Supporting Information

Facile synthesis and evaluation of tryptamine-piperazine-2,5-dione conjugates as anticancer agents

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General Information

^{1}H and ^{13}C NMR were recorded on Bruker AVANCE III 400MHz instrument with TMS as internal standard. ^{1}H NMR data are reported as follows: chemical shift in ppm (δ), multiplicity (s = singlet, d = doublet, t= triplet, m = multiplet), coupling constant (Hz), relative intensity. ^{13}C NMR data are reported as follows: chemical shift in ppm (δ). HPLC-MS analyses were performed on a Shimadzu-2020 LC-MS instrument using the following conditions: Shim-pack VPODS C18 column (reverse phase, 150 x 2.0mm); 80% acetonitrile and 20% water over 10.0 min; flow rate of 0.4 mL/min; UV photodiode array detection from 200 to 300nm. The products were purified by Biotage Isolera™ Spektra Systems and Hexane/EtOAc solvent systems. All reagents and solvents were obtained from commercial sources and used without further purification.

Microwave Irradiation Experiments

All microwave irradiation experiments were carried out in a Biotage® Initiator Classic microwave apparatus with continuous irradiation power from 0 to 400W with utilization of the standard absorbance level of 250W maximum power (external surface sensor for temperature monitoring). The reactions were carried out in 5 mL glass tubes, sealed with microwave cavity. The reaction was irradiated at a required ceiling temperature using maximum power for the stipulated time. Then it was cooled to 50 ^{o}C with gas jet cooling.

General procedures for compound 6.

A solution of benzaldehyde (1.00 mmol) and propiolic acid (1.00 mmol) in MeOH (2 mL) was stirred at room temperature for 5 min in a 10 mL microwave vial. Then, tryptamine (1.0 mL) and benzyl isonitrile (1.00 mmol) were added separately. The mixture was stirred overnight and monitored by TLC. When the reaction was completed, the solvent was removed under a nitrogen stream. The residue was dissolved in MeCN (3.0 mL), and then K_2CO_3 (2.0 mmol) was added, sealed and heated in microwave at 120 ^oC for 10 min. After the microwave vial was cooled to room temperature, the solvent was removed under reduced pressure and then diluted with EtOAc (15.0 mL) and washed with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography using a gradient of ethyl acetate/hexane (20-80%) to afford the relative targeted products **6a-j**.

Measurement of cell viability and proliferation

Human pancreatic cancer cell lines AsPC-1 and SW1990 were cultured under standard culture conditions at 37 ℃ and 5% CO_2 atmosphere in the specific medium (McCOY'S 5A and DMEM) supplemented with 10% fetal bovine serum, 100 UI/mL penicillin and 100 mg/L streptomycin. The short-term effects of compounds **6a-j** on cell growth were measured with the MTT assay. Briefly, AsPC-1 and SW1990 cancer cells were seeded into 96-well plates (3,000 cells/well) and incubated overnight at 37 °C, then treated with 0, 1, 5, 10, 20, 40 and 50 μmol/L test compound for 48 h. Next, 20 μL MTT solution (5 mg/mL) was added into each well and incubated for another 4 h followed by media removal and solubilization in 200 μL DMSO. The absorbance value was determined at 570 nm using a microplate reader (Bio-Tek, Winooski, VT, USA). The long-term effects of compound **6h** on cell growth were assessed with a clone formation assay. Cells were cultured at a density of 200 cells per well in 6-well plates for 36 h. Then, different concentrations of compound **6h** (0, 5, 10 and 20 μmol/L) were added. Cells were cultured for approximately 14 d until the cells grew

visible colonies. The medium was discarded, and the colonies were stained with crystal violet (C0121, Beyotime) for 15 min at room temperature. After carefully washing with PBS, the stained colonies in each well were photographed and the number of colonies with more than 50 cells was counted manually.

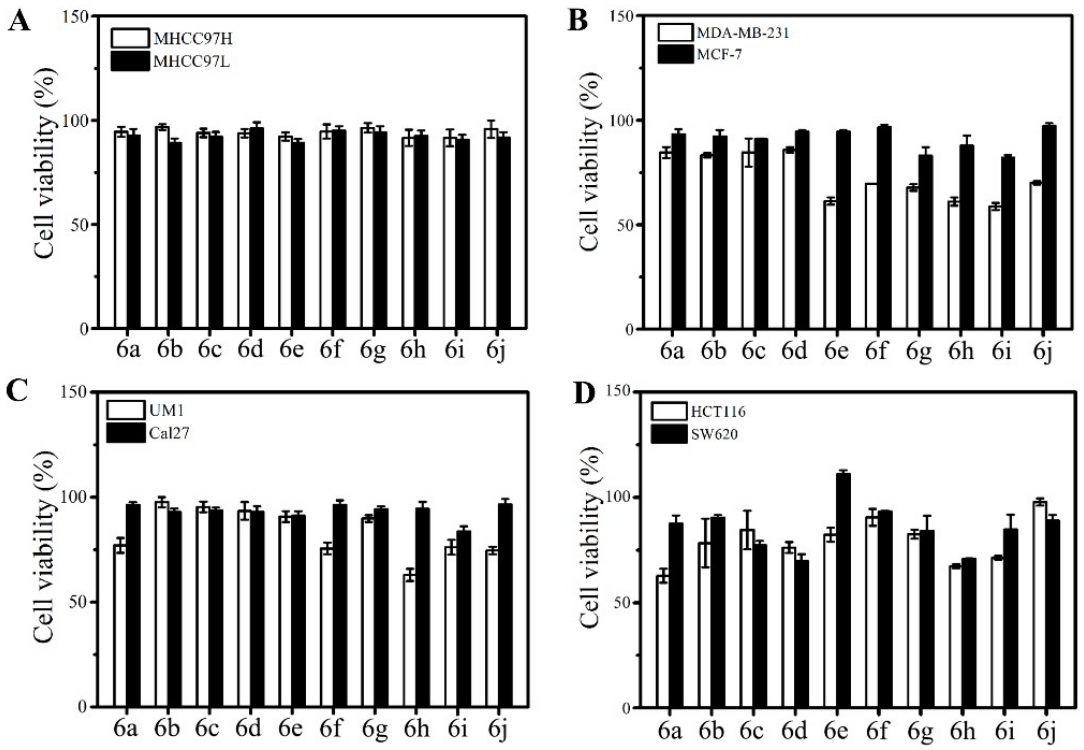
Materials and methods

Cell lines: The human cancer cell lines were purchased from Cobioer (Nanjing, China). Human pancreatic cancer cell lines AsPC-1, PANC-1, SW1990, HPDE and HPAC were culture in Roswell Park Memorial Institute (RPMI)-1640 medium. The MHCC97H, MHCC97L, MDA-MB-231, SW620 and Cal27 were culture in Dulbecco's modified Eagle's medium (DMEM). The MCF-7 was culture in Minimum Eagle's Medium (MEM). The MCF-7 and HCT116 were culture in MEM and MCCoy's 5A medium, respectively. All cell medium containing 10% fetal bovine serum and 1% PenStrep (100 U/ml Penicilium and 100 μg/ml Streptomycin). Human cancer cell lines was cultured at 37 °C in a humidified incubator supplied with 5% CO_2.

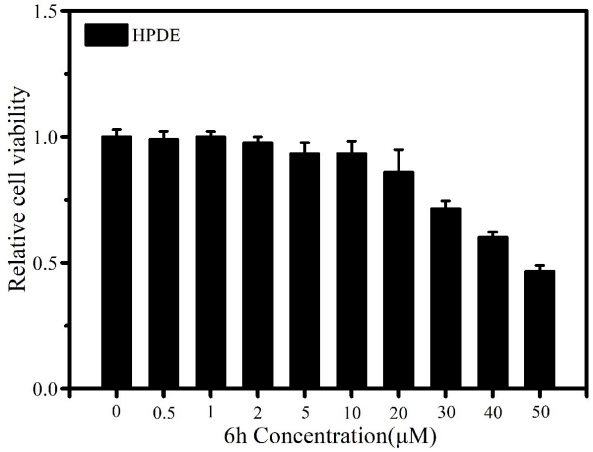
Reagents, antibodies, and standard assays: The compound **6h** was synthesized in our laboratory. All antibody were purchased from Cell Signaling (Beverly, MA). They include p-ERK, ERK, p-AKT, AKT, PARP and β-actin. Gemcitabine, erlotinib and cisplatin were purchased from Selleck (Shanghai, China). Cell viability was determined by MTT assay. For cell growth assays, 0.5% crystal violet was dissolved in 1% SDS after staining, and then measured at 570 nm (OD570) with a plate reader.

Statistical analysis: Statistical analyses were performed by unpaired Student's t test for two group comparisons and one-way analysis of variance (ANOVA) for multi-group comparisons at a significance level of $p< 0.05$. Data were presented as means ± SD from three or more independent experiments.

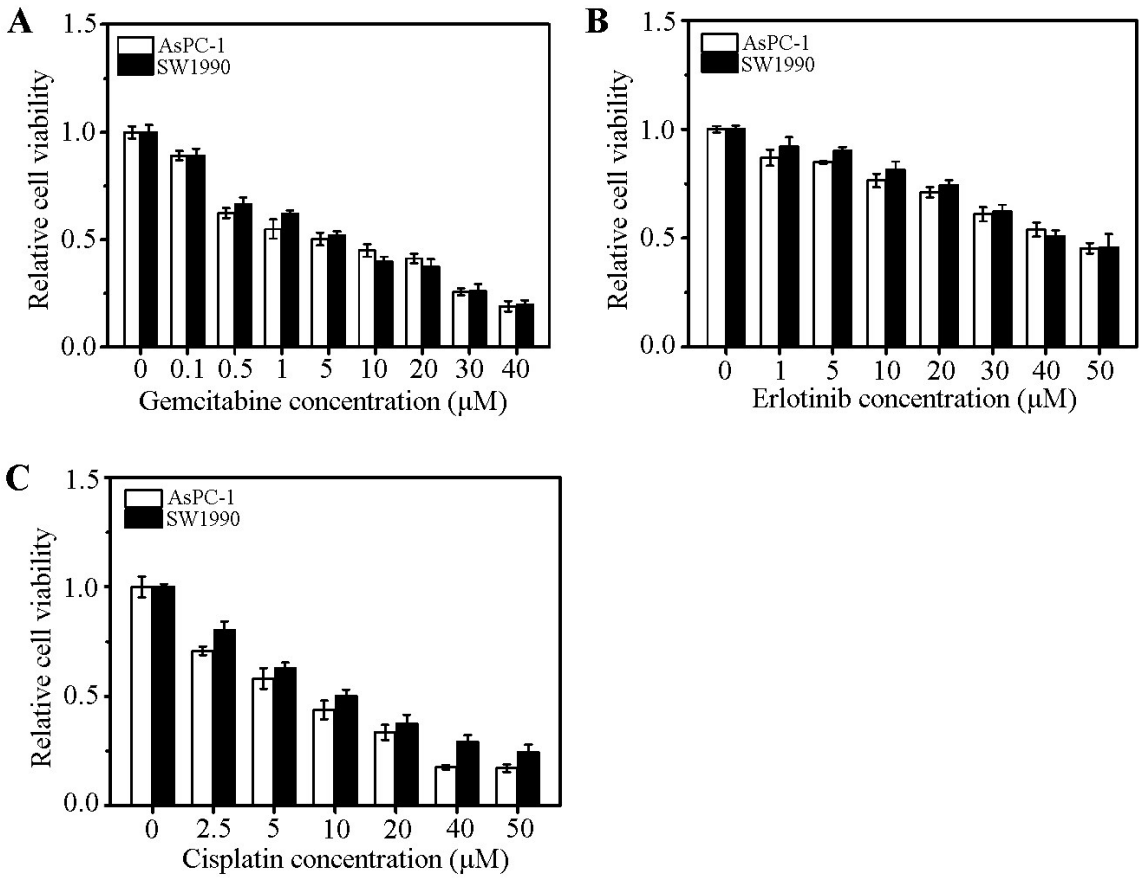
Supplementary Figures and Figure Legends



Supplementary Figure S1. The effect of compound 6 on cell viability in different cancer cell lines. The hepatoma carcinoma cell, breast cancer, head and neck squamous cell carcinoma and colon cancer cells were treated with 20 μM compound **6**, respectively, cell viability was determined by MTT assays (A-D).

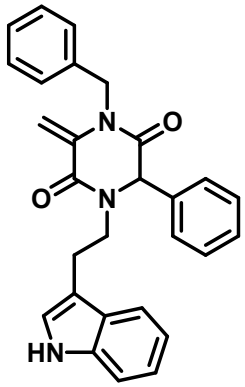


Supplementary Figure S2. The effect of compound 6 on cell viability in normal pancreatic ductal epithelial cell line. HPDE cell was treated with a range concentration 6h for 48 hours.



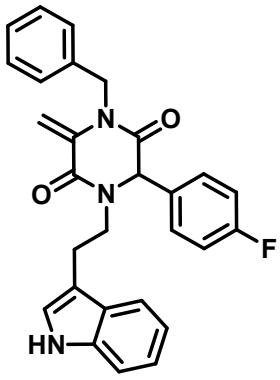
Supplementary Figure S3. The effect of pancreatic cancer clinical drugs on cell viability in pancreatic cancer cell line. AsPC-1 and SW1990 cells were treated with different concentration gemcitabine, erlotinib and cisplatin for 48 h, respectively (A-C).

1-(2-(1H-indol-3-yl)ethyl)-4-benzyl-3-methylene-6-phenylpiperazine-2,5-dione,

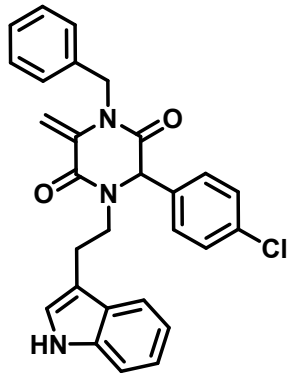


Compound **6a**, white solid, yield 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, NH), 7.55 (d, *J* = 7.9 Hz, 1H, ArH), 7.36 – 7.33 (m, 4H, ArH), 7.27 (s, 1H, ArH), 7.25 (d, *J* = 2.0 Hz, 1H, ArH), 7.21–7.17 (m, 4H, ArH), 7.12 – 7.08 (m, 1H, ArH), 6.98 (s, 1H, ArH), 6.94–6.92 (m, 2H, ArH), 5.90 (s, 1H, CH), 5.17 (d, *J* = 15.8 Hz, 1H, CH_2=), 4.94 (s, 2H, CH_2Ph), 4.61–4.57 (m, 1H, CH_2=), 4.29–4.23 (m, 1H, CH_2CH_2), 3.19–2.97 (m, 3H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 164.0, 159.0, 136.3, 135.4, 129.2 (2C), 129.1, 128.7 (3C), 127.4, 127.2 (2C), 126.8, 126.4, 122.3 (2C), 122.0, 119.6, 118.6, 112.5, 111.4 (2C), 104.6, 65.5, 47.0, 46.8, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{26}N_3O_2^+$ $(M+H)^+$ 436.20250, found 436.20212.

1-(2-(1H-indol-3-yl)ethyl)-4-benzyl-6-(4-fluorophenyl)-3-methylenepiperazine-2,5-dione, Compound **6b**, white solid, yield 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H, NH), 7.55 (d, *J* = 7.9 Hz, 1H, ArH), 7.35 (d, *J* = 8.2 Hz, 1H, ArH), 7.25 (s, 1H, ArH), 7.23–7.18 (m, 5H, ArH), 7.12–7.09 (m, 1H, ArH), 7.06–7.02 (m, 2H, ArH), 6.99 (s, 1H, ArH), 6.94 – 6.92 (m, 2H, ArH), 5.91 (s, 1H, CH), 5.18 (d, *J* = 16.2 Hz, 1H, CH_2=), 4.95 (s, 1H, CH_2Ph), 4.88 (s, 1H, CH_2Ph), 4.61 (d, *J* = 16.5 Hz, 1H, CH_2=), 4.29 – 4.22 (m, 1H, CH_2CH_2), 3.18 – 2.97 (m, 3H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 163.8, 158.8, 136.4, 136.1, 135.3, 132.1, 128.8 (2C), 128.6, 128.5, 127.5, 127.1, 126.4, 122.3 (2C), 122.0, 119.6, 118.5, 116.3, 116.1 (2C), 112.4, 111.4, 104.9, 64.8, 47.0, 46.7, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{25}FN_3O_2^+$ $(M+H)^+$ 454.19308, found 454.19339.

