-Supporting Information-

N-Aryl mercaptoacetamides as potential multi-target inhibitors of metallo-β-lactamases (MBLs) and the virulence factor LasB from *Pseudomonas aeruginosa*

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# Equal contribution

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Assays

Cytotoxicity assay[1,2]

Table S1. Previously published cytotoxicity data of compound **1** against HepG2, HEK293, and A549 cell lines.

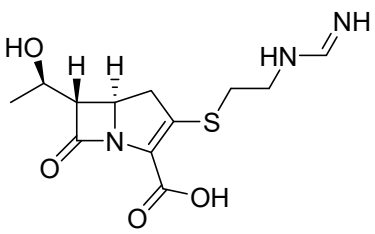
	IC_{50} (μM)[a]		
Cpd.	**HepG2**	**HEK293**	**A549**
1	>100	>100	>100
[a]Means and SD of at least two independent experiments			

In vivo zebrafish-embryo toxicity assay[1]

Table S2. Previously published results of zebrafish-embryo toxicity for compound **1**.

Cpd.	**Conc. (μM)**	**Observation after 1 day of incubation**	**Observation after 2 days of incubation**	**Observation after 3 days of incubation**	**Observation after 4 days of incubation**	**Final survival rate (%)**
1	100	all embryos dead, cpd precipitation	all embryos dead, cpd precipitation	all embryos dead, cpd precipitation	all embryos dead, cpd precipitation	0
	30	all embryos dead, cpd precipitation	all embryos dead, cpd precipitation	all embryos dead, cpd precipitation	all embryos dead, cpd precipitation	0
	10	no pigmentation	impaired pigmentation	impaired pigmentation	impaired pigmentation	90
	2	-	-	-	-	80

Growth-inhibition assay



Imipenem (Imi)

Figure S1. Structure of the carbapenem antibiotic imipenem used in the growth-curve assay.

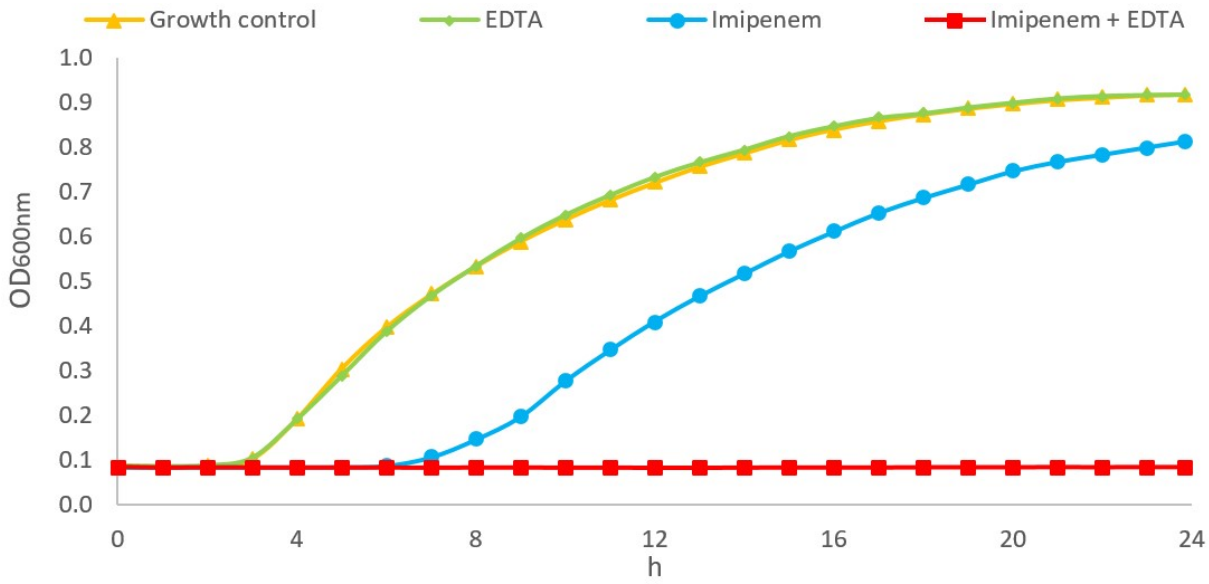
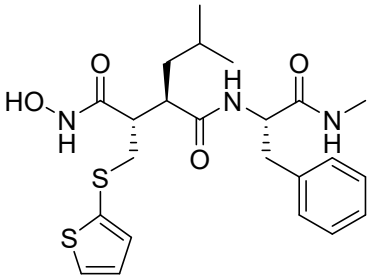


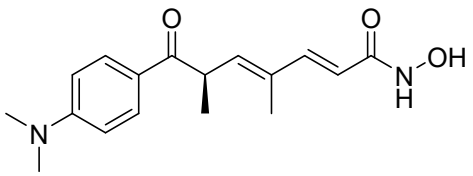
Figure S2. Growth curve of NDM-1-expressing *K. pneumoniae* (T2301) in the absence and presence of imipenem at 8 µg/mL (*i.e.*, 0.5x MIC) ± EDTA as MBL inhibitor at 50 µg/mL.

Selectivity assay over several human off-targets



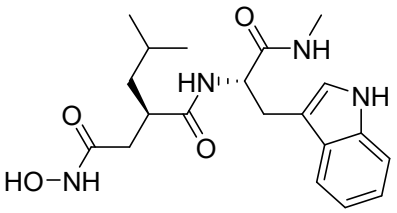
Batimastat

Figure S3. Structure of batimastat, an unselective MMP inhibitor used as reference in the MMP selectivity study.



Trichostatin A

Figure S4. Structure of trichostatin A, an HDAC inhibitor used as reference in the HDAC selectivity study.



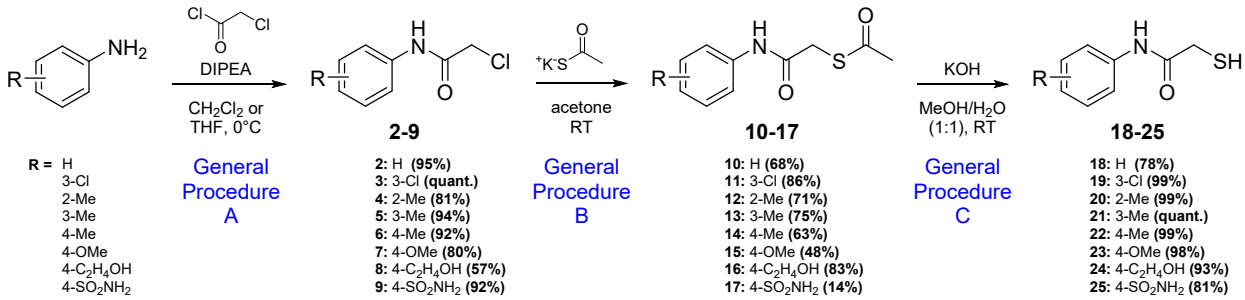
Ilomastat

Figure S5. Structure of ilomastat, an MMP and TACE inhibitor used as reference in the TACE selectivity study.

Chemistry

General. All chemicals were purchased from standard suppliers. Acetone and MeOH were purchased in HPLC quality, all other solvents were of technical quality and distilled before use. Deionized water was used throughout. The reactions were monitored by thin-layer chromatography (TLC) using aluminum plates precoated with silica gel 60 F_{254} (VWR) and visualized using UV light (254 nm) and staining under heating (CAM stain: 12 g ammonium molybdate, 0.5 g ceric ammonium molybdate, 235 mL H_2O, 15 mL conc. H_2SO_4). All organic extracts were dried over Na_2SO_4, and solvents were removed by a rotary evaporator equipped with a water bath at 40 °C. 300 MHz- and 500 MHz-^{1}H NMR spectra and 75 MHz- and 125 MHz-^{13}C NMR spectra were recorded on Bruker Fourier 300 or Bruker UltraShield™ 500 (Bruker Corporation, Billerica, MA, USA) spectrometers. All spectra were recorded at room temperature and were referenced internally to solvent reference frequencies. Chemical shifts (δ) are provided in parts per million (ppm) and coupling constants (J) are given in Hz. Low-resolution mass spectra were measured on a Thermo Finnigan Surveyor MSQ Plus mass spectrometer. High-resolution mass spectrometry (HRMS) was performed on a Thermo Scientific Q Exactive Orbitrap mass spectrometer equipped with an electrospray (ESI) source.

General procedures for the synthesis of compounds 2–25



General Procedure A. Aniline (1 equiv.) and DIPEA (1.2 equiv.) were dissolved in dry CH_2Cl_2 and cooled on ice to 0 °C. To this solution, acid chloride (1.2 equiv.) was added dropwise, and the resulting mixture was stirred at 0 °C for 1-2 h. The reaction mixture was quenched with 0.5 M $NaHCO_3$. After dilution with CH_2Cl_2, the mixture was extracted thrice with 0.5 M $NaHCO_3$ and 0.5 M HCl, as well as once with brine. The CH_2Cl_2 layer was dried with Na_2SO_4 and concentrated under reduced pressure to give the titled compounds in clean form without any further purification.

General Procedure B. Alpha-chloride (1 equiv.) was dissolved in acetone, and then potassium thioacetate (1.2 equiv.) was added. The resulting suspension was stirred at rt until the TLC indicated complete transformation. The reaction mixture was concentrated under reduced pressure. The resulting residue was diluted with EtOAc and extracted thrice with 0.5 M $NaHCO_3$ and 0.5 M HCl, as well as once with brine. The EtOAc layer was dried with Na_2SO_4 and concentrated under reduced pressure to give a solid or oily residue, which was further purified using flash chromatography on high performance silica gel, to yield the named thioacetates.

General Procedure C. The thioacetate compound was dissolved in methanol and heated to 35 °C. Then 2 M aqueous KOH was added to give a 1:1 mixture. The solution was stirred at this temperature until the TLC indicated complete transformation (0.5-2 h). The reaction mixture was diluted with EtOAc and extracted twice with 0.5 M $NaHCO_3$ and once with 0.5 M HCl and brine. The EtOAc layer was dried with Na_2SO_4 and concentrated under reduced pressure to give the final compounds without further purification.

Synthesis of alkyl chlorides 2 – 9

2-Chloro-*N*-phenylacetamide 2. Compound **2** was synthesized according to the general procedure **A**, using aniline (1.00 mL, 11.0 mmol), DIPEA (4.00 mL, 23.0 mmol) and chloroacetyl chloride (1.00 mL, 12.6 mmol) in 15 mL of dry CH_2Cl_2. The reaction was stirred for 2 h at 0 °C. After extraction, **2** was obtained as brown solid without further purification (1.76 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (br, 1 H, NH), 7.55 (dd, J = 1.1, 8.6 Hz, 2 H, 2'-H, 6'-H), 7.36 (dd, J = 7.6, 8.4 Hz, 2 H, 3'-H, 5'-H), 7.17 (t, J = 7.5 Hz, 1 H, 4'-H), 4.19 (s, 2 H, 2-H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.99 (1-C), 136.78 (1'-C), 129.25 (3'-C, 5'-C), 125.38 (4'-C), 120.27 (2'-C, 6'-C), 43.00 (2-C). HRMS (ESI) *m/z* calculated for C_8H_9ClNO 170.0367 $[M+H]^+$, found 170.0363. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.30.

2-Chloro-*N*-(3'-chlorophenyl)acetamide 3. Compound **3** was synthesized according to the general procedure **A**, using 3-chloroaniline (150 µL, 1.42 mmol), DIPEA (500 µL, 2.87 mmol) and 2-chloroacetyl chloride (130 µL, 1.63 mmol) in 10 mL of dry CH_2Cl_2. The reaction was stirred for 4 h 20 min at 0 °C. After extraction, **3** was obtained as brown solid without further purification (323 mg, quant.). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (br, 1 H, NH), 7.66 (t, J = 2.0 Hz, 1 H, 2'-H), 7.40 (ddd, J = 0.7, 1.9, 8.2 Hz, 1 H, 4'-H or 6'-H), 7.28 (t, J = 8.2 Hz, 1 H, 5'-H), 6.99 (ddd, J = 0.9, 1.9, 8.0 Hz, 1 H, 4'-H or 6'-H), 4.19 (s, 2 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.03 (1-C), 137.88 (1'-C or 3'-C), 134.95 (1'-C or 3'-C), 130.25 (5'-C), 125.45 (4'-C or 6'-C), 120.32 (2'-C), 118.17 (4'-C or 6'-C), 42.93 (2-C). HRMS (ESI) *m/z* calcd. for $C_8H_8Cl_2NO$ $[M+H]^+$ 203.9977, found: 203.9974. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.30.

2-Chloro-*N*-(*o*-tolyl)acetamide 4. Compound **4** was synthesized according to the general procedure **A**, using *o*-toluidine (500 µL, 4.70 mmol), DIPEA (1.70 mL, 9.76 mmol) and 2-chloroacetyl chloride (410 µL, 5.15 mmol) in 10 mL of dry CH_2Cl_2. The reaction was stirred for 6 h at 0 °C that slowly warmed up to room temperature. After extraction, **4** was obtained as brown solid without further purification (700 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (br, 1 H, NH), 7.87 (d, J = 8.2 Hz, 1 H, 6'-H), 7.26 – 7.20 (m, 2 H, 3'-H, 4'-H), 7.12 (td, J = 1.1, 7.5 Hz, 1 H, 5'-H), 4.24 (s, 2 H, 2-H), 2.31 (s, 3 H, 2'-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.92 (1-C), 134.76 (1'-C or 2'-C), 130.72 (3'-C), 129.14 (1'-C or 2'-C), 127.05 (4'-C), 125.92 (5'-C), 122.55 (6'-C), 43.28 (2-C), 17.62 (2'-CH_3). MS (ESI^+) *m/z* 184 $(M+H)^+$. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.30.

2-Chloro-*N*-(*m*-tolyl)acetamide 5. Compound **5** was synthesized according to the general procedure **A**, using *m*-toluidine (400 µL, 3.73 mmol), DIPEA (1.30 mL, 7.46 mmol) and chloroacetyl chloride (330 µL, 4.14 mmol) in 7 mL of dry CH_2Cl_2. The reaction was stirred for 3 h 30 min at 0 °C. After extraction, **5** was obtained as brown solid without further purification (642 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (br, 1 H, NH), 7.37 (s, 1 H, 2'-H), 7.33 (d, J = 8.3 Hz, 1 H, 6'-H), 7.23 (t, J = 7.7 Hz, 1 H, 5'-H), 6.99 (d, J = 7.5 Hz, 1 H, 4'-H), 4.17 (s, 2 H, 2-H), 2.35 (s, 3 H, 3'-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.91 (1-C), 139.26 (3'-C), 136.66 (1'-C), 129.07 (5'-C), 126.20 (4'-C), 120.88 (2'-C), 117.35 (6'-C), 43.02 (2-C), 21.57 (3'-CH_3). HRMS (ESI) *m/z* calcd. for $C_9H_{11}ClNO$ $[M+H]^+$ 184.0524, found: 184.0519. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.40.

2-Chloro-*N*-(*p*-tolyl)acetamide 6. Compound **6** was synthesized according to the general procedure **A**, using *p*-toluidine (499 mg, 4.66 mmol), DIPEA (1.70 mL, 9.76 mmol) and chloroacetyl chloride (450 µL, 5.65 mmol) in 10 mL of dry CH_2Cl_2. The reaction was stirred for 2 h 30 min at 0 °C. After extraction **6** was obtained as brown solid without further purification (784 mg, 92%). ^{1}H NMR (500 Hz, $CDCl_3$) δ 8.18 (br, 1 H, NH), 7.42 (d, J = 8.4 Hz, 2 H, aryl-H_a), 7.16 (d, J = 8.3 Hz, 2 H, aryl-H_b), 4.18 (s, 2 H, 2-H), 2.33 (s, 3 H, 4'-CH_3). ^{13}C NMR (126 Hz, $CDCl_3$) δ 163.84 (1-C), 135.15 (1'-C or 4'-C), 134.22 (1'-C or 4'-C), 129.77 (aryl-C_b), 120.36 (aryl-C_a), 43.01 (2-H), 21.05 (4'-CH_3). HRMS (ESI) *m/z* calcd. for $C_9H_{11}ClNO$ $[M+H]^+$ 184.0524, found: 184.0520. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.40.

2-Chloro-*N*-(4'-methoxyphenyl)acetamide 7. Compound **7** was synthesized according to the general procedure **A**, using 4-methoxyaniline (1.41 g, 11.5 mmol), DIPEA (4.00 mL, 23.0 mmol) and chloroacetyl chloride (1.00 mL, 12.56 mmol) in 15 mL of dry CH_2Cl_2. The reaction was stirred for 2 h 20 min at 0 °C. After extraction **7** was obtained as brown solid without further purification (1.83 g, 80%). ^{1}H NMR (500 Hz, $CDCl_3$) δ 8.18 (br, 1 H, NH), 7.43 (d, J = 9.0 Hz, 2 H, 3'-H, 5'-H), 6.88 (d, J = 9.0 Hz, 2 H, 2'-C, 6'-C), 4.18 (s, 2 H, 2-H), 3.80 (s, 3 H, 4'-OCH_3). ^{13}C NMR (126 Hz, $CDCl_3$) δ 163.90 (1-C), 157.22 (4'-C), 129.78 (1'-C), 122.22 (3'-C, 5'-C), 114.38 (2'-C, 6'-C), 55.60 (4'-OCH_3), 42.96 (2-C). HRMS (ESI) *m/z* calculated for $C_9H_{11}ClNO_2$ 200.0473 $[M+H]^+$, found 200.0466. TLC (petroleum ether-EtOAc, 2:8): R_f = 0.50.

2-Chloro-*N*-(4'-(2''-hydroxyethyl)phenyl)acetamide 8. Compound **8** was synthesized according to the general procedure **A**, using 4-(2'-hydroxyethyl)aniline (1.00 g, 7.30 mmol), DIPEA (1.40 mL, 8.04 mmol) and chloroacetyl chloride (640 µL, 8.04 mmol) in 20 mL of dry

THF. The reaction was stirred for 2 h 20 min at 0 °C. After extraction, **8** was obtained as white solid without further purification (885 mg, 57%). R_f = 0.06 (PE/EtOAc 65:35). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.21 (s, 1 H, NH), 7.47 (d, J = 8.5 Hz, 2 H, 3'-H, 5'-H), 7.16 (d, J = 8.5 Hz, 2 H, 2'-H, 6'-H), 4.62 (t, J = 5.1 Hz, 1 H, 2''-OH), 4.23 (s, 2 H, 2-H), 3.56 (td, J = 4.8, 7.0 Hz, 2 H, 2''-H), 2.67 (t, J = 7.1 Hz, 2 H, 1''-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.39 (1-C), 136.37 (1'-C), 135.07 (4'-C), 129.19 (2'-C, 6'-C), 119.31 (3'-C, 5'-C), 62.19 (2''-C), 43.56 (2-C), 38.46 (1''-C). MS (ESI$^+$) *m/z* 214 (M+H)$^+$. TLC (petroleum ether-EtOAc, 65:35): R_f = 0.05.

2-Chloro-*N*-(4'-sulfamoylphenyl)acetamide 9. Compound **9** was synthesized according to the general procedure **A**, using sulfanilamid (2.03 g, 11.8 mmol), DIPEA (2.30 mL, 13.2 mmol) and chloroacetyl chloride (1.10 mL, 13.8 mmol) in 45 mL of dry THF. The reaction was stirred for 1 h 30 min at 0 °C. After extraction **9** was obtained as orange solid without further purification (2.70 g, 92%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1 H, NH), 7.79 (d, J = 9.0 Hz, 2 H, aryl-H_a), 7.75 (d, J = 9.0 Hz, 2 H, aryl-H_b), 7.29 (s, 2 H, SO_2NH_2), 4.30 (s, 2 H, 2-C). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.18 (1-C), 141.34 (1'-C or 4'-C), 138.95 (1'-C or 4'-C), 126.81 (aryl-C_a), 118.97 (aryl-C_b), 43.55 (2-C). MS (ESI$^+$) *m/z* 249 (M+H)$^+$. TLC (CH_2Cl_2- MeOH, 95:5): R_f = 0.10.

Synthesis of thioacetates 10–17

2-*S*-(Acetylthio)-*N*-phenylacetamide 10. Compound **10** was synthesized according to the general procedure **B**, using intermediate **2** (242 mg, 1.43 mmol) and potassium thioacetate (200 mg, 1.75 mmol) in 10 mL acetone. The reaction was stirred for 3 h at room temperature. After extraction and column chromatography (PE/EtOAc 8:2) **10** was obtained as yellow solid (204 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (br, 1 H, NH), 7.49 (d, J = 7.6 Hz, 2 H, 2'-H, 6'-H), 7.29 (t, J = 7.7 Hz, 2 H, 3'-H, 5'-H), 7.11 (t, J = 7.5 Hz, 1 H, 4'-H), 3.64 (s, 2 H, 2-H), 2.43 (s, 3 H, S(CO)C$\underline{H}_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.26 (S($\underline{C}$O)CH_3), 166.46 (1-C), 137.73 (1'-C), 129.13 (3'-C, 5'-C), 124.68 (4'-C), 119.94 (2'-C, 6'-C), 34.38 (2-C), 30.40 (S(CO)$\underline{C}H_3$). HRMS (ESI) *m/z* calculated for $C_{10}H_{12}NO_2S$ 210.0583 $[M+H]^+$, found: 210.0576. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.20.

