SUPPORTING INFORMATION

Design and Synthesis of Isoxazole-functionalized Benzene Sulphonamides as novel inhibitors of *Mycobacterium tuberculosis* *β*-Carbonic Anhydrases

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1 Materials and Methods

1.1 Chemicals and Instrumentation

All the starting materials, chemicals, and reagents were purchased commercially and were used as such. Analytical TLC (thin layer chromatography) was performed on precoated Merck silica gel 60-F_{254} (0.5 mm) aluminium plates visualised under UV light. We used #60-120 or 100-200 mesh silica gel to perform the column chromatography. Nuclear magnetic resonance(^{1}H and ^{13}C NMR) spectra were recorded on a Bruker Advance 500MHz by preparing a sample solution in DMSO-d_6, which contains tetramethylsilane (TMS) as a reference standard. Chemical shifts for ^{1}H and ^{13}C NMR are provided in parts per million (ppm) downfield from tetramethylsilane. $CDCl_3$ (δ 7.26 for ^{1}H and 72.6 for ^{13}C NMR), and DMSO-d_6 (δ 2.50 for ^{1}H and 39.5 for ^{13}C NMR). Spin multiplicities were defined as s (singlet), d (doublet), brs (broad-singlet), dd (double doublet), and t (triplet), with coupling constants (*J*) values expressed in hertz (HZ). HRMS were determined using Agilent QTOF mass spectrometer 6540 series equipment and Electrospray Ionization (ESI) procedures at 70 eV. Melting points of all the compounds were recorded with a Stuart® SMP30 instrument. Where anhydrous conditions are required, the reactions were carried out under nitrogen pressure and with newly distilled solvents. Solvent evaporation was performed by using a rotating evaporator at temperatures below 50 °C. The names of all the synthesised compounds were taken from ChemDraw Professional, Version 20.

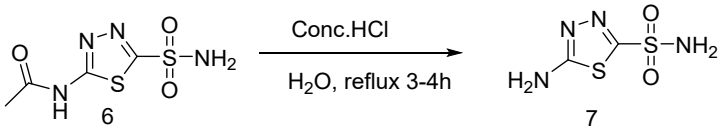
1.2 Experimental Procedures

1.2.5 General procedure A for the synthesis of compounds (7)

Step 1: Preparation of 5-Amino thiadiazole 2-sulphonamide

Acetazolamide (2.0 g, 9.3 mmol, 1 equiv.), concentrated HCl (10 mL, 120 mmol, 13 equiv.) and water added to the flask and stirred at room temperature to form a white suspension. The reaction was refluxed at 95 °C and the white suspension became a colorless solution. After completion of the reaction cooled to rt and a white precipitate began to form. The reaction was removed from the stirring and the white precipitate was allowed to settle. The excess HCl was

decanted off and the remaining solid suspension containing as little HCl as possible was cooled in ice bath. This suspension was neutralized to pH 7 with 5.0 M aqueous NaOH solution. The white precipitate was filtered out by vacuum filtration to afford 5-amino thiadiazole-2-sulphonamide as a white solid. The yield obtained were 70- 85%.



1.2.1 General procedure B for the Synthesis of substituted 5-phenyl isoxazole-3-carboxylic acid (5a-h)

Substituted acetophenone (1 equiv.) was dissolved in dry THF (15 mL), and the reaction mixture was stirred at 0 ºc. Diethyl oxalate (3.77 mmol) was added slowly, followed by the gradual addition of sodium ethoxide (5.65 mmol) over a period of 15 minutes while maintaining the temperature at 0ºc. After 30 minutes, the reaction mixture was slowly brought to room temperature and maintained for 2-4 hours. Upon completion of the reaction, dilute HCl solution (20-30 mL) was added under cold conditions to maintain a pH of 2-3. The reaction mixture was then extracted with EtOAc, washed with brine, dried over sodium sulphate, and evaporated to obtain the required intermediate, ethyl 4-(3-nitrophenyl-2,4-dioxobutanoate) (yield: 78-85%). The obtained intermediate (**3a-h**) was used as without further purification and suspended in ethanol (15 mL), to which hydroxylamine HCl (3.09 mmol) was added portion-wise and subjected to reflux for 6 hours, with TLC used to monitor the reaction progress. After the starting material was consumed, the reaction mixture was allowed to cool to room temperature. The liberated precipitate was filtered, washed with cold ethanol, and dried to obtain the intermediate substituted ethyl 5-phenylisoxazole-3-carboxylate (**4a-h**) (yield: 65-80%). This intermediate was then dissolved in an ethanol: water (4:1) mixture, to which lithium hydroxide monohydrate (3 equiv.) was added and stirred for 3-4 hours. Upon completion of the reaction, the mixture was cooled to room temperature. The ethanol was removed under vacuum, the residue was treated with ice-cold water, and the pH was adjusted to 7-8. The obtained precipitate was filtered and dried to yield the required intermediate substituted 5-phenylisoxazole-3-carboxylic acid (**5a-h&**) (yield: 60-70%).

1.2.2 General procedure C for the synthesis of compounds (8a-c, 10a-f, 12a-f, 14a-d & 19a-b)

Synthesised intermediate substituted 5-phenylisoxazole-3-carboxylic acids (**5a-h**)/5-(thiophen-2-yl) isoxazole-3-carboxylic acid (**17**) (1 equiv.) was taken and dissolved in anhydrous DMF. To this, HATU (1-[Bis(dimethylamino) methylene]-1*H*-1,2,3-triazolo [4,5-*b*]pyridinium 3-oxide hexafluorophosphate) (1.1 equiv.) and substituted amino benzene sulphonamides and substituted anilines (**7**, **9, 11&13a-d**) were added and stirred for 10 minutes. Then, diisopropylethylamine (DIPEA) (3.0 equiv.) was added and stirred at room temperature for 1 hour. After the completion of the reaction, as indicated by TLC, the reaction mass was added to crushed ice. The resulting crude precipitate was filtered and purified using column chromatography (40% EtOAc in hexane) or recrystallised from methanol to yield compounds (***8a-c, 10a-f, 12a-g, 14a-d & 19a-b***) (30-70% yields).

5-(4-chlorophenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)isoxazole-3carboxamide **(8a)**

Cream solid, Yield 65 %, mp: 236-238 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.02 (s, 1H), 8.35 (s, 2H), 7.94 (d, J = 8.5 Hz, 2H), 7.64 (s, 1H), 7.61 (d, J = 8.3 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.45, 165.63, 161.69, 158.72, 158.37, 136.33, 130.04, 128.15, 125.27, 101.47; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{12}H_9ClN_5O_4S_2$ 385.9784; found 385.9776.

5-(4-isobutylphenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)isoxazolecarboxamide **(8b)**

white color solid, Yield 55% 232-234 °C, ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.79 (s, 1H), 8.43 (s, 1H), 7.93 – 7.79 (m, 3H), 7.60 (s, 1H), 7.39 (d, J = 7.8 Hz, 2H), 2.57 – 2.53 (m, 2H), 1.90 (s, 1H), 0.89 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.88, 164.54, 163.66, 145.24, 145.18, 130.45, 130.23, 126.19, 125.92, 100.30, 44.82, 29.99, 22.57; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{18}N_5O_4S_2$ 408.0800; found 385.9776.

5-(3,4-dichlorophenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)isoxazole-carboxamide **(8c)**

White color solid, Yield 30 %, mp: 230-232°C, ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.10 (s, 1H), 8.41 (s, 2H), 8.29 (d, J = 1.5 Hz, 1H), 7.97 (dd, J = 8.3, 1.5 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.80 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.19, 160.28, 157.90, 141.33, 140.08, 127.08, 120.81, 119.43, 119.29, 116.13, 115.97, 101.69; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{12}H_7Cl_2N_5O_4S_2$ 419.9395; found 419.9985.

5-phenyl-N-(4-sulfamoylphenyl) isoxazole-3-carboxamide (**10a*****)***

white solid, yield 65%; mp: 237-239 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.11 (s, 1H), 8.00 (d, J = 8.3 Hz, 4H), 7.84 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 6.8 Hz, 3H), 7.54 (s, 1H), 7.33 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.23, 160.17, 158.12, 141.40, 140.03, 131.52,

129.88, 127.09, 126.64, 126.35, 120.78, 100.70; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{14}N_3O_4S$ 344.0740; observed 344.0732.

N-(4-sulfamoylphenyl)-5-(p-tolyl) isoxazole-3-carboxamide(*10b*)

Off white solid, yield 70%; mp: 242-244 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.05 (s, 1H), 7.97 (d, J = 8.9 Hz, 2H), 7.86 (d, J = 8.2 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 7.43 (s, 1H), 7.38 (d, J = 7.7 Hz, 2H), 7.30 (s, 2H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.40, 160.12, 158.18, 141.53, 141.42, 140.02, 130.40, 127.08, 126.30, 124.01, 120.75, 100.04, 21.54; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{17}H_{16}N_3O_4S$ 358.0862; observed 358.0863

5-(4-methoxyphenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide(*10c*)

Cream color solid, yield 65%; mp 255-257 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 10.98 (s, 1H), 7.91 (d, J = 8.9 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.76 (d, J = 8.8 Hz, 2H), 7.30 (s, 1H), 7.24 (s, 2H), 7.06 (d, J = 8.9 Hz, 2H), 3.78 (s, 3H).^{13}C NMR (125 MHz, DMSO-d_6) δ ^{13}C NMR (126 MHz, DMSO) δ ^{13}C NMR (126 MHz, DMSO) δ 171.31, 161.76, 160.08, 158.25, 141.43, 140.00, 128.13, 127.08, 120.75, 119.32, 115.28, 99.13, 55.94; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{17}H_{16}N_3O_4S$ 374.0811; observed 374.0816.

5-(4-chlorophenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide(*10d*)

Off white solid, yield 70%; mp: 251–253 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.10 (s, 1H), 8.01 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.58 (s, 1H), 7.31 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ^{13}C NMR (125 MHz, DMSO-d_6) δ ^{13}C NMR (126 MHz, DMSO) δ 170.11, 160.23, 157.97, 141.37, 140.05, 136.18, 129.98, 128.15, 127.09, 125.48, 120.79; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{13}ClN_3O_4S$ 378.0315; observed 378.0335.

5-(4-isobutylphenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide(*10e*)

white solid, yield 70%; mp: 260–262 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.08 (s, 1H), 7.98 (d, J = 8.4 Hz, 2H), 7.89 (d, J = 7.8 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 7.45 (s, 1H), 7.37 (d, J = 7.9 Hz, 2H), 7.31 (s, 2H), 2.53 (d, J = 7.2 Hz, 2H), 1.89 (hept, J = 6.7 Hz, 1H), 0.88 (d, J = 6.6 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) 171.42, 160.10, 158.18, 145.07, 141.43, 140.01, 130.38, 127.08, 126.20, 124.29, 120.77, 100.07, 44.83, 30.01, 22.60, 22.56; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{20}H_{22}N_3O_4S$ 400.1331; observed 400.1336.

***5-(3-nitrophenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide* (10f)**

Light brown solid, Yield 68%, mp: 228-230 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.17 (s, 1H), 8.76 (t, J = 2.1 Hz, 1H), 8.45 (d, J = 7.8 Hz, 1H), 8.41 (dd, J = 8.4, 2.3 Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.91 (t, J = 8.1 Hz, 1H), 7.84 (d, J = 4.6 Hz, 3H), 7.33 (s, 2H); ^{13}C NMR (125

MHz, DMSO-d_6) δ [1] 168.96, 160.39, 157.79, 148.93, 141.33, 140.12, 132.52, 131.67, 127.99, 127.10, 125.85, 120.92, 120.82, 102.7; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{14}N_4O_6S$ 389.0566; found 389.0558.

5-phenyl-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide (*12a*)

Off white solid, yield 60%; mp: 230-232 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.01 (s, 1H), 8.35 (s, 1H), 7.91 (d, J = 7.5 Hz, 2H), 7.88 (d, J = 8.5 Hz, 1H), 7.57 – 7.48 (m, 5H), 7.45 (s, 1H), 7.35 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.19, 160.18, 158.05, 145.18, 138.85, 131.50, 130.01, 129.88, 126.67, 126.35, 124.04, 122.06, 118.11, 100.66; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{14}N_3O_4S$ 344.0740; observed 344.0725.

N-(3-sulfamoylphenyl)-5-(p-tolyl)isoxazole-3-carboxamide(*12b*)

Off white solid, yield 60%; mp: 251–253 °C; 11.04 (s, 1H), 8.42 (t, J = 1.9 Hz, 1H), 7.93 (t, J = 8.4 Hz, 3H), 7.59 (dt, J = 15.6, 8.0 Hz, 2H), 7.41 (s, 2H), 7.35 (d, J = 3.4 Hz, 1H), 7.12 (dd, J = 8.8, 3.9 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.91, 159.63, 157.69, 141.04, 140.93, 139.53, 129.91, 126.59, 125.81, 123.52, 120.26, 99.55, 21.05; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{17}H_{16}N_3O_4S$ 358.0862; observed 358.0864

5-(4-methoxyphenyl)-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide(*12c*)

Off white solid, yield 55%; mp: 253–255 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.04 (s, 1H), 8.42 (t, J = 1.9 Hz, 1H), 7.93 (dd, J = 8.9, 2.1 Hz, 3H), 7.63 – 7.60 (m, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.42 (s, 2H), 7.36 (s, 1H), 7.15 – 7.11 (m, 2H), 3.85 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 171.26, 161.77, 160.10, 158.18, 145.19, 138.89, 129.96, 128.13, 124.02, 122.01, 119.36, 118.11, 115.29, 99.08, 55.96; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{17}H_{16}N_3O_4S$ 374.0811; observed 374.0819.

5-(4-chlorophenyl)-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide(*12d*)

Cream color solid, yield 70%; mp: 232–234 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.16 (s, 1H), 8.48 (s, 1H), 8.08 (d, J = 2.0 Hz, 1H), 8.07 (d, J = 2.0 Hz, 1H), 8.00 (ddd, J = 7.8, 2.1, 1.3 Hz, 1H), 7.73 (d, J = 2.0 Hz, 1H), 7.72 (d, J = 2.0 Hz, 1H), 7.70 – 7.67 (m, 1H), 7.65 (d, J = 7.9 Hz, 1H), 7.63 (s, 1H), 7.48 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) 170.06, 160.23, 157.91, 145.17, 138.82, 136.16, 130.00, 129.98, 128.15, 125.50, 124.05, 122.08, 118.12, 101.22; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{13}ClN_3O_4S$ 378.0315; observed 378.0335.

5-(4-isobutylphenyl)-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide(*12e*)

white solid, yield 58%; mp: 234–236 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.06 (s, 1H), 8.42 (s, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 2H), 7.61 (t, J = 7.4 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.43 (d, J = 14.2 Hz, 3H), 7.37 (d, J = 7.9 Hz, 2H), 2.54 (d, J = 7.2 Hz,

2H), 1.89 (hept, J = 6.7 Hz, 1H), 0.89 (d, J = 6.6 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) ^{13}C NMR (126 MHz, DMSO) δ 171.38, 160.11, 158.11, 145.16, 145.07, 138.86, 130.40, 129.99, 126.20, 124.31, 124.03, 122.04, 118.10, 100.03, 44.82, 30.01, 22.57; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{20}H_{22}N_3O_4S$ 400.1331; observed 400.1340.

5-(3-nitrophenyl)-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide **(12f)**

Light Brown solid, Yield 65 %, mp: 234-236 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.16 (s, 1H), 8.76 (s, 1H), 8.47 – 8.39 (m, 3H), 8.01 – 7.89 (m, 2H), 7.83 (s, 1H), 7.62 (dt, J = 16.2, 7.9 Hz, 2H), 7.44 (s, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.91, 160.40, 157.72, 148.93, 145.19, 138.80, 132.52, 131.66, 130.02, 128.01, 125.83, 124.06, 122.12, 120.92, 118.13, 102.69; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{14}N_4O_6S$ 389.0566; found 389.0564.

N-(4-fluorophenyl)-5-(3-nitrophenyl)isoxazole-3-carboxamide **(14a)**

Light yellow solid, Yield 66%, mp: 238-240 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.90 (s, 1H), 8.73 (t, J = 2.0 Hz, 1H), 8.44 – 8.41 (m, 1H), 8.39 (dd, J = 8.3, 2.3 Hz, 1H), 7.88 (t, J = 8.1 Hz, 1H), 7.85 – 7.82 (m, 2H), 7.80 (s, 1H), 7.23 (t, J = 8.8 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.79, 160.53, 157.28, 148.90, 134.81, 134.79, 132.48, 131.62, 128.03, 125.76, 123.03, 122.97, 120.84, 115.95, 115.77, 102.66; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{11}FN_3O_4$ 328.0734; found 328.0722.

5-(3-nitrophenyl)-N-(p-tolyl)isoxazole-3-carboxamide **(14b)**

Light yellow solid, Yield 70%, mp: 196-198 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.74 (s, 1H), 8.74 (t, J = 2.0 Hz, 1H), 8.43 (dt, J = 7.8, 1.3 Hz, 1H), 8.39 (ddd, J = 8.4, 2.4, 1.0 Hz, 1H), 7.89 (t, J = 8.0 Hz, 1H), 7.79 (s, 1H), 7.71 – 7.69 (m, 2H), 7.20 (d, J = 8.2 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.68, 160.69, 157.16, 148.90, 135.93, 134.04, 132.47, 131.62, 129.61, 128.07, 125.73, 121.05, 120.83, 102.63, 21.00; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{17}H_{14}N_3O_4$ 324.0984; found 324.0978.

N-(4-methoxyphenyl)-5-(3-nitrophenyl)isoxazole-3-carboxamide **(14c)**

Light yellow solid, Yield 70 %, mp: 186-188 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.76 (s, 1H), 8.48 (dt, J = 7.8, 1.3 Hz, 1H), 8.46 – 8.43 (m, 2H), 7.83 (s, 1H), 7.78 – 7.76 (m, 2H), 7.03 – 7.00 (m, 2H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.01, 161.70, 159.43, 158.87, 148.19, 129.52, 129.07, 128.19, 128.08, 122.63, 119.37, 115.26, 114.22, 98.91, 55.93; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{17}H_{14}N_3O_5$ 340.0933; found 340.0927.

5-(3-nitrophenyl)-N-phenylisoxazole-3-carboxamide (**14d*****)***

Light yellow solid, Yield 55%, mp: 195-197 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.83 (s, 1H), 8.74 (t, J = 2.0 Hz, 1H), 8.44 (dt, J = 7.8, 1.2 Hz, 1H), 8.41 – 8.38 (m, 1H), 7.89 (t, J = 8.0 Hz, 1H), 7.83 – 7.80 (m, 3H), 7.42 – 7.38 (m, 2H), 7.19 – 7.16 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.74, 160.64, 157.36, 148.90, 138.44, 132.47, 131.62, 129.23, 128.05, 125.75, 124.98, 121.10, 120.84, 102.66; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{16}H_{12}N_3O_4$ 310.0828; found 310.0820.

N-(4-sulfamoylphenyl)-5-(thiophen-2-yl)isoxazole-3-carboxamide(19a)

Off white solid, Yield 70 %, mp: 215-217 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 11.01 (s, 1H), 7.90 (d, J = 8.8 Hz, 2H), 7.84 (dd, J = 5.0, 1.2 Hz, 1H), 7.79 (dd, J = 3.7, 1.2 Hz, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.29 (s, 1H), 7.24 (d, J = 3.2 Hz, 2H), 7.23 – 7.22 (m, 1H).; ^{13}C NMR (125 MHz, DMSO-d_6) δ ^{13}C 166.66, 160.31, 157.95, 145.35, 138.99, 130.99, 130.17, 129.60, 129.47, 128.05, 124.20, 122.25, 118.28, 100.04; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{14}H_{12}N_3O_4S_2$ 350.0269; found 350.0272.

N-(3-sulfamoylphenyl)-5-(thiophen-2-yl)isoxazole-3-carboxamide(19b)

Light yellowish solid, Yield 75 %, mp: 205-207 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.05 (s, 1H), 8.40 (t, J = 2.0 Hz, 1H), 7.94 – 7.90 (m, 1H), 7.89 (dd, J = 5.0, 1.2 Hz, 1H), 7.84 (dd, J = 3.7, 1.2 Hz, 1H), 7.61 – 7.59 (m, 1H), 7.57 (t, J = 7.8 Hz, 1H), 7.40 (s, 2H), 7.33 (s, 1H), 7.28 (dd, J = 5.0, 3.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO) δ 166.49, 160.14, 157.78, 145.18, 138.82, 130.82, 130.00, 129.43, 129.30, 127.88, 124.03, 122.08, 118.10, 99.87; HRMS-QTOF (ESI): *m/z* calcd. for $[M+H]^+$ $C_{14}H_{12}N_3O_4S_2$ 350.0269; found 350.0275.

2. CA inhibition assay

For the synthesized compounds, the inhibition assay of selected CA isozymes was performed using SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow equipment [1]. For α-CAs 10−20 mM HEPES (pH 7.5), for β-CAs Tris (pH 8.3) as buffers, with 0.2 Mm phenol red as an indicator, maintaining a constant ionic strength by adding 20 mM Na_2SO_4 (for α-CAs) or 20 mM NaCl (for β-CAs) following the CA- catalyzed CO_2 hydration reaction for a period of 5–100 s working at the absorbance maximum of 557 nm [2]. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 mM (in DMSO/water 1:1, v/v), and dilutions up to 0.01 nM were done with the assay buffer described above. The inhibition assay, we used Acetazolamide as a positive control, and negative control is DMSO used and at least seven distinct inhibitor concentrations have been used to determine the inhibition constant. Before the experiment, inhibitor and enzyme

solutions were preincubated together for 10 min at room temperature to facilitate the formation of the E–I complex. Triplicate experiments were performed for each inhibitor concentration, and the values provided in this article are the average of these results. As previously stated, the inhibition constants were determined by nonlinear least squares methods with the Cheng–Prusoff equation, and represent the mean of at least three different determinations. All CA isozymes employed in this study were recombinant proteins obtained as reported earlier by our group [3,4].

2.1. Antimycobacterial activity

2.1.1 Bacterial strains, cell lines, drugs, and media

*Mycobacterium tuberculosis mc*² 6230 and its derivative strains were among the bacterial strains utilized to assess antitubercular activity. These bacteria were cultivated in Middlebrook 7H9 broth and 7H10 agar, which were both supplemented with 0.05% Tween 80 (BD Biosciences), 0.2% glycerol, and 10% oleic-albumin-dextrose-catalase (OADC). Furthermore, nutrients such leucine, D-pantothenate, methionine, and L-arginine (Sigma-Aldrich) were added to *M. tuberculosis mc*² 6230 cells. Dulbecco's Modified Eagle's Medium (DMEM) was used to cultivate the HepG2 cell line (ATCC# HB-8065), with 10% fetal bovine serum (FBS) added (Gibco).

2.1.2 Method

Using the microbroth dilution technique, the compounds' Minimum Inhibitory Concentrations (MICs) were determined (Wiegand et al., 2008). After being seeded at $5x10^5$ CFU/mL (OD600-0.005), the bacterial strain was grown to logarithmic phase in 7H9 broth supplemented with 10% OADC. While the control medications, isoniazid (INH) and rifampicin (RIF), were evaluated at concentrations ranging from 1 µg/mL to 0.001 µg/mL, the bacterial cultures were treated to each test compound at concentrations ranging from 128 to 0.25 µg/mL. These experiments were carried out in 96-well U-bottom microtiter plates, each of which held 200 µl of liquid. For ten days, the plates were sealed and incubated at 37°C. After the incubation period, the optical turbidity method was used to calculate MIC values, where MIC is the lowest drug concentration. MIC determinations were made three times independently, using duplicate samples each time.

4.4. In silico studies:

The crystal structure of MtCA1 (PDB 1YLK) [5], was downloaded by Protein Data Bank (RCSB.org).[6]. The homology model of MtCA3, built and reported in our previous work, was used.[7,8] All used structures were prepared using the Protein Preparation module implemented in the Maestro Schrödinger suite[9],. assigning bond orders, adding hydrogens, deleting water molecules, and optimizing H-bonding networks. Finally, energy minimization with a Root Means Square Deviation (RMSD) value of 0.30 was applied using an Optimized Potential for Liquid Simulation (OPLS4) force field.[10–15]. The 3D ligand structures were prepared by Maestro and evaluated for their ionization states at pH 7.3 ± 1.0 with Epik. The conjugate gradient method in Macromodel was used for energy minimization (maximum iteration number: 2500; convergence criterion: 0.05 Kcal/mol/Å2). Grids for docking were centered in the centroid of the complexed ligand. Docking studies were carried out with the program Glide using the standard precision (SP) mode[16]. Figures were generated with Maestro and Chimera.[9,17].

In Silico ADME Predictions.

The ADME *in silico* evaluation for the most active compounds **12e**, **12f** and **19b** was performed using the SwissADME web tool (http://www.swissadme.ch/index.php) in terms of molecular properties, pharmacokinetics, drug-likeness.

