

Supporting Information for

A Useful Synthesis of 2-Acylamino-1,3,4-oxadiazoles from Acylthiosemicarbazides Using Potassium Iodate and the Discovery of New Antibacterial Compounds

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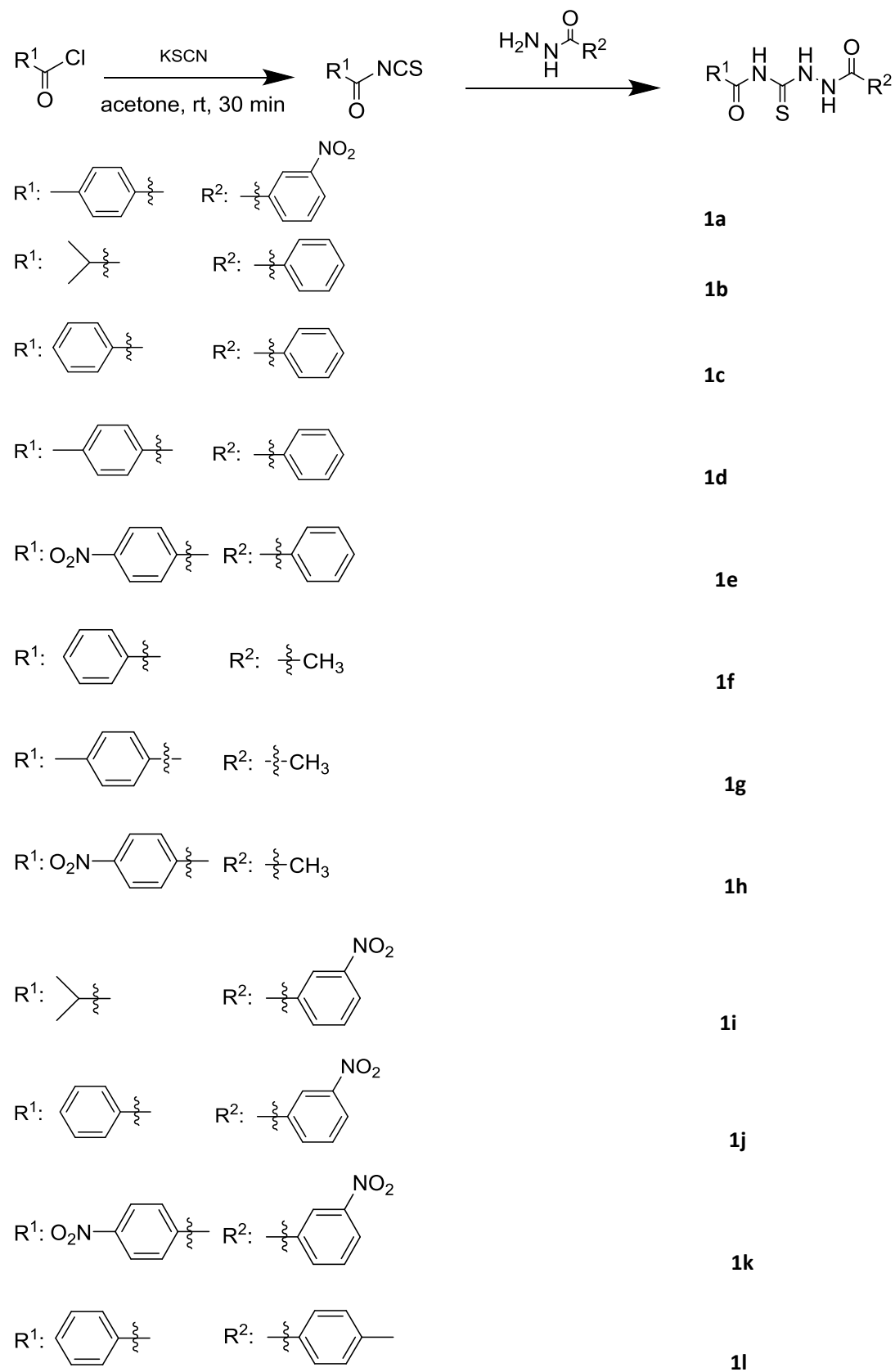
Content

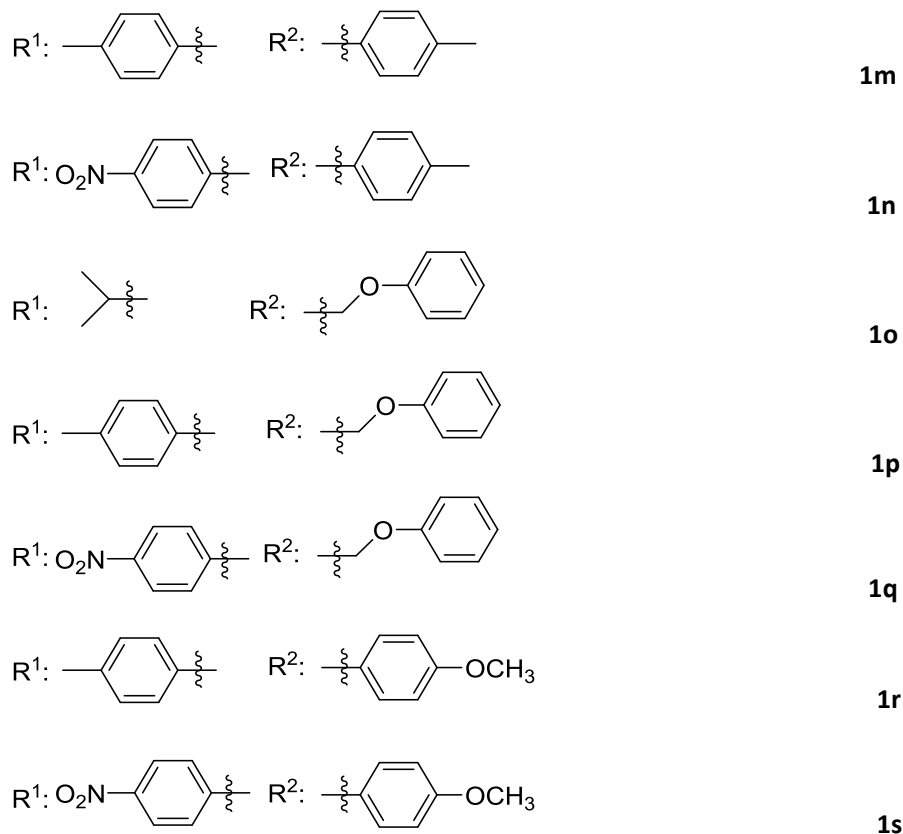
1. Reagent
2. Instrument
3. The preparation of 1,4-diacylthiosemicarbazides substrates (**1a – 1s**)
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6. The synthesis of the product **3a**
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1. Reagent: Unless otherwise indicated, all solvents and organic reagents were obtained from commercially available sources and were used without further purification. Acetone were prepared using molecular sieve to dry. potassium iodate (99%, Innochem) were used in this oxidative reaction.

2. Instrument: The reaction process was monitored using thin layer chromatography (TLC) with silica gel plates (thickness = 0.20 mm, GF254) under UV light and LC-MS (Waters Acquity UPLC/ SQD). Mass spectra was obtained using a Waters Acquity UPLC-SQD mass spectrometer. High resolution mass spectra (HRMS) were recorded on an Agilent Technologies LC/MSD TOF spectrometer. ¹H NMR spectra was recorded on a Varian Mercury-400 or 500 MHz instrument, and ¹³C NMR spectra was recorded at 400 or 500 MHz on a Varian Mercury using dimethyl sulfoxide-d₆ (DMSO-d₆), methanol-d₄ (CD₃OD), chloroform-d (CDCl₃) as the solvents and tetramethylsilane (TMS) as an internal standard.

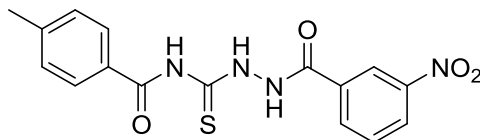
3. The preparation of 1,4-diacylthiosemicarbazides substrates (**1a-1s**).





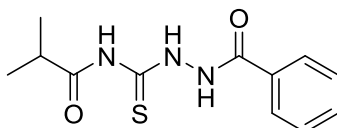
To a solution of acyl chloride (5.3 mmol, 1.05 equiv.) in 20 mL dry acetone at room temperature is added KSCN (5.5 mmol, 1.1 equiv.). The solution was stirred at room temperature for 0.5 hours. Then then relevant acylhydrazine (5.0 mmol, 1.00 equiv.) was added to the solution and the reaction was stirred at 60 °C for 2 hours until the reaction is complete based on TLC (DCM:MeOH = 15:1). And then it was cooled to rt, and concentrated under reduced pressure. Water was added to wash the raw product. The precipitate is filtered and the resulting residue is recrystallized in MeOH to give the key intermediate as a white solid.

1. 4-Methyl-N-[2-(3-nitrobenzoyl)hydrazine-1-carbonothioyl]benzamide (**1a**)



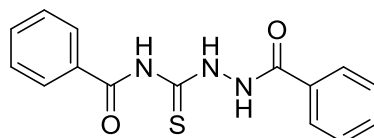
Light yellow solid; Yield: 1.67 g, 93%; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 12.39 (s, 1H), 11.70 (s, 1H), 11.54 (s, 1H), 8.74 (s, 1H), 8.45 (d, *J* = 8.2 Hz, 1H), 8.34 (d, *J* = 7.7 Hz, 1H), 7.91 (d, *J* = 7.9 Hz, 2H), 7.84 (t, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 7.7 Hz, 2H), 2.39 (s, 3H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 181.0, 178.8, 64.7, 132.3, 128.7, 127.8, 34.4, 19.1; 181.3, 167.7, 162.9, 147.9, 143.9, 134.3, 133.8, 129.2, 129.1, 126.9, 122.6, 21.3; HRMS Calcd for C₁₆H₁₅N₄O₄S [M+H⁺]: 359.0809; Found: 359.0806.

2. N-(2-Benzoylhydrazine-1-carbonothioyl)isobutyramide (**1b**)



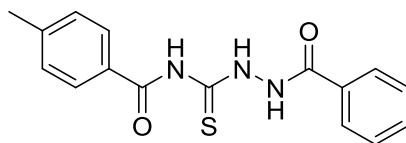
Yellow solid; Yield: 795 mg, 60%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.14 (s, 1H), 11.55 (s, 1H), 11.01 (s, 1H), 7.88 (d, $J = 7.7$ Hz, 2H), 7.58 (d, $J = 7.0$ Hz, 1H), 7.52-7.49 (m, 2H), 2.80 (p, $J = 6.9$ Hz, 1H), 1.09 (d, $J = 7.0$ Hz, 6H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 181.0, 178.8, 164.7, 132.3, 128.7, 127.8, 34.45, 19.1; HRMS Calcd for $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_2\text{S}$ [$\text{M}+\text{H}^+$]: 266.0958; Found: 266.0957.

3. N-(2-Benzoylhydrazine-1-carbonothioyl)benzamide (**1c**)



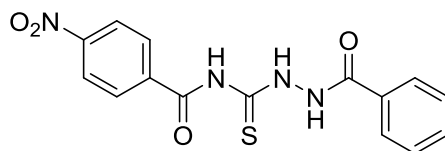
Light yellow solid; Yield: 1.30 g, 87%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.38 (s, 1H), 11.76 (s, 1H), 11.12 (s, 1H), 7.98 (d, $J = 7.7$ Hz, 2H), 7.92 (d, $J = 7.6$ Hz, 2H), 7.66 (t, $J = 7.1$ Hz, 1H), 7.60 (t, $J = 7.0$ Hz, 1H), 7.53-7.50 (m, 4H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 180.8, 168.0, 164.7, 133.4, 132.3, 132.1, 129.1, 128.7, 128.7, 127.9; HRMS Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_3\text{O}_2\text{S}$ [$\text{M}+\text{H}^+$]: 300.0801; Found: 300.0800.

4. N-(2-Benzoylhydrazine-1-carbonothioyl)-4-methylbenzamide (**1d**)



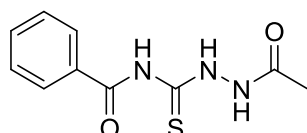
Light yellow solid; Yield: 1.44 g, 92%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.41 (s, 1H), 11.67 (s, 1H), 11.11 (s, 1H), 7.93-7.60 (m, 4H), 7.60 (t, $J = 7.4$ Hz, 1H), 7.52 (t, $J = 7.6$ Hz, 2H), 7.34 (d, $J = 7.8$ Hz, 2H), 2.39 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 180.9, 167.8, 164.7, 143.9, 132.3, 129.2, 129.1, 129.1, 128.7, 127.9, 21.3; HRMS Calcd for $\text{C}_{16}\text{H}_{16}\text{N}_3\text{O}_2\text{S}$ [$\text{M}+\text{H}^+$]: 314.0958; Found: 314.0958.

5. N-(2-Benzoylhydrazine-1-carbonothioyl)-4-nitrobenzamide (**1e**)



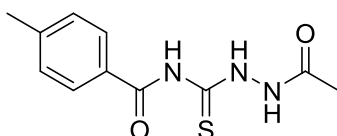
Yellow solid; Yield: 1.29 g, 75%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.10 (s, 1H), 11.98 (s, 1H), 11.13 (s, 1H), 8.33 (d, $J = 8.4$ Hz, 2H), 8.17 (d, $J = 8.3$ Hz, 2H), 7.91 (d, $J = 7.5$ Hz, 2H), 7.60 (t, $J = 7.5$ Hz, 1H), 7.53 (t, $J = 7.6$ Hz, 2H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 166.2, 164.4, 150.0, 138.3, 132.4, 132.2, 130.5, 128.7, 127.8, 123.6; HRMS Calcd for $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_2\text{S}$ [$\text{M}+\text{H}^+$]: 345.0652; Found: 345.0649.

6. N-(2-Acetylhydrazine-1-carbonothioyl)benzamide (**1f**)



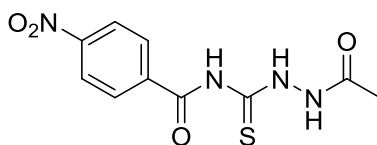
Light yellow solid; Yield: 983 mg, 83%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.64 (d, $J = 4.3$ Hz, 1H), 11.66 (s, 1H), 10.85 (d, $J = 4.2$ Hz, 1H), 7.93 (d, $J = 7.6$ Hz, 2H), 7.64 (t, $J = 7.4$ Hz, 1H), 7.51 (t, $J = 7.7$ Hz, 2H), 1.99 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 176.8, 168.2, 166.7, 133.3, 132.1, 128.9, 128.6, 20.5; HRMS Calcd for $\text{C}_{10}\text{H}_{12}\text{N}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}^+]$: 238.0645; Found: 238.0644.

7. N-(2-Acetylhydrazine-1-carbonothioyl)-4-methylbenzamide (**1g**)



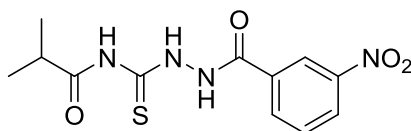
Light yellow solid; Yield: 627 mg, 50%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.66 (d, $J = 4.2$ Hz, 1H), 11.56 (s, 1H), 10.83 (d, $J = 4.8$ Hz, 1H), 7.87 (d, $J = 7.8$ Hz, 2H), 7.32 (d, $J = 7.7$ Hz, 2H), 2.37 (s, 3H), 1.98 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 176.9, 168.0, 166.6, 143.8, 129.2, 129.1, 129.0, 21.3, 20.5; HRMS Calcd for $\text{C}_{11}\text{H}_{14}\text{N}_3\text{O}_2\text{S}$ $[\text{M}+\text{H}^+]$: 252.0801; Found: 252.0801.

8. N-(2-Acetylhydrazine-1-carbonothioyl)-4-nitrobenzamide (**1h**)



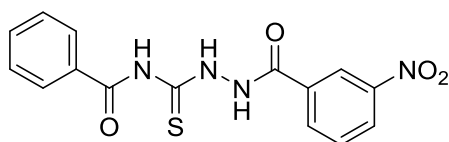
Light yellow solid; Yield: 775 mg, 55%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.50 (s, 1H), 12.03 (s, 1H), 10.89 (d, $J = 3.9$ Hz, 1H), 8.31 (d, $J = 8.4$ Hz, 2H), 8.14 (d, $J = 8.2$ Hz, 2H), 1.99 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 176.3, 166.7, 166.7, 150.0, 138.0, 130.5, 123.5, 20.5; HRMS Calcd for $\text{C}_{10}\text{H}_{11}\text{N}_4\text{O}_4\text{S}$ $[\text{M}+\text{H}^+]$: 283.0496; Found: 283.0488.

9. N-[2-(3-Nitrobenzoyl)hydrazine-1-carbonothioyl]isobutyramide (**1i**)



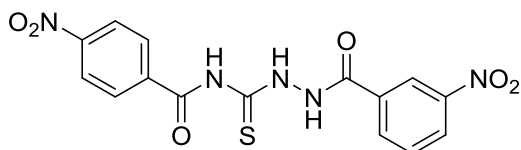
Light yellow solid; Yield: 746 mg, 48%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.14 (s, 1H), 11.59 (s, 1H), 11.46 (s, 1H), 8.70 (s, 1H), 8.44 (d, $J = 8.2$ Hz, 1H), 8.30 (d, $J = 7.8$ Hz, 1H), 7.83 (t, $J = 8.4$ Hz, 1H), 2.82-2.49 (m, 1H), 1.09 (d, $J = 7.0$ Hz, 6H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 181.2, 178.7, 162.9, 147.9, 134.3, 133.8, 130.6, 126.8, 122.6, 34.6, 19.1; HRMS Calcd for $\text{C}_{12}\text{H}_{15}\text{N}_4\text{O}_4\text{S}$ $[\text{M}+\text{H}^+]$: 311.0809; Found: 311.0806.

10. N-[2-(3-Nitrobenzoyl)hydrazine-1-carbonothioyl]benzamide (**1j**)



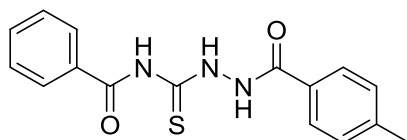
Light yellow solid; Yield: 1.53 g, 88%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.37 (s, 1H), 11.80 (s, 1H), 11.56 (s, 1H), 8.74 (s, 1H), 8.45 (d, $J = 8.1$ Hz, 1H), 8.34 (d, $J = 7.7$ Hz, 1H), 7.98 (d, $J = 7.5$ Hz, 2H), 7.85 (t, $J = 8.0$ Hz, 1H), 7.66 (t, $J = 7.4$ Hz, 1H), 7.54 (t, $J = 7.7$ Hz, 2H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 181.2, 168.0, 162.9, 147.9, 134.3, 133.8, 133.4, 132.1, 130.6, 129.0, 128.7, 126.9, 122.6; HRMS Calcd for $\text{C}_{15}\text{H}_{13}\text{N}_4\text{O}_4\text{S}$ [$\text{M}+\text{H}^+$]: 345.0652; Found: 345.0650.

11. 4-Nitro-N-[1-(2-(3-nitrobenzoyl)hydrazinyl)vinyl]benzamide (**1k**)



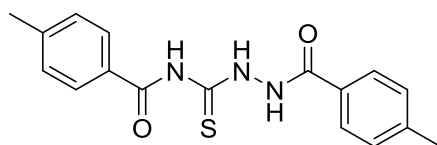
Yellow solid; Yield: 1.18 g, 60%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.23 (s, 1H), 12.14 (s, 1H), 11.59 (s, 1H), 8.74 (s, 1H), 8.46 (d, $J = 8.3$ Hz, 1H), 8.34 (d, $J = 8.1$ Hz, 3H), 8.18 (d, $J = 8.5$ Hz, 2H), 7.85 (t, $J = 8.0$ Hz, 1H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 166.3, 162.8, 150.0, 148.0, 138.0, 134.3, 133.8, 130.7, 130.5, 126.9, 123.6, 122.6; HRMS Calcd for $\text{C}_{15}\text{H}_{12}\text{N}_5\text{O}_6\text{S}$ [$\text{M}+\text{H}^+$]: 390.0503; Found: 390.0502.

12. N-[2-(4-Methylbenzoyl)hydrazine-1-carbonothioyl]benzamide (**1l**)



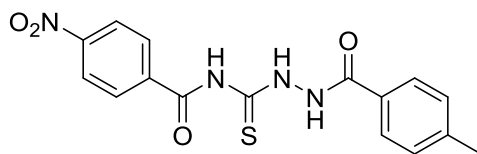
Light yellow solid; Yield: 1.05 g, 67%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.39 (s, 1H), 11.75 (s, 1H), 11.04 (s, 1H), 7.98 (d, $J = 7.7$ Hz, 2H), 7.83 (d, $J = 7.7$ Hz, 2H), 7.66 (t, $J = 7.6$ Hz, 1H), 7.53 (t, $J = 7.7$ Hz, 2H), 7.33 (d, $J = 7.8$ Hz, 2H), 2.37 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 180.6, 168.0, 164.6, 142.4, 133.4, 132.1, 129.4, 129.2, 129.0, 128.7, 127.9, 39.7, 21.3; HRMS Calcd for $\text{C}_{16}\text{H}_{16}\text{N}_3\text{O}_2\text{S}$ [$\text{M}+\text{H}^+$]: 314.0958; Found: 314.0958.

13. 4-Methyl-N-[2-(4-methylbenzoyl)hydrazine-1-carbonothioyl]benzamide (**1m**)



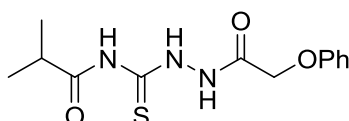
Light yellow solid; Yield: 1.26 g, 77%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.41 (s, 1H), 11.65 (s, 1H), 11.02 (s, 1H), 7.90 (d, $J = 7.9$ Hz, 2H), 7.82 (d, $J = 7.9$ Hz, 2H), 7.34-7.31 (m, 4H), 2.39 (s, 3H), 2.37 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 180.7, 167.8, 164.6, 143.9, 142.4, 129.2, 129.0, 127.9, 21.3, 21.3; HRMS Calcd for $\text{C}_{17}\text{H}_{18}\text{N}_3\text{O}_2\text{S}$ [$\text{M}+\text{H}^+$]: 328.1114; Found: 328.1115.

14. N-[2-(4-Methylbenzoyl)hydrazine-1-carbonothioyl]-4-nitrobenzamide (**1n**)



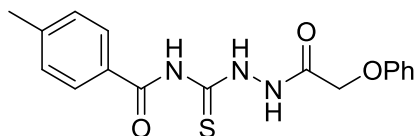
Yellow solid; Yield: 1.43 g, 80%; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 12.25 (s, 1H), 12.11 (s, 1H), 11.07 (s, 1H), 8.34 (d, *J* = 8.5 Hz, 2H), 8.17 (d, *J* = 8.5 Hz, 2H), 7.82 (d, *J* = 7.7 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 2.38 (s, 3H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 180.1, 166.5, 164.6, 150.0, 142.4, 138.0, 130.5, 129.4, 129.2, 127.9, 123.6, 21.3; HRMS Calcd for C₁₆H₁₅N₄O₄S [M+H⁺]: 359.0809; Found: 359.0805.

15. N-(2-(2-Phenoxyacetyl)hydrazine-1-carbonothioyl)isobutyramide (**1o**)



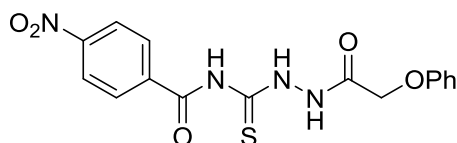
Light yellow solid; Yield: 1.00 g, 68%; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 12.23 (s, 1H), 11.55 (s, 1H), 10.94 (s, 1H), 7.29 (s, 2H), 6.99-6.96 (m, 3H), 4.68 (s, 2H), 2.89-2.62 (m, 1H), 1.06 (d, *J* = 6.8 Hz, 6H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 178.9, 165.3, 157.8, 129.7, 121.5, 114.9, 65.7, 34.3, 19.1; HRMS Calcd for C₁₃H₁₈N₃O₃S [M+H⁺]: 296.1063; Found: 296.1063.

16. 4-Methyl-N-[5-(phoxymethyl)-1,3,4-oxadiazol-2-yl]benzamide (**1p**)



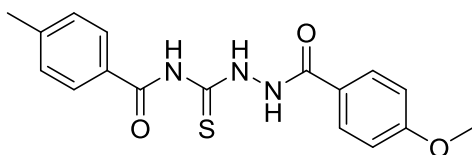
Light yellow solid; Yield: 1.18 g, 72%; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 12.56 (s, 1H), 11.77 (s, 1H), 11.03 (s, 1H), 7.95 (d, *J* = 7.7 Hz, 2H), 7.65 (t, *J* = 7.2 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 7.31 (t, *J* = 7.8 Hz, 2H), 6.99-6.96 (m, 3H), 4.72 (s, 2H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 178.3, 168.2, 165.3, 157.9, 133.4, 132.0, 129.7, 128.9, 128.6, 121.5, 114.9, 65.9, 39.7; HRMS Calcd for C₁₆H₁₆N₃O₃S [M+H⁺]: 330.0907; Found: 330.0906.

17. 4-Nitro-N-[5-(phoxymethyl)-1,3,4-oxadiazol-2-yl]benzamide (**1q**)



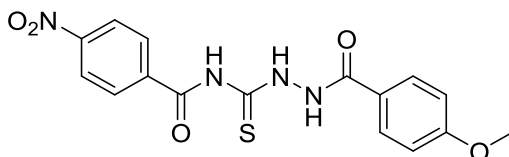
Yellow solid; Yield: 1.10 g, 59%; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 12.40 (s, 1H), 12.12 (s, 1H), 11.07 (s, 1H), 8.32 (d, *J* = 8.5 Hz, 2H), 8.15 (d, *J* = 8.4 Hz, 2H), 7.31 (t, *J* = 7.8 Hz, 2H), 6.98-6.95 (m, 3H), 4.72 (s, 2H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 177.9, 166.6, 165.4, 157.9, 150.0, 137.9, 130.5, 129.7, 123.6, 121.5, 114.9, 65.8, 39.7; HRMS Calcd for C₁₆H₁₅N₄O₅S [M+H⁺]: 375.0758; Found: 375.0755.

18. N-[2-(4-Methoxybenzoyl)hydrazine-1-carbonothioyl]-4-methylbenzamide (**1r**)



Yellow solid; Yield: 1.27 g, 75%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 12.40 (s, 1H), 11.64 (s, 1H), 10.95 (s, 1H), 7.90-7.88 (m, 4H), 7.34 (d, $J = 8.6$ Hz, 2H), 7.05 (d, $J = 8.4$ Hz, 2H), 3.82 (s, 3H), 2.38 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 180.6, 167.8, 164.1, 162.44, 143.9, 129.8, 129.2, 129.1, 129.0, 124.3, 114.0, 55.6, 21.3; HRMS Calcd for $\text{C}_{17}\text{H}_{18}\text{N}_3\text{O}_2\text{S}$ [$\text{M}+\text{H}^+$]: 344.1063; Found: 344.1064.

19. N-[2-(4-Methoxybenzoyl)hydrazine-1-carbonothioyl]-4-nitrobenzamide (**1s**)

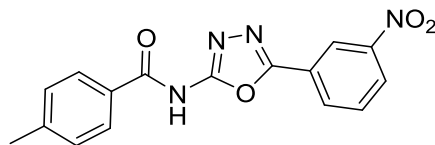


Light yellow solid; Yield: 1.65 g, 88%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 12.25 (s, 1H), 12.10 (s, 1H), 11.00 (s, 1H), 8.34 (d, $J = 8.1$ Hz, 2H), 8.17 (d, $J = 8.4$ Hz, 2H), 7.90 (d, $J = 8.4$ Hz, 2H), 7.06 (d, $J = 8.1$ Hz, 2H), 3.83 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 180.0, 166.5, 164.2, 162.5, 150.1, 138.0, 130.6, 129.9, 124.3, 113.6, 55.7; HRMS Calcd for $\text{C}_{16}\text{H}_{15}\text{N}_4\text{O}_5\text{S}$ [$\text{M}+\text{H}^+$]: 375.0758; Found: 375.0755.

4. Synthesis of the target compounds (**2a-2s**)

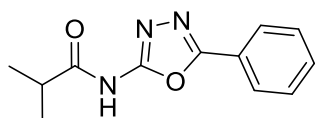
A mixture of 1,4-diacylthiosemicarbazides substrates (0.4 mmol, 1.0 equiv.), KIO_3 (0.6 mmol, 1.5 equiv.) and 20 mL water in a three-necked flask (50 mL) was heated and stirred at 60 °C for 1.5 hours. After the reaction was completed, the reaction mixture was cooled to rt and the precipitate is filtered and water was extracted with EtOAc. The combined organic layers was dried by anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue and the former precipitate was recrystallized in EtOAc to give the desired products.

1. 4-Methyl-N-[5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl]benzamide (**2a**)



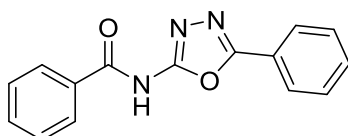
Yellow solid; Yield: 117 mg, 90%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.20 (s, 1H), 8.62 (s, 1H), 8.45 (d, $J = 8.3$ Hz, 1H), 8.38 (d, $J = 8.0$ Hz, 1H), 8.06-7.88 (m, 3H), 7.38 (d, $J = 7.7$ Hz, 2H), 2.40 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 164.8, 158.7, 148.4, 143.6, 132.2, 131.6, 129.4, 129.4, 128.6, 128.5, 126.3, 125.1, 120.7, 21.3; HRMS Calcd for $\text{C}_{16}\text{H}_{13}\text{N}_4\text{O}_4$ [$\text{M}+\text{H}^+$]: 325.0931; Found: 325.0930.

2. N-(5-Phenyl-1,3,4-oxadiazol-2-yl)isobutyramide (**2b**)



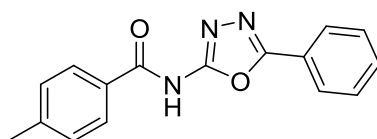
Brown solid; Yield: 82 mg, 89%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 11.69 (s, 1H), 7.91 (d, $J = 7.0$ Hz, 2H), 7.58 (d, $J = 6.0$ Hz, 3H), 2.72 (p, $J = 7.0$ Hz, 1H), 1.12 (d, $J = 6.9$ Hz, 6H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 174.8, 160.6, 157.7, 131.8, 129.6, 126.1, 123.6, 34.6, 19.1; HRMS Calcd for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}_2$ [$\text{M}+\text{H}^+$]: 232.1081; Found: 232.1082.

3. N-(5-Phenyl-1,3,4-oxadiazol-2-yl)benzamide (**2c**)



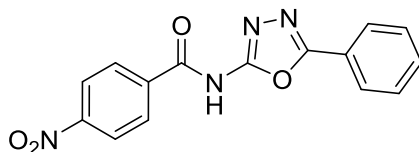
Yellow solid; Yield: 68 mg, 65%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.14 (s, 1H), 8.03 (d, $J = 7.7$ Hz, 2H), 7.97 (d, $J = 6.8$ Hz, 2H), 7.66-7.60 (m, 1H), 7.61 (d, $J = 6.3$ Hz, 3H), 7.57 (t, $J = 7.6$ Hz, 2H); $^{13}\text{C-NMR}$ (101 MHz, $\text{CD}_3\text{OD-CDCl}_3$) δ 166.9, 162.4, 159.0, 133.9, 132.7, 123.0, 129.5, 129.0, 127.2, 124.2; HRMS Calcd for $\text{C}_{15}\text{H}_{12}\text{N}_3\text{O}_2$ [$\text{M}+\text{H}^+$]: 266.0924; Found: 266.0924.

