Pyridazinone derivatives as Potential Anti-inflammatory Agents

Synthesis and Biological Evaluation as PDE4 inhibitors

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Experimental section

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1. Chemistry

General and protocols :

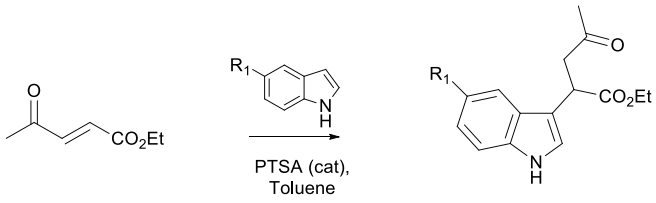
Solvents : Solvents were dried and purified by standard literature methods prior to use.
Reagents : Reagents were bought from Acros Organics and Sigma-Aldrich and were used without further purification.
Instrumentation : Reactions were monitored on silicagel plates (Silicagel 60F254 from Merck) and column chromatography was carried out on silica gel 60 (70-200 µm) and flash silica gel (35-70 µm). Melting points were determined on an hot-stage apparatus and are uncorrected.
Proton and Carbon Nuclear Magnetic Resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on either a Bruker DPX 300 apparatus (^{1}H 300 MHz; ^{13}C 75 MHz), a 500 MHz Bruker Avance Neo spectrometer equipped with a SP BB&19F/1H probe (^{1}H 500.28 MHz; ^{13}C 125.8 MHz) or a 600 MHz Bruker Avance III spectrometer equipped with a TCI cryoprobe (^{1}H 600.16 MHz; ^{13}C 150.9 MHz). Usual deuterated solvants as $CDCl_3$, CD_3OD or DMSO-*d*6 were used. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard followed by multiplicity in which s, bs, d, t, q, dd, dt, td, m are respectively singlet, broad singlet, doublet, triplet, quadruplet, dedoubled doublet, dedoubled triplet, detripled doublet and multiplet (or unwell-resolved signals). Coupling constants J are quoted in Hertz (Hz). Bidimensional NMRs (COSY, HSQC and HMBC) were used to assign signals.
Infrared spectra (IR) (thin film or KBr pastille) were measured on a Perkin-Elmer SPECTRUM BX/RX Fourier transform spectrometer. Principal absorption bands are given in cm^{-1}.
Electronic Impact (EI), Chemical Ionisation (CI), ElectroSpray Ionisation (ESI) Mass Spectra (MS) and High Resolution Mass Spectra (HRMS) were either recorded with an ESI-Q-TOF mass spectrometer from Waters or with a GCT CA 170 Micromass Waters apparatus. The (+)ESI Mass Spectra were performed in a positive mode with CH_3OH as solvent. The masses are measured in Dalton (Da).
LogP were calculated using ChemBioDrawUltra software.

Typical procedure for the formation of ethyl 4-oxopent-2-enoate:

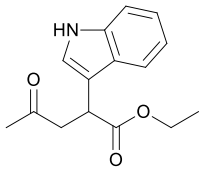
Ethyl 4-oxopent-2-enoate. Levulinic acid (1 eq) is dissolved in ethanol. Catalytic amount of sulfuric acid (0.1 eq) is added to the solution. The mixture is then stirred and headed at reflux. After 16 h, the reaction is stopped and the solvent is evaporated under reduced pressure to yield the ethyl levulinate in quantitative yield. Then bromine (1 eq) is dropwise added (during a period of 1 hour) to a cold (0°C) solution of ethyl levulinate (1 eq) in chloroform. After 30 minutes of stirring at 0°C, triethylamine (3 eq) is then dropwise added during 1 hour. The mixture is stirred for 1h30 after which water is added to the solution. The crude mixture is then extracted by dichloromethane and the combined organic phases are washed successively with aqueous solutions of hydrochloric acid (10 %) and saturated ammonium

chloride, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude mixture is purified by flash chromatography over silica gel using dichloromethane as eluent to yield 47 % of a yellow oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) = 1.33 (t, *J*= 7.1 Hz, 3 H), 2.37 (s, 3 H), 4.28 (q, *J*= 7.1 Hz, 2 H), 6.65 (d, *J*= 16.1 Hz, 1 H), 7.02 (d, *J*= 16.1 Hz, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 14.2, 27.6, 60.4, 135.3, 139.7, 165.7, 196.8. **IR** (KBr) ν (cm^{-1}) = 3052, 2982, 1721, 1701, 1422, 1291, 1263, 1157. **MS** (CI) *m/z* 143 $[M+H]^+$, 129. **HRMS** (CI; $[M+H]^+$) calculated for $C_7H_{11}O_3$ 143.0751, found 143.0708.

<u>General procedure for Friedel-Crafts alkylation:</u>

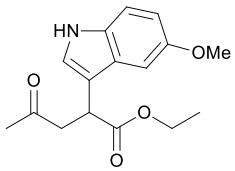


Ethyl 4-oxopenten-2-oate (1 eq (purity about 80%)) is dissolved in toluene. 0.6 to 1 equivalent of heterocyclic compound is added to the solution with a catalytic amount of *para*-toluenesulfonic acid (PTSA, 0.1 eq). The reaction is stirred at room temperature. After about 48 hours (monitoring by thin layer chromatography) the reaction is stopped and the solvent is evaporated under reduced pressure. The crude mixture is then purified by column chromatography and sometimes cristallized in diethyl ether.



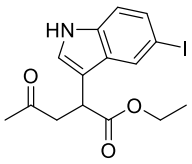
Ethyl 2-(1H-indol-3-yl)-4-oxo-pentanoate <u>2a</u>. According to the general procedure for Friedel-Crafts alkylation, starting from 147 mg of ethyl 4-oxopenten-2-oate and 120 mg of indole, the title compound is isolated after column chromatography (cyclohexane/AcOEt=7/3) followed by recrystallization in diethyl ether as a beige powder. Yield: 139 mg (61 %).

Mp: 116-118 °C. **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) = 1.18 (t, *J*= 7.1 Hz, 3 H), 2.17 (s, 3 H), 2.83 (dd, *J*= 17.9, 4.4 Hz, 1 H), 3.51 (dd, *J*= 18.0, 10.2 Hz, 1 H), 4.01-4.22 (m, 2 H), 4.39 (dd, *J*= 10.2, 4.5 Hz, 1 H), 7.05 (d, *J*= 2.7 Hz, 1 H), 7.10-7.22 (m, 2 H), 7.34 (d, *J*= 8.1 Hz, 1 H), 7.71 (d, *J*= 7.8 Hz, 1 H), 8.25 (bs, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 14.2, 30.1, 38.0, 46.3, 61.2, 111.5, 113.0, 119.3, 119.8, 122.2, 122.4, 126.2, 136.4, 173.9, 207.1. **IR** (KBr) ν (cm^{-1}) = 3405, 2919, 1721, 1708, 1457, 1413, 1361, 1336, 1323, 1258, 1232, 1173, 1090. **MS** (EI) *m/z* 259 $[M^{+\cdot}]$, 213, 186, 144, 115. **HRMS** (EI; $[M^{+\cdot}]$) calculated for $C_{15}H_{17}NO_3$ 259.1208, found 259.1212.



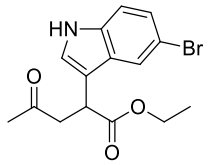
Ethyl 2-(5-methoxy-1H-indol-3-yl)-4-oxo-pentanoate <u>2b</u>. According to the general procedure for Friedel-Crafts alkylation using 154 mg of ethyl 4-oxopenten-2-oate and 151 mg of 5-methoxyindole, the title compound is isolated after column chromatography (cyclohexane/ ethyl acetate =7/3) as beige crystals. Yield: 198 mg (55 %).

Mp: 137-139 °C. **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) = 1.21 (t, *J*= 7.2 Hz, 3 H), 2.20 (s, 3 H), 2.83 (dd, *J*= 18.0, 4.4 Hz, 1 H), 3.50 (dd, *J*= 18.0, 10.4 Hz, 1 H), 3.87 (s, 3 H), 4.02-4.24 (m, 2 H), 4.35 (dd, *J*= 10.4, 4.4 Hz, 1 H), 6.87 (dd, *J*= 9.0, 2.4 Hz, 1 H), 7.06 (d, *J*= 2.4 Hz, 1 H), 7.16 (d, *J*= 2.4 Hz, 1 H), 7.24 (d, *J*= 8.7 Hz, 1 H), 8.01 (bs, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 14.2, 30.1, 37.9, 46.1, 56.0, 61.1, 100.9, 112.2, 112.5, 112.8, 122.8, 126.6, 131.4, 154.2, 173.9, 207.2. **IR** (KBr) ν (cm^{-1}) = 3333, 2925, 1718, 1708, 1620, 1581, 1483, 1457, 1364, 1302, 1266, 1199, 1170, 1023. **MS** (EI) *m/z* 289 [$M^{+\cdot}$], 243, 216, 174, 159, 130, 115. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{16}H_{19}NO_4$ 289.1314, found 289.1309.



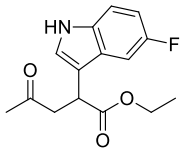
Ethyl 2-(5-iodo-1H-indol-3-yl)-4-oxopentanoate <u>2c</u>. According to the general procedure for Friedel-Crafts alkylation using 700 mg (4.9 mmol) of ethyl 4-oxopent-2-enoate and 600 mg (2.5 mmol) of 5-iodoindole, the title compound is isolated after 41 hours and after column chromatography (PE/EA=70/30) as a brownish-red sticky solid. Yield: 365 mg (39 %).

Mp: 144-146 °C. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) = 1.22 (t, *J*= 7.0 Hz, 3 H), 2.20 (s, 3 H), 2.82 (dd, *J*= 18.5, 4.5 Hz, 1 H), 3.47 (dd, *J*= 18.0, 10.0 Hz, 1H), 4.06-4.21 (m, 2H), 4.32 (dd, *J*= 10.5, 4.5 Hz, 1 H), 7.06 (d, *J*= 2.5 Hz, 1 H), 7.14 (d, *J*= 8.5 Hz, 1 H), 7.45 (dd, *J*= 8.5, 1.5 Hz, 1 H), 8.06 (d, *J*= 1.5 Hz, 1 H), 8.11 (s, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 14.2, 30.1, 37.8, 46.1, 61.4, 83.4, 112.3, 113.4, 123.1, 128.3, 128.8, 130.8, 135.4, 173.6, 206.9. **IR** (KBr) ν (cm^{-1}) = 3292, 2977, 1730, 1709, 1465, 1398, 1250, 1134. **MS** (EI) m/z 385 [$M^{+\cdot}$], 339, 311, 268, 185, 127. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{15}H_{16}NO_3I$ 385.0175, found 385.0181.



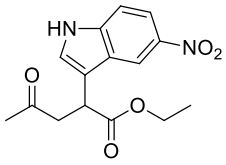
Ethyl 2-(5-bromo-1H-indol-3-yl)-4-oxopentanoate <u>2d</u>. According to the general procedure for Friedel-Crafts alkylation using 500 mg (3.5 mmol) of ethyl 4-oxopent-2-enoate and 686 mg (5 mmol) of 5-bromoindole, the title compound is isolated after column chromatography (PE/EA=70/30) as a beige powder. Yield: 947 mg (80 %).

Mp: 151-153 °C. **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) = 1.21 (t, *J*= 7.2 Hz, 3 H), 2.20 (s, 3 H), 2.83 (dd, *J*= 18.0, 4.5 Hz, 1 H), 3.48 (dd, *J*= 18.0, 10.2 Hz, 1H), 4.03-4.24 (m, 2 H), 4.33 (dd, *J*= 10.2, 4.5 Hz, 1 H), 7.08 (d, *J*= 2.7 Hz, 1 H), 7.22 (dd, *J*= 8.7, 0.9 Hz, 1 H), 7.28 (dd, *J*= 8.4, 1.8 Hz, 1 H), 7.85 (d, *J*= 1.8 Hz, 1 H), 8.18 (s, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 14.2, 30.1, 37.9, 46.1, 61.3, 112.8, 112.9, 113.2, 122.1, 123.4, 125.4, 128.0, 135.0, 173.6, 206.8. **IR** (KBr) ν (cm^{-1}) = 3464, 2974, 1724, 1580, 1483, 1447, 1172. **MS** (EI) m/z 337 [$M^{+\cdot}$], 291, 264, 220, 140, 115. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{15}H_{16}BrNO_3$ 337.0314, found 337.0298.



Ethyl 2-(5-fluoro-1H-indol-3-yl)-4-oxopentanoate <u>2e</u>. According to the general procedure for Friedel-Crafts alkylation using 1.052 g (7.4 mmol) of ethyl 4-oxopent-2-enoate and 1 g (7.4 mmol) of 5-fluoroindole, the title compound is isolated after 12 hours and after column chromatography (PE/EA=60/40) as a brown lacquer. Yield: 683 mg (33 %).

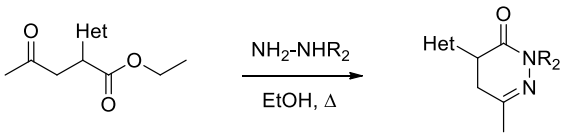
^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 1.20 (t, *J*= 7.0 Hz, 3 H), 2.19 (s, 3 H), 2.83 (dd, *J*= 18.0, 4.5 Hz, 1 H), 3.48 (dd, *J*= 18.0, 10.5 Hz, 1H), 4.05-4.21 (m, 2H), 4.32 (dd, *J*= 10.5, 4.5 Hz, 1 H), 6.94 (dt, *J*= 9.0, 2.5 Hz, 1 H), 7.12 (d, *J*= 2.5 Hz, 1 H), 7.25-7.27 (m, 1 H), 7.37 (dd, *J*= 9.5, 2.5 Hz, 1 H), 8.18 (s, 1H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) = 14.2, 30.1, 38.0, 46.1, 61.3, 104.5 (d, *J*= 23.8 Hz, 1 C), 111.0 (d, *J*= 26.4 Hz, 1 C), 112.1 (d, *J*= 9.4 Hz, 1 C), 126.7 (d, *J*= 9.9 Hz, 1 C), 132.9, 157.1, 158.9, 171.4, 173.6, 206.8. **IR** (KBr) ν (cm^{-1}) = 3333, 2920, 1733, 1707, 1456, 1367, 1239, 1171, 1150. **MS** (ESI+) m/z 300 [M+Na^+]. **HRMS** (ESI; [M+Na^+]) calculated for $C_{15}H_{16}NO_3FNa$ 300.1012, found 300.1011.



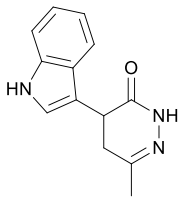
Ethyl 2-(5-nitro-1H-indol-3-yl)-4-oxopentanoate <u>2f</u>. According to the general procedure for Friedel-Crafts alkylation using 438 mg (3.1 mmol) of ethyl 4-oxopent-2-enoate and 500 mg (3.1 mmol) of 5-nitroindole, the title compound is isolated after 6 days and after column chromatography (CH_2Cl_2/MeOH=99/1) as a yellow lacquer. Yield: 187 mg (20 %).

^{1}H NMR (500 MHz, DMSO-*d*6) δ (ppm) = 1.10 (t, *J*= 7.0 Hz, 3 H), 2.15 (s, 3 H), 2.97 (dd, *J*= 18.0, 4.5 Hz, 1 H), 3.44 (dd, *J*= 18.5, 10.5 Hz, 1H), 3.96-4.08 (m, 2H), 4.28 (dd, *J*= 10.5, 4.5 Hz, 1 H), 7.54 (d, *J*= 13.0 Hz, 1 H), 7.54 (s, 1 H), 7.83 (d, *J*= 15.0 Hz, 1 H), 7.99 (dd, *J*= 9.0, 2.5 Hz, 1 H), 8.64 (d, *J*= 2.5 Hz, 1 H), 11.80 (s, 1H). **^{13}C NMR** (125 MHz, DMSO-*d*6) δ (ppm) = 13.9, 29.6, 37.3, 45.1, 60.4, 112.2, 114.2, 116.3, 116.7, 125.2, 127.2, 139.5, 140.5, 172.7, 206.5. **IR** (KBr) ν (cm^{-1}) = 3404, 1716, 1515, 1473, 1329, 1188. **MS** (ESI+) m/z 327 [M+Na^+]. **HRMS** (ESI; [M+Na^+]) calculated for $C_{15}H_{16}N_2O_5Na$ 327.0957, found 327.0960.

<u>General procedure for the formation of dihydropyridazinone cycle :</u>



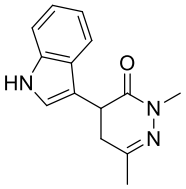
The hydrazine (1.5 to 2 eq) is added to a solution of the heterocyclic precursor (1 eq) in absolute ethanol. The mixture is stirred at room temperature and the reaction is monitored by TLC. Further additions of hydrazine are sometimes necessary to complete the reaction. After total conversion (from 5 h to 48 h), evaporation under reduced pressure furnished the corresponding dihydropyridazinone.



4-(1H-Indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3aa</u>. According to the general procedure for the formation of the dihydropyridazinone cycle starting from 70 mg of ethyl 2-(3-*1H*-indole)-4-oxo-pentanoate **<u>2a</u>** and 24 µL of hydrazine hydrate. The desired compound is obtained after flash column chromatography (dichloromethane/methanol=9.6/0.4) followed by a recrystallization in dichloromethane, as a brown powder. Yield: 42 mg (61 %).

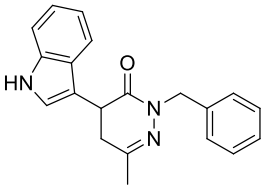
Mp: 244-246 °C. **^{1}H NMR** (300 MHz, DMSO-*d6*) δ (ppm) = 2.00 (s, 3 H), 2.71-2.87 (m, 2 H), 3.83 (dt, *J*= 7.4, 1.1 Hz, 1 H), 6.97 (dt, *J*= 7.1, 1.1 Hz, 1 H), 7.05-7.10 (m, 2 H), 7.34 (d, *J*= 8.1 Hz, 1 H), 7.61

(d, *J*= 7.8 Hz, 1 H), 10.51 (bs, 1 H), 10.95 (bs, 1 H). **^{13}C NMR** (75 MHz, DMSO-*d6*) δ (ppm) = 22.8, 32.4, 33.8, 111.3, 111.4, 118.5, 119.3, 121.2, 122.3, 126.4, 136.3, 151.9, 167.4. **IR** (KBr) ν (cm^{-1}) = 3366, 2914, 1667, 1455, 1416, 1374, 1330, 1248, 1222, 1173, 1101, 1013. **MS** (EI) *m/z* 227 [$M^{+\cdot}$], 198, 157, 143, 129, 115. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{13}H_{13}N_3O$ 227.1059, found 227.1057.



4-(1H-indol-3-yl)-2,6-dimethyl-4,5-dihydropyridazin-3(2H)-one ***<u>3ab</u>.*** According to the general procedure for the formation of dihydropyridazinone cycle starting from 100 mg (0.39 mmol) of ethyl 2-(1H-indol-3-yl)-4-oxopentanoate **<u>2a</u>**, 62 µl of methylhydrazine in two portions, the second one added after 24 hours (1.16 mmol, 1.5 eq x 2) in 5 mL of ethanol, the title compound is obtained after 48 h of reaction and purified by flash column chromatography using dichloromethane/methanol 99/1 as eluent system to yield 76 mg (81 %) of a yellow lacquer.

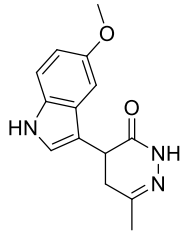
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.03 (s, 3 H), 2.71–2.86 (m, 2 H), 3.41 (s, 3 H), 3.97 (t, *J*= 7.2 Hz, 1 H), 6.72 (d, *J*= 2.4 Hz, 1 H), 7.07-7.18 (m, 2 H), 7.24 (d, *J*= 6.0 Hz, 1 H), 7.61 (d, *J*= 7.2 Hz, 1 H), 8.56 (s, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 23.5, 33.7, 34.8, 36.6, 111.6, 111.8, 119.0, 119.7, 121.8, 122.2, 126.3, 136.5, 153.8, 166.8. **IR** (KBr) ν (cm^{-1}) = 3315, 2948, 1635, 1426, 1345, 1261. **MS** (EI) m/z 241 [$M^{+\cdot}$], 212, 157, 143, 129, 115. **HRMS** (EI, [$M^{+\cdot}$]) calculated for $C_{14}H_{15}N_3O$ 241.1215, found 241.1217.



2-benzyl-4-(1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one ***<u>3ac</u>.*** According to the general procedure for the formation of dihydropyridazinone cycle starting from 100 mg (0.39 mmol) of ethyl 2-(1H-indol-3-yl)-4-oxopentanoate **<u>2a</u>**, 116 mg of benzylhydrazine (0.58 mmol, 1.5 eq) in 10 mL of ethanol, the title compound is obtained after 20 h of reaction and purified by flash column chromatography using dichloromethane/methanol 99.5/0.5 as eluent system to yield 45 mg (63 %) of an orange lacquer.

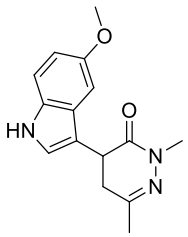
^{1}H NMR (500 MHz, CD_3OD) δ (ppm) = 2.02 (s, 3 H), 2.84-2.94 (m, 2 H), 4.05 (t, *J*= 7.0 Hz, 1 H), 4.88 (d, *J*= 15.0 Hz, 1 H), 4.95 (d, *J*= 15.0 Hz, 1 H), 6.91 (s, 1 H), 7.01 (dt, *J*= 7.0, 1.0 Hz, 1 H), 7.10 (dd, *J*= 7.0, 1.5 Hz, 1 H), 7.22-7.30 (m, 5 H), 7.34 (d, *J*= 8.0 Hz, 1 H), 7.59 (d, *J*= 8.0 Hz, 1 H). **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) = 23.3, 34.4, 36.1, 52.9, 112.2, 112.4, 119.9, 120.1, 122.8, 123.0, 127.5, 128.3, 129.0 (2 C), 129.4 (2 C), 138.2, 139.0, 157.0, 168.8. **IR** (KBr) ν (cm^{-1}) = 3469, 3050, 1721, 1679, 1478,

1423, 1452, 1261. **MS** (ESI+) m/z 318 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{20}H_{20}N_3O$ 318.1606, found 318.1608.



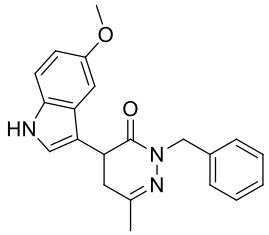
4-(5-Methoxy-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one 3ba. According to the general procedure for the formation of the dihydropyridazinone cycle starting from 80 mg of ethyl 2-(5-methoxy-*1H*-indol-3-yl)-4-oxo-pentanoate **2b** and 22 µL of hydrazine hydrate. The desired compound is obtained after flash column chromatography (dichloromethane/methanol=9.6/0.4) followed by a recrystallization in dichloromethane as a beige powder. Yield: 55 mg (76 %)

Mp: 225-227 °C. **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) = 2.05 (s, 3 H), 2.81-2.97 (m, 2 H), 3.82 (s, 3 H), 3.96 (dt, *J*= 6.9, 0.6 Hz, 1 H), 6.77 (dd, *J*= 8.7, 2.4 Hz, 1 H), 7.02 (s, 1 H), 7.12 (d, *J*= 2.4 Hz, 1 H), 7.23 (dd, *J*= 8.7, 0.6 Hz, 1 H). **^{13}C NMR** (75 MHz, DMSO-*d6*) δ (ppm) = 22.9, 32.3, 33.7, 55.3, 101.2, 111.0, 111.3, 112.0, 122.9, 126.8, 131.4, 152.0, 153.0, 167.4. **MS** (EI) *m/z* 257 [M$^{+\cdot}$], 187, 159. **HRMS** (EI; [M$^{+\cdot}$]) calculated for $C_{14}H_{15}N_3O_2$ 257.1164, found 257.1164.



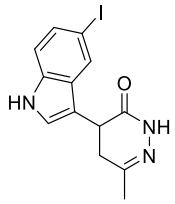
4-(5-methoxy-1H-indol-3-yl)-2,6-dimethyl-4,5-dihydropyridazin-3(2H)-one 3bb. According to the general procedure for the formation of dihydropyridazinone cycle starting from 200 mg (0.69 mmol) of ethyl 2-(5-methoxy-1H-indol-3-yl)-4-oxopentanoate **2b**, 116 µl of methylhydrazine, in two portions, the second one after 24 hours (2.08 mmol, 1.5 eq x 2) in 10 mL of ethanol, the title compound is obtained after 48 h of reaction and purified by flash column chromatography using dichloromethane/methanol 99/1 as eluent system to yield 37 mg (25 %) of a yellow lacquer.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.06 (s, 3 H), 2.72–2.93 (m, 2 H), 3.40 (s, 3 H), 3.85 (s, 3 H), 3.96 (t, *J*= 6.6 Hz, 1 H), 6.81-6.86 (m, 2 H), 7.10 (d, *J*= 2.7 Hz, 1 H), 7.19 (dd, *J*= 8.7, 1.5 Hz, 1 H), 8.35 (s, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 23.6, 33.7, 34.8, 36.6, 56.1, 101.2, 112.2, 112.8, 122.1, 124.0, 126.9, 131.6, 153.3, 154.4, 160.6. **IR** (KBr) ν (cm^{-1}) = 3310, 2923, 1731, 1629, 1488, 1459, 1208. **MS** (EI) m/z 271 [M$^{+\cdot}$], 187, 159. **HRMS** (EI, [M$^{+\cdot}$]) calculated for $C_{15}H_{17}N_3O_2$ 271.1321, found 271.1333.



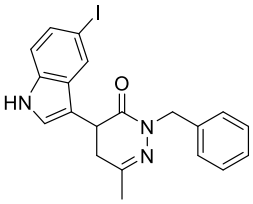
2-benzyl-4-(5-methoxy-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3bc</u>. According to the general procedure for the formation of dihydropyridazinone cycle starting from 100 mg (0.35 mmol) of ethyl 2-(5-methoxy-1H-indol-3-yl)-4-oxopentanoate ***<u>2b</u>***, 104 mg of benzylhydrazine (0.52 mmol, 1.5 eq) in 10 mL of ethanol, the title compound is obtained after 46 h of reaction and purified by flash column chromatography using dichloromethane/ethyl acetate 93/7 as eluent system to yield 49 mg (41 %) of a light brown solid.

Mp: 134-136 °C. **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) = 2.05 (s, 3 H), 2.76 – 2.92 (m, 2 H), 3.82 (s, 3 H), 4.00 (t, *J*= 6.6 Hz, 1 H), 4.96 (s, 2 H), 6.82 (dd, *J*= 6.9, 2.4 Hz, 1 H), 6.84 (s, 1 H), 7.06 (d, *J*= 2.4 Hz, 1 H), 7.21 – 7.35 (m, 7 H). **^{13}C NMR** (150 MHz, $CDCl_3$) δ (ppm) = 23.6, 33.6, 34.9, 52.3, 56.0, 100.9, 111.8, 112.2, 112.7, 122.4, 126.7, 127.4, 128.4 (2 C), 128.5 (2 C), 131.6, 137.9, 153.7, 154.2, 166.3. **IR** (KBr) ν (cm^{-1}) = 3334, 2985, 2832, 1717, 1707, 1652, 1485, 1375, 1208, 1171. **MS** (EI) m/z 347 [$M^{+\cdot}$], 213, 187, 174, 91. **HRMS** (EI, [$M^{+\cdot}$]) calculated for $C_{21}H_{21}N_3O_2$ 347.1634, found 347.1630.



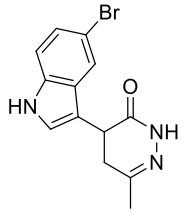
4-(5-iodo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ca</u>. According to the general procedure for the formation of dihydropyridazinone cycle starting from 78 mg (0.20 mmol) of ethyl 2-(5-iodo-1H-indol-3-yl)-4-oxopentanoate **<u>2c</u>**, 72 µL of hydrazine (35 % wt in water, 0.80 mmol, 4 eq in 2 portions, second 0.2 eq added after 6 hours) in 10 mL of ethanol, the title compound is obtained after 24 h of reaction and purified by flash column chromatography using dichloromethane/methanol 98/2 as eluent system to yield 55 mg (77 %) of a reddish-yellow oil.

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) = 2.06 (s, 3 H), 2.79-2.95 (m, 2 H), 3.95 (t, *J*= 7.2 Hz, 1 H), 7.06 (s, 1 H), 7.18 (d, *J*= 8.4 Hz, 1 H), 7.37 (dd, *J*= 8.7, 1.8 Hz, 1H), 7.98 (d, *J*= 1.8 Hz, 1H). **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) = 22.9, 34.0, 35.3, 83.1, 111.7, 114.6, 124.3, 129.0, 130.3, 131.1, 137.3, 156.0, 170.7. **IR** (KBr) ν (cm^{-1}) = 3252, 3114, 2915, 1668, 1642, 1456, 1375, 1312. **MS** (EI) m/z 353 [$M^{+\cdot}$], 324,283, 255. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{13}H_{12}N_3OI$ 353.0025, found 353.0038.



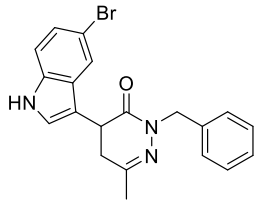
2-benzyl-4-(5-iodo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3cc</u>. According to the general procedure for the formation of dihydropyridazinone cycle starting from 150 mg (0.40 mmol) of ethyl 2-(5-iodo-1H-indol-3-yl)-4-oxopentanoate **<u>2c</u>**, 161 mg of benzylhydrazine (1.60 mmol, 4 eq in 2 portions, second portion added after 19 h) in 10 mL of ethanol, the title compound is obtained after 24 h of reaction and purified by flash column chromatography using dichloromethane/methanol 98/2 as eluent system to yield 79 mg (44 %) of a brownish-yellow solid.

Mp: 103-105 °C. **^{1}H NMR** (500 MHz, $CDCl_3$) δ (ppm) = 2.03 (s, 3 H), 2.71–2.80 (m, 2 H), 3.91 (t, *J*= 7.5 Hz, 1 H), 4.93 (d, *J*= 14.5 Hz, 1 H), 5.02 (d, *J*= 14.5 Hz, 1 H), 6.60 (d, *J*= 2.5 Hz, 1 H), 6.94 (d, *J*= 8.5 Hz, 1H), 7.27–7.40 (m, 6 H), 7.88 (d, *J*= 1.5 Hz, 1 H), 8.44 (s, 1 H). **^{13}C NMR** (500 MHz, $CDCl_3$) δ (ppm) = 23.6, 33.7, 34.8, 52.5, 83.4, 111.2, 113.6, 122.8, 127.6, 127.8, 128.4 (2 C), 128.6 (2 C), 128.8, 130.7, 136.5, 137.9, 153.9, 166.3. **IR** (KBr) ν (cm^{-1}) = 3404, 3063, 2922, 1717, 1639, 1452, 1250. **MS** (ESI +) m/z 466 [M+Na^+], 444 [M+H^+]. **HRMS** (ESI +; [M+H^+]) calculated for $C_{20}H_{19}N_3OI$ 444.0573, found 444.0572.



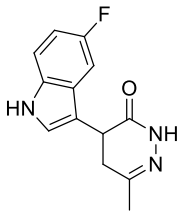
4-(5-bromo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3da</u>. According to the general procedure for the formation of dihydropyridazinone cycle starting from 212 mg of ethyl 2-(5-bromo-1H-indol-3-yl)-4-oxopentanoate (0.63 mmol) **<u>2d</u>**, 125 µL of hydrazine (35 % wt in water, 1.3 mmol, 2 eq) in 10 mL of ethanol, the title compound is obtained after 48 h of reaction and purified by column chromatography using dichloromethane/methanol 98/2 as eluent system to yield 157 mg (81 %) of a brown solid.

Mp: 215-217 °C. **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) = 2.06 (s, 3 H), 2.80-2.97 (m, 2 H), 3.96 (t, *J*= 7.2 Hz, 1 H), 7.11 (s, 1 H), 7.21 (dd, *J*= 8.7, 2.1 Hz, 1H), 7.28 (d, *J*= 8.4 Hz, 1H), 7.79 (d, *J*= 1.8 Hz, 1H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ (ppm) = 22.8, 32.0, 33.6, 111.1, 111.2, 113.5, 121.7, 123.7, 124.0, 128.3, 135.0, 152.1, 167.2. **IR** (KBr) ν (cm^{-1}) = 3333, 3239, 1669, 1645, 1460, 1384, 1321. **MS** (EI) m/z 305 [$M^{+\cdot}$], 235, 207. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{13}H_{12}N_3OBr$ 305.0164, found 305.0159.