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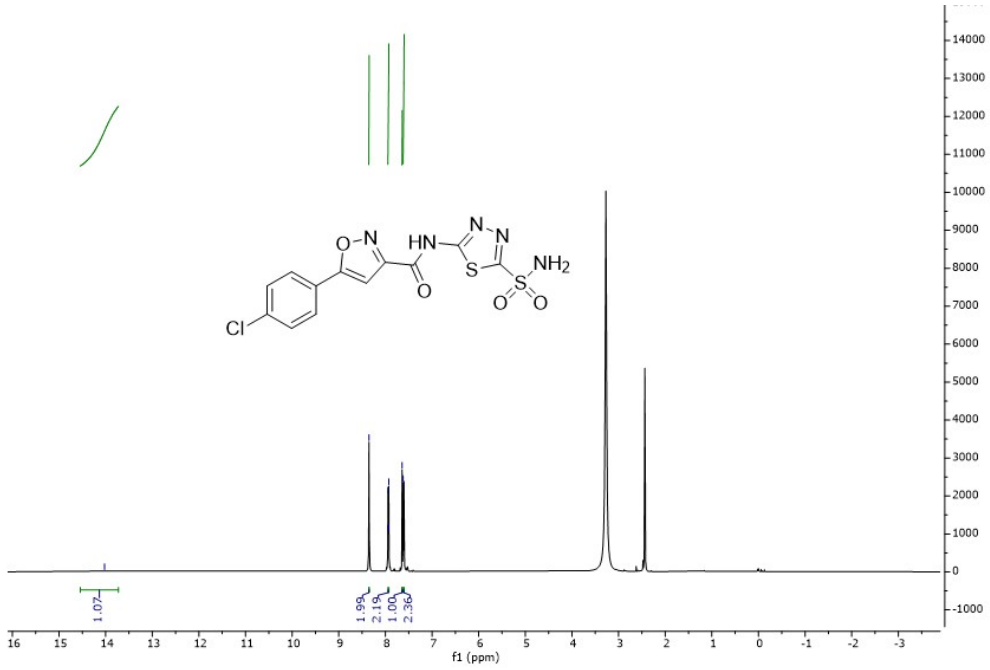
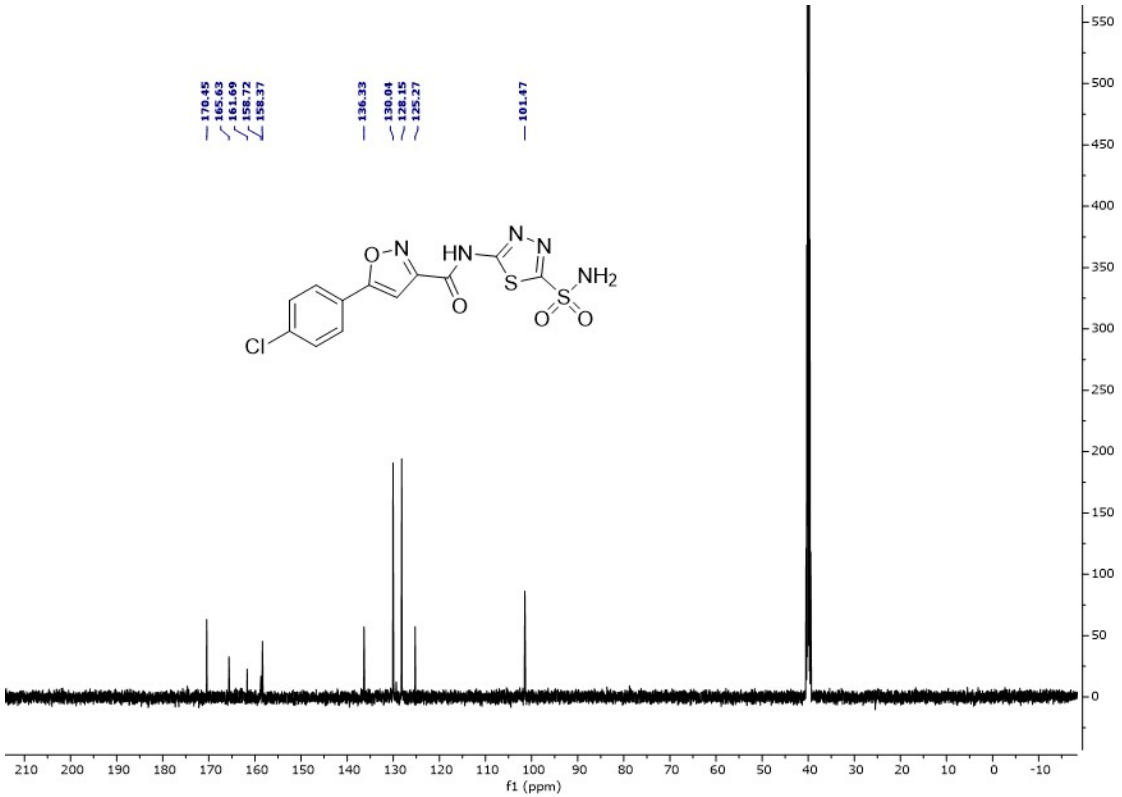
3. Spectral information

3.1 ^{1}H ,^{13}C NMR& Mass spectra for synthesized compounds (***8a-c,*****10a-f, 12a-g ,14a-d& 19a-b*)**

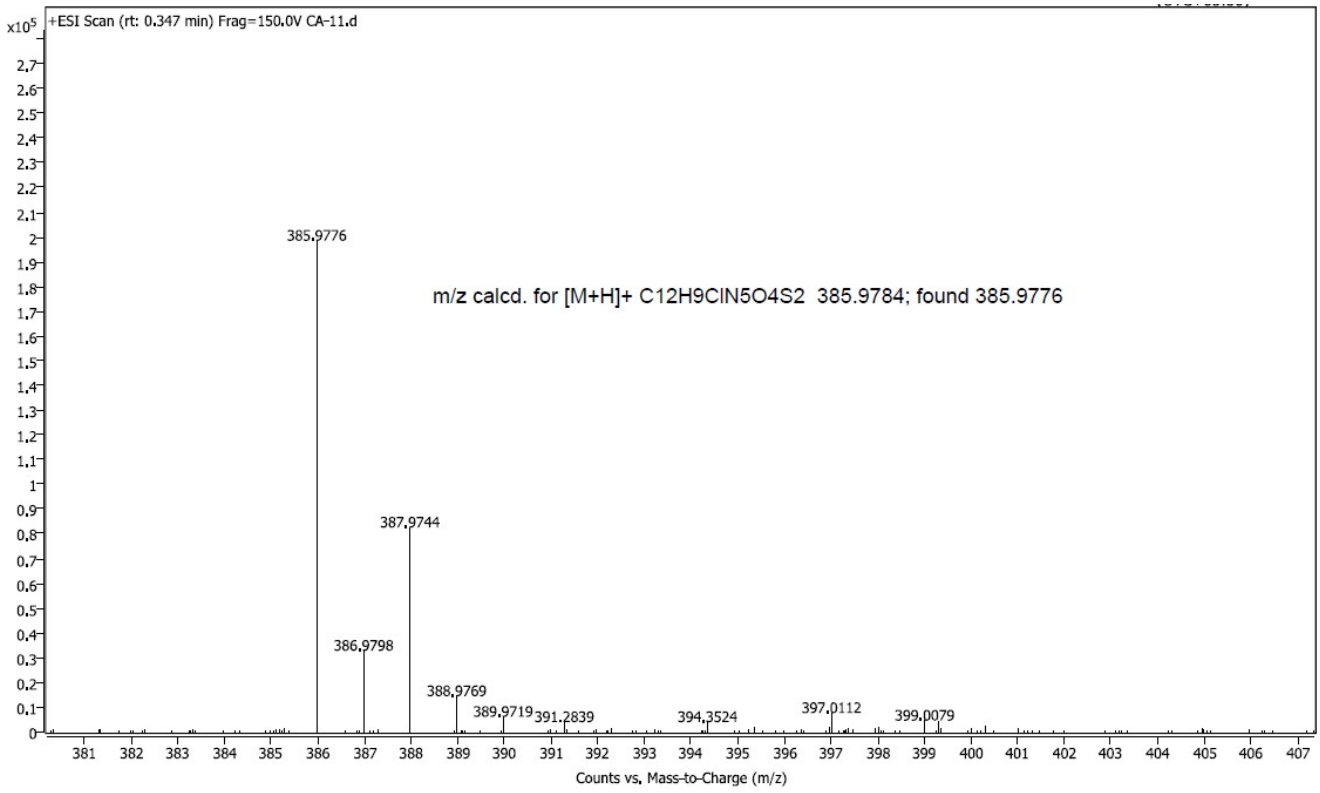
5-(4-chlorophenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)isoxazole-3-carboxamide(8a)



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **8a**

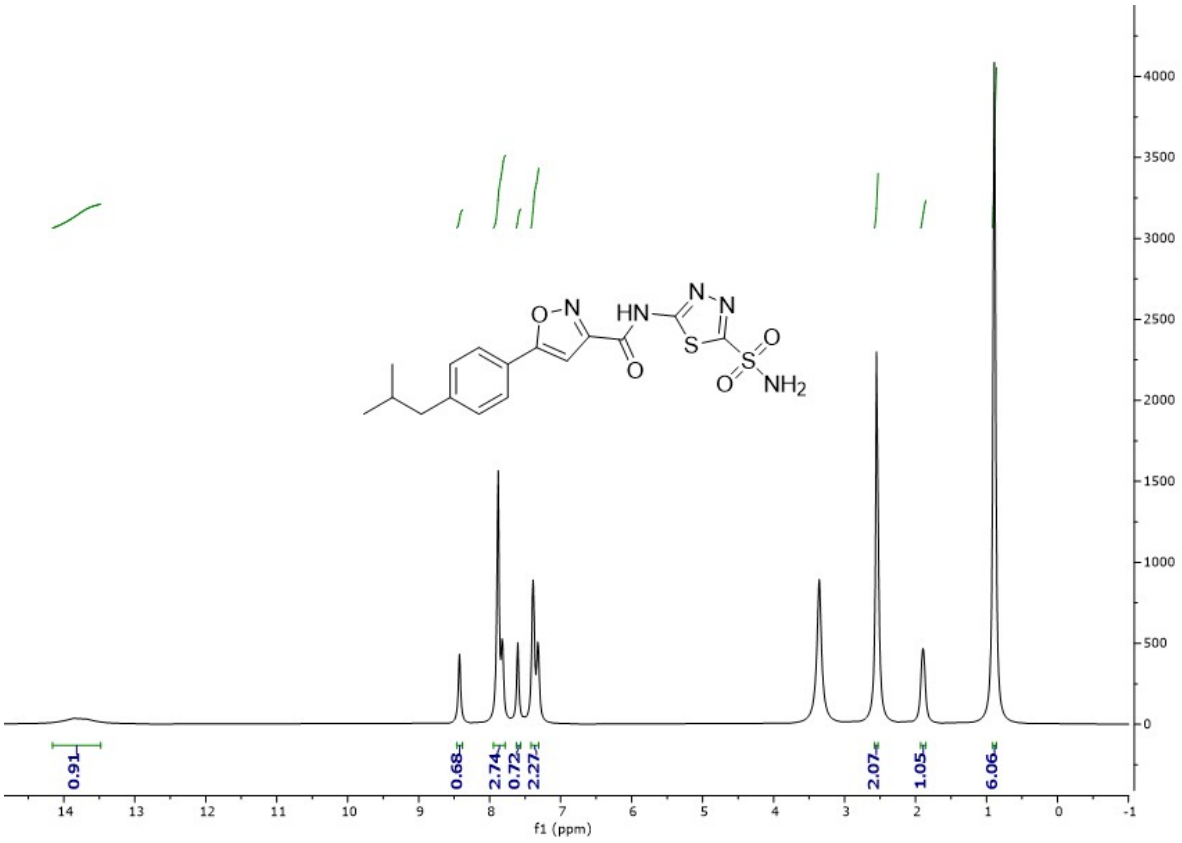


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **8a**

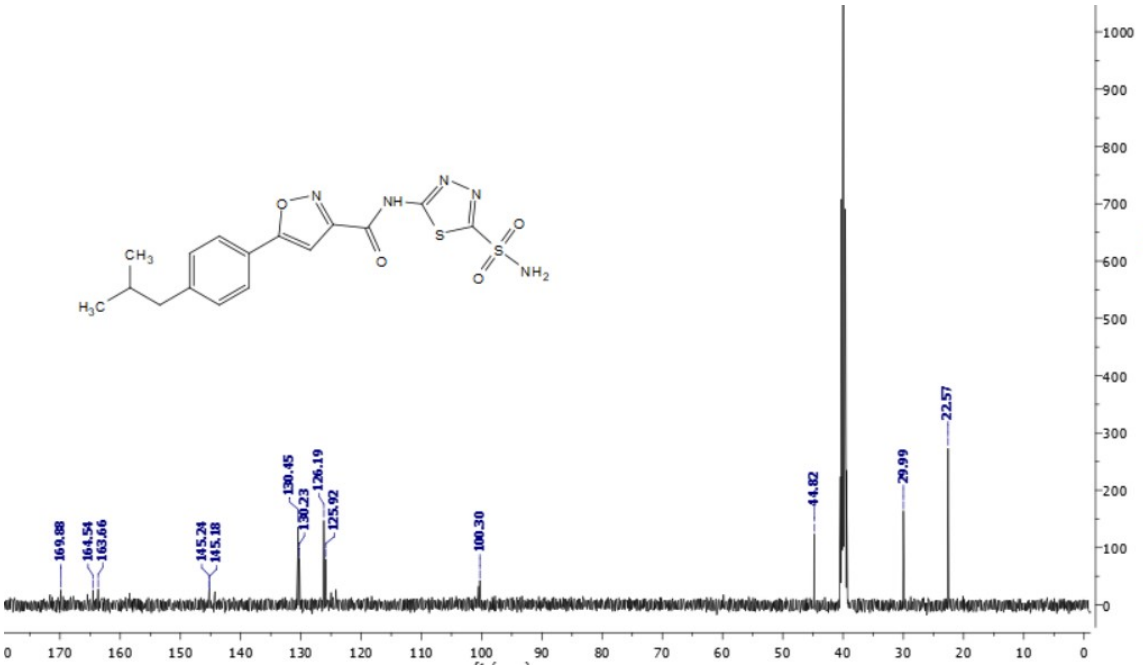


Mass spectrum of compound **8a**

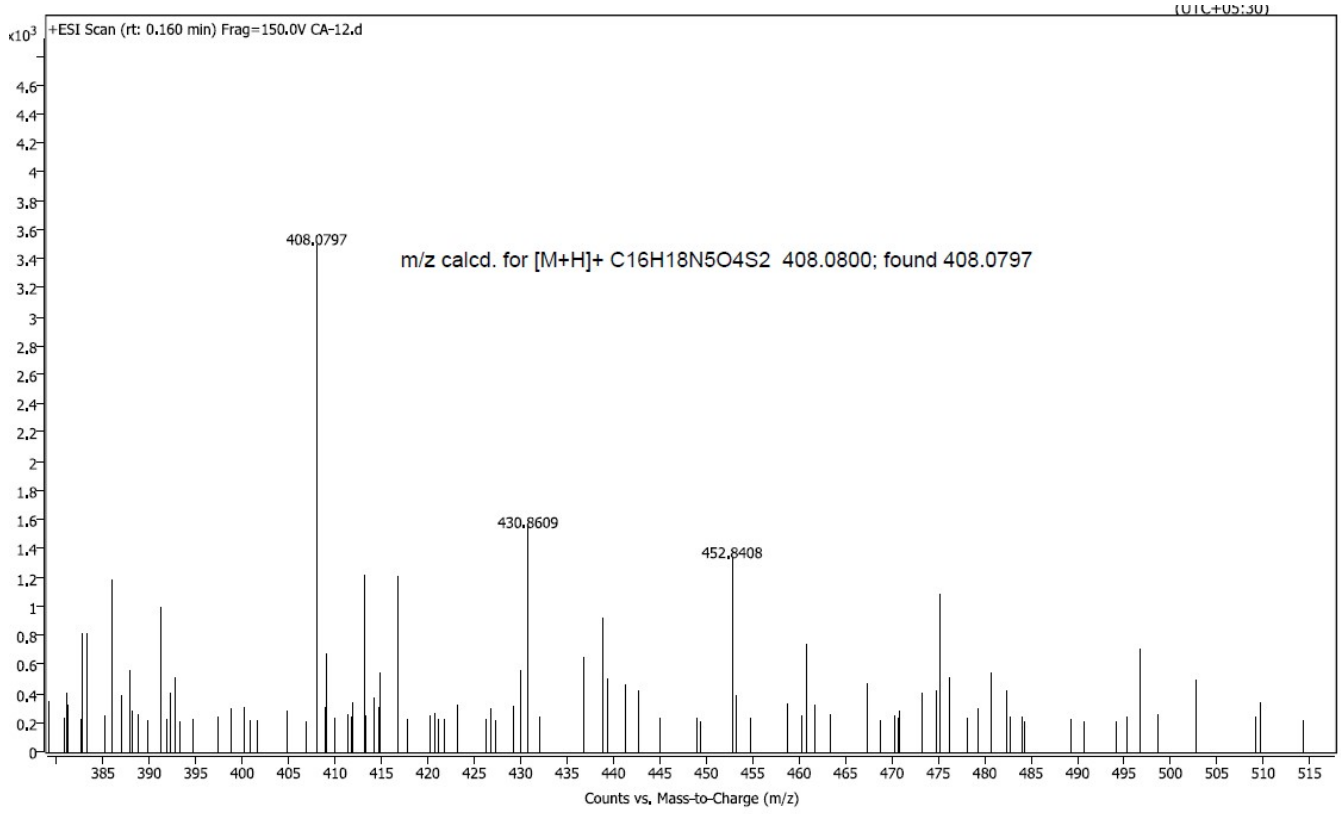
5-(4-isobutylphenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)isoxazole-3-carboxamide(8b)



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **8b**

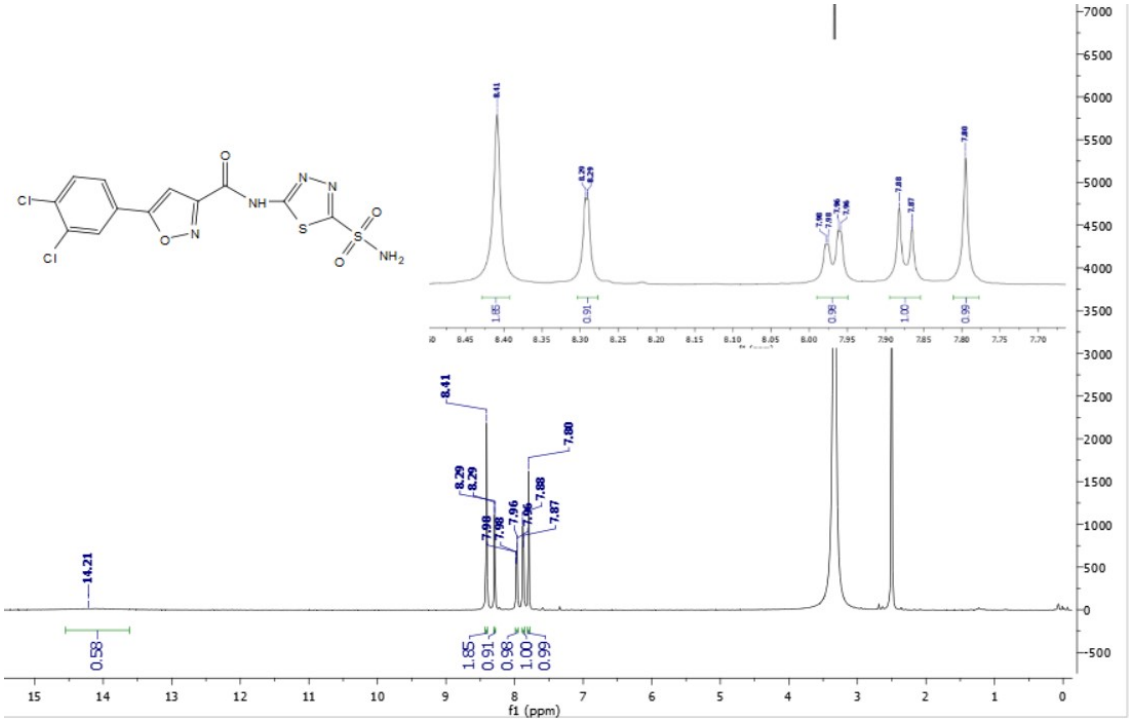


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **8b**

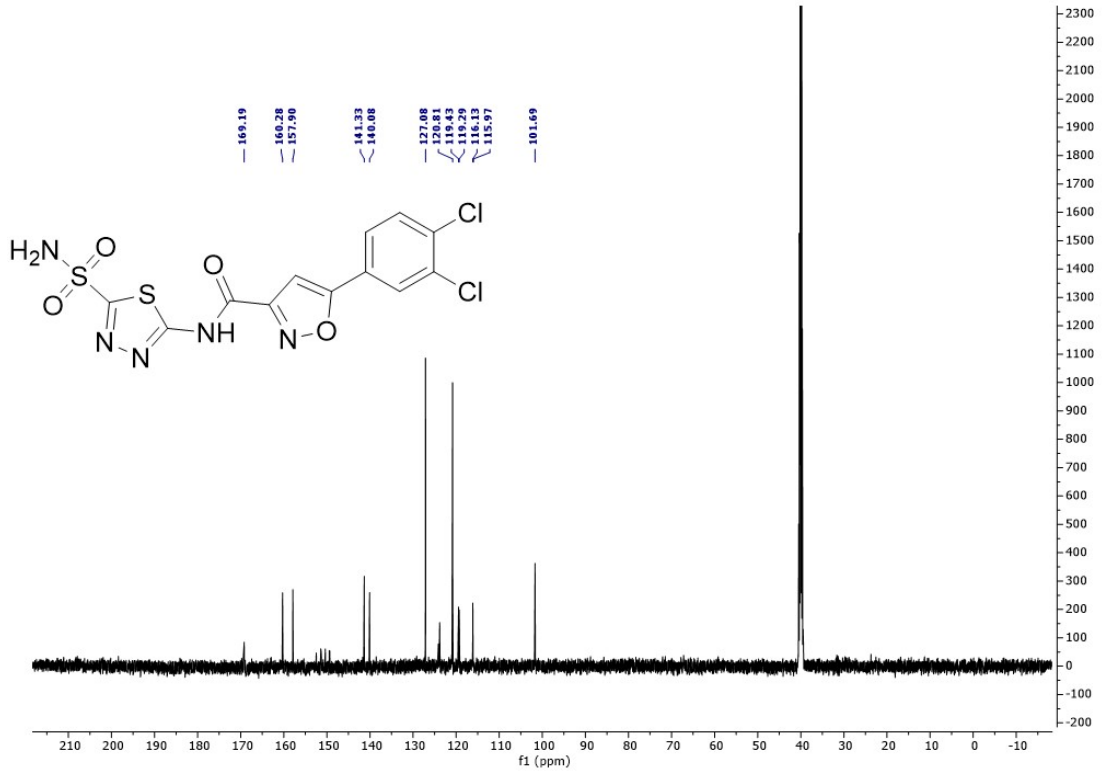


Mass spectrum of compound **8b**

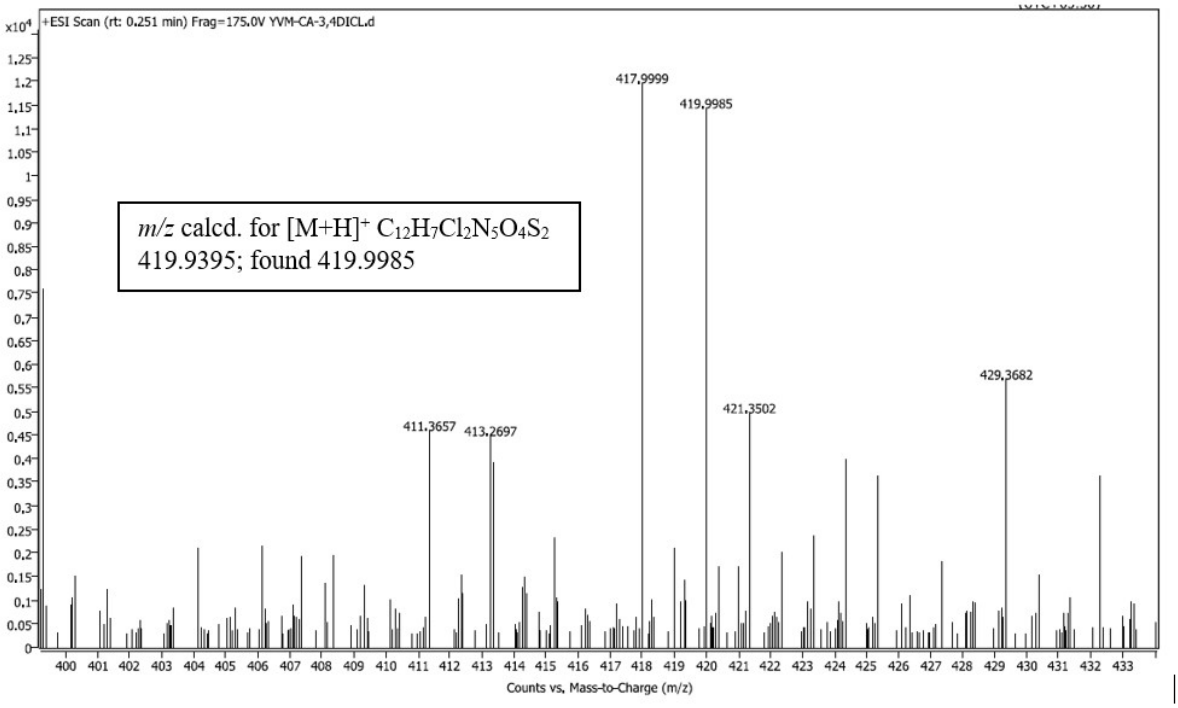
5-(3,4-dichlorophenyl)-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)isoxazole-3-carboxamide (8c)



