Synthesis of *cis*-stilbene based 1,2,4-triazole/1,3,4-oxadiazole conjugates as potential cytotoxic and tubulin polymerization inhibitors

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1. Chemistry

General Methods. All the reagents and solvents were obtained from commercially and were used without further purification. Analytical thin layer chromatography (TLC) was performed on MERCK pre-coated silica gel 60-F254 (0.5 mm) aluminum plates. Visualization of the spots on TLC plates was achieved by UV light. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz by making a solution of samples in the DMSO-d_6 as solvent using tetramethyl silane (TMS) as the internal standard. Chemical shifts for ^{1}H and ^{13}C are reported in parts per million (ppm) downfield from tetra methyl silane. Spin multiplicities are described as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constant (*J*) values are reported in hertz (Hz). HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument. Wherever required, column chromatography was performed using silica gel (60-120; 100-200). The reactions wherever anhydrous conditions required are carried under nitrogen positive pressure using freshly distilled solvents. All evaporation of solvents was carried out under reduced pressure using rotary evaporator below 45 °C. Melting points were determined with an electro thermal digital melting point apparatus IA9100 and are uncorrected. The names of all the compounds given in the experimental section were taken from ChemBioDraw Ultra, Version 12.0.

1.1. Synthesis of 5a-p and 6a-d

1.1.1 Synthesis of (E)-2-(3,4-dimethoxyphenyl)acrylohydrazides (4a-d)

To a solution of **3a-d** (1 equiv.) in dry THF, triethylamine (1.5 equiv.) was added and stirred for 15 min at 0 °C. To this reaction mixture, ethyl chloroformate (1.2 equiv.) was added and stirred at room temperature for 3 h. After the completion of reaction, hydrazine hydrate (2 equiv.) was added and stirred for 5 h at room temperature. Completion of reaction as observed on TLC was followed by simple filtration to obtain the desired products **4a-d**.

1.1.2. Synthesis of (E)-5-(1,2-bis(3,4-dimethoxyphenyl)vinyl)-4H-1,2,4-triazole-3-thiols (5a-p)

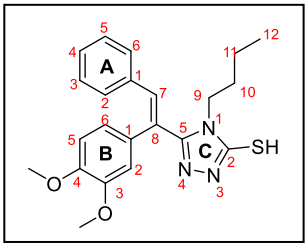
A mixture of **4a-d** (1.5 equiv.) and isothiocyanate (1.0 equiv.) in ethanol was refluxed for 1 h. After the completion of the reaction, the solvent was removed under reduced pressure. To this, a 2N NaOH solution was added and refluxed for 2 h at 100 °C. After reaction completion, the mixture was neutralized using 5M HCl solution. The precipitate so obtained was filtered off and washed with ethanol to obtain pure solid products **5a-p**.

1.1.3. Synthesis of (E)-5-(1-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole-2-thiols (6a-d)

A mixture of **4a-d** (1.0 equiv.), carbon disulphide (1.2 equiv.) and potassium hydroxide (1.2 equiv.) were refluxed for 10 h in ethanol. After completion of the reaction indicated by TLC, the reaction mixture was cooled, filtered and washed with water to obtain pure products **6a-d**.

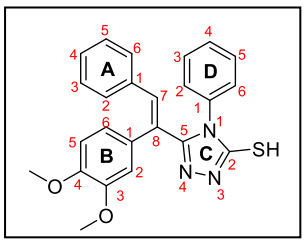
1.2. a. Characterization data

(E)-4-butyl-5-(2-(3,4-dimethoxyphenyl)-1-phenylvinyl)-4H-1,2,4-triazole-3-thiol ***(5a).*** Cream



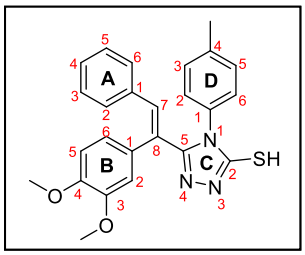
coloured solid; 71% yield; mp: 144-146 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.87 (s, 1H, SH), 7.30–7.25 (m, 3H, 3-H$_A$, 4-H$_A$, 5-H$_A$), 7.24–7.19 (m, 2H, 2-H$_B$, 6-H$_B$), 7.13 (s, 1H, 7-H$_{C=CH}$), 6.99 (d, J = 8.2 Hz, 1H, 5-H$_B$), 6.74 (dd, J = 10.3, 2.2 Hz, 2H, 2-H$_A$, 6-H$_A$), 3.78 (s, 3H, 3$_B$-OCH$_3$), 3.55-3.52 (m, 5H, 4$_B$-OCH$_3$, 9-CH$_2$), 1.29-1.24 (m, 2H, 10-CH$_2$), 1.10–0.97 (m, 2H, 11-CH$_2$), 0.71 (t, J = 7.3 Hz, 3H, 12-CH$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.6 (C-2$_C$), 153.1 (C-5$_C$), 149.7 (C-3$_B$), 149.4 (C-4$_B$), 135.4 (C-8), 135.0 (C-7), 130.0 (C-1$_A$,1$_B$), 128.9 (C-6$_A$, 2$_A$), 128.8 (C-5$_A$), 127.5 (C-3$_A$), 127.3 (C-4$_A$), 122.4 (C-6$_B$), 113.0 (C-2$_B$), 112.5 (C-5$_B$), 55.9 (C-3$_B$-OCH$_3$), 55.8 (C-4$_B$-OCH$_3$), 44.0 (C-9), 29.9 (C-10), 19.7 (C-11), 13.7 (C-12) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calc for $C_{22}H_{26}N_3O_2S$ 396.7400 found 396.7310. Purity: 97.79%

(E)-5-(2-(3,4-dimethoxyphenyl)-1-phenylvinyl)-4-phenyl-4H-1,2,4-triazole-3-thiol ***(5b).*** buff



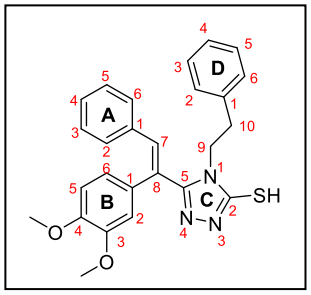
coloured solid; 91% yield, mp: 166-169 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.04 (s, 1H, SH), 7.34–7.29 (m, 3H, 6-H$_A$, 2-H$_A$, 5-H$_A$), 7.20–7.16 (m, 3H, 3-H$_A$, 3-H$_C$, 5-H$_C$), 7.13 (dd, J = 7.5, 2.0 Hz, 2H, 2-H$_D$, 6-H$_D$), 7.04–6.99 (m, 3H, 7-H$_{C=CH}$, 4-H$_D$, 4-H$_C$), 6.74 (d, J = 8.3 Hz, 1H, 5-H$_B$), 6.45 (dd, J = 8.2, 2.0 Hz, 1H, 6-H$_B$), 6.27 (d, J = 1.9 Hz, 1H, 2-H$_B$), 3.70 (s, 3H, 3$_B$-OCH$_3$), 3.40 (s, 3H, 4$_B$ -OCH$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.5 (C-2$_C$), 158.2 (C-5$_C$), 153.9 (C-3$_B$), 153.5 (C-4$_B$), 140.1 (C-8), 139.5 (C-1$_A$), 134.6 (C-7), 134.4 (C-1$_B$,1$_D$), 133.9 (C-2$_D$, 6$_D$), 133.5 (C-3$_A$), 133.4 (C-2$_A$, 6$_A$), 132.5 (C-4$_A$, 5$_A$), 132.2 (C-3$_D$, 5$_D$), 127.0 (C-6$_B$, 5$_A$), 118.0 (C-2$_B$), 117.0 (C-5$_B$), 60.7 (C-3$_B$-OCH$_3$), 60.4 (C-4$_B$-OCH$_3$) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calc for $C_{24}H_{22}N_3O_2S$ 416.1427 found 416.1429. Purity: 96.13%

(E)-5-(2-(3,4-dimethoxyphenyl)-1-phenylvinyl)-4-(p-tolyl)-4H-1,2,4-triazole-3-thiol ***(5c)***.



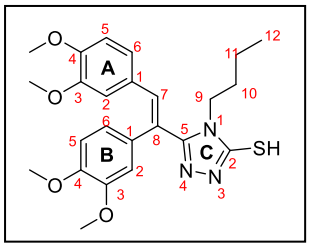
Cream coloured solid; 85% yield, mp: 174-176 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.99 (s, 1H, SH), 7.21–7.16 (m, 3H, 2-H$_A$, 6-H$_A$, 3-H$_A$), 7.11 (d, J = 8.1 Hz, 2H, 2-H$_C$, 6-H$_C$), 7.04-7.02 (m, 3H, 7-H$_{C=CH}$, 5-H$_C$, 4-H$_C$), 6.98 (d, J = 8.3 Hz, 2H, 3-H$_C$, 5-H$_C$), 6.76 (d, J = 8.3 Hz, 1H, 5-H$_A$), 6.45 (dd, J = 8.2, 2.0 Hz, 1H, 6-H$_B$), 6.23 (d, J = 1.9 Hz, 1H, 2-H$_B$), 3.71 (s, 3H, 3$_B$-OCH$_3$), 3.40 (s, 3H, 4$_B$-OCH$_3$), 2.26 (s, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.8 (C-2$_C$), 153.6 (C-5$_C$), 149.1 (C-3$_B$), 148.8 (C-4$_B$), 138.7 (C-8), 135.3 (C-1$_D$), 135.1 (C-1$_B$), 132.2 (C-7), 129.8 (C-2$_D$, 5$_D$), 129.7 (C-4$_D$), 128.8 (C-1$_A$), 128.7 (C-1$_B$, 6$_A$), 128.4 (C- 3$_D$, 5$_D$), 127.9 (C-3$_A$), 127.5 (C-4$_A$), 122.1 (C-6$_B$), 113.1 (C-2$_B$), 112.2 (C-5$_B$), 55.9 (C-3$_B$-OCH$_3$), 55.5 (C-4$_B$-OCH$_3$), 21.1(C-4$_D$-CH$_3$) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calcd. for $C_{25}H_{24}N_3O_2S$ 430.1584 found 430.1588. Purity: 96.93%

(E)-5-(2-(3,4-dimethoxyphenyl)-1-phenylvinyl)-4-phenethyl-4H-1,2,4-triazole-3-thiol ***(5d)***.



Cream coloured solid; 81% yield; mp: 182-184 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.93 (s, 1H, SH), 7.27–7.23 (m, 6H, 3-H$_A$, 4-H$_A$, 5-H$_A$, 3H$_D$, 4-H$_D$, 5-H$_D$), 7.13 (dd, J = 7.3, 2.0 Hz, 2H, 2-H$_D$, 6-H$_D$), 6.99 (d, J = 8.2 Hz, 1H, 5-H$_B$), 6.93 (dd, J = 7.7, 1.4 Hz, 2H, 6-H$_A$, 2-H$_A$), 6.81 (s, 1H, 7-H$_{C=CH}$), 6.70–6.67 (m, 2H, 2-H$_C$, 6-H$_C$), 3.78 (s, 3H, 3$_B$-OCH$_3$), 3.70 (t, 2H, 9-CH$_2$), 3.53 (s, 3H, 4$_B$-OCH$_3$), 2.67 (t, 2H, 10-CH$_2$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.6 (C-2$_C$), 153.3 (C-5$_C$), 149.7 (C-3$_B$), 149.4 (C-4$_B$), 137.9 (C-8), 135.4 (C-1$_A$), 134.9 (C-1$_D$), 130.0 (C-7), 129.1 (C-2D,6$_D$), 128.9 (C-4$_D$, 4$_A$), 128.7 (C-2$_A$), 128.7 (C-1$_B$), 127.6 (C-3$_A$,6$_A$), 127.2 (C-5$_D$), 126.9 (C-5$_A$), 122.4 (C-6$_B$), 113.3 (C-2$_B$), 112.6 (C-5$_B$), 55.9 (C-3$_B$-OCH$_3$), 55.9 (C-4$_B$-OCH$_3$), 45.7 (C-9), 33.3 (C-10) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calcd. for $C_{26}H_{26}N_3O_2S$ 444.1740 found 444.1756. Purity: 97.09%

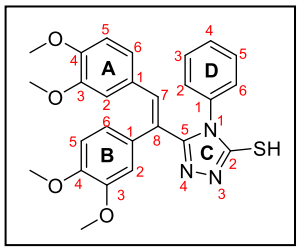
(E)-5-(1,2-bis(3,4-dimethoxyphenyl)vinyl)-4-butyl-4H-1,2,4-triazole-3-thiol ***(5e)***. Cream



coloured solid; 74% yield; mp: 155-158 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.81 (s, 1H, SH), 7.06 (s, 1H, 2-HA), 7.04 (d, J = 8.2 Hz, 1H, 5-HA), 6.89–6.82 (m, 3H, 7-H$_{C=CH}$, 5-HB, 6-HB), 6.80 (dd, J = 8.2, 2.1 Hz, 1H, 6-HA), 6.73 (d, J = 1.9 Hz, 1H, 2-HB), 3.79 (s, 3H, 3$_B$-OCH$_3$), 3.74 (s, 3H, 4$_B$-OCH$_3$), 3.62 (s, 3H, 3$_A$-OCH$_3$), 3.55 (t, J = 7.0, 2H, 9-CH$_2$), 3.45 (s, 3H, 4$_A$-OCH$_3$), 1.25 (m, 2H, 10-CH$_2$), 1.04 (m, 2H, 11-CH$_2$),

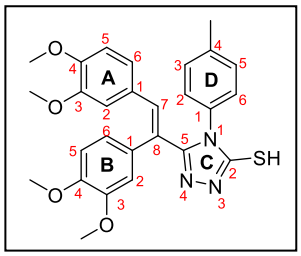
0.72 (t, J = 7.3 Hz, 3H, 12-CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.3 (C-2_C), 158.1 (C-5_C), 154.4 (C-3_B, 4_B), 153.2 (C-3_A, 4_A), 132.8 (C-8), 132.6 (C-7), 129.6 (C-1_A), 118.0 (C-1_B), 117.9 (C-6_A,6_B), 117.8 (C-5_A, 5_B), 116.6 (C-2_A, 2_B), 60.9 (C-3_B-OCH_3), 60.9 (C-4_B-OCH_3), 60.7 (C-3_A-OCH_3), 60.1 (C-4_A-OCH_3), 48.8 (C-9), 34.7(C-10), 24.5 (C-11), 18.6 (C-12) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{24}H_{30}N_3O_4S$ 456.1912 found 456.1900. Purity: 97.42%

(E)-5-(1,2-bis(3,4-dimethoxyphenyl)vinyl)-4-phenyl-4H-1,2,4-triazole-3-thiol ***(5f)***. Cream



white solid; 85% yield , mp: 240-243 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.99 (s, 1H, SH), 7.35–7.30 (m, 3H, 3-H_D, 4-H_D, 5-H_D), 7.11 (dd, J = 6.7, 2.9 Hz, 2H, 2-H_D, 6-H_D), 6.93 (s, 1H, 7-$H_{C=CH}$), 6.79 (dd, J = 10.6, 8.4 Hz, 2H, 5-H_A, 5-H_B), 6.64 (dd, J = 8.5, 1.8 Hz, 1H, 6-H_A), 6.50 (d, J = 1.9 Hz, 1H, 2-H_A), 6.46 (dd, J = 8.2, 2.0 Hz, 1H, 6-H_B), 6.32 (d, J = 1.9 Hz, 1H, 2-H_B), 3.70 (s, 3H, 3_B-OCH_3), 3.69 (s, 3H, 4_B-OCH_3), 3.47 (s, 3H, 3_A-OCH_3), 3.35 (s, 3H, 4_A-OCH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.7 (C-2C), 153.7 (C-5C), 149.6 (C-3_B), 149.0 (C-4_B), 149.0 (C-3_A), 148.3 (C-4_A), 135.2 (C-8), 134.9 (C-7), 129.2 (C-1_D, 1_B), 129.2 (C-1_A), 128.8 (C-2_D,6_D), 128.2 (C-4_D), 127.7 (C-5_D), 124.9 (C-3_D), 123.9 (C-6_B), 122.3 (C-6_A), 113.3 (C-2_A), 112.6 (C-2_B), 112.5 (C-5_A), 111.7 (C-5_B), 56.1 (C-3_B-OCH_3), 55.9 (C-4_B-OCH_3), 55.8 (C-3_A-OCH_3), 55.2 (C-4_A-OCH_3) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{26}H_{26}N_3O_4S$ 476.1566 found 476.1556. Purity: 98.13%

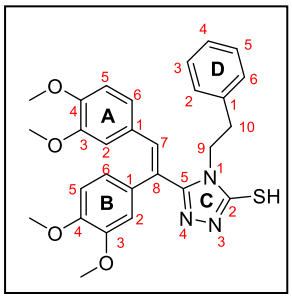
(E)-5-(1,2-bis(3,4-dimethoxyphenyl)vinyl)-4-(p-tolyl)-4H-1,2,4-triazole-3-thiol ***(5g)***. Cream



white solid; 80% yield; mp: 202-206 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.92 (s, 1H, SH), 7.12 (d, J = 8.0 Hz, 2H, 2-H_D, 5-H_D), 6.98–6.88 (m, 3H, 7-$H_{C=CH}$, 5-H_A, 5H_B), 6.80 (d, J = 8.2 Hz, 2H, 3-H_D, 5-H_D), 6.65 (dd, J = 8.5, 1.8 Hz, 1H, 6-H_A), 6.51 (d, J = 1.9 Hz, 1H, 2-H_A), 6.47 (dd, J = 8.2, 2.0 Hz, 1H, 6-H_B), 6.28 (d, J = 1.9 Hz, 1H, 2H_B), 3.71 (s, 3H, 3_B-OCH_3), 3.69 (s, 3H, 4_B-OCH_3), 3.46 (s, 3H, 3_A-OCH_3), 3.35 (s, 3H, 4_A-OCH_3), 2.28 (s, 3H, 4-CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.8 (C-2_C), 153.8 (C-5_C), 149.6 (C-3_B), 149.1 (C-4_B), 149.0 (C-3_A), 148.3 (C-4_A), 138.7 (C-8), 135.0 (C-1_D), 132.5 (C-7), 129.7 (C-2_D, 6D), 128.5 (C-3_D), 128.4 (C-5_D), 127.8 (C-1_A), 125.1 (C-1_B), 123.9 (C-6_B), 122.2 (C-6_A), 113.3 (C-2_A), 112.6 (C-2_B), 112.5 (C-5_A), 111.7 (C-5_B), 56.1 (C-3_B-OCH_3), 55.9 (C-4_B-OCH_3), 55.7 (C-3_A-OCH_3), 55.2 (C-4_A-OCH_3), 21.1 (C-4-CH_3) ppm;

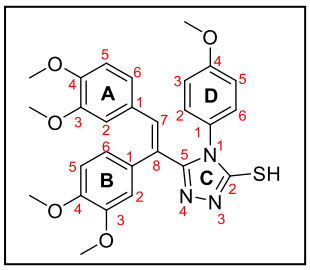
HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{27}H_{28}N_3O_4S$ 490.1795 found 490.1791.Purity: 98.25%

*(E)-5-(1,2-bis(3,4-dimethoxyphenyl)vinyl)-4-phenethyl-4H-1,2,4-triazole-3-thiol (**5h**).* Cream



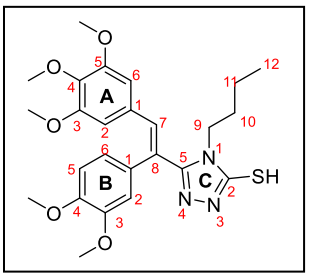
coloured solid; 83% yield; mp: 147-150 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.89 (s, 1H, SH), 7.29–7.21 (m, 3H, 3-H$_D$, 4-H$_D$, 5-H$_D$), 7.04 (d, J = 8.3 Hz, 1H, 5-H$_A$), 6.94 (d, J = 6.7 Hz, 2H, 2-H$_D$, 6-H$_D$), 6.86 (d, J = 8.5 Hz, 1H, 5-H$_B$), 6.80 (d, J = 2.0 Hz, 1H, 2-H$_A$), 6.78–6.73 (m, 3H, 7-H$_{C=CH}$, 6-H$_A$, 6-H$_B$), 6.65 (d, J = 1.9 Hz, 1H, 2-H$_B$), 3.78 (s, 3H, 3$_B$-OCH$_3$), 3.74 (m, 5H, 4$_B$-OCH$_3$, 9-CH$_2$), 3.61 (s, 3H, 3$_A$-OCH$_3$), 3.47 (s, 3H, 4$_A$-OCH$_3$), 2.68–2.62 (t, 2H, 10-CH$_2$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.5 (C-2$_C$), 153.5 (C-5$_C$), 149.7 (C-3$_B$), 149.6 (C-4$_B$), 149.5 (C-3$_A$), 148.3 (C-4$_A$), 137.9 (C-8), 135.0 (C-7), 129.1 (C-2$_D$,6$_D$), 129.0 (C-3$_D$, 5$_D$), 128.0 (C-4$_D$), 127.8 (C-1$_D$), 127.2 (C-1$_A$), 124.5 (C-1$_B$), 123.9 (C-6$_B$), 122.6 (C-6$_A$), 113.4 (C-2$_A$), 113.2 (C-2$_B$), 112.9 (C-5$_A$), 111.7 (C-5$_B$), 56.1 (C-3$_B$-OCH$_3$,4$_B$-OCH$_3$), 55.9 (C-3$_A$-OCH$_3$), 55.3 (C-4$_A$-OCH$_3$), 45.7 (C-9), 33.3 (C-10) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{28}H_{30}N_3O_4S$ 503.1879 found 503.1888. Purity: 97.99%

(E)-5-(1,2-bis(3,4-Dimethoxyphenyl)vinyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazole-3-thiol



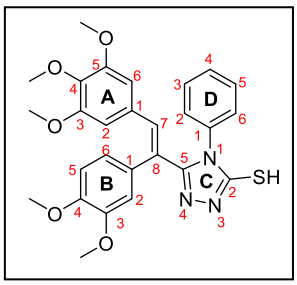
(5i). Cream coloured solid; 83% yield; mp: 200-220 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.92 (s, 1H, SH), 6.98 (d, J = 9.0 Hz, 2H, 2$_D$, 6$_D$), 6.95 (s, 1H, 7-H$_{C=CH}$), 6.85 (d, J = 8.9 Hz, 2H, 3-H$_D$, 5-H$_D$), 6.80 (d, J = 8.4 Hz, 2H, 5-H$_A$, 5-H$_B$), 6.66 (dd, J = 8.7, 2.0 Hz, 1H, 6-H$_A$), 6.51 (d, J = 2.0 Hz, 1H, 2-H$_A$), 6.47 (dd, J = 8.2, 2.0 Hz, 1H, 6-H$_B$), 6.30 (d, J = 2.0 Hz, 1H, 2-H$_B$), 3.73 (s, 3H, 3$_B$-OCH$_3$), 3.71 (s, 3H, 4$_B$-OCH$_3$), 3.69 (s, 3H, 3$_A$-OCH$_3$), 3.48 (s, 3H, 4$_A$-OCH$_3$), 3.35 (s, 3H, 4$_D$-OCH$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.9 (C-2$_C$), 159.6 (C-4$_D$), 153.9 (C-5$_C$), 149.6 (C-3$_B$), 149.1 (C-4$_B$), 149.0 (C-3$_A$), 148.3 (C-4$_A$), 135.1 (C-8), 129.9 (C-2$_D$, 6$_D$), 128.3 (C-7), 127.8 (C-1$_D$), 127.5 (C-1$_A$), 125.1 (C-1$_B$), 123.9 (C-6$_B$), 122.2 (C-6$_A$), 114.4 (C-3$_D$, 5$_D$), 113.3 (C-2$_A$), 112.6 (C-2$_B$), 112.5 (C-5$_A$), 111.7 (C-5$_B$), 56.1 (C-3$_B$-OCH$_3$), 55.8 (C-4$_A$-OCH$_3$), 55.7 (C-3$_A$-OCH$_3$), 55.2 (C-4$_A$-OCH$_3$) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{27}H_{28}N_3O_5S$ 506.1744 found 506.1749. Purity: 96.89%