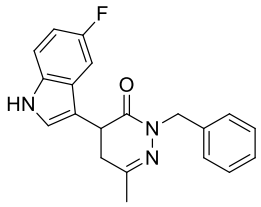
2-benzyl-4-(5-bromo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one ***<u>3dc</u>.*** According to the general procedure for the formation of dihydropyridazinone cycle starting from 100 mg (0.30 mmol) of ethyl 2-(5-bromo-1H-indol-3-yl)-4-oxopentanoate **<u>2d</u>**, 89 mg of benzylhydrazine (0.88 mmol, 3 eq in 2 portions, second portion added after 25 h) in 10 mL of ethanol, the title compound is obtained after 48 h of reaction and purified by flash column chromatography using dichloromethane/methanol 98/2 as eluent system to yield 51 mg (43 %) of a light brown lacquer.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 2.02 (s, 3 H), 2.72-2.75 (m, 2 H), 3.89 (t, *J*= 7.8 Hz, 1 H), 4.94 (d, *J*= 14.4 Hz, 1 H), 5.03 (d, *J*= 14.4 Hz, 1 H), 6.52 (d, *J*= 2.4 Hz, 1 H), 6.95 (d, *J*= 8.7 Hz, 1 H), 7.15 (dd, *J*= 8.4, 1.8 Hz, 1 H), 7.27-7.41 (m,5 H), 7.66 (d, *J*= 1.8 Hz, 1H), 8.59 (s, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 23.5, 33.6, 34.8, 52.5, 111.2, 112.9, 113.1, 121.5, 123.3, 125.0, 127.5, 127.9, 128.3 (2 C), 128.6 (2 C), 135.1, 137.8, 154.1, 166.5. **IR** (KBr) ν (cm^{-1}) = 3410, 3284, 2937, 1712, 1644, 1456, 1422, 1341, 1255. **MS** (EI) m/z 395 [$M^{+\cdot}$], 222, 143, 91, 77. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{20}H_{18}N_3OBr$ 395.0633, found 395.0626.



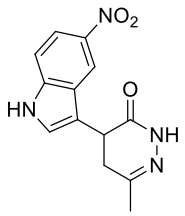
4-(5-fluoro-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one ***<u>3ea</u>.*** According to the general procedure for the formation of dihydropyridazinone cycle starting from 200 mg (0.72 mmol) of ethyl 2-(5-fluoro-1H-indol-3-yl)-4-oxopentanoate <u>2e</u>, 98 µL of hydrazine (35 % wt in water, 1.08 mmol, 1.5 eq) in 20 mL of ethanol, the title compound is obtained after 24 h of reaction and purified by flash column chromatography using dichloromethane/methanol 98/2 as eluent system to yield 120 mg (68 %) of a yellow solid.

Mp: 198-200 °C. **^{1}H NMR** (500 MHz, DMSO-*d6*) δ (ppm) = 2.01 (s, 3 H), 2.73-2.84 (m, 2 H), 3.80 (t, *J*= 7.5 Hz, 1 H), 6.92 (dt, *J*= 9.0, 2.5 Hz, 1 H), 7.18 (d, *J*= 2.5 Hz, 1H), 7.34 (dd, *J*= 8.5, 4.5 Hz, 1H), 7.36 (dd, *J*= 10.5, 2.5 Hz, 1H), 10.5 (s, 1 H), 11.1 (s, 1 H). **^{13}C NMR** (125 MHz, CD_3OD) δ (ppm) =23.0, 33.9, 35.4, 104.6 (d, *J*= 23.9 Hz, 1 C), 110.8 (d, *J*= 26.3 Hz, 1 C), 113.2 (d, *J*= 9.5 Hz, 1 C), 125.2, 127.9, 134.8, 156.1, 158.0, 159.9, 170.7. **IR** (KBr) ν (cm^{-1}) = 3425, 3239, 2918, 2379, 1668, 1480, 1375, 1320, 1265. **MS** (ESI+) m/z 246 [$M+H^+$]. **HRMS** (ESI; [$M+H^+$]) calculated for $C_{13}H_{13}N_3OF$ 246.1043, found 246.1045.



2-benzyl-4-(5-fluoro-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ec</u>. According to the general procedure for the formation of dihydropyridazinone cycle starting from 200 mg (0.72 mmol) of ethyl 2-(5-fluoro-1H-indol-3-yl)-4-oxopentanoate **<u>2e</u>**, 218 mg of benzylhydrazine (1.08 mmol, 1.5 eq) in 20 mL of ethanol, the title compound is obtained after 24 h of reaction and purified by flash column chromatography using dichloromethane/methanol 99/1 as eluent system to yield 97 mg (40 %) of an orange lacquer.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 2.01 (s, 3 H), 2.68–2.80 (m, 2 H), 3.89 (t, *J*= 7.5 Hz, 1 H), 4.97 (s, 2 H), 6.63 (d, *J*= 2.5 Hz, 1 H), 6.83 (dt, *J*= 9.0, 2.5 Hz, 1 H), 6.87 (dd, *J*= 9.0, 2.5 Hz, 1 H), 7.03 (dd, *J*= 8.5, 4.5 Hz, 1 H), 7.20 (dd, *J*= 10.0, 2.5 Hz, 1 H), 7.23–7.32 (m, 3 H), 7.35–7.36 (m, 1 H), 8.63 (s, 1 H). **^{13}C NMR** (500 MHz, $CDCl_3$) δ (ppm) = 23.5, 33.4, 34.9, 52.4, 103.9 (d, *J*= 23.8 Hz, 1 C), 110.6 (d, *J*= 26.1 Hz, 1 C), 112.2 (d, *J*= 9.6 Hz, 1 C), 123.7, 127.5, 128.3 (2 C), 128.5 (2 C), 133.0, 137.8, 154.0, 156.9, 158.7, 166.4, 207.0. **IR** (KBr) ν (cm^{-1}) = 3466, 3058, 1716, 1658, 1491, 1261. **MS** (ESI+) m/z 336 [M+H$^+$],. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{20}H_{19}N_3OF$ 336.1512, found 336.1514.



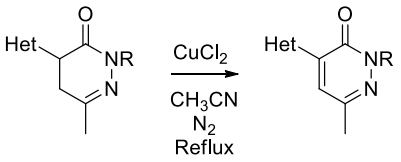
6-methyl-4-(5-nitro-1H-indol-3-yl)-4,5-dihydropyridazin-3(2H)-one <u>3fa</u>. According to the general procedure for the formation of dihydropyridazinone cycle starting from 50 mg (0.16 mmol) of ethyl 2-(5-nitro-1H-indol-3-yl)-4-oxopentanoate <u>2f</u>, 22 µL of hydrazine (35 % wt in water, 0.25 mmol) in 5 mL of ethanol, the title compound is obtained after 20 h of reaction and purified by flash column chromatography using dichloromethane/methanol 99/1 as eluent system to yield 33 mg (73 %) of pale yellow crystals.

Mp: 260-262 °C. **^{1}H NMR** (500 MHz, DMSO-*d*6) δ (ppm) = 2.04 (s, 3 H), 2.84 (d, *J*= 7.5 Hz, 2 H), 4.00 (t, *J*= 7.5 Hz, 1 H), 7.40 (d, *J*= 2.5 Hz, 1 H), 7.53 (d, *J*= 9.0 Hz, 1 H), 8.00 (dd, *J*= 9.0, 2.5 Hz, 1 H), 8.66 (d, *J*= 2.5 Hz, 1 H), 10.6 (s, 1 H), 11.7, (s, 1 H). **^{13}C NMR** (150 MHz, DMSO-*d*6) δ (ppm) = 22.8, 31.8, 33.5, 112.0, 114.3, 116.7, 117.1, 126.0, 126.4, 139.6, 140.4, 152.2, 167.1. **IR** (KBr) ν (cm^{-1}) = 3328, 3192, 1627, 1512, 1475, 1334, 1099. **MS** (ESI+) m/z 273 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{13}H_{13}N_4O_3$ 273.0988, found 273.0993.

Preparation of pyridazinone derivatives

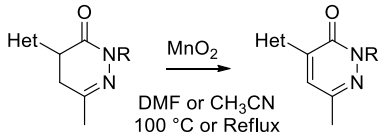
General procedure for the oxidation to pyridazinone:

Method A :

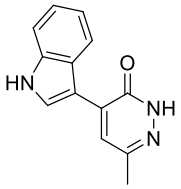


One equivalent of dihydropyridazinone is dissolved in anhydrous acetonitrile. Thus, 2 equivalents of anhydrous $CuCl_2$ are added to the solution. The mixture is stirred and heated at reflux under inert atmosphere (N_2 atmosphere) for 2 to 12 hours. The solvent is finally evaporated under reduced pressure and the crude mixture is purified by column chromatography.

Method B :



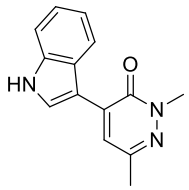
One equivalent of dihydropyridazinone is dissolved in anhydrous DMF or in acetonitrile. Thus, 17 equivalents of manganese dioxide are added to the solution. The mixture is stirred and heated respectively at 100 °C or at reflux for 3 to 12 hours (TLC monitoring). The solvent is finally evaporated under reduced pressure and the crude mixture is purified by cristallisation in methanol and/or column chromatography.



4-(1H-indol-3-yl)-6-methylpyridazin-3(2H)-one <u>4aa</u>. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 120 mg of 4-(1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **<u>3aa</u>** (0.54 mmol), 798 mg of MnO_2 in 2 mL of acetonitrile, the title compound is obtained after 20 hours of reaction at reflux and purification by crystallisation in methanol and flash column chromatography using dichloromethane/methanol 95/5 as eluent system to yield 60 mg (50 %) of a brown solid.

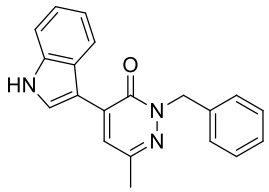
Mp: 282-284 °C. **^{1}H NMR** (300 MHz, DMSO-d_6) δ (ppm) = 2.34 (s, 3 H), 7.15-7.25 (m, 2 H), 7.51 (dd, *J*= 6.6, 1.8 Hz, 1 H), 7.71 (s, 1 H), 8.08 (dd, *J*= 6.9, 1.8 Hz, 1 H), 8.63 (d, *J*= 3.0 Hz, 1 H), 11.71 (s, 1 H), 12.71 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ (ppm) = 20.5, 107.6, 112.3, 120.1, 120.6, 122.1,

124.2, 125.0, 130.9, 133.9, 136.4, 144.7, 159.5 **IR** (KBr) ν (cm^{-1}) = 3241, 2961, 2878, 1643, 1601, 1556, 1441, 1125. **MS** (EI) m/z 225 [$M^{+\cdot}$], 169, 154. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{13}H_{11}N_3O$ 225.0902, found 225.0903. **Log P** 0.56



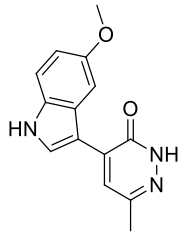
4-(1H-indol-3-yl)-2,6-dimethylpyridazin-3(2H)-one 4ab. According to the general procedure for the oxydation to pyridazinone (Method A) starting from 18 mg of 4-(1*H*-indol-3-yl)-2,6-dimethyl-4,5-dihydropyridazin-3(2*H*)-one ***3ab*** (0.07 mmol), 20 mg of anhydrous $CuCl_2$ in 2 mL of dried acetonitrile, the title compound is obtained after 24 hours of reaction at reflux and purification by column chromatography using dichloromethane/methanol 98/2 as eluent system to yield 6 mg (36 %) of a white lacquer.

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) = 2.40 (s, 3 H), 3.81 (s, 3 H), 7.19-7.23 (m, 2 H), 7.46-7.49 (m, 1 H), 7.74 (s, 1 H), 7.96-7.99 (m, 1 H), 8.48 (s, 1 H). **^{13}C NMR** (75 MHz, CD_3OD) δ (ppm) = 20.9, 41.1, 109.2, 113.1, 120.8, 122.0, 123.4, 125.9, 126.7, 131.8, 135.8, 138.3, 147.2, 161.1. **IR** (KBr) ν (cm^{-1}) = 3458, 3216, 2420, 1632, 1593, 1533, 1454, 1235. **MS** (EI) m/z 239 [$M^{+\cdot}$], 183, 168, 142. **HRMS** (EI, [$M^{+\cdot}$]) calculated for $C_{14}H_{13}N_3O$ 239.1059, found 239.1060. **Log P** 0.8



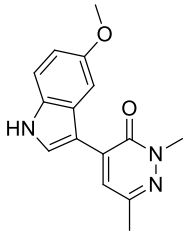
2-benzyl-4-(1H-indol-3-yl)-6-methylpyridazin-3(2H)-one 4ac. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 30 mg of 2-benzyl-4-(1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **3ac** (0.08 mmol), 112 mg of MnO_2 in 2 mL of dimethylformamide, the title compound is obtained after 24 hours of reaction at 100 °C and purification by flash column chromatography using dichloromethane/methanol 98/2 as eluent system to yield 12 mg (34 %) of a beige solid.

Mp: 229-231 °C. **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) = 2.43 (s, 3 H), 5.40 (s, 2 H), 7.16 (dd, *J*= 7.5, 2.1 Hz, 2 H), 7.28-7.36 (m, 6 H), 7.69 (s, 1 H), 7.99 (dd, *J*= 5.7, 2.1 Hz, 1 H), 8.78 (s, 1 H), 10.96 (s, 1 H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ (ppm) = 21.2, 50.8, 107.8, 111.1, 120.2, 121.5, 122.8, 124.9, 126.5, 126.9 (2 C), 128.0, 129.0 (3 C), 134.9, 136.6, 136.9, 146.1. **IR** (KBr) ν (cm^{-1}) = 2959, 2925, 2854, 1638, 1590, 1392, 1261. **MS** (EI) m/z 315 [$M^{+\cdot}$], 224, 91. **HRMS** (EI, [$M^{+\cdot}$]) calculated for $C_{20}H_{17}N_3O$ 315.1372, found 315.1387. **Log P** 2.53



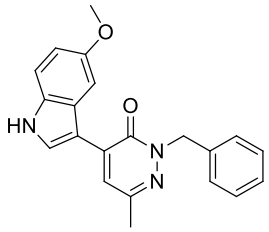
4-(5-methoxy-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one ***<u>4ba</u>.*** According to the general procedure for the oxydation to pyridazinone (Method B) starting from 50 mg of 4-(5-methoxy-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **<u>3ba</u>** (0.19 mmol), 281 mg of MnO_2 in 1 mL of anhydrous dimethylformamide, the title compound is obtained after 3 hours of reaction and purification by crystallisation in methanol to yield 30 mg (60 %) of a light brown solid.

Mp: 290-292 °C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ (ppm) = 2.35 (s, 3 H), 6.88 (d, *J*= 9.0 Hz, 1 H), 7.41 (d, *J*= 9.0 Hz, 1 H), 7.47 (s, 1 H), 7.62 (s, 1 H), 8.56 (s, 1 H), 11.56 (s, 1 H), 12.67 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ (ppm) = 20.5, 55.7, 103.1, 107.3, 111.3, 112.9, 124.0, 125.5, 131.3, 131.5, 134.1, 144.7, 154.6, 159.5. **IR** (KBr) ν (cm^{-1}) = 3427, 3163, 2956, 1643, 1598, 1551, 1486, 1141. **MS** (EI) m/z 255 [$M^{+\cdot}$], 240, 212, 198. **HRMS** (EI; [$M^{+\cdot}$]) calculated for $C_{14}H_{13}N_3O_2$ 255.1008, found 255.1015. **Log P** 0.43



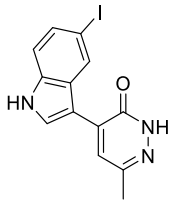
4-(5-methoxy-1H-indol-3-yl)-2,6-dimethylpyridazin-3(2H)-one ***<u>4bb</u>.*** According to the general procedure for the oxydation to pyridazinone (Method B) starting from 17 mg of 4-(5-methoxy-1*H*-indol-3-yl)-2,6-dimethyl-4,5-dihydropyridazin-3(2*H*)-one ***<u>3bb</u>*** (0.07 mmol), 104 mg of MnO_2 in 1.5 mL of dimethylformamide, the title compound is obtained after 6 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 99/1 as eluent system to yield 6 mg (35 %) of an orange lacquer.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 2.41 (s, 3 H), 3.86 (s, 3 H), 3.91 (s, 3 H), 6.93 (dd, *J*= 9.0, 2.5 Hz, 1 H), 7.35 (d, *J*= 9.0 Hz, 1 H), 7.41 (d, *J*= 2.5 Hz, 1 H), 7.56 (s, 1 H), 8.61 (d, *J*= 2.5 Hz, 1 H), 8.83 (s, 1 H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ (ppm) = 21.3, 40.8, 56.3, 103.7, 108.9, 111.5, 112.6, 124.4, 126.3, 126.7, 131.3, 131.6, 134.5, 144.7, 156.4. **IR** (KBr) ν (cm^{-1}) = 3432, 3220, 2940, 1627, 1583, 1483, 1441, 1292, 1214. **MS** (ESI+) m/z 270 [$M^{+\cdot}$]. **HRMS** (ESI+, [$M+H^{+\cdot}$]) calculated for $C_{15}H_{16}N_3O_2$ 270.1243, found 270.1244. **Log P** 0.67



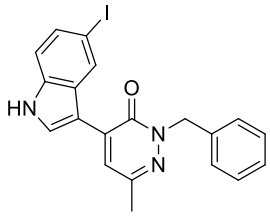
2-benzyl-4-(5-methoxy-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one <u>4bc</u>. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 20 mg of 2-benzyl-4-(5-methoxy-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **<u>3bc</u>** (0.06 mmol), 85 mg of MnO_2 in 1 mL of dimethylformamide, the title compound is obtained after 28 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 99/1 as eluent system followed by trituration in diethylether to yield 11 mg (46 %) of a beige solid.

Mp: 158-160 °C. **^{1}H NMR** (500 MHz, DMSO-*d*6) δ (ppm) = 2.38 (s, 3 H), 3.83 (s, 3 H), 5.32 (s, 2 H), 6.88 (dd, *J*= 8.5, 2.5 Hz, 1 H), 7.26-7.29 (m, 1 H), 7.32-7.35 (m, 5 H), 7.40 (d, *J*= 8.5 Hz, 1 H), 7.47 (d, *J*= 2.5 Hz, 1 H), 7.66 (s, 1 H), 11.61 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-*d*6) δ (ppm) = 21.2, 55.2, 56.1, 103.6, 107.9, 111.8, 113.4, 126.0, 127.8, 128.2 (2 C), 128.9 (2 C), 131.9, 132.1, 134.5, 138.0, 145.2, 150.1, 155.1. **IR** (KBr) ν (cm^{-1}) = 3448, 3299, 2926, 1621, 1579, 1475, 1430, 1286, 1234. **MS** (ESI+) m/z 346 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{21}H_{20}N_3O_2$ 346.1556, found 346.1558. **Log P** 0.2.4



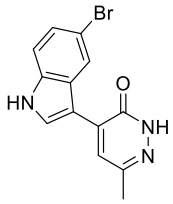
4-(5-iodo-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one <u>4ca</u>. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 60 mg (0.17 mmol) of 4-(5-iodo-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **<u>3ca</u>**, 251 mg of MnO_2 in 3 mL of dimethylformamide, the title compound is obtained after 2 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 95/5 as eluent system to yield 54 mg (90 %) of a beige powder.

Mp: 309-311 °C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ (ppm) = 2.35 (s, 3 H), 7.35 (d, *J*= 8.5 Hz, 1 H), 7.48 (dd, *J*= 8.0, 1.5 Hz, 1 H), 7.64 (s, 1 H), 8.35 (s, 1 H), 8.54 (d, *J*= 3.0 Hz, 1 H), 11.8 (s, 1 H), 12.7 (s, 1 H). **^{13}C NMR** (75 MHz, CD_3OD) δ (ppm) = 20.7, 85.1, 108.6, 115.2, 127.2, 128.4, 129.1, 129.7, 130.0, 135.9, 137.4, 148.0, 163.1. **IR** (KBr) ν (cm^{-1}) = 3166, 2953, 1646, 1594, 1485, 1440, 1213, 1134. **MS** (EI) m/z 351 [M$^{+\cdot}$], 224, 127. **HRMS** (EI; [M$^{+\cdot}$]) calculated for $C_{13}H_{10}N_3OI$ 350.9869, found 350.9885. **Log P** 1.92



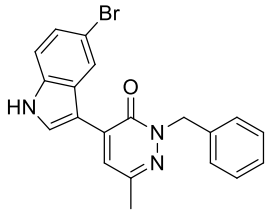
2-benzyl-4-(5-iodo-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one ***<u>4cc</u>.*** According to the general procedure for the oxydation to pyridazinone (Method B) starting from 26 mg of 2-benzyl-4-(5-iodo-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **<u>3cc</u>** (0.06 mmol), 174 mg of MnO_2 (in 2 portions, the second portion added after 4 hours) in 2 mL of dimethylformamide, the title compound is obtained after 48 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 99.5/0.5 as eluent system followed by trituration in diethylether to yield 25 mg (50 %) of beige crystals.

Mp: 255-257 °C. **^{1}H NMR** (500 MHz, DMSO-*d*6) δ (ppm) = 2.39 (s, 3 H), 5.31 (s, 2 H), 7.26-7.29 (m, 1 H), 7.31-7.36 (m, 5 H), 7.49 (dd, *J*= 8.5, 1.5 Hz, 1 H), 7.68 (s, 1 H), 8.35 (d, *J*= 2.0 Hz, 1 H), 8.52 (d, *J*= 2.5 Hz, 1 H), 11.89 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-*d*6) δ (ppm) = 20.7, 54.7, 85.0, 107.3, 114.6, 124.8, 127.4, 127.5, 127.7 (2 C), 128.2, 128.4 (2 C), 130.3, 131.6, 133.3, 135.5, 137.4, 144.8, 158.0. **IR** (KBr) ν (cm^{-1}) = 3320, 1632, 1588, 1449, 1420, 1109. **MS** (ESI+) m/z 442 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{20}H_{17}N_3OI$ 442.0416, found 442.0417.



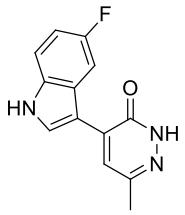
4-(5-bromo-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one ***<u>4da</u>.*** According to the general procedure for the oxydation to pyridazinone (Method B) starting from 60 mg of 4-(5-bromo-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **<u>3da</u>** (0.20 mmol), 290 mg of MnO_2 in 3 mL of dimethylformamide, the title compound is obtained after 2 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 95/5 as eluent system to yield 36 mg (60 %) of a beige powder.

Mp: 297-299 °C. **^{1}H NMR** (300 MHz, DMSO-d_6) δ (ppm) = 2.35 (s, 3 H), 7.33 (dd, *J*= 8.7, 1.8 Hz, 1 H), 7.47 (d, *J*= 8.7 Hz, 1 H), 7.67 (s, 1 H), 8.23 (d, *J*= 1.5 Hz, 1 H), 8.60 (s, 1 H), 11.89 (s, 1 H), 12.76 (s, 1 H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ (ppm) = 20.5, 107.5, 113.4, 114.2, 122.4, 124.7, 125.1, 126.7, 133.3, 135.0, 135.2, 144.8, 159.4. **IR** (KBr) ν (cm^{-1}) = 3195, 2926, 1644, 1592, 1548, 1459, 1420, 1294. **MS** (EI) m/z 303 [M$^{+\cdot}$], 245, 167, 139. **HRMS** (EI; [M$^{+\cdot}$]) calculated for $C_{13}H_{10}N_3OBr$ 303.0007, found 303.0019. **Log P** 1.39



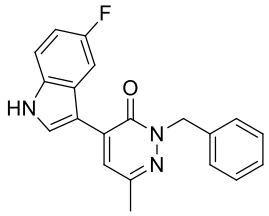
2-benzyl-4-(5-bromo-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one <u>4dc</u>. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 30 mg of 2-benzyl-4-(5-bromo-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one ***<u>3dc</u>*** (0.08 mmol), 112 mg of MnO_2 in 2 mL of dimethylformamide, the title compound is obtained after 28 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 98/2 as eluent system followed by trituration in diethylether to yield 14 mg (40 %) of a yellow solid.

Mp: 226-228 °C**. ^{1}H NMR** (500 MHz, DMSO-*d*6) δ (ppm) = 2.39 (s, 3 H), 5.31 (s, 2 H), 7.25-7.30 (m, 1 H), 7.32-7.35 (m, 5 H), 7.46 (d, *J*= 8.5 Hz, 1 H), 7.70 (s, 1 H), 8.22 (d, *J*= 1.5 Hz, 1 H), 8.58 (d, *J*= 3.0 Hz, 1 H), 11.92 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-*d*6) δ (ppm) = 20.6, 54.7, 107.6, 113.4, 114.2, 122.3, 124.7, 124.8, 126.7, 127.4, 127.7 (2 C), 128.4 (2 C), 132.1, 133.2, 135.2, 137.4, 144.9, 158.0. **IR** (KBr) ν (cm^{-1}) = 3283, 1630, 1585, 1457, 1428, 1114. **MS** (ESI+) m/z 394 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{20}H_{17}N_3OBr$ 394.0555, found 394.0554.



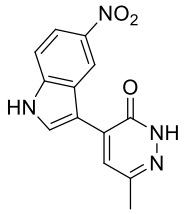
4-(5-fluoro-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one <u>4ea</u>. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 60 mg of 4-(5-fluoro-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **<u>3ea</u>** (0.25 mmol), 362 mg of MnO_2 in 3 mL of dimethylformamide, the title compound is obtained after 2 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 95/5 as eluent system to yield 17 mg (28 %) of a beige solid.

Mp: 294-296 °C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ (ppm) = 2.35 (s, 3 H), 7.06 (dt, *J*= 9.0, 2.5 Hz, 1 H), 7.50 (dd, *J*= 9.0, 5.0 Hz, 1 H), 7.66, (s, 1 H), 7.88 (dd, *J*= 11.0, 2.5 Hz, 1 H), 8.66 (d, *J*= 3.0 Hz, 1 H), 11.79 (s, 1 H), 12.71 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ (ppm) = 20.4, 105.5 (d, *J*= 24.6 Hz, 1 C), 107.9, 110.1 (d, *J*= 25.6 Hz, 1 C), 113.2 (d, *J*= 9.9 Hz, 1 C), 124.5, 132.4, 133.1, 133.4, 144.8, 157.0, 158.9, 159.4. **IR** (KBr) ν (cm^{-1}) 3223, 2959, 1658, 1630, 1590, 1475, 1439. **MS** (ESI+) m/z 244 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{13}H_{11}N_3OF$ 244.0886, found 244.0887. **Log P** 0.72



2-benzyl-4-(5-fluoro-1H-indol-3-yl)-6-methylpyridazin-3(2H)-one 4ec. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 40 mg of 2-benzyl-4-(5-fluoro-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **3ec** (0.06 mmol), 352 mg of MnO_2 (in 2 portions, the second portion added after 4 hours) in 3 mL of dimethylformamide, the title compound is obtained after 48 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 99.5/0.5 as eluent system followed by trituration in diethylether to yield 20 mg (45 %) of a white solid.

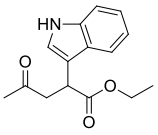
Mp: 250-252 °C. **^{1}H NMR** (500 MHz, DMSO-*d*6) δ (ppm) = 2.38 (s, 3 H), 5.31 (s, 2 H), 7.06 (dt, *J*= 9.0, 2.5 Hz, 1 H), 7.26-7.29 (m, 1 H), 7.32-7.35 (m, 4 H), 7.50 (dd, *J*= 8.5, 4.5 Hz, 1 H), 7.69 (s, 1 H), 7.89 (dd, *J*= 11.0, 2.5 Hz, 1 H), 8.64 (d, *J*= 3.0 Hz, 1 H), 11.83 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-*d*6) δ (ppm) = 20.6, 54.7, 105.5 (d, *J*= 24.6 Hz, 1 C), 108.0, 110.2 (d, *J*= 25.9 Hz, 1 C), 113.2 (d, *J*= 9.9 Hz, 1 C), 124.2, 127.3, 127.7 (2 C), 128.4 (2 C), 132.7, 133.0, 133.4, 137.4, 144.9, 157.1, 158.0, 158.9. **IR** (KBr) ν (cm^{-1}) = 3267, 1627, 1588, 1481, 1426, 1235. **MS** (ESI+) m/z 334 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{20}H_{17}N_3OF$ 334.1356, found 334.1358.



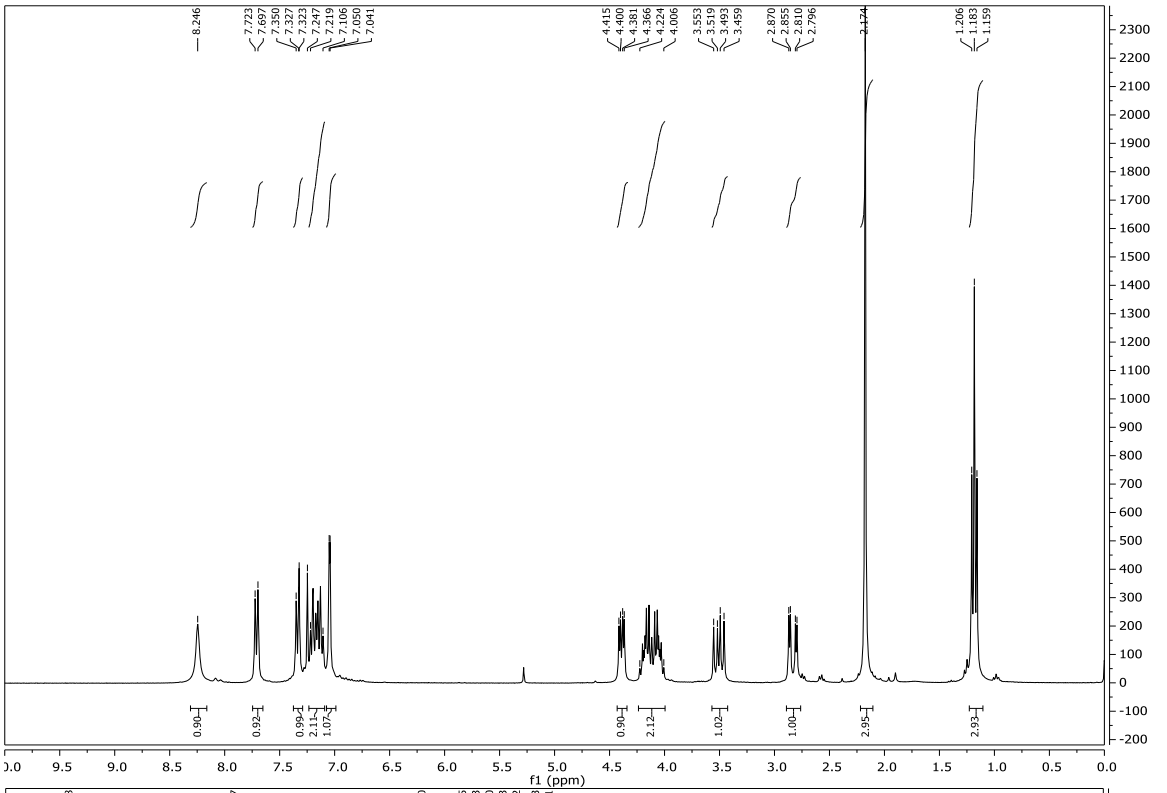
6-methyl-4-(5-nitro-1H-indol-3-yl)pyridazin-3(2H)-one 4fa. According to the general procedure for the oxydation to pyridazinone (Method B) starting from 20 mg of 4-(5-nitro-1*H*-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2*H*)-one **3fa** (0.07 mmol), 109 mg of MnO_2 in 1 mL of dimethylformamide, the title compound is obtained after 20 hours of reaction at reflux and purification by flash column chromatography using dichloromethane/methanol 95/5 as eluent system to yield 16 mg (80 %) of a bright yellow solid.