[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **8c**

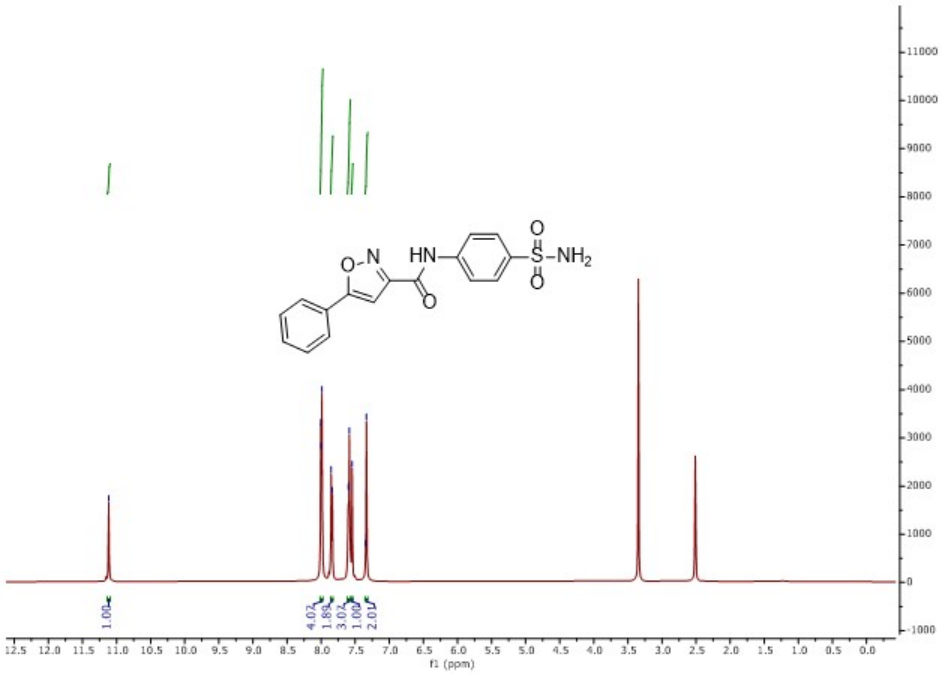


[13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **8c**

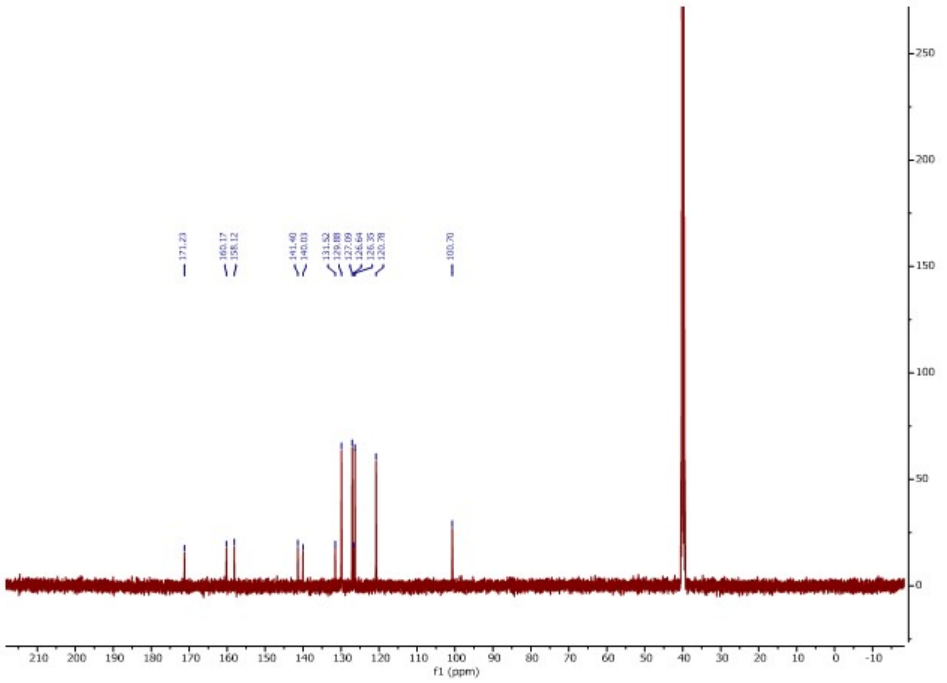


Mass spectrum of compound **8c**

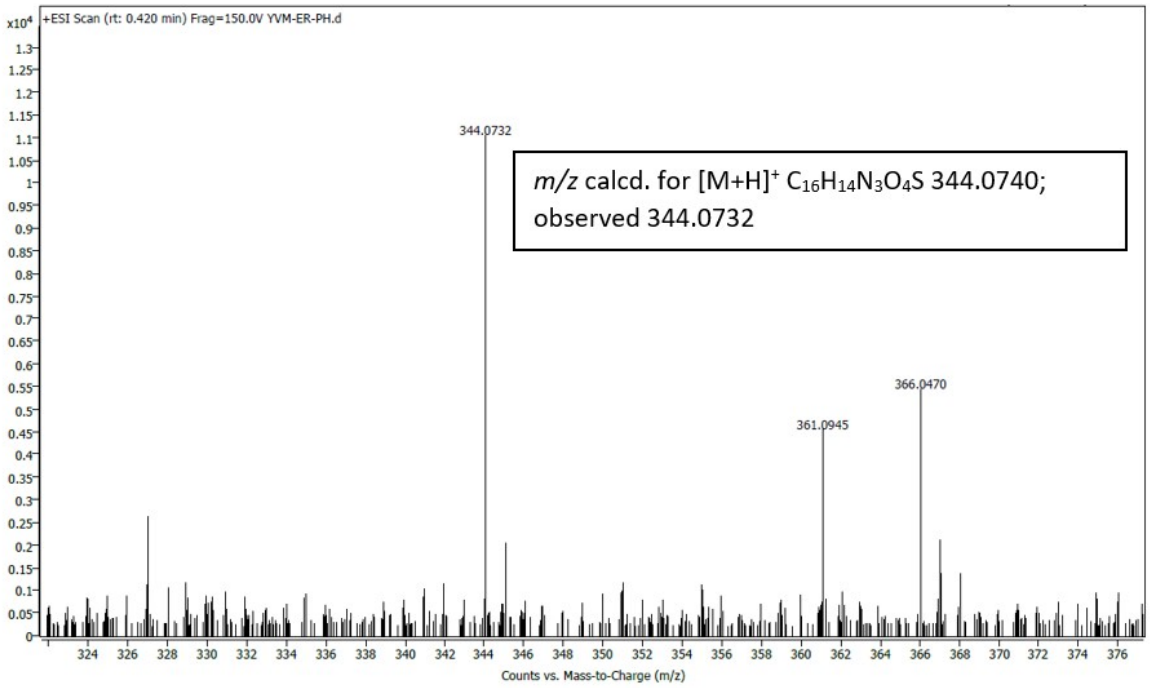
5-phenyl-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide (*10a*)



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **10a**

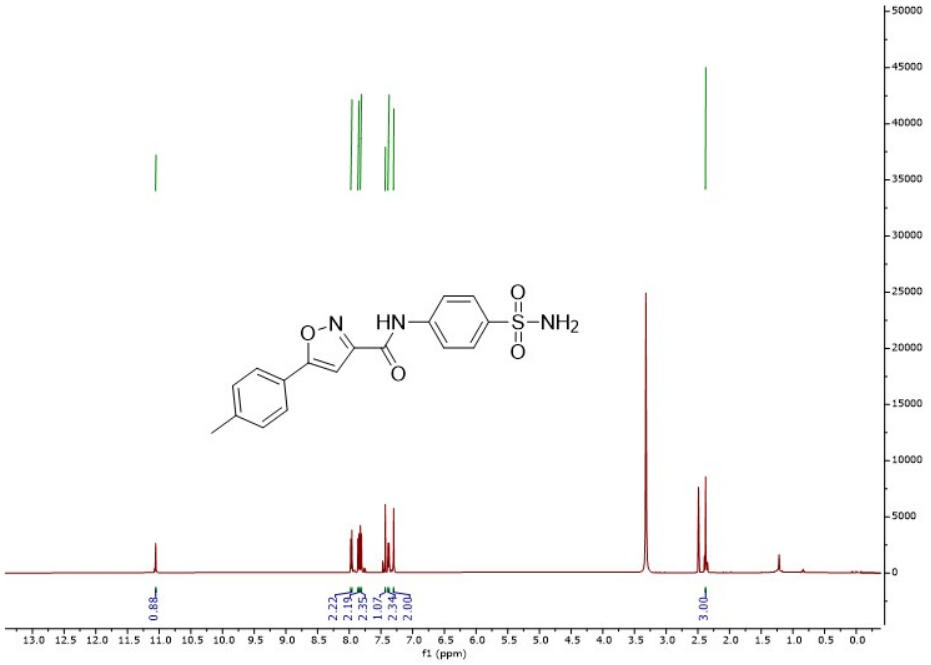


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **10a**

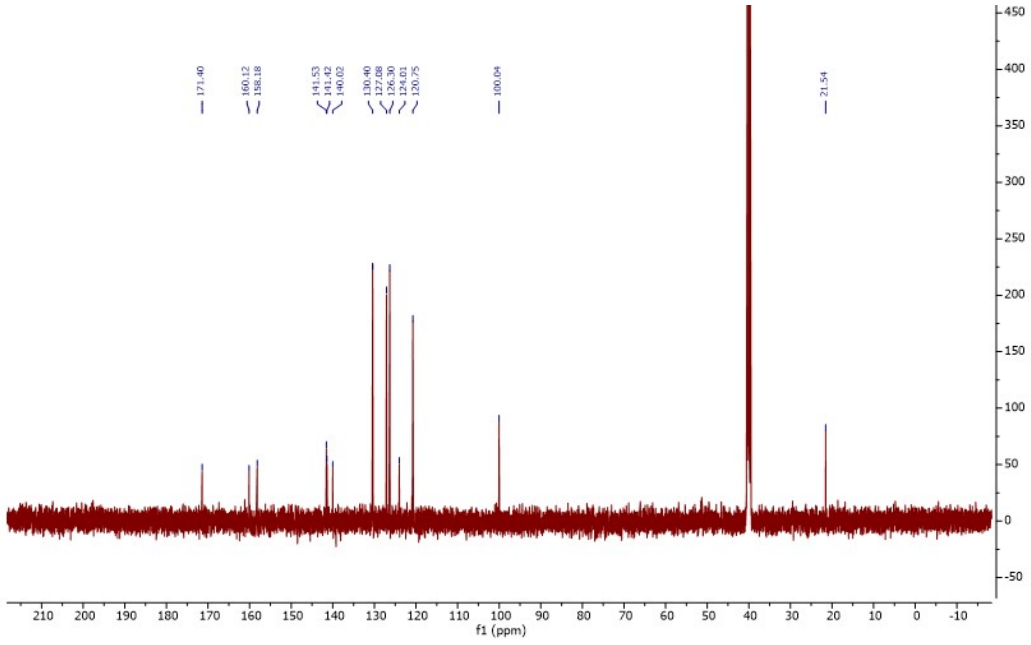


Mass spectrum of compound **10a**

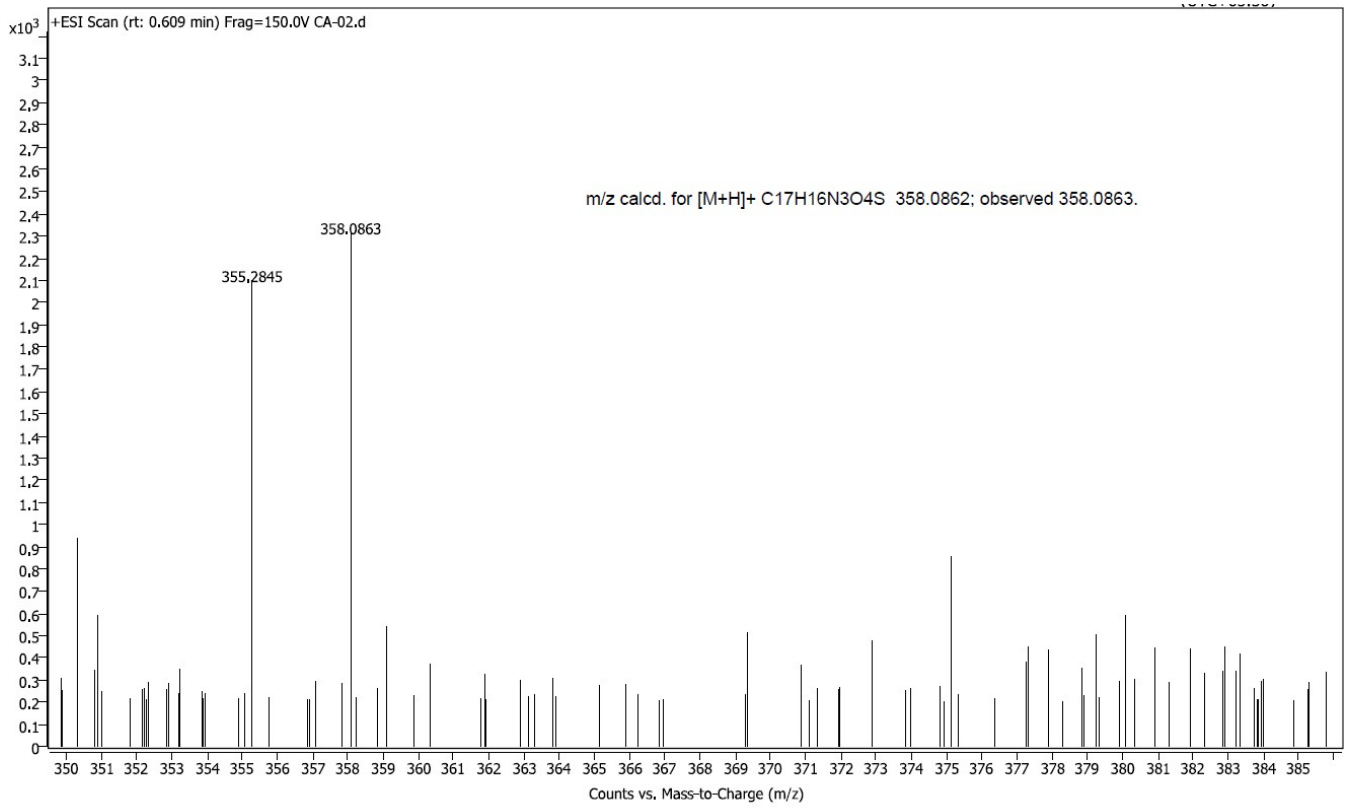
N-(4-sulfamoylphenyl)-5-(p-tolyl)isoxazole-3-carboxamide(*10b*)



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **10b**

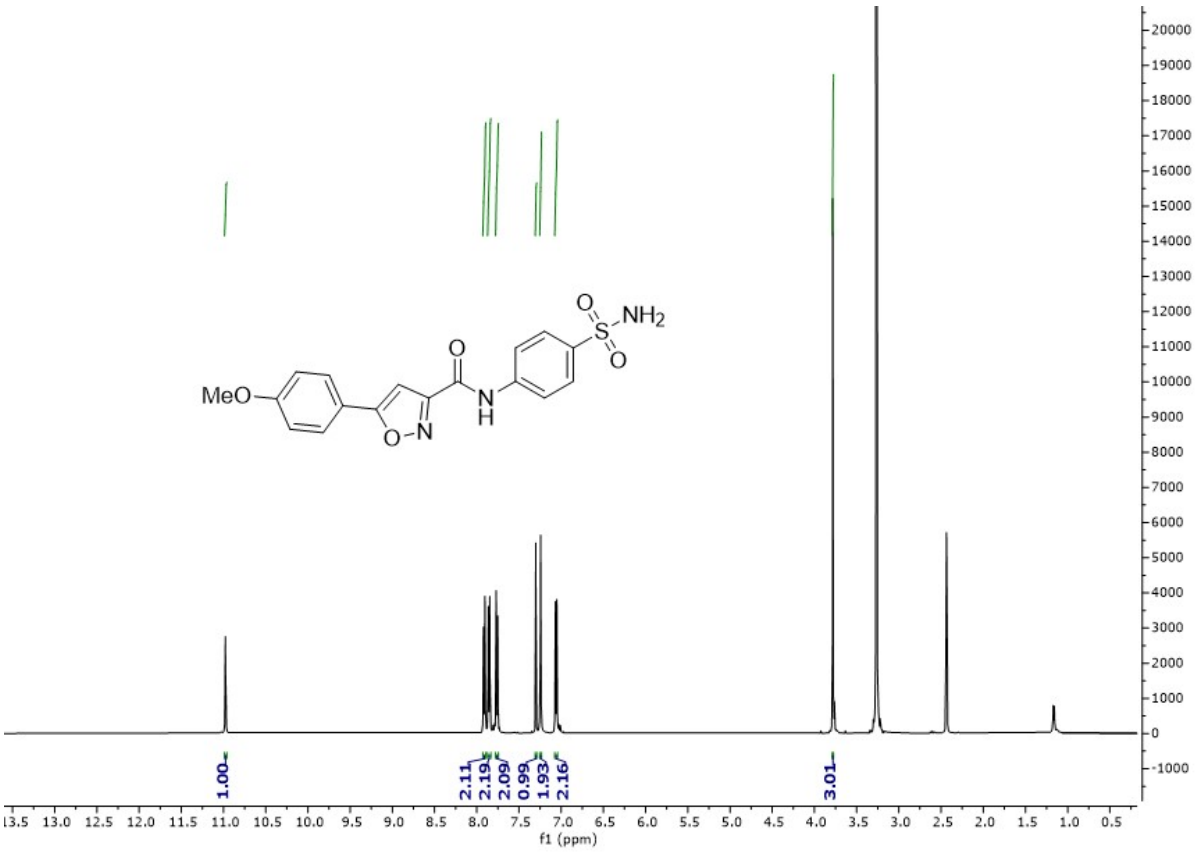


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **10b**

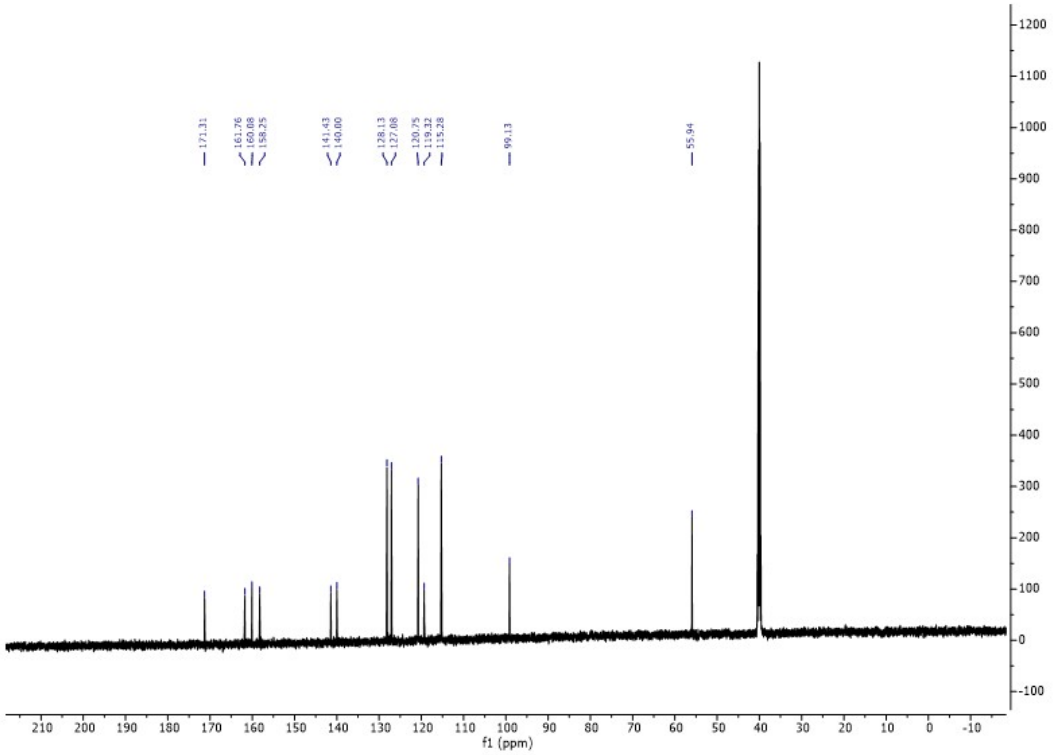


Mass spectrum of compound **10b**

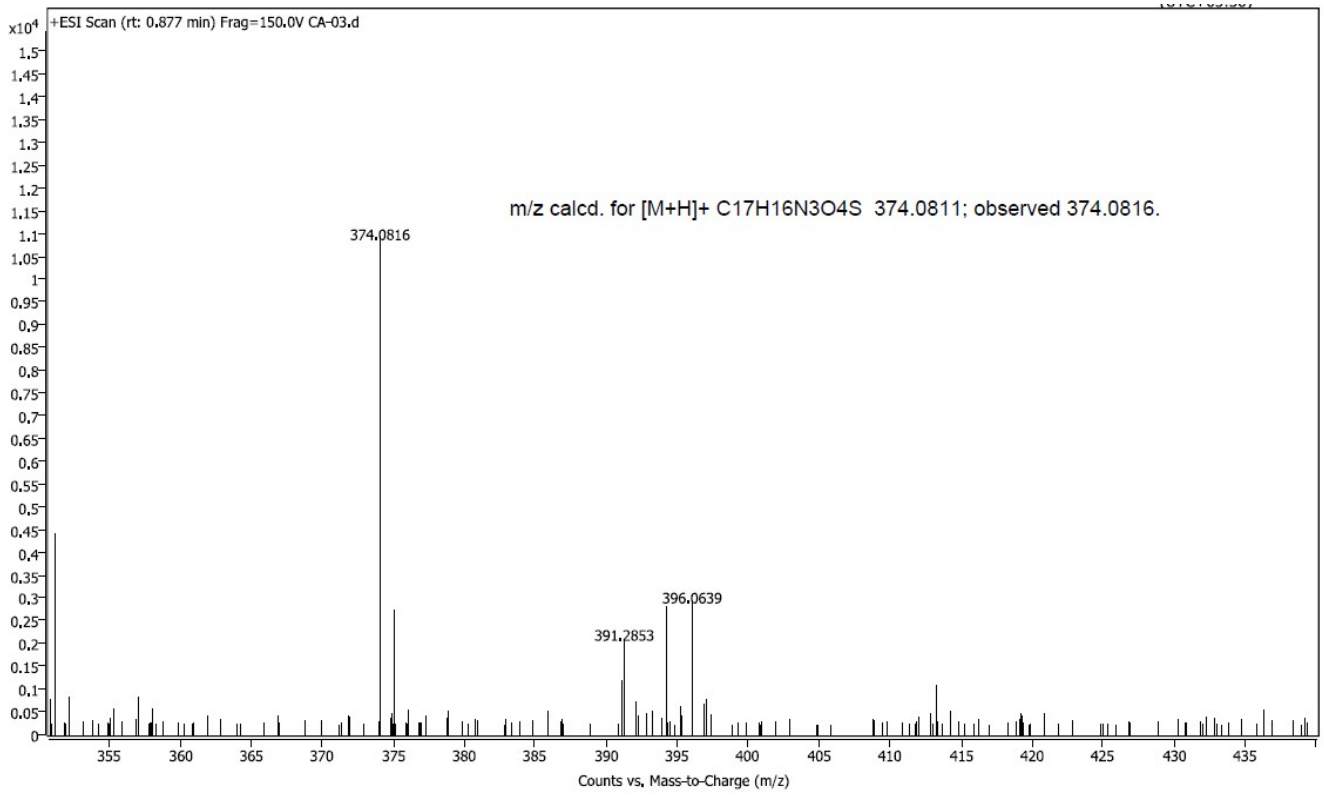
5-(4-methoxyphenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide* (10c*)



