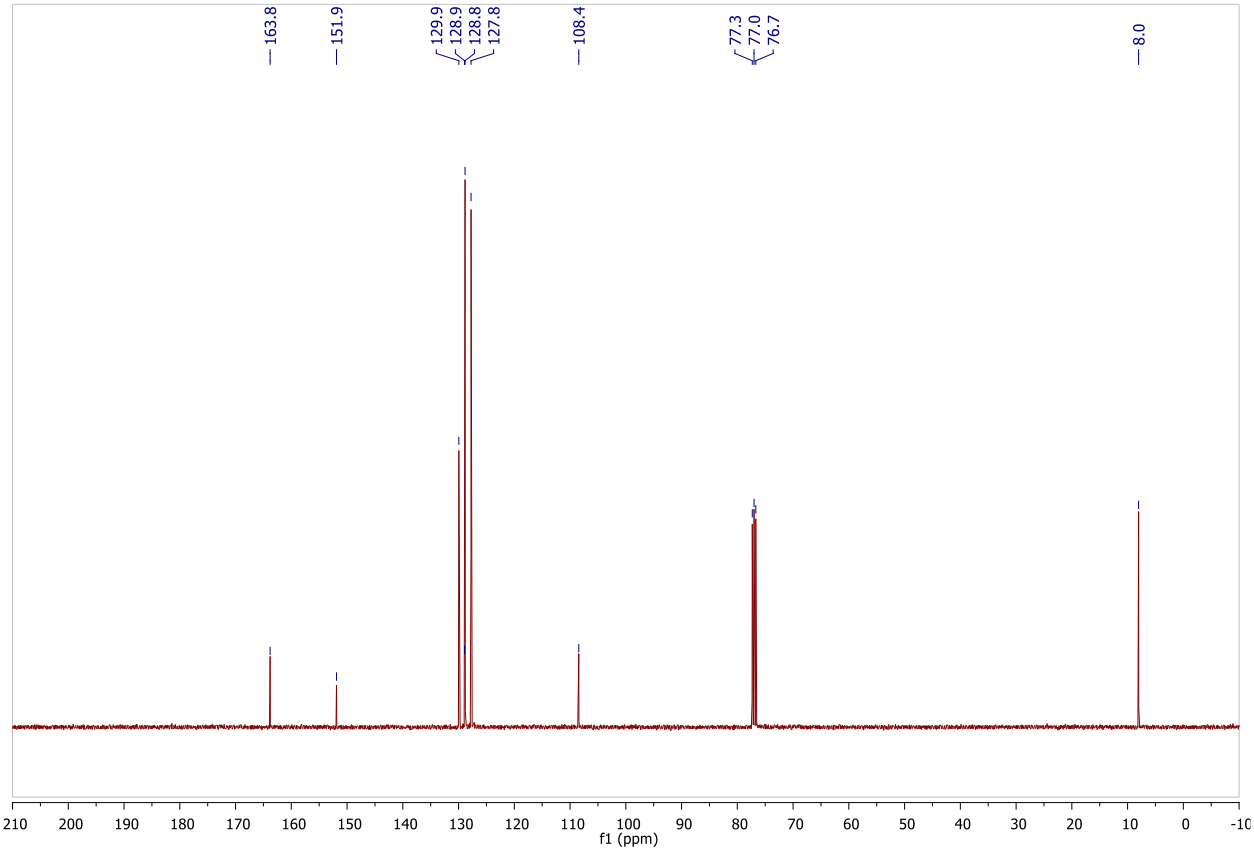
^{1}H and ^{13}C NMR spectra of compound **1r**



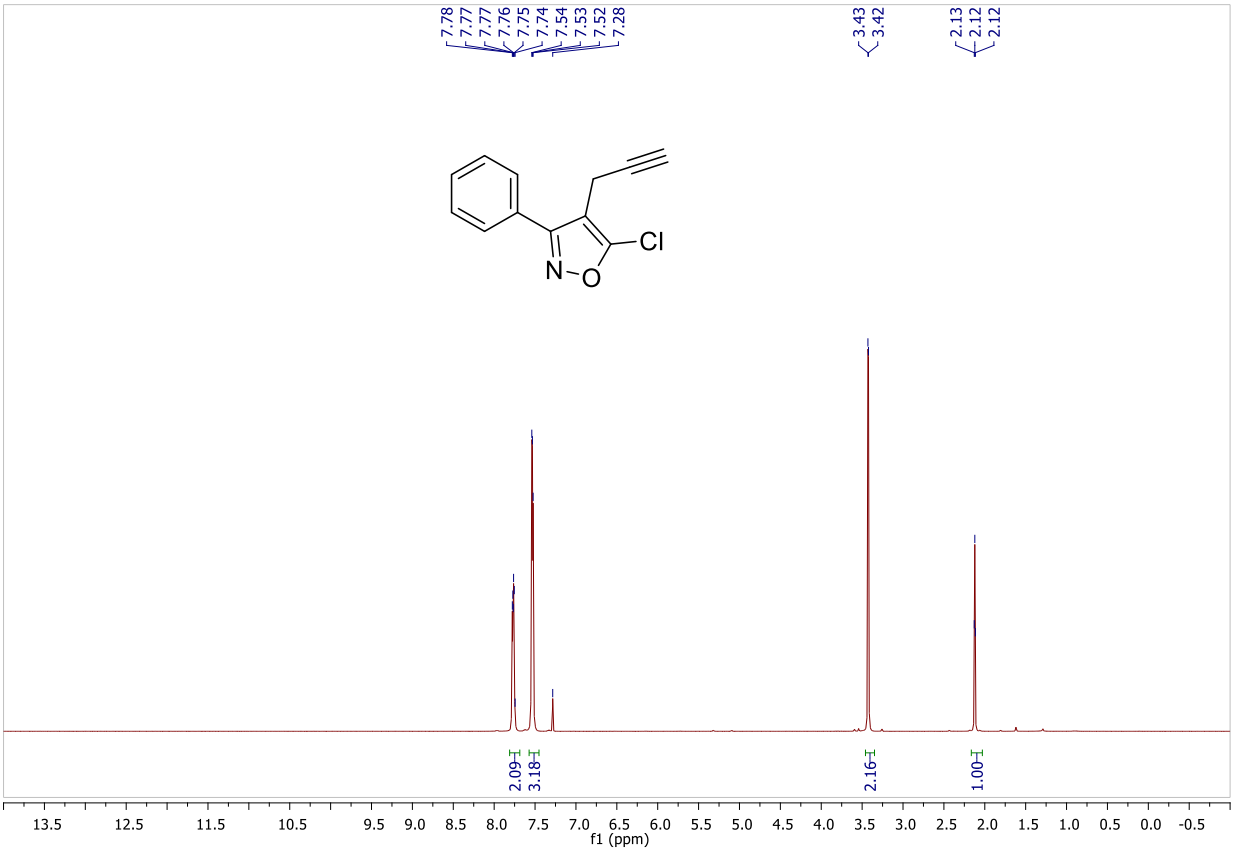


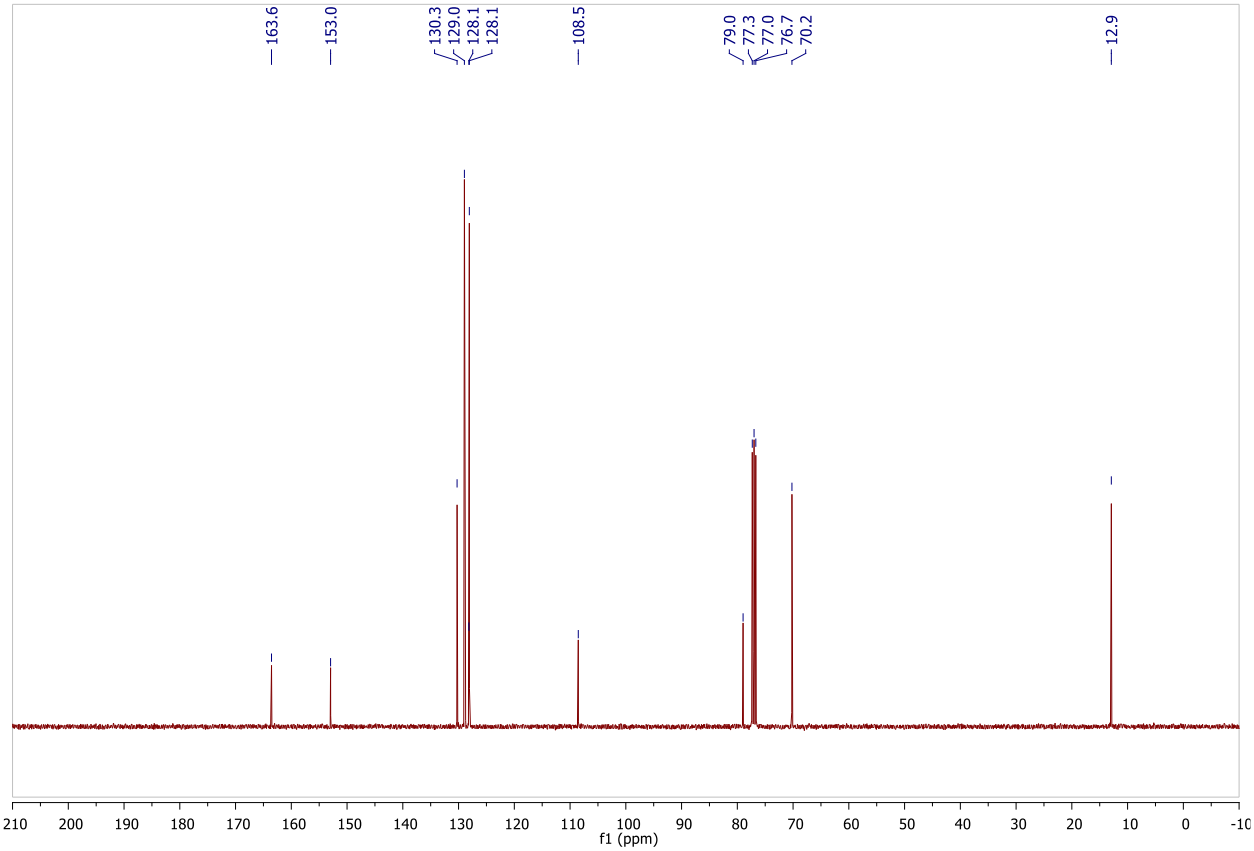
^{1}H and ^{13}C NMR spectra of compound **1s**





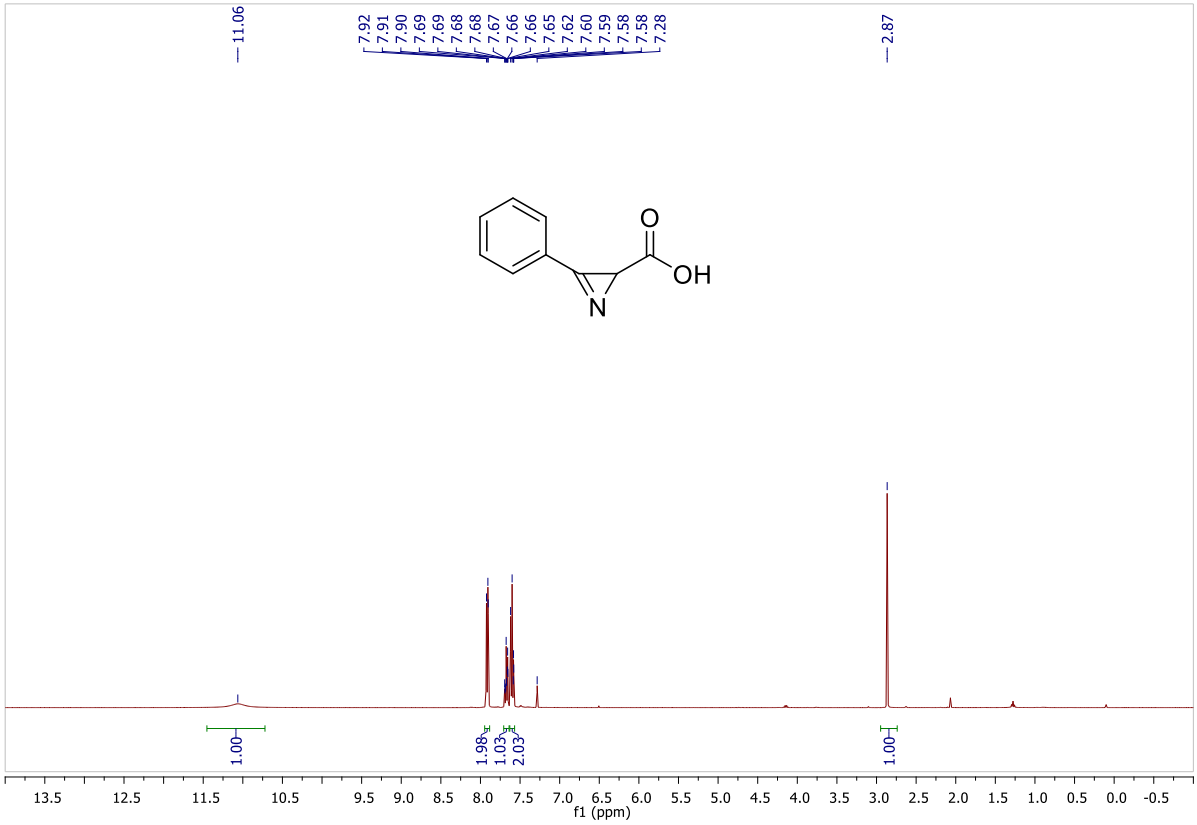
^{1}H and ^{13}C NMR spectra of compound **1t**

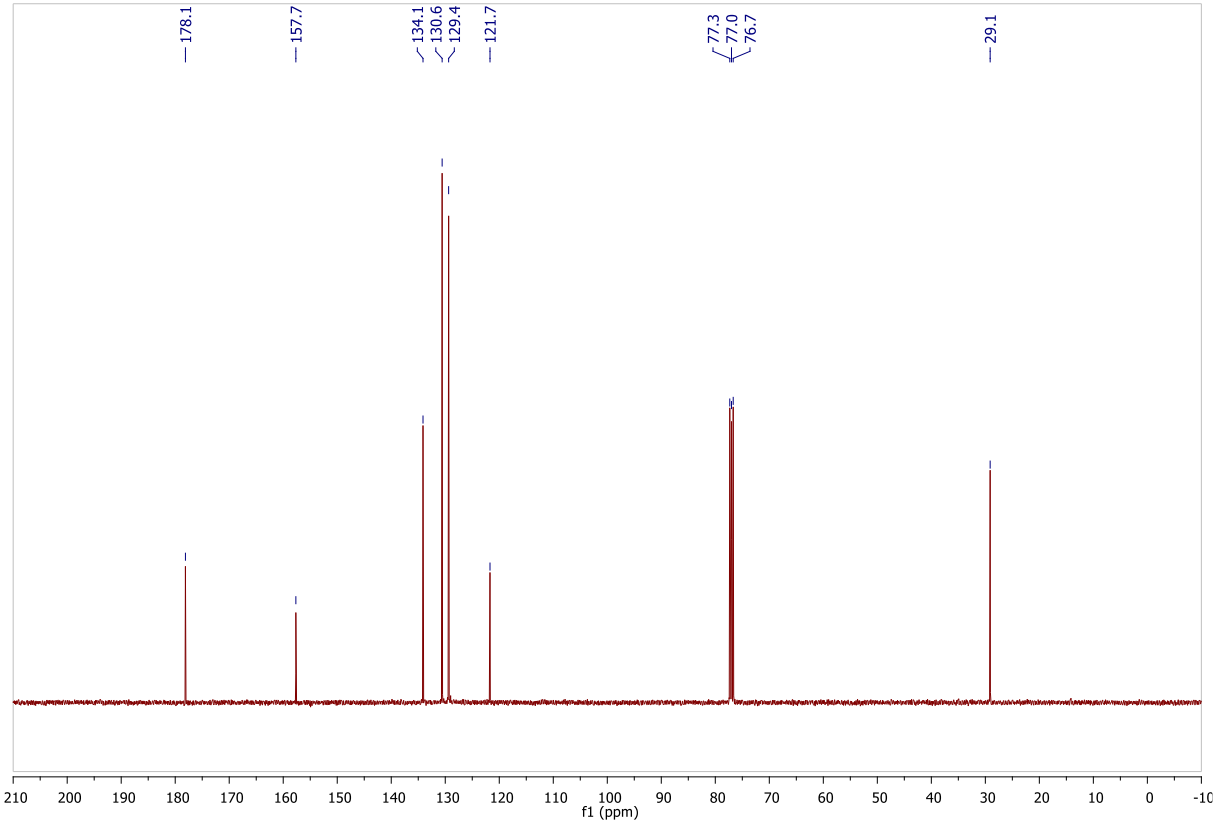




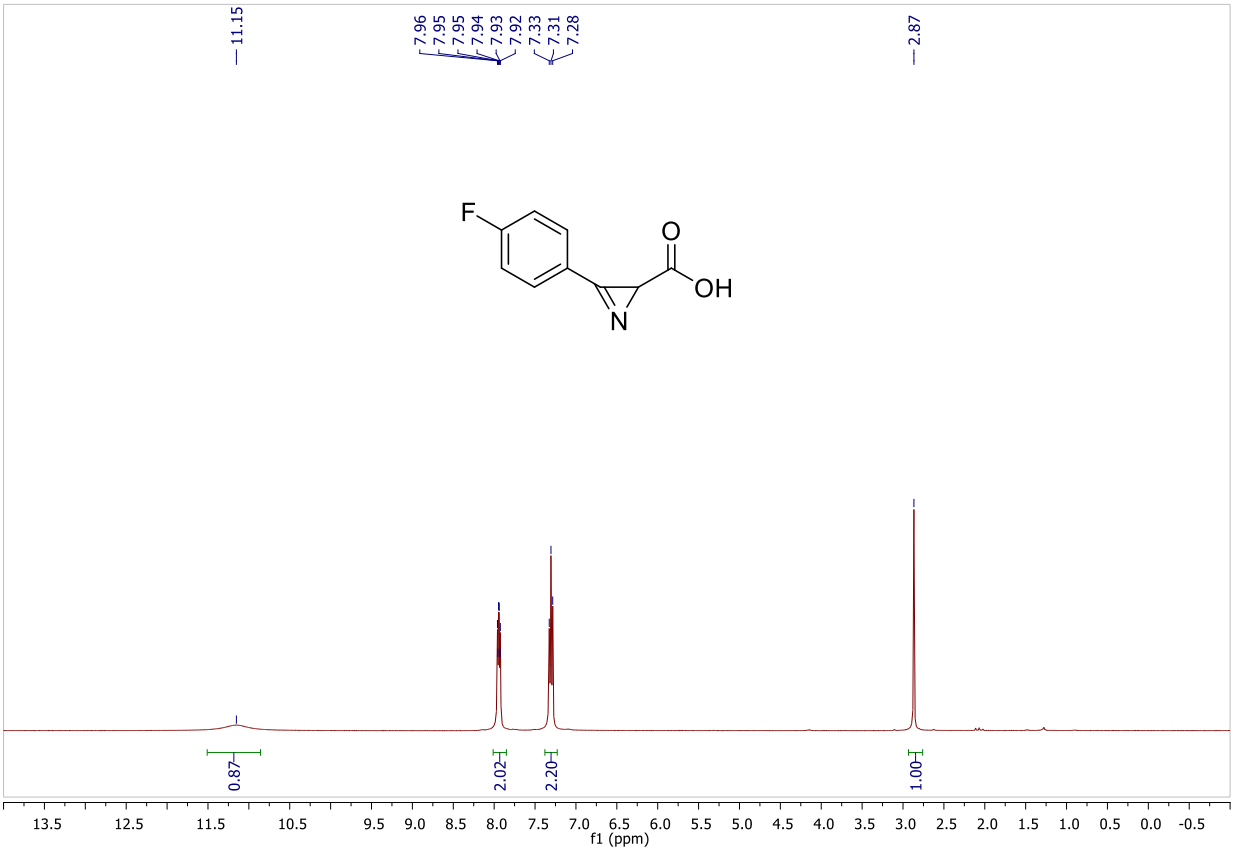
10 ^{1}H and ^{13}C NMR spectra of 2*H*-azirine-2-carboxylic acids