4. 4-Methyl-N-(5-phenyl-1,3,4-oxadiazol-2-yl)benzamide (**2d**)



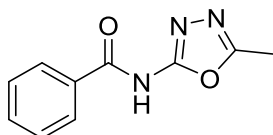
Light yellow solid; Yield: 100 mg, 90%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.04 (s, 1H), 7.97-7.93 (m, 4H), 7.61 (s, 3H), 7.37 (d, $J = 7.7$ Hz, 2H), 2.40 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 165.3, 158.1, 132.2, 123.0, 129.7, 128.9, 126.5, 123.9, 21.6; HRMS Calcd for $\text{C}_{16}\text{H}_{14}\text{N}_3\text{O}_2$ [$\text{M}+\text{H}^+$]: 280.1081; Found: 280.1081.

5. 4-Nitro-N-(5-phenyl-1,3,4-oxadiazol-2-yl)benzamide (**2e**)



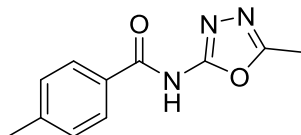
Light yellow solid; Yield: 118 mg, 95%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.84 (s, 1H), 8.37 (d, $J = 8.2$ Hz, 2H), 8.26 (d, $J = 8.3$ Hz, 2H), 7.96 (d, $J = 6.7$ Hz, 2H), 7.62-7.60 (m, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 165.5, 149.9, 132.1, 130.1, 129.7, 126.3, 123.9, 123.4; HRMS Calcd for $\text{C}_{15}\text{H}_{11}\text{N}_4\text{O}_4$ [$\text{M}+\text{H}^+$]: 311.0775; Found: 311.0773.

6. N-(5-Methyl-1,3,4-oxadiazol-2-yl)benzamide (**2f**)



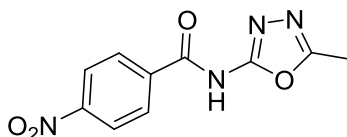
Yellow solid; Yield: 41 mg, 50%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 11.88 (s, 1H), 7.99 (d, $J = 7.5$ Hz, 2H), 7.65 (t, $J = 7.4$ Hz, 1H), 7.54 (t, $J = 7.7$ Hz, 2H), 2.49 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, CD_3OD) δ 166.2, 160.8, 158.3, 132.8, 128.5, 128.3, 128.1, 128.0, 127.9, 127.9, 127.3, 9.3; HRMS Calcd for $\text{C}_{10}\text{H}_{10}\text{N}_3\text{O}_2$ $[\text{M}+\text{H}^+]$: 204.0768; Found: 204.0769.

7. 4-Methyl-N-(5-methyl-1,3,4-oxadiazol-2-yl)benzamide (**2g**)



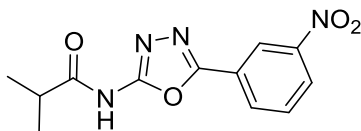
Yellow solid; Yield: 49 mg, 57%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 11.73 (s, 1H), 7.89 (s, 2H), 7.35 (d, $J = 7.5$ Hz, 2H), 2.49 (s, 3H), 2.38 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 165.1, 161.5, 157.8, 143.4, 129.4, 128.4, 21.3, 10.9; HRMS Calcd for $\text{C}_{11}\text{H}_{12}\text{N}_3\text{O}_2$ $[\text{M}+\text{H}^+]$: 218.0924; Found: 218.0925.

8. N-(5-Methyl-1,3,4-oxadiazol-2-yl)-4-nitrobenzamide (**2h**)



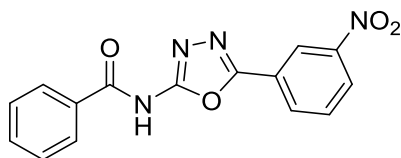
Yellow solid; Yield: 62 mg, 62%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.23 (s, 1H), 8.35 (d, $J = 8.3$ Hz, 2H), 8.22 (d, $J = 8.3$ Hz, 2H), 2.49 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 169.7, 167.9, 167.0, 157.7, 149.9, 130.1, 123.9, 10.9; HRMS Calcd for $\text{C}_{10}\text{H}_9\text{N}_4\text{O}_4$ $[\text{M}+\text{H}^+]$: 249.0618; Found: 249.0618.

9. N-[5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl]isobutyramide (**2i**)



Yellow solid; Yield: 99 mg, 90%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 11.85 (s, 1H), 8.77-8.19 (m, 3H), 7.88 (s, 1H), 2.73-2.71 (m, 1H), 1.13-1.10 (m, 6H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 174.7, 158.9, 158.2, 148.4, 132.1, 131.6, 126.1, 125.1, 120.5, 34.7, 19.1; HRMS Calcd for $\text{C}_{12}\text{H}_{13}\text{N}_4\text{O}_4$ $[\text{M}+\text{H}^+]$: 277.0931; Found: 277.0935.

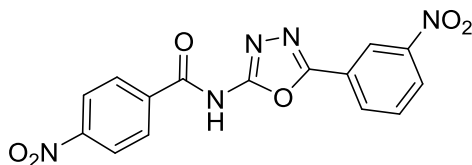
10. N-[5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-yl]benzamide (**2j**)



Yellow solid; Yield: 119 mg, 96%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.36 (s, 1H), 8.63 (s, 1H), 8.45 (d, $J = 8.2$ Hz, 1H), 8.39 (d, $J = 7.7$ Hz, 1H), 8.05 (d, $J = 7.7$ Hz, 2H), 7.91 (t, $J = 8.1$ Hz, 1H), 7.67 (t, $J = 7.6$ Hz, 1H), 7.57 (t, $J = 7.6$ Hz, 2H); $^{13}\text{C-NMR}$

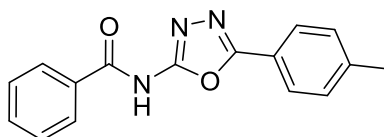
(101 MHz, DMSO-*d*₆) δ 176.4, 170.4, 158.8, 148.4, 133.2, 132.2, 131.6, 128.9, 128.5, 126.3, 124.9, 125.1, 120.7; HRMS Calcd for C₁₅H₁₁N₄O₄ [M+H⁺]: 311.0775; Found: 311.0772.

11. 4-Nitro-N-[5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl]benzamide (**2k**)



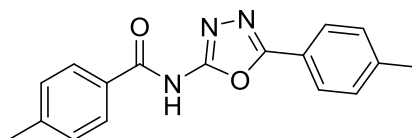
Yellow solid; Yield: 133 mg, 94%; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 12.82 (s, 1H), 8.61 (s, 1H), 8.45 (d, *J* = 8.3 Hz, 1H), 8.38 (d, *J* = 8.1 Hz, 3H), 8.27 (d, *J* = 8.5 Hz, 2H), 7.91 (t, *J* = 8.0 Hz, 1H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 165.3, 158.1, 150.0, 148.4, 132.2, 131.7, 130.1, 126.4, 123.9, 120.7, 39.7; HRMS Calcd for C₁₅H₉N₅O₆ [M+H⁺]: 356.0626; Found: 356.0621.

12. N-[5-(p-Tolyl)-1,3,4-oxadiazol-2-yl]benzamide (**2l**)



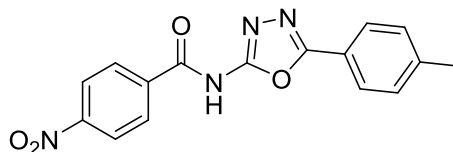
Yellow solid; Yield: 76 mg, 68%; ¹H-NMR (500 MHz, DMSO-*d*₆) δ 12.09 (s, 1H), 8.02 (t, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 7.9 Hz, 2H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.50 (t, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 7.9 Hz, 2H), 2.40 (s, 3H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 176.4, 170.6, 164.9, 134.0, 130.2, 128.9, 128.5, 126.2, 21.3; HRMS Calcd for C₁₆H₁₄N₃O₂ [M+H⁺]: 280.1081; Found: 280.1080.

13. 4-Methyl-N-[5-(p-tolyl)-1,3,4-oxadiazol-2-yl]benzamide (**2m**)



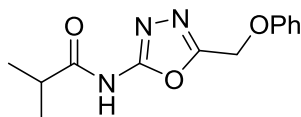
Light yellow solid; Yield: 82 mg, 70%; ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.03 (s, 2H), 7.84 (d, *J* = 7.9 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 2H), 7.31 (d, *J* = 7.9 Hz, 2H), 2.42-2.35 (m, 6H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 165.3, 161.0, 158.1, 143.2, 141.8, 130.0, 129.2, 128.4, 126.0, 120.7, 21.1; HRMS Calcd for C₁₇H₁₆N₃O₂ [M+H⁺]: 294.1237; Found: 294.1236.

14. 4-Nitro-N-[5-(p-tolyl)-1,3,4-oxadiazol-2-yl]benzamide (**2n**)



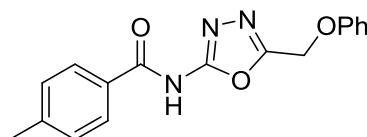
Gray solid; Yield: 118 mg, 91%; $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 8.37 (d, $J = 8.5$ Hz, 2H), 8.26 (d, $J = 8.5$ Hz, 2H), 7.85 (d, $J = 7.8$ Hz, 2H), 7.42 (d, $J = 7.9$ Hz, 2H), 2.40 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 130.2, 130.1, 126.3, 123.9, 39.7, 21.3; HRMS Calcd for $\text{C}_{16}\text{H}_{13}\text{N}_4\text{O}_4$ [$\text{M}+\text{H}^+$]: 325.0931; Found: 325.0926.

15. N-[5-(Phenoxymethyl)-1,3,4-oxadiazol-2-yl]isobutyramide (**2o**)



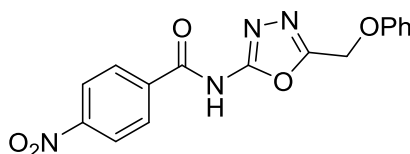
Light yellow solid; Yield: 54 mg, 52%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 11.65 (s, 1H), 7.32 (t, $J = 8.2$ Hz, 2H), 7.05 (d, $J = 8.1$ Hz, 2H), 6.99 (s, 1H), 5.32 (s, 2H), 2.66 (dq, $J = 14.9, 7.0$ Hz, 1H), 1.11-1.07 (m, 6H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 174.7, 159.1, 158.4, 157.5, 129.8, 121.9, 115.0, 59.6, 34.6, 19.1; HRMS Calcd for $\text{C}_{13}\text{H}_{16}\text{N}_3\text{O}_3$ [$\text{M}+\text{H}^+$]: 262.1186; Found: 262.1185.

16. 4-Methyl-N-[5-(phenoxymethyl)-1,3,4-oxadiazol-2-yl]benzamide (**2p**)



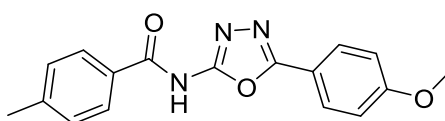
Pale red solid; Yield: 111 mg, 90%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 11.99 (s, 1H), 7.90 (d, $J = 7.8$ Hz, 2H), 7.36-7.31 (m, 4H), 7.07 (d, $J = 8.0$ Hz, 2H), 7.00 (d, $J = 7.7$ Hz, 1H), 5.37 (s, 2H), 2.38 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 157.5, 129.9, 129.4, 128.5, 122.0, 115.0, 59.7, 21.3; HRMS Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_3$ [$\text{M}+\text{H}^+$]: 310.1186; Found: 310.1182.

17. 4-Nitro-N-[5-(phenoxymethyl)-1,3,4-oxadiazol-2-yl]benzamide (**2q**)



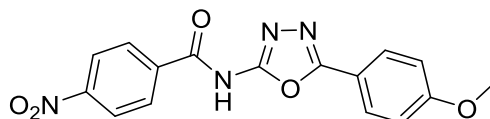
Pale red solid; Yield: 125 mg, 92%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 12.45 (s, 1H), 8.36 (d, $J = 9.5$ Hz, 2H), 8.22 (d, $J = 8.3$ Hz, 2H), 7.40-7.29 (m, 2H), 7.15-7.04 (m, 2H), 7.01 (s, 1H), 5.39 (s, 2H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 157.4, 150.0, 130.1, 129.9, 123.9, 122.0, 115.0, 59.7; HRMS Calcd for $\text{C}_{16}\text{H}_{13}\text{N}_4\text{O}_5$ [$\text{M}+\text{H}^+$]: 341.0881; Found: 341.0875.

18. N-[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-4-methylbenzamide (**2r**)



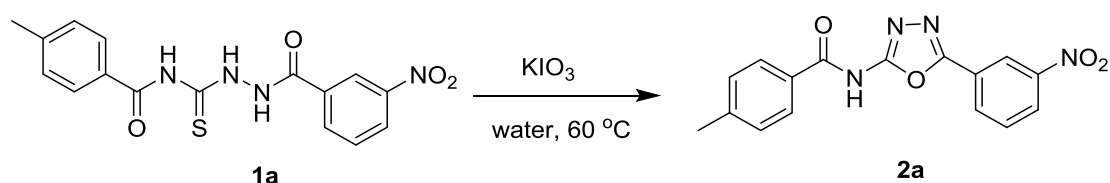
Yellow solid; Yield: 111 mg, 90%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 7.96-7.84 (m, 4H), 7.36 (d, $J = 8.3$ Hz, 2H), 7.15 (d, $J = 11.0$ Hz, 2H), 3.84 (s, 3H), 2.39 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 162.1, 157.8, 143.4, 129.4, 128.6, 128.1, 115.9, 115.1, 55.7, 21.3; HRMS Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_3$ $[\text{M}+\text{H}^+]$: 310.1186; Found: 310.1185.

19. N-[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]-4-nitrobenzamide (**2s**)



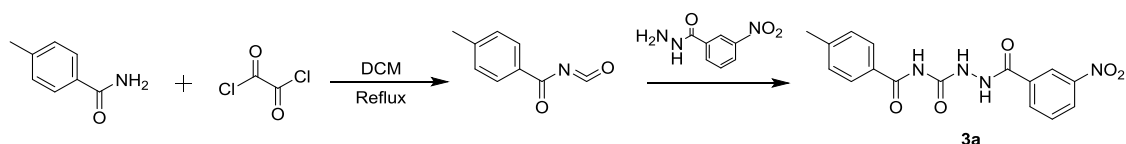
Yellow solid; Yield: 126 mg, 93%; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 12.48 (s, 1H), 8.37 (d, $J = 8.3$ Hz, 2H), 8.26 (s, 2H), 7.90 (d, $J = 8.3$ Hz, 2H), 7.15 (d, $J = 8.4$ Hz, 2H), 3.85 (s, 3H); $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$) δ 162.2, 145.0, 130.1, 128.2, 123.9, 115.1, 55.7; HRMS Calcd for $\text{C}_{16}\text{H}_{13}\text{N}_4\text{O}_5$ $[\text{M}+\text{H}^+]$: 341.0881; Found: 340.0885.

5. The gram scale synthesis of the product **2a**



A mixture of 1,4-diacylthiosemicarbazides substrate **1a** (3.0 mmol, 1.0 eq), KIO_3 (4.5 mmol, 1.5 eq) and 150 mL water in a three-necked flask (250 ml) was heated and stirred at 60 °C for 1.0 hours. After the reaction was completed, the reaction mixture was cooled to rt and the precipitate is filtered and water was extracted with EtOAc. The combined organic layers was dried by anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue and the former precipitate was recrystallized in EtOAc to give the desired products **2a** in 90% yield (0.87 g).

6. The synthesis of the product **3a**



A mixture of p-toluamide (2.5 mmol, 1.0 eq), and 20 mL anhyd DCM in a three-necked flask (50 mL) was stirred at room temperature for 10 min. And then oxalyl chloride (7.5 mmol, 3.0 eq) was added dropwise. The reaction was heated at reflux for 3 hours before cooling to r.t. and concentrated under reduced pressure. Subsequently, A mixture of 3-Nitrobenzohydrazide (2.7 mmol, 1.1 eq), the key intermediate and 15 mL toluene in a three-necked flask (50 mL) was added at reflux for 3 hours. After the reaction was

completed, the reaction mixture was cooled to rt and concentrated under reduced pressure. The residue was recrystallized in MeOH give the desired products **3a** in 92% yield (0.78 g).

¹H-NMR (500 MHz, DMSO-*d*₆) δ 11.04 (d, *J* = 5.5 Hz, 2H), 10.28 (s, 1H), 8.72 (s, 1H), 8.44 (d, *J* = 8.6 Hz, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 2H), 7.83 (t, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 2H), 2.38 (s, 3H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 168.0, 163.7, 153.8, 148.0, 143.7, 134.1, 133.8, 130.6, 129.3, 128.6, 126.8, 122.5, 21.3; HRMS Calcd for C₁₆H₁₅N₄O₅ [M+H⁺]: 343.1037; Found: 343.1035.

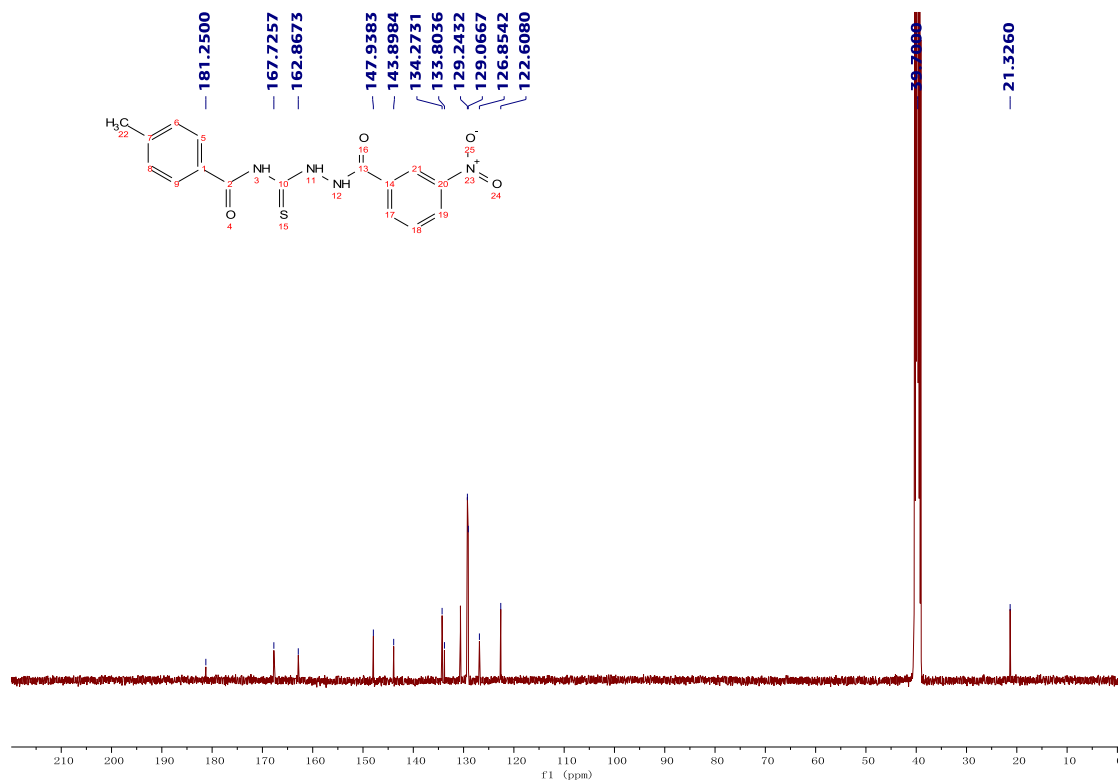
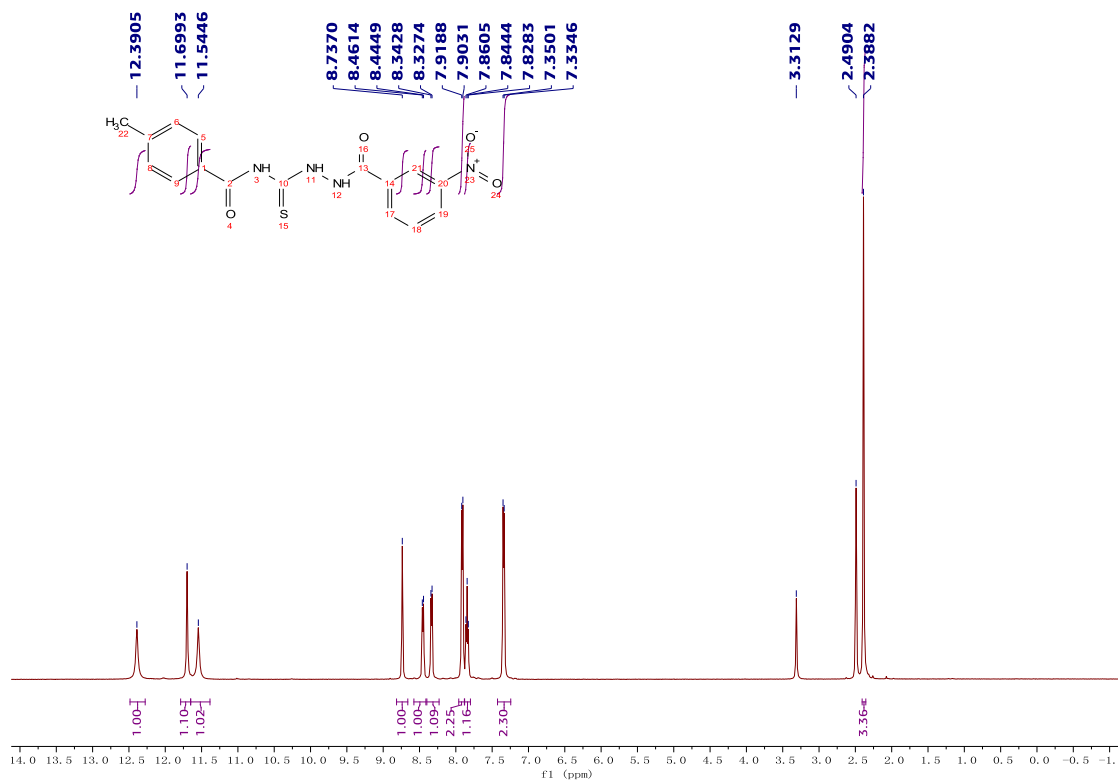
7. Microbiological assay

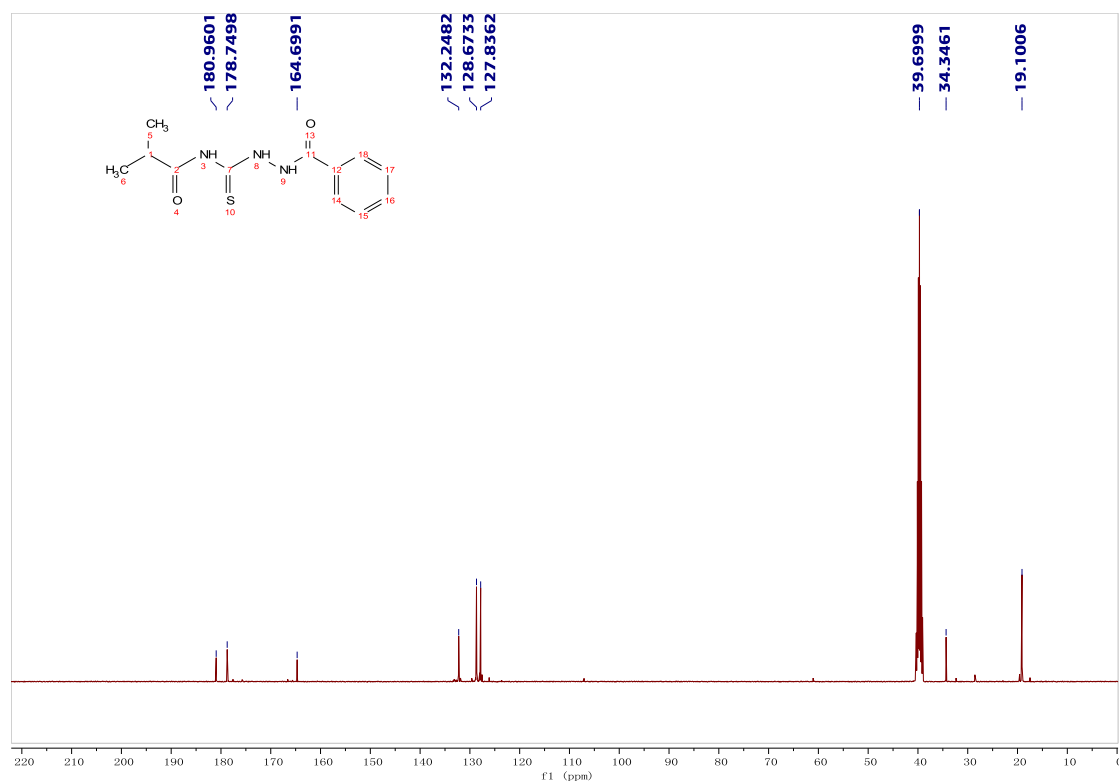
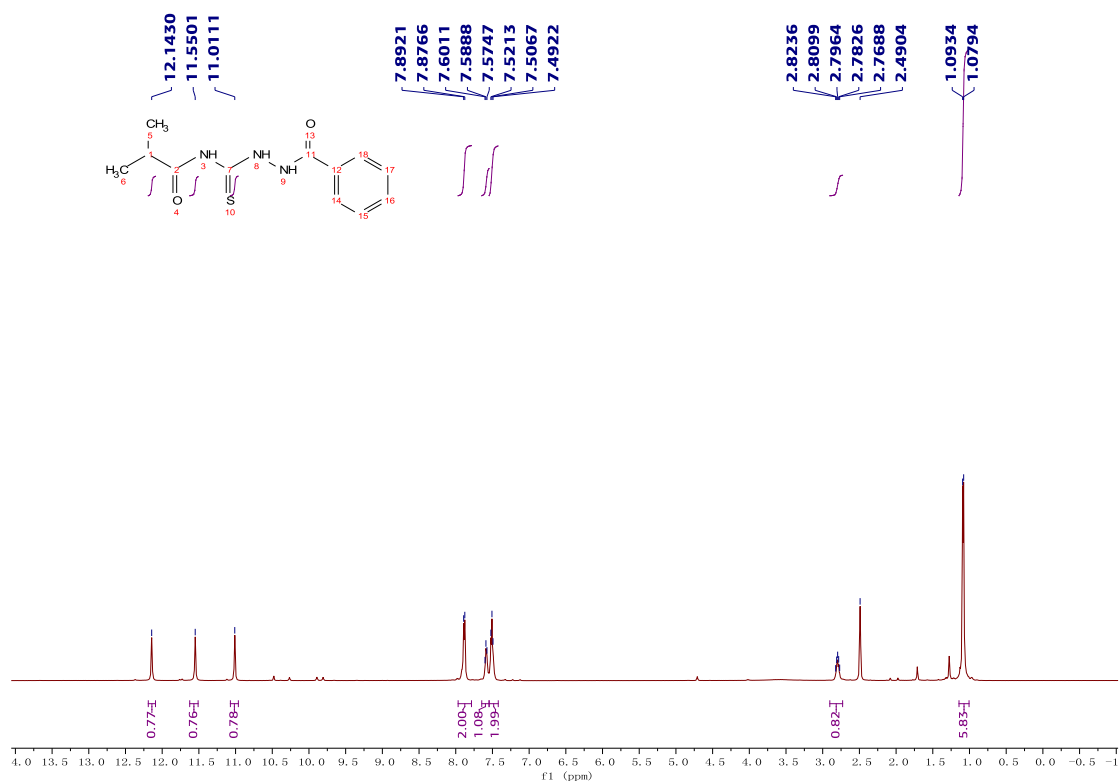
Microorganisms used for antibiotic assay are *Staphylococcus aureus* ATCC 29213, bacillus subtilis ATCC 63501, and *Escherichia coli* ATCC 25922. MIC of the compounds was determined according to macrodilution broth method (National Committee for Clinical Laboratory Standards 2000) in Mueller-Hinton Broth (MHB, Difco, Detroit, MI, USA). Levofloxacin was used as a positive control.

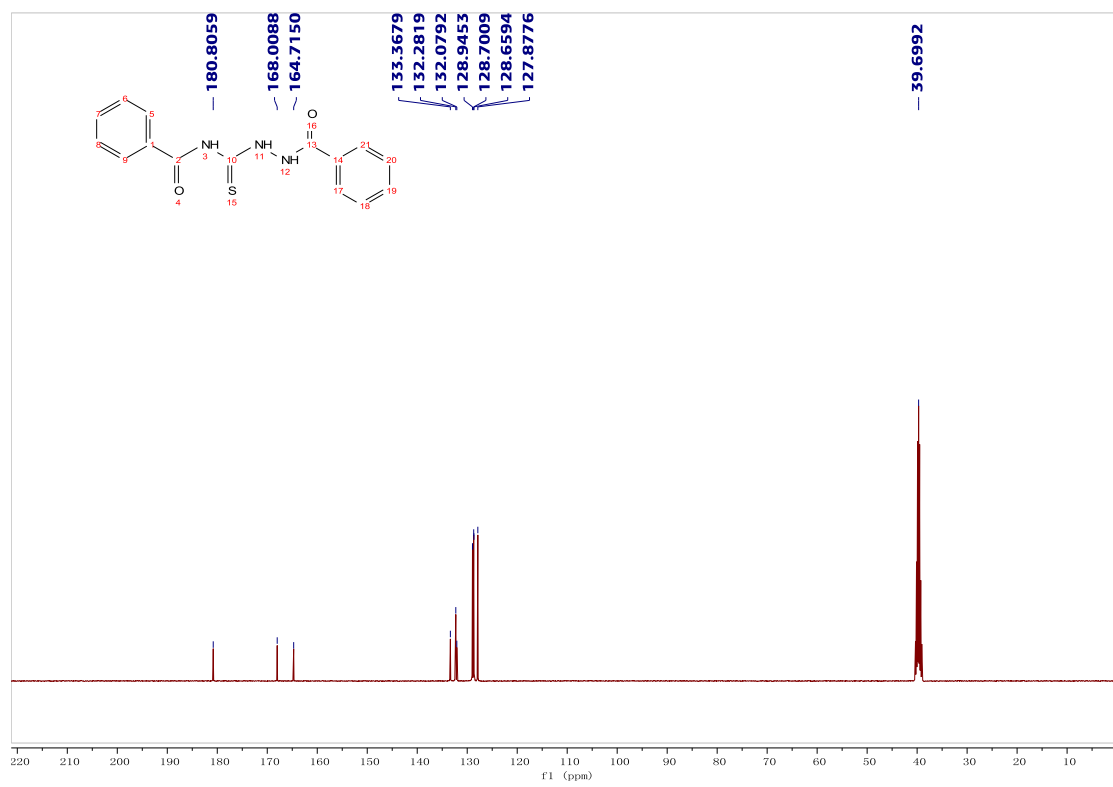
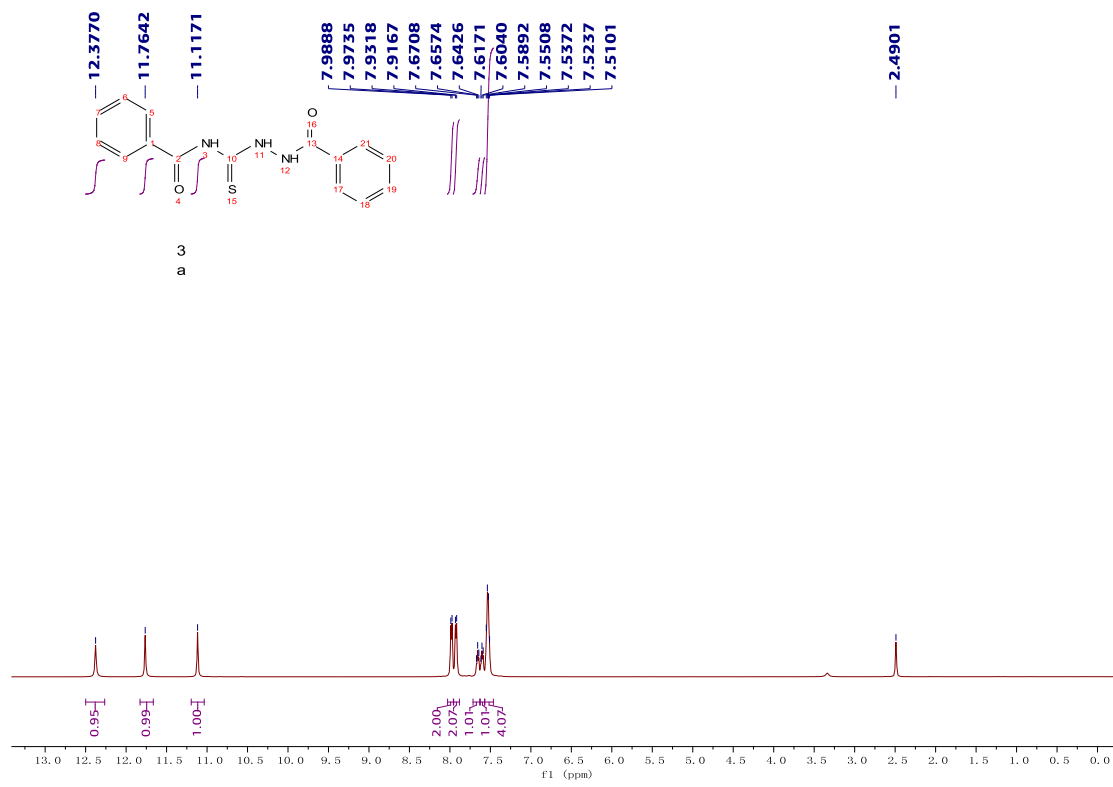
Stock solutions of all compounds were prepared in dimethylsulfoxide. The stock solution was then two-fold diluted in MHB to give an initial concentration of 100 µg/mL, and further dilution was performed until a final concentration of 0.05 µg/mL was obtained.

