Supporting Information

Hydroxamate and thiosemicarbazone: two highly promising scaffolds for the development of SARS-CoV-2 antivirals

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Expression and purification of M^{pro}

Materials

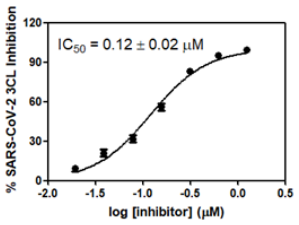
The HisTrapFF column and DEAE-Sepharose Fast Flow column were provided by Cytiva Technology Co (Shanghai, China). Fluorogenic substrate MCA-AVLQSGFR-Lys (Dnp)-Lys-NH2 (over 98% purity) was purchased from NJPeptide Ltd (Nanjing, China). PGEX-6P-1 vector and the SARS-CoV-2 M^{pro} gene were obtained from GENERAY (Shanghai, China). The other chemicals were purchased from Keluohuaboli Yiqi (Xian, China) and Sigma–Aldrich (St. Louis, MO, USA).

The expression and purification of M^{pro} were performed as reported method [1], PGEX-6P-1 plasmids with SARS-CoV-2 M^{pro} were transformed into *E. coli* BL21-Gold (DE3) cells. The single colony was picked to pre-culture at 37 ℃ in 15 ml Luria Broth (LB) supplemented with 100 ug/mL ampicillin overnight, and then subcultured into 4L LB medium. When the cells grow at 37℃ to an OD_{600} value in the range of 0.6-0.8, induced the protein expression via added isopropyl-*D*-thiogalactoside (1.0 mM). After 12 hours, induced cultures were centrifuged at 6000 x g for 15 min at 4 ℃ and resuspended with buffer A (20 mM Tris, pH 7.8, 150 mM NaCl). The cells were lysed by sonication on ice and cell fragments were removed by centrifugation at 8000 x g for 15 min at 4 ℃. The collected solution was loaded onto Ni-NTA affinity column, washed with buffer B (20 mM Tris, pH 7.8, 150 mM NaCl, 20 mM imidazole) and then eluted with buffer C (20 mM Tris, pH 7.8, 150 mM NaCl, 300 mM imidazole). The human rhinovirus (HRV) 3C protease was added to the eluted protein to remove the C-terminal hexahistidine-tag at 4˚C for 18 h. Then, the protein and His-tag was separated by His-trap column. Finally, the purified protein was identified by SDS–PAGE to determine size and purity.

IC_{50} measurement

The half maximal inhibitory concentration (IC_{50}) values of hydroxamates and thiosemicarbazones at a concentration between 0 and 80 μM on M^{pro} were measured. The protease (0.2 μM) was premixed with inhibitors at 37 °C for 2 h. The enzyme sample treated with 0.5% DMSO was used as negative control and ebselen was used as positive control. All experiments were performed in triplicate. The substrate was added to the mixture solutions, and then the hydrolysis rate of fluorescent substrate was monitored on Microplate Reader (Var ioskan flash, emission, 405 nm / excitation, 320 nm) for 1 min. The IC_{50} values were calculated by plotting the average percentage inhibition against inhibitor concentration and

fitted in GraphPad Prism 5 [2].



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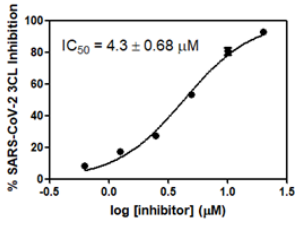

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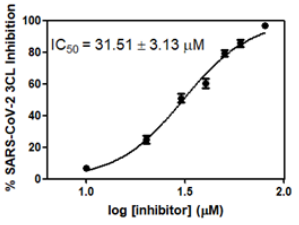

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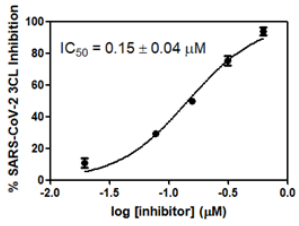

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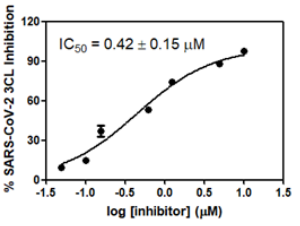

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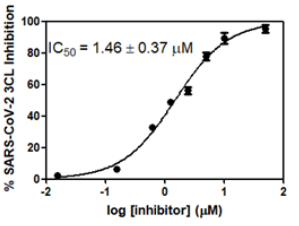

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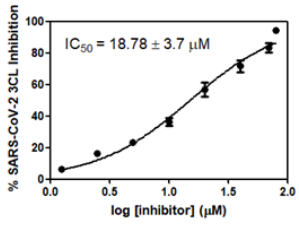

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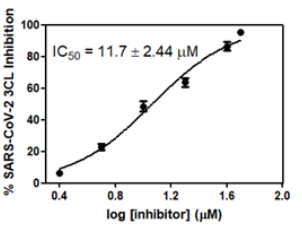

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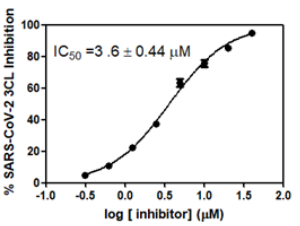

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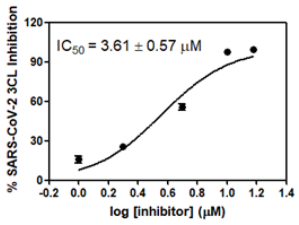

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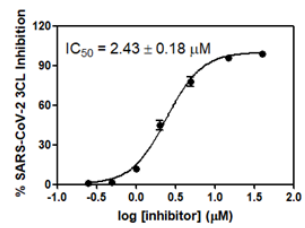

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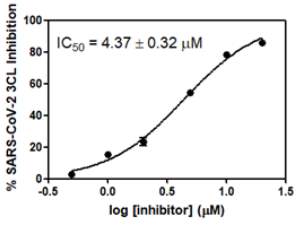

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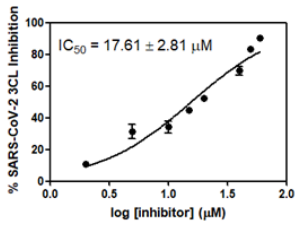

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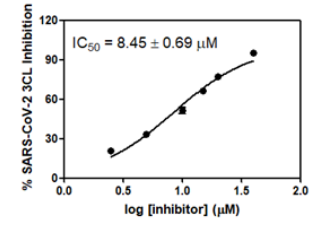

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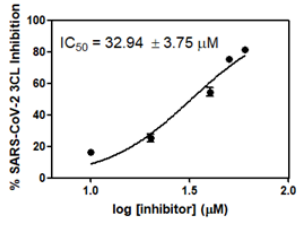

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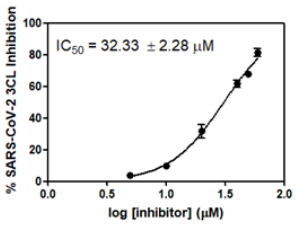

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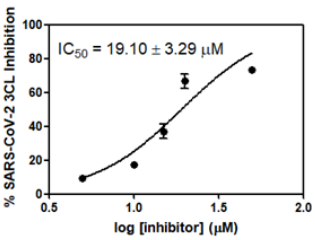

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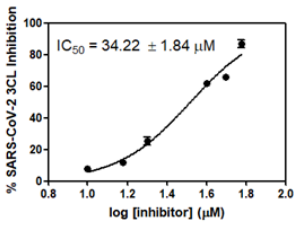

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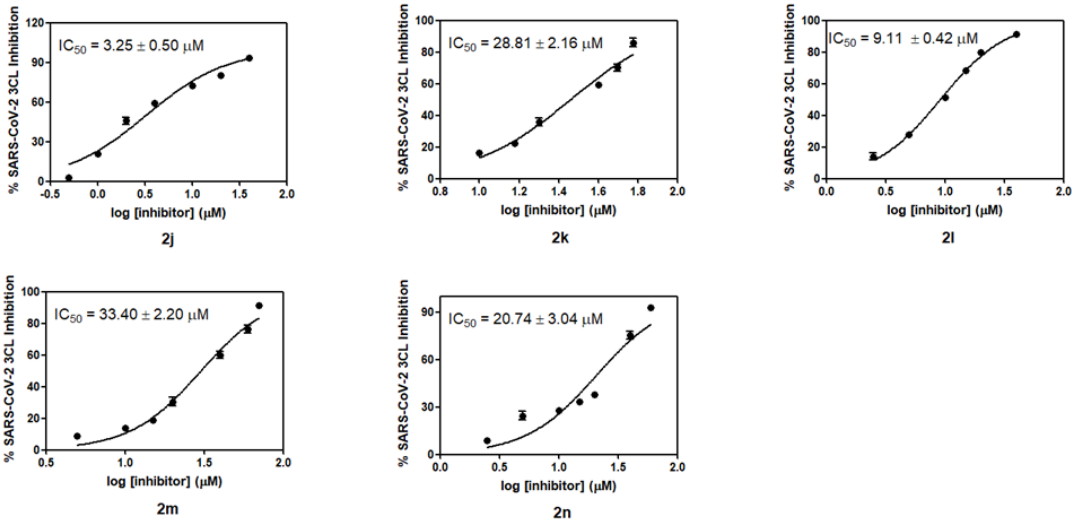


Docking study

Docking studies of hydroxamate **1a** and thiosemicarbazone **2b** into the active sites of the M^{pro} crystal structure (PDB ID: 6LU7) were performed by AutoDock 4.2 [3]. Before the simulation, the ligands and receptors were prepared. In detail, removing all water molecules, and then adding hydrogen atoms and charges. The dimension of the grid box was 50 x 50 x 50 grid points. The docking pose of the protein to the ligands was obtained by comparing the root mean square deviation (RMSD). All other parameters remain the default values. The structure, with the lowest binding free energy, was selected as the best docking posture. The minimized energy of **1a** and **2b** were calculated to -4.53 and -6.64 kcal/mol, respectively.

Cell fluorescence microscopy imaging and cytotoxicity assay

The cell fluorescence microscopy imaging can be used to observe the survival state of cells [4, 5]. The dyes used in this experiment were Calcein AM and propidium iodide (PI) and the cells were human breast cancer cells (MCF-7). In the dead cells, the PI-DNA complex release red fluorescence, but in the living cells, the Calcium-AM complex release green fluorescenc [4]. Firstly, the cells were cultured in Dulbecco modified eagle medium (DMEM) supplemented with 10% fetal bovine serum and penicillin-streptomycin (1000 ug/mL) at 37 ℃ in a 5% CO_2. Subsequently, the cells were premixed with inhibitors at 25 and 800 μM for 24 h, respectively. The cells without treatment with compounds were used as blank controls. After 24 h, the cell supernatant was removed and washed with buffer (PBS) 1 time, and then the cells were treated with Calcein AM and PI dyes under dark conditions at 37 ℃ for 30 min. Finally, the cells were imaged under a Confocal Microscope (Nikon A1).

As shown in Fig S1, more than 90% cells showed the green fluorescence in the presence

of hydroxamate **1a** and thiosemicarbazone **2b** at 25 μM, indicating that more than 90% cells tested maintained viability. However, a large number of cells exhibited red fluorescence at an inhibitor concentration up to 800 μM, indicating that these cells were dead. These assays suggest that the cells survival rate is dose-dependent.

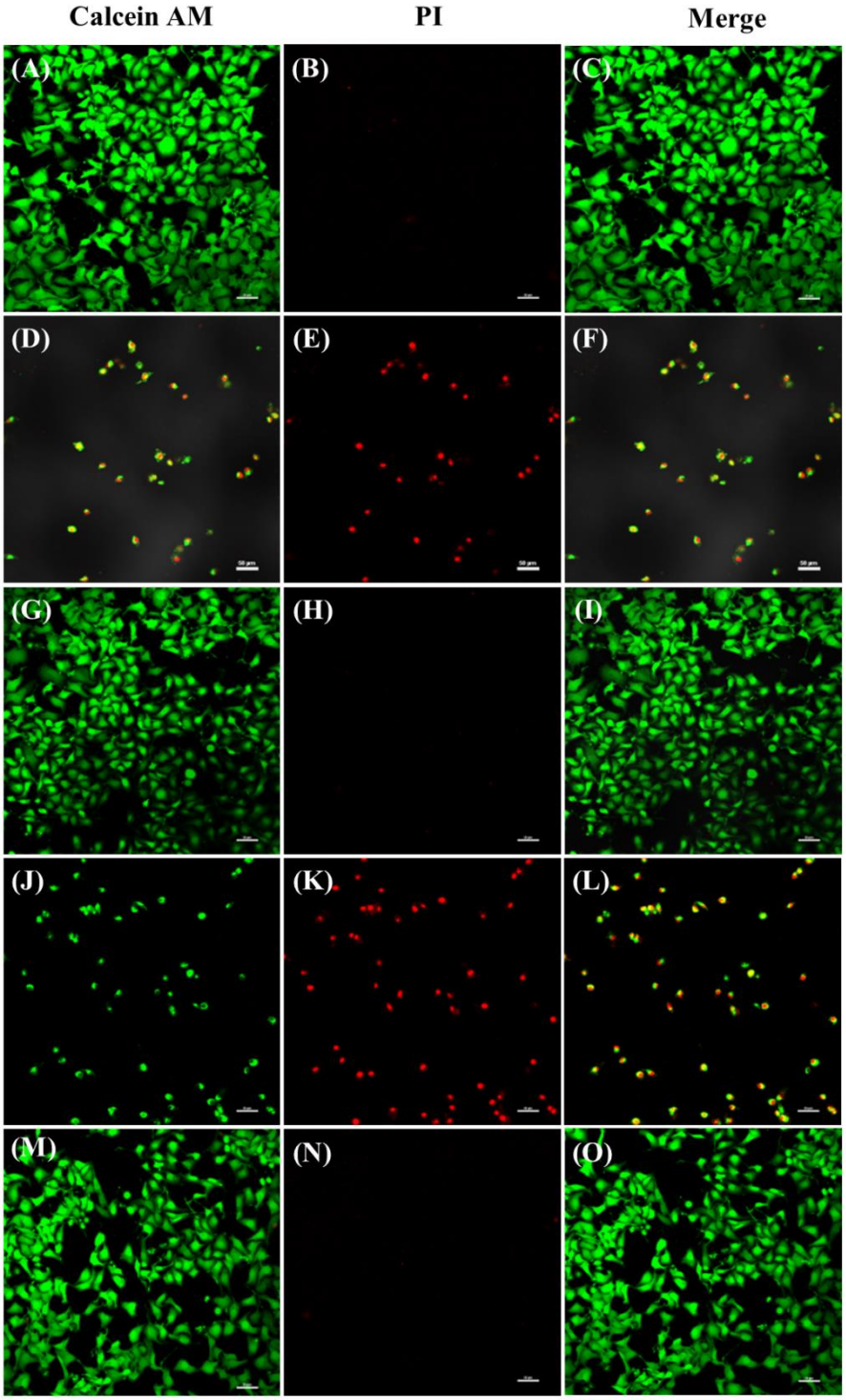


Fig. S1 Fluorescence microscopy images of MCF-7 cells treated with and without

hydroxamates and thiosemicarbazones. (A–C) non-treated cells; (D-F) cells treated with **1a** (800 μM); (G-I) cells treated with **1a** (25 μM); (G-L) cells treated with **2b** (800 μM); (M-O) cells treated with **2b** (25 μM)

The hydroxamates and thiosemicarbazones were reported to have anticancer potential [6-9]. Hence, the cytotoxic of the hydroxamate **1a** and thiosemicarbazone **2b** (1-800 μM) on the cancer cells (human breast cancer cells, MCF-7) were assayed. As shown in Fig 2, the cell viability was over 98% for 25 μM inhibitors, and the cell viability was over 85% for 100 μM inhibitors, indicating that both **1a** and **2b** have low cytotoxicity on the cells.

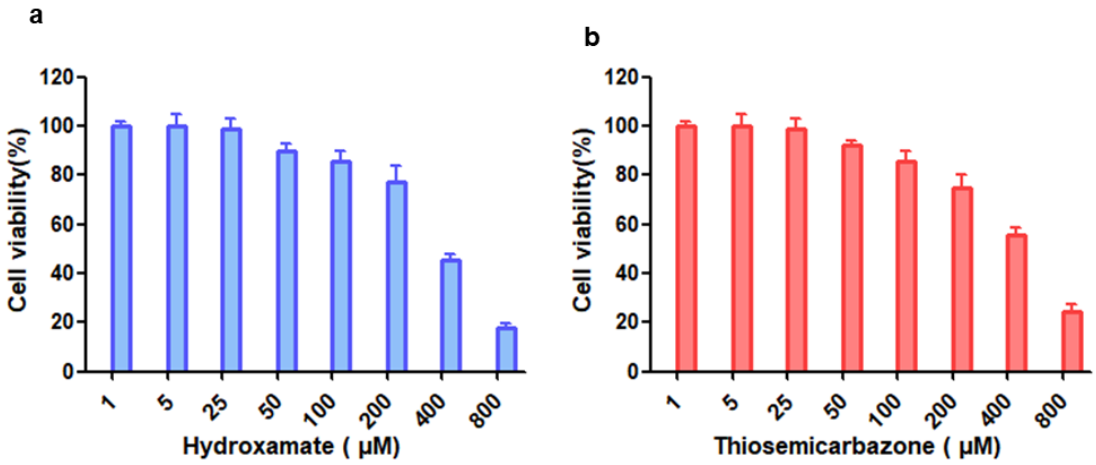


Fig. S2 The cytotoxicity of inhibitors (1-800 μM) hydroxamate **1a** (**a**) and thiosemicarbazone **2b** (**b**) on cancer cells (MCF-7).

Jump dilution assays

Jump dilution assays were performed as previously reported [10,11]. 20 μM M^{pro} (equivalent to 100-fold the concentration that needed for activity assay) was incubated with 6 μM **1a-i** and 122 μM **2b** (equivalent to 50 × IC_{50}). Two hours later, the reaction was initiated by addition of 2 μl of enzyme-inhibitor solution to 178 μL of assay buffer (20 mM Tris, pH 6.5, 0.4 mM EDTA, 20% glycerol, 120 mM NaCl) and 20 μl of the fluorescent substrate solution to make up a 200 μl total reaction solution per well in a 96 well. The mixtures solutions was diluted 100-fold and the inhibitors concentration thus goes from 50-fold IC_{50} (corresponding to 99% inhibition) to 0.5-fold the IC_{50} (corresponding to about 25% inhibition). Subsequently, the progress of the reaction was monitored on Microplate Reader

(Var ioskan flash, emission, 405 nm / excitation, 320 nm) for 4000 s and recorded, and M^{pro} was treated by 0.5% DMSO as blank control. The collected data analysis was performed with Graphpad Prism 5.0 software.

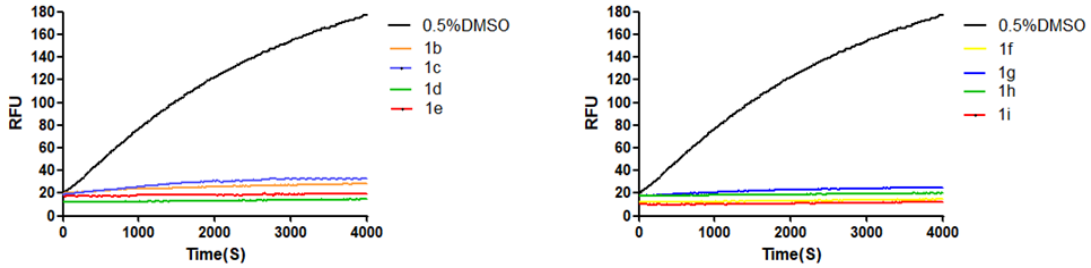


Fig. S3 Jump dilution: M^{pro} was soaked with hydroxamate **1b-i** (a concentration of 50 × IC_{50}) for 2 h, and then 100-fold diluted with enzymatic substrate solution and monitored for 4000 s. 0.5% DMSO was as the blank control.