[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **10c**

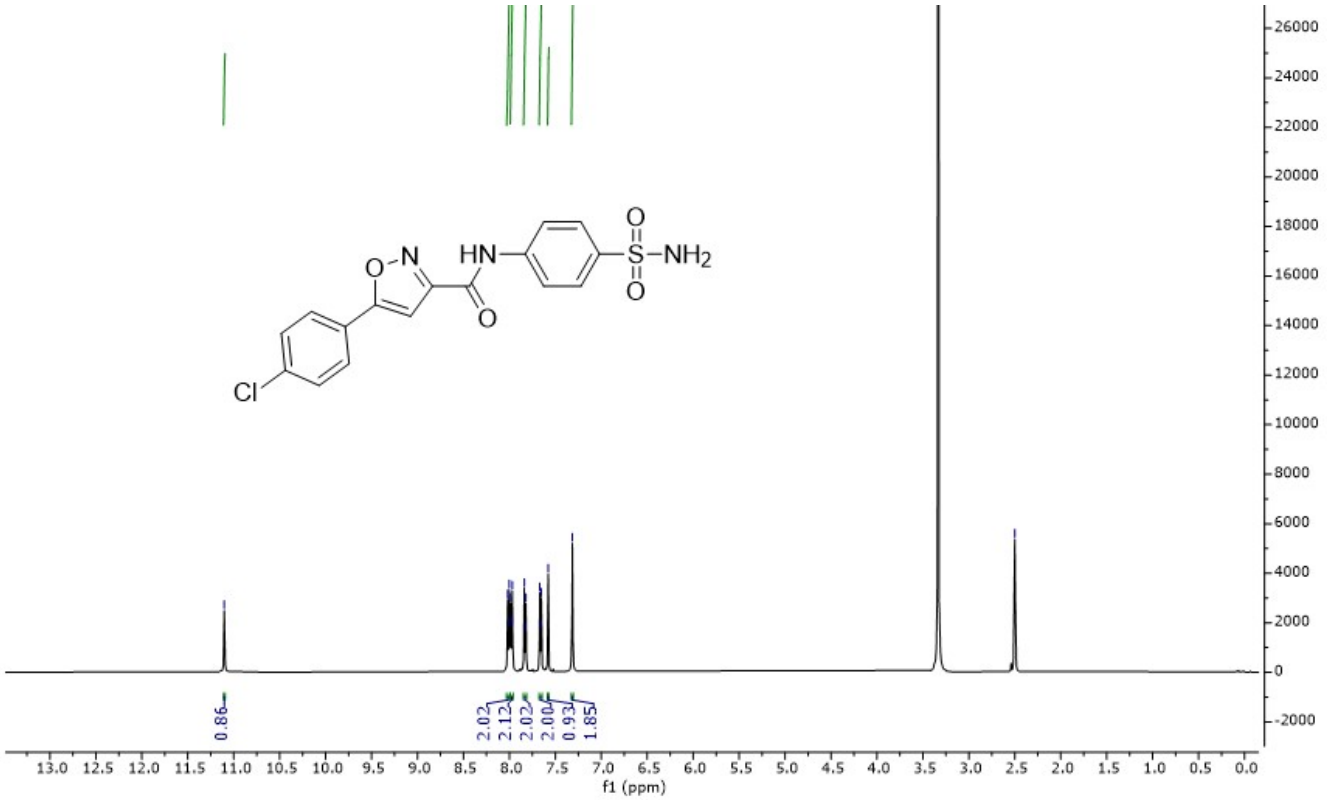


[13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **10c**



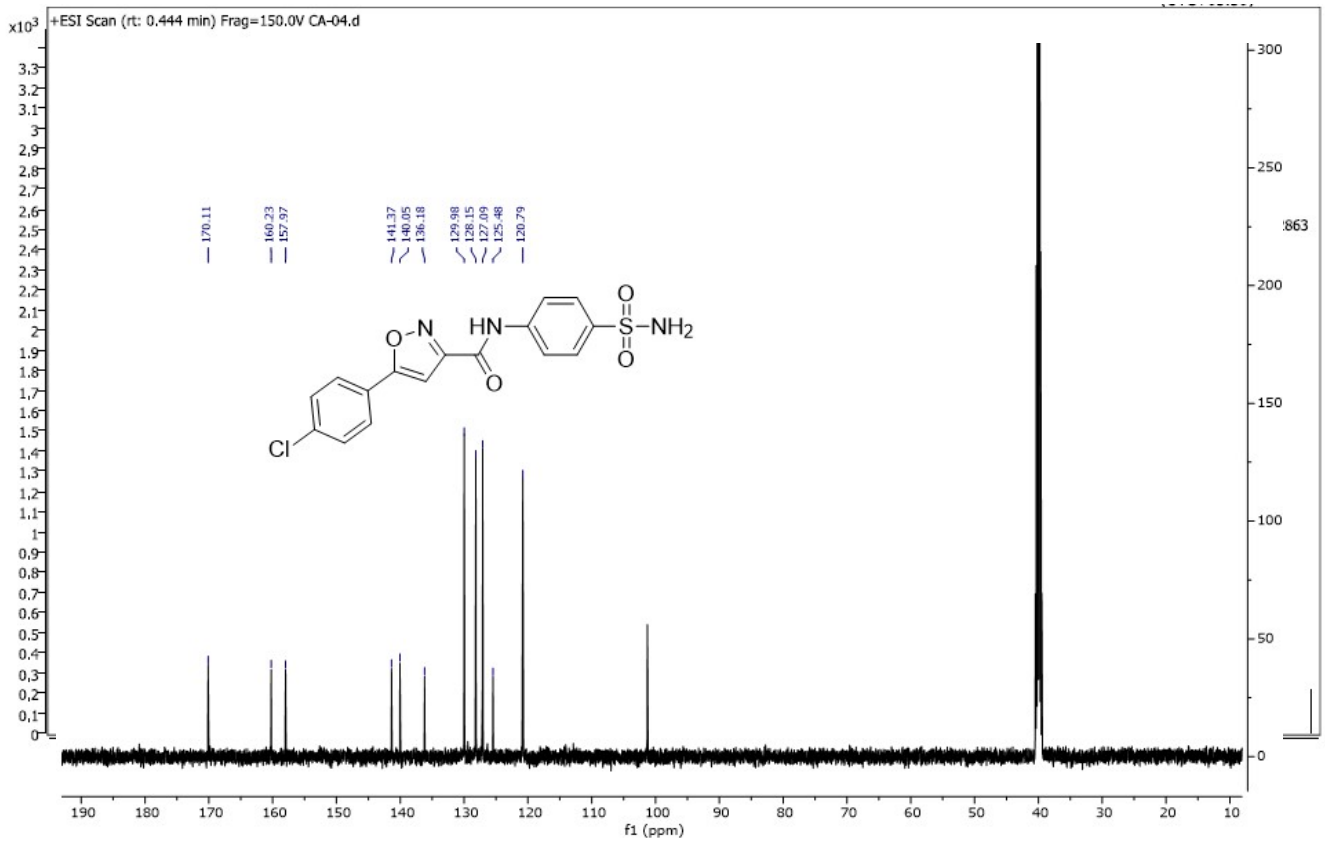
Mass spectrum of compound **10c**

5-(4-chlorophenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide (10d)



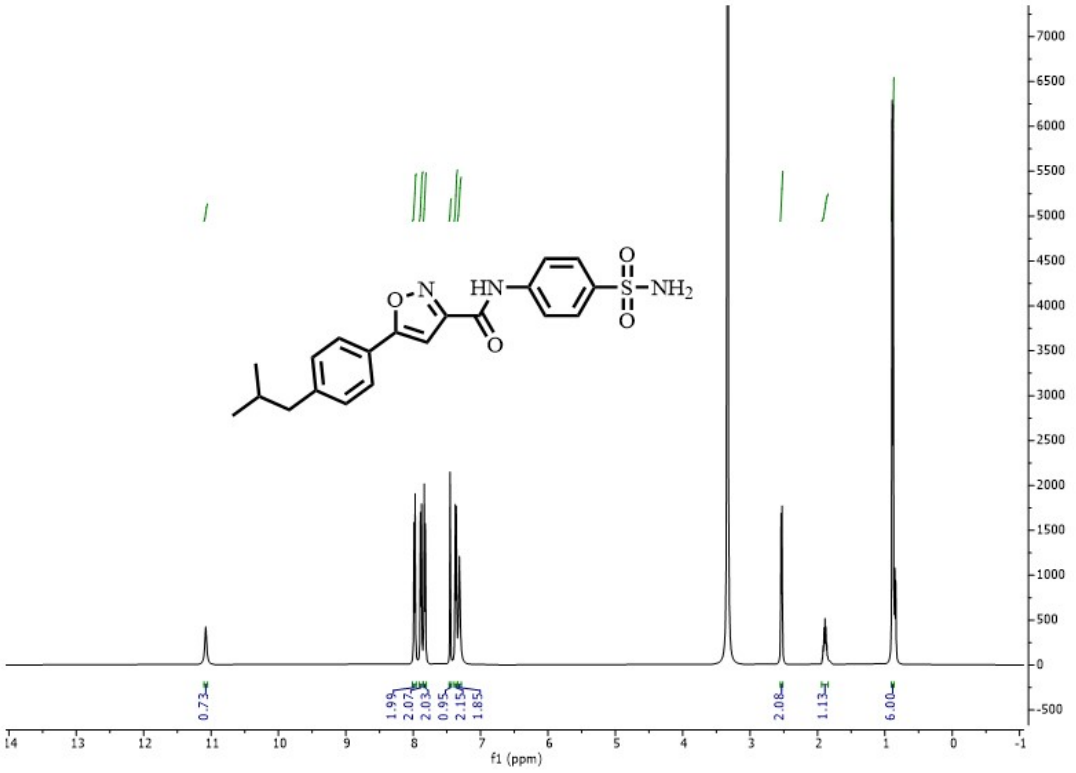
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **10d**

^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **10d**

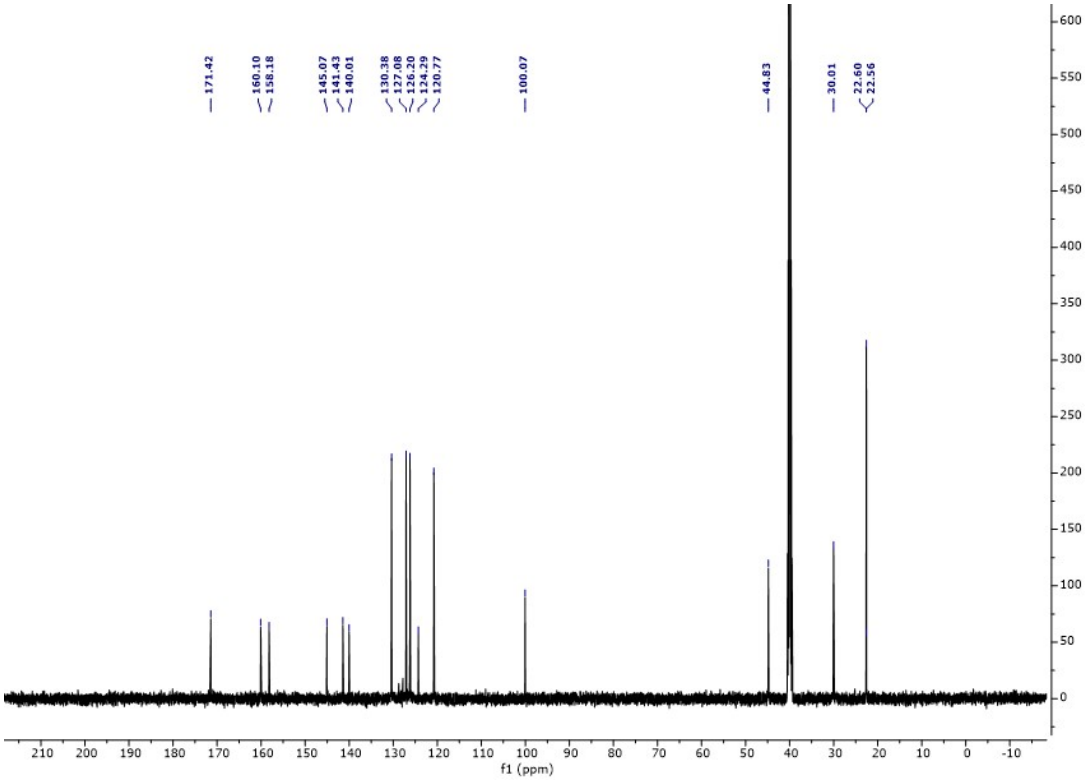


Mass spectrum of compound **10d**

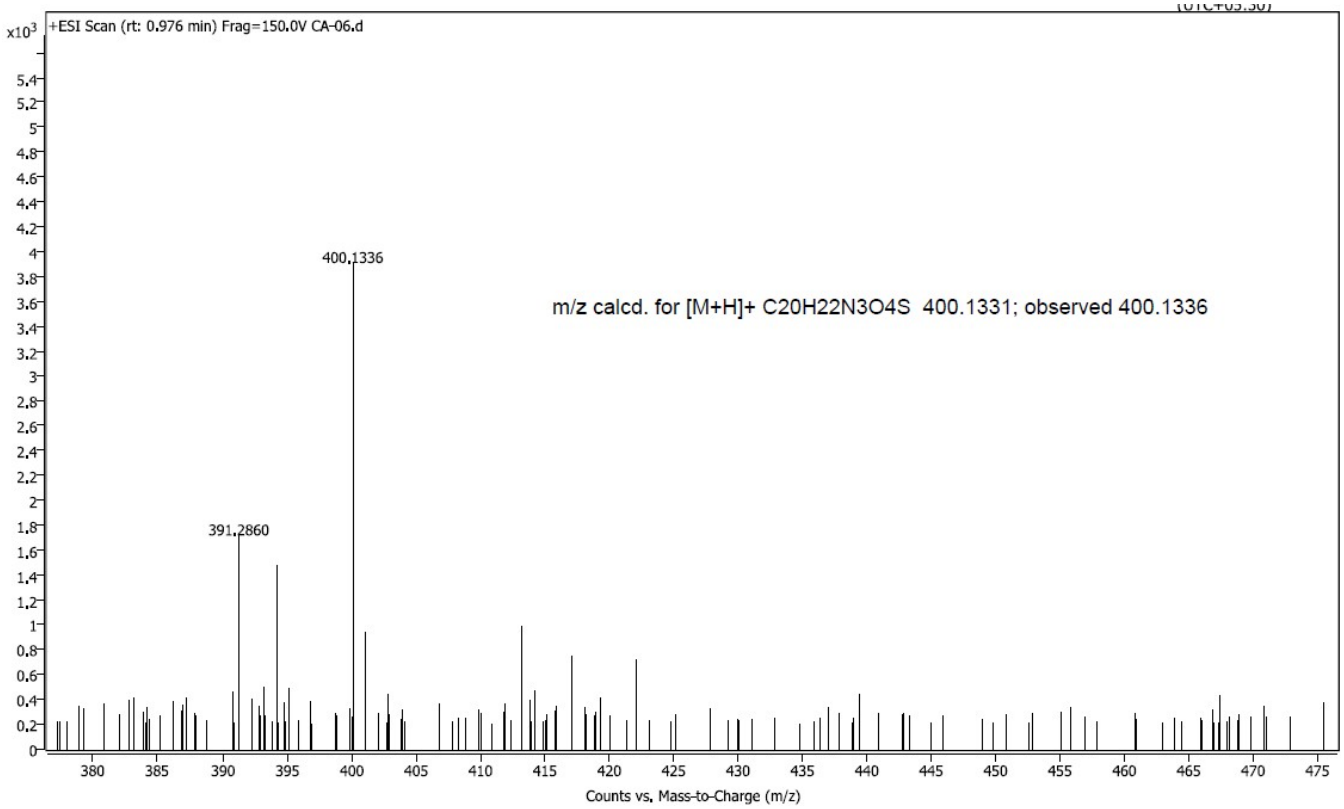
5-(4-isobutylphenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide(10e)



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **10e**

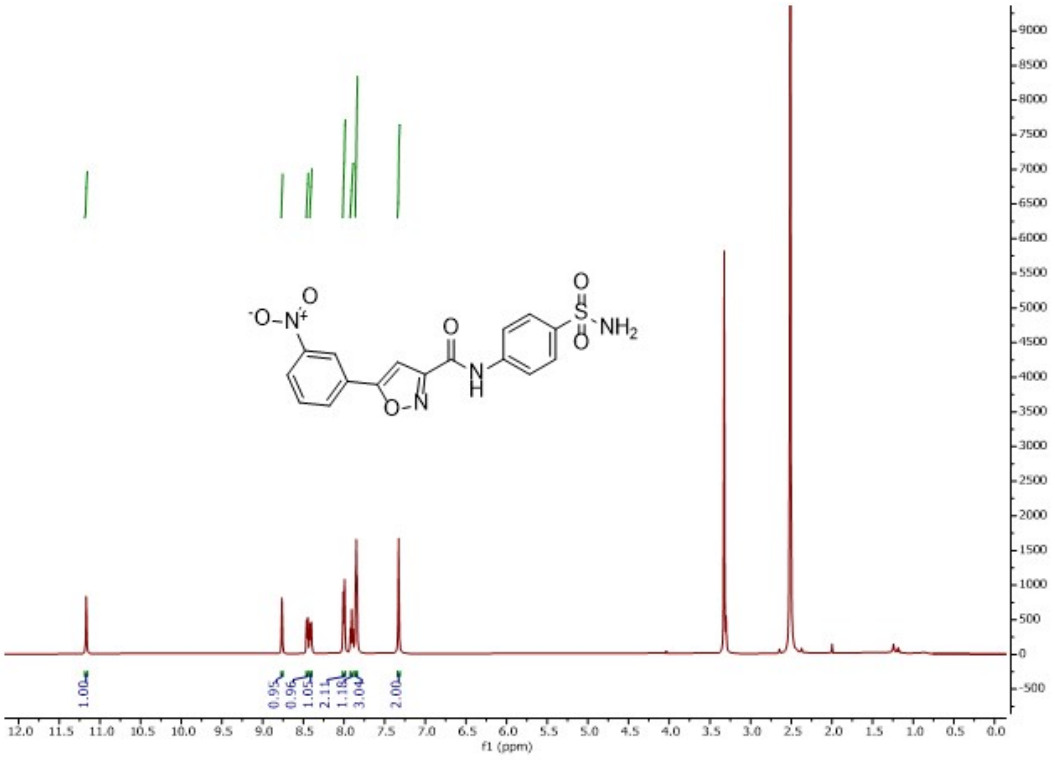


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **10e**

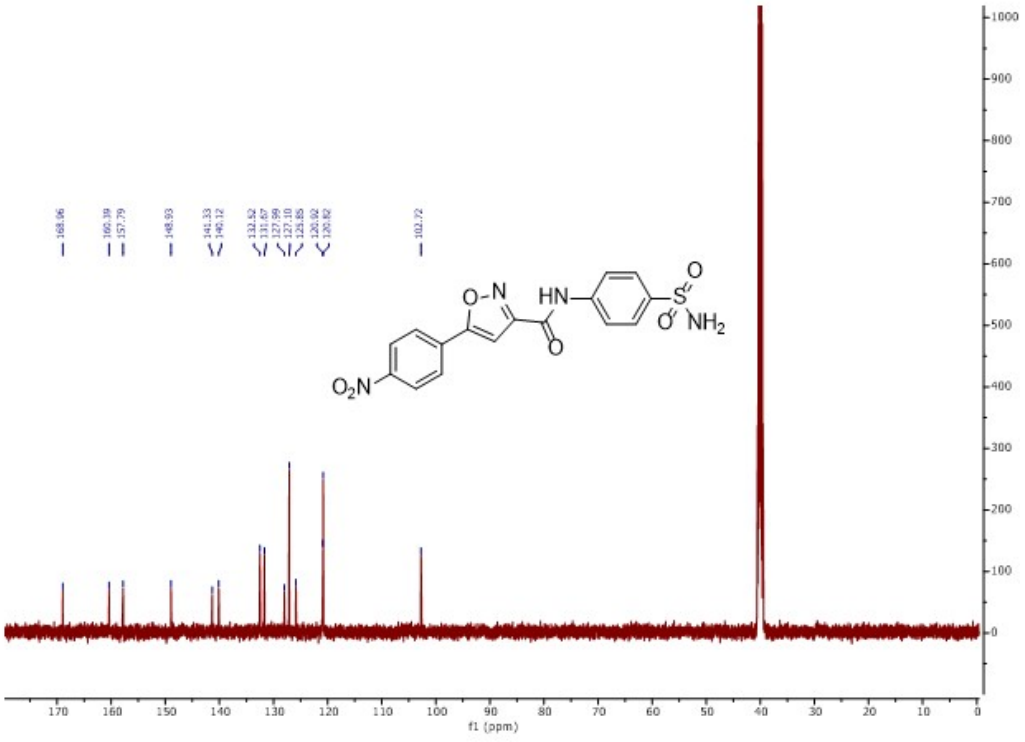


Mass spectrum of compound **10e**

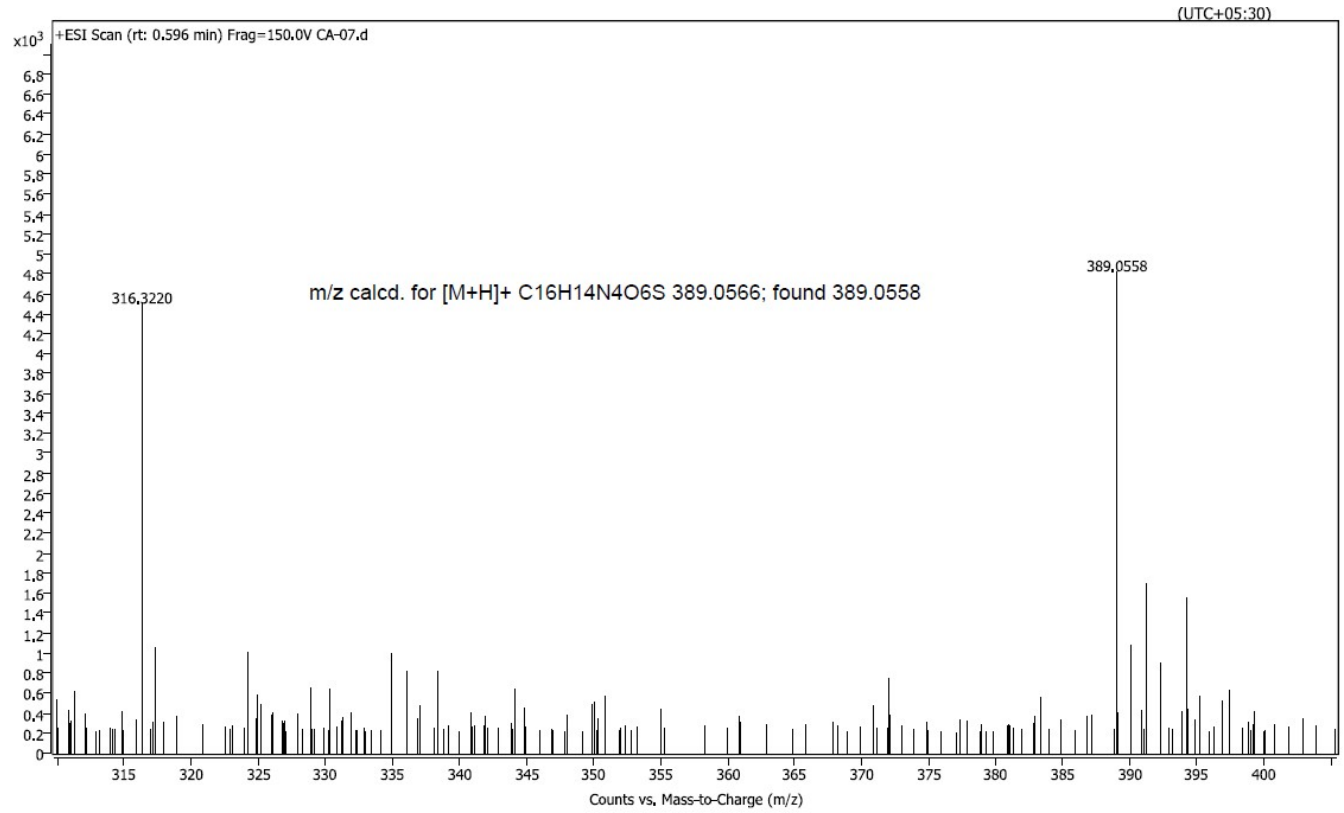
5-(3-nitrophenyl)-N-(4-sulfamoylphenyl)isoxazole-3-carboxamide **(10f)**