*(E)-4-butyl-5-(1-(3,4-dimethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)vinyl)-4H-1,2,4-triazole-3-thiol (**5j**).*



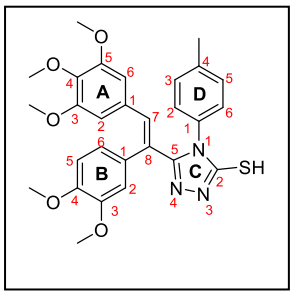
Yellow coloured solid; 75% yield, mp: 190-193 °C, ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.85 (s, 1H, SH), 7.08 (s, 1H, 6-H_A), 7.06 (d, J = 8.5 Hz, 1H, 5-H_B), 6.90–6.78 (m, 2H, 7-$H_{C=CH}$, 6-H_B), 6.54 (s, 2H, 2-H_A, 2-H_B), 3.78 (s, 3H, 3_B-OCH_3), 3.64 (s, 3H, 4_B-OCH_3), 3.63 (s, 3H, 4_A-OCH_3), 3.59–3.54 (m, 2H, 9-CH_2), 3.53 (s, 6H, 4_A-OCH_3, 5_A-OCH_3), 1.29–1.23 (m, 2H, 10-CH_2), 1.09–0.99 (m, 2H, 11-CH_2), 0.72 (t, J = 7.3 Hz, 3H, 12-CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.7 (C-2C), 153.1 (C-5C), 152.8 (C-3B, 4B, 3A), 149.8 (C-4A), 149.7 (C-5A), 138.3 (C-8), 135.1 (C-1A), 130.6 (C-7), 127.8 (C-1B), 126.4 (C-6B), 122.6 (C-6A), 113.3 (C-2A), 112.9 (C-2B), 107.9 (C-5B), 60.5 (C-5_A-OCH_3), 56.2 (C-3_B-OCH_3, 4_B-OCH_3), 55.9 (C-3_A-OCH_3, (C-4_A-OCH_3), 44.1 (C-9), 30.0 (C-10), 19.7 (C-11), 13.8 (C-12) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{25}H_{32}N_3O_5S$ 486.2057 found 486.2049. Purity: 98.13%

*(E)-5-(1-(3,4-dimethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)vinyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (**5k**).*



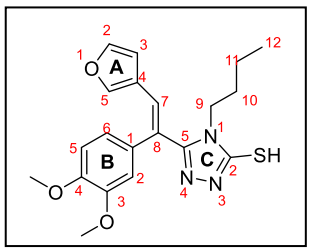
Yellow coloured solid; 90% yield; mp: 211-214 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.98 (s, 1H, SH), 7.34–7.32 (m, 2H, 3-H_D, 5-H_D), 7.14–7.08 (m, 2H, 6-H_A, 5-H_B), 6.93 (s, 1H, 7-$H_{C=CH}$), 6.79 (dd, J = 10.1, 8.4 Hz, 2H, 2-H_D, 6-H_D), 6.65 (dd, J = 8.7, 2.0 Hz, 1H, 4-H_D), 6.50 (d, J = 2.1 Hz, 1H, 2-H_A), 6.46 (dd, J = 8.2, 2.0 Hz, 1H, 6-H_B), 6.33 (d, J = 2.0 Hz, 1H, 2-H_B), 3.70 (s, 3H, 3_B-OCH_3), 3.69 (s, 3H, 4_B-OCH_3), 3.47 (s, 3H, 5_A-OCH_3), 3.35 (s, 3H, 3_A-OCH_3), 3.33 (s, 3H, C-4_A-OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.8 (C-2_C), 153.4 (C-5_C), 152.7 (C-3_A, 4_A, 5_A), 149.1 (C-3_B), 149.1 (C-4_B), 138.2 (C-8), 135.2 (C-1_D), 134.8, (C-7), 130.5 (C-1_B), 129.3 (C-2_D,6_D), 129.2 (C-1_B), 128.8 (C-1_A), 127.9 (C-3_D), 126.6 (C-5_D), 122.3 (C-4_D), 113.4 (C-2_A, C-2_B), 112.5 (C-6_A), 107.6 (C-5_B), 60.5 (C-3_B-OCH_3), 56.5 (C-4_B-OCH_3), 56.1 (C-5_A-OCH_3), 55.9 (C-3_A-OCH_3), 55.8 (C-4_A-OCH_3) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{27}H_{28}N_3O_5S$ 505.1671 found 505.1679. Purity: 98.16%

(E)-5-(1-(3,4-dimethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)vinyl)-4-(p-tolyl)-4H-1,2,4-



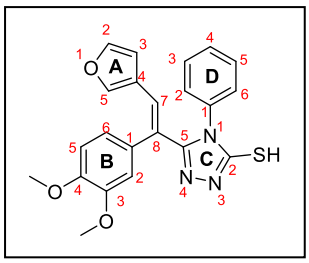
triazole-3-thiol (***5l***). cream coloured solid; 82% yield, mp: 192-195° C, ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.99 (s, 1H, SH), 7.13 (d, J = 8.1 Hz, 2H, 2-H$_D$, 6-H$_D$), 6.96 (m, 3H, 7-H$_{C=CH}$, 3-H$_D$, 5-H$_D$), 6.80 (d, J = 8.3 Hz, 1H, 5-H$_B$), 6.47 (dd, J = 8.2, 1.7 Hz, 1H, 6-H$_B$), 6.33 (s, 2H, 2-H$_A$, 2-H$_B$), 6.29 (d, J = 2.1 Hz, 1H, 6-H$_A$), 3.70 (s, 3H, 3$_B$-OCH$_3$), 3.60 (s, 3H, 4$_B$-OCH$_3$), 3.47 (s, 3H, 5$_A$-OCH$_3$), 3.46 (s, 6H, 3$_A$-OCH$_3$, 4$_A$-OCH$_3$), 2.28(s, 3H, 4$_D$-CH$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.9 (C-2$_C$), 153.5 (C-5$_C$), 152.7 (C-3$_A$, 4$_A$, 5$_A$), 149.1 (C-3$_B$, 4$_B$), 138.8 (C-8), 138.2 (C-4$_D$), 135.1 (C-1$_D$), 132.3 (C-7), 130.5 (C-1$_B$), 130.3 (C-2$_D$), 129.7 (C-6$_D$), 128.5 (C-1$_A$), 128.3 (C-3$_D$), 128.0 (C-5$_D$), 126.6 (C-6$_B$), 122.2 (C-6$_A$), 113.4 (C-2$_A$), 112.6 (C-2$_B$), 107.6 (C-5$_B$), 60.5 (C-5$_A$-OCH$_3$), 56.2 (C-3$_B$- OCH$_3$), 55.8 (C-3$_A$- OCH$_3$, 4$_A$-OCH$_3$) 55.8 (C-4$_B$- OCH$_3$), 21.1 (C-4$_D$-CH$_3$) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calcd. for C$_{28}$H$_{30}$N$_3$O$_5$S 519.1828 found 519.1820. Purity: 96.90%

(E)-4-butyl-5-(2-(3,4-dimethoxyphenyl)-1-(furan-3-yl)vinyl)-4H-1,2,4-triazole-3-thiol (***5m***).



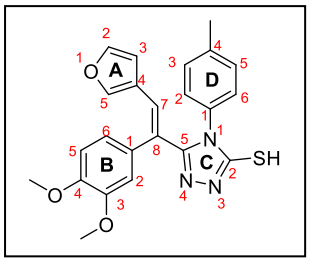
Cream coloured solid; 75%; mp: 226-229 °C; ^{1}H NMR (500 MHz, DMSO- d_6): δ 13.84 (s, 1H, SH), 7.68 (d, J = 1.7 Hz, 1H, 5-H$_A$), 7.04 (d, J = 8.3 Hz, 1H, 2-H$_A$), 6.97 (s, 1H, 7-H$_{C=CH}$), 6.96 (d, J = 1.9 Hz, 1H, 2-H$_B$), 6.82 (dd, J = 8.2, 2.0 Hz, 1H, 6-H$_B$), 6.51 (dd, J = 3.3, 1.8 Hz, 1H, 3-H$_A$), 6.36 (d, J = 3.4 Hz, 1H, 5-H$_B$), 3.81 (s, 3H, 3$_B$-OCH$_3$), 3.70 (s, 3H, 4$_B$-OCH$_3$), 3.52–3.47 (m, 2H, 9-CH$_2$), 1.26–1.21 (m, 2H, 10-CH$_2$), 1.07–0.99 (m, 2H, 11-CH$_2$), 0.71 (t, J = 7.3 Hz, 3H, 12-CH$_3$) ppm; ^{13}C (125 MHz, DMSO-d_6): δ 167.7 (C-2$_C$), 152.8 (C-5$_C$), 150.9 (C-5$_A$), 149.9 (C-3$_B$), 149.3 (C-4$_B$), 144.6 (C-8), 127.7 (C-7), 123.8 (C-2$_A$), 122.3 (C-1$_B$), 122.2 (C-4$_A$), 114.1 (C-3$_A$), 113.2 (C-6$_B$), 112.6 (C-5$_B$), 112.4 (C-2$_B$), 56.1 (C-3$_B$-OCH$_3$), 56.0 (C-4$_B$-OCH$_3$), 44.0 (C-9), 30.0 (C-10), 19.7 (C-11), 13.8 (C-12) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calcd. for C$_{20}$H$_{24}$N$_3$O$_3$S 386.1533 found 386.1533. Purity: 98.17%

(E)-5-(2-(3,4-dimethoxyphenyl)-1-(furan-3-yl)vinyl)-4-phenyl-4H-1,2,4-triazole-3-thiol ***(5n)***.



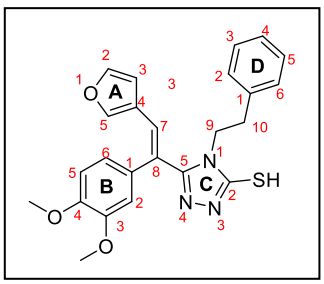
Cream coloured solid; 87% yield, mp: 186-188° C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.02 (s, 1H, SH), 7.58 (d, J = 1.7 Hz, 1H, 5-H$_A$), 7.35 (m, 3H, 2-H$_A$, 2-H$_D$, 6-H$_D$), 7.17–7.09 (m, 2H, 3-H$_D$, 5H$_D$), 6.81- 6.79 (m, 2H, 7-H$_{C=CH}$, 4-H$_D$), 6.52 (dd, J = 8.3, 1.3 Hz, 1H, 6-H$_B$), 6.43 (d, J = 1.8 Hz, 1H, 2-H$_B$), 6.41–6.38 (m, 1H, 3H$_A$), 5.99 (d, J = 3.4 Hz, 1H, 5H$_B$), 3.73 (s, 3H, 3$_B$-OCH$_3$), 3.53 (s, 3H, 4$_B$-OCH$_3$) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.9 (C-2$_C$), 153.0 (C-5$_C$), 150.8 (C-5$_A$), 149.2 (C-3$_B$), 148.7 (C-4$_B$), 144.4 (C-8), 134.9 (C-7), 129.3 (C-1$_D$), 129.3 (C-2$_A$), 128.8 (C-2$_D$, 6$_D$), 127.9 (C-4A), 124.2 (C-1$_B$), 122.6 (C-4$_D$), 121.9 (C-3$_D$, 5$_D$), 113.2 (C-3$_A$), 113.2 (C-6$_B$), 112.6 (C-5$_B$), 112.0 (C-2$_B$), 55.9 (C-3$_B$-OCH$_3$), 55.8 (C-4$_B$-OCH$_3$) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calcd. for $C_{22}H_{20}N_3O_3S$ 406.1220 found 406.1229. Purity: 96.30%

(E)-5-(2-(3,4-dimethoxyphenyl)-1-(furan-3-yl)vinyl)-4-(p-tolyl)-4H-1,2,4-triazole-3-thiol



(5o). Cream coloured solid; 82% yield; mp: 222-224 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.97 (s, 1H, SH), 7.59 (d, J = 1.8 Hz, 1H, 5-H$_A$), 7.14 (d, J = 8.1 Hz, 2H, 2-H$_D$, 6-H$_D$), 6.98 (d, J = 8.2 Hz, 2H, 3-H$_D$, 5-H$_D$), 6.81 (m, 2H, 7-H$_{C=CH}$, 2-H$_A$), 6.51 (dd, J = 8.2, 1.9 Hz, 1H, 6-H$_B$), 6.40 (dd, J = 3.3, 1.7 Hz, 1H, 3-H$_A$), 6.38 (d, J = 1.8 Hz, 1H, 2H$_B$), 5.97 (d, J = 3.4 Hz, 1H, 5-H$_B$), 3.74 (s, 3H, 3$_B$-OCH$_3$), 3.53 (s, 3H, 4$_B$-OCH$_3$), 2.30 (s, 3H, 4$_D$-CH$_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.0 (C-2$_C$), 153.1 (C-5$_C$), 150.8 (C-5$_A$), 149.2 (C-3$_B$), 148.8 (C-4$_B$), 144.4 (C-8), 138.8 (C-4$_D$), 132.3 (C-7), 129.8 (C-2$_A$) , 128.5, (C-2$_D$, 6$_D$) 128.1 (C-1$_B$), 124.4 (C-4$_A$), 122.6 (C-1$_D$), 121.8 (C-3$_D$, 5$_D$), 113.2 (C-3$_A$), 113.1 (C-6$_B$), 112.6 (C-5$_B$), 112.1 (C-2$_B$), 56.0 (C-3$_B$-OCH$_3$), 55.7 (C-4$_B$-OCH$_3$), 21.1 (C-4$_D$-CH$_3$) ppm; HRMS (ESI-QTOF): *m/z* [M+H]$^+$ calcd. for for $C_{23}H_{22}N_3O_3S$ 419.1304 found 419.1300. Purity: 97.88%

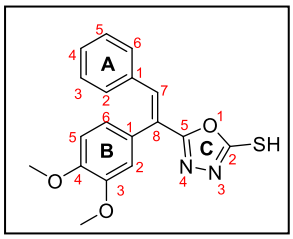
(E)-5-(1-(3,4-dimethoxyphenyl)-2-(furan-3-yl)vinyl)-4-phenethyl-4H-1,2,4-triazole-3-thiol



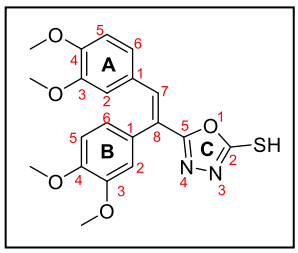
(5p). Cream coloured solid; 80% yield; mp: 166-169 °C, ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.94 (s, 1H, SH), 7.66 (d, J = 1.8 Hz, 1H, 5-H$_A$), 7.23 (m, 3H, 3-H$_D$, 4-H$_D$, 5-H$_D$), 7.05 (d, J = 7.9 Hz, 1H, 2-H$_A$), 6.96 (m, 3H, 7-H$_{C=CH}$, 2-H$_D$, 6-H$_D$), 6.80 (d, J = 7.2 Hz, 1H, 5-H$_B$), 6.70 (s, 1H, 2-H$_B$), 6.50 (d, J = 8.2 Hz, 1H, 6-H$_B$), 6.34 (d, J = 3.4 Hz, 1H, 3-H$_A$), 3.81 (s, 3H, 3$_B$-OCH$_3$), 3.71 (s, 5H, 4$_B$-

OCH_3, 9-CH_2), 2.62 (t, 2H, 10-CH_2) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 167.6 (C-2$_C$), 153.1 (C-5$_C$), 150.8 (C-5$_A$), 149.8 (C-3$_B$), 149.2 (C-4$_B$), 144.5 (C-8), 137.8 (C-7), 129.1 (C-2$_A$, 1$_D$), 128.9 (C-2$_D$, 6$_D$), 127.6 (C-1$_B$), 127.2 (C-4$_A$), 123.1 (C-4$_D$), 122.3 (C-3$_D$), 122.2 (C-5$_D$), 114.1 (C-3$_A$), 113.3 (C-6$_B$), 112.6 (C-2$_B$), 112.3 (C-5$_B$), 56.0 (C-3$_B$-OCH_3), 56.0 (C-4$_B$-OCH_3), 45.7 (C-9), 33.3 (C-10) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{24}H_{24}N_3O_3S$ 433.1460 found 433.1446. Purity: 98.19%

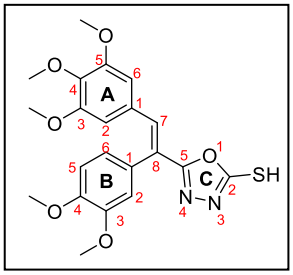
(E)-5-(2-(3,4-dimethoxyphenyl)-1-phenylvinyl)-1,3,4-oxadiazole-2-thiol ***(6a)***. Yellow coloured solid; 74% yield; mp: 166-168 °C, ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 14.59 (s, 1H, SH), 7.49 (s, 1H, 7-H$_{C=CH}$), 7.29–7.23 (m, 3H, 3-H$_A$, 4-H$_A$, 5-H$_A$), 7.20–7.16 (m, 2H, 2-H$_A$, 6-H$_A$), 7.02 (d, *J* = 8.3 Hz, 1H, 5-H$_B$), 6.92 (d, *J* = 2.0 Hz, 1H, 2-H$_B$), 6.85 (dd, *J* = 8.2, 2.0 Hz, 1H, 6-H$_B$), 3.80 (s, 3H, 3$_B$-OCH_3), 3.64 (s, 3H, 4$_B$-OCH_3); ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 177.7 (C-2$_C$), 162.9 (C-5$_C$), 149.6 (C-3$_B$), 149.4 (C-4$_B$), 135.0 (C-8), 134.8 (C-7), 130.5 (C-1$_A$), 129.5 (C-1$_B$), 128.8 (C-2$_A$, 6$_A$), 126.2 (C-4$_A$, 3$_A$), 124.7 (C-5$_A$), 122.7 (C-6$_B$), 113.8 (C-2$_B$), 112.5 (C-5$_B$), 56.0 (C-3$_B$-OCH_3), 55.9 (C-4$_B$-OCH_3) ppm; HR-MS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{18}H_{17}N_2O_3S$ 341.0954 found 341.0951. Purity: 96.41%



(E)-5-(1,2-bis(3,4-dimethoxyphenyl)vinyl)-1,3,4-oxadiazole-2-thiol ***(6b)***. Yellow coloured solid; 81% yield; mp: 220-223 °C, ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 14.49 (s, 1H, SH), 7.43 (s, 1H, 7-H$_{C=CH}$), 7.06 (d, *J* = 8.3 Hz, 1H, 5-H$_B$), 6.96 (d, *J* = 1.9 Hz, 1H, 2-H$_B$), 6.88 (m, 2H, 6-H$_A$, 6-H$_B$), 6.86 (d, *J* = 2.0 Hz, 1H, 2-H$_B$), 6.67 (d, *J* = 8.0 Hz, 1H, 5-H$_A$), 3.80 (s, 3H, 3$_B$-OCH_3), 3.73 (s, 3H, 4$_B$-OCH_3), 3.69 (s, 3H, 3$_A$-OCH_3), 3.41 (s, 3H, 4$_A$-OCH_3) ppm; ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ 177.6 (C-2$_C$), 163.2 (C-5$_C$), 150.2 (C-3$_B$), 149.7 (C-4$_B$), 149.5 (C-3$_A$), 148.4 (C-4$_A$), 135.1 (C-8), 127.3 (C-7), 126.7 (C-1$_A$), 124.9 (C-1$_B$), 122.7 (C-6$_B$), 122.0 (C-6$_A$), 113.8 (C-2$_B$), 113.2 (C-2$_A$), 112.8 (C-5$_B$), 111.8 (C-5$_A$), 56.2 (C-3$_B$-OCH_3), 56.1 (C-4$_B$-OCH_3), 55.9 (C-3$_A$-OCH_3), 55.2 (C-4$_A$-OCH_3)ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{20}H_{21}N_2O_5S$ 401.1166 found 401.1163. Purity: 98.26%

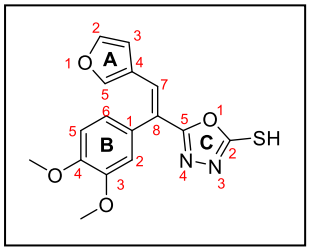


(E)-5-(1-(3,4-dimethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)vinyl)-1,3,4-oxadiazole-2-thiol



(**6c**). Yellow coloured solid; 78% yield; mp: 195-200 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.57 (s, 1H, SH), 7.45 (s, 1H, 7-$H_{C=CH}$), 7.08 (d, J = 8.3 Hz, 1H, 5-H_B), 6.98 (d, J = 1.9 Hz, 1H, 2-H_B), 6.88 (dd, J = 8.2, 1.9 Hz, 1H, 6-H_B), 6.55 (s, 2H, 2-H_A, 6-H_A), 3.80 (s, 3H, 3_B-OCH_3), 3.70 (s, 3H, 4_B-OCH_3), 3.63 (s, 3H, 5_A-OCH_3), 3.51 (s, 6H, 3_A-OCH_3, 4_A-OCH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 177.6 (C-2_C), 163.0 (C-5_C), 152.7 (C-5_A), 149.8 (C-3_B, 4_B), 149.6 (C-3_A,4_A), 138.8 (C-8), 135.1 (C-1_A), 130.0 (C-7), 126.5 (C-1_B), 123.6 (C-6_B), 122.7 (C-6_A), 113.9 (C-5_B), 112.9 (C-2_A), 108.4 (C-5_B), 60.5 (C-5_A), 56.2 (C-3_B), 56.2 (C-4_B), 55.8 (C-3_A, 4_A)ppm; HRMS (ESI): *m/z* $[M+H]^+$ calcd. for $C_{21}H_{23}N_2O_6S$ 430. 1199 found 430.1200. Purity: 96.08%

(E)-5-(1-(3,4-dimethoxyphenyl)-2-(furan-3-yl)vinyl)-1,3,4-oxadiazole-2-thiol ***(6d)***. Red



coloured solid; 70% yield, mp: 212-215 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.57 (s, 1H, SH), 7.73 (d, J =1.7 Hz, 1H, 5-H_A), 7.30 (s, 1H, 7-$H_{C=CH}$), 7.05 (d, J = 8.3 Hz, 1H, 5-H_B), 6.97 (d, J = 1.7 Hz, 1H, 2-H_B), 6.91 (dd, J = 8.2, 1.8 Hz, 1H, 6-H_B), 6.53-6.49 (m, 1H, 2-H_A), 6.20 (d, J = 3.4 Hz, 1H, 3-H_A), 3.82 (s, 3H, 3_B-OCH_3), 3.71 (s, 3H, 4_B-OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 177.6 (C-2_C), 162.7 (C-5_C), 150.6 (C-5_A), 149.5 (C-3_B), 149.1 (C-4_B), 145.6 (C-8), 126.1 (C-2_A), 122.5 (C-7), 122.4 (C-4_A), 120.9 (C-1_B), 115.5 (C-3_A), 113.5 (C-6_B), 113.1 (C-2_B), 112.2 (C-5_B), 56.1 (C-3_B-OCH_3), 55.9 (C-4_B-OCH_3) ppm; HRMS (ESI-QTOF): *m/z* $[M+H]^+$ calcd. for $C_{16}H_{14}N_2O_4S$ 331.0741 found 331.0749. Purity: 95.99%

1.2. b. 2-D NMR

The NOESY correlation was established to determine the (*E*)-configuration of the synthesized molecule. NOESY experiment was performed using compound **5j**. The experiment revealed the correlation between protons represented by multiplet at 6.90-6.78 and protons at 6.54. The correlation proves the presence of the phenyl rings on the same side and hence justify the (*E*)-configuration.