Mass data and NMR spectra of the synthetic hydroxamates and thiosemicarbazones

***N*- (phenethyloxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1a)**.

White solid, yield 53%, m.p. 63-64 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.16 (s, 1H), 7.37 (s, 5H), 7.22-7.15 (m, 5H), 5.22 (s, 2H), 2.81 (t, J = 7.7 Hz, 2H), 2.40 (t, J = 7.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 169.92, 154.50, 141.11, 135.27, 129.23, 129.12, 128.93, 128.87, 128.76, 126.61, 71.00, 33.98, 30.83. HRMS (ESI) m/z: (Calcd. for $C_{17}H_{17}NO_4$ [M+H] $^+$, 300.1230; found, 300.1247).

(*Z*) - (4-nitrophenylpropenylcarbonyl)-*O*-(ethyloxycarbonyl) hydroxamate (1b).

Light yellow solid, yield 45%, m.p. 143-144 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.36 (s, 1H), 8.22 (d, J = 8.5 Hz,2H), 7.89 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 15.9 Hz, 1H), 6.72 (d, J = 15.9 Hz, 1H), 4.24 (q, J = 7.0 Hz, 2H), 1.25 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 163.37, 154.33, 148.41, 141.21, 139.50, 129.58, 124.60, 121.78, 66.27, 14.51. HRMS (ESI) m/z: (Calcd. for $C_{12}H_{12}N_2O_6$ [M+H] $^+$, 281.0769; found, 281.0785).

***N*-(4-tert-butylphenoxycarbonyl)-*O*-(benzyloxycarbonyl)hydroxamate (1c)**.

White solid, yield 63%, m.p. 88-89 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.61 (s, 1H), 7.71 (d, *J* = 6.8 Hz, 2H), 7.51 (d, *J* = 6.9 Hz, 2H), 7.40 (s, 4H), 7.37 (s, 1H), 5.27 (s, 2H), 1.26 (s, 9H). ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 165.42, 156.01, 154.68, 135.29, 129.27, 129.14, 128.97, 128.42, 127.77, 126.06, 71.15, 35.32, 31.38. HRMS (ESI) m/z: (Calcd. for

$C_{19}H_{21}NO_4$ [M+Na] $^+$, 350.1397; found, 350.1362).

***N*-(phenethyloxycarbonyl)-*O*-(ethyloxycarbonyl)hydroxamate (1d)**.

White solid, yield 47%, m.p. 66-67 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 11.94 (s, 1H), 7.19 (m, 5H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.81 (t, *J* = 7.7 Hz, 2H), 2.38 (t, *J* = 7.7 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H). ^{13}CNMR (101 MHz, DMSO-*d* 6) δ 169.87, 154.47, 141.11, 128.87, 128.75, 126.61, 66.04, 33.96, 30.80, 14.50. HRMS (ESI) m/z: (Calcd. for $C_{12}H_{15}NO_4$ [M+H] $^+$, 238.1074; found, 238.1052).

(*Z*) - (2, 4-dichlorophenylpropenylcarbonyl)-*O*-(ethyloxycarbonyl) hydroxamate (1e).

White solid, yield 68%, m.p. 98-99 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.35 (s, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.75 (d, *J* = 15.9 Hz, 1H), 7.71 (d, *J* = 2.1 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 6.61 (d, *J* = 15.8 Hz, 1H), 4.24 (q, *J* = 7.1 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 162.99, 154.33, 135.83, 135.71, 134.91, 131.60, 130.04, 129.83, 128.65, 121.35, 66.29, 14.52. HRMS (ESI) m/z: (Calcd. for $C_{12}H_{11}Cl_2NO_4$ [M+H] $^+$, 304.0138; found, 304.0166).

***N*- (4-tert-butylphenoxycarbonyl)-*O*-(ethyloxycarbonyl) hydroxamate (1f)**.

White solid, yield 52%, m.p. 101-102 ℃; ^{1}H NMR (400 MHz, DMSO-*d* 6) δ 12.54 (s, 1H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 8.2 Hz, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 1.26 (s, 9H). ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 165.40, 155.96, 154.62, 128.49, 127.76, 126.04, 66.18, 35.31, 31.38, 14.56. HRMS (ESI) m/z: (Calcd. for $C_{14}H_{13}NO_4$ [M+Na] $^+$, 288.1206; found, 288.1273).

***N*- (2, 2-diphenylethylcarbonyl)-*O*-(ethyloxycarbonyl) hydroxamate (1g)**.

White solid, yield 57%, m.p. 94-95 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.00 (s, 1H), 7.24 (s, 4H), 7.23 (s, 4H), 7.14 (dd, *J* = 8.6, 4.1 Hz), 4.46 (t, *J* = 7.9 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 2.86 (d, *J* = 7.9 Hz, 2H), 1.16 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 168.53, 154.28, 144.31, 128.95, 128.06, 126.83, 65.99, 46.56, 38.07, 14.45. HRMS (ESI) m/z: (Calcd. for $C_{18}H_{19}NO_4$ [M+Na] $^+$, 336.1206; found 336.1228).

***N*- (phenoxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1h)**.

White solid, yield 70%, m.p. 114-115 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.72 (s, 1H), 7.78 (d, *J* = 5.8 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.41 (m, 5H), 5.29 (s, 2H). ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 163.54, 154.52, 134.22, 130.12, 126.98,

120.86. HRMS (ESI) m/z: (Calcd. for $C_{15}H_{13}NO_4$ $[M+H]^+$, 272.0917; found, 272.0959).

***N*- (phenoxycarbonyl)-*O*-(phenoxycarbonyl) hydroxamate (1i)**.

White solid, yield 63%, m.p. 59-60 ℃; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.82 (s, 2H), 7.80 (d, *J* = 1.5 Hz, 2H), 7.70 (t, *J* = 7.4 Hz, 2H), 7.61 (t, *J* = 7.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ^{13}C NMR (101 MHz, DMSO-*d* 6) δ 163.56, 154.52, 131.66, 130.12, 128.90, 127.39, 126.99, 120.86. HRMS (ESI) m/z: (Calcd. for $C_{14}H_{11}NO_4$ $[M+K]^+$, 296.0320; found, 296.0331).

***(E)*-1-(2-hydroxybenzylidene)-4-p-tolylthiosemicarbazone (2a)**

White solid, yield 74%, 1 H NMR (400 MHz, DMSO) δ: 11.72 (s, 1H), 9.98 (s, 2H), 8.50 (d, J = 6.5 Hz, 1H), 8.09 (s, 1H), 7.43 (d, J = 8.0 Hz, 2H), 7.30–7.05 (m, 3H), 6.86 (dt, J = 14.4, 7.6 Hz, 2H), 2.30 (s, 3H). 13C NMR (101 MHz, DMSO) δ: 175.87, 156.65, 140.05, 136.67, 134.42, 131.36, 128.59, 127.17, 125.76, 120.38, 119.31, 116.12, 20.69. HRMS (ESI) m/z: 308.0808 (Calcd. for [M + Na] + 308.0828).

***(E)*-1-(2-hydroxybenzylidene)-4-p-tolyl thiosemicarbazone (2b)**

White solid, yield 78%, 1H NMR (400 MHz, DMSO-d6) δ 11.51 (s, 1H), 9.74 (d, J = 11.7 Hz, 3H), 8.32 (s, 1H), 7.80 (d, J = 8.7 Hz, 1H), 7.27 (d, J = 8.8 Hz, 1H), 7.23 (s, 1H), 7.19 (s, 2H), 6.29 (s, 1H), 6.24 (d, J = 9.8 Hz, 1H), 2.57 (q, J = 7.6 Hz, 2H), 1.11 (t, J = 7.6 Hz, 3H).13C NMR (101 MHz, DMSO-D6) δ 176.81, 161.11, 158.65, 141.28, 138.20, 129.55, 129.20, 129.08, 128.76, 127.24, 126.28, 112.38, 108.31, 102.79, 24.64, 14.95, 14.73. HRMS (ESI) m/z: 338.0933 (Calcd. for [M + Na] + 338.0900).

***(E)*-1-(2-hydroxybenzylidene)-4-(4-chlorophenyl) thiosemicarbazone (2c)**

White solid, yield 82%, 1 H NMR (400 MHz, DMSO) δ: 11.73 (s, 1H), 9.98 (s, 2H), 8.50 (s, 1H), 8.24–8.03 (m, 1H), 7.46 (d, J = 8.1 Hz, 2H), 7.30–7.05 (m, 3H), 7.05–6.71 (m, 2H), 2.58 (d, J = 7.1 Hz, 2H), 1.55 (d, J = 6.8 Hz, 2H), 1.44–1.17 (m, 2H), 0.91 (q, J = 8.2, 7.1 Hz, 3H). 13C NMR (101 MHz, DMSO) δ : 175.81, 156.67, 140.02, 139.36, 136.85, 131.35, 127.92, 127.17, 125.69, 120.38, 119.31, 116.12, 34.47, 33.31, 21.84, 13.90. HRMS (ESI) m/z: 328.1465 (Calcd. for [M + H] + 328.1478).

***(E)*-1-(2-hydroxybenzylidene)-4-cyclohexyl thiosemicarbazone (2d)**

White solid, yield 85%, 1 H NMR (400 MHz, DMSO) δ: 11.35 (s, 1H), 9.92 (s, 1H), 8.38 (s, 1H), 7.92 (dd, J = 19.8, 8.0 Hz, 2H), 7.22 (t, J = 7.6 Hz, 1H), 6.85 (dd, J = 16.9,

8.1 Hz, 2H), 4.31–4.03 (m, 1H), 1.91–1.10 (m, 10H). 13C NMR (101 MHz, DMSO) δ: 175.64, 156.50, 139.39, 131.14, 126.74, 120.45, 119.30, 116.12, 52.63, 31.94, 25.02. HRMS (ESI) m/z: 300.1142 (Calcd. for [M + Na] + 300.1141).

***(E)*-1-(2-hydroxybenzylidene)-4-cyclohexyl thiosemicarbazone (2e)**

White solid, yield 85%, 1H NMR (400 MHz, DMSO-d6) δ 11.57 (s, 1H), 9.84 (d, J = 44.5 Hz, 3H), 8.33 (s, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.54 (d, J = 7.8 Hz, 2H), 7.31 (t, J = 7.8 Hz, 2H), 7.14 (t, J = 7.4 Hz, 1H), 6.34 – 6.22 (m, 2H). 13C NMR (101 MHz, DMSO-d 6) δ 175.54, 161.22, 158.75, 141.39, 139.77, 129.09, 128.52, 126.05, 125.51, 112.27, 108.28, 102.74. HRMS (ESI) m/z: 310.0520 (Calcd. for [M + Na] + 310.0594).

***(E)*-1-(2-hydroxybenzylidene)-4-(4-butylphenyl) thiosemicarbazone (2f)**

White solid, yield 69%, 1 H NMR (400 MHz, DMSO) δ: 11.86 (s, 1H), 10.04 (d, J = 45.0 Hz, 2H), 8.51 (t, J = 9.0 Hz, 1H), 8.19–7.96 (m, 1H), 7.63 (d, J = 7.5 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.34–7.13 (m, 1H), 6.87 (dt, J = 15.0, 7.7 Hz, 2H). 13C NMR (101 MHz, DMSO) δ: 175.77, 156.75, 140.50, 138.24, 131.52, 129.24, 127.99, 127.41, 127.16, 120.29, 119.32, 116.15. HRMS (ESI) m/z: 328.0256 (Calcd. for [M + Na] + 328.0282).

***(E)*-1-(4-hydroxybenzylidene)-4-cyclohexyl thiosemicarbazone (2g)**

White solid, yield 85%, 1 H NMR (400 MHz, DMSO) δ: 11.23 (s, 1H), 9.88 (s, 1H), 7.97 (s, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.60 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 4.18 (ddt, J = 15.3, 11.0, 4.7 Hz, 1H), 1.92–1.82 (m, 2H), 1.77–1.66 (m, 2H), 1.60 (d, J = 12.4 Hz, 1H), 1.42 (qd, J = 12.1, 2.8 Hz, 2H), 1.28 (q, J = 12.4 Hz, 2H), 1.13 (q, J = 12.3 Hz, 1H). 13C NMR (101 MHz, DMSO) δ: 175.40, 159.35, 142.64, 129.16, 125.13, 115.64, 52.55, 32.01, 25.26, 25.03. HRMS (ESI) m/z: 278.1340 (Calcd. for [M + H] + 278.1322).

***(E)*-1-(4-hydroxybenzylidene)-4-(2-ethylphenyl) thiosemicarbazone (2h)**

White solid, yield 82%, 1 H NMR (400 MHz, DMSO) δ: 11.62 (s, 1H), 9.86 (d, J = 23.1 Hz, 2H), 8.06 (s, 1H), 7.72 (d, J = 8.6 Hz, 2H), 7.39–7.11 (m, 4H), 6.80 (d, J = 8.6 Hz, 2H), 2.61 (q, J = 7.5 Hz, 2H), 1.15 (t, J = 7.6 Hz, 3H). 13C NMR (101 MHz, DMSO) δ: 176.77, 159.44, 142.92, 140.95, 137.72, 129.40, 128.31, 126.89, 125.86, 125.25, 115.63, 24.20, 14.29. HRMS (ESI) m/z: 322.0976 (Calcd. for [M + Na] + 322.0985).

***(E)*-1-(4-hydroxybenzylidene)-4-(4-butylphenyl) thiosemicarbazone (2i)**

White solid, yield 82%, 1 H NMR (400 MHz, DMSO) δ: 11.60 (s, 1H), 9.91 (s, 2H), 8.06 (s, 1H), 7.72 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 8.2 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 2.58 (t, J = 7.6 Hz, 2H), 1.57 (p, J = 8.0, 7.6 Hz, 2H), 1.31 (dt, J = 14.3, 7.3 Hz, 2H), 0.91 (d, J = 14.6 Hz, 3H). 13C NMR (101 MHz, DMSO) δ: 175.48, 159.51, 143.22, 139.30, 136.84, 129.50, 127.88, 125.67, 125.08, 115.60, 34.43, 33.29, 21.81, 13.88. HRMS (ESI) m/z: 328.1476 (Calcd. for [M + H] + 328.1478).

***(E)*-1-(4-hydroxybenzylidene)-4-p-tolyl thiosemicarbazone (2j)**

White soild, yield 69%, 1 H NMR (400 MHz, DMSO) δ: 11.61 (s, 1H), 9.92 (s, 2H), 8.07 (s, 1H), 7.73 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 7.16 (d, J = 8.1 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 2.30 (s, 3H). 13C NMR (101 MHz, DMSO) δ: 175.59, 159.54, 143.28, 136.68, 134.39, 129.54, 128.59, 125.73, 125.11, 115.65, 20.71. HRMS (ESI) m/z: 308.0815 (Calcd. for [M + Na] + 308.0828).

***(E)*-1-(4-hydroxybenzylidene)-4-o-tolyl thiosemicarbazone (2k)**

White solid, yield 76%, 1 H NMR (400 MHz, DMSO) δ: 11.62 (s, 1H), 9.89 (s, 1H), 9.84 (s, 1H), 8.06 (s, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.34–7.29 (m, 1H), 7.27 (dd, J = 6.6, 2.3 Hz, 1H), 7.25–7.16 (m, 2H), 6.80 (d, J = 8.7 Hz, 2H), 2.24 (s, 3H). 13C NMR (101 MHz, DMSO) δ: 176.42, 159.45, 142.93, 138.26, 135.42, 130.11, 129.43, 128.73, 126.63, 125.94, 125.24, 115.63, 17.95. HRMS (ESI) m/z: 308.0822 (Calcd. for [M + Na] + 308.0828).

***(E)*-1-(4-hydroxybenzylidene)-4-(4-chlorophenyl) thiosemicarbazone (2l)**

White soild, yield 74%, 1 H NMR (400 MHz, DMSO) δ: 11.75 (s, 1H), 10.03 (s, 1H), 9.94 (s, 1H), 8.08 (s, 1H), 7.73 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 8.8 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H). 13C NMR (101 MHz, DMSO) δ: 175.46, 159.65, 143.76, 138.24, 129.63, 129.20, 127.98, 127.35, 124.98, 115.66. HRMS (ESI) m/z: 328.0258 (Calcd. for [M + Na] + 328.0282).

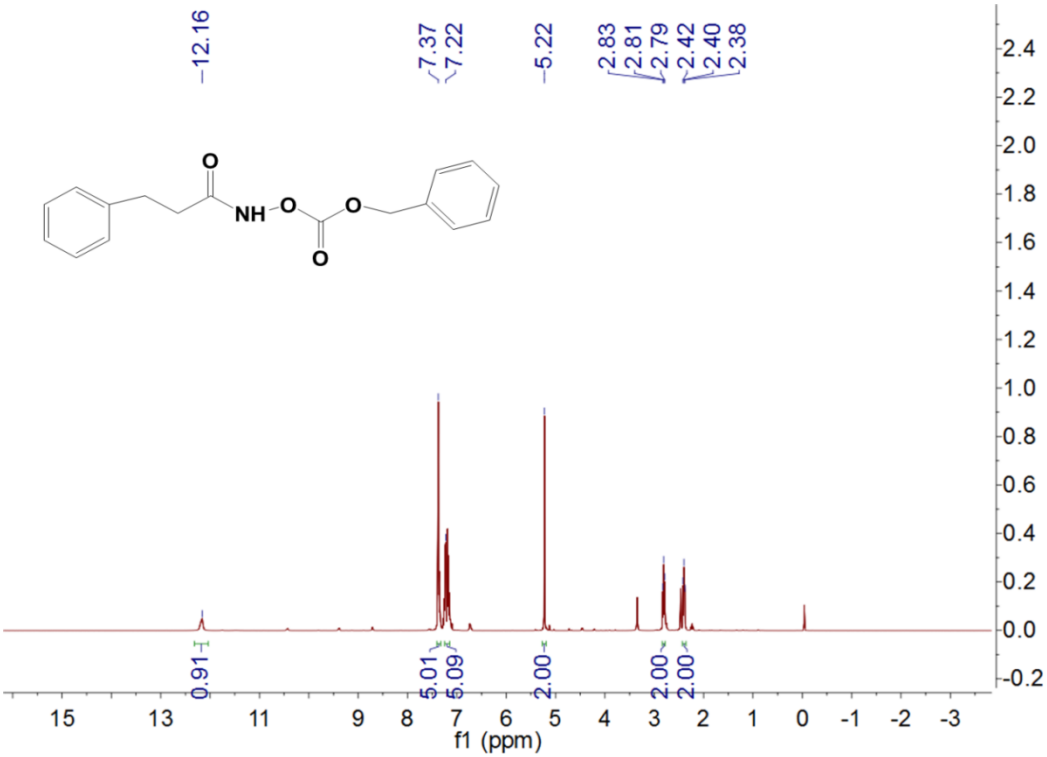
***(E)*-1-(2-hydroxybenzylidene)-4-(2-ethylphenyl) thiosemicarbazone (2m)**

Light yellow solid, yield 72%, 1 H NMR (400 MHz, DMSO) δ: 11.72 (s, 1H), 9.89 (s, 2H), 8.48 (s, 1H), 8.06 (d, J = 7.3 Hz, 1H), 7.25 (ddd, J = 24.1, 9.7, 5.0 Hz, 5H), 6.88 (d, J = 8.1 Hz, 1H), 6.82 (t, J = 7.5 Hz, 1H), 2.60 (q, J = 7.5 Hz, 2H), 1.15 (t, J = 7.6 Hz, 3H). 13C NMR (101 MHz, DMSO) δ: 176.99, 156.59, 140.95, 137.69, 131.25,

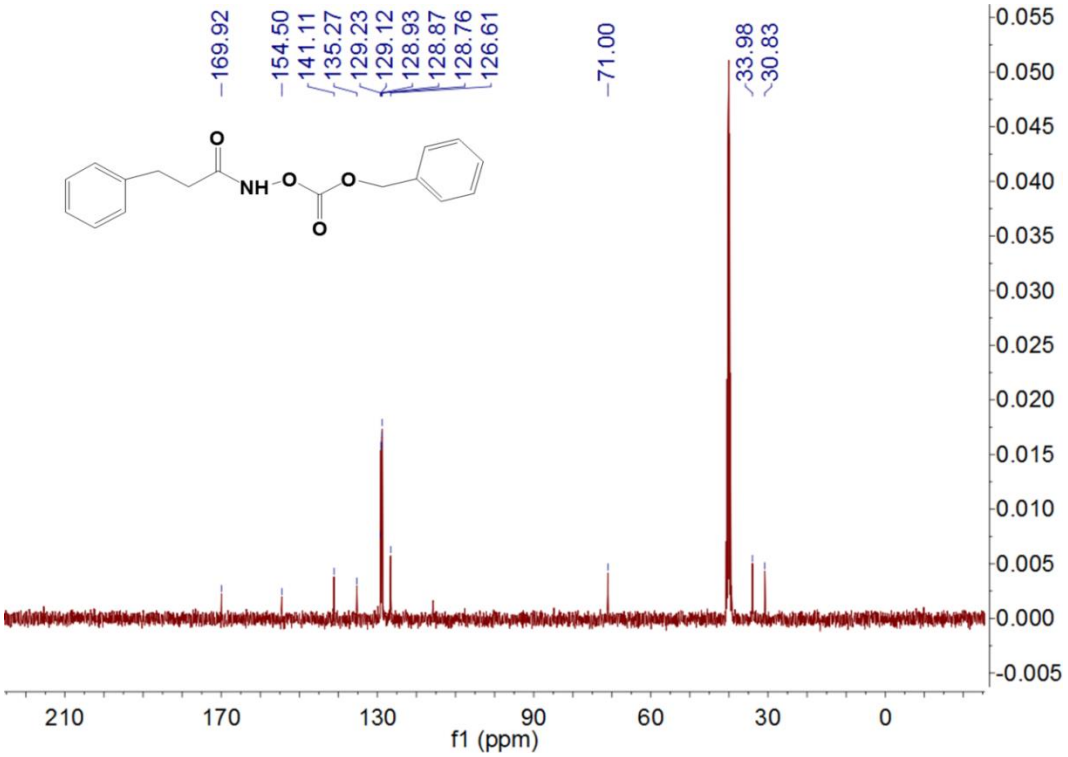
129.17, 128.33, 126.91, 125.87, 119.33, 116.12, 24.19, 14.28. HRMS (ESI) m/z: 300.1157 (Calcd. for [M + H] + 300.1165).