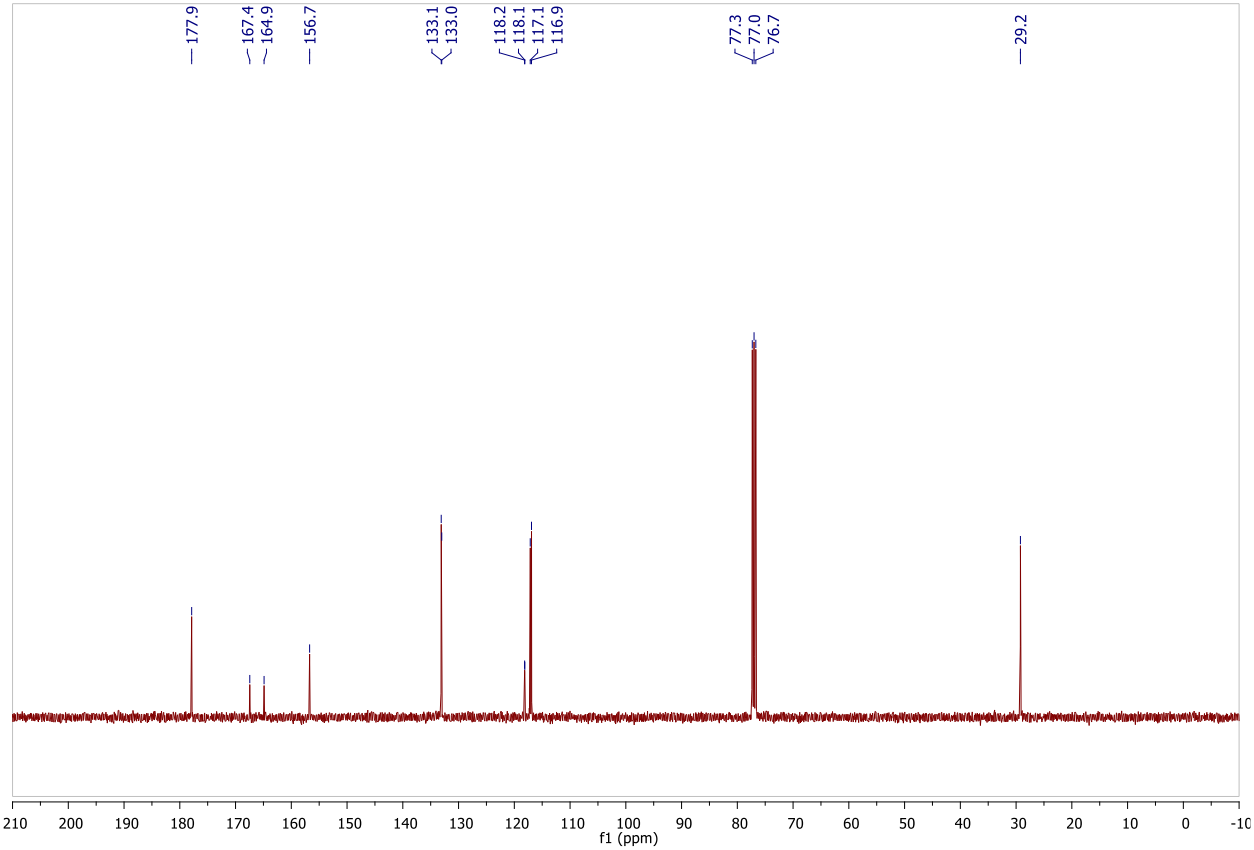
^{1}H and ^{13}C NMR spectra of compound **3a**



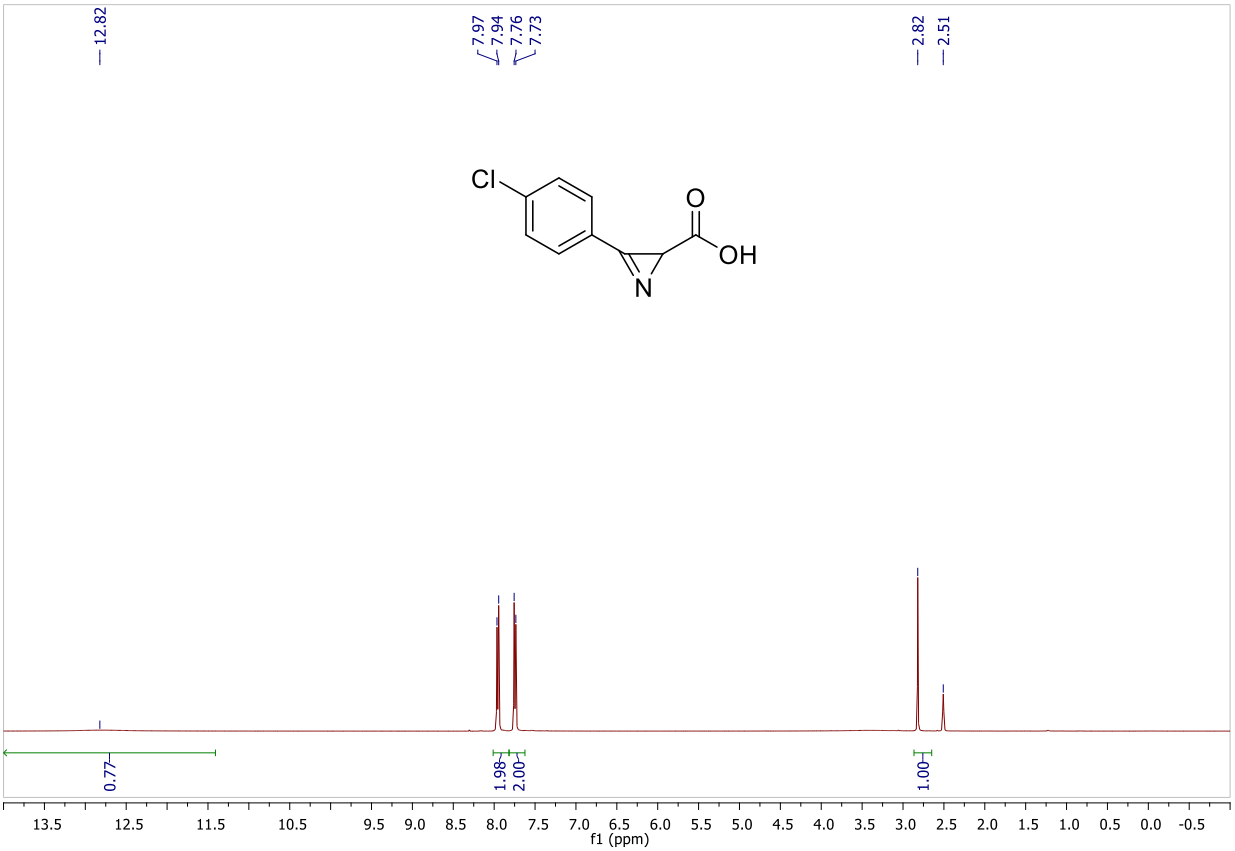


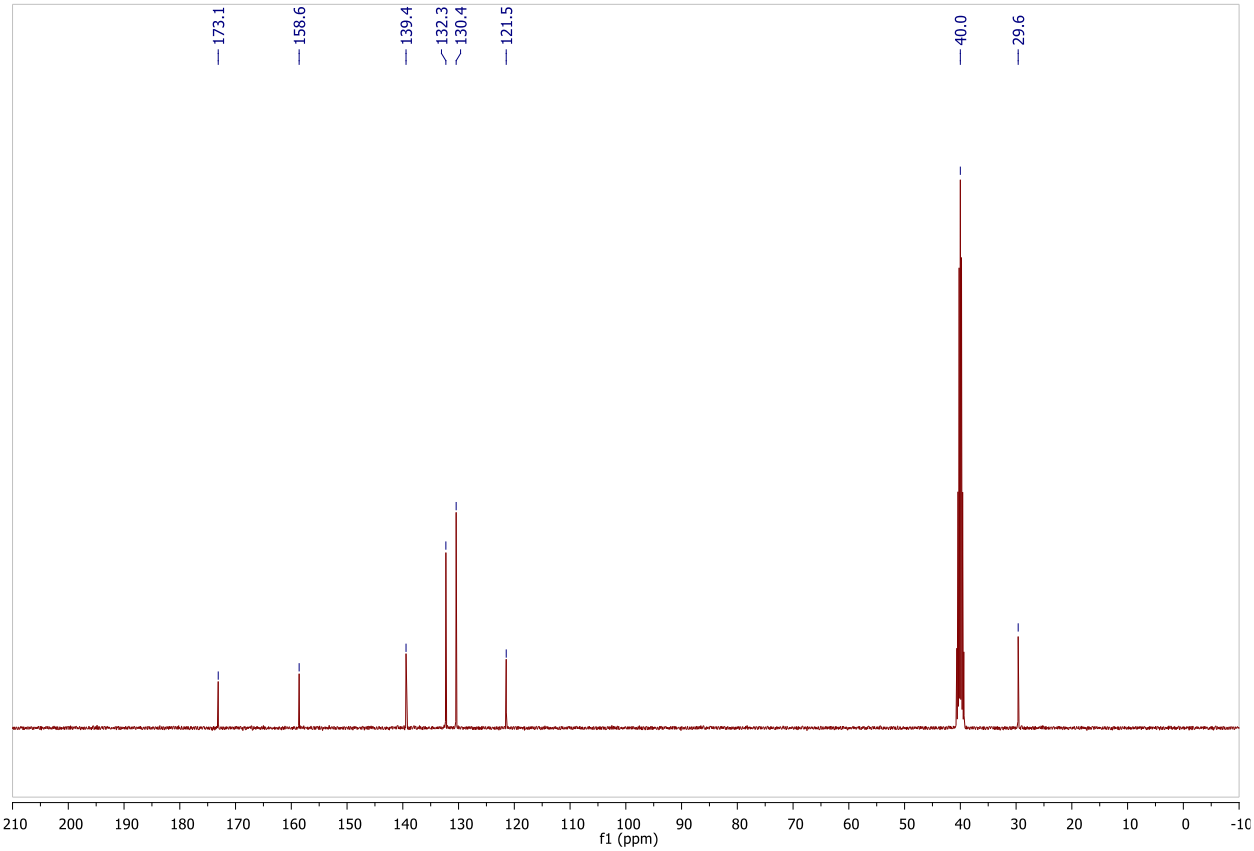
^{1}H and ^{13}C NMR spectra of compound **3b**



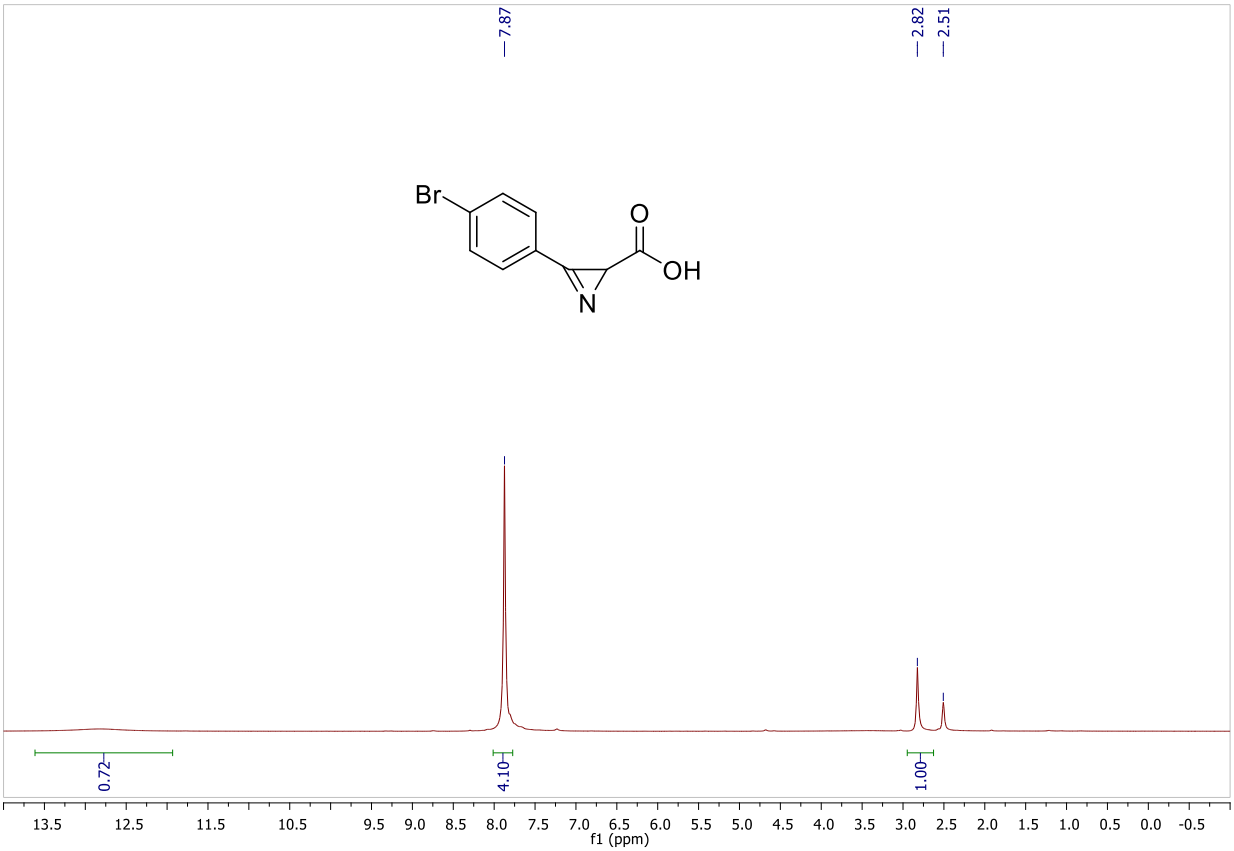


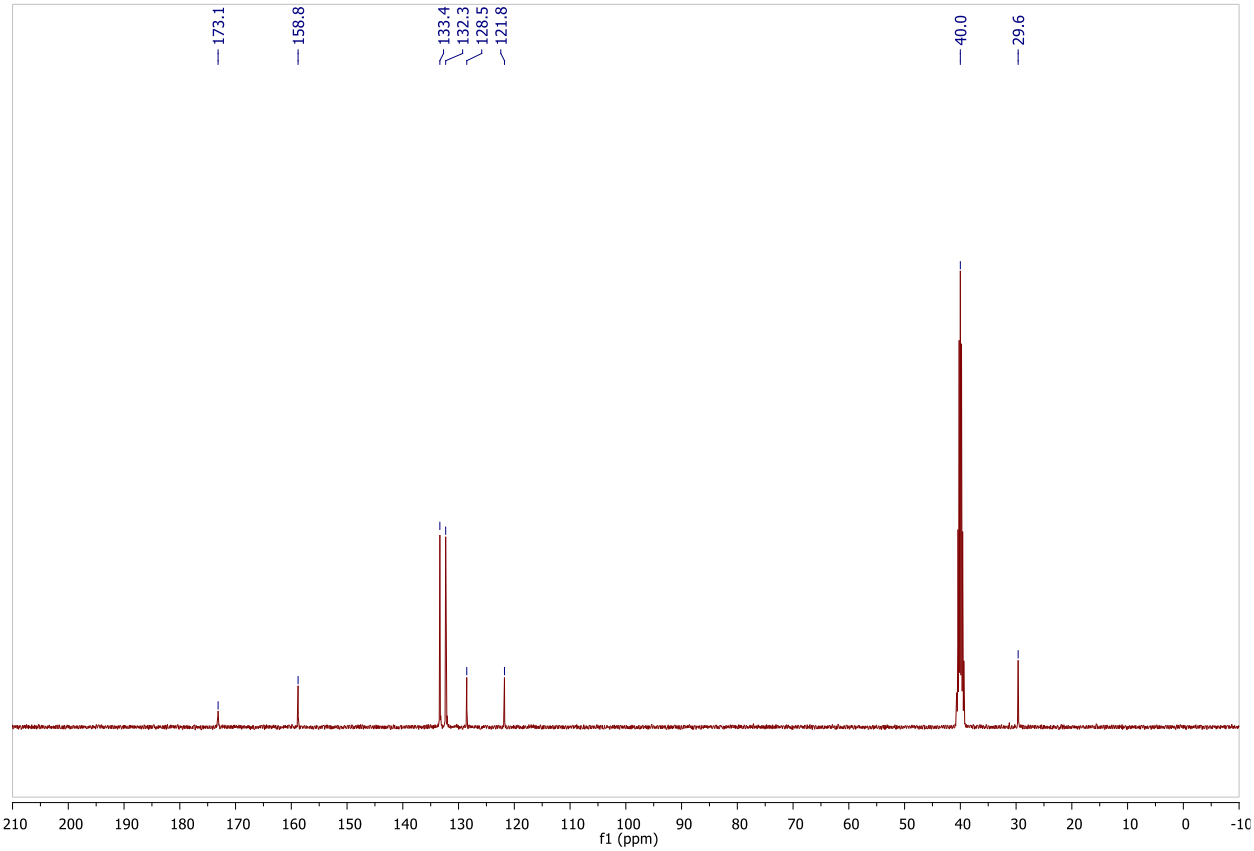
^{1}H and ^{13}C NMR spectra of compound **3c**



