

Supporting Information

Synthesis, biological evaluation and *in silico* studies of certain oxindole-indole conjugates as anticancer CDK inhibitors

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1. Anti-proliferative action toward human breast cell lines

The two examined human breast cancer cell lines (MCF-7 and Breast MDA-MB-231) have been obtained from American Type Culture Collection (ATCC). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 °C in humidified atmosphere containing 5% CO₂. Cells at a concentration of 0.50×10^6 were grown in a 25 cm² flask in 5 ml of culture medium.

The anti-proliferative activity of the tested hybrids **6a-i**, **9a-f** and **11a, b** was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay. Briefly, Cells were inoculated in 96-well microtiter plate (5×10^4 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentrations of tested compounds, doxorubicin and sorafenib were added to the cells (three wells were prepared for each individual dose). Cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO₂. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between percent of surviving fraction and drug concentration is plotted to get the survival curve for each cell line. The concentration required for 50% inhibition of cell viability (IC₅₀) was calculated.

2. Cell Cycle Analysis

Breast cancer MCF-7 cells were treated with hybrids **6e** and **6f** for 24 h (at their IC₅₀ concentration), and then cells were washed twice with ice-cold phosphate buffered saline (PBS). Subsequently, the treated cells were collected by centrifugation, fixed in ice-cold 70% (*v/v*) ethanol, washed with PBS, re-suspended with 100 µg/mL RNase, stained with 40 µg/mL PI, and analyzed by flow cytometry using FACS Calibur (Becton Dickinson, BD, Franklin Lakes, NJ, USA). The cell cycle distributions were calculated using CellQuest software 5.1 (Becton Dickinson).

3. ELISA Immunoassay

The levels of the pro-apoptotic markers (Bax, caspase-3 and p53) as well as the anti-apoptotic marker Bcl-2 were determined using ELISA colorimetric kits per the manufacturer's instructions. Breast cancer MCF-7 cells were cultured as a monolayer in T-25 flasks and were seeded to attain 30% confluency prior to treatment. Cells were then treated separately with hybrids **6e** and **6f** at their IC₅₀ concentrations for 48 h. At the end of treatment, cells were collected via trypsinization and centrifuged at 10,000 rpm. The pellet was then rinsed with PBS and lysed in RIPA lysis buffer at 4 °C for 45 min, then centrifuged at 14,000 rpm for 20 min to remove the cellular debris. Lysates were then collected and stored at -80 °C for later protein determination using Pierce BCA Protein Assay Kit according to manufacturer's recommendations.

The cell lysate was diluted 10 times, and 100 µL (50 mg protein) was added to the wells of four separate microtiter plates for the four ELISA kits that were pre-coated with primary antibodies specific to Bax, Bcl-2, caspase-3 and p53 proteins, respectively. A secondary biotin-linked antibody specific to the protein captured by the primary antibody was further added to bind the captured protein, forming a "sandwich" of specific antibodies around the desired protein in the cell lysate. The streptavidin-HRP complex was then used to bind the biotin-linked secondary antibody through its streptavidin portion. The HRP domain reacted with the added TMB substrate to form a colored product that measured at 450 nm by a plate reader ChroMate-4300 after the reaction was terminated *via* the addition of stop solution.

4. Annexin V-FITC Apoptosis Assay

Phosphatidylserine externalization was assayed using Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, USA) according to the manufacturer's instructions. Breast cancer MCF-7 cells were cultured to a monolayer then treated with hybrids **6e** and **6f** at their IC₅₀ concentration. Briefly, cells were then harvested *via* trypsinization, and rinsed twice in PBS followed by binding buffer. Moreover, cells were re-suspended in 100 µL of binding buffer with the addition of 1 µL of FITC-Annexin V followed by an incubation period of 30 min at 4 °C. Cells were then rinsed in binding buffer and resuspended in 150 µL of binding buffer with the addition of 1 µL of DAPI (1 µg/µL in PBS). Cells were then analyzed using the flow cytometer BD FACS Canto II and the results were interpreted with FlowJo7.6.4 software (Tree Star, Ashland, OR, USA).

5. CDK Kinase Inhibitory Activity

Reaction Biology Corp. Kinase HotSpotSM service (<http://www.reactionbiology.com>) was used for screening of tested compounds. CDK Kinase inhibitory activities were assessed by the HotSpot assay platform, which contained specific kinase/substrate pairs along with required cofactors. Base reaction buffer: 20 mM Hepes (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na₃VO₄, 2 mM DTT, 1% DMSO. Testing compounds were dissolved in 100% DMSO to specific concentration. The serial dilution was conducted by Integra Viaflo Assist in DMSO. The reaction mixture containing the examined compound and ³³P-ATP was incubated at room temperature for 2 h and radioactivity was detected by filter-binding method. Kinase activity data were expressed as the percent remaining kinase activity in test samples compared to vehicle (dimethyl sulfoxide) reactions.

6. 2D Diagrams for hybrids 6b-d and 6f-i showing their interactions with the CDK4 binding site

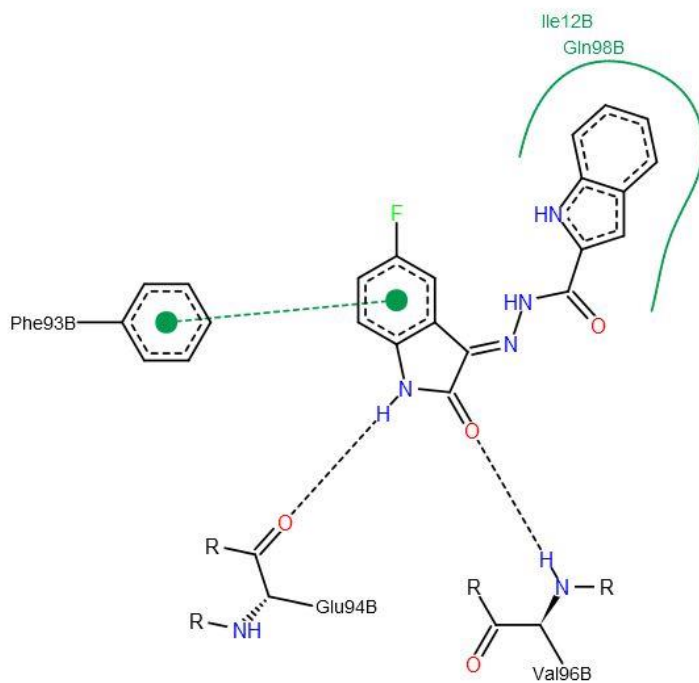


Figure S1. 2D diagram for hybrid **6b** showing its interaction with the CDK4 binding site.

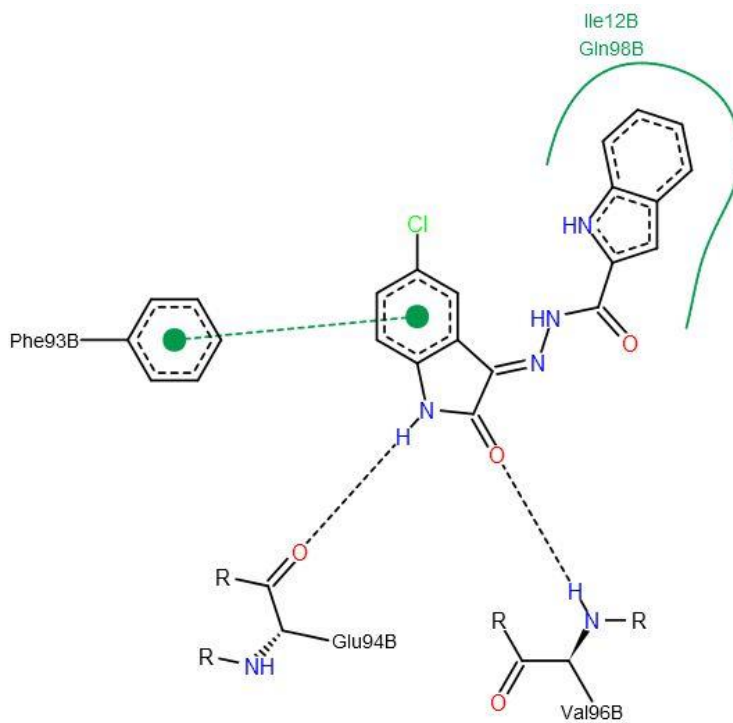


Figure S2. 2D diagram for hybrid **6c** showing its interaction with the CDK4 binding site.

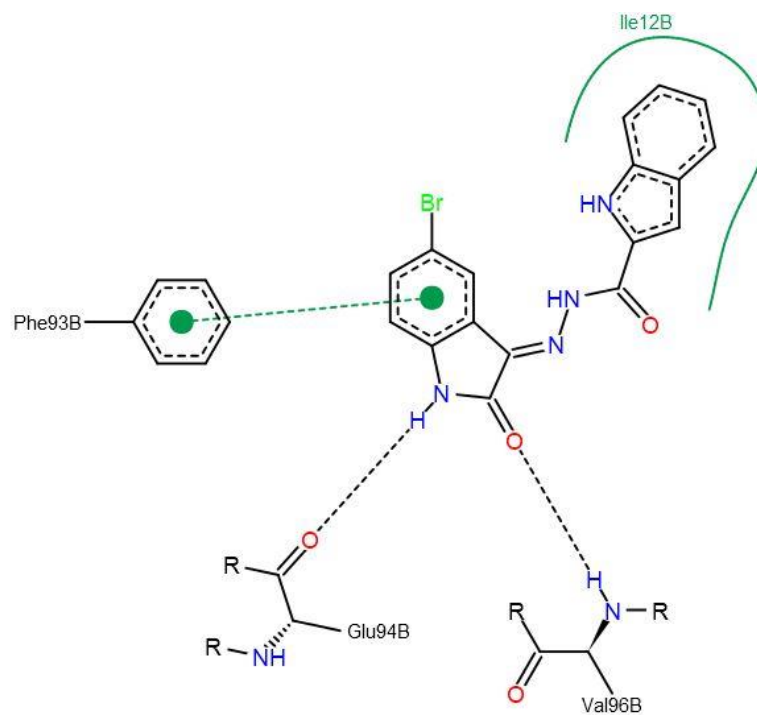


Figure S3. 2D diagram for hybrid **6d** showing its interaction with the CDK4 binding site.

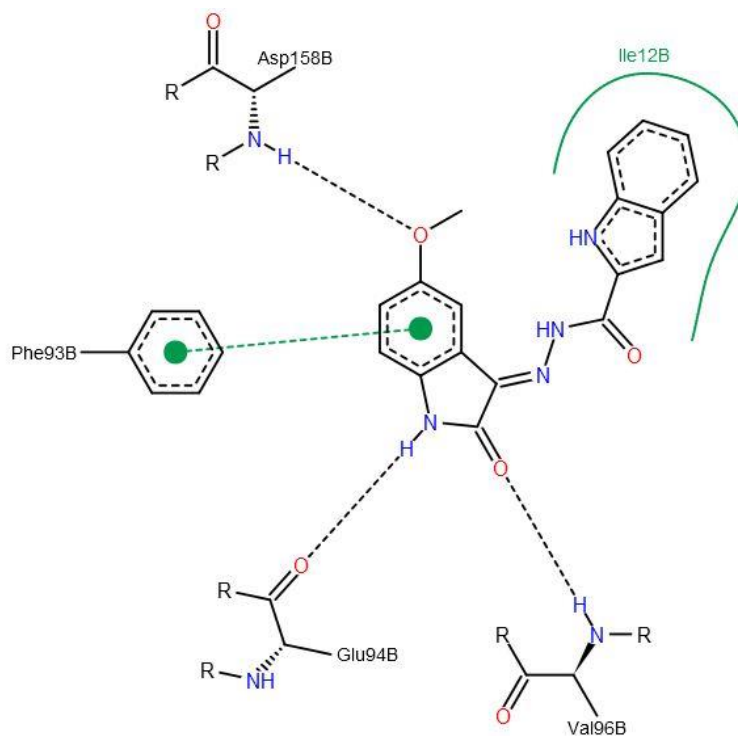


Figure S4. 2D diagram for hybrid **6f** showing its interaction with the CDK4 binding site.