***(E)*-1-(2-hydroxybenzylidene)-4-o-tolyl thiosemicarbazone (2n)**

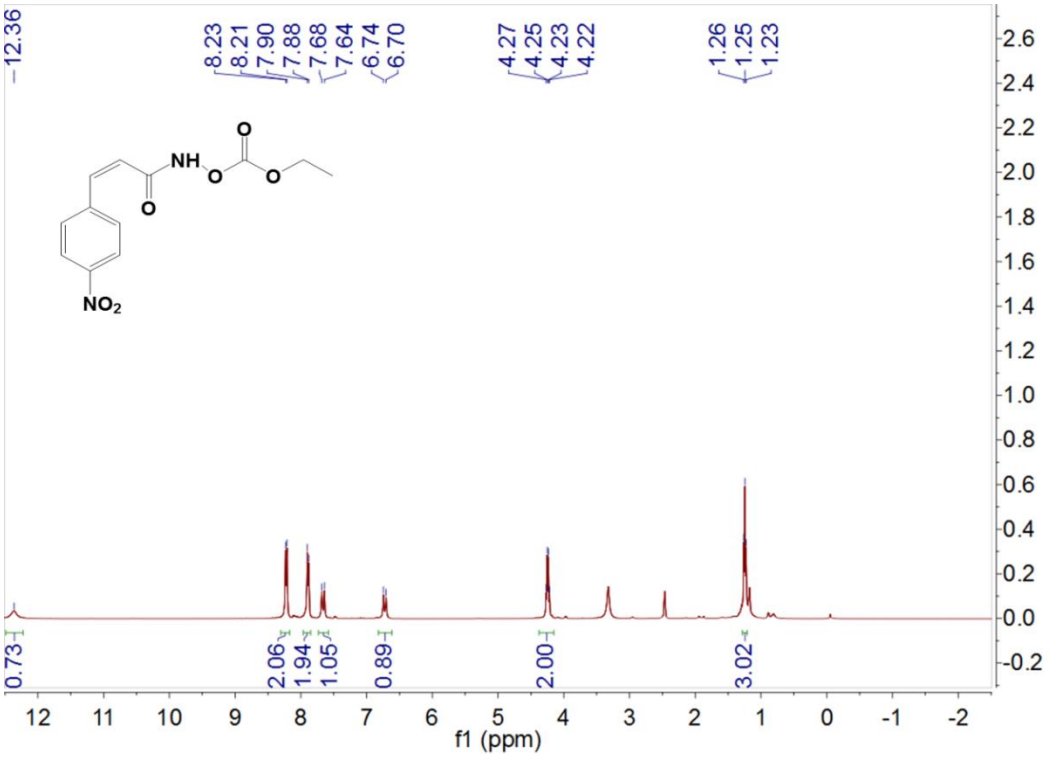
White solid, yield 76%, 1 H NMR (400 MHz, DMSO) δ: 11.73 (s, 1H), 9.90 (s, 2H), 8.48 (s, 1H), 8.08 (d, J = 6.8 Hz, 1H), 7.37–7.09 (m, 5H), 6.94–6.71 (m, 2H), 2.24 (s, 3H). 13C NMR (101 MHz, DMSO) δ: 161.22, 141.18, 122.82, 120.00, 115.85, 114.69, 113.29, 111.71, 111.25, 110.53, 105.05, 103.91, 100.70, 2.50. HRMS (ESI) m/z: 308.0827 (Calcd. for [M + Na] + 308.0828).



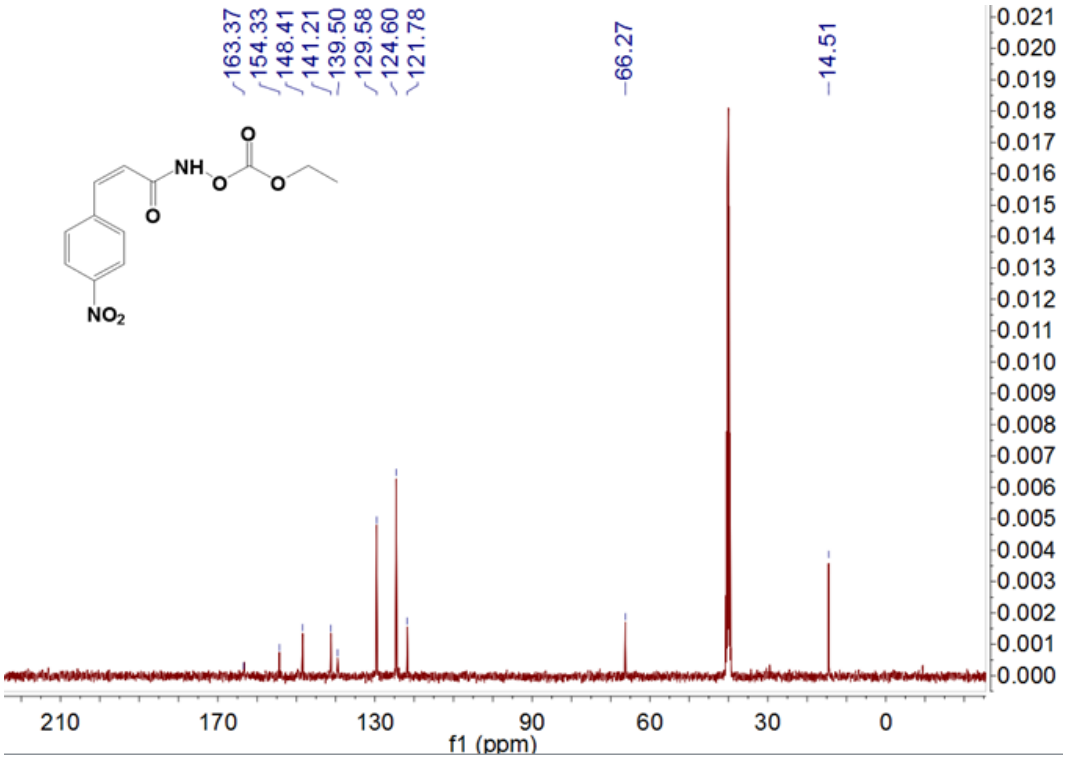
^{1}H NMR spectrum of *N*-(phenethyloxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1a)



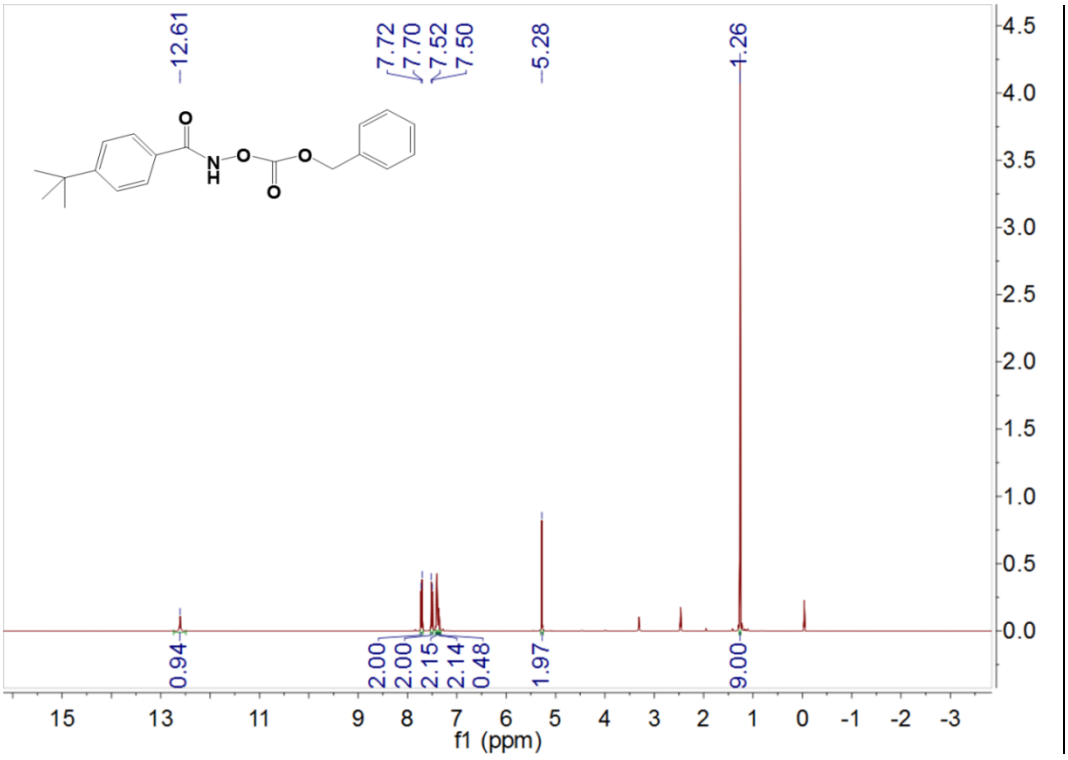
^{13}C NMR spectrum of *N*-(phenethyloxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1a)



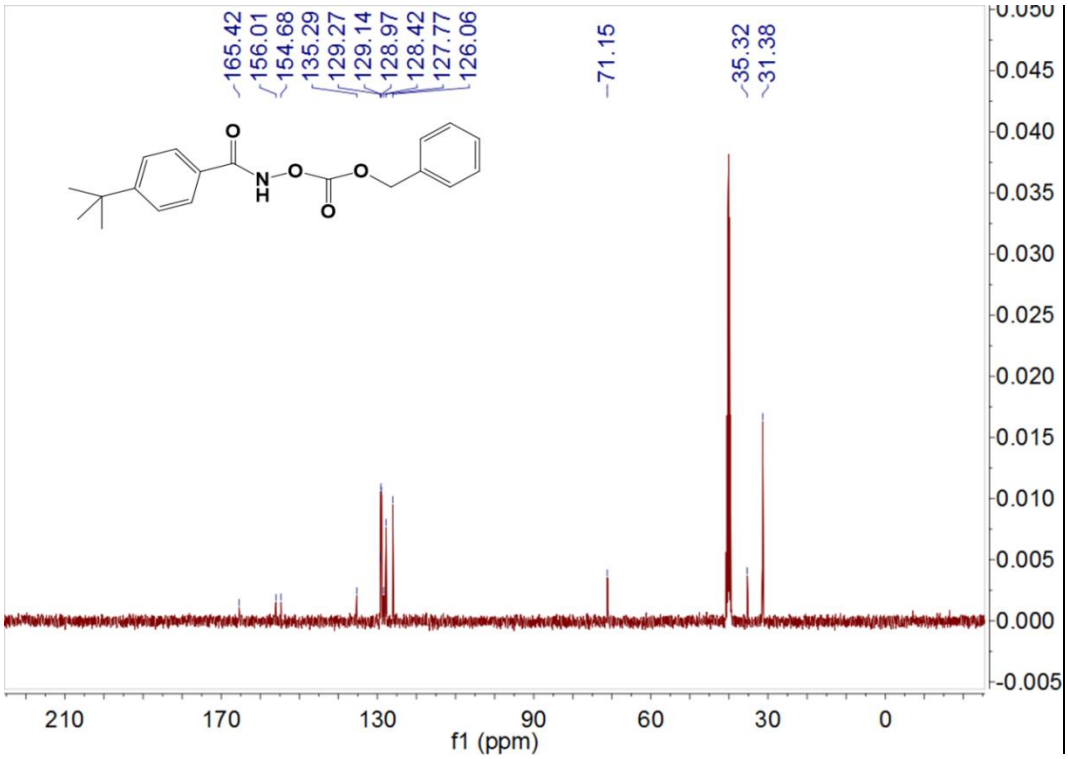
^{1}H NMR spectrum of (Z)- (4-nitrophenylpropenylcarbonyl)-O-(ethyloxycarbonyl) hydroxamate (1b)



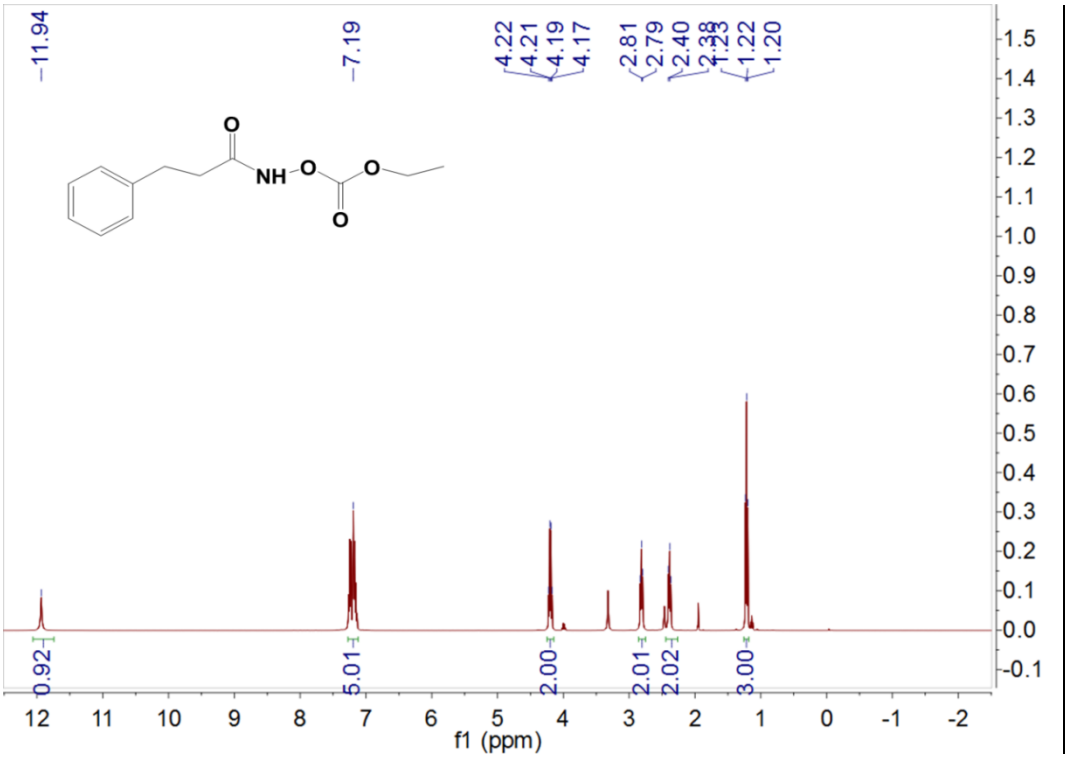
^{13}C NMR spectrum of (Z) - (4-nitrophenylpropenylcarbonyl)-O-(ethyloxycarbonyl) hydroxamate (1b)



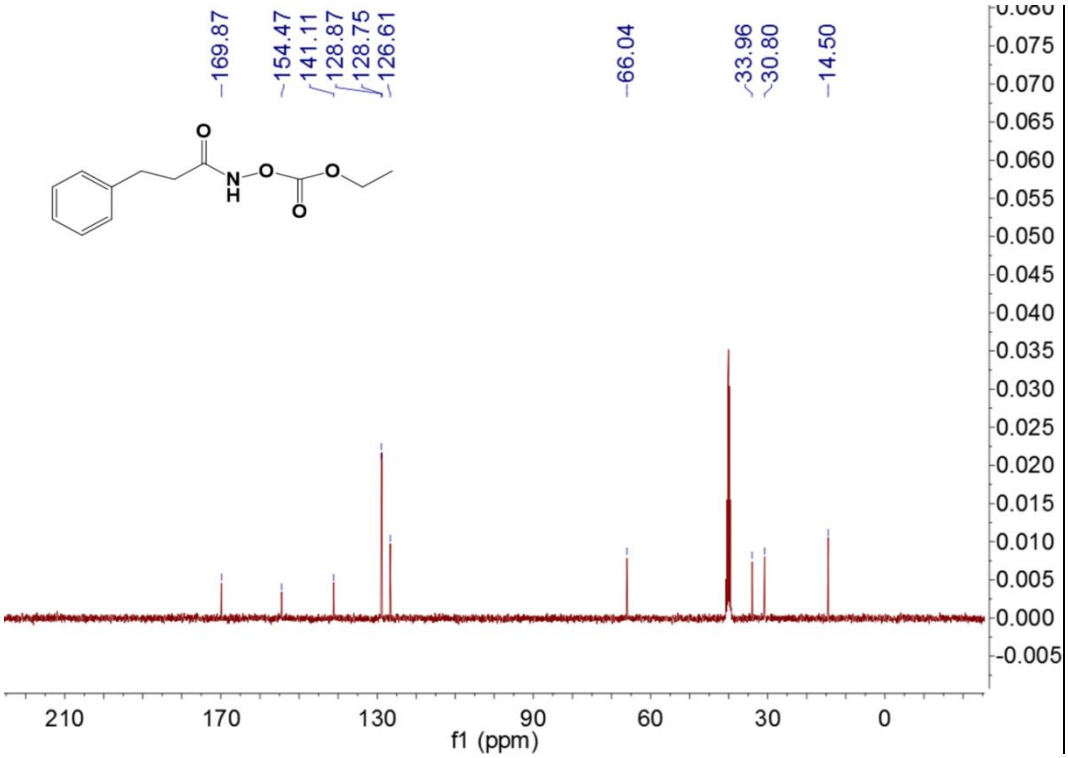
^{1}H NMR spectrum of *N*-(4-tert-butylphenoxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1c)



