Supporting Information for:

Development of small-molecule probes that selectively kill cells induced to express mutant *RAS*

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1. Materials and Reagents

All reagents and solvents were purchased from commercial vendors and used as received.

Phosphate-buffered saline (PBS; catalog no. 08J07A0022) was acquired from the Broad Institute Supply and Quality Management (SQM; Cambridge, MA). CellTiter Glo (catalog no. G7573 lot 268563) was purchased from Promega (Fitchburg, WI), and Alamar Blue (catalog no. DAL1025) was acquired from Biosource/Invitrogen (Grand Island, NY). White, sterile, TC-treated, 384-well plates (catalog no.3570) were acquired from Corning.

Growth Medium

Dulbecco's modified Eagle's medium (DMEM; catalog no. 11995, Lot no. 476124) with 4 mM L-glutamine and fetal bovine serum (FBS; catalog no. 26140-079, Lot no. 302496) were purchased from Gibco (Grand Island, NY). M199 (catalog no. M7528, Lot no. 028K2403) was acquired from Sigma (St. Louis, MO). Trypsin (catalog no. 25-053-Cl; Lot no. 25053204) was purchased from MediaTech (Manassas VA).

Cell Lines

Throughout the project, four different cell types derived from BJ human fibroblasts were used to determine the effect of compounds on either $HRAS^{V12}$-expressing or wild-type cell lines. The progenitor line was engineered into immortalized tumor lines by the method of Hahn (20,21,22,23,24), which uses the expression of human telomerase (hTERT) and the Simian Virus 40 (SV40) large T (LT) and small T (ST) oncoproteins.

Three versions of these cells were used for screening. The primary screen was performed in fully transformed cells also expressing an oncogenic RAS allele, $HRAS^{V12}$, a line referred to herein as BJeLR. For counterscreening, the isogenic cell line without $HRAS^{V12}$ was used, referred to as BJeH-LT. BJ fibroblasts with only hTERT expression were also used for non-$HRAS^{V12}$-expressing counterscreening

(BJeH). In addition, an alternative $HRAS^{V12}$-expressing line was generated with different immortalizing factors to eliminate the possibility of compounds acting in a synthetically lethal manner with one of these other factors. These cells (referred to as DRD) are BJ fibroblasts expressing hTERT, SV40 small T oncoprotein, dominant negative p53, cyclin D1, and a mutant form of CDK4, along with the gene of interest, $HRAS^{V12}$.

2. Assays

Primary Screen for BJeLR Cell Viability

All cell lines were generated by the Stockwell lab as described previously (17,18). Cells were maintained in 35 mL of growth medium (per 1 Liter: 730 ml DMEM with 4 mM L-glutamine, 210 ml M199, 150 ml heat-inactivated fetal bovine serum (FBS) in a T175 cell culture flask (Corning) and incubated in a TC incubator (Thermo-Fisher) at 95% humidity, 5% CO_2, 37ºC. To passage, cells were harvested by first aspirating the media and rinsing the flask with 10 ml sterile PBS. Next, PBS was aspirated and 5 mL trypsin was added to the flask and incubated for 5 minutes at 22ºC. Then 8 mL of growth medium was added to the trypsin to quench the reaction. The cells were resuspended, counted, and 4.5 million cells in approximately 2 mL were transferred to 33 mL fresh growth medium in a new T175 flask. The lines were carried for no more than twenty passages.

For screening, the cells were harvested, and the concentration was adjusted to 33,000/mL. While gently stirring, cells were dispensed with a Combi multidrop (Thermo-Fisher) by adding 30 µL of suspension per well to white, sterile, TC-treated, 384-well plates (Corning) for a total of 1000 cells per well. The plates were incubated overnight in an automated TC incubator (Liconic) at 95% humidity, 5% CO_2, 37ºC.

For compound screening, 50 nL or 100 nL of compound were added, depending on the desired final compound concentration, using slotted steel pins (V&P Scientific) on a pin tool (HiRes Biosolutions). The plates were returned to the incubator for 48 hours. To read viability, the cells were removed from the incubator and cooled to room temperature for 30 minutes. Lids were removed, and 30 µL of diluted CellTiter Glo (1:3 dilution with PBS) was added to each with a Combi Multidrop. The plates were incubated for 10 minutes, and luminescence was detected on an Envision (Perkin-Elmer) multimode reader (0.1 seconds per well).

Primary Retest for BJeLR Cell Viability

Repeat of primary screen at dose in BJeLR cells using Cell TiterGlo.

Secondary Counter screen for BJeH/LT/ST Cell Viability (Cell TiterGlo)

As described in the primary screen in BJeLR cells but using the BJeH/LT/ST cell line.

Secondary Screen for DRD Cell Viability (Cell TiterGlo)

As described in the primary screen in BJeLR cells but using the DRD cell line.

Secondary Counter screen for BJeH Cell Viability (Cell TiterGlo)

As described in the primary screen in BJeLR cells but using the BJeH cell line.

Secondary Screen for BJeLR Cell Viability (Alamar Blue)

The compounds were diluted into growth medium by adding 2 µL of DMSO compound solution to 148 µL of medium and mixing thoroughly. Dilutions were made in 384-well stock plates (Greiner, catalog no. 781270). Concentration-response curves were then made by further diluting this plate in series by adding 75 µL of solution to 75 µL of fresh growth medium, proceeding across the 384-well plate.

Next, 36 µL of cell suspension at 28,000 cells per well were added to the assay plates (1000 cells/well), and 4 µL of the medium containing the dilution series of compound were added to the cells. The cells were incubated for 48 hours in a TC incubator at 95% humidity, 5% CO_2, 37ºC. To measure viability, 10 µL Alamar Blue solution (50% in growth medium) was added to each well. The cells were incubated for 16 hours, and fluorescence intensity was read (544 nM excitation, 590 nM emission.)

Secondary Screen for BJeH-LT/ST Cell Viability (Alamar Blue)

As described in the secondary screen in BJeLR cells but using the BJeH-LT/ST cell line.

Secondary Screen for DRD Cell Viability (Alamar Blue)

As described in the secondary screen in BJeLR cells but using the DRD cell line.

Secondary Screen for BJeH Cell Viability (Alamar Blue)

As described in the primary screen in BJeLR cells but using the BJeH cell line.

3. Detailed Assay Protocols

Primary Screen for BJeLR Cell Viability

1) Maintain cells in 35 mL of growth medium (per 1 Liter: 730 ml DMEM with 4 mM L-glutamine, 210 ml M199, 150 ml heat-inactivated fetal bovine serum (FBS) in a T175 cell culture flask.
2) Incubate in a TC incubator at 95% humidity, 5% CO_2, 37ºC.
3) To passage, harvest cells by first aspirating the media and rinsing the flask with 10 ml sterile PBS.
4) Aspirate PBS and add 5 mL trypsin to the flask. Incubate for 5 minutes at 22ºC.
5) Add 8 mL of growth medium to the trypsin to quench the reaction.
6) Resuspend the cells, count, and transfer 4.5 million cells in approximately 2 mL to 33 mL fresh growth medium in a new T175 flask. Carry the lines for no more than 20 passages.
7) For screening, harvest the cells, and adjust the concentration to 33,000/mL. While gently stirring, disperse the cells with a Combi multidrop by adding 30 µL of suspension per well to white, sterile, TC-treated, 384-well plates for a total of 1000 cells per well. Incubate the plates overnight in an automated TC incubator at 95% humidity, 5% CO_2, 37ºC.
8) For compound screening, add 50 nL or 100 nL of compound, depending on the desired final compound concentration, using slotted steel pins on a pin tool.
9) Return the plates to the incubator for 48 hours. To read viability, remove the cells from the incubator and cool to room temperature for 30 minutes. Remove the lids, and add 30 µL of diluted CellTiter Glo (1:3 dilution with PBS) to each with a Combi Multidrop. Incubate the plates for 10 minutes, and detect luminescence on an Envision (Perkin-Elmer) multimode reader (0.1 seconds per well).

Secondary Screen for BJeLR Cell Viability (Alamar Blue)

1) Dilute the compounds into growth medium by adding 2 µL of DMSO compound solution to 148 µL of medium and mix thoroughly. Make dilutions in 384-well stock plates (Greiner, catalog no. 781270).
2) Further dilute these plates in series by adding 75 µL of solution to 75 µL of fresh growth medium, proceeding across the 384-well plate, and generate concentration-response curves.
3) Next, add 36 µL of cell suspension at 28,000 cells per well to the assay plates (1000 cells/well), and add 4 µL of the medium containing the dilution series of compound to the cells.
4) Incubate the cells for 48 hours in a TC incubator at 95% humidity, 5% CO_2, 37ºC.
5) To measure viability, add 10 µL Alamar Blue solution (50% in growth medium) to each well.
6) Incubate the cells for 16 hours, and read fluorescence intensity (544 nM excitation, 590 nM emission.)

4. Synthesis of analogs

General details. All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded on a Bruker 300 MHz or Varian UNITY INOVA 500 MHz spectrometer as indicated. Proton and carbon chemical shifts are reported in ppm (δ) relative to tetramethylsilane or $CDCl_3$ solvent (^1H δ 7.26, ^{13}C δ 77.0). NMR data are reported as follows: chemical shifts, multiplicity (obs. = obscured, br = broad, s = singlet, d = doublet, t = triplet, m = multiplet); coupling constant(s) in Hz; integration. Unless otherwise indicated NMR data were collected at 25°C. Flash chromatography was performed using 40-60 μm Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash R*f* system. Tandem Liquid Chromatography/Mass Spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with ultraviolet (UV) light and aqueous potassium permanganate ($KMnO_4$) stain followed by heating.

4.1 Representative synthesis of 1a and analogs

2-chloro-N-(3-chloro-4-methoxyphenyl)-N-(2-oxo-2-(phenethylamino)-1-(thiophen-2-yl)ethyl) acetamide (1a): 2-Thiophene carboxaldehyde (51 mg, 0.45 mmol) was dissolved in MeOH (189 μl) and 3-chloro-4-methoxyaniline (71.5 mg, 0.45 mmol) was added. The mixture was stirred for 15 minutes and 2-chloroacetic acid (35.7 mg, 0.38 mmol) and 2-phenethyl isocyanide (50 mg, 0.38 mmol) were added. After stirring at room temperature for 48 hours, the solvents were evaporated. The crude material was purified by column chromatography over silica gel (hexanes/ethyl acetate: 100/0 to 0/100) to afford 106 mg (0.22 mmol, 59% yield) of **1a** as a white solid. **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.29-7.13 (m, 7H), 6.90-6.70 (m, 4H), 6.09 (s, 1H), 6.03 (br. s, 1H), 3.89 (s, 3H), 3.82 (s, 2H), 3.60-3.50 (m, 2H), 2.90-2.75 (m, 2H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 167.92, 166.71, 155.54, 138.60, 134.80, 130.07, 128.81, 128.59, 128.23, 126.50, 111.73, 60.71, 56.26, 42.25, 41.04, 35.49. **HRMS (ESI):** calculated mass for $C_{23}H_{21}Cl_2N_2O_3S$ $[M-H]^-$ 475.0655, found 475.0669.

Preparative HPLC conditions used to separate the two enantiomers of 1a

Compound **1a** resolved using a Chiralcel OD-H 10*250 mm, 5-μm column (Chiral Technologies, West Chester, PA; catalog no. 14335) with a gradient of methanol in hexane. Both enantiomers were obtained with >99% ee (Figure 1b and 1c). To establish whether a pure enantiomer can racemize in the assay conditions, one enantiomer was subjected to a PBS stability assay. After, 48 hours of incubation in PBS (0.1% DMSO), the compound was extracted, concentrated, and injected on a chiral HPLC/MS. No detectable racemization could be observed (Figure 1d).

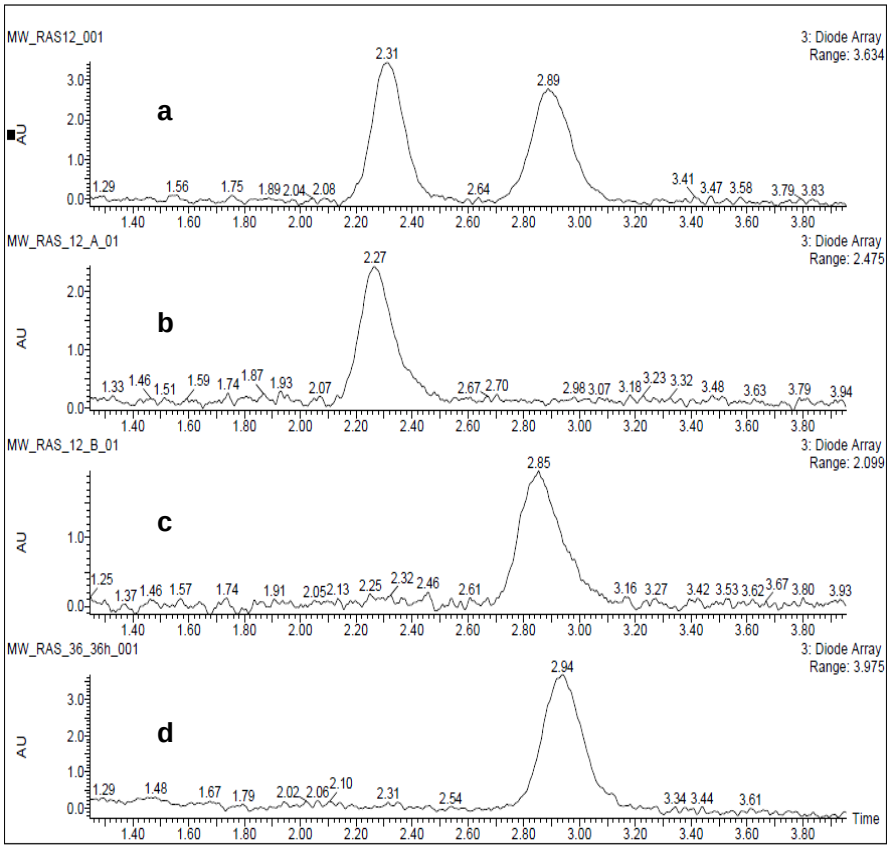


Figure 1. Racemic mixture of **1a** (***a***); single enantiomer A after purification on chiral HPLC (***b***); single enantiomer B after purification on chiral HPLC (***c***); and single enantiomer B after 48 hours incubation in PBS (***d***)

4.2 Representative Synthesis of 2y and analogs

5-methyl-4-nitroisoxazole-3-carboxylic acid (4): 5-methylisoxazole-3-carboxylic acid (1.5 g, 12.04 mmol) was added to a mixture of potassium nitrate (1.83 g, 18.06 mmol) and sulfuric acid (5 ml) at room temperature. After complete dissolution, the mixture was warmed to 50^{o}C and stirred for 4 hours. The mixture was then cooled to 0^{o}C, ice was added, and the solution was neutralized with sodium bicarbonate. The mixture was extracted with ethyl acetate (3 × 30 ml), dried over sodium sulfate, filtered and concentrated to give 1.45 g of **4** as a white solid (8.43 mmol, 70%). The product could be further recrystallized from dichloromethane. **^{1}H NMR (300 MHz, DMSO-d6):** δ 6.72 (s, br, 1H), 2.28 (s, 3H); **^{13}C NMR (75 MHz, DMSO-d6):** δ 187.4, 124.2, 117.7, 107.3, 29.5.

5-methyl-4-nitroisoxazole-3-carbonyl chloride (5): 5-methyl-4-nitroisoxazole-3-carboxylic acid (200 mg, 1.16 mmol) was dissolved in dichloromethane (4 ml) under argon atmosphere. Oxalyl chloride (202

uL, 2.32 mmol) was added dropwise at room temperature, followed by addition of one drop of DMF. After stirring overnight, the mixture was concentrated and co-evaporated with chloroform to afford 220 mg of **5** as a slightly yellow oil (1.15 mmol, 99%), which was carried on to the next step without further purification.

Bis(4-chlorophenyl)methanol (7y): Bis(4-chlorophenyl)methanone (3.0 g, 11.95 mmol) was dissolved in methanol/tetrahydrofuran 1:1 (100 ml) and cooled to 0°C. Sodium borohydride (452 mg, 11.95 mmol) was added in one portion, and the mixture was stirred for 30 minutes. After TLC showed complete conversion, the mixture was neutralized with acetic acid and concentrated. Dichloromethane was added, and the solution was washed with water (2 × 30 ml), dried on sodium sulfate and concentrated to afford 2.83 g of **7** as an off-white solid (11.18 mmol, 94%), which was carried to the next step without further purification. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.32 (d, *J* = 8.47 Hz, 4H), 7.28 (d, *J* = 7.28 Hz, 4H), 5.78 (s, 1H), 2.27 (s, 1H); **LRMS** $(M + HCO_2)^-$: 298.95.

4,4'-(Chloromethylene)bis(chlorobenzene) (8y): Bis(4-chlorophenyl)methanol (2.83 g, 11.18 mmol) was dissolved in dichloromethane (30 ml) at room temperature. Oxalyl chloride (975 uL, 11.18 mmol) was added followed by addition of a drop of dimethylformamide. After stirring overnight, the mixture was concentrated and co-evaporated with chloroform to afford 3.0 g of **8** as an off-white solid (11.05 mmol, 99%), which was carried to the next step without further purification. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.32 (m, 8H), 6.06 (s, 1H).

1-(bis(4-chlorophenyl)methyl)piperazine (9y): 4,4'-(Chloromethylene)bis(chlorobenzene) (3.0 g, 11.05 mmol) was dissolved in acetonitrile (100 ml) at room temperature. Piperazine (8.6 g, 100 mmol) was added, and the mixture was refluxed overnight. Acetonitrile was evaporated. The mixture was dissolved in ethyl acetate and washed three times with water to remove the excess piperazine. The organic phase was dried over sodium sulfate, filtered, and concentrated. The crude mixture was purified on silica gel using a gradient of (7N NH_3 in methanol) in dichloromethane to afford 2.4 g of **9** as an off-white solid (7.51 mmol, 68%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.32 (d, *J* = 8.50 Hz, 4H), 7.25 (d, *J* = 8.56 Hz, 4H), 4.18 (s, 1H), 2.87 (t, *J* = 4.79 Hz, 4H), 2.32 (br s, 4H), 1.55 (br s, 1H); **LRMS** $(M + H)^+$: 322.04.

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-methyl-4-nitroisoxazol-3-yl)methanone (2y): 5-methyl-4-nitroisoxazole-3-carbonyl chloride **5** (33 mg, 0.17 mmol) was dissolved in dichloromethane (1 ml) at room temperature and 1-(bis(4-chlorophenyl)methyl)piperazine **9** (55 mg, 0.17 mmol) was added, along with one drop of triethylamine. After stirring for 2 hours, TLC shows complete conversion. The mixture was dissolved in dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate (2 × 10 ml). The organic phase was dried over sodium sulfate, filtered, and concentrated. The crude mixture was purified on silica gel using a gradient of ethyl acetate in hexanes to afford the final product **2y** (61 mg) as a white solid (0.128 mmol, 74%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.32 (d, *J* = 8.58 Hz, 4H), 7.26 (d, *J* = 8.56 Hz, 4H), 4.25 (s, 1H), 3.84 (t, *J* = 4.96 Hz, 2H), 3.36 (t, *J* = 4.99 Hz, 2H), 2.85 (s, 3H), 2.52 (t, *J* = 5.08 Hz, 2H), 2.37 (t, *J* = 4.97 Hz, 2H); **^{13}C NMR (125 MHz, $CDCl_3$):** δ 171.7, 156.4, 153.0, 139.9, 133.2, 129.0, 128.9, 94.7, 74.2, 51.5, 50.9, 46.9, 42.2, 13.4; **HRMS (ESI):** calculated mass for $C_{22}H_{20}Cl_2N_4O_4$ [M+H] 475.0934, found 475.0953.

Analogs **2a**,**e**,**f**,**g**,**h**,**i**,**j**,**k**,**l**,**m**,**s**,**t**,**w**,**x**,**y**,**z** were synthesized using the same strategy.

Analogs **2n,o,p,q,r** were commercially available.

Reduction of the nitro group:

(4-amino-5-methylisoxazol-3-yl)(4-benzhydrylpiperazin-1-yl)methanone (2b):

A mixture of **2a** (22 mg, 0.054 mmol) and sodium dithionite (28 mg, 0.162 mmol) in water/THF 1/1 (1 ml) was heated at 90 ^{o}C for 1h. The mixture was cool to room temperature and extracted with ethyl acetate (ml). The organic phase was dried, concentrated and purified on silica gel using a gradient of methanol in DCM to afford **2b** as a white solid (18 mg, 88%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.42 (d, *J* = 8.6 Hz, 4H), 7.30-7.10 (m, 6H), 4.26 (s, 1H), 4.01 (m, 2H), 3.76 (m, 2H), 3.51 (bs, 2H), 2.50-2.40 (m, 4H), 2.31 (s, 3H). **LRMS** $(M + H)^+$: 377.31.

N-Acetylation of aminoisoxazole:

N-(3-(4-benzhydrylpiperazine-1-carbonyl)-5-methylisoxazol-4-yl)acetamide (2c):

Compound **2b** (18 mg, 0.048 mmol) was dissolved in dry DCM (2.3 ml) under nitrogen atmosphere. Triethylamine (33 μl, 0.239 mmol) was added followed by acetic anhydride (23 μl, 0.239 mmol), and the reaction mixture was stirred at room temperature for 2h. DCM (10 ml) was added and the mixture was washed with water (10 ml). The organic phase was dried, concentrated and purified on silica gel using a gradient of methanol in DCM to afford **2c** as a white solid (16 mg, 80%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 8.13 (s, 1H), 7.42 (d, *J* = 8.5 Hz, 4H), 7.30-7.10 (m, 6H), 4.28 (s, 1H), 3.94 (m, 2H), 3.76 (m, 2H), 2.55-2.30 (m, 7H), 2.11 (s, 3H). **LRMS** $(M + H)^+$: 419.31.

N-Mesylation of aminoisoxazole:

N-(3-(4-benzhydrylpiperazine-1-carbonyl)-5-methylisoxazol-4-yl)methanesulfonamide (2d):

Compound **2b** (10 mg, 0.027 mmol) was dissolved in dry DCM (1.3 ml) under nitrogen atmosphere. Triethylamine (7.4 μl, 0.053 mmol) was added followed by methanesulfonyl chloride (3 μl, 0.038 mmol), and the reaction mixture was stirred at room temperature for 2h. DCM (10 ml) was added and the mixture was washed with water (10 ml). The organic phase was dried, concentrated and purified on silica gel using a gradient of methanol in DCM to afford **2d** as a white solid (8 mg, 67%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.41 (d, *J* = 8.4 Hz, 4H), 7.35-7.15 (m, 6H), 4.27 (s, 1H), 4.01 (s, 2H), 3.78 (s, 2H), 2.89 (s, 3H), 2.55-2.35 (m, 7H). **LRMS** $(M + H)^+$: 455.26.

Homologation of 5-methyl-4-nitroisoxazole-3-carboxylic acid (4): To a solution of 291 mg (1.53 mmol) of 5-methyl-4-nitroisoxazole-3-carbonyl chloride in 2 mL of THF cooled on an ice bath was added 1.5 mL of a 2.0 M trimethylsilyldiazomethane in hexane solution and the reaction was stirred overnight warming to room temperature. Ether was added and the solution was rinsed with a solution of citric acid, the ether layer was dried, filtered and concentrated to a red oil which was dissolved in 20 mL of THF and cooled on an ice bath. A slurry of 60 mg of silver acetate in 5 mL Et3N was added and the dark mixture was stirred overnight. The mixture was decanted, concentrated, and partitioned between EtOAc and acidic water. The EtOAc was dried, filtered, and concentrated, and chromatographed with 2/8/90 HOAc/MeOH/CH_2Cl_2 to isolate the corresponding homologated carboxylic acid intermediate (114 mg, 40%). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 10.92 (s br, 1H), 4.06 (s, 2H), 2.85 (s, 3H).

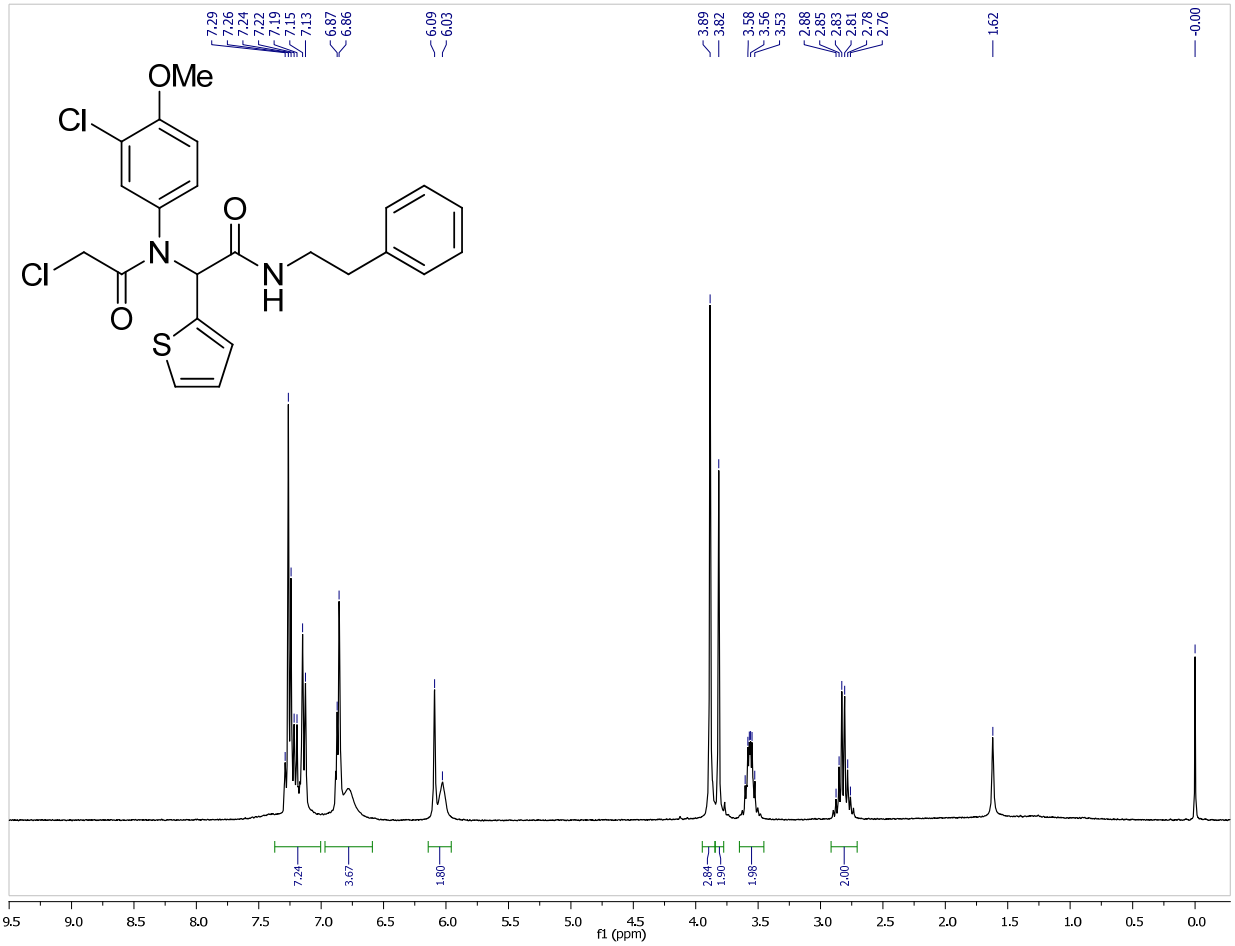
1-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)-2-(5-methyl-4-nitroisoxazol-3-yl)ethanone (2w'):

The obtained homologated carboxylic acid (72 mg, 0.39 mmol) was converted to the acid chloride as above, the acid chloride was dissolved in 2 mL of CH_2Cl_2 with 100 mL of Et_3N before addition of 135 mg (0.47 mmol) of 1-(bis(4-fluorophenyl)methyl)piperazine and stirring overnight. The next day more CH_2Cl_2 was added and the mixture was rinsed with water, the combined CH_2Cl_2 was dried, filtered, and concentrated before chromatography with 33% EtOAc in hexane. Further purification by loading onto an SCX (acidic) resin, rinsing with MeOH and then rinsing with 1 M NH_3/MeOH afforded **2w'** (27 mg, 13%). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.36 (dd, *J* = 5.5, 8.5, 4H), 6.99 (t, *J* = 8.6, 4H), 4.27 (s, 1H), 3.98 (s, 2H), 3.68 – 3.61 (m, 2H), 3.61 – 3.52 (m, 2H), 2.81 (s, 3H), 2.51 – 2.42 (m, 2H), 2.42 – 2.32 (m, 2H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 175.21, 171.85, 165.01, 163.58, 160.31, 154.35, 137.67, 129.20 (d, *J* = 7.92), 115.78, 115.64 (d, *J* = 21.33), 74.24, 51.73, 51.36, 46.06, 42.23, 30.75, 13.79.

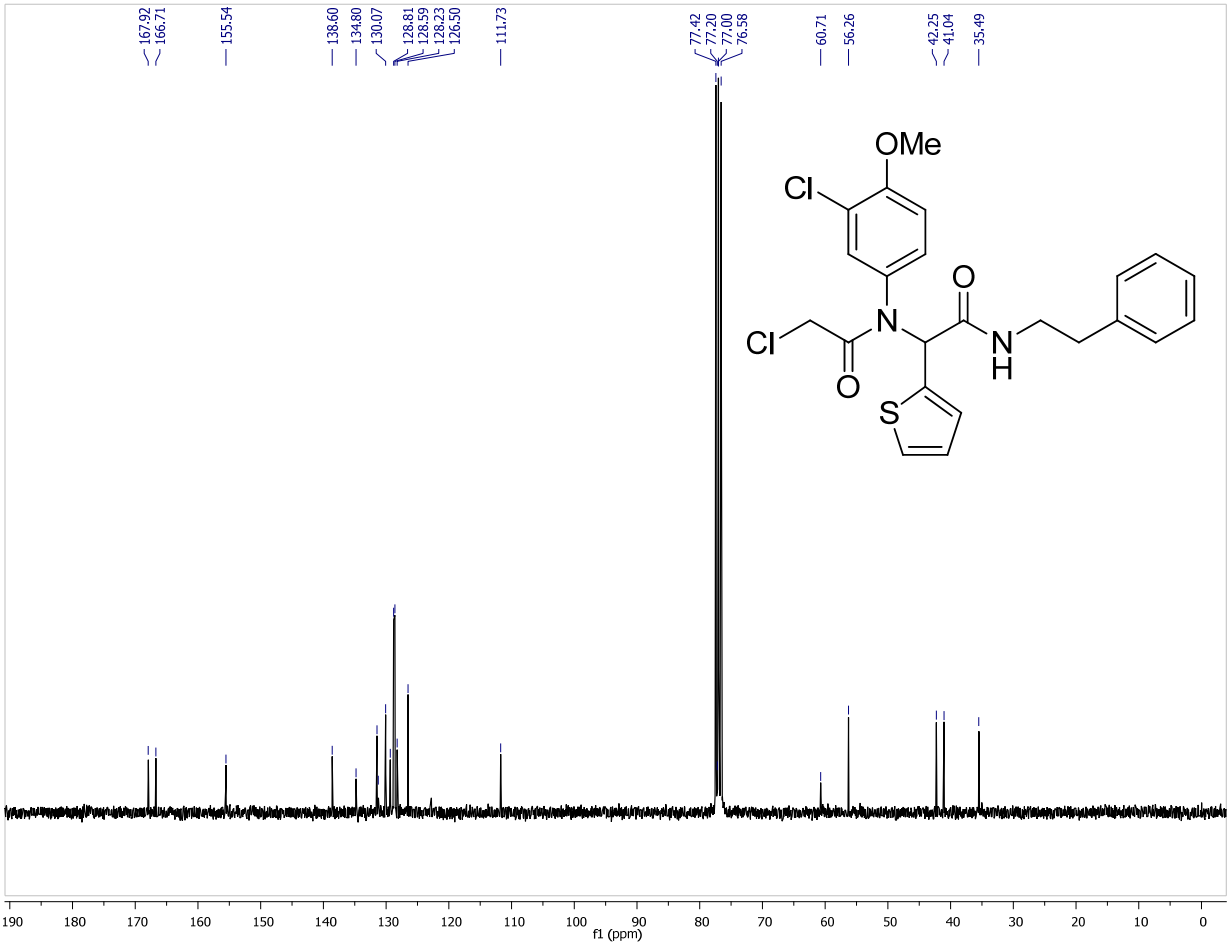
5. NMR and LCMS of all analogs

5.1 NMR and LCMS of 1a and analogs

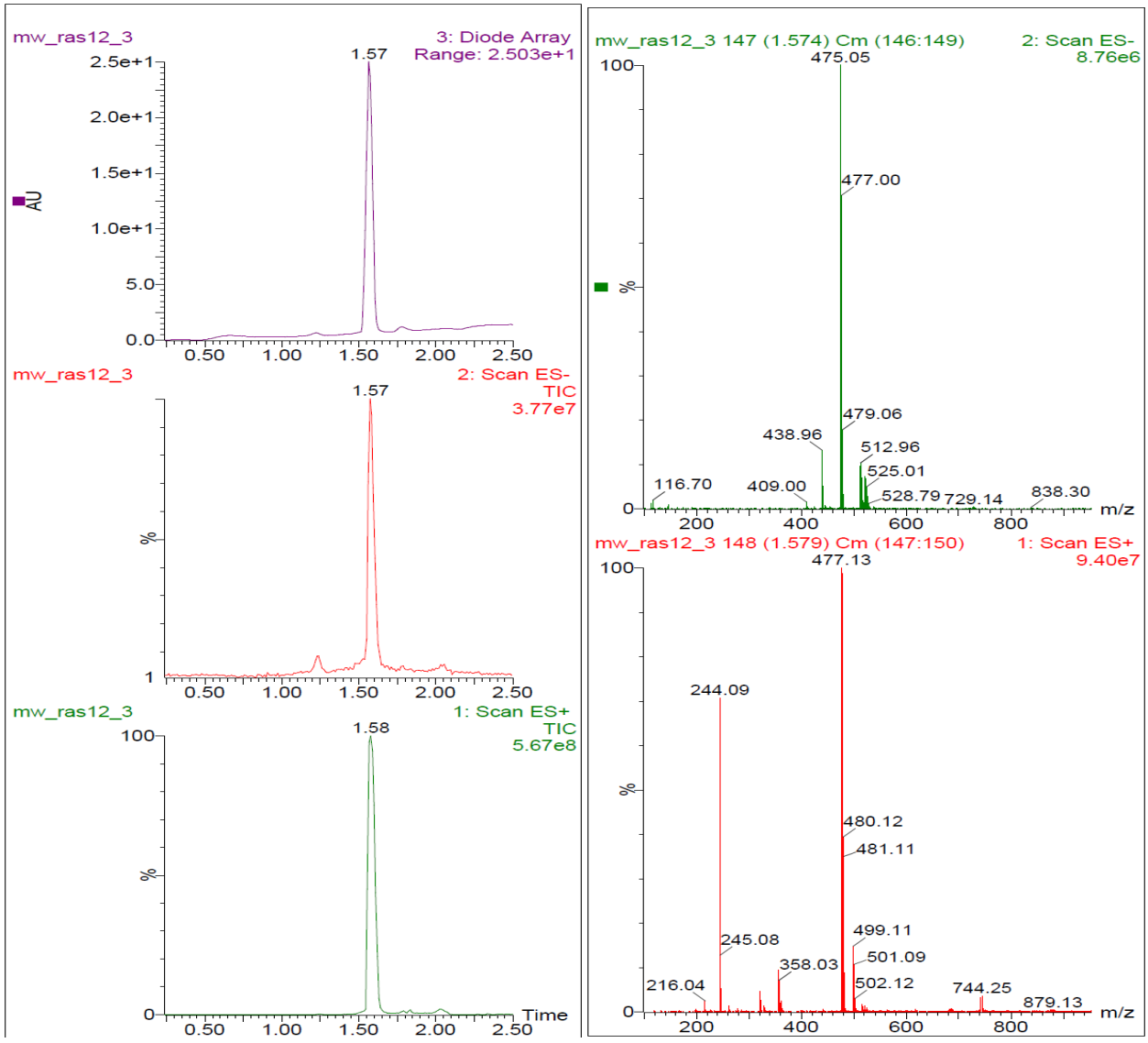
^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **1a**