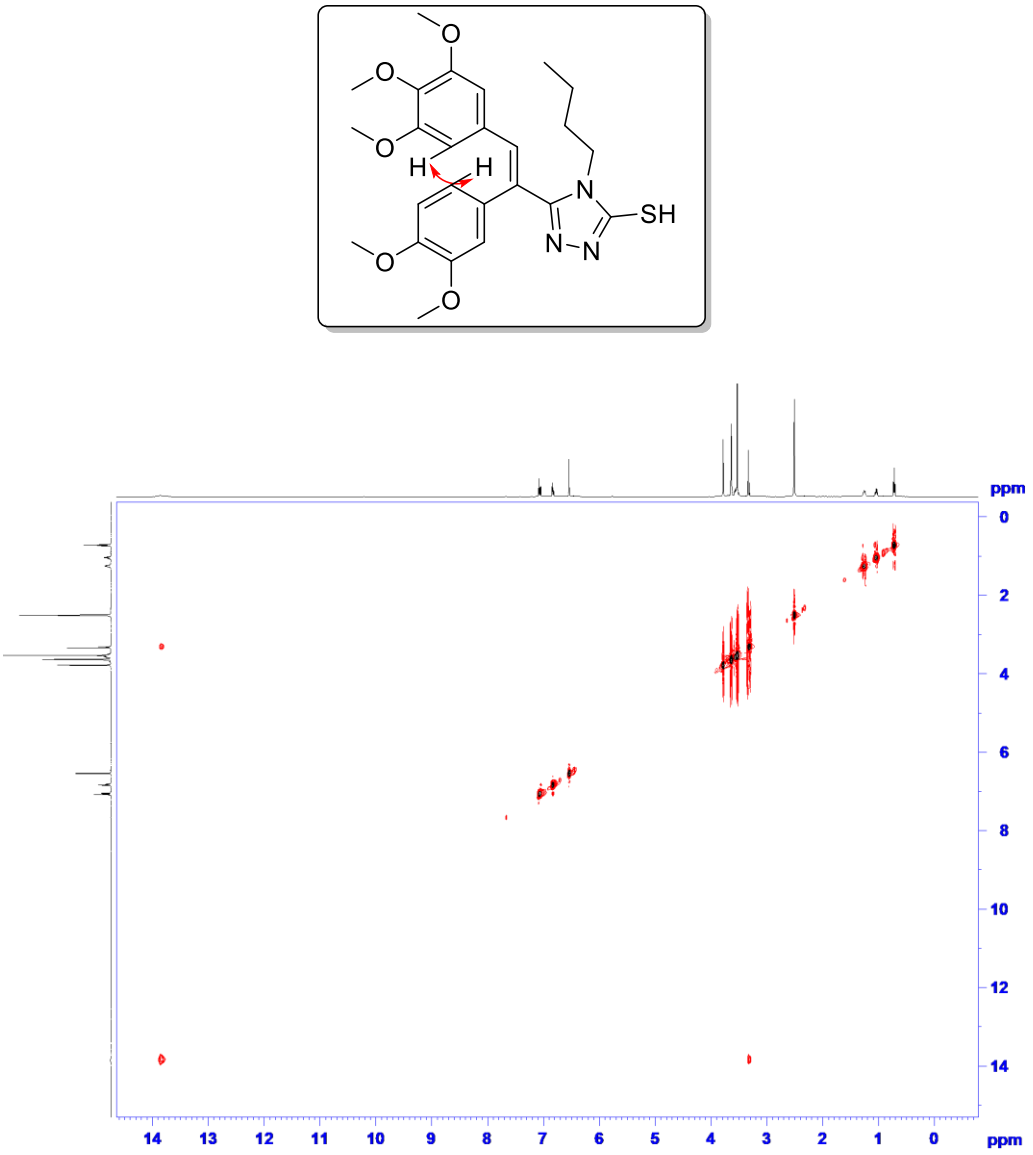


Figure S1. NOESY spectrum of compound **5j**

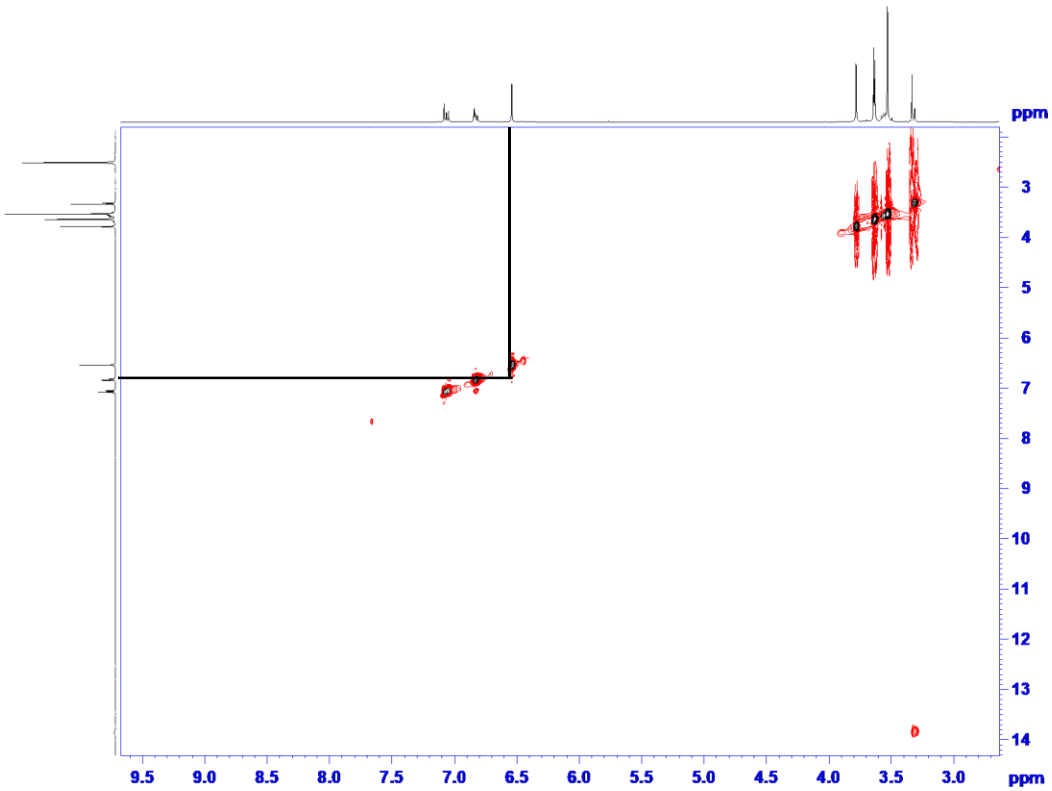


Figure S1. Expanded NOESY spectrum of compound **5j**

2. Biological evaluation

2.1. Cell culture

Human cancer cell lines such as breast cancer (MCF-7, MDA-MB-231), lung cancer (A549), colon cancer (HCT-116), skin cancer (SK-Mel-28) and normal human keratin cells (HaCat) were procured from National Centre for Cell Science, Pune, India. The Cells were maintained in the incubator at 37 °C with an appropriate media supplement of 10% fetal bovine serum (FBS) stabilized with 1% antibiotic-antimycotic solution (Sigma Aldrich). Sub-culturing was performed when the cells reached confluency upto 80–90%, using a 0.25% trypsin/1 mM EDTA solution for further passage. A 20 mM stock solution was prepared by dissolving compounds in a calculated quantity of DMSO and further respective media was used for the following dilutions to obtain the required concentration accordingly.

2.2. MTT assay

The MTT assay (colorimetric assay) was employed to determine the cytotoxicity of all newly synthesized compounds. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is a dye used for accessing the metabolic activity of the cells. The basic principle utilizes the reducing capability of the mitochondrial succinate dehydrogenase enzyme that converts MTT into insoluble formazan producing purple color.[1] In brief, the 96-well plates

were seeded with cells at a density of 10,000 to 12,000 cells/cm^2 in 100 µL of complete media and allowed to grow overnight. Before treatment, media was replaced with fresh media without supplemented with serum for 2 hrs. After 2 hrs the cells were treated with various concentrations of the test compounds in complete media for a period of 24 h. After 24 hr incubation, after the media was aspirated, 100 µL of MTT (0.5 mg/mL) was added and incubated at 37 °C for 4 h. Later, the removal of the MTT reagent was followed by the DMSO (100 µL) addition to dissolve the formazan. Finally, absorbance was measured 570 nm wavelength using a spectrophotometric microtiter plate reader. The compounds which exhibited >50% inhibition of cell viability (IC_{50}) at 50 µM in preliminary screening were further selected to evaluate dose response curve (DRC) analysis. IC_{50} values were determined from the DRC analysis by linear regression method. All the values were expressed as mean ± SEM of three independent experiments.

2.3. Apoptosis and DNA damage studies

2.3.1. DAPI staining

Nuclear morphological changes were observed through DAPI staining. After treatment of A549 cells with **5a** at different concentrations (4.5, 9, 18 µM) and colchicine (0.88 µM) for 24 h, cells were washed with PBS and then fixed using paraformaldehyde (4%) followed by permeabilization with 200 µL of 0.1% triton X for 10 min and finally stained with 1 µM DAPI for 15 min. The fluorescence microscope (Model: Nikon, Japan) with excitation at 359 nm and emission at 461 nm using DAPI filter at 40X magnification was used to observe the control and treated cells.

2.3.2. Acridine orange/Ethidium bromide

The A549 cells were plated at a concentration of 10,000 to 12,000 cells/cm^2 and treated with different concentration of compound **5a**. Plates were incubated in an a incubator having 5% CO_2 and temperature 37 °C for 24 h. Fluorescent dyes containing Acridine Orange (AO, 2 µM) and Ethidium Bromide (EB, 8µM) were added into each well and then the cells were visualized and images were captured using fluorescence microscope (Nikon, Inc. Japan) with excitation (488 nm) and emission (550 nm) at 40X magnification.

2.3.3. Reactive oxidative species (ROS) generation.

The A549 cells were plated at 10,000 to 12,000 cells/well in 24-well culture plates and allowed to adhere overnight. Cells were treated with colchicine and compound **5a** for 24 h, washed with PBS. Cells were exposed to Carboxy-DCFDA dye (life technologies) (10 µM) for

30 min in incubator at dark and washed with PBS to remove the excess dye. The increase in the intensity of fluorescence which is directly co-related with generation of reactive oxygen species was analysed using a fluorescence microscope (Nikon).

2.4. Flow cytometric analysis

2.4.1. Apoptosis detection study by Annexin V/PI dual staining assay

The assay was performed as described by Rieger *et al.* with slight modifications.[2] Briefly, 10,000 to 12,000 cell/cm^2 cells were seeded in a 12-well plate and treated with 4.5, 9 and 18 μM concentration of **5a** compound for 24 h. Additionally, cells were also treated with colchicine (0.88 μM). At the termination of experiment, cells were trypsinized and centrifuged along with media at 130g for 5 min. Then, the cells were incubated for 15 min. at room temperature in dark with 200 μL of 1×binding buffer containing 10 μL propidium iodide (PI) and 3 μL of annexin V-FITC. After incubation, flow cytometer (BD FACSVerse™, USA) was employed to analyse the cells for apoptosis. The quadrant statistics on propidium iodide-negative cells, fluorescein positive cells, and propidium iodide (PI)-positive cells, respectively enabled analysis of apoptosis and necrosis.

2.4.2. Cell cycle assay

To substantiate the results observed in MTT assay and to comprehend the effect of **5a** compound on cell cycle progression, cell cycle analysis was performed using flow cytometry experiments according to the reported protocol.[3] The A549 cells were treated with 4.5, 9, 18 μM of **5a** compound and colchicine (0.88 μM) and incubated for 24 h. The untreated A549 cells were used as a control. After 24 h, the untreated and treated cells were harvested, washed with phosphate-buffered saline (PBS), fixed in ice-cold with 70% alcohol overnight at 4 °C. The fixed cells were then stained with propidium iodide (PI) (Sigma-Aldrich) in dark and the cell cycle was analyzed using a Becton Dickinson (BD FACSVerseTM, USA) flow cytometer.

2.5. Tubulin polymerization assay

Tubulin polymerization kit was procured from Cytoskeleton, Inc. (BK011P). To study the effect of compound **5a**, fluorescence based *in vitro* tubulin polymerization assay was performed following the manufacturer's protocol. The reaction mixture having porcine brain tissue (2 mg/mL) in 80 mM PIPES at pH 6.9, 2.0 mM $MgCl_2$, 0.5 mM EGTA,1.0 mM GTP and glycerol in the presence and absence of test compound **5a** was prepared and added to each well of 96-well plate. The tubulin polymerization was assessed by a time dependent increase in fluorescence due to the insertion of a fluorescence reporter into microtubules as

polymerization takes place. The fluorescence emission was measured at 440 nm (excitation wavelength 360 nm) using Spectramax M4 Multi mode Micro plate Detection System. The positive control paclitaxel was used in the assay at 3 μM final concentration. The IC_{50} value was determined from the drug concentration required for inhibiting 50% of tubulin assembly compared to control.

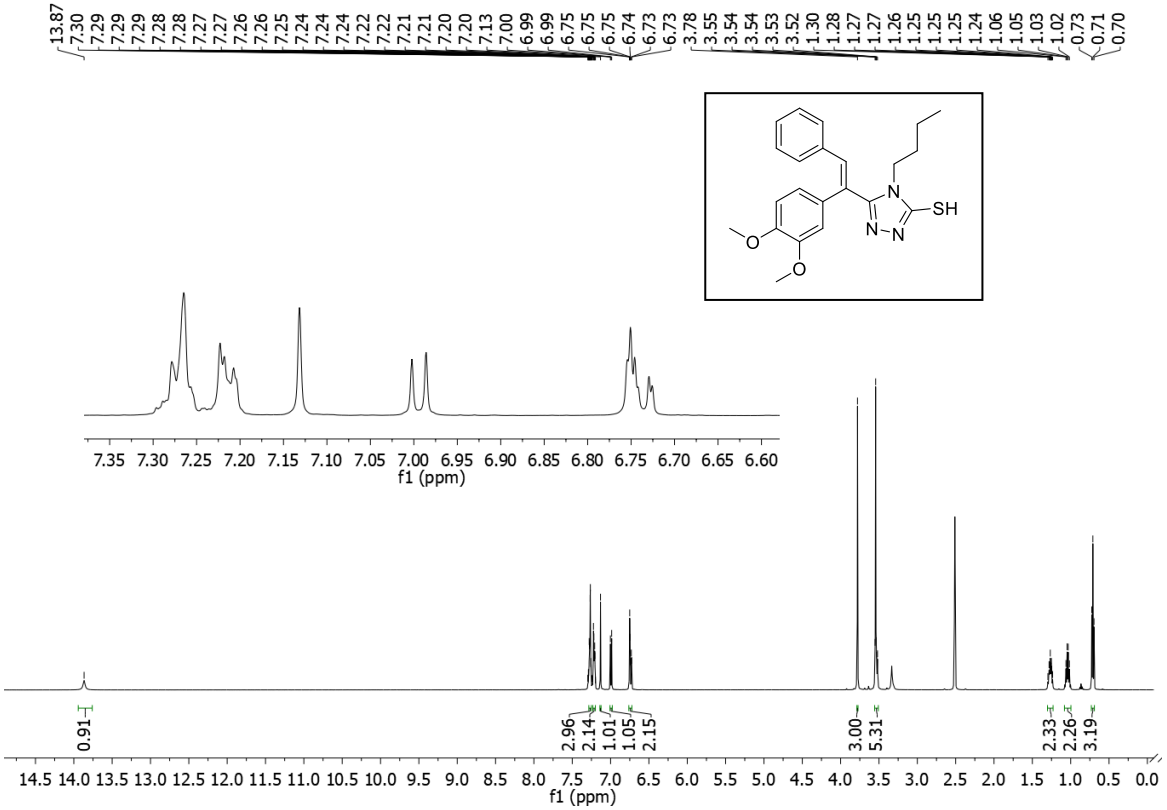
3. Molecular modelling

The tubulin crystal structure has been retrieved from the Protein Data Bank (PDB ID: 3E22). By using 2D sketcher the potent hybrids **5a**, **5d** and **5p** were sketched and energy minimized, ligand preparation was performed for the generation of different conformers (Schrödinger 2019–1). The protein preparation tool was used for the preparation of the tubulin protein. The grid is generated by picking the active site where the co-crystal is located and grid box of 10 × 10 × 10Å (Schrödinger 2019–1). The various conformers of **5a**, **5d** and **5p** thus obtained were subjected to molecular docking with SP Glide (Schrödinger 2019–1). The ligand-protein was analyzed for various interactions and the poses generated were assessed and the best one was reported.

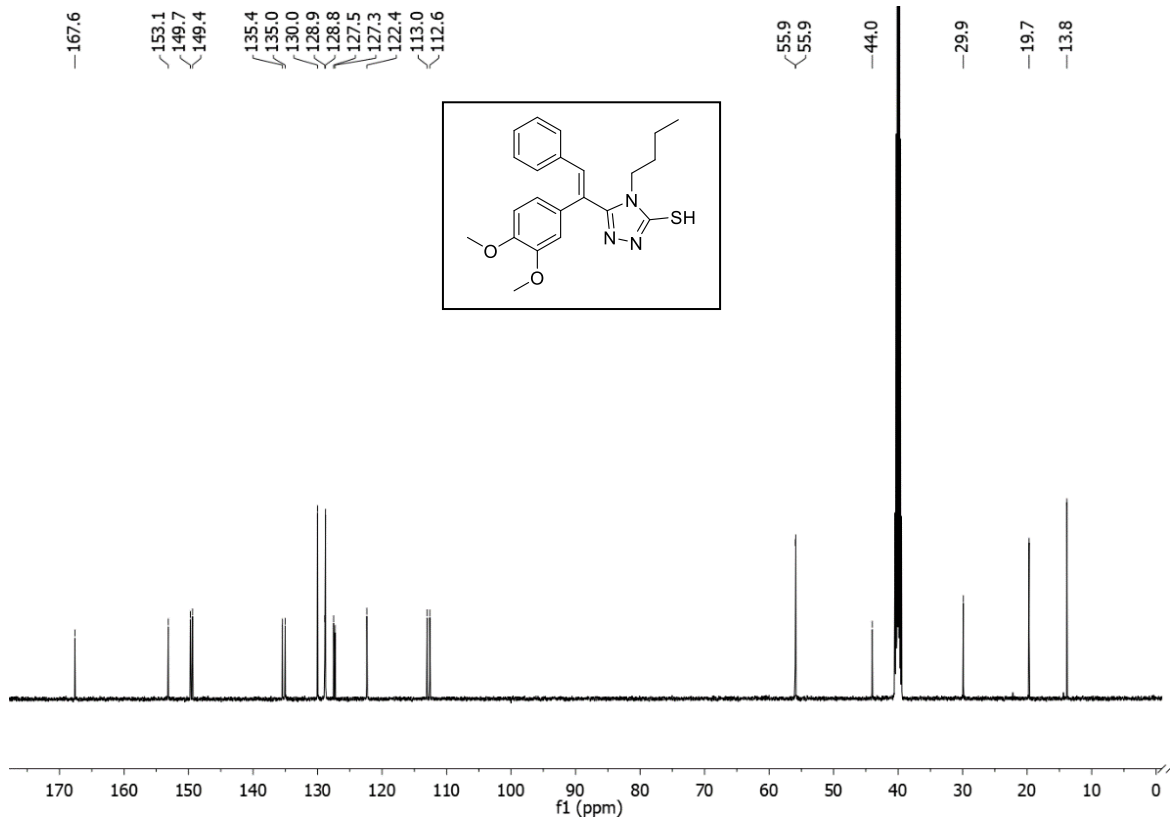
4. References

1. J. van Meerloo, G. J. L. Kaspers, J. Cloos, Cell Sensitivity Assays: The MTT Assay. In: I. Cree (Ed.), Cancer Cell Culture. Methods in Molecular Biology (Methods and Protocols), vol. 731, Humana Press, 2011. https://doi.org/10.1007/978-1-61779- 080-5_20.
2. A. M. Rieger, K. L. Nelson, J. D. Konowalchuk D. R. Barreda DR. Modified annexin V/ propidium iodide apoptosis assay for accurate assessment of cell death. *J Vis Exp.* **2011**, 50, 2597.
3. J. P. Johnpeter, G. Gupta, J. M. Kumar, G. Srinivas, N. Nagesh, B. Therrien, Biological studies of chalcogenolato-bridged dinuclear half-sandwich complexes. *Inorg Chem.*, **2013**, *52*, 13663.

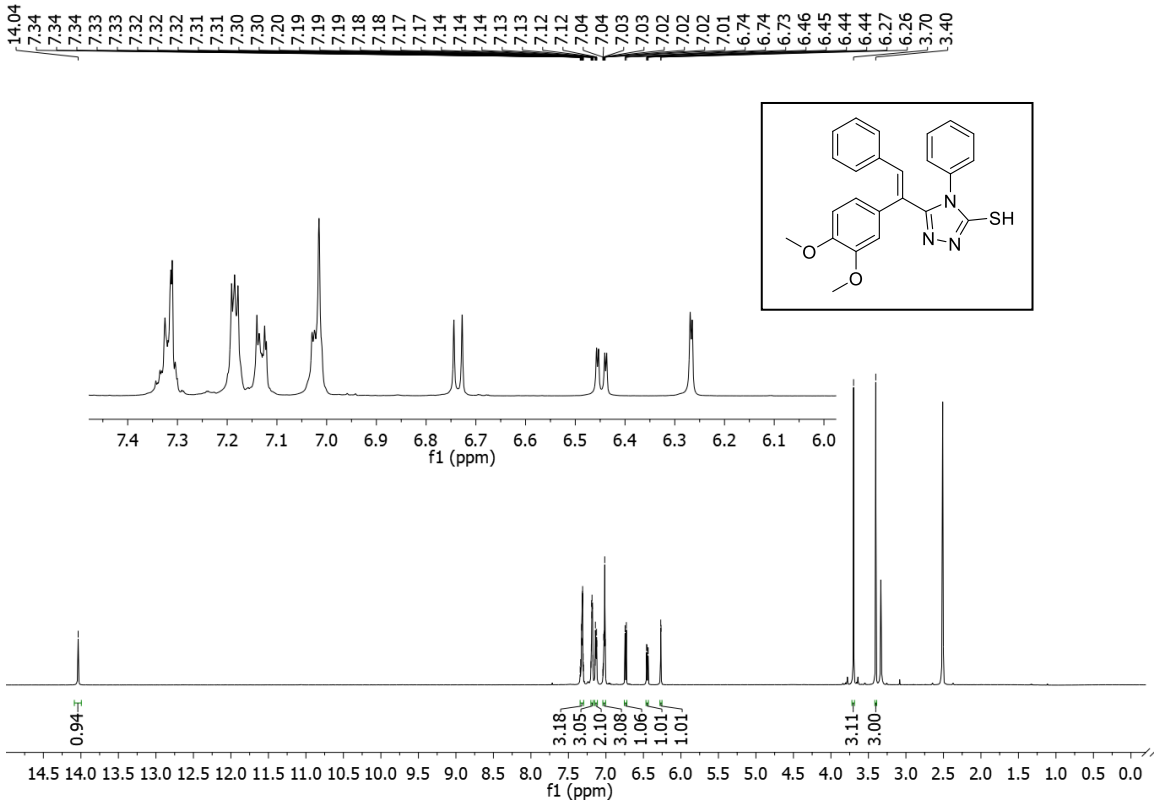
5. Spectral Data



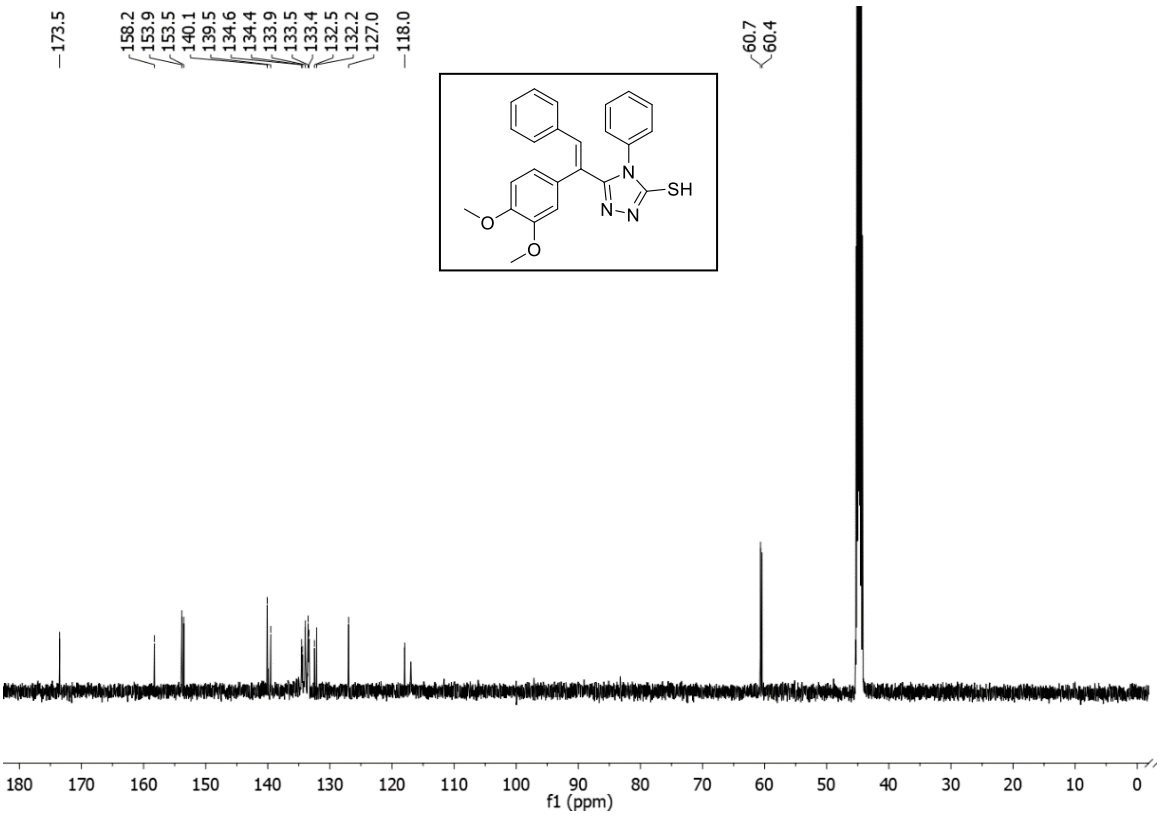
^{1}H NMR (500 MHz, DMSO-d_6) of compound **5a**