2-*S*-(Acetylthio)-*N*-(3'-chlorophenyl)acetamide 11. Compound **11** was synthesized according to the general procedure **B**, using intermediate **3** (125 mg, 0.61 mmol) and potassium thioacetate (90 mg, 0.79 mmol) in 5 mL acetone. The reaction was stirred for 3 h at room temperature. After extraction and column chromatography (PE/EtOAc 8:2) **11** was obtained as yellow solid (128 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (br, 1 H, NH), 7.60 (t, J = 2.0 Hz, 1 H, 2'-H), 7.34 (ddd, J = 0.9, 2.0, 8.2 Hz, 1 H, 4'-H or 6'-H), 7.23 (t, J = 8.1 Hz, 1 H, 5'-H), 7.06 (ddd, J = 0.9, 1.9, 8.0 Hz, 1 H, 4'-H or 6'-H), 3.64 (s, 2 H, 2-H), 2.46 (s, 3 H, S(CO)C$\underline{H}_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.62 (S($\underline{C}$O)CH_3), 166.60 (1-C), 138.87 (1'-C or 3'-C), 134.81 (1'-C or 3'-C), 130.12 (5'-C), 124.70 (4'-C or 6'-C), 119.99 (2'-C), 117.87 (4'-C or 6'-C), 34.36 (2-C), 30.41 (S(CO)$\underline{C}H_3$). HRMS (ESI) *m/z* calculated for $C_{10}H_{11}ClNO_2S$ 244.0194 $[M+H]^+$, found: 244.0183. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.20.

2-*S*-(Acetylthio)-*N*-(*o*-tolyl)acetamide 12. Compound **12** was synthesized according to the general procedure **B**, using intermediate **4** (458 mg, 2.29 mmol) and potassium thioacetate (321 mg, 2.81 mmol) in 10 mL acetone. The reaction was stirred for 3 h 30 min at room temperature. After extraction and column chromatography (PE/EtOAc 7:3) **12** was obtained as yellow solid (193 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (br, 1 H, NH), 7.88 (d, J = 8.1 Hz, 1 H, 6'-H), 7.21-7.13 (m, 2 H, 3'-H, 4'-H), 7.04 (td, J = 1.1, 7.4 Hz, 1 H, 5'-H), 3.67 (s, 2 H, 2-H), 2.44 (s, 3 H, S(CO)C$\underline{H}_3$), 2.23 (s, 3 H, 2'-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.21 (S($\underline{C}$O)CH_3), 166.63 (1-C), 135.76 (1'-C or 2'-C), 130.59 (3'-C), 128.57 (1'-C or 2'-C), 126.91 (4'-C), 125.21 (5'-C), 122.38 (6'-C), 34.21 (2-C), 30.37 (S(CO)$\underline{C}H_3$), 17.80 (2'-CH_3). MS (ESI^+) *m/z* 224 $(M+H)^+$. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.25.

2-*S*-(Acetylthio)-*N*-(*m*-tolyl)acetamide 13. Compound **13** was synthesized according to the general procedure **B**, using intermediate **5** (145 mg, 0.79 mmol) and potassium thioacetate (118 mg, 1.03 mmol) in 15 mL acetone. The reaction was stirred for 4 h at room temperature. After extraction and column chromatography (PE/EtOAc 9:1→8:2), **13** was obtained as yellow solid (132 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (br, 1 H, NH), 7.32 (s, 1 H, 2'-H), 7.29 (d, J = 8.0 Hz, 1 H, 6'-H), 7.19 (t, J = 7.8 Hz, 1 H, 5'-H), 6.92 (d, J = 7.5 Hz, 1 H, 4'-H), 3.65 (s, 2 H, 2-H), 2.45 (s, 3 H, S(CO)C$\underline{H}_3$), 2.33 (s, 3 H, 3'-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.20 (S($\underline{C}$O)CH_3), 166.40 (1-C), 139.06 (3'-C), 137.64 (1'-C), 128.97 (5'-C), 125.47 (4'-C), 120.54 (2'-C), 117.03 (6'-C), 34.39 (2-C), 30.38 (S(CO)$\underline{C}H_3$), 21.56 (3'-CH_3). MS (ESI$^+$) *m/z* 224 $(M+H)^+$. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.25.

2-*S*-(Acetylthio)-*N*-(*p*-tolyl)acetamide 14. Compound **14** was synthesized according to the general procedure **B**, using intermediate **6** (122 mg, 0.66 mmol) and potassium thioacetate (92 mg, 0.81 mmol) in 5 mL acetone. The reaction was stirred for 2 h 30 min at room temperature. After extraction and column chromatography (PE/EtOAc 8:2) **14**was obtained as yellow solid (94 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (br, 1 H, NH), 7.37 (d, J = 8.4 Hz, 2 H, aryl-H_a), 7.11 (d, J = 8.2 Hz, 2 H, aryl-H_b), 3.64 (s, 2 H, 2-H), 2.44 (s, 3 H, S(CO)C$\underline{H}_3$), 2.30 (s, 3 H, 4'-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.21 (S($\underline{C}$O)CH_3), 166.32 (1-C), 135.17 (1'-C or 4'-C), 134.36 (1'-C or 4'-C), 129.62 (aryl-C_b), 120.00 (aryl-C_a), 34.33 (2-C), 30.42 (S(CO)$\underline{C}H_3$), 21.02 (4'-CH_3). MS (ESI$^+$) *m/z* 224 $(M+H)^+$. TLC (petroleum ether-EtOAc, 7:3): R_f = 0.20.

2-*S*-(Acetylthio)-*N*-(4'-methoxyphenyl)acetamide 15. Compound **15** was synthesized according to the general procedure **B**, using intermediate **7** (458 mg, 2.29 mmol) and potassium thioacetate (321 mg, 2.81 mmol) in 10 mL acetone. The reaction was stirred for 3 h 30 min at room temperature. After extraction and column chromatography (PE/EtOAc 7:3) **15**was obtained as yellow solid (262 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1 H, NH), 7.39 (d, J = 9.0 Hz, 2 H, 3'-H, 5'-H), 6.85 (d, J = 9.0 Hz, 2 H, 2'-C, 6'-C), 3.78 (s, 3 H, 4'-OCH_3), 3.64 (s, 2 H, 2-H), 2.44 (s, 3 H, S(CO)C$\underline{H}_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.12 (S($\underline{C}$O)CH_3), 166.25 (1-C), 156.73 (4'-C), 130.85 (1'-C), 121.77 (3'-C, 5'-C), 114.28 (2'-C, 6'-C), 55.63 (4'-OCH_3), 34.21 (2-C), 30.42 (S(CO)$\underline{C}H_3$). HRMS (ESI) *m/z* calculated for $C_{11}H_{14}NO_3S$ 240.0689 $[M+H]^+$, found 240.0679. TLC (petroleum ether-EtOAc, 6:4): R_f = 0.20.

2-*S*-(Acetylthio)-*N*-(4'-(2''-hydroxyethyl)phenyl)acetamide 16. Compound **16** was synthesized according to the general procedure **B**, using intermediate **8** (201 mg, 0.94 mmol) and potassium thioacetate (136 mg, 1.19 mmol) in 7 mL acetone. The reaction was stirred for

5 h at room temperature. After extraction, **16** was obtained as white solid (197 mg, 83%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.14 (s, 1 H, NH), 7.44 (d, *J* = 8.5 Hz, 2 H, 3'-H, 5'-H), 7.14 (d, *J* = 8.5 Hz, 2 H, 2'-H, 6'-H), 4.60 (t, *J* = 5.2 Hz, 1 H, 2''-OH), 3.80 (s, 2 H, 2-H), 3.56 (td, *J* = 5.2, 7.1 Hz, 2 H, 2''-H), 2.66 (t, *J* = 7.1 Hz, 2 H, 1''-H), 2.38 (s, 3 H, S(CO)C$\underline{H}_3$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 194.57 (S($\underline{C}$O)CH_3), 165.43 (1-C), 136.75 (1'-C), 134.63 (4'-C), 129.08 (2'-C, 6'-C), 119.04 (3'-C, 5'-C), 62.20 (2''-C), 38.44 (1''-C), 33.78 (2-C), 30.11 (S(CO)$\underline{C}H_3$). MS (ESI$^+$) *m/z* 254 $(M+H)^+$. TLC (CH_2Cl_2-MeOH, 95:5): R_f = 0.15.

2-*S*-(Acetylthio)-*N*-(4'-sulfamoylphenyl)acetamide 17. Compound **17** was synthesized according to the general procedure **B**, using intermediate **9** (300 mg, 1.21 mmol) and potassium thioacetate (166 mg, 1.45 mmol) in 7 mL acetone. The reaction was stirred for 5 h at room temperature. After extraction, **17** was obtained as white solid (48 mg, 14%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.59 (s, 1 H, NH), 7.76 (d, *J* = 8.8 Hz, 2 H, aryl-H_a), 7.72 (d, *J* = 8.8 Hz, 2 H, aryl-H_b), 7.27 (s, 2 H, SO_2NH_2), 3.86 (s, 2 H, 2-C), 2.39 (s, 3 H, S(CO)C$\underline{H}_3$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 194.58 (S($\underline{C}$O)CH_3), 166.33 (1-C), 141.68 (1'-C or 4'-C), 138.57 (1'-C or 4'-C), 126.75 (aryl-C_a), 118.66 (aryl-C_b), 33.90 (2-C), 30.10 (S(CO)$\underline{C}H_3$). MS (ESI$^+$) *m/z* 289 $(M+H)^+$. TLC (petroleum ether-EtOAc, 4:6): R_f = 0.15.

Synthesis of thiols 18–25

2-mercapto-*N*-phenylacetamide 18. Compound **18** was synthesized according to the general procedure **C**, using thioacetate **10** (29 mg, 0.14 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 40 min. Extraction gave the final compound **18** without any further purification as white solid (17 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1 H, NH), 7.55 (dd, *J* = 0.9, 8.5 Hz, 2 H, 2'-H, 6'-H), 7.35 (t, *J* = 8.0 Hz, 2 H, 3'-H, 5'-H), 7.15 (t, *J* = 7.4 Hz, 1 H, 4'-H), 3.41 (d, *J* = 9.2 Hz, 2 H, 2-H), 2.03 (t, *J* = 9.2 Hz, 1 H, SH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.23 (1-C), 137.37 (1'-C), 129.22 (3'-C, 5'-C), 124.97 (4'-C), 119.96 (2'-C, 6'-C), 29.29 (2-C). HRMS (ESI) *m/z* calculated for $C_8H_{10}NOS$ 168.0478 $[M+H]^+$, found 168.0474.

2-mercapto-*N*-(3'-chlorophenyl)acetamide 19. Compound **19** was synthesized according to the general procedure **C**, using thioacetate **11** (28 mg, 0.12 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 1 h 20 min. Extraction gave the final compound **19** without any further purification as white solid (23 mg, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.26 (s, 1 H, NH), 7.80 (t, *J* = 2.0 Hz, 1 H, 2'-H), 7.40 (ddd, *J* = 1.1, 1.9, 8.2 Hz, 1 H, 4'-H or 6'-H), 7.34 (t, *J* = 8.0 Hz, 1 H, 5'-H), 7.12 (ddd, *J* = 1.1, 2.1, 7.8 Hz, 1 H, 4'-H or 6'-H), 3.30* (br, 2 H, 2-H), 2.97** (br, 1 H, SH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.06 (1-C), 140.46 (1'-C or 3'-C), 133.13 (1'-C or 3'-C), 130.57 (5'-C), 123.14 (4'-C or 6'-C), 118.52 (2'-C), 117.49 (4'-C or 6'-C), 28.32 (2-C). HRMS (ESI) *m/z* calculated for C_8H_9ClNOS 202.0088 $[M+H]^+$, found 202.0085.

* 3.30 (d, *J* = 7.7 Hz, 2H, 2-H), when measured at 500 MHz, DMSO-d_6

** 2.99 (t, *J* = 7.9 Hz, 1H, SH), when measured at 500 MHz, DMSO-d_6

2-mercapto-*N*-(*o*-tolyl)acetamide 20. Compound **20** was synthesized according to the general procedure **C**, using thioacetate **12** (32 mg, 0.14 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 45 min. Extraction gave the final compound **20** without any further purification as slightly yellow solid (26 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (br, 1 H, NH), 7.91 (d, *J* = 8.0 Hz, 1 H, 6'-H), 7.25–7.18 (m, 2 H, 3'-H, 4'-H), 7.09 (td, *J* = 0.9, 7.5 Hz, 1 H, 5'-H), 3.45 (d, *J* = 9.2 Hz, 2 H, 2-H), 2.31 (s, 3 H, 2'-CH_3), 2.04 (t, *J* = 9.3 Hz, 1 H, SH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.18 (1-C), 135.39 (1'-C or 2'-C), 130.66 (3'-C), 128.80 (1'-C or 2'-C), 127.04 (4'-C), 125.48 (5'-C), 122.25 (6'-C), 29.40 (2-C), 17.85 (2'-CH_3). HRMS (ESI) *m/z* calculated for $C_9H_{12}NOS$ 182.0634 $[M+H]^+$, found 182.0631.

2-mercapto-*N*-(*m*-tolyl)acetamide 21. Compound **21** was synthesized according to the general procedure **C**, using thioacetate **13** (30 mg, 0.13 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 45 min. Extraction gave the final compound **7** without any further purification as slightly yellow solid (27 mg, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (br, 1 H, NH), 7.39 (s, 1 H, 2'-H), 7.34 (d, J = 8.1 Hz, 1 H, 6'-H), 7.22 (t, J = 7.8 Hz, 1 H, 5'-H), 6.96 (d, J = 7.4 Hz, 1 H, 4'-H), 3.39 (d, J = 8.8 Hz, 2 H, 2-H), 2.34 (s, 3 H, 3'-CH_3), 2.03 (t, J = 9.1 Hz, 1 H, SH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.32 (1-C), 139.16 (3'-C), 137.26 (1'-C), 129.01 (5'-C), 125.77 (4'-C), 120.62 (2'-C), 117.07 (6'-C), 29.29 (2-C), 21.58 (3'-CH_3). HRMS (ESI) *m/z* calculated for $C_9H_{12}NOS$ 182.0634 $[M+H]^+$, found 182.0631.

2-mercapto-*N*-(*p*-tolyl)acetamide 22. Compound **22** was synthesized according to the general procedure **C**, using thioacetate **14** (31 mg, 0.14 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 45 min. Extraction gave the final compound **22** without any further purification as white solid (25 mg, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.97 (br, 1 H, NH), 7.46 (d, J = 8.4 Hz, 2 H, aryl-H_a), 7.11 (d, J = 8.3 Hz, 2 H, aryl-H_b), 3.27 (d, J = 8.0 Hz, 2 H, 2-C), 2.90 (t, J = 8.0 Hz, 1 H, SH), 2.24 (s, 3 H, 4'-CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.35 (1-C), 136.52 (1'-C or 4'-C), 132.32 (1'-C or 4'-C), 129.18 (aryl-C_b), 119.08 (aryl-C_a), 28.25 (2-C), 20.47 (4'-CH_3). HRMS (ESI) *m/z* calculated for $C_9H_{12}NOS$ 182.0634 $[M+H]^+$, found 182.0630.

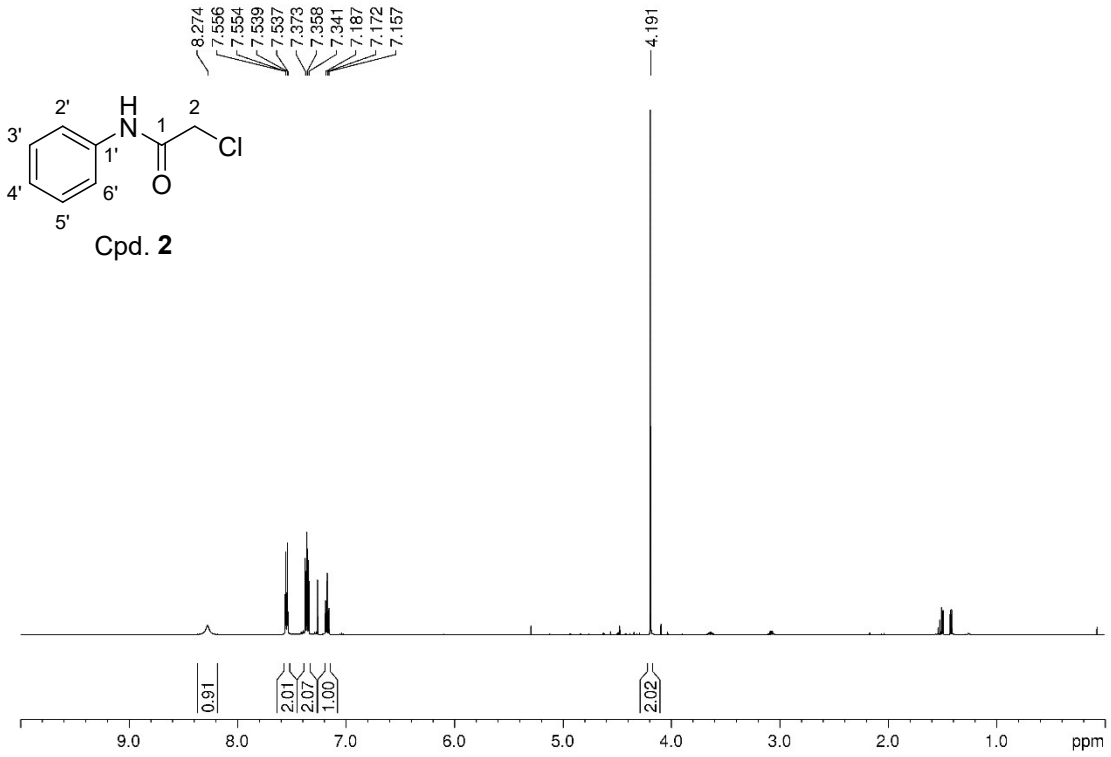
2-mercapto-*N*-(4'-methoxyphenyl)acetamide 23. Compound **23** was synthesized according to the general procedure **C**, using thioacetate **16** (37 mg, 0.15 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 45 min. Extraction gave the final compound **23** without any further purification as white solid (30 mg, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (br, 1 H, NH), 7.44 (d, J = 9.0 Hz, 2 H, 3'-H, 5'-H), 6.88 (d, J = 9.0 Hz, 2 H, 2'-C, 6'-C), 3.80 (s, 3 H, 4'-OCH_3), 3.39 (d, J = 9.3 Hz, 2 H, 2-H), 2.01 (t, J = 9.3 Hz, 1 H, SH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.09 (1-C), 156.92 (4'-C), 130.48 (1'-C), 121.87 (3'-C, 5'-C), 114.36 (2'-C, 6'-C), 55.62 (4'-OCH_3), 29.13 (2-C). HRMS (ESI) *m/z* calculated for $C_9H_{11}NO_2S$ 198.0583 $[M+H]^+$, found 198.0580.

2-mercapto-*N*-(4'-(2''-hydroxyethyl)phenyl)acetamide 24. Compound **24** was synthesized according to the general procedure **C**, using thioacetate **16** (36 mg, 0.14 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 1 h. Extraction gave **24** as white solid (28 mg, 93%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.99 (s, 1 H, NH), 7.46 (d, J = 8.5 Hz, 2 H, 3'-H, 5'-H), 7.14 (d, J = 8.5 Hz, 2 H, 2'-H, 6'-H), 4.63-4.58 (m, 1 H, 2''-OH),

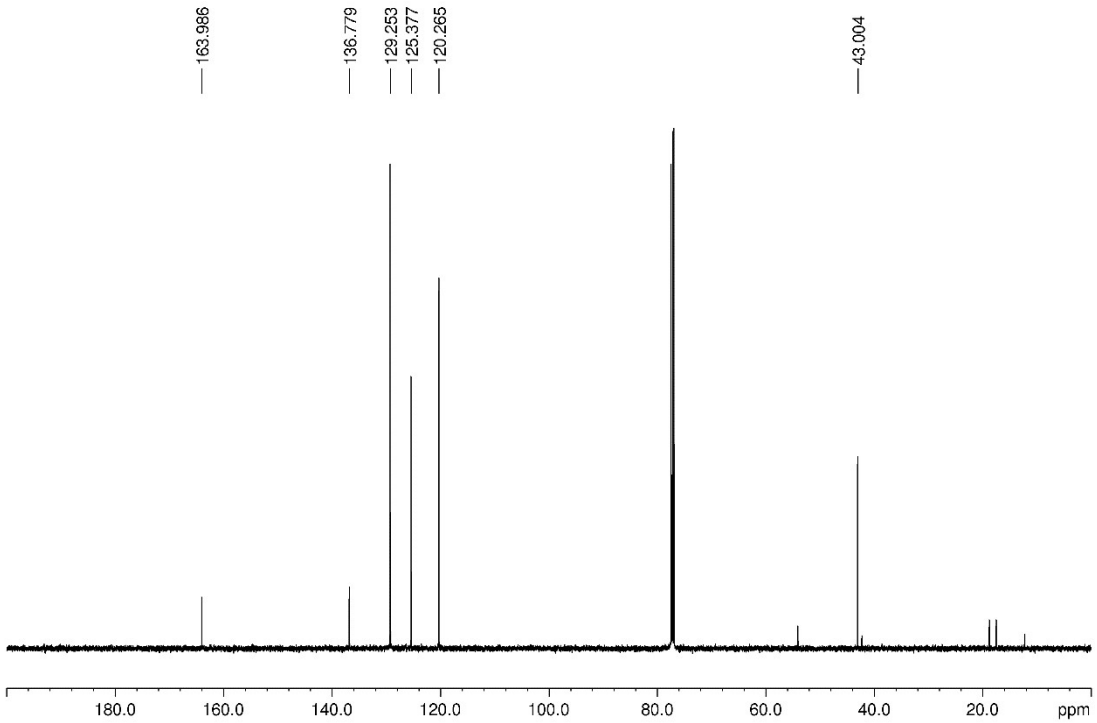
3.58-3.54 (m, 2 H, 2''-H), 3.27 (d, J = 8.0 Hz, 2 H, 2-H), 2.91 (t, J = 8.0 Hz, 1 H, SH), 2.66 (t, J = 7.1 Hz, 2 H, 1''-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.32 (1-C), 136.90 (1'-C), 134.56 (4'-C), 129.10 (2'-C, 6'-C), 119.04 (3'-C, 5'-C), 62.23 (2''-C), 38.46 (1''-C), 28.24 (2-C). HRMS (ESI) *m/z* calcd. for $C_{10}H_{14}NO_2S$ $[M+H]^+$ 212.0740, found 212.0733.