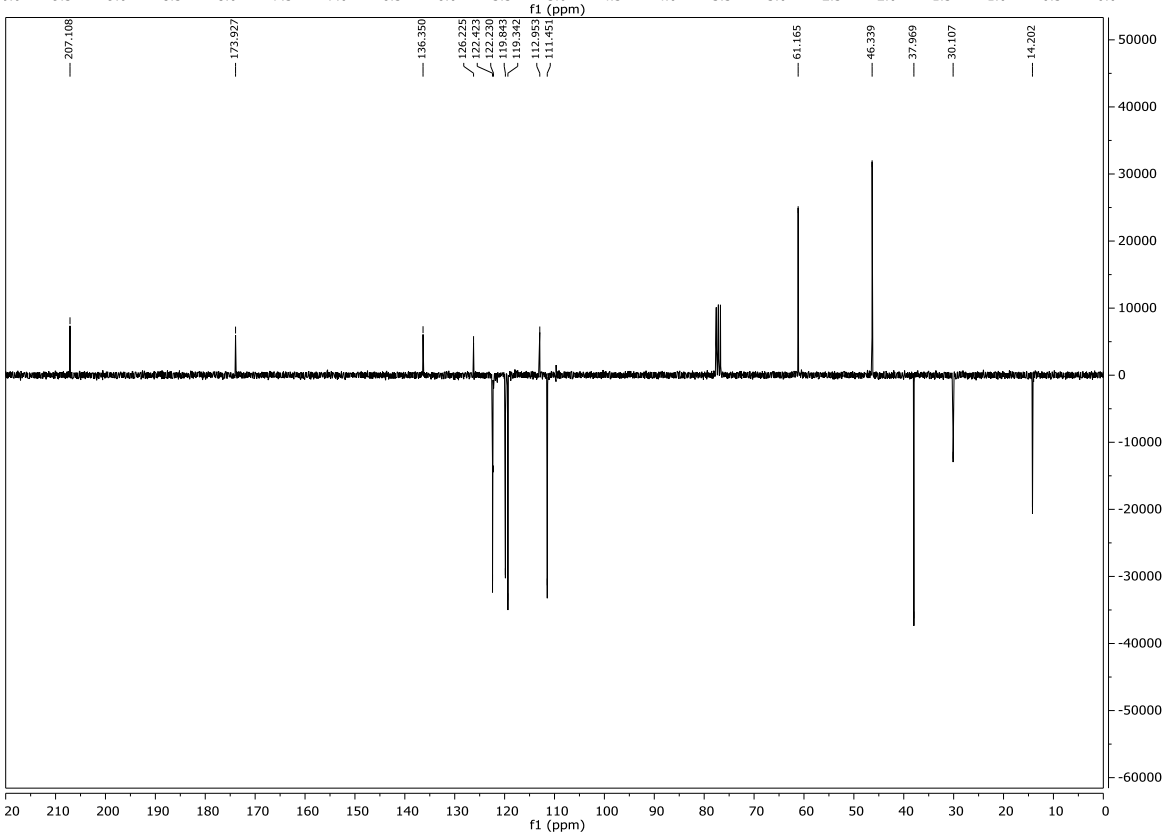
Mp: 338-340 °C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ (ppm) = 2.37 (s, 3 H), 7.67 (d, *J*= 9.0 Hz, 1 H), 7.73 (s, 1 H), 8.09 (dd, *J*= 9.0, 2.0 Hz, 1 H), 8.63 (d, *J*= 2.5 Hz, 1 H), 12.33 (s, 1 H), 12.86 (s, 1 H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ (ppm) = 20.5, 110.3, 112.7, 117.3, 117.5, 124.3, 126.6, 132.8, 133.2, 139.7, 141.6, 147.7, 159.3. **IR** (KBr) ν (cm^{-1}) 3307, 2961, 2886, 2472, 1643, 1601, 1470, 1316, 1295. **MS** (ESI+) m/z 271 [M+H$^+$]. **HRMS** (ESI; [M+H$^+$]) calculated for $C_{13}H_{11}N_4O$ 271.0831, found 271.0828. **Log P** 1.13

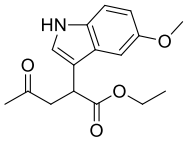
^{1}H and ^{13}C NMR spectra :



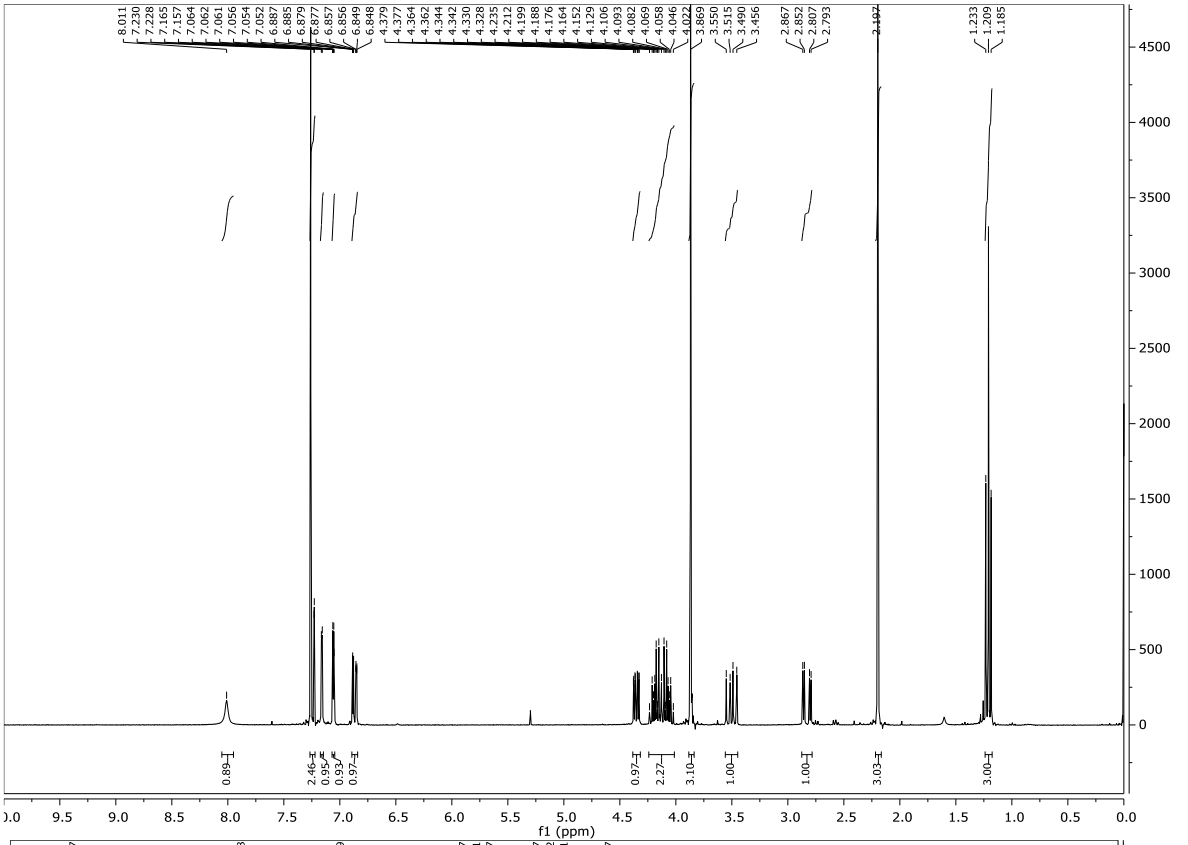
Ethyl 2-(1H-indol-3-yl)-4-oxo-pentanoate <u>2a</u>

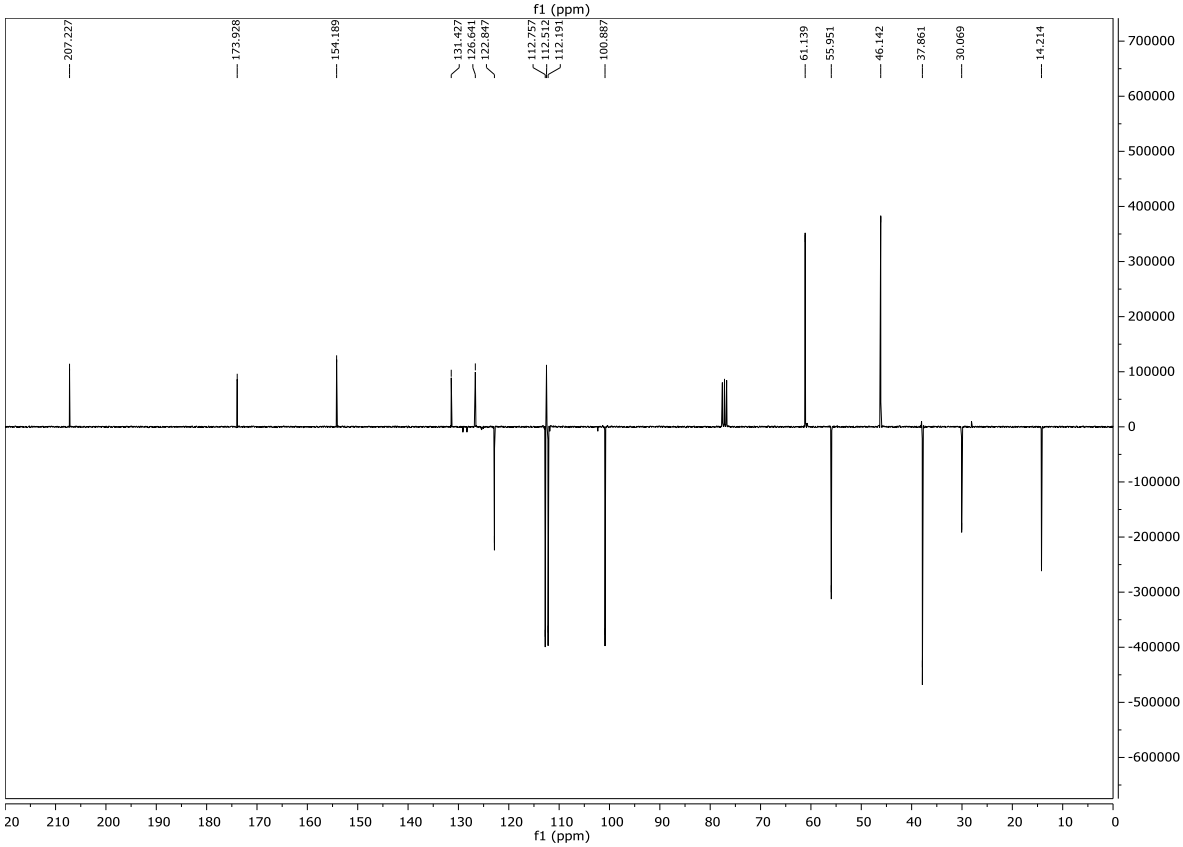


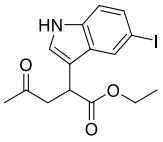




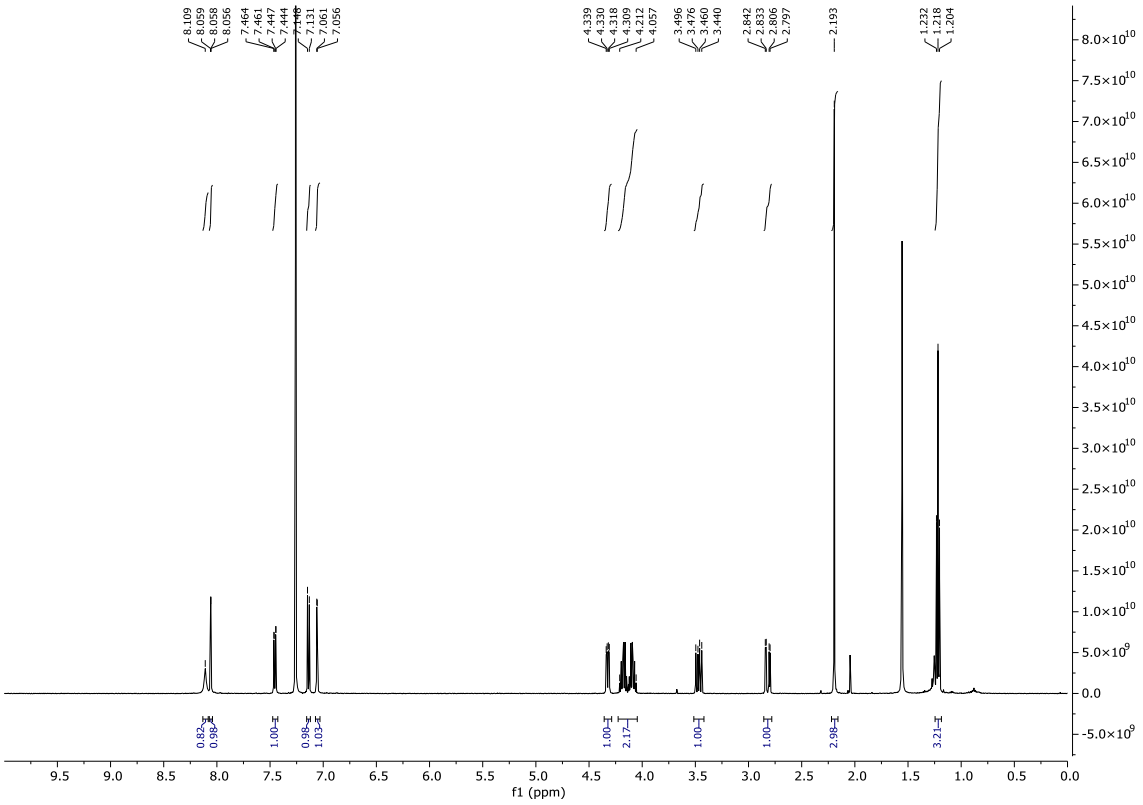
Ethyl 2-(5-methoxy-1H-indol-3-yl)-4-oxo-pentanoate <u>2b</u>

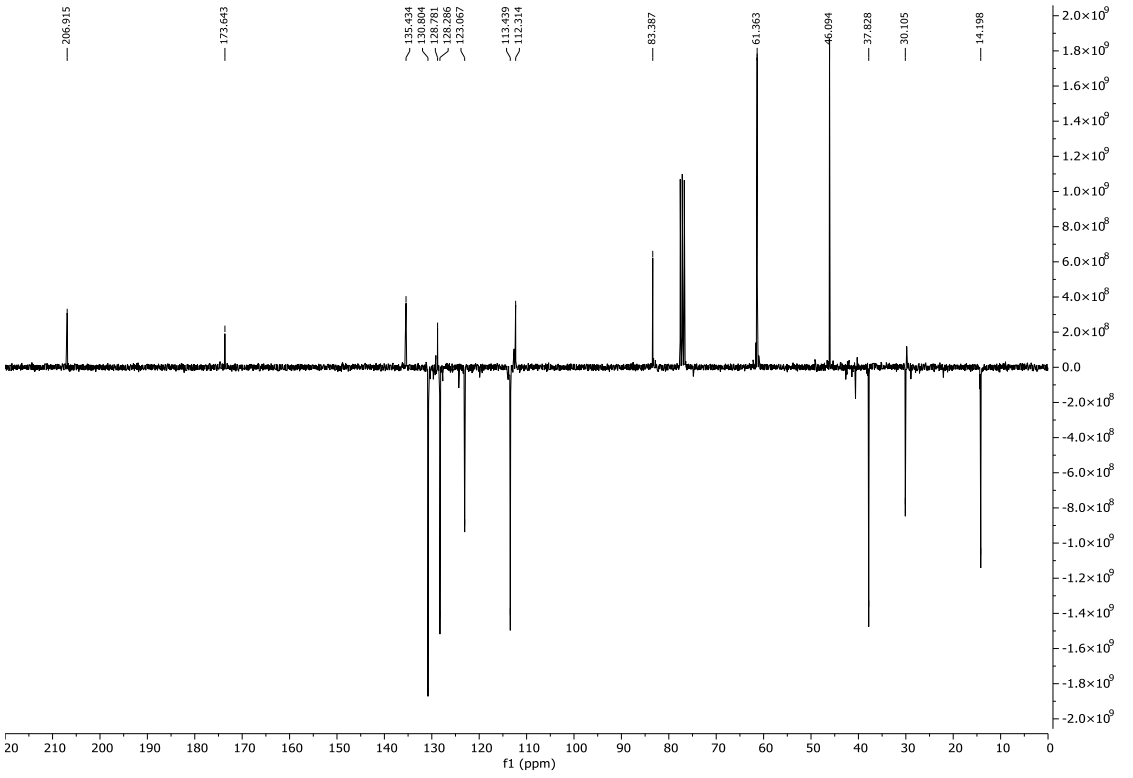


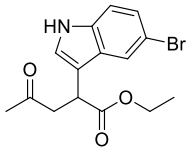




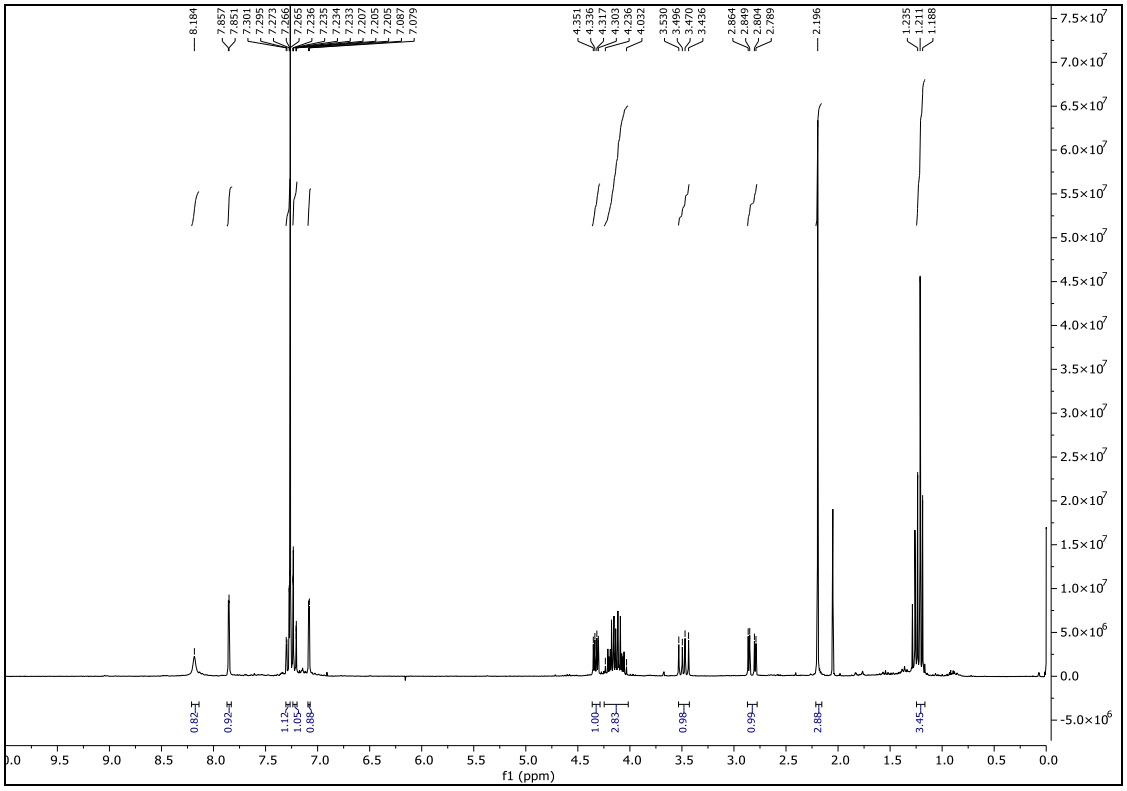
***Ethyl 2-(5-iodo-1H-indol-3-yl)-4-oxopentanoate* <u>2c</u>**

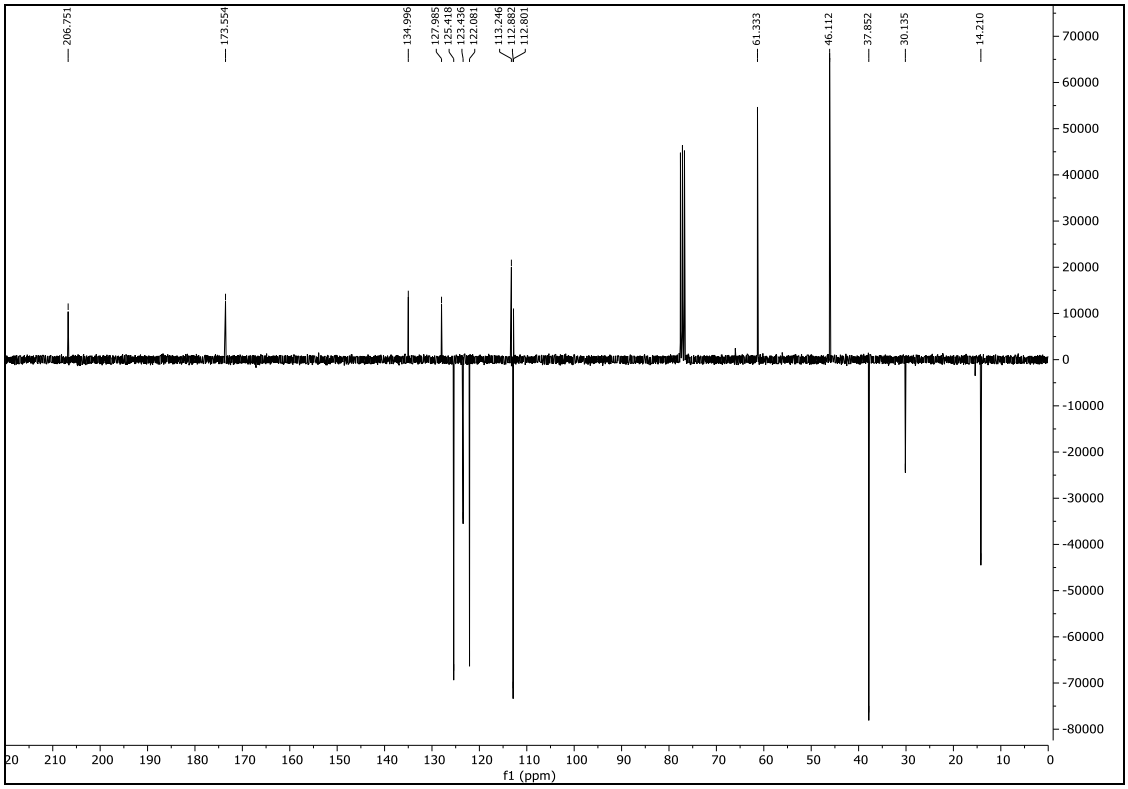


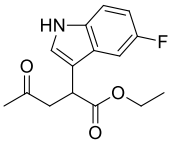




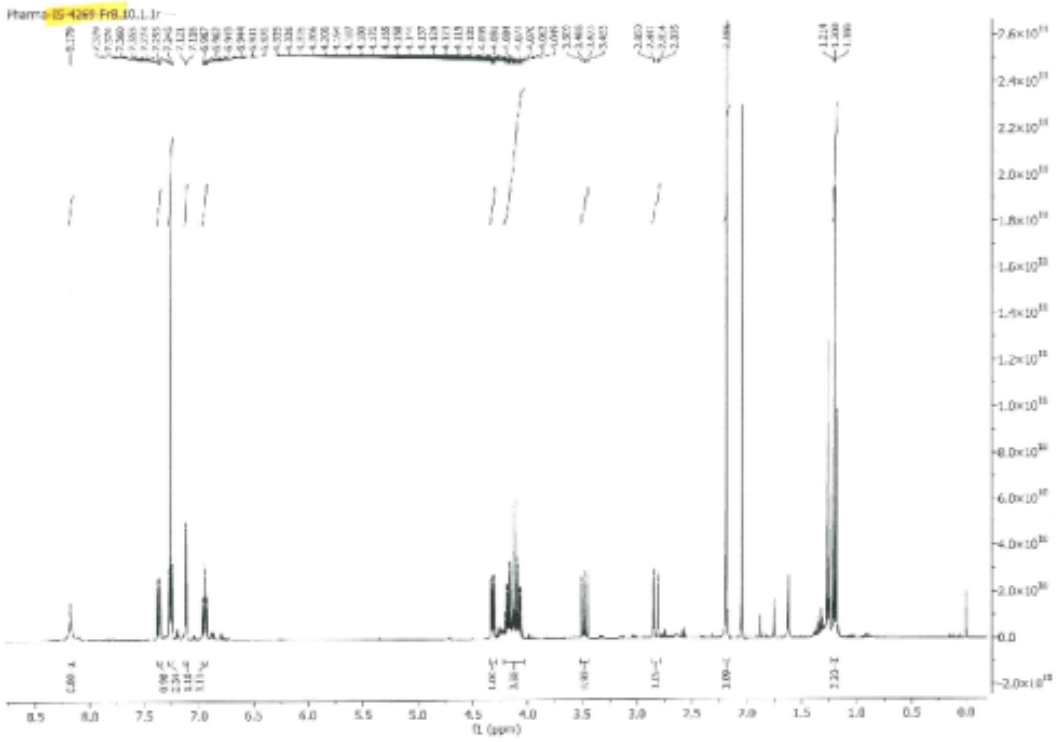
***Ethyl 2-(5-bromo-1H-indol-3-yl)-4-oxopentanoate* <u>2d</u>**

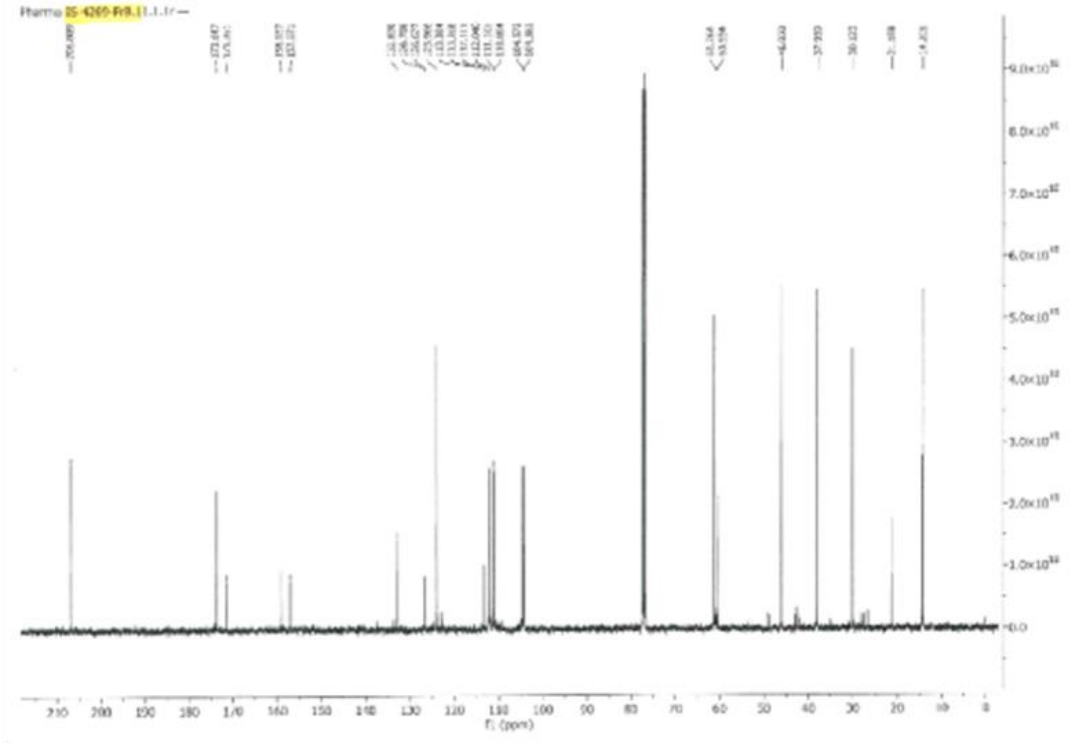


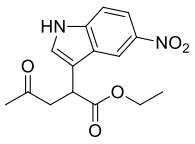




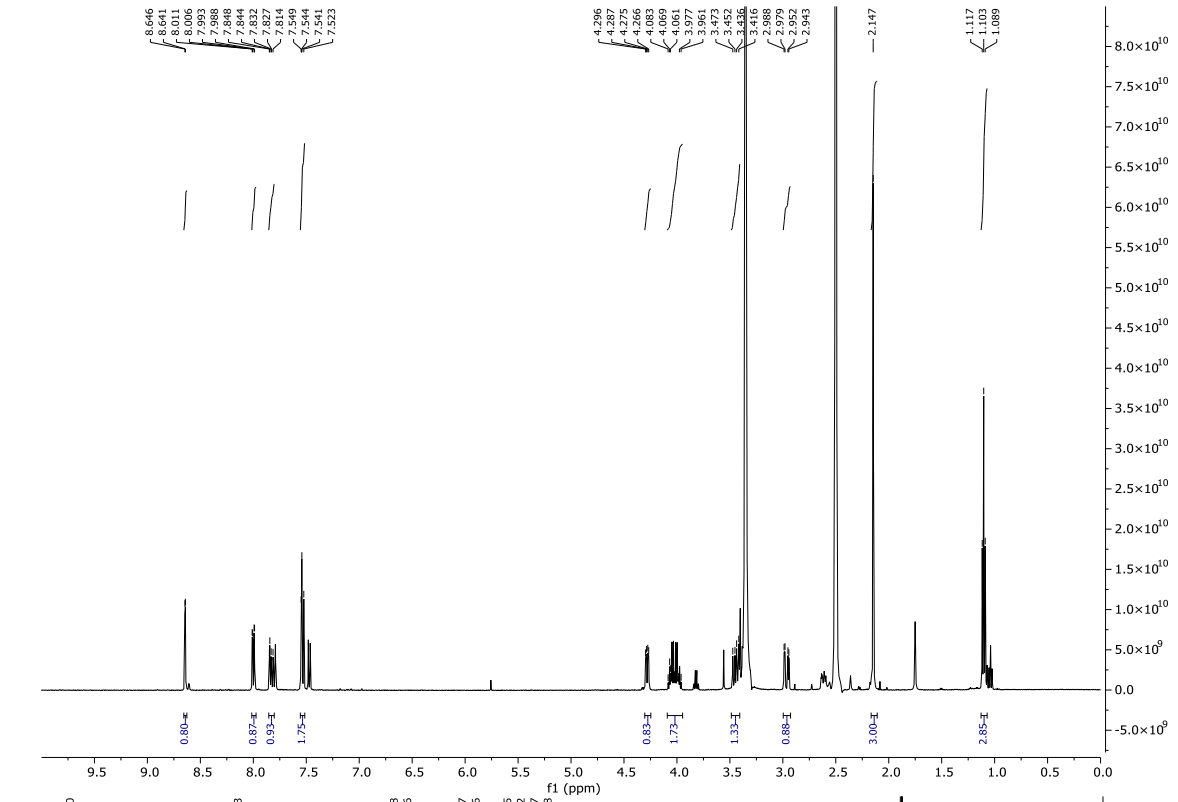
Ethyl 2-(5-fluoro-1H-indol-3-yl)-4-oxopentanoate <u>2e</u>

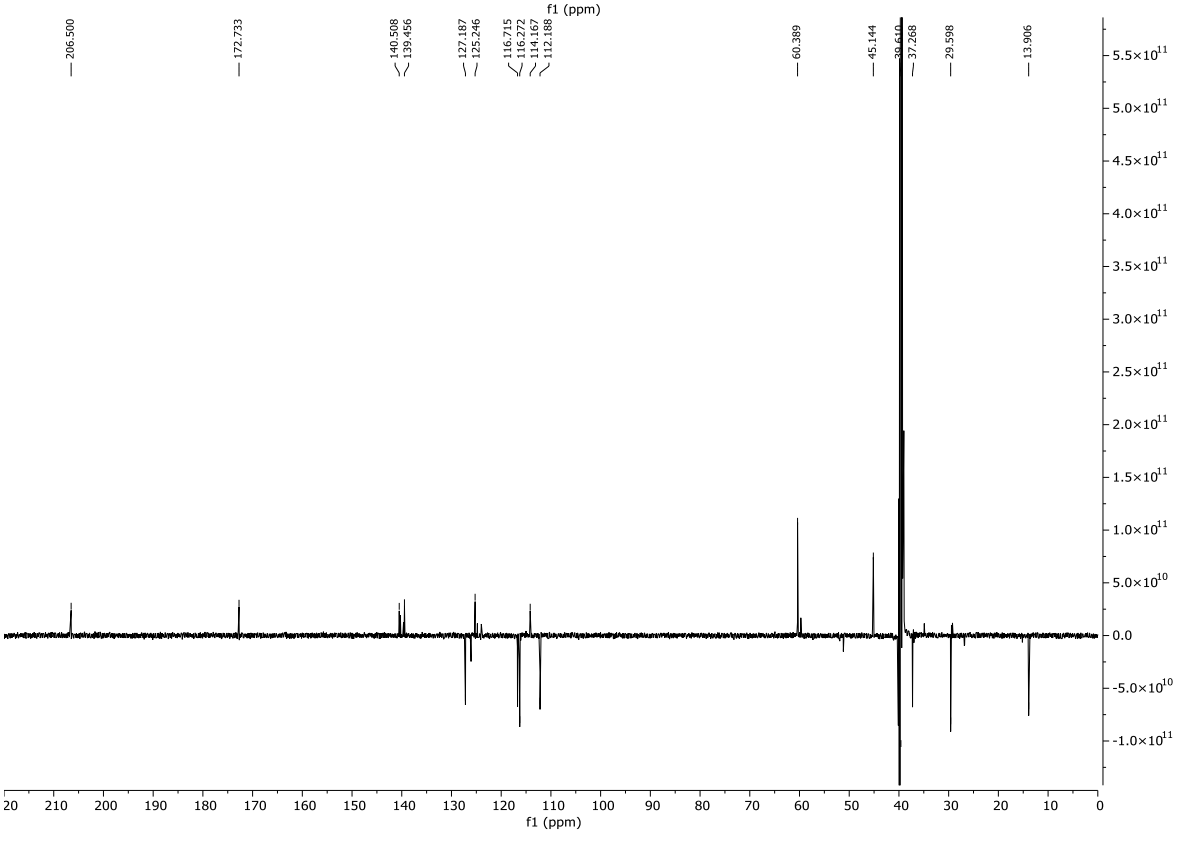


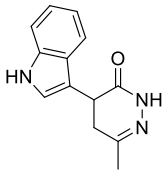




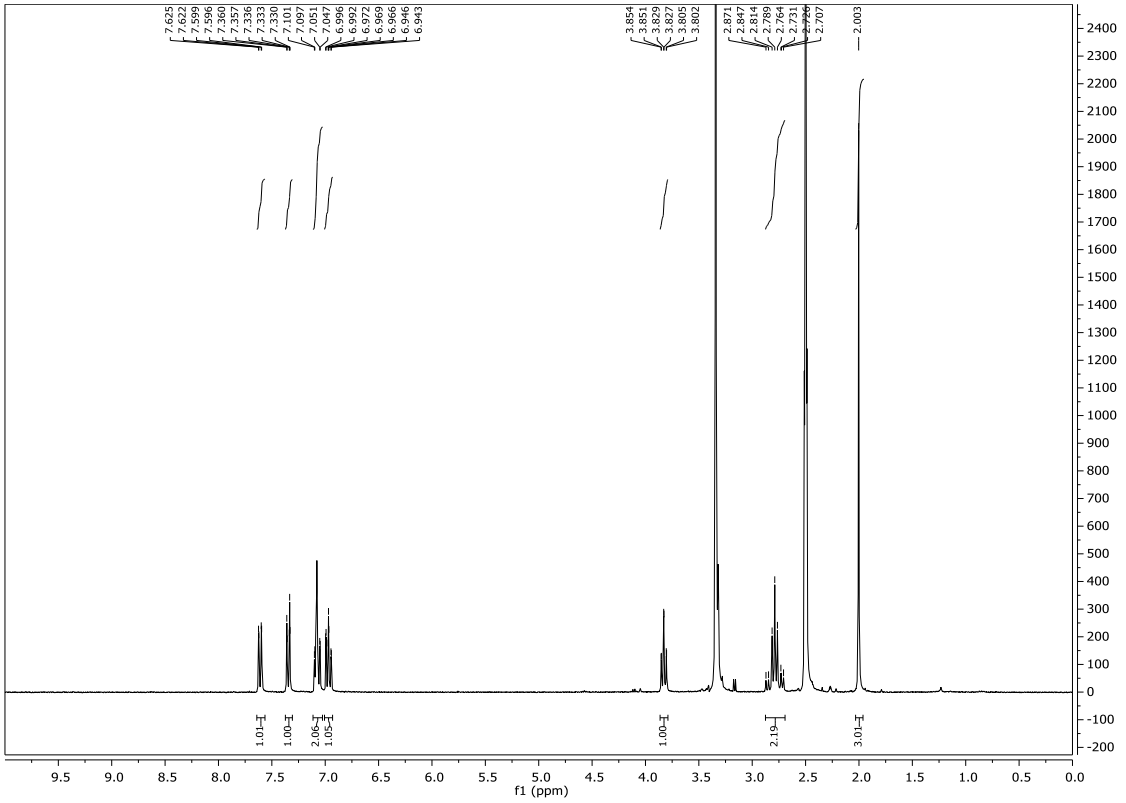
Ethyl 2-(5-nitro-1H-indol-3-yl)-4-oxopentanoate <u>2f</u>