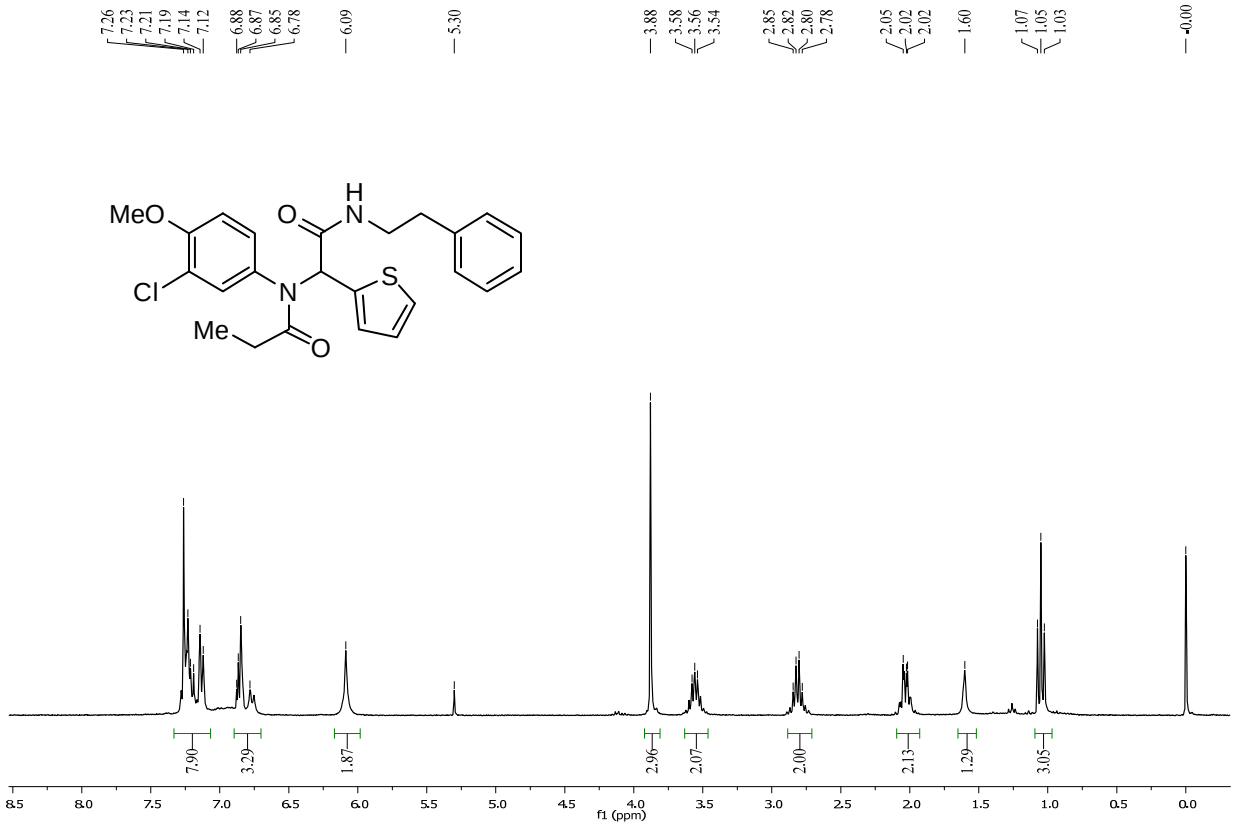
^{13}C NMR (125 MHz, $CDCl_3$) Spectrum of **1a**



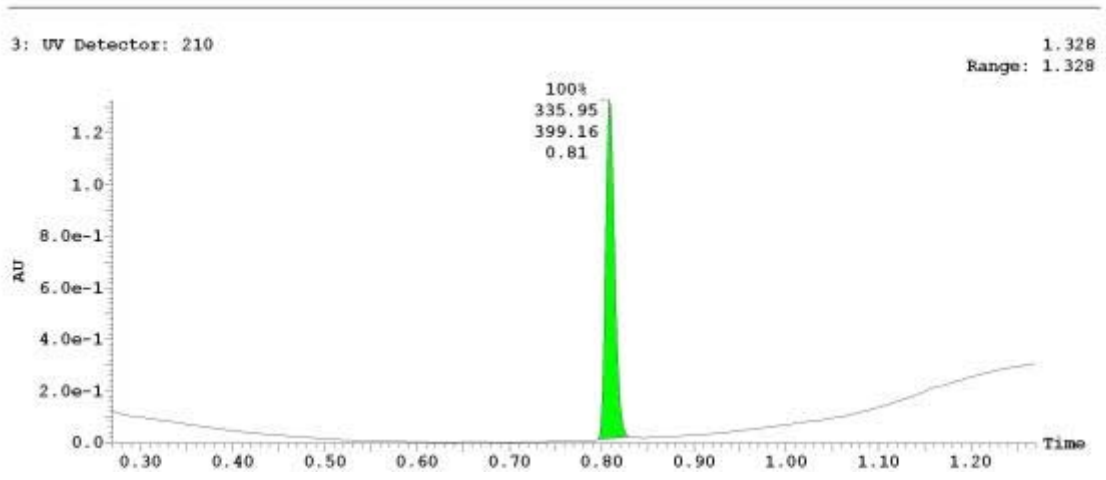
HPLC/MS Chromatogram of **1a**



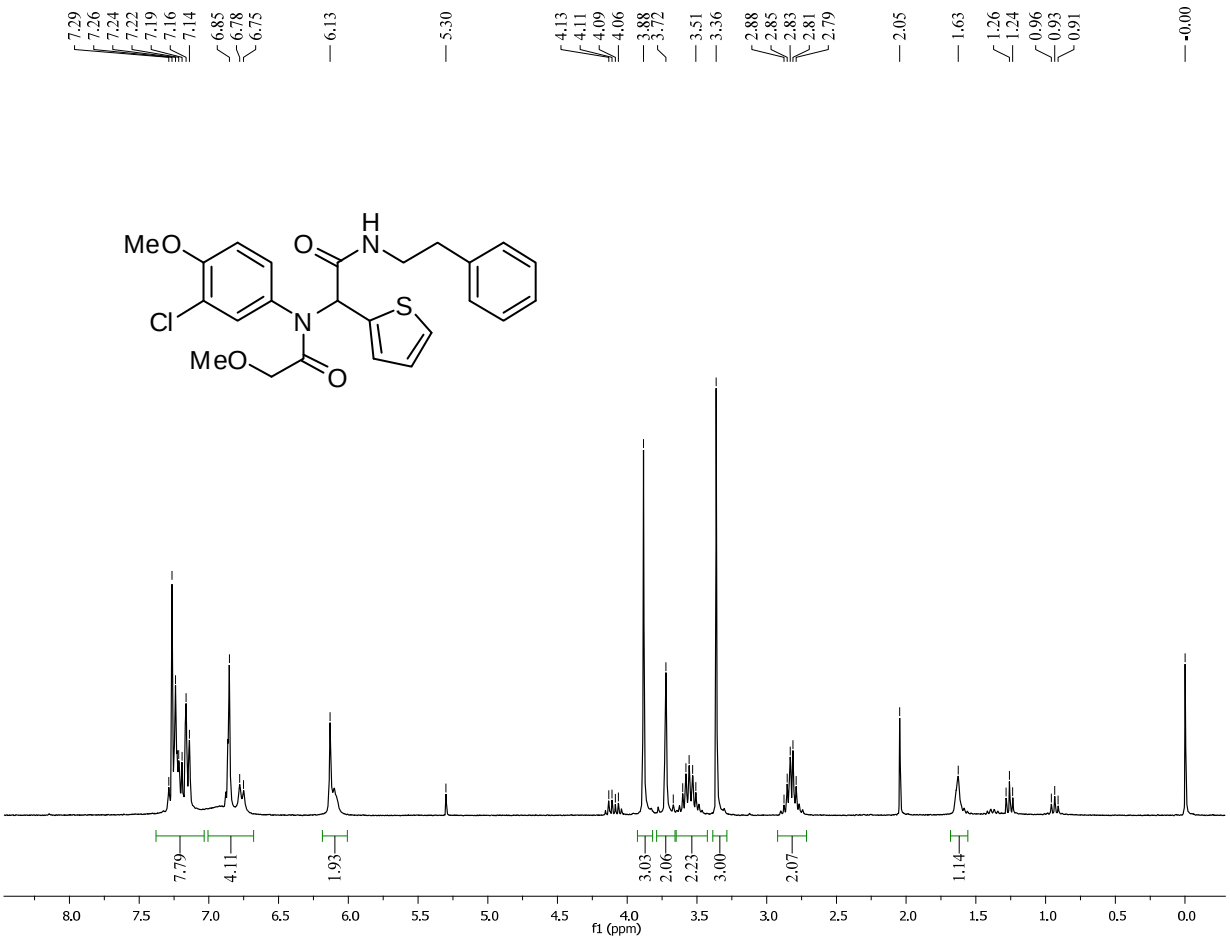
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1b**



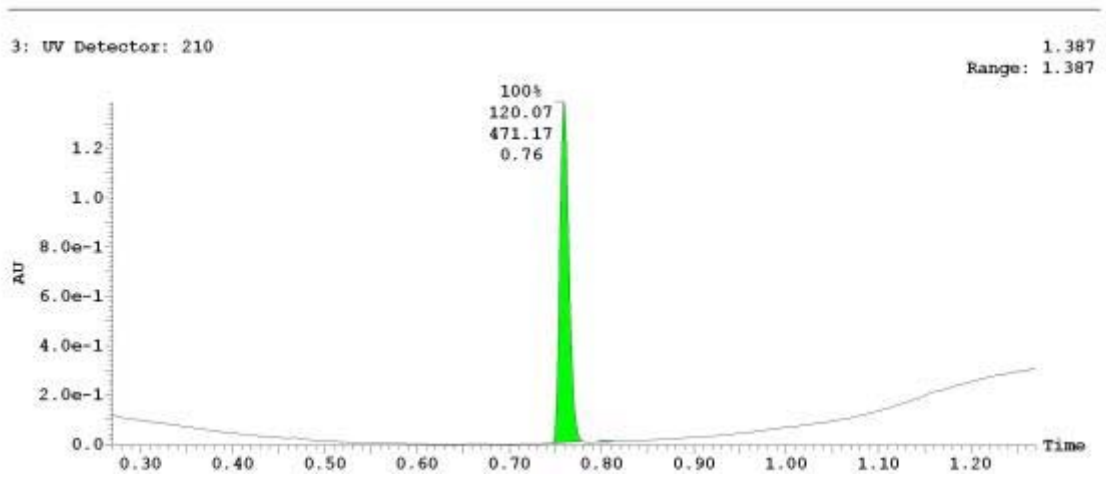
UPLC Chromatogram of **1b**



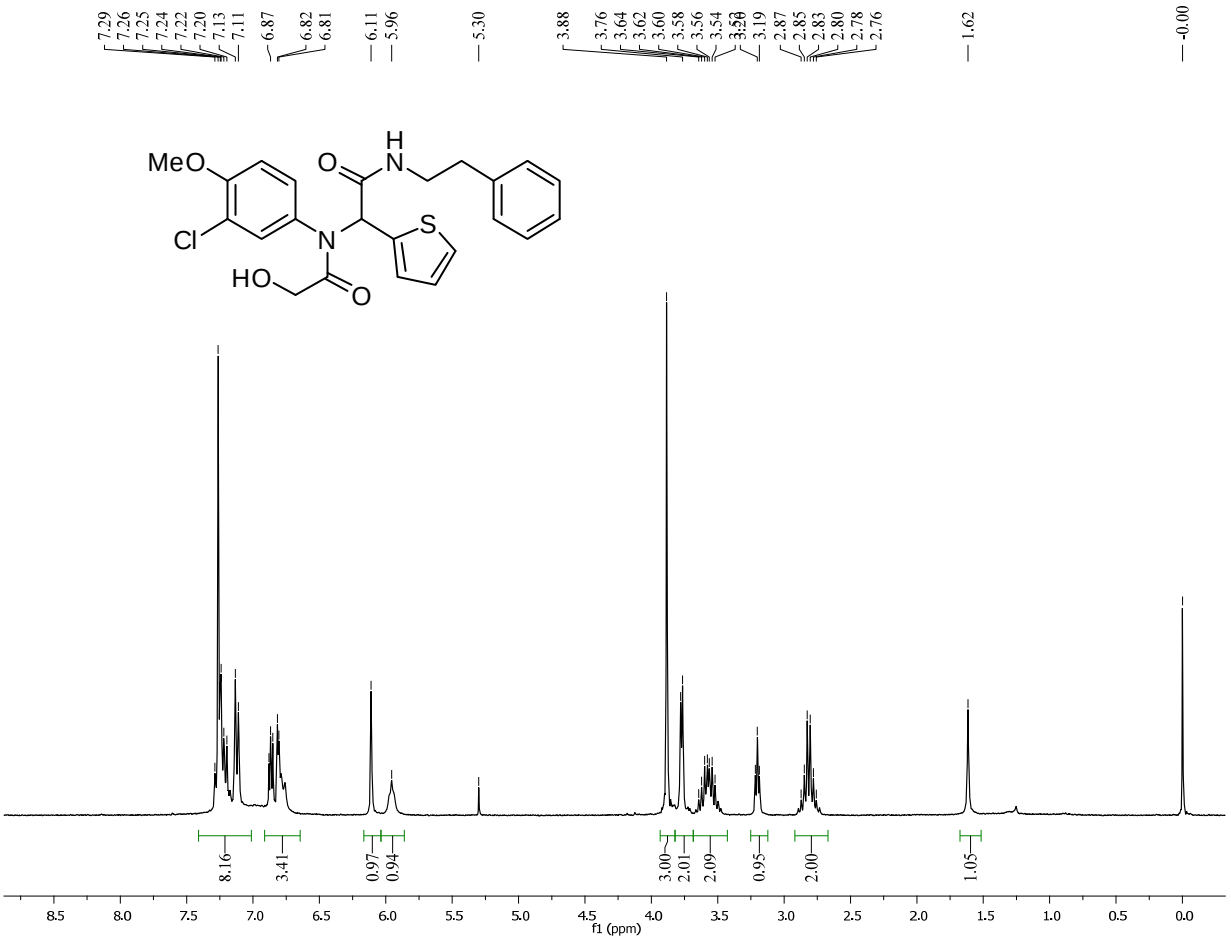
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1c**



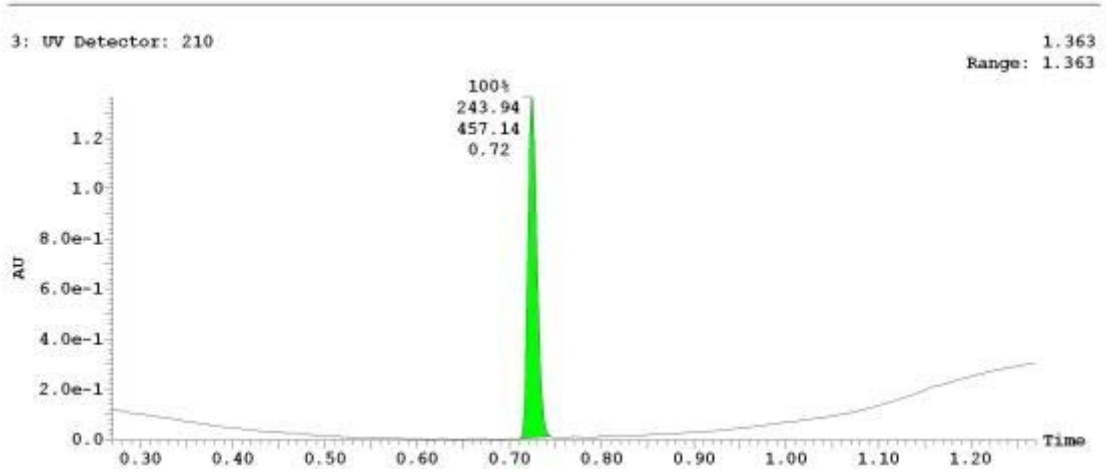
UPLC Chromatogram of **1c**



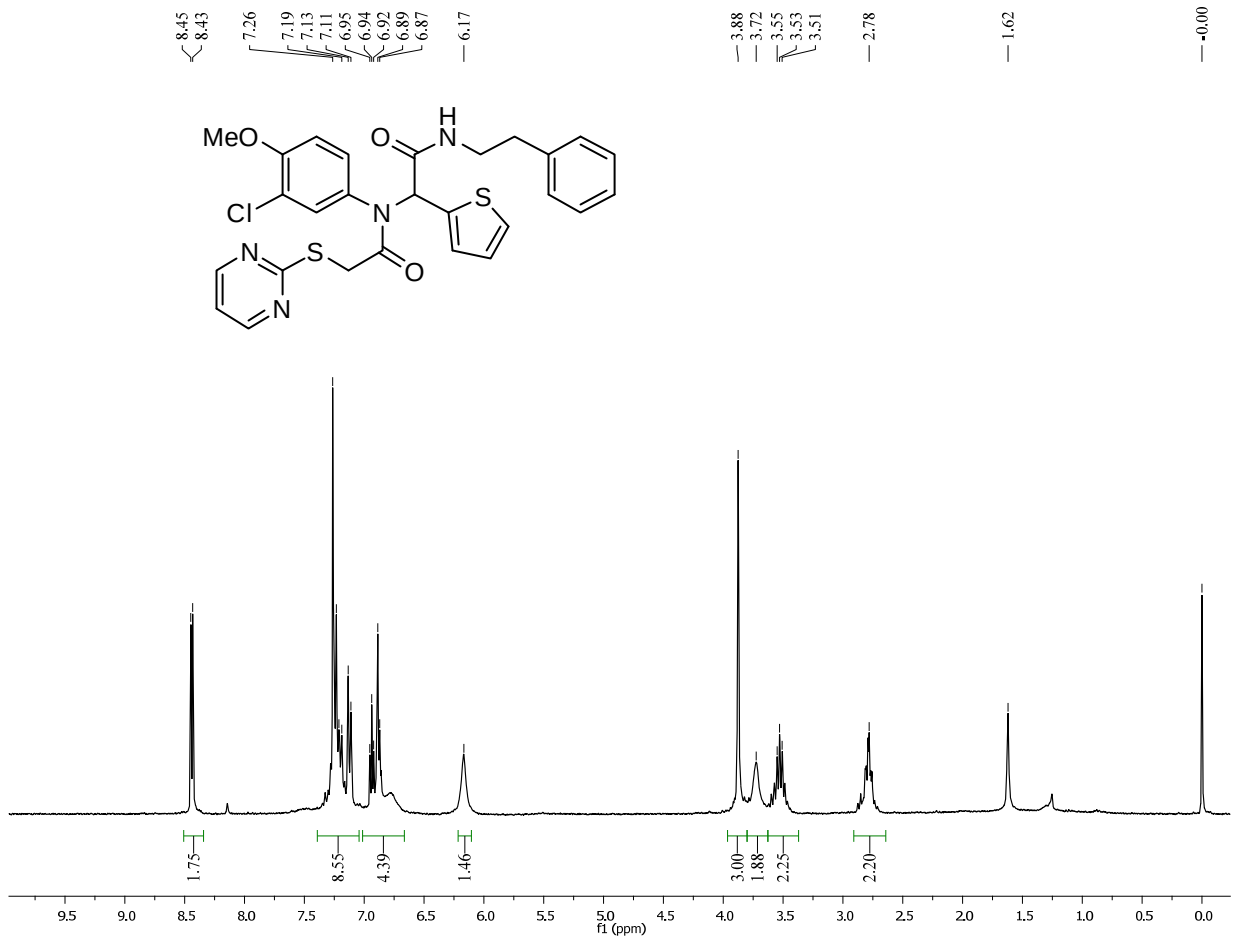
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1d**



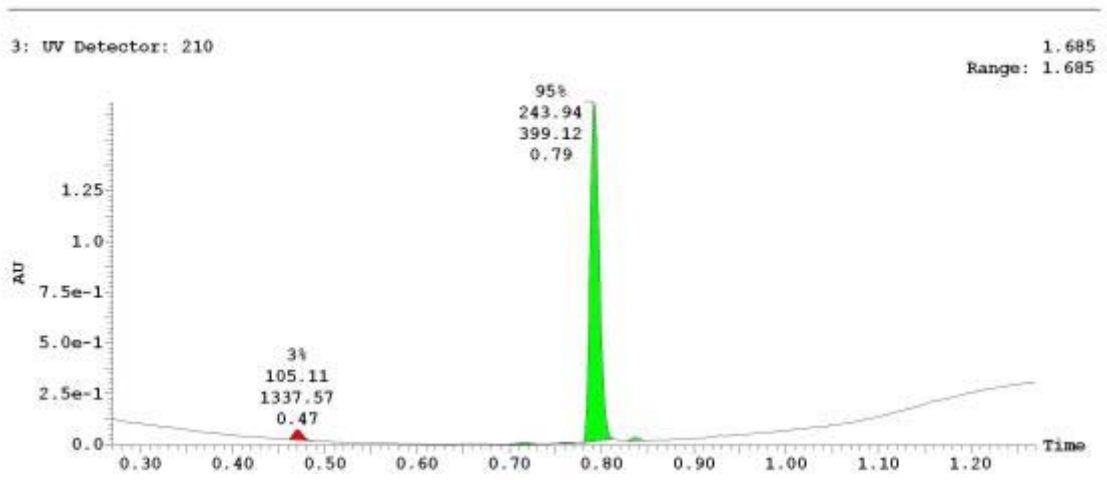
UPLC Chromatogram of **1d**



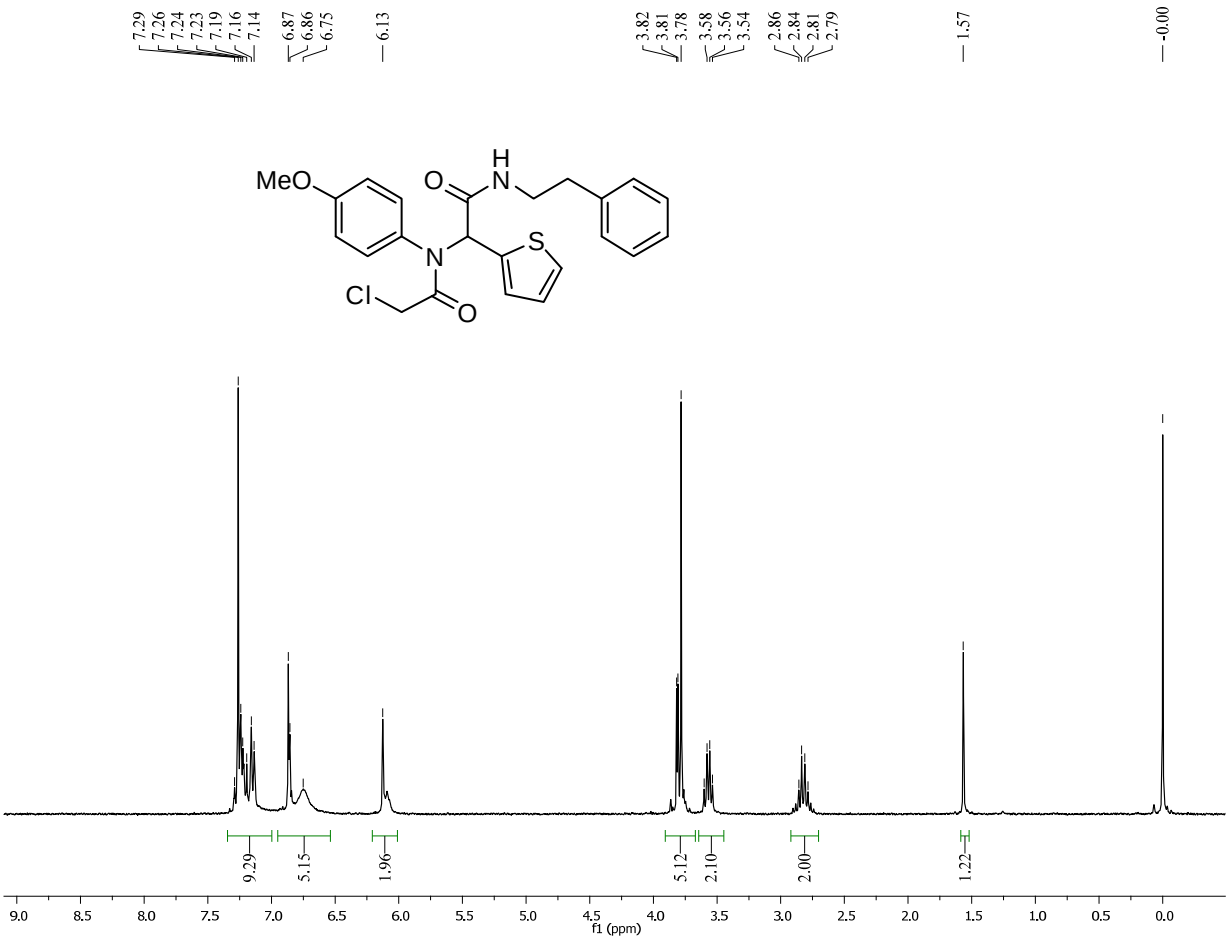
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1e**



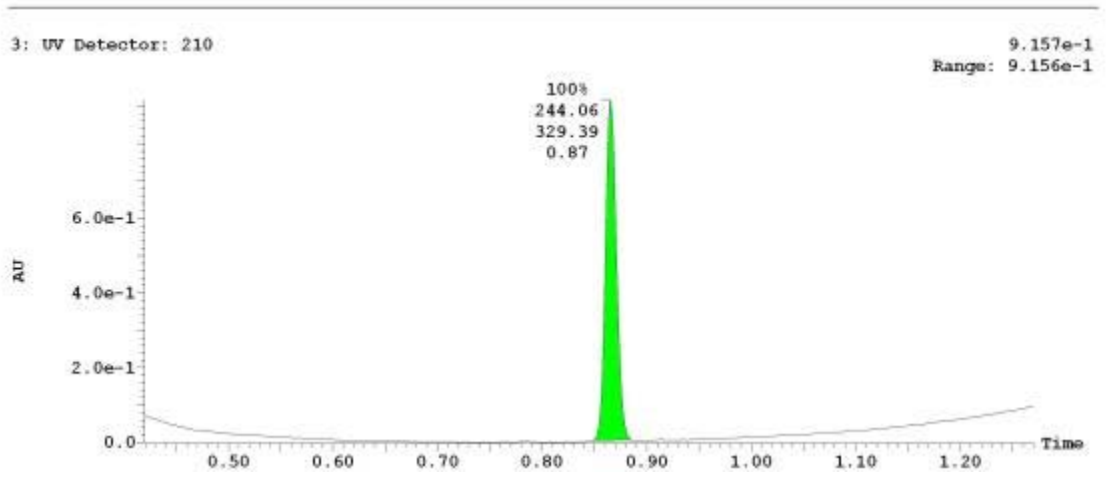
UPLC Chromatogram of **1e**



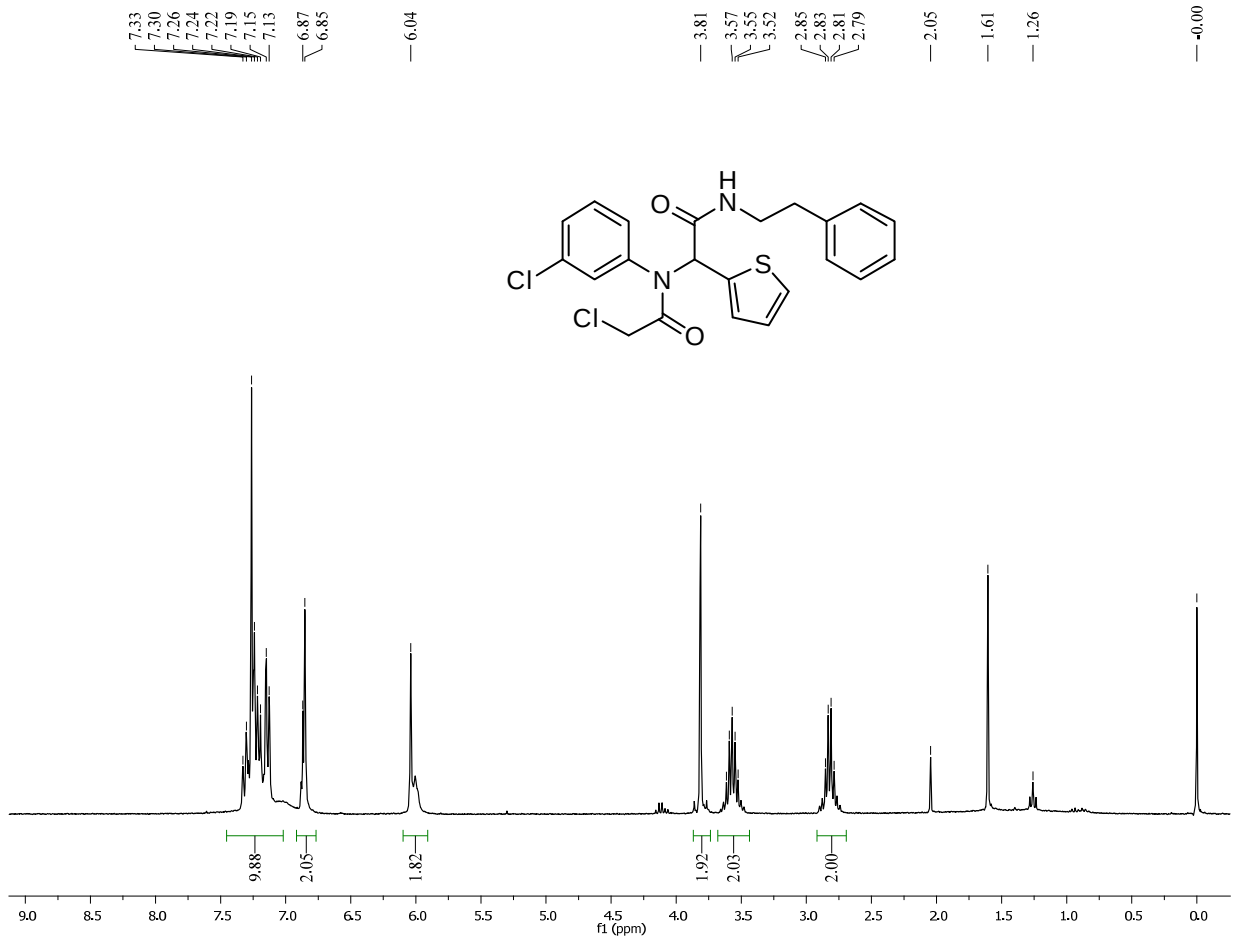
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1f**



