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Synthesis and cytotoxic evaluation of novel simplified plinabulin-quinoline derivatives

Pham T. Tham, Pham T. Chinh, Nguyen V. Tuyen, Duong N. Bang, Dinh T. Van, Vu T. Kien, Hoang T. Thanh, Duong H. Quynh, Vu D. Cuong, Nguyen H. Thanh and Alfonso Pérez-Encabo

Contents

Chemistry: All reactions were performed in the appropriate oven-dried glass apparatus and under nitrogen atmosphere. Unless otherwise stated, solvents and chemicals were obtained from commercial sources and used without further purification. Column chromatography was performed using silica gel (60Å, particle size 40-60 μm). NMR spectra were recorded on a Bruker Advance I (500 MHz). Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) in hertz (Hz). High resolution mass spectrometry analysis (HRMS) was recorded on a Q-executive or a Q-TOF$_2$ instrument. IR analysis was recorded on Perkin Elmer Spectrum Two.
Cell culture and cell viability assay: The synthesized compounds were evaluated for their cytotoxicity against four human cancer cell lines, including epidermoid carcinoma cell line (KB), hepatoma carcinoma cell line (HepG$_2$) and breast carcinoma cell line (MCF$_7$) obtained from the American Type Culture Collection (USA) ATCC and used for cytotoxic evaluation. The cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100μg/mL streptomycin at 37°C in a humidified atmosphere (95% air and 5% CO_2). The exponentially growing cells were used throughout the experiments. The inhibitory effects of the compounds on the growth of the human cancer cell lines were determined by measuring the metabolic activity using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-trazolium bromide (MTT) assay. Briefly, human cancer cell lines (1×10^5 cells/mL) were treated for 3 days with a series of concentrations of the compounds (in DMSO): 0.125; 0.5; 2.0; 8.0; 32.0 and 128.0 μg/mL. After incubation, 0.1 mg MTT solution (50μL of a 2 mg/mL solution) was added to each well, and the cells were then incubated at 37°C for 4h. The plates were centrifuged at 1000 rpm for 10 min at room temperature, and the media were then carefully aspirated. Dimethyl sulfoxide (150 μL) was added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (TECAN GENIOUS). All the experiments were performed three times, and the mean absorbance values were calculated. The results are expressed as the percentage of inhibition that produced a reduction in the absorbance by the treatment of the compounds compared to the untreated controls. A dose-response curve was generated, and the inhibitory concentration of 50% (IC_{50}) was determined for each compound as well as each cell line.

General procedure for the synthesis of compounds 2

A solution of 1,4-diacetylpiperazine-2,5-dione **(1)** (1.0 equiv.) and 4-chloro-8-methylquinoline-2-carbaldehyde derivatives (1.5 equiv.) in dry DMF (10 mL) was cooled to 0 °C. A solution of K_2CO_3 (3.0 equiv.) in dry DMF (10 mL) was then added dropwise over 30 minutes. After the addition was complete, the mixture was allowed to reach room temperature and was stirred for 24 h. Water (50 mL) was added, and the organics were extracted with ethyl acetate. The organic phase was washed with water and brine. Drying of the organic phase ($MgSO_4$), filtration of the drying agent, and evaporation of the solvent *in vacuo* afforded crude compound **2**, which was then purified by column chromatography on silica gel (Hexane-EtOAc, 8:2) to obtain pure compound **2**.

(*Z*)-1-Acetyl-3-((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-piperazine-2,5-dione **2a.** 60% yield, a yellow solid. IR (KBr) cm^{-1}: 3321; 3080; 3040; 2924; 2852; 1681; 1629; 1579; 1525; 1429; 1361; 1217; 1188; 835; 746. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 12.49 (1H, s); 8.22 (1H, s); 8.03 (1H, d, *J*=7.5 Hz); 7.87 (1H, d, *J*=7.5 Hz); 6.97 (1H; s); 4.41 (2H; s); 2.72 (3H, s); 2.55 (3H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 172.1; 166.7; 163.0; 158.5; 154.9; 144.3; 140.7; 138.2; 134.5; 130.3; 127.4; 123.5; 114.9; 106.5; 46.9; 27.0; 19.0. HRMS calc. for: $C_{17}H_{14}ClN_4O_5$: 389.0647 $[M+H]^+$; Found: 389.0648.

(*Z*)-1-Acetyl-3-((4-chloro-8-methylquinolin-2-yl)methylidene)piperazine-2,5-dione **2b.** 65% yield, a yellow solid. IR (KBr) cm^{-1}: 3383; 3062; 2983; 2904; 1693; 1683; 1627; 1575; 1546; 1431; 1085; 808; 744. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 12.96 (1H, s); 8.13 (1H, s); 8.07 (1H, d, *J*=7.0 Hz); 7.82 (1H, d, *J*=7.0 Hz); 7.68 (1H, t, *J*=8.0); 7.04 (1H, s); 4.42 (2H; s); 2.77 (3H, s); 2.56 (3H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 171.0; 166.5; 162.7; 158.7; 153.6; 145.7; 142.2; 135.6; 133.2; 131.5; 127.8; 123.9; 121.3; 107.6; 46.2; 26.4; 17.9. HRMS calc. for: $C_{17}H_{15}ClN_3O_3$: 344.0795 $[M+H]^+$; Found: 344.0795.

General procedure for the synthesis of compounds 3

A solution of compound **(2)** (1.0 equiv.) and 4-chloro-8-methylquinoline-2-carbaldehyde derivative and MeI (3.0 equiv.) in dry DMF (5 mL) was cooled to 0 °C. A solution of K_2CO_3 (3.0 equiv.) in dry DMF (5 mL) was then added dropwise over 30 minutes. After the addition was complete, the mixture was heated to 80 °C and stirred for 24 h. Afterward, the reaction mixture was treated with water (50 mL) and extracted with ethyl acetate. The organic phase was washed with water and brine. Drying of the organic phase ($MgSO_4$), filtration of the drying agent, and evaporation of the solvent *in vacuo* afforded crude compound **3**, which was then purified by column chromatography on silica gel (hexane-EtOAc, 8:2) to obtain pure compound **3**.

(3*Z*,6*Z*)-3,6-Bis((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-1,4-dimethylpiperazine-2,5-dione **3a**. 22% yield, a yellow solid. IR (KBr) cm^{-1}: 3035; 2922; 1683; 1618; 1577; 1529; 1431; 1338; 1311; 1157; 831; 761. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 8.28 (2H, s); 8.06 (2H, d, *J*=7.5 Hz); 7.86 (2H, d, *J*=7.5 Hz); 7.35 (2H, s); 3.06 (6H, s); 2.73 (6H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 160.1 (2xC); 158.9 (2xC); 153.2 (2xC); 146.8 (2xC); 142.2 (2xC); 137.2 (2xC); 135.5 (2xC); 129.7 (2xC); 127.6 (2xC); 123.4 (2xC); 123.1 (2xC); 119.1 (2xC); 115.3 (2xC); 36.4 (2xC); 18.5; 18.4. HRMS calc. for: $C_{28}H_{21}Cl_2N_6O_6$: 607.0894 $[M+H]^+$; Found: 609.0799; 607.0879.

(3*Z*,6*Z*)-3,6-Bis((4-chloro-8-methylquinolin-2-yl)methylidene)-1,4-dimethylpiperazine-2,5-dione **3b**. 21% yield, a yellow solid. IR (KBr) cm^{-1}: 3034; 2970; 2918; 1705; 1685; 1624; 1602; 1512; 1381; 1363; 1262; 1174; 1016; 792; 842; 763. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 8.05 (2H, d, *J*=7.5 Hz); 7.98 (2H, s); 7.74 (2H, d, *J*=7.5 Hz); 7.64 (2H, t, *J*=8.0 Hz); 7.30 (2H, s); 3.09 (6H, s); 2.71 (6H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 160.1 (2xC); 151.4 (2xC); 146.5 (2xC); 141.0 (2xC); 136.8 (2xC); 134.2 (2xC); 130.8 (2xC); 127.6 (2xC); 123.9 (2xC); 123.1 (2xC); 121.0 (2xC); 117.6 (2xC); 35.4 (2xC); 17.1 (2xC). HRMS calc. for: $C_{28}H_{23}Cl_2N_4O_2$: 517.1193$[M+H]^+$; Found: 517.1191

General procedure for the synthesis of compounds 4

A solution of compound **(2)** (1.0 equiv.), benzaldehyde derivative (1.5 equiv.) and MeI (3.0 equiv.) in dry DMF (5 mL) was cooled to 0 °C. A solution of K_2CO_3 (3.0 equiv.) in dry DMF (5 mL) was then added dropwise over 30 minutes. After the addition was complete, the mixture was heated to 80 °C and stirred for 24 h. Afterward, the reaction mixture was treated water (50 mL) and extracted with ethyl acetate. The organic phase was washed with water and brine. Drying of the organic phase ($MgSO_4$), filtration of the drying agent, and evaporation of the solvent *in vacuo* afforded crude compound **4**, which was then purified by column chromatography on silica gel (hexane-EtOAc, 8:2) to obtain pure compound **4**.

(*Z*)-3-((4-Chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-6-((*Z*)-4-methoxybenzylidene)-1,4-dimethylpiperazine-2,5-dione **4a**. 21% yield, a yellow solid. IR (KBr) cm^{-1}: 3074; 2922; 2848; 1716; 1688; 1583; 1527; 1508; 1300; 1249; 1097; 1029; 825; 729. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 8.14 (2H, d, *J*=8.5 Hz); 8.06 (1H, s); 8.03 (1H, d, *J*=7.5 Hz); 7.82 (1H, d, *J*=7.5 Hz); 7.25 (1H, s); 7.0 (2H, d, *J*=8.5 Hz); 6.59 (1H, s); 3.80 (3H, s); 3.65 (3H, s); 3.39 (1H, s); 2.76 (3H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 160.0; 159.3; 156.4; 153.0; 146.5; 144.4; 142.0; 135.4; 133.3 (2xC); 131.2; 129.1; 128.4; 127.4; 123.1; 115.0; 114.0 (2xC); 112.6; 109.8; 104.6. 55.2; 36.9; 29.8; 18.5. HRMS calc. for: $C_{25}H_{22}ClN_4O_5$: 493.1273 $[M+H]^+$; Found: 493.1267.

(*Z*)-3-((4-Chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-6-((*Z*)-4-chlorobenzylidene)-1,4-dimethylpiperazine-2,5-dione **4b**. 22% yield, a yellow solid. IR (KBr) cm^{-1}: 3065; 2932; 2853; 1712; 1679; 1580; 1520; 1307; 1240; 1086; 825; 723. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 8.15 (2H, d, *J*=8.0 Hz); 8.08 (1H, s); 8.04 (1H, d, *J*=7.5 Hz); 7.83 (1H, d, *J*=7.5 Hz); 7.49 (2H, d, *J*=8.0 Hz); 7.25 (1H, s); 6.73 (1H, s); 3.65 (3H, s); 3.40 (3H, s); 2.76 (3H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 160.1; 155.2; 153.1; 145.6; 144.2; 142.2; 135.6 (2xC); 135.4; 132.5 (2xC); 130.3; 129.2; 128.6; 127.6; 123.5; 117.2; 114.3; 112.7; 107.8; 104.5; 34.9; 30.8; 18.6. HRMS calc. for: $C_{24}H_{19}Cl_2N_4O_4$: 497.0778 $[M+H]^+$; Found: 497.00781; 499.0751.

(*Z*)-3-((4-Chloro-8-methylquinolin-2-yl)methylidene)-6-((*Z*)-4-methoxy-benzylidene)-1,4-dimethylpiperazine-2,5-dione **4c**. 23% yield, a yellow solid. IR (KBr) cm^{-1}: 3067; 2922; 2851; 1718; 1686; 1610; 1589; 1330; 1105; 1089; 818; 726. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 8.08 (2H, d, *J*=8.5 Hz); 7.23 (1H, s); 7.17 (2H, d, *J*=8.5 Hz); 6.93 (1H, s); 6.91 (1H, d, *J*=8.0 Hz); 6.89 (1H, d, *J*=8.5 Hz); 6.86 (1H, d, *J*=8.0 Hz); 6.78 (1H, s); 4.04 (3H, s); 3.84 (3H, s); 3.82 (3H, s); 2.99 (3H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 162.1; 159.3; 156.6; 144.2; 142.3; 133.2; 133.1 (2xC); 130.8 (2xC); 130.5; 128.3; 127.3;

127.2; 126.9; 114.7; 113.8 (2xC); 113.7 (2xC); 112.3; 55.3; 36.1; 35.3; 17.8. HRMS calc. for: $C_{25}H_{23}ClN_3O_3$: 448.1423 $[M+H]^+$; Found: 448.1431.

(*Z*)-3-((4-Chloro-8-methylquinolin-2-yl)methylidene)-6-((Z)-4-chloro-benzylidene)-1,4-dimethylpiperazine-2,5-dione **4d**. 22% yield, a yellow solid. IR (KBr) cm^{-1}: 3047; 2924; 2855; 1735; 1680; 1616; 1583; 1487; 1334; 1165; 1089; 819; 756. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 8.02 (1H, s); 7.45 (1H, d, *J*=8.0 Hz); 7.63 (1H, dd, *J*=1.0; 8.0 Hz); 7.38-7.50 (5H, m, overlap); 7.21 (1H, s); 7.11 (1H, s); 3.02 (3H, s); 2.88 (3H, s); 2.67 (3H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 160.8; 160.6; 151.8; 146.7; 141.3; 137.1; 134.7; 132.2; 132.2; 131.6 (2xC); 131.2; 128.3 (2xC); 128.0; 124.4; 123.7; 121.4; 119.1; 118.6; 117.2; 36.0; 35.3; 17.7. HRMS calc. for: $C_{24}H_{20}Cl_2N_3O_2$: 452.0927 $[M+H]^+$; Found: 452.0929; 454.0935.

General procedure for the synthesis of compounds 5

A solution of compound **(2)** (1.0 equiv.), benzaldehyde derivative (1.5 equiv.) and propargyl bromide (1.5 equiv.) in dry DMF (5 mL) was cooled to 0 °C. A solution of K_2CO_3 (3.0 equiv.) in dry DMF (5 mL) was then added dropwise over 30 minutes. After the addition was complete, the mixture was heated to 80 °C and stirred for 24 h. Afterwards, the reaction mixture was added water (50 mL) and extracted with ethyl acetate. The organic phase was washed with water and brine. Drying of the organic phase ($MgSO_4$), filtration of the drying agent, and evaporation of the solvent *in vacuo* afforded crude compound **5**, which was then purified by column chromatography on silica gel (hexane-EtOAc, 8:2) to obtain pure compound **5**.

(*Z*)-6-((4-Chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-3-((*Z*)-4-methoxybenzylidene)-1-(prop-2-yn-1-yl)piperazine-2,5-dione **5a**. 23% yield, a yellow solid. IR (KBr) cm^{-1}: 3066; 2926; 2850; 1734; 1681; 1668; 1620; 1585; 1558; 1336; 1163; 1087; 821; 758. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 12.82 (1H, s, NH); 8.11 (1H, s); 8.02 (1H, d, *J*=8.0 Hz); 7.72 (1H, dd, *J*=1.0; 8.0 Hz); 7.42 (2H, d, *J*=8.5 Hz); 7.36 (1H, s); 7.27 (1H, s); 7.00 (2H, d, *J*=8.5Hz); 4.87 (2H, d, *J*=2.5 Hz); 3.79 (3H, s); 3.09 (1H, t, *J*=2.5 Hz); 2.64 (3H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 161.2; 161.0; 159.9; 151.1; 146.6; 141.5; 137.2; 131.4 (2xC); 131.3; 131.2; 128.3; 125.9; 124.5; 124.2; 124.1; 121.1; 119.8; 114.2 (2xC); 113.4; 77.7; 75.4; 55.2; 34.4; 17.6. HRMS calc. for: $C_{26}H_{20}ClN_4O_5$: 503.1117 $[M+H]^+$; Found: 503.1123.

(*Z*)-6-((4-Chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-3-((*Z*)-4-chlorobenzylidene)-1-(prop-2-yn-1-yl)piperazine-2,5-dione **5b**. 20% yield, a yellow solid. IR (KBr) cm^{-1}: 3086; 2921; 2852; 1725; 1683; 1669; 1624; 1585; 1336; 1170; 1011; 827; 756. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 12.88 (1H, s, NH); 7.70 (1H, d, *J*=7.0 Hz); 7.66 (2H, overlap); 7.45 (1H, s); 7.39 (2H, d, *J*=7.5 Hz) 7.31 (2H, d, *J*=7.5 Hz); 6.91 (1H, s); 4.44 (2H, d, *J*=2.5 Hz); 2.94 (3H, s); 2.48 (1H, s). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 158.5; 157.8; 154.8; 147.1; 145.4; 141.6; 140.2; 135.1; 133.6; 132.0; 130.9; 130.5 (2xC); 129.8; 128.9 (2xC); 128.1; 126.6; 123.1; 121.9; 107; 73.5; 73.2; 35.9; 20.0. HRMS calc. for: $C_{25}H_{17}Cl_2N_4O_4$: 507.0622 $[M+H]^+$; Found: 507.0631; 509.0592.

General procedure for the synthesis of compounds 6

A solution of 1,4-diacetylpiperazine-2,5-dione **(1)** (1.0 equiv.), 4-(prop-2-yn-1-yloxy)benzaldehyde (1.5 equiv.) and MeI (1.5 equiv.) in dry DMF (10 mL) was cooled to 0 °C. A solution of K_2CO_3 (3.0 equiv.) in dry DMF (10 mL) was then added dropwise over 30 minutes. After the addition was complete, the mixture was allowed to reach room temperature and stirred for 24 h. Afterward, the reaction mixture was treated with water (50 mL) and extracted with ethyl acetate. The organic phase was washed with water and brine. Drying of the organic phase ($MgSO_4$), filtration of the drying agent, and evaporation of the solvent *in vacuo* afforded crude compound **6**, which was then purified by column chromatography on silica gel (hexane-EtOAc, 9:1) to obtain pure compound **6** (65%).

(*Z*)-1-Acetyl-4-methyl-3-(4-(prop-2-yn-l-yloxy)benzylidene)piperazine-2,5-dione **6**. 65% yield, a yellow solid. IR (KBr) cm^{-1}: 3331; 3060; 2922; 2853; 2130; 1710; 1680; 1666; 1602; 1580; 1330; 1117; 1011; 826; 758. ^{1}H-NMR ($CDCl_3$-*d1*, 500 MHz): 7.30 (2H, d, *J*=7.5 Hz); 7.27 (1H, s); 7.01 (2H, d, *J*=7.5 Hz); 4.74 (2H, d, *J*=2.5Hz); 4.51 (2H, s); 2.94 (3H, s); 2.62 (3H, s); 2.56 (1H, t, *J*=2.5 Hz). ^{13}C-NMR ($CDCl_3$-*d1*, 125 MHz): 171.4; 165.0; 164.1; 158.4; 131.4 (2xC); 130.3; 125.7; 125.4; 115.0 (2xC); 77.9; 76.1; 55.8; 45.3; 34.3; 26.6. HRMS calc. for: $C_{17}H_{17}N_2O_4$: 313.1183 $[M+H]^+$; Found: 313.1189.