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **10f**

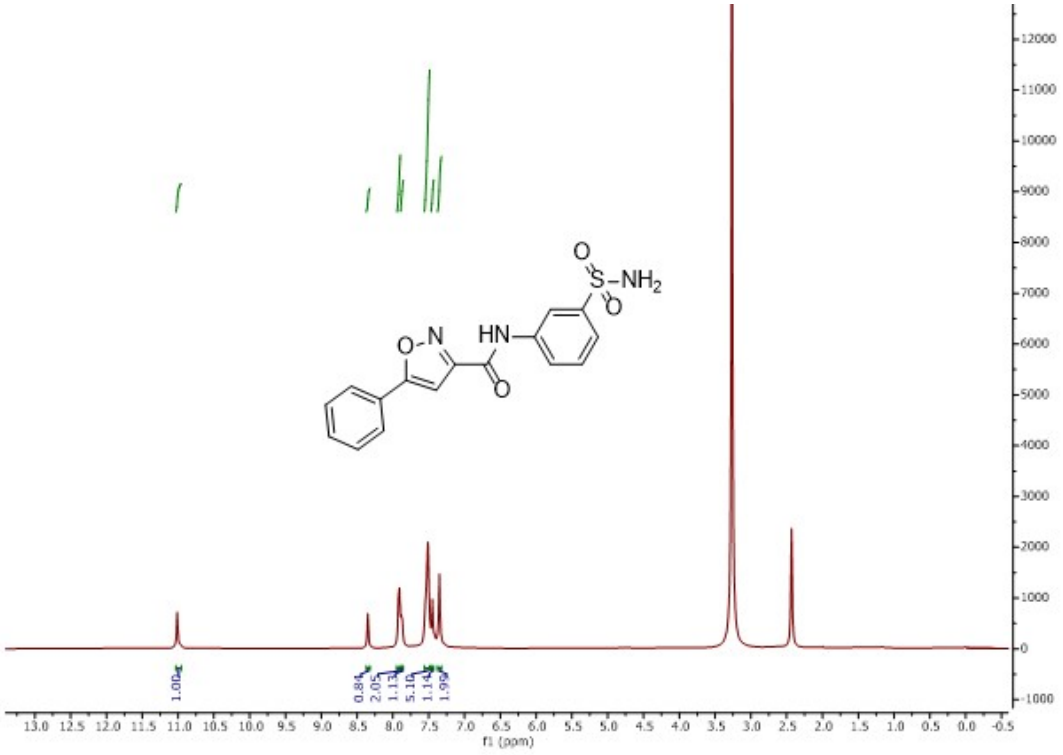


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **10f**

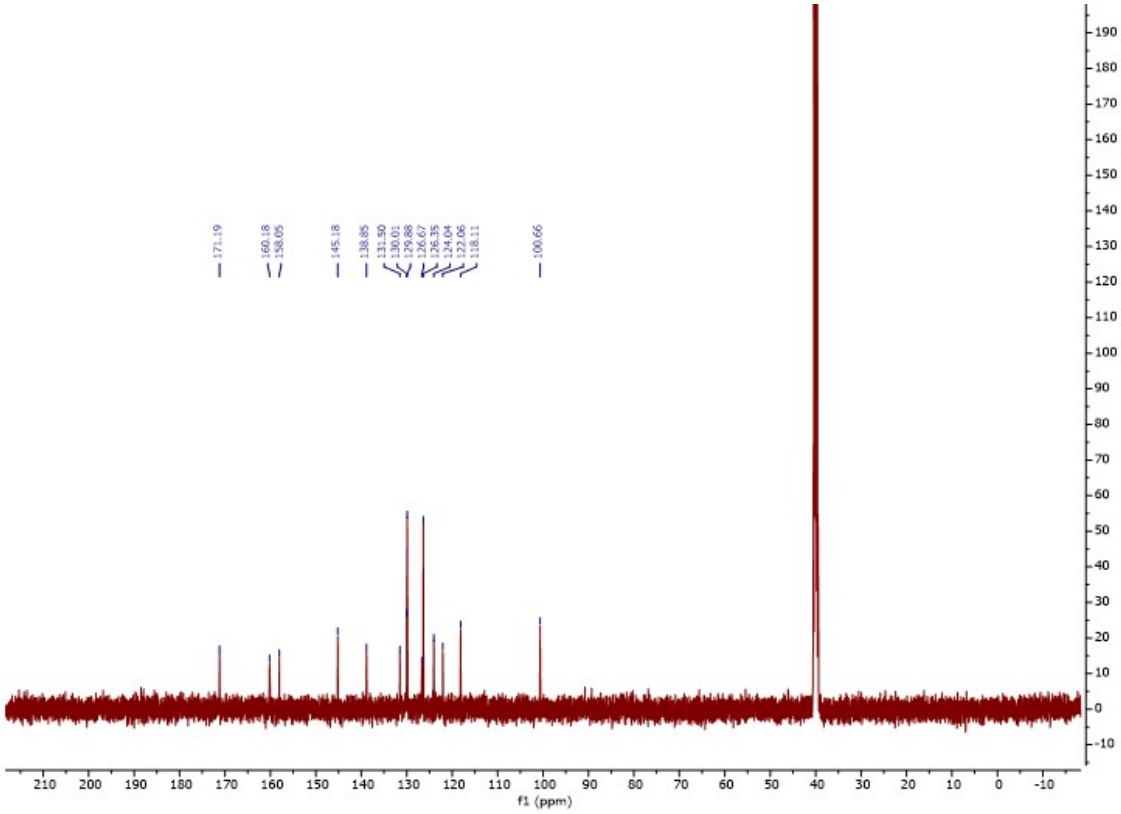


Mass spectrum of compound **10f**

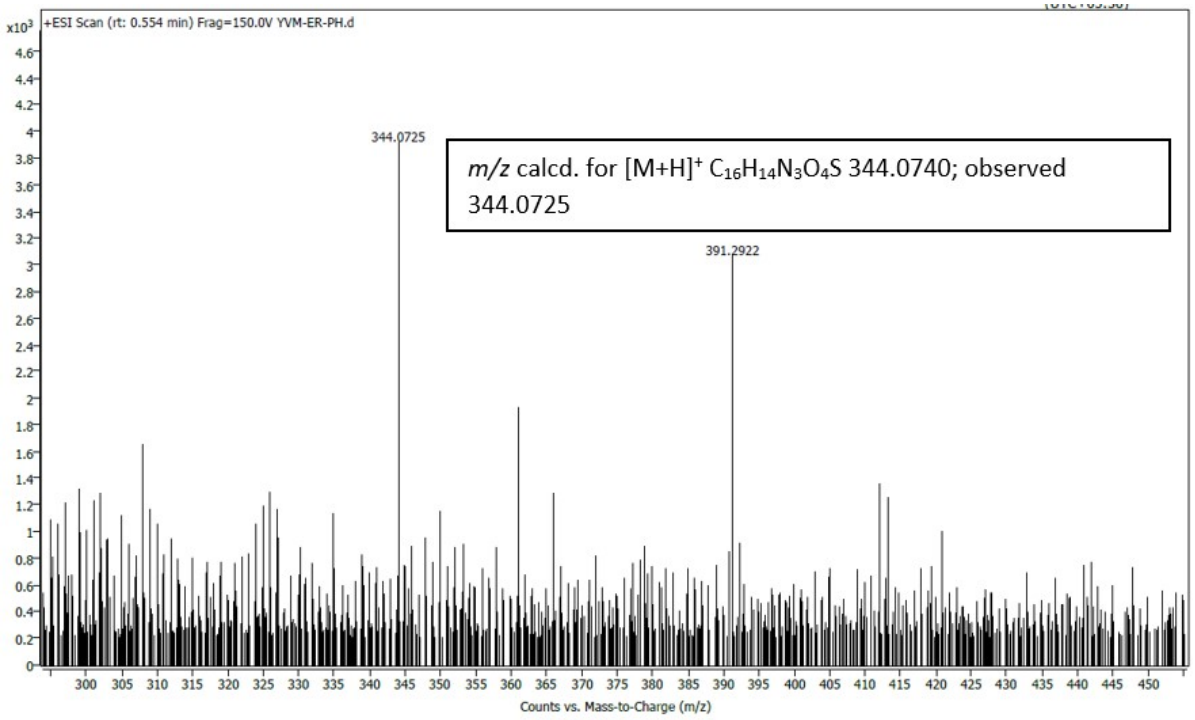
5-phenyl-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide (*12a*)



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **12a**

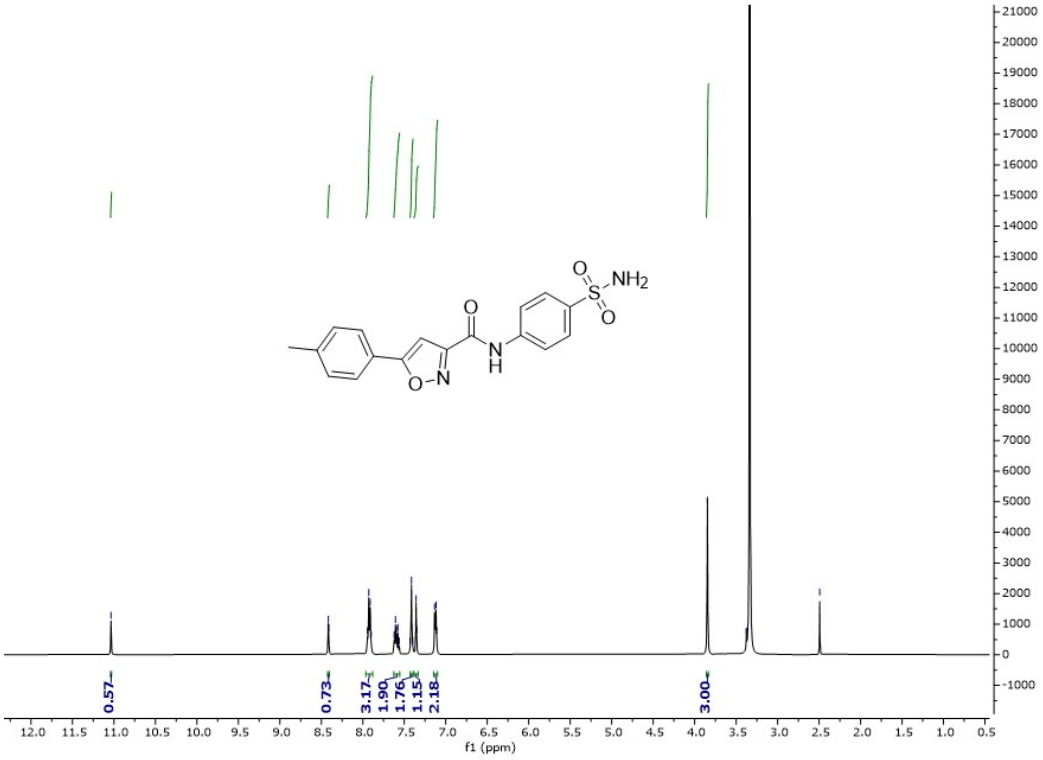


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **12a**

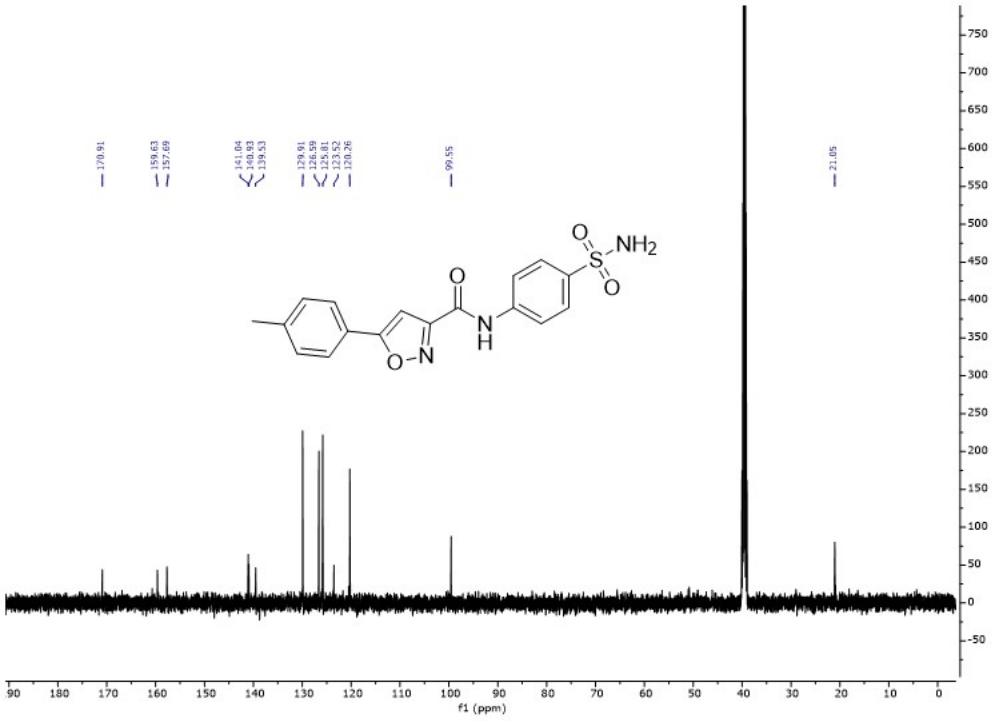


Mass spectrum of compound **12a**

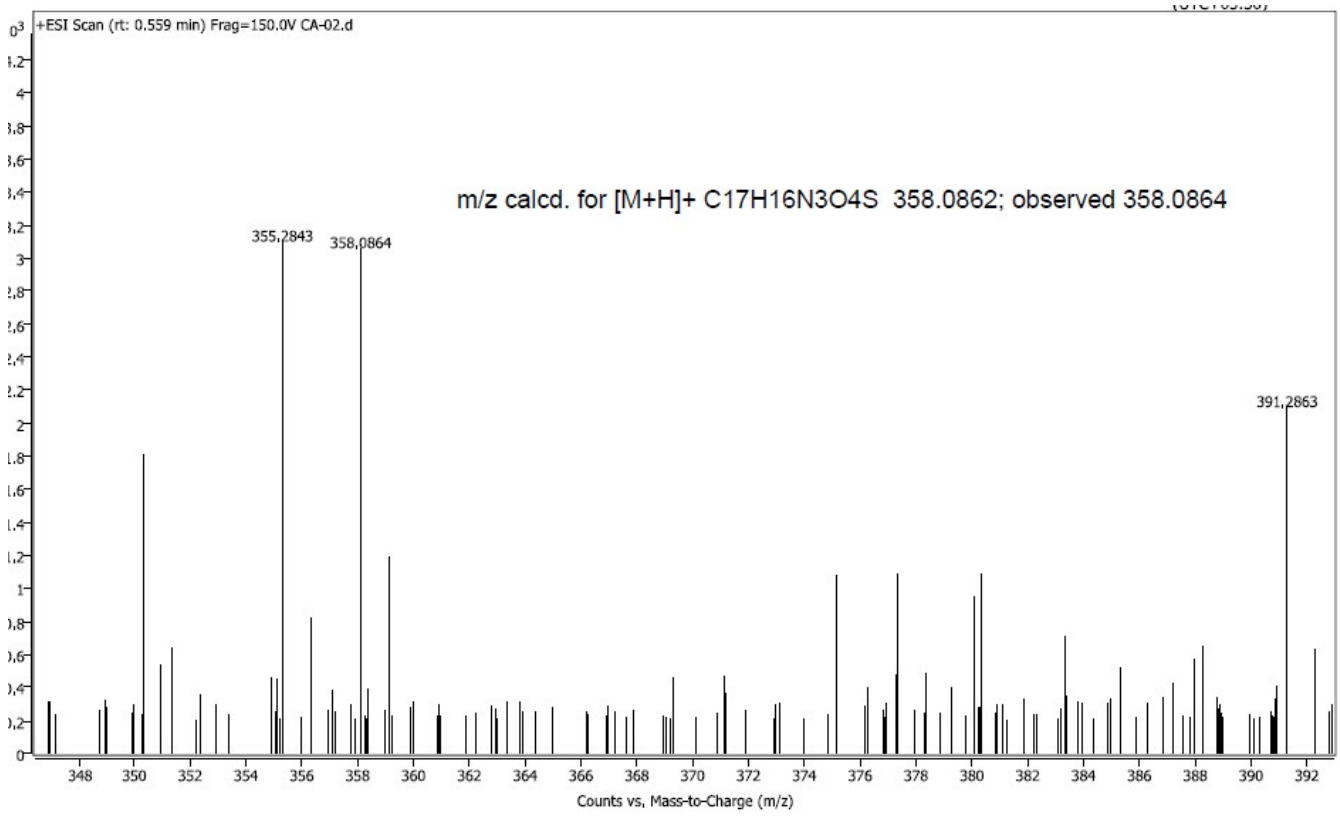
N-(4-sulfamoylphenyl)-5-(m-tolyl)isoxazole-3-carboxamide (12b)



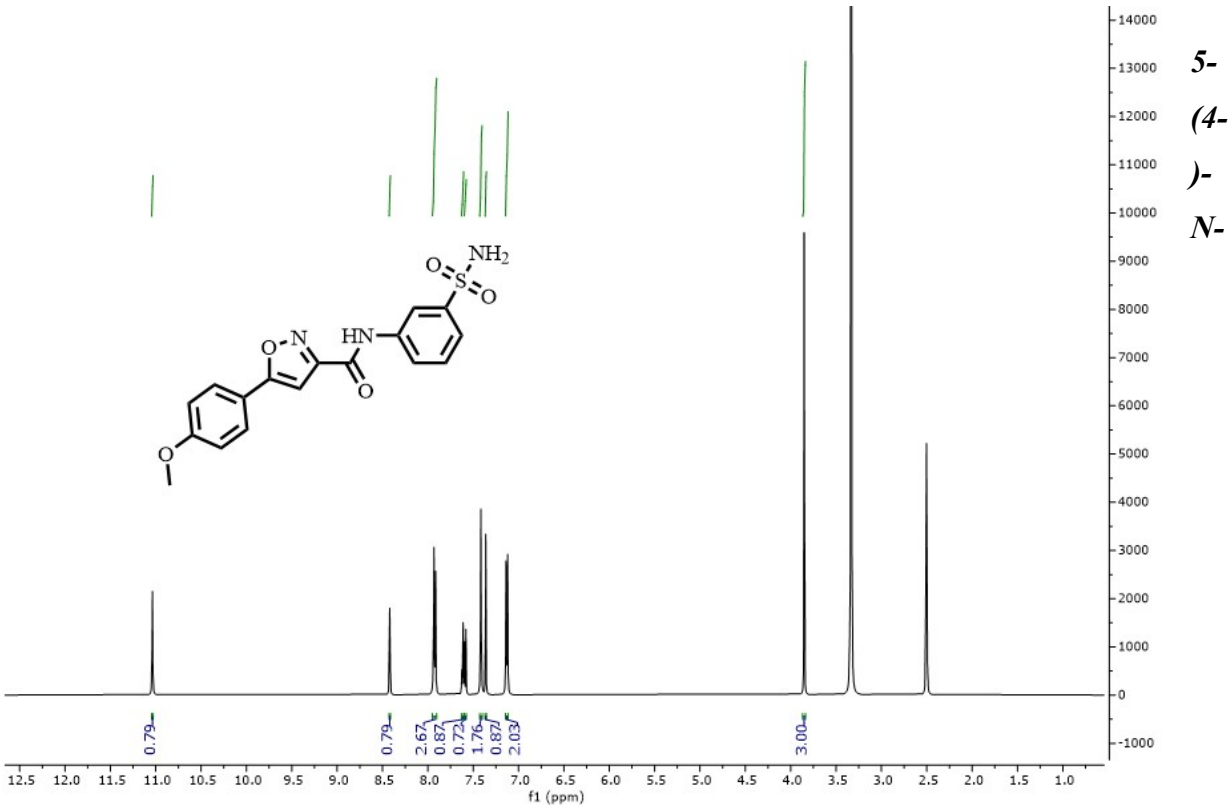
[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **12b**



^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **12b**



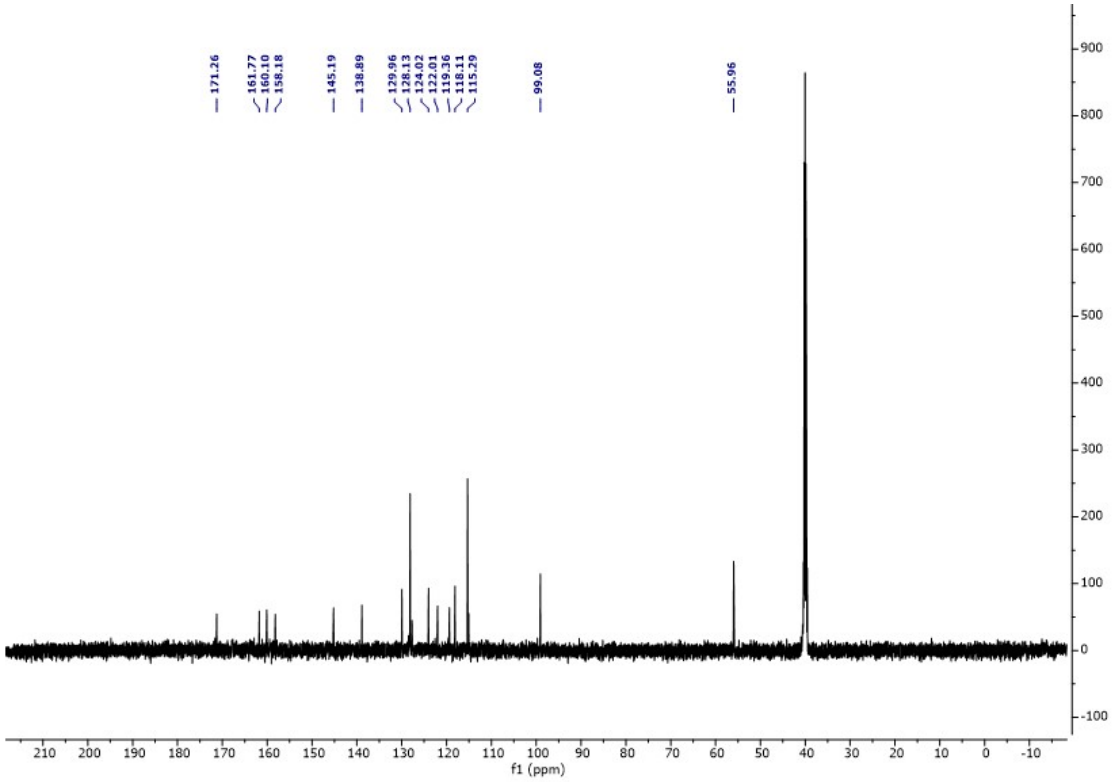
Mass spectrum of compound **12b**



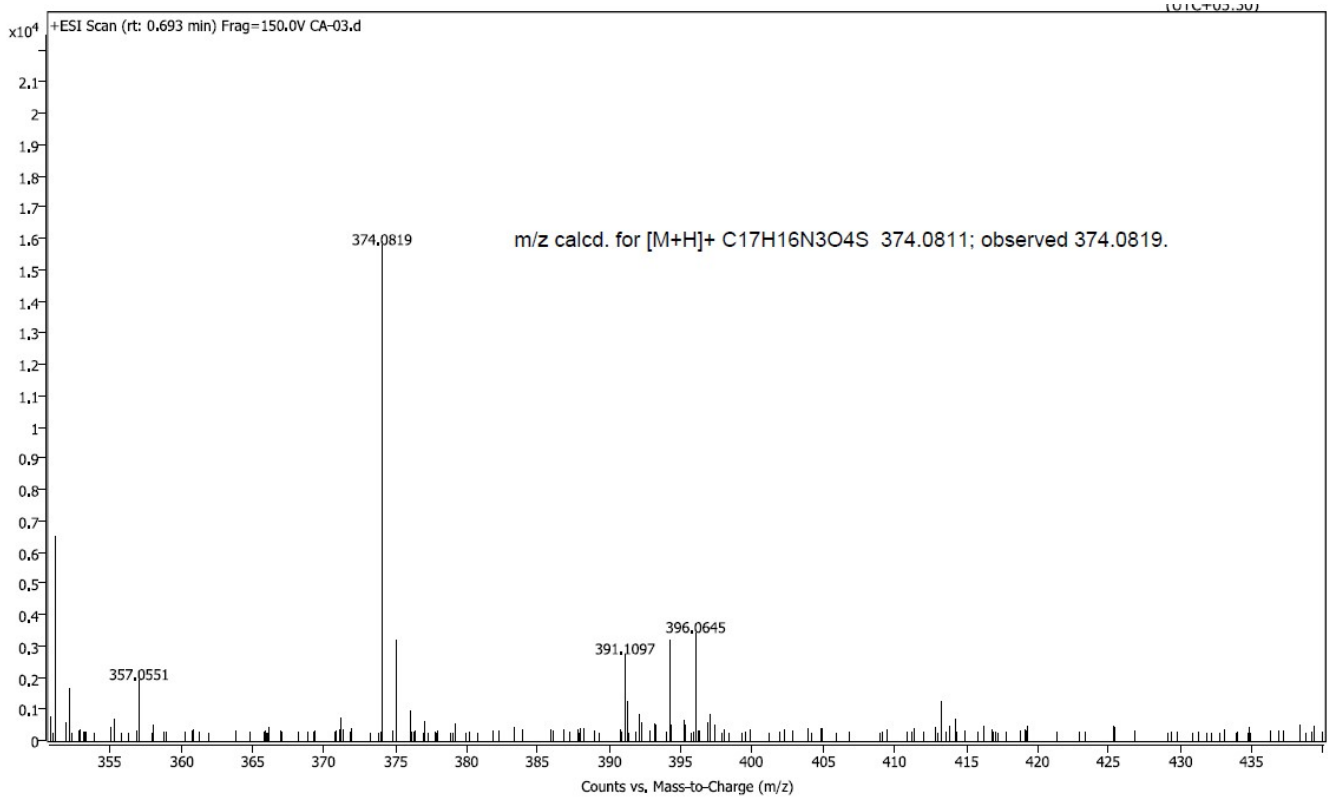
5-(4-)-N-

(3-sulfamoylphenyl)isoxazole-3-carboxamide (*12c*)