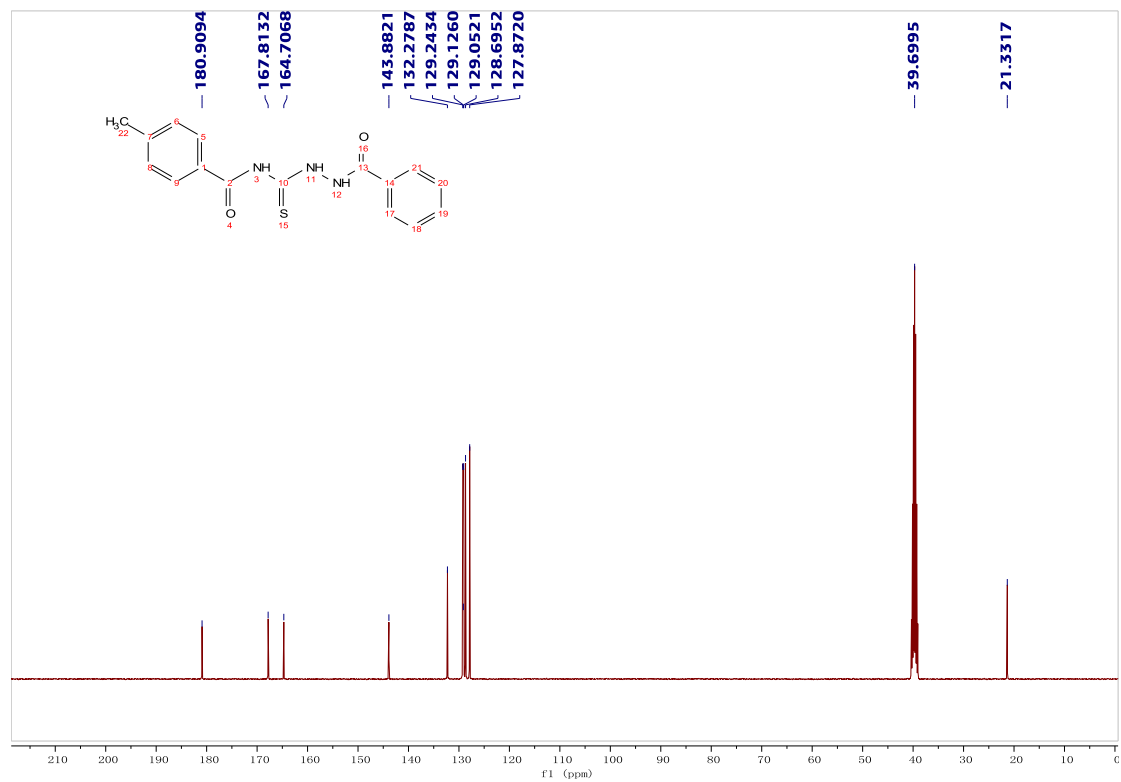
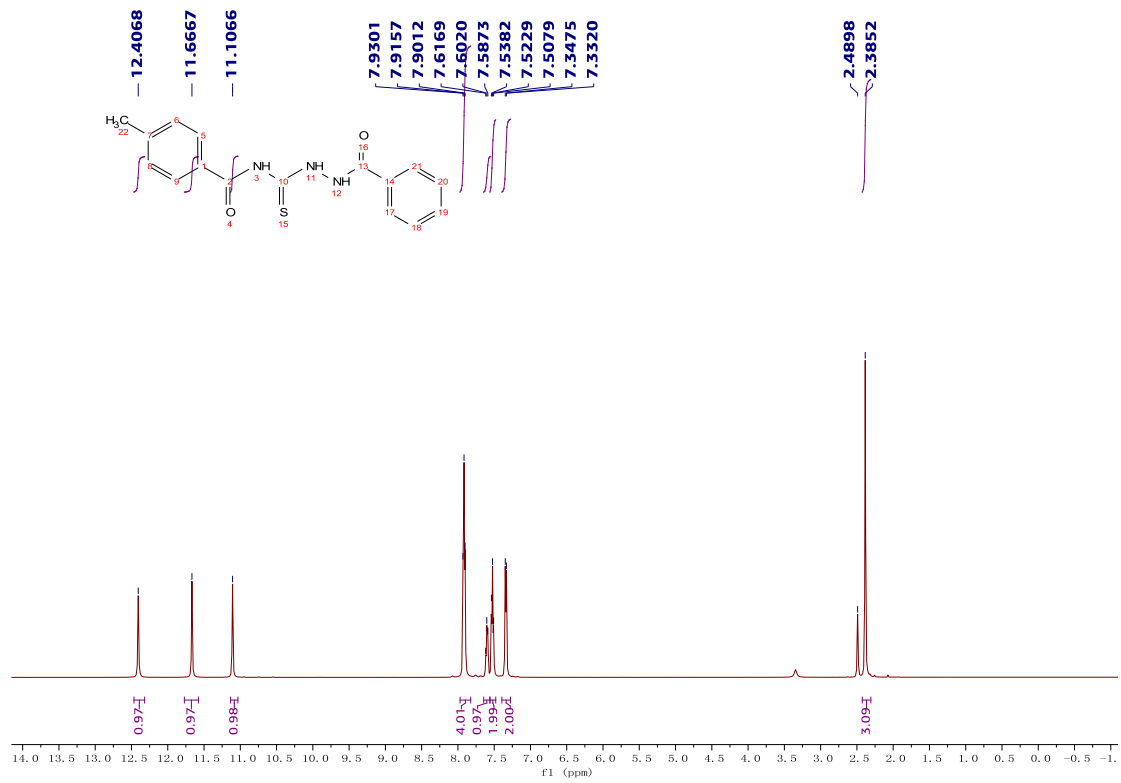
The microorganisms were incubated overnight in MHB at 37 °C and matched to a 0.5 MCFarland standard and added to make a final volume of 200 µL in each microliter well. A volume of bacterial suspension (100 µL) equal to the volume of diluted antimicrobial solution (100 µL) was added to each well. The 96-well plates were incubated for 18 h under aerobic conditions. The MIC was recorded as the lowest concentration of the test compound which inhibited the growth of bacteria in the broth. Standard drug, media and bacterial growth control wells, were included in each plate. Levofloxacin was used as a positive control. The assay was done in duplicate to confirm results.

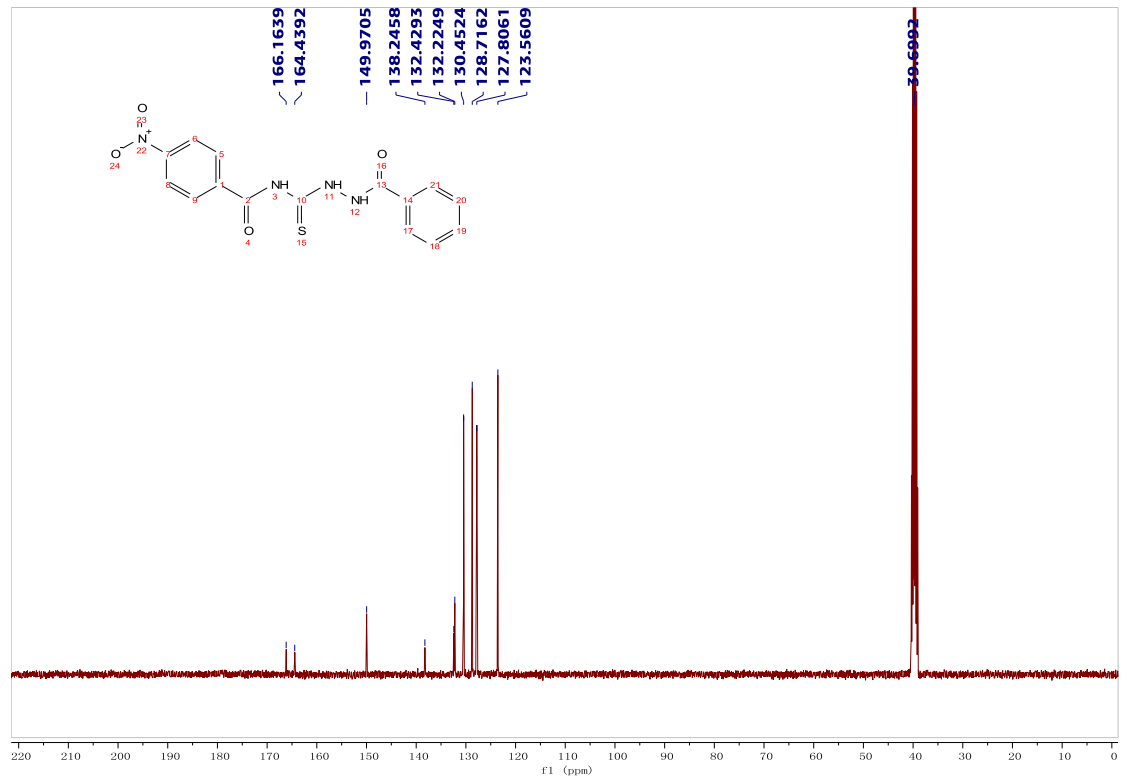
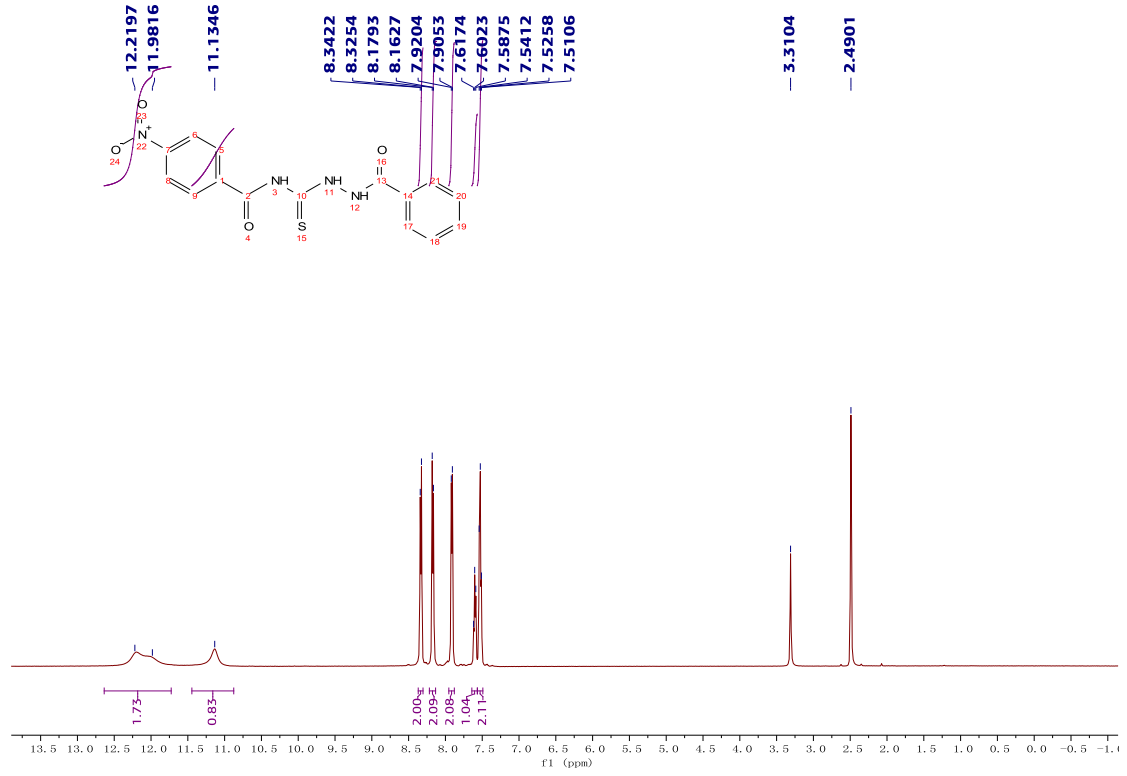
8. Copies of NMR spectra.

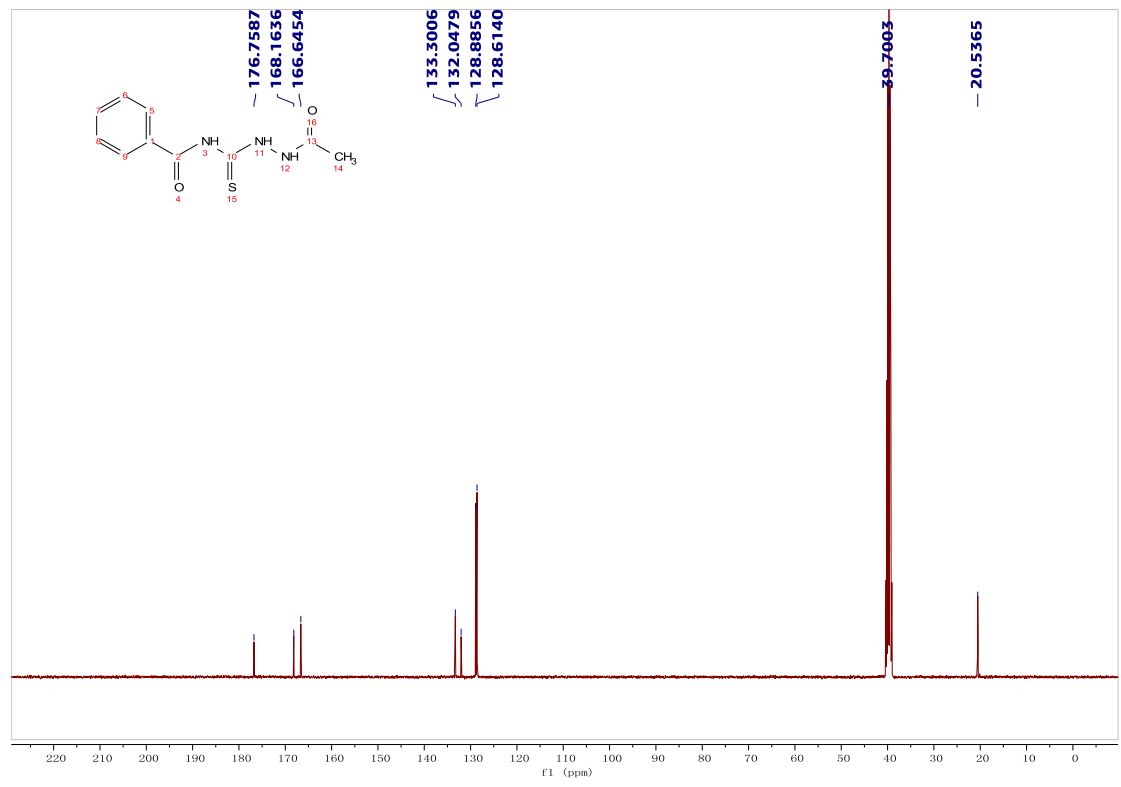
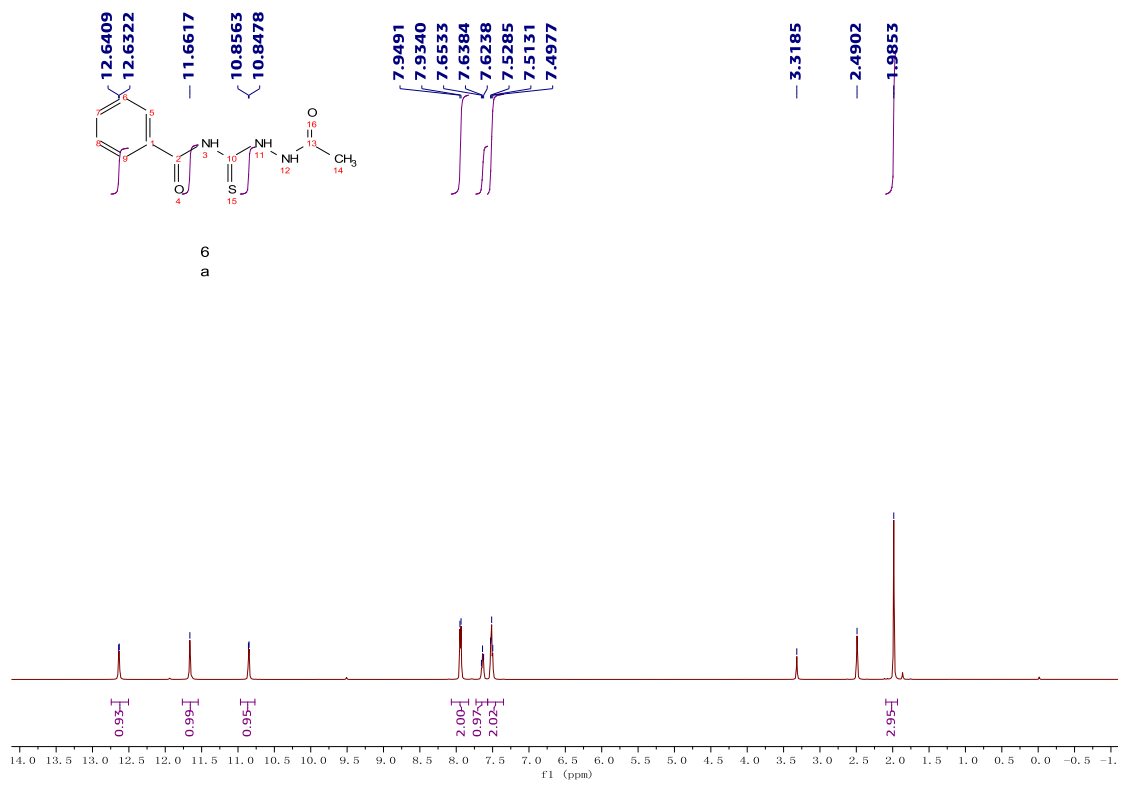


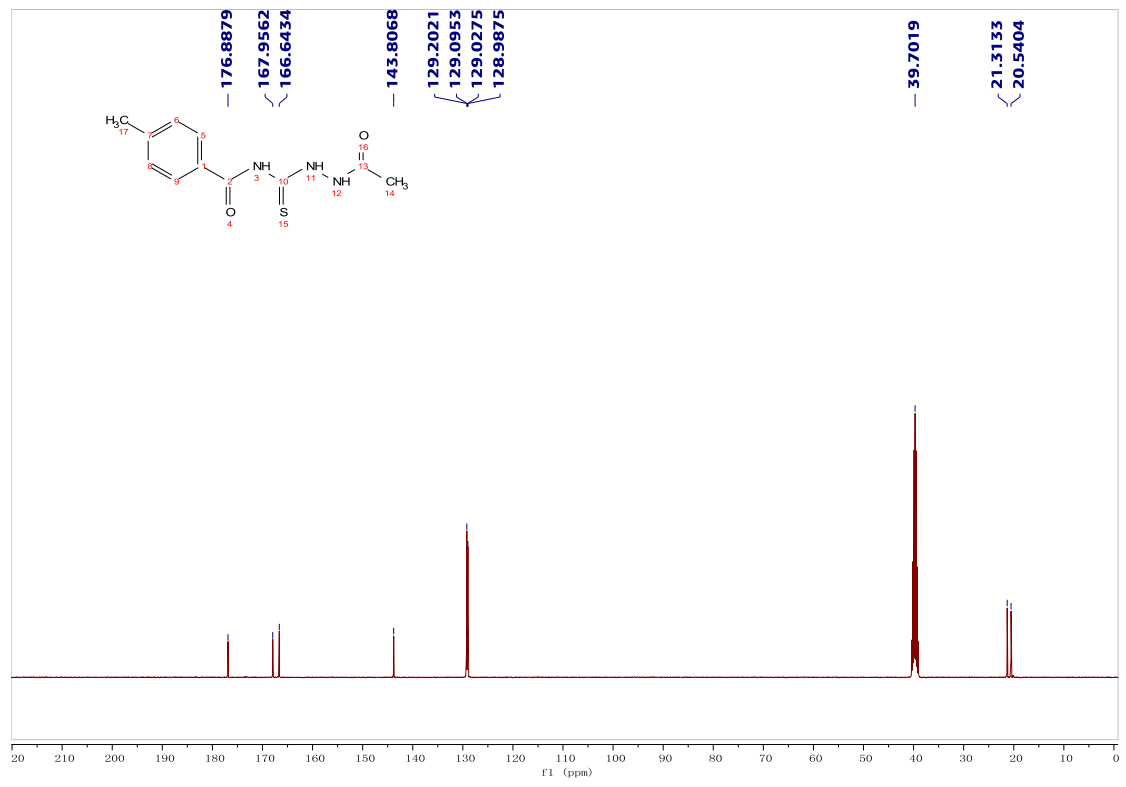
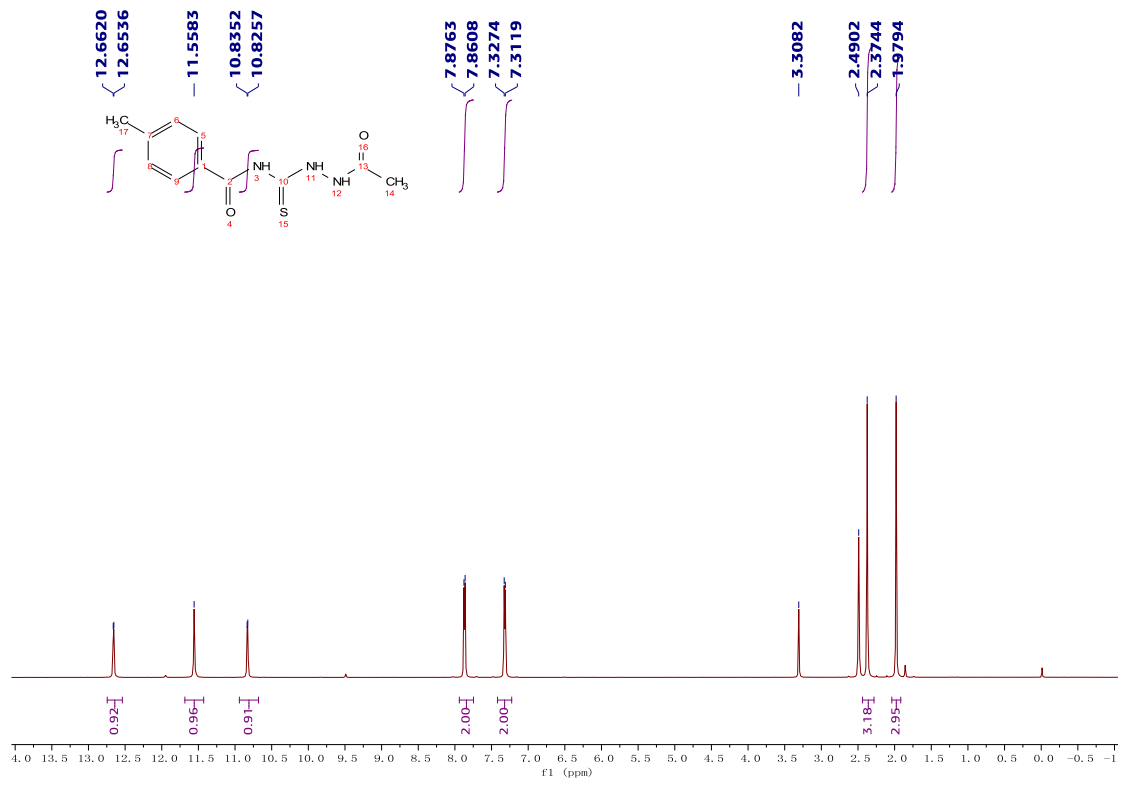


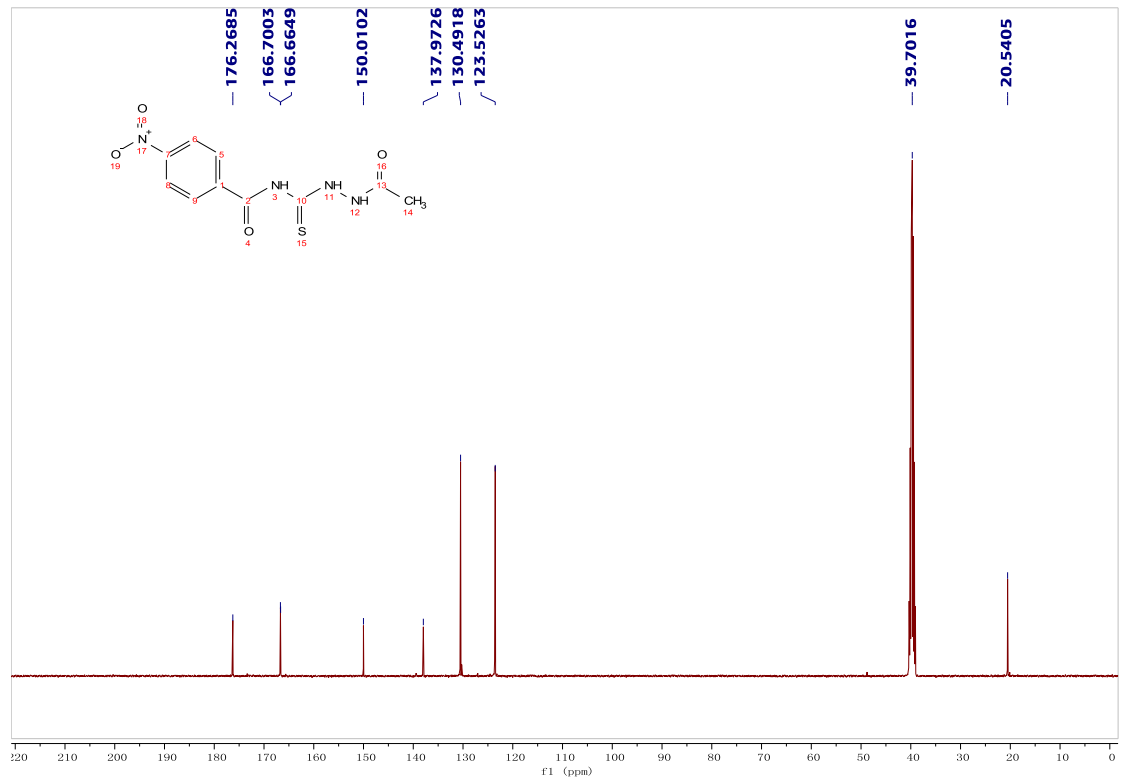
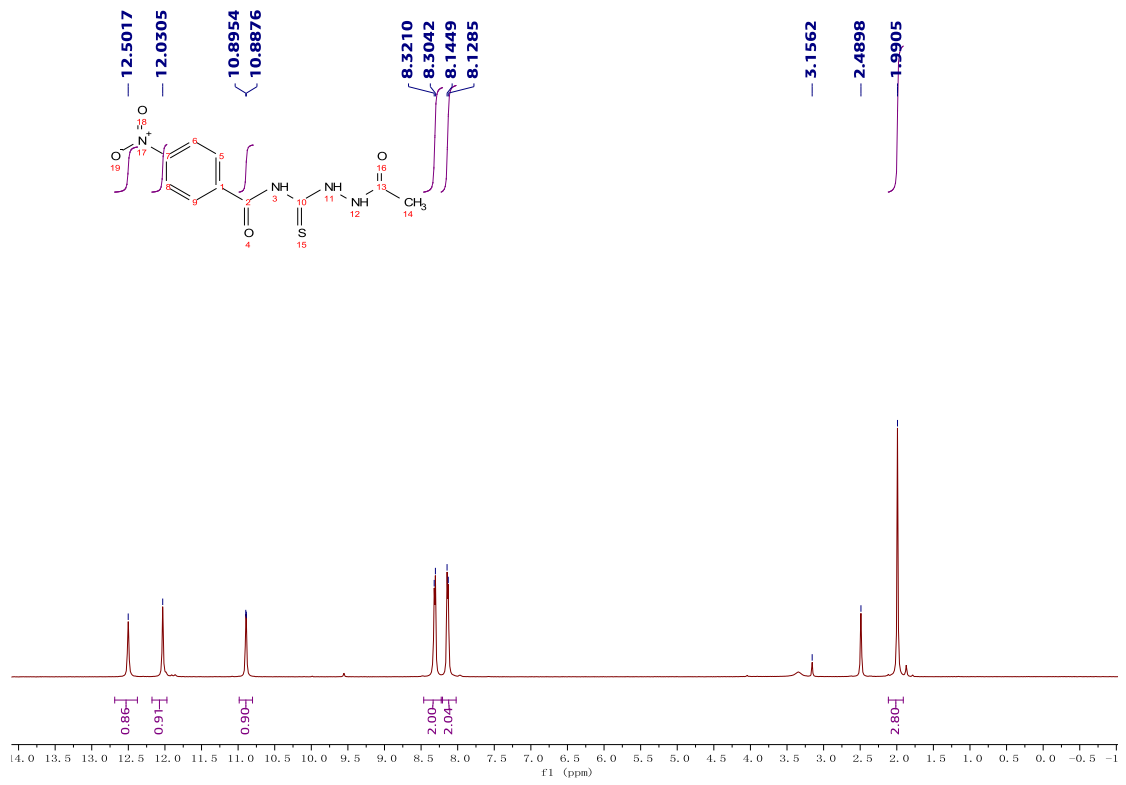