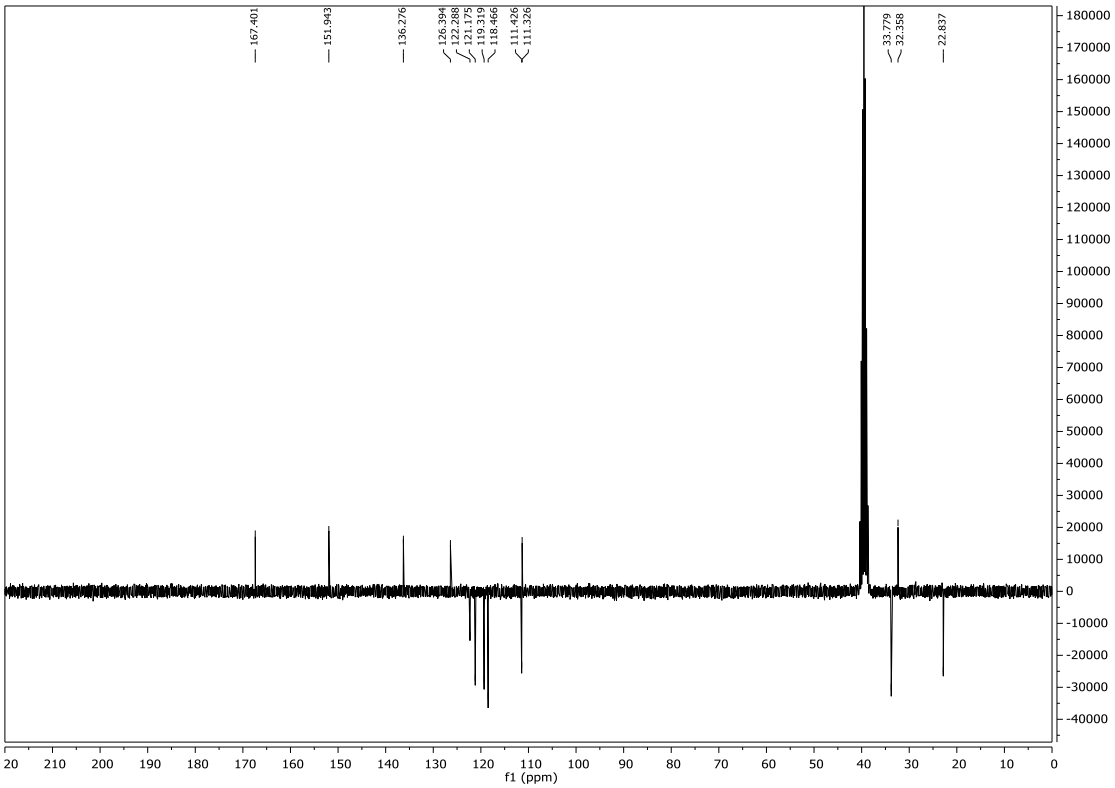


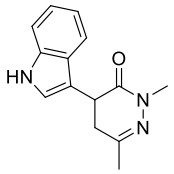




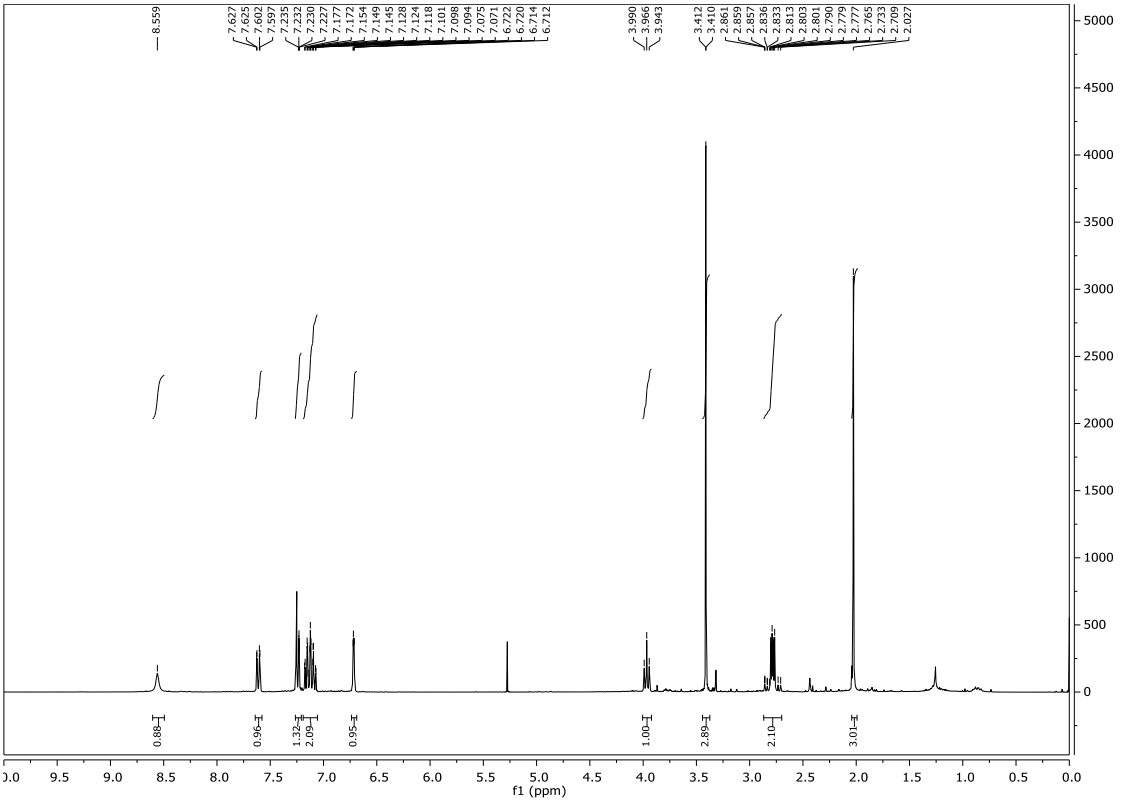
4-(1H-Indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3aa</u>

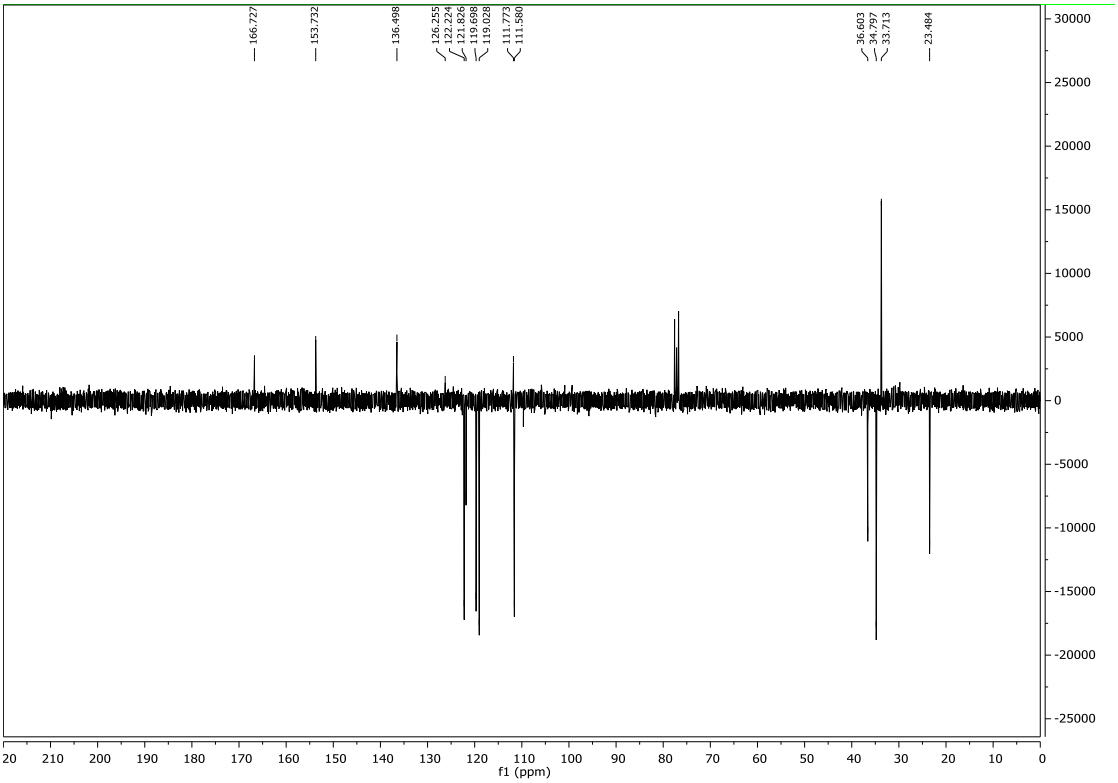


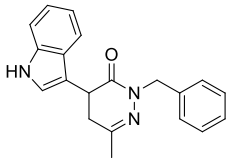




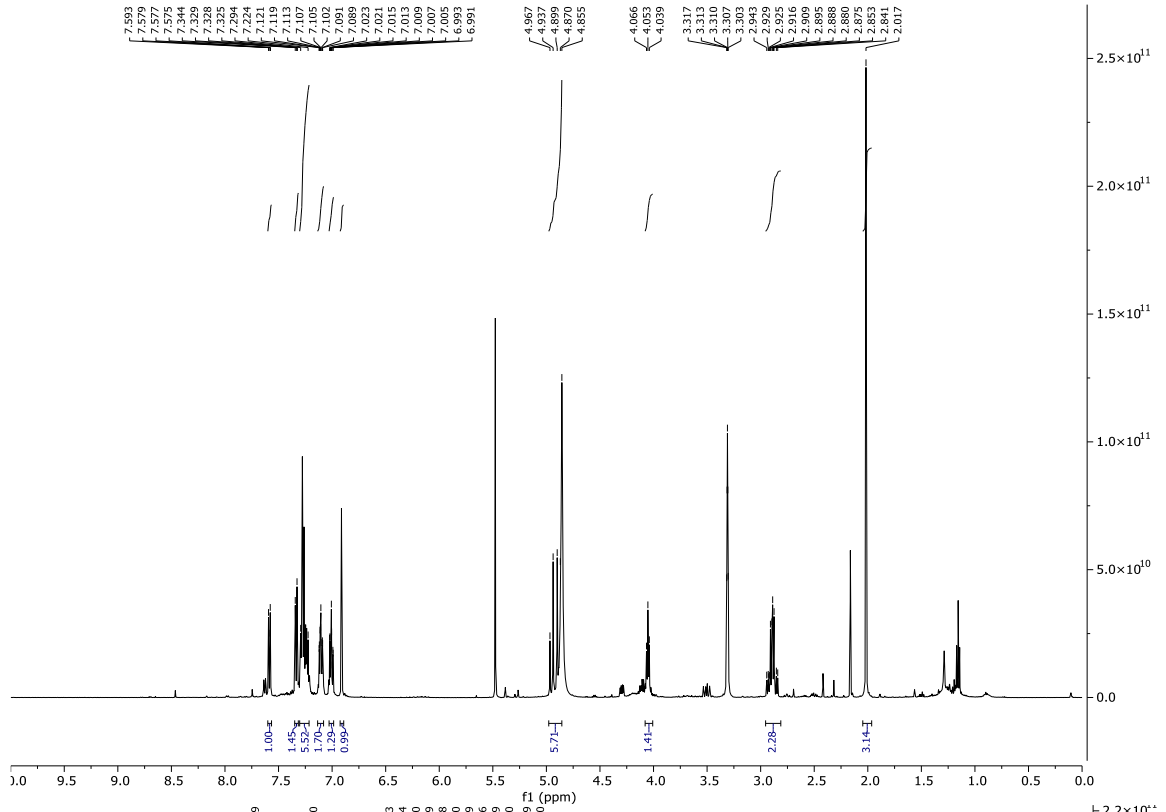
4-(1H-Indol-3-yl)-2,6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ab</u>

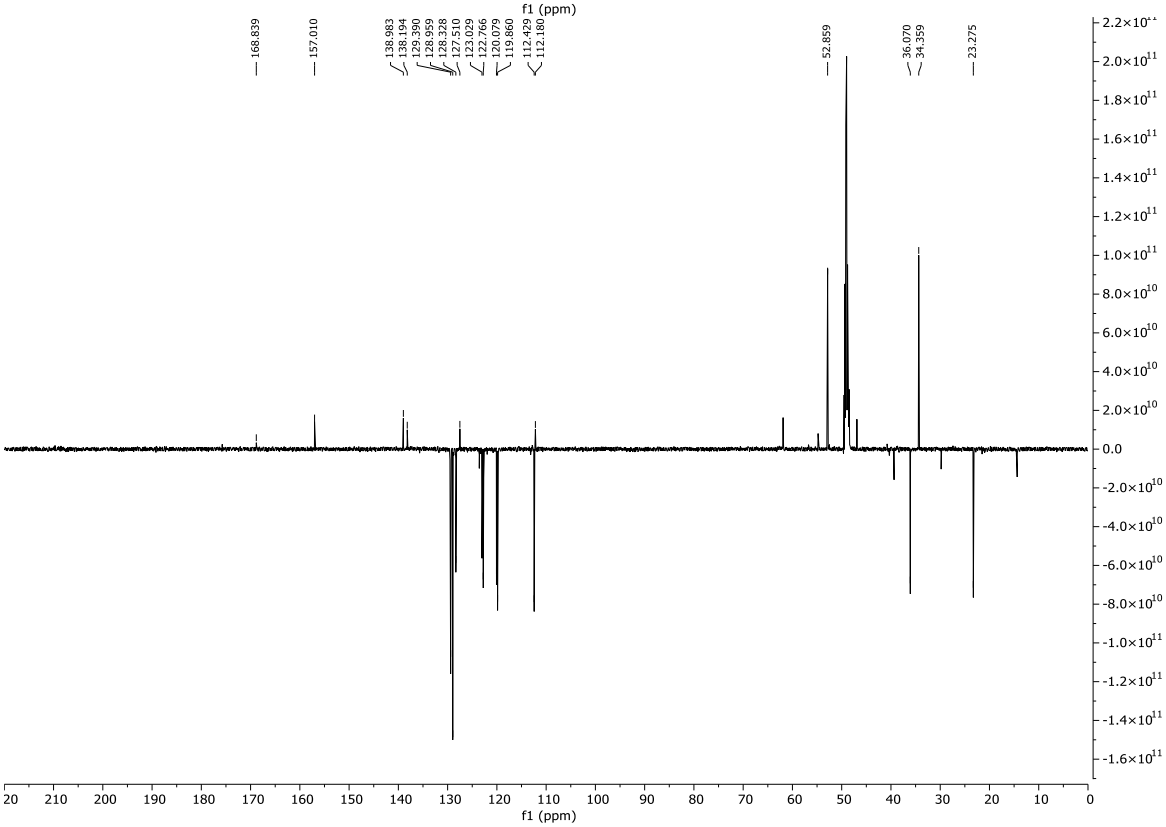


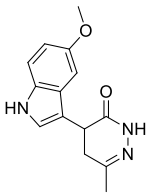




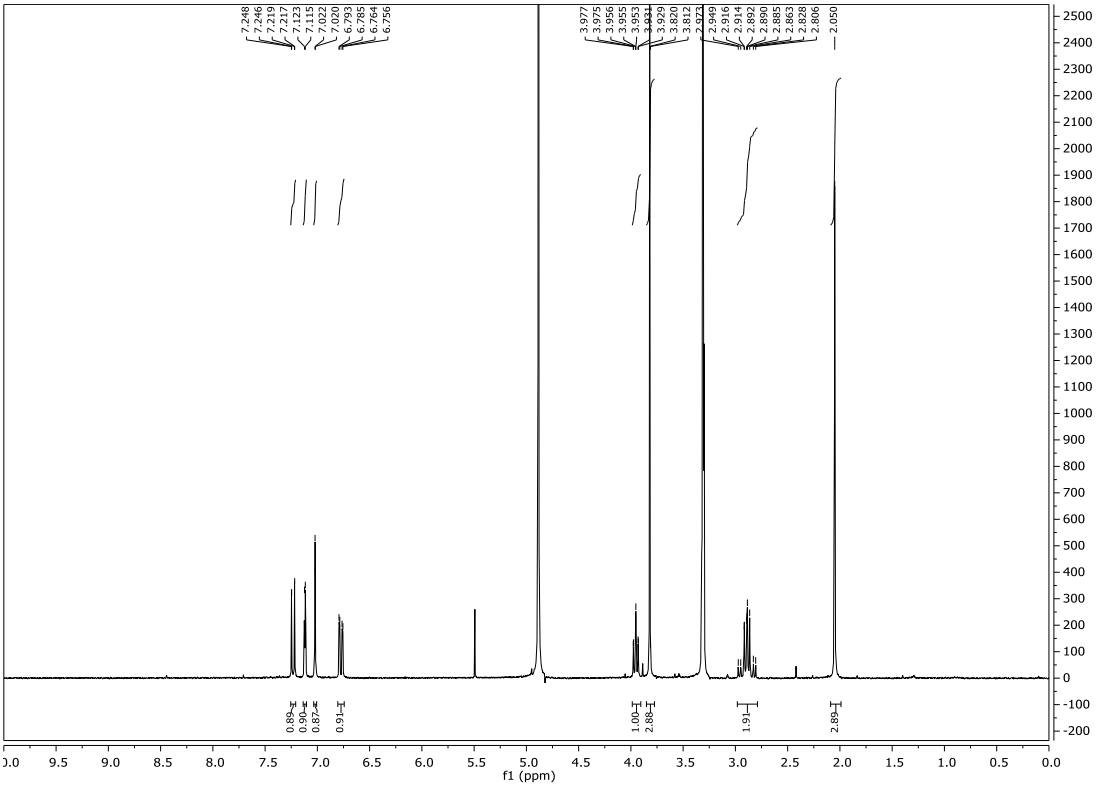
2-benzyl-4-(1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ac</u>

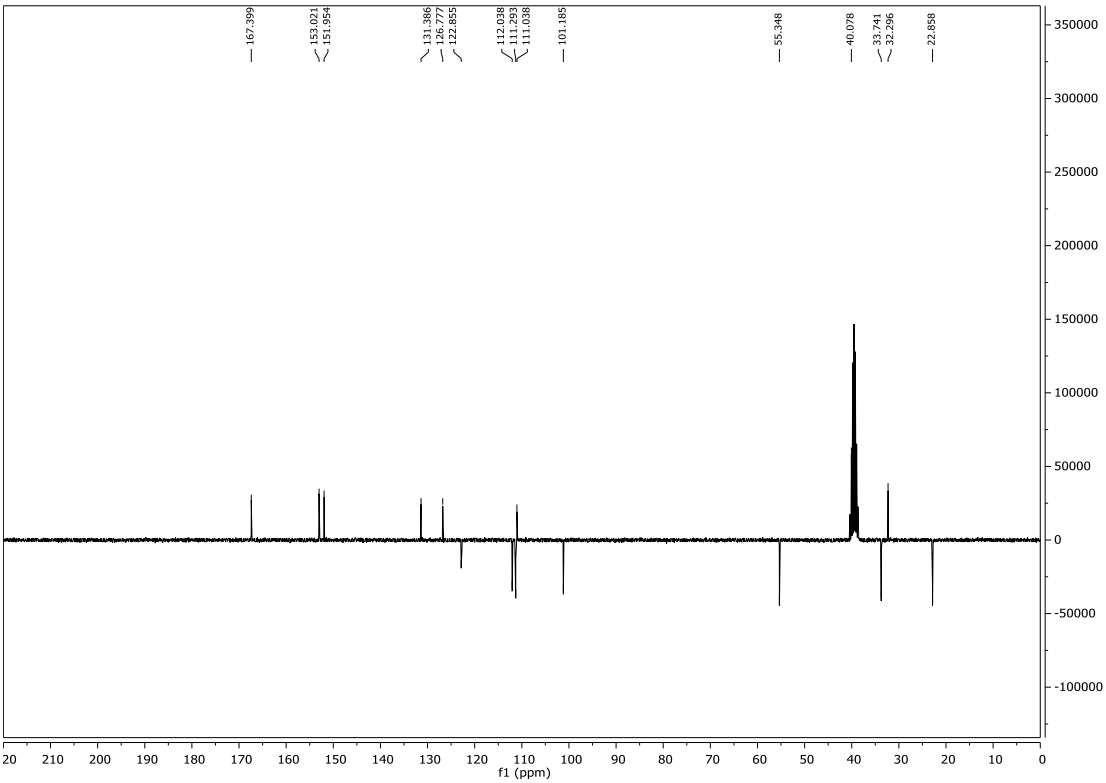


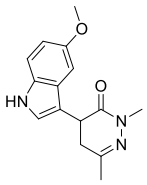




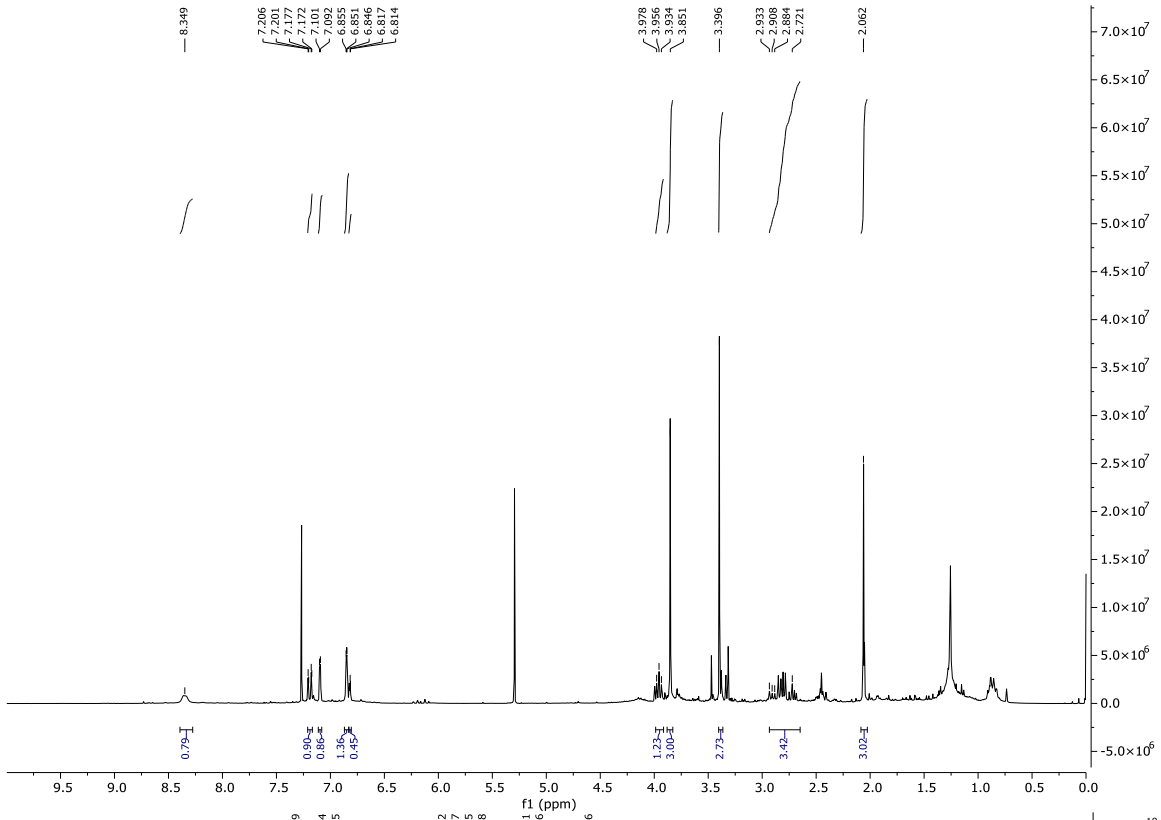
4-(5-Methoxy-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ba</u>

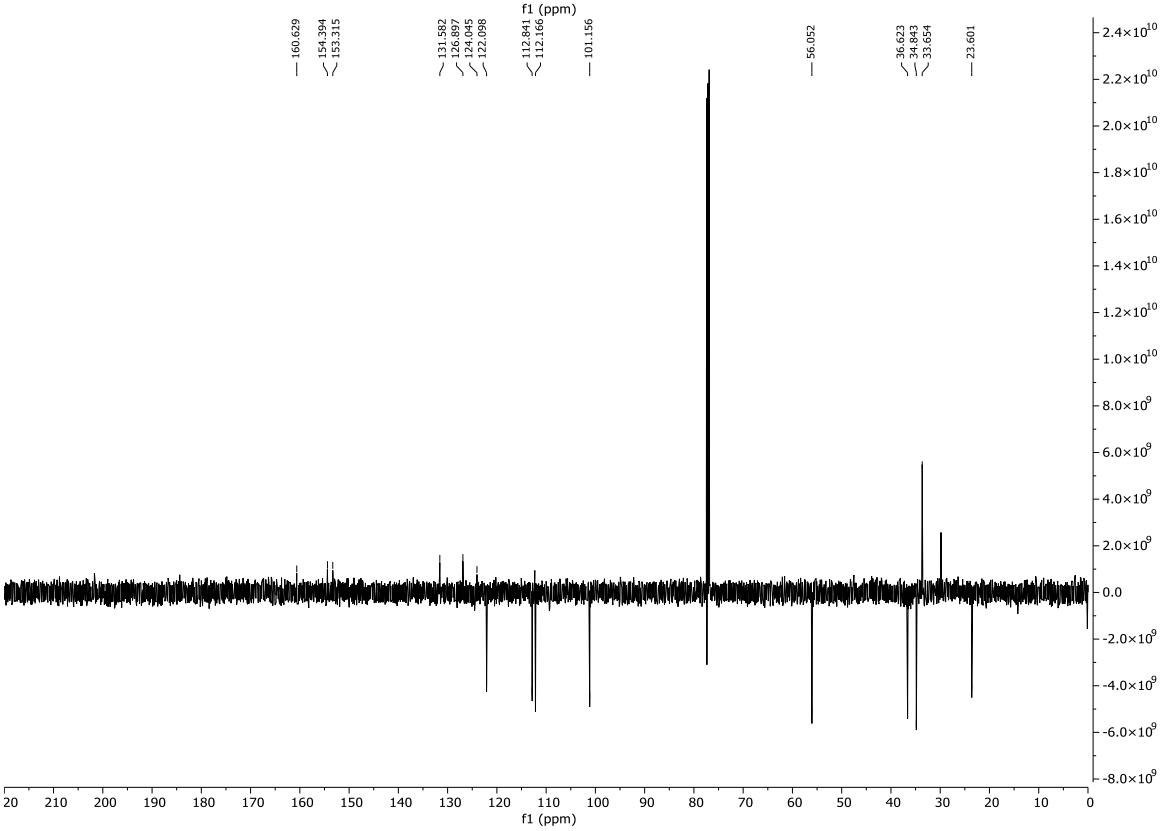


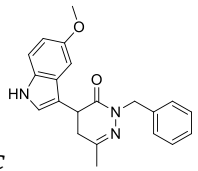




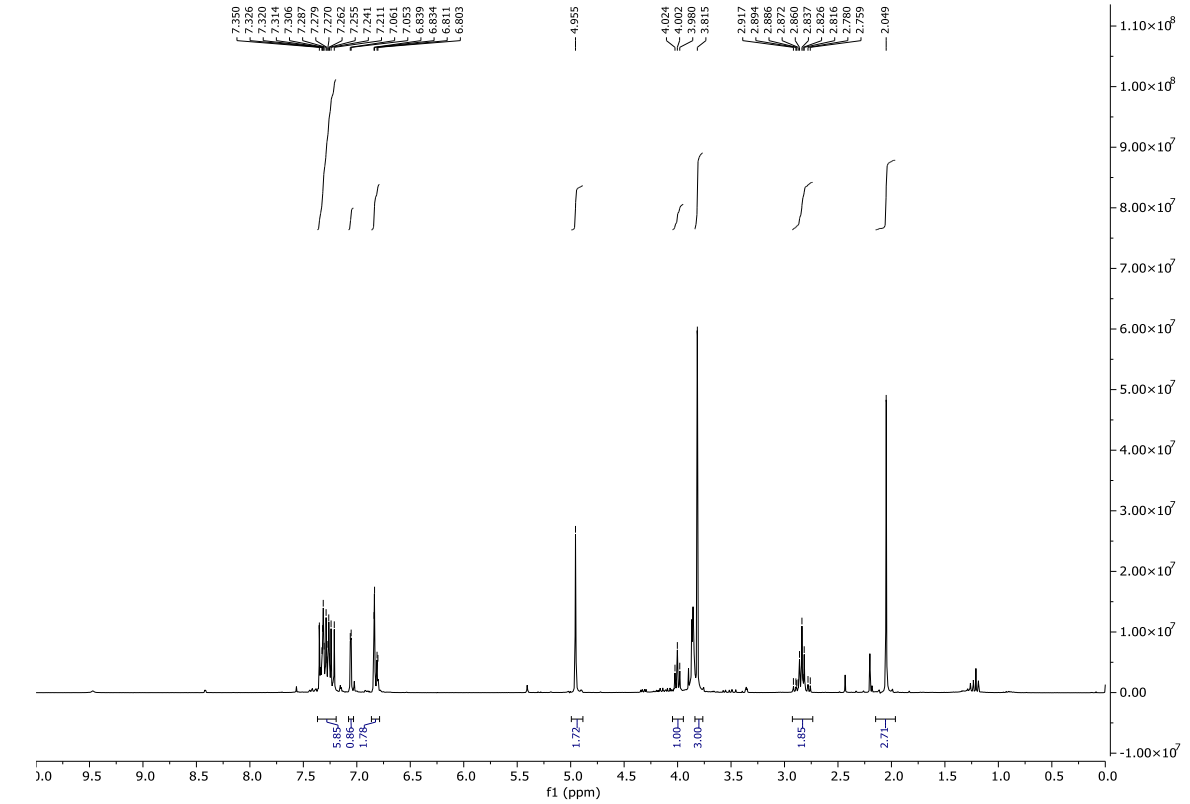
4-(5-methoxy-1H-indol-3-yl)-2,6-dimethyl-4,5-dihydropyridazin-3(2H)-one <u>3bb</u>