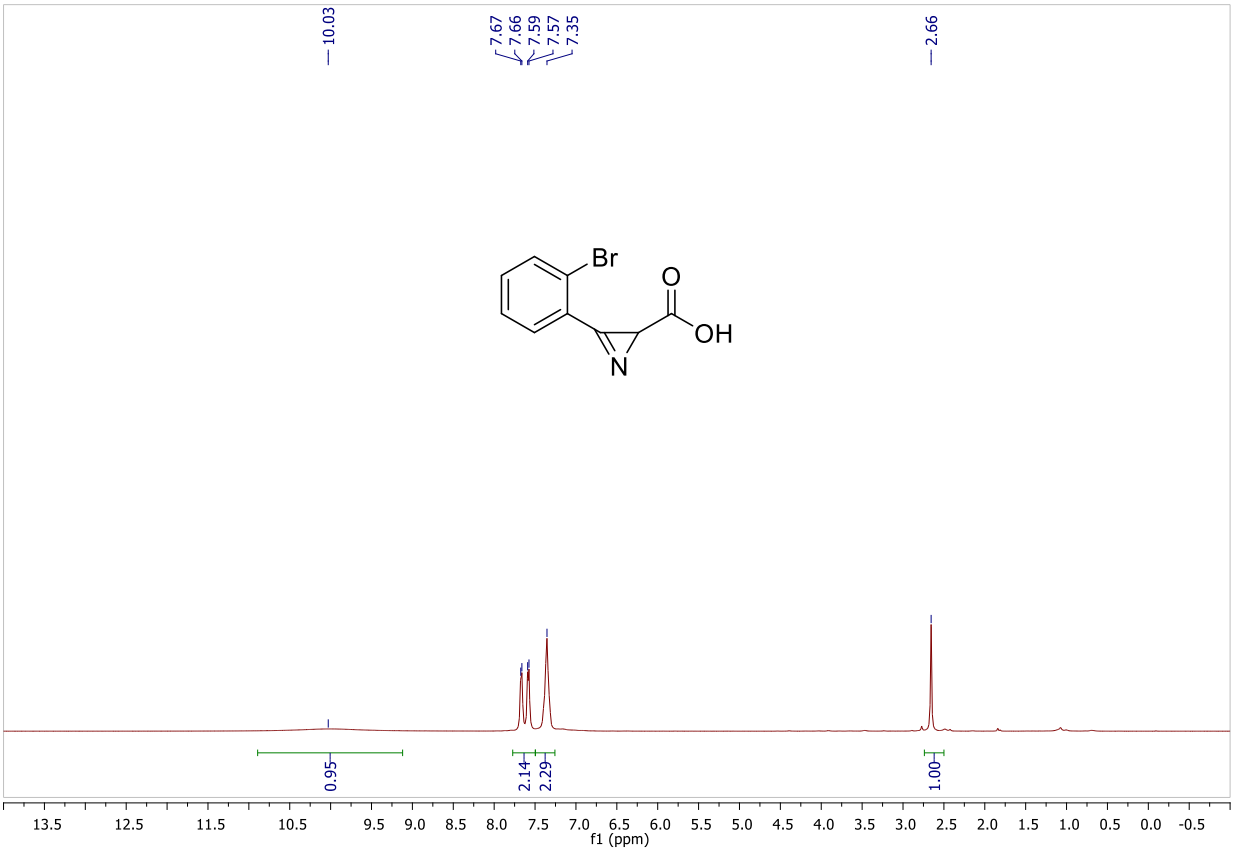


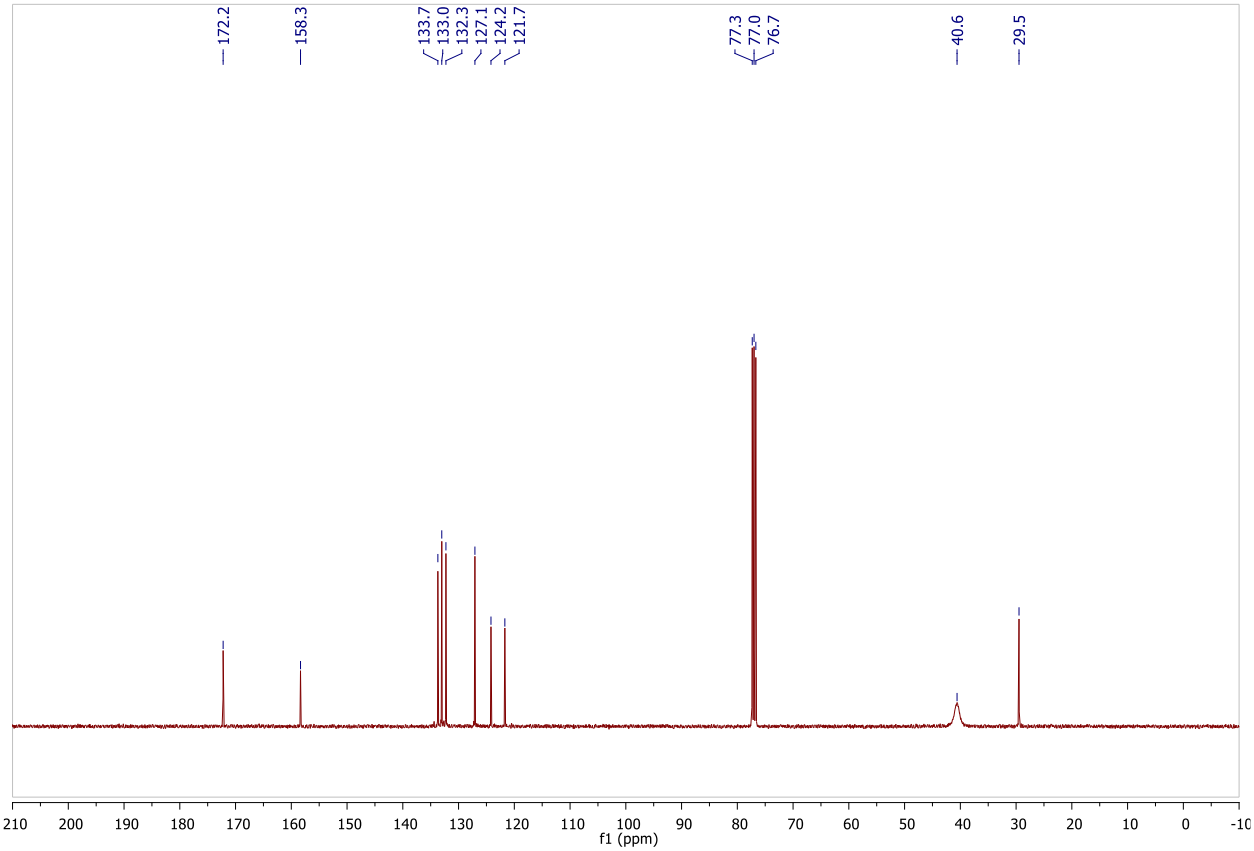
^{1}H and ^{13}C NMR spectra of compound **3d**



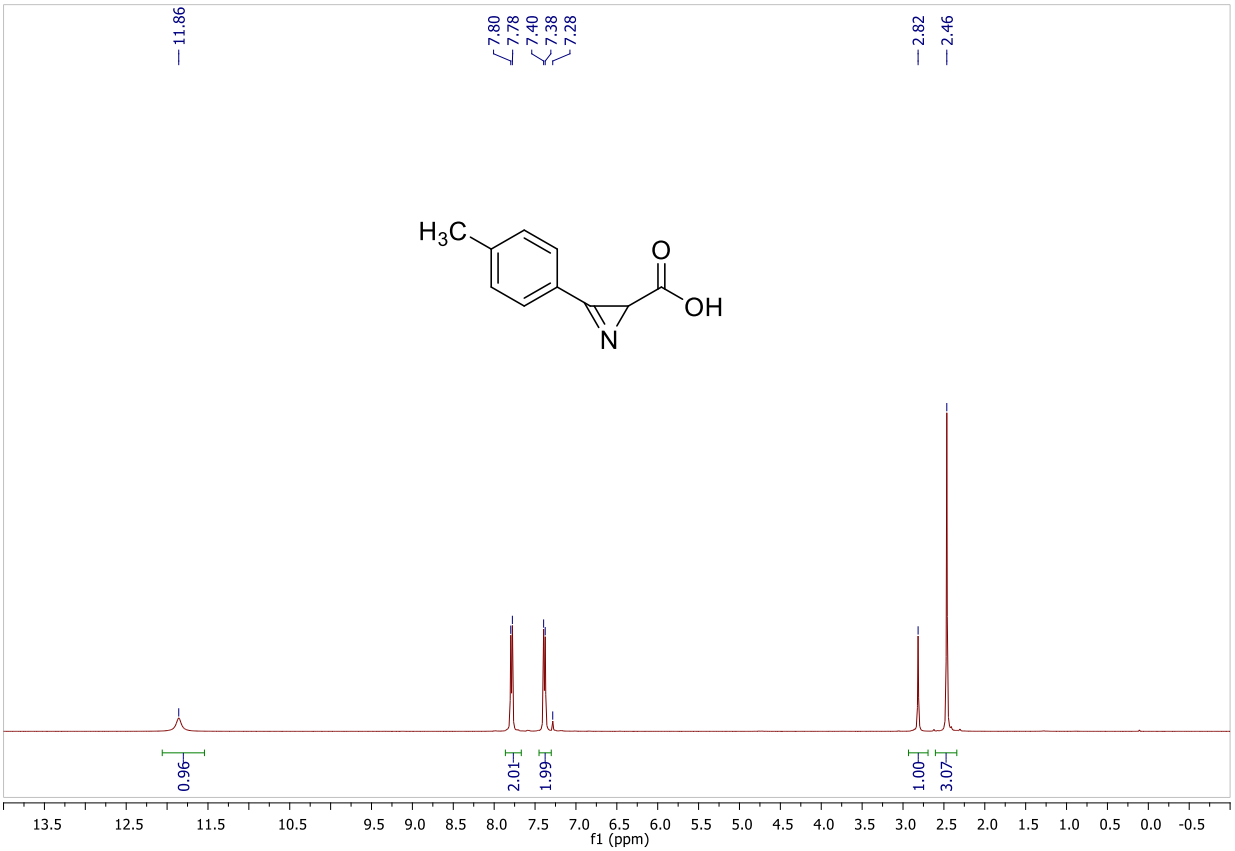


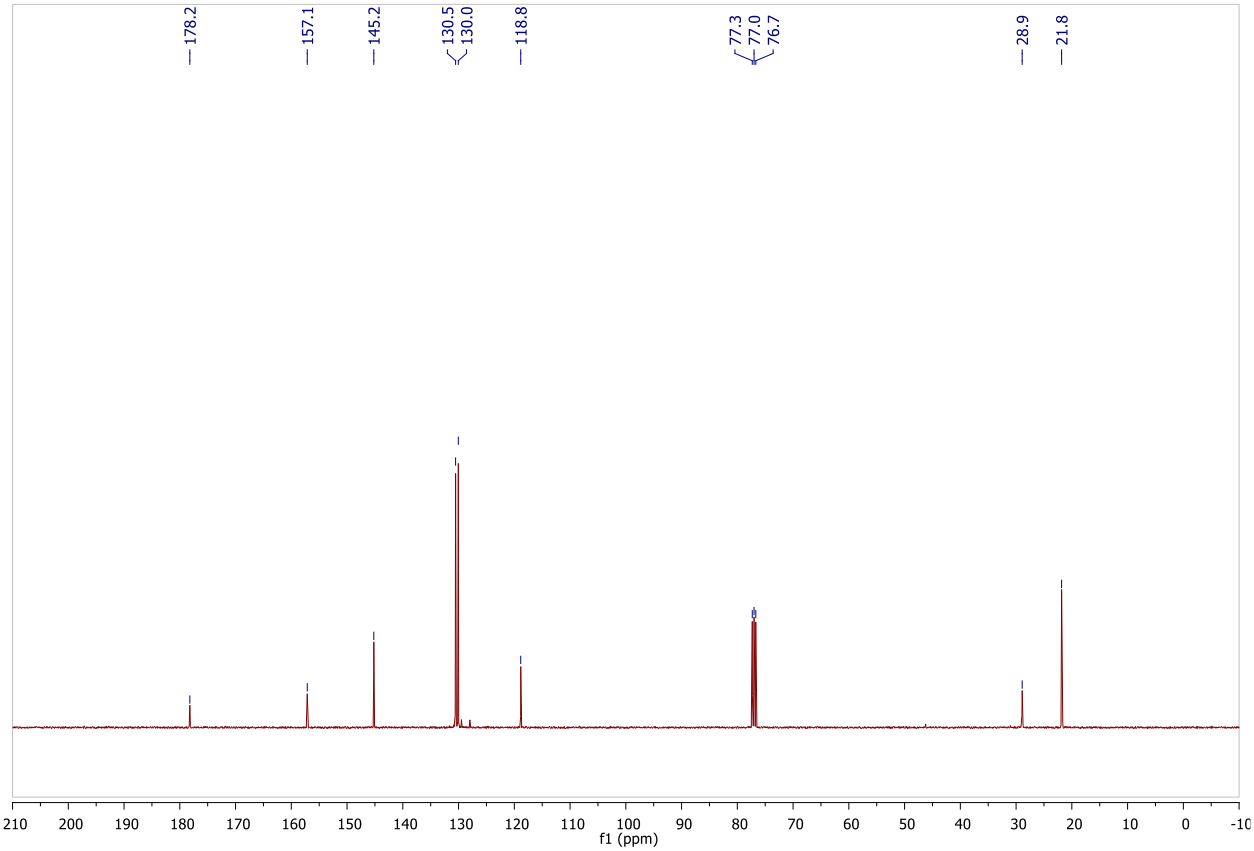
^{1}H and ^{13}C NMR spectra of compound **3e**



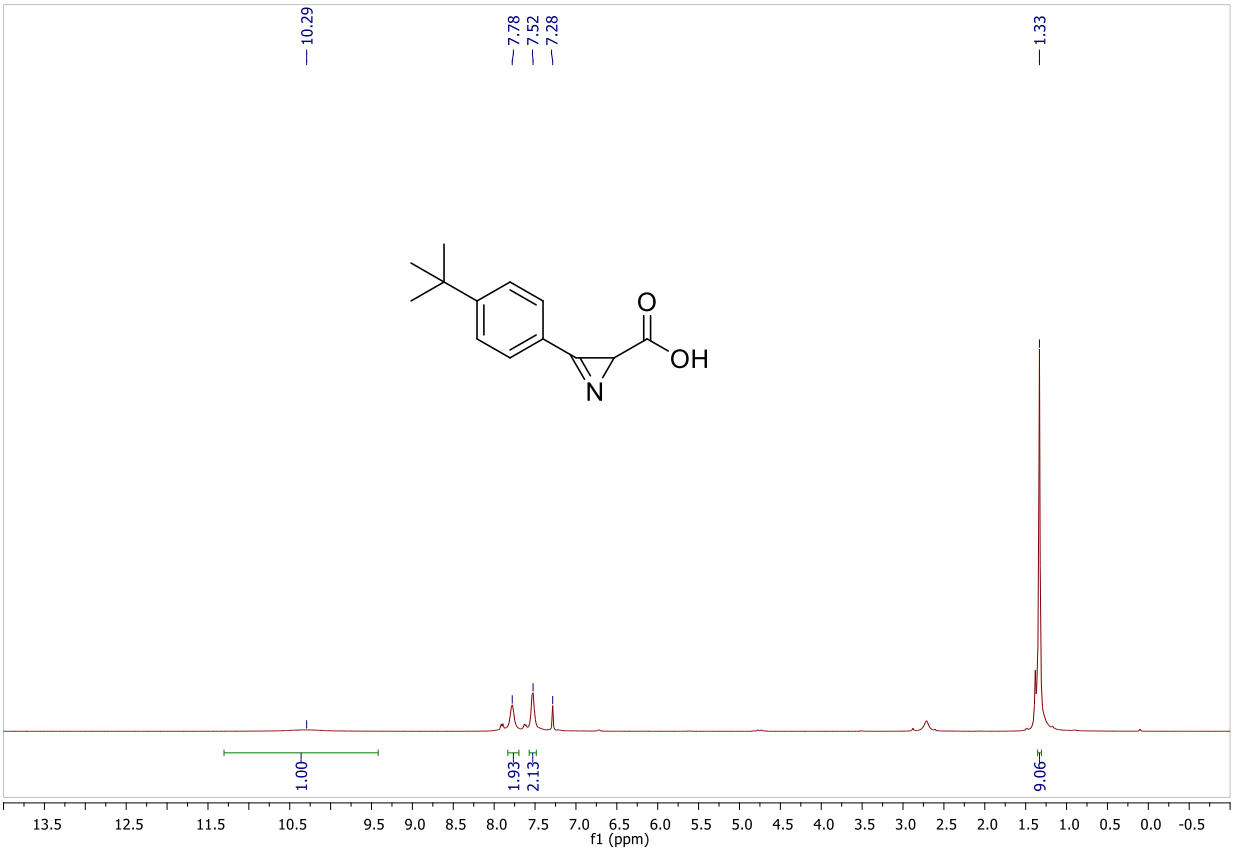


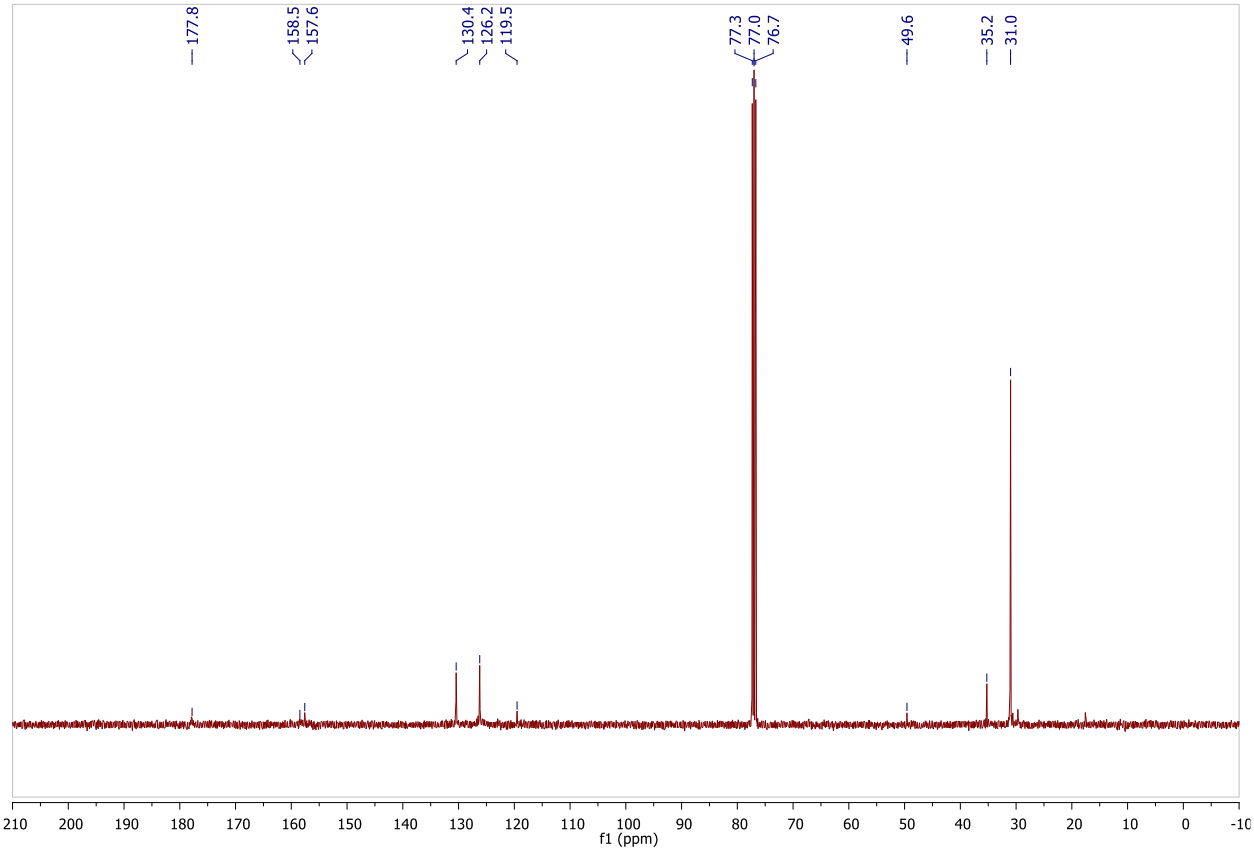
^{1}H and ^{13}C NMR spectra of compound **3f**