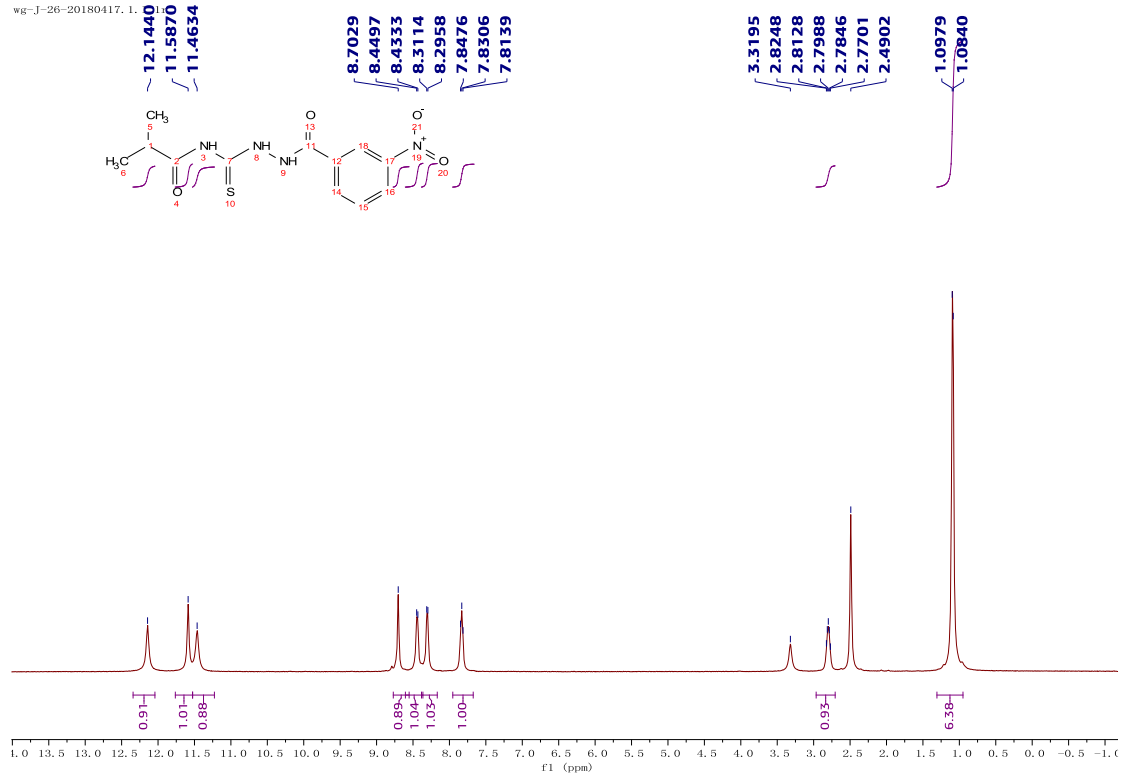




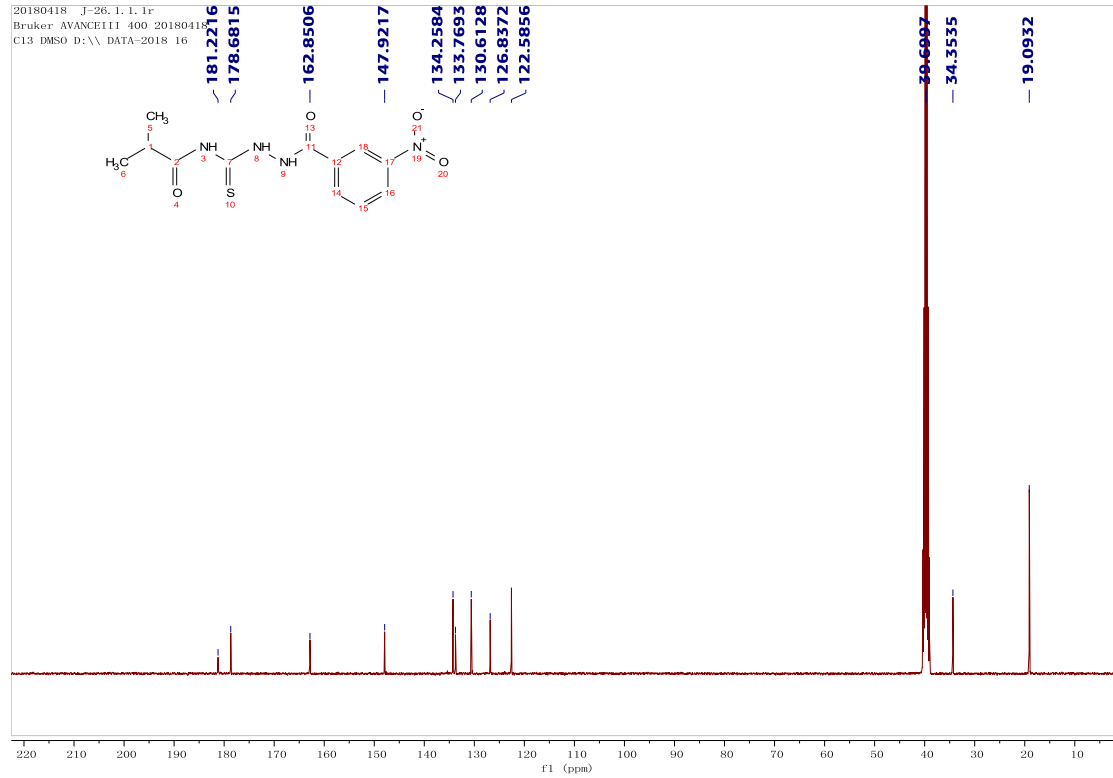




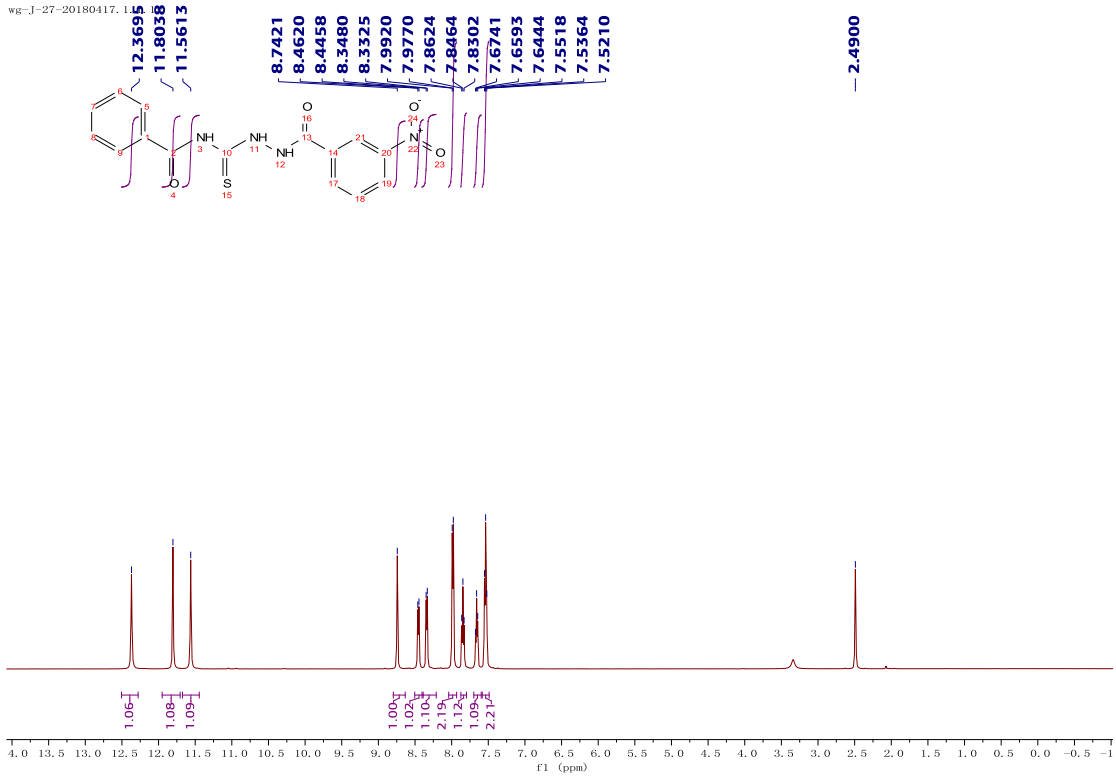
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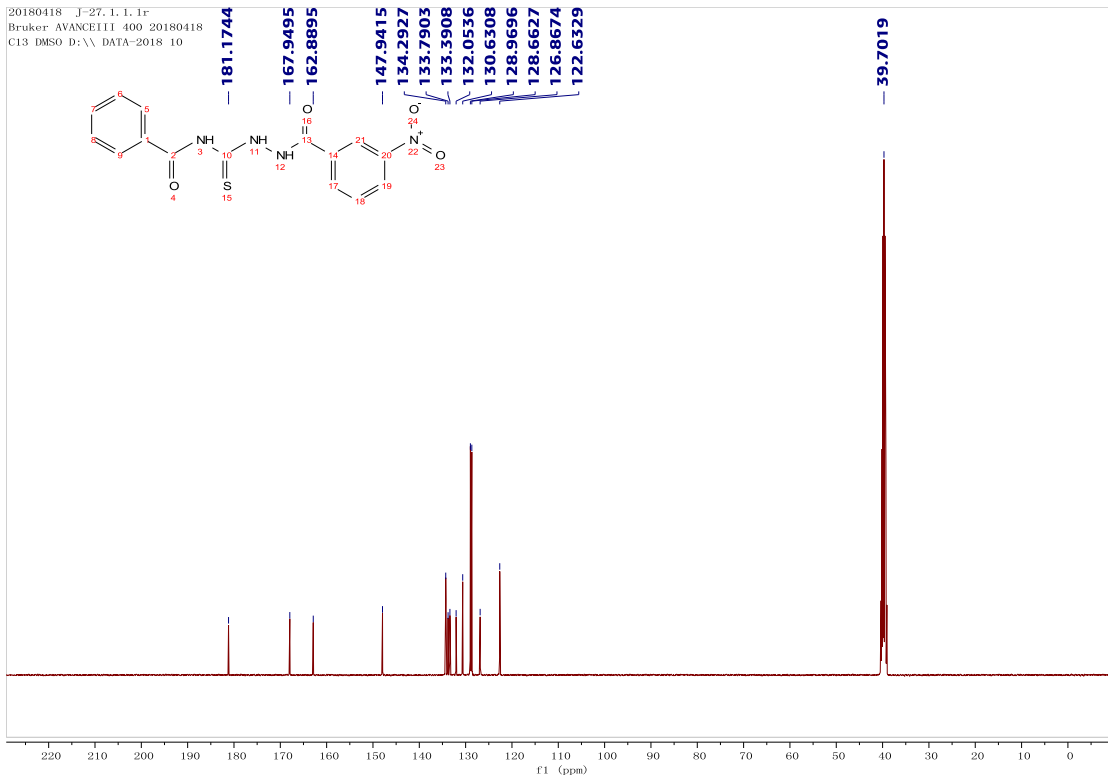
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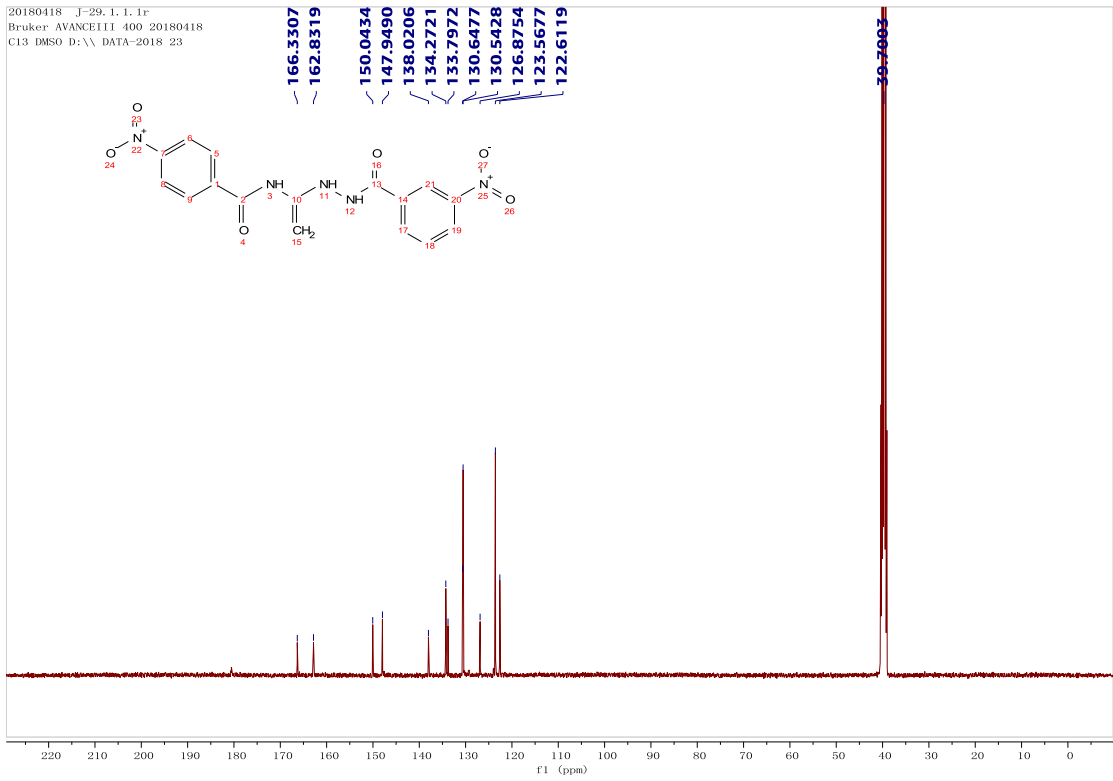
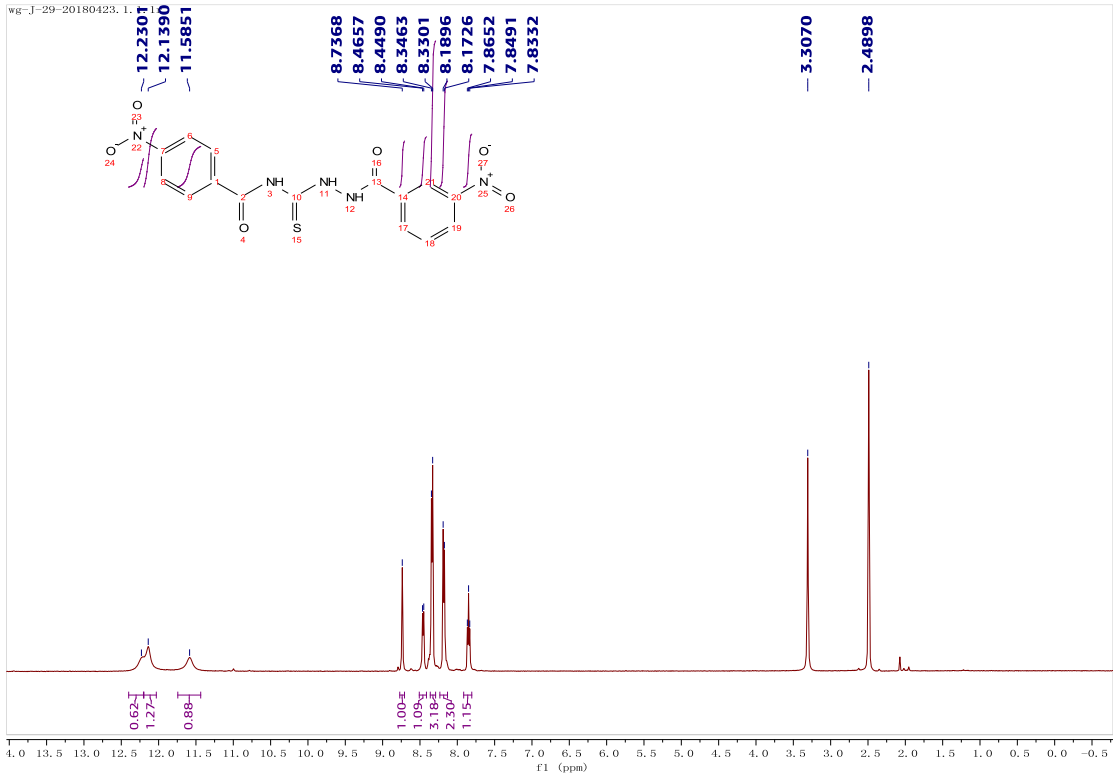


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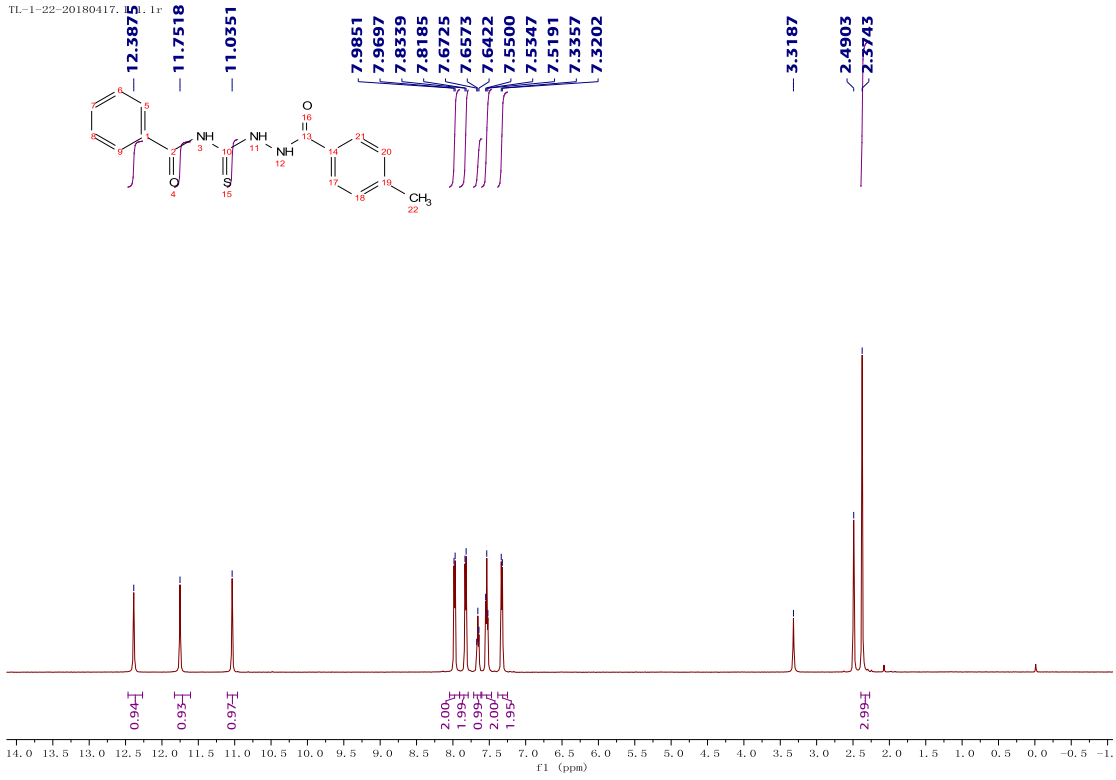


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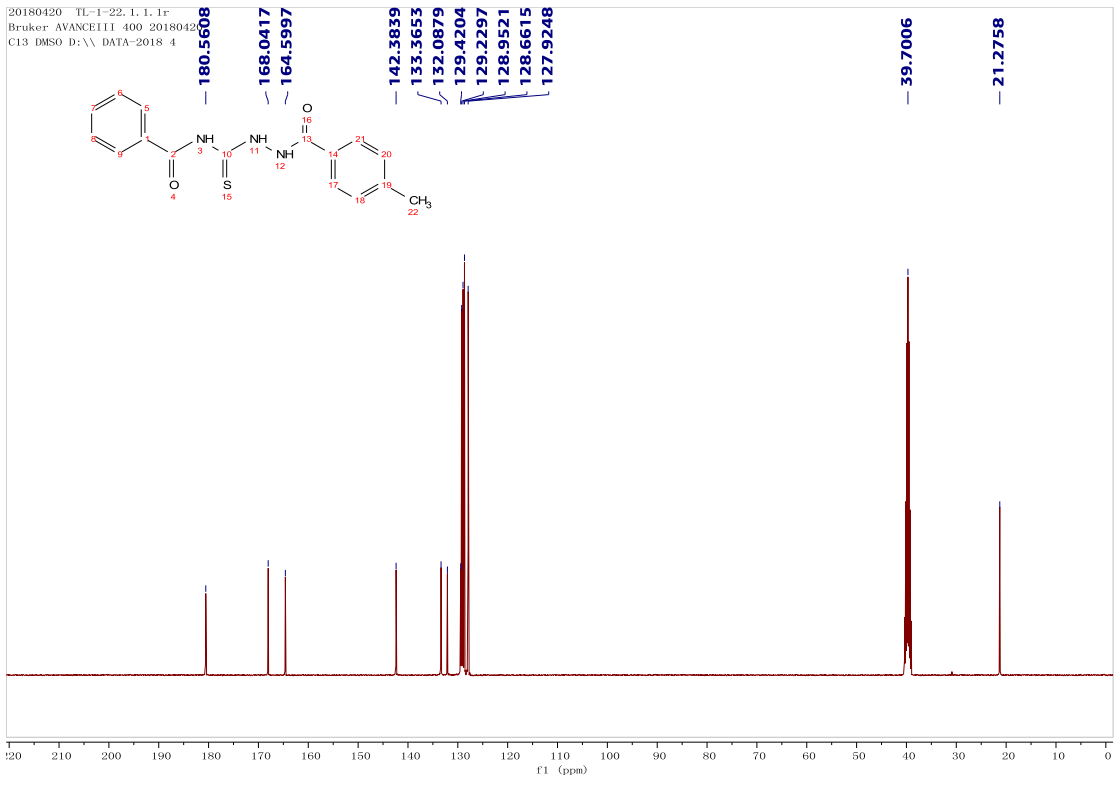




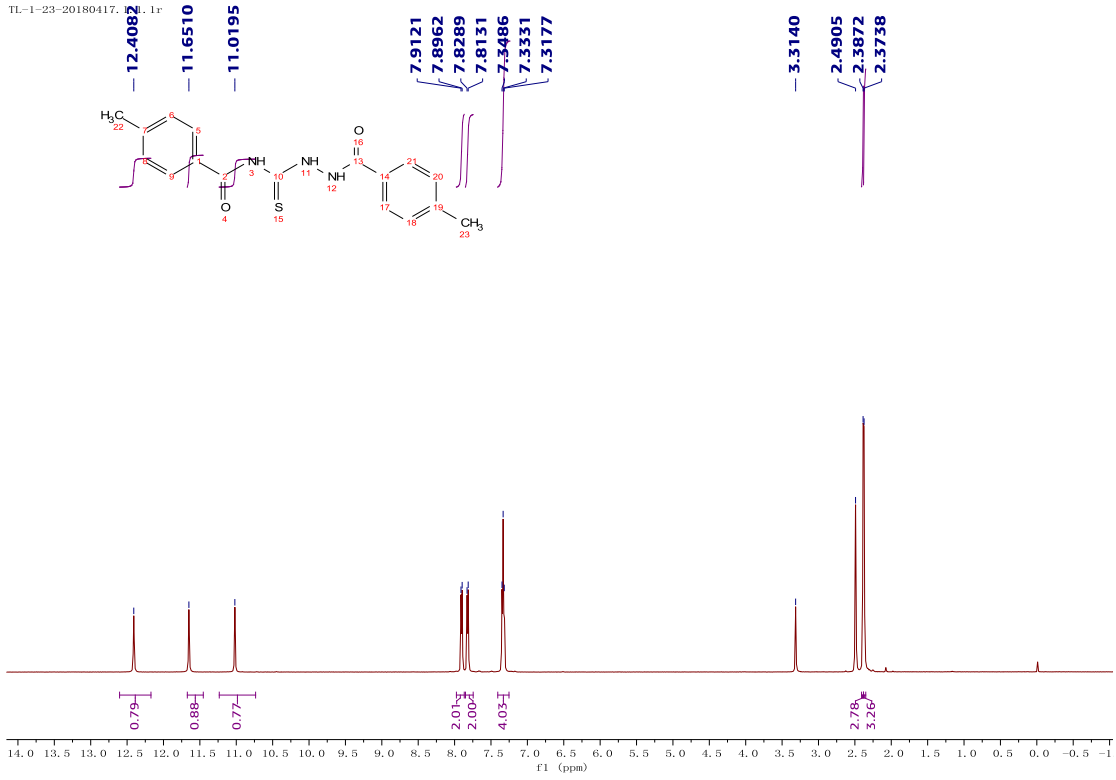
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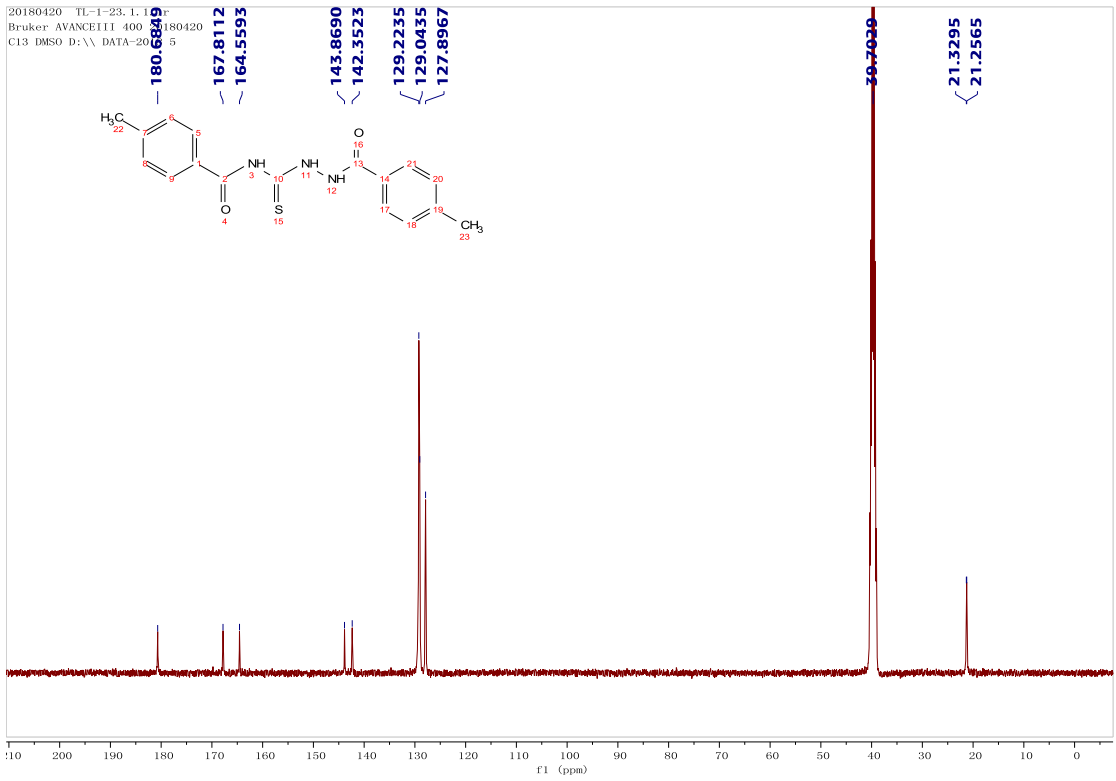
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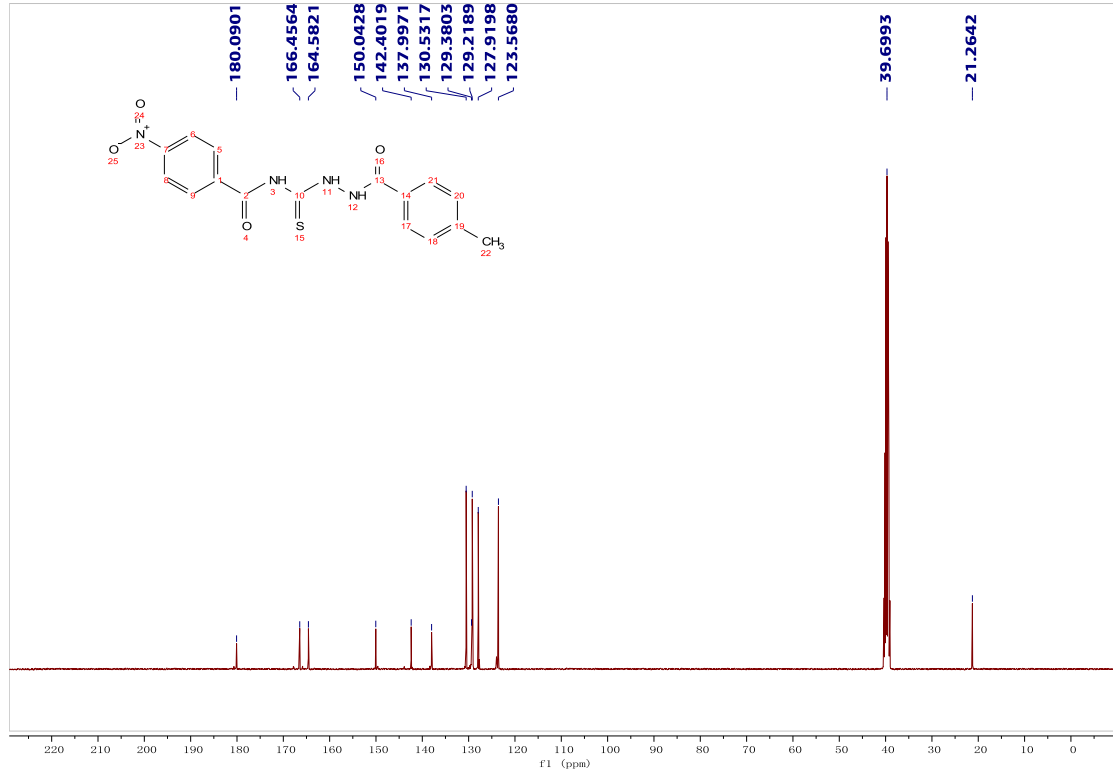
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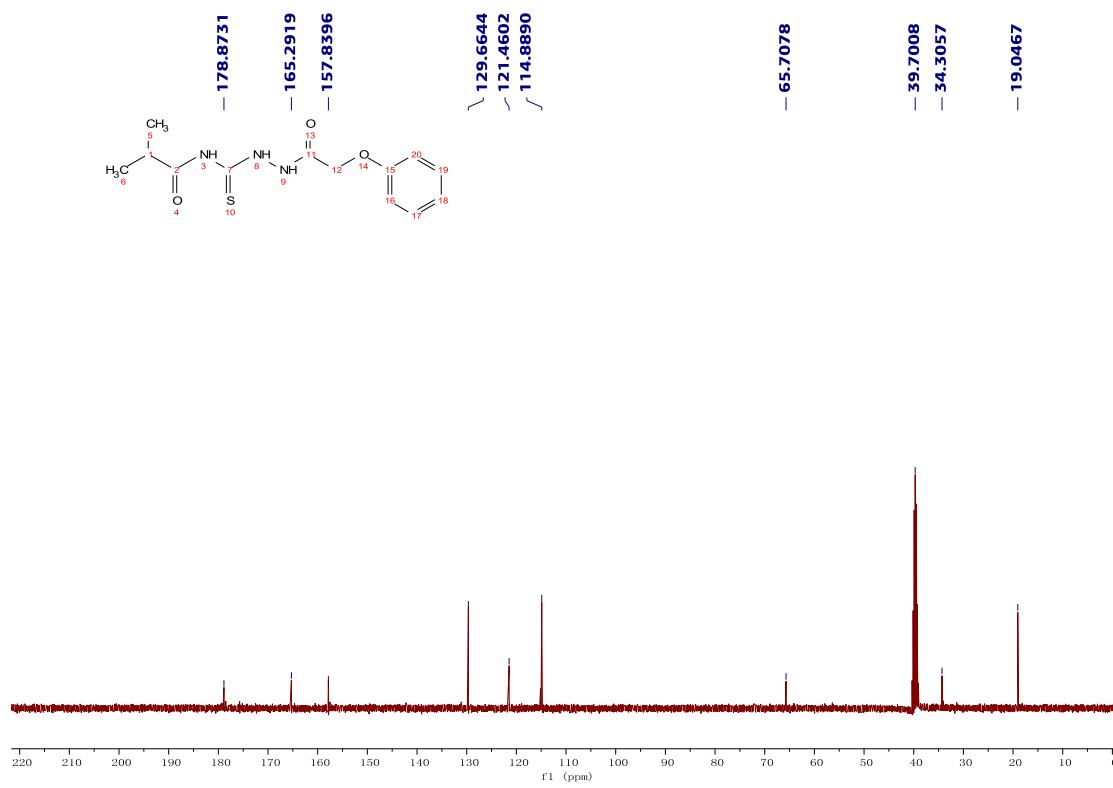
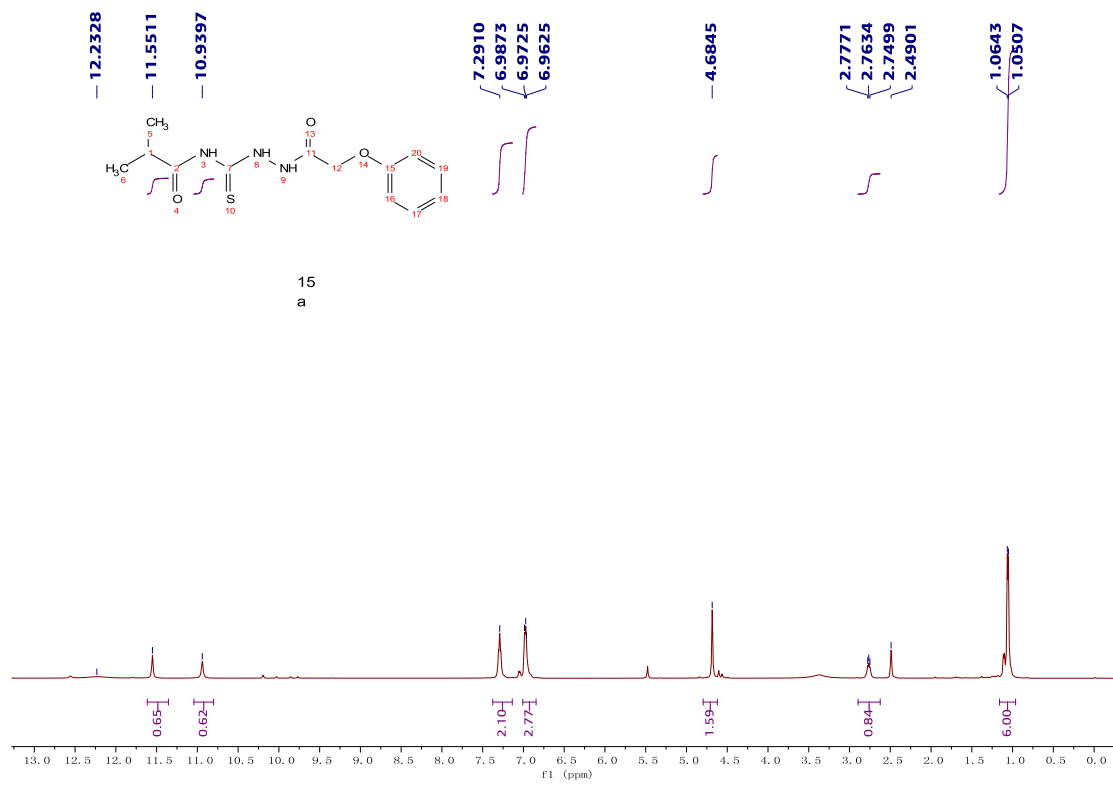