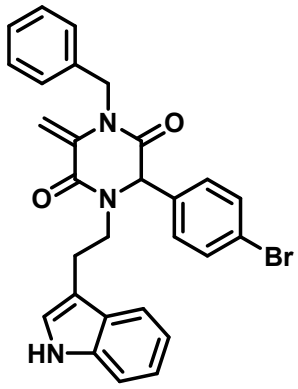


1-(2-(1H-indol-3-yl)ethyl)-4-benzyl-6-(4-chlorophenyl)-3-methylenepiperazine-2,5-dione, Compound **6c**, white solid, yield 64%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H, NH), 7.55 (d, *J* = 7.9 Hz, 1H, ArH), 7.35–7.31(m, 4H, ArH), 7.25–7.21 (m, 4H,

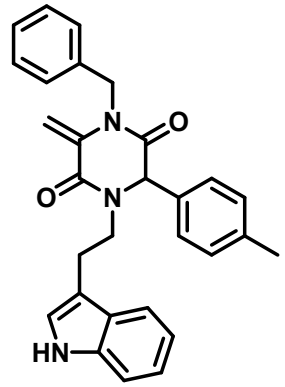


ArH), 7.16 (d, *J* = 6.5 Hz, 1H, ArH), 7.12 – 7.09 (m, 1H, ArH), 6.99 (s, 1H, ArH), 6.94–6.92 (m, 2H, ArH), 5.90 (s, 1H, CH), 5.14 (d, *J* = 15.7 Hz, 1H, CH_2=), 4.95 (s, 1H, CH_2Ph), 4.85 (s, 1H, CH_2Ph), 4.61 (d, *J* = 15.7 Hz, 1H, CH_2=), 4.30 – 4.23 (m, 1H, CH_2CH_2), 3.17 – 3.12 (m, 1H, CH_2CH_2), 3.08–2.98 (m, 2H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 163.5, 158.8, 136.4, 136.1, 135.2, 134.8, 129.4 (2C), 128.8, 128.1 (2C), 127.5 (2C), 127.1 (2C), 126.4, 122.4, 122.0, 119.7, 118.5 (2C), 112.4, 111.4, 105.0, 64.9, 47.1, 46.8, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{25}ClN_3O_2^+$ $(M+H)^+$ 470.16353, found 470.16422.

1-(2-(1H-indol-3-yl)ethyl)-4-benzyl-6-(4-bromophenyl)-3-methylenepiperazine-2,5-dione, Compound **6d**, white solid, yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H, NH), 7.48 – 7.46 (m, 3H, ArH), 7.36 (d, *J* = 8.1 Hz, 1H, ArH), 7.26–7.19 (m, 5H, ArH), 7.13–7.09 (m, 3H, ArH), 6.99–6.94 (m, 3H, ArH), 5.90 (s, 1H, CH), 5.15 (s, 1H, CH_2=), 4.95 (m, 1H, CH_2Ph), 4.83 (s, 1H, CH_2Ph), 4.61 – 4.57 (m, 1H, CH_2=), 4.29 – 4.25 (m, 1H, CH_2CH_2), 3.17–3.12 (m, 1H, CH_2CH_2), 3.08 – 2.99 (m, 2H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 163.4, 158.8, 136.3, 136.0, 135.4, 135.2, 132.3, 128.8 (2C), 128.4 (2C), 127.5 (2C), 127.1, 126.4, 123.3, 122.4, 122.0, 119.7 (2C), 118.5, 112.4, 111.4, 105.0, 65.0, 47.1, 46.8, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{25}BrN_3O_2^+$ $(M+H)^+$ 514.11301, found 514.11298.

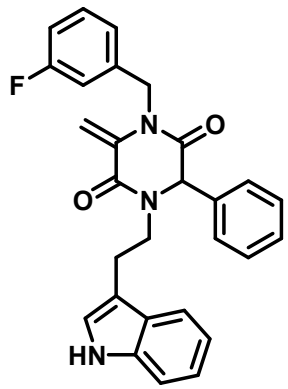


1-(2-(1H-indol-3-yl)ethyl)-4-benzyl-3-methylene-6-(p-tolyl)piperazine-2,5-dione, Compound **6e**, white solid, yield 88%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H, NH), 7.55 (d, *J* = 7.9 Hz, 1H, ArH), 7.34 (d, *J* = 8.1 Hz, 1H, ArH), 7.25 (s, 1H, ArH), 7.20 – 7.08 (m, 7H, ArH), 6.98 (s, 1H, ArH), 6.91–6.81 (m, 1H, ArH), 6.75 (d, *J* = 8.0 Hz, 1H, ArH), 6.58 (d, *J* = 8.0 Hz, 1H, ArH), 5.90 (s, 1H, CH), 5.10 (d, *J* = 16.0 Hz, 1H, CH_2=), 4.87 (s, 2H, CH_2Ph), 4.60 (d, *J* = 16.0 Hz, 1H, CH_2=), 4.28–4.22 (m, 1H,



CH_2CH_2), 3.20 – 3.06 (m, 2H, CH_2CH_2), 3.03–2.97 (m, 1H, CH_2CH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) 164.2, 158.8, 139.2, 138.0, 136.4, 136.2, 133.1, 130.3 (2C), 129.9, 127.2, 126.6, 122.3, 122.1, 122.0, 119.6, 118.5, 114.5, 114.3, 113.5, 112.5, 111.4 (2C), 104.4, 65.2, 46.8, 46.4, 23.3, 21.1. HRMS (ESI) m/z calcd for $C_{29}H_{28}N_3O_2^+$ $(M+H)^+$ 450.21815, found 450.21674.

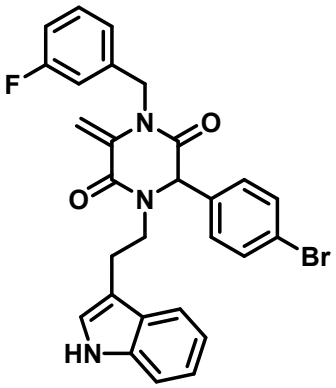
1-(2-(1H-indol-3-yl)ethyl)-4-(3-fluorobenzyl)-3-methylene-6-phenylpiperazine-2,5-dione, Compound **6f**, white solid, yield 90%. ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H, NH), 7.56 (d, *J* = 7.9 Hz, 1H, ArH), 7.41 – 7.36 (m, 4H, ArH), 7.26–7.22 (m, 3H, ArH), 7.21–7.09 (m, 3H, ArH), 7.01 (s, 1H, ArH), 6.92 – 6.88 (m, 1H, ArH), 6.73 (d, *J* = 8.0 Hz, 1H, ArH), 6.58 (d, *J* = 11.9 Hz, 1H, ArH), 5.91 (s, 1H, CH), 5.11 (d, *J* = 16.0 Hz, 1H, CH_2=), 4.89 (d, *J* = 9.2 Hz, 2H, CH_2Ph), 4.61 (d, *J* = 16.0 Hz, 1H, CH_2=), 4.31–4.25 (m, 1H, CH_2CH_2), 3.21 – 2.98 (m, 3H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 164.0, 158.8, 138.0, 137.9, 136.3, 136.1, 130.4, 130.3, 129.3, 129.2, 127.2, 126.6, 122.3, 122.1, 122.0, 119.6, 118.5, 114.6, 114.3, 113.4, 113.2, 112.5, 111.4, 104.5, 65.5, 46.8, 46.4, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{25}FN_3O_2^+$ $(M+H)^+$ 454.19308, found 454.19302.



1-(2-(1H-indol-3-yl)ethyl)-4-(3-fluorobenzyl)-6-(4-fluorophenyl)-3-methylenepiperazine-2,5-dione, Compound **6g**, white solid, yield 68%. ^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H, NH), 7.55 (d, *J* = 7.8 Hz, 1H, ArH), 7.36 (d, *J* = 8.1 Hz, 1H, ArH), 7.25–7.16 (m, 4H, ArH), 7.13–7.09 (m, 1H, ArH), 7.06–6.99 (m, 3H, ArH), 6.92 – 6.89 (m, 1H, ArH), 6.73 (d, *J* = 8.0 Hz, 1H, ArH), 6.60 – 6.57 (m, 1H, ArH), 5.91 (s, 1H, CH), 5.08 (d, *J* = 15.9 Hz, 1H, CH_2=), 4.87 (d, *J* =

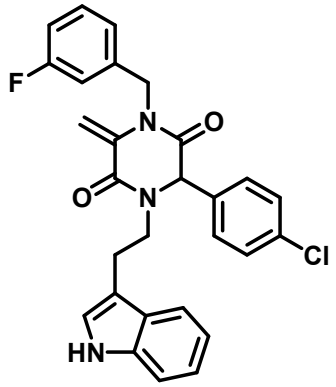
21.3 Hz, 2H, CH_2Ph), 4.60 (d, J = 16.1 Hz, 1H, CH_2=), 4.29–4.25 (m, 1H, CH_2CH_2), 3.19–3.14 (m, 1H, CH_2CH_2), 3.10–3.00 (m, 2H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 163.8, 158.7, 137.8 (2C), 136.4, 136.0, 131.9 (2C), 130.4, 128.5, 127.1, 122.4, 122.1, 119.7, 118.4, 116.4, 116.2, 114.7, 114.4, 113.4, 113.2, 112.3, 111.5, 104.8, 64.8, 46.7, 46.5, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{24}F_2N_3O_2^+$ $(M+H)^+$ 472.18366, found 472.18335.

1-(2-(1H-indol-3-yl)ethyl)-6-(4-bromophenyl)-4-(3-fluorobenzyl)-3-methylenepiperazine-2,5-dione, Compound **6h**, white solid, yield 75%. ^1H NMR (400



MHz, $CDCl_3$) δ 8.08 (s, 1H, NH), 7.54 (d, J = 7.8 Hz, 1H, ArH), 7.49 (t, J = 7.5 Hz, 2H, ArH), 7.37 (d, J = 8.1 Hz, 1H, ArH), 7.23 – 7.17 (m, 2H, ArH), 7.13–7.08 (m, 3H, ArH), 7.00 (s, 1H, ArH), 6.94–6.90 (m, 1H, ArH), 6.73 (d, J = 7.6 Hz, 1H, ArH), 6.60 (d, J = 9.4 Hz, 1H, ArH), 5.91 (s, 1H, CH), 5.04 (d, J = 16.0 Hz, 1H, CH_2=), 4.90 (s, 1H, CH_2Ph), 4.80 (s, 1H, CH_2Ph), 4.64 (d, J = 16.0 Hz, 1H, CH_2=), 4.32–4.25(m, 1H, CH_2CH_2), 3.22 – 3.14 (m, 1H, CH_2CH_2), 3.11 – 2.99 (m, 2H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 163.5, 158.7, 137.8 (2C), 136.4, 135.9, 135.1, 132.4 (2C), 130.5 (2C), 128.3, 127.1, 123.4, 122.4 (2C), 122.0, 119.7, 118.5, 114.7, 113.3, 112.4, 111.5, 104.9, 64.9, 46.8, 46.5, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{24}BrFN_3O_2^+$ $(M+H)^+$ 532.10359, found 532.10333.

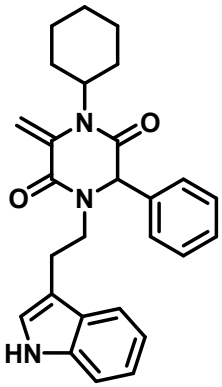
1-(2-(1H-indol-3-yl)ethyl)-6-(4-chlorophenyl)-4-(3-fluorobenzyl)-3-methylenepiperazine-2,5-dione, Compound **6i**, white solid, yield 62%. ^1H NMR (400



MHz, $CDCl_3$) δ 8.15 (s, 1H, NH), 7.54 (d, J = 7.9 Hz, 1H, ArH), 7.37 – 7.31 (m, 3H, ArH), 7.25 – 7.19 (m, 2H, ArH), 7.16–7.09 (m, 3H, ArH), 6.99 (s, 1H, ArH), 6.93–6.89 (m, 1H, ArH), 6.73 (d, J = 7.9 Hz, 1H, ArH), 6.60 (t, J = 7.8 Hz, 1H, ArH), 5.92 (s, 1H, CH), 5.05 (d, J = 16.0 Hz, 1H, CH_2=), 4.90 (s, 1H, CH_2Ph), 4.82 (s, 1H, CH_2Ph), 4.62 (d, J = 16.0 Hz, 1H, CH_2=), 4.31 – 4.24 (m,

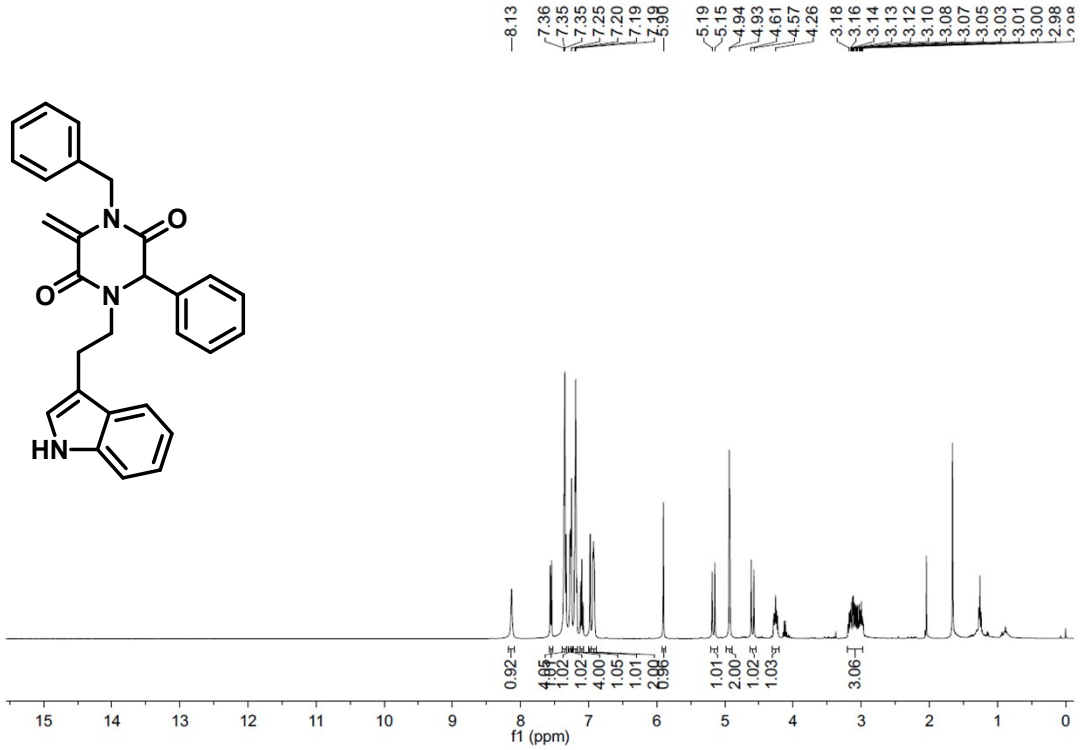
1H, CH_2CH_2), 3.22 – 3.14 (m, 1H, CH_2CH_2), 3.09 – 2.98 (m, 2H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) 163.6, 158.7, 137.8, 136.4, 135.9, 135.3, 134.6, 130.4 (2C), 129.4, 128.0, 127.1 (2C), 122.4, 122.0, 119.7, 118.4, 114.7, 114.5, 113.5, 113.3, 112.3, 111.5, 104.9, 64.9, 46.8, 46.5, 23.3. HRMS (ESI) m/z calcd for $C_{28}H_{24}ClFN_3O_2^+$ $(M+H)^+$ 488.15411, found 488.15320.