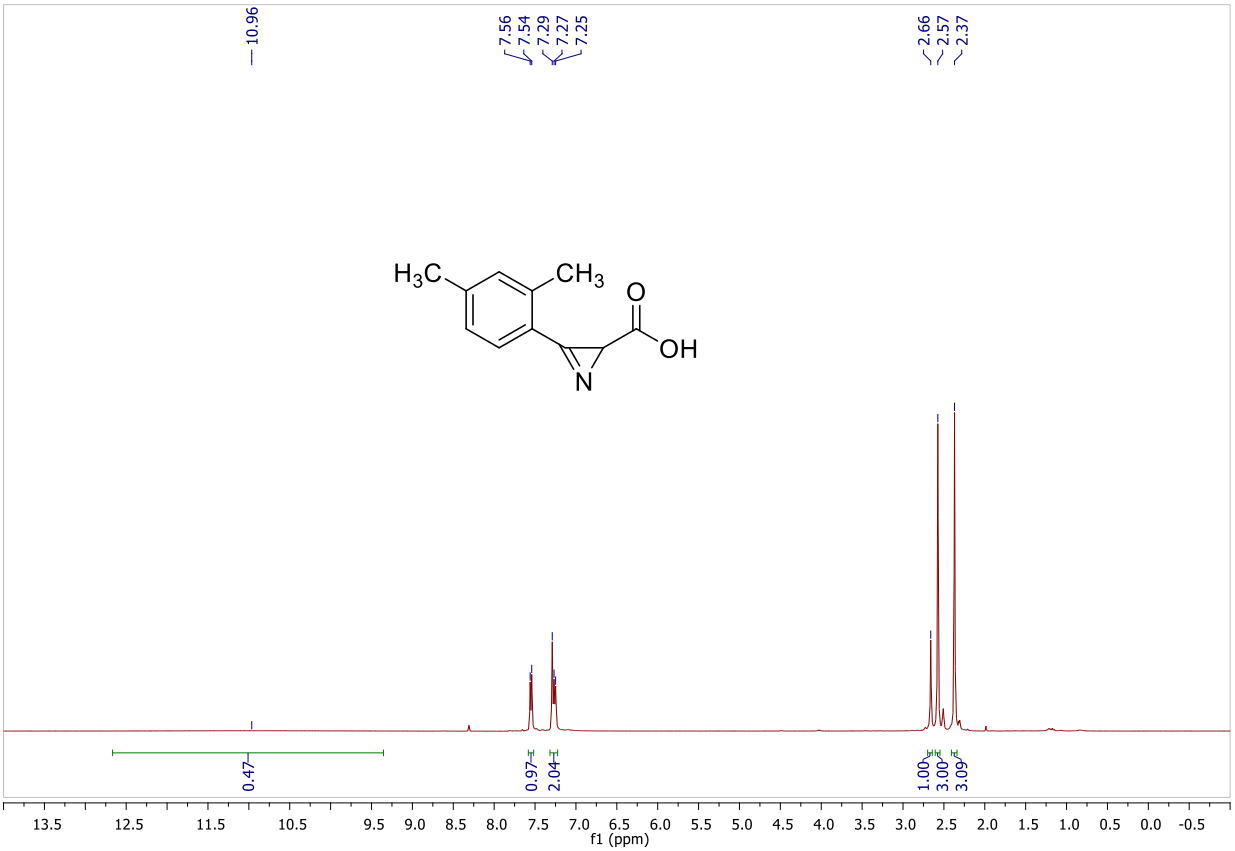


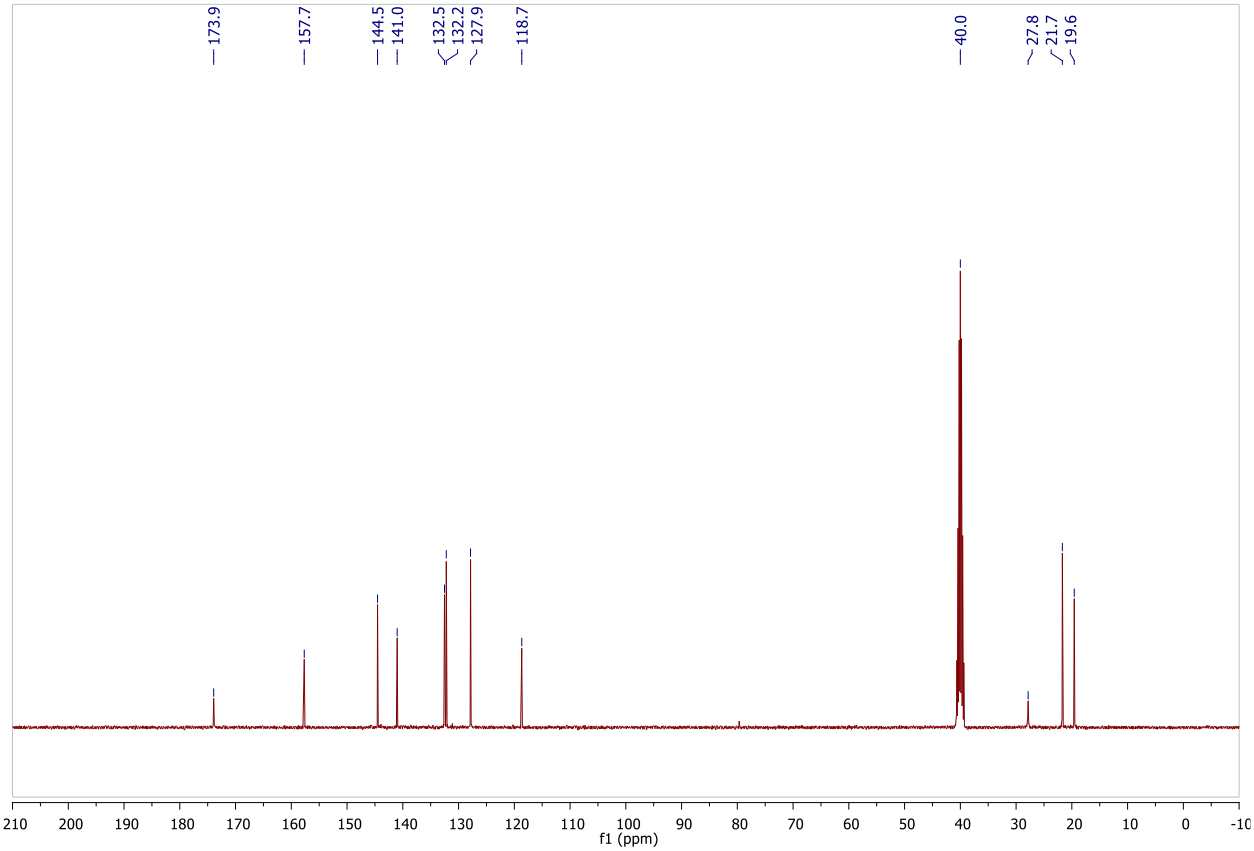
^{1}H and ^{13}C NMR spectra of compound **3g**



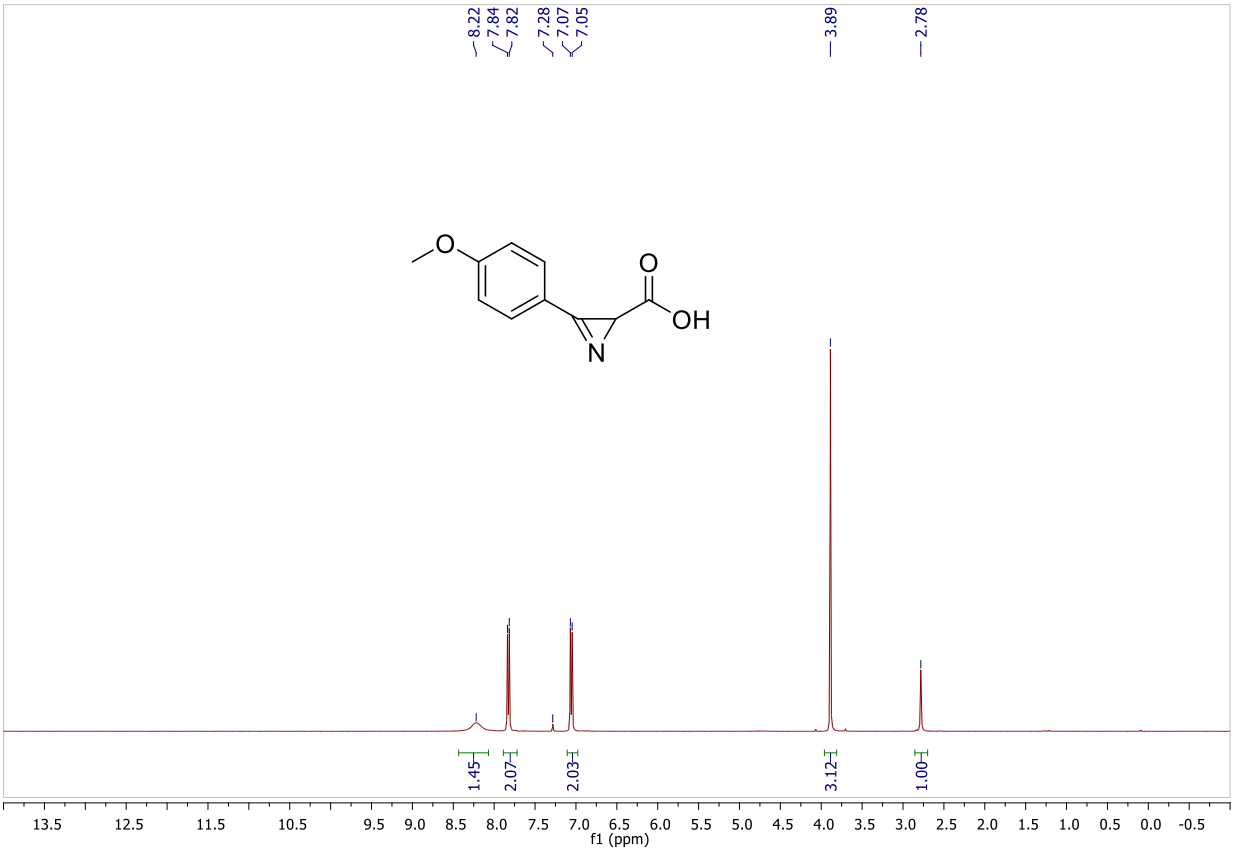


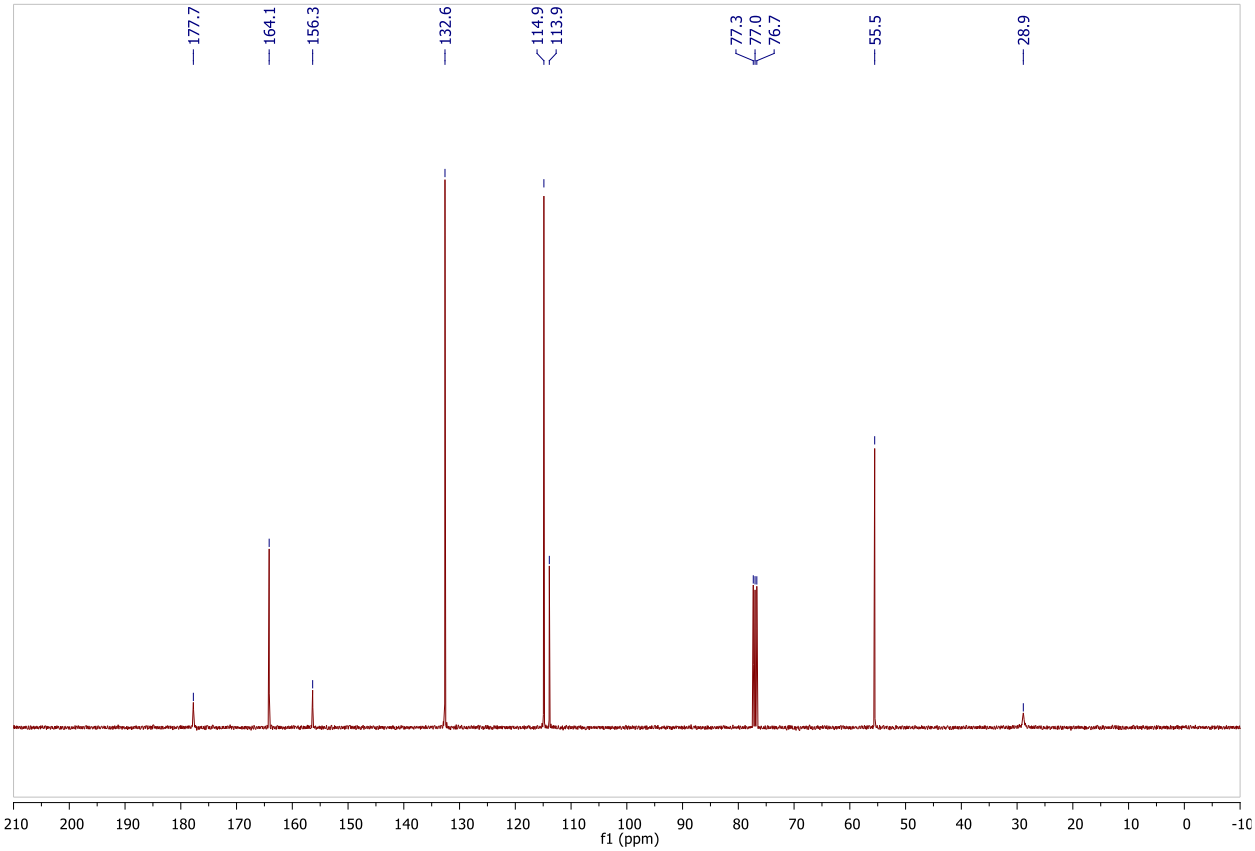
^{1}H and ^{13}C NMR spectra of compound **3h**



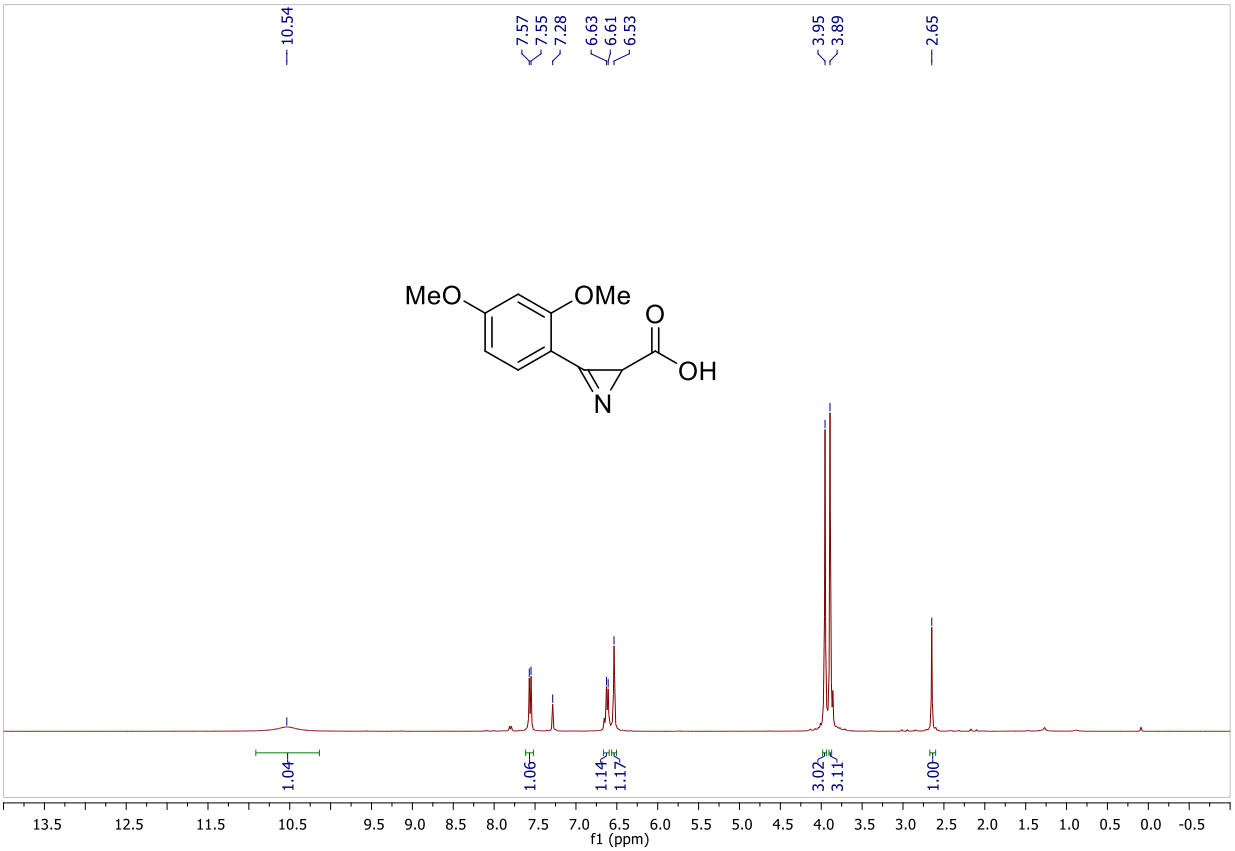


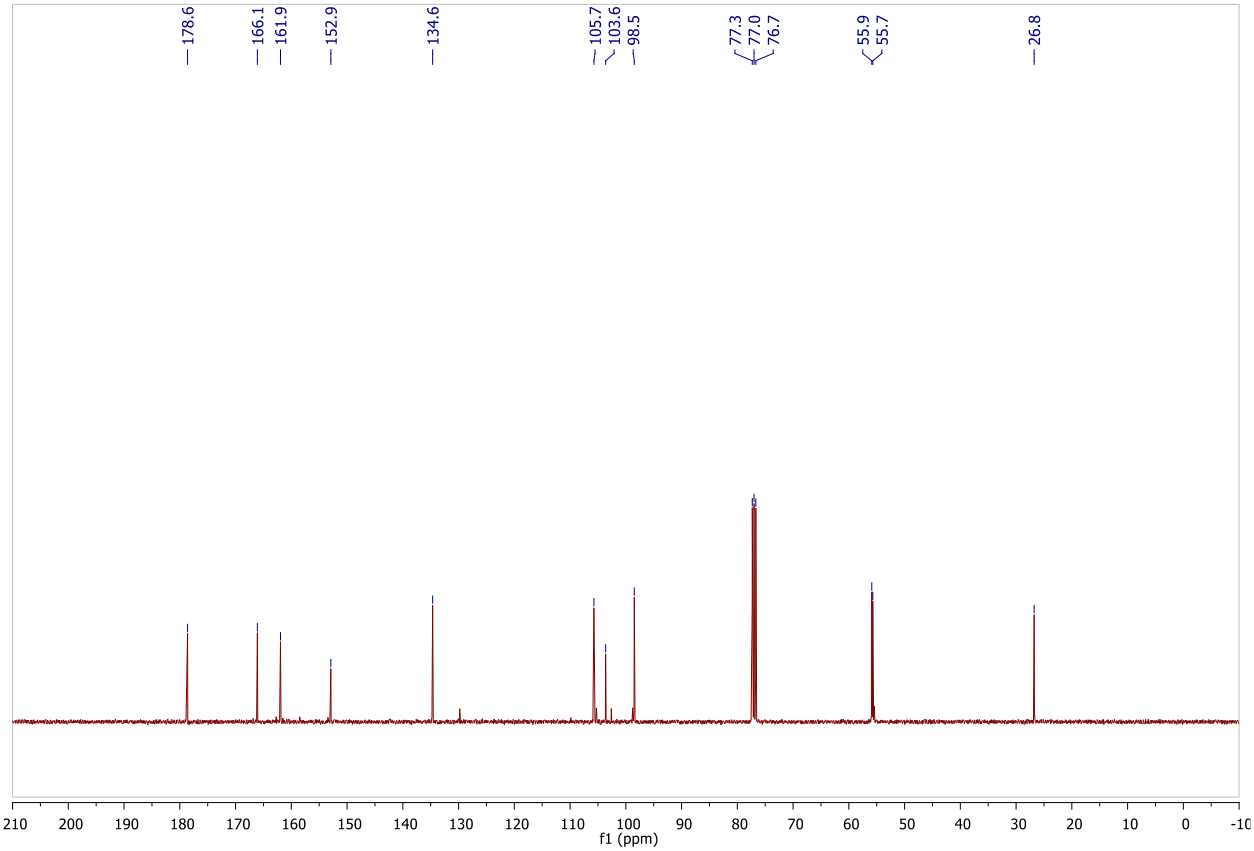
^{1}H and ^{13}C NMR spectra of compound **3i**