2-mercapto-*N*-(4'-sulfamoylphenyl)acetamide 25. Compound **25** was synthesized according to the general procedure **C**, using thioacetate **17** (23 mg, 0.08 mmol) and 5 mL KOH (1 M, H_2O/MeOH 1:1). The mix was stirred at room temperature for 1 h 15 min. After extraction, **25** was obtained as white solid (16 mg, 81%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.41 (s, 1 H, NH), 7.77 (d, J = 9.0 Hz, 2 H, aryl-H_a), 7.73 (d, J = 9.0 Hz, 2 H, aryl-H_b), 7.25 (s, 2 H, SO_2NH_2), 3.33 (m, 2H, 2-C (HMBC)), 2.99 (t, J = 8.0 Hz, 1 H, SH) ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.19 (1-C), 141.87 (1'-C or 4'-C), 138.52 (1'-C or 4'-C), 126.76 (aryl-C_a), 118.66 (aryl-C_b), 28.34 (2-C). HRMS (ESI) *m/z* calcd. for $C_8H_{11}N_2O_3S_2$ $[M+H]^+$ 247.0206, found 247.0201.

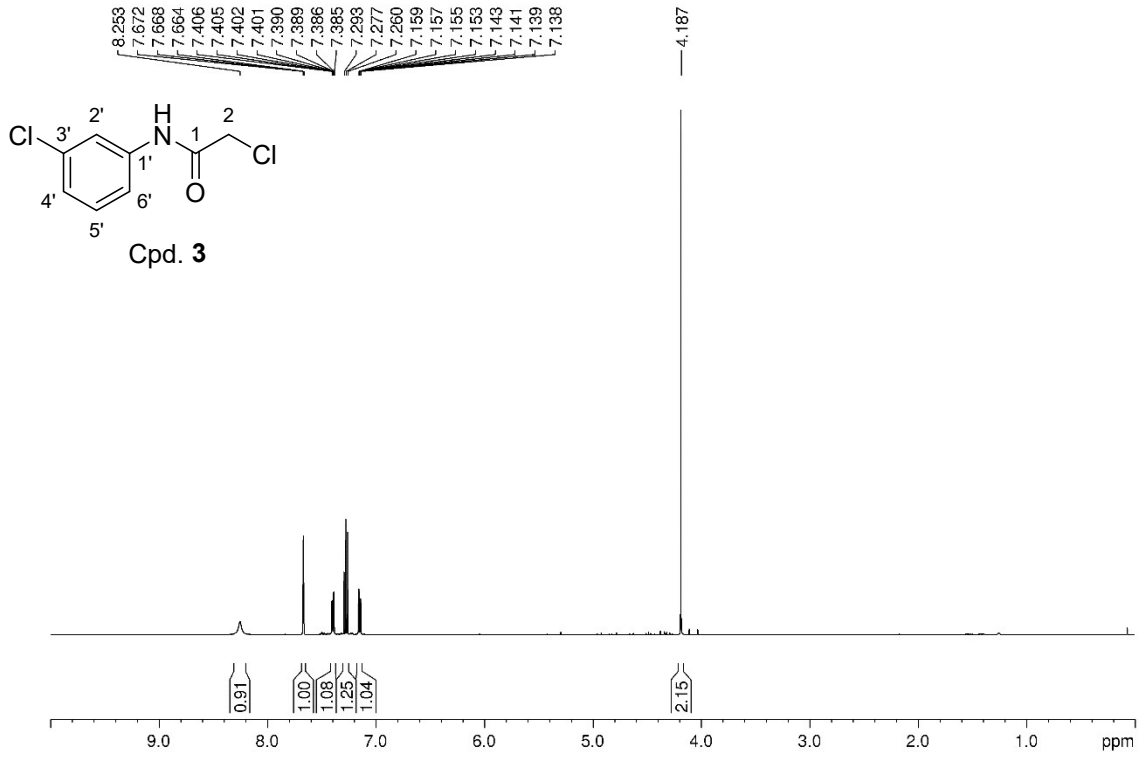
*[1]H and [13]C NMR spectra of compounds **2–25***



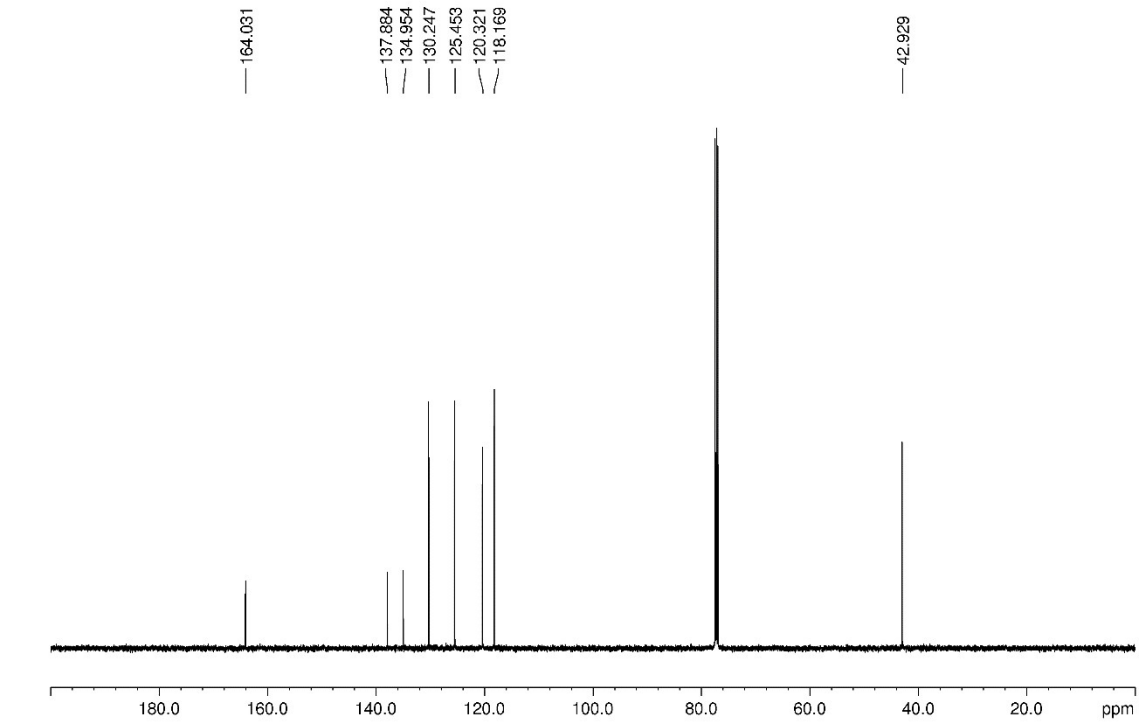
^{1}H NMR of Cpd. **2** (500 MHz, $CDCl_3$)



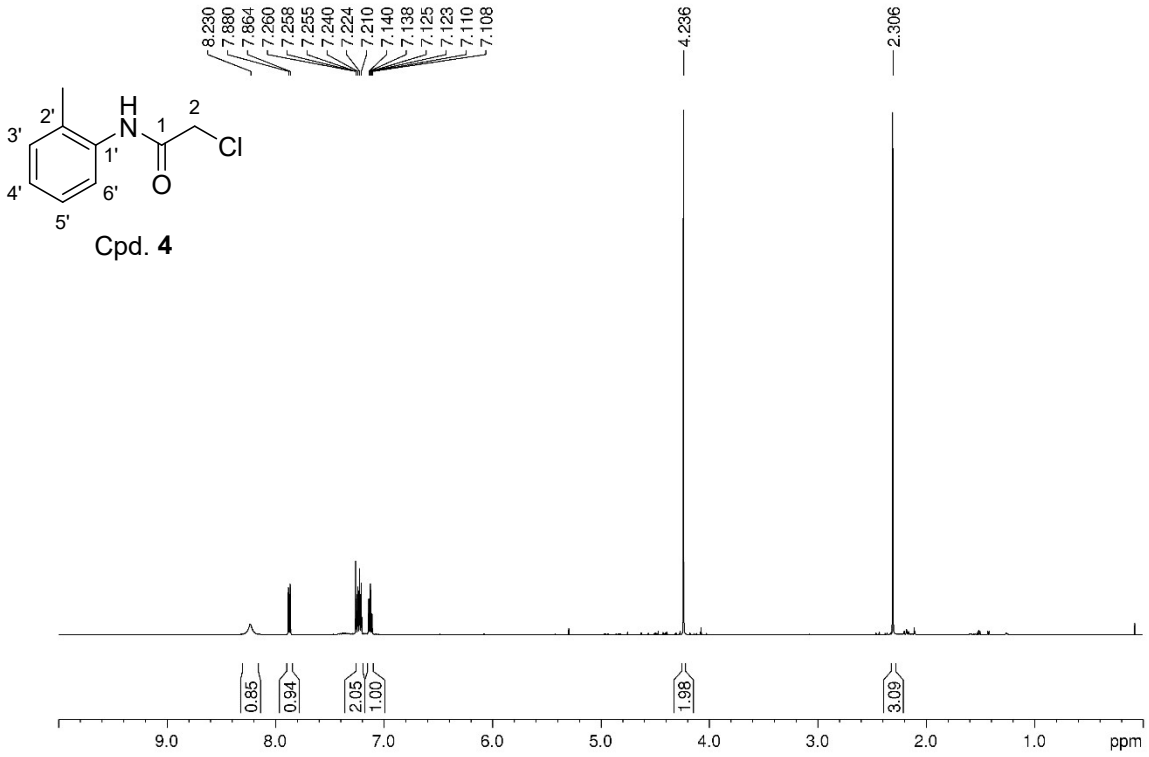
^{13}C NMR of Cpd. **2** (126 MHz, $CDCl_3$)



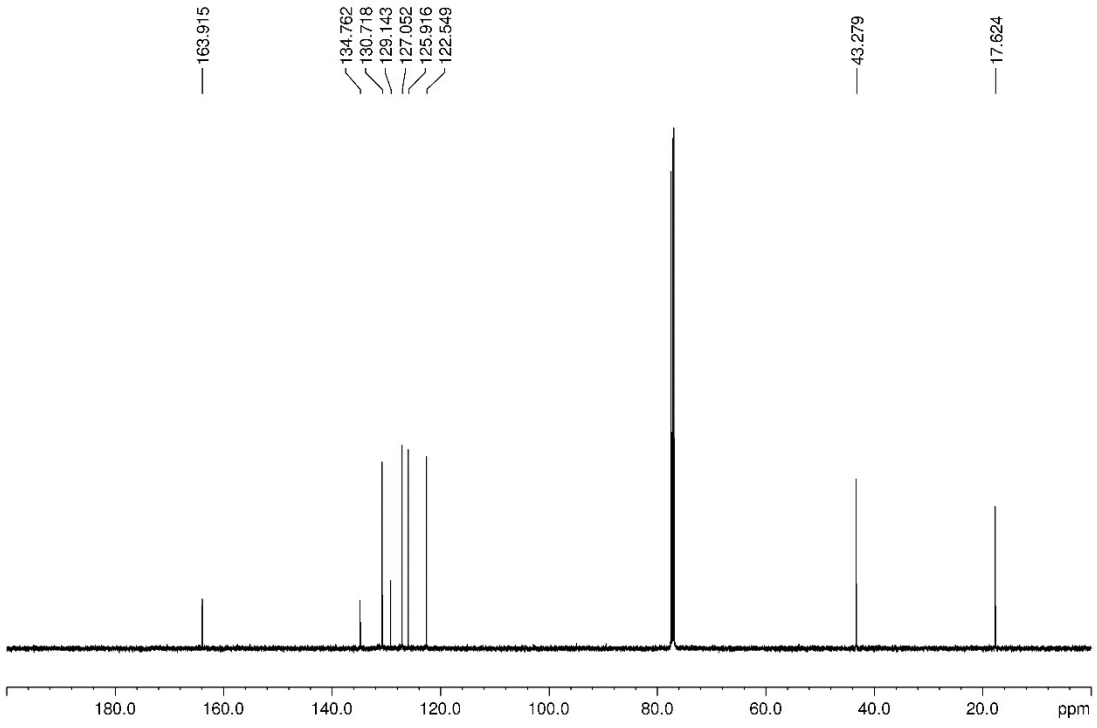
^{1}H NMR of Cpd. **3** (500 MHz, $CDCl_3$)



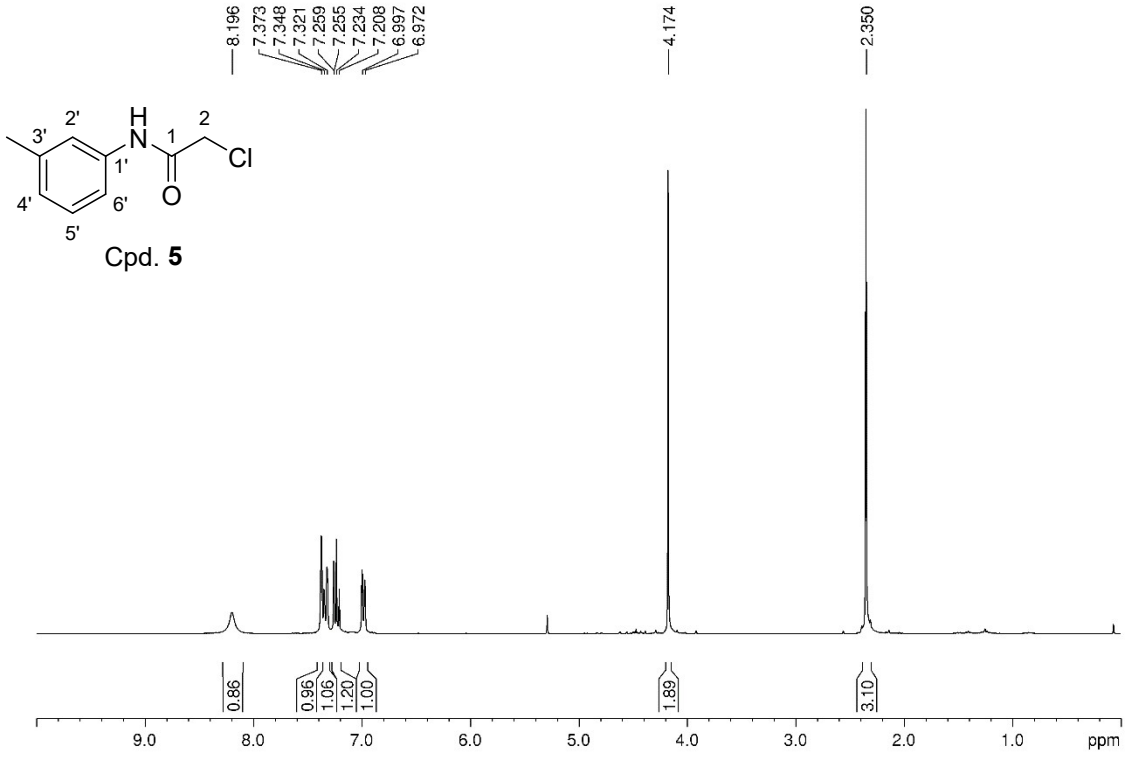
^{13}C NMR of Cpd. **3** (126 MHz, $CDCl_3$)



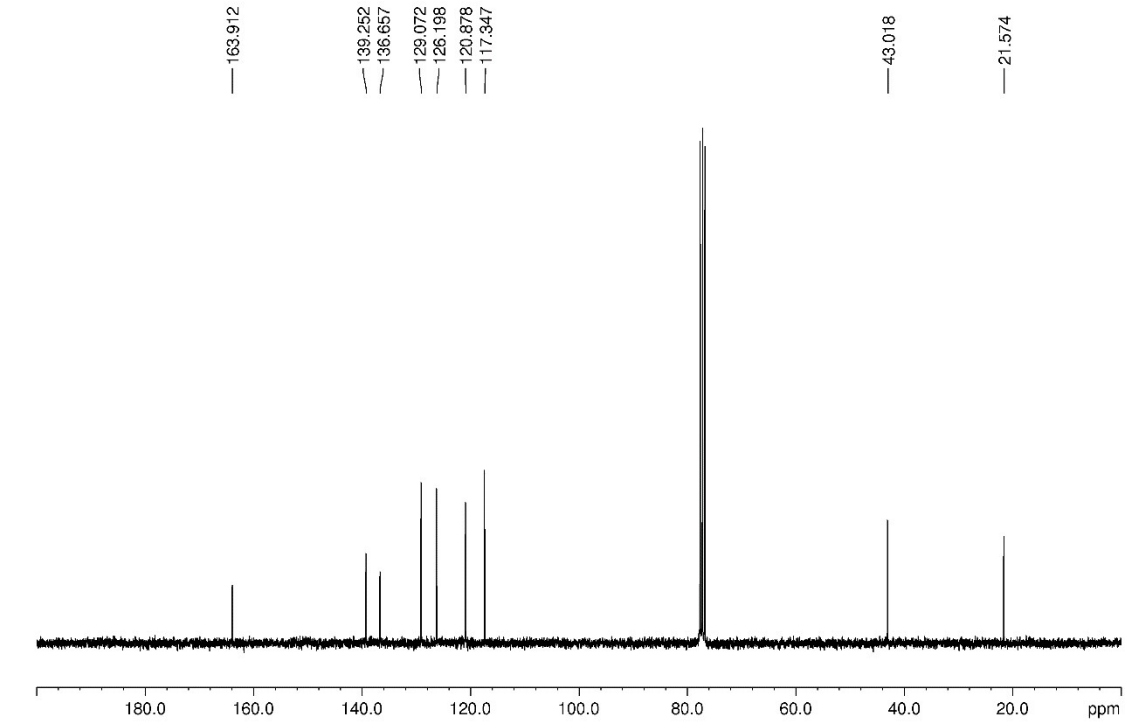
^{1}H NMR of Cpd. **4** (500 MHz, $CDCl_3$)



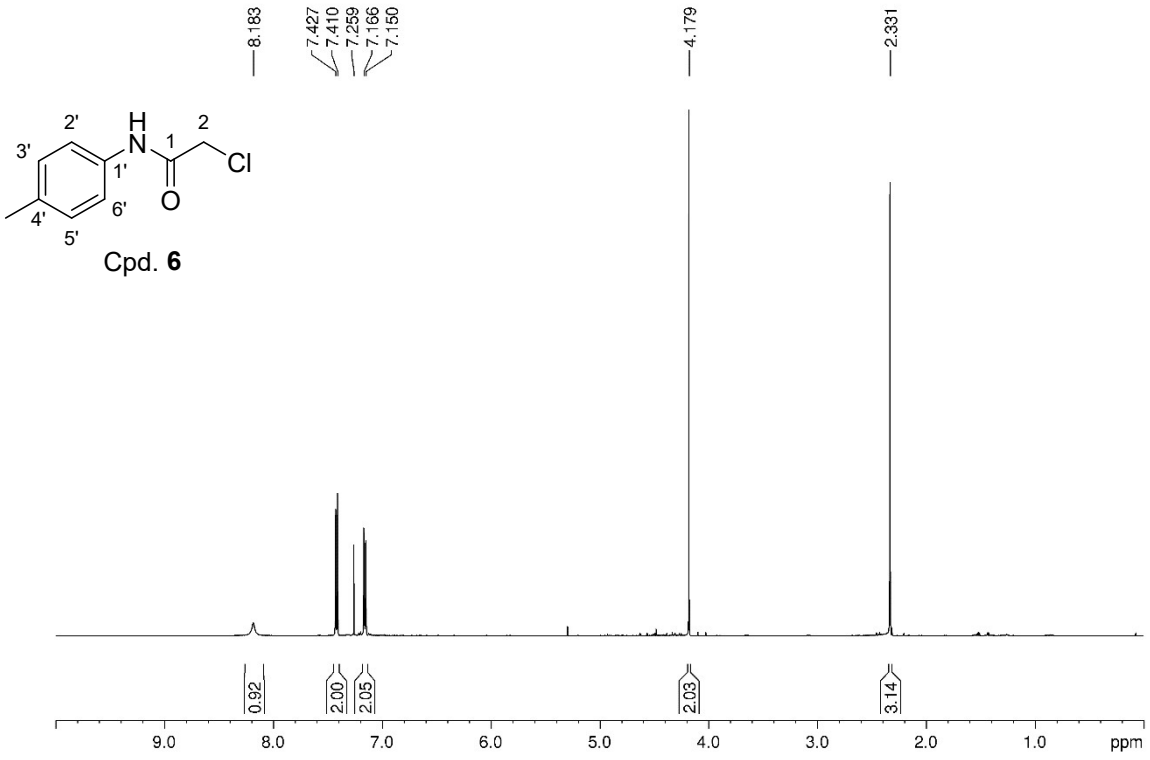
^{13}C NMR of Cpd. **4** (126 MHz, $CDCl_3$)