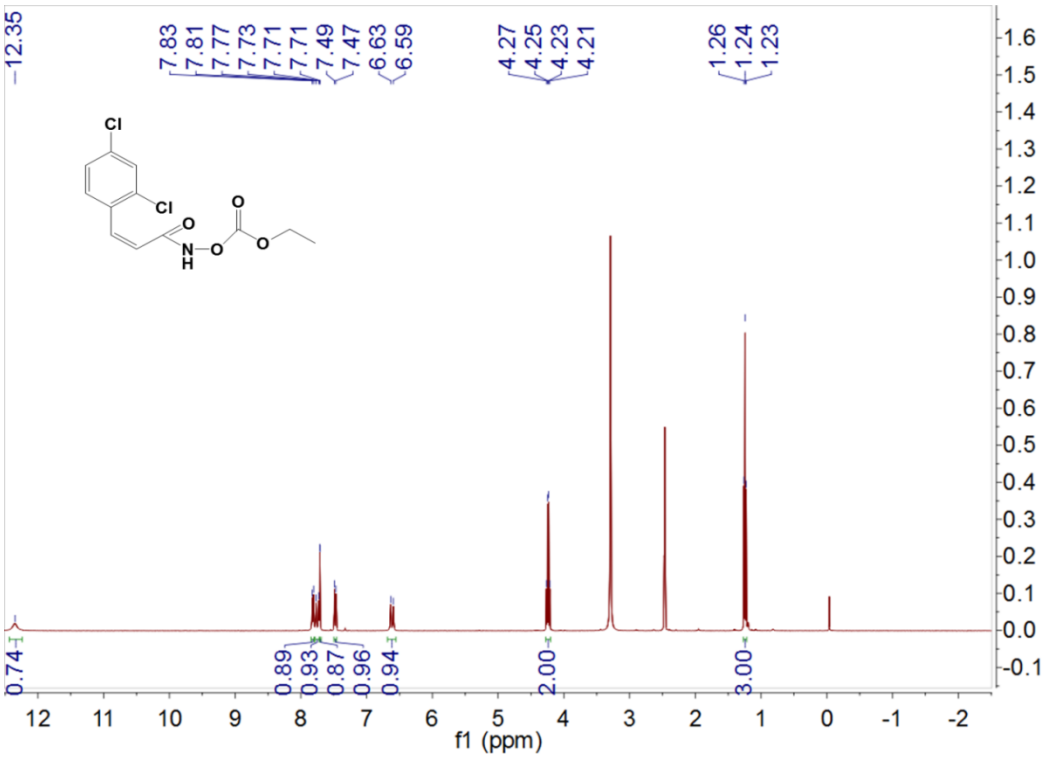
^{13}C NMR spectrum of *N*-(4-tert-butylphenoxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1c)



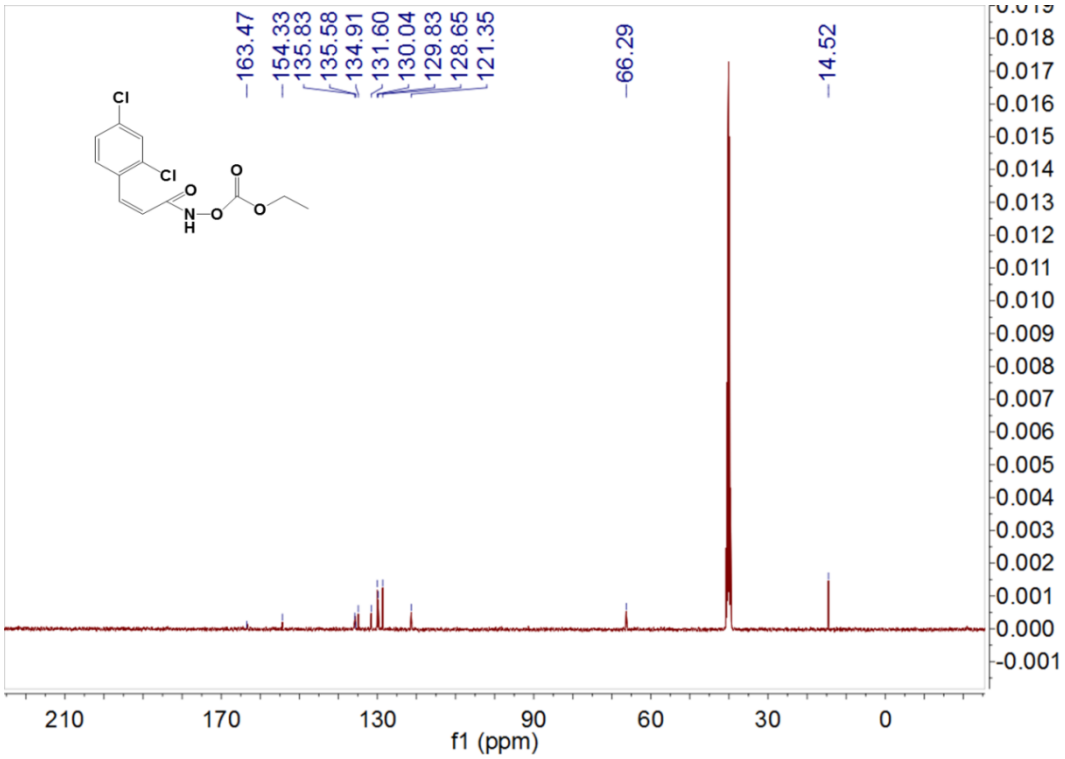
^{1}H NMR spectrum of N-(phenethyloxycarbonyl)-O-(ethylyloxycarbonyl) hydroxamate (1d)



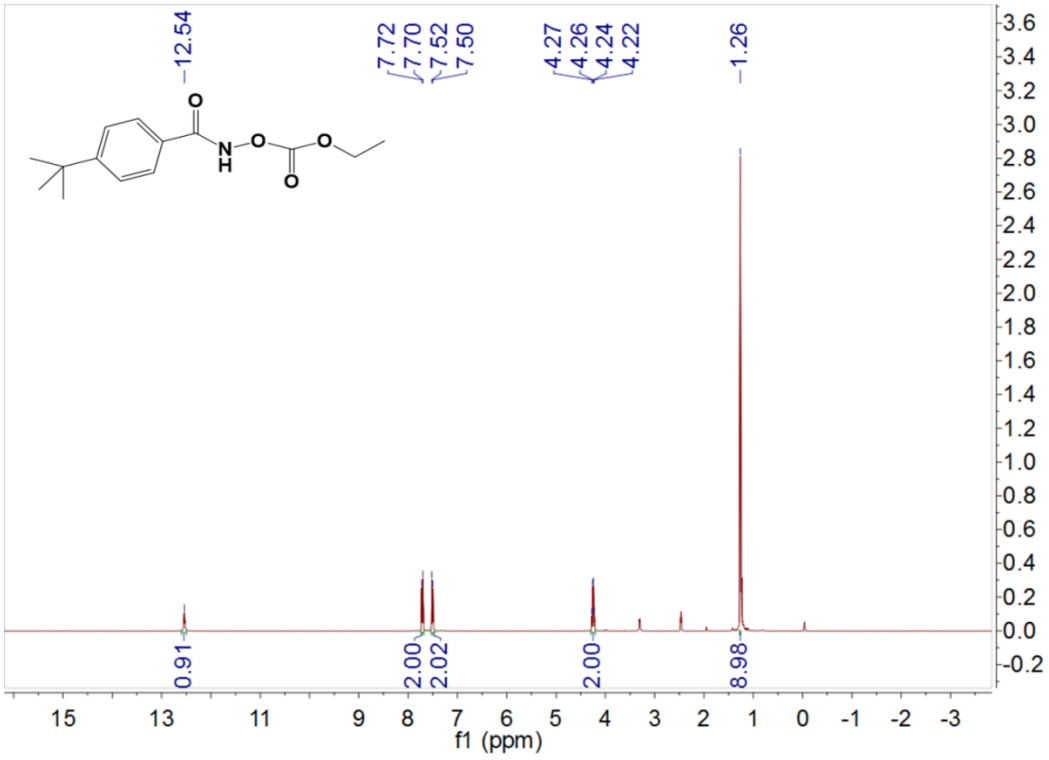
^{13}C NMR spectrum of N-(phenethyloxycarbonyl)-O-(ethylyloxycarbonyl) hydroxamate (1d)



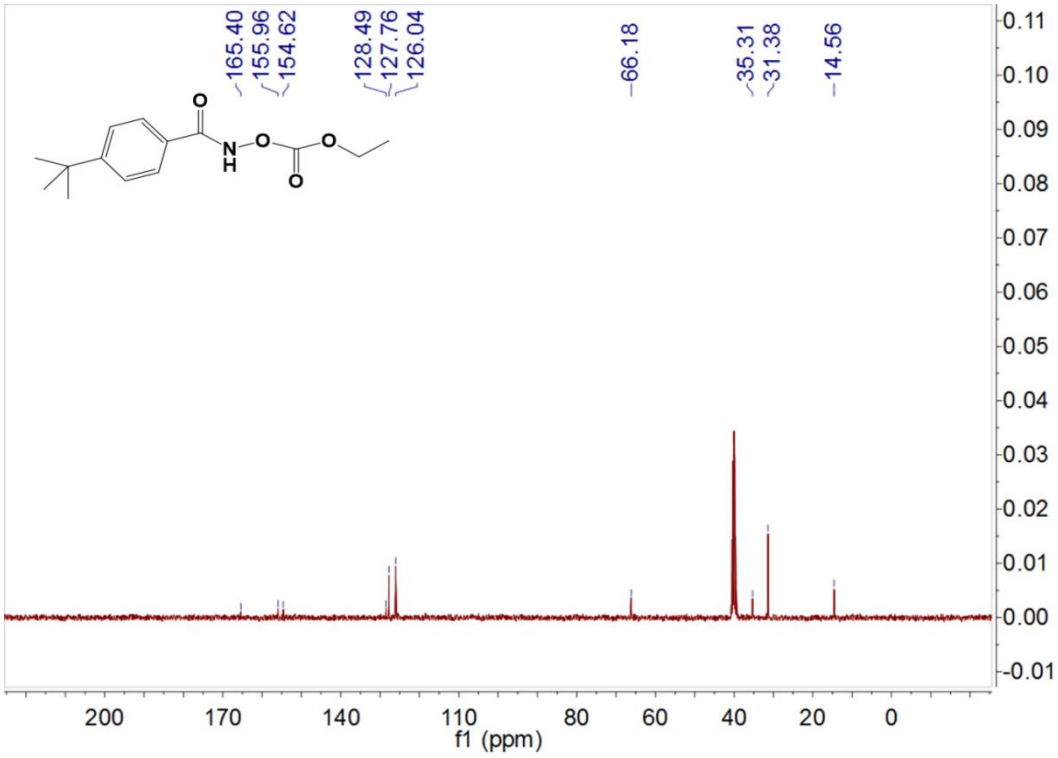
^{1}H NMR spectrum of (Z)-(2,4-dichlorophenylpropenylcarbonyl)-O-(ethyloxycarbonyl) hydroxamate (1e)



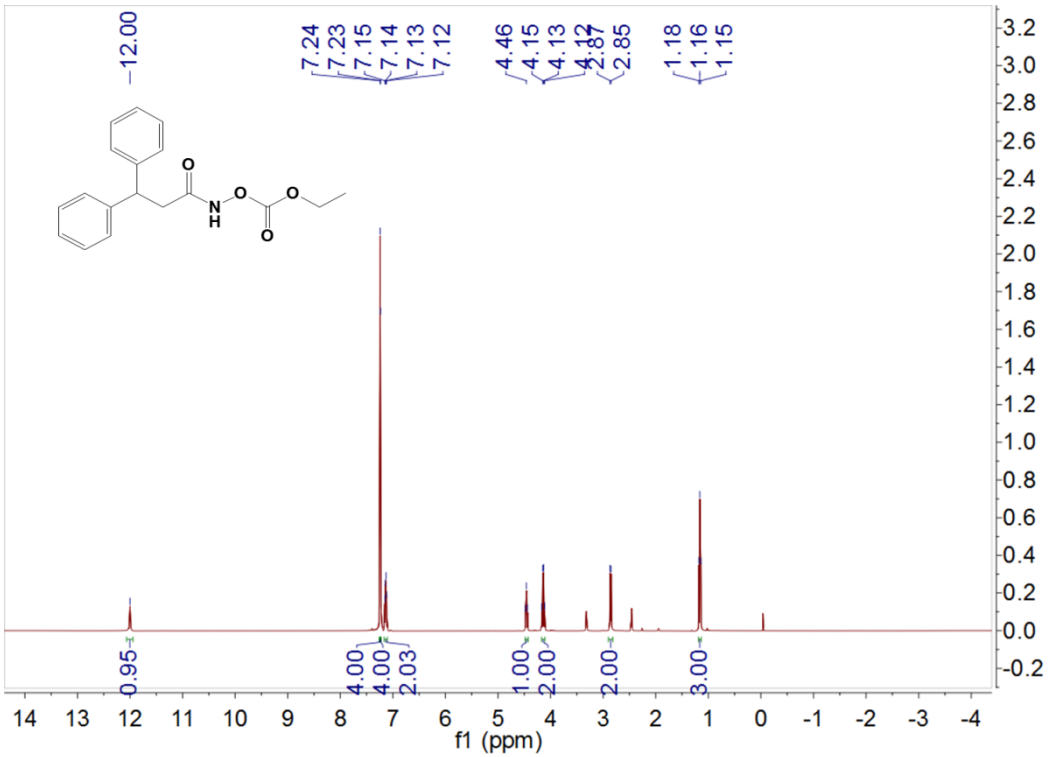
^{13}C NMR spectrum of (Z)-(2, 4-dichlorophenylpropenylcarbonyl)-O-(ethyloxycarbonyl) hydroxylamine (1e)



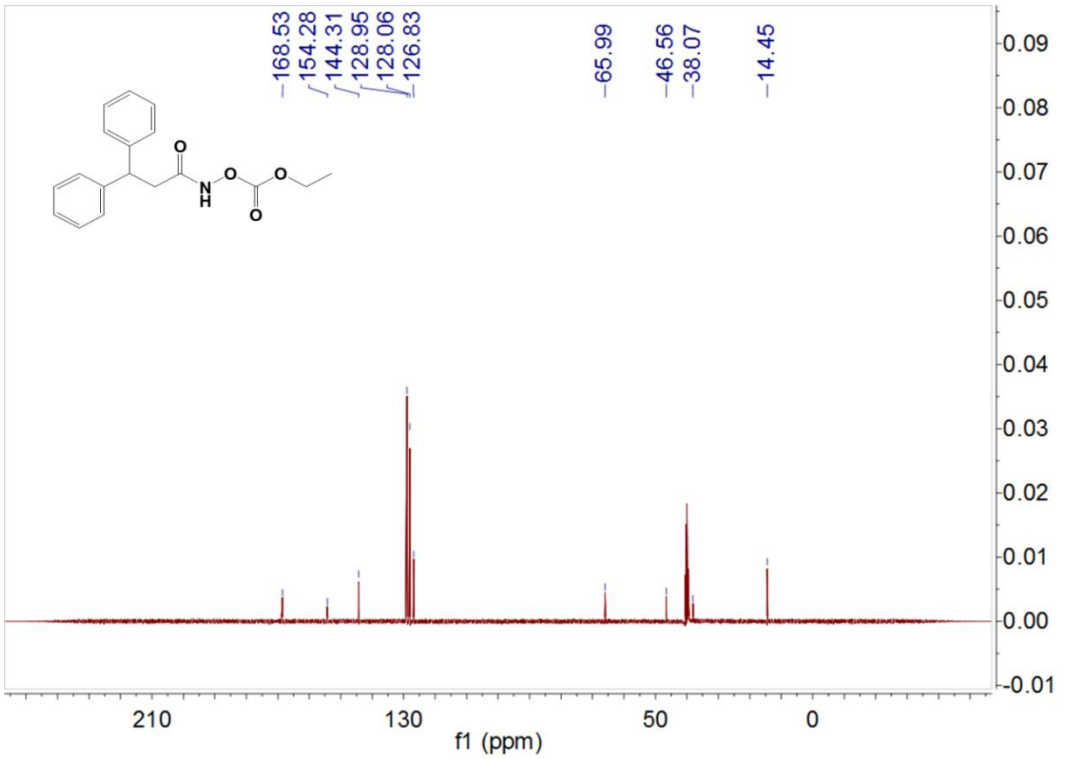
^{1}H NMR spectrum of N-(4-tert-butylphenoxycarbonyl)-O-(ethylyloxycarbonyl) hydroxamate (1f)



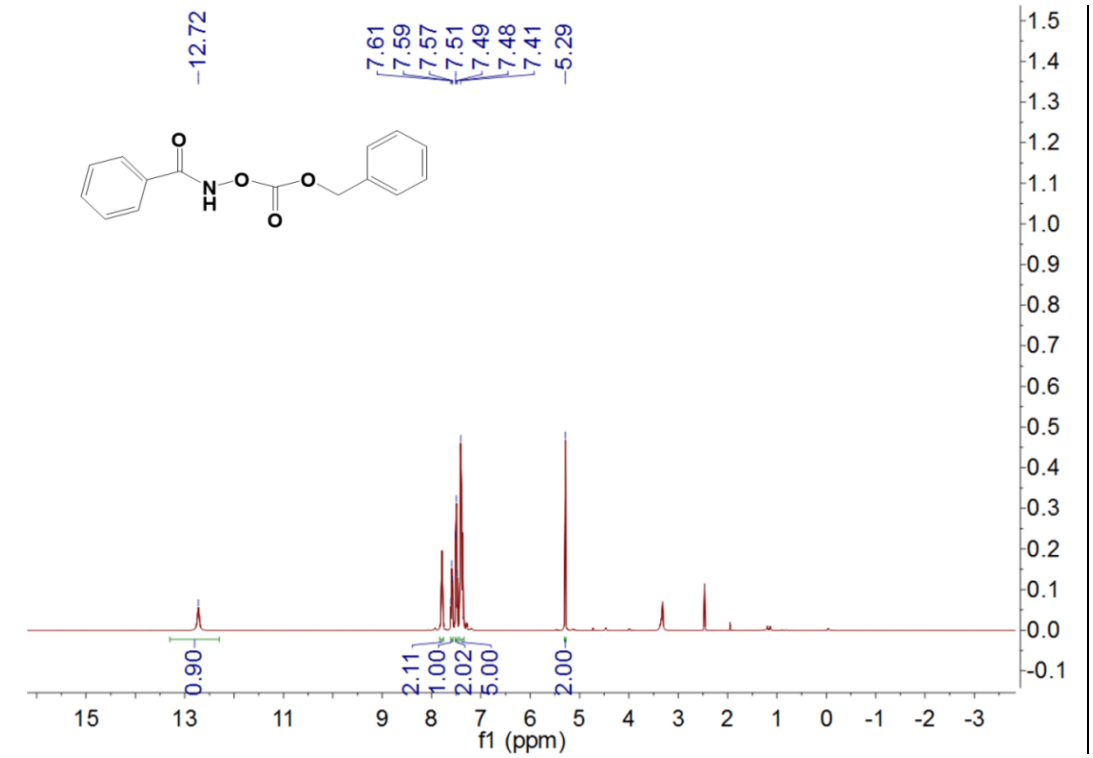
^{13}C NMR spectrum of N-(4-tert-butylphenoxycarbonyl)-O-(ethylyloxycarbonyl) hydroxamate (1f)



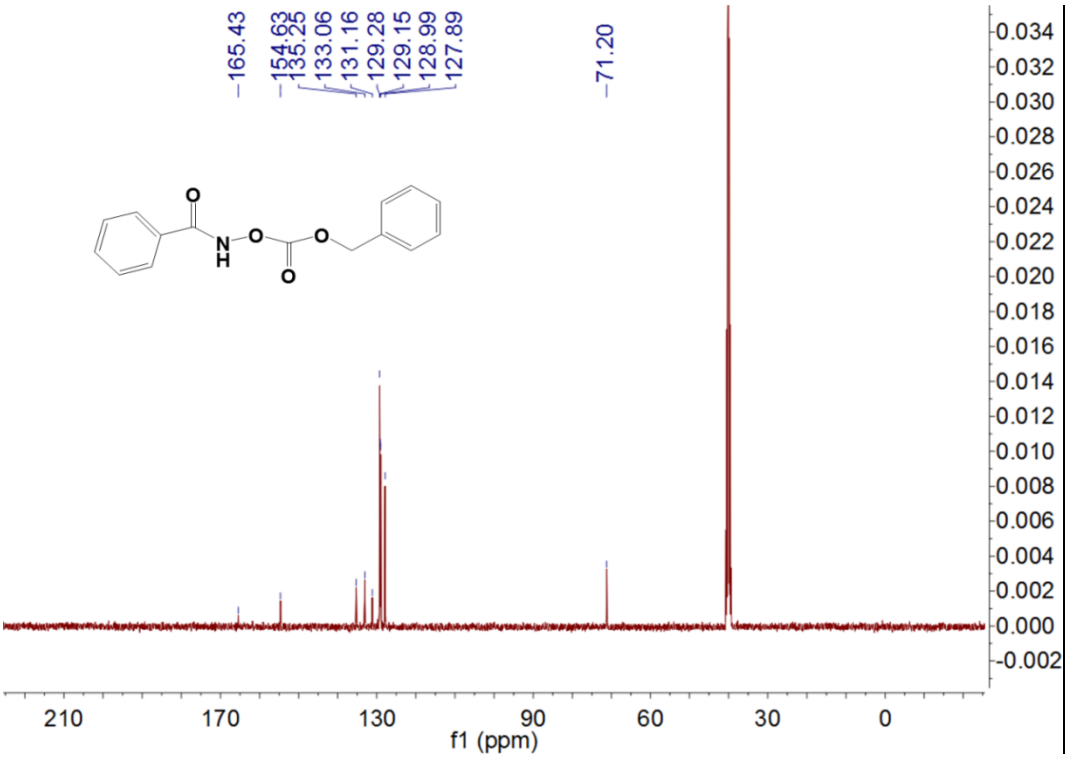
^{1}H NMR spectrum of N-(2, 2- diphenylethylcarbonyl)-O-(ethylyloxycarbonyl) hydroxamate(1g)



