Supporting Information

Potency and metabolic stability: a molecular hybrid case in the design of novel PF74-like small molecules targeting HIV-1 capsid protein

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Table of Content

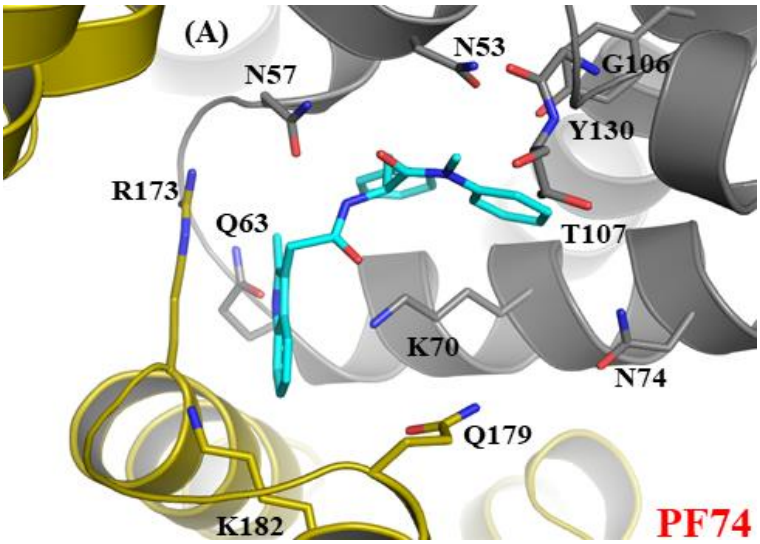
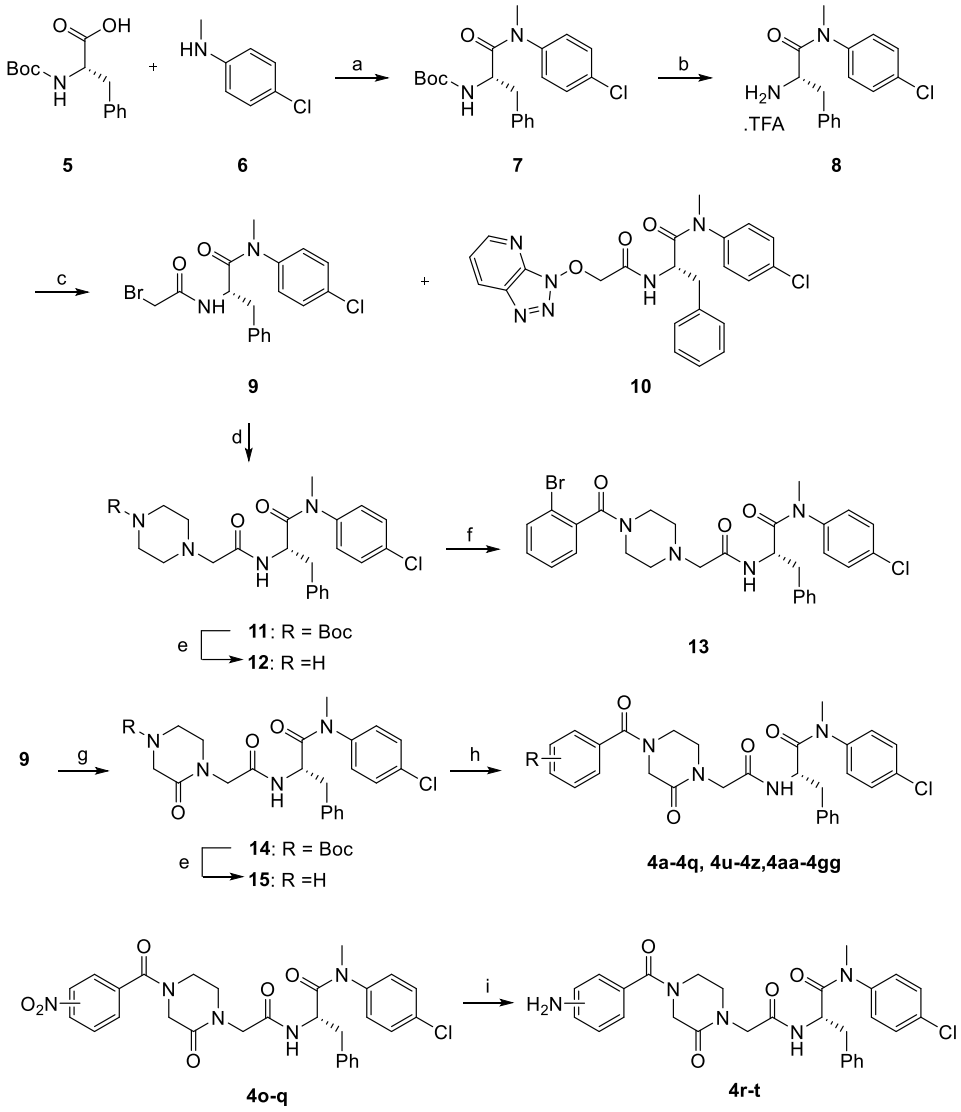


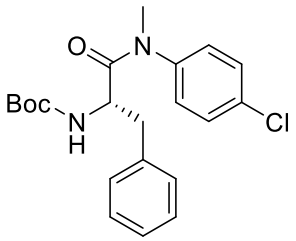
Figure S1 The control docking of PF74 to HIV-1 CA (PDB ID: 4XFZ). (A) The Glide score for the PF74 control docking was −5.8 kcal/mol. CANTD is shown in grey cartoon and adjacent CACTD in gold cartoon, with key residues around binding site shown as grey sticks, and ligands shown as cyan sticks. The nitrogen, and oxygen atoms are colored blue, and red, respectively.

Scheme S1: General synthetic scheme for the synthesis of intermediates **7-9, 11-12** and **14-15,** and final compounds **10, 13, 4a-4z,** and **4aa-4gg.**



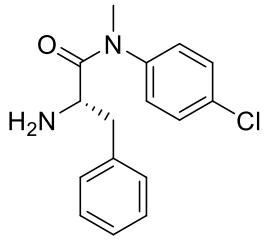
Reagents and conditions: a) HATU, DIPEA, DCM, rt, 12 h, 98%; b) TFA, DCM, 50 ºC, 12 h, 98%; c) Bromoacetic acid, HATU, DIPEA, DCM, rt, 12 h, **9** (61%), **10** (8%); d) 1-Boc-piperazine, K_2CO_3, DMF, 50 ºC, 12 h, 91%; e) TFA, DCM, 50 ºC, 12 h, 83%; f) 2-Bromobenzoic acid, HATU, DIPEA, DMF, rt, 12 h, 73%; g) 1-Boc-3-oxopiperazine, K_2CO_3, DMF, 50 ºC, 12 h, 55%; h) corresponding substituted benzoic acid, HATU, DIPEA, DMF, rt, 12 h or substituted benzoyl chloride, NEt_3, DCM, 12 h, **4a-4q**, **4u-4z**, **4aa-4gg** (50-98%); i) Fe, $CaCl_2$, EtOH/H_2O = 20:1, 70 ºC, 12 h, **4r-4t** (39-62%).

Synthesis of Intermediate 7: To a solution of N-Boc-phenyl alanine (**5**, 3 g, 12.03 mmol, 1.0 equiv.) in 50 mL of dichloromethane was added HATU (4.8 g, 12.63 mmol, 1.05 equiv.) at 0 ℃, and the reaction mixture was stirred for 30 mins. Followed by addition of 4-chloro-*N*-methylaniline (**6**, 1.6 mL, 13.23 mmol, 1.1 equiv.) and DIPEA (6.30 mL, 36.09 mmol, 3.0 equiv.). The reaction mixture was then slowly warmed to room temperature and stirred for 12 hours. Upon completion, confirmed by TLC, the reaction mixture was concentrated, diluted with water, and extracted with ethyl acetate (3 X 50mL). The combined organic layer was further washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified by Combi-flash on silica get using 5-15% EtOAc/Hexane to get amide intermediate as white solid (**7**, 4.34 g, 11.16 mmol, 98%).



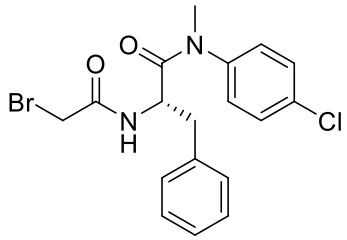
White solid, *tert-butyl (S)-(1-((4-chlorophenyl)(methyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate* **(7)**. Yield 98%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 – 7.21 (m, 5H), 6.98 – 6.93 (m, 2H), 6.63 (s, 2H), 5.17 (d, *J* = 9.2 Hz, 1H), 4.47 (q, *J* = 8.0 Hz, 1H), 3.14 (s, 3H), 2.87 (dd, *J* = 13.0, 8.6 Hz, 1H), 2.74 (dd, *J* = 13.1, 6.2 Hz, 1H), 1.39 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.9, 154.9, 141.1, 136.6, 133.9, 129.9, 129.6, 128.8, 128.6, 126.9, 79.8, 52.3, 40.2, 37.6, 28.4. HRMS (ESI) m/z calcd for $C_{21}H_{25}ClN_2O_3$ $[M - Na]^+$ 411.1446, found: 411.1446.

Synthesis of Intermediate 8: To a solution of amide intermediate (**7**, 4.34 g, 11.16 mmol, 1.0 equiv.) in 50 mL of dichloromethane was added Trifluoroacetic acid (8.5 mL, 111.6 mmol, 10.0 equiv.) at room temperature, and the reaction mixture was refluxed for at 50 ℃ for 12 hours. Upon completion, confirmed by TLC, the reaction mixture was carefully neutralized to pH 7 with a saturated sodium bicarbonate solution and the organic layer was separated. The aqueous layer was further extracted with dichloromethane (2 X 50mL). The combined organic layer was further washed with water and brine, dried over Na_2SO_4 and concentrated to get amine intermediate as yellow oil (**8**, 3.14 g, 10.90 mmol, 98%).



Yellow oil, *(S)-2-amino-N-(4-chlorophenyl)-N-methyl-3-phenylpropanamide* **(8)**. Yield 98%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.15 (m, 5H), 6.93 (dd, *J* = 7.0, 2.5 Hz, 2H), 6.63 (d, *J* = 7.6 Hz, 2H), 3.55 (s, 1H), 3.13 (s, 3H), 2.92 (dd, *J* = 12.9, 7.9 Hz, 1H), 2.67 (ddd, *J* = 13.0, 6.6, 2.7 Hz, 1H), 2.57 – 2.30 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 141.6, 137.5, 133.9, 129.9, 129.5, 128.8, 128.6, 126.9, 53.6, 42.7, 37.6. HRMS (ESI) m/z calcd for $C_{16}H_{17}ClN_2O$ $[M - H]^+$ 289.1102, found: 289.1103.

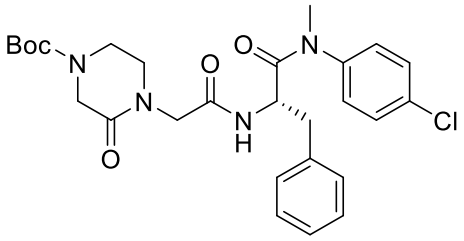
Synthesis of Intermediate 9 and final compound 10: To a solution of 2-bromoacetic acid (1.64 g, 11.99 mmol, 1.1 equiv.) in 20 mL of dichloromethane was added HATU (4.35 g, 11.45 mmol, 1.05 equiv.) at 0 °C, and the reaction mixture was stirred for 30 mins. Followed by addition of a solution of amine intermediate (**8**, 3.14 g, 10.90 mmol, 1.0 equiv.) in 10 mL dichloromethane and DIPEA (5.7 mL, 32.71 mmol, 3.0 equiv.). The reaction mixture was then slowly warmed to room temperature and stirred for 12 hours. Upon completion, confirmed by TLC, the reaction mixture was concentrated, diluted with water, and extracted with ethyl acetate (3 X 50mL). The combined organic layer was further washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified by Combi-flash on silica get using 12-20% EtOAc/hexane to get 2-bromoacetamide intermediate as white solid (**9**, 2.72 g, 6.65 mmol, 61%) and 35-40% EtOAc/hexane to get HOBT product (10, 0.41 g, 0.88 mmol, 8%).



White solid, *(S)-2-(2-bromoacetamido)-N-(4-chlorophenyl)-N-methyl-3-phenylpropanamide* **(9)**. Yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.20 (m, 5H), 7.06 (d, *J* = 6.5 Hz, 1H), 7.02 – 6.95 (m, 2H), 6.65 – 6.59 (m, 1H), 4.72 (td, *J* = 8.4, 6.2 Hz, 1H), 3.90 – 3.74 (m, 2H), 3.16 (s, 3H),

2.96 (dd, *J* = 13.0, 8.5 Hz, 1H), 2.78 (d, *J* = 6.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 164.9, 140.8, 135.9, 134.2, 130.0, 129.6, 128.8, 128.7, 127.3, 51.8, 39.5, 38.8, 37.7. HRMS (ESI) m/z calcd for $C_{18}H_{18}BrClN_2O_2$ $[M - H]^+$ 409.0313, found: 409.0317.

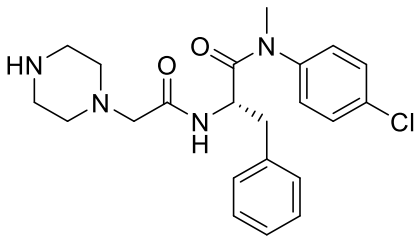
Synthesis of Intermediate 11, 14: To a solution of 2-bromoacetamide intermediate (**9**, 1 g, 2.44 mmol, 1.0 equiv.) in 10 mL of N,N-dimethyl formamide was added 1-Boc-3-oxopiperazine (0.59 g, 2.93 mmol, 1.2 equiv.), and K_2CO_3 (0.67 g, 4.88 mmol, 2.0 equiv.) at room temperature, and the reaction mixture was stirred at 50 ℃ for 12 hours. Upon completion, confirmed by TLC, the reaction mixture was concentrated, diluted with water, and extracted with ethyl acetate (3 X 20mL). The combined organic layer was further washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified by Combi-flash on silica get using 2-5% MeOH/DCM to get N-boc-3oxopiperazine acetamide intermediate as white solid (**14**, 0.72 g, 1.35 mmol, 55%).



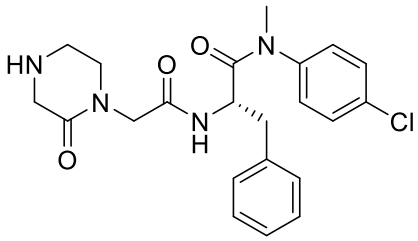
White solid, *tert-butyl (S)-4-(2-((1-((4-chlorophenyl)(methyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-3-oxopiperazine-1-carboxylate* **(14)**. Yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.20 (m, 5H), 6.93 (dd, *J* = 6.7, 2.9 Hz, 2H), 6.80 – 6.70 (m, 3H), 4.73 (q, *J* = 7.6 Hz, 1H), 4.11 (s, 2H), 4.00 (d, *J* = 2.4 Hz, 2H), 3.67 – 3.53 (m, 2H), 3.31 (tdd, *J* = 11.8, 9.0, 4.9 Hz, 2H), 3.17 (s, 3H), 2.90 (dd, *J* = 13.3, 7.7 Hz, 1H), 2.75 (dd, *J* = 13.3, 6.9 Hz, 1H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 167.3, 166.8, 153.8, 140.9, 136.0, 134.2, 130.1, 129.5, 128.8, 128.7, 127.2, 81.1, 77.4, 51.4, 50.4, 47.9, 39.1, 37.9, 28.5. HRMS (ESI) m/z calcd for $C_{27}H_{33}ClN_4O_5$ $[M - H]^+$ 529.2212, found: 529.2209.

Synthesis of Intermediate 12, 15: To a solution of N-boc-3oxopiperazine acetamide intermediate (**14**, 0.72 g, 1.35 mmol, 1.0 equiv.) in 10 mL of dichloromethane was added Trifluoroacetic acid (1.03 mL, 13.51 mmol, 10.0 equiv.) at room temperature, and the reaction mixture was refluxed for at 50 ℃ for 12 hours. Upon completion, confirmed by TLC, the reaction mixture was carefully neutralized to pH 7 with a saturated sodium bicarbonate solution and the organic layer was

separated. The aqueous layer was further extracted with dichloromethane (2 X 10mL). The combined organic layer was further washed with water and brine, dried over Na_2SO_4 and concentrated to get 3-oxopiperzine acetamide intermediate as white solid (**15**, 0.44 g, 1.03 mmol, 76%).

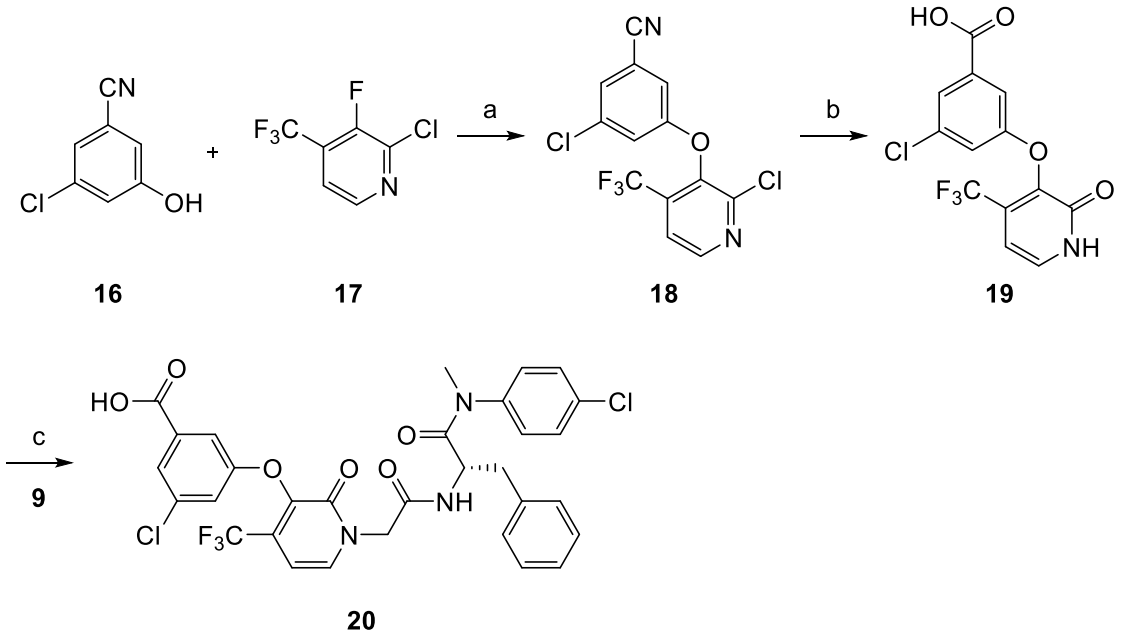


White solid, *(S)-N-(4-chlorophenyl)-N-methyl-3-phenyl-2-(2-(piperazin-1-yl)acetamido)propanamide* **(12)**. Yield 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 7.5 Hz, 2H), 7.22 (q, *J* = 2.9 Hz, 3H), 7.15 – 7.08 (m, 1H), 6.93 (dd, *J* = 6.6, 2.9 Hz, 2H), 6.78 (s, 2H), 4.70 (q, *J* = 7.6 Hz, 1H), 4.02 (s, 2H), 3.77 – 3.55 (m, 3H), 3.40 (ddt, *J* = 18.3, 12.6, 6.4 Hz, 2H), 3.23 (d, *J* = 17.9 Hz, 2H), 3.15 (s, 3H), 2.91 (dd, *J* = 13.3, 7.6 Hz, 1H), 2.84 – 2.70 (m, 1H). HRMS (ESI) m/z calcd for $C_{22}H_{27}ClN_4O_2$ $[M + H]^+$ 415.1817, found: 415.1815.



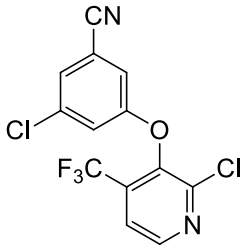
White solid, *(S)-N-(4-chlorophenyl)-N-methyl-2-(2-(2-oxopiperazin-1-yl)acetamido)-3-phenylpropanamide* **(15)**. Yield 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 7.5 Hz, 2H), 7.22 (q, *J* = 2.9 Hz, 3H), 7.15 – 7.08 (m, 1H), 6.93 (dd, *J* = 6.6, 2.9 Hz, 2H), 6.78 (s, 2H), 4.70 (q, *J* = 7.6 Hz, 1H), 4.02 (s, 2H), 3.77 – 3.55 (m, 3H), 3.40 (ddt, *J* = 18.3, 12.6, 6.4 Hz, 2H), 3.23 (d, *J* = 17.9 Hz, 2H), 3.15 (s, 3H), 2.91 (dd, *J* = 13.3, 7.6 Hz, 1H), 2.84 – 2.70 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 167.7, 167.4, 141.0, 136.3, 134.1, 129.9, 129.5, 128.9, 128.7, 127.1, 51.5, 50.5, 48.9, 48.2, 42.5, 38.9, 37.9. HRMS (ESI) m/z calcd for $C_{22}H_{25}ClN_4O_3$ $[M - H]^+$ 429.1688, found: 429.1691.

Scheme S2: General synthetic scheme for the synthesis of intermediates **18** and **19,** and final compounds **20.**



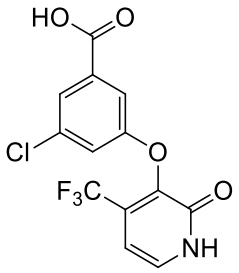
Reagents and conditions: a) K_2CO_3, NMP, 80 ºC, 12 h, 67%; b) KOH, t-BuOH, 100 ºC, 12 h, dil. HCl, 76%; c) K_2CO_3, DMF, 50 ºC, 12 h, 41%.

Synthesis of Intermediate 18: To a solution of 2-chloro-3-fluoro-4-(trifluoromethyl)pyridine (**17**, 0.2 g, 1.00 mmol, 1.0 equiv.) in 10 mL of N-methyl pyrrolidinone was added 3-chloro-5-hydroxybenzonitrile (**16**, 0.19 g, 1.20 mmol, 1.2 equiv.), and K_2CO_3 (0.17 g, 1.20 mmol, 1.2 equiv.) and the reaction mixture was stirred at 80 ℃ for 12 hours. Upon completion, confirmed by TLC, the reaction mixture was cooled to room temperature, 20 mL of ice water was added to it slowly, and the solid was filtered. The solid product was further washed with 5 mL of DMF/water (1:1) to get N-boc-3oxopiperazine acetamide intermediate as white solid (**18**, 0.29 g, 0.87 mmol, 67%).



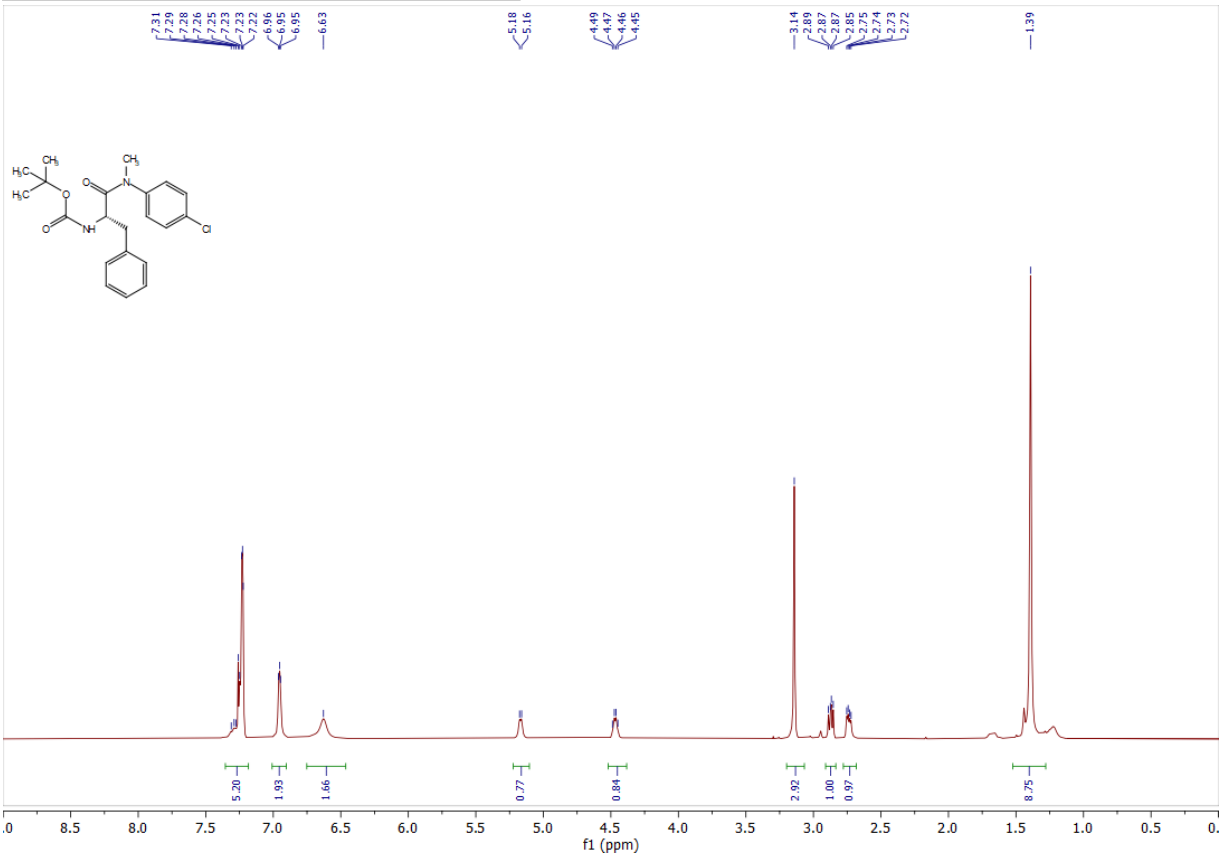
White solid, *3-chloro-5-((2-chloro-4-(trifluoromethyl)pyridin-3-yl)oxy)benzonitrile* **(18)**: Yield 67%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.57 (d, *J* = 5.0 Hz, 1H), 7.65 (d, *J* = 4.9 Hz, 1H), 7.41 (s, 1H), 7.09 (s, 1H), 6.94 (s, 1H). HRMS (ESI) m/z calcd for $C_{13}H_5Cl2F_3N2O$ $[M + H]^-$ 332.9804, found.

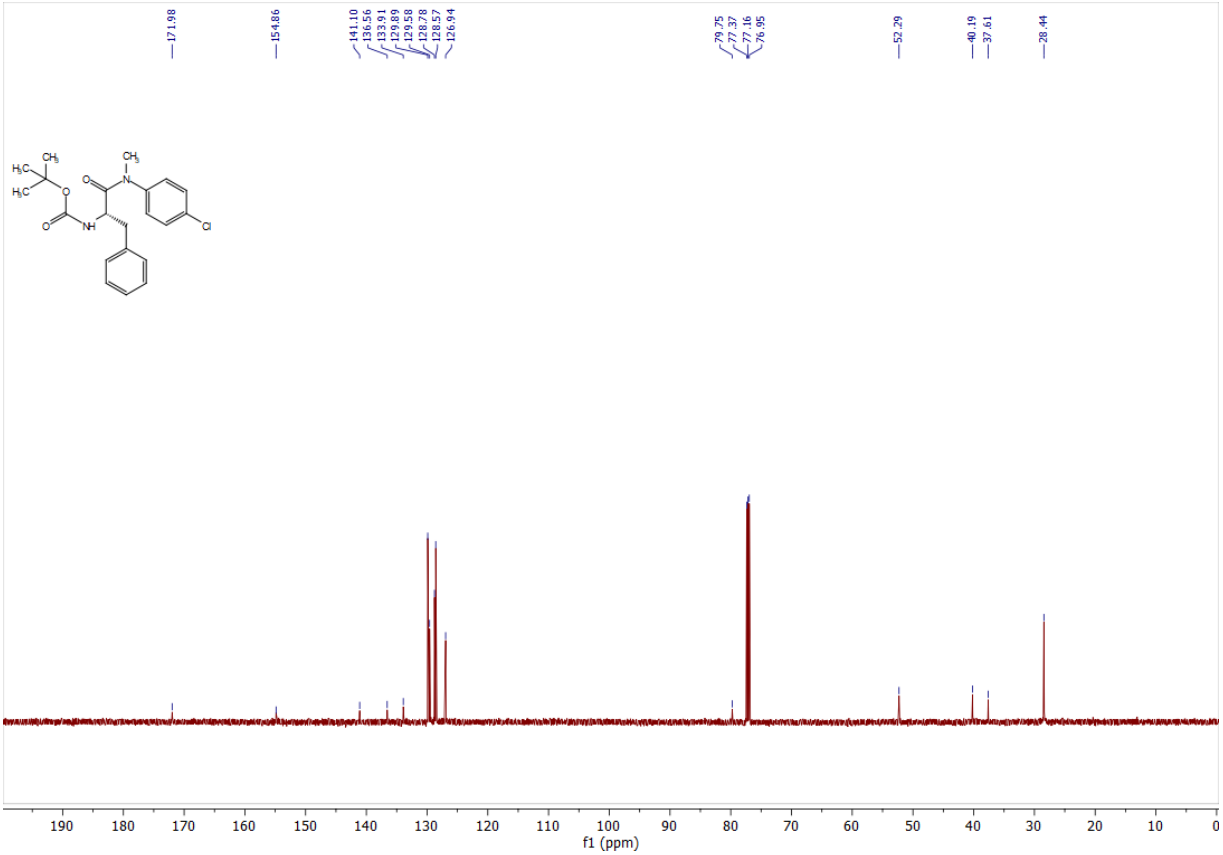
Synthesis of Intermediate 19: To a solution of substituted 2-chloro pyridine intermediate (**18**, 0.29 g, 0.87 mmol, 1.0 equiv.) in 5 mL of *t*-butanol was added KOH (0.15 g, 2.61 mmol, 3.0 equiv.) and the reaction mixture was stirred at 100 °C for 12 hours. Upon completion, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was diluted with water and extracted with dichloromethane to remove unreacted starting material. The aqueous layer was acidified with 10% HCl to pH 2 to precipitate out the hydrolyzed product, which was filtered and washed with DCM to get 2-pyridinone intermediate (**19**, 0.22 g, 0.66 mmol, 76%).



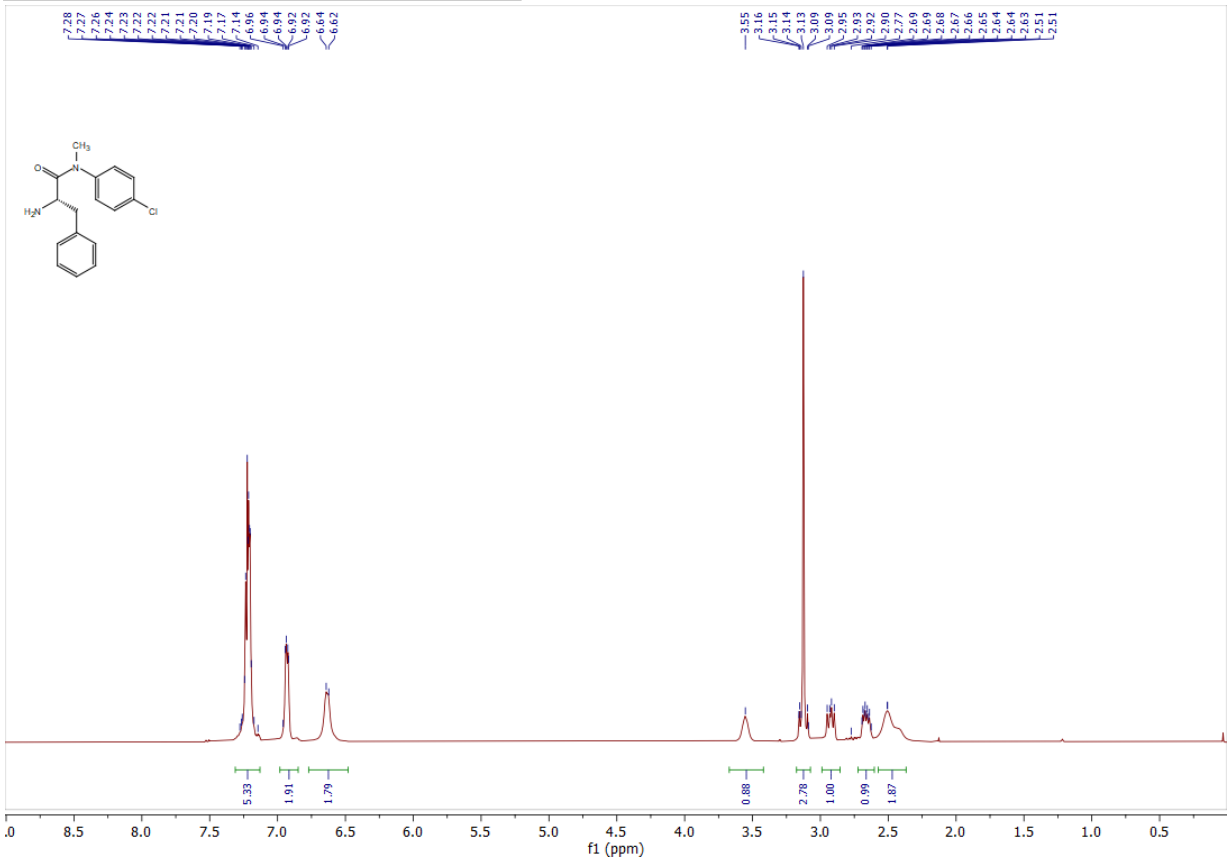
White solid, *3-chloro-5-((2-oxo-4-(trifluoromethyl)-1,2-dihydropyridin-3-yl)oxy)benzoic acid* **(19)**: Yield 76%. ^{1}H NMR (600 MHz, $DMSO_{d6}$) δ 12.74 (s, 1H), 7.63 – 7.58 (m, 2H), 7.38 (s, 1H), 7.26 (s, 1H), 6.50 (d, *J* = 6.9 Hz, 1H). HRMS (ESI) m/z calcd for $C_{13}H_7ClF_3NO_4$ $[M - H]^-$ 331.9943, found 331.9955.