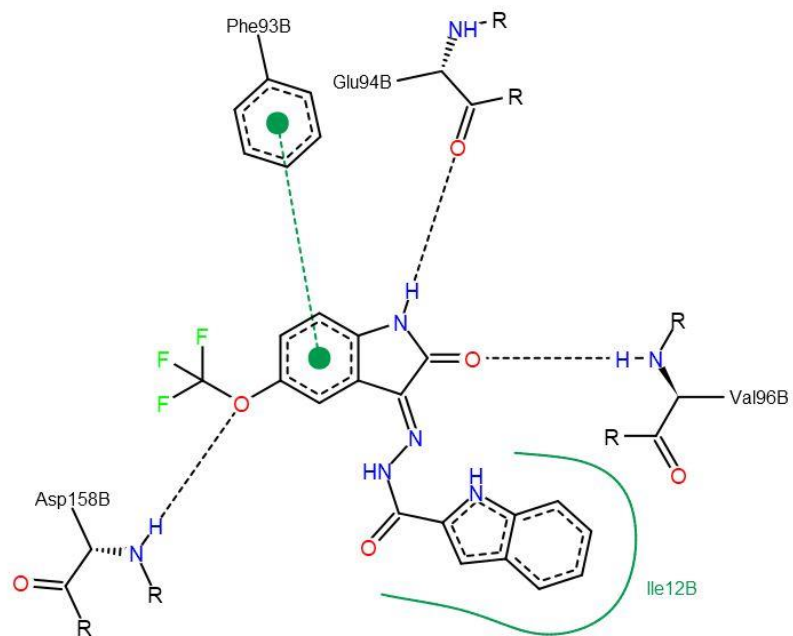


Figure S5. 2D diagram for hybrid **6g** showing its interaction with the CDK4 binding site.

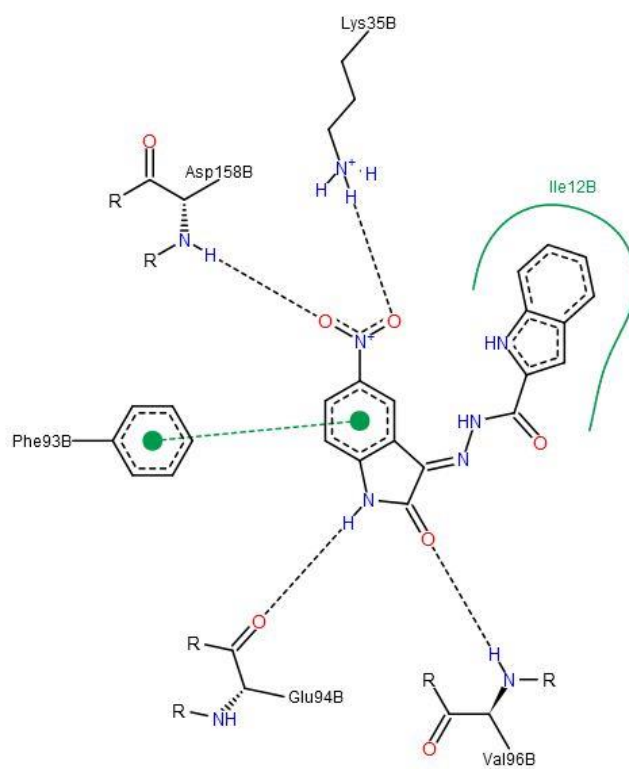


Figure S6. 2D diagram for hybrid **6h** showing its interaction with the CDK4 binding site.

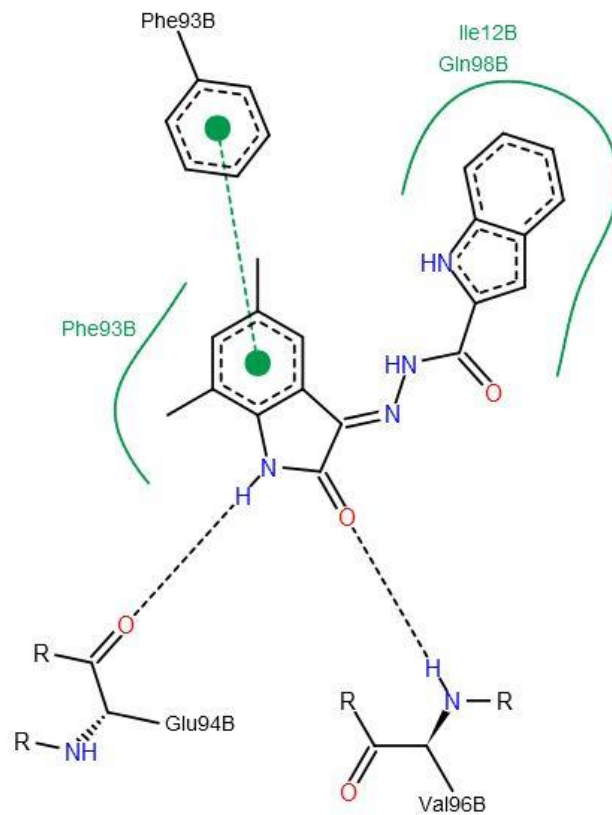


Figure S7. 2D diagram for hybrid **6i** showing its interaction with the CDK4 binding site.

7. Characterisation of intermediate (4) and the target hybrids (6a-i, 9a-f and 11a, b)

1H-indole-2-carbohydrazide 4

Yield 84 %, m.p. 249-251 °C (reported: 247-248 °C [48]); ¹H NMR (500 MHz) δ ppm: 4.51 (s, 2H, NH₂), 6.99 (t, 1H, *J* = 8.5 Hz, Ar_H), 7.07 (d, 1H, *J* = 2.0 Hz, Ar_H), 7.13 (dt, 1H, *J* = 1.5 and 8.5 Hz, Ar_H), 7.41 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.56 (d, 1H, *J* = 8.0 Hz, Ar_H), 9.78 (s, 1H, NH), 11.60 (s, 1H, NH); ¹³C NMR (125 MHz) δ ppm: 101.85, 112.28, 119.73, 121.43, 123.14, 127.13, 130.51, 136.33, 161.27

N'-(2-Oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6a.

Yield 81 %, m.p. > 300 °C (reported: > 300 °C [49]); Purity 97%; IR: 3362, 3168, 3070 (3NH), 1727, 1658 (2C=O); ¹H NMR (500 MHz) δ ppm: 6.91 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.07-7.11 (m, 2H, Ar_H), 7.24 (t, 1H, *J* = 8.0 Hz, Ar_H), 7.39 (t, 1H, *J* = 7.0 Hz, Ar_H), 7.47 (d, 1H, *J* = 8.5 Hz, Ar_H), 7.57 (s, 1H, Ar_H), 7.70 (d, 1H, *J* = 8.0 Hz, Ar_H), 8.01 (d, 1H, *J* = 7.5 Hz, Ar_H), 10.87 (s, 1H, NH), 11.68 (s, 1H, NH), 11.95 (s, 1H, NH); ¹³C NMR (125 MHz) δ ppm: 110.68, 111.25, 112.52, 112.61, 115.62, 119.87, 120.25, 120.49, 120.96, 121.89, 122.24, 122.77, 124.65, 124.87, 126.73, 126.95, 127.04, 128.77, 131.69, 132.78, 137.13, 137.47, 142.38, 143.93, 160.73, 161.01, 163.16, 164.79; MS *m/z* [%]: 304.2 [M⁺, 46.86], 144.02 [100]; Anal. calcd. For C₁₇H₁₂N₄O₂: C, 67.10; H, 3.97; N, 18.41; Found C, 66.83; H, 4.01; N, 18.53.

N'-(5-Fluoro-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6b.

Yield 82 %, m.p. > 300 °C (reported: > 300 °C [49]); Purity 96%; IR: 3453, 3372, 3189 (3NH), 1723, 1649 (2C=O); ¹H NMR (500 MHz) δ ppm: 6.89-6.92 (m, 1H, Ar_H), 7.07 (t, 1H, *J* = 8.0 Hz, Ar_H), 7.23-7.29 (m, 2H, Ar_H), 7.47 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.64 (s, 1H, Ar_H), 7.71 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.98 (d, 1H, *J* = 8.5 Hz, Ar_H), 10.88 (s, 1H, NH), 11.79 (s, 1H, NH), 11.95 (s, 1H,); ¹³C NMR (125 MHz) δ ppm: 111.38, 111.44, 112.30, 112.52, 112.63, 113.71, 113.92, 115.79, 115.87, 117.89, 118.08, 118.82, 119.01, 120.25, 120.52, 121.11, 122.35, 124.78, 124.98, 126.96, 127.05, 128.52, 137.12, 137.54, 138.61, 140.25, 156.50, 157.49, 158.38, 159.39, 161.26, 163.29, 164.94; MS *m/z* [%]: 322.02 [M⁺, 21.90], 144.05 [46.89], 89.19 [100]; Anal. calcd. For C₁₇H₁₁FN₄O₂: C, 63.35; H, 3.44; N, 17.38; Found C, 63.49; H, 3.42; N, 17.45.

N'-(5-Chloro-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6c.

Yield 85 %, m.p. > 300 °C (reported: > 300 °C [49]); Purity 98%; IR: 3442, 3347, 3137 (3NH), 1721, 1657 (2C=O); ¹H NMR (500 MHz) δ ppm: 6.91 (d, 1H, *J* = 8.0 Hz, Ar_H), 7.07 (t, 1H, *J* = 8.0 Hz, Ar_H), 7.25 (t, 1H, *J* = 7.5 Hz, Ar_H), 7.42-7.45 (m, 1H, Ar_H), 7.47 (d, 1H, *J* = 8.5 Hz, Ar_H), 7.63 (s, 1H, Ar_H), 7.71 (d, 1H, *J* = 8.0 Hz, Ar_H), 8.19 (s, 1H, Ar_H), 10.99 (s, 1H, NH), 11.85 (s, 1H, NH), 11.95 (s, 1H, NH); ¹³C NMR (125 MHz) δ ppm: 112.00, 112.52, 112.64, 112.74, 116.58, 120.26, 120.48, 120.52, 121.60, 122.36, 124.79, 124.99, 125.73, 126.15, 126.90, 126.96, 127.05, 128.50, 131.02, 131.98, 137.09, 137.56, 142.63, 160.84, 161.45, 162.98, 164.67; Anal. calcd. For C₁₇H₁₁ClN₄O₂: C, 60.28; H, 3.27; N, 16.54; Found C, 60.03; H, 3.31; N, 16.62.

N'-(5-Bromo-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6d.

Yield 85 %, m.p. > 300 °C (reported: > 300 °C [49]); Purity 96%; IR: 3433, 3344, 3161 (3NH), 1722, 1658 (2C=O); ¹H NMR (500 MHz) δ ppm: 6.87 (d, 1H, *J* = 8.5 Hz, Ar_H), 7.07 (t, 1H, *J* = 7.5 Hz, Ar_H), 7.25 (t, 1H, *J* = 7.5 Hz, Ar_H), 7.47 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.56 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.63 (s, 1H, Ar_H), 7.70 (d, 1H, *J* = 8.0 Hz, Ar_H), 8.31 (s, 1H, Ar_H), 11.00 (s, 1H, NH), 11.86 (s, 1H, NH), 11.95 (s, 1H, NH); Anal. calcd. For C₁₇H₁₁BrN₄O₂: C, 53.28; H, 2.89; N, 14.62; Found C, 53.13; H, 2.91; N, 14.72.