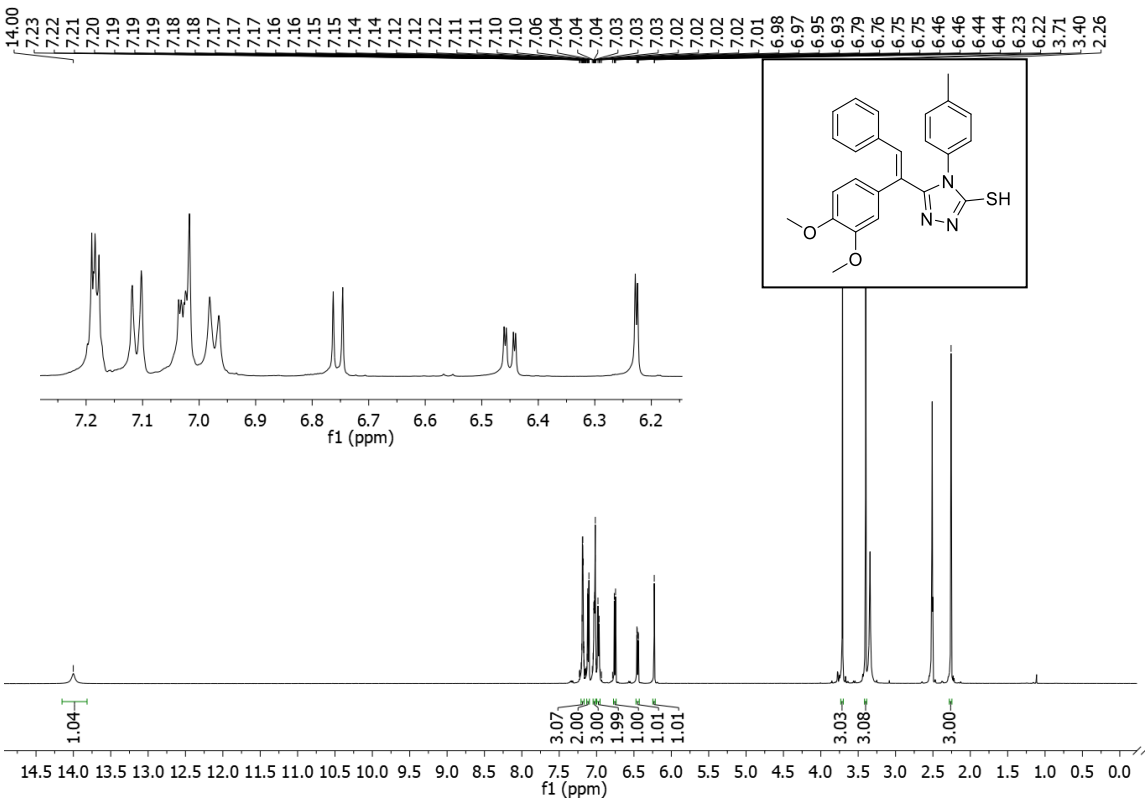
^{13}C NMR (125 MHz, DMSO-d_6) of compound **5a**



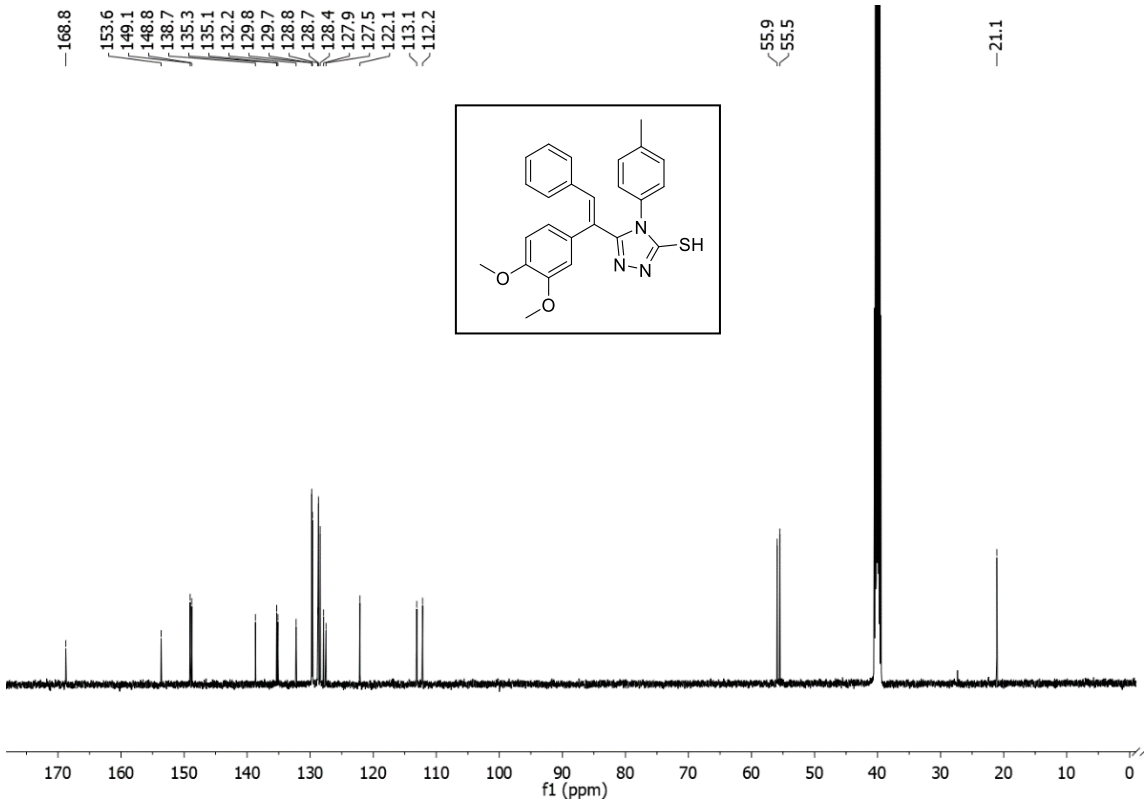
^{1}H NMR (500 MHz, DMSO-d_6) of compound **5b**



^{13}C NMR (125 MHz, DMSO-d_6) of compound **5b**



^{1}H NMR (500 MHz, DMSO-d_6) of compound **5c**



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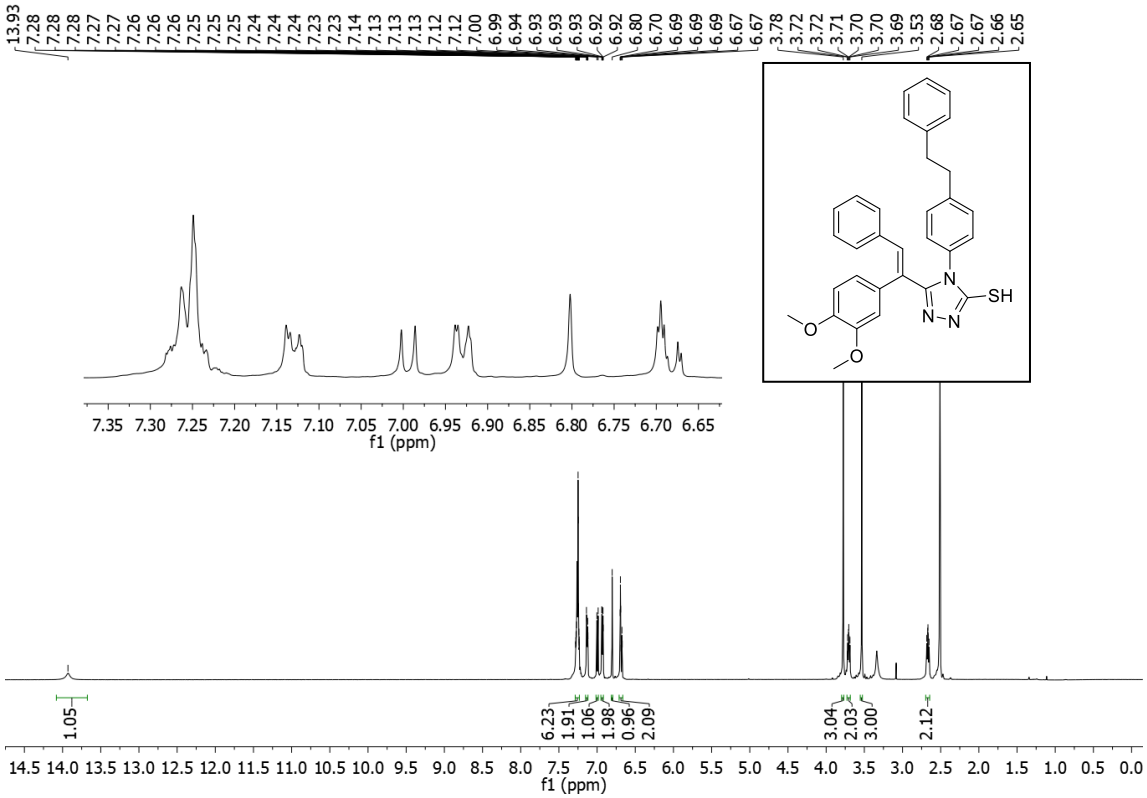
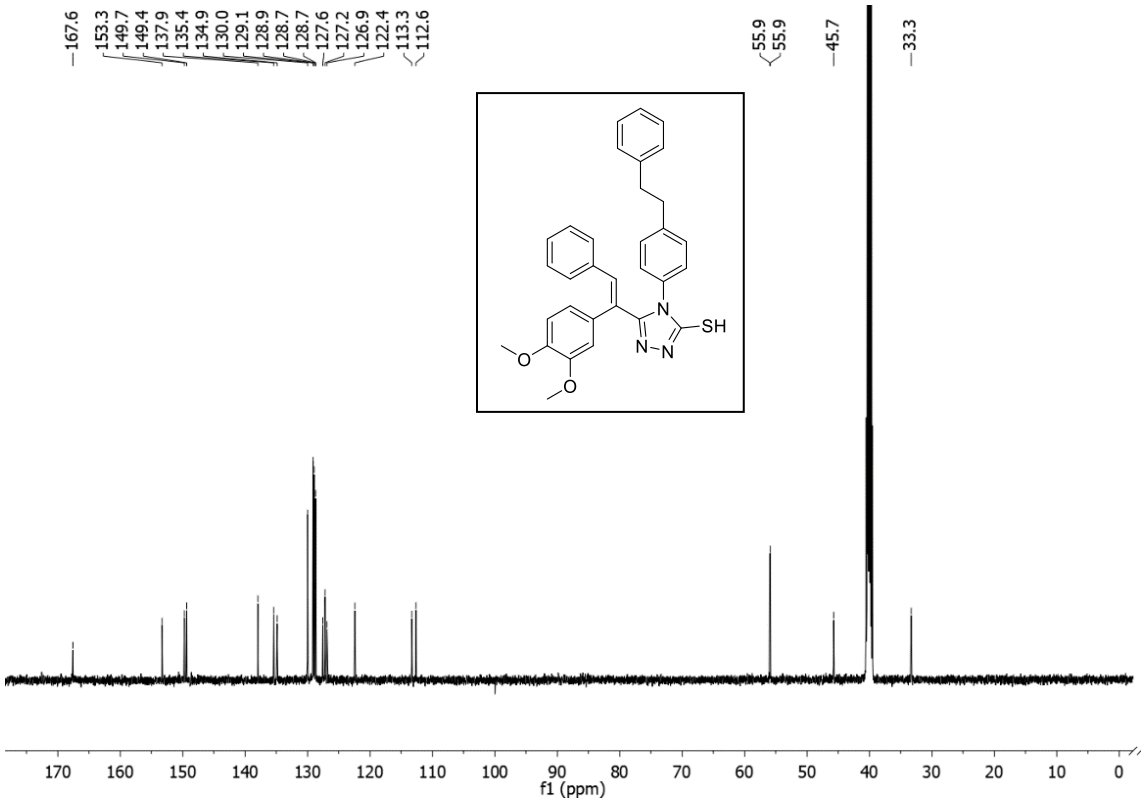
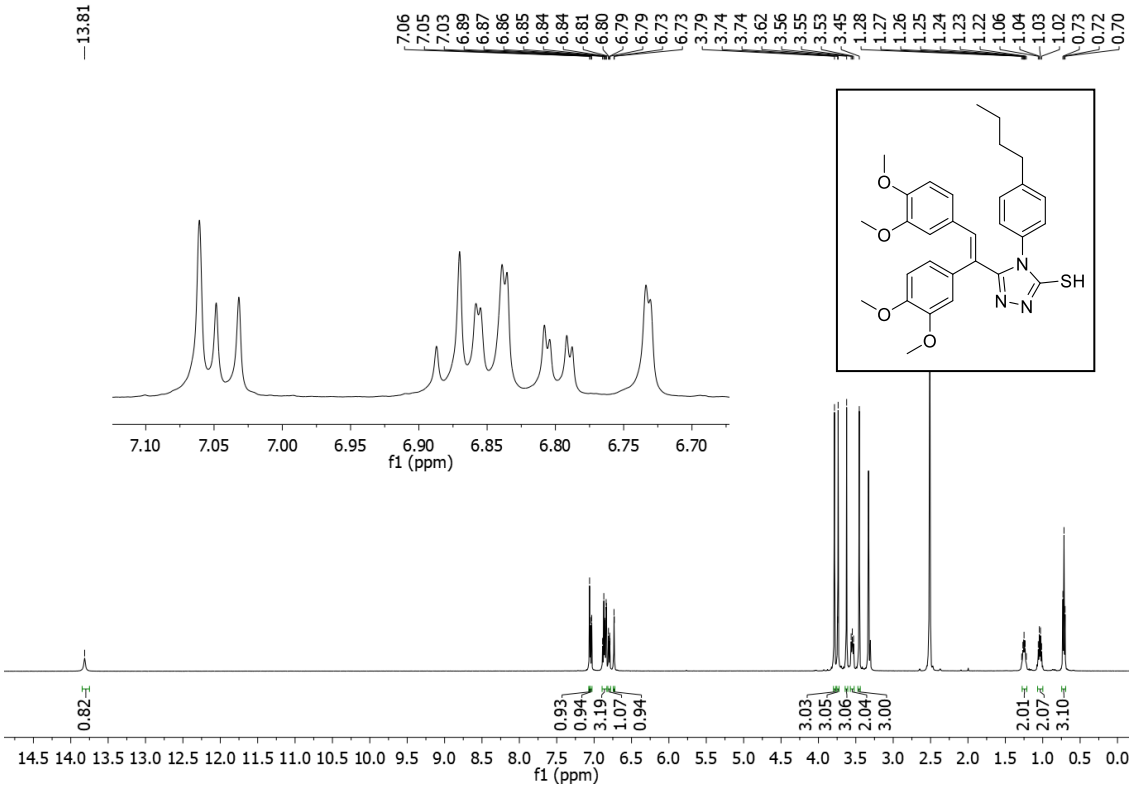


^{1}H NMR (500 MHz, DMSO-d_6) of compound **5d**



^{13}C NMR (125 MHz, DMSO-d_6) of compound **5d**



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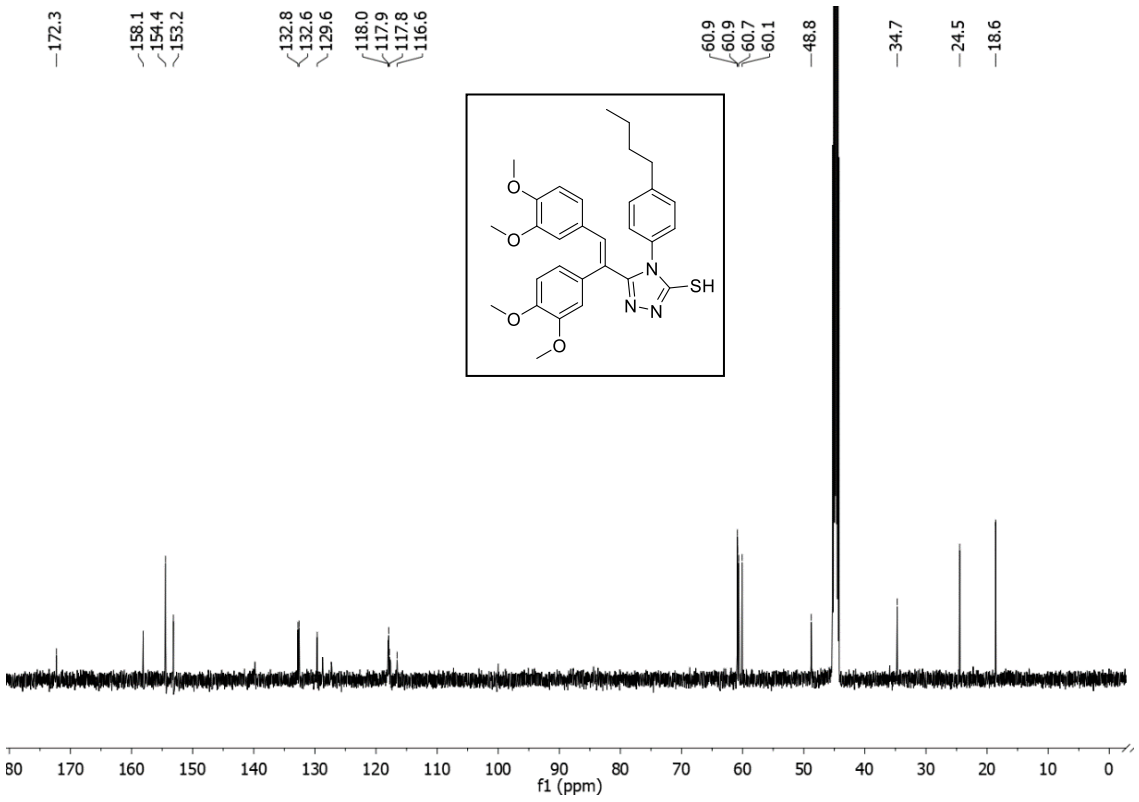