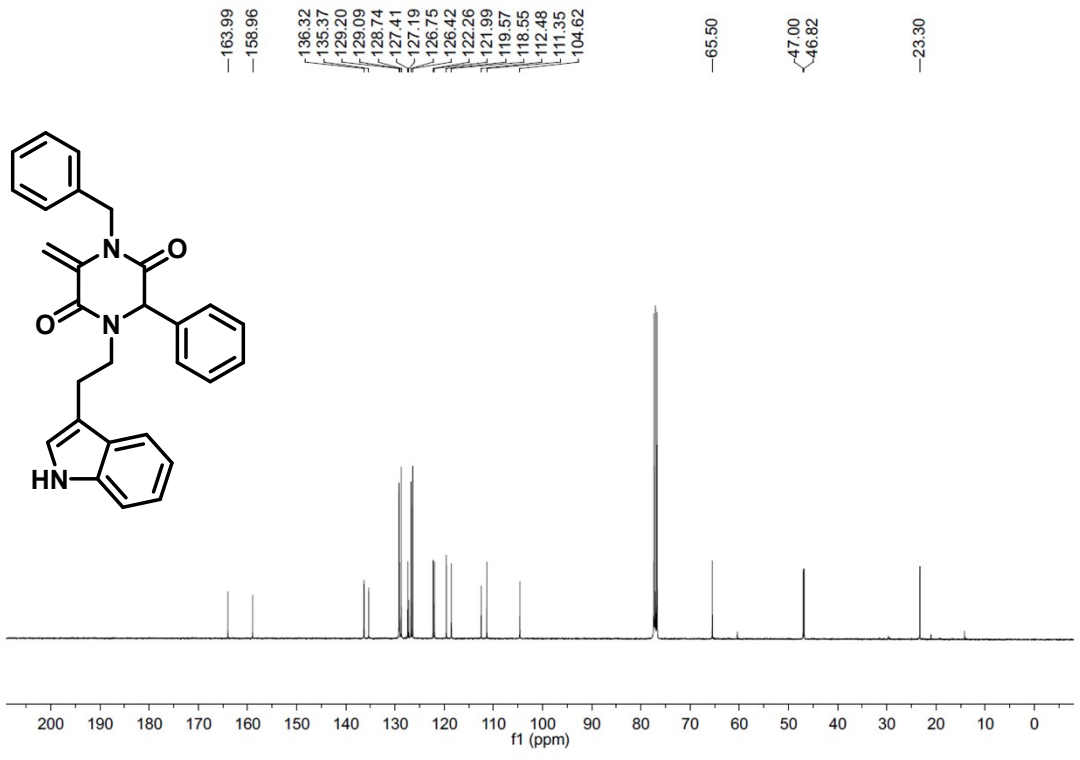
1-(2-(1H-indol-3-yl)ethyl)-4-cyclohexyl-3-methylene-6-phenylpiperazine-2,5-dione,

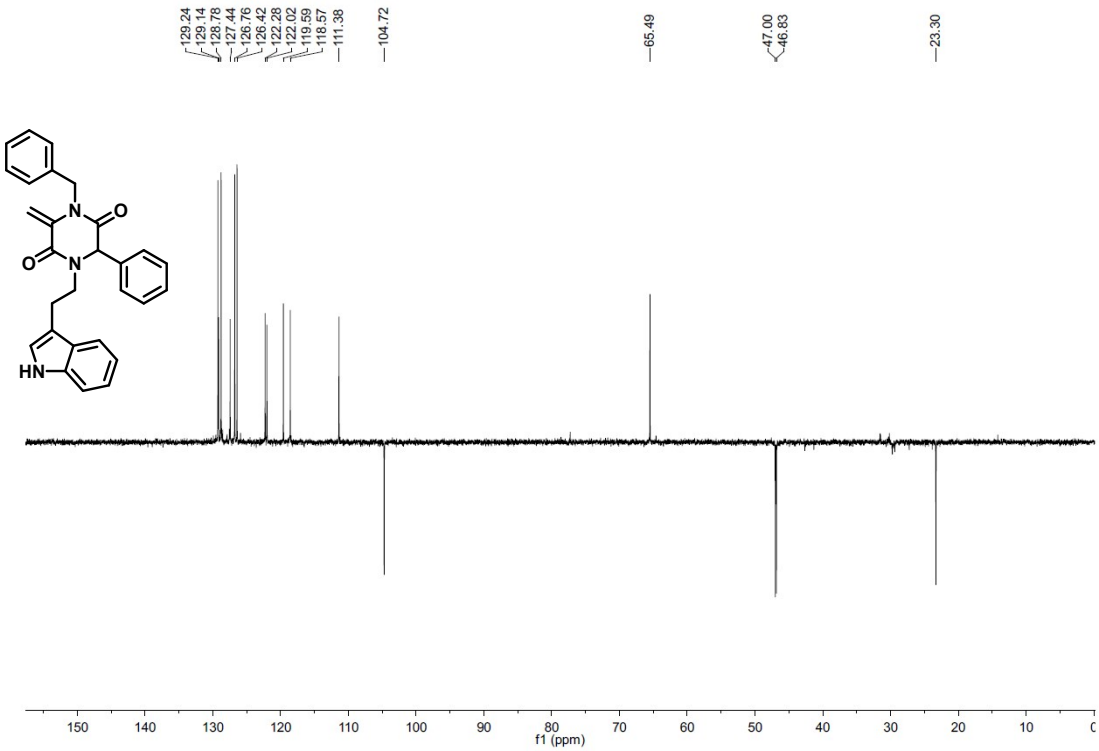


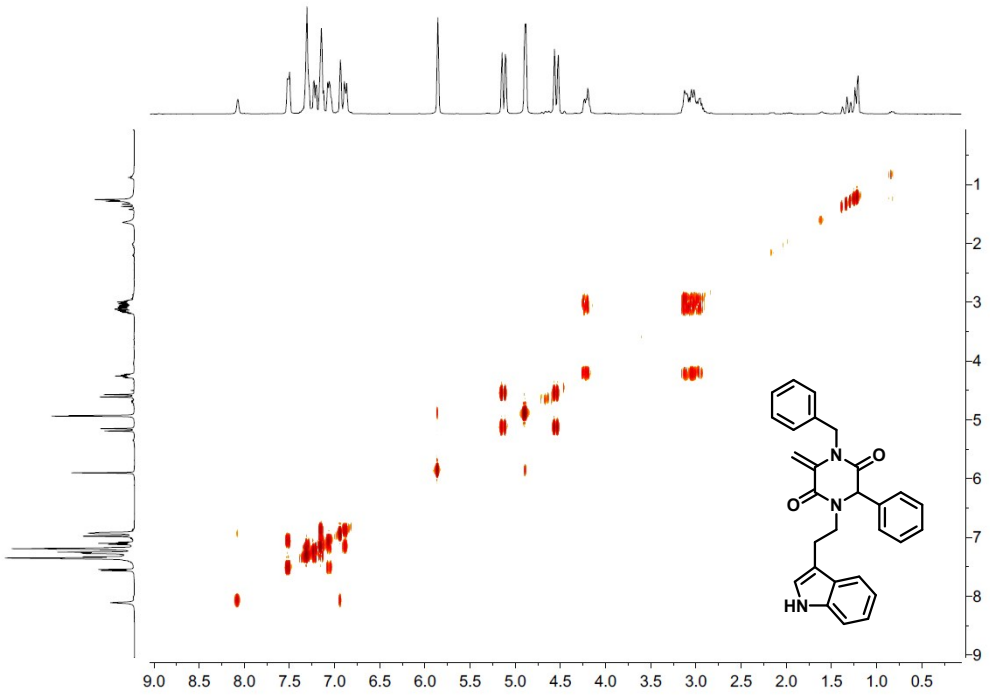
Compound **6j**, white solid, yield 63%.^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H, NH), 7.48 (d, *J* = 7.9 Hz, 1H, ArH), 7.37 (d, *J* = 3.7 Hz, 5H, ArH), 7.26 (s, 2H, ArH), 7.21 (t, *J* = 7.5 Hz, 1H, ArH), 7.12 (t, *J* = 7.5 Hz, 1H, ArH), 5.96 (d, *J* = 8.3 Hz, 1H, CH), 5.91 (d, *J* = 1.8 Hz, 1H, CH_2=), 5.46 (d, *J* = 1.8 Hz, 1H, CH_2=), 3.80 – 3.72 (m, 1H, CH_2CH_2), 3.64– 3.55 (m, 1H, C_6H_{11}), 3.22 – 3.11 (m, 2H, CH_2CH_2), 3.01 – 2.93 (m, 1H, CH_2CH_2), 1.52 – 1.41 (m, 4H, C_6H_{11}), 1. 81 – 1.11 (m, 2H, C_6H_{11}), 0.94 – 0.83 (m, 1H, C_6H_{11}), 0.77 – 0.67 (m, 1H, C_6H_{11}), 0.49 – 0.39 (m, 1H, C_6H_{11}), 0.29 – 0.20 (m, 1H, C_6H_{11}). ^{13}C NMR (100 MHz, $CDCl_3$) 168.2, 165.3, 150.4, 136.5, 134.8, 128.9 (2C), 127.9 (2C), 127.2 (2C), 122.5, 121.9, 119.7, 118.7, 112.3, 111.3 (2C), 74.3, 48.3, 41.3, 32.5, 31.8, 29.7, 25.0, 24.8, 23.9. HRMS (ESI) m/z calcd for $C_{27}H_{30}N_3O_2^+$ $(M+H)^+$ 428.23380, found 428.23352.

Compound **6a**

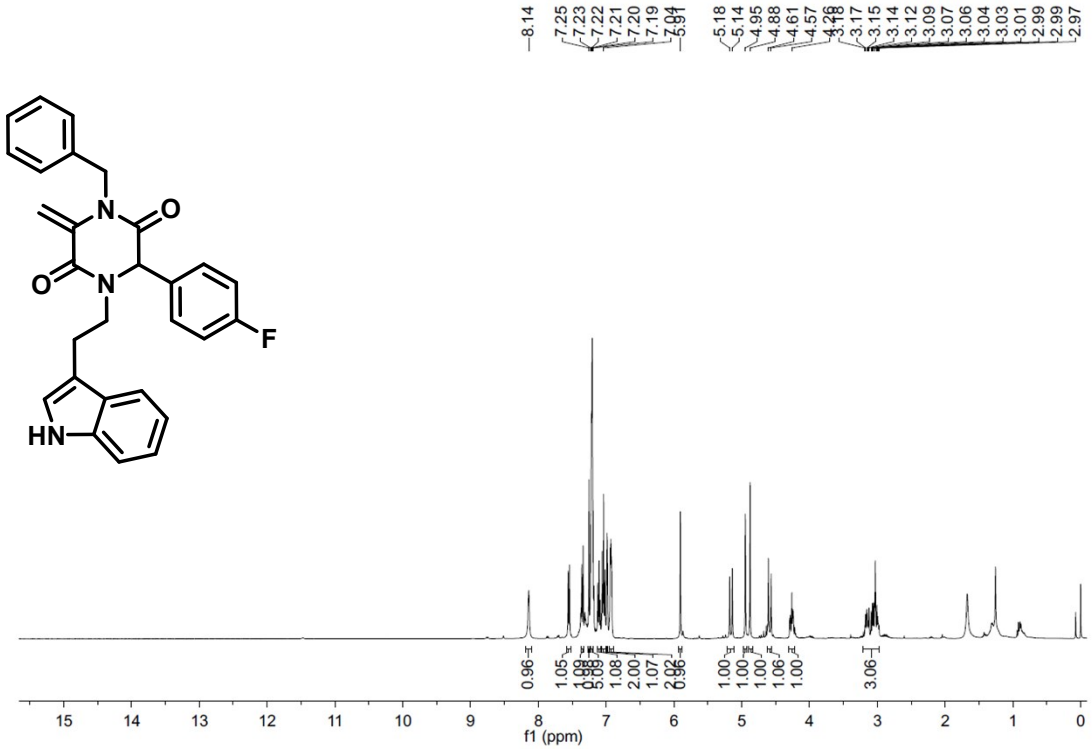

8.13
7.36
7.35
7.35
7.25
7.20
7.19
7.19
5.90
5.19
5.15
4.94
4.93
4.61
4.57
4.26
3.18
3.16
3.14
3.13
3.12
3.10
3.08
3.07
3.05
3.03
3.01
3.00
2.98
0.92
4.05
1.02
1.02
4.00
1.05
1.01
2.00
0.96
1.01
2.00
1.02
1.03
3.06
f1 (ppm)

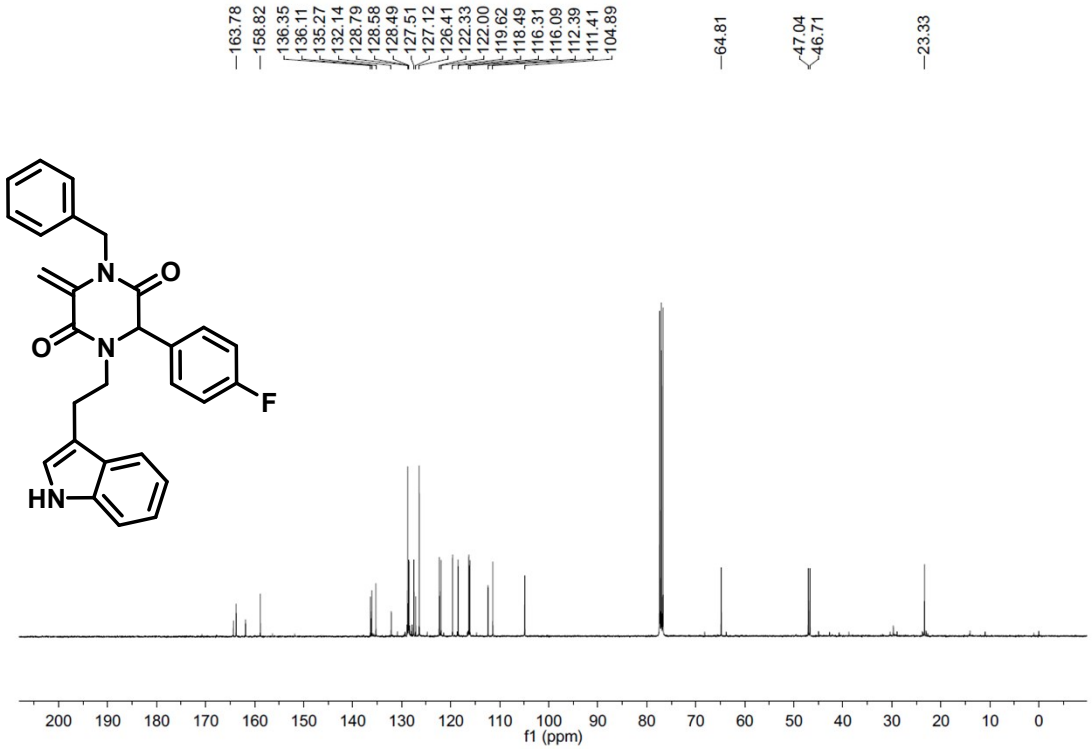

163.99
158.96
136.32
135.37
129.20
129.09
128.74
127.41
127.19
126.75
126.42
122.26
121.99
119.57
118.55
112.48
111.35
104.62
65.50
47.00
46.82
23.30
f1 (ppm)


129.24
129.14
128.78
127.44
126.76
126.42
122.28
122.02
119.59
118.57
111.38
104.72
65.49
47.00
46.83
23.30
HN
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

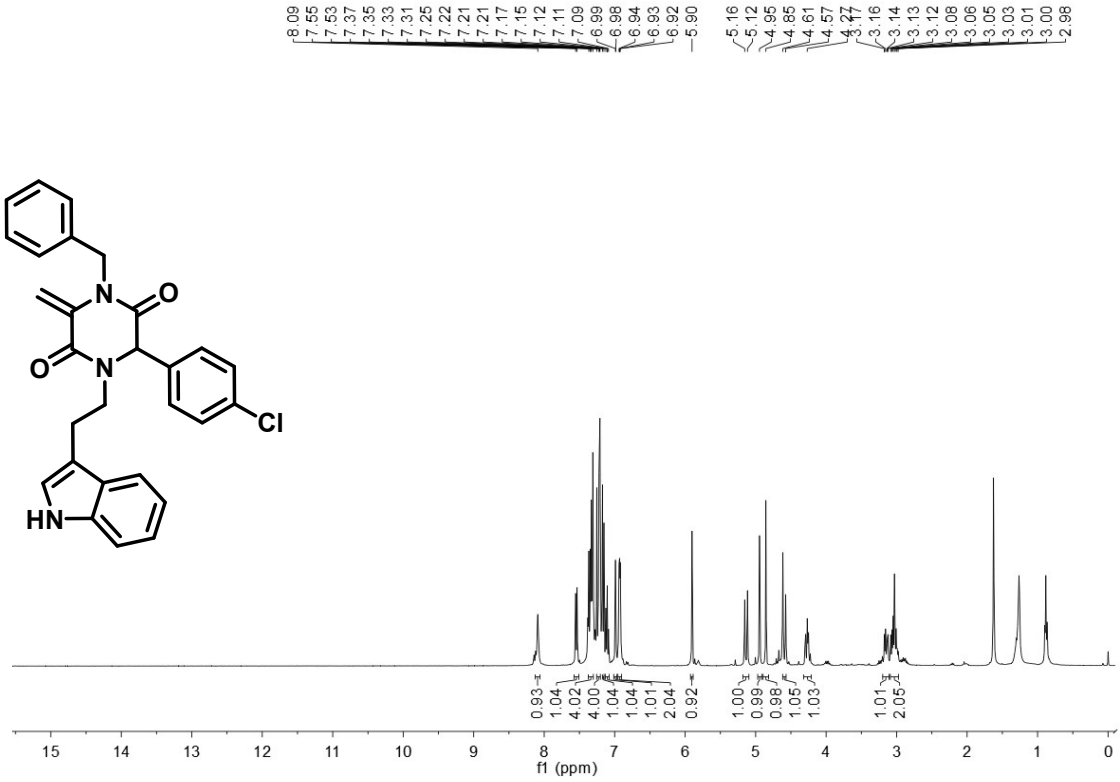

HN
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1 2 3 4 5 6 7 8 9

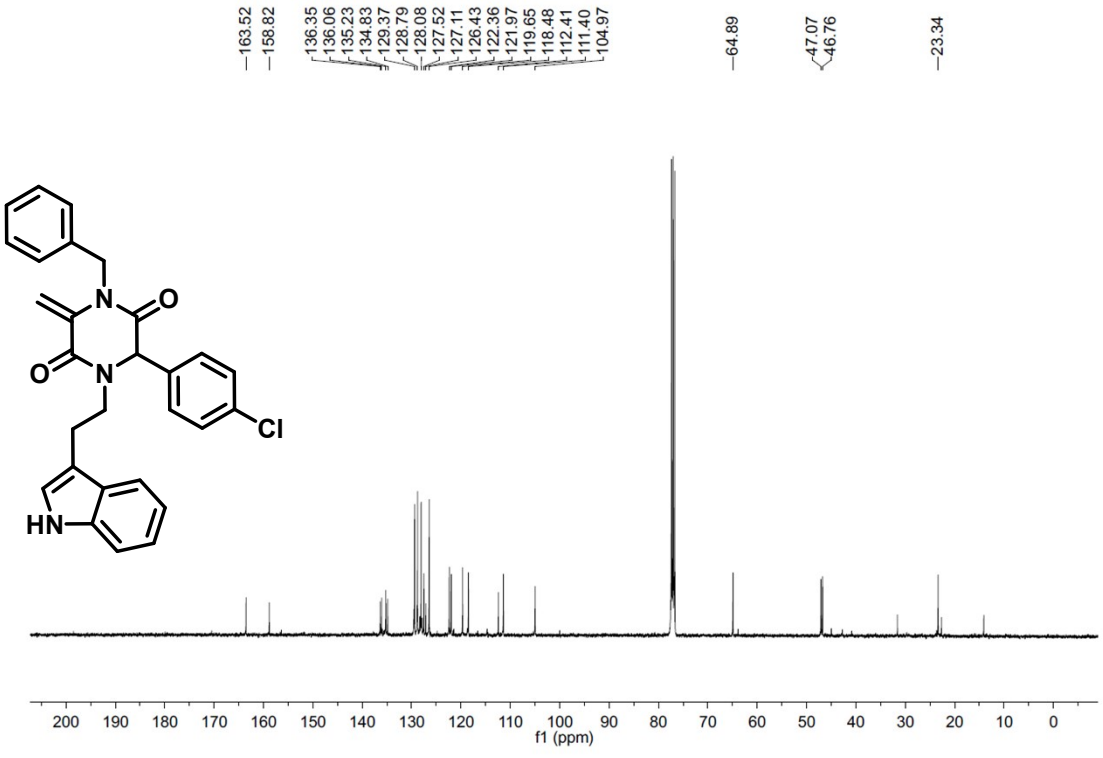
Compound **6b**


8.14
7.25
7.23
7.22
7.21
7.20
7.19
5.18
5.14
4.95
4.88
4.61
4.57
3.17
3.15
3.14
3.12
3.09
3.07
3.06
3.04
3.03
3.01
2.99
2.97
0.96
1.05
1.08
2.00
1.07
2.02
0.96
1.00
1.00
1.00
1.06
1.00
3.06
f1 (ppm)