N'-(5-Methyl-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6e.

Yield 69 %, m.p. > 300 °C; Purity 99%; IR: 3455, 3329, 3140 (3NH), 1720, 1661 (2C=O); ¹H NMR (500 MHz) δ ppm: 2.31 (s, 3H, CH₃), 6.80 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.07 (t, 1H, *J* = 8.0 Hz, Ar_H), 7.20 (d, 1H, *J* = 8.0 Hz, Ar_H), 7.24 (t, 1H, *J* = 7.5 Hz, Ar_H), 7.47 (d, 1H, *J* = 8.5 Hz, Ar_H), 7.61 (s, 1H, Ar_H), 7.70 (d, 1H, *J* = 8.0 Hz, Ar_H), 7.89 (s, 1H, Ar_H), 10.76 (s, 1H, NH), 11.62 (s, 1H, NH), 11.89 (s, 1H, NH); ¹³C NMR (125 MHz) δ ppm: 20.58, 20.67 (CH₃), 110.42, 111.02, 112.51, 112.61, 115.60, 119.87, 120.22, 120.49, 121.32, 122.32, 124.66, 124.84, 126.97, 127.03, 127.07, 128.69, 130.73, 131.86, 132.12, 133.07, 137.06, 137.47, 140.12, 141.66, 160.75, 161.07, 163.24, 164.90; Anal. calcd. For C₁₈H₁₄N₄O₂: C, 67.92; H, 4.43; N, 17.60; Found C, 68.22; H, 4.40; N, 17.69.

N'-(5-Methoxy-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6f.

Yield 71 %, m.p. > 300 °C (reported: > 300 °C [49]); Purity 98%; IR: 3407, 3345, 3177 (3NH), 1722, 1664 (2C=O); ¹H NMR (500 MHz) δ ppm: 3.80 (s, 3H, OCH₃), 6.83 (d, 1H, *J* = 8.5 Hz, Ar_H), 6.99-7.02 (m, 1H, Ar_H), 7.06 (t, 1H, *J* = 8.0 Hz, Ar_H), 7.24 (t, 1H, *J* = 8.0 Hz, Ar_H), 7.46 (d, 1H, *J* = 8.5 Hz, Ar_H), 7.60 (s, 1H, Ar_H), 7.67 (brs, 1H, Ar_H), 7.70 (d, 1H, *J* = 7.5 Hz, Ar_H), 10.68 (s, 1H, NH), 11.80 (s, 1H, NH), 11.94 (s, 1H, NH); ¹³C NMR (125 MHz) δ ppm: 55.65, 55.84 (OCH₃), 111.16, 112.11, 112.50, 112.65, 115.91, 118.21, 120.21, 120.50, 120.59, 122.32, 124.69, 124.87, 126.94, 127.05, 128.67, 136.02, 137.03, 137.50, 137.61, 154.49, 155.47, 160.45, 161.33, 163.29, 164.91; Anal. calcd. For C₁₈H₁₄N₄O₃: C, 64.67; H, 4.22; N, 16.76; Found C, 64.89; H, 4.18; N, 16.83.

N'-(2-Oxo-5-(trifluoromethoxy)indolin-3-ylidene)-1H-indole-2-carbohydrazide 6g.

Yield 78 %, m.p. > 300 °C; Purity 96%; IR: 3405, 3370, 3138 (3NH), 1728, 1653 (2C=O); ¹H NMR (500 MHz) δ ppm: 7.07-7.13 (m, 2H, Ar_H), 7.25-7.29 (m, 1H, Ar_H), 7.46 (t, 1H, *J* = 8.5 Hz, Ar_H), 7.65 (s, 1H, Ar_H), 7.71 (d, 1H, *J* = 8.0 Hz, Ar_H), 8.29-8.32 (m, 1H, Ar_H), 9.01 (s, 1H, Ar_H), 11.56 (s, 1H, NH), 11.97 (s, 1H, NH), 12.27 (s, 1H, NH); Anal. calcd. For C₁₈H₁₁F₃N₄O₃: C, 55.68; H, 2.86; N, 14.43; Found C, 55.83; H, 2.88; N, 14.54.

N'-(5-Nitro-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6h.

Yield 84 %, m.p. > 300 °C; Purity 97%; IR: 3418, 3337, 3141 (3NH), 1724, 1665 (2C=O); ¹H NMR (500 MHz) δ ppm: 6.98 (d, 1H, *J* = 8.5 Hz, Ar_H), 7.05-7.10 (m, 1H, Ar_H), 7.25 (t, 1H, *J* = 7.0 Hz, Ar_H), 7.41 (d, 1H, *J* = 9.5 Hz, Ar_H), 7.47 (d, 1H, *J* = 8.0 Hz, Ar_H), 7.64 (s, 1H, Ar_H), 7.70 (d, 1H, *J* = 8.5 Hz, Ar_H), 8.19 (s, 1H, Ar_H), 11.06 (s, 1H, NH), 11.94 (s, 2H, NH); ¹³C NMR (125 MHz) δ ppm: 109.36, 112.53, 114.94, 120.27, 120.62, 122.25, 122.40, 123.31, 124.69, 126.43, 127.03, 128.74, 132.70, 137.15, 144.99, 161.73, 163.47; Anal. calcd. For C₁₇H₁₁N₅O₄: C, 58.46; H, 3.17; N, 20.05; Found C, 58.25; H, 3.30; N, 19.93.

N'-(5,7-Dimethyl-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 6i.

Yield 86 %, m.p. > 300 °C; Purity 95%; IR: 3349, 3273, 3171 (3NH), 1720, 1668 (2C=O); ¹H NMR (500 MHz) δ ppm: 2.18 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 7.02-7.26 (m, 4H, Ar_H), 7.46 (d, 1H, *J* = 7.5 Hz, Ar_H), 7.60 (s, 1H, Ar_H), 7.70 (d, 1H, *J* = 8.0 Hz, Ar_H), 10.78 (s, 1H, NH), 11.56 (s, 1H, NH), 11.93 (s, 1H, NH); ¹³C NMR (125 MHz) δ ppm: 16.03 (CH₃), 20.55 (CH₃), 112.49, 112.60, 115.32, 119.64, 120.19, 122.30, 124.41, 124.63, 127.06, 128.70, 130.60, 134.48, 137.03, 140.19, 163.72, 165.63; MS *m/z* [%]: 332.07 [M⁺, 22.68], 144.13 [43.89], 89.10 [100]; Anal. calcd. For C₁₉H₁₆N₄O₂: C, 68.66; H, 4.85; N, 16.86; Found C, 68.49; H, 4.88; N, 16.76.

N'-(1-Methyl-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 9a.

Yield 76 %, m.p. 259-261 °C (reported: 258-260 °C [49]); Purity 98%; IR: 3363, 3181 (2NH), 1722, 1659 (2C=O); ¹H NMR (500 MHz) δ ppm: 3.21 (s, 3H, N-CH₃), 7.07-7.13 (m, 2H, Ar_H), 7.16-7.19 (m, 1H, Ar_H), 7.25 (t, 1H, *J* = 8.0 Hz, Ar_H), 7.47-7.52 (m, 2H, Ar_H), 7.58 (s, 1H, Ar_H), 7.70 (d, 1H, *J* = 8.0 Hz, Ar_H), 8.06

(d, 1H, $J = 7.5$ Hz, Ar_H), 11.75 (s, 1H, NH), 11.96 (s, 1H, NH); ^{13}C NMR (125 MHz) δ ppm: 42.01 (N-CH₂), 109.87, 112.53, 115.18, 115.47, 115.64, 120.29, 122.28, 122.64, 124.74, 126.65, 127.04, 128.66, 129.42, 129.49, 129.66, 132.45, 132.56, 137.17, 143.70, 160.56, 161.07, 162.49, 163.65; MS m/z [%]: 318.20 [M^+ , 24.32], 144.16 [67.97], 89.14 [100]; Anal. calcd. For C₁₈H₁₄N₄O₂: C, 67.92; H, 4.43; N, 17.60; Found C, 67.73; H, 4.46; N, 17.69.

N'-(2-Oxo-1-propylindolin-3-ylidene)-1H-indole-2-carbohydrazide 9b.

Yield 69 %, m.p. 229-231 °C; Purity 99%; IR: 3450, 3284 (2NH), 1716, 1681 (2C=O); ^1H NMR (500 MHz) δ ppm: 0.88 (t, 3H, $J = 7.5$ Hz, -CH₂-CH₃), 1.60-1.67 (m, 2H, -CH₂-CH₃), 3.70 (t, 2H, $J = 7.5$ Hz, N-CH₂), 7.08 (t, 1H, $J = 7.0$ Hz, Ar_H), 7.15-7.19 (m, 2H, Ar_H), 7.25 (t, 1H, $J = 8.5$ Hz, Ar_H), 7.47-7.50 (m, 2H, Ar_H), 7.59 (s, 1H, Ar_H), 7.70 (d, 1H, $J = 8.5$ Hz, Ar_H), 8.07 (d, 1H, $J = 8.0$ Hz, Ar_H), 11.74 (s, 1H, NH), 11.96 (s, 1H, NH); ^{13}C NMR (125 MHz) δ ppm: 11.17 (-CH₂-CH₃), 20.40 (N-CH₂-CH₂), 40.87 (N-CH₂), 109.55, 112.52, 114.99, 120.27, 122.26, 122.30, 124.70, 126.60, 127.04, 128.70, 132.69, 137.15, 144.33, 160.85, 163.47; Anal. calcd. For C₂₀H₁₈N₄O₂: C, 69.35; H, 5.24; N, 16.17; Found C, 69.08; H, 5.29; N, 16.08.

Ethyl 2-(3-(2-(1H-indole-2-carbonyl)hydrazono)-2-oxoindolin-1-yl)acetate 9c.

Yield 81 %, m.p. 261-263 °C; Purity 96%; IR: 3347, 3245 (2NH), 1726, 1687 (2C=O); ^1H NMR (500 MHz) δ ppm: 1.21 (t, 3H, $J = 7.0$ Hz, CH₂-CH₃), 4.17 (q, 2H, $J = 7.0$ Hz, CH₂-CH₃), 4.74 (s, 2H, N-CH₂-CO), 7.09 (t, 1H, $J = 8.0$ Hz, Ar_H), 7.20-7.23 (m, 3H, Ar_H), 7.26 (t, 1H, $J = 8.0$ Hz, Ar_H), 7.45 (t, 2H, $J = 8.0$ Hz, Ar_H), 7.71 (t, 2H, $J = 8.0$ Hz, Ar_H), 12.15 (s, 1H, NH), 13.69 (s, 1H, NH); ^{13}C NMR (125 MHz) δ ppm: 14.04 (CH₃), 40.96 (N-CH₂), 61.47 (CH₂-CH₃), 110.38, 112.61, 119.03, 120.52, 120.80, 122.35, 123.62, 124.98, 126.95, 128.59, 131.63, 135.59, 137.55, 142.59, 161.25, 167.48; Anal. calcd. For C₂₁H₁₈N₄O₄: C, 64.61; H, 4.65; N, 14.35; Found C, 64.78; H, 4.67; N, 14.24.

N'-(1-Benzyl-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 9d.