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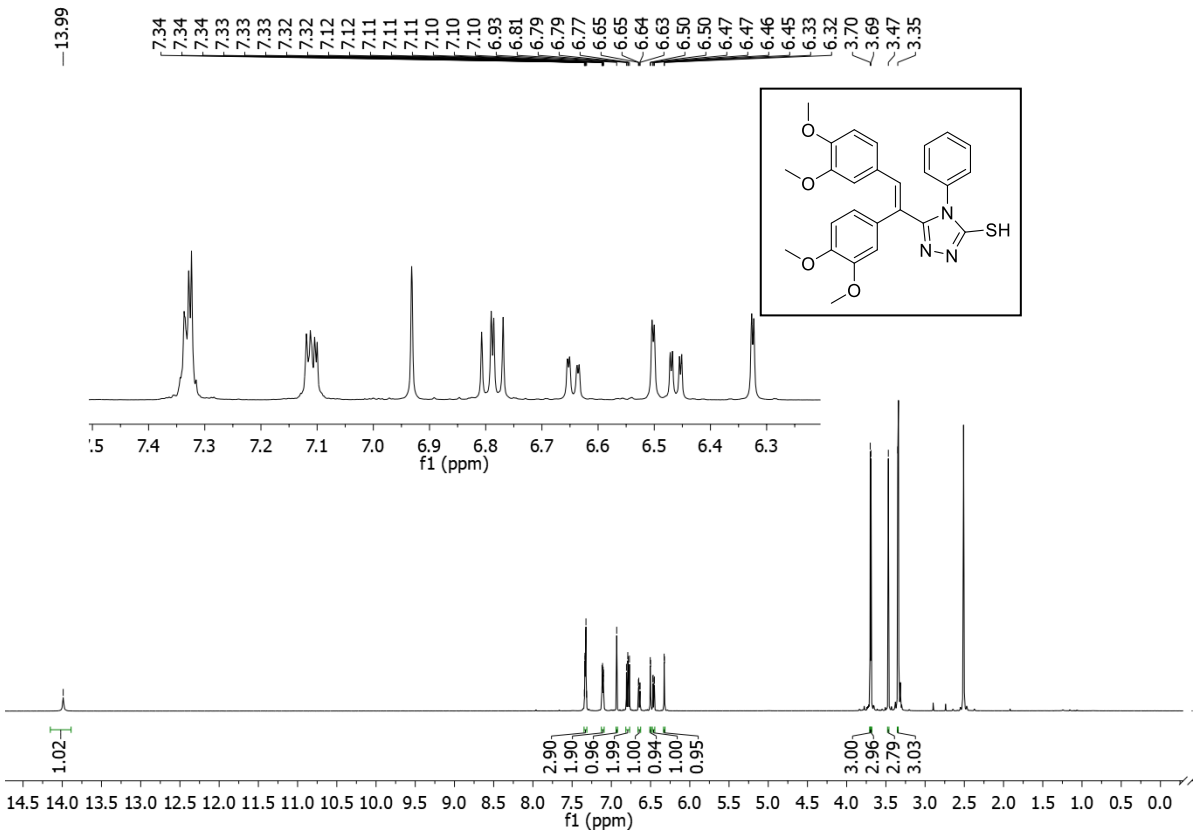
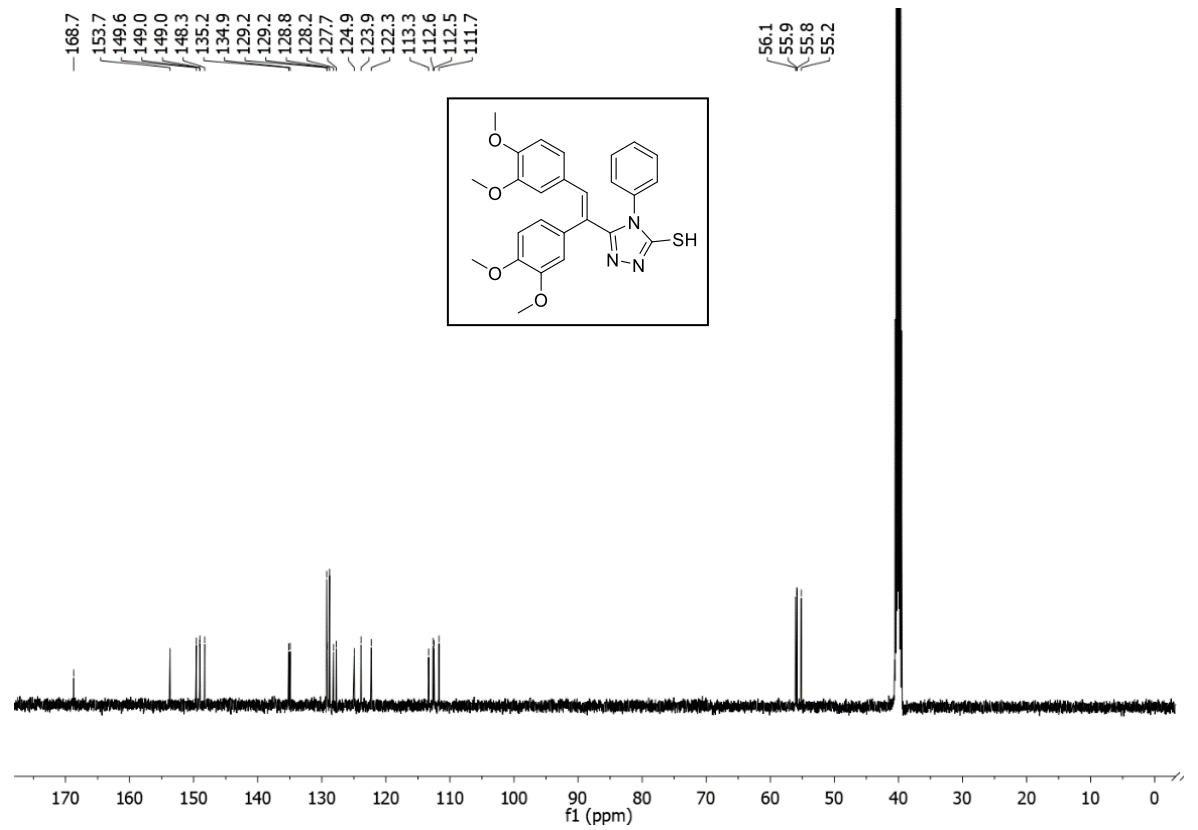
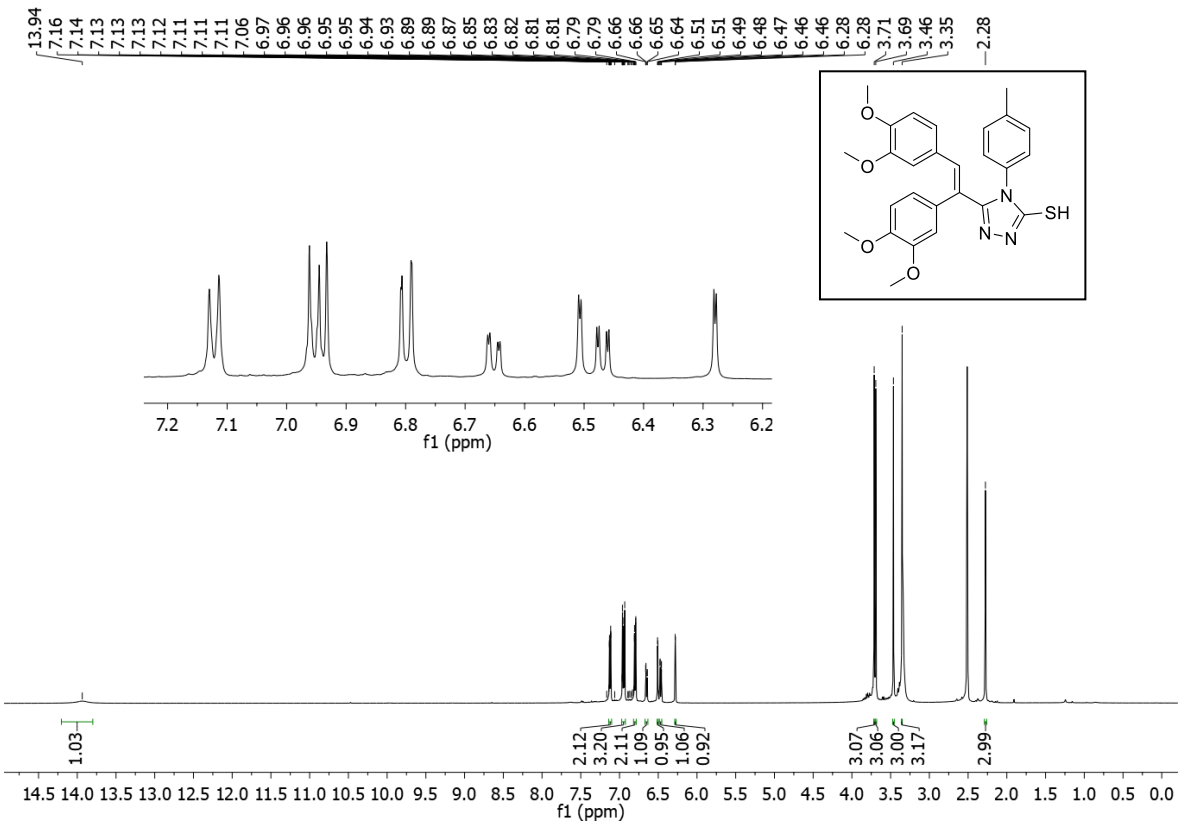


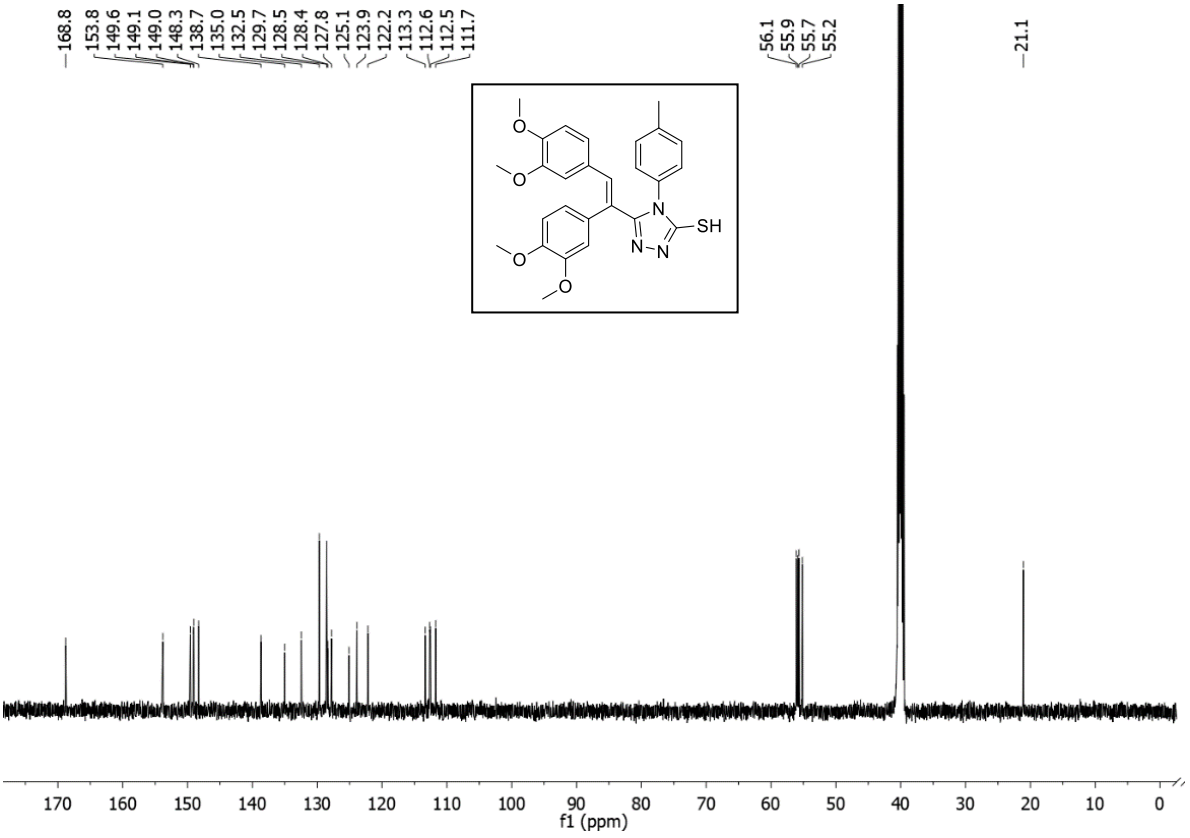
^{1}H NMR (500 MHz, DMSO-d_6) of compound **5f**



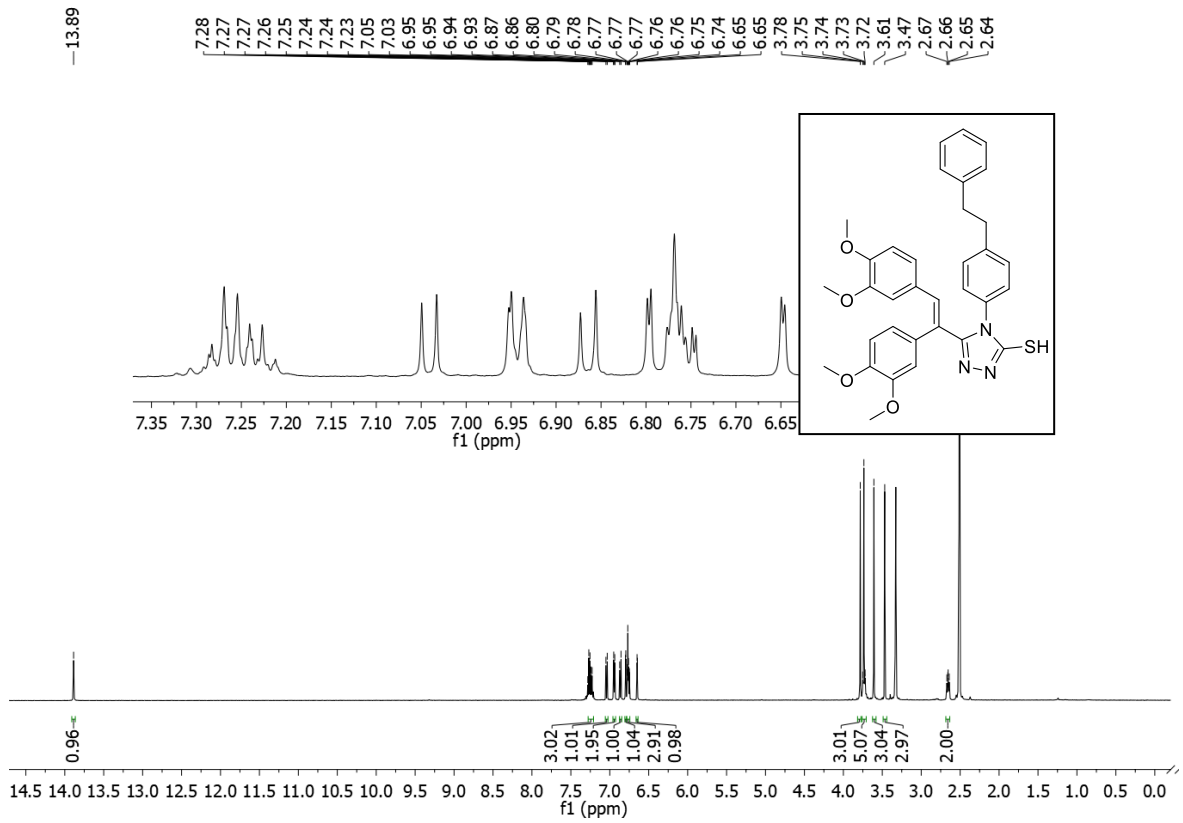
^{13}C NMR (125 MHz, DMSO-d_6) of compound **5f**



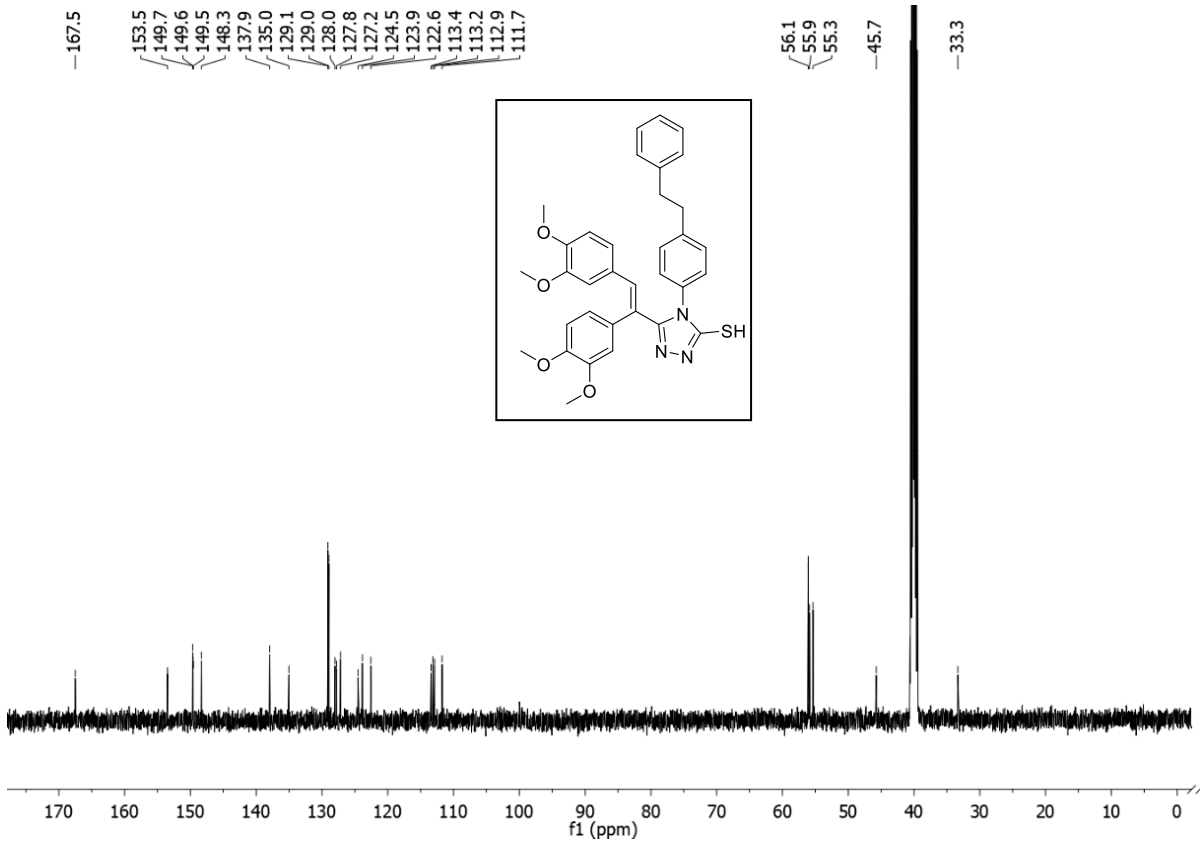
^{1}H NMR (500 MHz, DMSO-d_6) of compound **5g**



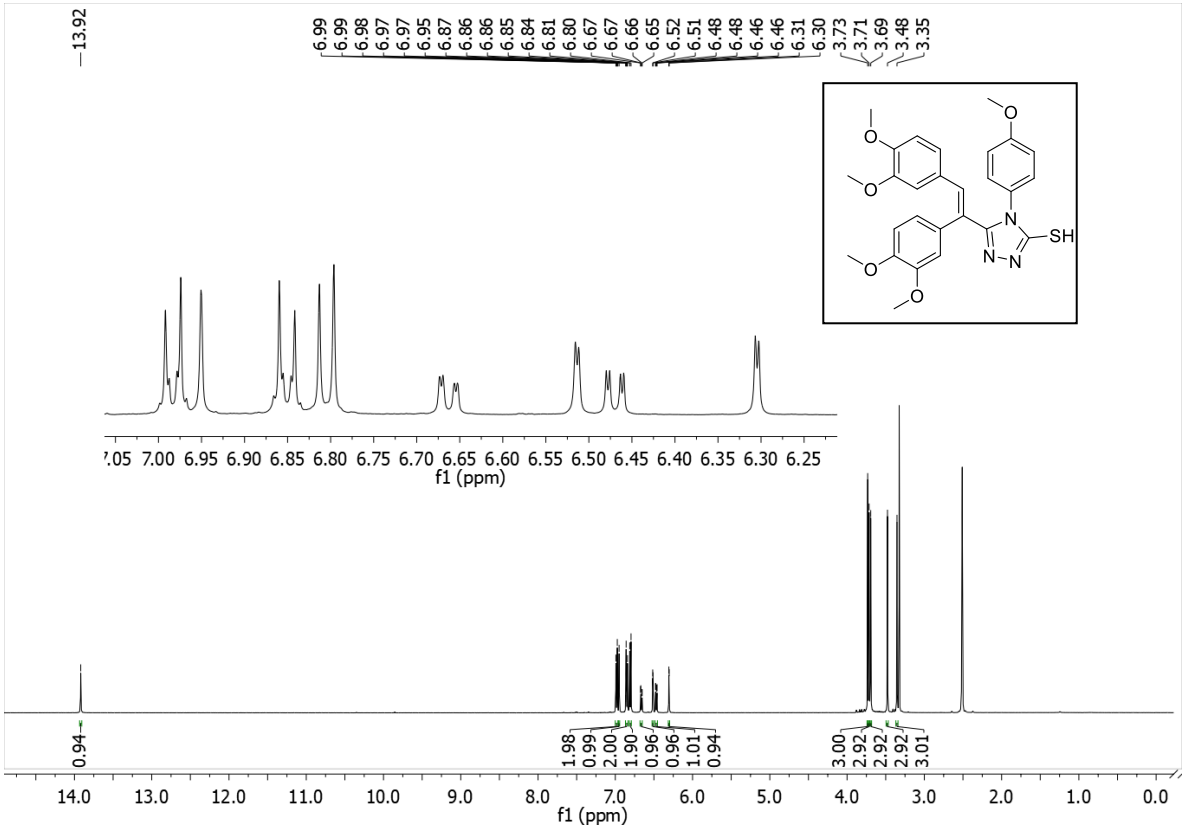
^{13}C NMR (125 MHz, DMSO-d_6) of compound **5g**



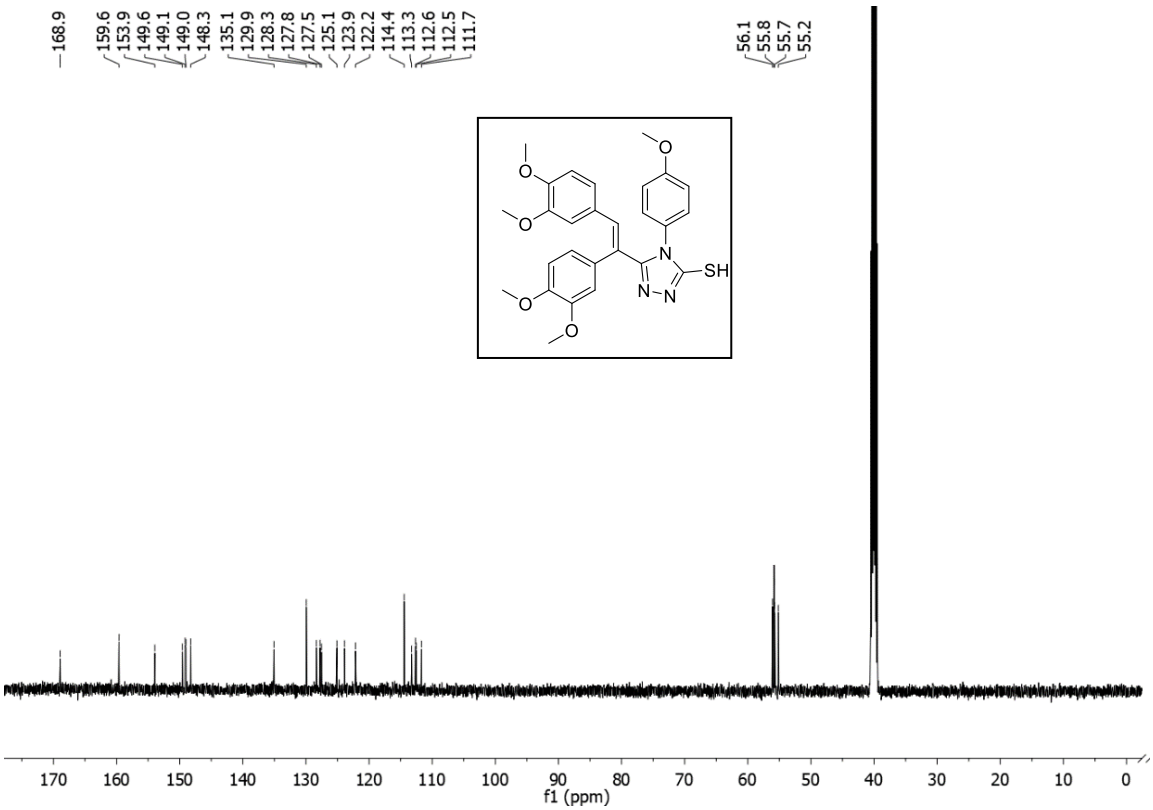
^{1}H NMR (500 MHz, DMSO-d_6) of compound **5h**



^{13}C NMR (125 MHz, DMSO-d_6) of compound **5h**



^{1}H NMR (500 MHz, DMSO-d_6) of compound **5i**



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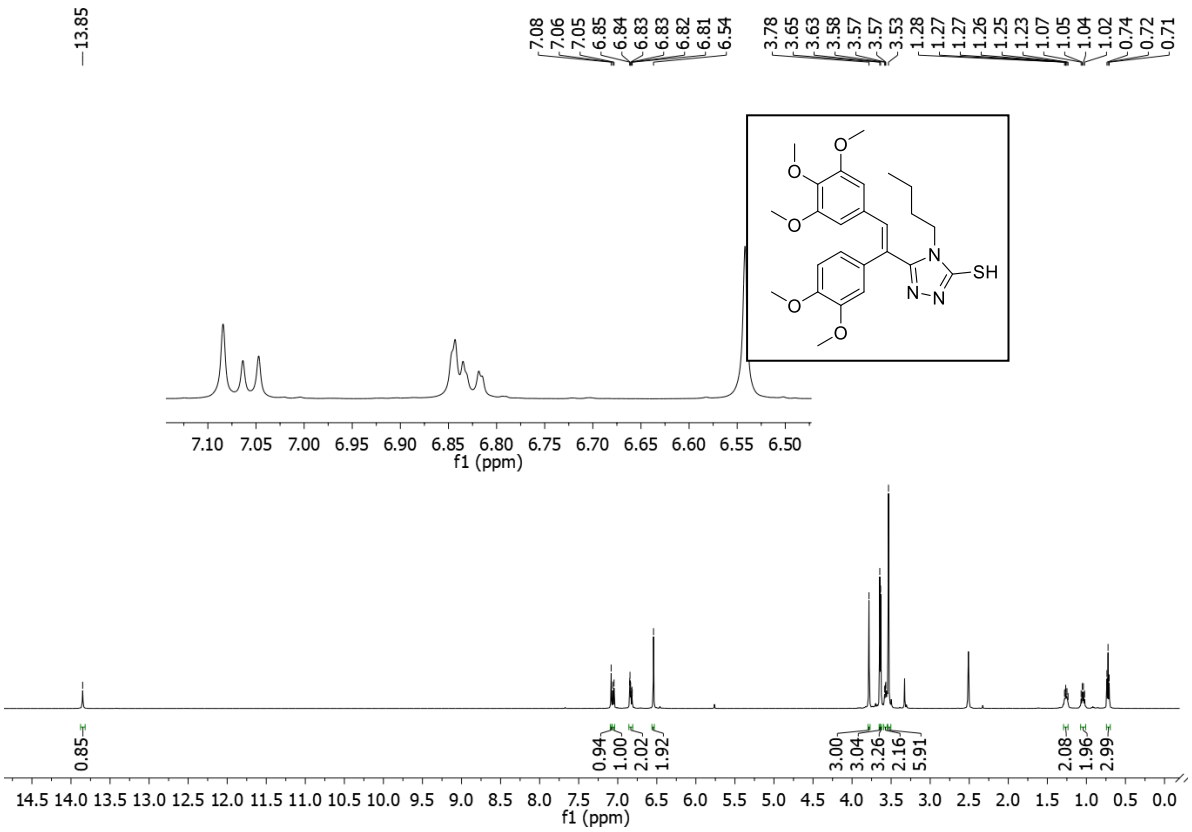