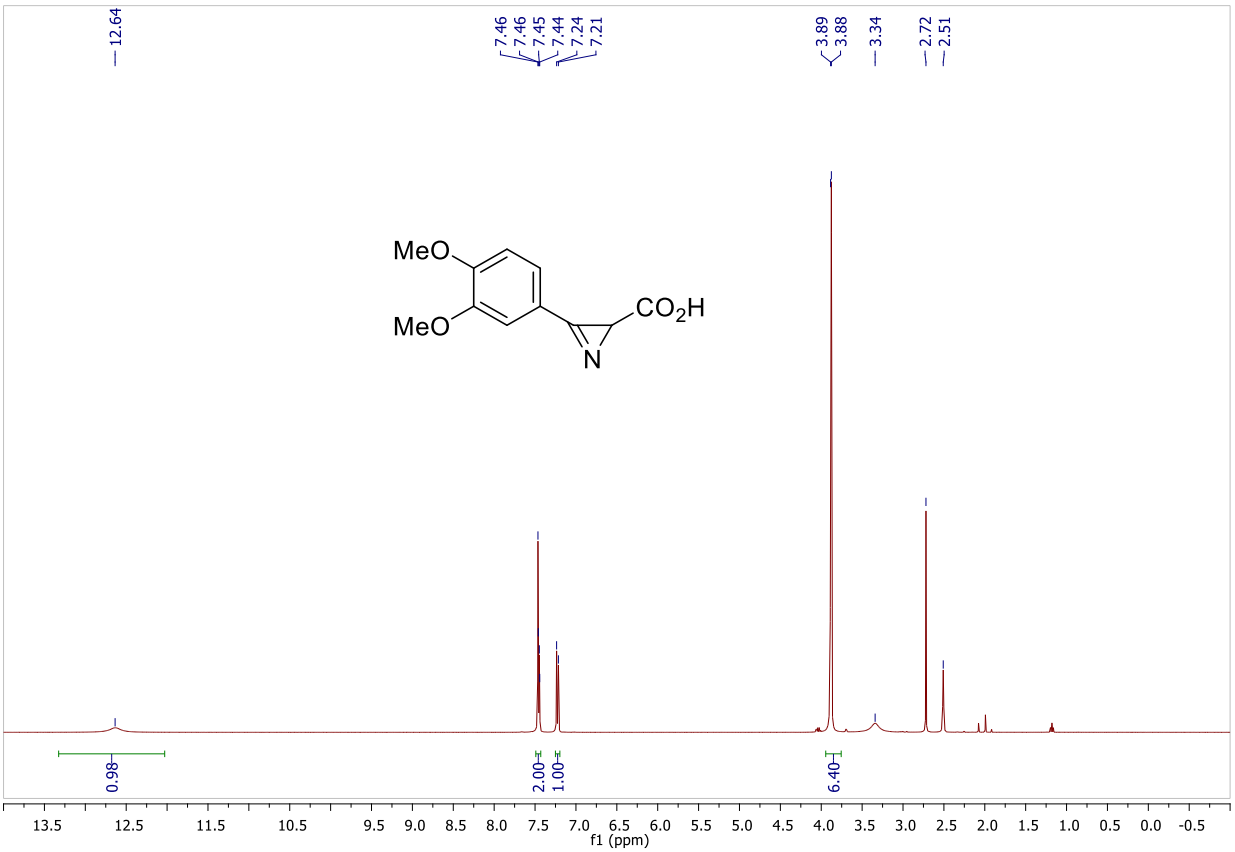


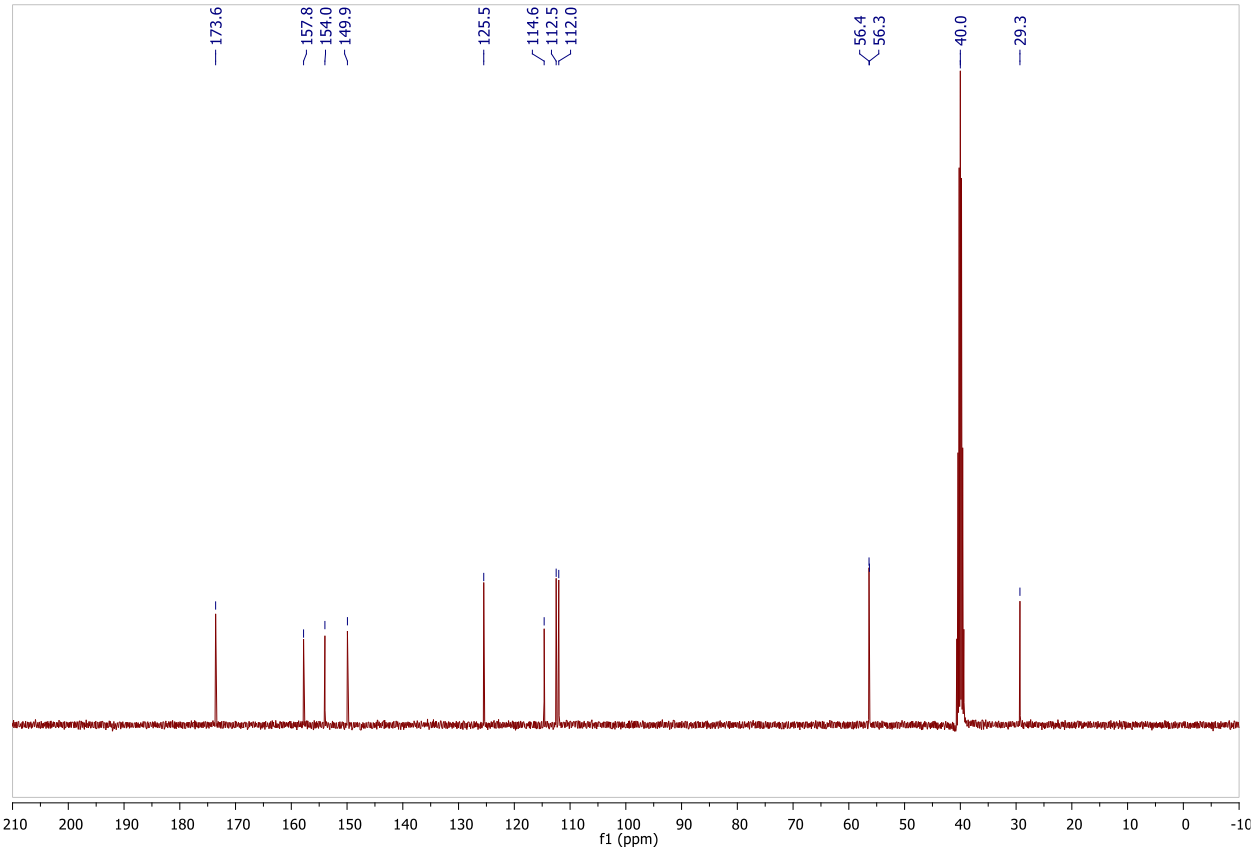
^{1}H and ^{13}C NMR spectra of compound **3j**



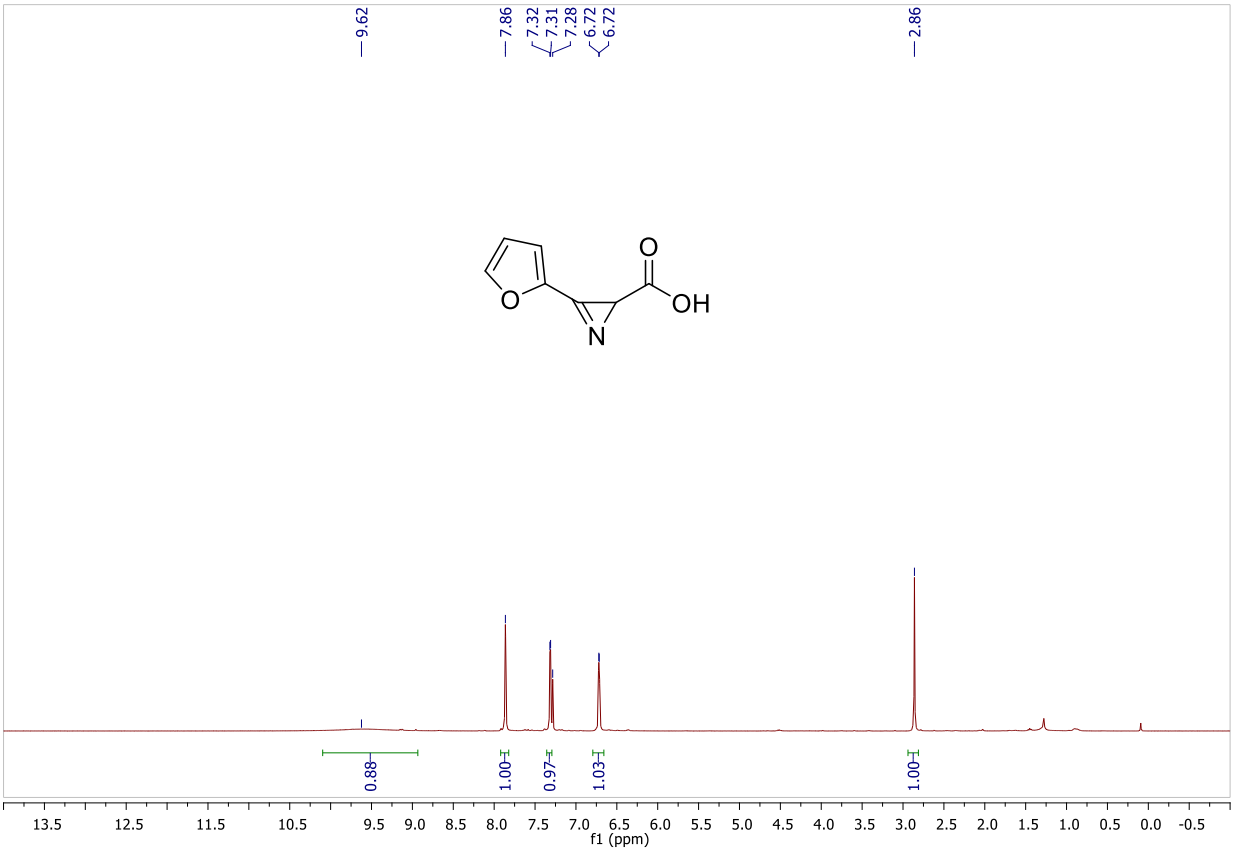


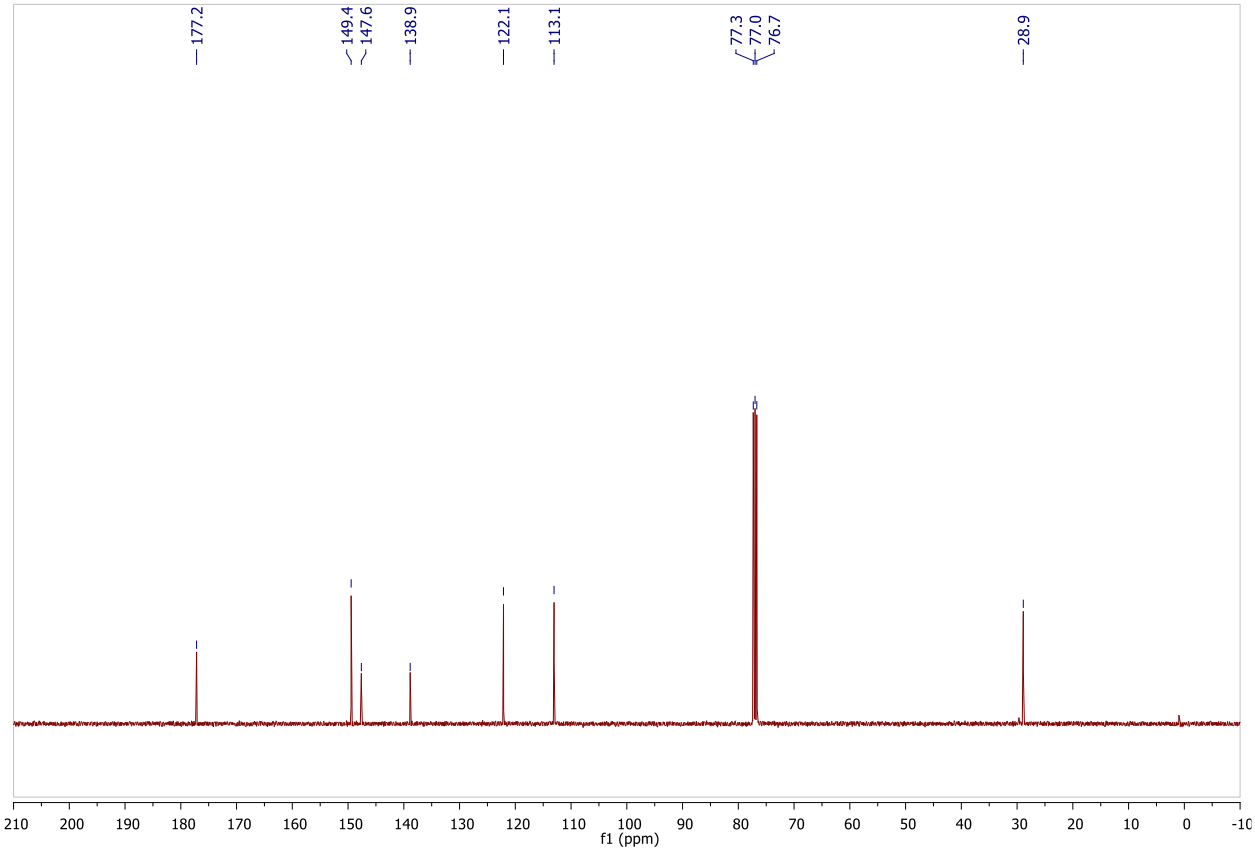
^{1}H and ^{13}C NMR spectra of compound **3k**



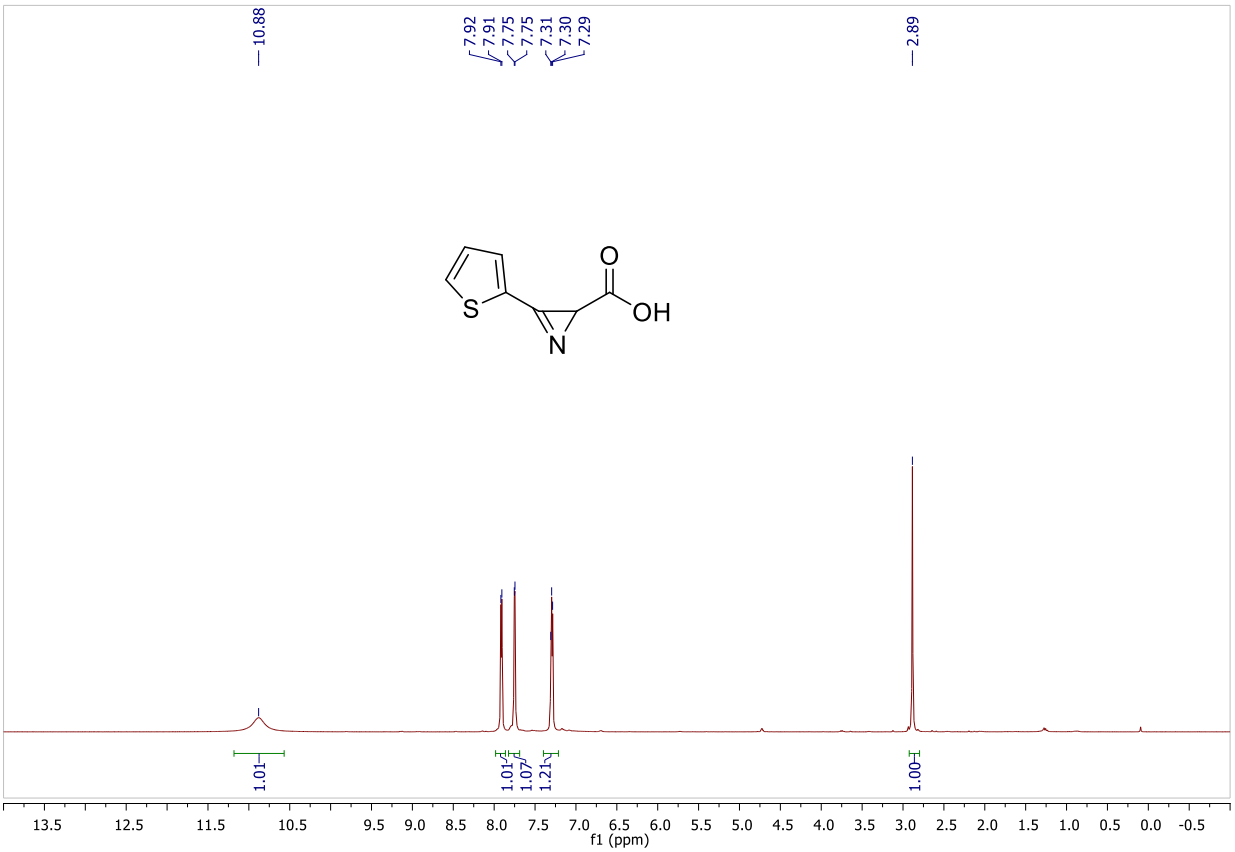


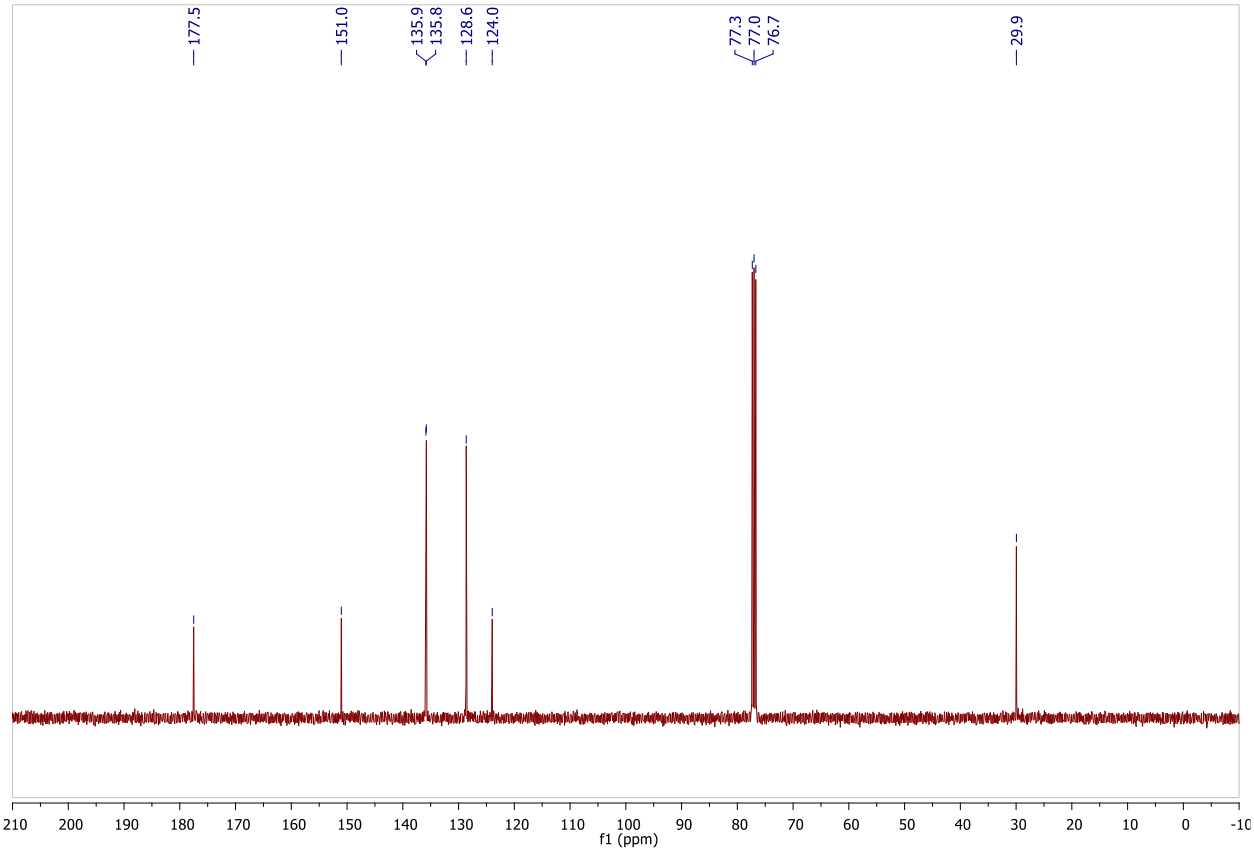
^{1}H and ^{13}C NMR spectra of compound **3l**