General procedure for the synthesis of compounds 7

A solution of compound **6** (1.0 equiv.) and benzaldehyde derivative (1.5 equiv.) in dry DMF (5 mL) was cooled to 0 °C. A solution of K_2CO_3 (3.0 equiv.) in dry DMF (5 mL) was then added dropwise over 30 minutes. After the addition was complete, the mixture was heated to 80 °C and stirred for 24 h. Afterward, the reaction mixture was treated with water (30 mL) and extracted with ethyl acetate. The organic phase was washed with water and brine. Drying of the organic phase ($MgSO_4$), filtration of the drying agent, and evaporation of the solvent *in vacuo* afforded crude compound **7**, which was then purified by column chromatography on silica gel (hexane-EtOAc, 8:2) to obtain pure compound **7**.

3-((*Z*)-4-Methoxybenzylidene)-1-methyl-6-((*Z*)-4-(prop-2-yn-1-yloxy)-benzylidene)piperazine-2,5-dione **7a**. 23% yield, a yellow solid. IR (KBr) cm^{-1}: 3332; 3066; 2926; 2855; 2137; 1718; 1680; 1668; 1600; 1580; 1551; 1333; 1169; 1077; 845. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 7.39 (2H, dd, *J*=2.0; 8.0 Hz); 7.37 (1H, s); 7.23 (2H, d, *J*=8.0 Hz); 7.00 (2H, dd, *J*=2.0; 8.0 Hz); 6.97 (2H, d, *J*=8.0Hz); 6.96 (1H, s); 4.75 (2H, d, *J*=2.5Hz); 3.84 (3H, s); 3.03 (3H, s); 2.55 (1H, t, *J*=2.5 Hz). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 159.9; 159.7; 157.7; 131.0 (2xC); 130.5; 129.5 (2xC); 127.0; 124.6; 120.6; 119.9; 117.2; 116.0; 114.9 (2xC); 114.7 (2xC); 78.1; 75.9; 55.8; 55.4; 36.6. HRMS calc. for: $C_{23}H_{21}N_2O_4$: 389.4303 $[M+H]^+$; Found: 389.4310.

3-((*Z*)-4-Chlorobenzylidene)-1-methyl-6-((*Z*)-4-(prop-2-yn-1-yloxy)benzylidene)piperazine-2,5-dione **7b.** 26% yield, a yellow solid. IR (KBr) cm^{-1}: 3336; 3076; 2924; 2851; 2133; 1734; 1683; 1700; 1610; 1583; 1558; 1336; 1179; 1088; 845. ^{1}H-NMR (DMSO-*d6*, 500 MHz): 7.43 (2H, d, *J*=7.5 Hz); 7.36 (2H, d, *J*=7.5 Hz); 7.27 (1H, s); 7.24 (2H, d, *J*=8.5 Hz); 7.00 (2H, d, *J*=8.5 Hz); 6.98 (1H, s); 4.72 (2H, d, *J*=2.5 Hz); 3.03 (3H, s); 2.53 (1H, t, *J*=2.5 Hz). ^{13}C-NMR (DMSO-*d6*, 125 MHz): 159.6; 159.4; 157.8; 134.7; 131.4; 131.0 (2xC); 129.8 (2xC); 129.7 (2xC); 129.2; 126.8; 126.4; 121.3; 115.5; 114.8 (2xC); 78.1; 75.9; 55.8; 36.7. HRMS calc. for: $C_{22}H_{18}ClN_2O_3$: 393.1001 $[M+H]^+$; Found: 393.10012.

^{1}H NMR and ^{13}C NMR Spectra

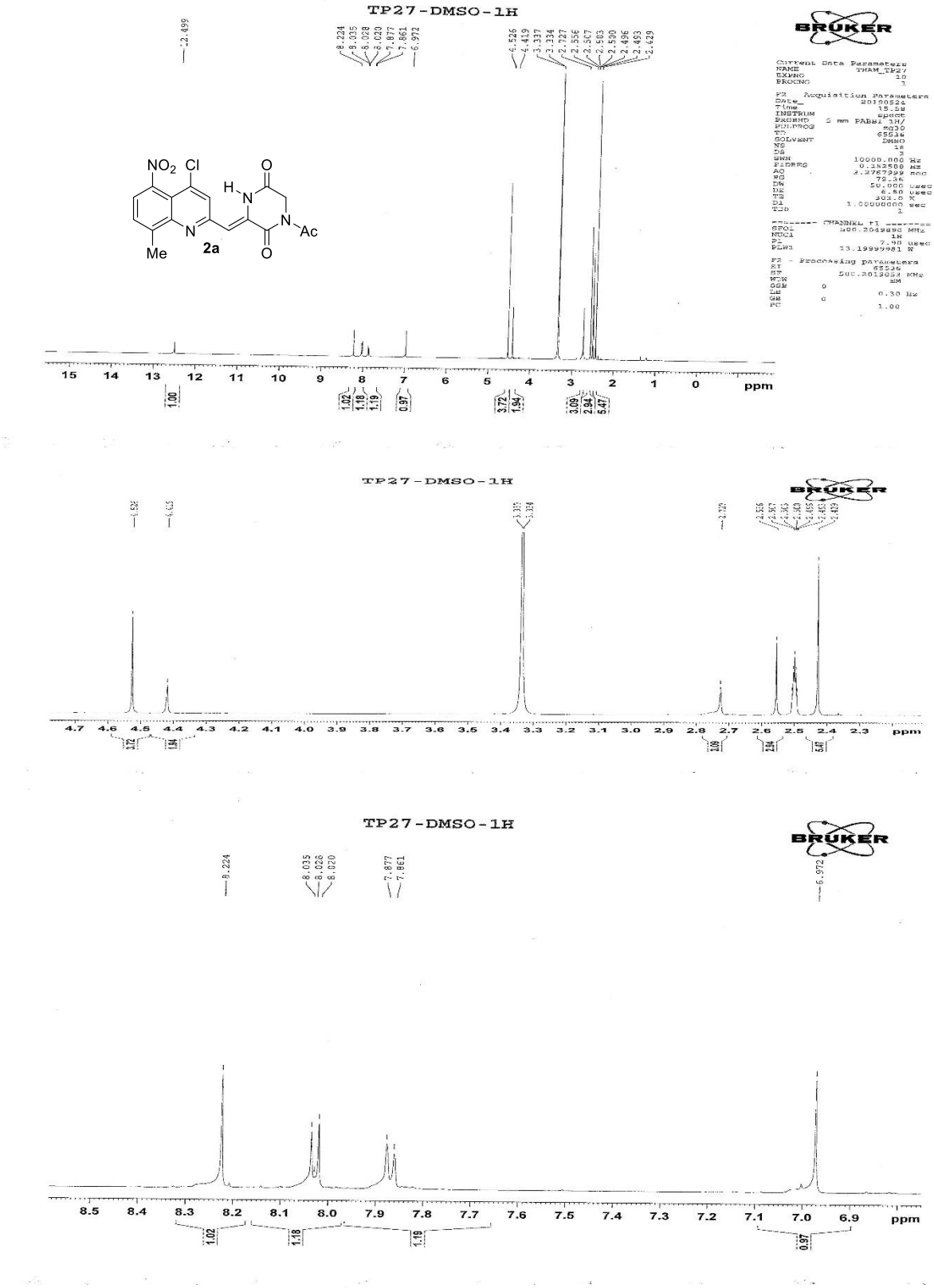

Figure S1. ^{1}H-NMR spectrum of compound **2a**
((Z)-1-acetyl-3-((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)piperazine-2,5-dione)