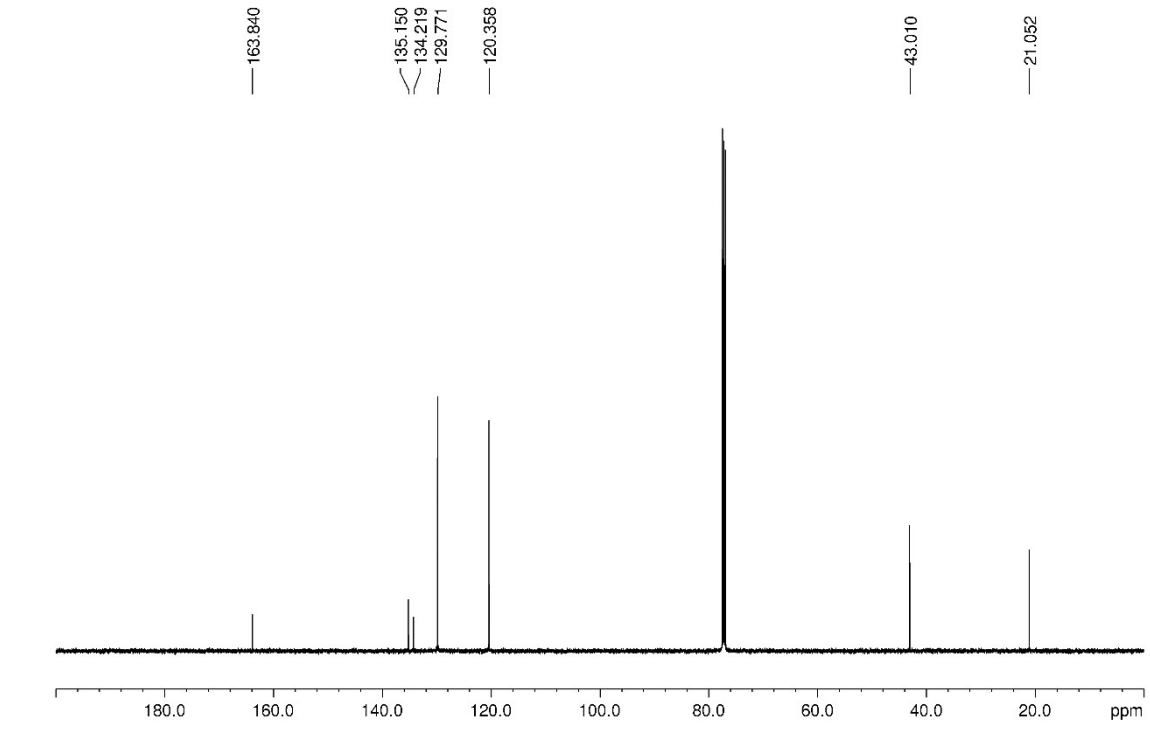
^{1}H NMR of Cpd. **5** (500 MHz, $CDCl_3$)



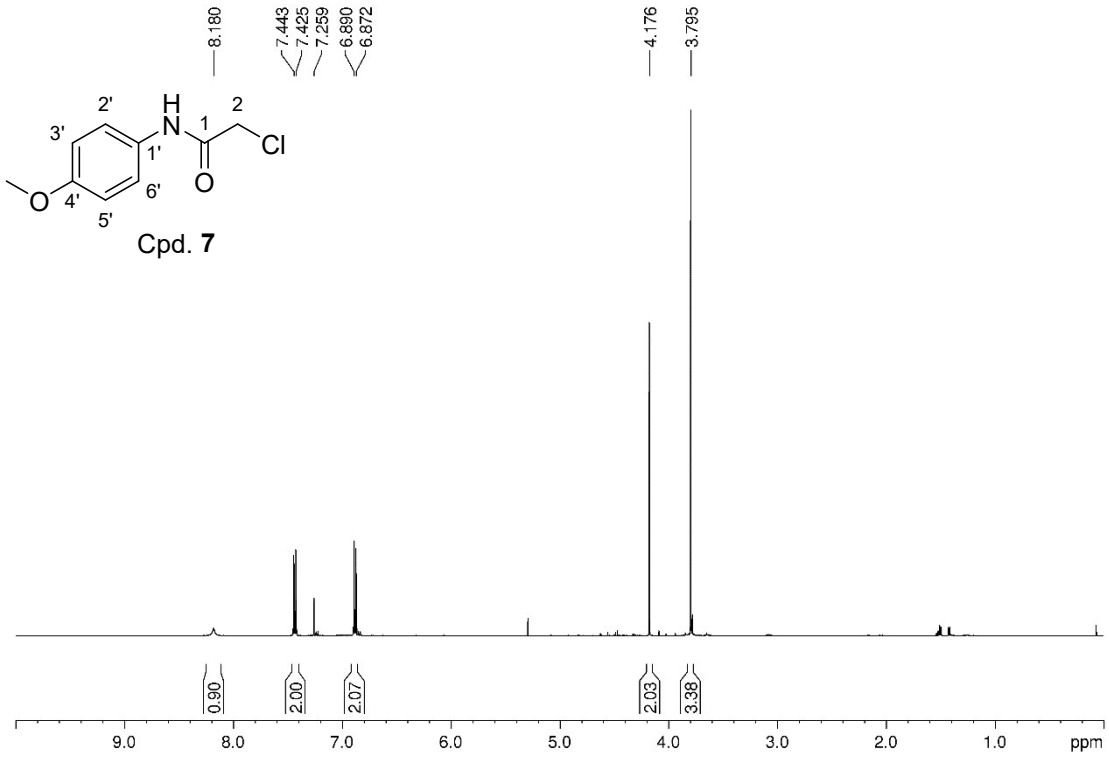
^{13}C NMR of Cpd. **5** (126 MHz, $CDCl_3$)



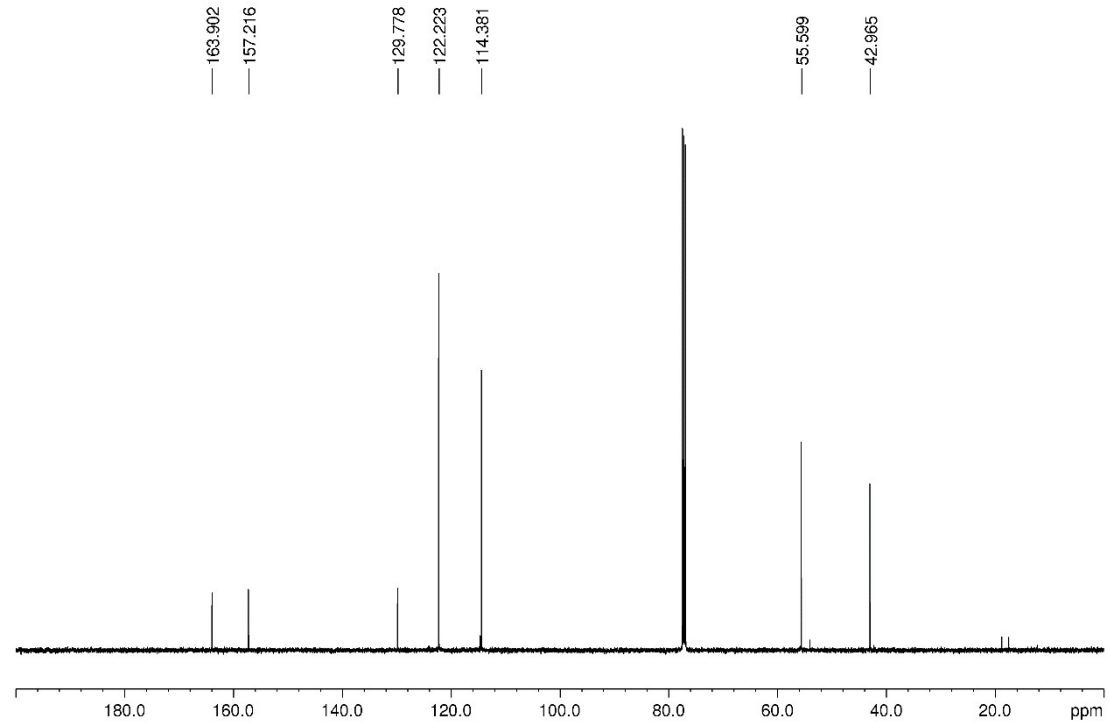
^{1}H NMR of Cpd. **6** (500 MHz, $CDCl_3$)



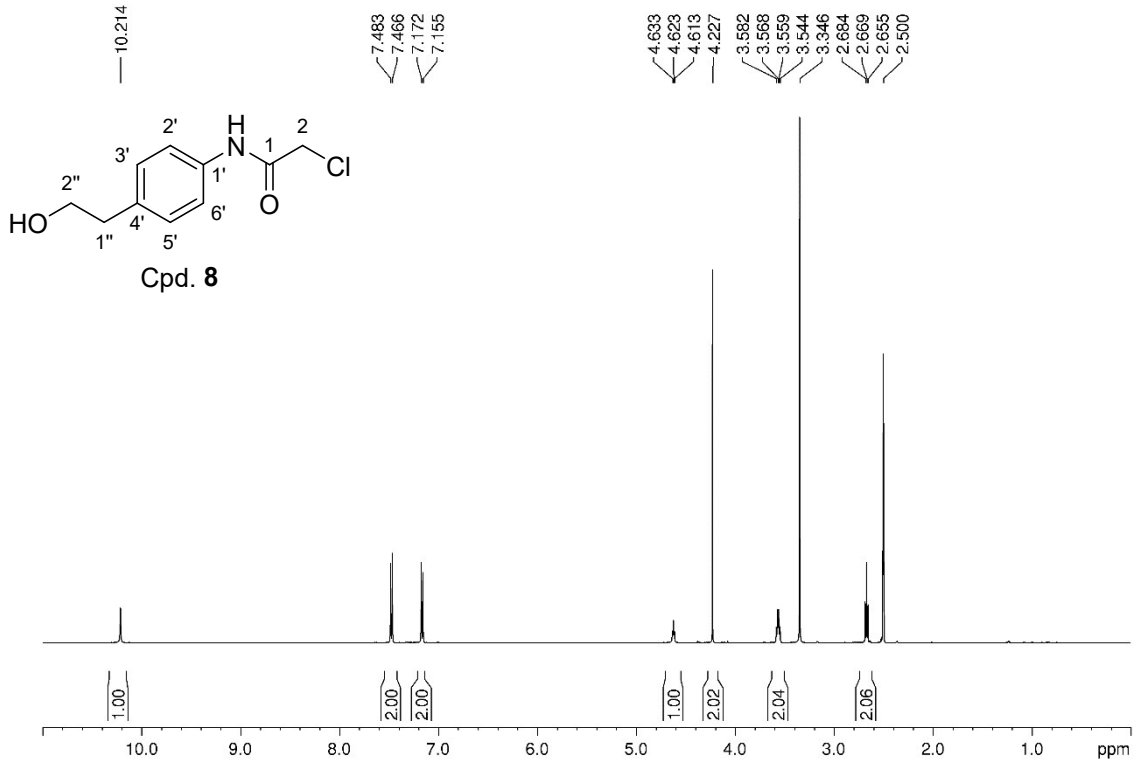
^{13}C NMR of Cpd. **6** (126 MHz, $CDCl_3$)



^{1}H NMR of Cpd. **7** (500 MHz, $CDCl_3$)

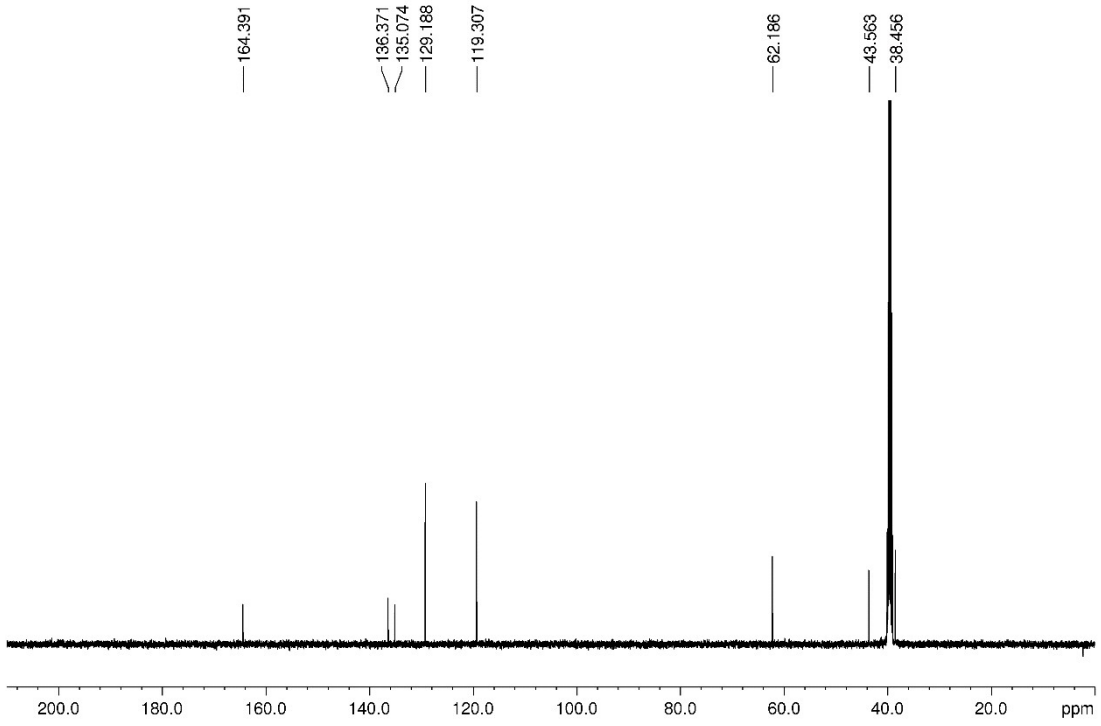


^{13}C NMR of Cpd. **7** (126 MHz, $CDCl_3$)

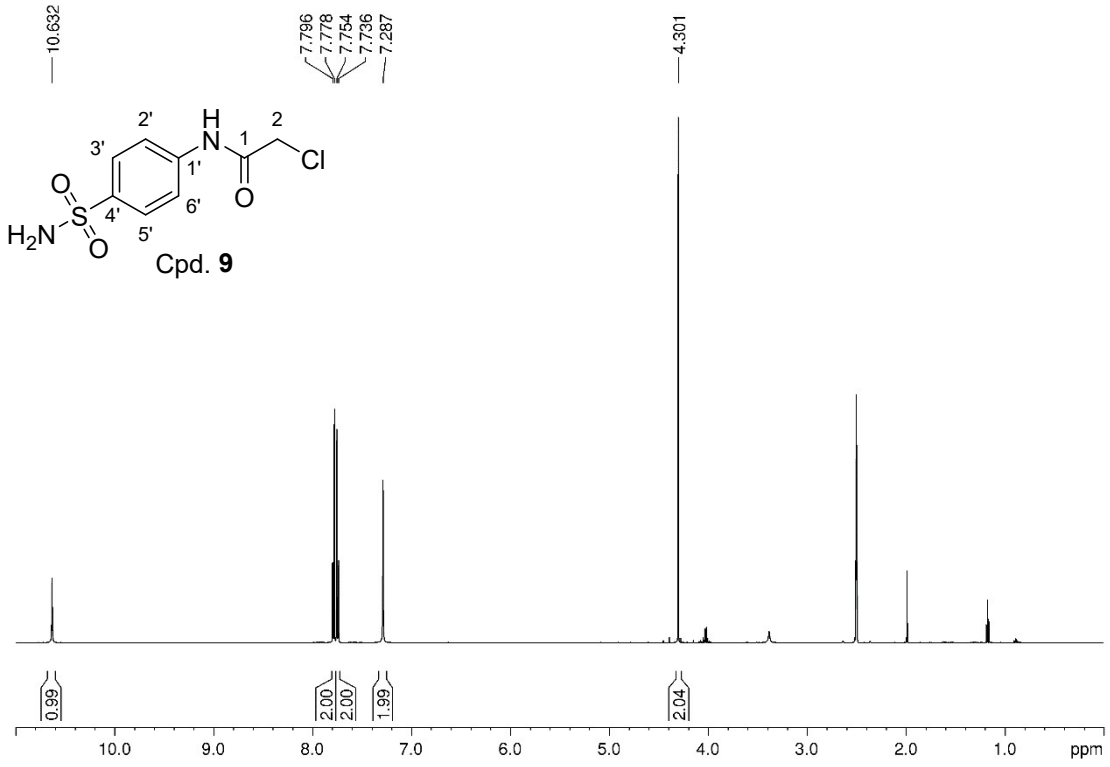


Cpd. **8**

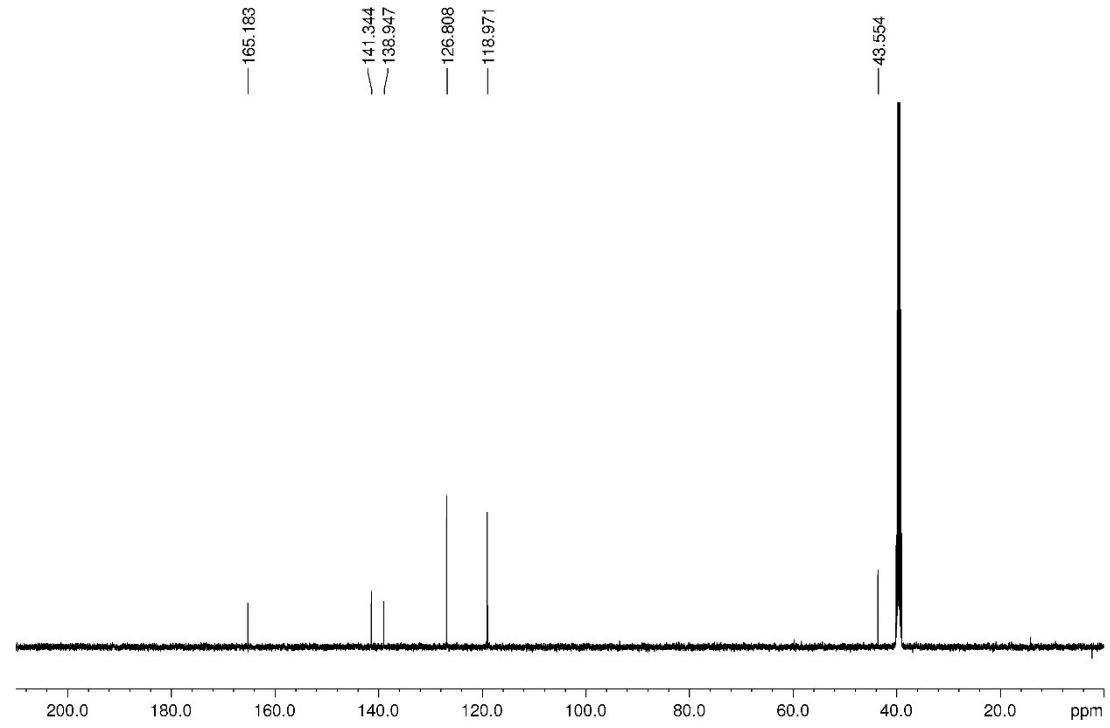
^{1}H NMR of Cpd. **8** (500 MHz, DMSO-d_6)



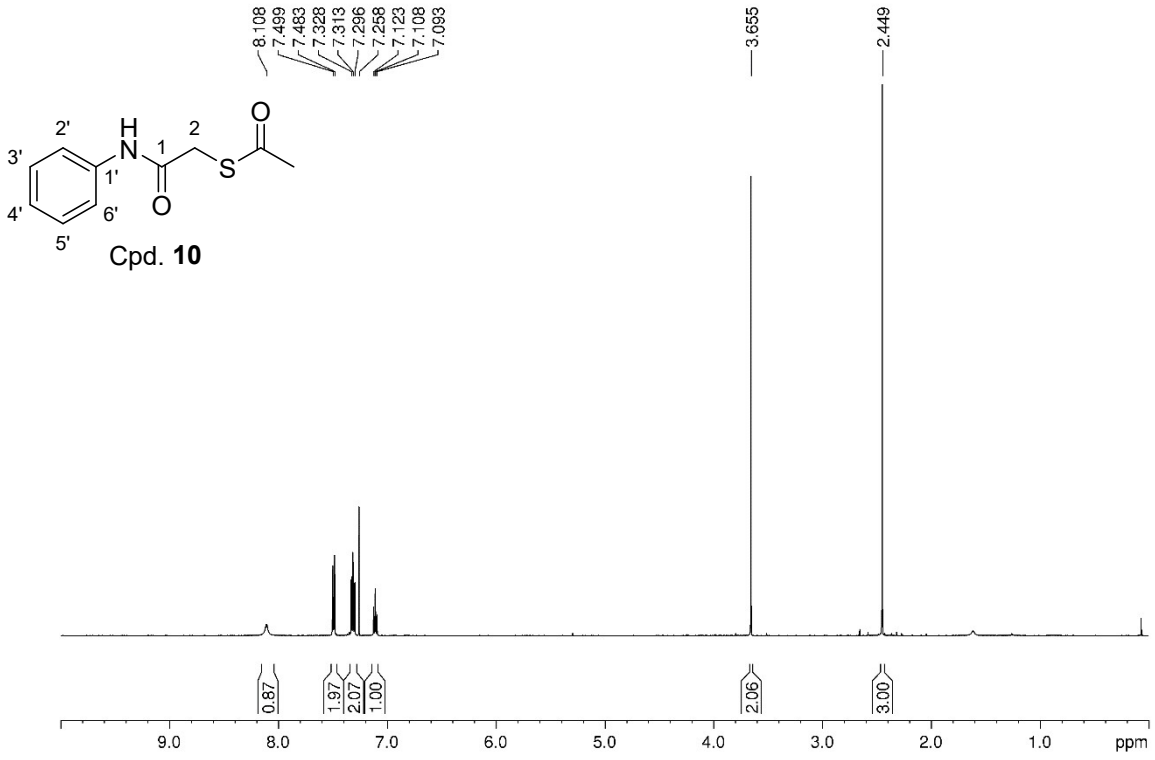
^{13}C NMR of Cpd. **8** (126 MHz, DMSO-d_6)