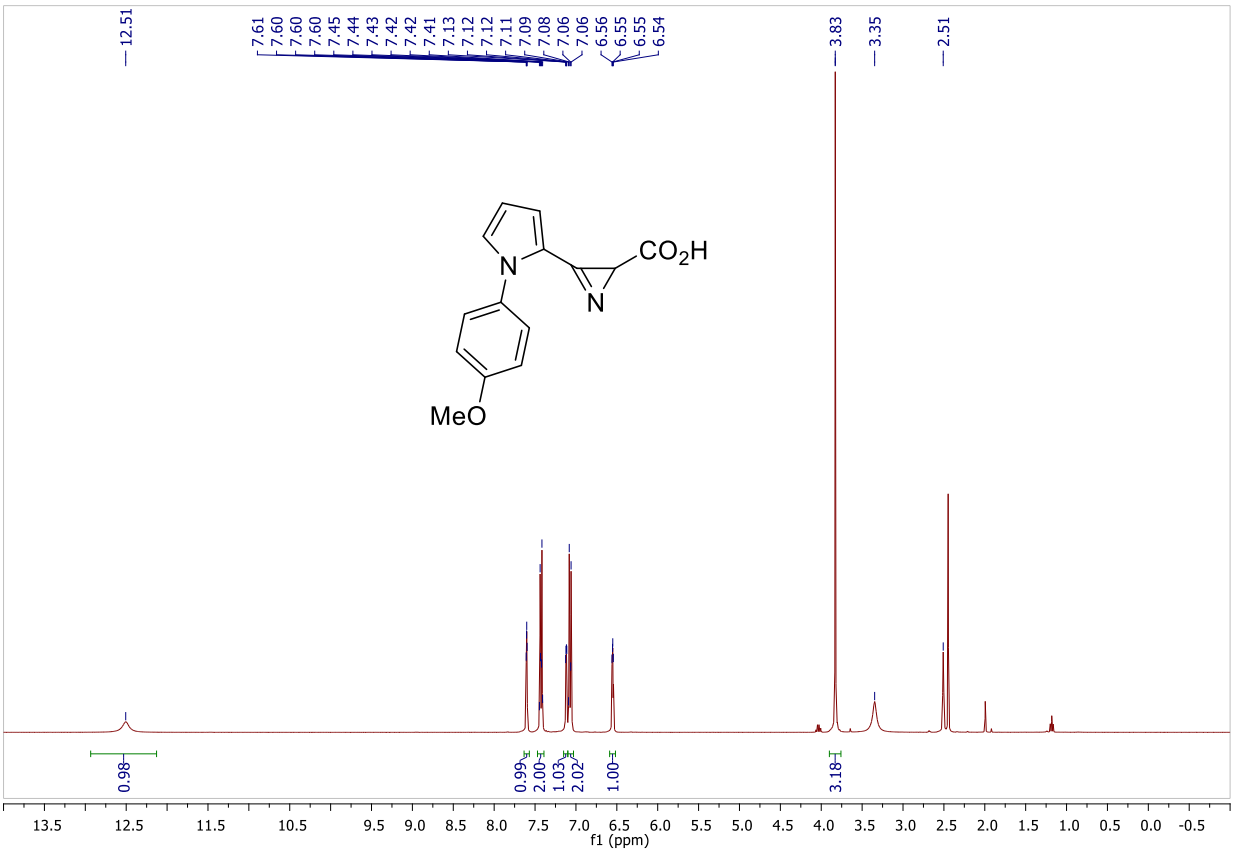


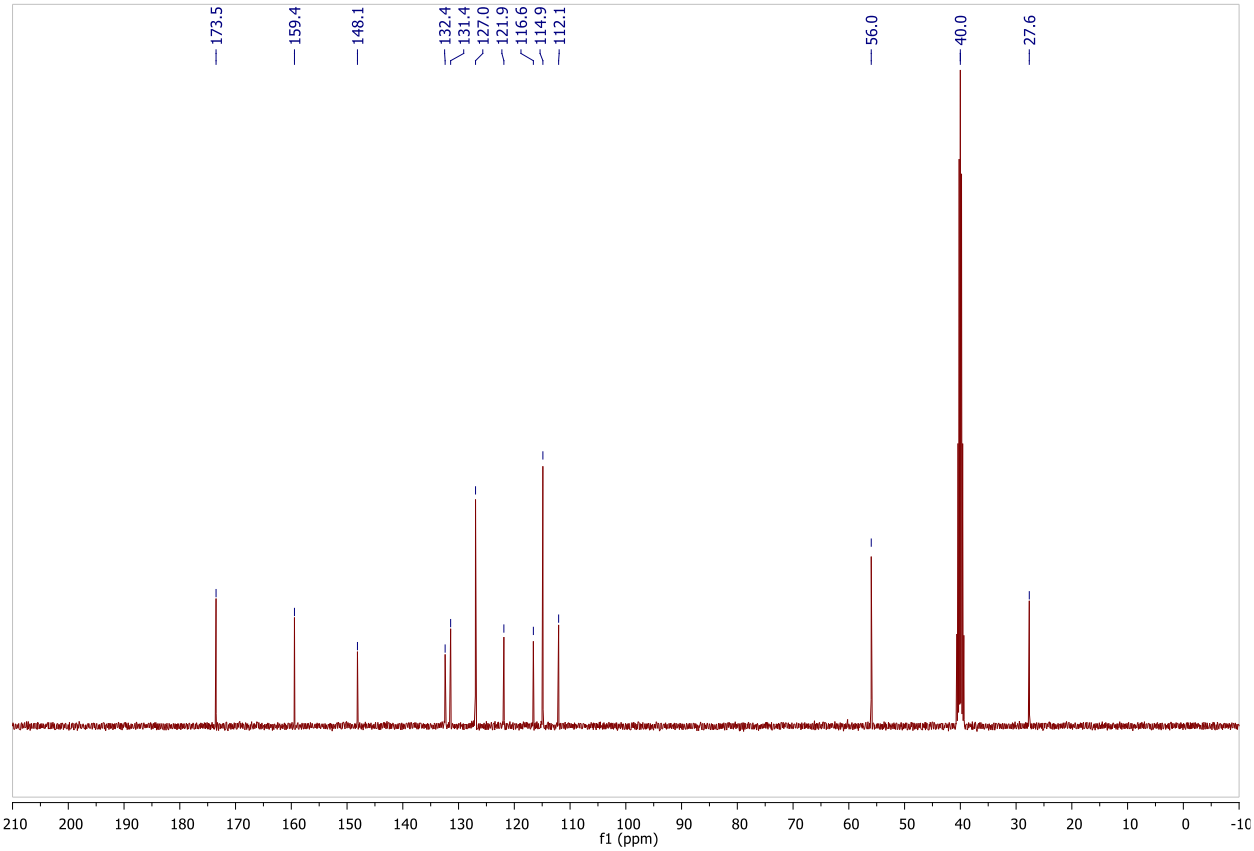
^{1}H and ^{13}C NMR spectra of compound **3m**



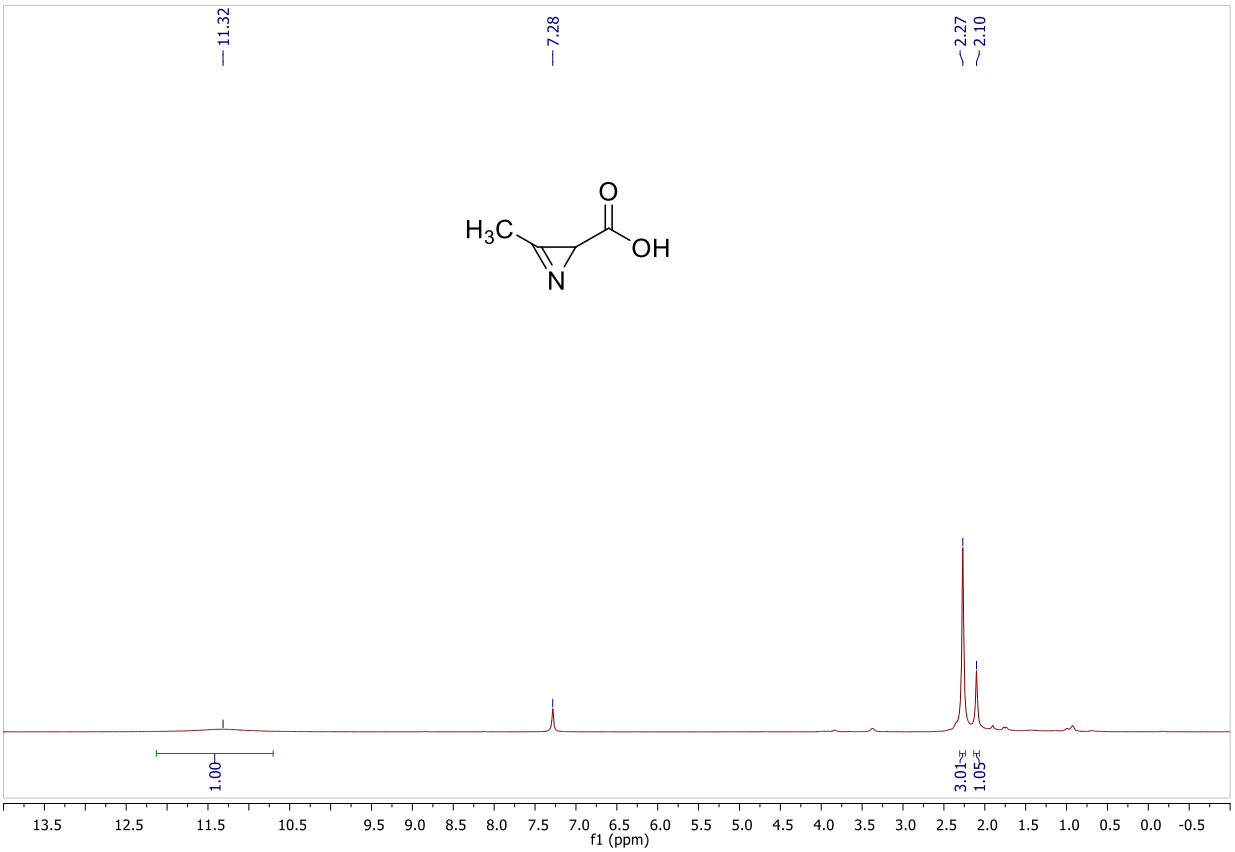


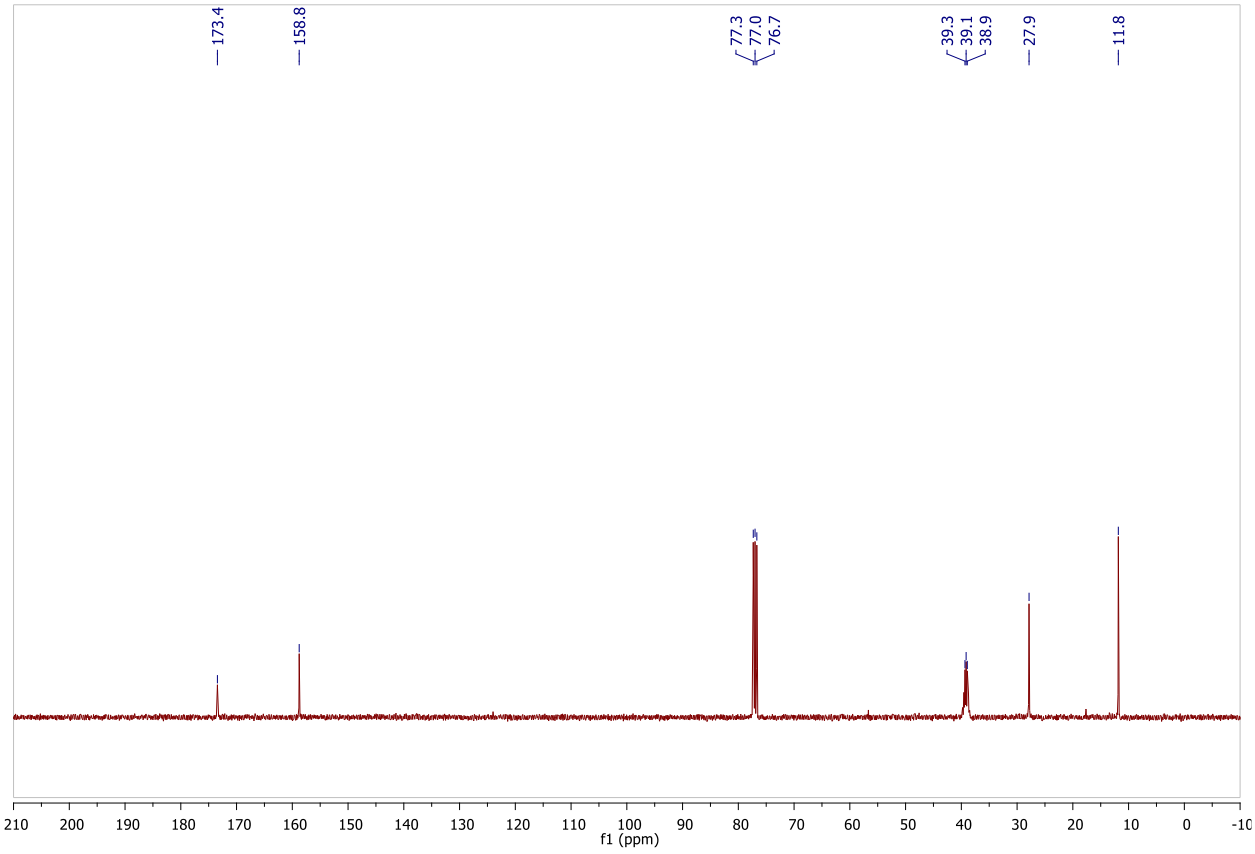
^{1}H and ^{13}C NMR spectra of compound **3n**



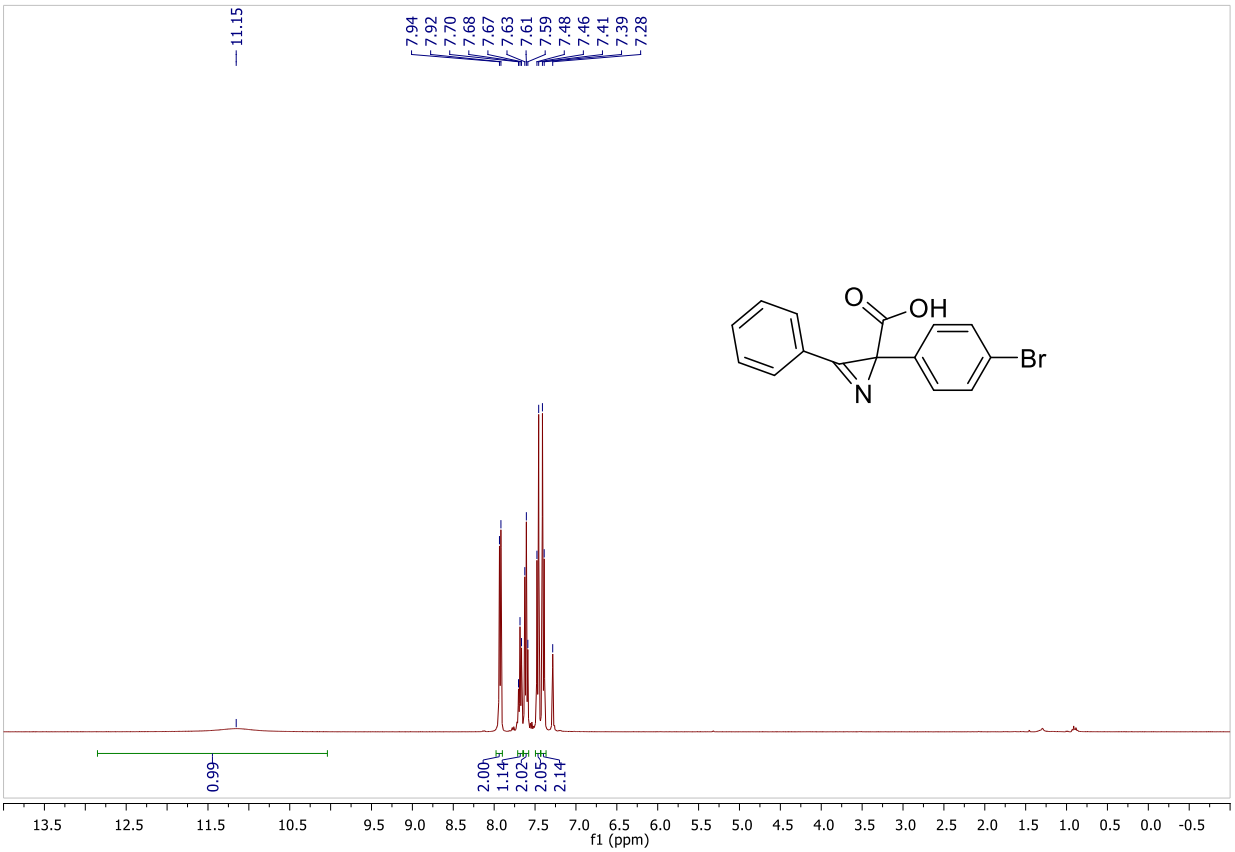


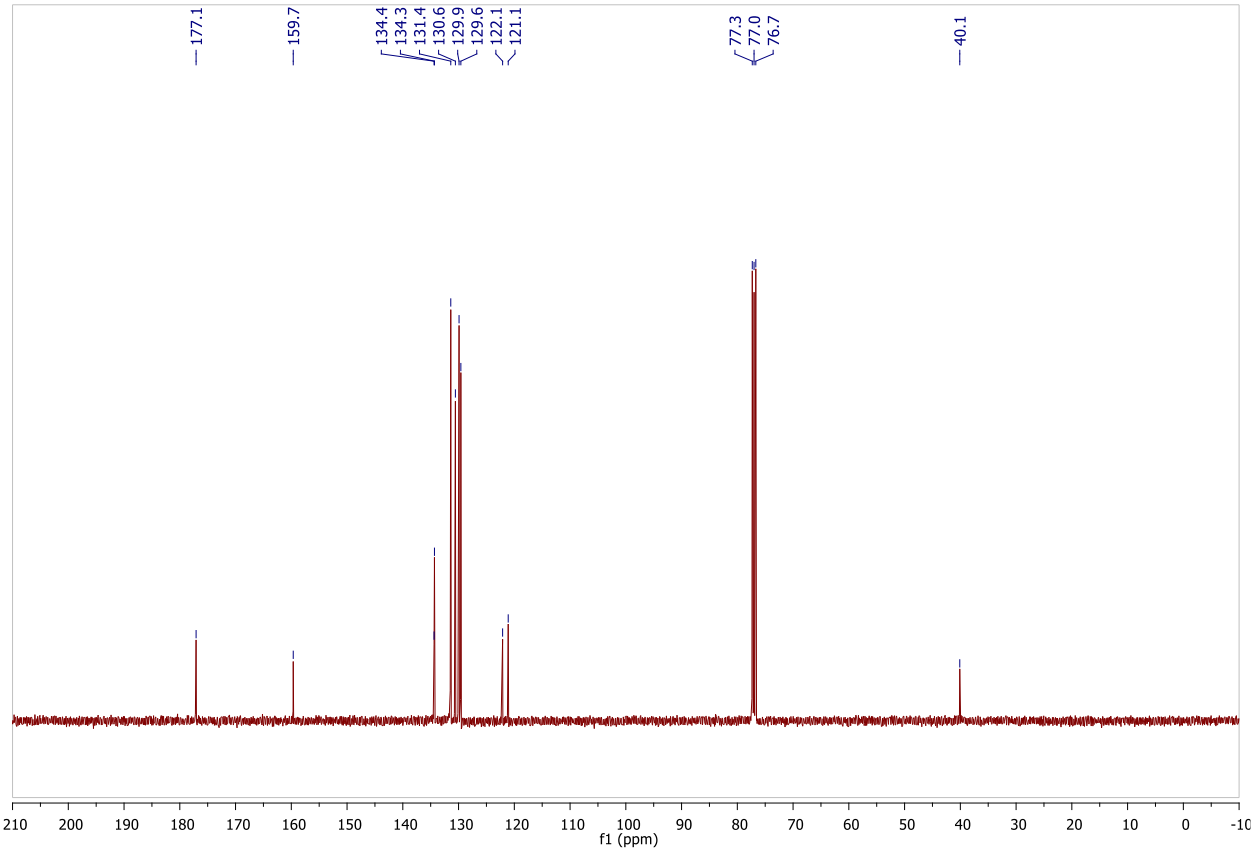
^{1}H and ^{13}C NMR spectra of compound **3o**