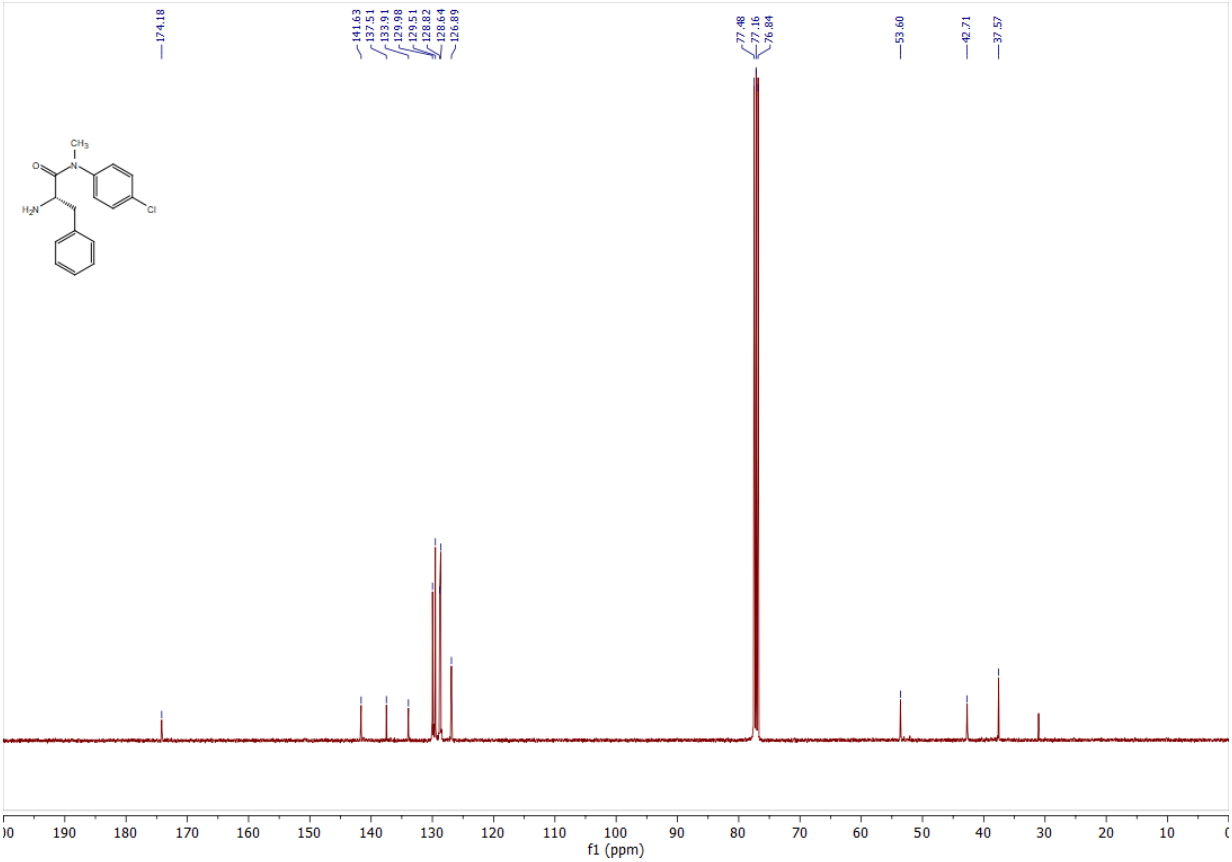
^{1}H and ^{13}C spectra of compound **7**



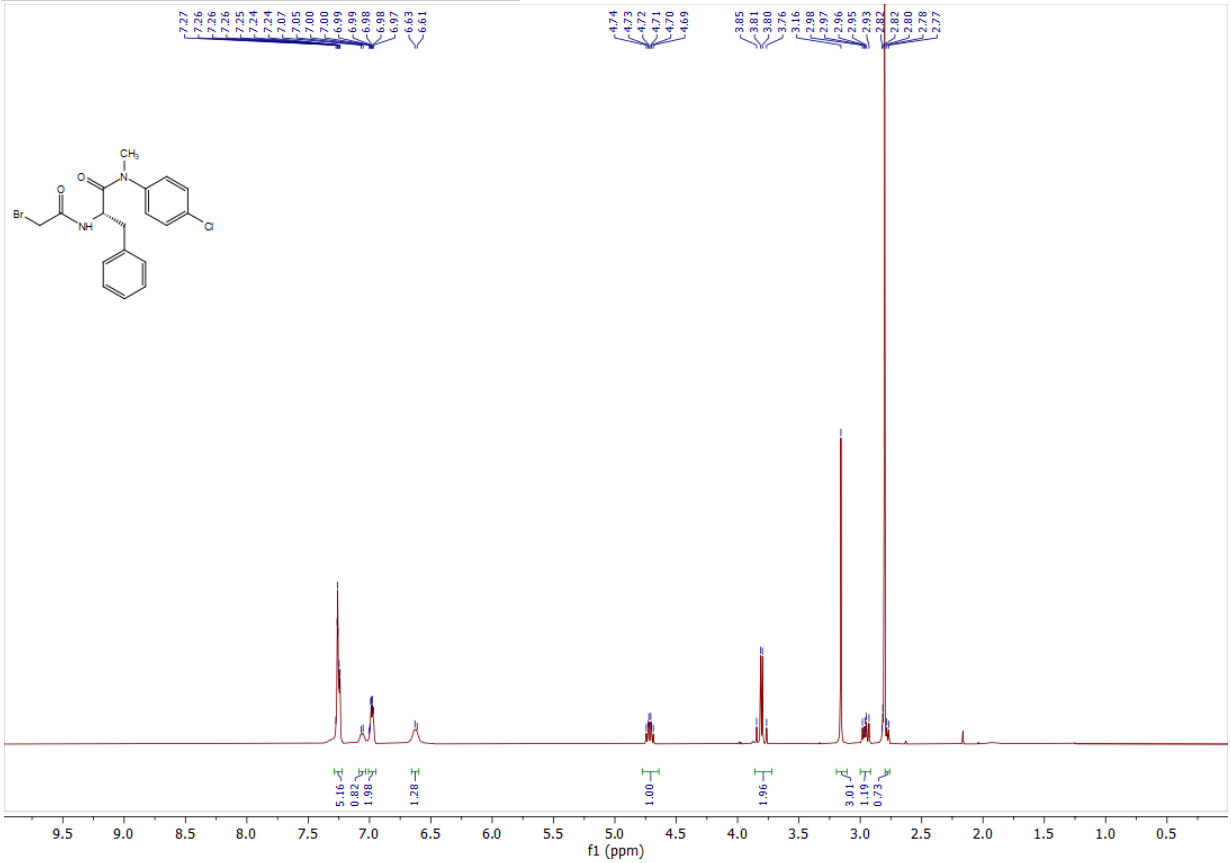


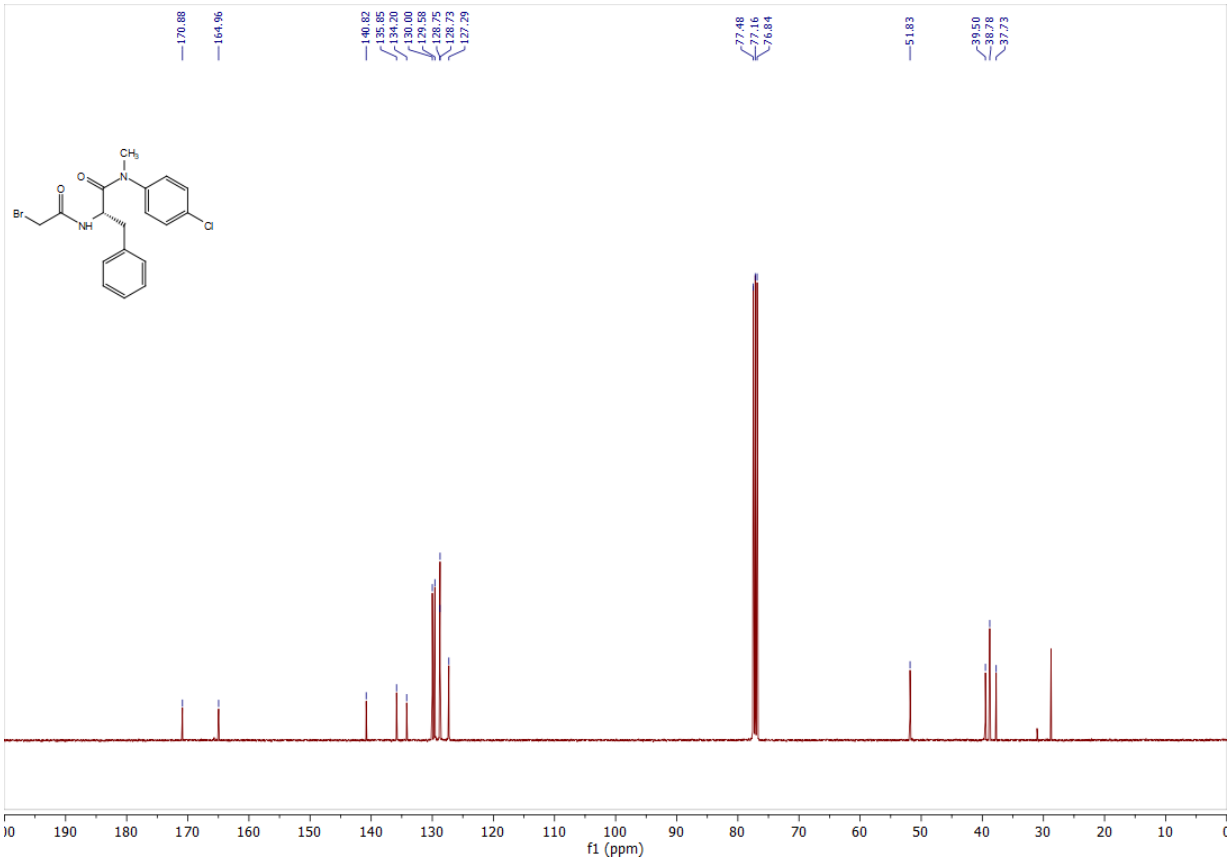
^{1}H and ^{13}C spectra of compound **8**



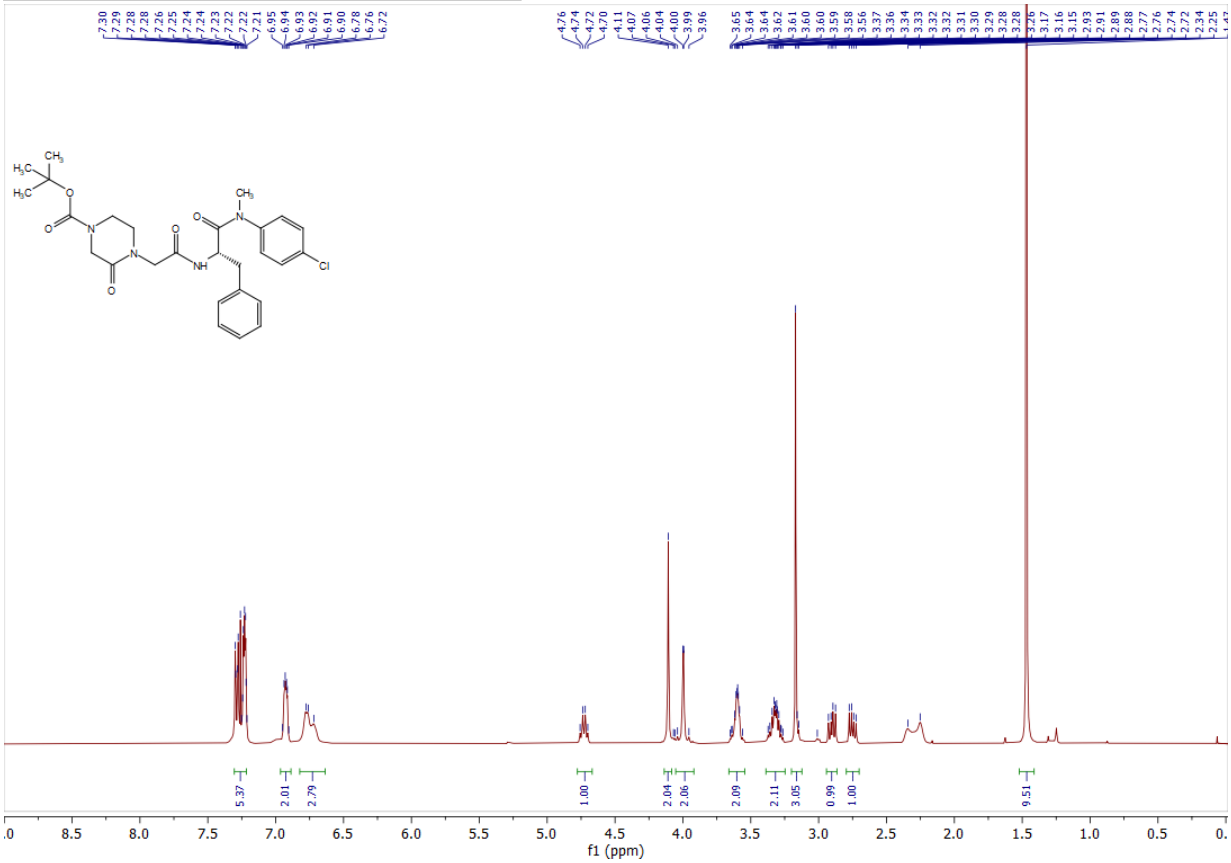


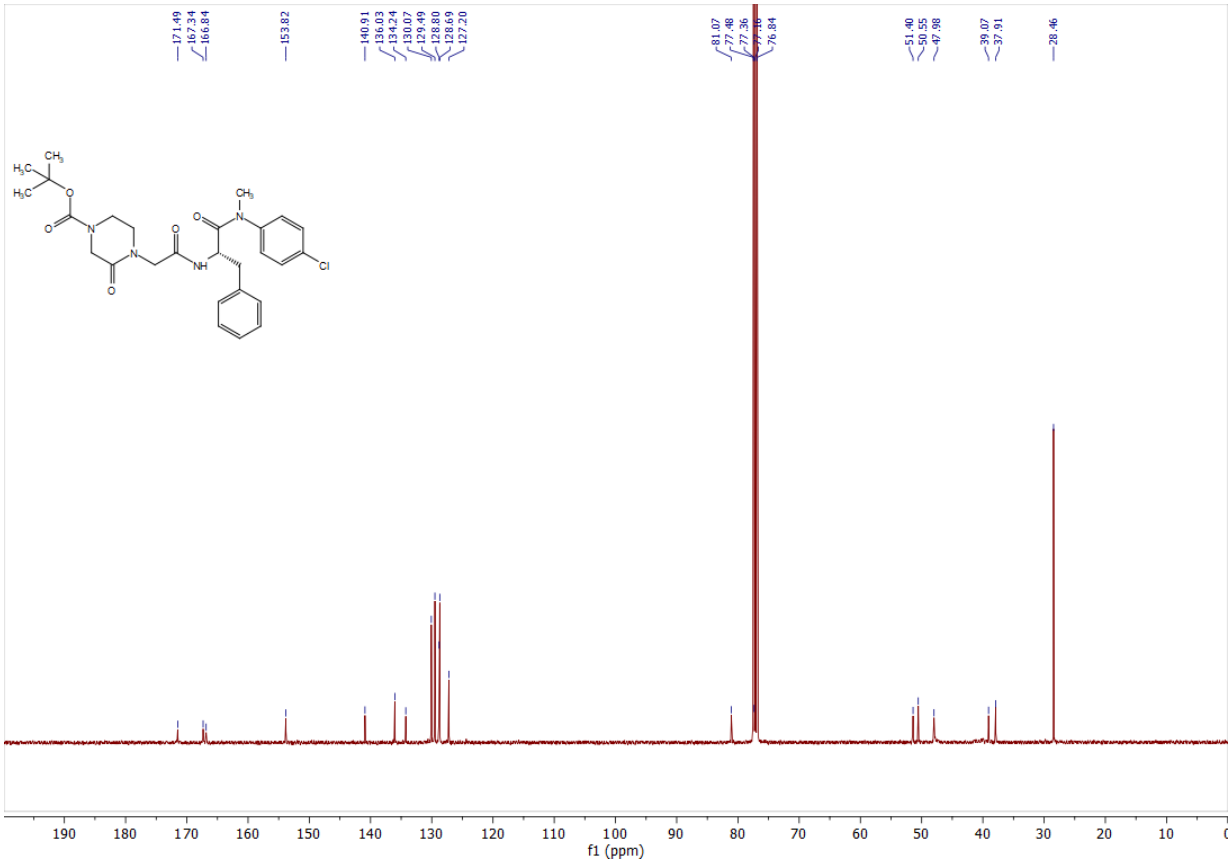
^{1}H and ^{13}C spectra of compound **9**



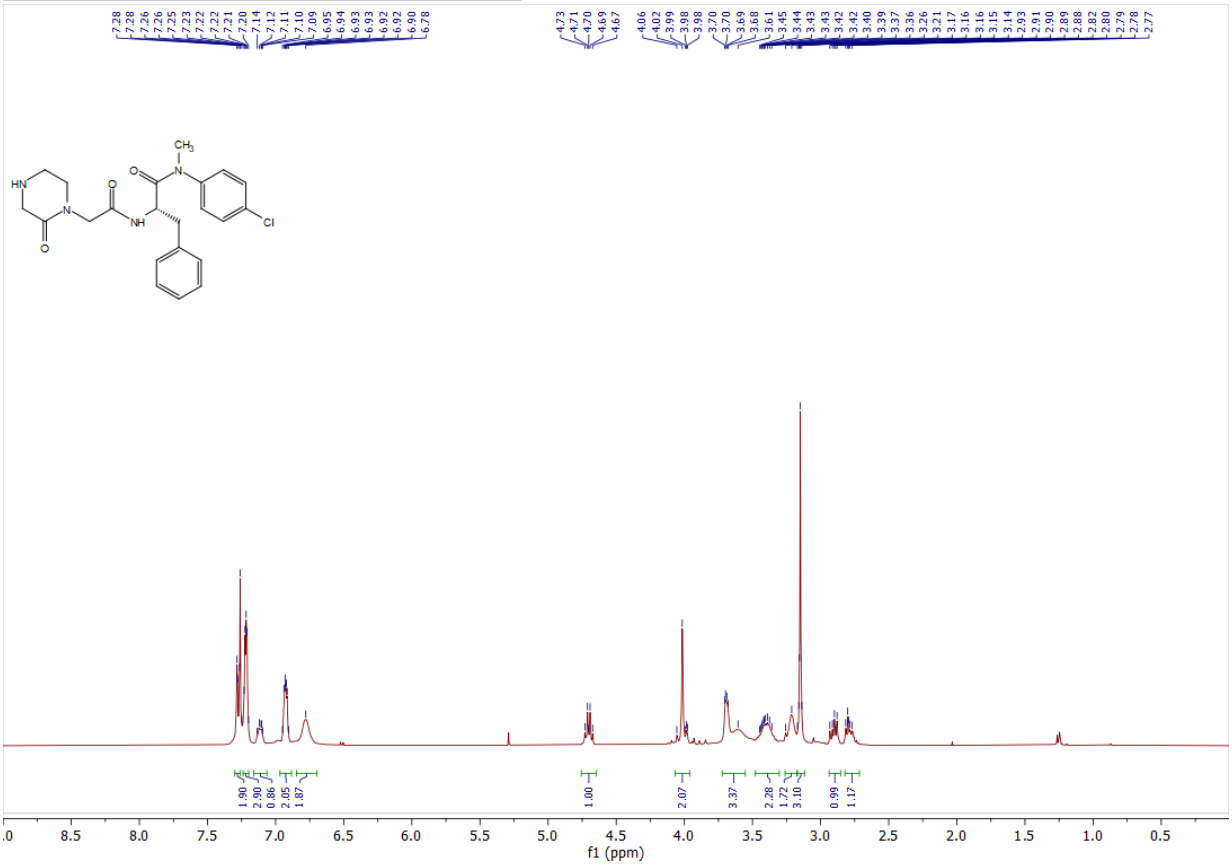


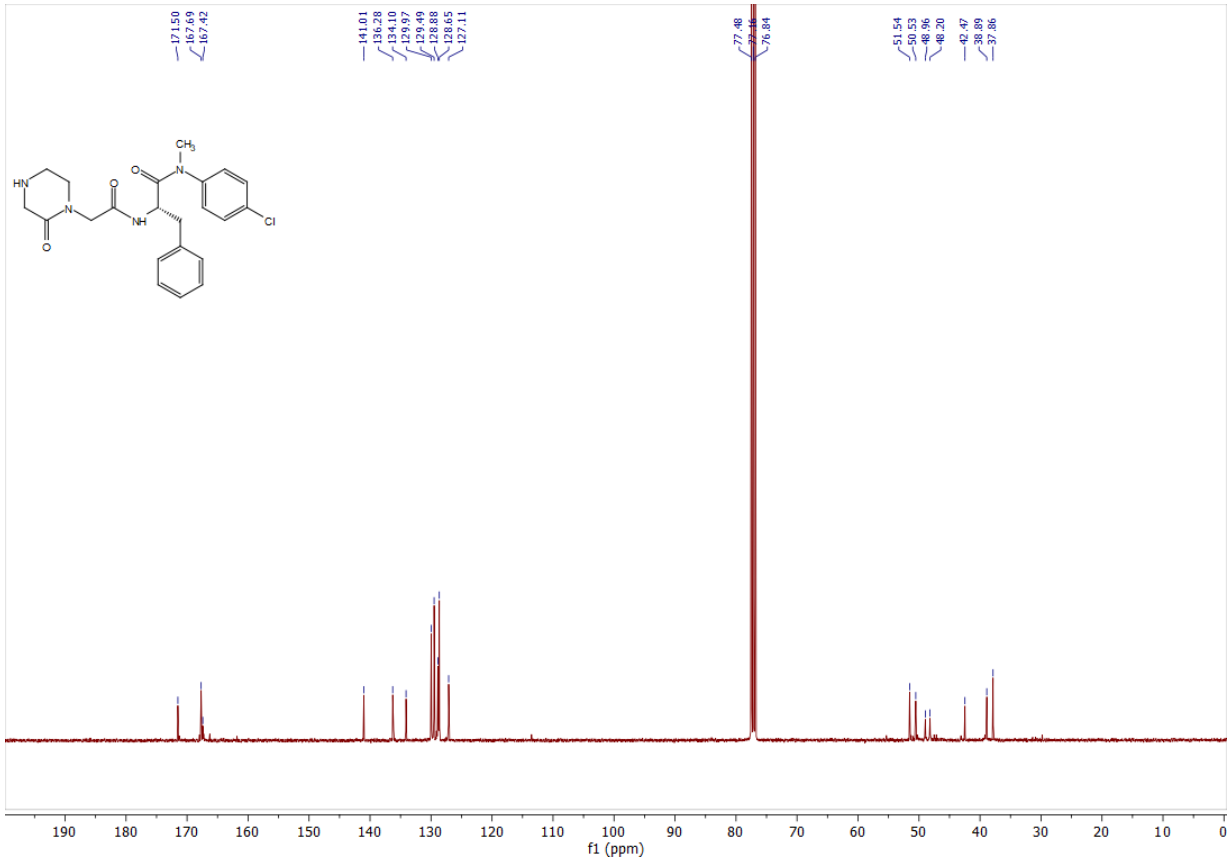
^{1}H and ^{13}C spectra of compound **14**



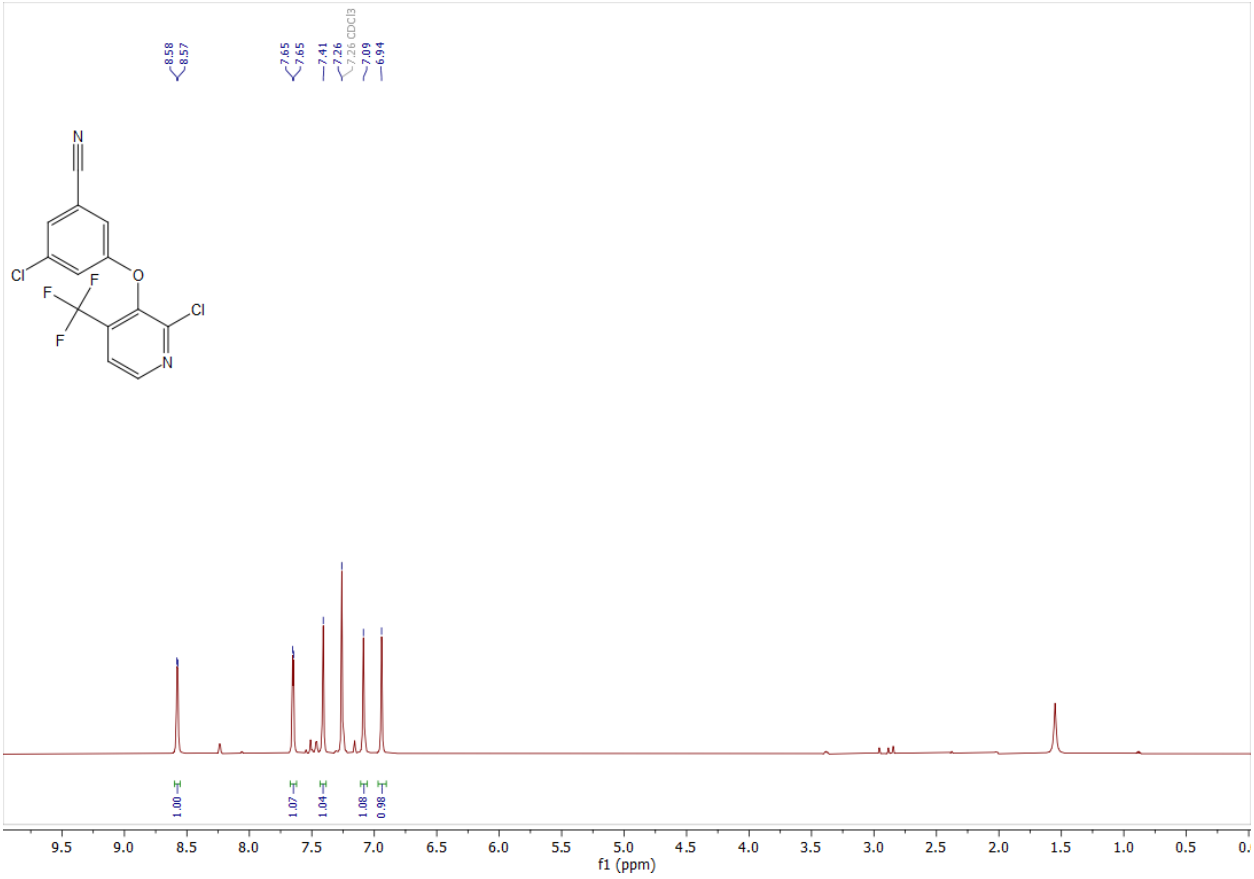


^{1}H and ^{13}C spectra of compound **15**





^{1}H and ^{13}C spectra of compound **18**



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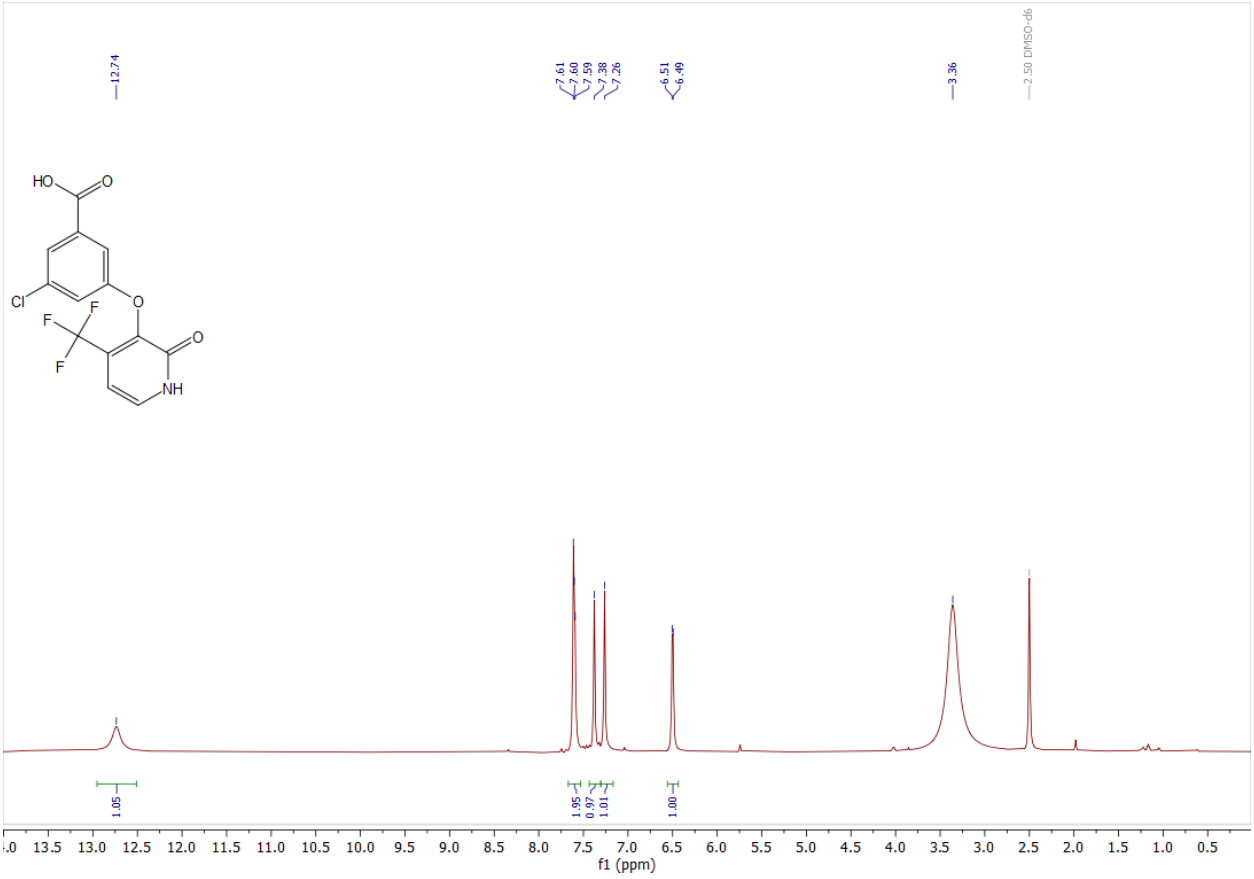
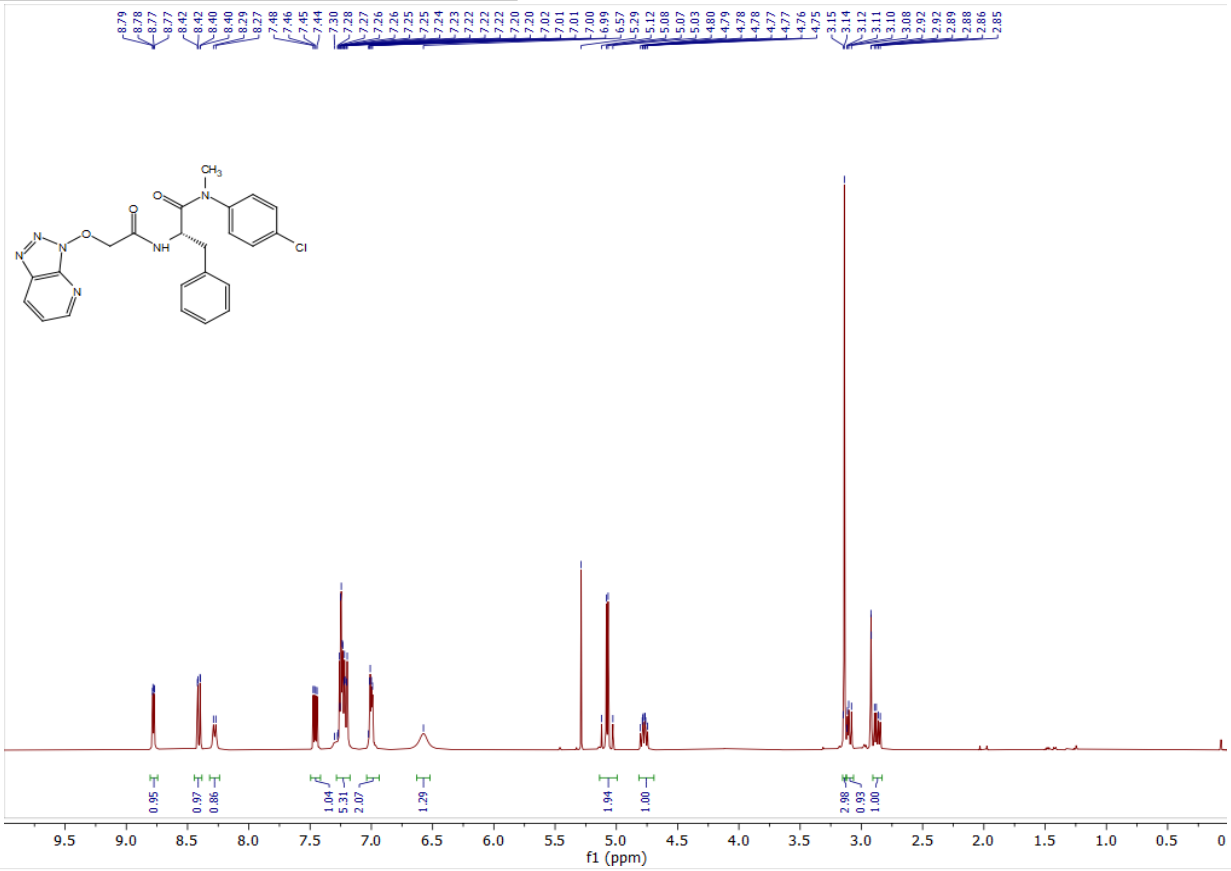
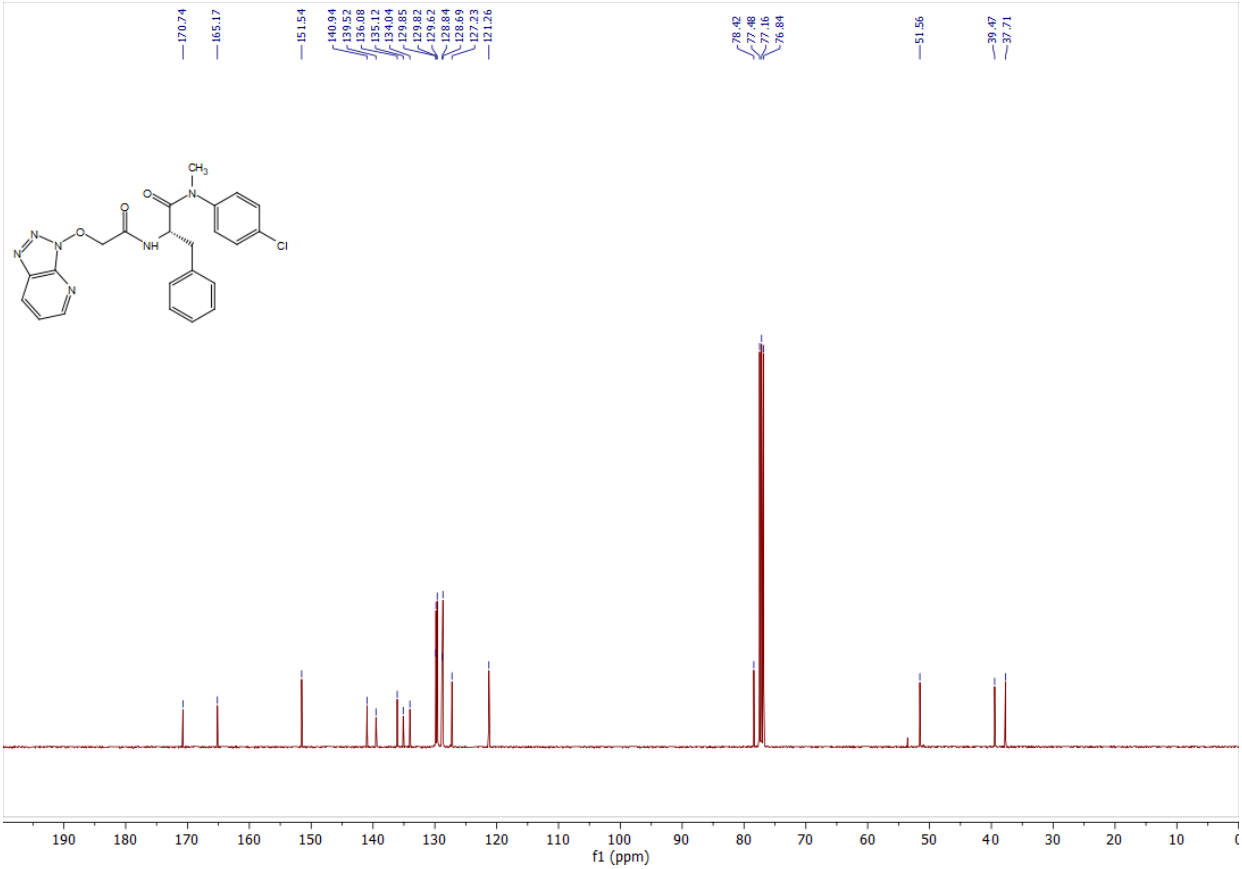
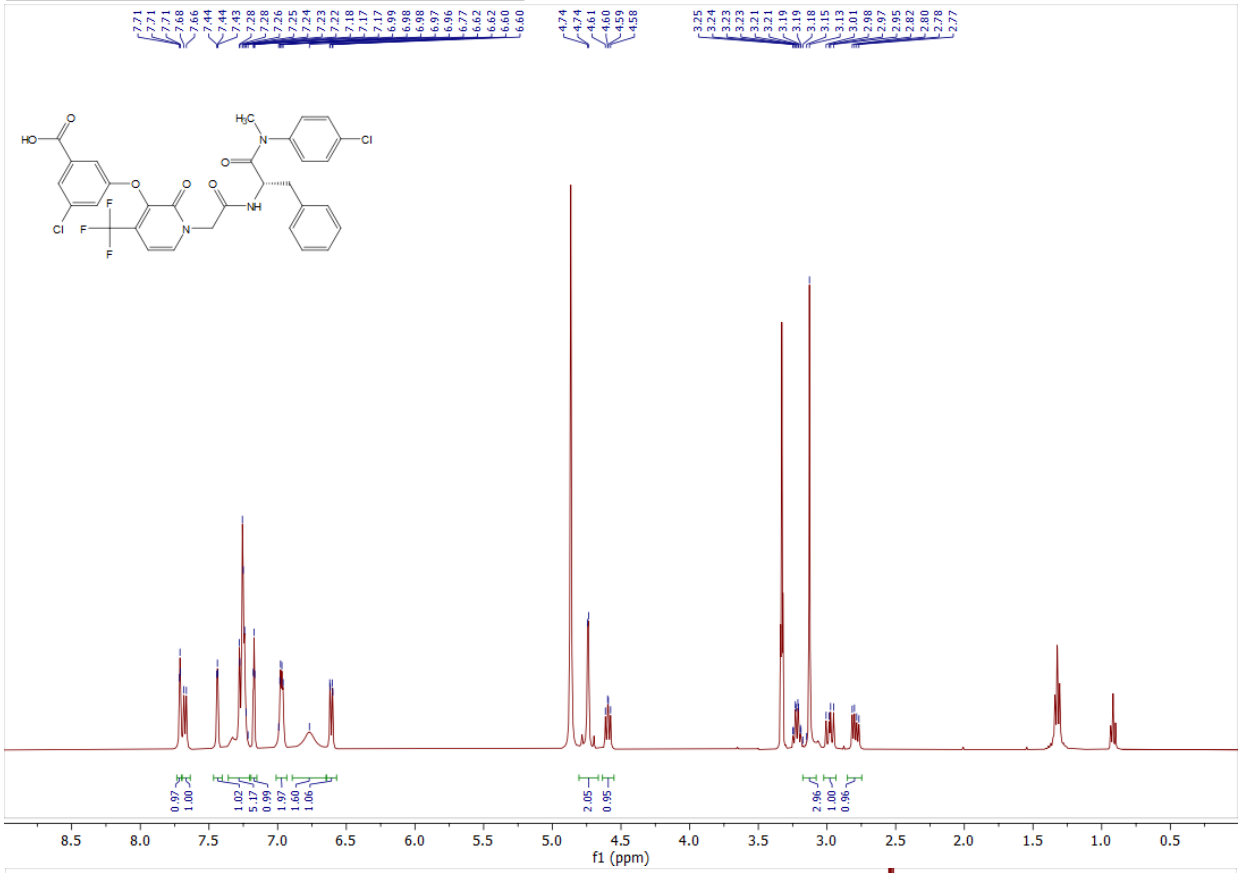


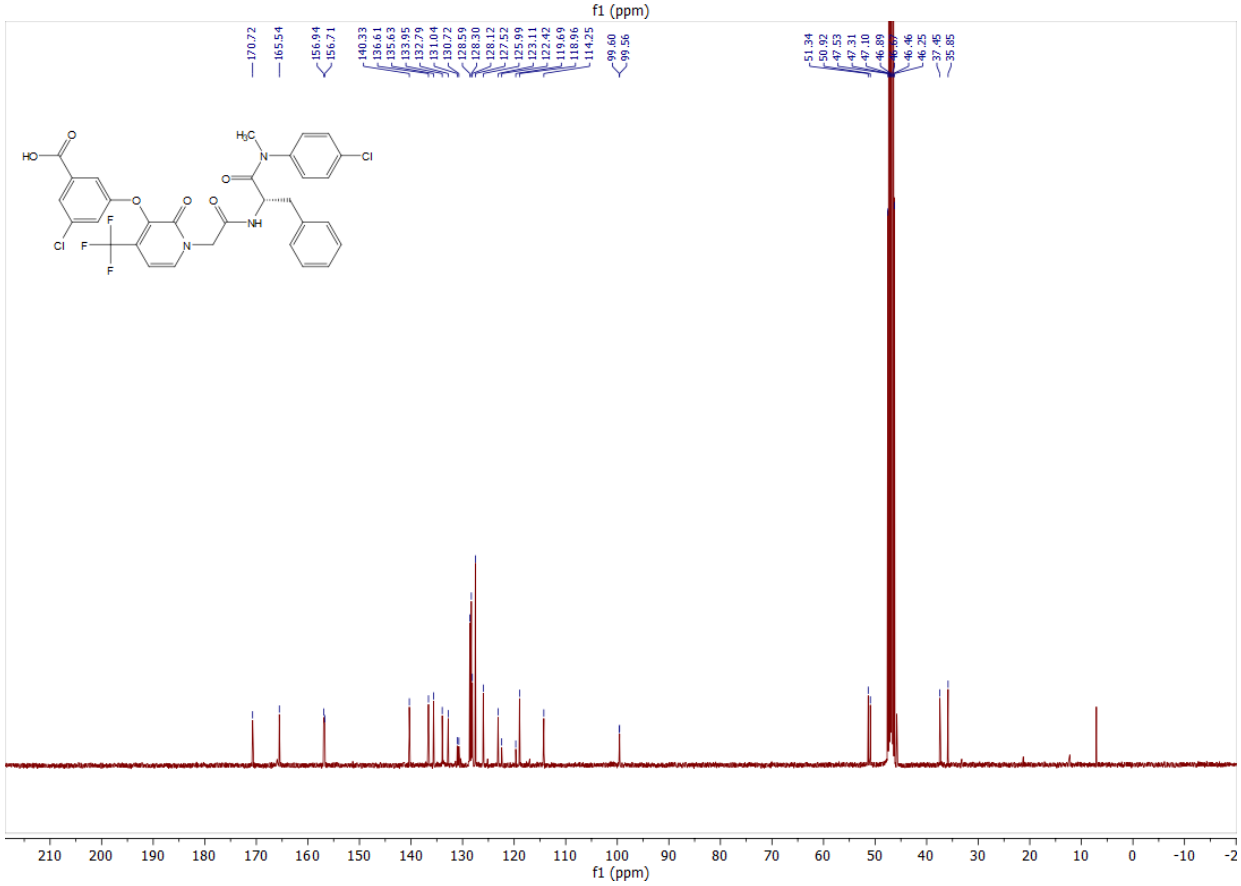
^{1}H and ^{13}C spectra of compound **10**