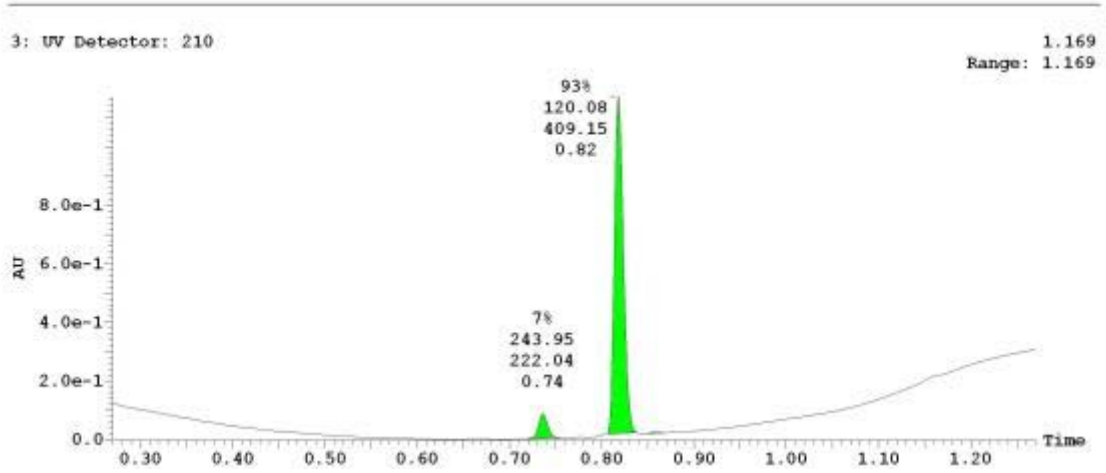
UPLC Chromatogram of **1f**



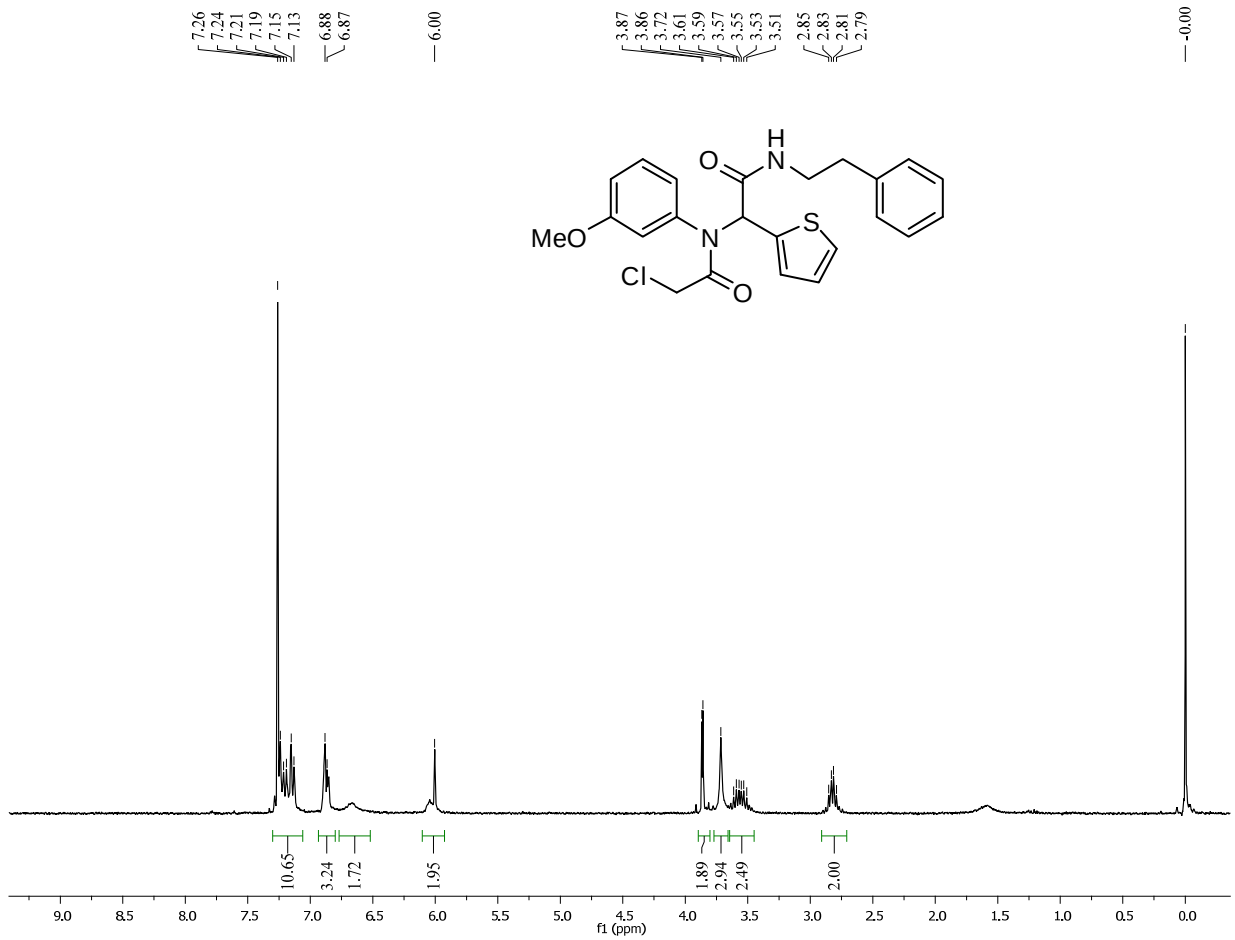
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1g**



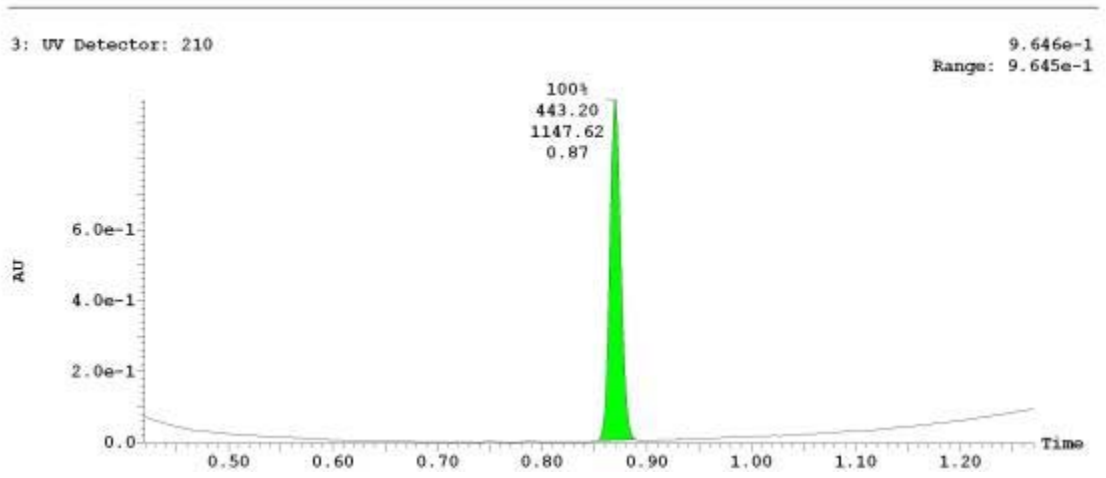
UPLC Chromatogram of **1g**



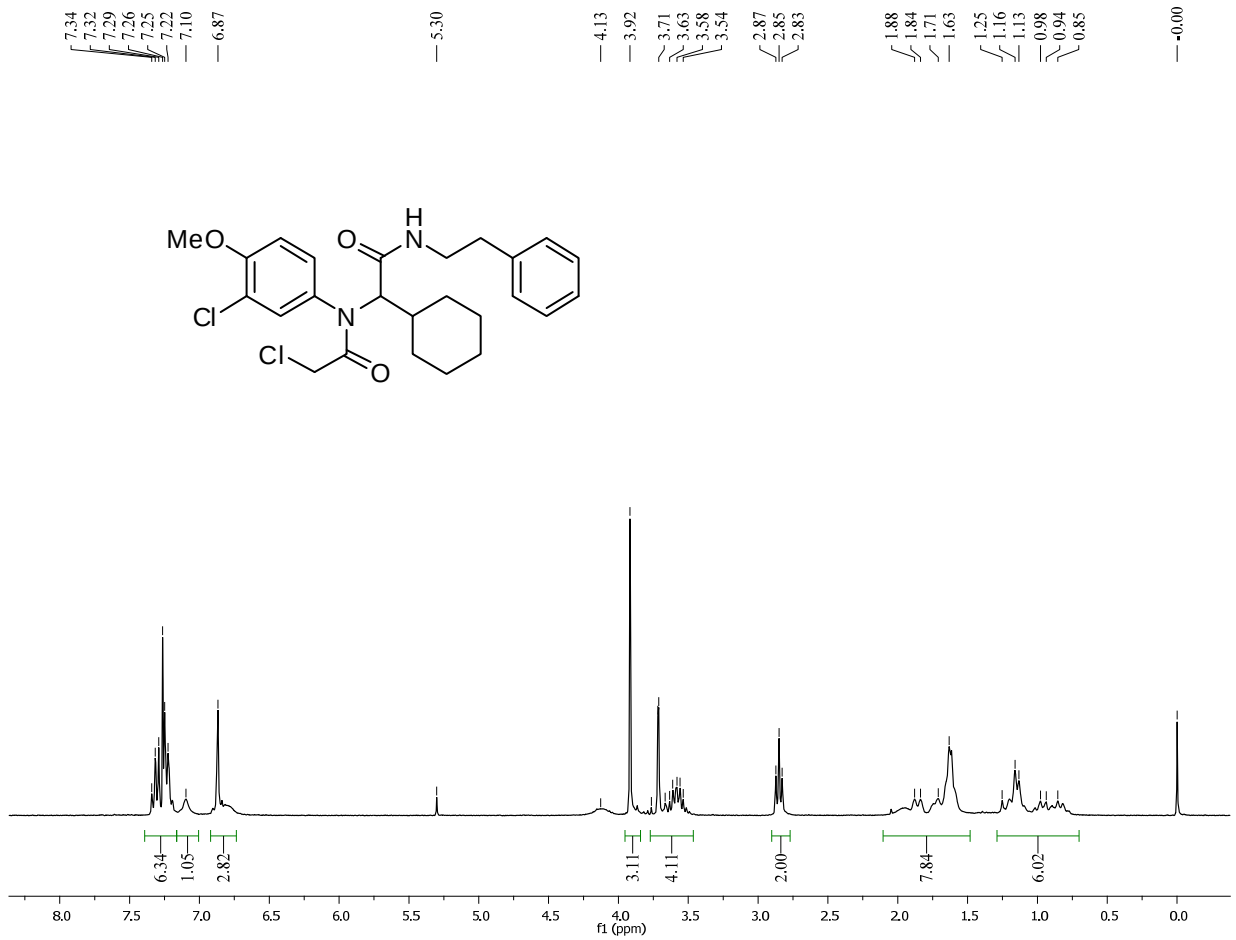
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1h**



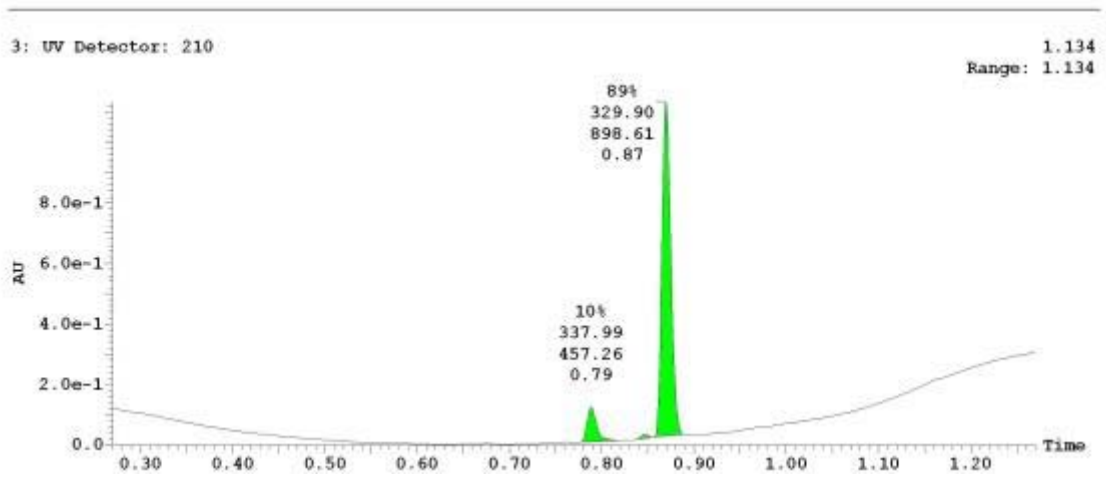
UPLC Chromatogram of **1h**



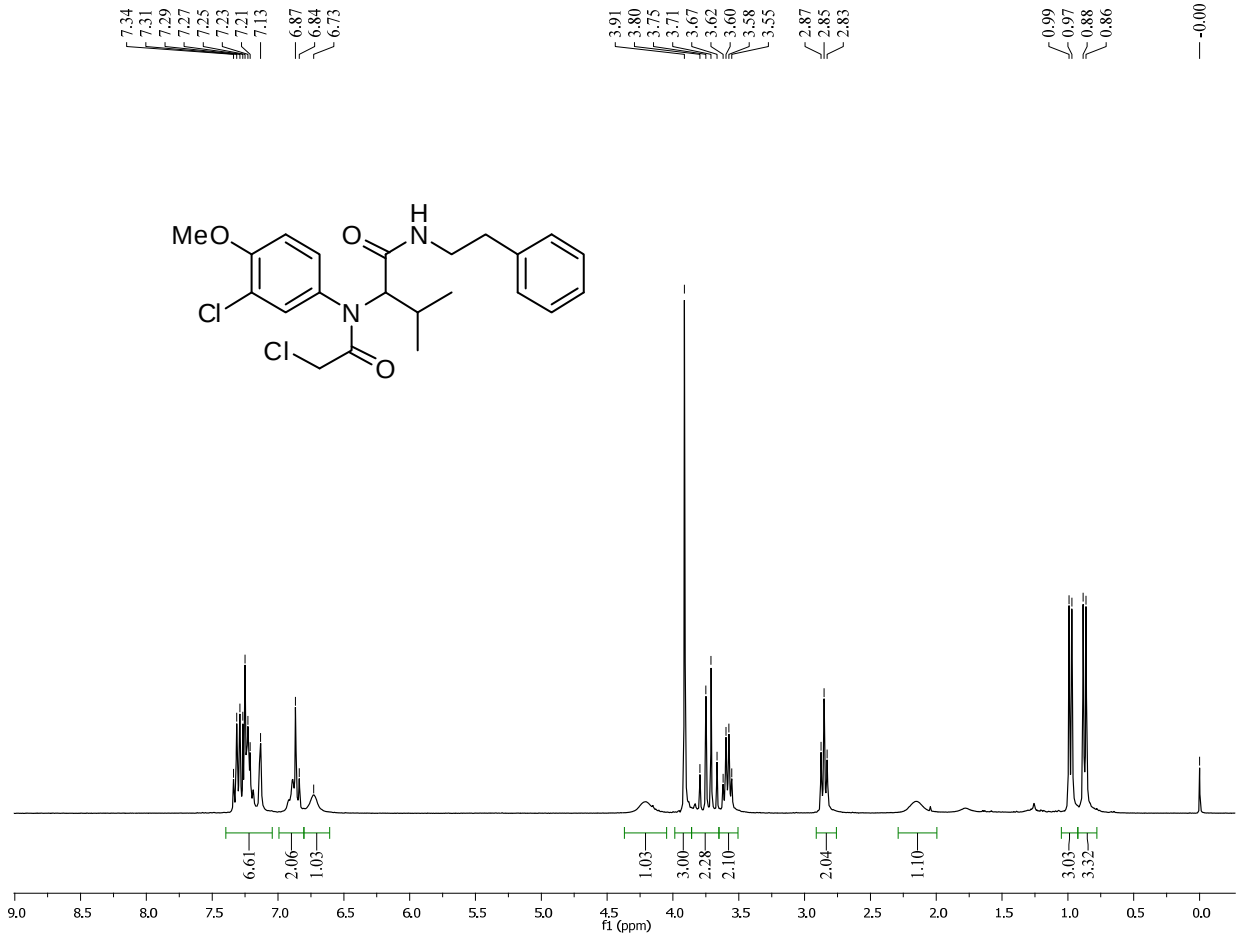
^{1}HNMR (300 MHz, $CDCl_3$) of **1i**



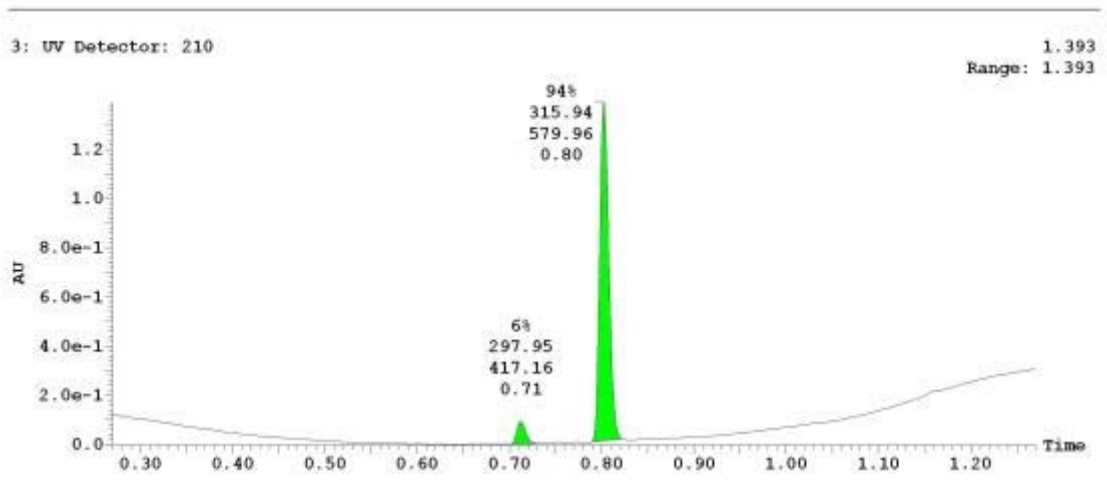
UPLC Chromatogram of **1i**



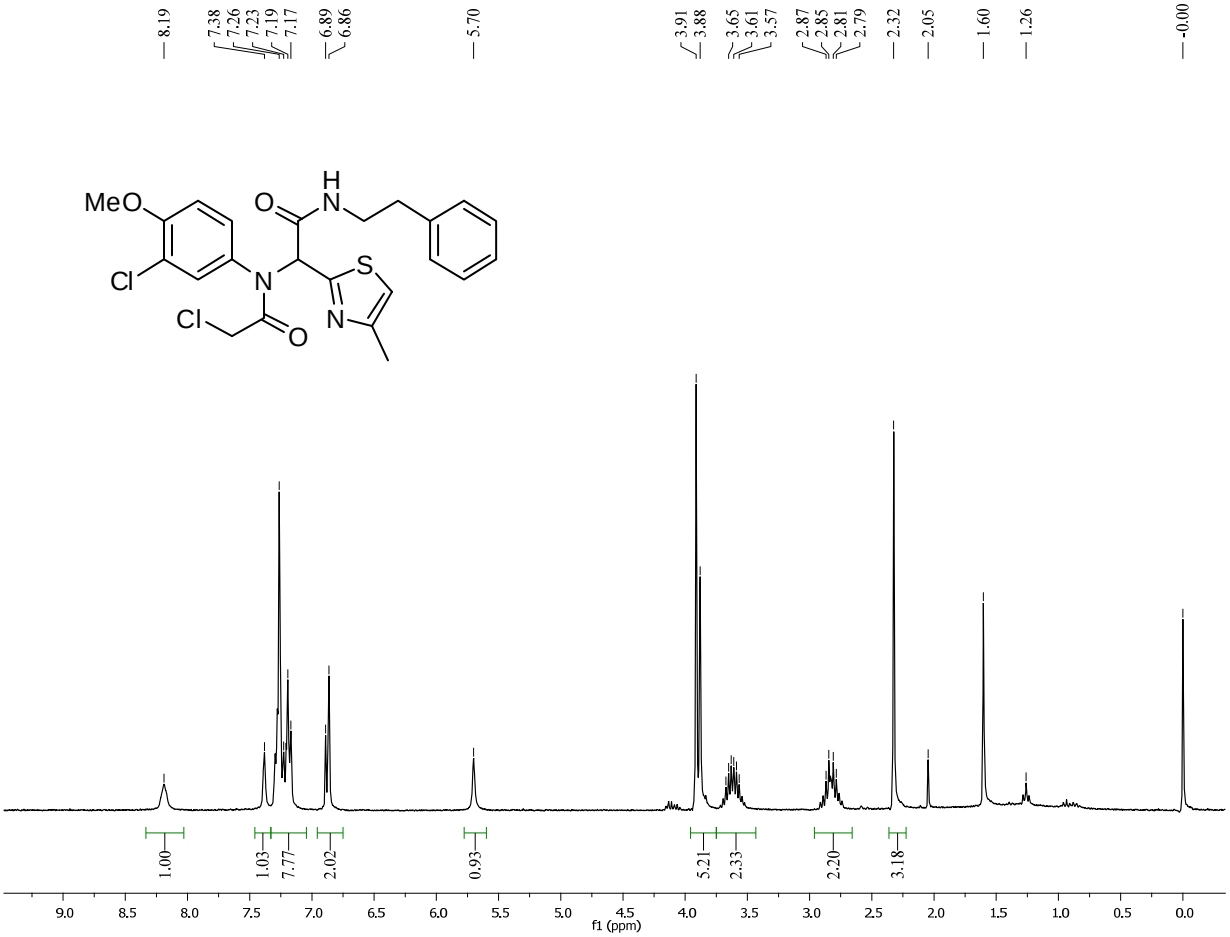
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1j**



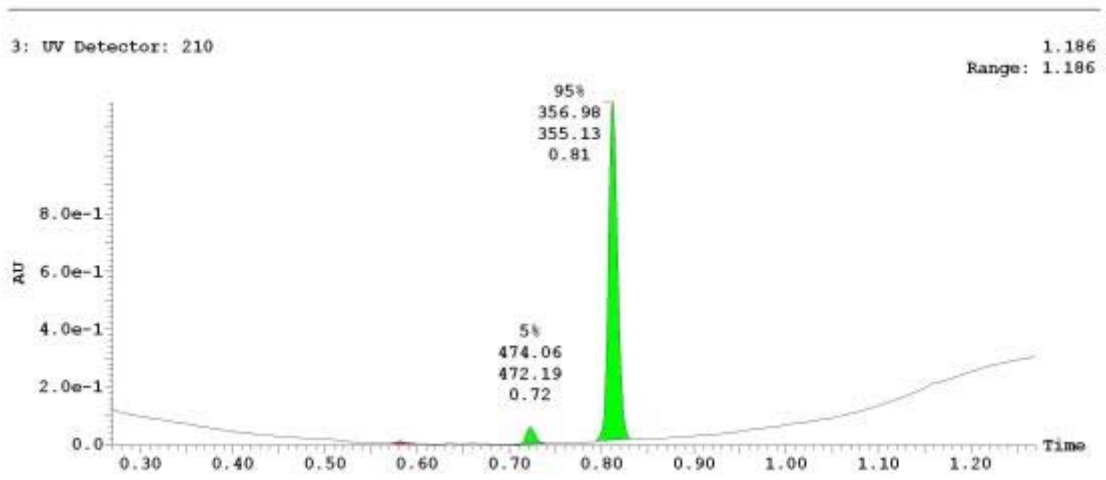
UPLC Chromatogram of **1j**



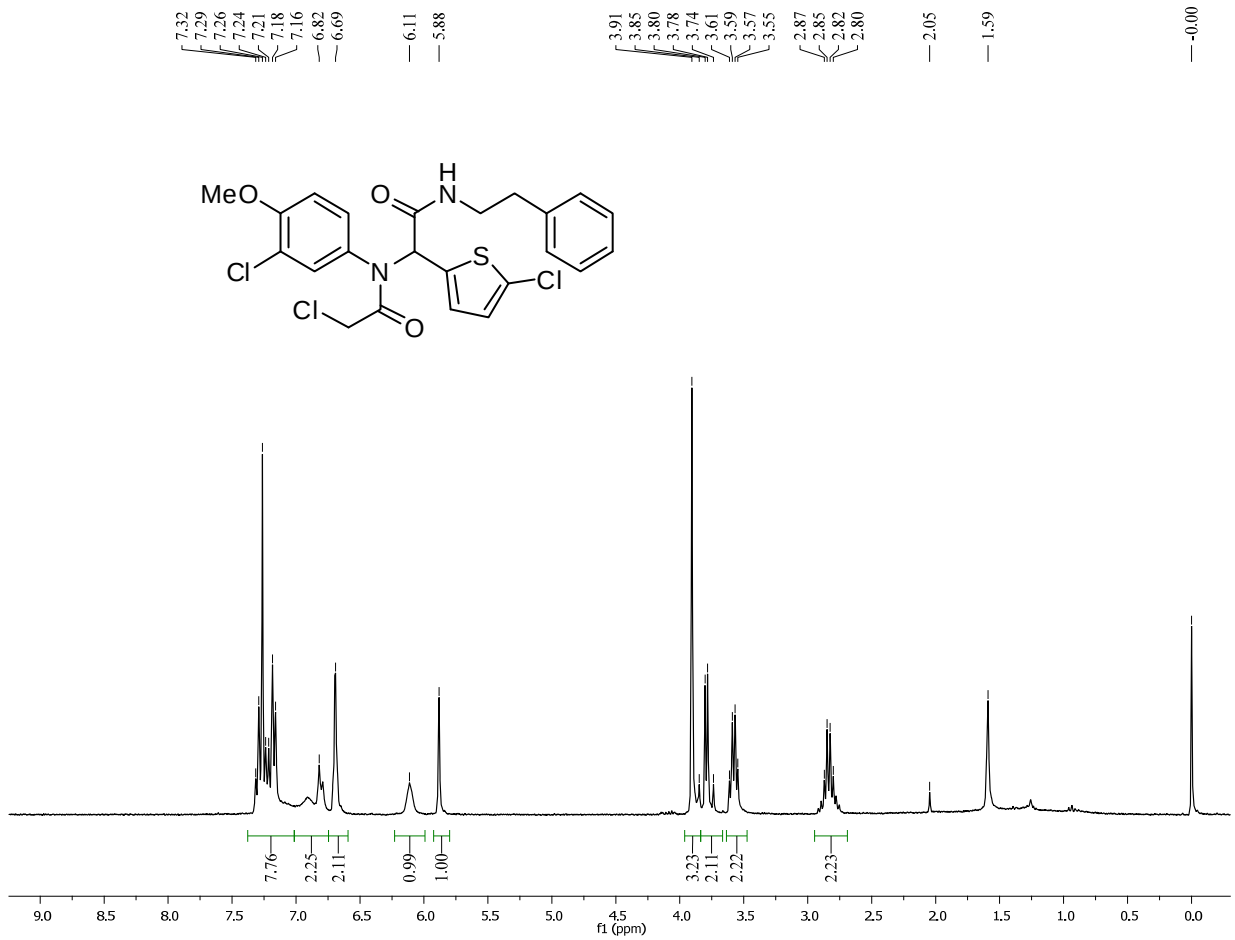
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1k**



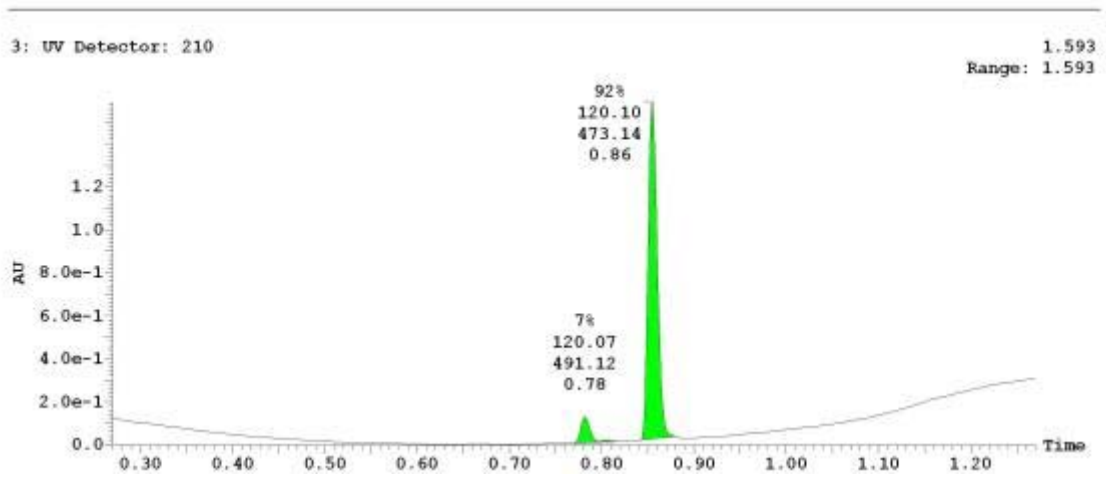
UPLC Chromatogram of **1k**



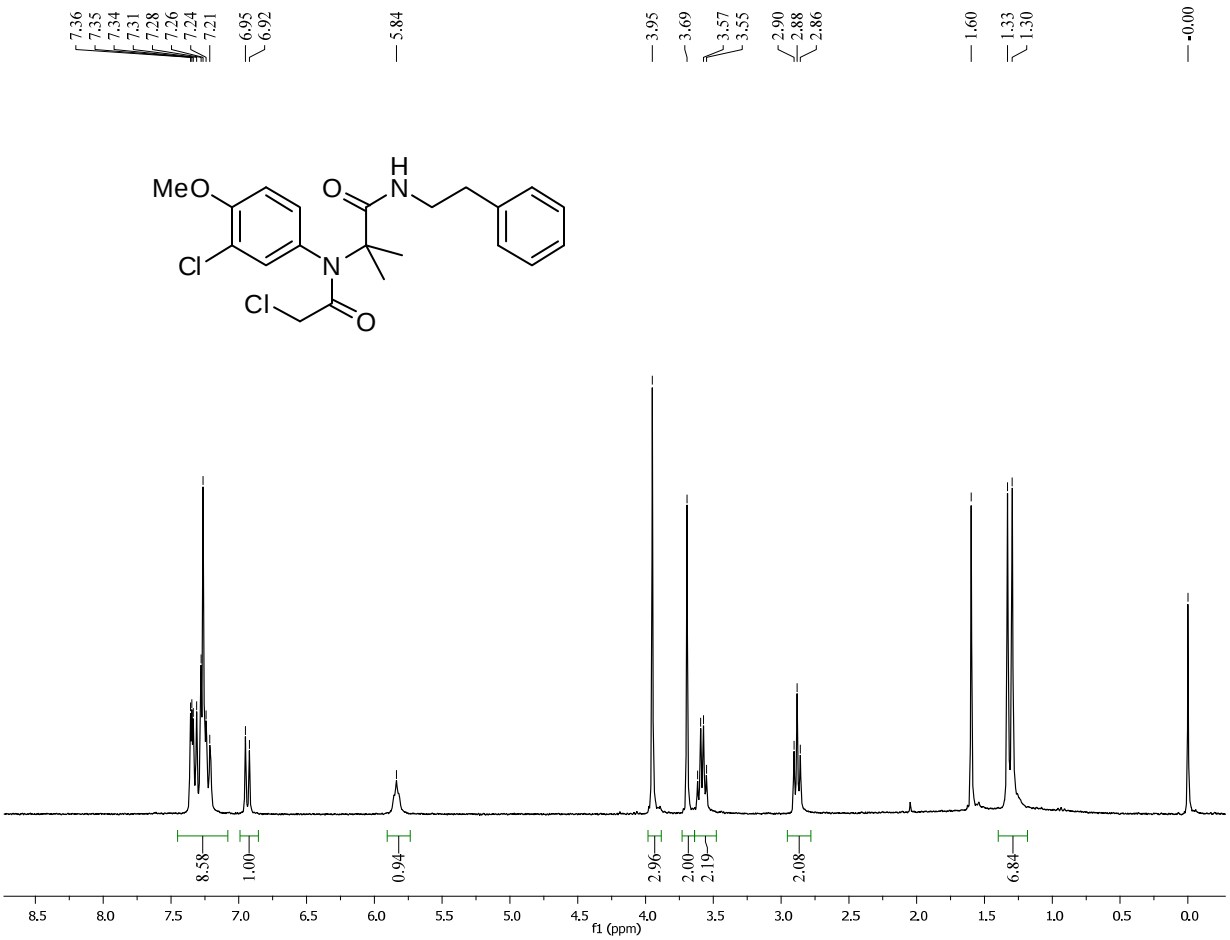
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1l**



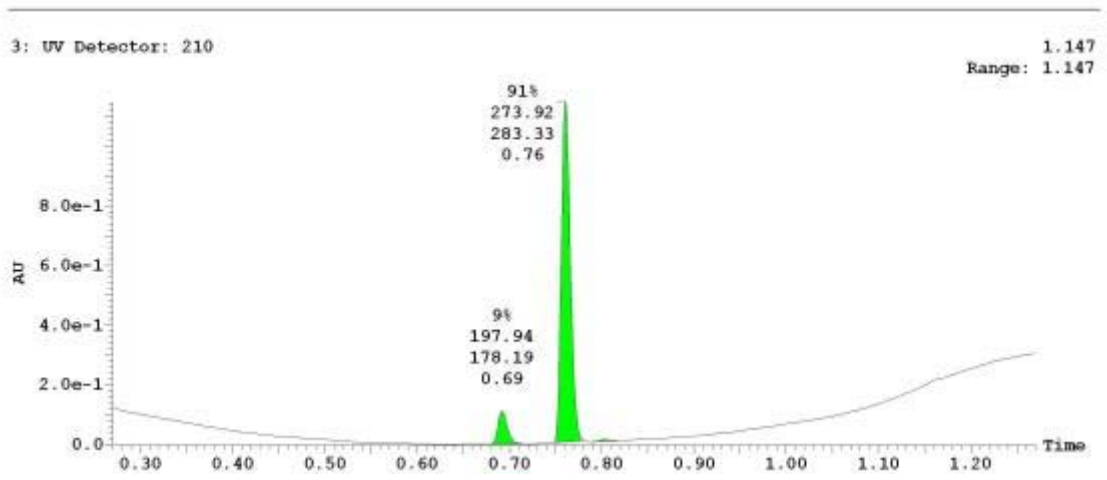
UPLC Chromatogram of **1l**



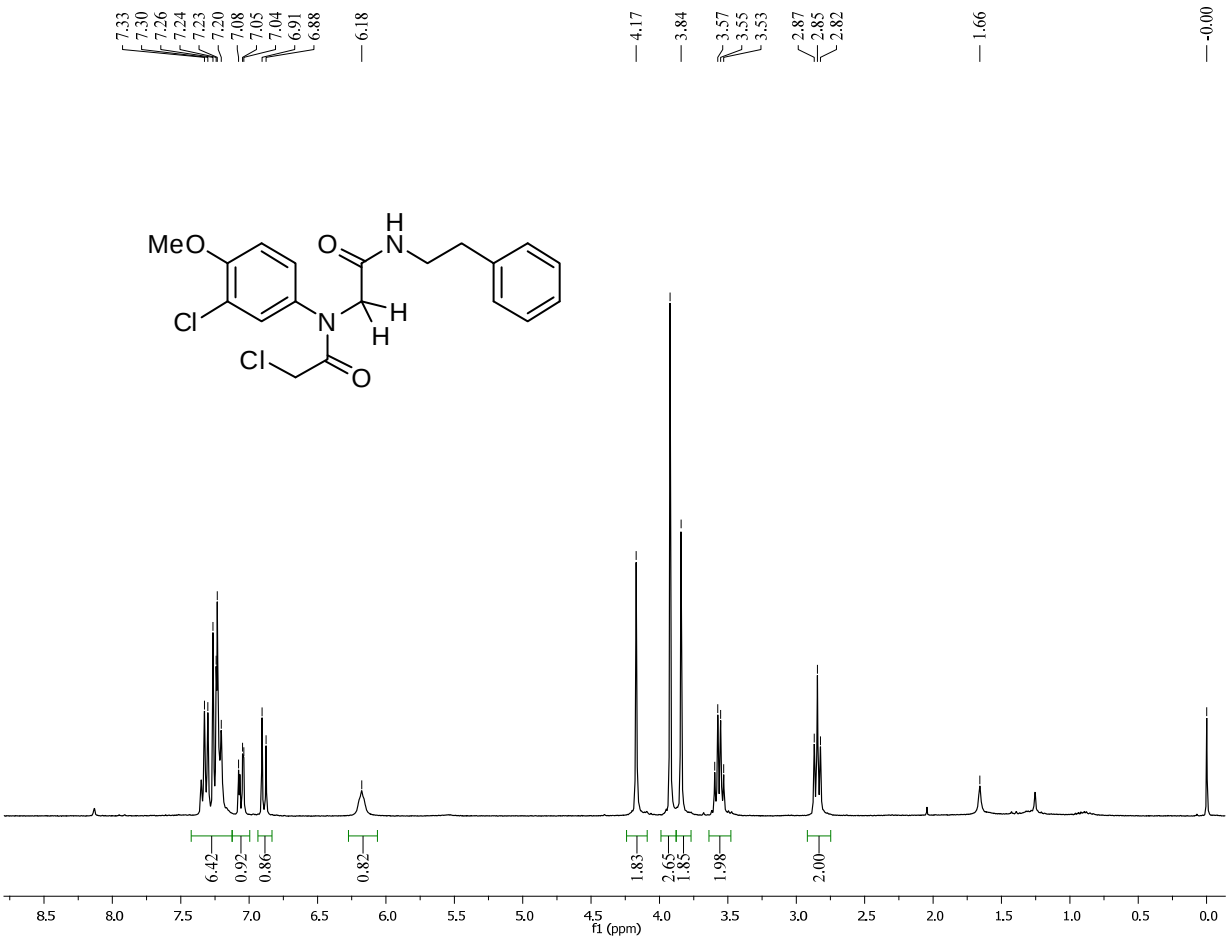
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1m**