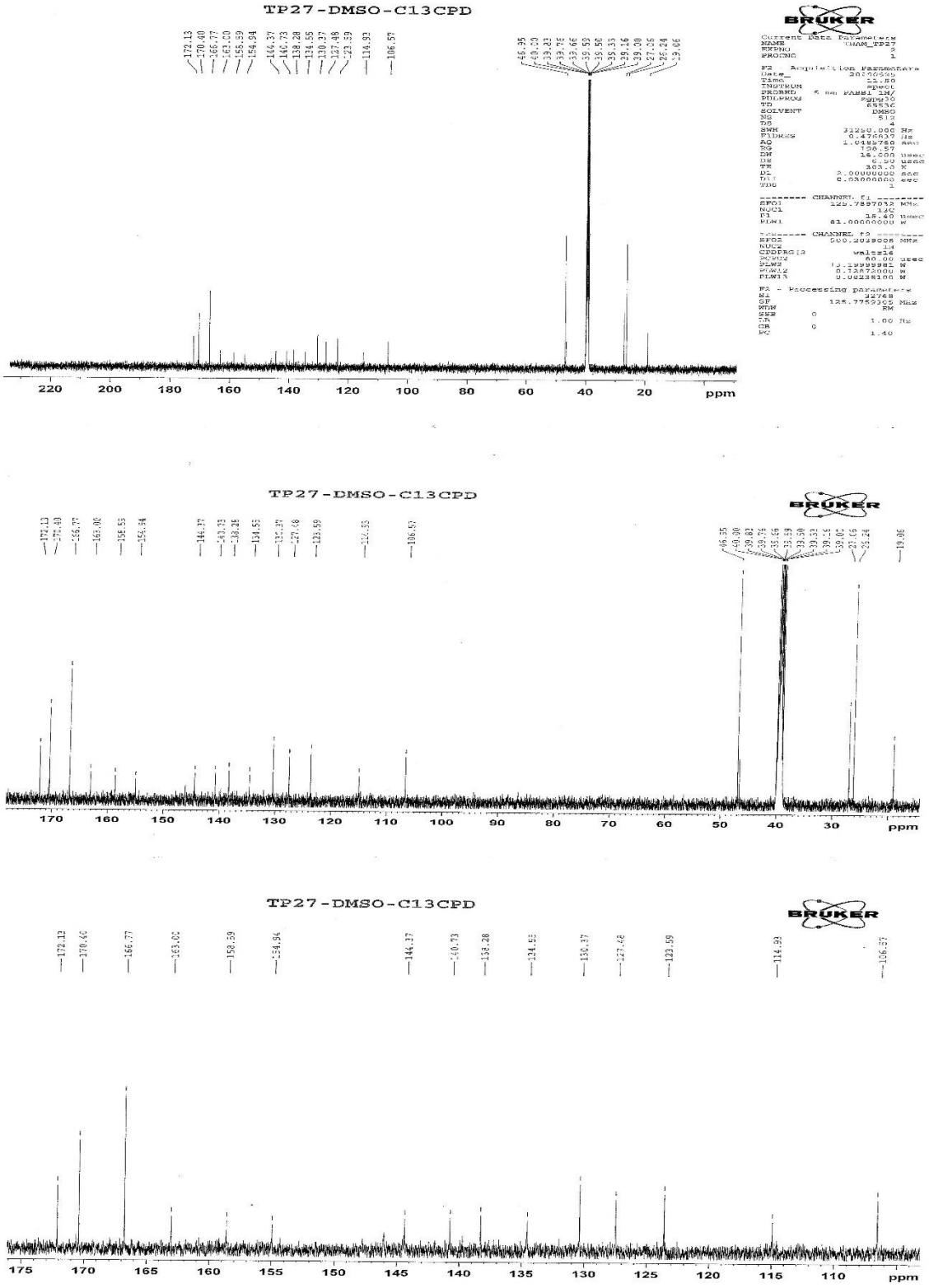

Figure S2. ^{13}C-NMR spectrum of compound **2a**

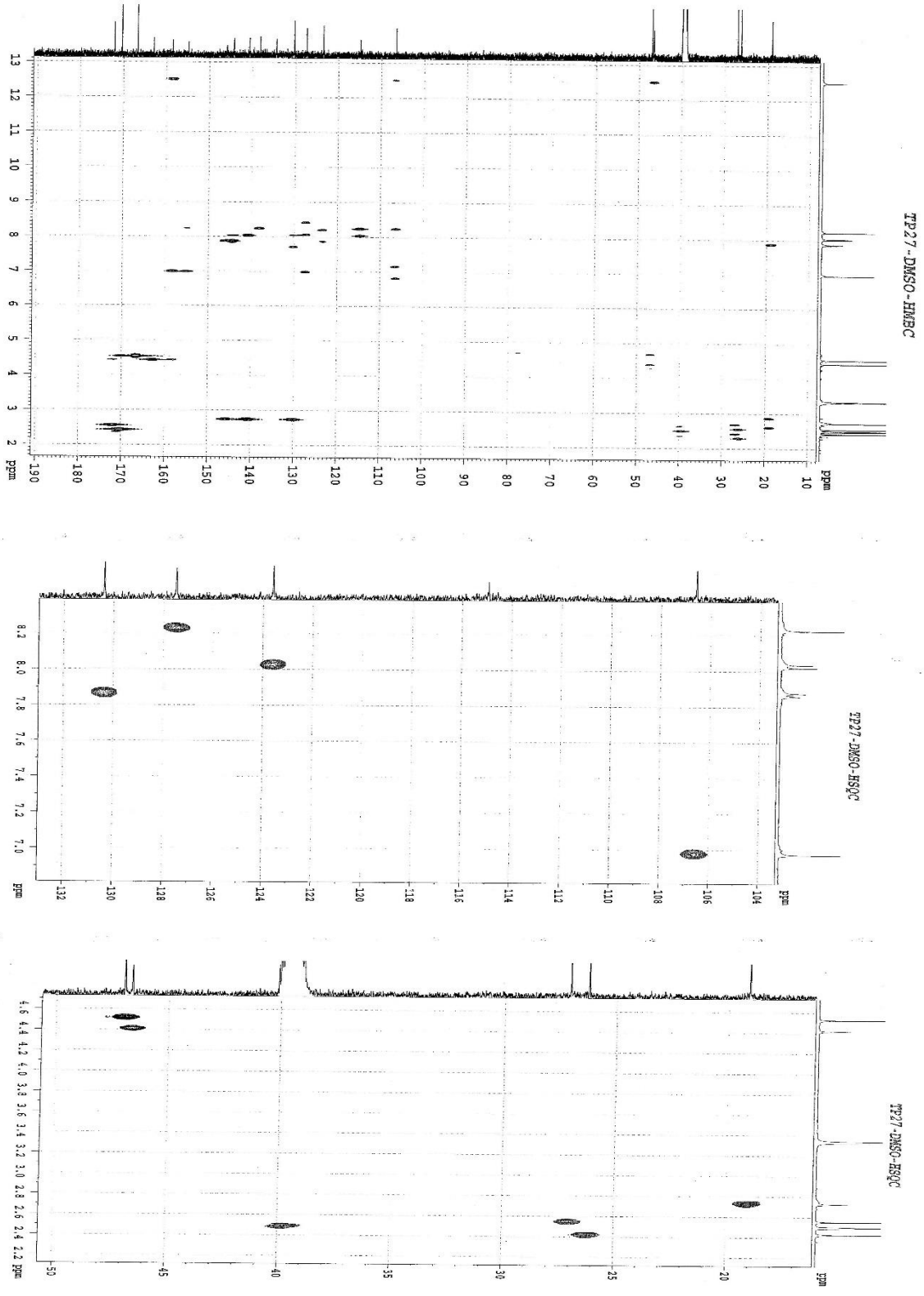

Figure S3. HSQC spectrum of compound **2a**

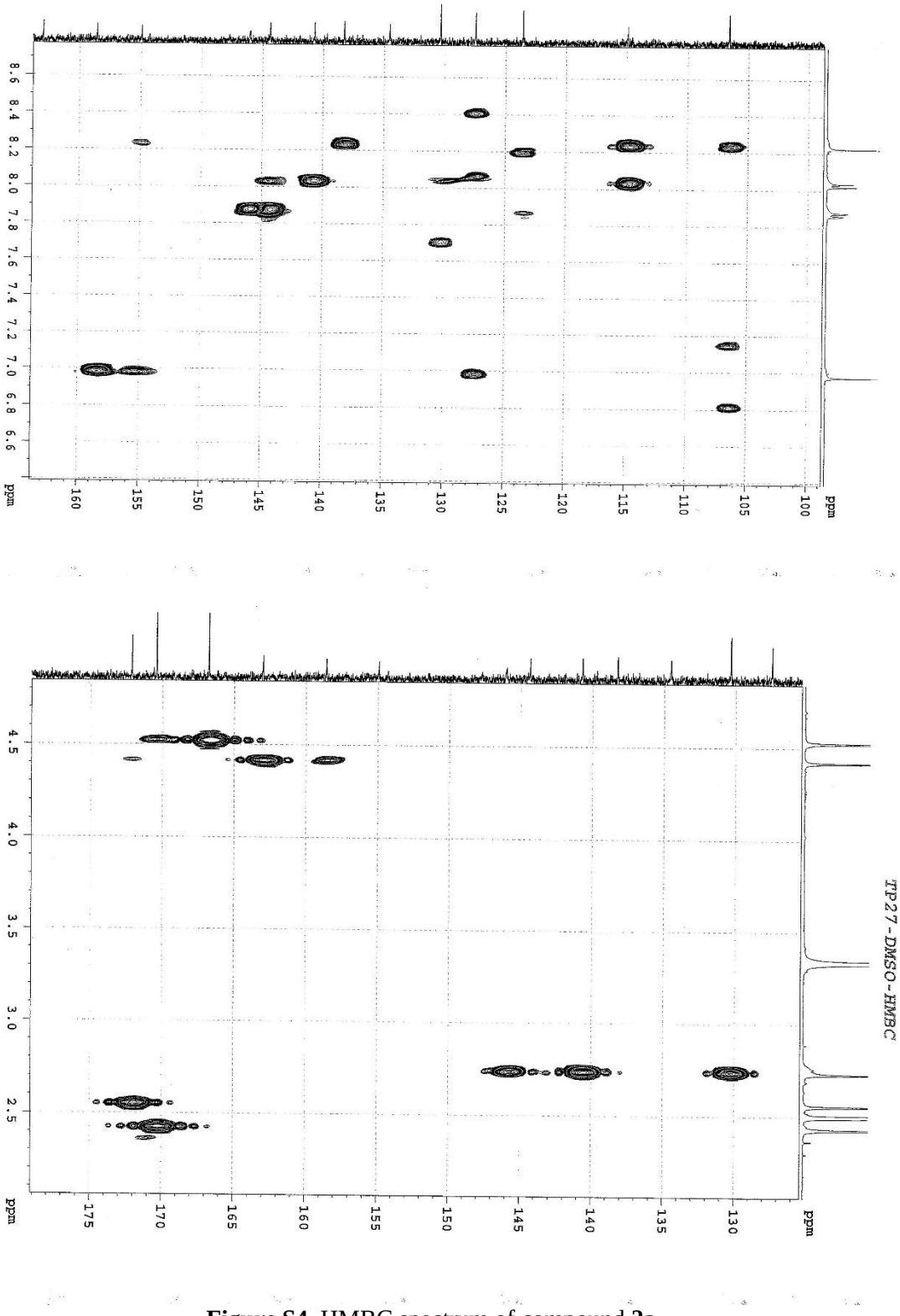

Figure S4. HMBC spectrum of compound **2a**

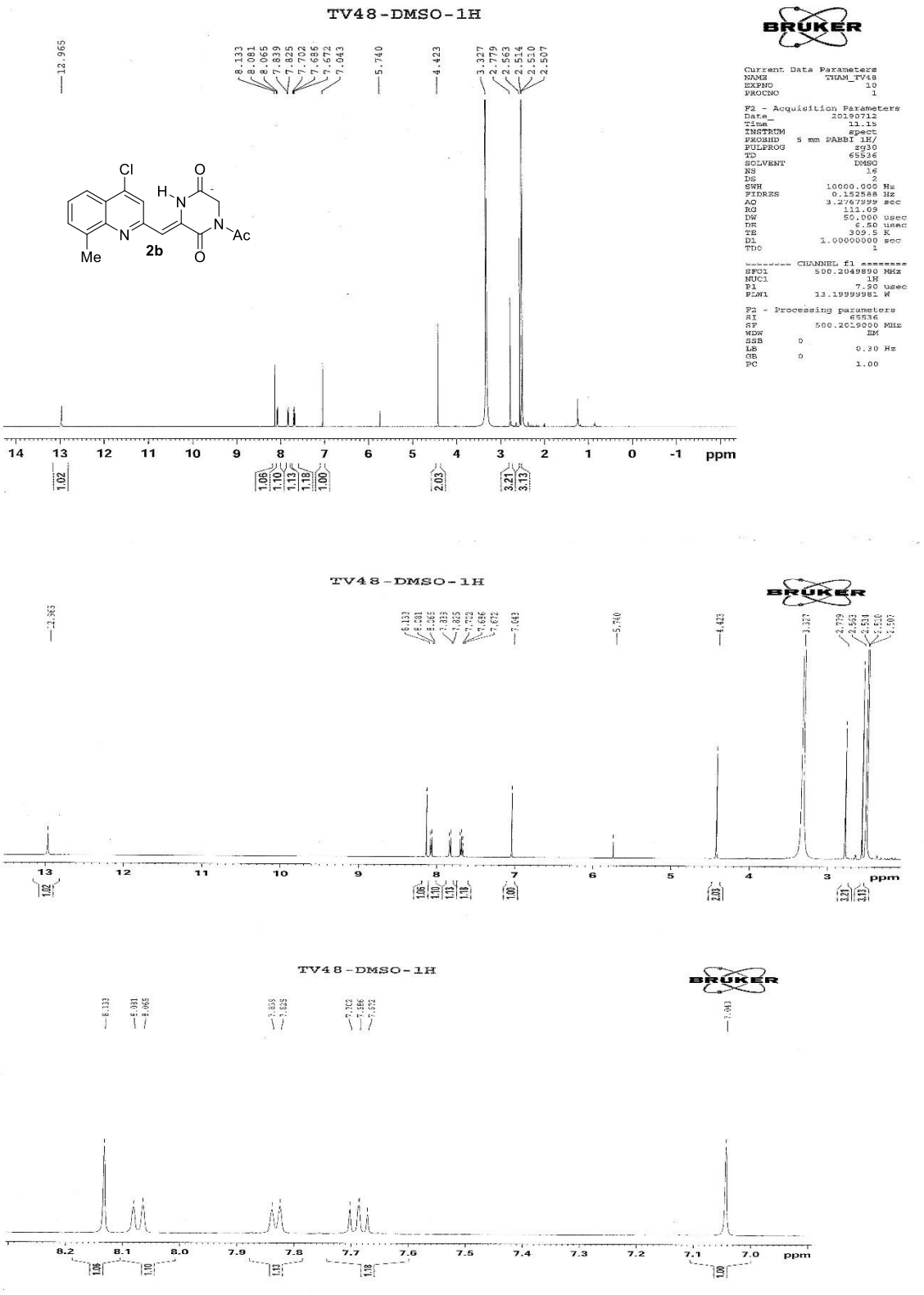

Figure S5. ^{1}H-NMR spectrum of compound **2b**
(Z)-1-acetyl-3-((4-chloro-8-methylquinolin-2-yl)methylidene)piperazine-2,5-dione

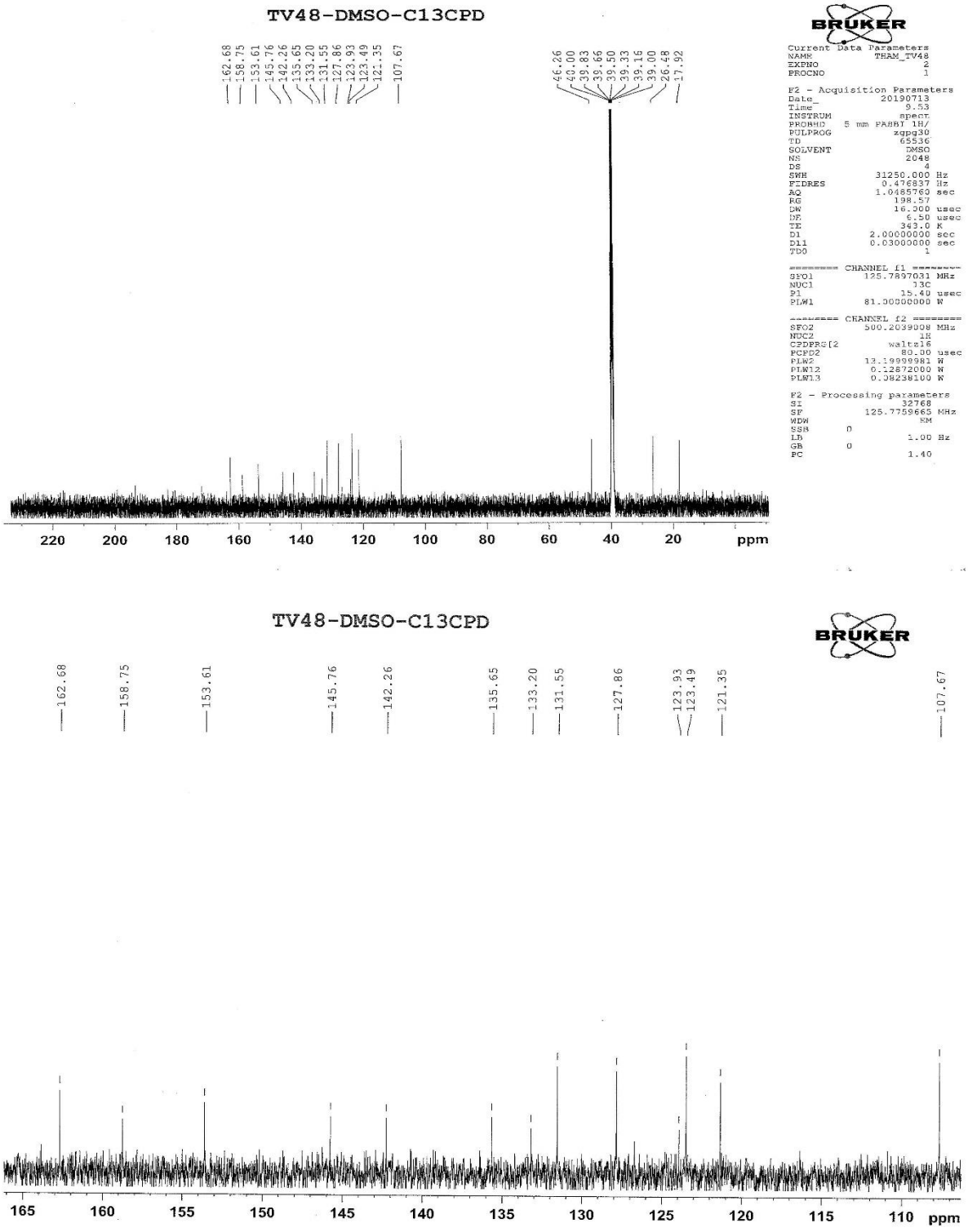

Figure S6. ^{13}C-NMR spectrum of compound **2b**

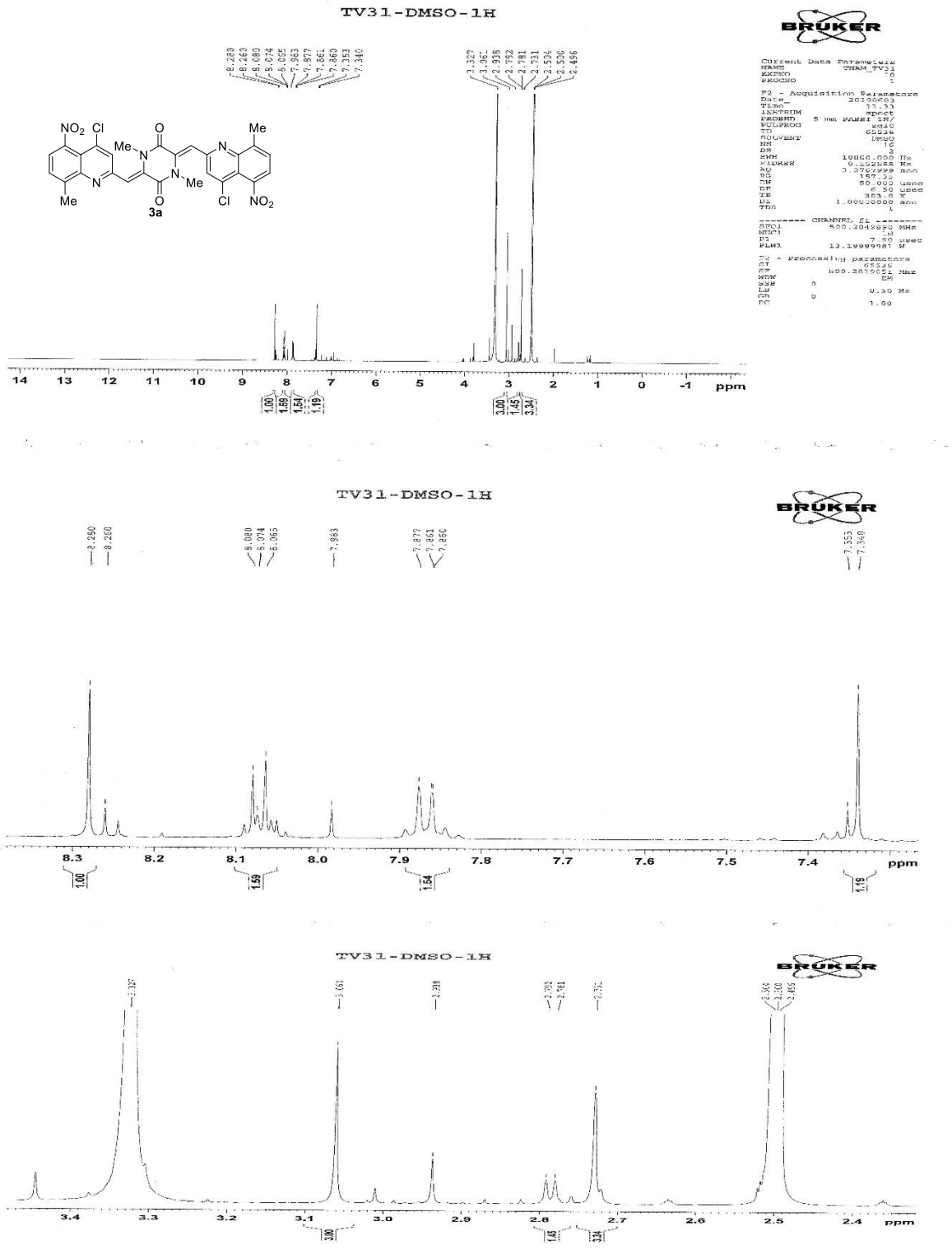

Figure S7. ^{1}H-NMR spectrum of compound **3a**
(3Z,6Z)-3,6-bis((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-1,4-dimethylpiperazine-2,5-dione

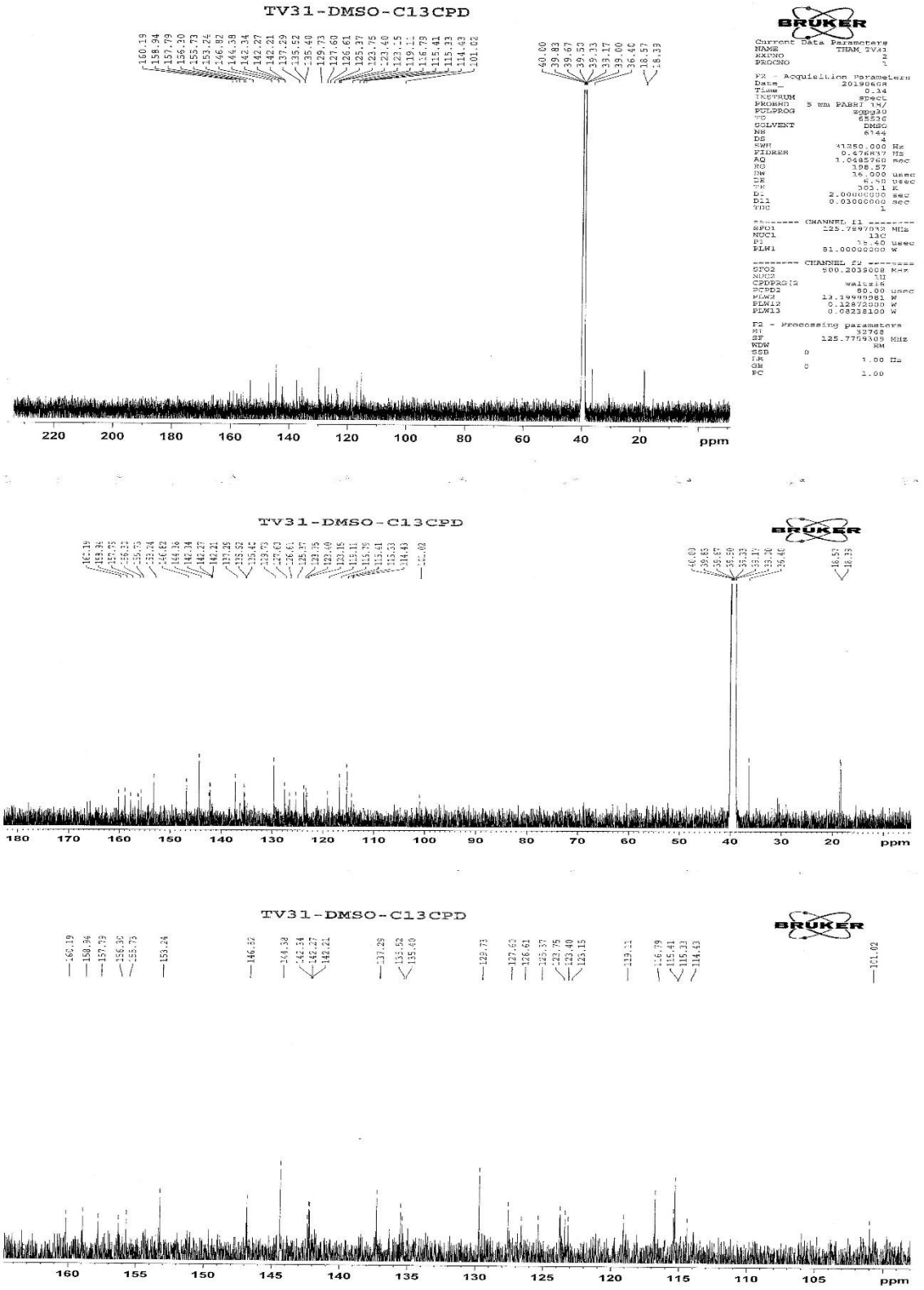

Figure S8. ^{13}C-NMR spectrum of compound **3a**

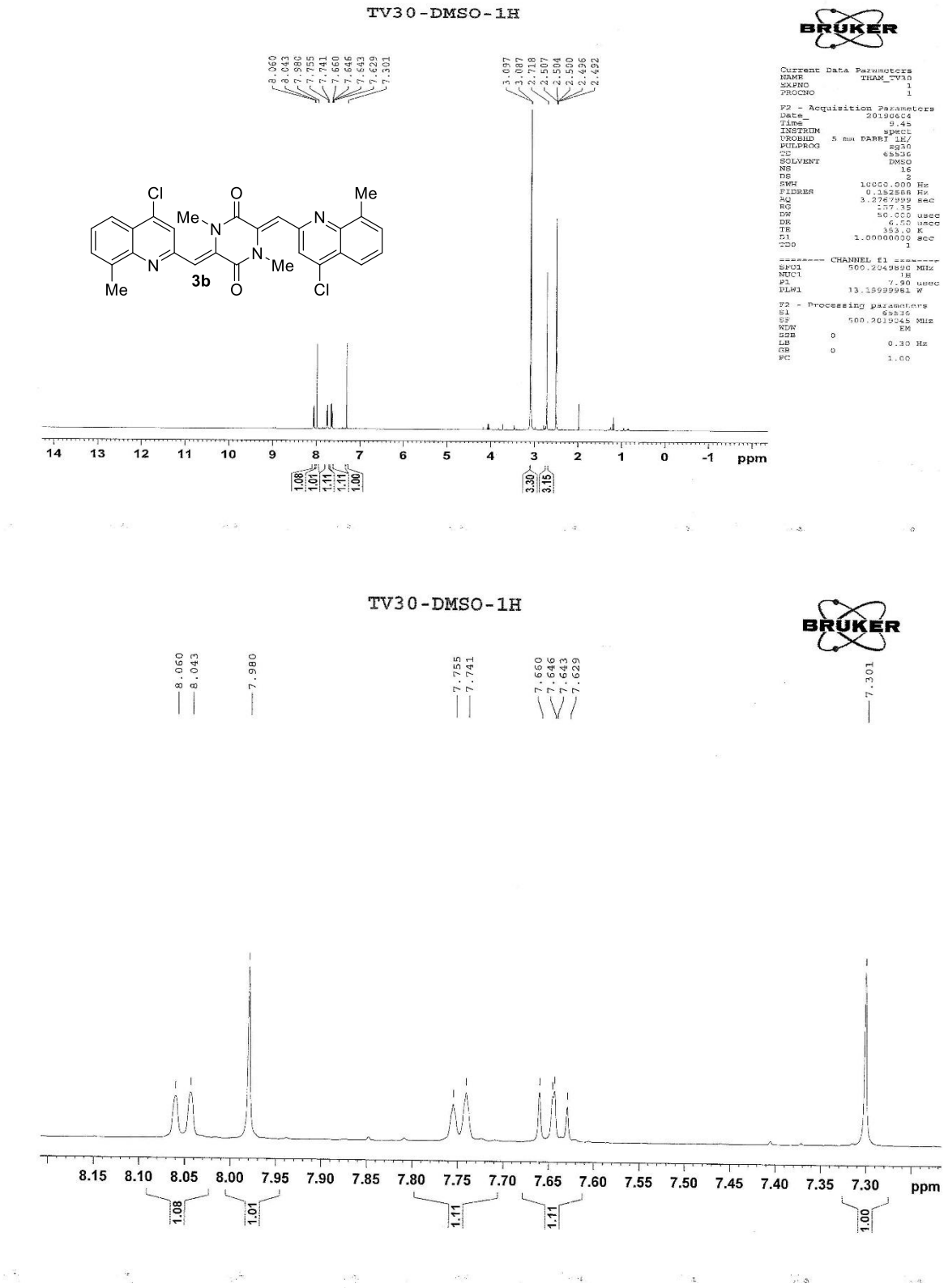

Figure S9. ^{1}H-NMR spectrum of compound **3b**

(3Z,6Z)-3,6-bis((4-chloro-8-methylquinolin-2-yl)methylidene)-1,4-dimethylpiperazine-2,5-dione