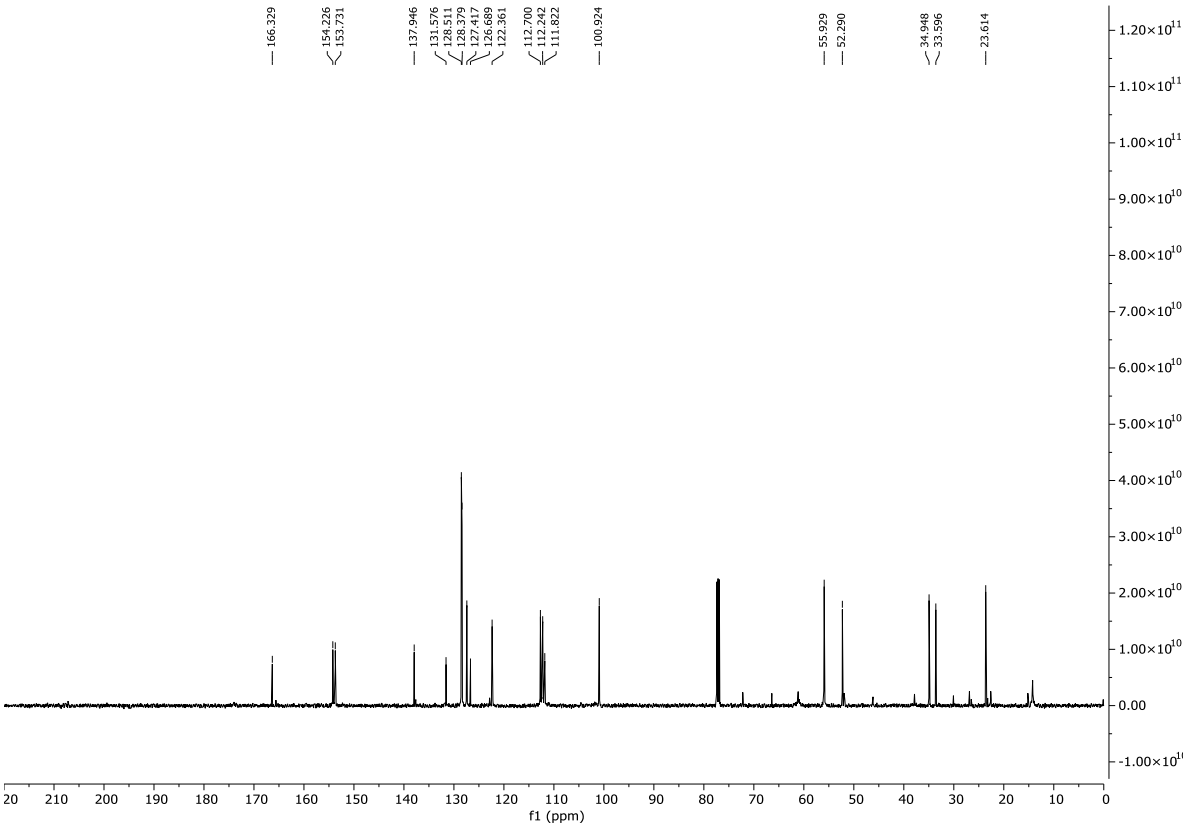


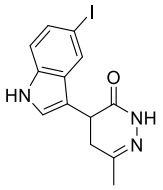




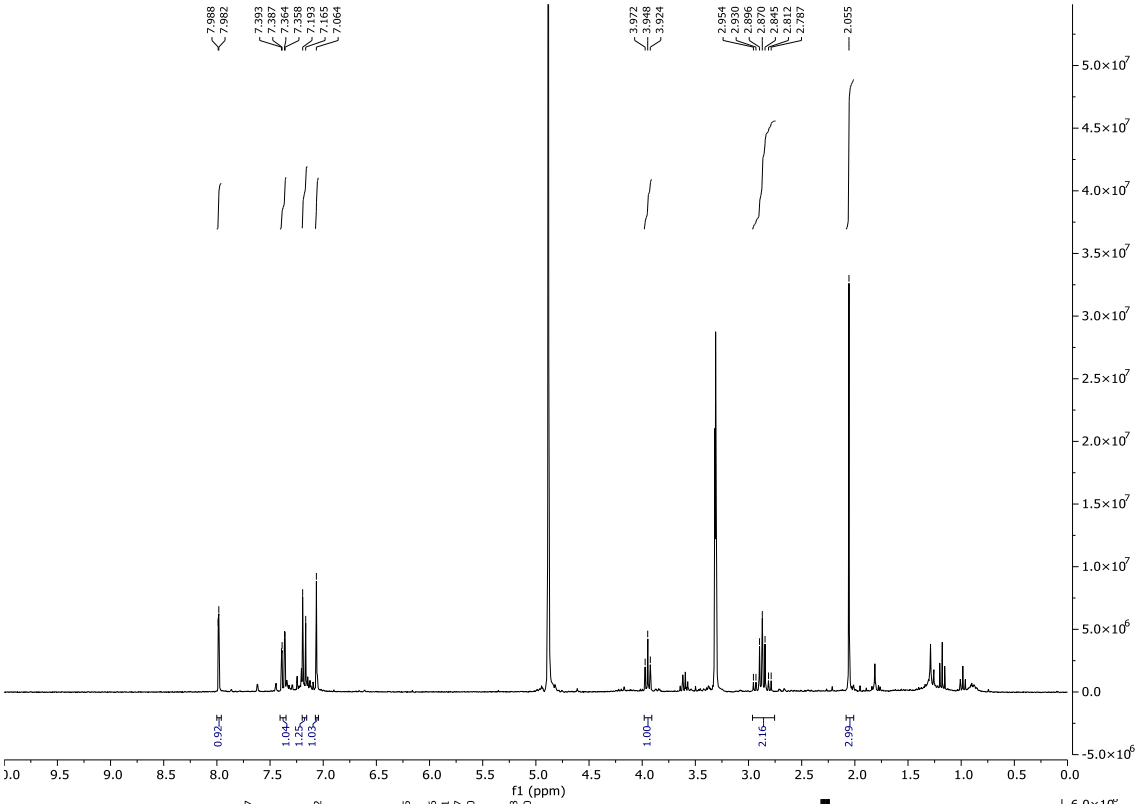
2-benzyl-4-(5-methoxy-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3bc</u>

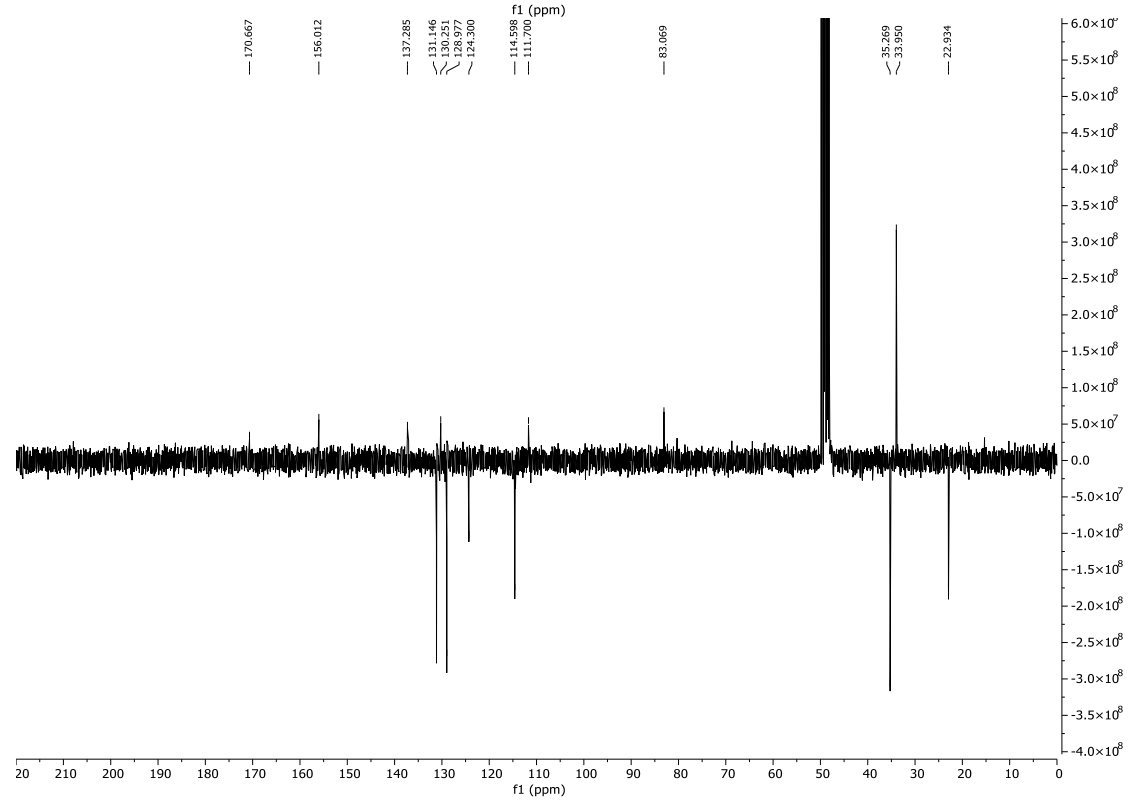


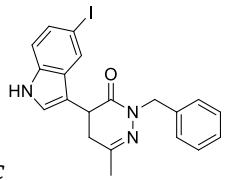




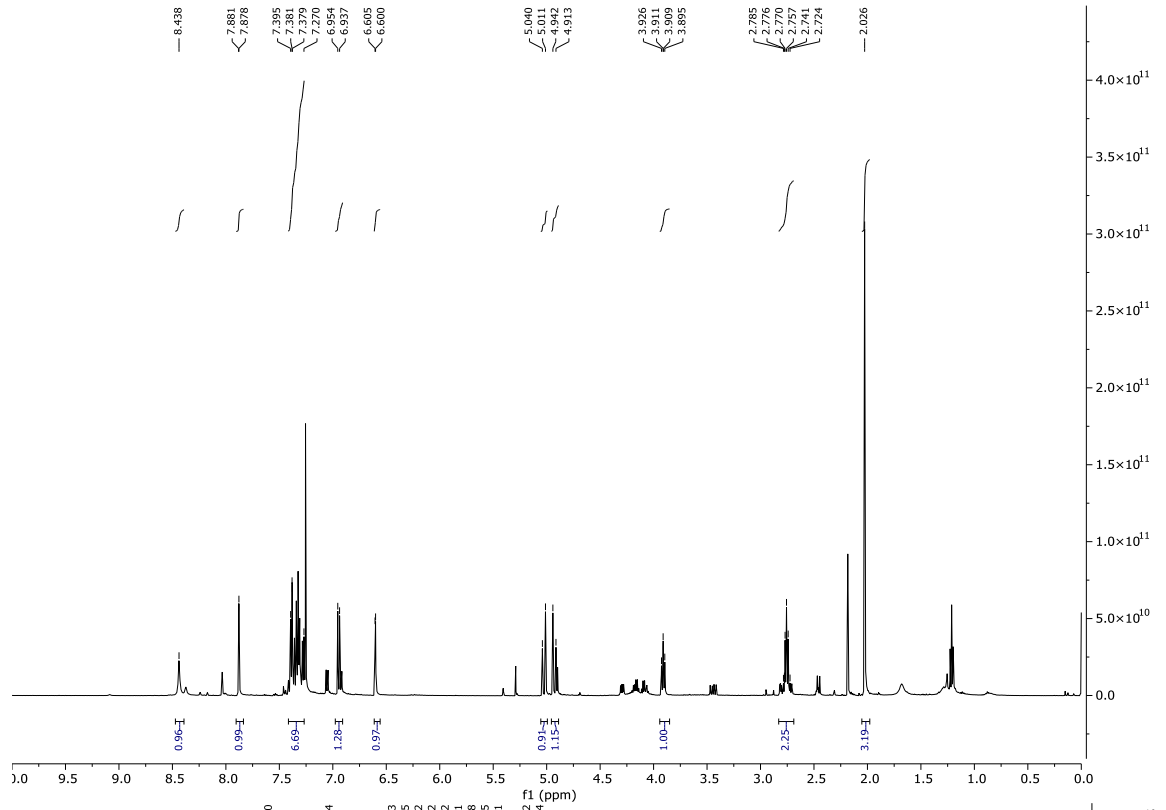
4-(5-iodo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ca</u>

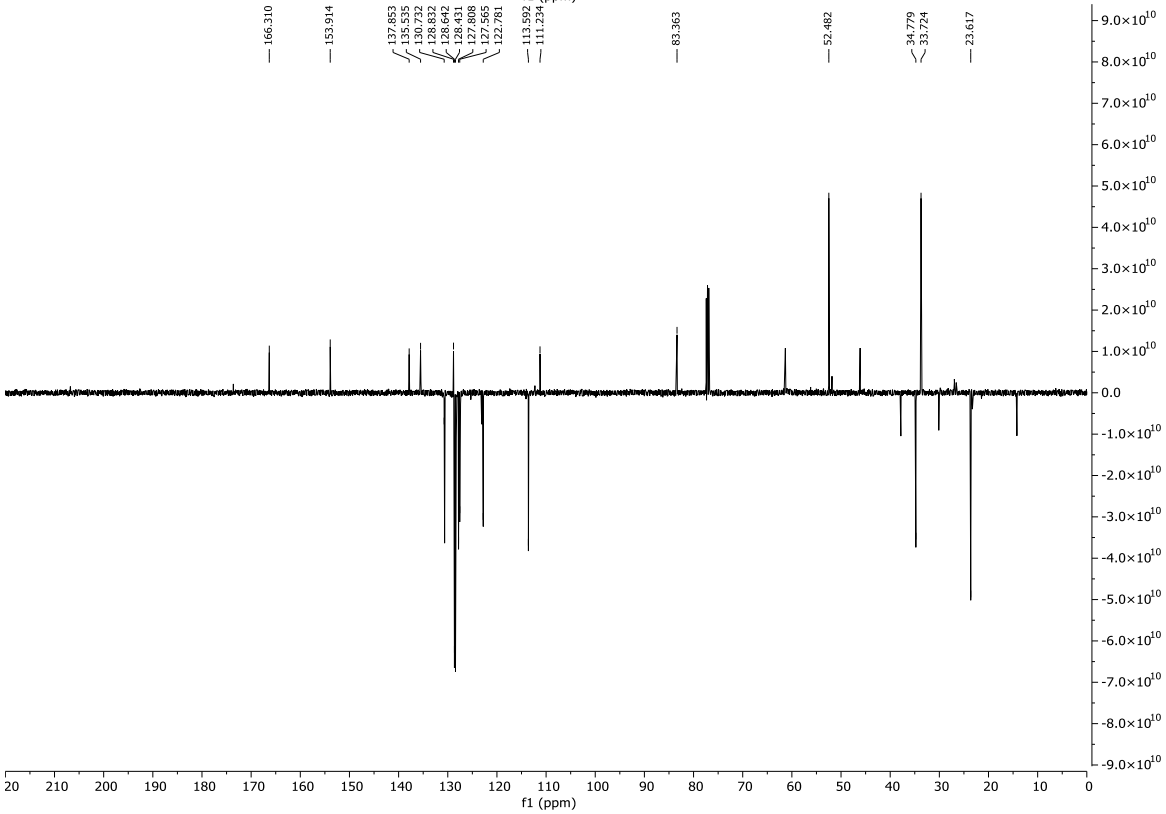


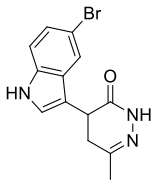




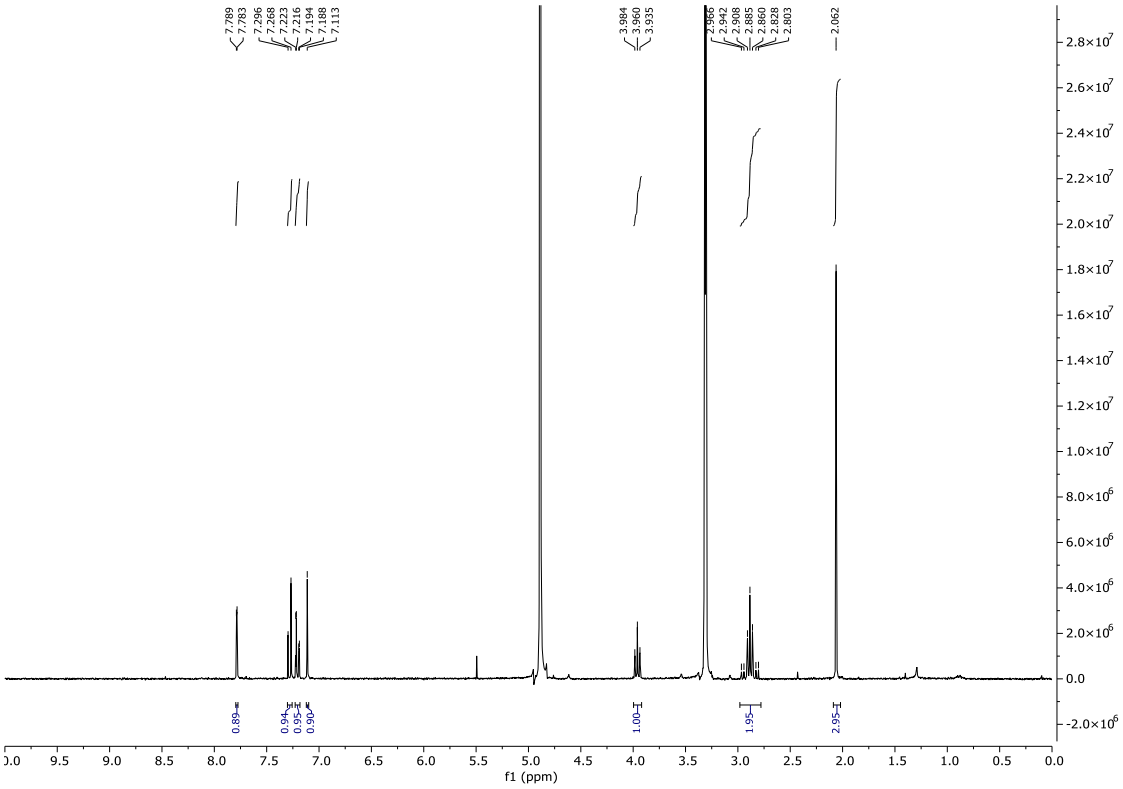
2-benzyl-4-(5-iodo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3cc</u>

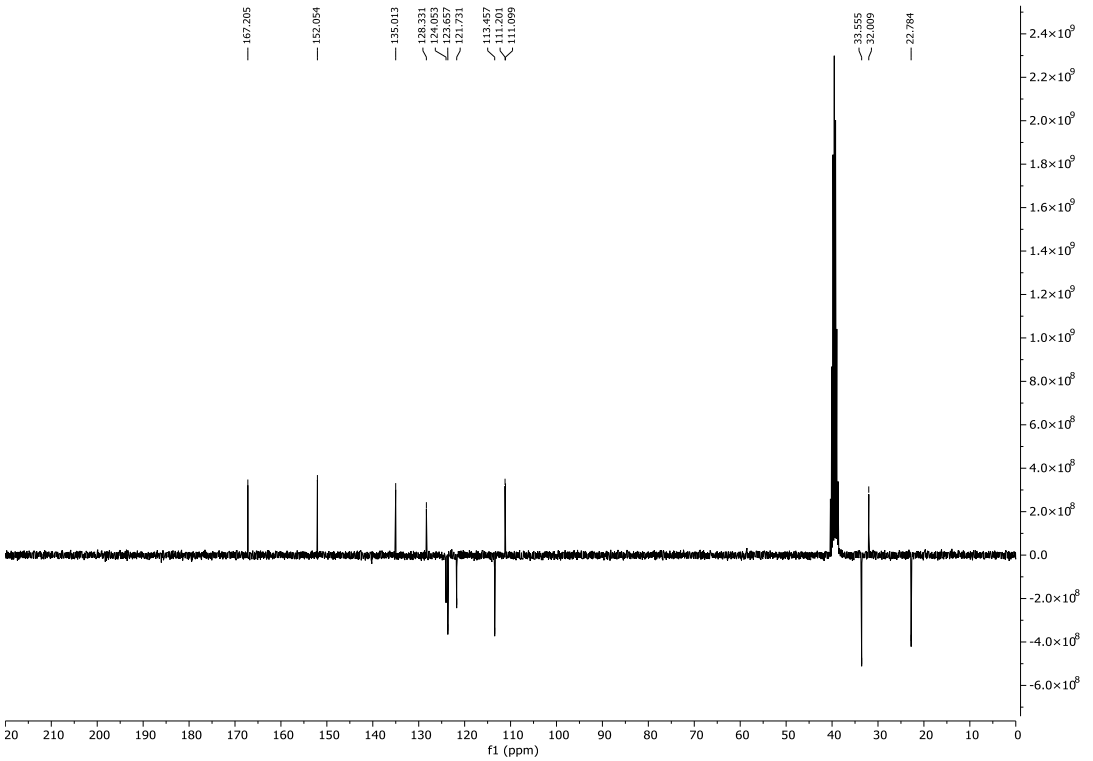


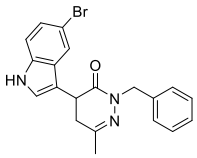




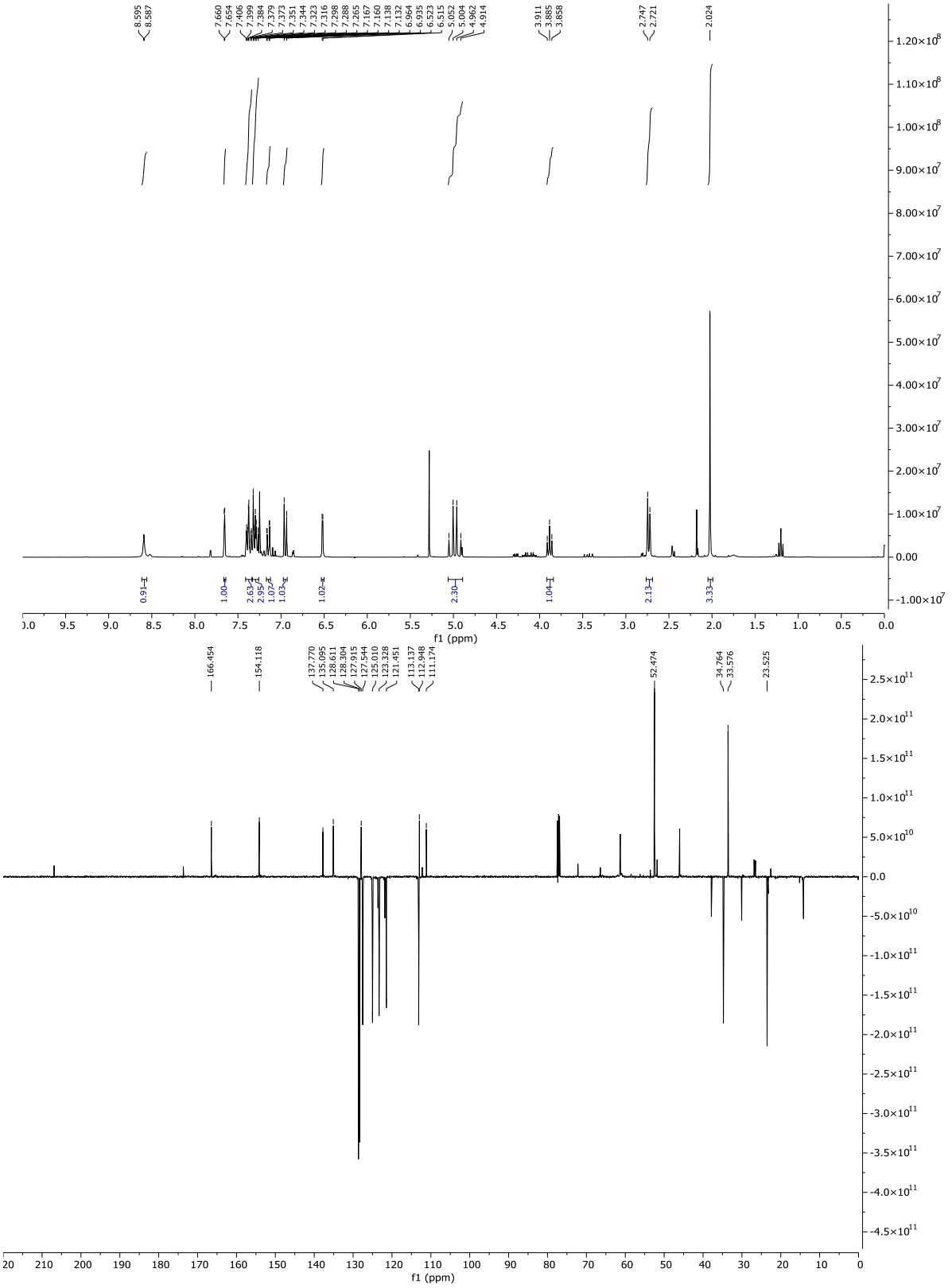
4-(5-bromo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3da</u>

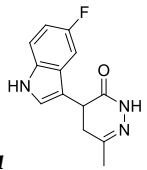




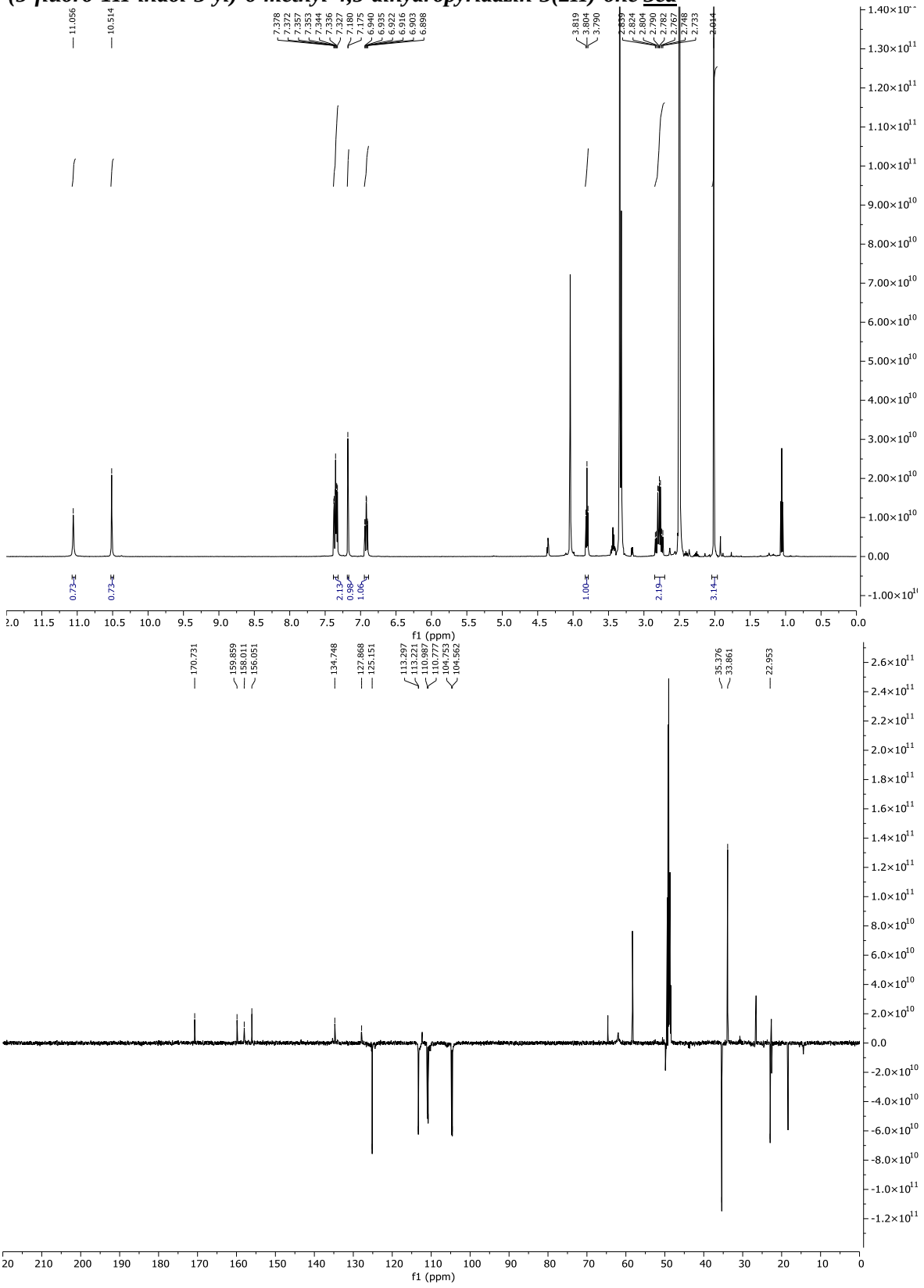


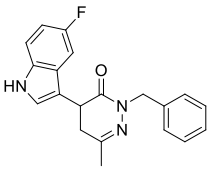
2-benzyl-4-(5-bromo-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3dc</u>



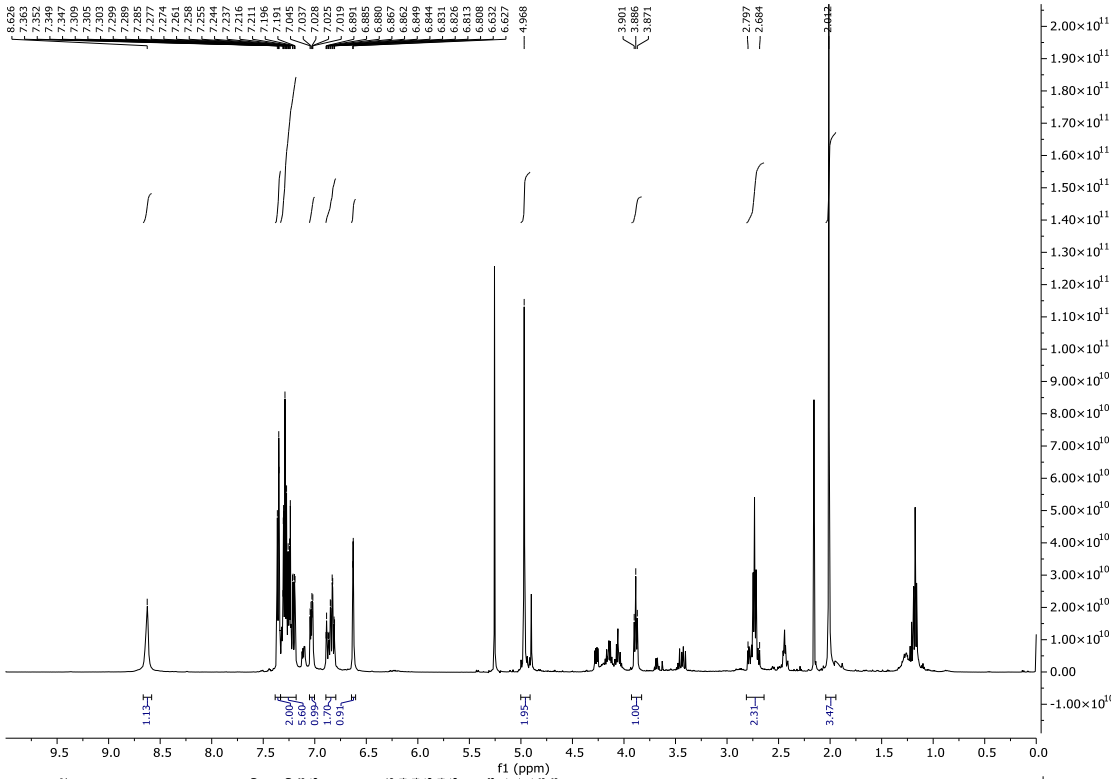


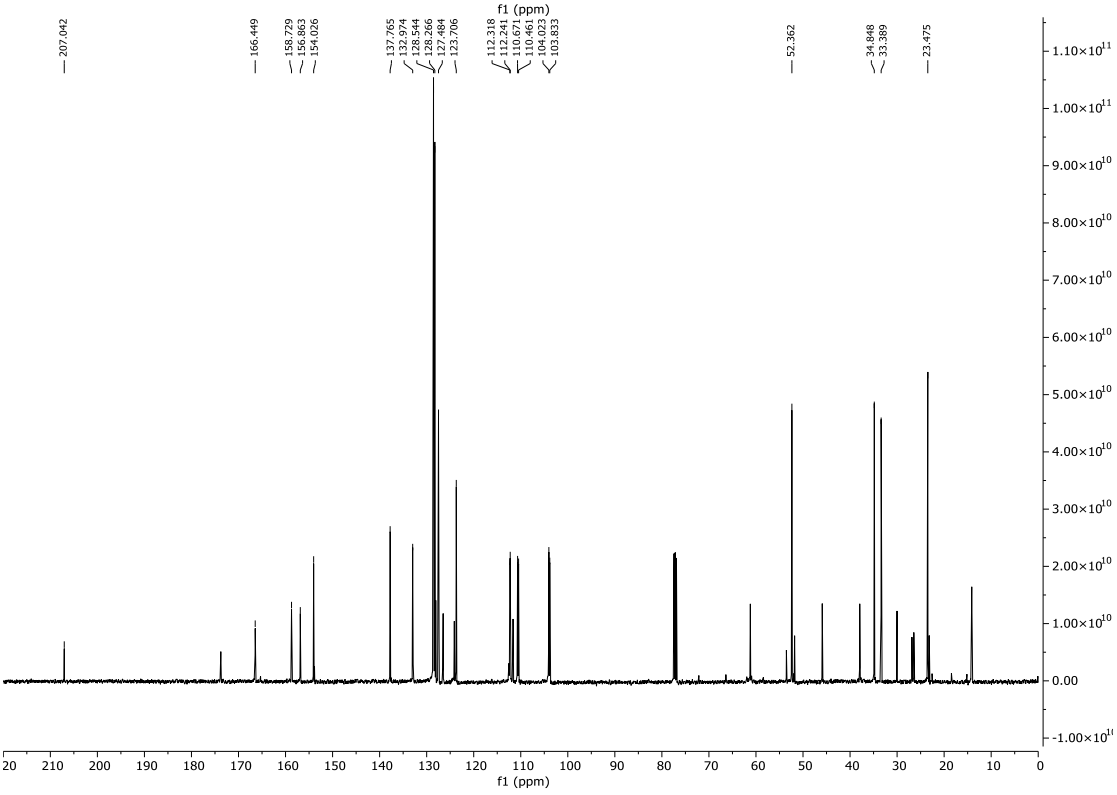
4-(5-fluoro-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ea</u>