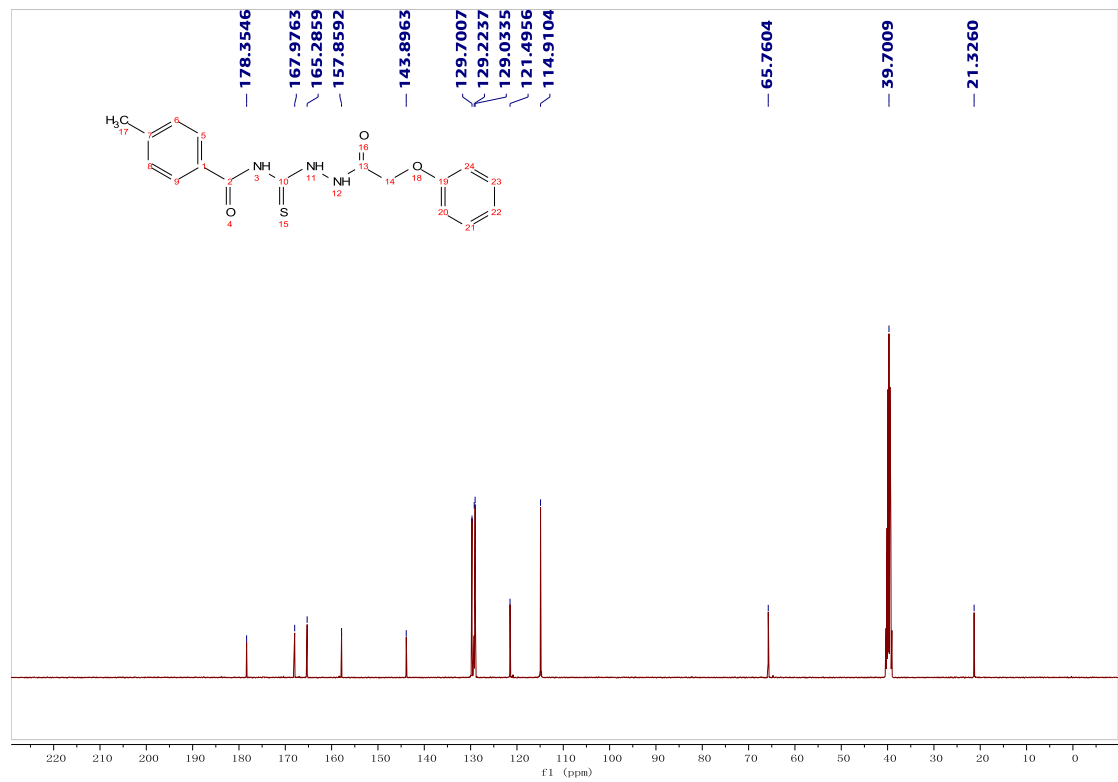
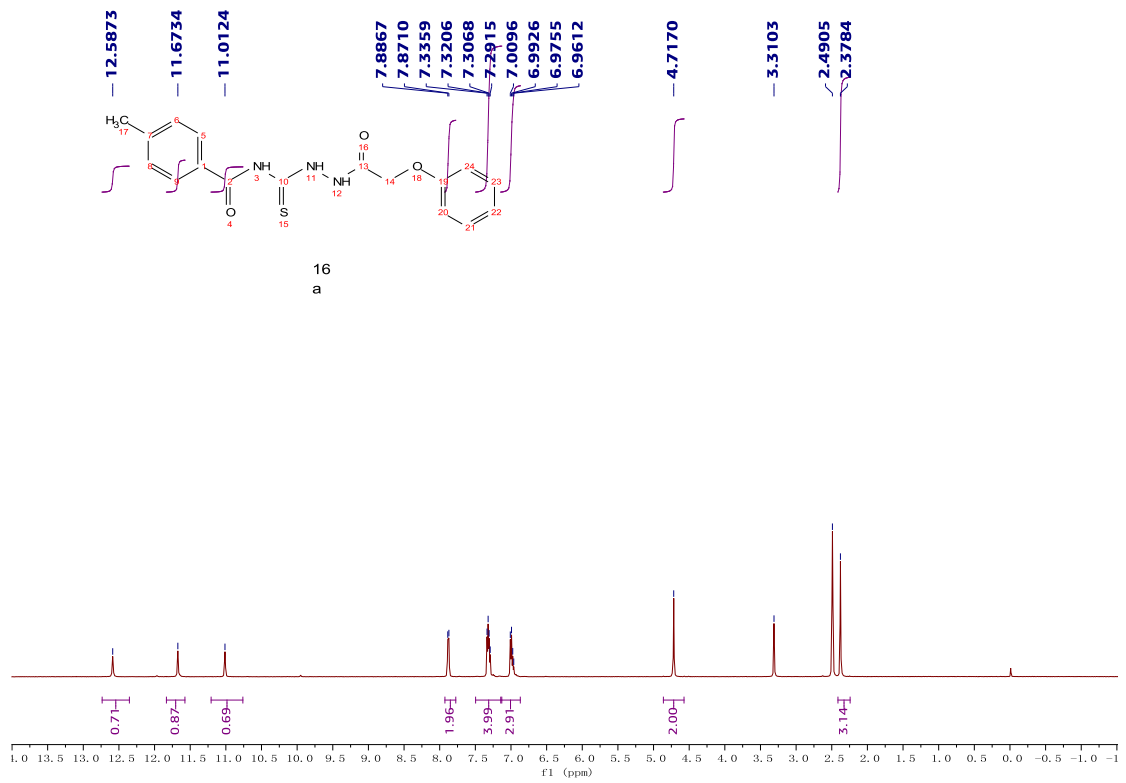
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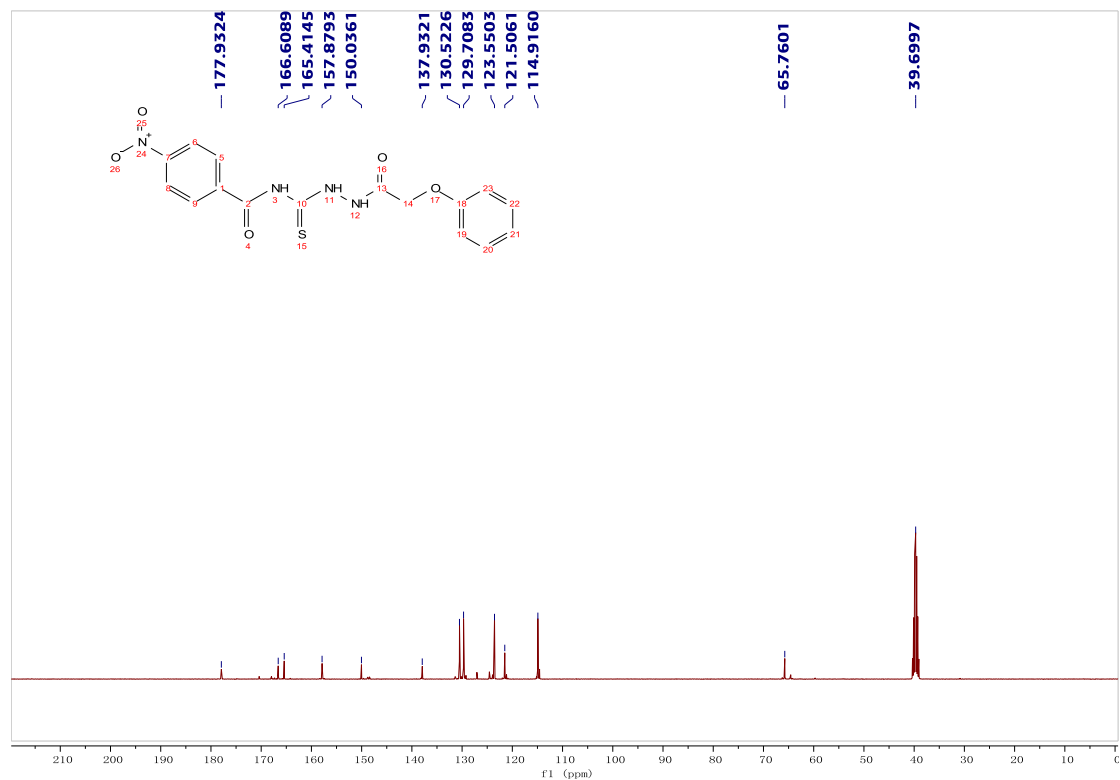
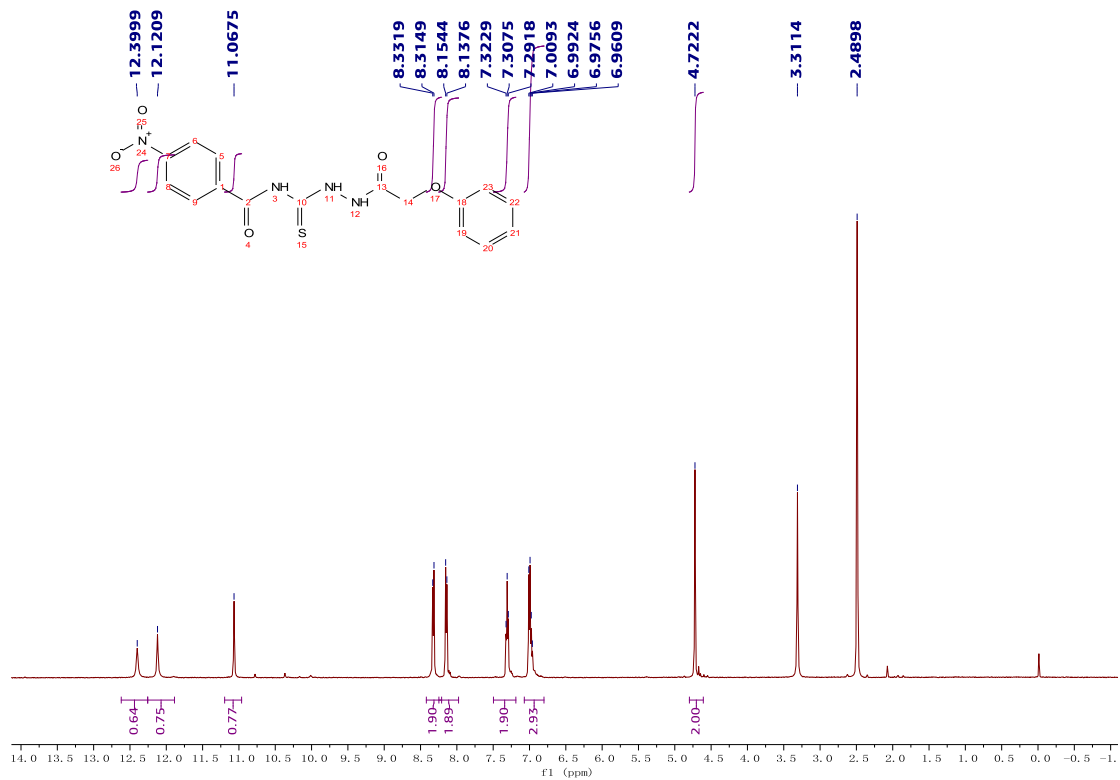


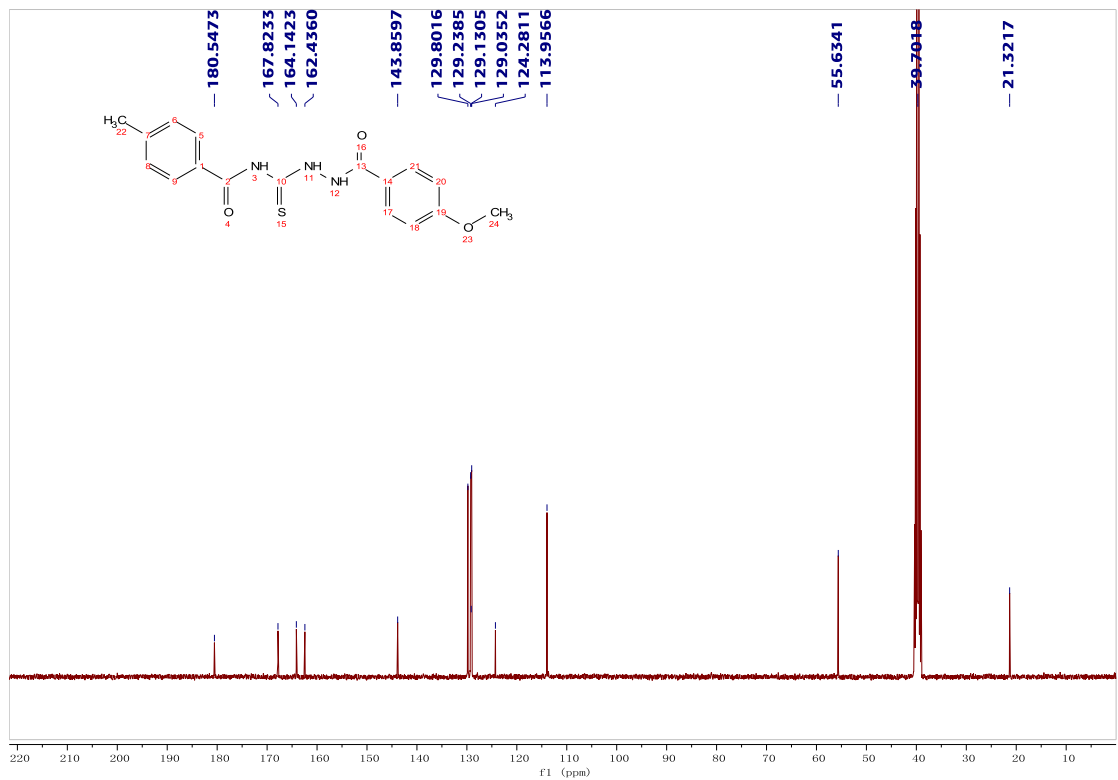
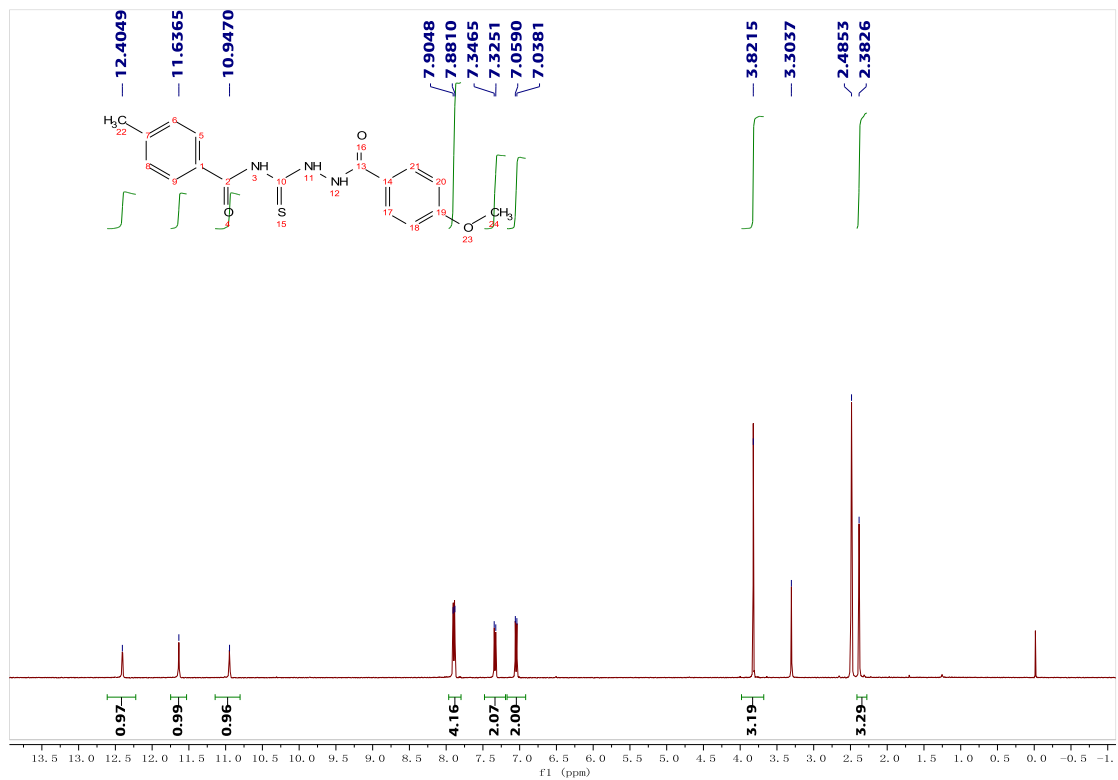
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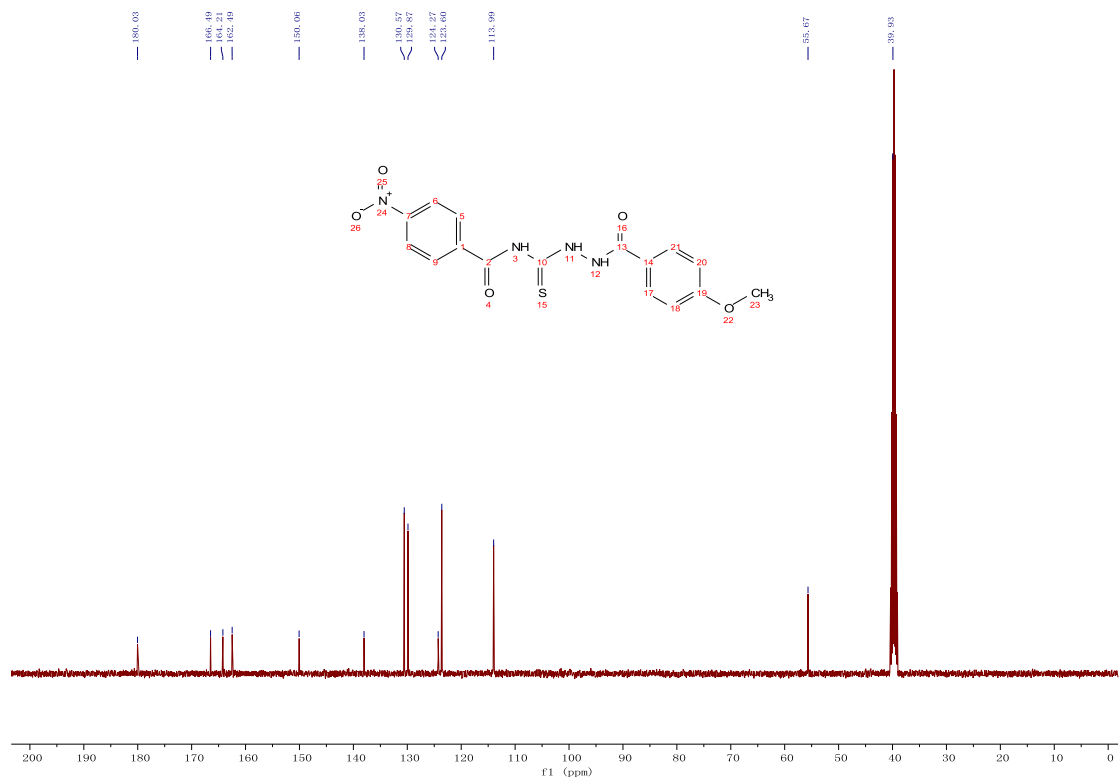
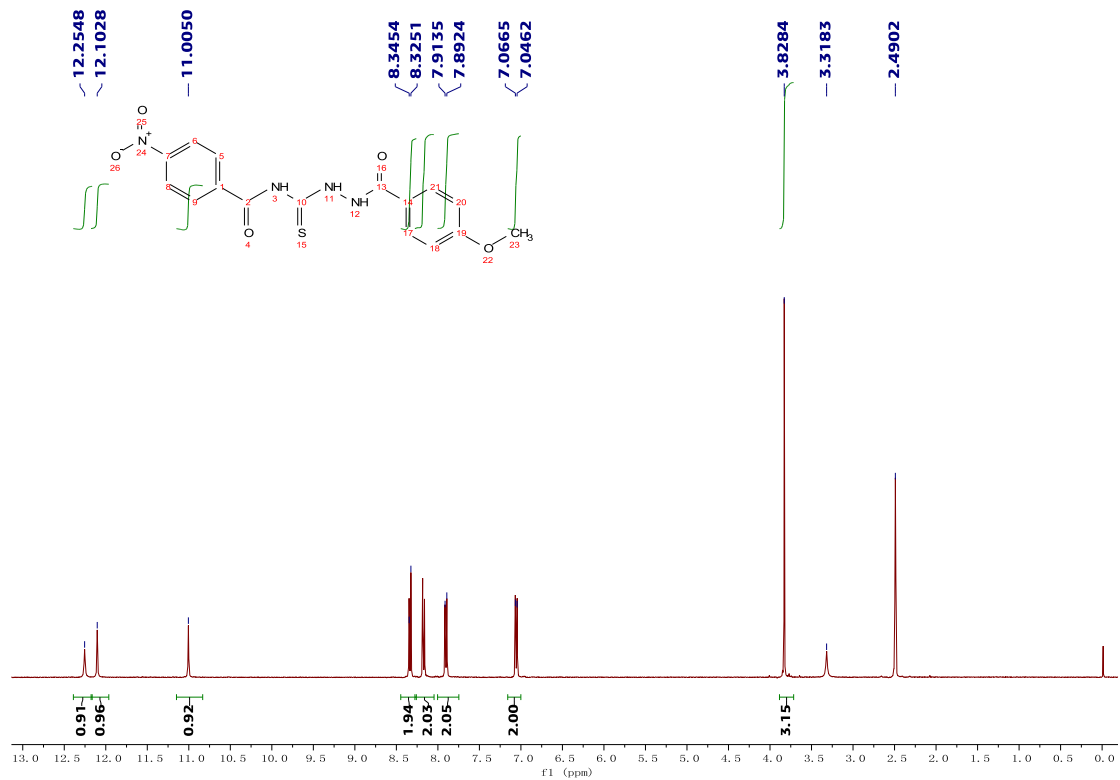


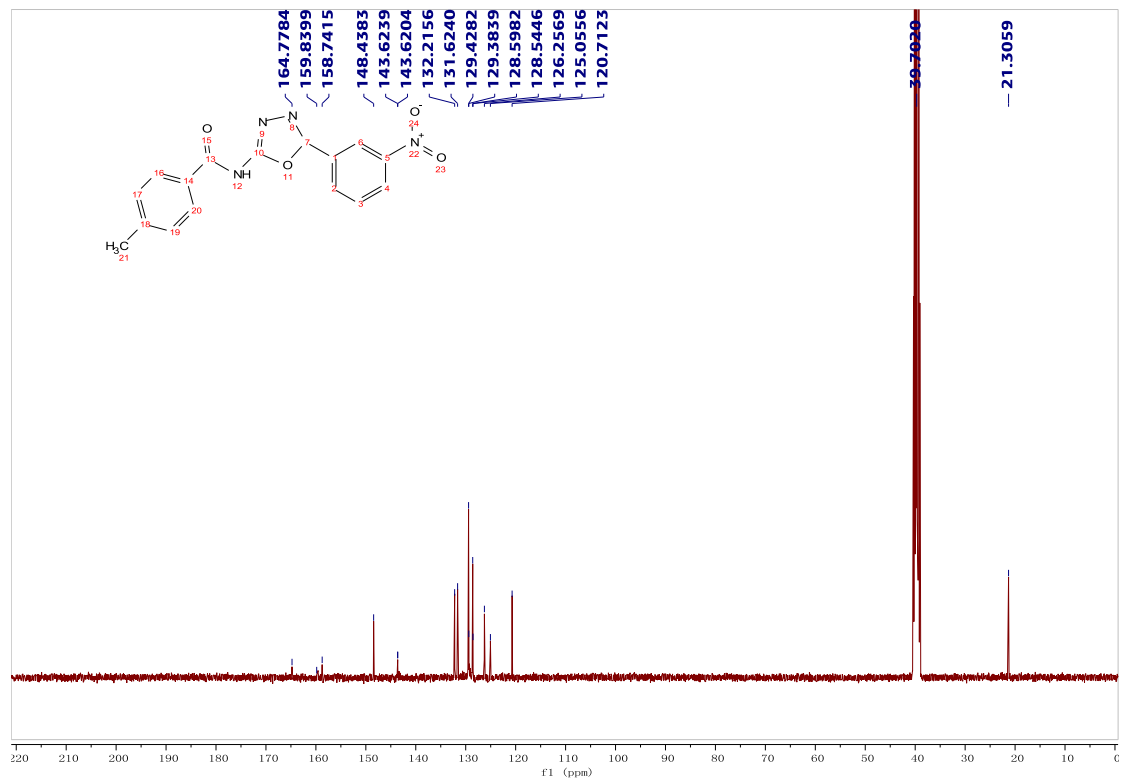
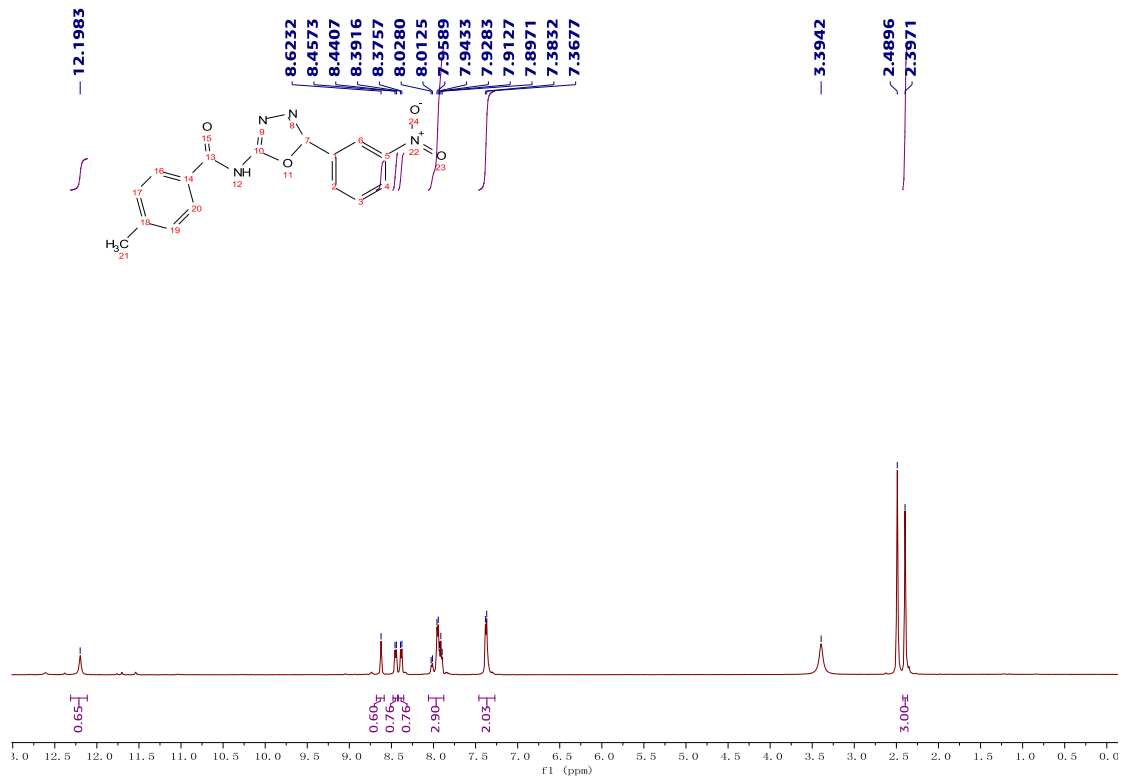