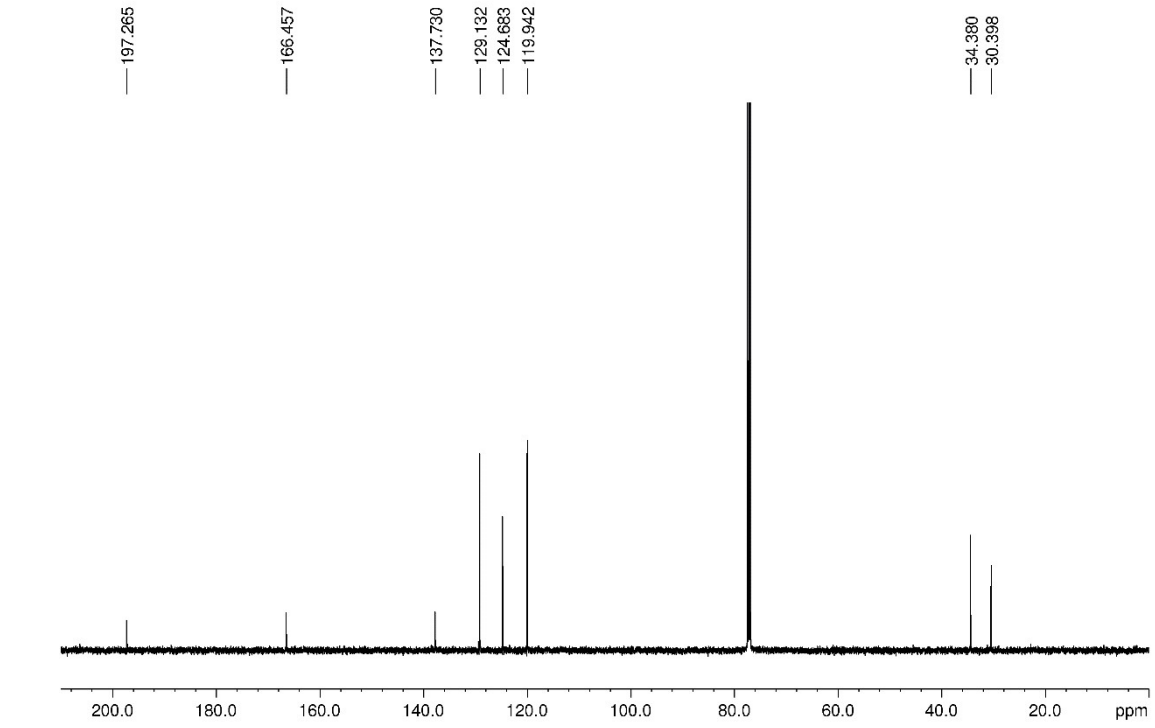
^{1}H NMR of Cpd. **9** (500 MHz, DMSO-d_6)



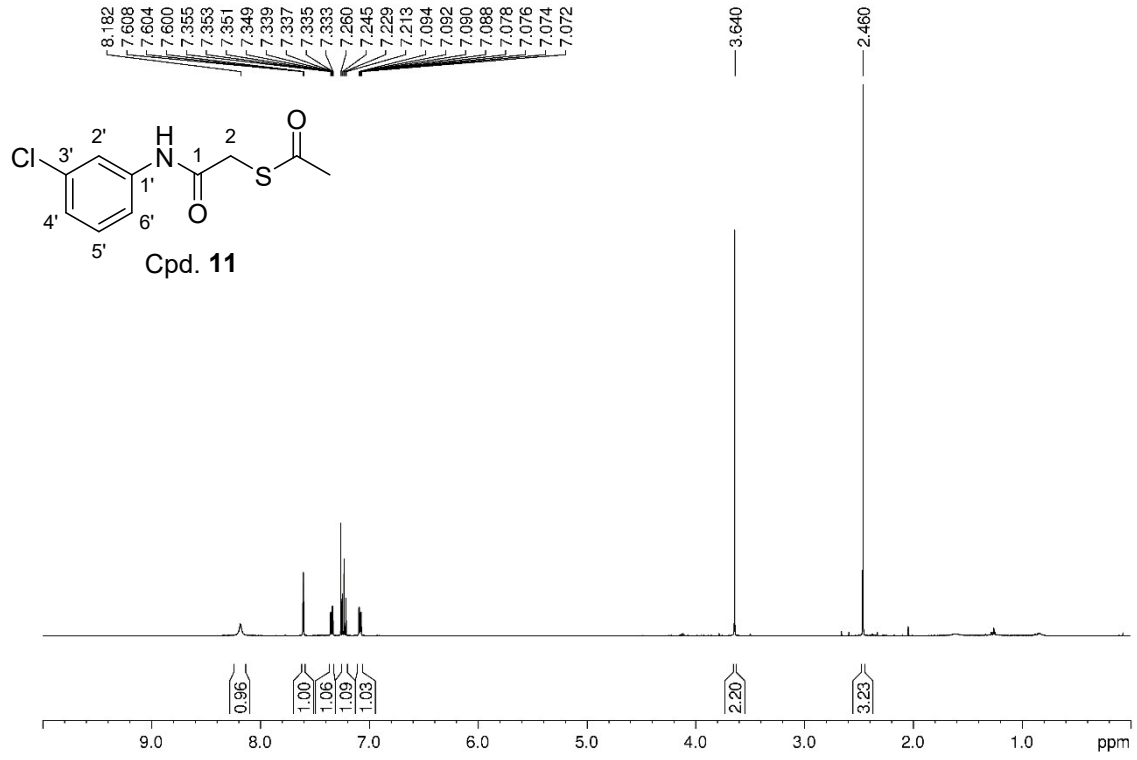
^{13}C NMR of Cpd. **9** (126 MHz, DMSO-d_6)



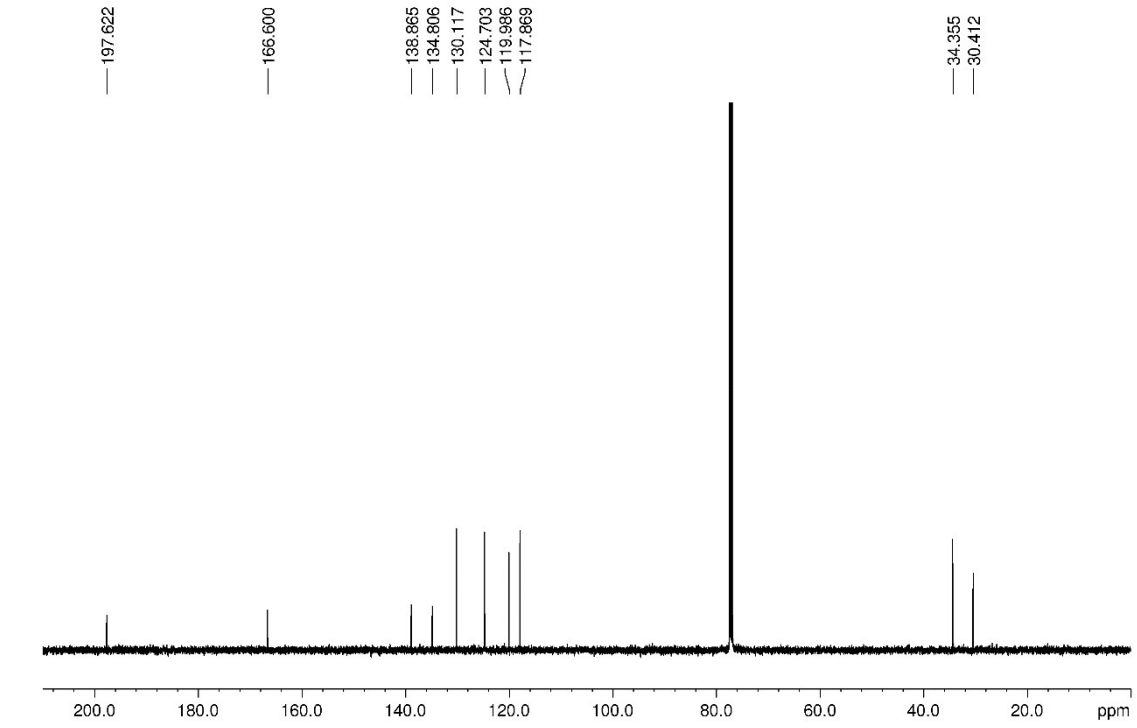
^{1}H NMR of Cpd. **10** (500 MHz, $CDCl_3$)



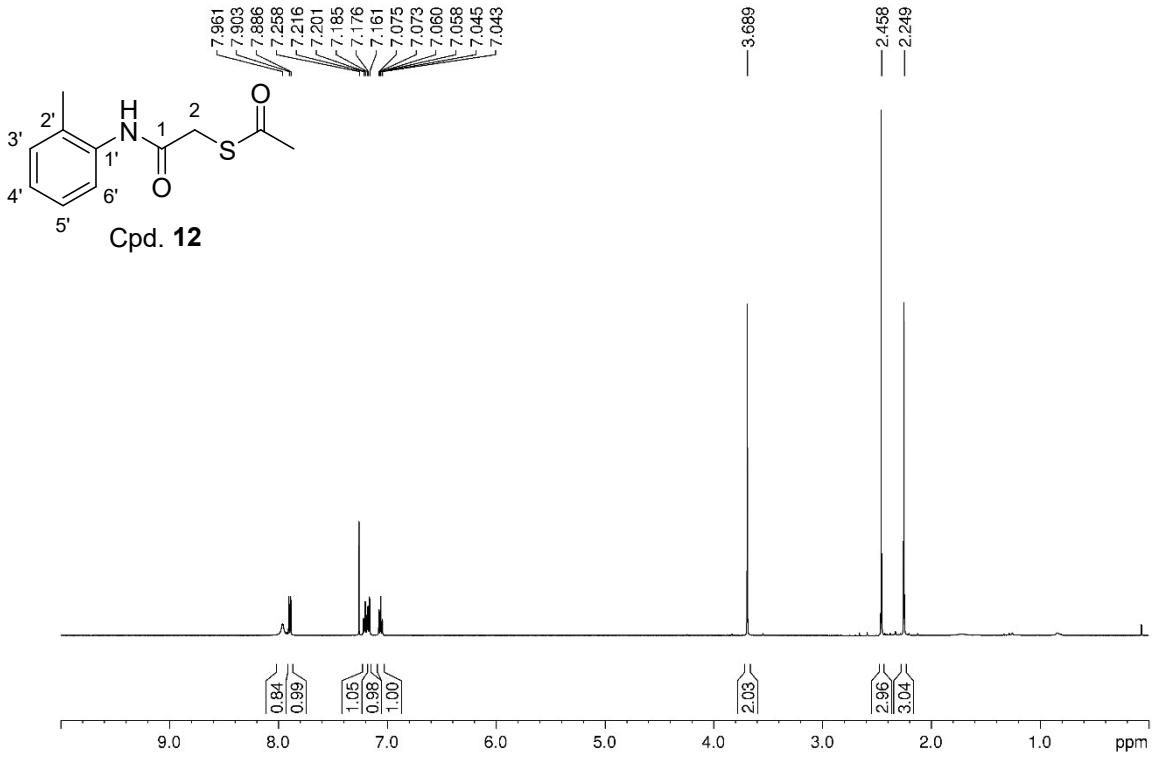
^{13}C NMR of Cpd. **10** (126 MHz, $CDCl_3$)



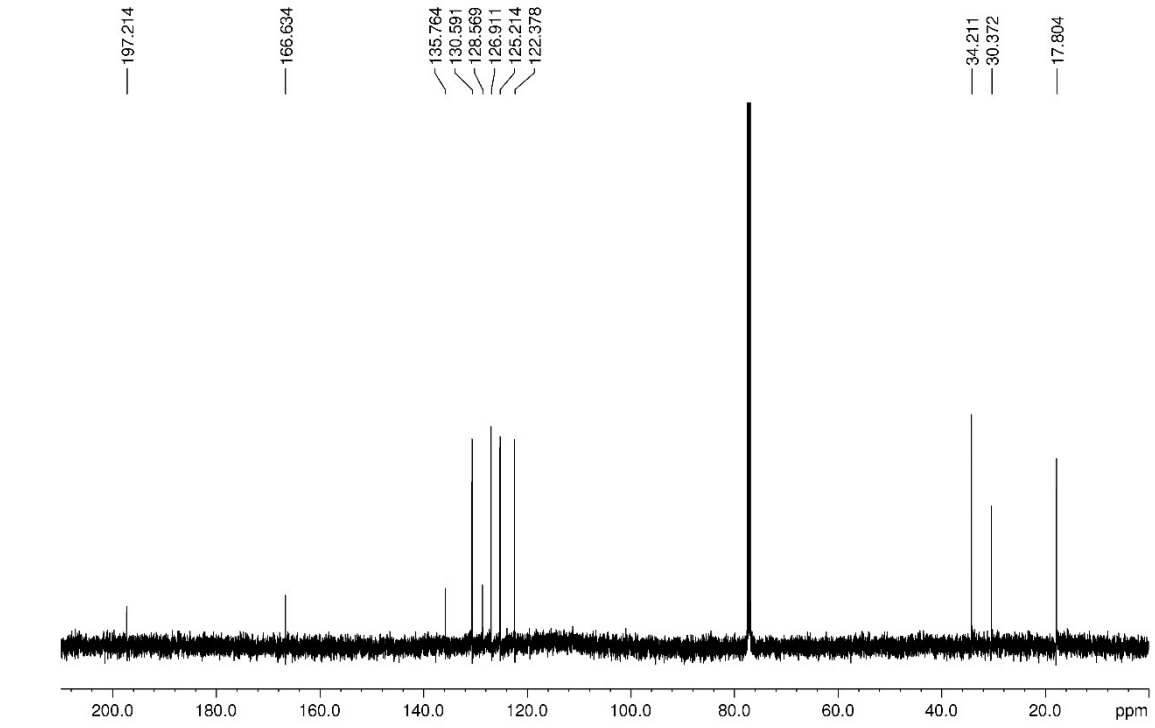
^{1}H NMR of Cpd. **11** (500 MHz, $CDCl_3$)



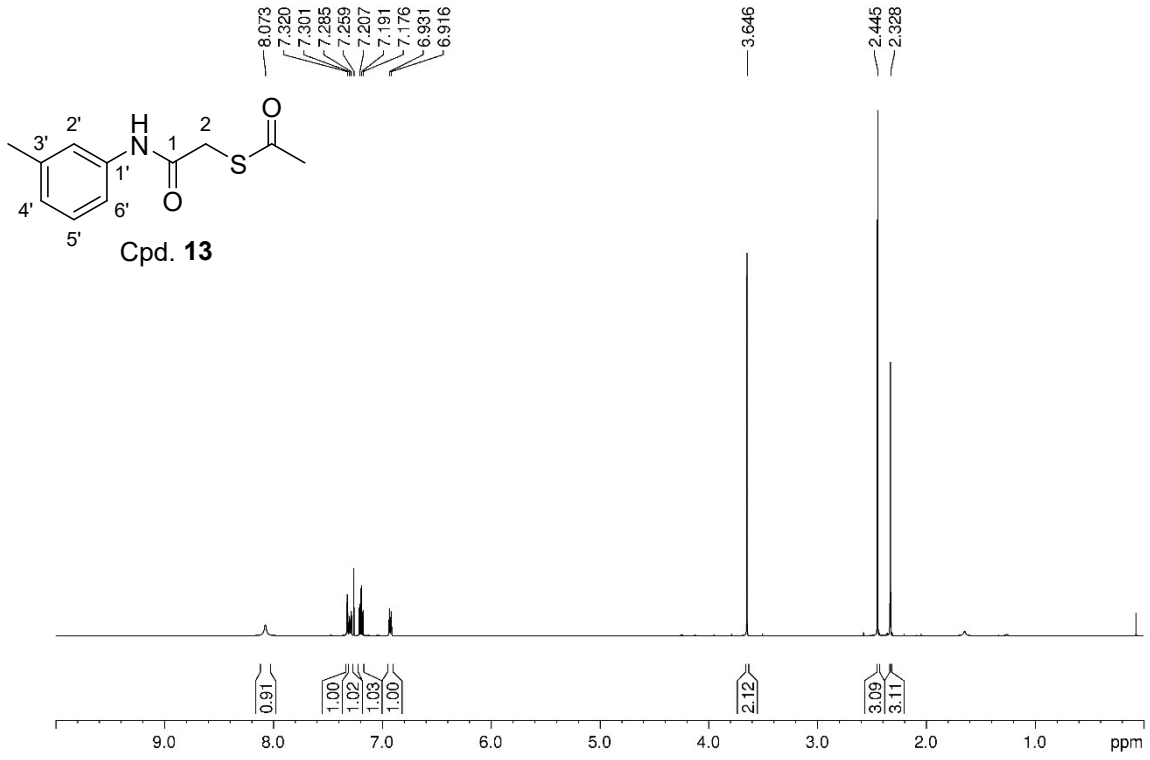
^{13}C NMR of Cpd. **11** (126 MHz, $CDCl_3$)



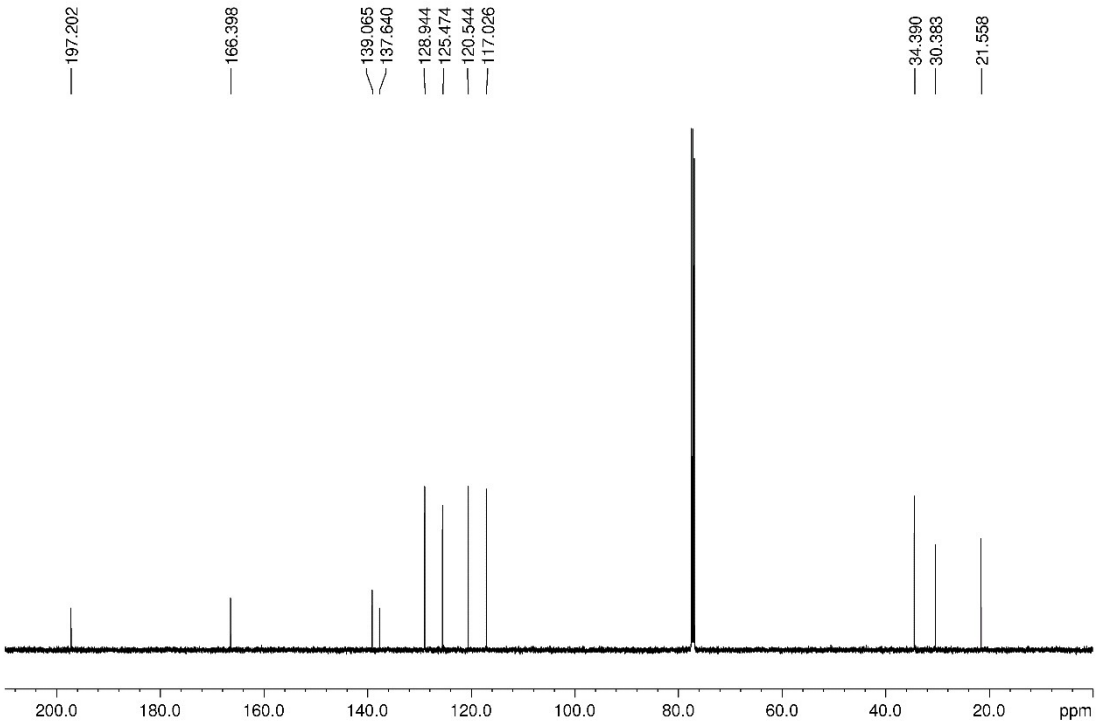
^{1}H NMR of Cpd. **12** (500 MHz, $CDCl_3$)



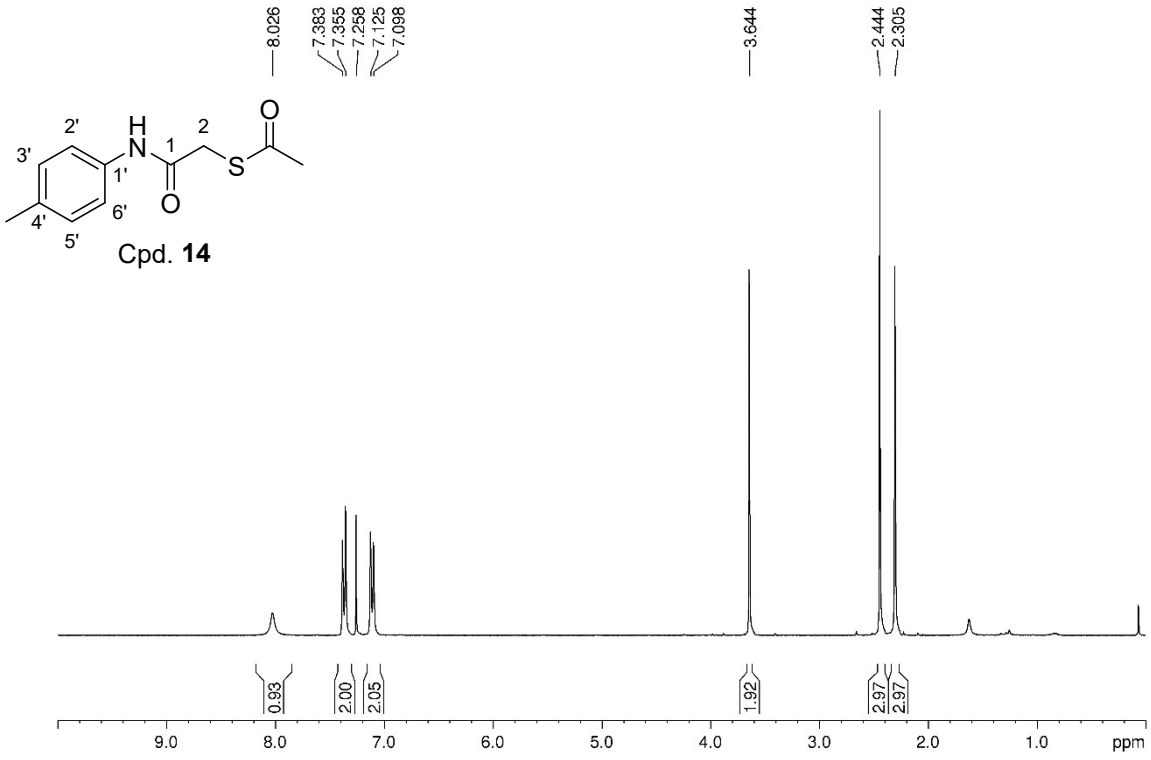
^{13}C NMR of Cpd. **12** (126 MHz, $CDCl_3$)