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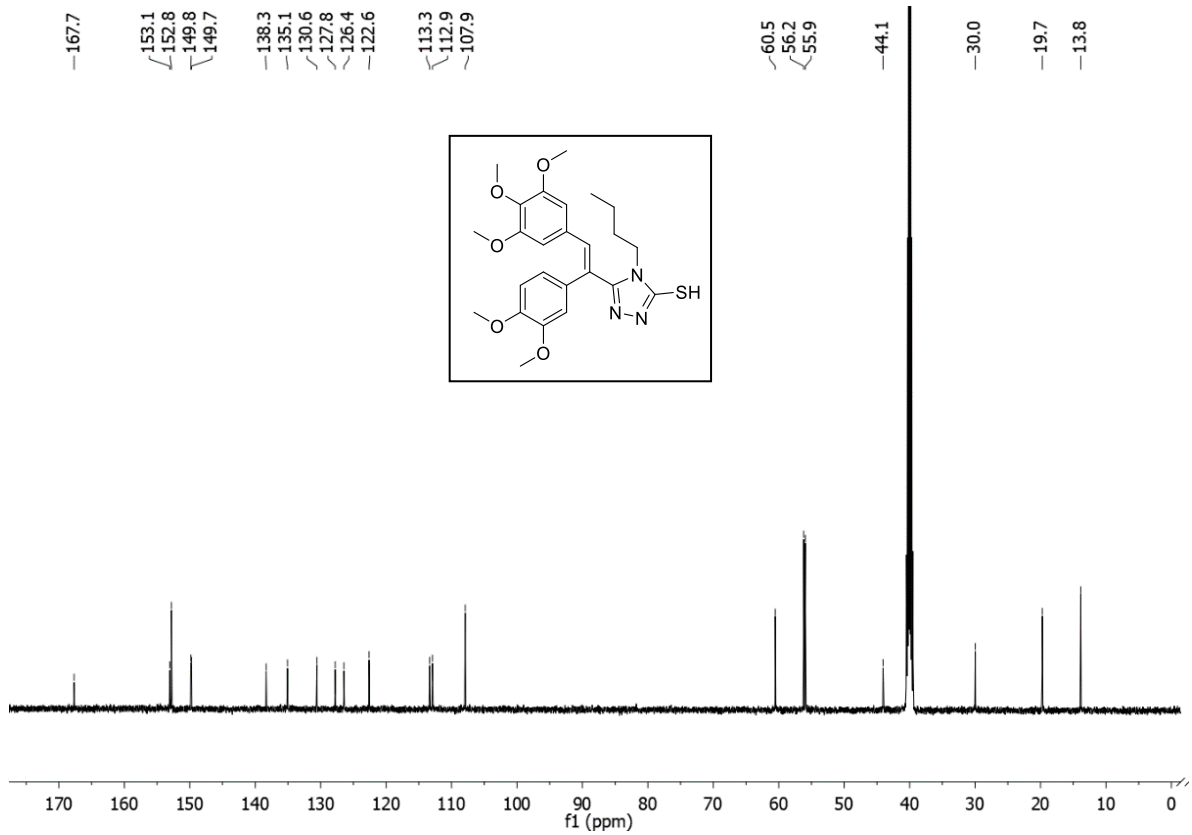

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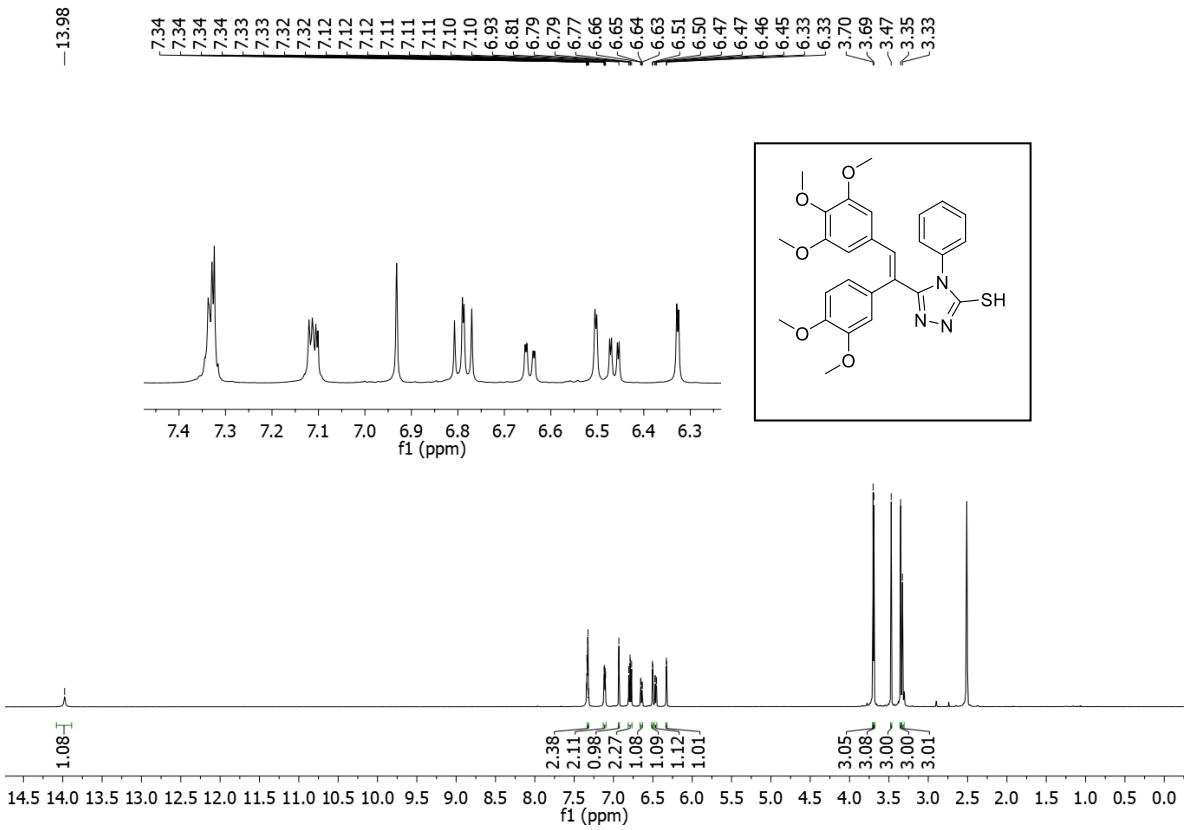

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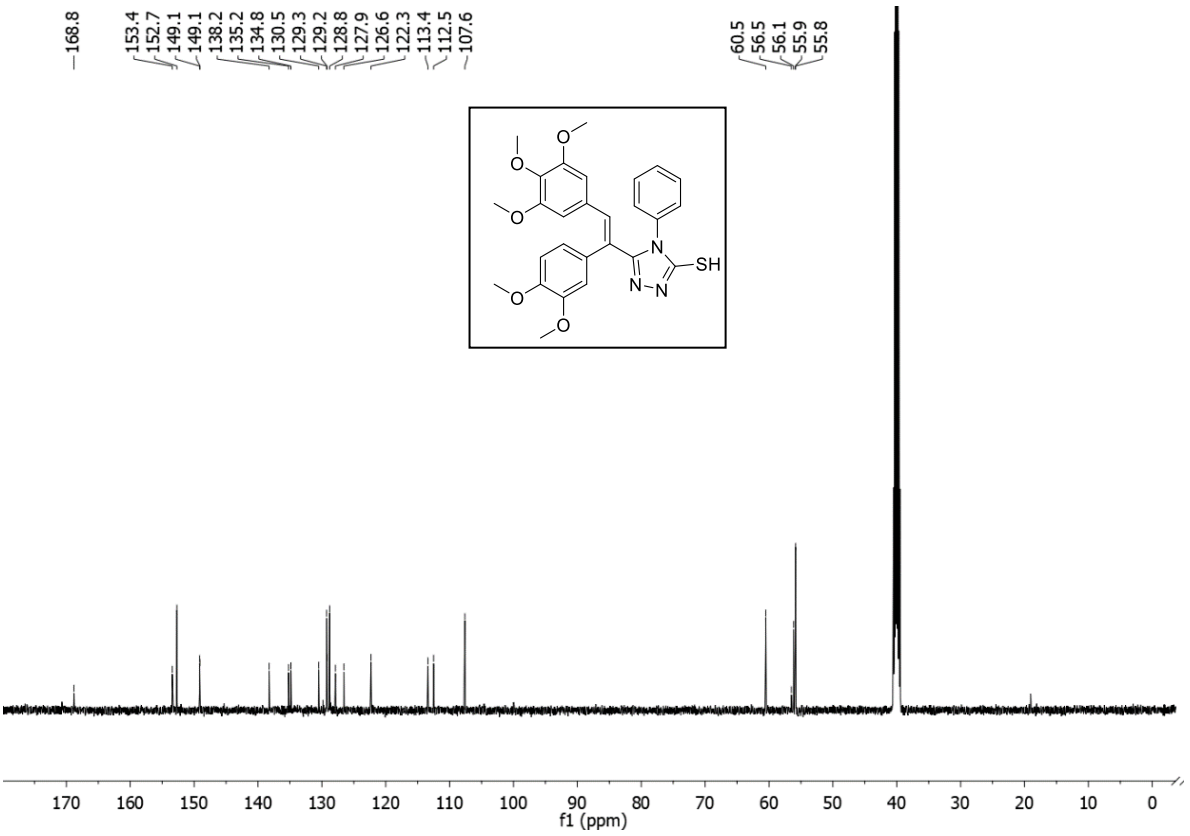

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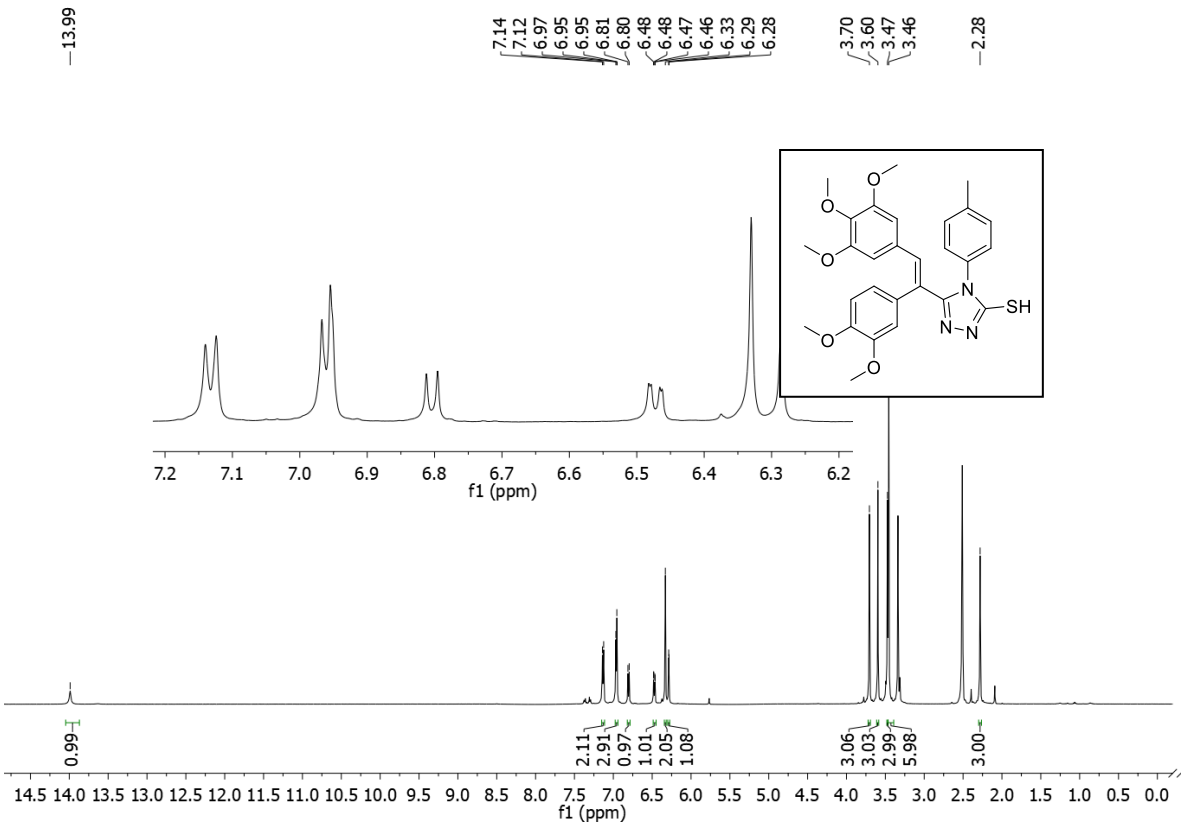
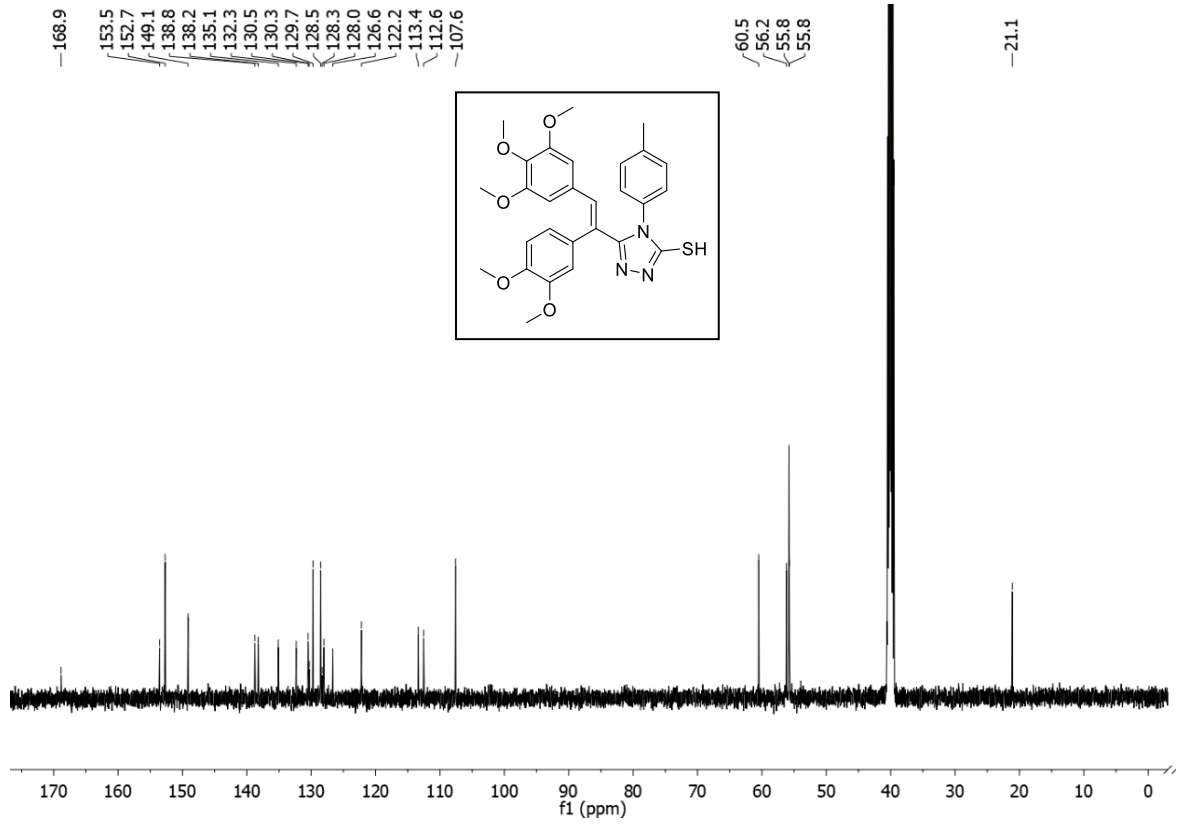


^{1}H NMR (500 MHz, DMSO-d_6) of compound **5l**



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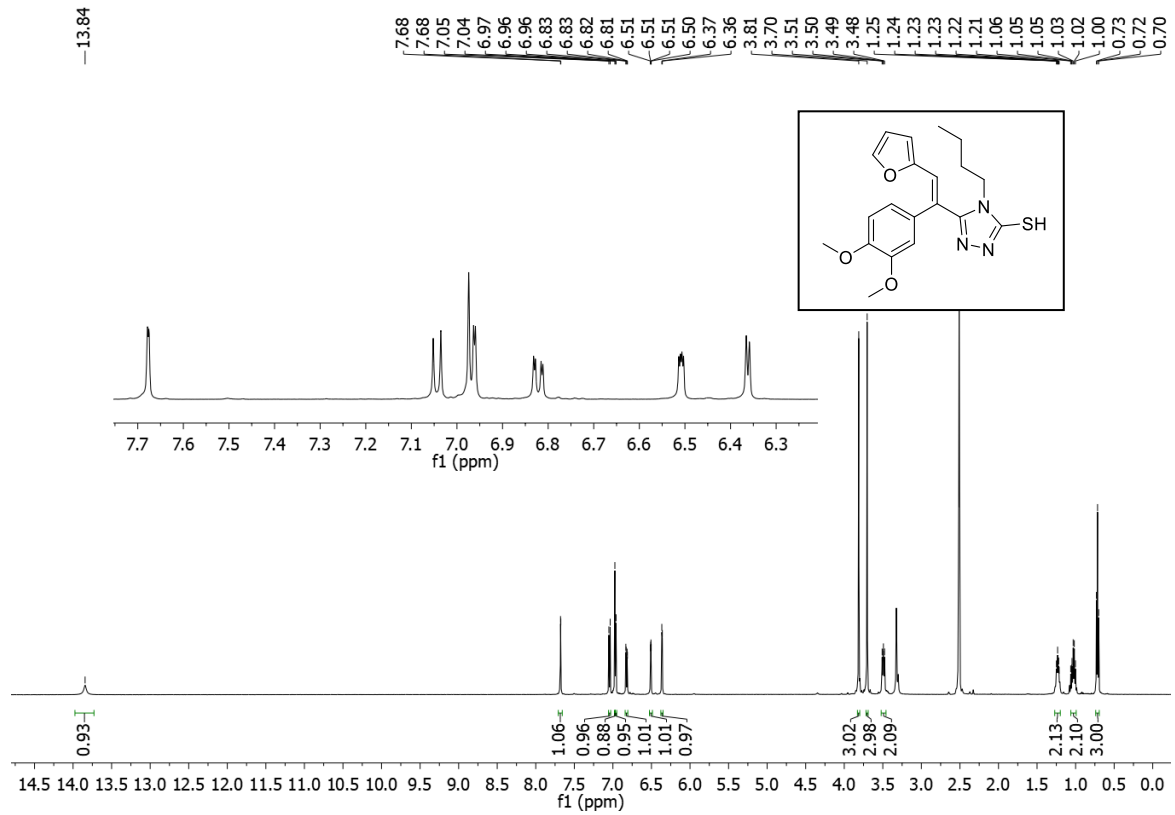
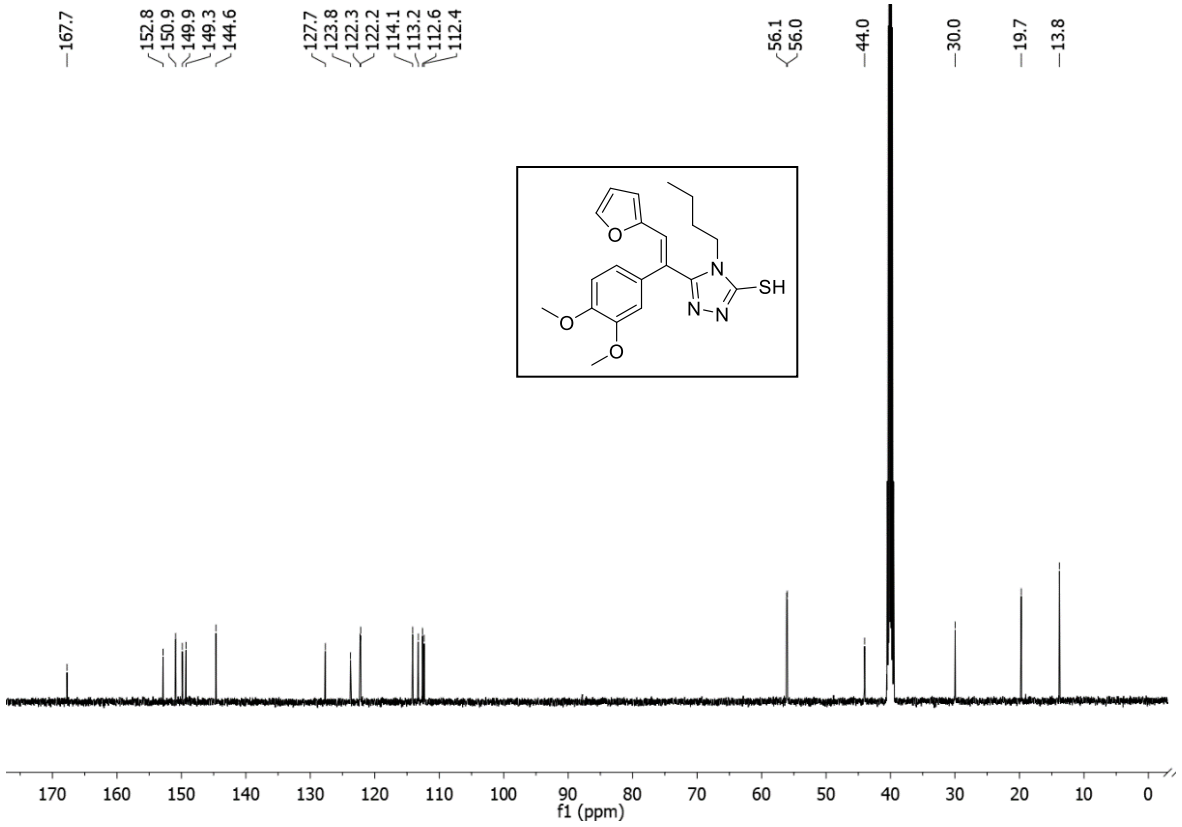