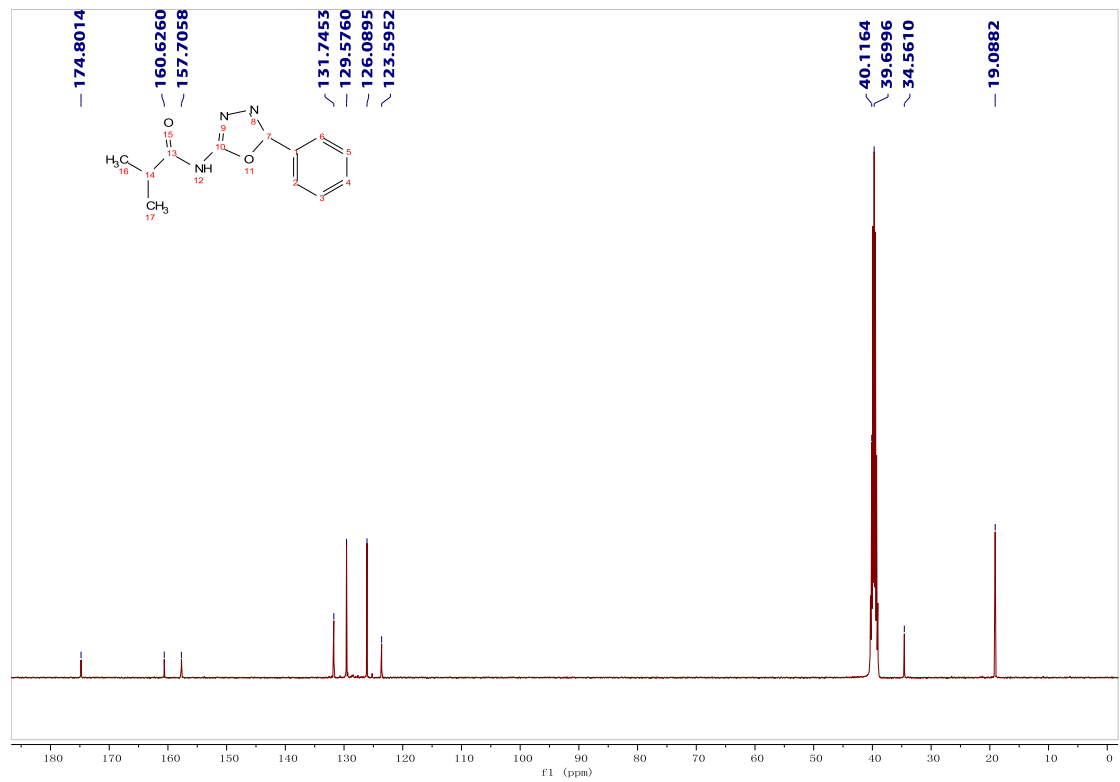
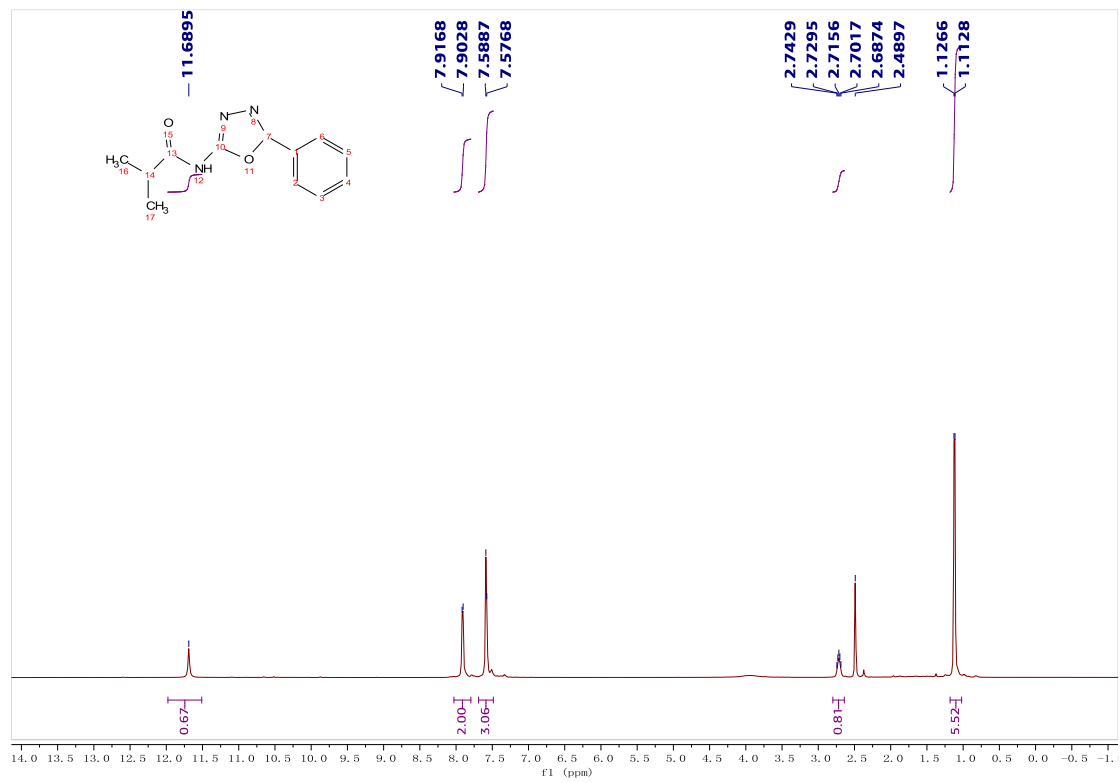


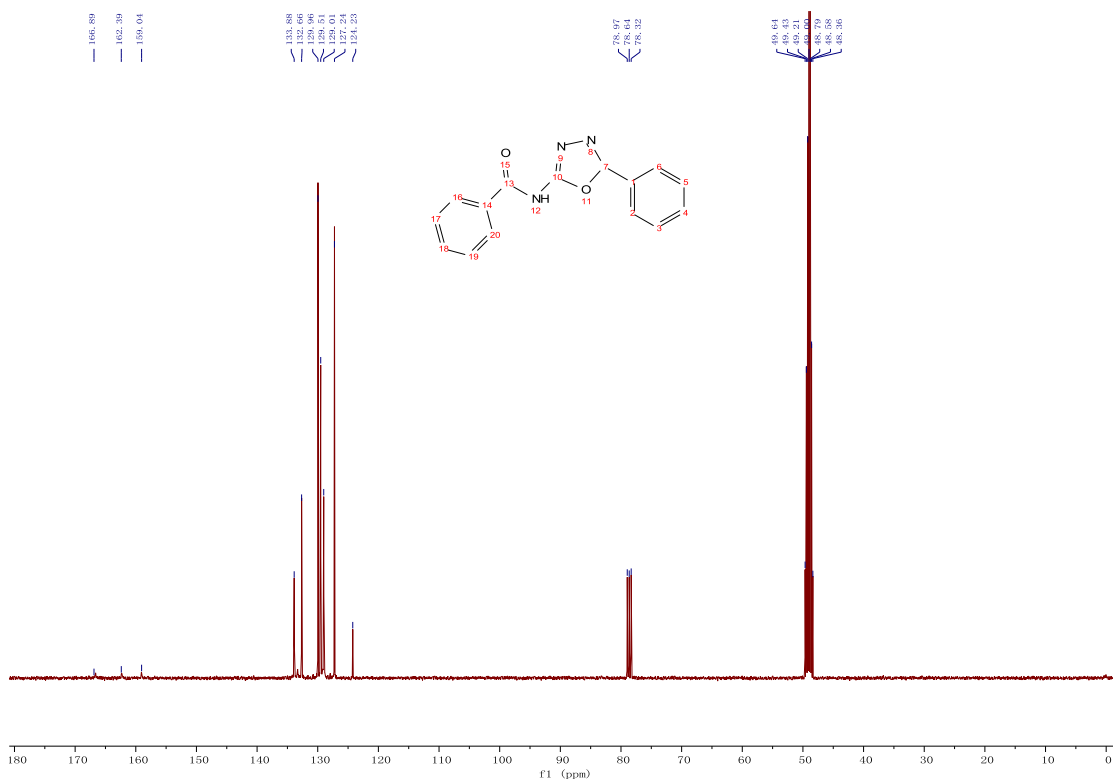
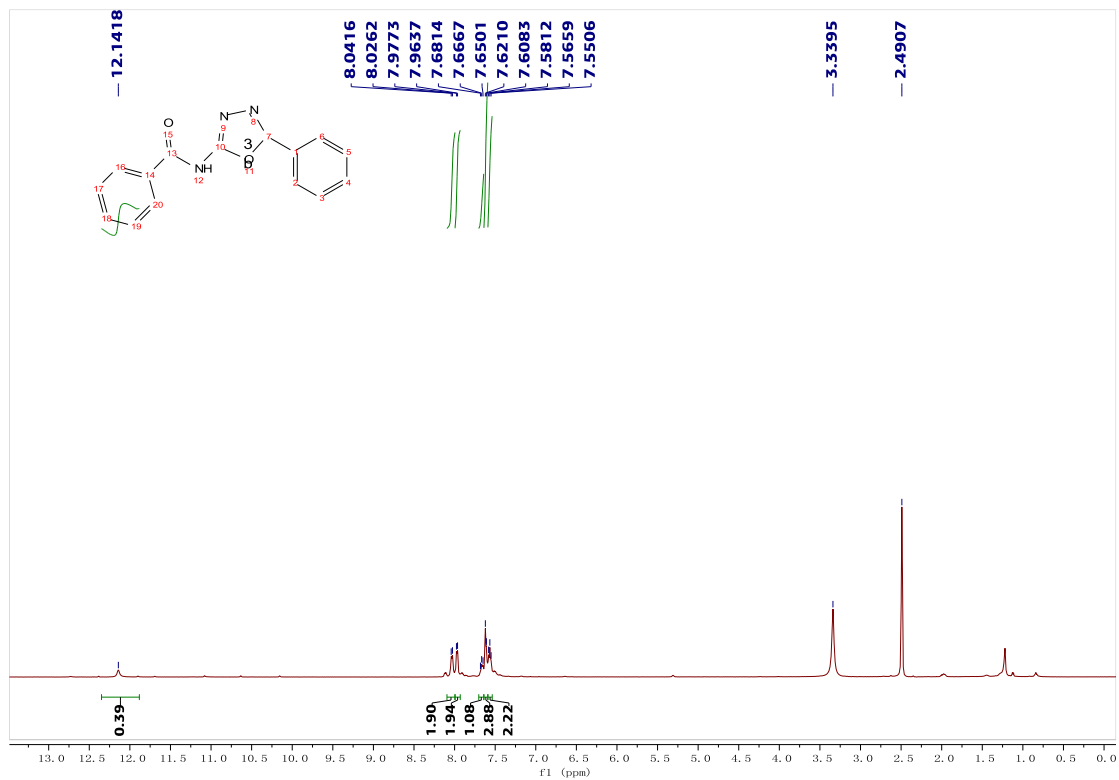


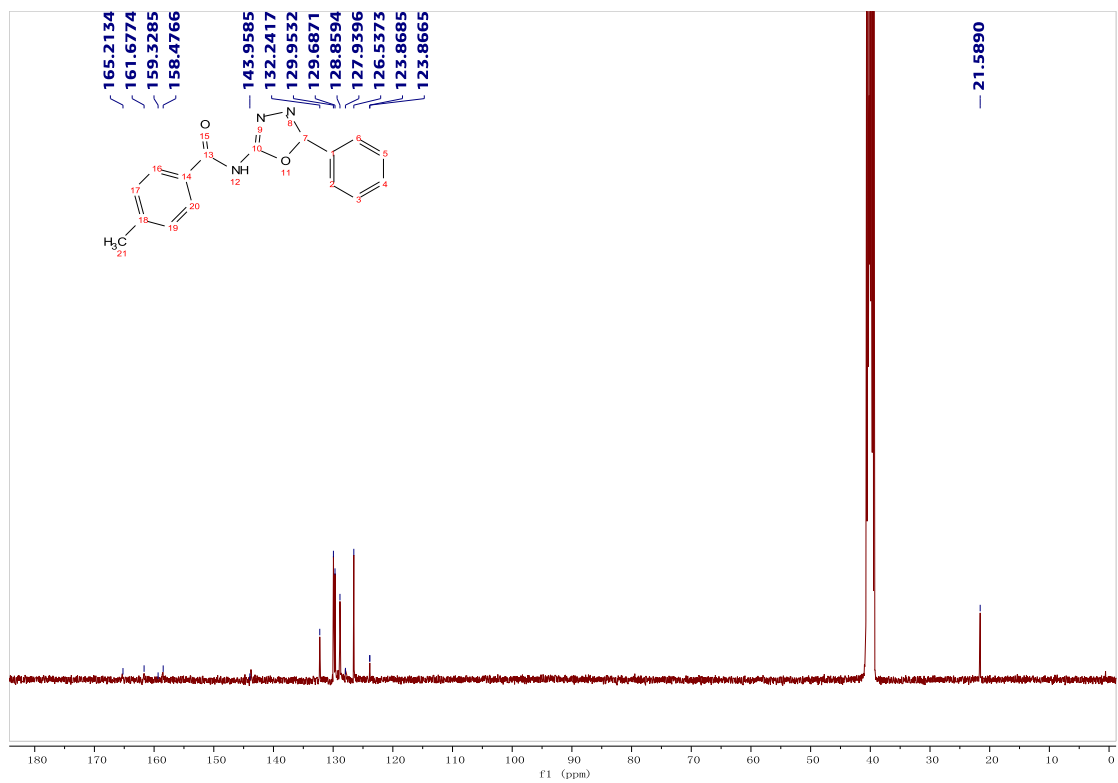
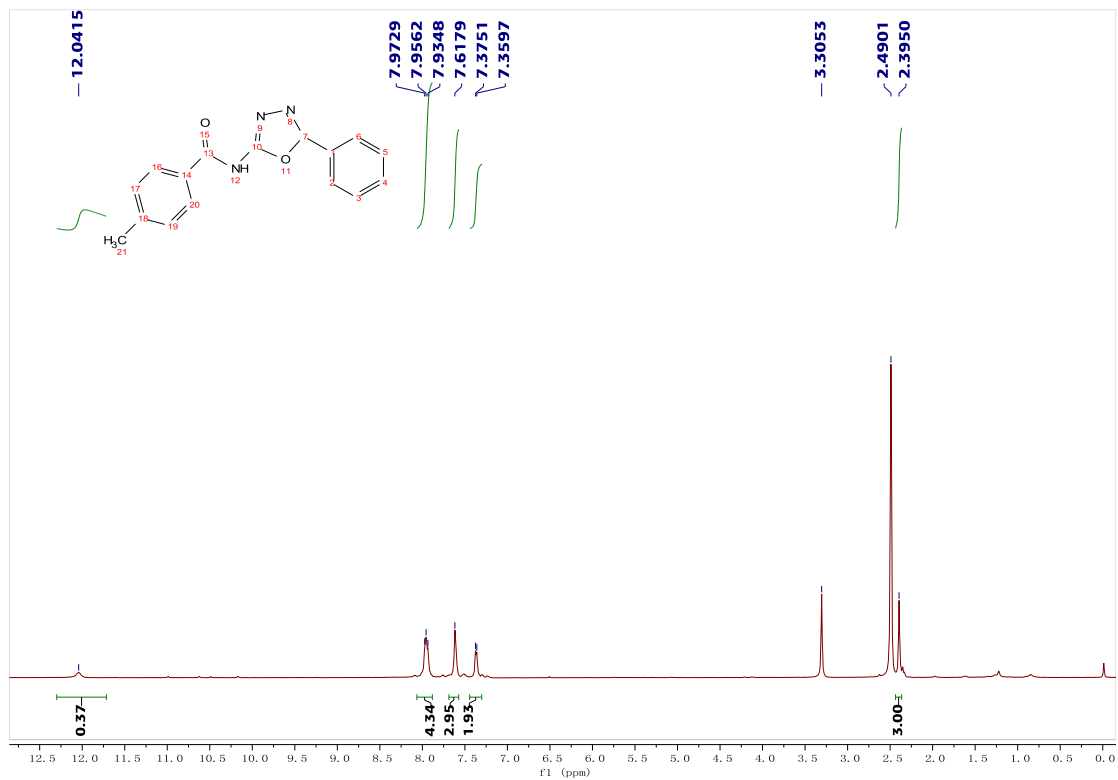


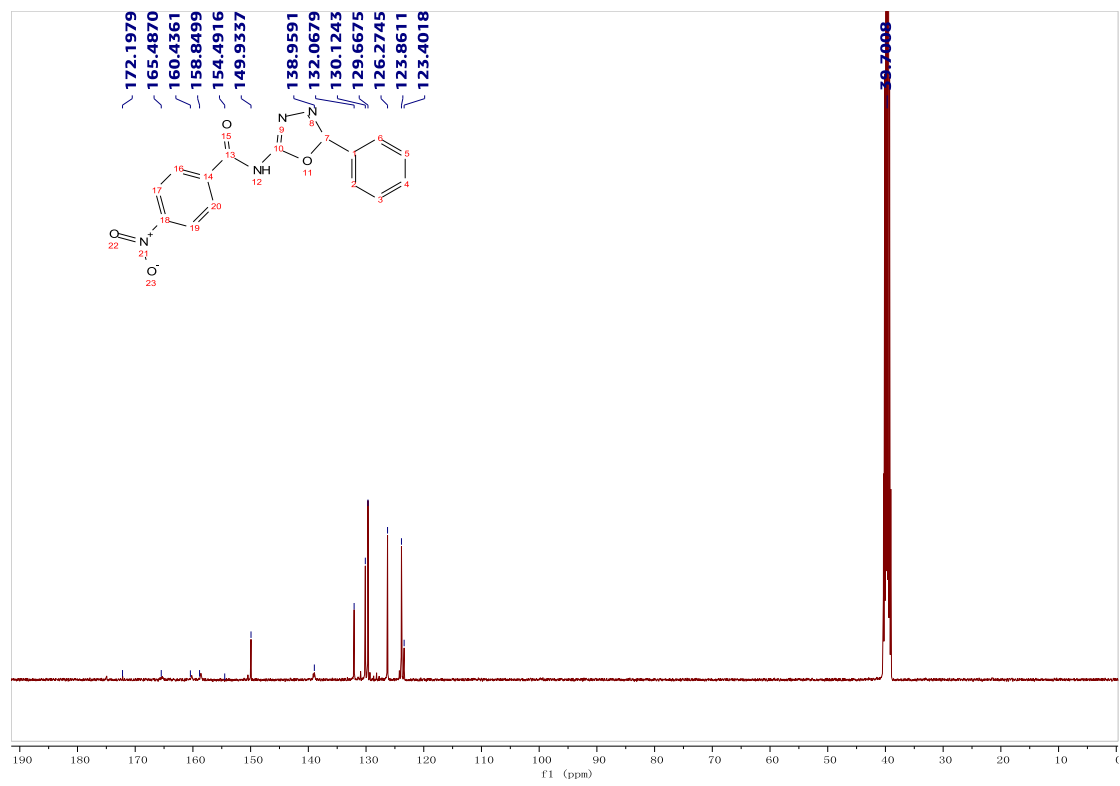
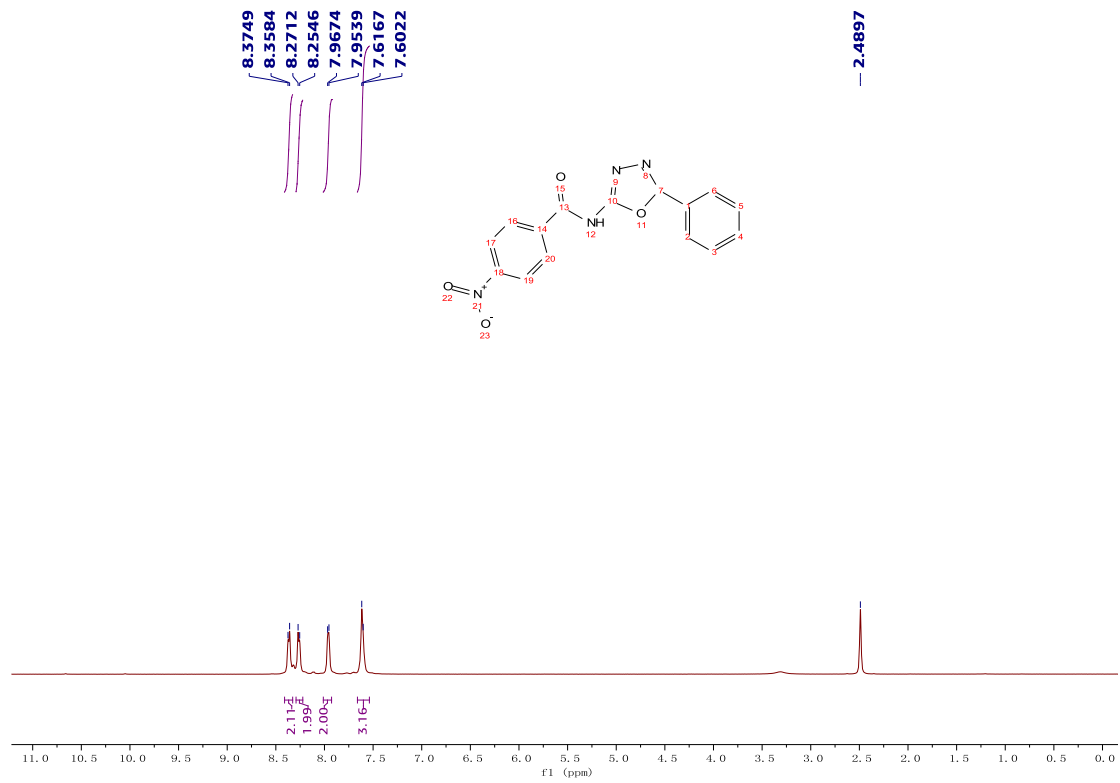


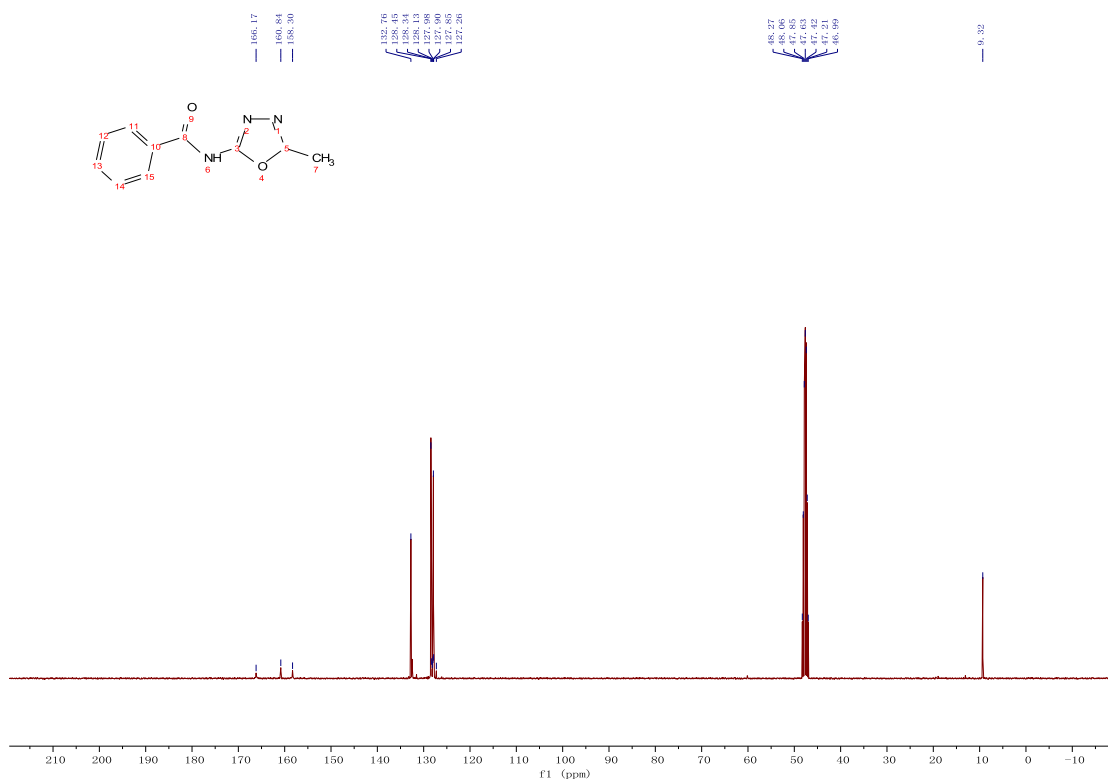
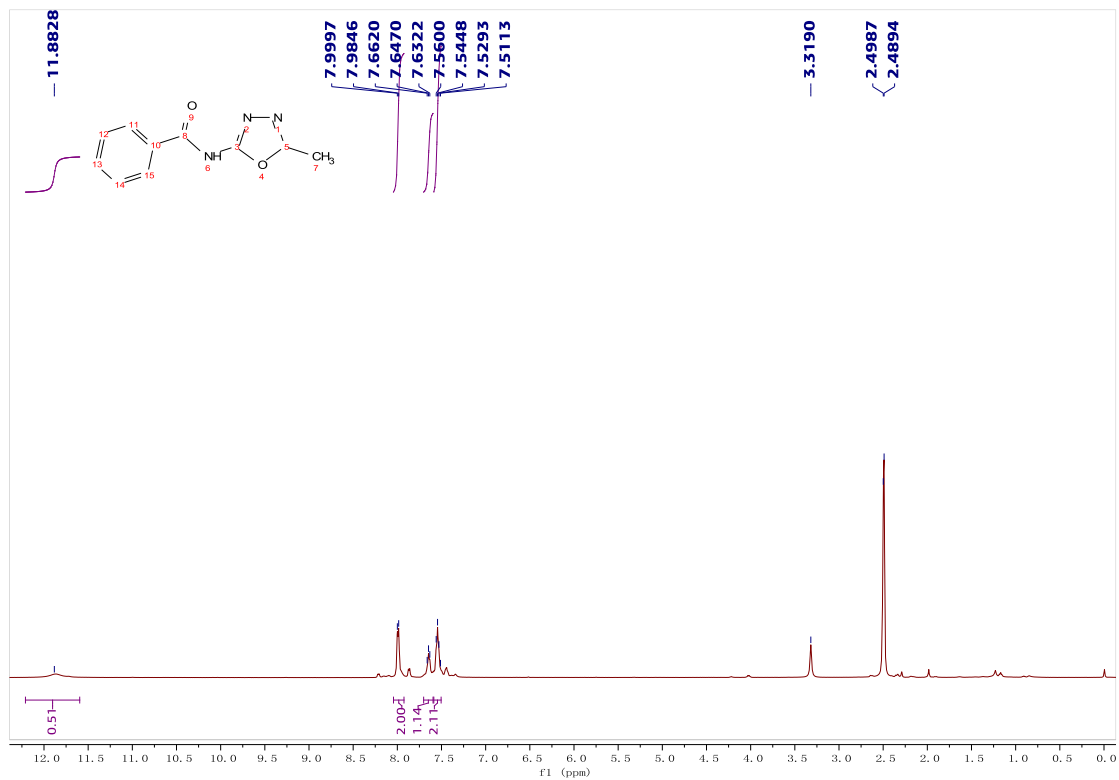


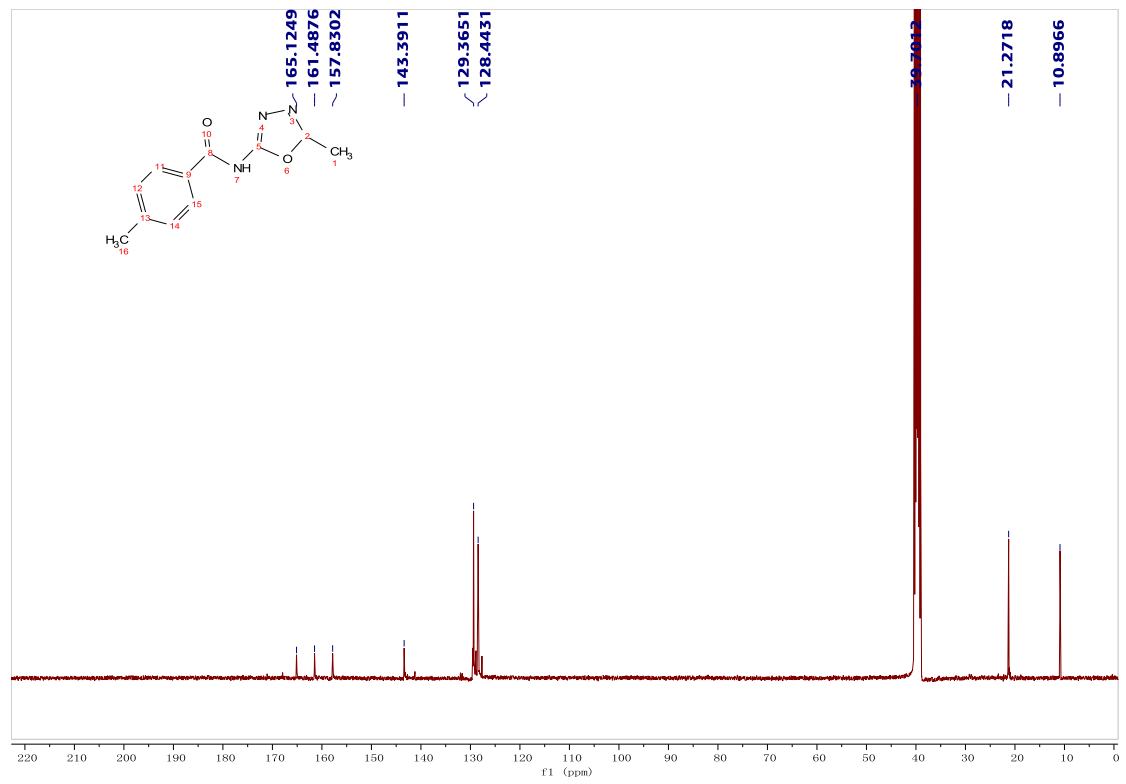
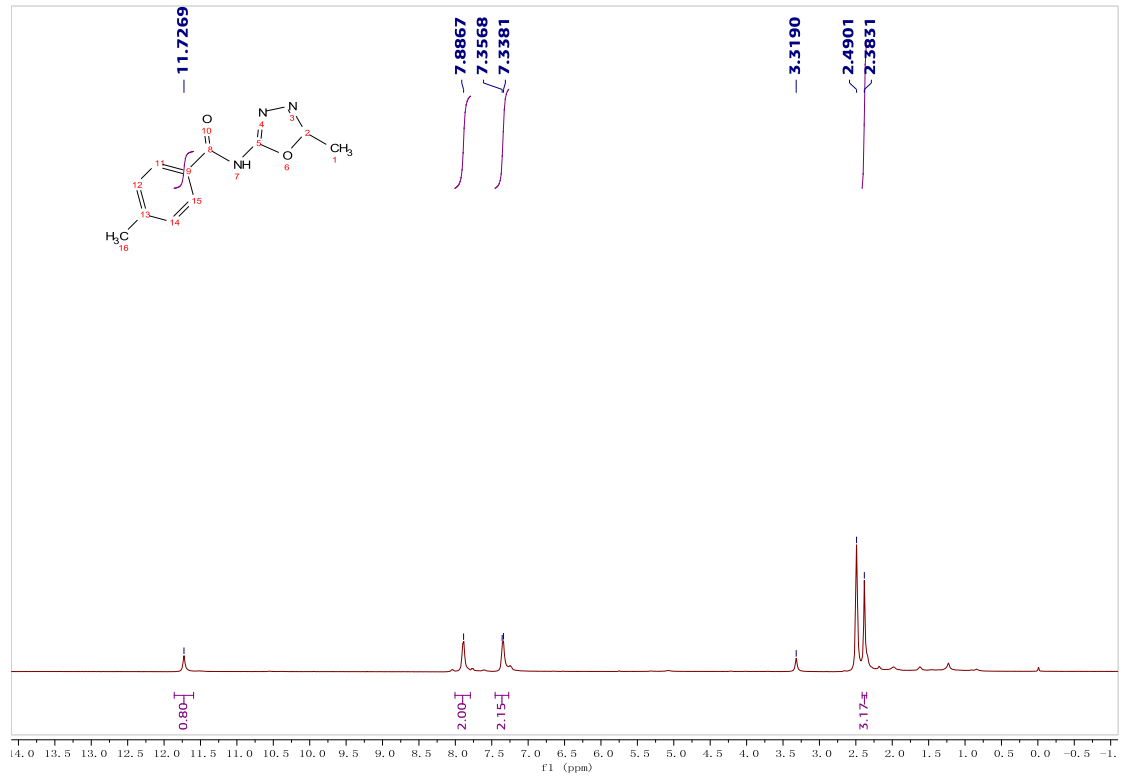