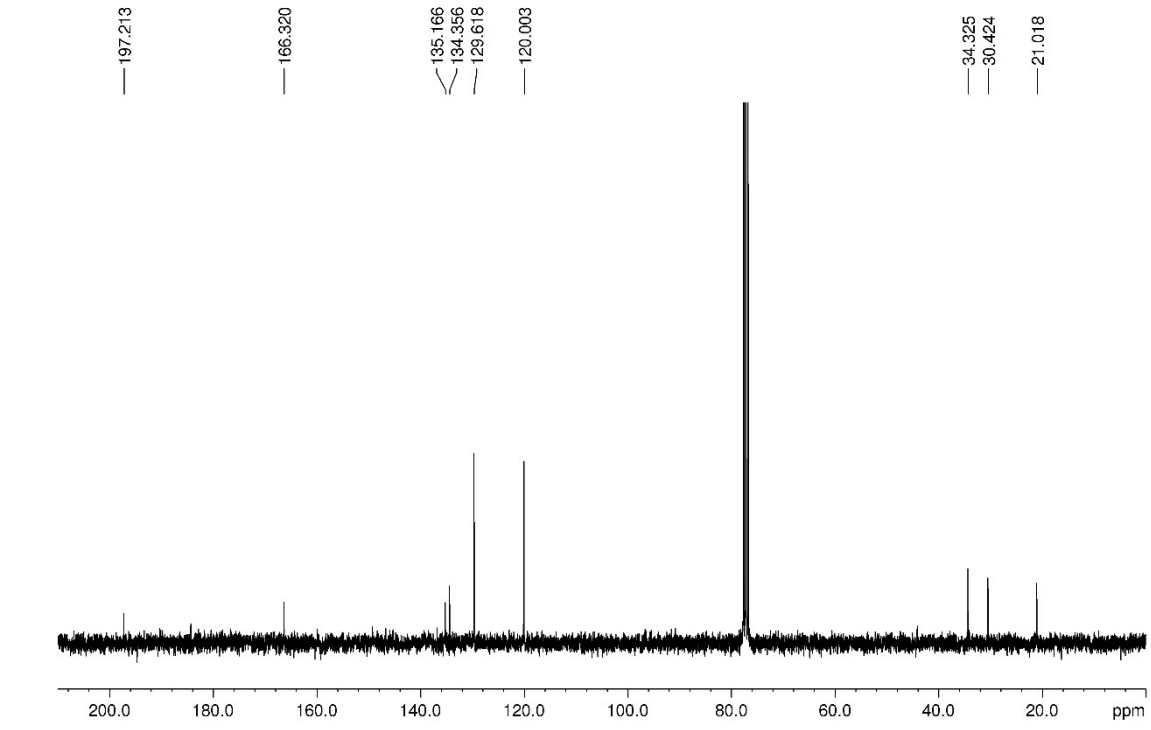
^{1}H NMR of Cpd. **13** (500 MHz, $CDCl_3$)



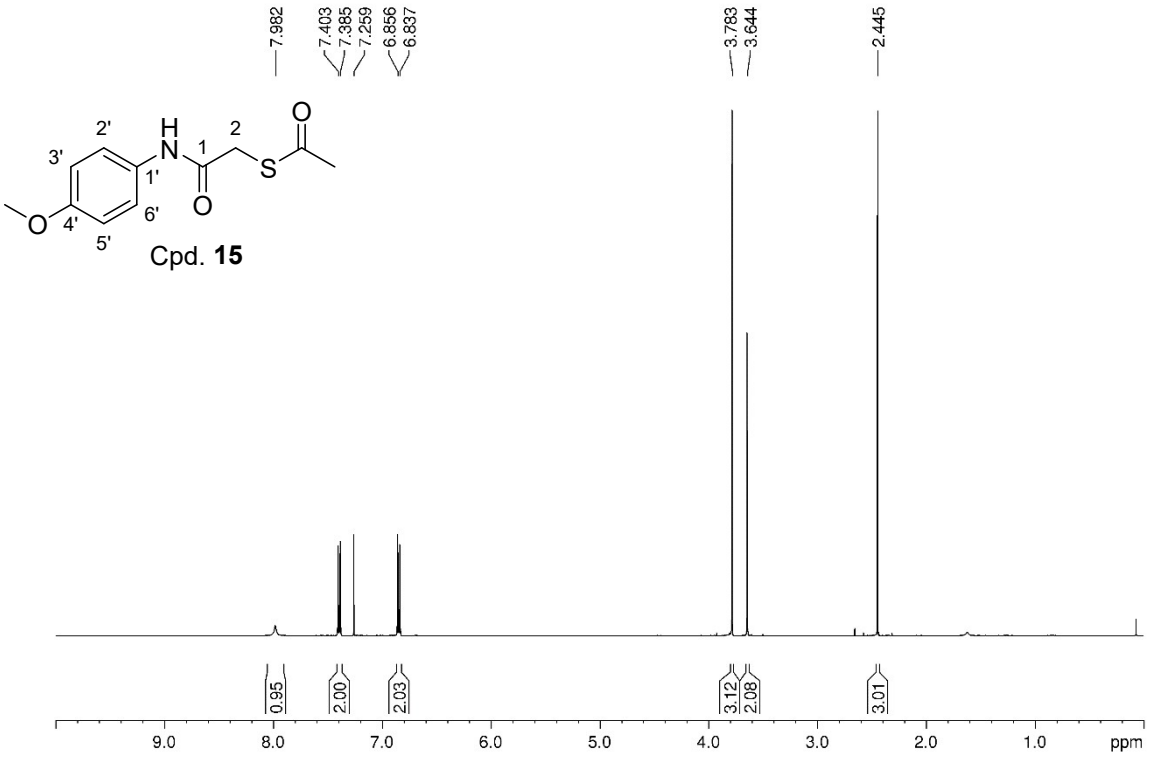
^{13}C NMR of Cpd. **13** (126 MHz, $CDCl_3$)



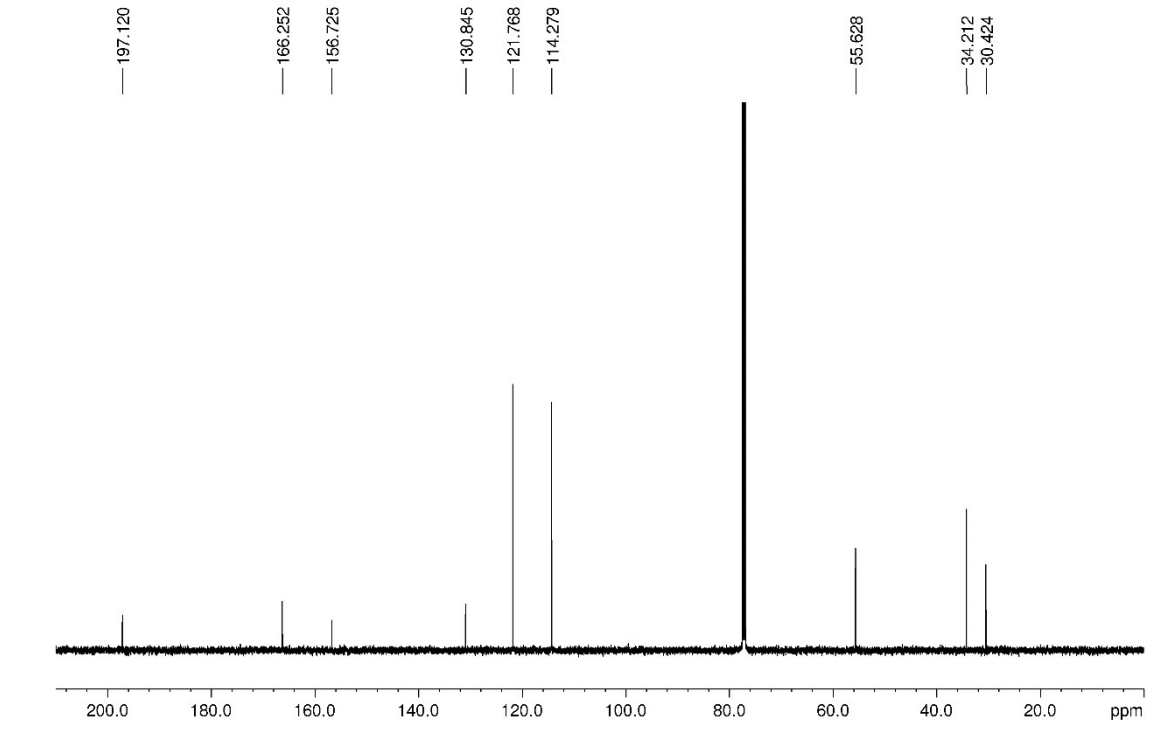
^{1}H NMR of Cpd. **14** (300 MHz, $CDCl_3$)



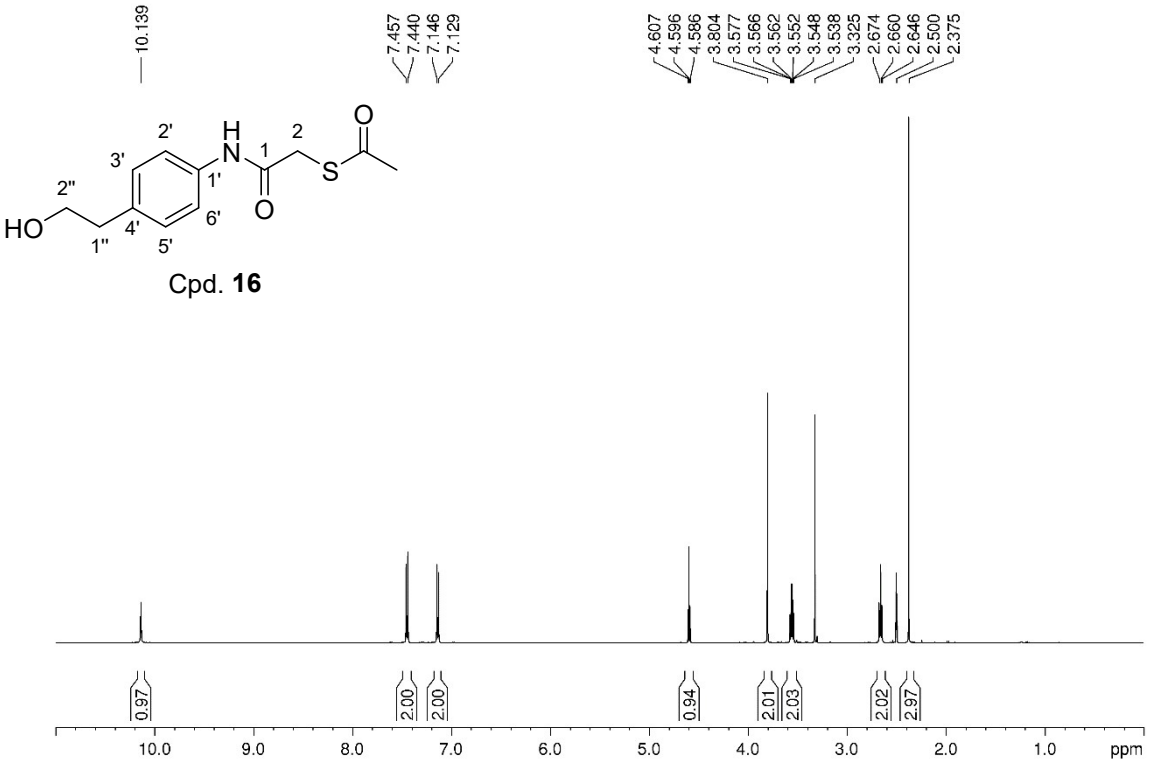
^{13}C NMR of Cpd. **14** (75 MHz, $CDCl_3$)



^{1}H NMR of Cpd. **15** (500 MHz, $CDCl_3$)

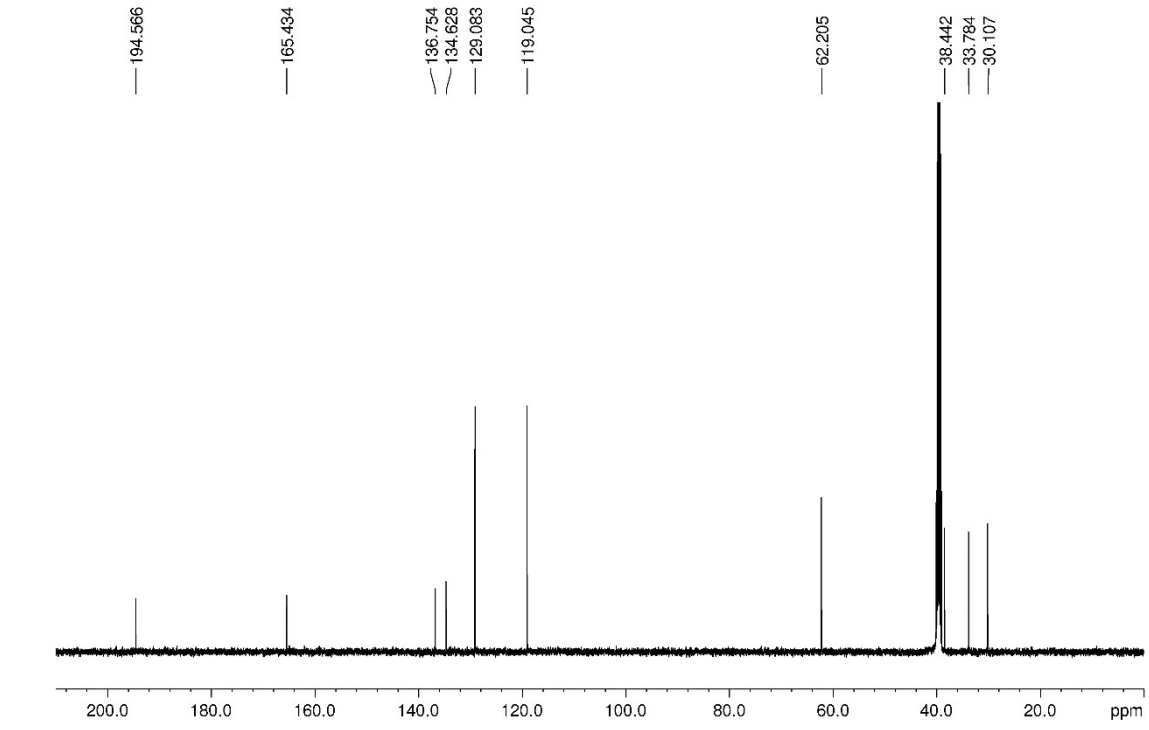


^{13}C NMR of Cpd. **15** (126 MHz, $CDCl_3$)

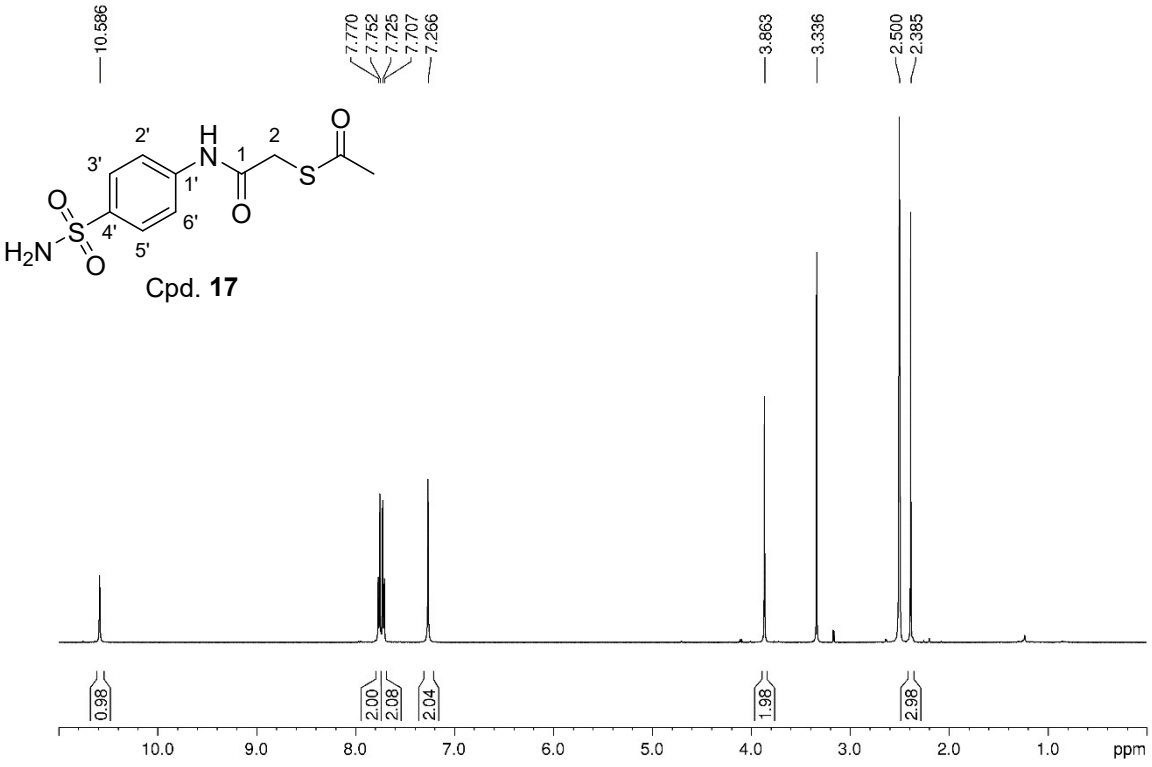


Cpd. **16**

^{1}H NMR of Cpd. **16** (500 MHz, DMSO-d_6)

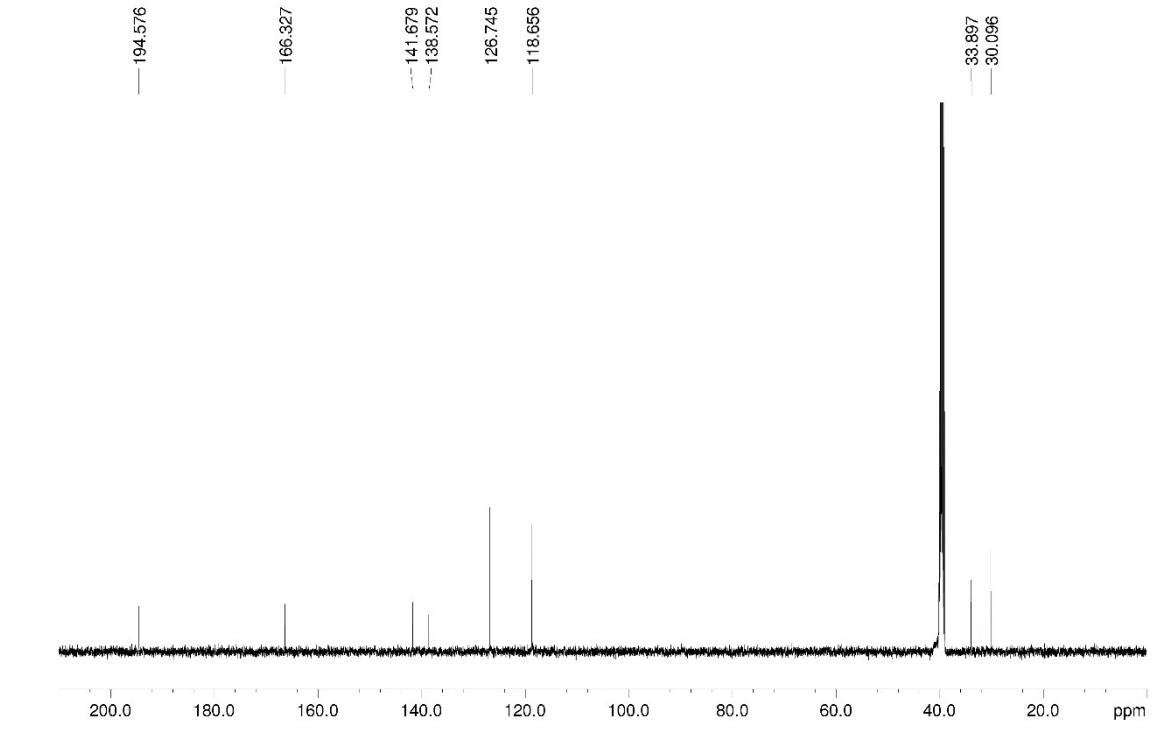


^{13}C NMR of Cpd. **16** (126 MHz, DMSO-d_6)