Yield 81 %, m.p. 253-255 °C (reported: 249-251 °C [49]); Purity 98%; IR: 3453, 3368 (2NH), 1717, 1665 (2C=O); ^1H NMR (500 MHz) δ ppm: 5.00 (s, 2H, benzylic H), 7.04 (d, 1H, $J = 8.5$ Hz, Ar_H), 7.08 (t, 1H, $J = 7.5$ Hz, Ar_H), 7.14 (t, 1H, $J = 7.5$ Hz, Ar_H), 7.25-7.28 (m, 1H, Ar_H), 7.32-7.36 (m, 5H, Ar_H), 7.40 (t, 1H, $J = 7.5$ Hz, Ar_H), 7.47 (d, 1H, $J = 8.5$ Hz, Ar_H), 7.62 (s, 1H, Ar_H), 7.70 (d, 1H, $J = 8.5$ Hz, Ar_H), 8.10 (d, 1H, $J = 7.5$ Hz, Ar_H), 11.79 (s, 1H, NH), 11.98 (s, 1H, NH); MS m/z [%]: 394.06 [M^+ , 66.21], 144.06 [27.98], 91.13 [100]; Anal. calcd. For C₂₄H₁₈N₄O₂: C, 73.08; H, 4.60; N, 14.20; Found C, 72.71; H, 4.63; N, 14.28.

N'-(1-(4-Fluorobenzyl)-2-oxoindolin-3-ylidene)-1H-indole-2-carbohydrazide 9e.

Yield 84 %, m.p. 233-235 °C; Purity 97%; IR: 3448, 3378 (2NH), 1714, 1663 (2C=O); ^1H NMR (500 MHz) δ ppm: 4.99 (s, 2H, benzylic H), 7.07-7.09 (m, 2H, Ar_H), 7.15-7.19 (m, 3H, Ar_H), 7.25 (t, 1H, $J = 7.0$ Hz, Ar_H), 7.40-7.44 (m, 3H, Ar_H), 7.47 (d, 1H, $J = 8.0$ Hz, Ar_H), 7.61 (s, 1H, Ar_H), 7.70 (d, 1H, $J = 8.0$ Hz, Ar_H), 8.10 (d, 1H, $J = 7.5$ Hz, Ar_H), 11.77 (s, 1H, NH), 11.97 (s, 1H, NH); ^{13}C NMR (125 MHz) δ ppm: 41.95, 42.01, 109.85, 112.53, 115.18, 115.46, 115.63, 119.54, 120.28, 120.58, 120.89, 122.27, 122.63, 123.41, 124.73, 124.91, 126.63, 127.04, 128.65, 129.41, 129.48, 129.64, 129.71, 131.75, 132.46, 132.54, 137.17, 143.70, 160.56, 160.83, 162.48, 163.64; MS m/z [%]: 412.14 [M^+ , 44.30], 109.03 [100]; Anal. calcd. For C₂₄H₁₇FN₄O₂: C, 69.89; H, 4.15; N, 13.59; Found C, 70.23; H, 4.17; N, 13.68.

N'-(5-Chloro-2-oxo-1-propylindolin-3-ylidene)-1H-indole-2-carbohydrazide 9f.

Yield 73 %, m.p. 242-243 °C; Purity 99%; IR: 3450, 3284 (2NH), 1716, 1681 (2C=O); ^1H NMR (500 MHz) δ ppm: 0.84 (t, 3H, $J = 6.8$ Hz, -CH₂-CH₃), 1.57-1.60 (m, 2H, -CH₂-CH₃), 3.64 (t, 2H, $J = 6.8$ Hz, N-CH₂), 6.99 (t, 1H, $J = 7.2$ Hz, Ar_H), 7.08 (t, 1H, $J = 7.2$ Hz, Ar_H), 7.16 (s, 1H, Ar_H), 7.37-7.60 (m, 5H, Ar_H), 10.99 (s, 1H, NH), 12.39 (s, 1H, NH); ^{13}C NMR (125 MHz) δ ppm: 11.55 (-CH₂-CH₃), 20.78 (N-CH₂-CH₂), 41.29 (N-CH₂),

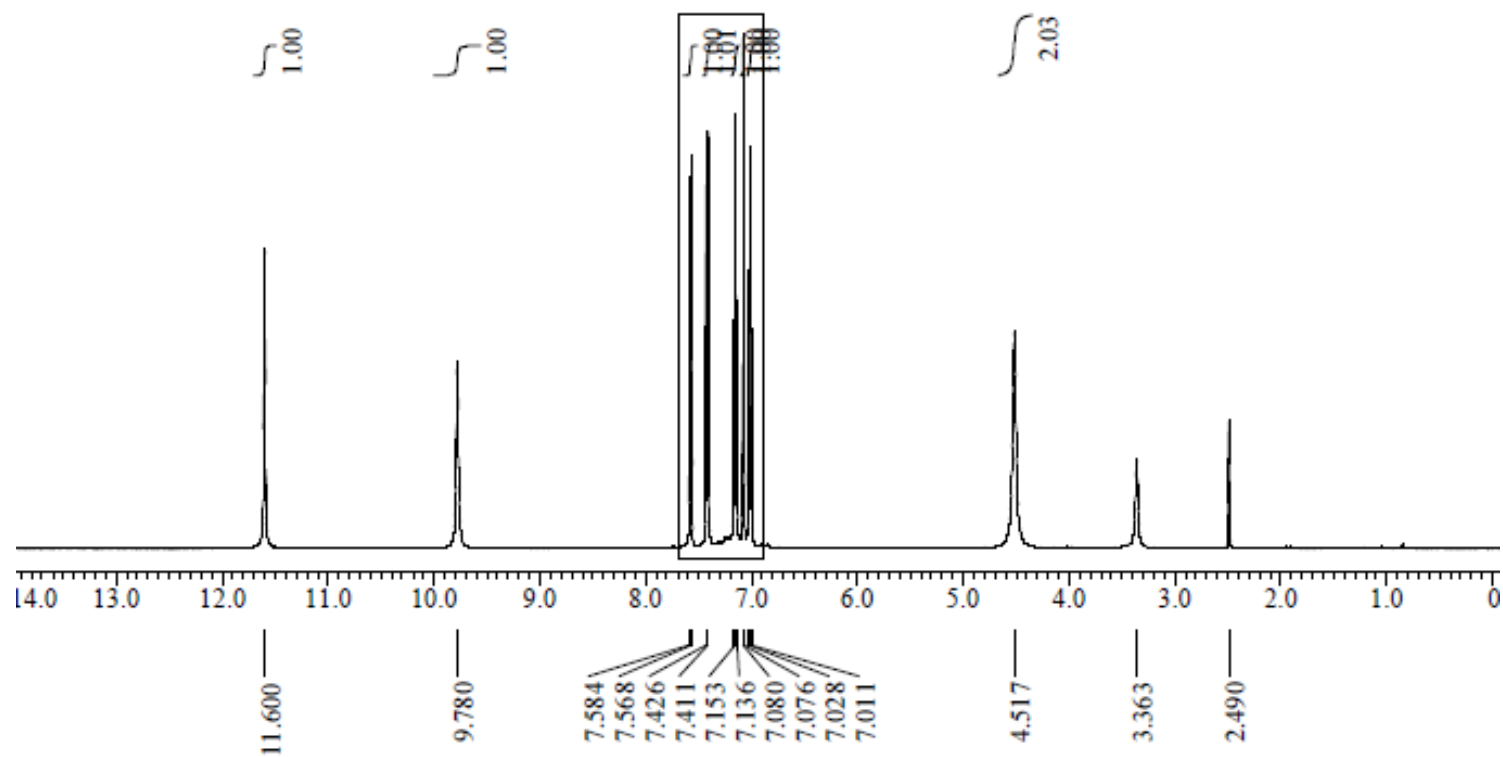
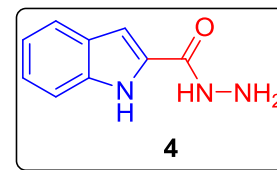
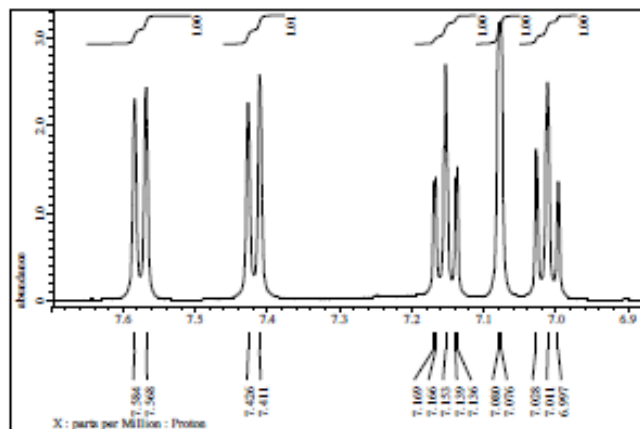
107.29, 111.95, 112.05, 119.00, 120.49, 121.45, 121.63, 125.01, 127.69, 131.05, 136.57, 142.02, 160.83; Anal. calcd. For $C_{20}H_{17}ClN_4O_2$: C, 63.08; H, 4.50; N, 14.71; Found C, 63.27; H, 4.53; N, 14.63.

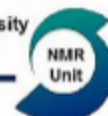
N'-(1,3-Dihydro-2H-inden-2-ylidene)-1H-indole-2-carbohydrazide 11a.

Yield 79 %, m.p. 219-212 °C; Purity 97%; IR: 3358, 3264 (2NH), 1689 (C=O); 1H NMR (400 MHz) δ ppm: 3.41 (s, 4H, 2 CH₂), 7.06 (t, 1H, J = 8.5 Hz, Ar_H), 7.20 (t, 1H, J = 8.5 Hz, Ar_H), 7.33 (d, 2H, J = 8.0 Hz, Ar_H), 7.37 (s, 1H, Ar_H), 7.41 (t, 2H, J = 8.0 Hz, Ar_H), 7.45 (d, 1H, J = 8.5 Hz, Ar_H), 7.65 (d, 1H, J = 8.5 Hz, Ar_H), 10.74 (s, 1H, NH), 11.92 (s, 1H, NH); Anal. calcd. For $C_{18}H_{15}N_3O$: C, 74.72; H, 5.23; N, 14.52; Found C, 75.11; H, 5.18; N, 14.47.

N'-(3,4-Dihydronaphthalen-1(2H)-ylidene)-1H-indole-2-carbohydrazide 11b.

Yield 74 %, m.p. 195-197 °C; Purity 98%; IR: 3329, 3283 (2NH), 1691 (C=O); 1H NMR (400 MHz) δ ppm: 1.84 (q, 2H, J = 6.5 Hz, CH₂-CH₂-CH₂), 2.78 (t, 4H, J = 6.5 Hz, CH₂-CH₂-CH₂), 7.04 (t, 1H, J = 8.0 Hz, Ar_H), 7.20 (t, 2H, J = 7.5 Hz, Ar_H), 7.28-7.33 (m, 2H, Ar_H), 7.39 (brs, 1H, Ar_H), 7.45 (d, 1H, J = 8.5 Hz, Ar_H), 7.65 (d, 1H, J = 8.5 Hz, Ar_H), 8.09 (d, 1H, J = 7.5 Hz, Ar_H), 10.63 (s, 1H, NH), 11.76 (s, 1H, NH); ^{13}C NMR (100 MHz) δ ppm: 21.44, 26.03, 28.93, 112.36, 119.88, 121.80, 123.84, 124.72, 126.35, 127.06, 128.70, 129.38, 132.31, 136.71, 140.18; MS m/z [%]: 303.13 [M^+ , 100], 144.18 [42.85]; Anal. calcd. For $C_{19}H_{17}N_3O$: C, 75.23; H, 5.65; N, 13.85; Found C, 75.32; H, 5.69; N, 13.77.