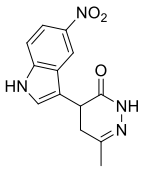




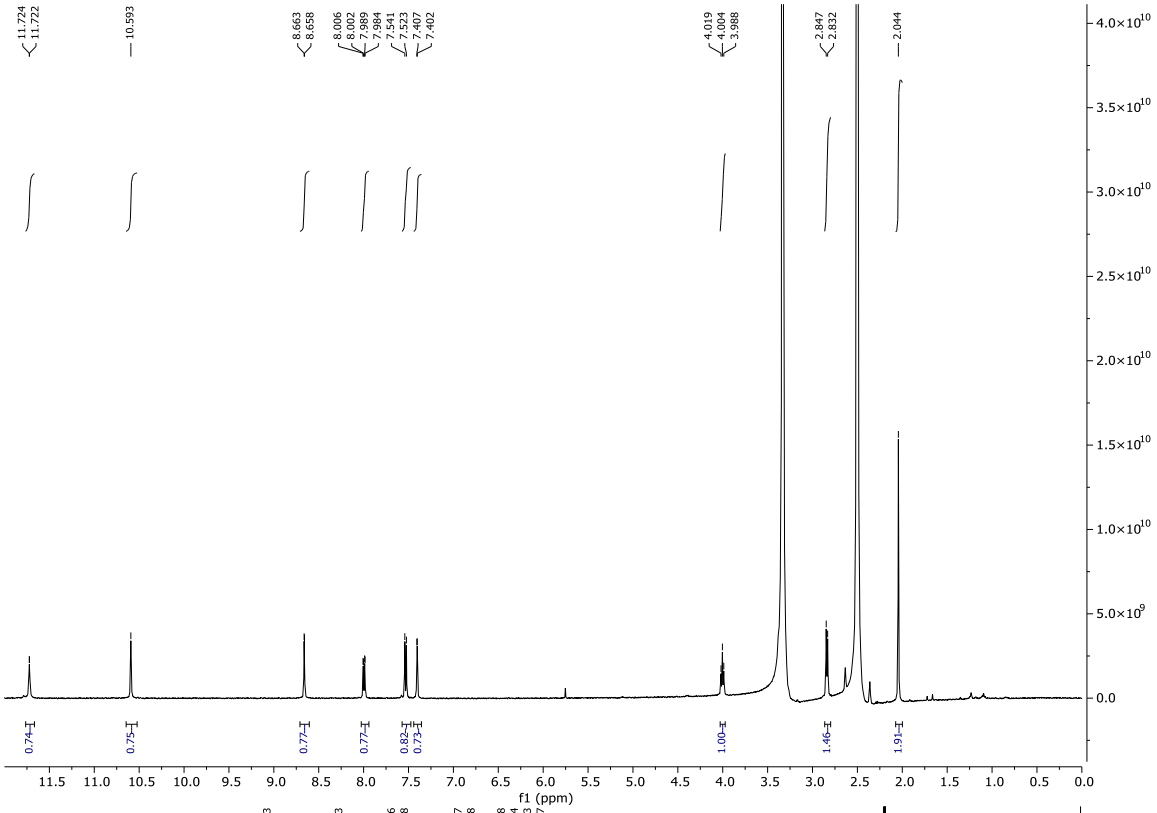
2-benzyl-4-(5-fluoro-1H-indol-3-yl)-6-methyl-4,5-dihydropyridazin-3(2H)-one <u>3ec</u>

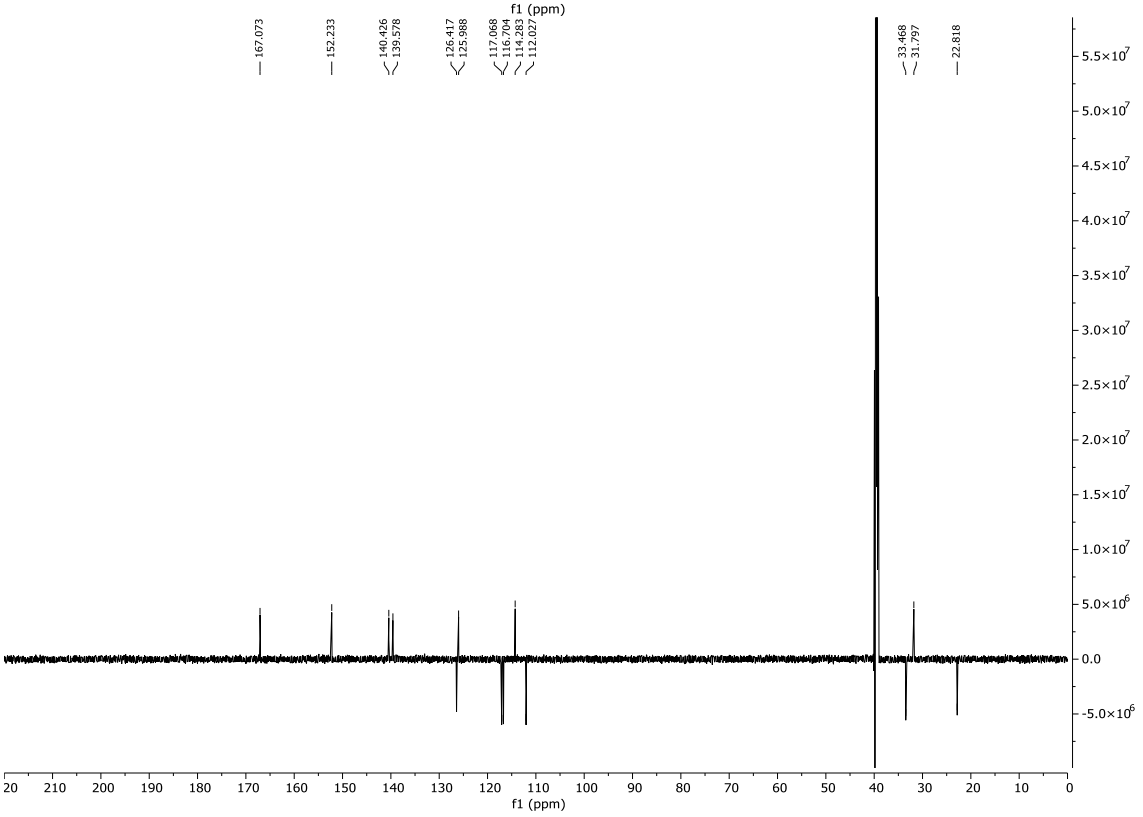


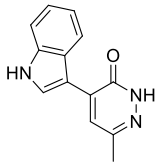




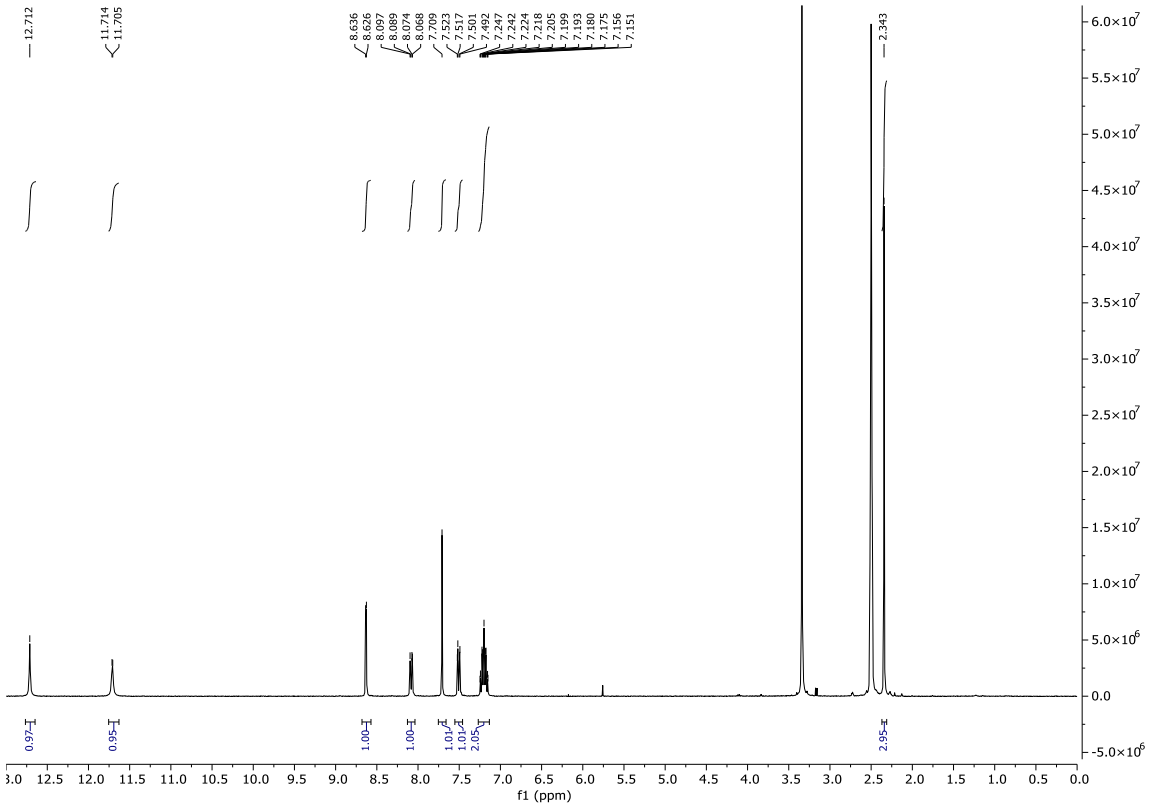
6-methyl-4-(5-nitro-1H-indol-3-yl)-4,5-dihydropyridazin-3(2H)-one <u>3fa</u>

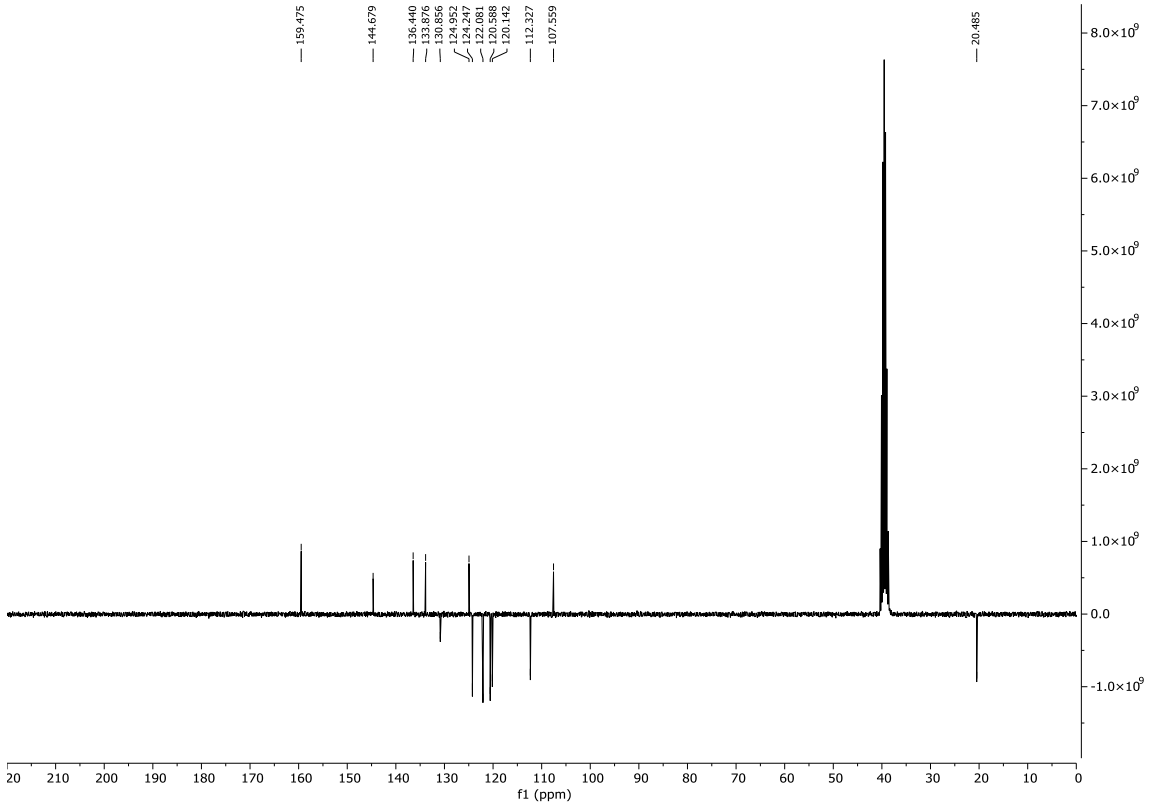


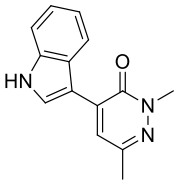




4-(1H-indol-3-yl)-6-methylpyridazin-3(2H)-one <u>4aa</u>







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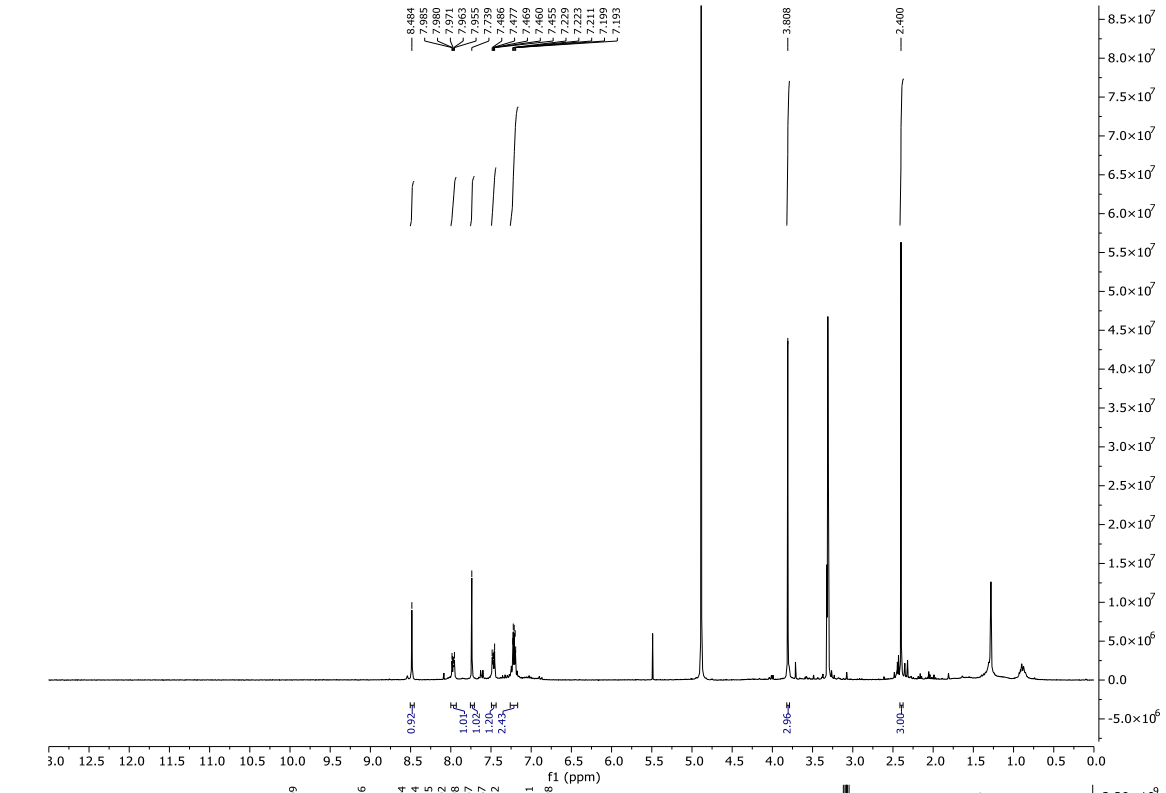

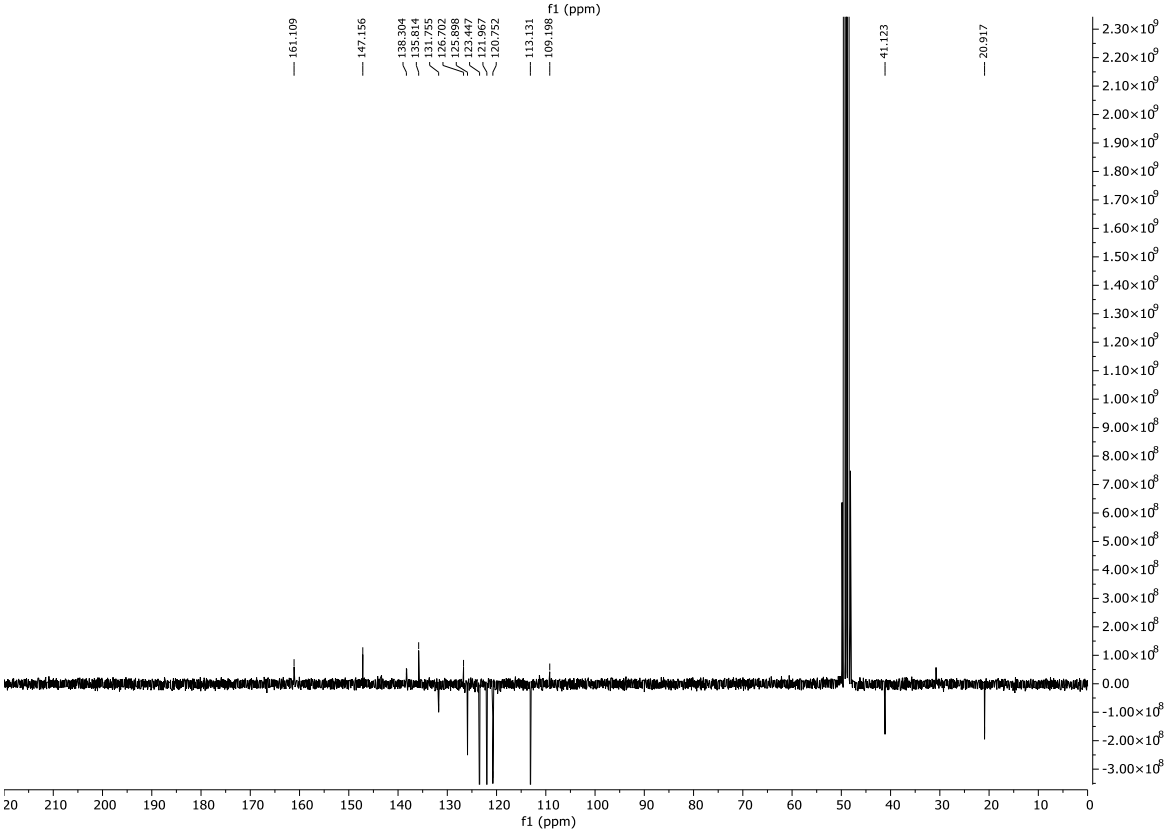

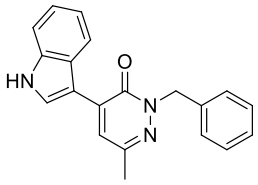

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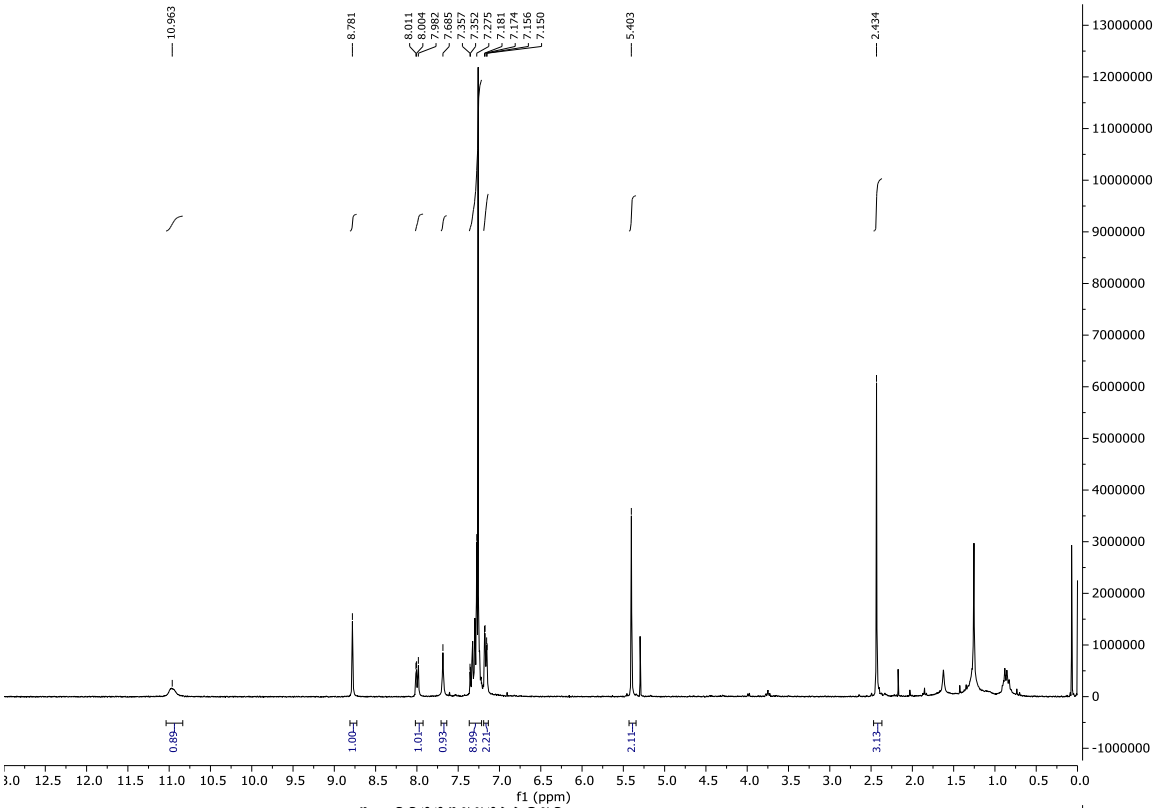

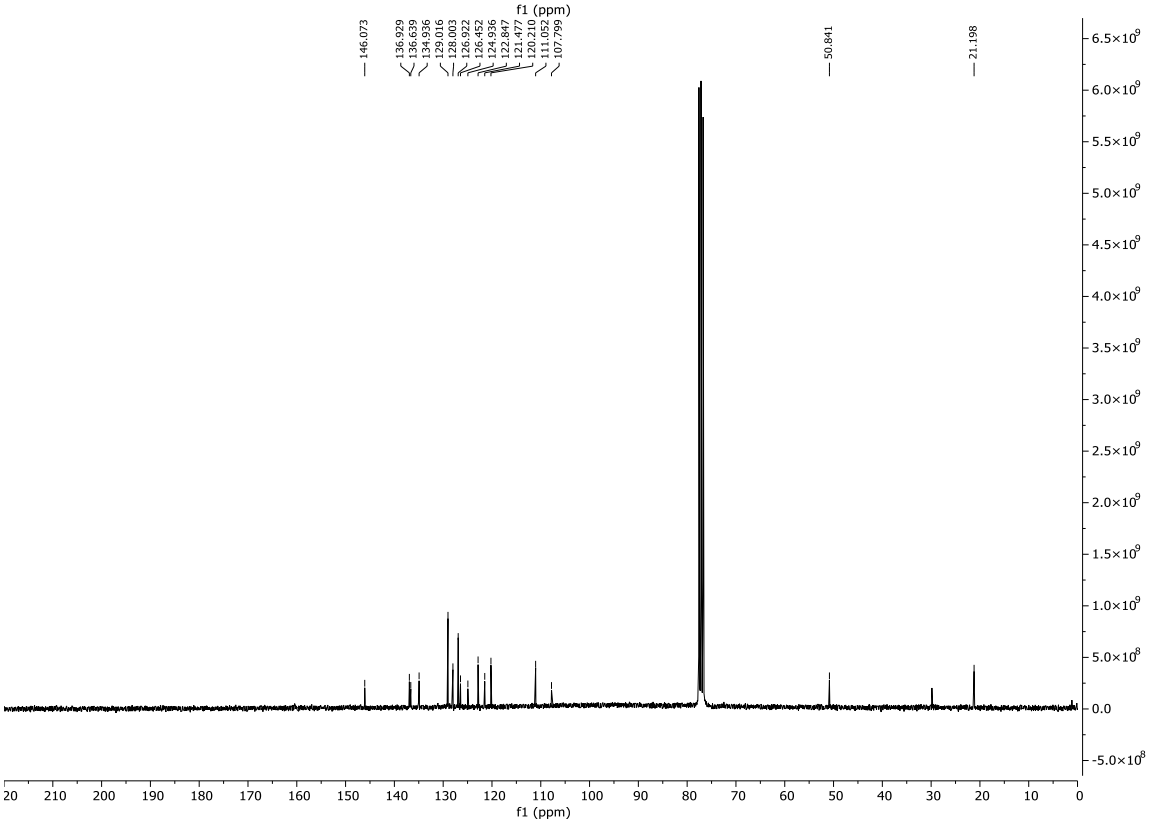

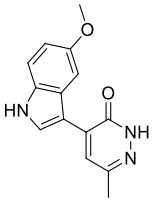

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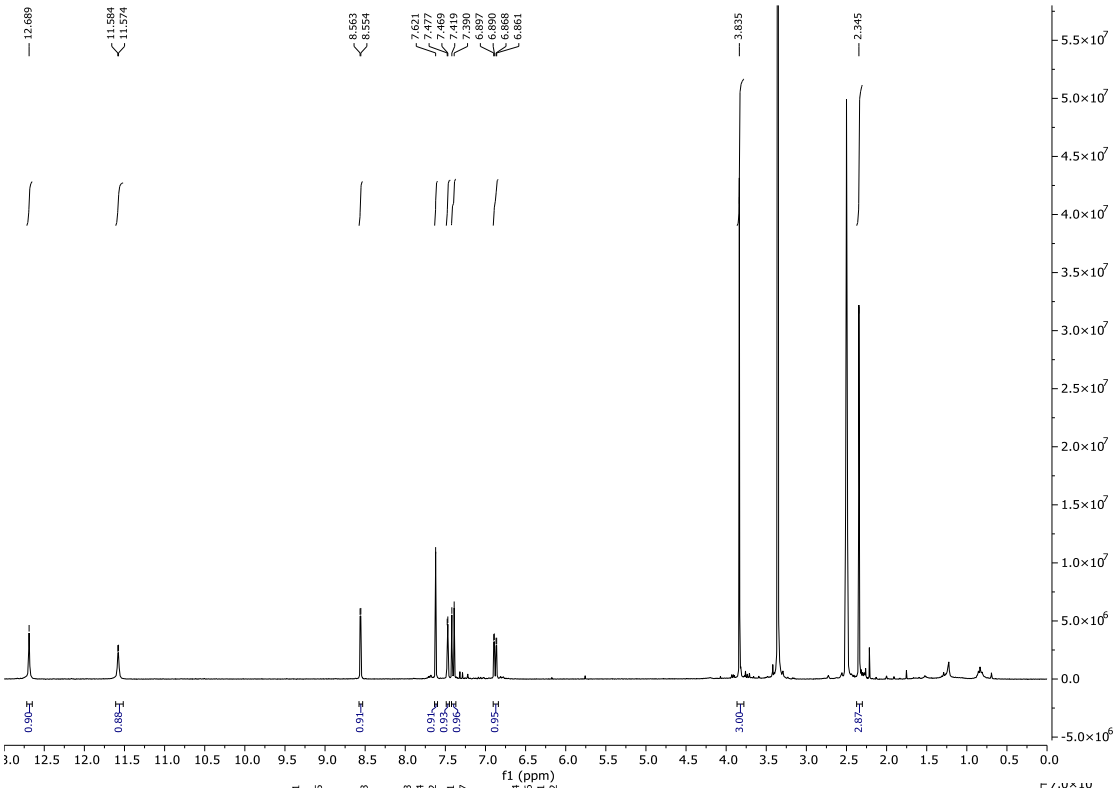

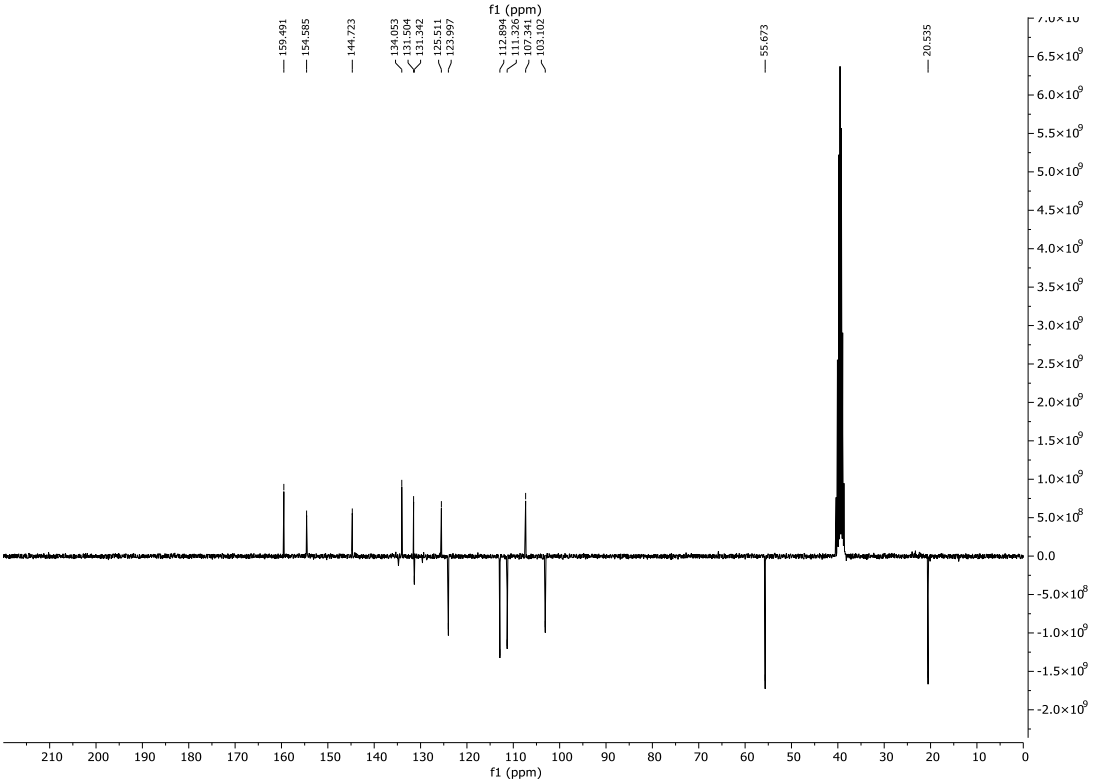

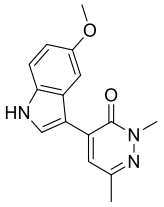

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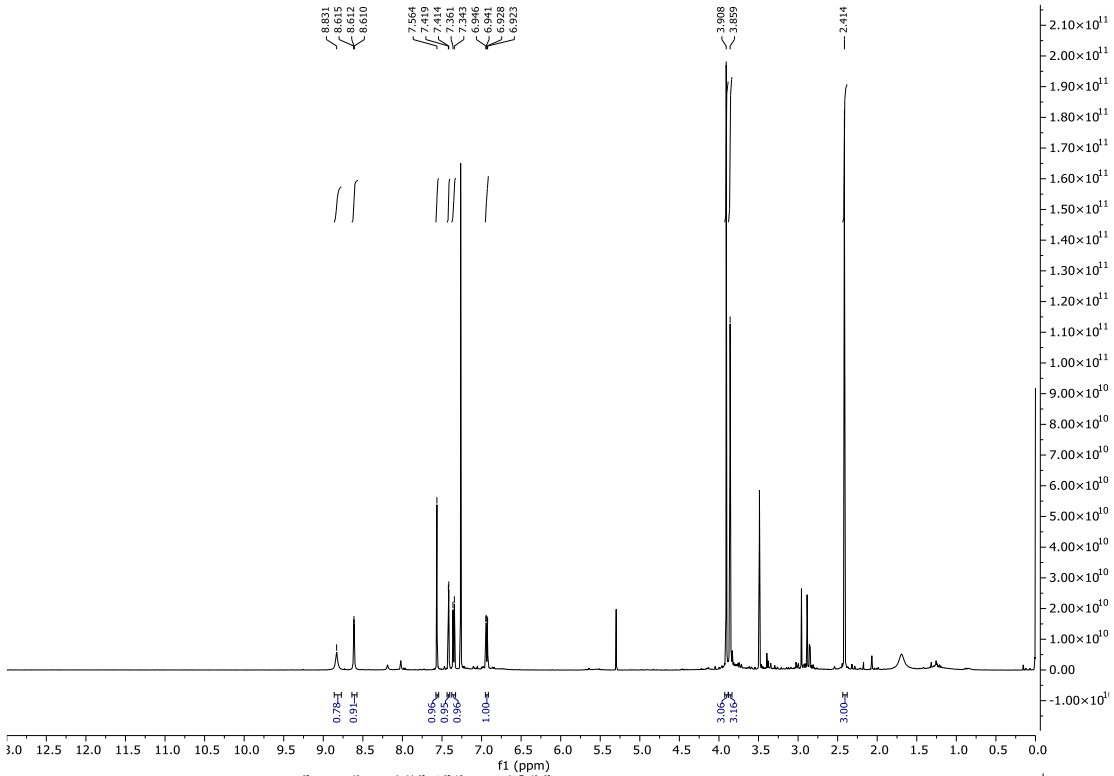