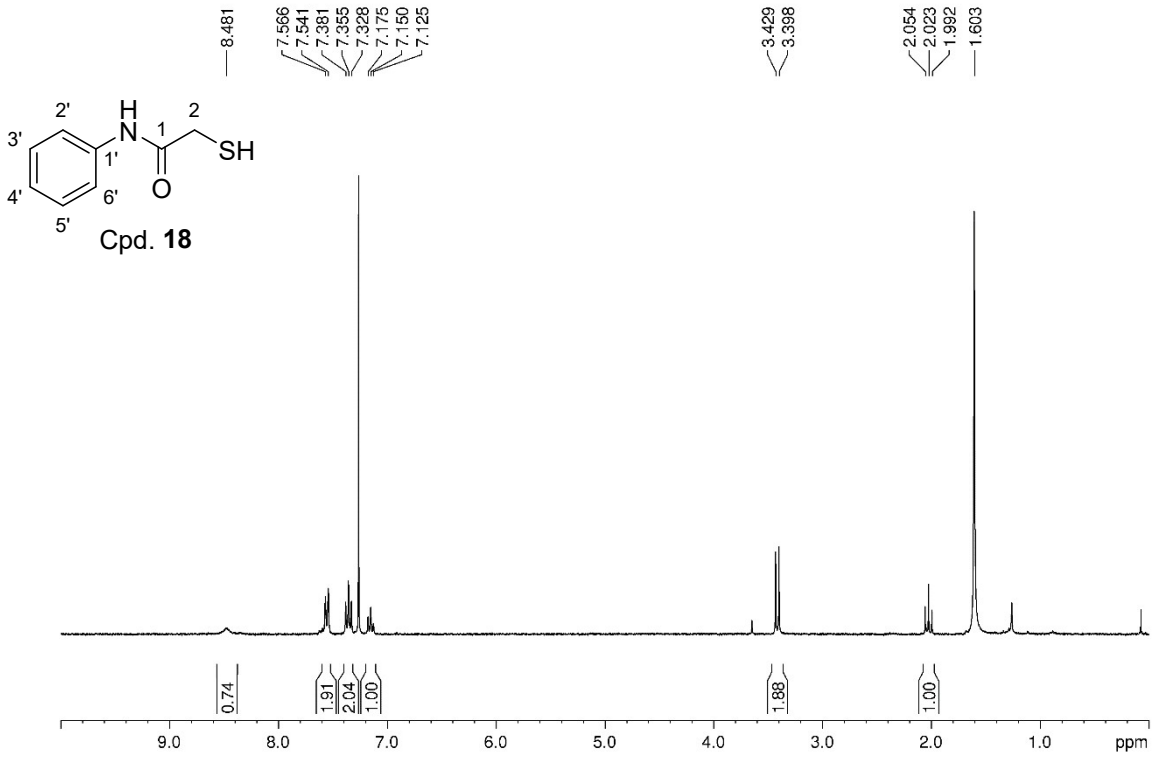


Cpd. **17**

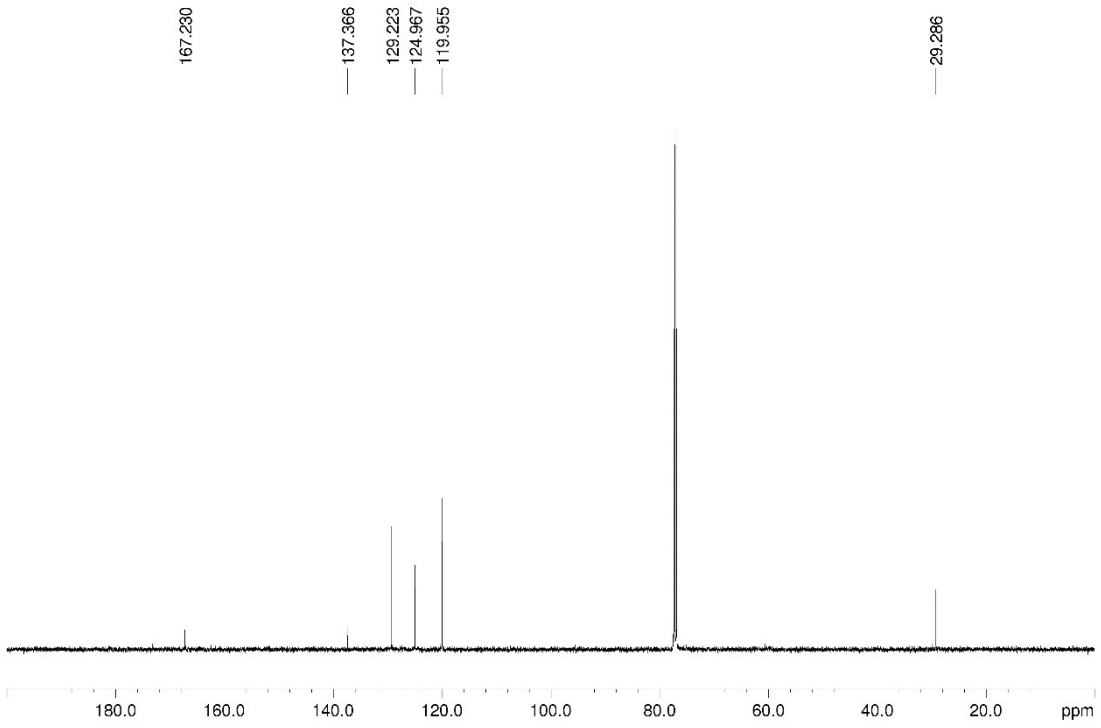
^{1}H NMR of Cpd. **17** (500 MHz, DMSO-d_6)



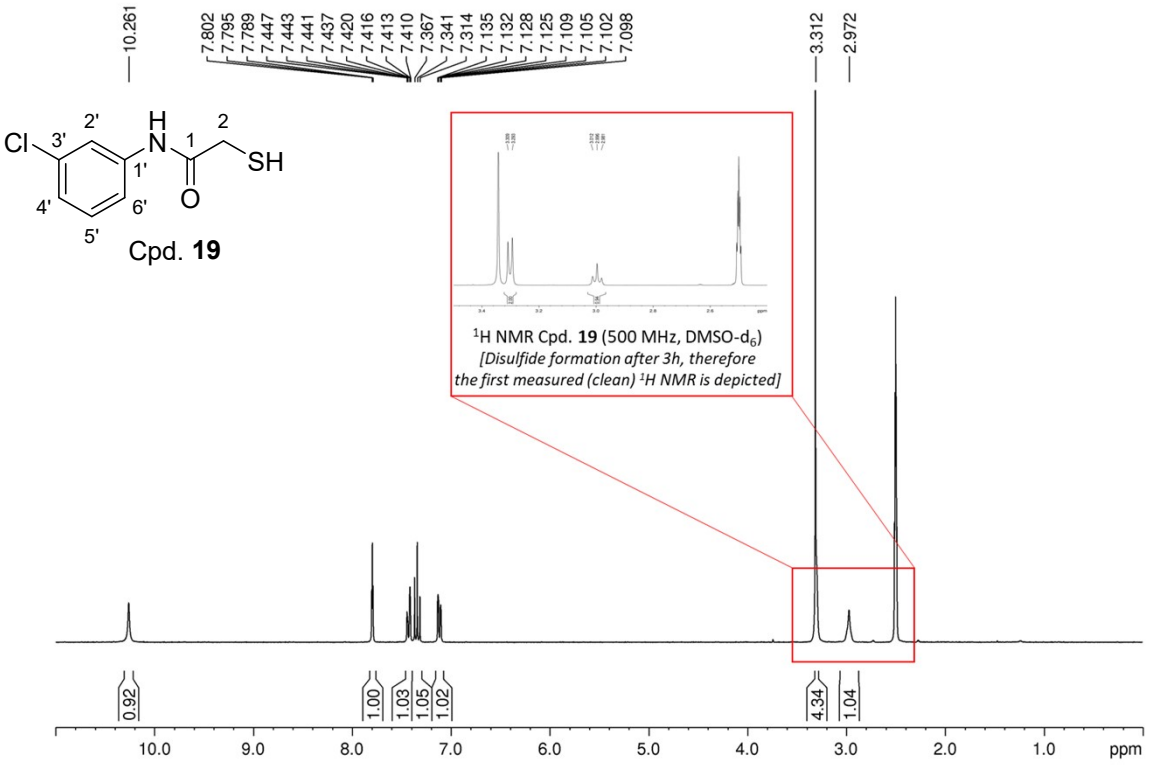
^{13}C NMR of Cpd. **17** (126 MHz, DMSO-d_6)



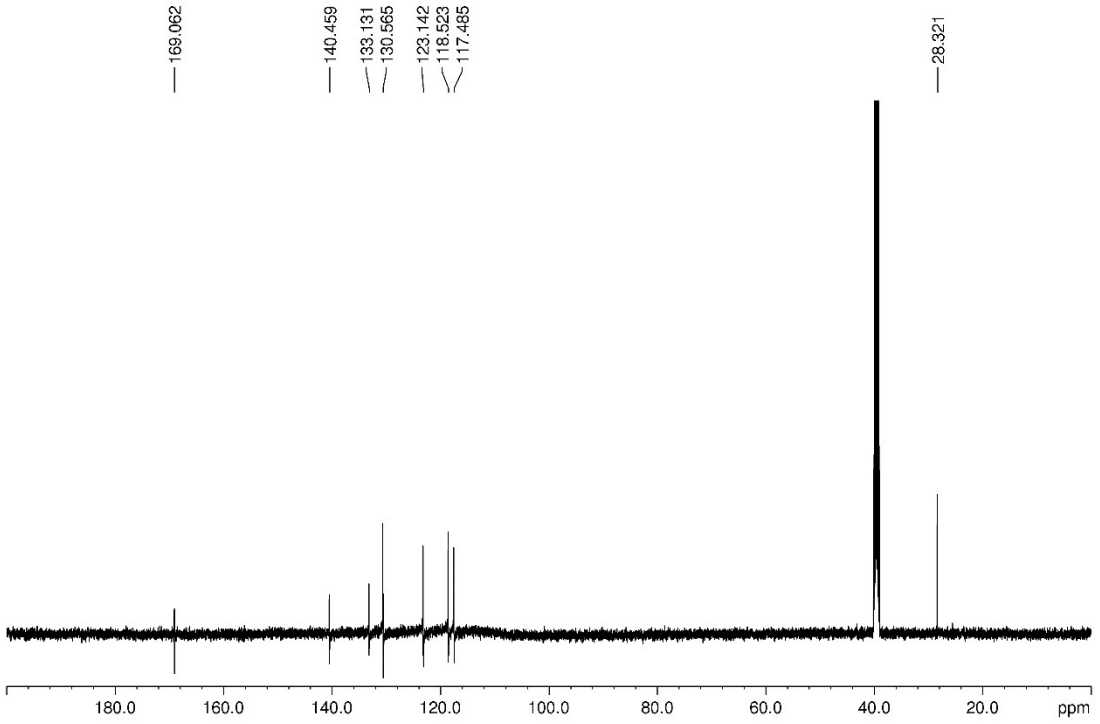
^{1}H NMR of Cpd. **18** (300 MHz, $CDCl_3$)



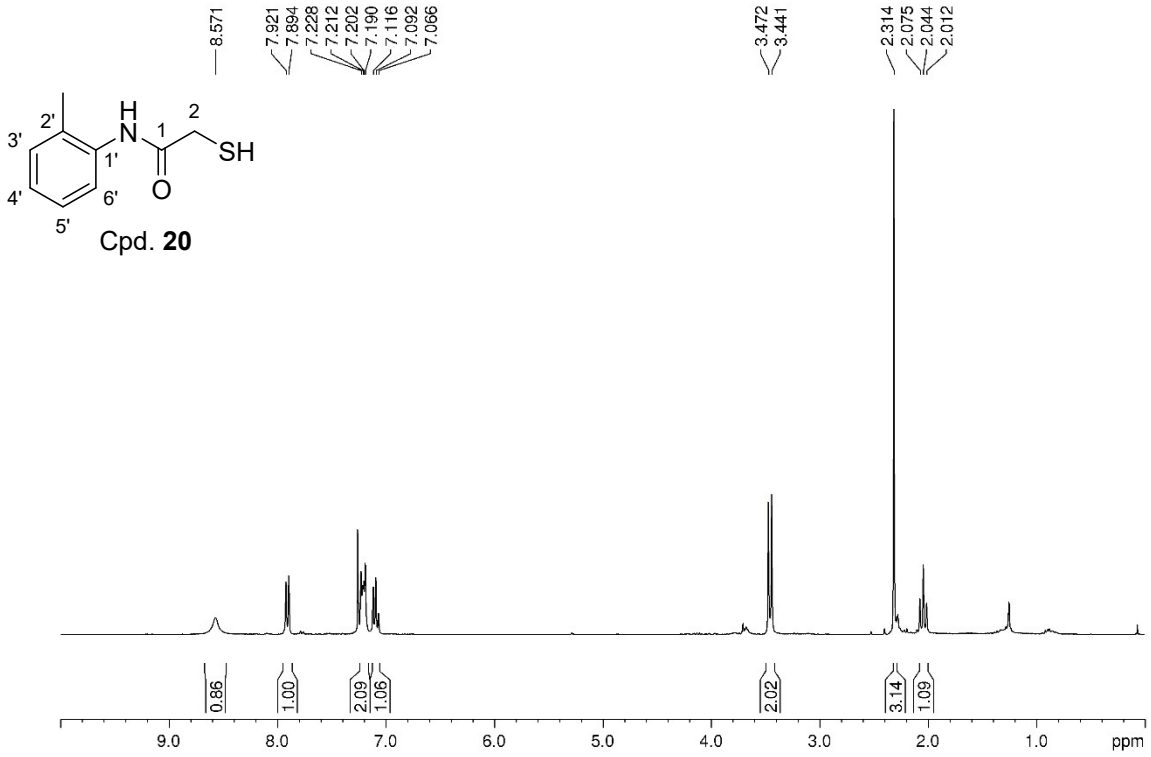
^{13}C NMR of Cpd. **18** (126 MHz, $CDCl_3$)



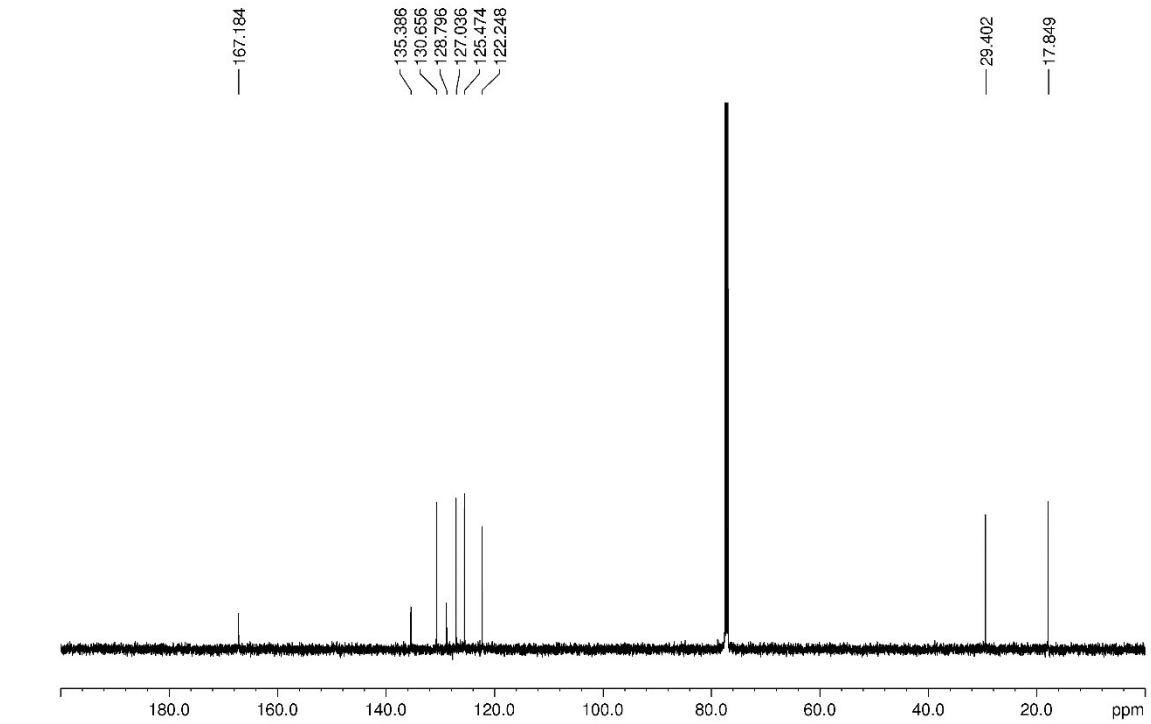
^{1}H NMR of Cpd. **19** (300 MHz, DMSO-d_6)



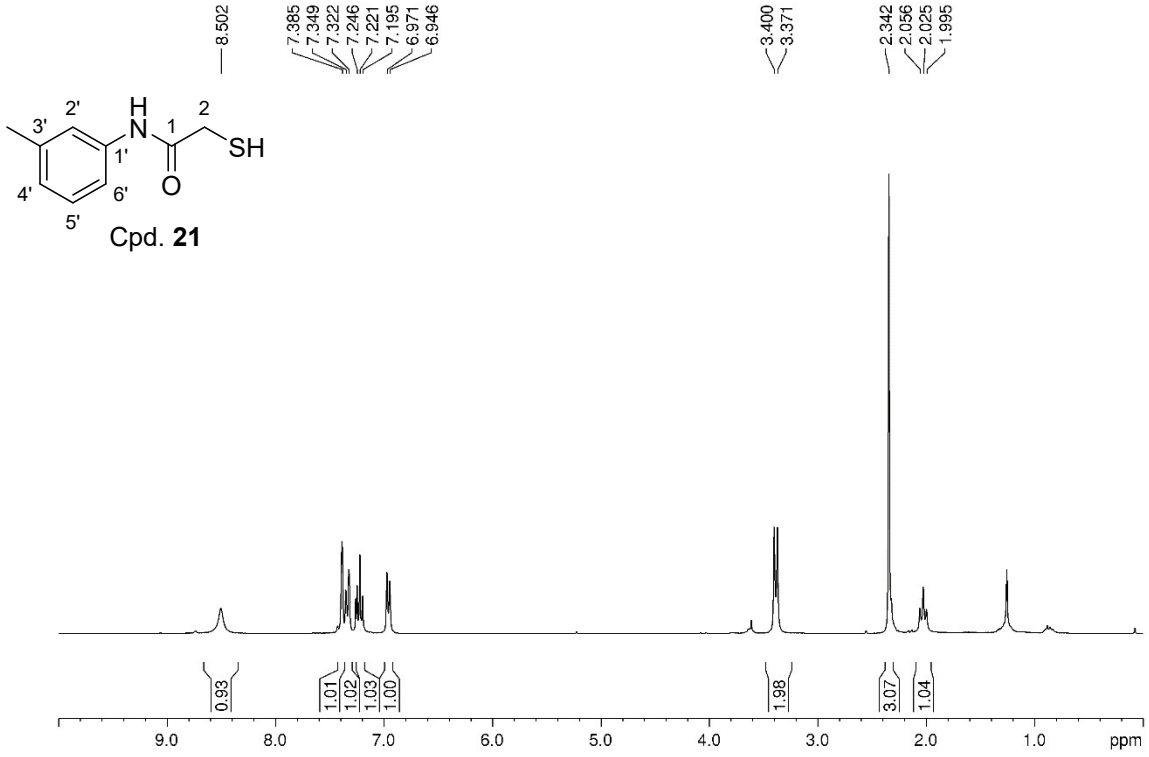
^{13}C NMR of Cpd. **19** (126 MHz, DMSO-d_6)



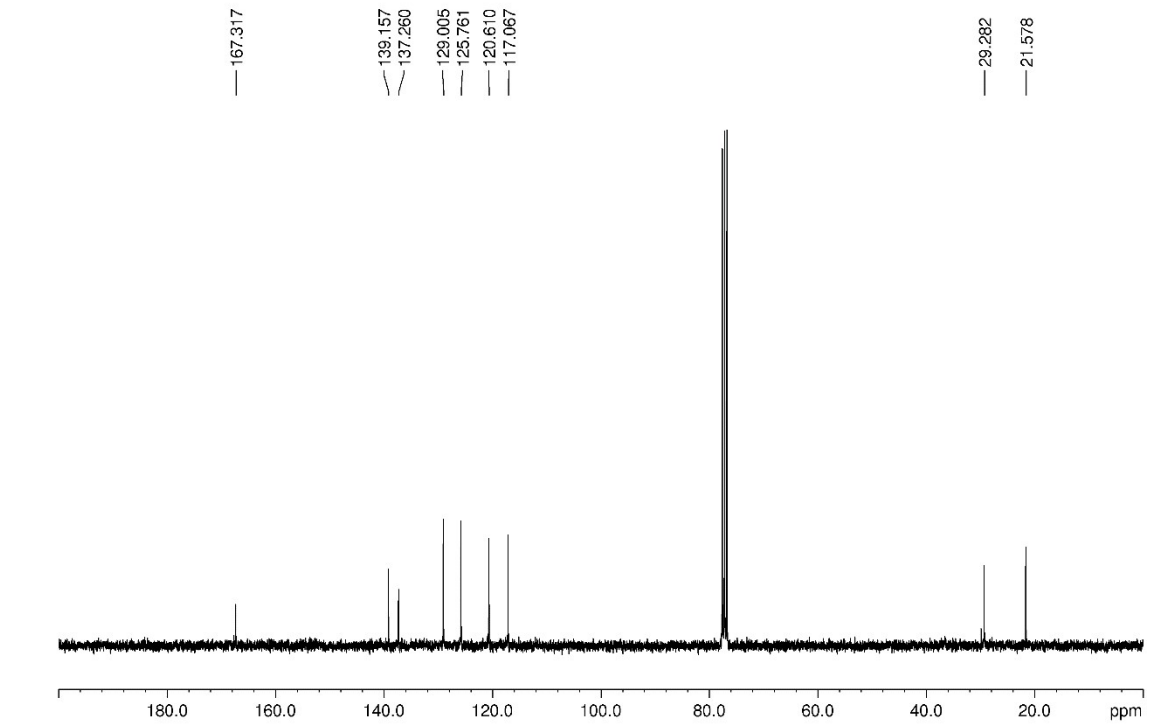
^{1}H NMR of Cpd. **20** (300 MHz, $CDCl_3$)



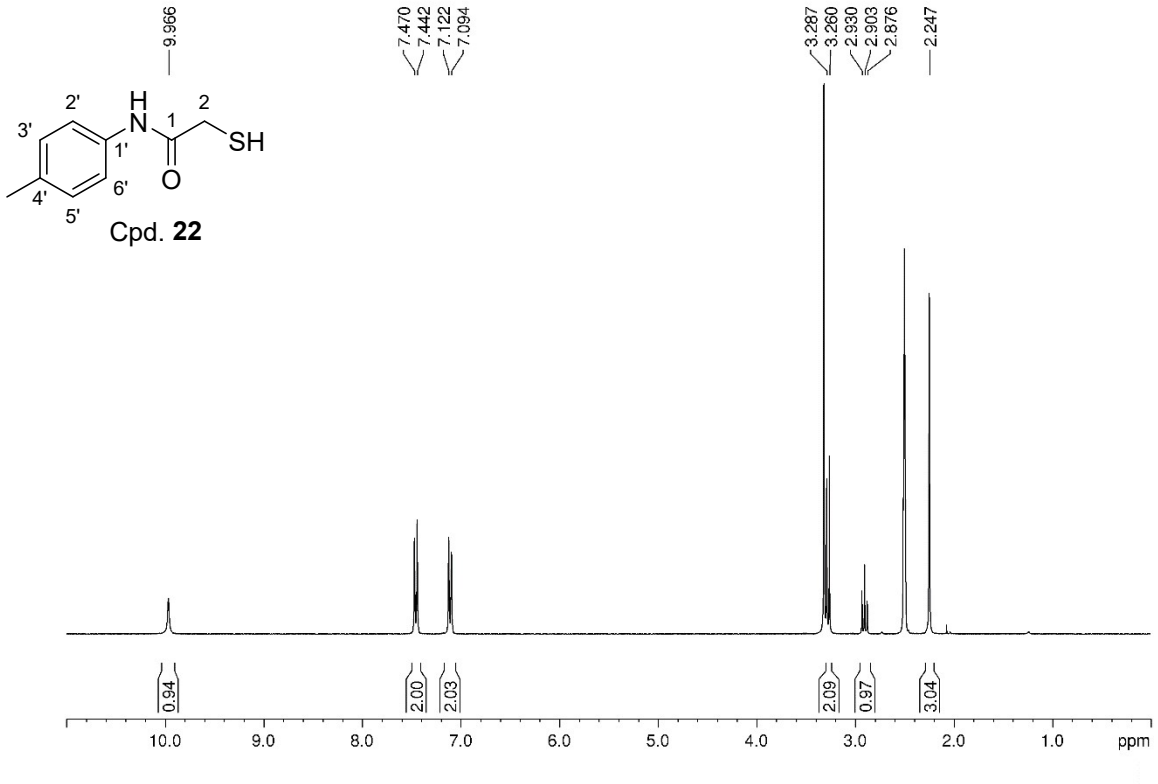
^{13}C NMR of Cpd. **20** (126 MHz, $CDCl_3$)