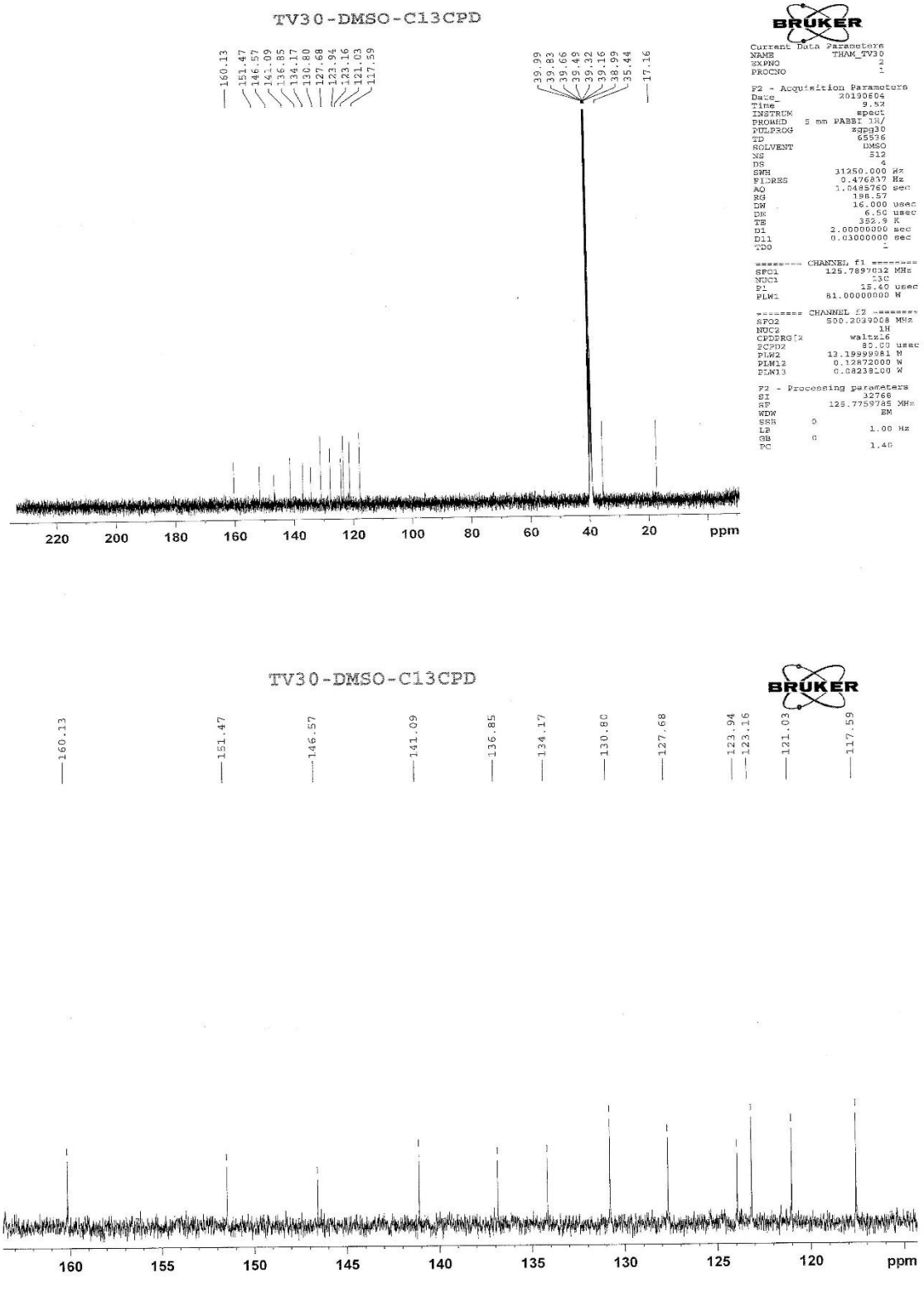

Figure S10. ^{13}C-NMR spectrum of compound **3b**

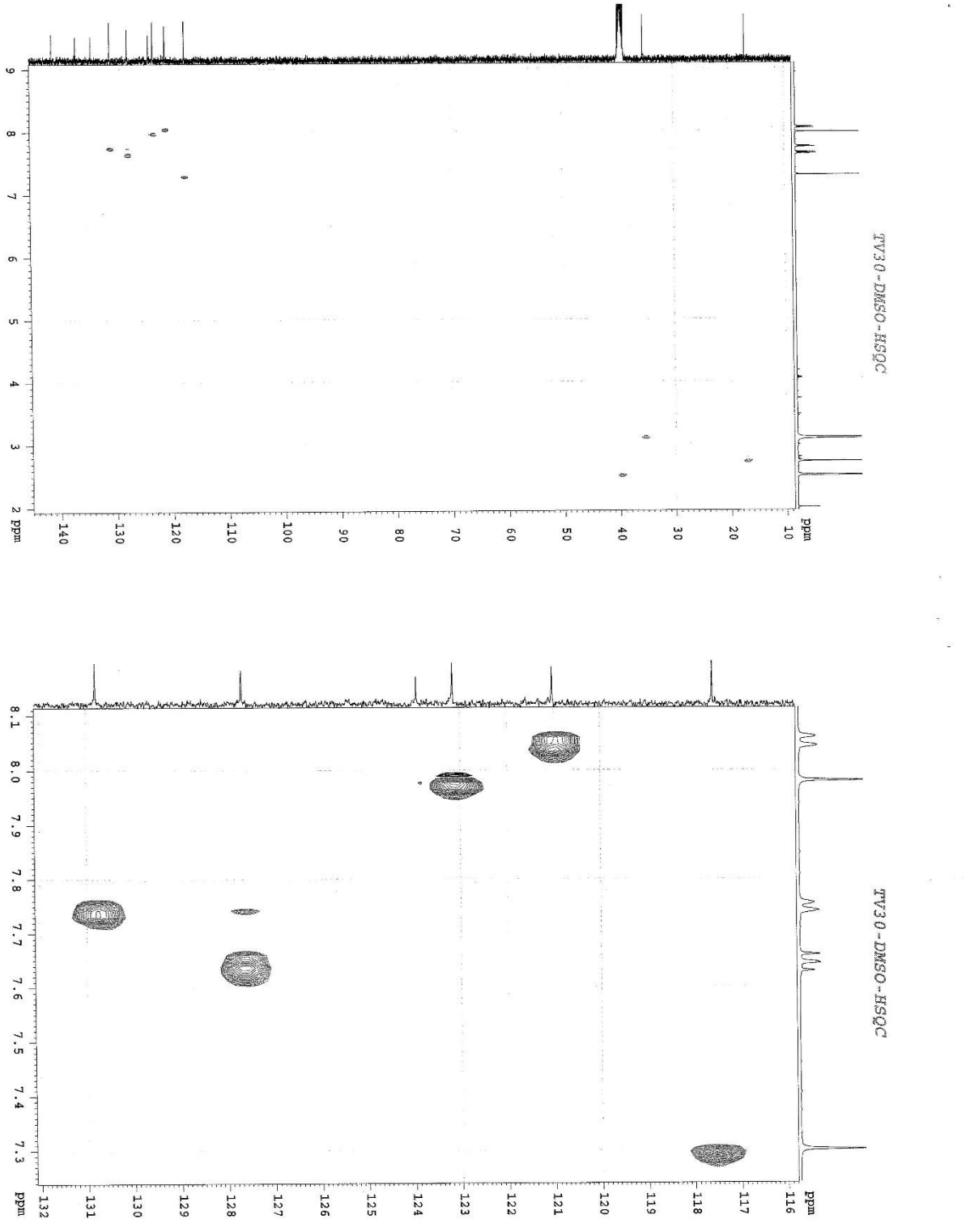

Figure S11. HSQC spectrum of compound **3b**

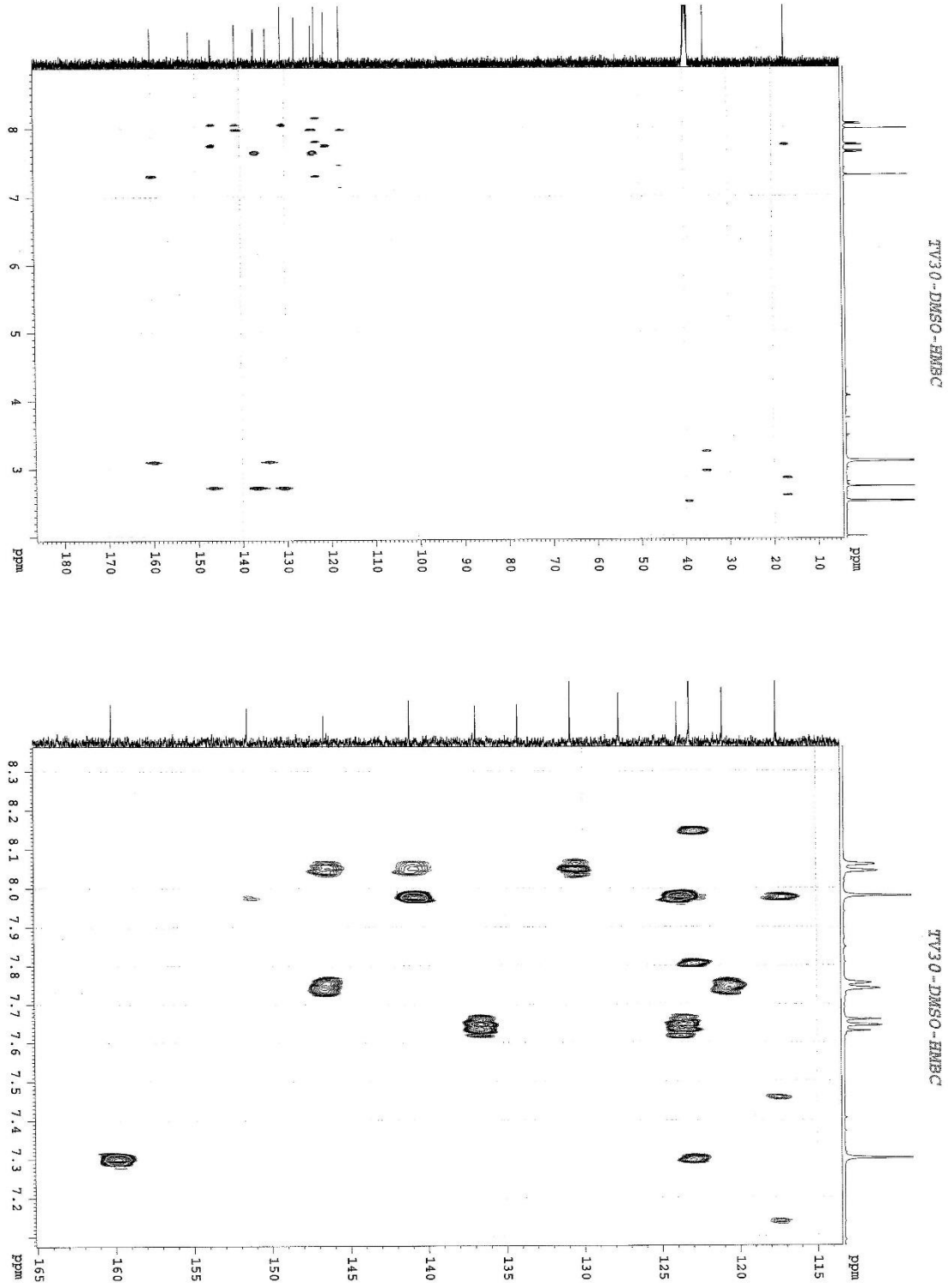

Figure S12. HMBC spectrum of compound **3b**

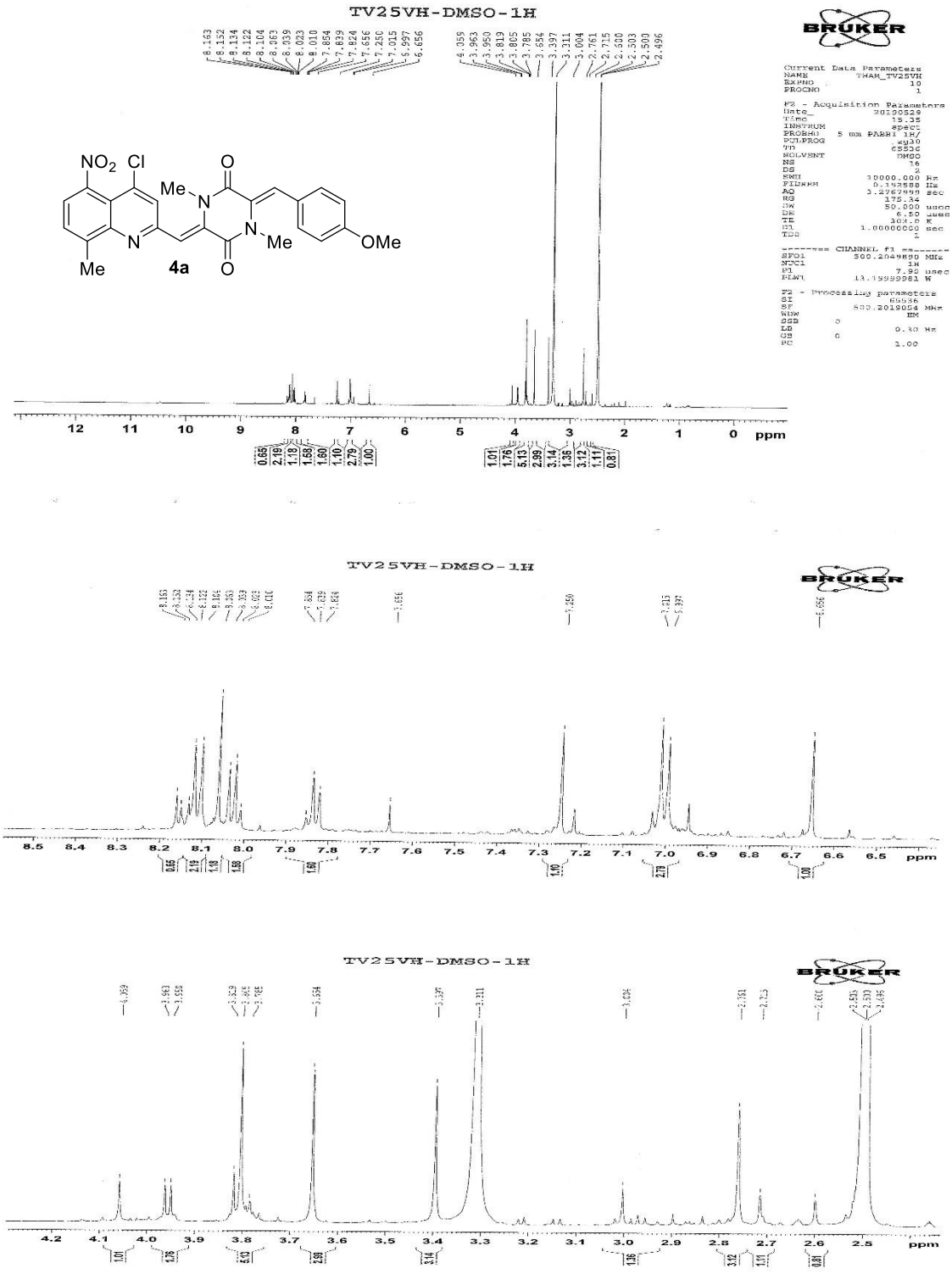

Figure S13. ^{1}H-NMR spectrum of compound **4a**
(Z)-3-((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-6-((Z)-4-methoxybenzylidene)-1,4-dimethylpiperazine-2,5-dione