^{1}H NMR (500 MHz, DMSO-d_6) of compound **5m**



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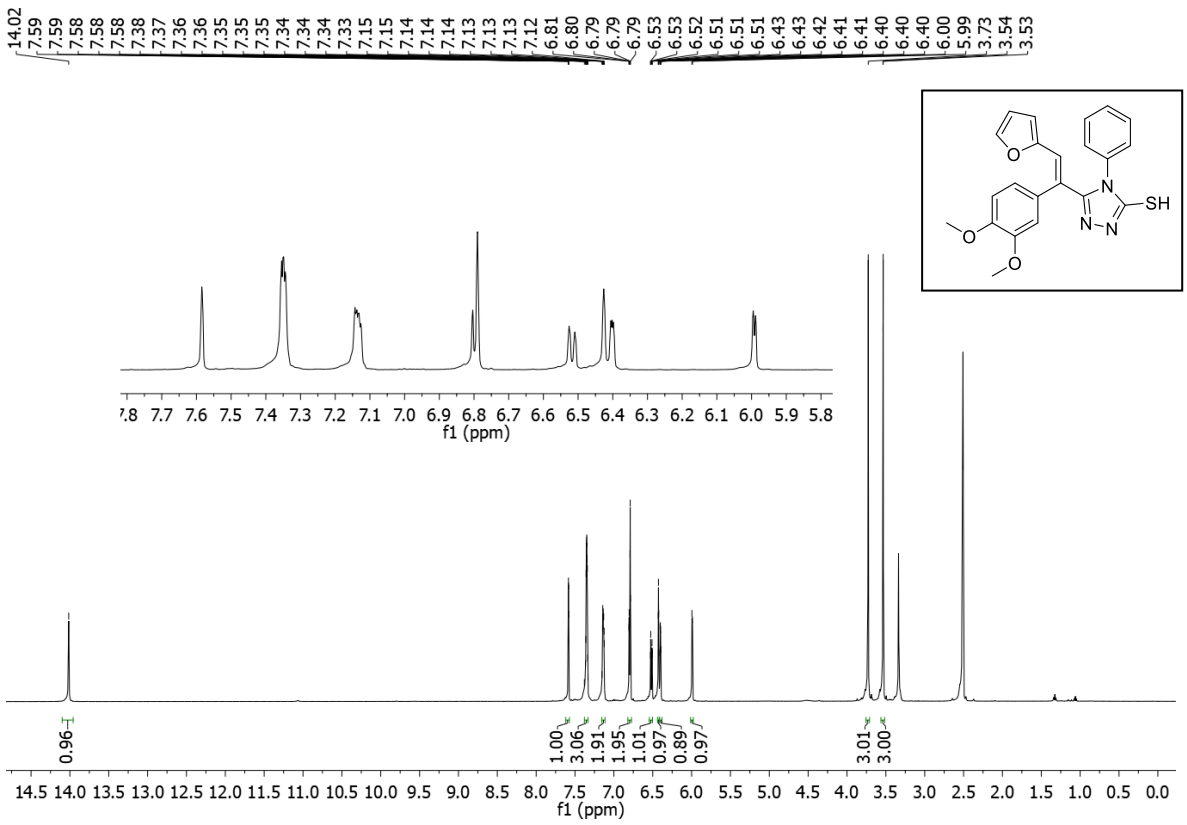

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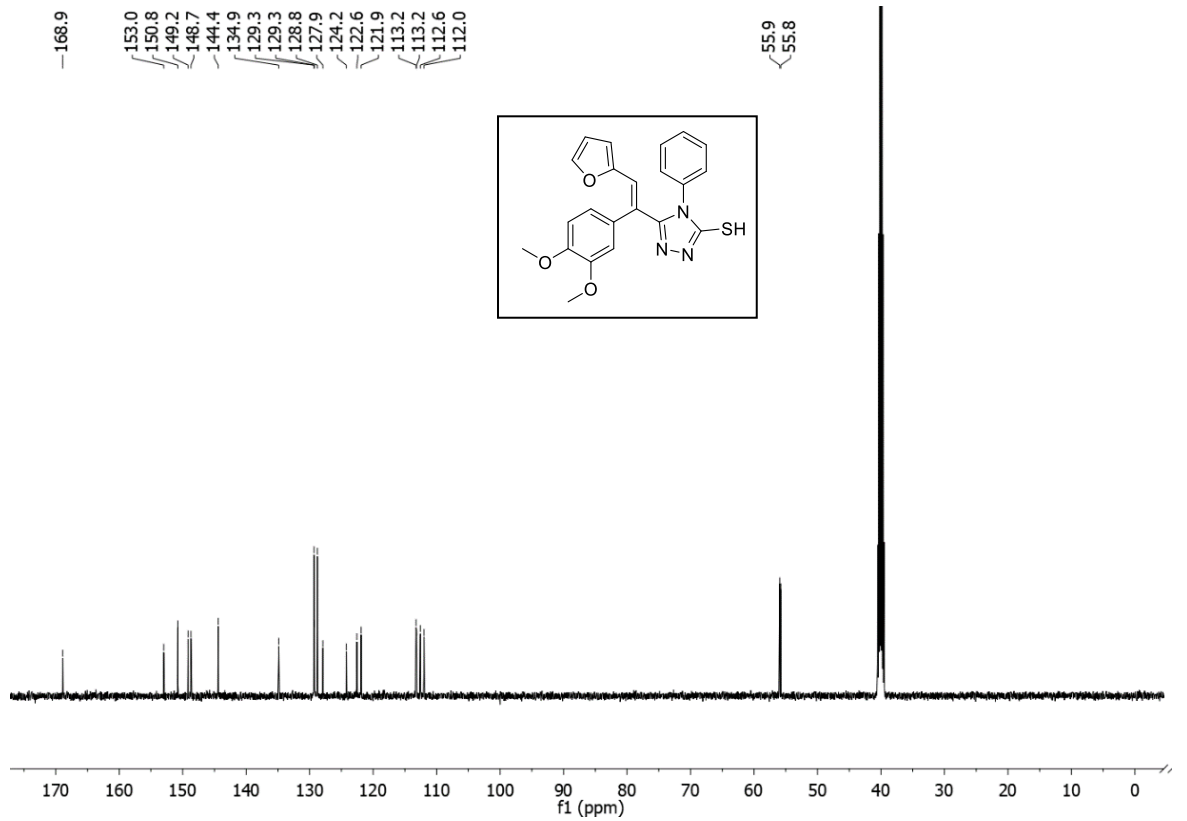
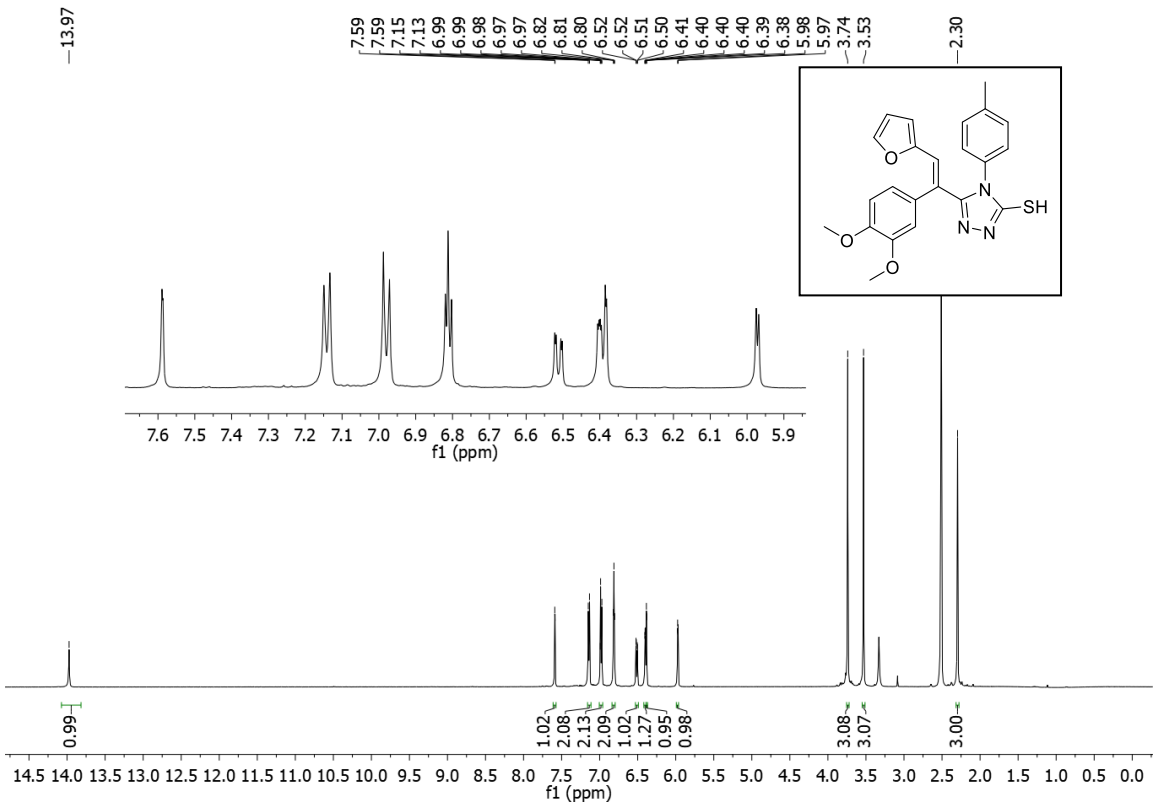
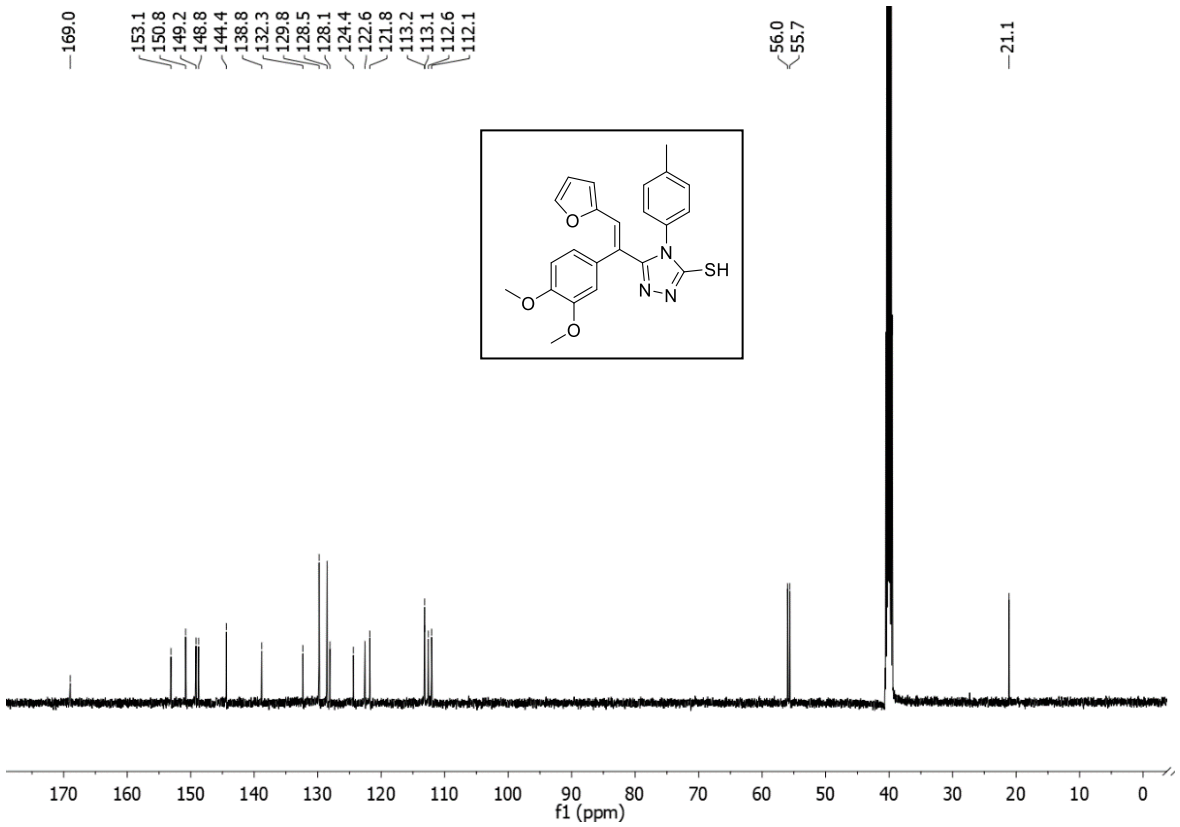


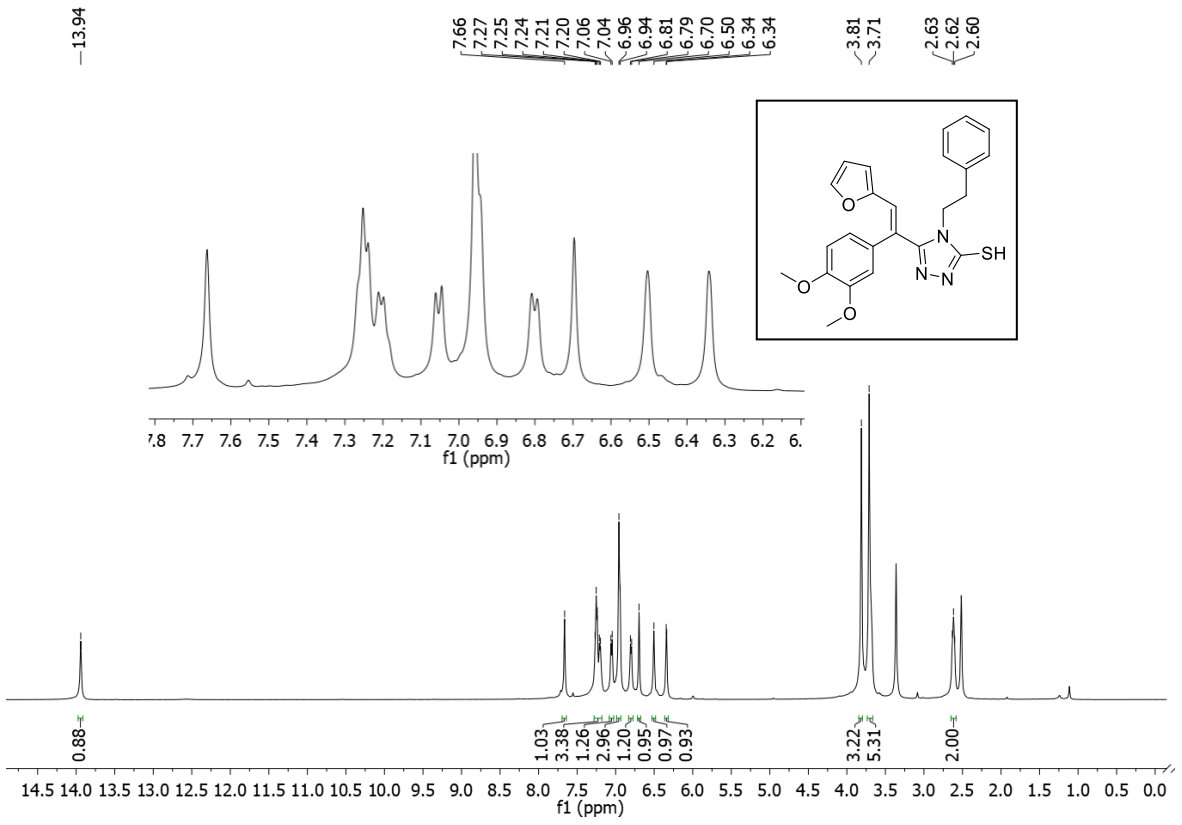
^{13}C NMR (125 MHz, DMSO-d_6) of compound **5n**



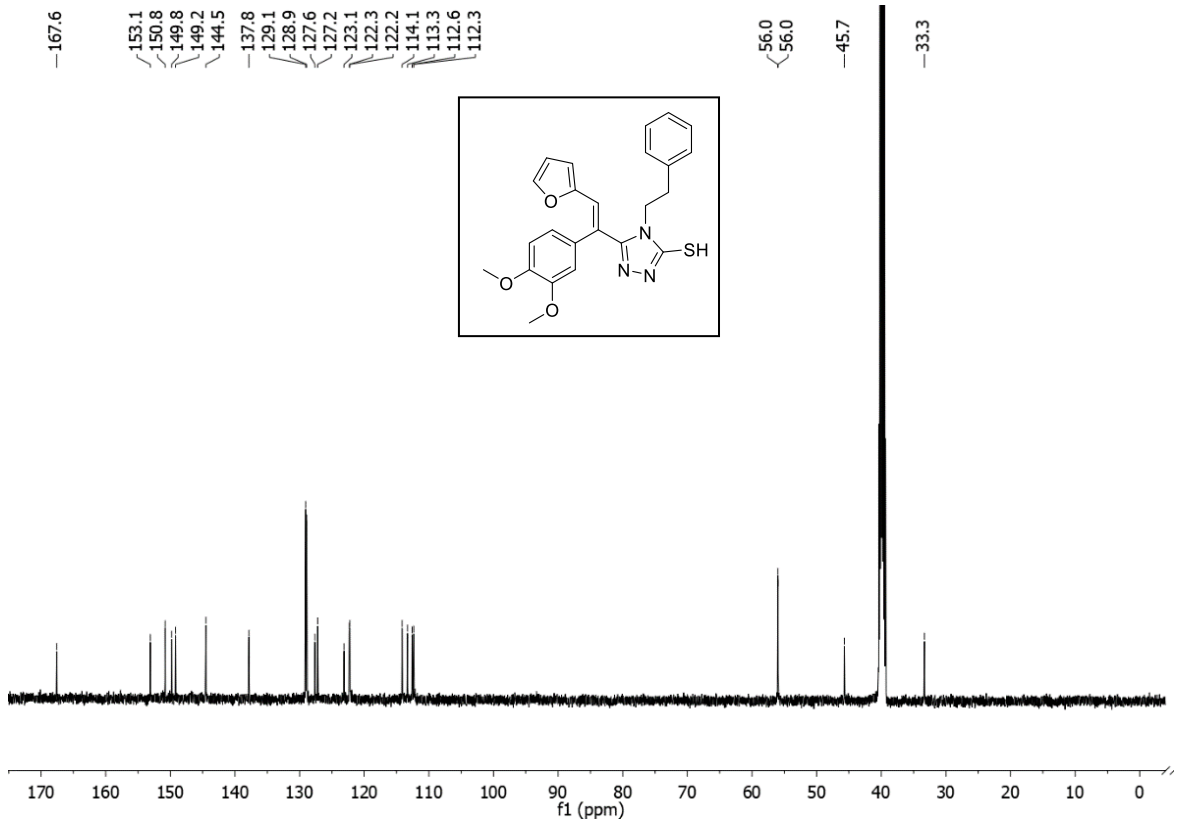
^{1}H NMR (500 MHz, DMSO-d_6) of compound **5o**



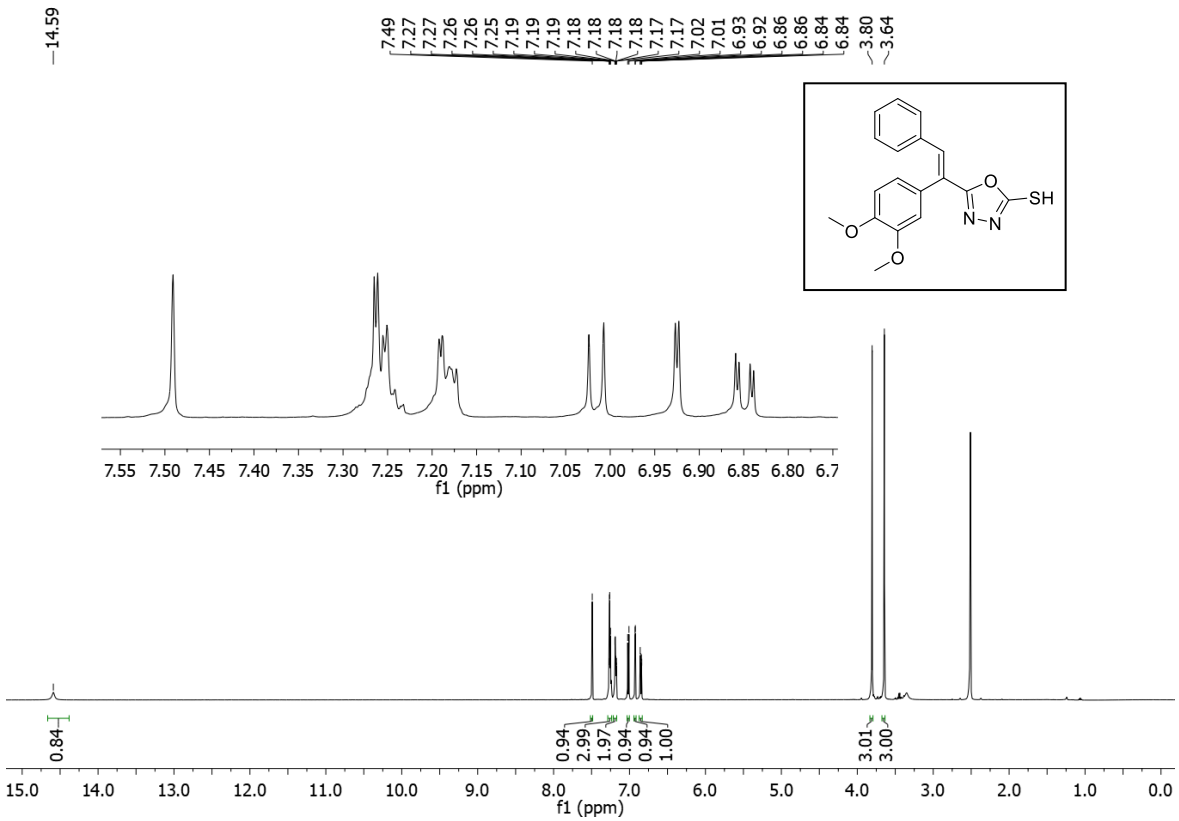
^{13}C NMR (125 MHz, DMSO-d_6) of compound **5o**



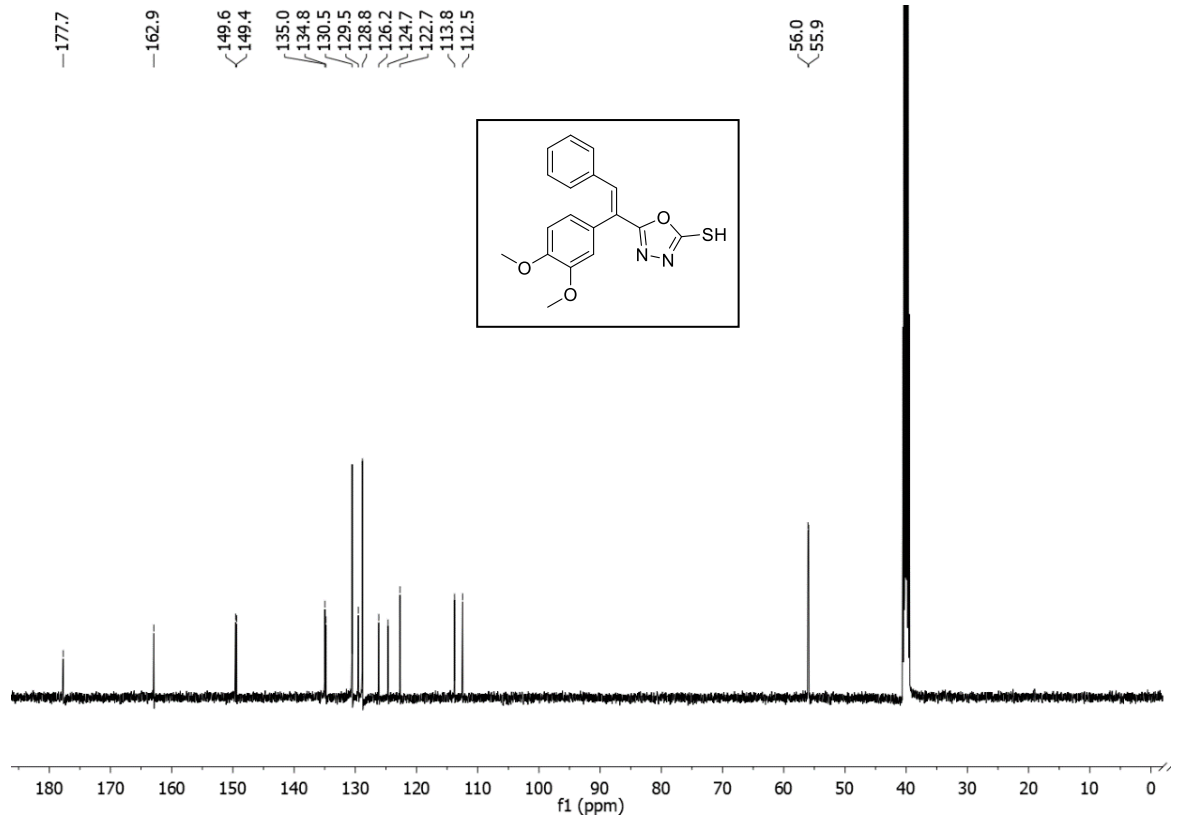
^{1}H NMR (500 MHz, DMSO-d_6) of compound **5p**