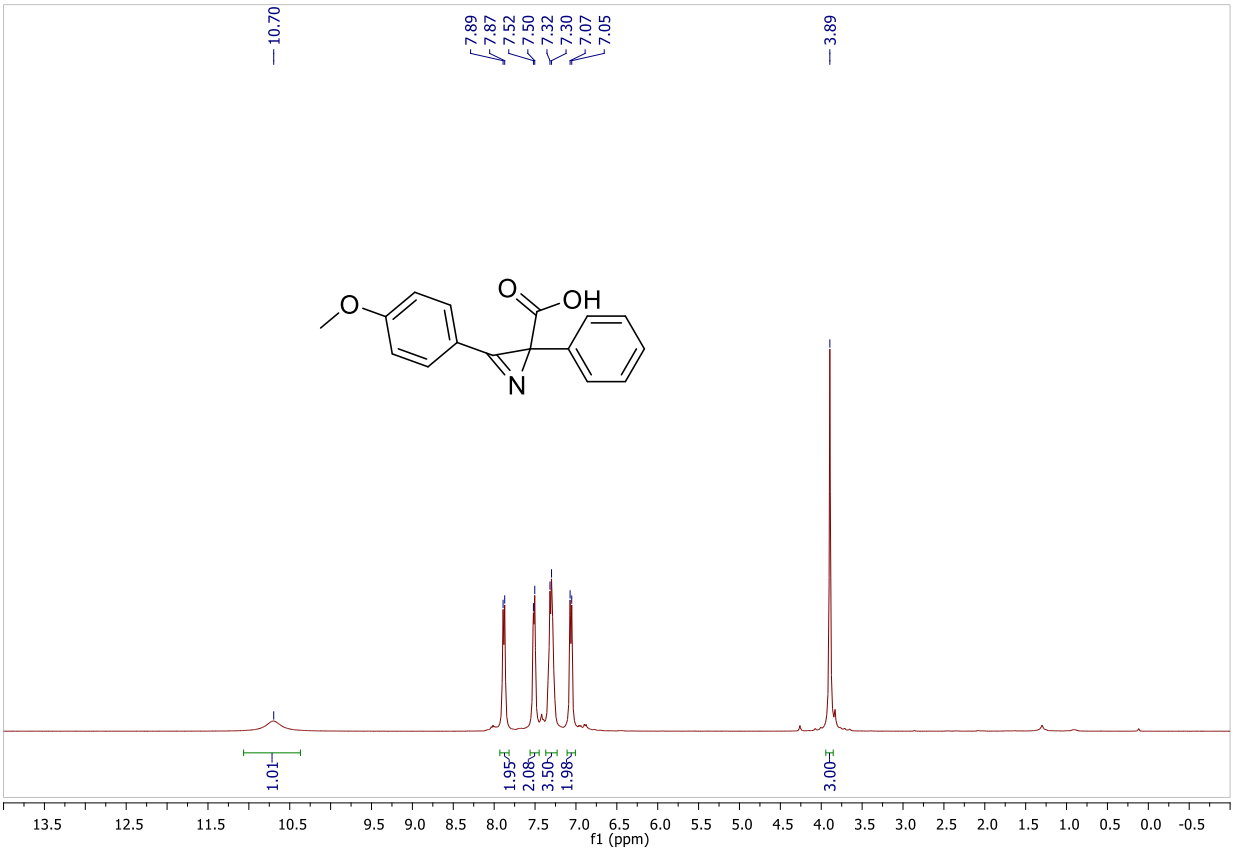


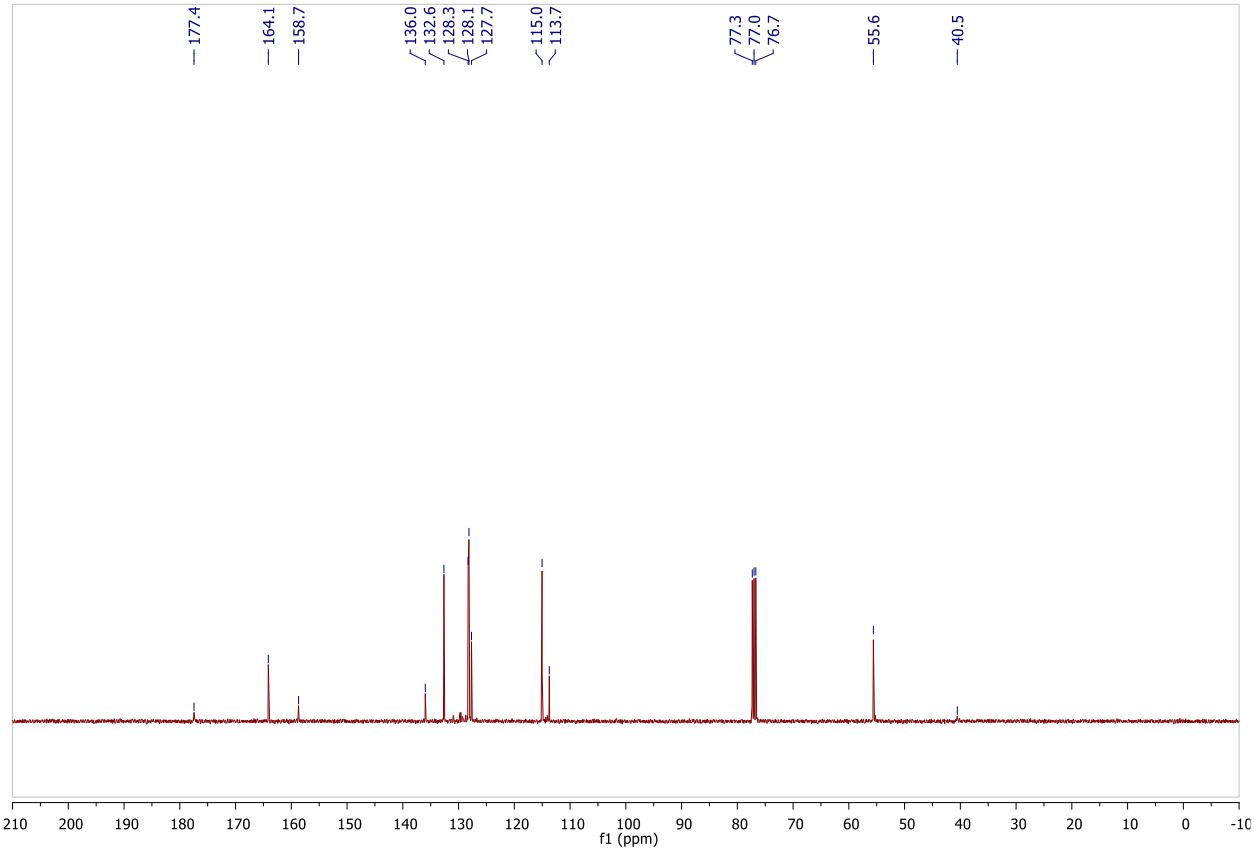
^{1}H and ^{13}C NMR spectra of compound **3p**



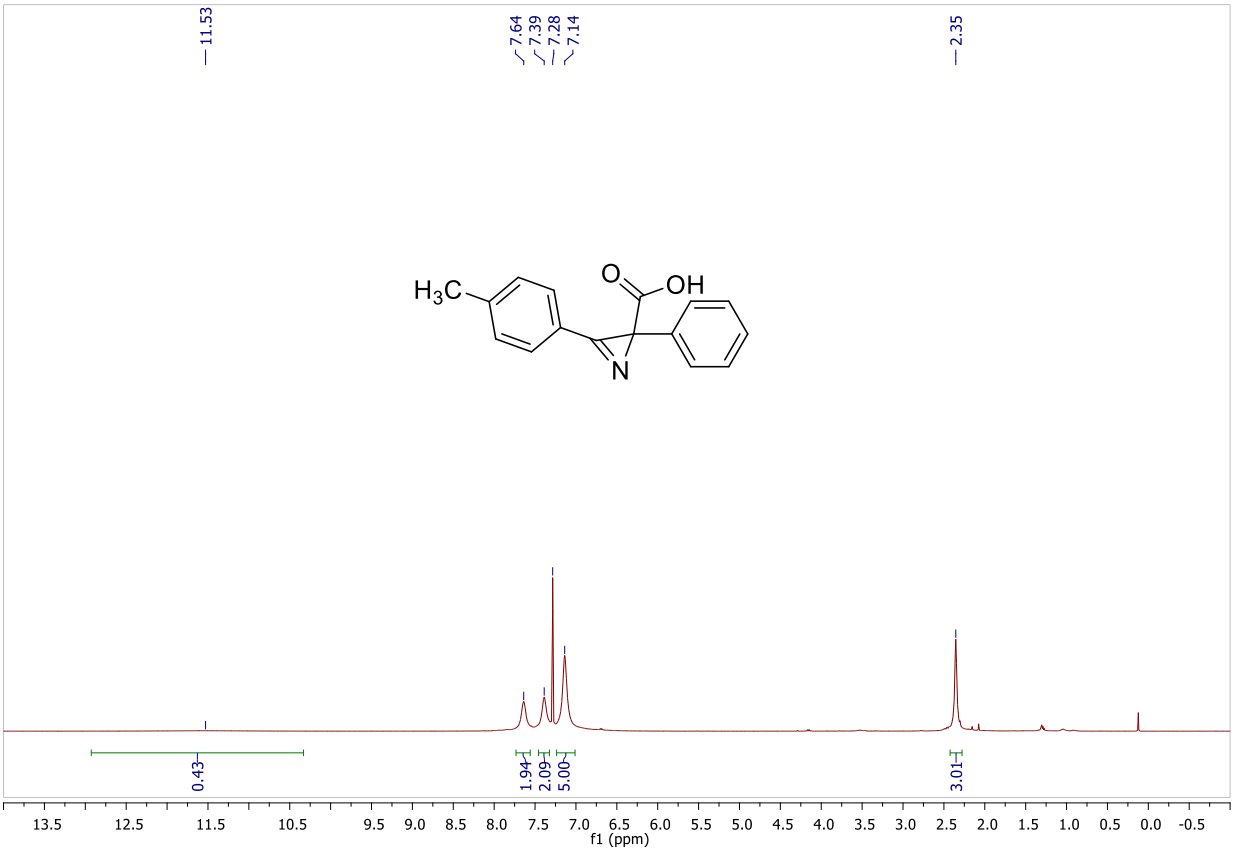


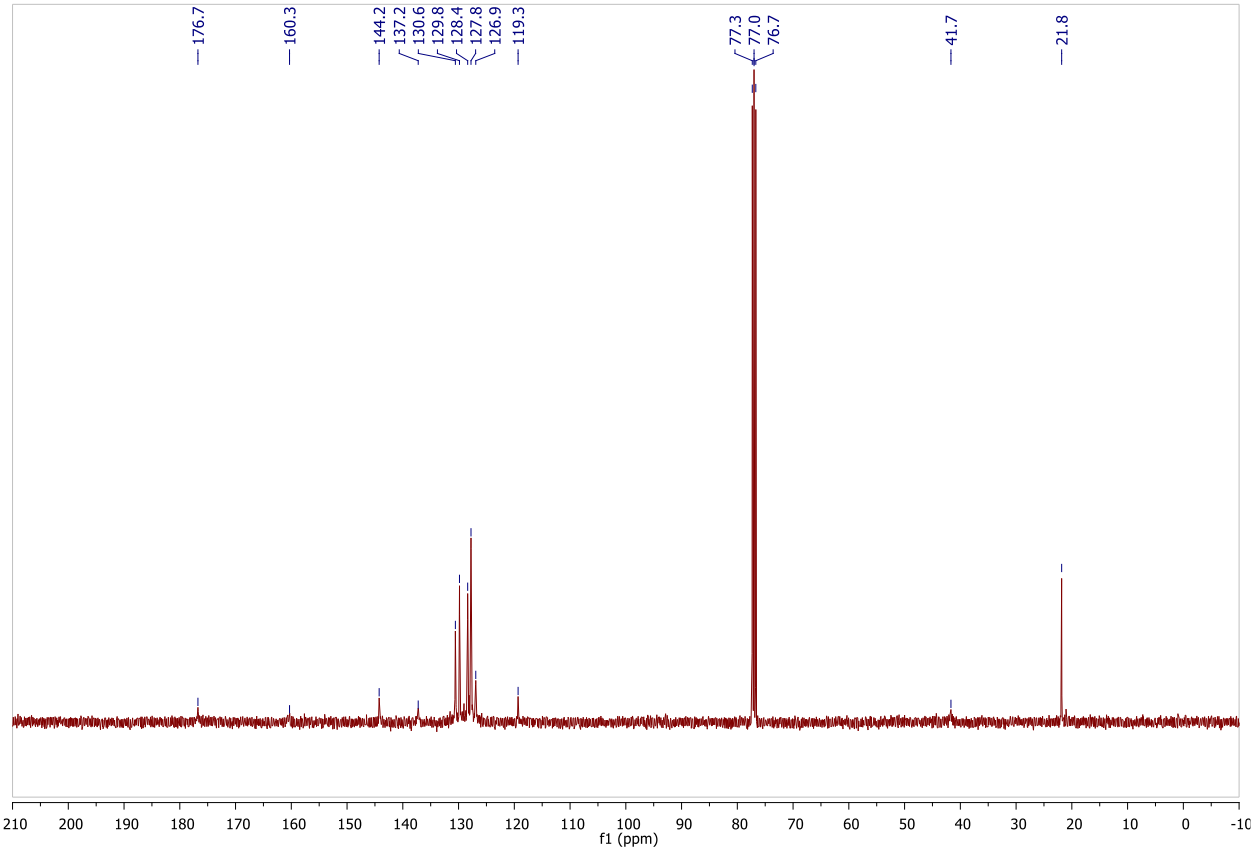
^{1}H and ^{13}C NMR spectra of compound **3q**



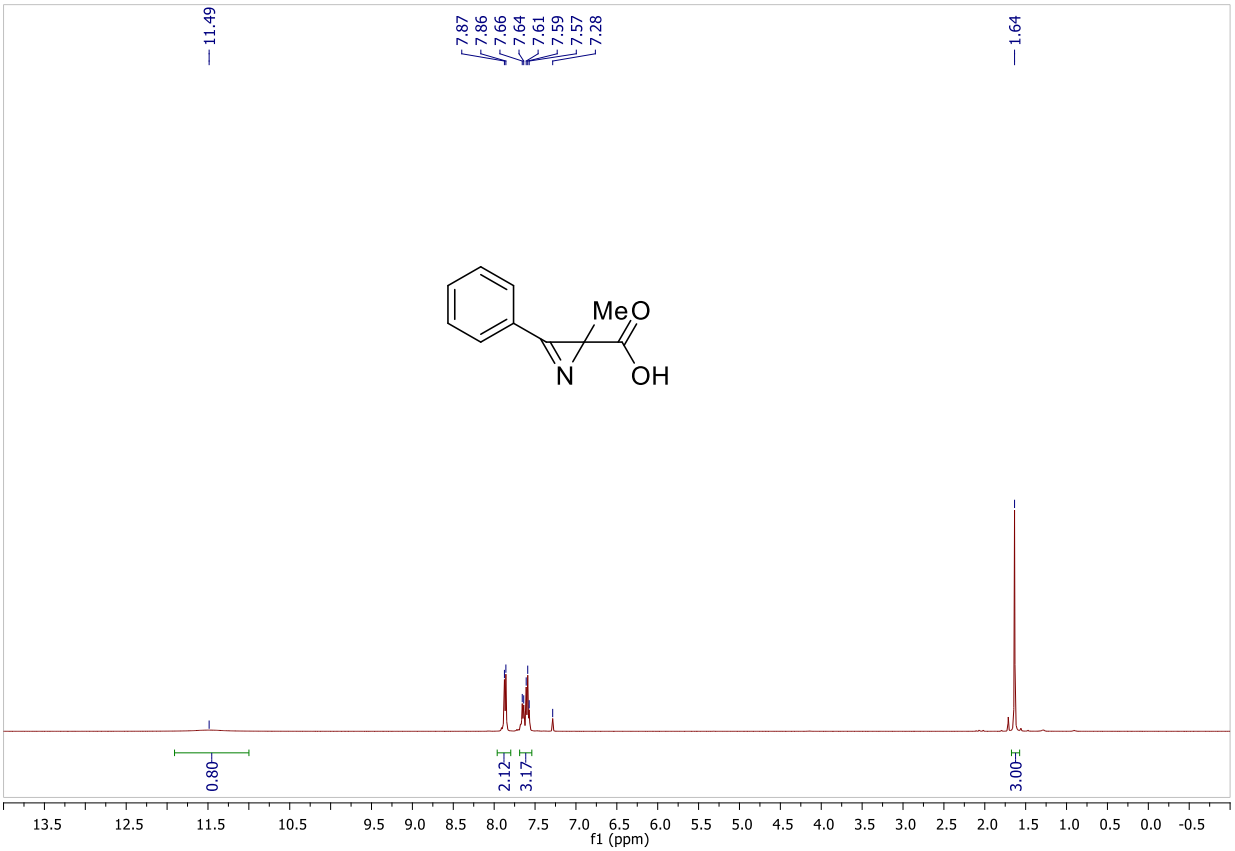


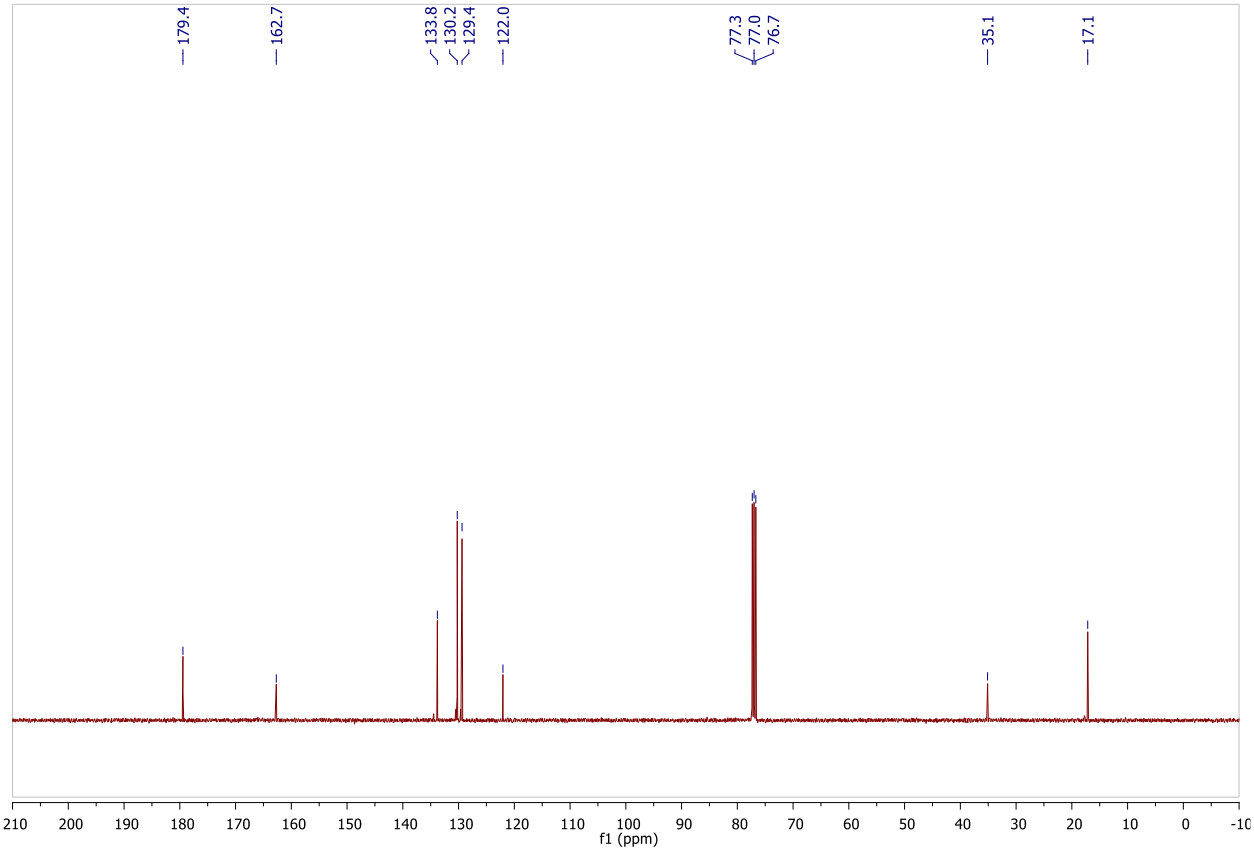
^{1}H and ^{13}C NMR spectra of compound **3r**