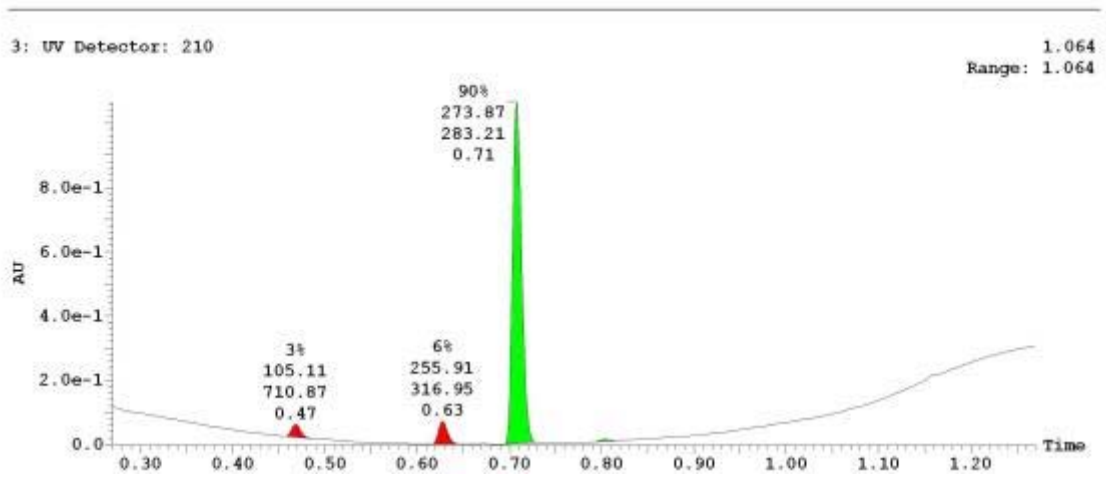
UPLC Chromatogram of **1m**



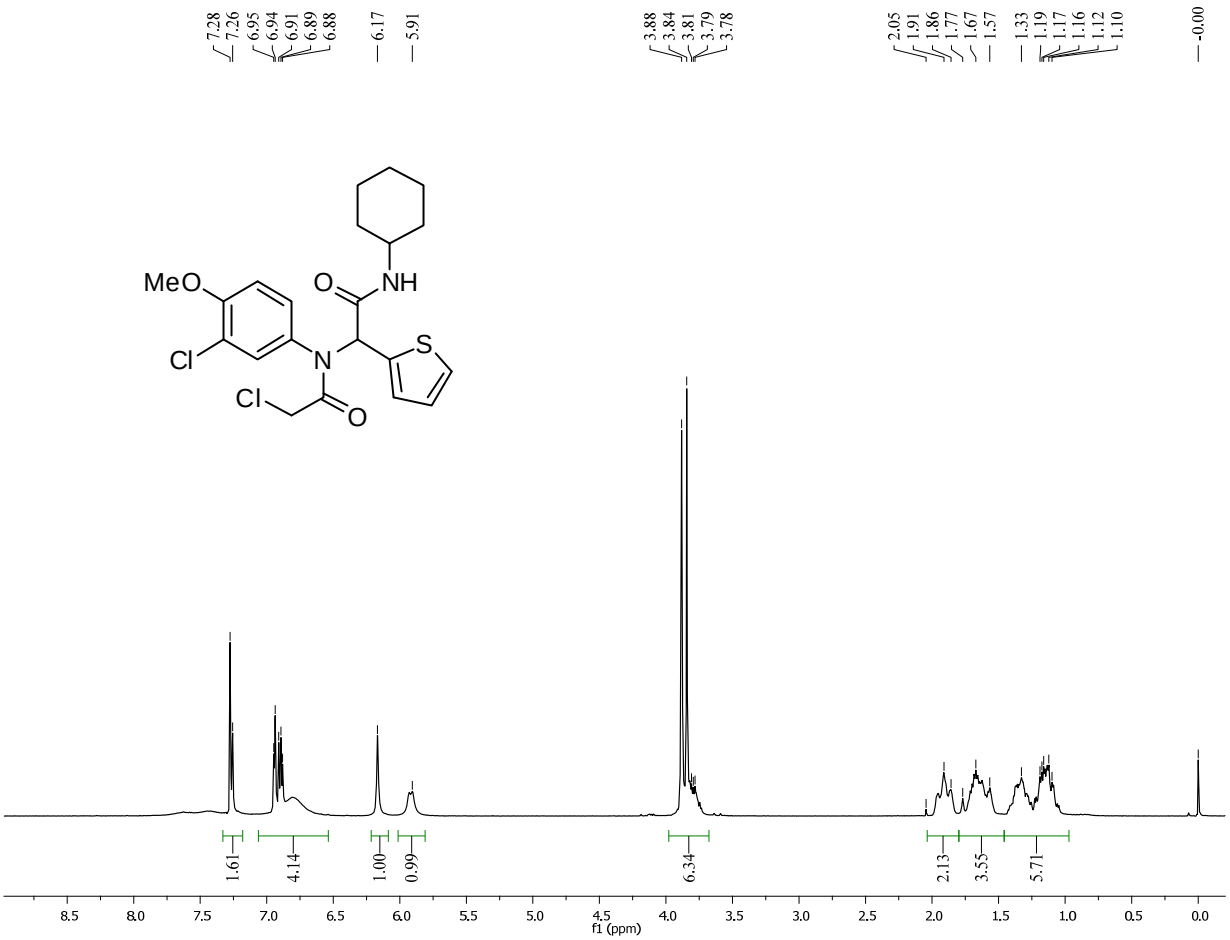
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1n**



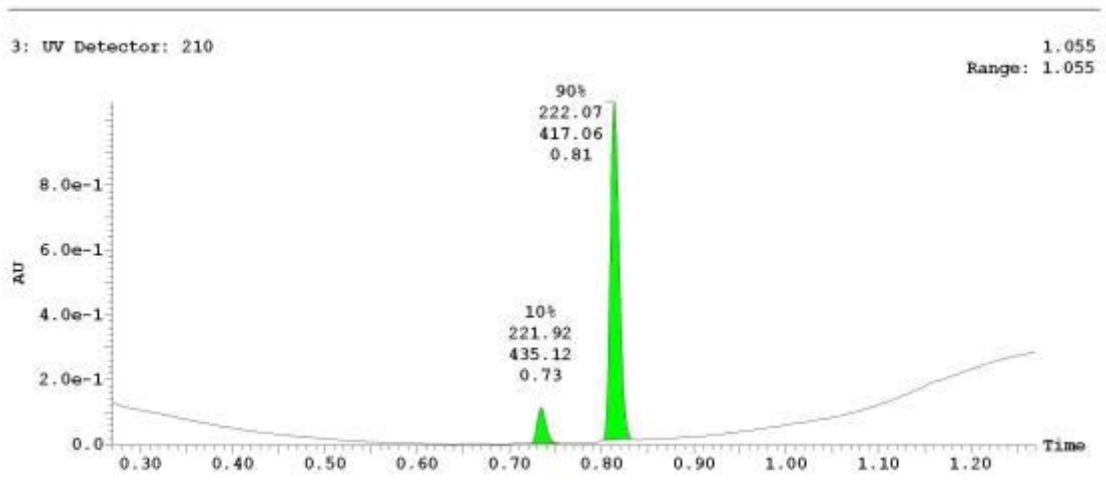
UPLC Chromatogram of **1n**



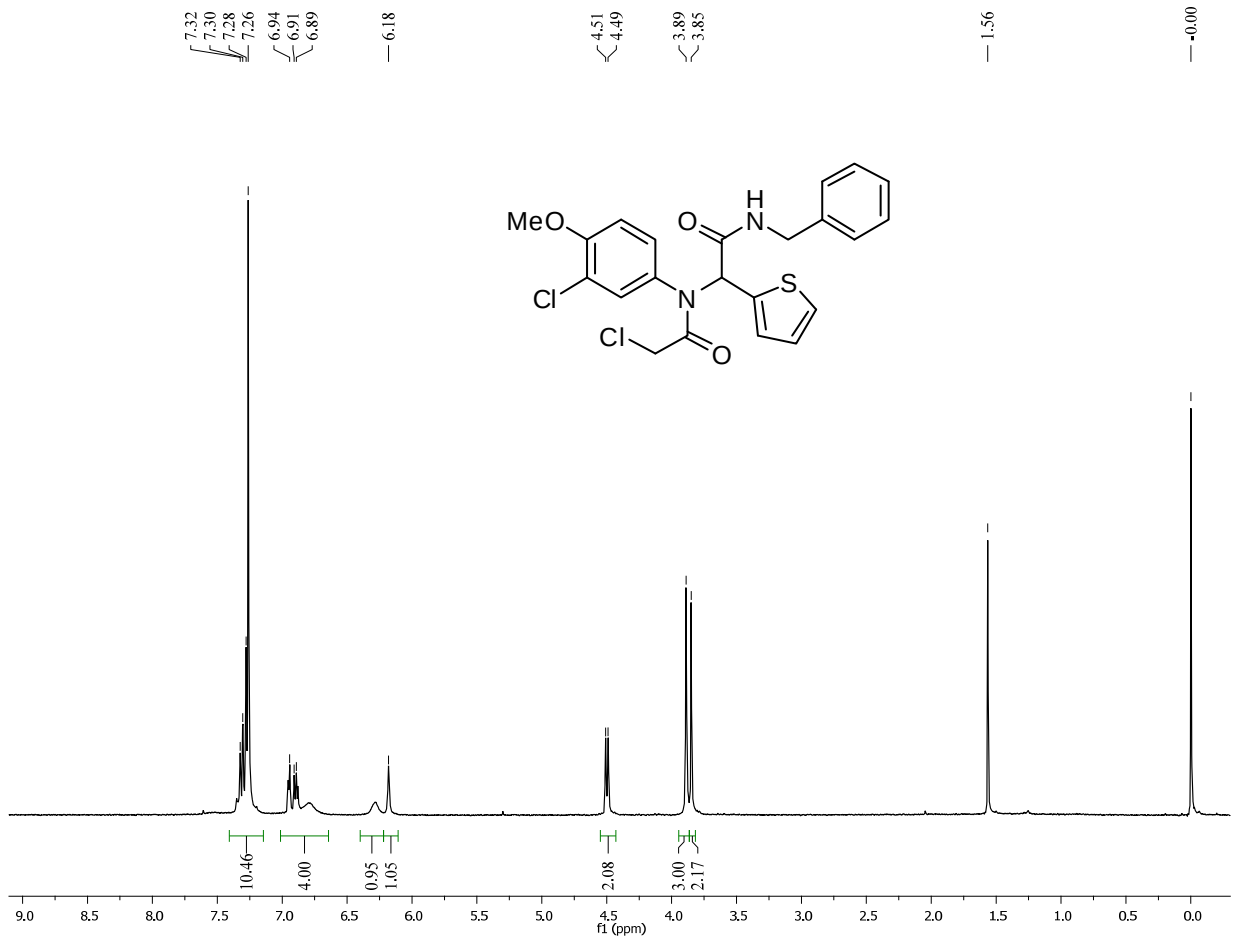
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1o**



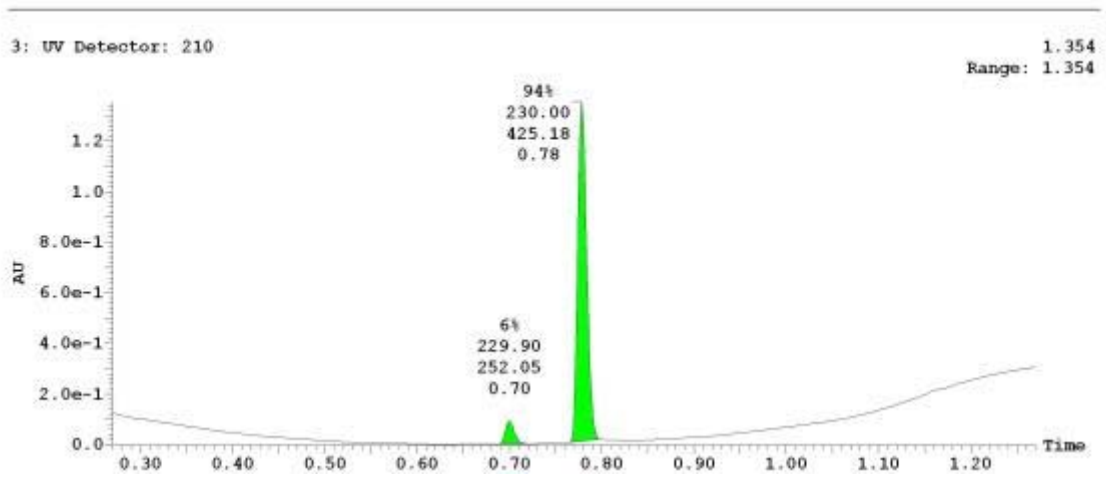
UPLC Chromatogram of **1o**



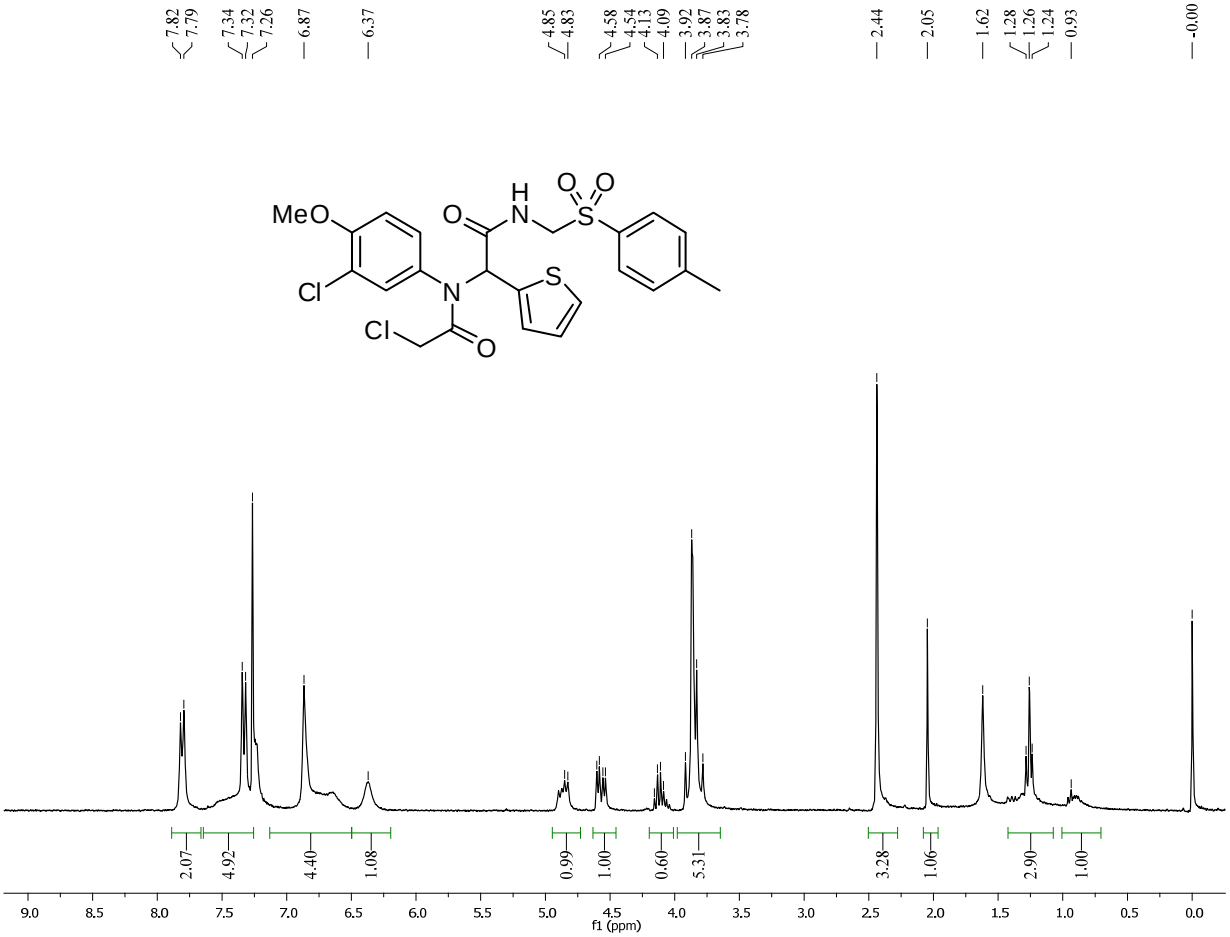
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1p**



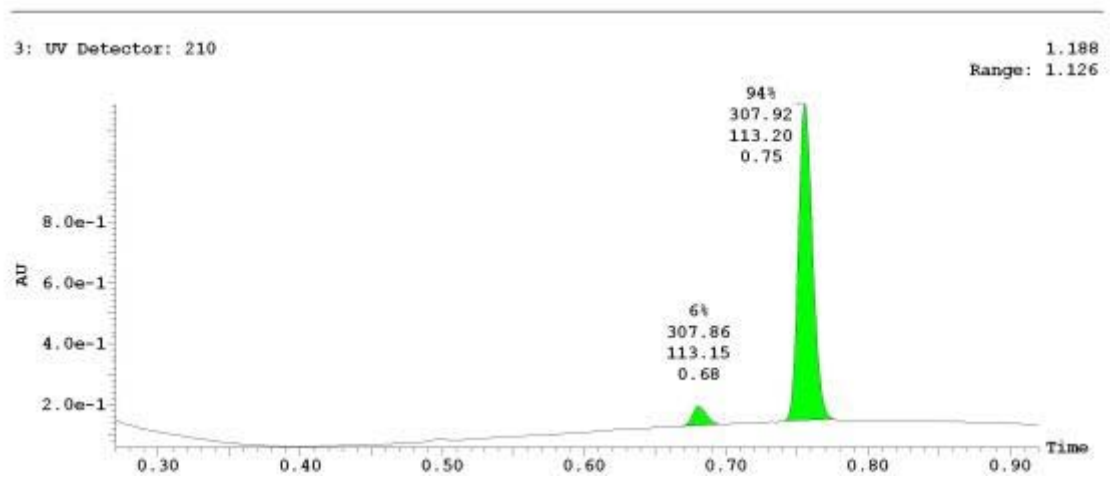
UPLC Chromatogram of **1p**



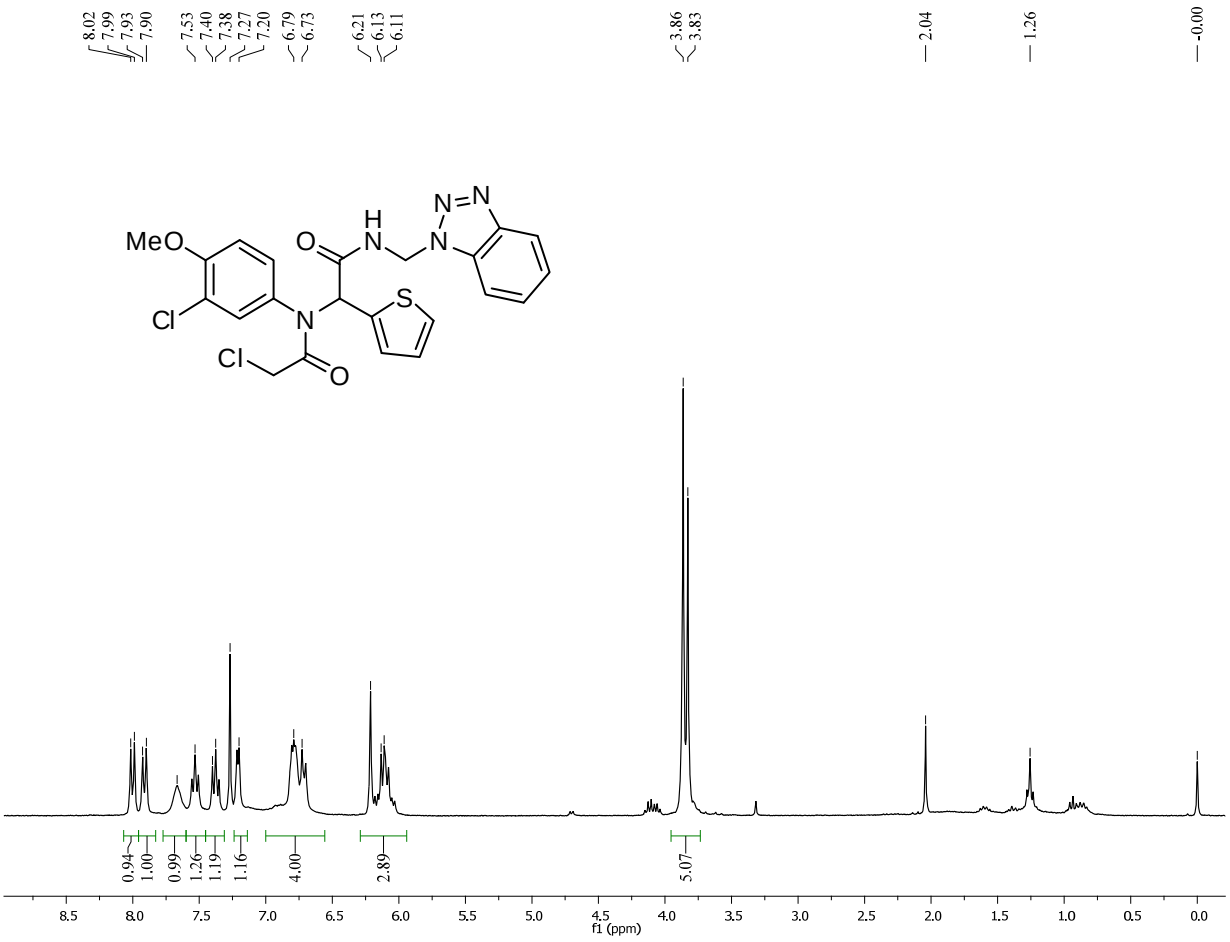
[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1q**



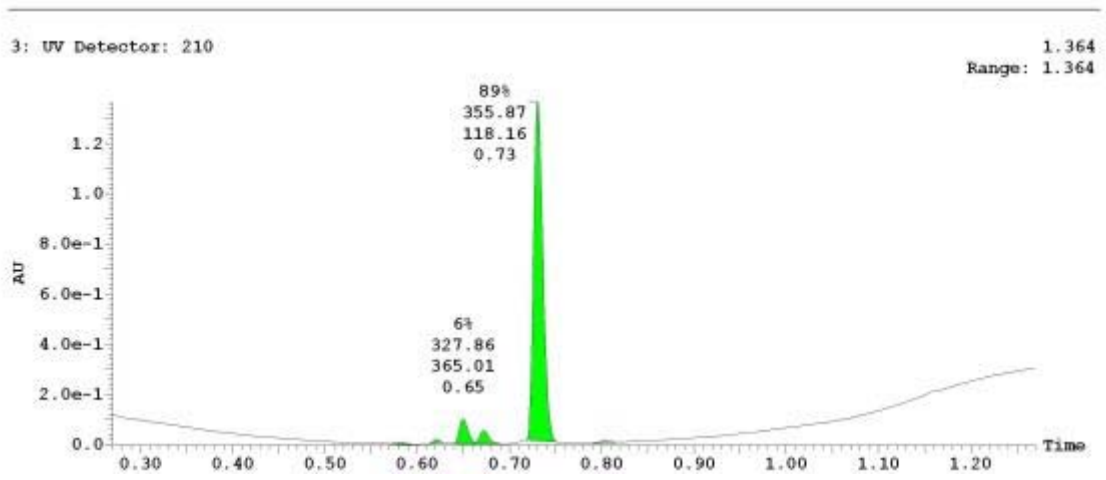
UPLC Chromatogram of **1q**



[1]HNMR Spectra (300 MHz, $CDCl_3$) of **1r**

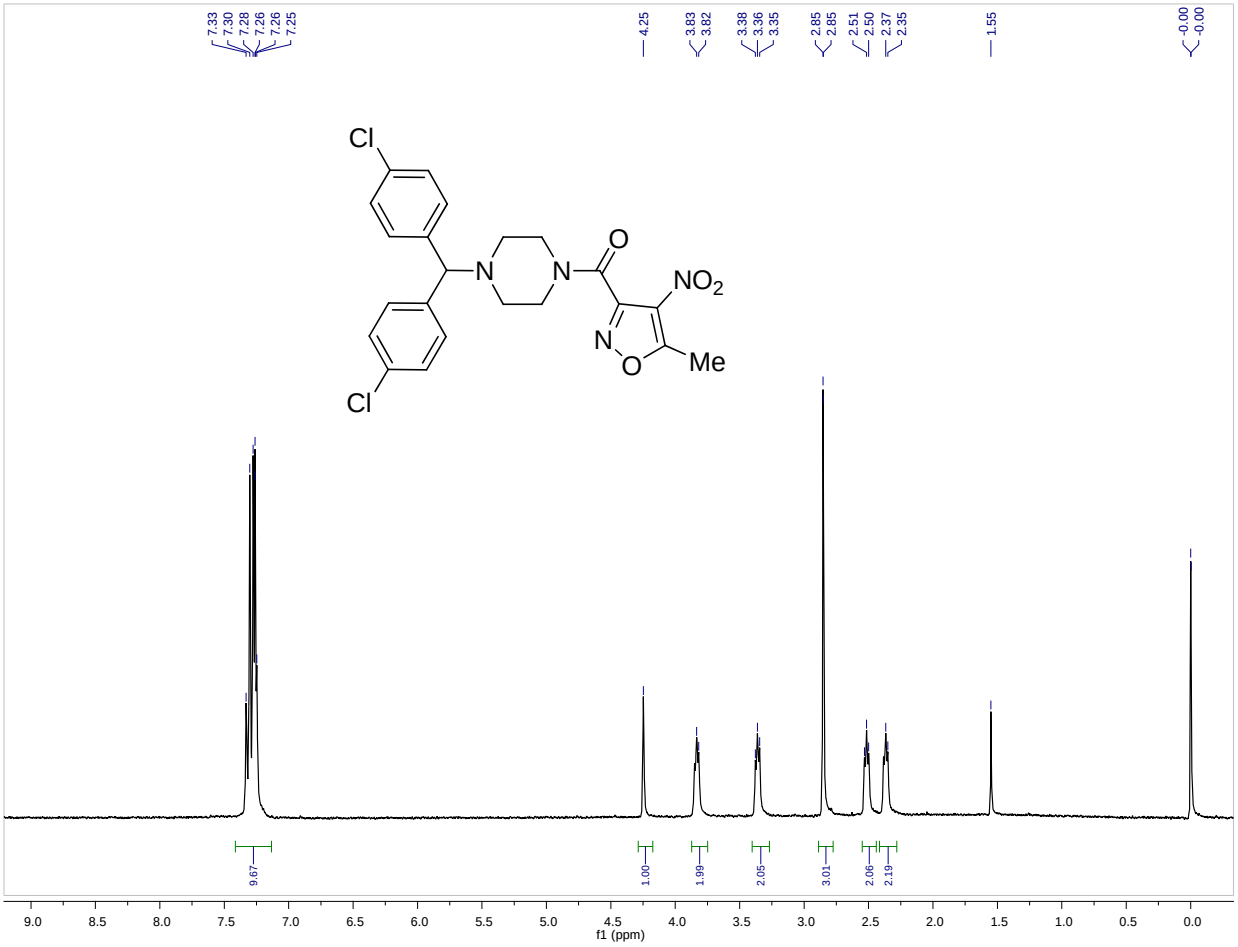


UPLC Chromatogram of **1r**

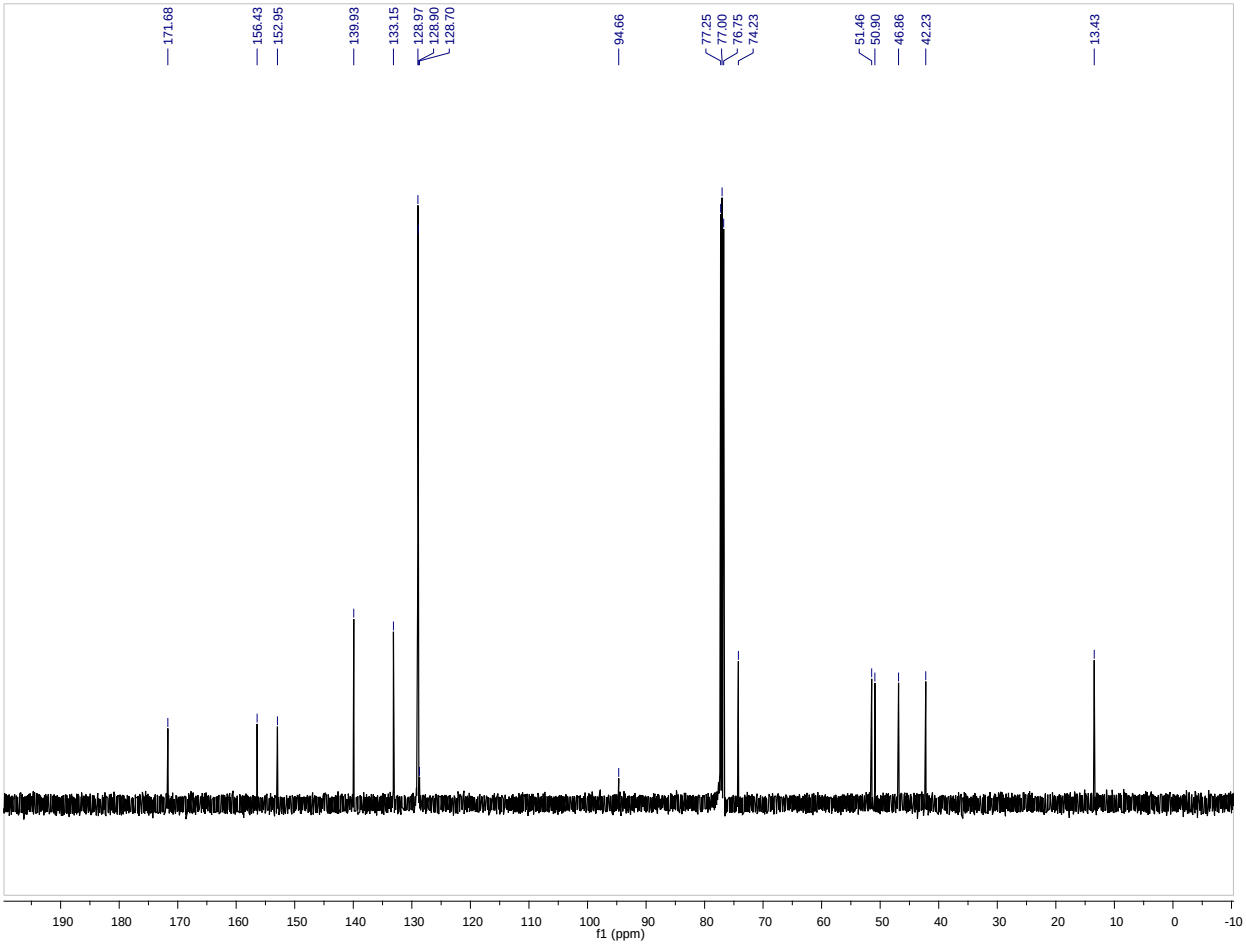


5.2 NMR and LCMS of 2a and analogs

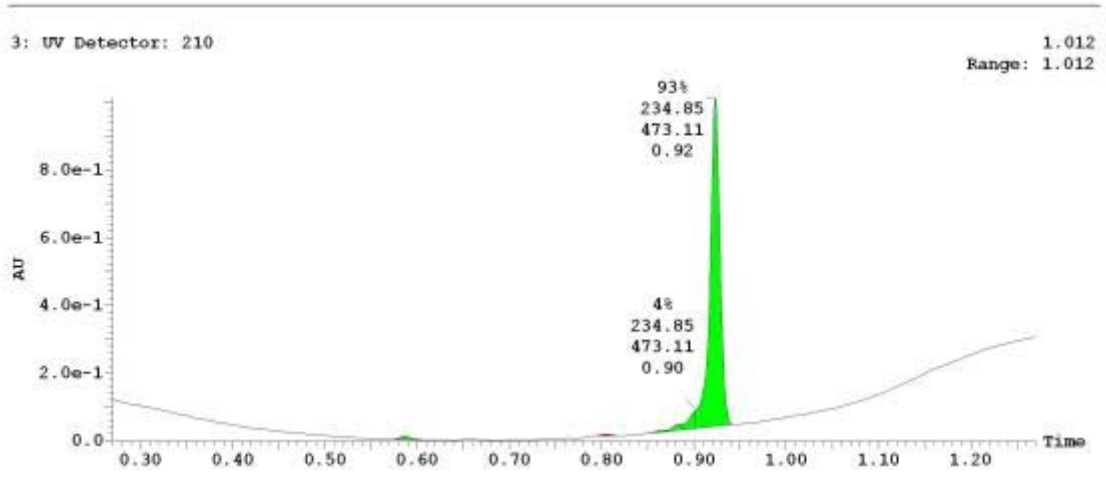
^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **2y**