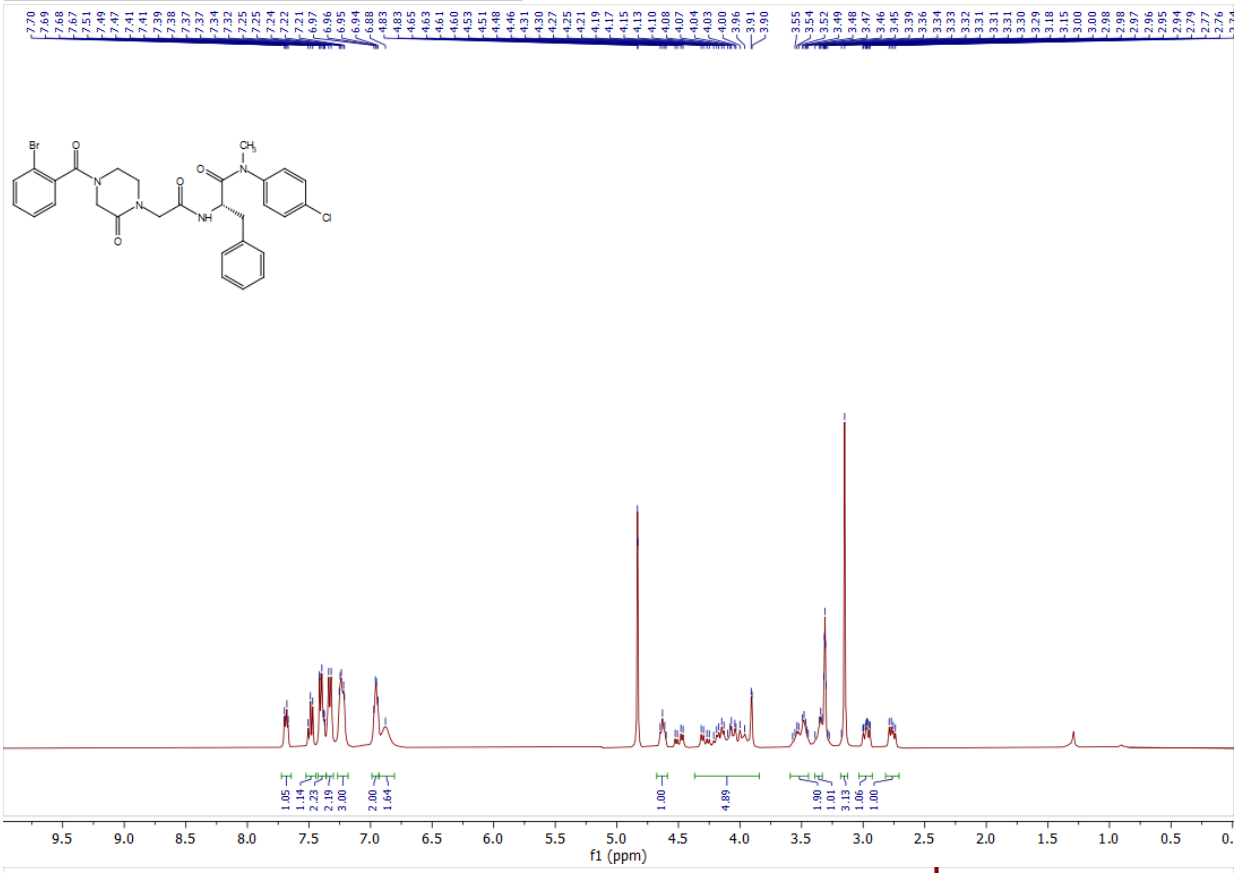


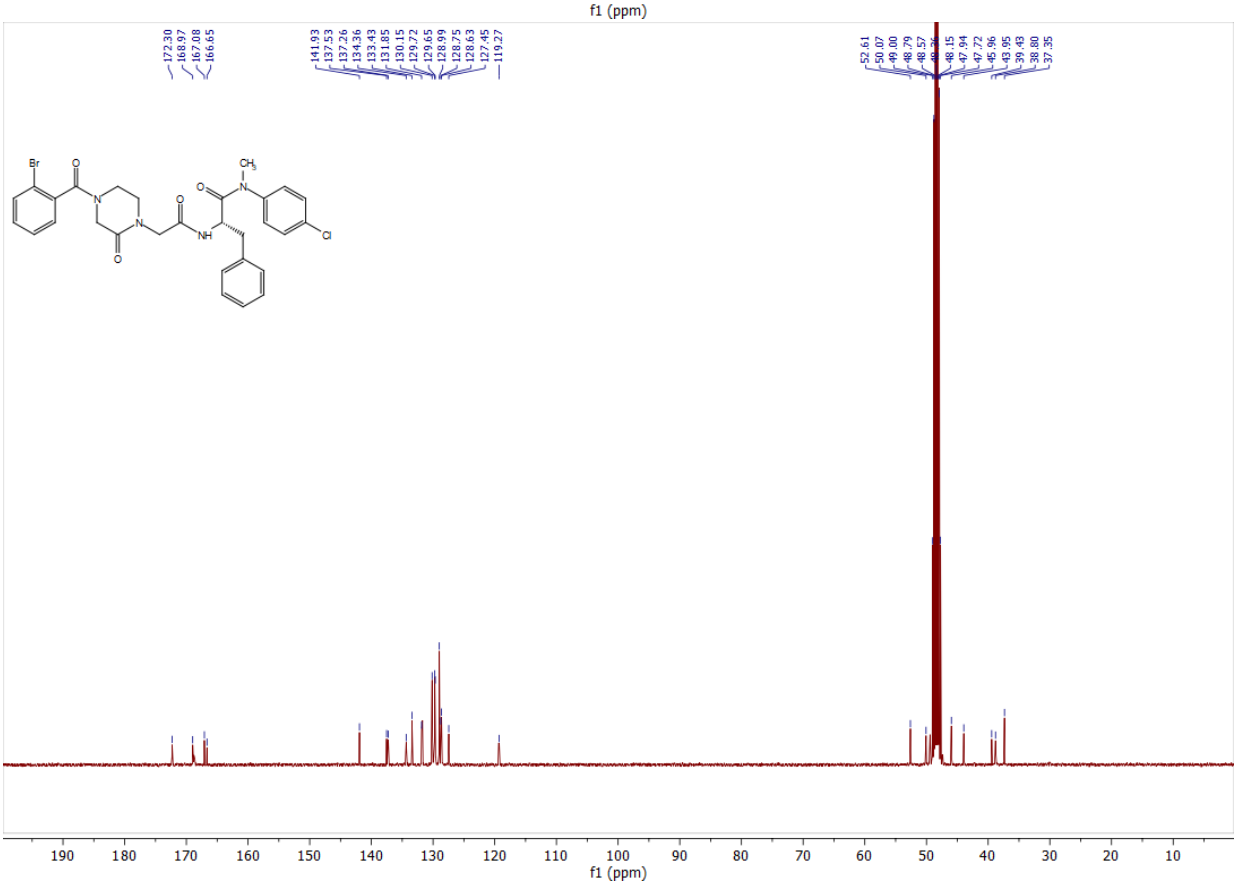
^{1}H and ^{13}C spectra of compound **20**



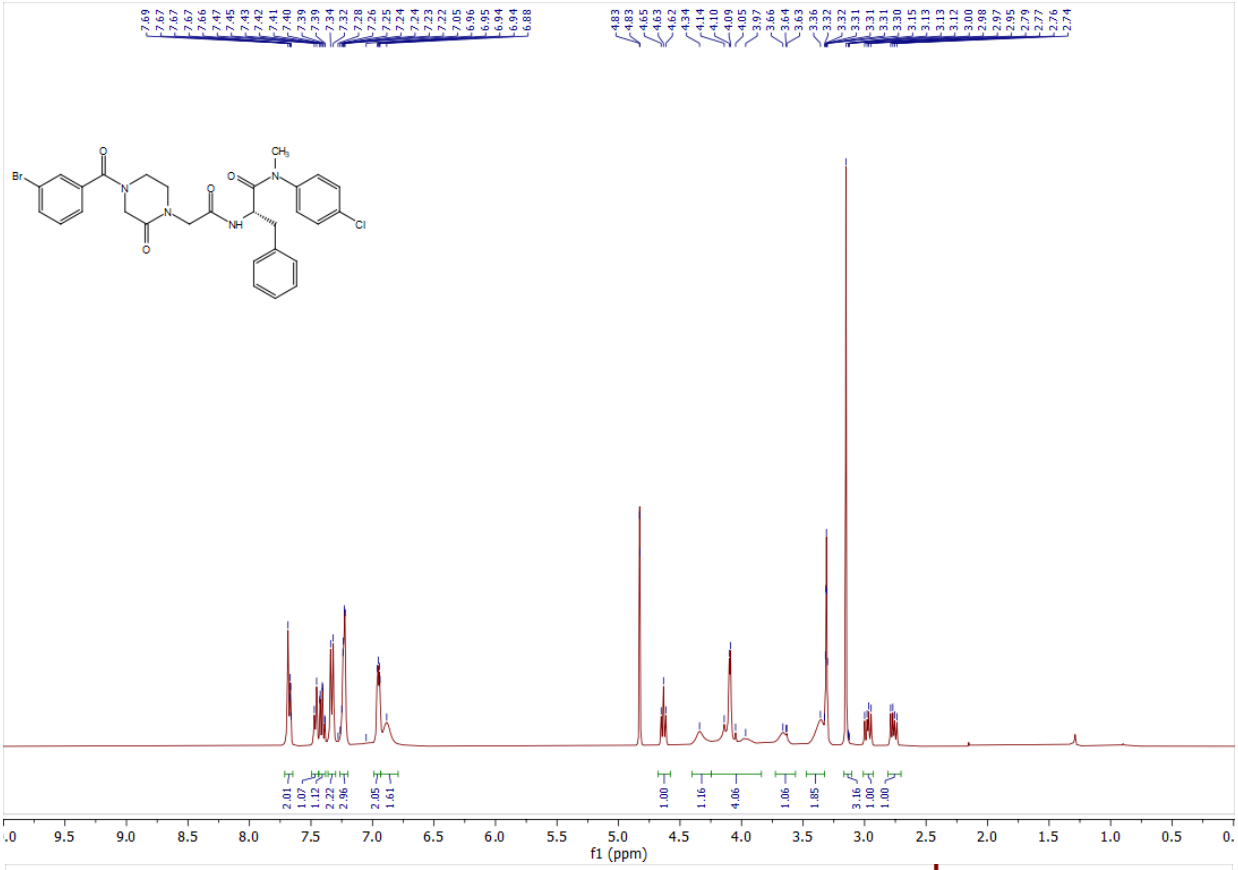


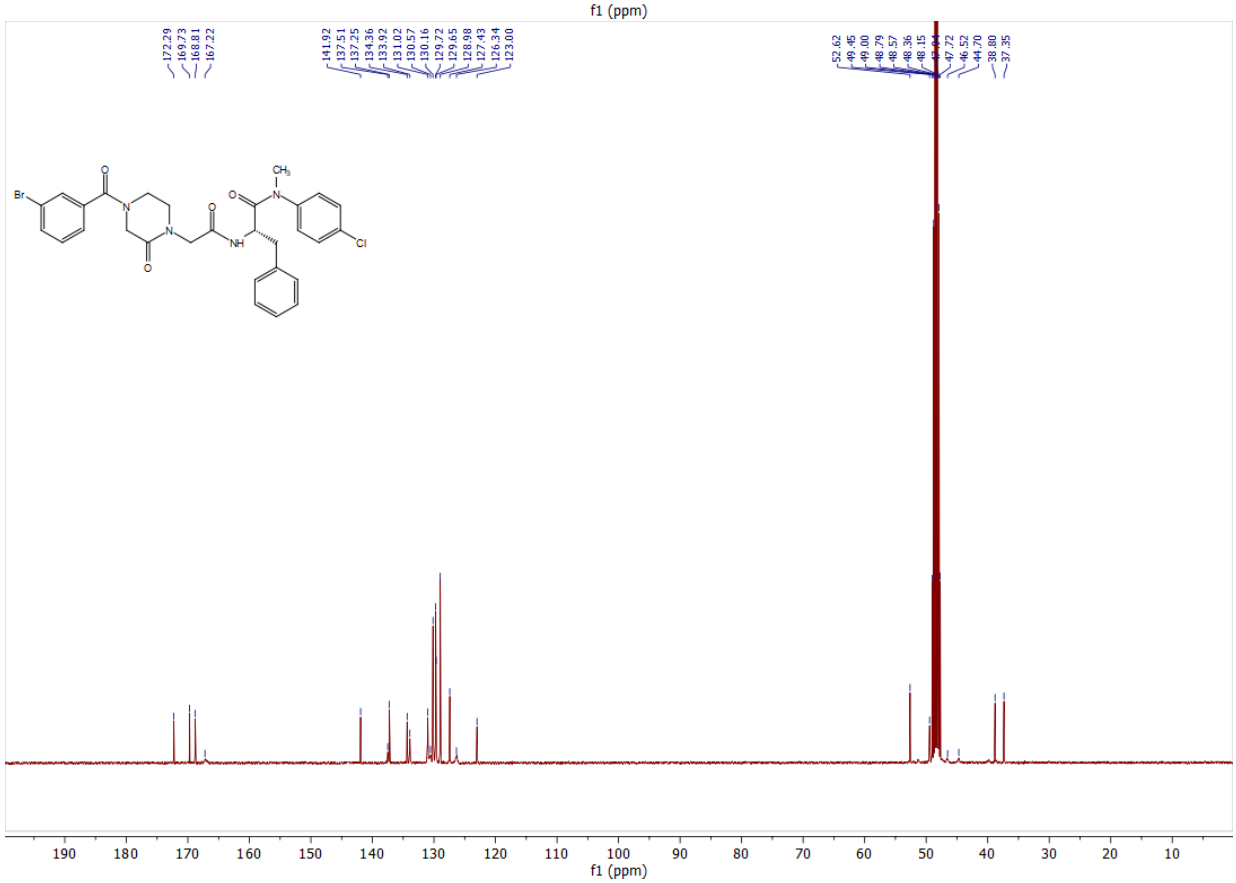
^{1}H and ^{13}C spectra of compound **4a**



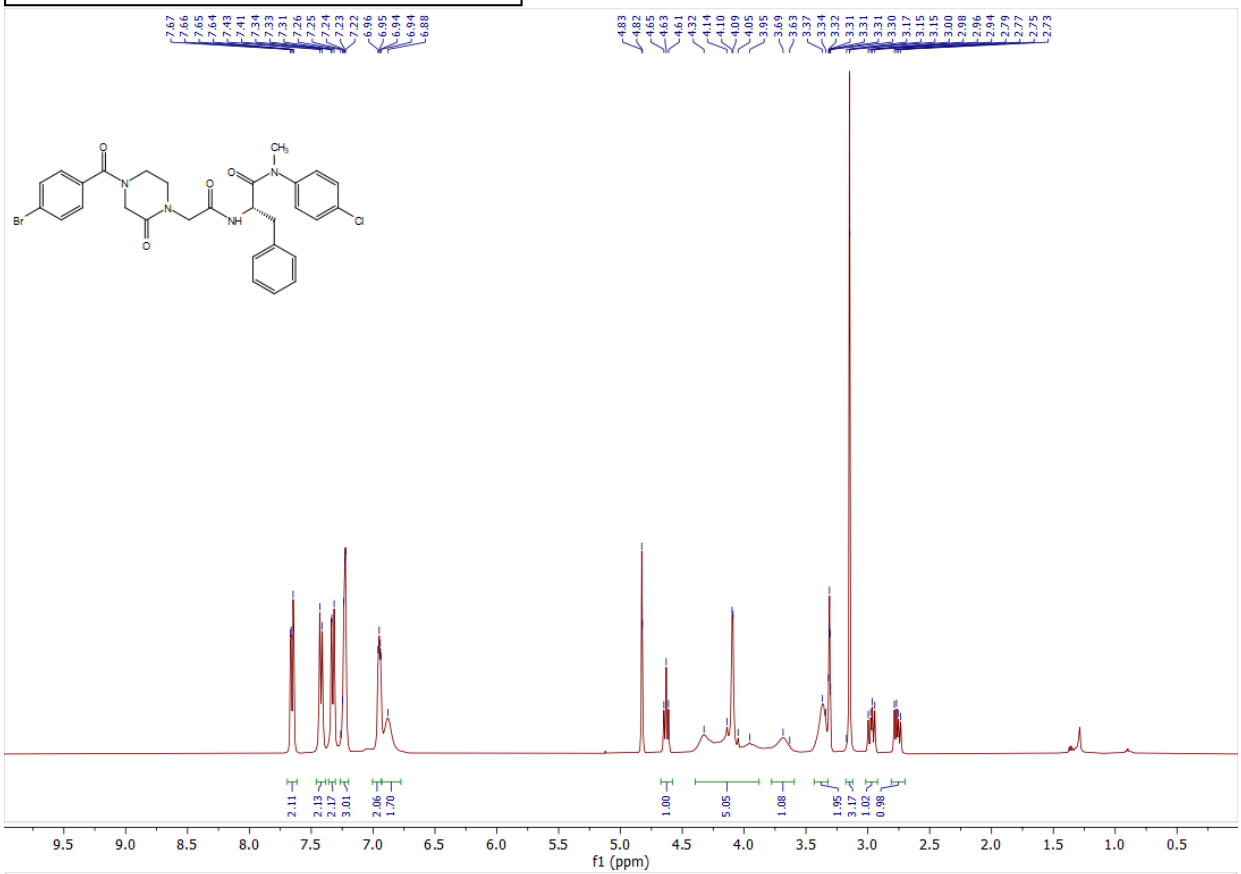


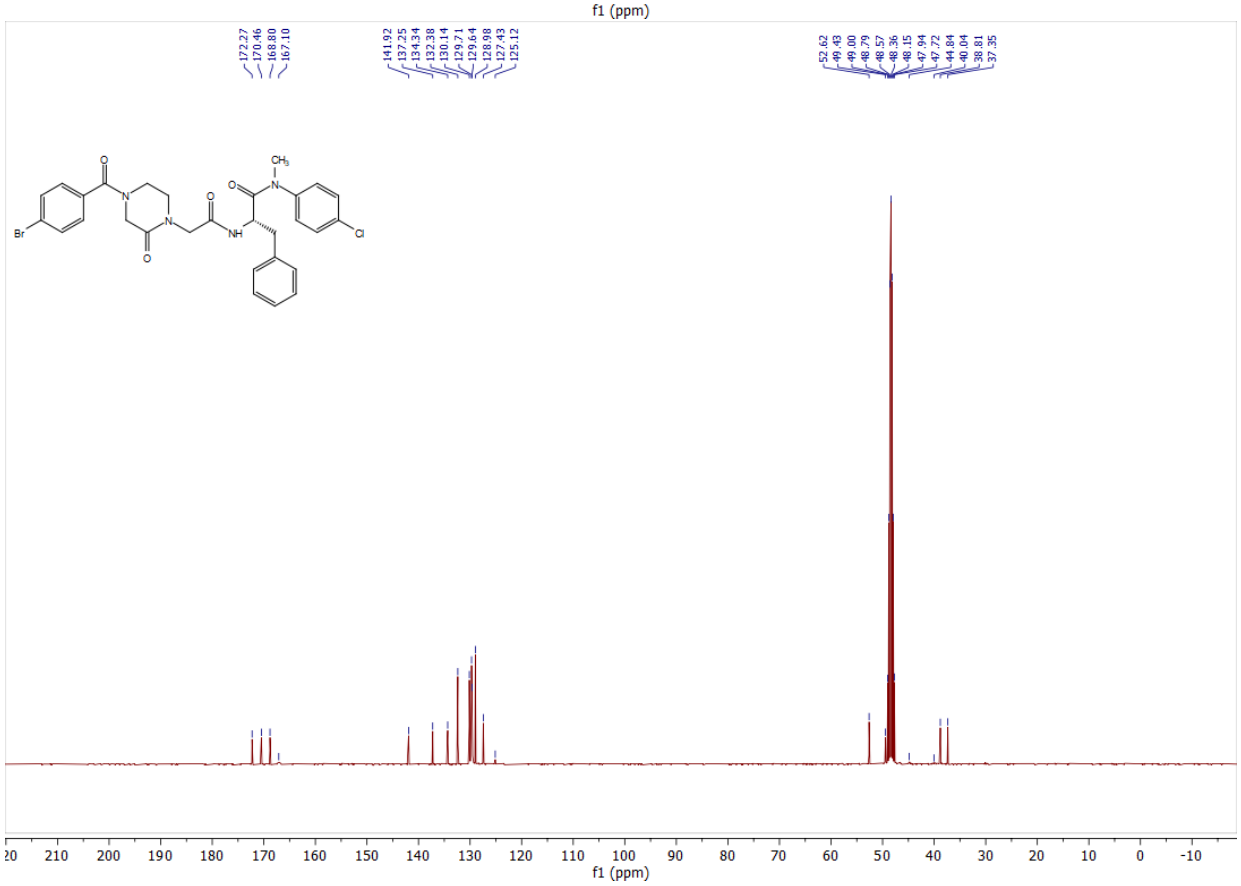
^{1}H and ^{13}C spectra of compound **4b**



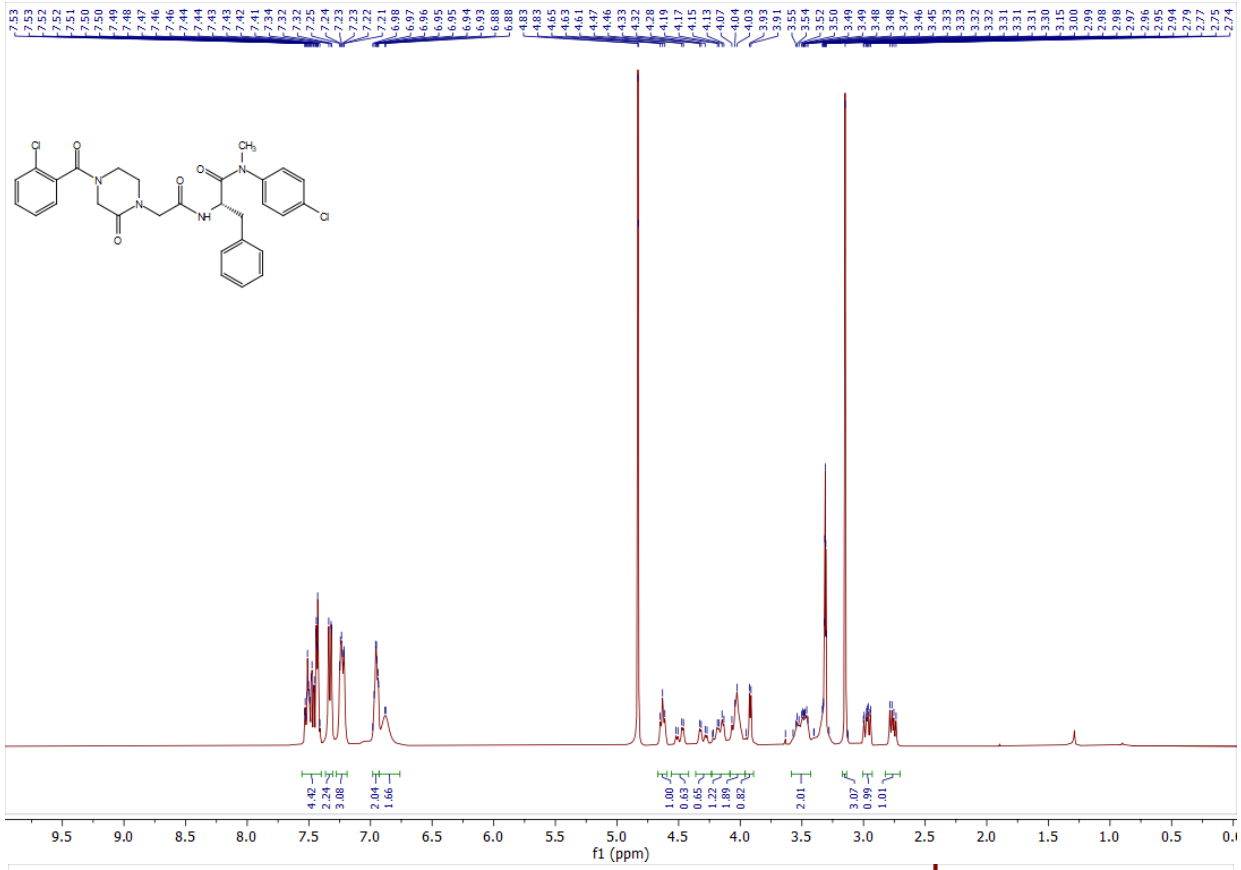


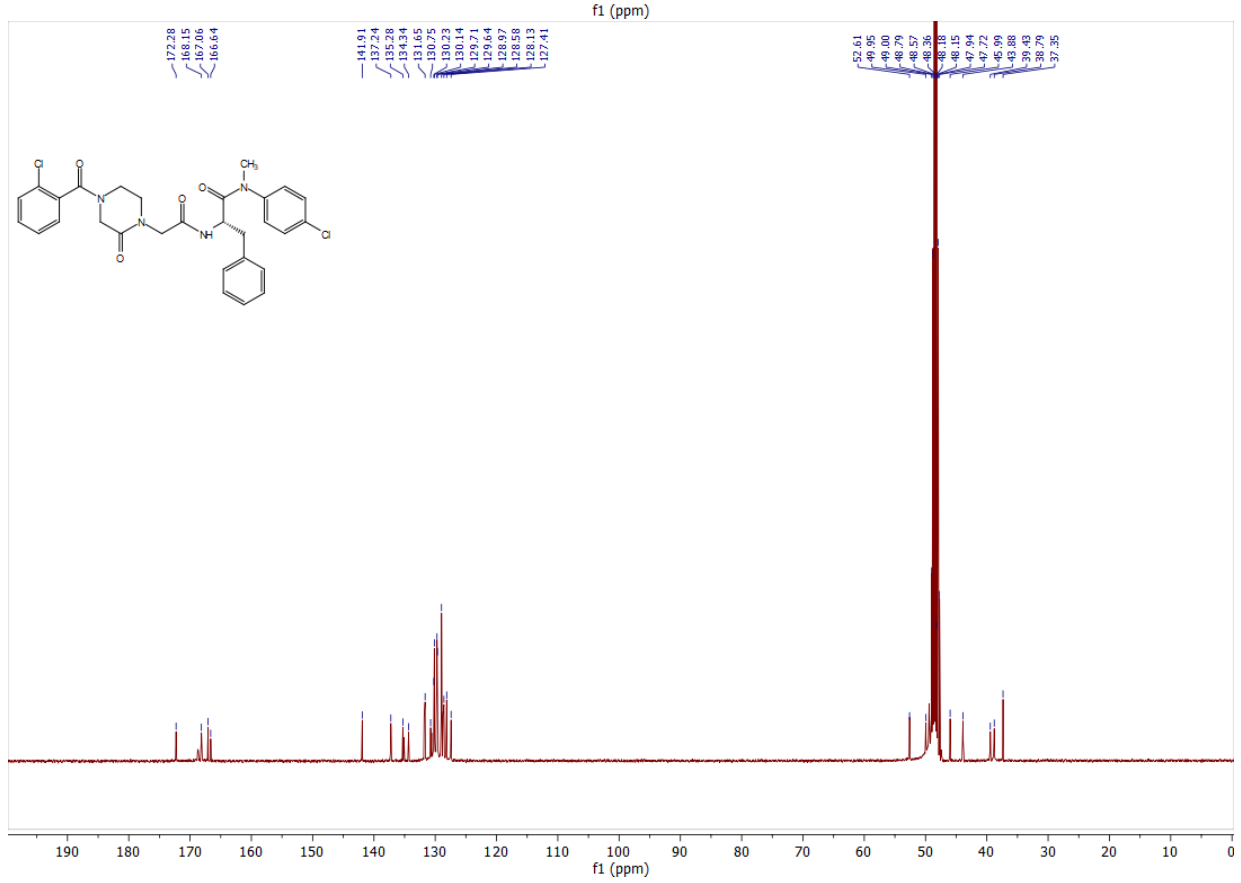
^{1}H and ^{13}C spectra of compound **4c**



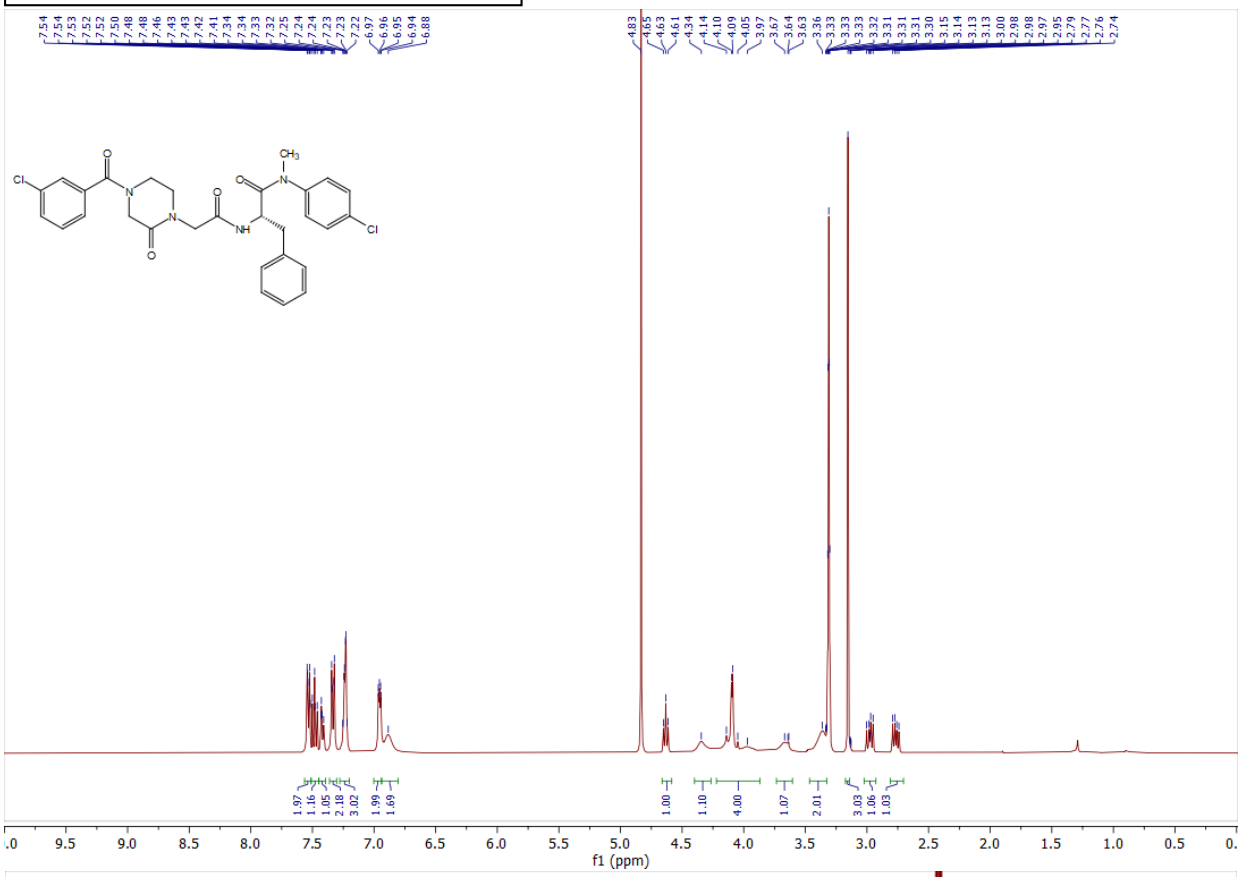


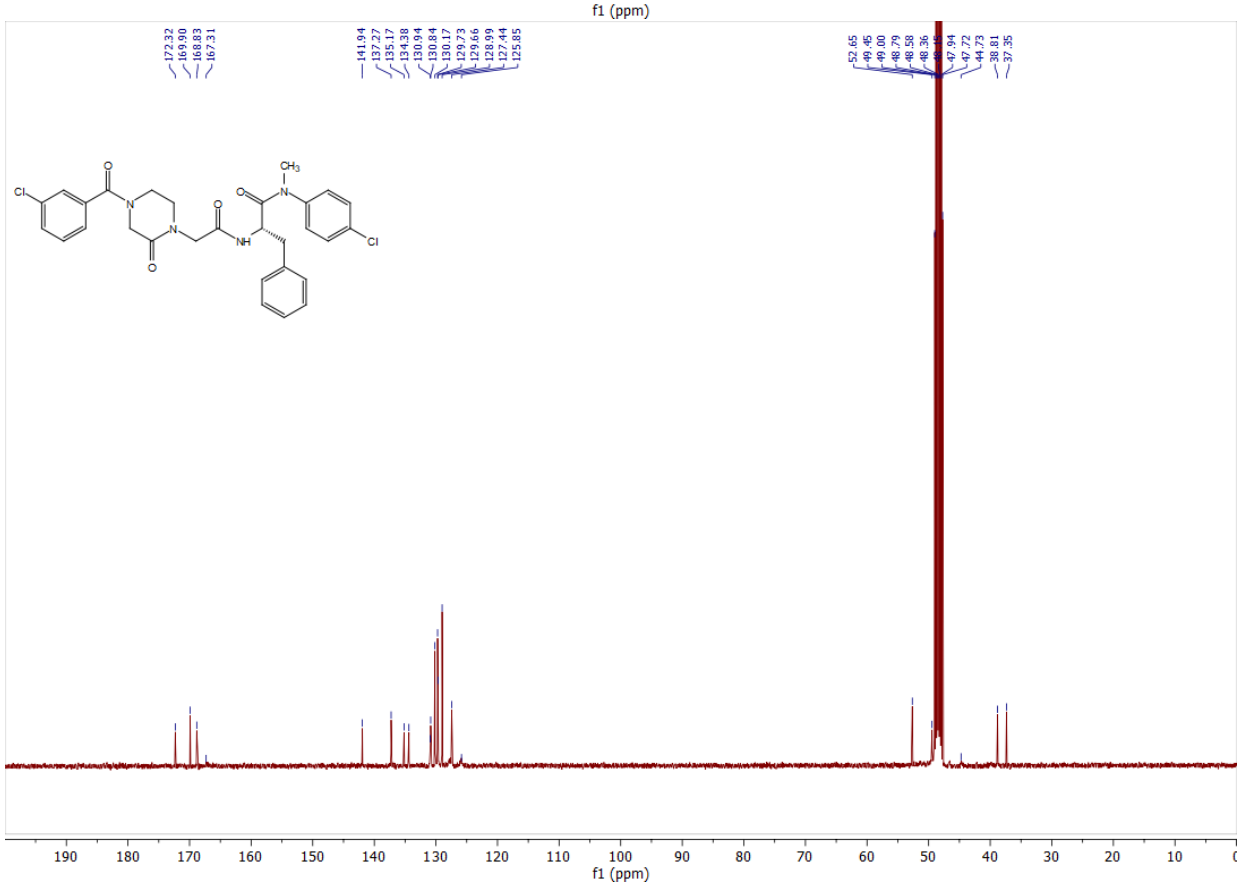
^{1}H and ^{13}C spectra of compound **4d**