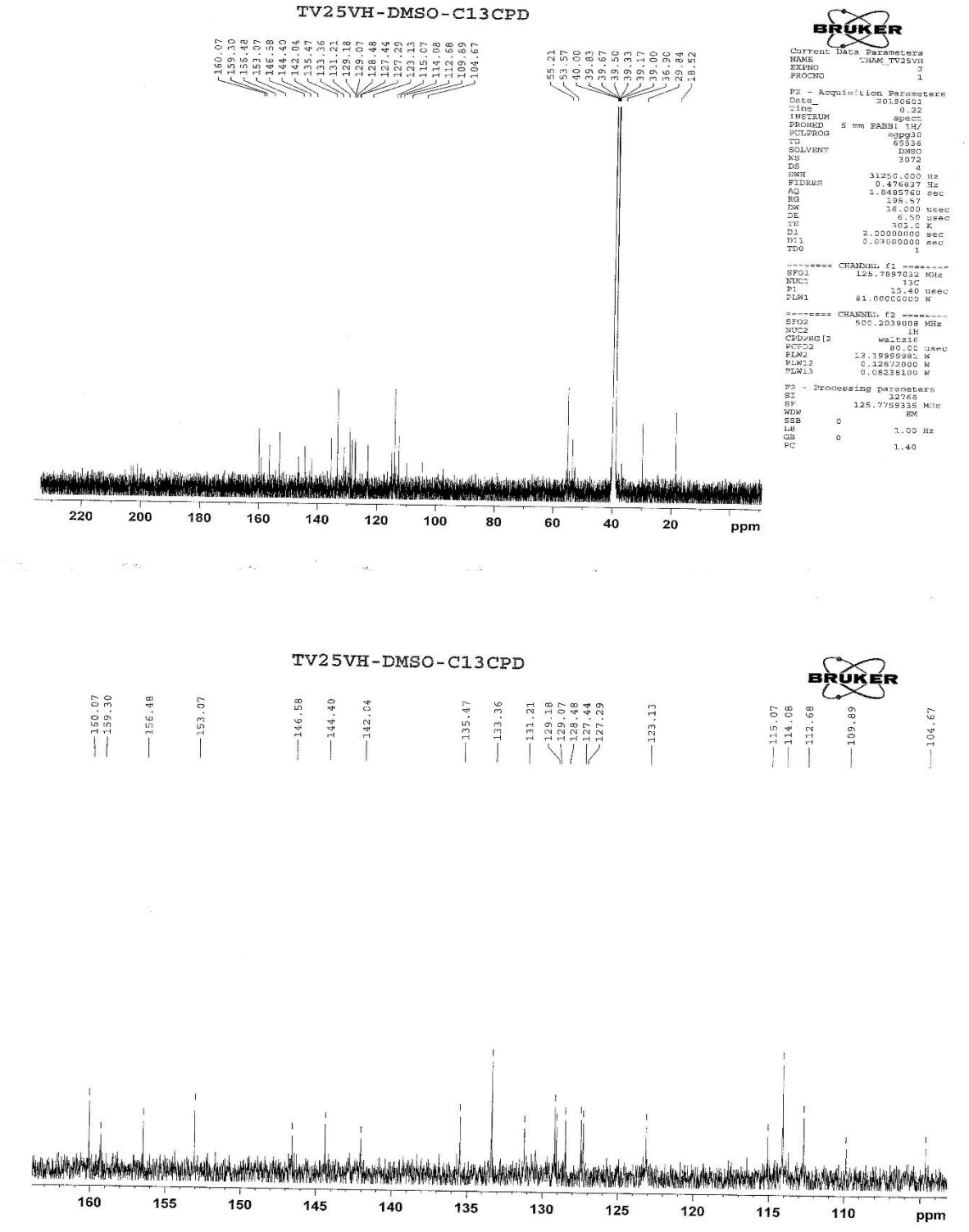

Figure S14. ^{13}C-NMR spectrum of compound **4a**

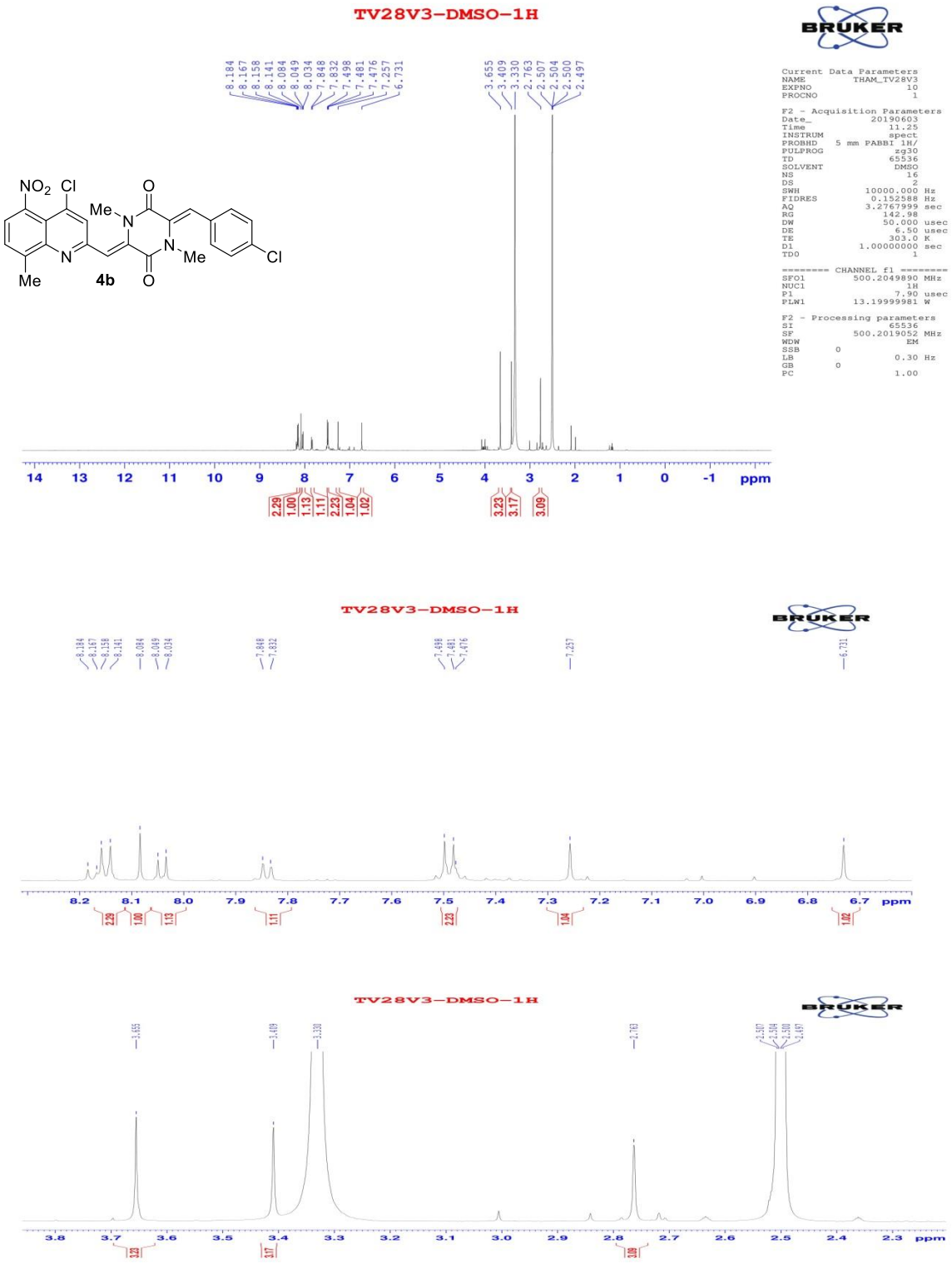

Figure S15. ^{1}H-NMR spectrum of compound **4b**
(Z)-3-((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-6-((Z)-4-chlorobenzylidene)-1,4-dimethylpiperazine-2,5-dione

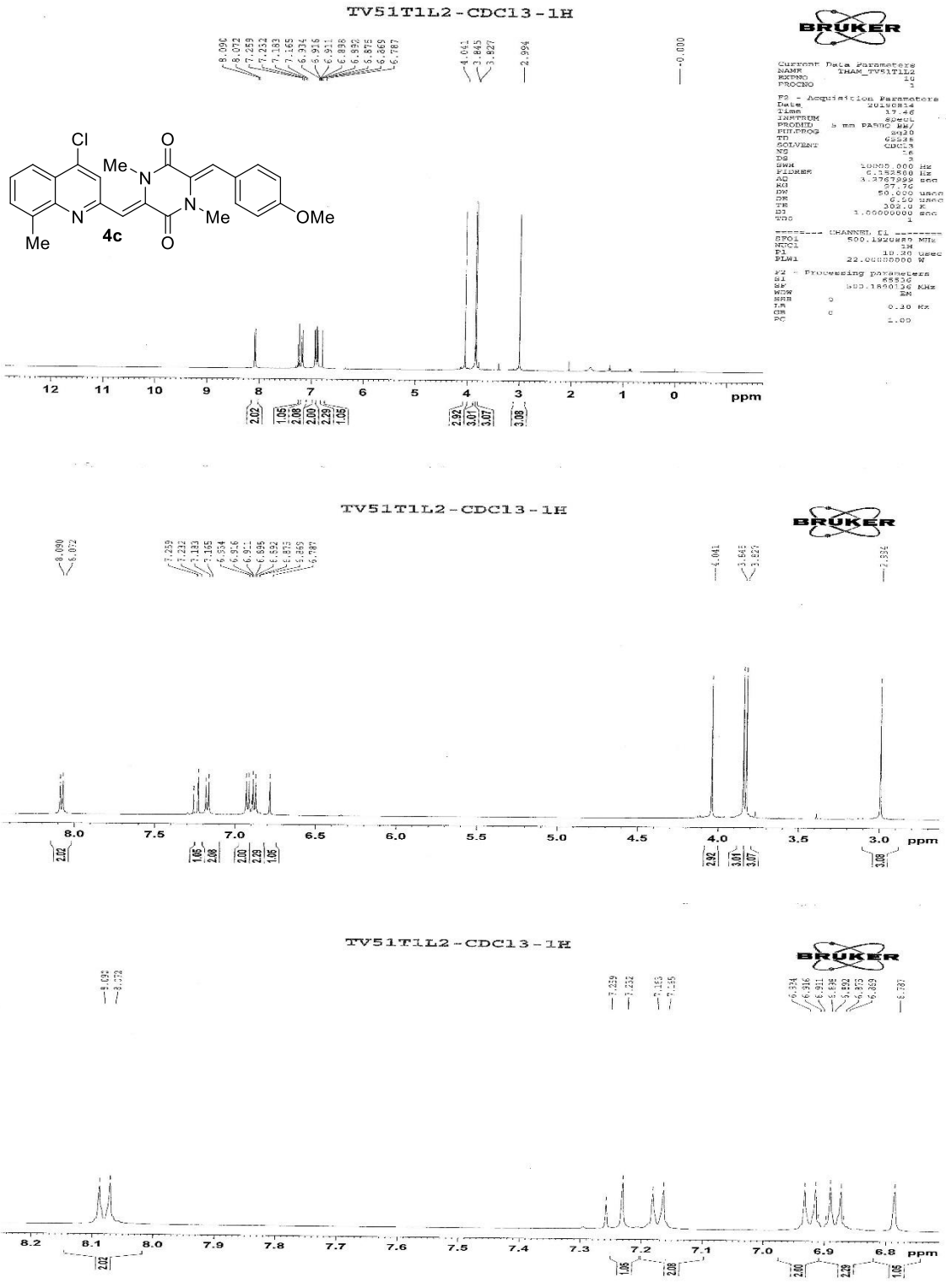

Figure S16. ^{1}H-NMR spectrum of compound **4c**
(Z)-3-((4-chloro-8-methylquinolin-2-yl)methylidene)-6-((Z)-4-methoxybenzylidene)-1,4-dimethylpiperazine-2,5-dione

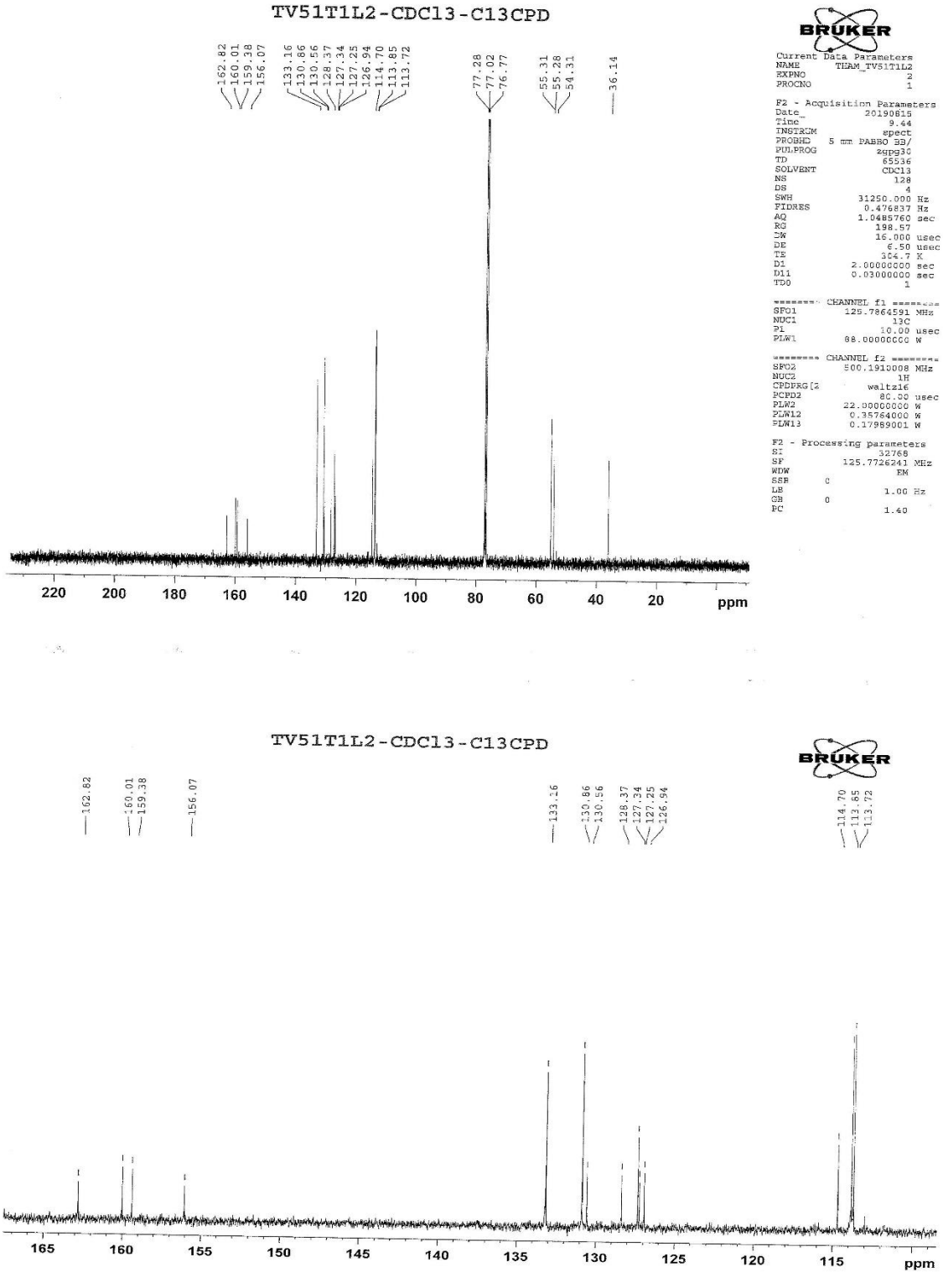

Figure S17. ^{13}C-NMR spectrum of compound **4c**

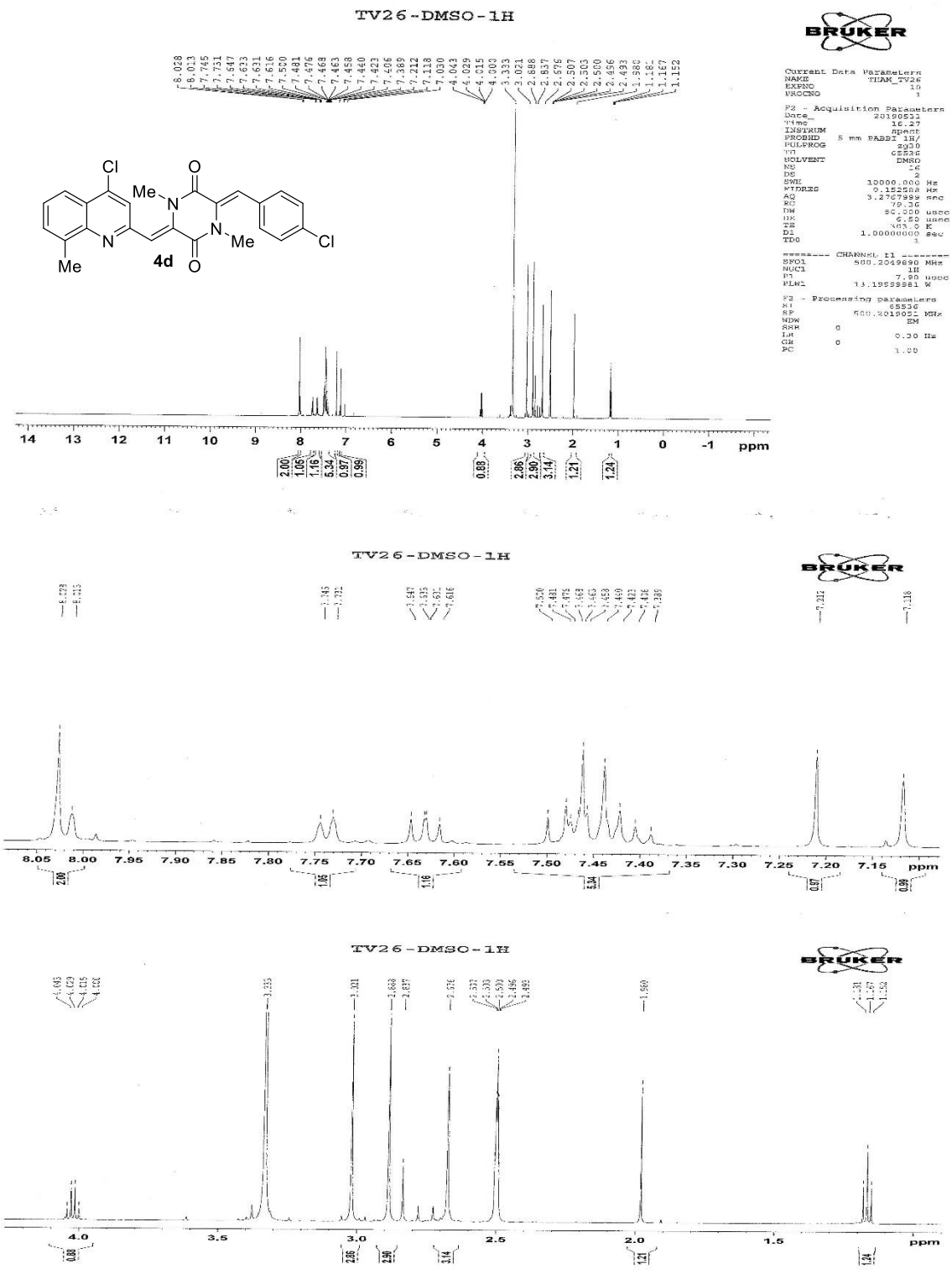

Figure S18. ^{1}H-NMR spectrum of compound **4d**
(Z)-3-((4-chloro-8-methylquinolin-2-yl)methylidene)-6-((Z)-4-chlorobenzylidene)-1,4-dimethylpiperazine-2,5-dione