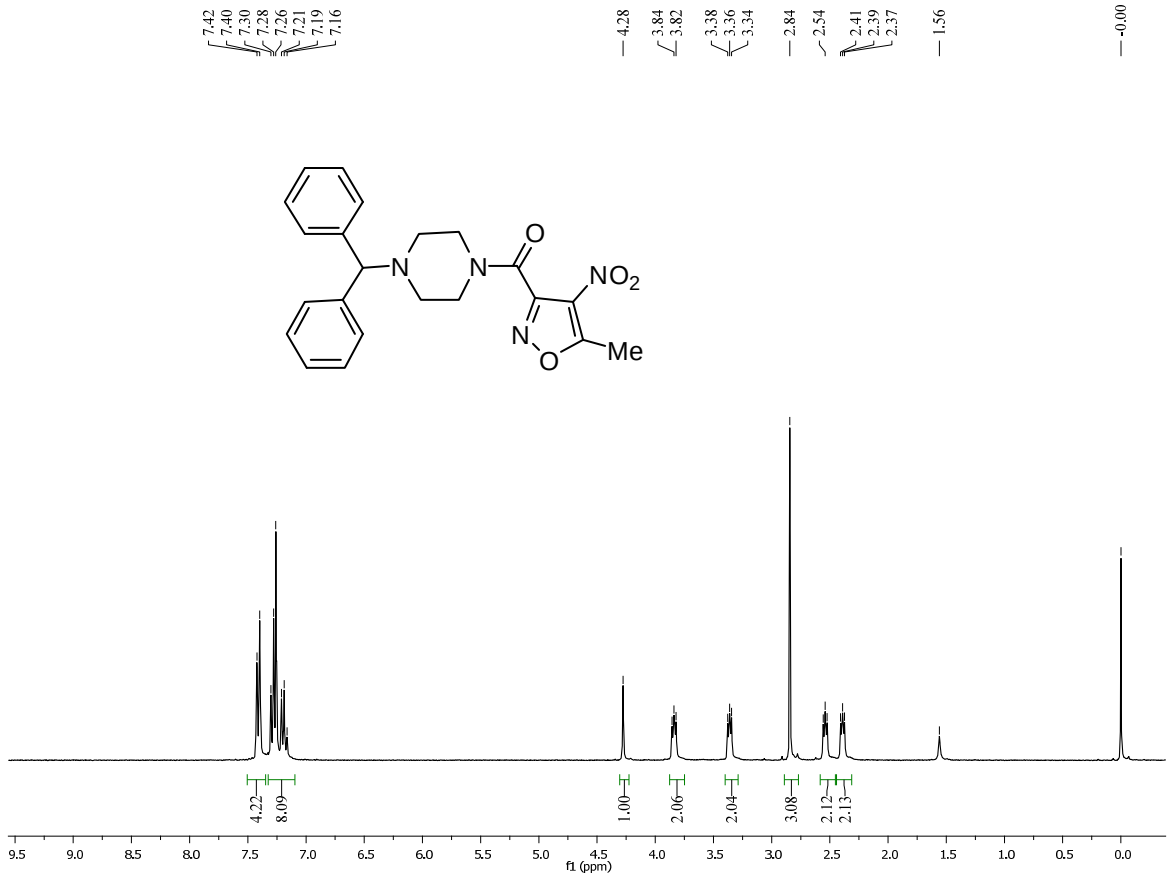
^{13}C NMR Spectrum (125 MHz, $CDCl_3$) of **2y**



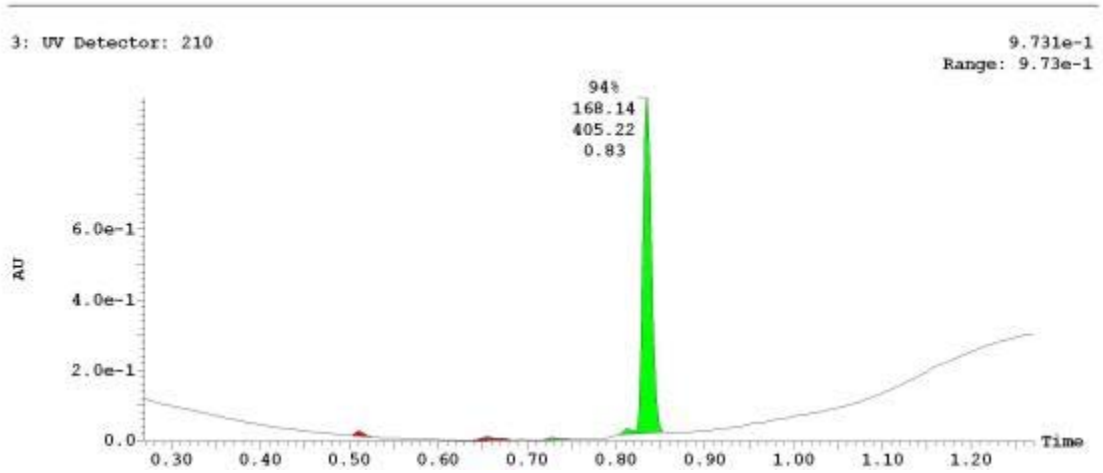
UPLC chromatogram of **2y**



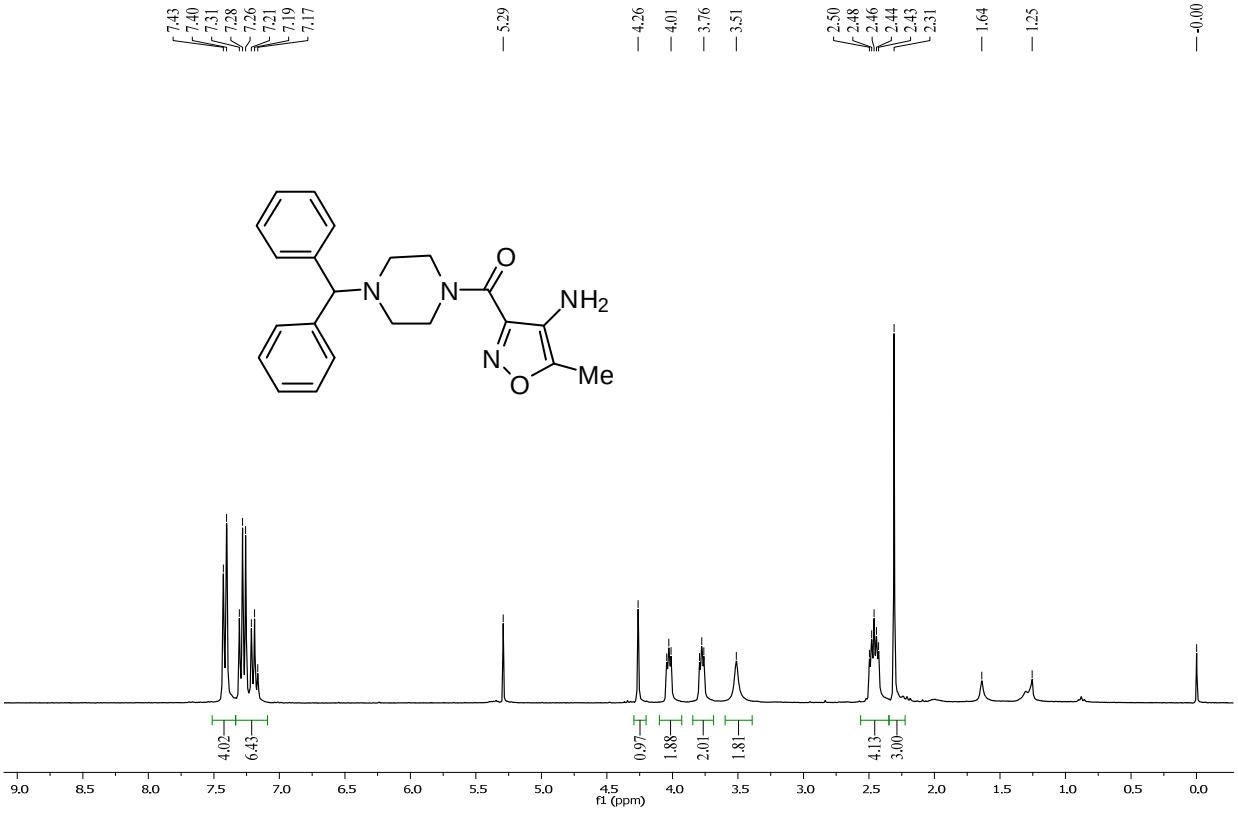
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2a**



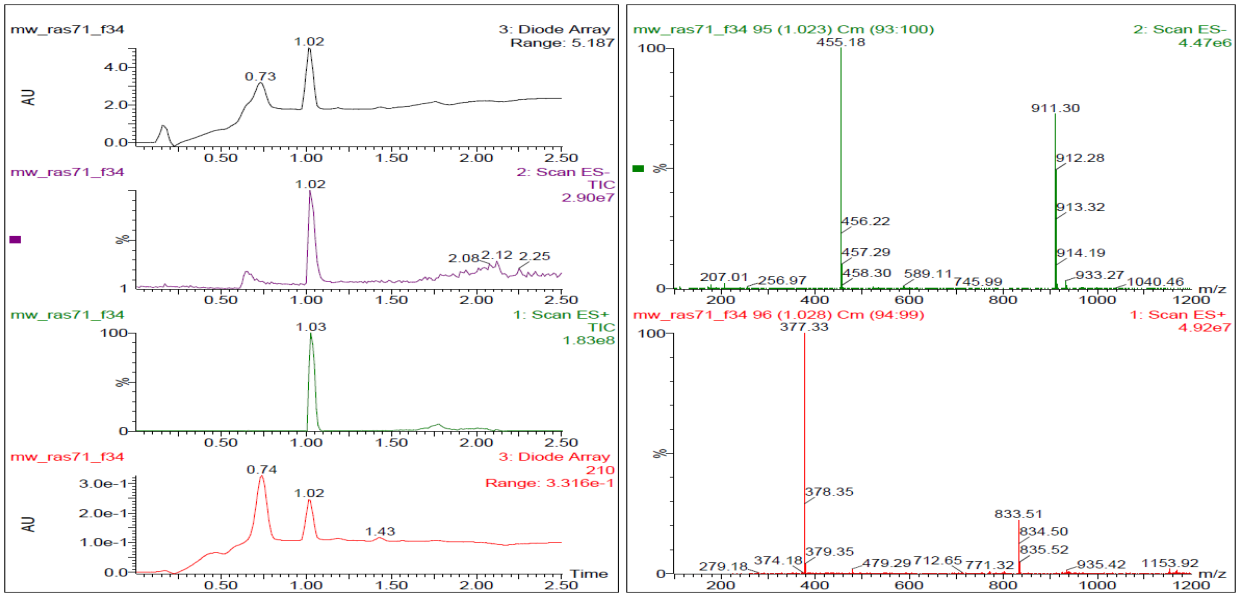
UPLC Chromatogram of **2a**



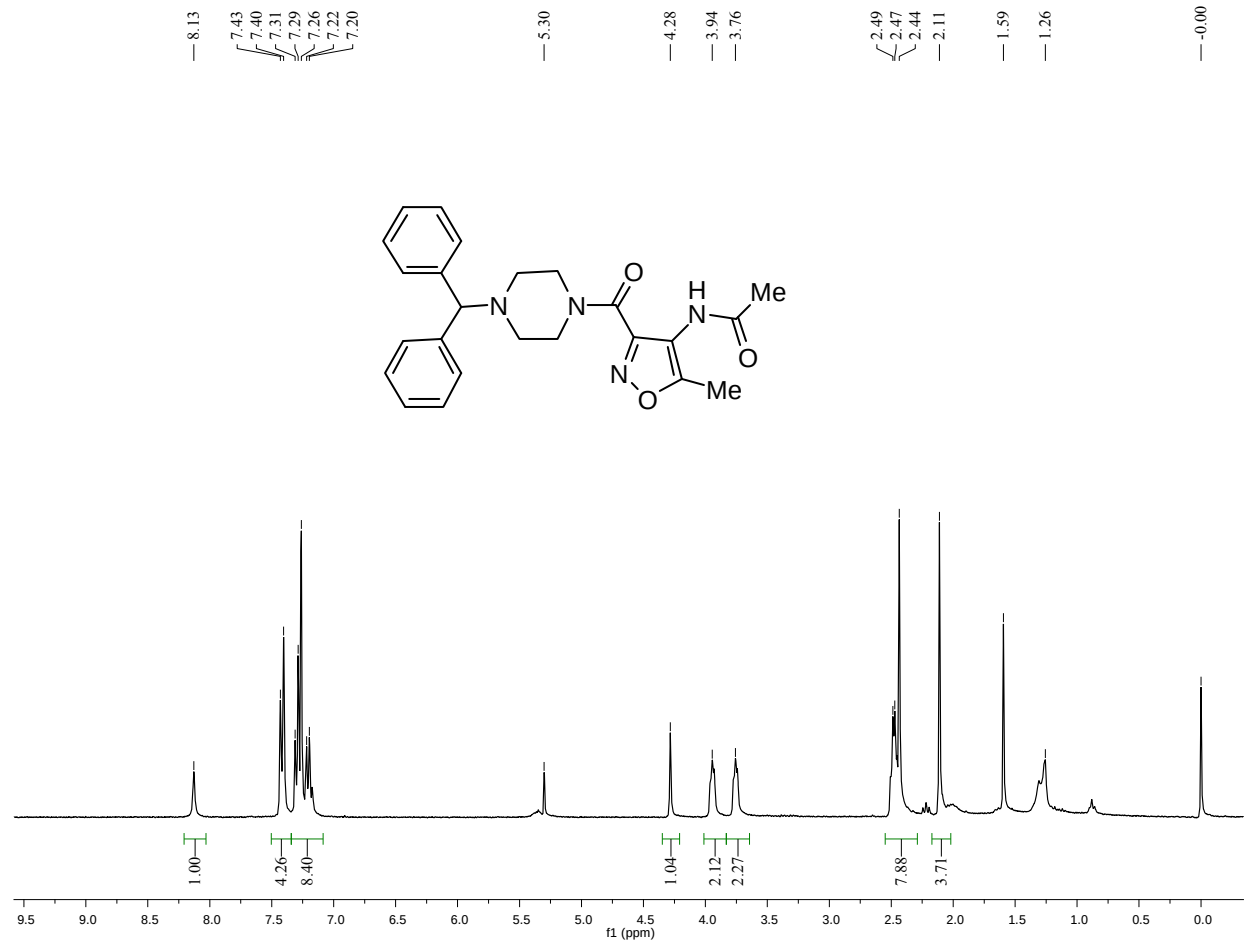
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2b**



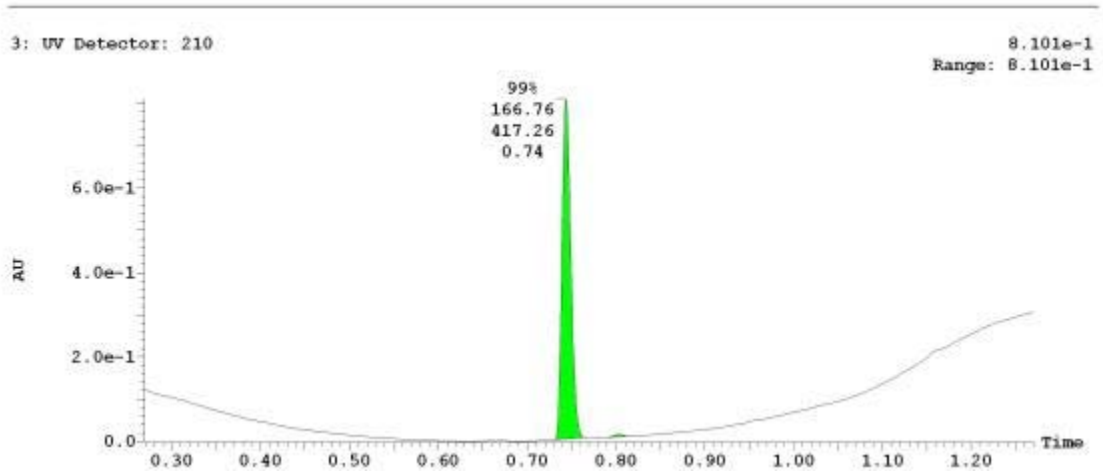
LCMS Chromatogram of **2b (**peak at 0.74 is methanol used to dissolve the sample)



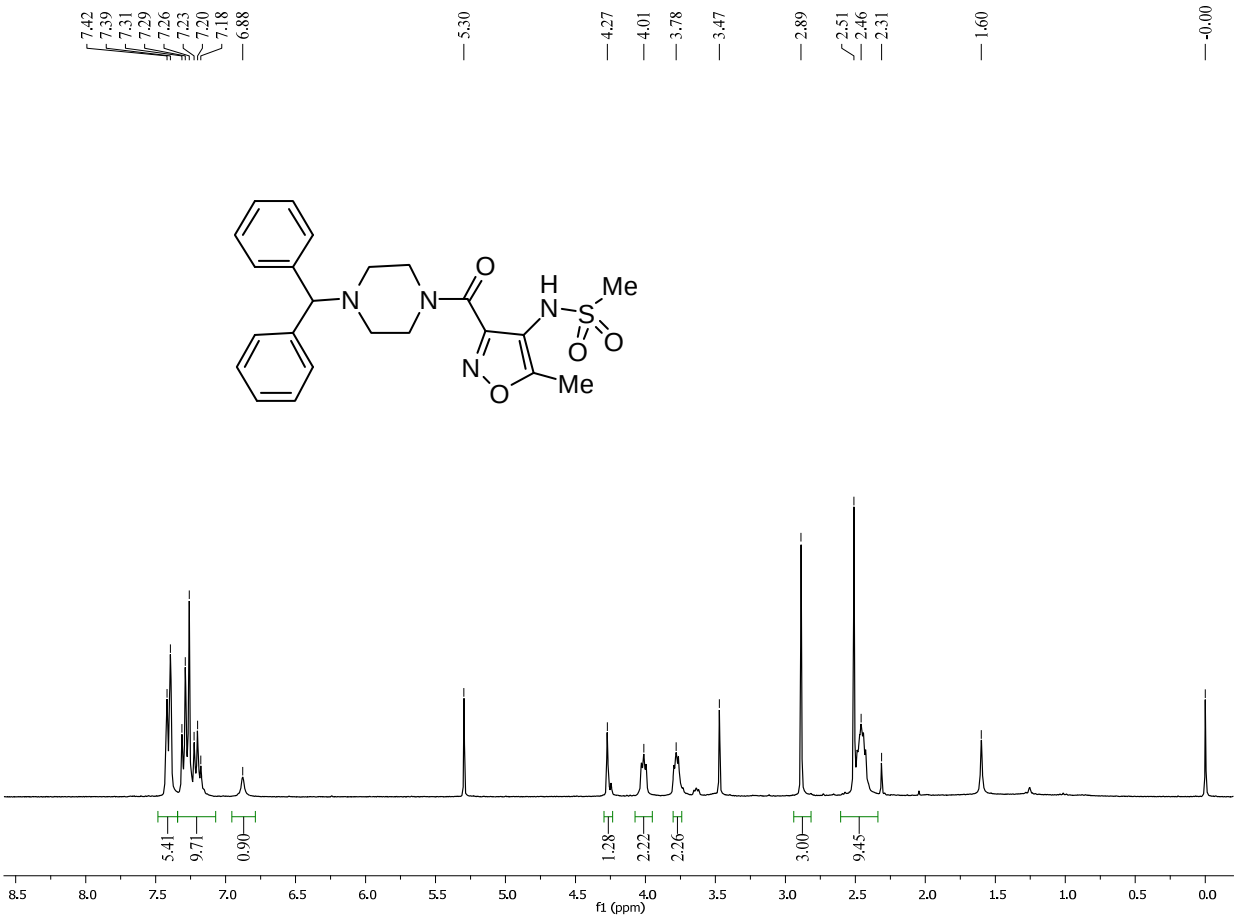
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2c**



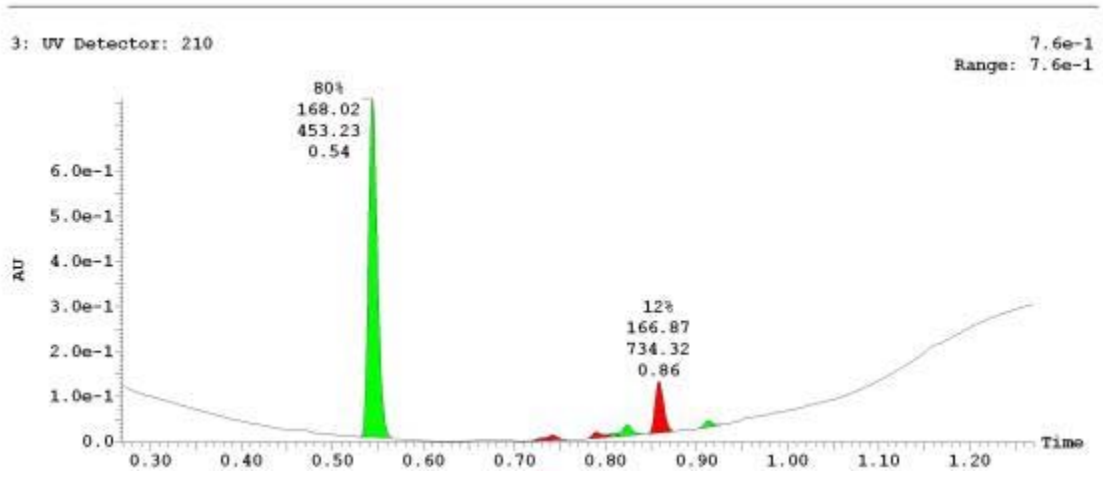
UPLC Chromatogram of **2c**



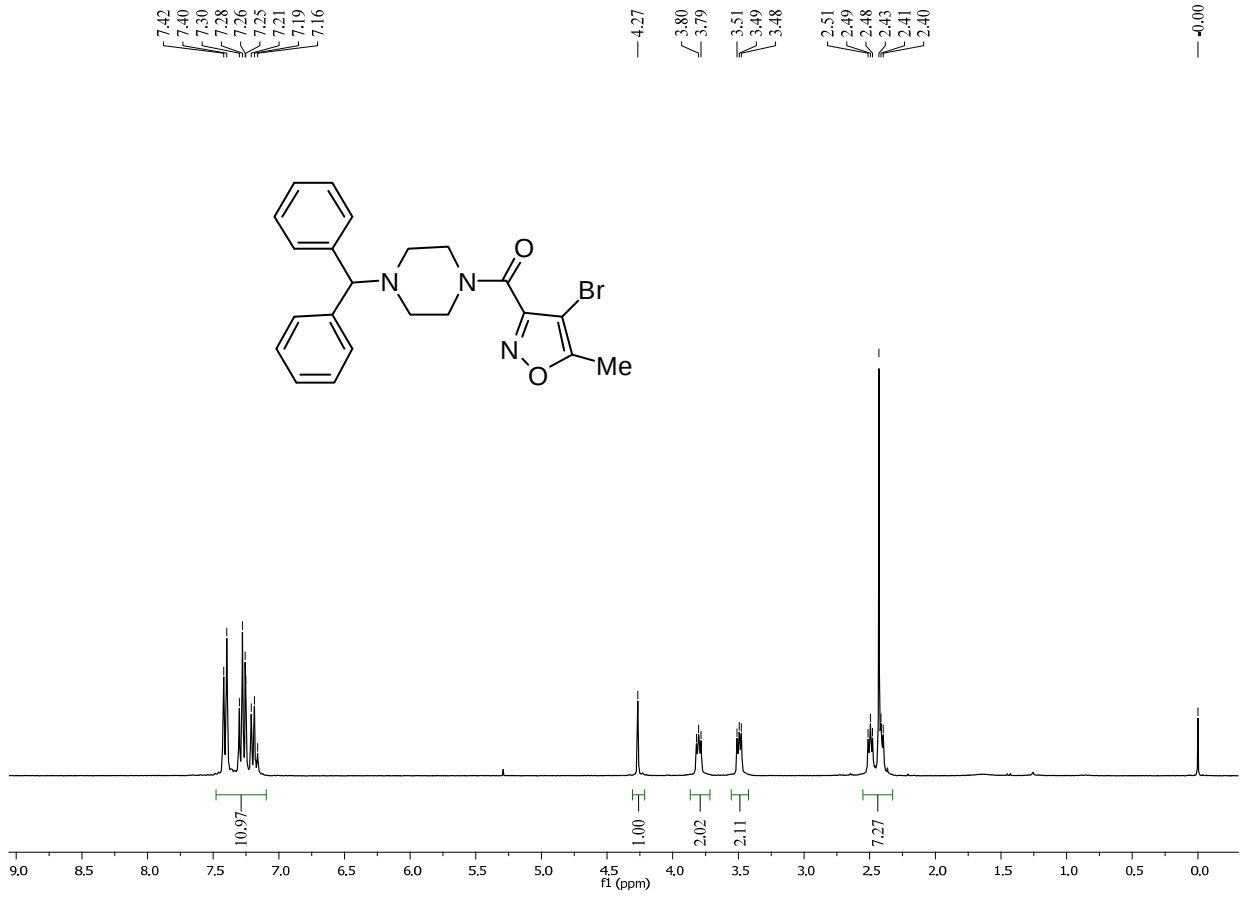
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2d**



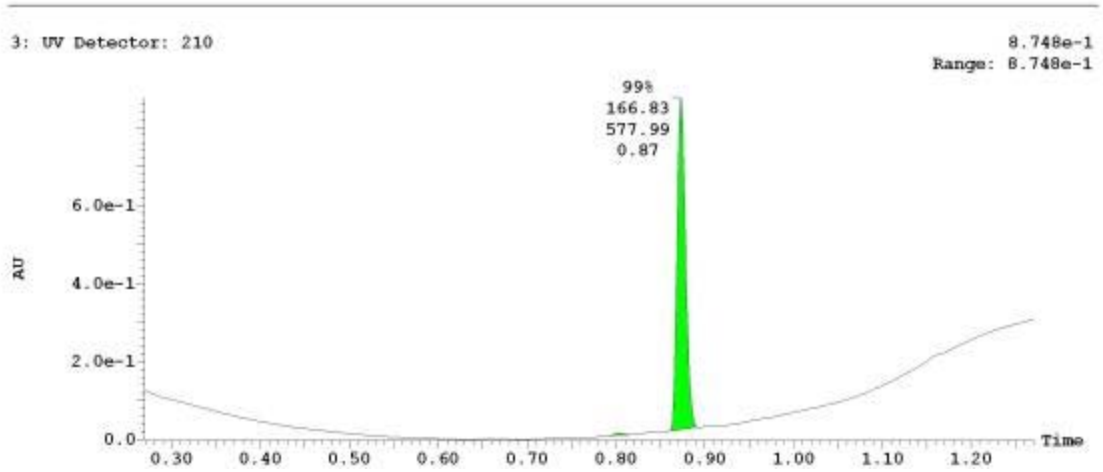
UPLC Chromatogram of **2d**



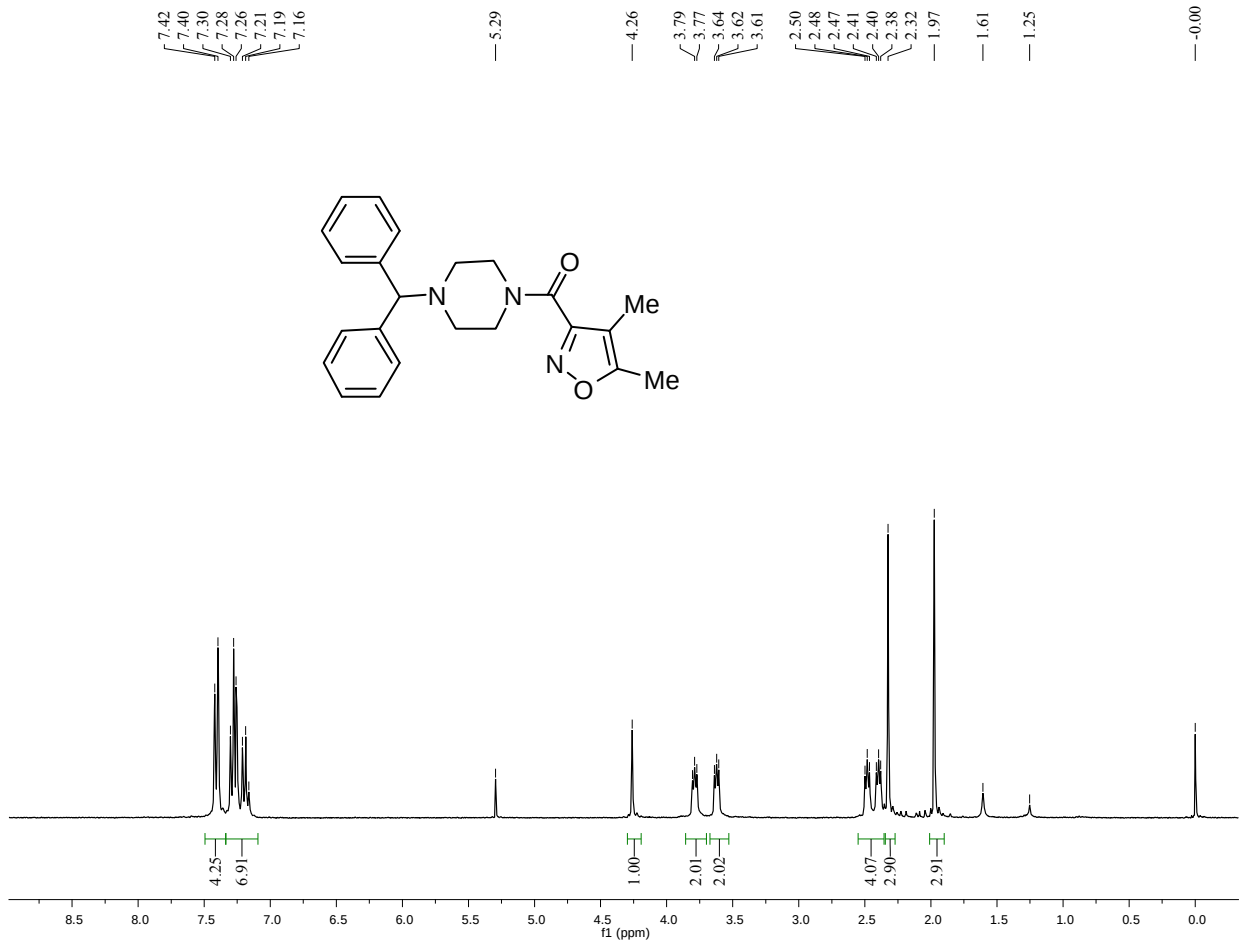
^{1}HNMR Spectrum (300 MHz, $CDCl_3$) of **2e**



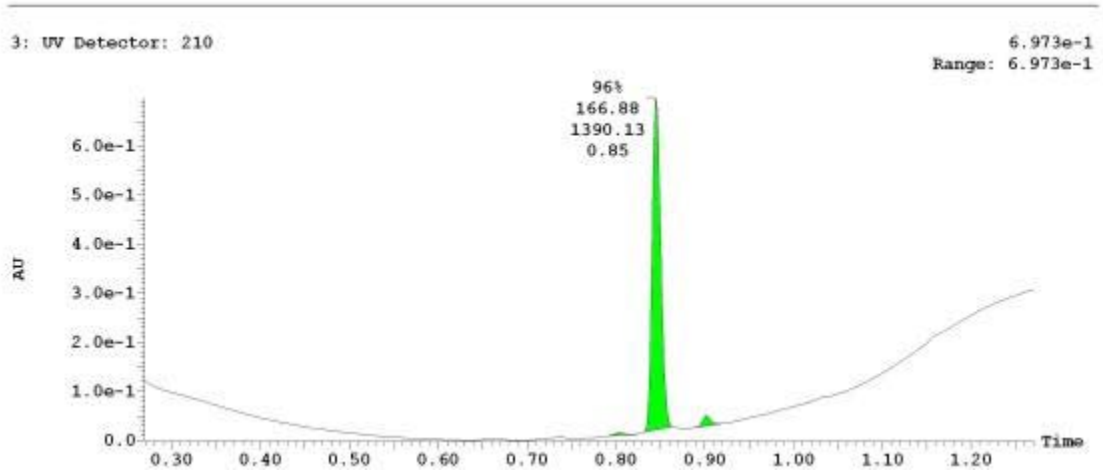
UPLC Chromatogram of **2e**



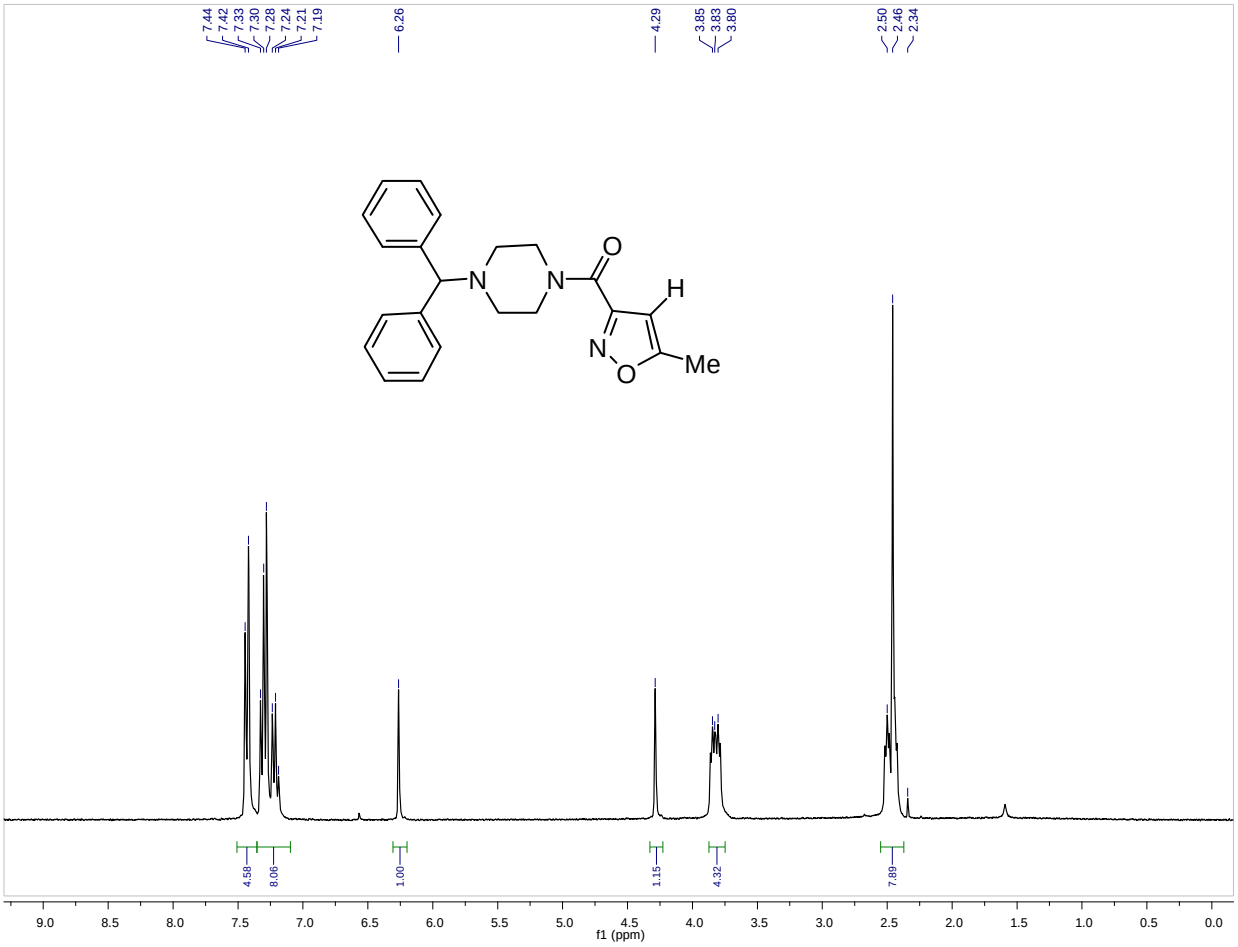
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2f**