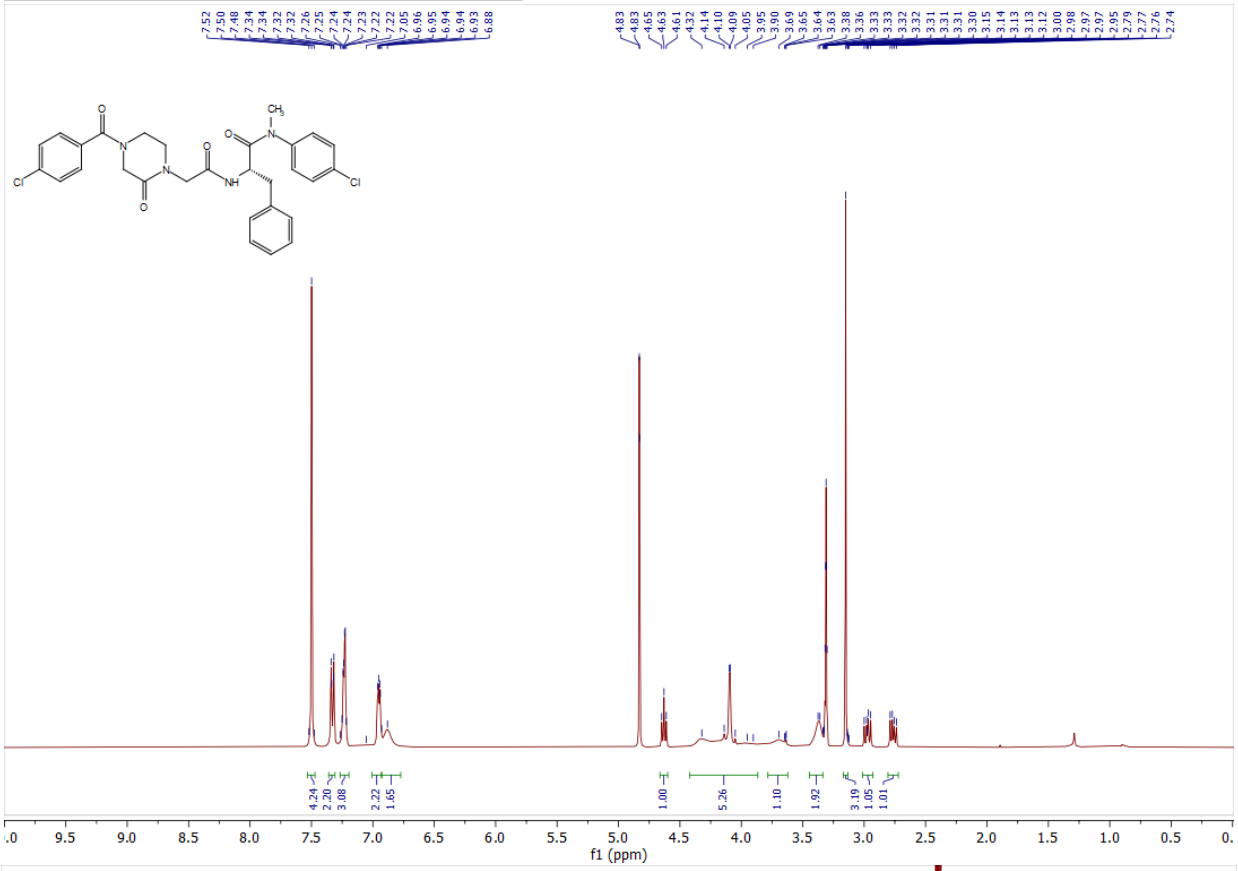


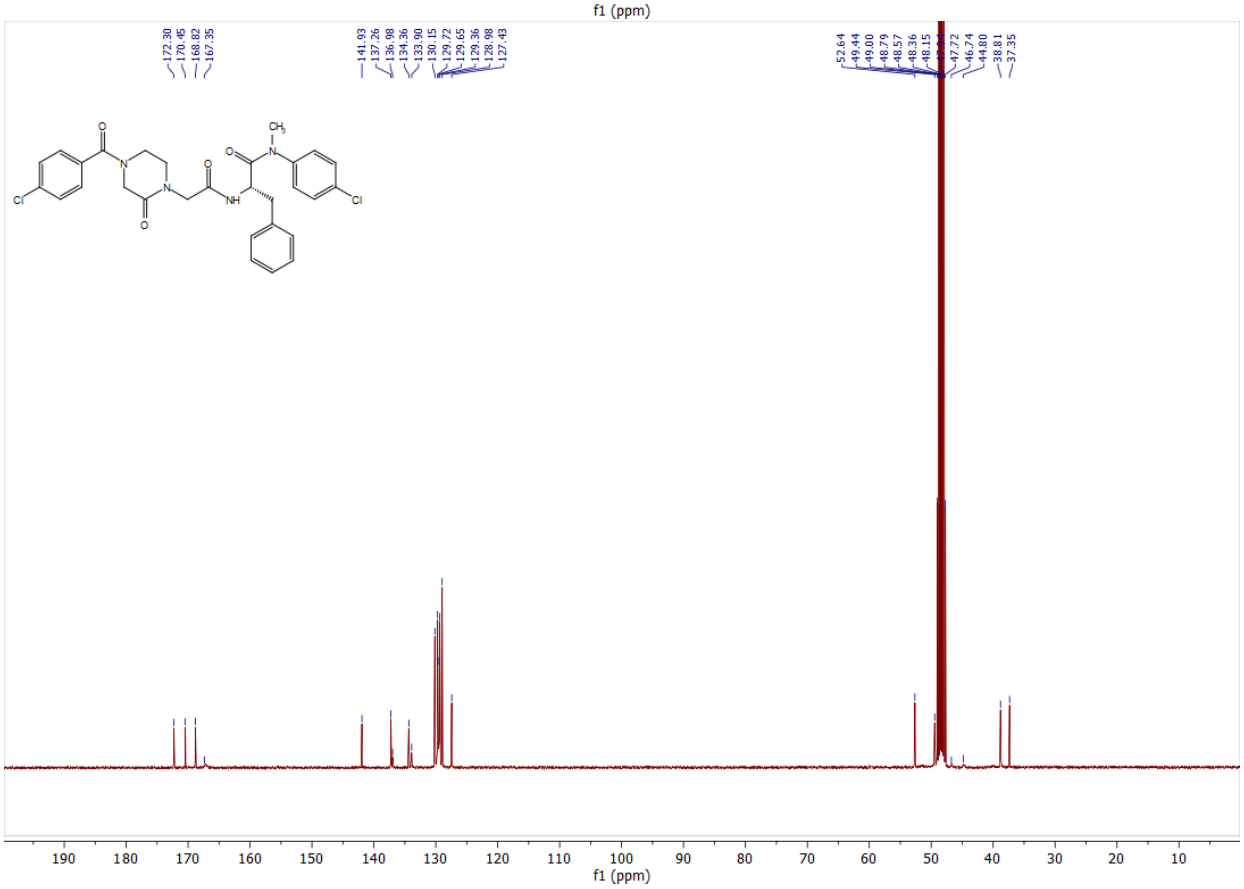
^{1}H and ^{13}C spectra of compound **4e**



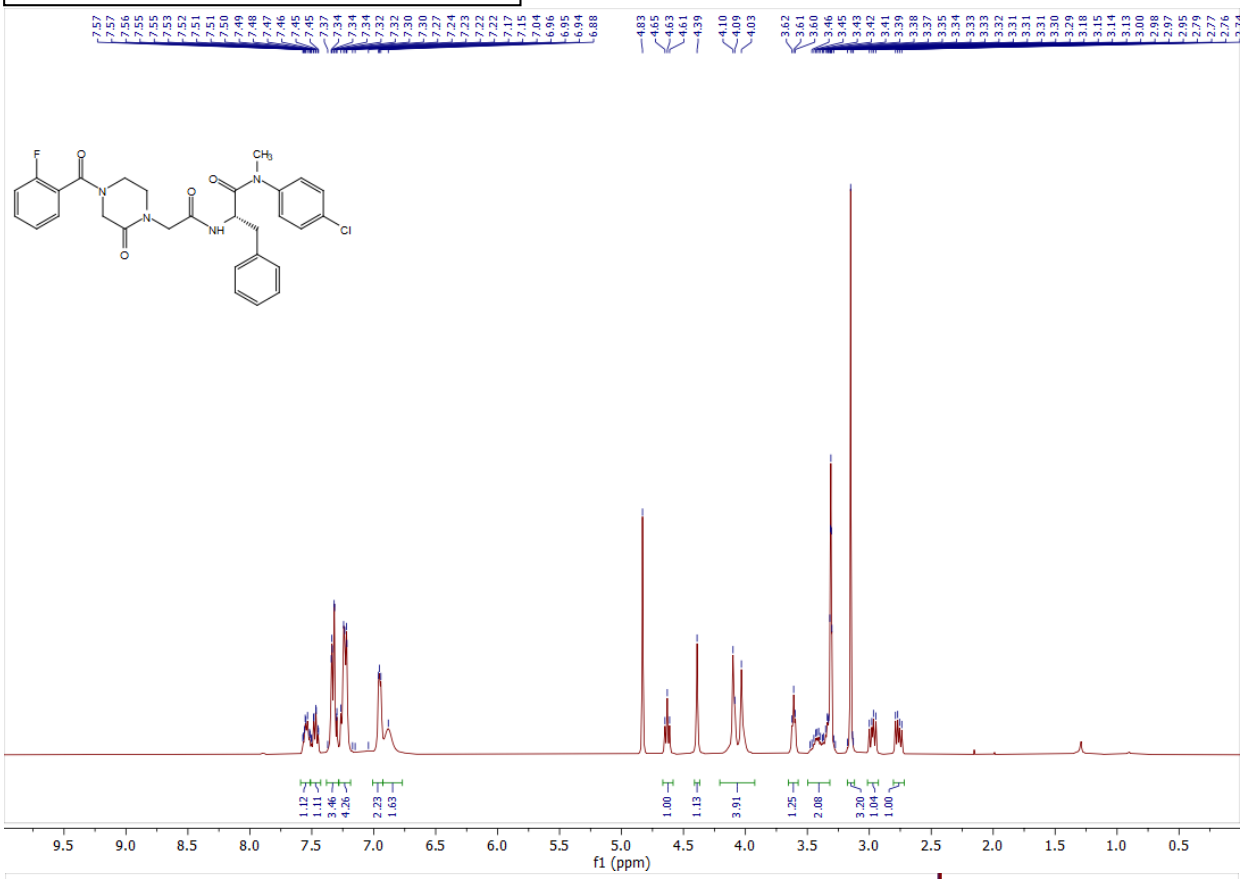


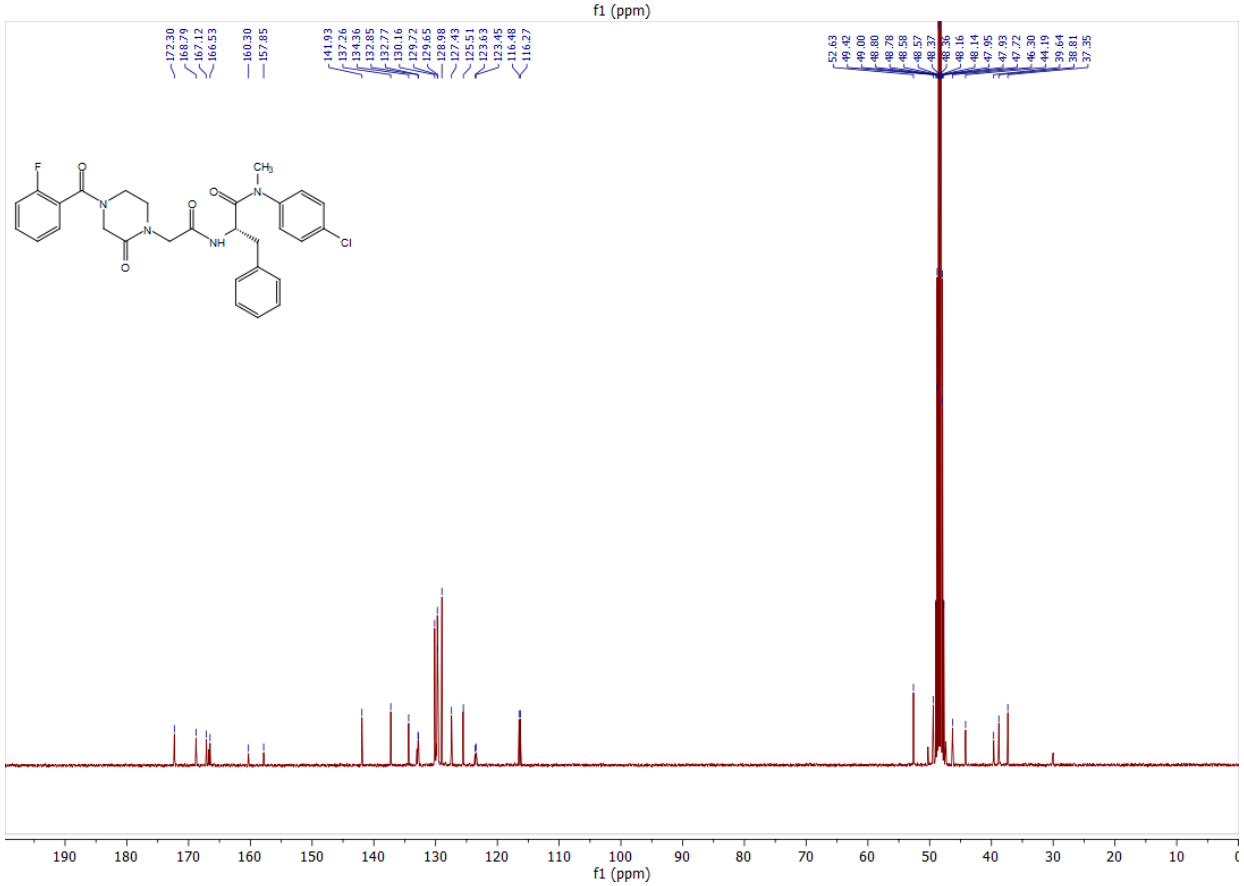
^{1}H and ^{13}C spectra of compound **4f**



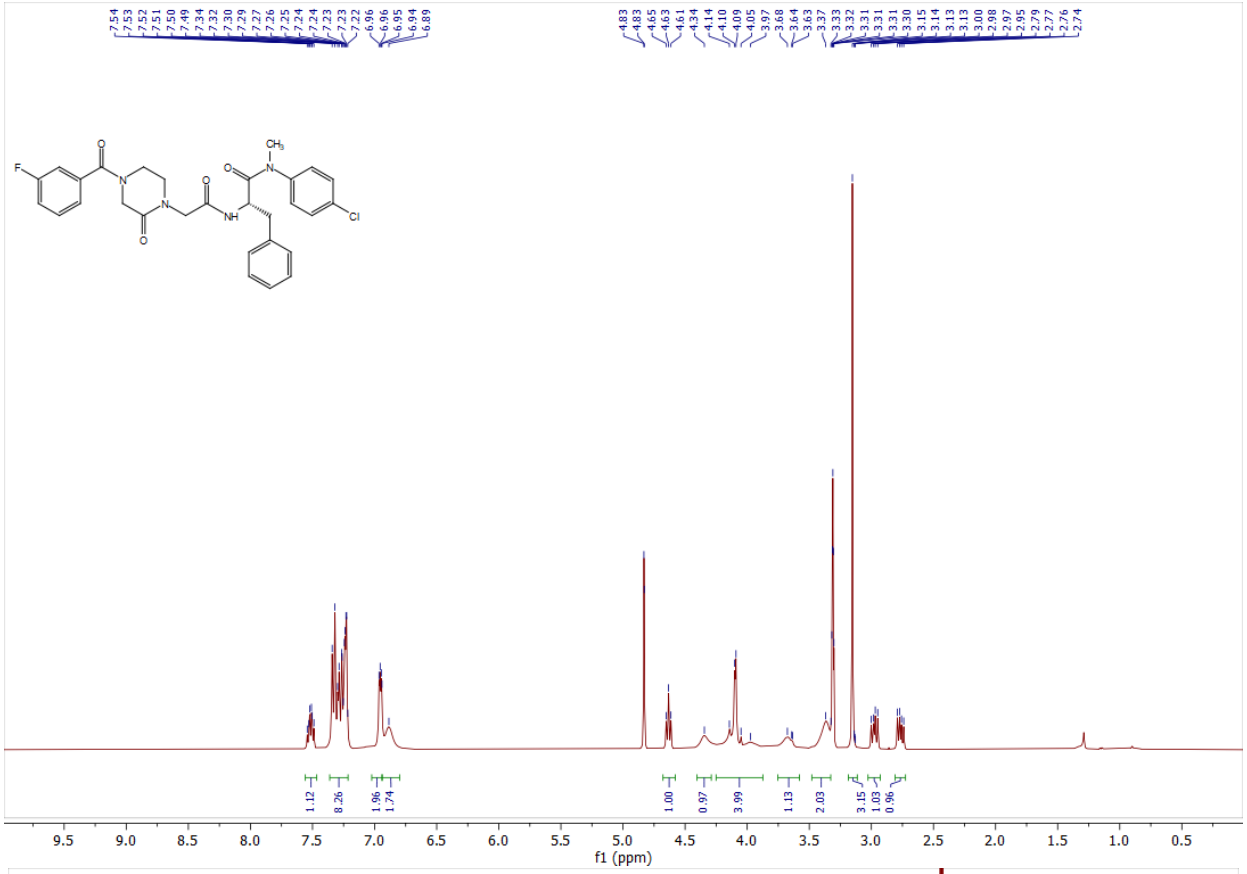


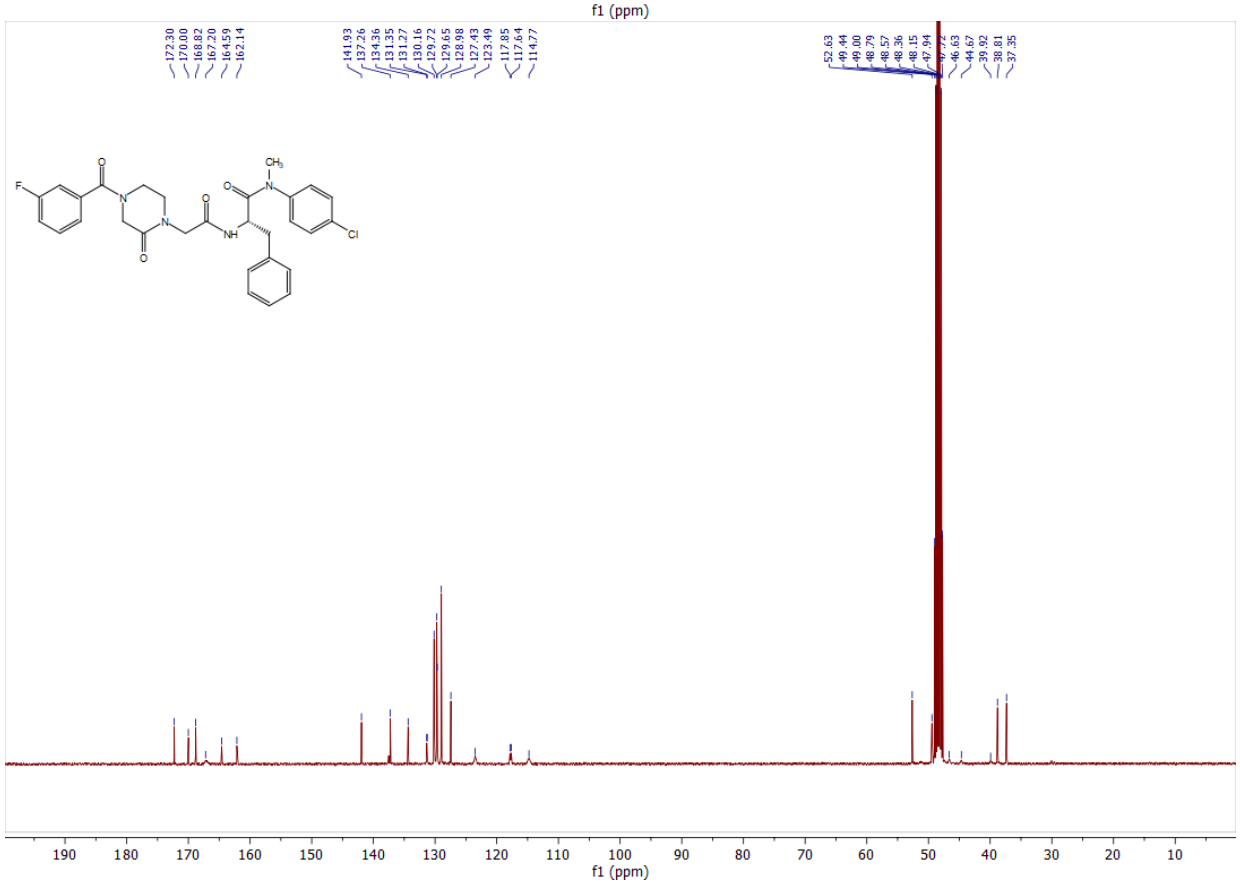
^{1}H and ^{13}C spectra of compound **4g**



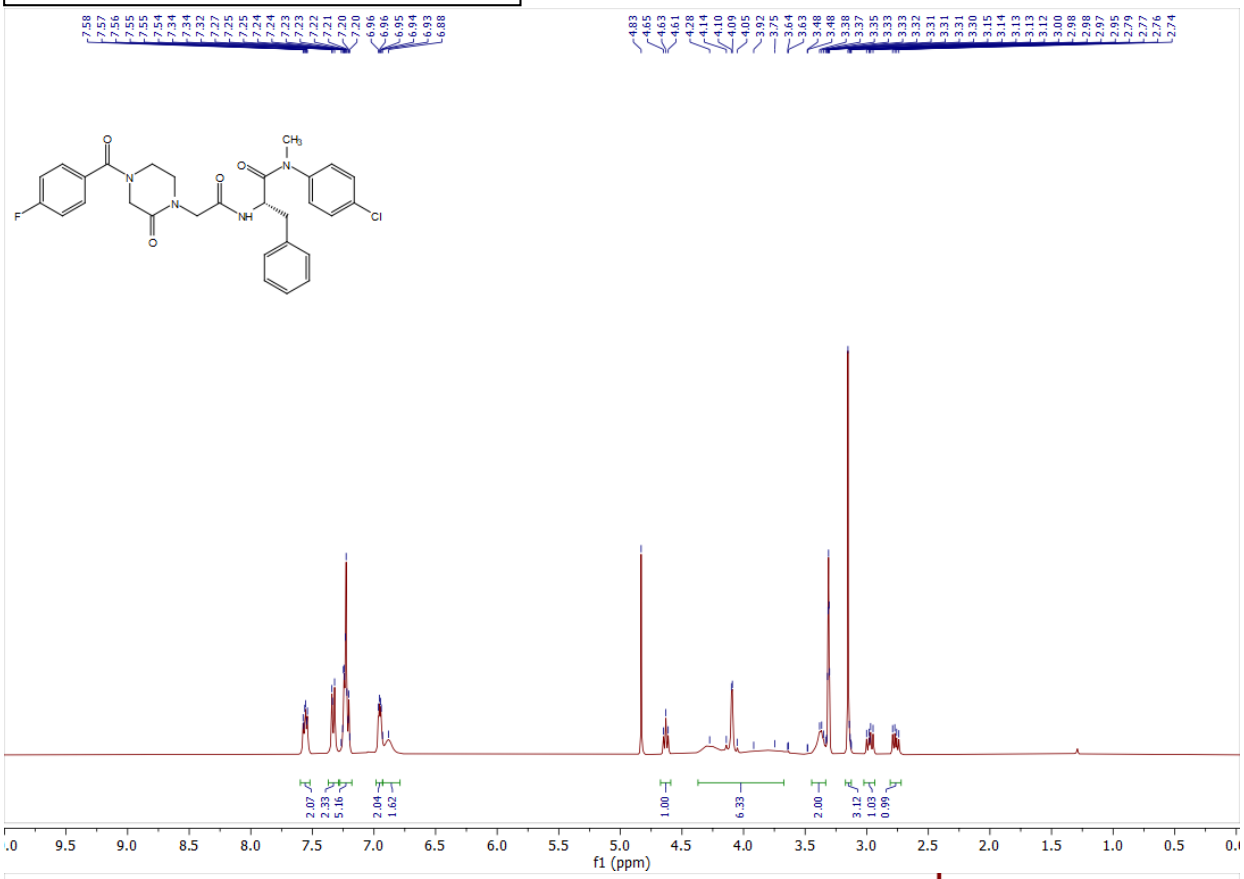


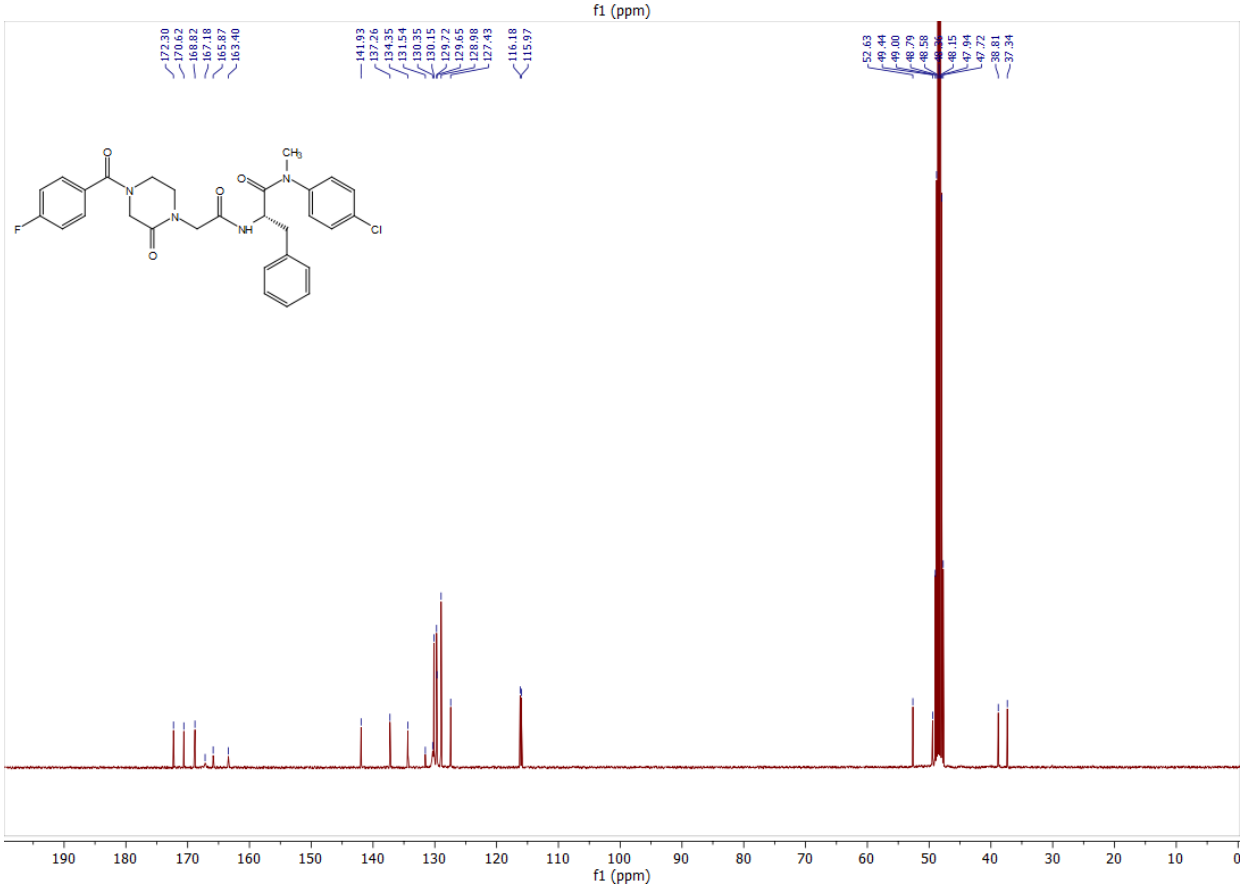
^{1}H and ^{13}C spectra of compound **4h**



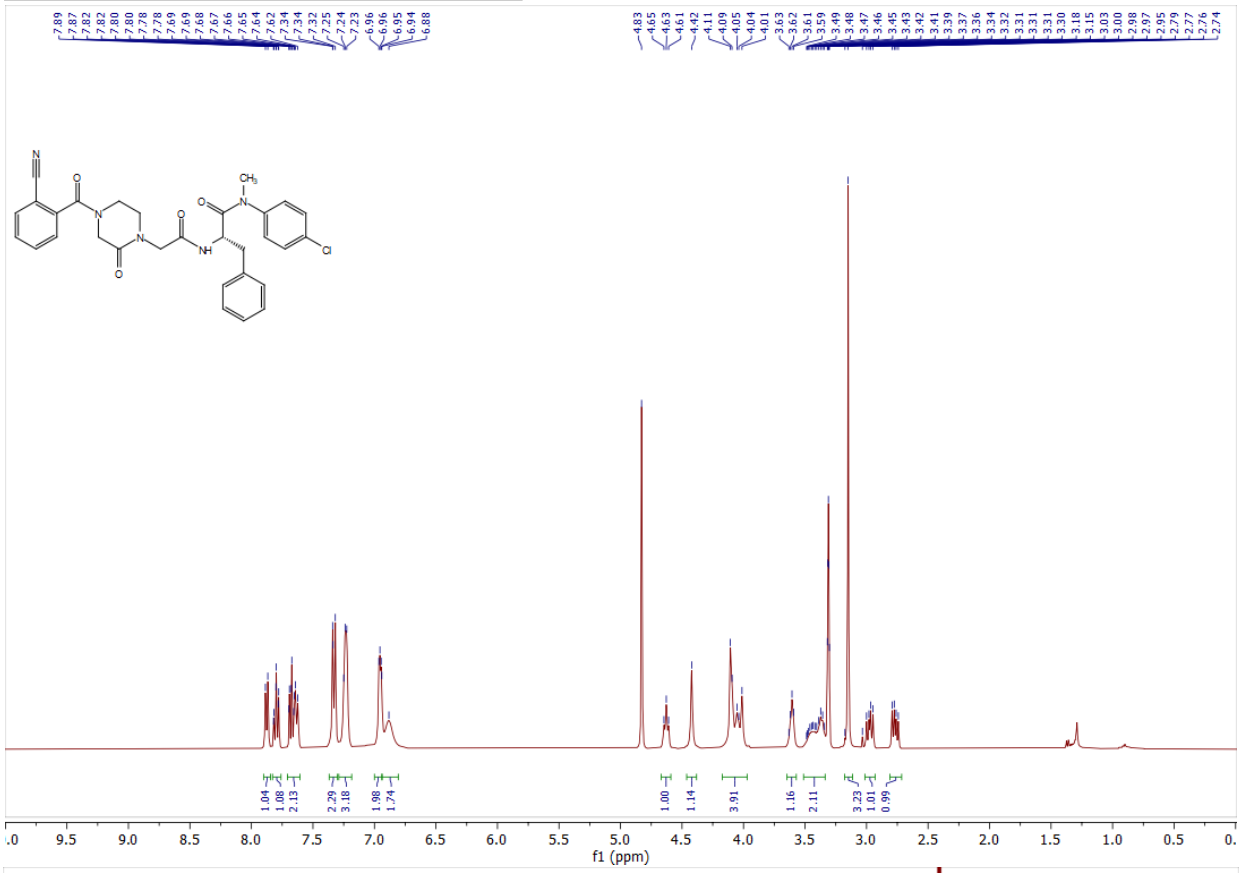


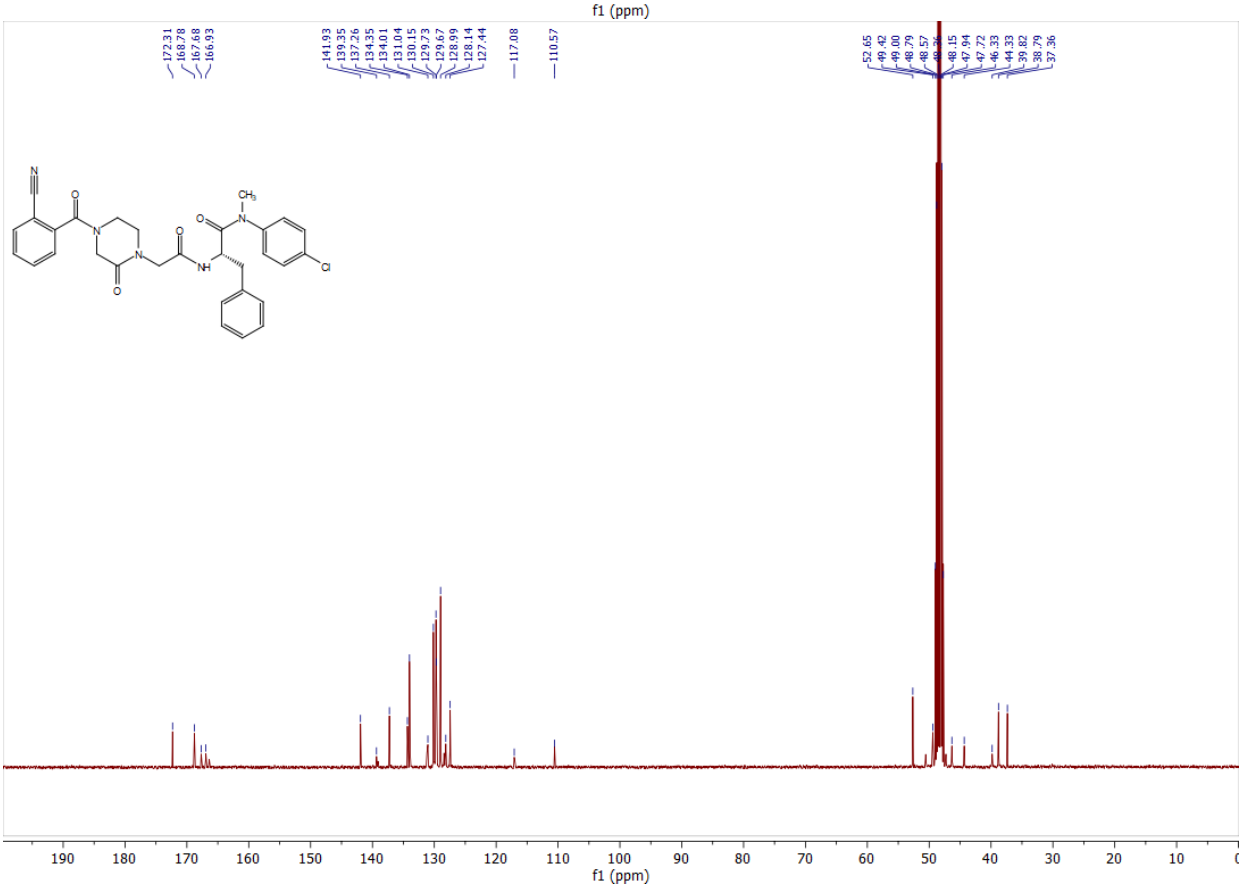
^{1}H and ^{13}C spectra of compound **4i**



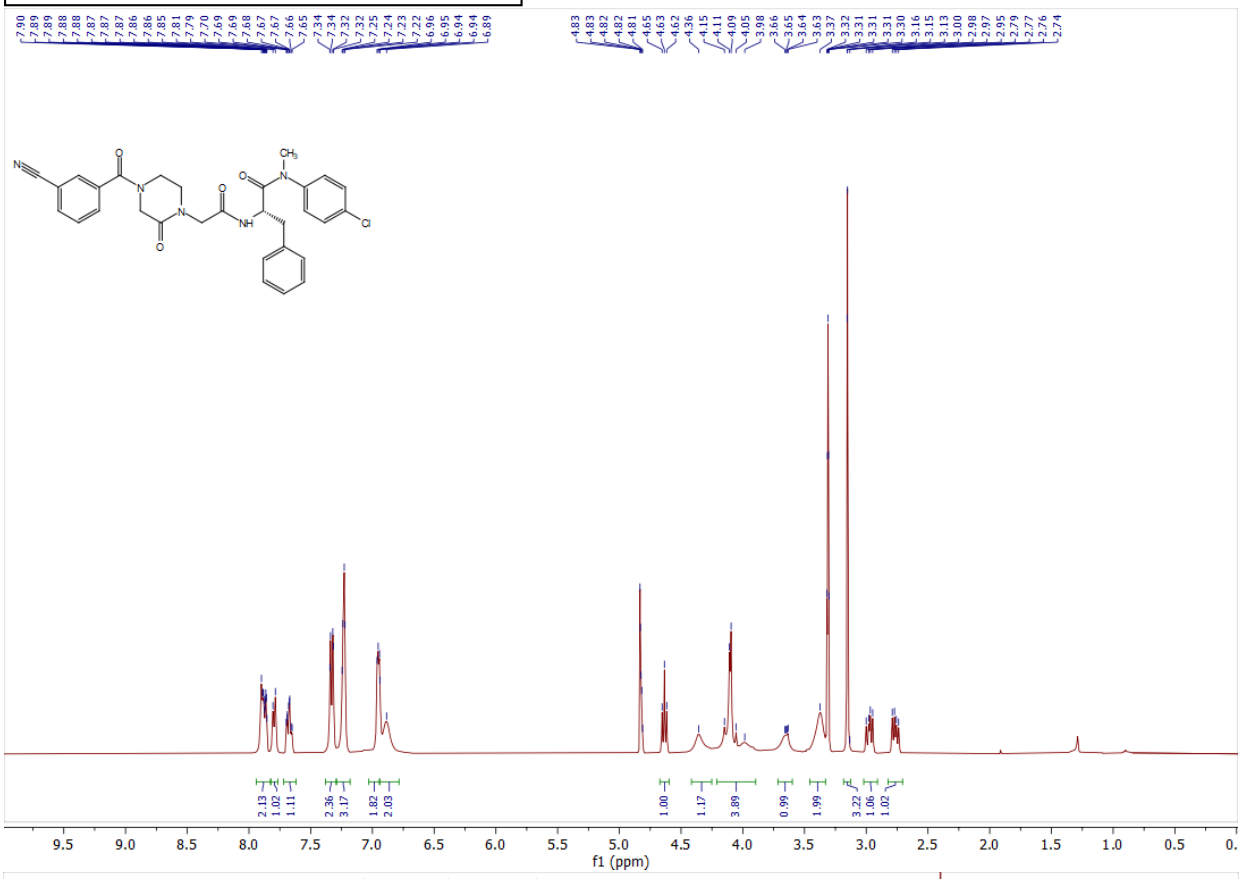


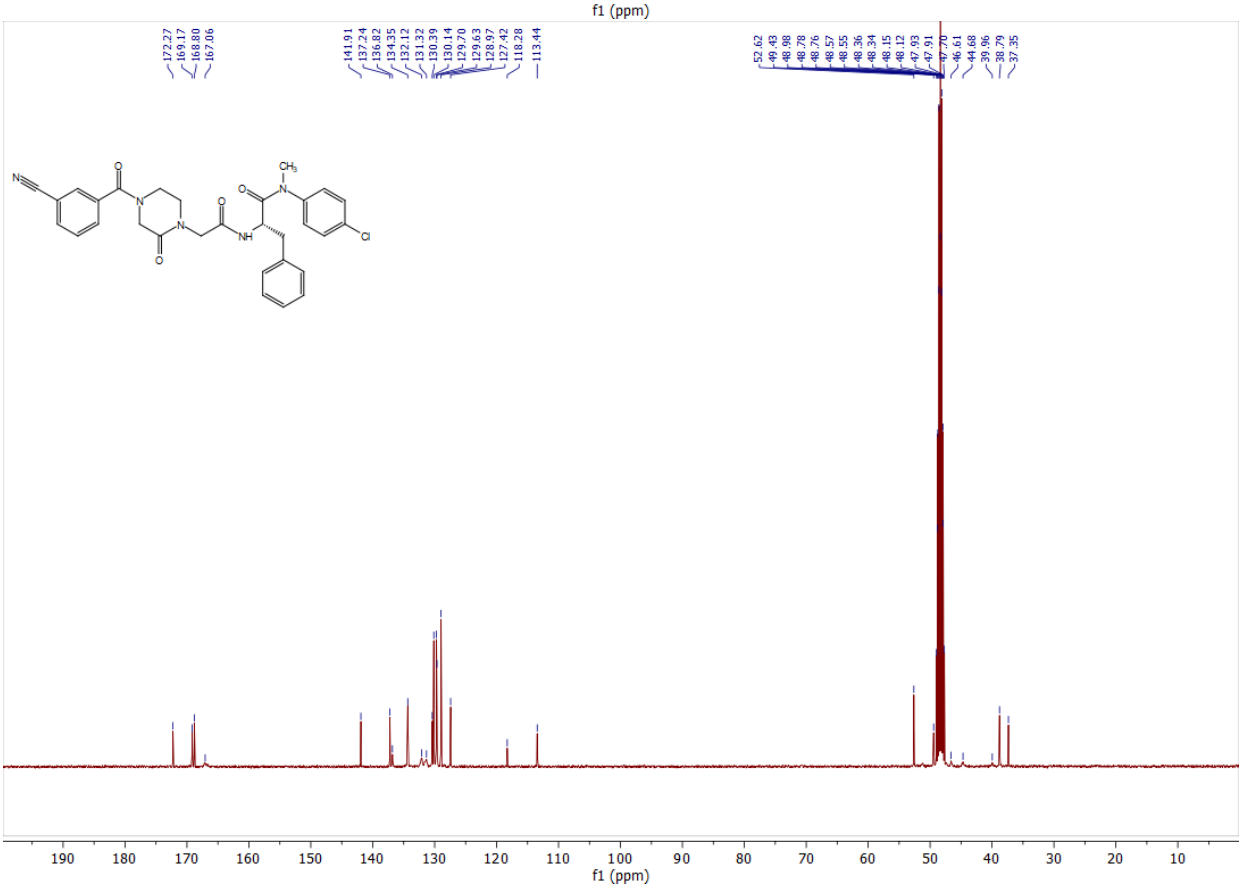
^{1}H and ^{13}C spectra of compound **4j**