^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **12c**

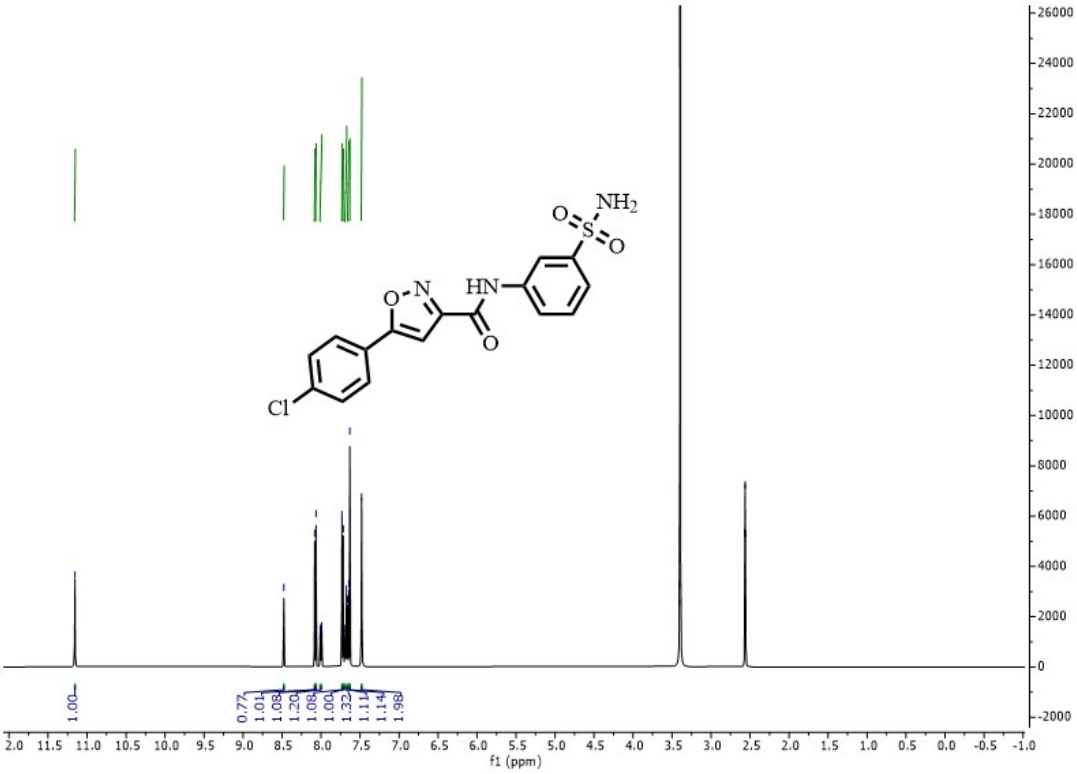


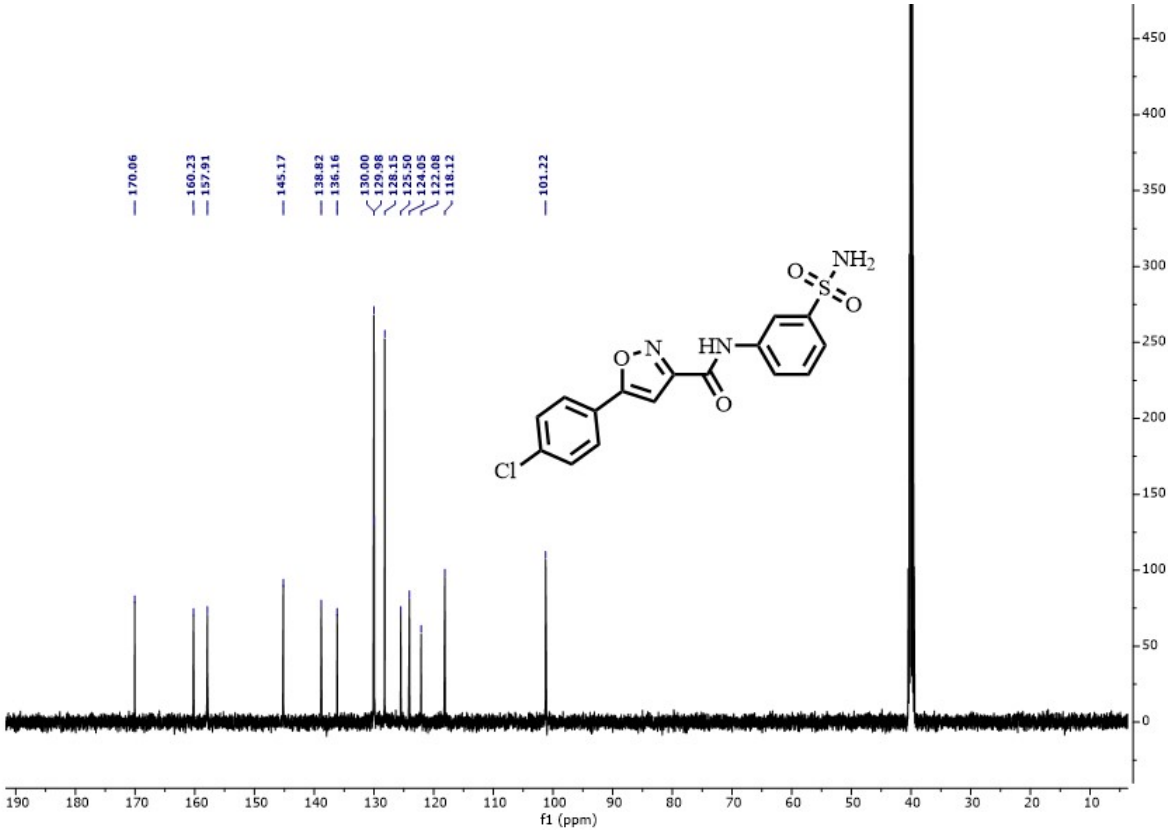
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **12c**



Mass spectrum of compound **12c**

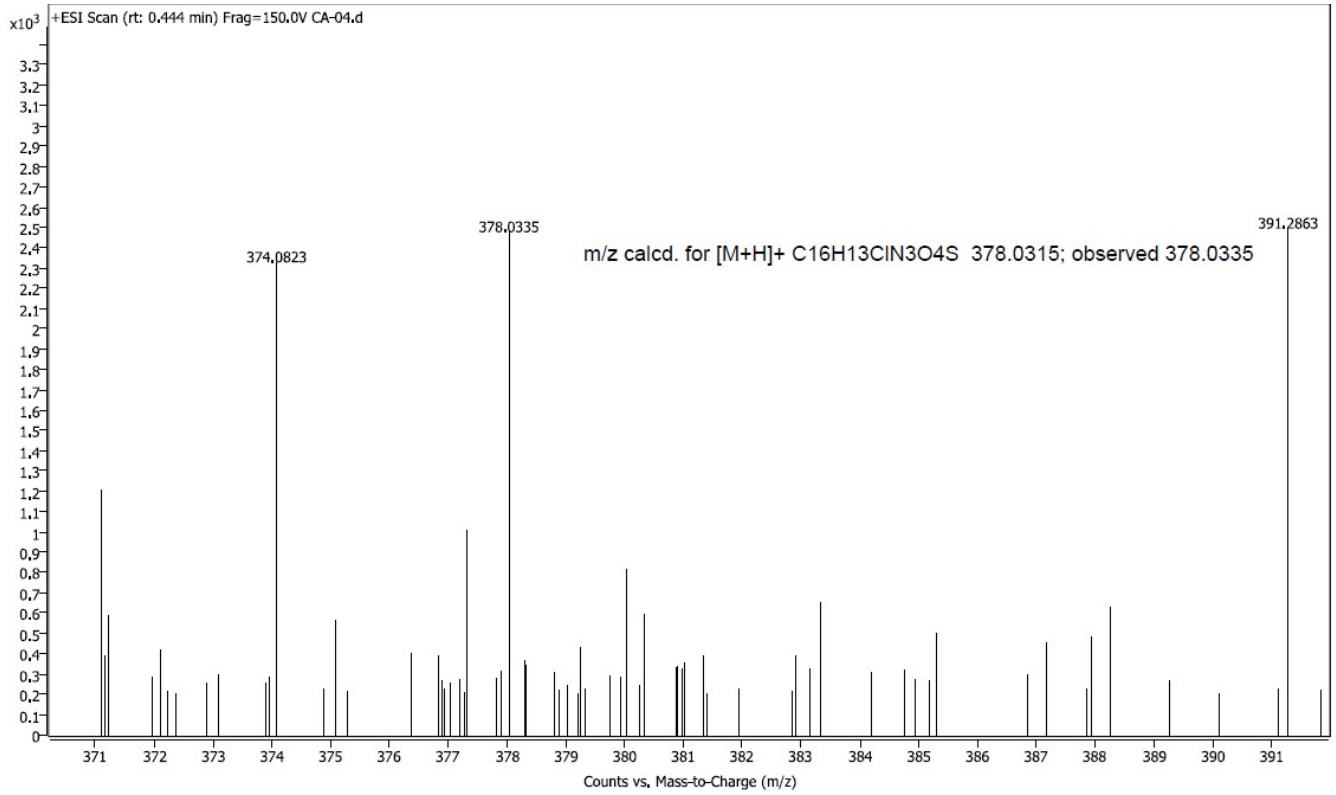
5-(4-chlorophenyl)-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide (*12d*)



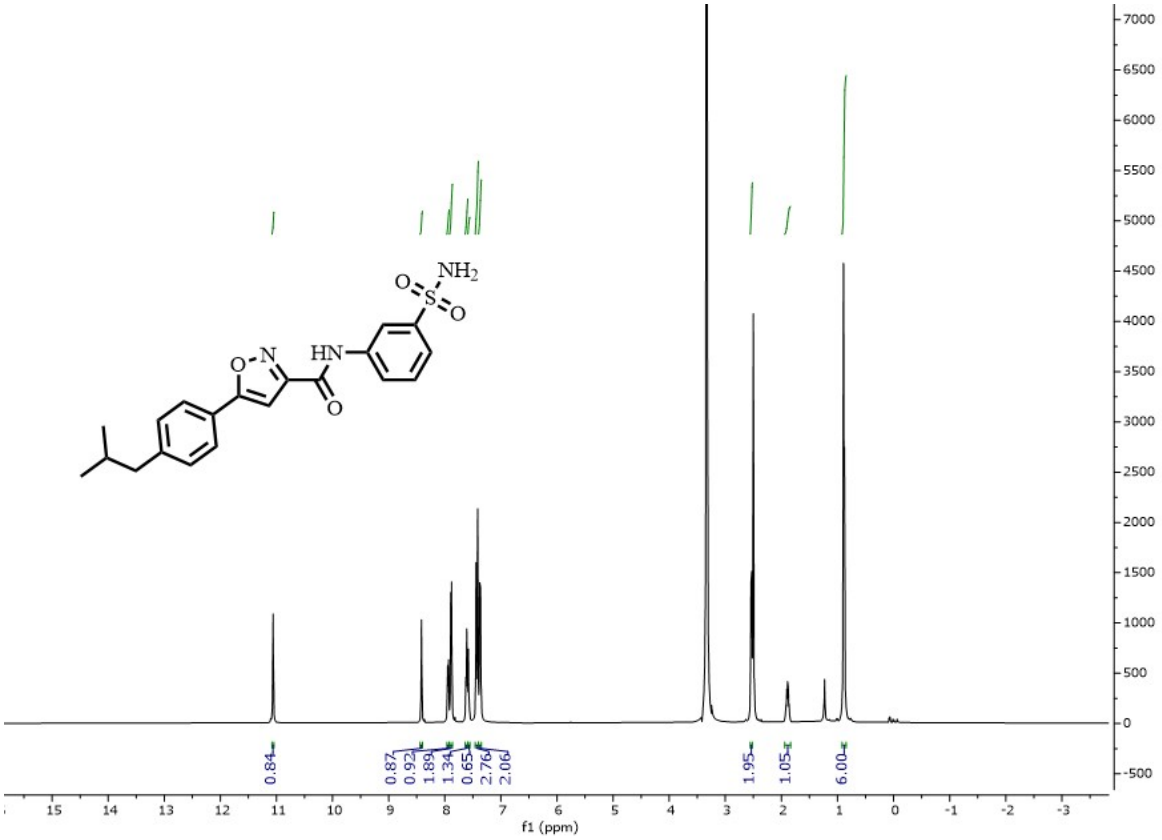


[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **12d**

^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **12d**



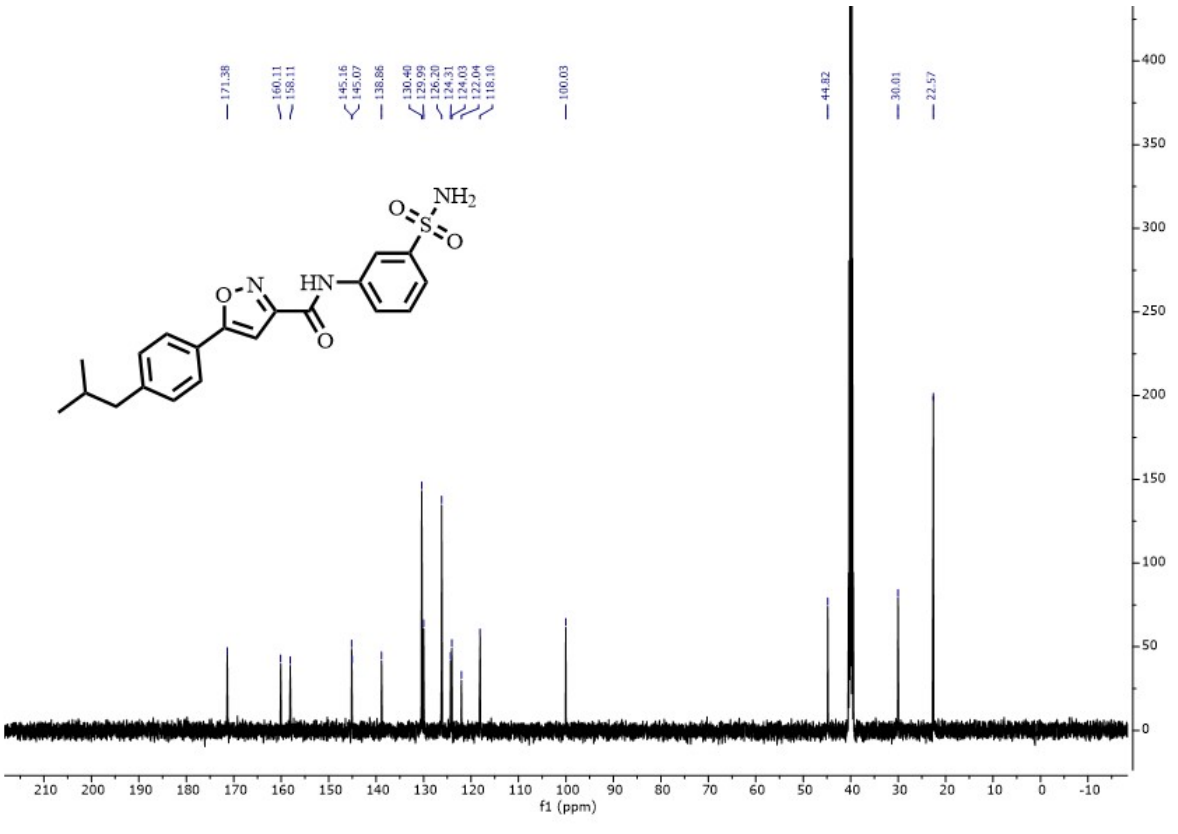
Mass spectrum of compound **12d**

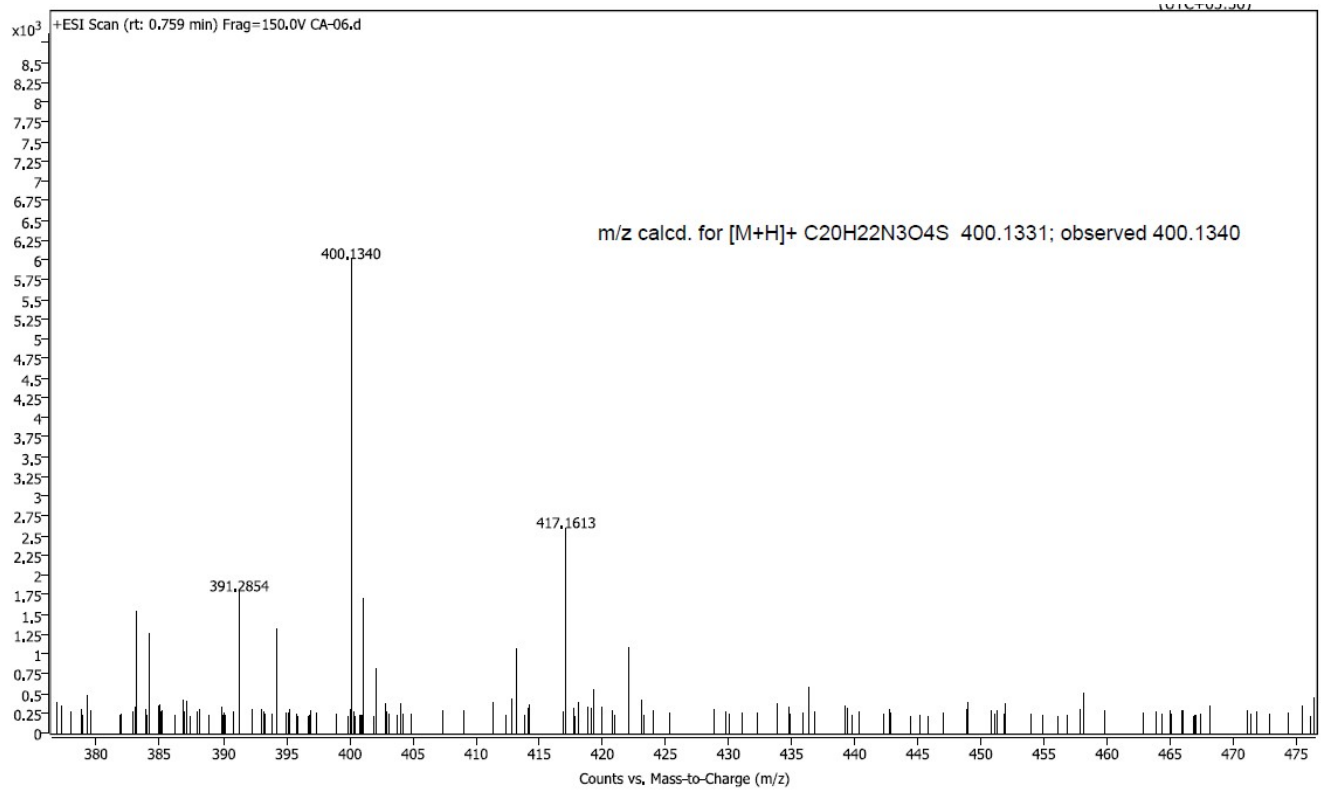


5-(4-isobutylphenyl)-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide(*12e*)

^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **12e**

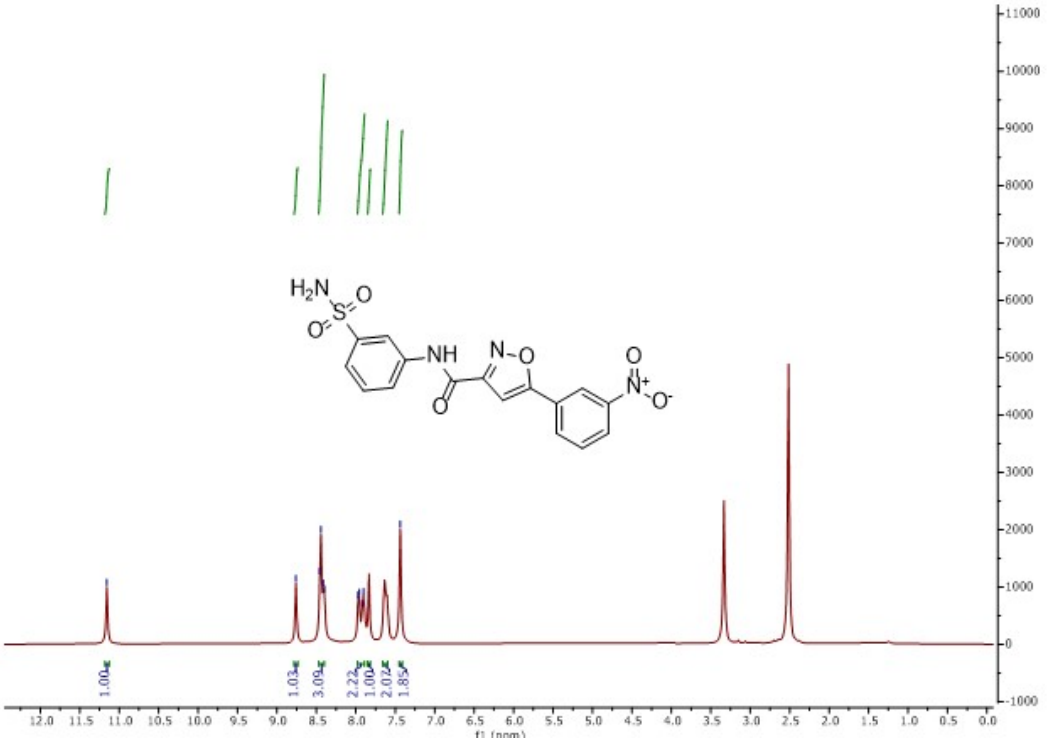
^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **12e**



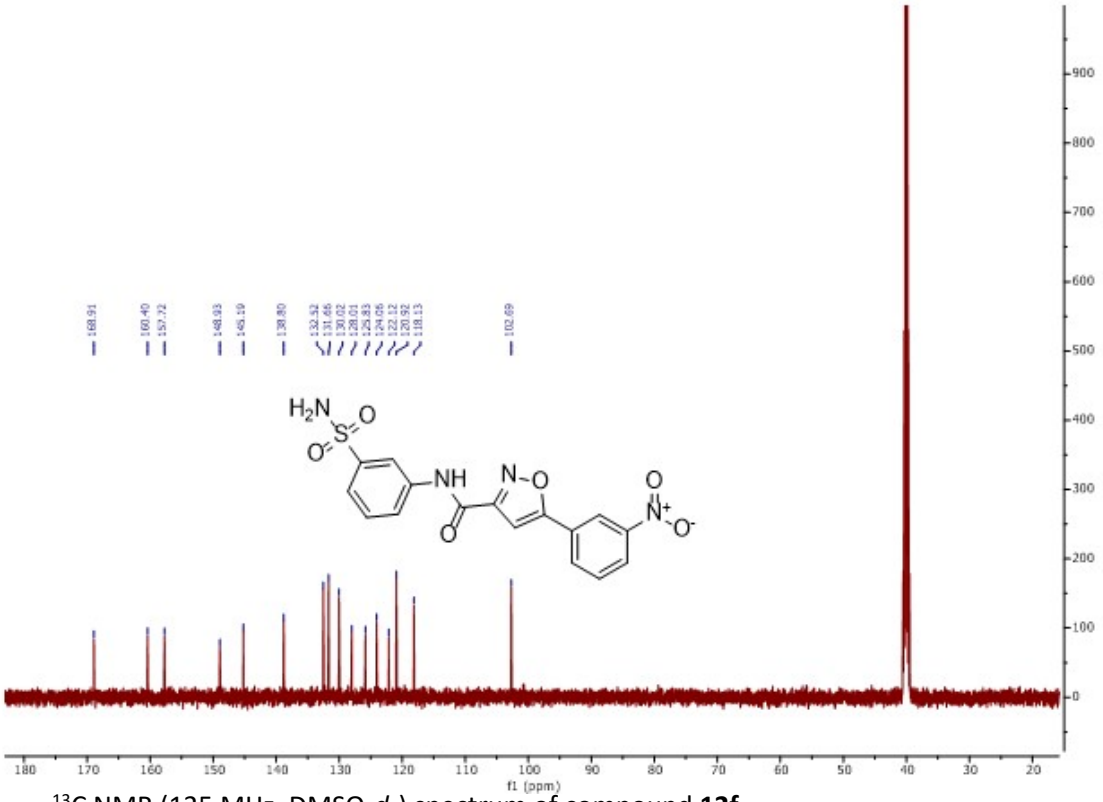


Mass spectrum of compound **12e**

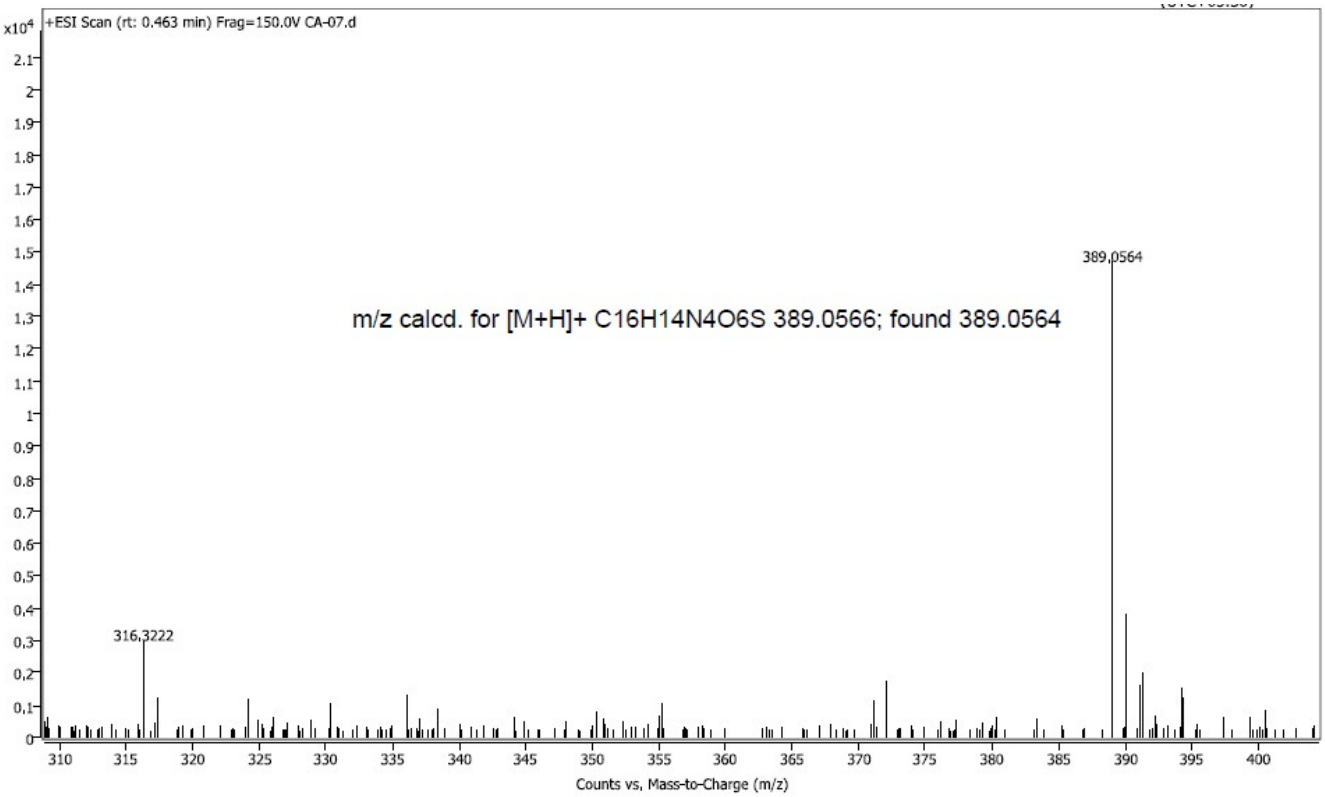
***5-(3-nitrophenyl)-N-(3-sulfamoylphenyl)isoxazole-3-carboxamide*(12f)**