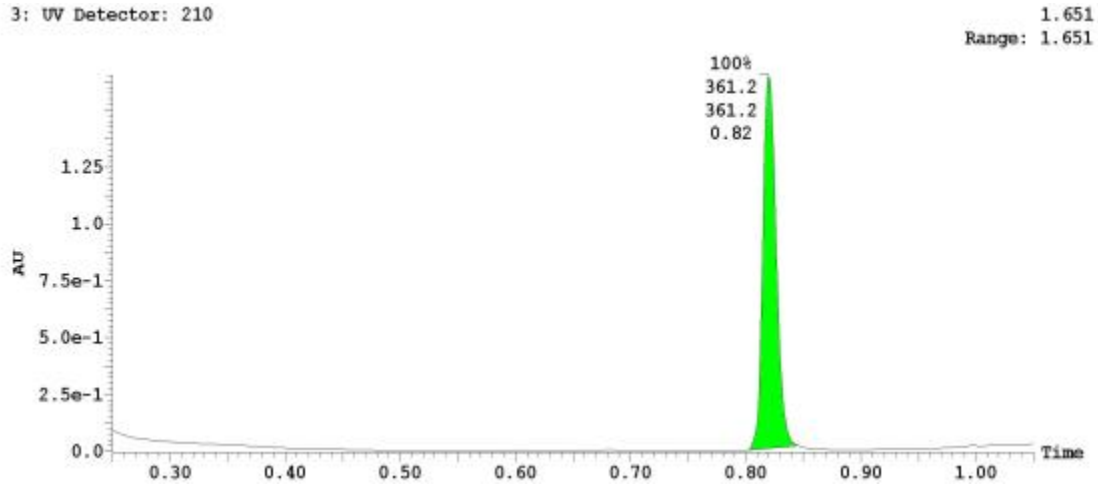
UPLC Chromatogram of **2f**



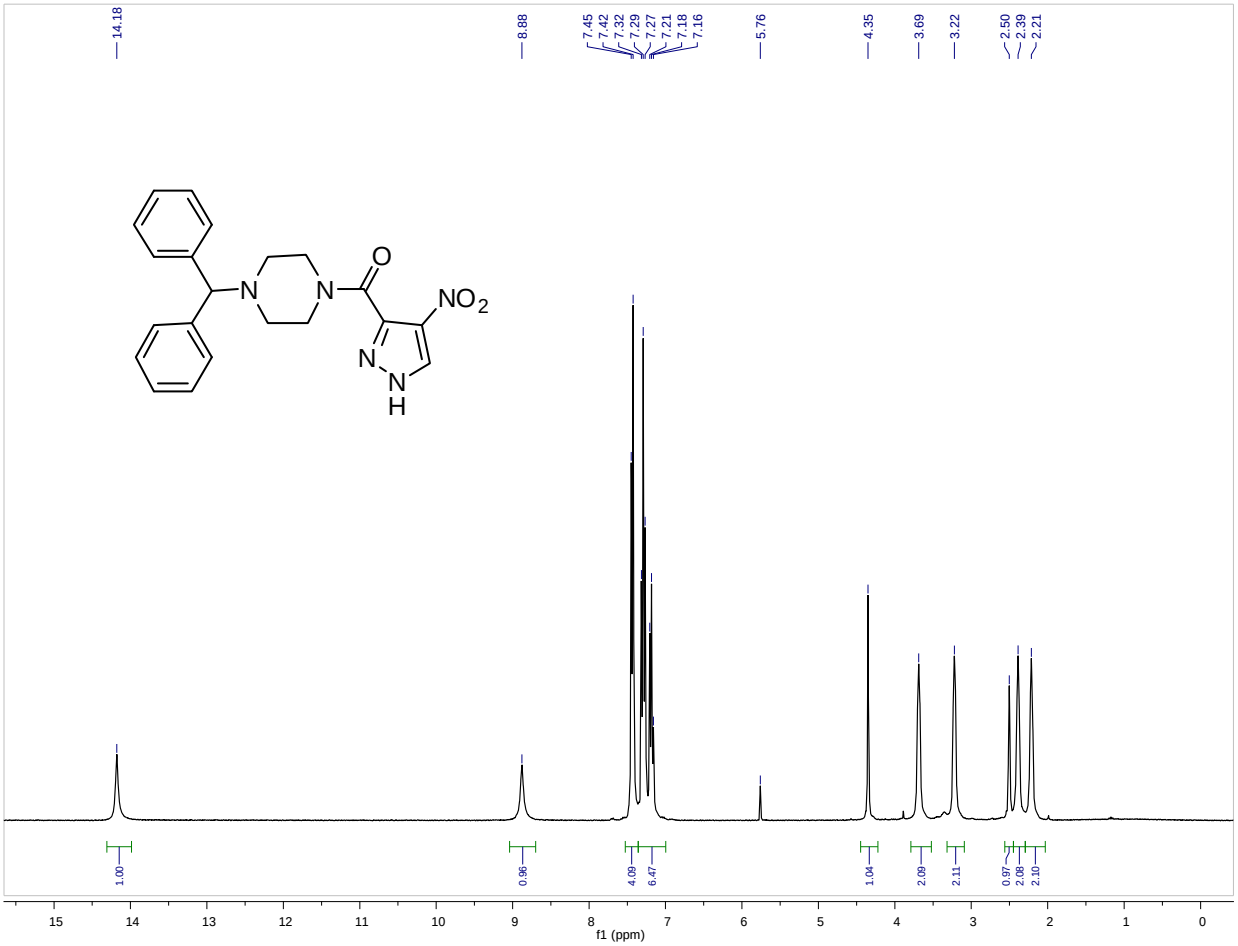
^{1}HNMR Spectrum (300 MHz, $CDCl_3$) of **2g**



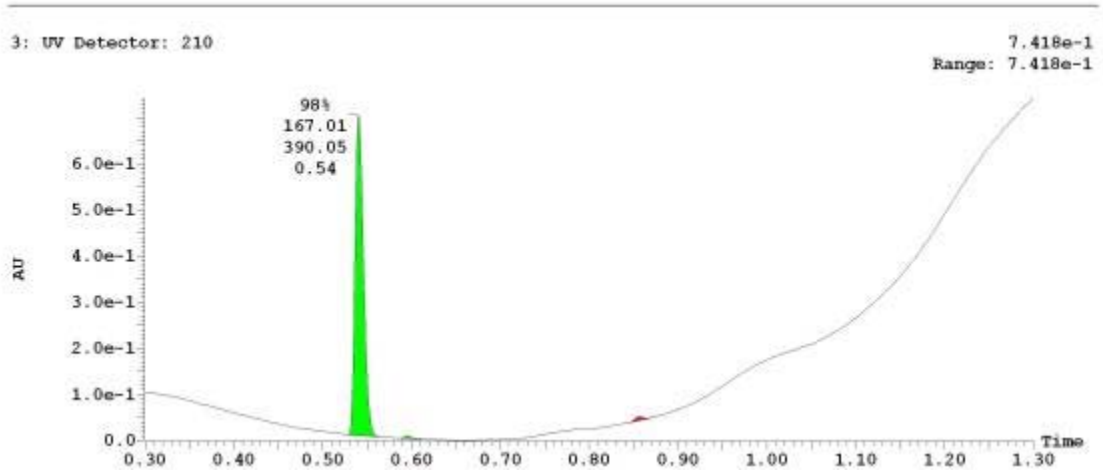
UPLC Chromatogram of **2g**



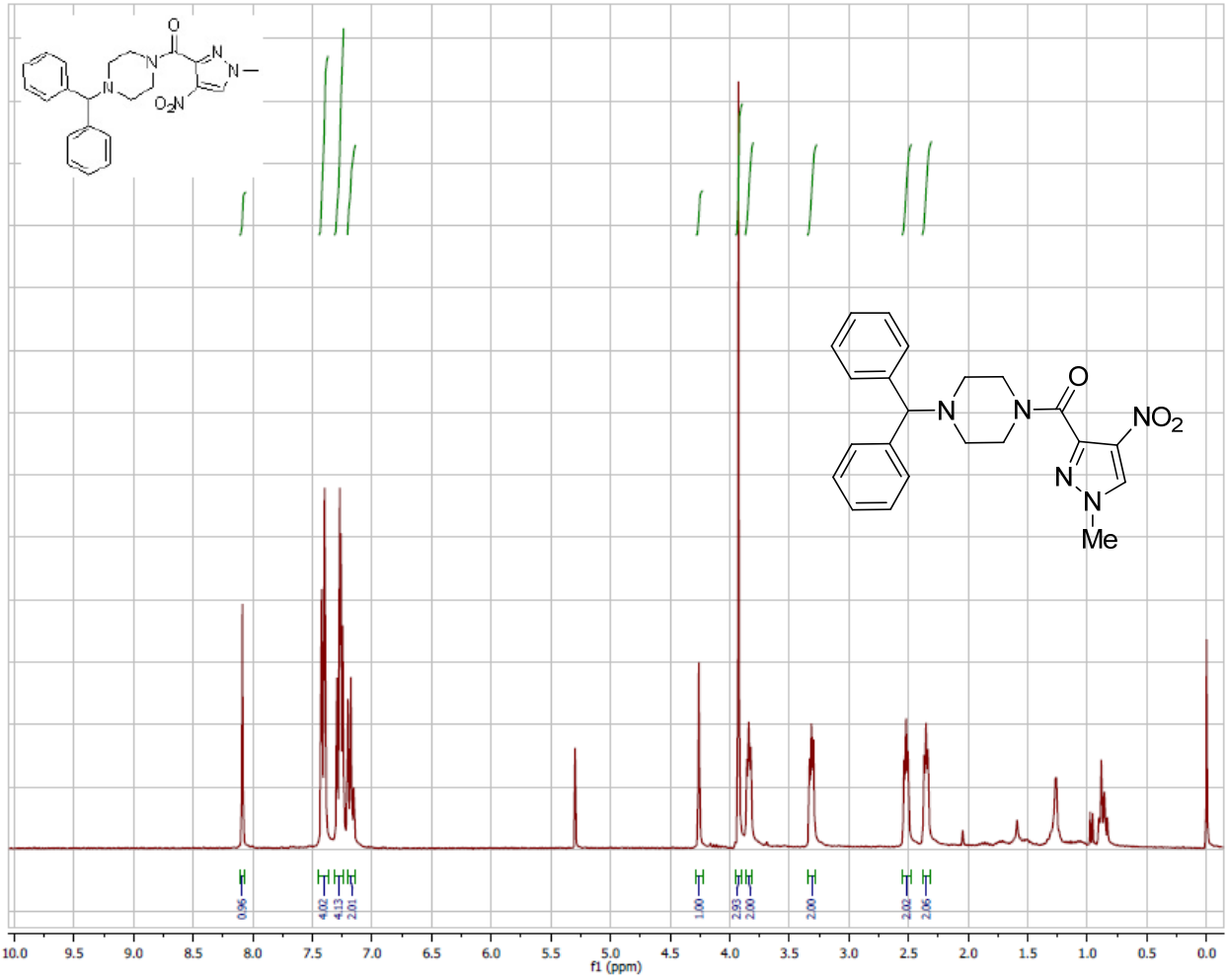
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2h**



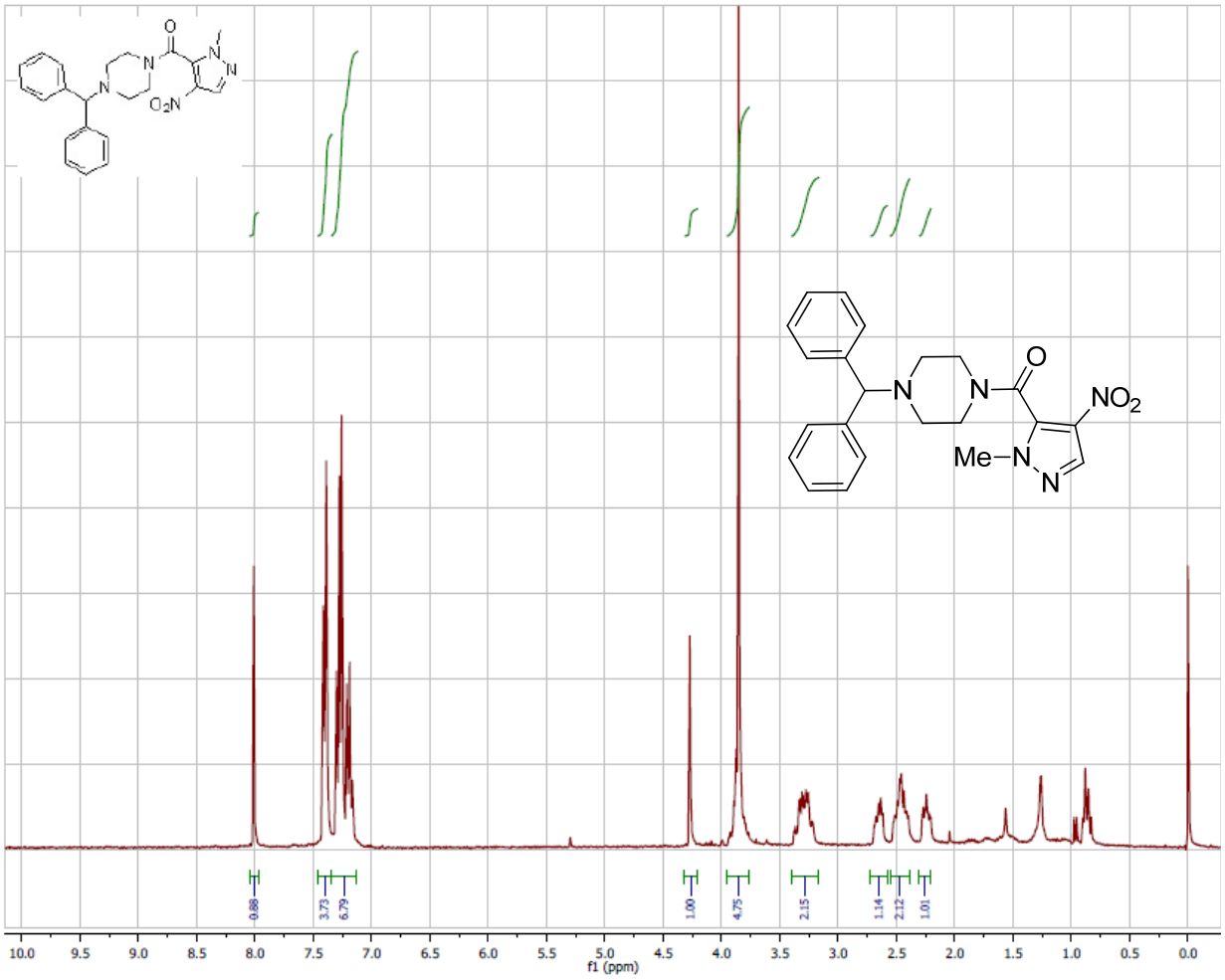
UPLC Chromatogram of **2h**



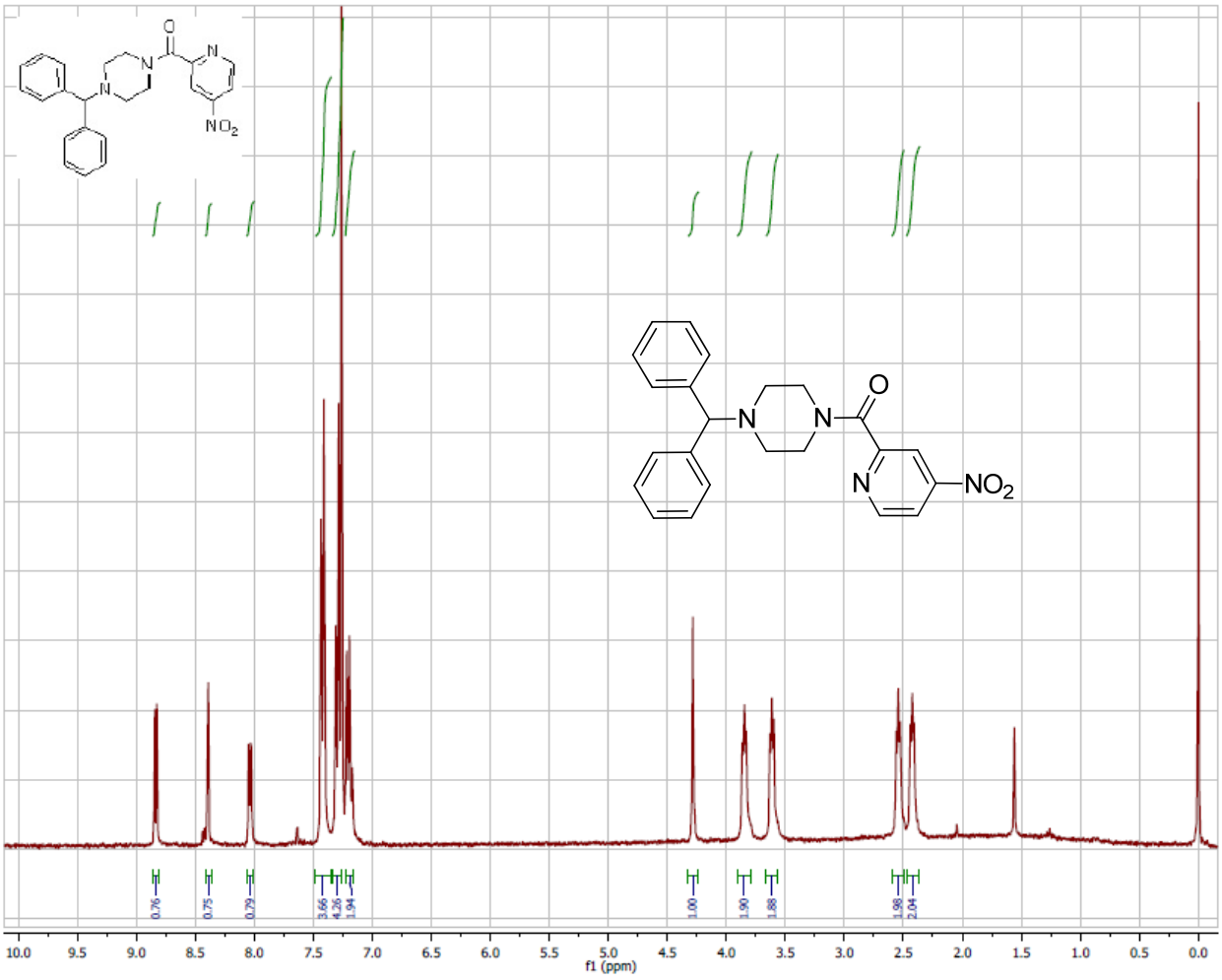
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2i**



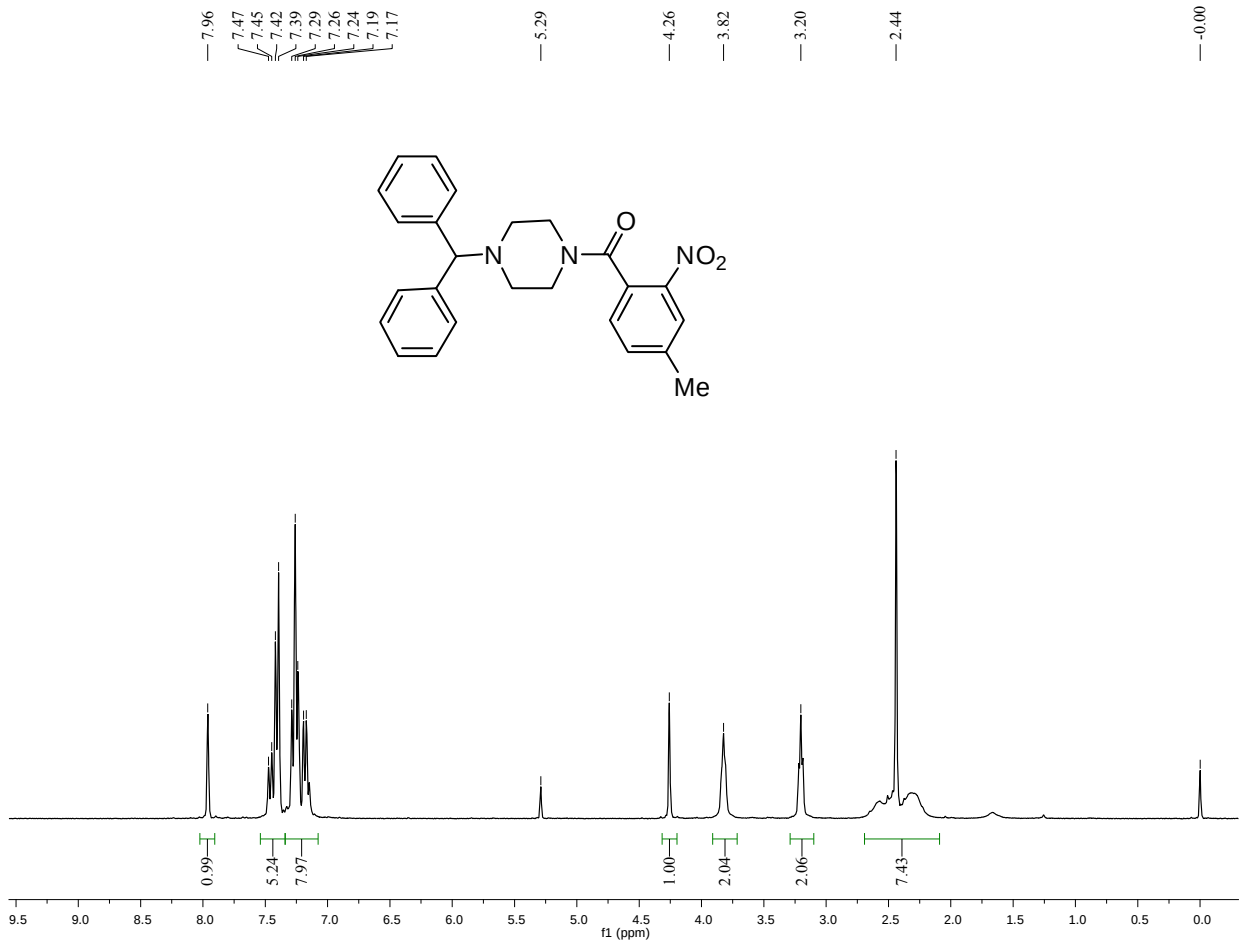
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2j**



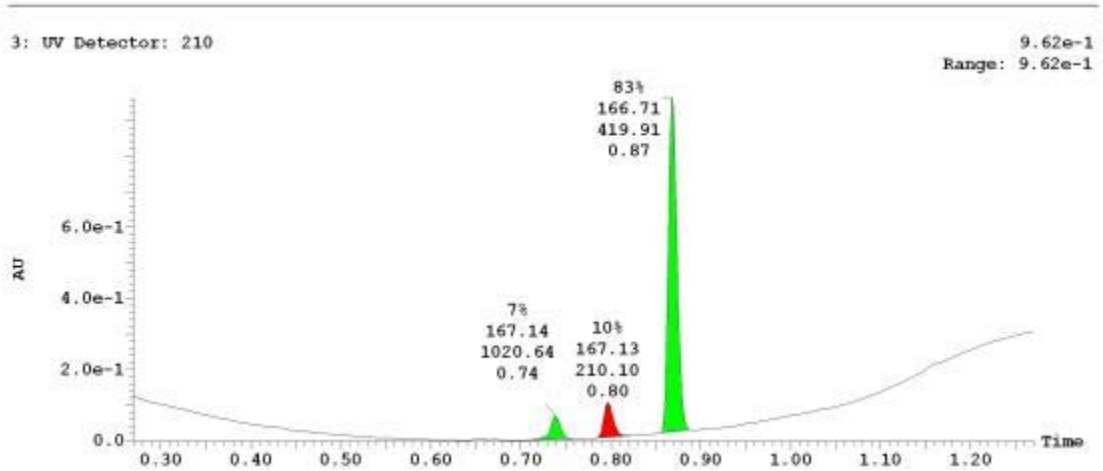
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2k**



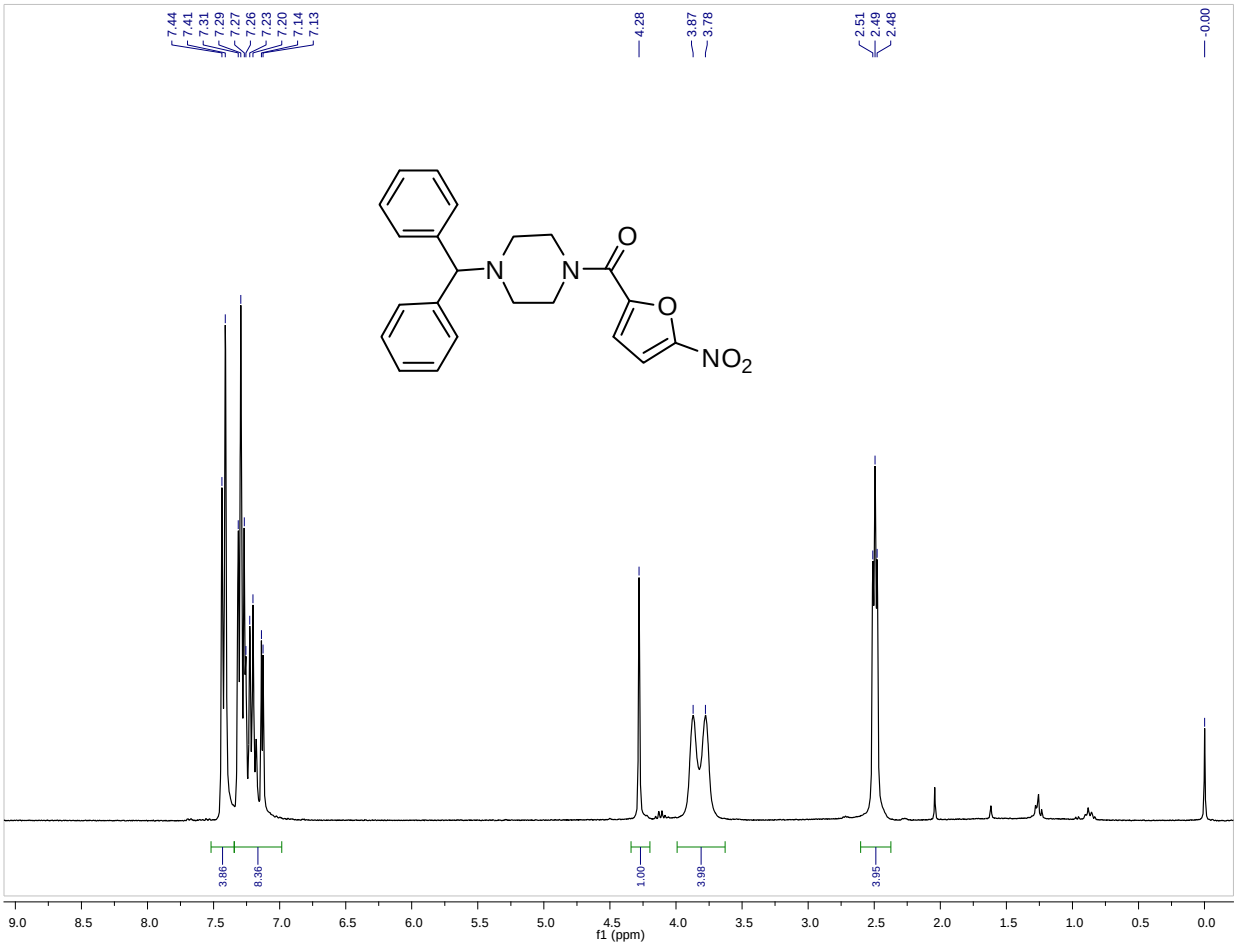
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2l**



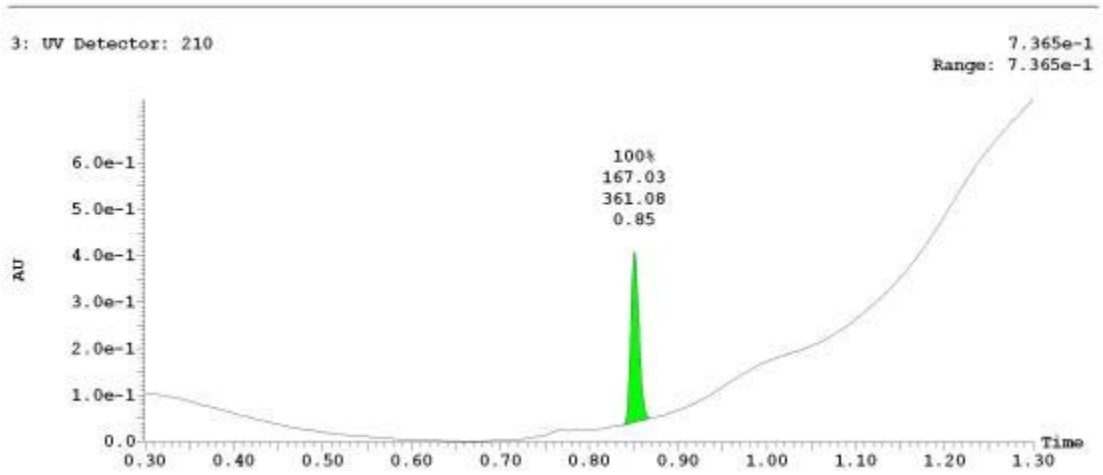
UPLC Chromatogram of **2l**



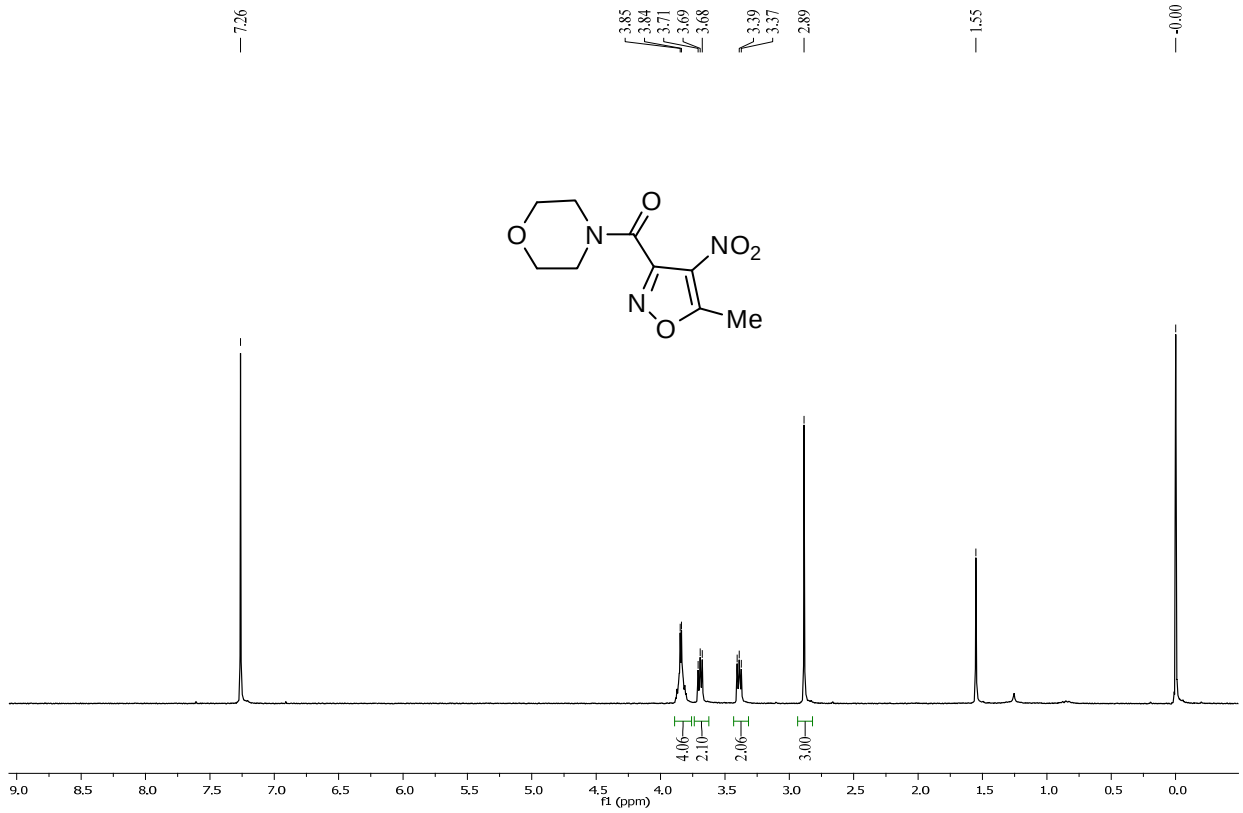
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2m**