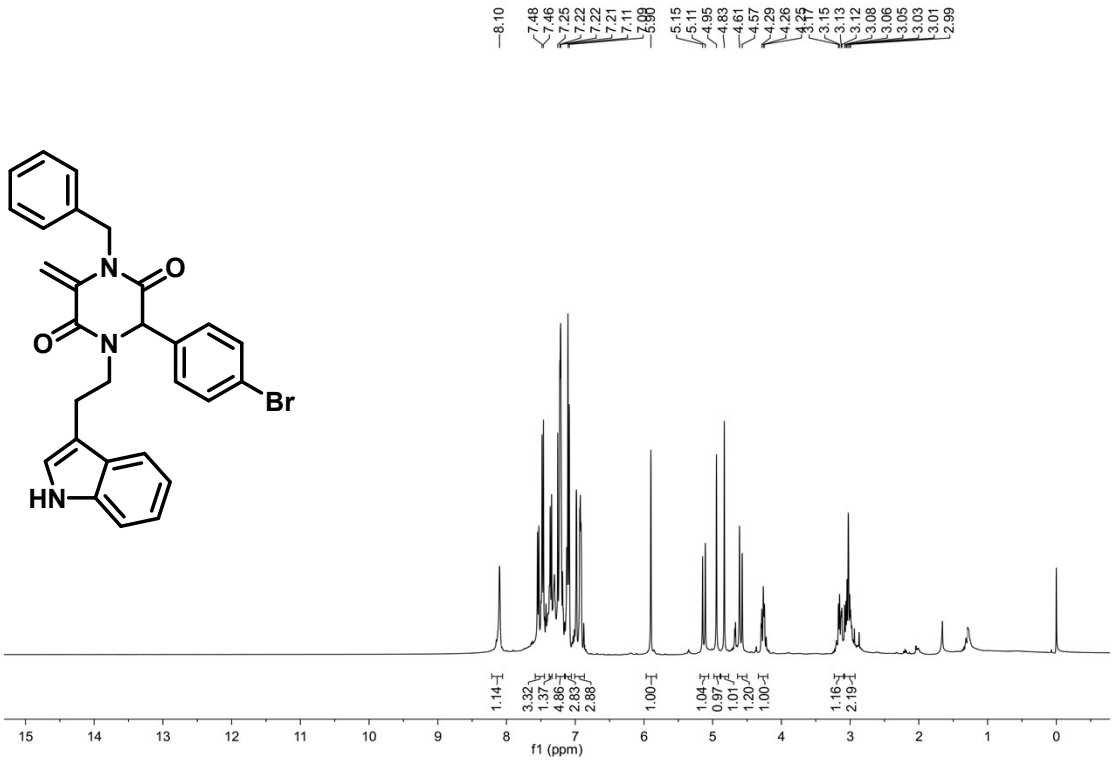

163.78
158.82
136.35
136.11
135.27
132.14
128.79
128.58
128.49
127.51
127.12
126.41
122.33
122.00
119.62
118.49
116.31
116.09
112.39
111.41
104.89
64.81
47.04
46.71
23.33
f1 (ppm)

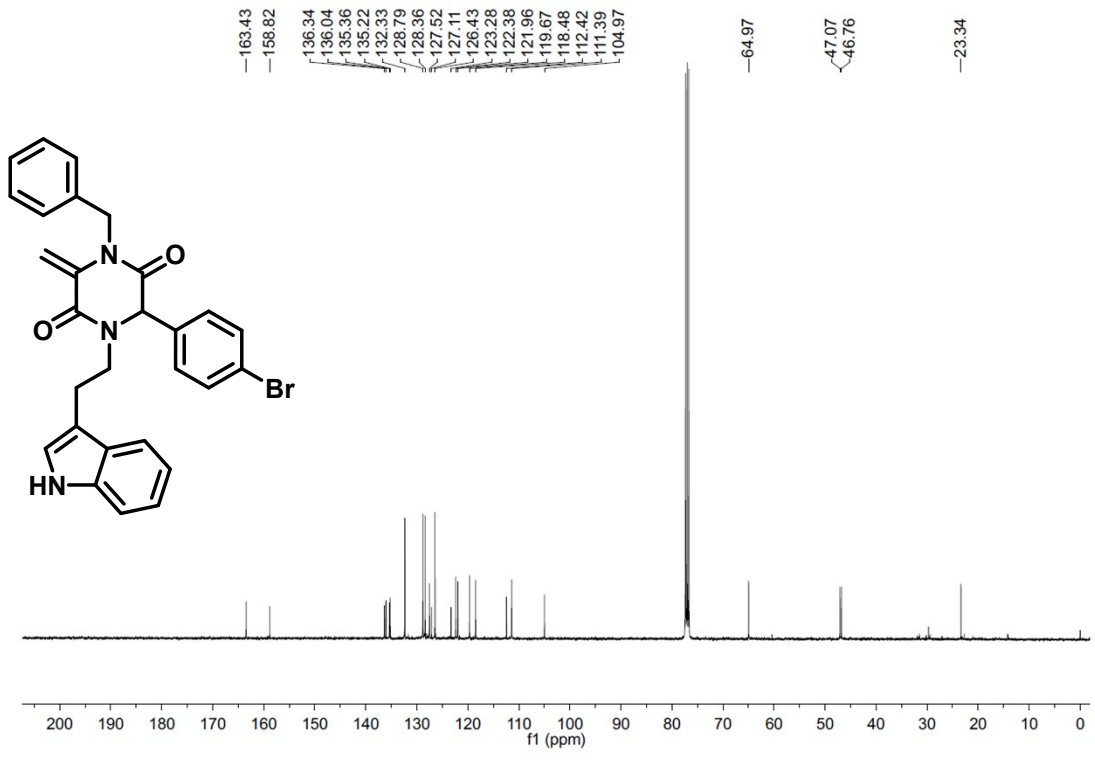
Compound **6c**


8.09
7.55
7.53
7.37
7.35
7.33
7.31
7.25
7.22
7.21
7.21
7.17
7.15
7.12
7.11
7.09
6.99
6.98
6.94
6.93
6.92
5.90
5.16
5.12
4.95
4.85
4.61
4.57
4.27
3.17
3.16
3.14
3.13
3.12
3.08
3.06
3.05
3.03
3.01
3.00
2.98
0.93
1.04
4.02
4.00
1.04
1.04
1.01
2.04
0.92
1.00
0.99
0.98
1.05
1.03
1.01
2.05
f1 (ppm)

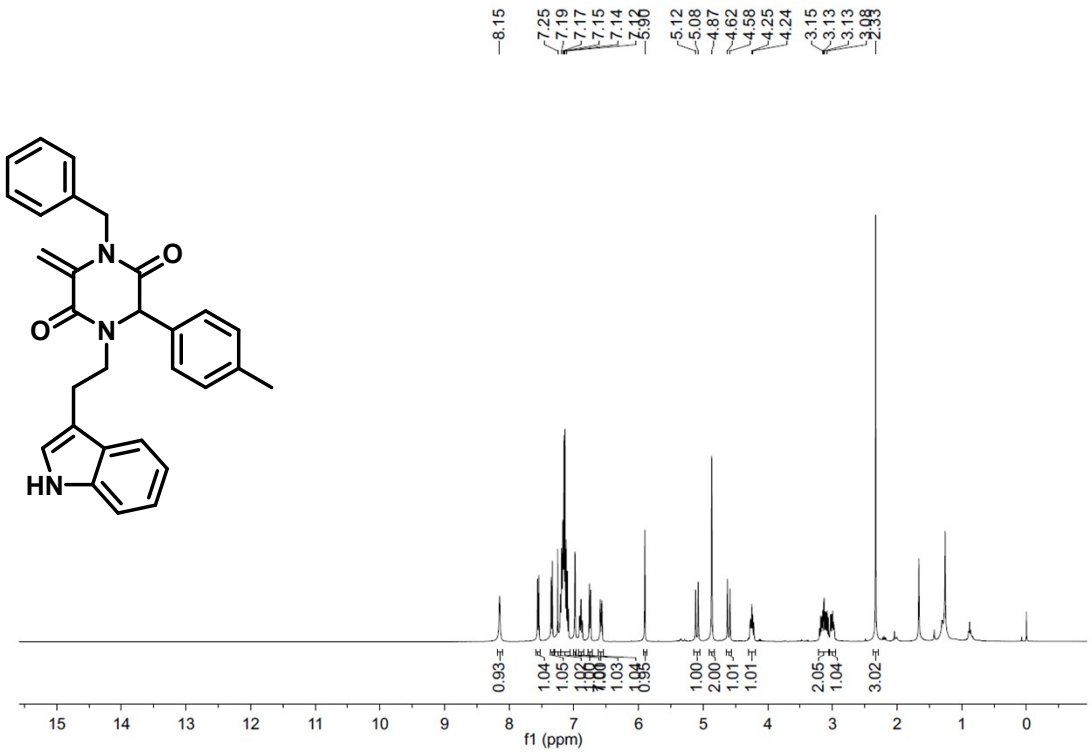

163.52
158.82
136.35
136.06
135.23
134.83
129.37
128.79
128.08
127.52
127.11
126.43
122.36
121.97
119.65
118.48
112.41
111.40
104.97
64.89
47.07
46.76
23.34
f1 (ppm)

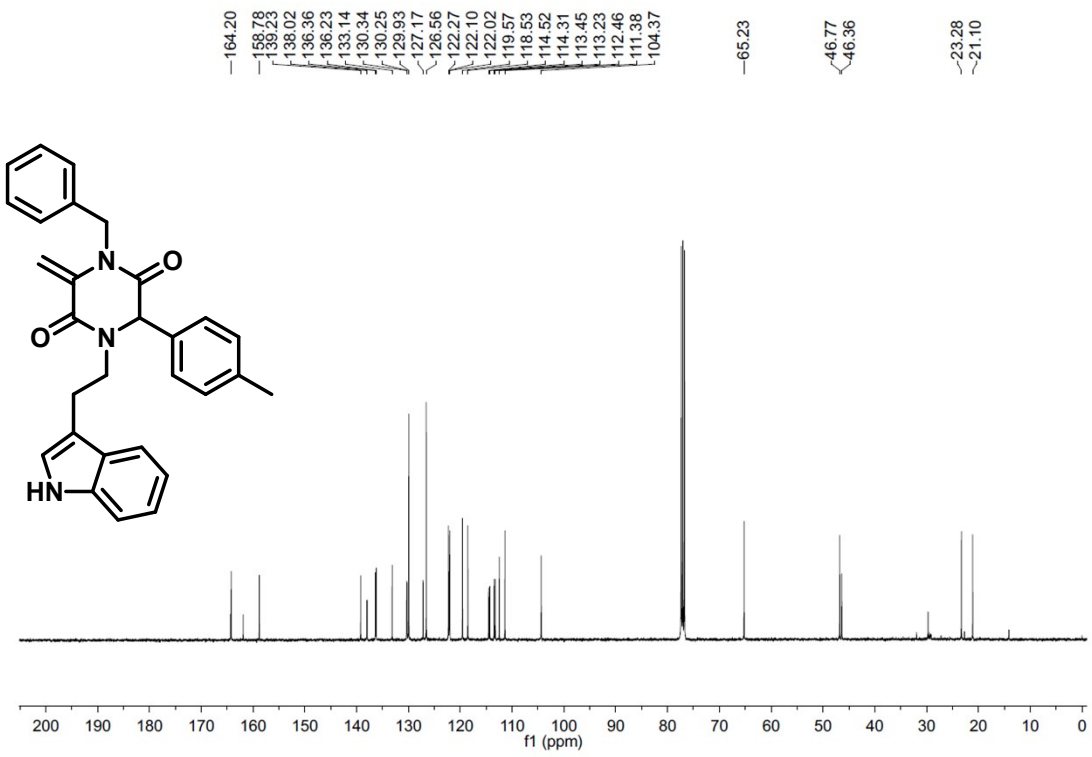
Compound **6d**

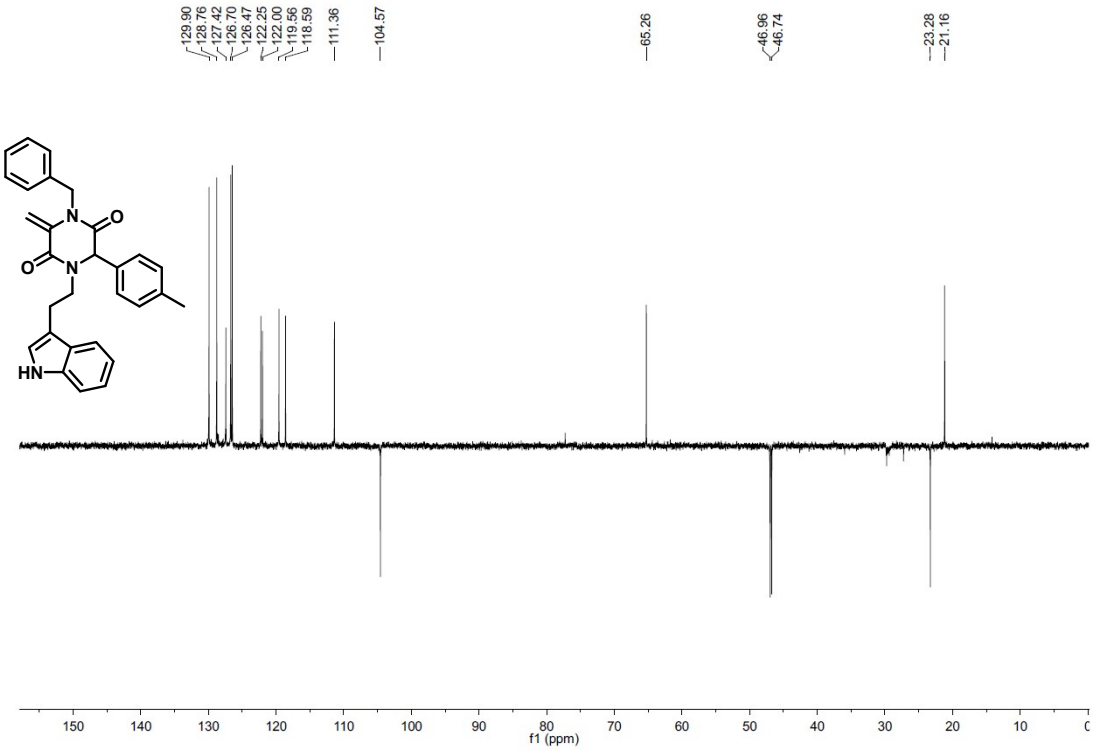

8.10
7.48
7.46
7.25
7.22
7.22
7.21
7.11
7.09
5.90
5.15
5.11
4.95
4.83
4.61
4.57
4.29
4.26
3.17
3.15
3.13
3.12
3.08
3.06
3.05
3.03
3.01
2.99
1.14
3.32
1.37
4.86
2.83
2.88
1.00
1.04
0.97
1.01
1.20
1.00
1.16
2.19
f1 (ppm)