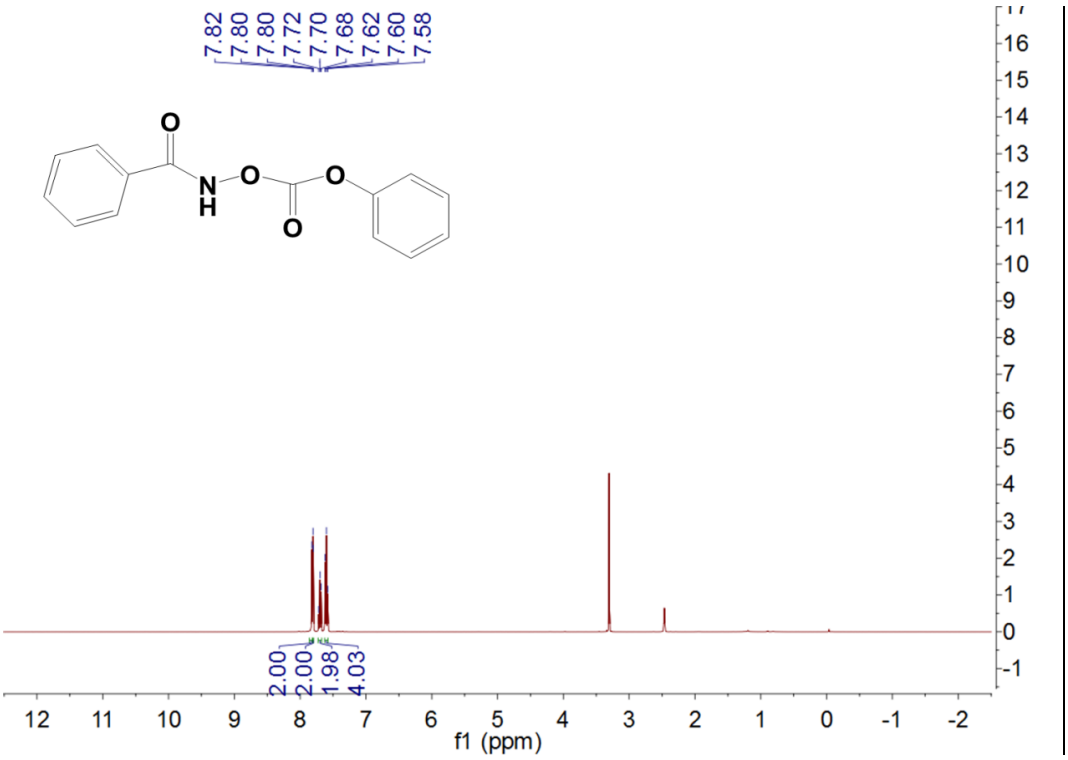
^{13}C NMR spectrum of N-(2, 2- diphenylethylcarbonyl)-O-(ethylyloxycarbonyl)hydroxamate(1g)



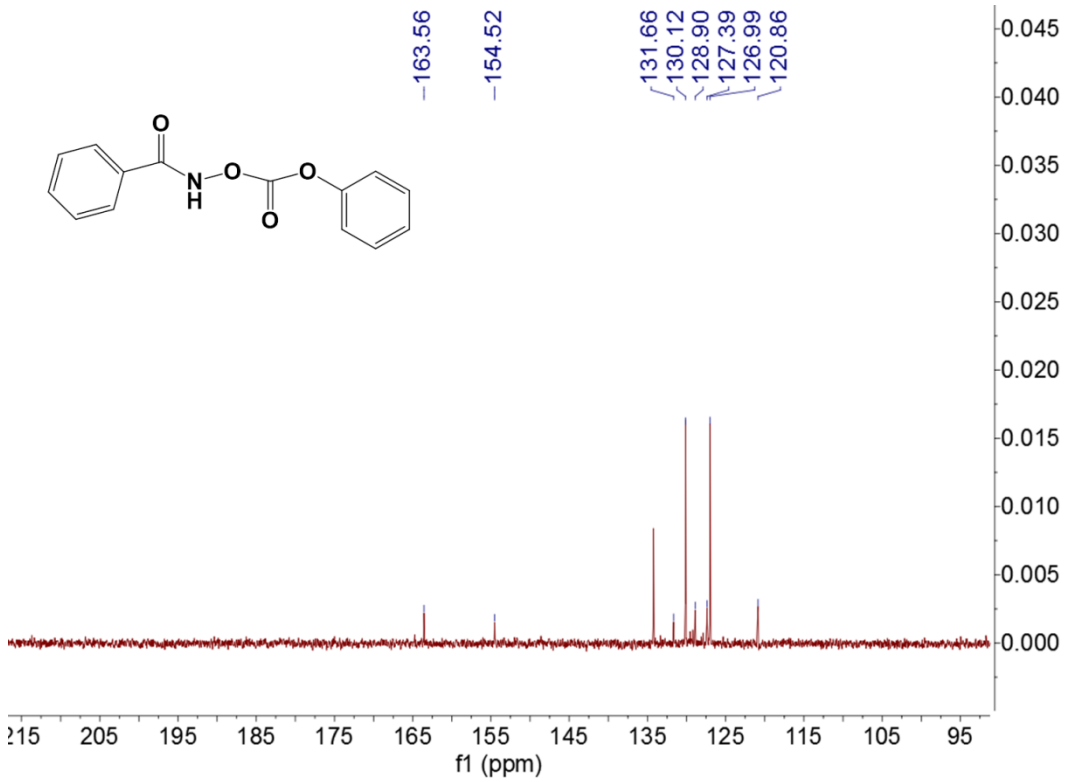
^{1}H NMR spectrum of *N*-(phenoxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1h)



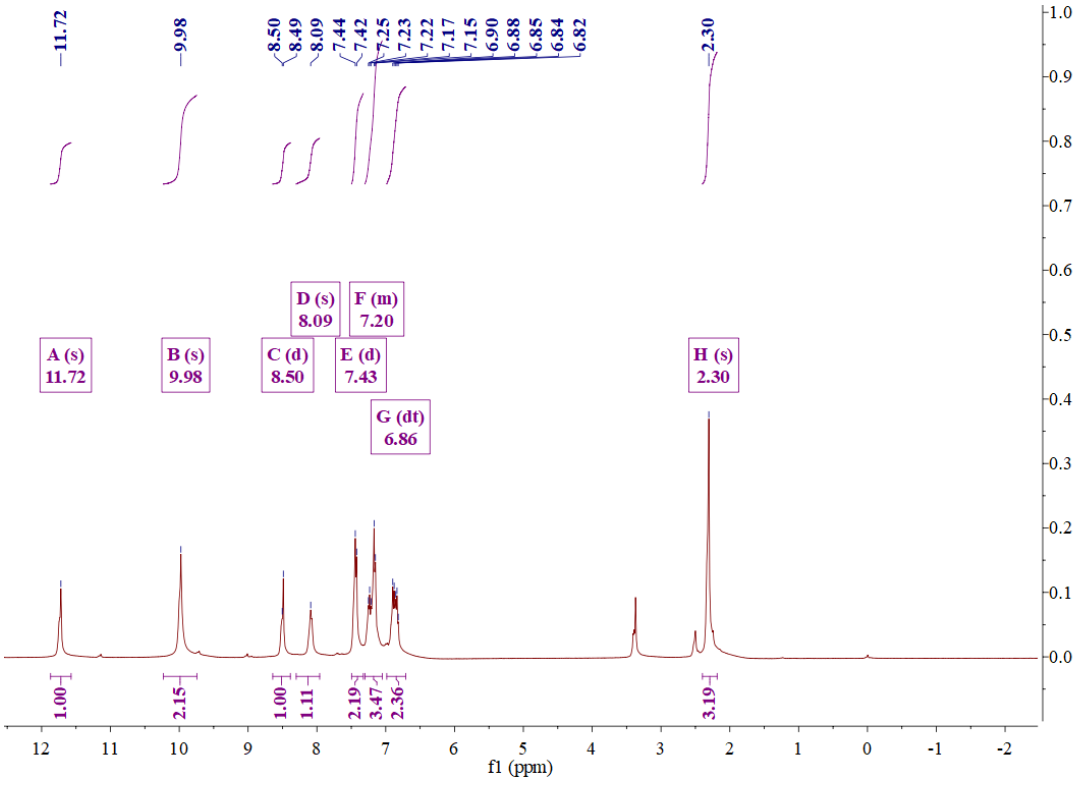
^{13}C NMR spectrum of *N*-(phenoxycarbonyl)-*O*-(benzyloxycarbonyl) hydroxamate (1h)



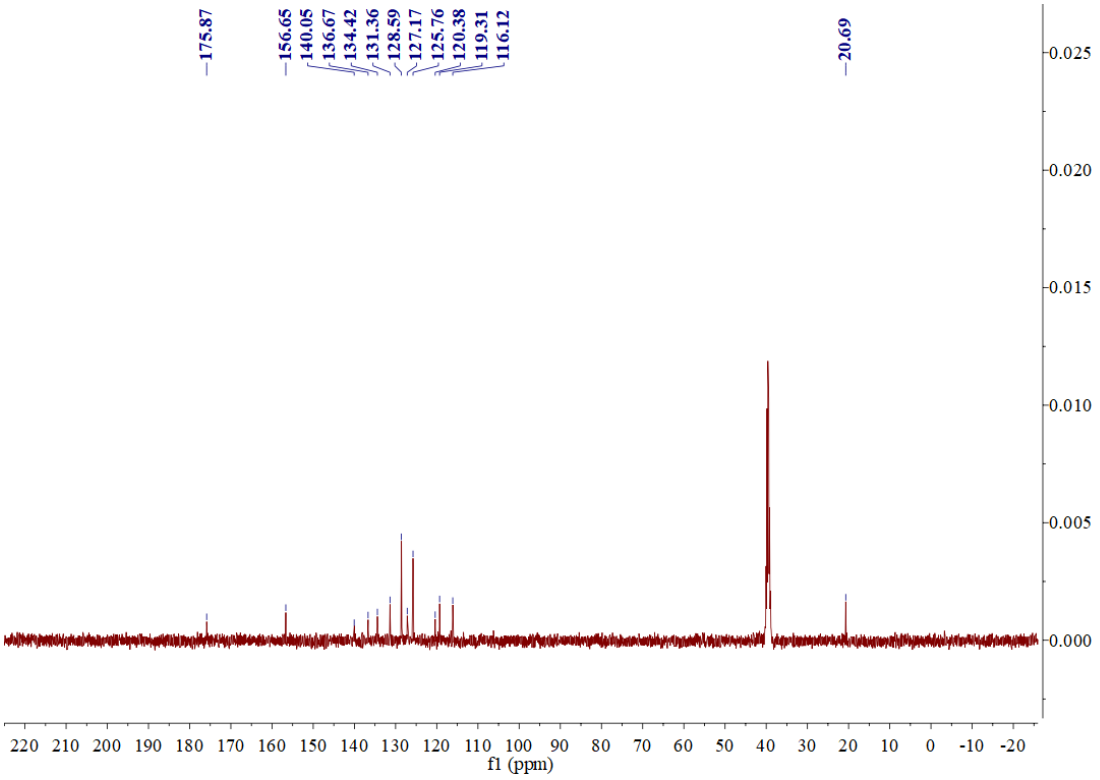
^{1}H NMR spectrum of N-(phenoxycarbonyl)-O-(phenoxycarbonyl) hydroxamate (1i)



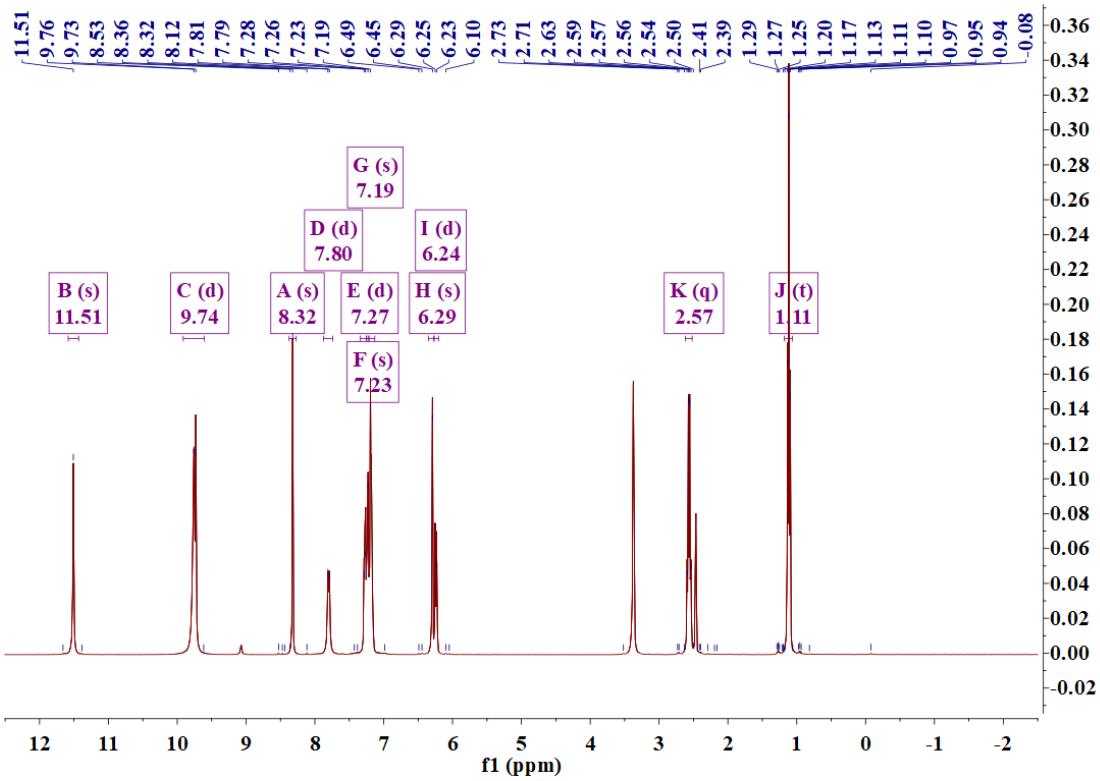
^{13}C NMR spectrum of N- (phenoxycarbonyl)-O-(phenoxycarbonyl) hydroxamate (1i)



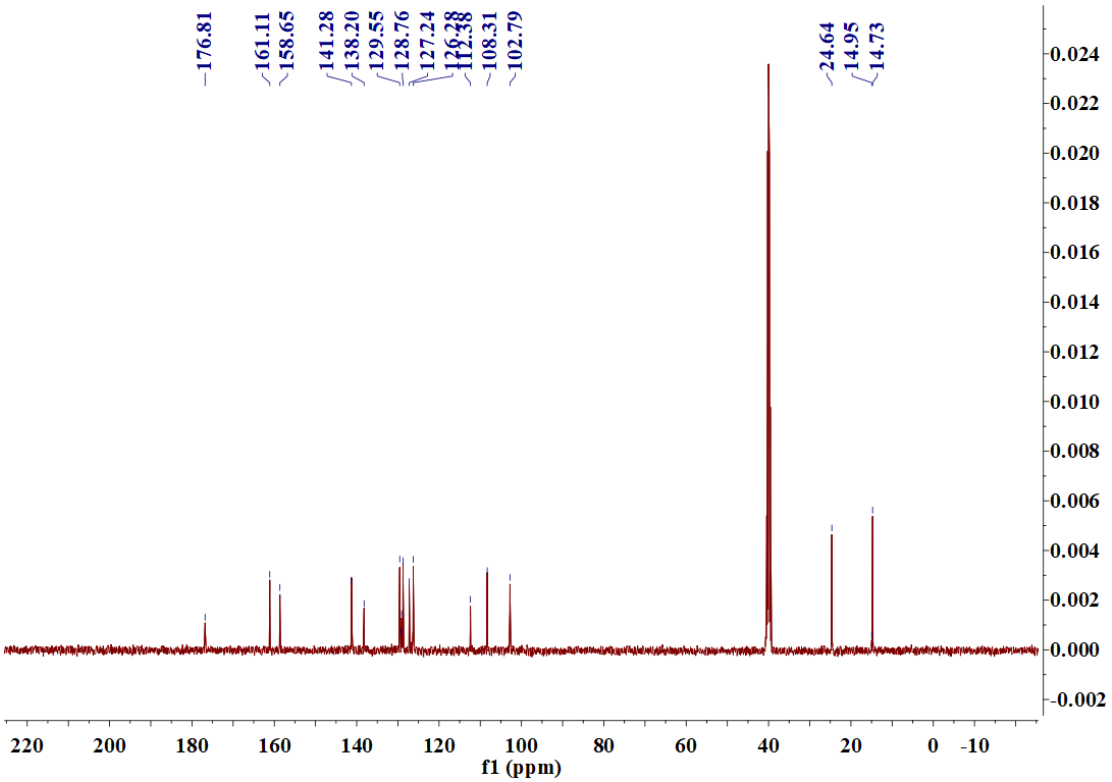
^{1}H NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-p-tolythiosemicarbazones (2a)



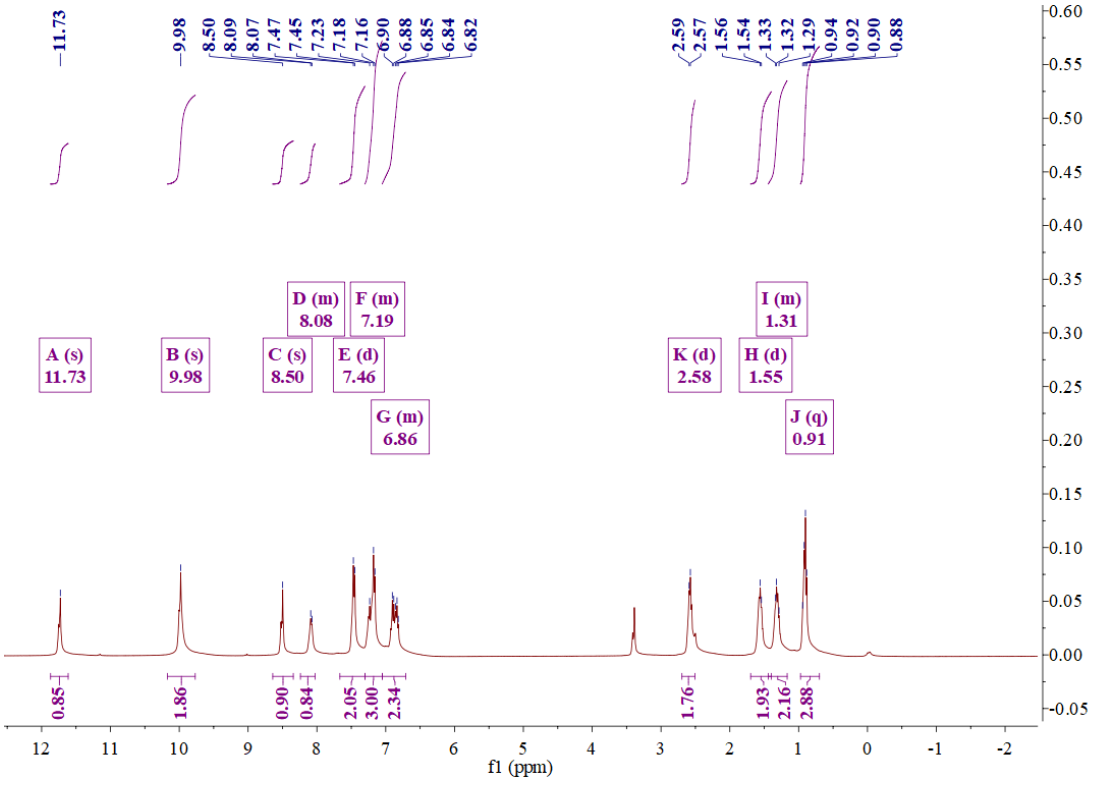
^{13}C NMR spectrum of (*E*)-1-(2-hydroxybenzylidene)-4-p-tolythiosemicarbazone (2a)