[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **12f**

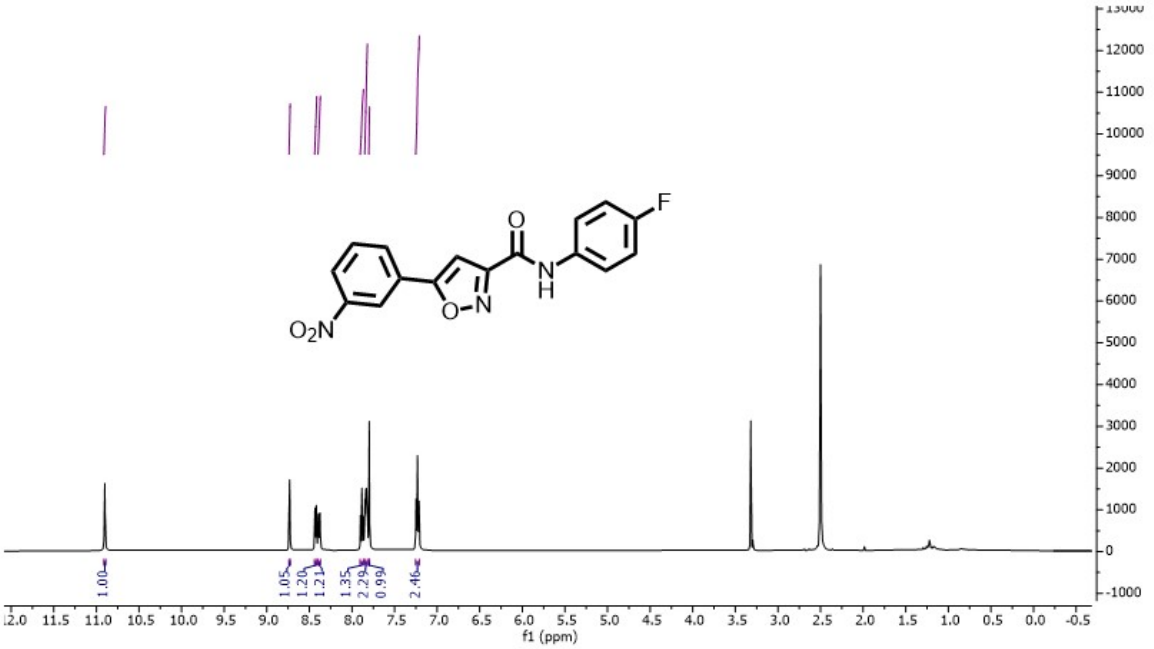


[13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **12f**

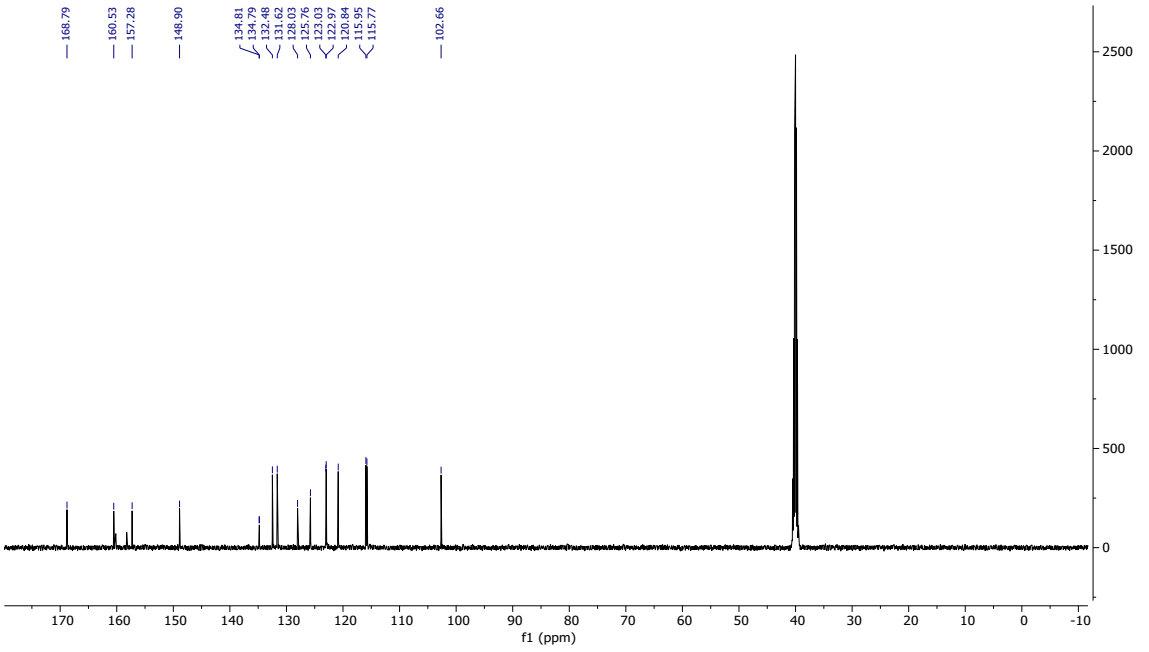


Mass spectra of compound **12f**

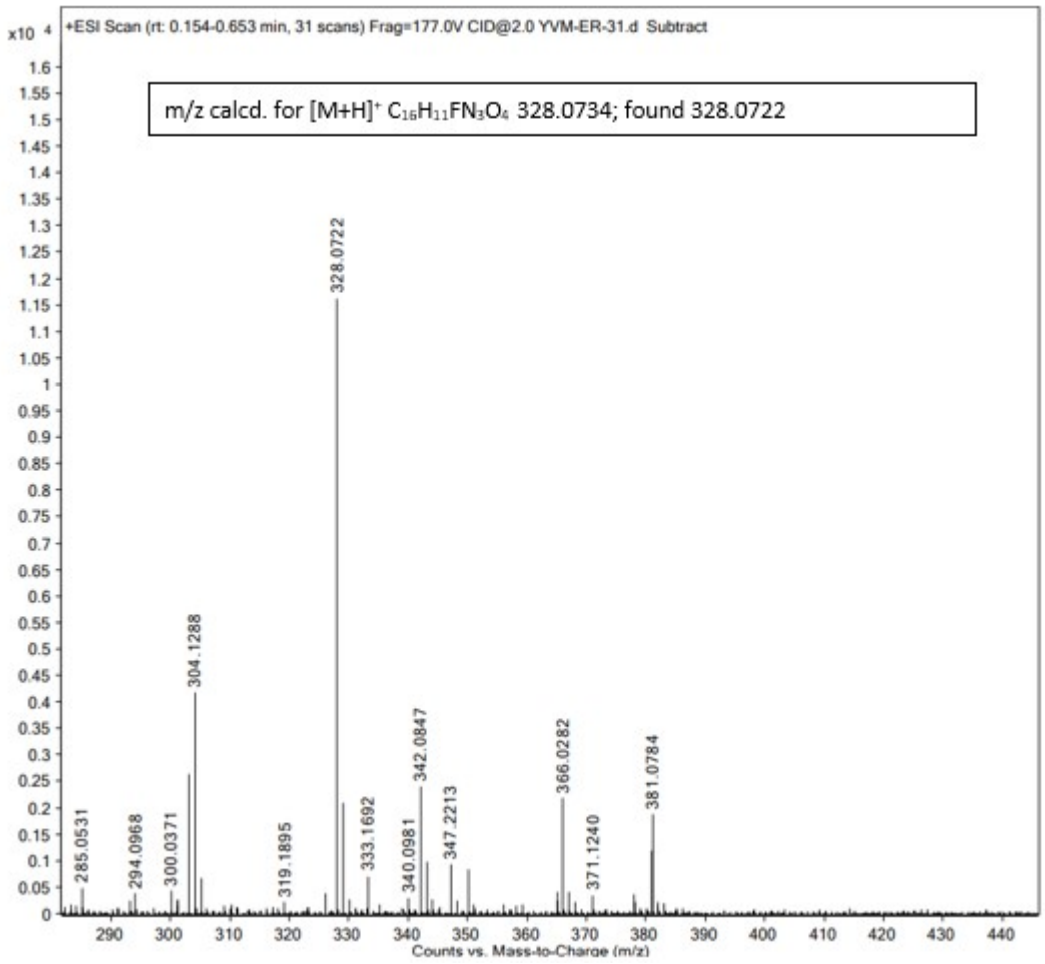
***N-(4-fluorophenyl)-5-(3-nitrophenyl)isoxazole-3-carboxamide*(14a)**



[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **14a**

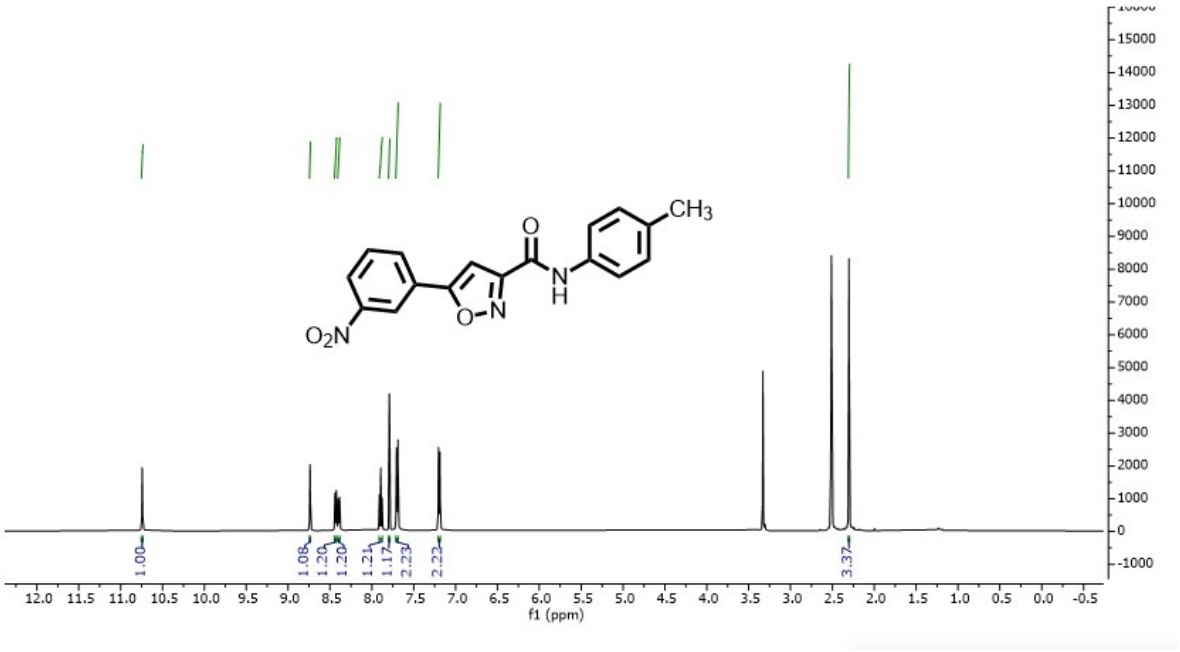


[13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **14a**

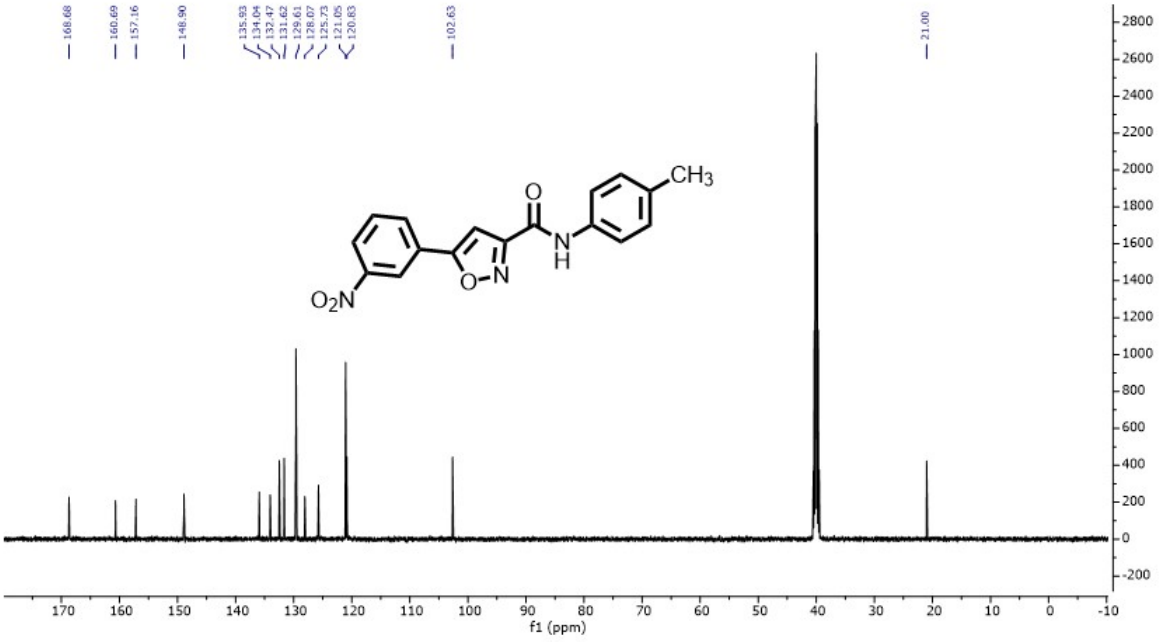


Mass spectrum of compound **14a**

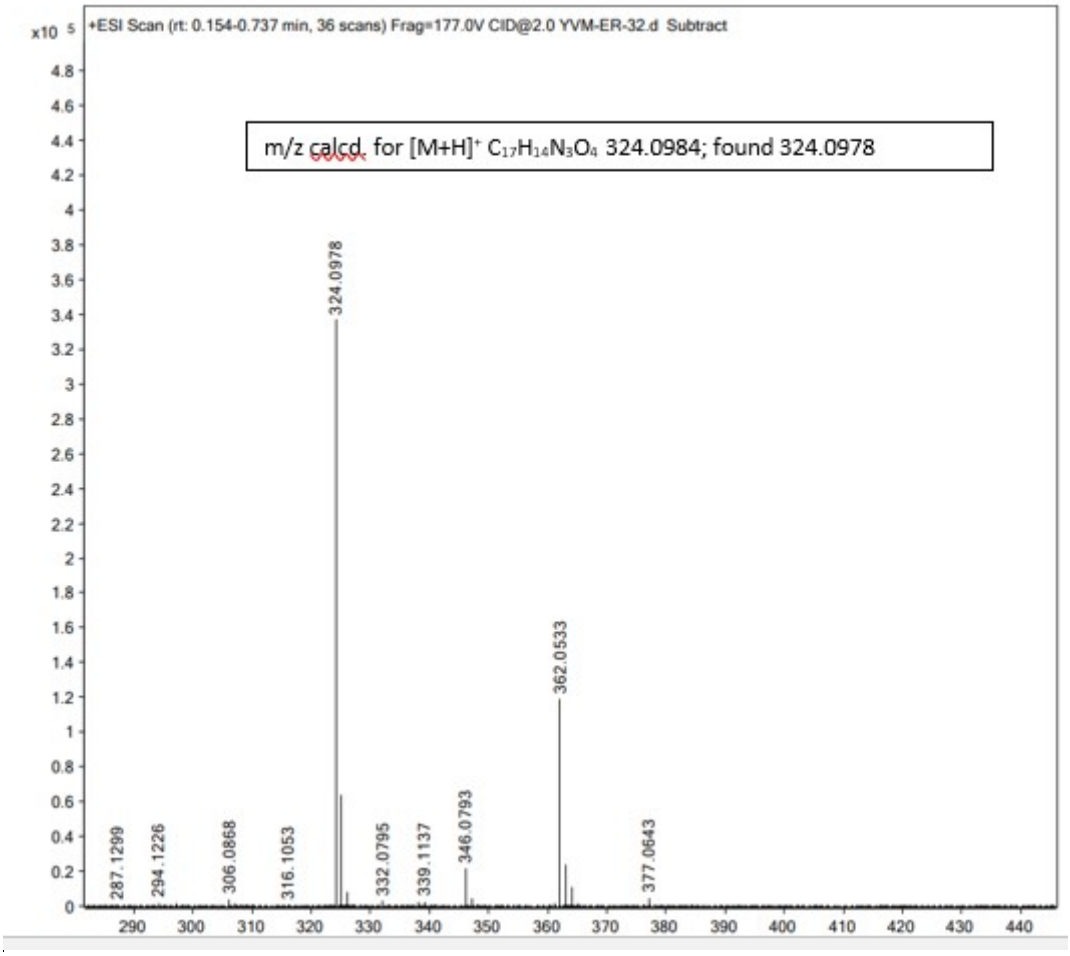
***5-(3-nitrophenyl)-N-(p-tolyl)isoxazole-3-carboxamide*(14b)**



[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **14b**

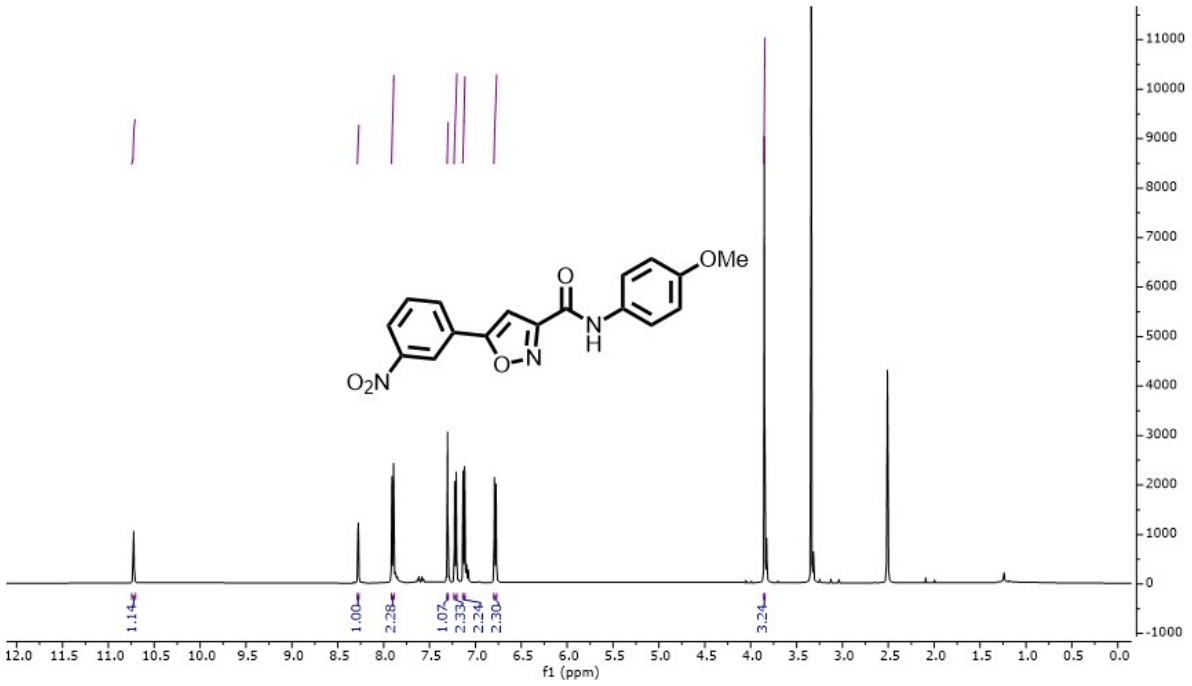


[13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **14b**

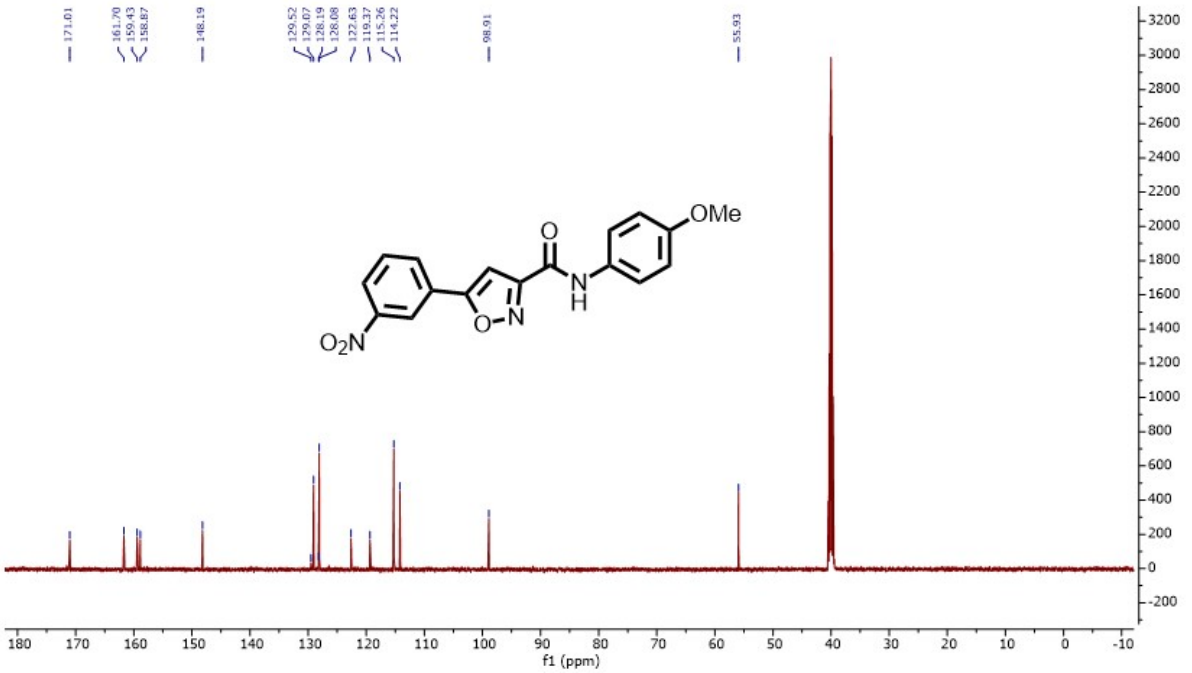


Mass spectra of compound 14b

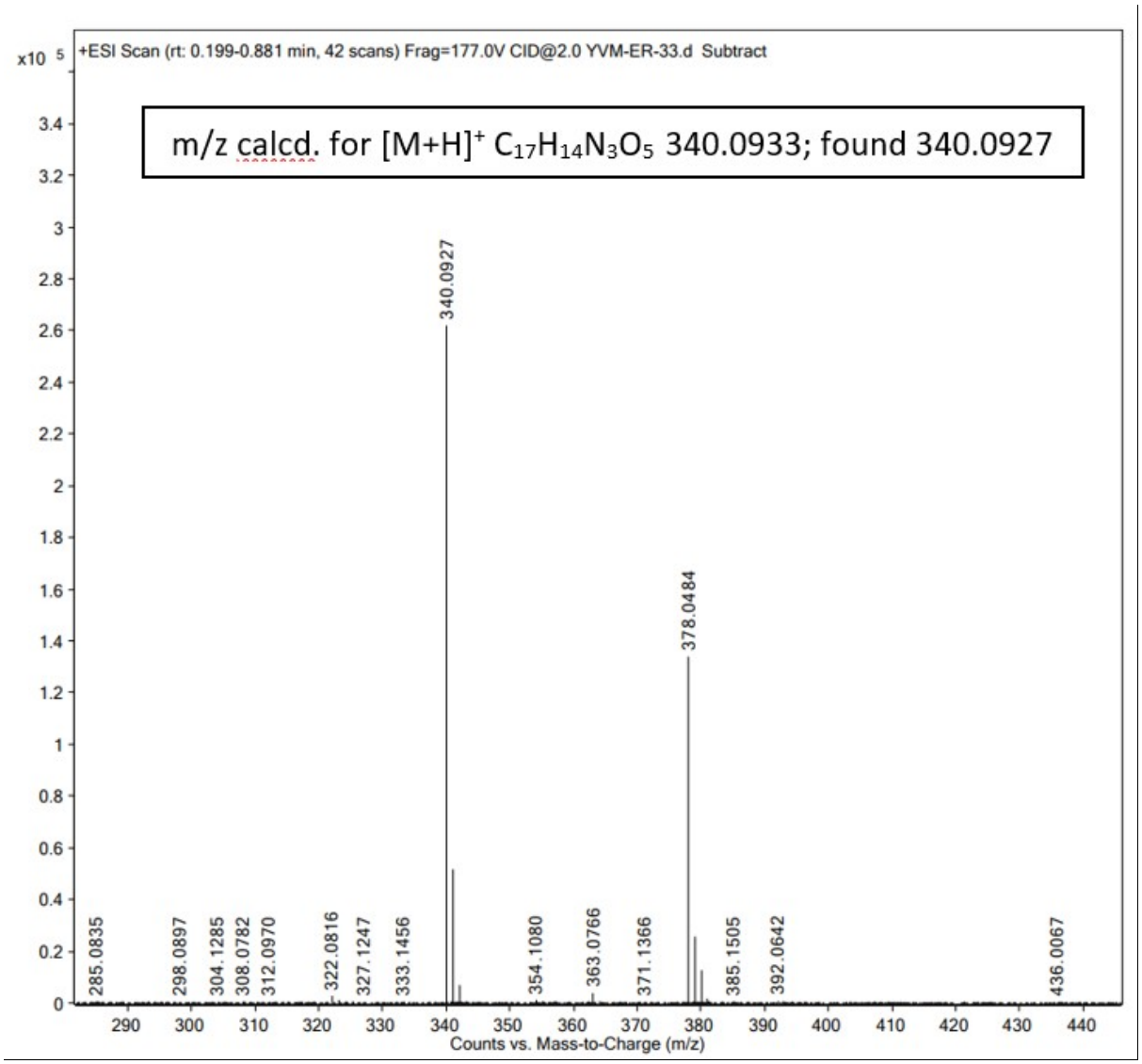
N-(4-methoxyphenyl)-5-(3-nitrophenyl)isoxazole-3-carboxamide **(14c)**