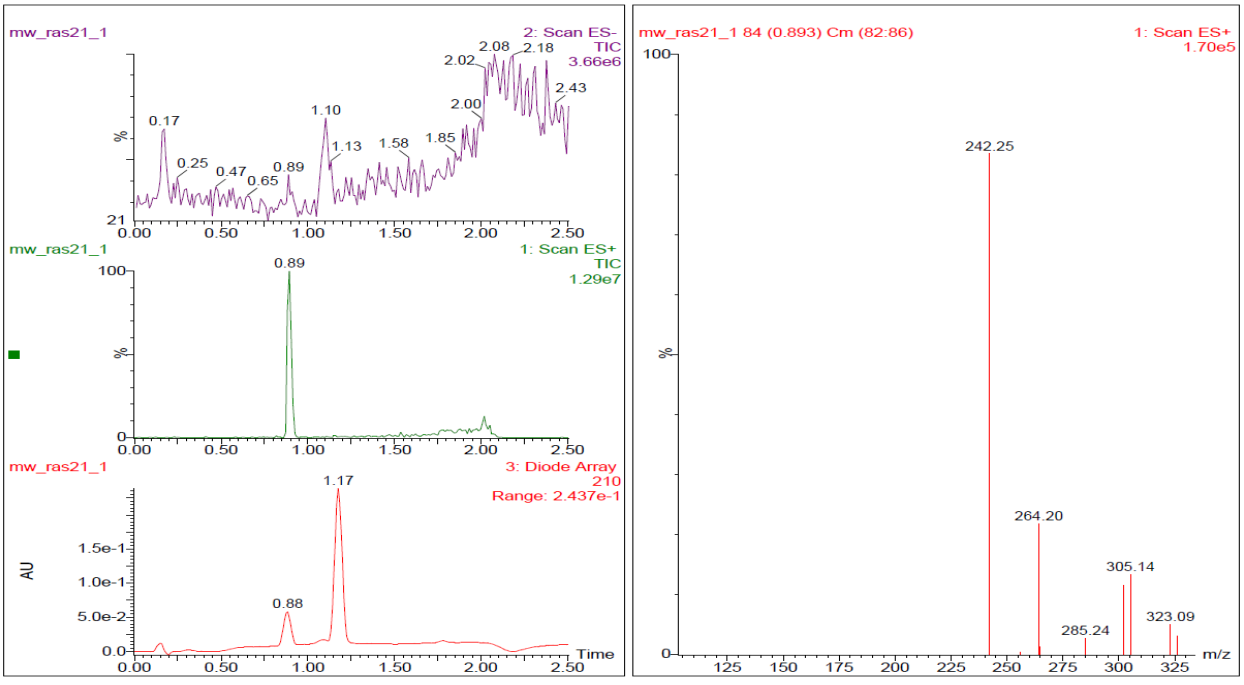
UPLC Chromatogram of **2m**



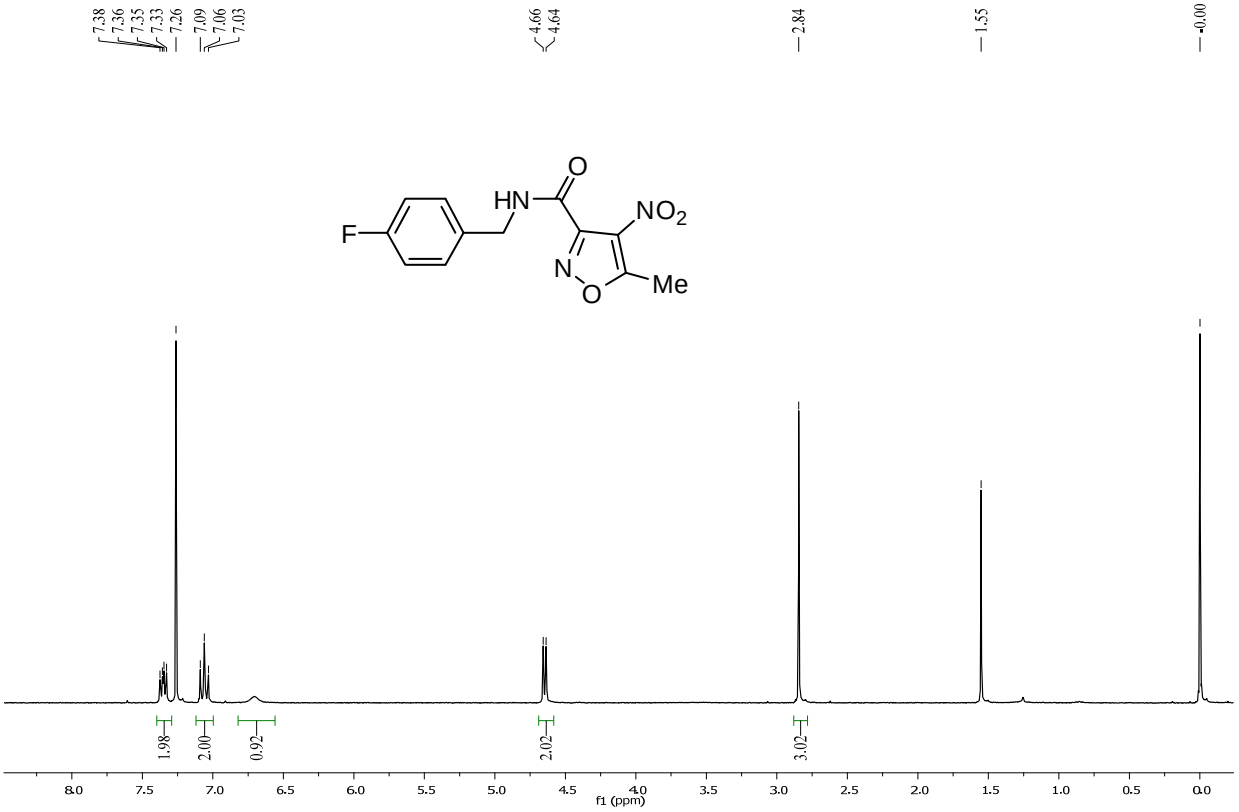
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2n**



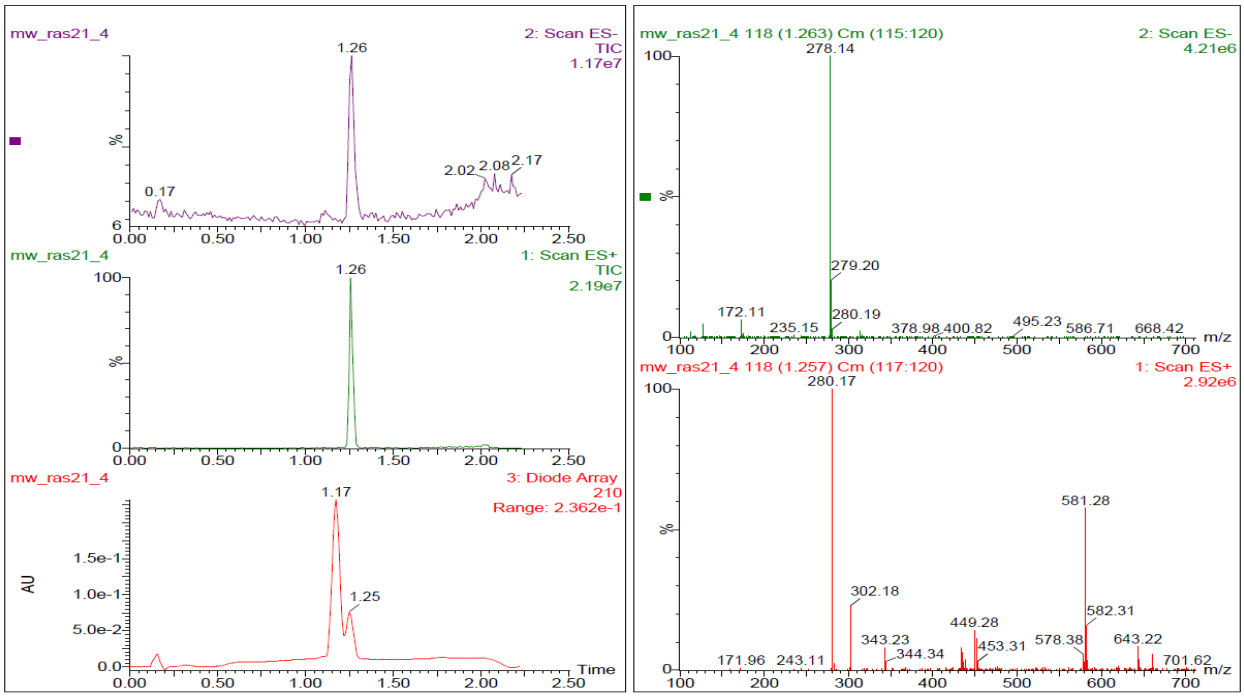
LCMS Chromatogram of **2n** (peak at 1.17 is chloroform used to dissolve the sample)



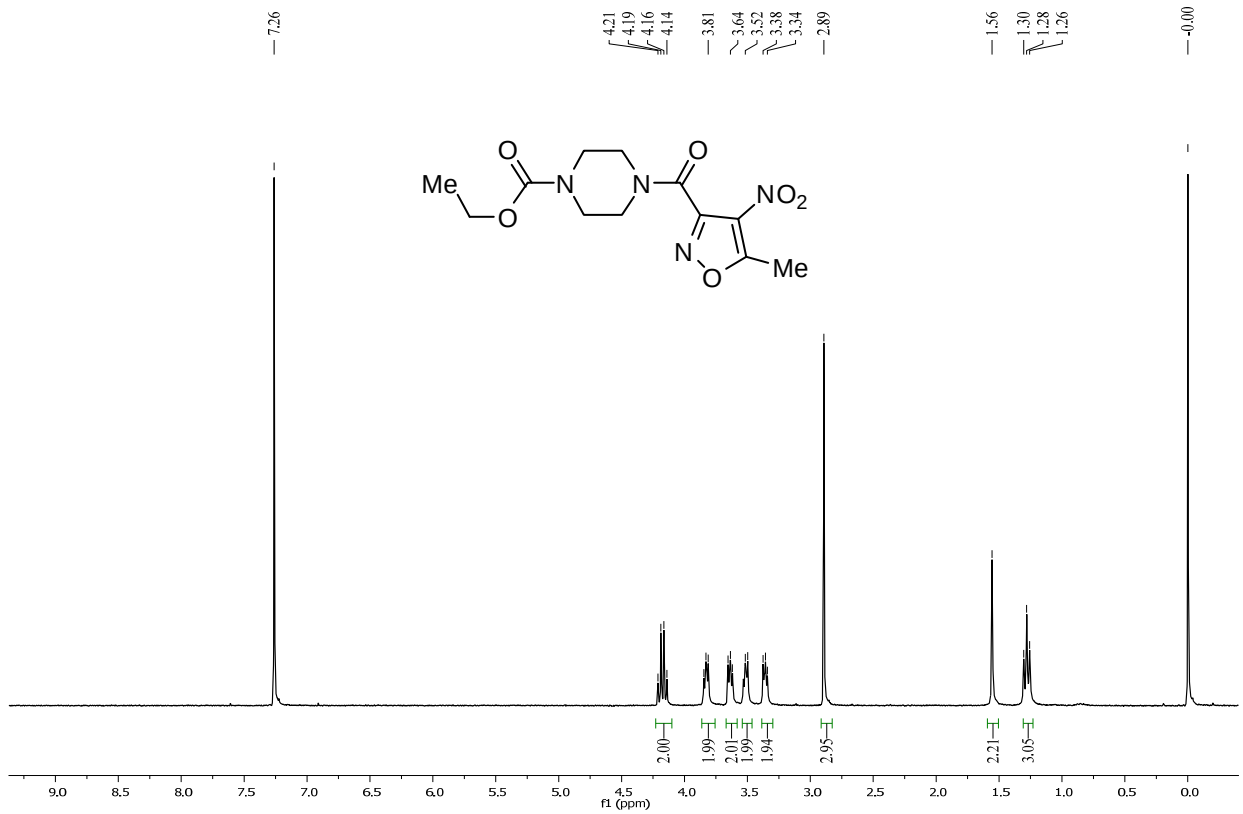
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2o**



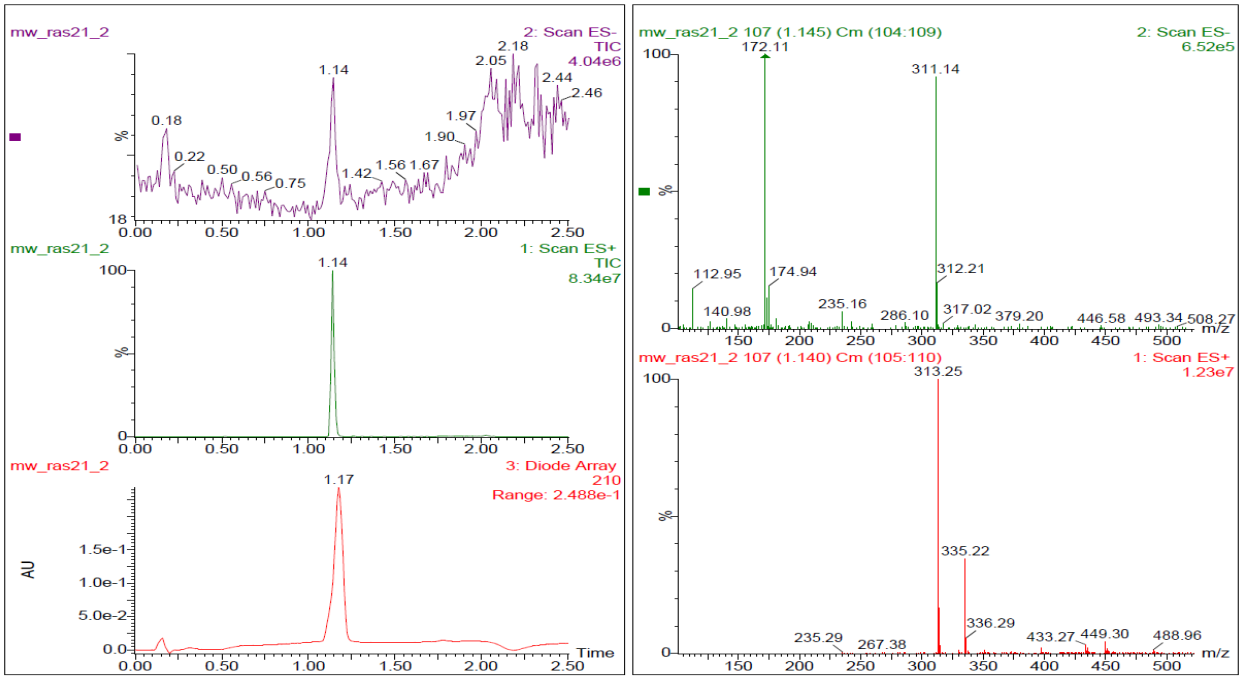
LCMS Chromatogram of **2o** (peak at 1.17 is chloroform used to dissolve the sample)



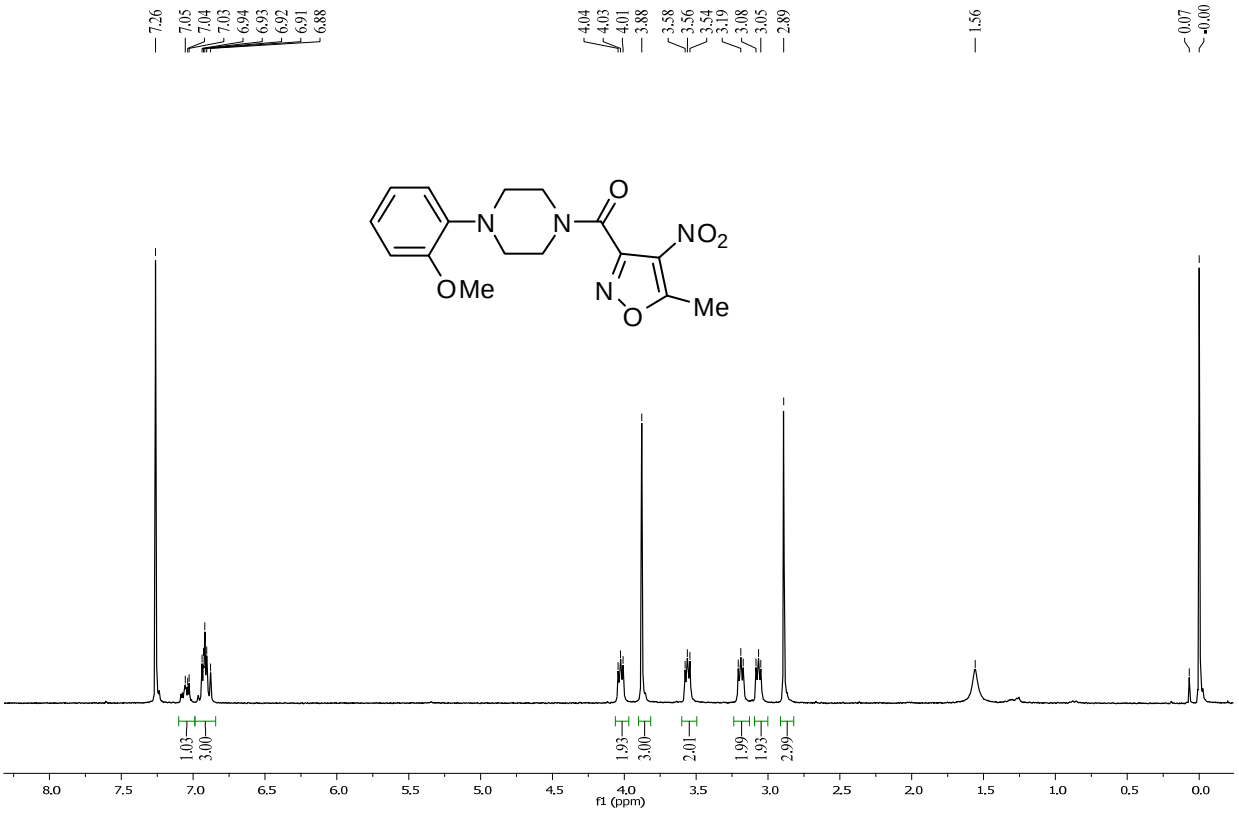
^{1}HNMR Spectrum (300 MHz, $CDCl_3$) of **2p**



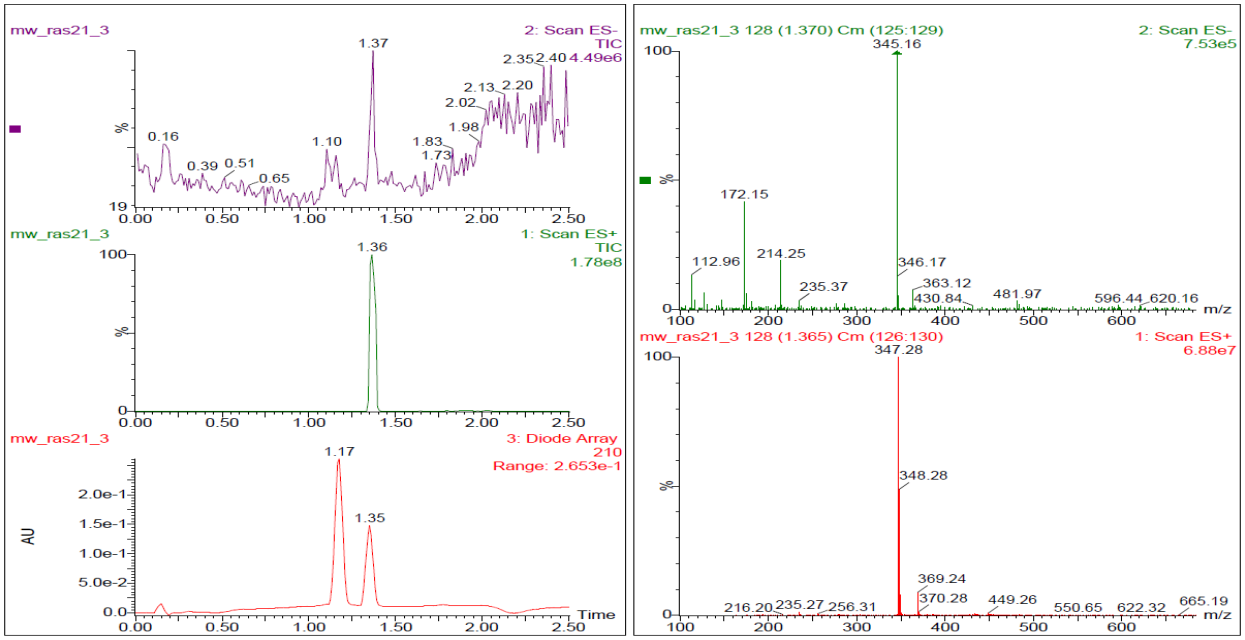
LCMS Chromatogram of **2p** (peak at 1.17 is chloroform used to dissolve the sample)



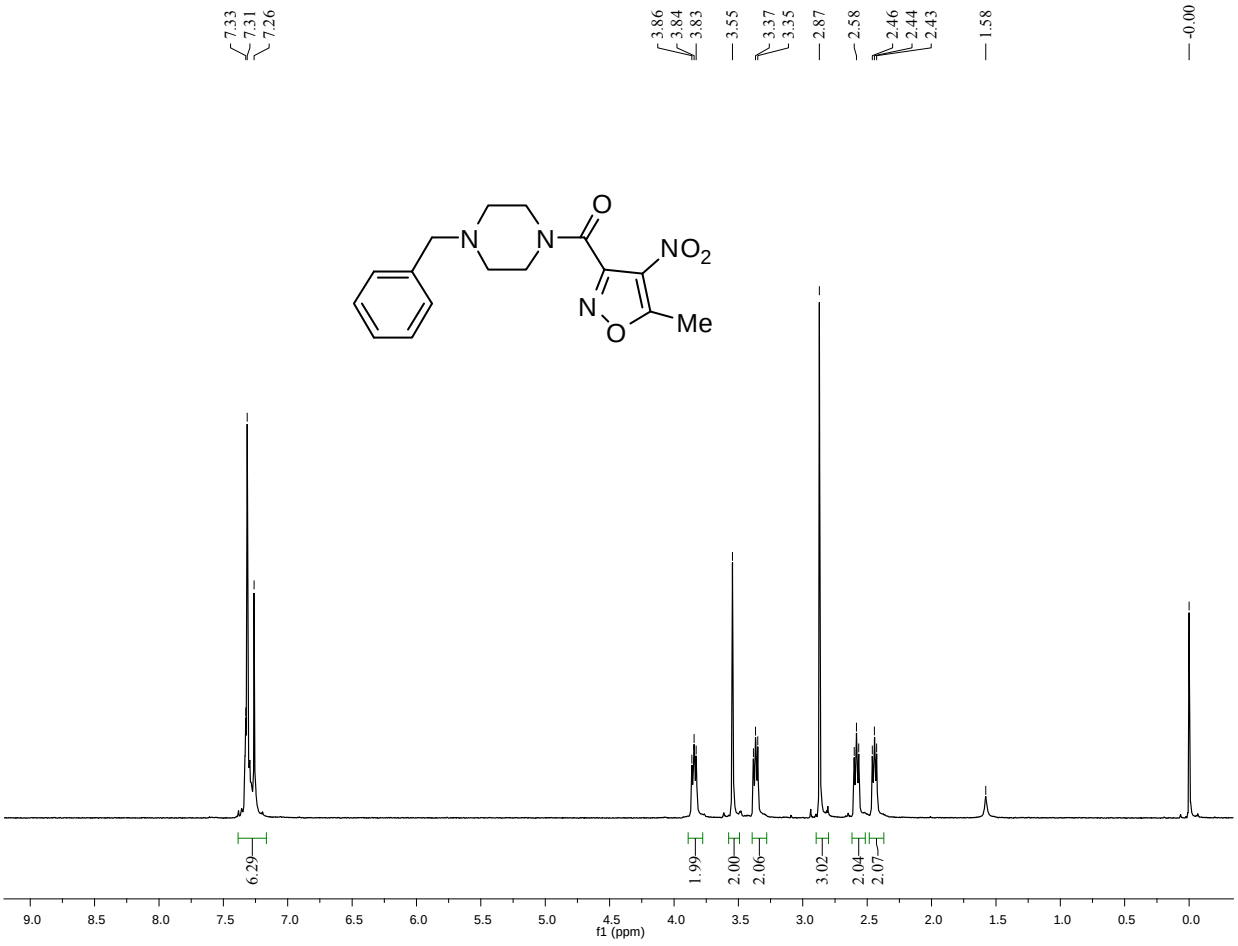
^{1}HNMR Spectrum (300 MHz, $CDCl_3$) of **2q**



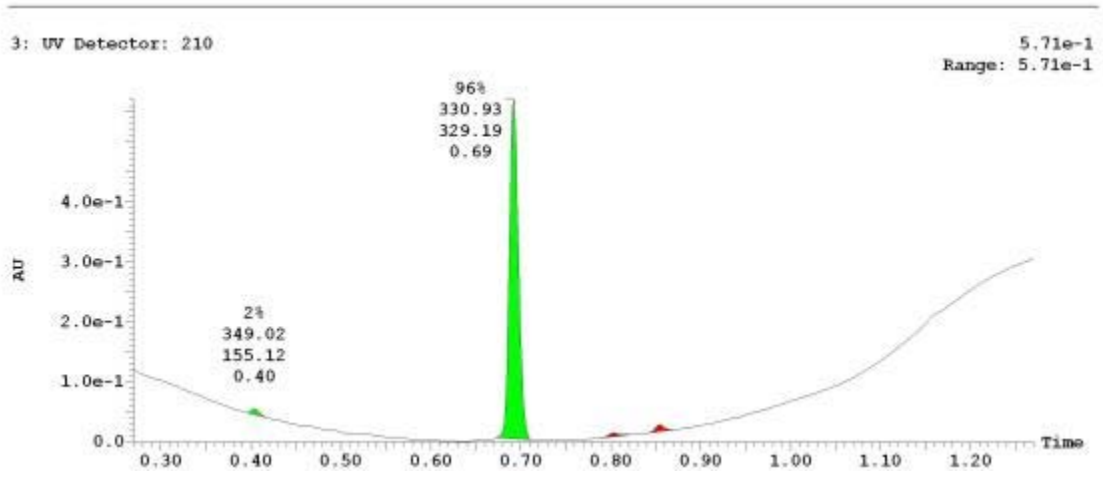
LCMS Chromatogram of **2q** (peak at 1.17 is chloroform used to dissolve the sample)



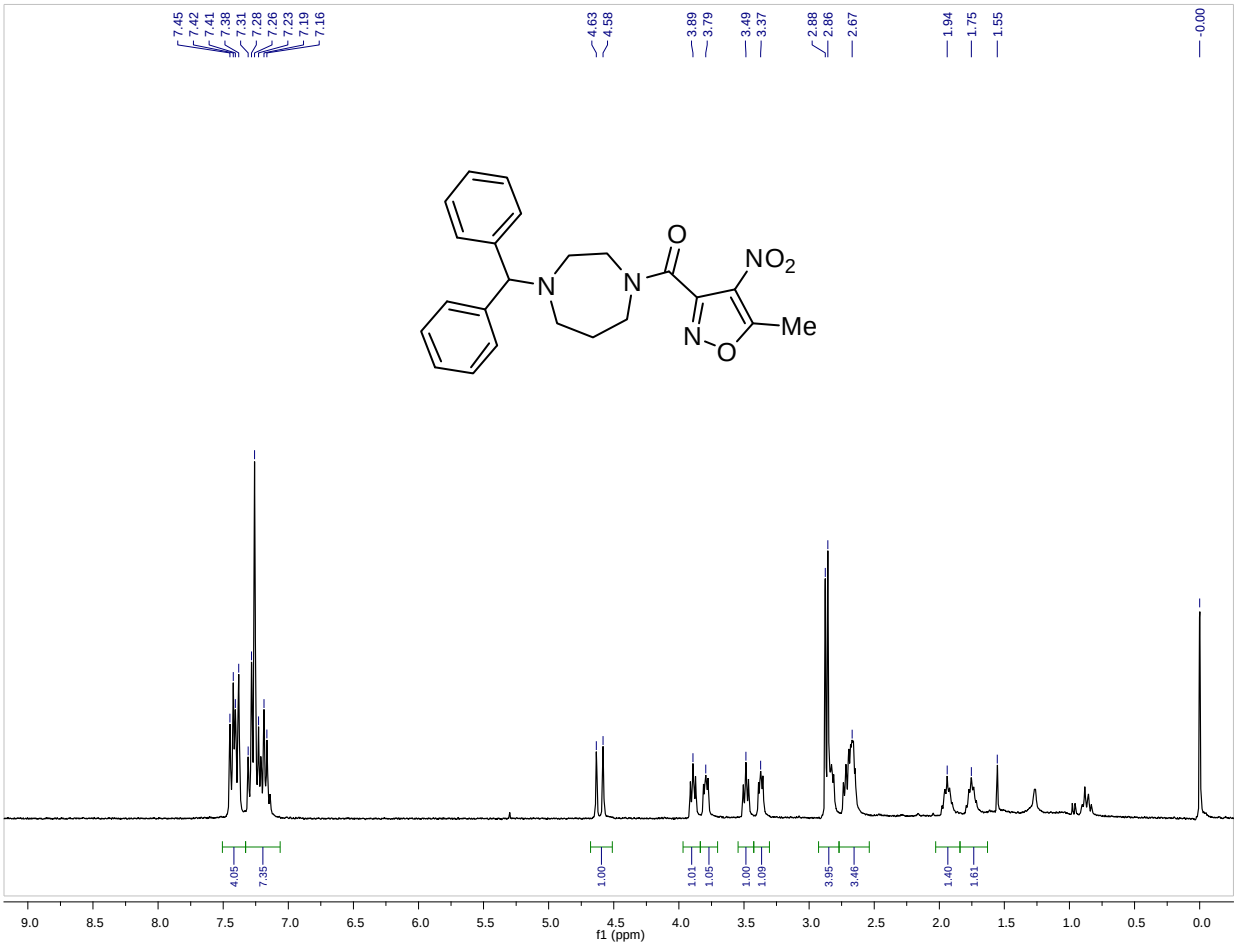
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2r**



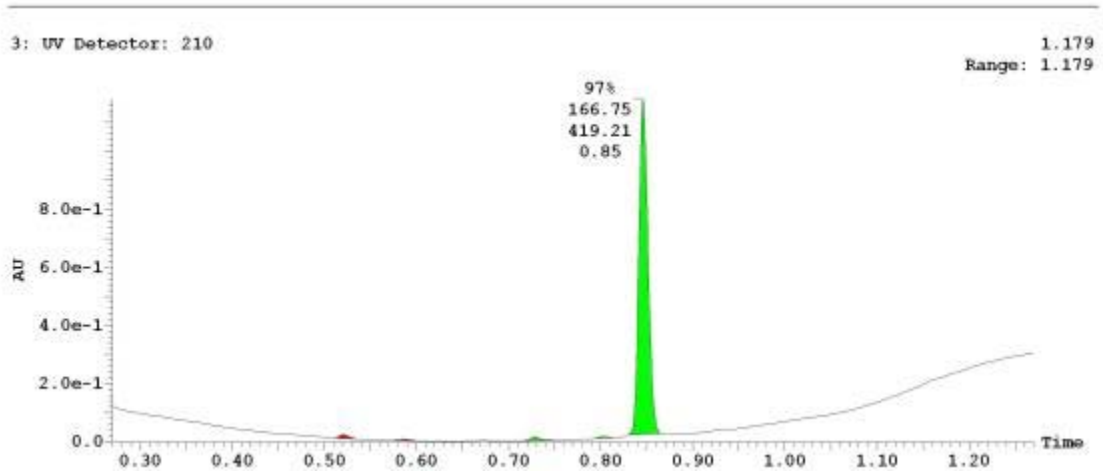
UPLC Chromatogram of **2r**



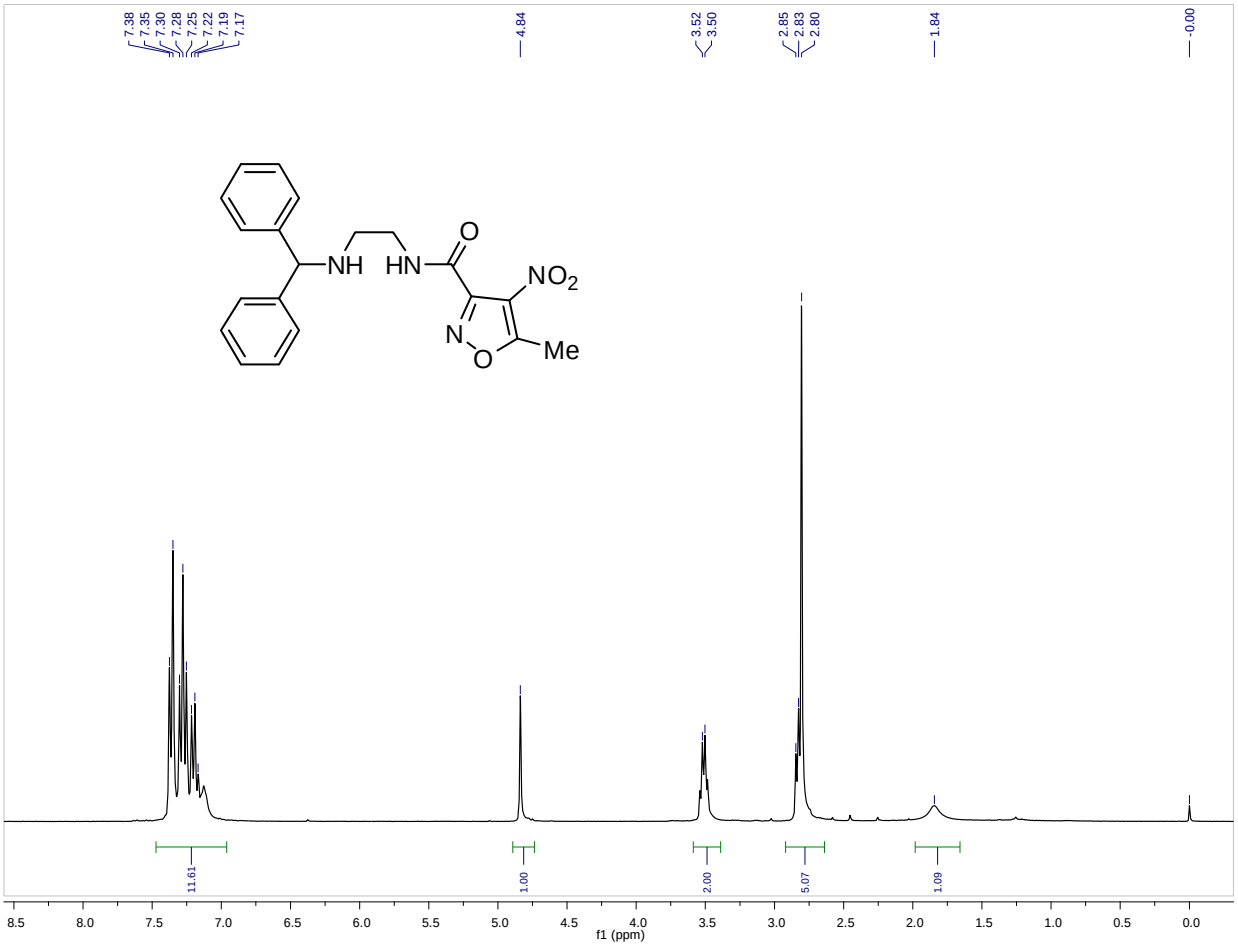
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2s**