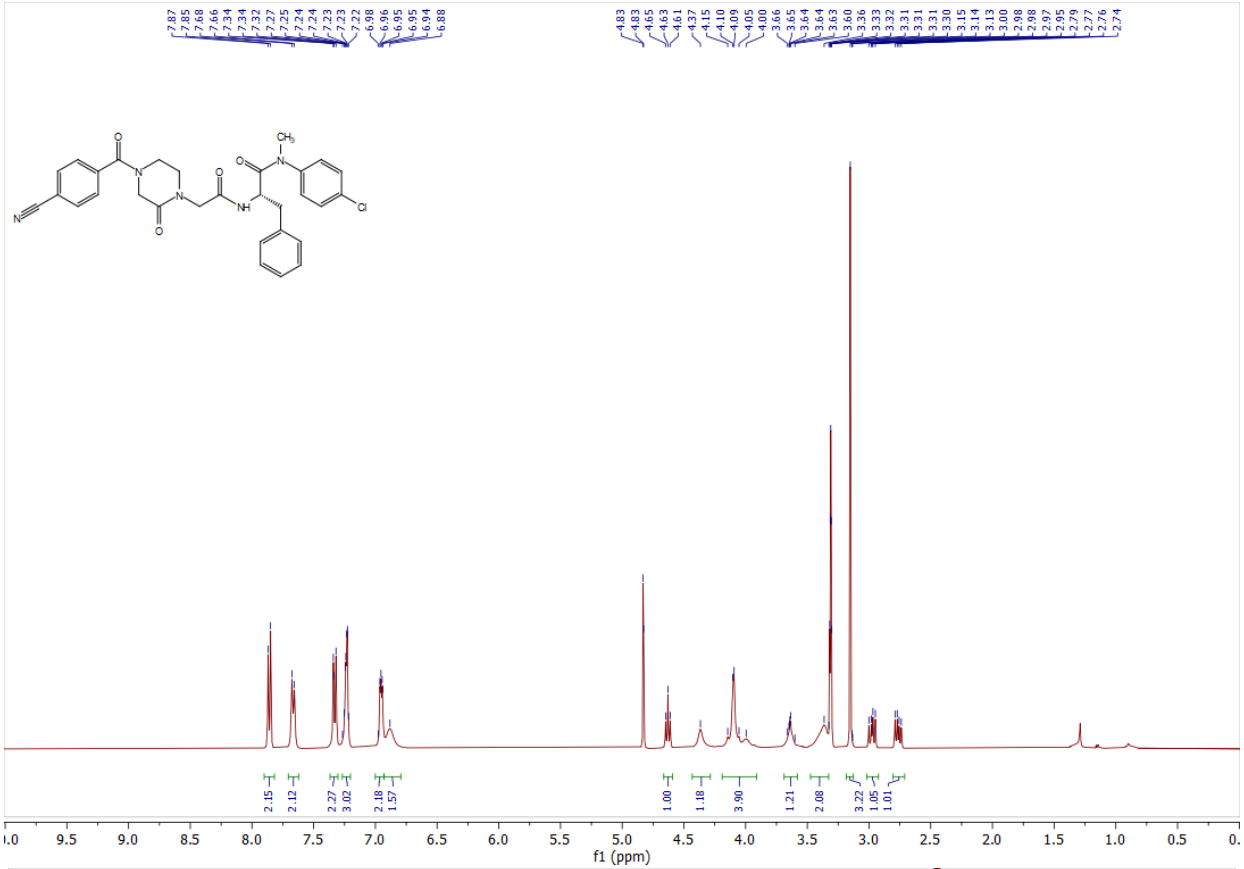


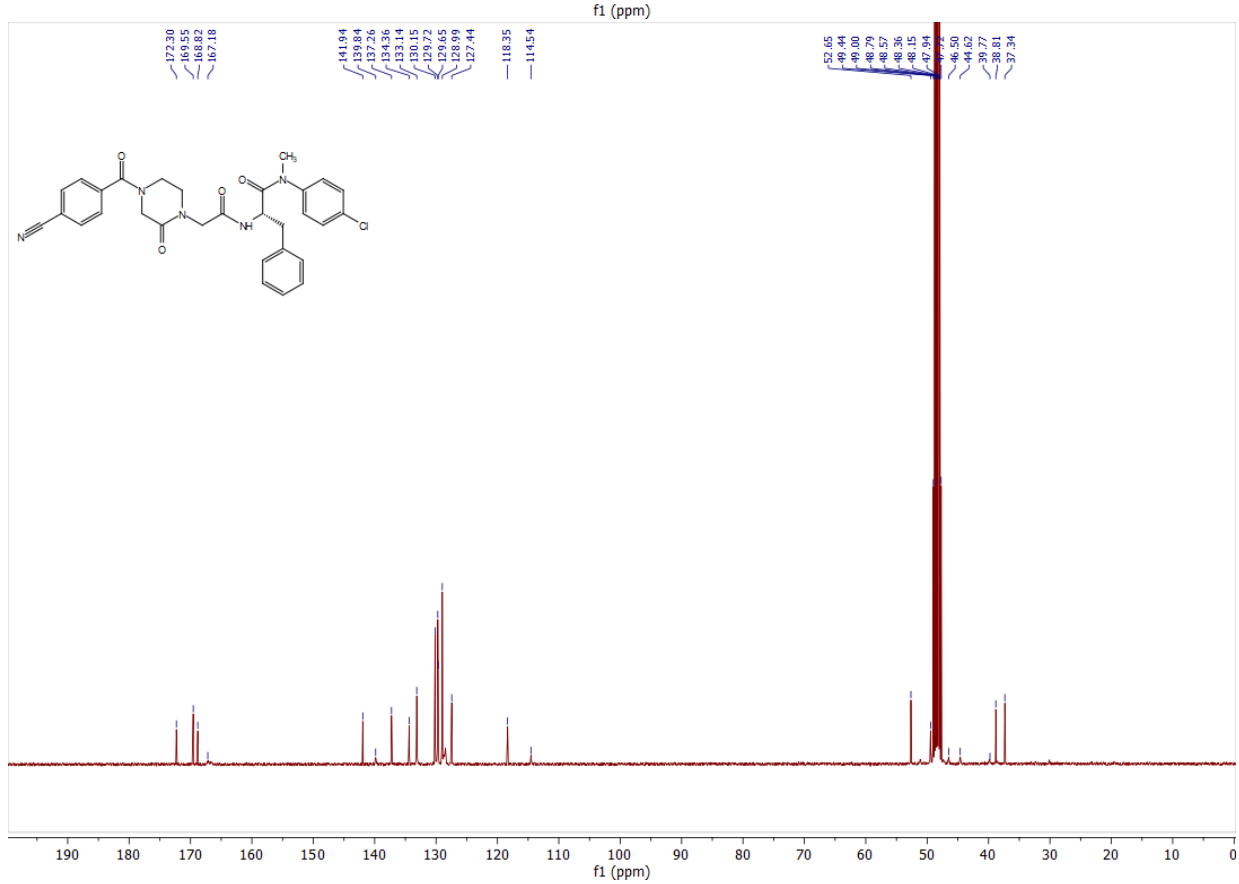
1H and ^{13}C spectra of compound **4k**



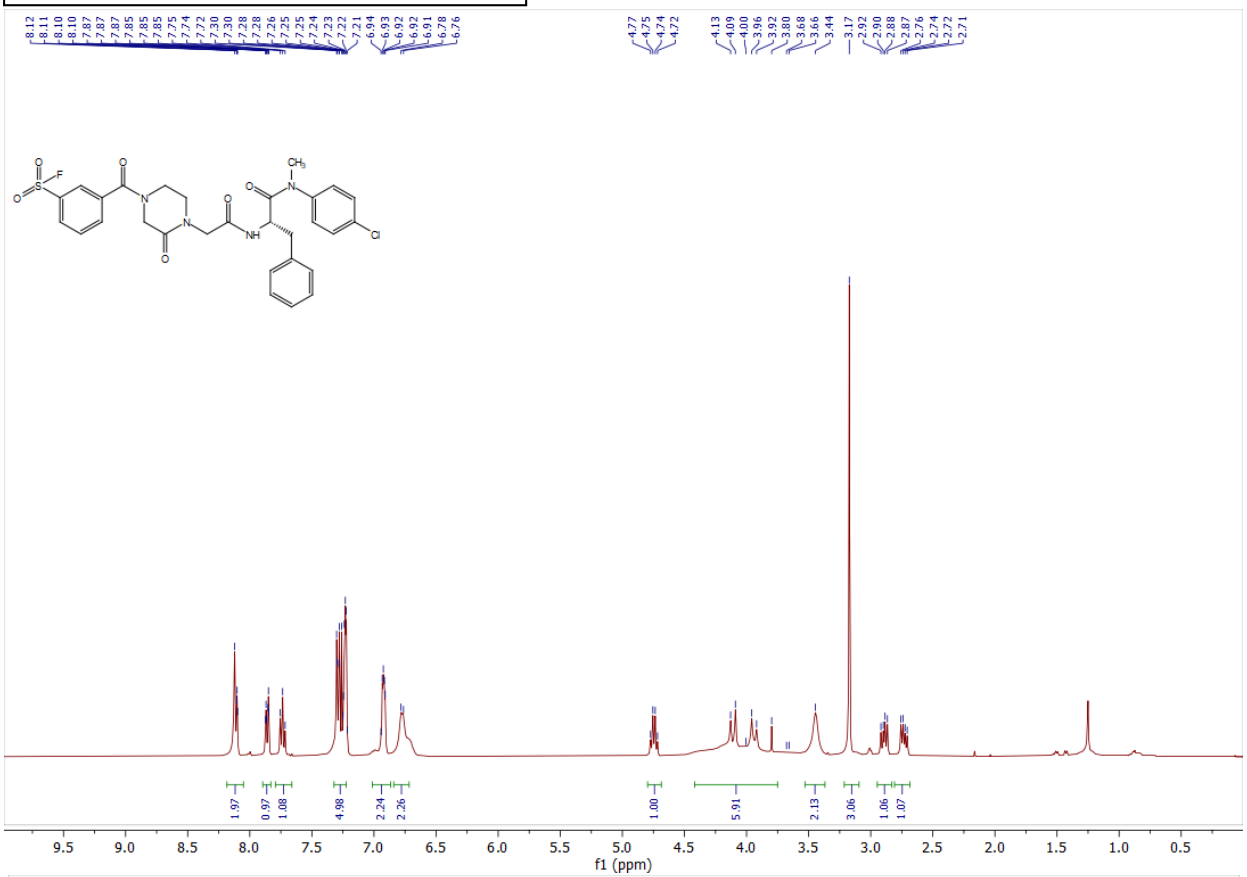


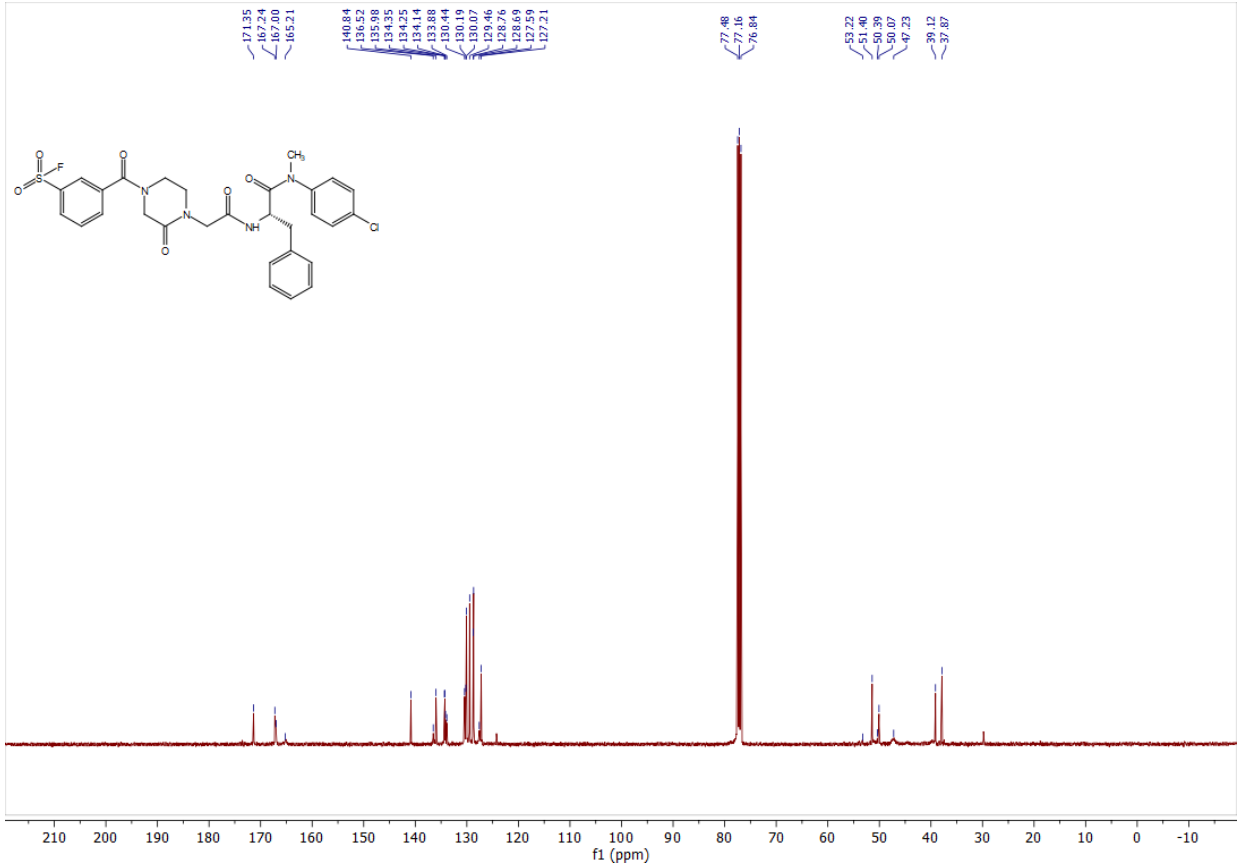
^{1}H and ^{13}C spectra of compound **4l**



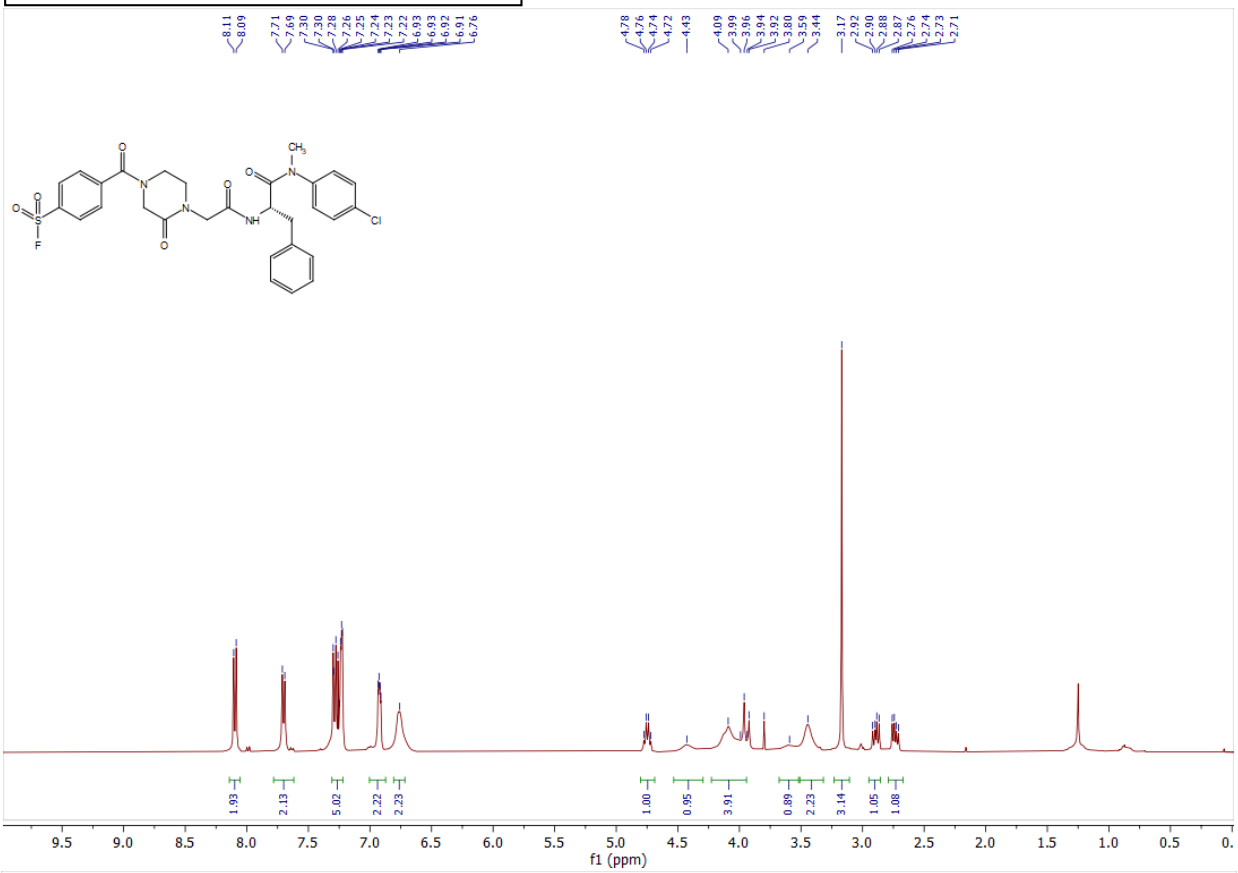


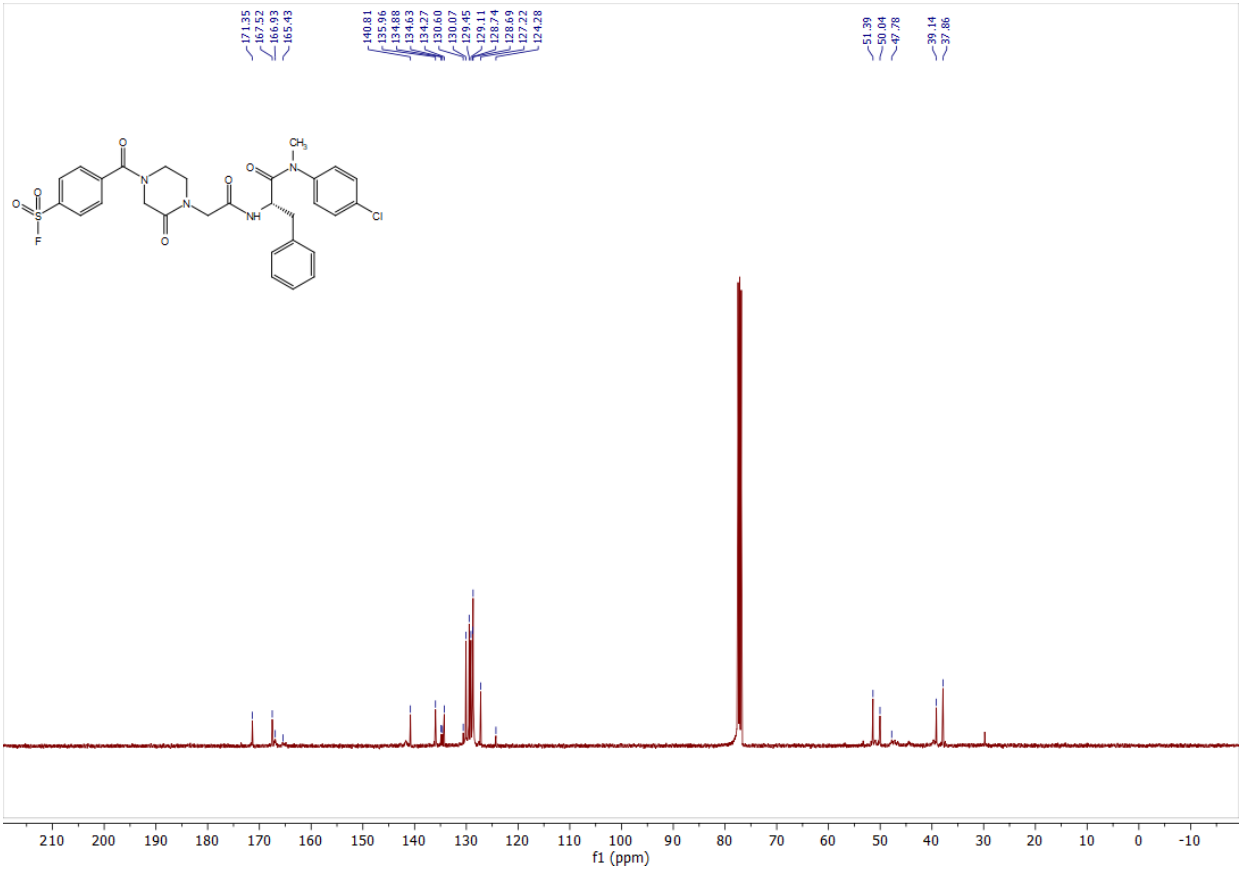
^{1}H and ^{13}C spectra of compound **4m**



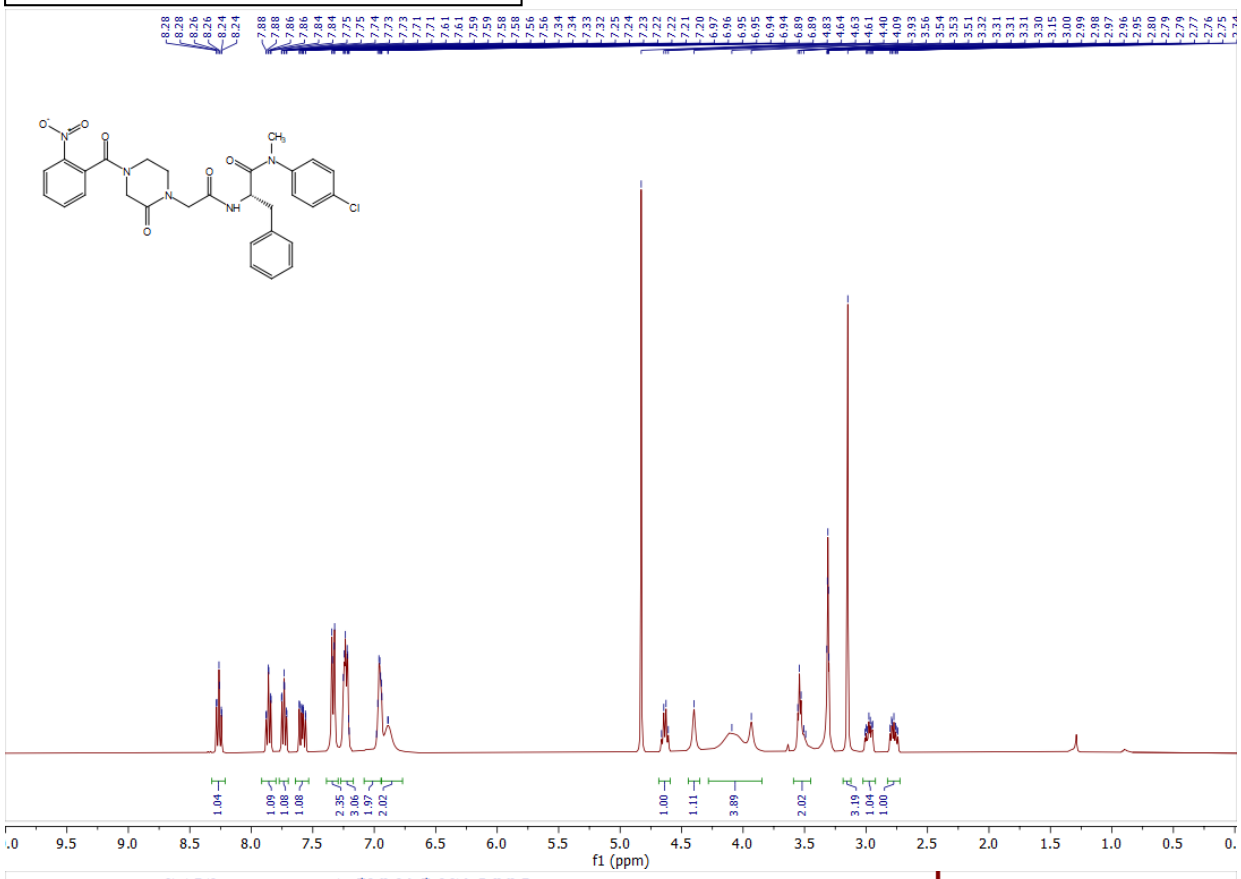


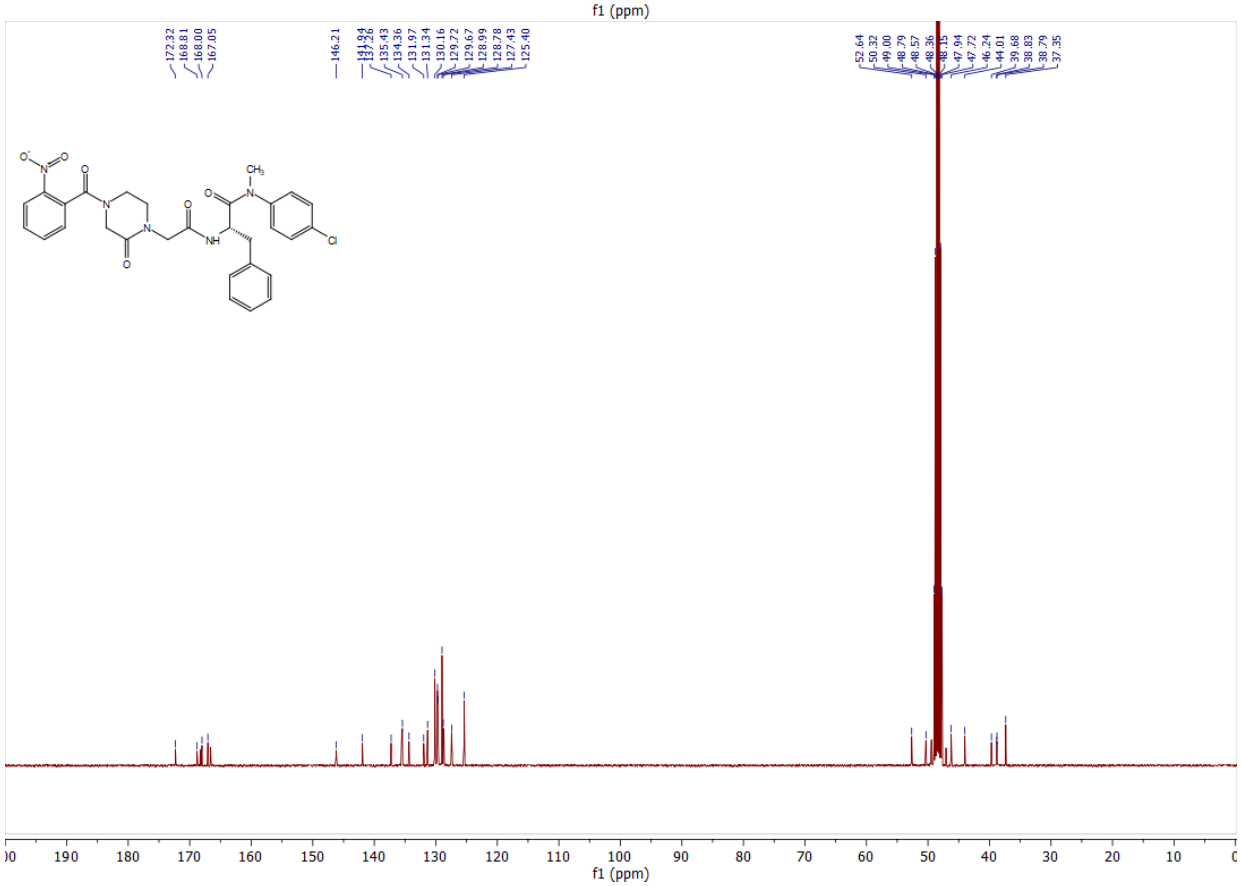
^{1}H and ^{13}C spectra of compound **4n**



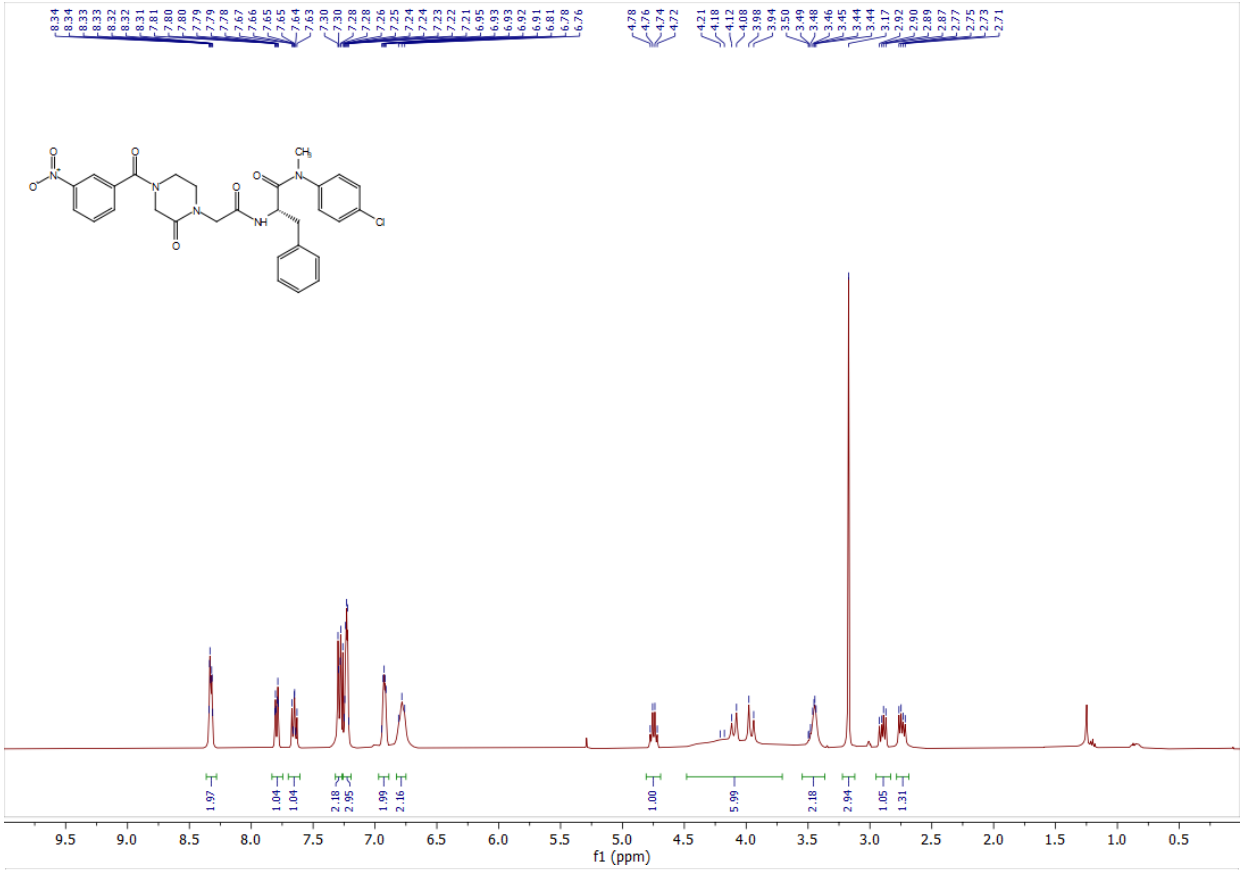


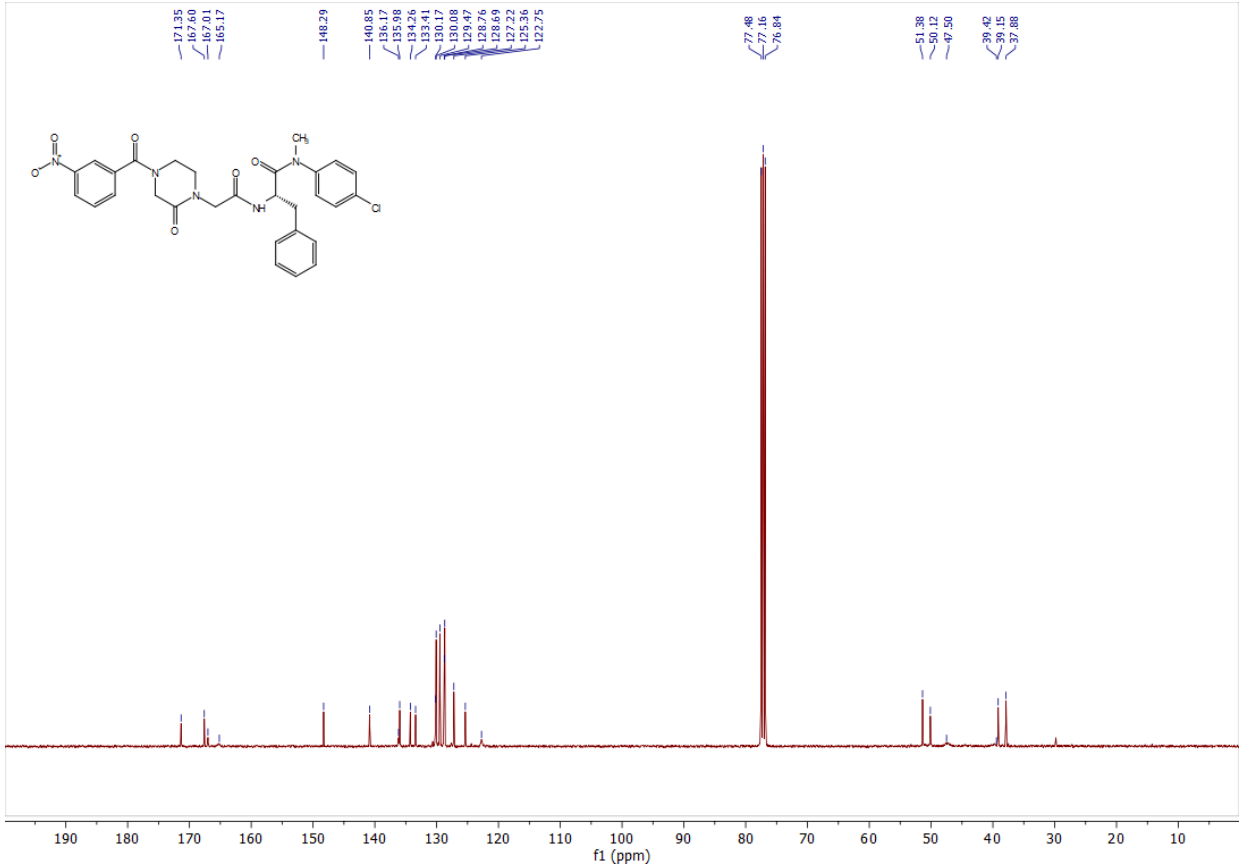
^{1}H and ^{13}C spectra of compound **4o**