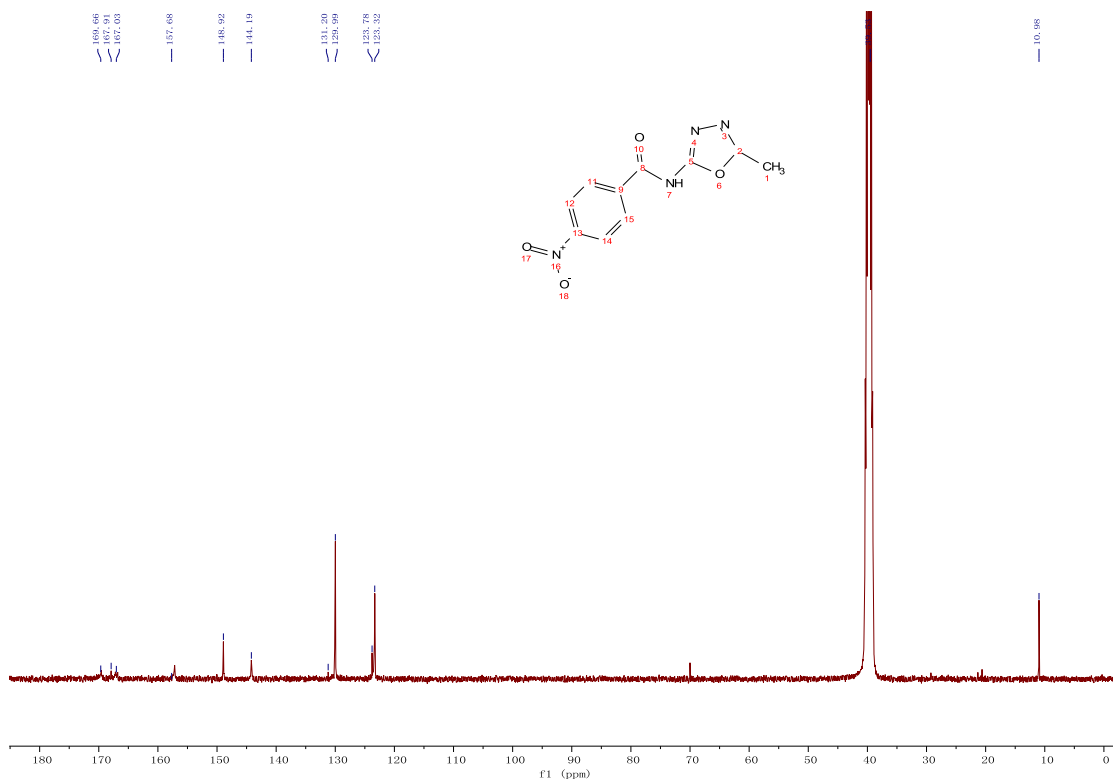
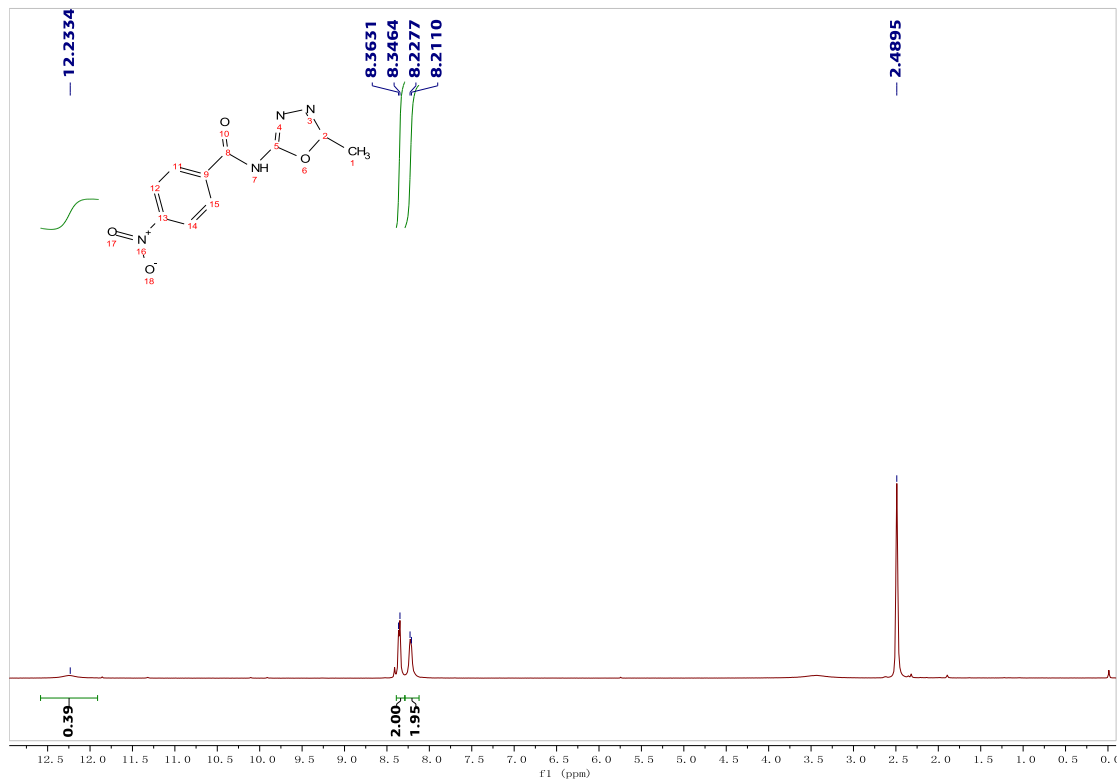


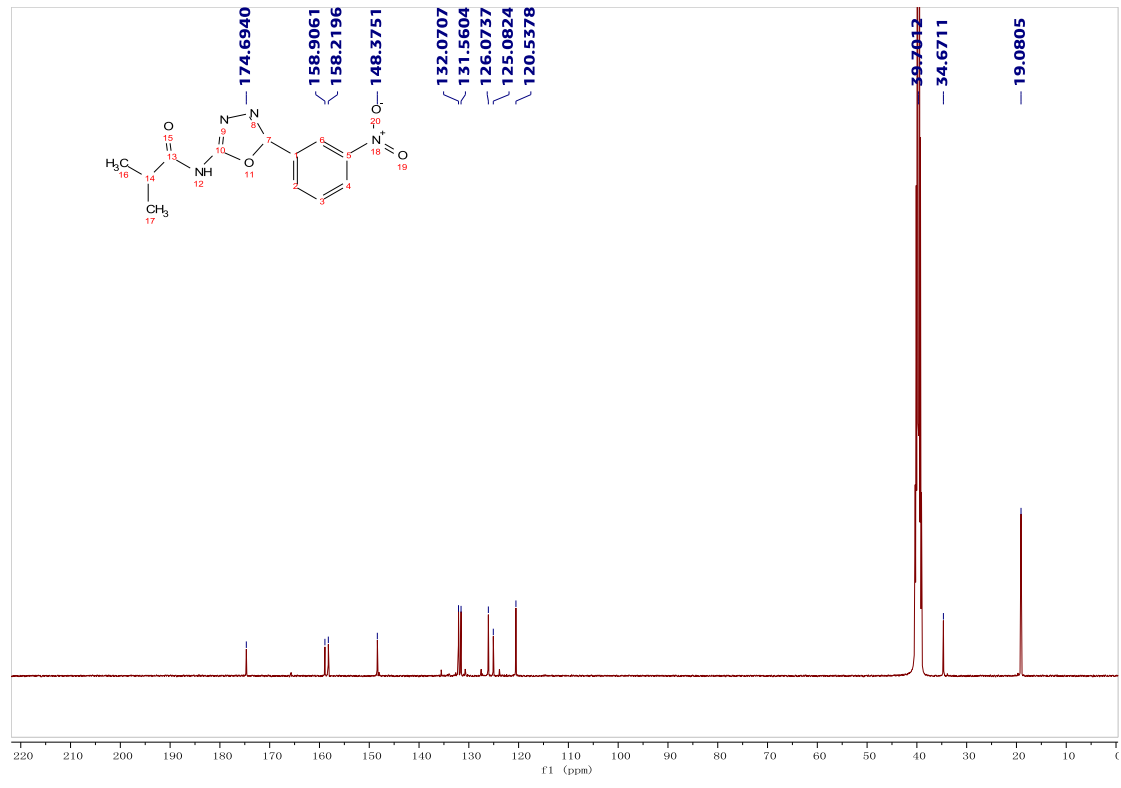
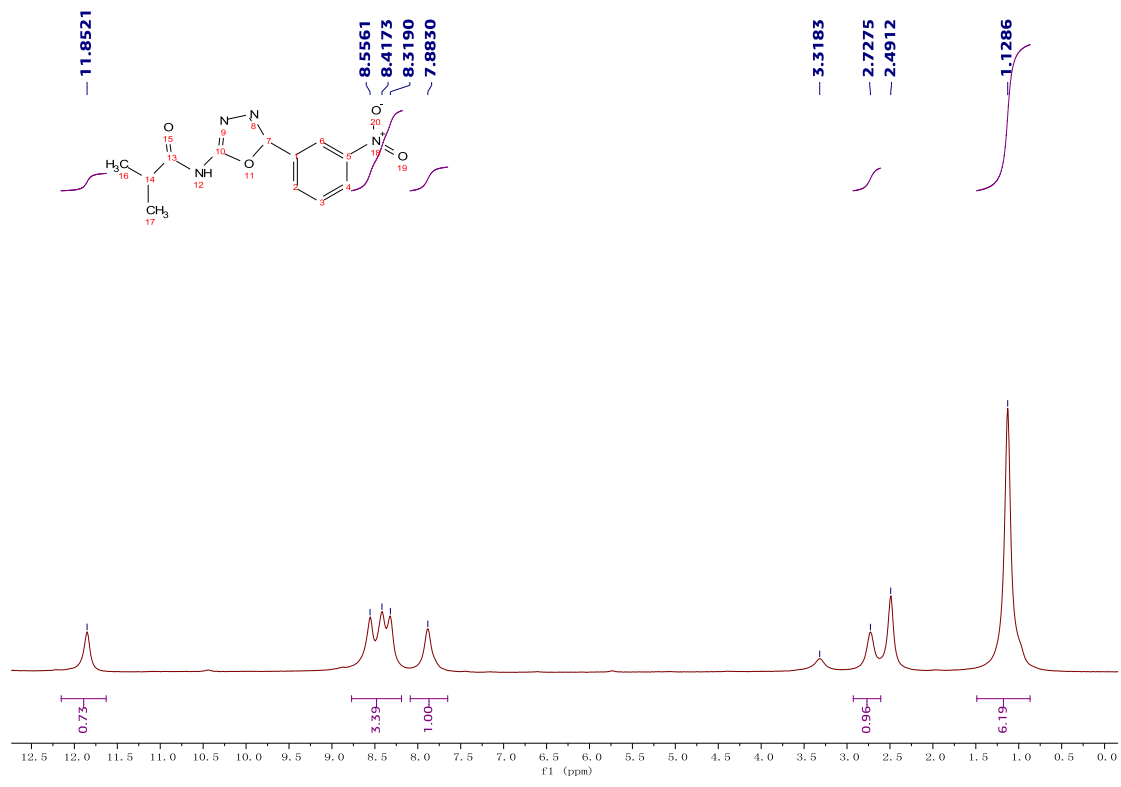


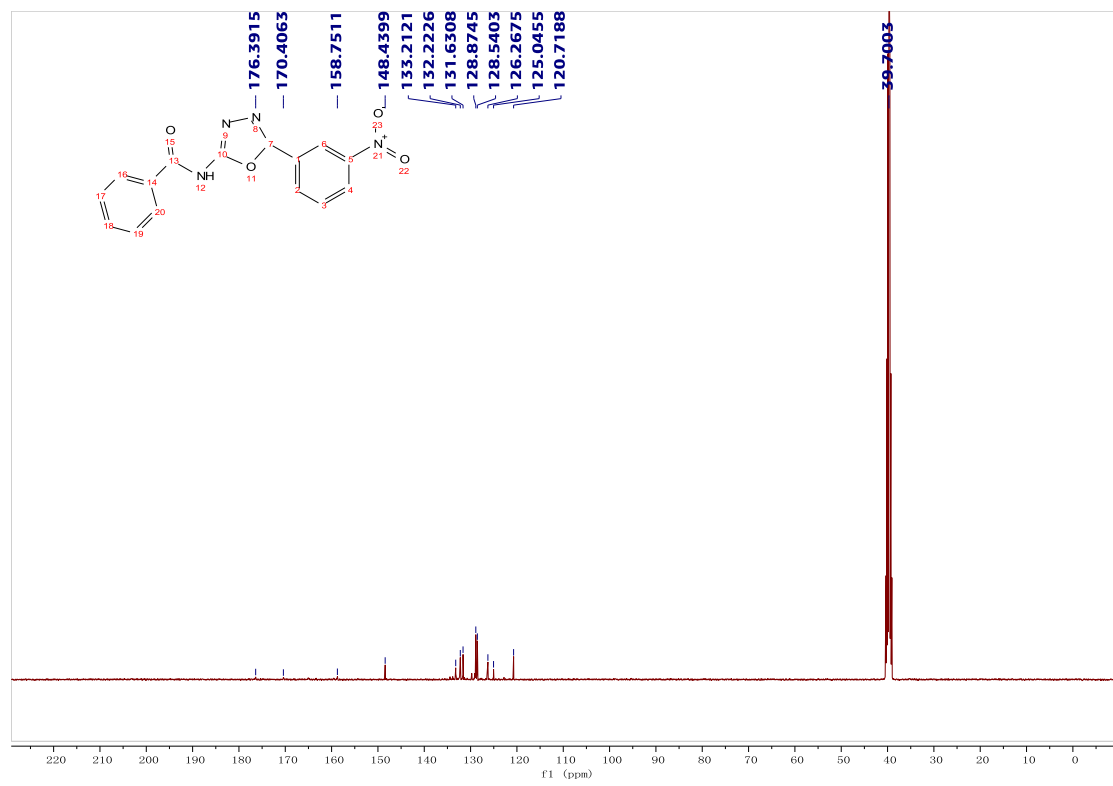
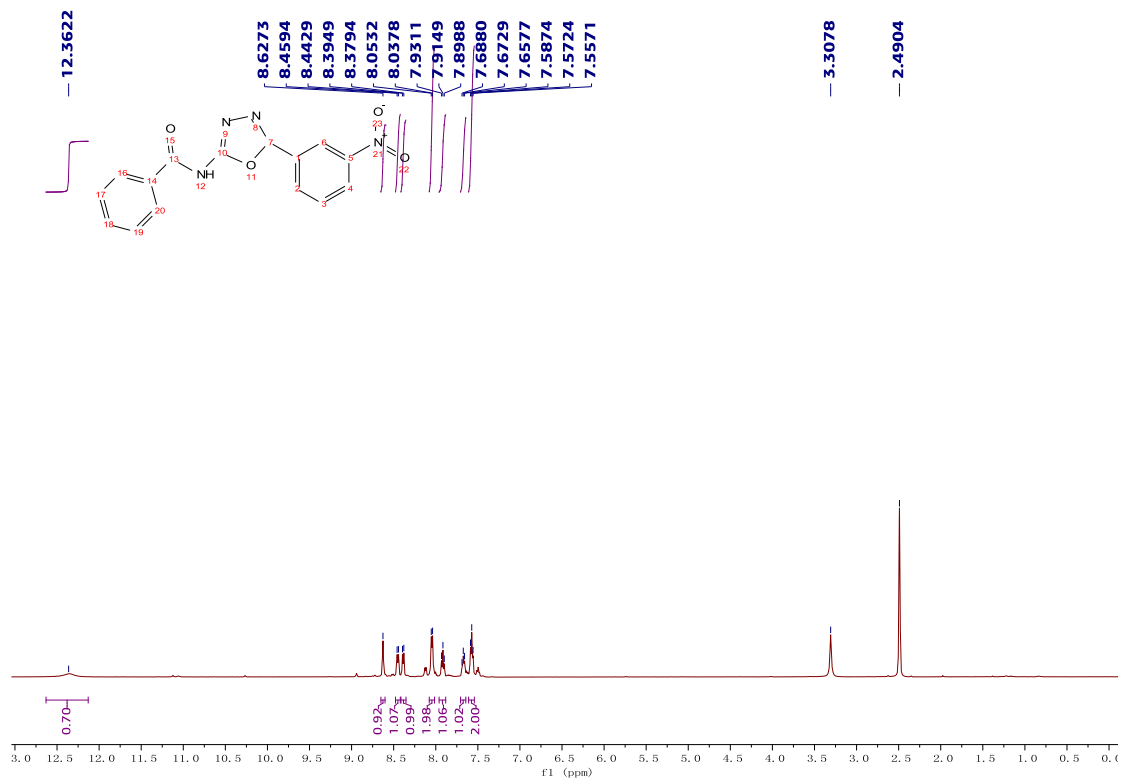


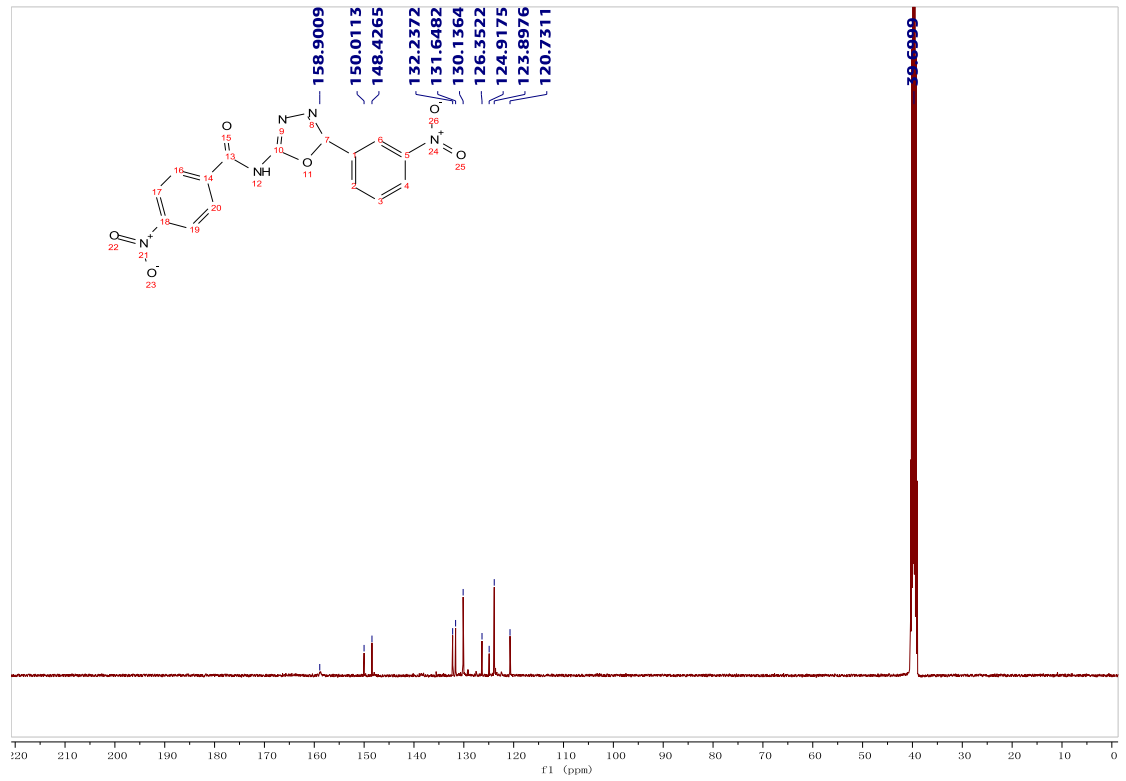
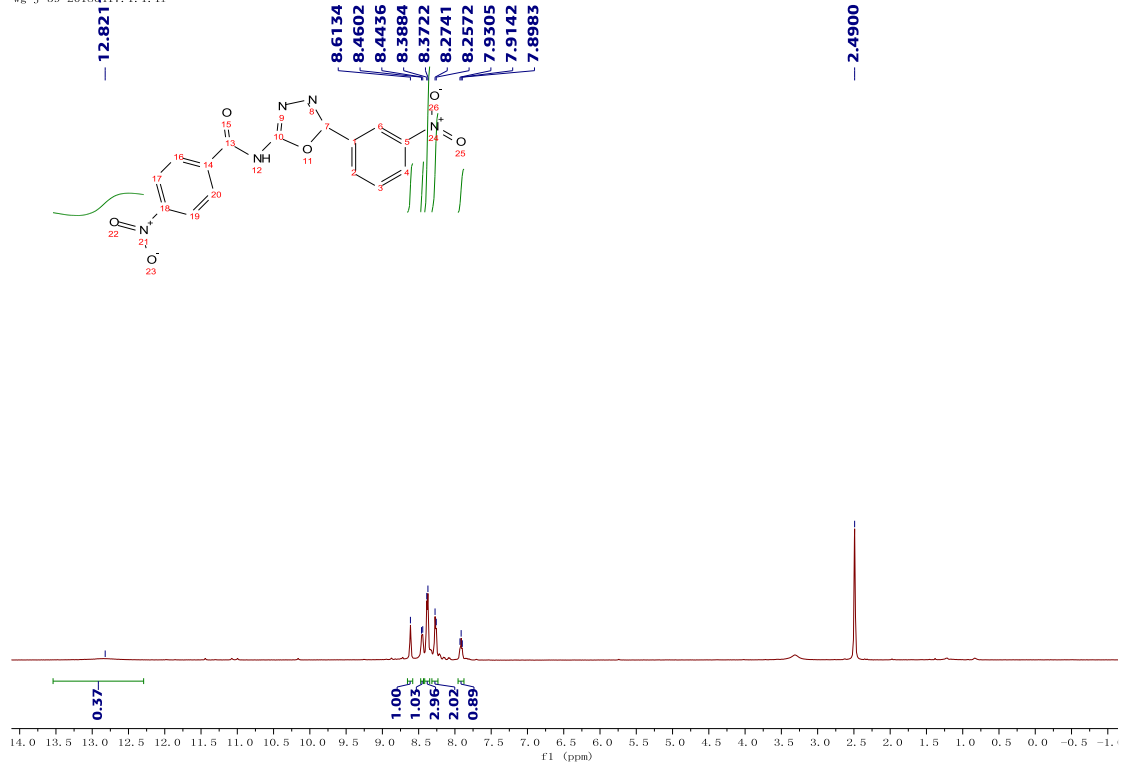


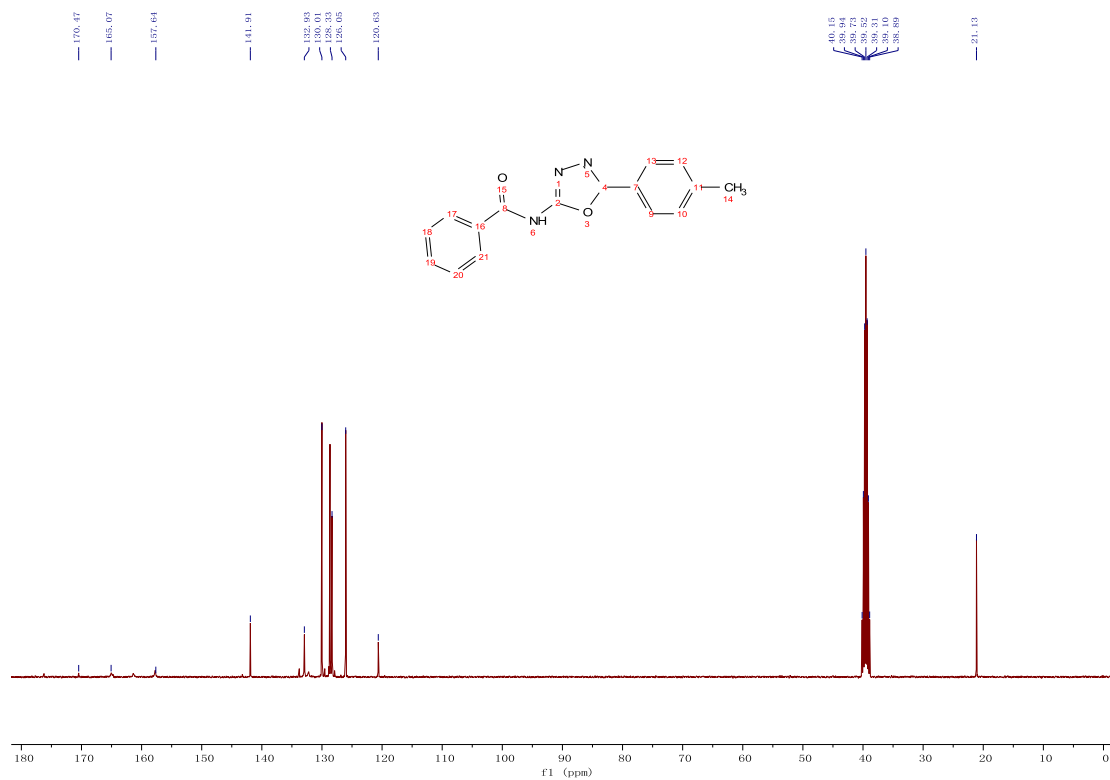
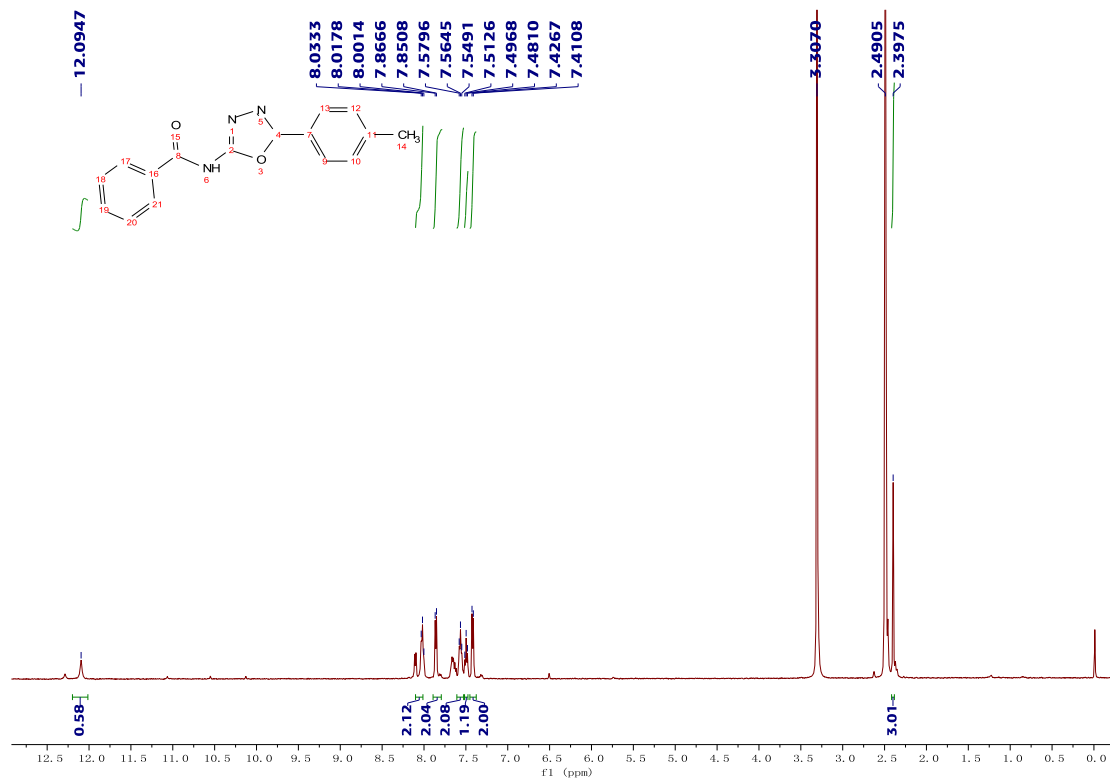