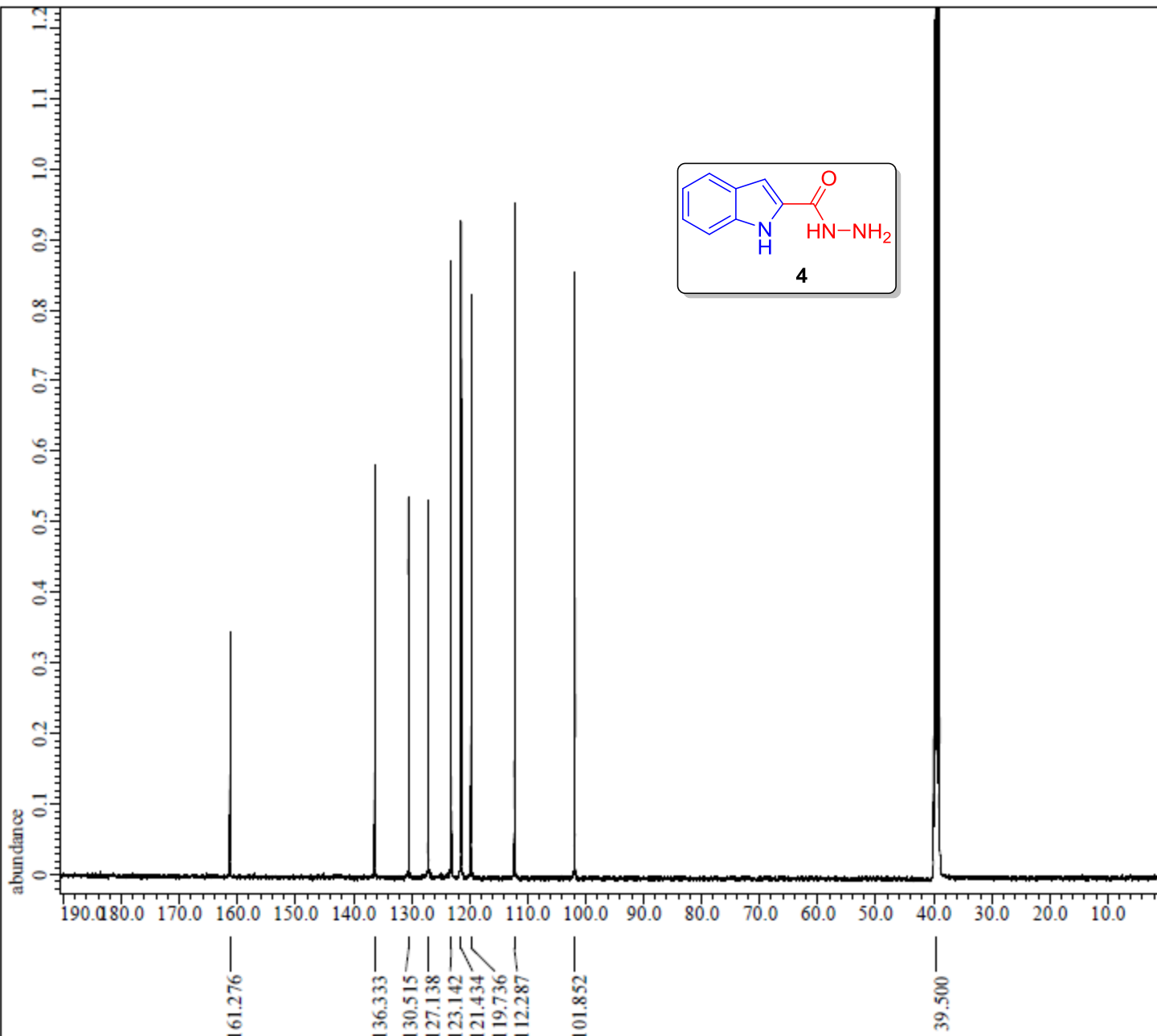
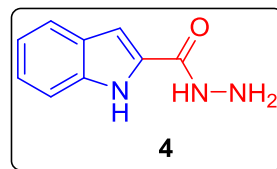