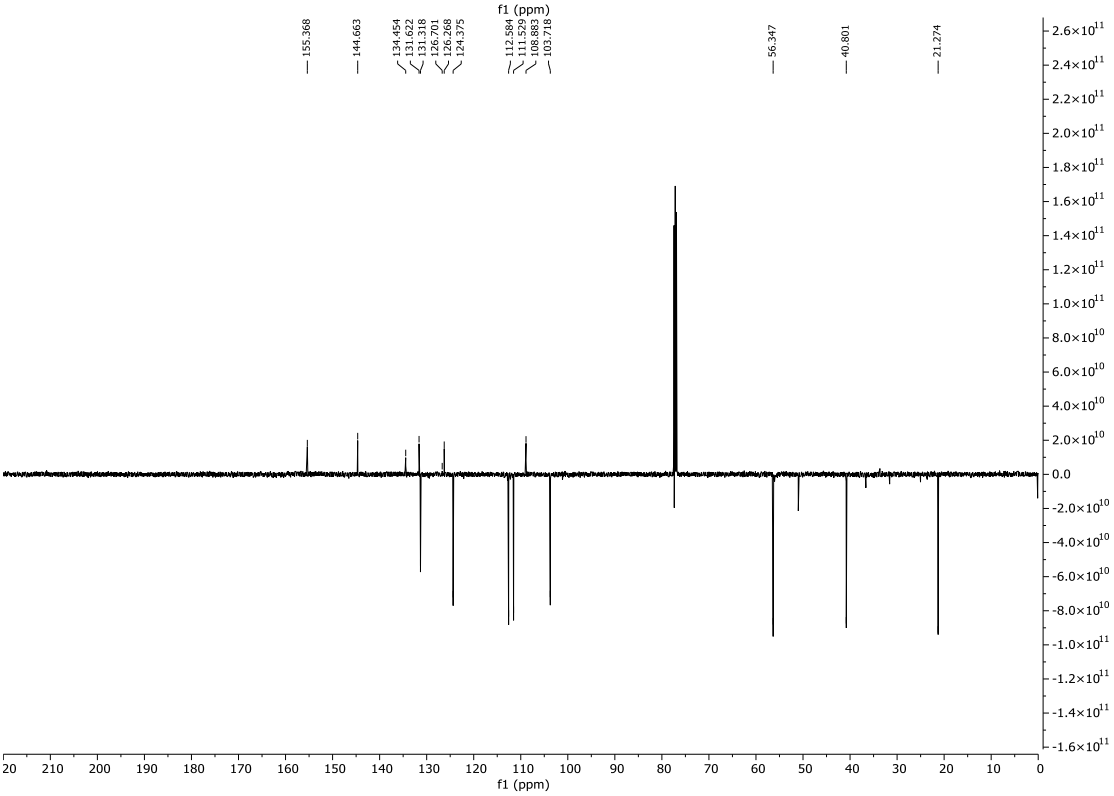

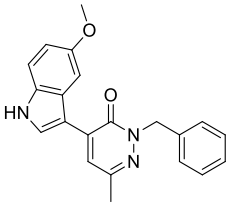

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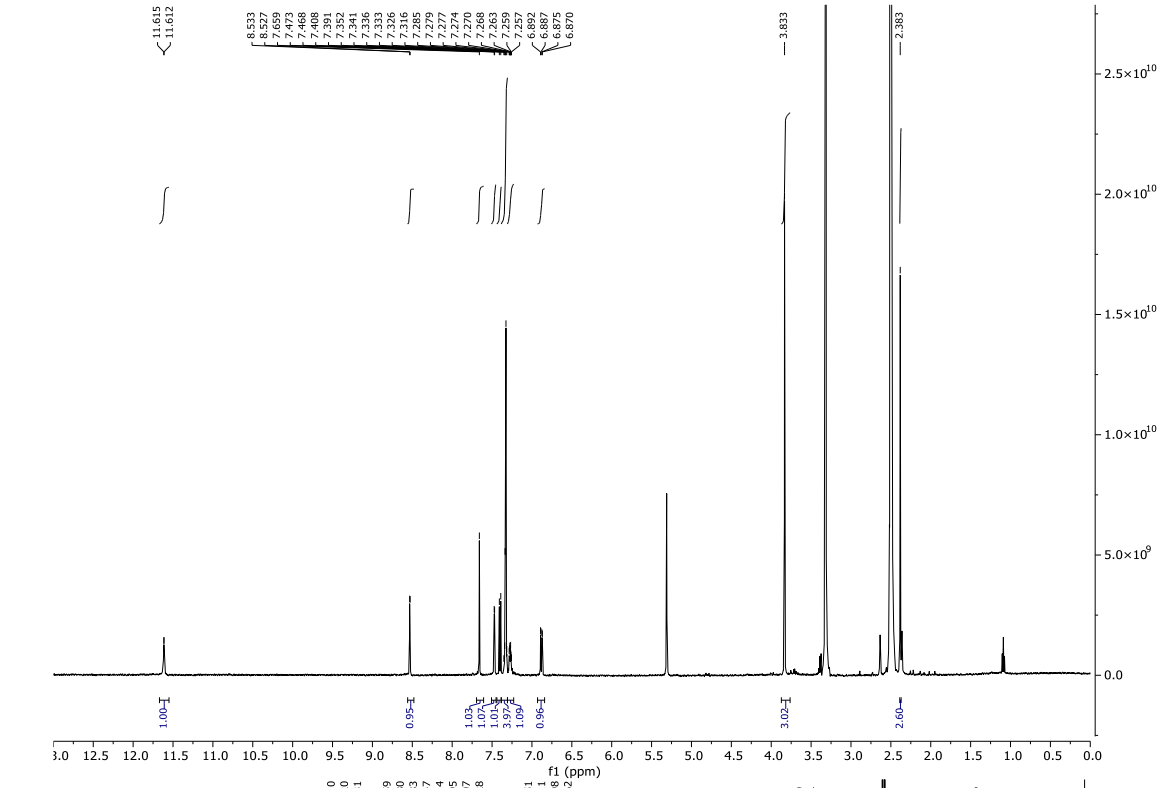

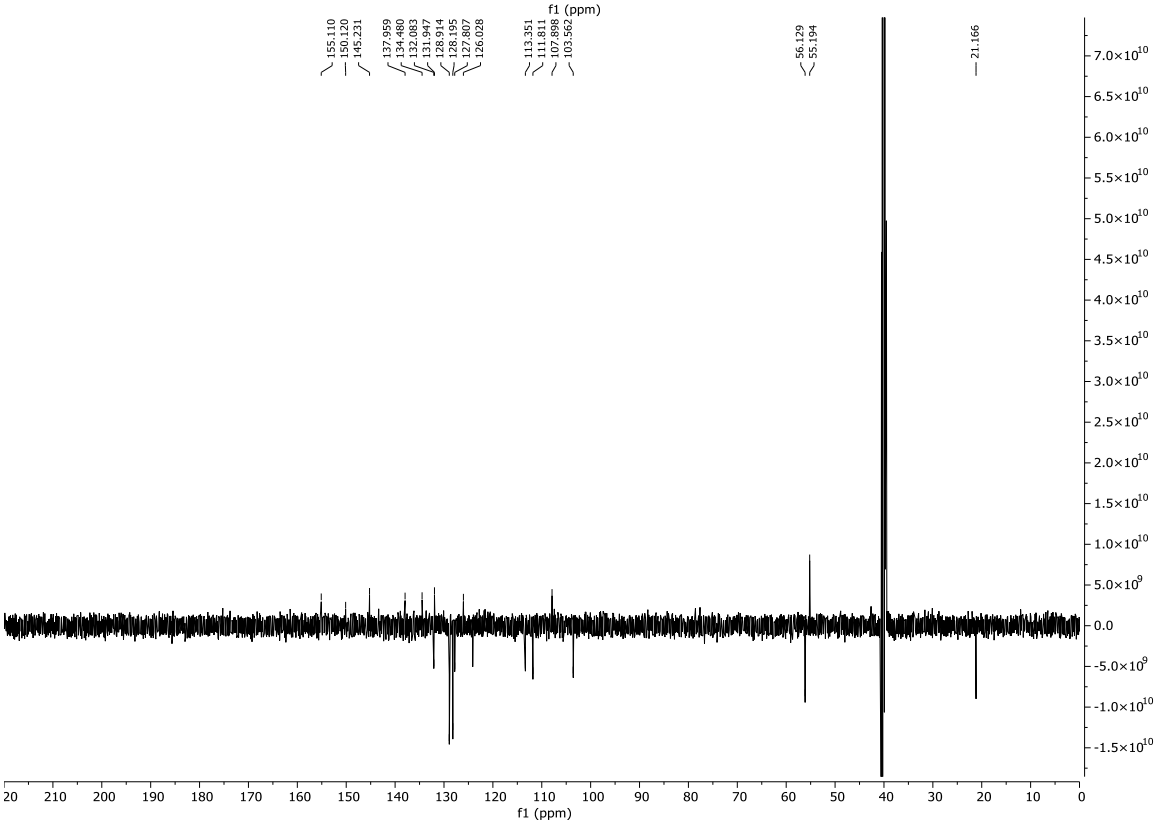

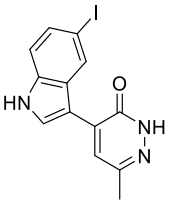

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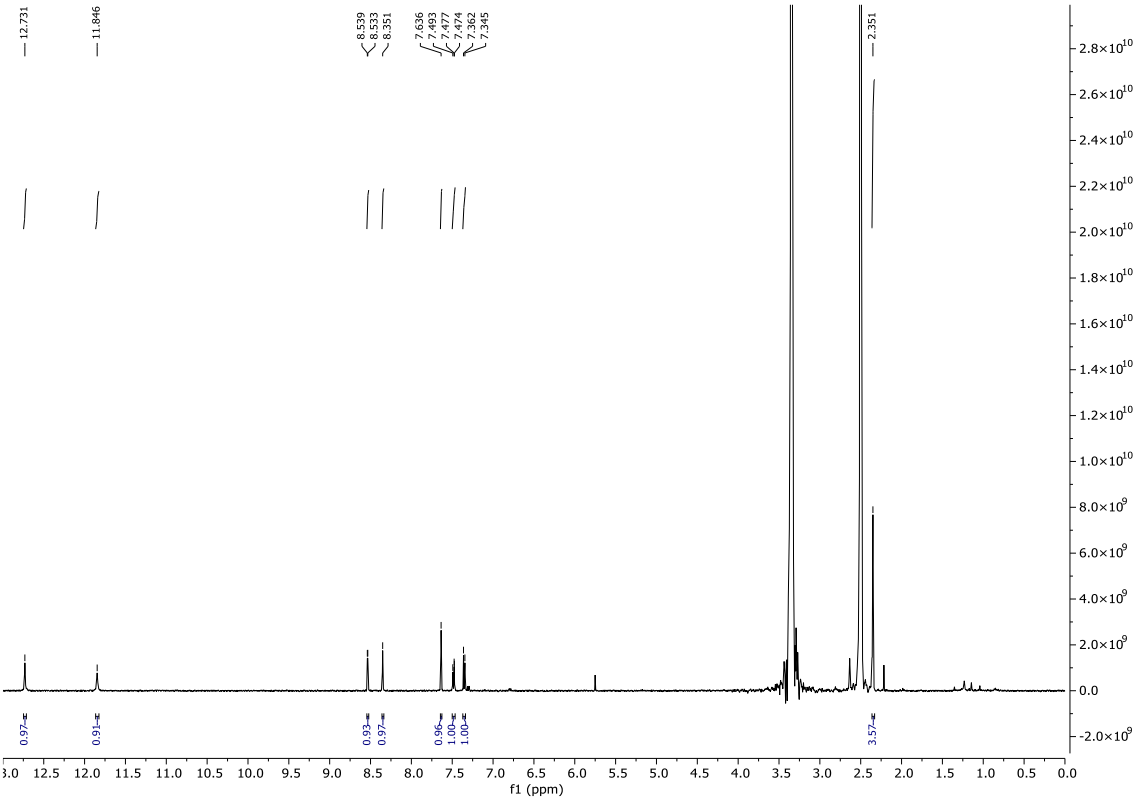

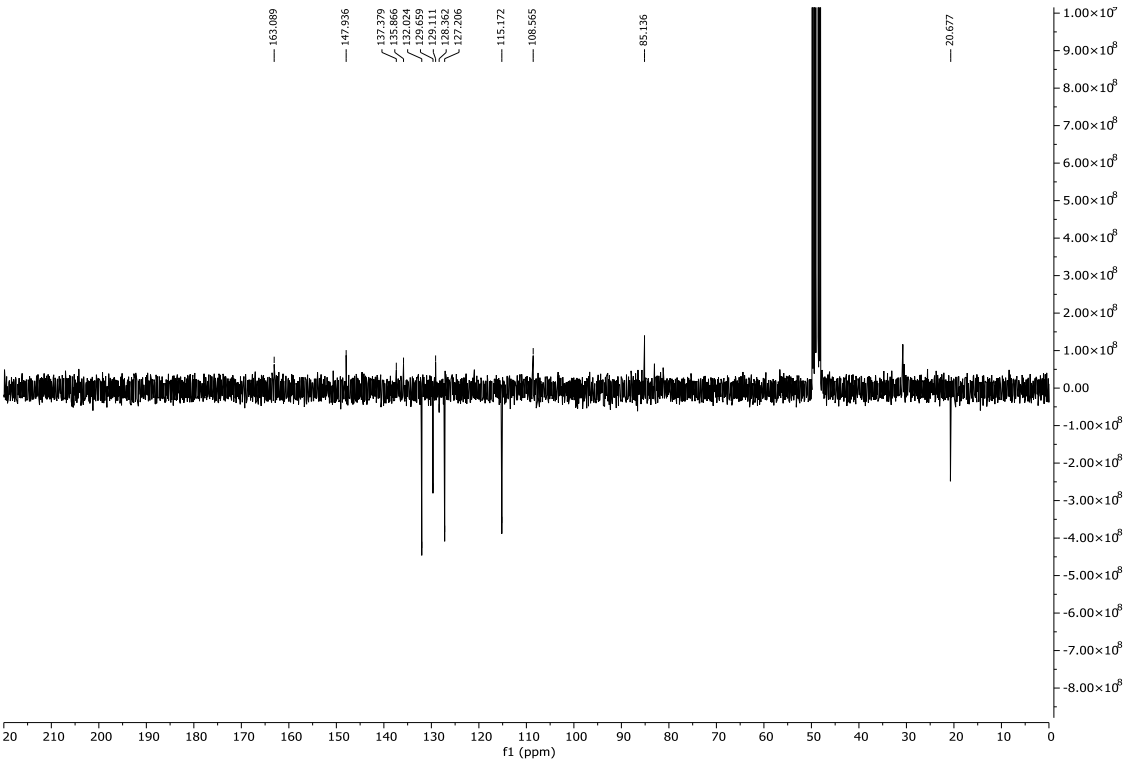

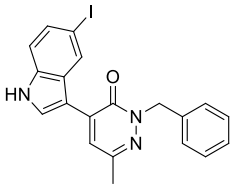

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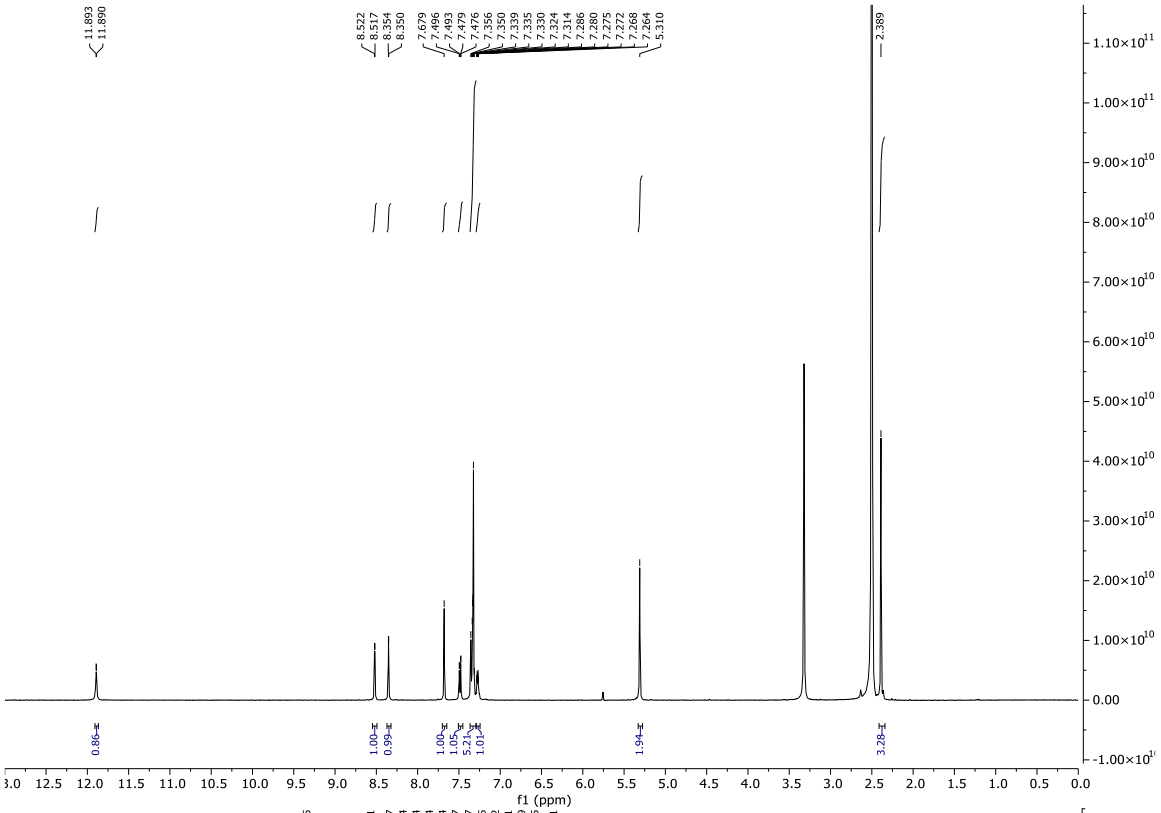

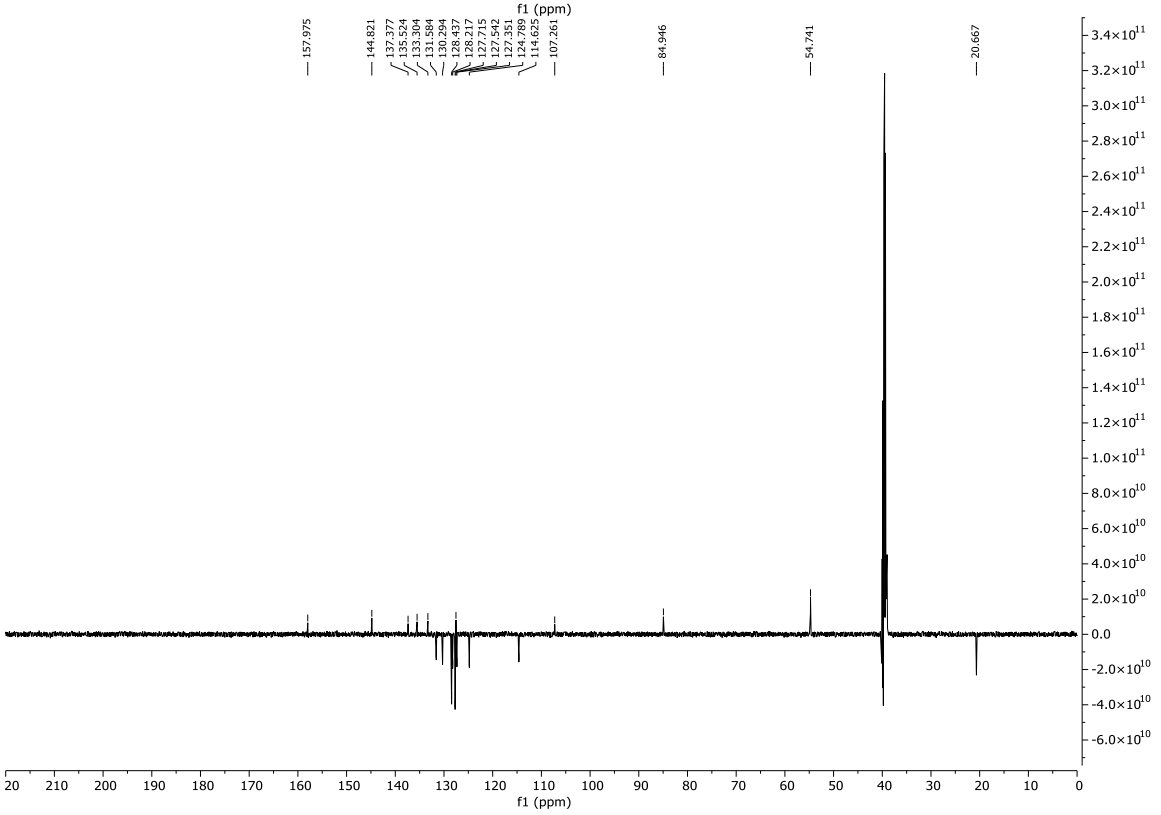

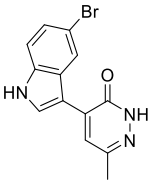

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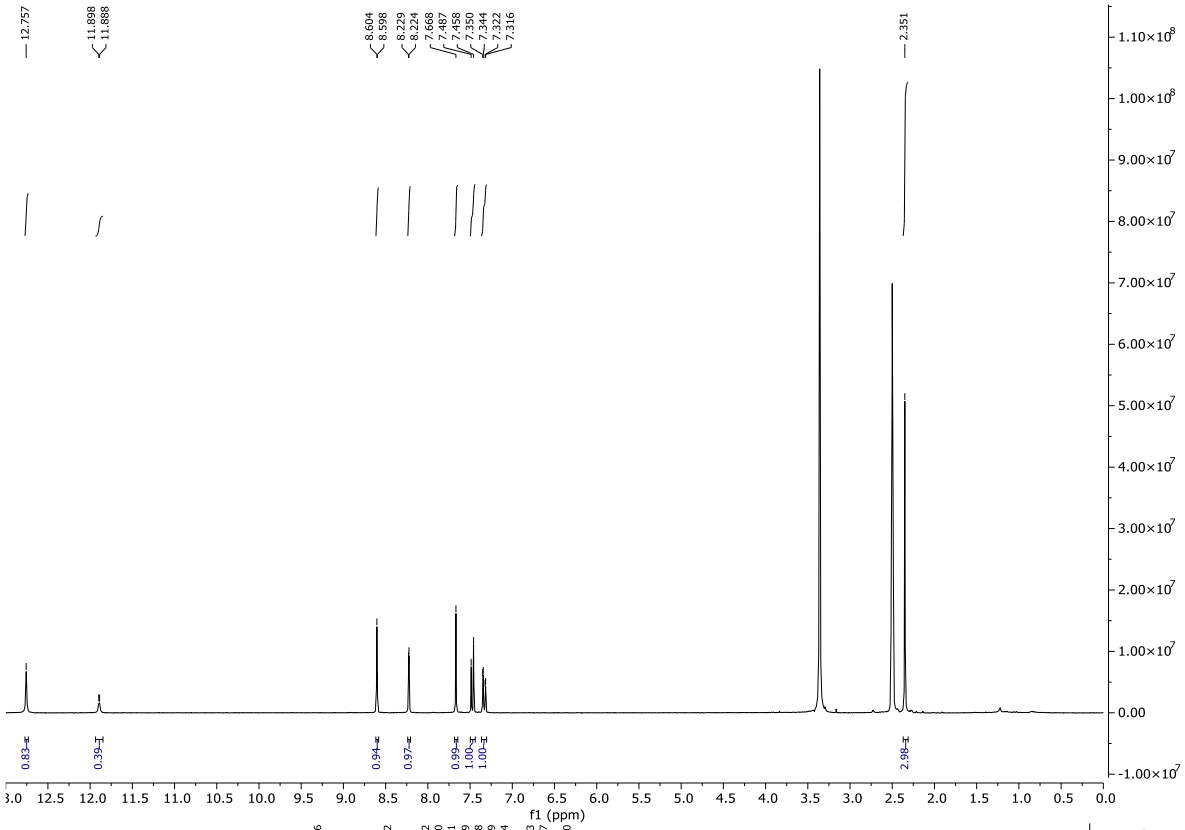

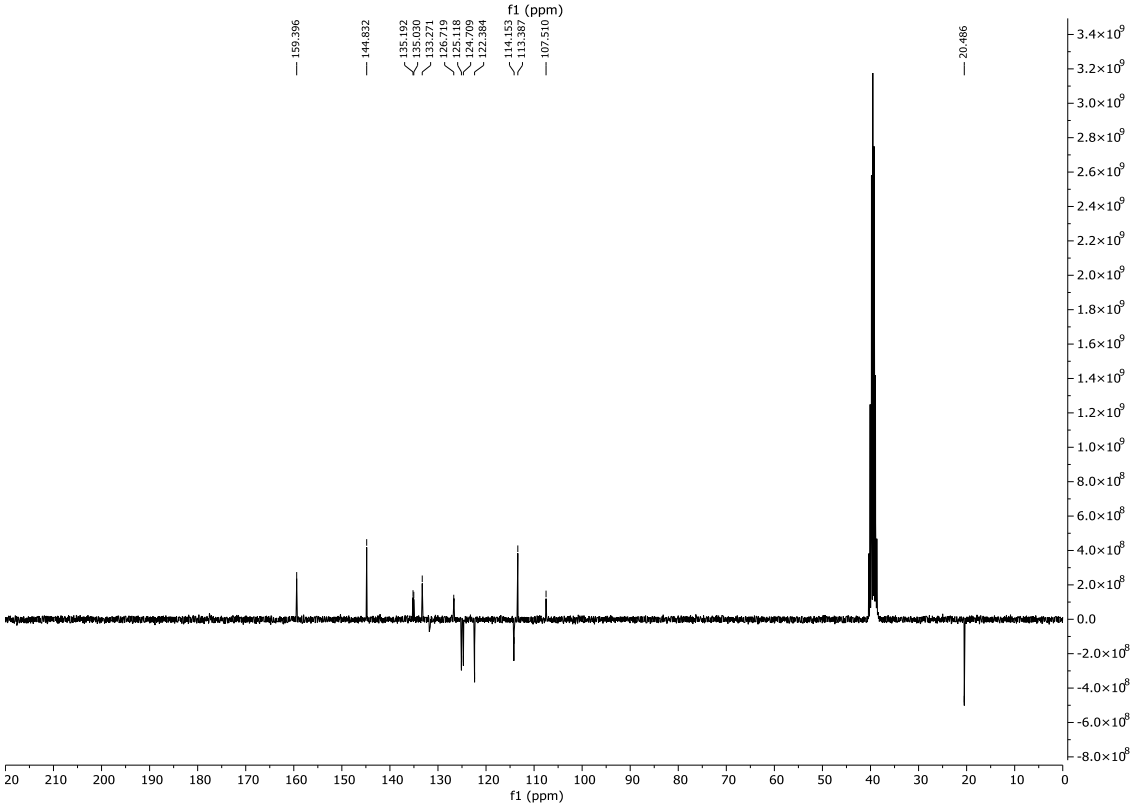

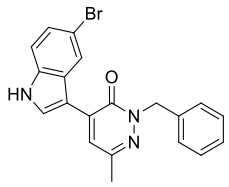

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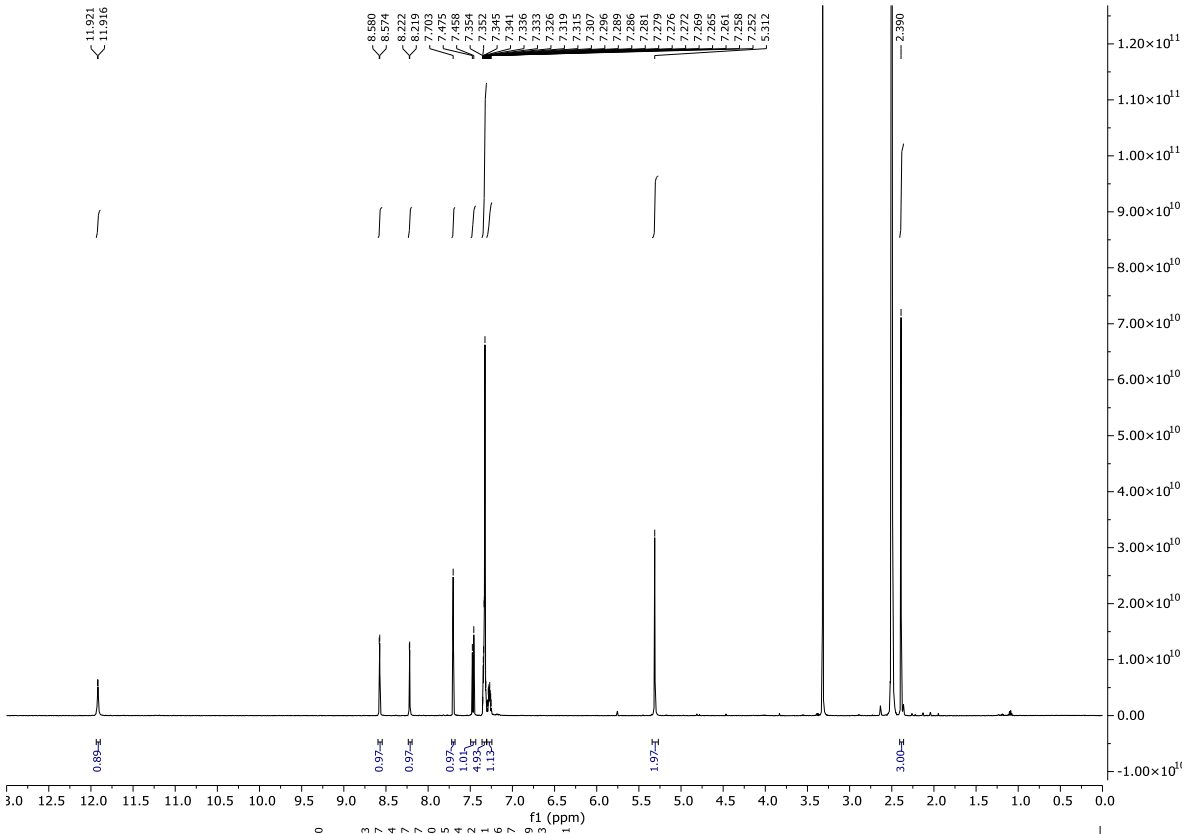