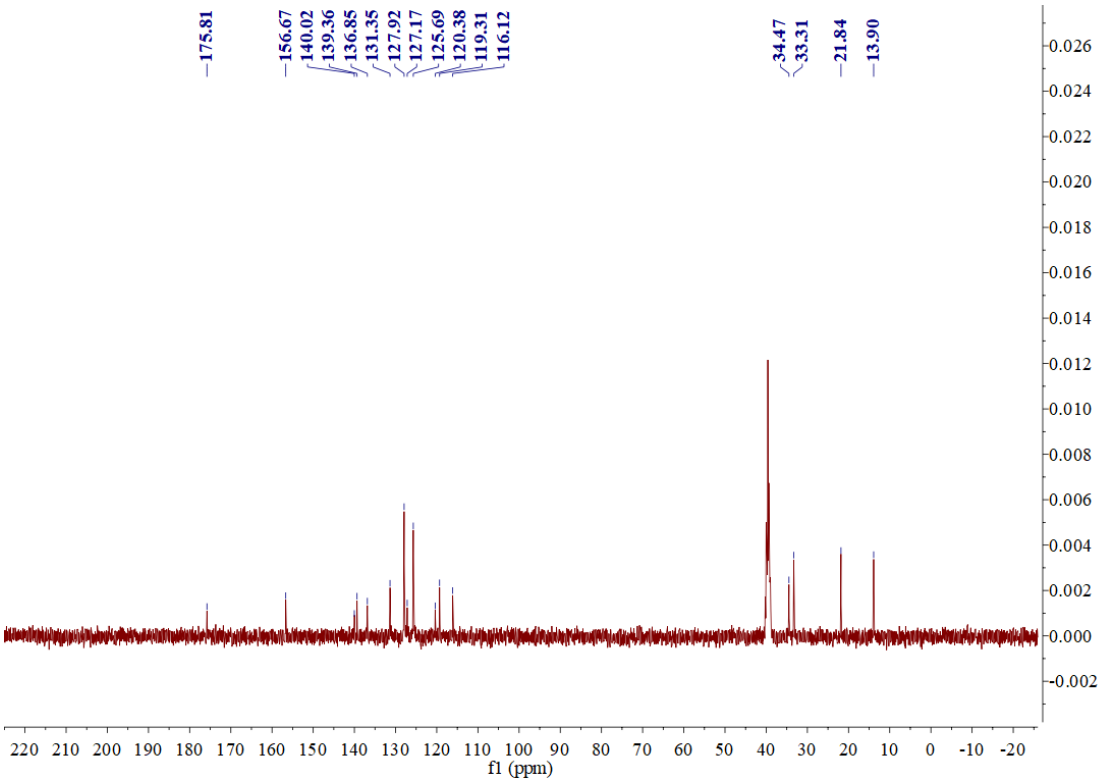
^{1}H NMR spectrum of (E)-2-(2, 4-dihydroxybenzylidene)-*N*-(2-ethylphenyl) hydrazine-1-carbothiosemicarbazones (2b)



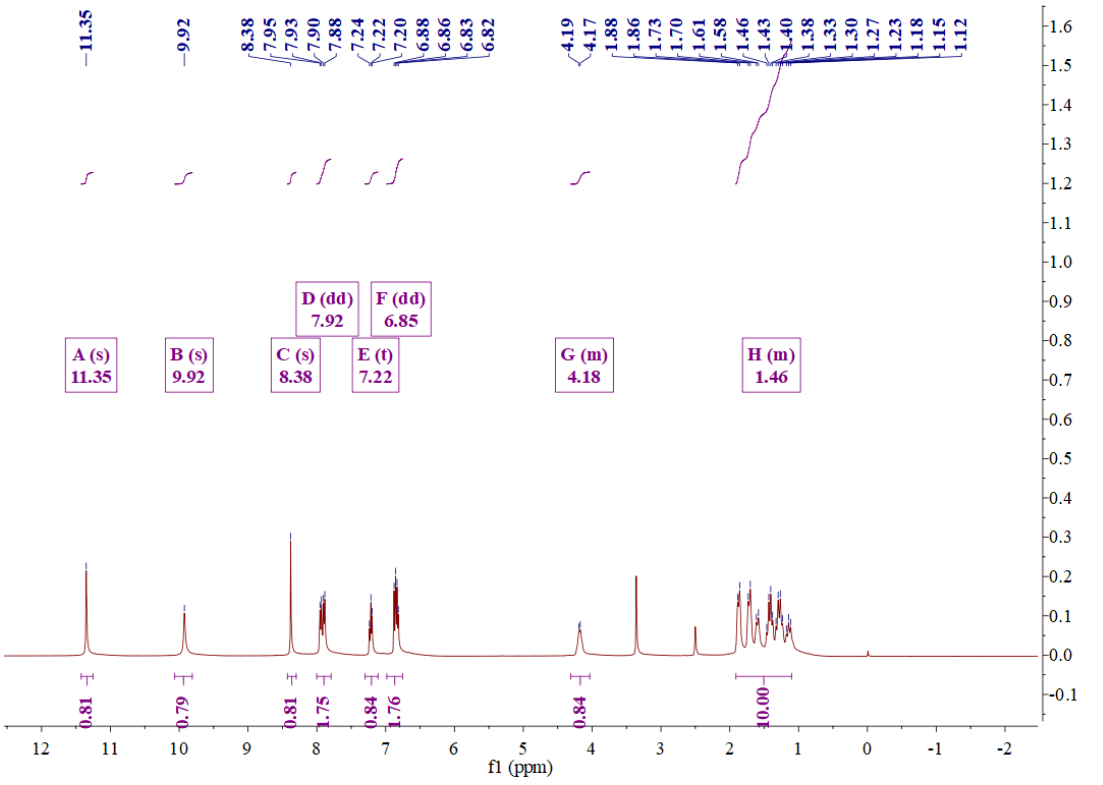
^{13}C NMR spectrum of (E)-2-(2, 4-dihydroxybenzylidene)-*N*-(2-ethylphenyl) hydrazine-1-carbothiosemicarbazones (2b)



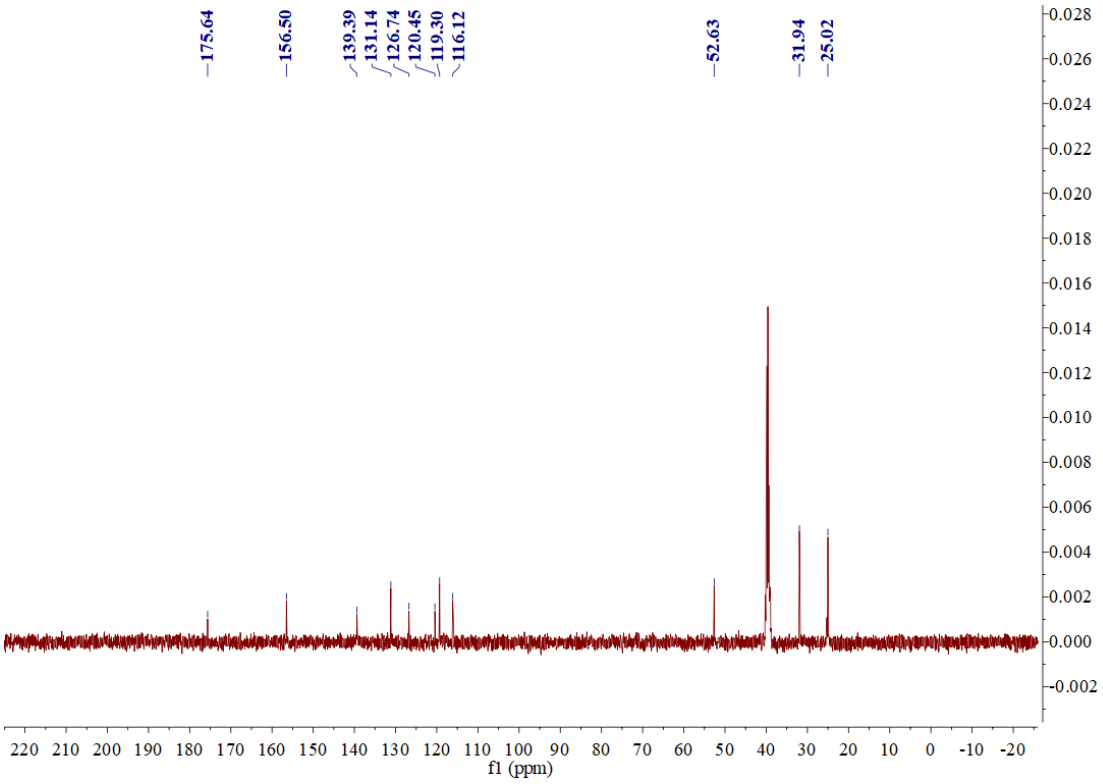
^{1}H NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-(4-butylphenyl) thiosemicarbazone (2c)



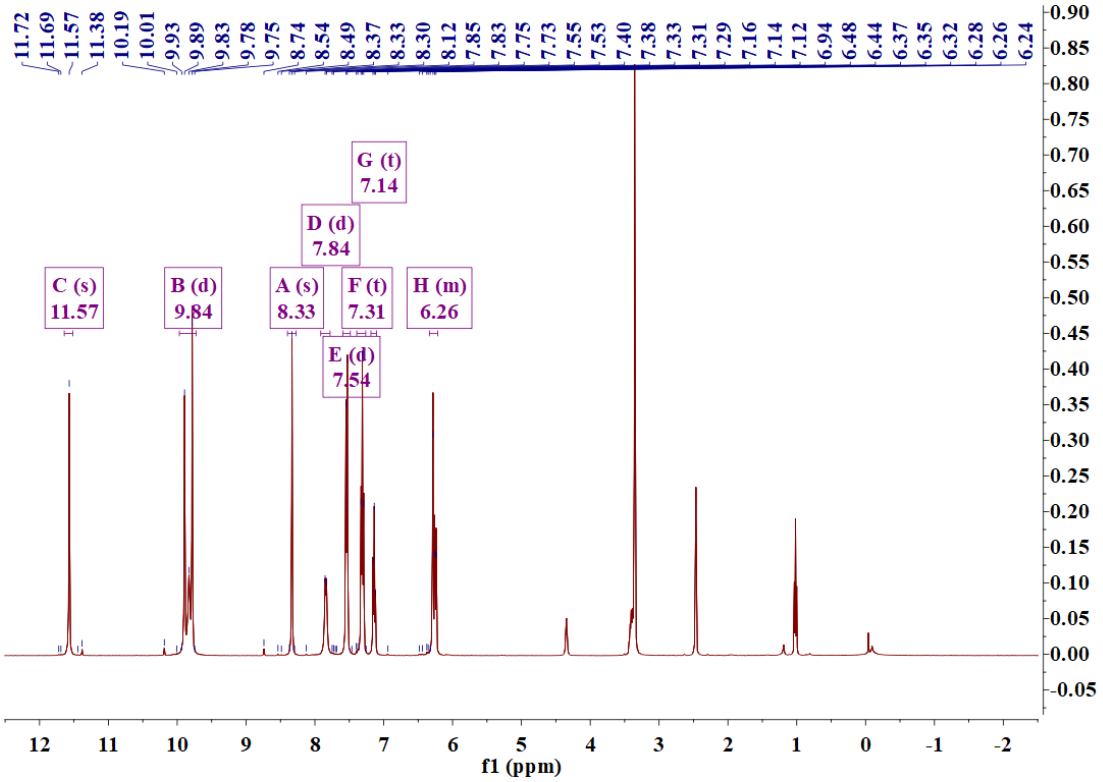
^{13}C NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-(4-butylphenyl) thiosemicarbazone (2c)



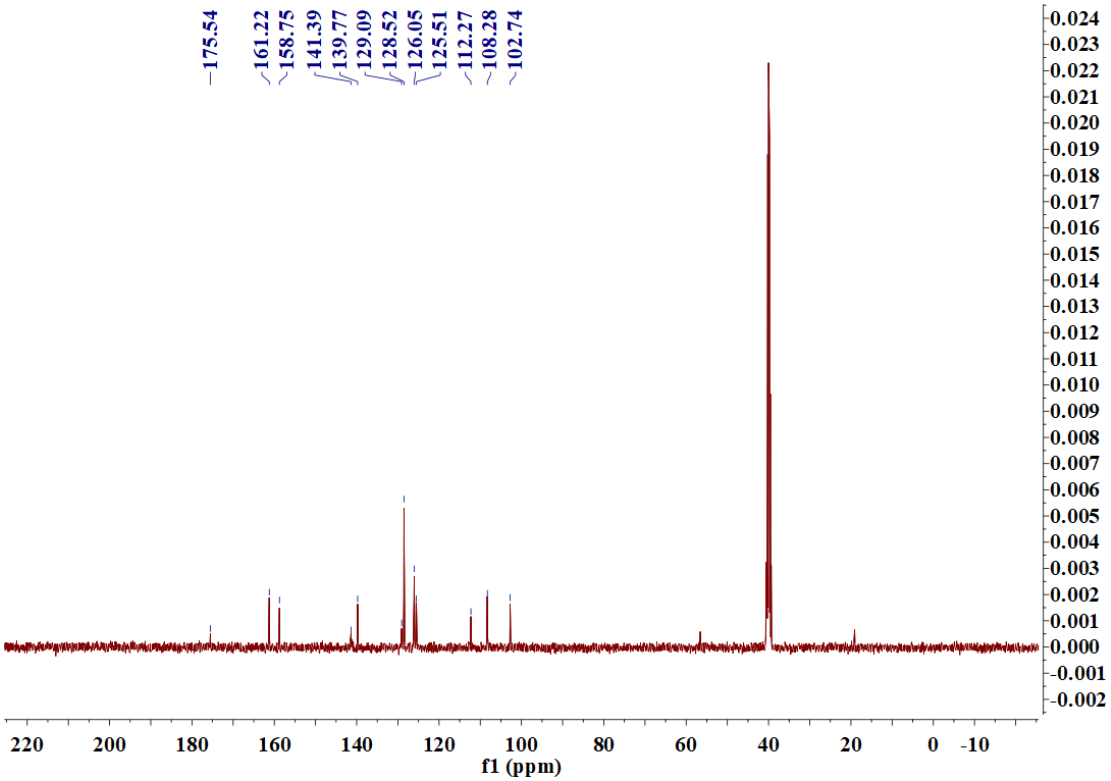
^{1}H NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-cyclohexylthiosemicarbazone (2d)



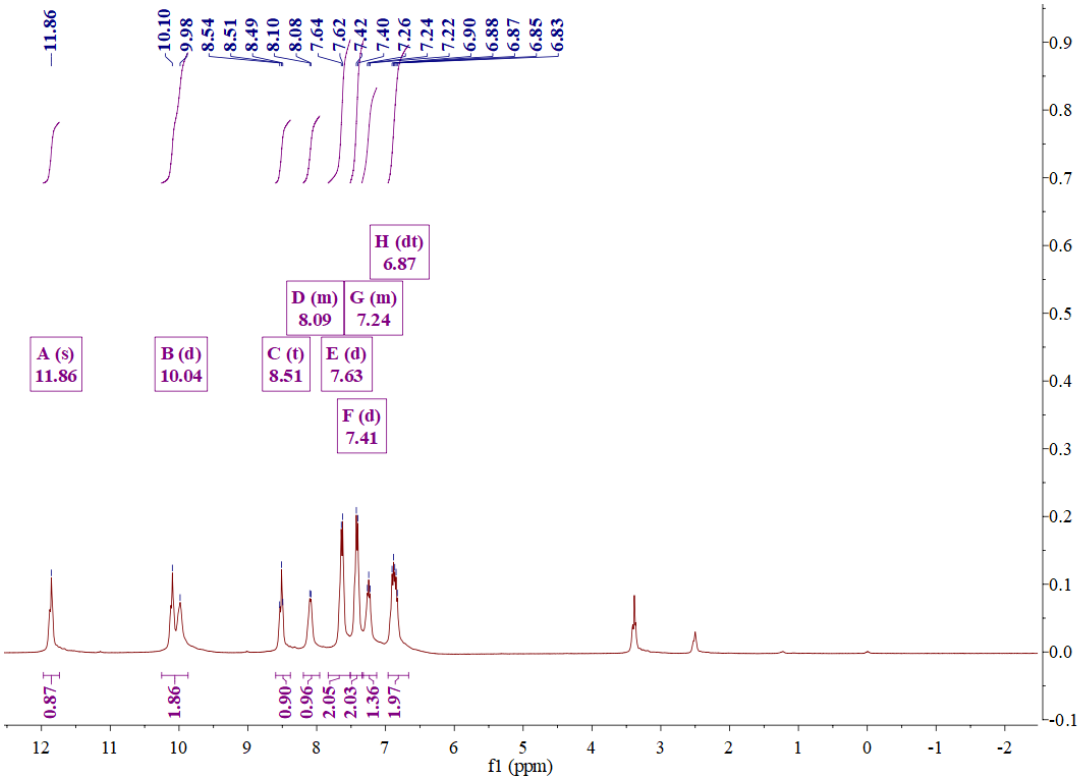
^{13}C NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-cyclohexyl thiosemicarbazone (2d)



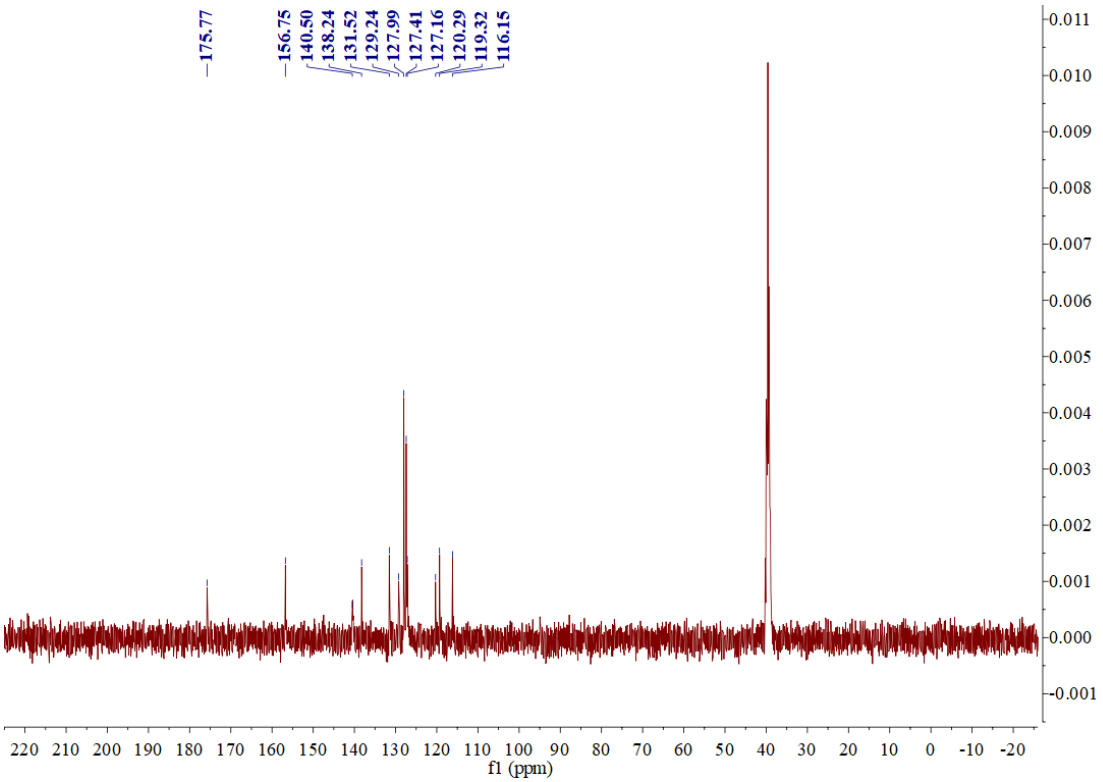
1H NMR spectrum of (E)-2-(2, 4-dihydroxybenzylidene)-N-phenylhydrazine-1-carbothiosemicarbazones (2e)