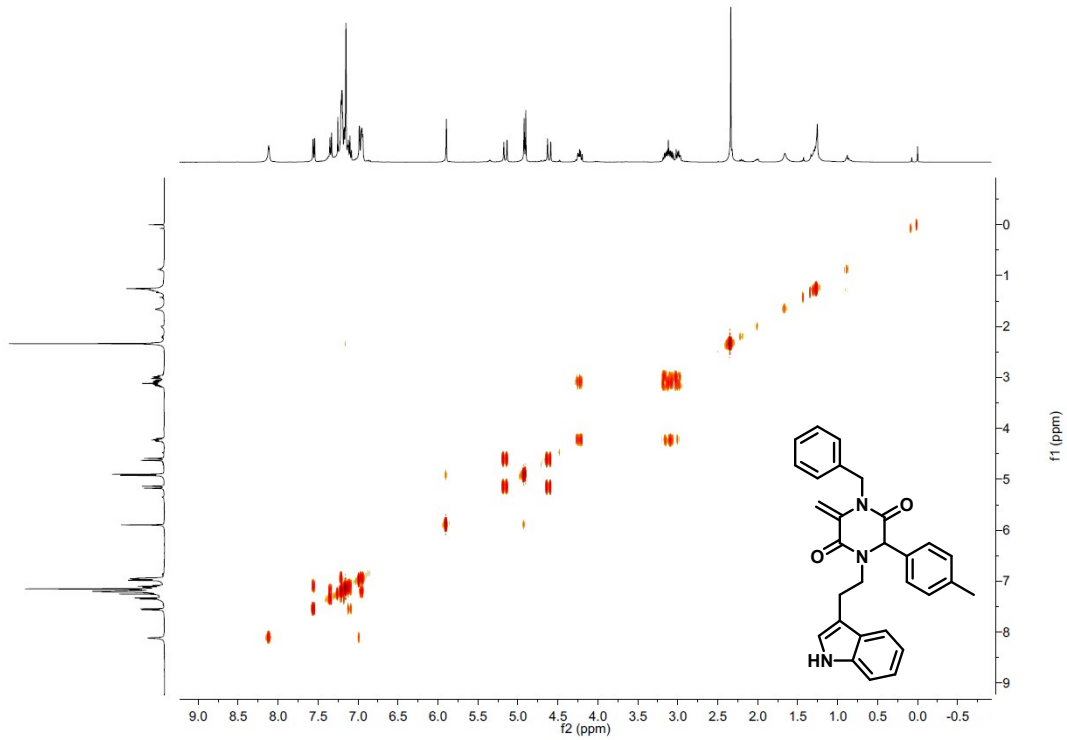

163.43
158.82
136.34
136.04
135.36
135.22
132.33
128.79
128.36
127.52
127.11
126.43
123.28
122.38
121.96
119.67
118.48
112.42
111.39
104.97
64.97
47.07
46.76
23.34
f1 (ppm)

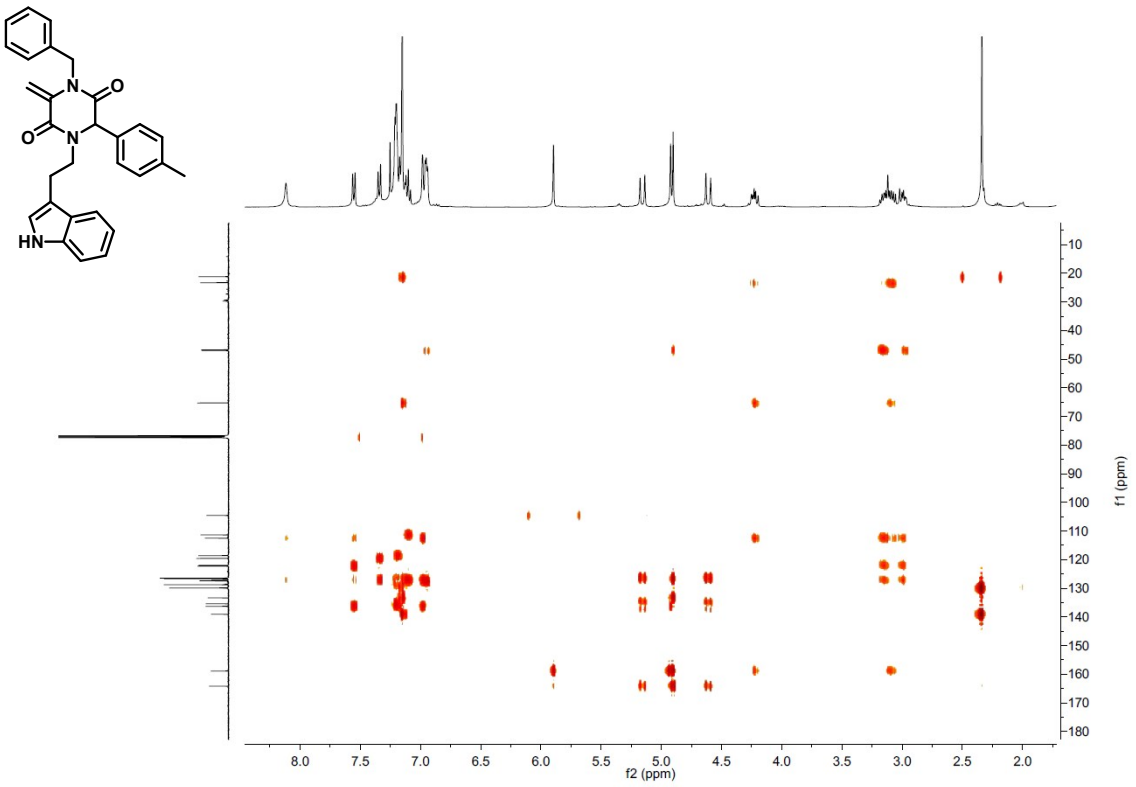
Compound **6e**


8.15
7.25
7.19
7.17
7.15
7.14
5.90
5.12
5.08
4.87
4.62
4.58
4.25
4.24
3.15
3.13
3.13
3.08
2.33
0.93
1.04
1.05
1.03
1.00
2.00
1.01
1.01
2.05
1.04
3.02
f1 (ppm)

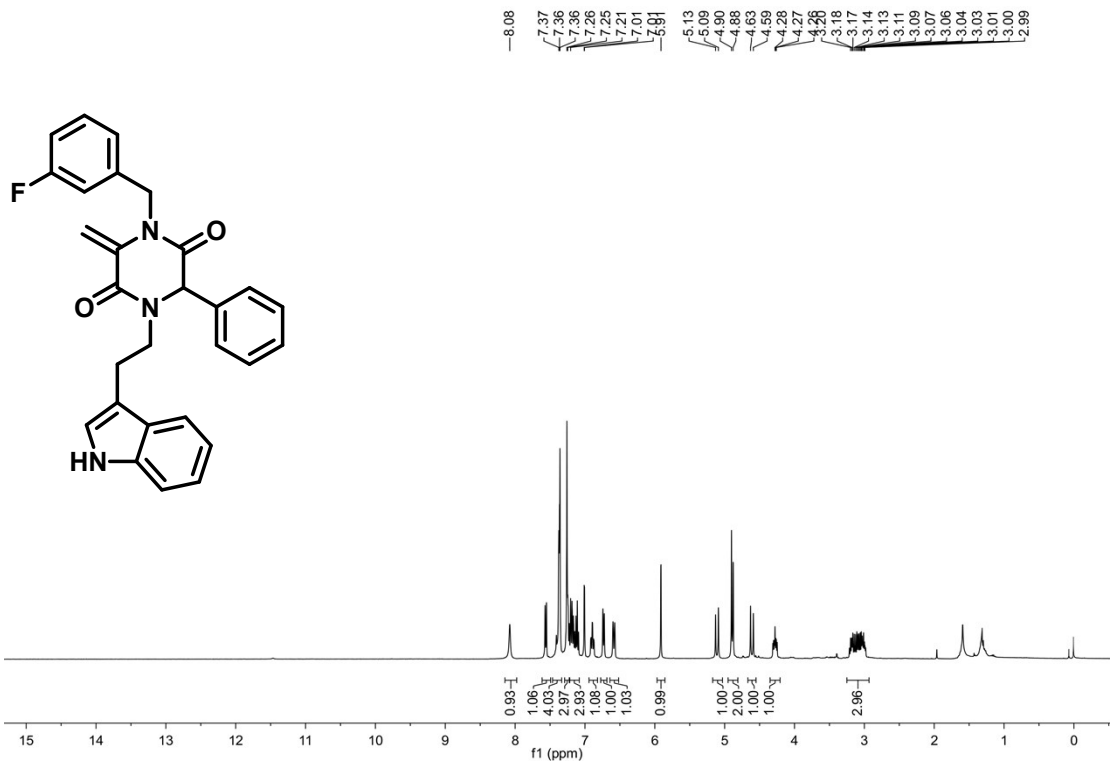

164.20
158.78
139.23
138.02
136.36
136.23
133.14
130.34
130.25
129.93
127.17
126.56
122.27
122.10
122.02
119.57
118.53
114.52
114.31
113.45
113.23
112.46
111.38
104.37
65.23
46.77
46.36
23.28
21.10
f1 (ppm)

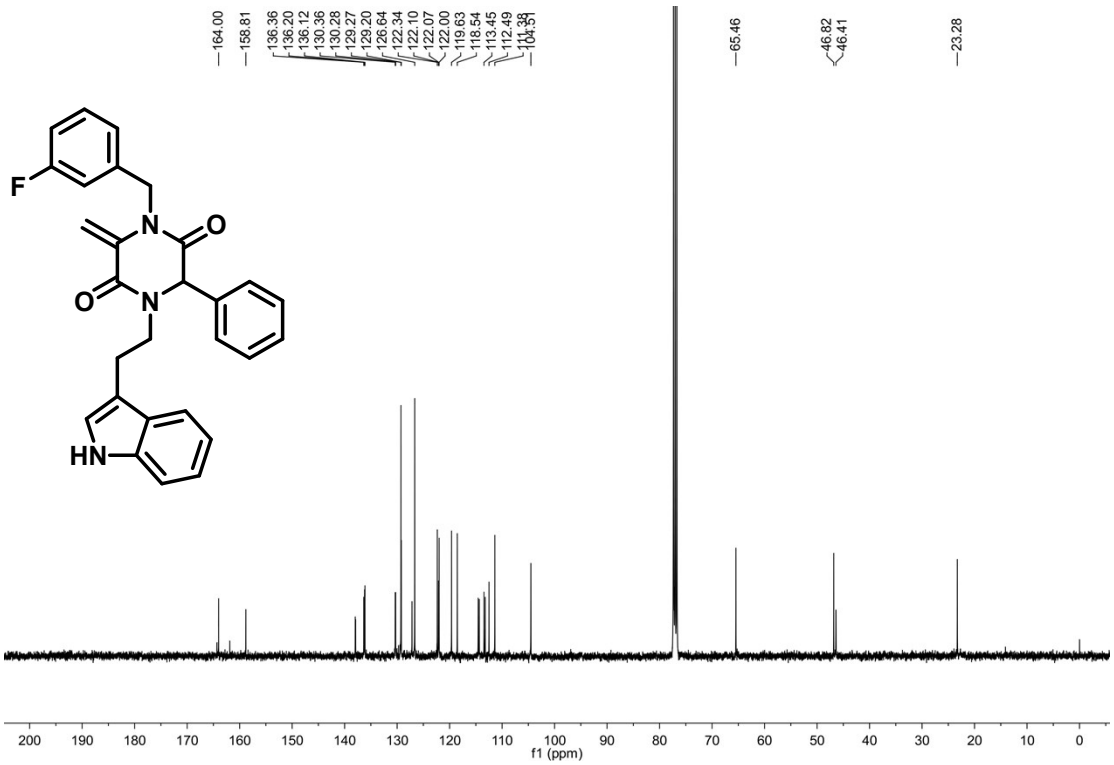

129.90
128.76
127.42
126.70
126.47
122.25
122.00
119.56
118.59
111.36
104.57
65.26
46.96
46.74
23.28
21.16
HN
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


HN
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f2 (ppm)
f1 (ppm)

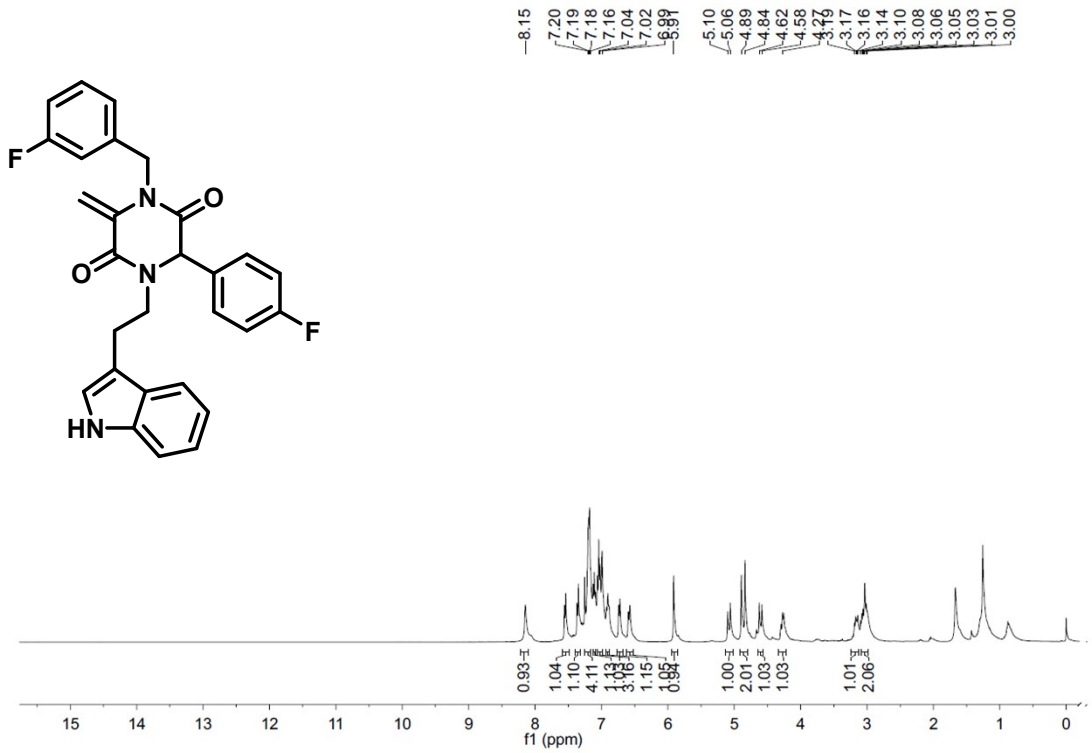

N
O
O
N
HN
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)

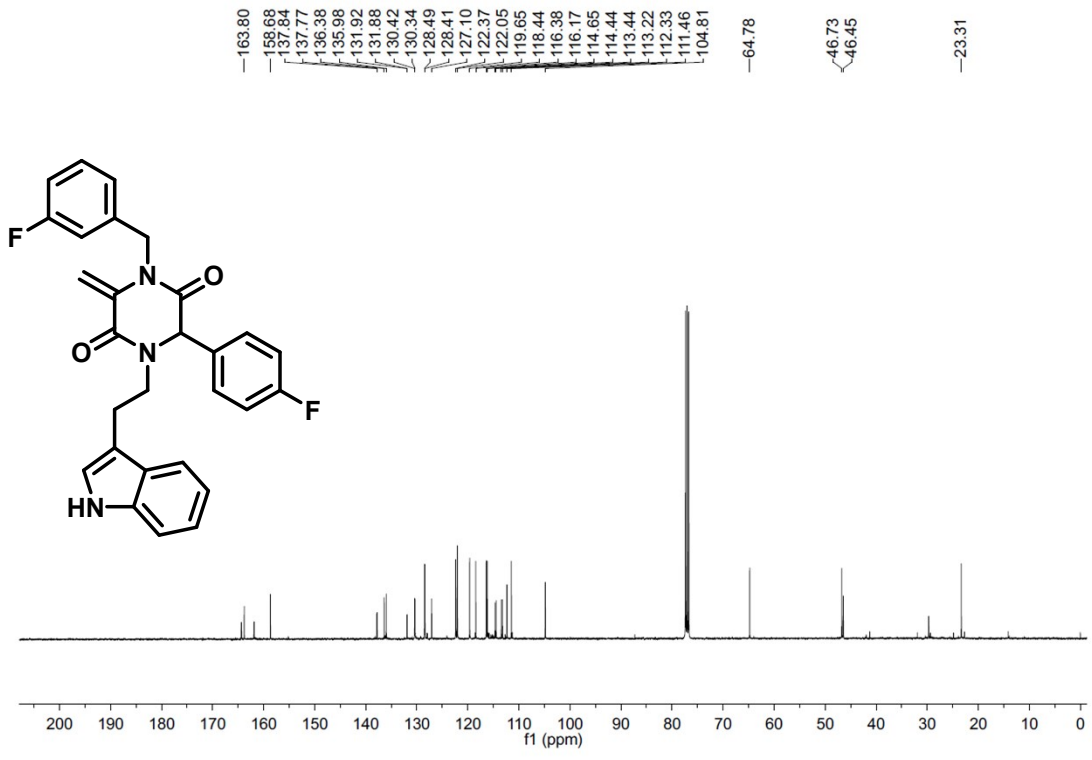
Compound 6f


8.08
7.37
7.36
7.36
7.26
7.25
7.21
7.01
7.01
5.91
5.13
5.09
4.90
4.88
4.63
4.59
4.28
4.27
4.26
3.18
3.17
3.14
3.13
3.11
3.09
3.07
3.06
3.04
3.03
3.01
3.00
2.99
F
N
O
O
N
HN
0.93
1.06
4.03
2.97
2.93
1.08
1.00
1.03
0.99
1.00
2.00
1.00
1.00
2.96
f1 (ppm)