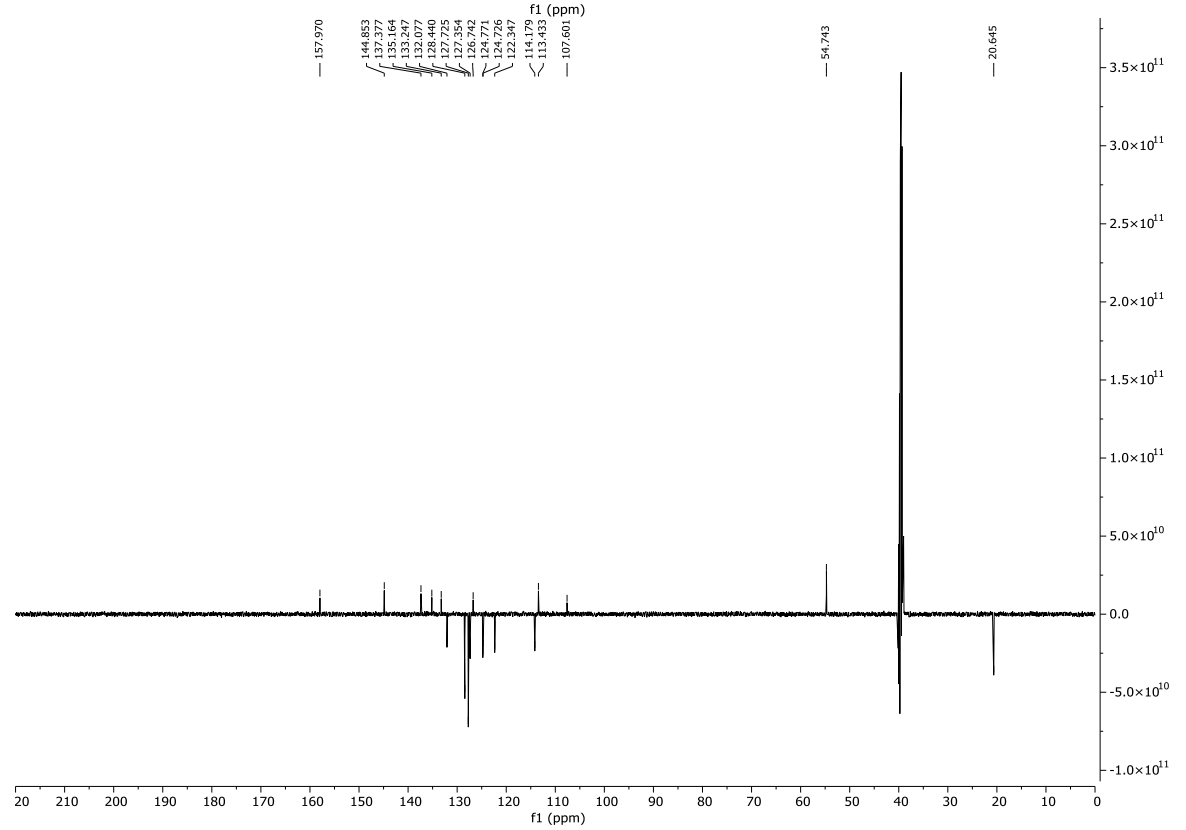

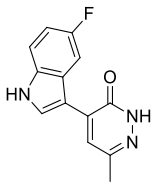

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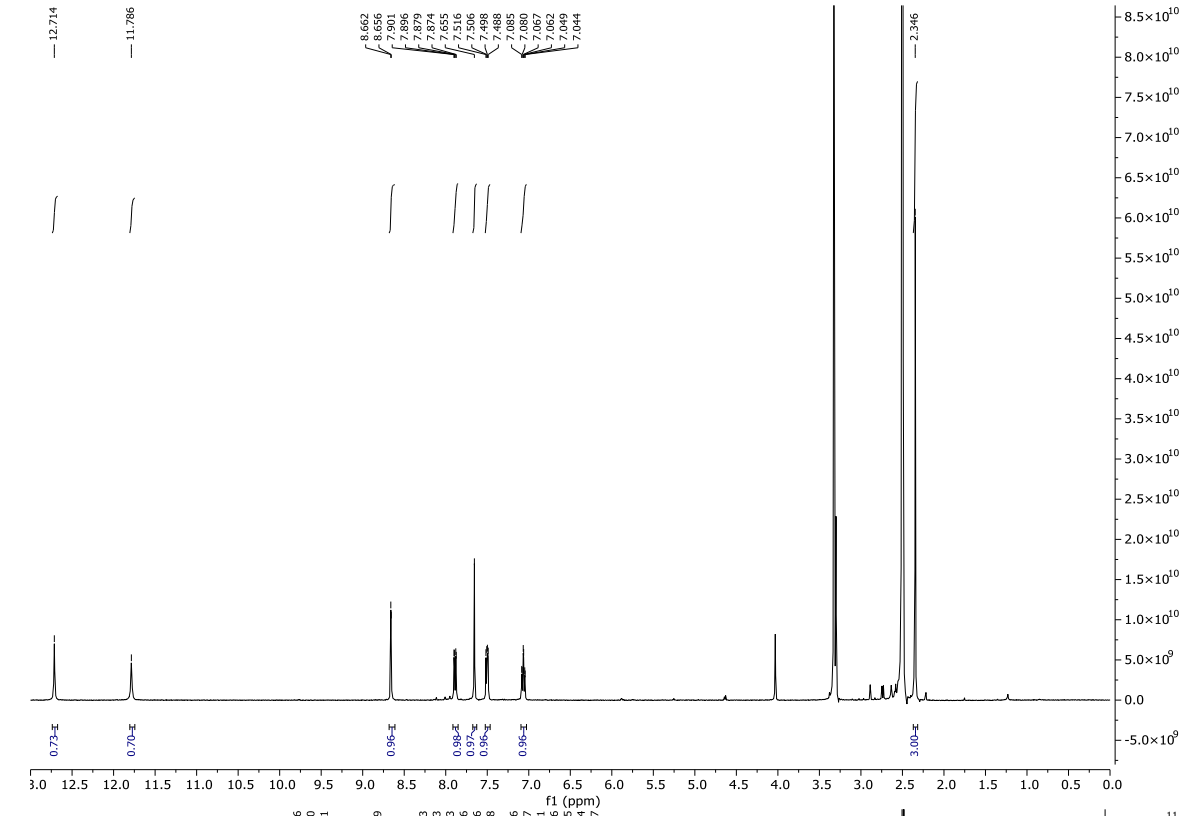

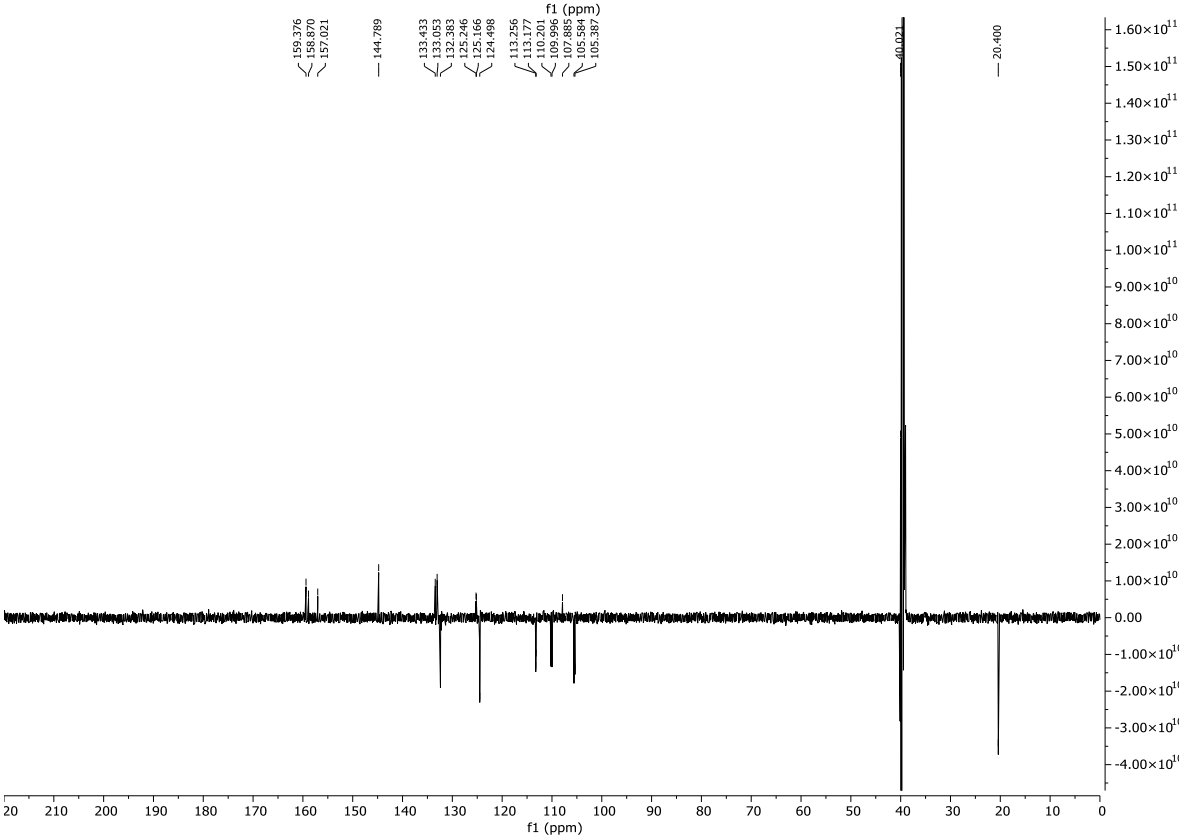

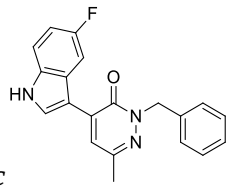

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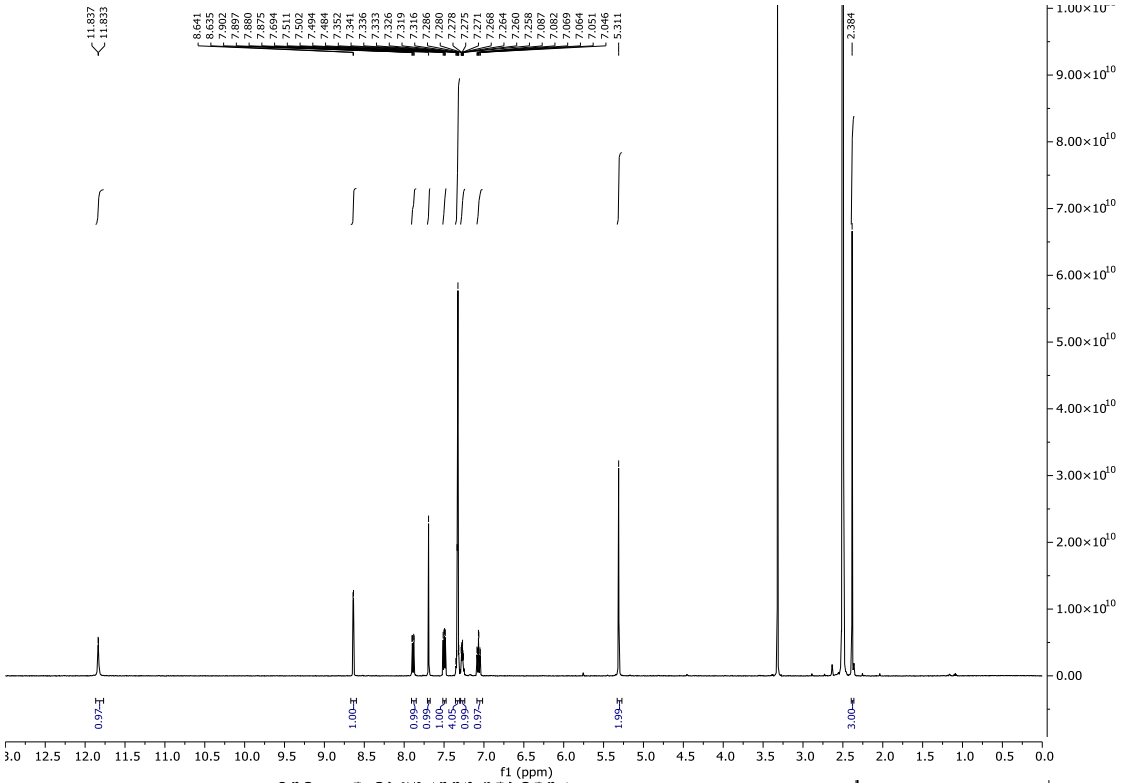
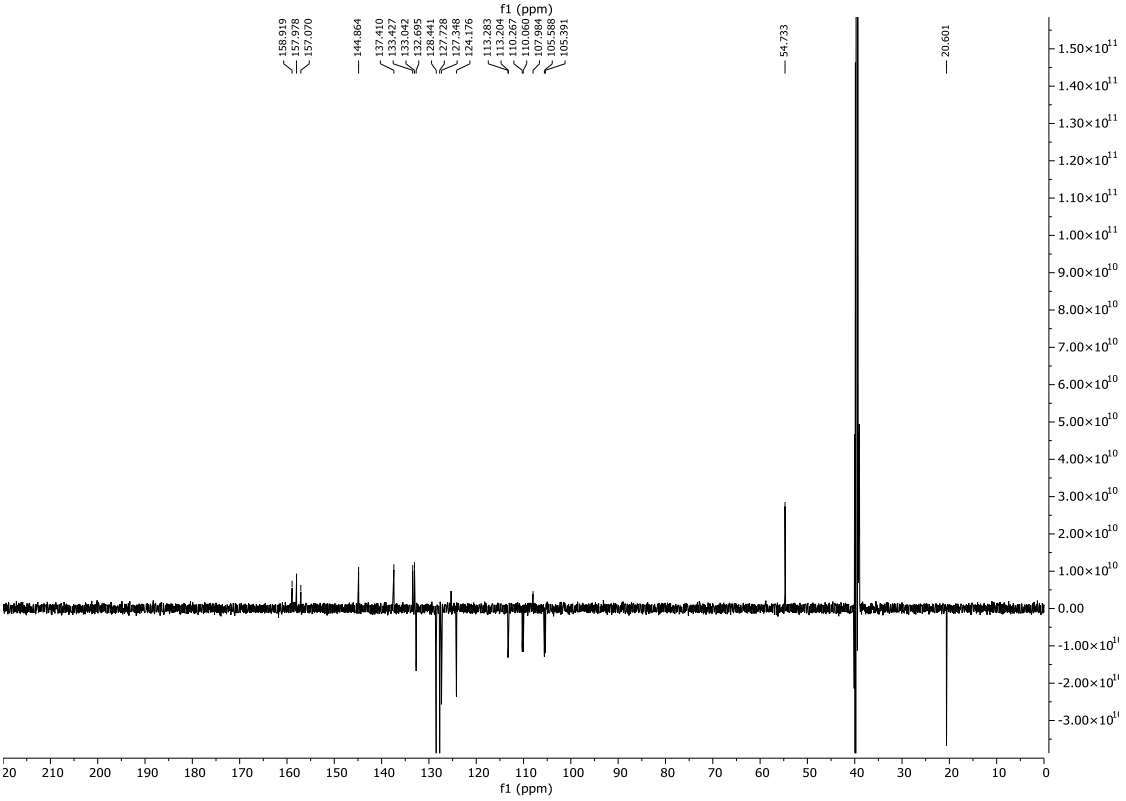
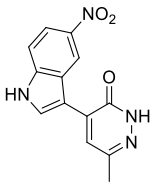
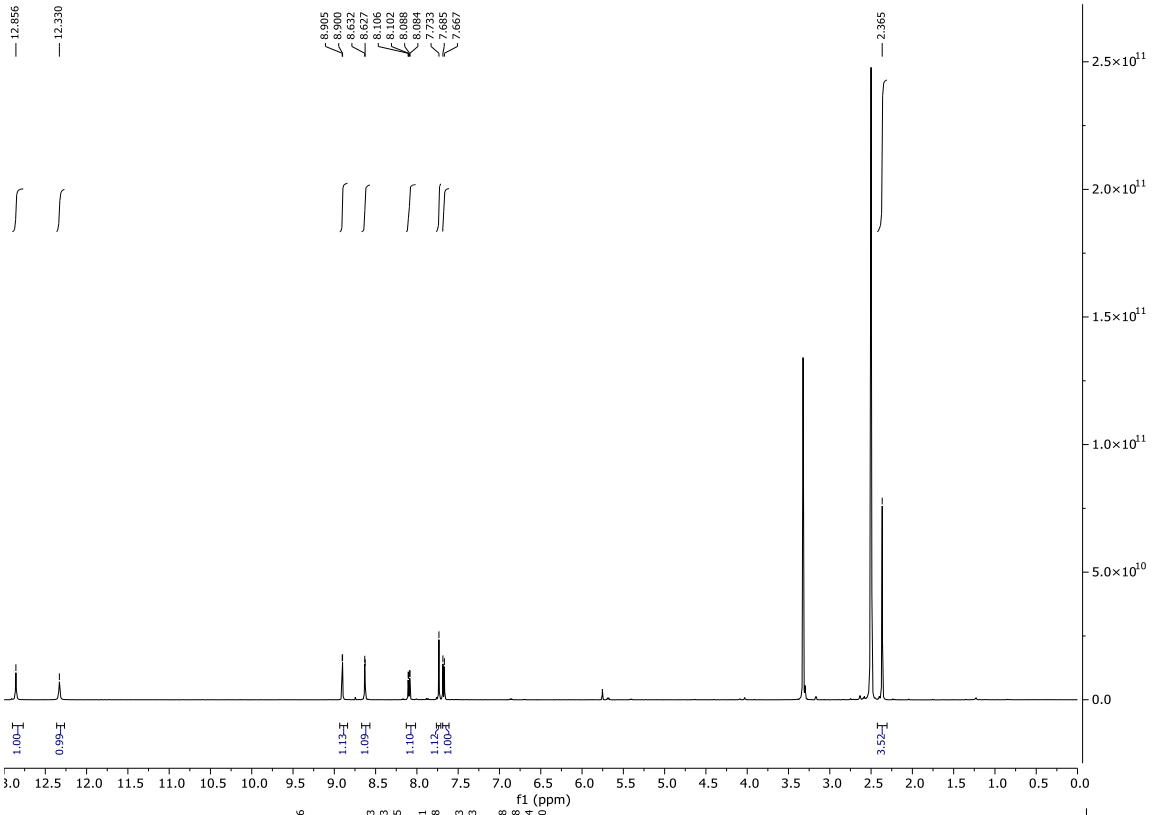
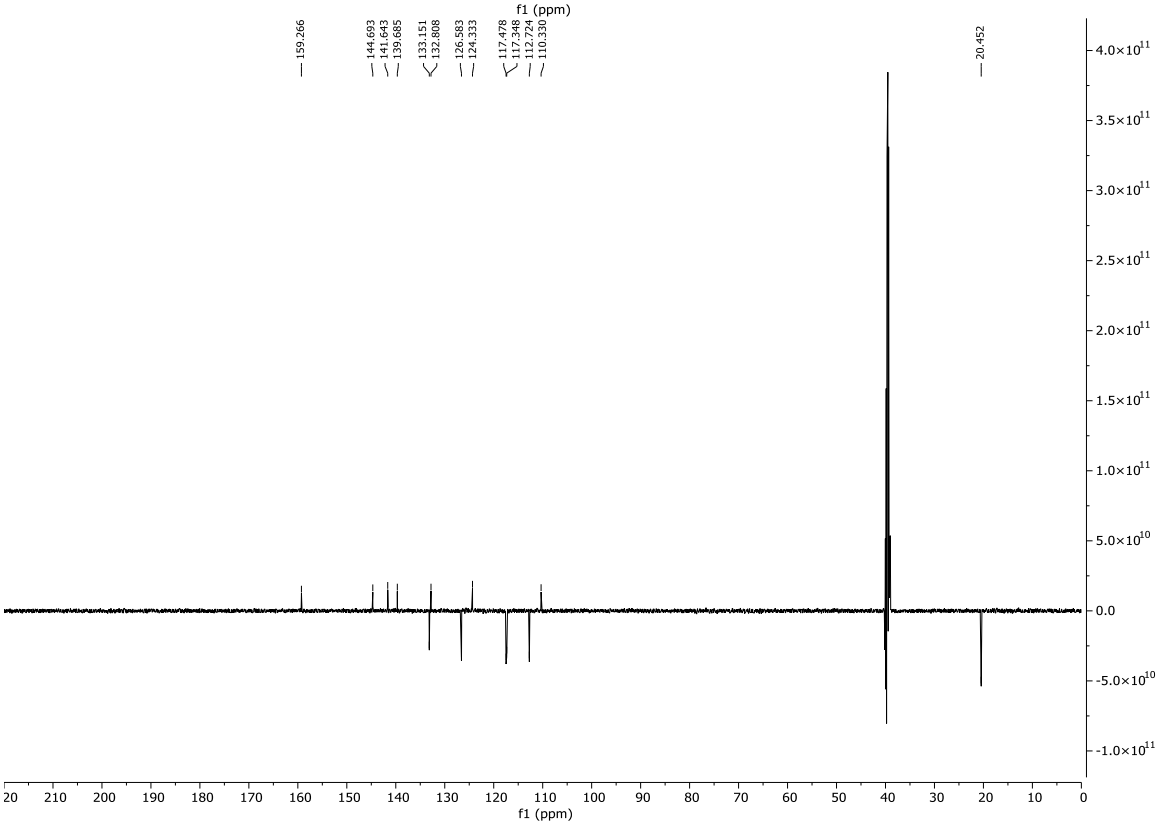






6-methyl-4-(5-nitro-1H-indol-3-yl)pyridazin-3(2H)-one <u>4fa</u>





2. Phosphodiesterase assay

PDE IV activity was monitored by using a PDE IV assay kit (Enzo) adapted to human recombinant phosphodiesterase isoform PDE IV expressed in E. coli. PDE I come from bovine brain and is available in PDE assay kit from Enzo. The basis for the assay is the cleavage of cAMP by the cyclic nucleotide phophodiesterase. The 5'-nucleotide released is further cleaved by 5'-nucleotidase into the nucleoside and phosphate which is quantified using BIOMOLGREEN® reagent. The assay mixture (50 µL/well) contained in the assay buffer (10mM Tris-HCl, pH 7.4), PDE incubating with cAMP, 5'-nucleotidase with or without inhibitor (compounds or control) at 30°C for 60 min. The reaction was stopped by addition of 100 µL of BIOMOLGREEN® reagent and the plate was incubated for another 30 min to allow color to develop before reading OD on a microplate reader.

Test compounds were dissolved in DMSO with a final concentration (2%) which did not significantly affect PDE activity. A non-specific PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX) and Zardaverine as reference, were included as a test control. The initial screening on PDE IV is conducted at 20 µM. For the most potent compounds, the inhibition study on PDE IV activity included five concentrations of the drug. The IC_{50} values were evaluated by nonlinear regression and represent the mean value of two or three independent determinations, each performed in triplicate.

Selectivity index correspond to the absolute value of the ratio : [(%inh PDE4B)-(%inh PDE4D)]/(%inh PDE4B).

3. Molecular modeling

Computational details

Ligand geometry optimizations without symmetry constraints and the corresponding vibrational frequency calculations were conducted with the M06-2X functional [i] and the 6-311G basis set, in the framework of the density functional theory (DFT) using the Gaussian16 software.[ii] To reproduce solvent effects, the polarizable continuum model (PCM) was employed during these optimizations.[iii,iv]

The molecular docking simulations have been conducted by using AutoDock VINA as implemented in the SAMSON software platform.[v] The PDB structures 4MYQ (PDE4B isoform) and 3G58 (PDE4D isoform) were employed to prepare the protein models. A protocol similar to our previous study was followed to add hydrogen atoms and to include a water coordination sphere for the metallic centers Mg^{2+} and Zn^{2+}.[vi] All torsional degrees of freedom of the ligands were then taken into account during the docking simulations. No protein flexibility was considered.

The post-docking noncovalent interaction analyses were conducted by using the IGMPlot code with promolecular electronic density.[vii,viii] This methodology provides IGM-δg^{inter} isosurfaces depicting the noncovalent interaction regions. The nature of the interaction is color-coded: blue for attractive interactions (generally hydrogen-bonding), green for weakly attractive or repulsive interactions (vdW) or red for steric repulsion. IGM also semi-quantitatively estimates the ligand-protein noncovalent interactions through the Δg^{inter} score (the integration of the IGM-δg^{inter} local descriptor). Two partition schemes have been considered: i) the ligand as the first fragment and the protein cavity (excluding the CR3 gating helix) as second fragment, and ii) the ligand as first fragment and the CR3 segment as second fragment. We also employed the atomic scheme decomposition provided within the IGM approach. This extension is able to emphasize the most relevant atomic contributions to the non-covalent interactions occurring between two fragments.[ix] To obtain the atomic contributions to these intermolecular interactions we considered a third partition scheme: iii) the ligand as first fragment and the whole protein cavity (including the CR3 gating helix) as the second fragment.

Complementary results

Table S1. Δg^{inter} score (a.u.) provided by the IGM approach for the two considered protein-ligand systems.

	PDE4B		**PDE4D**	
	Protein excluding CR3	*CR3*	*Protein excluding CR3*	*CR3*
	Δg^{inter}	Δg^{inter}	Δg^{inter}	Δg^{inter}
4ba	5.38	1.44	5.58	1.03
4bc	7.23	0.88	8.47	1.10
4aa	4.73	1.08	5.10	0.94
4ea	4.40	0.76	6.28	1.32

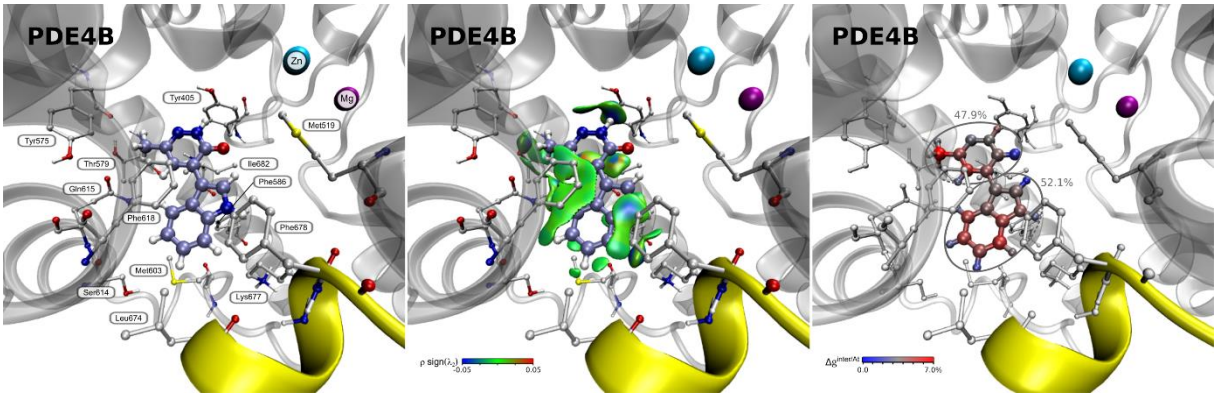


Figure S1. Molecular docking and IGM analysis of **4aa** inside: PDE4B-UCR cavity. IGM δg^{inter} isosurface of 0.07 a.u. and a BGR color code in the range -0.05 < $\rho\ sign\ (\lambda_2)$ < 0.05 a.u (center). IGM atomic decomposition with a ligand colored according to the $\Delta g^{inter/At}$ score using a BGryR color scale (right).

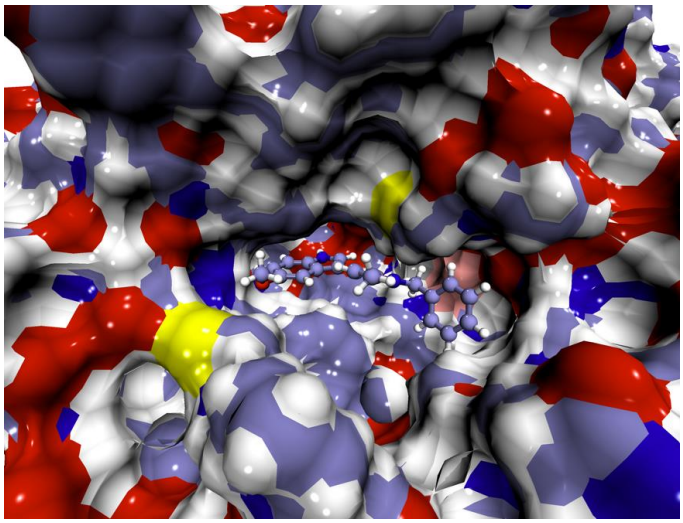


Figure S2. PDE4B-**4bc** docking pose with a benzyl group on the pyridazinone

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4. Anti-inflammatory effects evaluation

Collection of blood samples, cells isolation and culture

Blood samples were provided from "Etablissement Français du Sang Grand Est" and were collected on EDTA (BD Vacutainer® K2E). Peripheral blood mononuclear cells (PBMCs) were purified from whole human blood using Polymorphprep™ protocol (Abcys). Contaminating red blood cells were removed by a hypotonic shock. Purified PBMCs were resuspended in RPMI 1640 Glutamax (Life Technologies) with 10% heat-inactivated Fetal bovine serum (Dutscher) and 1% PenStrep® (Life Technologies). Cells were at least 95% viable. Cells were cultured in 0.5 mL of complete medium containing 25 ng/mL recombinant human M-CSF (Macrophages Colony-Stimulating-Factor, Millipore) for 24 h in 48-well Falcon plates (Becton Dickinson), or in 1 mL of complete medium on Thermanox™ coverslips (Dutscher) in 24-well Falcon plates (Becton Dickinson) for imagery purpose, both at 37°C in humidified atmosphere with 5% CO2. In both culture models, non adherent cells were then removed and media replenished, without M-CSF. Stimulation of the cells was performed using DMSO 0.5% (control condition), DMSO 0.5% plus *E. Coli* lipopolysaccharide 10 ng/mL (to mimic inflammatory environment, LPS B111:O4, Sigma Aldrich), or LPS and anti-PDE4 compounds: 10 µM zardaverine, 10 µM roflumilast and 50, 20, 10 or 5 µM **4ba** compound. At the end of this stimulation time (24 h), cells in 48-well plates were centrifuged at 500 g for 10 min, supernatants were collected and frozen at -20°C, and cells in 24-well plates were processed for scanning electron microscopy as described below.

Cell death

Cell death was determined by measuring lactate dehydrogenase (LDH) activity in cell culture supernatant according to the manufacturer's protocol (Cytotoxicity Detection Kit (LDH) Roche). The absorbance was read at 492 nm and corrected for background non-specific signal at 700 nm on a Fluostar Omega spectrophotometer (BMG Labtech).

ELISA

IL-1β, IL-6 and IL-10 concentrations in macrophages conditioned supernatants were measured using *DuoSet ELISA* kits (RnD Systems) following manufacturer's instructions. IL-8 and TNF-α concentration were measured using *ELISA-MAX™* kits (BioLegend) following manufacturer's instructions. Controls included non-stimulated cells and medium alone. Levels of cytokines were estimated using human recombinant cytokines standard curve. The detection

limit for the kits were 3.9 pg/mL for IL-1β, 7.8 pg/mL for IL-6 and TNF-α, 15.7 for IL-8 pg/ml and 31 pg/mL for IL-10.

Scanning electron microscopy

Cells cultured on Thermanox™ lamella were washed 2-times in PBS, then fixed in 2.5% (w/v) glutaraldehyde (Sigma-Aldrich) at room temperature for 1 h. After Cells were washed twice with distilled water then dehydrated in graded ethanol solutions (50, 70, 90, and 100% 2-times) for 10 min. Cells were finally desiccated in a drop of hexamethyldisilazane (HMDS, Sigma). After air-drying at room temperature, samples were sputtered with a thin gold-palladium film using a JEOL ion sputter JFC 1100 instrument. Cells were observed using a Schottky Field Emission Scanning Electron Microscope (JEOL JSM-7900F). Images were obtained at a primary beam energy of 2 kV (SM-EXG65 electron emitter).

Air pouch model of inflammation

Animal experiments were performed on mice housed in a controlled environment (temperature: 21 ± 2°C, relative humidity: 65 ± 15%, natural alternating light cycle/darkness, health university campus, Reims, France, agreement n°B514543) in accordance with protocols approved by the Regional Ethics Committee on Animal Experimentation (CEEA n°056) and the Ministry of Agriculture, under the direction of investigators certified for animal experiments following protocol APAFIS#13375-2017121218136235v9. Seven week-old BALB/cJRj mice were acclimated for 1 week before air pouch induction. Before each injection, mice were anesthetized using IsoFlo. At D0, three milliliters of 0.2 µm filtered air were injected with 10 mL syringe and 27G needle, subcutaneously, in the middle of the back of mice. At day 3, injection was repeated. At D7, inflammation was induced, injecting 2 mL of LPS (10 ng/mL) with 0.5% DMSO, zardaverine or compound 4c (at 2, 20 or 200 µM). Control mice were injected with 2 mL of 0.5% DMSO in saline only. After six hours, animals were sacrificed. To remove the exudate from the air pouch, 2 mL of PBS-EDTA at 5 mM were injected, and after a 30 s soft massage of the pouch, exudate was collected. Cells from the exudate were washed and counted using a Kova® slide (Dutsher, Brumath, France). To evaluate leukocyte subsets and discriminate monocytes and macrophages, cells were stained with fluorescent antibodies anti-Ly6G and anti-F4/80. After a wash, cells were fixed and used for flow cytometry acquisition with a BD LSRFortessa™ system. F4/80-BV421 was excited with the 405 nm laser and detected with the emission filter 450/50 nm. Ly6G-PE was excited with the 561 nm laser and detected with the emission filter 585/15 nm. One hundred thousand cells were collected per

sample. At the first stage, single cells were selected by a FSC-H/FSC-A gate. On this population, cells were selected by a FSC-A/SSC-A gate excluding subcellular debris. Then, monocytes and macrophages were selected by a Ly6G-PE$^-$ gate on a SSC-A/Ly6G-PE dot plot. Macrophages and monocytes were discriminated on a SSC-A/F4/80-BV421 dot plot. Data analysis was performed with FlowJo software.

Statistics

Each *in-vitro* experiment was performed on cells from 8 independent donors. For *in-vivo* experiments, 8 to 17 animals were used depending on condition. Owing to a lack of normal distribution of the assessed variables due to the small number of donors, non parametric exact Kruskal Wallis test followed by *post-hoc* exact and stratified (when appropriate) Wilcoxon-Mann-Whitney tests with the *p*-value fixed at 0.05, were carried out to determine the significance of the results (StatXact7.0, Cytel Inc.).