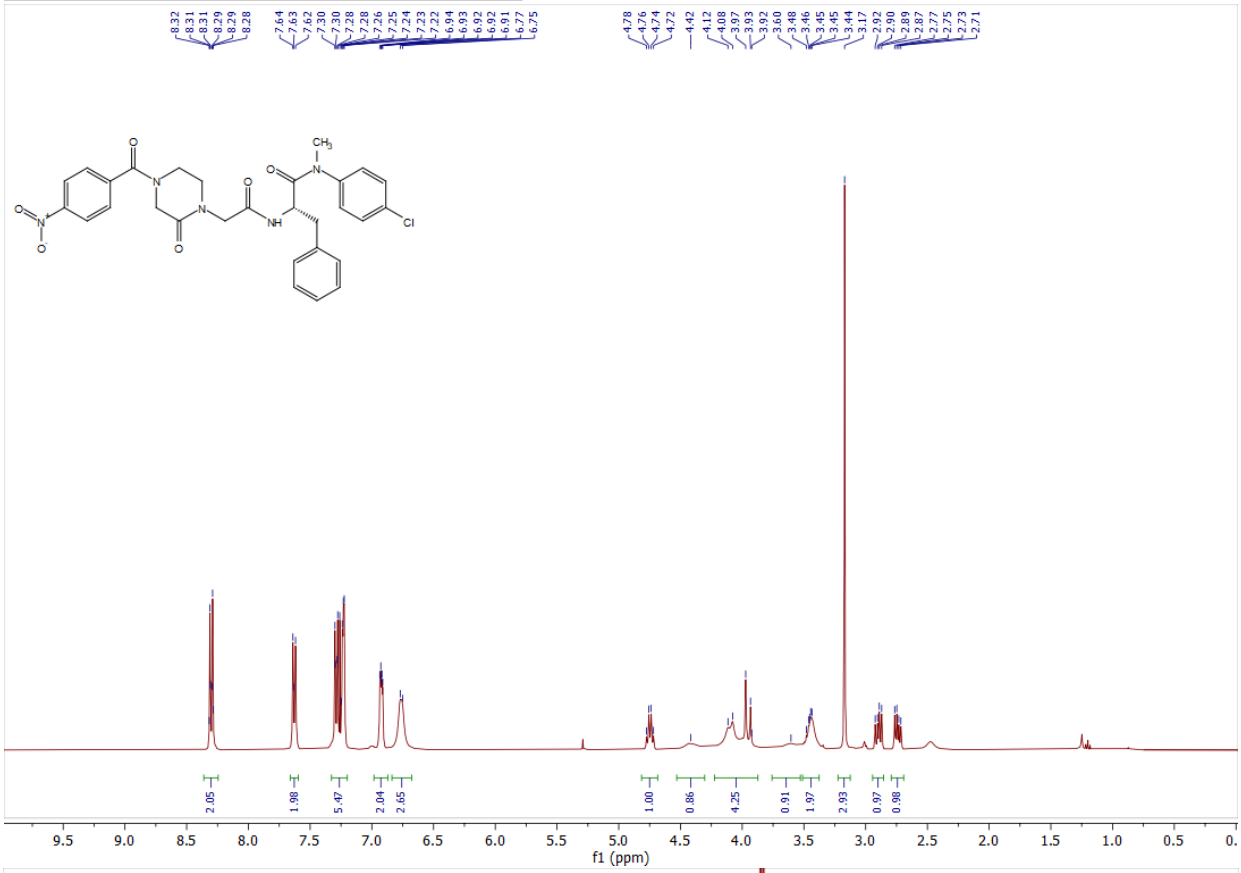


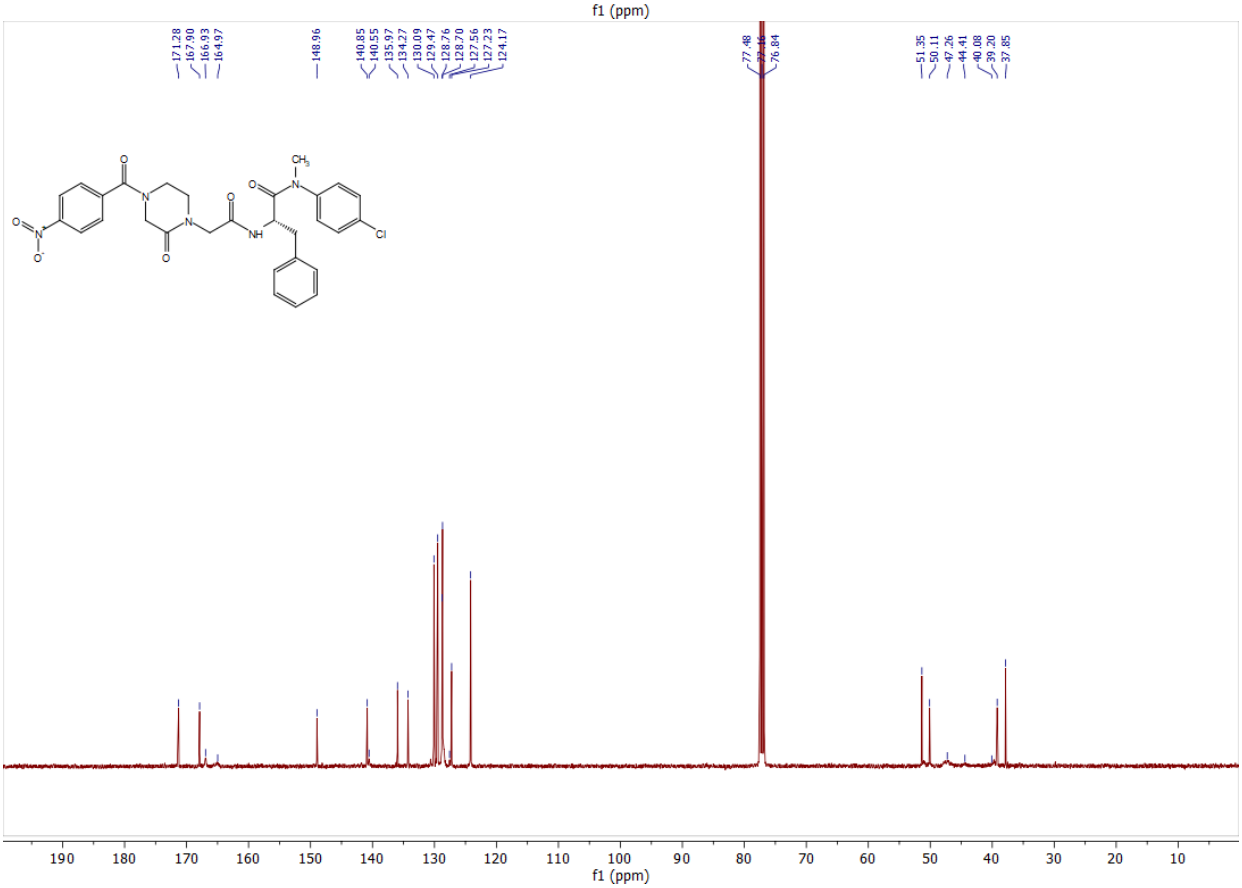
^{1}H and ^{13}C spectra of compound **4p**



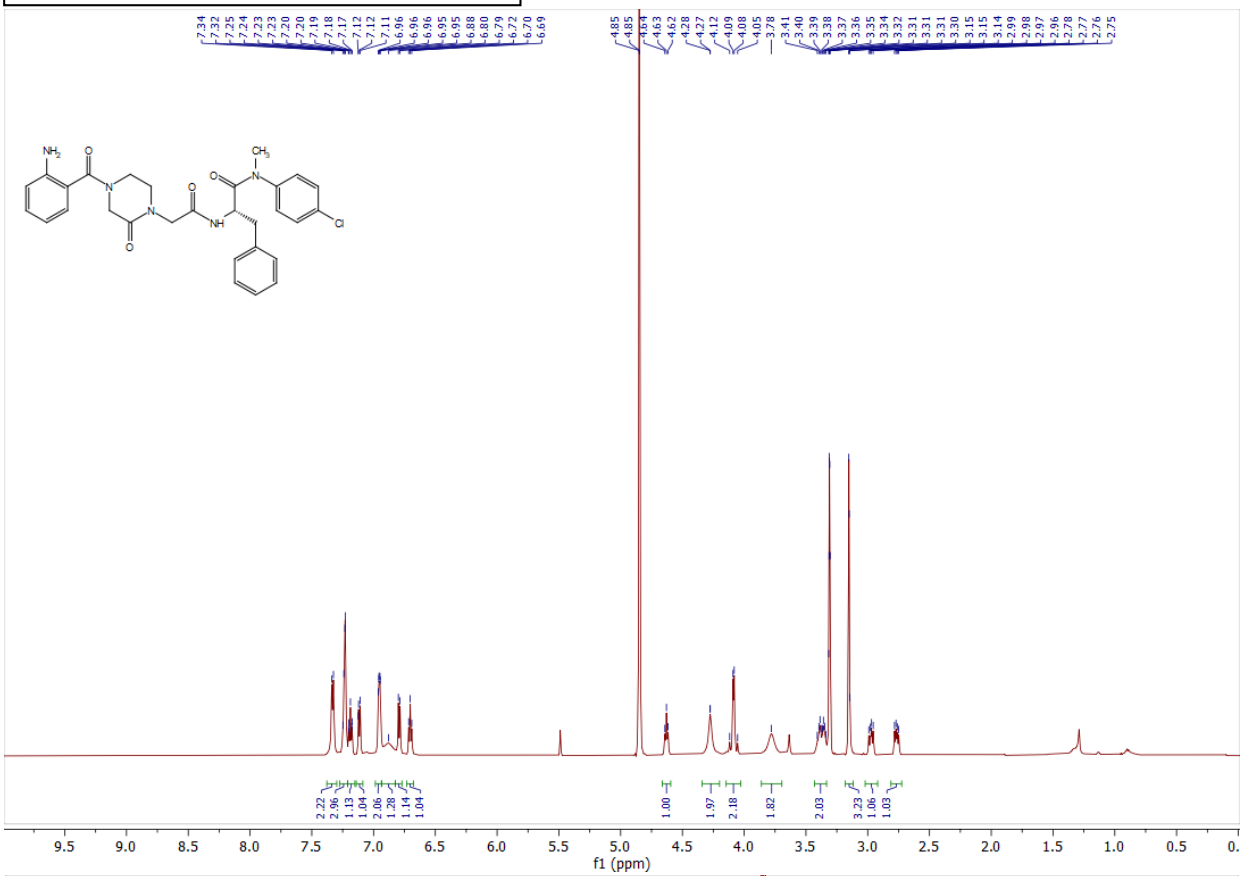


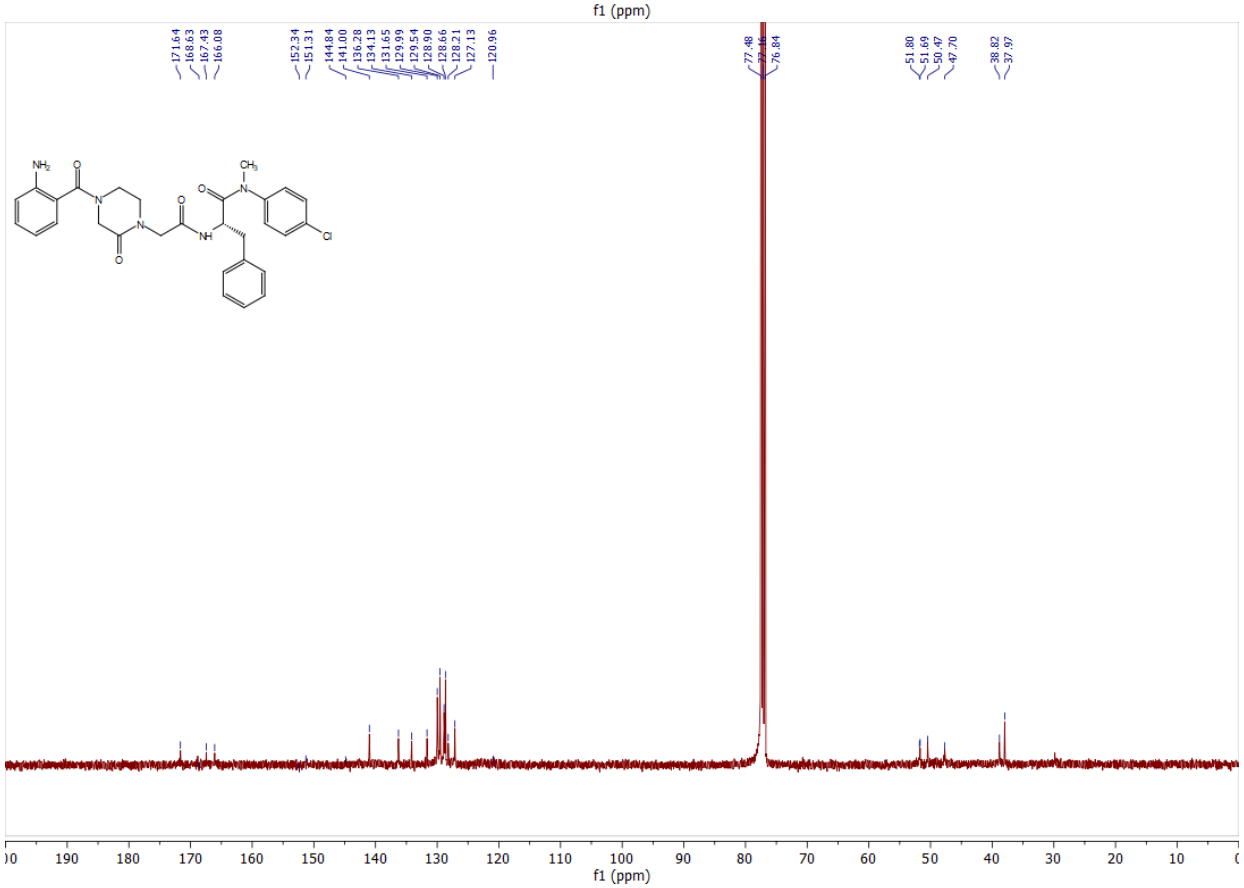
^{1}H and ^{13}C spectra of compound **4q**



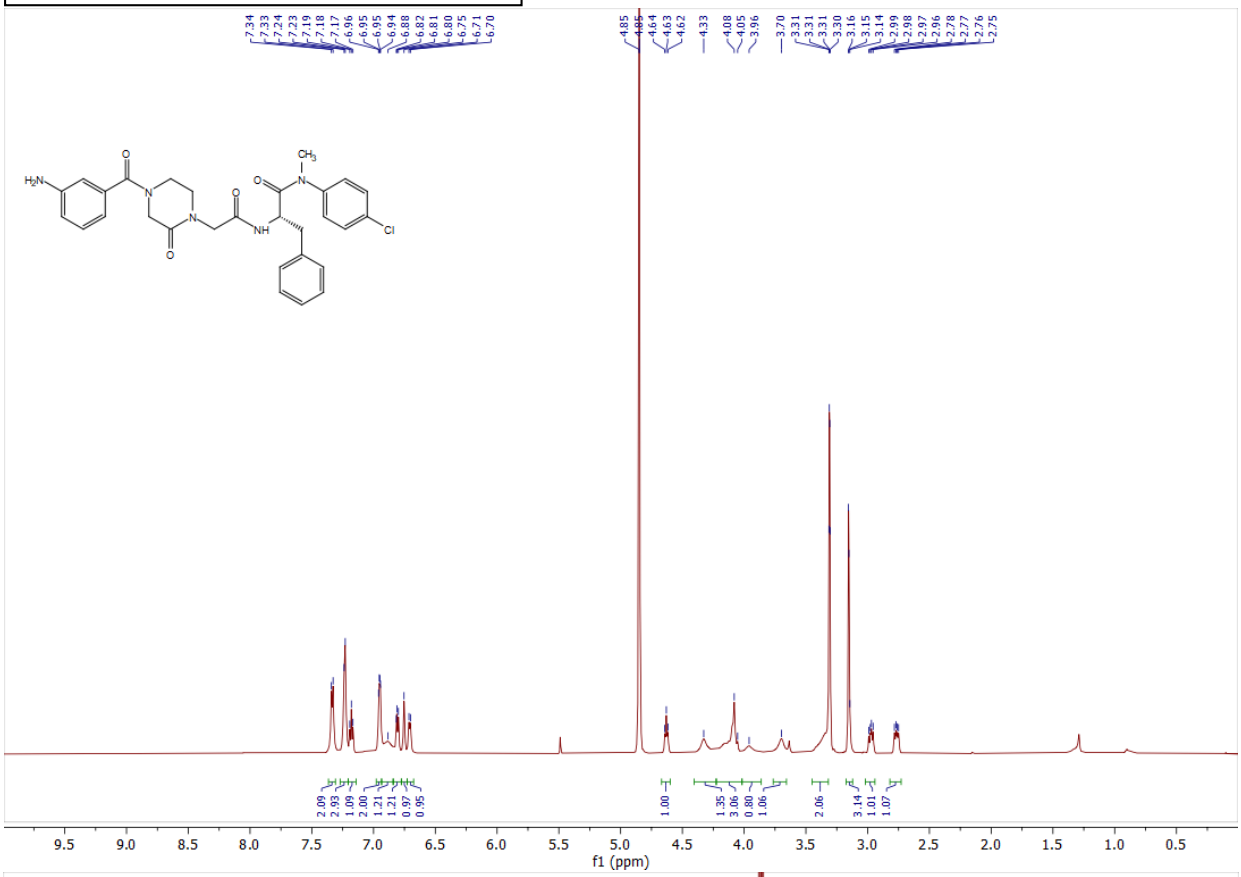


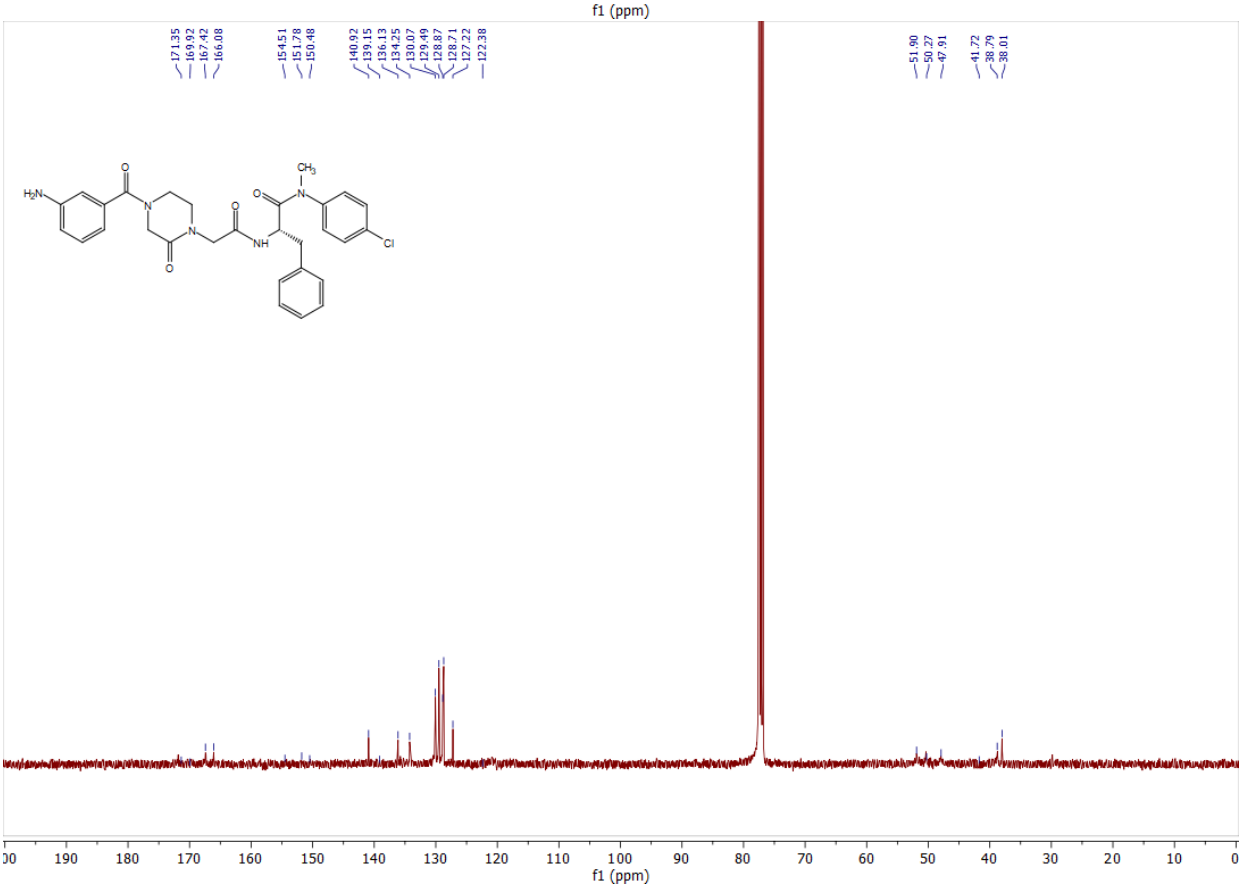
^{1}H and ^{13}C spectra of compound **4r**



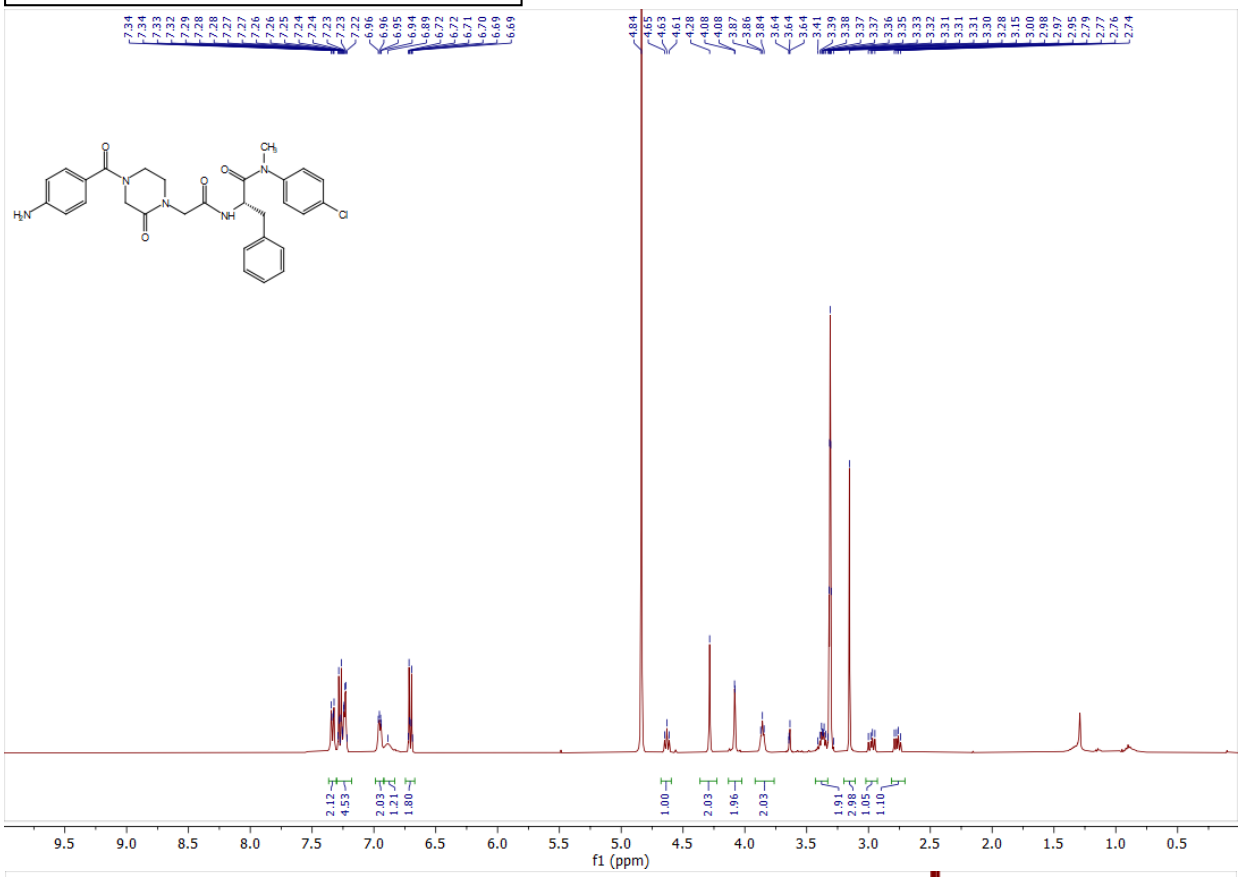


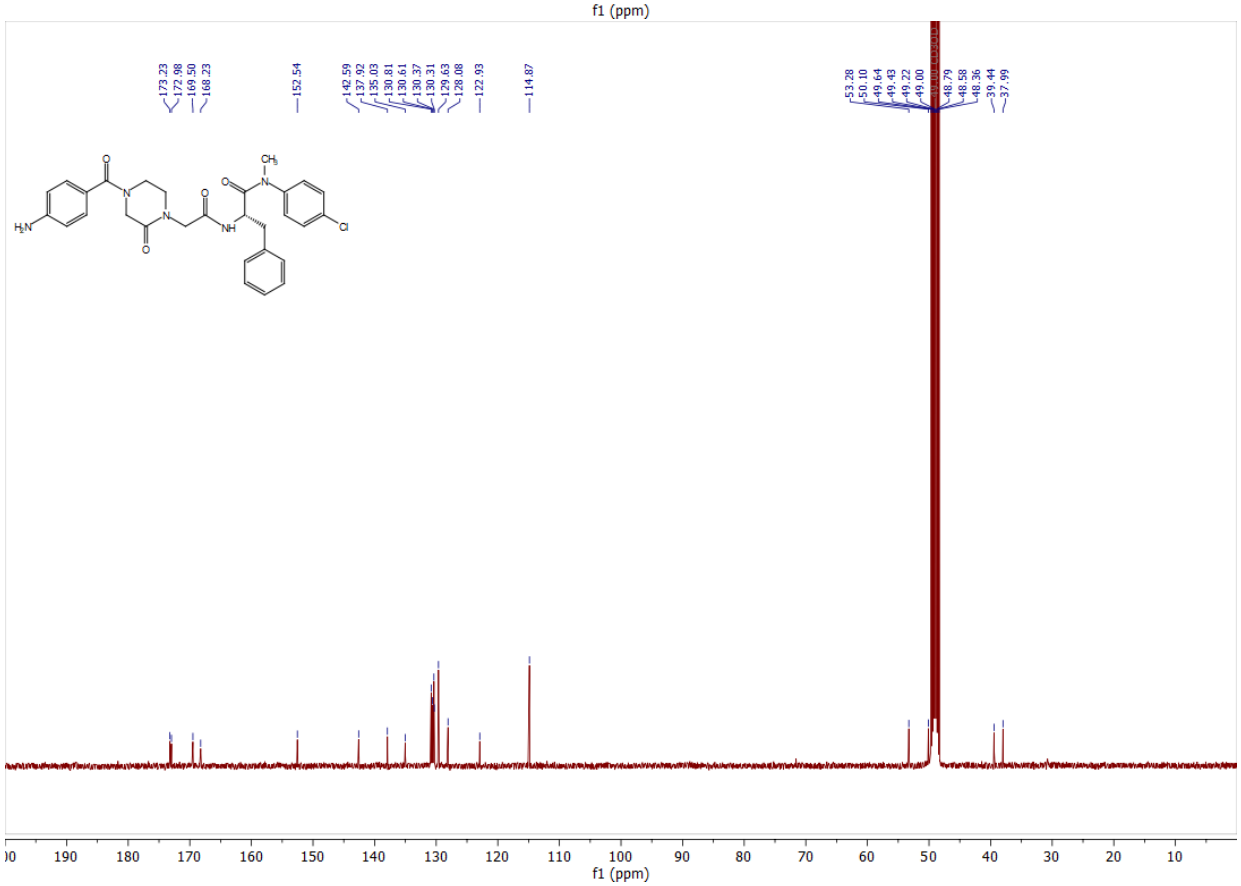
^{1}H and ^{13}C spectra of compound **4s**



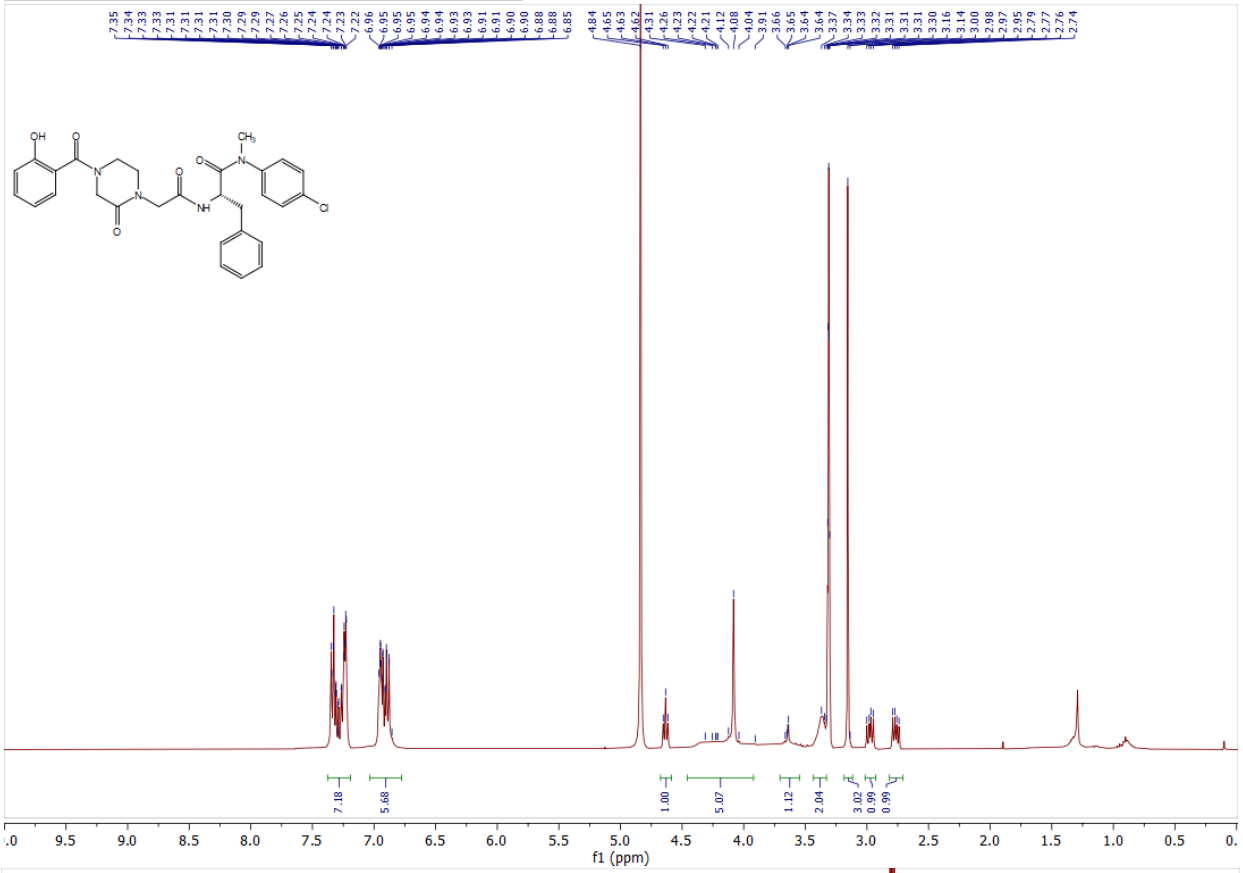


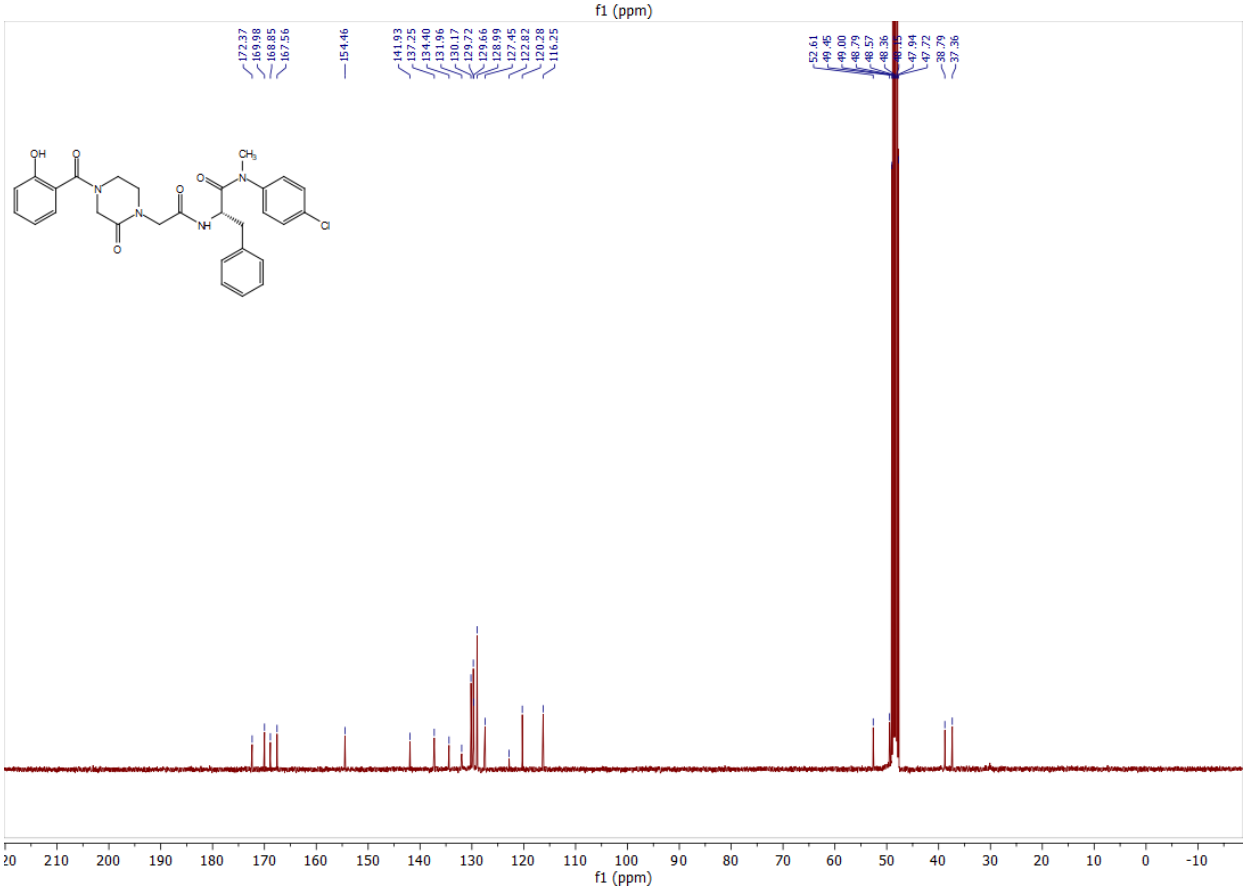
^{1}H and ^{13}C spectra of compound **4t**



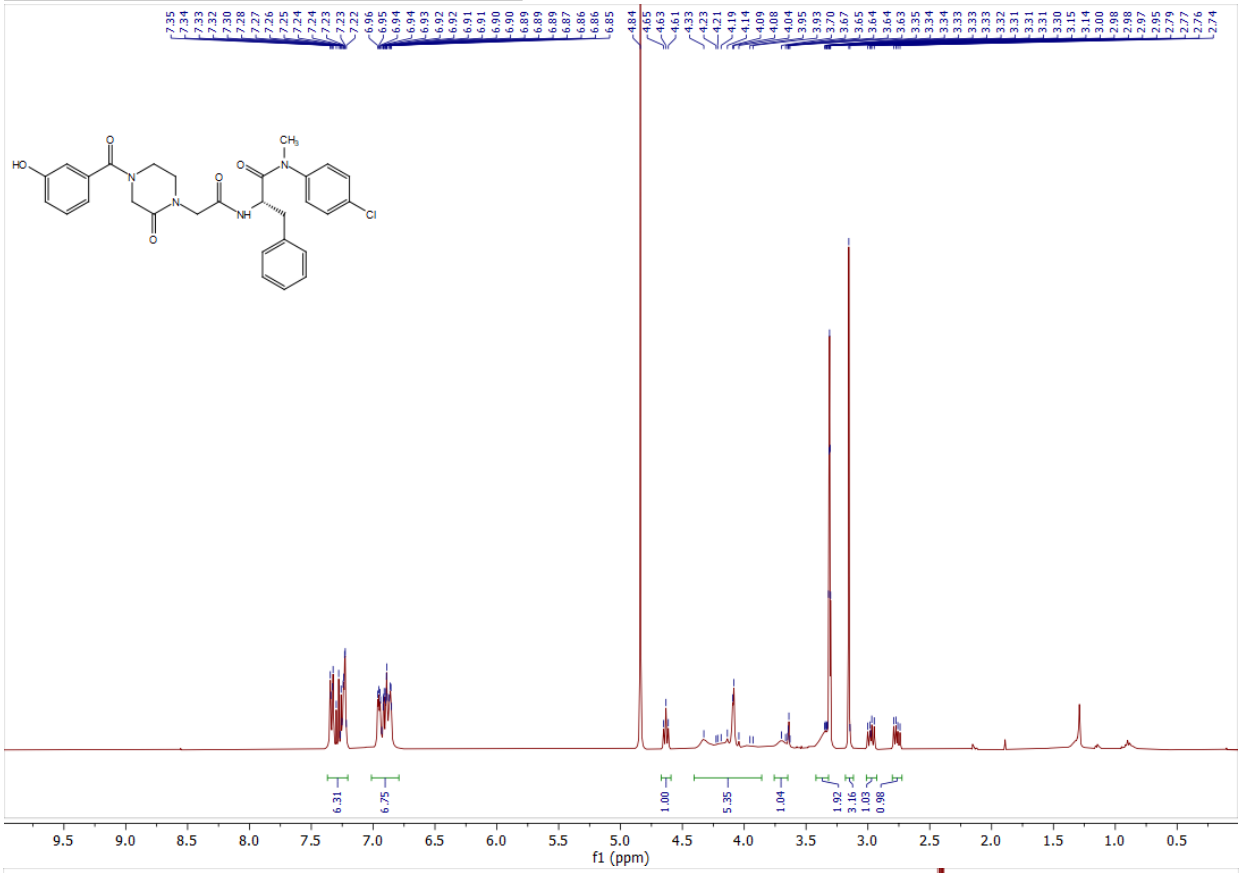


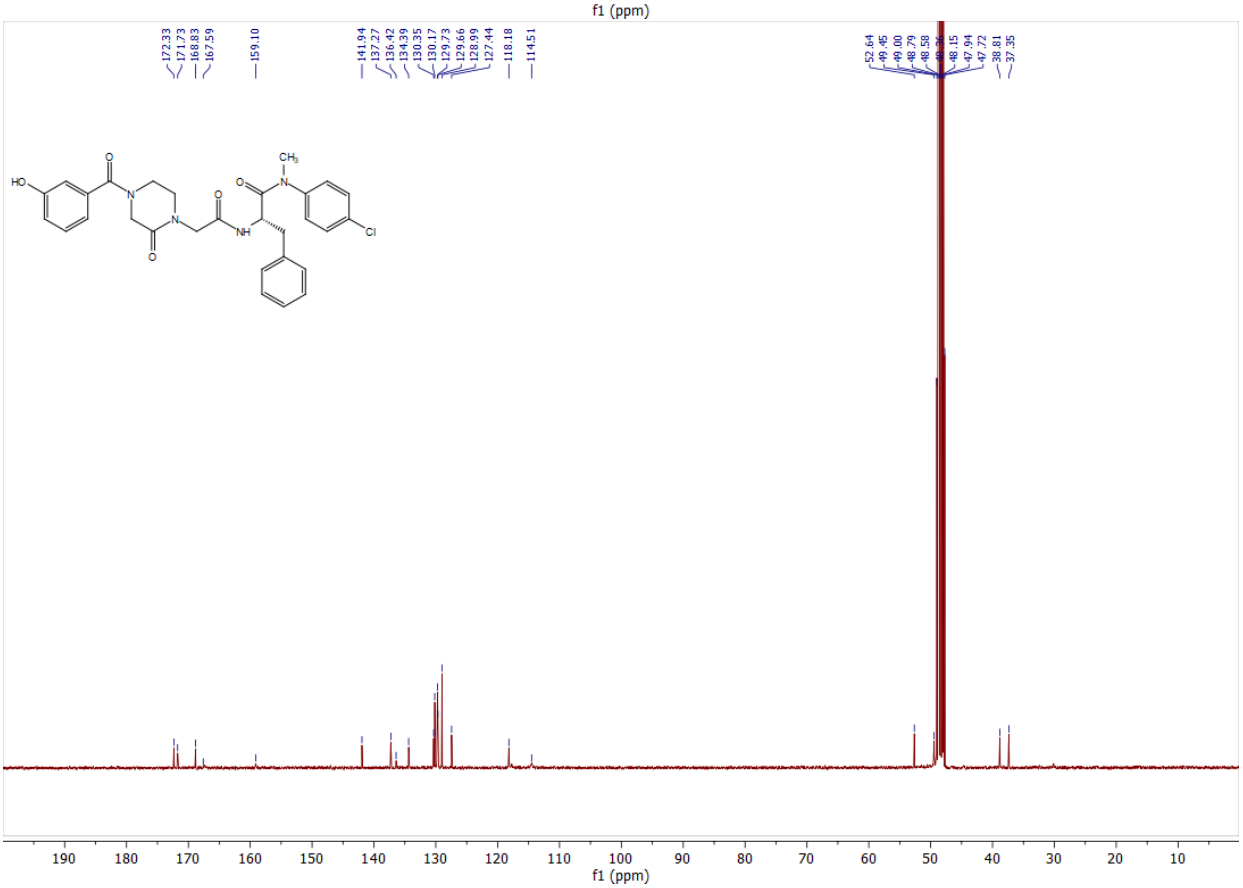
^{1}H and ^{13}C spectra of compound **4u**