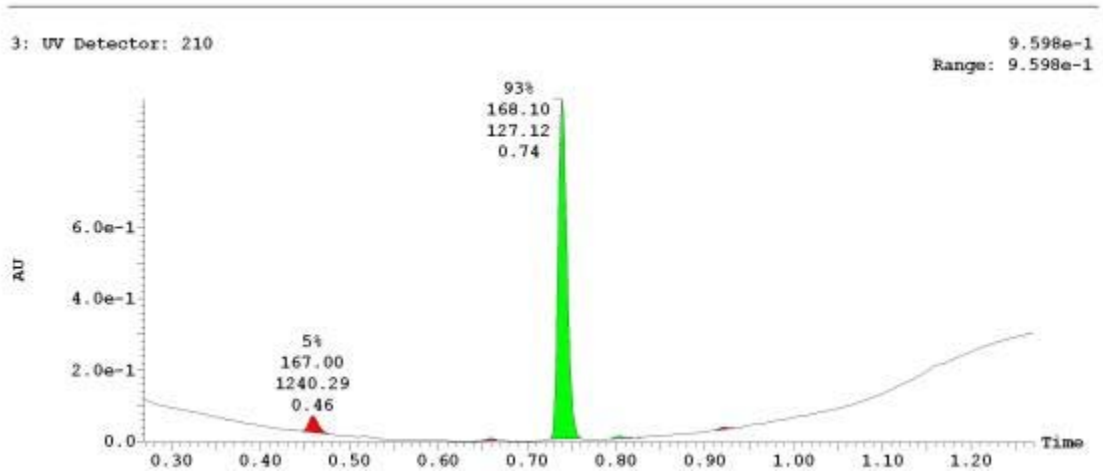
UPLC Chromatogram of **2s**



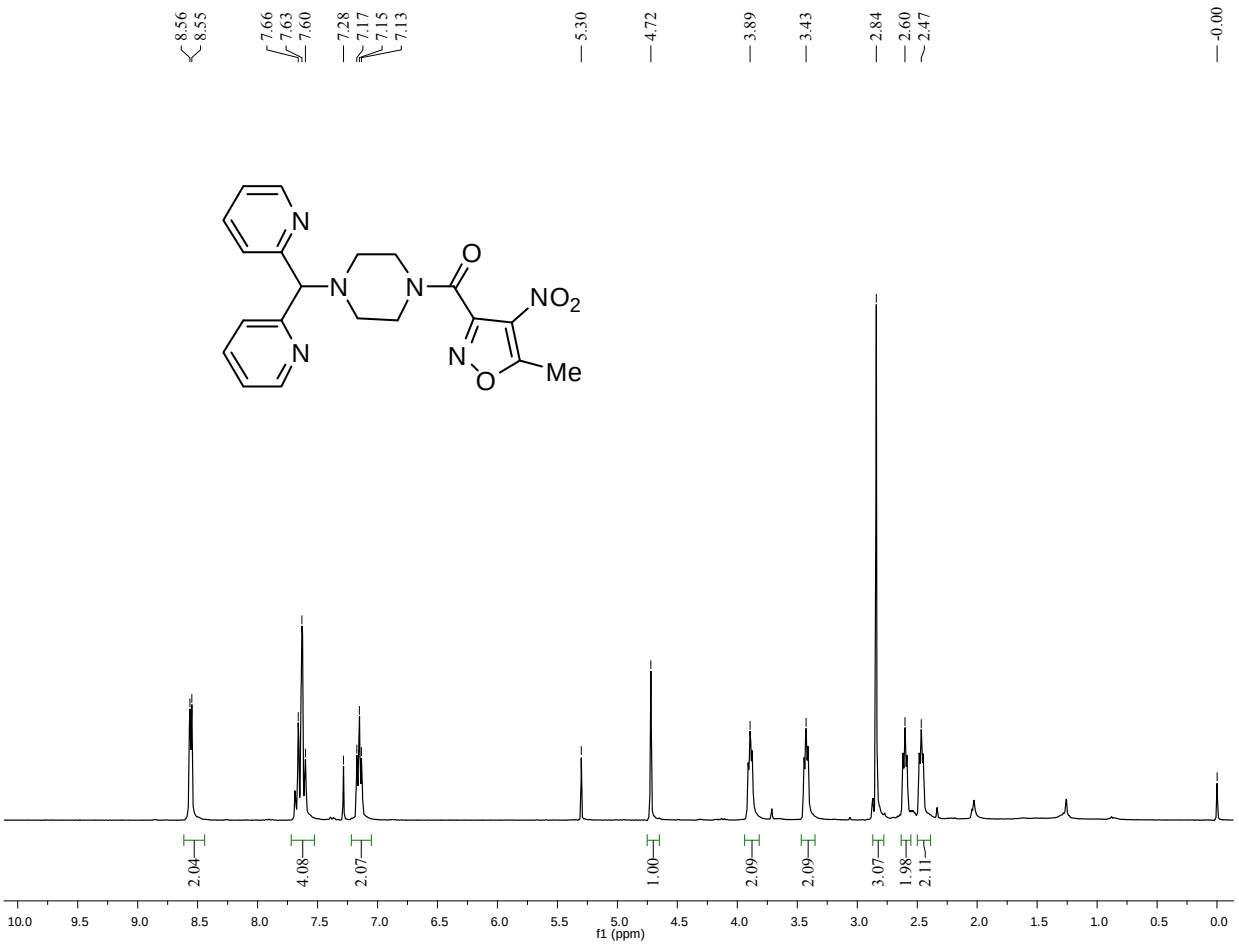
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2t**



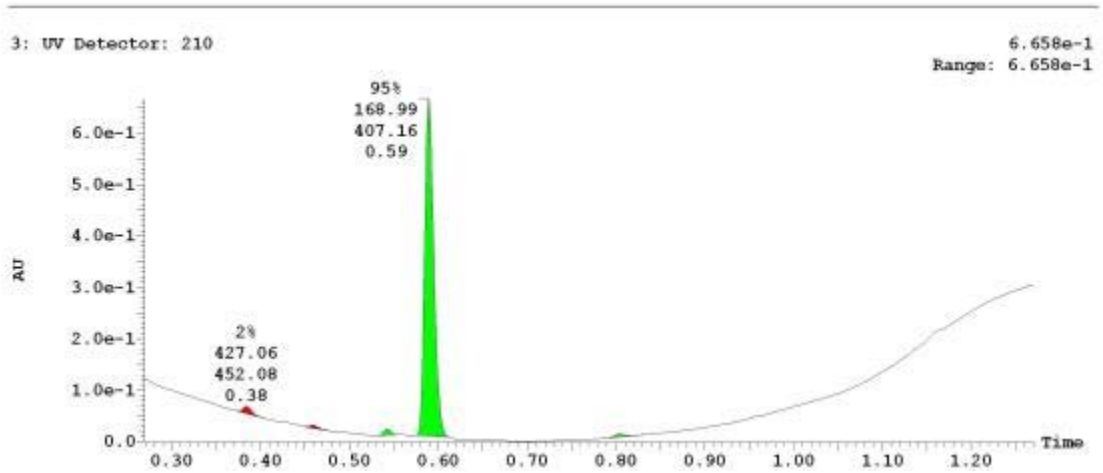
UPLC Chromatogram of **2t**



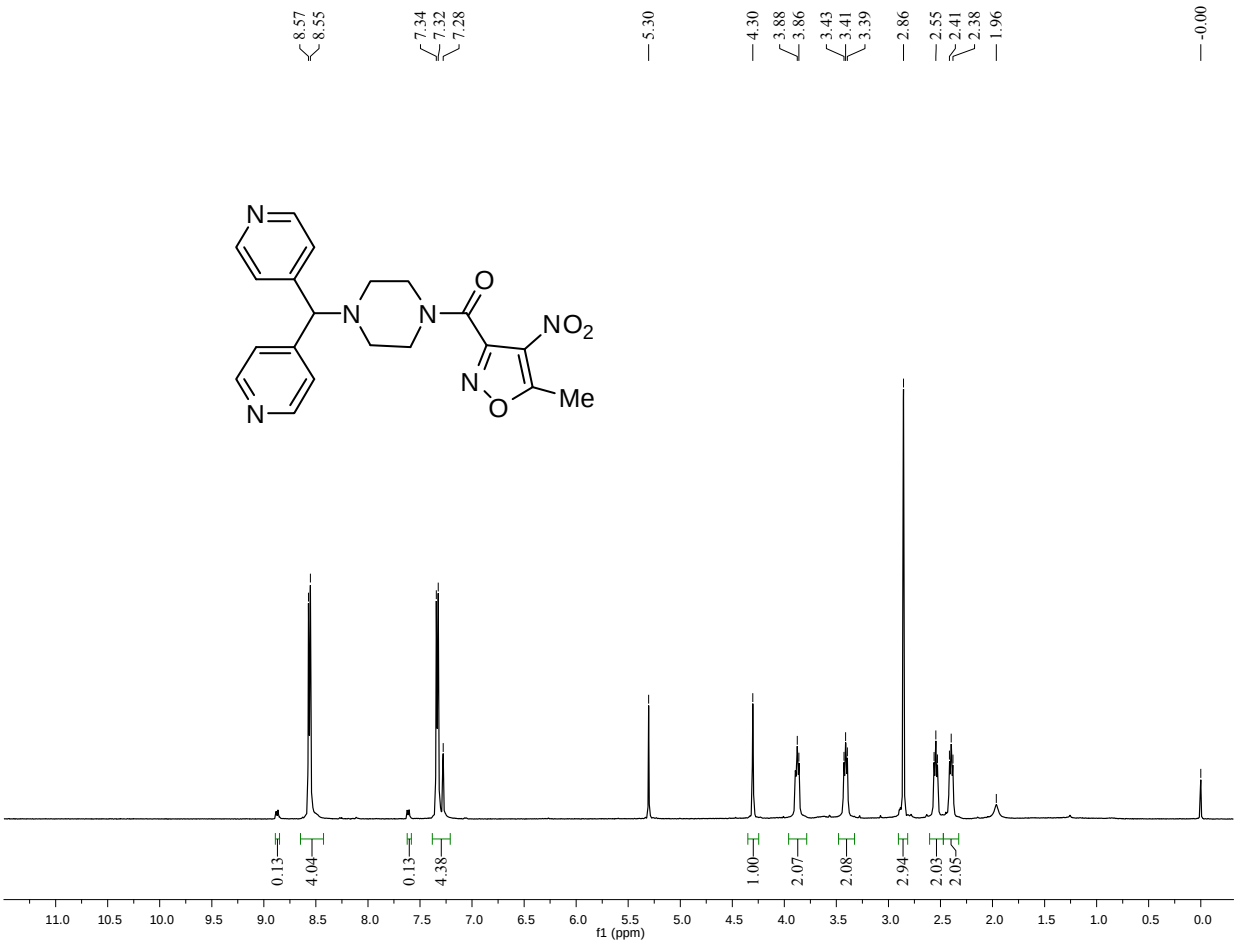
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2u**



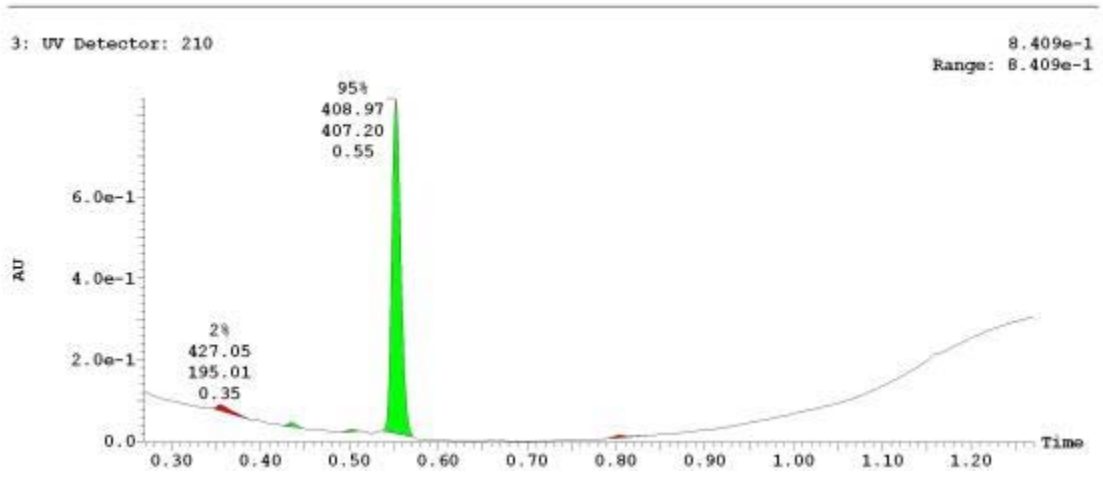
UPLC Chromatogram of **2u**



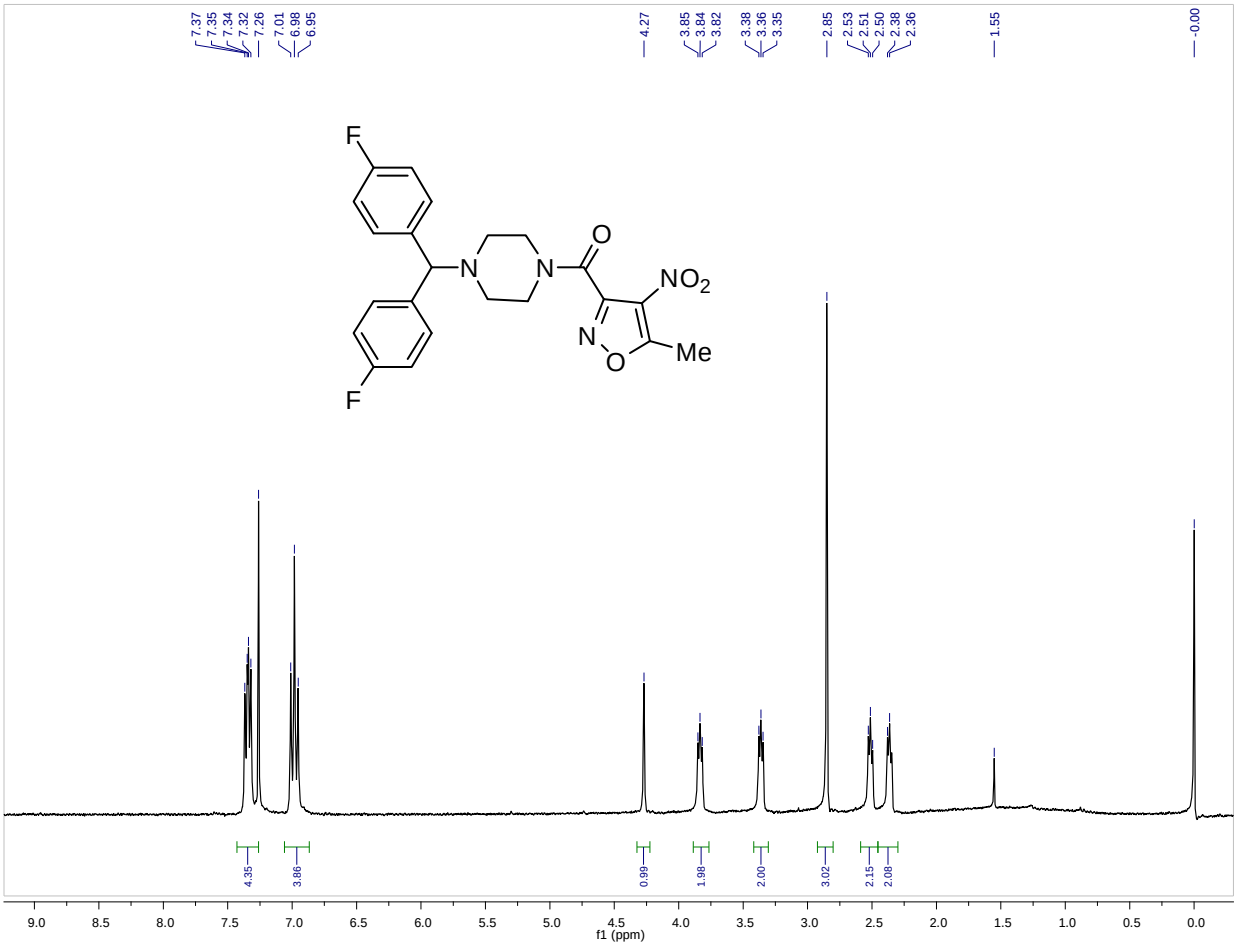
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2v**



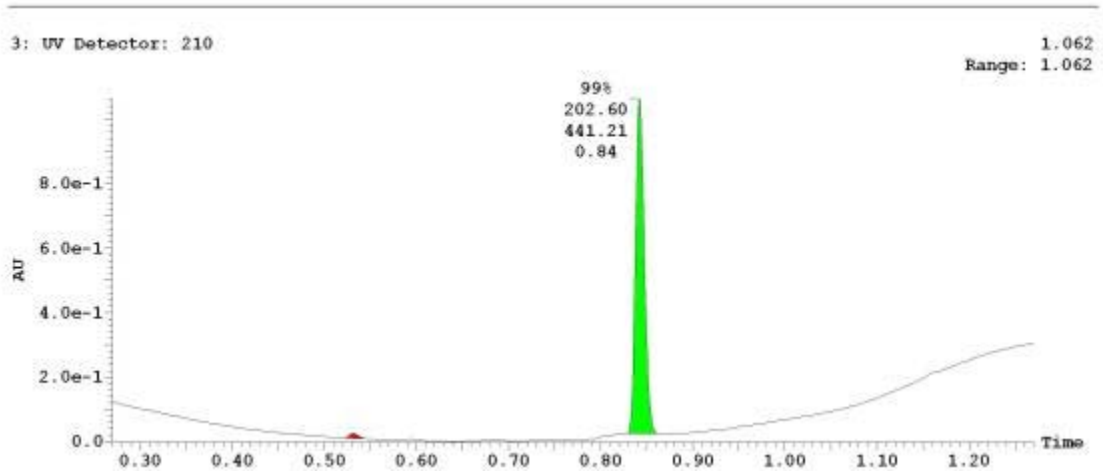
UPLC Chromatogram of **2v**



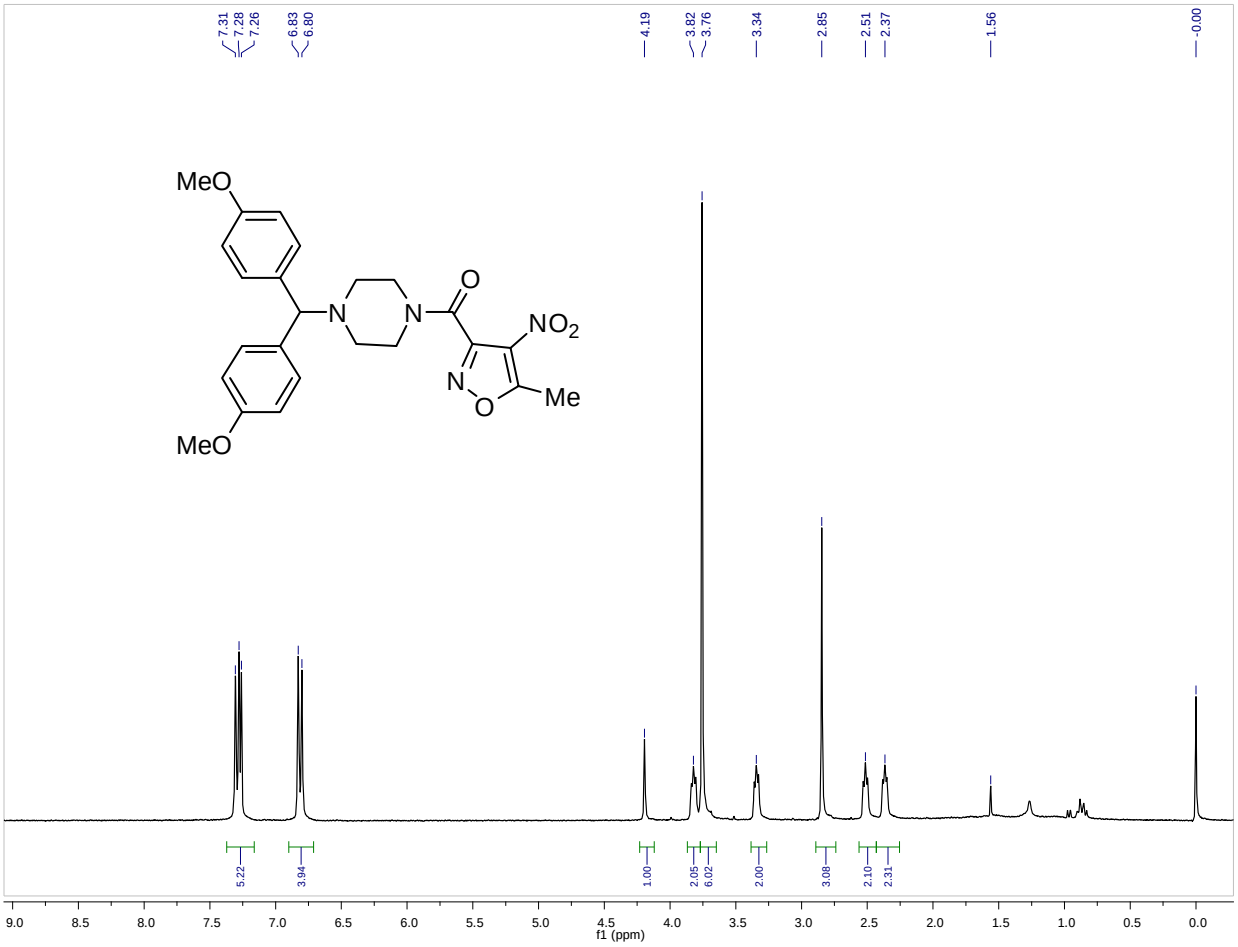
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2w**



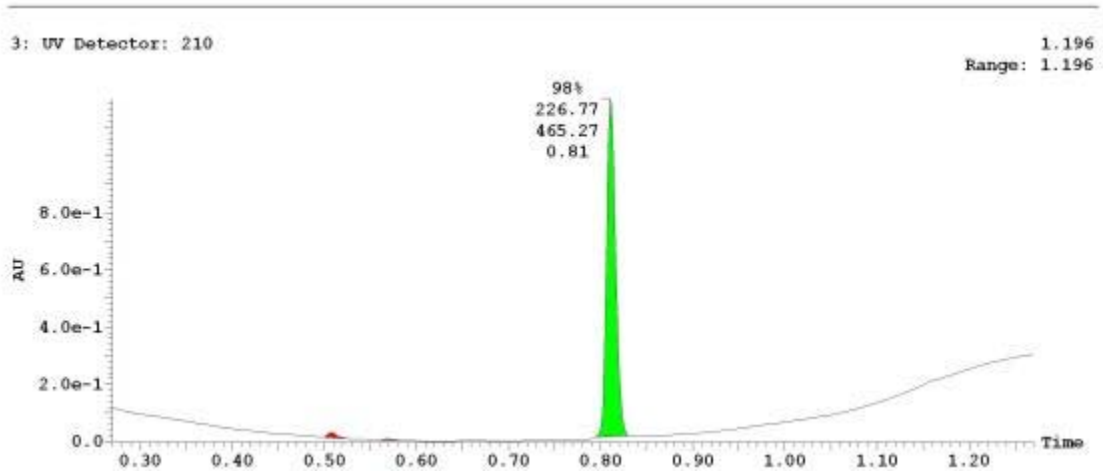
UPLC Chromatogram of **2w**



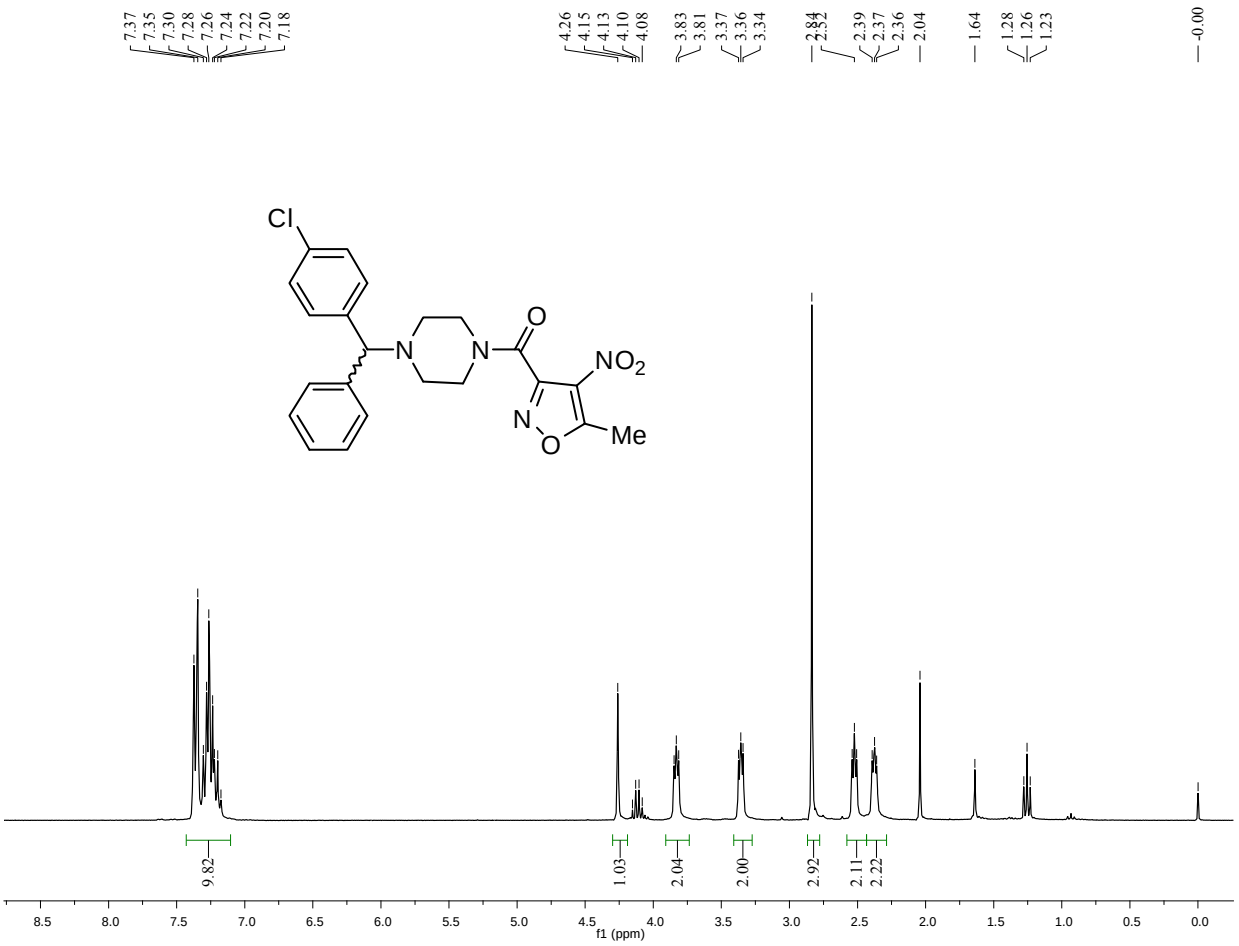
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2x**



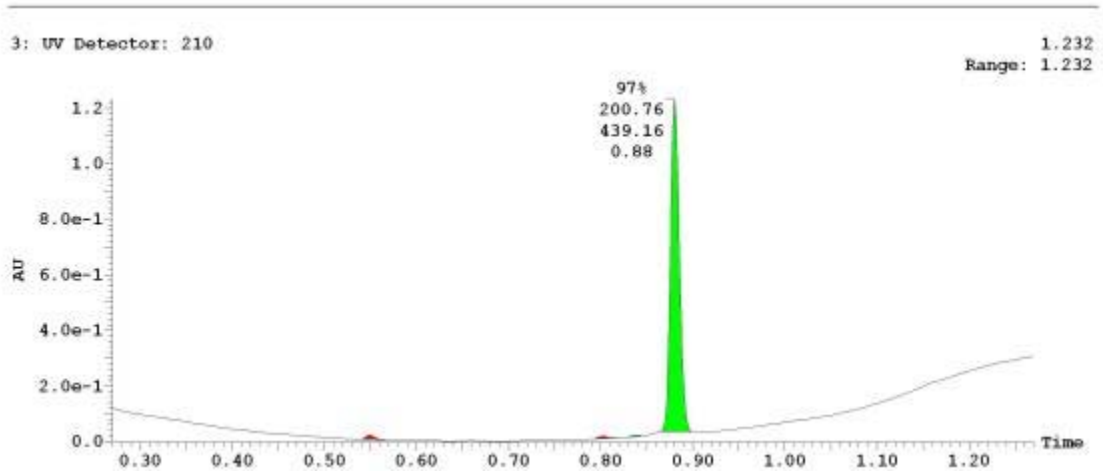
UPLC Chromatogram of **2x**



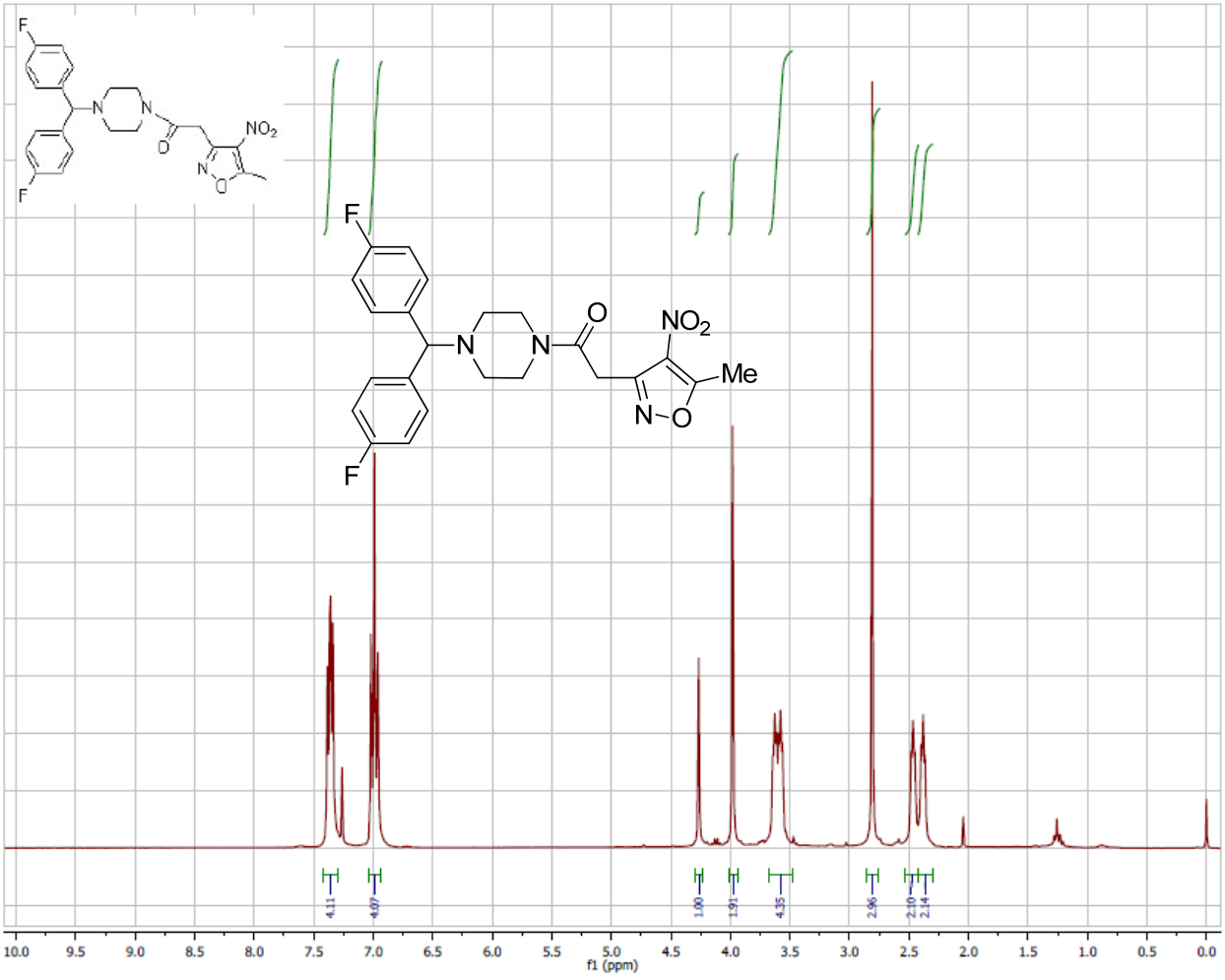
[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2z**



UPLC Chromatogram of **2z**



^{1}HNMR Spectrum (300 MHz, $CDCl_3$) of **2w'**



[1]HNMR Spectrum (300 MHz, $CDCl_3$) of **2y'**