13C NMR spectrum of (E)-2-(2, 4-dihydroxybenzylidene)-N-phenylhydrazine-1-carbothiosemicarbazones (2e)



[1]H NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-(4-chlorophenyl) thiosemicarbazone (2f)



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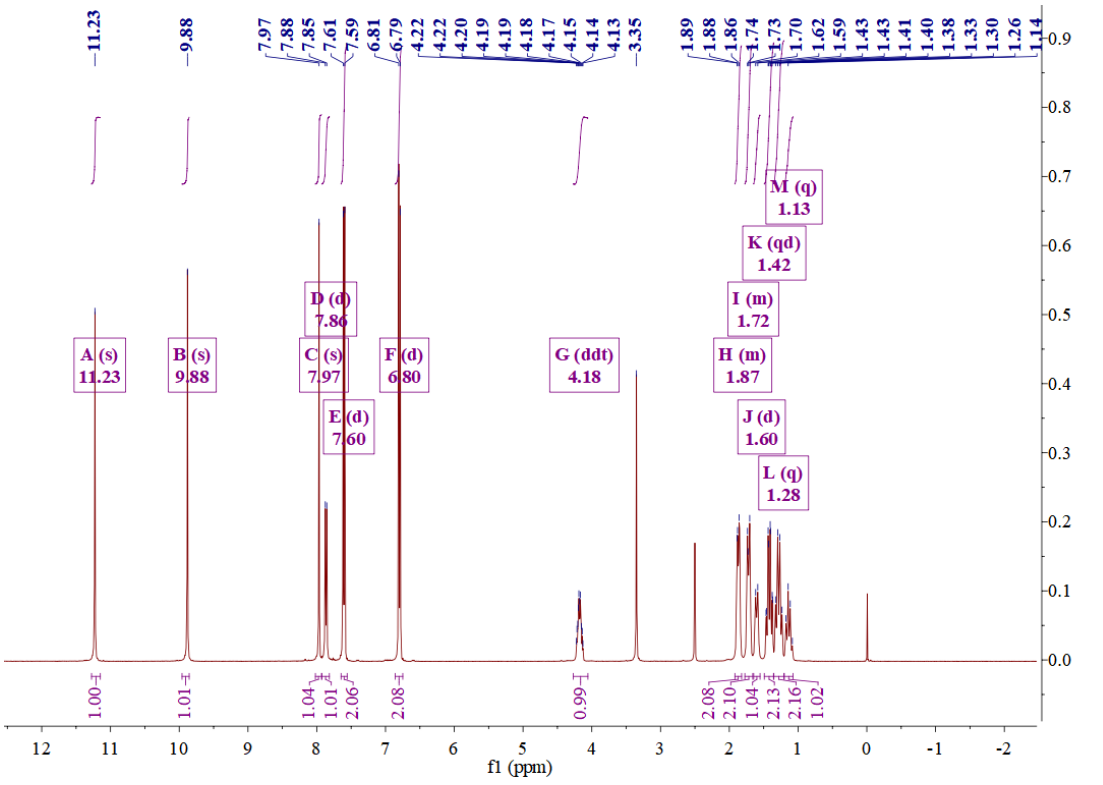

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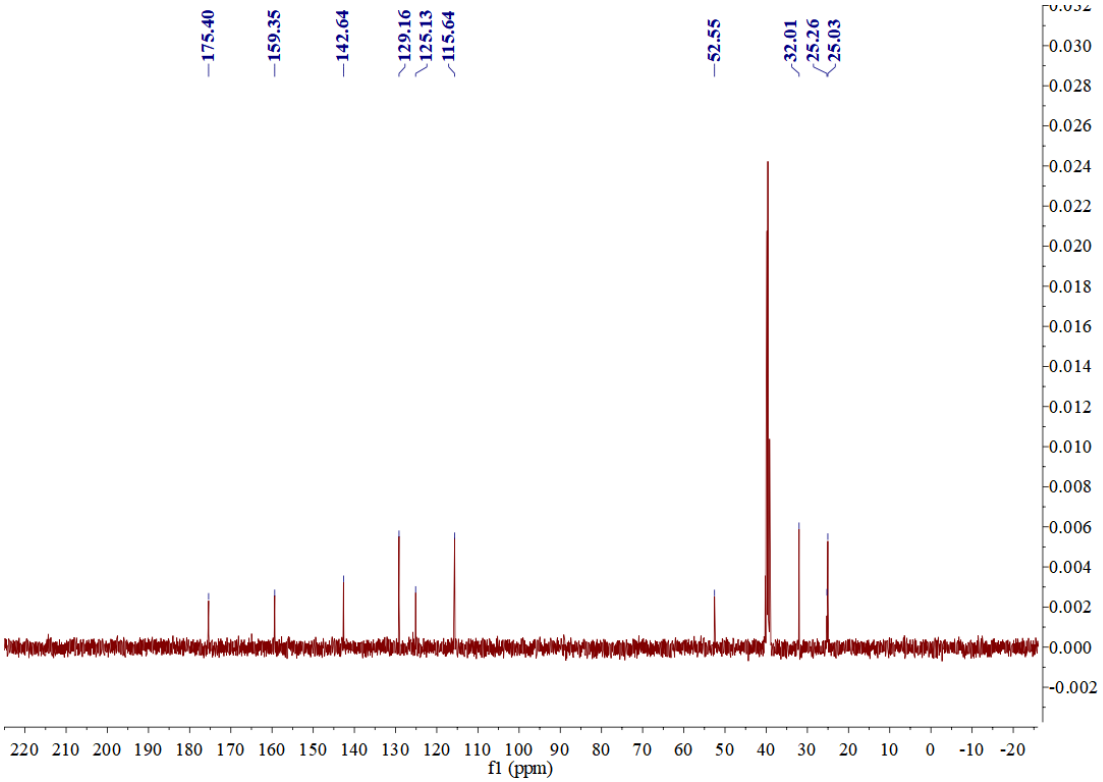

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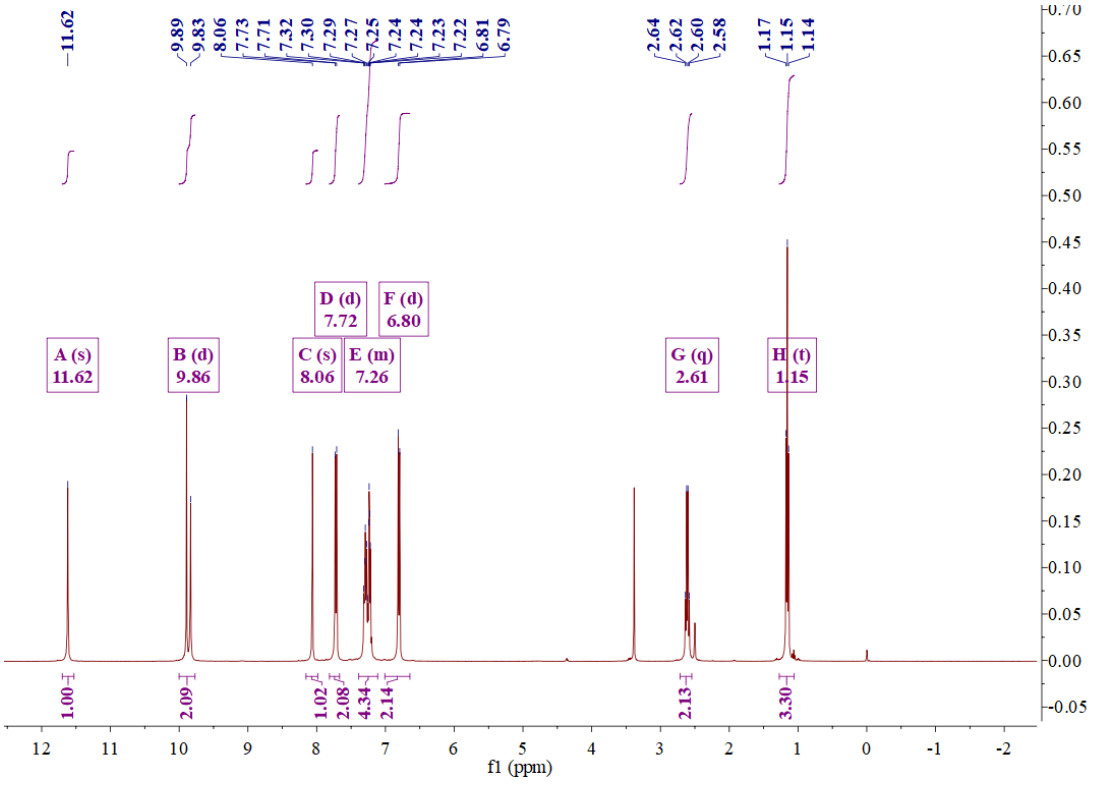

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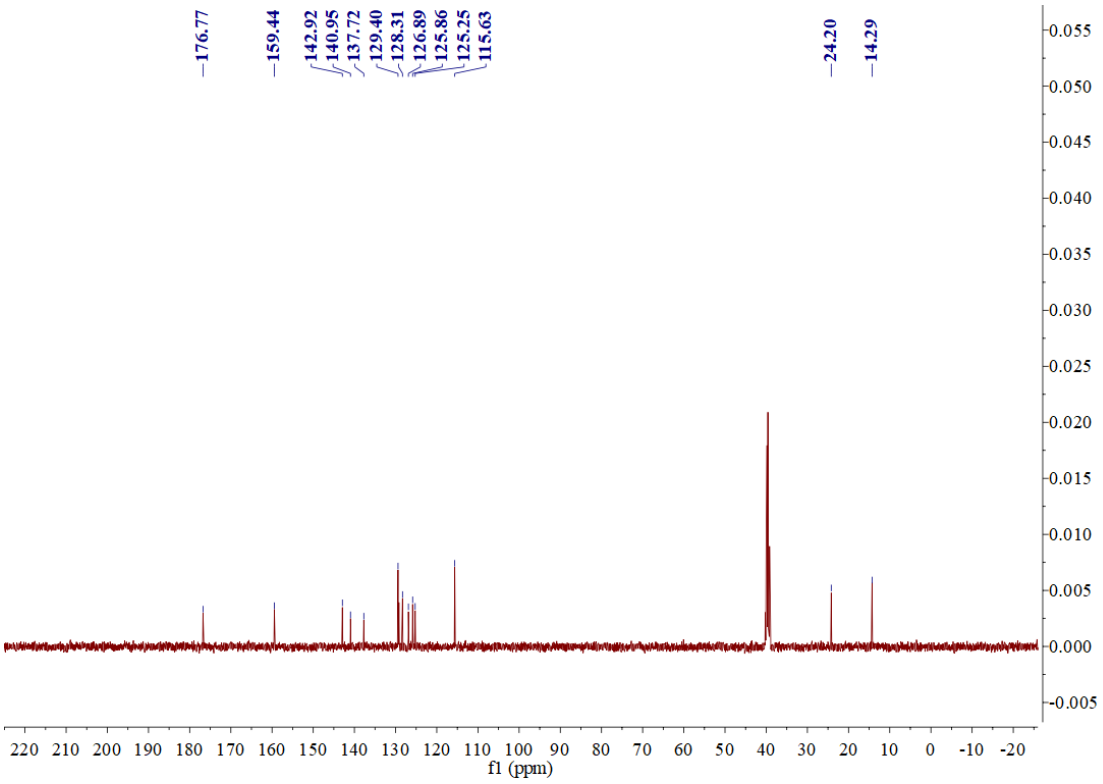

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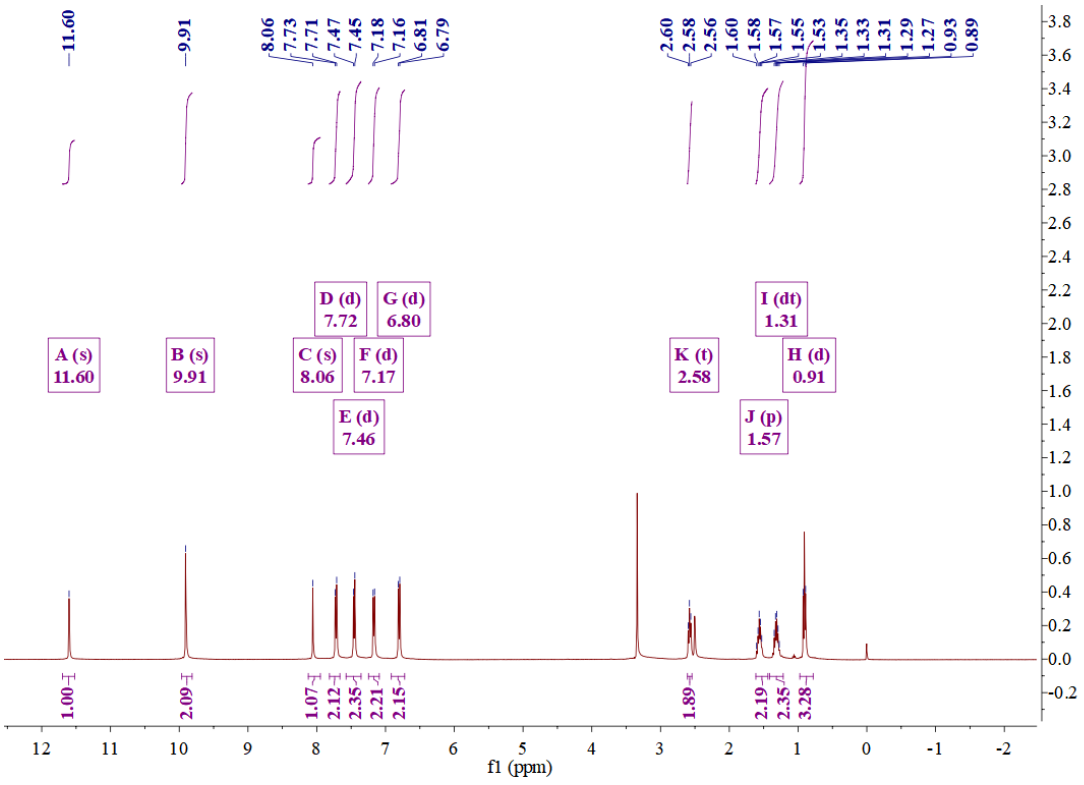
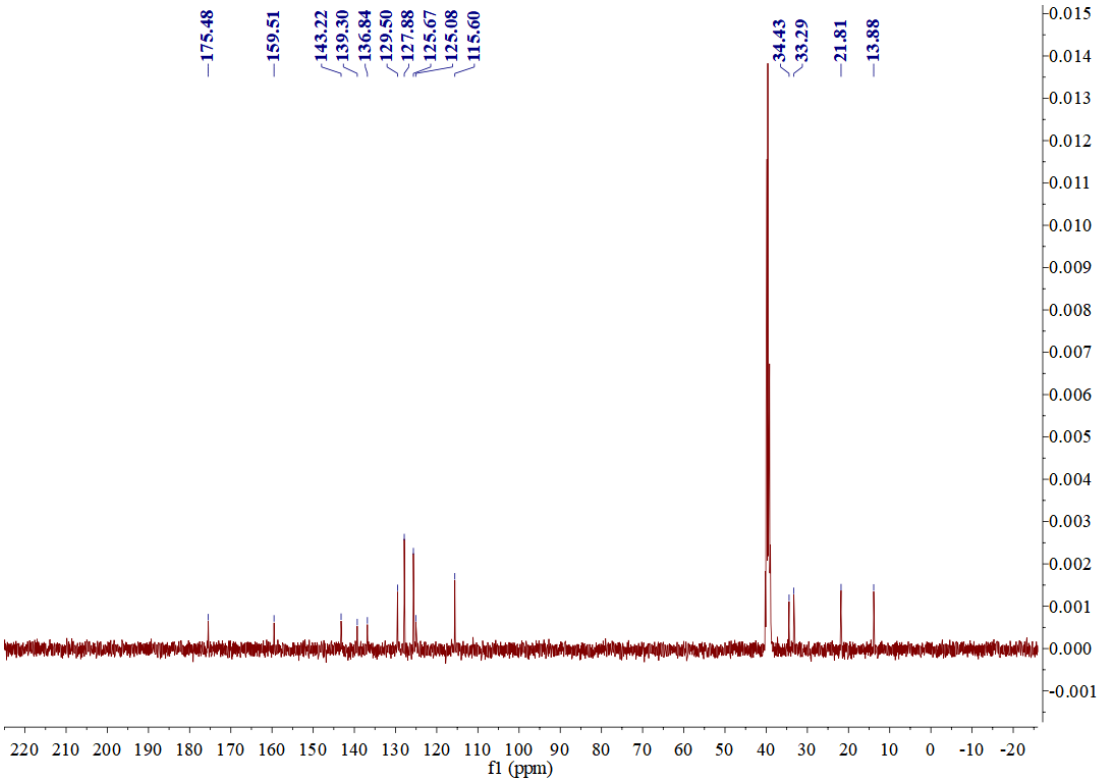


^{1}H NMR spectrum of (E)-1-(4-hydroxybenzylidene)-4-(4-butylphenyl) thiosemicarbazone (2i)



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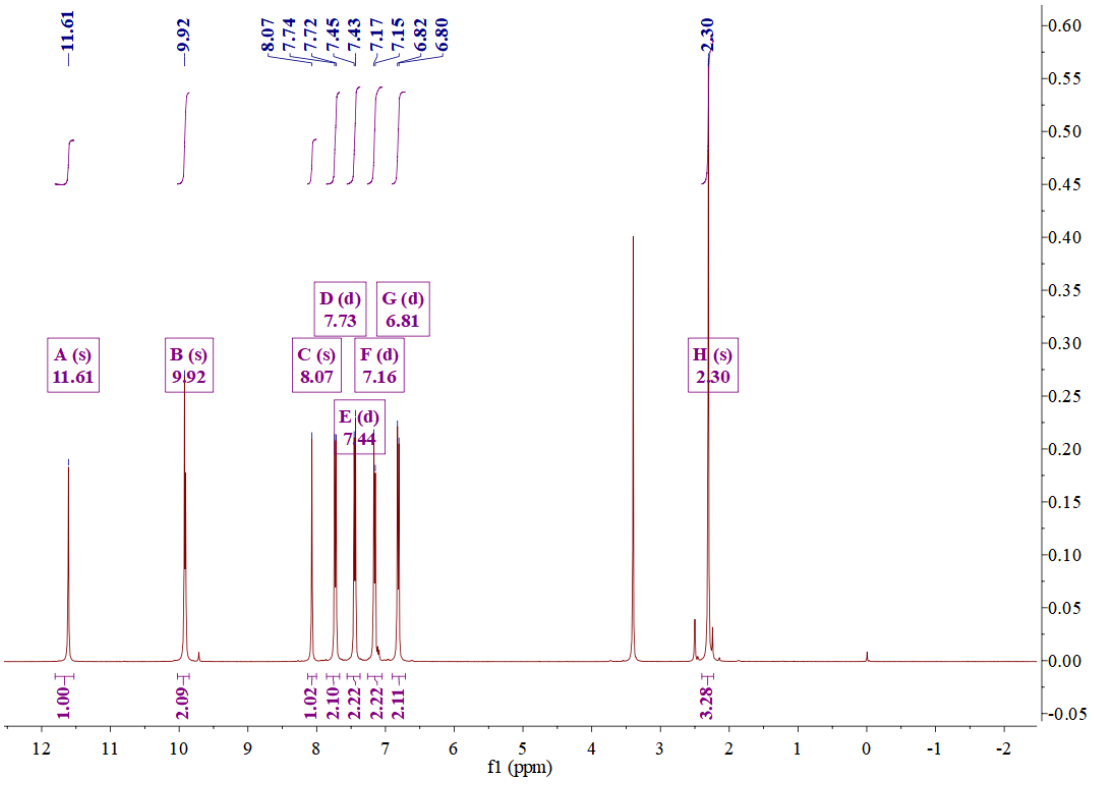