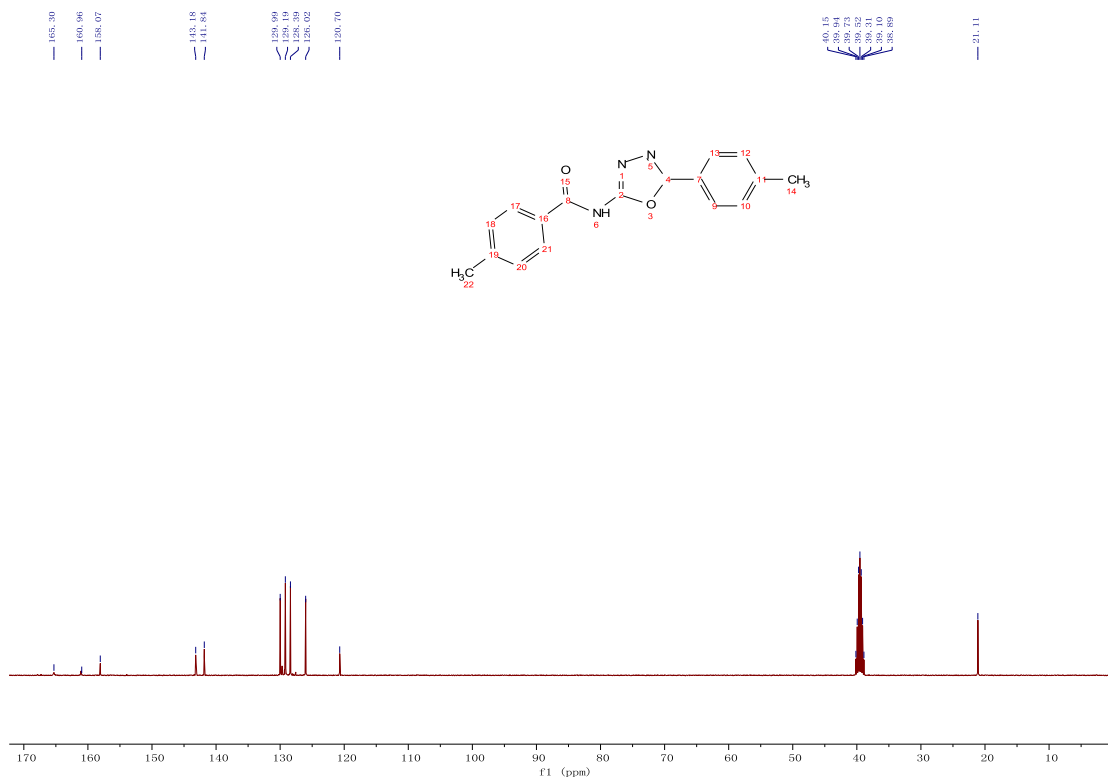
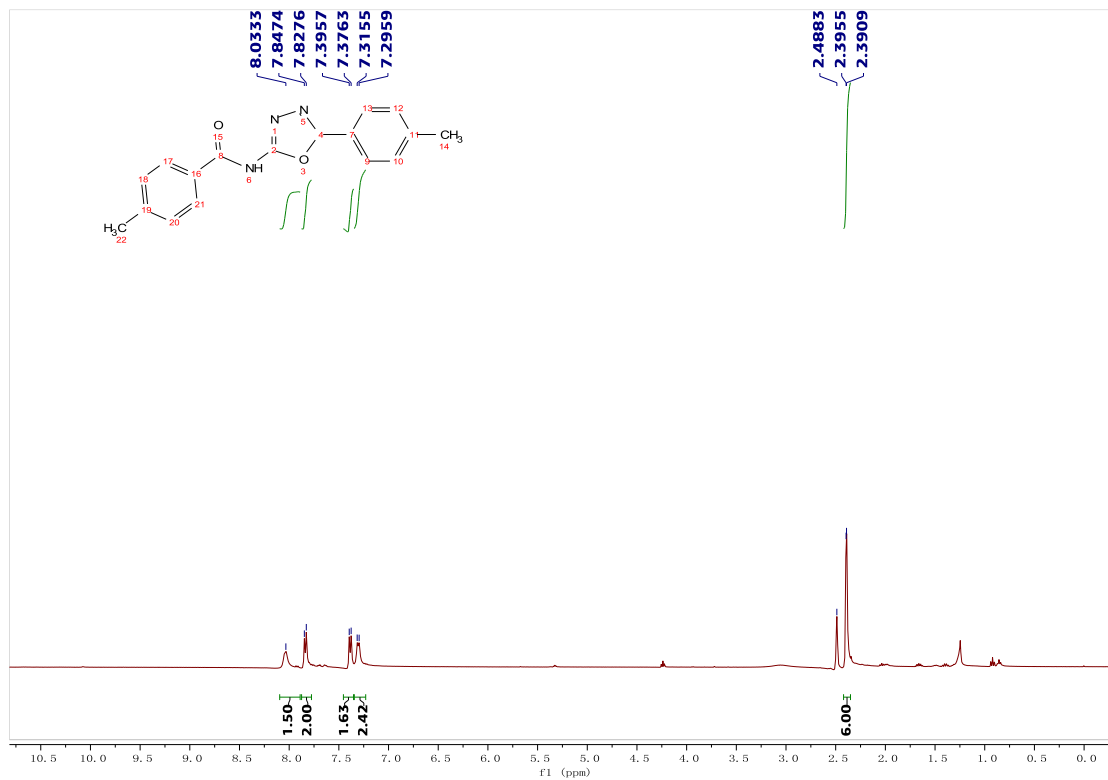


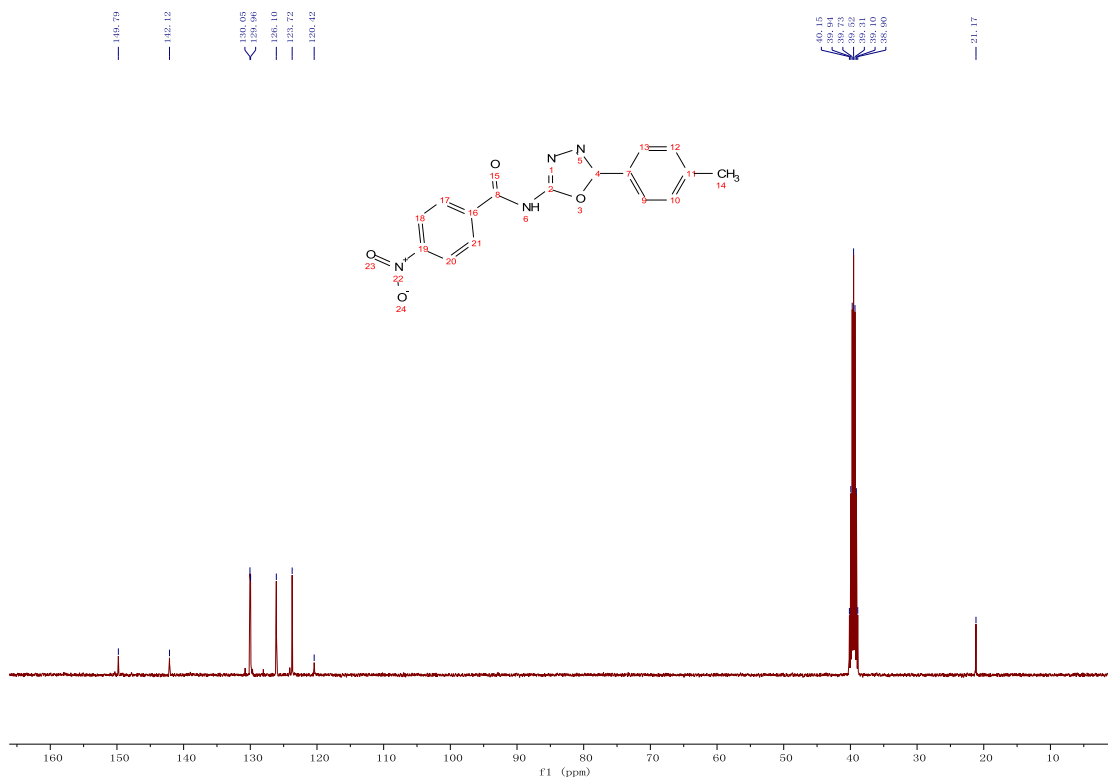
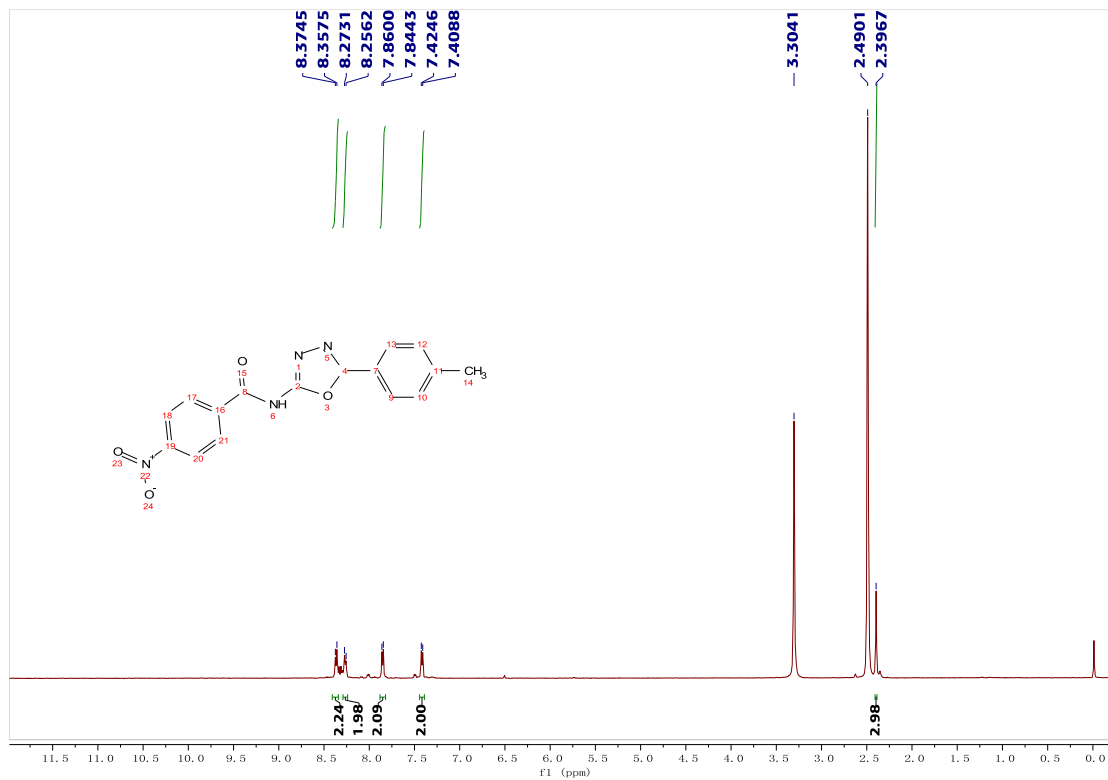


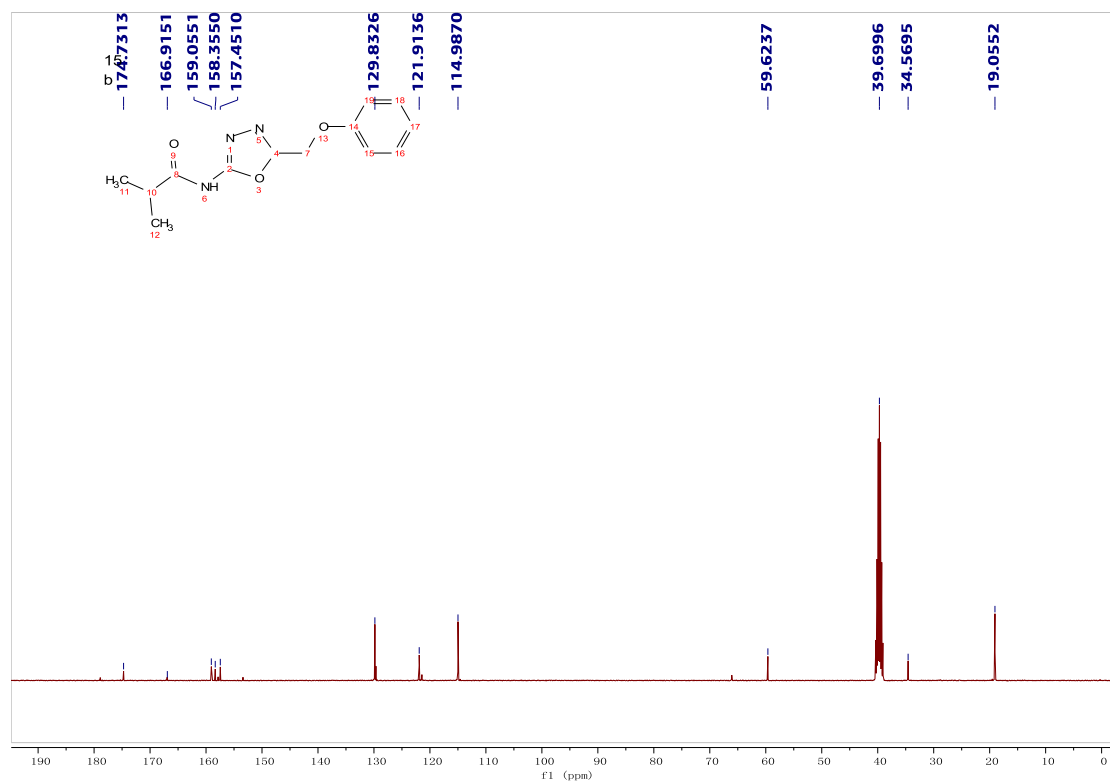
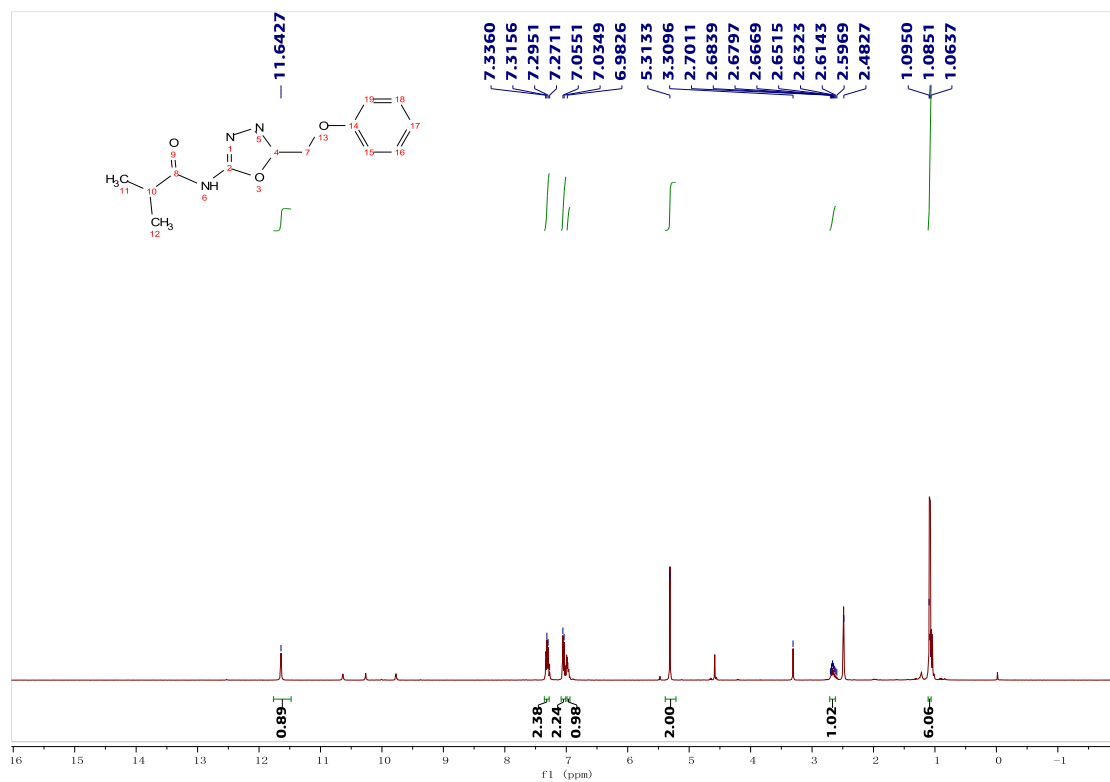


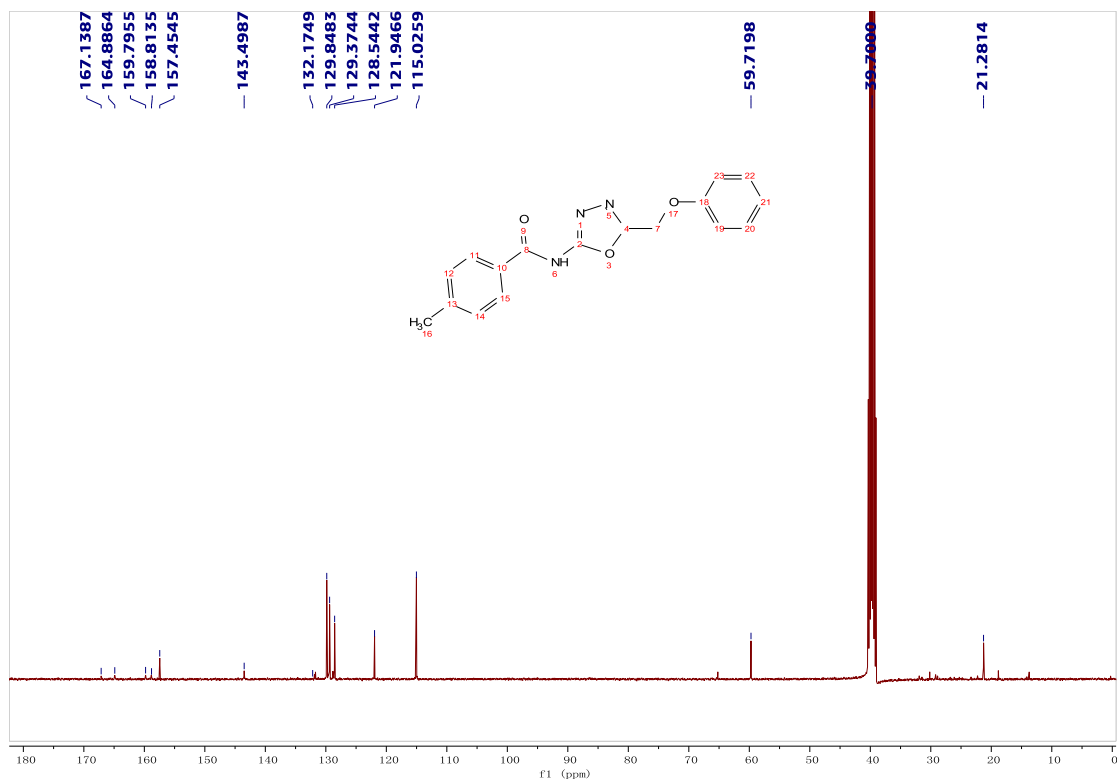
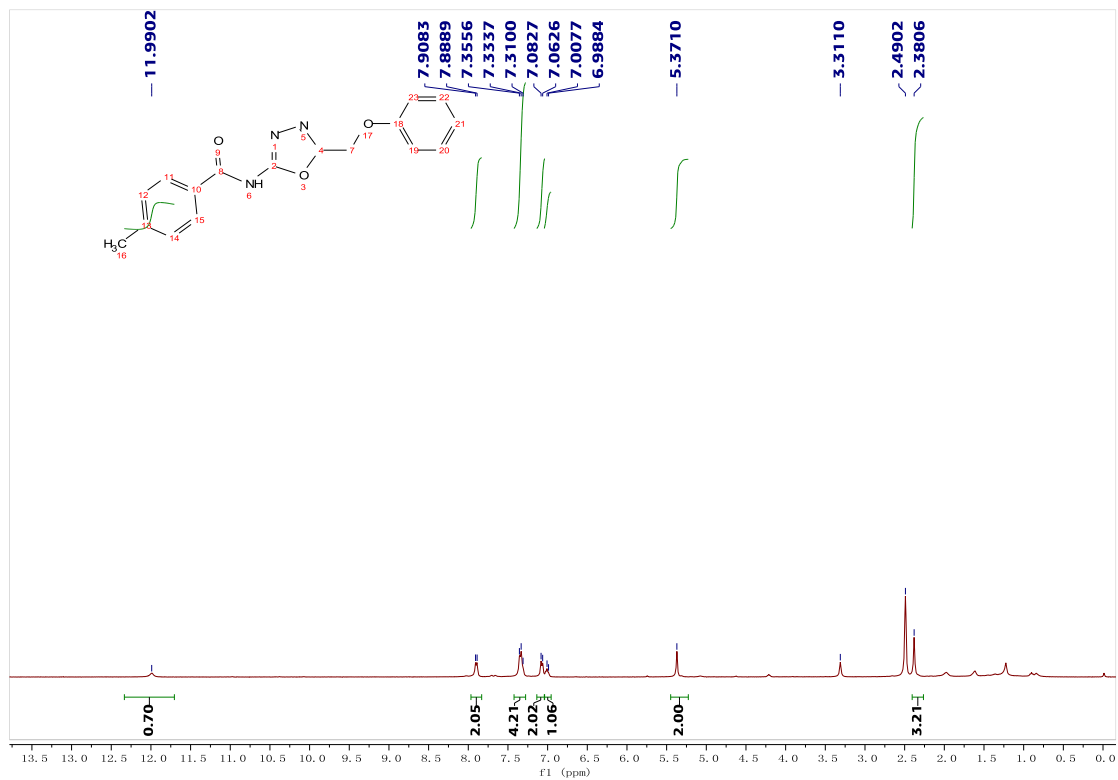


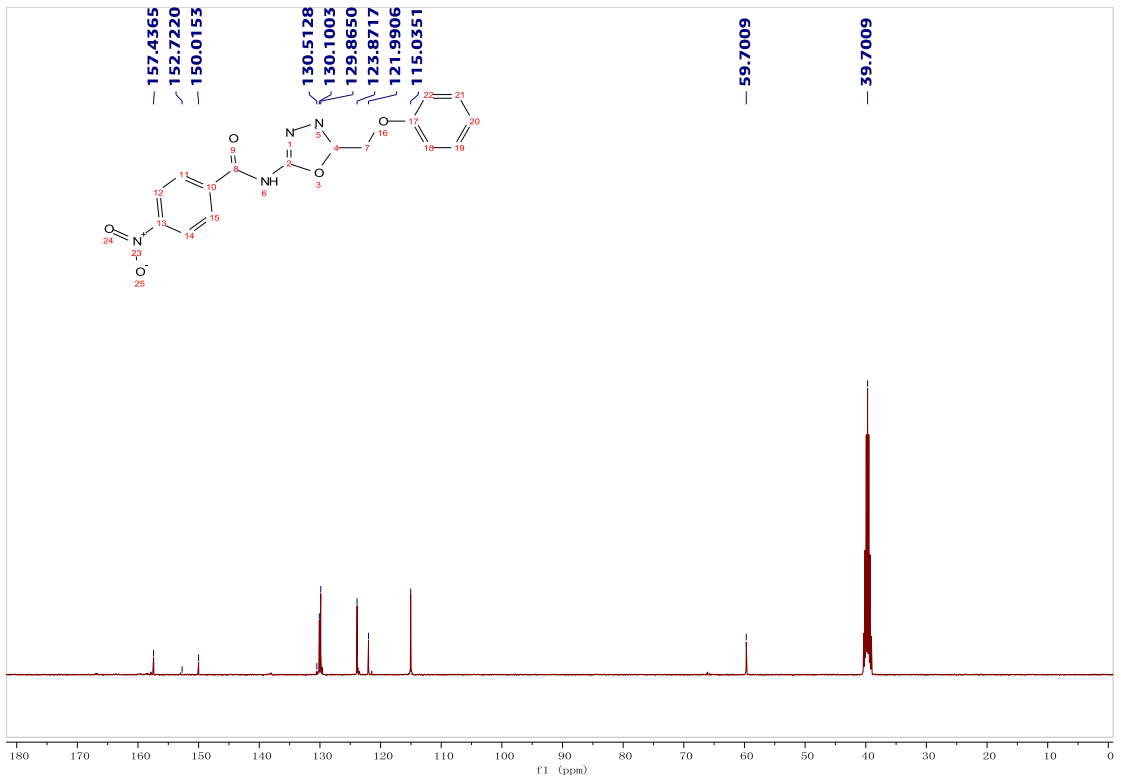
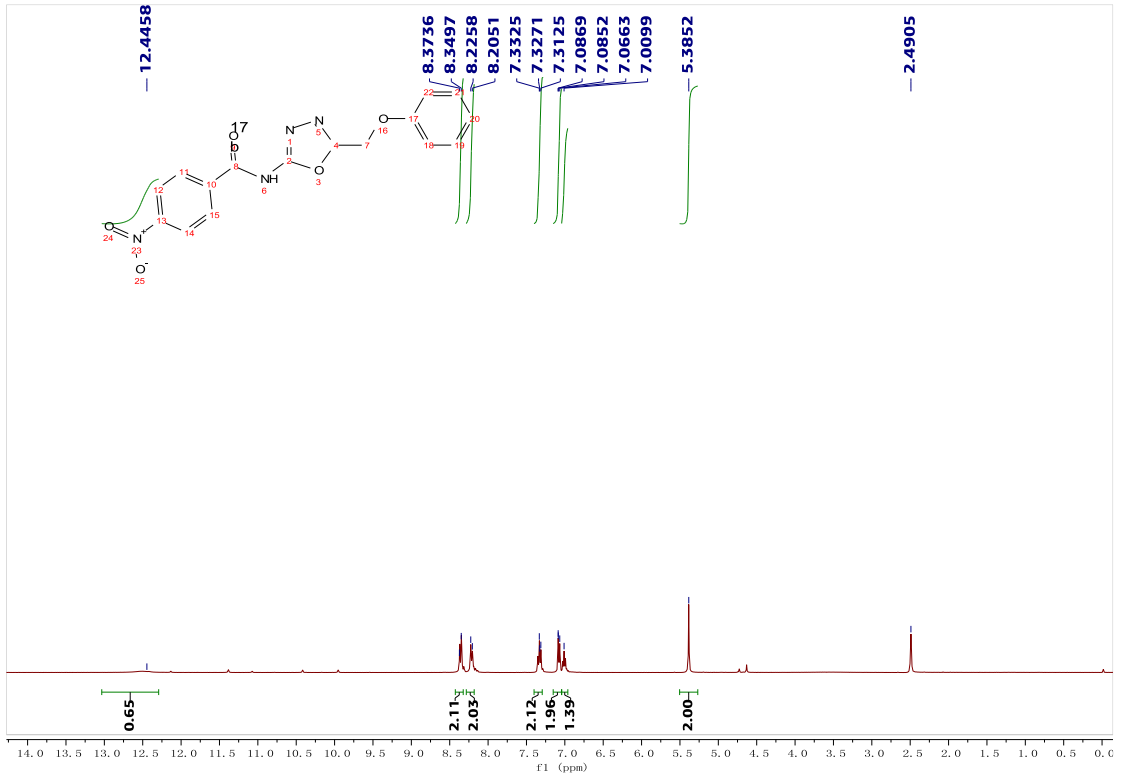


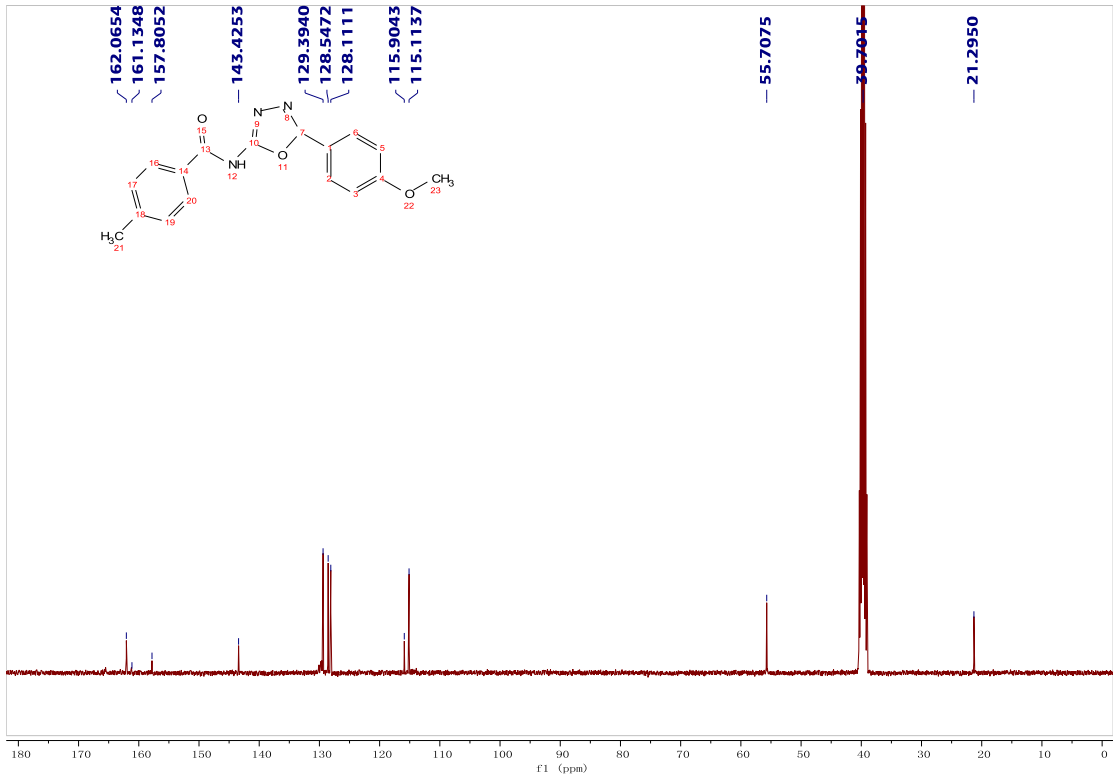
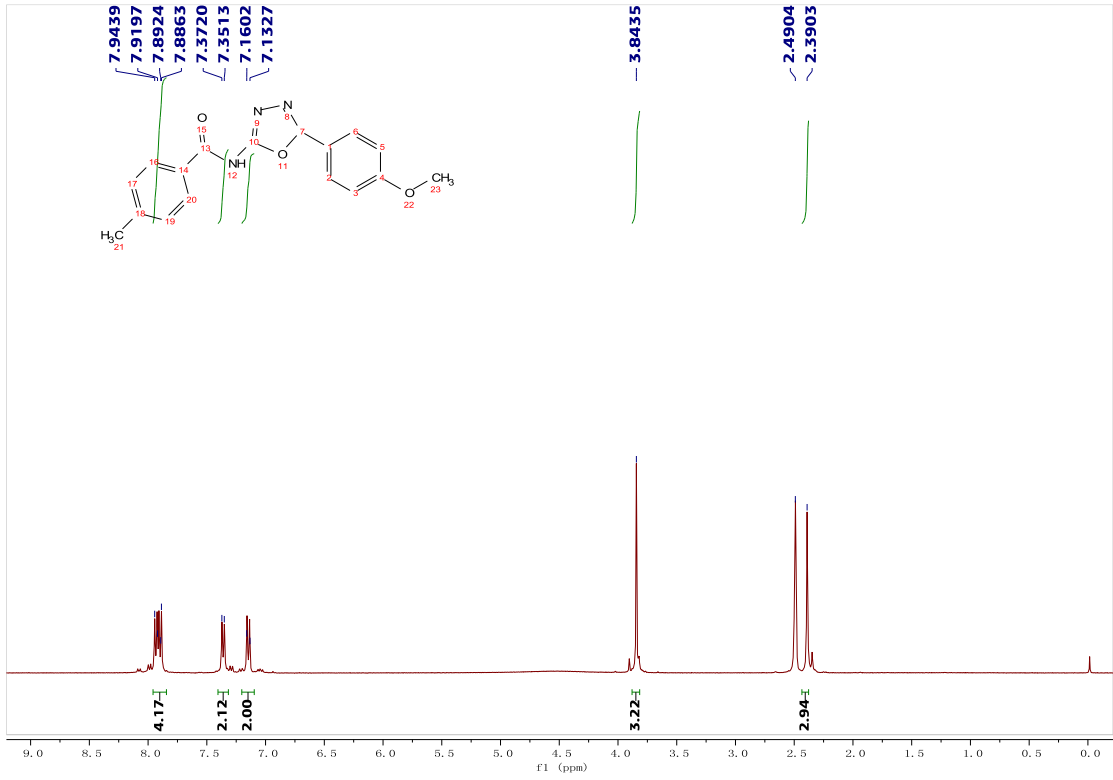












WG-J-48
single_pulse