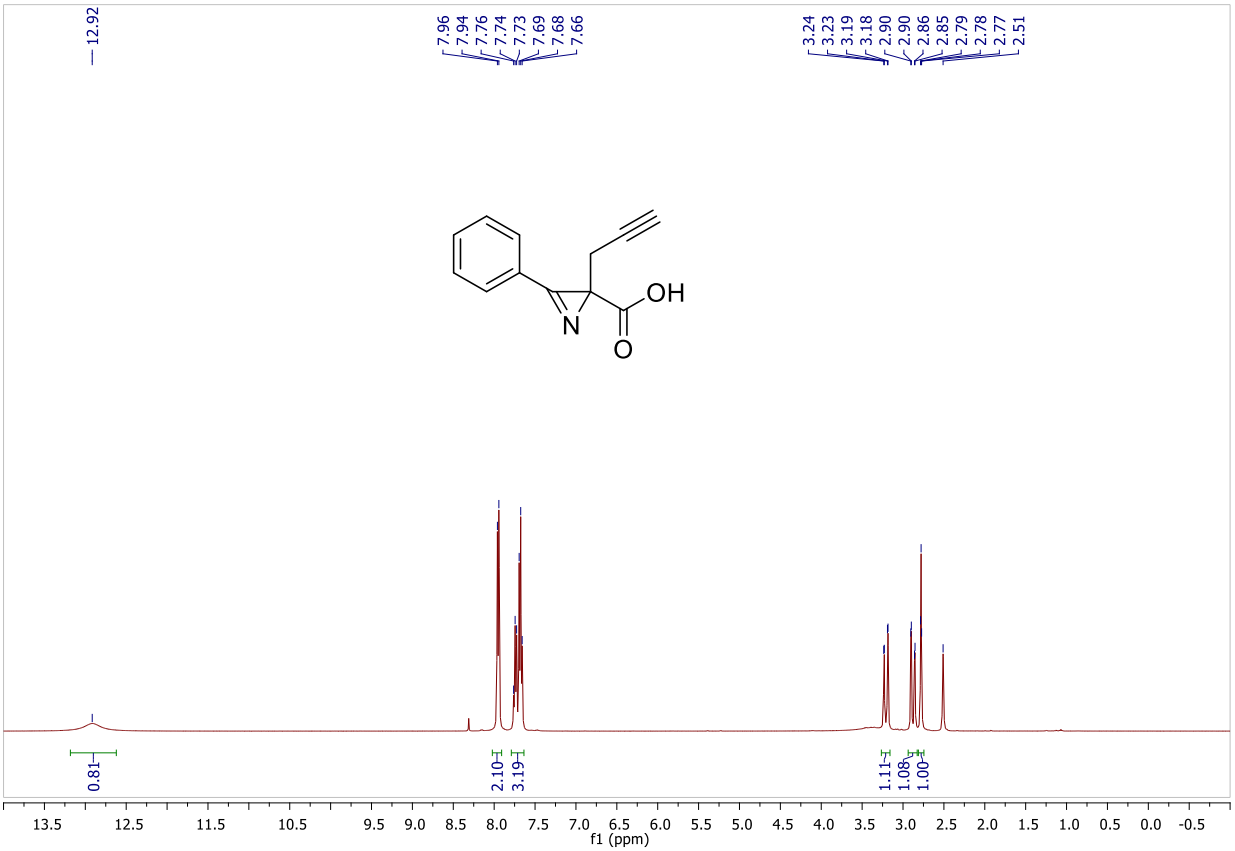


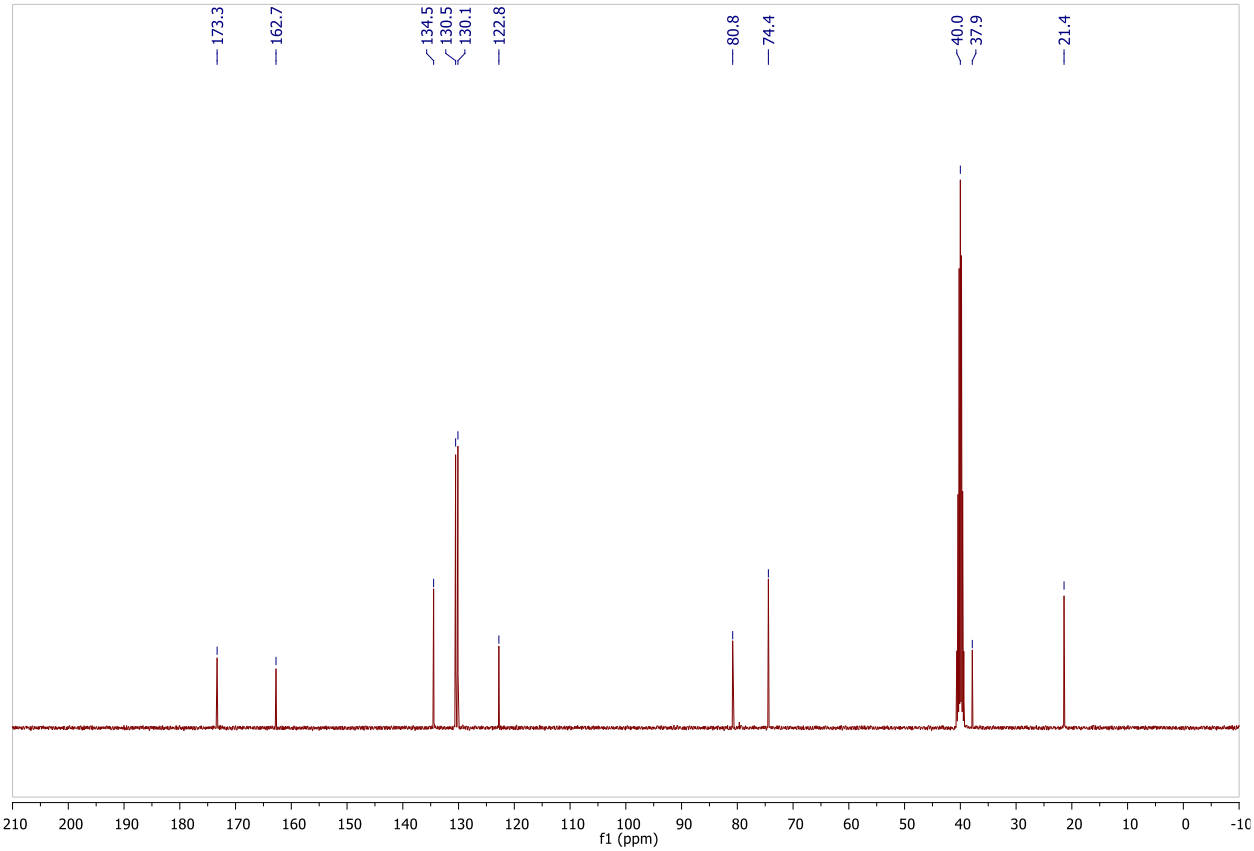
^{1}H and ^{13}C NMR spectra of compound **3s**



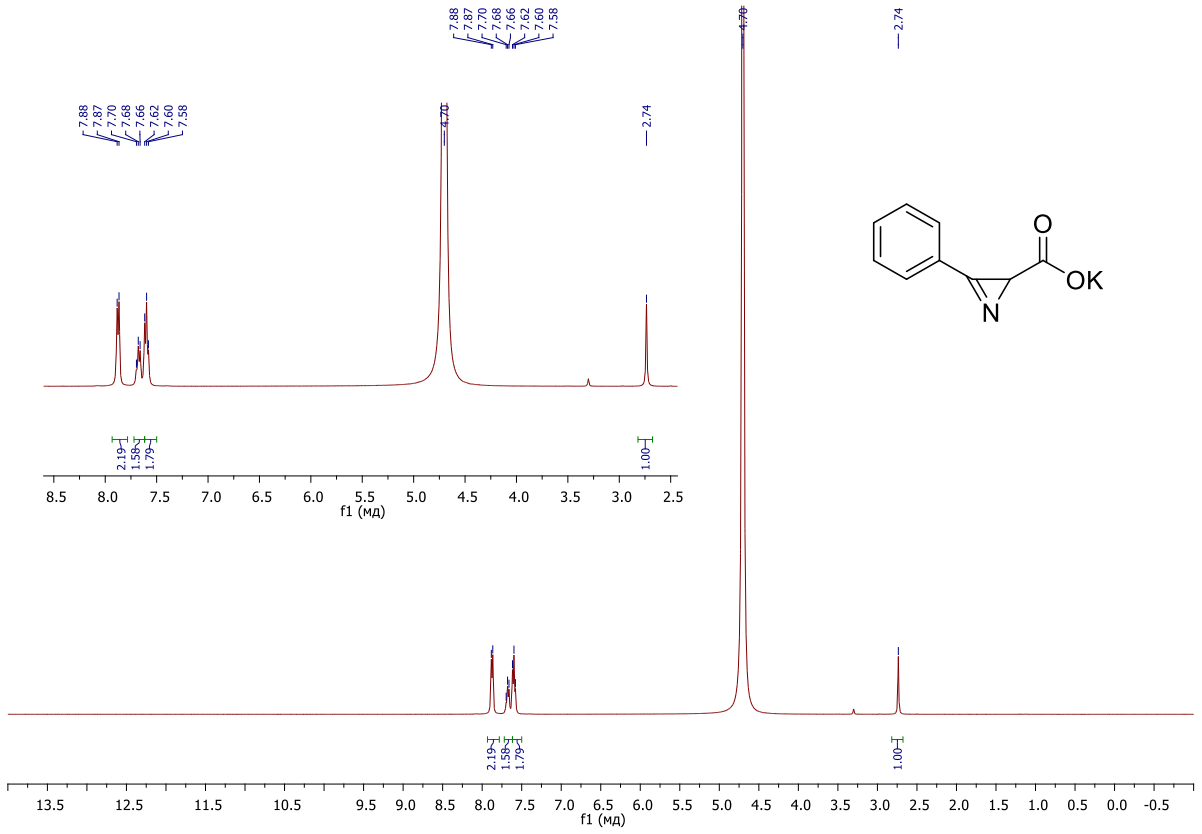


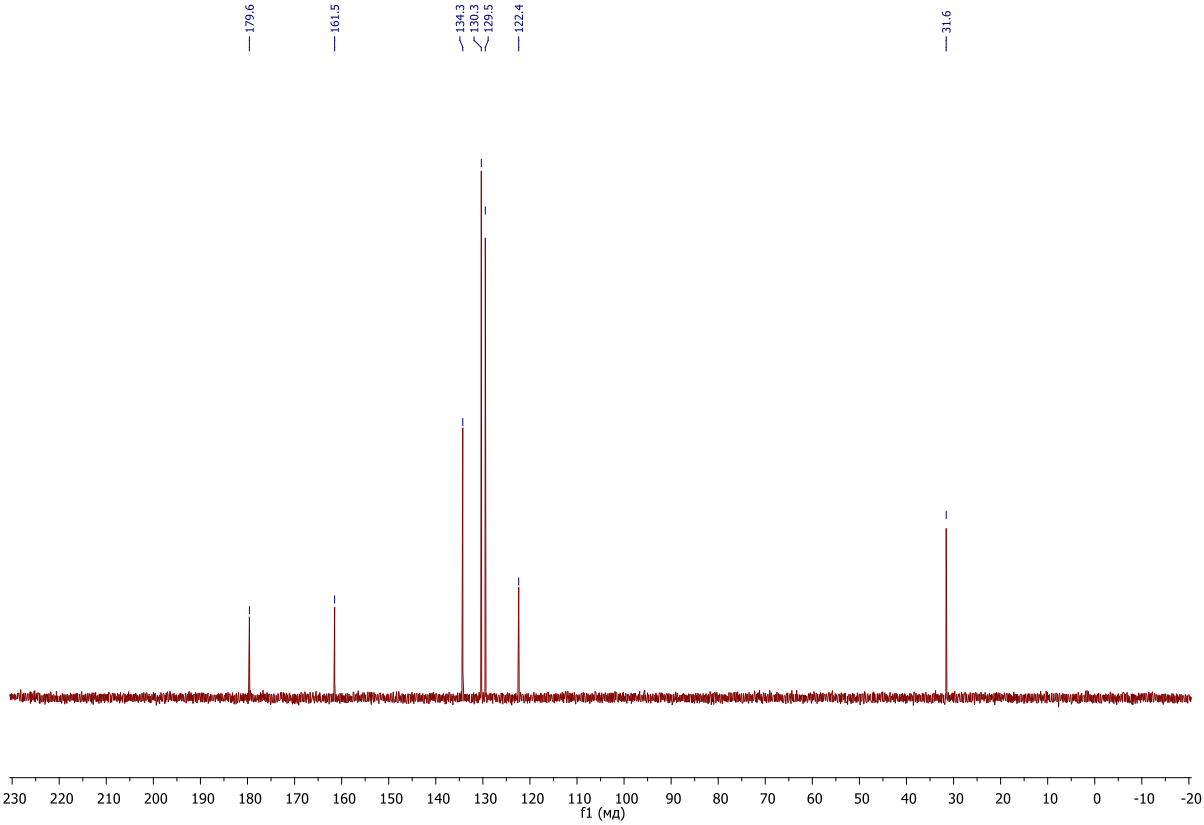
^{1}H and ^{13}C NMR spectra of compound **3t**





11 ^{1}H and ^{13}C NMR spectra of salt 4





12 X-Ray Data of Compound 3a

Figure S-1. X-Ray Crystal Structure of Compound **3a** with 50% Ellipsoid Probability (CCDC 1956437)

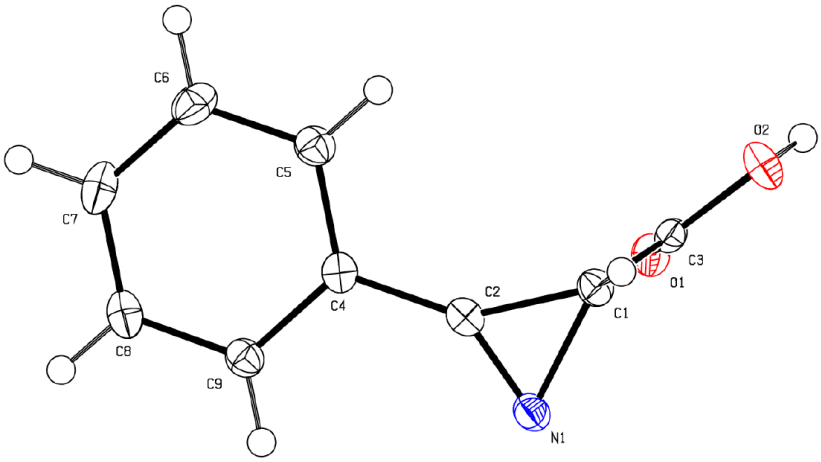
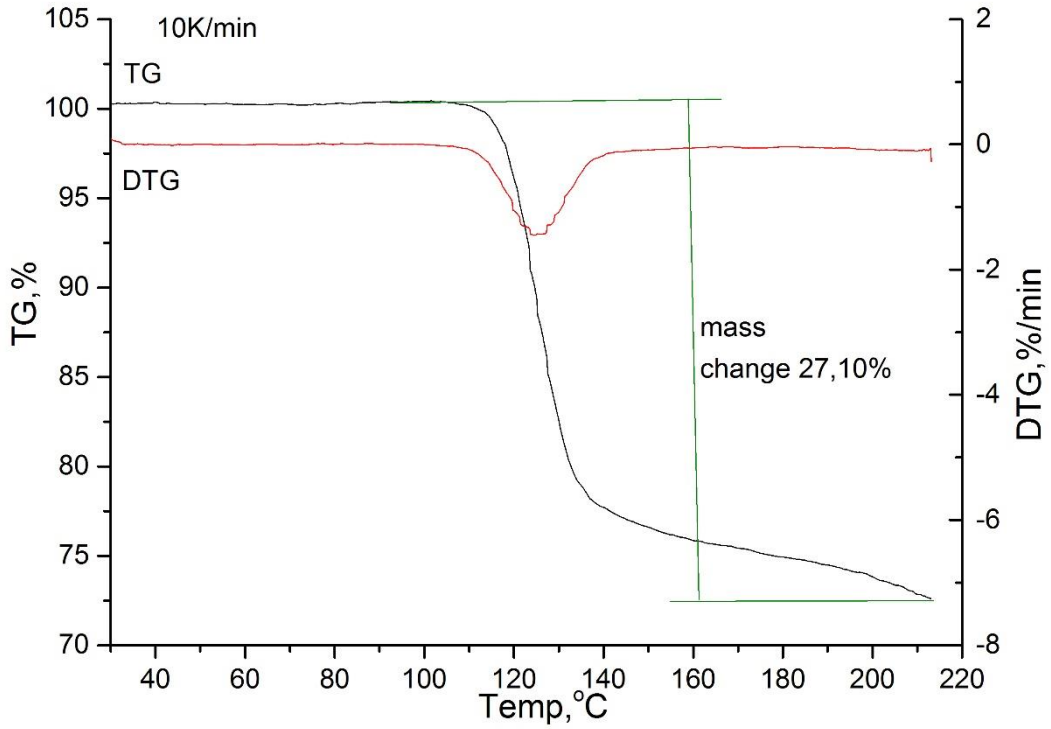


Table S-2. Crystal data and structure refinement for compound 3a.

Identification code	pas5
Empirical formula	$C_9H_7NO_2$
Formula weight	161.16
Temperature/K	100(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	8.0481(9)
b/Å	9.7876(9)
c/Å	10.8529(13)
α/°	90
β/°	111.525(13)
γ/°	90
Volume/Å^3	795.28(16)
Z	4
ρ_{calc}g/cm^3	1.346
μ/mm^{-1}	0.097
F(000)	336.0
Crystal size/mm^3	0.45 × 0.21 × 0.19
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.442 to 56.992
Index ranges	$-9 \le h \le 10$, $-13 \le k \le 13$, $-14 \le l \le 13$
Reflections collected	4098
Independent reflections	2004 [R_{int} = 0.0251, R_{sigma} = 0.0418]
Data/restraints/parameters	2004/0/110
Goodness-of-fit on F^2	1.042
Final R indexes [I>=2σ (I)]	R_1 = 0.0409, wR_2 = 0.0932
Final R indexes [all data]	R_1 = 0.0512, wR_2 = 0.1014
Largest diff. peak/hole / e Å^{-3}	0.33/-0.21

13 Thermogravimetric analysis of compound 3a



14 Differential scanning calorimetry analysis of compound 3a