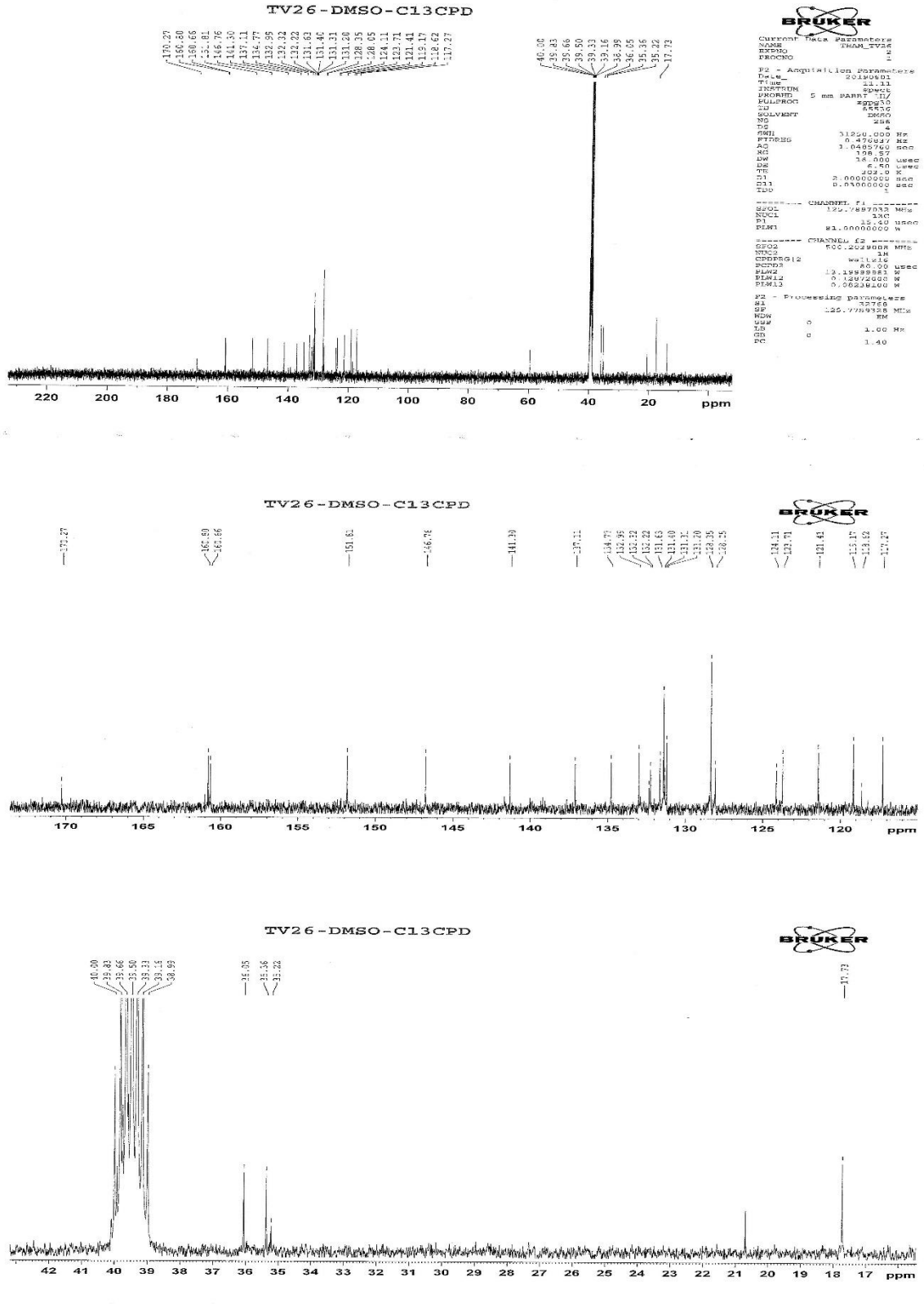

Figure S19. ^{13}C-NMR spectrum of compound **4d**

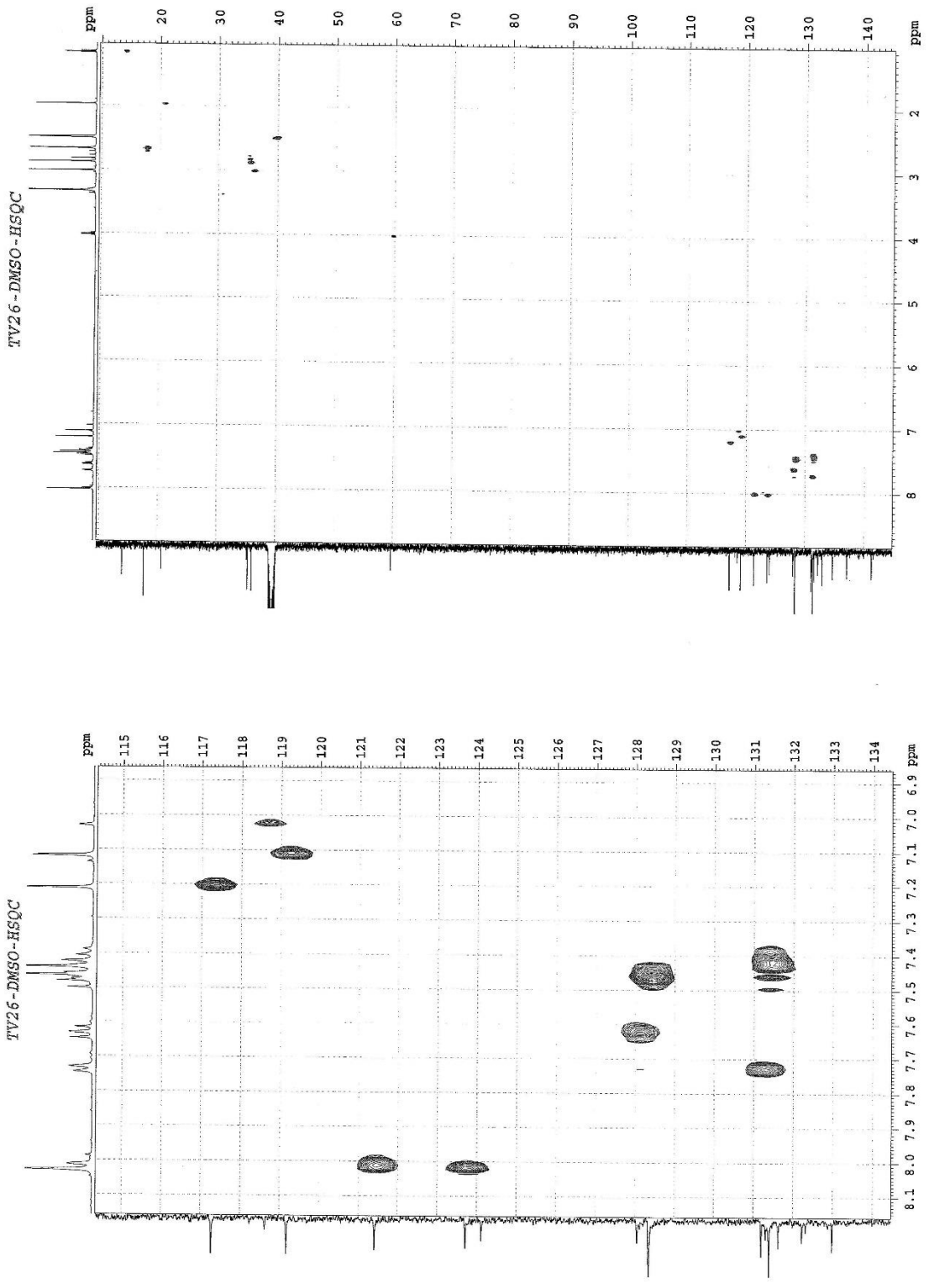

Figure S20. HSQC spectrum of compound **4d**

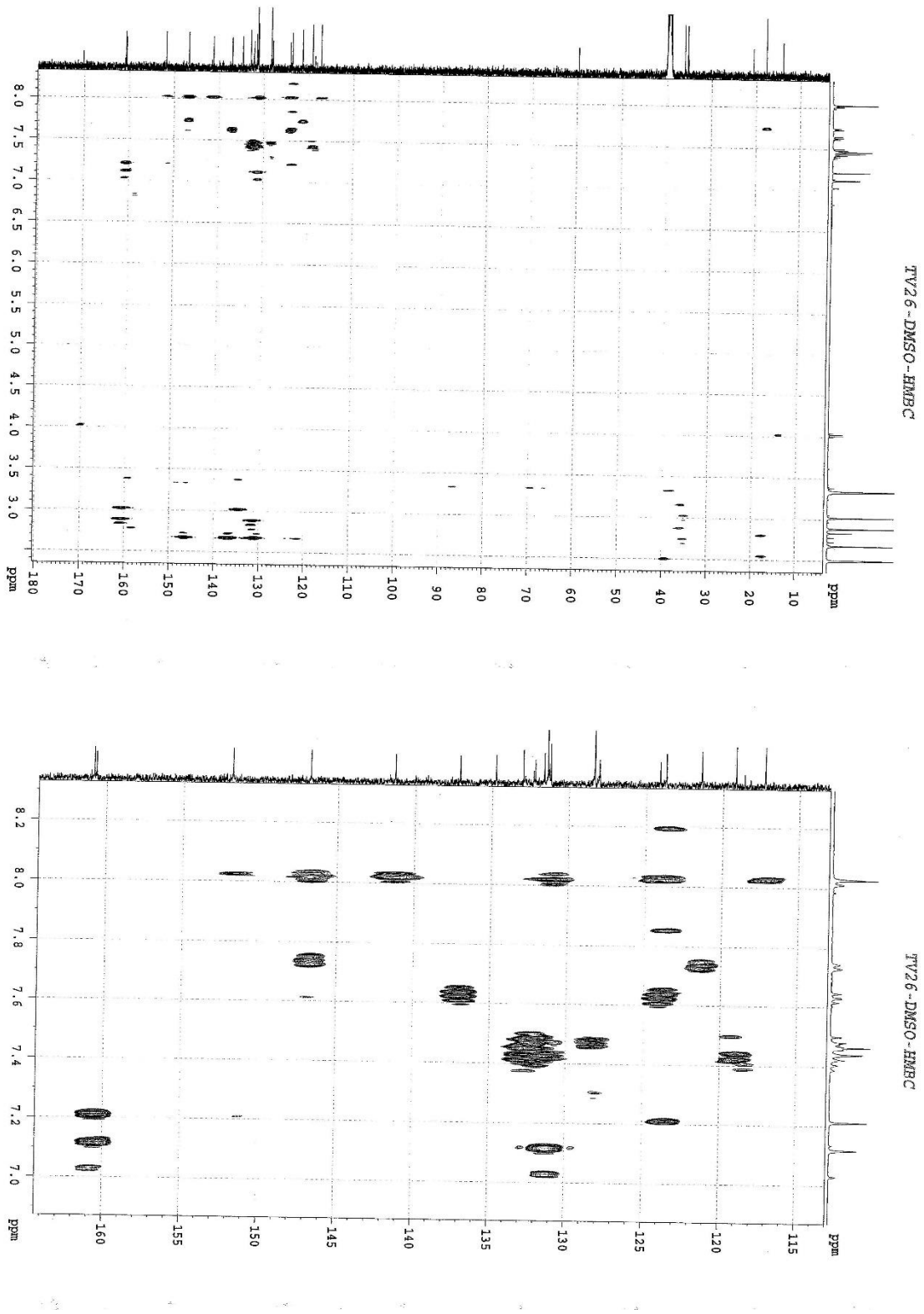

Figure S21. HMBC spectrum of compound **4d**

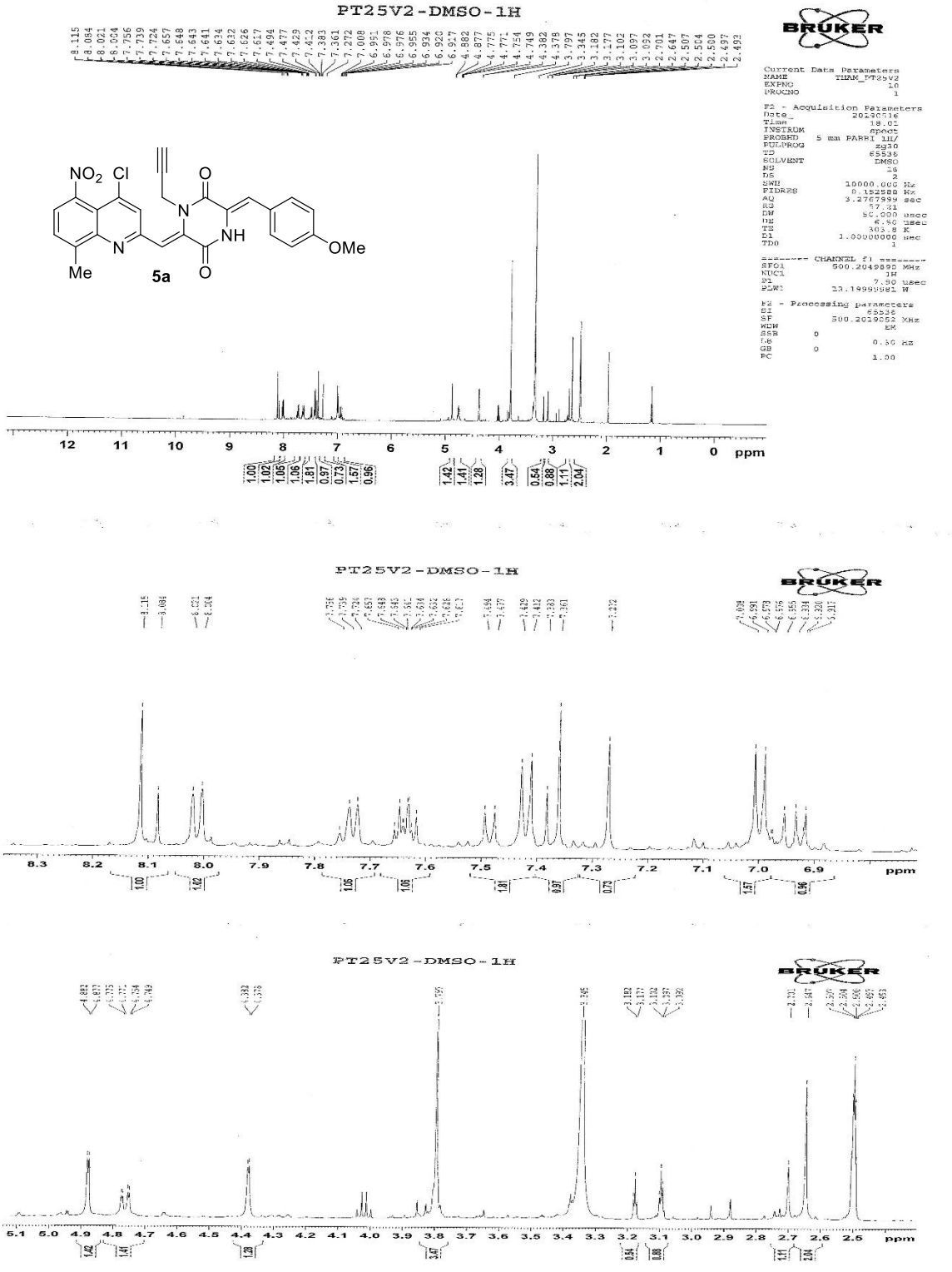

Figure S22. ^{1}H-NMR spectrum of compound **5a**
(Z)-6-((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-3-((Z)-4-methoxybenzylidene)-1-(prop-2-yn-1-yl)piperazine-2,5-dione