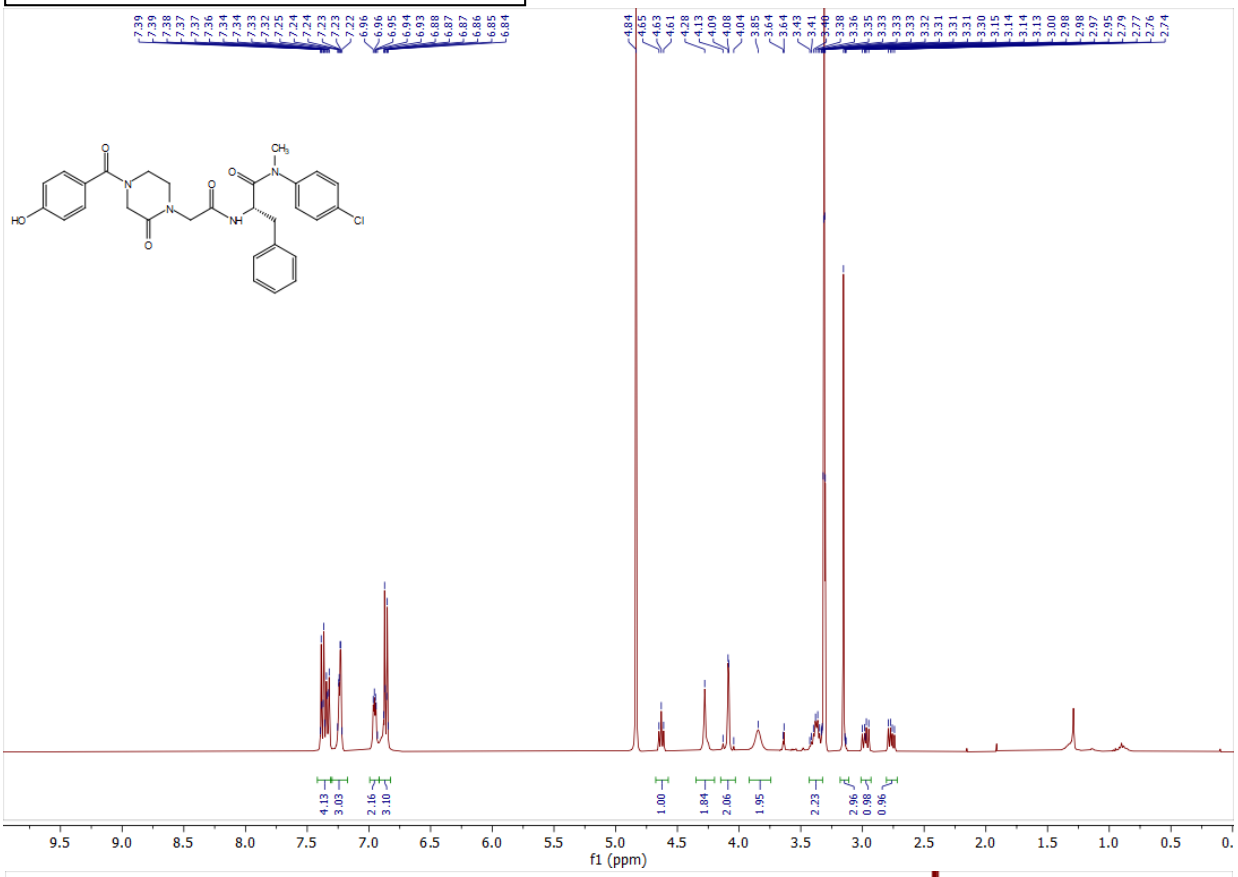


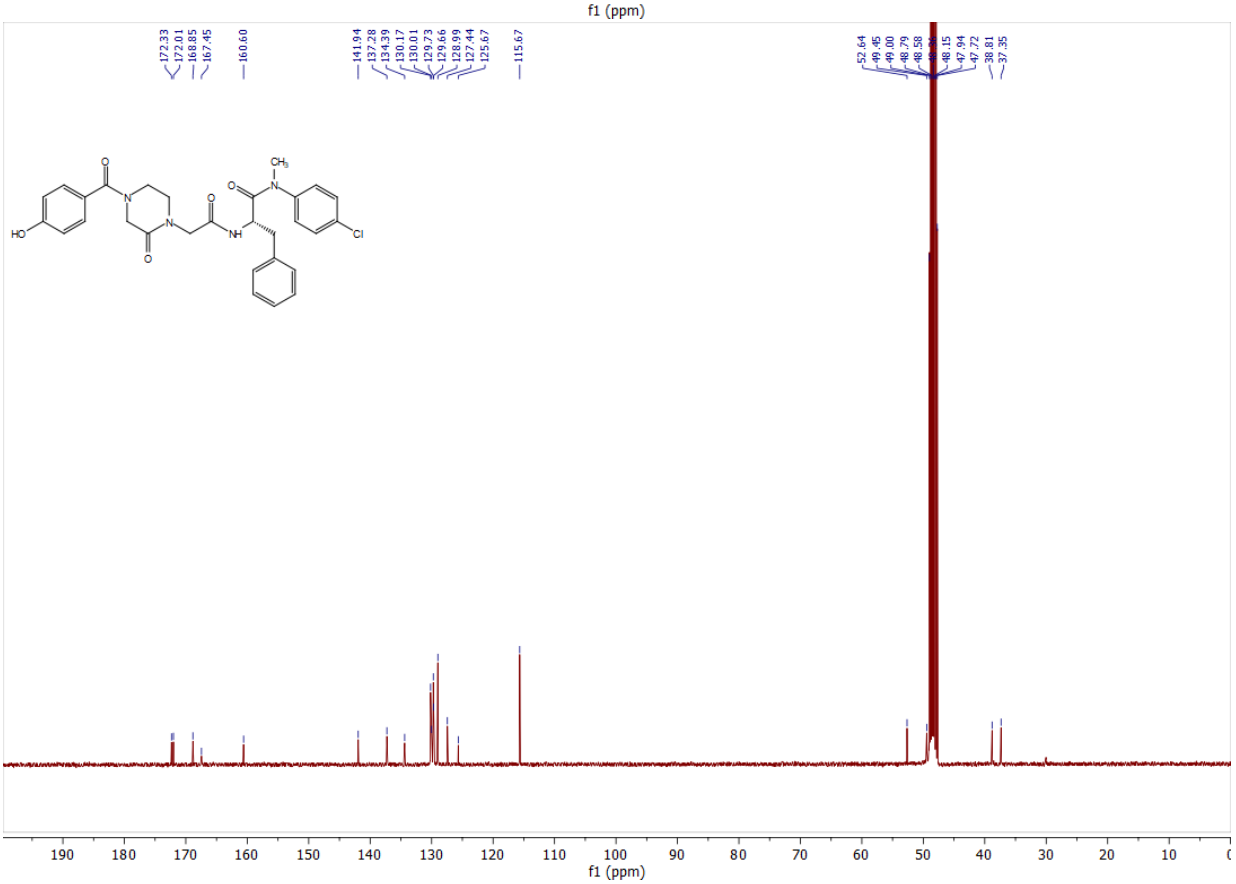
^{1}H and ^{13}C spectra of compound **4v**



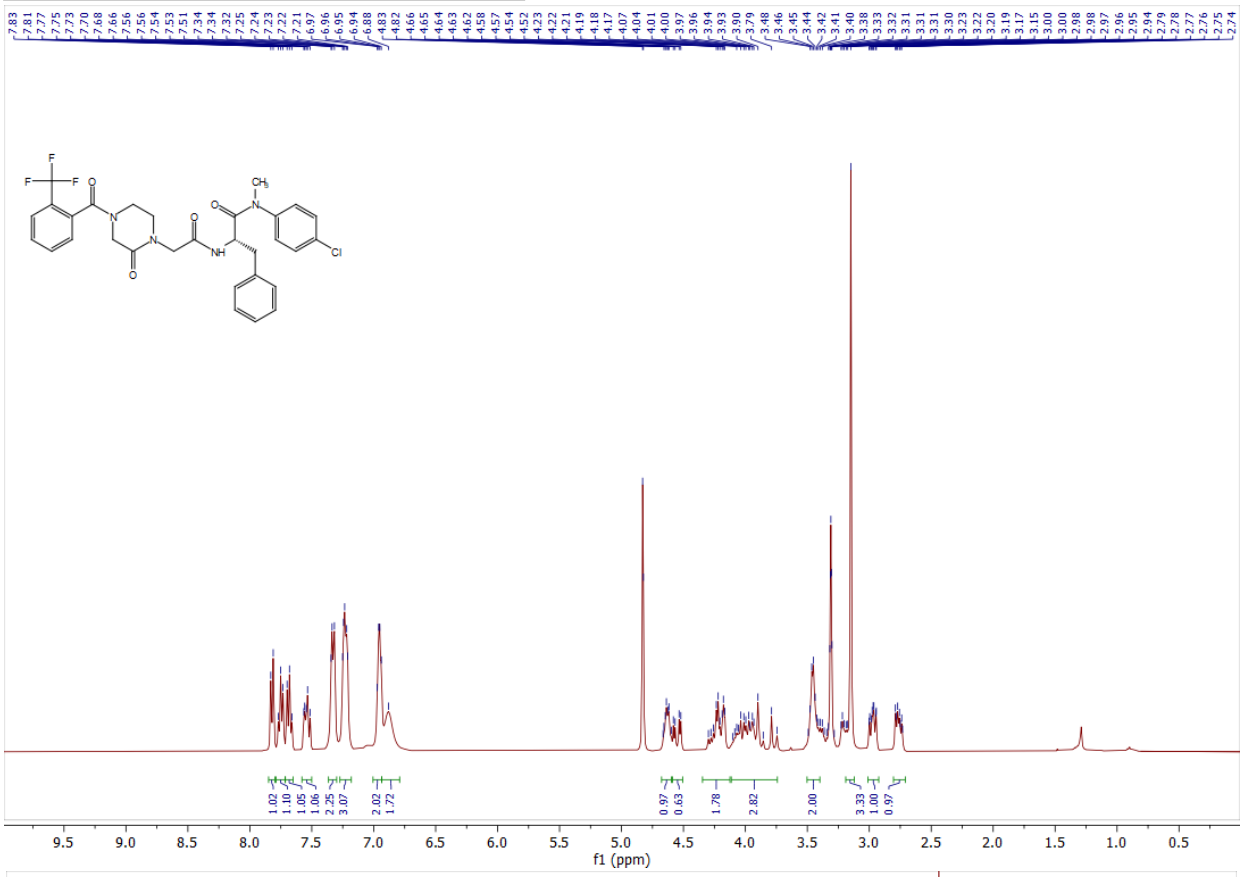


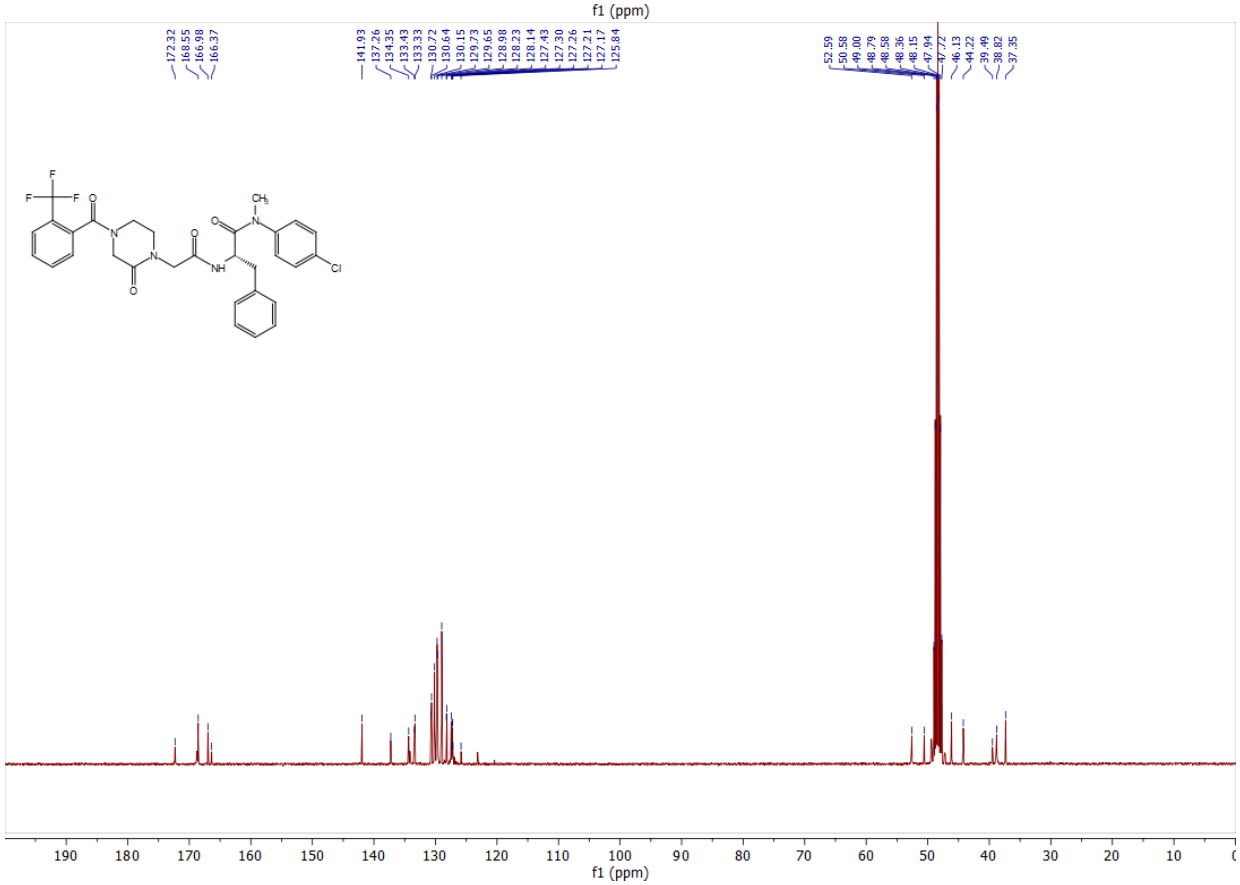
^{1}H and ^{13}C spectra of compound **4w**



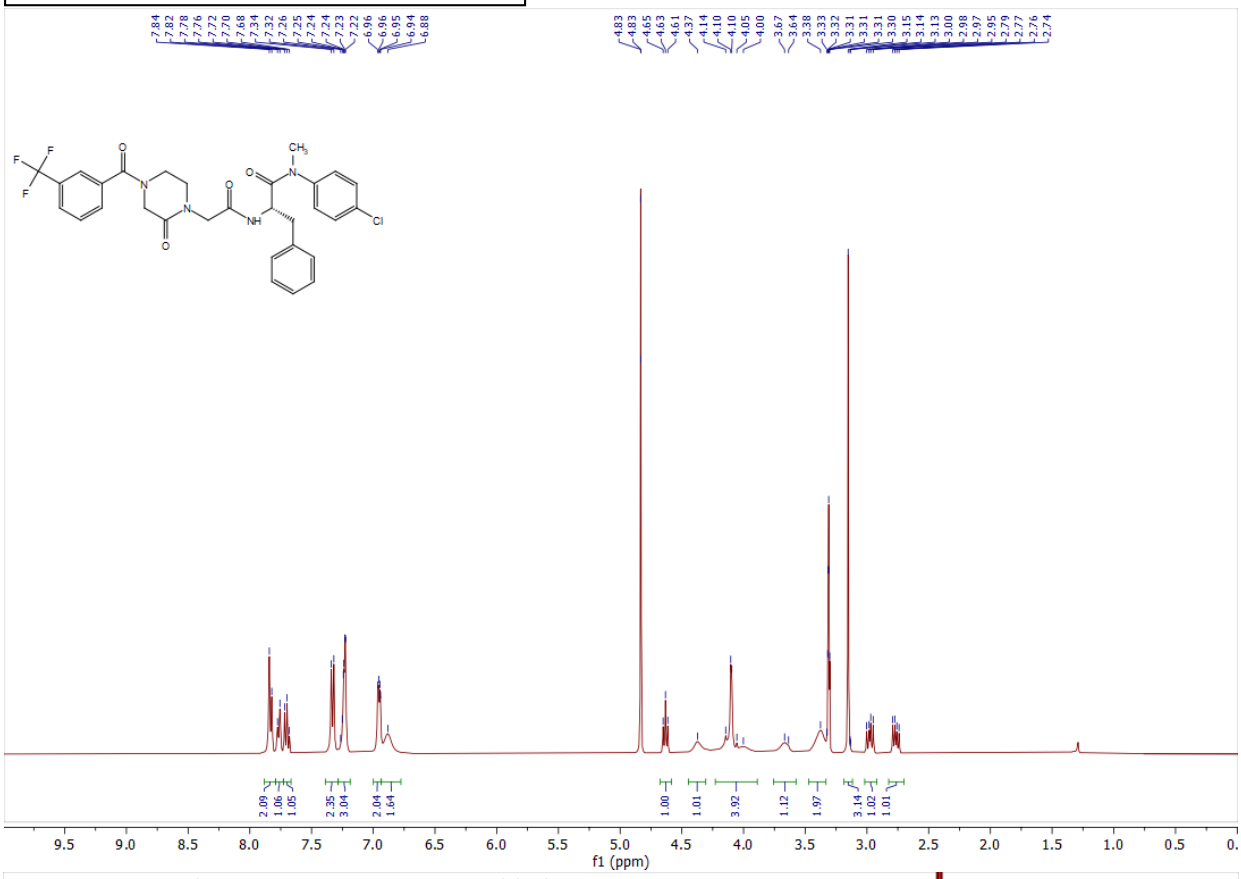


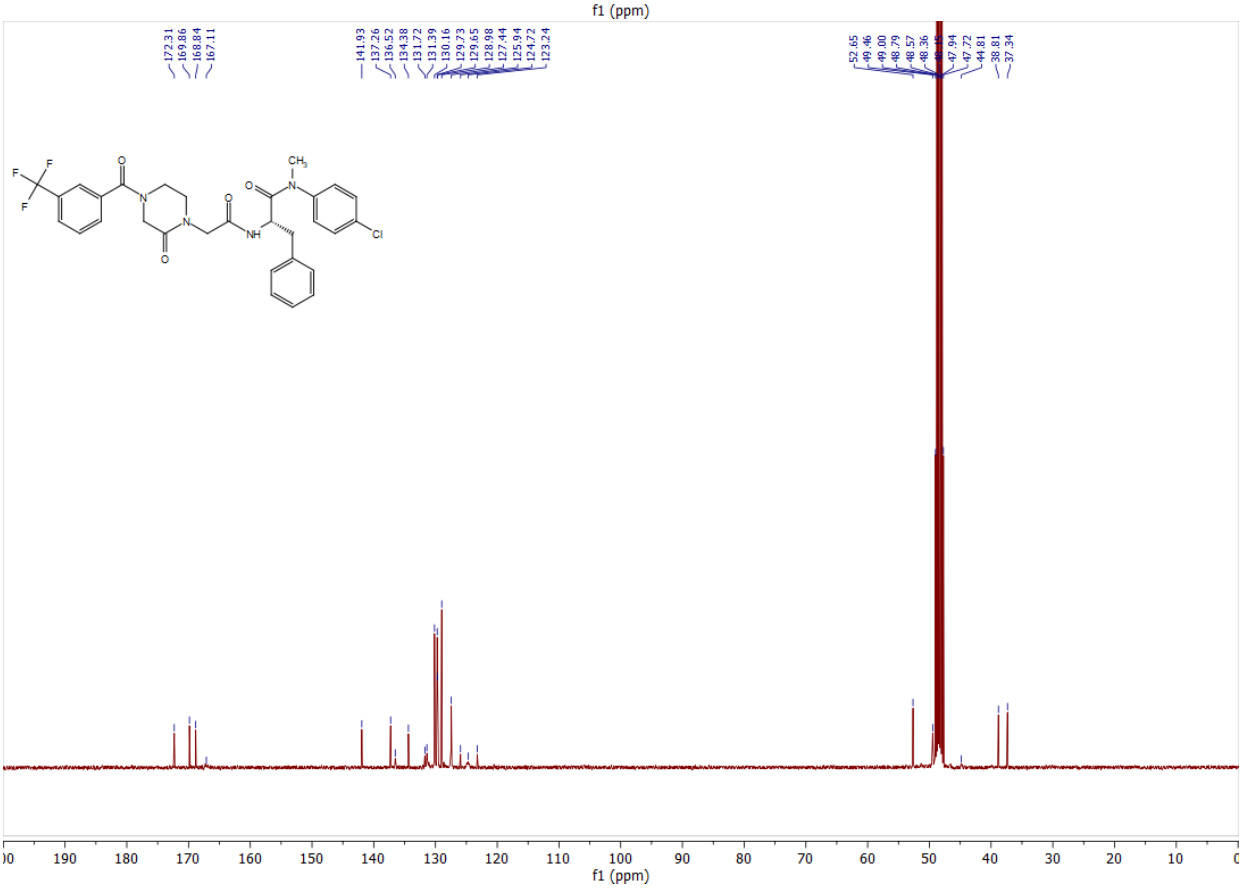
^{1}H and ^{13}C spectra of compound **4x**



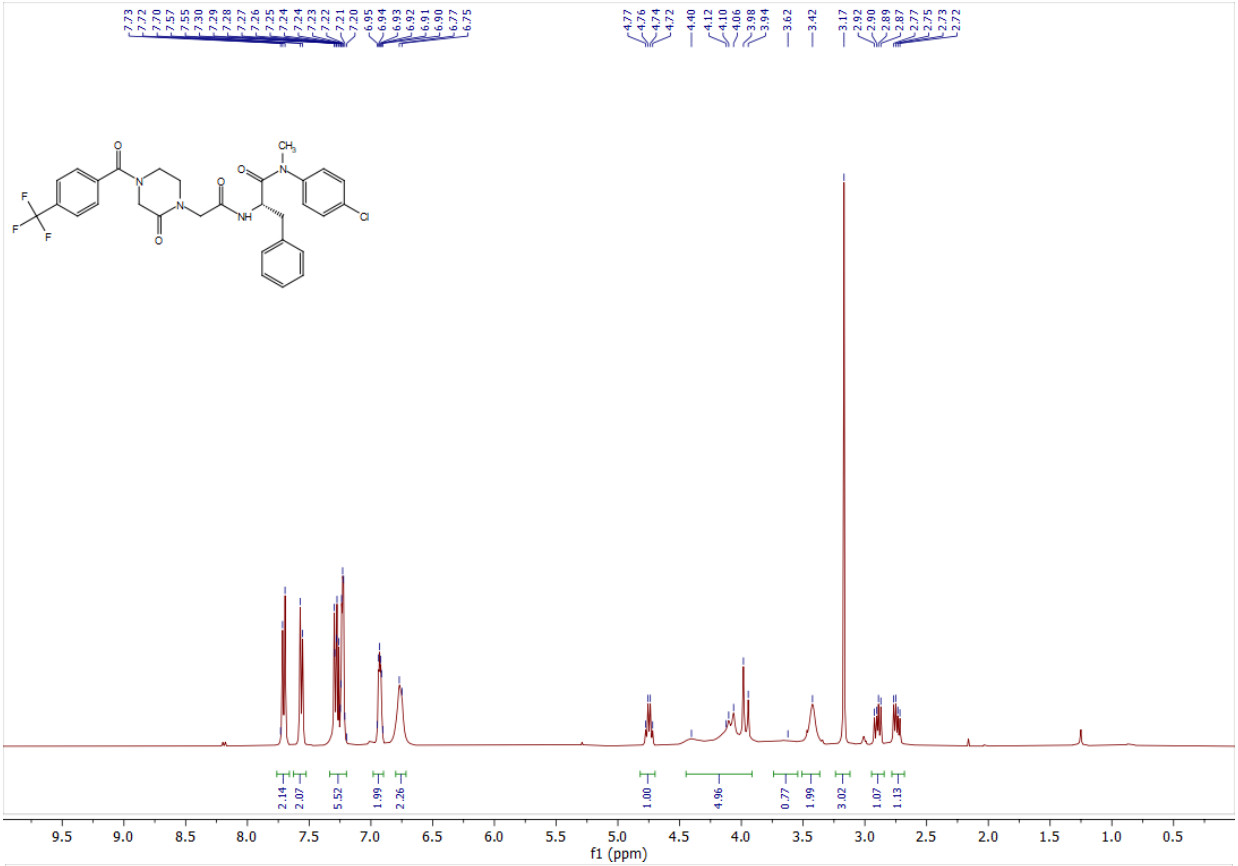


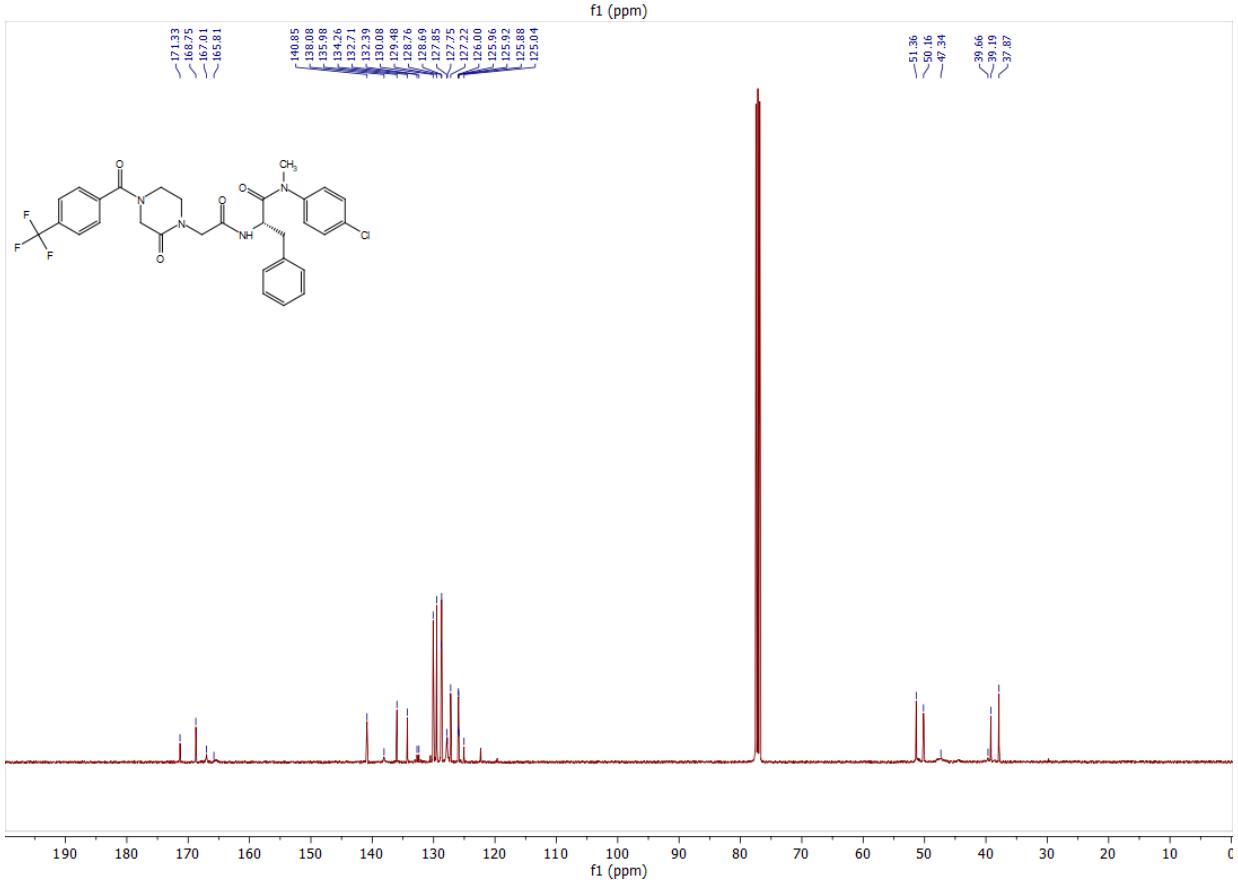
^{1}H and ^{13}C spectra of compound **4y**



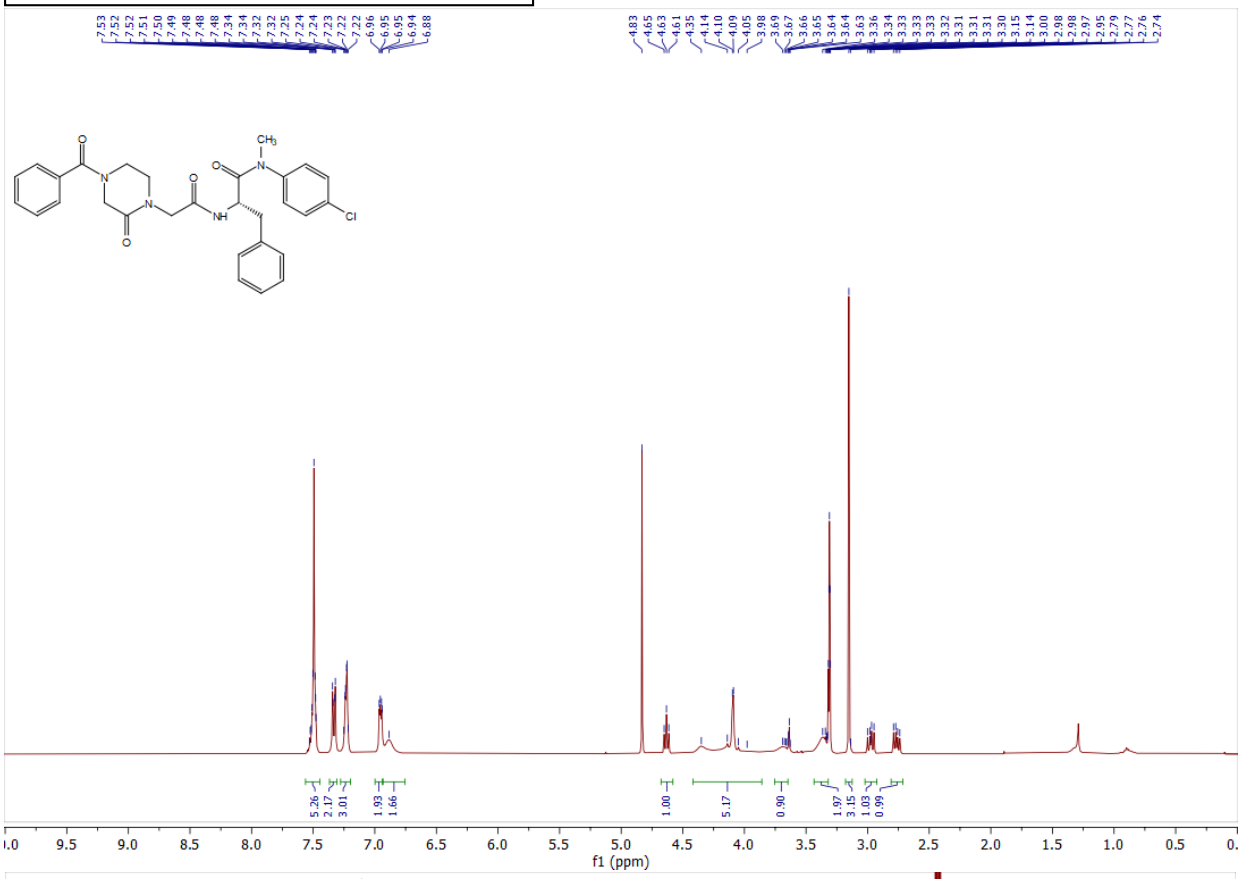


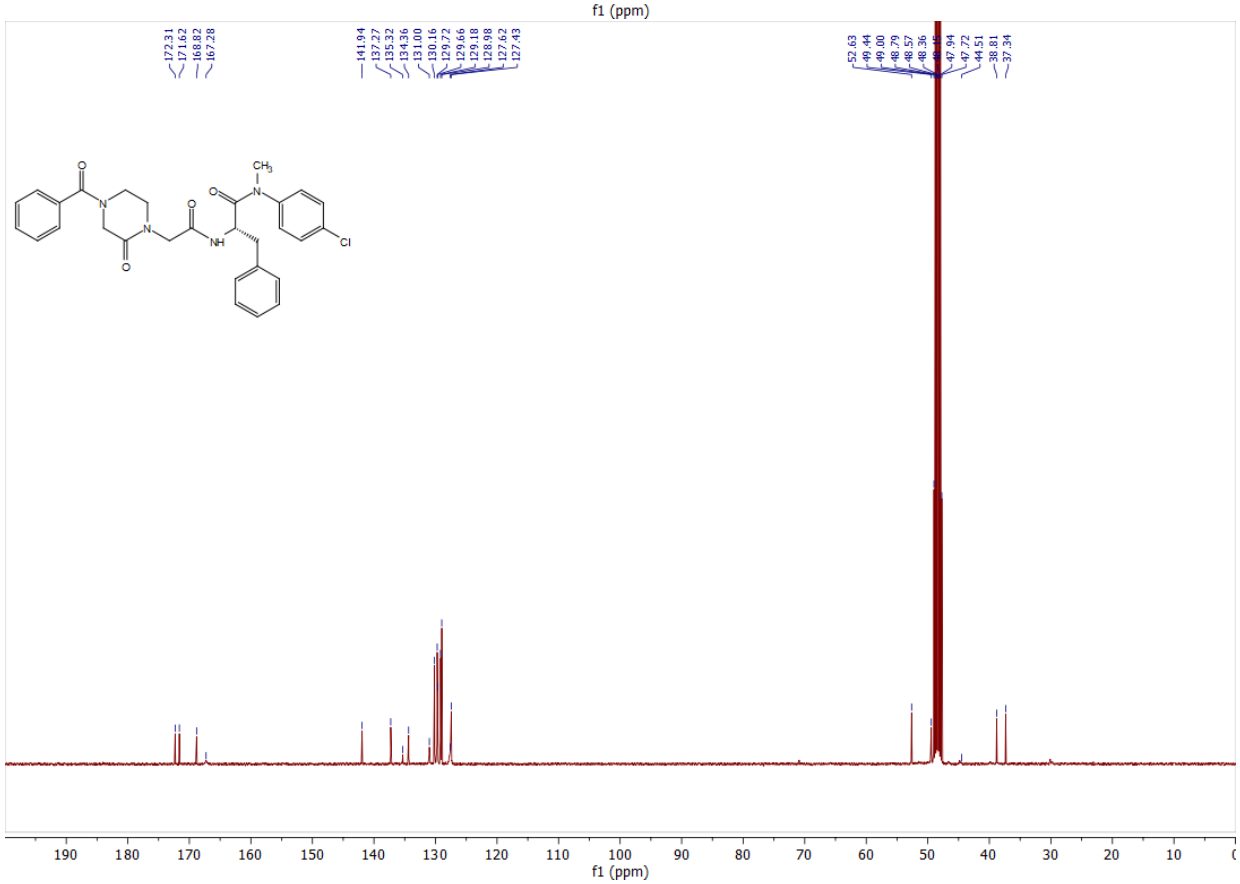
^{1}H and ^{13}C spectra of compound **4z**