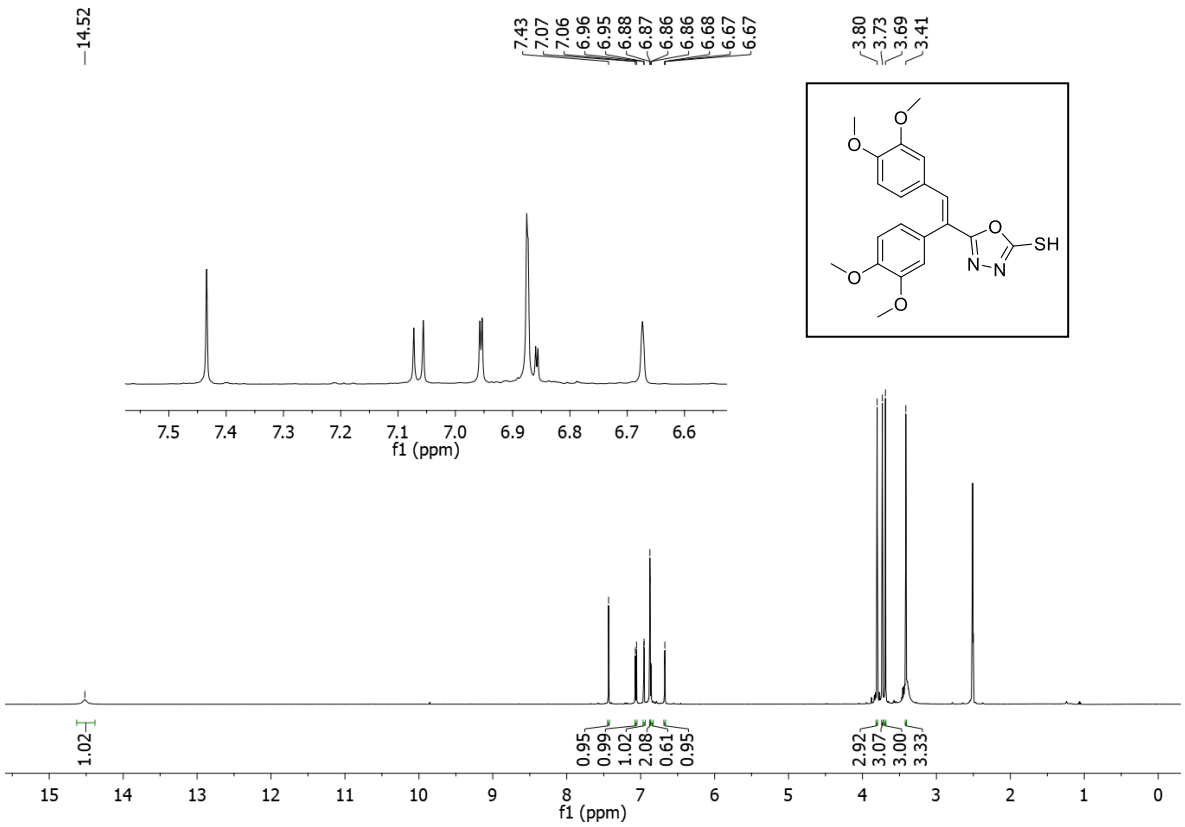
^{13}C NMR (125 MHz, DMSO-d_6) of compound **5p**



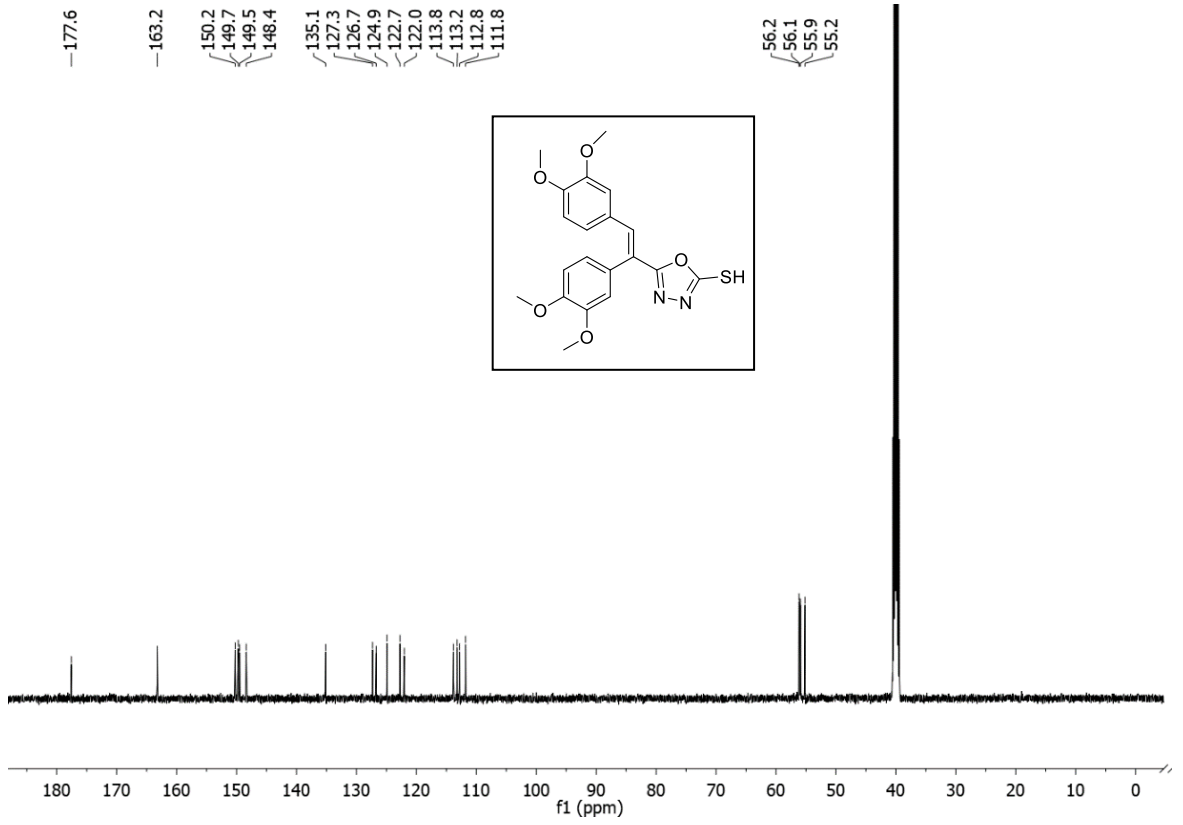
^{1}H NMR (500 MHz, DMSO-d_6) of compound **6a**



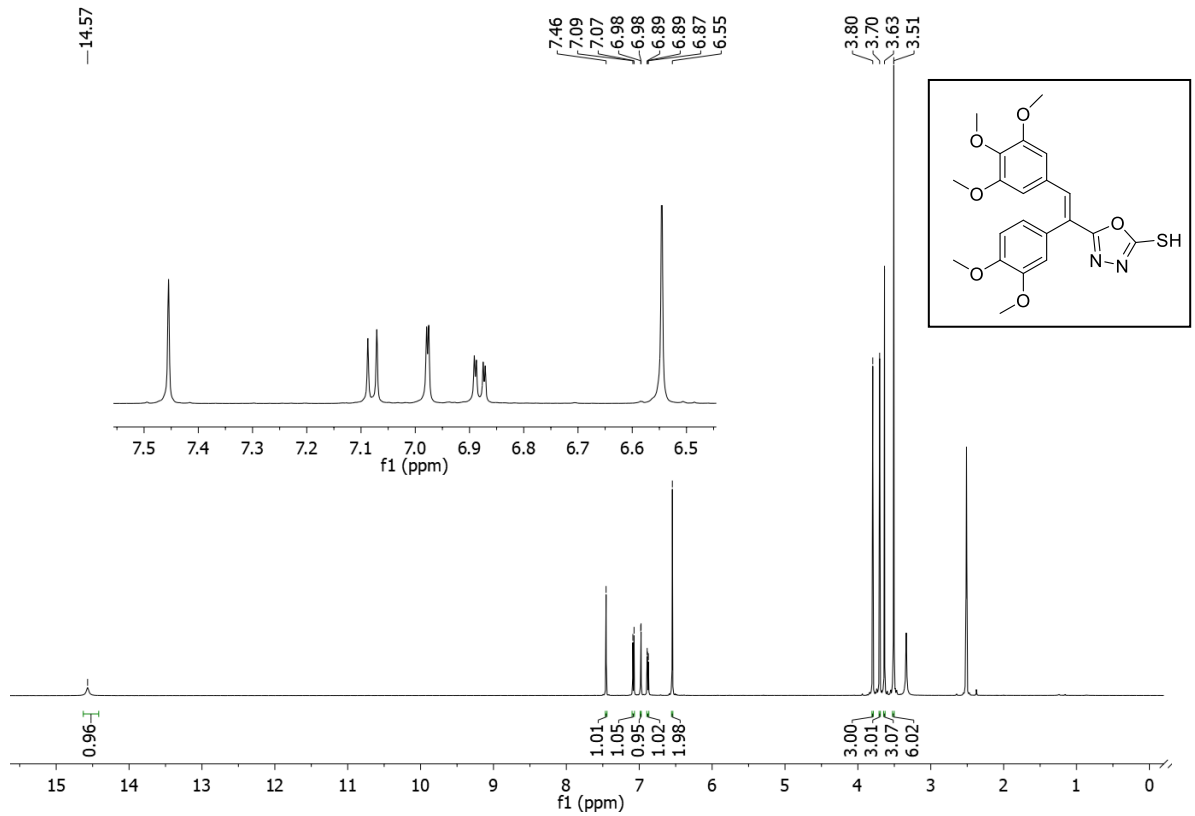
^{13}C NMR (125 MHz, DMSO-d_6) of compound **6a**



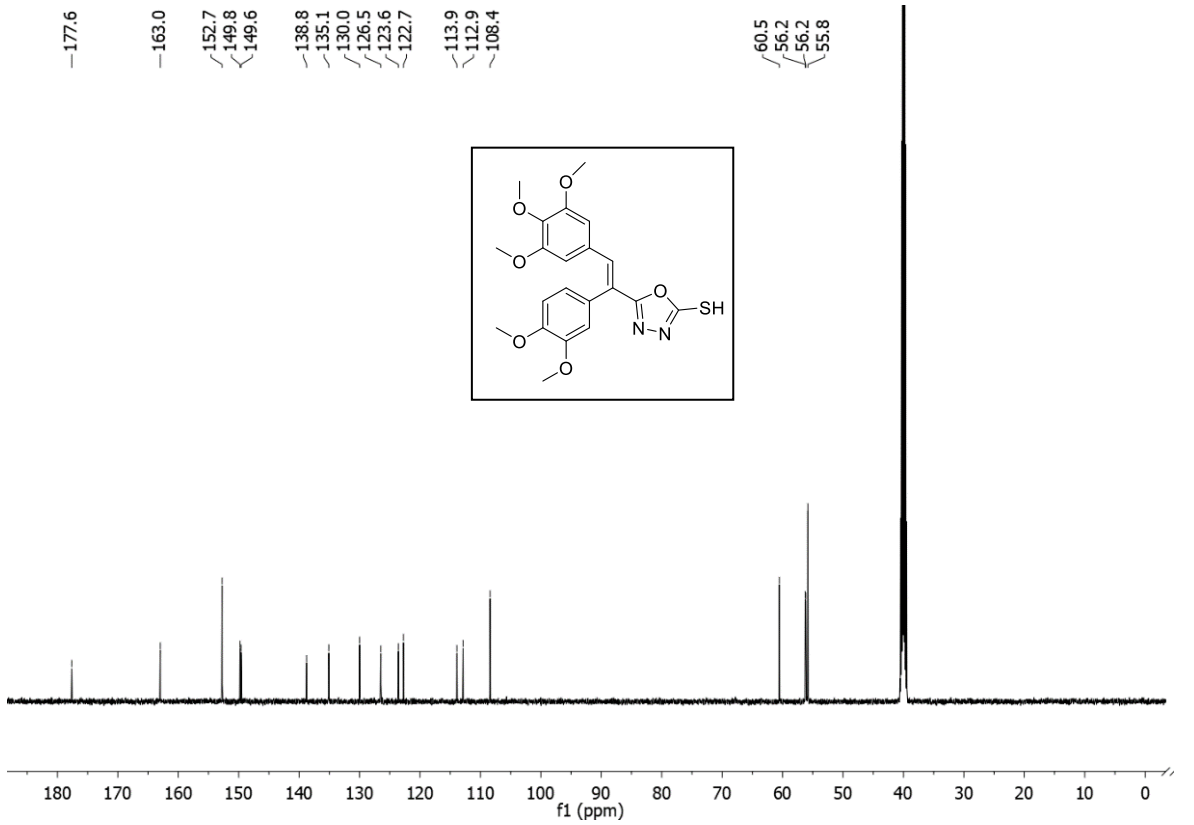
^{1}H NMR (500 MHz, DMSO-d_6) of compound **6b**



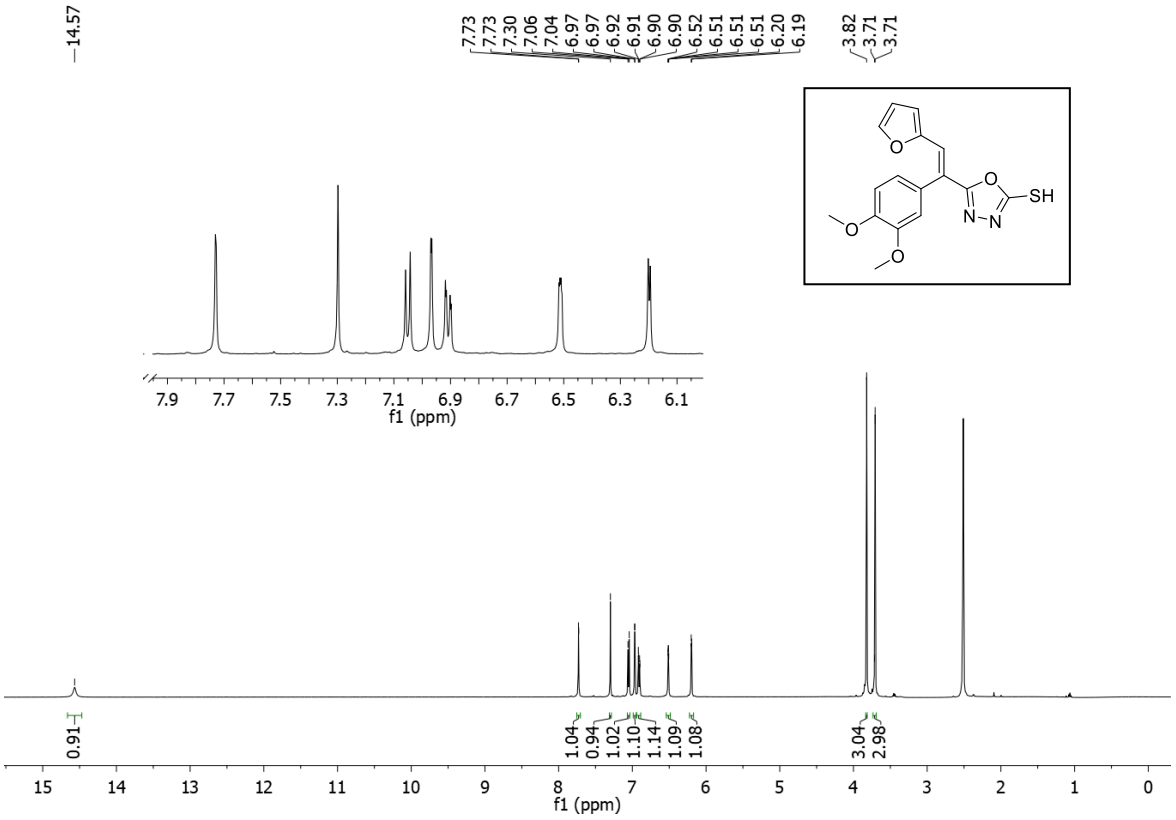
^{13}C NMR (125 MHz, DMSO-d_6) of compound **6b**



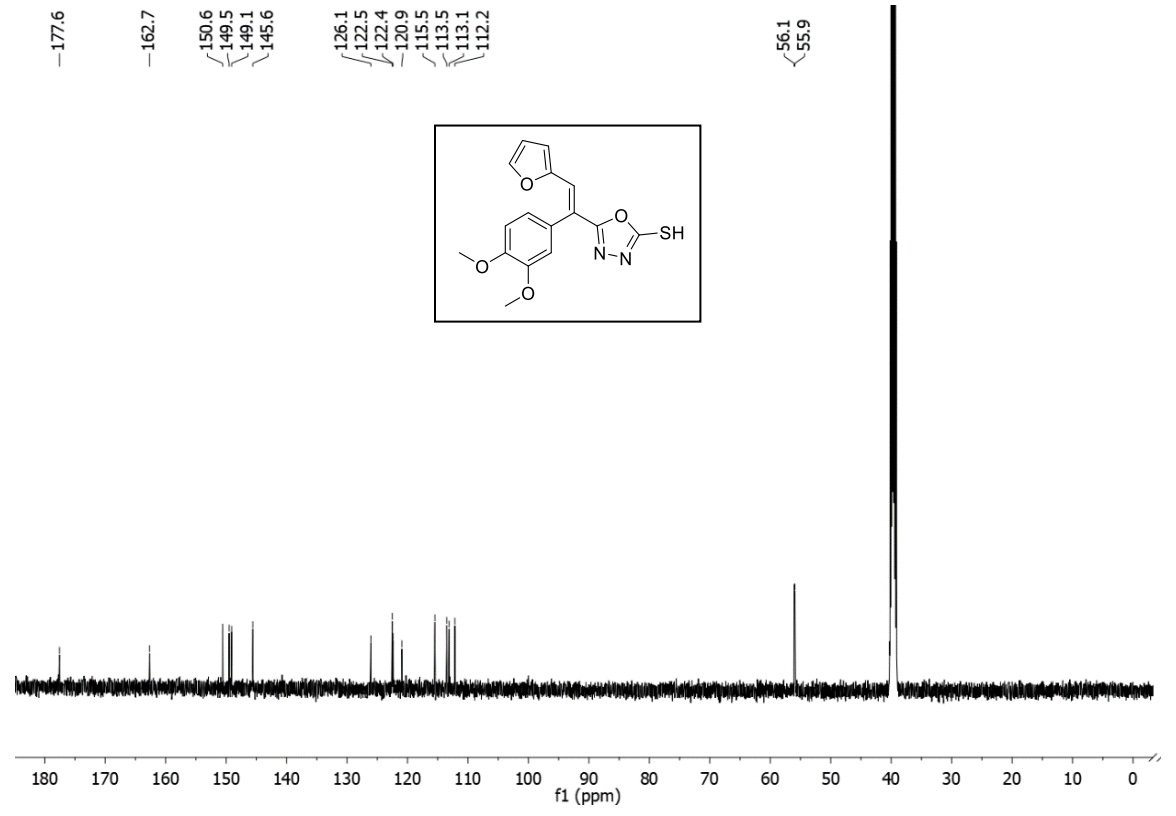
^{1}H NMR (500 MHz, DMSO-d_6) of compound **6c**



^{13}C NMR (125 MHz, DMSO-d_6) of compound **6c**



^{1}H NMR (500 MHz, DMSO-d_6) of compound **6d**



^{13}C NMR (125 MHz, DMSO-d_6) of compound **6d**

6. HPLC Data

HPLC Experimental description

Sample preparation: The compound is dissolved in the mixture of acetonitrile and H_2O (1:1) with the help of sonication.

Mobile Phase

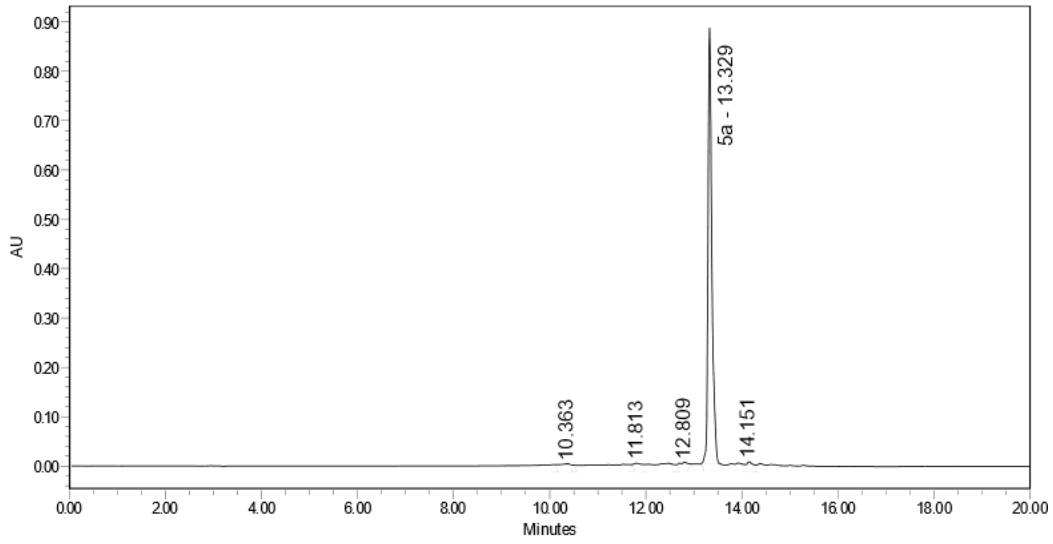
A) 0.1% Formic acid: dissolve 1 mL of formic acid into 1000 mL distilled water.

B) Acetonitrile

Chromatographic condition

1	**Column**	Xbridge C18, 5 µ 4.6 mm x 250 mm	
2	**Column temp**	30 °C	
3	**Sample temp**	15 °C	
4	**Flow rate**	1 ml/min	
5	**Injection volume**	10 µL	
6	**Wavelength**	254 nm	
7	**Gradient Program**	**Time**	**%B**
		0.1	10
		3	10
		8	50
		12	90
		14	90
		16	50
		20	10

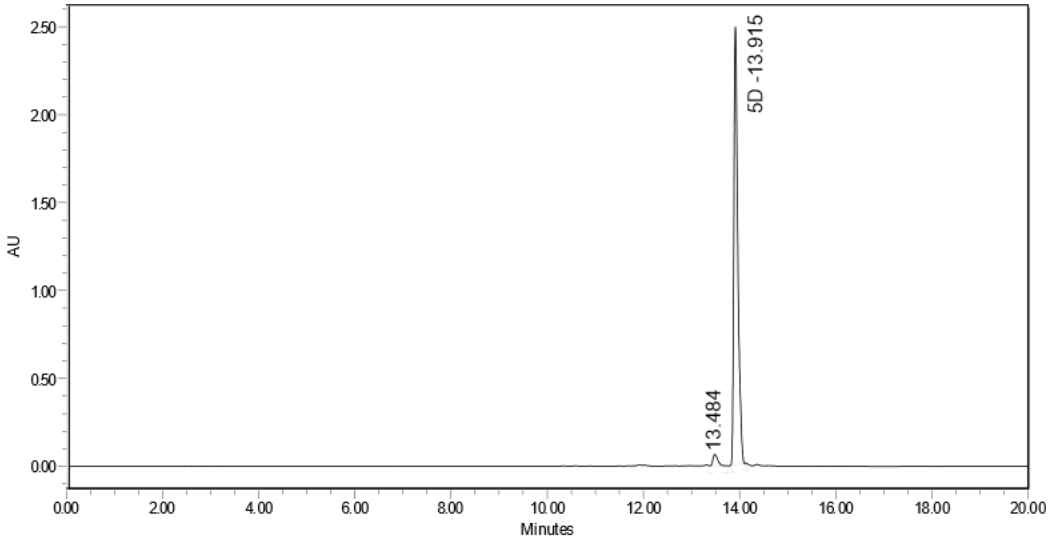
Auto-scaled Chromatogram



	Name	Retention Time	Area	% Area
1		10.363	25319	0.51
2		11.813	17303	0.35
3		12.809	35355	0.71
4	**5a**	13.329	4887210	97.79
5		14.151	32638	0.65

Compound 5a

Auto-scaled Chromatogram



	Name	Retention Time	Area	% Area
1		13.484	433672	2.97
2	**5d**	13.915	14175067	97.03

Compound 5d