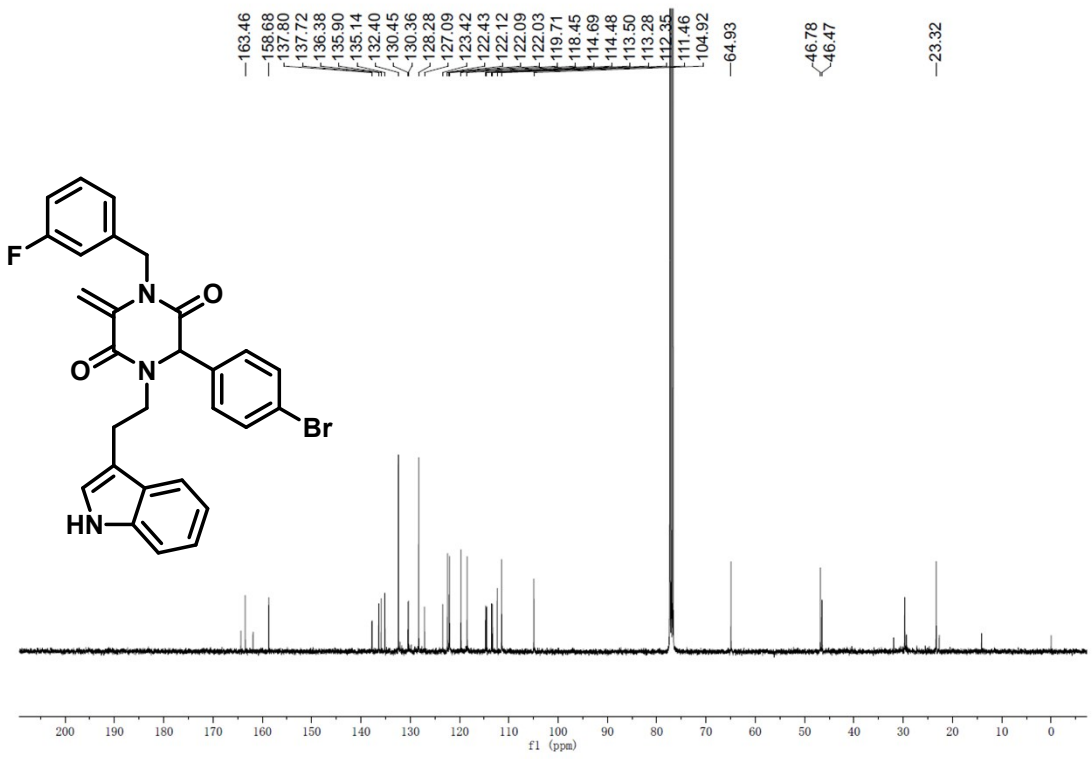

164.00
158.81
136.36
136.20
136.12
130.36
130.28
129.27
129.20
126.64
122.34
122.10
122.07
122.00
119.63
118.54
113.45
112.49
65.46
46.82
46.41
23.28
F
N
O
O
N
HN
f1 (ppm)

Compound **6g**

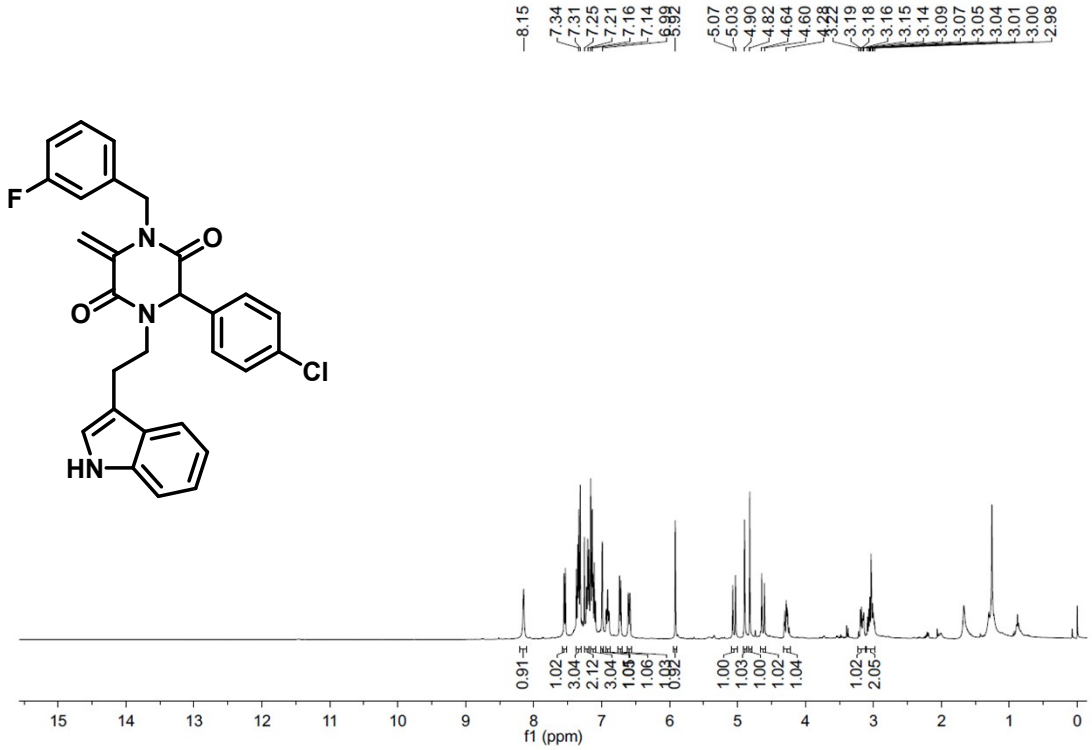

F
N
O
O
N
F
HN
8.15
7.20
7.19
7.18
7.16
7.04
7.02
6.99
6.91
5.10
5.06
4.89
4.84
4.62
4.58
4.27
3.19
3.17
3.16
3.14
3.10
3.08
3.06
3.05
3.03
3.01
3.00
0.93
1.04
1.10
4.11
1.13
3.16
1.15
0.94
1.00
2.01
1.03
1.03
1.01
2.06
f1 (ppm)

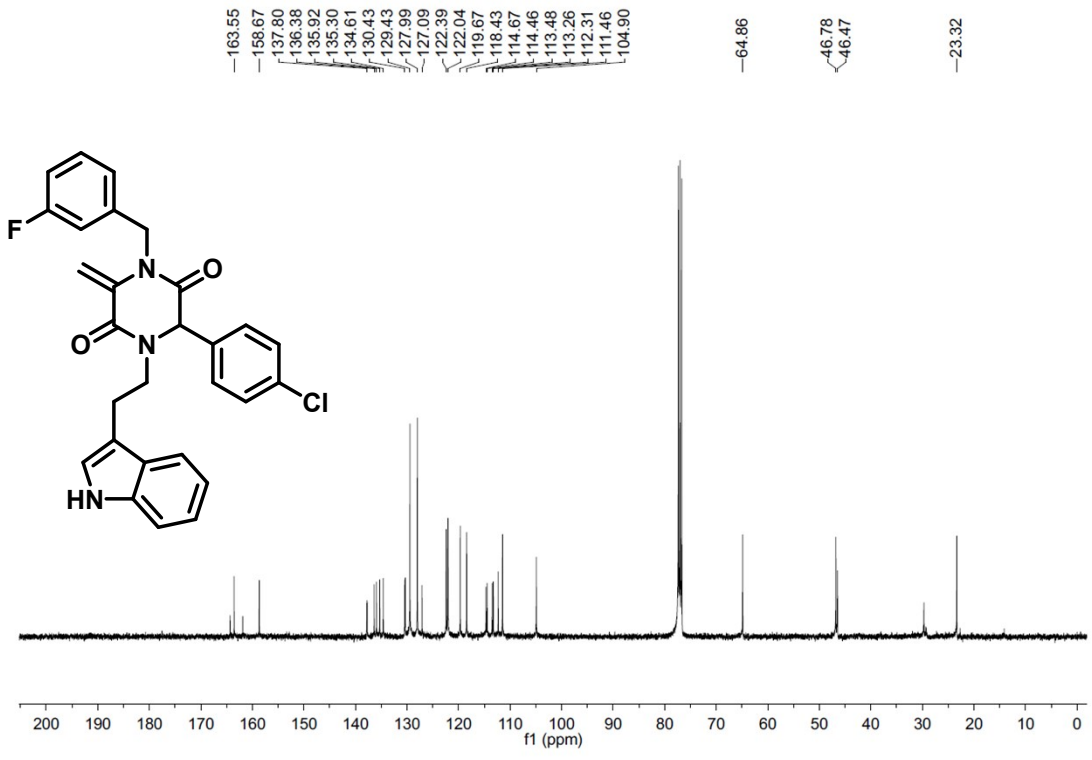

F
N
O
O
N
F
HN
163.80
158.68
137.84
137.77
136.38
135.98
131.92
131.88
130.42
130.34
128.49
128.41
127.10
122.37
122.05
119.65
118.44
116.38
116.17
114.65
114.44
113.44
113.22
112.33
111.46
104.81
64.78
46.73
46.45
23.31
f1 (ppm)

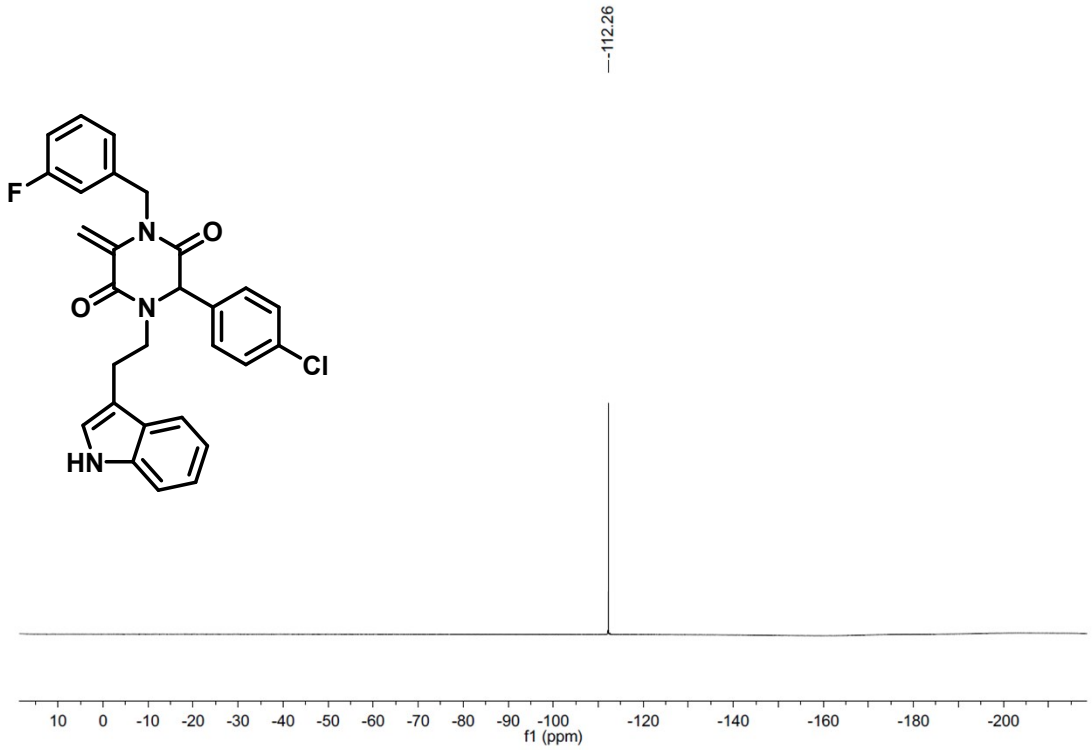
Compound **6h**



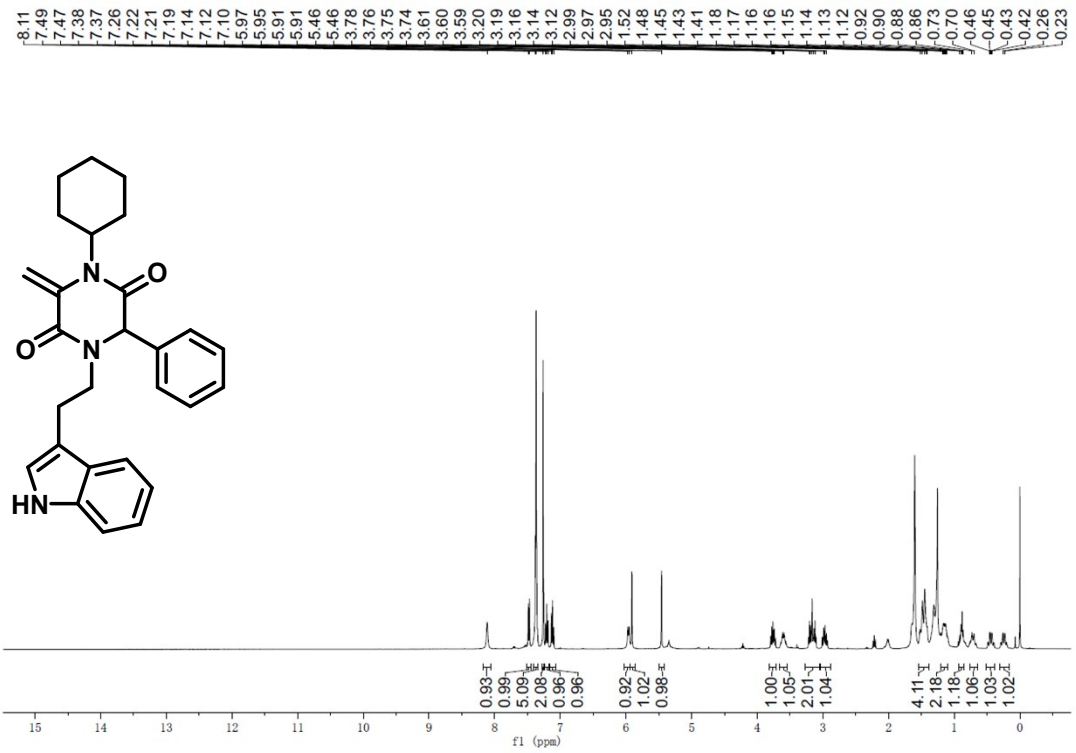
Compound **6i**

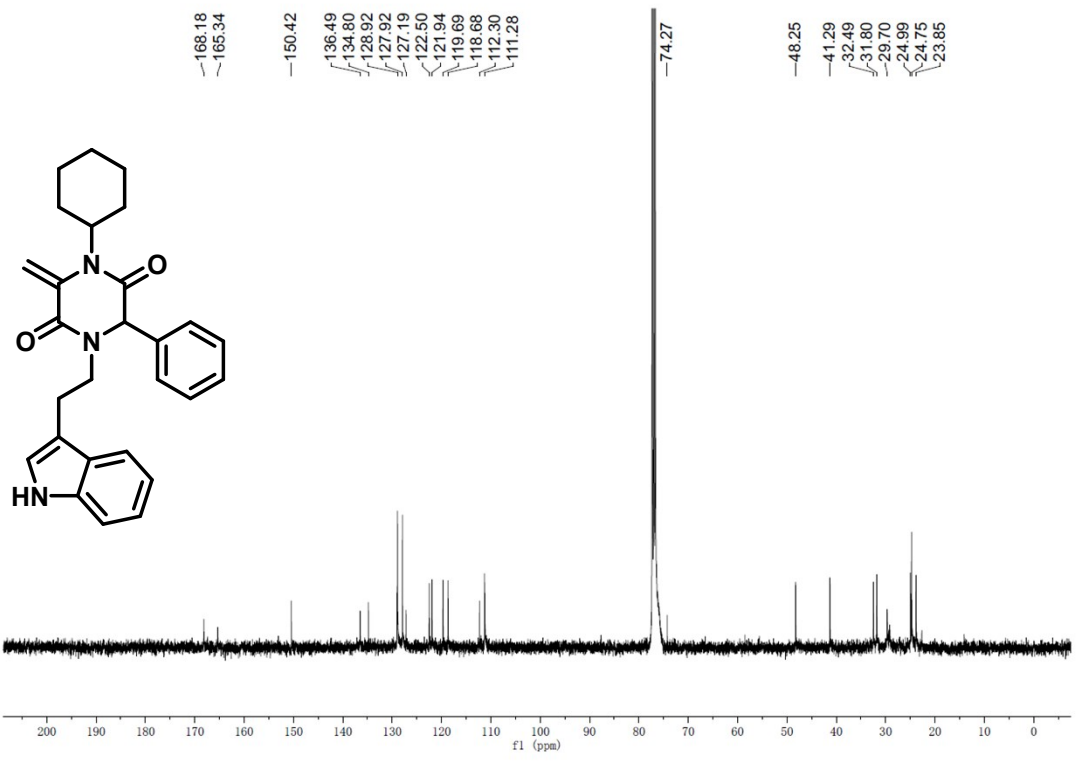

F
N
O
O
N
Cl
HN
8.15
7.34
7.31
7.25
7.21
7.16
7.14
6.99
5.92
5.07
5.03
4.90
4.82
4.64
4.60
4.28
3.22
3.19
3.18
3.16
3.15
3.14
3.09
3.07
3.05
3.04
3.01
3.00
2.98
0.91
1.02
3.04
2.12
1.00
1.03
1.00
1.02
1.04
1.02
2.05
f1 (ppm)