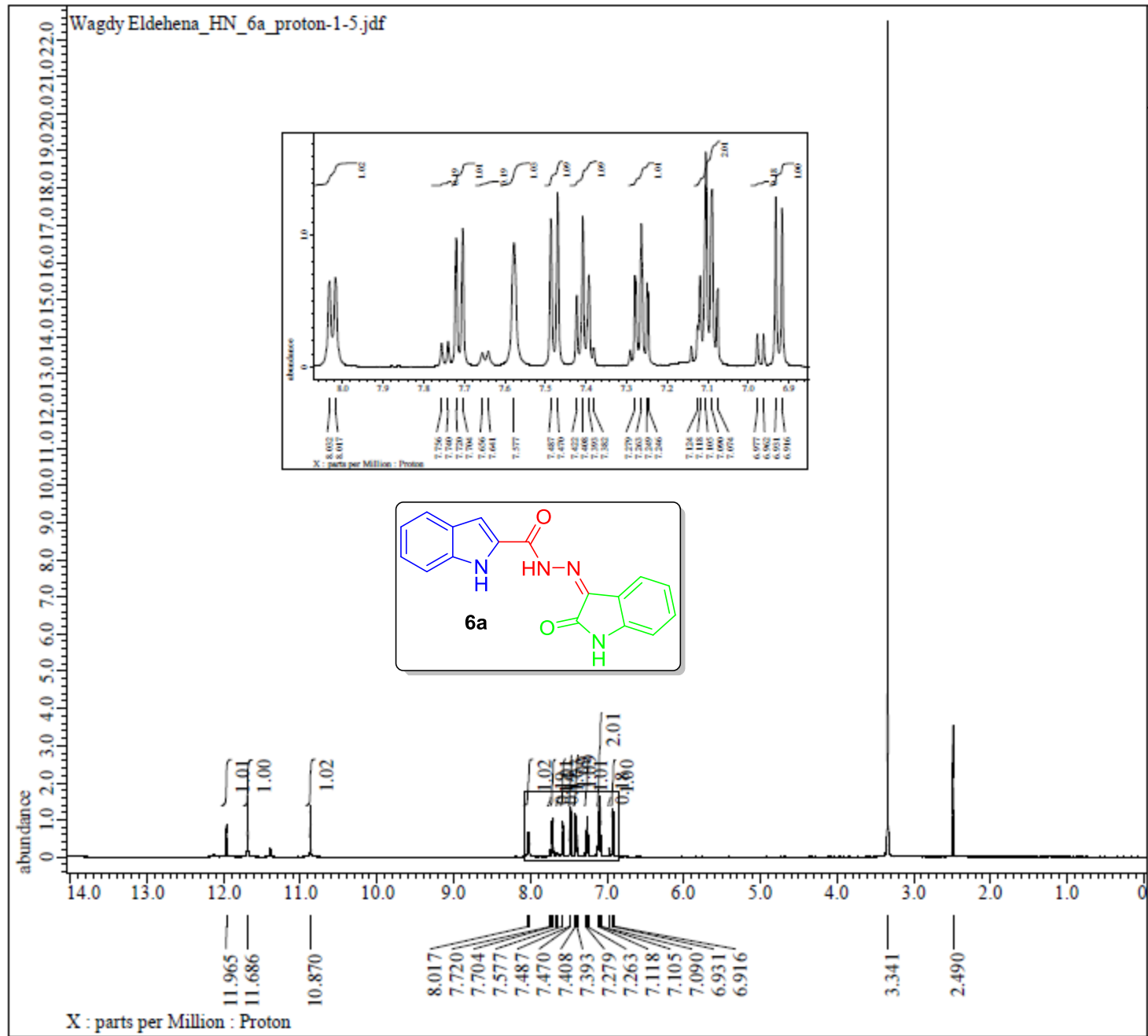
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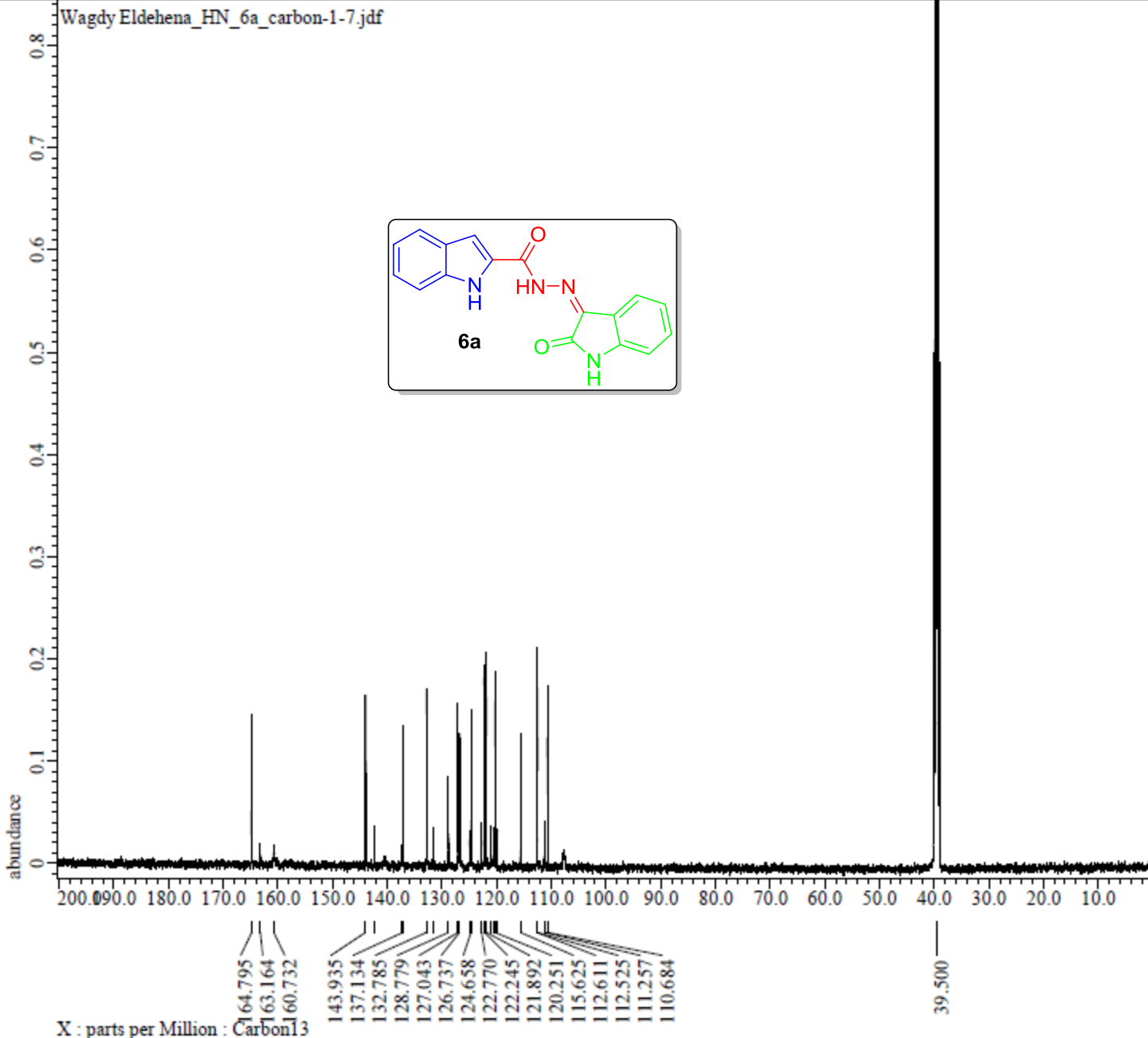
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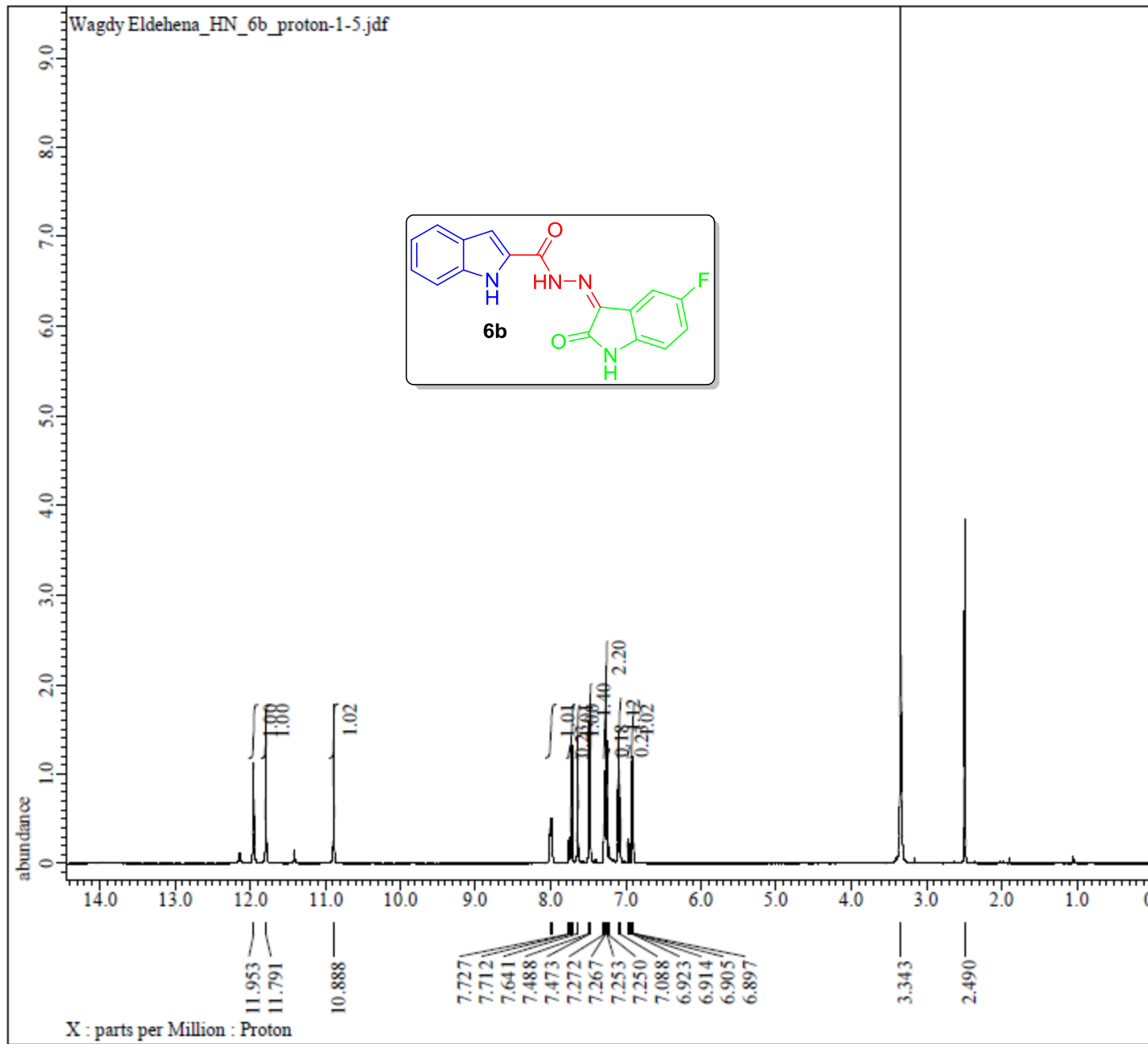
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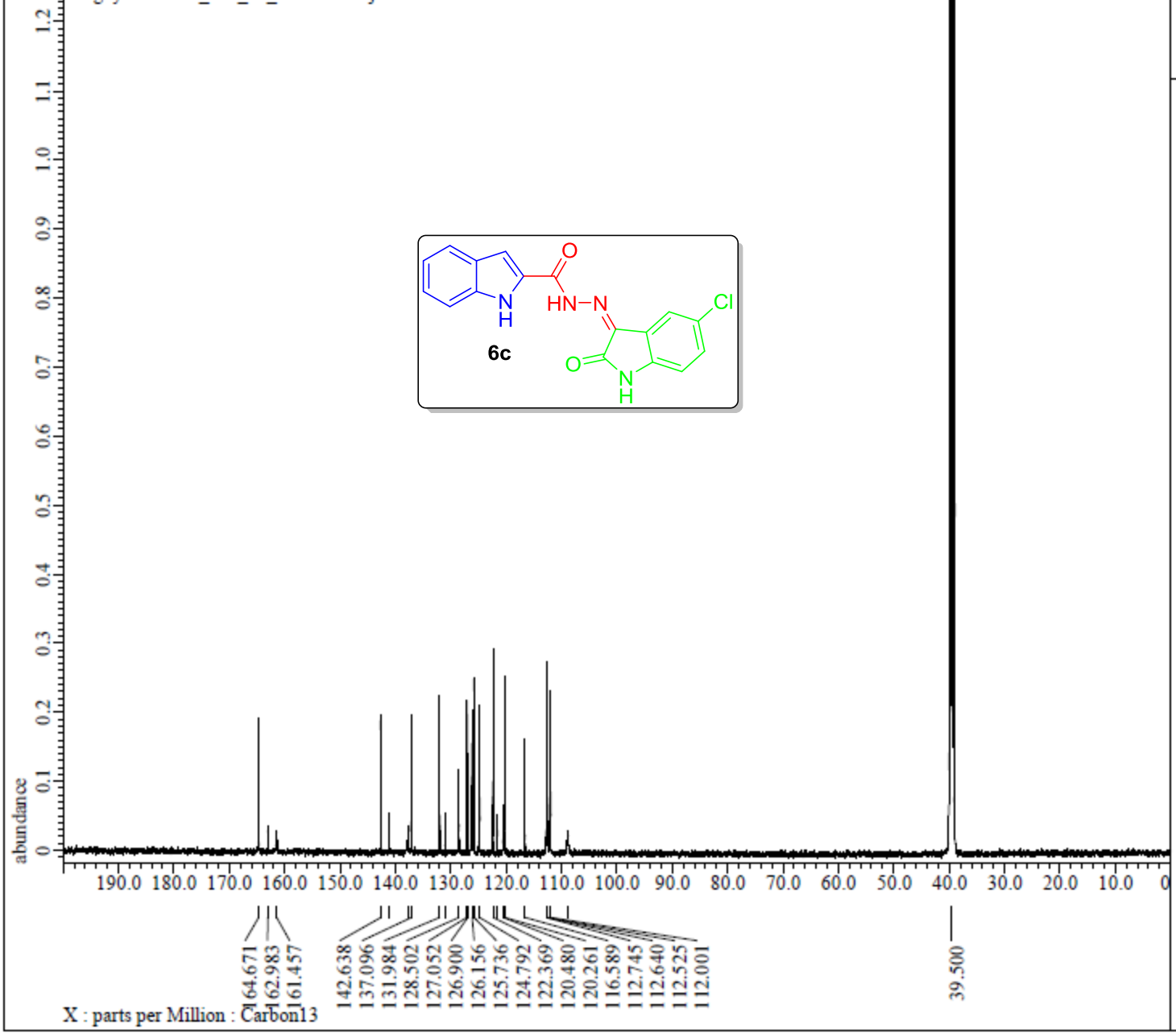
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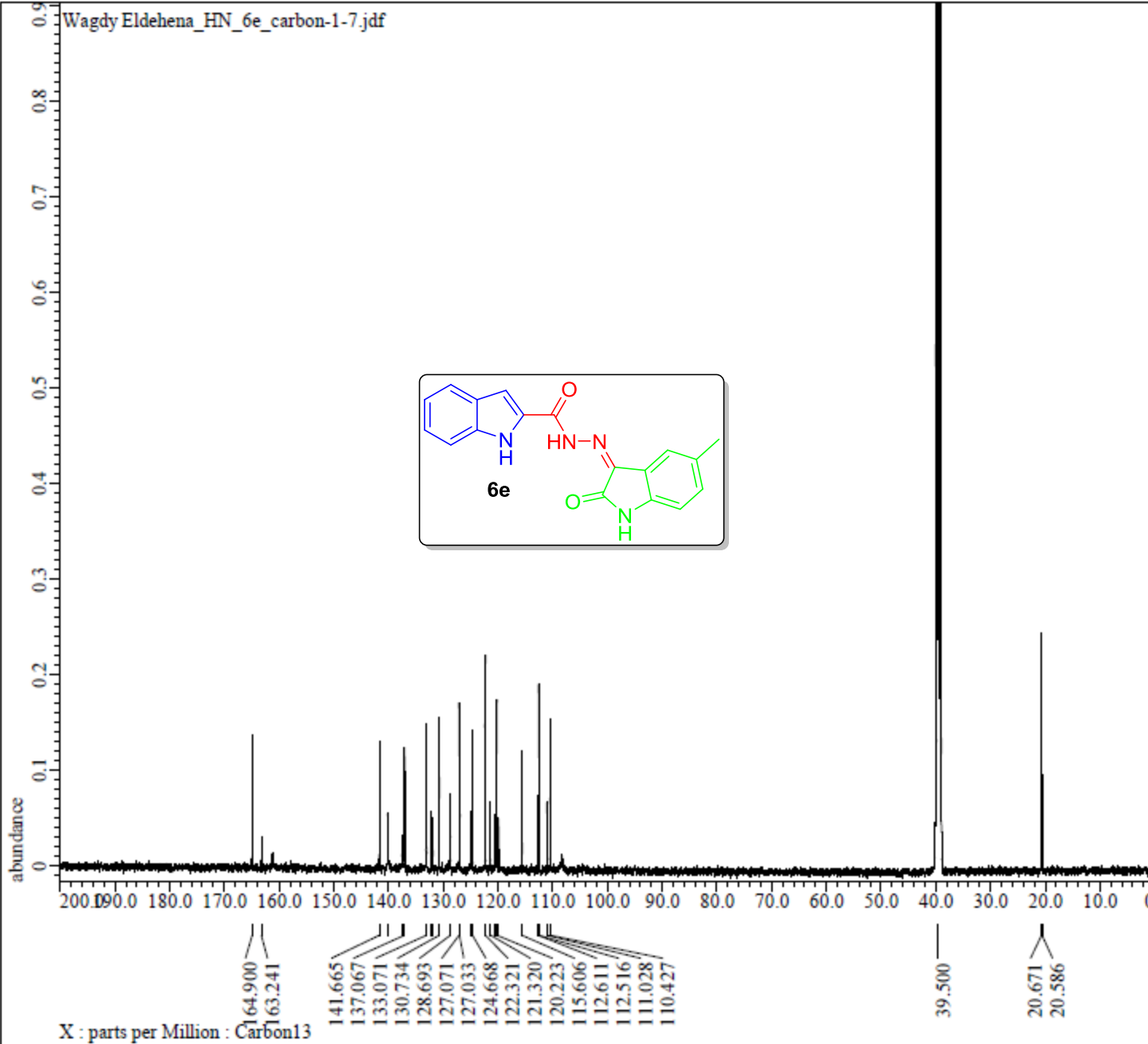