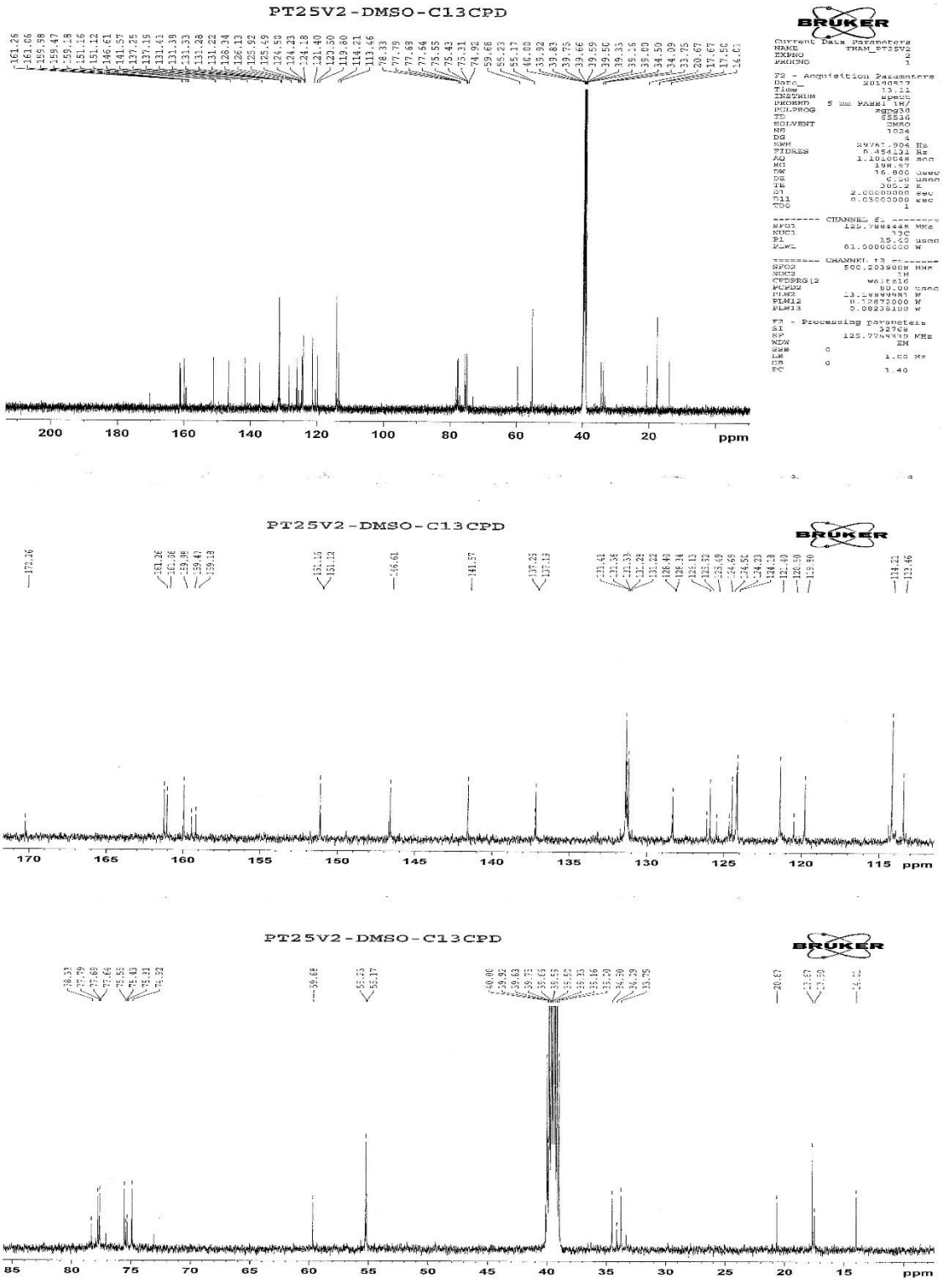

Figure S23. ^{13}C-NMR spectrum of compound **5a**

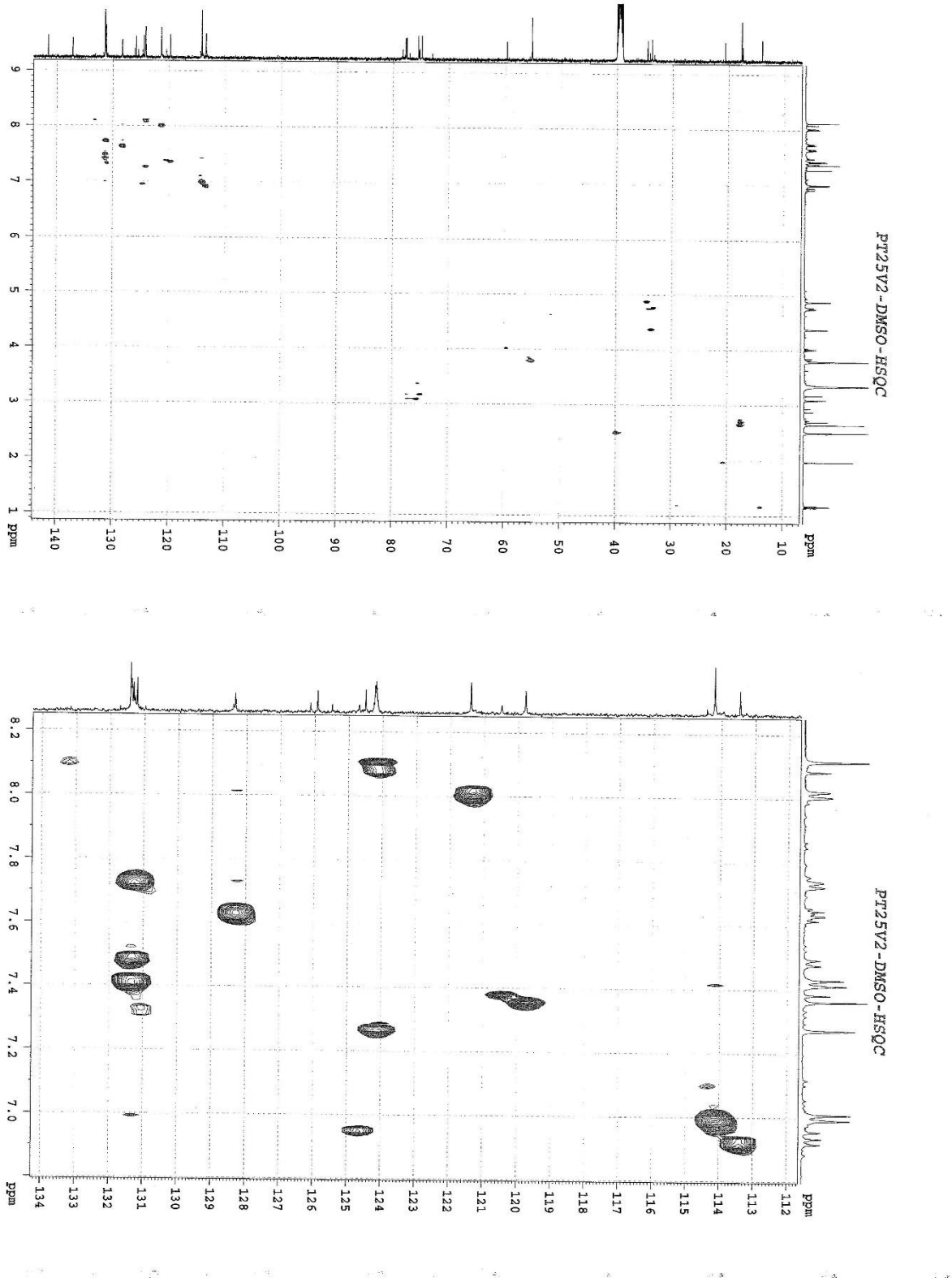

Figure S24. HSQC spectrum of compound **5a**

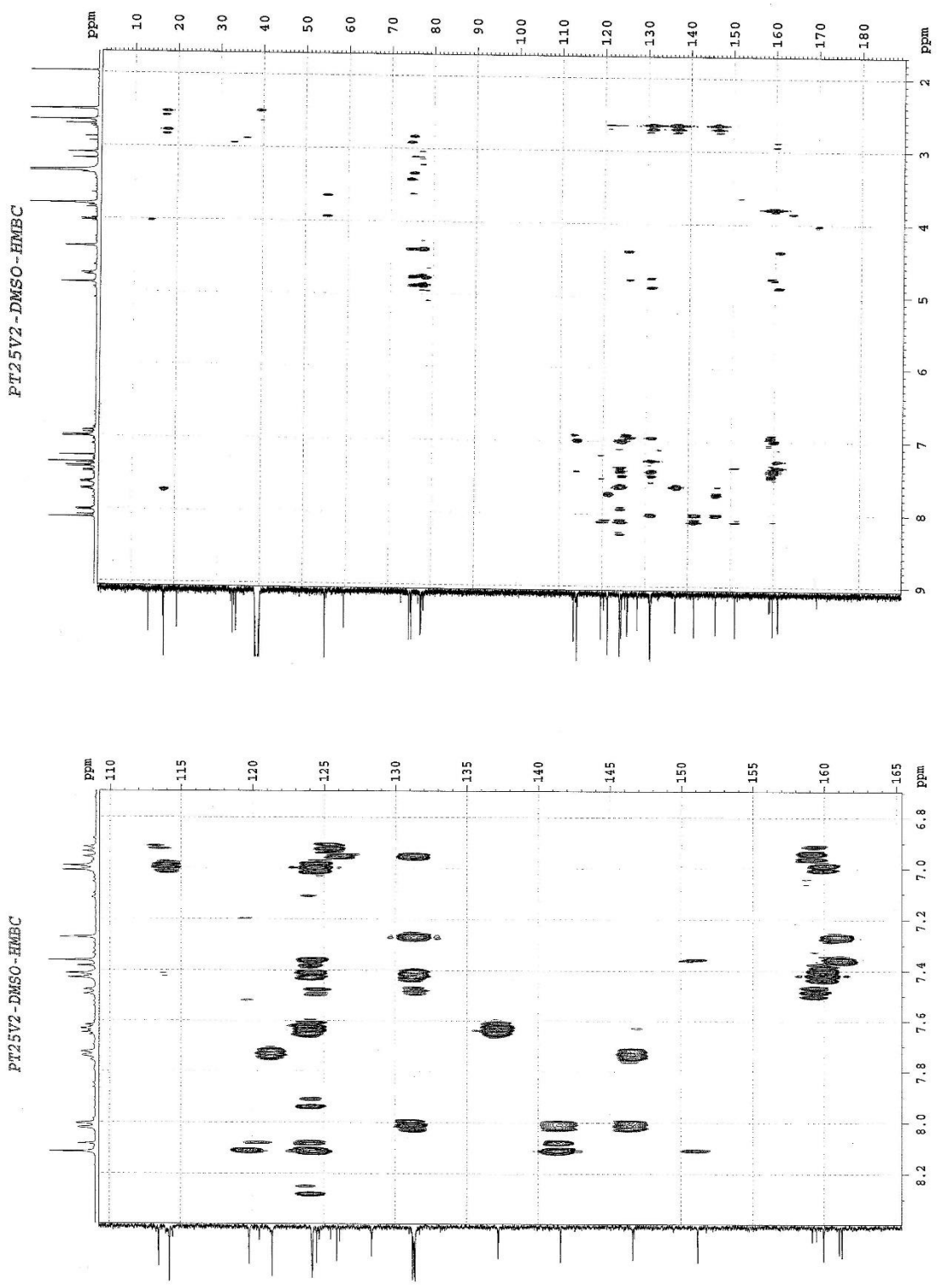

Figure S25. HMBC spectrum of compound **5a**

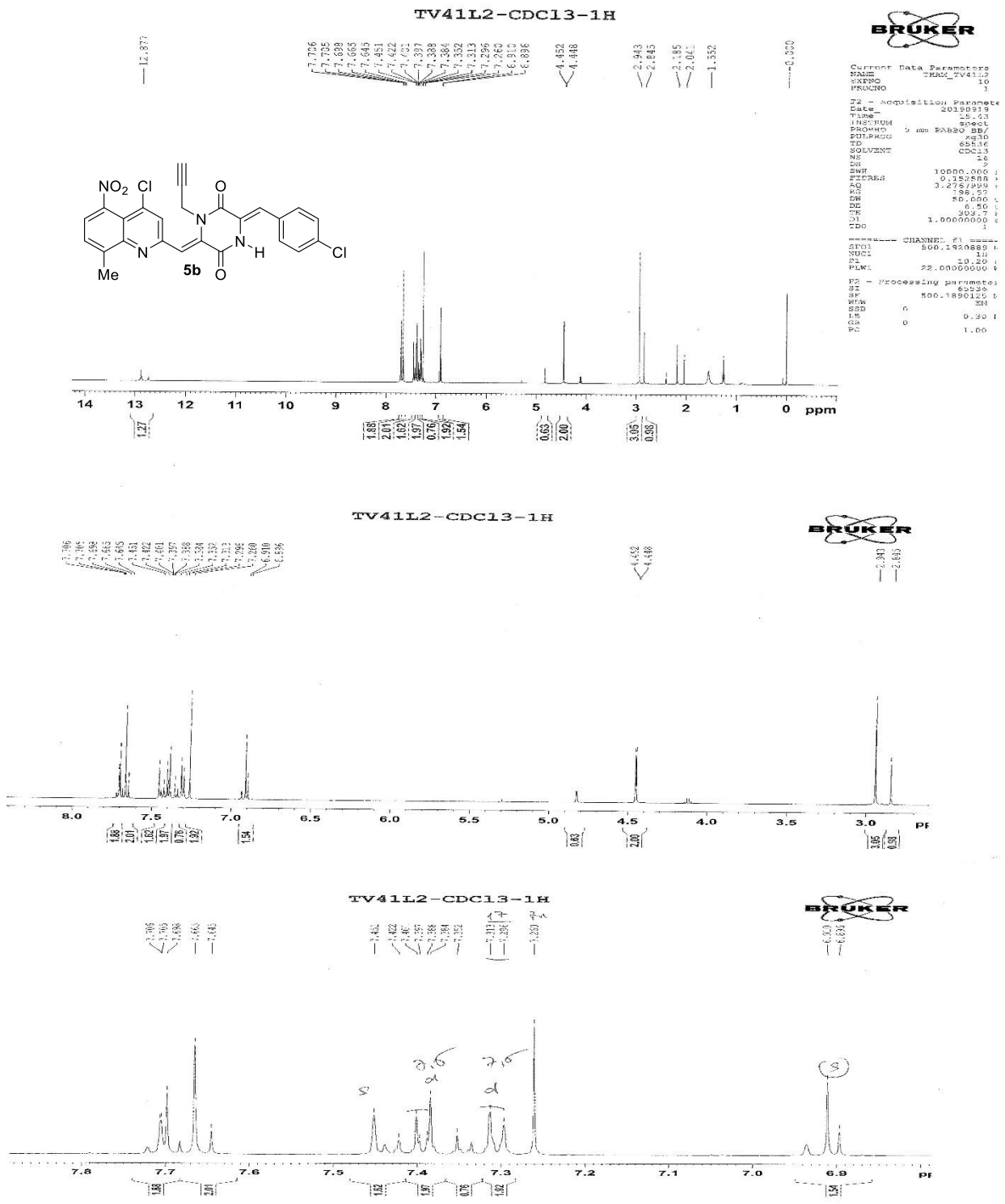

Figure S26. ^{1}H-NMR spectrum of compound **5b**
(Z)-6-((4-chloro-8-methyl-5-nitroquinolin-2-yl)methylidene)-3-((Z)-4-chlorobenzylidene)-1-(prop-2-yn-1-yl)piperazine-2,5-dione

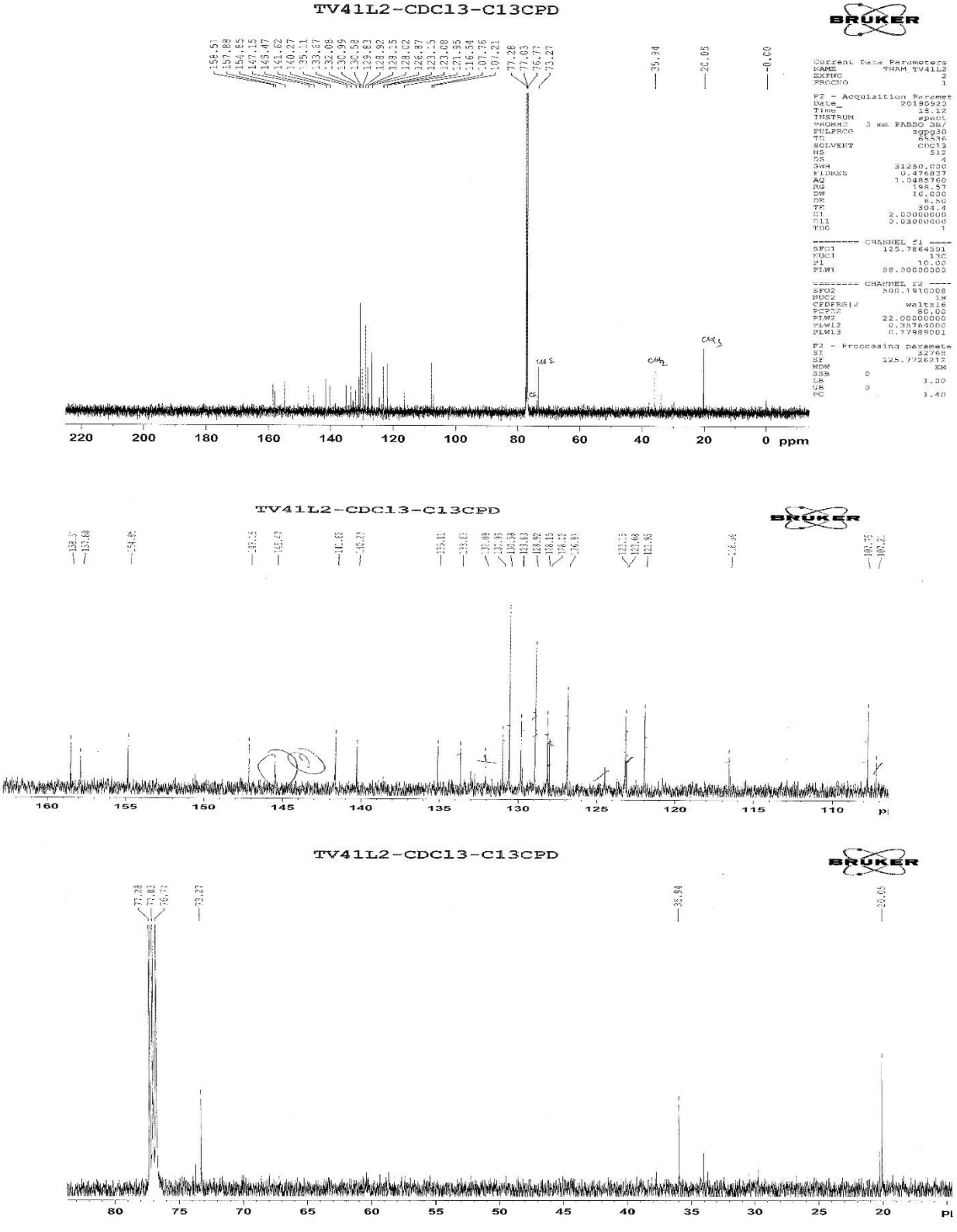

Figure S27. ^{13}C-NMR spectrum of compound **5b**

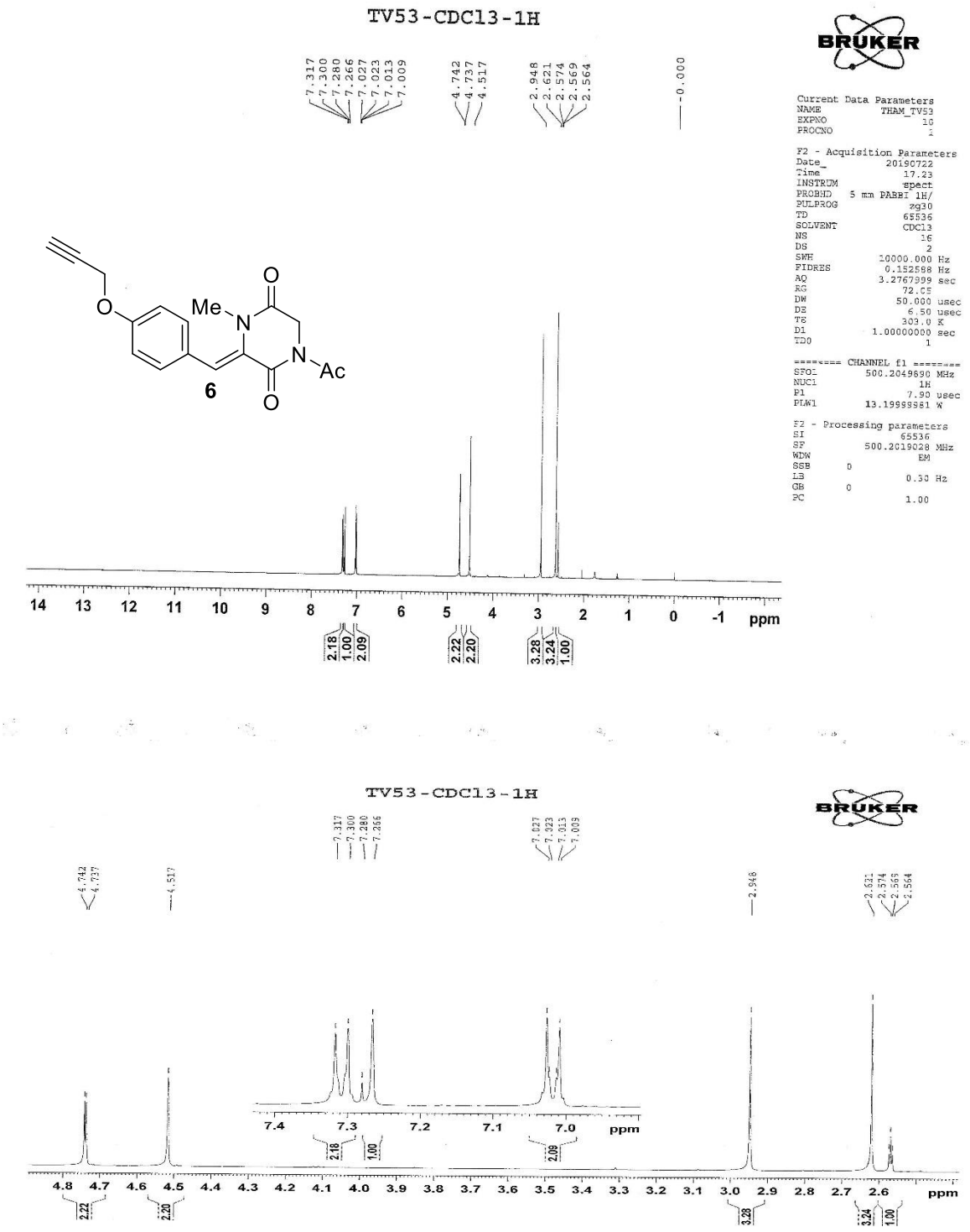

Figure S28. ^{1}H-NMR spectrum of compound **6**
(Z)-1-acetyl-4-methyl-3-(4-(prop-2-yn-1-yloxy)benzylidene)piperazine-2,5-dione