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **14c**

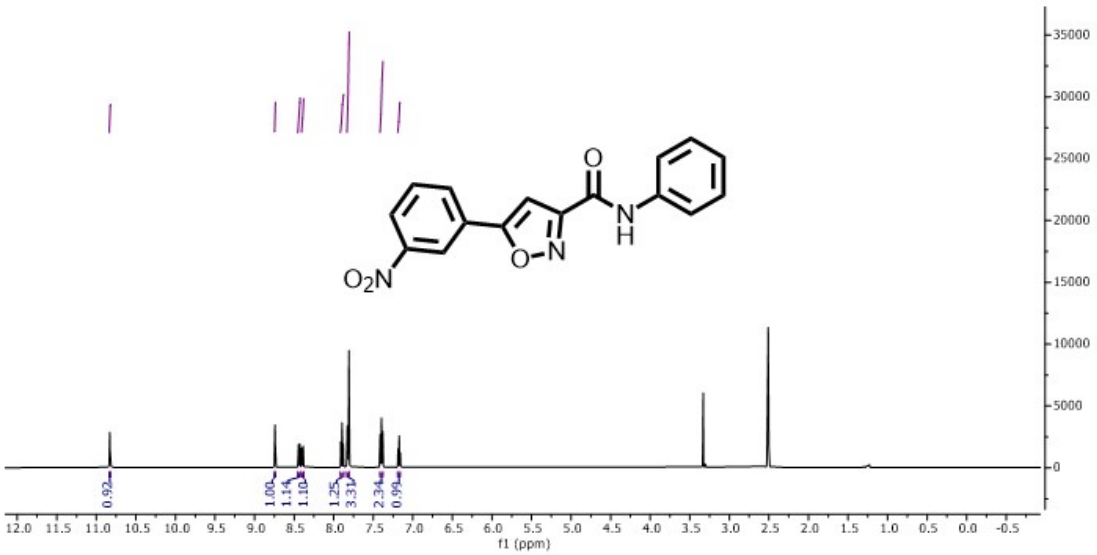


^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **14c**

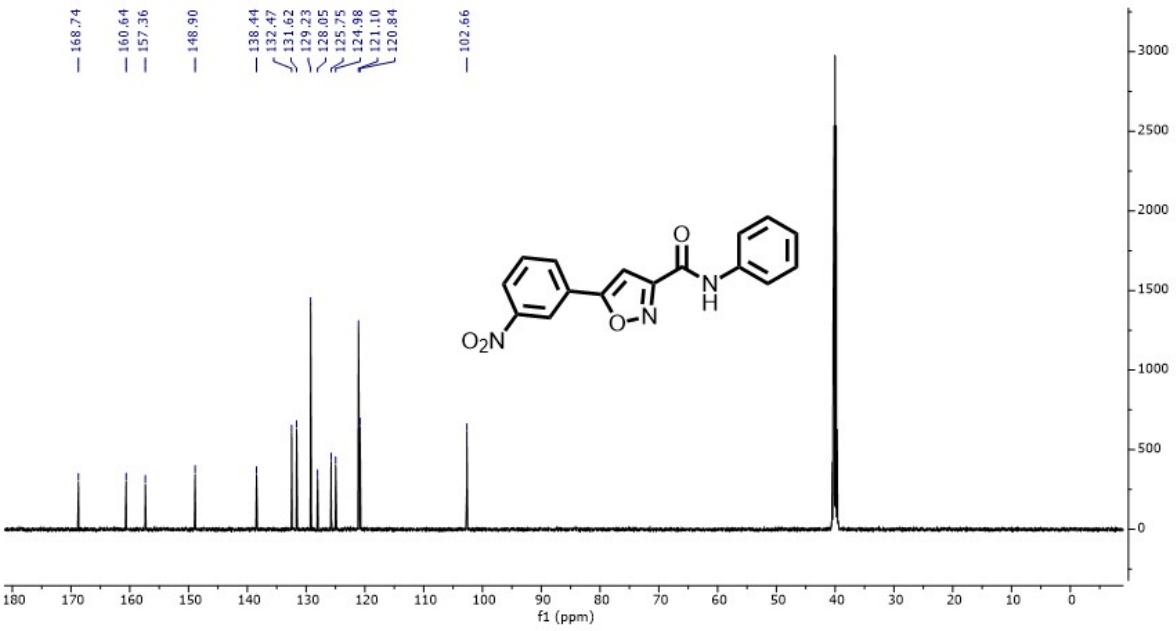


Mass spectra of compound **14c**

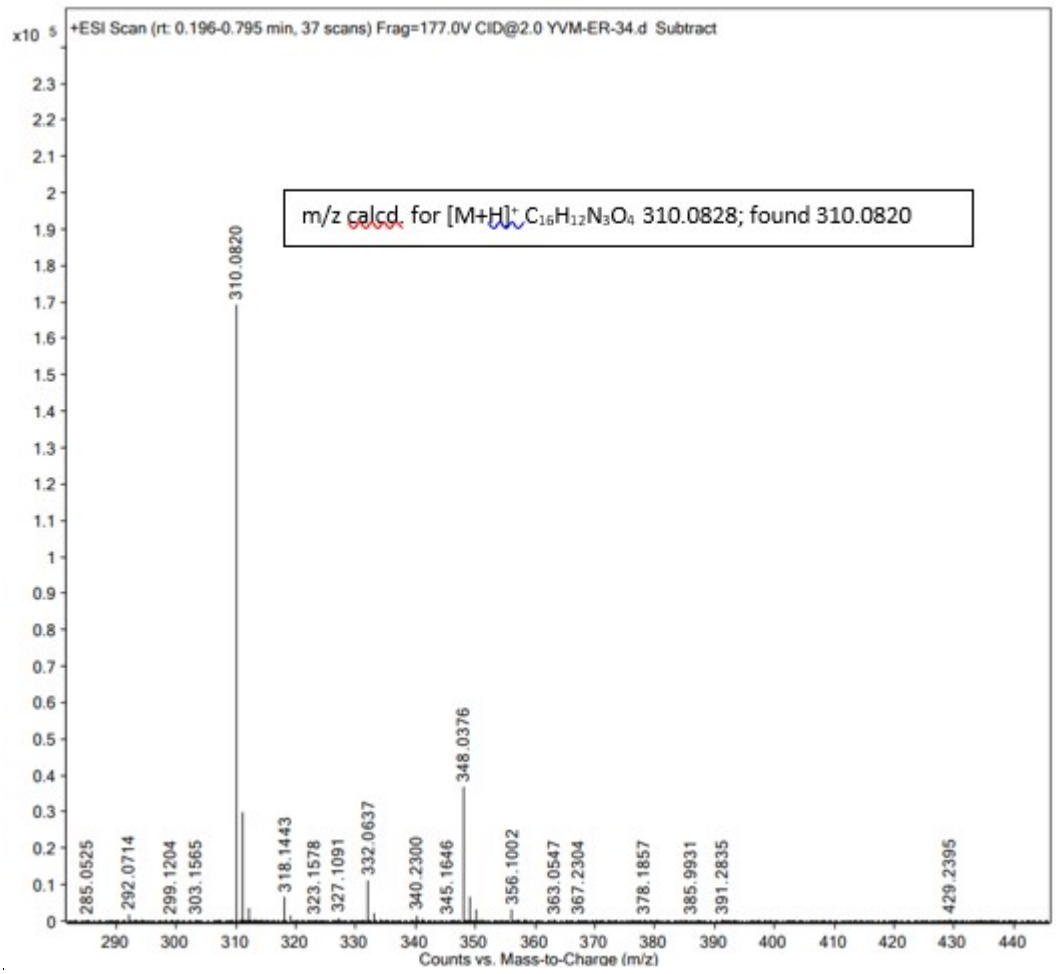
5-(3-nitrophenyl)-N-phenylisoxazole-3-carboxamide **(14d)**



[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **14d**

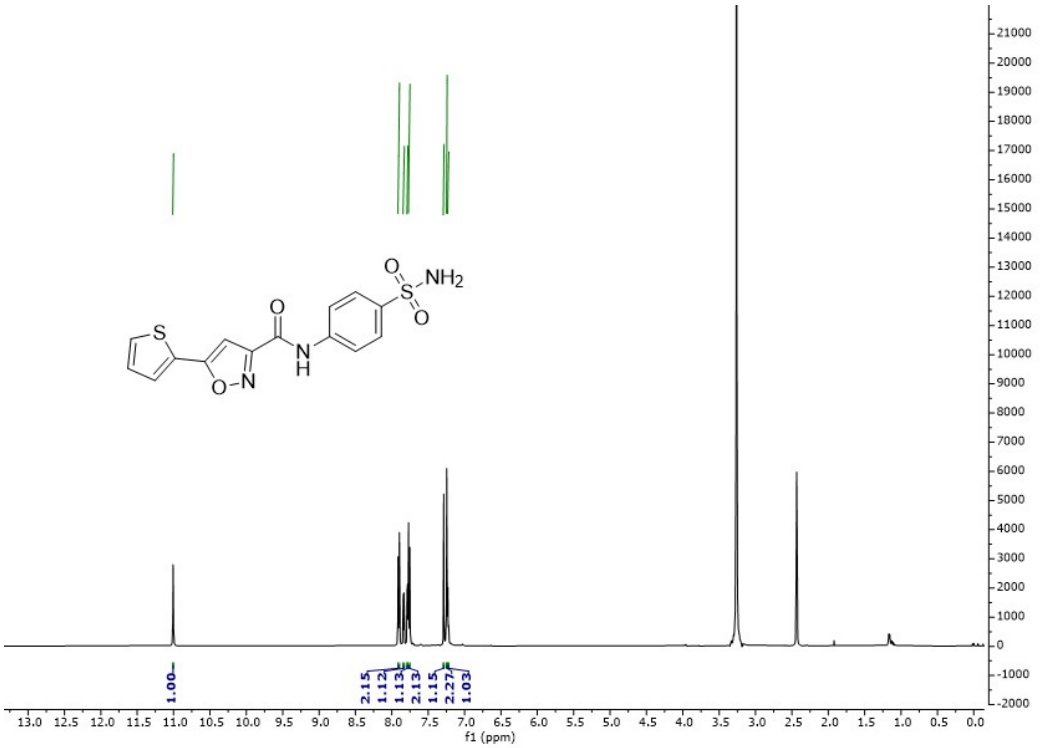


[13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **14d**

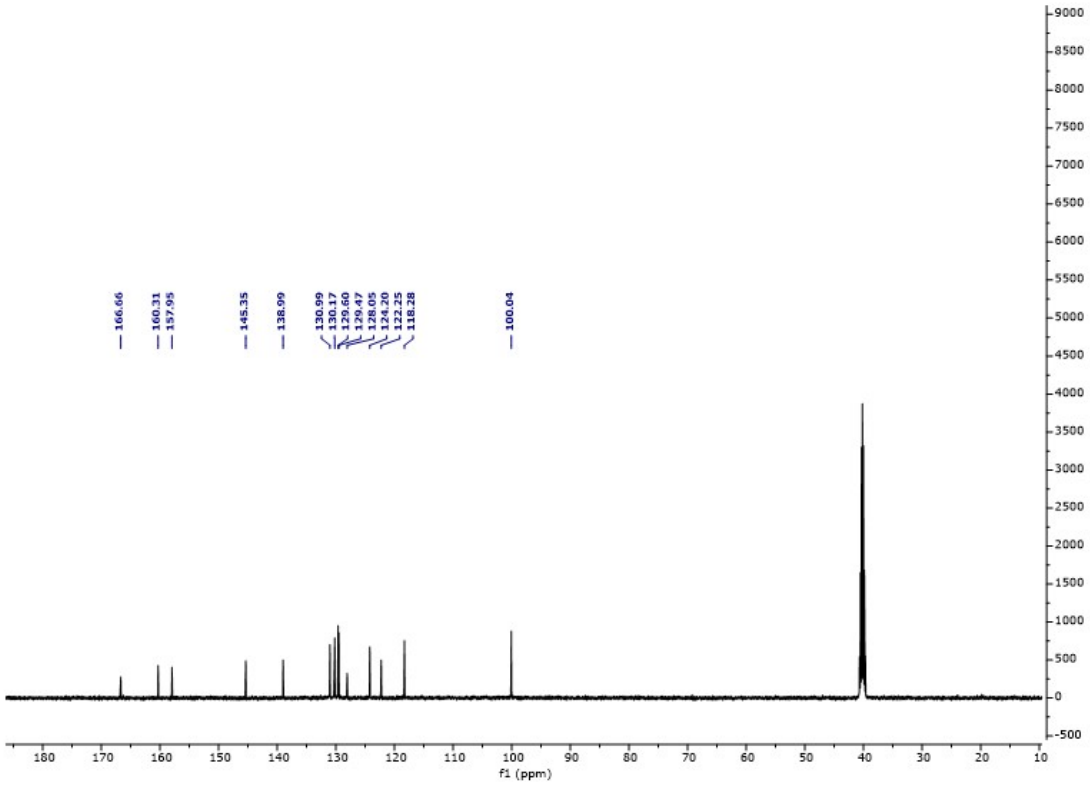


Mass spectra of compound **14d**

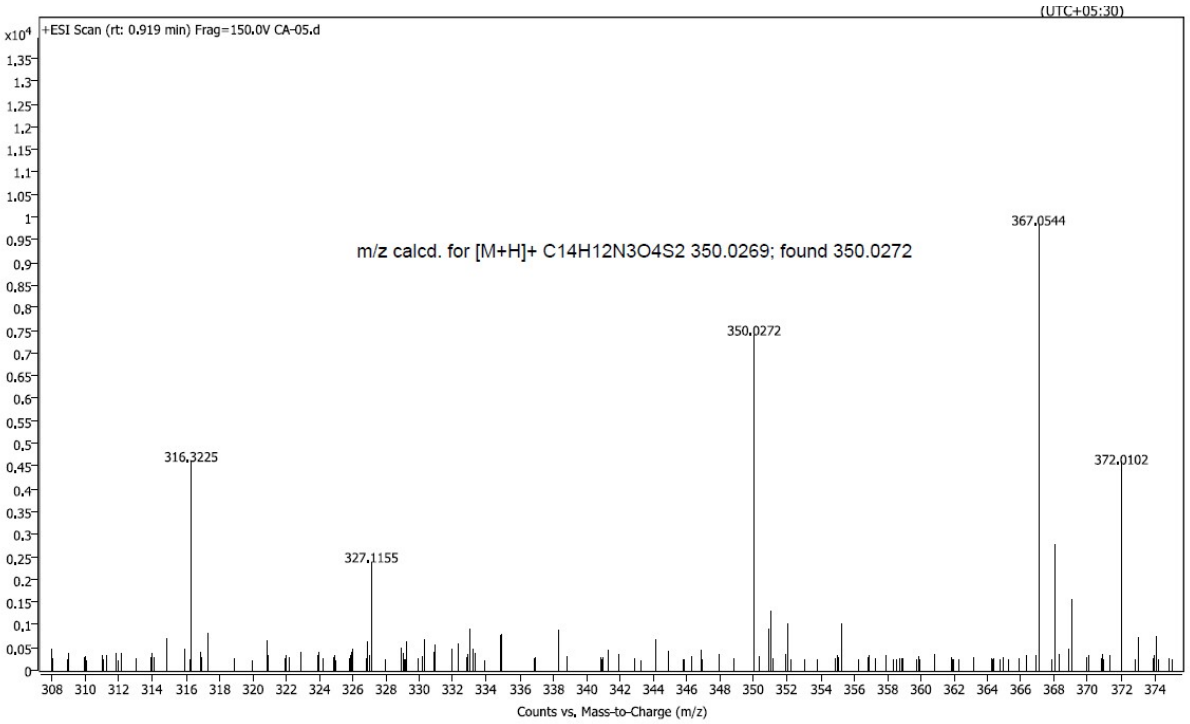
N-(4-sulfamoylphenyl)-5-(thiophen-2-yl)isoxazole-3-carboxamide (19a)



[1] H NMR (500 MHz, DMSO-d_6) spectrum of compound **19a**

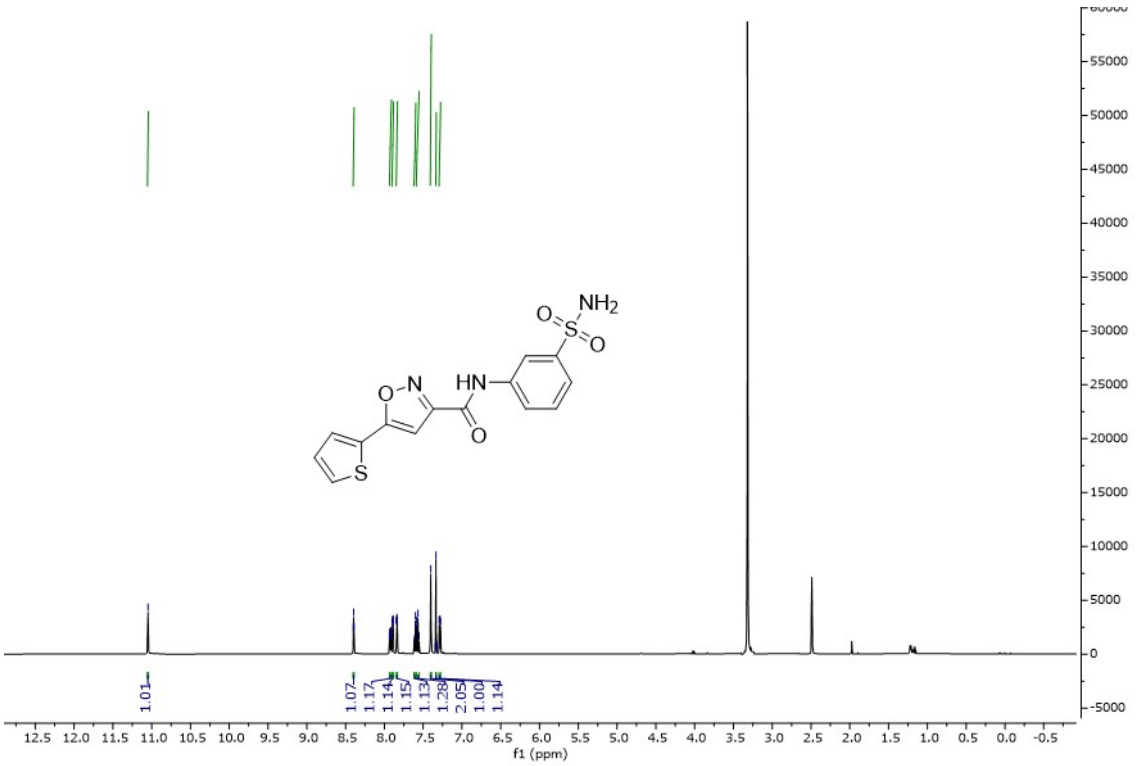


[13]C NMR (125 MHz, DMSO-d_6) spectrum of compound **19a**

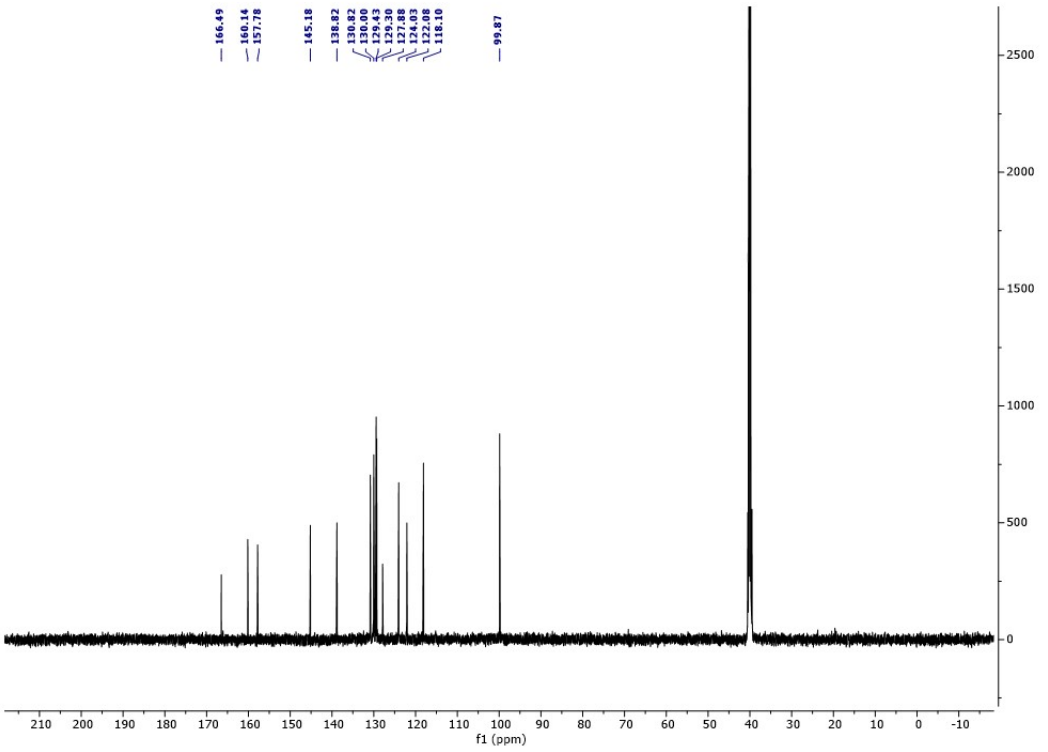


Mass spectra of compound **19a**

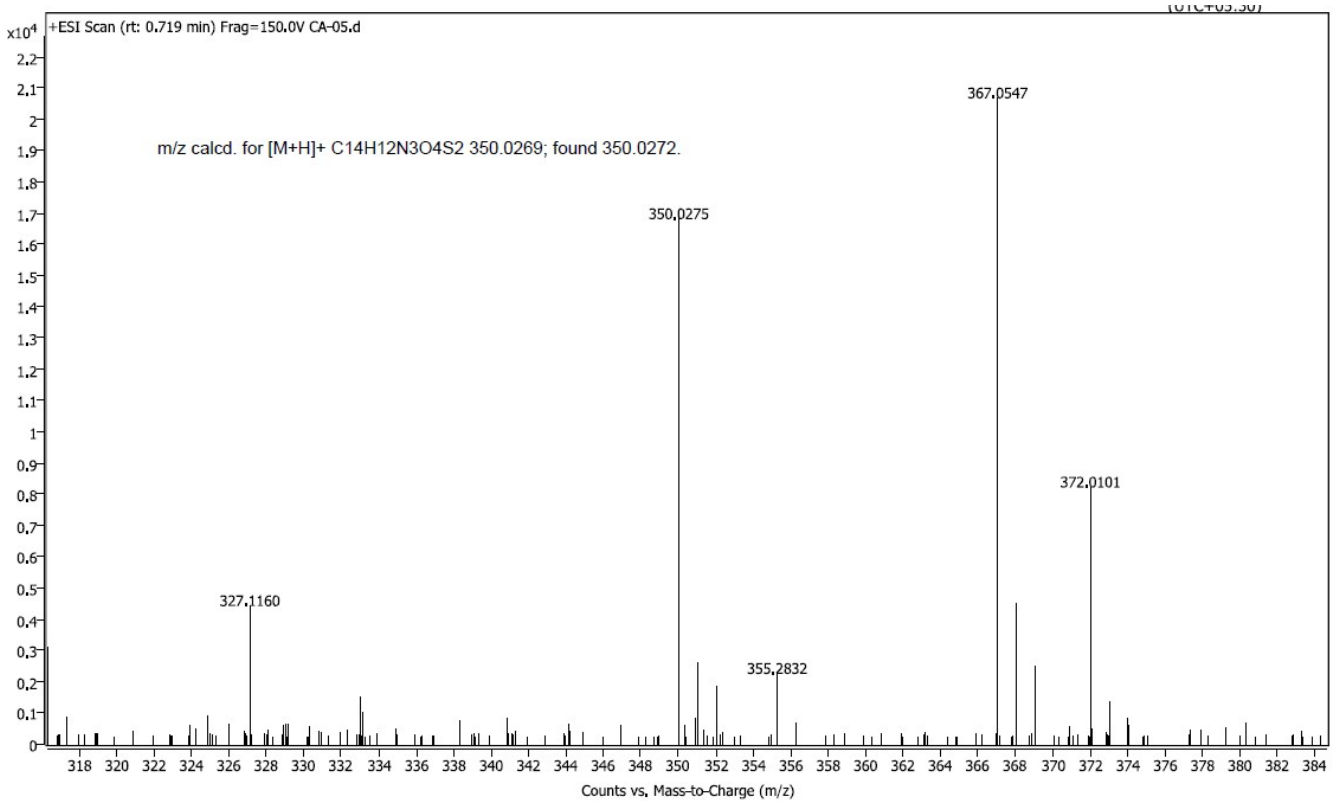
N-(3-sulfamoylphenyl)-5-(thiophen-2-yl)isoxazole-3-carboxamide (19b)



^{1} H NMR (500 MHz, DMSO-d_6) spectrum of compound **19b**



^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **19b**



Mass spectra of compound **19b**