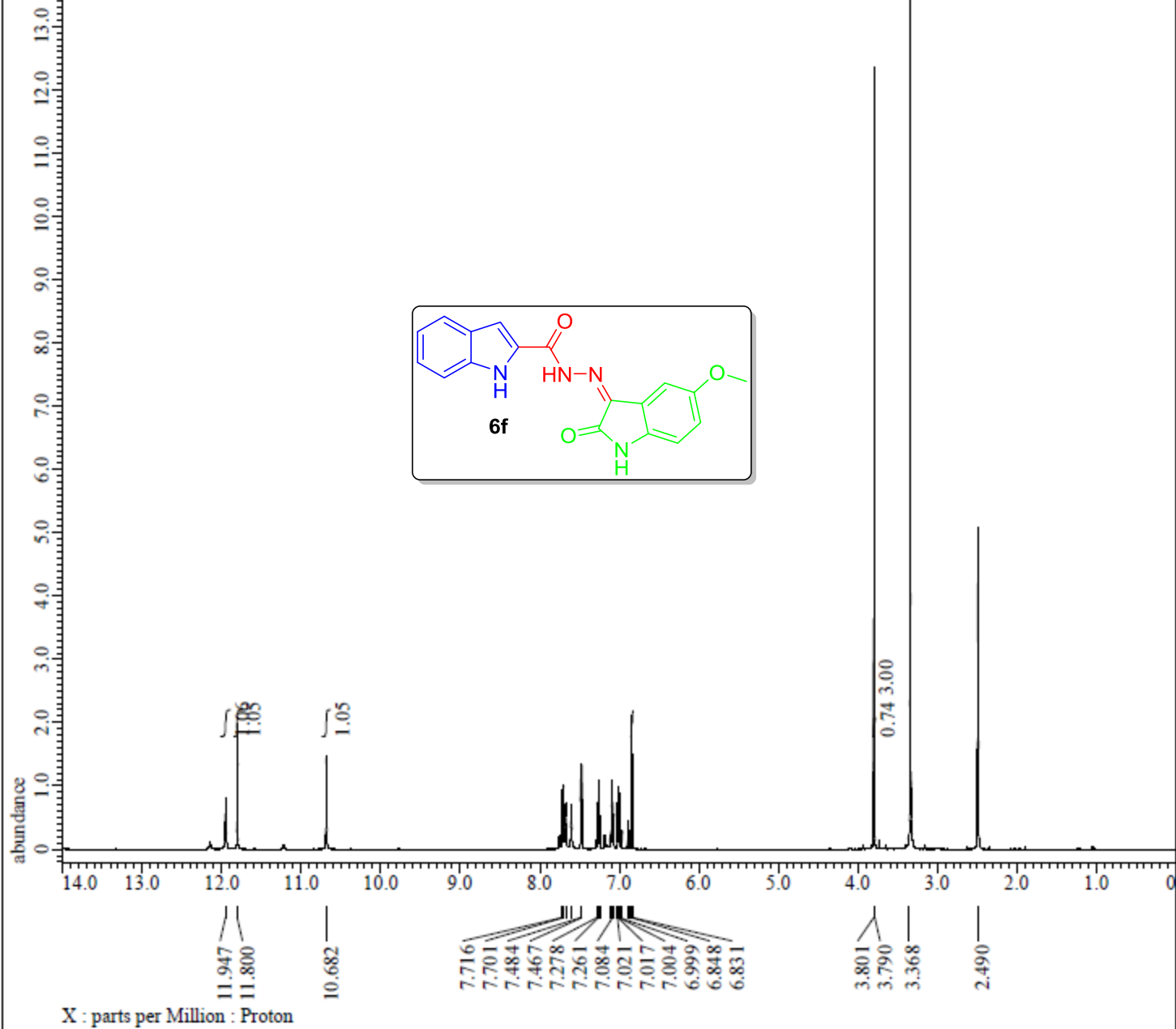


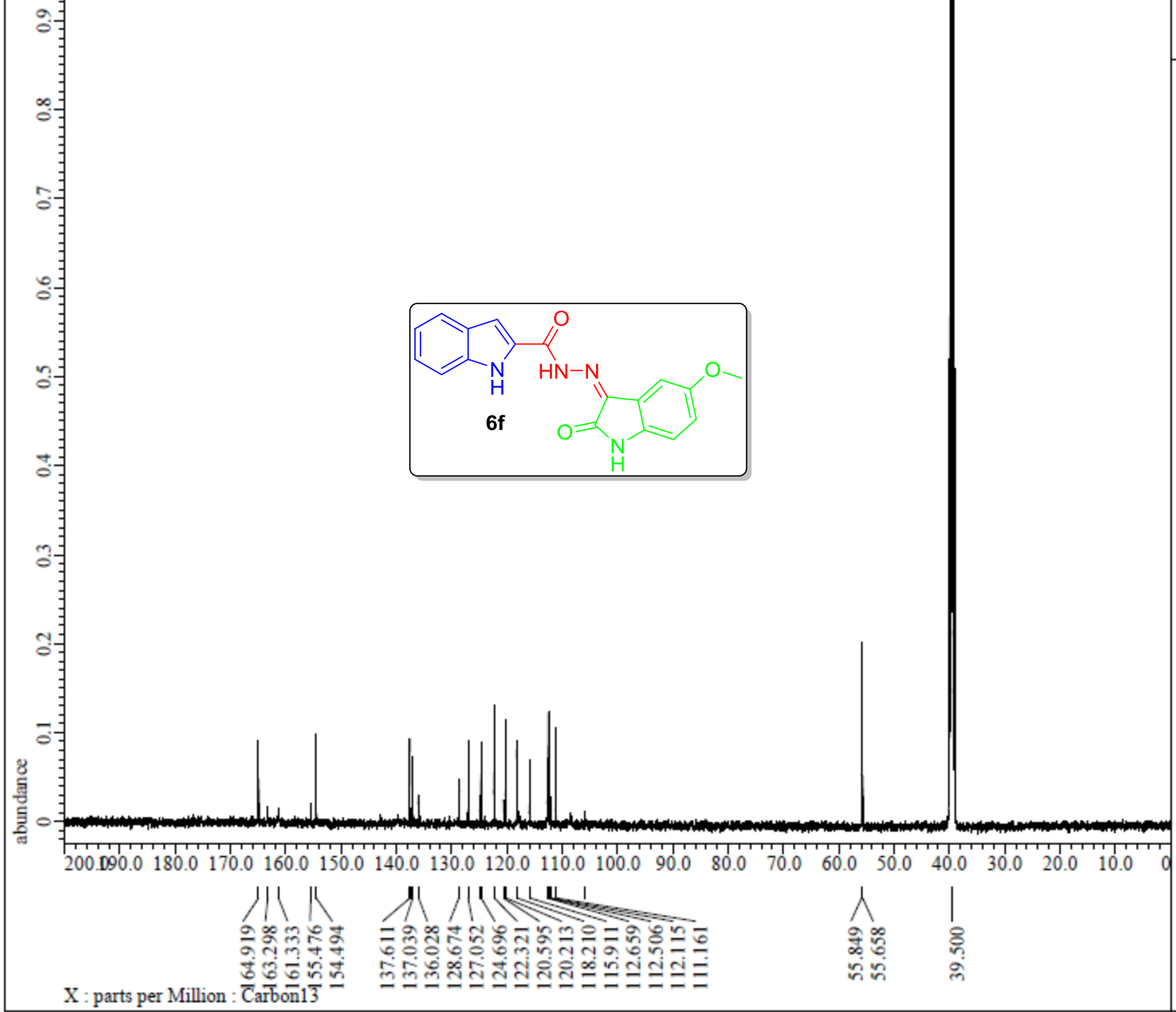


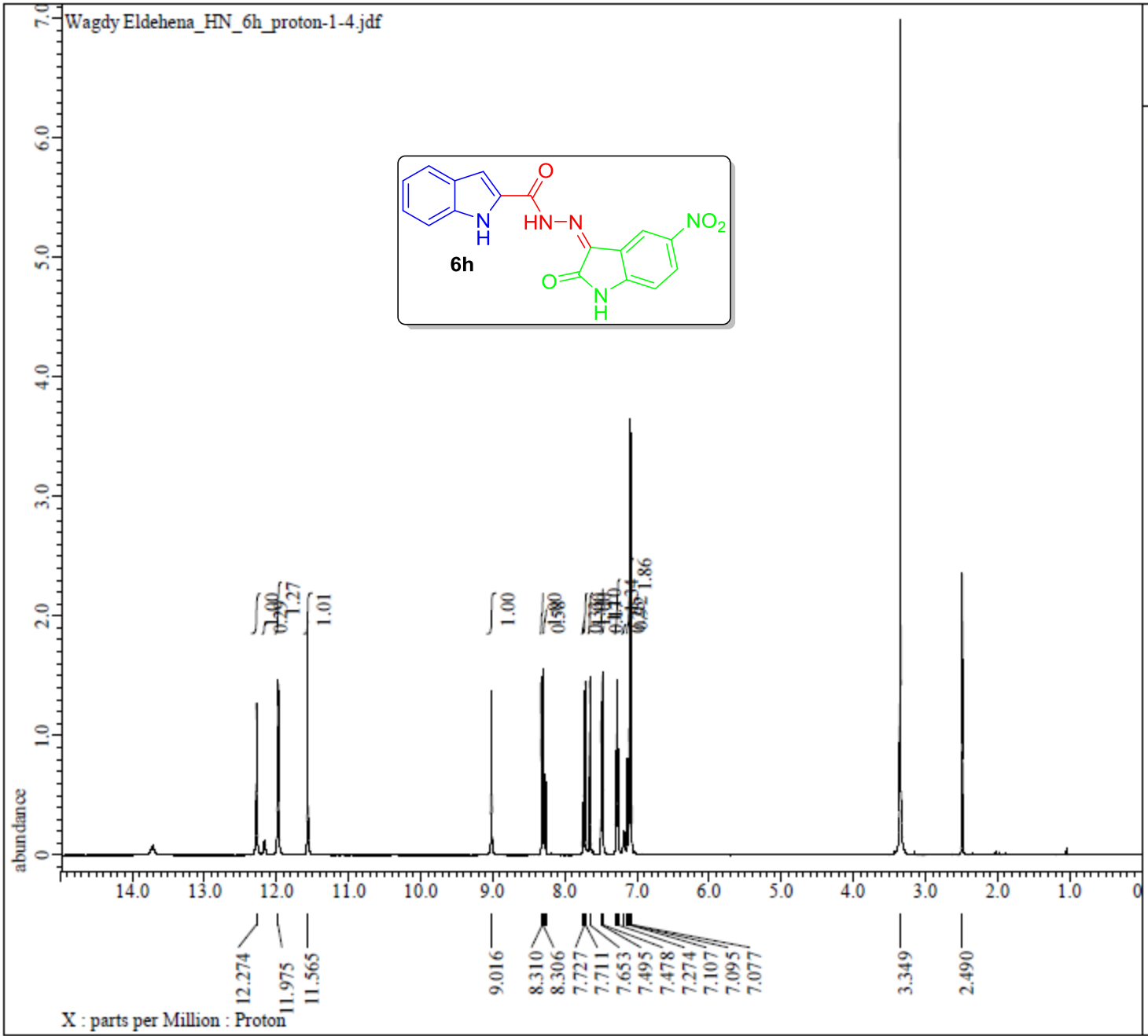


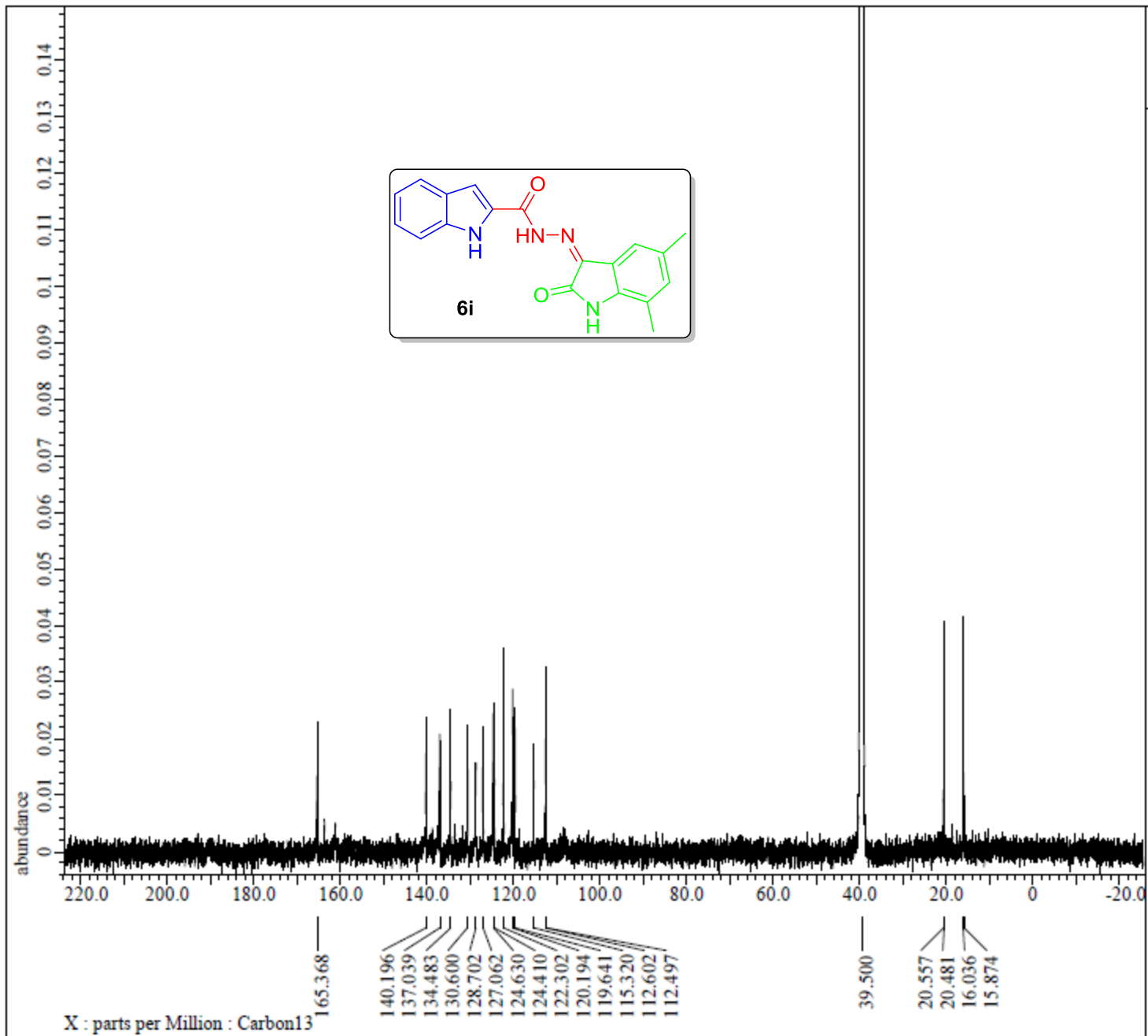


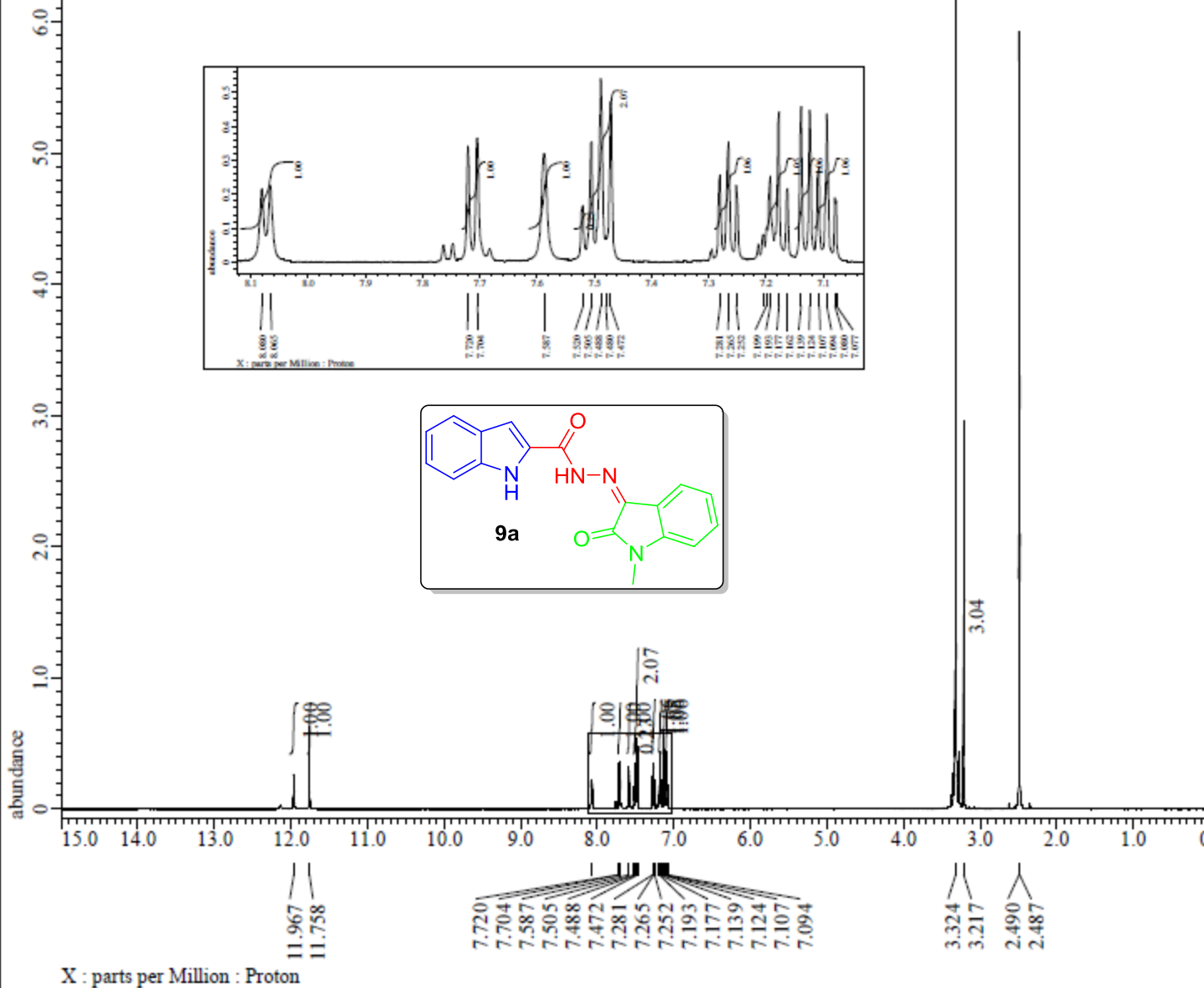


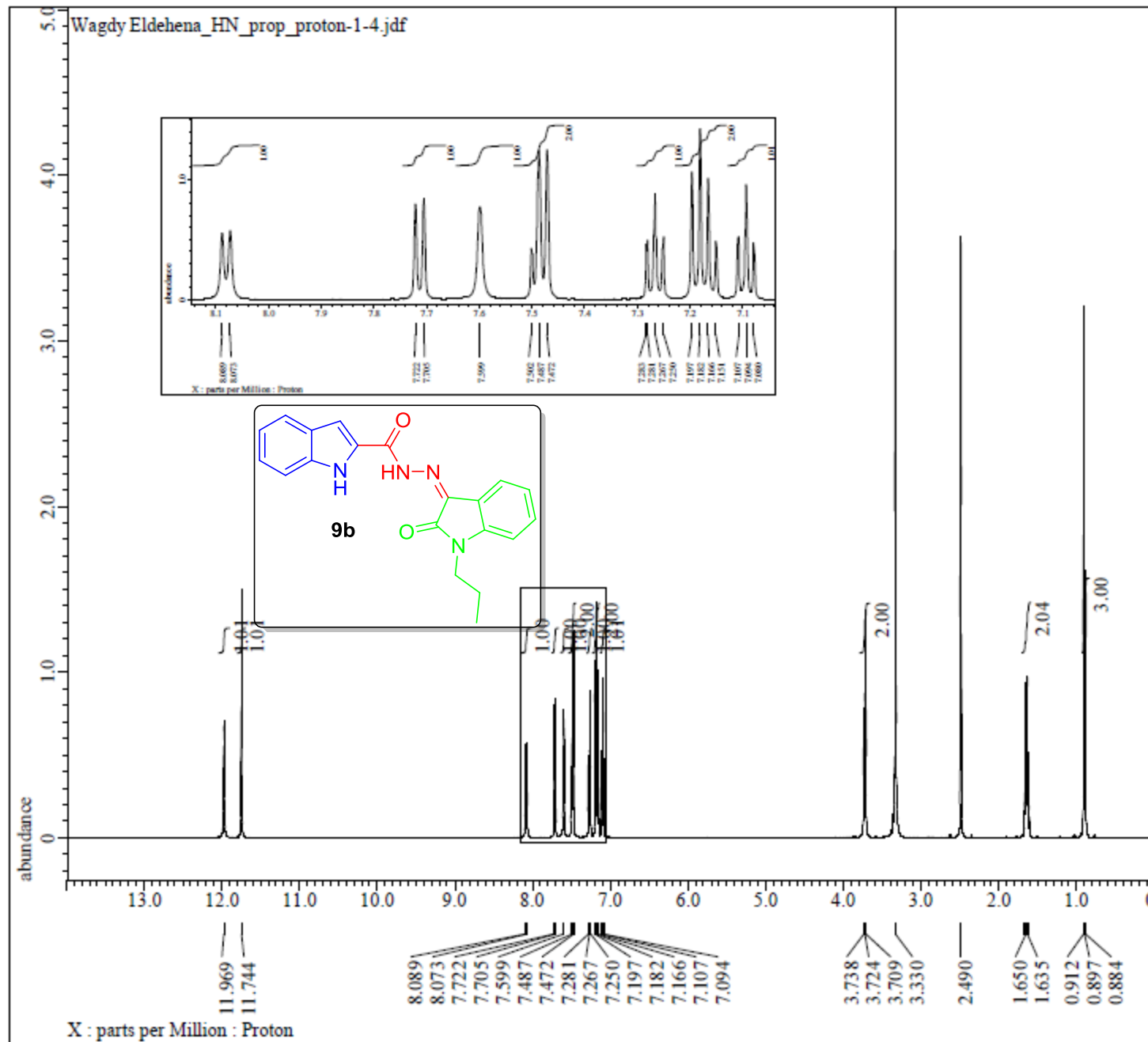








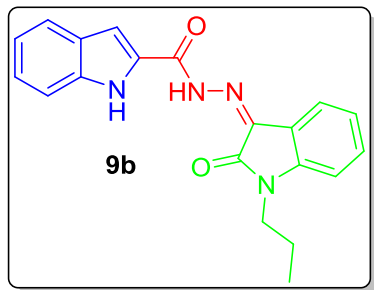




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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

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X : parts per Million : Carbon13

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- 144.336
- 137.153
- 132.699
- 128.702
- 127.043
- 126.604
- 124.706
- 122.302
- 122.264
- 120.271
- 114.996
- 112.525
- 109.559
- 40.874
- 39.500
- 20.404
- 11.171