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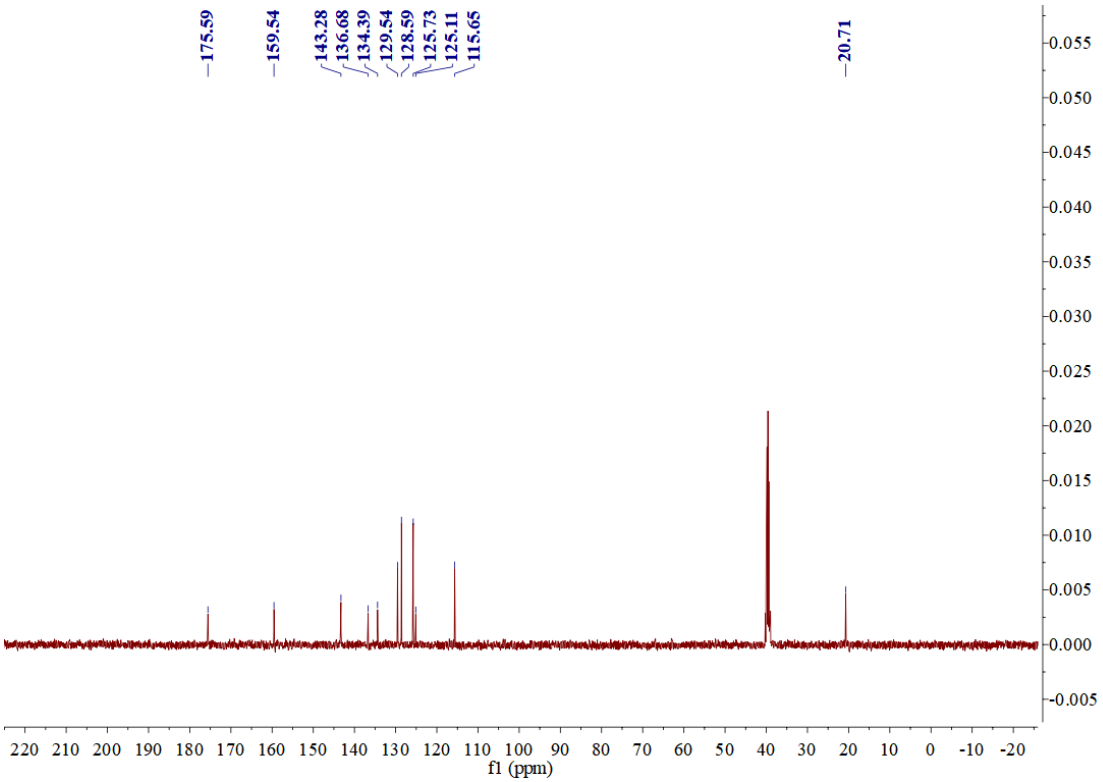
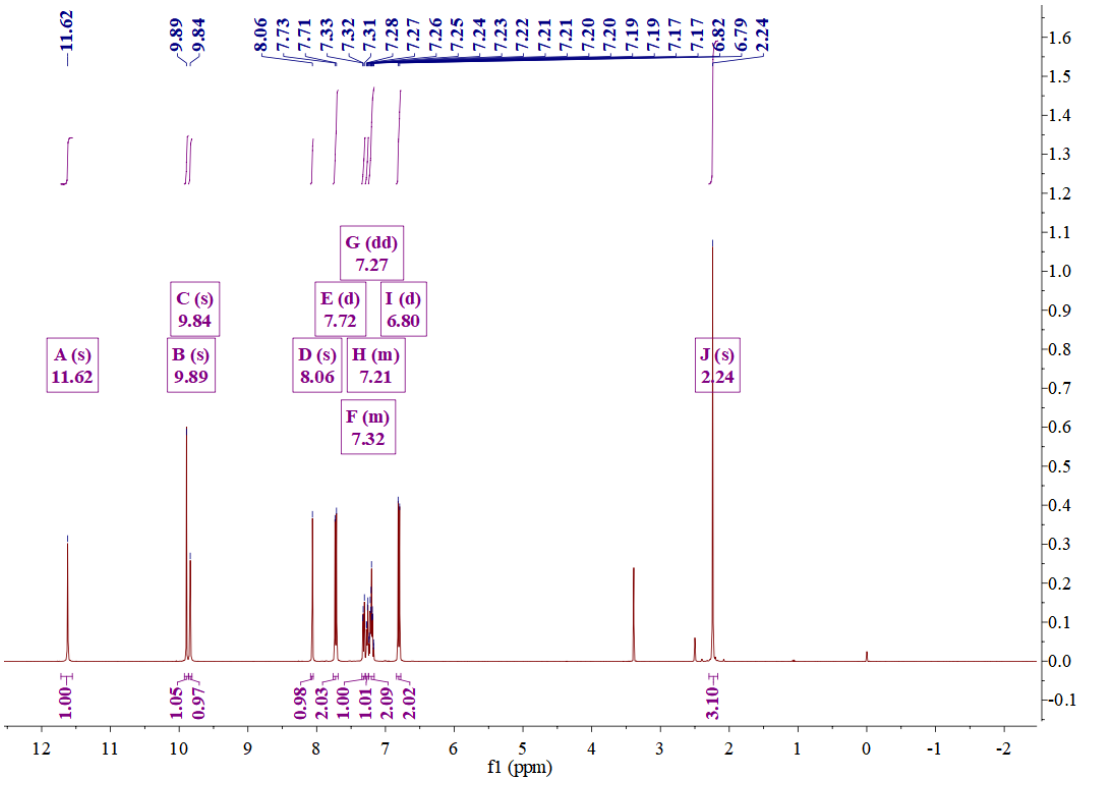


^{13}C NMR spectrum of (E)-1-(4-hydroxybenzylidene)-4-p-tolylthiosemicarbazide (2j)



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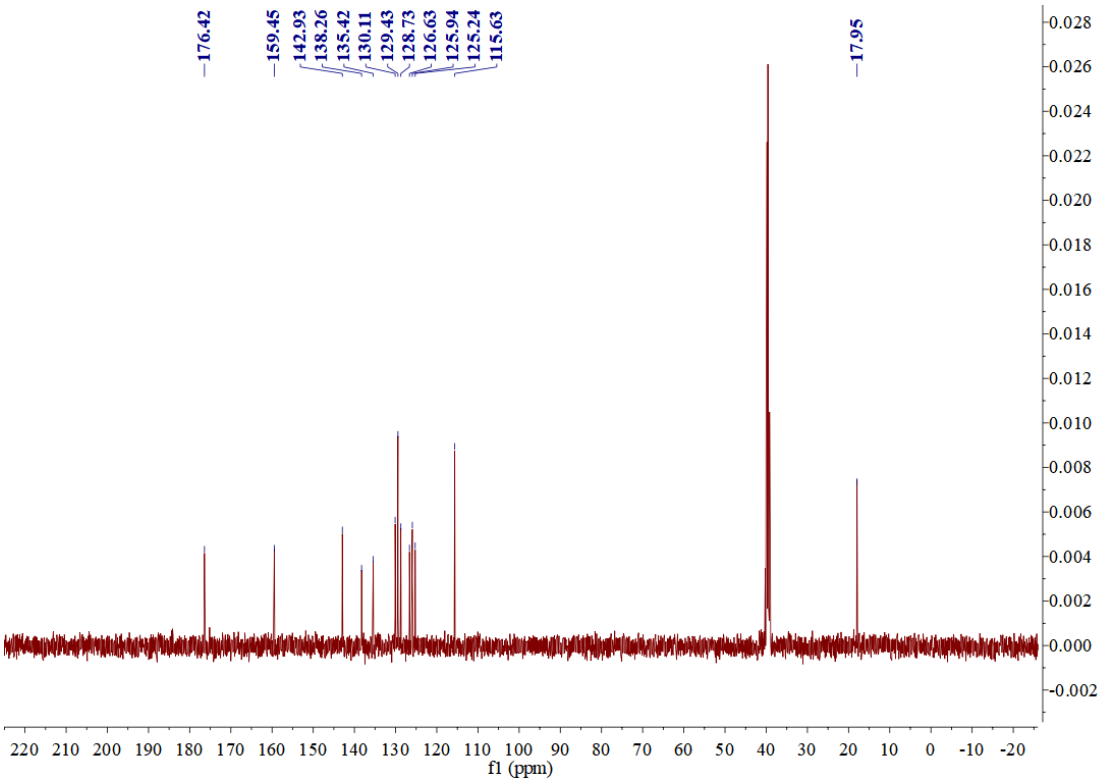
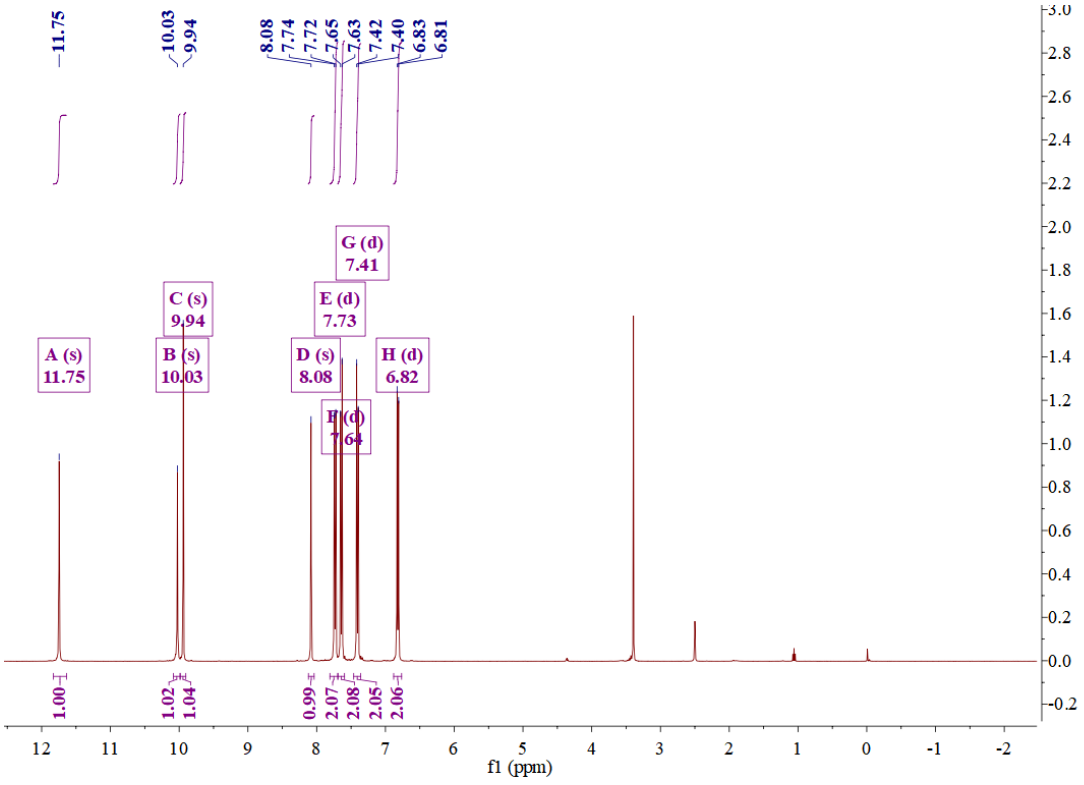
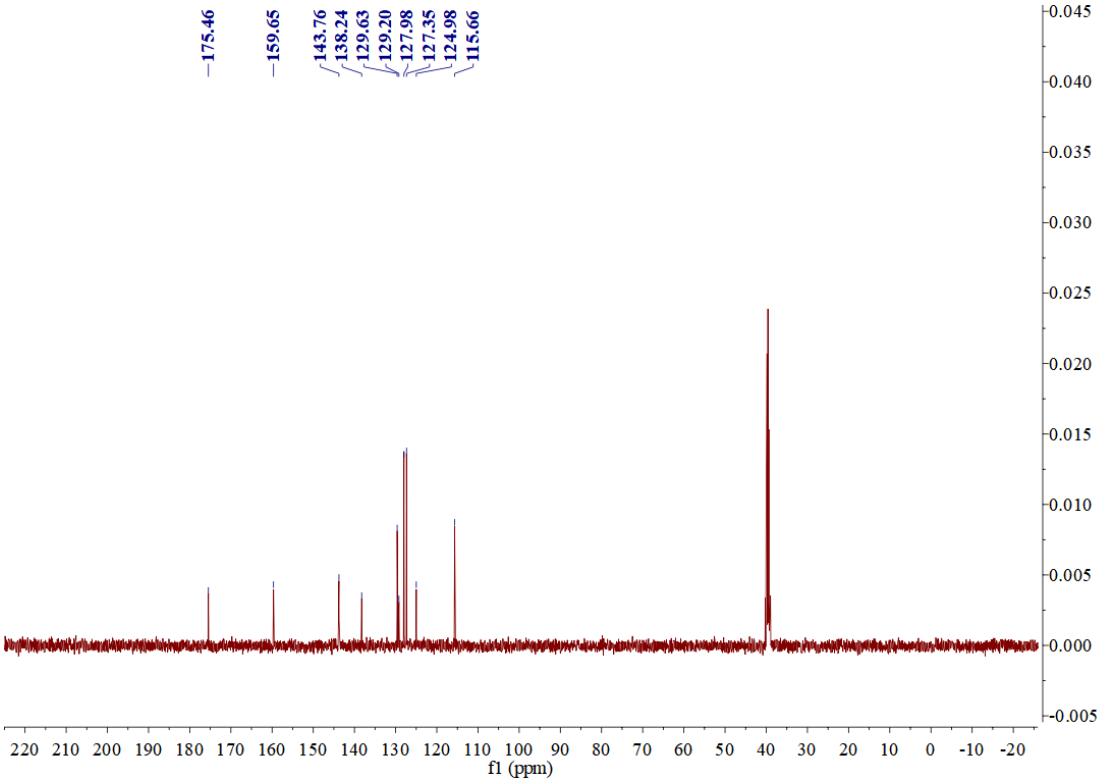


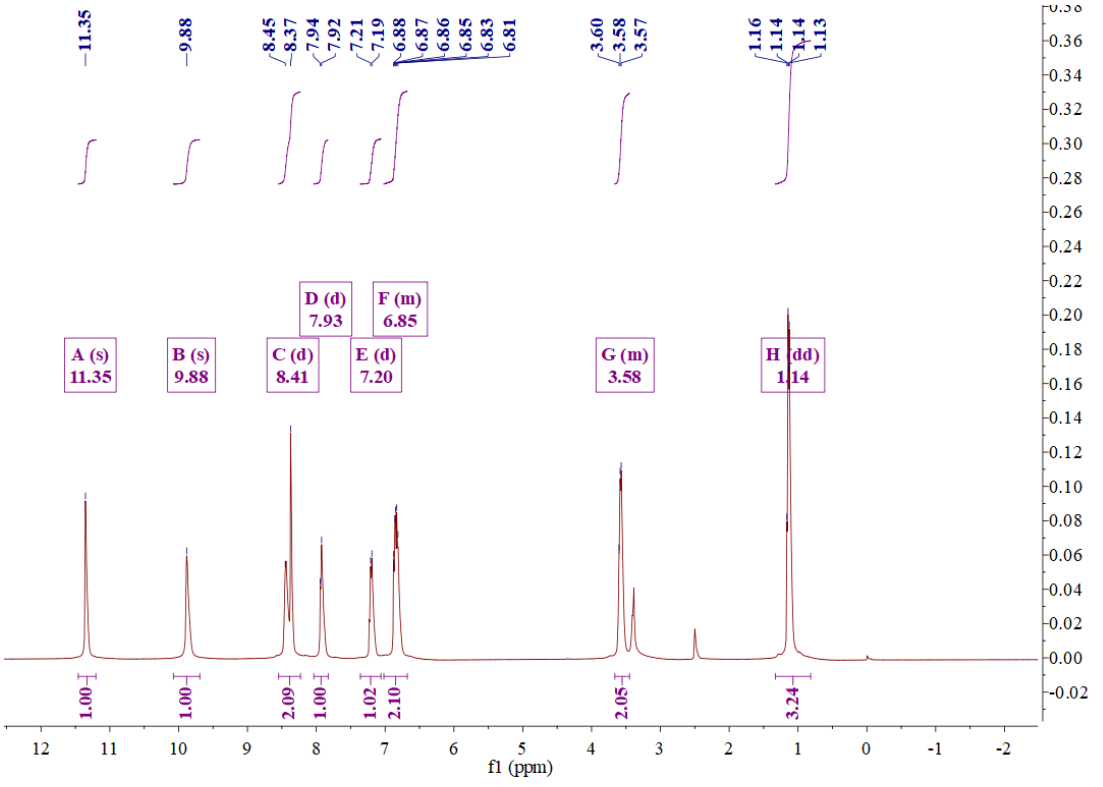
^{13}C NMR spectrum of (E)-1-(4-hydroxybenzylidene)-4-o-tolylthiosemicarbazide (2k)



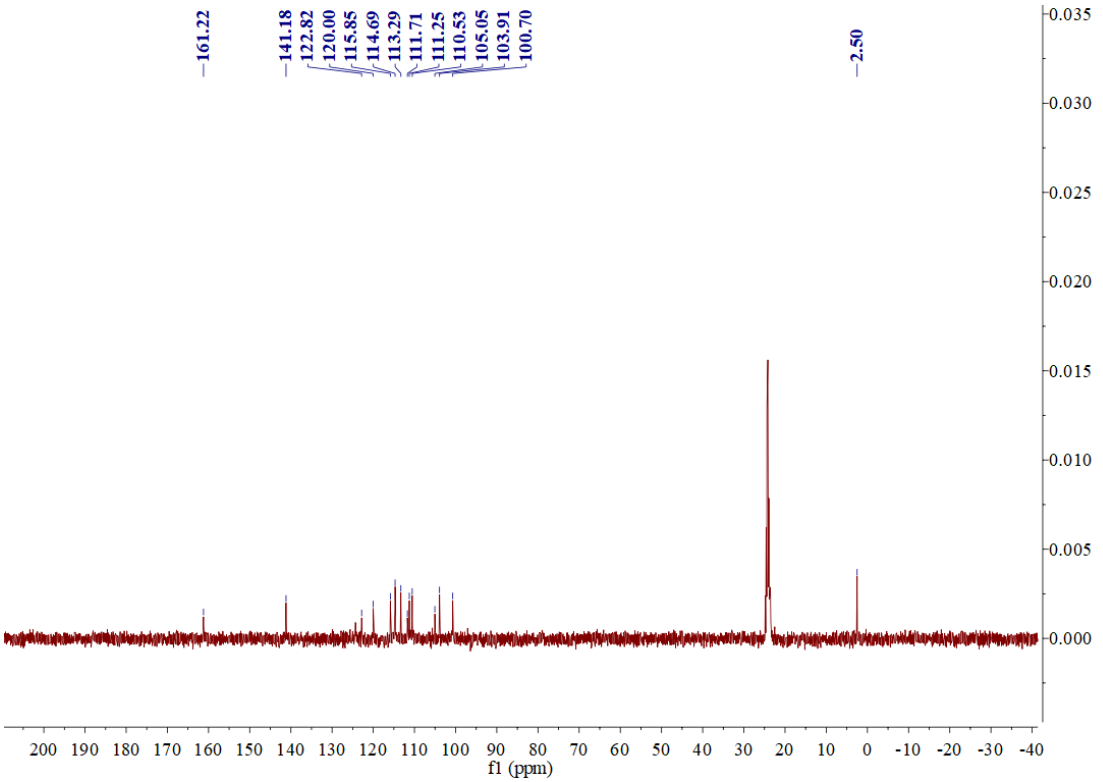
^{1}H NMR spectrum of (E)-1-(4-hydroxybenzylidene)-4-(4-chlorophenyl) thiosemicarbazide (2l)



^{13}C NMR spectrum of (E)-1-(4-hydroxybenzylidene)-4-(4-chlorophenyl) thiosemicarbazide (2l)



^{1}H NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-o-tolylthiosemicarbazide (2n)



^{13}C NMR spectrum of (E)-1-(2-hydroxybenzylidene)-4-o-tolylthiosemicarbazide (2n)

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