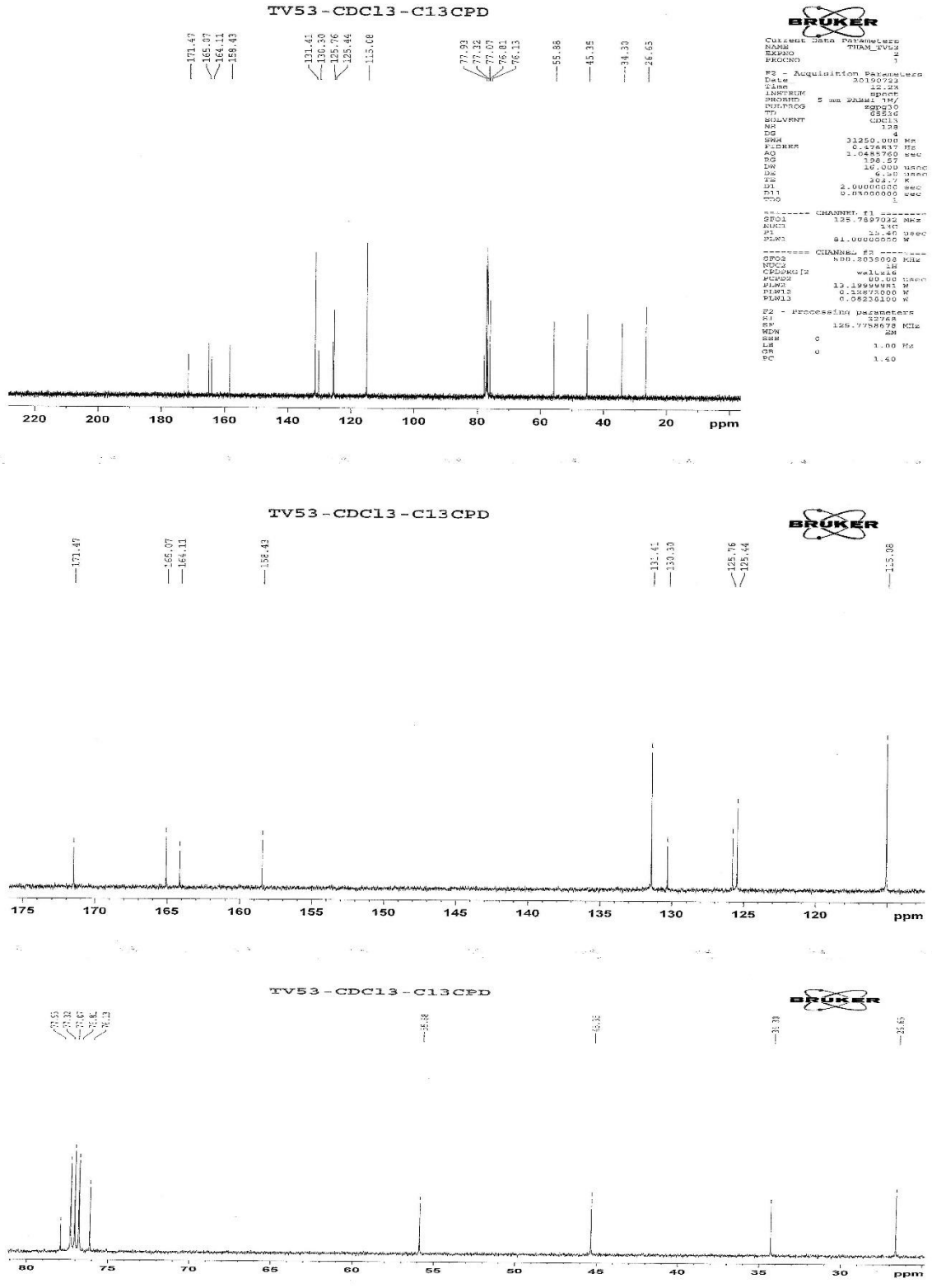

Figure S29. ^{13}C-NMR spectrum of compound **6**

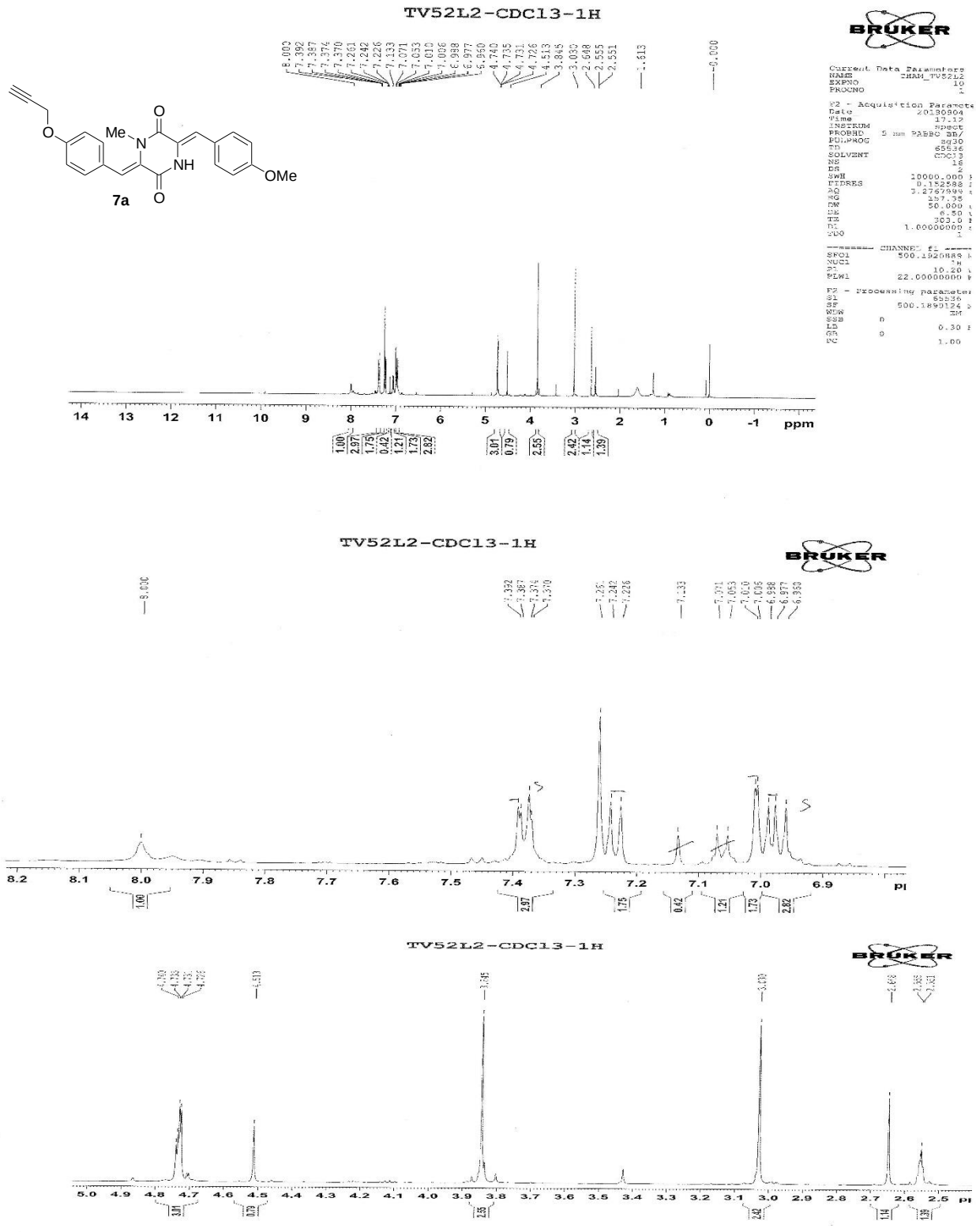

Figure S32. ^{1}H-NMR spectrum of compound **7a**
3-((Z)-4-methoxybenzylidene)-1-methyl-6-((Z)-4-(prop-2-yn-1-yloxy)benzylidene)piperazine-2,5-dione

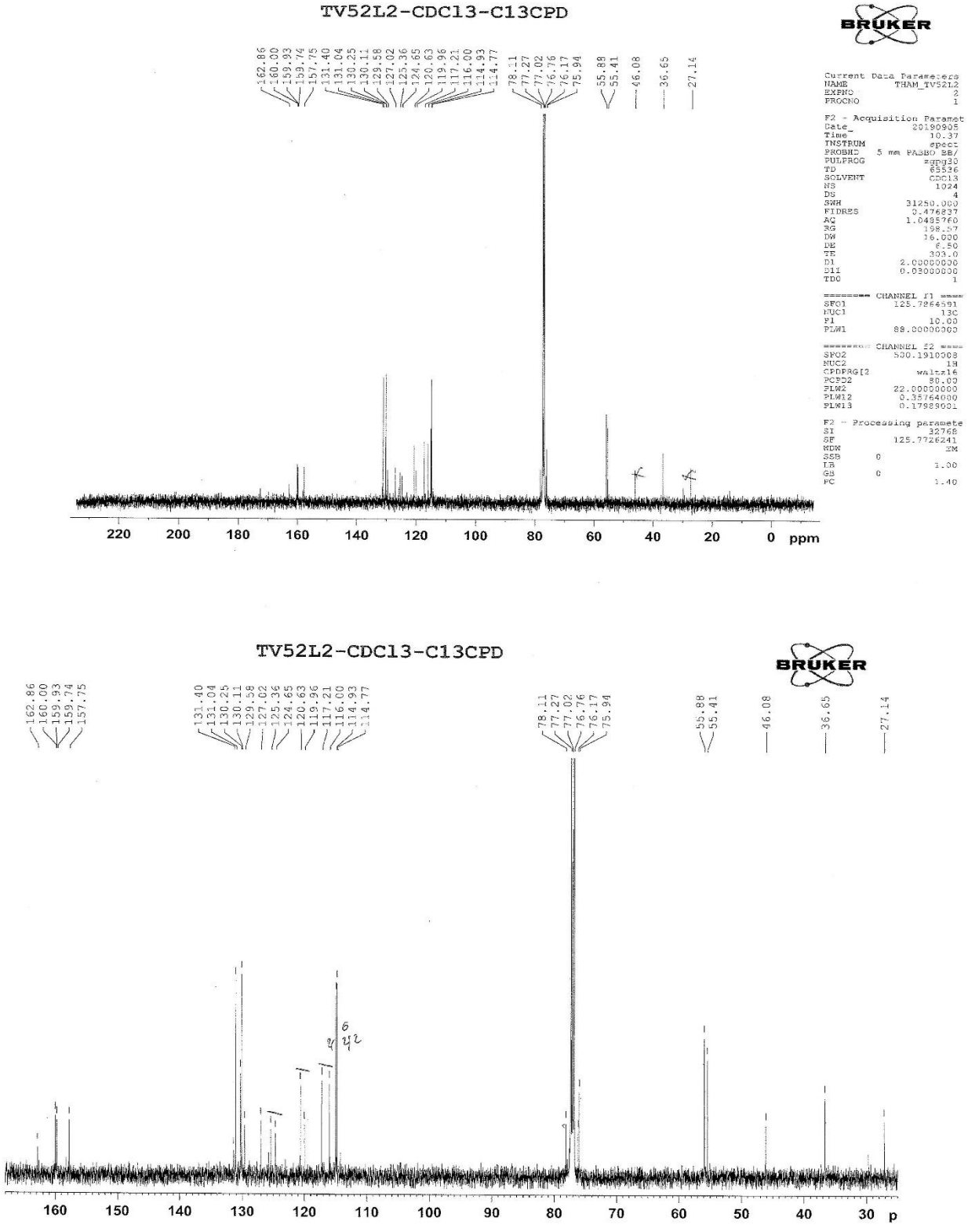

Figure S33. ^{13}C-NMR spectrum of compound **7a**

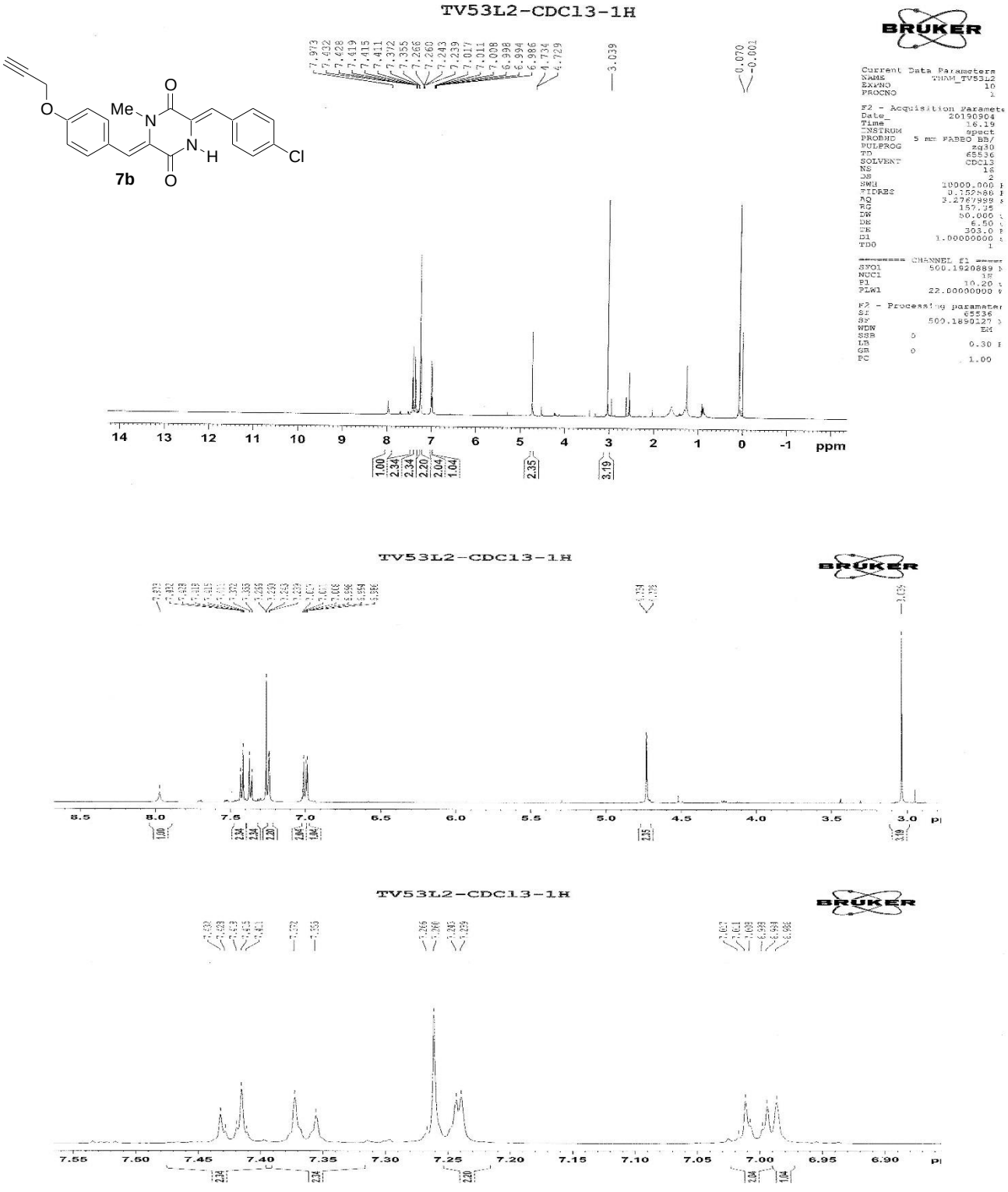

Figure S30. ^{1}H-NMR spectrum of compound **7b**
3-((Z)-4-chlorobenzylidene)-1-methyl-6-((Z)-4-(prop-2-yn-1-yloxy)benzylidene)piperazine-2,5-dione

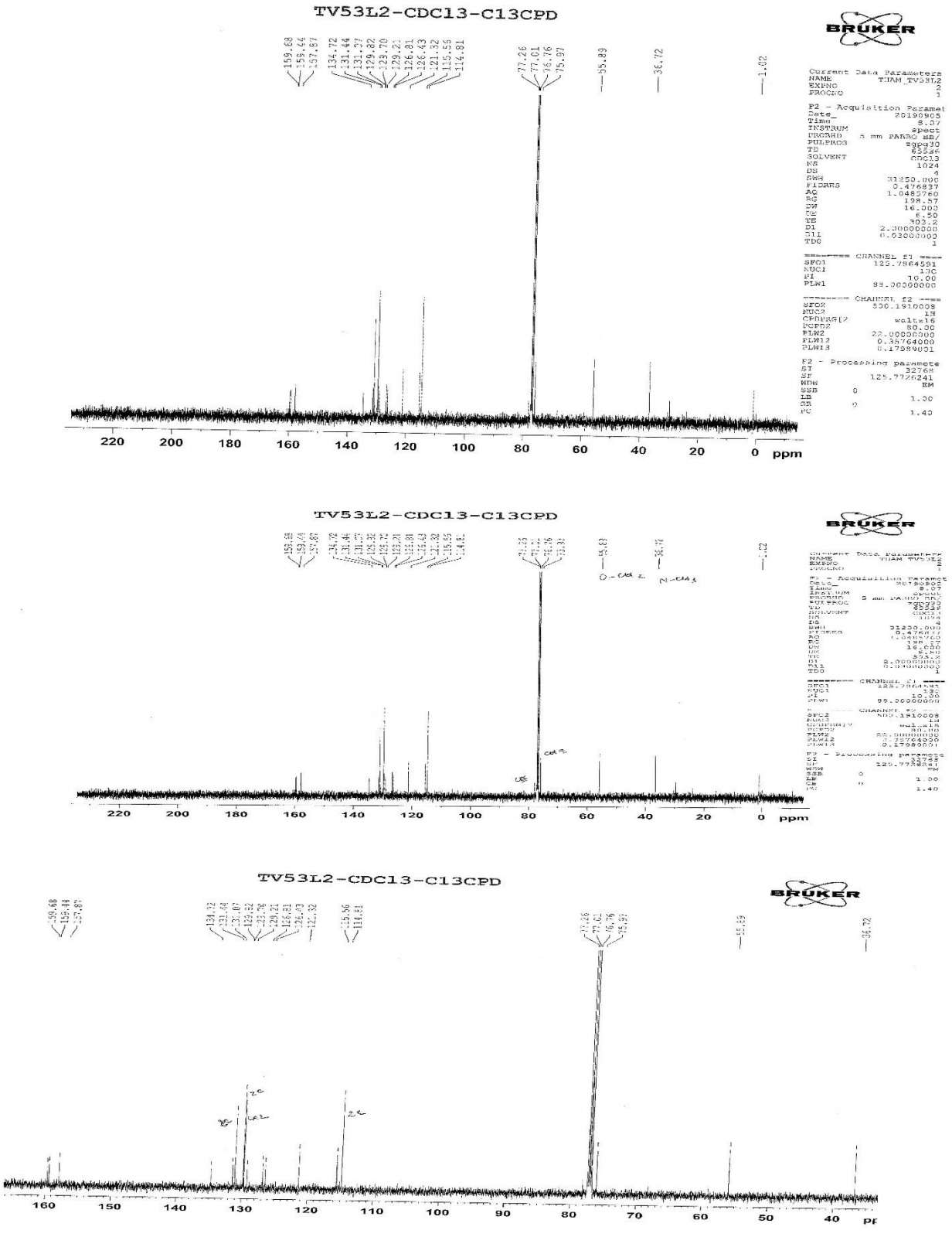

Figure S31. ^{13}C-NMR spectrum of compound **7b**