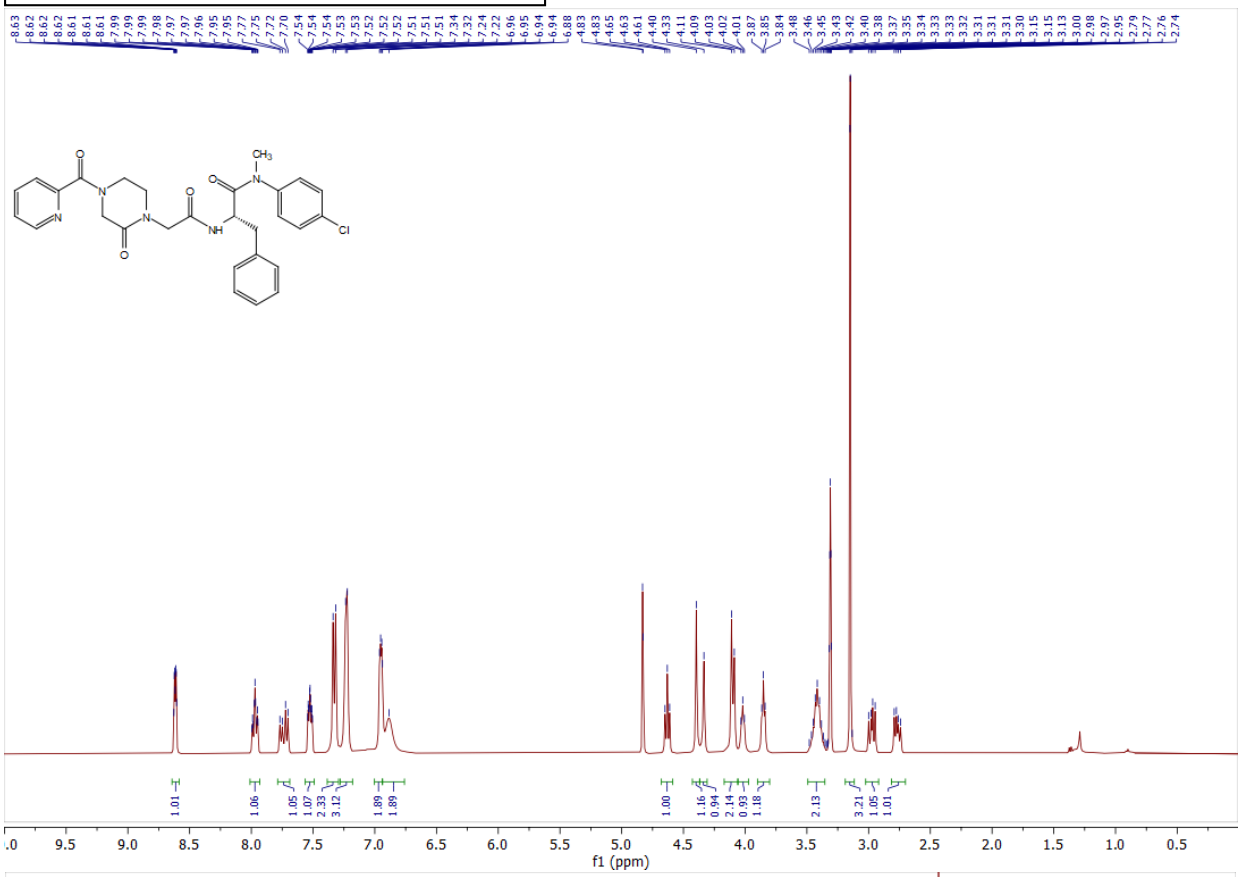


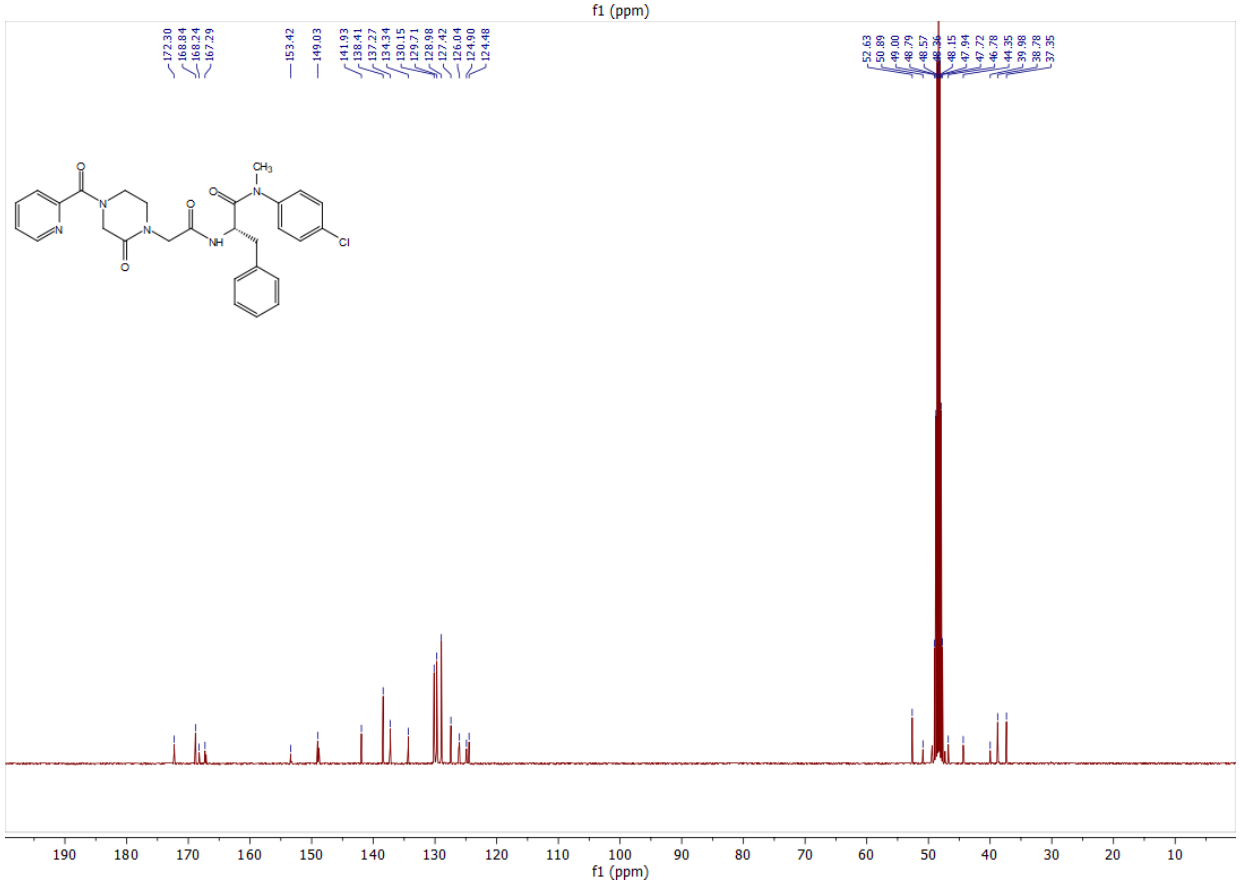
^{1}H and ^{13}C spectra of compound **4aa**



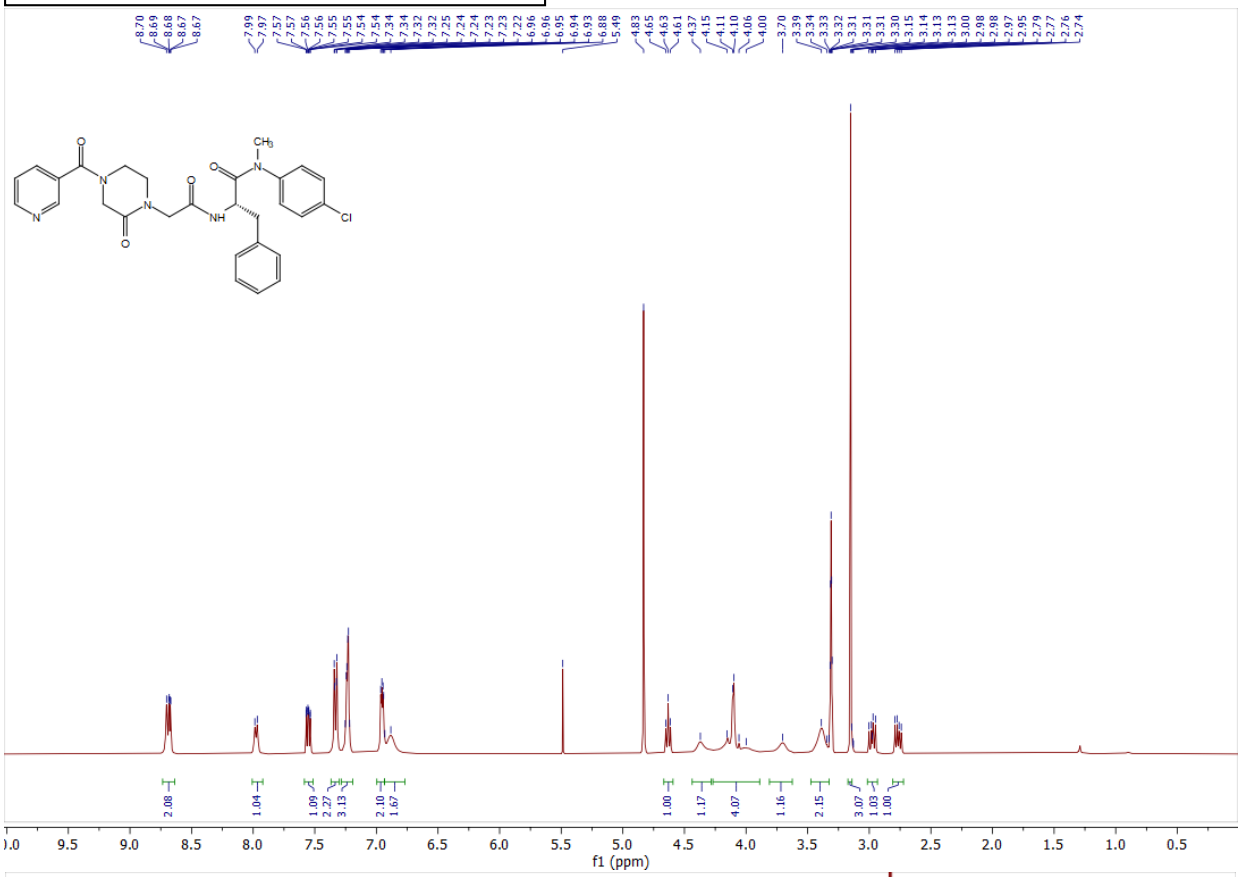


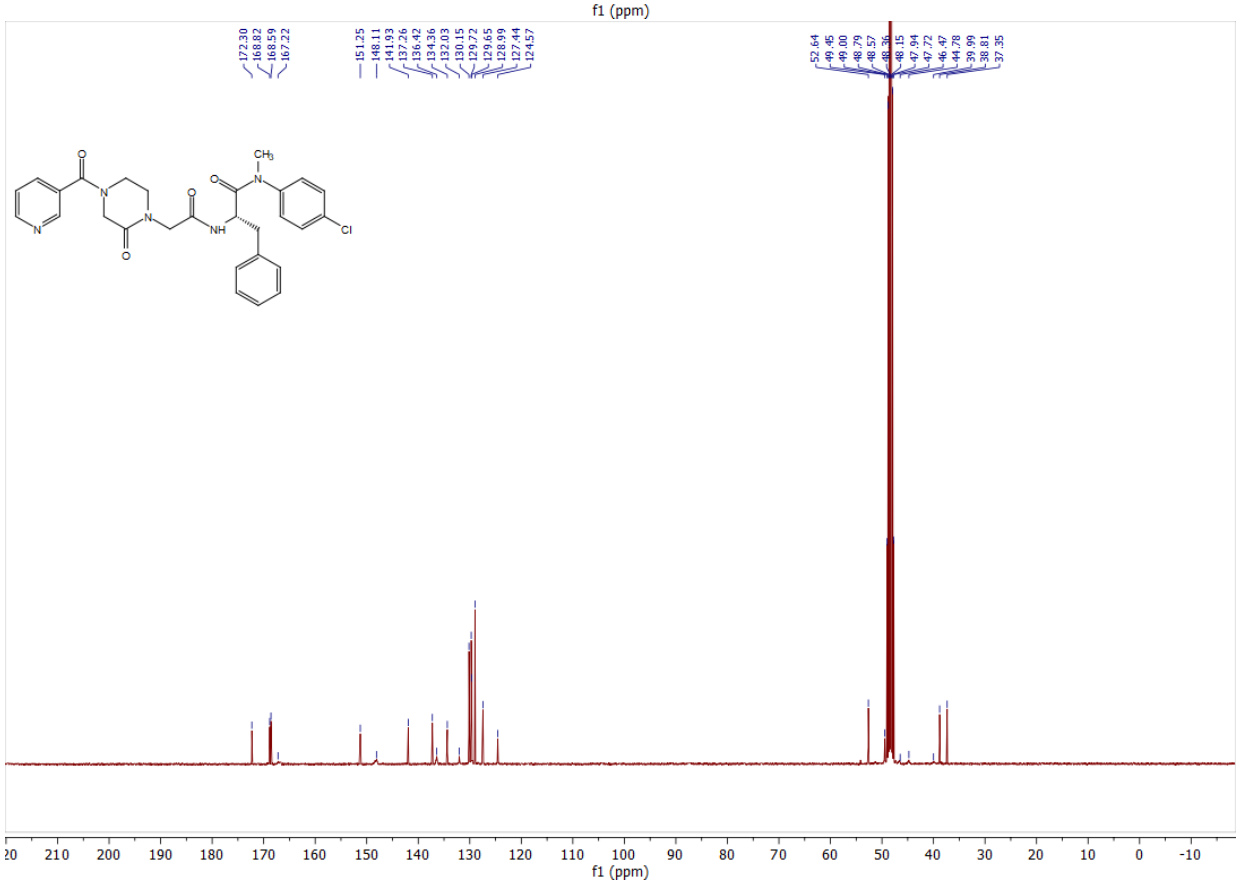
^{1}H and ^{13}C spectra of compound **4bb**



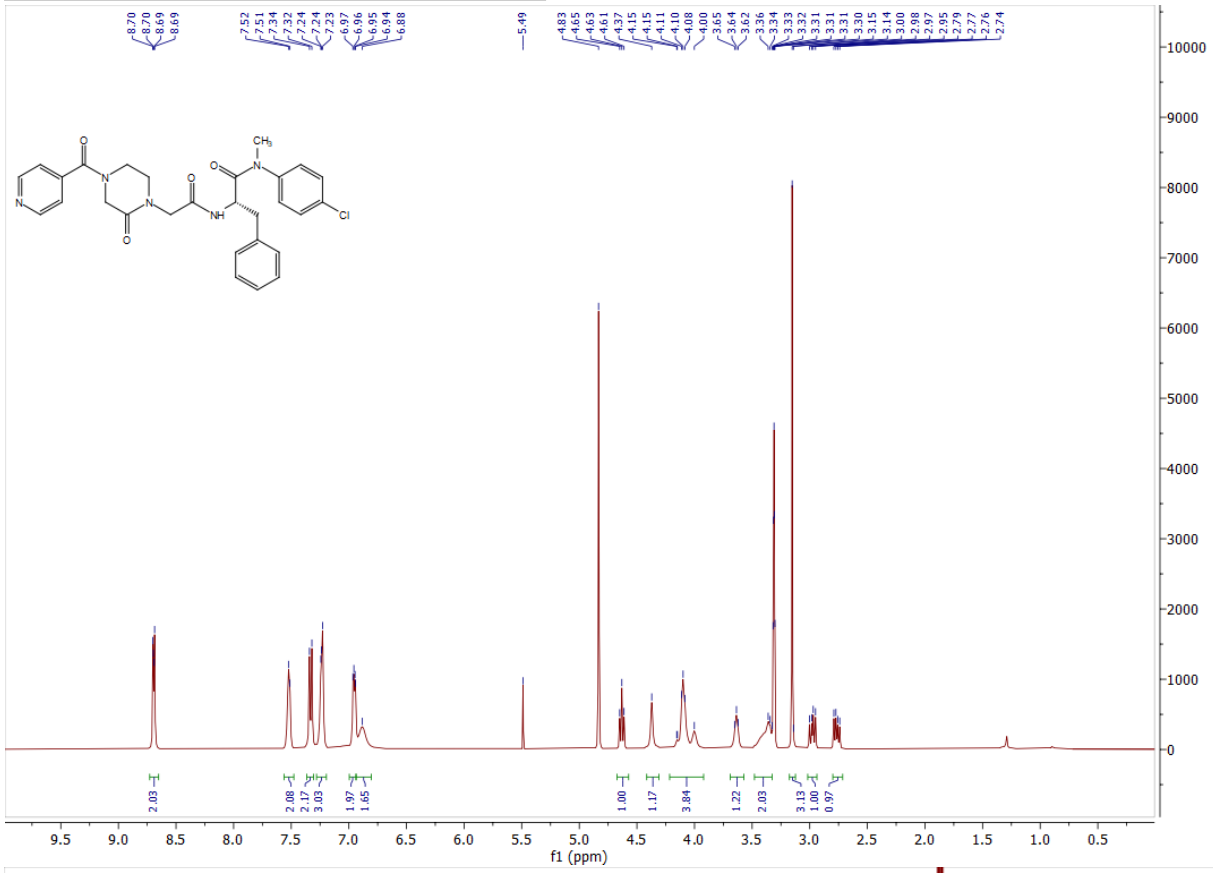


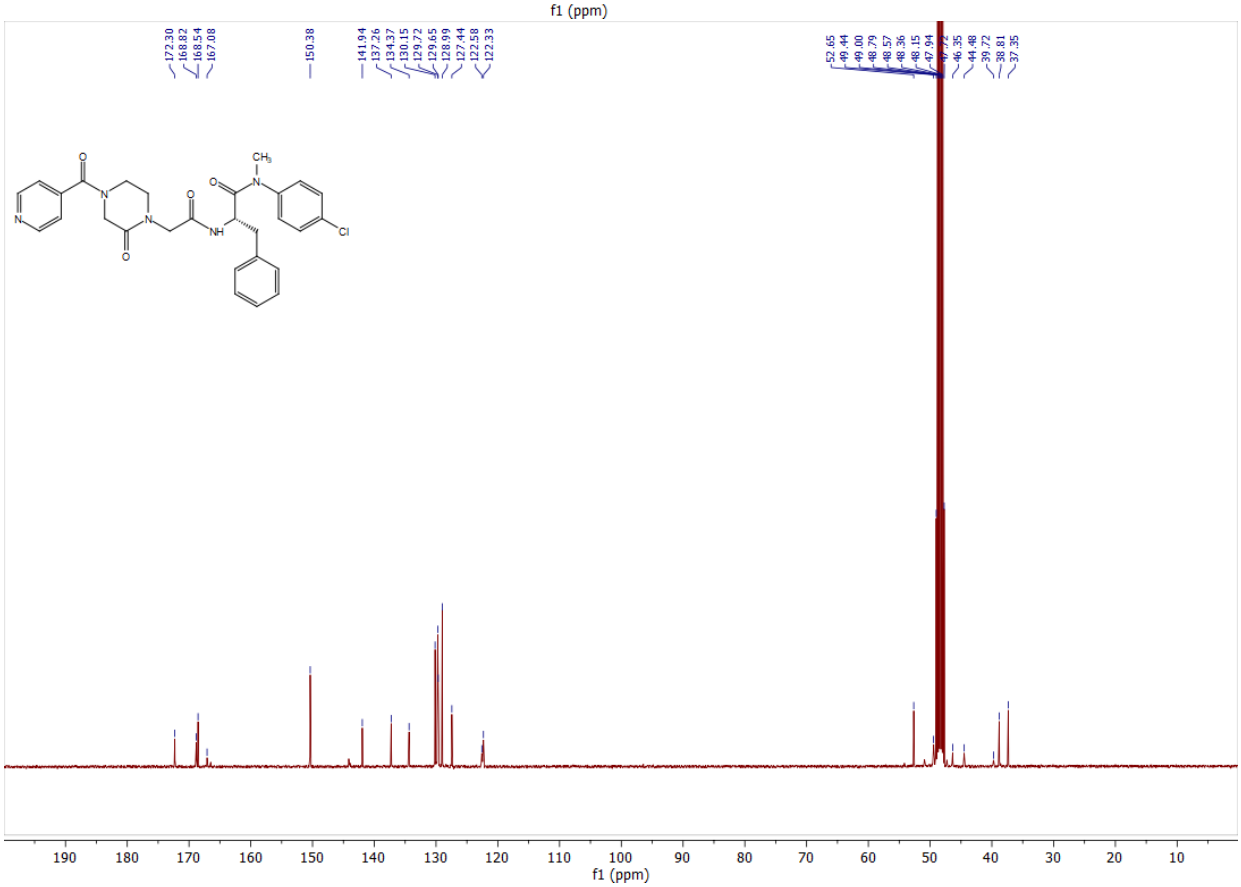
^{1}H and ^{13}C spectra of compound **4cc**



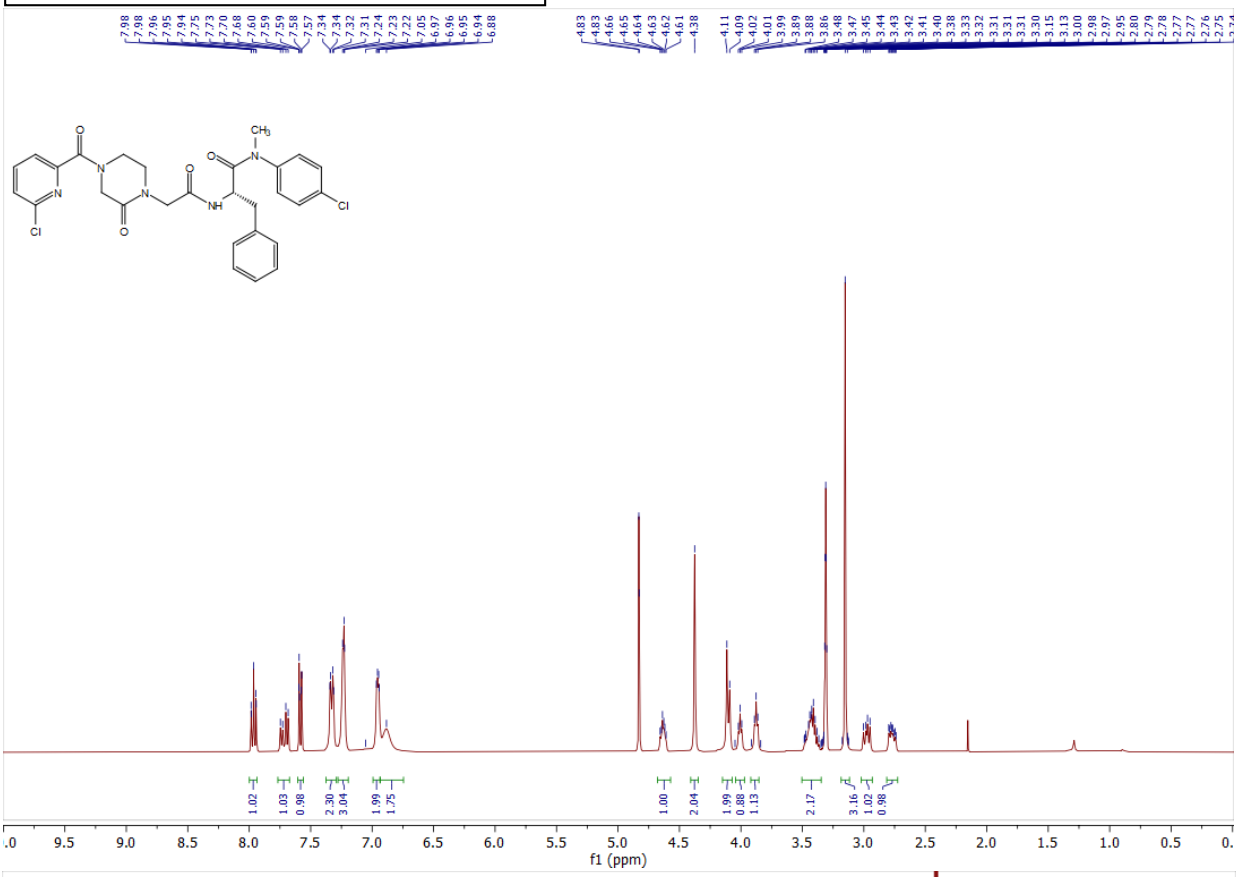


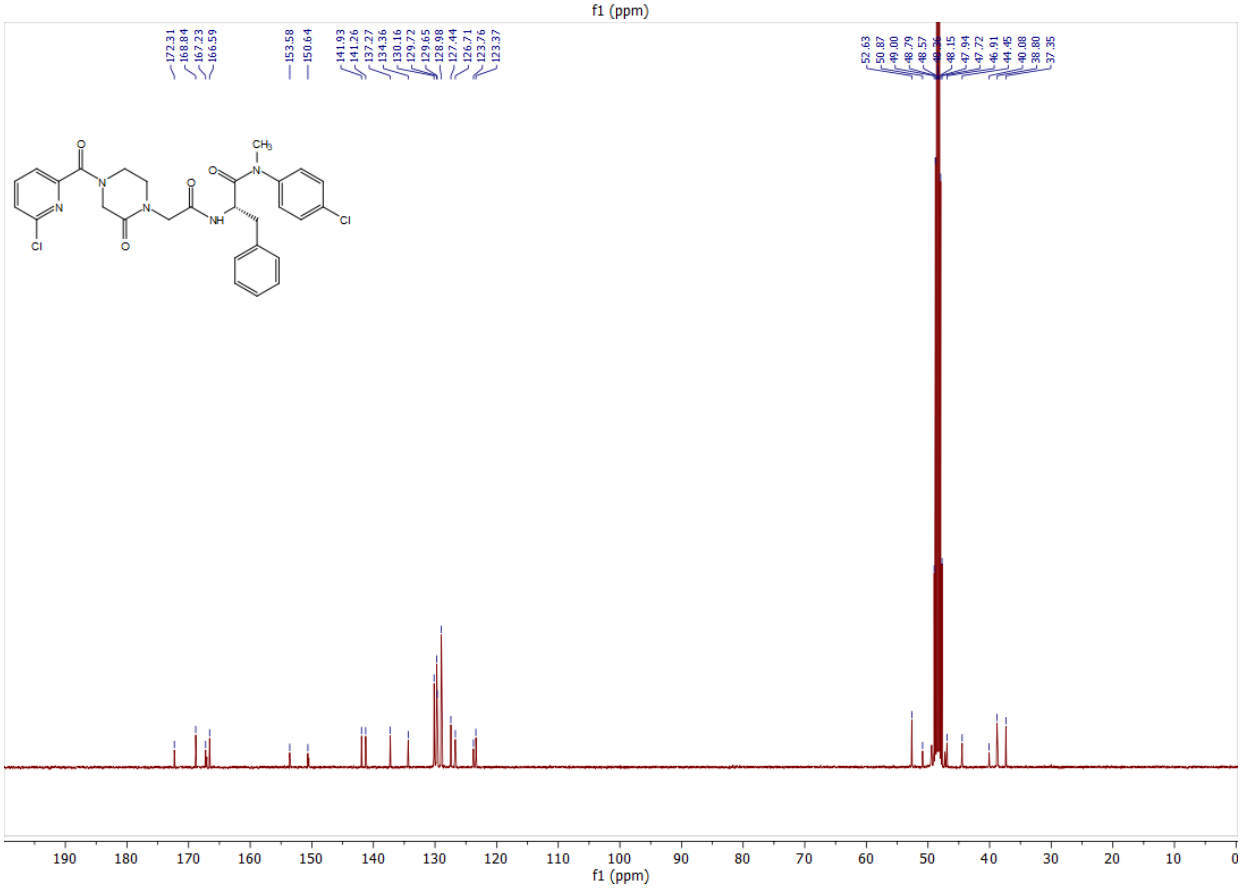
^{1}H and ^{13}C spectra of compound **4dd**



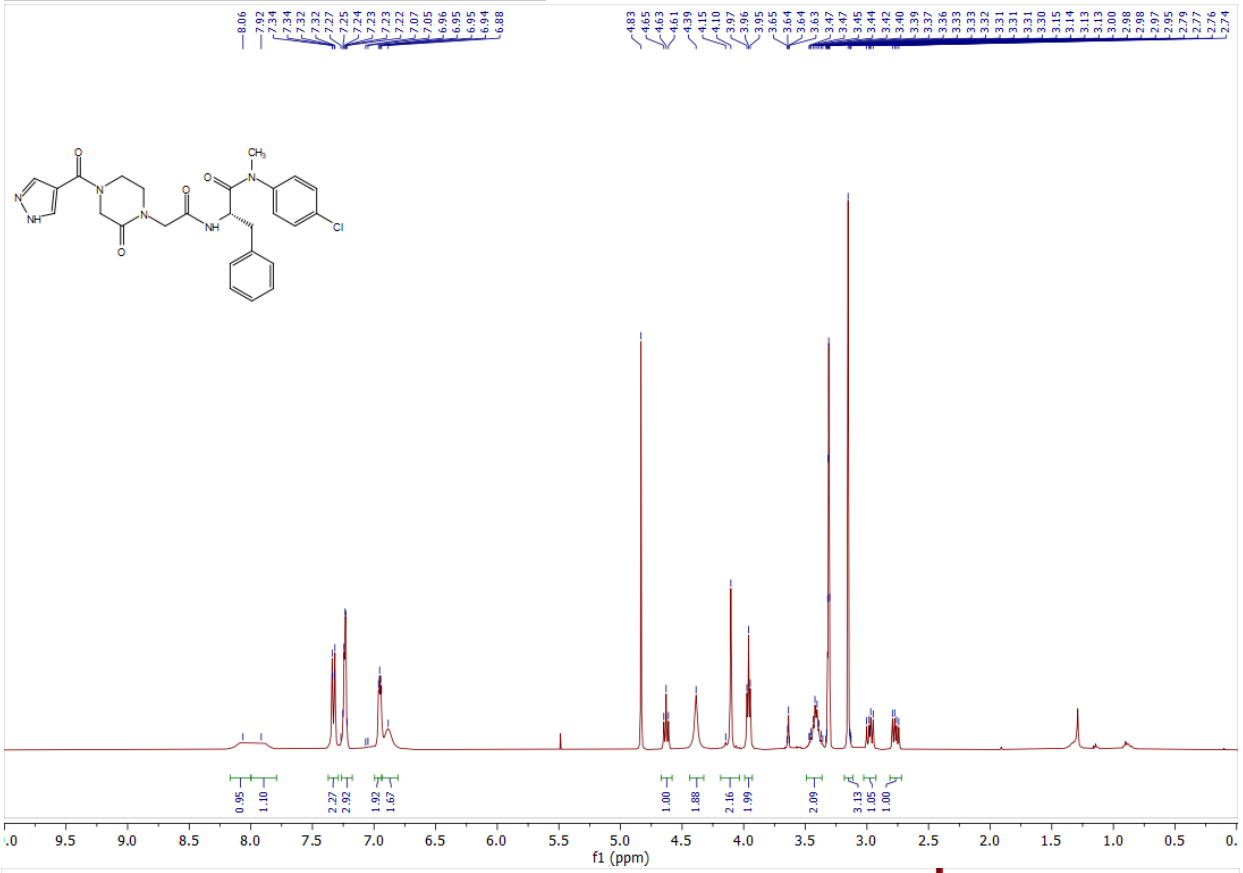


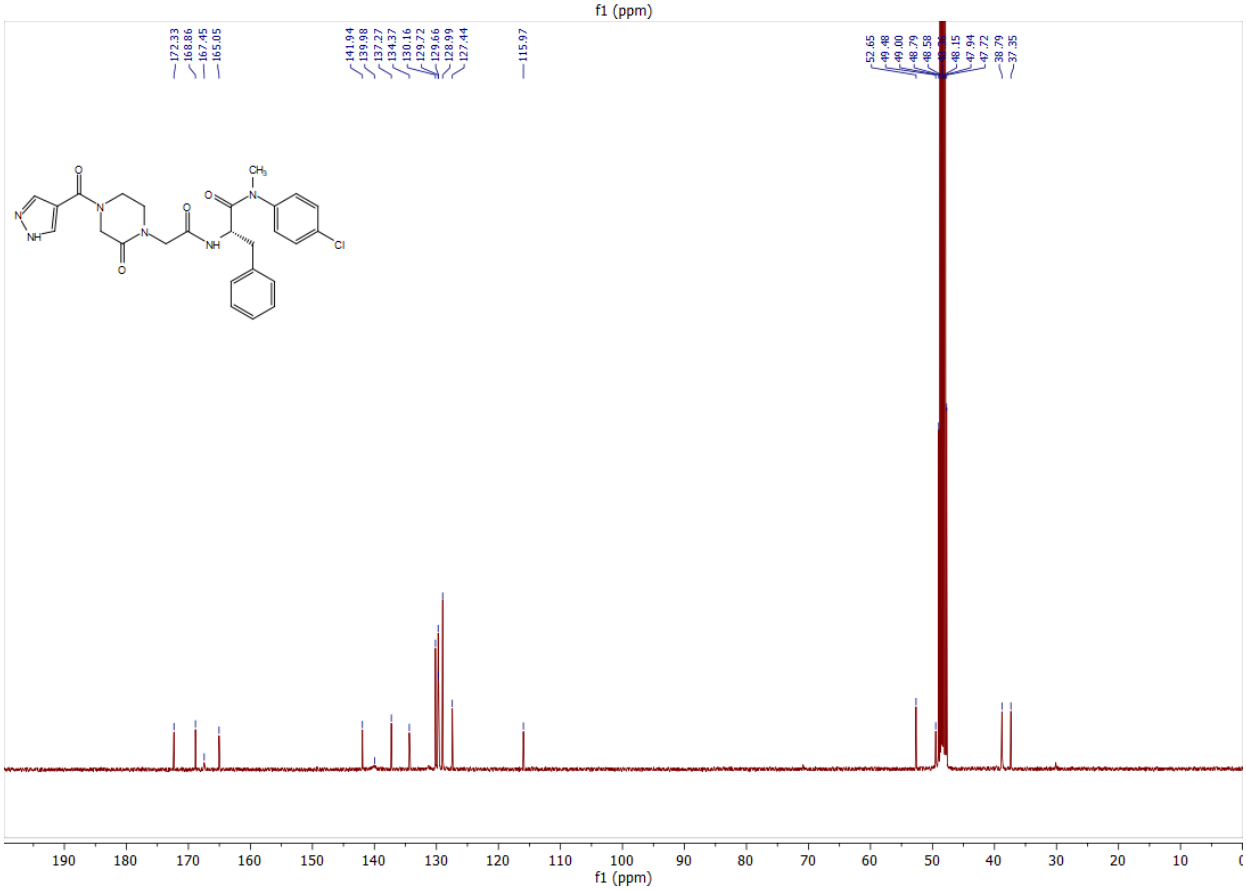
^{1}H and ^{13}C spectra of compound **4ee**



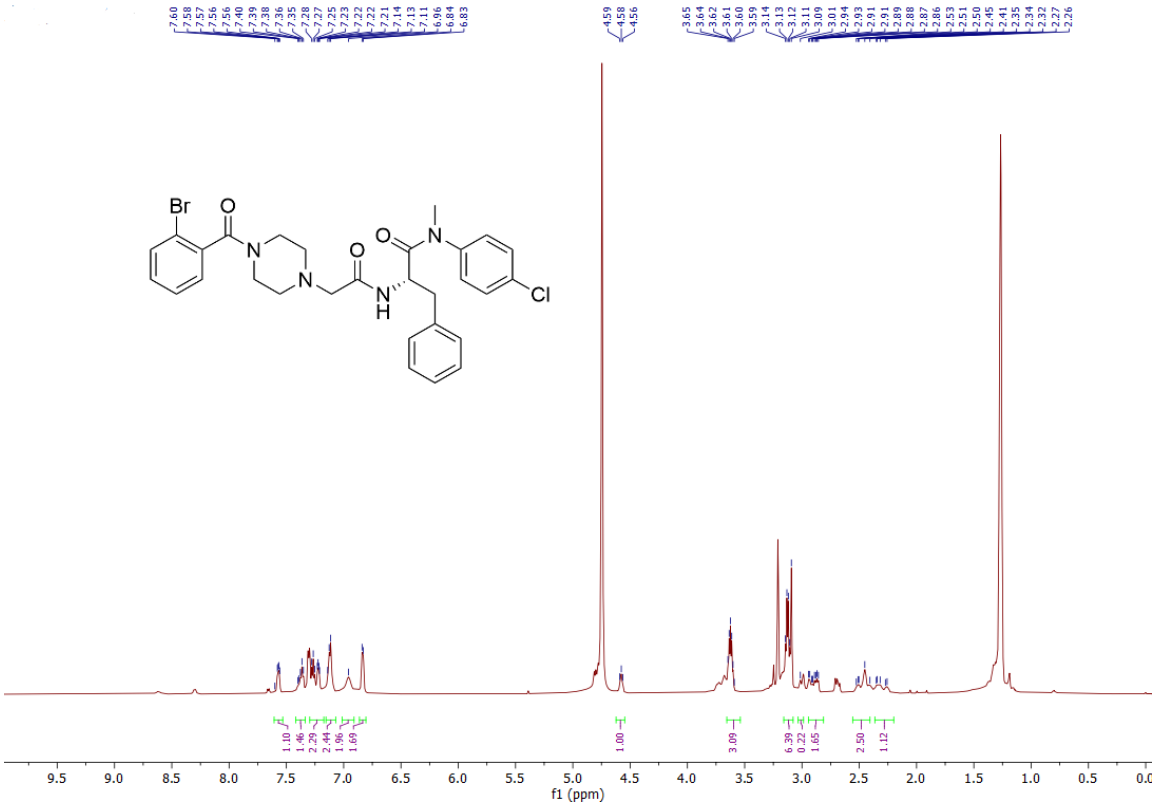


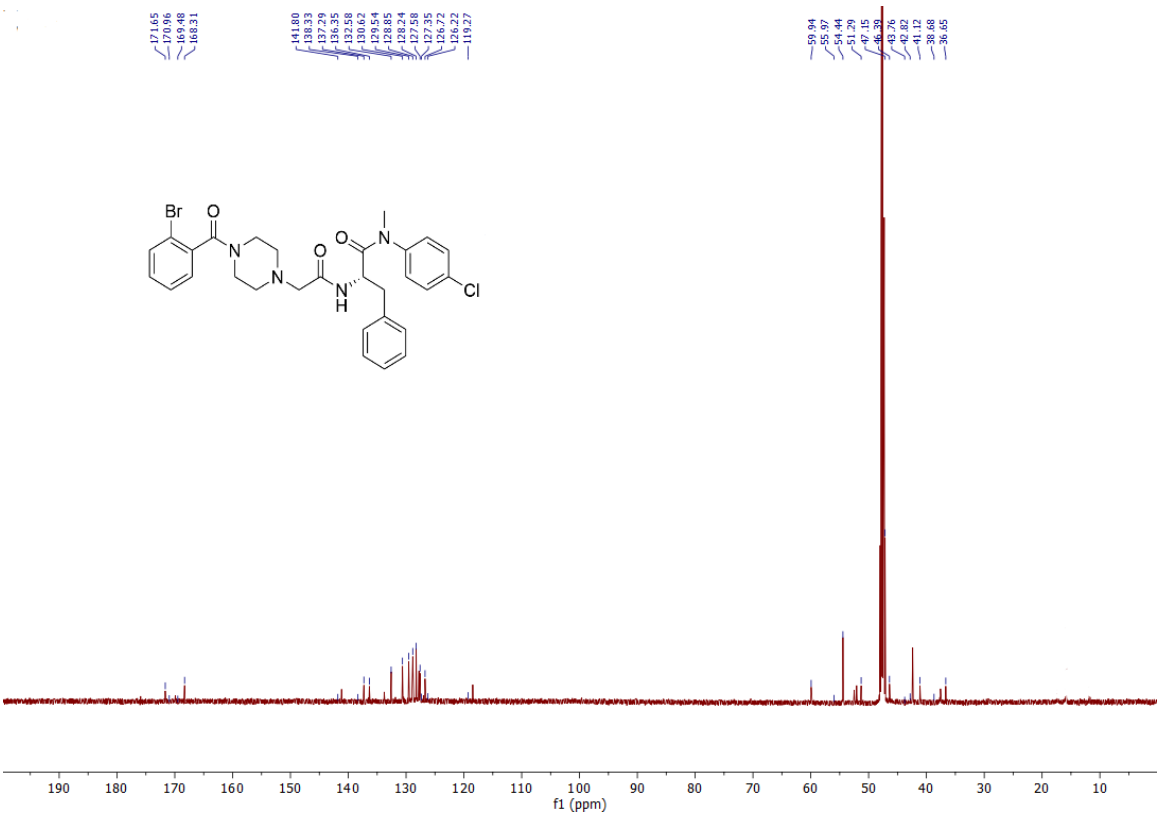
^{1}H and ^{13}C spectra of compound **4ff**





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





^{1}H and ^{13}C spectra of compound **4gg**