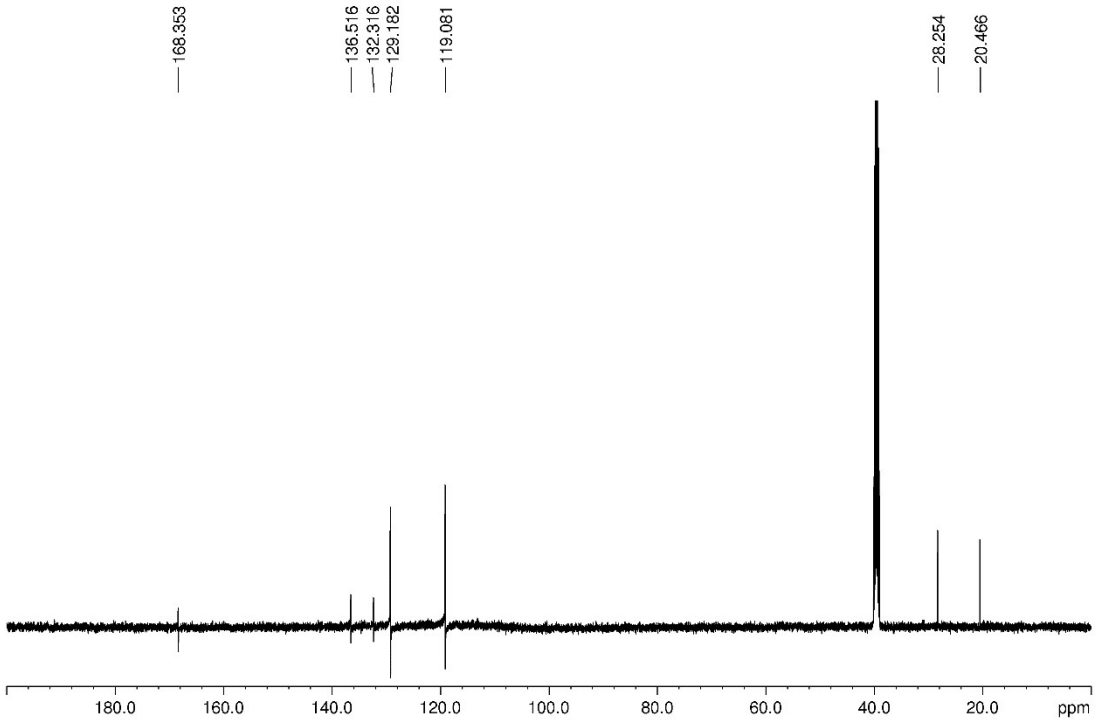
^{1}H NMR of Cpd. **21** (300 MHz, $CDCl_3$)



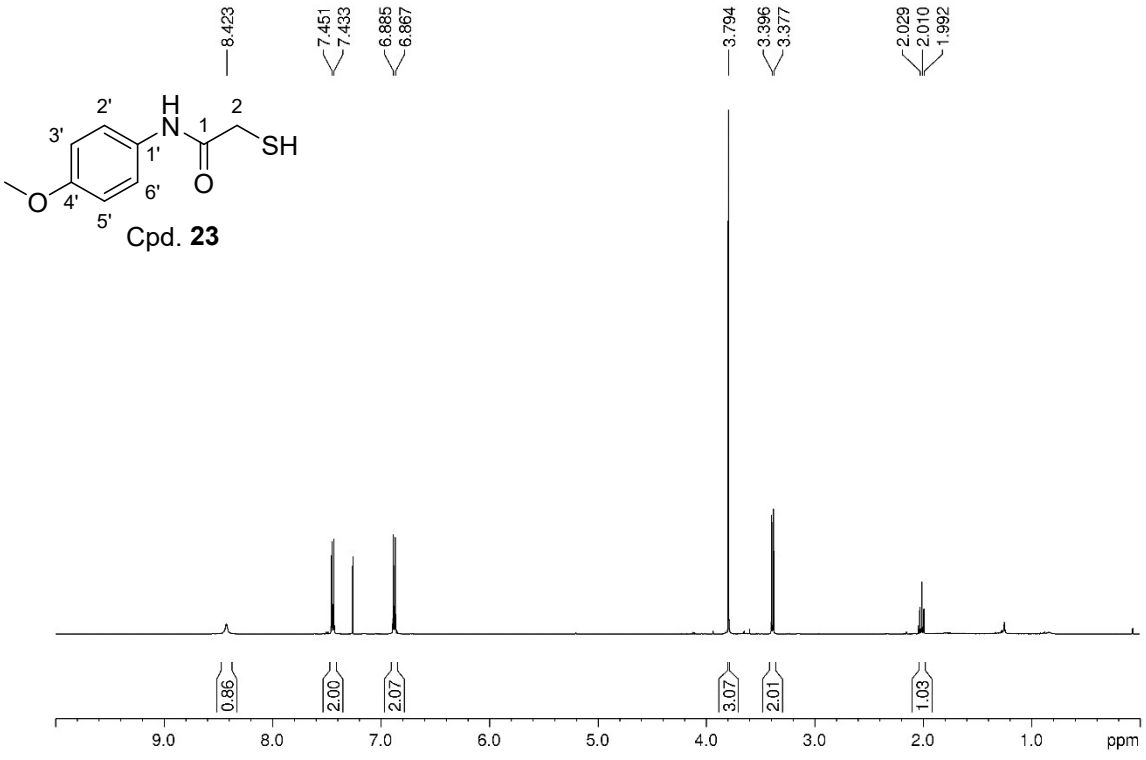
^{13}C NMR of Cpd. **21** (75 MHz, $CDCl_3$)



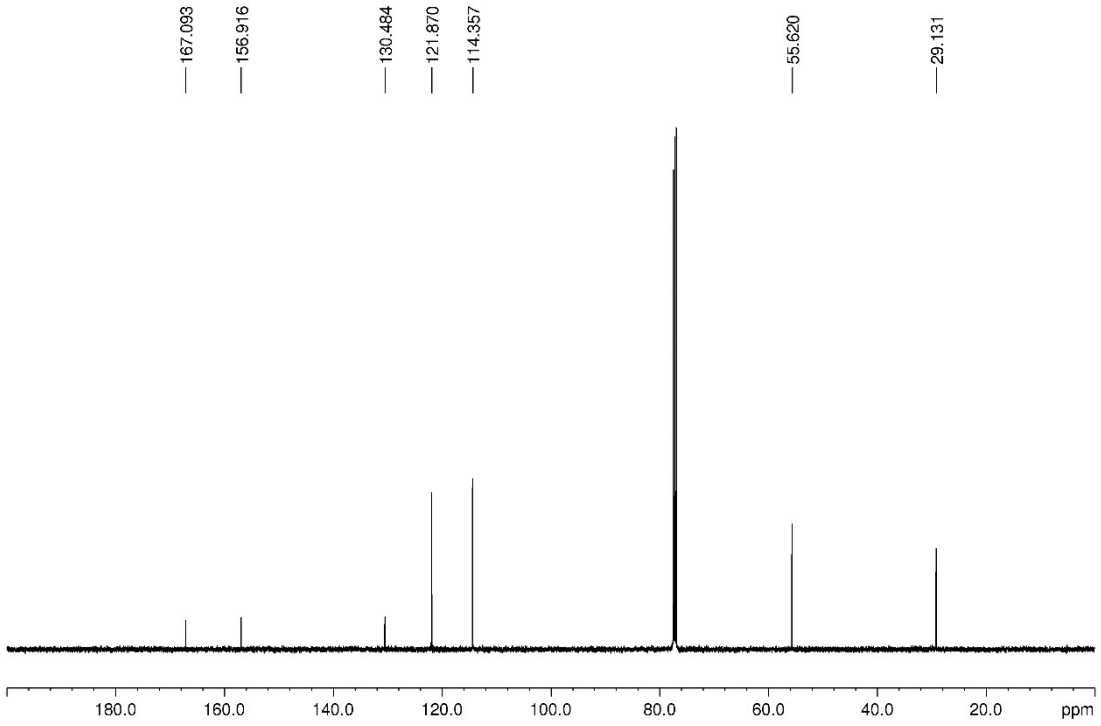
^{1}H NMR of Cpd. **22** (300 MHz, DMSO-d_6)



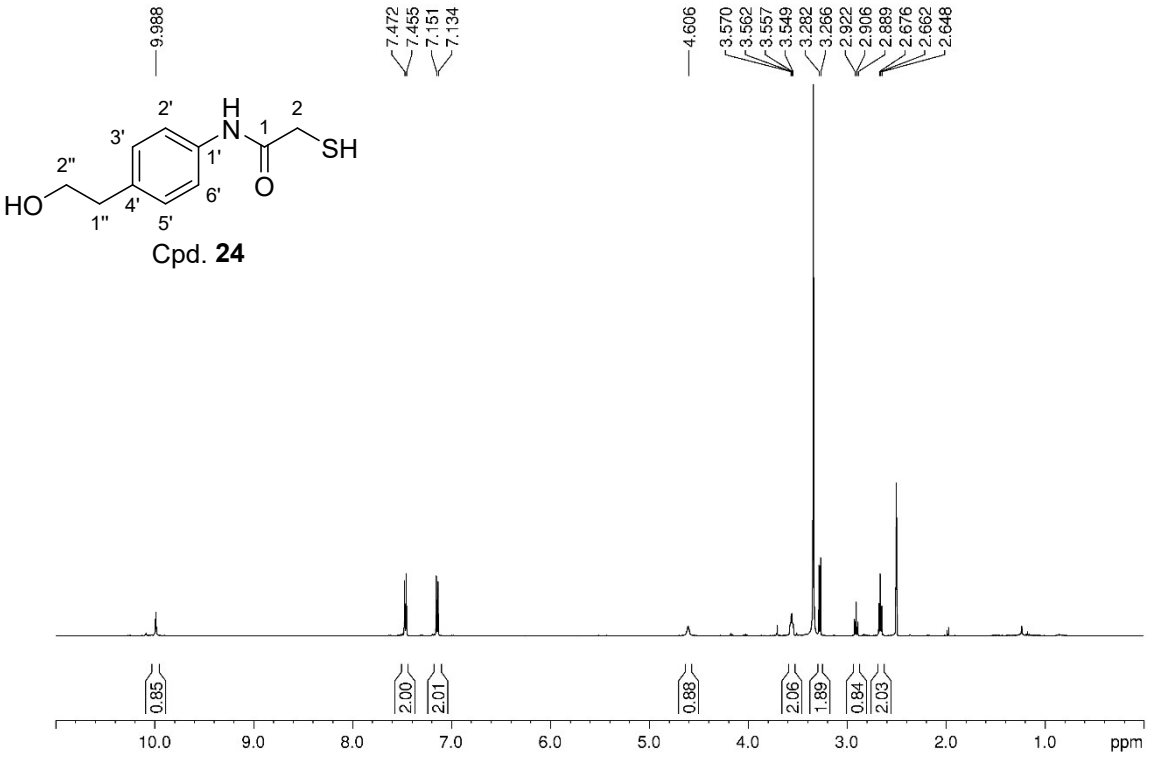
^{13}C NMR of Cpd. **22** (126 MHz, DMSO-d_6)



^{1}H NMR of Cpd. **23** (500 MHz, $CDCl_3$)

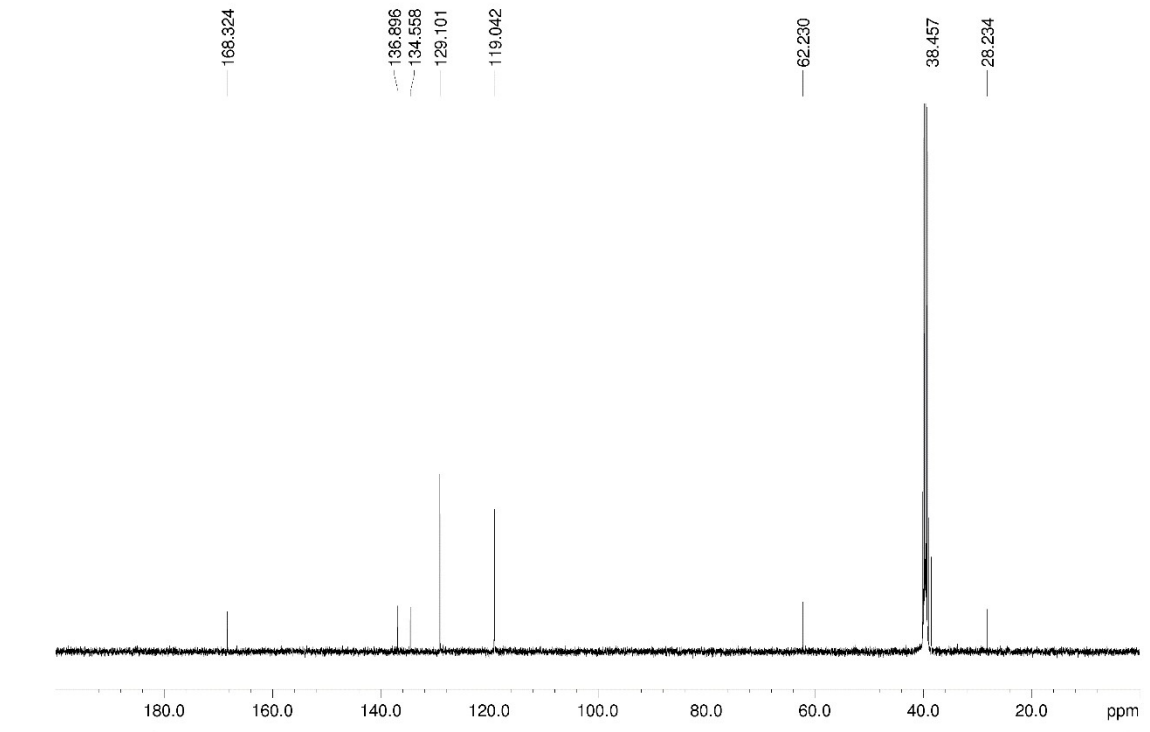


^{13}C NMR of Cpd. **23** (126 MHz, $CDCl_3$)

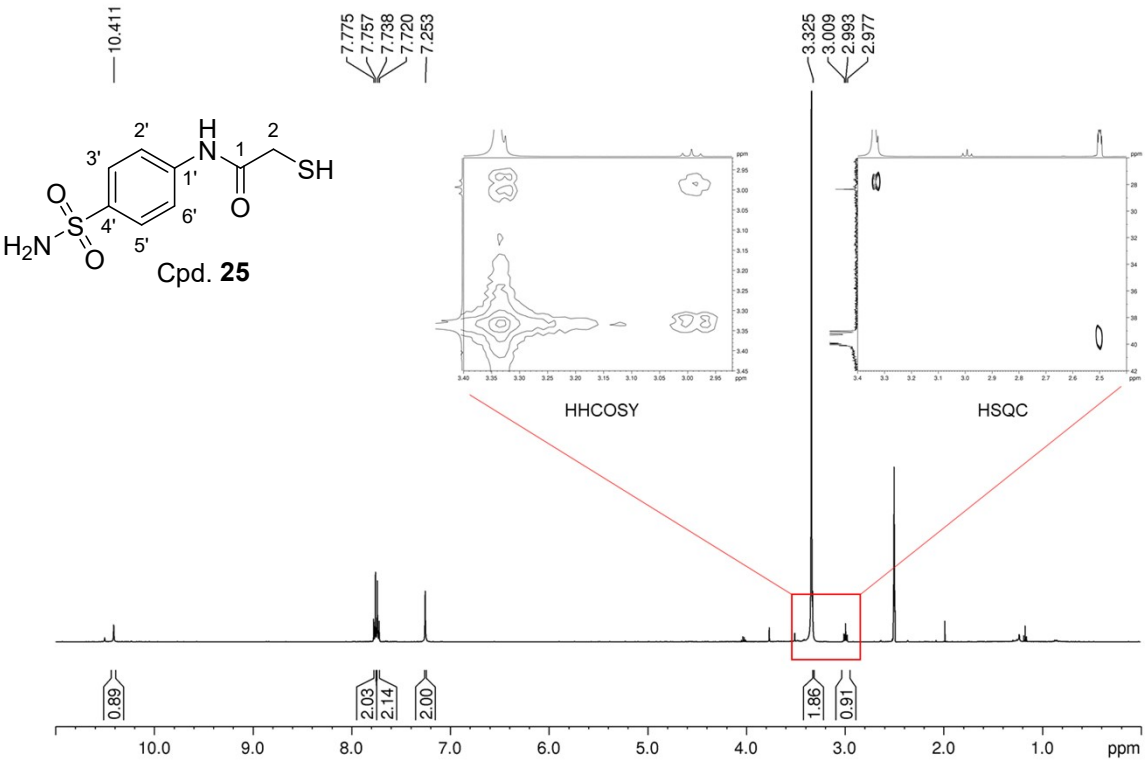


Cpd. **24**

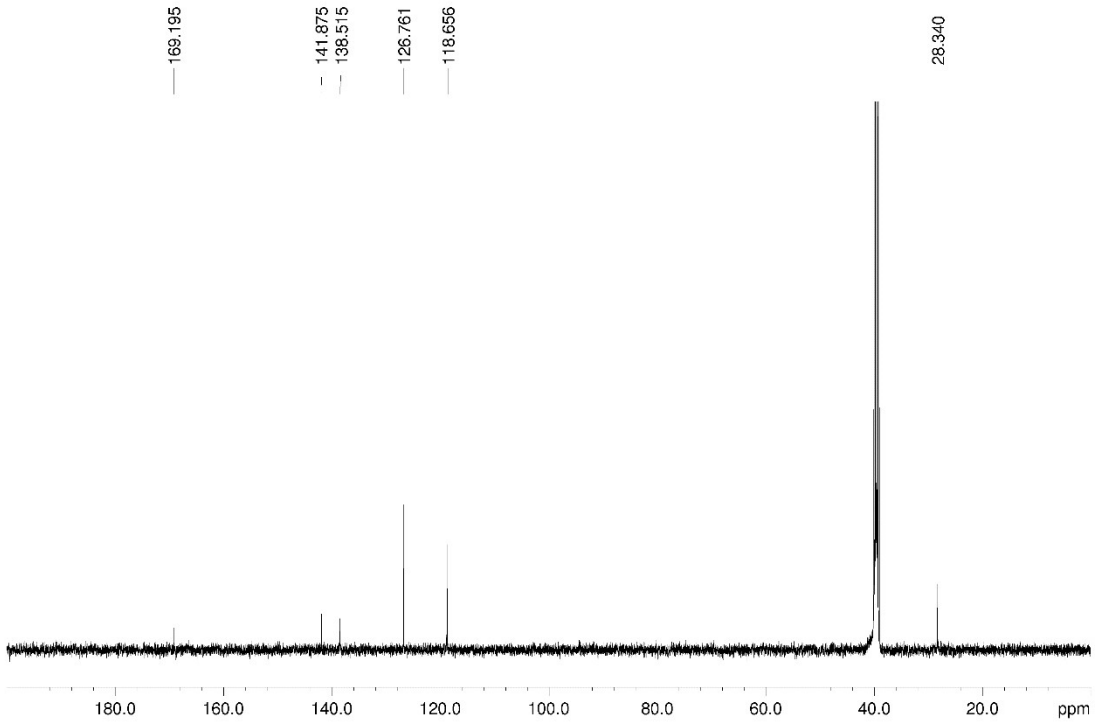
^{1}H NMR of Cpd. **24** (500 MHz, DMSO-d_6)



^{13}C NMR of Cpd. **24** (126 MHz, DMSO-d_6)



^{1}H NMR of Cpd. **25** (500 MHz, DMSO-d_6)



^{13}C NMR of Cpd. **25** (126 MHz, DMSO-d_6)

References

(1) Konstantinović, J.; Yahiaoui, S.; Alhayek, A.; Haupenthal, J.; Schönauer, E.; Andreas, A.; Kany, A. M.; Mueller, R.; Köhnke, J.; Berger, F.; Bischoff, M.; Hartmann, R. W.; Brandstetter, H.; Hirsch, A. K. H. N-Aryl-3-Mercaptosuccinimides as Antivirulence Agents Targeting Pseudomonas Aeruginosa Elastase and Clostridium Collagenases. *J. Med. Chem.* **2020**, *63* (15), 8359–8368. https://doi.org/10.1021/acs.jmedchem.0c00584.

(2) Kany, A. M.; Sikandar, A.; Haupenthal, J.; Yahiaoui, S.; Maurer, C. K.; Proschak, E.; Köhnke, J.; Hartmann, R. W. Binding Mode Characterization and Early *in Vivo* Evaluation of Fragment-Like Thiols as Inhibitors of the Virulence Factor LasB from *Pseudomonas* Aeruginosa. *ACS Infect. Dis.* **2018**, *4* (6), 988–997. https://doi.org/10.1021/acsinfecdis.8b00010.