F
N
O
O
N
Cl
HN
163.55
158.67
137.80
136.38
135.92
135.30
134.61
130.43
129.43
127.99
127.09
122.39
122.04
119.67
118.43
114.67
114.46
113.48
113.26
112.31
111.46
104.90
64.86
46.78
46.47
23.32
f1 (ppm)

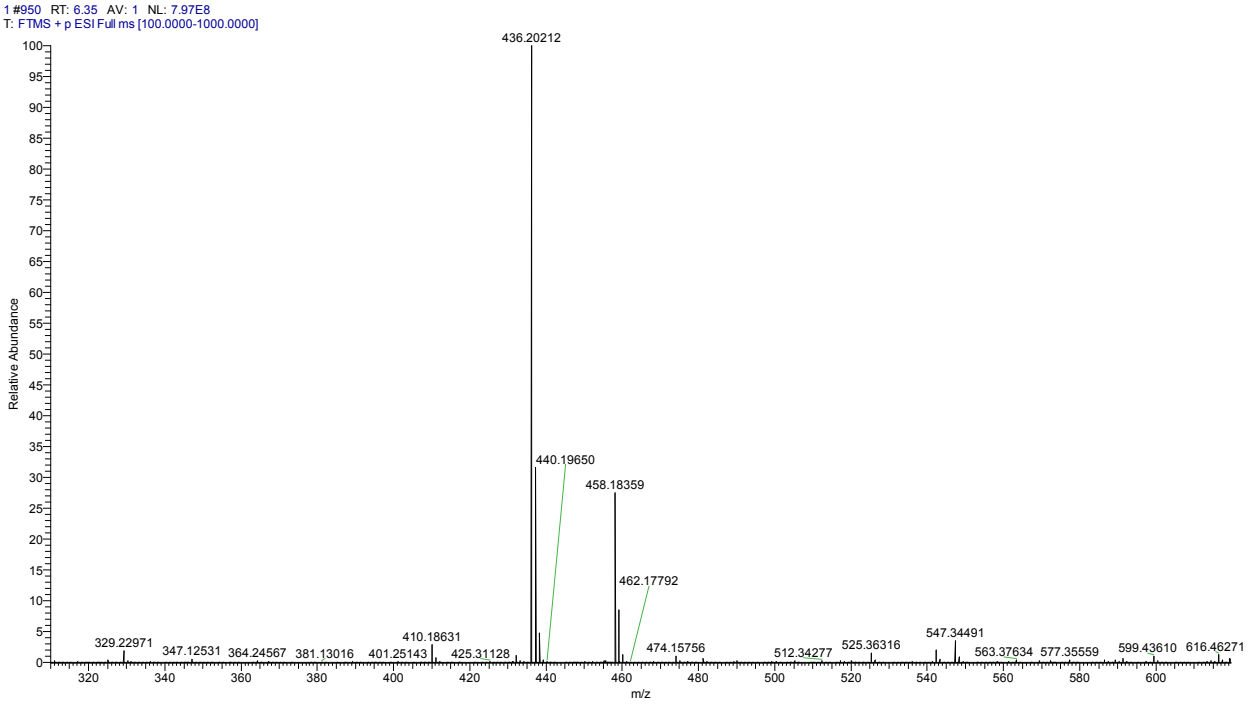

F
N
O
O
N
Cl
HN
-112.26
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-120
-140
-160
-180
-200
f1 (ppm)

Compound 6j

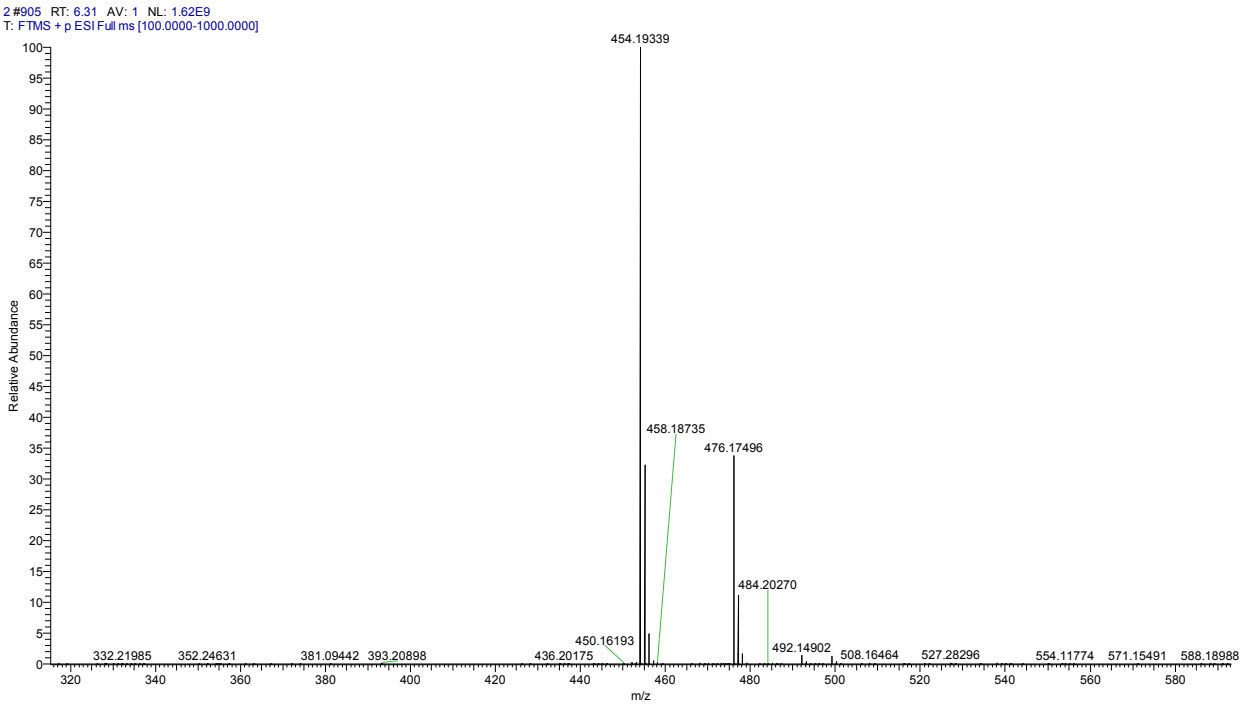

8.11
7.49
7.47
7.38
7.37
7.26
7.22
7.21
7.19
7.14
7.12
7.10
5.97
5.95
5.91
5.91
5.46
5.46
3.78
3.76
3.75
3.74
3.61
3.60
3.59
3.20
3.19
3.16
3.14
3.12
2.99
2.97
2.95
1.52
1.48
1.45
1.43
1.41
1.18
1.17
1.16
1.16
1.15
1.14
1.13
1.12
0.92
0.90
0.88
0.86
0.73
0.70
0.46
0.45
0.43
0.42
0.26
0.23
O
N
O
N
HN
0.93
0.99
5.09
2.08
0.96
0.96
0.92
1.02
0.98
1.00
1.05
2.01
1.04
4.11
2.18
1.18
1.06
1.03
1.02
f1 (ppm)


168.18
165.34
150.42
136.49
134.80
128.92
127.92
127.19
122.50
121.94
119.69
118.68
112.30
111.28
74.27
48.25
41.29
32.49
31.80
29.70
24.99
24.75
23.85
O
N
O
N
HN
f1 (ppm)

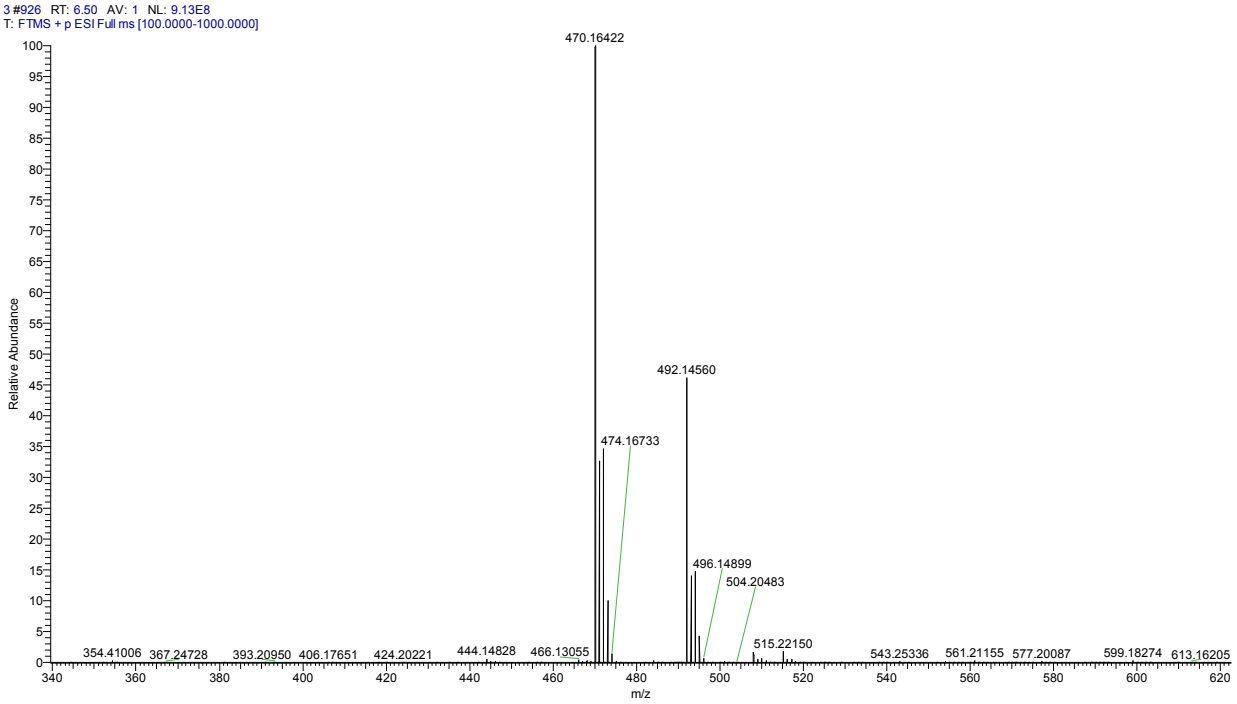
6a


1 #950 RT: 6.35 AV: 1 NL: 7.97E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]
436.20212
440.19650
458.18359
462.17792
329.22971
347.12531
364.24567
381.13016
401.25143
410.18631
425.31128
474.15756
512.34277
525.36316
547.34491
563.37634
577.35559
599.43610
616.46271
Relative Abundance
m/z

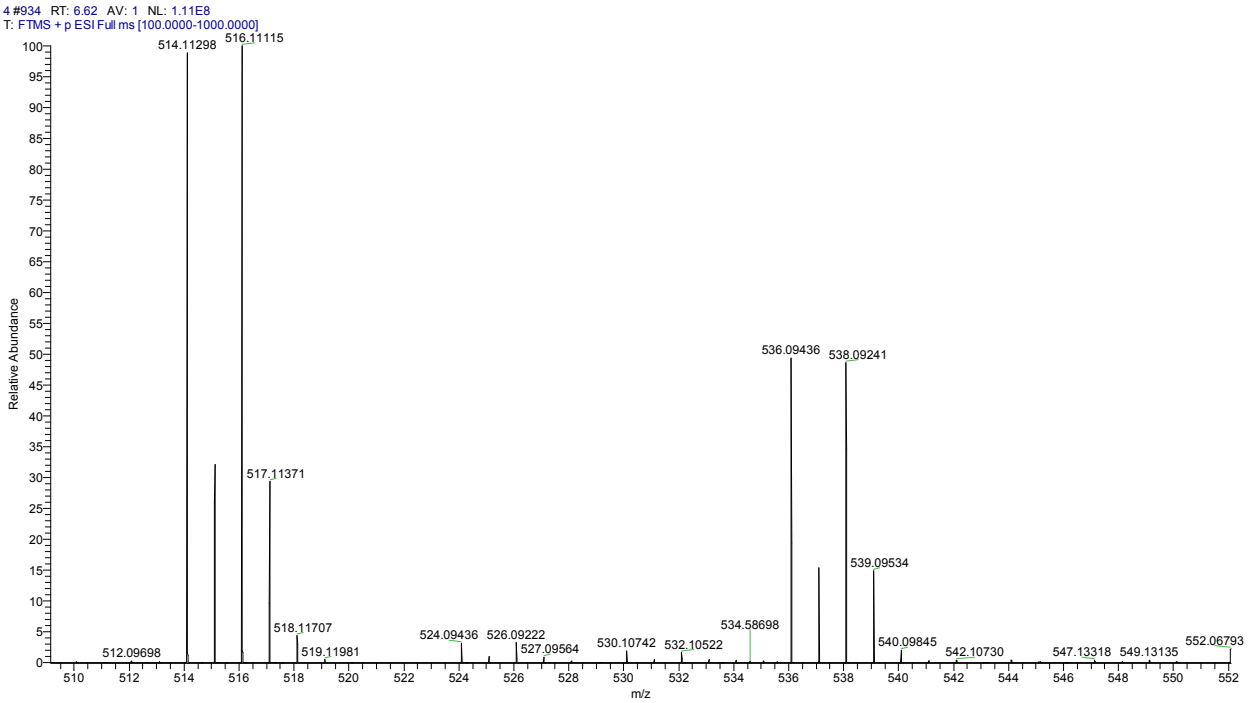
6b


2 #905 RT: 6.31 AV: 1 NL: 1.62E9
T: FTMS + p ESI Full ms [100.0000-1000.0000]
454.19339
458.18735
476.17496
484.20270
450.16193
436.20175
492.14902
332.21985
352.24631
381.09442
393.20898
508.16464
527.28296
554.11774
571.15491
588.18988
Relative Abundance
m/z

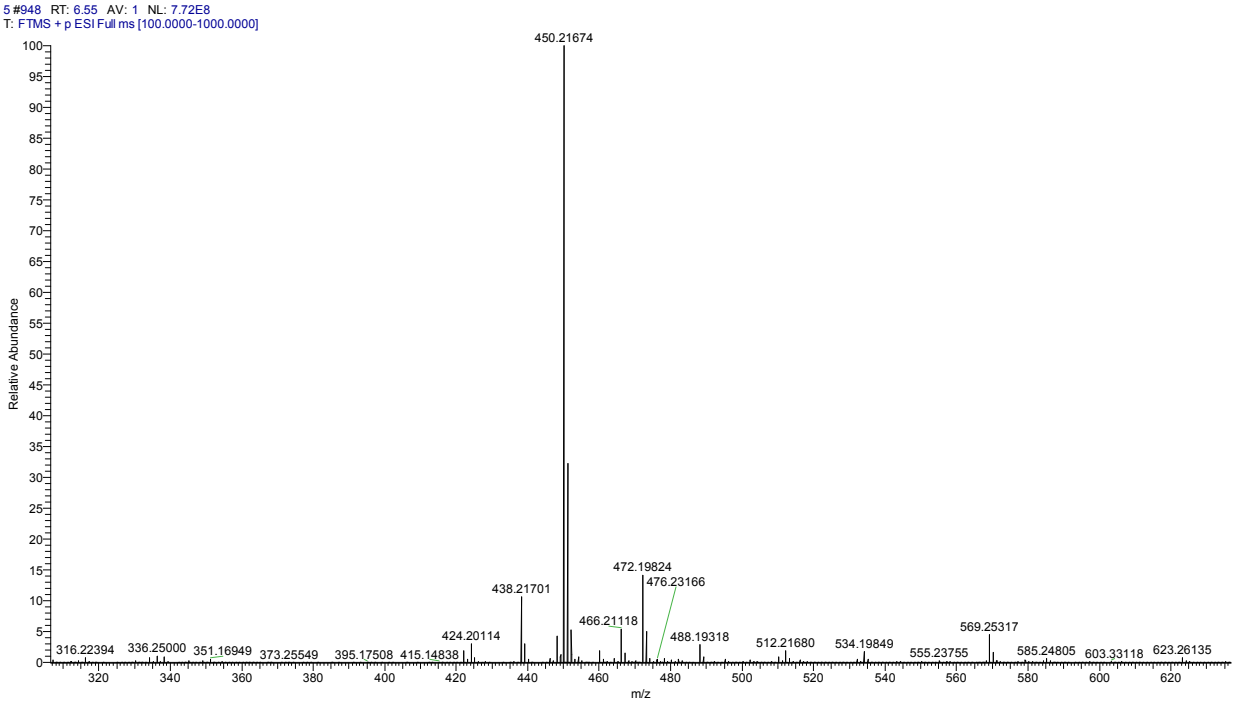
6c


3 #926 RT: 6.50 AV: 1 NL: 9.13E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]
470.16422
492.14560
474.16733
496.14899
504.20483
515.22150
354.41006
367.24728
393.20950
406.17651
424.20221
444.14828
466.13055
543.25336
561.21155
577.20087
599.18274
613.16205
Relative Abundance
m/z

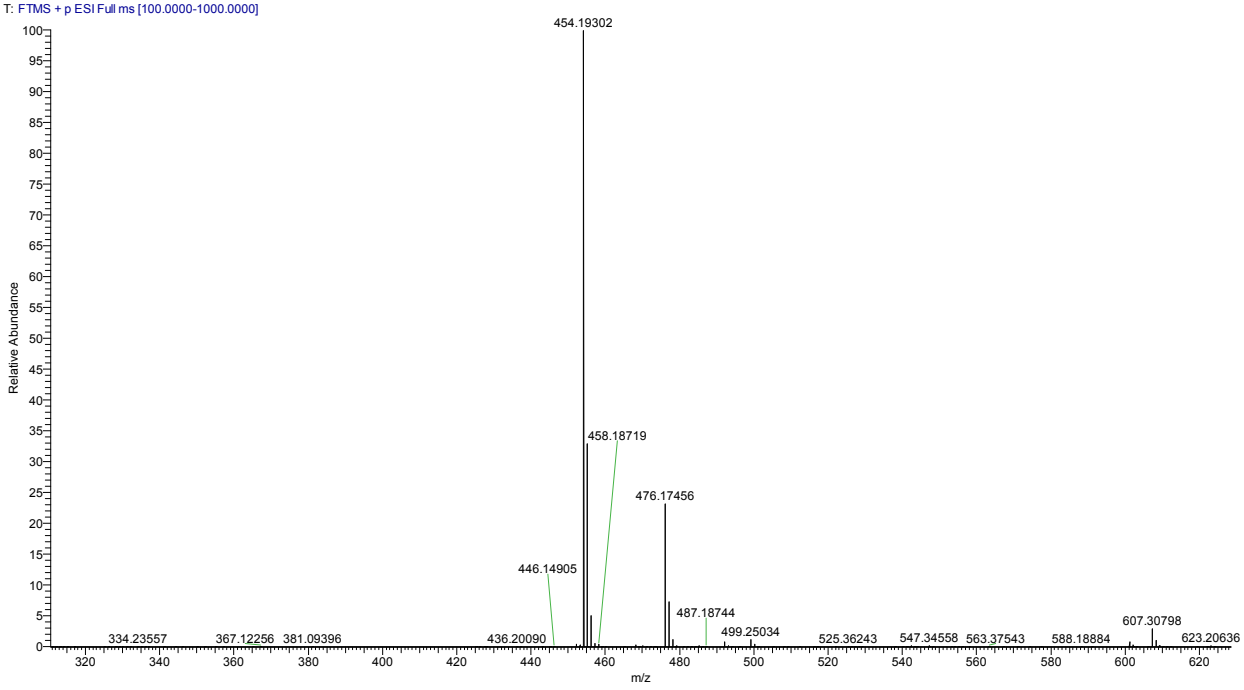
6d


4 #934 RT: 6.62 AV: 1 NL: 1.11E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]
514.11298
516.11115
536.09436
538.09241
517.11371
539.09534
518.11707
534.58698
512.09698
519.11981
524.09436
526.09222
527.09564
530.10742
532.10522
540.09845
542.10730
547.13318
549.13135
552.06793
Relative Abundance
m/z

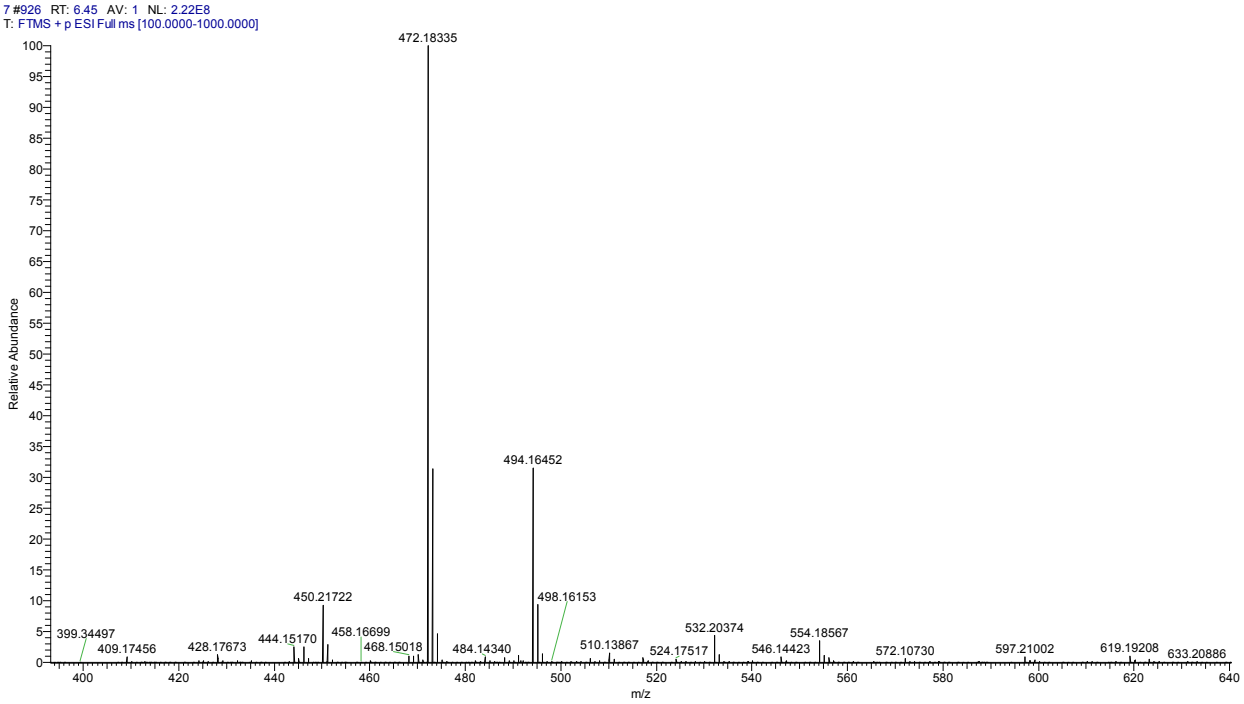
6e


5 #948 RT: 6.55 AV: 1 NL: 7.72E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]
450.21674
472.19824
476.23166
438.21701
466.21118
424.20114
488.19318
569.25317
316.22394
336.25000
351.16949
373.25549
395.17508
415.14838
512.21680
534.19849
555.23755
585.24805
603.33118
623.26135
Relative Abundance
m/z

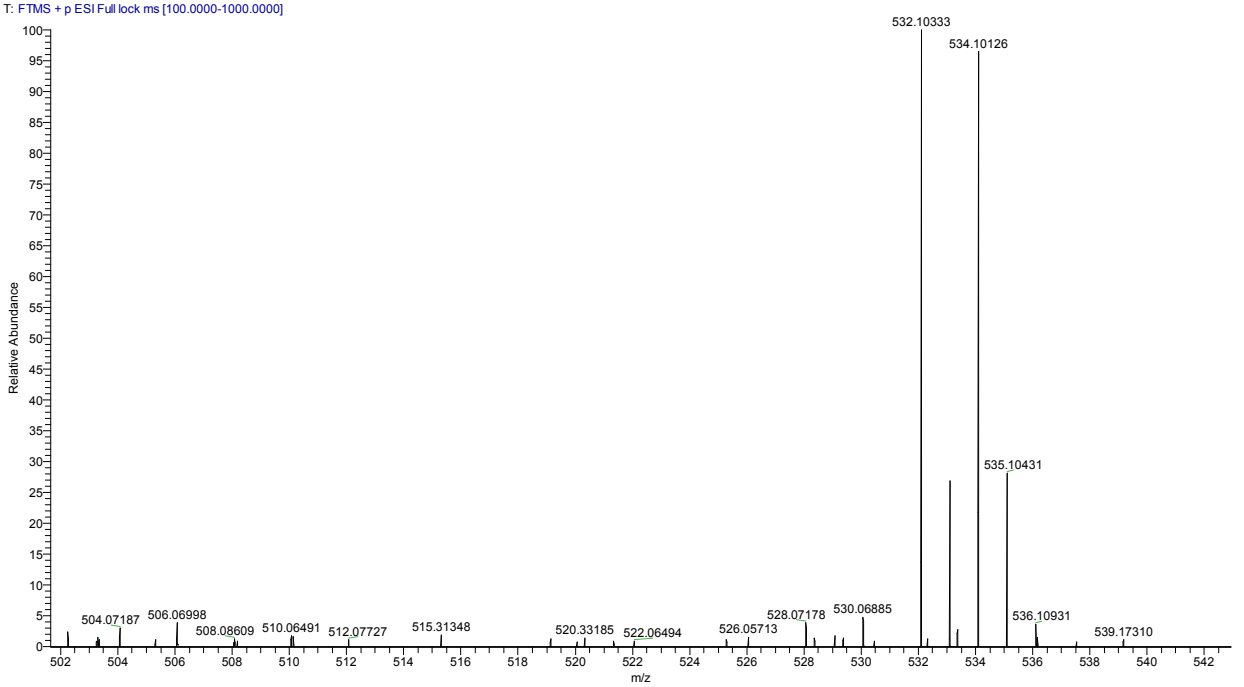
6f


6-2 #937 RT: 6.37 AV: 1 NL: 1.91E9
T: FTMS + p ESI Full ms [100.0000-1000.0000]
454.19302
458.18719
476.17456
446.14905
487.18744
499.25034
607.30798
334.23557
367.12256
381.09396
436.20090
525.36243
547.34558
563.37543
588.18884
623.20636
Relative Abundance
m/z

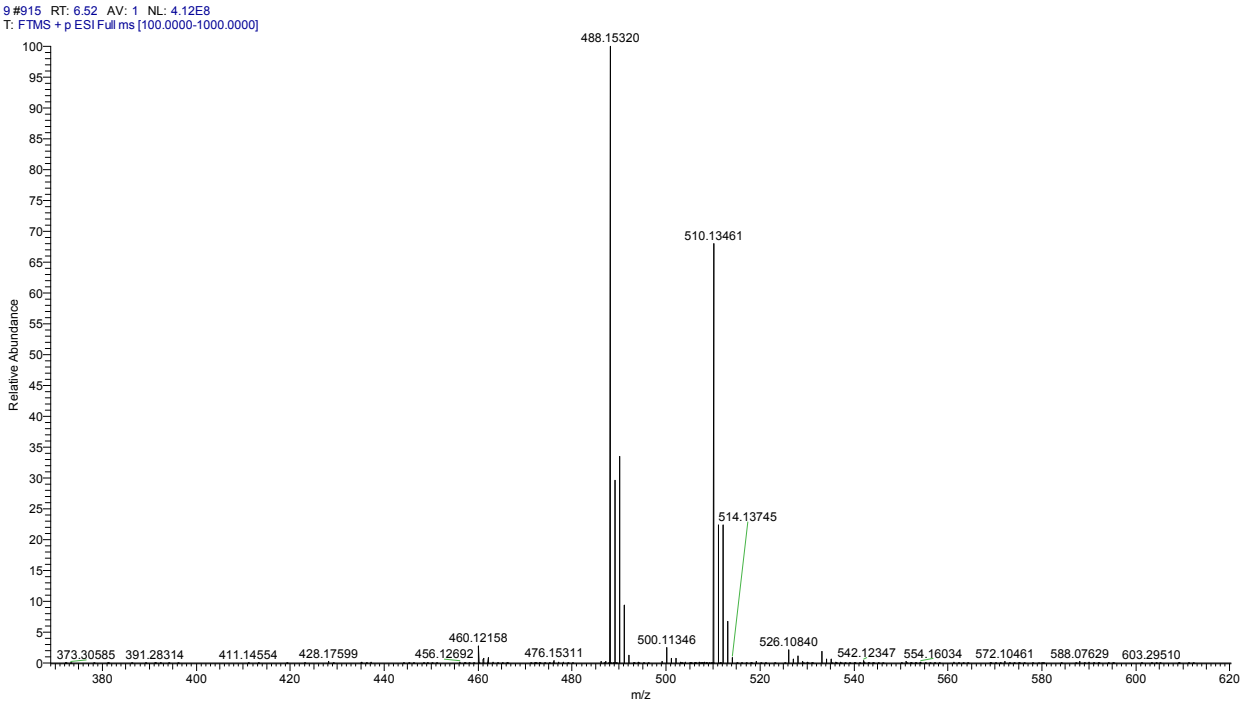
6g


7 #926 RT: 6.45 AV: 1 NL: 2.22E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]
472.18335
494.16452
450.21722
498.16153
399.34497
409.17456
428.17673
444.15170
458.16699
468.15018
484.14340
510.13867
524.17517
532.20374
546.14423
554.18567
572.10730
597.21002
619.19208
633.20886
Relative Abundance
m/z

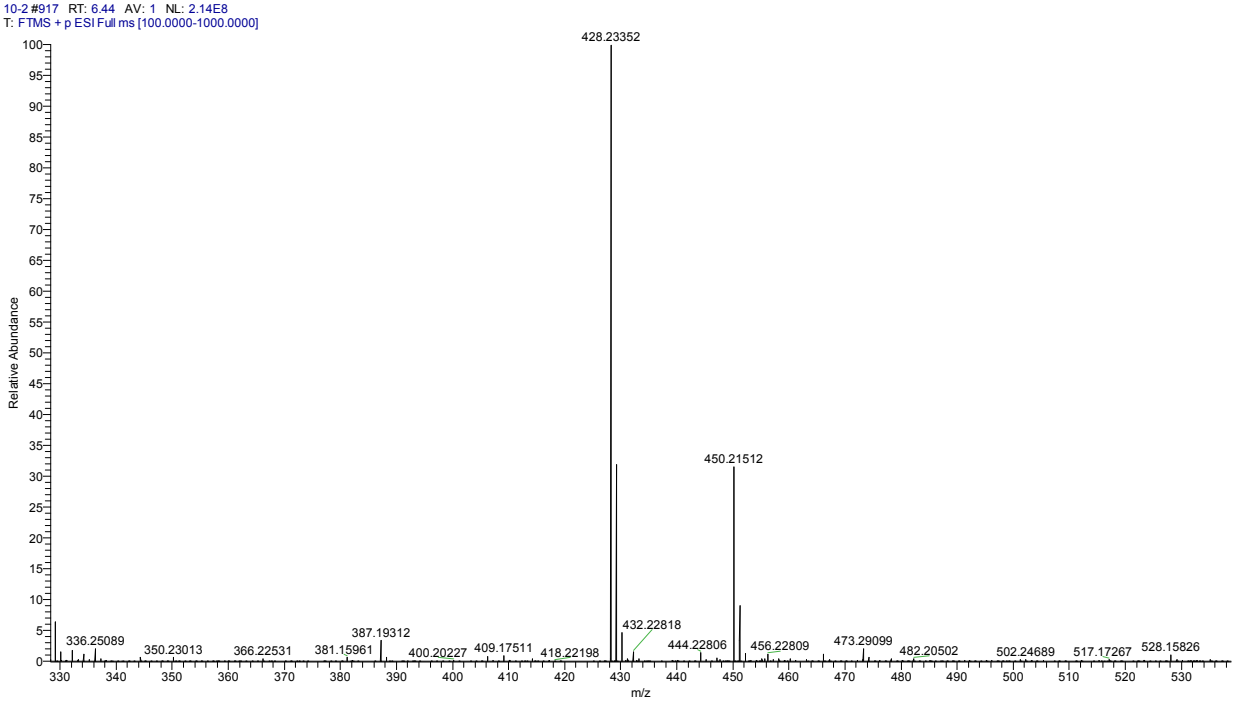
6h


8 #984 RT: 6.96 AV: 1 NL: 1.27E6
T: FTMS + p ESI Full lock ms [100.0000-1000.0000]
532.10333
534.10126
535.10431
504.07187
506.06998
508.08609
510.06491
512.07727
515.31348
520.33185
522.06494
526.05713
528.07178
530.06885
536.10931
539.17310
Relative Abundance
m/z

6i


9 #915 RT: 6.52 AV: 1 NL: 4.12E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]
488.15320
510.13461
514.13745
460.12158
500.11346
526.10840
373.30585
391.28314
411.14554
428.17599
456.12692
476.15311
542.12347
554.16034
572.10461
588.07629
603.29510
Relative Abundance
m/z

6j


10-2 #917 RT: 6.44 AV: 1 NL: 2.14E8
T: FTMS + p ESI Full ms [100.0000-1000.0000]
428.23352
450.21512
432.22818
387.19312
336.25089
350.23013
366.22531
381.15961
400.20227
409.17511
418.22198
444.22806
456.22809
473.29099
482.20502
502.24689
517.17267
528.15826
Relative Abundance
m/z